REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: BAS 762 02 F

Product name: Revydas

Chemical active substances:

Mefentrifluconazole, 100.0 g/L

Boscalid, 200.0 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: BASF

Submission date: March 2021

MS finalisation date: November 2021 (initial Core Assessment)

April 2022 (final Core Assessment)

Version history

When	What
March 2021	Applicant initial dRR
November 2021	Initial assessment by the zRMS The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~.
April 2022	Final report (Core Assessment after the commenting period) Additional information/assessments included by the zRMS in the report in response to comments recieved from the cMS and the Applicant are highlighted in yellow, while not agreed use pattern is ~~struck through and shaded~~.

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5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substance(s) and relevant impurities in the plant protection product.
Noticed data gaps are: none

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.
Noticed data gaps are: none

Commodity/crop	Supported/ Not supported
Wheat	Supported
Sunflower seed	Supported
Oilseed rape	Supported

zRMS comments:

BASF is the owner of both active substances.
Sufficiently sensitive and selective analytical methods for mefentrifluconazole and boscalid are available for all analytes included in the residue definitions.

Mefentrifluconazole
In EFSA Journal 2018;16(7):5379 it is stated that "*Adequate methods are available for the generation of pre-approval data required for the risk assessment. However, validation data for the analytical method used in the key toxicity studies, in particular the 1-year dog, and developmental toxicity studies in rats and rabbits were not provided (data gap).*
Mefentrifluconazole residues can be monitored in food and feed of plant origin by a quick, easy, cheap, effective and safe (QuEChERS) method using liquid chromatography with tandem mass spectrometry (LC–MS/MS) with a limit of quantification (LOQ) of 0.01 mg/kg in each commodity group.
Mefentrifluconazole residues in food of animal origin can be determined by LC–MS/MS with a LOQ of 0.01 mg/kg in all animal matrices.
Mefentrifluconazole residues in soil and in drinking and surface water can be monitored by LC–MS/ MS with LOQs 0.002 mg/kg and 0.030 µg/L, respectively.
An appropriate LC–MS/MS method exists for monitoring mefentrifluconazole residues in air with a LOQ of 0.01 ng/L.
A LC–MS/MS method can be used for monitoring of mefentrifluconazole residue in body fluids (urine and blood) with a LOQ of 0.01 mg/L. The method for monitoring of mefentrifluconazole in food of animal origin can be used for the determination of mefentrifluconazole in body tissues. However, it has been concluded that metabolites M750F015, M750F016 and M750F017 should be also included in the residue definition for body fluids, as a consequence a data gap for monitoring methods for their determination in body fluids was identified."

According to the EFSA Journal 2018;16(7):5379 following data gap in analytical methods area has been identified during the peer review process:

- Monitoring methods for determination of metabolites M750F015, M750F016 and M750F017 in body fluids.

Appropriate analytical monitoring method for the determination of metabolites M750F015, M750F016 and M750F017 in body fluids (plasma and urine) using LC-MS/MS with a LOQ of 0.01 mg/L have been provided by Applicant in the framework of this application. The details of the evaluation of the new study are referred in Appendix 2. The study has been accepted. This data gap (see EFSA Journal 2018;16(7):5379) has been fulfilled.

Additionally, the evaluation of methods for the generation of pre-authorization data is presented in Appendix 2:

- the analytical method APL0500/03 has been satisfactorily validated for the determination of residues of BAS 750 F (Mefentrifluconazole) and its metabolite M750F007 (Reg.No.6003432) in M4-Medium, OECD-water and mixing water by LC/MS with a LOQ of 0.001 mg/L,

- the slightly modified analytical method APL0500/03 has been satisfactorily validated for the determination of residues of BAS 750 F (Mefentrifluconazole) in test water by HPLC/MS with a LOQ of 0.001 mg/L.
- the analytical method L0361/01 has been satisfactorily validated for the determination of residues of BAS 750 F (Mefentrifluconazole) in tap water and M4-medium by LC-MS/MS with a LOQ of 0.1 µg/L,

and for post-authorization control and monitoring purposes:

- the analytical method L0359/01 has been satisfactorily validated for the determination of residues of BAS 750 F (Mefentrifluconazole) in drinking (ground) and surface water by LC-MS/MS with a LOQ of 0.03 µg/L.

Additionally, a residue study for the determination of BAS 750 F residues in honey has been performed (BASF DocID: 2020/2109990, Report Amendment N°1 DocID: 2021/2038566) and has been provided by Applicant. The study is a combination of residue analytics and a validation study in honey. Therefore, the method analytical part of the study is presented in Appendix 2 of Part B5.
Whole plant (no roots), inflorescences, pollen and honey specimens were analyzed for residues of BAS 750 F (Mefentrifluconazole) and for residues of the triazole metabolites: 1,2,4-Triazole (T), Triazolylalanine (TA), Triazole lactic acid (TLA) and Triazole acetic acid (TAA) using BASF method L0170/03. The limit of quantification (LOQ) for all analytes was 0.050 mg/kg. The study is acceptable.

Boscalid
Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is not identical. In the Draft Assessment Report, 2002, the residue definition was the following:

- for food of plant origin: boscalid
- for food of animal origin: boscalid, M510F01 (including its conjugates) calculated as boscalid.

The residue definition, stated in Regulation (EU) No. 2021/590 is the following:

- for food of plant origin: boscalid
- for food of animal origin: sum of boscalid and its hydroxy metabolite 2-chloro-N-(4′-chloro-5-hydroxybiphenyl-2-yl)nicotinamide (free and conjugated) expressed as boscalid.

Analytical methods for residues in plant and animal matrices provided in this dossier are therefore complying with the last residue definition stated in reg. (EU) 2021/590.

According to the EFSA Journal 2014;12(7):3799:
Methods for enforcement of residues in food of plant origin
"*During the peer review under Directive 91/414/EEC, an analytical method using GC-MS and its ILV was evaluated and validated for the determination of boscalid in plant matrices with an LOQ of 0.01 mg/kg in high water content (tomato), 0.02 mg/kg in high fat content (oilseed rape), and 0.01 mg/kg in acidic (lemon) and dry commodities (wheat grain) (Germany, 2002). The method is however not highly specific according to SANCO/825/00 rev 8.1 (EC, 2010b).*
In addition, an analytical method using HPLC-MS/MS was evaluated and validated for the determination of boscalid in plant matrices with an LOQ of 0.05 mg/kg in high water (apple, sour cherry, strawberry, carrot, onion, tomato, broccoli, white cabbage, leek, dwarf bean), high acid (grape) and high fat content (oilseed rape) commodities (Germany, 2002; FAO, 2006).
The multi-residue QuEChERS method in combination with HPLC-MS/MS, as described by CEN (2008), is also reported for analysis of parent boscalid with an LOQ of 0.01 mg/kg in dry commodities, high water content, high fat content and acidic commodities.
Hence it is concluded that boscalid can be enforced in food of plant origin with an LOQ of 0.01 mg/kg in high water content, high fat content, dry and acidic commodities.
No analytical method is available for hops, spices and herbal infusions. Considering that analytical methods are validated in four matrix groups and that dilutions of extracts before analysis should reduce the analysis difficulties inherent to those commodities, it is likely that validated methods presented above can be used to enforce the high residue levels observed in hops, spices and herbal infusions. Nevertheless, full validation data is in principle still required."

Methods for enforcement of residues in food of animal origin
"*During the peer review under Directive 91/414/EEC, an analytical method using GC-ECD, confirmed by GC-MS, and its ILV were evaluated and validated for the determination of boscalid and its metabolite M510F01 (free and conjugated) in food of animal origin with an LOQ of 0.01 mg/kg for each compound in milk and 0.025 mg/kg for each compound in meat, fat, liver, kidney and eggs (Germany, 2002). The method should be able to analyse the conjugates of the metabolite but the hydrolysis step has not been validated. Moreover, the method is not highly*

specific according to SANCO/825/00 rev 8.1 (EC, 2010b).
Nevertheless, an analytical method using HPLC-MS/MS was also evaluated and validated for the determination of boscalid and its metabolite M510F01 (free and conjugated) in animal matrices with an LOQ of 0.01 mg/kg for each compound in milk, cream and eggs, and 0.025 mg/kg for each compound in muscle, fat, kidney and liver. The method is able to analyse the conjugates of the metabolite and the hydrolysis step is considered as validated.
Hence it is concluded that boscalid can be enforced in food of animal origin with an LOQ of 0.01 mg/kg in milk and eggs, and an LOQ of 0.025 mg/kg in muscle and fat. Boscalid and its metabolite M510F01 (free and conjugated) can be enforced in liver and kidney with an LOQ of 0.025 mg/kg for each compound. Further validation of the hydrolytic step dissociating the conjugates of metabolite M510F01 should be required for GC-ECD method. However, considering the hydrolysis step was validated for another analytical method, this information is considered as desirable only (minor deficiency)."

The lack of an analytical method for hops, spices and herbal infusions has been identified during review of the Maximum Residue Levels (EFSA Journal 2014;12(7):3799). To close this data gap, a new method for the determination of boscalid in these matrices was developed and validated. The details of the evaluation of new study is referred in point A 2.2.2.1.2.1 of Appendix 2. Study has been accepted. The limit of quantitation was 0.01 mg/kg for boscalid in all matrices tested. **T**he method is suitable for data generation as well as monitoring purposes.

Additionally, the evaluation of methods for the generation of pre-authorization data and for monitoring purposes is presented in Appendix 2:

- the analytical method 535/1 has been satisfactorily validated for the determination of residues of boscalid in following plant matrices: wheat (grain, straw), lemon, lettuce, oilseed rape (seed), tomato and onion with an LOQ of 0.01 mg/kg using LC-MS/MS;
- the analytical method 471/0 has been successfully validated and fulfils the requirements with regard to specificity, linearity, limit of quantification, recoveries and repeatability for the analysis of BAS 510 F (boscalid) and its metabolite M510F01 in foodstuffs of animal origin (exemplified with milk, cream, egg, muscle, fat, liver and kidney);
- the analytical method L0096/01 has been successfully validated and fulfils the requirements with regard to specificity, linearity, limit of quantification, recoveries and repeatability for the analysis of BAS 510 F (boscalid) in soil with LOQ of 0.01 mg/kg using HPLC-MS/MS;
- the analytical method L0096/02 has been successfully validated and fulfils the requirements with regard to specificity, linearity, limit of quantification, recoveries and repeatability for the analysis of boscalid metabolites: M510F47 and M510F49 in soil with LOQ of 0.01 mg/kg using HPLC-MS/MS;
- the analytical method L0127/01 has been successfully validated and fulfils the requirements with regard to specificity, linearity, limit of quantification, recoveries and repeatability for the analysis of BAS 510 F (boscalid) in groundwater and surface water with LOQ of 0.03 µg/kg using LC-MS/MS;
- the analytical method L0127/02 has been successfully validated and fulfils the requirements with regard to specificity, linearity, limit of quantification, recoveries and repeatability for the analysis of boscalid metabolites M510F47 (Reg. No. 107371) and M510F49 (Reg. No. 391572) in groundwater and surface water with LOQ of 0.03 µg/L using LC-MS/MS;
- the analytical method L0336/01 has been successfully validated and fulfils the requirements with regard to specificity, linearity, limit of quantification, recoveries and repeatability for the analysis of boscalid in air with LOQ of 0.0012 µg/m³ using HPLC-MS/MS;
- the analytical method L0342/01 ((based on multi-residue method QuEChERS) has been satisfactorily validated for the determination of residues of BAS 510 F (Boscalid) and its metabolite M510F01 in body fluids (blood and urine) using LC-MS/MS with a LOQ of 0.01 mg/L.
- the standard stability of boscalid in methanol/acetate buffer solution (80/20, v/v) at concentration levels of 0.1 µg/mL and 1.0 ng/mL was determined in BASF DocID 2010/1046613. The standard solutions were stable over a time period of at least 30 days when stored under refrigerated conditions at 4°C in the dark;

No data are required.

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

The analytical method AFL0995/01 was developed for the determination of the active substances mefentrifluconazole (BAS 750 F, Reg. No. 5834378) and boscalid (BAS 510 F, Reg. No. 300355) in the SC formulation BAS 762 02 F and aqueous solutions of BAS 762 02 F. This method has not been previously reviewed and is provided in support of this assessment.

Comments of zRMS:	The AFL0995/01 method is acceptable for the determination of the content of Boscalid and Mefentrifluconazole in BAS 762 02 F formulation.

Reference:	CP 5.1.1/1
Report	AFL0995/01: Determination of the Active Ingredients Boscalid and Mefentrifluconazole in BAS 762 02 F and Aqueous Solutions of BAS 762 02 F by HPLC and UPLC, XXX, A., 2019 report No 2019/2034432 Authority registration No
Guideline(s):	No guidelines available
Deviations:	No
GLP:	No, not relevant for this subject type
Acceptability:	Yes

Materials and methods

The determination of the contents of the active ingredients in the BAS 762 02 F formulation and in aqueous suspensions of BAS 762 02 F is performed by reversed-phase HPLC and UPLC separation with DAD / UV detection. The amounts of the active ingredients are calculated using external calibrations with authentic reference items by applying bracketing. The identity of the test items is confirmed by comparing retention times and UV spectra of the test and reference items.

HPLC parameters

Column	Waters Xbridge C18 (100 mm x 4.6 mm; 3.5 µm)		
Column temperature	40 °C		
Injection volume	5 µL		
Detection wavelength	232 nm		
Flow rate	1.8 mL/min		
Eluent	A: 1000 mL water + 1 mL formic acid conc. B: 1000 mL acetonitrile + 1 mL formic acid conc.		
Approx. retention times	3.81 min Boscalid 5.58 min Mefentrifluconazole		
Gradient	Time [min]	A [%]	B [%]
	0.00	55	45
	6.50	55	45
	6.55	5	95
	10.50	5	95
	10.55	55	45
	15.00	55	45
Dwell volume	Ca. 1048 µL (Agilent 1200 SL Series, LC196)		

UPLC parameters

Column	Waters Acquity BEH C18 (50 mm x 2.1 mm; 1.7 µm)		
Column temperature	40 °C		
Injection volume	1 µL		
Detection wavelength	232 nm		
Flow rate	0.8 mL/min		
Eluent	A: 1000 mL water + 1 mL formic acid conc. B: 1000 mL acetonitrile + 1 mL formic acid conc.		
Approx. retention times	0.98 min Boscalid 1.40 min Mefentrifluconazole		
Gradient	Time [min]	A [%]	B [%]
	0.00	55	45
	1.50	55	45
	1.55	5	95
	2.45	5	95
	2.50	55	45
	3.50	55	45
Dwell volume	Ca. 344 µL (Agilent 1290 Series, LC152)		

Validation - Results and discussion

Comments of zRMS:	The analytical method AFL0995/01 was successfully validated for the determination of Boscalid and Mefentrifluconazole in BAS 762 02 F formulation according to the requirements laid down by SANCO3030/99 rev.4.

Reference:	CP 5.1.1/2
Report	Validation of the Analytical Method AFL0995/01: Determination of the Active Ingredients Boscalid and Mefentrifluconazole in BAS 762 02 F and Aqueous Solutions of BAS 762 02 F by HPLC and UPLC, XXX, A., 2019 report No 880403_1 2019/2034429 Authority registration No
Guideline(s):	ABNT NBR 14029, CIPAC Guidelines on method validation, EPA 830.1000, OPPTS 830.1800, SANCO/3029/99, SANCO/3030/99 rev. 4 (11 July 2000) (If none, give justification, e.g., " no guidelines available" or " methods used comparable to guideline(s) xxx")
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Mainz, Germany),
Acceptability:	Yes

Table 5.2-1: Method for the determination of the active substances mefentrifluconazole and boscalid in the plant protection product BAS 762 02 F

Parameter	**Mefentrifluconazole**	**Boscalid**
Author(s), year	Andrea XXX, 2019	
Principle of method	HPLC / UPLC with DAD / UV detection	
Linearity (linear between mg/L of the nominal concentration) (correlation coefficient expressed as R)	HPLC Range: 40.937-184.216 mg/L R: 1.0000 Slope: 0.1172 Intercept: -0.0149 UPLC Range: 40.937-184.216 mg/L R: 1.0000 Slope: 0.0493 Intercept: 0.0150	HPLC Range: 80.784-363.528 mg/L R: 1.0000 Slope: 0.1653 Intercept: -0.0145 UPLC Range: 80.784-363.528 mg/L R: 1.0000 Slope: 0.0698 Intercept: 0.0823
Precision n = 5 (Repeatability mean) (% RSD)	HPLC Mean: 8.515 % % RSD: 0.45 % H_r = 0.23 UPLC Mean: 8.548 % % RSD: 0.33 % H_r = 0.17	HPLC Mean: 18.253 % % RSD: 0.38 % H_r = 0.22 UPLC Mean: 18.258 % % RSD: 0.31 % H_r = 0.18

Parameter	Mefentrifluconazole	Boscalid
Accuracy **n = 5** **(% Recovery)**	HPLC Recoveries: 50 % of nominal concentration: 100.4 % (RSD = 0.38 %; H_r = 0.26) 100 % of nominal concentration: 100.6 % (RSD = 0.22 %; H_r = 0.16) 150 % of nominal concentration: 100.3 % (RSD = 0.25 %; H_r = 0.20) UPLC Recoveries: 50 % of nominal concentration: 99.6 % (RSD = 0.42 %; H_r = 0.28) 100 % of nominal concentration: 99.6 % (RSD = 0.34 %; H_r = 0.25) 150 % of nominal concentration: 99.8 % (RSD = 0.40 %; H_r = 0.32)	HPLC Recoveries: 50 % of nominal concentration: 100.7 % (RSD = 0.20 %; H_r = 0.13) 100 % of nominal concentration: 100.9 % (RSD = 0.27 %; H_r = 0.20) 150 % of nominal concentration: 100.5 % (RSD = 0.19 %; H_r = 0.15) UPLC Recoveries: 50 % of nominal concentration: 99.5 % (RSD = 0.44 %; H_r = 0.30) 100 % of nominal concentration: 99.8 % (RSD = 0.31 %; H_r = 0.23) 150 % of nominal concentration: 100.1 % (RSD = 0.42 %; H_r = 0.33)
Interference / specificity	No interfering signals and no co-elutions were observed.	
Comment	Suitable	

Conclusion

With respect to the conditions described in the analytical method AFL0995/01, all validation parameters (identity, specificity, linearity, accuracy and precision) are acceptable. Therefore, this method is valid without restrictions in the tested concentration range and is applicable to determine the contents of mefentrifluconazole and boscalid in the SC formulation BAS 762 02 F.

