FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: BAS 758 00 F

Product name(s): Revyflex Plus

Chemical active substance(s):

Mefentrifluconazole, 66.6 g/L

Metrafenone, 100 g/L

Pyraclostrobin, 80 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: BASF

Submission date: March 2022

MS Finalisation date: 27/01/2023

Version history

When	What
03/2022	Initial dRR – BASF DocID 2021/2050428
04/2022	Dossier sent for evaluation
10/2022	zRMS evaluation of dRR
January 2023	Post-comments update submission by the applicant (BASF DocID 2022/2060893)
January 2023	Final version prepared by zRMS after Commenting period

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5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are ~~not~~ available for the active substance(s) and relevant impurities in the plant protection product.
Noticed data gaps are: none

- ~~data gap 1~~
- ~~data gap 2~~
- ~~data gap 3~~

The applicant's report was not rewritten. The zRMS comments/corrections are on grey background.
Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.

Mefentrifluconazole
The analytical methods developed for mefentrifluconazole (BAS 750 F) in plant and animal matrices were already submitted and evaluated in the context of the previous process of Annex I Inclusion of mefentrifluconazole except of a new enforcement water method with its ILV, which are submitted with the current dossier.
For data generation in foodstuffs of plant origin BASF method L0076/09 based on LC-MS/MS. For enforcement purposes LC-MS/MS method L0295/01 based on QuEChERS.
For data generation and enforcement in cow liver, kidney, muscle, fat, milk and cream and hen egg BASF method L0272/01 based on LC-MS/MS. For data generation and enforcement of the metabolite M750F022 in animal matrices GC-MS method L0309/01
For enforcement purposes and data generation of BAS 750 F in soil LC-MS/MS method L0214/01 can be applied. In water LC/MS/MS method L0359/01 with a LOQ of 30 ng/L.
BAS 750 F in air can be determined with LC/MS-MS method L0327/01 at a LOQ of 0.01 µg/L and the method L0339/01 is available for body fluids.

Metrafenone
The analytical methods developed for metrafenone (BAS 650 F) in plant and animal matrices were already submitted and evaluated by the RMS in the context of the renewal for metrafenone.
In foodstuffs of plant origin LC-MS/MS method with an LOQ of 0.01 mg/kg (BASF Doc ID 2011/7007816). In addition BASF methods L0076/01 and L0339/02 were used in the residues trials submitted with this application. For enforcement and data generation in liver, kidney, muscle, fat, milk and egg an analytical LC-MS/MS method with an LOQ of 0.01 mg/kg (BASF Doc ID 2014/1181105) is available.
For enforcement and data generation in soil LC-MS/MS method (BASF Doc ID 2014/1181107) can be applied. In water with a limit of quantification of 0.05 µg/L LC-MS/MS method (BASF Doc ID 2014/1181109) is available. Metrafenone in air can be determined (BASF Doc ID 2014/1181110) by LC-MS/MS method with a LOQ of 0.03 mg/m^3 and in body fluids (BASF Doc ID 2018/1029049) with a LOQ of 0.05 mg/L

Pyraclostrobin
The analytical methods for the determination of pyraclostrobin in foodstuffs of plant and animal origin were evaluated in the previous Annex I inclusion process and during more recent evaluations performed by EFSA in the context of MRL applications. During the re-registration process of pyraclostrobin analytical methods for residues were submitted to RMS. All analytical methods provided for the EU review are active substance data.
For the determination of pyraclostrobin in foodstuffs of plant origin, a single residue method using LC-MS/MS (421/0) and an additional method (D9904) using HPLC-UV were developed. The general suitability of these methods has been already confirmed by EFSA (EJ 2011;9(8):2344). Moreover, a new LC-MS/MS methods (445/0, 535/1), was included in this submission. Also, for the determination of the metabolite 500M79 a new method using LC-MS/MS was validated.

For enforcement and data generation in animal matrices and body fluids LC-MS/MS method L0151/01 is available. Furthermore BASF methods no. 446/2, L0058/03 and D9902 were developed.
For soil, two new LC-MS/MS methods were submitted with LOQ of 0.001 mg/kg. A new LC-MS/MS method was submitted for water with a LOQ of 0.003 µg/L. A new LC-MS/MS method was submitted for air with a LOQ of 4.44 ng/L.
The methods have been considered acceptable and suitable for the determination of pyraclostrobin residues in the respective matrix.

Noticed data gaps for all are none.

Commodity/crop	**Supported/ Not supported**
Plan matrices	Supported
Animal matrices	Supported
Soil	Supported
Water	Supported
Air	Supported
Body fluids	Supported

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)

Study summary

The analytical method AFL1019/01 was developed for the determination of the active substances Mefentrifluconazole (Reg. 5834378), Pyraclostrobin (Reg. 304428) and Metrafenone (Reg. 4037710) in BAS 758 00 F (EC - formulation) and validated according SANCO/3030/99 rev. 5.

The samples are analyzed using liquid chromatographic procedure that employs DAD/UV detection and external calibration. The separation is achieved by reversed phase chromatography using gradient conditions with acetonitrile, methanol, tetrahydrofuran, formic acid and water on a RP-C18 or ODS-H80 columns. The evaluation of the UHPLC / HPLC analyses was carried out by comparison of the peak areas with an authentic external reference item by applying bracketing calibration.

The method was validated with regard to linearity, specificity, precision and accuracy of the analytical system.

Comments of zRMS:	The HPLC method is suitable to detect active substances Mefentrifluconazole, Pyraclostrobin, and Metrafenone in the PPP.

Reference:	CP 5.1.1/1
Report	Analytical method AFL1019/01: Quantitative determination of the active ingredients Mefentrifluconazole, Metrafenone and Pyraclostrobin in BAS 758 00 F by Liquid Chromatography, Barth, J., 2020 report No 2020/2091975 Authority registration No
Guideline(s):	None
Deviations:	No
GLP:	No
Acceptability:	Yes

Comments of zRMS:	The method is validated and can be used for analyzing the active substances in the PPP.

Reference:	CP 5.1.1/2
Report	Validation of the analytical method AFL1019/01: Determination of the active ingredients Mefentrifluconazole, Metrafenone and Pyraclostrobin in BAS 758 00 F by Liquid Chromatography, Barth, J., 2020 report No 868719_1 2020/2091974

	Authority registration No
Guideline(s):	CIPAC Guidelines on method validation, SANCO/3030/99 rev. 5 (22 March 2019)
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Mainz, Germany),
Acceptability:	Yes

Materials and methods

UHPLC parameters:

Column	YMC Triart C18 (3.0 mm x 100 mm; 1.9 µm), or equivalent		
Column temperature	40 °C		
Injection volume	1 µL		
Detection wavelength	230 nm Mefentrifluconazole 275 nm Pyraclostrobin 285 nm Metrafenone Note: device-specific can be switched the detection wavelength depending on the retention time		
Flow rate	0.8 mL/min.		
Eluent	A: Water + 0.1 % Formic Acid (1000/1 v/v) B: Acetonitrile + Methanol + Tetrahydrofuran + 0.1% Formic Acid (800/150/50/1 v/v/v/v) Eluent A/B = 25/75 Note: if necessary, a rinse gradient can be used		
Rinse Gradient	Time [min.]	A [%]	B [%]
	0.00	25	75
	2.45	25	75
	2.50	1	99
	3.90	1	99
	3.95	25	75
	5.00	25	75
Dwell Volume	ca. 176 µL (Agilent 1290 infinity II, LC339)		
Approx. retention times	approx 1.32 min. Mefentrifluconazole approx. 1.84 min. Pyraclostrobin approx. 2.30 min. Metrafenone Note: retention time can be different		
Runtime	5 minutes		

HPLC parameters:

Column	YMC J'sphere ODS H80 (4.6 mm x 150 mm; 4 µm)		
Column temperature	40 °C		
Injection volume	5 µL		
Detection wavelength	230 nm Mefentrifluconazole 275 nm Pyraclostrobin 285 nm Metrafenone Note: device specific can be switched the detection wavelength depending on the retention time		
Flow rate	1.0 mL/min.		
Eluent	A: Water + 0.1 % Formic Acid (1000/1 v/v) B: Acetonitrile + Methanol + Tetrahydrofuran + 0.1% Formic Acid (750/150/100/1 v/v/v/v)		
Gradient	Time [min.]	A [%]	B [%]
	0.00	30	70
	5.00	30	70
	5.05	5	99
	7.50	5	99
	7.55	30	70
	10.00	30	70
Dwell Volume	ca. 1.037 mL (Agilent 1200 SL, LC196)		
Approx. retention times	approx 3.32 min. Mefentrifluconazole approx. 4.12 min. Pyraclostrobin approx. 5.10 min. Metrafenone Note: retention time can be different		

Validation for content determination of total active ingredients in BAS 758 00 F - Results and discussion

Identity:

The identity of Reg.No. 4037710, Reg.No. 5834378 and Reg.No. 304428 in BAS 758 00 F were confirmed by comparing the retention time in combination with the MS-spectra andthe UV-spectra of the test item with the pure reference items.

Specificity:

A solution of the reference substances, a solution of the test item, a solution of the blank formulation and a solution of the test item fortified with the reference substances were measured. No interferences were detected.

Stability:

The solutions of the test items were measured again about 70 hours for UHPLC and HPLC after first injection of this solution. Also, a calibration solution was measured again about 74 hours for UHPLCand HPLC after first injection of this solution. The areas of the first injections were compared to the areas of the injection after storage. The recovery for the sample solution were found to be between 100.3 and 101.2%

for HPLC and 100.4 and 100.8% for UHPLC. The recovery for the calibration solution were found to be between 100.1 and 100.9 % for HPLC and 100.2 and 100 .7 % for UHPLC. All solutions were stored at room temperature.

Linearity:

The linearity of the detector response was determined by preparing a calibration series ofthe pure reference items. The linearities were demonstrated by preparing five calibration solutions, which were injected twice.

Accuracy (recovery):

The evaluation of the accuracy was done by analyzing five sample solutions for each concentration level containing BAS 758 00 F fortified with a concentration of approximately50%, 100% or 150% of Reg.No. 4037710, Reg.No. 5834378 and Reg.No. 304428 relative to the nominal active ingredient concentrations in the formulation. Each sample was injected twice. The accuracy was confirmed by the determination of therecovery rate by comparing of the found and the expected content.

Precision:

The content of Reg.No. 4037710, Reg.No. 5834378 and Reg.No. 304428 in BAS 758 00 F were determined by analyzing seven sample solutions and calculating the relative standard deviation of the recovery rates in %. The acceptability of the % RSD (coefficient of variation, CV) for precision was proven by the Horrat value.

Table 5.2- 1: Validation of the method AFL1019/01 for the determination of total active ingredients in BAS 758 00 F.

Parameter	**Mefentrifluconazole (Reg.No. 5834378)** ***Max. 6.1% pure active substance in BAS 758 00 F***	**Pyraclostrobin (Reg.No. 304428)** ***Max. 7.3% pure active substance in BAS 758 00 F***	**Metrafenone (Reg.No. 4037710)** ***Max. 9.2% pure active substance in BAS 758 00 F***
Author(s), year	Barth J., 2020		
Principle of method	UHPLC / HPLC with DAD/UV detection		
Linearity n = 5 (double injection) Mefentrifluconazole: Linear between 38.0 – 190.1% (calculated corresponding to the nominal content of approx. 6.1%) Pyraclostrobin: Linear between 37.4 – 187.0% (calculated corresponding to the nominal content of approx. 7.3%) Metrafenone: Linear between 38.0 – 190.1% (calculated corresponding to the nominal content of approx. 9.2%)	UHPLC parameters: Slope: 1.000 Y-axis intercept: -0.00004 r = 0.9999	UHPLC parameters: Slope: 1.000 Y-axis intercept: 0.00043 r = 1.0000	UHPLC parameters: Slope: 1.000 Y-axis intercept: -0.00004 r = 0.9999
	HPLC parameters: Slope: 1.000 Y-axis intercept: 0.0002 r = 0.9999	HPLC parameters: Slope: 1.000 Y-axis intercept: -0.0001 r = 1.0000	HPLC parameters: Slope: 1.000 Y-axis intercept: 0.0003 r = 0.9999

Parameter	Mefentrifluconazole (Reg.No. 5834378) *Max. 6.1% pure active substance in BAS 758 00 F*	Pyraclostrobin (Reg.No. 304428) *Max. 7.3% pure active substance in BAS 758 00 F*	Metrafenone (Reg.No. 4037710) *Max. 9.2% pure active substance in BAS 758 00 F*
(correlation coefficient expressed as r)			
Accuracy as recovery n = 5 (double injection)	UHPLC parameters: 99.8% for the first fortification level (corresponding to 54.44% of the nominal content in the formulation) 99.5% for the second fortification level (corresponding to 108.60% of the nominal content in the formulation) 100.1% for the third fortification level (corresponding to 163.76% of the nominal content in the formulation)	UHPLC parameters: 101.2% for the first fortification level (corresponding to 55.00% of the nominal content in the formulation) 99.1% for the second fortification level (corresponding to 107.66% of the nominal content in the formulation) 99.8% for the third fortification level (corresponding to 162.63% of the nominal content in the formulation)	UHPLC parameters: 100.1% for the first fortification level (corresponding to 54.31% of the nominal content in the formulation) 99.6% for the second fortification level (corresponding to 108.11% of the nominal content in the formulation) 100.2% for the third fortification level (corresponding to 163.22% of the nominal content in the formulation)
	HPLC parameters: 101.6% for the first fortification level (corresponding to 55.41% of the nominal content in the formulation) 101.3% for the second fortification level (corresponding to 108.98% of the nominal content in the formulation) 100.8% for the third fortification level (corresponding to 164.89% of the nominal content in the formulation)	HPLC parameters: 100.6% for the first fortification level (corresponding to 54.68% of the nominal content in the formulation) 100.6% for the second fortification level (corresponding to 109.33% of the nominal content in the formulation) 100.5% for the third fortification level (corresponding to 163.78% of the nominal content in the formulation)	HPLC parameters: 101.4% for the first fortification level (corresponding to 55.04% of the nominal content in the formulation) 100.8% for the second fortification level (corresponding to 109.45% of the nominal content in the formulation) 101.1% for the third fortification level (corresponding to 164.61% of the nominal content in the formulation)
Precision as repeatability n = 7 (double injection)	UHPLC parameters: The relative standard deviation (RSD) was found to be 1.046%. The Horrat value was calculated to be 0.51 at Mefentrifluconazole content of 6.03% (w/w).	UHPLC parameters: The relative standard deviation (RSD) was found to be 1.022%. The Horrat value was calculated to be 0.52 at Pyraclostrobin content of 7.44% (w/w).	UHPLC parameters: The relative standard deviation (RSD) was found to be 1.062%. The Horrat value was calculated to be 0.55 at Metrafenone content of 9.29% (w/w).

Parameter	Mefentrifluconazole (Reg.No. 5834378) *Max. 6.1% pure active substance in BAS 758 00 F*	Pyraclostrobin (Reg.No. 304428) *Max. 7.3% pure active substance in BAS 758 00 F*	Metrafenone (Reg.No. 4037710) *Max. 9.2% pure active substance in BAS 758 00 F*
	The RSD was acceptable since the Horrat value was ≤ 1.	The RSD was acceptable since the Horrat value was ≤ 1.	The RSD was acceptable since the Horrat value was ≤ 1.
	HPLC parameters: The relative standard deviation (RSD) was found to be 1.472%. The Horrat value was calculated to be 0.72 at Mefentrifluconazole content of 6.07% (w/w). The RSD was acceptable since the Horrat value was ≤ 1.	HPLC parameters: The relative standard deviation (RSD) was found to be 1.710%. The Horrat value was calculated to be 0.87 at Pyraclostrobin content of 7.57% (w/w). The RSD was acceptable since the Horrat value was ≤ 1.	HPLC parameters: The relative standard deviation (RSD) was found to be 1.550%. The Horrat value was calculated to be 0.81 at Metrafenone content of 9.33% (w/w). The RSD was acceptable since the Horrat value was ≤ 1.

Conclusion

With respect to the conditions described for the analytical method AFL1019/01 all validation parameters (accuracy, precision, identity, linearity, specificity and stability) are acceptable. Therefore, the method is valid without restriction in the tested concentration range and is suitable for the determination of Reg.No. 4037710, Reg.No. 5834378 and Reg.No. 304428 in BAS 758 00 F.

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

Analytical Method AFL0944 – determination of the relevant impurity N,N-Dimethylformamide (Reg.No. 159267) in formulations containing Mefentrifluconazole

The analytical method AFL0944 is used for the determination of the relevant impurity N,N-Dimethylformamide (Reg.No. 159267) in formulations containing Mefentrifluconazole including BAS 758 00 F. The samples are analyzed using a gas chromatographic procedure that employs external standard. The separation is achieved by using gradient conditions for detection and quantification. A RTX-200 column or equivalent is used. The analyses are detectedusing a MS detector and quantified by external linear regression using authentic reference item with known content. The method was validated with regards to specificity, identity, linearity, accuracy (recovery), precision (repeatability), Limit of Detection, Limit of Quantification and stability according SANCO 3030/99.

There are several versions of AFL0944 available. The analytical method AFL0944/01 is used for the determination of the relevant impurity N,N-Dimethylformamide (Reg.No. 159267) in BAS 750 01 F (EC - formulation) at a concentration level of approximately 10 – 1030 ppm of Reg. 159267 in formulation. In addition, the analytical method AFL0944/02 as another version is used for the determination of the relevant impurity N,N-Dimethylformamide (Reg.No. 159267) in BAS 758 00 F (at a concentration level of approximately 0.002% - 0.006% of Reg. 159267 in formulation) and BAS 751 00 F. Different to the method AFL0944/01 the weigh-in of the test item was adjusted (300 mg BAS 751 00 F and 450 mg BAS 758 00 F) and the MS transferline temperature was added (250°C). The concentration range of the determination was smaller: 0.185 mg/L to 1.530 mg/L for Reg.No.: 159267. The validation of BAS 751 00 F is not an object of this dossier and will be not presented here.

Comments of zRMS:	This study contains description of an analytical method used for DMS impurity

	determination. The information is accepted.

Reference:	CP 5.1.1/3
Report	Analytical Method AFL0944/01- Determination of Dimethylformamide (DMF) in Formulation containing Mefentrifluconazole (BAS 750 F) by GC/MS", Harsch M., 2017 report No 2017/1012360 Authority registration No
Guideline(s):	None
Deviations:	No
GLP:	No
Acceptability:	Yes

Comments of zRMS:	The study conains inrmation concerning the method's validation for DMF in BAS 750 F

Reference:	CP 5.1.1/4
Report	Validation of the Analytical Method AFL0944/01- Determination of Dimethylformamide (DMF) in Formulation containing Mefentrifluconazole (BAS 750 F) by GC/MS", Harsch M., 2017 report No 837633_1 2017/1012361 Authority registration No
Guideline(s):	OECD Principles of Good Laboratory Practice, CIPAC Guidelines on methoda validation, SANCO/3030/99, US EPA OPPTS Harmonized Test Guideline 830.1000, US EPA OPPTS Harmonized Test Guideline 830.1800, ABNT NBR 14029
Deviations:	No
GLP:	Yes (certified by Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Comments of zRMS:	The objective of this study was the additional validation of the analytical method AFL0944/01 to be used for the determination of Reg.No.:159267 in BAS 758 00 F and BAS 751 00 F. The method is validated and accepted for analyzing DMSF in the PPP.

Reference:	CP 5.1.1/5

Report	Additional Validation of the Analytical Method AFL0944/01: "Determination of Dimethylformamide (DMF) in Formulation containing Mefentrifluconazole (BAS 750 F) by GC/MS", Schubring, M., 2021 report No 897010_1 2020/2109404 Authority registration No
Guideline(s):	2004/10/EC, ABNT NBR 14029, CIPAC Guidelines on method validation, EC 1107/2009, US EPA OPPTS Harmonized Test Guideline 830.1000, US EPA OPPTS Harmonized Test Guideline 830.1800, SANCO/3030/99 rev. 5 (22 March 2019)
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Mainz, Germany),
Acceptability:	Yes

Materials and methods of Analytical Method AFL0944/01

GC parameters:

Column	Rtx-200; 60 m x 0.32 mm;1.5 µm		
Injector temperature	280 °C		
Oven temperature	Rate [°C/min.]	Value [°C]	Hold Time [min.]
	-	60	2
	10	130	2
	30	250	10
Carrier gas	Helium		
Detector	MSD (EI)		
Split ratio	Splitless		
Column flow	2.0 mL/minute (constant flow)		
Injection volume	1 µL		
Analysis time	25 min.		
Source temperature	230°C		
Quad temperature	200°C		

Target compound	Retention time [min.]	m/z [quantifier]	m/z [qualifier]
Reg.No.:159267	12.4	44	73

Validation of the Analytical Method AFL0944/01 - content determination of DMF (Reg.No. 159267) in formulation containing Mefentrifluconazole (BAS 750 F) - Results and discussion

Identity:

The comparison of the MS-spectra and retention times demonstrate that the analytes (RegNo. 159267) in the reference item are identical to the analytes in the test item.

Specificity:

A solution of Reg.No.:159267 and a solution of each blank formulation were measured. There were no interferences between the blank formulations and Reg.No.:159267.

Stability:

The calibration solutions were reanalyzed after storage period of about 40 hours at room temperature. The sample solutions were reanalysed after storage period of about 26 hours at room temperature. The test item was found to be stable.

LoQ:

The Limit of Quantification has been determined to be 10 ppm for Reg.No. 159267.

LoD:

The Limit of Detection has been set up to be 1 ppm for Reg.No. 159267.

Linearity:

Linearity was measured using the reference item Reg.No. 159267 in a series of eleven concentrations in a range of approximately 0.08 mg/L to 15.7 mg/L. The reference item was analyzed in each case in accordance with the present analytical method. The correlation coefficients were within the limit.

Accuracy (recovery):

The evaluation of the accuracy was done by analyzing for each concentration level five sample solutions containing BAS 750 01 F fortified with concentration levels about 10 ppm, 100 ppm, 500 ppm and 1000 ppm. Each fortification level was taken through the sample preparation procedure and quantified according to GC method AFL0944/01 in order to calculated % recovery of Reg.No. 159267. The recovery rate found at all fortified concentration levels are within the acceptable range.

Precision:

The content of Reg.No.:159267 in BAS 758 00 F was determined by analyzing seven sample solutions, each sample was injected twice and calculating the relative standard deviation of the recovery rates in %. The acceptability of the % RSD (coefficient of variation, CV) for precision was proven by the Horrat value.

Table 5.2- 2 Validation of the method AFL0944/01 for the determination of the relevant impurity of Reg.No. 159267 in formulation containing Mefentrifluconazole (BAS 750 F)

Parameter	**N,N-Dimethylformamide (Reg.No. 159267)**
Author(s), year	Harsch M., 2017
Principle of method	GC-MS
Formulation	BAS 750 01 F
Linearity n = 11 Reg.No. 159267: Linear between 0.08 – 15.7 mg/L (corresponding to approx.. 7 – 1300 ppm in formulation) (correlation coefficient expressed as r)	Slope: 45443 Y-axis intercept: -1691 r = 0.9999
Accuracy as recovery n = 5	The mean recovery for Reg.No. 159267 in the preparation was found to be: • 102% for the 10 ppm accuracy level, • 117% for the 103 ppm accuracy level, • 104% for the 515 ppm accuracy level, • 108% for the 1030 ppm accuracy level
Precision as repeatability n = 7 (double injection)	The relative standard deviations (RSD) for the whole procedure of sample preparation and measurement of the precisions for BAS 750 01 F were found to be: • Reg.No. 159267: Mean value: 68 ppm RSD= 1.27% (corresp. Conc. 0.000068), Horrat value of H_r = 0.22. The RSD was acceptable since the Horrat value was ≤ 1.
LoQ	10 ppm
LoD	1 ppm

Materials and methods of Analytical Method AFL0944/02

GC parameters:

Column	Rtx-200; 60 m x 0.32 mm; 1.5 µm		
Injector temperature	280 °C		
MS transferline temperature	250 °C		
Oven temperature	Rate [°C/min.]	Value [°C]	Hold Time [min.]
	-	60	2
	10	130	2
	30	250	10
Carrier qas	Helium		

Detector	MSD
Split ratio	10:1
Column flow	2.0 mL/minute (constant flow)
Injection volume	1 µL
Analysis time	25 min.
Source temperature	230°C
Quad temperature	150°C
Solvent delay	9 min.
MS off	After 16 min.

Target compound	Retention time [min.]	m/z [quantifier]	m/z [qualifier]
Reg.No.:159267	14.2	44	73

Additional Validation of the Analytical Method AFL0944/02 - content determination of DMF (Reg.No. 159267) in BAS 758 00 F - Results and discussion

Identity:

A solution of the reference substance and a solution of each test item fortified with the reference substance were measured. The identity was confirmed.

Specificity:

A solution of Reg.No.:159267 and a solution of eachblank formulation were measured. There were no interferences between the blank formulations and Reg.No.:159267.

Stability:

A solution of the reference item was measured again after 83h and a solution of each test item was measured again after 66h for BAS 758 00 F. All solutions were found to be stable.

LoQ:

The lowest accuracy level (0.002%) was used for the LoQ BAS 758 00 F: 0.324 mg/L.

Linearity:

The linearity of the detector response was determined by preparing a calibration series of the pure reference item. The linearities were demonstrated by preparing at least six calibration solutions, which were injected twice. Data evaluation confirmed a linear detector response at least between 0.185 mg/L and 1.530 mg/L

Accuracy (recovery):

The evaluation of the accuracy was done by analyzing for each concentration level five sample solutions containing BAS 758 00 F fortified with a concentration of approximately 0.006% and 0.002% Reg.No.:159267 relative to the nominal weigh in concentration of the formulations. Each sample was injected twice. The accuracy was confirmed by the determination of the recovery rate by comparing of the found and the expected content.

Precision:

The content of Reg.No.:159267 in BAS 758 00 F was determined by analyzing five sample solutions, each sample was injected twice and calculating the relative standard deviation of the recovery rates in %. The acceptability of the % RSD (coefficient of variation, CV) for precision was proven by the Horrat value.

Table 5.2- 3 Additional Validation of the method AFL0944/02 for the determination of the relevant impurity of Reg.No. 159267 in BAS 758 00 F.

Parameter	**N,N-Dimethylformamide (Reg.No. 159267)** ***Max. 0.031 g/kg (31 ppm) in BAS 758 00 F***
Author(s), year	Schubring M., Frohn D., 2021
Principle of method	GC-MS
Linearity n = 6 (double injection) Reg.No. 159267: Linear between 0.185 – 1.530 mg/L (corresponding to 0.01 – 0.085 g/kg in formulation) (correlation coefficient expressed as r)	Slope: 2.35e+04 Y-axis intercept: 941.52 r = 0.9947
Accuracy as recovery n = 5 (double injection)	The mean recovery for Reg.No. 159267 in the preparation was found to be: • 91.0% for the 0.002% accuracy level, • 82.1% for the 0.006% accuracy level, calculated corresponding to the nominal weigh in concentration of the formulation (18 g/L).
Precision as repeatability n = 5 (double injection)	The relative standard deviations (RSD) for the whole procedure of sample preparation and measurement of the precisions for BAS 758 00 F were found to be: • Reg.No. 159267: Mean value: 0.0024% RSD= 6.60% (limit 6.66%), Horrat value of H_r = 1.0. The RSD was acceptable since the Horrat value was ≤ 1.
LoQ	0.324 mg/L (corresponds to 0.002% accuracy concentration)

Conclusion

With respect to the conditions described for the analytical method AFL0944 all validation parameters (linearity, precision, accuracy, identity, specificity, stability and LoQ) are acceptable. Therefore, the method is valid without restriction in the tested concentration range and is suitable for the determination of Reg.No.:159267 in BAS 758 00 F.

Study summary

The analytical method AFL1023/01 was used for the determination of the relevant impurity Toluene (Reg.No. 4005250) in BAS 758 00 F (EC - formulation) and validated according CIPAC Guidelines on method validation, SANCO/3030/99 rev.5, EPA OPPTS 830.1000, EPA OPPTS 830.1800 and ABNT NBR 14029.

The samples are analyzed using a GC-MS procedure that employs external standard.The separation is achieved by using gradient conditions for detection and quantification.A RTX 200 column or equivalent is used. The analyses are detected using a MS detector and quantified by comparing the specific response ratio of the sample with those of the standard of known quality.

The method was validated with regard to linearity, specificity, precision, accuracy, LoQ and LoD of the analytical system.

Comments of zRMS:	This study covers the information that methodis applicable to the determine the content of Toluene in the PPP. (Reg.No.:4005250) in BAS 758 00 F EC-formulation containing Mefentrifluconazole, Metrafenone and Pyraclostrobin.

Reference:	CP 5.1.1/6
Report	Analytical Method AFL1023/01: Determination of Toluene in BAS 758 00 F (EC - Formulation) by GC-MS, Schubring, M., 2020 report No 2020/2094553 Authority registration No
Guideline(s):	None
Deviations:	No
GLP:	No
Acceptability:	Yes

Comments of zRMS:	The method is validated and accepted to analyse toluene in the PPP.

Reference:	CP 5.1.1/7
Report	Validation of the Analytical Method AFL1023/01: Determination of Toluene in BAS 758 00 F (EC - Formulation) by GC-MS, Schubring, M., 2020 report No 895582_1 2020/2094552 Authority registration No
Guideline(s):	EC 1107/2009, EPA 830.1000, OPPTS 830.1800, SANCO/3030/99 rev. 4 (11 July 2000)
Deviations:	No

GLP: Yes

(certified by Landesamt fuer Umwelt, Mainz, Germany),

Acceptability: Yes

Materials and methods

GC parameters:

Column	RTX-200; 30m x 0.32 mm; 1.5 µm		
Injector temperature	250 °C		
MS transferlinetemperature	250 °C		
Oven temperature	Rate [°C/min]	Value [°C]	Hold Time [min]
	-	100	4
	20	250	4
Carrier gas	Helium		
Detector	MSD		
Split ratio	10:1		
Column flow	1.5 mL/min (constant flow)		
Injection volume	1.5 µL		
Analysis time	15.5 min		
Source temperature	230 °C		
Quad temperature	150 °C		
Solvent delay	3.5 min		
MS off	After 5 min		

Target compound	Retention time [min]	m/z [quantifier]	m/z [qualifier]
Reg.No.:4005250	4	91	92

Validation for content determination of Toluene (Reg.No. 4005250) in BAS 758 00 F - Results and discussion

Identity:

A solution of the reference substance and a solution of the test item fortified with the reference substance were measured. Theidentity was confirmed.

Specificity:

A solution of Reg.No.:4005250, Reg.No.:5834378, Reg.No.:4037710, Reg.No.:304428 and a solution of the blank formulation weremeasured. There were no interferences between the blank formulation andToluene.

Stability:

A solution of the reference item was measured again after 116h and a solution of the test item was measured again after 115h. Both solutions were found to be stable.

LoQ:

The lowest accuracy level (0.000641%) was used for the LoQ.

LoD:

The lowest linearity level (0.00048%) was used for the LoD.

Linearity:

The linearity of the detector response was determined by preparing a calibration series of the pure reference item. The linearity was demonstrated by preparing at least six calibration solutions, which were injected twice. Data evaluation confirmed a linear detector response at least between 0.108 mg/L and 20.172 mg/L.

Accuracy (recovery):

The evaluation of the accuracy was done by analyzing for each concentration level five samplesolutions containing BAS 758 00 F fortified with a concentration of approximately 0.000641%,0.00482%, 0.0112% or 0.0700% Toluene (Reg.No.:4005250) relative to the nominal weigh in concentration of the formulation. Each sample was injected twice. The accuracy was confirmed by the determination of the recovery rate by comparing ofthe found and the expected content.

Precision:

The content of Toluene (Reg.No.:4005250) in BAS 758 00 F was determined by analyzing fivesample solutions, each sample was injected twice and calculating the relative standard deviation of the recovery rates in %. The acceptability of the % RSD (coefficient of variation, CV) for precision was proven by the Horrat value.

Table 5.2- 4 Validation of the method AFL1023/01 for the determination of the relevant impurity of Reg.No.:4005250 in BAS 758 00 F.

Parameter	**Toluene** **Reg.No.:4005250** ***Max. 0.063 g/kg (63 ppm) in BAS 758 00 F***
Author(s), year	Schubring M., 2020
Principle of method	GC-MS
Linearity n = 6 (double injection) Reg.No. 4005250: Linear between 0.108 – 20.172 mg/L (corresponding to 0.0048 – 0.9005 g/kg in formulation) Reg.No. 4005250: Linear between 0.108 –3.237 mg/L (corresponding to 0.0048 – 0.145 g/kg in formulation)	 Slope: 15006.0868 Y-axis intercept: -1308.3873 R^2 = 0.9994 Slope: 14299.4517 Y-axis intercept: 68.2746 r = 1.0000

(correlation coefficient expressed as r)	
Accuracy as recovery n = 5 (double injection)	The mean recovery for Reg.No. 4005250 in the preparation was found to be: • 113.6% for the 0.000641% accuracy level, • 109.4% for the 0.00482% accuracy level, • 104.9% for the 0.0112% accuracy level, • 99.3% for the 0.0700% accuracy level, calculated corresponding to the nominal weigh in concentration of the formulation (22.4 g/L).
Precision as repeatability n = 5 (double injection)	The relative standard deviations (RSD) for the whole procedure of sample preparation and measurement of the precisions for RegNo. 4005250 in BAS 758 00 F was found to be: • Reg.No. 4005250: Mean value: 0.0302% RSD= 0.49% (limit 4.54%), Horrat value of H_r = 0.11. The RSD was acceptable since the Horrat value was ≤ 1.
LoQ	0.144 mg/L (corresponds to 0.000641% accuracy concentration)
LoD	0.108 mg/L (corresponds to 0.00048% linearity concentration)

Conclusion

With respect to the conditions described for the analytical method AFL1023/01 all validation parameters (linearity, precision, accuracy, intermediate precision, identity, specificity, stability,LoQ and LoD) are acceptable. Therefore, the method is valid without restriction in the tested concentration range and is suitable for the determination of Reg.No.:4005250 in BAS 758 00 F.

Analytical Method AFL0977 – determination of the relevant impurity 1,2,4-(1H)-triazole (Reg.No. 87084) in formulations containing Mefentrifluconazole

The analytical method AFL0977 is used for the determination of the relevant impurity1,2,4-(1H)-triazole (Reg.No. 87084) in formulations containing Mefentrifluconazole including BAS 758 00 F. The determination of the content of Reg.No. 87084 is performed by high performance liquid chromatographic procedure. The analyte was detected using a MS detector.
The method was validated with regard to specificity, linearity precision, accuracy and LoQ of the analytical system according SANCO 3030/99.

There are several versions of AFL0977 available. The analytical method version AFL0977/04 is used for the determination of the relevant impurity 1,2,4-(1H)-triazole (Reg.No. 87084) in BAS 760 00 F at a concentration range from approximately 0,004 – 0.015% of Reg.No. 87084 in formulation using a high performance liquid chromatographic procedure that employs standard addition for quantification. The analytical

method AFL0977/06 as another version is used for the determination of the relevant impurity 1,2,4-(1H)-triazole (Reg.No. 87084) in BAS 758 00 F at a concentration range from approximately 0,0040 – 0.0075% of Reg.No. 87084 in formulation. The calculation of the test item concentration is carried out by comparison of the peak area of the sample with those of an external standard series applying a linear function. Within this new version, information about all former studies (test items, chromatorgrams, study numbers, DocIDs) were added.
The validation report of BAS 763 00 F does not include a linearity determination. For linearity please see additional validation (study code: 886096_1, DocID 2020/2037319).

Comments of zRMS:	This method is applicable to the determination of Reg.No. 87084 in Formulations containing Mefentrifluconazole. Considered as supportive study.

Reference:	CP 5.1.1/8
Report	Additional Validation of the Analytical Method AFL0977/03: "Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole", Nemitz, A., 2020 report No 886096_1 2020/2037319 Authority registration No
Guideline(s):	OECD Principles of Good Laboratory Practice, CIPAC Guidelines on method validation, SANCO/3030/99 rev. 5, US EPA OPPTS Harmonized Test Guideline 830.1000, US EPA OPPTS Harmonized Test Guideline 830.1800, ABNT NBR 14029
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Mainz, Germany),
Acceptability:	Yes

Comments of zRMS:	This study contains data on determining 1,2,4-(1H)-triazole in Formulations containing Reg.No. 5834378 (Mefentrifluconazole or BAS 750 F).

Reference:	CP 5.1.1/9
Report	Analytical Method AFL0977/06: “Determination of the impurity Reg.No.87084 in Formulations containing Mefentrifluconazole”, Frohn D. and Nemitz, A., 2021 report No 2020/2102073 Authority registration No
Guideline(s):	None
Deviations:	No
GLP:	No

Acceptability: Yes

Comments of zRMS:	The objective of the study is to provide an additional validation to the analytical method AFL0977/05. The results of the validation report include data to demonstrate the specificity, identity, accuracy, precision (repeatability), stability and LoQ of the analytical method. The method can be used for analysing the impurity in the PPP.

Reference: CP 5.1.1/10

Report: Additional Validation of the Analytical Method AFL0977/05: "Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole",

Frohn, D., 2021

report No 895584_1

2020/2101772

Authority registration No

Guideline(s): CIPAC Guidelines on method validation, EPA 830.1000, SANCO/3030/99 rev. 5 (22 March 2019), US EPA 830.1800

Deviations: No

GLP: Yes

(certified by Landesamt fuer Umwelt, Mainz, Germany),

Acceptability: Yes

Materials and methods of Analytical Method AFL0977/04

HPLC-Parameters:

Column:	Synergie Polar-RP 4µm, 150 mm x 4.6 mm
Column temperature:	40°C
Injection volume:	10 µL
Detection:	MS-Detection SIM-mode
MS Detection Signal:	70 m/z (monoisotopic mass M + H^+)
Eluent A:	1000 mL water + 1 mL formic acid
Eluent B:	1000 mL acetonitrile + 1 mL formic acid

Gradient

Time [min.]	Eluent A [%]	Eluent B [%]	Flow [mL/min.]
0.0	95	5	1.0
5.0	95	5	1.0
5.1	1	99	1.0
10.0	1	99	1.0
10.1	95	5	1.0
15.0	95	5	1.0

Approx. retention times:	Reg.No. 87084 2.1 min
Run time:	15 min

Validation of the Analytical Method AFL0977/04 for content determination of Reg.No. 87084 in formulations containing Mefentrifluconazole - Results and discussion

Specificity:

The specificity of the method was demonstrated by analyzing typical chromatograms of the pure solvent water, the reference item, the test item, the test item fortified and the blank formulation. No interferences with the peak of the analytical substance were observed.

LoQ:

The lowest accuracy level (0.004%) was used for the LoQ.

Linearity:

The linearity of the detector response was determined by preparing a calibration series of the pure reference item. The linearity was demonstrated by preparing at least five calibration solutions, which were injected twice. Data evaluation confirmed a linear detector response at least between 0.08 mg/L and 1.206 mg/L.

Accuracy (recovery):

The evaluation of the accuracy was done by analyzing for each concentration level five samplesolutions containing BAS 760 00 F fortified with a concentration of approximately 0.0040% and analyzing two sample solutions containing BAS 760 00 F fortified with approximately 0.0090% and 0.015%. Each sample was injected twice. The accuracy was confirmed by the determination of the recovery rate by comparison of the found and the expected content.

Precision:

The precision was determined by using the 0.0040% accuracy sample and calculating the relative standard deviation of the recovery rate in %, because in the formulation BAS 760 00 F a fewer content of Reg.No. 87084 was detected. The acceptability of the % RSD (coefficient of variation, CV) for precision was proven by Horrat value.

Table 5.2- 5 Validation of the method AFL0977/04 for the determination of the relevant impurity of Reg.No.:87084 in formulations containing Mefentrifluconazole

Parameter	**1,2,4-(1H)-Triazole Reg.No.:87084**
Author(s), year	Nemitz A., 2020
Principle of method	HPLC-MS
Formulation	BAS 760 00 F

Linearity n = 5 (double injection) Reg.No. 87084: Linear between 0.08 – 1.206 mg/L (corresponding to 0.0125 – 0.1884 g/kg in formulation) (correlation coefficient expressed as r)	Slope: 1352685 Y-axis intercept: 17353 R^2 = 0.9999
Accuracy as recovery (double injection)	The mean recovery for Reg.No. 87084 in the preparation was found to be: • 99.6% for the 0.004% accuracy level (n = 5), • 99.5% for the 0.009% accuracy level (n = 2), • 99.0% for the 0.015% accuracy level (n = 2), calculated corresponding to the nominal weigh in concentration of Mefentrifluconazole in the formulation (150 g/L).
Precision as repeatability n = 5	The relative standard deviations (RSD) for the whole procedure of sample preparation and measurement of the precisions for RegNo. 87084 in BAS 760 00 F was found to be: • Reg.No. 87084: Mean value: 0.004% RSD= 0.86% (limit 6.15%), Horrat value of H_r = 0.14 The RSD was acceptable since the Horrat value was ≤ 1.
LoQ	0.0040% for Reg.No. 87084 in formulation BAS 760 00 F.

Materials and methods of Analytical Method AFL0977/06

HPLC-Parameters:

As described in the validation report (study code: 886096_1, DocID 2020/2037319) for the analytical method AFL0977/04 with a slight difference in the retention time of the analyte Reg.No. 87084.

Additional Validation of the Analytical method AFL0977/06 for the content determination of Reg.No. 87084 in BAS 758 00 F - Results and discussion

Identity:

A solution of the reference substance and a solution of the test itemfortified with the reference substance were measured. The identity was confirmed.

Specificity:

The specificity of the method was demonstrated by analyzing typical chromatograms of the pure solvent water, the reference item, the testitem, the test item fortified and the blank formulation. No interferences with the peak of the analytical substance were observed.

Stability:

A solution of the test item fortified with the reference substance Reg.No. 87084 was measured. The sample solution was found to bestable for at least 50 hours at room temperature.

LoQ:

The lowest accuracy level (0.000641%) was used for the LoQ.

Accuracy (recovery):

The evaluation of the accuracy was done by analyzing for each concentration level five samplesolutions containing BAS 758 00 F fortified with a concentration of approximately 0.0040% or 0.0075% Reg.No. 87084 relative to the nominal weigh in concentration of the formulation. Each sample was injected twice. The accuracy was confirmed by the determination of the recovery rate by comparison of the found and the expected content.

Precision:

The precision was determined by using the 0.0040% accuracy sample and calculating the relative standard deviation of the recovery rate in %, because in the formulation BAS 758 00 F no content of Reg.No. 87084 was detected. The acceptability of the % RSD (coefficient of variation, CV) for precision was proven by Horrat value.

Table 5.2- 6 Additional Validation of the Analytical method AFL0977/06 for the determination of the relevant impurity of Reg.No.:87084 in BAS 758 00 F

Parameter	**1,2,4-(1H)-Triazole** **Reg.No.:87084** ***Max. 0.063 g/kg (63 ppm) in BAS 758 00 F***
Author(s), year	Frohn D., Nemitz A., 2021
Principle of method	HPLC-MS
Accuracy as recovery n = 5 (double injection)	The mean recovery for Reg.No. 87084 in the preparation was found to be: • 98.5% for the 0.0040% accuracy level, • 95.8% for the 0.0075% accuracy level, calculated corresponding to the nominal weigh in concentration of Mefentrifluconazole in the formulation.
Precision as repeatability	The precision was determined by using the 0.0040% accuracy sample and calculating the relative standard deviation of the recovery rate in %, because in the formulation BAS 758 00 Fno content of Reg.No. 87084 was detected. • Reg.No. 87084: Mean value: 0.004% RSD= 0.80% (limit 6.15%), Horrat value of H_r = 0.13. The RSD was acceptable since the Horrat value was ≤ 1.
LoQ	0.0040% for Reg.No. 87084 in formulation BAS 758 00 F.

Conclusion

The analytical method AFL0977 was developed and validated for the determination of Reg.No. 87084 in Formulations containing Mefentrifluconazole. With respect to the conditions described in the analytical method AFL0977/06 all validation parameters (specificity, identity, accuracy, precision, stability and LoQ) are acceptable. Therefore, the method is valid without restriction in the tested concentration range and is suitable for the determination of 1,2,4-(1H)-Triazole in BAS 758 00 F.

Study summary

A validated method for the determination of dimethyl sulfate in "BAS 758 00 F" could not be developed. Known amounts of dimethyl sulfate spiked to both the test item and the blank formulation in the range of 0.0488 mg/kg to 4.88 mg/kg could not be detected. The water content of the test item was found to be 0.4 g/100g (The water content of the blank formulation BAS 758 00 F, however, could not be determined due to insufficient amount of substance). Therefore, it can be assumed that fortifications of both dimethyl sulfate and dimethyl-D6 sulfate were hydrolysed by residual water. This, in turn, means that it is not possible to determine dimethyl sulfate concentrations as low as required (0.05 mg/kg) in this kind of sample matrix.

Comments of zRMS:	The information is acceptted as sufficient.

Reference:	CP 5.1.1/11
Report	Validation of the analytical method AFL1024/01: Determination of Dimethylsulfat in BAS 758 00 F
	Stegmaier, W., 2020
	report No 20L00161
	2020/2092912
	Authority registration No
Guideline(s):	None
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

GC-Parameters:

GC conditions A:

Method	Headspace gas chromatography-mass spectrometry (GC-MS), quantification by standard addition	
Apparatus	GC-MS system with headspace autosampler and data system	
Column:	Fused silical capillary	DB-225 (Agilent)
	Length	30 m
	Internal diameter	0.25 mm
	Film thickness	0.25 µm
Carrier gas	Helium	
	Column head pressure	0.6 bar (constant pressure)
	Septum purge	3 mL/min.

