REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: 102000012886

Product name: Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L)

Chemical active substance(s):

Fluopyram, 250 g/L

Trifloxystrobin, 250 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(Re-Authorisation)

Applicant: Bayer Crop Science Division

Submission date: 30/06/2020 updated February 2021

Finalisation date: September 2021 (initial Core Assessment)

February 2022 (final Core Assessment)

Version history

When	What
June 2020	Original Bayer submission
February 2021	Methods of analysis not necessary for the central zone removed on ZRMS request.
September 2021	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~.
February 2022	Final report (Core Assessment after the commenting period) No additional information or assessments after the commenting period.

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Table of Contents

The product fluopyram + trifloxystrobin SC 500 (250 + 250 g/L) (FLU + TFS SC 500 / Product Code 102000012886) was not the representative formulation during the renewal of approval of trifloxystrobine. All data and information assessed during the EU re-evaluation of trifloxystrobine is considered EU peer-reviewed data.

Non renewed substance Fluopyram: according to the guidance SANCO/2010/13170 rev. 14, 7 October 2016, for product containing two or more substances, there is no need to evaluate data related to the « non-renewed » substance(s). It is therefore our understanding that only data pertaining to combitox assessment will be taken into consideration.

5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substances and relevant impurities in the plant protection product.

Noticed data gaps are: none

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.

Noticed data gaps are: none

Commodity/crop	Supported/ Not supported
Asparagus / high water content commodity	supported
Grape / high acid content commodity	supported
Strawberry / high acid content commodity	supported
Canefruit / high acid content commodity	supported
Other berries / high acid content commodity	supported
Celeriac / high water content commodity	supported
Lettuce and salad plants / high water content commodity	supported
Chicory witloof / high water content commodity	supported
Bean, peas with pods / high water content commodities	supported
Bean and pea without pods / high water content commodities	supported
Chickpea / high water content commodity	supported
Lentil / dry commodities	supported
Hops / difficult group	supported

State whether or not submitted data are sufficient for evaluation and whether enforcement of all relevant MRLs/residue levels is possible. Data gaps should be listed and conditions for registration presented, if appropriate. Provide an overview on the commodities/crops that are supported/ not supported as outcome of the evaluation on the available analytical methods.

5.2 Methods used for the generation of pre-authorization data (KCP 5.1)

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

5.2.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of fluopyram and trifloxystrobin in plant protection product is provided as follows:

Comments of zRMS:	The analytical methods AM009707MF1 was successfully validated for the determination of the active substances fluopyram and trifloxystrobin in the plant protection product according to the requirements laid down by SANCO3030/99 rev.5.

Additionally, to the method(s) previously submitted and reviewed at European level, new methods have been developped and validated.

Analytical method for the determination of the active substance in formulation:

Analytical method AM009707MF1

Reference:	**KCP 5.1.1/01**
Title:	Determination of fluopyram and trifloxystrobin in formulations assay - GLC, internal standard
Report:	Schulz, F.; 2007; AM009707MF1; M-286825-01-2
Authority registration No:	--
Guideline(s):	Equivalent to U S EPA OPPTS Guideline No. 830.1800
Deviations:	Not specified
GLP/GEP:	No
Acceptability:	Yes
Duplication (if vertebrate study):	No

Reference:	**KCP 5.1.1/02**
Title:	Validation of GLC-method AM009707MF1 - Determination of fluopyram and trifloxystrobin in formulations - fluopyram + trifloxystrobin SC 500 (250+250 g/L)
Report:	Bastian-Bertrams, V.; Schulz, F.; 2015; VB1.1-AM009707MF1; M-286826-02-1
Authority registration No:	--
Guideline(s):	REGULATION (EC) No 1107/2009, Commission Regulation (EU) 284/2013, 5.1, SANCO/3030/99 rev. 4, US EPA OCSPP 830.1800
Deviations:	Not specified
GLP/GEP:	No
Acceptability:	Yes
Duplication (if vertebrate study):	No

Materials and methods
After the addition of a standard substance (dipentylphtalate) as internal standard and dilution with a suitable solvent (acetone), the active substance is determined by gas chromatography coupled to a flame ionisation detector (FID).

Equipment and operating conditions are as follows:
Gas chromatographic conditions:

Column : fused silica, i.d. 0.1 mm, length 10 m
Column coating : DB 17

Film thickness		:	0.2 µm	
Temperatures				
	Detector	:	300 °C	
	Injector	:	260 °C	
	Oven	:	200 °C → 10 °C/min → 300 °C	
Carrier gas		:	helium approx. 0.7 ml/min	
Split ratio		:	1 : 100	
Injection volume		:	1 µl	
Combustion gases:	Hydrogen	:	approx. 30 ml/min	
	Air	:	approx. 400 ml/min	
Make-up gas		:	helium approx. 30 ml/min	
Running time		:	approx. 10 min	
Total retention times:				
Standard substance		:	dipentylphtalate	approx. 3.7 min
Component to be determined		:	fluopyram	approx. 3.2 min
			trifloxystrobin	approx. 4.0 min

Analytical Method Validation

The method AM009707MF1 was validated for the determination of fluopyram and trifloxystrobin in the formulation fluopyram + trifloxystrobin SC 500 (250+250 g/L). This was accomplished by evaluating specificity, linearity, accuracy, and precision.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of the active substances fluopyram and trifloxystrobin in plant protection product FLU + TFS SC 500

Analyte	Fluopyram	Trifloxystrobin
Author(s), year	Bastian-Bertrams, V., Schulz, F., 2015	
Principle of method	GC-FID	
Linearity For each a.i.: 6 concentrations (single injections); Range: 50-150 % of expected concentration	The function is linear in the operation range. Correlation coefficient r_k: 0.9998 Regression equation: y = -0.0162 + 0.6946 x	The function is linear in the operation range. Correlation coefficient r_k: 0.9998 Regression equation: y = - 0.0214 + 0.8286 x
Precision 6 samples (single injection) from one batch; Assessment of repeatability	The precision is acceptable according to the Horwitz equation; the Horwitz ratio (H_r) with ≤ 1 is found to be acceptable. mean measured value: 21.697 RSD: 0.16 % Horwitz-Value RSDr(max)(%): 1.69% Hr: 0.095 No outliers have been detected.	The precision is acceptable according to the Horwitz equation; the Horwitz ratio (H_r) with ≤ 1 is found to be acceptable. mean measured value: 21.653 RSD: 0.16 % Horwitz-Value RSDr(max)(%): 1.69% Hr: 0.095 No outliers have been detected.
Accuracy For each a.i.: 6 samples of laboratory-prepared synthetic formulation containing known weight of analyte; Statistical assessment of the recovery results; Calculation of the confidence interval	Mean recovery: 100.7% Confidence interval of recovery: 100.70 ± 0.54 The method shows no constant systematic error. The method shows no proportional systematic error.	Mean recovery: 100.7% Confidence interval of recovery: 100.66 ± 0.59 The method shows no constant systematic error. The method shows no proportional systematic error.
Interference Comparison of reference item, sample and blank chromatograms with regard to interferences	No interferences observed.	No interferences observed.

Specificity Comparison of retention times and MS-spectra from reference substance and sample	GC: Retention times of active ingredients and reference items are identical. GC-MS: The MS-spectra of analyte from reference items and sample show no spectral difference, the retention times are identical	GC: Retention times of active ingredients and reference items are identical. GC-MS: The MS-spectra of analyte from reference items and sample show no spectral difference, the retention times are identical
Comment	No deviation from guideline SANCO/3030/99 rev.5.	

Conclusion
The method AM009707MF1 was validated with success for the determination of fluopyram and trifloxystrobin in the formulation fluopyram + trifloxystrobin SC 500 (250+250 g/L) according to the requirements laid down by SANCO/3030/99 rev.5, all criteria were met.

The method was employed with success on the formulation above. It may also be applied to other formulations containing the active ingredients fluopyram and trifloxystrobin if the absence of chromatographic interferences is ensured.

5.2.1.2 Description of analytical methods for the determination of relevant impurities (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of relevant impurities in plant protection product is provided as follows:

Comments of zRMS:	The analytical method AM033118MF1 was successfully validated for the determination of of the relevant impurity CGA 344605 in the preparation according to the requirements of SANCO/3030/99 rev. 5. and is considered fit for purpose.

Reference:	**KCP 5.1.1/03**
Title:	Determination of CGA 344605 in formulations - HPLC-UV external standard
Report:	Hein, E. M.; Bowen, T.; 2018; AM033118MF1; M-639504-01-1
Authority registration No:	--
Guideline(s):	Commission Regulation (EU) 284/2013 in accordance with Regulation (EC) No 1107/2009 (10/2009); US EPA OCSPP No. 830.1800 (08/1996)
Deviations:	None
GLP/GEP:	No
Acceptability:	Yes
Duplication (if vertebrate study):	No

Reference:	**KCP 5.1.1/04**
Title:	Amendment no. 1 to final report - Validation of analytical method AM033118MF1 - Determination of CGA 344605 in the formulation fluopyram + trifloxystrobin SC 500 (250+250 g/L)
Report:	Hein, E. M.; 2018; VB2-AM033118MF1; M-644196-02-1
Authority registration No:	--
Guideline(s):	SANCO/3030/99 rev. 4 (07/2000); Commission Regulation (EU) 284/2013 (03/2013) in accordance with Regulation (EC) No 1107/2009 (10/2009); US EPA OCSPP Test Guideline No. 830.1800 (08/1996)
Deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study):	No

Materials and methods
The method was developed for the determination of CGA 344605 in formulations. CGA 344605 is an impurity of the active substance trifloxystrobin.

The analyte CGA 344605 is separated from active substances and formulation components on a reversed phase column using gradient elution. After UV detection, the quantitative evaluation is carried out by comparing the peak areas with those of reference items, using either a single-point calibration or a multi-point calibration curve.

Equipment and operating conditions are as follows:
Chromatographic conditions:

Column	Ascentis Express C18; 2.7 µm, 100 x 4.6 mm			
Supplier	Supelco			
Flow rate	1.8 mL/min			
Temperature	35 °C			
Injection volume	5 µL			
Eluent A	purified water + 0.1% (v/v) phosphoric acid			
Eluent B	acetonitrile + 0.1% (v/v) phosphoric acid			
Gradient program				
	Time [min]	A[%]	B[%]	flow rate [mL/min]
•Separation	0.0	54	46	1.8
	6.0	40	60	1.8
•Rinsing Gradient	6.2	5	95	1.8
	8.0	5	95	1.8
	8.2	54	46	1.8
	13.0	54	46	1.8
Total run time	13.0 min			
Retention time	CGA 344605 approx. 1.7 min			
Measurement wavelength	210 nm			

Analytical Method Validation
The method AM033118MF1 was validated for the determination of CGA 344605 in the formulation fluopyram + trifloxystrobin SC 500 (250+250 g/L). This was accomplished by evaluating specificity, linearity, accuracy, and precision.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of CGA 344605 in plant protection product FLU + TFS SC 500

Analyte	**CGA 344605**
Author(s), year	Hein, E.-M.; 2018
Principle of method	HPLC-UV
Linearity regression equation correlation coefficient type of regression function concentration range [mg/50 mL] concentration range [% (w/w)][1] concentration range [% MAL][2]	n = 8 y = - 0.00029 + 3.09156 x 0.99999 linear (1st order) 0.0151 – 0.2540 0.00646 – 0.1086 7.55 – 127
Precision mean value [% (w/w)][1] RSD [%] outliers detected Horwitz-Value RSDr(max) [%] Horrat value (Horwitz ratio) Hr	n = 6 0.0470 2.81 No 4.25 0.66 The Horrat value (Horwitz ratio, Hr) is ≤ 1 and thus, the precision of the analytical method is assessed acceptable.
Accuracy • fortification level I mean recovery [%]	n = 6 106.0

RSD [%] concentration [mg/50 mL] concentration [% (w/w)][1] concentration [% MAL][2] • fortification level II mean recovery [%] RSD [%] concentration [mg/50 mL] concentration [% (w/w)][1] concentration [% MAL][2]	3.03 0.0221 0.00945 11.05 99.52 0.66 0.221 0.0945 110.5
Limit of Quantification LOQ [mg/50 mL] LOQ [% (w/w)][1] LOQ [% MAL][2]	 0.0221 0.00945 11.05
Interference Comparison of reference item, sample and blank chromatograms with regard to interferences	No interferences observed.
Specificity Comparison of retention times and UV-spectra from reference substance and sample	Retention time and UV-spectra from reference item and sample are identical.
Comment	No deviation from guideline SANCO/3030/99 rev.5.

1 Referred to a nominal test item concentration of 234 mg/50 mL.
2 Calculated based on a max. accepted level (MAL) of CGA 344605 of 0.0856% (w/w) in formulation.

Conclusion
The method AM033118MF1 was validated for the determination of CGA 344605 in the formulation fluopyram + trifloxystrobin SC 500 (250+250 g/L) according to the requirements laid down by SANCO/3030/99 rev.5, all criteria were met.

5.2.1.3 Description of analytical methods for the determination of formulants (KCP 5.1.1)

With respect to toxicological, eco-toxicological or environmental aspects the product FLU + TFS SC 500 does not contain any relevant formulants. Therefore, a special analytical method and validation is not needed.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is no CIPAC method available for the determination of trifloxystrobin, in formulation.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Fluopyram

zRMS comments:
Fluopyram
Not required according to Article 43 of Regulation (EC) No 1107/2009.
However the summaries and concurrent validations presented in the Table 5.2-3 and Appendix 2 refer to plant and ecotoxicity studies presented in the dRR B7/9 Core document. Due to the fact that the test item of these tests were several formulations containing fluopyram as one of the active substances and that fluopyram was the analyte of the analytical monitoring in these studies (chosen as representative substance for the whole product), these summaries are reported within the fluopyram section.

Table 5.2-3: Validated methods for the generation of pre-authorization data

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Component of residue definition:				
Plants, plant products (Residues)	Not relevant.			
Component of residue definition:				
Animal products, food of animal origin (Residues)	Not relevant.			
Body fluids (Exposure)	Not relevant.			
Component of residue definition:				
Soil, water,... (Efficacy)	Not Relevant			
Feed, body fluids,... (Toxicology)	Not Relevant			
Body fluids, air,.... (Exposure)	Not relevant.			
Leaf punch washings (Exposure) Apple	Primary **Method 01158/M001**	20 µg/L (for extraction of 400 cm² leaf surface; corresponds to 0.01 µg/cm²)	HPLC-MS/MS	Stuke, S., Diehl, P., 2014; M-502699-02-1; not EU agreed, see Appendix 2
Leaf punch washings (Exposure) Grape	Primary **Method 01158/M001 Supplementary validation**	20 µg/L (for extraction of 400 cm² leaf surface; corresponds to 0.01 µg/cm²)	HPLC-MS/MS	Stuke, S., Daniels, M., van Berkum, S., 2016; M-569303-01-1; not EU agreed, see Appendix 2
Leaf punch washings (Exposure) Lily	Primary **Method 01158/M001 Supplementary validation**	20 µg/L (for extraction of 400 cm² leaf surface; corresponds to 0.01 µg/cm²)	HPLC-MS/MS	Stuke, S., van Berkum, S., 2016; M-558518-01-1; not EU agreed, see Appendix 2
Leaf punch washings (Exposure) Raspberry	Primary **Method 01158/M001 Supplementary validation**	0.8 µg/L (for extraction of 400 cm² leaf surface; corresponds to 0.01 µg/cm²)	HPLC-MS/MS	Daniels, M., van Berkum, S., 2020; M-677729-01-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary **Method**	Fluopyram 0.05 µg/L	HPLC-MS/MS	Krebber, R., 2014; M-494841-02-1;

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	01387/M001			not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary **Method 01387/M001 Supplementary validation**	Fluopyram 0.4 µg/L	HPLC-MS/MS	xxx., 2018; M-636236-01-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary **Method 01387/M001 Supplementary validation**	Fluopyram 0.27 µg/L	HPLC-MS/MS	xxx, 2018; M-636231-01-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary **Method 01387/M001 Supplementary validation**	Fluopyram 0.0625 µg/L	HPLC-MS/MS	Kuhl, K., 2018; M-615579-01-1; not EU agreed, see Appendix 2 in support of Krebber, R., Braune, M., 2013; M-466732-01-1
Water (Ecotoxicology)	Primary **Method 01387/M001 Supplementary validation**	Fluopyram 0.2 µg/L	HPLC-MS/MS	xxx., 2018; M-636234-01-1; not EU agreed, see Appendix 2

Trifloxystrobin

An overview on the acceptable methods and possible data gaps for analysis of residues of trifloxystrobin for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Only methods used in support of the new residue studies and not previously evaluated in the EU peer review are reflected in the table below. Methods for determination of residues in plant and animal commodities which were evaluated in the DAR (UK, 2000 and 2017) and EFSA Conclusion on the peer review of Trifloxystrobin (EFSA, 2017) and which were used for analysis of the residue data package evaluated in the EU peer review are considered acceptable and are not included in the table below.

1. Re-Assessment Report on the active substance trifloxystrobin prepared by the rapporteur Member State UK in the framework of Regulation (EC) No 1107/2009, [RAR, 2017]
2. Conclusion on the peer review of the pesticide risk assessment of the active substance trifloxystrobin. EFSA Journal 2017;15(10):4989 [EFSA, 2017]

Table 5.2-4: Validated methods for the generation of pre-authorization data

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Component of residue definition: Trifloxystrobin (CGA 279202) and its metabolite CGA321113				
Plants, plant products (Residues) Citrus, fruit Peas, fruit Wheat, grain Rape, seed Corn, green material	Primary **Method 01013**	0.01 mg/kg for all analytes and matrices	HPLC-MS/MS	Brumhard, B., Stuke, S., 2007; M-283439-01-1; EU agreed, RAR, 2017
Asparagus, sticks	Primary **Method 01013** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Billian, P., 2010; M-359460-02-1; not EU agreed, see Appendix 2 in support of Brumhard, B., Stuke, S., 2007; M-283439-01-1
Asparagus, sticks	Primary **Method 01013** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Uceda, L., Ratajczak, M., 2011; M-415549-01-1; not EU agreed, see Appendix 2 in support of Brumhard, B., Stuke, S., 2007; M-283439-01-1
Grapes, bunch of grape	Primary **Method 01013** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Cavaillé, C., Uceda, L., 2011; M-415381-01-1; not EU agreed, see Appendix 2 in support of Brumhard, B., Stuke, S., 2007; M-283439-01-1
Hop, green cone Hop, kiln-dried cone	Primary **Method 01013** **Supplementary validation**	0.01 mg/kg for all analytes for hop, green cone 0.05 mg/kg for all analytes for hop, kiln-dried cone	HPLC-MS/MS	Noss, G., Ballmann, C., 2012; M-423507-02-1; not EU agreed, see Appendix 2 in support of Brumhard, B., Stuke, S., 2007; M-283439-01-1
Hops, green cone Hops, dried cone Hops, draff Hops, brewers yeast Hops, beer	Primary **Method 01013/M002**	0.10 mg/kg for all analytes and matrices	HPLC-MS/MS	Schmeer, K., Reineke, A., 2010; M-390173-01-1; not EU agreed, see Appendix 2

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Hops, green cone Hops, kiln-dried cone	Primary **Method 01013/M002** **Supplementary validation**	0.10 mg/kg for all analytes and matrices	HPLC-MS/MS	Noss, G., 2010; M-389144-01-1; not EU agreed, see Appendix 2 in support of Schmeer, K., Reineke, A., 2010; M-390173-01-1
Component of residue definition: Trifloxystrobin (CGA 279202), its isomers CGA 331409, CGA 357262, CGA 357261, its metabolite CGA 321113 and its isomer CGA 373466				
Plants, plant products (Residues) Corn, green material Bean, seed Wheat, grain Rape, seed Orange, fruit Hop, kiln dried cone	Primary **Method 01313**	0.01 mg/kg for all analytes and matrices, except hop: 0.05 mg/kg	HPLC-MS/MS	Stuke, S. 2011; M-411496-02-1; EU agreed, RAR, 2017
Chicory witloof, root Chicory witloof, leaf	Primary **Method 01313** **Supplementary validation**	0.01 mg/kg for all analytes and matrices	HPLC-MS/MS	Fargeix, G., 2013; M-448916-02-1; not EU agreed, see Appendix 2
Kidney bean, pod Kidney bean, green material	Primary **Method 01313** **Supplementary validation**	0.01 mg/kg for all analytes and matrices	HPLC-MS/MS	Noss, G., Ballmann, C., 2012; M-425357-01-1; not EU agreed, see Appendix 2
Kidney bean, pod Kidney bean, seed	Primary **Method 01313** **Supplementary validation**	0.01 mg/kg for all analytes and matrices	HPLC-MS/MS	Noss, G., Guerleyen, N., Ballmann, C., 2012; M-425362-02-1; not EU agreed, see Appendix 2 in support of Noss, G., Ballmann, C., 2012; M-425357-01-1
Field pea, pod Field pea, dry bean	Primary **Method 01313** **Supplementary validation**	0.01 mg/kg for all analytes and matrices	HPLC-MS/MS	Fargeix, G., 2013; M-444960-01-1; not EU agreed, see Appendix 2
Grapes, bunch of grape	Primary **Method 01313** **Supplementary validation**	0.01 mg/kg for all analytes and matrices	HPLC-MS/MS	Stuke, S., 2013; M-421645-02-1; not EU agreed, see Appendix 2
Hop, green cone Hop, kiln-dried cone	Primary **Method 01313** **Supplementary validation**	0.01 mg/kg for all analytes for hop, green cone 0.05 mg/kg for all analytes for hop, kiln-dried cone	HPLC-MS/MS	Noss, G., Ballmann, C., 2012; M-432715-01-1; not EU agreed, see Appendix 2
Plants, plant products (Residues) Broccoli, head Rape, seed Bean, seed Grape, bunch Wheat, grain	Primary **Method 01313/M001**	0.01 mg/kg for all analytes and matrices	HPLC-MS/MS	Stuke and Tebuner, 2013; M-448498-01-1; EU agreed, RAR, 2017
Lettuce, head	Primary **Method 01313/M001** **Supplementary**	0.01 mg/kg for all analytes	HPLC-MS/MS	Bellof, S., Kuester, S., 2015; M-530177-01-1; not EU agreed, see Appendix 2

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	validation			
Lettuce, head	Primary **Method 01313/M001** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Bellof, S., Kuester, S., 2015; M-536965-01-1; not EU agreed, see Appendix 2 in support of Bellof, S., Kuester, S., 2015; M-530177-01-1
Lettuce, head	Primary **Method 01313/M001** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Schulte, G., Sosniak, A., 2015; M-534202-01-1; not EU agreed, see Appendix 2 in support of Bellof, S., Kuester, S., 2015; M-530177-01-1
Strawberry, fruit	Primary **Method 01313/M001** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Stuke, S., Diehl, P., 2013; M-452140-01-1; not EU agreed, see Appendix 2
Strawberry, fruit	Primary **Method 01313/M001** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Noss, G., Czaja, C., Diehl, P., 2013; M-460009-01-1; not EU agreed, see Appendix 2
Strawberry, fruit	Primary **Method 01313/M001** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Stuke, S., 2013; M-453332-02-1; not EU agreed, see Appendix 2
Strawberry, fruit	Primary **Method 01313/M001** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Schulte, G., Sosniak, A., 2015; M-534577-01-1; not EU agreed, see Appendix 2 in support of Stuke, S., Diehl, P., 2013, M-452140-01-1; Noss, G., Czaja, C., Diehl, P., 2013, M-460009-01-1 and Stuke, S., 2013; M-453332-02-1
Strawberry, fruit	Primary **Method 01313/M001** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Braune, M, Eremeeva, T., 2020; M-684200-01-1; not EU agreed, see Appendix 2
Raspberry, fruit	Primary **Method 01313/M001** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Buchmueller, K., Holbein, J., 2019; M-675722-01-1; not EU agreed, see Appendix 2
French bean, pod French bean, green material	Primary **Method 01313/M001** **Supplementary validation**	0.01 mg/kg for all analytes and matrices	HPLC-MS/MS	Glaubitz, J., 2013; M-467728-01-1; not EU agreed, see Appendix 2
Field pea, pod Field pea, green seed Field pea, rest of plant Field pea, dry seed Field pea, straw	Primary **Method 01313/M001** **Supplementary validation**	0.01 mg/kg for all analytes and matrices	HPLC-MS/MS	Glaubitz, J., Ballmann, C., 2014; M-475814-01-1; not EU agreed, see Appendix 2

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Field pea, green material Field pea, pod Field pea, dry seed Field pea, green seed	Primary **Method 01313/M001** **Supplementary validation**	0.01 mg/kg for all analytes and matrices	HPLC-MS/MS	Noss, G., Czaja, C., 2017; M-566823-03-1; not EU agreed, see Appendix 2
Carrot (root)	Primary **Method 01313/M001** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Semrau, J., 2017; M-598289-01-1; not EU agreed, see Appendix 2
Carrot (root)	Primary **Method 01313/M001** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Braune, M., Cuesta-Pérez, J., 2020; M-682016-01-1; not EU agreed, see Appendix 2
Hop, green cone Hop, kiln-dried cone	Primary **Method 01313/M001** **Supplementary validation**	0.01 mg/kg for all analytes and matrices	HPLC-MS/MS	Buchmueller, K., van Berkum, S., 2020; M-681429-01-1; not EU agreed, see Appendix 2
Sweet pepper, fruit	Primary **Method 01313/M001** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Glaubitz, J, Czaja, C., 2014; M-491166-01-1; not EU agreed, see Appendix 2
Component of residue definition: Trifloxystrobin and metabolite CGA 321113				
Apple (fruit), orange (whole fruit), carrot (root), oilseed rape (seed) and dry bean (seeds)	Primary **Method 01207**	0.01 mg/kg for all analytes	HPLC-MS/MS	Lakaschus, S., Amann, S., Winter, O., Gizler, A., 2013 M-424756-02-1 not EU agreed, see Appendix 2
Raspberry (fruit)	Primary **Method 01207** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Loriau, P., 2012 M-433737-01-1 not EU agreed, see Appendix 2 in support of Lakaschus, S., Amann, S., Winter, O., Gizler, A., 2013; M-424756-02-1
Raspberry (fruit)	Primary **Method 01207** **Supplementary validation**	0.01 mg/kg for all analytes	HPLC-MS/MS	Oostingh, C., 2013 M-434309-02-1 not EU agreed, see Appendix 2 in support of Lakaschus, S., Amann, S., Winter, O., Gizler, A., 2013; M-424756-02-1
Raspberry	Primary	0.005 mg/kg for all analytes	HPLC-MS/MS	Malet, J. C., Allard, L., 2019 M-434818-01-2 not EU agreed, see Appendix 2
Raspberry	Primary	0.005 mg/kg for all analytes	HPLC-MS/MS	Malet, J. C., Allard, L., 2019 M-434815-01-2 not EU agreed, see Appendix 2 in support of Malet, J. C., Allard, L., 2019; M-434818-01-2
Animal products, food of animal origin (Residues)	Not relevant.			

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Body fluids (Exposure)	Not relevant.			
Component of residue definition: Trifloxystrobin (CGA 279202)				
Soil, water,... (Efficacy)	Not relevant			
Feed, body fluids,... (Toxicology)	Not relevant			
Body fluids, air,.... (Exposure)	Not relevant.			
Leaf punch washings (Exposure) Grape Strawberry	Primary **Method 01158/M002**	20 µg/L (for extraction of 400 cm² leaf surface; corresponds to 0.01 µg/cm²)	HPLC-MS/MS	Stuke, S., van Berkum, S., 2017; M-532610-02-1; not EU agreed, see Appendix 2
Leaf punch washings (Exposure) Grape	Primary **Method 01158/M002** **Supplementary validation**	20 µg/L (for extraction of 400 cm² leaf surface; corresponds to 0.01 µg/cm²)	HPLC-MS/MS	Stuke, S., Daniels, M., van Berkum, S., 2016; M-569303-01-1; not EU agreed, see Appendix 2
Leaf punch washings (Exposure) Lily	Primary **Method 01158/M002** **Supplementary validation**	20 µg/L (for extraction of 400 cm² leaf surface; corresponds to 0.01 µg/cm²)	HPLC-MS/MS	Stuke, S., van Berkum, S., 2016; M-558518-01-1; not EU agreed, see Appendix 2
Leaf punch washings (Exposure) Raspberry	Primary **Method 01158/M002** **Supplementary validation**	0.8 µg/L (for extraction of 400 cm² leaf surface; corresponds to 0.01 µg/cm²)	HPLC-MS/MS	Daniels, M., van Berkum, S., 2020; M-677729-01-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary **Method EBTF0035**	trifloxystrobin 0.1 µg/L	UHPLC-MS/MS Ultra-high-performance liquid chromatography with MS/MS detection	Kosak, L., Hennecke, S., 2018; M-637834-01-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary **Method EBTF0035** **Supplementary validation**	trifloxystrobin 0.1 µg/L	UHPLC-MS/MS	Kosak, L., Hennecke, S., 2018; M-638530-01-1; not EU agreed, see Appendix 2 in support of Kosak, L., Hennecke, S., 2018; M-637834-01-1
Water (Ecotoxicology)	Primary **Method EBTF0039**	trifloxystrobin 0.1 µg/L	UHPLC-MS/MS	Kosak, L., Hennecke, S., 2019; M-630875-02-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary **Method EBTF0039** **Supplementary validation**	trifloxystrobin 0.1 µg/L	UHPLC-MS/MS	Hommen, U., Hennecke, S., 2018; M-638527-01-1; not EU agreed, see Appendix 2 in support of Kosak, L., Hennecke, S., 2019; M-630875-02-1
Water (Ecotoxicology)	Primary **Method EBTF0039** **Supplementary**	trifloxystrobin 0.1 µg/L	UHPLC-MS/MS	Hommen, U., Hennecke, S., 2018; M-638519-01-1; not EU agreed, see Appendix 2

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	validation			in support of Kosak, L., Hennecke, S., 2019; M-630875-02-1
Water (Ecotoxicology)	Primary **Method EBTF0039 Supplementary validation**	trifloxystrobin 0.1 µg/L	UHPLC-MS/MS	Kosak, L., Hennecke, S., 2018; M-638524-01-1; not EU agreed, see Appendix 2 in support of Kosak, L., Hennecke, S., 2019; M-630875-02-1
Water (Ecotoxicology)	Primary **Method EBTF0039 Supplementary validation**	trifloxystrobin 0.1 µg/L	UHPLC-MS/MS	Kosak, L., Hennecke, S., 2018; M-637890-01-1; not EU agreed, see Appendix 2 in support of Kosak, L., Hennecke, S., 2019; M-630875-02-1
Water (Ecotoxicology)	Primary **Method EBTF0039 Supplementary validation**	trifloxystrobin 0.1 µg/L	UHPLC-MS/MS	Kosak, L., Hennecke, S., 2018; M-637847-01-1; not EU agreed, see Appendix 2 in support of Kosak, L., Hennecke, S., 2019; M-630875-02-1
Water (Ecotoxicology)	Primary **Method EBTF0039 Supplementary validation**	trifloxystrobin 0.1 µg/L	UHPLC-MS/MS	Kosak, L., Hennecke, S., 2018; M-638529-01-1; not EU agreed, see Appendix 2 in support of Kosak, L., Hennecke, S., 2019; M-630875-02-1
Water (Ecotoxicology)	Primary	BCS-AL58660 0.1 mg test item/L	HPLC-MS/MS	xxx., 2019; M-670324-02-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary	CGA 357261 2 µg test item/L	HPLC-MS/MS	xxx., 2019; M-670322-02-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary **Method 01555**	AE 1344181 AE 1393224 0.05 µg/L	HPLC-MS/MS	Krebber, R., Leppelt, L., 2018; M-623236-01-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary **Method 01555 Supplementary validation**	AE 1393224 0.05 µg/L	HPLC-MS/MS	Riebschläger, T., 2018; M-630021-01-1; not EU agreed, see Appendix 2 In support of Krebber, R., Leppelt, L., 2018; M-623236-01-1
Water (Ecotoxicology)	Primary **Method 01555 Supplementary validation**	AE 1393224 0.05 µg/L	HPLC-MS/MS	Kuhl, K., 2018; M-629680-01-1; not EU agreed, see Appendix 2 In support of Krebber, R., Leppelt, L., 2018; M-623236-01-1
Water (Ecotoxicology)	Primary **Method 01555 Supplementary**	AE 1344148 0.05 µg/L	HPLC-MS/MS	Kuhl, K., 2018; M-628915-01-1; not EU agreed, see Appendix 2

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	validation			In support of Krebber, R., Leppelt, L., 2018; M-623236-01-1
Water (Ecotoxicology)	Primary	trifloxystrobin-TFMAP ~~0.04 µg/L~~ 0.952 mg/L	HPLC-UV/VIS	Neuhahn, A., 2017; M-602375-01-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary	trifloxystrobin-TFMAP ~~0.04 µg/L~~ 0.952 mg/L	HPLC-UV/VIS	Spoo-Klöppel, M., 2017; M-602410-01-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary **Method 01556**	AE 1344132 0.05 mg/L	HPLC-UV	Krebber, R., Leppelt, L., 2018; M-621113-01-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary **Method 01556 Supplementary validation**	AE 1344132 0.05 mg/L	HPLC-UV	Kuhl, K., 2018; M-629159-02-1; not EU agreed, see Appendix 2 In support of Krebber, R., Leppelt, L., 2018; M-621113-01-1
Water (Ecotoxicology)	Primary	BCS-AB39835 0.4 µg test item/L	HPLC-MS/MS	xxx., 2019; M-670321-02-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary **Method 01387**	Trifloxystrobin 0.05 µg/L	HPLC-MS/MS	Krebber, R., Braune, M., 2013; M-466732-01-1; EU agreed, RAR & EFSA 2017
Water (Ecotoxicology)	Primary **Method 01387 Supplementary validation**	Trifloxystrobin 0.4 µg/L	HPLC-MS/MS	xxx., 2018; M-636236-01-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary **Method 01387 Supplementary validation**	Trifloxystrobin 0.86 µg/L	HPLC-MS/MS	xxx, 2018; M-636231-01-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary **Method 01387 Supplementary validation**	Trifloxystrobin 0.0625 µg/L	HPLC-MS/MS	Kuhl, K., 2018; M-615579-01-1; not EU agreed, see Appendix 2 in support of Krebber, R., Braune, M., 2013; M-466732-01-1
Water (Ecotoxicology)	Primary **Method 01387 Supplementary validation**	Trifloxystrobin 0.75 µg/L	HPLC-MS/MS	xxx, 2018; M-636234-01-1; not EU agreed, see Appendix 2
Water (Ecotoxicology)	Primary	AE 1344138 5 µg/L	HPLC-MS/MS	Egeler, P., Witte, A., 2018; M-630580-01-2; not EU agreed, see Appendix 2
Sediment (Ecotoxicology)	Primary	AE 1344138 0.1 mg/kg	HPLC-MS/MS	Egeler, P., Witte, A., 2018; M-630580-01-2; not EU agreed, see Appendix 2
Feeding soultion (Ecotoxicity)	Primary **Method 01013 Supplementary validation**	Trifloxystrobin technical 0.01 mg/kg	HPLC-MS/MS	Kleebaum, K., 2019; M-648913-01-1; not EU agreed, see Appendix 2

5.3 Methods for post-authorization control and monitoring purposes (KCP 5.2)

5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product shall be submitted, unless the applicant shows that these methods already submitted in accordance with the requirements set out in point 5.2.1 can be applied.

Methods already submitted in accordance with the requirements set out in point 5.2.1 can be applied.

5.3.2 Description of analytical methods for the determination of residues of Fluopyram (KCP 5.2)

Nothing submitted

5.3.3 Description of analytical methods for the determination of residues of Trifloxystrobin (KCP 5.2)

5.3.3.1 Overview of residue definitions and levels for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.

Table 5.3-1: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Trifloxystrobin	0.01 mg/kg (lowest MRL) / 0.01 mg/kg (LOQ)	Reg (EU) No. 2019/1791 Default MRL at (LOQ)
Plant, high acid content		0.01 mg/kg (lowest MRL) / 0.01 mg/kg (LOQ)	Reg (EU) No. 2019/1791 Default MRL at (LOQ)
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg (lowest MRL) / 0.01 mg/kg (LOQ)	Reg (EU) No. 2019/1791 Default MRL at (LOQ)
Plant, high oil content		0.01 mg/kg (lowest MRL) / 0.01 mg/kg (LOQ)	Reg (EU) No. 2019/1791 Default MRL at (LOQ)
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg (lowest MRL) / 0.05mg/kg (LOQ)	Reg (EU) No. 2019/1791 Default MRL at (LOQ)
Muscle	Sum of trifloxystrobin and CGA 321113, expressed as trifloxystrobin	0.04 mg/kg	Reg (EU) No. 2019/1791 Default MRL at (LOQ)
Milk		0.02 mg/kg	Reg (EU) No. 2019/1791 Default MRL at (LOQ)
Eggs		0.04 mg/kg	Reg (EU) No. 2019/1791 Default MRL at (LOQ)
Fat		0.06 mg/kg / 0.04 mg/kg	Reg (EU) No. 2019/1791 Default MRL at (LOQ)
Liver		0.07 mg/kg/ 0.04 mg/kg	Reg (EU) No. 2019/1791 Default MRL at (LOQ)
Kidney		0.04 mg/kg	Reg (EU) No. 2019/1791 Default MRL at (LOQ)
Soil (Ecotoxicology)	Trifloxystrobin	10 µg/kg (LOQ)	LOQ below lowest NOEC for soil organisms (3.5 mg/kg dw soil, EFSA 2017)
Drinking water (Human toxicology)	Trifloxystrobin (and the metabolite CGA 321113)	0.05 µg/L (LOQ)	LOQ below the general limit for drinking water

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
			(0. 1 µg/L)
Surface water (Ecotoxicology)	Trifloxystrobin	0.05 µg/L (LOQ)	LOQ below lowest NOEC for aquatic organisms (2.76 µg a.s./L, EFSA 2017)
Air	Trifloxystrobin	2 µg/L (LOQ)	AOEL sys: 0.06 mg/kg bw/d concentration c calculated from AOEL : 20 µg/m^3 (EFSA Journal 2017)
Body fluids and tissues	Sum of trifloxystrobin and CGA 321113, expressed as trifloxystrobin	not required LOQ 0.01 mg/kg (blood, urine) LOQ 50 µg/L (blood plasma)	Not classified as T/T+

5.3.3.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Trifloxystrobin in plant matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-2: Validated methods for food and feed of plant origin (required for all matrix types, "difficult" matrix only when indicated by intended GAP)

Component of residue definition (enforcement): Trifloxystrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary	0.02 mg/kg	HPLC-UV	Kissling, 1996, M-038781-01-1, method REM 177.02 EU agreed: EFSA 2017 [1]
	Primary	0.02 mg/kg	GC-ECD	Kissling, 1996, M-038798-01-1, method REM 177.03; M-060773-01-1, report no. 141/96 (validation data) EU agreed: EFSA 2017 [1]
	Primary	0.02 mg/kg	GC-NPD	Campbell, 1997, M-038841-01-1, method AG 659 EU agreed: EFSA 2017 [1]
	ILV	0.02 mg/kg	GC-NPD	Bandong, 1998, M-136732-01-1, ILV to method AG 659 EU agreed: EFSA 2017 [1]
	Primary (Quechers)	0.01 mg/kg	HPLC-MS/MS	EURL, 2013 [2] EU agreed: EFSA 2017 [1]
	Confirmatory (if required)	confirmation is included in above listed methods		
High acid content	Primary	0.02 mg/kg	HPLC-UV	Kissling, 1996, M-038781-01-1, method REM 177.02 EU agreed: EFSA 2017 [1]
	Primary	0.02 mg/kg	GC-NPD	Campbell, 1997, M-038841-01-1, method AG 659 EU agreed: EFSA 2017 [1]
	ILV	0.02 mg/kg	GC-NPD	Bandong, 1998, M-136732-01-1, ILV to method AG 659 EU agreed: EFSA 2017 [1]
	Primary	0.01 mg/kg	HPLC-MS/MS	EURL, 2013 [2] EU agreed: EFSA 2017 [1]

Component of residue definition (enforcement): Trifloxystrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Confirmatory (if required)	confirmation is included in above listed methods		
High oil content	Primary	0.02 mg/kg	GC-NPD	Campbell, 1997, M-038841-01-1, method AG 659 EU agreed: EFSA 2017 [1]
	Primary	0.01 mg/kg	HPLC-MS/MS	EURL, 2013 [2] EU agreed: EFSA 2017 [1]
	Primary	0.01 mg/kg	HPLC-MS/MS	Uceda, 2013, M-437196-02-1, method 01300/M002 EU agreed: EFSA 2017 [1]
	ILV	0.01 mg/kg	HPLC-MS/MS	Winter and Amann, 2013; M-467297-01-1, ILV to method 01300/M002 EU agreed: EFSA 2017 [1]
	Primary	0.01 mg/kg	HPLC-MS/MS	Winter and Gizler, 2013; M-466556-01-1, method 01300/M013 EU agreed: EFSA 2017 [1]
	Confirmatory (if required)	confirmation is included in above listed methods		
High protein/high starch content (dry)	Primary	0.02 mg/kg	HPLC-UV	Kissling, 1996, M-038781-01-1, method REM 177.02 EU agreed: EFSA 2017 [1]
	Primary	0.02 mg/kg	GC-ECD	Kissling, 1996, M-038798-01-1, method REM 177.03; M-060773-01-1, report no. 141/96 (validation data) EU agreed: EFSA 2017 [1]
	Primary	0.02 mg/kg	GC-NPD	Campbell, 1997, M-038841-01-1, method AG 659 EU agreed: EFSA 2017 [1]
	Primary	0.01 mg/kg	HPLC-MS/MS	EURL, 2013 [2] EU agreed: EFSA 2017 [1]
	Primary	0.01 mg/kg	HPLC-MS/MS	Uceda, 2013, M-437196-02-1, method 01300/M002 EU agreed: EFSA 2017 [1]
	ILV	0.01 mg/kg	HPLC-MS/MS	Winter and Amann, 2013; M-467297-01-1, ILV to method 01300/M002 EU agreed: EFSA 2017 [1]
	Primary	0.01 mg/kg	HPLC-MS/MS	Winter and Gizler, 2013; M-466556-01-1, method 01300/M013 EU agreed: EFSA 2017 [1]
	Confirmatory (if required)	confirmation is included in above methods		
Difficult (if required, depends on intended use)	Primary	0.01 / 0.05 mg/kg	HPLC-MS/MS	Uceda, 2013, M-437196-02-1, method 01300/M002 EU agreed: EFSA 2017 [1]
	ILV	0.01 / 0.05 mg/kg	HPLC-MS/MS	Winter and Amann, 2013; M-467297-01-1, ILV to method 01300/M002 EU agreed: EFSA 2017 [1]
	Primary	0.01 / 0.05 mg/kg	HPLC-MS/MS	Winter and Gizler, 2013; M-466556-01-1, method 01300/M013 EU agreed: EFSA 2017 [1]
	Confirmatory	confirmation is included in above listed methods		

Component of residue definition (enforcement): Trifloxystrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	(if required)			

[1] EFSA Journal 2017;15(10):4989 (Conclusion on pesticides peer review)
[2] EURL (European Union Reference Laboratories for Pesticide Residues), 2013. Data pool on method validation for pesticide residues. Status on 10 December 2013. Available online: www.eurl-pesticides-datapool.eu

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 2.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	The extraction of plant matrices with high water content, high acid content, high oil content, dry commodities (high protein content), relevant to this dossier, is based on the extraction conditions used in the cucumber, apple, peanut and wheat metabolism studies where sufficient extraction efficiency has been demonstrated. Additional extraction steps under harsher extraction conditions used in the metabolism studies do not contribute significantly to the overall extractability of the parent compound trifloxystrobin and thus the enforcement methods do not result in an underestimate of trifloxystrobin residues. The residue analytical methods can be considered capable to efficiently extract trifloxystrobin related residues from plant matrices with high water content, high acid content, high oil content and dry commodities.

For the detailed evaluation of (additional) studies on extraction efficiency, it is referred to Appendix 2.

5.3.3.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Trifloxystrobin in animal matrices is given in the following tables.

Table 5.3-4: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Trifloxystrobin and CGA 321113				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Meat, fat, liver, kidney, eggs, milk	Primary	0.02 mg/kg, except milk: 0.01 mg/kg	GC-NPD	Campbell, 1997, M-038841-01-1, method AG 659 EU agreed: DAR - United Kingdom, 2000 [1]
Liver, milk, eggs	ILV	0.02 mg/kg milk: 0.01 mg/kg	GC-NPD	Bandong, 1998, M-136732-01-1, ILV to method AG 659 EU agreed: DAR - United Kingdom, 2000 [1]
Animal matrices	Confirmatory	confirmation is included in above listed methods		
Milk	Primary	0.01 mg/kg	HPLC-MS/MS	Winter and Amann, 2016, M-453914-03-1, method 01300/M005 EU agreed: EFSA 2017 [2]
	ILV	0.01 mg/kg	HPLC-MS/MS	Amic, 2013, M-467489-03-1, ILV to method 01300/M005 EU agreed: EFSA 2017 [2]

Component of residue definition: Trifloxystrobin and CGA 321113				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
	Confirmatory (if required)	confirmation is included in above listed methods		
Eggs	Primary	0.01 mg/kg	HPLC-MS/MS	Winter and Amann, 2016, M-453914-03-1, method 01300/M005 EU agreed: EFSA 2017 [2]
	ILV	0.01 mg/kg	HPLC-MS/MS	Amic, 2013, M-467489-03-1, ILV to method 01300/M005 EU agreed: EFSA 2017 [2]
	Confirmatory (if required)	confirmation is included in above listed methods		
Muscle	Primary	0.01 mg/kg	HPLC-MS/MS	Winter and Amann, 2016, M-453914-03-1, method 01300/M005 EU agreed: EFSA 2017 [2]
	ILV	0.01 mg/kg	HPLC-MS/MS	Amic, 2013, M-467489-03-1, ILV to method 01300/M005 EU agreed: EFSA 2017 [2]
	Confirmatory (if required)	confirmation is included in above listed methods		
Fat	Primary	0.01 mg/kg	HPLC-MS/MS	Winter and Amann, 2016, M-453914-03-1, method 01300/M005 EU agreed: EFSA 2017 [2]
	ILV	0.01 mg/kg	HPLC-MS/MS	Amic, 2013, M-467489-03-1, ILV to method 01300/M005 EU agreed: EFSA 2017 [2]
	Confirmatory (if required)	confirmation is included in above listed methods		
Kidney, liver	Primary	0.01 mg/kg	HPLC-MS/MS	Winter and Amann, 2016, M-453914-03-1, method 01300/M005 EU agreed: EFSA 2017 [2]
	ILV	0.01 mg/kg	HPLC-MS/MS	Amic, 2013, M-467489-03-1, ILV to method 01300/M005 EU agreed: EFSA 2017 [2]
	Confirmatory (if required)	confirmation is included in above listed methods		

[1] United Kingdom, 2000. Draft assessment report on the active substance trifloxystrobin prepared by the rapporteur Member State United Kingdom in the framework of Council Directive 91/414/EEC, April 2000.
[2] EFSA Journal 2017;15(10):4989 (Conclusion on pesticides peer review)

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	The extraction of animal matrices is based on the extraction conditions used in the goat and hen metabolism studies, where sufficient extraction efficiency has been demonstrated. The residue analytical method can be considered capable to efficiently extract trifloxystrobin and CGA 321113 related residues from animal matrices.

5.3.3.4 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

Since trifloxystrobin is not classified as toxic or very toxic, methods for the analysis of trifloxystrobin in body fluids and tissues are not required (SANCO/825/00 rev.8.1).

Nevertheless, the validated analytical methods for the determination of residues in animal matrices can be considered as adequate for the analysis or monitoring of trifloxystrobin in human tissues by HPLC-MS/MS with a LOQ of 0.01 mg/kg.
For body fluids two methods were submitted and evaluated, see below.

An overview on the acceptable methods and possible data gaps for analysis of Trifloxystrobin in body fluids and tissues is given in the following table.

Table 5.3-6: Methods for body fluids and tissues (if appropriate)

Component of residue definition: Trifloxystrobin and CGA 321113			
Method type	**Method LOQ**	**Principle of method**	**Author(s), year / missing**
Primary Tissues	see above, methods for food and feed of animal origin		
Primary Body fluids	LOQ 0.01 mg/kg (blood, urine)	GC-ECD	Kissling, 1997. M-060777-01-1, REM 177.05 EU agreed: DAR, 2000
Primary Body fluids	LOQ 50 µg/L (blood plasma)	HPLC-MS/MS	Kaussmann, 2017. M-572949-02-1, 01513 EU agreed, RAR, 2017
Confirmatory	confirmation is included in above listed methods		

[1] EFSA Journal 2017;15(10):4989 (Conclusion on pesticides peer review)

5.3.3.5 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Trifloxystrobin in soil is given in the following tables.

Table 5.3-7: Validated methods for soil (if appropriate)

Component of residue definition: Trifloxystrobin (and metabolites CGA357261, CGA357262, CGA331409, CGA373466 and CGA321113)			
Method type	**Method LOQ**	**Principle of method**	**Author(s), year / missing**
Primary	10 µg/kg	HPLC-UV	Chamkasem, N.; 1996; M-038688-01-1. EU agreed: DAR, 2000.
Primary	5 µg/kg in soil (trifloxystrobin)	HPLC-MS/MS	Freitag, T.; Koch, V., 2014 M-476164-01-1; Method 01401 EU agreed: RAR & EFSA, 2017
Primary	0.1 µg/kg in soil (Trifloxystrobin and metabolites)	HPLC-MS/MS	Freitag, 2013 M-464872-01-1 Method 01327/M001 EU agreed: RAR & EFSA, 2017

5.3.3.6 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Trifloxystrobin in surface and drinking water is given in the following tables.

Table 5.3-8: Validated methods for water (if appropriate)

Component of residue definition: Trifloxystrobin (and the metabolite CGA 321113)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing**
Drinking water	Primary	0.05 µg/L	HPLC-MS/MS	Krebber, R.; Braune, M.; 2013; M-466732-01-1. Method 01387 EU agreed: RAR & EFSA 2017

Component of residue definition: Trifloxystrobin (and the metabolite CGA 321113)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing**
	ILV	0.05 µg/L	HLPC-MS/MS	Stanislowski, T.; 2013; M-470714-02-1. ILV of 01387 EU agreed: RAR 2017
Drinking water	Primary	0.05 µg/L	HPLC-UV	Kissling, M.; 1995; M-038601-01-1. EU agreed, DAR, 2000
	ILV	0.05 µg/L	HPLC-UV	Hohl, J.; 1997; M-038646-01-1. EU agreed, DAR, 2000
~~Drinking water~~	~~Primary~~	~~0.05 µg/L~~	~~GC-ECD~~	~~Kissling, M.; 1999; M-065375-01-1. EU agreed, DAR, 2000~~
	~~ILV~~	~~0.05 µg/L~~	~~GC-ECD~~	~~Kissling, M.; 1999. M-065341-01-1. EU agreed, DAR, 2000~~
Surface water	Primary	0.05 µg/L	HPLC-MS/MS	Krebber, R.; Braune, M.; 2013; M-466732-01-1. Method 01387 EU agreed: RAR & EFSA 2017
	ILV	0.05 µg/L	HPLC-MS/MS	Stanislowski, T.; 2013; M-470714-02-1. ILV of 01387 EU agreed: RAR 2017
Surface water	Primary	0.05µg/L	HLPC-UV	Kissling, M.; 1995; M-038601-01-1. EU agreed, DAR, 2000
	Confirmatory	0.1 ppb	HPLC-MS	Williams, R.W.; 1998; M-032937-01-1. EU agreed, DAR, 2000
	ILV	0.05 µg/L	HLPC-UV	Hohl, J.; 1997; M-038646-01-1. EU agreed, DAR, 2000
Surface water	Primary	1.0 µg/L	GC-ECD	Kissling, M.; 1999; M-065375-01-1. EU agreed, DAR, 2000
	ILV	1.0 µg/L	GC-ECD	Kissling, M.; 1999. M-065341-01-1. EU agreed, DAR, 2000

5.3.3.7 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of Trifloxystrobin in air is given in the following tables.

Table 5.3-9: Validated methods for air (if appropriate)

Component of residue definition: Trifloxystrobin			
Method type	**Method LOQ**	**Principle of method**	**Author(s), year / missing**
Primary	2 µg/m^3	GC-ECD	Tribolet, R.; 1997. M-038535-01-1. EU-agreed : DAR, 2000
Confirmatory	2 µg/m^3	HPLC-UV	Tribolet, R., Kissling, M.; 1999. M-038474-02-1. EU-agreed : DAR, 2000

5.3.3.8 Other studies/ information

No other study is required.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCP 5.1.1 / 01	Schulz, F.	2007	Determination of fluopyram and trifloxystrobin in formulations assay - GLC, internal standard Report No.: AM009707MF1, Edition Number: M-286825-01-2 Bayer CropScience AG, Monheim, Germany GLP/GEP: No unpublished	No	Bayer
KCP 5.1.1 / 02	Bastian-Bertrams, V.; Schulz, F.	2015	Validation of GLC-method AM009707MF1 - Determination of fluopyram and trifloxystrobin in formulations - fluopyram + trifloxystrobin SC 500 (250+250 g/L) Report No.: VB1.1-AM009707MF1, Edition Number: M-286826-02-1 Bayer CropScience AG, Monheim, Germany **... amended: 2015-02-12** GLP/GEP: No unpublished	No	Bayer
KCP 5.1.1 / 03	Hein, E. M.; Bowen, T.	2018	Determination of CGA 344605 in formulations - HPLC-UV external standard Report No.: AM033118MF1, Edition Number: M-639504-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: No unpublished	No	Bayer
KCP 5.1.1 / 04	Hein, E. M.	2018	Amendment no. 1 to final report - Validation of analytical method AM033118MF1 - Determination of CGA 344605 in the formulation fluopyram + trifloxystrobin SC 500 (250+250 g/L) Report No.: VB2-AM033118MF1, Edition Number: M-644196-02-1 Bayer AG, Crop Science Division, Monheim, Germany **... amended: 2018-12-14** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.4 / 01	Stuke, S.; Diehl, P.	2014	Modification 001 of analytical method 01158 for the determination of tebuconazole and fluopyram in leaf punches washing solution by HPLC-MS/MS Report No.: MR-14/016, Edition Number: M-502699-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 5.1.2.4 / 02 KCA 6.10 / 01	Stuke, S.; Daniela, M.; van Berkum, S.	2016	Determination of the dislodgeable foliar residues (DFR) of trifloxystrobin and AE C656948 in/on grape after spraying of AE C656948 & CGA279202 SC 500 in the field in the North of France Report No.: 15-2924, Edition Number: M-569303-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.4 / 03 KCA 6.10 / 03	Stuke, S.; van Berkum, S.	2016	Determination of the dislodgeable foliar residues (DFR) of trifloxystrobin and AE C656948 in/on lily after spraying of AE C656948 & CGA279202 SC 500 in the field in the Netherlands Report No.: 15-2925, Edition Number: M-558518-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.4 / 04 KCA 6.10 / 02	Daniels, M. ; van Berkum, S.	2020	Determination of the dislodgeable foliar residues (DFR) of trifloxystrobin and AE C656948 in/on raspberry after spray application of AE C656948 & CGA279202 SC 500 in the field in Italy Report No.: 18-2905, Edition Number: M-677729-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.4 / 05	Stuke, S.; van Berkum, S.	2017	Amendment no. 1 to the final report of study no.: P602155506 - Modification 002 of analytical method 01158 for the determination of tebuconazole, fluopyram and trifloxystrobin in leaf punches washing solution by HPLC-MS/MS Report No.: MR-15/032, Edition Number: M-532610-02-1 Bayer AG, Crop Science Division, Monheim, Germany **... amended: 2017-04-24** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 01 KCA 6.3.1.1 / 01	Billian, P.	2010	Determination of the residues of AE C656948 and trifloxystrobin in/on asparagus after spraying of AE C656948 & CGA279202 SC 500 in the field in France (North) and Germany Report No.: 08-2209, Edition Number: M-359460-02-1 Bayer CropScience AG, Monheim, Germany **... amended: 2010-07-12** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 02 KCA 6.3.1.1 / 02	Uceda, L.; Ratajczak, M.	2011	Determination of the residues of AE C656948 and trifloxystrobin in/on asparagus after spraying of AE C656948 & CGA279202 SC 500 in the field in France (north) and Netherlands Report No.: 09-2073, Edition Number: M-415549-01-1	No	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			Bayer S.A.S., Bayer CropScience, Lyon, France GLP/GEP: Yes unpublished		
KCP 5.1.2.5 / 03	Bellof, S.; Kuester, S.	2015	Determination of the residues of trifloxystrobin in/on lettuce after spraying of trifloxystrobin WG 50 in the greenhouse in the Netherlands, Belgium, Italy and Spain Report No.: 14-2144, Edition Number: M-530177-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 05 KCA 6.3.9.1 / 05	Schulte, G.; Sosniak, A.	2015	Determination of the residues of fluopyram and trifloxystrobin in/on lettuce after spray application of fluopyram & trifloxystrobin SC 500 in Belgium, Germany, the Netherlands and northern France Report No.: 14-2029, Edition Number: M-534202-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 07 KCA 6.3.9.1 / 06	Bellof, S.; Kuester, S.	2015	Determination of the residues of fluopyram and trifloxystrobin in/on lettuce after spray application of fluopyram & trifloxystrobin SC 500 in Germany, the Netherlands, Hungary and the United Kingdom Report No.: 14-2184, Edition Number: M-536965-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 10	Stuke, S.; Diehl, P.	2013	Determination of the residues of trifloxystrobin in/on strawberry after spraying of trifloxystrobin WG 50 in the field in Germany, the Netherlands, France (north) and Belgium Report No.: 12-2012, Edition Number: M-452140-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 11	Noss, G.; Czaja, C.; Diehl, P.	2013	Determination of the residues of trifloxystrobin in/on strawberry after spray application of trifloxystrobin WG 50 in Spain, Italy and Greece Report No.: 12-2013, Edition Number: M-460009-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 5.1.2.5 / 12	Stuke, S.	2013	Determination of the residues of trifloxystrobin in/on strawberry after spraying of trifloxystrobin WG 50 in the greenhouse in Belgium, France (North) and Germany Report No.: 12-2014, Edition Number: M-453332-02-1 Bayer CropScience AG, Monheim, Germany **... amended: 2013-06-20** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 13 KCA 6.3.3.1 / 01	Schulte, G.; Sosniak, A.	2015	Determination of the residues of fluopyram and trifloxystrobin in/on strawberry after spray application of fluopyram & trifloxystrobin SC 500 in Germany, northern France, the Netherlands and Belgium Report No.: 14-2026, Edition Number: M-534577-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 15 KCA 6.3.3.1 / 02	Szeley, C. M.; Sadler, C.	2016	Determination of the residues of fluopyram and trifloxystrobin in/on strawberry after spray application of AE C656948 & CGA 279202 SC 500 in Germany, Denmark, Spain, southern France and Italy Report No.: 15-2031, Edition Number: M-553855-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 16 KCA 6.3.3.1 / 03	Braune, M.; Eremeeva, T.	2020	Determination of the residues of trifloxystrobin and AE C656948 in/on strawberry after spray application of AE C656948 & CGA279202 SC 500 in Germany and Belgium Report No.: 18-2050, Edition Number: M-684200-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 21	Lakaschus, S.; Amann, S.; Winter, O.; Gizler, A.	2013	Validation of the BCS method no. 01207 (based on modified QuEChERS method) for the determination of selected BCS analytes and their metabolites in carrot, apple, orange, oilseed rape seed and beans Report No.: S10-00279, Edition Number: M-424756-02-1 Eurofins Agroscience Services Chem GmbH (EAS Chem), Hamburg, Germany **... amended: 2013-12-11** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 22	Loriau, P.	2012	Residues of fluopyram and trifloxystrobing in raspberry under plastic umbrella at intervals following two foliar applications of FLU+TFS 500 SC - Belgium, season 2011	No	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			Report No.: BCS-G401-11, Edition Number: M-433737-01-1 Redebel S.A., Saint Amand, Belgium GLP/GEP: Yes unpublished		
KCP 5.1.2.5 / 23	Oostingh, C.	2013	Amendment no. 1 to report no: PTZ-NLI-11797 - Residues of fluopyram + trifloxystrobin in red raspberry under plastic umbrella at intervals following two foliar applications of fluopyram & trifloxystrobin SC 500 Report No.: PTZ-NLI-11797, Edition Number: M-434309-02-1 Proeftuin Zwaagdijk, Zwaagdijk, Netherlands **... amended: 2013-03-06** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 24	Malet, J. C.; Allard, L.	2019	Residues of fluopyram and trifloxystrobine, after 2 applications of F413BCS in raspberry in support of the registration for use in this crop Report No.: RAFR03509, Edition Number: M-434818-01-2 Ministère de l'Agriculture et de la Pêche, Paris, France GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 25	Malet, J. C.; Allard, L.	2019	Mesure du niveau de résidu de fluopyram et de trifloxystrobine, après 2 applications de la préparation F413BCS sur framboisier dans le cadre d'une extension d'usage sur la culture - Residues of fluopyram and trifloxystrobine, after 2 applications of F413BCS in raspberry in support of the registration Report No.: RAFR00810, Edition Number: M-434815-01-2 Ministère de l'Agriculture et de la Pêche, Paris, France GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 26 KCA 6.3.4.1 / 01	Buchmueller, K.; Holbein, J.	2019	Determination of the residues of trifloxystrobin and AE C656948 in/on raspberry after spray application of AE C656948 & CGA279202 SC 500 in Hungary, Poland, Germany and northern France Report No.: 18-2051, Edition Number: M-675722-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 28 KCA 6.3.10.1 / 01	Fargeix, G.	2013	Amendment No.1 - Determination of the residues of AE C656948 and trifloxystrobin in/on chicory, witloof after dip and spraying of fluopyram SC 500 and AE C656948 & CGA279202 SC 500 in the field and room, hall, store, etc. in Germany, Belgium, northern France and the Netherlands Report No.: 11-2140, Edition Number: M-448916-02-1 Bayer S.A.S., Bayer CropScience, Lyon, France	No	Bayer

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			... amended: 2013-10-18 GLP/GEP: Yes unpublished		
KCP 5.1.2.5 / 29 KCA 6.3.11.1 / 04	Noss, G.; Ballmann, C.	2012	Determination of the residues of AE C656948 and trifloxystrobin in/on bean, kidney after spraying of AE C656948 & CGA279202 SC 500 in the field in Germany, Belgium, Spain, Italy, France (south) and Portugal Report No.: 10-2125, Edition Number: M-425357-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 30 KCA 6.3.11.1 / 01	Noss, G.; Guerleyen, N.; Ballmann, C.	2012	Determination of the residues of AE C656948 and trifloxystrobin in/on bean, kidney after spraying of AE C656948 & CGA279202 SC 500 in the field in France (north) Report No.: 10-2128, Edition Number: M-425362-02-1 Bayer CropScience AG, Monheim, Germany **... amended: 2012-03-12** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 31 KCA 6.3.11.1 / 02	Fargeix, G.	2013	Determination of the residues of fluopyram and trifloxystrobin in/on field pea after spray application of AE C656948 & CGA279202 SC 500 in northern France and Germany Report No.: 11-2000, Edition Number: M-444960-01-1 Bayer S.A.S., Bayer CropScience, Lyon, France GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 32 KCA 6.3.11.1 / 05	Glaubitz, J.	2013	Determination of the residues of AE C656948 and trifloxystrobin in/on French bean after spray application of AE C656948 & CGA279202 SC 500 in the field in Germany and northern France Report No.: 12-2030, Edition Number: M-467728-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: No unpublished	No	Bayer
KCP 5.1.2.5 / 33 KCA 6.3.11.1 / 03	Glaubitz, J.; Ballmann, C.	2014	Determination of the residues of fluopyram and trifloxystrobin in/on field pea after spray application of AE C656948 & CGA279202 SC 500 in the field in Germany, Northern France, Belgium and United Kingdom Report No.: 12-2031, Edition Number: M-475814-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 5.1.2.5 / 36 KCA 6.3.11.1 / 06	Noss, G.; Czaja, C.	2017	Determination of the residues of fluopyram and trifloxystrobin in/on field pea, after spray application of AE C656948 & CGA 279202 SC 500 in Denmark, Germany, Spain and Italy Report No.: 15-2030, Edition Number: M-566823-03-1 Bayer AG, Crop Science Division, Monheim, Germany **... amended: 2017-09-25** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 39 KCA 6.3.6.1 / 01	Semrau, J.	2017	Determination of residues of fluopyram and trifloxystrobin in/on carrots after spray application of fluopyram & trifloxystrobin SC 500 in Northern France, Austria and Germany Report No.: 16-2155, Edition Number: M-598289-01-1 Eurofins Agroscience Services GmbH, Stade, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 40 KCA 6.3.6.1 / 02	Braune, M.; Cuesta-Pérez, J.	2020	Determination of the residues of trifloxystrobin and AE C656948 in/on carrot after spray application of AE C656948 & CGA279202 SC 500 in Germany, the United Kingdom and northern France Report No.: 18-2044, Edition Number: M-682016-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 41 KCA 6.3.2.1 / 02	Cavaillé, C.; Uceda, L.	2011	Determination of the residues of AE C656948 and trifloxystrobin in/on grape after spraying and spraying, low-volume of AE C656948 CGA279202 SC 500 in the field in France (north), france (south), germany and Italy Report No.: 09-2077, Edition Number: M-415381-01-1 Bayer S.A.S., Bayer CropScience, Lyon, France GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 43 KCA 6.3.12.1 / 04 KCA 6.3.2.1 / 03 KCA 6.3.3.1 / 07	Stuke, S.	2013	Amendment no. 1 to report no: P 652 11 5503 - Determination of the residues of trifloxystrobin, CGA 357261, CGA 357262, CGA 331409, CGA 321113, and CGA 373466 in/on materials of plant origin by HPLCMS/MS Report No.: MR-11/044, Edition Number: M-421645-02-1 Bayer CropScience AG, Monheim, Germany **... amended: 2013-07-24** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 45	Schmeer, K.; Reineke, A.	2010	Modification M002 of the residue analytical method 01013 for the determination of trifloxystrobin and CGA 321113 in/on hops cone (green and dried) and processed materials (hops draff, brewers yeast, and beer) by HPLC-MS/MS Report No.: 01013/M002, Edition Number: M-390173-01-1	No	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			Method Report No.: MR-10/031 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished		
KCP 5.1.2.5 / 46 KCA 6.3.12.1 / 05	Noss, G.	2010	Determination of the residues of AE C656948 and trifloxystrobin in/on hop after spraying of AE C656948 & CGA 279202 SC 500 in the field in France (North) and Germany Report No.: 08-2086, Edition Number: M-389144-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 47 KCA 6.3.12.1 / 02	Noss, G.; Ballmann, C.	2012	Determination of the residues of AE C656948 and trifloxystrobin in/on hop after spraying of AE C656948 & CGA279202 SC 500 in the field in France (North) Report No.: 10-2127, Edition Number: M-432715-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 48 KCA 6.3.12.1 / 03	Noss, G.; Ballmann, C.	2012	Amendment No. 1 to report no: 09-2076 - Determination of the residues of AE C656948 and trifloxystrobin in/on hop after spraying of AE C656948 & CGA279202 SC 500 in the field in France (North) and Germany Report No.: 09-2076, Edition Number: M-423507-02-1 Bayer CropScience AG, Monheim, Germany **... amended: 2012-11-23** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 49 KCA 6.3.12.1 / 01	Buchmueller, K.; van Berkum, S.	2020	Determination of the residues of trifloxystrobin and AE C656948 in/on hop after spray application of AE C656948 & CGA279202 SC 500 in northern France, Germany and Czech Republic - Final report - Report No.: 18-2047, Edition Number: M-681429-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 51	Glaubitz, J.; Czaja, C.	2014	Determination of the residues of AE C656948 and trifloxystrobin in/on (sweet) pepper after spray application of AE C656948 & CGA279202 SC 500 in southern France, Spain, Italy, Portugal and Greece Report No.: 13-2122, Edition Number: M-491166-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 5.1.2.6 / 01 KCP 10.2.1 / 03	xxx	2018	Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L) - Acute toxicity to rainbow trout (Oncorhynchus mykiss) in a 96-hour semi-static test Report No.: 134621230, Edition Number: M-636236-01-1 xxx GLP/GEP: Yes unpublished	Yes	Bayer
KCP 5.1.2.6 / 02 KCP 10.2.1 / 16	xxx	2018	Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L): Acute toxicity to Daphnia magna in a semi-static 48-hour immobilisation test Report No.: 134621220, Edition Number: M-636231-01-1 xxx GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 03	Krebber, R.	2014	Modification M001 of the analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS Report No.: 01387/M001, Edition Number: M-494841-02-1 Method Report No.: MR-14/053 Bayer CropScience AG, Monheim, Germany **... amended: 2014-10-23** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 04 KCP 10.2.1 / 21	Kuhl, K.	2018	Pseudokirchneriella subcapitata growth inhibition test with fluopyram + trifloxystrobin SC 500 G - Final report Report No.: EBGM0016, Edition Number: M-615579-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 05 KCP 10.2.1 / 22	xxx	2018	Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L): Toxicity to Pseudokirchneriella subcapitata in an algal growth inhibition test Report No.: 134621210, Edition Number: M-636234-01-1 xxx GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 06 KCP 10.2.1 / 04	Kosak, L.; Hennecke, S.	2018	Trifloxystrobin - Acute toxicity test with Brachionus calyciflorus, basic test conditions following OECD TG 202 Report No.: EBTF0035, Edition Number: M-637834-01-1 Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany GLP/GEP: Yes	No	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			unpublished		
KCP 5.1.2.6 / 07 KCP 10.2.1 / 05	Kosak, L.; Hennecke, S.	2018	Trifloxystrobin - Acute toxicity test with Thamnocephalus platyurus, basic test conditions following OECD TG 202 - Report - Report No.: EBTF0036, Edition Number: M-638530-01-1 Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 08 KCP 10.2.1 / 07	Kosak, L.; Hennecke, S.	2019	1st report amendment - Trifloxystrobin - Acute toxicity test with Daphnia pulex, basic test conditions following OECD TG 202 Report No.: EBTF0039, Edition Number: M-630875-02-1 Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany **... amended: 2019-01-16** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 09 KCP 10.2.1 / 06	Hommen, U.; Hennecke, S.	2018	Trifloxystrobin - Acute toxicity test with Daphnia longispina, basic test conditions following OECD TG 202 - Report - Report No.: EBTF0038, Edition Number: M-638527-01-1 Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 10 KCP 10.2.1 / 08	Hommen, U.; Hennecke, S.	2018	Trifloxystrobin - Acute toxicity test with Chydorus spec., basic test conditions following OECD TG 202 - Report Report No.: EBTF0040, Edition Number: M-638519-01-1 Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 11 KCP 10.2.1 / 09	Kosak, L.; Hennecke, S.	2018	Trifloxystrobin - Acute toxicity test with Cyclopoidae, basic test conditions following OECD TG 202 - Report - Report No.: EBTF0041, Edition Number: M-638524-01-1 Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 12 KCP 10.2.1 / 10	Kosak, L.; Hennecke, S.	2020	Amendment no. 01: Trifloxystrobin - Acute toxicity test with Chaoborus crystallinus, basic test conditions following OECD TG 202 Report No.: EBTF0042, Edition Number: M-637890-02-1 Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany	No	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			... amended: 2020-01-23 GLP/GEP: Yes unpublished		
KCP 5.1.2.6 / 13 KCP 10.2.1 / 11	Kosak, L.; Hennecke, S.	2018	Trifloxystrobin - Acute toxicity test with Baetis rhodani, basic test conditions following OECD TG 202 Report No.: EBTF0043, Edition Number: M-637847-01-1 Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 14 KCP 10.2.1 / 12	Kosak, L.; Hennecke, S.	2018	Trifloxystrobin - Acute toxicity test with Gammarus sp., basic test conditions following OECD TG 202 - Report - Report No.: EBTF0044, Edition Number: M-638529-01-1 Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 15 KCP 10.2.2 / 01	xxx	2019	Metabolite of trifloxystrobin: BCS-AL58660: Influence to Daphnia magna in a semi-static reproduction test - 1st final report amendment Report No.: 140421221, Edition Number: M-670324-02-1 xxx **... amended: 2019-11-29** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 16 KCP 10.2.2 / 02	Egeler, P.; Witte, A.	2018	A study on the chronic toxicity to the sediment dweller Lumbriculus variegatus - AE 1344138, technical Report No.: 18P6LA, Edition Number: M-630580-01-2 ECT Oekotoxikologie GmbH, Floersheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 17 KCP 10.2.2 / 04	xxx	2019	Metabolite of trifloxystrobin: BCS-AR14200 - Influence to Daphnia magna in a semi-static reproduction test -1st final report amendment Report No.: 140441221, Edition Number: M-670322-02-1 xxx **... amended: 2019-11-29** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 18	Krebber, R.; Leppelt, L.	2018	Analytical method 01555 for the determination of AE1344148 (BCS-AL58690) and AE 1393224 (BCS-AR14200) in test water by HPLC-MS/MS	No	Bayer

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			Report No.: P604187027, Edition Number: M-623236-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: No unpublished		
KCP 5.1.2.6 / 19 KCP 10.2.1 / 13	Riebschläger, T.	2018	Acute toxicity of CGA357261 (technical metabolite) to the waterflea Daphnia magna in a static renewal laboratory test system Report No.: EBTF0037, Edition Number: M-630021-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 20 KCP 10.2.1 / 18	Kuhl, K.	2018	Desmodesmus subspicatus growth inhibition test with AE 1344148 (BCS-AL58690) Report No.: EBTF0047, Edition Number: M-628915-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 21 KCP 10.2.1 / 17	Kuhl, K.	2018	Desmodesmus subspicatus growth inhibition test with AE1393224 (BCS-AR14200) Report No.: EBTF0046, Edition Number: M-629680-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 22 KCP 10.2.1 / 14	Neuhahn, A.	2017	Daphnia sp., acute immobilisation test with trifloxystrobin - TFMAP Report No.: 2017/0043/03, Edition Number: M-602375-01-1 Currenta GmbH & Co. OHG, Leverkusen, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 23	Spoo-Klöppel, M.	2017	Alga, groth inhibition test with trifloxystrobin-TFMAP Report No.: 2017/0043/04, Edition Number: M-602410-01-1 Currenta GmbH & Co. OHG, Leverkusen, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 24	Krebber, R.; Leppelt, L.	2018	Analytical method 01556 for the determination of AE 1344132 (BCS-AB55122) in test water by HPLC-UV Report No.: 01556, Edition Number: M-621113-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: No	No	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			unpublished		
KCP 5.1.2.6 / 25 KCP 10.2.1 / 19	Kuhl, K.	2018	Amendment no. 1 to final report: Desmodesmus subspicatus growth inhibition test with AE 1344132 tech. (BCS-AB55122) Report No.: E 201 05127 - 8, Edition Number: M-629159-02-1 Bayer AG, Crop Science Division, Monheim, Germany **... amended: 2018-07-17** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 26 KCP 10.2.2 / 03	xxx	2019	Metabolite of trifloxystrobin: BCS-AB39835 - Influence to Daphnia magna in a semi-static reproduction test - 1st final report amendment Report No.: 140431221, Edition Number: M-670321-02-1 xxx **... amended: 2019-11-29** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 27 KCP 10.3.1.3 / 01	Kleebaum, K.	2019	Trifloxystrobin tech. - Repeated exposure to honey bee (Apis mellifera) larvae under laboratory conditions (in vitro) Report No.: 18 48 BLC 0044, Edition Number: M-648913-01-1 BioChem agrar GmbH, Gerichshain, Germany GLP/GEP: Yes unpublished	No	Bayer

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review
Please note that all data mentioned as part of DAR, RAR, or EFSA journals are considered as relied on.