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

Determination of the relevant impurity N,N-dimethylformamide (DMF)

Mefentrifluconazole contains ≤ 0.5 g/kg N,N-dimethylformamide (DMF), which is considered to be an impurity of toxicological concern (equivalent to ≤ 51.6 mg/L or 45.4 mg/kg DMF in the SC formulation BAS 762 02 F). The analytical method AFL1010/01 was developed for the determination of DMF (Reg. No. 159267) in SC formulations containing mefentrifluconazole and validated for the determination of DMF in BAS 762 02 F. This method has not been previously reviewed and is provided in support of this assessment.

Comments of zRMS:	The analytical method AFL 1010/01 is acceptable for determination of the content of Dimethylformamide in formulations containing Mefentrifluconazole (BAS 750 F).

Reference:	CP 5.1.1/3
Report	Analytical Method AFL 1010/01 - Determination of Dimethylformamide in Formulations containing Mefentrifluconazole (BAS 750 F), XXX, D., 2020 report No 862258_1 2020/2028497 Authority registration No
Guideline(s):	No guidelines available
Deviations:	No
GLP:	No, not relevant for this subject type
Acceptability:	Yes

Materials and methods

The samples are analysed using a gas chromatographic procedure that employs external standards. The separation is achieved by using gradient conditions for detection and quantification. An RTX-200 column or equivalent is used. DMF is detected using a mass spectrometer and quantified by comparing the specific response ratios of the sample with those of the standard.

GC parameters

Column	RTX-200, 30 m x 0.32 mm, 1.5 µm		
Injector temperature	250 °C		
MS transfer line temperature	250 °C		
Oven temperature	Rate [°C/min]	Value [°C]	Hold time [min]
	-	160	5
	30	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	12 min		
Source temperature	230 °C		
Quad temperature	150 °C		
Solvent delay	3 min		
MS off	After 5 min		

Target compound	Retention time [min]	m/z [quantifier]	m/z [qualifier]
DMF (Reg. No. 159267)	4.0	73	44

Validation - Results and discussion

Comments of zRMS:	The analytical method AFL1010/01 was successfully validated for the determination of Dimethylformamide in formulations containing Mefentrifluconazole (BAS 750 F) according to the requirements of SANCO/3030/99 rev. 5. and is considered fit for purpose. The additional validation study of analytical method AFL1010/01 is acceptable.

Reference:	CP 5.1.1/4
Report	Validation of the Analytical Methode AFL1010/01: "Determination of Dimethylformamide in Formulations containing Mefentrifluconazole (BAS 750 F)", XXX, D., 2020 report No 862258_1 2020/2032727 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

Table 5.2-2: Method for the determination of the relevant impurity DMF in the plant protection product BAS 762 02 F

Parameter	**N,N-dimethylformamide (DMF)** **Max. 0.5 g/kg in the formulation**
Author(s), year	Daniela XXX, 2020
Principle of method	GC with MS detection
Linearity **(linear between mg/L of the nominal concentration)** **(correlation coefficient expressed as R)**	Range: 0.06-12.89 mg/L R: 0.9995 Slope: 11900 Intercept: 416.48
Precision **n = 5** **(Repeatability mean)** **(% RSD)**	Mean: 0.0009 % % RSD: 5.91 % (limit 7.69 %) H_r = 0.77
Accuracy **n = 5** **(% Recovery)**	Recoveries: 0.001 % of nominal concentration: 90.8 % (RSD = 0.078 %; H_r = 0.021) 0.013 % of nominal concentration: 110.8 % (RSD = 0.051 %; H_r = 0.020) 0.04 % of nominal concentration: 93.8 % (RSD = 0.028 %; H_r = 0.013)
Interference / specificity	A solution of the reference substance, a solution of the test item, a solution of the blank formulation and a solution of the test item fortified with the reference substance were measured. No interferences were detected.
LOQ	The lowest accuracy level (0.001 %) was used for the LOQ. The LOQ was defined as

Parameter	**N,N-dimethylformamide (DMF)** **Max. 0.5 g/kg in the formulation**
	0.24 mg/L.
Comment	Suitable

Additional validation - Results and discussion

Reference:	CP 5.1.1/5
Report	Additional Validation of the Analytical Method AFL1010/01: "Determination of Dimethylformamide in Formulations containing Mefentrifluconazole (BAS 750 F)", XXX, A., 2020 report No 862256_1 2020/2085538 Authority registration No
Guideline(s):	No guideline available
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Mainz, Germany),
Acceptability:	Yes

Table 5.2-3: Method for the determination of the relevant impurity DMF in the plant protection product BAS 762 02 F

Parameter	**N,N-dimethylformamide (DMF)** **Max. 0.5 g/kg (equivalent to ≤ 51.6 mg/L or 45.4 mg/kg DMF in the SC formulation BAS 762 02 F)**
Author(s), year	Andrea XXX, 2020
Principle of method	GC with MS detection
Linearity **(linear between mg/L of the nominal concentration)** **(correlation coefficient expressed as R)**	Determined within the previous validation study (see above). Not determined within this additional validation study.
Precision **n = 5** **(Repeatability mean)** **(% RSD)**	Mean: 0.0010 % % RSD: 6.18 % (limit 7.57 %) H_r = 0.82

Parameter	**N,N-dimethylformamide (DMF)** **Max. 0.5 g/kg (equivalent to ≤ 51.6 mg/L or 45.4 mg/kg DMF in the SC formulation BAS 762 02 F)**
Accuracy **n = 5** **(% Recovery)**	Recoveries: 0.001 % of nominal concentration: 97.3 % (RSD = 0.055 %; H_r = 0.015) 0.006 % of nominal concentration: 104.2 % (RSD = 0.018 %; H_r = 0.006)
Interference / specificity	A solution of the reference substance, a solution of the test item, a solution of the blank formulation and a solution of the test item fortified with the reference substance were measured. No interferences were detected.
LOQ	The lowest accuracy level (0.001 %) was used for the LOQ. The LOQ was defined as 0.24 mg/L.
Comment	Suitable

Conclusion

With respect to the conditions described in the analytical method AFL1010/01, all validation parameters (identity, specificity, linearity, accuracy and precision) are acceptable. Therefore, this method is valid without restrictions in the tested concentration range and is applicable to determine the contents of DMF in the SC formulation BAS 762 02 F.

Determination of the relevant impurity toluene

Mefentrifluconazole contains ≤ 1.0 g/kg toluene, which is considered to be an impurity of toxicological concern (equivalent to ≤ 103.1 mg/L or 90.8 mg/kg toluene in the SC formulation BAS 762 02 F).
The analytical method AFL0948/01 was developed for the determination of toluene (Reg. No. 4005250) in the EC formulation BAS 751 05 F containing mefentrifluconazole.

Comments of zRMS:	The analytical method AFL0948/01 is acceptable for the determination of the content of toluene in formulations containing Mefentrifluconazole (BAS 750 F).

Reference:	CP 5.1.1/6
Report	Analytical method AFL0948/01 - Determination of Toluene in formulations containing Mefentrifluconazole (BAS 750 F), XXX, M., 2017 report No 2017/1077926 Authority registration No
Guideline(s):	No guideline available
Deviations:	No
GLP:	No, not relevant for this subject type
Acceptability:	Yes

Materials and methods

The samples are analysed using a gas chromatographic procedure that employs external standards. The separation is achieved by using gradient conditions for detection and quantification. An RTX-200 column or equivalent is used. Toluene is detected using a mass spectrometer and quantified by comparing the specific response ratios of the sample with those of the standard.

GC parameters

Column	RTX-200, 60 m x 0.32 mm, 1.5 µm		
Injector temperature	280 °C		
Detector temperature	300 °C		
Oven temperature	Rate [°C/min]	Value [°C]	Hold time [min]
	-	95	3
	15	280	10.7
Carrier gas	Helium		
Detector	MSD		
Split ratio	10:1		
Column flow	0.9 mL/min (constant flow)		
Injection volume	1.5 µL		
Analysis time	26 min		

Target compound	Retention time [min]	m/z [quantifier]	m/z [qualifier]
Toluene (Reg. No. 4005250)	9.8	91	92

Validation - Results and discussion

Comments of zRMS:	The analytical method AFL0948/01 was successfully validated for the determination of Toluene in formulations containing Mefentrifluconazole (BAS 750 F) according to the requirements of SANCO/3030/99 rev. 5. and is considered fit for purpose.

Reference:	CP 5.1.1/7
Report	Validation of the analytical method AFL0948/01: Determination of Toluene in formulations containing Mefentrifluconazole (BAS 750 F), XXX, M., 2017 report No 844268_1 2017/1078235 Authority registration No
Guideline(s):	2004/10/EC, ABNT NBR 14029, CIPAC Guidelines on method validation, EC 1107/2009, EPA 830.1000, EPA 830.1800, SANCO/3030/99
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Mainz, Germany),
Acceptability:	Yes

Table 5.2-4: Method for the determination of the relevant impurity toluene in the plant protection product BAS 751 05 F

Parameter	**Toluene** **Max. 1.0 g/kg (equivalent to ≤ 103.1mg/L or 90.8 mg/kg toluene in the SC formulation BAS 762 02 F)**
Author(s), year	Michael XXX, 2017
Principle of method	GC with MS detection
Linearity **(linear between mg/L of the nominal concentration)** **(correlation coefficient expressed as R)**	Range: 0.04-60 mg/L R: 1.0000 Slope: 32595 Intercept: -608
Precision **n = 7** **(Repeatability mean)** **(% RSD)**	Mean: 0.000017 % % RSD: 0.65 % H_r = 0.092
Accuracy **n = 5** **(% Recovery)**	Recoveries: 0.0011 % of nominal concentration: 79 % (RSD = 0.063 %; H_r = 0.017) 0.0111 % of nominal concentration: 90 % (RSD = 0.016 %; H_r = 0.006) 0.0529 % of nominal concentration: 101 % (RSD = 0.009 %; H_r = 0.004) 0.1059 % of nominal concentration: 103 % (RSD = 0.014 %; H_r = 0.008)
Interference / specificity	There were no indications of interferences due to other components.
LOQ	The lowest accuracy level (0.0011 %) was used for the LOQ. The LOQ was defined as 0.17 mg/L.
Comment	Suitable

The analytical method AFL0948/03, which is based on the method AFL0948/01, was developed for the determination of toluene in SC formulations containing mefentrifluconazole and validated for the determination of toluene in BAS 762 02 F. This method has not been previously reviewed and is provided in support of this assessment.

Comments of zRMS:	The analytical method AFL0948/03 is acceptable for the determination of the content of Toluene in formulations containing Mefentrifluconazole (BAS 750 F). The additional validation study of analytical method AFL0948/03 is acceptable.

Reference:	CP 5.1.1/8
Report	Analytical Method AFL0948/03 - Determination of Toluene in Formulations containing Mefentrifluconazole (BAS 750 F)., XXX, M., 2020 report No 2020/2080925 Authority registration No
Guideline(s):	No guideline available
Deviations:	No

GLP: No, not relevant for this subject type

Acceptability: Yes

Materials and methods

The samples are analysed using a gas chromatographic procedure that employs external standards. The separation is achieved by using gradient conditions for detection and quantification. An RTX-200 column or equivalent is used. Toluene is detected using a mass spectrometer and quantified by comparing the specific response ratios of the sample with those of the standard.
The method consists of two parts for the GC parameters (part A and part B). The GC parameters in part B are the same as in part A with the addition of a bake out step at the end of the temperature gradient.

GC parameters
Part A

Column	RTX-200, 60 m x 0.32 mm, 1.5 µm		
Injector temperature	280 °C		
Detector temperature	300 °C		
Oven temperature	Rate [°C/min]	Value [°C]	Hold time [min]
	-	95	3
	15	280	10.7
Carrier gas	Helium		
Detector	MSD		
Split ratio	10:1		
Column flow	0.9 mL/min (constant flow)		
Injection volume	1.5 µL		
Analysis time	26 min		

Target compound	Retention time [min]	m/z [quantifier]	m/z [qualifier]
Toluene (Reg. No. 4005250)	9.8	91	92

Part B

Column	RTX-200, 60 m x 0.32 mm, 1.5 µm		
Injector temperature	280 °C		
Detector temperature	300 °C		
Oven temperature	Rate [°C/min]	Value [°C]	Hold time [min]
	-	95	3
	15	280	11
	20	300	10
Carrier gas	Helium		
Detector	MSD		
Split ratio	10:1		
Column flow	0.9 mL/min (constant flow)		
Injection volume	1.5 µL		
Analysis time	37 min		
MS off	14 min		

Target compound	Retention time [min]	m/z [quantifier]	m/z [qualifier]
Toluene (Reg. No. 4005250)	10.1	91	92

Validation - Results and discussion

Reference:	CP 5.1.1/9
Report	Additional Validation to the Analytical Method AFL0948/03: Determination of Toluene in Formulations containing Mefentrifluconazole (BAS 750 F), XXX, M., 2020 report No 888037_1 2020/2085856 Authority registration No
Guideline(s):	ABNT NBR 14029, CIPAC 3807, EPA 830.1000, EPA 830.1800, SANCO/3030/99 rev. 5 (22 March 2019)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Mainz, Germany),
Acceptability:	Yes

Table 5.2-5: Method for the determination of the relevant impurity toluene in the plant protection product BAS 762 02 F

Parameter	**Toluene** **Max. 1.0 g/kg (equivalent to ≤ 103.1 mg/L or 90.8 mg/kg toluene in the SC formulation BAS 762 02 F)**
Author(s), year	Monika XXX, 2020
Principle of method	GC with MS detection
Linearity **(linear between mg/L of the nominal concentration)** **(correlation coefficient expressed as R)**	Determined within the previous validation study (see above). Not additionally determined within this validation study.
Precision **n = 5** **(Repeatability mean)** **(% RSD)**	Mean: 0.0072 % % RSD: 4.82 % H_r = 0.9
Accuracy **n = 5** **(% Recovery)**	Recoveries: 0.004 % of nominal concentration: 116.5 % (RSD = 0.042 %; H_r = 0.014) 0.01 % of nominal concentration: 104.5 % (RSD = 0.031 %; H_r = 0.011)
Interference / specificity	The specificity of the method was demonstrated by analysing typical chromatograms of the pure solvent acetonitrile, the reference item, the test item, the blank formulation, the blank formulation fortified with mefentrifluconazole and boscalid at both accuracy levels. No interferences with the peak of the analytical substance were observed.
LOQ	Determined within the previous validation study (see above). Not additionally determined within this validation study.
Comment	Suitable

Conclusion

With respect to the conditions described in the analytical method AFL0948/03, all validation parameters (identity, specificity, linearity, accuracy and precision) are acceptable. Therefore, this method is valid without restrictions in the tested concentration range and is applicable to determine the contents of toluene in the SC formulation BAS 762 02 F.

Determination of the relevant impurity 1,2,4-(1H)-triazole

Mefentrifluconazole contains ≤ 1.0 g/kg 1,2,4-(1H)-triazole, which is considered to be an impurity of toxicological concern (equivalent to ≤ 103.0 mg/L or 90.7 mg/kg 1,2,4-(1H)-triazole in the SC formulation BAS 762 02 F).
The analytical method AFL0977/01 was developed for the determination of 1,2,4-(1H)-triazole (Reg. No. 87084) in the EC formulation BAS 750 01 F containing mefentrifluconazole.

Comments of zRMS:	The analytical method AFL0977/01 is acceptable for the determination of the of the impurity Reg.No. 87084 in formulations containing Mefentrifluconazole.

Reference:	CP 5.1.1/10
Report	Analytical method AFL0977/01 - Determination of the impurity Reg.No. 87084 in formulations containing Mefentrifluconazole, XXX, D., 2018 report No 2018/1144189 Authority registration No
Guideline(s):	No guideline available
Deviations:	No
GLP:	No, not relevant for this subject type
Acceptability:	Yes

Materials and methods

The samples are analysed using a high-performance liquid chromatographic procedure that employs external standards. The separation is achieved by applying isocratic conditions with water, acetonitrile and formic acid for detection and quantification followed by a rinsing step with a high acetonitrile portion and an equilibration step. A Synergi Polar-RP (4 µm, 150 mm x 4.6 mm) column (or equivalent type) is used. 1,2,4-(1H)-triazole is detected using a mass spectrometer and quantified by comparing the specific response ratios of the sample with those of the standard.

HPLC-MS parameters

Column	Synergi Polar-RP: 4 µm, 150 mm x 4.6 mm (or equivalent)		
Mobile phase A	1000 mL water + 1 mL formic acid (100 %)		
Mobile phase B	1000 mL acetonitrile + 1 mL formic acid (100 %)		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	5.0	95	5
	5.1	1	99
	10.0	1	99

	10.1	95	5
	15.0	95	5
Flow	1.0 mL/min (constant flow)		
Column temperature	40 °C		
Injection volume	10 µL		
Detection	MS detection, SIR mode		
MS detection signal	70 m/z (monoisotopic mass M + H^+)		
Retention time	Approximately 2.2 min		

Specific Waters SQD2 tune parameters

Capillary voltage	1.00 kV
Cone voltage	50.00 V
RF voltage	2.50 V
Extractor voltage	3.00 V
Source temperature	150 °C
Desolvation temperature	600 °C
Cone gas flow	50 L/Hr
Desolvation gas flow	600 L/Hr

Validation – Results and discussion

Comments of zRMS:	The analytical method AFL0977/01 was successfully validated for the determination of the impurity Reg.No. 87084 in formulations containing Mefentrifluconazole according to the requirements of SANCO/3030/99 rev. 5.