	Split ratio	8:1
Temperatures	Oven: 50°C isothermal for 4 min. 50°C → 160°C, 15 K/min. 160°C → 240°C, 35 K/min. 240°C isothermal for 22 min.	
	Injector: 210°C	
	MS transfer line: 240°C	
Detector	MSD	
	Acquisition mode	SIM
	Multiplier voltage	1905V + 500V
	Ion source temperature	230°C
	Quadrupole temperature	150°C
Registered ions (in SIM)	Dimethyl-D6 sulfate	m/z 98, m/z 100, m/z 130
	Dimethyl sulfate	m/z 95, m/z 96, m/z 125 (Target ions underlined)

GC conditions B:
Gas chromatographic separations in system B were performed on a stationary phase different to the one which was used in system A. Apart from that GC conditions of system B were the same as for system A except from a slightly (but insignificant) different final oven temperature, transfer line temperature and multiplier voltage.

Method	Headspace gas chromatography-mass spectrometry (GC-MS), quantification by standard addition	
Apparatus	GC-MS system with headspace autosampler and data system	
Column:	Fused silical capillary	ZB-1701 (Phenomenex)
	Length	30 m
	Internal diameter	0.25 mm
	Film thickness	0.25 µm
Detector	MSD	
	Multiplier voltage	1839V + 500V

Validation for content determination of DMS in BAS 758 00 F - Results and discussion

Linearity:

Analysis of calibration solutions for external calibration yielded linear calibration curves in a concentration range from 0.0488 mg/kg to 4.88 mg/kg with coefficients of determination of $R^2 = 0.9999$ for system A as well as for system B. This demonstrates that technically it is possible to determine dimethyl sulfate in the required concentration range (0.05 mg/kg - 1000 mg/kg) under the chromatographic conditions described. Although system suitability and also linearity was confirmed it was not possible to validate the analytical method as intended, e.g. the target signal m/z 98 of dimethyl-DB sulfate could not be detected neither in the chromatogram of the test item nor in the chromatograms of the fortified test items even though approx. 15 mg/kg of internal standard had been added to these samples.

However, internal standard signals could be seen clearly when blank formulation or standard solutions were analyzed.

This indicates strongly that the test item's matrix reacted with the analyte. Most probably dimethyl sulfate was hydrolyzed by water contained in the test item (0.4 g/100 g determined with Karl-Fischer

titration). Loss of internal standard in samples was observed regardless of the chromatographic system applied.

The same was true for native dimethyl sulfate. 103 mg/kg fortified to the test item could be detect- ed clearly but a fortification of approx. one order of magnitude less - 8.9 mg/kg - could not be detected with certainty.

In general system A must be preferred to system B since chromatographic separation seems to be better. In system B components of the matrix interfered nearby the retention time of the analyte, sometimes pretending the presence of the analyte. However, a closer look at retention times reveals the misapprehension. Especially the chromatogram of the test item with a fortification of 103 mg/kg illustrates that the retention time of dimethyl sulfate was slightly higher than the one of the interfering components.

Conclusion

The analytical method AFL1024/01 is technically suitable for dimethyl sulfate determination. Although system suitability and also linearity was confirmed it was not possible to validate the analytical method as intended. Most probably dimethyl sulfate was hydrolyzed by water contained in the test item and could not be detected.

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

Not required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

No CIPAC method available.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

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

Mefentrifluconazole

Table 5.2- 7: Validated methods for the generation of pre-authorization data for mefentrifluconazole in plant and animal matrices

Component of residue definition: plants/plant products: mefentrifluconazole **Animal/food of animal origin: mefentrifluconazole + M750F022 +fatty acid conjugates of M750F022**				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products				
Citrus- fruit Coffee - beans Dry beans Soya beans Tomato - fruit Wheat – grain, straw (Residues)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	HPLC-MS/MS UPLC-MS/MS *(parent only)*	Paula Jose W.F. de, 2015 BASF DocID 2015/3001681 Method L0076/09 EU agreed
Animal products, food of animal origin				
Cow – meat, kidney, liver, fat, milk, cream Hen – egg (Residues)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Devine C., 2015 BASF DocID 2015/1106707 Method L0272/01 EU agreed *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.2.3)*
Cow – muscle, kidney, liver, fat, milk Hen – egg (Residues)	Primary (VAL) Confirmatory method not necessary (three ions used for confirmation)	0.01 mg/kg	GC-MS *(M750F022 only)*	Heger N., Taraschewski I., 2016 BASF DocID 2015/1106706 Method L0309/01 EU agreed *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.2.3)*
Hen – egg, muscle, liver, fat (Residues)	Primary (VAL) Confirmatory method not necessary (three ions used for confirmation)	0.01 mg/kg	GC-MS *(fatty acid conjugates of M750F022 only)*	Guedez Orozco A.A.,Heger N., 2016 BASF DocID 2016/1001326 Method L0309/02 EU agreed
Animal food				
Bee feeding solution	Primary (VAL) for quantification (one mass transition used for quantification)	0.1 mg/kg	HPLC-MS/MS *(parent only)*	**New study** KCP 5.1/4 not peer-reviewed Dressler, A., 2021 BASF DocID 2021/2008152 Method L0452/02

Table 5.2- 8: Validated methods for the generation of pre-authorization data for mefentrifluconazole in plant and animal matrices – Triazole derivative metabolites

Components on interests:	**1,2,4 triazole (T), triazole alanine (TA), triazole acetic acid (TAA) and triazole lactic acid (TLA)**			
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products				
Tomato - fruit Cucumber - fruit Lettuce - leaves Cereal – grain, straw, green plant Orange - fruit Melon – peel, fruit, pulp Sweet pepper - fruit Carrot – leaf, root Dry bean Oilseed rape Sunflower (Residues)	Primary (VAL)	0.01 mg/kg	LC-DMS/MS/MS	Class T., 2011 BASF DocID 2012/1294644 Method L0170/02 (01062) EU agreed
	Confirmatory	Different confirmatory method(s) are available. Monitoring a confirmatory mass transition (157→88 m/z, positive mode for triazolylalanine), a second LC (Hypercarb) column or monitoring in the negative ion modus (triazole acetic acid, triazole lactic acid), an additional stationary phase (e.g. Phenomenex Luna Synergi Polar-RP for 1,2,4-triazole) or multiple derivatisation with subsequent SPE clean-up were performed in former versions of the method (M001/M002 and M003). These versions were also submitted by the Triazole Derivative Metabolite Group to CRD. Therefore enough confirmatory methods are available.		
Animal products, food of animal origin				
Cow – whole milk, skimmed milk, cream, meat, liver, fat, kidney, Hen - whole egg, egg yolk, egg white (Residues)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Billian P.,Druskus M., 2009 BASF DocID 2010/1230632 Method L0293/01 (01132) EU agreed

Table 5.2- 9: Validated methods for the generation of pre-authorization data for mefentrifluconazole in soil matrices

Component of residue definition:	**mefentrifluconazole and M750F001 (1,2,4-triazole)**			
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Soil (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.002 mg/kg	LC-MS/MS *(also Reg.No 5924326)*	Studenroth S.,Lueer D, 2015 BASF DocID 2015/1039006 Report Amendment 1: 2016/1030227 Report Amendment 2: 2016/1215646 Method L0214/01 EU agreed

Table 5.2- 10: Validated methods for the generation of pre-authorization data for mefentrifluconazole and metabolites in surface water and sediment matrices

Component of residue definition: mefentrifluconazole + M750F001 (1,2,4-triazole) + M750F003 + M750F005 + M750F006 + M750F007 + M750F008				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Surface, drinking water (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	30 ng/L	LC-(ESI)-MS/MS *(except 1,2,4-triazole)*	Malinsky D.S., 2016 BASF DocID 2015/7001125 Report Amendment DocID: 2016/7010048 Method D1506/01 EU agreed
Surface, drinking water (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two columns used for confirmation)	0.05 ng/L	HPLC-MS/MS *(1,2,4-triazole only)*	Penning H. et al., 2013 BASF DocID 2012/1297158 Method L0199/01 EU agreed
M4-Medium, OECD-water and mixing water (Ecotoxicology)	Primary (VAL) Confirmatory method not necessary	0.001 mg/L	LC/MS (*BAS 750 F, M750F007)*	**New study** KCP 5.1/1, not peer-reviewed Ziegler G., 2017 BASF DocID 2017/1064882 Method APL0500/03
Test water (mixing water)	Primary (VAL) Confirmatory method not necessary	0.001 mg/L	HPLC-MS (BAS 750 F)	**New study** KCP 5.1/2, not peer-reviewed xxxxxxxxx., 2016 BASF DocID 2016/1155889 Method APL0500/03
Tap water or M4-medium	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.1 µg/L	LC-MS/MS	**New study** KCP 5.1/3 not peer-reviewed Andre, M., 2017 BASF DocID 2017/1065621 Method L0361/01

Table 5.2- 11: Validated methods for the generation of pre-authorization data for mefentrifluconazole in air

Component of residue definition: mefentrifluconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Air (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 ng/L	LC-MS/MS	Obermann M.,Studenroth S., 2015 BASF DocID 2015/1111330 Method L0327/01 EU agreed *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.2.6)*

Metrafenone

Table 5.2- 12: Validated methods for the generation of pre-authorization data for metrafenone in plants

Component of residue definition: Metrafenone				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Wheat whole plant, wheat grain, wheat straw, grape, tomato, dry pea, soybean (Residues)	Primary	0.1 mg/kg	LC-MS/MS	Lehmann A., Mackenroth C., 2012 Not peer reviewed BASF DocID: 2012/1166088 Appendix 2
Wheat whole plant, wheat grain, wheat straw, grape, lemon, oilseed rape seed (Residues)	Primary	0.01 mg/kg	LC-MS/MS	Benz A., Mackenroth C., 2005 Not peer reviewed BASF DocID: 2004/1010553 Appendix 2

Table 5.2- 13: Validated methods for the generation of pre-authorization data for metrafenone in ecotoxicology media and matrices

Component of residue definition: Metrafenone				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
ISO medium (Ecotox)	Primary	0.384 µg/L	LC-MS/MS	Eckenstein H., 2021 Not peer reviewed BASF DocID: 2020/2033902 Appendix 2
Reconstituted water (Ecotox)	Primary	0.136 µg/L	LC-MS/MS	Eckenstein H., 2021 Not peer reviewed BASF DocID: 2020/2033900 Appendix 2
AAP medium (Ecotox)	Primary	0.433 µg/L	LC-MS/MS	Eckenstein H., 2021 Not peer reviewed BASF DocID: 2020/2033904 Appendix 2
50% sucrose solution (Ecotox)	Primary	0.1 mg/kg	LC-MS/MS	Dreßler K., 2021 Not peer reviewed BASF DocID: 2021/2008152 Appendix 2
Flowerrs, nectar, pollen (Ecotox)	Primary and confirmatory	0.01 mg/kg	LC-MS/MS	Schneider J.Q. & Link, T., 2022 Not peer reviewed BASF DocID: 2021/2052011 Appendix 2

Pyraclostrobin

Table 5.2- 14: Validated methods for the generation of pre-authorization data (pyraclostrobin)

Component of residue definition: pyraclostrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Plants, plant products (Residues)				
wheat - forage, straw, grain	Primary; Confirmatory method not necessary (two mass transitions)	0.02 mg/kg	LC-MS/MS	Reinhard K., Mackenroth C. 1999a BASF DocID 1999/11134 Method no. 421/0 (Germany) / D9808 (USA) EU agreed *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.4.2 and A 2.3.2.1)*
grapes				
peanut - nutmeat				
orange				
beer	Primary	0.02 mg/kg	LC-MS/MS	Reinhard K., Mackenroth C. 1999b BASF DocID 1999/11135 Method no. 453/0 EU agreed
brewer's yeast				
brewing malt				
spent grains and flocs				
pod barley				
malt sprouts				
wheat - forage, grain, straw	Primary	0.02 mg/kg	HPLC-UV	Abdel-Baky S., Riley M.E., 2000 BASF DocID 1999/5179 Method no. D9904 EU agreed *The method is also used for monitoring purposes (see KCP 5.2 and chapter5.3.4.2 and A 2.3.2.1.2)*
grape				
orange				
peanut - nutmeat				
apple	Primary	0.02 mg/kg	LC-MS/MS	Benz A., Mackenroth C., 2001 BASF DocID 2000/1012405 Method no. 445/0 **New study**, not peer-reviewed see A 2.3.1.1.1.1
sour cherry				
grapes				
strawberry				
carrot				
onion				
tomato				
broccoli				
white cabbage				
leek				
dwarf bean				

Component of residue definition: pyraclostrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
oilseed rape wheat - plant, grain, straw				
coffee - grain soya bean - grain wheat - grain	Primary; Confirmatory method not necessary (two mass transitions)	0.02 mg/kg	LC-MS/MS	Leite R., 2005 BASF Doc ID 2005/1037978 Method no. 445/0 **New study**, not peer-reviewed see A 2.3.1.1.1.4
wheat - plant, grain, straw lemon lettuce oilseed rape tomato onion	Primary; Confirmatory method not necessary (two mass transitions)	0.01 mg/kg	LC-MS/MS	Lehmann A., Mackenroth C., 2007 BASF Doc ID 2006/1039427 Method no. 535/1 **New study**, not peer-reviewed see A 2.3.1.1.4
citrus - whole fruit dry beans - seed tomato - whole fruit soybeans - grain wheat -grain	Primary; Confirmatory method not necessary (two mass transitions)	0.01 mg/kg	LC-MS/MS	Jose W.F.P. de, 2015 BASF Doc ID 2015/3004795 Method no. L0076/09 **New study**, not peer-reviewed see A 2.3.1.1.3
Animal products, food of animal origin (Residues)				
milk - bovine muscle - bovine liver - bovine kidney - bovine fat - bovine egg - hen	Primary & Confirmatory	0.01 mg/kg 0.05 mg/kg	HPLC-UV primary: normal-phase HPLC-UV confirmatory: reversed-phase HPLC-UV	Kampke-Thiel K., 1999 BASF DocID 1999/11079 Method no. 439/0 EU agreed *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.4.3 and A 2.3.2.7.2)*
milk - bovine muscle - bovine liver - bovine kidney - bovine fat - bovine	Primary & Confirmatory	0.01 mg/kg 0.05 mg/kg	LC-MS/MS confirmatory for matrix milk: GC-MS	Tilting N., Lehmann W., 2000 BASF DocID 1999/11075 Method no. 446 EU agreed
muscle - bovine liver - bovine kidney - bovine fat - bovine	Primary; Confirmatory method not necessary (two mass transitions)	0.01 mg/kg	LC-MS/MS	Eilers B., Taraschewski I., 2014 BASF DocID 2013/1400972 Method no. 446/2 **New study**, not peer-reviewed see A 2.3.1.2.1

Component of residue definition: pyraclostrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
milk - bovine				
egg - hen				
muscle - bovine	Primary; Confirmatory method not necessary (two mass transitions)	0.01 mg/kg	LC-MS/MS	Hopf B., 2010 BASF DocID 2010/1018944 Method no. L0151/01 **New study**, not peer-reviewed see A 2.3.2.2.1.1 *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.4.2.3 and A 2.3.2.2.1.2)*
liver - bovine				
kidney - bovine				
fat - bovine				
milk				
skimmed milk				
cream				
blood - pig				
egg - hen				
egg - hen	Primary	0.05 mg/kg	LC-MS/MS	Malinsky D.S., Riley M.E., 2000 BASF DocID 2000/5004 Method no. D9902 **New study**, not peer-reviewed see A 2.3.1.2.2
liver - hen				
muscle - hen				
fat - hen				
Animal food				
Bee feeding solution	Primary (VAL) for quantification (one mass transitions used for quantification)	0.1 mg/kg	HPLC-MS/MS *(parent only)*	Dressler, A., 2021 BASF DocID 2021/2008152 Method no. L0452/02 **New study**, not peer-reviewed see A 2.3.1.2.3
Soil, water, air (Environmental fate)				
Soil	Primary; Confirmatory method not necessary (two mass transitions)	0.001 mg/kg	LC-MS/MS	Tilting N., Sopena-Vazquez F., 2014 BASF DocID 2013/1184817 Method no. L0166/01 **New study**, not peer-reviewed see A 2.3.1.3.1 *The method can be used for monitoring purposes if required (see KCP 5.2 and chapter 5.3.4.4 and A 2.3.2.3)*
Soil	Primary; Confirmatory method not necessary (two mass transitions)	0.001 mg/kg	LC-MS/MS	Zangmeister W., 2010 BASF DocID 2010/1075848 Method no. L0161/01 **New study**, not peer-reviewed see A 2.3.1.3.2
Water	Primary & Confirmatory	0.05 µg/L	LC-MS LC-MS/MS (confirmatory method)	Staab G., 1998 BASF DocID 1998/11182 Method no. 415 EU agreed

Component of residue definition: pyraclostrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Water	Primary; Confirmatory method not necessary (two mass transitions)	0.05 µg/L	LC-MS/MS	Zangmeister W., 1999 BASF DocID 1999/10701 Method no. 455 EU agreed
Water	Primary; Confirmatory method not necessary (two mass transitions)	0.003 µg/L	LC-MS/MS	Tilting N., 2012 BASF DocID 2012/1009641 Method no. L0182/01 **New study**, not peer-reviewed see A 2.3.2.4.1.1 *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.4.5 and A 2.3.2.4.1)*
Air	Primary & Confirmatory	0.0003 µg/L air	HPLC-UV LC-MS/MS (confirmation method)	Zangmeister W., 1999 BASF DocID 1999/10694 Method no. 447 EU agreed *The method is also used for monitoring purposes (see KCP 5.2 and chapter5.3.4.6)*
Air	Primary; Confirmatory method not necessary (two mass transitions)	4.44 ng/L air	LC-MS/MS	Penning H., 2012 BASF DocID 2012/1220256 Method no. L0197/01 **New study**, not peer-reviewed see A 2.3.2.5.1 *The method is also used for monitoring purposes (see KCP 5.2 and chapter A 2.3.2.5)*
Water (Ecotoxicology)				
Water	Primary (Identity was proved by coincidence of retention times and MS-detection)	0.001 mg/L	LC-MS	Obermann M., 2005 BASF DocID 2005/1026675 Method no. APL0500/01 **New study**, not peer-reviewed see A 2.3.1.4.1
Tap water or M4-medium	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.1 µg/L	LC-MS/MS	Andre, M., 2017 BASF DocID 2017/1065621 Method no. L0361/01 **New study**, not peer-reviewed see A 2.3.1.4.2

Table 5.2- 15: Validated methods for the generation of pre-authorization data (pyraclostrobin metabolite 500M07)

Additional component: 500M07				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Plants, plant products (Residues)				
wheat - forage, straw, grain grapes peanut - nutmeat orange	Primary; Confirmatory method not necessary (two mass transitions)	0.02 mg/kg	LC-MS/MS	Reinhard K., Mackenroth C. 1999a BASF DocID 1999/11134 Method no. 421/0 (Germany) / D9808 (USA) EU agreed *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.4.2 and A 2.3.2.1.1)*
beer brewer's yeast brewing malt spent grains and flocs pod barley malt sprouts	Primary	0.02 mg/kg	LC-MS/MS	Reinhard K., Mackenroth C. 1999b BASF DocID 1999/11135 Method no. 453/0 EU agreed
wheat - forage, grain, straw grape orange peanut - nutmeat	Primary	0.02 mg/kg	HPLC-UV	Abdel-Baky S., Riley M.E., 2000 BASF DocID 1999/5179 Method no. D9904 EU agreed *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.4.2 and A 2.3.2.1.2)*
apple sour cherry grapes strawberry carrot onion tomato broccoli white cabbage leek dwarf bean oilseed rape	Primary	0.02 mg/kg	LC-MS/MS	Benz A., Mackenroth Ch., 2001 BASF DocID 2000/1012405 Method no. 445/0 **New study**, not peer-reviewed see A 2.3.1.1.1.1

Additional component: 500M07				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Plants, plant products (Residues)				
wheat - plant, grain, straw				
coffee - grain	Primary; Confirmatory method not necessary (two mass transitions)	0.02 mg/kg	LC-MS/MS	Leite R., 2005 BASF Doc ID 2005/1037978 Method no. 445/0 **New study**, not peer-reviewed see A 2.3.1.1.1.4
soya bean - grain				
wheat - grain				
wheat - plant, grain, straw	Primary; Confirmatory method not necessary (two mass transitions)	0.01 mg/kg	LC-MS/MS	Lehmann A., Mackenroth C., 2007 BASF Doc ID 2006/1039427 Method no. 535/1 **New method**, not peer-reviewed see A 2.3.1.1.4
lemon				
lettuce				
oilseed rape				
tomato				
onion				
citrus - whole fruit	Primary; Confirmatory method not necessary (two mass transitions)	0.01 mg/kg	LC-MS/MS	Jose W.F.P. de, 2015 BASF Doc ID 2015/3004795 Method no. L0076/09 **New method**, not peer-reviewed see A 2.3.1.1.3
dry beans - seed				
tomato - whole fruit				
soybeans - grain				
wheat -grain				

Table 5.2- 16: Validated methods for the generation of pre-authorization data (pyraclostrobin me tabolite 500M04)

Component of residue definition: 500M04 *				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Animal products, food of animal origin				
muscle - bovine	Primary; Confirmatory method not necessary (two mass transitions)	0.01 mg/kg	HPLC-MS/MS	Eilers B., Taraschewski I., 2014 BASF DocID 2013/1400972 Method no. 446/2 **New study**, not peer-reviewed see A 2.3.1.2.1
liver - bovine				
kidney - bovine				
fat - bovine				
milk - bovine				
egg - hen				
Water (Environmental fate)				
Water	Primary; Confirmatory method not necessary (two mass transitions)	0.03 µg/L	LC-MS/MS	Obermann M., 2014 BASF DocID 2014/1004891 Method no. L0182/02 **New study**, not peer-reviewed see A 2.3.2.4.1.2 *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.4.5 and A 2.3.2.4.1.3)*

* Animal residue definition for risk assessment in liver (except poultry liver) and milk fat only: pyraclostrobin and its metabolites analysed as the hydroxy pyrazoles 500M04 and 500M85.

Table 5.2- 17: Validated methods for the generation of pre-authorization data (pyraclostrobin me tabolite 500M35)

Additional component: 500M35				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Animal products, food of animal origin (Residues)				
milk - bovine	Primary & Confirmatory	0.01 mg/kg	LC-MS/MS confirmatory for matrix milk: GC-MS	Tilting N., Lehmann W., 1999 BASF DocID 1999/11075 Method no. 446 EU agreed
muscle - bovine		0.05 mg/kg		
liver - bovine		0.05 mg/kg		
kidney - bovine		0.05 mg/kg		
fat - bovine		0.05 mg/kg		

Table 5.2- 18: Validated methods for the generation of pre-authorization data (pyraclostrobin me tabolite 500M77)

Additional component: 500M77				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Animal products, food of animal origin				
egg - hen	Primary	0.05 mg/kg	LC-MS/MS	Malinsky D.S., Riley M.E., 2000 BASF DocID 2000/5004 Method no. D9902 **New study**, not peer-reviewed see A 2.3.1.2.2
liver - hen				
muscle - hen				
fat - hen				

Table 5.2- 19: Validated methods for the generation of pre-authorization data (pyraclostrobin me tabolite 500M79)

Additional component: 500M79				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Plants (Residues)				
lettuce - head	Primary; Confirmatory method not necessary (two mass transitions)	0.01 mg/kg	LC-MS/MS	Courtois J., 2014 BASF Doc ID 2014/1001721 Method no. L0220/01 **New method**, not peer-reviewed see A 2.3.1.1.2
white cabbage				
leek				

Table 5.2- 20: Validated methods for the generation of pre-authorization data (pyraclostrobin me tabolites 500M85)

Component of residue definition: 500M85 *				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Animal products, food of animal origin				
muscle - bovine	Primary; Confirmatory method not necessary (two mass transitions)	0.01 mg/kg	LC-MS/MS	Eilers B., Taraschewski I., 2014 BASF DocID 2013/1400972 Method no. 446/2 **New study**, not peer-reviewed see A 2.3.1.2.1
liver - bovine				
kidney - bovine				
fat - bovine				
milk - bovine				
egg - hen				

* Animal residue definition for risk assessment in liver (except poultry liver) and milk fat only: pyraclostrobin and its metabolites analysed as the hydroxy pyrazoles 500M04 and 500M85.

Table 5.2- 21: Validated methods for the generation of pre-authorization data (pyraclostrobin me tabolites 500M01, 500M02 and 500M07)

Additional component: 500M01, 500M02, 500M07				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Soil (Environmental fate)				
Soil	Primary; Confirmatory method not necessary (two mass transitions)	0.001 mg/kg	LC-MS/MS	Tilting N., Sopena-Vazquez F., 2014 BASF DocID 2013/1184817 Method no. L0166/01 **New study**, not peer-reviewed see A 2.3.1.3.1 *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.4.4 and A 2.3.2.3)*

Table 5.2- 22: Validated methods for the generation of pre-authorization data (pyraclostrobin me tabolites 500M59, 500M60, 500M62, 500M76 and 500M78)

Additional components: 500M59, 500M60, 500M62, 500M76 and 500M78				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Water (Environmental fate)				
Water	Primary; Confirmatory method not necessary (two mass transitions)	0.05 µg/L	LC-MS/MS	Zangmeister W., 1999 BASF DocID 1999/10701 Method no. 455 EU agreed
Water	Primary; Confirmatory method not necessary (two mass transitions)	0.03 µg/L	LC-MS/MS	Tilting N., 2012 BASF DocID 2012/1009641 Method no. L0182/01 **New study**, not peer-reviewed see A 2.3.2.4.1.1 *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.4.5 and A 2.3.2.4.1)*

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.

5.3.2 Description of analytical methods for the determination of residues of

Mefentrifluconazole (KCP 5.2)

5.3.2.1 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	Mefentrifluconazole	0.01 mg/kg	Reg. (EU) No 977/2019
Plant, high acid content		0.01 mg/kg	Reg. (EU) No 977/2019
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Reg. (EU) No 977/2019
Plant, high oil content		0.01 mg/kg	Reg. (EU) No 977/2019
Plant, difficult matrices (hops, spices, tea)		0.01 mg/kg	Reg. (EU) No 977/2019
Muscle	Mefentrifluconazole	0.01 mg/kg	Reg. (EU) No 977/2019
Milk		0.01 mg/kg	Reg. (EU) No 977/2019
Eggs		0.01 mg/kg	Reg. (EU) No 977/2019
Fat		0.01 mg/kg	Reg. (EU) No 977/2019
Liver, kidney		0.01 mg/kg	Reg. (EU) No 977/2019
Soil (Ecotoxicology)	Mefentrifluconazole	0.05 mg/kg	Common limit
Drinking water (Human toxicology)	Mefentrifluconazole	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Mefentrifluconazole	10 µg/L	21 d NOEC *Daphnia magna*
Air	Mefentrifluconazole	5.314 mg/L	LC_{50} inhal (NOAEL sys: 25 mg/kg bw/d)
Tissue (meat or liver)	Mefentrifluconazole	0.01 mg/L	Not classified as T / T+
Body fluids	Mefentrifluconazole + M750F015, M750F016, M750F017	0.01 mg/L	Not classified as T / T+

5.3.2.2 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 mefentrifluconazole in plant matrices is given in the following tables.

Table 5.3-2: Validated methods for food and feed of plant origin

Component of residue definition: mefentrifluconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Primary (VAL)	0.01 mg/kg	LC-MS/MS	Klimmek S. et al., 2015

Table 5.3-2: Validated methods for food and feed of plant origin

Component of residue definition: mefentrifluconazole				
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 (tomato, whole fruit)	Confirmatory method not necessary (two mass transitions used for confirmation)			BASF DocID 2015/1106708 Method L0295/01 (QuEChERS) EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Richter S.,Schmiedt S., 2015 BASF DocID 2015/1240004 Method L0295/01 (QuEChERS) EU agreed
High acid content (orange, whole fruit)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Klimmek S. et al., 2015 BASF DocID 2015/1106708 Method L0295/01 (QuEChERS) EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Richter S.,Schmiedt S., 2015 BASF DocID 2015/1240004 Method L0295/01 (QuEChERS) EU agreed

Table 5.3-2: Validated methods for food and feed of plant origin

Component of residue definition: mefentrifluconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High oil content (soybeans, seeds)	Primary (VAL)Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Klimmek S. et al., 2015 BASF DocID 2015/1106708 Method L0295/01 (QuEChERS) EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Richter S.,Schmiedt S., 2015 BASF DocID 2015/1240004 Method L0295/01 (QuEChERS) EU agreed
High protein/high starch content (dry) (dry beans (seeds) / wheat (grain))	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Klimmek S. et al., 2015 BASF DocID 2015/1106708 Method L0295/01 (QuEChERS) EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Richter S.,Schmiedt S., 2015 BASF DocID 2015/1240004 Method L0295/01 (QuEChERS) EU agreed

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:	Extraction efficiency of data generation method (L0076/01 equivalent to L0076/09) and multi methods (QuEChERS, DFG S 19, and SweEt) in commodities of plant origin were tested with radio-labeled residues and compared to the metabolism studies. The results were submitted in the context of annex I inclusion (BASF DocID 2014/1261057).
Not required, because:	-

Conclusion on extraction efficiency of plant matrices

Efficient extraction for the analytical method, BASF data generation method L0076/01 was confirmed by comparison of residue amounts extracted in the metabolism study with the amounts extracted according to extraction procedures of a residue analytical method.
Extraction efficiencies generally were 90% or higher for all matrices investigated, namely wheat forage (98%), wheat straw (111%), soybean green pod (102%) and grapevine grape (93%). In contrast, with the multi-methods, extraction efficiency was lower for forage (QuEChERS 80%, DFG S 19 63%, SweEt 56%), and for straw (QuEChERS 59%, DFG S 19 52%, SweEt 65%) while similar high extraction efficiency was observed for soybean green pod and grapevine grape (88% or higher).

5.3.2.3 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 mefentrifluconazole 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: mefentrifluconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Devine C., 2015 BASF DocID 2015/1106707 Method L0272/01 EU agreed
		0.01 mg/kg	LC-MS/MS *(M750F022 only)*	Heger N.,Taraschewski I., 2016 BASF DocID 2015/1106706 Method L0309/01 EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Richter S.,Djedovic S., 2015 BASF DocID 2015/1240005 Method L0272/01 EU agreed
		0.01 mg/kg	LC-MS/MS *(M750F022 only)*	Bendig P.,Wabbel C., 2015 BASF DocID 2015/1240006 Method L0309/01 EU agreed
Eggs	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Devine C., 2015 BASF DocID 2015/1106707 Method L0272/01 EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Richter S.,Djedovic S., 2015 BASF DocID 2015/1240005 Method L0272/01 EU agreed

Component of residue definition: mefentrifluconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Muscle	Primary (VAL)Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Devine C., 2015 BASF DocID 2015/1106707 Method L0272/01 EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Richter S.,Djedovic S., 2015 BASF DocID 2015/1240005 Method L0272/01 EU agreed
Fat	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Devine C., 2015 BASF DocID 2015/1106707 Method L0272/01 EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Richter S.,Djedovic S., 2015 BASF DocID 2015/1240005 Method L0272/01 EU agreed
Kidney, liver	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Devine C., 2015 BASF DocID 2015/1106707 Method L0272/01 EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Richter S.,Djedovic S., 2015 BASF DocID 2015/1240005 Method L0272/01 EU agreed

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

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Extraction efficiency of data generation and post-authorization methods (L0272/01 for BAS 750 F, L0309/01 for M750F022) in commodities of animal origin were tested with radio-labeled residues and compared to the metabolism studies. The results were submitted in the context of annex I inclusion (BASF DocID 2015/1161960).
Not required, because:	-

Conclusion on extraction efficiency of animal matrices

Comparison of residue amounts extracted in the metabolism study with the amounts extracted by the extraction procedures of a residue analytical method confirms efficient extraction for the analytical methods, method L0272/01 for BAS 750 F and L0309/01 for metabolite M750F022.
For BAS 750 F, extraction efficiencies generally were 80% or higher for most matrices (milk, cream, muscle, kidney, fat, egg yolk), and lower for liver (46%). For M750F022, extraction efficiencies generally were 90% or higher for most matrices (milk, cream, kidney, fat) and lower for egg yolk (66%), for muscle (61%) and for liver (46-50%).

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

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

Table 5.3-6: Validated methods for soil (if appropriate)

Component of residue definition: mefentrifluconazole			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.002 mg/kg	LC-MS/MS *(also Reg.No 5924326, 1,2,4-triazole and M750F003)*	Studenroth S.,Lueer D, 2015 BASF DocID 2015/1039006 Report Amendment 1: 2016/1030227 Report Amendment 2: 2016/1215646 Method L0214/01 EU agreed

Soil types used: Field soil LUFA 2.2 (USDA: loamy fine sand / ISO 11277: loamy sand (Ss)) and Field soil LUFA 2.3 (USDA: sandy loam, ISO 11277: silty sand (Su3))

For any special comments or remarkable points concerning the analytical methods for soil please refer to Appendix 2.

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

An overview on the acceptable methods and possible data gaps for analysis of mefentrifluconazole in surface and drinking water is given in the following table. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

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

Component of residue definition: mefentrifluconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	30 ng/L	LC-MS/MS	**New study** KCP 5.2/1, not peer-reviewed Obermann M., 2017 BASF DocID 2017/1066523 Method L0359/01
	ILV Confirmatory method not necessary (two mass transitions used for confirmation	30 ng/L	LC-MS/MS	**New study** KCP 5.2/2, not peer-reviewed Stanislowski T., 2017, BASF DocID 2017/1066522 Method L0359/01
Surface water	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	30 ng/L	LC-MS/MS	**New study** KCP 5.2/1, not peer-reviewed Obermann M., 2017 BASF DocID 2017/1066523 Method L0359/01
	ILV Confirmatory method not necessary (two mass transitions used for confirmation	30 ng/L	LC-MS/MS	**New study** KCP 5.2/2, not peer-reviewed Stanislowski T., 2017, BASF DocID 2017/1066522 Method L0359/01
Surface, drinking water (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	30 ng/L	LC-(ESI)-MS/MS	Malinsky D.S., 2016 BASF DocID 2015/7001125 Report Amendment DocID: 2016/7010048 Method D1506/01 EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation	30 ng/L	LC-(ESI)-MS/MS	Guodong G., et al., 2016 BASF DocID 2015/7006199 Method D1506/01 EU agreed

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

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

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

Component of residue definition: mefentrifluconazole			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 ng/L	LC-MS/MS	Obermann M.,Studenroth S., 2015 BASF DocID 2015/1111330 Method L0327/01

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

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

An overview on the acceptable methods and possible data gaps for analysis of mefentrifluconazole in body fluids and tissues is given in the following table. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-9: Methods for body fluids and tissues (if appropriate)

Component of residue definition: mefentrifluconazole + M750F015 + M750F016 + M750F017 (body fluids), Mefentrifluconazole (body tissues)			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary (VAL) Confirmatory method not necessary (two mass transitions)	0.01 mg/L	LC-MS/MS	Wiesner F., Breyer N., 2016, BASF DocID 2016/1148911 Method L0339/01 EU agreed
Primary (VAL) Confirmatory method not necessary (two mass transitions)	0.01 mg/L	LC-MS/MS	**New study** KCP 5.2/3, not peer-reviewed Homazava N. 2019 BASF DocID 2019/1046404 Method L0339/02

Note: plasma and urine were the matrices used. In the case of the tissues see Table 5.3-4.

5.3.2.8 Other studies/ information

No further studies submitted.

5.3.3 Description of analytical methods for the determination of residues of metrafenone (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. All references are taken from the metrafenone RAR (October, 2019). No changes in residue definitions are proposed.

Table 5.3-10: 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	Metrafenone	LOQ: 0.01 mg/kg	Meyer, M., 2011 [2011/7007816]
Plant, high acid content		LOQ: 0.01 mg/kg	Meyer, M., 2011 [2011/7007816]
Plant, high protein/high starch content (dry commodities)		LOQ: 0.01 mg/kg	Meyer, M., 2011 [2011/7007816]

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high oil content		LOQ: 0.01 mg/kg	Meyer, M., 2011 [2011/7007816]
Plant, difficult matrices (hops, spices, tea)		LOQ: 0.01 mg/kg	Meyer, M., 2011 [2011/7007816]
Muscle	Metrafenone	LOQ: 0.01 mg/kg	Kuhn, T., 2014 [2014/1181105]
Milk		LOQ: 0.01 mg/kg	Kuhn, T., 2014 [2014/1181105]
Eggs		LOQ: 0.01 mg/kg	Kuhn, T., 2014 [2014/1181105]
Fat		LOQ: 0.01 mg/kg	Kuhn, T., 2014 [2014/1181105]
Liver, kidney		LOQ: 0.01 mg/kg	Kuhn, T., 2014 [2014/1181105]
Soil (Ecotoxicology)	Metrafenone	LOQ: 0.005 mg/kg	Austin, R., 2015b [2014/1181107]
Drinking water (Human toxicology)	Metrafenone	LOQ: 0.05 µg/L	Austin, R., 2014 [2014/1181109]
Surface water (Ecotoxicology)	Metrafenone	LOQ: 0.05 µg/L	Austin, R., 2014 [2014/1181109]
Air	Metrafenone	LOQ: 0.03 mg/m^3	Austin, R., 2015c [2014/1181110]
Tissue (meat or liver)	Metrafenone	LOQ: 0.01 mg/kg	Kuhn, T., 2014 [2014/1181105]

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Body fluids (blood)		LOQ: 0.05 mg/L	Turner, R., 2018 [2018/1029049]

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 metrafenone in plant matrices is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-11: 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: Metrafenone				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
High water content	Primary + confirmatory	LOQ: 0.01 mg/kg	HPLC-MS/MS	Meyer, M., 2011 [2011/7007816]
	ILV	LOQ: 0.01 mg/kg	HPLC-MS/MS	Weber, H., 2011 [2011/1124162]
High acid content	Primary + confirmatory	LOQ: 0.01 mg/kg	HPLC-MS/MS	Meyer, M., 2011 [2011/7007816]
	ILV	LOQ: 0.01 mg/kg	HPLC-MS/MS	Weber, H., 2011 [2011/1124162]
High oil content	Primary + confirmatory	LOQ: 0.01 mg/kg	HPLC-MS/MS	Meyer, M., 2011 [2011/7007816]
	ILV	LOQ: 0.01 mg/kg	HPLC-MS/MS	Weber, H., 2011 [2011/1124162]
High protein/high starch content (dry)	Primary + confirmatory	LOQ: 0.01 mg/kg	HPLC-MS/MS	Meyer, M., 2011 [2011/7007816]
	ILV	LOQ: 0.01 mg/kg	HPLC-MS/MS	Weber, H., 2011 [2011/1124162]
Difficult (if required, depends on intended use)	Primary + confirmatory	LOQ: 0.01 mg/kg	HPLC-MS/MS	Meyer, M., 2011 [2011/7007816]
	ILV	LOQ: 0.01 mg/kg	HPLC-MS/MS	Weber, H., 2011 [2011/1124162]

There are no special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices.

Table 5.3-12: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Extraction efficiency has been demonstrated to be acceptable. For details, refer to the metrafenone RAR, Volume B.7 (October, 2019).

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 metrafenone in animal matrices is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-13: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Metrafenone				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Milk	Primary + confirmatory	LOQ: 0.01 mg/kg	HPLC-MS/MS	Kuhn, T., 2014 [2014/1181105]
	ILV	LOQ: 0.01 mg/kg	HPLC-MS/MS	Austin, R., 2015a [2014/1181106]
Eggs	Primary + confirmatory	LOQ: 0.01 mg/kg	HPLC-MS/MS	Kuhn, T., 2014 [2014/1181105]
	ILV	LOQ: 0.01 mg/kg	HPLC-MS/MS	Austin, R., 2015a [2014/1181106]
Muscle	Primary + confirmatory	LOQ: 0.01 mg/kg	HPLC-MS/MS	Kuhn, T., 2014 [2014/1181105]
	ILV	LOQ: 0.01 mg/kg	HPLC-MS/MS	Austin, R., 2015a [2014/1181106]
Fat	Primary + confirmatory	LOQ: 0.01 mg/kg	HPLC-MS/MS	Kuhn, T., 2014 [2014/1181105]
	ILV	LOQ: 0.01 mg/kg	HPLC-MS/MS	Austin, R., 2015a [2014/1181106]
Kidney, liver	Primary + confirmatory	LOQ: 0.01 mg/kg	HPLC-MS/MS	Kuhn, T., 2014 [2014/1181105]
	ILV	LOQ: 0.01 mg/kg	HPLC-MS/MS	Austin, R., 2015a [2014/1181106]

Table 5.3-14: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Extraction efficiency has been demonstrated to be acceptable. For details, refer to the metrafenone RAR, Volume B.7 (October, 2019).

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

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

Table 5.3-15: Validated methods for soil (if appropriate)

Component of residue definition: Metrafenone			
Method type	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Primary + confirmatory	LOQ: 0.005 mg/kg	HPLC-MS/MS	Austin, R., 2015b

Component of residue definition: Metrafenone			
Method type	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
			[2014/1181107]

For any special comments or remarkable points concerning the analytical methods for soil please refer to Appendix 2.

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

An overview on the acceptable methods and possible data gaps for analysis of metrafenone in surface and drinking water is given in the following tables. For the detailed valuation of new/ additional studies it is referred to Appendix 2.

Table 5.3-16: Validated methods for water (if appropriate)

Component of residue definition: Metrafenone				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Drinking water	Primary + confirmatory	LOQ: 0.05 µg/L	HPLC-MS/MS	Austin, R., 2014 [2014/1181109]
	ILV	LOQ: 0.05 µg/L	HPLC-MS/MS	Richter, S., 2015 [20141181108]
Surface water	Primary + confirmatory	LOQ: 0.05 µg/L	HPLC-MS/MS	Austin, R., 2014 [2014/1181109]

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

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

An overview on the acceptable methods and possible data gaps for analysis of metrafenone in air is given in the following tables. For the detailed evaluation of new/ additional studies please refer to Appendix 2.

Table 5.3-17: Validated methods for air (if appropriate)

Component of residue definition: Metrafenone			
Method type	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Primary + confirmatory	LOQ: 0.03 mg/m^3	HPLC-MS/MS	Austin, R., 2015c [2014/1181110]

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

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

An overview on the acceptable methods and possible data gaps for analysis of metrafenone in body fluids and tissues is given in the following table. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-18: Methods for body fluids and tissues (if appropriate)

Component of residue definition: Metrafenone				
Matrix	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Blood	Primary + confirmatory	0.05 mg/L	HPLC-MS/MS	Turner, R., 2018 [2018/1029049]

Body tissues are covered by the methods for animal products detailed in Section 5.3.3.3 above.

For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 2.

5.3.3.8 Other studies/ information

None.

5.3.4 Description of analytical methods for the determination of residues of pyraclostrobin (KCP 5.2)

5.3.4.1 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 5.3-19: 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	Pyraclostrobin	0.02 mg/kg	Reg. (EU) 2017/1016
Plant, high acid content		0.02 mg/kg	Reg. (EU) 2017/1016
Plant, high protein/high starch content (dry commodities)		0.02 mg/kg	Reg. (EU) 2017/1016
Plant, high oil content		0.02 mg/kg	Reg. (EU) 2017/1016
Plant, difficult matrices (hops, spices, tea)		0.02 mg/kg	Reg. (EU) 2017/1016
Muscle	Pyraclostrobin	0.05 mg/kg	Reg. (EU) 2017/1016
Milk		0.01 mg/kg	Reg. (EU) 2017/1016
Eggs		0.05 mg/kg	Reg. (EU) 2017/1016
Fat		0.05 mg/kg	Reg. (EU) 2017/1016
Liver, kidney		0.05 mg/kg	Reg. (EU) 2017/1016
Soil (Ecotoxicology)	Pyraclostrobin The LOQ of the soil residue analytical methods covers the lowest ecotoxicological endpoint	0.05 mg/kg	Common limit
Drinking water (Human toxicology)	Pyraclostrobin	0.1 µg/L	general limit for drinking water

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Surface water (Ecotoxicology)	Pyraclostrobin	3 µg/L	NOEC of Daphnia magna as most sensitive species
Air	Pyraclostrobin	6 µg/m^3	AOEL sys: 0.02 mg/kg bw/d
Tissue (meat or liver)	Pyraclostrobin	~~0.1 mg/kg~~ 0.01 mg/kg	classified as T / T+
Body fluids		~~0.05 mg/L~~ 0.01 mg/L	classified as T / T+

5.3.4.2 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 pyraclostrobin in plant matrices is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-20: 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: pyraclostrobin and metabolite 500M07				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
High water content	Primary; Confirmatory method not necessary (two mass transitions)	0.02 mg/kg	LC-MS/MS	*Reinhard K., Mackenroth C. 1999a* *BASF DocID 1999/11134* Method no. 421/0 (Germany) / D9808 (USA) EU agreed
High acid content		0.02 mg/kg		
High oil content		0.02 mg/kg		
High protein/high starch content (dry)		0.02 mg/kg		
High water content	ILV	0.02 mg/kg	LC-MS/MS	Devine H.C., 2002a BASF DocID 2002/1007082 Method no. 421/0 (Germany) / D9808 (USA) New study, not peer-reviewed see A 2.3.2.1.1.3
High acid content		0.02 mg/kg		
High oil content		0.02 mg/kg		
High protein/high starch content (dry)		0.02 mg/kg		
High acid content	ILV	0.02 mg/kg	LC-MS/MS	*Perez R. and Perez S., 2000* *BASF DocID 1999/5187* Method no. 421/0 (Germany) / D9808 (USA) EU agreed
High protein/high starch content (dry)		0.02 mg/kg		

Component of residue definition: pyraclostrobin and metabolite 500M07				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
High water content	Primary; Confirmatory method not necessary (two mass transitions)	0.02 mg/kg	HPLC-UV	*Abdel-Baky S., Riley M.E., 2000* *BASF DocID 1999/5179* Method no. D9904 EU agreed
High acid content		0.02 mg/kg		
High oil content		0.02 mg/kg		
High protein/high starch content (dry)		0.02 mg/kg		
High water content	ILV	0.02 mg/kg (0.5 mg/kg for onions)	HPLC-UV	Devine H.C., 2002b BASF DocID 2002/1007083 Method no. D9904 New study, not peer-reviewed see A 2.3.2.1.2.2
High acid content		0.02 mg/kg		
High protein/high starch content (dry)		0.02 mg/kg		
High acid content	ILV	0.02 mg/kg	HPLC-UV	*Jordan J., 2000* *BASF DocID 1999/5184* Method no. D9904 EU agreed
Difficult (hops)	ILV	0.02 mg/kg	LC-MS/MS	Devine H.C., 2002a BASF DocID 2002/1007082 Method no. 421/0 (Germany) / D9808 (USA) New study, not peer-reviewed see A 2.3.2.1.1.3
Difficult (green tea, green coffee)	ILV	0.02 mg/kg (pyraclostrobin) 0.05 mg/kg (500M07)	LC-MS/MS	Scherthan D., 2011 BASF DocID 2011/1268146 Method no. 421/0 New study, not peer-reviewed see A 2.3.2.1.1.2

Table 5.3-21: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Extraction efficiency was investigated in the context of metabolism investigations in potato (BASF DocID 1999/11419 and 2000/1000048) which is already peer-reviewed in the DAR for the evaluation of pyraclostrobin (Germany, 2001) and summarized in a statement (Bross, M., Tilting, N., BASF DocID *1999/11136*, see Appendix 2, A 2.3.2.7.1).
Not required, because:	-

Comment:
The statement about the efficiency of the extraction procedure of plant origin compared the extraction procedure of the metabolism study with that of the data generation method 421/0, which is already peer-reviewed. Thereby, the extraction procedure of the data generation method 421/0 (Reinhard, K. and Mackenroth, C., DocID 1999/11134) using methanol/water (70/30, v/v) was shown to be equivalent to the one in

the metabolism study (Bross, M., Mackenroth, C., DocID 1999/11419) using methanol for the extraction of ^{14}C-labeled pyraclostrobin. Results were confirmed by HPLC analysis which showed qualitatively comparable patterns for both extraction solvents.