Trifloxystrobin

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCA 4.1.1 /10	Bowen, T.; Knorsch, S.	2013	Determination of AE C642802 (trifloxystrobin) in pure and technical grade materials of trifloxystrobin (AE C642802) by high performance liquid chromatography (HPLC) Bayer CropScience, Report No.: AM03512FP1, Edition Number: M-460723-01-1 Method Report No.: AM03512FP1 Date: 2013-07-26 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.1.1 /11	Bowen, T.; Schaefer, C.	2013	Validation of the HPLC - method AM035112FP1 - Determination of AE C642802 (trifloxystrobin) in trifloxystrobin (AE C642802) technical and pure material by high performance liquid chromatography (HPLC) Bayer CropScience, Report No.: PA12/091, Edition Number: M-460727-01-1 Method Report No.: PA12/091 Date: 2013-07-26 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.1.1 /12	Bowen, T.; Knorsch, S.	2013	Determination of the by-products in pure and technical grade Trifloxystrobin (AE C642802) by high performance liquid chromatography (HPLC) Bayer CropScience, Report No.: AM035212FP1, Edition Number: M-460719-01-1 Date: 2013-07-26 GLP/GEP: yes, unpublished confidential	N	Bayer
KCA 4.1.1 /13	Schaefer, C.; Bowen, T.	2013	Validation of the HPLC - method AM035212FP1 - Determination of the by-products in technical grade trifloxystrobin (AE C642802) by high performance liquid chromatography (HPLC) Bayer CropScience, Report No.: PA12/092, Edition Number: M-460716-01-1 Method Report No.: PA12/092 Date: 2013-07-26 GLP/GEP: yes, unpublished confidential	N	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCA 4.1.1 /14	Bowen, T.; Eckl, D.	2013	Trifloxystrobin (AE C642802) - Determination of methanol (AE F130989) in pure and technical grade trifloxystrobin (AE C642802) by gas chromatography (GC) Bayer CropScience, Report No.: AM036513FP1, Edition Number: M-464891-01-1 Method Report No.: AM036513FP1 Date: 2013-08-09 GLP/GEP: no, unpublished confidential	N	Bayer
KCA 4.1.1 /15	Eckl, D.; Bowen, T.	2013	Validation of GC method AM036513FP1 - Determination of the solvent methanol (AE F130989) in technical grade material of trifloxystrobin (AE C642802) by gas chromatography (GC) Bayer CropScience, Report No.: PA13/012, Edition Number: M-464890-01-1 Date: 2013-08-09 GLP/GEP: no, unpublished confidential	N	Bayer
KCA 4.1.2 /01 KCA 4.2 /02	Kissling, M.	1996	CGA 279202: Determination of parent compound by HPLC, fruits, vegetables and liquid processed commodities - Residue method including validation Ciba-Geigy Limited, Basel, Switzerland Bayer CropScience, Report No.: REM 177.02, Edition Number: M-038781-01-1 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.1.2 /02 KCA 4.2 /03 KCA 6.1 /04	Kissling, M.	1996	CGA 279202: Determination of parent compound and of metabolite CGA 321113 by GC -- cereals, bananas Ciba-Geigy Limited, Basel, Switzerland Bayer CropScience, Report No.: REM 177.03, Edition Number: M-038798-01-1 GLP/GEP: no, unpublished	N	Bayer

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 4.1.2 /03 KCA 4.2 /04	Kissling, M.	1996	Validation of Method REM 177.03: Validation by analysis of fortified specimens and determination of recoveries (including efficiency of extraction and accountability tests) Ciba-Geigy Limited, Basel, Switzerland Bayer CropScience, Report No.: 141/96, Edition Number: M-060773-01-1 Method Report No.: 141/96 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.1.2 /05 KCA 4.2 /06	Campbell, D. D.	1997	Analytical method for the determination of residues CGA 279202 and the acid metabolite, CGA 321113, in crops and animal substrates by gas chromatography Novartis Crop Protection, Inc., Greensboro, NC, USA Bayer CropScience, Report No.: AG-659, Edition Number: M-038841-01-1 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.1.2 /08 KCA 4.2 /07	Bandong, G. Q.	1998	Independent laboratory validation of the analytical method for the determination of residues of CGA-279202 and the acid metabolite, CGA-321113, in crops and animal substrates by gas chromatography The National Food Laboratory, Inc., Dublin, CA, USA Bayer CropScience, Report No.: 279202/564, Edition Number: M-136732-01-1 EPA MRID No.: 44527505 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.1.2 /10 KCA 4.2 /08	Chamkasem, N.	1996	Analytical method for the determination of CGA-279202 and its metabolites CGA 357261, CGA 357262, CGA 331409, CGA-373466 and CGA-321113 in soil by high performance liquid chromatography with UV detection including validation data Ciba-Geigy Corporation, Greensboro, NC, USA Bayer CropScience, Report No.: AG-654, Edition Number: M-038688-01-1 EPA MRID No.: 44496811 GLP/GEP: yes, unpublished	N	Bayer

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 4.1.2 /18 KCA 4.2 /13	Tribolet, R.; Kissling, M.	1999	CGA 279202: Determination of parent compound by gas chromatography, air Novartis Crop Protection AG, Basel, Switzerland Bayer CropScience, Report No.: REM 177.06, Edition Number: M-038474-02-1 GLP/GEP: no, unpublished	N	Bayer
KCA 4.1.2 /19 KCA 4.2 /14	Tribolet, R.	1997	Validation of method REM 177.06 in air: Validation by analysis of fortified specimens and evaluation of recoveries Novartis Crop Protection AG, Basel, Switzerland Bayer CropScience, Report No.: 167/97, Edition Number: M-038535-01-1 Method Report No.: 167/97 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.1.2 /20	Kissling, M.	1997	CGA 279202: Determination of parent compound and of metabolite CGA 321113 in body fluids (urine, blood) by GC Novartis Crop Protection AG, Basel, Switzerland Bayer CropScience, Report No.: REM 177.05, Edition Number: M-060777-01-1 GLP/GEP: no, unpublished	N	Bayer
KCA 4.1.2 /21	Kissling, M.	1997	Validation of Method REM 177.05: Validation by analysis of fortified specimens and determination of recoveries Novartis Crop Protection AG, Basel, Switzerland Bayer CropScience, Report No.: 164/97, Edition Number: M-060782-01-1 Method Report No.: 164/97 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.1.2 /24	Freitag, T.	2013	Modification M001 of the analytical method 01327 for the determination of trifloxystrobin and the metabolites CGA 279202 ZE-isomer, CGA 321113, CGA 373466, BCS-AB39835, BCS-CR74871, NOA 413161 and NOA 413163 in soil by HPLC-MS/MS Bayer CropScience, Report No.: MR-13/014, Edition Number: M-464872-01-1 Date: 2013-09-09 GLP/GEP: yes, unpublished	N	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCA 4.1.2 /25	Nuesslein, F.	2002	Method 00742 for the determination of residues of trifloxystrobin (parent compound) and CGA 321113 (metabolite) in/on sample materials of carrot, brussels sprouts, cabbage, tomato, red pepper and lettuce by HPLC-MS/MS Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: 00742, Edition Number: M-060431-01-1 Method Report No.: MR-078/02 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.1.2 /26	Nuesslein, F.	2003	Supplement E001 of the method 00742 for the determination of residues of Trifloxystrobin and CGA 321113 in/on the additional sample materials bean, broccoli, cauliflower, cherry, cucumber, currant, leek, melon, plum and strawberry Bayer CropScience, Report No.: 00742/E001, Edition Number: M-089461-01-1 Method Report No.: MR-052/03 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.1.2 /27	Nuesslein, F.	2004	Modification M002 to method 00742 for the determination of residues of trifloxystrobin and CGA 321113 (metabolite) in/on peach (fruit), apple (fruit), currant (fruit, jam, juice), barley (green material, grain, straw), wheat .... Bayer CropScience, Report No.: 00742/M002, Edition Number: M-121835-01-1 Method Report No.: MR-144/03 Date: 2004-02-06 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.1.2 /28	Nuesslein, F.	2004	Method 00839 for the determination of residues of Trifloxystrobin, CGA 31113 and Tebuconazole in/on sample materials of wheat, barley and rye by HPLC-MS/MS Bayer CropScience, Report No.: 00839, Edition Number: M-122162-01-1 Method Report No.: MR-155/03 GLP/GEP: yes, unpublished	N	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCA 4.1.2 /29	Zimmer, D.	2004	Supplement E001 of the method 00839 for the determination of residues of Trifloxystrobin, CGA 321113 and tebuconazole in/on the additional sample materials apple, pear, grape, broccoli, kidney bean, cauliflower, pepper, plum, Brussels ... Bayer CropScience, Report No.: 00839/E001, Edition Number: M-084249-01-1 Method Report No.: MR-051/04 Date: 2004-08-02 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.1.2 /30	Brumhard, B.; Stuke, S.	2007	Analytical method 01013 for the simultaneous determination of residues of the active Items BYF00587, prothioconazole, tebuconazole, trifloxystrobin and the metabolites BYF00587-desmethyl, JAU6476-desthio (SXX0665) and CGA321113 in/on ... Bayer CropScience, Report No.: 01013, Edition Number: M-283439-03-1 Method Report No.: MR-06/138 Date: 2007-01-25 **...Amended: 2008-02-18** GLP/GEP: yes, unpublished	N	Bayer
KCA 4.1.2 /31	Stuke, S.	2011	Amendment no. 1 to report no: P 602 11 5501 - Development of the residue analytical method 01313 for the determination of CGA279202, CGA357262, CGA357261, CGA331409, CGA321113, and CGA373466 by HPLC-MS/MS Bayer CropScience, Report No.: 01313, Edition Number: M-411496-02-1 Method Report No.: MR-11/179 Date: 2011-07-26 **...Amended: 2013-07-24** GLP/GEP: yes, unpublished	N	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCA 4.1.2 /32	Stuke, S.; Teubner, L.	2013	Modification M001 of the residue analytical method 01313 for the determination of trifloxystrobin (CGA279202) and its metabolites/isomers CGA357261, CGA357262, CGA331409, CGA321113, and CGA373466 in plant sample material at an LOQ of 0.01 mg/kg by HPLC-MS/MS Bayer CropScience, Report No.: 01313/M001, Edition Number: M-448498-01-1 Date: 2013-03-04 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2 /02 KCA 4.1.2 /01	Kissling, M.	1996	CGA 279202: Determination of parent compound by HPLC, fruits, vegetables and liquid processed commodities - Residue method including validation Ciba-Geigy Limited, Basel, Switzerland Bayer CropScience, Report No.: REM 177.02, Edition Number: M-038781-01-1 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2 /03 KCA 4.1.2 /02 KCA 6.1 /04	Kissling, M.	1996	CGA 279202: Determination of parent compound and of metabolite CGA 321113 by GC -- cereals, bananas Ciba-Geigy Limited, Basel, Switzerland Bayer CropScience, Report No.: REM 177.03, Edition Number: M-038798-01-1 GLP/GEP: no, unpublished	N	Bayer
KCA 4.2 /04 KCA 4.1.2 /03	Kissling, M.	1996	Validation of Method REM 177.03: Validation by analysis of fortified specimens and determination of recoveries (including efficiency of extraction and accountability tests) Ciba-Geigy Limited, Basel, Switzerland Bayer CropScience, Report No.: 141/96, Edition Number: M-060773-01-1 Method Report No.: 141/96 GLP/GEP: yes, unpublished	N	Bayer

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 4.2 /06 KCA 4.1.2 /05	Campbell, D. D.	1997	Analytical method for the determination of residues CGA 279202 and the acid metabolite, CGA 321113, in crops and animal substrates by gas chromatography Novartis Crop Protection, Inc., Greensboro, NC, USA Bayer CropScience, Report No.: AG-659, Edition Number: M-038841-01-1 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2 /07 KCA 4.1.2 /08	Bandong, G. Q.	1998	Independent laboratory validation of the analytical method for the determination of residues of CGA-279202 and the acid metabolite, CGA-321113, in crops and animal substrates by gas chromatography The National Food Laboratory, Inc., Dublin, CA, USA Bayer CropScience, Report No.: 279202/564, Edition Number: M-136732-01-1 EPA MRID No.: 44527505 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2 /08 KCA 4.1.2 /10	Chamkasem, N.	1996	Analytical method for the determination of CGA-279202 and its metabolites CGA 357261, CGA 357262, CGA 331409, CGA-373466 and CGA-321113 in soil by high performance liquid chromatography with UV detection including validation data Ciba-Geigy Corporation, Greensboro, NC, USA Bayer CropScience, Report No.: AG-654, Edition Number: M-038688-01-1 EPA MRID No.: 44496811 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2 /13 KCA 4.1.2 /18	Tribolet, R.; Kissling, M.	1999	CGA 279202: Determination of parent compound by gas chromatography, air Novartis Crop Protection AG, Basel, Switzerland Bayer CropScience, Report No.: REM 177.06, Edition Number: M-038474-02-1 GLP/GEP: no, unpublished	N	Bayer

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KCA 4.2 /14 KCA 4.1.2 /19	Tribolet, R.	1997	Validation of method REM 177.06 in air: Validation by analysis of fortified specimens and evaluation of recoveries Novartis Crop Protection AG, Basel, Switzerland Bayer CropScience, Report No.: 167/97, Edition Number: M-038535-01-1 Method Report No.: 167/97 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2 /16	Uceda, L.	2012	Validation of the method 01300/M002 (based on QuEChERS) for the determination of trifloxystrobin in/on plant materials by HPLC-MS/MS Bayer S.A.S., Bayer CropScience, Lyon, France Bayer CropScience, Report No.: 01300/M002, Edition Number: M-437196-02-1 Date: 2012-08-28 **...Amended: 2013-07-02** GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2 /17	Winter, O.; Amann, S.	2013	Independent laboratory validation of the method 01300/M002 (based on QuEChERS) for the determination of trifloxystrobin in/on plant materials by HPLC-MS/MS Eurofins Agroscience Services Chem GmbH, Hamburg, Germany Bayer CropScience, Report No.: S12-04450, Edition Number: M-467297-01-1 Method Report No.: S12-04450 Date: 2013-10-14 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2 /18	Winter, O.; Gizler, A.	2013	Validation of the BCS-method 01300/M013 (based on QuEChERS) for the determination of trifloxystrobin in/on plant materials by HPLC-MS/MS Eurofins Agroscience Services Chem GmbH (EAS Chem), Hamburg, Germany Bayer CropScience, Report No.: 01300/M013, Edition Number: M-466556-01-1 Method Report No.: S13-03763 Date: 2013-08-23 GLP/GEP: yes, unpublished	N	Bayer

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCA 4.2 /19	Winter, O.; Amann, S.	2013	Validation of the BCS-method-01300/M005 (based on QuEChERS) for the determination of residues of trifloxystrobin and its metabolite CGA 321113 and of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the metabolite HEC 7154 in animal tissues Eurofins Agroscience Services Chem GmbH (EAS Chem), Hamburg, Germany Bayer CropScience, Report No.: 01300/M005, Edition Number: M-453914-02-1 Method Report No.: 01300/M005 Date: 2013-05-08 **...Amended: 2013-06-06** GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2 /20	Amic, S.	2013	Independent laboratory validation of the BCS-method-01300/M005 (based on (QuEChERS) for the determination of residues of trifloxystrobin and its metabolite CGA 321113 and of fluoxastrobin (HEC 5725 E-isomer), HEC 5725 Z-isomer and the metabolite HEC 7154 in animal tissues Eurofins Agroscience Services Chem SAS, Vergeze, France Bayer CropScience, Report No.: S12-02570, Edition Number: M-467489-01-1 Method Report No.: S12-02570 Date: 2013-10-17 GLP/GEP: yes, unpublished	N	Bayer
KCA 4.2 /21	Krebber, R.; Braune, M.	2013	Analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS Bayer CropScience, Report No.: MR-13/085, Edition Number: M-466732-01-1 Method Report No.: MR-13/085 Date: 2013-10-09 GLP/GEP: yes, unpublished	N	Bayer

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for the active substance Fluopyram

The summaries and concurrent validations presented hereafter refer to plant and ecotoxicity studies presented in the dRR B7/9 Core document. Due to the fact that the test item of these tests were several formulations containing fluopyram as one of the active substances and that fluopyram was the analyte of the analytical monitoring in these studies (chosen as representative substance for the whole product), these summaries are reported within this fluopyram section.

A 2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 2.1.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.3 Description of analytical methods for the determination of residues in support to environmental fate studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.4 Description of analytical methods for the determination of residues in support to toxicological studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.5 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1)

A 2.1.1.5.1 Analytical method 01158/M001

A 2.1.1.5.1.1 Method validation

Comments of zRMS:	The objective of the study was to validate the analytical method 01158 for the determination of residues of fluopyram in leaf punches washing solutions. The method meets all guideline criteria to determine residues of fluopyram in leaf punches washing solution at the LOQ level of 10 µg/L (for 200 cm² of extractable leaf surface) and 20 µg/L (for 400 cm² of extractable leaf surface) corresponding to 0.01 µg/cm². Mean recoveries for each fortification level and the overall mean recovery were within the 70 - 110% range for all analytes. Relative standard deviations were below 20%. The correlation between the injected amount of substance and the detector response was proportional for tebuconazole and fluopyram in solvent standards ranging from 5.0 µg/L to 2000 µg/L. The correlation coefficients of the 1/x weighted linear regressions were > 0.999 for both investigated mass transitions of both investigated analytes. The study is acceptable.

Reference:	**KCP 5.1.2.4/01**
Title:	Modification 001 of analytical method 01158 for the determination of tebuconazole and fluopyram in leaf punches washing solution by HPLC-MS/MS
Report:	Stuke, S.; Diehl, P.; 2014; MR-14/016; M-502699-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The modification 001 of the analytical method 01158 (Freitag, T.; 2016; M-357292-02-1) has been developed for the determination of tebuconazole and fluopyram in washing solutions from punched leaves. In the following part, only data for fluopyram is presented.

Tebuconazole and fluopyram are washed off from the leaves punches surface twice with 100 mL of a 0.01% Aerosol® OT solution using a shaking machine. After decantation and combination of the extracts, 40 mL of acetonitrile are added. After filtration the sample solutions are subjected to reversed phase HPLC-MS/MS in positive mode for tebuconazole and fluopyram detection without any further clean-up step. Two MRM transitions were monitored in each matrix tested, m/z 396.7→ 172.7 for quantitation and m/z 396.7 → 207.7 for confirmation of fluopyram. Residues are quantified using internal stable-labelled standards.

The limit of quantitation (LOQ) is 10 µg/L (for extraction of 200 cm² of leaf surface) and 20 µg/L (for extraction of 400 cm² leaf surface) for fluopyram in leaf punches washing solution that corresponds to 0.01 µg/cm².

Results and discussions

Recovery rates were determined at fortification levels of 20.0 µg/L (=LOQ, corresponding to 400 cm² extracted leaf surface), 200 µg/L and 2000 µg/L which corresponds to fortification levels of 0.01, 0.1 and 1.0 µg/cm², respectively. Recovery experiments were conducted by fortification of untreated control material with defined amounts of trifloxystrobin prior to analysis.

The mean and overall mean recoveries per fortification level were within the range of 70 – 110%.

As a measure for the precision of the method, the intra-laboratory repeatability (n = 5) is given as relative standard deviation (% RSD) for all sample materials at fortification levels of 10.0 µg/L (= LOQ, corresponding to 200 cm² extracted leaf surface), 100 µg/L and 1000 µg/L (which correspond to fortification levels of 20.0 µg/L, 200 µg/L and 2000 µg/L for an extracted leaf surface of 400 cm²). The RSD of the repeatability tests at each recovery set ranged from 1.4 to 3.9% for fluopyram.

For confirmation of the individual residues a 2nd MRM transition was used. Results of the confirmation procedure showed comparable recovery rates for all compounds to the quantifier MRMs for all analytes.

Table A 1: Recovery rates and precision results (repeatability) of fluopyram

Analyte	Crop/Sample Material	FL [µg/cm²]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [µg/cm²]
fluopyram (m/z 396.7→ 172.7 for quantitation)	apple / leaf punch washings	0.01	101, 110, 109, 105, 111	107	3.9	0.01
		0.10	107, 110, 104, 112, 105	108	3.1	
		1.0	106, 108, 105, 105, 104	106	1.4	
			Overall recovery (n = 15)	**107**	**2.9**	
fluopyram (m/z 396.7 → 207.7 for confirmation)	apple / leaf punch washings	0.01	102, 108, 104, 99, 104	103	3.2	0.01
		0.10	103, 109, 106, 108, 107	107	2.2	
		1.0	106, 108, 104, 106, 106	106	1.3	
			Overall recovery (n = 15)	**105**	**2.6**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 2: Characteristics for the analytical method 01158/M002 used for validation of fluopyram

Specificity	HPLC-MS/MS method is highly specific. Two MRM transitions were monitored and an additional confirmatory method is not necessary. Blank values of all analytes were below 30% of the respective LOQ.	
	fluopyram (m/z 396.7→ 172.7 for quantitation)	**fluopyram (m/z 396.7 → 207.7 for confirmation)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 1.3 x + 0.00628, Correlation coefficient r: 0.9994, number of data points: 6	Individual calibration data is presented, calibration equation (1/x weighted quadratic): y = 0.961 x + 0.00303, Correlation coefficient r: 0.9995, number of data points: 6
Calibration range	5 – 2000 µg/L	
Limit of determination/quantification	LOQ = 20 µg/L when extracting 400 cm² corresponding to 0.01 µg/cm²	
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.	

Conclusion

The modification 001 of the analytical method 01158 was developed for the determination of tebuconazole and fluopyram in washing solutions from punched leaves at a limit of quantitation (LOQ) of 0.01 µg/cm². The analytical method complies with all guideline criteria according to SANCO/3029/99 rev. 4. The analytical method 01158/M001 is suitable for the determination of the magnitude of the dislodgeable foliar residues (DFR) of fluopyram in washings from apple leaf punches via HPLC-MS/MS.

A 2.1.1.5.2 Analytical method 01158/M001 in support of the study M-569303-01-1

A 2.1.1.5.2.1 Method validation

Comments of zRMS:	Dislodgeable foliar residues of AE C656948 and trifloxystrobin were determined according to the 01158/M002 method (S. Stuke, S. van Berkum, MR-15/032, 2015-09-07). During the set of analysis, a calibration curve was established for AE C656948 and trifloxystrobin with at least six concentration levels and used for the quantitation. For the calibration curves the correlation coefficients R were above 0.999. No residues above the LOQ were found in the control samples. The mean of the concurrent laboratory recoveries for AE C656948 amounted to 98% with a relative standard deviation of 6.3%. The mean of the concurrent laboratory recoveries for trifloxystrobin amounted to 102% with a relative standard deviation of 5.7%. The mean of the field recovery samples for AE C656948 amounted to 84% with a relative standard deviation of 8.5%. The mean of the field recovery samples for trifloxystrobin amounted to 80% with a relative standard deviation of 4.2%. All criteria according to SANCO/3029/99 rev. 4 were met. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.4/02**
Title:	Determination of the dislodgeable foliar residues (DFR) of trifloxystrobin and AE C656948 in/on grape after spraying of AE C656948 & CGA279202 SC 500 in the field in the North of France
Report:	Stuke, S.; Daniela, M.; van Berkum, S.; 2016; 15-2924; M-569303-01-1
Authority registration No:	
Guideline(s):	US EPA OPPTS 875.2100 Foliar Dislodgeable Residue Dissipation
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The magnitude of the dislodgeable foliar residues (DFR) of the substances AE C656948 (fluopyram, FLU) and CGA 279202 (trifloxystrobin, TFS) in washings from grape leaf punches was determined after two spray applications of the formulation AE C656948 & CGA 279202 SC 500 (containing 250 g/L AE C656948 and 250 g/L trifloxystrobin). In the following part, only data for AE C656948 is presented.

Full validation data is documented with the method 01158/M001 (Stuke, S.; Diehl, P.; 2014; M-502699-01-1) for AE C656948. For the matrix relevant to this study (grape, leaf punch washings), a limited set of additional validation recoveries was performed within the present study.

The test item was extracted from the leaf punches by adding 100 mL of a 0.01% Aerosol OT solution (i.e. docusate sodium salt) which corresponds to a surfactant. After shaking, the solution was decanted and the dislodging procedure was repeated for each sample with a fresh 100 mL aliquot of the Aerosol solution. The second rinse was again decanted and added to the first.

Due to observed adherence problems of CGA 279202 the samples were treated deviating to the cited method to reduce the adherence effect and, thus to obtain higher field recoveries. After thawing the samples the solution was filled into another bottle, the original bottle was washed with pure acetonitrile to avoid adherence of CGA 279202 to the vessel walls to a total of 25 mL for the field spike samples and 500 mL for the field samples. The resulting sample dilution factor was 2.5.

After adding an internal standard solution the samples were subjected to HPLC-MS/MS analysis without further clean-up or preparation steps. Residues were calculated using internal standard calibration.

Results and discussions
A limited set of additional validation recoveries was analysed and recoveries were determined at fortification levels of 0.01 µg/cm^2, 0.10 µg/cm^{2}and 1.0 µg/cm^2.

In order to check the performance of the method, recovery determinations were included in each set of analysis (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The limit of quantification (LOQ), defined as the lowest validated fortification level, was set to 20 µg/L when extracting 400 cm^2 corresponding to 0.01 µg/cm^2. Blank values in control samples were below 30% of the LOQ.

The average recoveries per fortification level were within the range of 70 – 110%, and the relative standard deviation (RSD) values were below 20%, if applicable (n ≥ 3).

Table A 3: Recovery rates and precision results (repeatability) of AE C656948

Analyte	Crop/Sample Material	FL [µg/cm^2]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [µg/cm^2]
AE C656948	grape / leaf punch washings	0.01	106; 110; 94; 95; 97	100	7.1	0.01
		0.10	97; 99; 95; 93	96	2.7	
		1.0	88; 102	95	-	
			Overall recovery (n = 11)	**98**	**6.3**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 4: Characteristics for the analytical method 01158/M001 used for validation of AE C656948

	AE C656948
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.0054834 x + 0.00039617, Correlation coefficient r: 0.9996, number of data points: 7
Calibration range	0.5 to 1000 µg/L
Limit of determination/quantification	LOQ = 20 µg/L when extracting 400 cm^2 corresponding to 0.01 µg/cm^2
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.

Conclusion
The analytical method 01158/M001 used in the present study for the determination of AE C656948 in leaf punches washing solution was fully validated during study M-502699-01-1 (Stuke, S.; Diehl, P.; 2014). For the matrix relevant to this study (grape, leaf punch washings) a limited set of additional validation recoveries was analyzed. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01158/M001 is suitable for the determination of the magnitude of the dislodgeable foliar residues (DFR) of AE C656948 in washings from grape leaf punches via HPLC-MS/MS.

A 2.1.1.5.3 Analytical method 01158/M001 in support of the study M-558518-01-1

A 2.1.1.5.3.1 Method validation

Comments of zRMS:	Dislodgeable foliar residues of AE C656948, and CGA 32113 and trifloxystrobin were determined according to the 01158/M002 method (S. Stuke, S. van Berkum, MR-15/032, 2015-09-07).

	During the set of analysis, a calibration curve was established for AE C656948 and trifloxystrobin and the additional analysis of CGA 321113 with at least six concentration levels and used for the quantitation. For the calibration curves the correlation coefficients R were above 0.998. No residues above the LOQ were found in the control samples. The mean of the concurrent laboratory recoveries for AE C656948 amounted to 98% with a relative standard deviation of 4.2%. The mean of the field recovery samples for AE C656948 amounted to 89% with a relative standard deviation of 7.9%. The mean of the concurrent laboratory recoveries for trifloxystrobin amounted to 85% with a relative standard deviation of 5.9%. The mean of the field recovery samples for trifloxystrobin amounted to 79% with a relative standard deviation of 10.0%. The results for the dislodgeable foliar residues for AE C656948 and trifloxystrobin in the field samples are not corrected for laboratory or field recoveries. All criteria according to SANCO/3029/99 rev. 4 were met. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.4/03**
Title:	Determination of the dislodgeable foliar residues (DFR) of trifloxystrobin and AE C656948 in/on lily after spraying of AE C656948 & CGA279202 SC 500 in the field in the Netherlands
Report:	Stuke, S.; van Berkum, S.; 2016; 15-2925; M-558518-01-1
Authority registration No:	
Guideline(s):	US EPA OPPTS 875.2100 Foliar Dislodgeable Residue Dissipation
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The magnitude of the dislodgeable foliar residues (DFR) of the substances AE C656948 (fluopyram, FLU) and CGA279202 (trifloxystrobin, TFS) in washings from lily leaf punches was determined after two spray applications with AE C656948 & CGA279202 SC 500 (containing 250 g/L fluopyram and 250 g/L trifloxystrobin).

Full validation data is documented with the method 01158/M001 (Stuke, S.; Diehl, P.; 2014; M-502699-01-1) for AE C656948. For the matrix relevant to this study (lily, leaf punch washings), a full set of additional validation recoveries was performed within the present study.

The test item was extracted from the leaf punches by adding 100 mL of a 0.01% Aerosol OT solution (i.e. docusate sodium salt) which corresponds to a surfactant. After shaking, the solution was decanted and the dislodging procedure was repeated for each sample with a fresh 100 mL aliquot of the Aerosol solution. The second rinse was again decanted and added to the first.

Due to observed adherence problems of CGA 279202 the samples were treated deviating to the cited method to reduce the adherence effect and, thus to obtain higher field recoveries. After thawing the samples the solution was filled into another bottle, the original bottle was washed with pure acetonitrile to avoid adherence of CGA 279202 to the vessel walls to a total of 25 mL for the field spike samples and 500 mL for the field samples. The resulting sample dilution factor was 2.5.

After adding an internal standard solution the samples were subjected to HPLC-MS/MS analysis without further clean-up or preparation steps. Residues were calculated using internal standard calibration.

Results and discussions

A full set of additional validation recoveries was analysed and recoveries were determined at fortification levels of 0.01 µg/cm^2, 0.10 µg/cm^{2}and 1.0 µg/cm^2.

In order to check the performance of the method, recovery determinations were included in each set of analysis (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The limit of quantification (LOQ), defined as the lowest validated fortification level, was set to 20 µg/L when extracting 400 cm^2 corresponding to 0.01 µg/cm^2. Blank values in control samples were below 30% of the LOQ.
The average recoveries per fortification level were within the range of 70 – 110%, and the relative standard deviation (RSD) values were below 20%, if applicable (n ≥ 3).

Table A 5: Recovery rates and precision results (repeatability) of AE C656948

Analyte	Crop/Sample Material	FL [µg/cm^2]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [µg/cm^2]
AE C656948	lily / leaf punch washings	0.01	91; 95; 95; 99; 105	97	5.5	0.01
		0.10	93; 97; 101; 101; 103	99	4.0	
		1.0	97; 97	97	-	
			Overall recovery (n = 12)	**98**	**4.2**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 6: Characteristics for the analytical method 01158/M001 used for validation of AE C656948

	AE C656948
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.0083690 x + 0.0033771, Correlation coefficient r: 0.9998, number of data points: 6
Calibration range	0.5 to 1000 µg/L
Limit of determination/quantification	LOQ = 20 µg/L when extracting 400 cm^2 corresponding to 0.01 µg/cm^2
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.

Conclusion
The analytical method 01158/M001 used in the present study for the determination of AE C656948 in leaf punches washing solution was fully validated during study M-502699-01-1 (Stuke, S.; Diehl, P.; 2014). For the matrix relevant to this study (lily, leaf punch washings) a full set of additional validation recoveries was analyzed. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01158/M001 is suitable for the determination of the magnitude of the dislodgeable foliar residues (DFR) of AE C656948 in washings from lily leaf punches via HPLC-MS/MS.

A 2.1.1.5.4 Analytical method 01158/M001 in support of the study M-677729-01-1

A 2.1.1.5.4.1 Method validation

Comments of zRMS:	Analytical Method: Stuke, S., van Berkum, S., Modification 002 of analytical method 01158 for the determination of tebuconazole, fluopyram and trifloxystrobin in leaf punches washing solution by HPLC-MS/MS, BAG report MR-15/032, dated 2015-09-07. The validity of this adapted methodology was ensured by 5 recoveries at the LOQ level, 5 recoveries at the 10x LOQ level and 5 recoveries at the 100x LOQ level. During each set of analysis, a calibration curve was established for trifloxystrobin with at least five concentration levels and used for the quantitation. For the calibration curve the correlation coefficient R was above 0.999.

	During each set of analysis, a calibration curve was established for AE C656948 with at least five concentration levels and used for the quantitation. For the calibration curve the correlation coefficient R was above 0.999. No residues of trifloxystrobin above the LOQ were found in the control samples. No residues of AE C656948 above the LOQ were found in the control samples. The mean of the concurrent laboratory recoveries for trifloxystrobin amounted to 96% with a relative standard deviation of 2.3%. The mean of the concurrent laboratory recoveries for AE C656948 amounted to 99% with a relative standard deviation of 6.7%. The mean of the field recovery samples for trifloxystrobin amounted to 69% with a relative standard deviation of 4.8%. The mean of the field recovery samples for AE C656948 amounted to 94% with a relative standard deviation of 4.2%. All criteria according to SANCO/3029/99 rev. 4 were met. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.4/04**
Title:	Determination of the dislodgeable foliar residues (DFR) of trifloxystrobin and AE C656948 in/on raspberry after spray application of AE C656948 & CGA279202 SC 500 in the field in Italy
Report:	Daniels, M. ; van Berkum, S.; 2020; 18-2905; M-677729-01-1
Authority registration No:	
Guideline(s):	US EPA OPPTS 875.2100 Foliar Dislodgeable Residue Dissipation
Deviations:	Yes (see report)
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The magnitude of the dislodgeable foliar residues (DFR) of the substances AE C656948 (FLU) and trifloxystrobin (TFS) in washings from raspberry leaf punches was determined after two spray applications with the suspension concentrate formulation AE C656948 & CGA279202 SC 500 (containing 250 g/L AE C656948 and 250 g/L trifloxystrobin).

Full validation data is documented with the method 01158/M001 (Stuke, S.; Diehl, P.; 2014; M-502699-01-1) for AE C656948. For the matrix relevant to this study (raspberry, leaf punch washings), a full set of additional validation recoveries was performed within the present study.

The test item was extracted from the leaf punches by adding 100 mL of a 0.01% Aerosol OT solution (i.e. docusate sodium salt) which corresponds to a surfactant. After shaking, the solution was decanted and the dislodging procedure was repeated for each sample with a fresh 100 mL aliquot of the Aerosol solution. The second rinse was again decanted and added to the first.

Conditions used in this study:

The following presented sample preparation scheme also considers experiences during past DFR studies with the active compound trifloxystrobin which showed the tendency to adhere to plastic surfaces (especially to the plastic surface of field spike pre-solution vials). To avoid those adherences the plastic bottles (and field spike vials inside) are flushed with pure acetonitrile and subsequently with pure dichloromethane according to the following described procedure.

200 mL thawed field sample is filled into a 500-mL volumetric flask and 10-mL thawed field spike or lab recovery sample is filled into a 25-mL volumetric flask. 100 mL of pure acetonitrile is added into the empty 200 mL plastic bottle (or 5 mL to the empty lab and field spike recoveries bottles). After ultra-sonicating, fill the solution into the corresponding volumetric flask. 100 mL of acetonitrile is added again into the field sample bottle (5 mL to the empty lab and field spike recoveries bottles). After ultra-sonicating, the washing solutions are combined with the corresponding sample solution in the volumetric flask. 50 mL of pure dichloromethane is added into the empty 200-mL plastic bottle (or 5 mL to the empty lab and field spike recoveries bottles). After shaking, evaporate the dichloromethane fractions to dryness applying a vacuum at a temperature of 40°C. Re-dissolve the dry residues; the former 50-mL fractions with 2x 25 mL pure

acetonitrile and the former 5-mL fractions with 2x 2 mL of pure acetonitrile. Combine the solutions with those in the corresponding volumetric flask. Fill the flask up to the mark with water. The total volume is 500 mL for field samples and 25 mL for lab or field spike recoveries (dilution factor of 2.5). Transfer an aliquot of 0.1 mL of the sample solution into a 1.8-mL HPLC vial, add 100 µL of an internal standard solution (containing 100 µg/L internal standard of each compound) and 0.8 mL of water. The total sample dilution factor is 25. Subject to Liquid Chromatography and MS/MS- determination. The final concentration at the LOQ is 0.8 µg/L in the final analytical extract which corresponds to 0.01 µg/cm^2.

Results and discussions

A full set of additional validation recoveries was analysed and 5 recoveries were determined each at fortification levels of 0.01 µg/cm^2 (LOQ), 0.10 µg/cm^2 (10xLOQ) and 1.0 µg/cm^2 (100xLOQ).
In order to check the performance of the method and the adaptation, at least five recovery determinations per fortification level were performed. Concurrent recoveries were included in each set of analysis (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.
Blank values in control samples were below 30% of the LOQ.
The average recoveries per fortification level were within the range of 70 – 110%, and the relative standard deviation (RSD) values were below 20%, if applicable (n ≥ 3).

Table A 7: Recovery rates and precision results (repeatability) of AE C656948

Analyte	Crop/Sample Material	FL [µg/cm^2]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [µg/cm^2]
AE C656948	raspberry / leaf punch washings	0.01	101; 97; 99; 97;93	97	3.0	0.01
		0.10	95; 93; 93; 93; 97	94	1.9	
		1.0	99; 95; 94; 95;101	97	3.1	
		2.0	115; 109; 111	112	2.7	
			Overall recovery (n = 18)	**99**	**6.7**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 8: Characteristics for the analytical method 01158/M001 used for validation of AE C656948

	AE C656948
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.14653 x + 0.0045164, Correlation coefficient r: 0.9999, number of data points: 7
Calibration range	0.2 to 100 µg/L (corresponding to 0.0025 – 1.25 µg/cm^2)
Limit of determination/quantification	LOQ = 0.8 µg/L when extracting 400 cm^2 corresponding to 0.01 µg/cm²
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.

Conclusion

The analytical method 01158/M001 used in the present study for the determination of AE C656948 in leaf punches washing solution was fully validated during study M-502699-01-1 (Stuke, S.; Diehl, P.; 2014). For the matrix relevant to this study (raspberry, leaf punch washings) a full set of additional validation recoveries was analyzed. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01158/M001 is suitable for the determination of the magnitude of the dislodgeable foliar residues (DFR) of AE C656948 in washings from raspberry leaf punches via HPLC-MS/MS.

A 2.1.1.6 Description of analytical methods for the determination of residues in ecotoxicology studies (KCP 5.1)

A 2.1.1.6.1 Analytical method 01387 in support of the study M-636236-01-1

A 2.1.1.6.1.1 Method validation

Comments of zRMS:	Concentrations of fluopyram and trifloxystrobin of the test item Fluopyram + Trifloxystrobin SC 500 (250 + 250 g/L) were determined in test media samples using liquid chromatography with MS/MS detection. Specificity: No significant (< 30 %) interference of total peak area for the target analyte was found for either analyte. Linearity: Calibration Range: Fluopyram: 0.1 – 45 µg reference item /L Trifloxystrobin: 0.1 – 45 µg reference item /L Linearity of Response: Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression. Correlation Coefficient: Fluopyram: r = 0.9995 at least Trifloxystrobin: r = 0.9988 at least Calibration Curves: Fluopyram: y = 23008 * x + 924 Trifloxystrobin: y = 59585 * x – 1465 Accuracy and Precision: Mean Recovery Rates in the Fortified Samples: Fluopyram: 94% (n = 15, RSD 7%) Trifloxystrobin: 84% (n = 15, RSD 7%) The values found for the precision (RSD) and for the accuracy (mean recovery rate) are acceptable. Limit of Quantification: Fluopyram: 0.4 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.004 mg test item/L) 90% (n = 5, RSD 12%) Trifloxystrobin: 0.4 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.004 mg test item/L) 86% (n = 5, RSD 8%) The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met. The study is acceptable.

Reference:	**KCP 5.1.2.6/01**
Title:	Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L) - Acute toxicity to rainbow trout (Oncorhynchus mykiss) in a 96-hour semi-static test
Report:	xxx
Authority registration No:	
Guideline(s):	- Commission Regulation (EC) No 440/2008, Annex, Part C, C.1: "Acute Toxicity for Fish", Official Journal of the European Union, May 30, 2008 - EPA Guideline 712-C-16-007:OCSPP 850.1075, " Freshwater and Saltwater Fish Acute Toxicity Test" October 2016 - Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-7-1-1, Fish acute toxicity studies, 2005 - OECD Guideline for Testing of Chemicals, Section 2, No. 203: "Daphnia sp., "Fish, Acute Toxicity Test" adopted July 17, 1992 - SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of Analysis in Support of preregistration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	No

Materials and methods

The purpose of the analytical part of this study was to verify the concentrations of the active ingredients fluopyram and trifloxystrobin of the test item fluopyram + trifloxystrobin SC 500 in the test medium by LC-MS/MS. In the following part, only the data for fluopyram is presented.
The analytical method 01387 was used in the present study which is fully validated and EU-agreed (Krebber, R.; Braune, M.; 2013; M-466732-01-1).
After appropriate dilution, the determination was conducted using high performance liquid chromatography (HPLC) with mass-spectrometric (MS/MS) detection (ESI positive, MRM used as quantifier m/z: 397.0 → 173.0, MRM used as qualifier m/z: 397.0 → 208.0).

Results and discussions

For fluopyram, recoveries were performed in test water spiked with test item at the fortification levels of 0.004, 0.01 and 3 mg test item/L. The mean and overall mean recovery values were within the acceptable range of 70 – 110%, all relative standard deviation (RSD) values were below 20%.

Table A 9: Recovery rates and precision results (repeatability) of fluopyram

	Concentration					
Sample description	**Nominal [mg test item/L]**	**Found [µg a.i./L][1]**	**DF**	**Concentration calculated [µg a.i./L][1]**	**Corrected nominal [µg a.i./L][2]**	**Recovery [%][1]**
Anayltical Blank	0	<LOQ	2	n.a.	0.000	n.a.
Anayltical Blank	0	<LOQ	2	n.a.	0.000	n.a.
Fortified Sample	0.004	0.371	2	0.741	0.839	88
	0.004	0.442	2	0.885	0.839	105
	0.004	0.351	2	0.702	0.845	83
	0.004	0.407	2	0.815	0.845	96
	0.004	0.332	2	0.665	0.845	79
					Mean value (n = 5):	**90**
					RSD (n = 5):	**12**
Fortified Sample	0.01	1.007	2	2.015	2.098	96
	0.01	0.994	2	1.988	2.098	95
	0.01	0.955	2	1.910	2.114	90
	0.01	0.964	2	1.928	2.114	91
	0.01	0.964	2	1.928	2.114	91
					Mean value (n = 5):	**93**
					RSD (n = 5):	**3**

Fortified Sample	3	30.729	20	614.582	629.020	98
	3	30.144	20	602.873	629.020	96
	3	31.803	20	636.051	641.774	99
	3	30.957	20	619.136	641.774	96
	3	32.063	20	641.774	641.774	100
					Mean value (n = 5):	**98**
					RSD (n = 5):	**2**
					Overall mean value (n = 15):	**94**
					RSD (n = 15):	**7**

LOQ: Limit of Quantification = 0.004 mg test item/L corresponding to 0.4 µg a.i./L after dilution by factor 2;
n.a.: not applicable; RSD: Relative Standard Deviation; DF: Dilution factor; a.i.: active ingredient

Table A 10: Characteristics for the analytical method used for validation of fluopyram

	fluopyram
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration equation: y = 23008 x - 924, Correlation coefficient r: 0.9995, number of data points: 9 The function is linear in the operating range.
Calibration range	0.1 – 45 µg reference item/L
Limit of determination/quantification	LOQ = 0.4 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.004 mg test item/L)
Assessment of matrix effects is presented	No effects observed.

Conclusion
The analytical method complies with all guideline criteria according to SANCO/3029/99 rev. 4 and can be seen as fit for purpose for the presented study.

A 2.1.1.6.2 Analytical method 01387 in support of the study M-636231-01-1

A 2.1.1.6.2.1 Method validation

Comments of zRMS:	Concentrations of fluopyram and trifloxystrobin of the test item Fluopyram + Trifloxystrobin SC 500 (250 + 250 g/L) were determined in test media samples using liquid chromatography with MS/MS detection. Specificity: No significant (< 30%) interference of total peak area for the target analyte was found for either analyte. Linearity: Calibration Range: Fluopyram: 0.1 – 35 µg reference item /L Trifloxystrobin: Two calibration curves were used in order to cover the wide concentration range of 0.1 – 35 µg reference item/L with high accuracy. 1. 0.1 – 10 µg a.i./L 2. 0.1 – 35 µg a.i./L Linearity of Response: Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression. Correlation Coefficient: Fluopyram: r = 0.9997 Trifloxystrobin: 1. r = 0.9997 2. r = 0.9999 Calibration Curves: Fluopyram:

	y = 12006 * x + 1154 Trifloxystrobin: 1. y = 31386 * x + 3204 2. y = 29688 * x + 6913 Accuracy and Precision: Mean Recovery Rates in the Fortified Samples: Fluopyram: 94% (n = 15, RSD 10%) Trifloxystrobin: 75% (n = 10, RSD 8%) The values found for the precision (RSD) and for the accuracy (mean recovery rate) are acceptable. Limit of Quantification: Fluopyram: 0.27 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.0025 mg test item/L) 86% (n = 5, RSD 12%) Trifloxystrobin: 0.86 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.008 mg test item/L) 78% (n = 5, RSD 9%) The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met. The study is acceptable.

Reference:	**KCP 5.1.2.6/02**
Title:	Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L): Acute toxicity to Daphnia magna in a semi-static 48-hour immobilisation test
Report:	xxxx
Authority registration No:	
Guideline(s):	- Commission Regulation (EC) No 440/2008, Annex, Part C, C.2: "Daphnia sp. Acute Immobilsisation Test", Official Journal of the European Union (EN), dated May 30, 2008 - EPA Guideline 712-C-16-013:OCSPP 850.1010, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids" October 2016 - Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-7-2-1, Daphnia acute immobilization studies, 2005 - OECD Guideline for Testing of Chemicals No. 202: "Daphnia sp., Acute Immobilisation Test" adopted Aprils 13, 2004 - SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of Analysis in Support of preregistration for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the analytical part of this study was to verify the concentrations of the active ingredients fluopyram and trifloxystrobin of the test item fluopyram + trifloxystrobin SC 500 in the test medium by LC-MS/MS. In the following part, only the data for fluopyram is presented.

The analytical method 01387 was used in the present study which is fully validated and EU-agreed (Krebber, R.; Braune, M.; 2013; M-466732-01-1).

After appropriate dilution, the determination was conducted using high performance liquid chromatography (HPLC) with mass-spectrometric (MS/MS) detection (ESI positive, MRM used as quantifier m/z: 409.1 → 186.0, MRM used as qualifier m/z: 409.1 → 145.0).

Results and discussions

For fluopyram, recoveries were performed in test water spiked with test item at the fortification levels of 0.004, 0.01 and 3 mg test item/L. The mean and overall mean recovery values were within the acceptable range of 70 – 110%, all relative standard deviation (RSD) values were below 20%.

Table A 11: Recovery rates and precision results (repeatability) of fluopyram

	Concentration					
Sample description	**Nominal [mg test item/L]**	**Found [µg a.i./L][1]**	**DF**	**Concentration calculated [µg a.i./L][1]**	**Corrected nominal [µg a.i./L][2]**	**Recovery [%][1]**
Anayltical Blank	0	<LOQ	2	n.a.	0.000	n.a.
Anayltical Blank	0	<LOQ	2	n.a.	0.000	n.a.
Fortified Sample	0.0025	0.190	2	0.379	0.540	70
	0.0025	0.258	2	0.516	0.540	96
	0.0025	0.221	2	0.442	0.538	82
	0.0025	0.250	2	0.501	0.538	93
	0.0025	0.240	2	0.481	0.538	89
					Mean value (n = 5):	**86**
					RSD (n = 5):	**12**
Fortified Sample	0.008	0.787	2	1.574	1.727	91
	0.008	0.762	2	1.524	1.727	88
	0.008	0.837	2	1.674	1.721	97
	0.008	0.862	2	1.723	1.721	100
	0.008	0.928	2	1.857	1.721	108
					Mean value (n = 5):	**97**
					RSD (n = 5):	**8**
Fortified Sample	0.2	20.394	2	40.787	43.176	94
	0.2	20.477	2	40.954	43.176	95
	0.2	21.560	2	43.119	43.023	100
	0.2	22.476	2	44.952	43.023	104
	0.2	22.393	2	44.785	43.023	104
					Mean value (n = 5):	**100**
					RSD (n = 5):	**5**
					Overall mean value (n = 15):	**94**
					RSD (n = 15):	**10**

LOQ: Limit of Quantification = 0.0025 mg test item/L, corresponding to 0.27 µg a.i./L after dilution by factor 2;
n.a.: not applicable; RSD: Relative Standard Deviation; DF: Dilution factor; a.i.: active ingredient;

Table A 12: Characteristics for the analytical method used for validation of fluopyram

	fluopyram
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration equation for lower calibration range: y = 12006 x + 1154, Correlation coefficient r: 0.9997, number of data points: 9 The function is linear in the operating range.
Calibration range	0.1 – 35 µg a.i./L
Limit of determination/quantification	LOQ = 0.27 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.0025 mg test item/L)
Assessment of matrix effects is presented	No effects observed.

Conclusion

The analytical method complies with all guideline criteria according to SANCO/3029/99 rev. 4 and can be seen as fit for purpose for the presented study.

A 2.1.1.6.3 Analytical method 01387/M001

A 2.1.1.6.3.1 Method validation

Comments of zRMS:	The objective of the study was to validate the analytical method 01387/M001 for the determination of concentrations of fluopyram in drinking and surface water by HPLC-MS/MS using two MRM transitions. The method/detector response was linear in the concentration range from 0.015 µg/L to 1 µg/L (correlation coefficient r ≥ 0.9993) for fluopyram for both MRM transitions. An untreated control sample in surface water was examined. The concentration was below 30% of the LOQ. Because of the direct measurement of the samples recovery rates cannot be calculated. The relative standard deviation for the peak area counts is ≤ 3.4 % for both MRM transitions. LOQ = 0.05 µg/L

Reference:	**KCP 5.1.2.6/03**
Title:	Modification M001 of the analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS
Report:	Krebber, R.; 2014; 01387/M001; M-494841-02-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EC Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1 of November 16, 2010 European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, July 11, 2000
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The modification M001 of the analytical method 01387 describes the determination of fluopyram (and other analytes) in drinking and surface water by HPLC-MS/MS using two MRM transitions.
The samples are added with of 0.1 mL formic acid per liter sample volume and directly injected into the HPLC-MS/MS instrument or after appropriate dilution with a mixture of river Rhine water / formic acid (1000 / 0.1, v/v). Water samples were determined without further clean-up using positive ion-mode. The MS/MS instrument was operated in the Multiple Reaction Monitoring mode (MRM). Concentrations were quantified using external matrix-matched standard solutions.

Results and discussions

Because of the direct measurement of the samples recovery rates cannot be calculated. Thus precision data are presented. The relative standard deviations for the peak areas were ≤ 20 % for all analytes and MRM transitions.

Table A 13: Recovery/repeatability results in surface water

Analyte	Matrix	Fortification level [µg/L]	Mean value [Peak Area]	RSD [%]	n
Fluopyram Quantitation ion: m/z 397 → m/z 173	Surface water	0.05 0.5	359167 3364824	3.0 3.4	10
Fluopyram Confirmatory ion: m/z 397 → m/z 208	Surface water	0.05 0.5	421924 3905793	3.0 1.1	10

Table A 14: Validation data for the determination of active substance fluopyram in surface water

	Fluopyram
Author(s), year	Krebber, R., Ruttmann, F., Leppelt, L., 2014
Principle of method	HPLC-MS/MS
Specificity	Two MRM transitions were monitored. HPLC-MS/MS method is highly specific. No signals/peaks interfering with the detection of the analytes were observed. Apparent concentrations in control samples were below 0.3 × LOQ.
Linearity	Quantitation MRM (m/z 397 → m/z 173): Individual calibration data is presented, calibration equation (1/x weighted): $y = 6.55 \cdot 10^6 x + 3.31 \cdot 10^4$, Correlation coefficient r: 0.9999, number of data points: 6 Confirmatory MRM (m/z 397 → m/z 208): Individual calibration data is presented, calibration equation (1/x weighted): $y = 7.47 \cdot 10^6 x + 4.54 \cdot 10^4$, Correlation coefficient r: 0.9997, number of data points: 6
Calibration range	0.015 µg/L to 1 µg/L
Limits of quantification (LOQ)	LOQ = 0.05 µg/L
Assessment of matrix effects is presented	No effects observed.
Comment	The analytical method was validated for surface water. A validation for drinking water was not necessary because the limit of quantitation for surface water is equal or below the drinking water limit.

Conclusion

The objective of the study was to validate the analytical method 01387/M001 for the determination of concentrations of fluopyram (and other analytes) in drinking and surface water by HPLC-MS/MS using two MRM transitions.
The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.
The method was used in support of the study Kuhl, K.; 2018; M-615579-01-1.

A 2.1.1.6.4 Concurrent validation of method 01387/M001 in support of the study M-615579-01-1

Comments of zRMS:	The water samples were analysed according to the following methods: Fluopyram: Modification M001 of analytical method 01387 for the determination of varius pesticides in drinking and surface water by HPLC-MS/MS, Report of Bayer CropScience AG, MR-14/053, dated 2014-10-23. Trifloxystrobin: Analytical method 01387 for the determination of varius pesticides in drinking and surface water by HPLC-MS/MS, Report of Bayer CropScience AG, MR-13/0085, dated 2013-10-09. In the present study the methods were validated concurrently with the sample analyses of the study by evaluation of the standard injections. The limit of quantitation (LOQ) : Fluopyram: LOQ = 0.0625 µg a.s./L Trifloxystrobin: LOQ = 0.0625 µg a.s./L

	The method is considered as fit for purpose and can be used in the evaluation.

Reference:	**KCP 5.1.2.6/04**
Title:	Pseudokirchneriella subcapitata growth inhibition test with fluopyram + trifloxystrobin SC 500 G - Final report
Report:	Kuhl, K.; 2018; EBGM0016; M-615579-01-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation1107/2009 (Europe) OECD Test Guideline 201 US EPA OCSPP 850.4500
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation

The purpose of the analytical part of this study was to verify the concentrations of the active ingredients fluopyram and trifloxystrobin of the test item fluopyram + trifloxystrobin SC 500 in the test water by LC-MS/MS. In the following part, only the data for fluopyram is presented.

For the determination of fluopyram, the analytical method 01387/M001 was used in the present study which was fully validated during study M-494841-02-1 (Krebber, R.; 2014). The method was validated concurrently with the test solution analyses. For this purpose, the fluopyram standard injections were evaluated.

The diluted water samples were directly injected into the HPLC-MS/MS instrument. The injection volume was 10 µL. Each sample was injected in duplicate.

Because of the direct measurement of the samples recovery rates cannot be calculated. Thus, the presented precision data is based on four to six injections of six different standard solutions. The relative standard deviations for the peak areas were <4% for all measured concentration levels.

Table A 15: Recovery rates and precision results (repeatability) of fluopyram

fluopyram standard concentration [µg/L]	n	Peak area		Retention Time	
		Mean Value	RSD	Mean Value	RSD
		[area counts]	[%]	[min]	[%]
0.0500	4	108215	2.2	2.57	0.2
0.100	4	204276	1.7	2.57	<0.1
0.500	4	948892	1.2	2.57	0.2
1.00	6	1881783	1.2	2.57	0.2
5.00	4	9036958	1.0	2.57	<0.1
10.0	4	16194491	1.5	2.58	0.2

Table A 16: Characteristics for the analytical method used for validation of fluopyram

	fluopyram
Specificity	HPLC-MS/MS method is highly specific. Blank values were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): $y = 1.6943 \cdot 10^{6}\ x + 39869$, Correlation coefficient r: 0.998142, number of data points: 6
Calibration range	0.0500 – 10.0 µg/L
Limit of determination/quantification	LOQ = 0.0625 µg a.s./L
Assessment of matrix effects is presented	No effects observed.

Conclusion
The applicability of the method 01387/M001 for the analysis of fluopyram in water samples was tested. The data presented demonstrate that the method allows the determination of this substance with satisfactory precision data given as the relative standard deviation was below 20% of four to six replicates per measured concentration level. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.1.1.6.5 Analytical method 01387 in support of the study M-636234-01-1

A 2.1.1.6.5.1 Method validation

Comments of zRMS:	Concentrations of fluopyram and trifloxystrobin of the test item Fluopyram + Trifloxystrobin SC 500 (250 + 250 g/L) were determined in the test water using liquid chromatography with MS/MS detection. Specificity: No significant (< 30%) interference of total peak area for the target analyte was found for either analyte. Linearity: Calibration Range: Fluopyram: Two calibration curves were used in order to cover the wide concentration range of 0.1 – 100 µg reference item/L with high accuracy. 1. 0.1 – 10 µg a.i./L 2. 0.1 – 100 µg a.i./L Trifloxystrobin: 0.1 – 100 µg a.i./L Linearity of Response: Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression. Correlation Coefficient: Fluopyram: 1. r = 1.0000 2. r = 0.9998 Trifloxystrobin: r = 0.9999 Calibration Curves: Fluopyram: 1. y = 27248 * x + 1087 2. y = 24682 * x + 10730 Trifloxystrobin: y = 56584 * x + 5193 Accuracy and Precision: Mean Recovery Rates in the Fortified Samples: Fluopyram: 92% (n = 15, RSD 10%) Trifloxystrobin: 77% (n = 15, RSD 4%) The values found for the precision (RSD) and for the accuracy (mean recovery rate) are acceptable. Limit of Quantification: Fluopyram: 0.2 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.002 mg test item/L) 86% (n = 5, RSD 13%) Trifloxystrobin: 0.75 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.007 mg test item/L) 79% (n = 5, RSD 3%) The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met. The study is acceptable.

Reference:	**KCP 5.1.2.6/05**
Title:	Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L): Toxicity to Pseudokirchneriella subcapitata in an algal growth inhibition test
Report:	xxx
Authority registration No:	
Guideline(s):	-OECD Guidelines for the Testing of Chemicals, Section 2, No. 201: "Feshwater Alga and Cyanobacteria, Growth Inhibition Test", adopted March 23, 2006, corrected July 28, 2011 - Commission Regulation (EC) No 761/2009, Annex, Part C, C.3: "Feshwater Alga and Cyanobacteria, Growth Inhibition Test", Official Journal of the European Union (EN), dated August 24, 2009 - EPA Guideline 712-C-006: OCSPP 850.4500, "Algal Toxicity", January 2012 - Japanese MAFF, Gueidelines for preparation of Study Results, Algae growth Inhibition studies. Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-7-7, Algae growth Inhibition, 2005 - SANCO/3029/99 rev. 4 11/07/00: Residues: Guidance for generating and reporting methods of Analysis in Support of preregistation data requiremetns for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the analytical part of this study was to verify the concentrations of the active ingredients fluopyram and trifloxystrobin of the test item fluopyram + trifloxystrobin SC 500 in the test water by LC-MS/MS. In the following part, only the data for fluopyram is presented.
The analytical method 01387 was used in the present study which is fully validated and EU-agreed (Krebber, R.; Braune, M.; 2013; M-466732-01-1).
After appropriate dilution, the samples were centrifuged (13,000 rpm, 3 minutes) before analysis. The determination was conducted using high performance liquid chromatography (HPLC) with mass-spectrometric (MS/MS) detection (ESI positive, MRM used as quantifier m/z: 397.0 → 173.0, MRM used as qualifier m/z: 397.0 → 208.0).

Results and discussions

For trifloxystrobin, recoveries were performed in test water spiked with test item at the fortification levels of 0.002, 0.007 and 3.6 mg test item/L. The mean and overall mean recovery values were within the acceptable range of 70 – 110%, all relative standard deviation (RSD) values were below 20%.