Reference:	CP 5.1.1/11
Report	Validation of the Analytical Method AFL0977/01: Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole, XXX, D., 2018 report No 869531_1 2018/1144190 Authority registration No
Guideline(s):	2004/10/EC, ABNT NBR 14029, CIPAC Guidelines on method validation, EC 1107/2009, EPA 830.1000, EPA 830.1800, SANCO/3030/99
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Mainz, Germany),
Acceptability:	Yes

Table 5.2-6: Method for the determination of the relevant impurity 1,2,4-(1H)-triazole in the plant protection product BAS 750 01 F

Parameter	**1,2,4-(1H)-triazole** **Max. 1.0 g/kg (equivalent to ≤ 103.1 mg/L or 90.8 mg/kg 1,2,4-(1H)-triazole in the SC formulation BAS 762 02 F)**
Author(s), year	Daniela XXX, 2018

Parameter	**1,2,4-(1H)-triazole** **Max. 1.0 g/kg (equivalent to ≤ 103.1 mg/L or 90.8 mg/kg 1,2,4-(1H)-triazole in the SC formulation BAS 762 02 F)**
Principle of method	HPLC with MS detection
Linearity **(linear between mg/L of the nominal concentration)** **(correlation coefficient expressed as R)**	Range: 0.002-0.25 mg/L (2-250 µg/L) R: 0.9994 Slope: 1402093417 Intercept: 4072486
Precision **n = 5** **(Repeatability mean)** **(% RSD)**	Mean: 0.000108 % % RSD: 2.14 % H_r = 0.40
Accuracy **n = 5** **(% Recovery)**	Recoveries: 0.0002 % of nominal concentration: 100 % (RSD = 0.007 %; H_r = 0.001) 0.001 % of nominal concentration: 108 % (RSD = 0.020 %; H_r = 0.005) 0.005 % of nominal concentration: 95 % (RSD = 0.007 %; H_r = 0.002)
Interference / specificity	There were no indications of interferences due to other components.
LOQ	The lowest accuracy level (0.0002 %) was used for the LOQ. The LOQ was defined as 0.01 mg/L.
Comment	Suitable

The analytical method AFL0977/04, which is based on the method AFL0977/01, was developed for the determination of 1,2,4-(1H)-triazole in formulations containing mefentrifluconazole and validated for the determination of 1,2,4-(1H)-triazole in BAS 762 02 F. This method has not been previously reviewed and is provided in support of this assessment.

Comments of zRMS:	The analytical method AFL0977/04 was successfully validated for the determination of the impurity Reg.No. 87084 in formulations containing Mefentrifluconazole according to the requirements of SANCO/3030/99 rev. 5. The additional validation study of analytical method AFL0977/04 is acceptable.

Reference:	CP 5.1.1/12
Report	AFL0977/04: Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole, XXX, A., 2020 report No 886096_1 2020/2037327 Authority registration No
Guideline(s):	No guideline available
Deviations:	No
GLP:	No, not relevant for this subject type
Acceptability:	Yes

Materials and methods

The samples are analysed using a high-performance liquid chromatographic procedure that employs external standards. The separation is achieved by applying isocratic conditions with water, acetonitrile and formic acid for detection and quantification followed by a rinsing step with a high acetonitrile portion and an equilibration step. A Synergi Polar-RP (4 µm, 150 mm x 4.6 mm) column (or equivalent type) is used. 1,2,4-(1H)-triazole is detected using a mass spectrometer and quantified by comparing the specific response ratios of the sample with those of the standard.
This method consists of three parts with different calculation methods (parts A, B and C). Part A: 1,2,4-(1H)-triazole is quantified by comparing the specific response ratios of the sample with those of the standard. Parts B and C: 1,2,4-(1H)-triazole is quantified by the standard addition method.

HPLC-MS parameters

Column	Synergi Polar-RP: 4 µm, 150 mm x 4.6 mm (or equivalent)		
Mobile phase A	1000 mL water + 1 mL formic acid (100 %)		
Mobile phase B	1000 mL acetonitrile + 1 mL formic acid (100 %)		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	5.0	95	5
	5.1	1	99
	10.0	1	99
	10.1	95	5
	15.0	95	5
Flow	1.0 mL/min (constant flow)		
Column temperature	40 °C		
Injection volume	10 µL		
Detection	MS detection, SIR mode		
MS detection signal	70 m/z (monoisotopic mass M + H^+)		
Retention time	Approximately 2.2 min		

Specific Waters SQD2 tune parameters

Capillary voltage	1.00 kV
Cone voltage	50.00 V
RF voltage	2.50 V
Extractor voltage	3.00 V
Source temperature	150 °C
Desolvation temperature	600 °C
Cone gas flow	50 L/Hr
Desolvation gas flow	600 L/Hr

Specific Agilent G6130 MSD parameters

Capillary voltage	4.0 kV
Gas temperature	300 °C
Gas flow	11 L/min
Capillary current	17 nA
Nebuliser pressure	15 psi
Fragmentor voltage	135 V
Chamber current	0.07 µA
MS off	After 3 min

Validation - Results and discussion

Reference:	CP 5.1.1/13
Report	Additional Validation to the Analytical Method AFL0977/04: Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole, XXX, A., 2020 report No 886097_1 2020/2080849 Authority registration No
Guideline(s):	ABNT NBR 14029, EPA 830.1000, EPA 830.1800, SANCO/3030/99 rev. 5 (22 March 2019)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Mainz, Germany),
Acceptability:	Yes

Table 5.2-7: Method for the determination of the relevant impurity 1,2,4-(1H)-triazole in the plant protection product BAS 762 02 F

Parameter	**1,2,4-(1H)-triazole** **Max. 1.0 g/kg (equivalent to ≤ 103.1 mg/L or 90.8 mg/kg 1,2,4-(1H)-triazole in the SC formulation BAS 762 02 F)**
Author(s), year	Andrea XXX, 2020
Principle of method	HPLC with MS detection using standard addition for calculation
Linearity **(linear between mg/L of the nominal concentration)** **(correlation coefficient expressed as R)**	Determined within the previous validation study (see above). Not additionally determined within this validation study.
Precision **n = 5** **(Repeatability mean)** **(% RSD)**	Mean: 0.002 % % RSD: 0.73 % (limit 6.83 %) H_r = 0.11
Accuracy **n = 5** **(% Recovery)**	Recoveries: 0.002 % of nominal concentration: 99.1 % (RSD = 0.73 %; H_r = 0.21) 0.005 % of nominal concentration: 98.8 % (RSD = 0.005 %; H_r = 0.002) 0.010 % of nominal concentration: 100.4 % (RSD = 0.005 %; H_r = 0.002)
Interference / specificity	The specificity of the method was demonstrated by evaluating typical chromatograms of the pure solvent water, the reference item, the test item, the fortified test item and the blank formulation. No interferences with the peak of the analytical substance were observed.
LOQ	Based on the lowest fortification level, the limit of quantification (LOQ) was defined to be 0.002 % (0.128 mg/L) for 1,2,4-(1H)-triazole in the SC formulation BAS 762 02 F.
Comment	Suitable

Conclusion

With respect to the conditions described in the analytical method AFL0977/04, all validation parameters (identity, specificity, linearity, accuracy and precision) are acceptable. Therefore, this method is valid without restrictions in the tested concentration range and is applicable to determine the contents of 1,2,4-(1H)-triazole in the SC formulation BAS 762 02 F.

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

Under current EU legislation, analytical methods for the determination of co-formulants are not required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is no CIPAC method available for the simultaneous determination of mefentrifluconazole and boscalid in the plant protection product BAS 762 02 F.
There is no CIPAC method available for the analysis of mefentrifluconazole in technical or formulated material.
A CIPAC method was developed for the determination of boscalid in TC, WG, SE and SC formulations (CIPAC Handbook N, p. 4 et seq).

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 and boscalid 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.
Already evaluated studies are written in italics.

Mefentrifluconazole

Table 5.2- 8: 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

Table 5.2- 9: 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- 10: 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)*	XXX 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- 11: 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)*	XXX 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 XXX G., 2017 BASF DocID 2017/1064882 Method APL0500/03 see A 2.1.1
Test water (mixing water)	Primary (VAL) Confirmatory meth-od not necessary	0.001 mg/L	HPLC-MS (BAS 750 F)	**New study** KCP 5.1/2, not peer-reviewed XXX E., 2016 BASF DocID 2016/1155889 Method APL0500/03 see A 2.1.1
Tap water or M4-medium	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.1 µg/L	LC-MS/MS	**New study** KCP 5.1/3 not peer-reviewed XXX, M., 2017 BASF DocID 2017/1065621 Method L0631/01 see A 2.1.1

Table 5.2- 12: 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	XXX M.,XXX 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)*

Boscalid

Table 5.2- 13: Validated methods for the generation of pre-authorization data (boscalid)

Component of residue definition: boscalid				
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 (residues)				
Wheat - plant without root, grain, straw	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	XXX A., XXX C., 2007 BASF DocID 2006/1039427 Method no. 535/1 (L0076/01) not EU peer-reviewed see A 2.2.1.1.1
Lettuce - plant				
Lemon - fruit				
Oilrape - seed				
Tomato - fruit				
Onion - bulb				
Hops – dried hops	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	XXX R., 2015 BASF DocID 2015/1091103 Method no. L0076/01 not EU peer-reviewed see A 2.2.2.1.2 *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.3.2)*
Spices - white and black pepper mix				
Herbal infusions - green tea leaves				
Plants, plant products (residues) – Information on stability in solvents and extractability				
Solvent (acetonitrile)	Primary (no confirmatory required as spiked AI in solvent analysed, hence the source of origin is known)	10 µg/mL	HPLC-UV	Funk H., XXX D., 2001 BASF DocID 2000/1014856 EU agreed (DAR, 8th Nov 2002)
Solvent (acetonitrile)	Primary (no confirmatory required as spiked AI in solvent analysed, hence the source of origin is known)	10 µg/mL	HPLC-UV	XXX F., 2001 BASF DocID 2000/1017225 EU agreed (DAR, 8th Nov 2002)
Various plant matrices (grape, lettuce, wheat grain, wheat straw radish root)	Primary & Confirmatory as ^{14}C-label used	not applicable	^{14}C-HPLC	Bross M., 2001 BASF DocID 2001/1001739 (Extractability for 445/0, 535/1 and L0076/01) EU agreed (DAR, 8th Nov 2002)
Animal products, food of animal origin (Residues)				
Milk – bovine	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	XXX F., 2001 BASF DocID 2000/1017223 Method no. 471/0 EU agreed (DAR, 8th Nov 2002) Amendment 1 BASF DocID 2003/1021922 not EU peer-reviewed Amendment 2 BASF DocID 2015/1174463 not EU peer-reviewed see A 2.2.2.2.1
Cream – bovine		0.01 mg/kg		
Eggs - hen		0.01 mg/kg		
Muscle - bovine		0.025 mg/kg		
Liver - bovine		0.025 mg/kg		
Kidney - bovine		0.025 mg/kg		

Component of residue definition: boscalid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.3.3)
Soil, sediment (Environmental fate)				
Soil, sediment	Primary & Confirmatory (2 ions and full scan mode)	0.01 mg/kg	GC-MS	Keller W., 1998 BASF DocID 1998/11314 Method no. 408/1 EU agreed (DAR, 8th Nov 2002) Amendment 1 adding uncorrected recoveries BASF DocID 2003/1000977 EU agreed (Addendum 2 to DAR, May 2006) *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.3.4)*
Soil	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	XXX M., 2009 BASF DocID 2008/1084832 Method no. L0096/01 not EU peer-reviewed see A 2.2.2.3.1 Amendment 1 BASF DocID 2015/1174527 not EU peer-reviewed *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.3.4)*

Component of residue definition: boscalid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Soil, sediment (Environmental fate) - Information on stability in standard solutions and extractability				
Methanol/acetate buffer pH 4.6 (80/20, v/v)	Primary & Confirmatory	0.01 mg/kg	LC-MS/MS	XXX H., 2010 BASF DocID 2010/1046613 not EU peer-reviewed see A 2.2.2.7.1
Soil	Primary & Confirmatory	not applicable	LC-MS/MS	XXX T., XXX S., 2015 BASF DocID 2015/1109589 (Extractability for L0096/01) not EU peer-reviewed see A 2.2.2.7.2
Water (Environmental fate)				
Tapwater, leaching water	Primary & Confirmatory (2 ions)	0.05 µg/L	GC-MS	Keller W., 1998 BASF DocID 1998/10922 Method no. 411/0 EU agreed (DAR, 8th Nov 2002) Amendment 1 adding uncorrected recoveries BASF DocID 2003/1000976 EU agreed (Addendum 2 to DAR, May 2006)
Surface water	Primary & Confirmatory (2 ions)	0.05 µg/L	GC-MS	Grote C., 2001 BASF DocID 2001/1008955 Method no. 411/0 EU agreed (DAR, 8th Nov 2002) Amendment 1 adding corrected recoveries BASF DocID 2003/1000975 EU agreed (Addendum 2 to DAR, May 2006)
Surface water, groundwater	Primary & Confirmatory (2 mass transitions)	0.03 µg/L	LC-MS/MS	XXX H., 2009 BASF DocID 2008/1086809 Method no. L0127/01 not EU peer-reviewed see A 2.2.2.4.1 Amendment 1 information on 2nd mass transition and matrix effects BASF DocID 2015/1174526 not EU peer-reviewed *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.3.5)*

Table 5.2- 14: Validated methods for the generation of pre-authorization data (boscalid metabolite M510F01)

Component of residue definition: M510F01				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Animal products, food of animal origin (Residues)				
Milk – bovine	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	XXX F., 2001 BASF DocID 2000/1017223 Method no. 471/0 EU agreed (DAR, 8th Nov 2002) Amendment 1 BASF DocID 2003/1021922 not EU peer-reviewed Amendment 2 BASF DocID 2015/1174463 not EU peer-reviewed see A 2.2.2.2.1 *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.3.3)*
Cream – bovine		0.01 mg/kg		
Eggs - hen		0.01 mg/kg		
Muscle - bovine		0.025 mg/kg		
Liver - bovine		0.025 mg/kg		
Kidney - bovine		0.025 mg/kg		
Fat - bovine		0.025 mg/kg		

Table 5.2- 15: Validated methods for the generation of pre-authorization data (boscalid metabolites M510F47 and M510F49)

Component of residue definition: M510F47 and M510F49				
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)				
Soil	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	XXX D., 2013 BASF DocID 2013/1415720 not EU peer-reviewed see A 2.2.2.3.2 *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.3.4)*
Water (Environmental fate)				
Surface water, groundwater	Primary & Confirmatory (2 mass transitions)	0.03 µg/L	LC-MS/MS	XXX T., XXX S., 2015 BASF DocID 2015/1109588 Method no. L0127/02 not EU peer-reviewed see A 2.2.2.4.2 *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.3.5)*

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 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~~ 2021/590
Plant, high acid content		0.01 mg/kg	Reg. (EU) No ~~977/2019~~ 2021/590
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Reg. (EU) No ~~977/2019~~ 2021/590
Plant, high oil content		0.01 mg/kg	Reg. (EU) No ~~977/2019~~ 2021/590
Plant, difficult matrices (hops, spices, tea)		0.01 mg/kg	Reg. (EU) No ~~977/2019~~ 2021/590
Muscle	Mefentrifluconazole	0.01 mg/kg	Reg. (EU) No ~~977/2019~~ 2021/590
Milk		0.01 mg/kg	Reg. (EU) No ~~977/2019~~ 2021/590
Eggs		0.01 mg/kg	Reg. (EU) No ~~977/2019~~ 2021/590
Fat		0.01 mg/kg	Reg. (EU) No ~~977/2019~~ 2021/590
Liver, kidney		0.01 mg/kg	Reg. (EU) No ~~977/2019~~ 2021/590
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**
High water content (tomato, whole fruit)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	XXX 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 acid content (orange, whole fruit)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	XXX 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	XXX 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	XXX 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

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**
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 table.

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)*	XXX 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	30 ng/L	LC-MS/MS	**New study** KCP 5.2/1, not peer-reviewed

	method not necessary (two mass transitions used for confirmation)			XXX 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 XXX 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 XXX 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 XXX 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 table.

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	XXX M.,XXX S., 2015 BASF DocID 2015/1111330 Method L0327/01 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 XXX 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.~~

A residue study for the determination of BAS 750 F residues in honey has been performed (BASF DocID: 2020/2109990, Report Amendment N°1 DocID: 2021/2038566) and has been provided by Applicant. The following study is a combination of residue analytics and a validation study in honey. Therefore, the method analytical part of the study is presented in Appendix 2 of Part B5.

Whole plant (no roots), inflorescences, pollen and honey specimens were analyzed for residues of BAS 750 F (Mefentrifluconazole) and for residues of the triazole metabolites 1,2,4-Triazole (T), Triazolylalanine (TA), Triazole lactic acid (TLA) and Triazole acetic acid (TAA) using BASF method L0170/03.
The limit of quantification (LOQ) for all analytes was 0.050 mg/kg.