5.3.4.3 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 pyraclostrobin in animal matrices is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-22: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: pyraclostrobin				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Milk	Primary & Confirmatory	0.01 mg/kg	HPLC-UV primary: normal-phase HPLC-UV confirmatory: reversed-phase HPLC-UV	*Kampke-Thiel K., 1999* *BASF DocID 1999/11079* Method no. 439/0 EU agreed
Eggs		0.05 mg/kg		
Muscle		0.05 mg/kg		
Fat		0.05 mg/kg		
Kidney, liver		0.05 mg/kg		
Milk	ILV	0.01 mg/kg	HPLC-UV (normal-phase	*Levsen K., 1999* *BASF DocID 1999/11369* Method no. 439/0 EU agreed
Muscle		0.05 mg/kg		
Milk	Primary; Confirmatory method not necessary (two mass transitions)	0.01 mg/kg	LC-MS/MS	Hopf B., 2010 BASF DocID 2010/1018944 Method no. L0151/01 New study, not peer-reviewed see A 2.3.2.2.1
Eggs		0.01 mg/kg		
Muscle		0.01 mg/kg		
Fat		0.01 mg/kg		
Kidney, liver		0.01 mg/kg		
Milk	ILV	0.01 mg/kg	LC-MS/MS	Schacherl A., 2010 BASF DocID 2010/1123694 Method no. L0151/01 New study, not peer-reviewed see A 2.3.2.2.1.2
Eggs		0.01 mg/kg		
Muscle		0.01 mg/kg		
Fat		0.01 mg/kg		
Kidney, liver		0.01 mg/kg		

Table 5.3-23: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	BASF DocID *2001/1001011* (see Appendix 2)
Not required, because:	-

Comment:

A statement about the efficiency of the extraction procedure of animal origin exist about the data generation methods 439/0, 446/0 and 446/1, which is already peer-reviewed. The conclusions drawn therein are:

1) Acetonitrile is a suitable solvent for extraction of incurred residues as demonstrated in hen metabolism and for milk and fat also in goat metabolism.
2) No residues will remain in the hexane phase during partitioning or extraction.
3) The efficiency of acetonitrile + hexane as extraction solvent was compared to methanol used in metabolism during method development with incurred residues from a goat muscle sample and the efficiency was similar.

The data generation methods 439/0, 446/0, 446/1 and 446/2 use acetonitrile and iso-hexane or cyclohexane for extraction.

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

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

Table 5.3-24: Validated methods for soil (if appropriate)

Component of residue definition: pyraclostrobin Additional Components: pyraclostrobin metabolites 500M04, 500M59, 500M60, 500M62, 500M76 and 500M78			
Method type	**Method LOQ**	**Principle of method**	**Author(s), year / missing**
Primary; Confirmatory method not necessary (two mass transitions)	0.001 mg/kg	LC-MS/MS	Tilting N., Sopena-Vazquez F., 2014 BASF DocID 2013/1184817 Method no. L0166/01 New study, not peer-reviewed see A 2.3.1.3.1

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

An overview on the acceptable methods and possible data gaps for analysis of pyraclostrobin in surface and drinking water is given in the following table. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-25: Validated methods for water (if appropriate)

Component of residue definition: pyraclostrobin Additional Components: pyraclostrobin metabolites 500M04, 500M59, 500M60, 500M62, 500M76 and 500M78				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Drinking water, Surface water	Primary; Confirmatory method not necessary (two mass transitions)	0.003 µg/L (pyraclostrobin) 0.03 µg/L (500M59, 500M60, 500M62, 500M76 and 500M78)	LC-MS/MS	Tilting N., 2012 BASF DocID 2012/1009641 Method no. L0182/01 New study, not peer-reviewed see A 2.3.2.4.1
	Primary; Confirmatory method not necessary (two mass transitions)	0.03 µg/L (500M04)	LC-MS/MS	Obermann M., 2014 BASF DocID 2014/1004891 Method no. L0182/02 New study, not peer-reviewed see A 2.3.2.4.1.2

Component of residue definition: pyraclostrobin Additional Components: pyraclostrobin metabolites 500M04, 500M59, 500M60, 500M62, 500M76 and 500M78				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
	ILV (Primary, no confirmatory method required, two mass transitions employed)	0.003 µg/L (pyraclostrobin) 0.03 µg/L (500M04, 500M59, 500M60, 500M62, 500M76 and 500M78)	LC-MS/MS	Rutt D., Jones G., 2014 BASF DocID 2015/7001873 Method no. L0182/02 New study, not peer-reviewed see A 2.3.2.4.1.3

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

An overview on the acceptable methods and possible data gaps for analysis of pyraclostrobin in air is given in the following table. For the detailed evaluation of new/ additional studies please refer to Appendix 2.

Table 5.3-26: Validated methods for air (if appropriate)

Component of residue definition: pyraclostrobin			
Method type	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Primary & Confirmatory	0.0003 µg/L air	HPLC-UV LC-MS/MS (confirmatory method)	*Zangmeister W., 1999* *BASF DocID 1999/10694* Method no. 447 EU agreed
Primary; Confirmatory method not necessary (two mass transitions)	0.5 ng/L air	LC-MS/MS	Bloss K., 2018 BASF DocID 2018/1128631 Method no. L0197/02 New study, not peer-reviewed see A 2.3.2.5.2

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

An overview on the acceptable methods and possible data gaps for analysis of pyraclostrobin in body fluids and tissues is given in the following table.
As pyraclostrobin is classified as toxic, a residue analytical method in body fluids and tissues is required. Therefore, method L0151/01 developed for animal matrices was adapted for body fluids and validated for blood. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-27: Methods for body fluids and tissues (if appropriate)

Component of residue definition: pyraclostrobin			
Method type	**Method LOQ**	**Principle of method**	**Author(s), year / missing**
Primary; Confirmatory method not necessary (two mass transitions)	0.01 mg/kg	HPLC-MS/MS	Hopf B., 2010 BASF DocID 2010/1018944 Method No. L0151/01 New study, not peer-reviewed see A 2.3.2.2.1
ILV	0.01 mg/kg	HPLC-MS/MS	Schacherl A., 2010 BASF DocID 2010/1123694

Component of residue definition: pyraclostrobin			
Method type	**Method LOQ**	**Principle of method**	**Author(s), year / missing**
			Method No. L0151/01 New study, not peer-reviewed see A 2.3.2.2.1.2

5.3.4.8 Other studies/ information

Supplementary data on residue analytical methods presenting an overview of mass transitions of pyraclostrobin analytes useful for final determination is given in an additional document (BASF DocID 2015/1197842). An executive summary of the document can be found in appendix 2, A 2.3.2.7.3.

Supplementary data concerning the stability of standard solutions of pyraclostrobin, metabolite 500M07 and two derivatives MMP (Reg. No. 342878) and DMP (Reg. No. 412041) in several solvents and solvent mixtures stored under different conditions is presented in an additional study (BASF DocID *1999/11136*). For the detailed evaluation of the study, it is referred to appendix 2, A 2.3.2.7.1.

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	Ziegler, G.	2017	Validation of analytical method APL0500/03 for the determination of BAS 750 F (Reg.No. 5834378) and its metabolite M750F007 (Reg.No. 6003432) in M4-Medium, OECD-water and mixing water by LC/MS 2017/1064882 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1/2	xxxxxxxxxxx	2016	BAS 750 F - Acute toxicity study in the fathead minnow (Pimephales promelas) 2016/1155889 xxxxxxxxxxxxxxxxxxxxxxxxxxx yes Unpublished	Yes	BASF
KCP 5.1/3	Andre, M.	2017	Validation of BASF Method L0361/01 for the determination of pesticides in water by LC-MS/MS 2017/1065621 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1/4	Dressler, K.	2021	Chronic toxicity of BAS 758 00 F to the honey bee Apis mellifera L. under laboratory conditions 2021/2008152 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1/5	Benz-Birck, A., Mackenroth, C.	2005	Validation of the analytical method No. 535/0: Determination of Metrafenone BAS 560 F (Reg.No. 4037710) in plant matrices 2004/1010553 BASF AG, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF

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 5.1/6	Link, T.	2022	Validation of analytical method L0372/05 for the determination of BAS 560 F in bee matrices 2021/2052011 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/1	Barth, J.	2020	Analytical method AFL1019/01: Quantitative determination of the active ingredients Mefentrifluconazole, Metrafenone and Pyraclostrobin in BAS 758 00 F by Liquid Chromatography 2020/2091975 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 5.1.1/2	Barth, J.	2020	Validation of the analytical method AFL1019/01: Determination of the active ingredients Mefentrifluconazole, Metrafenone and Pyraclostrobin in BAS 758 00 F by Liquid Chromatography 2020/2091974 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/3	Harsch, M.	2017	Analytical method AFL0944/01: Determination of Dimethylformamide (DMF) in formulation containing Mefentrifluconazole (BAS 750 F) by GC/MS 2017/1012360 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 5.1.1/4	Harsch, M.	2017	Validation of the analytical method AFL0944/01: Determination of Dimethylformamide (DMF) in formulation containing Mefentrifluconazole (BAS 750 F) by GC/MS 2017/1012361 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF

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 5.1.1/5	Schubring, M.	2021	Additional Validation of the Analytical Method AFL0944/01: "Determination of Dimethylformamide (DMF) in Formulation containing Mefentrifluconazole (BAS 750 F) by GC/MS" 2020/2109404 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/6	Schubring, M.	2020	Analytical Method AFL1023/01: Determination of Toluene in BAS 758 00 F (EC - Formulation) by GC-MS 2020/2094553 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 5.1.1/7	Schubring, M.	2020	Validation of the Analytical Method AFL1023/01: Determination of Toluene in BAS 758 00 F (EC - Formulation) by GC-MS 2020/2094552 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/8	Nemitz, A.	2020	Additional Validation to the Analytical Method AFL0977/03: Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole 2020/2037319 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/9	Frohn, D.	2021	Analytical Method AFL0977/06: Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole 2020/2102073 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF

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 5.1.1/10	Frohn, D.	2021	Additional Validation of the Analytical Method AFL0977/05: "Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole" 2020/2101772 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/11	Stegmaier, W.	2020	Validation of the analytical method AFL1024/01: Determination of Dimethylsulfat in BAS 758 00 F 2020/2092912 BASF SE - GKA Competence Center Analytics, Ludwigshafen, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/1	Obermann, M.	2017	Validation of analytical method L0359/01 for the determination of BAS 750 F and its metabolites M750F003, M750F005, M750F006 (Reg.No.5863469), M750F007 (Reg.No.6003432) and M750F008 (Reg.No.6010286) in drinking and surface water by LC-MS/MS 2017/1066523 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/2	Stanislowski, T.	2017	Independent laboratory validation (IVL) of method L0359/01 for the determination of BAS 750 F and its metabolites M750F005, M750F006, M750F007 and M750F008 in drinking water and surface water by LC-MS/MS 2017/1066522 EAG Laboratories PTRL Europe, Ulm, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/3	Homazava, N.	2019	Validation of BASF Analytical Method L0339/02 for the determination of M750F015, M750F016 and M750F017 in body fluids 2019/1046404 IES - Innovative Environmental Services Ltd., Witterswil, Switzerland yes Unpublished	No	BASF

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 5.2/4	Benz-Birck, A., Mackenroth, C.	2001	Validation of BASF Method No. 445/0: Determination of BAS 500 F and BF 500-3 in various plant matrices 2000/1012405 BASF AG, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/5	Leite, R.	2005	Validation study of the SOP-PA.0243 for determination of Pyraclostrobin and its metabolite (BF 500-3) residues in coffee (grain), soybean (grain) and wheat (grain) 2005/1037978 BASF SA, Resende, Brazil yes Unpublished	No	BASF
KCP 5.2/6	Courtois, J.	2014	Validation of analytical method L0220/01 for the determination of metabolite 500M79 (Reg.No. 5937091) in plant matrices by LC-MS/MS 2014/1001721 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/7	Jose, W	2015	Validation of BASF Method Number L0076/09 for the determination of BAS 500 F and its metabolite 500M07 in citrus (whole fruit), dry beans (seeds), tomato (whole fruit), soybeans (grain) and wheat (grain) using HPLC-MS/MS and UPLC-MS/MS 2015/3004795 BASF SA, Guaratingueta, Brazil yes Unpublished	No	BASF
KCP 5.2/8	Lehmann, A., Mackenroth, C.	2007	Validation of BASF method No. 535/1 in plant matrices 2006/1039427 BASF AG, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF

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 5.2/9	Eilers, B., Tarasche-wski, I.	2014	Validation of analytical method 446/2 (L0058/03) for the determination of BAS 500 F (Reg.No. 304428) and its metabolites 500M04 (Reg.No. 298327) and 500M85 (Reg.No. 399530) in animal matrices by LC-MS/MS 2013/1400972 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/10	Riley, M., Malinsky, S.	2000	Method validation of BASF analytical method D9902: Method for determination of residues of BAS 500F and its metabolite BF 500-16 in hen tissues using LC/MS/MS 2000/5004 BASF Corp., Research Triangle Park NC, United States of America yes Unpublished	No	BASF
KCP 5.2/11	Dressler, K.	2021	Chronic toxicity of BAS 758 00 F to the honey bee Apis mellifera L. under laboratory conditions 2021/2008152 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/12	Tilting, N.	2014	Validation of analytical method L0166/01: Determination of BAS 500 F (Pyraclostrobin) and its metabolites Reg.No. 364380 (500M01), Reg.No. 369315 (500M02) and Reg.No. 340266 (500M07) in soil using LC/MS/MS 2013/1184817 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/13	Zangmeister, W.	2010	Validation of analytical method L0161/01: Determination of BAS 500 F in soil at LOQ 0.001 mg/kg 2010/1075848 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/14	Obermann, M.	2005	Validation of analytical method APL0500/01: Determination of pesticides in water by HPLC/MS 2005/1026675 BASF AG, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF

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 5.2/15	Andre, M.	2017	Validation of BASF Method L0361/01 for the determination of pesticides in water by LC-MS/MS 2017/1065621 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/16	Scherthan, D.	2011	Independent laboratory validation of the BASF analytical method 421/0: Method for determination of BAS 500 F and its metabolite BF 500-3 residues in plant matrices using LC/MS/MS 2011/1268146 RLP AgroScience GmbH, Neustadt/Weinstrasse, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/17	Devine, C.	2002	Independent laboratory validation of BASF method Number D9808 (USA), 421/0 (Germany), an analytical method for the determination of residues of BAS 500 F and its metabolite BF 500-3 2002/1007082 CEMAS - CEM Analytical Services Ltd., North Ascot Berkshire SL5 8JB, United Kingdom yes Unpublished	No	BASF
KCP 5.2/18	Devine, C.	2002	Independent laboratory validation of BASF method D9904, an analytical method for the determination of residues of BAS 500 F and its metabolite BF 500-3 2002/1007083 CEMAS - CEM Analytical Services Ltd., North Ascot Berkshire SL5 8JB, United Kingdom yes Unpublished	No	BASF
KCP 5.2/19	Eilers, B.	2010	Validation of the analytical method L0151/01: Method for the determination of BAS 500 F (Reg.No. 304428) in animal matrices 2010/1018944 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF

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 5.2/20	Schacherl, A.	2010	Independent laboratory validation (ILV) of an analytical method for the determination of BAS 500 F (Reg.No. 304428) in animal matrices 2010/1123694 Eurofins Agroscience Services GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/21	Tilting, N.	2012	Validation of method L0182/01: Determination of BAS 500 F and its metabolites Reg.No. 412053 (500M59), Reg.No. 411847 (500M60), Reg.No. 412785 (500M62), Reg.No. 413038, and Reg.No. 377613 in ground- surface- and tapwater using LC-MS/MS 2012/1009641 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/22	Obermann, M.	2014	Validation of analytical method L0182/02 for the determination of BF 500-5 (Reg.No. 298327), metabolite of BAS 500 F, in ground- and surface water by LC-MS/MS 2014/1004891 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/23	Bianca, C.	2015	Independent laboratory validation of BASF method L0182/02: BAS 500 F (Pyraclostrobin) and its metabolites BF 500-5 (Reg.No. 298327), BF 500-12, BF 500-11, BF 500-13, BF 500-14, BF 500-15 in ground- and surface- water by LC/MS/MS 2015/7001873 JRF America, Audubon PA, United States of America yes Unpublished	No	BASF
KCP 5.2/24	Penning, H.	2012	Validation of analytical method L0197/01: Method for the determination of BAS 500 F (Pyraclostrobin) in air by LC-MS/MS 2012/1220256 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF

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 5.2/25	Bloss, K.	2018	Validation of Analytical Method L0197/02: Method for the Determination of BAS 500 F (Pyraclostrobin) in air by LC-MS/MS 2018/1128631 Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/26	Reinhard, K.	1999	Determination of the stability of BAS 500 F and of relevant metabolites and derivatives thereof in different solutions 1999/11136 BASF AG, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/27	Bross, M., Tilting, N.	2001	Efficiency of the extraction procedure in methods for the determination of Pyraclostrobin in matrices of animal origin 2001/1001011 BASF AG, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 5.2/28	Obermann, M.	2015	Supplementary data on residue analytical methods for Pyraclostrobin - Compilation of Mass Transitions/Product Ion Scans, used within residue analytical methods for the determination of BAS 500 F - Pyraclostrobin Analytes 2015/1197842 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 10.2.1/6	Eckenstein, H.	2021	BAS 758 00 F - Effect on Daphnia magna in a static 48-Hour Immobilization Test 2020/2033902 IES - Innovative Environmental Services Ltd., Witterswil, Switzerland yes Unpublished	No	BASF
KCP 10.2.1/4	Eckenstein, H.	2021	BAS 758 00 F – Acute Toxicity to Rainbow trout (Oncorhynchus mykiss) in a static 96-Hour Test 2020/2033900 IES - Innovative Environmental Services Ltd., Witterswil, Switzerland yes Unpublished	No	BASF

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 10.2.1/8	Eckenstein, H.	2021	BAS 758 00 F - Effect on Pseudokirchneriella subcapitata in a 72 Hour Algal Growth Inhibition Test 2020/2033904 IES - Innovative Environmental Services Ltd., Witterswil, Switzerland yes Unpublished	No	BASF

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Please refer to Part A

Appendix 2 detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Mefentrifluconazole

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

Comments of zRMS:	The study has been accepted. The objective of this validation study was to validate the LC/MS method APL500/03 for the determination of BAS 750 F, M750F007 in M4 Medium, OECD- Water and Mixing Water. The method was validated at 2 fortification levels (0.001 mg/L and 0.01 mg/L) for water. For each fortification level and water type, five replicates were done. Quantification was done using MS-detection at m/z 398 for BAS 750 F and at m/z 338 for M750F007. The LOQ for both analytes were set at 0.001mg/L. Significant peak interference (> 30 % of the LOQ) were not observed at the retention time of BAS 750 F and M750F007. Good linearity (r > 0.995) was observed in the range of 0.25 ng/mL to 5 ng/mL for BAS 750 F and its metabolite M750F007. No significant matrix effects were identified. The mean recovery values ranged between 86 % and 109 % of the nominal values. The relative standard deviations (RSD, %) for both fortification levels were below 20%. The method APL0500/03 fulfils the requirements regarding specificity, linearity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BAS 750 F (Mefentrifluconazole) and its metabolite M750F007 in water.

Reference:	CP 5.1/1
Report	Validation of analytical method APL0500/03 for the determination of BAS 750 F (Reg.No. 5834378) and its metabolite M750F007 (Reg.No. 6003432) in M4-Medium, OECD-water and mixing water by LC/MS, Ziegler, G., 2017 report No 838449 BASF DocID 2017/1064882 Authority registration No
Guideline(s):	EFSA Panel on Plant Protection Products and their Residues (PPR), OECD-ENV/JM/MONO/(2007)17, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany),
Acceptability:	Yes

Principle of the Method Samples are diluted with acetonitrile, acidified with formic acid, and analysed by LC-MS. Separation is achieved by a YMC Pro C18 column (50 mm x 3 mm, 3 µm for mefentrifluconazole and 150 mm x 4.6 mm, 3µm for M750F007) and a gradient mixture of water/formic acid (1000/1, v/v) and acetonitrile/formic acid (1000/1, v/v) at a flow rate of 0.7 mL/min. Detection is accomplished by MS measurement in ESI positive mode.

Recovery Findings The method proved to be suitable to determine mefentrifluconazole and

M750F007 in water. Samples were spiked with the analytes at LOQ and 10x LOQ. All average recovery values (mean of 5 replicates per fortification level, analyte and matrix) were between 70% and 110%. The detailed results are given in the table below (Table A 1).

Table A 1 Results of the Method Validation for the Determination of Mefentrifluconazole and M750F007 in Water

Analyte	Matrix	Fortification Level [mg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]	Overall Mean Recovery [%]	Overall RSD [%]
Mefentrifluconazole	M4-Medium	0.001	5	108	1.0	108	2.2	106	2.4
		0.01	5	109	3.2				
	Mixing-Water	0.001	5	106	0.5	105	2.0		
		0.01	5	104	2.5				
	OECD-Medium	0.001	5	103	0.7	103	0.8		
		0.01	5	103	0.9				
M750F007	M4-Medium	0.001	5	86	1.4	96	1.3	93.4	5.1
		0.01	5	86	1.3				
	Mixing-Water	0.001	5	93	1.6	95	2.6		
		0.01	5	97	2.0				
	OECD-Medium	0.001	5	93	1.8	96	3.9		
		0.01	5	98	3.3				

RSD = Relative standard deviation

Linearity

Linearity of detector response was tested using five calibration standard concentrations in the range of 0.25 ng/mL to 5 ng/mL with correlation coefficients of > 0.995. The calibration standards were prepared in water/acetonitrile/formic acid (80:20:0.1, v/v/v).
The following calibration curves were received:
y=15565.5x-2470.15, R=0.9998774 for Mefentrifluconazole
y=5915.26x+5590.4, R=0.9998544 for M750F007

Specificity

The method is specific for analysis of the test items in water. Quantification was done by reversed phase HPLC using MS detection at *m/z* 398 $[M+H]^+$ mefentrifluconazole and at *m/z* 338 $[M+H]^+$ for M750F007 and external calibration calculated from a linear regression line. The retention times of the test items in samples matched the retention times in calibration solutions. No peak interferences occurred at the retention times of mefentrifluconazole and its metabolite M750F007.

Matrix Effects

Solvent standards as well as matrix-matched standards were analysed to assess potential matrix effects. As no significant matrix effects were identified, solvent-standards, prepared in water/acetonitrile/formic acid (80:20:0.1, v/v/v), were used for calibration and quantification of the analyte mefentrifluconazole and its metabolite M750F007.

Interference

No significant interferences (> 30% LOQ) were observed at the appropriate retention time and using the given detector.

Limit of Quantification

The method has a limit LOQ of 0.001 mg/L, corresponding to the lowest fortification level successfully tested. The LOQ is therefore well within the range of linearity calibration tests.

Limit of Detection The method has a limit of detection (LOD) of 0.00025 mg/L, corresponding to the lowest calibration level used.

Stability Working Solutions Stability of working solutions was not determined within this validation study hence calibration solutions and matrix matched standard solutions were prepared freshly before the analytical determination.

Repeatability The relative standard deviations (RSD, %) for all fortification levels were < 20%.

Reproducibility Reproducibility of the method was not determined within the validation study.

Conclusion **It could be demonstrated that the analytical method APL500/03 fulfils the requirements with regard to linearity, specificity, repeatability, LOQ and recoveries and is therefore applicable to correctly determine residues of mefentrifluconazole and its metabolite M750F007 in M4-medium, OECD-water and mixing water with a LOQ of 0.001 mg/L.**

Comments of zRMS:	The method has been accepted. To support a fish acute toxicity study in the Fathead Minnow concentration control analysis of BAS 750 F in test water was performed using HPLC-MS. The analytical method APL0500/03 was used and slightly modified with respect to the chromatographic conditions to determine BAS 750 F in test water. Quantification was done using MS-detection at *m/z* 398 for BAS 750 F. 2 fortification levels in test water with 5 replicates each cover the range of the analyzed samples. Mean recovery rates of 106 % for the lower fortification level and 103% for the higher fortification level were determined. The relative standard deviation (RSD) was < 10 % for both levels investigated. Linearity was shown over a range of 0.0002 mg/L – 0.004 mg/L BAS 750 F with correlation coefficients (r) 0.99. The LOQ for BAS 750 F was 0.001 mg/L. Significant peak interference (> 30 % of the LOQ) was not observed in the control samples at the retention time of BAS 750 F. The modified method was validated with regard to recovery, repeatability, limit of quantification, specificity and linearity.

Reference:	CP 5.1/2
Report	BAS 750 F - Acute toxicity study in the fathead minnow (Pimephales promelas), xxxxxxxxxxxxxxxxxx, 2016 report No 805877 BASF DocID 2016/1155889 Authority registration No
Guideline(s):	EC 440/2008 C.1, EPA 72-1, EPA 850.1075, OECD 203
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Principle of the Method

The method used for the determination of BAS 750 F in test water is based on BASF method APL0500/03 and was validated within the current study. Fortified samples and test samples were directly dissolved in 0.5 % formic acid in acetonitrile and if necessary, further diluted with a mixture of test water/acetonitrile/formic (80:20:0.1, v/v/v) into the range of the calibration solutions.
Quantification of residues of mefentrifluconazole (BAS 750 F) was done by reversed phase UHPLC on a BEH C18 column using MS-detection at m/z 398 ($[M+H]^+$) and external calibration calculated from a linear regression line. The identity of the test item was confirmed by comparison of the mean retention time of the reference item with the mean retention time of the corresponding peak of the test item during UHPLC-MS analysis.

Recovery findings

The analytical method APL0500/03 was used and slightly modified with respect to the chromatographic conditions to determine BAS 750 F in test water. The modified method was validated with regard to recovery, repeatability, limit of quantification, linearity and specificity. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4 (11/07/2000). Mean recovery rates of 106 % for the lower fortification level (0.001 mg/L) as well as 103 % for the higher fortification level (5.0 mg/L) were found. The relative standard deviation (RSD) was <10 % for both levels investigated. This confirms the validity of the method for the determination of the test item in test water.

Table A 2: Recovery results from method validation of mefentrifluconazole using the analytical method

Matrix	Analyte	Fortification level (mg a.s./L)	Number of Replicates	Mean recovery (%)	RSD (%)	Overall recovery [%]	RSD [%]	Comments
Test water	BAS 750 F	0.000972	2	111	0	106	4.3	Quantitation m/z 398 ([M+H]+)
			2	109	1.4			
			2	108	0			
		0.000976	2	101	1.3			
			2	101	1.3			
		4.86	2	104	0.5	103	1.8	
			2	101	0			
			2	105	0			
		4.88	2	101	0			
			2	102	0			

RSD = Relative standard deviation

Linearity

Calibration standards, ranging from 0.0002 mg/L – 0.004 mg/L, were prepared in test water/acetonitrile/formic acid mixture (80:20:0.1, v/v/v). Five calibration points were used and individual calibration data was presented. Linear correlations with coefficients r ≥0.99 were obtained, thus demonstrating satisfactory linearity.
The following calibration curve was received:

	y=979801x-445.713, R=0.9999468 for Mefentrifluconazole
Specificity	Significant peak interference (>30% of the LOQ) was not observed in the control samples at the retention time of BAS 750 F.
Matrix Effects	Not relevant for water matrix.
Interference	No significant interferences (> 30% LOQ) were observed at the appropriate retention time and using the given detector.
Limit of Quantification	The limit of quantification (LOQ) representing the lowest validated level with sufficient recovery and precision was 0.001 mg/L. The LOQ is therefore well within the range of linearity calibration tests.
Limit of Detection	The limit of detection (LOD) is 0.0002 mg/L corresponding to the lowest calibration standard.
Stability Working Solutions	Stability of working solutions was not determined within this validation study hence calibration solutions and matrix matched standard solutions were prepared freshly before the analytical determination.
Extract Stability	Not relevant as no extract available (only direct dissolving of water in several solvents).
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were < 20%.
Reproducibility	Reproducibility of the method was not determined within the validation study.
Conclusion	The method uses highly specific UHPLC-MS for final determination of mefentrifluconazole with a limit of quantitation of 0.001 mg/kg. Thereby, it could be demonstrated that the method fulfils the requirements with regards to recovery, repeatability, limit of quantitation, linearity and specificity.

Comments of zRMS:	The method validation has been accepted. The objective of this study was to validate LC-MS/MS method L0361/01 for the determination of **BAS 500 F (Pyraclostrobin),** BAS 510 F (Boscalid), BAS 550 F (Dimethomorph), BAS 700 F (Fluxapyroxad), **BAS 750 F (Mefentrifluconazole),** BAS 656 H (Dimethenamid-P), BAS 684 H (Cinmethylin), BAS 720 H (Imazamox), BAS 9178 H (Clomazone), BAS 395 I (Dinotefuran), BAS 550 I and Prothioconazole in tap water and M4-medium. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary. The quantification of the twelve analytes is based on the monitoring of two mass transitions. Recovery data are reported for each mass transition and matrix considered. The method has a limit of quantitation of 0.1 µg/L in tap water and M4-medium for each analyte of interest. Good linearity (r > 0.998) was observed in the range of 0.01 ng/mL to 0.8 ng/mL for both mass transitions of the analytes. The mean recovery values of the validation experiments over all tested analytes were between 83% and 106%, which fulfils the legal requirements for mean recovery values. The relative standard deviation (RSD, %) for all fortification levels was below 20%.

	Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of the analytes. It could be demonstrated that method L0361/01 fulfils the requirements with regard to specificity, linearity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BAS500F (**Pyraclostrobin**), and BAS 750 F (**Mefentrifluconazole**).

Reference:	CP 5.1/3
Report	Validation of BASF Method L0361/01 for the Determination of Pesticides in Water by LC-MS/MS, Andre, M., 2017 report No EU-IF-17/04022633,EU-783160,IF-17/04022633 BASF DocID 2017/1065621 Authority registration No
Guideline(s):	EFSA Panel on Plant Protection Products and their Residues (PPR), OECD-ENV/JM/MONO/(2007)17, SANCO/3029/99 rev. 4 (11 July 2000)
Deviations:	No
GLP:	yes (certified by Hess. Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden, Germany)
Acceptability:	Yes

Principle of the Method

A 5 g tap water or M4-medium specimen aliquot is extracted by shaking with Acetonitrile/Water/HCOOH, 400/600/2, v/v/v. An aliquot of the extract is then used for determination by LC-MS/MS. Analysis was accomplished using a Pinnacle DB AQ C18 column and a water-acetonitrile gradient with formic acid as modifier at a flow rate of 600 µL/min. Samples were analysed at mass transition *m/z* 398 → 70 for quantitation and *m/z* 398 → 182 for confirmation for mefentrifluconazole (corresponding MS-spectra are attached below).

Recovery findings

Fortification levels of 0.1 µg/L, 1.0 µg/L and 10 µg/L were validated for BAS 750 F. Method validation acceptance criteria were fully met with mean recovery values between 94% and 106% in all matrices tested.

Table A 3: Recovery results from method validation of mefentrifluconazole using the analytical method

Matrix	Analyte	Fortification level (µg a.s./L)	Number of replicates	Mean recovery [%]	RSD (%)	Overall recovery [%]	RSD (%)	Comments
Tap water	**BAS 750 F**	0.1	5	94	4.3	95	2.8	Mass transition m/z 398→70*
		1.0	5	96	2.0			
		10	5	97	0.6			

Matrix	Analyte	Fortification level (µg a.s./L)	Number of replicates	Mean recovery [%]	RSD (%)	Overall recovery [%]	RSD (%)	Comments
		0.1	5	99	5.2	96	3.9	Mass transition m/z 398→182
		1.0	5	94	2.4			
		10	5	96	1.5			
M4-medium		0.1	5	101	2.9	100	2.5	Mass transition m/z 398→70*
		1.0	5	101	2.1			
		10	5	99	2.4			
		0.1	5	106	3.9	104	3.3	Mass transition m/z 398→182
		1.0	5	105	2.3			
		10	5	101	1.8			

*used as quantification transition
RSD = Relative standard deviation

Linearity

Good linearity (r > 0.9995) was observed in the range of 0.01 ng/mL to 0.8 ng/mL for the two mass transitions of BAS 750 F At least six calibration levels, prepared as matrix matched standards, distributed over the tested concentration range were used.
The following calibration curves were given in the study report:
y=144494.9x+13911 (transition 398→70) in Tap Water
y=16888x+1389 (transition 398→182) in Tap Water
y=145322.8x+7220 (transition 398→70) in M4-Medium
y=16493x+860.4 (transition 398→182) in M4-Medium

Specificity

The method allows the specific determination of BAS 750 F in tap water and M4-Medium using LC-MS/MS. Detection is accomplished by high selective MS/MS-detection using two mass transitions.

Matrix Effects

The results demonstrate that the matrix-load in the tested matrix-matched standards had negligible influence on the detection. But as the matrices were used for fortification and control specimens, the matrices were used also for preparation of standard solutions.

Interference

No significant interferences (> 30% LOQ) were observed at the appropriate retention time and using the given detector.

Limit of Quantification

The limit of quantification (LOQ) representing the lowest validated level with sufficient recovery and precision was 0.1 µg/L. The LOQ is therefore well within the range of linearity calibration tests.

Limit of Detection

The limit of detection (LOD) is 0.02 µg/L corresponding to the lowest calibration standard.

Stability Working Solutions

Stability tests confirmed that the analytes were stable for at least 28 days in calibration solutions in tap water matrix and 29 days in calibration solutions in M4-medium matrix when stored refrigerated at approximately 2 – 8 °C in the dark. For fortification solutions stability was proven for 28 days. Mean uncorrected recoveries for all analytes were in an acceptable range 85% to 110% for calibration solutions over the tested time period. As the

stability was confirmed over all concentrations investigated, it can be concluded that concentration dependency is not given.

Extract Stability The stability of specimen final volumes was not investigated during this study, as storage stability of matrix matched standards was proven and composition of matrix matched standards and specimen final volume is equal.

Repeatability The relative standard deviations (RSD, %) for all fortification levels were < 20%.

Reproducibility Reproducibility of the method was not determined within the validation study.

Conclusion It could be demonstrated that analytical method L0361/01 fulfils the requirements with regard to specificity, linearity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BAS 750 F (Mefentrifluconazole).

Comments of zRMS:	The analytical method has been accepted. The concentration of Metrafenone, Mefentrifluconazole, and Pyraclostrobin in the highest and lowest test item feeding solution applied on the first and last day of application (D 0 and D 9) was determined with the LC-MS/MS method no. L0452/02 within the study. Results for the concurrent procedural recovery samples showed values in an acceptable range with overall mean recoveries of 74.3 % to 96.6 % for the three analytes at both fortification levels with RSD values ≤8.4 %. These results fulfill the requirements according to current guidelines; therefore, the suitability of the applied methodology is proven. The analytical results of concentration control samples for BAS 758 00 F in bee feeding solution confirmed its nominal concentrations of 204 mg /kg and 3261 mg /kg, with recoveries of 90.9 % and 99.8 % for day 0 samples, and 82.4 % and 88.4 % for day 9 samples when determined via the active ingredient BAS 750 F. The concentrations were also confirmed via the active ingredient BAS 560 F with recoveries of 82.7% and 101% for day 0 samples, and 81.1% and 89.5 % for day 9 samples. The concentrations were also confirmed via the active ingredient BAS 500 F with recoveries of 88.3 % and 104 % for day 0 samples, and 94.8 % and 85.1 % for day 9 samples. The mean measured concentrations of untreated control were always below the LOQ for all sampling intervals. No significant peak interferences occurred at the retention times and mass transitions of all analytes. Furthermore, no residues of the active ingredients were found in the control samples, i.e., the concentrations of active ingredients were below 30% of the LOQ.

Reference:	CP 5.1/4
Report	Chronic toxicity of BAS 758 00 F to the honey bee Apis mellifera L. under laboratory conditions Dreßler, A., 2021 Report No. 892147,2148BAC0053 BASF DocID 2021/2008152
Guideline(s):	OECD TG 245 (2017)

Deviations:	No
GLP:	yes (Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)
Acceptability:	Yes

Principle of the Method 0.25 g bee feeding solution samples were extracted by shaking with acetonitrile/water/formic acid (50/50/1, v/v/v). After addition of QuEChERS-Salt the samples were shaken, vortexed and centrifugated. 1 mL aliquots of the acetonitrile-phase were diluted with acetonitrile/water/formic acid (50/50/1, v/v/v) and analyzed by LC-MS/MS. Samples were analysed at mass transition *m/z* 398 → 133 for quantitation of mefentrifluconazole.

Recovery findings Fortification levels of 0.1 mg/kg and 400 mg/kg were validated for BAS 750 F. Method validation acceptance criteria were fully met with mean recovery values between 76.6% and 106% in bee feeding solution matrices.

Table A 4: Recovery results of mefentrifluconazole using the analytical method L0452/02

Matrix	Analyte	Fortification level (mg/kg)	Number of replicates	Mean recovery [%]	RSD (%)	Overall recovery [%]	RSD (%)	Comments
Bee feeding solution	**BAS 750 F**	0.10	5	94.5	8.4	90.7	8.5	Mass transition m/z 398→133
		400	5	86.9	6.8			

RSD = Relative standard deviation

Linearity Good linearity (r = 0.9990) was observed in the range of 0.01 ng/mL to 1.0 ng/mL for one matrix-matched calibration standards for quantifier mass transitions of BAS 750 F
The following calibration curve was given in the study report:
$y=2.31\cdot10^{5}x+6.21\cdot10^{3}$ (transition 398→133)

Specificity The method allows the specific determination of BAS 750 F in bee feeding solution using LC-MS/MS.

Matrix Effects Potential matrix effects were compensated by using matrix-matched calibration standards.

Interference No significant peak interferences occurred at the retention time and mass transition of the analyte.

Limit of Quantification The limit of quantification (LOQ) representing the lowest validated level with sufficient recovery and precision was 0.1 mg/kg. The LOQ is therefore well within the range of linearity calibration tests.

Limit of Detection The limit of detection (LOD) is 0.01 ng/mL (being equivalent to 0.008 mg/kg) corresponding to the lowest calibration standard.

Stability Working Solutions All test samples were measured within 30 days of sampling and therefore

	further studies were not required.
Extract Stability	All test samples were measured within one day after extraction and therefore further studies were not required.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were < 20%.
Reproducibility	Reproducibility of the method was not determined within the validation study.
Conclusion	It could be demonstrated that analytical method L0452/02 fulfils the requirements with regard to specificity, linearity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BAS 750 F (mefentrifluconazole).

A 2.1.1.1.1.1 Confirmatory method

A confirmatory technique is not required since the detection by MS/MS with two characteristic mass transitions is regarded to be highly specific.

A 2.1.2 Methods for post-authorization 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 Soil (KCP 5.2)

No new or additional studies have been submitted

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

A 2.1.2.4.1 Analytical method L0359/01 for the determination of mefentrifluconazole in water

A 2.1.2.4.1.1 Method validation 1

Comments of zRMS:	The study is accepted. The purpose of this evaluated study was to validate analytical method L0359/01 for the determination of mefentrifluconazole (BAS 750 F) and its metabolites in ground and surface water by LC-MS/MS. Concluding, the method L0359/01 can be considered highly specific for analysis of the test items (mass transitions from the positively charged molecule ions to typical fragment ions in MS/MS mode). Thus, no further confirmatory method is required. It could be demonstrated that this method fulfils the requirements with regard to specificity, linearity, repeatability, limit of quantification and recoveries consistently with SANCO/825/00 rev. 8.1 (16/11/2010) and SANCO/3029/99 rev. 4 (11/07/2000). Reproducibility of the method was not determined within this validation study, but an ILV has been successfully conducted without any recommendations or

	deviations to the Technical Procedure L0359/01. Therefore, this method fulfils also the reproducibility requirements as defined in (SANCO/825/00 rev. 8.1) and it can be applicable as an enforcement method. Essential details of the study: For mefentrifluconazole and the metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 two mass transitions detection was employed (mefentrifluconazole 398 → 70 for quantification, 400 → 70 for confirmation); the relevant interferences were < 30% of LOQ at the retention times and mass transitions of the analytes; 6 calibration levels were used, good linearity $r \geq 0.995$ within the range of 0.03 ng/mL to 1 ng/mL was obtained for all transitions, the mean recovery and RSD values for both fortification levels ranged from 83% to 103% and below 10%. Recovery data are reported for each mass transition and matrix considered. For the recovery experiments, untreated ground and surface water samples were fortified with 0.03 µg/L (LOQ) and 0.3 µg/L (10x LOQ) of mefentrifluconazole and each analyte. Repeatability of the method was tested for 6 analytes in both water types using 5 replicates per fortification level. Quantification of the analytes was performed for both mass transitions. Control samples were handled the same way as fortified samples, except that no analyte was spiked. All results obtained from measurements of control samples were below the LOD. The impact of concentrated water matrix in matrix-matched standards in comparison to solvent standards within one analytical queue was assessed. The calculated mean response factors obtained from matrix-matched standards were between 93% and 101% (differences < 20%) demonstrating that the matrix-load in the tested matrix-matched standards had negligible influence on the target detection. Therefore, no matrix-matched standards were needed for further experiments. Detailed stability tests confirmed that mefentrifluconazole as well as the metabolites investigated were stable for a maximum 30 days in stock and calibration solutions, when stored at 4°C in the dark; in sample extracts over a period of 7 days in case of surface water and 8 days in case of ground water, when stored at 4°C in the dark. The method validation data (according to a need of the residue definition) was also sufficiently described and concluded below by the applicant. The study was already evaluated in PL.

Reference:	CP 5.2/1
Report	Validation, analytical method L0359/01, BAS 750 F (Reg.No.5834378) and metabolites M750F003 (Reg.No.5924326), M750F005 (Reg.No.6003433), M750F006 (Reg.No.5863469), M750F007 (Reg.No.6003432) and M750F008 (Reg.No.6010286) in drinking and surface water by LC-MS/MS Obermann, M., 2017 report No 836940 BASF DocID 2017/1066523
Guideline(s):	EPA 850.6100 (2012), SANCO/3029/99 rev. 4 (11 July 2000),

	SANCO/825/00 rev. 8.1 (16 November 2010)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Please note that the method was validated for mefentrifluconazole and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008. Only results for parent are presented in the summary below as this is the only compound relevant for residue definition.

Materials and methods

Residues of mefentrifluconazole (BAS 750 F) are extracted from water with ethyl acetate. An aliquot of the organic phase is evaporated to dryness using a nitrogen evaporator at 40°C and the obtained residues are reconstituted in acetonitrile/water (50/50, v/v) prior to final determination by LC-MS/MS. Analysis was accomplished using a Waters Xbridge C18 column and a water-acetonitrile gradient with formic acid as modifier at a flow rate of 800 µL/min. Samples were analysed at mass transition 398 → 70 for quantitation and 400 → 70 for confirmation for mefentrifluconazole.

Results and discussions

Method validation acceptance criteria were fully met with mean recovery values between 70% and 110% in all matrices tested. The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%. Method validation data are summarised in the table below.