Table A 17: Recovery rates and precision results (repeatability) of fluopyram

	Concentration					
Sample description	**Nominal [mg test item/L]**	**Found [µg a.i./L][1]**	**DF**	**Concentration calculated [µg a.i./L][1]**	**Corrected nominal [µg a.i./L][2]**	**Recovery [%][1]**
Anayltical Blank	0	<LOQ	2	n.a.	0.000	n.a.
Anayltical Blank	0	<LOQ	2	n.a.	0.000	n.a.
Fortified Sample	0.002	0.189	2	0.378	0.441	86
	0.002	0.152	2	0.303	0.441	69
	0.002	0.197	2	0.394	0.431	91
	0.002	0.182	2	0.364	0.431	85
	0.002	0.213	2	0.425	0.431	99
					Mean value (n = 5):	**86**
					RSD (n = 5):	**13**
Fortified Sample	0.007	0.845	2	1.689	1.544	109
	0.007	0.716	2	1.432	1.544	93
	0.007	0.731	2	1.462	1.509	97
	0.007	0.690	2	1.381	1.509	92
	0.007	0.690	2	1.381	1.509	92

					Mean value (n = 5):	**96**
					RSD (n = 5):	**8**
Fortified Sample	3.6	70.061	10	700.613	794.156	88
	3.6	70.061	10	700.613	794.156	88
	3.6	73.302	10	733.025	775.944	94
	3.6	74.518	10	745.179	775.944	96
	3.6	74.923	10	749.230	775.944	97
					Mean value (n = 5):	**93**
					RSD (n = 5):	**4**
					Overall mean value (n = 15):	**92**
					RSD (n = 15):	**10**

LOQ: Limit of Quantification = 0.002 mg test item/L corresponding to 0.2 µg a.i./L after dilution by factor 2;
n.a.: not applicable; RSD: Relative Standard Deviation; DF: Dilution factor; a.i.: active ingredient

Table A 18: Characteristics for the analytical method used for validation of fluopyram

	fluopyram	
Specificity	HPLC-MS/MS method is highly specific. Blank values were below 30 % of the respective LOQ.	
Calibration (type, number of data points)	Individual calibration data is presented calibration equation for the lower calibration range: y = 27248 x + 1087, Correlation coefficient r: 1.0000, number of data points: 9 The function is linear in the operating range.	Individual calibration data is presented calibration equation for the upper calibration range: y = 24682 x + 10730, Correlation coefficient r: 0.9998, number of data points: 9 The function is linear in the operating range.
Calibration range	0.1– 10 µg a.i./L	0.1– 100 µg a.i./L
Limit of determination/quantification	LOQ = 0.2 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.002 mg test item/L)	
Assessment of matrix effects is presented	No effects observed.	

Conclusion
The analytical method complies with all guideline criteria according to SANCO/3029/99 rev. 4 and can be seen as fit for purpose for the presented study.

A 2.1.1.7 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1)

No specific method developed in support of studies summarized in B1,2,4 section 2 & Appendix 2.

A 2.1.2 Methods for post-authorisation control and monitoring purposes (KCP 5.2)

A 2.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.4 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.5 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.6 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted.

A 2.2 Analytical methods for the active substance Trifloxystrobin

A 2.2.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 2.2.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.1)

A 2.2.1.1.1 Concurrent validation of method 01013 in support of the study M-359460-02-1

Comments of zRMS:	The analytical method 01013 was developed for the determination of residues of BYF00587, Prothioconazole, Tebuconazole, Trifloxystrobin and the metabolites BYF00587-desmethyl, JAU6476-desthio (SXX0665) and CGA 321113 in/on plant materials ((Dr. B. Brumhard, S. Stuke, 2007). A confirmation of the validity of the analytical method was demonstrated in this study on the following sample material asparagus (sticks). The average recoveries were within the acceptable range of 70 – 110% and the relative standard deviations were below 20% for all the substances analysed. The Limits Of Quantification (LOQ) were 0.01 mg/kg for trifloxystrobin and its metabolite in/on asparagus (stick). The storage period of deep-frozen samples for trifloxystrobin and its metabolite ranged between 167 and 175 days. Only the residues of trifloxystrobin and the CGA 321113 metabolite were determined. The 3 isomers of trifloxystrobin (CGA 357262, CGA 357261 and CGA 331409) were not analysed in this study, so only the existing plant residue definition for monitoring can be followed (Reg. (EU) 2019/1791). The study is acceptable.

Reference:	**KCP 5.1.2.5/01**
Title:	Determination of the residues of AE C656948 and trifloxystrobin in/on asparagus after spraying of AE C656948 & CGA279202 SC 500 in the field in France (North) and Germany
Report:	Billian, P.; 2010; 08-2209; M-359460-02-1
Authority registration No:	
Guideline(s):	91/414/EEC of July 15, 1991, 7029/VI/95 rev. 5 (1997-07-22)
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation

The purpose of the presented study was to determine the magnitude of residues of AE C656948, AE C656948-benzamide, AE C656948-pyridyl-acetic acid, AE C656948-pyridyl-carboxylic acid, trifloxystrobin (CGA 279202) and CGA 321113 in/on asparagus (sticks) harvested after two spraying applications with AE C656948 & CGA279202 SC 500 on asparagus in northern Europe (Germany and France).

Full validation data is documented with the method 01013 itself (Brumhard, B.; Stuke, S.; 2007; M-283439-01-1) for the determination of residues of CGA 279202 and CGA321113 (beside other analytes) in/on plant materials. For concurrent validation purposes, the method performance was checked during the present study.

Residues of CGA 279202 and its metabolite CGA 321113 were extracted from the samples (5 g) with a mixture of acetonitrile/water (4/1; v/v, containing cystein hydrochloride) using a blender. After filtration of the extract, the stable isotopically labeled analytes were added. The solution was made up to volume, diluted and subjected to reversed phase LC-MS/MS without a further clean-up step (ESI positive, MRMs: CGA

279202 m/z: 409 → 186, CGA 321113 m/z 395 → 186). Residues were quantified using internal stable labeled standards.
In order to check the performance of the method, recovery determinations were analysed. The mean of the concurrent recoveries for all compounds and fortification levels were within the acceptable range of 70 – 110% and the RSD values were below 20%.

Table A 19: Recovery rates and precision results (repeatability) of CGA 279202 and its metabolite CGA 321113

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Asparagus sticks	0.01	91, 97, 92, 95	94	2.9	0.01
		1.0	93, 93, 92, 93	93	0.5	
			Overall Recovery (n = 8)	**93**	**2.0**	
CGA 321113	Asparagus sticks	0.01	83, 80, 81, 84	82	2.2	0.01
		0.10	83, 100, 98, 96	94	8.1	
			Overall Recovery (n = 8)	**88**	**9.5**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 20: Characteristics for the analytical method 01013 used for validation of CGA 279202 and its metabolite CGA 321113 in/on asparagus sticks

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.	
Calibration range	0.02 µg/L – 1.0 µg/L (corresponds to 0.002 mg/kg – 0.1 mg/kg) for each compound	
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound	
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.	
	CGA 279202	**CGA 321113**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.8737 x + 0.004097, Correlation coefficient r: 0.99980 number of data points: 3	Individual calibration data is presented calibration equation (1/x weighted): y = 0.9818 x + 0.01722, Correlation coefficient r: 0.99899 number of data points: 3

Conclusion
The analytical method 01013 used in the present study for the determination of residues of CGA 279202 and CGA321113 (beside other analytes) in/on plant materials was fully validated during study M-283439-01-1 (Brumhard, B.; Stuke, S.; 2007). For concurrent validation purposes, the method performance of the analytical method 01013 was tested during the present study for the matrix asparagus sticks. The data presented demonstrate that the method allows the determination of these substances with satisfactory precision given that the overall mean relative standard deviation was below 5% of eight measured replicates for CGA 279202 and below 10% of eight measured replicates for CGA 321113. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.2.1.1.2 Concurrent validation of method 01013 in support of the study M-415549-01-1

Comments of zRMS:	The analytical method 01013 was developed for the determination of residues of BYF00587, Prothioconazole, Tebuconazole, Trifloxystrobin and the metabolites BYF00587-desmethyl, JAU6476-desthio (SXX0665) and CGA 321113 in/on plant materials ((Dr. B. Brumhard, S. Stuke, 2007). A confirmation of the validity of the analytical method was demonstrated in this study on the following sample material asparagus (sticks). The average recoveries were within the acceptable range of 70 – 110% and the relative standard deviations were below 20% for all the substances analysed.

	The Limits Of Quantification (LOQ) were 0.01 mg/kg for trifloxystrobin and its metabolite in/on asparagus (stick). The storage period of deep-frozen samples for trifloxystrobin and its metabolite ranged between 63 and 91 days. Only the residues of trifloxystrobin and the CGA 321113 metabolite were determined. The 3 isomers of trifloxystrobin (CGA 357262, CGA 357261 and CGA 331409) were not analysed in this study, so only the existing plant residue definition for monitoring can be followed (Reg. (EU) 2019/1791). The study is acceptable.

Reference:	**KCP 5.1.2.5/02**
Title:	Determination of the residues of AE C656948 and trifloxystrobin in/on asparagus after spraying of AE C656948 & CGA279202 SC 500 in the field in France (north) and Netherlands
Report:	Uceda, L.; Ratajczak, M.; 2011; 09-2073; M-415549-01-1
Authority registration No:	
Guideline(s):	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8; Residues in or on Treated Products, Food and Feed; EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation
The purpose of the study 09-2073 was to determine the magnitude of the relevant residues of AE C656948, AE C656948-benzamide, AE C656948-pyridyl-acetic acid, AE C656948-pyridyl-carboxylic acid, CGA 321113 and trifloxystrobin (CGA 279202) in/on asparagus (sticks) after two spraying applications with AE C656948 & CGA279202 SC 500 a SC formulation containing trifloxystrobin and AE C656948.
Full validation data is documented with the method 01013 itself (Brumhard, B.; Stuke, S.; 2007; M-283439-01-1) for the determination of residues of CGA 279202 and CGA321113 (beside other analytes) in/on plant materials. For concurrent validation purposes, the method performance was checked during the present study.
The analytical method 01013 was developed for the determination of residues of BYF00587, Prothioconazole, Tebuconazole, CGA 279202 and the metabolites BYF00587-desmethyl, JAU6476-desthio (SXX0665) and CGA 321113 in/on plant materials. The above mentioned test items were extracted from the samples with a mixture of acetonitrile/water (4/1; v/v, containing cysteine hydrochloride) using a blender. After filtration of the extract, the stable isotopically labeled analytes were added. The solution was made up to volume, diluted and subjected to reversed phase HPLC-MS/MS without a further clean-up step. BYF00587, Tebuconazole, CGA 279202, JAU6476-desthio and CGA 321113 were detected using electrospray ionization in the positive ion mode (ESI+), Prothioconazole and BYF00587-desmethyl were detected using electrospray ionization in the negative ion mode (ESI-). Residues were quantified using internal stable labeled standards. Due to an unsatisfying chromatographic separation of the seven test items two injections, one in the positive ion mode and another in the negative ion mode, are necessary.

Conditions used in this study:
Residues of the following compounds were determined: trifloxystrobin and its metabolite CGA 321113.
The chromatographic system used was: a high performance liquid chromatograph with a reversed phase chromatography (on a Luna C18 column) coupled with tandem mass spectrometry (MS/MS) with Electrospray ionisation (Applied Biosystems API 4000 Triple Quadruple Mass Spectrometer, Analyst version 1.4.1.) (ESI positive, MRMs: CGA 279202 m/z: 409 → 186, CGA 321113 m/z 395 → 186). The quantification was carried out by internal standardization using trifloxystrobin-methyl-d^3 and trifloxystrobin acid-methoxy- d^3, as internal stable labeled standards.

For the preparation of the samples, some modifications were carried out concerning the internal standard adding without impact on the quality of the study. First, the 100 mL volumetric flask was made to volume with the mixture acetonitrile : water (4/1 ; v/v). This was the extract A. Second, an aliquot to the extract A was filtered on Anotop 25, 0.2 µm. Then the extract A filtered was diluted five times with the adding of the internal standards. At the end, as in the method 01013, the LOQ concentration was: 0.1/1 µg/L test item/ISTD.
The quantification was done by internal standardisation in pure solvent.
In order to check the performance of the method, recovery determinations were included in each set of analysis (at least one recovery for twelve study samples). Control samples from the study were fortified for the use as the recovery samples. All the recovery determinations were performed in parallel to the analyses of control and treated samples from the study.
The apparent residues in the control sample used for fortification were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

Table A 21: Recovery rates and precision results (repeatability) of CGA 279202 and its metabolite CGA 321113

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Asparagus sticks	0.01	96; 95; 96	96	0.6	0.01
		0.1	97; 98; 99	98	1.0	
		1	96	96	-	
			Overall Recovery (n = 7)	**97**	**1.4**	
CGA 321113	Asparagus sticks	0.01	89; 87; 87	88	1.3	0.01
		0.1	94; 93; 96	94	1.6	
		1	96	96	-	
			Overall Recovery (n = 7)	**92**	**4.3**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 22: Characteristics for the analytical method 01013 used for validation of CGA 279202 and its metabolite CGA 321113 in/on asparagus sticks

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.	
Calibration range	0.05 µg/L – 10.0 µg/L (corresponds to 0.005 mg/kg – 1 mg/kg) for each compound	
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound	
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.	
	CGA 279202	**CGA 321113**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.97983 x + 0.0038255, Correlation coefficient r: 1.0000 number of data points: 7	Individual calibration data is presented calibration equation (1/x weighted): y = 0.70055 x – 5.2663 · 10^{-4}, Correlation coefficient r: 1.0000 number of data points: 7

Conclusion

The analytical method 01013 used in the present study for the determination of residues of trifloxystrobin and CGA321113 (beside other analytes) in/on plant materials was fully validated during study M-283439-01-1 (Brumhard, B.; Stuke, S.; 2007). For concurrent validation purposes, the method performance of the analytical method 01013 was tested during the present study for the matrix asparagus sticks. The data presented demonstrate that the method allows the determination of these substances with satisfactory precision given that the overall mean relative standard deviation was below 5% of seven measured replicates of each analyte. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.2.1.1.3 Analytical method 01313/M001 in support of the study M-530177-01-1

A 2.2.1.1.3.1 Method validation

Comments of zRMS:	Residues of trifloxystrobin and its isomers / metabolites were determined by LC-MS/MS according to method 01313/M001. Method 01313/M001 (Stuke, S.; Teubner, L.; 2013; M-448498-01-1): Full validation data is documented with the method 01313/M001 itself for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix relevant to this study (lettuce) but not included in the original validation, a set (one control sample, 3 repetitions each at two fortification levels) of additional validation recoveries were analysed within the course of the study. The limits of quantitation (LOQ) for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 were 0.01 mg/kg. No residues above the LOQ were found in the control samples. The average recoveries were within the acceptable range of 70 – 110%. RSD values were below 20%. All method validation data are in compliance with the guideline requirements for data collection methods. The validation of method 01313/M001 can therefore be considered successful for the new matrix (lettuce). The stydy is acceptable.

Reference:	**KCP 5.1.2.5/03**
Title:	Determination of the residues of trifloxystrobin in/on lettuce after spraying of trifloxystrobin WG 50 in the greenhouse in the Netherlands, Belgium, Italy and Spain
Report:	Bellof, S.; Kuester, S.; 2015; 14-2144; M-530177-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market „h OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) „h US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the study 14-2144 was to determine the magnitude of the residues of trifloxystrobin (CGA 279202) in/on lettuce (head, loose leaf varieties) after three spray applications with Trifloxystrobin WG 50, a water dispersible granule formulation containing 50% trifloxystrobin.

Full validation data is documented with the method 01313/M001 itself (Stuke, S.; Teubner, L.; 2013; M-448498-01-1) for CGA 279202, CGA 357261, CGA 357262, CGA 331409, CGA 321113 and CGA 373466 in broccoli (head), kidney bean (dry seed), rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix lettuce head, relevant to this study but not included in the original validation, a limited set (one control sample, 3 repetitions each at two fortification levels) of additional validation recoveries were analysed within the present study.

Residues of trifloxystrobin (CGA 279202), its isomers CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466 were extracted from the homogenised sample material with a mixture of acetonitrile/water (4/1; v/v) by shaking. After filtration, addition of ammonium acetate solution to adjust the pH value and addition of internal standard solution the extracts were made up to volume, diluted if necessary, filtered and subjected to HPLC-MS/MS measurement. The quantification was done by external standardisation in pure solvent.

The examination samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen and the remaining sample was immediately returned to the freezer.

Results and discussions

A limited set (one control sample, 3 repetitions each at two fortification levels) of additional validation recoveries were analysed for the matrix lettuce (head) at fortification levels of 0.01 mg/kg and 0.10 mg/kg for each analyte.

Apparent residues in control samples were below 30% of the LOQ for trifloxystrobin parent compound as well as for the isomers/metabolites. The mean of the concurrent recoveries at each fortification level and overall were within the range of 70 – 110% for all analytes. Wherever applicable (n ≥ 3), the RSD values were below 20%.

Table A 23: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method validation

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Lettuce / head	0.01	99; 106; 108	104	4.5	0.01
		0.10	98; 102; 108	103	4.9	
			Overall recovery (n = 6)	**104**	**4.3**	
CGA 357261	Lettuce / head	0.01	93; 95; 96	95	1.6	0.01
		0.10	98; 104; 106	103	4.1	
			Overall recovery (n = 6)	**99**	**5.3**	
CGA 357262	Lettuce / head	0.01	102; 105; 110	106	3.8	0.01
		0.10	101; 105; 109	105	3.8	
			Overall recovery (n = 6)	**105**	**3.4**	
CGA 331409	Lettuce / head	0.01	101; 102; 106	103	2.6	0.01
		0.10	103; 104; 107	105	2.0	
			Overall recovery (n = 6)	**104**	**2.2**	
CGA 321113	Lettuce / head	0.01	95; 99; 102	99	3.6	0.01
		0.10	99; 101; 104	101	2.5	
			Overall recovery (n = 6)	**100**	**3.1**	
CGA 373466	Lettuce / head	0.01	92; 104; 107	101	7.9	0.01
		0.10	96; 98; 103	99	3.6	
			Overall recovery (n = 6)	**100**	**5.6**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

Table A 24: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method performance

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Lettuce / head	0.01	99; 106; 108	104	4.5	0.01
		0.10	98; 102; 108	103	4.9	
		1.0	102	-	-	
		25	94	-	-	
			Overall recovery (n = 8)	**102**	**4.9**	
CGA 357261	Lettuce / head	0.01	93; 95; 96	95	1.6	0.01
		0.10	98; 104; 106	103	4.1	
		1.0	103	-	-	
			Overall recovery (n = 7)	**99**	**5.1**	
CGA 357262	Lettuce / head	0.01	102; 105; 110	106	3.8	0.01
		0.10	101; 105; 109	105	3.8	
		1.0	103	-	-	
			Overall recovery (n = 7)	**105**	**3.3**	
CGA 331409	Lettuce / head	0.01	101; 102; 106	103	2.6	0.01
		0.10	103; 104; 107	105	2.0	
		1.0	106	-	-	
			Overall recovery (n = 7)	**104**	**2.2**	
CGA 321113	Lettuce / head	0.01	95; 99; 102	99	3.6	0.01
		0.10	99; 101; 104	101	2.5	
		1.0	101	-	-	
			Overall recovery (n = 7)	**100**	**2.8**	
CGA 373466	Lettuce / head	0.01	92; 104; 107	101	7.9	0.01
		0.10	96; 98; 103	99	3.6	
		1.0	106	-	-	
			Overall recovery (n = 7)	**101**	**5.6**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 25: Characteristics for the analytical method 01313/M001 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on lettuce (head)

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.		
Calibration range	0.2 µg/L - 100 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg) for each compound		
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound		
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.		
	CGA 279202	**CGA 357262**	**CGA 357261**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted):	Individual calibration data is presented calibration equation (1/x weighted):	Individual calibration data is presented calibration equation (1/x weighted):

	y = 0.022641 x + 1.6258 · 10^{-4}, Correlation coefficient r: 0.9998 number of data points: 6	y = 0.029382 x – 3.1584 · 10^{-4}, Correlation coefficient r: 0.9998 number of data points: 6	y = 0.040902 x + 0.0021371, Correlation coefficient r: 0.9998 number of data points: 6
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 0.074243 x + 0.0013013, Correlation coefficient r: 0.9998 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): y = 0.055849 x – 4.4998 · 10^{-5}, Correlation coefficient r: 0.9999 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): y = 0.086172 x - 0.0014297, Correlation coefficient r: 0.9999 number of data points: 6

Conclusion

The analytical method 01313/M001 was fully validated during study M-448498-01-1 (Stuke, S.; Teubner, L.; 2013). For the matrix relevant to this study (lettuce (head)) a limited set of validation recoveries was analysed within the course of the present study. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01313/M001 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on lettuce (head) via HPLC-MS/MS.

A 2.2.1.1.4 Concurrent validation of method 01313/M001 in support of the studies M-534202-01-1 and M-536965-01-1

Comments of zRMS:	Method 01313/M001: Full validation data is documented with the method 01313/M001 itself for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix relevant to this study (lettuce) but not included in the original validation, a set (one control sample, 3 repetitions each at two fortification levels) of additional validation recoveries were analysed within the course of the study 14-2144. For concurrent validation purposes, the recoveries of the method performance were performed during the conduct of the studies 14-2029 (Schulte, G.; Sosniak, A.; 2015; M-534202-01-1) and 14-2184 (Bellof, S.; Kuester, S.; 2015; M-536965-01-1). The limits of quantitation (LOQ) for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 are 0.01 mg/kg, corresponding to the lowest fortification level of successfully conducted recovery experiments. Blank values in control samples were below 30% of the LOQ for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%. No residues above the LOQ were found in the control samples. All method validation data are in compliance with the guideline requirements for data collection methods. The validation of method 01313/M001 can therefore be considered successful for the new matrix - lettuce. The study is acceptable.

Reference:	**KCP 5.1.2.5/05**
Title:	Determination of the residues of fluopyram and trifloxystrobin in/on lettuce after spray application of fluopyram & trifloxystrobin SC 500 in Belgium, Germany, the Netherlands and northern France
Report:	Schulte, G.; Sosniak, A.; 2015; 14-2029; M-534202-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Reference:	**KCP 5.1.2.5/07**
Title:	Determination of the residues of fluopyram and trifloxystrobin in/on lettuce after spray application of fluopyram & trifloxystrobin SC 500 in Germany, the Netherlands, Hungary and the United Kingdom
Report:	Bellof, S.; Kuester, S.; 2015; 14-2184; M-536965-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market; OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009); US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation

The analytical method used in this study was fully validated during study M-448498-01-1 (Stuke, S.; Teubner, L.; 2013) for CGA 279202, CGA 357261, CGA 357262, CGA 331409, CGA 321113 and CGA 373466 in broccoli (head), kidney bean (dry seed), rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix lettuce head, relevant to this study but not included in the original validation, a limited set (one control sample, 3 repetitions each at two fortification levels) of additional validation recoveries were analysed within the study 14-2144 (Bellof, S.; Kuester, S.; 2015; M-530177-01-1). For concurrent validation purposes, the recoveries of the method performance were performed during the conduct of the studies 14-2029 (Schulte, G.; Sosniak, A.; 2015; M-534202-01-1) and 14-2184 (Bellof, S.; Kuester, S.; 2015; M-536965-01-1).

Residues of trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 were extracted from the homogenised sample material with a mixture of acetonitrile/water (4/1; v/v) by shaking. After filtration, addition of ammonium acetate solution to adjust the pH value and addition of internal standard solution the extracts were made up to volume, diluted, filtered and subjected to HPLC-MS/MS measurement. The quantification was done using isotopically labelled internal standards in pure solvent.

Conditions used for studies 14-2029 and 14-2184:

Slight adaptions were made to the sample preparation procedure described within the analytical method 01313/M001 which are as follows: The sample dilution step (step 9 in method 01313/M001) was omitted and in parallel the injection volume was reduced to 1 µL.

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses

of control and treated samples from the study. The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

Table A 26: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Lettuce / head	0.01	76; 83; 89; 93; 96; 98; 101; 102; 102; 108	95	10.3	0.01
		0.10	93; 93; 95; 96; 99; 99	96	2.8	
		1.0	94; 98; 98; 99; 100	98	2.3	
		15	91	-	-	
			Overall recovery (n = 22)	**96**	**7.1**	
CGA 357261	Lettuce / head	0.01	83; 89; 91; 93; 93; 95; 95; 99; 102; 104	94	6.6	0.01
		0.10	91; 93; 94; 95; 96; 101	95	3.6	
		1.0	92; 93; 93; 96; 96	94	2.0	
		15	92	-	-	
			Overall recovery (n = 22)	**94**	**4.8**	
CGA 357262	Lettuce / head	0.01	89; 90; 92; 94; 94; 95; 96; 102; 103; 104	96	5.6	0.01
		0.10	91; 94; 94; 97; 97; 97	95	2.6	
		1.0	90; 94; 95; 97; 99	95	3.6	
		15	88	-	-	
			Overall recovery (n = 22)	**95**	**4.5**	
CGA 331409	Lettuce / head	0.01	91; 91; 92; 95; 95; 95; 96; 96; 97; 103	95	3.7	0.01
		0.10	92; 94; 96; 97; 98; 99	96	2.7	
		1.0	95; 97; 98; 98; 99	97	1.6	
		15	92	-	-	
			Overall recovery (n = 22)	**96**	**3.1**	
CGA 321113	Lettuce / head	0.01	83; 89; 89; 99; 99; 104; 106; 107; 107; 111	99	9.5	0.01
		0.10	89; 92; 93; 93; 100; 102	95	5.3	
		1.0	93; 94; 95; 96; 97	95	1.7	
		15	87	-	-	
			Overall recovery (n = 22)	**97**	**7.6**	
CGA 373466	Lettuce / head	0.01	76; 79; 82; 83; 89; 92; 96; 96; 105; 107	91	11.8	0.01
		0.10	89; 92; 93; 94; 96; 101	94	4.3	
		1.0	94; 96; 97; 98; 99	97	2.0	
		15	88	-	-	
			Overall recovery (n = 22)	**93**	**8.4**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification

Table A 27: Characteristics for the analytical method 01313/M001 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on lettuce (head) for studies 14-2028, 14-2029, 14-2030, 14-2184 and 14-2185

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.		
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound		
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.		
	Study 14-2029		
	CGA 279202	**CGA 357262**	**CGA 357261**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): $y = 0.019172 x + 5.1748 \cdot 10^{-4}$, Correlation coefficient r: 0.9996 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.040858 x + 7.7359 \cdot 10^{-6}$, Correlation coefficient r: 0.9997 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.043227 x – 4.6785 \cdot 10^{-4}$, Correlation coefficient r: 0.9996 number of data points: 6
Calibration range	0.2 µg/L - 100 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg) for each compound		
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.090937 x + 4.6155 \cdot 10^{-4}$, Correlation coefficient r: 0.9998 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.067256 x – 0.0094092$, Correlation coefficient r: 0.9996 number of data points: 5	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.095504 x - 0.0029416$, Correlation coefficient r: 0.9996 number of data points: 6
Calibration range	0.2 µg/L - 100 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg) for each compound	1.00 µg/L - 100 µg/L (corresponds to 0.01 mg/kg – 1 mg/kg) for each compound	0.2 µg/L - 100 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg) for each compound
	Study 14-2184		
	CGA 279202	**CGA 357262**	**CGA 357261**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): $y = 0.019143 x + 0.0019163$, Correlation coefficient r: 0.9997 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.041408 x - 2.4194 \cdot 10^{-5}$, Correlation coefficient r: 0.9998 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.043580 x – 1.0930 \cdot 10^{-4}$, Correlation coefficient r: 0.9994 number of data points: 6
Calibration range	0.2 µg/L - 100 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg) for each compound		
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.091497 x + 0.0029719$, Correlation coefficient r: 0.9998 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.067356 x – 0.0089817$, Correlation coefficient r: 0.9989 number of data points: 5	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.096139 x + 0.0013648$, Correlation coefficient r: 0.9983 number of data points: 6
Calibration range	0.2 µg/L - 100 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg) for each compound	1.00 µg/L - 100 µg/L (corresponds to 0.01 mg/kg – 1 mg/kg) for each compound	0.2 µg/L - 100 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg) for each compound

Conclusion
The analytical method used in this study was fully validated during study M-448498-01-1 (Stuke, S.; Teubner, L.; 2013). For the matrix lettuce head, a limited set (one control sample, 3 repetitions each at two fortification levels) of additional validation recoveries was analysed within the study 14-2144. For concurrent validation purposes, the recoveries of the method performance were performed during the conduct of the studies 14-2029 and 14-2184. The method 01313/M001 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on lettuce (head) via HPLC-MS/MS in the presented studies and can therefore be regarded as fit for purpose.

A 2.2.1.1.5 Analytical method 01313/M001 in support of the studies M-452140-01-1, M-460009-01-1 and M-453332-02-1

A 2.2.1.1.5.1 Method validation

Comments of zRMS:	Residues of Trifloxystrobin and its isomers / metabolites were determined by LC-MS/MS according to method 01313/M001. Method 01313/M001 (Stuke, S.; Teubner, L.; 2013): Full validation data is documented with the method 01313/M001 itself for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix relevant to this study (strawberry, fruit) but not included in the original validation, a set (minimum 4 repetitions each at three fortification levels) of additional validation recoveries were analysed within the course of the study 12-2012, 12-2013 and 12-2014. The limits of quantitation (LOQ) for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 are 0.01 mg/kg, for all sample materials tested. Blank values in control samples were below 30% of the LOQ for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%. No residues above the LOQ were found in the control samples. All method validation data are in compliance with the guideline requirements for data collection methods. The validation of method 01313/M001 can therefore be considered successful for the new matrix - strawberry. The study is acceptable.

Reference:	**KCP 5.1.2.5/10**
Title:	Determination of the residues of trifloxystrobin in/on strawberry after spraying of trifloxystrobin WG 50 in the field in Germany, the Netherlands, France (north) and Belgium
Report:	Stuke, S.; Diehl, P.; 2013; 12-2012; M-452140-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial US EPA OCSPP Guideline No. 860.1500
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Reference:	**KCP 5.1.2.5/11**
Title:	Determination of the residues of trifloxystrobin in/on strawberry after spray application of trifloxystrobin WG 50 in Spain, Italy and Greece
Report:	Noss, G.; Czaja, C.; Diehl, P.; 2013; 12-2013; M-460009-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EC guidance working document 7029/VI/95 rev. 5 (July 22, 1997) OECD 509 Adopted 2009-09-07, OECD Guideline for the testing of chemicals, Crop Field Trial EC guidance working document 7029/VI/95 rev. 5 (July 22, 1997) US EPA OCSPP Guideline No. 860.1500
Deviations:	not applicable
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Reference:	**KCP 5.1.2.5/12**
Title:	Determination of the residues of trifloxystrobin in/on strawberry after spraying of trifloxystrobin WG 50 in the greenhouse in Belgium, France (North) and Germany
Report:	Stuke, S.; 2013; 12-2014; M-453332-02-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, EC Guidance working document 7029/VI/95 rev.5 (1997-07-22), OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial US EPA OCSPP Guideline No. 860.1500
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The analytical method used in this study was fully validated during study M-448498-01-1 (Stuke, S.; Teubner, L.; 2013) for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix relevant to this study (strawberry (fruit)) but not included in the original validation, a set (minimum 4 repetitions each at three fortification levels) of additional validation recoveries was analysed within the course of the studies 12-2012 (Stuke, S.; Diehl, P.; 2013; M-452410-01-1), 12-2013 (Noss, G.; Czaja, C.; Diehl, P.; 2013; M-460009-01-1) and 12-2014 (Stuke, S.; 2013; M-453332-02-1).

Residues of trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 were extracted from the homogenized sample material with a mixture of acetonitrile/water (4/1; v/v) by shaking. After filtration, addition of ammonium acetate solution to adjust the pH value and addition of internal standard solution the extracts were made up to volume, diluted, filtered and subjected to HPLC-MS/MS measurement. The quantification was done by external standardisation in pure solvent.

Results and discussions

A set (minimum 4 repetitions each at three fortification levels) of additional validation recoveries were analysed. Recoveries were determined at fortification levels of 0.01 mg/kg (LOQ), 0.1 mg/kg and 0.50 mg/kg.

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples.
The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

Table A 28: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	strawberry / fruit	0.01	85; 87; 95; 95; 96; 97; 98; 102; 111	96	8.0	0.01
		0.10	71; 84; 88; 89; 102; 107; 109	93	14.8	
		0.50	64; 79; 81; 84	77	11.6	
			Overall recovery (n = 20)	**91**	**13.5**	
CGA 357261	strawberry / fruit	0.01	75; 78; 79; 82; 82; 84; 85; 89; 92	83	6.5	0.01
		0.10	79; 80; 83; 83; 88; 91; 96	86	7.2	
		0.50	76; 76; 80; 82	79	3.8	
			Overall recovery (n = 20)	**83**	**6.9**	
CGA 357262	strawberry / fruit	0.01	70; 84; 85; 86; 91; 93; 94; 97; 118	91	14.2	0.01
		0.10	73; 75; 79; 83; 87; 97; 108	86	14.7	
		0.50	67; 74; 81; 84	77	9.9	
			Overall recovery (n = 20)	**86**	**14.6**	
CGA 331409	strawberry / fruit	0.01	70; 75; 76; 77; 78; 78; 81; 85; 96	80	9.3	0.01
		0.10	73; 75; 82; 83; 91; 92; 96	85	10.4	
		0.50	72; 74; 77; 78	75	3.7	
			Overall recovery (n = 20)	**80**	**9.7**	
CGA 321113	strawberry / fruit	0.01	70; 71; 72; 72; 73; 78; 86; 90; 97	79	12.5	0.01
		0.10	72; 76; 83; 83; 85; 86; 101	84	10.9	
		0.50	75; 77; 77; 79	77	2.1	
			Overall recovery (n = 20)	**80**	**10.8**	
CGA 373466	strawberry / fruit	0.01	72; 73; 75; 78; 78; 86; 87; 92; 95	82	10.3	0.01
		0.10	72; 74; 77; 78; 79; 79; 85	78	5.3	
		0.50	71; 72; 75; 80	75	5.4	
			Overall recovery (n = 20)	**79**	**8.7**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification;
These recoveries were performed during the conduct of the studies 12-2012, 12-2013 and 12-2014.

Table A 29: Characteristics for the analytical method 01313/M001 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on strawberry (fruit) for studies 12-2012, 12-2013 and 12-2014

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.

Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound		
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.		
	Study 12-2012		
	CGA 279202	**CGA 357262**	**CGA 357261**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation: $y = -0.013901\ x^2 + 0.37269\ x + 3.1816 \cdot 10^{-4}$, Correlation coefficient r: 1.0000, number of data points: 5 Remark: Due to a wide range of concentrations the regression is not linear.	Individual calibration data is presented calibration equation: $y = 0.80561\ x + 0.010933$, Correlation coefficient r: 0.9992, number of data points: 6 Function linear in the operating range	Individual calibration data is presented calibration equation: $y = 1.0747\ x + 0.0077458$, Correlation coefficient r: 0.9984, number of data points: 6 Function linear in the operating range
Calibration range	0.1 µg/L - 10 µg/L (corresponds to 0.001 – 0.1 mg/kg)	0.02 µg/L - 5 µg/L (corresponds to 0.0002 – 0.05 mg/kg)	0.02 µg/L - 5 µg/L (corresponds to 0.0002 – 0.05 mg/kg)
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation: $y = 1.1687\ x + 0.0073664$, Correlation coefficient r: 0.9999, number of data points: 7 Function linear in the operating range	Individual calibration data is presented calibration equation: $y = 0.64827\ x + 0.011225$, Correlation coefficient r: 0.9995, number of data points: 7 Function linear in the operating range	Individual calibration data is presented calibration equation: $y = 1.1298\ x + 0.0018682$, Correlation coefficient r: 0.9999, number of data points: 7 Function linear in the operating range
Calibration range	0.02 µg/L - 10 µg/L (corresponds to 0.0002 – 0.1 mg/kg)	0.02 µg/L - 10 µg/L (corresponds to 0.0002 – 0.1 mg/kg)	0.02 µg/L - 10 µg/L (corresponds to 0.0002 – 0.1 mg/kg)
	Study 12-2013		
	CGA 279202	**CGA 357262**	**CGA 357261**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation: $y = -0.034176\ x^2 + 0.46919\ x + 0.0029221$, Correlation coefficient r: 0.9999, number of data points: 5 Remark: Due to a wide range of concentrations the regression is not linear.	Individual calibration data is presented, calibration equation: $y = -0.069978\ x^2 + 1.0557\ x - 0.015655$, Correlation coefficient r: 0.9999, number of data points: 5 Remark: Due to a wide range of concentrations the regression is not linear.	Individual calibration data is presented, calibration equation: $y = -0.050487\ x^2 + 1.0587\ x + 0.0087130$, Correlation coefficient r: 1.0000, number of data points: 6 Remark: Due to a wide range of concentrations the regression is not linear.
Calibration range	0.02 µg/L - 5 µg/L (corresponds to 0.0002 – 0.05 mg/kg)	0.1 µg/L - 5 µg/L (corresponds to 0.001 – 0.05 mg/kg)	0.02 µg/L - 10 µg/L (corresponds to 0.0002 – 0.1 mg/kg)
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation: $y = 1.0892\ x + 0.010734$, Correlation coefficient r: 0.9992, number of data points: 6 Function linear in the operating range	Individual calibration data is presented calibration equation: $y = 0.58342\ x – 8.6626 \cdot 10^{-4}$, Correlation coefficient r: 0.9998, number of data points: 4 Function linear in the operating range	Individual calibration data is presented calibration equation: $y = 1.0292\ x + 0.0016147$, Correlation coefficient r: 0.9998, number of data points: 6 Function linear in the operating range
Calibration range	0.02 µg/L - 5 µg/L (corresponds to 0.0002 – 0.05 mg/kg)	0.02 µg/L - 10 µg/L (corresponds to 0.0002 – 0.1 mg/kg)	0.02 µg/L - 10 µg/L (corresponds to 0.0002 – 0.1 mg/kg)

	Study 12-2014		
	CGA 279202	**CGA 357262**	**CGA 357261**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation: $y = -0.067959 x^2 + 0.66389 x - 0.0098467$, Correlation coefficient r: 1.0000, number of data points: 5 Remark: Due to a wide range of concentrations the regression is not linear.	Individual calibration data is presented, calibration equation: $y = -0.043366 x^2 + 0.99561 x - 4.0814 \cdot 10^{-4}$, Correlation coefficient r: 1.0000, number of data points: 6 Remark: Due to a wide range of concentrations the regression is not linear.	Individual calibration data is presented, calibration equation: $y = -0.084688 x^2 + 1.1461 x + 0.0025501$, Correlation coefficient r: 1.0000, number of data points: 6 Remark: Due to a wide range of concentrations the regression is not linear.
Calibration range	0.1 µg/L - 5 µg/L (corresponds to 0.001 – 0.05 mg/kg)	0.02 µg/L - 10 µg/L (corresponds to 0.0002 – 0.1 mg/kg)	0.02 µg/L - 5 µg/L (corresponds to 0.0002 – 0.05 mg/kg)
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation: $y = 1.0397 x + 0.0099429$, Correlation coefficient r: 0.9986, number of data points: 6 Function linear in the operating range	Individual calibration data is presented, calibration equation: $y = -0.0070883 x^2 + 0.66300 x + 0.018653$, Correlation coefficient r: 1.0000, number of data points: 6 Remark: Due to a wide range of concentrations the regression is not linear.	Individual calibration data is presented calibration equation: $y = 1.0839 x + 0.016469$, Correlation coefficient r: 0.9999, number of data points: 6 Function linear in the operating range
Calibration range	0.02 µg/L - 5 µg/L (corresponds to 0.0002 – 0.05 mg/kg)	0.02 µg/L - 10 µg/L (corresponds to 0.0002 – 0.1 mg/kg)	0.02 µg/L - 5 µg/L (corresponds to 0.0002 – 0.05 mg/kg)

Conclusion

The analytical method used in this study was fully validated during study M-448498-01-1 (Stuke, S.; Teubner, L.; 2013). For the matrix relevant to this study (strawberry (fruit)), a set of additional validation recoveries was analysed within the course of the studies 12-2012, 12-2013 and 12-2014. The analytical method complies with all guideline criteria according to SANCO 3029/99 rev. 4 with the minor exception of the calibration data. Here quadratic calibrations were used for some analytes. According to SANCO 3029/99 rev. 4 where a non-linear calibration is used this should be justified. It is stated that due to a wide range of concentrations the regression is not linear. In addition, the calibration curves show that the data points fall exactly on the line, representing the best-fit line. Therefore, the analytical method 01313/M001 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on strawberry (fruit) via HPLC-MS/MS.

A 2.2.1.1.6 Concurrent validation of method 01313/M001 in support of the study M-534577-01-1

Comments of zRMS:	Method 01313/M001: Full validation data is documented with the method 01313/M001 itself for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix relevant to this study (strawberry) but not included in the original validation, a set (minimum 4 repetitions each at three fortification levels) of additional validation recoveries were analysed within the course of the studies 12-2012, 12-2013 and 12-2014. The limits of quantitation (LOQ) for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 were 0.01 mg/kg. Blank values in control samples were below 30% of the LOQ for all compounds. The mean of the concurrent recoveries were within the acceptable range of 70 - 110%. RSD values were below 20%. All method validation data are in compliance with the guideline requirements for data

	collection methods. The validation of method 01313/M001 can therefore be considered successful for the new matrix. The stydy is acceptable.

Reference:	**KCP 5.1.2.5/13**
Title:	Determination of the residues of fluopyram and trifloxystrobin in/on strawberry after spray application of fluopyram & trifloxystrobin SC 500 in Germany, northern France, the Netherlands and Belgium
Report:	Schulte, G.; Sosniak, A.; 2015; 14-2026; M-534577-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation

The analytical method used in this study was fully validated during study M-448498-01-1 (Stuke, S.; Teubner, L.; 2013) for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix relevant to this study (strawberry (fruit)) but not included in the original validation, a set (minimum 4 repetitions each at three fortification levels) of additional validation recoveries was analysed within the course of the studies 12-2012 (Stuke, S.; Diehl, P.; 2013; M-452410-01-1), 12-2013 (Noss, G.; Czaja, C.; Diehl, P.; 2013; M-460009-01-1) and 12-2014 (Stuke, S.; 2013; M-453332-02-1). For concurrent validation purposes, the method performance was checked during the study 14-2026 (Schulte, G.; Sosniak, A.; 2015; M-534577-01-1).
Residues of trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 were extracted from the homogenized sample material with a mixture of acetonitrile/water (4/1; v/v) by shaking. After filtration, addition of ammonium acetate solution to adjust the pH value and addition of internal standard solution the extracts were made up to volume, diluted, filtered and subjected to HPLC-MS/MS measurement. The quantification was done using isotopic labelled internal standards in pure solvent.

Conditions used for these studies:

Slight adaptions were made to the sample preparation procedure described within the analytical method 01313/M001 which are as follows: The sample dilution step (step 9 in method 01313/M001) was omitted and in parallel the injection volume was reduced to 1 µL.
In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.
The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

Table A 30: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
	strawberry / fruit	0.01	96; 106; 110; 113	106	7.0	0.01

CGA 279202		0.10	95; 96; 96; 97	96	0.9	
		1.0	95; 98	97	-	
			Overall recovery (n = 10)	**100**	**6.8**	
CGA 357261	strawberry / fruit	0.01	90; 93; 94; 97	94	3.1	0.01
		0.10	91; 94; 99; 102	97	5.1	
		1.0	91; 98	95	-	
			Overall recovery (n = 10)	**95**	**4.2**	
CGA 357262	strawberry / fruit	0.01	84; 105; 107; 107	101	11.1	0.01
		0.10	96; 98; 100; 103	99	3.0	
		1.0	91; 95	93	-	
			Overall recovery (n = 10)	**99**	**7.5**	
CGA 331409	strawberry / fruit	0.01	90; 95; 96; 97	95	3.3	0.01
		0.10	94; 97; 97; 100	97	2.5	
		1.0	88; 94	91	-	
			Overall recovery (n = 10)	**95**	**3.7**	
CGA 321113	strawberry / fruit	0.01	94; 98; 103; 106	100	5.3	0.01
		0.10	99; 101; 103; 104	102	2.2	
		1.0	88; 99	94	-	
			Overall recovery (n = 10)	**100**	**5.3**	
CGA 373466	strawberry / fruit	0.01	87; 90; 90; 110	94	11.2	0.01
		0.10	94; 97; 103; 103	99	4.5	
		1.0	89; 95	92	-	
			Overall recovery (n = 10)	**96**	**7.8**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
These recoveries were performed during the conduct of the studies 14-2026 and 14-2189.

Table A 31: Characteristics for the analytical method 01313/M001 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on strawberry (fruit) for study 14-2026

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30% of the respective LOQ.		
Calibration range	0.2 µg/L - 100 µg/L (corresponds to 0.002 – 1 mg/kg)		
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound		
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.		
	Study 14-2026		
	CGA 279202	**CGA 357262**	**CGA 357261**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.018886 x + 5.1146 \cdot 10^{-4}$, Correlation coefficient r: 0.9991 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.039796 x - 9.0903 \cdot 10^{-4}$, Correlation coefficient r: 0.9997 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.042229 x - 4.6239 \cdot 10^{-4}$, Correlation coefficient r: 0.9997 number of data points: 6
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.092830 x - 2.7559 \cdot 10^{-4}$, Correlation coefficient r: 0.9997 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.063383 x – 0.0014508$, Correlation coefficient r: 0.9996 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.10009 x + 0.0017962$, Correlation coefficient r: 0.9997 number of data points: 6

Conclusion

The analytical method used in this study was fully validated during study M-448498-01-1 (Stuke, S.; Teubner, L.; 2013). For the matrix relevant to this study (strawberry (fruit)), a set of additional validation recoveries was analysed within the course of the studies 12-2012, 12-2013 and 12-2014. For concurrent validation purposes, the recoveries of the method performance were performed during the conduct of the study 14-2026. The method 01313/M001 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on strawberry (fruit) via HPLC-MS/MS in the presented studies and can therefore be regarded as fit for purpose.

A 2.2.1.1.7 Analytical method 01313/M001 in support of the study M-553855-01-1

A 2.2.1.1.7.1 Method validation

Comments of zRMS:	Method 01313/M001: Full validation data is documented with the method 01313/M001 itself for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix relevant to this study (strawberry) but not included in the original validation, a set (minimum 3 repetitions each at three fortification levels) of additional validation recoveries were analysed within the course of the study 15-2031. The limits of quantitation (LOQ) for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 are 0.01 mg/kg, corresponding to the lowest fortification level of successfully conducted recovery experiments. Blank values in control samples were below 30% of the LOQ for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466. All method validation data are in compliance with the guideline requirements for data collection methods. The validation of method 01313/M001 can therefore be considered successful for the new matrix.

	The mean of the concurrent recoveries were within the acceptable range of 70 - 110%. RSD values were below 20%. The study is acceptable.

Reference:	**KCP 5.1.2.5/15**, Also as KCA 6.3.3.1/02
Title:	Determination of the residues of fluopyram and trifloxystrobin in/on strawberry after spray application of AE C656948 & CGA 279202 SC 500 in Germany, Denmark, Spain, southern France and Italy
Report:	Szeley, C. M.; Sadler, C.; 2016; 15-2031; M-553855-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market; OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009); US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Deviations:	
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the study 15-2031 was to determine the magnitude of the residues of fluopyram (AE C656948) and its metabolite AE C656948-benzamide and of trifloxystrobin (CGA 279202), its isomers CGA 331409, CGA 357262, CGA 357261 and the metabolite CGA 321113 and its isomer CGA 373466 in/on strawberry (fruit) after two spray applications with AE C656948 & CGA 279202 SC 500, a suspension concentrate formulation containing 250 g/L AE C656948 and 250 g/L trifloxystrobin.

Full validation data is documented with the method 01313/M001 itself (Stuke, S.; Teubner, L.; 2013; M-448498-01-1) for CGA 279202, CGA 357261, CGA 357262, CGA 331409, CGA 321113 and CGA 373466 in broccoli (head), kidney bean (dry seed), rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix relevant to this study (strawberry (fruit)) a limited set (1 control, 3 repetitions each at three fortification levels) of additional validation recoveries was analyzed within the course of the present study.

Residues of trifloxystrobin and its isomers / metabolites were determined by LC-MS/MS according to method 01313/M001.

Residues of trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 were extracted from the homogenised sample material with a mixture of acetonitrile/water (4/1; v/v) by shaking. After filtration, addition of ammonium acetate solution to adjust the pH value and addition of internal standard solution the extracts were made up to volume, diluted, filtered and subjected to HPLC-MS/MS measurement.

Conditions used for this Study:

Slight adaptions were made to the sample preparation procedure described within the analytical method 01313/M001 which are as follows: The sample dilution step (step 9 in method 01313/M001) was omitted and in parallel the injection volume was reduced to 1 µL.

Results and discussions

A limited set of validation recoveries was analysed and recovery rates were determined at fortification levels of 0.01 mg/kg, 0.1 mg/kg and 1.0 mg/kg for each analyte.

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries.

The average recoveries were within the range of 70 – 110%. The RSD values were below 20%.

Table A 32: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	strawberry / fruit	0.01	97; 104; 106	102	4.6	0.01
		0.10	82; 100; 100	94	11.1	
		1.0	81; 92; 96	90	8.7	
			Overall recovery (n = 9)	**95**	**9.3**	
CGA 357261	strawberry / fruit	0.01	81; 92; 95	89	8.3	0.01
		0.1	80; 92; 97	90	9.7	
		1.0	80; 96; 99	92	11.1	
			Overall recovery (n = 9)	**90**	**8.6**	
CGA 357262	strawberry / fruit	0.01	82; 92; 93	89	6.8	0.01
		0.1	86; 94; 100	93	7.5	
		1.0	79; 93; 99	90	11.4	
			Overall recovery (n = 9)	**91**	**7.9**	
CGA 331409	strawberry / fruit	0.01	80; 88; 89	86	5.8	0.01
		0.1	82; 93; 99	91	9.4	
		1.0	81; 93; 96	90	8.8	
			Overall recovery (n = 9)	**89**	**7.7**	
CGA 321113	strawberry / fruit	0.01	75; 77; 82	78	4.6	0.01
		0.1	88; 89; 93	90	2.9	
		1.0	83; 99; 101	94	10.5	
			Overall recovery (n = 9)	**87**	**10.4**	
CGA 373466	strawberry / fruit	0.01	78; 91; 92	87	9.0	0.01
		0.1	81; 96; 97	91	9.8	
		1.0	85; 92; 97	91	6.6	
			Overall recovery (n = 9)	**90**	**7.8**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 33: Characteristics for the analytical method 01313/M001 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on strawberry (fruit)

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30% of the respective LOQ.		
Calibration range	0.2 – 100 µg/L (corresponds to 0.0025 – 1.25 mg/kg)		
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound		
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.		
	CGA 279202	**CGA 357262**	**CGA 357261**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted):	Individual calibration data is presented calibration equation (1/x weighted):	Individual calibration data is presented calibration equation (1/x weighted):

	y = 0.022905 x + 0.00096992, Correlation coefficient r: 0.9997, number of data points: 8	y = 0.086837 x + 0.0012355, Correlation coefficient r: 1.0000, number of data points: 8	y = 0.037639 x + 0.00076513, Correlation coefficient r: 0.9998, number of data points: 8
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 0.035963 x + 0.0010620, Correlation coefficient r: 0.9998, number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 0.091273 x - 0.010027, Correlation coefficient r: 0.9999, number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 0.093641 x - 0.0043887, Correlation coefficient r: 0.9999, number of data points: 8

Conclusion

The analytical method 01313/M001 was fully validated during study (Stuke, S.; Teubner, L.; 2013; M-448498-01-1). For the matrix relevant to this study (strawberry (fruit)) a limited set of validation recoveries was analysed within the course of the present study. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01313/M001 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on strawberry (fruit) via HPLC-MS/MS.

A 2.2.1.1.8 Analytical method 01313/M001 in support of the study M-684200-01-1

A 2.2.1.1.8.1 Method validation

Comments of zRMS:	Method 01313/M001: Full validation data is documented with the method 01313/M001 itself for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix relevant to this study (strawberry) but not included in the original validation, a set (minimum 3 repetitions each at two fortification levels) of additional validation recoveries were analysed within the course of the study 18-2050. The limits of quantitation (LOQ) for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 are 0.01 mg/kg, corresponding to the lowest fortification level of successfully conducted recovery experiments. Blank values in control samples were below 30% of the LOQ for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%. No residues above the LOQ were found in the control samples. All method validation data are in compliance with the guideline requirements for data collection methods. The validation of method 01313/M001 can therefore be considered successful for the new matrix. The study is acceptable.

Reference:	**KCP 5.1.2.5/16**
Title:	Determination of the residues of trifloxystrobin and AE C656948 in/on strawberry after spray application of AE C656948 & CGA279202 SC 500 in Germany and Belgium
Report:	Braune, M.; Eremeeva, T.; 2020; 18-2050; M-684200-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the study 18-2050 was to determine the magnitude of the residues of trifloxystrobin (CGA 279202)(comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) and AE C656948 (comprising AE C656948 and its metabolite AE C656948-benzamide) in/on strawberry (fruit) after two spray applications with AE C656948 & CGA279202 SC 500, a suspension concentrate formulation containing 250 g/L trifloxystrobin and 250 g/L AE C656948.

Full validation data is documented with the method 01313/M001 itself (Stuke, S.; Teubner, L.; 2013; M-448498-01-1) for CGA 279202, CGA 357261, CGA 357262, CGA 331409, CGA 321113 and CGA 373466 in broccoli (head), kidney bean (dry seed), rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix relevant to this study (strawberry (fruit)) a limited set (1 control, 3 repetitions each at two fortification levels) of additional validation recoveries was analyzed within the course of the present study.

Residues of CGA 279202, its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 were extracted from the homogenised sample material with a mixture of acetonitrile/water (4/1; v/v) by shaking. After filtration, addition of ammonium acetate solution to adjust the pH value and addition of internal standard solution the extracts were made up to volume, diluted, filtered and subjected to HPLC-MS/MS measurement.

Conditions used in this study:

Slight adaptions were made to the sample preparation procedure described within the analytical method modification 01313/M001 which are as follows: 0.25 mL of an internal standard mixture with 1000 µg/L of each analyte-ISTD is added, the extract is filtered through a 0.45 µm syringe filter into a HPLC vial and subjected to HPLC-MS/MS determination or the sample is centrifuged for 5 minutes at 4750 rpm. A volume of 1 – 2 µL of this final extract is injected for HPLC-MS/MS analysis. The quantification was done by using isotopic labelled internal standards.

Samples containing high analyte concentrations were diluted until their concentrations were within the linearity range of the corresponding calibration curve.

Results and discussions

A limited set of validation recoveries was analysed and recovery rates were determined at fortification levels of 0.01 mg/kg and 0.50 mg/kg for each analyte.

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery per ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The limits of quantitation (LOQ) for each analyte is 0.01 mg/kg in strawberry (fruit), corresponding to the lowest fortification level of successfully conducted recovery experiments.

Apparent residues in control samples were below 30% of the LOQ. The mean and overall mean recoveries were within the range of 70 – 110%. The RSD values were below 20%.

Table A 34: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	strawberry / fruit	0.01	92; 96; 96	95	2.4	0.01
		0.50	85; 102; 103	97	10.5	
			Overall recovery (n = 6)	**96**	**7.0**	
CGA 357261	strawberry / fruit	0.01	97; 100; 101	99	2.1	0.01
		0.50	89; 100; 103	97	7.6	
			Overall recovery (n = 6)	**98**	**5.1**	
CGA 357262	strawberry / fruit	0.01	96; 102; 105	101	4.5	0.01
		0.50	84; 100; 101	95	10.0	
			Overall recovery (n = 6)	**98**	**7.6**	
CGA 331409	strawberry / fruit	0.01	98; 100; 102	100	2.0	0.01
		0.50	87; 98; 102	96	8.1	
			Overall recovery (n = 6)	**98**	**5.7**	
CGA 321113	strawberry / fruit	0.01	107; 107; 109	108	1.1	0.01
		0.50	88; 101; 106	98	9.4	
			Overall recovery (n = 6)	**103**	**7.6**	
CGA 373466	strawberry / fruit	0.01	90; 102; 105	99	8.0	0.01
		0.50	86; 101; 102	96	9.3	
			Overall recovery (n = 6)	**98**	**7.9**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 35: Characteristics for the analytical method 01313/M001 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on strawberry (fruit)

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.		
Calibration range	0.2 – 100 µg/L (corresponds to 0.0025 – 1.25 mg/kg)		
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound		
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.		
	CGA 279202	**CGA 357262**	**CGA 357261**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.031042 x + 0.0014849, Correlation coefficient r: 0.9999, number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 0.037990 x + 0.00021728, Correlation coefficient r: 1.0000, number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 0.039576 x + 0.00028211, Correlation coefficient r: 1.0000, number of data points: 8
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 0.076891 x + 0.00062839,	Individual calibration data is presented calibration equation (1/x weighted): y = 0.10450 x - 0.00021141,	Individual calibration data is presented calibration equation (1/x weighted): y = 0.10485 x - 0.0013286,

	Correlation coefficient r: 1.0000, number of data points: 8	Correlation coefficient r: 0.9999, number of data points: 8	Correlation coefficient r: 1.0000, number of data points: 8

Conclusion
The analytical method 01313/M001 was fully validated during study (Stuke, S.; Teubner, L.; 2013; M-448498-01-1). For the matrix relevant to this study (strawberry (fruit)) a limited set of validation recoveries was analysed within the course of the present study. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01313/M001 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on strawberry (fruit) via HPLC-MS/MS.

A 2.2.1.1.9 Analytical method 01207
A 2.2.1.1.9.1 Method validation

Comments of zRMS:	The QuEChERS method was validated for the determination of trifloxystrobin and the metabolite CGA 321113 in/on plant materials: carrot, apple, orange, oilseed rape seed and beans. Two MRM transitions were monitored for each analyte and each matrix tested. Therefore, the HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. The limit of quantitation (LOQ) for each single analyte was 0.01 mg/kg in all matrices tested. The limit of detection (LOD) was by definition 30 % of the LOQ or 0.003 mg/kg. Mean recoveries for each fortification level and the overall mean recovery were within the 70 - 110% range for all matrices. Relative standard deviations were below 20% for all analytes and sample materials. All method validation data are in compliance with the guideline requirements of SANCO/3029/99 rev. 4. The validation of method 01207 can therefore be considered successful for carrot, apple, orange, oilseed rape seed and beans. The study is acceptable.

Reference:	**KCP 5.1.2.5/21**
Title:	Validation of the BCS method no. 01207 (based on modified QuEChERS method) for the determination of selected BCS analytes and their metabolites in carrot, apple, orange, oilseed rape seed and beans
Report:	Lakaschus, S.; Amann, S.; Winter, O.; Gizler, A.; 2013; S10-00279; M-424756-02-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 US EPA Residue Chemistry Test Guideline OCSSP 860.1340: Residue Analytical Method
Deviations:	Not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods
The QuEChERS multi residue method 01207 was validated for the determination of trifloxystrobin (CGA 279202) and CGA 321113 and other analytes in/on plant materials. The analytes were extracted from apple (fruit), orange (whole fruit), carrot (root), oilseed rape (seed) and bean (dry seeds) with acetonitrile/water

(4/1, v/v). An aliquot of the extract was taken and the internal stable labeled standards were added. The solution was subjected to LC-MS/MS. The internal standard procedure, using stable isotopically labelled internal standards was used for calibration.
The Limit of Quantification for this method was 0.01 mg/kg for each analyte and sample material.
An aliquot of the sample solution was injected into the high performance liquid chromatograph and subjected to reversed phase chromatography coupled with tandem mass spectrometry (MS/MS) with electrospray ionisation. The MS/MS instrument was operated in the Multiple Reaction Monitoring mode (MRM). The pseudomolecular ions of the analytes ([M+H]+, [M-H]- or any adducts) were selected by the first quadrupole. These precursor ions were impulsed with nitrogen in the collision cell (second quadrupole) and the resulting fragment ions (product ions) were separated according to their m/z ratio in the third quadrupole. Two of these product ions per analyte were selected: one product ion (MRM-transition) serving for quantitation and the second for confirmation (MRMs used for quantification: CGA 279202 m/z: 409 → 186, CGA 321113 m/z 395 → 186; MRMs used for confirmation: CGA 279202 m/z: 409 → 145, CGA 321113 m/z 395 → 148).

Results and discussions
Recovery rates were determined at fortification levels of 0.01 mg/kg (= LOQ level), and 0.10 mg/kg. Metabolite fortification levels are not converted into parent equivalents. The lowest fortification level providing a mean recovery between 70 and 110% with a relative standard deviation of < 20% per definition corresponding to the Limit of Quantitation (LOQ), provided that the blank values were below 30% at this level. Recovery experiments were conducted by fortifications of untreated control samples with defined amounts of each analyte prior to analysis. Mean recoveries for each fortification level and the overall mean recovery were within the 70 - 110% range for all matrices.

Up to three untreated control samples of different origin were examined. For all analytes the residues found were below the LOD (< 0.003 mg/kg).

Using internal standard procedure, the correlation between the injected amount of substance and the detector response was linear for solvent standards ranging from 0.5ng/mL to 50 ng/mL for trifloxystrobin and CGA 321113. The coefficients of determination were > 0.98.

As a measure for the precision, the intra-laboratory repeatability (n=5) is given as relative standard deviation (% RSD) for all sample materials at fortification levels of 0.01 and 0.10 mg/kg. Relative standard deviations were below 20%.

Two MRM transitions were monitored for each analyte and each matrix tested. For each compound, 2nd MRM transitions are considered to be suitable for confirmatory purposes in all tested sample materials at the LOQ level of 0.01 mg/kg.

Table A 36: Recovery rates and precision results (repeatability) of CGA 279202 and its metabolite CGA 321113

Analyte	Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Apple	0.01	88, 85, 80, 86, 86	85	3.5	~~0.10~~ 0.01
		0.10	77, 87, 84, 81, 78	81	5.1	
			Overall Recovery (n = 10)	**83**	**4.7**	
	Orange	0.01	83, 91, 74, 86, 79	83	7.9	~~0.10~~ 0.01
		0.10	85, 83, 86, 76, 81	82	4.8	
			Overall Recovery (n = 10)	**82**	**6.2**	
	Carrot	0.01	84, 68, 90, 90, 99	86	13	~~0.10~~ 0.01
		0.10	82, 85, 78, 83, 82	82	3.1	
			Overall Recovery (n = 10)	**84**	**9.7**	
	Dry bean	0.01	89, 88, 83, 84, 83	85	3.4	~~0.10~~ 0.01
		0.10	81, 78, 80, 78, 79	79	1.6	
			Overall Recovery (n = 10)	**82**	**4.7**	
	Oilseed rape	0.01	86, 83, 87, 89, 83	86	3.0	~~0.10~~

		0.10	83, 80, 82, 83, 84	82	1.8	0.01
			Overall Recovery (n = 10)	**84**	**3.1**	
CGA 321113	Apple	0.01	97, 96, 100, 105, 96	99	3.9	~~0.10~~ 0.01
		0.10	98, 104, 105, 101, 96	101	3.8	
			Overall Recovery (n = 10)	**100**	**3.8**	
	Orange	0.01	100, 104, 101, 101, 101	101	1.5	~~0.10~~ 0.01
		0.10	103, 99, 103, 86, 107	100	8.1	
			Overall Recovery (n = 10)	**101**	**5.6**	
	Carrot	0.01	105, 86, 105, 115, 108	104	10	~~0.10~~ 0.01
		0.10	103, 102, 101, 110, 105	104	3.4	
			Overall Recovery (n = 10)	**104**	**7.3**	
	Dry bean	0.01	88, 87, 87, 90, 88	88	1.4	~~0.10~~ 0.01
		0.10	86, 80, 83, 85, 83	83	2.8	
			Overall Recovery (n = 10)	**86**	**3.5**	
	Oilseed rape	0.01	84, 85, 88, 87, 88	86	2.1	~~0.10~~ 0.01
		0.10	90, 87, 89, 87, 90	89	1.7	
			Overall Recovery (n = 10)	**88**	**2.2**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 37: Characteristics for the QuEChERS multi residue method 01207 used for validation of CGA 279202 and its metabolite CGA 321113

Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ	
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound in each matrix	
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.	
	CGA 279202	**CGA 321113**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.0046 x + 0.0006, Correlation coefficient r: 0.9999 number of data points: 6	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.0368 x – 0.0023, Correlation coefficient r: 0.9999 number of data points: 6
Calibration range	0.5 ng/mL to 50 ng/mL (corresponds to 0.01 mg/kg to 1 mg/kg)	

Conclusion

The QuEChERS multi residue method 01207 was validated for the determination of trifloxystrobin, CGA 321113, and other analytes in/on plant materials by conducting recovery experiments with a broad number of plant matrices (commodity groups with high water, high acid, high protein and high oil content). All results are in accordance with the criteria set by the guideline SANCO/3029/99 rev. 4. It meets all necessary performance criteria to determine residues of trifloxystrobin and its metabolite CGA 321113 in plants with an LOQ of 0.01 mg/kg. Therefore, the analytical method 01207 has been successfully validated.

A 2.2.1.1.10 Analytical method 01207 in support of the study M-433737-01-1

A 2.2.1.1.10.1 Method validation

Comments of zRMS:	Analysis of fluopyram (AE C656948) and its metabolites AE C656948-benzamide, AE C656948-pyridyl-acetic acid and AE C656948-pyridyl-carboxylic acid and trifloxystrobin (CGA 279202) and its metabolite CGA321113 was carried out according to the method validated in BCS Study 01207 based on QuEChERS Method for carrot, apple, orange, oilseed rape seed and beans. For the matrix relevant to this study (raspberry) but not included in the original validation, a set (one control, 3 repetitions each at two fortification levels) of additional validation recoveries were analysed within the course of the study BCS-G401-11. The limits of quantitation (LOQ) for this method was 0.01 mg/kg each compound in raspberry (fruits). The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%. No residues above the LOQ were found in the control samples. All method validation data are in compliance with the guideline requirements for data

	collection methods. The study is acceptable.

Reference:	**KCP 5.1.2.5/22**
Title:	Residues of fluopyram and trifloxystrobing in raspberry under plastic umbrella at intervals following two foliar applications of FLU+TFS 500 SC - Belgium, season 2011
Report:	Loriau, P.; 2012; BCS-G401-11; M-433737-01-1
Authority registration No:	
Guideline(s):	EC Guideline SANCO 1607NI/97 rev.2, 10.06.99: Guidelines for the generation of data concerning residues as provided in Annex II, Part A, Section 6 and Annex III, Part A, Section 8 of Directive 91/414/EEC and Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market - Appendix B: EC Guideline SANCO 7029NI/95 rev.5, 22/07/97 General recommendations for the design, preparation and realization of residue trial - Appendix D: EC Guideline SANCO 7525NI/95 rev.9, March 2011 Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs. FAO Guidelines on Producing Pesticide Residues Data from Supervised Trials, Rome 1990 other appropriate SOP's or OECD Guidelines US EPA OCPP Guideline No. 860.1500
Deviations:	Not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the study BCS-G401-11 was to determine the magnitude of the relevant residues of fluopyram (AE C656948) [and its metabolites AE C656948-benzamide, AE C656948-pyridyl-acetic acid and AE C656948-pyridyl-carboxylic acid] and trifloxystrobin (CGA 279202) [and its metabolite CGA321113] in/on raspberry (fruit) after two foliar applications with FLU+TFS 500 SC, an SC formulation containing 250 g/L of fluopyram and 250 g/L of trifloxystrobin.