For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

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

In addition to already EU peer-reviewed residue analytical methods, the applicant has developed and validated new analytical methods for the determination of all relevant analytes in plant and animal matrices as well as in matrices relevant for environmental fate, such as soil, water and air. These methods are, in general, based on the determination of the individual analytes and not on the plant protection product itself. New analytical methods are described in detail for the relevant matrices in Appendix 2. The methods allow the determination of the relevant analytes at the required limit of quantification in the matrix types as listed below.

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

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

Table 5.3-10: 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	Boscalid	~~0.6 mg/kg~~ 0.01* mg/kg	Reg. (EU) ~~2016/156~~ 2021/590
Plant, high acid content		~~2 mg/kg~~ 0.01* mg/kg	Reg. (EU) ~~2016/156~~ 2021/590
Plant, high protein/high starch content (dry commodities)		0.15 mg/kg	Reg. (EU) ~~2016/156~~ 2021/590
Plant, high oil content		~~0.6 mg/kg~~ 0.01* mg/kg	Reg. (EU) ~~2016/156~~ 2021/590
Plant, difficult matrices (hops, spices, tea)		~~0.4 mg/kg~~ 0.01* mg/kg	Reg. (EU) ~~2016/156~~ 2021/590
Muscle	Boscalid	~~0.2 mg/kg~~ 0.01* mg/kg	Reg. (EU) ~~2016/156~~ 2021/590
Milk		0.02 mg/kg	Reg. (EU) ~~2016/156~~ 2021/590
Eggs		0.01* mg/kg	Reg. (EU) ~~2016/156~~ 2021/590
Fat		0.07 mg/kg	Reg. (EU) ~~2016/156~~ 2021/590
Liver, kidney	Boscalid and hydroxylation product M510F01 (including its conjugates)	~~0.15 mg/kg~~ 0.05* mg/kg	Reg. (EU) ~~2016/156~~ 2021/590
Soil (Ecotoxicology)	Boscalid	0.01 mg/kg The LOQ of the soil residue analytical methods covers the lowest ecotoxicological endpoints	$NOEC_{CORR}$ = 12.5 mg/kg dry soil (lowest value for boscalid) NOEC = 250 mg/kg dry soil (M510F047) NOEC = 62.5 mg/kg dry soil (M510F49) $NOEC_{CORR}$ = 31.25 mg/kg dry soil
Drinking water (Human toxicology)	Boscalid, M510F47, M510F49	0.03 µg/L covers the lowest aquatic endpoint as well as endpoints in view of human toxicology	general limit for drinking water applying a safety factor of 3
Surface water (Ecotoxicology)	Boscalid, M510F47, M510F49		Ecotoxicology: 125 µg/L EU Review Report 2008
Air	Boscalid	0.0012 µg/m^3	based on the ADI of 0.04 mg/kg

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
			bw/d, applying an additional safety factor of 10
Tissue (meat or liver)	Boscalid	not required according to SANCO 825/00 rev.8.1	not classified as T / T+
Body fluids		not required according to SANCO 825/00 rev.8.1	not classified as T / T+

* indicates lower limit of analytical determination/quantification

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 boscalid 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: boscalid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary & Confirmatory (3 ions monitored)	0.01 mg/kg	GC-MS	Weeren R.D., Pelz S., 1999 BASF DocID 1999/11461 (DFG S19 based method) EU agreed (DAR, 8th Nov 2002)
High acid content		0.01 mg/kg		
High oil content		0.02 mg/kg		
High protein/ High starch content (dry)		0.01 mg/kg		
High water content	ILV	0.01 mg/kg	LC-MS/MS	Reichert N., 2001 BASF DocID 2000/1014886 Remark: Matrices investigated were white cabbage, rape seed, hops, and lettuce EU agreed (DAR, 8th Nov 2002)
High oil content		0.02 mg/kg in rape (seed) 0.05 mg/kg in hops		
High water content	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	XXX N., 2015 BASF DocID 2015/1114667 (QuEChERS based method) not EU peer-reviewed see A 2.2.2.1.1
High acid content		0.01 mg/kg		
High oil content		0.01 mg/kg		
High protein/ High starch content (dry)		0.01 mg/kg		
High oil, high water, high acid, high, high protein, high starch	ILV	n.a.	n.a.	*not applicable as over 1200 acceptable recoveries are available on all relevant crop commodities in the EURL database for QuEChERS method*

Component of residue definition: boscalid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Difficult (hops, spices, herbal infusions)	Primary & Confirmatory (2 mass transitions evaluated)	0.01 mg/kg	LC-MS/MS	XXX R., 2015 BASF DocID 2015/1091103 Method no. L0076/01 not EU peer-reviewed see A 2.2.2.1.2
	ILV	-	-	*not applicable as sufficient data available on all relevant crop commodities in the EURL database*

Table 5.3-12: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Extraction efficiency was investigated in study BASF DocID 2001/1001739 (Bross M., 2001) which is already peer-reviewed in the DAR of boscalid (8th of November 2002).
Comment on Extraction efficiency	The study demonstrated that the extraction solvent used in residue analytical method 445/0 (mixture of methanol, water and hydrochloric acid) as well as acetone and water used in the multimethod approach DFG S19 (Weeren R.D., Pelz S., 1999) removed comparable amounts of residues than the metabolism extraction scheme applied in several metabolism studies (e.g. 2000/1014861, 2000/1014860, 2000/1014862 and 1999/11240). The newly submitted residue analytical method L0076/01 (XXX R., 2015) uses an identical extraction liquid than method 445/0, hence suitability of this extraction liquid is also fully confirmed. The extraction efficiency of the QuEChERS method (XXX N., 2015) was confirmed by the vast number of validated methods available on the EURL (European Reference Laboratories for Residues of Pesticides) Data Pool.

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 boscalid and boscalid metabolite M510F01 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 (boscalid and M510F01)

Component of residue definition: boscalid and M510F01				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Milk	Primary	0.01 mg/kg	GC-ECD	Class T., 2001 BASF DocID 2000/1017227 (DFG S19 based method) EU agreed (DAR, 8th Nov 2002)
Eggs		0.025 mg/kg		
Muscle		0.025 mg/kg		
Fat		0.025 mg/kg		
Kidney, liver		0.025 mg/kg		
Milk	Confirmatory	0.01 mg/kg	GC-MS	Class T., 2001 BASF DocID 2000/1017227 (DFG S19 based method) EU agreed (DAR, 8th Nov 2002)
Eggs		0.025 mg/kg		
Muscle		0.025 mg/kg		

Component of residue definition: boscalid and M510F01				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Fat		0.025 mg/kg		
Kidney, liver		0.025 mg/kg		
Milk	ILV	0.01 mg/kg	GC-ECD	Kampke-Thiel K., 2001 BASF DocID 2000/1017226 (DFG S19 based method) EU agreed (DAR, 8th Nov 2002)
Liver		0.025 mg/kg		
Milk	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	XXX F., 2001 BASF DocID 2000/1017223 Method no. 471/0 (L0041/01) EU agreed (DAR, 8th Nov 2002) Amendment 1 BASF DocID 2003/1021922 not EU peer-reviewed see A 2.2.2.2.1 Amendment 2 BASF DocID 2015/1174463 not EU peer-reviewed see A 2.2.2.2.1
Eggs		0.01 mg/kg		
Muscle		0.025 mg/kg		
Fat		0.025 mg/kg		
Kidney, liver		0.025 mg/kg		
Milk	ILV	0.01 mg/kg	LC-MS/MS	XXX H., Zetsch A., 2015 BASF DocID 2015/1114666 Method no. 471/0 (L0041/01) not EU peer-reviewed see A 2.2.2.2.1.2 Amendment 1 BASF DocID 2015/1251211 not EU peer-reviewed
Eggs		0.01 mg/kg		
Muscle		0.01 mg/kg		
Fat		0.01 mg/kg		
Kidney, liver		0.01 mg/kg		

Table 5.3-14: Statement on extraction efficiency

	Method for products of animal origin
Not required, because:	No separate bridging study required as the same extraction solvent has been used in the animal metabolism study.
Comment on Extraction efficiency	In both methods, methanol is used as extraction solvent. Extractability of the analytes (boscalid and its metabolite M510F01) from animal matrices by methanol and efficiency of enzymatic deconjugation was demonstrated in a related animal metabolism study (lactating goats) using radio-labelled compounds (BASF DocID 2000/1017221).

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 boscalid and boscalid metabolites M510F47 and M510F49 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 (boscalid)

Component of residue definition: boscalid			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary & Confirmatory (Confirmatory: ^{37}Cl isotopic mass m/z 142 and the entire MS-	0.01 mg/kg	GC-MS	Keller, 1998 BASF DocID 1998/11314

Component of residue definition: boscalid			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
spectrum)			Method no. 408/1 EU agreed (DAR, 8th Nov 2002) Amendment 1 adding uncorrected recoveries BASF DocID 2003/1000977 EU agreed (Addendum 2 to DAR, May 2006)
Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	XXX M., 2009 BASF DocID 2008/1084832 Method no. L0096/01 not EU peer-reviewed see A 2.2.2.3.1 Amendment 1 BASF DocID 2015/1174527 not EU peer-reviewed

Table 5.3-16: Validated methods for soil (boscalid metabolites M510F47 and M510F49)

Component of residue definition: M510F47 and M510F49			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	XXX D., 2013 BASF DocID 2013/1415720 not EU peer-reviewed see A 2.2.2.3.2

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 boscalid and boscalid metabolites M510F4 and M510F49 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 5.3-17: Validated methods for water (if appropriate)

Component of residue definition: boscalid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Surface water, groundwater	Primary & Confirmatory (2 mass transitions)	0.03 µg/L	LC-MS/MS	XXX H., 2009 BASF DocID 2008/1086809 Method no. L0127/01 not EU peer-reviewed see A 2.2.2.4.1 Amendment 1 information on 2nd mass transition and matrix effects BASF DocID 2015/1174526 not EU peer-reviewed
	ILV	0.03 µg/L	LC-MS/MS	Göcer M., 2015 BASF DocID 2016/1112645 Method no. L0127/01 and L0127/02 not EU peer-reviewed see A 2.2.2.4.1.2

Table 5.3-18: Validated methods for water (boscalid metabolites M510F47 and M510F49)

Component of residue definition: M510F47, M510F49				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Surface water, groundwater	Primary & Confirmatory (2 mass transitions)	0.03 µg/L	LC-MS/MS	XXX T., XXX S., 2015 BASF DocID 2015/1109588 Method no. L0127/02 not EU peer-reviewed see A 2.2.2.4.1
	ILV	0.03 µg/L	LC-MS/MS	Göcer M., 2015 BASF DocID 2016/1112645 Method no. L0127/01 and L0127/02 not EU peer-reviewed see A 2.2.2.4.1.2

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 boscalid in air is given in the following tables. For the detailed evaluation of new/additional studies please refer to Appendix 2.

Table 5.3-19: Validated methods for air

Component of residue definition: boscalid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Air	Primary & Confirmatory	1.5 µg/m^3	GC-MS	Zangmeister W., 2000 BASF DocID 2000/1014992 Method no. 460 EU agreed
Air	Primary & Confirmatory (2 mass transitions)	0.0012 µg/m^3	LC-MS/MS	Göcer M.., 2016 BASF DocID 2016/1037754 Method no. L0336/01 not EU peer-reviewed see A 2.2.2.5.1

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 boscalid and boscalid metabolite M510F01 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 5.3-20: Methods for body fluids and tissues

Component of residue definition: boscalid			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Primary & Confirmatory (2 mass transitions)	0.01 mg/L	LC-MS/MS	XXX S., XXX S., 2016 BASF DocID 2016/1193046 Method no. L0342/01 not EU peer-reviewed see A 2.2.2.6.1

5.3.3.8 Other studies/ information

A new study assessing the stability of boscalid (BAS 510 F) in standard solutions in methanol / acetate buffer solution (80/20, v/v; pH 4.65) was tested in BASF study DocID 2010/1046613. A detailed summary of the new study is presented in Appendix 2, chapter A 2.2.2.7.1.

A new study (BASF DocID 2015/1109589) assessing the extractability of boscalid (BAS 510 F) from soil has been conducted and is presented in detail in Appendix 2, chapter A 2.2.2.7

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	XXX, 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	XXX, E.	2016	BAS 750 F - Acute toxicity study in the fathead minnow (Pimephales promelas) 2016/1155889 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	Yes	BASF
KCP 5.1/3	XXX, 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 5.1.1/1	XXX, A.	2019	AFL0995/01: Determination of the Active Ingredients Boscalid and Mefentrifluconazole in BAS 762 02 F and Aqueous Solutions of BAS 762 02 F by HPLC and UPLC 2019/2034432 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 5.1.1/2	XXX, A.	2019	Validation of the Analytical Method AFL0995/01: Determination of the Active Ingredients Boscalid and Mefentrifluconazole in BAS 762 02 F and Aqueous Solutions of BAS 762 02 F by HPLC and UPLC 2019/2034429 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 5.1.1/3	XXX, D.	2020	Analytical Method AFL 1010/01 - Determination of Dimethylformamide in Formulations containing Mefentrifluconazole {BAS 750 F) 2020/2028497 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 5.1.1/4	XXX, D.	2020	Validation of the Analytical Methode AFL1010/01: "Determination of Dimethylformamide in Formulations containing Mefentrifluconazole (BAS 750 F)" 2020/2032727 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/5	XXX, A.	2020	Additional Validation of the Analytical Method AFL1010/01: "Determination of Dimethylformamide in Formulations containing Mefentrifluconazole (BAS 750 F)" 2020/2085538 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/6	XXX, M.	2017	Analytical method AFL0948/01 - Determination of Toluene in formulations containing Mefentrifluconazole (BAS 750 F) 2017/1077926 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 5.1.1/7	XXX, M.	2017	Validation of the analytical method AFL0948/01: Determination of Toluene in formualtions containing Mefentrifluconazole (BAS 750 F) 2017/1078235 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 5.1.1/8	XXX, M.	2020	Analytical Method AFL0948/03 - Determination of Toluene in Formulations containing Mefentrifluconazole (BAS 750 F). 2020/2080925 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 5.1.1/9	XXX, M.	2020	Additional Validation to the Analytical Method AFL0948/03: Determination of Toluene in Formulations containing Mefentrifluconazole (BAS 750 F) 2020/2085856 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/10	XXX, D.	2018	Analytical method AFL0977/01 - Determination of the impurity Reg.No. 87084 in formulations containing Mefentrifluconazole 2018/1144189 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 5.1.1/11	XXX, D.	2018	Validation of the Analytical Method AFL0977/01: Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole 2018/1144190 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/12	XXX, A.	2020	AFL0977/04: Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole 2020/2037327 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 5.1.1/13	XXX, A.	2020	Additional Validation to the Analytical Method AFL0977/04: Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole 2020/2080849 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 5.1.2/1	XXX, A., XXX, C.	2007	Validation of BASF method No. 535/1 in plant matrices 2006/1039427 BASF AG, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/1	XXX, 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	XXX, 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	XXX, 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
IIA 4.3/7	XXX, M.	2021	Determination of residues of BAS 750 F (Mefentrifluconazole) in oilseed rape and rapeseed honey (unripe) after one application of BAS 750 05 F under simi-field conditions in Germany, 2018 BASF DocID 2020/2109990 yes Unpublished	No	BASF
IIA 4.3/8	XXX, M.	2021	Amendment N°1 to final report Determination of residues of BAS 750 F (Mefentrifluconazole) in oilseed rape and rapeseed honey (unripe) after one application of BAS 750 05 F under simi-field conditions in Germany, 2018 BASF DocID 2021/2038566 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	XXX, N.	2015	Validation of the analytical method QuEChERS for the determination of BAS 510 F (Boscalid) in foodstuff of plant origin 2015/1114667 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/5	XXX, R.	2015	Validation of BASF analytical method number L0076/01 for the determination of BAS 510 F (Boscalid) in hops, spices and herbal infusions 2015/1091103 Battelle UK Ltd., Chelmsford Essex CM2 5LB, United Kingdom yes Unpublished	No	BASF
KCP 5.2/6	XXX, F.	2001	The validation of BASF method 471/0: The determination of BAS 510 F and the metabolite M510F01 in animal matrices 2000/1017223 BASF AG, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/7	XXX, F.	2004	Report amendment No. 1: The validation of BASF method 471/0: The determination of BAS 510 F and the metabolite M510F01 in animal matrices 2003/1021922 BASF AG, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/8	XXX, J.	2015	Report Amendment No.2: The validation of BASF method 471/0: The determination of BAS 510 F and the metabolite M510F01 in animal matrices 2015/1174463 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	XXX, H., XXX, A.	2015	Independent laboratory validation of the BASF method L0041/01 (471/0) for the determination of BAS 510 F (Boscalid) and metabolite M510F01 in animal matrices 2015/1114666 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/10	XXX, H.	2015	Amendment No. 1 - Independent laboratory validation of the BASF method L0041/01 (471/0) for the determination of BAS 510 F (Boscalid) and metabolite M510F01 in animal matrices 2015/1251211 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/11	XXX, M.	2009	Validation of analytical method L0096/01: Determination of Boscalid Reg.No. 300355 in soil using HPLC/MS-MS 2008/1084832 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/12	XXX, M.	2015	Report amendment no. 1 - Validation of analytical method L0096/01: Determination of Boscalid Reg.No. 300355 in soil using HPLC/MS-MS 2015/1174527 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/13	XXX D.	2013	Validation of an Analytical Method for Determination of Residues of M510F47 and M510F49 in Soil 2013/1415720 Eurofins Agroscience Services yes Unpublished	No	BASF
KCP 5.2/14	XXX, H.	2009	Validation of analytical method L0127/1 for the determination of BAS 510 F (Boscalid) residues in surface water and groundwater 2008/1086809 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 5.2/15	XXX, T.	2015	Report Amendment No. 1 to final report: Validation of analytical method L0127/1 for the determination of BAS 510 F (Boscalid) residues in surface water and groundwater 2015/1174526 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/16	XXX, M.	2016	Independent laboratory validation (ILV) of the BASF method L0127 for the determination of Boscalid (BAS 510 F) and two of its metabolites M510F47 (Reg.No. 107371) and M510F49 (Reg.No. 391572) in surface water and groundwater 2016/1112645 Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/17	XXX, T.	2015	Validation of analytical method L0127/02 for the determination of M510F47 (Reg.No. 107371) and M510F49 (Reg.No. 391572) in surface and groundwater 2015/1109588 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/18	XXX, M.	2016	Validation of analytical method L0336/01: Determination of BAS 510 F (Boscalid) in Air 2016/1037754 Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/19	XXX, S., XXX, S.	2016	Validation of BASF analytical method L0342/01 for the determination of BAS 510 F (Boscalid) and its metabolite M510F01 in body fluids 2016/1193046 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/20	XXX, H.	2010	Standard stability of BAS 510 F in methanol / acetate buffer solution 2010/1046613 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 5.2/21	XXX, T.	2015	Comparative analysis of extraction procedures on Boscalid (BAS 510 F) originating from a field accumulation and dissipation study in Northern Italy 2015/1109589 BASF SE, Limburgerhof, Germany Fed.Rep. 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.