Table A 5: Recovery results from method validation of mefentrifluconazole using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Ground water	BAS 750 F	0.03 (n=5)	95	2.1	Quantitation
		0.3 (n=5)	98	2.0	m/z 398→70
		0.03 (n=5)	96	2.7	Confirmation
		0.3 (n=5)	98	2.8	m/z 400→70
Surface water	BAS 750 F	0.03 (n=5)	103	1.3	Quantitation
		0.3 (n=5)	102	1.5	m/z 398→70
		0.03 (n=5)	101	3.2	Confirmation
		0.3 (n=5)	98	2.7	m/z 400→70

Table A 6: Characteristics for the analytical method used for validation of mefentrifluconazole residues in water

	Mefentrifluconazole
Specificity	The method L0359/01 determines residues of

	Mefentrifluconazole
	mefentrifluconazole in water. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered.
Calibration (type, number of data points)	Calibration standards were prepared in acetonitrile / water (50/50, v/v). Six calibration points were used and individual calibration data was presented. Linear correlations with coefficients ≥0.99 were obtained.
Calibration range	Calibration points distributed over a concentration range of 0.03 to 1 ng/mL were used. This covers the tested concentration range.
Assessment of matrix effects is presented	Solvent- as well as matrix-matched standards were analysed to assess potential matrix effects. As no significant matrix effects were identified, solvent standards, prepared in acetonitrile/water (50/50, v/v), were used for calibration and quantification of BAS 750 F.
Limit of determination/quantification	The limit of quantification (LOQ) representing the lowest validated level with sufficient recovery and precision was 0.03 µg/L.
Standard stability	BAS 750 F was stable for a maximum duration of 30 days in stock and calibration solutions, when stored refrigerated at approximately 4°C in the dark. Stock solutions were prepared in acetonitrile, while calibration solutions were prepared in acetonitrile/water (50/50, v/v). BAS 750 F was stable in final water-sample extracts, prepared in acetonitrile/water, 50/50, v/v), over a time period of 7 days in case of surface water and 8 days in case of ground water, when stored refrigerated at approximately 4°C in the dark.

Conclusion

The method uses highly specific LC-MS/MS for final determination of mefentrifluconazole with a limit of quantitation of 0.03 µg/L. Thereby, it could be demonstrated that the method fulfils the requirements with regards to specificity, linearity, repeatability, limit of quantitation and recoveries.

A 2.1.2.4.1.2 Independent laboratory validation 1

Comments of zRMS:	The study is accepted. The purpose of this study was to independently validate the analytical method L0359/01 for the determination of mefentrifluconazole and its metabolites in surface and in drinking water, according to Technical Procedure L0359/01 (see the validation study). The target LOQ was 0.03 µg/L per analyte, using LC-MS/MS with two mass transitions: 398 m/z -> 70 m/z for mefentrifluconazole quantification and 400 m/z -> 70 m/z for mefentrifluconazole qualification. The ILV was performed at 2 fortification levels (0.03 and 0.3 µg/L), each with 5 replicates and 2 untreated control samples per matrix. Highly selective LC-MS/MS using two mass transition ions was applied per analyte. As a consequence of the high selectivity of the method (2 transitions monitored) no further confirmatory method is required. The matrices of drinking and surface water show no significant matrix interferences. The interferences in the control samples were found below 20 % of LOQ for each matrix and each mass transition. Calibration of at least 5 points was used. Good linearity (r > 0.993) was observed in the range of 0.03 ng/mL to 1.0

	ng/mL for both mass transitions of each analyte. The mean recovery values obtained at 2 fortification levels (each with at least 5 replicates and 2 untreated control samples) per matrix were between 87.2 % and 110 %. The RSD for both fortification levels were ≤ 13 %. No significant matrix effect was observed for each matrix and analyte. Mefentrifluconazole and its five metabolites indicated sufficient stability (less than 10 % difference) in stock solutions for 16 days as well as in solutions used for fortification and calibration (less than 20 % difference for mefentrifluconazole or less than 10 % difference for the five metabolites) when stored at ≤ 8°C in the dark. Final sample extracts stability can be considered sufficiently proven for at least 7, 11 or 15 days under refrigerated storage conditions (≤ 8°C). For additional details see the applicant study report below. Concluding, the method L0359/01 can be considered independently validated. This method fulfils the reproducibility requirements, and it can be applicable as an enforcement method. The study was already evaluated in PL.

Reference:	CP 5.2/2
Report	Independent laboratory validation (IVL) of method L0359/01 for the determination of BAS 750 F and its metabolites M750F005, M750F006, M750F007 and M750F008 in drinking water and surface water by LC-MS/MS Stanislowski, T., 2017 report No EU-836906,P 4262 G BASF DocID 2017/1066522
Guideline(s):	EPA 850.6100, EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 8.1 (16 November 2010)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)
Acceptability:	Yes

Please note that the ILV was performed for mefentrifluconazole and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008. Only results for parent are presented in the summary below as this is the only compound relevant for residue definition.

Materials and methods

There were no significant deviations from the primary method.

Results and discussions

Method validation acceptance criteria were fully met with mean recovery values between 70% and 110% in all matrices tested. The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%. Method validation data are summarised in the table below.

Table A 7: Recovery results from independent laboratory validation of mefentrifluconazole using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Drinking water	BAS 750 F	0.03 (n=5)	87.7	5.6	Quantitation m/z 398→70
		0.3 (n=5)	102	10	
		0.03 (n=5)	92.2	2.6	Confirmation m/z 400→70
		0.3 (n=5)	108	7.4	
Surface water	BAS 750 F	0.03 (n=5)	108	7.8	Quantitation m/z 398→70
		0.3 (n=5)	108	8.3	
		0.03 (n=5)	110	2.8	Confirmation m/z 400→70
		0.3 (n=5)	108	2.3	

Table A 8: Characteristics for the analytical method used for independent laboratory validation of mefentrifluconazole residues in water

	Mefentrifluconazole
Specificity	The method L0359/01 determines residues of mefentrifluconazole in water. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered.
Calibration (type, number of data points)	Calibration standards were prepared in acetonitrile/water (50:50, v/v). Six (or three injected in at least duplicate for storage stability determination) calibration points were used and individual calibration data was presented. Linear correlations with coefficients ≥0.99 were obtained.
Calibration range	Calibration points distributed over a concentration range of 0.03 to 1 ng/mL were used. This covers the tested concentration range.
Assessment of matrix effects is presented	The matrix effect was tested for each matrix. No significant matrix effect was observed.
Limit of determination/quantification	The limit of quantification (LOQ) representing the lowest validated level with sufficient recovery and precision was 0.03 µg/L.
Standard stability	BAS 750 F indicated sufficient stability (less than 10 % difference) in stock solution (acetonitrile) for 16 days as well as in acetonitrile/water (1/1, v/v) solutions used for fortification and calibration (<20% difference for BAS 750 F) when stored refrigerated in the dark. Final sample extracts in acetonitrile/water (1/1, v/v) were re-injected after 11 (for surface water) or 15 days (for drinking water) of storage under refrigerated conditions. No significant decrease (80.4-98.6% of initial value) or increase (101-114% of initial value) in recovery in the stored final extracts was observed when the results were evaluated with freshly prepared calibration solutions in solvent. Thus, stability of final extracts is considered sufficiently proven for at least 11 or 15 days under refrigerated storage conditions.

Conclusion

The method uses highly specific LC-MS/MS for final determination of mefentrifluconazole with a limit of quantitation of 0.03 µg/L. Thereby, it could be demonstrated that the method fulfils the requirements with regards to specificity, linearity, repeatability, limit of quantitation and recoveries. The method is acceptable as ILV for the primary method.

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

No new or additional studies have been submitted

A 2.1.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

Comments of zRMS:	The study has been accepted. The purpose of the study was to validate the analytical method for the determination of M750F015, M750F016 and M750F017 in body fluids using LC-MS/MS (BASF Method Number L0339/02). The method is highly sensitive and selective. Two characteristic mass transitions (quantification and confirmation transitions) were selected for each analyte. Untreated control samples were free from interference and residues above 30% of the LOQ for each analyte and each mass transition. The BASF Method L0339/02 is suitable to determine M750F015, M750F016 and M750F017 in body fluids at a LOQ of 0.010 mg/L. The mean recovery values were between 70% and 110% for both mass transitions for each analyte in all matrices tested. The relative standard deviations (RSD, %) for all fortification levels were below 20%. No significant matrix effects were observed for M750F015, M750F016 and M750F017 in any of the body fluid matrices tested.

Reference:	CP 5.2/3
Report	Validation of BASF Analytical Method L0339/02 for the determination of M750F015, M750F016 and M750F017 in body fluids, Homazava, N., 2019 report No EU-20180309,EU-867704,20180309 BASF DocID 2019/1046404 Authority registration No
Guideline(s):	EPA 860.1340 (1996), OECD-ENV/JM/MONO/(2007)17, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010)
Deviations:	No
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)
Acceptability:	Yes

Materials and methods

The analytical method L0339/02 was validated for the determination of M750F015, M750F016 and M750F017 (metabolites of BAS 750 F) in body fluids (bovine plasma and human urine) by LC-MS/MS.

Residues of M750F015, M750F016 and M750F017 are extracted from body fluids with acetonitrile. A salt mixture containing magnesium sulfate, sodium chloride and sodium citrate is added, and the extract is shaken. After centrifugation, an aliquot of the acetonitrile phase is cleaned up using primary secondary amine (PSA) and magnesium sulphate mixture. The final determination of M750F015, M750F016 and M750F017 is performed by LC-MS/MS, monitoring two mass transitions for each analyte in positive ion ESI mode. For quantification, the mass transition m/z 414→70 (M750F015, M750F016 and M750F017) is proposed and for confirmation, the mass transitions m/z 414→143 (M750F015 and M750F017) and m/z 414→182 (M750F016) are proposed. Analysis is accomplished on a Waters Acquity C18 BEH column (150 mm x 2.1 mm, 1.7 µm) applying a gradient mixture of water and acetonitrile with 0.1% formic acid as modifier at a flow rate of 0.4 mL/min.

Results and discussions

The results show that the method is suitable to determine residues of M750F015, M750F016 and M750F017 in body fluids. Samples were spiked with the analytes at the limit of quantification (0.01 mg/L) and 10x LOQ (0.1 mg/L). The overall recovery values (mean of five replicates per fortification level, matrix, analyte and mass transition) were between 70% and 110%. The detailed results are given in the table below.

Table A 9: Results of the method validation for the determination of M750F015, M750F016 and M750F017 in body fluids

Analyte	Matrix	m/z	Fortification level [mg metabolite/L]	Number of replicates	Mean recovery [%]	RSD [%]	Overall recovery [%]	Overall RSD [%]
M750F015	Plasma	414→70	0.010	5	94.7	1.7	92.2	3.2
			0.10	5	89.8	1.6		
		414→182	0.010	5	95.4	1.9	92.7	3.6
			0.10	5	90.0	2.1		
	Urine	414→70	0.010	5	99.5	2.8	97.1	3.6
			0.10	5	94.7	2.0		
		414→182	0.010	5	102	3.1	98.2	4.7
			0.10	5	94.6	2.5		
M750F016	Plasma	414→70	0.010	5	87.4	3.2	85.4	3.9
			0.10	5	83.4	3.3		
		414→182	0.010	5	86.8	2.7	85.2	3.5
			0.10	5	83.7	3.5		
	Urine	414→70	0.010	5	97.6	2.0	95.3	3.0
			0.10	5	93.1	1.7		
		414→182	0.010	5	100	3.9	96.2	5.0
			0.10	5	92.5	1.9		
M750F017	Plasma	414→70	0.010	5	91.5	1.3	89.7	2.8
			0.10	5	87.9	2.6		
		414→143	0.010	5	90.5	1.8	88.1	3.2
			0.10	5	85.8	1.6		
	Urine	414→70	0.010	5	98.8	2.0	95.9	3.6
			0.10	5	93.0	1.6		
		414→143	0.010	5	99.1	2.4	95.2	4.9
			0.10	5	91.3	2.4		

RSD = Relative standard deviation

Table A 10: Characteristics for the analytical method used M750F015, M750F016, M750F017 in body fluids

	M750F015, M750F016, M750F017
Specificity	The method L0359/02 determines residues of mefentrifluconazole metabolites in body fluids. Significant interferences (> 30% of LOQ) were not observed at the retention times and

	M750F015, M750F016, M750F017
	mass transitions considered. LC-MS/MS is a highly specific self-confirmatory technique. Under the described conditions the method is specific for the determination of M750F015, M750F016 and M750F017 in plasma and urine matrices.
Calibration (type, number of data points)	Good linearity of $r \geq 0.99$ was observed in the calibration range of 0.10 ng/mL to 10 ng/mL for all analytes. Seven calibration standards, prepared in acetonitrile/water (1/1, v/v), distributed over the tested concentration range were used. The LOQ falls within the calibration range determined. The following calibration curves were received: M750F015 $y= 7.48 \cdot 10^5x+1.7710^4$ (transition 414 → 70), R=0.9996 $y=5.79 \cdot 10^4x+980$ (transition 414 → 143), R=0.9996 M750F016: $y=7.37 \cdot 10^5x+1.97 \cdot 10^4$ (transition 414 → 70), R=0.9996 $y=8.15 \cdot 10^4x+2.32 \cdot 10^3$ (transition 414 → 182), R=0.9994 M750F017: $y=7.9 \cdot 10^5x+1.81 \cdot 10^4$ (transition 414 → 70), R=0.9997 $y=5.54 \cdot 10^4x+1.55 \cdot 10^3$ (transition 414 → 143), R=0.9996
Calibration range	Calibration points distributed over a concentration range of 0.10 ng/mL to 10 ng/mL were used. This covers the tested concentration range.
Assessment of matrix effects is presented	No significant matrix effects (i.e > ±20% signal suppression or signal enhancement) were observed for M750F015, M750F016 and M750F017 in any of the body fluid matrices tested. Therefore, solvent calibration standards were used for the quantification for all matrices.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.01 mg/L, corresponding to the lowest fortification level successfully tested. The limit of detection (LOD) is 0.10 ng/mL, corresponding to the lowest calibration standard.
Standard stability	Stability tests showed that M750F015, M750F016 and M750F017 stock, fortification and calibration solutions in acetonitrile and acetonitrile/water (1/1, v/v) were stable for 11 days, when stored refrigerated (2 – 8°C) in the dark. Raw extracts and final volume samples fortified at LOQ and 10x LOQ were shown to be stable for 8 days when stored refrigerated (2 – 8°C) in the dark for all body fluid matrices tested. Final volume samples were re-injected after 8 days of storage and raw extracts were carried through the complete work-up procedure and injected after 8 days of storage.

Conclusion

The method for analysis of M750F015, M750F016 and M750F017 in body fluids uses LC-MS/MS for final determination, which is a highly specific technique.
It could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of M750F015, M750F016 and M750F017 in body fluids.

A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted

A 2.2 Analytical methods for Metrafenone

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

New methods for the generation of pre-authorization data of metrafenone (BAS 560 F) have been submitted in the framework of this application and are summarized below.

A 2.2.1.1.1 Analytical Method 1

Comments of zRMS:	The analytical method has been accepted (the study is presented also in the section B7). The deep freeze stability of BAS 560 F in plant matrices was investigated over a period of two years. Samples were analysed with BASF method no. L0076/01 (also referred to as 535/1) which allows the quantitation of BAS 560 F residues to a limit of 0.01 mg/kg in different matrices. Procedural recoveries averaged at 93 %. The results are expressed as average percentage of the nominal fortification level. The sufficient metrafenone stability was shown in wheat (whole plant, grain and straw), grape, tomato, dried pea and soybean seeds.

Reference:	Cross Reference to KCA 6.1/1
Report	Investigation of the storage stability of BAS 560 F in plant matrices Lehmann A., Mackenroth C., 2012 BASF DocID 2012/1166088
Guideline(s):	EEC 7032/VI/97 rev. 5, EEC 1607/VI/97 rev. 2 10.06.1999, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EPA 860.1380
Deviations:	No assessment of matrix effects, extraction efficiency or stability of solutions / extracts
GLP:	Yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Materials and methods

The analytical method L0076/01 (also known as 535/1) was validated for the determination of metrafenone in plant matrices by HPLC-MS/MS.

Samples (5g) are weighed into wide neck bottles (250 mL) and methanol/water/2N HCL, 70:25:5, v/v/v (100 mL) is added. The samples are homogenized for 2 minutes at 5000 rpm, before an aliquot (10 mL) is centrifuged for 5 minutes at 4000 rpm. An aliquot (1 mL) of the supernatant is transferred to a culture tube (10 mL) containing 0.2 N NaOH (1 mL), cyclohexane (5 mL) is added and the samples are shaken for 15 minutes. An aliquot (2 mL) of the cyclohexane phase is transferred to a culture tube and evaporated to dryness using a nitrogen steam at 40°C. The residues are dissolved in methanol (0.5 mL) and water is added (0.5 mL). The samples are analyzed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive polarity mode, using a Betasil C18 column (100 mm x 2 mm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external standards. The ion transition m/z 411.0 > 209.0 is used for quantification and the ion transition m/z 411.0 > 229.0 is used for confirmation.

Results and discussions

The results show that the method is suitable to determine residues of metrafenone in plant matrices. Samples were spiked with the analyte at the limit of quantification (0.1 mg/kg). The overall recovery values (mean of 10 replicates per matrix, analyte and mass transition) were between 70% and 120%. The detailed results

are given in the table below.

Table A 11: Results of the method validation for the determination of metrafenone in plant matrices

Analyte	Matrix	m/z	Fortification level [mg/kg]	Number of Replicates	Mean Recovery [%]	RSD [%]
Metrafenone	Wheat whole plant	411→209	0.1	10	85.1	9.6
	Wheat grain	411→209	0.1	10	97.3	7.3
	Wheat straw	411→209	0.1	10	88.0	6.5
	Grape	411→209	0.1	10	89.8	8.8
	Tomato	411→209	0.1	10	90.3	9.0
	Dried peas	411→209	0.1	10	98.9	3.6
	Soybean	411→209	0.1	10	99.8	5.0
	Overall	411→209	0.1	10	93.2	9.0

Table A 12: Characteristics for the analytical method used for metrafenone in plant matrices

	Metrafenone
Specificity	LC-MS/MS monitoring multiple ion transitions. Retention time match with analytical standard. No significant interferences (> LOQ) were observed at the retention time of interest in the control matrices.
Calibration (type, number of data points)	Linear fit Correlation coefficient, r = 0.9995 Slope=1.65 x 10^5 Intercept= 1.17 x 10^3 n=6
Assessment of matrix effects is presented	No assessment of matrix effects was made.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.1 mg/kg, corresponding to the lowest fortification level successfully tested.
Standard and extract stability	No assessment of standard stability is presented

Conclusion

The study was performed according to the guidelines that were in force at the time. Determination of extraction efficiency and extract / standard stability are not reported, as the experimental work pre-dates SANTE/2020/12830 rev.1. The method satisfies the minimum validation requirements for an existing risk assessment method under SANTE/2020/12830 rev.1. The study complies with the data requirements given in Commission Regulation No 284/2013.

A 2.2.1.1.2 Analytical Method 2

Comments of zRMS:	The study has been accepted. The results of the study show that BASF method No. 535/0 is suitable to determine residues of metrafenone in plant matrices such as wheat whole plant, grain and straw, grape and lemon fruit and oilseed rape seeds. The LOQ was defined by the lowest fortification level successfully tested which was 0.01 mg /kg. The repetition of the HPLC -MS /MS measurement after storage of the final volume over 7 days lead to equivalent result. This proves that the analyte is stable over the time investigated under refrigerated and room temperature conditions. The standard solutions containing metrafenone in methanol /water (1 + 1, v/v) can

	be used over a time of 31 days if kept refrigerated.

Reference:	KCP 5.1/5
Report	Validation of the analytical method No. 535/0: Determination of Metrafenone BAS 560 F (Reg.No. 4037710) in plant matrices Benz A., Mackenroth C., 2005a BASF DocID 2004/1010553 Study Code: 135235
Guideline(s):	EPA 860.1340, EEC 96/46, SANCO/825/00 rev. 6 (20 June 2000), SANCO/3029/99 rev. 4 (11 July 2000)
Deviations:	No assessment of matrix effects, extraction efficiency or extract stability.
GLP:	Yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Materials and methods

The analytical method L0339/02 (also known as 535/0) was validated for the determination of metrafenone in plant matrices by HPLC-MS/MS.

Samples (5g) are weighed into wide neck bottles (250 mL) and methanol/water/2N HCL, 70:25:5, v/v/v (100 mL) is added. The samples are homogenized for 2 minutes at 5000 rpm, before an aliquot (10 mL) is centrifuged for 5 minutes at 4000 rpm. An aliquot (1 mL) of the supernatant is transferred to a culture tube (10 mL) containing 0.2 N NaOH (1 mL), cyclohexane (5 mL) is added and the samples are shaken for 15 minutes. An aliquot (2 mL) of the cyclohexane phase is transferred to a culture tube and evaporated to dryness using a nitrogen steam at 40°C. The residues are dissolved in methanol (0.5 mL) and water is added (0.5 mL). The samples are analyzed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive polarity mode, using a Betasil C18 column (100 mm x 2 mm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external standards. The ion transition m/z 411.0 > 209.0 is used for quantification and the ion transition m/z 411.0 > 229.0 is used for confirmation.

Results and discussions

The results show that the method is suitable to determine residues of metrafenone in plant matrices. Samples were spiked with the analytes at the limit of quantification (0.01 mg/kg) and 50 x LOQ (5.0 mg/kg). The overall recovery values (mean of five replicates per fortification level, matrix, analyte and mass transition) were between 70% and 120%. The detailed results are given in the table below.

Table A 13: Results of the method validation for the determination of metrafenone in plant ma trices

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Wheat whole plant	**Ion transition m/z 411 > 209 (quantification)**			
	0.01	98.0, 101.2, 93.3, 89.2, 83.8	93.1	7.4
	5.0	92.9, 91.9, 96.0, 92.0, 93.9	93.3	1.8
	Ion transition m/z 411 > 229 (confirmation)			
	0.01	94.7, 93.4, 92.0, 95.5, 94.3	94.0	1.4

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
	5.0	90.4, 94.0, 95.7, 93.8, 95.4	93.9	2.3
Wheat grain	**Ion transition m/z 411 > 209 (quantification)**			
	0.01	88.4, 86.4, 83.7, 86.0, 89.3	86.8	2.5
	0.1	85.0, 86.8, 84.9, 85.3, 83.0	85.0	1.6
	Ion transition m/z 411 > 229 (confirmation)			
	0.01	90.0, 90.8, 88.8, 87.4, 85.4	88.5	2.4
	0.1	84.1, 93.1, 90.8, 80.0, 74.4	84.5	9.1
Wheat straw	**Ion transition m/z 411 > 209 (quantification)**			
	0.01	87.1, 89.7, 90.3 91.5*, 90.0	89.3	1.6
	5.0	97.1, 93.3, 94.1, 93.2, 93.0	94.1	1.8
	Ion transition m/z 411 > 229 (confirmation)			
	0.01	94.4, 86.7, 86.4, 90.8, 90.5	89.5	4.2
	5.0	96.7, 96.2, 91.8, 87.6, 93.2	93.1	4.0
Grape, fruit	**Ion transition m/z 411 > 209 (quantification)**			
	0.01	91.7, 89.0, 89.1, 87.9, 86.5	88.8	2.1
	0.1	78.4, 81.7, 82.7, 84.2, 89.9	83.4	5.1
	Ion transition m/z 411 > 229 (confirmation)			
	0.01	82.8, 89.0, 89.5, 96.6, 86.7	88.9	5.7
	0.1	82.7, 76.1, 85.8, 79.2, 81.0	80.9	4.5
Lemon fruit	**Ion transition m/z 411 > 209 (quantification)**			
	0.01	88.1, 87.8, 87.7, 90.2, 83.7	87.5	2.7
	0.1	89.4, 90.1, 88.2, 89.8, 92.5	90.0	1.8
	Ion transition m/z 411 > 229 (confirmation)			
	0.01	81.9, 78.2, 88.5, 88.5, 82.2	83.9	5.4
	0.1	89.0, 88.8, 92.1, 88.5, 88.6	89.4	1.7
Oilseed, rape seed	**Ion transition m/z 411 > 209 (quantification)**			
	0.01	84.6, 85.8, 83.4, 83.5, 89.5	85.4	3.0
	0.1	83.4, 83.9, 82.8, 84.3, 86.3	84.1	1.6
	Ion transition m/z 411 > 229 (confirmation)			
	0.01	85.1, 81.7, 82.4, 88.4, 88.0	85.1	3.6
	0.1	79.4, 84.3, 85.5, 77.7, 80.9	81.6	4.0

Table A 14: Characteristics for the analytical method used for metrafenone (BAS 560 F) in plant matrices

	BAS 560 F
Specificity	LC-MS/MS monitoring multiple ion transitions. Retention time match with analytical standard. No significant interferences were observed at the retention time of interest in the control matrices.

	BAS 560 F
Calibration (type, number of data points)	Linear fit Correlation coefficient, r = 0.9995 Slope = 5960.6633 Intercept = 995.7479 n = 5
Calibration range	Calibration points distributed over a concentration range of 0.05 to 1.0 ng/mL were used. This covers the tested concentration range.
Assessment of matrix effects is presented	No assessment of matrix effects was made.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.01 mg/kg, corresponding to the lowest fortification level successfully tested. The limit of detection (LOD) is 0.05 ng/mL, corresponding to the lowest calibration standard.
Standard and extract stability	Stability tests showed that metrafenone stock solution was stable for 7 days, when stored refrigerated (4°C) in the dark. Fortification and calibration solutions in methanol / water (1/1, v/v) were stable for 31 days, when stored refrigerated (2 – 8°C) and over 8 days at room temperature.

Conclusion

The study was performed according to the guidelines that were in force at the time. Determination of matrix effects, extraction efficiency and extract stability are not reported, as the experimental work pre-dates SANTE/2020/12830 rev.1. The method satisfies the minimum validation requirements for an existing risk assessment method under SANTE/2020/12830 rev.1. The study complies with the data requirements given in Commission Regulation No 284/2013.

A 2.2.1.1.3 Analytical Method 3

Comments of zRMS:	The method has been accepted. The objective of the study analytical part was to perform dose verification of water samples from an ecotoxicology test with the test item BAS 758 00 F based on quantification of the active substances BAS 500 F, BAS 750 F and BAS 560 F. BASF LC-MS/MS Method L0361/01 with some adaptations and BASF analytical method number PTRL P3309 G with some adaptations were used for quantification purposes. The method has a LOQ of 0.328µg BAS500F/L, 0.270µg BAS 750 F/L and 0.384 µg BAS 560 F/L, corresponding to 4.42 µg BAS 758 00 F/L. Samples were fortified on two levels. 5 replicates were done. The recovery efficiency and repeatability were within the acceptable range of 70% to 110% of the intended concentrations for BAS 500 F, BAS 750 F and BAS 560 F, with relative standard deviations RSD < 20%. No significant peak interferences occurred at the retention time and mass transitions of BAS 500 F, BAS 750 F and BAS 560 F. No residues above the LOQ were found during analysis of untreated control samples. The suitability of the applied methodology was verified by means of procedural recoveries and the instrument performance was tested during each analytical run, using quality control samples. Additionally, the validity of analytical method L0361/01 for the analysis of BAS 750 F and BAS 500F and method number PTRL P3309 G for the analysis of BAS 560 F were proven by procedural recovery experiments performed within this study.

Reference:	Cross Reference to KCP 10.2.1/6
Report	BAS 758 00 F - Effect on *Daphnia magna* in a static 48-Hour Immobilization Test Eckenstein H., 2021 BASF DocID: 2020/2033902
Guideline(s):	SANCO/3029/99 Rev.4 (2000)
Deviations:	No determination of calibration solution and extract stability
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method PTRL P 3309 G was validated for the determination of BAS 560F in ISO medium by HPLC-MS/MS.

The samples (10 mL) are completely diluted with methanol (10 mL) and shaken mechanically. If necessary, aliquots of the samples are further diluted into the calibration range with 1/1, v/v, water/methanol and analyzed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive polarity mode, using a Thermo Betasil C18 column (100 mm x 2.1 mm , 5 µm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external standards. The ion transition m/z 409.0 → 209.0 is used for quantification and the ion transition m/z 409.0 → 227.0 is used for confirmation.

Results and discussions

The results show that the method is suitable to determine residues of metrafenone in water matrices. Samples were spiked with the analyte at the limit of quantification (0.384 µg/L) and a higher fortification level (73.2 µg/L). The mean recovery values (five replicates per fortification level) were between 70% and 120%. The detailed results are given in the table below.

Table A 15: Results of the method validation for the determination of metrafenone (BAS 560 F) in ISO Medium

Analyte	Matrix	m/z	Fortification Level [µg a.i./L]	Number of Replicates	Mean Recovery [%]	RSD [%]
Metrafenone	ISO medium	409 > 209	0.384	5	94.1	0.9
			73.2	5	96.2	1.1
			Overall	10	95.1	1.5

Table A 16: Characteristics for the analytical method used for metrafenone (BAS 560 F) in ISO Medium

	BAS 560 F
Specificity	LC-MS/MS monitoring multiple ion transitions. Retention time match with analytical standard. No significant interferences (>30% of LOQ) were observed at the retention time of interest in the control matrix.
Calibration (type, number of data points)	Linear fit Coefficient of determination (R^2) = 0.9995 Slope = 229895

	BAS 560 F
	Intercept = 2094 n = 5
Calibration range	Calibration points distributed over a concentration range of 0.126 µg/L to 5.03 µg/L were used. This covers the tested concentration range.
Assessment of matrix effects is presented	Potential matrix effects were addressed by the use of matrix matched calibration standards.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.384 µg/L in ISO medium, corresponding to the lowest fortification level successfully tested. The limit of detection (LOD) is 0.126 µg/L, corresponding to the lowest calibration standard.
Standard and extract stability	Not determined.

Conclusion

The study was performed according to the guidelines that were in force at the time. Determination of calibration solution and extract stability is not reported, as the experimental work pre-dates SANTE/2020/12830 rev.1. The method satisfies the minimum validation requirements for an existing risk assessment method under SANTE/2020/12830 rev.1. The study complies with the data requirements given in Commission Regulation No 284/2013.

A 2.2.1.1.4 Analytical Method 4

Comments of zRMS:	The method has been accepted. The objective of the study analytical part was to perform dose verification of water samples from of an ecotoxicology test with the test item BAS 758 00 F based on quantification of the active substances BAS 500 F, BAS 750 F and BAS 560 F. LC-MS/MS Method L0361/01 with some adaptations and BASF analytical method number PTRL P3309 G with some adaptations were used for quantification purposes. The methods have a LOQ of 0.116 µg BAS 500 F /L, 0.0954 µg BAS 750 F /L and 0.136 µg BAS 560 F /L, corresponding to 1.56 µg BAS 758 00 F/L. The fortifications were done on 2 levels with 5 replicates. Results of the procedural recovery experiments obtained during analysis showed that the recovery efficiency and repeatability was within the acceptable range of 70% to 110% of the intended concentrations for BAS 500 F, BAS 750 F and BAS 560 F, with relative standard deviations RSD < 20%. No significant peak interferences occurred at the retention time and mass transitions of BAS 500 F, BAS 750 F and BAS 560 F. No residues above the LOQ were found during analysis of untreated control samples. The suitability of the applied methodology was verified by means of procedural recoveries and the instrument performance was tested during each analytical run, using quality control samples. Additionally, the validity of analytical method L0361/01 for the analysis of BAS 750 F and BAS 500 F and method number PTRL P3309 G for the analysis of BAS 560 F were proven by procedural recovery experiments performed within this study.

Reference:	Cross Reference to KCP 10.2.1/4
Report	BAS 758 00 F – Acute Toxicity to Rainbow trout (*Oncorhynchus mykiss*) in a static 96-Hour Test Eckenstein H., 2021

	BASF DocID: 2020/2033900
Guideline(s):	SANCO/3029/99 Rev.4 (2000)
Deviations:	No determination of calibration solution and extract stability
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method PTRL P 3309 G was validated for the determination of BAS 560F in reconstituted water by HPLC-MS/MS.

The samples (10 mL) are completely diluted with methanol (10 mL) and shaken mechanically. If necessary, aliquots of the samples are further diluted into the calibration range with 1/1, v/v, water/methanol and analyzed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive polarity mode, using a Thermo Betasil C18 column (100 mm x 2.1 mm , 5 µm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external standards. The ion transition m/z 409.0 → 209.0 is used for quantification and the ion transition m/z 409.0 → 227.0 is used for confirmation.

Results and discussions

The results show that the method is suitable to determine residues of metrafenone in reconstituted water. Samples were spiked with the analyte at the limit of quantification (0.433 µg/L) and a higher fortification level (528 µg/L). The mean recovery values (five replicates per fortification level) were between 70% and 120%. The detailed results are given in the table below.

Table A 17: Results of the method validation for the determination of metrafenone (BAS 560 F) in reconstituted water

Analyte	Matrix	m/z	Fortification Level [µg a.i./L]	Number of Replicates	Mean Recovery [%]	RSD [%]
Metrafenone	Reconstituted water	409 > 209	0.136	5	95.2	8.4
			26.6	5	92.3	0.9
			Overall	10	93.7	5.9

Table A 18: Characteristics for the analytical method used for metrafenone (BAS 560 F) in reconstituted water

	BAS 560 F
Specificity	LC-MS/MS monitoring multiple ion transitions. Retention time match with analytical standard. No significant interferences (>30% of LOQ) were observed at the retention time of interest in the control matrix.
Calibration (type, number of data points)	Linear fit Coefficient of determination (R^2) = 0.9910 Slope = 487989 Intercept = 3253 n = 5
Calibration range	Calibration points distributed over a concentration range of 0.251 µg/L to 5.02 µg/L were used. This covers the tested

	BAS 560 F
	concentration range.
Assessment of matrix effects is presented	Potential matrix effects were addressed by the use of matrix matched calibration standards.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.136 µg/L for reconstituted water, corresponding to the lowest fortification level successfully tested. The limit of detection (LOD) is 0.0251 µg/L, corresponding to the lowest calibration standard.
Standard and extract stability	Not determined.

Conclusion

The study was performed according to the guidelines that were in force at the time. Determination of calibration solution and extract stability is not reported, as the experimental work pre-dates SANTE/2020/12830 rev.1. The method satisfies the minimum validation requirements for an existing risk assessment method under SANTE/2020/12830 rev.1. The study complies with the data requirements given in Commission Regulation No 284/2013.

A 2.2.1.1.5 Analytical Method 5

Comments of zRMS:	The method has been accepted. The objective of the study analytical part was to perform dose verification of water samples from an ecotoxicology test with the test item BAS 758 00 F based on quantification of the active substances BAS 500 F, BAS 750 F and BAS 560 F. LC-MS/MS BASF Method L0361/01 with some adaptations and BASF analytical method number PTRL P3309 G with some adaptations were used for quantification purposes. The methods have a LOQ of 0.370µg BAS500F/L, 0.305µg BAS 750 F /L and 0.433 µg BAS 560 F /L, corresponding to 0.00499 mg BAS 758 00 F/L. The fortifications were done on 2 levels with 5 replicates. Results of the procedural recovery experiments obtained during analysis showed that the recovery efficiency and repeatability was within the acceptable range of 70% to 110% of the intended concentrations for BAS 500 F, BAS 750 F and BAS 560 F, with relative standard deviations RSD < 20%. No significant peak interferences occurred at the retention time and mass transitions of BAS 500 F, BAS 750 F and BAS 560 F. No residues above the LOQ were found during analysis of untreated control samples. The validity of the analytical method L0361/01 for the analysis of BAS 750 F and BAS 500 F and method number PTRL P3309 G for the analysis of BAS 560 F were proven by procedural recovery experiments performed within this study. The method is suitable for the intended purposes.

Reference:	Cross Reference to KCP 10.2.1/8
Report	BAS 758 00 F - Effect on *Pseudokirchneriella subcapitata* in a 72 Hour Algal Growth Inhibition Test Eckenstein H., 2021 BASF DocID: 2020/2033904
Guideline(s):	SANCO/3029/99 Rev.4 (2000)
Deviations:	No determination of calibration solution and extract stability

GLP: Yes

Acceptability: Yes

Materials and methods

The analytical method PTRL P 3309 G was validated for the determination of BAS 560F in AAP medium by HPLC-MS/MS.

The samples (10 mL) are completely diluted with methanol (10 mL) and shaken mechanically. Test samples from day 3 are additionally centrifuged due to the presence of the algae (5 min, 2465g) in addition to an aliquot from one sample of each spike level. If necessary, aliquots of the samples are further diluted into the calibration range with 1/1, v/v, water/methanol and analyzed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive polarity mode, using a Thermo Betasil C18 column (100 mm x 2.1 mm , 5 µm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external standards. The ion transition m/z 409.0 → 209.0 is used for quantification and the ion transition m/z 409.0 → 227.0 is used for confirmation.

Results and discussions

The results show that the method is suitable to determine residues of metrafenone in AAP medium. Samples were spiked with the analyte at the limit of quantification (0.328 µg/L, 0.433 µg/L and 0.136 µg/L in *Daphnia magna, Pseudokirchneriella subcapitata* and *Oncorhynchus mykiss* test medium respectively) and a higher fortification level (62.5 µg/L, 528 µg/L and 26.6 µg/L respectively). The overall recovery values (mean of five replicates per fortification level, matrix, analyte and mass transition) were between 70% and 110%. The detailed results are given in the table below.

Table A 19: Results of the method validation for the determination of metrafenone (BAS 560 F) in AAP Medium

Analyte	Matrix	m/z	Fortification Level [µg a.i./L]	Number of Replicates	Mean Recovery [%]	RSD [%]
Metrafenone	AAP medium	409 > 209	0.433	5	99.4	1.3
			528	5	113	2.5
			Overall	10	106	7.0

Table A 20: Characteristics for the analytical method used for metrafenone (BAS 560 F) in AAP medium

	BAS 560 F
Specificity	LC-MS/MS monitoring multiple ion transitions. Retention time match with analytical standard. No significant interferences (>30% of LOQ) were observed at the retention time of interest in the control matrix.
Calibration (type, number of data points)	Linear fit Coefficient of determination (R2) = 0.9999 Slope = 178929 Intercept = 7924 n = 5
Calibration range	Calibration points distributed over a concentration range of 0.252 µg/L to 5.04 µg/L were used. This covers the tested concentration range.

	BAS 560 F
Assessment of matrix effects is presented	Potential matrix effects were addressed by the use of matrix matched calibration standards.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.433 µg/L for AAP medium, corresponding to the lowest fortification level successfully tested. The limit of detection (LOD) is 0.0252 µg/L, corresponding to the lowest calibration standard.
Standard and extract stability	Not determined.

Conclusion

The study was performed according to the guidelines that were in force at the time. Determination of calibration solution and extract stability is not reported, as the experimental work pre-dates SANTE/2020/12830 rev.1. The method satisfies the minimum validation requirements for an existing risk assessment method under SANTE/2020/12830 rev.1. The study complies with the data requirements given in Commission Regulation No 284/2013.

A 2.2.1.1.6 Analytical Method 6

Comments of zRMS:	The analytical method has been accepted. (See also page 80) The concentration of Metrafenone, Mefentrifluconazole, and Pyraclostrobin in the highest and lowest test item feeding solution applied on the first and last day of application (D 0 and D 9) was determined with the LC-MS/MS method no. L0452/02 within the study. Results for the concurrent procedural recovery samples showed values in an acceptable range with overall mean recoveries of 74.3 % to 96.6 % for the three analytes at both fortification levels with RSD values ≤8.4 %. These results fulfill the requirements according to current guidelines; therefore, the suitability of the applied methodology is proven. The analytical results of concentration control samples for BAS 758 00 F in bee feeding solution confirmed its nominal concentrations of 204 mg /kg and 3261 mg /kg, with recoveries of 90.9 % and 99.8 % for day 0 samples, and 82.4 % and 88.4 % for day 9 samples when determined via the active ingredient BAS 750 F. The concentrations were also confirmed via the active ingredient BAS 560 F with recoveries of 82.7% and 101% for day 0 samples, and 81.1% and 89.5 % for day 9 samples. The concentrations were also confirmed via the active ingredient BAS 500 F with recoveries of 88.3 % and 104 % for day 0 samples, and 94.8 % and 85.1 % for day 9 samples. The mean measured concentrations of untreated control were always below the LOQ for all sampling intervals. No significant peak interferences occurred at the retention times and mass transitions of all analytes. Furthermore, no residues of the active ingredients were found in the control samples, i.e., the concentrations of active ingredients were below 30% of the LOQ.

Reference:	Cross Reference to KCP 10.3.1.2/4
Report	Chronic toxicity of BAS 758 00 F to the honey bee Apis mellifera L. under laboratory conditions. Dreßler K., 2021 BASF DocID: 2021/2008152
Guideline(s):	SANTE/2020/12830, Rev.1, 24 Feb 2021, OECD Guidance Document ENV/JM/MONO(2007)17

Deviations:	No determination of calibration solution and extract stability
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method was validated for the determination of BAS 560F in 50% sucrose solution by LC-MS/MS.

50% sucrose solution samples (0.25g) are extracted with acetonitrile/water/formic acid (50/50/1, v/v/v). Samples are shaken for 15 minutes at 300 rpm and a portion of the BEKOlut-citrate-kit1/5 (QuEChERS-Salt) is added and the solution is shaken immediately by hand and vortexed for at least 30 seconds and centrifugation for 5 minutes at 4000 rpm. An aliquot of the acetonitrile upper phase (1.0mL) is taken and diluted with acetonitrile/water/formic acid (50/50/1, v/v/v) to a final volume of 10 mL.

The samples are analysed by liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive polarity mode, using a Restek Pinnacle DB AQ C18 (50 mm x 2.1 mm , 1.9 µm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Quantification is performed using external standards. The ion transition m/z 411 → 209 is used for quantification and the ion transition m/z 411 → 229 is used for confirmation.

Results and discussions

The results show that the method is suitable to determine residues of metrafenone in bee diet matrices. Samples were spiked with the analytes at the limit of quantification (0.1 mg/kg) and a higher fortification level (400 mg/kg). The mean recovery values (five replicates per fortification level) were between 70% and 120%. The detailed results are given in the table below.

Table A 21: Results of the method validation for the determination of metrafenone (BAS 560 F) in 50% sucrose solution

Analyte	Matrix	m/z	Fortification Level [mg/kg]	Number of Replicates	Mean Recovery [%]	RSD [%]
Metrafenone	50% sucrose solution	411 > 209	0.10	5	96.6	6.0
			400	5	85.9	6.2
			Overall	10	91.3	8.4

Table A 22: Characteristics for the analytical method used for metrafenone (BAS 560 F) in 50% sucrose solution

	BAS 560 F
Specificity	LC-MS/MS monitoring multiple ion transitions. Retention time match with analytical standard. No significant interferences (>30% of LOQ) were observed at the retention time of interest in the control matrix.
Calibration (type, number of data points)	Linear fit Coefficient of determination (R^2) = 0.9999 Slope = 2.35×10^6 Intercept = 2550 n = 7
Calibration range	Calibration points distributed over a concentration range of 0.01 ng/mL to 1.0 ng/mL (corresponding to 0.008 mg/kg to

	BAS 560 F
	0.8 mg/kg) were used for the bee diet calibration solutions. This covers the tested concentration ranges.
Assessment of matrix effects is presented	Potential matrix effects were addressed by the use of matrix matched calibration standards.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.10 mg/kg in 50% sucrose solution, corresponding to the lowest fortification level successfully tested. The limit of detection (LOD) is 0.01 ng/mL, corresponding to the lowest calibration standard.
Standard and extract stability	Not determined.

Conclusion

The study was performed according to the guidelines that were in force at the time. Determination of calibration solution and extract stability is not reported. The method satisfies the minimum validation requirements for a risk assessment methods under SANTE/2020/12830 rev.1. The study complies with the data requirements given in Commission Regulation No 284/2013.

A 2.2.1.1.7 Analytical Method 7

Comments of zRMS:	The analytical study has been accepted. The objective of the study was to validate the QuEChERS/ LC-MS/MS method L0372/05 for the determination of BAS 560 F (Metrafenone) in flowers, nectar, and pollen. Fortification levels of 0.010 mg/kg (LOQ) and 0.50 mg/kg (50 x LOQ) were validated. For each fortification level and matrix, five replicates were prepared and analysed. Additionally, at least two replicates of unfortified samples per matrix and one reagent blank were analysed in one set. Two mass transitions were evaluated for quantitation and confirmation for the analyte and each matrix. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered. Good linearity ($r \geq 0.998$) for BAS 560 F was observed. The obtained mean recovery values were: 409 m/z → 209 m/z between 85.4% and 99.3% with RSDs equal or below 6.1% 409 m/z → 227 m/z between 86.4% and 102% with RSDs equal or below 6.5% for all fortification levels and matrices. The method L0372/05 is suitable for the determination of BAS 560 F in bee-relevant matrices at a LOQ of 0.010 mg/kg.