Analysis of trifloxystrobin and the metabolite CGA321113 was carried out according to the method 01207 validated in study M-424756-02-1 (Lakaschus, S.; Amann, S.; Winter, O.; Gizler, A.; 2013) based on QuEChERS Method for apple, carrot, whole orange, oilseed rape and dry bean. For the matrix relevant to this study but not included in the original validation, a limited set (one control, 3 repetitions each at two fortification levels) of recoveries of the sample material raspberry (fruits) was analysed within the course of this study.

The weight of specimen per analysis was 5 g. The analytes were extracted from the specimen material by using a mixture of acetonitrile/water (4/1; v/v).

Conditions used for this study:

The chromatographic system used was: a high performance liquid chromatograph with a reversed phase chromatography (Phenomenex Luna 5µ C18 150x2.0mm, 5µm column) coupled with tandem mass spectrometry (MS/MS) with Electrospray ionisation (ESI positive, MRMs used for quantification: CGA 279202 m/z: 409 → 186, CGA 321113 m/z 395 → 186; Applied Biosystems API 4000 Triple Quadruple Mass Spectrometer Perkin-Elmer Sciex Instruments Analyst version 1.5) for all analytes. The quantification was performed according to the external bracketing procedure using stable-labelled internal standards. For trifloxystrobin and CGA321113 solvent standards were used.

The examination specimens were kept deep-frozen until their analysis. The quantity 5 g was weighed while the specimen was still deep-frozen and the remaining specimen was immediately returned to the freezer.

Results and discussions

For the matrix relevant to this study but not included in the original validation, a limited set (one control, 3 repetitions each at two fortification levels) of recoveries of the sample material raspberry (fruits) was analysed within the course of this study. Recovery rates were determined at fortification levels of 0.01 mg/kg (LOQ) and 0.10 mg/kg (10xLOQ) for each analyte. The recovery experiments were conducted by fortification of untreated control samples with defined amounts of the analytes prior to analysis.

No residues above the LOQ were found in the control specimens. The overall mean recoveries per fortification level were within the range of 70 – 110%. The RSD values were below 20%, if applicable ($n \geq 3$).

Table A 38: Recovery rates and precision results (repeatability) of CGA 279202 and its metabolite CGA 321113 for method validation (using the 1st MRM)

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	raspberry (fruits)	0.01	103, 107, 107	106	2.2	0.01
		0.1	88, 88, 91	89	1.9	
			Overall Recovery (n = 6)	**97**	**9.6**	
CGA 321113	raspberry (fruits)	0.01	91, 99, 94	95	4.3	0.01
		0.1	85, 86, 88	86	1.8	
			Overall Recovery (n = 6)	**91**	**5.9**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

In order to check the performance of the method, recovery determinations were included in each set of analysis. Control specimens from the study were fortified for the use as recovery specimens. All the recovery determinations were performed in parallel to the analyses of control and treated specimens from the study.
The apparent residues in the control specimen used for fortification were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control specimens used for these recoveries, except for CGA 279202, where recoveries were corrected for the residues found in the corresponding control specimens. The means of the concurrent recoveries were for all analytes and for all fortification levels, within the acceptable range of 70 – 120%.

Table A 39: Recovery rates and precision results (repeatability) of CGA 279202 and its metabolite CGA 321113 for method performance

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	raspberry (fruits)	0.01	94*, 108*	101	-	0.01
		0.1	93*	-	-	
		2.0	92*	-		
			Overall Recovery	**97**	**7.8**	
CGA 321113	raspberry (fruits)	0.01	108	-	-	0.01
		0.10	95	-	-	
			Overall Recovery	**102**	**-**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation;
* Recoveries were corrected for the residues found in the corresponding control specimens.

Table A 40: Characteristics for the analytical method 01207 used for validation of CGA 279202 and its metabolite CGA 321113

Specificity	For each compound and each matrix: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.	
Calibration range	0.5 ng/mL - 50 ng/mL (corresponds to 0.01 mg/kg – 1 mg/kg) for each compound	
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound in each matrix	
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.	
	CGA 279202	**CGA 321113**

Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.013 x + 0.0031, Correlation coefficient r: 0.9997 number of data points: 6	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.0239 x + 0.0007, Correlation coefficient r: 1.0000 number of data points: 6

Conclusion
The analytical method 01207 was validated during study M-424756-02-1 (Lakaschus, S.; Amann, S.; Winter, O.; Gizler, A.; 2013) based on QuEChERS Method for apple, carrot, whole orange, oilseed rape and dry bean. For the matrix relevant to this study, a limited set (one control, 3 repetitions each at two fortification levels) of recoveries of the sample material raspberry (fruits) was analysed within the course of this study. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01207 is suitable for the determination of residues of CGA 279202 and its metabolite CGA 321113 in/on raspberry (fruits) via HPLC-MS/MS.

A 2.2.1.1.11 Analytical method 01207 in support of the study M-434309-02-1

A 2.2.1.1.11.1 Method validation

Comments of zRMS:	The analyses for trifloxystrobin and the metabolite CGA321113 were conducted according to the 01207 method validated in study M-424756-02-1 (Lakaschus, S.; Amann, S.; Winter, O.; Gizler, A.; 2013). The method performance was checked during the present study. No residue above LOQ were detected in the control specimens. The limit of quantification for this method was 0.01 mg/kg for each compound in raspberry (fruit). The mean recovery rates for each spike level and analyte were within the acceptable range of 70 and 110%. Remark: It should be noted that a limited set (only 2 repetitions at LOQ level, one repetition at two another fortification levels (0.1 mg/kg and 1.5 mg/kg) for trifloxystrobin and only one repetition at two fortification levels (LOQ and 0.1 mg/kg) for CGA 321113) of recoveries of the sample material raspberry (fruits) was analysed within the course of this study. The method is fit for purpose.

Reference:	**KCP 5.1.2.5/23**
Title:	Amendment no. 1 to report no: PTZ-NLI-11797 - Residues of fluopyram + trifloxystrobin in red raspberry under plastic umbrella at intervals following two foliar applications of fluopyram & trifloxystrobin SC 500
Report:	Oostingh, C.; 2013; PTZ-NLI-11797; M-434309-02-1
Authority registration No:	
Guideline(s):	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22) OECD Guideline for testing of Chemicals; Resdiues in rotational crops (limited field studies), No. 504, 8 Jan. 2007 US EPA OCSPP Guideline No. 860.1500
Deviations:	Not specified
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

Materials and methods
The purpose of the study was to determine the amount of residues of fluopyram + trifloxystrobin (and metabolites) in raspberry after two foliar applications with fluopyram & trifloxystrobin SC 500.
Analysis of trifloxystrobin and the metabolite CGA321113 was carried out according to the method 01207 validated in study M-424756-02-1 (Lakaschus, S.; Amann, S.; Winter, O.; Gizler, A.; 2013) based on QuEChERS Method for apple, carrot, whole orange, oilseed rape and dry bean. For concurrent validation purposes, the method performance was checked during the present study.

The weight of specimen per analysis was 5 g. The analytes were extracted from the sample material by using a high speed blender with a mixture of acetonitrile/water (4/1; v/v).

Conditions used for this study:
The chromatographic system used was a high performance liquid chromatograph with a reversed phase chromatography (Phenomenex Luna 5µ C18 150x2.0mm, 5µm column) coupled with tandem mass spectrometry (MS/MS) with Electrospray ionisation (Applied Biosystems API 4000 Triple Quadruple Mass Spectrometer Perkin-Elmer Sciex Instruments Analyst version 1.5) for all analytes except AE C656948-pyridyl-carboxylic acid (AE C657188). In addition a high performance liquid chromatograph with a reversed phase chromatography (ZORBAX Eclipse C8 150x4.6mm, 5µm column) coupled with tandem mass spectrometry (MS/MS) with Electrospray ionisation (ESI positive, MRMs used for quantification: CGA 279202 m/z: 409 → 186, CGA 321113 m/z 395 → 186; Applied Biosystems API 5000 Triple Quadruple Mass Spectrometer Perkin-Elmer Sciex Instruments Analyst version 1.5) for AE C656948-pyridyl-carboxylic acid (AE C657188) was used.
The quantification was performed using an external standardisation using solvent standards with internal standards for trifloxystrobin and CGA 321113.
The examination specimens were kept deep-frozen until their analysis. The quantity 5 g was weighed while the specimen was still deep-frozen and the remaining specimen was immediately returned to the freezer.

Results and discussions
In order to check the performance of the method, recovery determinations were included in each set of analysis. Control samples from the study were fortified for the use as recovery samples. All the recovery determinations were performed in parallel to the analyses of control and treated samples from the study.
The apparent residues in the control sample used for fortification were below the LOD. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The means of the concurrent recoveries were for all analytes and for all fortification levels, within the acceptable range of 70 – 120%.

Table A 41: Recovery rates and precision results (repeatability) of CGA 279202 and its metabolite CGA 321113

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	raspberry (fruits)	0.01	98, 102	100	-	0.01
		0.10	97	-	-	
		1.5	80	-	-	
			Overall Recovery	**94**	**10**	
CGA 321113	raspberry (fruits)	0.01	90	-	-	0.01
		0.10	91	-	-	
			Overall Recovery	**91**	**-**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 42: Characteristics for the analytical method 01207 used for validation of CGA 279202 and its metabolite CGA 321113

Specificity	For each compound and each matrix: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.	
Calibration range	0.5 ng/mL - 50 ng/mL (corresponds to 0.01 mg/kg – 1 mg/kg) for each compound	
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound in each matrix	
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.	
	CGA 279202	**CGA 321113**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted):	Individual calibration data is presented, calibration equation (1/x weighted):

	y = 0.014 x – 0.0007, Correlation coefficient r: 1.0000 number of data points: 6	y = 0.0246 x - 0.0009, Correlation coefficient r: 1.0000 number of data points: 6

Conclusion
The analytical method 01207 was validated during study M-424756-02-1 (Lakaschus, S.; Amann, S.; Winter, O.; Gizler, A.; 2013) based on QuEChERS Method for apple, carrot, whole orange, oilseed rape and dry bean. For concurrent validation purposes, the method performance was tested during the present study. The data presented demonstrate that the method allows the determination of these substances with satisfactory precision. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.2.1.1.12 Analytical method in support of the study M-434818-01-2

A 2.2.1.1.12.1 Method validation

Comments of zRMS:	The objective of the analytical phase of this study was to determine the residues of fluopyram and trifloxystrobin after 2 applications of the product F413BCS in raspberry in support of the registration for use in this crop. The method was validated as per document SANCO/3029/99 for fluopyram and for trifloxystrobin and its metabolite CGA321113. The analytical method used was validated by performing 10 recovery experiments, 5 spiked at the limit of quantification (LOQ) and, 5 spiked at ten times the LOQ, and 2 control specimens. The mean recovery rates for each spike level and the general mean is between 70 and 110% with a coefficient of variation and a general coefficient of variation less than 20%. The limit of quantification is set at 0.01 mg/kg for fluopyram. The limit of quantification for trifloxystrobin and its metabolite CGA321113, both together expressed as trifloxystrobin, is 0.010 mg/kg (i.e. 0.005 mg/kg for trifloxystrobin and 0.005 mg/kg for metabolite CGA321113, expressed as trifloxystrobin). No residue was detected in the control specimens. The method was validated according to the SANCO/3029/99 guideline. The study is acceptable.

Reference:	**KCP 5.1.2.5/24**
Title:	Residues of fluopyram and trifloxystrobine, after 2 applications of F413BCS in raspberry in support of the registration for use in this crop
Report:	Malet, J. C.; Allard, L.; 2019; RAFR03509; M-434818-01-2
Authority registration No:	
Guideline(s):	OECD 91/414/ CEE US EPA OCSPP Guideline no. 860.SUPP
Deviations:	Not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods
The objective of the present study was to determine the residues of fluopyram and trifloxystrobin (CGA 279202) after 2 applications of the product F413BCS in raspberry in support of the registration for use in this crop.
The analytical method, GIRPA reference GIR/MET/TRIFLOXY/01V1, was used in the present study to determine residues of CGA 279202 and its metabolite CGA 321113.
Residues of trifloxystrobin and its metabolite CGA 321113 are extracted by homogenisation with an acetonitrile/water (80/20, v/v) mixture with the addition of L-cysteine hydrochloride. The assay is performed by liquid chromatography coupled with triple quadrupole tandem mass spectrometry (ESI positive, MRMs used for quantification: CGA 279202 m/z: 409 → 186, CGA 321113 m/z 395 → 186).

Results and discussions
Repeatability testing comprises the performance of at least 5 recovery tests at the limit of quantification (LOQ) and 5 recovery tests at ten times the limit of quantification. The recovery rates were determined

from a specimen spiked by the addition of a known quantity of trifloxystrobin and its metabolite CGA321113, expressed as trifloxystrobin, to an aliquot of an untreated specimen. The LOQ of trifloxystrobin and its metabolite CGA 321113, expressed together as trifloxystrobin, is 0.010 mg/kg (i.e. 0.005 mg/kg for trifloxystrobin and 0.005 mg/kg for its metabolite CGA321113, expressed as trifloxystrobin).
Blank values in control samples were below 30% of the LOQ. The mean recovery rates for each spike level and the general mean is between 70 and 110% with a relative standard deviation (RSD) less than 20%.

Table A 43: Recovery rates and precision results (repeatability) of CGA 279202 and its metabolite CGA 321113 for method validation (using the 1st MRM)

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Raspberry	0.005	98; 100; 86; 77; 80; 95	89	11	0.005
		0.05	86; 105; 75; 73; 71	82	17	
			Overall Recovery (n = 11)	**86**	**14**	
CGA 321113 expressed as CGA 279202	Raspberry	0.005	88; 88; 79; 80; 75; 81	82	6	0.005
		0.050	82; 92; 74; 72; 68	78	12	
			Overall Recovery (n = 11)	**80**	**9**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 44: Characteristics for the analytical method used for validation of CGA 279202 and its metabolite CGA 321113

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.	
Calibration range	Not reported; obtaind from the graph: approx.. 0.025 µg/L to 0.3 µg/L	
Limit of determination/quantification	LOQ = 0.005 mg/kg for each compound	
Assessment of matrix effects is presented	No effects observed	
	CGA 279202	**CGA 321113**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation: y = 101037 x + 881.51, Correlation coefficient r: 0.9984 number of data points: 7	Individual calibration data is presented, calibration equation : y = 36454 x + 320.94, Correlation coefficient r: 0.9989 number of data points: 7

Conclusion
The analytical method, GIRPA reference GIR/MET/TRIFLOXY/01V1, was used in the present study to determine residues of CGA 279202 and its metabolite CGA 321113 in raspberry. It can be regarded as fit for purpose with regard to the present study.

A 2.2.1.1.13 Concurrent validation in support of the study M-434815-01-2

A 2.2.1.1.13.1 Method validation

Comments of zRMS:	The method was validated on raspberry specimens during the course of a similar study (Malet, J. C.; Allard, L.; 2019; RAFR03509; M-434818-01-2) in the laboratory as per document SANCO/3029/99 for fluopyram and for trifloxystrobin and its metabolite CGA321113. A complementary validation was realized during this analytical study in order to validate the found high residue level: Fluopyram: The limit of quantification is set at 0.01 mg/kg for fluopyram. Repeatability tests (3 or 5 recoveries at each fortification level) were performed at LOQ level at 10 x LOQ and at 300 x LOQ.

	The mean recovery were within the range 70-110% and with a RSD less than or equal to 20%. Trifloxystrobin and its metabolite CGA321113 The limit of quantification for trifloxystrobin and its metabolite CGA321113, both together expressed as trifloxystrobin, is 0.010 mg/kg (i.e. 0.005 mg/kg for trifloxystrobin and 0.005 mg/kg for metabolite CGA321113, expressed as trifloxystrobin). For each item, repeatability tests (3 or 5 recoveries at each fortification level) were performed at LOQ level, at 10 x LOQ and at 300 X LOQ (for trifloxystrobin) or 40 X LOQ (for metabolite CGA321113 expressed as trifloxystrobin). The mean recovery were within the range 70-110% and with a RSD less than or equal to 20%. The method described was successfully validated according to the guidance document SANCO/3029/99 rev.4. The study is acceptable.

Reference:	**KCP 5.1.2.5/25**
Title:	Mesure du niveau de résidu de fluopyram et de trifloxystrobine, après 2 applications de la préparation F413BCS sur framboisier dans le cadre d'une extension d'usage sur la culture - Residues of fluopyram and trifloxystrobine, after 2 applications of F413BCS in raspberry in support of the registration
Report:	Malet, J. C.; Allard, L.; 2019; RAFR00810; M-434815-01-2
Authority registration No:	
Guideline(s):	OECD 91/414/ CEE
Deviations:	Not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The objective of the present study was to determine the residues of fluopyram and trifloxystrobin (CGA 279202), after 2 applications of F413BCS in raspberry in support of the registration.

The analytical method used in the present study to determine residues of CGA 279202 and its metabolite CGA 321113 was validated in study M-434818-01-2 (Malet, J. C.; Allard, L.; 2019). A complementary validation was realized during this analytical study in order to validate the found high residue level: 3 recovery experiments fortified at 300 X LOQ (1.5 mg/kg for trifloxystrobin) or fortified at 40 X LOQ (0.2 mg/kg for metabolite CGA321113 expressed as trifloxystrobin) were performed.

Residues of trifloxystrobin and its metabolite CGA 321113 are extracted by homogenisation with an acetonitrile/water (80/20, v/v) mixture with the addition of L-cysteine hydrochloride. The assay is performed by liquid chromatography coupled with triple quadrupole tandem mass spectrometry (ESI positive, MRMs used for quantification: CGA 279202 m/z: 409 → 186, CGA 321113 m/z 395 → 186).

Results and discussions

A complementary validation with recovery experiments fortified at 300 X LOQ (for trifloxystrobin) or fortified at 40 X LOQ (for metabolite CGA321113 expressed as trifloxystrobin) were performed with 3 repetitions each.

The LOQ of trifloxystrobin and its metabolite CGA 321113, expressed together as trifloxystrobin, is 0.010 mg/kg (i.e. 0.005 mg/kg for trifloxystrobin and 0.005 mg/kg for its metabolite CGA321113, expressed as trifloxystrobin).

Blank values in control samples were below 30% of the LOQ. The mean recovery rates for each spike level and the general mean is between 70 and 110% with a relative standard deviation (RSD) less than 20%.

Table A 45: Recovery rates and precision results (repeatability) of CGA 279202 and its metabolite CGA 321113 for high residue level

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]

CGA 279202	Raspberry	1.5	83; 85; 82	83	2	0.005
			Overall Recovery (n= 3)	**85**	**12**	
CGA 321113 expressed as CGA 279202	Raspberry	0.2	96; 76; 82	85	12	0.005
			Overall Recovery (n = 3)	**81**	**10**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 46: Characteristics for the analytical method used for validation of CGA 279202 and its metabolite CGA 321113

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.	
Calibration range	trifloxystrobin matrix matched standard injections: 0.02 to 0.1 µg/L or 0.04 to 0.15 µg/L metabolite CGA321113 expressed as trifloxystrobin matrix matched standardinjections: 0.02 to 0.2 µg/L trifloxystrobin and metabolite CGA321113 expressed as trifloxystrobin solvent standard injections: 0.02 to 0.2 µg/L	
Limit of determination/quantification	LOQ = 0.005 mg/kg for each compound	
Assessment of matrix effects is presented	No effects observed	
	CGA 279202	**CGA 321113**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation in raspberry: y = 77898 x + 628.08, Correlation coefficient r: 0.9994 number of data points: 7	Individual calibration data is presented, calibration equation in raspberry: y = 25114 x + 216.02, Correlation coefficient r: 0.9979 number of data points: 6

Conclusion

The analytical method used in the present study to determine residues of CGA 279202 and its metabolite CGA 321113 was validated in study M-434818-01-2 (Malet, J. C.; Allard, L.; 2019). A complementary validation was realized during this analytical study in order to validate the found high residue level. The analytical method can be regarded as fit for purpose with regard to the present study.

A 2.2.1.1.14 Analytical method 01313/M001 in support of the study M-675722-01-1

A 2.2.1.1.14.1 Method validation

Comments of zRMS:	Method 01313/M001: Full validation data is documented with the method 01313/M001 itself for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix relevant to this study (raspberry) but not included in the original validation, a set (minimum 3 repetitions each at three fortification levels (0.01, 0.50 and 5.0 mg/kg)) of additional validation recoveries were analysed within the course of the study 18-2051. The limits of quantitation (LOQ) for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 are 0.01 mg/kg, corresponding to the lowest fortification level of successfully conducted recovery experiments. Blank values in control samples were below 30% of the LOQ for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%. No residues above the LOQ were found in the control samples. All method validation data are in compliance with the guideline requirements for data collection methods. The validation of method 01313/M001 can therefore be considered successful for the new matrix. The study is acceptable.

Reference:	**KCP 5.1.2.5/26**
Title:	Determination of the residues of trifloxystrobin and AE C656948 in/on raspberry after spray application of AE C656948 & CGA279202 SC 500 in Hungary, Poland, Germany and northern France
Report:	Buchmueller, K.; Holbein, J.; 2019; 18-2051; M-675722-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market US EPA OCSPP 860.1500, Crop Field Trial OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009)
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the study 18-2051 was to determine the magnitude of the residues of trifloxystrobin (comprising of trifloxystrobin (CGA279202), CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on raspberry (fruit) after two spray applications with AE C656948 & CGA279202 SC 500, a suspension concentrate (SC) formulation containing 250 g/L AE C656948 and 250 g/L trifloxystrobin.

Full validation data is documented within the method 01313/M001 itself (Stuke, S.; Teubner, L.; 2013; M-448498-01-1) for CGA279202, CGA 357261, CGA 357262, CGA 331409, CGA 321113 and CGA 373466 in/on broccoli (head), kidney bean (dry seed), rape (seed), grapes (bunches of grapes) and wheat (grain). As the validation of the method 01313/M001 was demonstrated for the high acid content group in/on grape in the method itself, only a limited set of validation (1 control, 3 recoveries at the LOQ level and 3 recoveries at 50 times the LOQ level) was performed in/on raspberry (fruit) within the present study. Additional recoveries were performed at 500 times the LOQ (5mg/kg).

Residues of CGA 279202, its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 were extracted from the homogenised sample material with a mixture of acetonitrile/water (4/1; v/v) by shaking. After filtration, addition of ammonium acetate solution to adjust the pH value and addition of internal standard solution the extracts were made up to volume, diluted, filtered and subjected to HPLC-MS/MS measurement.

Conditions used in this study:

Slight adaptions were made to the sample preparation procedure described within the analytical method modification 01313//M001 which are as follows:

2 g of homogenized, deep-frozen sample is weighed into a 50 mL tube and 16 ml of acetonitrile/ water 4/1 (v/v) is added. After shaking, 1 mL ammonium acetate buffer (1mol/L) and 0.25 mL of an internal standard mixture with 1000 µg/L of each analyte-ISTD are added and the volume is adjusted to 25 mL with acetonitrile/water 4/1 (v/v). The extract is filtered through a 0.45 µm syringe into a HPLC vial and 1-2 µL are injected for LC-MS/MS analysis (ESI positive, MRMs: CGA 279202 m/z: 409 → 145, CGA 357261 m/z 409 → 206, CGA 357262 m/z 409 → 206, CGA 331409 m/z 409 → 206, CGA 321113 m/z 395 → 186, CGA 373466 m/z 395 → 148). The quantification was done by external standardisation in pure solvent. The examination samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen and the remaining sample was immediately returned to the freezer.

Samples containing high analyte concentrations were diluted until their concentrations were within the linearity range of the corresponding calibration curve.

Results and discussions

A limited set of validation (1 control, 3 recoveries at the LOQ level and 3 recoveries at 50 times the LOQ level) was performed in/on raspberry (fruit) within the present study. Additional recoveries were performed at 500 times the LOQ (5mg/kg).

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The average recoveries per fortification level were within the range of 70 – 110% and the RSD values were below 20%, if applicable ($n \geq 3$).

Table A 47: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method performance and method validation

Analyte	Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	raspberry / fruit	0.01	100; 101; 101	101	0.6	0.01
		0.50	100; 100; 102; 102	101	1.1	
		5.0	94; 96; 97; 98; 99; 102	98	2.8	
			Overall recovery (n = 13)	**99**	**2.5**	
CGA 331409	raspberry / fruit	0.01	93; 93; 98	95	3.0	0.01
		0.50	97; 99; 100; 101	99	1.7	
		5.0	95; 96; 98; 98; 102; 104	99	3.5	
			Overall recovery (n = 13)	**98**	**3.4**	
CGA 357262	raspberry / fruit	0.01	96; 99; 101	99	2.6	0.01
		0.50	99; 101; 101; 103	101	1.6	
		5.0	93; 94; 95; 99; 100; 103	97	4.0	
			Overall recovery (n = 13)	**99**	**3.3**	
CGA 357261	raspberry / fruit	0.01	96; 98; 102	99	3.1	0.01
		0.50	100; 101; 101; 101	101	0.5	
		5.0	94; 94; 97; 98; 101; 104	98	4.0	
			Overall recovery (n = 13)	**99**	**3.1**	
CGA 321113	raspberry / fruit	0.01	93; 104; 108	102	7.6	0.01
		0.50	96; 100; 101; 102	100	2.6	
		5.0	87; 91; 91; 98; 101; 103	95	6.7	
			Overall recovery (n = 13)	**98**	**6.2**	
CGA 373466	raspberry / fruit	0.01	106; 107; 107	107	0.5	0.01
		0.50	96; 98; 98; 99	98	1.3	
		5.0	89; 93; 97; 97; 100; 105	97	5.7	
			Overall recovery (n = 13)	**99**	**5.6**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification

Table A 48: Characteristics for the analytical method 01313/M001 used for validation of CGA 279202, its three isomers CGA 357261, CGA 357262 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in raspberry (fruit)

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.
Calibration range	0.2 µg/L – 100 µg/L (corresponds to 0.0025 to 1.25 mg/kg)

Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound		
Assessment of matrix effects is not presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.		
	CGA 279202	**CGA 357261**	**CGA 357262**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.031459 x + 0.00028709, Correlation coefficient r: 0.9999 number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 0.039725 x + 0.00045138, Correlation coefficient r: 1.0000 number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 0.038044 x - 0.00058736, Correlation coefficient r: 1.0000 number of data points: 8
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 0.076309 x + 0.0013450, Correlation coefficient r: 0.9999 number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 0.10619 x - 0.0022025, Correlation coefficient r: 0.9999 number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 0.10448 x - 0.0039912, Correlation coefficient r: 1.0000 number of data points: 8

Conclusion

The analytical method 01313/M001 was fully validated during study M-448498-01-1 (Stuke, S.; Teubner, L.; 2013). For the matrices relevant to this study (raspberry/fruit) limited sets of validation recoveries were analysed within the course of the present study. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01313/M001 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on raspberry (fruit) via HPLC-MS/MS.

A 2.2.1.1.15 Analytical method 01313/M001 in support of the study M-448916-02-1

A 2.2.1.1.15.1 Method validation

Comments of zRMS:	Method 01313: Full validation data is documented with the method 01313 itself for matrices representing 5 major crop groups, including corn (green material), rape (seed), wheat (grain), kidney bean and orange (fruit). For the matrices relevant to this study (chicory, witloof) but not included in the original validation, a limited set (3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of study 11-2140. The limits of quantitation (LOQ) for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 are 0.01 mg/kg, corresponding to the lowest fortification level of successfully conducted recovery experiments. Blank values in control samples were below 30% of the LOQ for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%. No residues above the LOQ were found in the control samples. All method validation data are in compliance with the guideline requirements for data collection methods. The validation of method 01313 can therefore be considered successful for the new matrix (chicory – root and leaf). The study is acceptable.

Reference:	**KCP 5.1.2.5/28**
Title:	Amendment No.1 - Determination of the residues of AE C656948 and trifloxystrobin in/on chicory, witloof after dip and spraying of fluopyram SC 500 and AE C656948 & CGA279202 SC 500 in the field and room, hall, store, etc. in Germany, Belgium, northern France and the Netherlands
Report:	Fargeix, G.; 2013; 11-2140; M-448916-02-1
Authority registration No:	
Guideline(s):	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8; Residues in or on Treated Products, Food and Feed; EC Guidance working document 7029/VI/95 rev.5 (1997-07-22); US EPA OCSPP Guideline No. 860.1500.SUPP
Deviations:	see page 16
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods
The purpose of the study 11-2140 was to determine the magnitude of the relevant residues of AE C656948 (comprising AE C656948 and AE C656948-benzamide) and, trifloxystrobin (CGA 279202) (comprising CGA 279202, CGA 321113, CGA 331409, CGA 357262, CGA 357261 and CGA 373466) in/on chicory, witloof (leaf and root). In the following part, only the validation for the analysis of CGA 279202, its isomers CGA 331409, CGA 357262, CGA 357261 and the metabolite CGA 321113 and its isomer CGA 373466 is presented.
Full validation data is documented with the method 01313 itself (Stuke, S.; Bauer, J.; 2011; M-411496-02-1) for matrices representing 5 major crop groups, including corn (green material), rape (seed), wheat (grain), kidney bean and orange (fruit). For the matrices relevant to this study but not included in the original validation, a limited set (3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of the present study.
Residues of trifloxystrobin, CGA 357261, CGA 357262, CGA 331409, CGA 321113 and CGA 373466 are extracted from 5 g of sample material by using a high speed blender with a mixture of acetonitrile/water (80/20). The extract pH is adjusted to 6 by addition of 1 mL of ammonium acetate solution (1 mol/L). After filtration and dilution, the extract was quantified by reversed phase HPLC with Electrospray and MS/MS detection (API 4000 system, AB MDS Sciex Instruments, software Analyst 1.5.1). The quantification is carried out by external standardization using matrix-matched standard.

Conditions used in this study:
The chromatographic system used was: a high performance liquid chromatograph with a reversed phase chromatography (on a Ascentis Express C18 column) coupled with tandem mass spectrometry (MS/MS) with Electrospray ionisation (Applied Biosystems API 5500 Q-Trap Mass Spectrometer, software Analyst version 1.5.2.). The quantification is carried out by external standardization using matrix-matched standards. To reduce the number of injection, an injection standard is used.

Results and discussions
Limited sets of validation recoveries were analysed and recovery rates were determined at fortification levels of 0.01 mg/kg and 0.10 mg/kg for each analyte and matrix.

The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries.

The average recoveries were within the acceptable range of 70 – 110% except for: Trifloxystrobin at the LOQ level for Chicory, Witloof/Leaf (111%); CGA 331409 at the LOQ level for Chicory, Witloof/Leaf (114%); CGA 331409 at the LOQ level for Chicory, Witloof/Root (115%); CGA 357262 at the LOQ level for Chicory, Witloof/Leaf (112%); CGA 357262 at the LOQ level for Chicory, Witloof/Root (116%). Wherever applicable ($n \geq 3$), the RSD values were below 20%.

Table A 49: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method validation

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	chicory, witloof (leaf)	0.01	116; 112; 109	112	3.1	0.01
		0.10	101; 107; 104	104	2.9	
			Overall recovery (n = 6)	**108**	**5.0**	
	chicory, witloof (root)	0.01	103; 110; 108	107	3.4	0.01
		0.10	96; 98; 99	98	1.6	
			Overall recovery (n = 6)	**102**	**5.6**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357261	chicory, witloof (leaf)	0.01	101; 113; 103	106	6.1	0.01
		0.10	100; 107; 105	104	3.5	
			Overall recovery (n = 6)	**105**	**4.5**	
	chicory, witloof (root)	0.01	100; 91; 97	96	4.8	0.01
		0.10	95; 102; 100	99	3.6	
			Overall recovery (n = 6)	**98**	**4.1**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357262	chicory, witloof (leaf)	0.01	109; 107; 107	108	1.1	0.01
		0.10	102; 108; 107	106	3.0	
			Overall recovery (n = 6)	**107**	**2.3**	
	chicory, witloof (root)	0.01	112; 115; 120	116	3.5	0.01
		0.10	95; 105; 101	100	5.0	
			Overall recovery (n = 6)	**108**	**8.6**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 331409	chicory, witloof (leaf)	0.01	114; 116; 108	113	3.7	0.01
		0.10	103; 112; 107	107	4.2	
			Overall recovery (n = 6)	**110**	**4.4**	
	chicory, witloof (root)	0.01	116; 112; 114	114	1.8	0.01
		0.10	100; 107; 105	104	3.5	
			Overall recovery (n = 6)	**109**	**5.6**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 321113	chicory, witloof (leaf)	0.01	113; 110; 103	109	4.7	0.01
		0.10	94; 98; 97	96	2.2	
			Overall recovery (n = 6)	**103**	**7.4**	
	chicory, witloof (root)	0.01	106; 104; 93	101	6.9	0.01
		0.10	89; 99; 90	93	5.9	

			Overall recovery (n = 6)	**97**	**7.5**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 373466	chicory, witloof (leaf)	0.01	95; 117; 93	102	13.1	0.01
		0.10	94; 95; 96	95	1.1	
			Overall recovery (n = 6)	**98**	**9.4**	
	chicory, witloof (root)	0.01	112; 94; 101	102	8.9	0.01
		0.10	88; 90; 92	90	2.2	
			Overall recovery (n = 6)	**96**	**9.3**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for twelve study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

Table A 50: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method performance

Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 279202	chicory, witloof (leaf)	0.01	108; 109; 112; 116	111	3.2	0.01
		0.10	97; 101; 104; 107	102	4.2	
		0.80	86	-	-	
			Overall recovery (n = 9)	**104**	**8.6**	
	chicory, witloof (root)	0.01	100; 103; 108; 110	105	4.3	0.01
		0.10	95; 96; 98; 99	97	1.9	
		0.80	96	-	-	
			Overall recovery (n = 9)	**101**	**5.4**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357261	chicory, witloof (leaf)	0.01	101; 103; 110; 113	107	5.3	0.01
		0.10	98; 100; 105; 107	103	4.1	
		0.80	85	-	-	
			Overall recovery (n = 9)	**102**	**7.9**	
	chicory, witloof (root)	0.01	91; 97; 99; 100	97	4.2	0.01
		0.10	95; 97; 100; 102	99	3.2	
		0.80	95	-	-	
			Overall recovery (n = 9)	**97**	**3.4**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357262	chicory, witloof (leaf)	0.01	107; 107; 109; 125	112	7.8	0.01
		0.10	102; 104; 107; 108	105	2.6	
		0.80	88	-	-	
			Overall recovery (n = 9)	**106**	**8.9**	

	chicory, witloof (root)	0.01	112; 115; 115; 120	116	2.9	0.01
		0.10	95; 98; 101; 105	100	4.3	
		0.80	100	-	-	
			Overall recovery (n = 9)	**107**	**8.3**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 331409	chicory, witloof (leaf)	0.01	108; 114; 116; 119	114	4.1	0.01
		0.10	103; 107; 109; 112	108	3.5	
		0.80	92	-	-	
			Overall recovery (n = 9)	**109**	**7.4**	
	chicory, witloof (root)	0.01	112; 114; 116; 119	115	2.6	0.01
		0.10	100; 102; 105; 107	104	3.0	
		0.80	103	-	-	
			Overall recovery (n = 9)	**109**	**6.2**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 321113	chicory, witloof (leaf)	0.01	103; 104; 110; 113	108	4.5	0.01
		0.10	92; 94; 97; 98	95	2.9	
		0.80	87	-	-	
			Overall recovery (n = 9)	**100**	**8.5**	
	chicory, witloof (root)	0.01	93; 94; 104; 106	99	6.8	0.01
		0.10	85; 89; 90; 99	91	6.5	
		0.80	93	-	-	
			Overall recovery (n = 9)	**95**	**7.3**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 373466	chicory, witloof (leaf)	0.01	93; 95; 106; 117	103	10.8	0.01
		0.10	94; 95; 96; 97	96	1.4	
		0.80	83	-	-	
			Overall recovery (n = 9)	**97**	**9.7**	
	chicory, witloof (root)	0.01	94; 101; 102; 112	102	7.2	0.01
		0.10	86; 88; 90; 92	89	2.9	
		0.80	92	-	-	
			Overall recovery (n = 9)	**95**	**8.7**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 51: Characteristics for the analytical method 01313 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on chicory, witloof (root and leaf)

Specificity	For each compound and each matrix: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound in each matrix
Assessment of matrix effects is presented	Matrix matched standards were used.

	CGA 279202 in chicory (root)	**CGA 279202 in chicory (leaf)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 780600 x + 4475.8, Correlation coefficient r: 0.9991 number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 825910 x + 1003.9, Correlation coefficient r: 0.9996 number of data points: 7
Calibration range	0.02 µg/L - 10.0 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg)	0.02 µg/L - 10.0 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg)
	CGA 357261 in chicory (root)	**CGA 357261 in chicory (leaf)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 967630 x + 2967.7, Correlation coefficient r: 0.9984 number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 1008200 x – 2089.2, Correlation coefficient r: 0.9991 number of data points: 8
Calibration range	0.02 µg/L - 10.0 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg)	0.02 µg/L - 10.0 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg)
	CGA 357262 in chicory (root)	**CGA 357262 in chicory (leaf)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 844600 x – 1519.6, Correlation coefficient r: 0.9990 number of data points: 8	Individual calibration data is presented, calibration equation (1/x weighted): y = 904860 x - 13188, Correlation coefficient r: 0.9993 number of data points: 7
Calibration range	0.02 µg/L - 10.0 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg)	0.05 µg/L – 10.0 µg/L (corresponds to 0.005 mg/kg – 10 mg/kg)
	CGA 331409 in chicory (root)	**CGA 331409 in chicory (leaf)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 562810 x – 584.86, Correlation coefficient r: 0.9996 number of data points: 7	Individual calibration data is presented, calibration equation (1/x weighted): y = 638200 x – 936.69, Correlation coefficient r: 0.9993 number of data points: 5
Calibration range	0.02 µg/L - 10.0 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg)	0.02 µg/L - 10.0 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg)
	CGA 321113 in chicory (root)	**CGA 321113 in chicory (leaf)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 80984 x + 826.61, Correlation coefficient r: 0.9995 number of data points: 7	Individual calibration data is presented, calibration equation (1/x weighted): y = 83320 x + 892.72, Correlation coefficient r: 0.9997 number of data points: 7
Calibration range	0.02 µg/L - 10.0 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg)	0.05 µg/L – 10.0 µg/L (corresponds to 0.005 mg/kg – 1 mg/kg)
	CGA 373466 in chicory (root)	**CGA 373466 in chicory (leaf)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 80271 x + 62.854, Correlation coefficient r: 0.9993 number of data points: 8	Individual calibration data is presented, calibration equation (1/x weighted): y = 81010 x – 35.879, Correlation coefficient r: 0.9997 number of data points: 8
Calibration range	0.02 µg/L - 10.0 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg)	0.02 µg/L - 10.0 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg)

Conclusion

The analytical method 01313 was fully validated during study M-411496-02-1 (Stuke, S.; Bauer, J.; 2011). For the matrices relevant to this study (chicory, witloof (root and leaf)) but not included in the original validation, a limited set (3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of the present study. The criteria according to SANCO/3029/99 rev. 4 were met with the minor exception of the accuracy data. Not all mean recovery values were within the acceptable range of 70 – 110%. But as the values are close to 110% and the corresponding RSD values very low (< 5%), the results are considered to be acceptable. In addition, the overall mean recoveries for each matrix and analyte are within the acceptable range. Therefore, the analytical method 01313 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on chicory, witloof (root and leaf) via HPLC-MS/MS.

A 2.2.1.1.16 Analytical method 01313 in support of the study M-425357-01-1

A 2.2.1.1.16.1 Method validation

Comments of zRMS:	Residues of CGA 279202, its isomers and its metabolites (CGA 357262, CGA 357261, CGA 331409, CGA 321113 and CGA 373466), were determined by HPLC-MS/MS according to method 01313. The Limit of Quantification (LOQ) defined as the lowest validated fortification level, was 0.01 mg/kg for all analytes in/on the kidney bean matrices. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%. No residues above the LOQ were found in the control samples. All method validation data are in compliance with the guideline requirements for data collection methods. The validation of method 01313 can therefore be considered successful for kidney bean – pod and green material. The study is acceptable.

Reference:	**KCP 5.1.2.5/29**
Title:	Determination of the residues of AE C656948 and trifloxystrobin in/on bean, kidney after spraying of AE C656948 & CGA279202 SC 500 in the field in Germany, Belgium, Spain, Italy, France (south) and Portugal
Report:	Noss, G.; Ballmann, C.; 2012; 10-2125; M-425357-01-1
Authority registration No:	
Guideline(s):	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22) EPA OCSPP Guideline Number 860.1500
Deviations:	Not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the study 10-2125 was to determine the magnitude of the relevant residues of fluopyram (comprising fluopyram, AE C656948-pyridyl-acetic acid, AE C656948-benzamide and AE C656948-pyridyl-carboxylic acid) and trifloxystrobin (CGA 279202) (comprising CGA 279202, CGA 321113, CGA 357261, CGA 357262, CGA 331409 and CGA 373466) in/on a specific variety of kidney beans (green material and pod), after two spray applications with Fluopyram & Trifloxystrobin SC 500, a SC formulation containing 250 g/L trifloxystrobin and 250 g/L AE C656948.

Full validation data is documented with the method 01313 itself (Stuke, S.; Bauer, J.; 2011; M-411496-02-1) for matrices representing 5 major crop groups, including corn (green material), rape (seed), wheat (grain), kidney bean and orange (fruit). For the matrices relevant to this study (kidney bean (green material and pod)) but not included in the original validation, a limited set (one control, 3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of the present study to confirm the method.

Residues of these compounds were extracted from 5 g of sample material by extraction using a high speed blender with a mixture of acetonitrile/water (80/20, v/v, 40 mL). After adding celite, the extract was filtered, then the pH was adjusted to 6 with ammonium acetate and the volume was adjusted to 100 mL with acetonitrile/water (80/20, v/v). An aliquot was then diluted with water/methanol (80/20, v/v) prior to quantification by high performance liquid chromatograph using Ascentis Express C18 50 mmx2.1mm, i.d. 2.7 µm column coupled with tandem mass spectrometry (MS/MS) with Electrospray ionisation (Applied Biosystems API 4000 or API5500 QTRAP, Analyst version 1.5.1.): One injection in positive electrospray ionization allowed the determination of all the analytes (transitions monitored for quantification: CGA 279202 in pod 1st MRM m/z: 409 → 186 or 3rd MRM m/z: 409 → 206, CGA 279202 in green material 1st MRM m/z: 409 → 186, CGA 357261 in pod and green material 2nd MRM m/z 409 → 116, CGA 357262 in pod and green material 1st MRM m/z 409 → 206, CGA 331409 in pod and green material 1st MRM m/z 409 → 186, CGA 321113 in pod and green material 1st MRM m/z 395 → 186, CGA 373466 in pod and green material 1st MRM m/z 395 → 148).

Conditions used in this study:
No modification was made to original method, except that no pre-heating was used for the mobile phase during the chromatography phase.
The quantitation was done by external standardisation in solvent.

Results and discussions
The analysis of one control sample, three specimens fortified at the limit of quantification and three specimens fortified at 10 times the limit of quantification were taken through the method.
Residues in control samples were found to be lower than 30% of the LOQ, and acceptable mean recoveries between 70% and 110% with a relative standard deviation lower than 20% were found for trifloxyxtrobin and its metabolites for the three corresponding MRM transitions. Single recoveries were between 60 and 120%.
No matrix effect was determined as matrix matched standards were used.
Due to the unsufficient HPLC resolution of Trifloxystrobin and CGA357262, the contribution of CGA357262 in the Trifloxystrobin peak was tested and found to be 1% of Trifloxystrobin. Because for both compounds the recoveries are in acceptable ranges this error is negligible.

Table A 52: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method validation

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Green material (1st MRM)	Blank	ND	-	-	0.01
		Control	ND	-	-	
		0.01	98, 95, 94	96	2.0	
		0.10	106, 103, 110	106	2.9	
			Overall recovery (n = 6)	**101**	**6.3**	
	Pod (1st MRM)	Blank	ND	-	-	0.01
		Control	ND	-	-	
		0.01	93, 87, 91	90	3.0	
		0.10	101, 101, 105	102	2.6	
			Overall recovery (n = 6)	**96**	**7.3**	
	Pod (3rd MRM)	Blank	ND	-	-	
		Control	ND	-	-	
		0.01	85, 89, 85	87	2.7	
		0.10	91, 90, 94	92	2.1	
			Overall recovery (n = 6)	**89**	**3.8**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357261	Green material (2nd MRM)	Blank	ND	-	-	0.01
		Control	ND	-	-	
		0.01	97, 82, 91	90	8.8	
		0.10	94, 97, 99	97	2.4	
			Overall recovery (n = 6)	**93**	**6.8**	
	Pod (2nd MRM)	Blank	ND	-	-	0.01
		Control	ND	-	-	

		0.01	85, 93, 91	90	4.9	
		0.10	94, 91, 94	93	1.8	
			Overall recovery (n = 6)	**91**	**3.9**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357262	Green material (1st MRM)	Blank	ND	-	-	0.01
		Control	ND	-	-	
		0.01	95, 92, 90	92	2.7	
		0.10	102, 102, 110	104	4.4	
			Overall recovery (n = 6)	**98**	**7.5**	
	Pod (1st MRM)	Blank	ND	-	-	0.01
		Control	ND	-	-	
		0.01	87, 87, 86	86	0.7	
		0.10	97, 96, 102	98	3.2	
			Overall recovery (n = 6)	**92**	**7.5**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 331409	Green material (1st MRM)	Blank	ND	-	-	0.01
		Control	ND	-	-	
		0.01	91, 86, 84	87	3.8	
		0.10	88, 86, 93	89	4.0	
			Overall recovery (n = 6)	**88**	**3.8**	
	Pod (1st MRM)	Blank	ND	-	-	0.01
		Control	ND	-	-	
		0.01	84, 81, 84	83	1.7	
		0.10	86, 86, 90	87	2.9	
			Overall recovery (n = 6)	**85**	**3.6**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 321113	Green material (1st MRM)	Blank	ND	-	-	0.01
		Control	ND	-	-	
		0.01	89, 83, 93	88	5.4	
		0.10	89, 89, 90	89	0.9	
			Overall recovery (n = 6)	**89**	**3.5**	
	Pod (1st MRM)	Blank	ND	-	-	0.01
		Control	ND	-	-	
		0.01	84, 94, 85	88	6.6	
		0.10	84, 89, 91	88	4.1	
			Overall recovery (n = 6)	**88**	**4.9**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
		Blank	ND	-	-	0.01

CGA 373466	Green material (1st MRM)	Control	ND	-	-	
		0.01	96, 97, 96	97	0.7	
		0.10	88, 94, 92	91	3.4	
			Overall recovery (n = 6)	**94**	**3.9**	
	Pod (1st MRM)	Blank	ND	-	-	0.01
		Control	ND	-	-	
		0.01	86, 95, 90	90	5.1	
		0.10	86, 87, 90	88	1.8	
			Overall recovery (n = 6)	**89**	**3.8**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation; ND = not detectable Residue (< 30% LOQ)

In order to check the performance of the method, recovery determinations were included in each set of analysis (at least one recovery for ten study samples). Control samples from this study were fortified for the use as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.
The apparent residues in the control sample used for fortification were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20% with two exceptions for pod (for CGA 331409 and CGA 357262). Since all single values were in an acceptable range and the overall RSD (%) for pod and green material was in an acceptable range for all analytes, the analytical series were considered valid.
For the calculation of the mean values and RSDs rounded values were used. Therefore, minor deviations may occur when the values given in this recovery tables are compared to the values given in the analytical phase report.

Table A 53: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method performance

Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 279202	bean, kidney / green material	0.01	95; 96	96	-	0.01
		1.0	97; 99	98	-	
			Overall recovery (n = 4)	**97**	**1.8**	
	bean, kidney / pod	0.01	83; 93; 97	91	7.9	0.01
		0.10	71	-	-	
		1.0	106	-	-	
		10	94	-	-	
			Overall recovery (n = 6)	**91**	**13.4**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357261	bean, kidney / green material	0.01	97	-	-	0.01
		1.0	94	-	-	
			Overall recovery (n = 2)	**96**	**-**	
	bean, kidney / pod	0.01	80; 98	89	-	0.01
		0.10	75	-	-	

		1.0	104	-	-	
			Overall recovery (n = 4)	**89**	**15.6**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357262	bean, kidney / green material	0.01	97	-	-	0.01
		1.0	95	-	-	
			Overall recovery (n = 2)	**96**	**-**	
	bean, kidney / pod	0.01	80; 102	91	-	0.01
		0.10	71	-	-	
		1.0	114	-	-	
			Overall recovery (n = 4)	**92**	**21.5***	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 331409	bean, kidney / green material	0.01	95	-	-	0.01
		1.0	82	-	-	
			Overall recovery (n = 2)	**89**	**-**	
	bean, kidney / pod	0.01	88; 108	98	-	0.01
		0.10	68	-	-	
		1.0	110	-	-	
			Overall recovery (n = 4)	**94**	**21.1***	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 321113	bean, kidney / green material	0.01	99	-	-	0.01
		1.0	90	-	-	
			Overall recovery (n = 2)	**95**	**-**	
	bean, kidney / pod	0.01	85; 89	87	-	0.01
		0.10	94	-	-	
		1.0	89	-	-	
			Overall recovery (n = 4)	**89**	**4.1**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 373466	bean, kidney / green material	0.01	92	-	-	0.01
		1.0	89	-	-	
			Overall recovery (n = 2)	**91**	**-**	
	bean, kidney / pod	0.01	85; 95	90	-	0.01
		0.10	96	-	-	
		1.0	92	-	-	
			Overall recovery (n = 4)	**92**	**5.4**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation; * Since all single values were in an acceptable range and the overall RSD (%) for pod was in an acceptable range, the analytical series was considered valid

During each set of analysis, a calibration curve was established with at least seven concentration levels and used for the quantitation, for each analyte/ and each sample material. For each calibration curve, the correlation coefficient R was above 0.99. All values for the linearity of response for each analyte in each matrix are given in the study. Example linearity graphs for the matrix pod are given.

Table A 54: Characteristics for the analytical method 01313 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on kidney bean (green material and pod)

Specificity	For each compound and matrix: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.		
Calibration range	0.00002 µg/mL – 0.0120 µg/mL (corresponds to 0.002 mg/kg – 1.2 mg/kg) for each compound and matrix		
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound and matrix		
Assessment of matrix effects is presented	No matrix effect was determined as matrix matched standards were used.		
	CGA 279202 (pod)	**CGA 357262 (pod)**	**CGA 357261 (pod)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 473037825 x + 1396, Correlation coefficient r: 0.9991 number of data points: 7	Individual calibration data is presented calibration equation (1/x weighted): y = 970428294 x + 5899, Correlation coefficient r: 0.9976 number of data points: 7	Individual calibration data is presented calibration equation (1/x weighted): y = 464022965 x + 843, Correlation coefficient r: 0.9992 number of data points: 7
	CGA 331409 (pod)	**CGA 321113 (pod)**	**CGA 373466 (pod)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 449837837 x + 525, Correlation coefficient r: 0.9992 number of data points: 7	Individual calibration data is presented calibration equation (1/x weighted): y = 188747457 x - 137, Correlation coefficient r: 0.9998 number of data points: 7	Individual calibration data is presented calibration equation (1/x weighted): y = 169499227 x + 212, Correlation coefficient r: 0.9996 number of data points: 7

Conclusion

Full validation data is documented with the method 01313 itself (Stuke, S.; Bauer, J.; 2011; M-411496-02-1) for matrices representing 5 major crop groups, including corn (green material), rape (seed), wheat (grain), kidney bean and orange (fruit). For the matrices relevant to this study (kidney bean (green material and pod)), a limited set (one control, 3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of the present study to confirm the method. The analytical method complies with all guideline criteria according to SANCO 3029/99 rev. 4 and SANCO/825/00 rev. 8.1 with the minor exception of the calibration data. All values for the linearity of response for each analyte in each matrix are given in the study, but only example linearity graphs for the matrix pod are presented. Nevertheless, it is confirmed that the correlation coefficient R was above 0.99 for all calibration curves. In addition, the provided accuracy and precision data clearly demonstrate that this method is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on kidney bean (green material and pod) via HPLC-MS/MS.

A 2.2.1.1.17 Analytical method 01313 in support of the study M-425362-02-1

A 2.2.1.1.17.1 Method validation

Comments of zRMS:	Method 01313: Full validation data is documented with the method 01313 itself for matrices representing 5 major crop groups, including corn (green material), rape (seed), wheat (grain), kidney bean and orange (fruit). For trifloxystrobin the method validation for pod was included in study 10-2125. In this case the sample material pod covers also the sample material green seed. The method

	performance was checked in the present study. The Limit of Quantification (LOQ) defined as the lowest validated fortification level, was 0.01 mg/kg for all analytes in/on the kidney bean matrices. No residues above the LOQ were found in the control samples, except for samples 10- 2128-01-0009E and 10-2128-01-0018E for CGA 331409 (0.01 mg/kg, in pod and seed). In order to check the performance of the method, recovery determinations were included in each set of analysis, but only one recovery for each fortification level. The mean of the concurrent recoveries were within the acceptable range of 70 - 110%. The study is acceptable.

Reference:	**KCP 5.1.2.5/30**
Title:	Determination of the residues of AE C656948 and trifloxystrobin in/on bean, kidney after spraying of AE C656948 & CGA279202 SC 500 in the field in France (north)
Report:	Noss, G.; Guerleyen, N.; Ballmann, C.; 2012; 10-2128; M-425362-02-1
Authority registration No:	
Guideline(s):	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations:	Not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the study 10-2128 was to determine the magnitude of the relevant residues of fluopyram (comprising fluopyram, AE C656948-pyridyl-acetic acid, AE C656948-benzamide and AE C656948-pyridyl-carboxylic acid) and trifloxystrobin (comprising trifloxystrobin, CGA 321113, CGA 357261, CGA 357262, CGA 331409 and CGA 373466) in/on a specific variety of kidney bean (pod and green seed) called flageolet, after two spray applications with Fluopyram & Trifloxystrobin SC 500, a SC formulation containing 250 g/L fluopyram and 250 g/L trifloxystrobin.

Full validation data is documented with the method 01313 itself (Stuke, S.; Bauer, J.; 2011; M-411496-02-1) for matrices representing 5 major crop groups, including corn (green material), rape (seed), wheat (grain), kidney bean and orange (fruit). For the matrix kidney bean, pod, the method validation is included in the study 10-2125 (Noss, G.; Ballmann, C.; 2012; M-425357-01-1). In this case the sample material pod covers also the sample material green seed relevant to the present study. For concurrent validation purposes, the method performance was checked in the present study.

Residues of these compounds were extracted from 5 g of sample material by extraction using a high speed blender with a mixture of acetonitrile/water (80/20, v/v, 40 mL). After adding celite, the extract was filtered, then the pH was adjusted to 6 with ammonium acetate and the volume was adjusted to 100 mL with acetonitrile/water (80/20, v/v). An aliquot was then diluted with water/methanol (80/20, v/v) prior to quantification by high performance liquid chromatograph using Ascentis Express C18 50mmx2.1mm i.d. 2.7µm column coupled with tandem mass spectrometry (MS/MS) with Electrospray ionisation (Applied Biosystems API 4000 or API5500 QTRAP, Analyst version 1.5.1.): One injection in positive electrospray ionization allowed the determination of all the analytes (transitions monitored for quantification: CGA 279202 in pod 3rd MRM m/z: 409 → 206, CGA 279202 in seeds 1st MRM m/z: 409 → 186, CGA 357261 in pod and seeds 2nd MRM m/z 409 → 116, CGA 357262 in pod and seeds 1st MRM m/z 409 → 206, CGA 331409 in pod and seeds 1st MRM m/z 409 → 186, CGA 321113 in pod and seeds 1st MRM m/z 395 → 186, CGA 373466 in pod and seeds 1st MRM m/z 395 → 148).

Conditions used in this study:

No modification was made to original method, except that no pre-heating was used for the mobile phase during the chromatography phase.

The quantitation was done in the matrices.

Results and discussions

In order to check the performance of the method, recovery determinations were included in each set of analysis (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The apparent residues in the control sample were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The average recoveries were within the acceptable range of 70 – 110%. The RSD value was below 20%.

For the calculation of the mean values and RSDs rounded values were used. Therefore, minor deviations may occur when the values given in this recovery tables are compared to the values given in the analytical phase report.

Table A 55: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Kidney bean / pod	0.01	90	-	-	0.01
		0.10	94	-	-	
			Overall recovery (n = 2)	**92**	**-**	
	Kidney bean / seed	0.01	88	-	-	0.01
		0.10	98	-	-	
			Overall recovery (n = 2)	**93**	**-**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357261	Kidney bean / pod	0.01	90	-	-	0.01
		0.10	94	-	-	
			Overall recovery (n = 2)	**92**	**-**	
	Kidney bean / seed	0.01	85	-	-	0.01
		0.10	94	-	-	
			Overall recovery (n = 2)	**90**	**-**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357262	Kidney bean / pod	0.01	100	-	-	0.01
		0.10	111	-	-	
			Overall recovery (n = 2)	**106**	**-**	
	Kidney bean / seed	0.01	87	-	-	0.01
		0.10	95	-	-	
			Overall recovery (n = 2)	**91**	**-**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 331409	Kidney bean / pod	0.01	95	-	-	0.01
		0.10	108	-	-	

			Overall recovery (n = 2)	**102**	**-**	
	Kidney bean / seed	0.01	80, 89	85	-	0.01
		0.10	85, 88	87	-	
			Overall recovery (n = 4)	**86**	**4.7**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 321113	Kidney bean / pod	0.01	96	-	-	0.01
		0.10	92	-	-	
			Overall recovery (n = 2)	**94**	**-**	
	Kidney bean / seed	0.01	99	-	-	0.01
		0.10	92	-	-	
			Overall recovery (n = 2)	**96**	**-**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 373466	Kidney bean / pod	0.01	91	-	-	0.01
		0.10	92	-	-	
			Overall recovery (n = 2)	**92**	**-**	
	Kidney bean / seed	0.01	87	-	-	0.01
		0.10	91	-	-	
			Overall recovery (n = 2)	**89**	**-**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 56: Characteristics for the analytical method 01313 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on kidney bean (pod and seeds)

Specificity	For each compound and matrix: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.	
Calibration range	0.00002 µg/mL – 0.0120 µg/mL (corresponds to 0.002 mg/kg – 1.2 mg/kg) for each compound and matrix	
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound and matrix	
Assessment of matrix effects is presented	No matrix effect was determined as matrix matched standards were used.	
	CGA 279202 (pod)	**CGA 279202 (seeds)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 473553650 x + 3081, Correlation coefficient r: 0.9985 number of data points: 7	Individual calibration data is presented, calibration equation (1/x weighted): y = 1293937687 x + 15721, Correlation coefficient r: 0.9975 number of data points: 7
	CGA 357262 (pod)	**CGA 357262 (seeds)**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 1154688774 x + 13257, Correlation coefficient r: 0.9979 number of data points: 7	Individual calibration data is presented calibration equation (1/x weighted): y = 923123692 x + 7987, Correlation coefficient r: 0.9979 number of data points: 7
	CGA 357261 (pod)	**CGA 357261 (seeds)**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 427912625 x + 3321,	Individual calibration data is presented calibration equation (1/x weighted): y = 399927207 x + 2388,

	Correlation coefficient r: 0.9983 number of data points: 7	Correlation coefficient r: 0.9999 number of data points: 7
	CGA 331409 **(pod)**	**CGA 331409** **(seeds)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 497975930 x + 4256, Correlation coefficient r: 0.9996 number of data points: 7	Individual calibration data is presented, calibration equation (1/x weighted): y = 248936763 x + 356, Correlation coefficient r: 0.9992 number of data points: 7
	CGA 321113 **(pod)**	**CGA 321113** **(seeds)**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 263021586 x - 294, Correlation coefficient r: 0.9998 number of data points: 7	Individual calibration data is presented calibration equation (1/x weighted): y = 264225320 x + 596, Correlation coefficient r: 0.9999 number of data points: 7
	CGA 373466 **(pod)**	**CGA 373466** **(seeds)**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 236205393 x - 400, Correlation coefficient r: 0.9998 number of data points: 7	Individual calibration data is presented calibration equation (1/x weighted): y = 232544693 x + 1322, Correlation coefficient r: 1.0000 number of data points: 7

Conclusion

Full validation data is documented with the method 01313 itself (Stuke, S.; Bauer, J.; 2011; M-411496-02-1) for matrices representing 5 major crop groups, including corn (green material), rape (seed), wheat (grain), kidney bean and orange (fruit). For the matrix kidney bean, pod, the method validation is included in the study 10-2125. In this case the sample material pod covers also the sample material green seed relevant to the present study. For concurrent validation purposes, the method performance was checked in the present study. The analytical method complies with all guideline criteria according to SANCO 3029/99 rev. 4 and SANCO/825/00 rev. 8.1 . The analytical method 01313 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on kidney bean (green material and pod) via HPLC-MS/MS.

A 2.2.1.1.18 Analytical method 01313 in support of the study M-444960-01-1

A 2.2.1.1.18.1 Method validation

Comments of zRMS:	Method 01313: Full validation data is documented with the method 01313 itself for matrices representing 5 major crop groups, including corn (green material), rape (seed), wheat (grain), kidney bean and orange (fruit). For the matrices relevant to this study (field pea - pod and dry seed) but not included in the original validation, a limited set (3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of study 11-2000. Residues of trifloxystrobin, stereo-isomers and metabolites were determined by HPLC-MS/MS. The quantification is carried out by external standardization using matrix-matched standard. The Limit of Quantification (LOQ) defined as the lowest validated fortification level, was 0.01 mg/kg for all analytes in/on the peas matrices. No residues above the LOQ were found in the control samples. The average recoveries were within the acceptable range of 70 – 110% except for: - Trifloxystrobin at the LOQ level in dry seed (116%) - CGA 331409 at the LOQ level in dry seed (118%) - CGA 357262 at the LOQ level in dry seed (112%) - CGA 321113 at the LOQ level in dry seed (111%). As residues in the study samples were all below the LOQ for dry seed, there is no major impact on the study results: RSD values are below 20%. All method validation data are in compliance with the guideline requirements for data collection methods. The validation of method 01313 can therefore be considered successful for the new matrix (field pea - pod and dry seed). The study is acceptable.

Reference:	**KCP 5.1.2.5/31**
Title:	Determination of the residues of fluopyram and trifloxystrobin in/on field pea after spray application of AE C656948 & CGA279202 SC 500 in northern France and Germany
Report:	Fargeix, G.; 2013; 11-2000; M-444960-01-1
Authority registration No:	
Guideline(s):	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed, EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) US EPA OCSPP Guideline No. 860.1500.SUPP
Deviations:	see page 146
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the study 11-2000 was to determine the magnitude of the relevant residues of AE C656948 (comprising AE C656948 and AE C656948-benzamide) and trifloxystrobin (CGA 279202) (comprising CGA 279202, CGA 321113, CGA 331409, CGA 357262, CGA 357261 and CGA 373466) in/on field pea (pod and dry seed) after two spraying applications with AE C656948 & CGA279202 SC 500, an SC formulation containing 250 g/L trifloxystrobin and 250 g/L AE C656948.

Full validation data is documented with the method 01313 itself (Stuke, S.; Bauer, J.; 2011; M-411496-02-1) for matrices representing 5 major crop groups, including corn (green material), rape (seed), wheat (grain), kidney bean and orange (fruit). For the matrices relevant to this study (field pea (pod and dry seed)) but not included in the original validation, a limited set (3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of the present study.

Residues of trifloxystrobin, CGA 357261, CGA 357262, CGA 331409, CGA 321113 and CGA 373466 are extracted from 5 g of sample material by using a high speed blender with a mixture of acetonitrile/water (80/20). The extract pH is adjusted to 6 by addition of 1 mL of ammonium acetate solution (1 mol/L). After filtration and dilution, the extract was quantified by reversed phase HPLC with Electrospray and MS/MS detection (API 4000 system, AB MDS Sciex Instruments, software Analyst 1.5.1).

Conditions used in this study:

The chromatographic system used was: a high performance liquid chromatograph with a reversed phase chromatography (on a Ascentis Express C18 column) coupled with tandem mass spectrometry (MS/MS) with Electrospray ionisation (Applied Biosystems API 5500 Q-Trap Mass Spectrometer, software Analyst version 1.5.2.).

The quantification is carried out by external standardization using matrix-matched standards. To reduce the number of injections, an injection standard is used.

Results and discussions

Limited sets of validation recoveries were analysed and recovery rates were determined at fortification levels of 0.01 mg/kg and 0.10 mg/kg for each analyte and matrix.

Blank values in control samples were below 30% of the LOQ for Trifloxystrobin parent compound as well as for the isomers / metabolites CGA 321113, CGA 331409, CGA 357262, CGA 357261 and CGA 373466.

The average recoveries were within the acceptable range of 70 – 110% except for: Trifloxystrobin at the LOQ level in dry seed (116%); CGA 331409 at the LOQ level in dry seed (118%); CGA 357262 at the LOQ level in dry seed (112%); CGA 321113 at the LOQ level in dry seed (111%). The RSD values were below 20%.

Table A 57: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method validation

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Field pea (pod)	0.01	105 ; 99 ; 98	101	3.8	0.01
		0.1	98 ; 104 ; 102	101	3.0	
			Overall recovery (n = 6)	**101**	**3.1**	
	Field pea (seed, dry)	0.01	115 ; 117 ; 114	115	1.3	0.01
		0.1	106 ; 105 ; 107	106	0.9	
			Overall recovery (n = 6)	**111**	**4.7**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357261	Field pea (pod)	0.01	95 ; 103 ;98	99	4.1	0.01
		0.1	90 ; 98 ; 100	96	5.5	
			Overall recovery (n = 6)	**97**	**4.6**	
	Field pea (seed, dry)	0.01	98 ; 96 ; 113	102	9.1	0.01
		0.1	101 ; 101 ; 104	102	1.7	
			Overall recovery (n = 6)	**102**	**5.9**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357262	Field pea (pod)	0.01	105 ; 103 ; 101	103	1.9	0.01
		0.1	97 ; 108 ; 102	102	5.4	
			Overall recovery (n = 6)	**103**	**3.6**	
	Field pea (seed, dry)	0.01	112 ; 110 ; 112	111	1.0	0.01
		0.1	107 ; 105 ; 107	106	1.1	
			Overall recovery (n = 6)	**109**	**2.7**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 331409	Field pea (pod)	0.01	106 ; 107 ; 99	104	4.2	0.01
		0.1	99 ; 108 ; 107	105	4.7	
			Overall recovery (n = 6)	**104**	**4.0**	
	Field pea (seed, dry)	0.01	114 ; 116 ; 123	118	4.0	0.01
		0.1	112 ; 109 ; 108	110	1.9	
			Overall recovery (n = 6)	**114**	**4.8**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 321113	Field pea (pod)	0.01	94 ; 95 ; 102	97	4.5	0.01
		0.1	88 ; 99 ; 96	94	6.0	
			Overall recovery (n = 6)	**96**	**5.0**	
	Field pea (seed, dry)	0.01	97 ; 124 ; 112	111	12.2	0.01
		0.1	100 ; 97 ;102	100	2.5	
			Overall recovery (n = 6)	**105**	**10.1**	

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 373466	Field pea (pod)	0.01	96 ; 104 ; 99	100	4.1	0.01
		0.1	91 ; 99 ; 97	96	4.4	
			Overall recovery (n = 6)	**98**	**4.4**	
	Field pea (seed, dry)	0.01	112 ; 96 ; 97	102	8.8	0.01
		0.1	104 ; 112 ; 106	107	3.9	
			Overall recovery (n = 6)	**105**	**6.7**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for twelve study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.
The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries.