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Mefentrifluconazole

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

A 2.1.2

Comments of zRMS:	The analytical method APL0500/03 has been satisfactorily validated for the determination of residues of BAS 750 F (Mefentrifluconazole) and its metabolite M750F007 (Reg.No.6003432) in M4-Medium, OECD-water and mixing water by LC/MS with a LOQ of 0.001 mg/L (corresponding to a concentration of 1 ng/mL in the water samples). The mean recovery values of the validation experiments over all tested analytes were between 86% and 109% with the relative standard deviations <20% for all tested matrices. The study is acceptable.

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, XXX, G., 2017 report No 838449 BASF DocID 2017/1064882 Authority registration No
Guideline(s):	EFSA Panel on Plant Prptection 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 [%]
Mefentriflu-conazole	M4-Medium	0.001	5	108	1.0	108	2.2
		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
		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).

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.

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 slightly modified analytical method APL0500/03 has been satisfactorily validated for the determination of residues of BAS 750 F (Mefentrifluconazole) in test water by HPLC/MS with a LOQ of 0.001 mg/L. Mean recovery rates of 106% for the lower fortification level as well as 103% for the higher fortification level were found. The relative standard deviation (RSD) was < 10% for both levels investigated. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4. The study is acceptable.

Reference:	CP 5.1/2
Report	BAS 750 F - Acute toxicity study in the fathead minnow (Pimephales promelas), XXX, E., 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/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 $r \geq 0.99$ were obtained, thus demonstrating satisfactory linearity.

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.

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 analytical method L0361/01 has been satisfactorily validated for the determination of residues of BAS 750 F (Mefentrifluconazole) and BAS 510 F (Boscalid) in tap water and M4-medium by LC-MS/MS with a LOQ of 0.1 µg/L. The mean recovery values of the validation experiments for mefentrifluconazole were between 94% and 106% with the relative standard deviations <20% for all tested matrices. The mean recovery values of the validation experiments for boscalid were between 95% and 99% with the relative standard deviations <20% for all tested matrices. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4. The study is acceptable.

Reference:	CP 5.1/3
Report	Validation of BASF Method L0361/01 for the Determination of Pesticides in Water by LC-MS/MS, XXX, 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.

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/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			
		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.

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.

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).

A 2.1.2.1.1.1 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 2.1.3 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.1.3.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.1.3.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted

A 2.1.3.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 2.1.3.4 Description of Methods for the Analysis of Water (KCP 5.2)

A 2.1.3.4.1 Analytical method L0359/01 for the determination of mefentrifluconazole in water

A 2.1.3.4.1.1 Method validation 1

Comments of zRMS:	The analytical method L0359/01 has been satisfactorily validated for the determination of residues of BAS 750 F (Mefentrifluconazole) and its metabolites M750F003 (Reg. No. 5924326), M750F005 (Reg. No. 6003433), M750F006 (Reg. No. 5863469), M750F007 (Reg. No. 6003432) and M750F008 (Reg. No. 6010286) in drinking (ground) and surface water by LC-MS/MS with a LOQ of 0.03 µg/L. The mean recovery values of the validation experiments for mefentrifluconazole were between 95% and 103% with the relative standard deviations <10% for all tested matrices. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4. The study is acceptable.

Reference:	CP 5.2/1
Report	Validation of analytical method L0359/01 for the Determination of 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 (Ground) and surface-water by LC-MS/MS XXX, 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 4: 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 5: Characteristics for the analytical method used for 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 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 2.1.3.4.1.2 Independent laboratory validation 1

Comments of zRMS:	The method L0359/01 was successfully independently validated for the determination of mefentrifluconazole (BAS 750 F) and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 in drinking water and surface water with the limit of quantification of 0.03 µg/L using LC-MS/MS with two mass transitions per analyte. The mean recovery values were between 70% and 110% in all matrices tested. The relative standard deviation (RSD) did not exceed 20% at any fortification level for either of the analytes. The study is acceptable.

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 XXX, 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 6: 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
		0.3 (n=5)	102	10	m/z 398→70
		0.03 (n=5)	92.2	2.6	Confirmation
		0.3 (n=5)	108	7.4	m/z 400→70
Surface water	BAS 750 F	0.03 (n=5)	108	7.8	Quantitation
		0.3 (n=5)	108	8.3	m/z 398→70
		0.03 (n=5)	110	2.8	Confirmation
		0.3 (n=5)	108	2.3	m/z 400→70

Table A 7: 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 2.1.3.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.3.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

Comments of zRMS:	The analytical method L0339/02 has been satisfactorily validated for the determination of residues of metabolites of BAS 750 F: M750F015, M750F016 and M750F017 in body fluids (plasma and urine) using LC-MS/MS with a LOQ of 0.01 mg/L. The LOD was set at 0.002 mg/L. The mean recovery values were between 70% and 110% of the nominal value for both mass transitions for each analyte in all matrices tested. The relative standard deviations (RSD, %) for all fortification levels were below 20%. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1. The study is acceptable.

Reference: CP 5.2/3

Report	Validation of BASF Analytical Method L0339/02 for the determination of M750F015, M750F016 and M750F017 in body fluids, XXX, 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 8: Results of the method validation for the determination of M750F015, M750F016 and M750F017 in body fluids

Analyte	Matrix	m/z	Fortification level [mg/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→143	0.010	5	99.5	2.8	97.1	3.5
			0.10	5	94.7	2.0		
	Urine	414→70	0.010	5	95.4	1.9	92.7	3.6
			0.10	5	90.0	2.1		
		414→143	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 9: 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 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.
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 2.1.3.7 Other Studies/ Information

~~No new or additional studies have been submitted~~

A residue study for the determination of BAS 750 F residues in honey has been performed (BASF DocID:

2020/2109990, Report Amendment N°1 DocID: 2021/2038566) and has been provided by Applicant. The following study is a combination of residue analytics and a validation study in honey. Therefore, the following summary is restricted to the method analytical part of the study.

Comments of zRMS:	Whole plant (no roots), inflorescences, pollen and honey specimens were analyzed for residues of BAS 750 F (Mefentrifluconazole) and for residues of the triazole metabolites 1,2,4-Triazole (T), Triazolylalanine (TA), Triazole lactic acid (TLA) and Triazole acetic acid (TAA) using BASF method L0170/03. The limit of quantification (LOQ) for all analytes was 0.050 mg/kg. The mean recovery values were between 70% and 110% of the nominal value for each analyte in all matrices tested. The relative standard deviations (RSD, %) for all fortification levels were below 20%. The study is acceptable.

Report:	IIA 4.3/7 XXX, M., 2021 Determination of residues of BAS 750 F (Mefentrifluconazole) in oilseed rape and rapeseed honey (unripe) after one application of BAS 750 05 F under semi-field conditions in Germany, 2018 2020/2109990
Guidelines:	EC 1107/2009, EEC 7525/VI/95 rev. 10.3, OECD 509 (2009), SANTE/11956/2016 rev. 9
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Report:	IIA 4.3/8 XXX, M., 2021 Amendment 1: Determination of residues of BAS 750 F (Mefentrifluconazole) in oilseed rape and rapeseed honey (unripe) after one application of BAS 750 05 F under semi-field conditions in Germany, 2018 2021/2038566
Guidelines:	EC 1107/2009, EEC 7525/VI/95 rev. 10.3, OECD 509 (2009), SANTE/11956/2016 rev. 9
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

For the analysis of mefentrifluconazole (BAS 750 F) and the TDMs (1,2,4-T, TA, TAA and TLA) method no. L0170/03 was used which determined the analyte by means of LC-MS/MS.

Principle of the method:	Method No. L0170/03: Specimens were extracted with a mixture of methanol and water. For the determination of BAS 750 F, an aliquot was taken and filtered. Then the isotopically labelled internal standard was added for quantitation using HPLC-MS/MS. Another aliquot, for determination of 1,2,4-triazole (T), triazolylalanine (TA), triazole acetic acid (TAA) and triazole lactic acid (TLA) was filtered, concentrated, and cleaned-up by a simple dispersive C18-SPE step. The analytes were determined by LC-DMS/MS/MS, using isotopically labelled internal standards for quantitation.
	Higher residues were accordingly diluted to fit into the calibration curve and matrix effects were compensated by using labelled internal standards. The method has a limit of quantitation of 0.050 mg/kg for each analyte. The limit of detection for each analyte is at least 10 % of the LOQ and therefore corresponds to 0.005 mg/kg.

Recovery findings: As shown in the table below, the mean recovery values were between 70% and 110% of the nominal value for each analyte.

Table 4.3-1: Recovery Results for BAS 750 F, 1,2,4-T, TA, TAA and TLA in oilseed rape and rapeseed honey

Method ID	Matrix	Level (mg/kg)	No of samples per fortification level	Individual recoveries (%)	Min recoveries (%)	Max recoveries (%)	Mean recoveries (%)	RSD (%)	Comments
BAS 750 F									
L0170/03	OSR[1)], Whole plant (no roots	0.050	3	99.4, 99.9, 97.0	97.0	99.9	98.8	1.6	Overall Recovery: 97.3%
		0.50	3	98.5, 100, 96.2	96.2	100	98.2	1.9	
		5	1	89.9	89.9	89.9	89.9	n.a.	RSD: 3.7%
	OSR[1)], Honey	0.050	3	91.8, 90.2, 89.4	89.4	91.8	90.5	1.4	Overall Recovery: 91.5%
		0.50	3	91.2, 93.0, 95.2	91.2	95.2	93.1	2.2	
		5	1	89.8	89.8	89.8	89.8	n.a.	RSD: 2.2%
	OSR[1)], Inflorescences	0.050	3	110, 101, 108	101	110	106.3	4.4	Overall Recovery: 98.9%
		0.50	3	88.1, 97.1, 98.0	88.1	98.0	94.4	5.8	
		5	1	92.6	92.6	92.6	92.6	n.a.	RSD: 7.4%
		20	1	96.3	96.3	96.3	96.3	n.a.	
	OSR[1)], Pollen	0.050	3	116, 88.9, 112	88.9	116	105.6	13.8	Overall Recovery: 105%
		0.50	3	107, 111, 103	103	111	107	3.7	
		10	1	100	100	100	100	n.a.	RSD: 8.6%
	OSR[1)], Overall	-	29	-	-	-	98.3	7.8	-
1,2,4-Triazole (1,2,4-T)									
L0170/03	OSR[1)], Whole plant (no roots	0.050	5	92.1, 94.0, 92.9, 88.9, 92.0	88.9	94.0	92.0	2.1	Overall Recovery: 92.5%
		0.50	5	91.6, 87.6, 92.5, 95.1, 98.3	87.6	98.3	93.0	4.3	RSD: 3.2%
	OSR[1)], Honey	0.050	5	92.0, 102, 91.4, 113, 110	91.4	110	101.7	9.8	Overall Recovery: 99.6%
		0.50	5	101, 92.4, 95.8, 96.5, 102	92.4	102	97.5	4.0	RSD: 7.5%
	OSR[1)], Inflorescences	0.050	5	100, 83.8, 97.0, 96.7, 90.5	83.8	100	93.6	6.9	Overall Recovery: 94.8%
		0.50	5	101, 94.4, 92.5, 95.8, 97.8	84.9	101	96.3	3.4	RSD: 5.1%
		2.0	1	93.5	93.5	93.5	93.5	n.a.	
	OSR[1)], Pollen	0.050	8	110, 108, 103, 104, 109, 107, 114, 109	103	114	108	3.2	Overall Recovery: 106%
		0.50	8	103, 99.0, 103, 96.5, 105, 109, 107, 104	96.5	109	103.3	3.9	RSD: 4.0%
		2.0	1	104	104	104	104	n.a.	

Method ID	Matrix	Level (mg/kg)	No of samples per fortification level	Individual recoveries (%)	Min recoveries (%)	Max recoveries (%)	Mean recoveries (%)	RSD (%)	Comments
	OSR[1)], Overall	-	48	-	-	-	99.1	7.3	-
Triazole alanine (TA)									
L0170/03	OSR[1)], Whole plant (no roots	0.050	5	90.7, 94.6, 114, 94.4, 103	90.7	114	99.3	9.4	Overall Recovery: 103%
		0.50	5	102, 101, 118, 106, 105	101	118	106.4	6.4	RSD: 8.3%
	OSR[1)], Honey	0.050	5	102, 105, 107, 105, 96.2	96.2	107	103	4.1	Overall Recovery: 100%
		0.50	5	105, 97.3, 93.6, 92.3, 100	92.3	105	97.6	5.2	RSD: 5.2%
	OSR[1)], Inflorescences	0.050	5	97.0, 94.9, 100, 124, 133	94.9	133	109.8	15.9	Overall Recovery: 105%
		0.50	5	106, 99.4, 98.7, 99.9, 101	98.7	106	101	2.9	RSD: 11%
		2.0	1	103	103	103	103	n.a.	
	OSR[1)], Pollen	0.050	8	102, 124, 133, 92.1, 93.9, 96.4, 87.2, 88.7	87.2	133	102.2	16.7	Overall Recovery: 103%
		0.50	8	109, 113, 116, 96.8, 104, 97.6, 98.2, 98.7	96.8	116	104.2	7.3	RSD: 12%
		2.0	1	102	102	102	102	n.a.	
	OSR[1)], Overall	-	48	-	-	-	103	10	-
Triazole acetic acid (TAA)									
L0170/03	OSR[1)], Whole plant (no roots	0.050	5	100, 106, 100, 95.6, 106	95.6	106	101.5	4.4	Overall Recovery: 101%
		0.50	5	100, 101, 100, 101, 102	100	102	100.8	0.8	RSD: 3.0%
	OSR[1)], Honey	0.050	5	99.3, 108, 101, 112, 103	99.3	108	104.7	5.5	Overall Recovery: 101%
		0.50	5	99.8, 96.6, 99.7, 93.6, 99.9	93.6	99.7	97.9	2.8	RSD: 5.3%
	OSR[1)], Inflorescences	0.050	5	107, 107, 106, 102, 103	102	107	105	2.2	Overall Recovery: 106%
		0.50	5	111, 107, 102, 107, 108	102	111	107	3.0	RSD: 2.6%
		2.0	1	107	107	107	107	n.a.	
	OSR[1)], Pollen	0.050	8	92.6, 92.7, 102, 98.2, 101, 95.4, 98.6, 95.0	92.6	102	96.9	3.7	Overall Recovery: 101%
		0.50	8	105, 109, 101, 112, 106, 105, 114, 105	101	114	107.1	4.0	RSD: 6.6%
		2.0	1	92.5	92.5	92.5	92.5	n.a.	

Method ID	Matrix	Level (mg/kg)	No of samples per fortification level	Individual recoveries (%)	Min recoveries (%)	Max recoveries (%)	Mean recoveries (%)	RSD (%)	Comments
	OSR[1)], Overall	-	48	-	-	-	102	5.2	-
Triazole lactic acid (TLA)									
L0170/03	OSR[1)], Whole plant (no roots	0.050	5	106, 108, 104, 92.3, 102	92.3	108	102.5	6.0	Overall Recovery: 103%
		0.50	5	107, 107, 108, 95.4, 96.1	95.4	108	102.7	6.2	RSD: 5.7%
	OSR[1)], Honey	0.050	5	104, 106, 108, 113, 116	104	116	109.4	4.6	Overall Recovery: 106%
		0.50	5	102, 102, 103, 102, 105	102	105	102.8	1.3	RSD: 4.6%
	OSR[1)], Inflorescences	0.050	5	96.7, 103, 98.6, 99.4, 101	96.7	103	99.7	2.4	Overall Recovery: 99.1%
		0.50	5	106, 101, 99.7, 92.4, 94.2	94.2	106	98.7	5.5	RSD: 3.9%
		2.0	1	97.6	97.6	97.6	97.6	n.a.	
	OSR[1)], Pollen	0.050	8	91.2, 93.7, 76.6, 92.8, 95.4, 164, 95.1, 121	76.6	164	103.7	26.2	Overall Recovery: 103%
		0.50	8	104, 99.1, 104, 91.8, 104, 111, 105, 105	91.8	111	103	5.4	RSD: 18%
		2.0	1	97.5	97.5	97.5	97.5	n.a.	
	OSR[1)], Overall	-	48	-	-	-	103	11	-

RSD = relative standard deviation

Linearity: The instrument calibration standards for this study were prepared by making appropriate dilutions of the stock solution. The calibration curve was constituted from the result of at least five different concentration levels. The correlation was performed using a linear function. The correlation coefficient (r) obtained was always ≥ 0.9981.