Reference:	KCP 5.1/6
Report	Validation of analytical method L0372/05 for the determination of BAS 560 F in bee matrices Schneider J.Q. & Link, T., 2022 BASF DocID: 2021/2052011
Guideline(s):	SANTE/2020/12830, Rev.1, 24 Feb 2021, OECD Guidance Document ENV/JM/MONO(2007)17
Deviations:	No
GLP:	Yes

Acceptability: Yes

Materials and methods

The analytical method was validated for the determination of BAS 560F in flowers, nectar and pollen matrices by LC-MS/MS.
Samples (0.2g) are weighed into a 15 mL centrifuge tube and fortified as appropriate for procedural recoveries. Methanol and water (5mL 75/25, v/v) are added and the solution is shaken for 30 minutes at 300 rpm on a mechanical shaker and then centrifuged for 5 minutes at 4000 rpm. The supernatant is decanted into a 10 mL volumetric flask and filled up to the mark with Methanol/water mixture (75/25 v/v). In the case of flower and honey matrices the extraction is repeated one additional time and the extracts are filled up to mark with methanol water mixture (75/25 v/v). A 1 mL aliquot of this is transferred into a QuEChERS dSPE 2 mL tube and vortexed for 30 seconds and then centrifuged for 5 minutes at 10,000 rpm. 400 µL of the aliquot is transferred to the sample vial and diluted with 600 µL of methanol water mixture. The samples are analysed by liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive polarity mode, using a Phenomenex Synergi Fusion-RP 80Å (50 mm x 2 mm , 4 µm) and gradient elution with mobile phases of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external standards. The ion transition m/z 409 → 209 is used for quantification and the ion transition m/z 409 → 227 is used for confirmation.

Results and discussions

The results show that the method is suitable to determine residues of metrafenone in flowers, nectar and pollen matrices. Samples were spiked with the analyte at the limit of quantification (0.01 mg/kg) and a higher fortification level (0.5 mg/kg). The mean recovery values (five replicates per fortification level) were between 70% and 120%. The detailed results are given in the table below.

Table A 23: Results of the method validation for the determination of metrafenone (BAS 560 F) in bee matrices

Analyte	Matrix	m/z	Fortification Level [mg/kg]	Number of Replicates	Mean Recovery [%]	RSD [%]
Metrafenone	Flowers	409 > 209	0.01	5	99.3	2.3
			0.5	5	96.6	1.2
			Overall	10	97.9	2.3
		409 > 227	0.01	5	102	2.3
			0.5	5	96.5	1.6
			Overall	10	99.0	3.3
	Nectar	409 > 209	0.01	5	97.0	3.0
			0.5	5	86.6	1.7
			Overall	10	92.4	6.4
		409 > 227	0.01	5	92.3	3.1
			0.5	5	86.4	1.9
			Overall	10	89.7	4.3
	Pollen	409 > 209	0.01	5	85.4	3.2
			0.5	5	89.4	6.1
			Overall	10	87.4	5.3
		409 > 227	0.01	5	95.7	5.2
			0.5	5	88.8	6.5
			Overall	10	92.2	6.7

Table A 24: Characteristics for the analytical method used for metrafenone (BAS 560 F) in bee

matrices

	BAS 560 F
Specificity	LC-MS/MS monitoring multiple ion transitions. Retention time match with analytical standard. No significant interferences (>30% of LOQ) were observed at the retention time of interest in the control matrices.
Calibration (type, number of data points)	Linear fit 409 > 209: Coefficient of determination (R^2) = 0.9985 Slope = 1222910 Intercept = -6566 n = 9 409 > 227: Coefficient of determination (R^2) = 0.9989 Slope = 729363 Intercept = -2861 n = 9
Calibration range	Calibration points distributed over a concentration range of 0.02 ng/mL to 3.0 ng/mL (0.0025 mg/kg to 0.375 mg/kg) were used. This covers the tested concentration range.
Assessment of matrix effects is presented	Solvent and matrix-matched standards were analysed to assess potential matrix effects for BAS 560 F in flowers, nectar and pollen. Investigations on matrix effects were performed at three different concentration levels. No significant matrix effect was observed for any of the matrices (matrix effect <20%). Therefore, solvent standards were used for calibration purposes.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.01 mg/kg in flowers, nectar and pollen, corresponding to the lowest fortification level successfully tested. The limit of detection (LOD) is 0.020 ng/mL, corresponding to the lowest calibration standard.
Standard and extract stability	Recovery data was used to prove the stability of the test item in stock/fortification solutions prepared in MeOH and calibration solutions preprared in MeOH/H2O (75/25, v/v). The mean recovery value of stock/fortification solutions was 96.1% and the mean recovery values of calibration solutions were in an acceptable range between 94.3-99.8%. This demonstrates that BAS 560 F is stable in MeOH and MeOH/H2O (75/25, v/v) for 30 days, when stored at 2-8°C in the dark. For extracts and final volumes, the stability tests confirmed that the analytes were stable for at least 7 days in all bee-related matrices, when stored at 2-8°C in the dark. The mean recoveries were in the range from 93.1 – 95.9% for flowers, 91.5 – 107% for nectar and 80.4 – 103% for pollen.

Conclusion

The study was performed according to the guidelines that were in force at the time. The method is fully validated in accordance with all of the requirements of SANTE/2020/12830 rev.1. The study complies with the data requirements given in Commission Regulation No 284/2013.

A 2.2.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

No new studies have been submitted.

A 2.3 Analytical methods for Pyraclostrobin

A 2.3.1 Methods used for the generation of pre-authorization data (KCP 5.1)

New methods for the generation of pre-authorization data of BAS 500 F and its metabolites have been submitted in the framework of this application and are summarized below.

A 2.3.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

A 2.3.1.1.1 Analytical method 445/0 for the determination of pyraclostrobin and its metabolite 500M07 in plant matrices

A 2.3.1.1.1.1 Method validation 1

Comments of zRMS:	The study is accepted. The purpose of this study was to validate the analytical BASF method No. 445/0 for the determination of pyraclostrobin (BAS 500 F) and its metabolite BF 500-3 (aka 500M07) in apple, sour cherry, grapes, strawberry, carrot, onion, tomato, broccoli, white cabbage, leek, dwarf bean, oilseed rape and wheat plant without roots, wheat grain and straw according to Technical Procedure (BASF DOC ID 2000\1014864; Method for the determination of BAS 480 F, BAS 490 F, BAS 500 F, BAS 505 F and BAS 510 F in plant matrices – BASF Method Number 445/0). The procedure points the transitions 388 → 194 and 388 → 163 as suitable for pyraclostrobin and 358 →164 and 358 → 132 for BF 500-3, however in the validation study, only the transition 388 → 194 for pyraclostrobin and 358 → 164 for BF 500-3 are reported. In turn, for the same method (the next study BASF DocID 2005/1037978) in coffee grain, soya bean and wheat grain both mass transitions are validated. However, it is minor concern. The present validation was performed at 2 fortification levels, each in quintuplicates (except OSR at 0,02) and 2 untreated control samples per matrix. Interferences > 30% of LOQ were not observed at the retention times for the transition monitored for each analyte. For calibration 6 points were used. Good linearity ((r) ≥0.99) were obtained for pyraclostrobin and BF 500-3. The LOQ was set at 0.02 mg/kg in all matrices. The study shows that mean recovery values as well as the RSD at both fortification levels were in acceptable range for all matrices and both analytes for one transition. The applicant claims also that the stability of the analytes is sufficient (see the end of the below study summary written by the applicant). The method with regard to specificity, linearity, repeatability, limit of quantitation and recoveries fulfils the requirements of generation of pre-authorisation data. The method is accepted. The study was already evaluated in PL.

Reference:	CP 5.2/4
Report	Validation of BASF Method No. 445/0: Determination of BAS 500 F and BF 500-3 in various plant matrices, Benz A.,Mackenroth C., 2001 report No 78593

	2000/1012405
Guideline(s):	EPA 860.1340, EEC 96/46, Guidance Document of Residue Analytical Methods 8064/VI/97 rev. 4 15.12.1998
Deviations:	No
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)
Acceptability:	Yes

Materials and methods

Residues of pyraclostrobin (BAS 500 F) and its metabolite 500M07 are extracted from plant matrices with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned against cyclohexane. After evaporation to dryness residues are dissolved in mobile phase for determination. The final determination of pyraclostrobin and its metabolite 500M07 is performed by HPLC-MS/MS. Analysis was accomplished using a Betasil C18 column and a methanol-water gradient with formic acid as modifier at a flow rate of 600 µL/min. Samples were analysed at mass transition 388 → 194 for quantitation and 388 → 163 for confirmation for pyraclostrobin and for 500M07 at mass transition 358 → 164 for quantitation and 358 → 132 for confirmation.

Results and discussions

Method validation acceptance criteria were fully met with mean recovery values between 70% and 110% in all matrices tested. The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%. Method validation data are summarised in the table below.

Table A 25: Recovery results from method validation of pyraclostrobin and its metabolite 500M07 using the analytical method 445/0

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Apple, fruit	BAS 500 F	0.02 (n=5)	92.0	4.8	Quantitation
		0.2 (n=5)	77.1	3.0	m/z 388→194
	500M07	0.02 (n=5)	94.4	6.0	Quantitation
		0.2 (n=5)	83.8	7.1	m/z 358→164
Sour cherry, fruit	BAS 500 F	0.02 (n=5)	90.6	8.9	Quantitation
		0.2 (n=5)	86.6	3.2	m/z 388→194
	500M07	0.02 (n=5)	84.7	2.8	Quantitation
		0.2 (n=5)	83.4	4.3	m/z 358→164
Grapes, fruit	BAS 500 F	0.02 (n=5)	97.5	5.6	Quantitation
		1.0 (n=5)	87.3	3.6	m/z 388→194
	500M07	0.02 (n=5)	97.5	8.9	Quantitation
		1.0 (n=5)	86.2	3.6	m/z 358→164
Strawberry, fruit	BAS 500 F	0.02 (n=5)	99.6	5.7	Quantitation
		0.2 (n=5)	103.1	9.6	m/z 388→194

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
	500M07	0.02 (n=5)	102.9	2.7	Quantitation
		0.2 (n=5)	113.4	10.5	m/z 358→164
Carrot, root	BAS 500 F	0.02 (n=5)	93.9	2.5	Quantitation
		0.2 (n=5)	89.8	6.1	m/z 388→194
	500M07	0.02 (n=5)	95.6	7.5	Quantitation
		0.2 (n=5)	81.1	4.3	m/z 358→164
Onion, bulb	BAS 500 F	0.02 (n=5)	87.5	3.1	Quantitation
		0.2 (n=5)	93.7	3.5	m/z 388→194
	500M07	0.02 (n=5)	75.2	10.0	Quantitation
		0.2 (n=5)	87.0	5.7	m/z 358→164
Tomato, fruit	BAS 500 F	0.02 (n=5)	96.6	12.1	Quantitation
		0.2 (n=5)	90.8	4.4	m/z 388→194
	500M07	0.02 (n=5)	96.4	14.7	Quantitation
		0.2 (n=5)	84.7	2.2	m/z 358→164
Broccoli, plant without root	BAS 500 F	0.02 (n=5)	91.6	17.9	Quantitation
		0.2 (n=5)	91.0	4.0	m/z 388→194
	500M07	0.02 (n=5)	100.5	17.4	Quantitation
		0.2 (n=5)	94.3	3.0	m/z 358→164
White cabbage, head	BAS 500 F	0.02 (n=5)	91.0	7.5	Quantitation
		0.2 (n=5)	82.9	2.5	m/z 388→194
	500M07	0.02 (n=5)	89.5	8.0	Quantitation
		0.2 (n=5)	70.2	7.6	m/z 358→164
Leek, plant without root	BAS 500 F	0.02 (n=5)	103.3	6.8	Quantitation
		1.0 (n=5)	93.7	3.5	m/z 388→194
	500M07	0.02 (n=5)	96.1	3.5	Quantitation
		1.0 (n=5)	93.0	1.7	m/z 358→164
Dwarf beans, pods with beans	BAS 500 F	0.02 (n=5)	97.4	12.0	Quantitation
		0.2 (n=5)	90.9	7.9	m/z 388→194
	500M07	0.02 (n=5)	94.1	8.0	Quantitation
		0.2 (n=5)	90.0	5.0	m/z 358→164
Oilrape, seed	BAS 500 F	0.02 (n=4)	84.9	17.6	Quantitation
		0.2 (n=5)	89.2	7.4	m/z 388→194
	500M07	0.02 (n=4)	89.9	17.4	Quantitation
		0.2 (n=5)	76.9	3.7	m/z 358→164
Wheat, plant without root	BAS 500 F	0.02 (n=5)	90.5	8.9	Quantitation
		5.0 (n=5)	79.2	11.4	m/z 388→194
	500M07	0.02 (n=5)	101.9	8.2	Quantitation

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
		5.0 (n=5)	78.4	9.8	m/z 358→164
Wheat, grain	BAS 500 F	0.02 (n=5)	78.2	10.4	Quantitation
		0.2 (n=5)	86.7	4.0	m/z 388→194
	500M07	0.02 (n=5)	81.7	14.7	Quantitation
		0.2 (n=5)	92.4	2.6	m/z 358→164
Wheat, straw	BAS 500 F	0.02 (n=5)	97.8	8.1	Quantitation
		5.0 (n=5)	95.2	7.0	m/z 388→194
	500M07	0.02 (n=5)	95.1	8.2	Quantitation
		5.0 (n=5)	90.7	9.0	m/z 358→164

Table A 26: Characteristics for the analytical method used for validation of pyraclostrobin residues and its metabolite 500M07 in plant matrices

	Pyraclostrobin and 500M07
Specificity	The method 445/0 determines residues of pyraclostrobin and its metabolites 500M07 plant matrices. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered for each analyte. According the technical procedure, a second mass transition for pyraclostrobin and 500M07 is suitable, but in this study only the quantitative mass transitions were measured.
Calibration (type, number of data points)	Calibration standards were prepared in methanol / water (80/20, v/v). Six calibration points were used and individual calibration data was presented. Linear correlations with coefficients ≥0.99 were obtained for pyraclostrobin and its metabolites 500M07.
Calibration range	Calibration points distributed over a concentration range of 0.10 to 10 ng/ml were used.
Assessment of matrix effects is presented	No matrix effects were investigated within an independent experiment, therefore instrument recovery sampels would be implemented within each analytical series, if no further information on matrix effects of the investigated matrix is available. Alternatively matrix-matched standards would be used.
Limit of determination/quantification	The limit of quantification (LOQ) representing the lowest validated level with sufficient recovery and precision was 0.02 mg/kg.
Standard stability	In another study pyraclostrobin and 500M07 are shown to be stable in methanol and in the HPLC solvent over a time interval of 60 days, if stored refrigerated.

Conclusion

The method uses highly specific LC-MS/MS for final determination of pyraclostrobin and its metabolite 500M07 with a limit of quantitation of 0.02 mg/kg. Thereby, it could be demonstrated that the method

fulfils the requirements with regard to specificity, linearity, repeatability, limit of quantitation and recoveries.

A 2.3.1.1.1.2 Confirmatory method

According to the technical procedure, a second mass transition for pyraclostrobin and 500M07 is suitable, but in the study BASF DocID 2000/1012405 only the quantitative mass transitions were measured. In the validation of the same method (445/0) for coffee grain, soya bean and wheat grain both mass transitions are validated (BASF DocID 2005/1037978).

A 2.3.1.1.1.3 Extraction efficiency

There are no new study on extraction efficiency of the method for the determination of residues in plant matrices. For more information see section 5.3.4.2.

A 2.3.1.1.1.4 Method validation 2

Comments of zRMS:	The study is accepted. The purpose of this study was to validate the analytical BASF method No. 445/0 according to analytical procedure SOP-PA.0002 for the determination of pyraclostrobin (BAS 500 F) and its metabolite BF 500-3 (aka 500M07) in coffee (grain), soybean (grain) and wheat (grain). The transitions 388 → 194, 388 → 163 pointed out as suitable for pyraclostrobin and 358 →164, 358 → 132 pointed out for BF 500-3 were monitored in this study. No confirmatory method is needed. For each matrix 2 fortification levels with 5 replicates and 2 blanks were applied. No interferences ≥ 30% of the LOQ were found in the range of the retention time of the analytes. 5 points for calibration were used. The working range of the method was considered linear with a correlation coefficient (r) ≥ 0,99. Obtained recoveries ranged 70 – 110% on each fortification level with RSD < 20% for both transitions. The LOQ of the method was set at 0,02 mg/kg in coffee grain, soybean grain and wheat grain. It can be concluded that the method is validated for determination of pyraclostrobin and BF 500-3 in coffee grain, soya bean grain and wheat grain consistently with SANCO/3029/99 rev.4 requirements. The study was already evaluated in PL.

Reference:	CP 5.2/5
Report	Validation study of the SOP-PA.0243 for determination of Pyraclostrobin and its metabolite (BF 500-3) residues in coffee (grain), soybean (grain) and wheat (grain), Leite R., 2005 report No 216583 2005/1037978
Guideline(s):	NBR ISO/IEC 17025/2001
Deviations:	No
GLP:	yes (certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)
Acceptability:	Yes

Materials and methods

Residues of pyraclostrobin (BAS 500 F) are extracted from plant matrices with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned against cyclohexane. After evaporation to dryness residues are dissolved in mobile phase for determination. The final determination of pyraclostrobin and its metabolite 500M07 (BF 500-3) is performed by HPLC-MS/MS. Analysis was accomplished using a Betasil C18 column and a methanol-water gradient with formic acid as modifier at a flow rate of 600 µL/min. Samples were analysed at mass transition 388 → 194 for quantitation and 388 → 163 for confirmation for pyraclostrobin and for 500M07 at mass transition 358 → 164 for quantitation and 358 → 132 for confirmation.

Results and discussions

Method validation acceptance criteria were fully met with mean recovery values between 70% and 110% in all matrices tested. The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%. Method validation data are summarised in the table below.

Table A 27: Recovery results from method validation of BAS 500 F and its metabolite 500M07 using the analytical method 445/0 (SOP-PA.0243)

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Coffee, grain	BAS 500 F	0.02 (n=5)	95	6.4	Quantitation
		2.0 (n=5)	106	2.1	m/z 388→194
		0.02 (n=5)	100	7.1	Confirmation
		2.0 (n=5)	108	4.1	m/z 388→163
	500M07	0.02 (n=5)	89	2.5	Quantitation
		2.0 (n=5)	102	2.7	m/z 358→164
		0.02 (n=5)	91	4.6	Confirmation
		2.0 (n=5)	101	4.1	m/z 358→132
Soya bean, grain	BAS 500 F	0.02 (n=5)	86	6.4	Quantitation
		2.0 (n=5)	97	2.8	m/z 388→194
		0.02 (n=5)	88	6.5	Confirmation
		2.0 (n=5)	96	2.3	m/z 388→163
	500M07	0.02 (n=5)	82	7.0	Quantitation
		2.0 (n=5)	88	3.1	m/z 358→164
		0.02 (n=5)	80	6.3	Confirmation
		2.0 (n=5)	89	2.5	m/z 358→132
Wheat, grain	BAS 500 F	0.02 (n=5)	96	9.3	Quantitation
		2.0 (n=5)	109	3.8	m/z 388→194
		0.02 (n=5)	102	11.8	Confirmation
		2.0 (n=5)	109	2.1	m/z 388→163
	500M07	0.02 (n=5)	90	7.9	Quantitation
		2.0 (n=5)	109	3.8	m/z 358→164
		0.02 (n=5)	92	8.2	Confirmation
		2.0 (n=5)	107	2.6	m/z 358→132

Table A 28: Characteristics for the analytical method used for validation of pyraclostrobin residues and its metabolite 500M07 in plant matrices (coffee grain, soya bean grain, wheat grain)

	Pyraclostrobin and 500M07
Specificity	HPLC-MS/MS, using two mass transitions, is a highly specific detection technique and therefore a confirmatory technique is not required.Mass spectrum is provided. There were no known interferences (blank value < 30 % LOQ).
Calibration (type, number of data points)	Five calibration points were used. Good linearity was observed with correlation coefficients of ≥0.99).
Calibration range	Calibration points distributed over a concentration range of 0.25 to 5.0 ng/ml were used.
Assessment of matrix effects is presented	No matrix effects were investigated within an independent experiment, therefore instrument recovery sampels would be implemented within each analytical series, if no further information on matrix effects of the investigated matrix is available. Alternatively matrix-matched standards would be used.
Limit of determination/quantification	The limit of quantification (LOQ) representing the lowest validated level with sufficient recovery and precision was 0.02 mg/kg
Reproducibility	The main purpose of an independent laboratory validation is to show that a method developed in one laboratory can be successfully used in another facility. The method is thought as data generation method and not for MRL enforcement purposes. Reproducibility was not determined within this validation study.

Conclusion

The method uses highly specific LC-MS/MS for final determination of pyraclostrobin and its metabolite 500M07 with a limit of quantitation of 0.02 mg/kg. The method is sufficiently validated for the commodities coffee grain, soya bean grain and wheat grain. Thereby, it could be demonstrated that the method fulfills the requirements with regard to specificity, linearity, repeatability, limit of quantitation and recoveries.

A 2.3.1.1.1.5 Confirmatory method

No confirmatory technique is required as two different, highly specific mass transitions are used for quantitation and qualification.

A 2.3.1.1.1.6 Extraction efficiency

There are no new study on extraction efficiency of the method for the determination of residues in plant matrices. For more information see section 5.3.4.2.

A 2.3.1.1.2 Analytical method L0220/01 for the determination of metabolite 500M79 in plant matrices

The method L0220/01was developed for the detection of the metabolite 500M04 and its glycoside conjugates 500M79.

A 2.3.1.1.2.1 Method validation

Comments of zRMS:	The study has been accepted. The objective of this validation study was to demonstrate the applicability and repeatability of method L0220/01 for the determination 500M79, one of the metabolites of pyraclostrobin in lettuce, leek and cabbage by using LC-MS/MS. The metabolite 500M79 is determined only for risk assessments and is not included in the residue definition for enforcement. Therefore, no independent laboratory validation is conducted with this method. Metabolite 500M79 was extracted from plant matrices and hydrolyzed using enzymatic cleavage to 500M04 (BF 500-5). The results show that 500M79 can be fully hydrolyzed to 500M04 with BASF method No. L0220/01. The results are expressed in 500M04. The method was validated at 2 fortification levels (at LOQ and at 0.10 mg/kg (10xLOQ)) for lettuce, leek and cabbage. For each fortification level and matrix, five replicates were analyzed. Additionally, at least two replicates of unfortified samples were examined. No separate confirmation technique was necessary as two parent-daughter ion transitions were used for quantitation during LC-MS/MS determination. No significant interferences were found. The use of matrix-matched standards was not necessary. Good linearity ($r > 0.99$) was observed in the method working range for the two mass transitions of 500M04 in standard solutions. Validation criteria with mean recoveries between 70-110%, relative standard deviation $\leq$ 20% and blank values $\leq$ 30% were always met. During method development, it was found that the fortification and calibration solutions of each analyte were stable (less than 20% decline) for at least one month refrigerated. The mean corrected stored recovery values found during the final volume stability experiments ranged between 100 and 103%. This demonstrates that 500M79 is stable in the extracts of lettuce, leek over the tested time period of 5 days and cabbage over the tested time period of 3 days. It was proven that the method L0220/01 is suitable to determine residues of 500M79 in lettuce, leek and cabbage. The mean recovery values were found to be 78—87% for lettuce, leek and cabbage using solvent-based standard. The overall relative standard deviations (RSD, %) for all fortification levels were below 20%. The limit of quantitation (LOQ) of the method for 500M04 is 0.01 mg/kg. The limit of detection was estimated at 20% of the limit of quantification. Thus, the common moiety method BASF method No. L0220/01 is suitable to determine residues of BAS 500F via the 500M04 in the matrices tested. The study was already evaluated in PL.

Reference: CP 5.2/6

Report	Validation of analytical method L0220/01 for the detrmination of metabolite 500M79 (Reg.No. 5937091) in plant matrices by LC-MS/MS, Courtois J., 2014 report No 423099 2014/1001721
Guideline(s):	SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), SANCO/825/00 rev. 8.1 (16 November 2010), OECD-ENV/JM/MONO/(2007)17 (OECD No. 72)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Materials and methods

Metabolite 500M79 is extracted from plant matrices using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is evaporated to dryness and dissolved in water. After hydrolysis to 500M04 by enzymatic cleavage reversed phase C18-column clean-up is conducted. The final determination of 500M04 is performed by UPLC-MS/MS. The results are expressed in 500M04. Analysis was accomplished using a Waters Acquity CSH Phenyl-Hexyl column and an acetonitrile-water gradient with formic acid as modifier at a flow rate of 600 µL/min. The mass transition 195 → 153 is used for quantitation and 195 → 150 for confirmation.

Results and discussions

The method proved to be suitable for analysis of metabolite 500M79 in plant matrices at a limit of quantitation of 0.01 mg/kg. In all matrices tested (lettuce heads, white cabbage, leek), the mean recovery values were between 70 and 110% and relative standard deviations (RSD) were well below 20%. Method validation data are summarised in the table below.

Table A 29: Recovery results from method validation of the metabolite 500M79 using the analytical method L0220/01

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Lettuce, head	500M79	0.01 (n=5)	81.9	2.5	Quantitation
		0.1 (n=5)	82.1	1.3	m/z 195→153
		0.01 (n=5)	82.5	8.6	Confirmation
		0.1 (n=5)	84.7	4.8	m/z 195→150
White cabbage	500M79	0.01 (n=5)	76.2	6.3	Quantitation
		0.1 (n=5)	80.3	1.9	m/z 195→153
		0.01 (n=5)	74.1	7.4	Confirmation
		0.1 (n=5)	79.6	3.0	m/z 195→150

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Leek, whole	500M79	0.01 (n=5)	89.3	1.5	Quantitation
		0.1 (n=5)	84.6	3.8	m/z 195→153
		0.01 (n=5)	97.4	4.8	Confirmation
		0.1 (n=5)	84.3	4.1	m/z 195→150

Table A 30: Characteristics for the analytical method used for validation of pyraclostrobin residues in plant matrices (lettuce head, white cabbage and leek)

	500M79
Specificity	HPLC-MS/MS, using two mass transitions, is a highly specific detection technique and therefore a confirmatory technique is not required. There were no interferences at the retention times for 500M79 (blank value < 30 % LOQ)
Calibration (type, number of data points)	Calibration standards were prepared in methanol/water, (50/50, v/v). At least 7 data points were used and individual calibration data was presented. Good linearity was observed with a correlation coefficient of ≥0.99.
Calibration range	Calibration points distributed over a concentration range of 0.025 and 2.5 ng/mL.
Assessment of matrix effects is presented	Yes, matrixatrix- and solvent-matched standards were analyzed within the study to check tor possible matrix effects. No significant differences have been identified.
Limit of quantitation	The limit of quantification representing the lowest validated level with sufficient recovery and precision was 0.01 mg/kg for 500M79. The limit of detection was estimated at 20% of the limit of quantitation, equivalent to 0.002 mg/kg.
Standard stability	Fortification solutions in acetonitrile and calibration standards in acetonitrile/water (50/50, v/v) of each analyte were stable (less than 20% decline) for at least one month refrigerated.
Final volume stability	The mean corrected stored recovery values found during the final volume stability experiments ranged between 100 and 103%. This demonstrates that 500M79 is stable in extracts of lettuce, leek over the tested time period of 5 days and cabbage over the tested time period of 3 days.

Conclusion

The method uses highly specific LC-MS/MS for determination of the metabolite 500M79 hydrolyzed to metabolite 500M04. The method is sufficiently validated for the commodities lettuce head, white cabbage and leek. Thereby, it could be demonstrated that the method fulfills the requirements with regard to linearity, specificity, repeatability, limit of quantitation and recoveries.

A 2.3.1.1.2.2 Confirmatory method

No confirmatory technique is required as two different, highly specific mass transitions are used for quantitation and qualification.

A 2.3.1.1.2.3 Extraction efficiency

There are no new study on extraction efficiency of the method for the determination of residues in plant matrices. For more information see section 5.3.4.2.

A 2.3.1.1.3 Analytical method L0076/09 (former method no. 353/1) for the determination of BAS 500 F and its metabolite 500M07 in plant matrices

The method L0076/09 was validated for the detection of BAS 500 F and its metabolite 500M07 in citrus (whole fruit), dry beans (seeds), tomato (whole fruit), soybeans (grain) and wheat (grain) using HPLC-MS/MS and UPLC-MS/MS.

A 2.3.1.1.3.1 Method validation

Comments of zRMS:	The study has been accepted. The objective of this study was the validation of BASF Method Number L0076/09 for determination of pyraclostrobin and its metabolite 500M07 in citrus (whole fruit), dry beans (seeds), tomato (whole fruit), soybeans (grain) and wheat (grain) using HPLC-MS/MS and UPLC-MS/MS. The results show that this method is suitable to determine residues of BAS 500 F and its metabolite 500M07 in plant matrices such as citrus, dry beans, tomato, soybeans and wheat with regard to specificity, repeatability, LOQ of 0.01 mg/kg, and recoveries according to SANCO/3029/99 rev.4 and is therefore applicable to correctly determine residues of BAS 500 F and its metabolite 500M07 in plant matrices. Confirmatory method is no needed. The residues were determined by high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC) - tandem mass spectrometer (MS/MS) monitoring ion transitions at m/z 388 → 194 (proposed as the primary transition for quantitation) and m/z 388 → 163 (typically for confirmatory purposes) for BAS 500 F and at m/z 358 → 164 (proposed as the primary transition for quantitation) and m/z 358 → 132 (typically for confirmatory purposes) for its metabolite 500M07. For each matrix at least one blank of reagents, two controls, five replicates fortified with the analytes at the LOQ, and five replicates fortified at 100x the LOQ were used. The only exception was tomato matrix (6 replicates, at 1000x LOQ). At least 5 points calibration was done; acceptable linearity was observed for the standard range and the mass transitions tested for each analyte. The method-detector response was linear over the 0.0400 ng/mL to 2.00 ng/mL range ($r \geq 0.9900$). All individual results of recovery were between (70 – 120) % and the average of each fortification level / matrix of the validation experiments were between (70 – 110) % with RSD < 20% in each fortification level. It was also demonstrated that matrix effects using both mass transitions in the investigated matrices were only significant for BAS 500 F in soybeans by UPLC. Therefore, the use of matrix matched standards was no needed in other cases. Furthermore, it could be proved the stability of BAS 500 F and its metabolite 500M07 in stock and fortification standard solutions for up to 68 days and calibration standard solutions for up to 34 days; whereas for the extract and final volume solutions, the analytes has shown to be stable for up to 7 days for all matrices. The study was already evaluated in PL.

Reference:	CP 5.2/7
Report	Validation of BASF Method Number L0076/09 for the determination of BAS 500 F and its metabolite 500M07 in citrus (whole fruit), dry beans (seeds), tomato (whole fruit), soybeans (grain) and wheat (grain) using HPLC-MS/MS and UPLC-MS/MS, Jose W.F.P. de, 2015 report No 761365 2015/3004795
Guideline(s):	Resolucao RDC No. 4 - ANVISA (18/01/2012)
Deviations:	No
GLP:	yes (certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)
Acceptability:	Yes

Materials and methods

Residues of pyraclostrobin and its metabolite 500M07 are extracted from plant matrices with a mixture of methanol, water and HCl (2 mol/L) / (70/25/5, v/v/v). After centrifugation, an aliquot is alkalized and partitioned against cyclohexane. Then, after evaporation to dryness, residues are dissolved in methanol/water (50/50, v,v) and filtered before injection. Final determination was performed by HPLC- and UPLC-MS/MS monitoring ion transition at m/z 388→194 (for quantitation) and at m/z 388→163 (for confirmation) for BAS 500 F and at m/z 358→164 (for quantitation) and at m/z 358→132 (for confirmation) for its metabolite 500M07 in ESI positive mode. Analysis by HPLC was accomplished on a Thermo Scientific, Betasil C18 column applying a methanol-pure water gradient using 0.1% formic acid as modifier. Analysis by UPLC was accomplished on an Acquity BEH C18 column applying an acetonitrile-pure water gradient using 0.1% formic acid as modifier.

Results and discussions

Method validation acceptance criteria were fully met with mean recovery values between 70 and 110%. The relative standard deviations (RSD, %) for all commodities and all fortification levels were well below 20%. Method validation data are summarised in the table below.

Table A 31: Recovery results from method validation of BAS 500 F using the analytical method L0076/09

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
HPLC					
Dry beans	BAS 500 F	0.01 (n=6)	91.6	3.6	Quantitation
		1.0 (n=6)	94.9	1.9	m/z 388→194
		0.01 (n=6)	85.6	6.7	Confirmation
		1.0 (n=6)	93.0	3.6	m/z 388→163
	500M07	0.01 (n=6)	91.3	6.7	Quantitation
		1.0 (n=6)	96.2	3.2	m/z 358→164
		0.01 (n=6)	85.7	5.3	Confirmation
		1.0 (n=6)	99.9	2.1	m/z 358→132
Soya bean, grain	BAS 500 F	0.01 (n=6)	101	3.7	Quantitation
		1.0 (n=6)	81.8	2.3	m/z 388→194
		0.01 (n=6)	98.3	3.9	Confirmation
		1.0 (n=6)	82.8	3.6	m/z 388→163
	500M07	0.01 (n=6)	83.5	4.2	Quantitation
		1.0 (n=6)	82.3	3.3	m/z 358→164
		0.01 (n=6)	75.5	3.1	Confirmation
		1.0 (n=6)	78.1	6.3	m/z 358→132
Citrus	BAS 500 F	0.01 (n=6)	94.3	2.8	Quantitation
		1.0 (n=6)	96.7	2.4	m/z 388→194
		0.01 (n=6)	91.8	3.0	Confirmation
		1.0 (n=6)	96.6	5.6	m/z 388→163
	500M07	0.01 (n=6)	93.5	2.0	Quantitation
		1.0 (n=6)	97.7	4.6	m/z 358→164
		0.01 (n=6)	90.9	4.3	Confirmation
		1.0 (n=6)	98.5	3.4	m/z 358→132
Wheat	BAS 500 F	0.01 (n=6)	100	4.7	Quantitation
		1.0 (n=6)	92.1	2.6	m/z 388→194
		0.01 (n=6)	99.5	4.2	Confirmation
		1.0 (n=6)	91.0	3.4	m/z 388→163
	500M07	0.01 (n=6)	100	8.3	Quantitation
		1.0 (n=6)	95.1	2.8	m/z 358→164
		0.01 (n=6)	95.5	9.4	Confirmation
		1.0 (n=6)	94.6	1.8	m/z 358→132

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Tomato	BAS 500 F	0.01 (n=5)	85.5	13	Quantitation
		1.0 (n=6)	97.0	5.1	m/z 388→194
		10 (n=6)	84.7	3.1	
		0.01 (n=5)	86.9	13	Confirmation
		1.0 (n=6)	92.8	4.6	m/z 388→163
		10 (n=6)	85.5	3.5	
	500M07	0.01 (n=5)	85.6	12	Quantitation
		1.0 (n=6)	99.2	6.7	m/z 358→164
		0.01 (n=5)	83.4	15	Confirmation
		1.0 (n=6)	96.1	10	m/z 358→132
UPLC					
Dry beans	BAS 500 F	0.01 (n=6)	85.0	3.9	Quantitation
		1.0 (n=6)	88.7	6.7	m/z 388→194
		0.01 (n=6)	88.5	3.4	Confirmation
		1.0 (n=6)	90.9	5.2	m/z 388→163
	500M07	0.01 (n=6)	85.3	4.9	Quantitation
		1.0 (n=6)	93.2	5.0	m/z 358→164
		0.01 (n=6)	81.3	4.9	Confirmation
		1.0 (n=6)	91.8	4.1	m/z 358→132
Soya bean, grain	BAS 500 F	0.01 (n=6)	89.8	7.8	Quantitation
		1.0 (n=6)	82.3	7.3	m/z 388→194
		0.01 (n=6)	89.6	6.6	Confirmation
		1.0 (n=6)	79.4	5.5	m/z 388→163
	500M07	0.01 (n=6)	76.2	2.0	Quantitation
		1.0 (n=6)	81.0	5.6	m/z 358→164
		0.01 (n=6)	77.3	5.1	Confirmation
		1.0 (n=6)	77.1	5.1	m/z 358→132
Citrus	BAS 500 F	0.01 (n=6)	94.1	5.2	Quantitation
		1.0 (n=6)	94.5	1.6	m/z 388→194
		0.01 (n=6)	85.2	6.3	Confirmation
		1.0 (n=6)	93.4	4.2	m/z 388→163
	500M07	0.01 (n=6)	87.1	4.7	Quantitation
		1.0 (n=6)	99.1	5.9	m/z 358→164
		0.01 (n=6)	88.0	2.5	Confirmation
		1.0 (n=6)	103	6.1	m/z 358→132

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Wheat	BAS 500 F	0.01 (n=6)	109	7.2	Quantitation
		1.0 (n=6)	92.4	5.8	m/z 388→194
		0.01 (n=6)	108	2.4	Confirmation
		1.0 (n=6)	93.3	4.2	m/z 388→163
	500M07	0.01 (n=6)	102	5.9	Quantitation
		1.0 (n=6)	92.5	5.0	m/z 358→164
		0.01 (n=6)	101	7.7	Confirmation
		1.0 (n=6)	94.2	4.1	m/z 358→132
Tomato	BAS 500 F	0.01 (n=6)	92.8	6.7	Quantitation
		1.0 (n=6)	101	7.5	m/z 388→194
		10 (n=6)	92.1	7.6	
		0.01 (n=6)	105	6.0	Confirmation
		1.0 (n=6)	102	6.6	m/z 388→163
		10 (n=6)	92.3	4.9	
	500M07	0.01 (n=6)	106	7.2	Quantitation
		1.0 (n=6)	108	4.3	m/z 358→164
		0.01 (n=6)	109	2.4	Confirmation
		1.0 (n=6)	107	4.2	m/z 358→132

Table A 32: Characteristics for the analytical method used for validation of pyraclostrobin residues in plant matrices (citrus, dry beans, tomato, soya bean and wheat grain)

	Pyraclostrobin and metabolite 500M07
Specificity	Highly selective determination of BAS 500 F and its metabolite 500M07 using LC-MS/MS monitoring two mass transitions. There were no interferences at the retention time corresponding to BAS 500 F and its metabolite 500M07 in any of the control specimens of citrus, dry beans, tomato, soya bean and wheat grain (blank value < 30 % LOQ).
Calibration (type, number of data points)	Calibration standards were prepared in methanol/water (50/50, v/v). At least six calibration points were used and individual calibration data was presented. Good linearity was observed in the range of 0.04 to 2 ng/mL (external reference standard, injected in triplicate) with a correlation coefficient ≥0.99.
Calibration range	Calibration points distributed over a concentration range of 0.04 to 2.0 ng/mL.
Assessment of matrix effects is presented	Yes. Analysis of matrix-matched standards and solvent standards showed no significant matrix effects, except for BAS 500 F in soya beans. Therefore, solvent-based calibration standard solutions were used for quantification, except for BAS 500 F in soya beans, where matrix-matched calibration standard solutions were used.
Limit of determination/quantification	The limit of quantitation representing the lowest validated fortification level with sufficient recovery and precision was 0.01 mg/kg for BAS 500 F and the metabolite 500M07.
Reproducibility	The reproducibility of the method was not estimated as identical samples were not evaluated by an independent laboratory. However based on the performance of the method, its reproducibility is expected to be good.
Standard stability	BAS 500 F and its metabolite 500M07 have been shown to be stable in methanol, the solvent used for preparation of stock solution, intermediate solutions and fortification standard solutions for up to 68 days, and in calibration solutions prepared by serial dilution of the intermediate solutions with methanol:water (50/50, v/v) for up to 34 days, when stored under refrigerator conditions.
Extract and final volume stability	BAS 500 F and its metabolite 500M07 were shown to be stable after extraction with a mixture consisting of methanol/Milli-Q water/2 mol/L HCl solution (70/25/5, v/v/v) for a time interval of 0 to 7 days for all matrices, when stored under refrigerator conditions. The final volume dissolved with a mixture of methanol:Milli-Q water (50/50, v/v) was also investigated and BAS 500 F and its metabolite 500M07 were shown to be stable for up to 7 days.

Conclusion

The method L0076/09 uses highly specific LC-MS/MS for final determination of pyraclostrobin and its metabolite 500M07. The method is sufficiently validated for the commodities citrus (whole fruit), dry beans (seeds), tomato (whole fruit), soya bean (grain) and wheat (grain). Thereby, it could be demonstrated that

the method fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantitation and recoveries.

A 2.3.1.1.3.2 Confirmatory method

No confirmatory technique is required as two different, highly specific mass transitions are used for quantitation and qualification.

A 2.3.1.1.3.3 Extraction efficiency

There are no new study on extraction efficiency of the method for the determination of residues in plant matrices. For more information see section 5.3.4.2.

A 2.3.1.1.4 Analytical method 535/1 for the determination of pyraclostrobin and its metabolite 500M07 in plant matrices

A 2.3.1.1.4.1 Method validation

Comments of zRMS:	Study has been accepted. The purpose of the study was the validation of BASF method No. 535/1 for determination of BAS 500 F and BF 500-3 (aka 500M07) residues in wheat, oilseed rape seed, tomato, onion, lemon and lettuce according to the technical procedure (BASF DocID 2006/1039426) which was used without any deviations and is given in the study appendix. The BASF method No. 535/1 is also suitable to determine residues of BAS 421 F, BAS 480 F, BAS 510 F and BAS 555 F in plant matrices. The final determination was performed by HPLC-MS/MS with 2 mass transitions for quantitation and confirmation. No confirmatory method needed. The results show that BASF method No. 535/1 is suitable to determine residues of BAS 500 F and BF 500-3 in plant matrices such as wheat (plant without root, grain, and straw), lemon, lettuce, oilseed rape seed, tomato and onion. In validation 2 fortification levels were used with 5 replicates on each. The calibrations with 4 points were done (should be 5 or 3 twice). Good linearity was obtained. In all cases, the mean recovery values per fortification level were between 70% and 110%. The RSD for all matrices and all fortification was below 20%. The LOQ defined by the lowest fortification level successfully tested was 0.01 mg/kg in all matrices. The limit of detection was not determined. Stability of pyraclostrobin and 500M07 in the relevant solutions is mentioned below in the applicant text. The study was already evaluated in PL.

Reference:	CP 5.2/8
Report	Validation of BASF method No. 535/1 in plant matrices, Mackenroth C.,Lehmann A., 2007 report No 246631 2006/1039427
Guideline(s):	EPA 860.1340, SANCO/825/00 rev. 6 (20 June 2000), SANCO/3029/99 rev. 4 (11 July 2000), EEC 6/46, EEC 91/414 Annex III (Part A Section 5)
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht,

Mainz, Germany Fed.Rep.)

Acceptability: Yes

Materials and methods

Residues of pyraclostrobin (BAS 500 F) and its metabolite 500M07 are extracted from plant matrices with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. After evaporation to dryness residues are dissolved in methanol / water (50/50, v/v) for determination. The final determination of pyraclostrobin and its metabolite 500M07 is performed by HPLC-MS/MS. Analysis was accomplished using a Betasil C18 column and a methanol-water gradient with formic acid as modifier at a flow rate of 600 µL/min. Samples were analysed at mass transition 388 → 194 for quantitation and 388 → 163 for confirmation for pyraclostrobin and for 500M07 at mass transition 358 → 164 for quantitation and 358 → 132 for confirmation.

Results and discussions

Method validation acceptance criteria were fully met with mean recovery values between 70% and 110% in all matrices tested. The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%. Method validation data are summarised in the table below.