Table A 58: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method performance

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Field pea (pod)	0.01	98; 99; 105; 116	105	7.9	0.01
		0.10	98; 102; 104	101	3.0	
		1.0	88	-	-	
			Overall recovery (n = 8)	**101**	**7.8**	
	Field pea (seed, dry)	0.01	114; 115; 117; 119	116	1.9	0.01
		0.10	105; 106; 107	106	0.9	
		1.0	105	-	-	
			Overall recovery (n = 8)	**111**	**5.3**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357261	Field pea (pod)	0.01	95; 98; 103; 105	100	4.6	0.01
		0.10	90; 98; 100	96	5.5	
		1.0	86	-	-	
			Overall recovery (n = 8)	**97**	**6.6**	
	Field pea (seed, dry)	0.01	96; 98; 104; 113	103	7.4	0.01
		0.10	101; 101; 104	102	1.7	
		1.0	103	-	-	
			Overall recovery (n = 8)	**103**	**5.0**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357262	Field pea (pod)	0.01	101; 103; 105; 119	107	7.6	0.01
		0.10	97; 102; 108	102	5.4	
		1.0	91	-	-	

			Overall recovery (n = 8)	**103**	**7.9**	
	Field pea (seed, dry)	0.01	110; 112; 112; 115	112	1.8	0.01
		0.10	105; 107; 107	106	1.1	
		1.0	104	-	-	
			Overall recovery (n = 8)	**109**	**3.5**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 331409	Field pea (pod)	0.01	99; 106; 107; 122	109	8.9	0.01
		0.10	99; 107; 108	105	4.7	
		1.0	92	-	-	
			Overall recovery (n = 8)	**105**	**8.4**	
	Field pea (seed, dry)	0.01	114; 116; 117; 123	118	3.3	0.01
		0.10	108; 109; 112	110	1.9	
		1.0	107	-	-	
			Overall recovery (n = 8)	**113**	**4.8**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 321113	Field pea (pod)	0.01	94; 95; 100; 102	98	4.0	0.01
		0.10	88; 96; 99	94	6.0	
		1.0	91	-	-	
			Overall recovery (n = 8)	**96**	**4.9**	
	Field pea (seed, dry)	0.01	97; 111; 112; 124	111	10.0	0.01
		0.10	97; 100; 102	100	2.5	
		1.0	100	-	-	
			Overall recovery (n = 8)	**105**	**9.0**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 373466	Field pea (pod)	0.01	96; 99; 104; 106	101	4.5	0.01
		0.10	91; 97; 99	96	4.4	
		1.0	81	-	-	
			Overall recovery (n = 8)	**97**	**8.1**	
	Field pea (seed, dry)	0.01	67; 96; 100; 112	94	20.4	0.01
		0.10	104; 106; 112	107	3.9	
		1.0	106	-	-	
			Overall recovery (n = 8)	**100**	**14.5**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 59: Characteristics for the analytical method 01313 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on field pea (pod and dry seed)

Specificity	For each compound and each matrix: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30% of the respective LOQ.
Calibration range	0.02 µg/L - 10.0 µg/L (corresponds to 0.002 mg/kg – 1.0 mg/kg) for each compound and matrix

Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound in each matrix	
Assessment of matrix effects is presented	Matrix matched standards were used.	
	CGA 279202 in field pea (pod)	**CGA 279202 in field pea (dry seed)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 888570 x + 4319.5, Correlation coefficient r: 0.9982 number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 744090 x + 2403.7, Correlation coefficient r: 0.9999 number of data points: 8
	CGA 357261 in field pea (pod)	**CGA 357261 in field pea (dry seed)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 774390 x – 659.95, Correlation coefficient r: 0.9986 number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 730550 x – 89.440, Correlation coefficient r: 0.9999 number of data points: 8
	CGA 357262 in field pea (pod)	**CGA 357262 in field pea (dry seed)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 998760 x + 1172.1, Correlation coefficient r: 0.9986 number of data points: 8	Individual calibration data is presented, calibration equation (1/x weighted): y = 792790 x + 205.01, Correlation coefficient r: 0.9997 number of data points: 7
	CGA 331409 in field pea (pod)	**CGA 331409 in field pea (dry seed)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 757760 x + 6805.0, Correlation coefficient r: 0.9981 number of data points: 8	Individual calibration data is presented, calibration equation (1/x weighted): y = 582600 x + 4035.8, Correlation coefficient r: 0.9995 number of data points: 8
	CGA 321113 in field pea (pod)	**CGA 321113 in field pea (dry seed)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 113170 x + 442.08, Correlation coefficient r: 0.9992 number of data points: 8	Individual calibration data is presented, calibration equation (1/x weighted): y = 79065 x + 16.678, Correlation coefficient r: 0.9997 number of data points: 8
	CGA 373466 in field pea (pod)	**CGA 373466 in field pea (dry seed)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 103630 x – 26.050, Correlation coefficient r: 0.9980 number of data points: 8	Individual calibration data is presented, calibration equation (1/x weighted): y = 75246 x + 541.00, Correlation coefficient r: 0.9996 number of data points: 8

Conclusion

The analytical method 01313 was fully validated during study M-411496-02-1 (Stuke, S.; Bauer, J.; 2011). For the matrices relevant to this study (field pea (pod and dry seed)) but not included in the original validation, a limited set (3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of the present study. The criteria according to SANCO/3029/99 rev. 4 were met with the minor exception of the accuracy data. Not all mean and overall mean recovery values were within the acceptable range of 70 – 110%. But as the values are close to 110% and the corresponding RSD values very low (< 5%), the results are considered to be acceptable. Therefore, the analytical method 01313 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on field pea (pod and dry seed) via HPLC-MS/MS.

A 2.2.1.1.19 Analytical method 01313/M001 in support of the study M-467728-01-1

A 2.2.1.1.19.1 Method validation

Comments of zRMS:	Full validation data is documented with the method 01313/M001 itself for matrices representing 5 commodity categories. For the matrices relevant to this study but not included in the original validation, a limited set (3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of study 12-2030.

	The limits of quantitation (LOQ) for trifloxystrobin and its isomers / metabolites are 0.01 mg/kg, for all sample materials tested, corresponding to the lowest fortification level of successfully conducted recovery experiments. Blank values in control samples were below 30% of the LOQ for Trifloxystrobin parent compound as well as for the metabolites. The average recoveries were within the acceptable range of 70 – 110% and the RSD values were below 20%. All method validation data are in compliance with the guideline requirements for data collection methods. The validation of method 01313/M001 can therefore be considered successful for the new matrix.

Reference:	**KCP 5.1.2.5/32**
Title:	Determination of the residues of AE C656948 and trifloxystrobin in/on French bean after spray application of AE C656948 & CGA279202 SC 500 in the field in Germany and northern France
Report:	Glaubitz, J.; 2013; 12-2030; M-467728-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, EC Guidance working document 7029/VI/95 rev.5 (1997-07-22), OECD 509 Adopted 2009-09-07 OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial, US EPA US EPA OCSPP Guideline No. 860.1500
Deviations:	not specified
GLP/GEP:	~~No~~ yes
Acceptability:	Yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the study 12-2030 was to determine the magnitude of the relevant residues of trifloxystrobin (CGA 279202) (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) and AE C656948 (comprising AE C656948 and AE C656948-benzamide) in/on French bean (green material and pod) after two spray applications with AE C656948 & CGA279202 SC 500 (SC 500), a SC formulation containing 250 g/L trifloxystrobin and 250 g/L AE C656948.

Full validation data is documented with the method 01313/M001 itself (Stuke, S.; Teubner, L.; 2013; M-448498-01-1) for matrices representing 5 commodity categories. For the matrices relevant to this study but not included in the original validation (french bean (pod and green material)), a limited set (3 repetitions each at two fortification levels) of additional validation recoveries was analyzed within the course of the present study.

Residues of trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 were extracted from the homogenized sample material with a mixture of acetonitrile/water (4/1; v/v) by shaking. After filtration, addition of ammonium acetate solution to adjust the pH value and addition of internal standard solution the extracts were made up to volume, diluted, filtered and subjected to HPLC-MS/MS measurement (ESI positive, MRMs: CGA 279202 m/z: 409 → 145, CGA 357261 m/z 409 → 206, CGA 357262 m/z 409 → 206, CGA 331409 m/z 409 → 206, CGA 321113 m/z 395 → 186, CGA 373466 m/z 395 → 148). The quantification was done by external standardisation in pure solvent, using the analytical standard together with isotopically labeled internal standard for isotopic dilution analysis.

Results and discussions

Limited sets of validation recoveries were analysed and recovery rates were determined at fortification levels of 0.01 mg/kg and 0.10 mg/kg for each analyte and matrix. The limits of quantitation (LOQ) for CGA 279202 and its isomers / metabolites are 0.01 mg/kg, for all sample materials tested, corresponding to the lowest fortification level of successfully conducted recovery experiments.

Blank values in control samples were below 30% of the LOQ for all analytes. The average recoveries were within the acceptable range of 70 – 110% and the RSD values were below 20%.

Table A 60: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method validation

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	French bean / green material	0.01	69; 83; 86	79	11.4	0.01
		0.10	73; 75; 81	76	5.5	
			Overall recovery (n = 6)	**78**	**8.4**	
	French bean / pod	0.01	77; 83; 89	83	7.2	0.01
		0.10	82; 89; 91	87	5.4	
			Overall recovery (n = 6)	**85**	**6.3**	
CGA 357261	French bean / green material	0.01	68; 76; 80	75	8.2	0.01
		0.10	71; 72; 82	75	8.1	
			Overall recovery (n = 6)	**75**	**7.3**	
	French bean / pod	0.01	75; 77; 85	79	6.7	0.01
		0.10	76; 80; 82	79	3.9	
			Overall recovery (n = 6)	**79**	**4.9**	
CGA 357262	French bean / green material	0.01	87; 93; 96	92	5.0	0.01
		0.10	75; 82; 84	80	5.9	
			Overall recovery (n = 6)	**86**	**8.9**	
	French bean / pod	0.01	80; 93; 107	93	14.5	0.01
		0.10	87; 89; 98	91	6.4	
			Overall recovery (n = 6)	**92**	**10.2**	
CGA 331409	French bean / green material	0.01	71; 74; 78	74	4.7	0.01
		0.10	75; 75; 75	75	0.0	
			Overall recovery (n = 6)	**75**	**3.0**	
	French bean / pod	0.01	69; 81; 81	77	9.0	0.01
		0.10	82; 86; 96	88	8.2	
			Overall recovery (n = 6)	**83**	**10.6**	
CGA 321113	French bean / green material	0.01	73; 81; 81	78	5.9	0.01
		0.10	72; 75; 80	76	5.3	
			Overall recovery (n = 6)	**77**	**5.4**	
	French bean / pod	0.01	67; 72; 75	71	5.7	0.01
		0.10	71; 76; 77	75	4.3	
			Overall recovery (n = 6)	**73**	**5.1**	
CGA 373466	French bean / green material	0.01	75; 80; 81	79	4.1	0.01
		0.10	77; 78; 81	79	2.6	
			Overall recovery (n = 6)	**79**	**3.1**	
	French bean / pod	0.01	74; 76; 78	76	2.6	0.01
		0.10	74; 78; 78	77	3.0	
			Overall recovery (n = 6)	**76**	**2.6**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples.
The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

Table A 61: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method performance

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	French bean / green material	0.01	69; 83; 86	79	11.4	0.01
		0.10	73; 75; 81	76	5.5	

		0.20	80	-	-	
		1.0	78	-	-	
		10	88	-	-	
			Overall recovery (n = 9)	**79**	**7.8**	
	French bean / pod	0.01	77; 83; 89	83	7.2	0.01
		0.10	82; 89; 91	87	5.4	
		0.50	67	-	-	
			Overall recovery (n = 7)	**83**	**10.2**	
CGA 357261	French bean / green material	0.01	68; 76; 80	75	8.2	0.01
		0.10	71; 72; 82	75	8.1	
		0.20	73	-	-	
			Overall recovery (n = 7)	**75**	**6.7**	
	French bean / pod	0.01	75; 77; 85	79	6.7	0.01
		0.10	76; 80; 82	79	3.9	
		0.50	70	-	-	
			Overall recovery (n = 7)	**78**	**6.4**	
CGA 357262	French bean / green material	0.01	87; 93; 96	92	5.0	0.01
		0.10	75; 82; 84	80	5.9	
		0.20	79	-	-	
			Overall recovery (n = 7)	**85**	**8.8**	
	French bean / pod	0.01	80; 93; 107	93	14.5	0.01
		0.10	87; 89; 98	91	6.4	
		0.50	73	-	-	
			Overall recovery (n = 7)	**90**	**12.6**	
CGA 331409	French bean / green material	0.01	71; 74; 78	74	4.7	0.01
		0.10	75; 75; 75	75	0.0	
		0.20	71	-	-	
			Overall recovery (n = 7)	**74**	**3.3**	
	French bean / pod	0.01	69; 81; 81	77	9.0	0.01
		0.10	82; 86; 96	88	8.2	
		0.50	69	-	-	
			Overall recovery (n = 7)	**81**	**11.7**	
CGA 321113	French bean / green material	0.01	73; 81; 81	78	5.9	0.01
		0.10	72; 75; 80	76	5.3	
		0.20	71	-	-	
			Overall recovery (n = 7)	**76**	**5.8**	
	French bean / pod	0.01	67; 72; 75	71	5.7	0.01
		0.10	71; 76; 77	75	4.3	
		0.50	71	-	-	
			Overall recovery (n = 7)	**73**	**4.8**	
CGA 373466	French bean / green material	0.01	75; 80; 81	79	4.1	0.01
		0.10	77; 78; 81	79	2.6	
		0.20	71	-	-	
			Overall recovery (n = 7)	**78**	**4.7**	
	French bean / pod	0.01	74; 76; 78	76	2.6	0.01
		0.10	74; 78; 78	77	3.0	
		0.50	73	-	-	
			Overall recovery (n = 7)	**76**	**2.9**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 62: Characteristics for the analytical method 01313/M001 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on French bean (pod and green material)

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.		
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound		
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.		
	CGA 279202	**CGA 357261**	**CGA 357262**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation:	Individual calibration data is presented calibration equation:	Individual calibration data is presented calibration equation:

	y = 0.56339 x + 0.0079926, Correlation coefficient r: 0.9997, number of data points: 5	y = 0.59989 x + 0.0041805, Correlation coefficient r: 0.9994, number of data points: 5	y = 0.54795 x + 0.0020525, Correlation coefficient r: 0.9995, number of data points: 5
Calibration range	0.019 µg/L – 4.9 µg/L (corresponds to 0.00019 mg/kg – 0.049 mg/kg)	0.019 µg/L – 2.0 µg/L (corresponds to 0.00019 mg/kg – 0.02 mg/kg)	0.019 µg/L – 2.0 µg/L (corresponds to 0.00019 mg/kg – 0.02 mg/kg)
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation: y = 1.1285 x + 0.0090805, Correlation coefficient r: 0.9991, number of data points: 5	Individual calibration data is presented calibration equation: y = 0.60063 x + 0.0039267, Correlation coefficient r: 0.9993, number of data points: 5	Individual calibration data is presented calibration equation: y = 0.95645 x + 0.0078252, Correlation coefficient r: 0.9984, number of data points: 5
Calibration range	0.019 µg/L – 2.0 µg/L (corresponds to 0.00019 mg/kg – 0.02 mg/kg)	0.018 µg/L – 1.9 µg/L (corresponds to 0.00018 mg/kg – 0.019 mg/kg)	0.017 µg/L – 1.9 µg/L (corresponds to 0.00017 mg/kg – 0.019 mg/kg)

Conclusion

The analytical method 01313/M001 was fully validated during study M-448498-01-1 (Stuke, S.; Teubner, L.; 2013). For the matrices relevant to this study (french bean (pod and green material)), a limited set (3 repetitions each at two fortification levels) of additional validation recoveries was analyzed within the course of the present study. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01313/M001 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on french bean (pod and green material) via HPLC-MS/MS.

A 2.2.1.1.20 Analytical method 01313/M001 in support of the study M-475814-01-1

A 2.2.1.1.20.1 Method validation

Comments of zRMS:	Method 01313/M001: Full validation data is documented with the method 01313/M001 itself for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrices relevant to this study (field pea (pod, green seed, rest of plant, dry seed and straw) but not included in the original validation, a limited set (3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of study 12-2031. Residues of trifloxystrobin, stereo-isomers and metabolites were determined by HPLC-MS/MS. The quantification is carried out by external standardization using matrix-matched standard. The Limit of Quantification (LOQ) defined as the lowest validated fortification level, was 0.01 mg/kg for all analytes in/on the field peas matrices. No residues above the LOQ were found in the control samples. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20% with one exception for CGA 321113 and rest of plant at the LOQ level, where it was 21.3%. This value was considered acceptable with reference to the OECD guidance document ENV/JM/MONO(2007)17. Overall recovery (n = 10) for CGA 321113 and rest of plant was 18.5%. All method validation data are in compliance with the SANCO/3029/99 rev. 4 requirements. The validation of method 01313/M001 can therefore be considered successful for the field pea matrices. The study is acceptable.

Reference:	**KCP 5.1.2.5/33**
Title:	Determination of the residues of fluopyram and trifloxystrobin in/on field pea after spray application of AE C656948 & CGA279202 SC 500 in the field in Germany, Northern France, Belgium and United Kingdom
Report:	Glaubitz, J.; Ballmann, C.; 2014; 12-2031; M-475814-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, EC Guidance working document 7029/VI/95 rev.5 (1997-07-22), OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial, US EPA OCSPP Guideline No. 860.1500
Deviations:	not applicable
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the study 12-2031 was to determine the magnitude of the relevant residues of Fluopyram (comprising AE C656948 and AE C656948-benzamide) and trifloxystrobin (CGA 279202) (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on field pea (pod, green seed, rest of plant, dry seed and straw) after two spray applications with AE C656948 & CGA279202 SC 500, a SC formulation containing 250 g/L AE C656948 and 250 g/L trifloxystrobin.

Full validation data is documented with the method 01313/M001 itself (Stuke, S.; Teubner, L.; 2013; M-448498-01-1) for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrices relevant to this study but not included in the original validation (field pea (pod, green seed, rest of plant, dry seed and straw)), a limited set (3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of the present study.

Residues of CGA 279202, its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 were extracted from the homogenised sample material with a mixture of acetonitrile/water (4/1; v/v) by shaking. After filtration, addition of ammonium acetate solution to adjust the pH value and addition of internal standard solution the extracts were made up to volume, diluted, filtered and subjected to HPLC-MS/MS measurement (ESI positive, MRMs: CGA 279202 m/z: 409 → 145, CGA 357261 m/z 409 → 206, CGA 357262 m/z 409 → 206, CGA 331409 m/z 409 → 206, CGA 321113 m/z 395 → 186, CGA 373466 m/z 395 → 148). The quantification was done by external standardisation in pure solvent.

The examination samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen and the remaining sample was immediately returned to the freezer.

Results and discussions

Limited sets of validation recoveries were analysed and recovery rates were determined at fortification levels of 0.01 mg/kg and 0.10 mg/kg for each analyte and matrix. The recovery experiments were conducted by fortification of untreated control samples with defined amounts of the analytes prior to analysis.

Blank values in control samples were below 30% of the LOQ. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20% with two exceptions for CGA 357262 and rest of plant at the LOQ level, where it was 21.3% (this value was considered acceptable with reference to the OECD guidance document ENV/JM/MONO(2007)17) and for the overall mean RSD which was 20.9%.

Table A 63: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method validation

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]

CGA 279202	pea / pod	0.01	72; 74; 75	74	2.1	0.01
		0.10	82; 84; 85	84	1.8	
			Overall recovery (n = 6)	**79**	**7.2**	
	pea / rest of plant	0.01	73, 84, 86	81	8.6	0.01
		0.10	73; 79; 81	78	5.4	
			Overall recovery (n = 6)	**79**	**6.9**	
	pea / dry seed	0.01	82; 87; 104	91	12.7	0.01
		0.10	78; 85; 92	85	8.2	
			Overall recovery (n = 6)	**88**	**10.4**	
	pea / green seed	0.01	74, 78, 92	81	11.6	0.01
		0.10	71; 73; 77	74	4.1	
			Overall recovery (n = 6)	**78**	**9.8**	
	pea / straw	0.01	96; 105; 114	105	8.6	0.01
		0.10	100; 100; 104	101	2.3	
			Overall recovery (n = 6)	**103**	**6.0**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357261	pea / pod	0.01	81; 81; 81	81	0.0	0.01
		0.10	76; 78; 79	78	2.0	
			Overall recovery (n = 6)	**79**	**2.6**	
	pea / rest of plant	0.01	86; 86; 99	90	8.3	0.01
		0.10	75; 77; 78	77	2.0	
			Overall recovery (n = 6)	**84**	**10.7**	
	pea / dry seed	0.01	97, 99, 92	96	3.8	0.01
		0.10	79; 83; 84	82	3.2	
			Overall recovery (n = 6)	**89**	**9.2**	
	pea / green seed	0.01	74, 81, 83	79	6.0	0.01
		0.10	76; 76; 79	77	2.2	
			Overall recovery (n = 6)	**78**	**4.4**	
	pea / straw	0.01	106; 110; 114	110	3.6	0.01
		0.10	100; 110; 119	110	8.7	
			Overall recovery (n = 6)	**110**	**5.9**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357262	pea / pod	0.01	72; 79; 83	78	7.1	0.01
		0.10	70; 75; 75	73	3.9	
			Overall recovery (n = 6)	**76**	**6.2**	
	pea / rest of plant	0.01	73; 98; 113	95	21.3*	0.01
		0.10	69; 73; 78	73	6.1	
			Overall recovery (n = 6)	**84**	**20.9****	
	pea / dry seed	0.01	76, 79, 95	83	12.3	0.01
		0.10	90; 92; 98	93	4.5	
			Overall recovery (n = 6)	**88**	**10.0**	
	pea / green seed	0.01	68, 78, 91	79	14.6	0.01
		0.10	73; 76; 77	75	2.8	
			Overall recovery (n = 6)	**77**	**10.0**	
	pea / straw	0.01	86; 91; 109	95	12.7	0.01
		0.10	97; 111; 112	107	7.9	
			Overall recovery (n = 6)	**101**	**11.1**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 331409	pea / pod	0.01	70; 75; 78	74	5.4	0.01
		0.10	73; 74; 77	75	2.8	
			Overall recovery (n = 6)	**75**	**3.9**	
	pea / rest of plant	0.01	74, 83, 86	81	7.7	0.01
		0.10	88; 94; 96	93	4.5	
			Overall recovery (n = 6)	**87**	**9.2**	
	pea / dry seed	0.01	86, 86, 87	86	0.7	0.01
		0.10	96; 99; 101	99	2.6	
			Overall recovery (n = 6)	**93**	**7.5**	
	pea / green seed	0.01	66, 71, 73	70	5.2	0.01
		0.10	70; 74; 77	74	4.8	

			Overall recovery (n = 6)	**72**	**5.2**	
	pea / straw	0.01	89; 98; 113	100	12.1	0.01
		0.10	95; 101; 115	104	9.9	
			Overall recovery (n = 6)	**102**	**10.1**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 321113	pea / pod	0.01	71; 73; 78	74	4.9	0.01
		0.10	78; 78; 79	78	0.7	
			Overall recovery (n = 6)	**76**	**4.3**	
	pea / rest of plant	0.01	99; 100; 108	102	4.8	0.01
		0.10	75; 76; 77	76	1.3	
			Overall recovery (n = 6)	**89**	**16.6**	
	pea / dry seed	0.01	92; 92; 98	94	3.7	0.01
		0.10	69; 71; 84	75	10.9	
			Overall recovery (n = 6)	**84**	**14.2**	
	pea / green seed	0.01	98; 98; 107	101	5.1	0.01
		0.10	76; 77; 83	79	4.8	
			Overall recovery (n = 6)	**90**	**14.3**	
	pea / straw	0.01	102; 104; 109	105	3.4	0.01
		0.10	88; 93; 100	94	6.4	
			Overall recovery (n = 6)	**99**	**7.7**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 373466	pea / pod	0.01	71; 74; 79	75	5.4	0.01
		0.10	78; 80; 88	82	6.5	
			Overall recovery (n = 6)	**78**	**7.4**	
	pea / rest of plant	0.01	79; 86; 92	86	7.6	0.01
		0.10	72; 72; 74	73	1.6	
			Overall recovery (n = 6)	**79**	**10.4**	
	pea / dry seed	0.01	83; 98; 98	93	9.3	0.01
		0.10	73; 76; 79	76	3.9	
			Overall recovery (n = 6)	**85**	**13.0**	
	pea / green seed	0.01	83; 93; 98	91	8.4	0.01
		0.10	75; 76; 77	76	1.3	
			Overall recovery (n = 6)	**84**	**11.6**	
	pea / straw	0.01	91; 96; 103	97	6.2	0.01
		0.10	96; 108; 109	104	6.9	
			Overall recovery (n = 6)	**101**	**7.3**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation
* This value was considered acceptable with reference to the OECD guidance document ENV/JM/MONO(2007)17.
** This value was considered acceptable since it is close to 20% and is therefore accepted by PI Analysis.

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.
The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20% with one exception for CGA 321113 and rest of plant at the LOQ level, where it was 21.3%. This value was considered acceptable with reference to the OECD guidance document ENV/JM/MONO(2007)17.

Table A 64: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method performance

Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 279202	pea / pod	0.01	72; 74; 75	74	2.1	0.01
		0.10	82; 84; 85	84	1.8	

			Overall recovery (n = 6)	**79**	**7.2**	
	pea / rest of plant	0.01	73; 81; 83; 84; 86; 90; 98; 100; 101	88	10.8	0.01
		0.10	73; 79; 81	78	5.4	
		0.50	76	-	-	
		5.0	96	-	-	
			Overall recovery (n = 14)	**86**	**11.3**	
	pea / dry seed	0.01	70; 70; 77; 80; 82; 82; 87; 104	82	13.3	0.01
		0.10	78; 82; 85	82	4.3	
		0.50	80	-	-	
			Overall recovery (n = 12)	**81**	**10.8**	
	pea / green seed	0.01	74; 78; 92; 99; 108; 109	93	15.9	0.01
		0.10	71; 73; 77	74	4.1	
		1.0	79	-	-	
			Overall recovery (n = 10)	**86**	**17.1**	
	pea / straw	0.01	96; 105; 114	105	8.6	0.01
		0.10	100; 100; 104	101	2.3	
		1.0	102	-	-	
		10	100	-	-	
			Overall recovery (n = 8)	**103**	**5.2**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357261	pea / pod	0.01	81; 81; 81	81	0.0	0.01
		0.10	76; 78; 79	78	2.0	
			Overall recovery (n = 6)	**79**	**2.6**	
	pea / rest of plant	0.01	73; 74; 82; 86; 86; 99	83	11.5	0.01
		0.10	75; 77; 78	77	2.0	
		0.50	65	-	-	
			Overall recovery (n = 10)	**80**	**11.7**	
	pea / dry seed	0.01	64; 68; 70; 72; 74; 76; 92; 97; 99	79	16.7	0.01
		0.10	79; 83; 84	82	3.2	
		0.50	73	-	-	
			Overall recovery (n = 13)	**79**	**14.0**	
	pea / green seed	0.01	74; 81; 83; 90; 99; 101	88	12.1	0.01
		0.10	76; 76; 79	77	2.2	
		1.0	72	-	-	
			Overall recovery (n = 10)	**83**	**12.4**	
	pea / straw	0.01	106; 110; 114	110	3.6	0.01
		0.10	100; 110; 119	110	8.7	
		1.0	107	-	-	
		10	108	-	-	
			Overall recovery (n = 8)	**109**	**5.1**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357262	pea / pod	0.01	72; 79; 83	78	7.1	0.01
		0.10	70; 75; 75	73	3.9	
			Overall recovery (n = 6)	**76**	**6.2**	
	pea / rest of plant	0.01	73; 73; 78; 80; 98; 113	86	18.9	0.01
		0.10	69; 73; 78	73	6.1	
		0.50	73	-	-	
			Overall recovery (n = 10)	**81**	**17.2**	
	pea / dry seed	0.01	75; 76; 79; 80; 93; 94; 95; 98; 99	88	11.3	0.01
		0.10	90; 92; 98	93	4.5	
		0.50	81	-	-	
			Overall recovery (n = 13)	**88**	**10.1**	
	pea / green seed	0.01	68; 78; 86; 91; 101; 104	88	15.6	0.01
		0.10	73; 76; 77	75	2.8	
		1.0	80	-	-	
			Overall recovery (n = 10)	**83**	**14.3**	
	pea / straw	0.01	86; 91; 109	95	12.7	0.01
		0.10	97; 111; 112	107	7.9	
		1.0	112	-	-	
		10	109	-	-	
			Overall recovery (n = 8)	**103**	**10.1**	

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 331409	pea / pod	0.01	70; 75; 78	74	5.4	0.01
		0.10	73; 74; 77	75	2.8	
			Overall recovery (n = 6)	**75**	**3.9**	
	pea / rest of plant	0.01	74; 83; 86; 96; 96; 97; 102; 103; 103	93	10.9	0.01
		0.10	88; 94; 96	93	4.5	
		0.50	76	-	-	
			Overall recovery (n = 13)	**92**	**10.6**	
	pea / dry seed	0.01	86; 86; 87; 87; 94; 98; 101; 102; 105	94	8.2	0.01
		0.10	96; 99; 101	99	2.6	
			Overall recovery (n = 12)	**95**	**7.3**	
	pea / green seed	0.01	66; 71; 73; 94; 99; 100	84	18.4	0.01
		0.10	70; 74; 77	74	4.8	
		1.0	80	-	-	
			Overall recovery (n = 10)	**80**	**15.7**	
	pea / straw	0.01	89; 98; 113	100	12.1	0.01
		0.10	95; 101; 115	104	9.9	
		1.0	106	-	-	
		10	99	-	-	
			Overall recovery (n = 8)	**102**	**8.7**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 321113	pea / pod	0.01	71; 73; 78	74	4.9	0.01
		0.10	78; 78; 79	78	0.7	
			Overall recovery (n = 6)	**76**	**4.3**	
	pea / rest of plant	0.01	65; 71; 73; 99; 100; 108	86	21.3*	0.01
		0.10	75; 76; 77	76	1.3	
		0.50	70	-	-	
			Overall recovery (n = 10)	**81**	**18.5**	
	pea / dry seed	0.01	66; 66; 68; 92; 92; 98	80	18.9	0.01
		0.10	69; 71; 84	75	10.9	
		0.50	68	-	-	
			Overall recovery (n = 10)	**77**	**16.4**	
	pea / green seed	0.01	98; 98; 107	101	5.1	0.01
		0.10	76; 77; 83	79	4.8	
		1.0	82	-	-	
			Overall recovery (n = 7)	**89**	**13.7**	
	pea / straw	0.01	102; 104; 109	105	3.4	0.01
		0.10	88; 93; 100	94	6.4	
		1.0	109	-	-	
		10	111	-	-	
			Overall recovery (n = 8)	**102**	**8.0**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 373466	pea / pod	0.01	71; 74; 79	75	5.4	0.01
		0.10	78; 80; 88	82	6.5	
			Overall recovery (n = 6)	**78**	**7.4**	
	pea / rest of plant	0.01	66; 67; 71; 79; 86; 92	77	13.9	0.01
		0.10	72; 72; 74	73	1.6	
		0.50	68	-	-	
			Overall recovery (n = 10)	**75**	**11.4**	
	pea / dry seed	0.01	68; 78; 80; 83; 98; 98	84	14.1	0.01
		0.10	73; 76; 79	76	3.9	
		0.50	71	-	-	
			Overall recovery (n = 10)	**80**	**12.8**	
	pea / green seed	0.01	63; 70; 70; 83; 93; 98	80	17.7	0.01
		0.10	75; 76; 77	76	1.3	
		1.0	74	-	-	
			Overall recovery (n = 10)	**78**	**13.8**	
	pea / straw	0.01	91; 96; 103	97	6.2	0.01
		0.10	96; 108; 109	104	6.9	

		1.0	105	-	-	
		10	104	-	-	
			Overall recovery (n = 8)	**102**	**6.3**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation
* This value was considered acceptable with reference to the OECD guidance document ENV/JM/MONO(2007)17.

Table A 65: Characteristics for the analytical method 01313/M001 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on field pea (pod, green seed, rest of plant, dry seed and straw)

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30% of the respective LOQ.		
Calibration range	0.02 µg/L - 5.0 µg/L (corresponds to 0.0002 mg/kg – 0.05 mg/kg) for each compound		
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound		
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.		
	CGA 279202	**CGA 357261**	**CGA 357262**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation: y = 0.56216 x + 0.015403, Correlation coefficient r: 0.9988, number of data points: 6	Individual calibration data is presented calibration equation: y = 0.59335 x + 0.011834, Correlation coefficient r: 0.9983, number of data points: 6	Individual calibration data is presented calibration equation: y = 0.59677 x + 0.018240, Correlation coefficient r: 0.9995, number of data points: 6
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation: y = 0.98877 x + 0.033399, Correlation coefficient r: 0.9981, number of data points: 6	Individual calibration data is presented calibration equation: y = 0.53878 x + 0.015053, Correlation coefficient r: 0.9986, number of data points: 6	Individual calibration data is presented calibration equation: y = 0.93822 x + 0.013375, Correlation coefficient r: 0.9986, number of data points: 6

Conclusion
The analytical method 01313/M001 was fully validated during study M-448498-01-1 (Stuke, S.; Teubner, L.; 2013). For the matrices relevant to this study (field pea (pod, green seed, rest of plant, dry seed and straw)) limited sets of validation recoveries were analysed within the course of the present study. All criteria according to SANCO/3029/99 rev. 4 were met with the minor exception of the precision data. The RSD values were below 20% with two exceptions for CGA 357262 and rest of plant at the LOQ level, where it was 21.3% and for the overall mean RSD which was 20.9%. But since these two values are close to 20% and all other values are within the acceptable range, these values are also considered acceptable. The analytical method 01313/M001 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on field pea (pod, green seed, rest of plant, dry seed and straw) via HPLC-MS/MS.

A 2.2.1.1.21 Analytical method 01313/M001 in support of the study M-566823-03-1

A 2.2.1.1.21.1 Method validation

Comments of zRMS:	Method 01313/M001: Full validation data is documented with the method 01313/M001 itself for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrices relevant to this study but not included in the original validation, a limited set (at least one control sample, 3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of this study. For green material a full validation (at least one control sample, 5 repetitions each at two fortification levels) was done. Residues of trifloxystrobin, stereo-isomers and metabolites were determined by HPLC-MS/MS.

	The Limit of Quantification (LOQ) defined as the lowest validated fortification level, was 0.01 mg/kg for all analytes in/on the field peas matrices. No residues above the LOQ were found in the control samples. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%. All method validation data are in compliance with the SANCO/3029/99 rev. 4 requirements. The validation of method 01313/M001 can therefore be considered successful for the field pea matrices. The study is acceptable.

Reference:	**KCP 5.1.2.5/36**
Title:	Determination of the residues of fluopyram and trifloxystrobin in/on field pea, after spray application of AE C656948 & CGA 279202 SC 500 in Denmark, Germany, Spain and Italy
Report:	Noss, G.; Czaja, C.; 2017; 15-2030; M-566823-03-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Deviations:	yes, see report
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the study 15-2030 was to determine the magnitude of the residues of fluopyram (AE C656948) and its metabolites AE C656948-benzamide as well as the residues of trifloxystrobin (CGA 279202), its isomers CGA 331409, CGA 357262, CGA 357261 and the metabolite CGA 321113 and its isomer CGA 373466 in/on pea, field (green material, pod, seed, dry and seed, green) after two spray applications with Fluopyram & Trifloxystrobin SC 500, a suspension concentrate formulation containing 250 g/L AE C656948 and 250 g/L trifloxystrobin.

Full validation data is documented with the method 01313/M001 itself (Stuke, S.; Teubner, L.; 2013; M-448498-01-1) for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For green material a full validation (at least one control sample, 5 repetitions each at two fortification levels) was done within the present study. Limited sets of validation recoveries (at least one control sample, 3 repetitions each at two fortification levels) were analysed for the matrices field pea (pod, dry seed, green set) within the study 12-2031 (Glaubitz, J.; Ballmann, C.; 2014; M-475814-01-1).

Residues of CGA 279202, its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 were extracted from the homogenised sample material with a mixture of acetonitrile/water (4/1; v/v) by shaking. After filtration, addition of ammonium acetate solution to adjust the pH value and addition of internal standard solution the extracts were made up to volume, diluted, filtered and subjected to HPLC-MS/MS measurement (ESI positive, MRMs: CGA 279202 m/z: 409 → 145, CGA 357261 m/z 409 → 206, CGA 357262 m/z 409 → 206, CGA 331409 m/z 409 → 206, CGA 321113 m/z 395 → 186, CGA 373466 m/z 395 → 148). The quantification was done by external standardisation in pure solvent.

The examination samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen and the remaining sample was immediately returned to the freezer.

Conditions used for this Study:

Slight adaptions were made to the sample preparation procedure described within the analytical method modification 01313/M001 which are as follows: 0.25 mL of an internal standard mixture with 1000 µg/L of each analyte-ISTD is added and the volume is adjusted to 25 mL with acetonitrile/water 4/1 (v/v). The

extract is filtered through a 0.45 µm syringe into a HPLC vial and 1-2 µL are injected for LC-MS/MS analysis.

Results and discussions

For green material a full validation (at least one control sample, 5 repetitions each at two fortification levels) was done within the present study. Limited sets of validation recoveries (at least one control sample, 3 repetitions each at two fortification levels) were analysed for the matrices field pea (pod, dry seed, green set) within the study 12-2031. Recovery rates were determined at fortification levels of 0.01 mg/kg and 0.10 mg/kg for each analyte and matrix. The recovery experiments were conducted by fortification of untreated control samples with defined amounts of the analytes prior to analysis.

Blank values in control samples were below 30% of the LOQ. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

Table A 66: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method validation

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	pea, field / green material[a]	0.01	95; 100; 100; 100; 106	100	3.9	0.01
		0.10	98; 99; 99; 101; 104	100	2.4	
			Overall recovery (n = 10)	**100**	**3.0**	
	pea, field / pod[b]	0.01	72; 74; 75	74	2.1	0.01
		0.10	82; 84; 85	84	1.8	
			Overall recovery (n = 6)	**79**	**7.2**	
	pea, field / seed, dry[b]	0.01	82; 87; 104	91	12.7	0.01
		0.10	78; 85; 92	85	8.2	
			Overall recovery (n = 6)	**88**	**10.4**	
	pea, field / seed, green[b]	0.01	74, 78, 92	81	11.6	0.01
		0.10	71; 73; 77	74	4.1	
			Overall recovery (n = 6)	**78**	**9.8**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357261	pea, field / green material[a]	0.01	93; 97; 101; 101; 101	99	3.6	0.01
		0.10	98; 100; 100; 100; 101	100	1.1	
			Overall recovery (n = 10)	**99**	**2.6**	
	pea, field / pod[b]	0.01	81; 81; 81	81	0.0	0.01
		0.10	76; 78; 79	78	2.0	
			Overall recovery (n = 6)	**79**	**2.6**	
	pea, field / seed, dry[b]	0.01	97, 99, 92	96	3.8	0.01
		0.10	79; 83; 84	82	3.2	
			Overall recovery (n = 6)	**89**	**9.2**	
	pea, field / seed, green[b]	0.01	74, 81, 83	79	6.0	0.01
		0.10	76; 76; 79	77	2.2	
			Overall recovery (n = 6)	**78**	**4.4**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357262	pea, field / green material[a]	0.01	85; 96; 97; 100; 108	97	8.5	0.01
		0.10	98; 100; 100; 101; 103	100	1.8	
			Overall recovery (n = 10)	**99**	**6.0**	
	pea, field / pod[b]	0.01	72; 79; 83	78	7.1	0.01
		0.10	70; 75; 75	73	3.9	
			Overall recovery (n = 6)	**76**	**6.2**	
	pea, field / seed, dry[b]	0.01	76, 79, 95	83	12.3	0.01
		0.10	90; 92; 98	93	4.5	
			Overall recovery (n = 6)	**88**	**10.0**	
	pea, field / seed, green[b]	0.01	68, 78, 91	79	14.6	0.01
		0.10	73; 76; 77	75	2.8	
			Overall recovery (n = 6)	**77**	**10.0**	

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 331409	pea, field / green material[a]	0.01	89; 98; 102; 103; 104	99	6.2	0.01
		0.10	97; 100; 102; 103; 103	101	2.5	
			Overall recovery (n = 10)	**100**	**4.5**	
	pea, field / pod[b]	0.01	70; 75; 78	74	5.4	0.01
		0.10	73; 74; 77	75	2.8	
			Overall recovery (n = 6)	**75**	**3.9**	
	pea, field / seed, dry[b]	0.01	86, 86, 87	86	0.7	0.01
		0.10	96; 99; 101	99	2.6	
			Overall recovery (n = 6)	**93**	**7.5**	
	pea, field / seed, green[b]	0.01	66, 71, 73	70	5.2	0.01
		0.10	70; 74; 77	74	4.8	
			Overall recovery (n = 6)	**72**	**5.2**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 321113	pea, field / green material[a]	0.01	88; 89; 97; 108; 114	99	11.6	0.01
		0.10	95; 99; 101; 103; 106	101	4.1	
			Overall recovery (n = 10)	**100**	**8.2**	
	pea, field / pod[b]	0.01	71; 73; 78	74	4.9	0.01
		0.10	78; 78; 79	78	0.7	
			Overall recovery (n = 6)	**76**	**4.3**	
	pea, field / seed, dry[b]	0.01	92; 92; 98	94	3.7	0.01
		0.10	69; 71; 84	75	10.9	
			Overall recovery (n = 6)	**84**	**14.2**	
	pea, field / seed, green[b]	0.01	98; 98; 107	101	5.1	0.01
		0.10	76; 77; 83	79	4.8	
			Overall recovery (n = 6)	**90**	**14.3**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 373466	pea, field / green material[a]	0.01	92; 92; 98; 102; 106	98	6.3	0.01
		0.10	100; 100; 101; 101; 101	101	0.5	
			Overall recovery (n = 6)	**99**	**4.4**	
	pea, field / pod[b]	0.01	71; 74; 79	75	5.4	0.01
		0.10	78; 80; 88	82	6.5	
			Overall recovery (n = 6)	**78**	**7.4**	
	pea, field / seed, dry[b]	0.01	83; 98; 98	93	9.3	0.01
		0.10	73; 76; 79	76	3.9	
			Overall recovery (n = 6)	**85**	**13.0**	
	pea, field / seed, green[b]	0.01	83; 93; 98	91	8.4	0.01
		0.10	75; 76; 77	76	1.3	
			Overall recovery (n = 6)	**84**	**11.6**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation
These recoveries were performed during the conduct of the studies 15-2030[a] and 12-2031[b].

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.
The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The average recoveries were within the range of 70 – 110%. The RSD values were below 20%.

Table A 67: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method performance

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	pea, field / green	0.01	95; 100; 100; 100; 106	100	3.9	0.01

	material	0.10	98; 99; 99; 101; 104	100	2.4	
		5.0	84	-	-	
		15	102	-	-	
			Overall recovery (n = 12)	**99**	**5.5**	
	pea, field / pod	0.01	93; 100; 105	99	6.1	0.01
		0.10	106; 106; 111	108	2.7	
		1.0	107	-	-	
			Overall recovery (n = 7)	**104**	**5.6**	
	pea, field / seed, dry	0.01	96; 102; 103	100	3.8	0.01
		0.10	101; 103; 103	102	1.1	
		5.0	93	-	-	
			Overall recovery (n = 7)	**100**	**4.0**	
	pea, field / seed, green	0.01	104; 110; 110	108	3.2	0.01
		0.10	103; 104; 105	104	1.0	
		5.0	84	-	-	
			Overall recovery (n = 7)	**103**	**8.6**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357261	pea, field / green material	0.01	93; 97; 101; 101; 101	99	3.6	0.01
		0.10	98; 100; 100; 100; 101	100	1.1	
		5.0	87	-	-	
			Overall recovery (n = 11)	**98**	**4.5**	
	pea, field / pod	0.01	94; 101; 103	99	4.8	0.01
		0.10	103; 108; 109	107	3.0	
		1.0	104	-	-	
			Overall recovery (n = 7)	**103**	**4.8**	
	pea, field / seed, dry	0.01	101; 102; 108	104	3.7	0.01
		0.10	102; 104; 106	104	1.9	
		5.0	95	-	-	
			Overall recovery (n = 7)	**103**	**4.1**	
	pea, field / seed, green	0.01	105; 105; 112	107	3.8	0.01
		0.10	99; 103; 105	102	3.8	
		5.0	83	-	-	
			Overall recovery (n = 7)	**102**	**9.0**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 357262	pea, field / green material	0.01	85; 96; 97; 100; 108	97	8.5	0.01
		0.10	98; 100; 100; 101; 103	100	1.8	
		5.0	87	-	-	
			Overall recovery (n = 11)	**98**	**6.8**	
	pea, field / pod	0.01	96; 100; 101	99	2.7	0.01
		0.10	102; 105; 108	105	2.9	
		1.0	103	-	-	
			Overall recovery (n = 7)	**102**	**3.7**	
	pea, field / seed, dry	0.01	104; 105; 107	105	1.5	0.01
		0.10	101; 105; 106	104	2.5	
		5.0	99	-	-	
			Overall recovery (n = 7)	**104**	**2.7**	
	pea, field / seed, green	0.01	95; 100; 101	99	3.3	0.01
		0.10	102; 103; 105	103	1.5	
		5.0	85	-	-	
			Overall recovery (n = 7)	**99**	**6.9**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 331409	pea, field / green material	0.01	89; 98; 102; 103; 104	99	6.2	0.01
		0.10	97; 100; 102; 103; 103	101	2.5	
		5.0	87	-	-	
			Overall recovery (n = 11)	**99**	**5.9**	
	pea, field / pod	0.01	102; 105; 105	104	1.7	0.01
		0.10	105; 106; 113	108	4.0	
		1.0	104	-	-	
			Overall recovery (n = 7)	**106**	**3.3**	

	pea, field / seed, dry	0.01	100; 105; 110	105	4.8	0.01
		0.10	102; 105; 107	105	2.4	
		5.0	95	-	-	
			Overall recovery (n = 7)	**103**	**4.8**	
	pea, field / seed, green	0.01	105; 106; 107	106	0.9	0.01
		0.10	98; 100; 107	102	4.6	
		5.0	83	-	-	
			Overall recovery (n = 7)	**101**	**8.6**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 321113	pea, field / green material	0.01	88; 89; 97; 108; 114	99	11.6	0.01
		0.10	95; 99; 101; 103; 106	101	4.1	
		5.0	86	-	-	
			Overall recovery (n = 11)	**99**	**9.0**	
	pea, field / pod	0.01	85; 88; 100	91	8.7	0.01
		0.10	98; 104; 109	104	5.3	
		1.0	102	-	-	
			Overall recovery (n = 7)	**98**	**8.8**	
	pea, field / seed, dry	0.01	104; 104; 112	107	4.3	0.01
		0.10	101; 103; 108	104	3.5	
		5.0	96	-	-	
			Overall recovery (n = 7)	**104**	**4.9**	
	pea, field / seed, green	0.01	87; 102; 103	97	9.2	0.01
		0.10	98; 101; 107	102	4.5	
		5.0	88	-	-	
			Overall recovery (n = 7)	**98**	**7.8**	
Analyte	**Crop/Sample Material**	**FL [mg/kg]**	**Single Values [%]**	**Mean Value [%]**	**RSD [%]**	**LOQ [mg/kg]**
CGA 373466	pea, field / green material	0.01	92; 92; 98; 102; 106	98	6.3	0.01
		0.10	100; 100; 101; 101; 101	101	0.5	
		5.0	84	-	-	
			Overall recovery (n = 11)	**98**	**6.3**	
	pea, field / pod	0.01	98; 108; 118	108	9.3	0.01
		0.10	100; 100; 102	101	1.1	
		1.0	106	-	-	
			Overall recovery (n = 7)	**105**	**6.6**	
	pea, field / seed, dry	0.01	96; 108; 108	104	6.7	0.01
		0.10	97; 101; 101	100	2.6	
		5.0	93	-	-	
			Overall recovery (n = 7)	**101**	**5.8**	
	pea, field / seed, green	0.01	94; 107; 116	106	10.5	0.01
		0.10	100; 108; 108	105	4.4	
		5.0	89	-	-	
			Overall recovery (n = 7)	**103**	**9.0**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 68: Characteristics for the analytical method 01313/M001 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on field pea (pod, green material, green seed and dry seed)

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.		
Calibration range	0.2 µg/L - 100 µg/L (corresponds to 0.002 mg/kg – 1 mg/kg) for each compound		
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound		
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.		
	CGA 279202	**CGA 357261**	**CGA 357262**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted):	Individual calibration data is presented calibration equation (1/x weighted):	Individual calibration data is presented calibration equation (1/x weighted):

	y = 0.022261 x + 0.0011928, Correlation coefficient r: 0.9999, number of data points: 8	y = 0.036646 x + 0.0013270, Correlation coefficient r: 0.9999, number of data points: 8	y = 0.043373 x + 0.0012154, Correlation coefficient r: 0.9998, number of data points: 8
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 0.082486 x + 0.0018014, Correlation coefficient r: 0.9996, number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 0.097176 x + 0.0048341, Correlation coefficient r: 0.9995, number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 0.095290 x + 0.0041754, Correlation coefficient r: 0.9990, number of data points: 8

Conclusion
The analytical method 01313/M001 was fully validated during study M-448498-01-1 (Stuke, S.; Teubner, L.; 2013). For green material a full validation (at least one control sample, 5 repetitions each at two fortification levels) was done within the present study. Limited sets of validation recoveries (at least one control sample, 3 repetitions each at two fortification levels) were analysed for the matrices field pea (pod, dry seed, green set) within the study 12-2031. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01313/M001 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on field pea (pod, green material, green seed and dry seed) via HPLC-MS/MS.

A 2.2.1.1.22 Analytical method 01313/M001 in support of the study M-598289-01-1

A 2.2.1.1.22.1 Method validation

Comments of zRMS:	Method 01313/M001: Full validation data is documented with the method 01313/M001 itself for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix relevant to this study (carrot) but not included in the original validation, a set (1 control, three LOQ and three 10xLOQ samples) of additional validation recoveries were analysed within the course of the study 16-2155. The limits of quantitation (LOQ) for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 are 0.01 mg/kg, corresponding to the lowest fortification level of successfully conducted recovery experiments. Blank values in control samples were below 30% of the LOQ for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%. No residues above the LOQ were found in the control samples. All method validation data are in compliance with the guideline requirements for data collection methods. The validation of method 01313/M001 can therefore be considered successful for the new matrix. The study is acceptable.

Reference:	**KCP 5.1.2.5/39**
Title:	Determination of residues of fluopyram and trifloxystrobin in/on carrots after spray application of fluopyram & trifloxystrobin SC 500 in Northern France, Austria and Germany
Report:	Semrau, J.; 2017; 16-2155; M-598289-01-1
Authority registration No:	
Guideline(s):	REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1500
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the present study was to determine the magnitude of the relevant residues of AE C656948 (fluopyram) and its metabolite AE C656948-benzamide and trifloxystrobin (CGA 279202) and its isomers / metabolites (CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on carrot (root) after two spray application with Fluopyram + Trifloxystrobin SC 500, a suspension concentrate containing 250 g/L fluopyram (nominal) and 250 g/L trifloxystrobin (nominal). In the following part, only the method validation for CGA 279202 and its isomers / metabolites is presented.

Full validation data is documented with the method 01313/M001 itself (Stuke, S.; Teubner, L.; 2013; M-448498-01-1) for CGA 279202 and its isomers and metabolites CGA 331409, GCA 357262, CGA 357261, CGA 321113 and CGA 373466 in plant sample material including broccoli (head), rape (seed), kidney bean (dry seed), grape (bunches of grape), wheat (root), cabbage (head), olive (fruit), hop (cone, kiln-dried). For carrot (root) a limited validation set (1 control, three LOQ and three 10xLOQ samples) was analysed within the course of the present study.

The weight of specimen per analysis was 2 g. The residues were extracted with acetonitrile/water (4/1, v/v). The final determination was performed with HPLC-MS/MS. All samples were analysed by single extraction and single injection to the detection system using internal standards.

Conditions used in this study:

The examination samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen and the remaining sample was immediately returned to the freezer.

The quantification of CGA 279202 and its isomers and metabolites was done by internal standardisation using stable-labelled internal standards.

The chromatographic system used for the determination of CGA 279202 and its isomers and metabolites was a high performance liquid chromatograph with a reversed phase chromatography coupled with tandem mass spectrometry (MS/MS) with electrospray ionization (ESI positive, MRMs used for quantification: CGA 279202 m/z: 409 → 186 used for quantification, m/z: 409 → 145 used for confirmation; CGA 357261 m/z 409 → 206 for quantification, m/z: 409 → 116 used for confirmation; CGA 357262 m/z 409 → 206 for quantification, m/z: 409 → 146 used for confirmation; CGA 331409 m/z 409 → 206 for quantification, m/z: 409 → 145 used for confirmation; CGA 321113 m/z 395 → 186 for quantification, m/z: 395 → 145 used for confirmation; CGA 373466 m/z 395 → 148 for quantification, m/z: 395 → 116 used for confirmation).

Results and discussions

A limited validation set (1 control, three LOQ and three 10xLOQ samples) was analysed and recovery rates were determined at fortification levels of 0.01 mg/kg and 0.10 mg/kg for each analyte.

The apparent residues in the control sample used for the determination of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations. The mean of the concurrent recoveries at each fortification level and overall per matrix were within the range of 70 – 110% for all analytes. Wherever applicable (n ≥ 3), the relative standard deviation (RSD) values were below 20%.

Table A 69: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method validation

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202 (MRM 409/186)	Carrot (root)	0.01	83, 80, 75	79	5.1	0.01
		0.1	80, 85, 86	84	3.8	
			Overall recovery (n = 6)	**82**	**5.0**	
CGA 279202 (MRM 409/145)	Carrot (root)	0.01	89, 80, 76	82	8.2	0.01
		0.1	80, 82, 84	82	2.4	
			Overall recovery (n = 6)	**82**	**5.4**	
CGA 357261 (MRM 409/206)	Carrot (root)	0.01	85, 85, 90	87	3.3	0.01
		0.1	82, 86, 85	84	2.5	
			Overall recovery (n = 6)	**86**	**3.0**	
CGA 357261 (MRM 409/116)	Carrot (root)	0.01	86, 74, 80	80	7.5	0.01
		0.1	83, 89, 90	87	4.3	
			Overall recovery (n = 6)	**84**	**7.2**	
CGA 357262 (MRM 409/206)	Carrot (root)	0.01	83, 84, 82	83	1.2	0.01
		0.1	100, 89, 95	95	5.8	
			Overall recovery (n = 6)	**89**	**8.2**	
CGA 357262 (MRM 409/146)	Carrot (root)	0.01	85, 90, 93	89	4.5	0.01
		0.1	95, 88, 92	92	3.8	
			Overall recovery (n = 6)	**91**	**4.0**	
CGA 331409 (MRM 409/206)	Carrot (root)	0.01	83, 88, 77	83	6.7	0.01
		0.1	88, 85, 90	88	2.9	
			Overall recovery (n = 6)	**85**	**5.5**	
CGA 331409 (MRM 409/145)	Carrot (root)	0.01	88, 85, 75	83	8.2	0.01
		0.1	79, 79, 90	83	7.7	
			Overall recovery (n = 6)	**83**	**7.1**	
CGA 321113 (MRM 395/186)	Carrot (root)	0.01	108, 97, 102	102	5.4	0.01
		0.1	85, 93, 103	94	9.6	
			Overall recovery (n = 6)	**98**	**8.4**	
CGA 321113 (MRM 395/145)	Carrot (root)	0.01	77, 99, 80	85	14	0.01
		0.1	88, 91, 93	91	2.8	
			Overall recovery (n = 6)	**88**	**9.4**	
CGA 373466	Carrot (root)	0.01	86, 82, 100	90	10	0.01
		0.1	100, 84, 99	94	9.5	

(MRM 395/148)			**Overall recovery (n = 6)**	**92**	**9.2**	
CGA 373466 (MRM 395/116)	Carrot (root)	0.01	89, 86, 75	83	8.8	0.01
		0.1	88, 84, 100	91	9.2	
			Overall recovery (n = 6)	**87**	**9.3**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

In order to check the performance of the method, recovery determinations were included in each set of analysis. Control samples from the study were fortified for the use as recovery samples. All recovery determinations were performed in parallel to the analyses of control and treated samples from the study. Procedural recoveries were handled and stored in the same way and for the same time period as the analytical specimens that were prepared within the same analytical set.
The apparent residues in the control sample used for the determination of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations. The mean of the concurrent recoveries at each fortification level and overall per matrix were within the range of 70 – 110% for all analytes. Wherever applicable ($n \geq 3$), the RSD values were below 20%.

Table A 70: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method performance

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Carrot (root)	0.01	83, 80, 75, 88, 96, 81	84	8.7	0.01
		0.1	80, 85, 86, 90, 92, 99	89	7.4	
			Overall Recovery (n = 12)	**86**	**8.2**	
CGA 357261	Carrot (root)	0.01	85, 85, 90, 94, 89, 79	87	6.0	0.01
		0.1	82, 86, 85, 87, 90, 88	86	3.2	
			Overall Recovery (n = 12)	**87**	**4.6**	
CGA 357262	Carrot (root)	0.01	83, 84, 82, 86, 95, 82	85	5.8	0.01
		0.1	100, 89, 95, 91, 87, 91	92	5.1	
			Overall Recovery (n = 12)	**89**	**6.6**	
CGA 331409	Carrot (root)	0.01	83, 88, 77, 90, 86, 85	85	5.3	0.01
		0.1	88, 85, 90, 83, 98, 89	89	5.8	
			Overall Recovery (n = 12)	**87**	**5.9**	
CGA 321113	Carrot (root)	0.01	108, 97, 102, 81, 97, 87	95	10	0.01
		0.1	85, 93, 103, 92, 90, 86	92	7.1	
			Overall Recovery (n = 12)	**93**	**8.8**	
CGA 373466	Carrot (root)	0.01	86, 83, 100, 75, 90, 96	88	10	0.01
		0.1	100, 84, 99, 78, 90, 79	88	11	
			Overall Recovery (n = 12)	**88**	**10**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 71: Characteristics for the analytical method 01313/M001 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on carrot (root)

Specificity	For each compound: HPLC-MS/MS method is highly specific.

	Blank values of all analytes were below 30 % of the respective LOQ.		
Calibration range	0.03 ng/mL - 2.0 ng/mL (corresponds to 0.003 mg/kg - 0.2 mg/kg) for each compound		
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound		
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.		
	CGA 279202 (m/z 409/186)	**CGA 357262 (m/z 409/206)**	**CGA 357261 (m/z 409/206)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 3.92693 x + 0.01547, Correlation coefficient r: 0.9996 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): y = 5.09287 x + 0.03221, Correlation coefficient r: 0.9973 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): y = 5.39642 x + 0.00035, Correlation coefficient r: 0.9981 number of data points: 6
	CGA 331409 (m/z 409/206)	**CGA 321113 (m/z 395/186)**	**CGA 373466 (m/z 395/148)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 3.79865 x + 0.01202, Correlation coefficient r: 0.9997 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): y = 3.81887 x - 0.01990, Correlation coefficient r: 0.9955 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): y = 5.88366 x - 0.04737, Correlation coefficient r: 0.9919 number of data points: 6
	CGA 279202 (m/z 409/145)	**CGA 357262 (m/z 409/146)**	**CGA 357261 (m/z 409/116)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 1.26751 x + 0.00940, Correlation coefficient r: 0.9978 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): y = 1.09598 x – 0.00224, Correlation coefficient r: 0.9990 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): y = 1.11467 x + 0.00533, Correlation coefficient r: 0.9987 number of data points: 6
	CGA 331409 (m/z 409/145)	**CGA 321113 (m/z 395/145)**	**CGA 373466 (m/z 395/116)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 1.97778 x + 0.00478, Correlation coefficient r: 0.9996 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): y = 1.25107 x + 0.00853, Correlation coefficient r: 0.9967 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): y = 1.82817 x - 0.02646, Correlation coefficient r: 0.9962 number of data points: 6

Conclusion

The analytical method 01313/M001 was fully validated during study M-448498-01-1 (Stuke, S.; Teubner, L.; 2013). For carrot (root) a limited validation set (1 control, three LOQ and three 10xLOQ samples) was analysed within the course of the present study. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01313/M001 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on carrot (root) via HPLC-MS/MS.

A 2.2.1.1.23 Analytical method 01313/M001 in support of the study M-682016-01-1

A 2.2.1.1.23.1 Method validation

Comments of zRMS:	Residues of trifloxystrobin, CGA 357261, CGA 357262, CGA 331409, CGA 321113 and CGA 373466 in/on carrot (root) were determined according to the method 01313/M001. Method 01313/M001: Full validation data is documented with the method 01313/M001 itself for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). A reduced validation set (1 control sample, three repetitions at LOQ and three repetitions at 0.50 mg/kg fortification

	level) for the sample material carrot (root) was performed in this study (18-2044). The limits of quantitation (LOQ) for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 are 0.01 mg/kg, corresponding to the lowest fortification level of successfully conducted recovery experiments. No residues above the LOQ were found in the control samples. The individual recoveries per fortification level were within the range of 70 – 110% and the RSD values were below 20%. All method validation data are in compliance with the guideline requirements for data collection methods. The validation of method 01313/M001 can therefore be considered successful for carrot (root). The study is acceptable.

Reference:	**KCP 5.1.2.5/40**
Title:	Determination of the residues of trifloxystrobin and AE C656948 in/on carrot after spray application of AE C656948 & CGA279202 SC 500 in Germany, the United Kingdom and northern France
Report:	Braune, M.; Cuesta-Pérez, J.; 2020; 18-2044; M-682016-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the study 18-2044 was to determine the magnitude of the residues of AE C656948 (comprising AE C656948 and AE C656948-benzamide) and trifloxystrobin (CGA 279202) (comprising of CGA 279202 and its isomers/metabolites CGA 357261, CGA 357262, CGA 331409, CGA 321113, and CGA 373466) in/on carrot (root) after two spray applications with AE C656948 & CGA279202 SC 500, a suspension concentrate formulation containing 250 g/L fluopyram and 250 g/L trifloxystrobin. In the following part, only the method validation for CGA 279202 and its isomers / metabolites is presented.

Full validation data is documented in method 01313/M001 (Stuke, S.; Teubner, L.; 2013; M-448498-01-1) for CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466 in broccoli (head), kidney bean (dry seed), rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrices relevant to this study (carrot (root)) but not included in the original validation, a limited set (one control sample, 3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of the present study.

Residues of trifloxystrobin, its isomers CGA 357261, CGA 357262, CGA 331409, and metabolite CGA 321113 and its isomer CGA 373466 were extracted from the homogenised sample material with a mixture of acetonitrile/water (4/1; v/v) by shaking. After filtration, addition of ammonium acetate solution to adjust the pH value and addition of internal standard solution, the extracts were made up to volume, diluted, filtered and subjected to HPLC-MS/MS measurement.

Conditions used for this study:

Slight adaptions were made to the sample preparation procedure described within the analytical method modification 01313/M001 which are as follows:

The weight of specimen per analysis was 2 g. After sample extraction with acetonitrile/ water (4/1; v/v) by shaking, 1mL of 1 mol/L ammonium acetate buffer and 0.25 mL internal standard mixture with 1000 µg/L of each analyte-ISTD were added. Afterwards, the volume was adjusted to 25 mL with acetonitrile/ water 4/1 (v/v). The extract is filtered through a 0.45 µm syringe into a HPLC vial and 1 or 2 µL of the final

extract was injected for determining the residue concentration using HPLC-MS/MS analysis (ESI positive, MRMs: CGA 279202 m/z: 409 → 145, CGA 357261 m/z 409 → 206, CGA 357262 m/z 409 → 206, CGA 331409 m/z 409 → 206, CGA 321113 m/z 395 → 186, CGA 373466 m/z 395 → 148). The quantification was done by external standardisation in pure solvent.
The examination samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen and the remaining sample was immediately returned to the freezer.
Samples containing high analyte concentrations were diluted until their concentrations were within the linearity range of the corresponding calibration curve.

Results and discussions
In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery per ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study. A limited validation set (one control sample, 3 repetitions each at two fortification levels) was analysed and recovery rates were determined at fortification levels of 0.01 mg/kg and 0.50 mg/kg for each analyte.
The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The average / individual recoveries per fortification level were within the range of 70 – 110%. The RSD values were below 20%, if applicable ($n \geq 3$).

Table A 72: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method validation

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Carrot (root)	0.01	96; 105; 106	102	5.4	0.01
		0.50	81; 96; 99	92	10.5	
			Overall recovery (n = 6)	**97**	**9.3**	
CGA 357261	Carrot (root)	0.01	91; 102; 102	98	6.5	0.01
		0.50	81; 95; 100	92	10.7	
			Overall recovery (n = 6)	**95**	**8.6**	
CGA 357262	Carrot (root)	0.01	89; 90; 99	93	5.9	0.01
		0.50	80; 96; 99	92	11.1	
			Overall recovery (n = 6)	**92**	**8.0**	
CGA 331409	Carrot (root)	0.01	89; 101; 104	98	8.1	0.01
		0.50	80; 95; 99	91	11.0	
			Overall recovery (n = 6)	**95**	**9.4**	
CGA 321113	Carrot (root)	0.01	89; 91; 99	93	5.7	0.01
		0.50	83; 97; 101	94	10.1	
			Overall recovery (n = 6)	**93**	**7.4**	
CGA 373466	Carrot (root)	0.01	91; 95; 99	95	4.2	0.01
		0.50	81; 97; 100	93	11.0	
			Overall recovery (n = 6)	**94**	**7.5**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 73: Characteristics for the analytical method 01313/M001 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on carrot (root)

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.		
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound		
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.		
	CGA 279202	**CGA 357262**	**CGA 357261**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): $y = 0.034142\ x + 4.8027 \cdot 10^{-4}$, Correlation coefficient r: 0.9998 number of data points: 8	Individual calibration data is presented, calibration equation (1/x weighted): $y = 0.033996\ x + 5.6137 \cdot 10^{-4}$, Correlation coefficient r: 0.9999 number of data points: 8	Individual calibration data is presented, calibration equation (1/x weighted): $y = 0.037421\ x + 2.3319 \cdot 10^{-4}$, Correlation coefficient r: 1.0000 number of data points: 8
Calibration range	0.200 – 100 µg/L corresponds to 0.00250 – 1.00 mg/kg of trifloxystrobin, expressed as trifloxystrobin	0.200 – 100 µg/L corresponds to 0.00250 – 1.00 mg/kg of CGA 357262, expressed as CGA 357262	0.200 – 100 µg/L corresponds to 0.00250 – 1.00 mg/kg of CGA 357261, expressed as CGA 357261
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): $y = 0.084655\ x – 6.6731 \cdot 10^{-4}$, Correlation coefficient r: 0.9999 number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.10977\ x + 0.0018935$, Correlation coefficient r: 0.9999 number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.11147\ x - 0.0010344$, Correlation coefficient r: 0.9999 number of data points: 8
Calibration range	0.200 – 100 µg/L corresponds to 0.00250 – 1.00 mg/kg of CGA 331409, expressed as CGA 331409	0.200 – 100 µg/L corresponds to 0.00250 – 1.00 mg/kg of CGA 321113, expressed as CGA 321113	0.200 – 100 µg/L corresponds to 0.00250 – 1.00 mg/kg of CGA 373466, expressed as CGA 373466

Conclusion

The analytical method 01313/M001 was fully validated during study M-448498-01-1 (Stuke, S.; Teubner, L.; 2013). For carrot (root) a limited validation set (one control sample, 3 repetitions each at two fortification levels) was analysed within the course of the present study. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01313/M001 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on carrot (root) via HPLC-MS/MS.