Specificity: LC-MS/MS is a highly specific detection technique and therefore no confirmatory technique is required. Analysis is possible at two different mass transitions, hence no additional confirmatory analytical technique is required.

Matrix effects: Higher residues were accordingly diluted to fit into the calibration curve and matrix effects were compensated by using labelled internal standards.

Interference: Interference was not determined in this study.

A 2.2 Analytical methods for Boscalid

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

A 2.2.1.1 Description of analytical methods for the determination of residues in plant

matrices (KCP 5.1.2)

The currently proposed data generation method for the determination of boscalid in plant matrices is BASF residue analytical method 535/1 (BASF DocID 2006/1017227); for difficult plant matrices, such as hops, spices and herbal infusions, method L0076/01 (BASF DocID 2015/1091103), is the proposed data generation method for determination of boscalid. The method is also suitable as method for monitoring purposes and is summarized in appendix section A 2.2.2.1.2.

A 2.2.1.1.1 BASF method No. 535/1: Determination of boscalid in plant matrices

A 2.2.1.1.1.1 Method validation

Comments of zRMS:	The analytical method 535/1 has been satisfactorily validated for the determination of residues of boscalid in following plant matrices: wheat (grain, straw), lemon, lettuce, oilseed rape (seed), tomato and onion with an LOQ of 0.01 mg/kg using LC-MS/MS. Average recoveries at each fortification level were all within the acceptance range of 70-120%. The relative standard deviation (RSD) did not exceed 20% at any fortification level for all matrices. The analytical method fulfils the requirements of guideline SANCO/825/00 rev. 8.1. The study is acceptable.

Reference:	CP 5.1.2/1
Report	Validation of BASF method No. 535/1 in plant matrices, XXX C.,XXX A., 2007 Report No 246631 BASF DocID 2006/1039427 Authority registration No.
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
In method 535/1 residues of boscalid (BAS 510 F) are extracted from wheat (plant, straw, grain), lemon, lettuce, oilseed rape (seed), tomato and onion using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. After evaporation of cyclohexane, the residues are dissolved in methanol/water (50/50, v/v). The determination of boscalid is performed by HPLC-MS/MS using a HP 1100 Series HPLC system (Betasil Cl8 100*2.1 mm column), equipped with PE Sciex API 3000 and applying a water/methanol gradient with 0.1% formic acid as modifier. The detection is accomplished by electrospray ionization in positive mode at mass transition 343→271 for quantification and 343→307 for confirmation. The results are calculated by direct comparison of the sample peak responses with external standards prepared in methanol/water.

Results and discussions
In all matrices tested, the mean recovery values 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.

The stability of boscalid in methanol and in the sample solvent over a time period of 60 days was shown during validation of method 445/0 (BASF DocID *2000/1012404*) and solvent stability study BASF DocID *2000/1014856* (already EU-peer-reviewed study; refer to chapter 5.2.2). Standard and extract stability was also addressed during validation of method L0076/01 (BASF DocID 2015/1091103): Analytical standards are stable in solvent for at least 62 days and extracts in a mixture of methanol, water and hydrochloric acid for at least 8 days. No independent laboratory validation was conducted, as the methods purpose is pre-registration data generation.

Table A 10: Recovery results from method validation of boscalid using analytical method 535/1

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Wheat plant*	Boscalid (BAS 510 F)	0.01	93	5.2	Quantitation m/z 343 → 307
		0.1	83	5.6	
		0.01	92	16.6	Confirmation m/z 343 → 271
		0.1	84	7.7	
Wheat grain	Boscalid (BAS 510 F)	0.01	84	7.3	Quantitation m/z 343 → 307
		0.1	84	4.3	
		0.01	100	5.2	Confirmation m/z 343 → 271
		0.1	82	13.9	
Wheat straw	Boscalid (BAS 510 F)	0.01	86	7.3	Quantitation m/z 343 → 307
		0.1	87	11.6	
		0.01	86	11.1	Confirmation m/z 343 → 271
		0.1	82	9.4	
Lemon	Boscalid (BAS 510 F)	0.01	88	2.1	Quantitation m/z 343 → 307
		0.1	82	8.7	
		0.01	89	17.4	Confirmation m/z 343 → 271
		0.1	77	14.8	
Lettuce	Boscalid (BAS 510 F)	0.01	82	6.0	Quantitation m/z 343 → 307
		0.1	82	8.8	
		0.01	92	5.3	Confirmation m/z 343 → 271
		0.1	81	3.0	
Oilseed rape seed	Boscalid (BAS 510 F)	0.01	80	7.1	Quantitation m/z 343 → 307
		0.1	84	6.5	
		0.01	80	12.9	Confirmation m/z 343 → 271
		0.1	86	18.2	
Tomato	Boscalid (BAS 510 F)	0.01	86	5.0	Quantitation m/z 343 → 307
		0.1	86	3.2	
		0.01	81	18.4	Confirmation m/z 343 → 271
		0.1	82	9.2	
Onion	Boscalid (BAS 510 F)	0.01	88	7.0	Quantitation m/z 343 → 307
		0.1	88	8.8	
		0.01	83	10.3	Confirmation m/z 343 → 271
		0.1	81	9.2	

* without roots

Table A 11: Characteristics for the analytical method used for validation of boscalid residues in plant matrices

	Boscalid
Specificity	Boscalid is determined by HPLC-MS/MS monitoring two different mass transitions. The mass spectrum of boscalid is not provided in the study report but is provided in BASF DocID 2015/1114667. No significant interference was observed at the elution time of boscalid in blank samples (interference <30% LOQ).
Calibration (type, number of data points)	The standards used for calibration were prepared in methanol/water. Four standard concentrations were injected in triplicate and the response was plotted against the concentration. Linear correlations with coefficients of r≥0.99 were obtained.
Calibration range	0.05 - 0.5 ng/mL
Assessment of matrix effects is presented	No matrix matched standards have been used as matrix effects were below 20%.
Limit of determination/quantification	The limit of quantitation was defined by the lowest fortification level successfully tested and was 0.01 mg/kg in all plant matrices.

Conclusion
Analytical method 535/1 is considered fully suitable for the analysis of boscalid in plant matrices wheat (grain, straw), lemon, lettuce, oilseed rape (seed), tomato and onion with a limit of quantification of 0.01 mg/kg.

A 2.2.1.1.1.2 Confirmatory method
A confirmatory technique is not required as method 535/1 uses two different mass transitions of boscalid for quantitation and confirmation.

A 2.2.1.1.1.3 Extraction efficiency
Residues of boscalid are extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. Extraction efficiency was assessed in the already peer-reviewed study BASF DocID *2001/1001739* (Bross M., 2001). This study demonstrated that a mixture of methanol, water and hydrochloric acid removed comparable amounts of boscalid residues than the metabolism extraction scheme applied in several metabolism studies (e.g. BASF DocIDs 2000/1014861, 2000/1014860, 2000/1014862 and 1999/11240). Thus, comparability of extraction efficiency of residue analytical method 535/1 and the metabolism extraction scheme has been fully confirmed.

A 2.2.1.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.1.2)

Method 471/0 (BASF DocID *2000/1017223*) applied for the determination of boscalid in animal matrices is used to generate pre-authorization data but can also be used for monitoring purposes. The method is already peer-reviewed. As two amendments to the method were issued (BASF DocIDs *2003/1021922* and *2015/1174463*) which are not EU reviewed, the amendments are summarized together with the original study report in appendix section A 2.2.2.2.1 for completeness of information.

A 2.2.1.3 Description of analytical methods for the determination of residues in soil (KCP 5.1.2)

Method L0096/01 (BASF DocID 2008/1084832; amendment: BASF DocID 2015/1174527) for determination of boscalid and method L0096/02 (BASF DocID 2013/1415720) for determination of boscalid metabolites M510F47 and M510F49 in soil samples are applied to generate pre-authorization data. Both methods can also be used for monitoring purposes and are summarized in appendix sections A 2.2.2.3.1 and A 2.2.2.3.2.

A 2.2.1.4 Description of analytical methods for the determination of residues in water (KCP 5.1.2)

Method L0127/01 (BASF DocID 2008/1086809) for determination of boscalid and method L0127/02 (BASF DocID 2015/1109588) for determination of boscalid metabolites M510F47 and M510F49 in surface water and groundwater samples are applied to generate pre-authorization data. Both methods can also be used for monitoring purposes and are summarized in appendix sections A 2.2.2.4.1 and A 2.2.2.4.2.

A 2.2.1.5 Description of analytical methods for the determination of residues in air (KCP 5.1.2)

A new residue analytical method for the determination of boscalid in air has been developed (BASF DocID 2016/1037754). As the study's main purpose is post-authorisation, a summary of the method is given in appendix section A 2.2.2.5.1.

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

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

A 2.2.2.1.1 Method L0328/01: Determination of BAS 510 F in plant matrices

A 2.2.2.1.1.1 Method validation

Comments of zRMS:	The analytical method QuEChERS (L0328/01) has been satisfactorily validated for the determination of residues of boscalid in plant matrices of high water, high acid, dry high protein and high fat content (exemplified by wheat grain, lemon, dry peas, oilseed rape seed and tomato) with an LOQ of 0.01 mg/kg using HPLC-MS/MS. The mean recovery values of the validation experiments were between 70% and 110%. The relative standard deviation (RSD) did not exceed 10% at any fortification level for all matrices. The analytical method fulfils the requirements of guideline SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4. The study is acceptable.

Reference:	CP 5.2/4
Report	Validation of the analytical method QuEChERS for the determination of BAS 510 F (Boscalid) in foodstuff of plant origin, XXX N., 2015 Report No 783712 BASF DocID 2015/1114667 Authority registration No.
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), OECD-ENV/JM/MONO/(2007)17, EPA 860.1340 (1996)
Deviations:	No
GLP:	yes (certified by Freie und Hansestadt Hamburg, Behoerde fuer Gesundheit und Verbraucherschutz, Hamburg, Germany)
Acceptability:	Yes

Materials and methods

Samples of homogenized plant (wheat grain, lemon, dry peas, oilseed rape seed and tomato) were extracted with acetonitrile after addition of water to the plant matrix. After addition of a buffer salt mixture, containing magnesium sulphate, sodium chloride and sodium citrate, the extract was shaken. After centrifugation, an aliquot of the acetonitrile phase was cleaned by dispersive solid phase extraction, using primary secondary amine (PSA). The determination of boscalid is performed by HPLC-MS/MS using a 1200 Binary Rapid Resolution LC System equipped with an API 5000 detector system and applying a water/methanol gradient with 0.05% acetic acid as modifier. Detection was accomplished by electrospray ionization in positive mode at mass transition 343→307 for quantification and 343→271 for confirmation. The results are calculated by direct comparison of the sample peak responses with external standard prepared in a mixture of acetonitrile and formic acid (1/1, v/v).

Results and discussions

The mean recovery values of the validation experiments were between 70% and 110%, which fulfils the legal requirements for mean recovery values per fortification level. The relative standard deviations (RSD, %) for all fortification levels were below 10%. The detailed results are given in the table below.

Analytical standards of boscalid prepared in acetonitrile/0.1% formic acid (1/1, v/v) were found to be stable for at least 10 days when stored refrigerated (5 ± 4 °C) in the dark. Boscalid was found to be stable in the final extract of all matrices for at least 9 days when stored refrigerated (5 ± 4 °C) in the dark.
As a vast number of validated enforcement methods using the QuEChERS approach is available from the EURL (European Reference Laboratories for Residues of Pesticides) Data Pool, no additional independent laboratory validation is required.

Table A 12: Recovery results from method validation of boscalid using the analytical method L0328/01 (QuEChERS)

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Tomato (Fruit)	Boscalid (BAS 510 F)	0.01	109	3.7	Quantitation m/z 343 → 307
		0.1	102	5.8	
		0.01	109	3.8	Confirmation m/z 343 → 271
		0.1	103	4.3	
Lemon (Fruit)	Boscalid (BAS 510 F)	0.01	103	2.6	Quantitation m/z 343 → 307
		0.1	101	1.5	
		0.01	105	3.5	Confirmation m/z 343 → 271
		0.1	101	2.2	
Wheat (Grain)	Boscalid (BAS 510 F)	0.01	94.4	2.8	Quantitation m/z 343 → 307
		0.1	93.4	4.3	
		0.01	97.3	1.6	Confirmation m/z 343 → 271
		0.1	93.4	3.9	
Dry Pea	Boscalid (BAS 510 F)	0.01	107	3.6	Quantitation m/z 343 → 307
		0.1	106	2.4	
		0.01	106	3.2	Confirmation m/z 343 → 271
		0.1	104	3.4	
Oilseed rape (Seed)	Boscalid (BAS 510 F)	0.01	86.6	3.2	Quantitation m/z 343 → 307
		0.1	83.8	6.1	
		0.01	87.9	3.5	Confirmation m/z 343 → 271
		0.1	83.7	5.6	

Table A 13: Characteristics for the analytical method used for validation of boscalid residues in plant matrices

	Boscalid
Specificity	LC-MS/MS monitoring two mass transitions. The mass spectrum of boscalid is provided in the study report. No significant interference above 30% of LOQ was detected at the retention times and mass transitions of any of the control specimen extracts of each matrix (wheat grain, lemon, dry peas, oilseed rape seed and tomato).
Calibration (type, number of data points)	Good linearity was observed in the range tested (0.15 ng/mL to 10.0 ng/mL). Linear correlations with coefficients ≥0.99 were obtained. Seven calibration standards were used and were prepared in a mixture of acetonitrile and formic acid (1/1, v/v).
Calibration range	0.15 - 10.0 ng/mL
Assessment of matrix effects is presented	Matrix effects on the detection of boscalid in extracts of plant (wheat grain, lemon, dry peas, oilseed rape seed and tomato) were found to be insignificant (<20%).
Limit of determination/quantification	The limit of quantitation was 0.01 mg/kg in all matrices tested, corresponding to a concentration of 0.5 ng/mL in the extract.

Conclusion
The analytical method L0328/01 (QuEChERS methodology) fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore considered fully suitable for the analysis of boscalid in different plant matrices (wheat grain, lemon, dry peas, oilseed rape seed and tomato) with an LOQ of 0.01 mg/kg for enforcement purposes.

A 2.2.2.1.1.2 Independent laboratory validation
As a vast number of validated enforcement methods using QuEChERS approach are available from the EURL (European Reference Laboratories for Residues of Pesticides) Data Pool, no independent laboratory validation is required.

A 2.2.2.1.1.3 Confirmatory method
A confirmatory technique is not required as method L0328/01 uses two different mass transitions of boscalid for quantitation and confirmation.

A 2.2.2.1.1.4 Extraction efficiency
Samples of homogenized plant were extracted with acetonitrile after addition of water to the plant matrix. After addition of a buffer salt mixture, containing magnesium sulphate, sodium chloride and sodium citrate, the extract was shaken (QuEChERS approach). As QuEChERS is an accepted enforcement method and a vast number of validated methods are available on the EURL (European Reference Laboratories for Residues of Pesticides) Data Pool, no additional study assessing extraction efficiency was conducted.

A 2.2.2.1.2 BASF method No. L0076/01: Determination of boscalid in hops, spices and herbal infusions

A 2.2.2.1.2.1 Method validation

Comments of zRMS:	Analytical method L0076/01 has been successfully validated and fulfils the requirements with regard to specificity, linearity, limit of quantification, recoveries and repeatability for the analysis of BAS 510 F (boscalid) in difficult plant matrices according to the SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4. The method is considered suitable for the determination of residues of boscalid in hops, spices and herbal infusions over the concentration range tested 0.01 - 0.1 mg/kg. The limit of quantitation was 0.01 mg/kg for boscalid in all matrices tested. The limit of detection (LOD) was set at 0.002 mg/kg for boscalid in all matrices. No confirmatory technique is required as two different, selective mass transitions have been validated. The study is acceptable.

Reference:	CP 5.2/5
Report	Validation of BASF analytical method number L0076/01 for the determination of BAS 510 F (Boscalid) in hops, spices and herbal infusions, XXX R., 2015 Report No 773279 BASF DocID 2015/1091103 Authority registration No.
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), OECD-ENV/JM/MONO/(2007)17, EPA 860.1340
Deviations:	No
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
Acceptability:	Yes

Materials and methods

Residues of boscalid (BAS 510 F) are extracted from hops, spices and herbal infusions (green tea) with a mixture of methanol, water and hydrochloric acid (70:25:5, v/v/v). An aliquot of the extract was centrifuged and partitioned in alkaline conditions against cyclohexane, evaporated to dryness and dissolved in methanol/water (1:1, v/v) for analysis. The final determination of boscalid was performed by LC-MS/MS monitoring selective ion mass transitions using positive electrospray ionization. In Table A 14 the mass transitions for all matrices regarding quantitation and confirmation are given. Analysis of hops and herbal infusions was accomplished on a Betasil C18 column applying a methanol/water gradient using 0.1% formic acid as modifier, whereas, chromatography of spice matrices was performed on a Synergi Polar-RP 100 Å column. The results are calculated by direct comparison of the sample peak responses with external standards prepared in solvent (methanol/water) or matrix.

Results and discussions

In all matrices tested, the mean recovery values 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.

Analytical standards of boscalid prepared in methanol or methanol/water (50/50, v/v) were demonstrated to be stable for at least 62 days when stored refrigerated. The final sample extracts were re-injected after at least 8 days of storage under refrigerated conditions. Re-injection of final extracts resulted in recoveries

within the acceptable range of 70-110%. No independent laboratory validation was conducted as sufficient validated methods are available for the difficult matrices on the EURL data pool.