Table A 33: Recovery results from method validation of pyraclostrobin and its metabolite 500M07 using the analytical method 535/1

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Wheat plant without root	BAS 500 F	0.01 (n=5)	101	7.7	Quantitation
		0.1 (n=5)	86	4.8	m/z 388→194
		0.01 (n=5)	103	9.6	Confirmation
		0.1 (n=5)	88	6.4	m/z 388→163
	500M07	0.01 (n=5)	95	7.0	Quantitation
		0.1 (n=5)	81	6.6	m/z 358→164
		0.01 (n=5)	93	8.4	Confirmation
		0.1 (n=5)	84	5.3	m/z 358→132
Wheat, grain	BAS 500 F	0.01 (n=5)	95	4.2	Quantitation
		0.1 (n=5)	98	5.0	m/z 388→194
		0.01 (n=5)	101	3.8	Confirmation
		0.1 (n=5)	96	7.4	m/z 388→163
	500M07	0.01 (n=5)	100	3.1	Quantitation
		0.1 (n=5)	93	7.5	m/z 358→164
		0.01 (n=5)	99	2.2	Confirmation
		0.1 (n=5)	98	7.7	m/z 358→132
Wheat, straw	BAS 500 F	0.01 (n=5)	92	5.0	Quantitation
		0.1 (n=5)	86	4.8	m/z 388→194
		0.01 (n=5)	95	7.7	Confirmation
		0.1 (n=5)	80	4.7	m/z 388→163

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
	500M07	0.01 (n=5)	90	6.7	Quantitation
		0.1 (n=5)	81	5.8	m/z 358→164
		0.01 (n=5)	95	9.7	Confirmation
		0.1 (n=5)	79	3.4	m/z 358→132
Lemon	BAS 500 F	0.01 (n=5)	97	7.0	Quantitation
fruit		0.1 (n=5)	88	6.1	m/z 388→194
		0.01 (n=5)	97	4.7	Confirmation
		0.1 (n=5)	89	5.4	m/z 388→163
	500M07	0.01 (n=5)	101	3.3	Quantitation
		0.1 (n=5)	88	5.1	m/z 358→164
		0.01 (n=5)	99	3.4	Confirmation
		0.1 (n=5)	85	2.9	m/z 358→132
Lettuce, head	BAS 500 F	0.01 (n=5)	93	4.1	Quantitation
		0.1 (n=5)	87	4.8	m/z 388→194
		0.01 (n=5)	92	6.2	Confirmation
		0.1 (n=5)	88	6.0	m/z 388→163
	500M07	0.01 (n=5)	97	1.9	Quantitation
		0.1 (n=5)	85	2.2	m/z 358→164
		0.01 (n=5)	102	2.2	Confirmation
		0.1 (n=5)	85	4.4	m/z 358→132
Oilseed rape,	BAS 500 F	0.01 (n=5)	96	6.4	Quantitation
seed		0.1 (n=5)	90	5.6	m/z 388→194
		0.01 (n=5)	94	2.4	Confirmation
		0.1 (n=5)	91	3.3	m/z 388→163
	500M07	0.01 (n=5)	83	10.6	Quantitation
		0.1 (n=5)	81	3.1	m/z 358→164
		0.01 (n=5)	82	9.8	Confirmation
		0.1 (n=5)	81	4.4	m/z 358→132
Tomato, fruit	BAS 500 F	0.01 (n=5)	92	5.0	Quantitation
		0.1 (n=5)	90	3.5	m/z 388→194
		0.01 (n=5)	92	4.2	Confirmation
		0.1 (n=5)	87	3.3	m/z 388→163
	500M07	0.01 (n=5)	92	3.3	Quantitation
		0.1 (n=5)	91	5.4	m/z 358→164
		0.01 (n=5)	91	4.4	Confirmation
		0.1 (n=5)	91	5.8	m/z 358→132
Onion,	BAS 500 F	0.01 (n=5)	99	3.5	Quantitation

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
bulb		0.1 (n=5)	93	3.8	m/z 388→194
		0.01 (n=5)	96	2.7	Confirmation
		0.1 (n=5)	85	6.7	m/z 388→163
	500M07	0.01 (n=5)	104	2.6	Quantitation
		0.1 (n=5)	96	2.4	m/z 358→164
		0.01 (n=5)	104	3.6	Confirmation
		0.1 (n=5)	95	3.0	m/z 358→132

Table A 34: Characteristics for the analytical method used for validation of pyraclostrobin residues and its metabolite 500M07 in plant matrices

	Pyraclostrobin and 500M07
Specificity	The method 535/1 determines residues of pyraclostrobin and its metabolites 500M07 plant matrices. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered for each analyte. HPLC-MS/MS, using two mass transitions, is a highly specific detection technique and therefore a confirmatory technique is not required.
Calibration (type, number of data points)	Calibration standards were prepared in methanol / water (80/20, v/v). Four calibration points were used and individual calibration data was presented. Linear correlations with coefficients ≥0.99 were obtained for pyraclostrobin and its metabolites 500M07.
Calibration range	Calibration points distributed over a concentration range of 0.05 to 0.5 ng/ml were used.
Assessment of matrix effects is presented	No matrix effects were investigated within an independent experiment, therefore instrument recovery sampels would be implemented within each analytical series, if no further information on matrix effects of the investigated matrix is available. Alternatively matrix-matched standards would be used.
Limit of determination/quantification	The limit of quantification (LOQ) representing the lowest validated level with sufficient recovery and precision was 0.01 mg/kg.
Standard stability	In another study pyraclostrobin and 500M07 are shown to be stable in methanol and in the HPLC solvent over a time interval of 60 days, if stored refrigerated.

Conclusion

The method uses highly specific LC-MS/MS for final determination of pyraclostrobin and its metabolite 500M07 with a limit of quantitation of 0.01 mg/kg. Thereby, it could be demonstrated that the method fulfills the requirements with regard to specificity, linearity, repeatability, limit of quantitation and recoveries.

A 2.3.1.1.4.2 Confirmatory method

No confirmatory technique is required as two different, highly specific mass transitions are used for quantitation and qualification.

A 2.3.1.1.4.3 Extraction efficiency

There are no new study on extraction efficiency of the method for the determination of residues in plant matrices. For more information see section 5.3.4.2.

A 2.3.1.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

A 2.3.1.2.1 Analytical method 446/2 (L0058/03) for the determination of pyraclostrobin and its metabolites 500M04 and 500M85 in animal matrices

A 2.3.1.2.1.1 Method validation

Comments of zRMS:	The study is acceptable. The purpose of this study was to validate BASF Analytical Method No. 446/2 (L0058/03) for the determination of BAS 500 F and its metabolites 500M04 and 500M85 in animal matrices by HPLC-MS/MS. The method can be considered suitable to determine residues of BAS 500 F and its metabolites 500M04 (BF 500-5) and 500M85 (BF 500-8) in animal matrices at LOQ of 0.01 mg/kg. Despite the recoveries requirement is formally not fulfilled for 500M85 in kidney, fat and muscle, the precision of the method was good for 500M85 in these matrices and both transitions. Therefore, this deviation from the requirement can be accepted. 2 fortification levels per matrix were used. The mean recovery values of the validation experiments were acceptable (70-100%) for BAS 500 F and its metabolites 500M04 and 500M85 in all matrices, except for 500M85 in fat, kidney, and muscle (60 - 70%). The RSD for both fortification levels was below 20% for all analytes. 6-point calibration was done. Linear correlations with coefficients >0.99 were obtained for BAS 500 F and its metabolites. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered for each analyte. The use of matrix matched standards was not needed. It was also shown that the fortification and calibration solutions of 500M04 and 500M85 were stable for at least 28 days, if stored refrigerated. As two mass transitions were monitored a confirmatory technique is not required. The study was already evaluated in PL.

Reference:	CP 5.2/9
Report	Validation of analytical method 446/2 (L0058/03) for the determination of BAS 500 F (Reg.No. 304428) and its metabolites 500M04 (Reg.No. 298327) and 500M85 (Reg.No. 399530) in animal matrices by LC-MS/MS, Eilers B.,Taraschewski I., 2014 report No 428212 2013/1400972
Guideline(s):	SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), SANCO/825/00 rev. 8.1 (16 November 2010), EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, OECD-ENV/JM/MONO/(2007)17 (OECD No. 72)
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und

Gewerbeaufsicht, Mainz, Germany)

Acceptability: Yes

Materials and methods

After a partition into acetonitrile/iso-hexane, the total residues were cleaved by boiling in aqueous sodium hydroxide to yield hydroxpyrazole(s), which can be extracted using ethyl acetate. After acidification and phase separation, the organic layer was taken. The final determination of 500M04 and 500M85 is performed by HPLC-MS/MS. Analysis was accomplished using a Phenomenex, Synergi 4 µm MAX-RP 80A column and an acetonitrile-water gradient with formic acid as modifier at a flow rate of 600 µL/min.
Pyraclostrobin was determined as 500M04; for 500M04 the ion transitions *m/z* = 195 → 117 and *m/z* = 195 → 153 and for 500M85 the transitions *m/z* = 211 → 138 and *m/z* = 211 → 166 can be used for quantification and confirmation. Detection was accomplished in a positive ionization mode.

Results and discussions

In all matrices tested, the mean recovery values were between 70% and 110% at both fortification levels and both mass transitions of all three analytes, except for 500M85 with recoveries between 60 and 70% in the matrices muscle, kidney and fat. The relative standard deviations (RSD, %) for all commodities and all fortification levels were well below 20%. The recovery data are summarized in the table below.

Table A 35: Recovery results from method validation of pyraclostrobin, 500M04 and 500M85 using the analytical method L0058/03

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Muscle	pyraclostrobin	0.01 (n = 5)	79.8	2.6	Quantitation
		0.1 (n = 5)	75.6	4.1	m/z 195 → 117
		0.01 (n = 5)	79.4	2.2	Confirmation
		0.1 (n = 5)	75.5	4.5	m/z 195 → 153
	500M04	0.01 (n = 5)	81.6	5.5	Quantitation
		0.1 (n = 5)	92.1	2.8	m/z 195 → 117
		0.01 (n = 5)	81.5	4.0	Confirmation
		0.1 (n = 5)	91.8	2.5	m/z 195 → 153
	500M85	0.01 (n = 5)	62.4	2.6	Quantitation
		0.1 (n = 5)	61.7	3.2	m/z 211 → 138
		0.01 (n = 5)	62.2	3.0	Confirmation
		0.1 (n = 5)	61.2	3.5	m/z 211 → 166
Kidney	BAS 500 F	0.01 (n = 5)	89.4	3.1	Quantitation
		0.2 (n = 5)	87.6	4.6	m/z 195 → 117
		0.01 (n = 5)	85.3	1.9	Confirmation
		0.2 (n = 5)	84.3	3.5	m/z 195 → 153
	500M04	0.01 (n = 5)	87.5	5.6	Quantitation
		0.2 (n = 5)	74.7	7.7	m/z 195 → 117
		0.01 (n = 5)	87.1	3.8	Confirmation
		0.2 (n = 5)	75.2	5.8	m/z 195 → 153

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
	500M85	0.01 (n = 5)	63.7	8.2	Quantitation
		0.2 (n = 5)	60.8	8.4	m/z 211 → 138
		0.01 (n = 5)	63.4	6.5	Confirmation
		0.2 (n = 5)	60.9	10.9	m/z 211 → 166
Liver	BAS 500 F	0.01 (n = 5)	86.7	7.5	Quantitation
		1.0 (n = 5)	89.5	6.1	m/z 195 → 117
		0.01 (n = 5)	94.1	9.8	Confirmation
		1.0 (n = 5)	90.2	9.2	m/z 195 → 153
	500M04	0.01 (n = 5)	88.9	6.1	Quantitation
		1.0 (n = 5)	78.1	5.6	m/z 195 → 117
		0.01 (n = 5)	90.2	5.4	Confirmation
		1.0 (n = 5)	78.2	5.7	m/z 195 → 153
	500M85	0.01 (n = 5)	80.4	6.7	Quantitation
		1.0 (n = 5)	77.0	9.1	m/z 211 → 138
		0.01 (n = 5)	81.3	8.4	Confirmation
		1.0 (n = 5)	78.8	7.1	m/z 211 → 166
Fat	BAS 500 F	0.01 (n = 5)	88.9	4.5	Quantitation
		0.1 (n = 5)	78.0	2.9	m/z 195 → 117
		0.01 (n = 5)	87.2	6.2	Confirmation
		0.1 (n = 5)	80.1	3.1	m/z 195 → 153
	500M04	0.01 (n = 5)	72.4	4.4	Quantitation
		0.1 (n = 5)	75.2	10.2	m/z 195 → 117
		0.01 (n = 5)	72.8	1.7	Confirmation
		0.1 (n = 5)	75.8	10.5	m/z 195 → 153
	500M85	0.01 (n = 5)	66.6	5.9	Quantitation
		0.1 (n = 5)	67.9	10.6	m/z 211 → 138
		0.01 (n = 5)	67.0	6.0	Confirmation
		0.1 (n = 5)	67.8	10.1	m/z 211 → 166
Milk	BAS 500 F	0.01 (n = 5)	91.7	4.4	Quantitation
		0.1 (n = 5)	91.5	5.4	m/z 195 → 117
		0.01 (n = 5)	88.5	5.8	Confirmation
		0.1 (n = 5)	91.7	3.7	m/z 195 → 153
	500M04	0.01 (n = 5)	91.4	3.1	Quantitation
		0.1 (n = 5)	88.1	6.2	m/z 195 → 117
		0.01 (n = 5)	99.8	2.2	Confirmation
		0.1 (n = 5)	89.3	5.2	m/z 195 → 153
	500M85	0.01 (n = 5)	95.1	4.7	Quantitation

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
		0.1 (n = 5)	85.3	7.7	m/z 211 → 138
		0.01 (n = 5)	94.4	4.8	Confirmation
		0.1 (n = 5)	85.7	7.6	m/z 211 → 166
Egg	BAS 500 F	0.01 (n = 5)	77.1	6.9	Quantitation
		0.1 (n = 5)	75.6	8.6	m/z 195 → 117
		0.01 (n = 5)	78.7	3.8	Confirmation
		0.1 (n = 5)	75.1	6.7	m/z 195 → 153
	500M04	0.01 (n = 5)	88.0	3.5	Quantitation
		0.1 (n = 5)	87.1	7.7	m/z 195 → 117
		0.01 (n = 5)	86.6	5.5	Confirmation
		0.1 (n = 5)	88.6	7.5	m/z 195 → 153
	500M85	0.01 (n = 5)	77.6	2.0	Quantitation
		0.1 (n = 5)	82.9	8.1	m/z 211 → 138
		0.01 (n = 5)	77.8	1.3	Confirmation
		0.1 (n = 5)	81.5	6.5	m/z 211 → 166

Table A 36: Characteristics for the analytical method used for validation of pyraclostrobin residues and its metabolites in animal matrices

	BAS 500 F, 500M04, 500M85
Specificity	The method L0058/03 determines residues of pyraclostrobin and its metabolites 500M04 and 500M85 in animal matrices. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered for each analyte. HPLC-MS/MS, using two mass transitions, is a highly specific detection technique and therefore a confirmatory technique is not required.
Calibration	Calibration standards were prepared in acetonitrile / water (50/50, v/v) .Six calibration points were used and individual calibration data was presented. Linear correlations with coefficients ≥0.99 were obtained for pyraclostrobin and its metabolites 500M04 and 500M85.
Calibration range	Calibration points distributed over a concentration range of 0.05 to 2.5 ng/mL were used.
Assessment of matrix effects is presented	Yes, in the matrix milk. Analysis of matrix-matched standards and solvent standards showed no significant matrix effects. Therefore, solvent standards were used for quantification.
Standard stability	The stability of 500M04 and 500M85 in fortification (methanol) and calibration (acetonitrile / water) solutions for at least 28 days has been determined, if stored refrigerated.
Limit of quantitation	The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was determined to be 0.01 mg/kg in various animal matrices. The limit of detection was estimated at 20% of the limit of quantitation, equivalent to 0.002 mg/kg for all analytes.

Conclusion

The method for analysis of pyraclostrobin and its metabolites in animal matrices uses LC-MS/MS for final determination, which is a highly specific technique. The limit of quantitation is 0.01 mg/kg for each analyte. It could be demonstrated that method L0058/03 fulfills the requirements with regard to linearity, specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine residues of pyraclostrobin and its metabolites 500M04 and 500M85 in animal matrices.

A 2.3.1.2.1.2 Confirmatory method

No confirmatory technique is required as two different, highly specific mass transitions are used for quantitation and qualification.

A 2.3.1.2.1.3 Extraction efficiency

There is no new study on extraction efficiency of the method for the determination of residues in animal matrices. For more information, see section 5.3.4.3.

A 2.3.1.2.2 Analytical method D9902 for the determination of pyraclostrobin and its metabolite 500M77 in animal matrices

A 2.3.1.2.2.1 Method validation

Comments of zRMS:	The study has been accepted. The method (D9902) can be considered adequate for measuring residues of BAS 500 F and BF 500-16 (aka 500M77) in hen tissues and eggs. This method is provided only as supplemental information, to allow a comprehensive evaluation of all basic studies. The validation was conducted on four hen tissues, including egg, liver, muscle, and fat. The method involves a common moiety approach for total residue determination, thus BAS 500 F and its metabolite BF 500-16 were base hydrolyzed into BF 500-5 and BF 500-9, respectively, which were the final analytes of the method. For all matrices BF 500-5 and BF 500-9 were determined by LC/MS/MS. Only quantitation transitions were used in each matrix i.e., m/z 195 → 153 for BF 500-5 (aka 500M04) and m/z 211 → 169 for BF 500-9 (aka 500M90). The LOQ of the method, for all matrices, was 0.05 for each analyte. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered for each analyte. 4 points for calibration were used. 2 fortification levels were used. The overall average recoveries of BAS 500 F and BF 500-16 in hen matrices were l03% ± 17 (n=32) and 65% ± 10 (n=32), respectively. Generally, lower recoveries were obtained for BF 500-9 as compared to BF 500-5. This was expected based on the adsorption of BF 500-9 which was observed during the development of the chromatographic purification steps. This is common for phenolic types of compounds. However, despite the lower recoveries, the precision of the method for BF 500-16 was good, with RSD at or below 11% for each matrix. The method is accepted. The general requirement for an analytical method in animal matrices is covered by the provided method L0151/01. The study was already evaluated in PL.

Reference:	CP 5.2/10
Report	Method validation of BASF analytical method D9902: Method for determination of residues of BAS 500F and its metabolite BF 500-16 in hen tissues using LC/MS/MS, Malinsky D.S.,Riley M.E., 2000 report No 60916

	2000/5004
Guideline(s):	EPA 860.1340
Deviations:	No
GLP:	Yes (certified by United States Environmental Protection Agency)
Acceptability:	Yes

Materials and methods

The method involves a common moiety approach for total residue determination. Pyraclostrobin and its metabolite 500M77 are base hydrolyzed into metabolite 500M04 and 500M90 (a derivative used for determination only and therefore not included in the metabolite list), respectively, so 500M04 and 500M90 are the final analytes of the method. Other compounds sharing the 500M04 and 500M90 moieties are also expected to undergo the same hydrolytic conversion to the analytes mentioned.
As a first step, egg, liver, and muscle samples are extracted as well as hydrolyzed in a sodium hydroxide solution. Fat is first partitioned with hexane and acetonitrile before hydrolysis. After hydrolysis, an aliquot of the fat extract is purified by partitioning with ethyl acetate. For egg, liver and muscle aliquots after hydrolysis, samples are purified by ENV and silica solid phase extraction column chromatography. For all matrices, the final analytes 500M04 and 500M90 are measured by LC-MS/MS. Analysis was accomplished using a Metachem Inertsil 3 µm ODS3 and a gradient of 4 Mm ammonium formate in water and in methanol, respectively with 0.1% formic acid as modifier in both solvents (flow rate: 250 µL/min). The residues are determined by LC-MS/MS in the positive ionization mode monitoring the mass transition 195 → 152.8 for 500M04 and mass transition 211 → 169.3 for 500M90.

Results and discussions

Good recoveries of pyraclostrobin were obtained in the four hen matrices over the fortification range tested (0.05 mg/kg and 0.10 mg/kg). Generally, the overall recoveries for the parent were higher, averaging near 100%. The 500M77 overall recoveries were lower, averaging between 58-71% for the various matrices. The relative standard deviations (RSD, %) for all commodities and all fortification levels were below 20%, except for pyraclostrobin in hen liver at the low fortification level with 32 %. The recovery data are summarized in the table below.

Table A 37: Recovery results from method validation of pyraclostrobin and 500M77 using the analytical method D9902

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Hen egg	pyraclostrobin	0.05 (n = 4)	115	12	Quantitation
		0.1 (n = 4)	93.0	14	m/z 195 → 153
		Overall	**104**	**16**	
	500M77	0.05 (n = 4)	61.0	11	Quantitation
		0.1 (n = 4)	63.0	14	m/z 211 → 169
		Overall	**62**	**11**	
Hen liver	pyraclostrobin	0.05 (n = 4)	110	32	Quantitation
		0.1 (n = 4)	99.3	13	m/z 195 → 153
		Overall	**104**	**24**	
	500M77	0.05 (n = 4)	67.8	10	Quantitation
		0.1 (n = 4)	74.0	2.9	m/z 211 → 169

		Overall	**71**	**8**	
Hen muscle	pyraclostrobin	0.05 (n = 4)	112	16	Quantitation m/z 195 → 153
		0.1 (n = 4)	108	5.6	
		Overall	**110**	**12**	
	500M77	0.05 (n = 4)	55.3	7.0	Quantitation m/z 211 → 169
		0.1 (n = 4)	60.0	4.5	
		Overall	**58**	**7**	
Hen fat	pyraclostrobin	0.05 (n = 4)	95.3	4.0	Quantitation m/z 195 → 153
		0.1 (n = 4)	91.5	4.9	
		Overall	**93**	**4**	
	500M77	0.05 (n = 4)	72.3	18	Quantitation m/z 211 → 169
		0.1 (n = 4)	64.5	28	
		Overall	**68**	**15**	

Table A 38: Characteristics for the analytical method D9902 used for validation of pyraclostrobin residues in animal matrices

	Pyraclostrobin and 500M77
Specificity	The method D9902 allows the specific determination of pyraclostrobin and its metabolites 500M77 in hen matrices. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered for each analyte.
Calibration	Calibration standards were prepared in methanol / water (90/10, v/v). Four calibration points were used and individual calibration data was presented. Linear correlations with coefficients ≥0.99 were obtained for pyraclostrobin and 500M77.
Calibration range	Calibration points distributed over a concentration range of 2.5 to 25 pg/µL were used.
Assessment of matrix effects is presented	No matrix effects were investigated within an independent experiment, therefore instrument recovery sampels would be implemented within each analytical series, if no further information on matrix effects of the investigated matrix is available. Alternatively matrix-matched standards would be used.
Standard stability	The stability in standard solutions of pyraclostrobin and 500M77 for at least 85 days and the stability of 500M04 and 500M90 for at least 102 days was shown in another study (study code 61117).
Limit of determination/quantitation	The limit of quantitation was defined by the lowest fortification level successfully tested, which was 0.05 mg/kg for the analytes in hen tissues.

Conclusion

The results of the method validation study demonstrate that BASF analytical method D9902 fulfills the requirements with regard to linearity, specificity, repeatability, limit of quantitation, and recoveries and is, therefore, applicable to correctly determine residues of pyraclostrobin and 500M77 in hen matrices.

A 2.3.1.2.2.2 Confirmatory method

No confirmatory technique is required as two different, highly specific mass transitions are used for quantitation and qualification.

A 2.3.1.2.2.3 Extraction efficiency

There is no new study on extraction efficiency of the method for the determination of residues in animal matrices. For more information, see section 5.3.4.3.

A 2.3.1.2.3 Analytical method L0452/02 for the determination of pyraclostrobin in animal food

A 2.3.1.2.3.1 Method validation

Comments of zRMS:	The analytical method has been accepted. (See also page 103) The concentration of Metrafenone, Mefentrifluconazole, and Pyraclostrobin in the highest and lowest test item feeding solution applied on the first and last day of application (D 0 and D 9) was determined with the LC-MS/MS method no. L0452/02 within the study. Results for the concurrent procedural recovery samples showed values in an acceptable range with overall mean recoveries of 74.3 % to 96.6 % for the three analytes at both fortification levels with RSD values ≤8.4 %. These results fulfill the requirements according to current guidelines; therefore, the suitability of the applied methodology is proven. The analytical results of concentration control samples for BAS 758 00 F in bee feeding solution confirmed its nominal concentrations of 204 mg /kg and 3261 mg /kg, with recoveries of 90.9 % and 99.8 % for day 0 samples, and 82.4 % and 88.4 % for day 9 samples when determined via the active ingredient BAS 750 F. The concentrations were also confirmed via the active ingredient BAS 560 F with recoveries of 82.7% and 101% for day 0 samples, and 81.1% and 89.5 % for day 9 samples. The concentrations were also confirmed via the active ingredient BAS 500 F with recoveries of 88.3 % and 104 % for day 0 samples, and 94.8 % and 85.1 % for day 9 samples. The mean measured concentrations of untreated control were always below the LOQ for all sampling intervals. No significant peak interferences occurred at the retention times and mass transitions of all analytes. Furthermore, no residues of the active ingredients were found in the control samples, i.e., the concentrations of active ingredients were below 30% of the LOQ.

Reference:	CP 5.2/11
Report	Chronic toxicity of BAS 758 00 F to the honey bee Apis mellifera L. under laboratory conditions Dressler, A., 2021 Report No. 892147, 2148BAC0053 BASF DocID 2021/2008152
Guideline(s):	OECD TG 245 (2017)

Deviations: No

GLP: yes

(Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Acceptability: Yes

Principle of the Method 0.25 g bee feeding solution samples were extracted by shaking with acetonitrile/water/formic acid (50/50/1, v/v/v). After addition of QuEChERS-Salt the samples were shaken, vortexed and centrifugated. 1 mL aliquots of the acetonitrile-phase were diluted with acetonitrile/water/formic acid (50/50/1, v/v/v) and analyzed by LC-MS/MS. Samples were analysed at mass transition *m/z* 388 → 163 for quantitation of pyraclostrobin.

Recovery findings Fortification levels of 0.10 mg/kg and 400 mg/kg were validated for BAS 500 F. Method validation acceptance criteria were met with overall mean recovery values of 74.3% at fortification level 0.10 mg/kg and 88.0% at 400 mg/kg in bee feeding solution matrices.

Table A 39: Recovery results of pyraclostrobin using the analytical method L0452/02

Matrix	Analyte	Fortification level (mg/kg)	Number of replicates	Mean recovery [%]	RSD (%)	Overall recovery [%]	RSD (%)	Comments
Bee feeding solution	**BAS 500 F**	0.10	5	74.3	6.1	81.2	10.6	Mass transition m/z 388→163
		400	5	88.0	5.9			

RSD = Relative standard deviation

Linearity Good linearity (r = 0.9996) was observed in the range of 0.01 ng/mL to 1.0 ng/mL for one matrix-matched calibration standard for quantifier mass transitions of BAS 500 F.
The following calibration curve was given in the study report:
$y=3.10\cdot10^{6}x+8.83\cdot10^{3}$ (transition 388→163)

Specificity The method allows the specific determination of BAS 500 F in bee feeding solution using LC-MS/MS.

Matrix Effects Potential matrix effects were compensated by using matrix-matched calibration standards.

Interference No significant peak interferences occurred at the retention time and mass transition of the analyte.

Limit of Quantification The limit of quantification (LOQ) representing the lowest validated level with sufficient recovery and precision was 0.1 mg/kg. The LOQ is therefore well within the range of linearity calibration tests.

Limit of Detection The limit of detection (LOD) is 0.01 ng/mL corresponding to the lowest calibration standard.

Stability Working Solutions All test samples were measured within 30 days of sampling and therefore

further studies were not required.

Extract Stability All test samples were measured within one day after extraction and therefore further studies were not required.

Repeatability The relative standard deviations (RSD, %) for all fortification levels were < 20%.

Reproducibility Reproducibility of the method was not determined within the validation study.

Conclusion It could be demonstrated that analytical method L0452/02 fulfils the requirements with regard to specificity, linearity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BAS 500 F (pyraclostrobin).

A 2.3.1.3 Description of Methods for the Analysis of Soil (KCP 5.2)

A 2.3.1.3.1 Analytical method L0166/01 for determination of pyraclostrobin and metabolites in soil

Analytical method L0166/01 for the determination of residues of pyraclostrobin and its metabolites in soil is summarized below. The method can also be used for post-authorization control and monitoring purposes.

A 2.3.1.3.1.1 Method validation

Comments of zRMS:	The study has been accepted. The method was validated at two fortification levels for two soils (LUFA 2.2 and LUFA 5M) and two soils freshly collected from the field. For each fortification level and matrix, five replicates were analysed. Additionally, at least two replicates of unfortified control samples were examined per analytical sample set. For each analyte, two mass transitions were also evaluated. As matrix effects were observed during method development, matrix-matched standards were used for the calibration. Six calibration levels were injected. Recovery data was reported for each mass transition and matrix considered. The mean recovery values of the validation experiments were between 70 % and 120 %. Good linearity (r > 0.977) was observed in the working range for both mass transitions of BAS 500 F, 500M01, 500M02 and 500M07. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered for each analyte. The LOQ defined by the lowest fortification level successfully tested was set at 0.001 mg/kg for all analytes. The RSD for all fortification levels were below 20%. It was found that calibration solutions of each analyte were stable (less than 10% decline) for at least 3 weeks refrigerated. BAS 500 F and its metabolites are stable in tested soil extracts over the period of 7 days. The method L0166/01 is accepted. It fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BAS 500 F (pyraclostrobin) and its metabolites (500M01,500M02, 500M07) in all the tested soils. Since for every compound the quantitation was possible at two different transitions, no additional confirmatory technique is required. The study was already evaluated in PL.

Reference: CP 5.2/12

Report	Validation of analytical method L0166/01: Determination of BAS 500 F (Pyraclostrobin) and its metabolites Reg.No. 364380 (500M01), Reg.No. 369315 (500M02) and Reg.No. 340266 (500M07) in soil using LC/MS/MS, Tilting N.,Sopena-Vazquesz F., 2014 report No 370957 2013/1184817
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Materials and methods

Soil samples of 5 g are placed into a PP tube and extracted with 25 mL acetonitrile by mechanical shaking for 30 min at 225 rpm. Subsequently, the sample is centrifuged and the liquid phase is decanted and evaporated to dryness. The extraction is repeated with 25 mL acetonitrile/water (80/20, v/v). After centrifugation, the liquid phase from the second extraction is separated and used to re-dissolve the dried residue from the first extraction. An additional dilution with acetonitrile/water (80/20, v/v) is performed when necessary prior to analysis.
Final determination is performed by LC-MS/MS using an Acquity BEH C18 analytical column and a gradient mixture of water – acetonitrile with formic acid as modifier at a flow rate of 500 µL/min. Detection is accomplished using positive ion electrospray ionization tandem mass spectrometry (MS/MS-ESI+) monitoring mass transitions 388 → 194, 611 → 223, 595 → 207 and 358 → 132 for quantification and 388 → 163, 611 → 417, 595 → 401 and 358 → 164 for confirmation of analytes pyraclostrobin and metabolites 500M01, 500M02 and 500M07, respectively.

Results and discussions

The method L0166/01 was proved to be suitable to determine residues of pyraclostrobin and its metabolites 500M01, 500M02 and 500M07 in soil using LC-MS/MS. Samples were fortified with the analytes at the limit of quantification of 0.001 mg/kg and 10 times higher (0.01 mg/kg). The mean recovery values were between 70% and 110% and the relative standard deviations (RSD) for all fortification levels were below 20%. The recovery data are summarized in the table below.

Table A 40: Recovery results from method validation of pyraclostrobin and its metabolites using the analytical method L0166/01 (LUFA 5M and LUFA 2.2)

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
LUFA 5M	pyraclostrobin	0.001 (n = 5)	100.1	2.4	388 → 194
		0.01 (n = 5)	96.5	2.8	Quantifier transition
		0.001 (n = 5)	101.4	2.5	388 → 163
		0.01 (n = 5)	95.7	1.6	Qualifier transition
	500M01	0.001 (n = 5)	102.0	4.5	611 → 223
		0.01 (n = 5)	100.1	4.7	Quantifier transition
		0.001 (n = 5)	101.9	8.9	611 → 417

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
		0.01 (n = 5)	98.4	3.8	Qualifier transition
	500M02	0.001 (n = 5)	101.5	3.1	595 → 207
		0.01 (n = 5)	97.7	5.0	Quantifier transition
		0.001 (n = 5)	98.2	3.2	595 → 401
		0.01 (n = 5)	96.3	3.6	Qualifier transition
	500M07	0.001 (n = 5)	98.7	5.3	358 → 132
		0.01 (n = 5)	94.9	4.6	Quantifier transition
		0.001 (n = 5)	103.4	4.4	358 → 164
		0.01 (n = 5)	96.9	4.4	Qualifier transition
LUFA 2.2	pyraclostrobin	0.001 (n = 5)	99.3	2.2	388 → 194
		0.01 (n = 5)	98.9	1.7	Quantifier transition
		0.001 (n = 5)	102.8	2.1	388 → 163
		0.01 (n = 5)	98.0	3.1	Qualifier transition
	500M01	0.001 (n = 5)	99.7	1.8	611 → 223
		0.01 (n = 5)	98.8	5.1	Quantifier transition
		0.001 (n = 5)	95.6	2.7	611 → 417
		0.01 (n = 5)	96.3	7.5	Qualifier transition
	500M02	0.001 (n = 5)	96.5	3.2	595 → 207
		0.01 (n = 5)	95.3	3.2	Quantifier transition
		0.001 (n = 5)	89.2	1.5	595 → 401
		0.01 (n = 5)	87.8	1.8	Qualifier transition
	500M07	0.001 (n = 5)	100.5	2.9	358 → 132
		0.01 (n = 5)	98.8	2.5	Quantifier transition
		0.001 (n = 5)	100.1	4.1	358 → 164
		0.01 (n = 5)	97.2	3.0	Qualifier transition

Table A 41: Recovery results from method validation of pyraclostrobin and its metabolites using the analytical method L0166/01 (Field Soil Italy and Field Soil Germany)

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Field Soil Italy	pyraclostrobin	0.001 (n = 5)	102.1	2.5	388 → 194
		0.01 (n = 5)	103.6	2.2	Quantifier transition
		0.001 (n = 5)	101.9	2.4	388 → 163
		0.01 (n = 5)	104.2	4.7	Qualifier transition
	500M01	0.001 (n = 5)	96.6	4.2	611 → 223
		0.01 (n = 5)	97.3	3.8	Quantifier transition
		0.001 (n = 5)	97.0	5.8	611 → 417
		0.01 (n = 5)	98.6	3.7	Qualifier transition
	500M02	0.001 (n = 5)	102.4	1.9	595 → 207
		0.01 (n = 5)	96.8	1.7	Quantifier transition
		0.001 (n = 5)	100.6	2.3	595 → 401
		0.01 (n = 5)	95.6	4.0	Qualifier transition
	500M07	0.001 (n = 5)	100.7	3.4	358 → 132
		0.01 (n = 5)	103.1	4.4	Quantifier transition
		0.001 (n = 5)	97.6	2.9	358 → 164
		0.01 (n = 5)	99.1	5.2	Qualifier transition
Field Soil Germany	pyraclostrobin	0.001 (n = 5)	100.2	3.5	388 → 194
		0.01 (n = 5)	97.9	5.6	Quantifier transition
		0.001 (n = 5)	98.3	1.5	388 → 163
		0.01 (n = 5)	98.5	8.4	Qualifier transition
	500M01	0.001 (n = 5)	94.6	3.6	611 → 223
		0.01 (n = 5)	88.8	8.9	Quantifier transition
		0.001 (n = 5)	94.7	3.6	611 → 417
		0.01 (n = 5)	88.8	7.6	Qualifier transition
	500M02	0.001 (n = 5)	101.0	3.5	595 → 207
		0.01 (n = 5)	95.2	9.9	Quantifier transition
		0.001 (n = 5)	97.4	3.4	595 → 401
		0.01 (n = 5)	89.4	10.9	Qualifier transition
	500M07	0.001 (n = 5)	96.8	2.8	358 → 132
		0.01 (n = 5)	95.8	6.0	Quantifier transition
		0.001 (n = 5)	101.5	4.9	358 → 164
		0.01 (n = 5)	100.3	8.0	Qualifier transition

Table A 42: Characteristics for the analytical method used for validation of pyraclostrobin and its metabolites residues in soil

	Pyraclostrobin and metabolites 500M01, 500M02 and 500M07
Specificity	The method L0166/01 allows the specific determination of pyraclostrobin and its metabolites 500M01, 500M02 and 500M07 in soil. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered for each analyte.
Calibration (type, number of data points)	Linear regression was used for calibration using six calibration levels. In case of metabolite 500M02 sum of E- and Z-isomer were used for calibration and calculation of residues. Matrix-matched standards were used for calibration.
Calibration range	Good linearity (regression coefficients ≥ 0.977) was observed in the range of 0.01 ng/mL to 0.75 ng/mL for pyraclostrobin and its metabolites 500M01, 500M02 and 500M07.
Assessment of matrix effects is presented	Matrix effects were observed during method development, therefore, matrix-matched standards were used for the calibration.
Standard stability	Fortification and calibration solutions of each analyte (prepared in acetonitrile/water, 80/20, v/v) were stable for at least 2 weeks when stored under refrigerated conditions at 4°C in the dark.
Extract stability	Pyraclostrobin and its metabolites were stable in soil extracts over a tested time period of 7 days when stored under refrigerated conditions at 4°C in the dark.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.001 mg/kg, corresponding to the lowest fortification level. The limit of detection (LOD) is 0.0002 mg/kg, corresponding to the lowest calibration standard.

Conclusion

The method L0166/01 used LC MS/MS for the final determination of pyraclostrobin and its metabolites 500M01, 500M02 and 500M07 in different soils with a limit of quantification (LOQ) of 0.001 mg/kg for pyraclostrobin and each metabolite.

It could be demonstrated that method L0166/01 fulfills the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of pyraclostrobin and its metabolites 500M01, 500M02 and 500M07 in all tested soils.

A 2.3.1.3.2 Analytical method L0161/01 for determination of pyraclostrobin in soil

A 2.3.1.3.2.1 Method Validation

Comments of zRMS:	The study has been accepted. The purpose of this study was a validation of the method L0161/01 for determination of pyraclostrobin in soil using HPLC-MS/MS at the LOQ of 0.001 mg/kg. To validate this method for each matrix 2 fortification levels were analysed in 5 replicates. In addition, at least two untreated control samples have been analysed per analytical sample set. The method allows the specific determination of pyraclostrobin in soil using two transitions per analyte in each matrix. No significant interferences between analyte and matrix were observed. The calibration was done with 7 points. Good linearity was observed. All single mean recovery values (10 replicates) for BAS 500 F are in the range 91.3 - 95.2 % for both mass transitions validated. The mean recovery values were between 70% and 110%. For each fortification level the RSD values are < 20%. Based on these results method L0161/01 is accepted. It can be considered valid for the determination of pyraclostrobin in two different types of soils Lufa 2.2 and 5M with the LOQ of 0.001 mg/kg. The study was already evaluated in PL.

Reference:	CP 5.2/13
Report	Validation of analytical method L0161/01: Determination of BAS 500 F in soil at LOQ 0.001 mg/kg, Zangmeister W., 2010 report No 370957_1 2010/1075848
Guideline(s):	SANCO/825/00 rev. 7 (17 March 2004), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Materials and methods

Soil samples of 5 g are placed into a PP tube and extracted with 25 mL methanol/water (80/20, v/v) by mechanical shaking for 30 min at 225 rpm. Subsequently, the sample is centrifuged. The extract is diluted with appropriate amount of methanol/water (80/20, v/v) for HPLC-MS/MS analysis.
Final determination is performed by LC-MS/MS using a Betasil C18 analytical column and a gradient mixture of water – acetonitrile with formic acid as modifier at a flow rate of 600 µL/min. Detection is accomplished using positive ion electrospray ionization tandem mass spectrometry (MS/MS-ESI+) monitoring mass transitions 388 → 194 for quantification and 388 → 163 for confirmation of pyraclostrobin.

Results and discussions

The method L0161/01 was proved to be suitable to determine residues of pyraclostrobin in soil using LC-MS/MS. Samples were fortified with the analyte at the limit of quantification of 0.001 mg/kg and 10

times higher (0.01 mg/kg). The mean recovery values were between 70% and 110% and the relative standard deviations (RSD) for all fortification levels were below 20%. The recovery data are summarized in the table below.

Table A 43: Recovery results from method validation of pyraclostrobin using the analytical method L0161/01

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
LUFA 2.2	pyraclostrobin	0.001 (n = 5)	96.6	1.1	388 → 194
		0.01 (n = 5)	93.7	4.4	Quantifier transition
		0.001 (n = 5)	95.6	2.7	388 → 163
		0.01 (n = 5)	94.0	3.5	Qualifier transition
LUFA 5M	pyraclostrobin	0.001 (n = 5)	93.0	3.0	388 → 194
		0.01 (n = 5)	89.5	1.0	Quanitier transition
		0.001 (n = 5)	93.1	1.0	388 → 163
		0.01 (n = 5)	90.6	3.8	Qualifier transition

Table A 44: Characteristics for the analytical method used for validation of pyraclostrobin residues in soil

	Pyraclostrobin
Specificity	The method L0161/01 allows the specific determination of pyraclostrobin in soil. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered for pyraclostrobin.
Calibration (type, number of data points)	Linear regression was used for calibration using seven calibration levels, which were prepared in methanol/water (80/20, v/v).
Calibration range	Good linearity (regression coefficients ≥ 0.999) was observed in the range of 0.025 ng/mL to 1.0 ng/mL for pyraclostrobin.
Assessment of matrix effects is presented	No matrix effects were investigated within an independent experiment, therefore instrument recovery sampels would be implemented within each analytical series, if no further information on matrix effects of the investigated matrix is available. Alternatively matrix-matched standards would be used.
Standard stability	In a previous study, stability of standard solutions prepared in methanol/water (80/20, v/v) was proved to be stable for at least 28 days, when stored under refrigerated conditions.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.001 mg/kg, corresponding to the lowest fortification level. The limit of detection (LOD) is 0.025 ng/mL, corresponding to the lowest calibration standard.

Conclusion

The method L0161/01 used LC MS/MS for the final determination of pyraclostrobin in different soils with a limit of quantification (LOQ) of 0.001 mg/kg.
It could be demonstrated that method L0161/01 fulfills the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of pyraclostrobin in all tested soils.

A 2.3.1.4 Description of Methods for the Analysis of Water (KCP 5.2)

Analytical methods L0182/01 (BASF DocID 2012/1009641) and its add-on L0182/02 (BASF DocID 2014/1004891) used for the determination of pyraclostrobin and its metabolites in water are also used for post-authorization control and monitoring purposes und are summarized in appendix section A 2.3.2.4.1.1 (KCP 5.3.4).
Analytical method APL0500/01 is used in ecotoxicological studies for the analysis of pesticides in aqueous solutions. Several active ingredients were validated during the course of the validation study, however, only the validation results of pyraclostrobin are summarized below.
Meanwhile, to this method version further analytes have been included and validated, resulting in version 02 and 03 of the method (APL0500/02+03). The last version covers all analytes, independent in which version they have been validated. Therefore, it could be possible, that version 02 or version 03 might be cited in studies. Both is correct, as this is a multi-component method, validated within multiple validation studies.

A 2.3.1.4.1 Analytical method APL0500/01 for determination of pyraclostrobin in water

A 2.3.1.4.1.1 Method validation

Comments of zRMS:	The study has been accepted. For the support of eco-toxicological studies, the analytical method APL0500/01 was validated for pesticides determination in water by HPLC with MS-detection at LOQ of 0.001 mg/L. The method is suitable for dose verification of known substances and known nominal concentrations of BAS 325 F, BAS 421 F, BAS 480 F, BAS 500 F, BAS 550 F, BAS 560 F, BAS 600 F, BAS 800 H and Reg.No.4993353. The analyte was identified by retention time confirmation with the reference item and then by MS-detection (m/z 388). Samples were prepared and analyzed on 2 fortification levels with 5 replicates; no relevant interferences have been detected. Calibration with 5 points; good linearity was obtained with correlation coefficients > 0.995. The recoveries were in acceptable range with RSDs < 20%. This method is accepted according to the purpose of the method. It can be concluded that it is specific, and no confirmatory method is required. The study was already evaluated in PL.

Reference:	CP 5.2/14
Report	Validation of analytical method APL0500/01: Determination of pesticides in water by HPLC/MS, Obermann M., 2005 report No 230119_1 2005/1026675
Guideline(s):	SANCO/825/00 rev. 7 (17 March 2004)
Deviations:	No
GLP:	yes

(certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany Fed.Rep.)

Acceptability: Yes

Materials and methods

The samples are diluted with a mixture of acetonitrile/water and acidified with formic acid prior to analysis. Final determination is performed by HPLC/MS using a YMC Pro C18 analytical column and a gradient mixture of water – acetonitrile with formic acid as modifier at a flow rate of 700 µL/min. Detection is accomplished using positive ion electrospray ionization mass spectrometry. The identity of the test item was proved by coincidence of the retention times with the retention times of the authentic reference item peaks and furthermore by MS-detection (m/z 388).

Results and discussions

The method APL0500/01 was proved to be suitable to determine residues of pyraclostrobin in aqueous solutions using HPLC/MS. Samples were fortified with the analyte at the limit of quantification of 0.001 mg/L and 100 times higher (0.1 mg/L). The mean recovery values were between 70% and 110% and the relative standard deviations (RSD) for all fortification levels were below 20%. The recovery data are summarized in the table below.

Table A 45: Recovery results from method validation of pyraclostrobin using the analytical method APL0500/01

Matrix	Analyte	Fortification level (mg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Mix-water of Frankenthal (Tap-water)	pyraclostrobin	0.001 (n = 5)	99.4	2.2	HPLC/MS (retention time ~ 20 min, m/z 388)
		0.1 (n = 5)	104.9	3.0	

Table A 46: Characteristics for the analytical method used for validation of pyraclostrobin residues in water

	Pyraclostrobin
Specificity	The identification and quantification of pyraclostrobin were based on the retention time and the use of reference items and by MS-detection. Under the described conditions, the method is specific for the determination of pyraclostrobin in water. Under the given chromatographic conditions no significant co-elution of one of the active ingredient peaks with an unknown component was observed. As the method is only used for dose verification of known substances and known nominal concentrations, no additional confirmatory technique is necessary.
Calibration (type, number of data points)	Linear regression was used for calibration using five calibration levels, which were prepared in water/acetonitrile/ formic acid (80/20/0.1, v/v/v).
Calibration range	Good linearity (regression coefficients > 0.995) was observed in the range of 0.5 ng/mL to 130 ng/mL for all compounds in the study.
Assessment of matrix effects is presented	No matrix effects were investigated within an independent experiment, therefore instrument recovery sampels would be implemented within each analytical series, if no further information on matrix effects of the investigated matrix is available. Alternatively matrix-matched standards would be used.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.001 mg/L, corresponding to the lowest fortification level. The limit of detection (LOD) is 0.0005 mg/L, corresponding to the lowest calibration standard.

Conclusion

The method APL0500/01 used HPLC/MS for the final determination of different pesticides in water with a limit of quantification (LOQ) of 0.001 mg/L for each compound including pyraclostrobin.
The described method is suitable for the determination of residues of pesticides including pyraclostrobin in water.

A 2.3.1.4.2 Analytical method L0361/01 for the determination of pyraclostrobin in in water

A 2.3.1.4.2.1 Method validation

Comments of zRMS:	The method validation has been accepted. (See also a 2.1.1.1 page 77) The objective of this study was to validate LC-MS/MS method L0361/01 for the determination of **BAS 500 F (Pyraclostrobin),** BAS 510 F (Boscalid), BAS 550 F (Dimethomorph), BAS 700 F (Fluxapyroxad), **BAS 750 F (Mefentrifluconazole),** BAS 656 H (Dimethenamid-P), BAS 684 H (Cinmethylin), BAS 720 H (Imazamox), BAS 9178 H (Clomazone), BAS 395 I (Dinotefuran), BAS 550 I and Prothioconazole in tap water and M4-medium. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary. The quantification of the twelve analytes is based on the monitoring of two mass transitions. Recovery data are reported for each mass transition and matrix considered. The method has a limit of quantitation of 0.1 µg/L in tap water and M4-medium for each analyte of interest.