A 2.2.1.1.24 Concurrent validation of method 01013 in support of the study M-415381-01-1

Comments of zRMS:	The analytical method 01013 was developed for the determination of residues of BYF00587, Prothioconazole, Tebuconazole, Trifloxystrobin and the metabolites BYF00587-desmethyl, JAU6476-desthio (SXX0665) and CGA 321113 in/on plant materials. A confirmation of the validity of the analytical method was demonstrated in this study on the following sample material grape (bunch of grapes). The average recoveries were within the acceptable range of 70 – 110% and the relative standard deviations were below 20% for all the substances analysed. The Limits Of Quantitation (LOQ), were 0.01 mg/kg for trifloxystrobin and its metabolite

	in /on grape (bunch of grapes). For each calibration curve, the correlation coefficient R was above 0.99. Only the residues of trifloxystrobin and the CGA 321113 metabolite were determined. The 3 isomers of trifloxystrobin (CGA 357262, CGA 357261 and CGA 331409) were not analysed in this study, so only the existing plant residue definition for monitoring can be followed (Reg. (EU) 2019/1791). The study is acceptable.

Reference:	**KCP 5.1.2.5/41**
Title:	Determination of the residues of AE C656948 and trifloxystrobin in/on grape after spraying and spraying, low-volume of AE C656948 CGA279202 SC 500 in the field in France (north), france (south), germany and Italy
Report:	Cavaillé, C.; Uceda, L.; 2011; 09-2077; M-415381-01-1
Authority registration No:	
Guideline(s):	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation
The purpose of the study 09-2077 was to determine the magnitude of the relevant residues of AE C656948, AE C656948-benzamide, AE C656948-pyridyl-acetic acid, AE C656948-pyridyl-carboxylic acid, CGA 321113 and trifloxystrobin (CGA 279202) in/on grape (bunch of grapes) after two spraying applications or two spraying, low-volume applications with AE C656948 & CGA279202 SC 500.
Full validation data is documented with the method 01013 itself (Brumhard, B.; Stuke, S.; 2007; M-283439-01-1) for the determination of residues of CGA 279202 and CGA321113 (beside other analytes) in/on plant materials. For concurrent validation purposes, the method performance was checked during the present study.
The analytical method 01013 was developed for the determination of residues of BYF00587, Prothioconazole, Tebuconazole, Trifloxystrobin and the metabolites BYF00587-desmethyl, JAU6476-desthio (SXX0665) and CGA 321113 in/on plant materials. The above mentioned test items were extracted from the samples with a mixture of acetonitrile/water (4/1; v/v, containing cysteine hydrochloride) using a blender. After filtration of the extract, the stable isotopically labeled analytes were added. The solution was made up to volume, diluted and subjected to reversed phase HPLC-MS/MS without a further clean-up step. BYF00587, Tebuconazole, Trifloxystrobin, JAU6476-desthio and CGA 321113 were detected using electrospray ionization in the positive ion mode (ESI+), Prothioconazole and BYF00587-desmethyl were detected using electrospray ionization in the negative ion mode (ESI-). Residues were quantified using internal stable labeled standards. Due to an unsatisfying chromatographic separation of the seven test items two injections, one in the positive ion mode and another in the negative ion mode, are necessary.

Conditions used in this study for the analytical method 01013:
Residues of the following compounds were determined: trifloxystrobin and its metabolite CGA 321113.
The chromatographic system used was: a high performance liquid chromatograph with a reversed phase chromatography (on a Luna C18 column) coupled with tandem mass spectrometry (MS/MS) with Electrospray ionisation (ESI positive, MRMs: CGA 279202 m/z: 409 → 186, CGA 321113 m/z 395 → 186; Applied Biosystems API 4000 Triple Quadruple Mass Spectrometer, Analyst version 1.4.1.).
The quantification was carried out by internal standardization using trifloxystrobin-methyl-d3 and trifloxystrobin acid-methoxy- d3, as internal stable labeled standards.
For the preparation of the samples, some modifications were carried out concerning the internal standard adding without impact on the quality of the study. First, the 100 mL volumetric flask was made to volume with the mixture acetonitrile : water (4/1 ; v/v). This was the extract A. Second, an aliquot to the extract A was filtered on Anotop 25, 0.2 µm. Then the extract A filtered was diluted five times with the adding of the

internal standards. At the end, as in the method 01013, the LOQ concentration was: 0.1/1 µg/L test item/ISTD.
The quantification was done by internal standardisation in pure solvent.
In order to check the performance of the method, recovery determinations were included in each set of analysis (at least one recovery for twelve study samples). Control samples from the study were fortified for the use as the recovery samples. All the recovery determinations were performed in parallel to the analyses of control and treated samples from the study.
The apparent residues in the control sample used for fortification were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

Table A 74: Recovery rates and precision results (repeatability) of CGA 279202 and its metabolite CGA 321113

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Grape (bunch of grapes)	0.01	96; 100; 94; 102	98	3.7	0.01
		0.1	96; 97; 98; 98	97	1.0	
		1	90	90	-	
			Overall Recovery (n = 9)	**97**	**3.6**	
CGA 321113	Grape (bunch of grapes)	0.01	91; 96; 96; 97	95	2.9	0.01
		0.1	94; 94; 96; 96	95	1.2	
		1	92	92	-	
			Overall Recovery (n = 9)	**95**	**2.2**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 75: Characteristics for the analytical method 01013 used for validation of CGA 279202 and its metabolite CGA 321113 in/on grape (bunch of grapes)

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.	
Calibration range	0.05 µg/L – 10.0 µg/L (corresponds to 0.005 mg/kg – 1 mg/kg) for each compound	
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound	
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.	
	CGA 279202	**CGA 321113**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.98351 x + 0.0029261, Correlation coefficient r: 0.9999 number of data points: 7	Individual calibration data is presented calibration equation (1/x weighted): y = 0.68183 x – 0.0010998, Correlation coefficient r: 1.0000 number of data points: 7

Conclusion
The analytical method 01013 used in the present study for the determination of residues of CGA 279202 and CGA321113 (beside other analytes) in/on plant materials was fully validated during study M-283439-01-1 (Brumhard, B.; Stuke, S.; 2007). For concurrent validation purposes, the method performance of the analytical method 01013 was tested during the present study. The data presented demonstrate that the method allows the determination of these substances with satisfactory precision given that the overall mean relative standard deviation was below 5% of nine measured replicates for CGA 279202 and below 5% of nine measured replicates for CGA 321113. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.2.1.1.25 Analytical method 01313 in support of the study M-421645-02-1

A 2.2.1.1.25.1 Method validation

Comments of zRMS:	The analytical method 01313 was validated for the determination of residues of trifloxystrobin (CGA 279202) and its stereo-isomers (E/Z-isomers) CGA 357261, 357262, 331409 as well as its metabolite CGA 321113 and its stereoisomer CGA 373466 in/on plant materials in study of Stuke, S.; Bauer, J.; 2011; M-411496-02-1. For grapes relevant to this study but not included in the original validation, a limited set (3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of the present study. The LOQ for all compounds, defined as the lowest validated fortification level, was 0.01 mg/kg for grapes samples. No residues above the LOQ were found in the control samples. The average recoveries were within the acceptable range of 70 – 110% and the RSD values below 20%. For each calibration curve, the correlation coefficient R was above 0.998. All criteria according to SANCO/3029/99 rev. 4 were met. The study is acceptable.

Reference:	**KCP 5.1.2.5/43**
Title:	Amendment no. 1 to report no: P 652 11 5503 - Determination of the residues of trifloxystrobin, CGA 357261, CGA 357262, CGA 331409, CGA 321113, and CGA 373466 in/on materials of plant origin by HPLCMS/MS
Report:	Stuke, S.; 2013; MR-11/044; M-421645-02-1
Authority registration No:	
Guideline(s):	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the study P 652 11 5503 was to determine the magnitude of the relevant residues of trifloxystrobin (CGA 279202) and its stereo-isomers CGA 357261, CGA 357262, CGA 331409 as well as its metabolite CGA 321113 and its stereo-isomer CGA 373466 in/on samples from plant origin after spraying applications with Trifloxystrobin containing formulations.

In the following part only the matrix grapes (bunches of grape) is reported.

Full validation data is documented with the method 01313 itself (Stuke, S.; Bauer, J.; 2011; M-411496-02-1) for matrices representing 5 major crop groups, including corn (green material), rape (seed), wheat (grain), kidney bean and orange (fruit). For the matrix relevant to this study but not included in the original validation, a limited set (3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of the present study.

The analytical method 01313 was developed for the determination of residues of trifloxystrobin (CGA 279202) and its stereo-isomers (E/Z-isomers) CGA 357261, 357262, 331409 as well as its metabolite CGA 321113 and its stereoisomer CGA 373466 in/on plant materials.

The above mentioned test items were extracted from the samples with a mixture of acetonitrile/water (4/1; v/v) using a blender. After filtration the extract was adjusted to pH ≥ 6 with ammonia acetate and the solution was made up to volume, diluted, filtered and subjected to reversed phase HPLC-MS/MS without any further clean-up step. Residues were quantified using external calibration with matrix-matched standards to compensate possible matrix effects.

Results and discussions

Limited sets of validation recoveries were analysed and recovery rates were determined at fortification levels of 0.01 mg/kg and 0.10 mg/kg for each analyte and matrix.

In order to check the performance of the method, recovery determinations were included in each set of analysis (at least one recovery for ten study samples). Control samples from the study were fortified to be

used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.
The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries.
The average recoveries were within the acceptable range of 70 – 110% and wherever applicable (n ≥ 3), the RSD values were below 20%.

Table A 76: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Grapes, bunches of grape	0.01	102; 91; 95; **92; 94; 88**	94	5.1	0.01
		0.10	**86; 82; 84**	84	2.4	
		0.20	109; 93; 100	101	8.0	
			Overall recovery (n = 12)	**93**	**8.4**	
CGA 357261	Grapes, bunches of grape	0.01	100; 103; 99; **92; 97; 88**	97	5.7	0.01
		0.10	**90; 86; 86**	87	2.6	
		0.20	97; 95; 98	97	1.6	
			Overall recovery (n = 12)	**94**	**6.1**	
CGA 357262	Grapes, bunches of grape	0.01	101; 72; 99; **80; 87; 80**	87	13.3	0.01
		0.10	79; 83, **69; 82; 97**	82	12.3	
		0.20	82; 74; 75	77	5.7	
			Overall recovery (n = 14)	**83**	**12.0**	
CGA 331409	Grapes, bunches of grape	0.01	100; 104; 101; **80; 84; 75**	91	13.7	0.01
		0.10	**83; 76; 82**	80	4.7	
		0.20	93; 105; 107	102	7.4	
			Overall recovery (n = 12)	**91**	**13.3**	
CGA 321113	Grapes, bunches of grape	0.01	96; 88; 94; **98; 92; 84**	92	5.7	0.01
		0.10	**91; 90; 84**	88	4.3	
		0.20	93; 92; 98	94	3.4	
			Overall recovery (n = 12)	**92**	**5.1**	
CGA 373466	Grapes, bunches of grape	0.01	99; 89; 77; **98; 90; 85**	90	9.2	0.01
		0.10	**95; 90; 82**	89	7.4	
		0.20	97; 94; 91	94	3.2	
			Overall recovery (n = 12)	**91**	**7.4**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation; Single recovery values in **bold** are validation recoveries (3 replicates per level), not-bold are concurrent recoveries.

Table A 77: Characteristics for the analytical method 01313 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on grapes (bunches of grape)

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.
Calibration range	0.1 µg/L - 10.0 µg/L (corresponds to 0.001 mg/kg – 1 mg/kg) for each compound

Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound		
Assessment of matrix effects is presented	Matrix matched standards were used.		
	CGA 279202	**CGA 357261**	**CGA 357262**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 80100 x - 361, Correlation coefficient r: 0.9982 number of data points: 3	Individual calibration data is presented, calibration equation (1/x weighted): y = 111000 x + 4640, Correlation coefficient r: 0.9959 number of data points: 3	Individual calibration data is presented calibration equation (1/x weighted): y = 41000 x - 229, Correlation coefficient r: 0.9974 number of data points: 3
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 34600 x - 226, Correlation coefficient r: 0.9990 number of data points: 3	Individual calibration data is presented, calibration equation (1/x weighted): y = 203000 x + 4260, Correlation coefficient r: 0.9998 number of data points: 3	Individual calibration data is presented calibration equation (1/x weighted): y = 271000 x + 3800, Correlation coefficient r: 0.9992 number of data points: 3

Conclusion

The analytical method 01313 was fully validated during study M-411496-02-1 (Stuke, S.; Bauer, J.; 2011). For the matrix relevant to this study (grapes (bunches of grape)) but not included in the original validation, a limited set (3 repetitions each at two fortification levels) of additional validation recoveries was analyzed within the course of the present study. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01313 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on grapes (bunches of grape) via HPLC-MS/MS.

A 2.2.1.1.26 Analytical method 01013/M002

A 2.2.1.1.26.1 Method validation

Comments of zRMS:	The analytical method 01013/M002 was developed for the determination of residues of trifloxystrobin and CGA32113 in/on hops and processed products. The LOQ for all compounds, defined as the lowest validated fortification level, was 0.10 mg/kg for all sample materials. The overall mean recoveries were within the acceptable range of 70 – 110%, RSD ≤ 20%. All method validation data for trifloxystrobin and CGA 321113 are in compliance with the guideline requirements for residue data generation and enforcement. The study is acceptable.

Reference:	**KCP 5.1.2.5/45**
Title:	Modification M002 of the residue analytical method 01013 for the determination of trifloxystrobin and CGA 321113 in/on hops cone (green and dried) and processed materials (hops draff, brewers yeast, and beer) by HPLC-MS/MS
Report:	Schmeer, K.; Reineke, A.; 2010; 01013/M002; M-390173-01-1
Authority registration No:	
Guideline(s):	91/414/EEC, 96/68/EC 91/414, SANCO/3029/99 SANCO/825/00 rev. 7, US EPA Residue Chemistry Test Guideline OPPTS 860.1340:
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The analytical method presented in this report was designed to measure trifloxystrobin and its metabolite CGA 321113 in/on hops cone (green and dried) and processed materials (hops draff, brewers yeast, and beer) by HPLC-MS/MS. The analytical work was based on method 01013.

Trifloxystrobin and CGA 321113 were extracted from the homogenized sample material of hops cone (green and dried) and processed materials (hops draff, brewers yeast, and beer) with a mixture of acetonitrile/water (4/1; v/v) and cysteine hydrochloride by high-speed blending. After filtration using celite and addition of internal standard solution the extract was made up to volume. The extract is diluted by a factor of 50 and subjected to HPLC-MS/MS measurement.

The analytes were chromatographed by reversed-phase HPLC on a C18-column using a gradient methanol/water eluent containing ammonium formate and formic acid. A triple-stage mass spectrometer with an electrospray interface operated in the positive ion mode for trifloxystrobin and CGA 321113 under multiple-reaction monitoring (MRM) conditions. In the positive ion mode the protonated molecular ion of trifloxystrobin was separated and fragmented into its characteristic product ion (trifloxystrobin: m/z = 409 → m/z = 186). The product ion m/z = 186 was used for quantitation of trifloxystrobin. For CGA 321113 the protonated molecular ion was separated and fragmented into its characteristic product ion (CGA 321113: m/z = 395 → m/z = 186). The product ion m/z = 186 was used for quantitation of CGA 321113.

Trifloxystrobin and CGA 321113 are quantified using isotopically stable labeled internal standards.

Results and discussions

Recovery rates were determined at fortification levels of 0.10 mg/kg (= LOQ level), and 1.0 mg/kg for trifloxystrobin and CGA 321113 in green cone, dried cone, hops draff, brewers yeast, and beer.

The lowest fortification level (0.10 mg/kg) providing a mean recovery between 70 and 110% with a relative standard deviation of <20% per definition corresponding to the Limit of quantitation (LOQ), provided that the blank values were below 30% at this level. The limit of detection (LOD) was estimated on 30% of the LOQ (0.03 mg/kg). The blank values in control samples were below 20% of the respective LOQ for trifloxystrobin and CGA 321113. The recoveries were not corrected for interferences.

Recovery experiments were conducted by separate fortification of untreated control samples with defined amounts of trifloxystrobin and CGA 321113 prior to analysis.

As a measure for the precision of the method, the intra-laboratory repeatability (n > 5) is given as relative standard deviation (% RSD) for all sample materials. Relative standard deviations at each fortification level were below 20% for trifloxystrobin and CGA 321113 in all matrices tested.

The mean of RSD of the repeatability tests at each recovery set (both fortification level) ranged from 3.4 to 6.7% for trifloxystrobin and from 5.0 to 7.1 for CGA 321113 for the matrices tested within this study.

Table A 78: Recovery rates and precision results (repeatability) of CGA 279202 and its metabolite CGA 321113

Analyte	Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Green cone	0.10	96, 99, 90, 108, 90	97	7.7	0.10
		1.0	94, 95, 91, 95, 97	94	2.3	

			Overall Recovery (n = 10)	**96**	**5.6**	
	Dried cone	0.10	112, 113, 104, 107, 96	106	6.5	0.10
		1.0	95, 97, 96, 98, 99	97	1.6	
			Overall Recovery (n = 10)	**102**	**6.7**	
	Hops draff	0.10	99, 94, 99, 99, 91	96	3.9	0.10
		1.0	92, 91, 96, 95, 96	94	2.5	
			Overall Recovery (n = 10)	**95**	**3.4**	
	Brewers yeast	0.10	110, 103, 106, 110, 103	106	3.3	0.10
		1.0	102, 101, 100, 97, 98	100	2.1	
			Overall Recovery (n = 10)	**103**	**4.4**	
	Beer	0.10	100, 104, 99, 101, 105	102	2.5	0.10
		1.0	110, 108, 106, 107, 109	108	1.5	
			Overall Recovery (n = 10)	**105**	**3.7**	
CGA 321113	Green cone	0.10	79, 99, 99, 101, 94	94	9.5	0.10
		1.0	94, 92, 88, 91, 94	92	2.7	
			Overall Recovery (n = 10)	**93**	**6.8**	
	Dried cone	0.10	84, 85, 82, 79, 84	83	2.9	0.10
		1.0	75, 75, 70, 76, 74	74	3.2	
			Overall Recovery (n = 10)	**78**	**6.6**	
	Hops draff	0.10	89, 77, 99, 91, 94	90	9.1	0.10
		1.0	88, 100, 88, 90, 89	91	5.6	
			Overall Recovery (n = 10)	**91**	**7.1**	
	Brewers yeast	0.10	95, 107, 96, 99, 95	98	5.2	0.10
		1.0	106, 104, 104, 109,109	106	2.4	
			Overall Recovery (n = 10)	**102**	**5.5**	
	Beer	0.10	90, 101, 99, 92, 99	96	5.1	0.10
		1.0	97, 101, 106, 104, 98	101	3.8	
			Overall Recovery (n = 10)	**99**	**5.0**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 79: Characteristics for the analytical method 01013/M002 used for validation of CGA 279202 and its metabolite CGA 321113

Specificity	For each compound and matrix: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ	
Limit of determination/quantification	LOQ = 0.10 mg/kg for each compound and each matrix	
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.	
	CGA 279202	**CGA 321113**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): $y = 1.75 \cdot 10^6 x + 1.14 \cdot 10^4$, Correlation coefficient r: 0.9992 number of data points: 5	Individual calibration data is presented, calibration equation (1/x weighted): $y = 3.46 \cdot 10^5 x - 1.13 \cdot 10^3$, Correlation coefficient r: 0.9998 number of data points: 6
Calibration range	0.02 µg/L to 5 µg/L (corresponds to 0.02 mg/kg to 5 mg/kg)	0.02 µg/L to 10 µg/L (corresponds to 0.02 mg/kg to 10 mg/kg)

Conclusion

The modification 002 of the analytical method 01013 complies with criteria set by the guideline SANCO/3029/99 rev. 4 and was validated successfully.

A 2.2.1.1.27 Concurrent validation of method 01013/M002 in support of the study M-389144-01-1

Comments of zRMS:	The analytical method 01013/M002 was developed for the determination of residues of trifloxystrobin and CGA32113 in/on hops and processed products. The LOQ for all compounds, defined as the lowest validated fortification level, was 0.10 mg/kg for all sample materials.

	The overall mean recoveries were within the acceptable range of 70 – 110%, RSD ≤ 20%. In report it is stated that "*For trifloxystrobin residues in control samples of trial 08-2086-01, 08-2086-02 and 08- 2086-03 ranged from the < 0.10 mg/kg (LOQ) to 1.5 mg/kg whereas in control samples of trial 08-2086-04 no residues above the LOQ were found.* *For CGA321113 no residues in control samples above the LOQ = 0.1 mg/kg were found with the exception of trial 08-2086-02 where the residue for cone, kiln-dried at DALT 14 and 21 was 0.12 mg/kg.* *Control samples in trials 08-2086-01, 08-2086-02 and 08-2086-03 exhibited considerable residue levels of trifloxystrobin, but none of AE C656948. This is not characteristic of a contamination of the samples with the test item, nor do the maintenance treatments on the plots give any indication of why trifloxystrobin is present (with the exception of trial 08-2086-01 where trifloxystrobin was applied for maintenance on July 06, 2008 and July 14, 2008, 30 and 22 days before the start of trial). Thus it is assumed that the farmers whose land was used for the trials applied Flint or a similar trifloxystrobin-containing product on their adjacent fields and that the product deposited on the test plots via spray drift."* The study is acceptable.

Reference:	**KCP 5.1.2.5/46**
Title:	Determination of the residues of AE C656948 and trifloxystrobin in/on hop after spraying of AE C656948 & CGA 279202 SC 500 in the field in France (North) and Germany
Report:	Noss, G.; 2010; 08-2086; M-389144-01-1
Authority registration No:	
Guideline(s):	EU: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8; EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

Concurrent validation

The purpose of the study 08-2086 was to determine the magnitude of the relevant residues of AE C656948 (AE C656948, AE C656948-benzamide, AE C656948-pyridylacetic acid, AE C656948-pyridyl-carboxylic acid) and trifloxystrobin (CGA 279202) (CGA 279202, CGA321113) in/on hop (green cone and kiln-dried cone) after two spraying applications with AE C656948 & CGA 279202 SC 500 in/on hop.

Full validation data is documented with the method 01013/M002 itself (Schmeer, K.; Reineke, A.; 2010; M-390173-01-1). For concurrent validation purposes, the method performance was checked during the present study.

The analytical method 01013/M002 was developed for the determination of residues of CGA 279202 and CGA32113 in/on hops and processed products. The test items were extracted from the samples with a mixture of acetonitrile/water (4/1; v/v, containing cysteine hydrochloride) using a blender. After filtration of the extract, the stable isotopically labeled analytes were added. The solution was made up to volume, diluted and subjected to reversed phase HPLC-MS/MS without a further clean-up step (ESI positive, MRMs: CGA 279202 m/z: 409 → 186, CGA 321113 m/z 395 → 186). Residues were quantified using internal stable labeled standards. The LOQ for all compounds, defined as the lowest validated fortification level, was 0.10 mg/kg for all sample materials.

The method performance was checked at the fortification levels of 0.10 mg/kg, 1.0 mg/kg and 2.0 mg/kg. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

Table A 80: Recovery rates and precision results (repeatability) of CGA 279202 and its metabolite CGA 321113

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
	Hop; cone, green	0.10	88; 87; 80; 87	86	4.3	0.10

CGA 279202		1.0	94; 93; 94; 91	93	1.5	
		2.0	99	99	-	
			Overall Recovery (n = 9)	**90**	**6.1**	
	Hop; cone, kilndried	0.10	83; 87	85	-	0.10
		1.0	94; 93	94	-	
		2.0	92	92	-	
			Overall Recovery (n = 5)	**90**	**5.2**	
CGA 321113	Hop; cone, green	0.10	79; 88; 95; 86	87	7.6	0.10
		1.0	76; 77; 81; 76	78	3.1	
		2.0	76	76	-	
			Overall Recovery (n = 9)	**82**	**8.3**	
	Hop; cone, kilndried	0.10	93; 88	91	-	0.10
		1.0	79; 78	79	-	
		2.0	72	72	-	
			Overall Recovery (n = 5)	**82**	**10.2**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 81: Characteristics for the analytical method 01013/M002 used for validation of CGA 279202 and its metabolite CGA 321113 in/on hop (green cone and kiln-dried cone)

Specificity	For each compound and matrix: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.	
Limit of determination/quantification	LOQ = 0.10 mg/kg for each compound and matrix	
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.	
	CGA 279202	**CGA 321113**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 1.0222 x + 0.0075827, Correlation coefficient r: 0.9999 number of data points: 4	Individual calibration data is presented calibration equation (1/x weighted): y = 0.80941 x + 0.0031088, Correlation coefficient r: 0.9998 number of data points: 5
Calibration range	0.1 µg/L – 5.0 µg/L (corresponds to 0.1 mg/kg – 5.0 mg/kg)	0.01 µg/L – 10 µg/L (corresponds to 0.01 mg/kg – 10 mg/kg)

Conclusion

The analytical method 01013/M002 used in the present study for the determination of residues of CGA 279202 and CGA321113 was fully validated during study M-390173-01-1 (Schmeer, K.; Reineke, A.; 2010). For concurrent validation purposes, the method performance of the analytical method 01013 was tested during the present study. The data presented demonstrate that the method allows the determination of these substances with satisfactory precision given that the overall mean relative standard deviation was below 11% of all measured replicates for both analytes and matrices. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.2.1.1.28 Analytical method 01313 in support of the study M-432715-01-1

A 2.2.1.1.28.1 Method validation

Comments of zRMS:	The analytical method 01313 was validated for the determination of residues of trifloxystrobin (CGA 279202) and its stereo-isomers (E/Z-isomers) CGA 357261, 357262, 331409 as well as its metabolite CGA 321113 and its stereoisomer CGA 373466 in/on plant materials in study of Stuke, S.; Bauer, J.; 2011; M-411496-02-1. For hop relevant to this study but not included in the original validation, a limited set (3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of the present study. The LOQ for all compounds, defined as the lowest validated fortification level, was 0.05

	mg/kg for cone, kiln-dried samples. No residues above the LOQ were found in the control samples. The average recoveries were within the acceptable range of 70 – 110% and the RSD values below 20%. For each calibration curve, the correlation coefficient R was above 0.99. All criteria according to SANCO/3029/99 rev. 4 were met. The study is acceptable.

Reference:	**KCP 5.1.2.5/47**
Title:	Determination of the residues of AE C656948 and trifloxystrobin in/on hop after spraying of AE C656948 & CGA279202 SC 500 in the field in France (North)
Report:	Noss, G.; Ballmann, C.; 2012; 10-2127; M-432715-01-1
Authority registration No:	
Guideline(s):	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations:	the soil characterization, the weather data recording, the irrigation recording, the pesticide history, the cultural practices and the applications for maintenance (if relevant) which were not conducted under GLP
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods
The purpose of the study 10-2127 was to determine the magnitude of the relevant residues of fluopyram (comprising fluopyram, AE C656948-pyridyl-acetic acid, AE C656948-benzamide and AE C656948-pyridyl-carboxylic acid) and trifloxystrobin (CGA 279202) (comprising CGA 279202, CGA 321113, CGA 357261, CGA 357262, CGA 331409 and CGA 373466) in/on hop (green cone and kiln-dried cone) after two spray applications with Fluopyram & Trifloxystrobin SC 500, an SC formulation containing 250 g/L trifloxystrobin and 250 g/L AE C656948.
Full validation data is documented with the method 01313 itself (Stuke, S.; Bauer, J.; 2011; M-411496-02-1) for matrices representing 5 major crop groups, including corn (green material), rape (seed), wheat (grain), kidney bean and orange (fruit). For the matrix relevant to this study but not included in the original validation, a limited set (3 repetitions each at two fortification levels) of additional validation recoveries were analyzed within the course of the present study.
Residues of CGA 279202, its metabolite and isomers (CGA 357262, CGA 357261, CGA 331409, CGA 321113 and CGA 373466), were determined by HPLC-MS/MS according to method 01313.
Residues of these compounds were extracted from 5 g of sample material by extraction using a high speed blender with a mixture of acetonitrile/water (80/20, v/v, 40 mL). After adding celite, the extract was filtered, then the pH was adjusted to 6 with ammonium acetate and the volume was adjusted to 100 mL with acetonitrile/water (80/20, v/v). An aliquot was then diluted with water/methanol (80/20, v/v) prior to quantification by high performance liquid chromatograph using Ascentis Express C18 50mmx2.1mm i.d. 2.7µm column coupled with tandem mass spectrometry (MS/MS) with Electrospray ionisation (Applied Biosystems API 4000 or API5500 QTRAP, Analyst version 1.5.1.): One injection in positive electrospray ionization allowed the determination of all the analytes.

Conditions used for this study:
No modification was made to original method, except that no pre-heating was used for the mobile phase during the chromatography phase. The quantitation was done by external standardisation in matrix.

Results and discussions
Limited sets of validation recoveries were analysed and recovery rates were determined at fortification levels of 0.01 mg/kg and 0.10 mg/kg for hop (green cone) and at fortification levels of 0.05 mg/kg and 5.0 mg/kg for hop (kiln-dried cone). The limits of quantitation (LOQ) for trifloxystrobin and its metabolites are

0.01 mg/kg for green cone and 0.05 mg/kg for kiln-dried cone, corresponding to the lowest fortification level of successfully conducted recovery experiments.
Blank values in control samples were well below 30% of the LOQ for all samples. The average recoveries were within the acceptable range of 70 – 110% and wherever applicable ($n \geq 3$), the RSD values were below 20%.

Table A 82: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method validation

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	hop / cone, green	0.01	97, 95, 92	95	2.7	0.01
		1.0	86, 87, 89	87	1.7	
			Overall recovery (n = 6)	**91**	**4.9**	
	hop / cone, kiln-dried	0.05	95, 81, 101	92	11.1	0.05
		5.0	104, 107, 107	106	1.6	
			Overall recovery (n = 6)	**99**	**10.1**	
CGA 357261	hop / cone, green	0.01	101, 103, 102	102	1.0	0.01
		1.0	98, 100, 99	99	1.0	
			Overall recovery (n = 6)	**101**	**1.9**	
	hop / cone, kiln-dried	0.05	100, 101, 103	101	1.5	0.05
		5.0	105, 108, 107	107	1.4	
			Overall recovery (n = 6)	**104**	**3.1**	
CGA 357262	hop / cone, green	0.01	107, 91, 91	96	9.6	0.01
		1.0	98, 97, 96	97	1.0	
			Overall recovery (n = 6)	**97**	**6.1**	
	hop / cone, kiln-dried	0.05	83, 96, 93	91	7.5	0.05
		5.0	102, 106, 111	106	4.2	
			Overall recovery (n = 6)	**99**	**10.2**	
CGA 331409	hop / cone, green	0.01	112, 107, 95	105	8.3	0.01
		1.0	88, 80, 79	82	6.0	
			Overall recovery (n = 6)	**94**	**14.7**	
	hop / cone, kiln-dried	0.05	86, 86, 85	86	0.7	0.05
		5.0	91, 102, 102	98	6.5	
			Overall recovery (n = 6)	**92**	**8.7**	
CGA 321113	hop / cone, green	0.01	98, 93, 97	96	2.8	0.01
		1.0	84, 86, 85	85	1.2	
			Overall recovery (n = 6)	**91**	**6.9**	
	hop / cone, kiln-dried	0.05	87, 88, 87	87	0.7	0.05
		5.0	93, 88, 91	91	2.8	
			Overall recovery (n = 6)	**89**	**2.8**	
CGA 373466	hop / cone, green	0.01	92, 97, 97	95	3.0	0.01
		1.0	86, 85, 83	85	1.8	
			Overall recovery (n = 6)	**90**	**6.9**	
	hop / cone, kiln-dried	0.05	81, 87, 88	85	4.4	0.05
		5.0	89, 89, 91	90	1.3	

			Overall recovery (n = 6)	**88**	**3.9**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

In order to check the performance of the method, concurrent recovery determinations were included in each set of analysis (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.
The apparent residues in the control sample were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The average (concurrent) recoveries were within the acceptable range of 70 – 110% except for the LOQ level for trifloxystrobin, kiln-dried-cone, where it was (111%). This value was accepted as it is close to 110%. The RSD values were below 20%.

Table A 83: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method performance

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	hop / cone, green	0.01	88	-	-	0.01
		1.0	93	-	-	
			Overall recovery (n = 2)	**91**	**-**	
	hop / cone, kiln-dried	0.05	104; 108; 110; 120	111	6.2	0.05
		5.0	99	-	-	
			Overall recovery (n = 5)	**108**	**7.2**	
CGA 357261	hop / cone, green	0.01	98	-	-	0.01
		1.0	98	-	-	
			Overall recovery (n = 2)	**98**	**-**	
	hop / cone, kiln-dried	0.05	103; 107; 107; 115	108	4.7	0.05
		5.0	112	-	-	
			Overall recovery (n = 5)	**109**	**4.3**	
CGA 357262	hop / cone, green	0.01	94	-	-	0.01
		1.0	94	-	-	
			Overall recovery (n = 2)	**94**	**-**	
	hop / cone, kiln-dried	0.05	81; 88; 90; 91	88	5.2	0.05
		5.0	105	-	-	
			Overall recovery (n = 5)	**91**	**9.6**	
CGA 331409	hop / cone, green	0.01	97	-	-	0.01
		1.0	95	-	-	
			Overall recovery (n = 2)	**96**	**-**	
	hop / cone, kiln-dried	0.05	93; 96; 103; 106	100	6.1	0.05
		5.0	108	-	-	
			Overall recovery (n = 5)	**101**	**6.4**	
CGA 321113	hop / cone, green	0.01	86	-	-	0.01
		1.0	94	-	-	
			Overall recovery (n = 2)	**90**	**-**	
	hop / cone, kiln-dried	0.05	80; 81; 88; 93	86	7.2	0.05
		5.0	79	-	-	

			Overall recovery (n = 5)	**84**	**7.2**	
CGA 373466	hop / cone, green	0.01	115	-	-	0.01
		1.0	93	-	-	
			Overall recovery (n = 2)	**104**	**-**	
	hop / cone, kiln-dried	0.05	88; 89; 94; 95	92	3.8	0.05
		5.0	84	-	-	
			Overall recovery (n = 5)	**90**	**5.0**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 84: Characteristics for the analytical method 01313 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on hop (green cone and kiln-dried cone)

Specificity	For each compound and each matrix: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30% of the respective LOQ.	
Calibration range	For cone (kiln-dried): 0.0001 µg/mL - 0.06 µg/mL (corresponds to 0.01 mg/kg – 6 mg/kg) for each analyte For cone (green): 0.00002 µg/mL - 0.012 µg/mL (corresponds to 0.002 mg/kg – 1.2 mg/kg)	
Limit of determination/quantification	For cone (kiln-dried) LOQ = 0.05 mg/kg For cone (green) LOQ = 0.01 mg/kg	
Assessment of matrix effects is presented	No matrix effect was determined as matrix matched standards were used.	
	CGA 279202 in hop (kiln-dried cone)	**CGA 357261 in hop (green cone)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 27773198 x + 1739, Correlation coefficient r: 0.9981 number of data points: 5	Individual calibration data is presented, calibration equation (1/x weighted): y = 223404474 x + 512, Correlation coefficient r: 0.9999 number of data points: 7
	CGA 357261 in hop (kiln-dried cone)	**CGA 357261 in hop (green cone)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 36665411 x + 1270, Correlation coefficient r: 0.9986 number of data points: 5	Individual calibration data is presented, calibration equation (1/x weighted): y = 674639391 x + 911, Correlation coefficient r: 0.9997 number of data points: 7
	CGA 357262 in hop (kiln-dried cone)	**CGA 357262 in hop (green cone)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 28794088 x + 2035, Correlation coefficient r: 0.9967 number of data points: 6	Individual calibration data is presented, calibration equation (1/x weighted): y = 556037792 x + 104, Correlation coefficient r: 0.9998 number of data points: 7
	CGA 331409 in hop (kiln-dried cone)	**CGA 331409 in hop (green cone)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 45981051 x + 118, Correlation coefficient r: 0.9944 number of data points: 6	Individual calibration data is presented, calibration equation (1/x weighted): y = 341016100 x - 995, Correlation coefficient r: 0.9999 number of data points: 7
	CGA 321113 in hop (kiln-dried cone)	**CGA 321113 in hop (green cone)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 8298884 x - 68, Correlation coefficient r: 0.9988 number of data points: 6	Individual calibration data is presented, calibration equation (1/x weighted): y = 59647974 x - 162, Correlation coefficient r: 0.9994 number of data points: 7
	CGA 373466 in hop (kiln-dried cone)	**CGA 373466 in hop (green cone)**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 22297582 x - 592, Correlation coefficient r: 0.9997 number of data points: 6	Individual calibration data is presented, calibration equation (1/x weighted): y = 51723450 x + 131, Correlation coefficient r: 0.9994 number of data points: 7

Conclusion
The analytical method 01313 was fully validated during study M-411496-02-1 (Stuke, S.; Bauer, J.; 2011). For the matrix relevant to this study (hop (green cone and kiln-dried cone)) but not included in the original validation, a limited set (3 repetitions each at two fortification levels) of additional validation recoveries was analyzed within the course of the present study. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01313 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on hop (green cone and kiln-dried cone) via HPLC-MS/MS.

A 2.2.1.1.29 Concurrent validation of method 01013 in support of the study M-423507-02-1

Comments of zRMS:	Residues of the following compounds were determined: trifloxystrobin and its metabolite CGA 321113. A confirmation of the validity of the analytical method 01013 was shown in this study on the following sample materials: hop (cone, green) with a LOQ of 0.01 mg/kg and hop (cone, kiln-dried) with a LOQ of 0.05 mg/kg. The mean of the concurrent recoveries were for all matrices and for all fortification levels, within the acceptable range of 70 – 110%. No residues above the LOQ were found in the control samples. All method validation data are in compliance with the guideline requirements for data collection methods. The study is acceptable.

Reference:	**KCP 5.1.2.5/48**
Title:	Amendment No. 1 to report no: 09-2076 - Determination of the residues of AE C656948 and trifloxystrobin in/on hop after spraying of AE C656948 & CGA279202 SC 500 in the field in France (North) and Germany
Report:	Noss, G.; Ballmann, C.; 2012; 09-2076; M-423507-02-1
Authority registration No:	
Guideline(s):	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation
The purpose of the study 09-2076 was to determine the magnitude of the relevant residues of trifloxystrobin (CGA 279202) (comprising CGA 279202 and CGA 321113) and fluopyram (comprising AE C656948, AE C656948-pyridyl-acetic acid, AE C656948-benzamide and AE C656948-pyridyl-carboxylic acid) in/on hop (green cone and kiln-dried cone) after two spray applications with AE C656948 & CGA279202 SC 500, an SC formulation containing 250 g/L trifloxystrobin and 250 g/L AE C656948.
Full validation data is documented with the method 01013 itself (Brumhard, B.; Stuke, S.; 2007; M-283439-01-1) for the determination of residues of CGA 279202 and CGA321113 (beside other analytes) in/on plant materials. For concurrent validation purposes, the method performance was checked during the present study.
The analytical method 01013 was developed for the determination of residues of BYF00587, Prothioconazole, Tebuconazole, Trifloxystrobin and the metabolites BYF00587-desmethyl, JAU6476-desthio (SXX0665) and CGA 321113 in/on plant materials. The above mentioned test items were extracted from the samples with a mixture of acetonitrile/water (4/1; v/v, containing cysteine hydrochloride) using a blender. After filtration of the extract, the stable isotopically labeled analytes were added. The solution was made up to volume, diluted and subjected to reversed phase HPLC-MS/MS without a further clean-up step. BYF00587 and JAU6476-desthio were detected using electrospray ionization in the positive ion mode (ESI+), Prothioconazole and BYF00587-desmethyl were detected using electrospray ionization in the

negative ion mode (ESI-). Residues were quantified using internal stable labeled standards. Due to an unsatisfying chromatographic separation of the seven test items two injections, one in the positive ion mode and another in the negative ion mode, are necessary. The LOQ for all compounds, defined as the lowest validated fortification level, was 0.01 mg/kg for all sample materials.

Conditions used in this study for the analytical method 01013:
Residues of the following compounds were determined: trifloxystrobin and its metabolite CGA 321113.
The chromatographic system used was: a high performance liquid chromatograph with a reversed phase chromatography (on a Luna C18 column) coupled with tandem mass spectrometry (MS/MS) with Electrospray ionisation (ESI positive, MRMs: CGA 279202 m/z: 409 → 186, CGA 321113 m/z 395 → 186; Applied Biosystems API 4000 Triple Quadruple Mass Spectrometer, Analyst version 1.4.1.).
The quantification was carried out by internal standardization using trifloxystrobinmethyl- d3 and trifloxystrobin acid-methoxy-d3, as internal stable labeled standards.
For the preparation of the samples, some modifications were carried out concerning the internal standard adding without impact on the quality of the study. First, the 100 mL volumetric flask was made to volume with the mixture acetonitrile: water (4/1, v/v). This was the extract A. Second, an aliquot to the extract A was filtered on Anotop 25, 0.2 µm. Then the extract a filtered was diluted five times with the adding of the internal standards. At the end, as in the method 01013, the LOQ concentration was: 0.1/1 µg/L test item/ISTD.
A confirmation of the validity of the analytical method 01013 was shown in this study on the following sample materials: hop (cone, green) with a LOQ of 0.01 mg/kg and hop (cone, kiln-dried) with a LOQ of 0.05 mg/kg. The modification of the method 01013/M002 exists for hop and its processed fractions in which the LOQ is 0.10 mg/kg for all the matrices tested and so on hop (cone, green) and hop (cone, kiln-dried). This modification has been performed due to contamination encountered in these type of sample materials which doesn't allow to obtain a LOQ at 0.01 mg/kg. In the present study 09-2076, the contamination in the control samples is low enough to allow a LOQ at 0.01 mg/kg for cone, green and 0.05 mg/kg for cone, kiln-dried but it was not the case in the method 01013/M002 which proposed a LOQ at 0.10 mg/kg.
In order to check the performance of the method, recovery determinations were included in each set of analysis (at least one recovery for twelve study samples). Control samples from the study were fortified for the use as the recovery samples. All the recovery determinations were performed in parallel to the analyses of control and treated samples from the study.
The apparent residues in the control sample used for fortification were below 30% of the LOQ, except for 09-2076-03-0037E (47% in trifloxystrobin) and for 09-2076-04-0037E (100% in trifloxystrobin and 80% in CGA 321113) used for fortification at the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries, except for the two fortifications done with the samples above.
The means of the concurrent recoveries were for all matrices and for all fortification levels, within the acceptable range of 70 – 110%.

Table A 85: Recovery rates and precision results (repeatability) of CGA 279202 and its metabolite CGA 321113

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	Hop; cone, green	0.01	85[2]; 94; 95; 97; 100[1]	94	6.0	0.01
		0.1	95; 96; 97; 98	97	1.3	
		2	89	-	-	
			Overall recovery (n = 10)	**95**	**4.7**	
	Hop; cone, kiln-dried	0.05	95; 101; 103; 103; 121	105	9.3	0.05
		0.5	100; 100; 102	101	1.1	
		5	88	-	-	
			Overall recovery (n = 9)	**101**	**8.6**	
CGA 321113	Hop; cone, green	0.01	88; 115; 75[3]; 99; 94	94	15.6	0.01
		0.1	95; 94; 93; 95	94	1.0	
		2	88	-	-	

			Overall Recovery (n = 10)	**94**	**10.7**	
	Hop; cone, kiln-dried	0.05	89; 95; 82; 93; 93	90	5.7	0.05
		0.5	92; 90; 92	91	1.3	
		5	87	-	-	
			Overall Recovery (n = 9)	**90**	**4.4**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation
(1) This result of 100% in trifloxystrobin is the result corrected with the value for apparent residues in the control sample used (47%), value not corrected of 147%.
(2) This result of 85% in trifloxystrobin is the result corrected with the value for apparent residues in the control sample used (100%), value not corrected of 185%.
(3) This result of 75% in CGA 321113 is the result corrected with the value for apparent residues in the control sample used (80%), value not corrected of 155%.

Table A 86: Characteristics for the analytical method 01013 used for validation of CGA 279202 and its metabolite CGA 321113 in/on hop (green cone and kiln-dried cone)

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.	
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.	
	CGA 279202	**CGA 321113**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.98350 x + 0.0041802, Correlation coefficient r: 0.9999 number of data points: 7	Individual calibration data is presented calibration equation (1/x weighted): $y = 0.69796\ x - 7.8646 \cdot 10^{-4}$, Correlation coefficient r: 1.0000 number of data points: 7
Calibration range	0.05 µg/L – 10.0 µg/L (corresponds to 0.005 mg/kg – 1.0 mg/kg)	0.05 µg/L – 10.0 µg/L (corresponds to 0.005mg/kg – 1.0 mg/kg)
Limit of determination/quantification	LOQ = 0.01 mg/kg for hop, green cone for each compound LOQ = 0.05 mg/kg for hop, kiln-dried cone for each compound	

Conclusion
The analytical method 01013 used in the present study for the determination of residues of CGA 279202 and CGA321113 (beside other analytes) in/on plant materials was fully validated during study M-283439-01-1 (Brumhard, B.; Stuke, S.; 2007). For concurrent validation purposes, the method performance of the analytical method 01013 was tested during the present study. The data presented demonstrate that the method allows the determination of these substances with satisfactory precision. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.2.1.1.30 Analytical method 01313/M001 in support of the study M-681429-01-1

A 2.2.1.1.30.1 Method validation

Comments of zRMS:	Method 01313/M001: Full validation data is documented with the method 01313/M001 itself for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix relevant to this study (hop) but not included in the original validation, a set (1 control, at least three repetitions each at two fortification levels) of additional validation recoveries were analysed within the course of the study 18-2047. The limits of quantitation (LOQ) for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 are 0.01 mg/kg, corresponding to the lowest fortification level of successfully conducted recovery experiments. The average recoveries per fortification level were within the range of 70 – 110 %, except for the mean recoveries of CGA 331409, CGA 357262, CGA 357261, and CGA 373466 in hop, cone, green at the fortification level 0.5 mg/kg (50xLOQ). This is acceptable since there are no residues of these compounds in the samples at this residue level. The RSD values were below 20 %, if applicable (n ≥ 3). No residues above the LOQ were found in the control samples. All method validation data are in compliance with the guideline requirements for data

	collection methods. The validation of method 01313/M001 can therefore be considered successful for the new matrix - hop. The study is acceptable.

Reference:	**KCP 5.1.2.5/49**
Title:	Determination of the residues of trifloxystrobin and AE C656948 in/on hop after spray application of AE C656948 & CGA279202 SC 500 in northern France, Germany and Czech Republic - Final report -
Report:	Buchmueller, K.; van Berkum, S.; 2020; 18-2047; M-681429-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the study 18-2047 was to determine the magnitude of the residues of AE C656948 (fluopyram) and its metabolite AE C656948-benzamide as well as trifloxystrobin (CGA 279202) and its isomers / metabolites (CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on hop (cone, green and cone, kiln-dried) after two spray applications with AE C656948 & CGA279202 SC 500, a suspension concentrate formulation containing 250 g/L AE C656948 and 250 g/L trifloxystrobin.
Full validation data is documented with the method 01313/M001 itself (Stuke, S.; Teubner, L.; 2013; M-448498-01-1) for CGA 279202, CGA 357261, CGA 357262, CGA 331409, CGA 321113 and CGA 373466 in broccoli (head), kidney bean (dry seed), rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix relevant to this study (hop, kiln-dried and hop, green cone) a limited set (1 control, at least 3 repetitions each at two fortification levels) of additional validation recoveries was analyzed within the course of the present study.
Residues of trifloxystrobin, its isomers CGA 357261, CGA 357262, CGA 331409, and metabolite CGA 321113 and its isomer CGA 373466 were extracted from the homogenised sample material with a mixture of acetonitrile/water (4/1; v/v) by shaking. Ammonium acetate solution was added to adjust the pH value and then internal standard solution was added, the extracts were made up to volume, filtered and subjected to HPLC-MS/MS measurement (ESI positive, MRMs used for quantification: CGA 279202 m/z: 409 → 145, CGA 357261 m/z 409 → 206, CGA 357262 m/z 409 → 206, CGA 331409 m/z 409 → 206, CGA 321113 m/z 395 → 186, CGA 373466 m/z 395 → 148).

Conditions used in this study:

The examination samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen, and the remaining sample was immediately returned to the freezer.
The quantification of trifloxystrobin and its isomers and metabolites was done using stable-labelled internal standards.
Samples containing high analyte concentrations were diluted until their concentrations were within the linearity range of the corresponding calibration curve.

Results and discussions

A limited set (1 control, at least 3 repetitions each at two fortification levels) of additional validation recoveries was analyzed and recoveries were determined at fortification levels of 0.01 mg/kg, 0.50 mg/kg and 5.0 mg/kg.

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.
Apparent residues in control samples of trifloxystrobin, CGA 357261, CGA 357262, CGA 331409, CGA 321113 and CGA 373466 were below 30% of the LOQ.
The average recoveries per fortification level were within the range of 70 – 110 %, except for the mean recoveries of CGA 331409, CGA 357262, CGA 357261, and CGA 373466 in hop, cone, green at the fortification level 0.5 mg/kg (50xLOQ). This is acceptable since there are no residues of these compounds in the samples at this residue level. The RSD values were below 20 %, if applicable ($n \geq 3$).

Table A 87: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	hop / cone, green	0.01	93; 94; 96; 105	97	5.6	0.01
		0.50	73; 77; 79	76	4.0	
		5.0	90; 92; 92	91	1.3	
			Overall recovery (n = 10)	**89**	**11.0**	
	hop / cone, kiln-dried	0.01	86; 91; 101	93	8.2	0.01
		0.50	76; 81; 87; 93; 94; 96	88	9.1	
		5.0	99; 99; 101	100	1.2	
			Overall recovery (n = 12)	**92**	**8.8**	
CGA 357261	hop / cone, green	0.01	78; 85; 86; 86; 89; 98	87	7.5	0.01
		0.50	63; 63; 72	66	7.9	
		5.0	97; 98; 100	98	1.6	
			Overall recovery (n = 12)	**85**	**15.6**	
	hop / cone, kiln-dried	0.01	79; 83; 104	89	15.1	0.01
		0.50	88; 90; 92	90	2.2	
		5.0	101; 102; 106	103	2.6	
			Overall recovery (n = 9)	**94**	**10.4**	
CGA 357262	hop / cone, green	0.01	74; 82; 85; 89; 95; 97	87	9.8	0.01
		0.50	66; 67; 73	69	5.5	
		5.0	95; 97; 98	97	1.6	
			Overall recovery (n = 12)	**85**	**14.4**	
	hop / cone, kiln-dried	0.01	90; 105; 114	103	11.8	0.01
		0.50	79; 81; 87; 109; 110; 110	96	15.8	
		5.0	102; 102; 105	103	1.7	
			Overall recovery (n = 12)	**100**	**12.1**	
CGA 331409	hop / cone, green	0.01	85; 92; 95; 96; 97; 97	94	4.9	0.01
		0.50	66; 68; 73	69	5.2	
		5.0	93; 99; 101	98	4.3	
			Overall recovery (n = 12)	**89**	**14.1**	
	hop / cone, kiln-dried	0.01	87; 90; 95	91	4.5	0.01
		0.50	79; 79; 84; 90; 92; 94	86	7.6	
		5.0	103; 107; 108	106	2.5	
			Overall recovery (n = 12)	**92**	**10.6**	
	hop / cone, green	0.01	84; 90; 95; 110	95	11.7	0.01

CGA 321113		0.50	67; 73; 76	72	6.4	
		5.0	94; 95; 96	95	1.1	
			Overall recovery (n = 10)	**88**	**14.7**	
	hop / cone, kiln-dried	0.01	80; 80; 93	84	8.9	0.01
		0.50	70; 71; 78; 86; 88; 89	80	10.6	
		5.0	93; 94; 98	95	2.8	
			Overall recovery (n = 12)	**85**	**10.8**	
CGA 373466	hop / cone, green	0.01	87; 89; 93; 94; 98; 114	96	10.1	0.01
		0.50	61; 67; 72	67	8.3	
		5.0	94; 97; 98	96	2.2	
			Overall recovery (n = 12)	**89**	**16.9**	
	hop / cone, kiln-dried	0.01	81; 86; 90	86	5.3	0.01
		0.50	76; 80; 85; 86; 87; 88	84	5.6	
		5.0	97; 100; 102	100	2.5	
			Overall recovery (n = 12)	**88**	**9.0**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 88: Characteristics for the analytical method 01313/M001 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on hop (green cone and kiln-dried cone)

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.		
Calibration range	0.2 to 100 µg/L (corresponds to 0.0025 to 1.25 mg/kg) for each compound		
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound		
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.		
	CGA 279202	**CGA 357262**	**CGA 357261**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.092511 x + 0.0018568, Correlation coefficient r: 0.9999 number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 0.091801 x + 0.0019175, Correlation coefficient r: 0.9998 number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 0.086825 x + 0.0014182, Correlation coefficient r: 1.0000 number of data points: 8
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 0.11125 x + 6.4810 · 10^{-4}, Correlation coefficient r: 1.0000 number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 0.10110 x + 9.4661 · 10^{-4}, Correlation coefficient r: 0.9997 number of data points: 8	Individual calibration data is presented calibration equation (1/x weighted): y = 0.10556 x + 1.8262 · 10^{-4}, Correlation coefficient r: 0.9999 number of data points: 8

Conclusion

The analytical method 01013 used in the present study for the determination of residues of CGA 279202 and CGA321113 (beside other analytes) in/on plant materials was fully validated during study M-283439-01-1 (Brumhard, B.; Stuke, S.; 2007). For the matrix relevant to this study (hop, kiln-dried and hop, green cone) a limited set of additional validation recoveries was analyzed. The criteria according to SANCO/3029/99 rev. 4 were met with the minor exception of the accuracy data. The average recoveries per fortification level were within the range of 70 – 110 %, except for the mean recoveries of CGA 331409, CGA 357262, CGA 357261, and CGA 373466 in hop, cone, green at the fortification level 0.5 mg/kg (50xLOQ). This is acceptable since there are no residues of these compounds in the samples at this residue

level. In addition, the overall mean recoveries for each matrix and analyte are within the acceptable range and the RSD values are < 20%. Therefore, the analytical method 01313/M001 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on ~~lettuce (head)~~ hop via HPLC-MS/MS.

A 2.2.1.1.31 Analytical method 01313/M001 in support of the study M-491166-01-1

A 2.2.1.1.31.1 Method validation

Comments of zRMS:	Residues of trifloxystrobin and its isomers / metabolites were determined by LC-MS/MS according to method 01313/M001. Method 01313/M001: Full validation data is documented with the method 01313/M001 itself for matrices representing 5 major crop groups, including broccoli (head), kidney bean, rape (seed), grapes (bunches of grapes) and wheat (grain). For the matrix relevant to this study (pepper, sweet) but not included in the original validation, a set (1 control, at least three repetitions each at two fortification levels) of additional validation recoveries were analysed within the course of the study 13-2122. The limits of quantitation (LOQ) for trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 are 0.01 mg/kg, corresponding to the lowest fortification level of successfully conducted recovery experiments. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%. No residues above the LOQ were found in the control samples. All criteria according to SANCO/3029/99 rev. 4 were met. The validation of method 01313/M001 can therefore be considered successful for the new matrix – sweet pepper. The study is acceptable.

Reference:	**KCP 5.1.2.5/51**
Title:	Determination of the residues of AE C656948 and trifloxystrobin in/on (sweet) pepper after spray application of AE C656948 & CGA279202 SC 500 in southern France, Spain, Italy, Portugal and Greece
Report:	Glaubitz, J.; Czaja, C.; 2014; 13-2122; M-491166-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial US EPA OCSPP Guideline No. 860.1500
Deviations:	not applicable
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the study 13-2122 was to determine the magnitude of the relevant residues of AE C656948 (comprising AE C656948 and AE C656948-benzamide) and trifloxystrobin (CGA 279202) (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on pepper, sweet (fruit) after two spraying applications with AE C656948 & CGA279202 SC 500, a suspension concentrate formulation containing 250 g/L AE C656948 and 250 g/L trifloxystrobin (nominal content).
The analytical method 01313/M001 used in this study was fully validated during study M-448498-01-1 (Stuke, S.; Teubner, L.; 2013). For the matrices relevant to this study but not included in the original validation, a limited set (one control sample, 3 repetitions each at two fortification levels) of additional validation recoveries was analyzed within the course of the present study.
Residues of CGA 279202, its isomers CGA 357262, CGA 331409, CGA 357261 and metabolite CGA 321113 and its isomer CGA 373466 were extracted from the homogenised sample material with a mixture

of acetonitrile/water (4/1; v/v) by shaking. After filtration, addition of ammonium acetate solution to adjust the pH value and addition of internal standard solution the extracts were made up to volume, diluted, filtered and subjected to HPLC-MS/MS measurement (ESI positive, MRMs: CGA 279202 m/z: 409 → 145, CGA 357261 m/z 409 → 206, CGA 357262 m/z 409 → 206, CGA 331409 m/z 409 → 206, CGA 321113 m/z 395 → 186, CGA 373466 m/z 395 → 148). The quantification was done by external standardisation in pure solvent.
The examination samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen and the remaining sample was immediately returned to the freezer.

Results and discussions
A limited set (one control sample, 3 repetitions each at two fortification levels) of additional validation recoveries were analyzed for each analyte for the new matrix sweet pepper (fruit). Recovery rates were determined at fortification levels of 0.01 mg/kg and 0.10 mg/kg for each analyte and matrix. The recovery experiments were conducted by fortification of untreated control samples with defined amounts of the analytes prior to analysis.
Blank values in control samples were below 30% of the LOQ for parent compound as well as for the metabolite. The mean of the concurrent recoveries at each fortification level and overall per matrix were within the range of 70 – 110% for all analytes. Wherever applicable (n ≥ 3), the relative standard deviation (RSD) values were below 20%.

Table A 89: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method validation

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	pepper, sweet / fruit	0.01	88; 91; 102	94	7.9	0.01
		0.10	94; 97; 99	97	2.6	
			Overall recovery (n = 6)	**95**	**5.5**	
CGA 357261	pepper, sweet / fruit	0.01	85; 93; 96	91	6.2	0.01
		0.10	85; 93; 97	92	6.7	
			Overall recovery (n = 6)	**92**	**5.8**	
CGA 357262	pepper, sweet / fruit	0.01	81; 84; 96	87	9.1	0.01
		0.10	89; 91; 91	90	1.3	
			Overall recovery (n = 6)	**89**	**6.1**	
CGA 331409	pepper, sweet / fruit	0.01	82; 90; 103	92	11.6	0.01
		0.10	90; 91; 92	91	1.1	
			Overall recovery (n = 6)	**91**	**7.4**	
CGA 321113	pepper, sweet / fruit	0.01	87; 91; 95	91	4.4	0.01
		0.10	99; 99; 101	100	1.2	
			Overall recovery (n = 6)	**95**	**5.7**	
CGA 373466	pepper, sweet / fruit	0.01	81; 95; 95	90	8.9	0.01
		0.10	94; 100; 102	99	4.2	
			Overall recovery (n = 6)	**95**	**7.8**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for ten study samples). Control samples from the study were fortified to be

used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.
The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

Table A 90: Recovery rates and precision results (repeatability) of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 for method performance

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
CGA 279202	pepper, sweet / fruit	0.01	82; 84; 86; 87; 88; 90; 91; 92; 93; 95; 98; 98; 102	91	6.5	0.01
		0.10	81; 82; 86; 90; 91; 94; 97; 99; 106	92	8.9	
		0.50	85; 88; 92; 93; 98; 98	92	5.7	
		1.0	96	-	-	
		10	81	-	-	
			Overall recovery (n = 30)	**91**	**7.1**	
CGA 357261	pepper, sweet / fruit	0.01	83; 85; 88; 89; 92; 93; 94; 96; 96; 97; 98; 98	92	5.5	0.01
		0.10	83; 85; 93; 93; 94; 96; 97; 97	92	5.8	
		0.50	86; 88; 90; 96; 100; 100	93	6.6	
			Overall recovery (n = 26)	**93**	**5.6**	
CGA 357262	pepper, sweet / fruit	0.01	81; 81; 84; 84; 84; 86; 87; 87; 87; 90; 96; 99	87	6.3	0.01
		0.10	82; 89; 91; 91; 91; 91; 92; 93	90	3.8	
		0.50	84; 90; 91; 94; 96; 101	93	6.2	
			Overall recovery (n = 26)	**89**	**6.0**	
CGA 331409	pepper, sweet / fruit	0.01	79; 82; 83; 86; 87; 89; 90; 95; 97; 103; 105; 108	92	10.4	0.01
		0.10	82; 88; 90; 91; 92; 99; 100; 105	93	8.0	
		0.50	86; 95; 99; 101; 106; 106	99	7.7	
			Overall recovery (n = 26)	**94**	**9.2**	
CGA 321113	pepper, sweet / fruit	0.01	82; 86; 86; 87; 88; 89; 91; 91; 91; 92; 95; 100	90	5.2	0.01
		0.10	89; 93; 94; 98; 99; 99; 101; 103	97	4.8	
		0.50	86; 94; 95; 99; 100; 113	98	9.1	
			Overall recovery (n = 26)	**94**	**7.3**	
CGA 373466	pepper, sweet / fruit	0.01	81; 84; 87; 89; 90; 94; 95; 95; 99; 99; 99; 102	93	7.1	0.01
		0.10	89; 91; 91; 92; 94; 94; 100; 102	94	4.9	
		0.50	87; 88; 91; 95; 95; 97	92	4.5	
			Overall recovery (n = 26)	**93**	**5.8**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation;
These recoveries were performed during the conduct of the studies 13-2122 and 13-2123.

Table A 91: Characteristics for the analytical method 01313/M001 used for validation of CGA 279202, its three isomers CGA 357262, CGA 357261 and CGA 331409, metabolite CGA 321113 and its isomer CGA 373466 in/on sweet pepper (fruit)

Specificity	For each compound: HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.		
Limit of determination/quantification	LOQ = 0.01 mg/kg for each compound		
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.		
	CGA 279202	**CGA 357262**	**CGA 357261**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.67164 x + 6.1974 · 10^{-4}, Correlation coefficient r: 0.9997 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): y = 0.57452 x + 0.0059009, Correlation coefficient r: 0.9999 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): y = 0.62281 x + 0.0025749, Correlation coefficient r: 0.9997 number of data points: 6
Calibration range	0.02 µg/L - 5.0 µg/L (corresponds to 0.0002 mg/kg - 0.05 mg/kg)	0.02 µg/L - 5.0 µg/L (corresponds to 0.0002 mg/kg - 0.05 mg/kg)	0.02 µg/L - 5.0 µg/L (corresponds to 0.0002 mg/kg - 0.05 mg/kg)
	CGA 331409	**CGA 321113**	**CGA 373466**
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 1.0794 x + 0.015836, Correlation coefficient r: 0.9998 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted): y = 0.56481 x + 0.014823, Correlation coefficient r: 0.9998 number of data points: 5	Individual calibration data is presented calibration equation (1/x weighted): y = 0.89582 x + 0.021709, Correlation coefficient r: 0.9991 number of data points: 6
Calibration range	0.02 µg/L - 5.0 µg/L (corresponds to 0.0002 mg/kg - 0.05 mg/kg)	0.02 µg/L - 2.0 µg/L (corresponds to 0.0002 mg/kg - 0.02 mg/kg)	0.02 µg/L - 5.0 µg/L (corresponds to 0.0002 mg/kg - 0.05 mg/kg)

Conclusion

The analytical method 01313/M001 was fully validated during study M-448498-01-1 (Stuke, S.; Teubner, L.; 2013). In the present study, a limited set (one control sample, 3 repetitions each at two fortification levels) of additional validation recoveries was analyzed for the matrix sweet pepper (fruit). All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01313/M001 is suitable for the determination of residues of CGA 279202 (comprising CGA 279202, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on sweet pepper (fruit) via HPLC-MS/MS.

A 2.2.1.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.1)

No new or additional studies have been submitted.

A 2.2.1.3 Description of analytical methods for the determination of residues in support to environmental fate studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.2.1.4 Description of analytical methods for the determination of residues in support to toxicological studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.2.1.5 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP

5.1)

A 2.2.1.5.1 Analytical method 01158/M002

A 2.2.1.5.1.1 Method validation

Comments of zRMS:	The objective of the study was to validate the modification 002 of the analytical method 01158 for the determination of residues of trifloxystrobin in leaf punches washing solutions. Analytical Method: Stuke, S., van Berkum, S., Modification 002 of analytical method 01158 for the determination of tebuconazole, fluopyram and trifloxystrobin in leaf punches washing solution by HPLC-MS/MS, BAG report MR-15/032, dated 2015-09-07. The analytical part of this method described in this report delivered very good recovery rates with mean values > 70 % and relative standard deviation of < 20 % (n = 5) at all fortification levels. The used detector showed quadratic response (due to high sensitivity of the analyte) in the required concentration range with correlation coefficients > 0.999 at 7 calibration levels. Residues in control samples were below 30% of the LOQ. LOQ = 20 µg/L when extracting 400 cm^2 corresponding to 0.01 µg/cm^2. All results could be confirmed by a second MRM. All presented method validation data are in compliance with the guideline requirements and, thus the method suitable for residue data generation. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.4/05**
Title:	Amendment no. 1 to the final report of study no.: P602155506 - Modification 002 of analytical method 01158 for the determination of tebuconazole, fluopyram and trifloxystrobin in leaf punches washing solution by HPLC-MS/MS
Report:	Stuke, S.; van Berkum, S.; 2017; MR-15/032; M-532610-02-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The modification 002 of the analytical method 01158 (Freitag, T.; 2016; M-357292-02-1) has been validated to implement trifloxystrobin into the method 01158/M001 (Stuke, S.; Diehl, P.; 2014; M-502699-01-1) to allow an compiled analysis of tebuconazole, fluopyram and trifloxystrobin in washing solutions from punched leaves in one analytical run if necessary.

Trifloxystrobin is washed off from the leaves punches surface twice with 100 mL each of an aqueous 0.01% Aerosol® OT solution using a shaking machine. After decantation and combination of the extracts, the samples are stored frozen. (During DFR field studies this part is done at the field test site).

After thawing, 40 mL of acetonitrile are added to the 200-mL field sample (or 2 mL to the 10-mL lab and field spike recoveries) as a solubilizer to avoid adherence to the vessel wall. The bottles are washed again with 40 mL (2 mL) acetonitrile and the solutions are combined. After adding an isotope-labelled internal standard the sample solutions are filtered and subjected to reversed phase HPLC-MS/MS in positive ion mode for trifloxystrobin detection without any further clean-up step. Two MRM transitions were monitored

for each analyte and in each matrix tested, m/z 409.0 → 145 for quantitation and m/z 409.0 → 206.0 for confirmation of trifloxystrobin. Residues are quantified using internal stable-labelled standard.
The limit of quantitation (LOQ) for trifloxystrobin in leaf punches washing solution is 20 µg/L (for extraction of 400 cm² leaf surface) that corresponds to 0.01 µg/cm².

Results and discussions
Recovery rates were determined at fortification levels of 20.0 µg/L (=LOQ, corresponding to 400 cm² extracted leaf surface), 200 µg/L and 2000 µg/L which corresponds to fortification levels of 0.01, 0.1 and 1.0 µg/cm², respectively. Recovery experiments were conducted by fortification of untreated control material with defined amounts of trifloxystrobin prior to analysis.
The mean and overall mean recoveries per fortification level were within the range of 70 – 110%.
As a measure for the precision of the method, the intra-laboratory repeatability (n = 5) is given as relative standard deviation (% RSD) for all sample materials at fortification levels of 10.0 µg/L (= LOQ, corresponding to 200 cm² extracted leaf surface), 100 µg/L and 1000 µg/L. The RSD of the repeatability tests at each recovery set ranged from 1.7 to 11.1% for the quantitation of trifloxystrobin.
For confirmation of the individual residues a 2nd MRM transition was used. Results of the confirmation procedure showed comparable recovery rates for all compounds to the quantifier MRMs for all analytes.