Table A 14: Recovery results from method validation of boscalid using the analytical method L0076/01

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Hops	Boscalid (BAS 510 F)	0.01	83.2	5.8	Quantitation m/z 343 → 272
		0.1	90.2	2.1	
		0.01	79.5	4.2	Confirmation m/z 343 → 140
		0.1	86.8	3.0	
Spices	Boscalid (BAS 510 F)	0.01	80.7	4.6	Quantitation m/z 343 → 271
		0.1	77.1	2.5	
		0.01	82.5	4.4	Confirmation m/z 343 → 140
		0.1	77.1	1.6	
Herbal infusions	Boscalid (BAS 510 F)	0.01	83.0	2.3	Quantitation m/z 343 → 307
		0.1	89.9	8.7	
		0.01	84.8	2.9	Confirmation m/z 343 → 271
		0.1	89.7	9.2	

Table A 15: Characteristics for the analytical method used for validation of boscalid residues in plant matrices

	Boscalid
Specificity	HPLC-MS/MS is a highly specific analytical technique. Two mass transitions of boscalid were evaluated. The mass spectrum of boscalid is provided in the study report. Significant interferences (> 30% of the limit of quantification) measured in the control samples were not observed at the retention time of boscalid.
Calibration (type, number of data points)	The linearity was tested using at least 5 standards prepared in an aqueous mixture of methanol and water (1:1, v/v). Linear correlations with coefficients of r≥0.99 were obtained. For hops and spices samples matrix-matched standards were used for calibration.
Calibration range	0.04 - 10.0 ng/mL
Assessment of matrix effects is presented	Significant matrix effects (≥ ±20%) on LC-MS/MS response were observed in hops and spices and therefore, calibration standards prepared in matrix were used for quantification of boscalid. In contrast, solvent standards were used for quantification of boscalid in herbal infusions as matrix effects were insignificant (<±20%).
Limit of determination/quantification	The limit of quantitation was defined by the lowest fortification level successfully tested and was 0.01 mg/kg in all plant matrices.

Conclusion
Analytical method L0076/01 fulfils the requirements with regard to specificity, linearity, limit of quantification, recoveries and repeatability for the analysis of BAS 510 F (boscalid) in difficult plant matrices. The method is considered suitable for the determination of boscalid residues in hops, spices and herbal infusions over the concentration range tested (0.01 - 0.1 mg/kg).

A 2.2.2.1.2.2 Confirmatory method
A confirmatory technique is not required as method L0076/01 uses two different mass transitions of boscalid for quantitation and confirmation.

A 2.2.2.1.2.3 Extraction efficiency
Residues of boscalid are extracted from hops, spices and herbal infusions (green tea) with a mixture of methanol, water and hydrochloric acid (70:25:5, v/v/v). Extraction efficiency was assessed in the already peer-reviewed study BASF DocID *2001/1001739* (Bross M., 2001). This study demonstrated that a mixture of methanol, water and hydrochloric acid removed comparable amounts of boscalid residues than the metabolism extraction scheme applied in several metabolism studies (e.g. BASF DocIDs 2000/1014861, 2000/1014860, 2000/1014862 and 1999/11240). Thus, comparability of extraction efficiency of residue analytical method L0076/01 and the metabolism extraction scheme has been fully confirmed.

A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

A 2.2.2.2.1 BASF Method 471/0: Determination of BAS 510 F and its metabolite M510F01 in animal matrices

A 2.2.2.2.1.1 Method validation

The validation study for method 471/0 (DocID *2000/1017223*) is already peer-reviewed. Two amendments were issued after the peer-review (amendment 1: DocID *2003/1021922*, amendment 2: DocID *2015/1174463*). For reasons of completeness, the original validation report and both amendments are summarized below.

Comments of zRMS:	Analytical method 471/0 has been successfully validated and fulfils the requirements with regard to specificity, linearity, limit of quantification, recoveries and repeatability for the analysis of BAS 510 F (boscalid) and its metabolite M510F01 in foodstuffs of animal origin (exemplified with milk, cream, egg, muscle, fat, liver and kidney). The limit of quantitation of the analytical method is 0.01 mg/kg in eggs, milk and cream and 0.025 mg/kg for muscle, fat, kidney and liver. The average recoveries for the two parent-daughter ion transitions monitored were within the acceptable range of 70% to 110%. The relative standard deviations for all fortification levels were below 20%. The study is acceptable.

Reference:	CP 5.2/6
Report	*The validation of BASF method 471/0: The determination of BAS 510 F and the metabolite M510F01 in animal matrices,* *XXX F., 2001* *Report No 42392* *BASF DocID 2000/1017223* *Authority registration No.*
Guideline(s):	*EPA 860.1340, Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4)*
Deviations:	*No*
GLP:	*yes* *(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)*
Acceptability:	Yes

Comments of zRMS:	Statement concerning the recommended maximum storage period of reference solutions was made in the revised version of the Technical procedure to fulfill EPA requirements. Accepted.

Reference: *CP 5.2/7*

Report *Report amendment No. 1: The validation of BASF method 471/0: The determination of BAS 510 F and the metabolite M510F01 in animal matrices,*
XXX F., 2004
Report No 42392
BASF DocID 2003/1021922
Authority registration No.

Guideline(s): *EPA 860.1340, Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4)*

Deviations: *No*

GLP: *yes*
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz

Acceptability: *Yes*

Comments of zRMS:	New information on second mass transitions and on influence of the matrix effect has been provided in the report amendment. The additional information is requested according to the guidelines SANCO/3029/99 rev. 4 and SANCO/825/OO rev. 8.1. In this report amendment, the second mass transitions are additionally reported for BAS 510 F m/z =343/140 and for M510F01 m/z =359/140. Since the matrix kidney was analysed on a different HPLC-MS/MS system than all other matrices, another second mass transition (m/z = 343/271) was measured for BAS 510 F. It could be demonstrated that BASF method 471/0 fulfils the requirements of the gu deline SANCO/3029/99 rev. 4 with regard to recoveries of the second mass transitions. Therefore, both mass transitions can be used for quantification of BAS 510 F and M510F01. The influence of the matrix on the response of the analyte in the mass spectrometer was determined by means of the quality control samples (QCS), measured in each analytical sequence. By comparing the measured concentrations of soivent-based and matrix-matched standards, the matrix effect in the method for BAS 510 F was shown to be s 20%, except for milk with the mass transition 343/307, where the matrix effect accounted for 24%. The use of matrix—matched standards is therefore not considered imperative. For M51OFO1 the matrix effect was shown to be ≤20 %. The use of matrix-matched standards is therefore not required. The study is acceptable.

Reference: *CP 5.2/8*

Report *Report Amendment No.2: The validation of BASF method 471/0: The determination of BAS 510 F and the metabolite M510F01 in animal matrices,*
XXX J. et al., 2015
Report No 42392
BASF DocID 2015/1174463
Authority registration No.

Guideline(s): *EPA 860.1340, Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4)*

Deviations: *No*

GLP: *yes*

(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Acceptability: *Yes*

Materials and methods

BAS 510 F (boscalid) and its metabolite M510F01 were extracted from animal matrices using methanol. An aliquot of the extract was evaporated to dryness, redissolved in buffer solution and incubated with ß-glucuronidase/arylsulfatase to cleave the glucuronide M510F02 to M510F01. Then a liquid/liquid partition with ethyl acetate is carried out and the organic phase is purified on SPE C18 and if necessary on SPE silica gel columns. The final determination of the analytes BAS 510 F and M510F01 was performed by HPLC-MS/MS using a water/acetonitrile gradient with ammonium formate as modifier. Detection of both analytes was accomplished by electrospray ionization in positive mode at two mass transitions for quantification and confirmation. In Table A 16 the analyzed mass transitions for all matrices are given. The results are calculated by direct comparison of the sample peak responses with external standard prepared in acetonitrile.

Results and discussions

In all matrices tested, the mean recovery values were between 70% and 110%. The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed results are given in the tables below.

Stability of standards as well as extract stability, storage and volume stability were not investigated during the study, but within study BASF DocID *2000/1017225* (already EU-peer-reviewed study; refer to chapter 5.2.2) as well as in the method's ILV (BASF DocID 2015/1114666, amendment: 2015/1251211). An independent laboratory validation has been successfully conducted and is reported below.

Table A 16: Recovery results from method validation of boscalid using the analytical method 471/0

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Cow, milk	Boscalid (BAS 510 F)	0.01	86.0	3.6	Quantitation m/z 343 → 307
		0.1	88.7	7.7	
		0.01	86.2	6.7	Confirmation m/z 343 → 140
		0.1	87.6	11.9	
Cow, cream	Boscalid (BAS 510 F)	0.01	72.2	1.5	Quantitation m/z 343 → 307
		0.1	89.9	4.7	
		0.01	73.4	9.6	Confirmation m/z 343 → 140
		0.1	83.5	8.7	
Cow, muscle	Boscalid (BAS 510 F)	0.025	86.4	4.0	Quantitation m/z 343 → 307
		0.25	94.5	1.5	
		0.025	107	3.4	Confirmation m/z 343 → 140
		0.25	93.1	1.8	
Cow, fat	Boscalid (BAS 510 F)	0.025	80.0	5.4	Quantitation m/z 343 → 307
		0.25	81.0	8.5	
		0.025	79.9	5.8	Confirmation m/z 343 → 140
		0.25	80.9	6.6	
Cow, liver	Boscalid (BAS 510 F)	0.025	86.7	6.3	Quantitation m/z 343 → 307
		0.25	96.0	8.7	
		0.025	74.2	8.7	Confirmation m/z 343 → 140
		0.25	90.9	8.8	
Hen, egg	Boscalid (BAS 510 F)	0.01	82.5	3.8	Quantitation m/z 343 → 307
		0.1	93.1	3.1	
		0.01	88.2	4.9	Confirmation m/z 343 → 140
		0.1	93.3	5.3	
Cow, kidney	Boscalid (BAS 510 F)	0.025	83.3	1.9	Quantitation m/z 343 → 307
		0.25	90.6	3.9	
		0.025	84.5	27.7	Confirmation m/z 343 → 271
		0.25	92.3	7.6	

Table A 17: Recovery results from method validation of M510F01 using the analytical method 471/0

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Cow, milk	M510F01	0.01	88.4	5.8	Quantitation m/z 359 → 323
		0.1	84.9	8.6	
		0.01	83.3	5.6	Confirmation m/z 359 → 140
		0.1	82.8	11.5	
Cow, cream	M510F01	0.01	89.5	1.7	Quantitation m/z 359 → 323
		0.1	94.2	2.3	
		0.01	83.9	3.2	Confirmation m/z 359 → 140
		0.1	93.9	6.0	
Cow, muscle	M510F01	0.025	89.3	2.1	Quantitation m/z 359 → 323
		0.25	86.3	1.4	
		0.025	106	2.9	Confirmation m/z 359 → 140
		0.25	88.6	4.0	
Cow, fat	M510F01	0.025	81.0	4.0	Quantitation m/z 359 → 323
		0.25	82.6	7.4	
		0.025	79.5	2.6	Confirmation m/z 359 → 140
		0.25	82.3	5.5	
Cow, kidney	M510F01	0.025	81.6	2.5	Quantitation m/z 359 → 323
		0.25	82.2	4.6	
		0.025	73.2	7.0	Confirmation m/z 359 → 140
		0.25	78.7	4.2	
Cow, liver	M510F01	0.025	90.9	10.3	Quantitation m/z 359 → 323
		0.25	91.5	6.2	
		0.025	91.9	11.6	Confirmation m/z 359 → 140
		0.25	91.0	5.1	
Hen, egg	M510F01	0.01	82.7	6.1	Quantitation m/z 359 → 323
		0.1	89.1	8.2	
		0.01	82.5	4.2	Confirmation m/z 359 → 140
		0.1	88.5	6.8	

Table A 18: Characteristics for the analytical method used for validation of boscalid and M510F01 residues in animal matrices

	Boscalid	**M510F01**
Specificity	LC-MS/MS monitoring two mass transitions. Mass spectra of boscalid and M510F01 are not provided in the study report, but the mass spectra are provided the method's ILV, BASF DocID 2015/1114666. At elution time of the signals of interest, interference was below the significant limit of 30% LOQ in all cases.	
Calibration (type, number of data points)	Calibration standards were prepared in acetonitrile. Four calibration points were used. Good linearity was observed over the concentration range tested with correlation coefficients >0.95.	
Calibration range	Linearity was observed in the range of 0.1 - 1.0 ng/mL (for milk, liver, muscle) or 0.25 ng/mL - 2.5 ng/mL (for egg, muscle, cream, fat and kidney).	
Assessment of matrix effects is presented	No matrix effects (>20%) were observed, except for milk at the mass transition 343→307, where the matrix effect accounted for 24%. The use of matrix-matched standards is therefore not considered imperative.	No matrix effects (>20%) were observed.
Limit of determination/quantification	The limit of quantitation of the analytical method is 0.01 mg/kg in eggs, milk and cream and 0.025 mg/kg for muscle, fat, kidney and liver.	

Conclusion

It could be demonstrated that the method 471/0 fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification, and recoveries and is therefore applicable and suitable to correctly determine residues of boscalid (BAS 510 F) and its metabolite M510F01 in foodstuff of animal origin (exemplified with milk, cream, egg, muscle, fat, liver and kidney).

A 2.2.2.2.1.2 Independent laboratory validation

Comments of zRMS:	The method L0041/01 (471/0) was successfully independently validated for the determination of boscalid and its metabolite M510F01 in animal matrices (muscle, kidney, liver, fat, milk, cream and egg) with the limit of quantification of 0.01 mg/kg using LC-MS/MS with two mass transitions per analyte. The mean recovery values were between 70% and 100% in all matrices tested. The relative standard deviation (RSD) did not exceed 20% at any fortification level for either of the analytes. The study is acceptable.

Reference:	CP 5.2/9
Report	Independent laboratory validation of the BASF method L0041/01 (471/0) for the determination of BAS 510 F (Boscalid) and metabolite M510F01 in animal matrices, XXX H., XXX A., 2015 Report No 783714 BASF DocID 2015/1114666 Authority registration No.
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07, EPA 860.1340 (1996)
Deviations:	No
GLP:	yes (certified by Freie und Hansestadt Hamburg, Behoerde fuer Gesundheit und Verbraucherschutz, Hamburg, Germany)
Acceptability:	Yes

Comments of zRMS:	Due to a wrong study identification in the header (page 2 to 167) of the original analytical phase report of study 783714 (DocID 2015/1114666), the analytical phase report has to be amended. The correct study identification in the header is S15-04210 / BASF 783714 (instead of S14-00201 / BASF 413363).

Reference:	CP 5.2/10
Report	Amendment No. 1 - Independent laboratory validation of the BASF method L0041/01 (471/0) for the determination of BAS 510 F (Boscalid) and metabolite M510F01 in animal matrices, XXX H., 2015 Report No 783714 BASF DocID 2015/1251211 Authority registration No.
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07, EPA 860.1340 (1996)
Deviations:	No
GLP:	yes (certified by Freie und Hansestadt Hamburg, Behoerde fuer Gesundheit und Verbraucherschutz, Hamburg, Germany)
Acceptability:	Yes

Remark: The amendment does not contain any scientific information, but corrects a typing error.

Materials and methods
The independent validation study followed the analytical steps of the primary method. The determination of boscalid and its metabolite M510F01 is performed by LC-MS/MS (1200 Binary Rapid Resolution LC System) equipped with a Nucleosil 100-5 C18 column and an API 4000 ESI detector (AB Sciex). Detection of both analytes is accomplished in ESI+ mode at mass transitions 343→307 and 359→323 for quantitation and 343→271 and 359→140 for confirmation, respectively.

Results and discussions
The results of the recovery experiments indicate that the independent laboratory validation was successfully completed. In all matrices tested, the mean recovery values were between 70% and 110%. The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed results are given in the tables below.

Analytical standards of boscalid and M510F01 prepared in acetonitrile were found to be stable for at least 21 days when stored refrigerated (5 ± 4 °C) in the dark. After extraction, both analytes were found to be stable in the final extract of all matrices for at least 9 days when stored refrigerated (5 ± 4 °C) in the dark.