	Good linearity (r > 0.998) was observed in the range of 0.01 ng/mL to 0.8 ng/mL for both mass transitions of the analytes. The mean recovery values of the validation experiments over all tested analytes were between 83% and 106%, which fulfils the legal requirements for mean recovery values. The relative standard deviation (RSD, %) for all fortification levels was below 20%. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of the analytes. It could be demonstrated that method L0361/01 fulfils the requirements with regard to specificity, linearity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BAS500F (**Pyraclostrobin**), and BAS 750 F (**Mefentrifluconazole**).

Reference:	CP 5.1.2/15
Report	Validation of BASF Method L0361/01 for the Determination of Pesticides in Water by LC-MS/MS, Andre, M., 2017 report No 783160, IF-17/04022633 BASF DocID 2017/1065621 Authority registration No
Guideline(s):	EFSA Panel on Plant Protection Products and their Residues (PPR), OECD-ENV/JM/MONO/(2007)17, SANCO/3029/99 rev. 4 (11 July 2000)
Deviations:	No
GLP:	yes (certified by Hess. Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden, Germany)
Acceptability:	Yes

Principle of the Method

A 5 g tap water or M4-medium specimen aliquot is extracted by shaking with Acetonitrile/Water/HCOOH, 400/600/2, v/v/v. An aliquot of the extract is then used for determination by LC-MS/MS. Analysis was accomplished using a Pinnacle DB AQ C18 column and a water-acetonitrile gradient with formic acid as modifier at a flow rate of 600 µL/min. Samples were analysed at mass transition *m/z* 388 → 194 for quantitation and *m/z* 388 → 163 for confirmation for pyraclostrobin.

Recovery findings

Fortification levels of 0.1 µg/L, 1.0 µg/L and 10 µg/L were validated for BAS 500 F. Method validation acceptance criteria were fully met with mean recovery values between 95% and 102% in all matrices tested.

Table A 47: Recovery results from method validation of pyraclostrobin using the analytical method L0361/01

Matrix	Analyte	Fortification level (µg a.s./L)	Number of replicates	Mean recovery [%]	RSD (%)	Overall recovery [%]	RSD (%)	Comments
Tap water	BAS 500 F	0.1	5	97	2.2	98	2.0	Mass transition m/z 388→194*
		1.0	5	96	2.0			
		10	5	99	0.6			
		0.1	5	95	2.6	96	2.3	Mass transition m/z 388→163
		1.0	5	96	1.5			
		10	5	98	2.4			
M4-medium		0.1	5	99	1.8	98	2.2	Mass transition m/z 388→194*
		1.0	5	98	1.5			
		10	5	97	3.0			
		0.1	5	96	1.9	99	3.1	Mass transition m/z 388→163
		1.0	5	102	2.4			
		10	5	99	1.1			

*used as quantification transition
RSD = Relative standard deviation

Linearity

Good linearity ($r \geq 0.99993$) was observed in the range of 0.01 ng/mL to 0.8 ng/mL for the two mass transitions of BAS 500 F. At least six calibration levels, prepared as matrix matched standards, distributed over the tested concentration range were used.
The following calibration curves were given in the study report:
y=173485.1x-194.5 (transition 388→194) in Tap Water
y=123703.1x+3123 (transition 388→163) in Tap Water
y=183468.3x-3903 (transition 388→194) in M4-Medium
y=127694.9x+6251 (transition 388→163) in M4-Medium

Specificity

The method allows the specific determination of BAS 500 F in tap water and M4-Medium using LC-MS/MS. Detection is accomplished by highly selective MS/MS-detection using two mass transitions.

Matrix Effects

It was demonstrated that the matrix-load in the tested matrix-matched standards had no significant influence on the detection of the analytes.

Interference

No significant interferences (> 30% LOQ) were observed at the appropriate retention time and using the given detector.

Limit of Quantification

The limit of quantification (LOQ) representing the lowest validated level with sufficient recovery and precision was 0.1 µg/L. The LOQ is therefore well within the range of linearity calibration tests.

Limit of Detection

The limit of detection (LOD) is 0.02 µg/L corresponding to the lowest calibration standard.

Stability Working Solutions

Stability tests confirmed that the analytes were stable for at least 28 days in calibration solutions in tap water matrix and 29 days in calibration solutions in M4-medium matrix when stored refrigerated at approximately 2 – 8 °C in the dark. For fortification solutions stability was proven for 28 days. Mean uncorrected recoveries were in an acceptable range 85% to 110% for

	calibration solutions over the tested period. As the stability was confirmed over all concentrations investigated, it can be concluded that concentration dependency is not given
Extract Stability	The stability of specimen final volumes was not investigated during this study, as storage stability of matrix matched standards was proven and composition of matrix matched standards and specimen final volume is equal.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were < 20%.
Reproducibility	Reproducibility of the method was not determined within the validation study.
Conclusion	It could be demonstrated that analytical method L0361/01 fulfils the requirements with regard to specificity, linearity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BAS 500 F (pyraclostrobin).

A 2.3.1.5 Description of Methods for the Analysis of Air (KCP 5.2)

Analytical method L0197/01 (BASF DocID 2012/1220256) used for the determination of pyraclostrobin in air is also used for post-authorization control and monitoring purposes und is summarized in appendix section A 2.3.2.5.1 (KCP 5.3.4).

A 2.3.1.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

A 2.3.1.7 Other Studies/ Information

No new or additional studies have been submitted

A 2.3.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.3.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

A 2.3.2.1.1 Analytical method 421/0 for determination of BAS 500 F and its metabolite 500M07 residues in plant matrices

A 2.3.2.1.1.1 Method validation

During the peer review under Directive 91/414/EEC, method no. 421/0 was already evaluated (DAR 2001).

A 2.3.2.1.1.2 Independent laboratory validation (green coffee and green tea)

In context of the re-evaluation of existing MRLs, EFSA identified the lack of an enforcement method for coffee as data gap. For proving that the residue analytical method 421/0, which is recommended as enforcement method in other plant matrices, is suitable for coffee and tea, the subsequent independent laboratory validation study has been performed. Due to their matrix constituents, coffee and tea are considered as matrices which are difficult to analyze.

Comments of zRMS:	The study has been accepted. This study was conducted to independently validate the method 421/0 for determination of pyraclostrobin and desmethoxy pyraclostrobin (BF 500-3) in green tea and green coffee. The final determination was performed by HPLC-MS/MS. 2 transitions were evaluated per analyte. Therefore, no confirmatory technique is needed.

	For both matrices 2 fortification levels with 5 replicates and 2 blanks were used. The mean recovery values for both analytes were between 70-110% with RSDs for all matrices and fortification levels < 20%. The LOQ defined as the lowest fortification level was successfully validated for pyraclostrobin at 0.02 mg/kg, and for BF 500-3 at 0.05 mg/kg. 5 calibration points were used. Good linearity was observed with a correlation coefficient ≥ 0.99. The ILV in green tea and coffee is accepted. The analytical methods for the determination of pyraclostrobin in foodstuffs of plant origin were evaluated in the previous Annex I inclusion process and during more recent evaluations performed by EFSA in context of MRL applications. The BASF methods 421/0, 445/0 and 535/1 (synonym: L0076) allow the determination of pyraclostrobin and its metabolite 500M07 (BF 500-3) in multiple crops. The data generation methods are fully validated in separate GLP studies; furthermore, an extensive set of concurrent fortification experiments exist from supervised field trials or processing studies, which equals independent lab validation. The general suitability of the residue analytical methods provided for data generation and risk assessment has also been confirmed by EFSA in the recently published Reasoned Opinion on MRLs (Review of established MRLs according to Reg. 396/2005 (Art. 12); EFSA Journal 2011;9(8):2344). Therefore, the provided data package can be seen as "fit for purpose". In addition, a new validation of method L0076 has been provided (A 2.2.1.1.3), following the same method principle, and applicable as additional/independent validation. The study was already evaluated in PL.

Reference:	CP 5.1.2/16
Report	Independent laboratory validation of the BASF analytical method 421/0: Method for determination of BAS 500 F and its metabolite BF 500-3 residues in plant matrices using LC/MS/MS, Scherthan D., 2011 report No 252652 2011/1268146
Guideline(s):	EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), EEC 96/46 (16.07.1996)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Materials and methods

Pyraclostrobin and its metabolite 500M07 were extracted from plant matrices (green coffee and green tea) using a mixture of methanol/water 70/30. A 0.5% aliquot of the extract was removed and cleaned by C18 Polar Plus micro-column. The final determination of BAS 500 F and its metabolite 500M07 was performed by HPLC-MS/MS using an Inertsil Phenyl 5µm column. Detection of BAS 500 F was accomplished in ESI+ mode at mass transitions *m/z* 388 → *m/z* 194 for quantitation and *m/z* 388 → *m/z* 163 for confirmation. Metabolite 500M07 is quantified on transition *m/z* 358 → *m/z* 164, and *m/z* 358 → *m/z* 132 serves for confirmatory purpose.

Results and discussions

In all matrices tested, the mean recovery values of pyraclostrobin and its metabolite 500M07 were between 70% and 110%. The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%. The detailed results are given in the table below.

Table A 48: Recovery results from method validation of BAS 500 F and 500M07 using the analytical method 421/0

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Green tea	BAS 500 F	0.02 (n=5)	79.9	8.6	Quantitation
		0.2 (n=5)	80.3	2.6	m/z 388→194
		0.02 (n=5)	78.9	8.9	Confirmation
		0.2 (n=5)	79.6	2.8	m/z 388→163
	500M07	0.05 (n=5)	72.6	4.5	Quantitation
		0.5 (n=5)	97.0	7.2	m/z 358→164
		0.05 (n=5)	87.5	9.6	Confirmation
		0.5 (n=5)	86.8	1.3	m/z 358→132
Green coffee	BAS 500 F	0.02 (n=5)	87.7	4.0	Quantitation
		0.2 (n=5)	85.4	1.8	m/z 388→194
		0.02 (n=5)	87.0	4.4	Confirmation
		0.2 (n=5)	85.2	1.6	m/z 388→163
	500M07	0.05 (n=5)	84.8	5.7	Quantitation
		0.5 (n=5)	84.2	8.6	m/z 358→164
		0.05 (n=5)	81.7	10.8	Confirmation
		0.5 (n=5)	89.4	1.4	m/z 358→132

Table A 49: Characteristics for the analytical method used for validation of pyraclostrobin residues in plant matrices (coffee grain, soya bean grain, wheat grain)

	Pyraclostrobin and metabolite 500M07
Specificity	Highly selective determination of BAS 500 F and its metabolite 500M07 using LC-MS/MS monitoring two mass transitions. There were interferences at the retention time corresponding to BAS 500 F and its metabolite 500M07 in the control specimens of green tea and green coffee. Therefore, the recovery data were corrected for interference from matrix compounds of the appropriate unfortified sample.
Calibration (type, number of data points)	Calibration standards were prepared in methanol/99:1 of 4 mM arnmonium formate in water and 01% formic acid (80/20, v/v). Five calibration points were used and individual calibration data was presented. Good linearity was observed with a correlation coefficient ≥0.99.
Calibration range	Calibration points distributed over a concentration range of 0.05 to 5.0 ng/mL.
Assessment of matrix effects is presented	Analysis of matrix-matched standards compared to solvent standards showed significant matrix effects. Therefore, matrix-matched calibration standard solutions were used for quantification.
Limit of determination/quantification	The limit of quantitation representing the lowest validated level with sufficient recovery and precision was 0.02 mg/kg for BAS 500 F and 0.05 mg/kg for the metabolite 500M07
Reproducibility	The ILV confirmed that the analytical method 421/0 is suitable for coffee and tea.

Conclusion

The method uses highly specific LC-MS/MS for final determination for analysis of pyraclostrobin and its metabolite 500M07. The method is sufficiently validated for the commodities green tea and green coffee. Thereby, it could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantitation and recoveries.

A 2.3.2.1.1.3 Independent laboratory validation (cabbage, onion, orange, wheat grain, hops and oilseed rape)

Comments of zRMS:	The study has been accepted. The purpose of the study was an independent validation of the method 421/0 for determination the residues of pyraclostrobin and desmethoxy pyraclostrobin (BF 500-3) in head cabbage, onions, oranges (flesh), wheat grain, hops and rape seed performed according to the attached in this study Technical Procedure of BASF Method Number D9808 (USA), 421/0 (Germany). The final determination of BAS 500 F and BF 500-3 was performed by HPLC-MS/MS at mass transition *m/z* 388 → *m/z* 194 for pyraclostrobin quantitation and *m/z* 358 → *m/z* 164 for its desmethoxy derivative (consistently with the technical procedure). The LOQ was set 0.02 mg/kg in all matrices. 4 points were used for calibration. Acceptable linearity with correlation coefficients > 0.995 was obtained for all determinations. For all matrices 2 fortification levels with 5 replicates and a control per each were tested. No matrix interferences were observed. In all matrices the obtained mean recovery values can be accepted. Also, RSDs for all matrices and both fortification levels were < 20%. The method 421/0 is accepted as independently validated in head cabbage, onions,

	oranges (flesh), wheat grain, hops, and rape seed. However, the further confirmatory technique is needed. The study was already evaluated in PL.

Reference:	CP 5.2/17
Report	Independent laboratory validation of BASF method Number D9808 (USA), 421/0 (Germany), an analytical method for the determination of residues of BAS 500 F and its metabolite BF 500-3, Devine H.C., 2002 report No CEMR-1655 2002/1007082
Guideline(s):	EEC 91/414, EEC 96/68, Guidance Document on Residue Analytical Methods (SANCO/825/00rev.6)
Deviations:	No
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
Acceptability:	Yes

Materials and methods

Pyraclostrobin and its metabolite 500M07 were extracted from plant matrices (wheat grain and head cabbage) using a mixture of methanol/water (70/30, v/v). After filtration, an aliquot of the extract was cleaned by C18 Polar Plus micro column eluted with dichloromethane.
From onion, orange and hops specimens residues were extracted using a mixture of methanol/water (70/30, v/v) and filtered. An aliquot of the extract was cleaned by C18 Polar Plus micro column, eluted with dichloromethane followed by an additional clean-up stage using micro silica columns.
Extraction of residues from rape seed was performed by homogenization with acetonitrile followed by addition of hexane. After filtration, the acetonitrile layer of the extract was partitioned with hexane. An aliquot was concentrated to dryness and then re-dissolved in methanol/water (70/30, v/v). The extracts were purified by C18 Polar Plus micro column clean-up eluted with dichloromethane followed by micro silica column as additional clean-up step.
The final determination of BAS 500 F and its metabolite 500M07 was performed by HPLC-MS/MS using an Inertsil Phenyl 5µm column. Detection of BAS 500 F was accomplished in ESI+ mode at mass transitions *m/z* 388 → *m/z* 194 for quantitation. Metabolite 500M07 is quantified on mass transition *m/z* 358 → *m/z* 164.

Results and discussions

The method proved to be suitable for analysis of pyraclostrobin and metabolite 500M07 in plant matrices at a limit of quantitation of 0.02 mg/kg for each analyte.
In all matrices tested (cabbage, onion, orange, wheat grain, hops and oilseed rape), the mean recovery values were between 70 and 110%. The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%, except one single outlier for orange at the fortification level of 0.02 mg/kg. The detailed results are given in the table below.

Table A 50: Recovery results from method validation of BAS 500 F and 500M07 using the analytical method 421/0

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Cabbage, head	BAS 500 F	0.02 (n=5)	82	3.4	Quantitation
		1.0 (n=5)	86	3.4	m/z 388→194
	500M07	0.02 (n=5)	85	16.2	Quantitation
		0.2 (n=5)	90	4.1	m/z 358→164
Onion	BAS 500 F	0.02 (n=5)	86	6.3	Quantitation
		1.0 (n=5)	88	5.0	m/z 388→194
	500M07	0.02 (n=5)	79	5.6	Quantitation
		0.2 (n=5)	93	4.4	m/z 358→164
Orange flesh	BAS 500 F	0.02 (n=4)	85 (74)*	28.0 (4.3)*	Quantitation
		1.0 (n=5)	86	8.3	m/z 388→194
	500M07	0.02 (n=5)	83	12.9	Quantitation
		0.2 (n=5)	94	10.9	m/z 358→164
Wheat, grain	BAS 500 F	0.02 (n=4)	83	1.5	Quantitation
		0.5 (n=5)	90	2.6	m/z 388→194
	500M07	0.02 (n=4)	77	3.1	Quantitation
		0.2 (n=5)	87	8.0	m/z 358→164
Hops	BAS 500 F	0.02 (n=5)	71	7.1	Quantitation
		10.0 (n=5)	92	3.4	m/z 388→194
	500M07	0.02 (n=5)	94	7.4	Quantitation
		0.2 (n=5)	91	12.4	m/z 358→164
Rape, seed	BAS 500 F	0.02 (n=5)	89	8.9	Quantitation
		0.5 (n=5)	84	7.2	m/z 388→194
	500M07	0.02 (n=5)	70	14.1	Quantitation
		0.2 (n=5)	79	9.3	m/z 358→164

*One single statistical outlier according to Dixons test. In brackets, mean result after rejection of the outlier.

Table A 51: Characteristics for the analytical method 421/0 used for inter-laboratory validation of pyraclostrobin residues in plant matrices (cabbage, onion, orange, wheat grain, hops and oilseed rape)

	Pyraclostrobin and metabolite 500M07
Specificity	Selective determination of BAS 500 F and its metabolite 500M07 using LC-MS/MS monitoring one mass transitions. There were no known interferences from plant components or from reagents, solvents and glassware used.
Calibration (type, number of data points)	Calibration standards were prepared in 80/20 (v/v) methanol/buffer solution (99.9% of 4 mM ammonium formate in water and 0.1% formic acid). Four calibration points were used and individual calibration data was presented (external reference standard). Good linearity was observed with a

	Pyraclostrobin and metabolite 500M07
	correlation coefficient ≥0.99.
Calibration range	Calibration points distributed over a concentration range of 0.5 to 5.0 ng/mL.
Assessment of matrix effects is presented	No matrix effects were investigated within an independent experiment, therefore instrument recovery sampels would be implemented within each analytical series, if no further information on matrix effects of the investigated matrix is available. Alternatively matrix-matched standards would be used.
Limit of determination/quantification	The limit of quantitation representing the lowest validated level with sufficient recovery and precision was 0.02 mg/kg for BAS 500 F and 0.02 mg/kg for the metabolite 500M07
Reproducibility	The ILV confirmed that the analytical method 421/0 is suitable for cabbage, onion, orange, wheat grain, hops and oilseed rape.

Conclusion

The method uses LC-MS/MS for final determination for analysis of pyraclostrobin and its metabolite 500M07. The method is sufficiently validated for the commodities cabbage, onion, orange, wheat grain, hops and oilseed rape. Thereby, it could be demonstrated that the method fullfils the requirements with regard to repeatability, limit of quantitation and recoveries. In addition, it could be shown that the method is highly reproducible. The method is regarded as suitable enforcement method for MRL monitoring.

A 2.3.2.1.2 Analytical method D9904 for determination of BAS 500 F and its metabolite 500M07 residues in plant matrices

A 2.3.2.1.2.1 Method validation

During the peer review under Directive 91/414/EEC, method no. D9904 was already evaluated (DAR 2001).

A 2.3.2.1.2.2 Independent laboratory validation (head cabbage, onions, oranges, and wheat grain)

Comments of zRMS:	The study has been accepted. The reported in the study ILV was originally dedicated to validating the method D9904 in head cabbage, onions, oranges (flesh), wheat grain, hops and rape seed. The final determination of the analytes was performed by HPLC/UV. However due to the unacceptable chromatography the independent validation results for hops and OSR was rejected (see ILV of the method 421/0 for these matrices). For head cabbage, onions, oranges, and wheat grain no interferences at the LOQ were observed. For calibration 4 points were applied. The linearity of the detector response was acceptable with a correlation coefficient greater than 0.995 for all analytical determinations. 2 fortification levels with 5 replicates and control blank were tested. The mean recovery range obtained for these matrices was between 79% and 102% for BAS 500 F and 84% and 110% for BF 500-3. The RSD values were significantly below 20 %. For pyraclostrobin and desmethoxy pyraclostrobin in head cabbage, oranges and wheat grain the LOQ of the method was set at 0.02 mg/kg. In onions the LOQ was 0.5 mg/kg for pyraclostrobin and 0.2 mg/kg for BF 500-3 (0,5 value is consistent with the relevant MRL). This ILV of the method D9904 can be accepted for head cabbage, onions, oranges, and wheat grain. The confirmatory technique proposed by the technical procedure of the method is an application of a different chromatographic system (conditions

	are proposed). However, such confirmation is not reported in the study. Thus, the confirmatory technique is required. The analytical methods for the determination of pyraclostrobin in foodstuffs of plant origin were evaluated in the previous Annex I inclusion process and during more recent evaluations performed by EFSA in context of MRL applications. The BASF methods 421/0, 445/0 and 535/1 (synonym: L0076) allow the determination of pyraclostrobin and its metabolite 500M07 (BF 500-3) in multiple crops. The data generation methods are fully validated in separate GLP studies; furthermore, an extensive set of concurrent fortification experiments exist from supervised field trials or processing studies, which equals independent lab validation. The general suitability of the residue analytical methods provided for data generation and risk assessment has also been confirmed by EFSA in the recently published Reasoned Opinion on MRLs (Review of established MRLs according to Reg. 396/2005 (Art. 12); EFSA Journal 2011;9(8):2344). Therefore, the provided data package can be seen as "fit for purpose". In addition, a new validation of method L0076 has been provided (A 2.2.1.1.3), following the same method principle, and applicable as additional/independent validation. The study was already evaluated in PL.

Reference:	CP 5.2/18
Report	Independent laboratory validation of BASF method D9904, an analytical method for the determination of residues of BAS 500 F and its metabolite BF 500-3, Devine H.C., 2002 report No CEMR-1656 2002/1007083
Guideline(s):	EEC 94/414, EEC 96/68, Guidance Document on Residue Analytical Methods (SANCO/825/00rev.6)
Deviations:	No
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
Acceptability:	Yes

Materials and methods

Pyraclostrobin and its metabolite 500M07 were extracted from plant matrices (wheat grain and head cabbage) using a mixture of methanol/water (70/30, v/v). After filtration, an aliquot of the extract was cleaned by C18 Polar Plus micro column eluted with dichloromethane.
From onion, orange and hops specimens residues were extracted using a mixture of methanol/water (70/30, v/v) and filtered. An aliquot of the extract was cleaned by C18 Polar Plus micro column, eluted with dichloromethane followed by an additional clean-up stage using micro silica columns.
Extraction of residues from rape seed was performed by homogenization with acetonitrile followed by addition of hexane. After filtration, the acetonitrile layer of the extract was partitioned with hexane. An aliquot was concentrated to dryness and then re-dissolved in methanol/water (70/30, v/v). The extracts were purified by C18 Polar Plus micro column clean-up eluted with dichloromethane followed by micro silica column as additional clean-up step.

The final determination of BAS 500 F and its metabolite 500M07 was performed by HPLC/UV using column switching liquid chromatography with UV detection and Luna Phenyl hexyl, 3 µm as column 1 and Betasil 5 C18, 5 µm as column 2.

Results and discussions

The method proved to be suitable for analysis of pyraclostrobin and metabolite 500M79 in plant matrices such as cabbage, orange, onion and wheat grain at a limit of quantitation of 0.02 mg/kg for each analyte. Analytes from the matrices hops and oilseed rape were not able to be determined with sufficient results by means of HPLC/UV.
In all matrices successfully tested, the mean recovery values were between 70 and 110%. The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%, except one single outlier for head cabbage at the fortification level of 1.0 mg/kg (BAS 500 F) and 0.2 mg/kg (500M07), respectively. The detailed results are given in the table below.

Table A 52: Recovery results from method validation of BAS 500 F and 500M07 using the analytical method D9904

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Cabbage, head	BAS 500 F	0.02 (n=5)	86	2.7	-
		1.0 (n=5)	75 (93)*	55.2 (2.9)*	
	500M07	0.02 (n=5)	110	5.3	-
		0.2 (n=5)	71 (89)*	56 (3.5)*	
Onion	BAS 500 F	0.5 (n=5)	90	2.8	-
		1.0 (n=5)	94	3.8	
	500M07	0.2 (n=5)	84	4.7	-
		0.5 (n=5)	89	3.3	
Orange flesh	BAS 500 F	0.02 (n=5)	102	12.2	-
		1.0 (n=5)	89	1.8	
	500M07	0.02 (n=5)	100	3.7	-
		0.2 (n=5)	90	4.8	
Wheat, grain	BAS 500 F	0.02 (n=5)	79	18.1	-
		0.5 (n=5)	88	7.5	
	500M07	0.02 (n=5)	84	8.4	-
		0.2 (n=5)	84	15.3	

*One single statistical outlier according to Dixons test. In brackets, mean result after rejection of the outlier.

Table A 53: Characteristics for the analytical method D9904 used for inter-laboratory validation of pyraclostrobin residues in plant matrices (cabbage, onion, orange, wheat grain, hops and oilseed rape)

	Pyraclostrobin and metabolite 500M07
Specificity	Determination of BAS 500 F and its metabolite 500M07 using HPLC-UV. For the matrices cabbage, orange, onion and wheat grain, there were no known interferences from plant components or from reagents, solvents and glassware used. A second chromatographic system as confirmatory method is provided.
Calibration (type, number of data points)	Calibration standards were prepared in acetonitrile. Four calibration points were used and individual calibration data was presented (external reference standard). Good linearity was observed with a correlation coefficient ≥0.99.
Calibration range	Calibration points distributed over a concentration range of 2 to 20 ng/mL.
Assessment of matrix effects is presented	No. Since the detecion is based on UV-measurement, no signal supression/enhancement based on co-elution is expected, as specifity is given.
Limit of determination/quantification	The limit of quantitation representing the lowest fortification level with sufficient recovery and precision was 0.02 mg/kg for each analyte in cabbage, orange and wheat grain. In onion, the lowest fortification level successfully tested was 0.5 mg/kg for BAS 500 F and 0.2 mg/kg for 500M07.
Reproducibility	The ILV confirmed that the analytical method D9904 is suitable for the plant matrices cabbage, orange, onion and wheat grain.

Conclusion

The method uses HPLC/UV for final determination of pyraclostrobin and its metabolite 500M07. The method is sufficiently validated for the commodities cabbage, orange and wheat grain. For onion, the method is suitable at 0.5 mg/kg for BAS 500 F and at 0.2 mg/kg for 500M07. The HPLC/UV method does not yield satisfactory results for the matrices hops and oilseed rape and is therefore unsuitable for the use as an enforcement method for hops and rape seed.
For the commodities head cabbage, onions, oranges and wheat grain it could be demonstrated that the method fullfills the requirements with regard to specificity, repeatability, limit of quantitation and recoveries. In addition, it could be shown that the method is reproducible. Therefore, the method is regarded as suitable enforcement method for MRL monitoring of the matrices head cabbage, onions, oranges and wheat grain.

A 2.3.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

A 2.3.2.2.1 Analytical method L0151/01: Method for the determination of pyraclostrobin in animal matrices

A 2.3.2.2.1.1 Method validation

Comments of zRMS:	The study has been accepted. The purpose of the study was the validation of the BASF method No. L0151/01 for the determination of BAS 500 F in matrices of animal origin by means of LC-MS/MS monitoring mass transition 388 → 194 for quantification and mass transition 388 → 163 for confirmation. The validation was performed according

	to the technical procedure for the method attached to the study. Significant interferences (> 30% of LOQ) were not observed. For calibration 6 points were used. Good linearity with coefficients ≥0.99 were obtained for pyraclostrobin. 2 fortification levels with 2 blanks and 5 replicates were tested. In all matrices tested, the mean recovery values were between 70% and 110%. The RSDs for all commodities and all fortification levels were well below 20%. The LOQ defined by the lowest fortification level successfully tested was at 0.01 mg/kg for cow muscle, kidney, fat, liver, milk, skim milk, cream as well as hen egg and 0.01 mg/L for swine blood. Good linearity was observed in the range of 0.025 to 1.0 ng/mL. Furthermore, the study shows that the analyte was stable in the extract solvent and in the solution used as final volume. Also, the fortification solutions and calibration standards proved to be stable over a period of 30 days. The validation results show that BASF method No. L0151/01 is suitable to determine residues of pyraclostrobin in matrices of animal origin such as cow muscle, kidney, fat and liver, milk, skim milk, cream as well as swine blood and hen egg. The confirmatory technique is not required. The study was already evaluated in PL.

Reference:	CP 5.2/19
Report	Validation of the analytical method L0151/01: Method for the determination of BAS 500 F (Reg.No. 304428) in animal matrices, Hopf B., 2010 report No 357556 2010/1018944
Guideline(s):	EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 7 (17 March 2004), SANCO/3029/99 rev. 4 (11 July 2000)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Materials and methods

Pyraclostrobin (BAS 500 F) is extracted with acetonitrile. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. An aliquot of the organic phase is evaporated to dryness and dissolved in a mixture of acetonitrile and water. The final determination of pyraclostrobin is performed by HPLC-MS/MS. Analysis was accomplished using a Betasil C18 column and an acetonitrile-water gradient with formic acid as modifier at a flow rate of 600 µL/min. The residues are determined by LC-MS/MS in ESI positive mode monitoring mass transition 388 → 194 for quantification and mass transition 388 → 163 for confirmation.

Results and discussions

In all matrices tested, the mean recovery values were between 70% and 110% at both fortification levels and both mass transitions. The relative standard deviations (RSD, %) for all commodities and all fortification levels were well below 20%. The recovery data are summarized in the table below.

Table A 54: Recovery results from method validation of pyraclostrobin using the

analytical method L0151/01

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Cow muscle	Pyraclostrobin	0.01 (n = 5)	101	1.6	Quantitation
		0.1 (n = 5)	97.0	1.7	m/z 388 → 194
		0.01 (n = 5)	102	1.2	Confirmation
		0.1 (n = 5)	95.8	1.5	m/z 388 → 163
Cow kidney		0.01 (n = 5)	106	1.4	Quantitation
		0.1 (n = 5)	97.3	1.8	m/z 388 → 194
		0.01 (n = 5)	105	1.6	Confirmation
		0.1 (n = 5)	97.0	0.8	m/z 388 → 163
Cow fat		0.01 (n = 5)	103	1.5	Quantitation
		0.1 (n = 5)	99.7	1.3	m/z 388 → 194
		0.01 (n = 5)	102	1.3	Confirmation
		0.1 (n = 5)	98.7	1.1	m/z 388 → 163
Cow liver		0.01 (n = 5)	98.4	4.2	Quantitation
		0.1 (n = 5)	96.7	2.1	m/z 388 → 194
		0.01 (n = 5)	99.3	4.0	Confirmation
		0.1 (n = 5)	95.1	1.4	m/z 388 → 163
Cow milk		0.01 (n = 5)	103	1.8	Quantitation
		0.1 (n = 5)	96.1	1.4	m/z 388 → 194
		0.01 (n = 5)	101	1.6	Confirmation
		0.1 (n = 5)	96.4	1.6	m/z 388 → 163
Cow skimmed milk		0.01 (n = 5)	94.4	1.4	Quantitation
		0.1 (n = 5)	90.9	2.3	m/z 388 → 194
		0.01 (n = 5)	95.2	1.0	Confirmation
		0.1 (n = 5)	90.6	1.5	m/z 388 → 163
Cow cream		0.01 (n = 5)	99.3	5.0	Quantitation
		0.1 (n = 5)	95.5	3.8	m/z 388 → 194
		0.01 (n = 5)	99.8	3.5	Confirmation
		0.1 (n = 5)	94.9	3.1	m/z 388 → 163

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Hen egg		0.01 (n = 5)	94.4	7.7	Quantitation
		0.1 (n = 5)	98.5	1.8	m/z 388 → 194
		0.01 (n = 5)	93.6	8.9	Confirmation
		0.1 (n = 5)	98.0	1.6	m/z 388 → 163
Matrix	**Analyte**	**Fortification level (mg/L) (n = x)**	**Mean recovery (%)**	**RSD (%)**	**Comments**
Swine blood		0.01 (n = 5)	81.4	3.7	Quantitation
		0.1 (n = 5)	76.5	1.6	m/z 388 → 194
		0.01 (n = 5)	81.9	3.5	Confirmation
		0.1 (n = 5)	77.6	2.5	m/z 388 → 163

Table A 55: Characteristics for the analytical method used for validation of pyraclostrobin residues in animal matrices

	Pyraclostrobin
Specificity	Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered for each analyte. HPLC-MS/MS, using two mass transitions, is a highly specific detection technique and therefore a confirmatory technique is not required.
0Calibration	Calibration standards were prepared in acetonitrile / water (50/50, v/v) .Six calibration points were used and individual calibration data was presented. Linear correlations with coefficients ≥0.99 were obtained for pyraclostrobin.
Calibration range	Calibration points distributed over a concentration range of 0.025 to 1.0 ng/mL were used.
Assessment of matrix effects is presented	Matrix effects were investigated by instrument recovery samples (QCS) and have been identified as non relevant.
Standard stability	Pyraclostrobin was stable in acetonitrile (fortification solutions) as well as in acetonitrile /water (50/50, v/v) (calibration solutions) for up to 30 days, if stored refrigerated in the dark.
Extract stability	Extract solutions of cow liver and hen egg which were stored for 7 days and further worked-up thereafter showed that the analyte was stable under refrigerated conditions.
Final volume stability	Solutions prepared for the final determination by HPLC-MS/MS had been kept in the refrigerator for 7 days. Their re-analysis after storage led to quite comparable recoveries.
Limit of determination/quantification	The limit of quantitation was defined by the lowest fortification level successfully tested, which was 0.01 mg/kg for the analyte in cow liver, kidney, fat, muscle, milk, skim milk, cream and hen egg and 0.01 mg/L for swine blood.

Conclusion

The results of the method validation study demonstrate that BASF analytical method L0151/01 fulfills the requirements with regard to linearity, specificity, repeatability, limit of quantitation, and recoveries and is, therefore, applicable to correctly determine residues of pyraclostrobin in animal matrices.

A 2.3.2.2.1.2 Independent laboratory validation

Comments of zRMS:	The study has been accepted. The purpose of the study was the independent validation of the BASF method No. L0151/01 for the determination of BAS 500 F in matrices of animal origin by means of LC-MS/MS monitoring mass transition 388 → 194 for quantification and mass transition 388 → 163 for confirmation. For each matrix 2 fortification levels with 2 blanks and 5 replicates were tested. In all matrices tested, the mean recovery values were between 70% and 110%. The RSDs for all matrices and all fortification levels were well below 20%. The LOQ defined by the lowest fortification level successfully tested was at 0.01 mg/kg for bovine muscle, kidney, fat, milk as well as hen egg and 0.01 mg/L for swine blood. Significant interferences (> 30% of LOQ) were not observed in blanks. For calibration 12 points were used. Good linearity with r > 0.999 were obtained for pyraclostrobin in the range of 0.02 ng/mL to 100 ng/mL. Furthermore, the study shows that the analyte was stable in the extract solvent and in the solution used as final volume. Also, the fortification solutions and calibration standards proved to be stable over a period of 30 days. The ILV is accepted. The validation results show that BASF method No. L0151/01 is suitable to enforce residues of pyraclostrobin in meat, fat, kidney, milk, and egg with the LOQ of 0.01 mg/kg and in blood with the LOQ of 0.01 mg/L. The confirmatory technique is not required. The study was already evaluated in PL.

Reference:	CP 5.2/20
Report	Independent laboratory validation (ILV) of an analytical method for the determination of BAS 500 F (Reg.No. 304428) in animal matrices, Schacherl A., 2010 report No 371244 2010/1123694
Guideline(s):	SANCO/825/00 rev. 7 (17 March 2004), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07
Deviations:	No
GLP:	yes (certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)
Acceptability:	Yes

Materials and methods

Pyraclostrobin (BAS 500 F) is extracted with acetonitrile. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. An aliquot of the organic phase is evaporated to dryness and dissolved in a mixture of acetonitrile and water. The final determination of pyraclostrobin is performed

by HPLC-MS/MS, monitoring two parent-daughter ion transitions. Analysis was accomplished using a Phenomenex Luna C18 column and an acetonitrile-water gradient with acetic acid as modifier at a flow rate of 750 µL/min. The residues are determined by LC-MS/MS in ESI positive mode monitoring mass transition 388 → 194 for quantification and mass transition 388 → 163 for confirmation.

Results and discussions

The method is suitable to determine residues of pyraclostrobin in animal matrices. Samples were fortified at the limit of quantification of 0.01 mg/kg (respectively 0.01 mg/L) and 10 times higher. The mean recovery values were between 70% and 110% and relative standard deviations (RSD) of < 10%. The recovery data are summarized in the table below.

Table A 56: Recovery results from independent laboratory validation of pyraclostrobin using the analytical method L0151/01

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Bovine muscle	pyraclostrobin	0.01 (n = 5)	70	2	Quantitation
		0.1 (n = 5)	73	5	m/z 388 → 194
		0.01 (n = 5)	76	4	Confirmation
		0.1 (n = 5)	72	3	m/z 388 → 163
Bovine fat		0.01 (n = 5)	71	4	Quantitation
		0.1 (n = 5)	73	3	m/z 388 → 194
		0.01 (n = 5)	71	4	Confirmation
		0.1 (n = 5)	72	3	m/z 388 → 163
Bovine kidney		0.01 (n = 5)	71	6	Quantitation
		0.1 (n = 5)	72	2	m/z 388 → 194
		0.01 (n = 5)	73	1	Confirmation
		0.1 (n = 5)	73	2	m/z 388 → 163
Bovine milk		0.01 (n = 5)	77	4	Quantitation
		0.1 (n = 5)	82	2	m/z 388 → 194
		0.01 (n = 5)	77	3	Confirmation
		0.1 (n = 5)	83	3	m/z 388 → 163
Hen egg		0.01 (n = 5)	104	3	Quantitation
		0.1 (n = 5)	105	3	m/z 388 → 194
		0.01 (n = 5)	104	4	Confirmation
		0.1 (n = 5)	105	3	m/z 388 → 163
Swine blood	pyraclostrobin	0.01 (n = 5)	71	4	Quantitation
		0.1 (n = 5)	73	4	m/z 388 → 194
		0.01 (n = 5)	72	2	Confirmation
		0.1 (n = 5)	73	5	m/z 388 → 163

Table A 57: Characteristics for the analytical method used for independent laboratory validation of pyraclostrobin residues in animal matrices

	Pyraclostrobin
Specificity	Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered for each analyte. HPLC-MS/MS, using two mass transitions, is a highly specific detection technique and therefore a confirmatory technique is not required.
Calibration	Calibration standards were prepared in acetonitrile / water (50/50, v/v) .12 calibration points were used and individual calibration data was presented. Linear correlations with coefficients ≥0.999 were obtained for pyraclostrobin.
Calibration range	Calibration points distributed over a concentration range of 0.02 to 100 ng/mL were used.
Assessment of matrix effects is presented	Yes matrix effects were investigated. No significant matrix effect was present for muscle, fat, kidney milk and blood. For egg the matrix effect was significant, therefore matrix-matched standards were used for analysis.
Final extract stability	It was shown that pyraclostrobin is stable for at least 3 days in egg and fat extracts when stored at approximately 15°C.
Limit of determination/quantification	The limit of quantitation (LOQ) of the method for cow liver, kidney, fat, muscle, milk, skim milk, cream and hen egg is 0.01 mg/kg. The LOQ for swine blood is 0.01 mg/L. The limit of detection (LOD) was defined as 30% of LOQ, 0.003 mg/kg pyraclostrobin for meat, fat, kidney, milk and egg and 0.003 mg/L for blood.

Conclusion

The ILV was completed successfully on the first trial for the determination of residues of pyraclostrobin, in animal matrices.
The results of the method validation study demonstrate that BASF analytical method L0151/01 fulfills the requirements with regard to linearity, specificity, repeatability, limit of quantitation, and recoveries and is, therefore, applicable to correctly determine residues of pyraclostrobin in animal matrices.

A 2.3.2.2.1.3 Confirmatory method

No confirmatory technique is required as two different, highly specific mass transitions are used for quantitation and qualification.

A 2.3.2.2.1.4 Extraction efficiency

There is no new study on extraction efficiency of the method for the determination of residues in animal matrices. For more information, see section 5.3.4.3 and A 2.3.2.7.2.

A 2.3.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

Analytical method L0166/01 is used for the determination of pyraclostrobin residues in soil, but can also be used for post-authorization control and monitoring purposes and is summarized in section A 2.3.1.3.1 (KCP 5.2.2).

A 2.3.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

A 2.3.2.4.1 Analytical method L0182/01 for determination of pyraclostrobin and metabolites in water

A 2.3.2.4.1.1 Method validation 1

BASF analytical method L0182/01 was developed for the determination of residues of pyraclostrobin and its metabolites 500M59, 500M60, 500M62, 500M76 and 500M78 in water. Even though analytical methods for determination of metabolites in water are not needed, because pyraclostrobin is considered as the only relevant analyte for monitoring purposes, the results of the metabolites are additionally presented, as the method is also used to generate pre-authorization data.

Comments of zRMS:	The study has been accepted. The purpose of the study was the validation of the BASF method No. L0182/01 for the determination of pyraclostrobin and its metabolites 500M59, 500M60, 500M62, 500M76 and 500M78 in ground- surface- and tap-water by means of LC-MS/MS monitoring 2 mass transitions per analyte for quantification and confirmation. The validation was done according to the Technical Procedure of L0182/01 attached to the study. For each matrix 2 fortification levels were analysed in 5 replicates. ln addition at least two blanks have been analysed per analytical sample set. In all matrices tested, the recovery data were within the acceptable range (70-110%). The RSDs for all matrices and all fortification levels were below 20%. The LOQ defined by the lowest fortification level successfully tested was at 0.003 µg/L for pyraclostrobin and 0.03 µg/L for each metabolite. Significant interferences (> 30% of LOQ) were not observed in the blanks. For calibration 6 points were used. Good linearity with $r \geq 0.99$ were obtained in the method working range. Furthermore, the study shows that standards of each analyte were stable for at least 2 weeks when stored under refrigerated conditions in the dark. Pyraclostrobin and its metabolites were stable in matrix extracts over a tested period of 7 days when stored at final dilution level under refrigerated conditions in the dark (all recoveries obtained were still above 70%). The validation is accepted. The validation results show that BASF method No. L0182/01 is suitable to determine residues of pyraclostrobin in ground- surface- and tap-water with the LOQ of 0.003 µg/L and each metabolite with the LOQ of 0.03 µg/L The confirmatory technique is not required. The study was already evaluated in PL.

Reference:	CP 5.2/21
Report	Validation of method L0182/01: Determination of BAS 500 F and its metabolites Reg.No. 412053 (500M59), Reg.No. 411847 (500M60), Reg.No. 412785 (500M62), Reg.No. 413038, and Reg.No. 377613 in ground- surface- and tapwater using LC-MS/MS, Tilting N., 2012 report No 370958 2012/1009641
Guideline(s):	SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), SANCO/825/00 rev. 8 (30 June 2010)
Deviations:	No

GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Materials and methods

A 50 mL water sample aliquot is acidified with formic acid and extracted by SPE. After washing with 5 mL acidified water, the SPE column is dried. The column is eluted with 2 x 5 mL ethyl acetate and the combined extracts are evaporated at 40°C.
Final determination is performed by LC-MS/MS using an Atlantis T3 analytical column and a gradient mixture of water – acetonitrile with formic acid as modifier at a flow rate of 500 µL/min. Detection is accomplished using positive ion electrospray ionization tandem mass spectrometry (MS/MS-ESI+) monitoring two mass transitions for quantification and confirmation: 388 → 194 and 388 → 163 for pyraclostrobin, 370 → 194 and 370 → 278 for 500M59, 278 → 194 and 278 → 149 for 500M60, 248 → 132 and 248 → 216 for 500M62, 388 → 241 and 388 → 300 for 500M76 and 177 → 135 and 177 → 132 for 500M78.

Results and discussions

The method L0182/01 was proved to be suitable to determine residues of pyraclostrobin and its metabolites 500M59, 500M60, 500M62, 500M76 and 500M78 in water using LC-MS/MS. Samples were spiked with the analytes at the limit of quantification (0.003 µg/L for pyraclostrobin, 0.03 µg/L for the metabolites) and 10 times higher (0.03 µg/L for pyraclostrobin, 0.3 µg/L for the metabolites). The mean recovery values were between 70% and 110% and the relative standard deviations (RSD) for all fortification levels were below 20%. The recovery data are summarized in the tables below.