Table A 92: Recovery rates and precision results (repeatability) of trifloxystrobin

Analyte	Crop/Sample Material	FL [µg/cm²]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [µg/cm²]
trifloxystrobin (m/z 409.0 → 145 for quantitation)	grape / leaf punch washings	0.01	70, 93, 87, 90, 93	87	11.1	0.01
		0.10	88, 88, 95, 92, 95	92	3.8	
		1.0	96, 95, 86, 87, 84	90	6.1	
			Overall recovery (n = 15)	**89**	**7.4**	
trifloxystrobin (m/z 409.0 → 145 for quantitation)	strawberry / leaf punch washings	0.01	89, 92, 90, 92, 89	90	1.7	0.01
		0.10	87, 88, 90, 93, 86	89	3.1	
		1.0	92, 91, 93, 86, 89	90	3.1	
			Overall recovery (n = 15)	**90**	**2.6**	
trifloxystrobin (m/z 409.0 → 206.0 for confirmation)	grape / leaf punch washings	0.01	75, 95, 89, 93, 90	88	8.9	0.01
		0.10	94, 91, 97, 92, 96	94	2.7	
		1.0	101, 101, 84, 76, 79	88	13.6	
			Overall recovery (n = 15)	**90**	**9.2**	
trifloxystrobin (m/z 409.0 → 206.0 for confirmation)	strawberry / leaf punch washings	0.01	94, 91, 89, 93, 85	90	4.0	0.01
		0.10	89, 90, 93, 96, 86	91	4.2	
		1.0	106, 98, 105, 84, 116	102	11.6	
			Overall recovery (n = 15)	**94**	**9.4**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 93: Characteristics for the analytical method 01158/M002 used for validation of trifloxystrobin

Specificity	HPLC-MS/MS method is highly specific. Two MRM transitions were monitored and an additional confirmatory method is not necessary. Blank values of all analytes were below 30% of the respective LOQ.	
	trifloxystrobin (m/z 409.0 → 145 for quantitation)	**trifloxystrobin (m/z 409.0 → 206.0 for confirmation)**
Calibration (type, number of data points)	Individual calibration data is presented; the used detector showed quadratic response (due to high sensitivity of the analyte) in the required concentration	Individual calibration data is presented; the used detector showed quadratic response (due to high sensitivity of the analyte) in the required concentration

	range with correlation coefficients > 0.999 at 7 calibration levels. Calibration equation (1/x weighted quadratic): $y = -0.00836 x^2 + 0.425 x – 0.000205$, Correlation coefficient r: 1.0000, number of data points: 7	range with correlation coefficients > 0.999 at 7 calibration levels. Calibration equation (1/x weighted quadratic): $y = -0.0196 x^2 + 0.697 x + 0.000153$, Correlation coefficient r: 0.9999, number of data points: 7
Calibration range	2.0 – 1500 µg/L	
Limit of determination/quantification	LOQ = 20 µg/L when extracting 400 cm^2 corresponding to 0.01 µg/cm^2	
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.	

Conclusion

The modification 002 of the analytical method 01158 was developed for the determination of trifloxystrobin in washing solutions from punched leaves at a limit of quantitation (LOQ) of 0.01 µg/cm^2. The modification 002 allows the compiled determination of trifloxystrobin, tebuconazole and fluopyram in one analytical run. The analytical method complies with all guideline criteria according to SANCO/3029/99 rev. 4 with the minor exception of the calibration data. While SANCO/3029/99 rev. 4 prefers the use of linear regression, a quadratic function was used for regression analysis.

In this study, the correlation between amount of the compounds injected and response by the detector was evaluated on the basis of a quadratic term ($y = ax^2+bx+c$) rather than linear regression ($y = ax+b$) preferred as the standard approach. The use of a quadratic regression curve in this case is regarded as justified when considering the following: In general, the primary purpose of a calibration curve is to assess whether a constant and predictable response is generated by the detector under the conditions of determination. In this study the graph showing the calibration data indicated that a curve with a rather high correlation coefficient best describes the measured against estimated values with consistent results. In comparison to linear regression the quadratic regression was thus able to better describe the process and should therefore be applied. It is noteworthy that the phenomenon is well known in HPLC-MS/MS determination using electrospray ionization. There are several potential explainations for this observation. One explanation comes from rather complex ionization mechanisms. For example, within the electrospray droplets there can be a competition among analytes for space and charge to result in ion suppression finally leading to a reduced signal at the detector, typically at higher analyte concentrations. Moreover, and depending on the analyte concentration, other components originating from the sample matrix can have similar effects to also result in a reduced signal. Consequently, detector signals of solvent standards followed more a less a straight line while matrix matched standards give lower signals, especially at higher concentrations. It is therefore reasonable to assume that factors as given above have caused the effect. However, the overall excellent correlation coefficients as well as the comparison of the different calibrations curves demonstrate that a reliable determination of the analytes is nevertheless possible using this approach in evaluation of detector results.

The analytical method 01158/M002 is suitable for the determination of the magnitude of the dislodgeable foliar residues (DFR) of trifloxystrobin in washings from grape and strawberry leaf punches via HPLC-MS/MS.

A 2.2.1.5.2 Analytical method 01158/M002 in support of the study M-569303-01-1

A 2.2.1.5.2.1 Method validation

Comments of zRMS:	Dislodgeable foliar residues of AE C656948 and trifloxystrobin were determined according to the 01158/M002 method (S. Stuke, S. van Berkum, MR-15/032, 2015-09-07). During the set of analysis, a calibration curve was established for AE C656948 and trifloxystrobin with at least six concentration levels and used for the quantitation. For the calibration curves the correlation coefficients R were above 0.999. No residues above the LOQ were found in the control samples. The mean of the concurrent laboratory recoveries for AE C656948 amounted to 98% with a relative standard deviation of 6.3%.

	The mean of the concurrent laboratory recoveries for trifloxystrobin amounted to 102% with a relative standard deviation of 5.7%. The mean of the field recovery samples for AE C656948 amounted to 84% with a relative standard deviation of 8.5%. The mean of the field recovery samples for trifloxystrobin amounted to 80% with a relative standard deviation of 4.2%. All criteria according to SANCO/3029/99 rev. 4 were met. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.4/02**
Title:	Determination of the dislodgeable foliar residues (DFR) of trifloxystrobin and AE C656948 in/on grape after spraying of AE C656948 & CGA279202 SC 500 in the field in the North of France
Report:	Stuke, S.; Daniela, M.; van Berkum, S.; 2016; 15-2924; M-569303-01-1
Authority registration No:	
Guideline(s):	US EPA OPPTS 875.2100 Foliar Dislodgeable Residue Dissipation
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods
The magnitude of the dislodgeable foliar residues (DFR) of the substances AE C656948 (fluopyram, FLU) and CGA 279202 (trifloxystrobin, TFS) in washings from grape leaf punches was determined after two spray applications of the formulation AE C656948 & CGA 279202 SC 500 (containing 250 g/L AE C656948 and 250 g/L trifloxystrobin). In the following part, only data for CGA 279202 is presented.

Full validation data is documented with the method 01158/M002 (Stuke, S.; van Berkum, S.; 2017; M-532610-02-1) for CGA 279202. For the matrix relevant to this study (grape, leaf punch washings), a full set of additional validation recoveries was performed within the present study.

The test item was extracted from the leaf punches by adding 100 mL of a 0.01% Aerosol OT solution (i.e. docusate sodium salt) which corresponds to a surfactant. After shaking, the solution was decanted and the dislodging procedure was repeated for each sample with a fresh 100 mL aliquot of the Aerosol solution. The second rinse was again decanted and added to the first.

Due to observed adherence problems of CGA 279202 the samples were treated deviating to the cited method to reduce the adherence effect and, thus to obtain higher field recoveries. After thawing the samples the solution was filled into another bottle, the original bottle was washed with pure acetonitrile to avoid adherence of CGA 279202 to the vessel walls to a total of 25 mL for the field spike samples and 500 mL for the field samples. The resulting sample dilution factor was 2.5.

After adding an internal standard solution the samples were subjected to HPLC-MS/MS analysis without further clean-up or preparation steps. Residues were calculated using internal standard calibration.

Results and discussions
A full set of additional validation recoveries was analysed and recoveries were determined at fortification levels of 0.01 µg/cm², 0.10 µg/cm²and 1.0 µg/cm².

In order to check the performance of the method, recovery determinations were included in each set of analysis (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The limit of quantification (LOQ), defined as the lowest validated fortification level, was set to 20 µg/L when extracting 400 cm² corresponding to 0.01 µg/cm². Blank values in control samples were below 30%

of the LOQ.

The average recoveries per fortification level were within the range of 70 – 110%, and the relative standard deviation (RSD) values were below 20%, if applicable (n ≥ 3).

Table A 94: Recovery rates and precision results (repeatability) of CGA 279202

Analyte	Crop/Sample Material	FL [µg/cm²]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [µg/cm²]
CGA 279202	grape / leaf punch washings	0.01	102; 105; 105; 99; 95	101	4.2	0.01
		0.10	110; 108; 112; 103; 94	105	6.8	
		1.0	99; 97	98	-	
			Overall recovery (n = 12)	**102**	**5.7**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 95: Characteristics for the analytical method 01158/M002 used for validation of CGA 279202

	CGA 279202
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.0035672 x + 0.0012451, Correlation coefficient r: 0.9993, number of data points: 6
Calibration range	0.5 to 1000 µg/L
Limit of determination/quantification	LOQ = 20 µg/L when extracting 400 cm² corresponding to 0.01 µg/cm²
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.

Conclusion
The analytical method 01158/M002 used in the present study for the determination of CGA 279202 in leaf punches washing solution was fully validated during study M-532610-02-1 (Stuke, S.; van Berkum, S.; 2017). For the matrix relevant to this study (grape, leaf punch washings) a full set of additional validation recoveries was analyzed. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01158/M002 is suitable for the determination of the magnitude of the dislodgeable foliar residues (DFR) of CGA 279202 in washings from grape leaf punches via HPLC-MS/MS.

A 2.2.1.5.3 Analytical method 01158/M002 in support of the study M-558518-01-1

A 2.2.1.5.3.1 Method validation

Comments of zRMS:	Dislodgeable foliar residues of AE C656948, and CGA 32113 and trifloxystrobin were determined according to the 01158/M002 method (S. Stuke, S. van Berkum, MR-15/032, 2015-09-07). During the set of analysis, a calibration curve was established for AE C656948 and trifloxystrobin and the additional analysis of CGA 321113 with at least six concentration levels and used for the quantitation. For the calibration curves the correlation coefficients R were above 0.998. No residues above the LOQ were found in the control samples. The mean of the concurrent laboratory recoveries for AE C656948 amounted to 98% with a relative standard deviation of 4.2%. The mean of the field recovery samples for AE C656948 amounted to 89% with a relative standard deviation of 7.9%. The mean of the concurrent laboratory recoveries for trifloxystrobin amounted to 85% with a relative standard deviation of 5.9%. The mean of the field recovery samples for trifloxystrobin amounted to 79% with a relative standard deviation of 10.0%.

	The results for the dislodgeable foliar residues for AE C656948 and trifloxystrobin in the field samples are not corrected for laboratory or field recoveries. All criteria according to SANCO/3029/99 rev. 4 were met. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.4/03**
Title:	Determination of the dislodgeable foliar residues (DFR) of trifloxystrobin and AE C656948 in/on lily after spraying of AE C656948 & CGA279202 SC 500 in the field in the Netherlands
Report:	Stuke, S.; van Berkum, S.; 2016; 15-2925; M-558518-01-1
Authority registration No:	
Guideline(s):	US EPA OPPTS 875.2100 Foliar Dislodgeable Residue Dissipation
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The magnitude of the dislodgeable foliar residues (DFR) of the substances AE C656948 (fluopyram, FLU) and CGA279202 (trifloxystrobin, TFS) in washings from lily leaf punches was determined after two spray applications with AE C656948 & CGA279202 SC 500 (containing 250 g/L fluopyram and 250 g/L trifloxystrobin).

Full validation data is documented with the method 01158/M002 (Stuke, S.; van Berkum, S.; 2017; M-532610-02-1) for CGA 279202. For the matrix relevant to this study (lily, leaf punch washings), a full set of additional validation recoveries was performed within the present study.

The test item was extracted from the leaf punches by adding 100 mL of a 0.01% Aerosol OT solution (i.e. docusate sodium salt) which corresponds to a surfactant. After shaking, the solution was decanted and the dislodging procedure was repeated for each sample with a fresh 100 mL aliquot of the Aerosol solution. The second rinse was again decanted and added to the first.

Due to observed adherence problems of CGA 279202 the samples were treated deviating to the cited method to reduce the adherence effect and, thus to obtain higher field recoveries. After thawing the samples the solution was filled into another bottle, the original bottle was washed with pure acetonitrile to avoid adherence of CGA 279202 to the vessel walls to a total of 25 mL for the field spike samples and 500 mL for the field samples. The resulting sample dilution factor was 2.5.

After adding an internal standard solution the samples were subjected to HPLC-MS/MS analysis without further clean-up or preparation steps. Residues were calculated using internal standard calibration.

Due to adherence problems with trifloxystrobin in the field spike samples the empty sample bottles with the pre-solution vials inside were again rinsed with pure acetonitrile to obtain good recovery results. Additionally, field spike samples were analyzed for the hydrolysis product of trifloxystrobin, CGA 321113. Residues were determined using matrix matched standard solutions and double injection.

Results and discussions

A full set of additional validation recoveries was analysed and recoveries were determined at fortification levels of 0.01 µg/cm^2, 0.10 µg/cm^{2}and 1.0 µg/cm^2.

In order to check the performance of the method, recovery determinations were included in each set of analysis (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The limit of quantification (LOQ), defined as the lowest validated fortification level, was set to 20 µg/L when extracting 400 cm^2 corresponding to 0.01 µg/cm^2. Blank values in control samples were below 30% of the LOQ.

The average recoveries per fortification level were within the range of 70 – 110%, and the relative standard deviation (RSD) values were below 20%, if applicable ($n \geq 3$).

Table A 96: Recovery rates and precision results (repeatability) of CGA 279202

Analyte	Crop/Sample Material	FL [µg/cm²]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [µg/cm²]
CGA 279202	lily / leaf punch washings	0.01	76; 79; 79; 82; 82	80	3.2	0.01
		0.10	86; 87; 88; 89; 90	88	1.8	
		1.0	89; 91	90	-	
			Overall recovery (n = 12)	**85**	**5.9**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

In order to check the performance of the method and the stability of the field samples, recovery determinations were performed from control samples spiked with trifloxystrobin during the field part.
The field spike recoveries for trifloxystrobin are the sum of results from re-analysis after additional flushing of the sample bottles with acetonitrile and a separate analysis for the hydrolysis product of trifloxystrobin, CGA 321113 (expressed as trifloxystrobin).

Table A 97: Recovery rates and precision results (repeatability) of CGA 279202 in field spikes

Analyte	Crop/Sample Material	FL [µg/cm²]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [µg/cm²]
CGA 279202	lily / leaf punch washings	0.01	80, 94, 104	93	13.0	0.01
		0.10	90, 82, 88	87	4.8	
		1.0	87, 89, 86	87	1.7	
			Overall recovery (n = 9)	**89**	7.9	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 98: Characteristics for the analytical method 01158/M002 used for validation of CGA 279202 and CGA 321113

Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.	
	CGA 279202	**CGA 321113**
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.0041043 x + 0.0013742, Correlation coefficient r: 0.9998, number of data points: 6	Individual calibration data is presented, calibration equation (1/x weighted): y = 1328.75437 x – 995.40615, Correlation coefficient r: 0.99839, number of data points: 6
Calibration range	2 to 1000 µg/L	2 – 500 µg/L
Limit of determination/quantification	LOQ = 20 µg/L when extracting 400 cm² corresponding to 0.01 µg/cm²	
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.	

Conclusion
The analytical method 01158/M002 used in the present study for the determination of CGA 279202 in leaf punches washing solution was fully validated during study M-532610-02-1 (Stuke, S.; van Berkum, S.; 2017). For the matrix relevant to this study (lily, leaf punch washings) a full set of additional validation recoveries was analyzed. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01158/M002 is suitable for the determination of the magnitude of the dislodgeable foliar residues (DFR) of CGA 279202 and CGA 321113 in washings from lily leaf punches via HPLC-MS/MS.

A 2.2.1.5.4 Analytical method 01158/M002 in support of the study M-677729-01-1

A 2.2.1.5.4.1 Method validation

Comments of zRMS:	Analytical Method: Stuke, S., van Berkum, S., Modification 002 of analytical method 01158 for the determination of tebuconazole, fluopyram and trifloxystrobin in leaf punches washing solution by HPLC-MS/MS, BAG report MR-15/032, dated 2015-09-07. The

	validity of this adapted methodology was ensured by 5 recoveries at the LOQ level, 5 recoveries at the 10x LOQ level and 5 recoveries at the 100x LOQ level. During each set of analysis, a calibration curve was established for trifloxystrobin with at least five concentration levels and used for the quantitation. For the calibration curve the correlation coefficient R was above 0.999. During each set of analysis, a calibration curve was established for AE C656948 with at least five concentration levels and used for the quantitation. For the calibration curve the correlation coefficient R was above 0.999. No residues of trifloxystrobin above the LOQ were found in the control samples. No residues of AE C656948 above the LOQ were found in the control samples. The mean of the concurrent laboratory recoveries for trifloxystrobin amounted to 96% with a relative standard deviation of 2.3%. The mean of the concurrent laboratory recoveries for AE C656948 amounted to 99% with a relative standard deviation of 6.7%. The mean of the field recovery samples for trifloxystrobin amounted to 69% with a relative standard deviation of 4.8%. The mean of the field recovery samples for AE C656948 amounted to 94% with a relative standard deviation of 4.2%. All criteria according to SANCO/3029/99 rev. 4 were met. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.4/04**
Title:	Determination of the dislodgeable foliar residues (DFR) of trifloxystrobin and AE C656948 in/on raspberry after spray application of AE C656948 & CGA279202 SC 500 in the field in Italy
Report:	Daniels, M. ; van Berkum, S.; 2020; 18-2905; M-677729-01-1
Authority registration No:	
Guideline(s):	US EPA OPPTS 875.2100 Foliar Dislodgeable Residue Dissipation
Deviations:	Yes (see report)
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The magnitude of the dislodgeable foliar residues (DFR) of the substances AE C656948 (FLU) and trifloxystrobin (TFS) in washings from raspberry leaf punches was determined after two spray applications with the suspension concentrate formulation AE C656948 & CGA279202 SC 500 (containing 250 g/L AE C656948 and 250 g/L trifloxystrobin).

Full validation data is documented with the method 01158/M002 (Stuke, S.; van Berkum, S.; 2017; M-532610-02-1) for CGA 279202. For the matrix relevant to this study (raspberry, leaf punch washings), a full set of additional validation recoveries was performed within the present study.

The test item was extracted from the leaf punches by adding 100 mL of a 0.01% Aerosol OT solution (i.e. docusate sodium salt) which corresponds to a surfactant. After shaking, the solution was decanted and the dislodging procedure was repeated for each sample with a fresh 100 mL aliquot of the Aerosol solution. The second rinse was again decanted and added to the first, then the sample was frozen.

Conditions used in this study:

The following presented sample preparation scheme also considers experiences during past DFR studies with the active compound trifloxystrobin which showed the tendency to adhere to plastic surfaces (especially to the plastic surface of field spike pre-solution vials). To avoid those adherences the plastic bottles (and field spike vials inside) are flushed with pure acetonitrile and subsequently with pure dichloromethane according to the following described procedure.

200 mL thawed field sample is filled into a 500-mL volumetric flask and 10-mL thawed field spike or lab recovery sample is filled into a 25-mL volumetric flask. 100 mL of pure acetonitrile is added into the empty 200 mL plastic bottle (or 5 mL to the empty lab and field spike recoveries bottles). After ultra-sonicating, fill the solution into the corresponding volumetric flask. 100 mL of acetonitrile is added again into the field sample bottle (5 mL to the empty lab and field spike recoveries bottles). After ultra-sonicating, the washing

solutions are combined with the corresponding sample solution in the volumetric flask. 50 mL of pure dichloromethane is added into the empty 200-mL plastic bottle (or 5 mL to the empty lab and field spike recoveries bottles). After shaking, evaporate the dichloromethane fractions to dryness applying a vacuum at a temperature of 40°C. Re-dissolve the dry residues; the former 50-mL fractions with 2x 25 mL pure acetonitrile and the former 5-mL fractions with 2x 2 mL of pure acetonitrile. Combine the solutions with those in the corresponding volumetric flask. Fill the flask up to the mark with water. The total volume is 500 mL for field samples and 25 mL for lab or field spike recoveries (dilution factor of 2.5). Transfer an aliquot of 0.1 mL of the sample solution into a 1.8-mL HPLC vial, add 100 µL of an internal standard solution (containing 100 µg/L internal standard of each compound) and 0.8 mL of water. The total sample dilution factor is 25. Subject to Liquid Chromatography and MS/MS- determination. The final concentration at the LOQ is 0.8 µg/L in the final analytical extract which corresponds to 0.01 µg/cm^2.

Results and discussions

A full set of additional validation recoveries was analysed and 5 recoveries were determined each at fortification levels of 0.01 µg/cm^2 (LOQ), 0.10 µg/cm^2 (10xLOQ) and 1.0 µg/cm^2 (100xLOQ).
In order to check the performance of the method and the adaptation, at least five recovery determinations per fortification level were performed. Concurrent recoveries were included in each set of analysis (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.
Blank values in control samples were below 30% of the LOQ.
The average recoveries per fortification level were within the range of 70 – 110%, and the relative standard deviation (RSD) values were below 20%, if applicable (n ≥ 3).

Table A 99: Recovery rates and precision results (repeatability) of CGA 279202

Analyte	Crop/Sample Material	FL [µg/cm^2]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [µg/cm^2]
CGA 279202	raspberry / leaf punch washings	0.01	99; 99; 93; 98; 93	96	3.2	0.01
		0.10	96; 96; 95; 97; 99	97	1.6	
		1.0	99; 97; 93; 95; 97	96	2.4	
			Overall recovery (n = 15)	**96**	**2.3**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 100: Characteristics for the analytical method 01158/M002 used for validation of CGA 279202

	CGA 279202
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.034500 x + 0.00042172, Correlation coefficient r: 1.0000, number of data points: 7
Calibration range	0.2 to 100 µg/L (corresponding to 0.0025 – 1.25 µg/cm^2)
Limit of determination/quantification	LOQ = 0.8 µg/L when extracting 400 cm^2 corresponding to 0.01 µg/cm^2
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.

Conclusion

The analytical method 01158/M002 used in the present study for the determination of CGA 279202 in leaf punches washing solution was fully validated during study M-532610-02-1 (Stuke, S.; van Berkum, S.; 2017). For the matrix relevant to this study (raspberry, leaf punch washings) a full set of additional validation recoveries was analyzed. All criteria according to SANCO/3029/99 rev. 4 were met. The analytical method 01158/M002 is suitable for the determination of the magnitude of the dislodgeable foliar residues (DFR) of CGA 279202 in washings from raspberry leaf punches via HPLC-MS/MS.

A 2.2.1.6 Description of analytical methods for the determination of residues in ecotoxicology studies (KCP 5.1)

A 2.2.1.6.1 Analytical method EBTF0035 in support of the study M-637834-01-1

A 2.2.1.6.1.1 Method validation

Comments of zRMS:	The validation of the analytical method for the determination of trifloxystrobin in aqueous samples for an 'Acute toxicity test with *Brachionus calyciflorus* using UHPLC-MS/MS was successfully performed following the EU guideline SANCO/3029/99 rev.4 (11/07/00). The limit of quantification (LOQ) of the method for matrix charged water samples was 0.1 µg/L. The quantitative measurements of trifloxystrobin were achieved by Ultra-High Performance Liquid Chromatography (UHPLC) coupled to a triple quadrupole mass spectrometer (MS); the MS was operated in the tandem mass spectrometry mode (MS/MS). The basic calibration standards covered concentrations from 0.05 µg/L to 5.0 µg/L. As the calculated correlation coefficient (r^2) for trifloxystrobin was close to 1 [r^2 = 0.999703], the applicability of the linear calibration function was accepted. The mean recovery of each fortification level and the overall mean recovery value was between 70 – 110% with an RSD < 20%. LOQ = 0.1 µg/L The validation parameters are acceptable. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.6/06**
Title:	Trifloxystrobin - Acute toxicity test with Brachionus calyciflorus, basic test conditions following OECD TG 202
Report:	Kosak, L.; Hennecke, S.; 2018; EBTF0035; M-637834-01-1
Authority registration No:	
Guideline(s):	The basic test conditions were according to the OECD guideline 202 and EC method C.2
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The following method validation of the method EBTF0035 was carried out during the study M-637834-01-1. The analytical method describes the determination of trifloxystrobin in aqueous samples using UHPLC-MS/MS. In addition to the analysis of fortified samples for validation purposes, recoveries of quality control (QC) samples were determined to ensure the applicability of the method.

A sample volume of 5 mL of each sample were transferred in 15 mL PP-vials prepared with 5 mL methanol including 0.2 % formic acid. The samples of the test vessels were diluted with methanol / medium including 0.1 % formic acid (50:50 v:v) if necessary and analysed.

The water samples are analysed by direct injection in an HPLC-UV instrument after appropriate dilution.

The quantitative measurements of trifloxystrobin were achieved by Ultra-High Performance Liquid Chromatography (UHPLC) coupled to a triple quadrupole mass spectrometer (MS); the MS was operated in the tandem mass spectrometry mode (MS/MS).

Mass transitions

Substance	Parent ion [m/z]	Daughter ion [m/z]	Dwell [sec]	Cone voltage [V]	Collision energy [eV]
Trifloxystrobin (Quantifier)	409	186	0.163	30	15
Trifloxystrobin (Qualifier)	409	206	0.163	30	15

Results and discussions

Recovery rates were determined at fortification levels of 0.1 µg/L (LOQ) and 1.0 µg/L (10 x LOQ).

For each fortification level five separate fortification samples were prepared. In addition, two untreated

control samples (blank samples) were measured using UHPLC-MS/MS.
The mean recoveries and the overall mean recovery values are in the defined range between 70 – 110% with an RSD < 20% (n = 10). The results are presented in the following table.

Table A 101: Recovery/ repeatability results from method validation of trifloxystrobin

Fortification level		Analytical result, Cw	Recovery	Mean Recovery	RSD
Name	**Concentration in final sample**	**[µg/L]**	**[%]**	**[%]**	**[%]**
0	(Blank)	<LOQ	-	n.a.	n.a.
0		<LOQ	-		
LOQ	**0.1 µg/L** (LOQ level)	0.098	98.0	100.9	3.8
LOQ		0.099	99.1		
LOQ		0.105	104.5		
LOQ		0.097	97.4		
LOQ		0.105	105.5		
10x LOQ	**1.0 µg/L** (10x LOQ level)	0.963	96.3	96.9	2.5
10x LOQ		0.963	96.3		
10x LOQ		0.936	93.6		
10x LOQ		0.981	98.1		
10x LOQ		1.000	100.0		
Overall mean (2 fortification levels):				98.9	3.7

Cw = water concentration

In addition, six QC samples were analyzed during the study, six each at the 0.3 µg/L level and the 30.0 µg/L level. The individual recoveries ranged from 95.5% to 102.3%. The overall mean recovery of the six QC samples was at 98.6% with a corresponding RSD of 2.6%.

Table A 102: Recovery results from QC sample analysis for trifloxystrobin

Sample	Nominal conc. [µg/L]	Measured conc. [µg/L]	Relative recovery of nominal [%]
QC1	3.0	0.307	102.3
QC2	30.0	3.007	100.2
QC3	3.0	0.294	98.1
QC4	30.0	2.985	99.5
QC5	3.0	0.289	96.2
QC6	30.0	2.866	95.5
Overall mean recovery [%] (n = 6):			**98.6**
Overall mean RSD [%] (n = 6):			**2.6**

Table A 103: Characteristics for the analytical method for trifloxystrobin

	trifloxystrobin
Specificity	UHPLC-MS/MS method is highly specific. Blank values of analyte were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration line equation (1/x weighted): y = 6405.36 x - 36.5082, Correlation coefficient r^2 = 0.999703 number of data points: 9
Calibration range	0.05 µg/L to 5 µg/L
Limit of determination/quantification	LOQ = 0.1 µg/L
Assessment of matrix effects is presented	The LC-MS system used was capable to determine the analyte trifloxystrobin in the worked-up injection-solutions without interference of matrix compounds.

Conclusion
The analytical method complies with all guideline criteria according to guideline SANCO/3029/99 rev.4 and is suitable for the determination of trifloxystrobin in water samples via UHPLC-MS/MS.

A 2.2.1.6.2 Concurrent validation of method EBTF0035 in support of the study M-638530-01-1

Comments of zRMS:	The analytical method EBTF0035 for the determination of trifloxystrobin in test water by UHPLC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4 (11/07/00) by Kosak, L. and Hennecke, S. (2018; M-638530-01-1). In the present study the method was validated concurrently with the sample analyses of the study by evaluation of quality control (QC) samples. LOQ = 0.1 µg/L The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.6/07**
Title:	Trifloxystrobin - Acute toxicity test with Thamnocephalus platyurus, basic test conditions following OECD TG 202 - Report -
Report:	Kosak, L.; Hennecke, S.; 2018; EBTF0036; M-638530-01-1
Authority registration No:	
Guideline(s):	OECD guideline 202 and EC method C.2
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation
For the determination of trifloxystrobin in test water by UHPLC-MS/MS, the previously validated analytical method EBTF0035 (Kosak, L.; Hennecke, S.; 2018; M-637834-01-1) was used. In the present study the method was validated concurrently with the sample analyses of the study by evaluation of quality control (QC) samples.
The quantitative measurements of trifloxystrobin were achieved by Ultra-High Performance Liquid Chromatography (UHPLC) coupled to a triple quadrupole mass spectrometer (MS); the MS was operated in the tandem mass spectrometry mode (MS/MS).

Mass transitions

Substance	Parent ion [m/z]	Daughter ion [m/z]	Dwell [sec]	Cone voltage [V]	Collision energy [eV]
Trifloxystrobin (Quantifier)	409	186	0.163	30	15
Trifloxystrobin (Qualifier)	409	206	0.163	30	15

QC samples were run alongside each batch to insure the applicability of the calibration. Four QC samples were analysed during the study, two each at the 0.3 µg/L and 3.0 µg/L concentration levels. The individual recoveries for trifloxystrobin ranged from 99.9% to 104.3%. The overall mean recovery from 4 samples was 102.0% with an overall relative standard deviation (RSD) of 2.3%.

Table A 104: Recovery/ repeatability results from concurrent method validation of trifloxystrobin

Sample	Nominal conc. [µg/L]	Measured conc. [µg/L]	Relative recovery of nominal [%]
QC1	0.3	0.311	103.7
QC2	3.0	3.00	99.9

QC3	0.3	0.313	104.3
QC4	3.0	3.00	100.1
		Overall mean recovery [%] (n = 4):	102.0
		Overall mean RSD [%] (n = 4):	2.3

Table A 105: Characteristics for the analytical method for trifloxystrobin

	trifloxystrobin
Specificity	UHPLC-MS/MS method is highly specific. Blank values of analyte were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration line equation (1/x weighted): y = 6398.32 x – 36.5667, Correlation coefficient r^2 = 0.999417 number of data points: 9
Calibration range	0.05 µg/L to 5 µg/L
Limit of determination/quantification	LOQ = 0.1 µg/L
Assessment of matrix effects is presented	The LC-MS system used was capable to determine the analyte trifloxystrobin in the worked-up injection-solutions without interference of matrix compounds.

Conclusion

The applicability of the UHPLC-MS/MS method for the analysis of trifloxystrobin in water samples was tested. The data presented demonstrate that the method allows the determination of this substance with satisfactory precision data given as the overall mean relative standard deviation was below 5% of six measured replicates. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.2.1.6.3 Analytical method EBTF0039 in support of the study M-630875-02-1

A 2.2.1.6.3.1 Method validation

Comments of zRMS:	The validation of the analytical method for the determination of trifloxystrobin in aqueous samples for an 'Acute toxicity test with *Daphnia pulex*' was successfully performed following the EU guideline SANCO/3029/99 rev.4 (11/07/00). The limit of quantification (LOQ) of the method for matrix charged water samples was 0.1 µg/L. The quantitative measurements of trifloxystrobin were achieved by Ultra-High Performance Liquid Chromatography (UHPLC) coupled to a triple quadrupole mass spectrometer (MS); the MS was operated in the tandem mass spectrometry mode (MS/MS). The individual recoveries of trifloxystrobin ranged from 95.4% to 102.0%. The mean recovery for the fortification level 0.1 µg/L was 99.3% with a relative standard deviation (RSD) of 2.3% (n = 5). The mean recovery for the fortification level 1.0 µg/L was 97.4% with an RSD of 1.5% (n = 5). The overall mean recovery from 10 replicates was 98.4% with an overall RSD of 2.1%. LOQ = 0.1 µg/L The validation parameters are acceptable. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.6/08**
Title:	1st report amendment - Trifloxystrobin - Acute toxicity test with Daphnia pulex, basic test conditions following OECD TG 202
Report:	Kosak, L.; Hennecke, S.; 2019; EBTF0039; M-630875-02-1
Authority registration No:	
Guideline(s):	OECD 202 (13 April 2004): Guideline for Testing of Chemicals - Daphnia sp., Acute Immobilisation Test
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The following method validation of the method EBTF0039 was carried out during the study M-630875-02-1. Test media from the study were analyzed using UHPLC-MS/MS to determine the concentrations of the test item trifloxystrobin in aqueous samples. In addition to the analysis of fortified samples for validation purposes, recoveries of quality control (QC) samples were determined.

The quantitative measurements of trifloxystrobin were achieved by Ultra-High Performance Liquid Chromatography (UHPLC) coupled to a triple quadrupole mass spectrometer (MS); the MS was operated in the tandem mass spectrometry mode (MS/MS). The mass spectrometer was operated in the positive ionization mode using the mass transitions m/z 409 → 186 for the quantitation of trifloxystrobin and m/z 409 → 206 for confirmation. The water samples are analysed by direct injection in an UHPLC-MS/MS instrument after appropriate dilution.

Results and discussions

Recovery rates were determined at fortification levels of 0.1 µg/L (LOQ) and 1.0 µg/L (10 x LOQ). The recovery experiments were conducted by fortification of untreated control samples with defined amounts of the analytes prior to analysis.

The individual recoveries of trifloxystrobin ranged from 95.4% to 102.0%. The mean recovery for the fortification level 0.1 µg/L was 99.3% with a relative standard deviation (RSD) of 2.3% (n = 5). The mean recovery for the fortification level 1.0 µg/L was 97.4% with an RSD of 1.5% (n = 5). The overall mean recovery from 10 replicates was 98.4% with an overall RSD of 2.1%.

Table A 106: Recovery/ repeatability results from method validation of trifloxystrobin

Fortification level		**Analytical result, Cw**	**Recovery**	**Mean Recovery**	**RSD**
Name	**Concentration in final sample**	**[µg/L]**	**[%]**	**[%]**	**[%]**
0	(Blank)	<LOQ	-	n.a.	n.a.
0		<LOQ	-		
LOQ	**0.1 µg/L** (LOQ level)	0.098	97.7	99.3	2.3
LOQ		0.102	102.0		
LOQ		0.101	101.1		
LOQ		0.099	99.3		
LOQ		0.096	96.5		
10x LOQ	**1.0 µg/L** (10x LOQ level)	0.967	96.7	97.4	1.5
10x LOQ		0.990	99.0		
10x LOQ		0.984	98.4		
10x LOQ		0.976	97.6		
10x LOQ		0.954	95.4		
Overall mean (2 fortification levels):				**98.4**	**2.1**

In addition, 12 QC samples were analyzed during the study, six each at the 0.3 µg/L level and the 3.0 µg/L level. The individual recoveries ranged from 91.7% to 102.1%. The overall mean recovery of the 12 QC samples was at 97.2% with a corresponding RSD of 3.5%.

Table A 107: Recovery results from QC sample analysis for trifloxystrobin

Sample	Nominal conc. [µg/L]	Measured conc. [µg/L]	Relative recovery of nominal [%]
QC1	0.3	0.306	102.1
QC2	3.0	2.95	98.2
QC3	0.3	0.286	95.2
QC4	3.0	2.88	95.9
QC5	0.3	0.286	95.5
QC6	3.0	2.85	94.8
QC7	0.3	0.306	101.9
QC8	3.0	3.02	100.5
QC9	0.3	0.278	92.8
QC10	3.0	2.99	99.5
QC11	0.3	0.275	91.7
QC12	3.0	2.94	97.9
Overall mean recovery [%] (n = 12):			**97.2**
Overall mean RSD [%] (n = 12):			**3.5**

Table A 108: Characteristics for the analytical method used for concurrent validation of trifloxystrobin

	trifloxystrobin
Specificity	UHPLC-MS/MS method is highly specific. Blank values of analyte were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 11551 x – 23.6037, Correlation coefficient r: 0.997810 number of data points: 9
Calibration range	0.05 µg/L to 5.0 µg/L
Limit of determination/quantification	LOQ = 0.1 µg/L
Assessment of matrix effects is presented	The LC-MS system used was capable to determine the analyte trifloxystrobin in the worked-up injection-solutions without interference of matrix compounds.

Conclusion

The analytical method complies with all guideline criteria according to SANCO/3029/99 rev. 4 to determine trifloxystrobin in water samples and was successfully validated. Thus, this method can be regarded as fit for purpose.

A 2.2.1.6.4 Concurrent validation of method EBTF0039 in support of the study M-638527-01-1

Comments of zRMS:	The analytical method EBTF0039 for the determination of trifloxystrobin in test water by UHPLC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4 (11/07/00) by Kosak, L. and Hennecke, S. (2019; M-630875-02-1). In the present study the method was validated concurrently with the sample analyses of the study by evaluation of quality control (QC) samples. LOQ = 0.1 µg/L The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.6/09**
Title:	Trifloxystrobin - Acute toxicity test with Daphnia longispina, basic test conditions following OECD TG 202 - Report -
Report:	Hommen, U.; Hennecke, S.; 2018; EBTF0038; M-638527-01-1
Authority registration No:	
Guideline(s):	OECD guideline 202 and EC method C.2
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation

For the determination of trifloxystrobin in test water by UHPLC-MS/MS, the previously validated analytical method EBTF0039 (Kosak, L.; Hennecke, S.; 2019; M-630875-02-1) was used. In the present study the method was validated concurrently with the sample analyses of the study by evaluation of quality control (QC) samples.

The quantitative measurements of trifloxystrobin were achieved by Ultra-High Performance Liquid Chromatography (UHPLC) coupled to a triple quadrupole mass spectrometer (MS); the MS was operated in the tandem mass spectrometry mode (MS/MS).

Mass transitions

Substance	Parent ion [m/z]	Daughter ion [m/z]	Dwell [sec]	Cone voltage [V]	Collision energy [eV]
Trifloxystrobin (Quantifier)	409	186	0.163	30	15
Trifloxystrobin (Qualifier)	409	206	0.163	30	15

QC samples were run alongside each batch to insure the applicability of the calibration. Six QC samples were analysed during the study, three each at the 0.3 µg/L and 3.0 µg/L concentration levels. The individual recoveries for trifloxystrobin ranged from 94.4% to 99.8%. The overall mean recovery from 6 samples was 96.7% with an overall relative standard deviation (RSD) of 2.1%.

Table A 109: Recovery/ repeatability results from concurrent method validation of trifloxystrobin

Sample	Nominal conc. [µg/L]	Measured conc. [µg/L]	Relative recovery of nominal [%]
QC1	0.3	0.299	99.8
QC2	3.0	2.958	98.6
QC3	0.3	0.286	95.3
QC4	3.0	2.893	96.4
QC5	0.3	0.288	95.9
QC6	3.0	2.831	94.4
		Overall mean recovery [%] (n = 6):	**96.7**
		Overall mean RSD [%] (n = 6):	**2.1**

Table A 110: Characteristics for the analytical method used for concurrent validation of trifloxystrobin

	trifloxystrobin
Specificity and blanks	UHPLC-MS/MS method is highly specific. Blank values of analyte were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration line equation (1/x weighted): y = 4536.52 x + 117.931, correlation coefficient r^2 = 0.999429 number of data points: 9
Calibration range	0.05 µg/L to 5 µg/L
Limit of determination/quantification	LOQ = 0.1 µg/L

Assessment of matrix effects is presented	The LC-MS system used was capable to determine the analyte trifloxystrobin in the worked-up injection-solutions without interference of matrix compounds.

Conclusion
The applicability of the UHPLC-MS/MS method for the analysis of trifloxystrobin in test water was tested. The data presented demonstrate that the method allows the determination of this substance with satisfactory precision data given as the overall mean relative standard deviation was below 5% of six measured replicates. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.2.1.6.5 Concurrent validation of method EBTF0039 in support of the study M-638519-01-1

Comments of zRMS:	The analytical method EBTF0039 for the determination of trifloxystrobin in test water by UHPLC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4 (11/07/00) by Kosak, L. and Hennecke, S. (2019; M-630875-02-1). In the present study the method was validated concurrently with the sample analyses of the study by evaluation of quality control (QC) samples. LOQ = 0.1 µg/L The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.6/10**
Title:	Trifloxystrobin - Acute toxicity test with Chydorus spec., basic test conditions following OECD TG 202 - Report
Report:	Hommen, U.; Hennecke, S.; 2018; EBTF0040; M-638519-01-1
Authority registration No:	
Guideline(s):	OECD guideline 202 and EC method C.2
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation
For the determination of trifloxystrobin in test water by UHPLC-MS/MS, the previously validated analytical method EBTF0039 (Kosak, L.; Hennecke, S.; 2019; M-630875-02-1) was used. In the present study the method was validated concurrently with the sample analyses of the study by evaluation of quality control (QC) samples.

The quantitative measurements of trifloxystrobin were achieved by Ultra-High Performance Liquid Chromatography (UHPLC) coupled to a triple quadrupole mass spectrometer (MS); the MS was operated in the tandem mass spectrometry mode (MS/MS). The mass spectrometer was operated in the positive ionization mode using the mass transitions m/z 409 → 186 for the quantitation of trifloxystrobin and m/z 409 → 206 for confirmation. The water samples are analysed by direct injection in an UHPLC-MS/MS instrument after appropriate dilution.

QC samples were run alongside each batch to insure the applicability of the calibration. Eight QC samples were analysed during the study, four each at the 0.3 µg/L and 3.0 µg/L concentration levels.

The individual recoveries for trifloxystrobin ranged from 96.6% to 109.3%. The overall mean recovery from 8 samples was 103.1% with an overall relative standard deviation (RSD) of 4.7%.

Table A 111: Recovery/ repeatability results from concurrent method validation of trifloxystrobin

Sample	Nominal conc. [µg/L]	Measured conc. [µg/L]	Relative recovery of nominal [%]

QC1	0.3	0.316	105.5
QC2	3.0	3.113	103.8
QC3	0.3	0.325	108.3
QC4	3.0	2.991	99.7
QC5	0.3	0.328	109.3
QC6	3.0	3.128	104.3
QC7	0.3	0.291	97.1
QC8	3.0	2.899	96.6
		Overall mean recovery [%] (n = 8):	**103.1**
		Overall mean RSD [%] (n = 8):	**4.7**

Table A 112: Characteristics for the analytical method used for concurrent validation of trifloxystrobin

	trifloxystrobin
Specificity	UHPLC-MS/MS method is highly specific. Blank values of analyte were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 4306.78 x + 44.7853, Correlation coefficient r: 0.999581 number of data points: 9
Calibration range	0.05 µg/L to 5.0 µg/L
Limit of determination/quantification	LOQ = 0.1 µg/L
Assessment of matrix effects is presented	The LC-MS system used was capable to determine the analyte tri-floxystrobin in the worked-up injection-solutions without interference of matrix compounds.

Conclusion

The applicability of the UHPLC-MS/MS method for the analysis of trifloxystrobin in water samples was tested. The data presented demonstrate that the method allows the determination of this substance with satisfactory precision data given as the overall mean relative standard deviation was below 5% of eight measured replicates. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.2.1.6.5.1 Concurrent validation of method EBTF0039 in support of the study M-638524-01-1

Comments of zRMS:	The analytical method EBTF0039 for the determination of trifloxystrobin in test water by UHPLC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4 (11/07/00) by Kosak, L. and Hennecke, S. (2019; M-630875-02-1). In the present study the method was validated concurrently with the sample analyses of the study by evaluation of quality control (QC) samples. LOQ = 0.1 µg/L The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.6/11**
Title:	Trifloxystrobin - Acute toxicity test with Cyclopoidae, basic test conditions following OECD TG 202 - Report -
Report:	Kosak, L.; Hennecke, S.; 2018; EBTF0041; M-638524-01-1
Authority registration No:	
Guideline(s):	OECD guideline 202 and EC method C.2
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation

For the determination of trifloxystrobin in test water by UHPLC-MS/MS, the previously validated analytical method EBTF0039 (Kosak, L.; Hennecke, S.; 2019; M-630875-02-1) was used. In the present study the method was validated concurrently with the sample analyses of the study by evaluation of quality control (QC) samples.

The quantitative measurements of trifloxystrobin were achieved by Ultra-High Performance Liquid Chromatography (UHPLC) coupled to a triple quadrupole mass spectrometer (MS); the MS was operated in the tandem mass spectrometry mode (MS/MS). The mass spectrometer was operated in the positive ionization mode using the mass transitions m/z 409 → 186 for the quantitation of trifloxystrobin and m/z 409 → 206 for confirmation. The water samples are analysed by direct injection in an UHPLC-MS/MS instrument after appropriate dilution.

QC samples were run alongside each batch to insure the applicability of the calibration. Six QC samples were analysed during the study, three each at the 0.3 µg/L and 3.0 µg/L concentration levels.

The individual recoveries for trifloxystrobin ranged from 95.8% to 106.4%. The overall mean recovery from 6 samples was 102.1% with an overall relative standard deviation (RSD) of 4.5%.

Table A 113: Recovery/ repeatability results from concurrent method validation of trifloxystrobin

Sample	Nominal conc. [µg/L]	Measured conc. [µg/L]	Relative recovery of nominal [%]
QC1	0.3	0.291	97.0
QC2	3.0	2.873	95.8
QC3	0.3	0.317	105.6
QC4	3.0	3.100	103.3
QC5	0.3	0.319	106.4
QC6	3.0	3.137	104.6
		Overall mean recovery [%] (n = 6):	**102.1**
		Overall mean RSD [%] (n = 6):	**4.5**

Table A 114: Characteristics for the analytical method used for concurrent validation of trifloxystrobin

	trifloxystrobin
Specificity	UHPLC-MS/MS method is highly specific. Blank values of analyte were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 4134.38 x + 76.2201, Correlation coefficient r: 0.999863 number of data points: 9
Calibration range	0.05 µg/L to 5.0 µg/L
Limit of determination/quantification	LOQ = 0.1 µg/L
Assessment of matrix effects is presented	The LC-MS system used was capable to determine the analyte trifloxystrobin in the worked-up injection-solutions without interference of matrix compounds.

Conclusion
The applicability of the UHPLC-MS/MS method for the analysis of trifloxystrobin in water samples was tested. The data presented demonstrate that the method allows the determination of this substance with satisfactory precision data given as the overall mean relative standard deviation was below 5% of six measured replicates. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.2.1.6.6 Concurrent validation of method EBTF0039 in support of the study M-637890-01-1

Comments of zRMS:	The analytical method EBTF0039 for the determination of trifloxystrobin in test water by UHPLC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4 (11/07/00) by Kosak, L. and Hennecke, S. (2019; M-630875-02-1). In the present study the method was validated concurrently with the sample analyses of the study by evaluation of quality control (QC) samples. LOQ = 0.1 µg/L The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.6/12**
Title:	Amendment no. 01: Trifloxystrobin - Acute toxicity test with Chaoborus crystallinus, basic test conditions following OECD TG 202
Report:	Kosak, L.; Hennecke, S.; 2020; EBTF0042; M-637890-02-1
Authority registration No:	
Guideline(s):	OECD guideline 202 and EC method C.2
Deviations:	Not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation
For the determination of trifloxystrobin in test water by UHPLC-MS/MS, the previously validated analytical method EBTF0039 (Kosak, L.; Hennecke, S.; 2019; M-630875-02-1) was used. In the present study the method was validated concurrently with the sample analyses of the study by evaluation of quality control (QC) samples.
The quantitative measurements of trifloxystrobin were achieved by Ultra-High Performance Liquid Chromatography (UHPLC) coupled to a triple quadrupole mass spectrometer (MS); the MS was operated in the tandem mass spectrometry mode (MS/MS). The mass spectrometer was operated in the positive ionization mode using the mass transitions m/z 409 → 186 for the quantitation of trifloxystrobin and m/z 409 → 206 for confirmation. The water samples are analysed by direct injection in an UHPLC-MS/MS instrument after appropriate dilution.
QC samples were run alongside each batch to insure the applicability of the calibration. Six QC samples were analysed during the study, three each at the 0.3 µg/L and 3.0 µg/L concentration levels.
The individual recoveries for trifloxystrobin ranged from 101.7% to 115.9%. The overall mean recovery from 6 samples was 108.7% with an overall relative standard deviation (RSD) of 4.4%.

Table A 115: Recovery/ repeatability results from concurrent method validation of trifloxystrobin

Sample	Nominal conc. [µg/L]	Measured conc. [µg/L]	Relative recovery of nominal [%]
QC1	0.3	0.318	106.1
QC2	3.0	3.052	101.7
QC3	0.3	0.325	108.4
QC4	3.0	3.256	108.5

QC5	0.3	0.348	115.9
QC6	3.0	3.347	111.6
		Overall mean recovery [%] (n = 6):	**108.7**
		Overall mean RSD [%] (n = 6):	**4.4**

Table A 116: Characteristics for the analytical method used for concurrent validation of trifloxystrobin

	trifloxystrobin
Specificity	UHPLC-MS/MS method is highly specific. Blank values of analyte were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 4331.58 x + 115.581, Correlation coefficient r: 0.999890 number of data points: 9
Calibration range	0.05 µg/L to 5.0 µg/L
Limit of determination/quantification	LOQ = 0.1 µg/L
Assessment of matrix effects is presented	The LC-MS system used was capable to determine the analyte trifloxystrobin in the worked-up injection-solutions without interference of matrix compounds.

Conclusion
The applicability of the UHPLC-MS/MS method for the analysis of trifloxystrobin in test water was tested. The data presented demonstrate that the method allows the determination of this substance with satisfactory precision data given as the overall mean relative standard deviation was below 5% of six measured replicates. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.2.1.6.7 Concurrent validation of method EBTF0039 in support of the study M-637847-01-1

Comments of zRMS:	The analytical method EBTF0039 for the determination of trifloxystrobin in test water by UHPLC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4 (11/07/00) by Kosak, L. and Hennecke, S. (2019; M-630875-02-1). In the present study the method was validated concurrently with the sample analyses of the study by evaluation of quality control (QC) samples. LOQ = 0.1 µg/L The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.6/13**
Title:	Trifloxystrobin - Acute toxicity test with Baetis rhodani, basic test conditions following OECD TG 202
Report:	Kosak, L.; Hennecke, S.; 2018; EBTF0043; M-637847-01-1
Authority registration No:	
Guideline(s):	OECD guideline 202 and EC method C.2
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation
For the determination of trifloxystrobin in test water by UHPLC-MS/MS, the previously validated analytical method EBTF0039 (Kosak, L.; Hennecke, S.; 2019; M-630875-02-1) was used. In the present study the method was validated concurrently with the sample analyses of the study by evaluation of quality control (QC) samples.

The quantitative measurements of trifloxystrobin were achieved by Ultra-High Performance Liquid Chromatography (UHPLC) coupled to a triple quadrupole mass spectrometer (MS); the MS was operated in the tandem mass spectrometry mode (MS/MS). The mass spectrometer was operated in the positive ionization mode using the mass transitions m/z 409 → 186 for the quantitation of trifloxystrobin and m/z 409 → 206 for confirmation. The water samples are analysed by direct injection in an UHPLC-MS/MS instrument after appropriate dilution.
QC samples were run alongside each batch to insure the applicability of the calibration. Six QC samples were analysed during the study, three each at the 0.3 µg/L and 3.0 µg/L concentration levels.
The individual recoveries for trifloxystrobin ranged from 94.5% to 102.2%. The overall mean recovery from 6 samples was 98.4% with an overall relative standard deviation (RSD) of 2.9%.

Table A 117: Recovery/ repeatability results from concurrent method validation of trifloxystrobin

Sample	Nominal conc. [µg/L]	Measured conc. [µg/L]	Relative recovery of nominal [%]
QC1	0.3	0.284	94.5
QC2	3.0	2.97	99.1
QC3	0.3	0.293	97.5
QC4	3.0	3.07	102.2
QC5	0.3	0.289	96.3
QC6	3.0	3.03	100.9
		Overall mean recovery [%] (n = 6):	**98.4**
		Overall mean RSD [%] (n = 6):	**2.9**

Table A 118: Characteristics for the analytical method used for concurrent validation of trifloxystrobin

	trifloxystrobin
Specificity	UHPLC-MS/MS method is highly specific. Blank values of analyte were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 4650.13 x – 83.0797, Correlation coefficient r: 0.999536 number of data points: 9
Calibration range	0.05 µg/L to 5.0 µg/L
Limit of determination /quantification	LOQ = 0.1 µg/L
Assessment of matrix effects is presented	The LC-MS system used was capable to determine the analyte tri-floxystrobin in the worked-up injection-solutions without interference of matrix compounds.

Conclusion

The applicability of the UHPLC-MS/MS method for the analysis of trifloxystrobin in water samples was tested. The data presented demonstrate that the method allows the determination of this substance with satisfactory precision data given as the overall mean relative standard deviation was below 3% of six measured replicates. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.2.1.6.8 Concurrent validation of method EBTF0039 in support of the study M-638529-01-1

Comments of zRMS:	The analytical method EBTF0039 for the determination of trifloxystrobin in test water by UHPLC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4 (11/07/00) by Kosak, L. and Hennecke, S. (2019; M-630875-02-1). In the present study the method was validated concurrently with the sample analyses of the study by evaluation of quality control (QC) samples. LOQ = 0.1 µg/L The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.6/14**
Title:	Trifloxystrobin - Acute toxicity test with Gammarus sp., basic test conditions following OECD TG 202 - Report -
Report:	Kosak, L.; Hennecke, S.; 2018; EBTF0044; M-638529-01-1
Authority registration No:	
Guideline(s):	the OECD guideline 202 and EC method C.2
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation

For the determination of trifloxystrobin in test water by UHPLC-MS/MS, the previously validated analytical method EBTF0039 (Kosak, L.; Hennecke, S.; 2019; M-630875-02-1) was used. In the present study the method was validated concurrently with the sample analyses of the study by evaluation of quality control (QC) samples.

The quantitative measurements of trifloxystrobin were achieved by Ultra-High Performance Liquid Chromatography (UHPLC) coupled to a triple quadrupole mass spectrometer (MS); the MS was operated in the tandem mass spectrometry mode (MS/MS). The mass spectrometer was operated in the positive ionization mode using the mass transitions m/z 409 → 186 for the quantitation of trifloxystrobin and m/z 409 → 206 for confirmation. The water samples are analysed by direct injection in an UHPLC-MS/MS instrument after appropriate dilution.

QC samples were run alongside each batch to insure the applicability of the calibration. Six QC samples were analysed during the study, three each at the 0.3 µg/L and 3.0 µg/L concentration levels.

The individual recoveries for trifloxystrobin ranged from 97.0% to 100.9%. The overall mean recovery from 6 samples was 98.95% with an overall relative standard deviation (RSD) of 1.5%.

Table A 119: Recovery/ repeatability results from concurrent method validation of trifloxystrobin

Sample	Nominal conc. [µg/L]	Measured conc. [µg/L]	Relative recovery of nominal [%]
QC1	0.3	0.291	97.0
QC2	3.0	2.977	99.2
QC3	0.3	0.293	97.5
QC4	3.0	3.026	100.9
QC5	0.3	0.298	99.4
QC6	3.0	2.990	99.7
Overall mean recovery [%] (n = 6):			**98.95**
Overall mean RSD [%] (n = 6):			**1.5**

Table A 120: Characteristics for the analytical method used for concurrent validation of trifloxystrobin

	trifloxystrobin
Specificity	UHPLC-MS/MS method is highly specific. Blank values of analyte were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 4024.26 x + 107.009, Correlation coefficient r: 0.999033 number of data points: 9
Calibration range	0.05 µg/L to 5.0 µg/L
Limit of determination /quantification	LOQ = 0.1 µg/L
Assessment of matrix effects is presented	The LC-MS system used was capable to determine the analyte trifloxystrobin in the worked-up injection-solutions without interference of matrix compounds.

Conclusion

The applicability of the UHPLC-MS/MS method for the analysis of trifloxystrobin in test water was tested. The data presented demonstrate that the method allows the determination of this substance with satisfactory precision data given as the overall mean relative standard deviation was below 2% of six measured replicates. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.2.1.6.9 Analytical method in support of the study M-670324-02-1

A 2.2.1.6.9.1 Method validation

Comments of zRMS:	The analytical method was validated for the determination of concentration the test item, metabolite of trifloxystrobin (BCS-AL58660), in the test medium by LC-MS/MS. Final analysis: Analysis by HPLC with MS/MS detection. Limit of quantification: 0.1 mg test item/L (99%, n = 5, RSD 1%) Limit of detection: 0.04 µg test item/L Linearity: Calibration Range: 2 – 30 µg test item /L Linearity of Response: Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression Correlation Coefficient: r = 1.0000 Calibration Curve: y = 7094 * x – 170 Specificity: No interference of total peak area for the target analyte was found. Accuracy and Precision: Mean Recovery Rates in the Fortified Samples: 102% (n = 10, RSD 4%). The values found for the precision (RSD) and for the accuracy (mean recovery rate) are acceptable. The validation parameters are acceptable. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.6/15**
Title:	Metabolite of trifloxystrobin: BCS-AL58660: Influence to Daphnia magna in a semi-static reproduction test - 1st final report amendment
Report:	xxx
Authority registration No:	
Guideline(s):	Commission Regulation (EC) No 440/2008, Annex, Part C, C.20.: "Daphnia magna Reproduction Test", Official Journal of the European Union (EN), dated May 30, 2008 EPA Guideline 712-C-16-005: OCSPP 850.1300, "Daphnid Chronic Toxicity Test", October 2016 EPA Guideline 712-C-16-014: OCSPP 850.1000, "Background and Special Considerations-Tests with Aquatic and Sediment-Dwelling Fauna and Aquatic Microcosms", October 2016 OECD Guideline for Testing of Chemicals, No. 211: "Daphnia magna Reproduction Test", adopted October 02, 2012 SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of Directive 91/414
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the analytical part of this study was to verify the concentrations of the test item, metabolite of trifloxystrobin (BCS-AL58660), in the test medium by LC-MS/MS.
After appropriate dilution, the samples were centrifuged (13,000 rpm, 3 minutes) and then analysed.
The determination was conducted using high performance liquid chromatography (HPLC) with mass-spectrometric (MS/MS) detection (ESI positive, MRM m/z: 395 → 186).

Results and discussions

For BCS-AL58660 recoveries were performed in test medium spiked with test item at the fortification levels of 0.1 mg test item/L (LOQ) and at 13 mg test item/L. The individual recovery values at the low fortification level ranged between 98 and 100% with a mean of 99%. The corresponding relative standard deviation (RSD) was 1% (n = 5). The individual recovery values at the high fortification level ranged between 102 and 108% with a mean of 105%. The corresponding relative standard deviation (RSD) was 2% (n = 5). The overall mean recovery was 102% with a corresponding relative standard deviation (RSD) of 4% (n = 10).

Table A 121: Recovery rates and precision results (repeatability) of BCS-AL58660

	Concentration					
Sample description	**Nominal [mg test item/L]**	**Found [µg metabolite/L]**	**DF**	**Concentration calculated [µg metabolite/L]**	**Corrected nominal [µg metabolite/L]**	**Recovery [%]**
Anayltical Blank	0	<LOD	1.25	n.a.	0.000	n.a.
Anayltical Blank	0	<LOD	1.25	n.a.	0.000	n.a.
Fortified Sample	0.1	9.822	10	98.217	99.894	98
	0.1	9.949	10	99.485	99.894	100
	0.1	9.892	10	98.921	99.795	99
	0.1	9.836	10	98.358	99.795	99
	0.1	9.808	10	98.076	99.795	98
					Mean value (n = 5):	**99**
					RSD (n = 5):	**1**
Fortified Sample	13	18.632	750	13974.361	12986.27	108
	13	18.210	750	13657.172	12986.27	105
	13	18.492	750	13868.631	12973.38	107
	13	17.646	750	13234.252	12973.38	102

	13	18.210	750	13657.172	12973.38	105
					Mean value (n = 5):	**105**
					RSD (n = 5):	**2**
					Overall mean value (n = 10):	**102**
					RSD (n = 10):	**4**

LOD: Limit of Detection = 0.04 µg test item/L
n.a.: not applicable
RSD: Relative Standard Deviation
DF: Dilution factor

Table A 122: Characteristics for the analytical method used for validation of BCS-AL58660

	BCS-AL58660
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 7094 x – 170, Correlation coefficient r: 1.0000 number of data points: 7
Calibration range	2 - 30 µg test item/L (corresponds to 0.002 – 0.03 mg test item/L)
Limit of determination/quantification	LOQ = 0.1 mg test item/L
Assessment of matrix effects is presented	No effects observed.

Conclusion
The analytical method complies with all guideline criteria according to SANCO/3029/99 rev. 4. It was validated successfully and can be seen as fit for purpose with regard to the presented study.

A 2.2.1.6.10 Analytical method in support of the study M-630580-01-2

A 2.2.1.6.10.1 Method validation

Comments of zRMS:	Water and sediment specimens from an ecotoxicity study were analysed for their content of the test item AE 1344138. The specimens were derived from a study which was conducted to determine the chronic toxicity of the test item to the sediment dweller *Lumbriculus variegatus.* The analytical method was validated according to SANCO/3029/99 rev.4. Final analysis: Analysis by HPLC with MS/MS detection. Limit of quantification: LOQ was 5 µg/L for water and 0.1 mg/kg for sediment. Limit of detection: LOD was defined as 30% of the LOQ, i.e. 1.5 µg/L for water and 0.03 mg/kg for sediment. Precision: The relative standard deviations per fortification level were 0.8% to 5.8 % (quantifier SRM) for AE 1344138 in water and 5.6% to 9.1% in sediment. Linearity: The calibration graph for AE 1344138 in water was linear from 1 µg/L - 200 µg/L with a correlation coefficient of r ≥ 0.9985 (for the quantifier). The calibration graph for AE 1344138 in sediment was linear from 5 µg/L - 500 µg/L with a correlation coefficient of r ≥ 0.9996 (for the quantifier). Specificity: AE 1344138 parent compound with mass transitions SRM 395 → 145 (used for quantification), SRM 395 → 186 (used for confirmation). Blanks: Analysis of control water and sediment specimens with HPLCMS/ MS during method validation yielded no residues above 30 % of the LOQ, indicating that no significant interferences were present. Accuracy (Recovery): Fortification experiments for water were performed at the limit of quantification of 5 µg/L and at 5000 µg/L and 25000 µg/L. Fortification experiments for sediment were performed at the limit of quantification of 0.1 mg/kg and at 150 mg/kg. Mean recovery values obtained by HPLC-MS/MS for AE 1344138 at the fortification levels comply with the standard acceptance criteria of SANCO/3029/99 rev.4. The study is acceptable.

Reference:	**KCP 5.1.2.6/16**
Title:	A study on the chronic toxicity to the sediment dweller Lumbriculus variegatus - AE 1344138, technical
Report:	Egeler, P.; Witte, A.; 2018; 18P6LA; M-630580-01-2
Authority registration No:	
Guideline(s):	OECD Guideline 225, "Sediment-water Lumbriculus toxicity test using spiked sediment", October 2007
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The analytical method was developed for the determination of AE 1344138 in test medium (overlying water and sediment) deriving from a toxicity test to the sediment dweller *Lumbriculus variegatus* (Egeler, P.; Witte, A.; 2018; M-630580-01-2) with accuracy, precision and repeatability.

Water samples were analysed by direct injection into the HPLC-MS/MS instrument without further clean-up. Sediment samples were extracted with acetonitrile/water and phase is separated by addition of sodium chloride. Thereafter, filtration of acetonitrile extract took place. Finally, the samples were analysed by HPLC-MS/ MS.

Identification and quantitation of AE 1344138 was performed by HPLC using MS/MS detection in the multiple reaction monitoring (MRM) mode and external matrix-matched standard solutions. A second MRM transition was used for confirmation.

The following MRM transitions were used for quantitation and confirmation of AE 1344138:

m/z 395 → *m/z* 145 (quantitation)

m/z 395 → *m/z* 186 (confirmation)

Results and discussions

Fortification experiments for water were performed at the limit of quantification of 5 µg/L and at 5000 µg/L and 25000 µg/L. The fortification level at 25000 µg/L was additionally tested to cover the highest concentrations found in the test specimens of the ecotoxicity test. The water samples were used directly for analysis by HPLC-MS/MS. The amount of acetonitrile added in the samples and standard solutions was identical. All concentrations for determination of concentrations in water samples were calculated on the water volume of the sample / standard solution, therefore the dilution step cause by addition of acetonitrile has not to be considered. The concentrations determined in the final samples are therefore equivalent to the concentrations of the test solutions.

Fortification experiments for sediment were performed at the limit of quantification of 0.1 mg/kg and at 150 mg/kg. If necessary, final extracts were diluted with control sediment extract to achieve final concentrations falling within the calibrated range of detector response.

The following recoveries were obtained with HPLC-MS/MS for samples fortified with AE 1344138:

Table A 123: Results of accuracy, precision and repeatability in water

Matrix	Fortification Level [µg/L]	Recoveries Single Values [%]	Mean [%]	RSD [%]	No. of Analyses
AE 1344138 SRM 395 → 145 (quantification)					
water*	5	93 / 93 / 94 / 103 / 104	97	5.8	5
	5000	106 / 108 / 108 / 110 / 110	108	1.5	5
	25000	105 / 107 / 105 / 106 / 106	106	0.8	5

RSD = Relative Standard Deviation * test medium from ecotoxicity test

Table A 124: Results of accuracy, precision and repeatability in sediment

Matrix	Fortification Level	Recoveries Single Values	Mean	RSD	No. of Analyses

	[mg/kg]	[%]	[%]	[%]	
AE 1344138 SRM 395 → 145 (quantification)					
sediment*	0.1	105 / 98 / 90 / 96 / 96	97	5.6	5
	150	96 / 110 / 87 / 91 / 97	96	9.1	5

RSD = Relative Standard Deviation; * from ecotoxicity test

Table A 125: Characteristics for the analytical method used for validation of AE 1344138 residues in test water and sediment

	AE 1344138
Specificity	Analysis of control water and sediment specimens with HPLCMS/ MS during method validation yielded no residues above 30 % of the LOQ, indicating that no significant interferences were present.
Calibration (type, number of data points)	individual calibration data is presented calibration line equations are presented: m/z 145: y = 8.10288e+006*x, r^2 = 0.9970 (water) m/z 145: y = 8.44475e+006*x, r^2 = 0.9992 (sediment) number of data points: 6
Calibration range	1 – 200 µg/L (water) 5 – 500 µg/L (sediment)
Assessment of matrix effects is presented	The MS/MS detection of AE 1344138 was not affected by the matrix.
Limit of determination/quantification	LOQ = 5 µg/L (water) LOQ = 0.1 mg/kg (sediment)

Conclusion

The method was successfully validated according to the guideline SANCO/3029/99 rev.4. and is suitable for the determination of AE 1344138 in test water and sediment samples via HPLC-MS/MS. The method was used in the present study and can be regarded as fit for purpose.

A 2.2.1.6.11 Analytical method in support of the study M-670322-02-1

A 2.2.1.6.11.1 Method validation

Comments of zRMS:	The analytical method was validated for the determination of concentration of metabolite of trifloxystrobin (BCS-AR14200 or CGA 357261) in test medium by HPLC-MS/MS using two MRM transitions. Specificity: No interference of total peak area for the target analyte was found. Linearity: Calibration Range: Two calibration curves were used in order to cover the wide concentration range of 0.1 – 12 µg test item/L with high accuracy. 1. 0.6 – 12 µg test item/L 2. 0.1 – 6 µg test item/L Linearity of Response: Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression Correlation Coefficient: 1. r = 1.0000 2. r = 1.0000 Calibration Curves: 1. y = 14263 * x + 406 2. y = 14433 * x + 87 LOQ: 2 µg test item/L Accuracy and Precision: The individual recovery values at 0.002 mg test item/L ranged between 68 and 70% with a mean of 70% with RSD of 1% (n = 5). The individual recovery values at 2 mg test item/L ranged between 104 and 112% with a mean of 107% with RSD of 3% (n = 5). Mean Recovery Rates in the Fortified Samples: 88% (n = 10, RSD 22%) The values found for the precision (RSD of each individual fortification level) and for the accuracy (mean recovery rate) are acceptable. The validation parameters are acceptable. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.6/17**
Title:	Metabolite of trifloxystrobin: BCS-AR14200 - Influence to Daphnia magna in a semi-static reproduction test -1st final report amendment
Report:	xxxx
Authority registration No:	
Guideline(s):	Commission Regulation (EC) No 440/2008, Annex, Part C, C.20.: "Daphnia magna Reproduction Test", Official Journal of the European Union (EN), dated May 30, 2008 EPA Guideline 712-C-16-005: OCSPP 850.1300, "Daphnid Chronic Toxicity Test", October 2016 EPA Guideline 712-C-16-014: OCSPP 850.1000, "Background and Special Considerations-Tests with Aquatic and Sediment-Dwelling Fauna and Aquatic Microcosms", October 2016 OECD Guideline for Testing of Chemicals, No. 211: "Daphnia magna Reproduction Test", adopted October 02, 2012 OECD Series on Testing and Assessment, No. 23, "Guidance Document on Aqueous-phase Aquatic Toxicity Testing of Difficult Test Chemicals", 2nd Ed., February 08, 2019 SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of Directive 91/414
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the analytical part of this study was to verify the concentrations of the test item, metabolite of trifloxystrobin (BCS-AR14200 or CGA 357261), in the test medium by LC-MS/MS.
After appropriate dilution, the samples were centrifuged (13,000 rpm, 3 minutes) and then analysed.
The determination was conducted using high performance liquid chromatography (HPLC) with mass-spectrometric (MS/MS) detection (ESI positive, mass transitions m/z 409 → 206 for the quantitation of CGA 357261 and m/z 409 → 116 for confirmation).

Results and discussions

For CGA 357261 recoveries were performed in test medium spiked with test item at the fortification levels of 0.002 mg test item/L (LOQ) and at 2 mg test item/L. The individual recovery values at the low fortification level ranged between 68 and 70% with a mean of 70%. The corresponding relative standard deviation (RSD) was 1% (n = 5). The individual recovery values at the high fortification level ranged between 104 and 112% with a mean of 107%. The corresponding RSD was 3% (n = 5). The overall mean recovery was 88% with a corresponding RSD of 22% (n = 10).

Table A 126: Recovery rates and precision results (repeatability) of CGA 357261

	Concentration					
Sample description	**Nominal [mg test item/L]**	**Found [µg metabolite/L]**	**DF**	**Concentration calculated [µg metabolite/L]**	**Corrected nominal [µg metabolite/L]**	**Recovery [%]**
Anayltical Blank	0	<LOD	1.25	n.a.	0.000	n.a.
Anayltical Blank	0	<LOD	1.25	n.a.	0.000	n.a.
Fortified Sample	0.002	1.019	1.25	1.274	1.845	69
	0.002	1.033	1.25	1.292	1.845	70
	0.002	1.012	1.25	1.266	1.850	68
	0.002	1.033	1.25	1.292	1.850	70
	0.002	1.040	1.25	1.300	1.850	70
					Mean value (n = 5):	**70**
					RSD (n = 5):	**1**
	2	6.127	312.5	1914.702	1844.892	104

Fortified Sample	2	6.302	312.5	1969.474	1844.892	107
	2	6.246	312.5	1951.947	1850.430	105
	2	6.632	312.5	2072.445	1850.430	112
	2	6.225	312.5	1945.374	1850.430	105
					Mean value (n = 5):	**107**
					RSD (n = 5):	**3**
					Overall mean value (n = 10):	**88**
					RSD (n = 10):	**22**

LOD: Limit of Detection = 0.04 µg test item/L
n.a.: not applicable
RSD: Relative Standard Deviation
DF: Dilution factor

Table A 127: Characteristics for the analytical method used for validation of CGA 357261

	CGA 357261	
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.	
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted) for the lower range: y = 14433 x + 87, Correlation coefficient r: 1.0000 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted) for the higher range: y = 14264 x + 406, Correlation coefficient r: 1.0000 number of data points: 6
Calibration range	0.1 - 6 µg test item/L	0.6 - 12 µg test item/L
Limit of determination/quantification	LOQ = 2 µg test item/L	
Assessment of matrix effects is presented	No effects observed.	

Conclusion
The analytical method complies with all guideline criteria according to SANCO 3029/99 rev. 4. It was validated successfully and can be seen as fit for purpose for the presented study.

A 2.2.1.6.12 Analytical method 01555

A 2.2.1.6.12.1 Method validation

Comments of zRMS:	The analytical method 01555 was validated for the determination of concentration of trifloxystrobin metabolites of AE 1344148 and AE 1393224 in test water by HPLC-MS/MS using two MRM transitions. Specificity: Apparent concentrations in control samples were below 0.3 × LOQ. Two MRM transitions for AE 1344148 (m/z 395 → m/z 148 for quantitation and m/z 395 → m/z 116 for confirmation) and AE 1393224 (m/z 409 → m/z 206 for quantitation and m/z 409 → m/z 116 for confirmation) were monitored. Therefore, the HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. Linearity: The correlation between the injected amount of substance and the detector response was linear (1/x weighted) for standard solutions in test water/acetonitrile (80/20, v/v) ranging from 0.012 µg/L to at least 10 µg/L. The correlation coefficient was 0.9999 for both MRM transitions and both compounds. Untreated Control Samples: The concentration was below 30% of the LOQ of 0.04 µg/L for AE 1344148 and AE 1393224. LOQ: The limit of quantitation (LOQ) for AE 1344148 and AE 1393224 is 0.05 µg/L (corresponding to 0.04 µg/L in standard solution). Repeatability (Precision): Because of the direct measurement of the samples recovery rates cannot be calculated. Thus precision data based on 10 injections of a standard solution of 0.04 µg/L and 10 injections of a standard solution of 0.4 µg/L (corresponding to 0.05 µg/L and 0.5 µg/L in test water) are presented. The relative standard deviations for the peak areas were ≤ 4.9 % for all fortification levels and MRM transitions. The validation parameters are acceptable.This analytical method is suitable for the determination of trifloxystrobin metabolites of AE 1344148 and AE 1393224 in test water samples via HPLC-UV. Remark: no GLP.

Reference:	**KCP 5.1.2.6/18**
Title:	Analytical method 01555 for the determination of AE1344148 (BCS-AL58690) and AE 1393224 (BCS-AR14200) in test water by HPLC-MS/MS
Report:	Krebber, R.; Leppelt, L.; 2018; P604187027; M-623236-01-1
Authority registration No:	
Guideline(s):	None
Deviations:	None
GLP/GEP:	no
Acceptability:	The method is considered as fit for purpose
Duplication (if vertebrate study):	

Materials and methods

The analytical method 01555 describes the determination of the trifloxystrobin metabolites of AE 1344148 and AE 1393224 in test water by HPLC-MS/MS using two MRM transitions.
The water samples are added with acetonitrile (25% of the sample volume) and analysed by direct injection into the HPLC-MS/MS instrument using the positive ion mode without further clean-up.
The following MRM transitions were used for quantitation and confirmation of AE 1344148:

m/z 395 → *m/z* 148 (quantitation)

m/z 395 → *m/z* 116 (confirmation)

The following MRM transitions were used for quantitation and confirmation of AE 1393224:

m/z 409 → *m/z* 206 (quantitation)

m/z 409 → *m/z* 116 (confirmation)

Results and discussions

Because of the direct measurement of the samples recovery rates cannot be calculated. Thus precision data based on 10 injections of a standard solution of 0.04 µg/L and 10 injections of a standard solution of 0.4 µg/L (corresponding to 0.05 µg/L and 0.5 µg/L in test water) are presented.
The relative standard deviations for the peak areas were ≤ 4.9 % for all fortification levels and MRM transitions.

Table A 128: Repeatability for AE 1344148 1. MRM (Quantitation Ion m/z 395 → m/z 148)

Fortification Level [µg/L]	n	Peak area		Retention Time	
		Mean Value	RSD [%]	Mean Value [min]	RSD [%]
0.04	10	5270	3.0	2.80	< 0.1
0.4	10	54036	1.4	2.80	< 0.1

Table A 129: Repeatability for AE 1344148 2. MRM (Confirmatory Ion m/z 395 → m/z 116)

Fortification Level [µg/L]	n	Peak area		Retention Time	
		Mean Value	RSD [%]	Mean Value [min]	RSD [%]
0.04	10	2251	4.9	2.80	0.1
0.4	10	23408	2.0	2.80	< 0.1

Table A 130: Repeatability for AE 1393224 1. MRM (Quantitation Ion m/z 409 → m/z 206)

Fortification Level [µg/L]	n	Peak area		Retention Time	
		Mean Value	RSD [%]	Mean Value [min]	RSD [%]
0.04	10	40749	1.3	3.13	0.1

0.4	10	409527	1.4	3.13	< 0.1

Table A 131: Repeatability for AE 1393224 2. MRM (Confirmatory Ion m/z 409 → m/z 116)

Fortification Level [µg/L]	n	Peak area		Retention Time	
		Mean Value	RSD [%]	Mean Value [min]	RSD [%]
0.04	10	9240	1.0	3.13	0.1
0.4	10	94873	1.4	3.13	0.1

RSD: relative standard deviation

Table A 132: Characteristics for the analytical method used for validation of AE 1344148 and AE 1393224 residues in test water

	AE 1344148	AE 1393224
Specificity	Mass spectra available. Apparent concentrations in control samples were below 0.3 × LOQ. The HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary.	
Calibration (type, number of data points)	individual calibration data is presented calibration line equations are presented (1/x weighted): m/z 148: y = 133261.9x – 41.116 r = 0.9999 m/z 116: y = 59035.06x – 64.939 r = 0.9998 number of data points: 8 The method/detector response was linear.	individual calibration data is presented calibration line equations are presented (1/x weighted): m/z 206: y = 987688.9x + 1340.30 r = 0.9998 m/z 116: y = 234447.4x + 140.258 r = 0.9999 number of data points: 8 The method/detector response was linear.
Calibration range	0.012 – 10.0 µg/L	
Assessment of matrix effects is presented	The MS/MS detection of both substances was not affected by the matrix.	
Limit of determination/quantification	LOQ = 0.05 µg/L (corresponding to 0.04 µg/L in standard solution)	

Conclusion

The method was successfully validated according to the guideline SANCO/3029/99 rev.4. and is suitable for the determination of AE 1344148 and AE 1393224 in test water samples via HPLC-MS/MS. The method was used in the studies Riebschlaeger, T.; 2018; M-630021-01-1; Kuhl, K.; 2018; M-629680-01-1, Kuhl, K.; 2018; M-628915-01-1 and can be regarded as fit for purpose.

A 2.2.1.6.12.2 Concurrent validation of method 01555 in support of study M-630021-01-1

Comments of zRMS:	The analytical method 01555, Report of Bayer CropScience AG, P 604 187027, dated 2018-05-08 (non-GLP) for the determination of AE 1393224 (BCSAR14200) in test water by HPLC-MS/MS was used in the study. In the present study the method was validated concurrently with the sample analyses of the study by evaluation of the standard injections. The linearity of MS detection was determined in the concentration range from 0.012 µg/L to 10 µg/L. The correlation coefficient was 0.9999 (1/x weighted). CGA 357261 (technical metabolite, AE 1393224) was not detected in the control samples in a concentration higher than 0.0156 mg/L, which was used as the lowest standard concentration during this study (multiplied with the dilution factor). The validation parameters are acceptable according to SANCO/3029/99 rev. 4. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.6/19**
Title:	Acute toxicity of CGA357261 (technical metabolite) to the waterflea Daphnia magna in a static renewal laboratory test system
Report:	Riebschläger, T.; 2018; EBTF0037; M-630021-01-1
Authority registration No:	
Guideline(s):	OECD guideline 202,(2004); EC Council Regulation No 440/2008, Method C.2 (2008); U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72-2 (1982); OCSPP Guideline 850.1010, public draft (2016), modified; JMAFF 12 Nousan No. 8147 (2000).
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation

For the determination of AE 1393224 the analytical method 01555 (Krebber R., Leppelt L.; 2018; M-623236-01-1) was used and validated concurrently.

The linearity of MS detection was determined for AE 1393224 in the concentration range from 0.501 µg/L to 20.1 µg/L and was shown to be linear (y= 2.4543e+005 – 1193.5). The correlation coefficient was 0.9999 (1/x weighted). 5 concentrations were measured. If necessary, samples were diluted to achieve final concentrations falling within the calibrated range of detector response.

Because of the direct measurement of the samples recovery rates cannot be calculated. The evaluation of measurements based on HPLC-MS/MS for precision was done by comparison of the peak areas of the samples with the peak areas of the external standard solutions. For this purpose, standard solutions of AE 1393224 in test water/acetonitrile (80/20, v/v) were used. The relative standard deviation of AE 1393224 peak areas and retention times are shown in the table below.

Table A 133: Validation of Method 01555 for AE 1393224 by HPLC-MS/MS

AE 1393224 Standard concentration		AE 1393224			
		Peak area		Retention time	
[µg/L]	n	Mean value [area counts]	Rel. std. dev. [%]	Mean value [min]*	Rel. std. dev. [%]
0.501	3	128691	8.6	3.16	<0.1
0.501	4	116877	1.3	2.98	0.2
0.501	4	120522	0.9	2.99	0.2
0.501	6	117253	1.3	2.99	0.2
1.00	4	239664	1.5	3.16	<0.1
1.00	4	235251	1.4	2.98	<0.1
1.00	4	236794	0.8	2.98	<0.1
1.00	6	226611	1.4	2.99	0.2
5.01	4	1198508	0.8	3.16	<0.1
5.01	4	1161390	1.1	2.99	0.2
5.01	4	1199286	1.0	2.98	0.2
5.01	6	1120134	1.8	2.99	0.2
10.0	4	2427714	0.4	3.16	<0.1
10.0	4	2368552	0.3	2.98	0.2
10.0	4	2426502	1.6	2.98	<0.1
10.0	6	2309436	1.5	2.99	0.2
20.1	4	4986686	0.8	3.16	<0.1
20.1	4	4811625	0.8	2.98	<0.1
20.1	4	4973220	1.8	2.98	<0.1
20.1	6	4542690	1.8	2.99	0.1

* : different retention times due to different oven temperatures

Conclusion

The applicability of the HPLC-MS/MS method 01555 for the analysis of AE 1393224 in water samples was tested. The data presented demonstrate that the method allows the determination of AE 1393224 with satisfactory accuracy, precision and repeatability according to guideline SANCO/3029/99 rev.4 and can be

regarded as fit for purpose.

A 2.2.1.6.13 Concurrent validation of method 01555 in support of study M-628915-01-1

Comments of zRMS:	The analytical method 01555, Report of Bayer CropScience AG, P 604 187027, dated 2018-05-08 (non-GLP) for the determination of AE 1344148 (BCS-AL58690) in test water by HPLC-MS/MS was used in the study. In the present study the method was validated concurrently with the sample analyses of the study by evaluation of the standard injections. For this purpose the AE 1344148 (BCS-AL58690) standard injections were evaluated. The linearity of MS detection was determined for AE 1344148 (BCS-AL58690) in the concentration range from 0.012 µg/L to 10µg/L. The correlation coefficient was 0.9999 (1 /x weighted). AE 1344148 (BCS-AL58690) was not detected in the control samples in a concentration higher than 0.000626 mg/L, which was used as the lowest standard concentration during this study (multiplied with the dilution factor). The validation parameters are acceptable according to SANCO/3029/99 rev. 4. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.6/20**
Title:	Desmodesmus subspicatus growth inhibition test with AE 1344148 (BCS-AL58690)
Report:	Kuhl, K.; 2018; EBTF0047; M-628915-01-1
Authority registration No:	
Guideline(s):	OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (July 28, 2011) , OCSPP Guideline 850.4500: Algal Toxicity (January 2012)
Deviations:	OECD 201 recommends an initial cell number of 2 to 5 x 103 cells/mL for Desmodesmus subcapitatus. OCSPP 850.4500 stated that no test should be started with less than 10,000 cells/mL. Since an initial cell number of 10,000 cells/mL result in an acceptable population density after 72 it was decided to deviate from the OECD recommendation and adapt the initial cell number to the recommendation of OCSPP 850.4500. Since all validity criteria were fulfilled, this deviation is not considered to impact the quality of the study.
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation

For the determination of AE 1344148 the analytical method 01555 (Krebber R., Leppelt L.; 2018; M-623236-01-1) was used and validated concurrently.

The linearity of MS detection was determined for AE 1344148 in the concentration range from 0.501 µg/L to 20 µg/L and was shown to be linear (y= 31807.49x + 369.024). The correlation coefficient was 0.9999 (1/x weighted). 5 concentrations in duplicate were measured. If necessary, samples were diluted to achieve final concentrations falling within the calibrated range of detector response.

Because of the direct measurement of the samples recovery rates cannot be calculated. The evaluation of measurements based on HPLC-MS/MS for precision was done by comparison of the peak areas of the samples with the peak areas of the external standard solutions. For this purpose, standard solutions of AE 1344148 in test water/acetonitrile (80/20, v/v) were used. The relative standard deviation of AE 1344148 peak areas and retention times are shown in the table below.

Table A 134: Validation of Method 01555 for AE 1344148 by HPLC-MS/MS

AE 1344148 Standard concentration		AE 1344148			
		Peak area		Retention time	
[µg/L]	n	Mean value [area counts]	Rel. std. dev. [%]	Mean value [min]*	Rel. std. dev. [%]
0.501	6	16152	1.7	2.79	<0.1
1.00	4	32101	1.8	2.79	<0.1
5.01	6	160374	0.6	2.79	<0.1

10.0	6	322975	0.8	2.79	<0.1
20.1	6	631531	1.2	2.79	<0.1

* : different retention times due to different oven temperatures

Conclusion

The applicability of the HPLC-MS/MS method 01555 for the analysis of AE 1344148 in water samples was tested. The data presented demonstrate that the method allows the determination of AE 1344148 with satisfactory precision and repeatability according to guideline SANCO/3029/99 rev. 4 and can be regarded as fit for purpose.

A 2.2.1.6.14 Concurrent validation of method 01555 in support of study M-629680-01-1

Comments of zRMS:	The analytical method 01555, Report of Bayer CropScience AG, P 604 187027, dated 2018-05-08 (non-GLP) for the determination of AE 1393224 (BCSAR14200) in test water by HPLC-MS/MS was used in the study. In the present study the method was validated concurrently with the sample analyses of the study by evaluation of the standard injections. The linearity of MS detection was determined for AE 1393224 (BCS-AR14200) in the concentration range from 0.012 µg/L to 10µg/L. The correlation coefficient was 0.9999 (1 /x weighted). AE 1393224 (BCS-AR14200) was not detected in the control samples in a concentration higher than 0.000626 mg/L, which was used as the lowest standard concentration during this study (multiplied with the dilution factor). The validation parameters are acceptable according to SANCO/3029/99 rev. 4. The method is considered as fit for purpose.

Reference:	**KCP 5.1.2.6/21**
Title:	Desmodesmus subspicatus growth inhibition test with AE1393224 (BCS-AR14200)
Report:	Kuhl, K.; 2018; EBTF0046; M-629680-01-1
Authority registration No:	
Guideline(s):	OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (July 28, 2011), OCSPP Guideline 850.4500: "Algal Toxicity" (January 2012)
Deviations:	Yes (see report)
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation

For the determination of AE 1393224 the analytical method 01555 (Krebber R., Leppelt L.; 2018; M-623236-01-1) was used and validated concurrently.

The linearity of MS detection was determined for AE 1393224 in the concentration range from 0.501 µg/L to 20 µg/L and was shown to be linear (y = 180731.2 x + 11429.83). The correlation coefficient was 0.9990 (1/x weighted). 5 concentrations were measured in duplicate. If necessary, samples were diluted to achieve final concentrations falling within the calibrated range of detector response.

Because of the direct measurement of the samples recovery rates cannot be calculated. The evaluation of measurements based on HPLC-MS/MS for precision was done by comparison of the peak areas of the samples with the peak areas of the external standard solutions. For this purpose, standard solutions of AE 1393224 in test water/acetonitrile (80/20, v/v) were used. The relative standard deviation of AE 1393224 peak areas and retention times are shown in the table below.

Table A 135: Validation of Method 01555 for AE 1344148 by HPLC-MS/MS

AE 1393224 standard concentration [µg/L]	n	Peak area		Retention Time	
		Mean Value	RSD	Mean Value	RSD
		[area counts]	[%]	[min]	[%]
0.501	4	97064	5.2	2.89	<0.1

1.00	4	192786	5.5	2.89	<0.1
5.01	4	953269	3.7	2.89	<0.1
10.0	4	1888726	2.3	2.89	<0.1
20.0	4	3542056	1.6	2.89	<0.1

Conclusion
The applicability of the HPLC-MS/MS method 01555 for the analysis of AE 1393224 in water samples was tested. The data presented demonstrate that the method allows the determination of AE 1393224 with satisfactory precision and repeatability according to guideline SANCO/3029/99 rev. 4 and can be regarded as fit for purpose.

A 2.2.1.6.15 Analytical method in support of the study M-602375-01-1

A 2.2.1.6.15.1 Method validation

Comments of zRMS:	The quantification of the test item was performed by HPLC analysis and UV/VIS-detection. LOQ = 0.952 mg/L. Despite the fact that not all validation parameters according to SANCO/3029/99 rev. 4 are met, the method is considered as fit for purpose and can be used in the evaluation.

Reference:	**KCP 5.1.2.6/22**
Title:	Daphnia sp., acute immobilisation test with trifloxystrobin - TFMAP
Report:	Neuhahn, A.; 2017; 2017/0043/03; M-602375-01-1
Authority registration No:	
Guideline(s):	Council Regulation (EC) No 440/2008, Method C.2 ‘Acute toxicity for Daphnia’ (2008) which is equivalent to OECD Guideline for Testing of Chemicals No. 202 'Daphnia sp., Acute Immobilisation Test' (adopted April 13, 2004).
Deviations:	none
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

Materials and methods
The analytical method was developed for the determination of trifloxystrobin-TFMAP in test water by HPLC-UV/VIS in support of this toxicity study to *Daphnia magna*.
Aliquots of the samples from the biological test were directly analysed by HPLC and UV/VIS-detection. The samples were diluted with acetonitrile / millipore water (1/1, v/v) to match the calibration range, if necessary.

Results and discussions

Table A 136: Recovery results for trifloxystrobin – TFMAP using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 6)	Recovery (%)	Overall mean value
test water	Trifloxystrobin - TFMAP	0.9520	100.5	Recovery: 100.1 % RSD : 0.28 %
		0.9520	100.7	
		0.9520	100.1	
		0.9520	100.2	
		0.9520	99.6	
		0.9520	99.7	

Table A 137: Characteristics for the analytical method for the determination of trifloxystrobin – TFMAP residues in test water

	Trifloxystrobin - TFMAP
Specificity	An untreated control sample was examined. The concentration was below 30% of the

	LOQ, indicating that no significant interferences were present.
Calibration (type, number of data points)	individual calibration data is presented calibration line equation: y = 304.53163 * x – 6.15216, r = 0.9999 number of data points: 7
Calibration range	0.04 – 2.59 mg/L
Assessment of matrix effects is presented	No effects observed.
Limit of determination/quantification	~~LOQ = 0.04 mg/L (The concentration of the lowest used calibration solution was employed as the limit of quantification.)~~ LOQ = 0.952 mg/L.

Conclusion
Although not all validation parameters according to SANCO/3029/99 rev. 4 are met, the analytical method can be regarded as fit for purpose with regard to this toxicity study. The recoveries were performed in a concentration range, which is appropriate for the studies and showed good results with an average recovery over all determinations of 100.1%. The precision is with a relative standard deviation of 0.28% over all concentrations also well within the acceptable range.

A 2.2.1.6.16 Analytical method in support of the study M-602410-01-1

A 2.2.1.6.16.1 Method validation

Comments of zRMS:	The quantification of the test item was performed by HPLC analysis and UV/VIS-detection. LOQ = 0.952 mg/L. Despite the fact that not all validation parameters according to SANCO/3029/99 rev. 4 are met, the method is considered as fit for purpose and can be used in the evaluation.

Reference:	**KCP 5.1.2.6/23**
Title:	Alga, groth inhibition test with trifloxystrobin-TFMAP
Report:	Spoo-Klöppel, M.; 2017; 2017/0043/04; M-602410-01-1
Authority registration No:	
Guideline(s):	Commission Regulation (EC) No 761/2009 amending Regulation No 440/2008, Method C.3 ‘Freshwater Alga and Cyanobacteria, Growth inhibition test’ (2009) which is equivalent to OECD Guideline for Testing of Chemicals No. 201 (2006)
Deviations:	none
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

Materials and methods
The analytical method was developed for the determination of trifloxystrobin-TFMAP in test water by HPLC-UV/VIS in support of this toxicity study to alga *(Desmodesmus subspicatus*).
Aliquots of the samples from the biological test were directly analysed by HPLC and UV/VIS-detection. The samples were diluted with acetonitrile / millipore water (1/1, v/v) to match the calibration range, if necessary.

Results and discussions

Table A 138: Recovery results for trifloxystrobin – TFMAP using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 6)	Recovery (%)	Overall mean value
test water	Trifloxystrobin - TFMAP	0.9520	100.1	Recovery: 99.9 % RSD : 0.17 %
		0.9520	100.4	
		0.9520	100.0	
		0.9520	100.3	
		0.9520	99.6	
		0.9520	99.9	

		0.9520	99.5	
		0.9520	99.7	

Table A 139: Characteristics for the analytical method for the determination of trifloxystrobin – TFMAP residues in test water

	Trifloxystrobin - TFMAP
Specificity	An untreated control sample was examined. The concentration was below 30% of the LOQ, indicating that no significant interferences were present.
Calibration (type, number of data points)	individual calibration data is presented calibration line equation: y = 304.53163 * x – 6.15216, r = 0.9999 number of data points: 7
Calibration range	0.04 – 2.59 mg/L
Assessment of matrix effects is presented	No effects observed.
Limit of determination/quantification	~~LOQ = 0.04 mg/L (The concentration of the lowest used calibration solution was employed as the limit of quantification.)~~ LOQ = 0.952 mg/L.

Conclusion
Although not all validation parameters according to SANCO/3029/99 rev. 4 are met, the analytical method can be regarded as fit for purpose with regard to this toxicity study. The recoveries were performed in a concentration range, which is appropriate for the studies and showed good results with an average recovery over all determinations of 99.9 %. The precision is with a relative standard deviation of 0.17 % over all concentrations also well within the acceptable range.

A 2.2.1.6.17 Analytical method 01556

A 2.2.1.6.17.1 Method validation

Comments of zRMS:	The analytical method 01556 was validated for the determination of concentration of AE 1344132 in test water by HPLC-UV. The linearity of UV detection was determined for AE 1344132 in the concentration range from 0.012 mg/L to 2.50 mg/L. The correlation coefficient was 0.9990 (1/x weighted). An untreated control sample was examined. The concentration was below 30% of the LOQ of AE1344132 The limit of quantitation (LOQ) for AE 1344132 is 0.05 mg/L (corresponding to 0.04 mg/L in standard solution). The relative standard deviation for the peak area of AE1344132 was 1.7 % at 0.04 mg/L and 0.8 % at 0.4 mg/L. The relative standard deviation for the retention time was ≤ 0.1 % for both fortification levels. This analytical method is suitable for the determination of AE 1344132 in test water samples via HPLC-UV. The validation parameters are acceptable.

Reference:	**KCP 5.1.2.6/24**
Title:	Analytical method 01556 for the determination of AE 1344132 (BCS-AB55122) in test water by HPLC-UV
Report:	Krebber, R.; Leppelt, L.; 2018; 01556; M-621113-01-1
Authority registration No:	
Guideline(s):	not specified
Deviations:	None
GLP/GEP:	no
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods
The analytical method 01556 describes the determination of the trifloxystrobin metabolite of AE 1344132 in test water by HPLC-UV.

The water samples are added with acetonitrile (25% of the sample volume) and analysed by direct injection into the HPLC-UV instrument without further clean-up.

Results and discussions
Because of the direct measurement of the samples recovery rates cannot be calculated. Thus precision data based on 10 injections of a standard solution of 0.04 mg/L and 10 injections of a standard solution of 0.4 mg/L (corresponding to 0.05 mg/L and 0.5 mg/L in test water) are presented.
The relative standard deviations for the peak areas were ≤ 1.7 % for all fortification levels.

Table A 140: Repeatability for AE 1344132

Fortification Level [mg/L]	n	Peak area		Retention Time	
		Mean Value	RSD [%]	Mean Value [min]	RSD [%]
0.04	10	103	1.7	2.73	< 0.1
0.4	10	999	0.8	2.73	0.1

RSD: relative standard deviation

Table A 141: Characteristics for the analytical method used for validation of AE 1344132 residues in test water

	AE 1344132
Specificity	An untreated control sample was examined. The concentration was below 30% of the LOQ of AE1344132, indicating that no significant interferences were present.
Calibration (type, number of data points)	individual calibration data is presented calibration line equation is presented: y = 2.44e+003x + 8.92, r = 0.9990 number of data points: 6 in duplicate
Calibration range	0.0120 mg/L to 2.5 mg/L.
Assessment of matrix effects is presented	None – direct sample injection
Limit of determination/quantification	LOQ = 0.05 mg/L

Conclusion
The method was successfully validated according to the guideline SANCO/3029/99 rev.4. and is suitable for the determination of AE 1344132 in test water samples via HPLC-UV. The method was used in the study Kuhl, K.; 2018; M-629159-01-1 and can be regarded as fit for purpose.

A 2.2.1.6.18 Concurrent validation of method 01556 in support of study M-629159-02-1

Comments of zRMS:	The analytical method no. 01556, Report of Bayer CropScience AG, P 604 177071 dated 2018-04-18 was used in the study. The linearity of UV detection was determined for AE 1344132 in the concentration range from 0.012 mg/L to 2.50 mg/L. The correlation coefficient was 0.9990 (1/x weighted). In the present study the method was validated concurrently with the sample analyses of the study by evaluation of the standard injections. The limit of quantitation (LOQ) for AE 1344132 is 0.05 mg/L (corresponding to 0.04 mg/L in standard solution). The validation parameters are acceptable according to SANCO/3029/99 rev. 4.

Reference:	**KCP 5.1.2.6/25**
Title:	Amendment no. 1 to final report: Desmodesmus subspicatus growth inhibition test with AE 1344132 tech. (BCS-AB55122)
Report:	Kuhl, K.; 2018; E 201 05127 - 8; M-629159-02-1
Authority registration No:	
Guideline(s):	OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (July 28, 2011) , OCSPP Guideline 850.4500: "Algal Toxicity" (January 2012)
Deviations:	According to OCSPP 850.4500 the measured test substance concentration at test initiation is considered appropriate to use for unstable test items. However, in this study the ECx calculations after 96 hours were performed using the mean measured values to follow the recommendations from OPPTS 850.1000. OECD 201 recommends an initial cell number of 2 to 5 x 103 cells/mL for Desmodesmus subcapitatus. OCSPP 850.4500 stated that no test should be started with less than 10,000 cells/mL. Since an initial cell number of 10,000 cells/mL result in an acceptable population density after 72 and 96 hours, it was decided to deviate from the OECD recommendation and adapt the initial cell number to the recommendation of OCSPP 850.4500.
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

Concurrent validation

For the determination of AE 1344132 the analytical method 01556 (Krebber R.;Leppelt L.; 2018; M-6233236-01-1) was used and validated concurrently.

The linearity of MS detection was determined for AE 1393224 in the concentration range from 0.0501 mg/L to 2.50 mg/L and was shown to be linear (y= 2295.01x + 18.6137). The correlation coefficient was 0.9980 (1/x weighted). 5 concentrations in triplicate were measured. If necessary, samples were diluted to achieve final concentrations falling within the calibrated range of detector response.

Because of the direct measurement of the samples recovery rates cannot be calculated. The evaluation of measurements based on HPLC-UV for precision was done by comparison of the peak areas of the samples with the peak areas of the external standard solutions. For this purpose, standard solutions of AE 1344132 in test water/acetonitrile (80/20, v/v) were used. The relative standard deviation of AE 1344132 peak areas and retention times are shown in the table below.

Table A 142: Validation of Method 01556 for AE 1344132 by HPLC-MS/MS

AE 1344132 Standard concentration		AE 1344132			
		Peak area		Retention time	
[mg/L]	n	Mean value [area counts]	Rel. std. dev. [%]	Mean value [min]*	Rel. std. dev. [%]
0.0501	6	125	5.0	2.75	0.2
0.100	4	253	4.9	2.75	0.2
0.501	4	1248	3.7	2.75	0.2
1.00	6	2390	6.2	2.75	0.1
2.50	4	5570	3.7	2.75	0.2

* : different retention times due to different oven temperatures

Conclusion

The applicability of the HPLC-UV method 01556 for the analysis of AE 1344132 in water samples was tested. The data presented demonstrate that the method allows the determination of AE 1344132 with satisfactory precision and repeatability according to guideline SANCO/3029/99 rev. 4 and can be regarded as fit for purpose.

A 2.2.1.6.19 Analytical method in support of the study M-670321-02-1

A 2.2.1.6.19.1 Method validation

Comments of zRMS:	The quantification of the test item Metabolite of Trifloxystrobin: BCSAB39835 in the test samples was performed using liquid chromatography with MS/MS detection.

	Specificity: No interference of total peak area for the target analyte was found. Linearity: Two calibration curves were used in order to cover the wide concentration range of 0.1 – 65 µg test item/L with high accuracy. 1. 0.1 – 5 µg test item/L 2. 1 – 65 µg test item/L. Linearity of Response: Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression. Correlation Coefficient: 1. r = 0.9999 2. r = 0.9999 Calibration Curves: 1. y = 6830 * x + 25 2. y = 7026 * x + 350 Accuracy and Precision: Mean Recovery Rates in the Fortified Samples: 104 % (n = 15, RSD 4 %). The values found for the precision (RSD) and for the accuracy (mean recovery rate) are acceptable. LOQ = 0.4 µg test item/L. The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met.

Reference:	**KCP 5.1.2.6/26**
Title:	Metabolite of trifloxystrobin: BCS-AB39835 - Influence to Daphnia magna in a semi-static reproduction test - 1st final report amendment
Report:	xxx
Authority registration No:	
Guideline(s):	Commission Regulation (EC) No 440/2008, Annex, Part C, C.20.: "Daphnia magna Reproduction Test", Official Journal of the European Union (EN), dated May 30, 2008 EPA Guideline 712-C-16-005: OCSPP 850.1300, "Daphnid Chronic Toxicity Test", October 2016 EPA Guideline 712-C-16-014: OCSPP 850.1000, "Background and Special Considerations-Tests with Aquatic and Sediment-Dwelling Fauna and Aquatic Microcosms", October 2016 OECD Guideline for Testing of Chemicals, No. 211: "Daphnia magna Reproduction Test", adopted October 02, 2012 OECD Series on Testing and Assessment, No. 23, "Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures", December 15, 2000 SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of Directive 91/414
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the analytical part of this study was to verify the concentrations of the test item, metabolite of trifloxystrobin (BCS-AB39835), in the test medium by LC-MS/MS.
After appropriate dilution, the samples were centrifuged (13,000 rpm, 3 minutes) and then analysed.
The determination was conducted using high performance liquid chromatography (HPLC) with mass-spectrometric (MS/MS) detection (ESI positive, MRM used as quantifier m/z: 319.2 → 185.9, MRM used as qualifier m/z: 319.2 → 145.0).

Results and discussions

For BCS-AB39835 recoveries were performed in test water spiked with test item at the fortification levels of 0.4 µg test item/L (LOQ), at 1.5 and at 140 µg test item/L. The individual recovery values at the low fortification level ranged between 94 and 107% with a mean of 102%. The corresponding relative standard deviation (RSD) was 5% (n = 5). The individual recovery values at the fortification level of 1.5 µg/L ranged

between 102 and 108% with a mean of 105%. The corresponding RSD was 3% (n = 5). The individual recovery values at the high fortification level ranged between 101 and 111% with a mean of 106%. The corresponding RSD was 3% (n = 5). The overall mean recovery was 104% with a corresponding RSD of 4% (n = 15).

Table A 143: Recovery rates and precision results (repeatability) of BCS-AB39835

	Concentration					
Sample description	**Nominal [mg test item/L]**	**Found [µg metabolite/L]**	**DF**	**Concentration calculated [µg metabolite/L]**	**Corrected nominal [µg metabolite/L]**	**Recovery [%]**
Anayltical Blank	0	<LOD	1.25	n.a.	0.000	n.a.
Anayltical Blank	0	<LOD	1.25	n.a.	0.000	n.a.
Fortified Sample	0.4	0.293	1.25	0.367	0.389	94
	0.4	0.317	1.25	0.396	0.389	102
	0.4	0.327	1.25	0.409	0.384	107
	0.4	0.324	1.25	0.405	0.384	106
	0.4	0.307	1.25	0.383	0.384	100
					Mean value (n = 5):	**102**
					RSD (n = 5):	**5**
Fortified Sample	1.5	1.188	1.25	1.485	1.458	102
	1.5	1.187	1.25	1.483	1.458	102
	1.5	1.244	1.25	1.555	1.439	108
	1.5	1.228	1.25	1.534	1.439	107
	1.5	1.242	1.25	1.553	1.439	108
					Mean value (n = 5):	**105**
					RSD (n = 5):	**3**
Fortified Sample	140	37.950	1.25	142.313	136.071	105
	140	36.812	1.25	138.043	136.071	101
	140	39.658	1.25	148.717	134.319	111
	140	38.804	1.25	145.515	134.319	108
	140	37.808	1.25	141.779	134.319	106
					Mean value (n = 5):	**106**
					RSD (n = 5):	**3**
					Overall mean value (n = 15):	**104**
					RSD (n = 15):	**4**

LOD: Limit of Detection = 0.04 µg test item/L
n.a.: not applicable
RSD: Relative Standard Deviation
DF: Dilution factor

Table A 144: Characteristics for the analytical method used for validation of BCS-AB39835

	BCS-AB39835	
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.	
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted) for lower calibration range: y = 6830 x + 25, Correlation coefficient r: 0.9999 number of data points: 6	Individual calibration data is presented calibration equation (1/x weighted) for higher calibration range: y = 7026 x + 350, Correlation coefficient r: 0.9999 number of data points: 7
Calibration range	Lower range: 0.1 – 5 µg test item/L	Higher range: 1 – 65 µg test item/L
Limit of determination/quantification	LOQ = 0.4 µg test item/L	
Assessment of matrix effects is presented	No effects observed.	

Conclusion
The analytical method complies with all guideline criteria according to SANCO/3029/99 rev. 4. It was validated successfully and can be seen as fit for purpose for the presented study.

A 2.2.1.6.20 Analytical method 01013 in support of the study M-648913-01-1

A 2.2.1.6.20.1 Method validation

Comments of zRMS:	Concentrations of trifloxystrobin were determined in final diets. The method 01013 with minor modifications using reversed phase - high performance liquid chromatography (HPLC) with mass-spectrometric (MS/MS) detection was used to determine residues of trifloxystrobin. The LOQ was 0.01 mg/kg of trifloxystrobin (corresponding to 0.178 pg/L in diluted extracts). The recovery and precision data show that the influences of test medium were within the limits of the guidance document SANCO/3029/99; all criteria were fulfilled: - blank values did not exceed 30% of the lowest verified concentration, - mean recoveries for each level were in the range 70-110%, - the RSD was < 20% per level. The mean recoveries of trifloxystrobin in the final diets were between 83-90%. In the control specimens, the concentration of active ingrendient was below 30% of LOQ. Thus the concentrations of the specimens of the biological part of the study were verified. The study is acceptable.

Reference:	**KCP 5.1.2.6/27**
Title:	Trifloxystrobin tech. - Repeated exposure to honey bee (Apis mellifera) larvae under laboratory conditions (in vitro)
Report:	Kleebaum, K.; 2019; 18 48 BLC 0044; M-648913-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 (2009) Directive 2003-01 (CANADA/PMRA) US EPA OCSPP 850.SUPP OECD Guidance Document 239 (2016)
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the analytical phase of the study was the determination of the concentration of active ingredient trifloxystrobin in final diets. The determination was conducted according to the analytical method 01013 (Brumhard, B.; Stuke, S.; 2007; M-283439-01-1) with minor modifications using reversed phase - high performance liquid chromatography (HPLC) with mass-spectrometric (MS/MS) detection. (ESI positive, MRM m/z: 412 → 186 (internal standard); 409 → 206; 409 → 186; 409 → 145; 409 → 116). The final diet specimens of the biological part of the study as well as the respective procedural recovery samples were extracted prior to sample measurement. Samples were homogenized by vortexing for 5 minutes. 5 mL of acetonitrile/water (4/1, v/v) were added to a sample aliquot of 0.5 g. It was vortexed at 2500 rpm for 30 minutes and centrifuged at 3000 rpm for 2 min. The samples were diluted and injected into the HPLC.

The method was verified with test medium spiked with test item at 0.01 mg/kg and at approx. 153% of nominal test concentration (485.4 mg/kg, corresponding to 20.22 µg/L in diluted extracts).

An external calibration with the test item was performed from 8% of the lowest procedural recovery measuring concentration to 484% of the highest procedural recovery measuring concentration (0.014 to 97.915 µg/L). Matrix effects were taken into account by spiking the calibration solutions with 50% blank extract.

Results and discussions

For trifloxystrobin recoveries were performed in test medium spiked with test item at the LOQ of 0.01 mg/kg and at approx. 153% of nominal test concentration (485.4 mg/kg, corresponding to 20.22 µg/L in diluted extracts). Fortification levels and recovery data are given in the following table. Blank values did not exceed 30% of the lowest verified concentration. The individual recovery values for trifloxystrobin at the low fortification level ranged between 84 and 94% with an overall mean of 89%. The corresponding relative standard deviation (RSD) was 4.6% (n = 5). The individual recovery values for trifloxystrobin at

the high fortification level ranged between 96 and 98% with an overall mean of 97%. The corresponding relative standard deviation (RSD) was 1.1% (n = 5).

Table A 145: Recovery rates and precision results (repeatability) of trifloxystrobin

Sample Name	Nominal c of a.i. [mg/kg]	Nominal c of a.i. for analysis [µg/L]	Measured c of a.i. [µg/L]	RCF	DF	Analysed c of a.i. [mg/kg]	REC [%]
18 CRB 0083-proc. rec. Blank 1	0.000	0.000	0.002	-	-	<30 % LOQ	-
18 CRB 0083-proc. rec. Blank 2	0.000	0.000	0.002	-	-	<30 % LOQ	-
18CRB 0083-Reagent Blank	0.000	0.000	0.004	-	-	<30 % LOQ	-
18 CRB 0083-Cal 3	-	0.110	0.113	-	-	-	103
18 CRB 0083-proc rec low 1	0.0109	0.178	0.171	0.96	61	0.0101	92
18 CRB 0083-proc rec low 2	0.0109	0.178	0.164	0.96	61	0.0097	88
18 CRB 0083-proc rec low 3	0.0109	0.178	0.175	0.96	61	0.0103	94
18 CRB 0083-proc rec low 4	0.0109	0.178	0.156	0.96	61	0.0092	84
18 CRB 0083-proc rec low 5	0.0109	0.178	0.164	0.96	61	0.0097	89
						Mean recovery	**89**
						Mean RSD [%]	**4.6**
18 CRB 0083-Cal 3	-	0.110	0.115	-	-	-	105
18 CRB 0083-Cal 8	-	19.58	19.24	-	-	-	98
18 CRB 0083-proc rec high 1	485.39	20.22	19.02	1.02	24000	465.62	96
18 CRB 0083-proc rec high 2	485.39	20.22	18.96	1.02	24000	464.12	96
18 CRB 0083-proc rec high 3	485.39	20.22	19.28	1.02	24000	471.87	97
18 CRB 0083-proc rec high 4	485.39	20.22	19.19	1.02	24000	469.70	97
18 CRB 0083-proc rec high 5	485.39	20.22	19.46	1.02	24000	476.46	98
						Mean recovery	**97**
						Mean RSD [%]	**1.1**
18 CRB 0083-Cal 8	-	19.58	19.16	-	-	-	98

LOQ: 0.01 mg/kg, corresponding to 0. 1 78 µg/L in diluted extracts

Table A 146: Characteristics for the analytical method 01013 used for validation of trifloxystrobin

	trifloxystrobin
Specificity	HPLC-MS/MS method is highly specific. Blank values of analyte were below 30 % of the respective LOQ
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 1.064497 x + 0.000603, Correlation coefficient r: 0.99929792 number of data points: 12
Calibration range	0.014 µg/L to 97.915 µg/L (corresponds to 0.00079 mg/kg – 5.5 mg/kg)
Limit of determination/quantification	LOQ = 0.01 mg/kg
Assessment of matrix effects is presented	Matrix effects were taken into account by spiking the calibration solutions with 50% blank extract. No effects observed.

Conclusion
The analytical method complies with all guideline criteria according to SANCO 3029/99 rev. 4 with the minor exception of precision data. The fortification levels should be performed at LOQ and 10x LOQ, but the recoveries were performed at LOQ and approx. 153% of nominal test concentration (485.4 mg/kg, corresponding to 20.22 µg/L in diluted extracts) which is appropriate for the study. However, this deviation can be regarded as acceptable due to the fact that the overall relative standard deviation of the high fortification level is with 1.1% far lower than the highest acceptable value of 20%. The method is suitable for the determination of trifloxystrobin in feeding diets and can be regarded as fit for purpose with regard to the present study.

A 2.2.1.6.21 Analytical method 01387 in support of the study M-636236-01-1

A 2.2.1.6.21.1 Method validation

Comments of zRMS:	Concentrations of fluopyram and trifloxystrobin of the test item Fluopyram + Trifloxystrobin SC 500 (250 + 250 g/L) were determined in test media samples using liquid chromatography with MS/MS detection. Specificity: No significant (< 30 %) interference of total peak area for the target analyte was found for either analyte. Linearity: Calibration Range: Fluopyram: 0.1 – 45 µg reference item /L Trifloxystrobin: 0.1 – 45 µg reference item /L Linearity of Response: Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression. Correlation Coefficient: Fluopyram: r = 0.9995 at least Trifloxystrobin: r = 0.9988 at least Calibration Curves: Fluopyram: y = 23008 * x + 924 Trifloxystrobin: y = 59585 * x – 1465 Accuracy and Precision: Mean Recovery Rates in the Fortified Samples: Fluopyram: 94% (n = 15, RSD 7%) Trifloxystrobin: 84% (n = 15, RSD 7%) The values found for the precision (RSD) and for the accuracy (mean recovery rate) are acceptable. Limit of Quantification: Fluopyram: 0.4 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.004 mg test item/L) 90% (n = 5, RSD 12%) Trifloxystrobin: 0.4 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.004 mg test item/L) 86% (n = 5, RSD 8%) The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met. The study is acceptable.

Reference:	**KCP 5.1.2.6/01**
Title:	Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L) - Acute toxicity to rainbow trout (Oncorhynchus mykiss) in a 96-hour semi-static test
Report:	xxx
Authority registration No:	
Guideline(s):	- Commission Regulation (EC) No 440/2008, Annex, Part C, C.1: "Acute Toxicity for Fish", Official Journal of the European Union, May 30, 2008 - EPA Guideline 712-C-16-007:OCSPP 850.1075, " Freshwater and Saltwater Fish Acute Toxicity Test" October 2016 - Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-7-1-1, Fish acute toxicity studies, 2005 - OECD Guideline for Testing of Chemicals, Section 2, No. 203: "Daphnia sp., "Fish, Acute Toxicity Test" adopted July 17, 1992 - SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of Analysis in Support of preregistration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	No

Materials and methods

The purpose of the analytical part of this study was to verify the concentrations of the active ingredients fluopyram and trifloxystrobin of the test item fluopyram + trifloxystrobin SC 500 in the test medium by LC-MS/MS. In the following part, only the data for trifloxystrobin is presented.

The analytical method 01387 was used in the present study which is fully validated and EU-agreed (Krebber, R.; Braune, M.; 2013; M-466732-01-1).

After appropriate dilution, the determination was conducted using high performance liquid chromatography (HPLC) with mass-spectrometric (MS/MS) detection (ESI positive, MRM used as quantifier m/z: 409.1 → 186.0, MRM used as qualifier m/z: 409.1 → 145.0).

Results and discussions

For trifloxystrobin, recoveries were performed in test water spiked with test item at the fortification levels of 0.004, 0.01 and 3 mg test item/L. The individual, mean and overall mean recovery values were within the acceptable range of 70 – 110%, all relative standard deviation (RSD) values were below 20%.

Table A 147: Recovery rates and precision results (repeatability) of trifloxystrobin

	Concentration					
Sample description	**Nominal [mg test item/L]**	**Found [µg a.i./L][1]**	**DF**	**Concentration calculated [µg a.i./L][1]**	**Corrected nominal [µg a.i./L][2]**	**Recovery [%][1]**
Anayltical Blank	0	<LOQ	2	n.a.	0.000	n.a.
Anayltical Blank	0	<LOQ	2	n.a.	0.000	n.a.
Fortified Sample	0.004	0.360	2	0.720	0.846	85
	0.004	0.401	2	0.801	0.846	95
	0.004	0.352	2	0.704	0.852	83
	0.004	0.389	2	0.778	0.852	91
	0.004	0.333	2	0.667	0.852	78
					Mean value (n = 5):	**86**
					RSD (n = 5):	**8**
Fortified Sample	0.01	0.828	2	1.657	2.115	78
	0.01	0.891	2	1.781	2.115	84
	0.01	0.800	2	1.600	2.131	75
	0.01	0.847	2	1.694	2.131	79
	0.01	0.817	2	1.633	2.131	77
					Mean value (n = 5):	**79**
					RSD (n = 5):	**4**
Fortified Sample	3	28.157	20	563.145	634.078	89
	3	25.714	20	514.280	634.078	81
	3	27.808	20	556.164	646.935	86
	3	27.983	20	559.654	646.935	87
	3	28.157	20	563.145	646.935	87
					Mean value (n = 5):	**86**
					RSD (n = 5):	**3**
					Overall mean value (n = 15):	**84**
					RSD (n = 15):	**7**

LOQ: Limit of Quantification = 0.004 mg test item/L corresponding to 0.4 µg a.i./L after dilution by factor 2;
n.a.: not applicable; RSD: Relative Standard Deviation; DF: Dilution factor; a.i.: active ingredient

Table A 148: Characteristics for the analytical method used for validation of trifloxystrobin

	trifloxystrobin
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration equation: y = 59585 x - 1465, Correlation coefficient r: 0.9998, number of data points: 9 The function is linear in the operating range.
Calibration range	0.1 – 45 µg reference item/L
Limit of determination/quantification	LOQ = 0.4 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.004 mg test item/L)
Assessment of matrix effects is presented	No effects observed.

Conclusion

The analytical method complies with all guideline criteria according to SANCO/3029/99 rev. 4 and can be seen as fit for purpose for the presented study.

A 2.2.1.6.22 Analytical method 01387 in support of the study M-636231-01-1

A 2.2.1.6.22.1 Method validation

Comments of zRMS:	Concentrations of fluopyram and trifloxystrobin of the test item Fluopyram + Trifloxystrobin SC 500 (250 + 250 g/L) were determined in test media samples using liquid chromatography with MS/MS detection.

	Specificity: No significant (< 30%) interference of total peak area for the target analyte was found for either analyte. Linearity: Calibration Range: Fluopyram: 0.1 – 35 µg reference item /L Trifloxystrobin: Two calibration curves were used in order to cover the wide concentration range of 0.1 – 35 µg reference item/L with high accuracy. 1. 0.1 – 10 µg a.i./L 2. 0.1 – 35 µg a.i./L Linearity of Response: Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression. Correlation Coefficient: Fluopyram: r = 0.9997 Trifloxystrobin: 1. r = 0.9997 2. r = 0.9999 Calibration Curves: Fluopyram: y = 12006 * x + 1154 Trifloxystrobin: 1. y = 31386 * x + 3204 2. y = 29688 * x + 6913 Accuracy and Precision: Mean Recovery Rates in the Fortified Samples: Fluopyram: 94% (n = 15, RSD 10%) Trifloxystrobin: 75% (n = 10, RSD 8%) The values found for the precision (RSD) and for the accuracy (mean recovery rate) are acceptable. Limit of Quantification: Fluopyram: 0.27 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.0025 mg test item/L) 86% (n = 5, RSD 12%) Trifloxystrobin: 0.86 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.008 mg test item/L) 78% (n = 5, RSD 9%) The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met. The study is acceptable.

Reference:	**KCP 5.1.2.6/02**
Title:	Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L): Acute toxicity to Daphnia magna in a semi-static 48-hour immobilisation test
Report:	xxx
Authority registration No:	
Guideline(s):	- Commission Regulation (EC) No 440/2008, Annex, Part C, C.2: "Daphnia sp. Acute Immobilsisation Test", Official Journal of the European Union (EN), dated May 30, 2008 - EPA Guideline 712-C-16-013:OCSPP 850.1010, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids" October 2016 - Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-7-2-1, Daphnia acute immobilization studies, 2005 - OECD Guideline for Testing of Chemicals No. 202: "Daphnia sp., Acute Immobilisation Test" adopted Aprils 13, 2004 - SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of Analysis in Support of preregistration for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the analytical part of this study was to verify the concentrations of the active ingredients fluopyram and trifloxystrobin of the test item fluopyram + trifloxystrobin SC 500 in the test medium by LC-MS/MS. In the following part, only the data for trifloxystrobin is presented.
The analytical method 01387 was used in the present study which is fully validated and EU-agreed (Krebber, R.; Braune, M.; 2013; M-466732-01-1).
After appropriate dilution, the determination was conducted using high performance liquid chromatography (HPLC) with mass-spectrometric (MS/MS) detection (ESI positive, MRM used as quantifier m/z: 409.1 → 186.0, MRM used as qualifier m/z: 409.1 → 145.0).

Results and discussions

For trifloxystrobin, recoveries were performed in test water spiked with test item at the fortification levels of 0.004, 0.01 and 3 mg test item/L. The mean and overall mean recovery values were within the acceptable range of 70 – 110%, all relative standard deviation (RSD) values were below 20%.

Table A 149: Recovery rates and precision results (repeatability) of trifloxystrobin

	Concentration					
Sample description	**Nominal [mg test item/L]**	**Found [µg a.i./L][1]**	**DF**	**Concentration calculated [µg a.i./L][1]**	**Corrected nominal [µg a.i./L][2]**	**Recovery [%][1]**
Anayltical Blank	0	<LOQ	2	n.a.	0.000	n.a.
Anayltical Blank	0	<LOQ	2	n.a.	0.000	n.a.
Fortified Sample	*0.0025*	*0.210*	*2*	*0.419*	*0.544*	*77*
	0.0025	*0.255*	*2*	*0.510*	*0.544*	*94*
	0.0025	*0.217*	*2*	*0.433*	*0.542*	*80*
	0.0025	*0.187*	*2*	*0.374*	*0.542*	*69*
	0.0025	*0.239*	*2*	*0.478*	*0.542*	*88*
					Mean value (n = 5):	***82***
					RSD (n = 5):	***12***
Fortified Sample	0.008	0.653	2	1.306	1.741	75
	0.008	0.647	2	1.293	1.741	74
	0.008	0.647	2	1.293	1.735	75
	0.008	0.647	2	1.293	1.735	75
	0.008	0.787	2	1.574	1.735	91
					Mean value (n = 5):	**78**

					RSD (n = 5):	**9**
Fortified Sample	0.2	15.127	2	30.253	43.523	70
	0.2	15.901	2	31.803	43.523	73
	0.2	14.992	2	29.984	43.369	69
	0.2	15.969	2	31.938	43.369	74
	0.2	15.969	2	31.938	43.369	74
					Mean value (n = 5):	**72**
					RSD (n = 5):	**3**
					Overall mean value (n = 15):	**75**
					RSD (n = 15):	**8**

LOQ: Limit of Quantification = 0.008 mg test item/L corresponding to 0.86 µg a.i./L after dilution by factor 2;
n.a.: not applicable; RSD: Relative Standard Deviation; DF: Dilution factor; a.i.: active ingredient;
italic: The fortified samples of 0.0025 mg test item/L did not yield valid results with respect to interference withblank matrix due to carry-over effects of the method. Samples are excluded from evaluation.

Table A 150: Characteristics for the analytical method used for validation of trifloxystrobin

	trifloxystrobin	
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30 % of the respective LOQ.	
Calibration (type, number of data points)	Individual calibration data is presented calibration equation for lower calibration range: y = 31386 x + 3204, Correlation coefficient r: 0.9997, number of data points: not presented The function is linear in the operating range.	Individual calibration data is presented calibration equation for upper calibration range: y = 29688 x + 6913, Correlation coefficient r: 0.9999, number of data points: 9 The function is linear in the operating range.
Calibration range	0.1 – 10 µg a.i./L	0.1 – 35 µg a.i./L
Limit of determination/quantification	LOQ = 0.86 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.008 mg test item/L)	
Assessment of matrix effects is presented	No effects observed.	

Conclusion
The analytical method complies with all guideline criteria according to SANCO/3029/99 rev. 4 and can be seen as fit for purpose for the presented study.

A 2.2.1.6.23 Concurrent validation of method 01387 in support of the study M-615579-01-1

Comments of zRMS:	The water samples were analysed according to the following methods: Fluopyram: Modification M001 of analytical method 01387 for the determination of varius pesticides in drinking and surface water by HPLC-MS/MS, Report of Bayer CropScience AG, MR-14/053, dated 2014-10-23. Trifloxystrobin: Analytical method 01387 for the determination of varius pesticides in drinking and surface water by HPLC-MS/MS, Report of Bayer CropScience AG, MR-13/0085, dated 2013-10-09. In the present study the methods were validated concurrently with the sample analyses of the study by evaluation of the standard injections. The limit of quantitation (LOQ) : Fluopyram: LOQ = 0.0625 µg a.s./L Trifloxystrobin: LOQ = 0.0625 µg a.s./L The method is considered as fit for purpose and can be used in the evaluation.

Reference:	**KCP 5.1.2.6/04**
Title:	Pseudokirchneriella subcapitata growth inhibition test with fluopyram + trifloxystrobin SC 500 G - Final report
Report:	Kuhl, K.; 2018; EBGM0016; M-615579-01-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC Regulation1107/2009 (Europe) OECD Test Guideline 201 US EPA OCSPP 850.4500
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation

The purpose of the analytical part of this study was to verify the concentrations of the active ingredients fluopyram and trifloxystrobin of the test item fluopyram + trifloxystrobin SC 500 in the test water by LC-MS/MS. In the following part, only the data for trifloxystrobin is presented.
The analytical method 01387 was used in the present study which is fully validated and EU-agreed (Krebber, R.; Braune, M.; 2013; M-466732-01-1). The method was validated concurrently with the test solution analyses. For this purpose, the trifloxystrobin standard injections were evaluated.
The diluted water samples were directly injected into the HPLC-MS/MS instrument. The injection volume was 10 µL. Each sample was injected in duplicate.
Because of the direct measurement of the samples recovery rates cannot be calculated. Thus, the presented precision data is based on four to six injections of six different standard solutions. The relative standard deviations for the peak areas were <4% for all measured concentration levels.

Table A 151: Recovery rates and precision results (repeatability) of trifloxystrobin

trifloxystrobin standard concentration [µg/L]	n	Peak area		Retention Time	
		Mean Value	RSD	Mean Value	RSD
		[area counts]	[%]	[min]	[%]
0.0500	4	107838	1.1	2.91	0.2
0.100	4	216654	1.4	2.90	<0.1
0.500	4	1054547	3.2	2.91	0.2
1.00	6	2100124	2.0	2.91	0.2
5.00	4	10132284	1.6	2.91	0.2
10.0	4	17261809	1.5	2.91	<0.1

Table A 152: Characteristics for the analytical method used for validation of trifloxystrobin

	trifloxystrobin
Specificity	HPLC-MS/MS method is highly specific. Blank values were below 30% of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): $y = 1.8465 \cdot 10^6 \; x + 39443$, Correlation coefficient r: 0.996213, number of data points: 6
Calibration range	0.0500 – 10.0 µg/L
Limit of determination/quantification	LOQ = 0.0625 µg a.s./L
Assessment of matrix effects is presented	No effects observed.

Conclusion

The applicability of the method 01387 for the analysis of trifloxystrobin in water samples was tested. The data presented demonstrate that the method allows the determination of this substance with satisfactory

precision data given as the relative standard deviation was below 20% of four to six replicates per measured concentration level. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.2.1.6.24 Analytical method 01387 in support of the study M-636234-01-1

A 2.2.1.6.24.1 Method validation

Comments of zRMS:	Concentrations of fluopyram and trifloxystrobin of the test item Fluopyram + Trifloxystrobin SC 500 (250 + 250 g/L) were determined in the test water using liquid chromatography with MS/MS detection. Specificity: No significant (< 30%) interference of total peak area for the target analyte was found for either analyte. Linearity: Calibration Range: Fluopyram: Two calibration curves were used in order to cover the wide concentration range of 0.1 – 100 µg reference item/L with high accuracy. 1. 0.1 – 10 µg a.i./L 2. 0.1 – 100 µg a.i./L Trifloxystrobin: 0.1 – 100 µg a.i./L Linearity of Response: Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression. Correlation Coefficient: Fluopyram: 1. r = 1.0000 2. r = 0.9998 Trifloxystrobin: r = 0.9999 Calibration Curves: Fluopyram: 1. y = 27248 * x + 1087 2. y = 24682 * x + 10730 Trifloxystrobin: y = 56584 * x + 5193 Accuracy and Precision: Mean Recovery Rates in the Fortified Samples: Fluopyram: 92% (n = 15, RSD 10%) Trifloxystrobin: 77% (n = 15, RSD 4%) The values found for the precision (RSD) and for the accuracy (mean recovery rate) are acceptable. Limit of Quantification: Fluopyram: 0.2 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.002 mg test item/L) 86% (n = 5, RSD 13%) Trifloxystrobin: 0.75 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.007 mg test item/L) 79% (n = 5, RSD 3%) The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met. The study is acceptable.

Reference:	**KCP 5.1.2.6/05**
Title:	Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L): Toxicity to Pseudokirchneriella subcapitata in an algal growth inhibition test
Report:	xxx
Authority registration No:	
Guideline(s):	-OECD Guidelines for the Testing of Chemicals, Section 2, No. 201: "Feshwater Alga and Cyanobacteria, Growth Inhibition Test", adopted March 23, 2006, corrected July 28, 2011 - Commission Regulation (EC) No 761/2009, Annex, Part C, C.3: "Feshwater Alga and Cyanobacteria, Growth Inhibition Test", Official Journal of the European Union (EN), dated August 24, 2009 - EPA Guideline 712-C-006: OCSPP 850.4500, "Algal Toxicity", January 2012 - Japanese MAFF, Gueidelines for preparation of Study Results, Algae growth Inhibition studies. Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-7-7, Algae growth Inhibition, 2005 - SANCO/3029/99 rev. 4 11/07/00: Residues: Guidance for generating and reporting methods of Analysis in Support of preregistation data requiremetns for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the analytical part of this study was to verify the concentrations of the active ingredients fluopyram and trifloxystrobin of the test item fluopyram + trifloxystrobin SC 500 in the test water by LC-MS/MS. In the following part, only the data for trifloxystrobin is presented.
The analytical method 01387 was used in the present study which is fully validated and EU-agreed (Krebber, R.; Braune, M.; 2013; M-466732-01-1).
After appropriate dilution, the samples were centrifuged (13,000 rpm, 3 minutes) before analysis. The determination was conducted using high performance liquid chromatography (HPLC) with mass-spectrometric (MS/MS) detection (ESI positive, MRM used as quantifier m/z: 409.1 → 186.0, MRM used as qualifier m/z: 409.1 → 145.0).

Results and discussions

For trifloxystrobin, recoveries were performed in test water spiked with test item at the fortification levels of 0.002, 0.007 and 3.6 mg test item/L. The mean and overall mean recovery values were within the acceptable range of 70 – 110%, all relative standard deviation (RSD) values were below 20%.

Table A 153: Recovery rates and precision results (repeatability) of trifloxystrobin

	Concentration					
Sample description	**Nominal [mg test item/L]**	**Found [µg a.i./L][1]**	**DF**	**Concentration calculated [µg a.i./L][1]**	**Corrected nominal [µg a.i./L][2]**	**Recovery [%][1]**
Anayltical Blank	0	<LOQ	2	n.a.	0.000	n.a.
Anayltical Blank	0	<LOQ	2	n.a.	0.000	n.a.
Fortified Sample	*0.002*	*0.147*	*2*	*0.294*	*0.445*	*66*
	0.002	*0.156*	*2*	*0.311*	*0.445*	*70*
	0.002	*0.152*	*2*	*0.304*	*0.435*	*70*
	0.002	*0.145*	*2*	*0.290*	*0.435*	*67*
	0.002	*0.168*	*2*	*0.336*	*0.435*	*77*
					Mean value (n = 5):	***70***
					RSD (n = 5):	***6***
Fortified Sample	0.007	0.605	2	1.209	1.557	78
	0.007	0.631	2	1.262	1.557	81
	0.007	0.328	2	1.255	1.521	83
	0.007	0.599	2	1.198	1.521	79

	0.007	0.582	2	1.163	1.521	76
					Mean value (n = 5):	**79**
					RSD (n = 5):	**3**
Fortified Sample	3.6	58.582	10	585.816	800.543	73
	3.6	58.405	10	584.049	800.543	73
	3.6	59.465	10	594.652	782.184	76
	3.6	59.819	10	598.187	782.184	76
	3.6	61.232	10	612.325	782.184	78
					Mean value (n = 5):	**75**
					RSD (n = 5):	**3**
					Overall mean value (n = 15):	**77**
					RSD (n = 15):	**4**

LOQ: Limit of Quantification = 0.007 mg test item/L corresponding to 0.75 µg a.i./L after dilution by factor 2;
n.a.: not applicable; RSD: Relative Standard Deviation; DF: Dilution factor; a.i.: active ingredient;
italic: Fortification level of 0.002 mg test item/L did not yield valid results due to interference from analytical blanks. Samples displayed for information only and excluded from evaluation.

Table A 154: Characteristics for the analytical method used for validation of trifloxystrobin

	trifloxystrobin
Specificity	HPLC-MS/MS method is highly specific. Blank values were below 30 % of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented calibration equation (1/x weighted): y = 56584 x + 5193, Correlation coefficient r: 0.9999, number of data points: 9
Calibration range	0.1– 100 µg a.i./L
Limit of determination/quantification	LOQ = 0.75 µg a.i./L after dilution by factor 2 (corresponding to fortification level of nominal 0.007 mg test item/L)
Assessment of matrix effects is presented	No effects observed.

Conclusion
The analytical method complies with all guideline criteria according to SANCO/3029/99 rev. 4 and can be seen as fit for purpose for the presented study.

A 2.2.1.7 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1)

No specific method developed in support of studies summarized in B1,2,4 section 2 & Appendix 2.

A 2.2.2 Methods for post-authorisation control and monitoring purposes (KCP 5.2)

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.4 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.5 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.6 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.7 Other Studies/ Information

No new or additional studies have been submitted