Table A 58: Recovery results from method validation of pyraclostrobin and its metabolites using the analytical method L0182/01 (Groundwater)

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Groundwater	pyraclostrobin	0.003 (n = 5)	87.9	7.4	388 → 194
		0.03 (n = 5)	94.2	2.6	Quantifier transition
		0.003 (n = 5)	92.6	7.4	388 → 163
		0.03 (n = 5)	99.0	5.3	Qualifier transition
	500M59	0.03 (n = 5)	95.9	1.7	370 → 194
		0.3 (n = 5)	97.8	2.8	Quantifier transition
		0.03 (n = 5)	98.9	2.6	370 → 278
		0.3 (n = 5)	95.8	3.9	Qualifier transition
	500M60	0.03 (n = 5)	95.7	3.8	278 → 194
		0.3 (n = 5)	98.8	2.7	Quantifier transition
		0.03 (n = 5)	95.2	2.4	278 → 149
		0.3 (n = 5)	95.6	3.4	Qualifier transition
	500M62	0.03 (n = 5)	97.0	1.4	248 → 132
		0.3 (n = 5)	95.4	3.9	Quantifier transition
		0.03 (n = 5)	99.8	3.1	248 → 216
		0.3 (n = 5)	97.7	4.0	Qualifier transition
	500M76	0.03 (n = 5)	97.4	5.7	388 → 241
		0.3 (n = 5)	102.8	3.1	Quantifier transition
		0.03 (n = 5)	103.4	3.3	388 → 300
		0.3 (n = 5)	99.3	5.3	Qualifier transition
	500M78	0.03 (n = 5)	92.2	1.0	177 → 135
		0.3 (n = 5)	103.1	3.4	Quantifier transition
		0.03 (n = 5)	93.5	3.0	177 → 132
		0.3 (n = 5)	99.8	4.2	Qualifier transition

Table A 59: Recovery results from method validation of pyraclostrobin and its metabolites using the analytical method L0182/01 (Surface water)

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Surface water	pyraclostrobin	0.003 (n = 5)	89.3	4.1	388 → 194
		0.03 (n = 5)	97.5	4.6	Quantifier transition
		0.003 (n = 5)	93.8	6.1	388 → 163
		0.03 (n = 5)	100.4	2.6	Qualifier transition
	500M59	0.03 (n = 5)	97.1	1.8	370 → 194
		0.3 (n = 5)	96.9	1.7	Quantifier transition
		0.03 (n = 5)	98.6	3.0	370 → 278
		0.3 (n = 5)	97.8	2.3	Qualifier transition
	500M60	0.03 (n = 5)	86.7	1.8	278 → 194
		0.3 (n = 5)	85.0	2.4	Quantifier transition
		0.03 (n = 5)	87.6	3.4	278 → 149
		0.3 (n = 5)	88.4	1.9	Qualifier transition
	500M62	0.03 (n = 5)	91.2	1.9	248 → 132
		0.3 (n = 5)	83.8	1.6	Quantifier transition
		0.03 (n = 5)	91.1	4.7	248 → 216
		0.3 (n = 5)	82.0	2.4	Qualifier transition
	500M76	0.03 (n = 5)	97.6	3.7	388 → 241
		0.3 (n = 5)	97.7	6.2	Quantifier transition
		0.03 (n = 5)	104.0	4.7	388 → 300
		0.3 (n = 5)	102.1	3.4	Qualifier transition
	500M78	0.03 (n = 5)	95.0	3.7	177 → 135
		0.3 (n = 5)	97.2	5.2	Quantifier transition
		0.03 (n = 5)	100.3	2.0	177 → 132
		0.3 (n = 5)	103.3	4.8	Qualifier transition

Table A 60: Recovery results from method validation of pyraclostrobin and its metabolites using the analytical method L0182/01 (Tap water)

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Tap water	pyraclostrobin	0.003 (n = 5)	89.6	4.9	388 → 194
		0.03 (n = 5)	103.0	5.2	Quantifier transition
		0.003 (n = 5)	92.1	3.1	388 → 163
		0.03 (n = 5)	104.4	9.4	Qualifier transition
	500M59	0.03 (n = 5)	98.9	1.3	370 → 194
		0.3 (n = 5)	101.3	2.9	Quantifier transition
		0.03 (n = 5)	98.8	2.6	370 → 278
		0.3 (n = 5)	101.8	3.2	Qualifier transition
	500M60	0.03 (n = 5)	97.3	2.1	278 → 194
		0.3 (n = 5)	96.4	3.4	Quantifier transition
		0.03 (n = 5)	95.7	2.9	278 → 149
		0.3 (n = 5)	95.9	3.1	Qualifier transition
	500M62	0.03 (n = 5)	99.3	1.6	248 → 132
		0.3 (n = 5)	93.5	6.1	Quantifier transition
		0.03 (n = 5)	98.0	5.5	248 → 216
		0.3 (n = 5)	94.8	7.6	Qualifier transition
	500M76	0.03 (n = 5)	100.6	9.7	388 → 241
		0.3 (n = 5)	101.0	4.7	Quantifier transition
		0.03 (n = 5)	100.6	6.1	388 → 300
		0.3 (n = 5)	105.0	5.6	Qualifier transition
	500M78	0.03 (n = 5)	96.7	2.3	177 → 135
		0.3 (n = 5)	99.2	1.7	Quantifier transition
		0.03 (n = 5)	95.8	2.8	177 → 132
		0.3 (n = 5)	99.4	3.1	Qualifier transition

Table A 61: Characteristics for the analytical method used for validation of pyraclostrobin and its metabolites residues in water

	Pyraclostrobin and metabolites 500M59, 500M60, 500M62, 500M76 and 500M78
Specificity	The method L0182/01 allows the specific determination of pyraclostrobin and its metabolites 500M59, 500M60, 500M62, 500M76 and 500M78 in water. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary. The tested untreated water samples showed no significant interferences (< 30%) at the retention time of the analytes.
Calibration (type, number of data points)	Linear regression was used for calibration using six calibration levels. Matrix-matched standards were used for calibration.
Calibration range	Good linearity ($r \geq 0.99$) was observed in the range of 0.005 ng/mL to 1.0 ng/mL for the two mass transitions of pyraclostrobin and its metabolites in three different water types (ground-, surface- and tap water).
Assessment of matrix effects is presented	Matrix effects were observed during method development, therefore, matrix-matched standards were used for the calibration.
Standard stability	Standard solutions of each analyte (prepared in acetonitrile/water, 20/80, v/v) were stable for at least 2 weeks when stored under refrigerated conditions in the dark.
Extract stability	Pyraclostrobin and its metabolites were stable in extracts of water samples over a tested time period of 7 days when stored at final dilution level under refrigerated conditions in the dark.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.003 µg/L for pyraclostrobin and 0.03 µg/L for each metabolite, corresponding to the lowest fortification level. The limit of detection (LOD) is estimated as 20% of the LOQ with 0.0006 µg/L for pyraclostrobin and 0.006 µg/L for each metabolite.

Conclusion

The method L0182/01 for the analysis of pyraclostrobin and its metabolites 500M59, 500M60, 500M62, 500M76 and 500M78 in ground-, surface- and tap water used LC-MS/MS for final determination, with limit of quantification of 0.003 µg/L for pyraclostrobin and 0.03 µg/L for each metabolite.

It could be demonstrated that the method fulfills the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine pyraclostrobin and its metabolites in ground- surface- and tap water samples.

A 2.3.2.4.1.2 Method validation 2

Analytical method L0182/02 is an add-on to method L0182/01 and covers the validation for the determination of metabolite 500M04 in water. Even though methods for the determination of metabolites are not needed, because pyraclostrobin is considered as the only relevant analyte for monitoring purposes, the results of the metabolite are additionally presented, as the method is also used to generate pre-authorization data.

Comments of zRMS:	The study has been accepted. The purpose of the study was the validation of the BASF method No. L0182/02 for the determination of pyraclostrobin metabolite 500M04 (BF 500-5) in ground-

	and surface-water by means of LC-MS/MS monitoring 2 mass transitions for quantification and confirmation. For each matrix 2 fortification levels were applied. For each fortification level and water type, five replicates were analysed. Additionally, at least two replicates of untreated samples were examined. In all matrices tested, the recovery data were within the acceptable range (70-110%). The RSDs for all matrices and 2 fortification levels were below 10%. The LOQ defined by the lowest fortification level successfully tested was for BF 500-5 at 0.03 µg/L. Significant interferences (> 30% of LOQ) were not observed in the blanks. For calibration 6 points were used. Good linearity with r > 0.995 were obtained in the method working range. Furthermore, the study shows that standard solutions of 500M04 were stable for at least 4 weeks when stored under refrigerated conditions in the dark. 500M04 was stable in extracts of water samples over a tested period of 7 days when stored under refrigerated conditions in the dark. The validation is accepted. The validation results show that BASF method No. L0182/02 is suitable to determine residues of BF 500-5 in ground- and surface-water with the LOQ of 0.03 µg/L. The confirmatory technique is not required. The study was already evaluated in PL.

Reference:	CP 5.2/22
Report	Validation of analytical method L0182/02 for the determination of BF 500-5 (Reg.No. 298327), metabolite of BAS 500 F, in ground- and surface water by LC-MS/MS, Obermann M., 2014 report No 439916 2014/1004891
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Materials and methods

A 50 mL water sample aliquot is acidified with formic acid and concentrated on a C18 SPE column. After eluting with ethyl acetate, the eluate is evaporated to dryness and reconstituted with acetonitrile/water (20/80, v/v) prior to analysis.
Final determination is performed by LC-MS/MS using an Atlantis T3 analytical column and a gradient mixture of water – acetonitrile with formic acid as modifier at a flow rate of 500 µL/min. Detection is accomplished using positive ion electrospray ionization tandem mass spectrometry (MS/MS-ESI+) monitoring mass transitions 195 → 117 for quantification and 195 → 153 for confirmation.

Results and discussions

The method L0182/02 was proved to be suitable to determine residues of metabolite 500M04 (BF500-5) in water using LC-MS/MS. Samples were spiked with the analyte at the limit of quantification of 0.03 µg/L and 10 times higher (0.3 µg/L). The mean recovery values were between 70% and 110% and the relative

standard deviations (RSD) for all fortification levels were below 20%. The recovery data are summarized in the table below.

Table A 62: Recovery results from method validation of metabolite 500M04 using the analytical method L0182/02

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Groundwater	500M04	0.03 (n = 5)	98	5.9	195 → 117
		0.3 (n = 5)	102	6.0	Quantifier transition
		0.03 (n = 5)	103	7.9	195 → 153
		0.3 (n = 5)	101	6.7	Qualifier transition
Surface water	500M04	0.03 (n = 5)	110	5.6	195 → 117
		0.3 (n = 5)	110	4.0	Quantifier transition
		0.03 (n = 5)	108	7.2	195 → 153
		0.3 (n = 5)	105	1.9	Qualifier transition

Table A 63: Characteristics for the analytical method used for validation of metabolite 500M04 residues in water

	Metabolite 500M04
Specificity	The method L0182/02 allows the specific determination of metabolite 500M04 in water. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary. The tested untreated water samples showed no significant interferences (< 30%) at the retention time of the analyte.
Calibration (type, number of data points)	Linear regression was used for calibration using six calibration levels. Standards prepared in acetonitrile/water (20/80, v/v) were used for calibration.
Calibration range	Good linearity ($r \geq 0.996$) was observed in the range of 0.05 ng/mL to 1.0 ng/mL for the two mass transitions of 500M04 in two different water types (ground- and surface water).
Assessment of matrix effects is presented	No significant influence (differences below 20%) of the matrix load on the analysis of analyte 500M04 was observed.
Standard stability	Standard solutions of 500M04 (prepared in acetonitrile/water, 20/80, v/v) were stable for at least 4 weeks when stored under refrigerated conditions in the dark.
Extract stability	500M04 was stable in extracts of water samples over a tested time period of 7 days when stored under refrigerated conditions in the dark.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.03 µg/L, corresponding to the lowest fortification level. The limit of detection (LOD) is 0.006 µg/L, corresponding to the lowest calibration level.

Conclusion

The method L0182/02 for the analysis of 500M04 in ground- and surface water used HPLC-MS/MS for final determination, with limit of quantification of 0.03 µg/L.

It could be demonstrated that the method fulfills the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of 500M04 in ground- and surface water samples.

A 2.3.2.4.1.3 Independent laboratory validation

Comments of zRMS:	The study has been accepted. The purpose of the study was the independent validation of the BASF method No. L0182/02 (updated after additional validation of BF 500-5 to No. L0782/02) for the determination of parent pyraclostrobin and its metabolites BF 500-5, BF 500-12, BF 500-11, BF 500-13, BF 500-14, and BF 500-15 in drinking and surface water by means of LC-MS/MS monitoring 2 mass transitions for quantification and confirmation. The ILV in surface water was completed successfully for all analytes, but in drinking water except for analyte BF 500-15 (500M78). As BF 500-15 is not relevant for the enforcement residue definition in drinking water, it was eliminated in the ILV study in drinking water. For each matrix 2 fortification levels were applied. For each fortification level and water type, five replicates were analysed. 2 replicates of untreated samples also were examined. In all matrices tested, the recovery data were within the acceptable range (70-110%). The RSDs for all matrices and 2 fortification levels were below 10%. The LOQ defined by the lowest fortification level successfully tested was for pyraclostrobin at 0.003 µg/L and for each metabolite at 0.03 µg/L. Significant interferences (> 30% of LOQ) were not observed in the blanks. For calibration 6 points were used. For all analytes good linearity was observed in the method working range. The ILV is accepted. The independent validation results confirm that BASF method No. L0182/02 is suitable to enforce residues of pyraclostrobin in drinking- and surface-water with the LOQ of 0.003 µg/L. The ILV confirms also that the method No. L0182/02 is suitable to determine residues of BF 500-5, BF 500-12, BF 500-11, BF 500-13, BF 500-14, and BF 500-15 in surface-water with the LOQ of 0.03 µg/L and that the method No. L0182/02 is suitable to determine residues of BF 500-5, BF 500-12, BF 500-11, BF 500-13 and BF 500-14 in drinking-water with the LOQ of 0.03 µg/L. The confirmatory technique is not required. The study was already evaluated in PL.

Reference:	CP 5.2/23
Report	Independent laboratory validation of BASF method L0182/02: BAS 500 F (Pyraclostrobin) and its metabolites BF 500-5 (Reg.No. 298327), BF 500-12, BF 500-11, BF 500-13, BF 500-14, BF 500-15 in ground- and surface- water by LC/MS/MS, Bianca C.M., 2015 report No 714839 2015/7001873

Guideline(s):	EPA 850.6100, SANCO/825/00 rev. 8.1 (16 November 2010)
Deviations:	No
GLP:	yes (certified by United States Environmental Protection Agency)
Acceptability:	Yes

Analytical method L0182/01 and its add-on L0182/02 follow the same principle. The independent laboratory validation (BASF DocID 2015/7001873) covers both versions of the method.

Materials and methods

A 50 mL water sample aliquot is extracted by acidification with formic acid followed by solid phase extraction on a C18 SPE column. Residues are then eluted twice with ethyl acetate. The extracts are concentrated to dryness and reconstituted in acetonitrile/water (20/80, v/v).
Final determination is performed by LC-MS/MS using an Atlantis T3 analytical column and a gradient mixture of water – acetonitrile with formic acid as modifier at a flow rate of 500 µL/min. Detection is accomplished using positive ion electrospray ionization tandem mass spectrometry (MS/MS-ESI+) monitoring two mass transitions for quantification and confirmation: 389 → 195 and 389 → 164 for pyraclostrobin, 196 → 154 and 196 → 118 for 500M04, 370 → 194 and 370 → 278 for 500M59, 278 → 194 and 278 → 149 for 500M60, 248 → 132 and 248 → 164 for 500M62, 389 → 242 and 389 → 301 for 500M76 and 177 → 135 and 177 → 132 for 500M78.

Results and discussions

The method L0182/02 was proved to be suitable to determine residues of pyraclostrobin and its metabolites 500M04 (BF500-5), 500M59 (BF500-12), 500M60 (BF500-11), 500M62 (BF500-13), 500M76 (BF500-14) and 500M78 (BF500-15) in water using LC-MS/MS. Samples were spiked with the analytes at the limit of quantification (0.003 µg/L for pyraclostrobin, 0.03 µg/L for the metabolites) and 10 times higher (0.03 µg/L for pyraclostrobin, 0.3 µg/L for the metabolites). For drinking water, the method was validated for all analytes, with the exception of analyte 500M78. However, as only the parent molecule is relevant for the residue definition for enforcement in drinking water for Europe, 500M78 could be eliminated in the ILV study in the drinking water. The mean recovery values were between 70% and 110% and the relative standard deviations (RSD) for all fortification levels were below 20%. The recovery data are summarized in the tables below.

Table A 64: Recovery results from method validation of pyraclostrobin and its metabolites using the analytical method L0182/02 (Drinking water)

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Drinking water	pyraclostrobin	0.003 (n = 5)	88.9	10.8	389 → 195
		0.03 (n = 5)	102	5.4	Quantifier transition
		0.003 (n = 5)	89.8	10.5	389 → 164
		0.03 (n = 5)	105	7.8	Qualifier transition
	500M04	0.03 (n = 5)	100	9.3	196 → 154
		0.3 (n = 5)	101	8.8	Quantifier transition
		0.03 (n = 5)	87.0	19.8	196 → 118
		0.3 (n = 5)	98.2	5.4	Qualifier transition
	500M59	0.03 (n = 5)	86.6	9.2	370 → 194
		0.3 (n = 5)	98.3	2.9	Quantifier transition
		0.03 (n = 5)	83.8	10.6	370 → 278
		0.3 (n = 5)	94.6	8.9	Qualifier transition
	500M60	0.03 (n = 5)	94.7	3.0	278 → 194
		0.3 (n = 5)	101	4.2	Quantifier transition
		0.03 (n = 5)	90.5	5.0	278 → 149
		0.3 (n = 5)	96.5	2.7	Qualifier transition
	500M62	0.03 (n = 5)	95.4	3.8	248 → 132
		0.3 (n = 5)	97.4	5.3	Quantifier transition
		0.03 (n = 5)	95.9	3.0	248 → 164
		0.3 (n = 5)	98.9	2.1	Qualifier transition
	500M76	0.03 (n = 5)	86.3	19.4	389 → 242
		0.3 (n = 5)	91.0	7.9	Quantifier transition
		0.03 (n = 5)	83.6	8.8	389 → 301
		0.3 (n = 5)	103	8.9	Qualifier transition

Table A 65: Recovery results from method validation of pyraclostrobin and its metabolites using the analytical method L0182/02 (Surface water)

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Surface water	pyraclostrobin	0.003 (n = 5)	103	6.6	389 → 195
		0.03 (n = 5)	104	3.3	Quantifier transition
		0.003 (n = 5)	107	5.2	389 → 164
		0.03 (n = 5)	106	3.3	Qualifier transition
	500M04	0.03 (n = 5)	78.2	13.6	196 → 154
		0.3 (n = 5)	90.8	12.1	Quantifier transition
		0.03 (n = 5)	95.2	16.6	196 → 118
		0.3 (n = 5)	95.0	11.0	Qualifier transition
	500M59	0.03 (n = 5)	86.7	3.9	370 → 194
		0.3 (n = 5)	93.1	3.4	Quantifier transition
		0.03 (n = 5)	85.4	3.1	370 → 278
		0.3 (n = 5)	88.1	1.0	Qualifier transition
	500M60	0.03 (n = 5)	101	2.9	278 → 194
		0.3 (n = 5)	103	3.0	Quantifier transition
		0.03 (n = 5)	101	4.5	278 → 149
		0.3 (n = 5)	101	2.2	Qualifier transition
	500M62	0.03 (n = 5)	95.6	3.1	248 → 132
		0.3 (n = 5)	99.8	3.2	Quantifier transition
		0.03 (n = 5)	97.0	3.1	248 → 164
		0.3 (n = 5)	99.9	2.7	Qualifier transition
	500M76	0.03 (n = 5)	99.5	7.7	389 → 242
		0.3 (n = 5)	104	11.9	Quantifier transition
		0.03 (n = 5)	108	7.1	389 → 301
		0.3 (n = 5)	104	6.2	Qualifier transition
	500M78	0.03 (n = 5)	91.2	8.4	177 → 135
		0.3 (n = 5)	116	2.9	Quantifier transition
		0.03 (n = 5)	94.2	7.5	177 → 132
		0.3 (n = 5)	114	5.7	Qualifier transition

Table A 66: Characteristics for the analytical method used for independent laboratory validation of pyraclostrobin and its metabolites residues in water

	Pyraclostrobin and metabolites 500M04, 500M59, 500M60, 500M62, 500M76 and 500M78
Specificity	The method L0182/02 allows the specific determination of pyraclostrobin and its metabolites 500M04, 500M59, 500M60, 500M62, 500M76 and 500M78 in water. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary. The tested untreated water samples showed no significant interferences (< 30%) at the retention time of the analytes.
Calibration (type, number of data points)	Linear regression was used for calibration using six calibration levels. Matrix-matched standards were used for calibration.
Calibration range	For pyraclostrobin, good linearity ($r \geq 0.99$) was observed in matrix-matched standards in the range of 0.005 ng/mL to 0.1 ng/mL in drinking and surface water. For metabolites 500M04, 500M59, 500M60, 500M62 and 500M76, good linearity ($r \geq 0.99$) was observed in the range of 0.05 ng/mL to 1.0 ng/mL in drinking and surface water. For metabolite 500M78, good linearity ($r \geq 0.98$) was observed in matrix-matched standards in the range of 0.05 ng/mL to 1.0 ng/mL in surface water.
Assessment of matrix effects is presented	Matrix-matched standards were used for the calibration of validation experiments.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.003 µg/L for pyraclostrobin and 0.03 µg/L for each metabolite, corresponding to the lowest fortification level. The limit of detection (LOD) is estimated as 20% of the LOQ with 0.0006 µg/L for pyraclostrobin and 0.006 µg/L for each metabolite.

Conclusion

The ILV was completed successfully on the first trial for pyraclostrobin and its metabolites 500M04, 500M59, 500M60, 500M62, 500M76 and 500M78 in surface water and was completed successfully on the second trial in drinking water for all analytes with exception of analyte 500M78. The method L0182/02 for the analysis of pyraclostrobin and its metabolites in water used HPLC-MS/MS for final determination, with limit of quantification of 0.003 µg/L for pyraclostrobin and 0.03 µg/L for each metabolite.

It was demonstrated that the method L0182/02 fulfills the requirements with regards to linearity, specificity, repeatability, limit of quantification, and recoveries and is therefore applicable to correctly determine pyraclostrobin and its metabolites 500M04, 500M59, 500M60, 500M62 and 500M76 in drinking and surface water. Additionally, method L0182/02 was proved to be suitable to determine residues of metabolite 500M78 in surface water.

A 2.3.2.4.1.4 Confirmatory method

A confirmatory technique is not required as methods L0182/01 and L0182/02 use two different mass transitions of pyraclostrobin and its metabolites 500M59, 500M60, 500M62, 500M76, 500M78 and 500M04 for quantitation and confirmation.

A 2.3.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

A 2.3.2.5.1 Analytical method L0197/01 for determination of pyraclostrobin in air

A 2.3.2.5.1.1 Method validation

Comments of zRMS:	The study has been accepted. The objective of this validation study was to demonstrate the method L0197/01 consistency with the requirements of an enforcement analytical method intended for residues determination in air. The method was validated at two fortification levels of air samples (according to SANCO/825/00 rev.8.1). The quantification was based on two mass transitions for the analyte. For each fortification 6 replicates were analysed. Recovery data was reported for both mass transitions and matrix considered. The mean recovery values ranged between 70 % and 110 %. The RSDs for both fortification levels were below 20%. For calibration 5 points were used. Good linearity (r > 0.99) was observed in the working range for both mass transitions of BAS 500 F. Significant interferences (> 30% of LOQ) were not observed at the retention time and mass transitions of BAS 500 F. Pyraclostrobin LOQ defined by the lowest fortification level successfully tested was set at of 4.44 ng/L. Pyraclostrobin sufficient stability on Tenax ® was confirmed, but in properly stored standard solutions was known from previous studies (see supplementary stability study). The method L0197/01 validation is accepted. It fulfils the requirements regarding linearity, specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly enforce residues of pyraclostrobin in air. No additional confirmatory technique is required. The study was already evaluated in PL.

Reference:	CP 5.2/24
Report	Validation of analytical method L0197/01: Method for the determination of BAS 500 F (Pyraclostrobin) in air by LC-MS/MS, Penning H., 2012 report No 370959 2012/1220256
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Materials and methods

Pyraclostrobin is spiked onto Tenax® adsorption tubes. After sucking air through the glass tubes at 35°C and a relative humidity of > 80% for 6 hours at approximately 90 L/h, the tube content is extracted by ultrasonication using acetone.
Final determination is performed by LC-MS/MS using a Betasil C18 analytical column and a gradient mixture of water – methanol with formic acid as modifier at a flow rate of 600 µL/min. Detection is

accomplished using positive ion electrospray ionization tandem mass spectrometry (MS/MS-ESI+) monitoring mass transitions 388 → 194 for quantification and 388 → 163 for confirmation of pyraclostrobin.

Results and discussions

The method L0197/01 was proved to be suitable to determine residues of pyraclostrobin in air using LC-MS/MS. Adsorption tubes were fortified with the analyte at the limit of quantification of 4.44 ng/L air and 10 times higher (44.4 ng/L air). The mean recovery values were between 70% and 110% and the relative standard deviations (RSD) for all fortification levels were below 20%. The recovery data are summarized in the table below.

Table A 67: Recovery results from method validation of pyraclostrobin using the analytical method L0197/01

Matrix	Analyte	Fortification level (ng/L air) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Air	pyraclostrobin	4.44 (n = 6)	103	4.1	388 → 194
		44.4 (n = 6)	108	1.5	Quantifier transition
		4.44 (n = 6)	103	4.3	388 → 163
		44.4 (n = 6)	108	1.4	Qualifier transition

Table A 68: Characteristics for the analytical method used for validation of pyraclostrobin residues in air

	Pyraclostrobin
Specificity	Under the described conditions method L0197/01 is specific for the determination of pyraclostrobin in air. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary. Two mass transitions of pyraclostrobin were quantified. Significant interferences (> 30% of LOQ) were not observed at the retention time and mass transitions of pyraclostrobin.
Calibration (type, number of data points)	Linear regression was used for calibration using five calibration levels, which were prepared in acetonitrile/water (80/20, v/v).
Calibration range	Good linearity (r > 0.99) was observed in the range of 0.24 ng/mL to 2.4 ng/mL for both mass transitions of pyraclostrobin.
Assessment of matrix effects is presented	Even no matrix effects should be expected because of the simple matrix air, it has been tested by instrument recovery samples (QCS) and determined as not significant.
Storage stability on adsorber material	Pyraclostrobin was stable on Tenax ® adsorber material for 3 days of storage under refrigerated conditions at 4°C.
Standard Stability	In a previous study, the stability of pyraclostrobin in standard solutions, prepared in acteonitrile/water (20/80, v/v), was proved to be stable for at least 16 days, when stored under refrigerated conditions in the dark.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 4.44 ng/L air, corresponding to the lowest fortification level. The limit of detection (LOD) is 0.9 ng/L air.

Conclusion

The method L0197/01 used LC-MS/MS for the final determination of pyraclostrobin in air with a limit of quantification (LOQ) of 4.44 ng pyraclostrobin per L air.

The method fulfills the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of pyraclostrobin in air.

A 2.3.2.5.2 Analytical method L0197/02 for determination of pyraclostrobin in air

A 2.3.2.5.2.1 Method validation

Comments of zRMS:	The method has been accepted. The objective of this study was to validate LC-MS/MS method L0197/02 for determination of BAS 500 F (Pyraclostrobin) in air. Repeatability of the method was tested for the analyte BAS 500 F using 5 replicates per fortification level. Quantification of the analyte was done for 2 mass transitions. The mean recoveries for BAS 500 F ranged from 83 % to 92 %, which fulfils the requirements for recovery values. The relative standard deviations (RSD, %) over all fortification levels were below 20 %. The LOQ of 0.5 ng/L (0.5 µg/m^3) was set. Good linearity (r > 0.999) was observed in the range of 0.04 ng/mL to 5 ng/mL for the two mass transitions of BAS 500 F. Significant interferences (> 30 % of LOQ) were not observed at the retention times and mass transitions of the analyte. The method L0197/02 fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of Pyraclostrobin in air.

Reference:	CP 5.2/25
Report	Validation of Analytical Method L0197/02: Method for the Determination of BAS 500 F (Pyraclostrobin) in air by LC-MS/MS Bloss, K., 2018 report No 865278, S18-04959 2018/1128631 Authority registration No
Guideline(s):	EPA 850.6100, EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010)
Deviations:	No
GLP:	yes (certified by Landesanstalt fuer Umwelt, Baden-Wuerttemberg, Karlsruhe, Germany)
Acceptability:	Yes

Materials and methods

The purpose of the study was to validate the analytical method L0197/02 for the determination of pyraclostrobin (BAS 500 F) in air.
Pyraclostrobin is loaded onto the front filter of a polymer based adsorbent tube (Tenax). Air is passed over the filter and through the tube. Residues of pyraclostrobin in air are trapped in the adsorbent tube. Thereafter, the adsorbent material is extracted with acetone, the extract is evaporated to dryness and reconstituted with acetonitrile/water (80/20, v/v). The reconstituted extract is analyzed by HPLC-MS/MS using an Agilent Zorbax RRHD Eclipse Plus C18 column (50 mm x 2.1 mm, 1.8 µm) with a UHPLC guard column (AJ0-9000, Phenomenex) with 2.1 mm C18 cartridge (AJ0-8782, Phenomenex) and gradient elution mode. The mobile phases are water and methanol, each acidified with formic acid.
Integrated peak areas from MS detection are used for quantification. The mass transitions 388 → 194 m/z is used for quantification and the transition 388 → 163 m/z is used for qualification. Linear regression is used for calibration with external solvent-matched calibration standards.

Results and discussions

Method L0197/02 proved to be suitable for the determination of pyraclostrobin in air. The accuracy of the method was determined at two fortification levels appropriate to the levels of LOQ (0.5 µg/m³) and 10x LOQ (5.0 µg/m³). All mean recovery values (mean of five replicates per fortification level) were between 70 and 110%. The recovery data are summarized in the table below.

Table A 69: Recovery results from method validation of pyraclostrobin using the analytical method L0197/02

Matrix	Analyte	Fortification level (µg/m³) (n = x)	Mean recovery (%)	RSD (%)	Comments
Air	pyraclostrobin	0.5 (n = 5)	83	1.0	Quantitation
		5.0 (n = 5)	92	3.2	m/z 388 → 194
		0.5 (n = 5)	85	2.8	Confirmation
		5.0 (n = 5)	92	2.7	m/z 388 → 163

Table A 70: Characteristics for the analytical method used for validation of pyraclostrobin residues in air

	Pyraclostrobin
Specificity	The HPLC-MS/MS method is highly specific with two mass transitions monitored for each analysis. Target analyte concentrations in controls (blanks) did not exceed 30% of the LOQ. No interfering peaks were observed at the retention time of pyraclostrobin.
Calibration	Six calibration standards, prepared in acetonitrile / water (80/20, v/v), were used. Linear regression with weighting 1/x was applied with coefficients of correlation being 0.9992.
Calibration range	Calibration points distributed over a concentration range of 0.04 to 5.0 ng/mL (corresponding to 0.15 ng/L to 18.5 ng/L at test sample level) were used. The LOQ and 10x LOQ level fall within the calibration range used.
Assessment of matrix effects	Matrix effects were assessed by means of comparison of average response factors determined for both solvent-matched and matrix-matched calibration standards. Matrix effects were found to be insignificant, thus, solvent-standards were used for calibration.
Standard stability	Pyraclostrobin was stable for a maximum duration of 35 days in stock and fortification solutions, prepared in acetone, and 29 days in calibration solutions, prepared in acetonitrile/water (80/20, v/v), when stored refrigerated at 1 – 10°C in the dark.
Stability in tubes and extracts	Pyraclostrobin was stable in tubes and extracts over a time period of 7 days, when stored tubes at ambient temperature, in a refrigerator or in a freezer and extracts in a refrigerator or in a freezer in the dark.
Sorbent capacity	No breakthrough of pyraclostrobin was observed at a concentration of 50 ng/L (50 µg/m³, 100x LOQ) over a period of 9 h at a mean temperature of 35.2°C and a relative mean humidity >80%.
Limit of quantitation/determination	The limit of quantification (LOQ) is 0.5 ng/L (0.5 µg/m³) for pyraclostrobin in air resulting from the lowest fortification level successfully tested. The corresponding concentration in the reconstituted extract is 0.135 ng/mL. The limit of detection (LOD) is 0.15 ng/L (0.15 µg/m³) and corresponds to the lowest calibration level of 0.04 ng/mL.

Conclusion

The method for analysis of pyraclostrobin in air uses LC-MS/MS for final determination, which is a highly specific technique. The limit of quantitation is 0.5 µg/m³.

It could be demonstrated that method L0197/02 fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine residues of pyraclostrobin in air.

A 2.3.2.5.2.2 Confirmatory method

A confirmatory technique is not required as method L0197/01 uses two different mass transitions of pyraclostrobin for quantitation and confirmation.

A 2.3.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

Analytical method L0151/01, BASF DocID 2010/1018944, was validated for animal matrices, including swine blood. Also an ILV is provided, BASF DocID 2010/1123694. This method is covering the requirement for an analytical method on body fluids and tissues. For details see appendix section A 2.3.2.2.1.2.

A 2.3.2.7 Other Studies/ Information

A 2.3.2.7.1 Stability of standard solutions

Comments of zRMS:	The study has been accepted. The objective of this study was the stability determination of pyraclostrobin standards and its relevant metabolites/derivatives in different solutions. In plant matrices the parent pyraclostrobin and its desmethoxy metabolite (BF 500-3) were determined by method No. 421/0. In animal matrices the method No. 439/0 and method No. 446/0 for derivatives MMP [1-(4-chlorophenyl)-3-methoxy-1H-pyrazole] and DMP [1-(4-chloro-3-methoxy-phenyl)-3-methoxy-1H-pyrazole] determination was employed. The storage stability of the parent and desmethoxy metabolite was investigated in methanol, methanol/buffer solution and acetonitrile. The parent substance was also examined in a mixture of iso-octane/methanol/iso-propanol. The stability of the derivatives MMP and DMP was investigated in iso-octane. For the final determination of the parent and desmethoxy metabolite in methanol, methanol/buffer solution and acetonitrile LC-MS/MS (1 transition monitored) was applied. Determination of pyraclostrobin in solvent mixture iso-octane/methanol/iso-propanol was performed by HPLC-UV. MMP and DMP derivatives were determined by GC-MS. The results obtained from this study allow the calculation of the degradation of the test compounds in the respective solvents over a time of 120 days. The degradation figures were calculated as percent of day 0 concentration. The results show that pyraclostrobin is stable in methanol, methanol/buffer solution and iso-octane/methanol/iso-propanol over the whole interval of 120 days. In acetonitrile however, stability of pyraclostrobin is given for 30 days. BF 500-3 is stable in all solvents/mixtures investigated over 120 days. The derivatives MMP and DMP show a slight degradation if exposed to room temperature and daylight. In general, it is recommended to store standard solutions in the refrigerator and renew them after 30 days. The study was already evaluated in PL.

Reference:	CP 5.2/26
Report	Determination of the stability of BAS 500 F and of relevant metabolites and derivatives thereof in different solutions, Reinhard K.,Mackenroth C., 1999 report No 35511

	1999/11136
Guideline(s):	None (no guidelines available)
Deviations:	No
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)
Acceptability:	Supplementary

Executive Summary

The stability of standard solutions of pyraclostrobin, metabolite 500M07 and two derivatives MMP (Reg. No. 342878) and DMP (Reg. No. 412041) was determined in several solvents and solvent mixtures.

Standard solutions of each tested compound were prepared and stored in four volumetric flasks for each solvent or solvent mixture to be tested. Two flasks of each set were kept in the refrigerator in the dark at 4°C, while two flaks were exposed to daylight at room temperature.

After about 0, 1, 7, 14, 30, 60 and 120 days, aliquots were taken and analyzed using HPLC-MS/MS, HPLC-UV or GC-MS. The degradation figures were calculated as percent of day 0 concentration and are summarized in the table below.

Table A 71: Degradation after 120 days (in % of starting concentration)

Compound	Solvent	4°C, in the dark [%]	Room temperature, exposed to daylight [%]
Pyraclostrobin	Methanol	0.2	-12.8 [a]
	Acetonitrile	15.4	15.0
	Methanol/buffer solution (80/20, v/v)	-6.6 [a]	-8.0 [a]
	Iso-octane/methanol/iso-propanol (99/0.5/0.5, v/v/v)	-1.9 [a]	-4.6 [a]
500M07	Methanol	-42.0 [a]	-41.5 [a]
	Acetonitrile	-4.2 [a]	-1.7 [a]
	Methanol/buffer solution (80/20, v/v)	-2.4 [a]	-1.7 [a]
MMP	Iso-octane	-2.3 [a]	8.7
DMP	Iso-octane	4.8	15.8

[a] Negative figure indicates calculated “increase” of the concentration.

The results show that pyraclostrobin is stable in methanol, methanol/buffer solution (80/20, v/v) and iso-octane/methanol/iso-propanol (99/0.5/0.5, v/v/v) over 120 days under both storage conditions. In acetonitrile, the stability of pyraclostrobin is given for 30 days.

Metabolite 500M07 is stable in all tested solvents (methanol, acetonitrile and methanol/ buffer solution (80/20, v/v) over 120 days under both storage conditions.

The derivatives MMP and DMP show a slight degradation, when stored at room temperature and exposed to daylight. Under refrigerated conditions at 4°C in the dark, both compounds are stable over 120 days.

In general, it is recommended to store standard solutions in the refrigerator and renew them after 30 days.

A 2.3.2.7.2 Statement on Extraction Efficiency

Comments of zRMS:	The study has been accepted. It can be concluded here that the extraction procedures used in the residue analytical methods are equivalent with the extraction procedures applied in the metabolism investigation. And furthermore, the extraction procedure used in the method 439/0 (see Table 5.2-23) is suitable to extract incurred residues of pyraclostrobin completely from sample material of animal origin. The detailed conclusions below were sufficiently drawn. The study was already evaluated in PL.

Reference:	CP 5.2/27
Report	Efficiency of the extraction procedure in methods for the determination of Pyraclostrobin in matrices of animal origin, Bross M.,Tilting N., 2001 2001/1001011
Guideline(s):	one (presented data were extracted from validation studies, which were conducted according to the legal requirements of the actual guidelines)
Deviations:	not applicable
GLP:	No (The presented data, is a compilation of data from multiple GLP-studies, but has not been additionally QAU-audited.)
Acceptability:	Supplementary

Executive Summary

The aim of this statement was to describe the extraction efficiency of the procedure in the methods used for the determination of pyraclostrobin in plant matrices and matrices of animal origin. These investigations were performed in the course of two studies. In one study, in addition to the extractions with methanol, extractions with methanol/water (v/v) were performed. In the second study the extraction procedures applied in method number 421/0 were investigated.
For the determination of pyraclostrobin in matrices of animal origin two methods were used. Method 446/0 (GC-MS, for milk) or its variation 446/1 (HPLC-MS-MS, used for tissues and milk) was used for the determination of the total residues of pyraclostrobin and its metabolites and method 439/0 for the parent compound.
The results of the both studies in plant matrices show that the extraction procedures used in the residue analytical methods are equivalent with the extraction procedures applied in the metabolism investigation.
From the experiments in matrices of animal origin performed during the metabolism studies and the method development it can be concluded that the extraction procedure used in method 439/0 is suitable to extract incurred residues of pyraclostrobin completely from sample material of animal origin.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** active ingredient BAS 500 F and its metabolites
   Description: BAS 500 F
   Lot/Batch #: 00937-128, PCP03995
   Purity: n/a
   CAS#: 17013-18-0

Development code: BAS 500 F
Spiking levels: n/a

2. **Test Commodity:** Cereals, Root crop, Berries & small fruit, Hen, Goat,
Crop: wheat, sugarbeet, grape
Type: n/a
Variety: n/a
Botanical and zoological name: *Triticum vulgare, Vitis vinifera, Solanum tuberosum, Capra hircus, Gallus gallus*
Crop part(s), animal part(s) or processed commodity: wheat (forage, straw, grain), potato (green matter, tuber), grapes, milk, eggs, liver, muscle, fat, kidney
Sample size: n/a

B. STUDY DESIGN

The investigations on the extractability in accordance with the residue analytical methods used for the determination of BAS 500 F in plant matrices were performed in the course of two studies. In one study, in addition to the extractions with methanol, extractions with methanol/water (v/v) were performed. In the second study the extraction procedures applied in method number 421/0 were investigated.
For the determination of pyraclostrobin in matrices of animal origin, two methods were used. Method 446/0 (GC-MS, for milk) or its variation 446/1 (HPLC-MS-MS, used for tissues and milk) was used for the determination of the total residues of pyraclostrobin and its metabolites and method 439/0 for the parent compound.

II. RESULTS AND DISCUSSION

Considering the study "The metabolism of ^{14}C-BAS 500 F in Potato" shows that in the case of tolyl label, the extraction with methanol/water (v/v) led to mean extractability of 57.3% of the TRR, which is slightly higher than the 41.6% achieved with pure methanol. This slightly better extractability could be explained by the higher solubility of tryptophan and tryptophan containing proteins in water. With the chlorophenyl label, the aqueous methanol extraction resulted in an average extractability of 66.5%, which corresponds very well with the result of 67.5% extracted with methanol.

The results of the second study about the extractability of BAS 500 F in plant matrices show that 83.3% of the TRR from wheat forage, 61.6% from wheat grain and 85.9% from wheat straw as well as 86.3% of the TRR from grapes were released by methanol/water (7/3, v/v). This corresponds to the extraction yields from the metabolism studies.

Method 446/0 accounted for 152% of the residues identified as parent and metabolites oxygenated in the 2 position of the chlorophenyl ring when compared to the metabolism study results after ^{12}C and recovery correction. Correspondingly, LC-MS/MS method 446/1 accounted for 98%, 720% and 112% of the residues identified in milk, liver and muscle, respectively. These results support the use of these methods in determining BAS 500 F residues in animal products.

The extraction efficiency test performed during the method 439/0 development phase, with a ^{14}C muscle sample from the goat metabolism study extracted with methanol, yielding an extraction efficiency of 84.3% TRR, consisting mainly on pyraclostrobin. The extraction test for method 439 with acetonitrile + hexane was performed at an early stage of method development. It could be shown that by extracting either 20 g or 50 g of muscle with 200 mL of acetonitrile and 100 mL of hexane, 90.6% or 88.1% of the total radioactivity could be extracted. So the extraction efficiency was even slightly better than with methanol.

III. CONCLUSION

The results of the two studies in plant matrices show that the extraction procedures used in the residue analytical methods are equivalent with the extraction procedures applied in the metabolism investigation.
From the experiments in matrices of animal origin performed during the metabolism studies and the method development the following conclusions can be drawn:
1) Acetonitrile is a suitable solvent for extraction of incurred residues as demonstrated in hen metabolism and for milk and fat also in goat metabolism.
2) No residues will remain in the hexane phase during partitioning or extraction.
3) The efficiency of acetonitrile + hexane as extraction solvent was compared to methanol used in metabolism during method development with incurred residues from a goat muscle sample and the efficiency was similar.

This proves that the extraction procedure used in method 439/0 is suitable to extract incurred residues of pyraclostrobin completely from sample material of animal origin.

A 2.3.2.7.3 Compilation of Mass Transitions / Product Ion Scans of pyraclostrobin and pyraclostrobin analytes

Comments of zRMS:	This supplementary data has been accepted. This is basically a list of useful mass transitions of pyraclostrobin and its metabolites that can be used for further methods. The data has been extracted from validation studies, conducted under GLP and according to the legal requirements of the actual guidelines. The data was already evaluated in PL.

Reference:	CP 5.2/28
Report	Supplementary data on residue analytical methods for Pyraclostrobin - Compilation of Mass Transitions/Product Ion Scans, used within residue analytical methods for the determination of BAS 500 F - Pyraclostrobin Analytes, Obermann M., 2015 2015/1197842
Guideline(s):	None (presented data were extracted from validation studies, which were conducted according to the legal requirements of the actual guidelines)
Deviations:	not applicable
GLP:	No (The presented data, is a compilation of data from multiple GLP-studies, but has not been additionally QAU-audited.)
Acceptability:	Supplementary

Executive Summary

In course of the development of residue analytical methods, which are based on tandem mass spectrometry-detection (MS/MS), product ion scans of the individual analytes were generated, to identify useful mass transitions for the final determination. As the analytes are included in multiple analytical methods, the product ion scans are not always generated within each individual method validation, but the identified mass transitions also used for further methods.

Therefore, a compilation has been prepared to list mass transitions of pyraclostrobin and several metabolites with product ion scans, used in one or multiple residue analytical methods. The listed mass transitions can be used for further methods.

The mass transitions, which can be used for quantification and confirmation of the pyraclostrobin analytes, are summarized in the following table:

Table A 72: Overview of commonly used mass transitions of pyraclostrobin analytes

Analyte	Commonly used mass transition (s)			Comment (Spectrum generated within BASF DocID)
Pyraclostrobin	388 → 163	388 → 194		2013/1184817
	389 → 195	389 → 164		2015/7001873
500M01	611 → 223	611 → 417		2013/1184817
500M02	595 → 207	595 → 401		2013/1184817
500M04	195 → 153	195 → 117	195 → 150	2013/1400972
	196 → 154	196 → 118		2015/7001873
500M07	358 → 132	358 → 164		2013/1184817
500M59	370 → 194	370 → 278		2015/7001873
500M60	278 → 194	278 → 149		2015/7001873
500M62	248 → 132	248 → 164	248 → 216	2015/7001873
500M76	389 → 242	389 → 301		2015/7001873
500M78	177 → 135	177 → 132		2015/7001873
500M79	357 → 195	357 → 153		2014/1001721
500M85	211 → 138	211 → 166		2013/1400972

A 2.3.2.8 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

Analytical method L0151/01, BASF DocID 2010/1018944, was validated for animal matrices, including swine blood. Also an ILV is provided, BASF DocID 2010/1123694. This method is covering the requirement for an analytical method on body fluids and tissues. For details see appendix section A 2.3.2.2.1.2.