Table A 19: Recovery results from independent laboratory validation of boscalid using the analytical method 471/0

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Muscle	Boscalid (BAS 510 F)	0.01	83.3	4.8	Quantitation m/z 343 → 307
		0.025	78.8	3.5	
		0.25	80.0	4.7	
		0.01	80.7	5.1	Confirmation m/z 343 → 271
		0.025	80.7	6.1	
		0.25	80.2	4.7	
Kidney	Boscalid (BAS 510 F)	0.01	77.7	4.6	Quantitation m/z 343 → 307
		0.025	78.1	4.0	
		0.25	74.0	2.9	
		0.01	77.5	8.3	Confirmation m/z 343 → 271
		0.025	78.9	4.8	
		0.25	72.5	3.2	
Liver	Boscalid (BAS 510 F)	0.01	71.6	3.1	Quantitation m/z 343 → 307
		0.025	70.8	8.2	
		0.25	71.9	5.4	
		0.01	78.3	4.9	Confirmation m/z 343 → 271
		0.025	73.4	11	
		0.25	72.8	4.4	
Fat	Boscalid (BAS 510 F)	0.01	87.6	8.2	Quantitation m/z 343 → 307
		0.025	84.4	6.2	
		0.25	80.9	7.3	
		0.01	87.5	8.0	Confirmation m/z 343 → 271
		0.025	83.9	9.8	
		0.25	80.4	9.5	
Cream	Boscalid (BAS 510 F)	0.01	73.5	3.3	Quantitation m/z 343 → 307
		0.10	76.2	5.8	
		0.01	71.2	4.2	Confirmation m/z 343 → 271
		0.10	78.2	6.3	
Milk	Boscalid (BAS 510 F)	0.01	75.6	5.3	Quantitation m/z 343 → 307
		0.10	85.9	2.6	
		0.01	72.5	5.0	Confirmation m/z 343 → 271
		0.10	86.8	5.6	
Egg	Boscalid (BAS 510 F)	0.01	75.7	2.1	Quantitation m/z 343 → 307
		0.10	89.9	3.0	
		0.01	74.7	5.5	Confirmation m/z 343 → 271
		0.10	89.1	2.4	

Table A 20: Recovery results from independent laboratory validation of M510F01 using the analytical method 471/0

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Muscle	M510F01	0.01	78.3	6.3	Quantitation m/z 359 → 323
		0.025	81.7	4.7	
		0.25	82.6	4.8	
		0.01	76.4	7.0	Confirmation m/z 359 → 140
		0.025	82.6	3.8	
		0.25	83.0	4.1	
Kidney	M510F01	0.01	83.1	2.1	Quantitation m/z 359 → 323
		0.025	79.6	4.3	
		0.25	73.8	2.2	
		0.01	81.9	6.7	Confirmation m/z 359 → 140
		0.025	82.0	5.5	
		0.25	75.3	3.0	
Liver	M510F01	0.01	75.1	3.5	Quantitation m/z 359 → 323
		0.025	74.7	5.6	
		0.25	80.9	6.6	
		0.01	79.6	5.7	Confirmation m/z 359 → 140
		0.025	75.4	6.3	
		0.25	82.2	6.1	
Fat	M510F01	0.01	84.5	6.6	Quantitation m/z 359 → 323
		0.025	86.8	1.8	
		0.25	80.6	3.3	
		0.01	82.1	6.4	Confirmation m/z 359 → 140
		0.025	84.0	3.8	
		0.25	80.9	4.2	
Cream	M510F01	0.01	79.3	3.3	Quantitation m/z 359 → 323
		0.10	86.7	6.6	
		0.01	83.4	5.0	Confirmation m/z 359 → 140
		0.10	87.0	6.8	
Milk	M510F01	0.01	85.4	3.2	Quantitation m/z 359 → 323
		0.10	86.8	11	
		0.01	80.8	5.3	Confirmation m/z 359 → 140
		0.10	85.8	10	
Egg	M510F01	0.01	81.7	6.0	Quantitation m/z 359 → 323
		0.10	94.5	2.8	
		0.01	79.3	2.2	Confirmation m/z 359 → 140
		0.10	96.7	1.9	

Table A 21: Characteristics for the analytical method used for independent laboratory validation of boscalid and M510F01 residues in animal matrices

	Boscalid	**M510F01**
Specificity	LC-MS/MS monitoring two mass transitions. Mass spectra of boscalid and M510F01 are provided in the study report. No significant interference above 30% of LOQ was detected at the retention times and mass transitions of any of the control specimen extracts of each matrix (muscle, kidney, liver, fat, cream milk and egg).	
Calibration (type, number of data points)	At least 5 standard concentrations were injected and the response plotted against concentration. Linear correlations with coefficients ≥0.99 were obtained for boscalid and M510F01. The calibration standards were prepared in acetonitrile.	
Calibration range	1.25 – 500 ng/mL	
Assessment of matrix effects is presented	No matrix effects (>20%) were observed on the detection of boscalid and its metabolite M510F01 in extracts of all tested animal matrices.	
Limit of determination/quantification	The limit of quantitation is 0.01 mg/kg (lowest fortification level) in all animal matrices tested.	

Conclusion

The results of the independent laboratory validation confirm the results of the validation study reported above (BASF DocID *2000/1017223*) and demonstrate that analytical method 471/0 fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and can therefore be considered applicable to correctly determine residues of boscalid (BAS 510 F) and its metabolite M510F01 in foodstuff of animal origin (muscle, kidney, liver, fat, milk, cream and egg).

A 2.2.2.2.1.3 Confirmatory method

A confirmatory technique is not required as method 471/0 uses two different mass transitions of boscalid and M510F01 for quantitation and confirmation.

A 2.2.2.2.1.4 Extraction efficiency

BAS 510 F (boscalid) and its metabolite M510F01 were extracted from animal matrices using methanol. As methanol was also used for extraction of boscalid and M510F01 residues in the metabolism studies, no separate assessment of extraction efficiency is required.

A 2.2.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

A 2.2.2.3.1 Method L0096/01: Determination of Boscalid in soil

A 2.2.2.3.1.1 Method validation

Comments of zRMS:	Analytical method L0096/01 has been successfully validated and fulfils the requirements with regard to specificity, linearity, limit of quantification, recoveries and repeatability for the analysis of BAS 510 F (boscalid) in soil with LOQ of 0.01 mg/kg using HPLC-MS/MS. The average recoveries were within the acceptable range of 70% to 110%. The relative standard deviations for all fortification levels were below 20%. The analytical method fulfils the requirements for residue analytical methods as outlined in SANCO/825/00 rev. 7 and SANCO/3029/99 rev. 4. The study is acceptable.

Reference:	CP 5.2/11
Report	Validation of analytical method L0096/01: Determination of Boscalid Reg.No. 300355 in soil using HPLC/MS-MS, XXX M., 2009 Report No 346429 BASF DocID 2008/1084832 Authority registration No.
Guideline(s):	EEC 91/414 Annex IIA, EEC 91/414 Annex IIIA, 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

Comments of zRMS:	Additional information are provided to demonstrate the influence of matrix load on the analysis using quality control samples and additional information on the second mass transition of the LC-MS/MS method applied. Additional information are required for re-registration purposes according to SANCO/825/OO rev 8.1 and SANCO/3029/99 rev. 4. Accepted.

Reference:	CP 5.2/12
Report	Report amendment no. 1 - Validation of analytical method L0096/01: Determination of Boscalid Reg.No. 300355 in soil using HPLC/MS-MS, XXX M.,XXX S., 2015 Report No 346429 BASF DocID 2015/1174527 Authority registration No.
Guideline(s):	EEC 91/414 Annex IIA, EEC 91/414 Annex IIIA, 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

Remark: The study information of BASF DocIDs 2008/1084832 and 2015/1174527 is summarised and presented together in one study summary. The amendment issued (2015/1174527) provides additional information on the second mass transition of the LC-MS/MS method applied, as well as information on the assessment of matrix effects, which were not included in the originally issued final report.

Materials and methods

Boscalid is extracted from 5 g soil samples with 50 mL of methanol/aqueous acetate buffer (80/20, v/v) by shaking at 225 rpm for about 60 min. Approximately 5 mL of the suspension is centrifuged for 5 min at 4000 rpm and 20 °C. Final determination is performed by HPLC-MS/MS using a Betasil C_{18} analytical column and a gradient mixture of water/formic acid (1000/1) and methanol/formic acid (1000/1) at a flow rate of 600 µL min^{-1}. Detection is accomplished using the protonated molecular ion at mass transitions 343→271 and 343→307 for quantification and confirmation.

Results and discussions

The mean recovery values of the validation experiment were between 70% and 110%. The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed results are given in the table below.

The standard stability of boscalid in methanol/acetate buffer solution (80/20, v/v) at concentration levels of 0.1 µg mL^{-1} and 1.0 ng mL^{-1} was determined within BASF DocID 2010/1046613 (see appendix section A 2.2.2.7.1): The standard solutions were stable over a time period of at least 30 days when stored under refrigerated conditions at 4 °C in the dark. The extract stability was determined within BASF DocID 2015/1109589 (see appendix section A 2.2.2.7.2): Soil extracts are stable over a time period of 6 weeks, when stored refrigerated at 4 °C in methanol/acetate buffer pH 4.65 (80/20, v/v).

Table A 22: Recovery results from method validation of boscalid using analytical method L0096/01

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5 or 7)	Mean recovery (%)	RSD (%)	Comments
soil	Boscalid	0.01 (n=7)	94.6	2.2	Quantitation

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5 or 7)	Mean recovery (%)	RSD (%)	Comments
LUFA 2.2	(BAS 510 F)	0.1 (n=5)	93.7	1.9	m/z 343 → 271
		0.01 (n=7)	94.1	1.6	Confirmation m/z 343 → 307
		0.1 (n=5)	92.1	1.8	
soil LUFA 2.3	Boscalid (BAS 510 F)	0.01 (n=7)	97.1	4.1	Quantitation m/z 343 → 271
		0.1 (n=5)	93.1	1.6	
		0.01 (n=7)	97.0	2.5	Confirmation m/z 343 → 307
		0.1 (n=5)	92.8	2.3	

Table A 23: Characteristics for the analytical method used for validation of boscalid residues in soil

	Boscalid
Specificity	HPLC-MS/MS is a highly specific self-confirmatory technique. Two mass transitions were analyzed for quantitation and confirmation of boscalid. Mass spectrum of boscalid is not provided in the study report but in BASF DocID 2016/1037754. As the analytical conditions, such as the mobile phase, are identical, the mass spectrum, hence fragmentation will not change. Significant interferences (> 30% of LOQ) were not observed at the retention time of boscalid in the untreated soil control samples.
Calibration (type, number of data points)	The calculation of residue concentrations was based on linear regression. A linear correlation coefficient >0.999 was obtained. At least five calibration standards prepared in methanol/acetate buffer solution (80/20, v/v) were used.
Calibration range	0.25 ng/mL - 5 ng/mL
Assessment of matrix effects is presented	Yes. Matrix effects were tested by comparing solvent-based standard solutions (prepared in methanol/acetate buffer solution, 80/20, v/v) with matrix-matched standards at a concentration of 0.5 ng/mL. The findings showed a negligible influence of the matrix on the analysis of boscalid
Limit of determination/quantification	The method has a limit of quantification of 0.01 mg/kg, corresponding to the lowest validated concentration level with sufficient recovery and precision. The limit of detection (LOD) is 0.25 ng/mL, which is the lowest calibration standard tested.

Conclusion
It could be demonstrated that method L0096/01 fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine boscalid in soils.

A 2.2.2.3.1.2 Confirmatory method
A confirmatory technique is not required as method L0096/01 uses two different mass transitions of boscalid for quantitation and confirmation.

A 2.2.2.3.1.3 Extraction efficiency
Soil samples were extracted with 50 mL of methanol/aqueous acetate buffer (80/20, v/v). Extraction efficiency was assessed in detail in study BASF DocID 2015/1109589 (see A 2.2.2.7.2).

A 2.2.2.3.2 Method L0096/02: Determination of residues of M510F47 and M510F49 in soil

A 2.2.2.3.2.1 Method validation

Comments of zRMS:	Analytical method L0096/02 has been successfully validated and fulfils the requirements with regard to specificity, linearity, limit of quantification, recoveries and repeatability for the analysis of boscalid metabolites M510F47 and M510F49 in soil with LOQ of 0.01 mg/kg using HPLC-MS/MS. The average recoveries were within the acceptable range of 70% to 110%. The relative standard deviations for all fortification levels were below 20%. The analytical method fulfils the requirements for residue analytical methods as outlined in SANCO/825/00 rev. 8.1. The study is acceptable.

Reference:	CP 5.2/13
Report	Validation of analytical method for determination of residues of M510F47 and M510F49 in soil, XXX D., 2013 Report No 417346 BASF DocID 2013/1415720 Authority registration No
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010)
Deviations:	No
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)
Acceptability:	Yes

Remark: This method was not used for data generation purposes, but is considered as additional information of the analytical methodology. If required, this method is fully suitable to be used as monitoring method for enforcement purposes. At time of development and validation, the residue method has not been allocated a BASF method number. As unique identifier, the method was allocated the BASF method number L0096/02.

Materials and methods
10 g soil samples are extracted with methanol by shaking for 30 min on a horizontal shaker, followed by centrifugation for 5 min at 3000 rpm. After decantation of the supernatant into a volumetric flask, the extraction is repeated once with methanol/water (1/1, v/v) and the extracts are combined. 5 mL of the combined extract are diluted with 3 mL water including 0.1% formic acid and 4 mM ammonium formate, sonicated, and shaken for homogenization. An aliquot thereof is filtered with a single-use syringe and analyzed by LC-MS/MS using a Phenomenex Prodigy ODS3 column (with guard column) and a gradient mixture of water (0.1% formic acid), 10 mM ammonium formate and methanol (0.1% formic acid). Detection is accomplished in ESI negative mode at mass transitions 156→112 (M510F47) and 323→94 (M510F49) for quantification and 155→112 (M510F47) and 323→202 (M510F49) for confirmation.

Results and discussions
The mean recovery values of the validation experiment were between 70% and 110%. The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed results are given in the table below.

The test items are stable in fortified combined methanol/water extracts of matrix soils for at least 7 days when stored refrigerated (nominally 1 – 10 °C) in the dark. The stability of standard solutions was not

determined as standard solutions and extracts contained the same solvent, except that no matrix was present. Hence, standard stability was confirmed to be at least as long as extract stability (7 days).

Table A 24: Recovery results from method validation of boscalid metabolites M510F47 and M510F49 using analytical method L0096/02

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
soil LUFA 2.2	M510F47	0.01	85	2	Quantitation m/z 156 → 112
		0.10	84	4	
		0.01	83	6	Confirmation m/z 155 → 112
		0.10	86	3	
	M510F49	0.01	88	7	Quantitation m/z 323 → 94
		0.10	85	5	
		0.01	86	6	Confirmation m/z 323 → 202
		0.10	88	4	

Table A 25: Characteristics for the analytical method used for validation of boscalid metabolites M510F47 and M510F49 residues in soil

	M510F47	M510F49
Specificity	LC-MS/MS is a highly specific self-confirmatory technique. Two mass transitions were analyzed for quantitation and confirmation of M510F47 and M510F49. Mass spectra of both analytes are provided in the study report. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of both metabolites.	
Calibration (type, number of data points)	The calculation of concentrations of M510F47 was based on linear regression. A linear correlation coefficient ≥0.999 was obtained. At least eight calibration standards prepared in water including 0.1% formic acid and 4 mM ammonium formate/methanol (40/60; v/v) were used.	The calibration for M510F49 was second order with a correlation coefficient of ≥0.999. At least eight calibration standards prepared in water including 0.1% formic acid and 4 mM ammonium formate/methanol (40/60; v/v) were used.
Calibration range	0.5 ng/mL - 100 ng/mL	
Assessment of matrix effects is presented	Yes. No significant matrix effects occurred.	
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.01 mg/kg for both metabolites, corresponding to the lowest validated concentration level with sufficient recovery and precision. The limit of detection was defined as 30% of the LOQ, which equals 0.003 mg/kg for both metabolites.	

Conclusion
Method L0096/02 for analysis of boscalid metabolites M510F47 (Reg. No. 107371) and M510F49 (Reg. No. 391572) in soil uses LC-MS/MS for final determination. 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 boscalid metabolites M510F47 and M510F49 in soil.

A 2.2.2.3.2.2 Confirmatory method
A confirmatory technique is not required as method L0096/02 uses two different mass transitions of M510F47 and M510F49 for quantitation and confirmation.

A 2.2.2.3.2.3 Extraction efficiency
Soil samples are extracted with methanol. The extraction is repeated with methanol/water (1/1, v/v). The extracts were combined.

No data was generated using this method as the metabolites are not required to be monitored in soil. Furthermore, no data for pre-authorization purposes was generated using this method. The soil residue analytical method to quantify M510F47 and M510F49 is considered as additional information only. Hence, no separate study assessing the extraction efficiency has been conducted.

A 2.2.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

A 2.2.2.4.1 Method L0127/01: Determination of Boscalid residues in surface water and groundwater

A 2.2.2.4.1.1 Method validation

Comments of zRMS:	Analytical method L0127/01 has been successfully validated and fulfils the requirements with regard to specificity, linearity, limit of quantification, recoveries and repeatability for the analysis of BAS 510 F (boscalid) in groundwater and surface water with LOQ of 0.03 µg/kg using LC-MS/MS. The present method L0127/01 is also accepted as confirmatory technique. The average recoveries were within the acceptable range of 70% to 110%. The relative standard deviations for all fortification levels were below 20%. The analytical method fulfils the requirements for residue analytical methods as outlined in SANCO/3029/99 rev. 4. The study is acceptable.

Reference:	CP 5.2/14
Report	Validation of analytical method L0127/1 for the determination of BAS 510 F (Boscalid) residues in surface water and groundwater, XXX H., 2009 Report No 357249 BASF DocID 2008/1086809 Authority registration No
Guideline(s):	EEC 91/414 Annex IIA, EEC 91/414 Annex IIIA, SANCO/3029/99 rev. 4 (11 July 2000)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Comments of zRMS:	Additional information are provided to demonstrate the influence of matrix load on the analysis using quality control samples. Additional information are required for re-registration purposes according to SANCO/825/OO rev 8.1 and SANCO/3029/99 rev. 4. The findings demonstrate that the matrix load in the tested quality control samples had only negligible influence on the analysis of BAS 510 F, therefore the use of matrix-matched standards is not required. Accepted.

Reference:	CP 5.2/15
Report	Report Amendment No. 1 to final report: Validation of analytical method L0127/1 for the determination of BAS 510 F (Boscalid) residues in surface water and groundwater, XXX T., XXX S., 2015 Report No 357249 BASF DocID 2015/1174526 Authority registration No
Guideline(s):	EEC 91/414 Annex IIA, EEC 91/414 Annex IIIA, SANCO/3029/99 rev. 4 (11 July 2000)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Remark: The Amendment issued in 2015 contains additional information on matrix effects and reports the results of the second mass transition. Information from the originally issued report and the amendment are summarised together.

Materials and methods
10 mL of a water sample is extracted on a pre-conditioned C18 SPE column. After drying the column with a stream of N_2 at 30°C for 45 min, the column is prewashed with cyclohexane, which is discarded. Then, boscalid is eluted from the column with 2 x 2.5 mL cyclohexane/ethyl acetate (1/1, v/v). The collected eluates are evaporated to dryness in the evaporator at 40°C water bath temperature. The residues are dissolved in methanol/water (80/20, v/v). Final determination is performed by HPLC-MS/MS using a Betasil C_{18} analytical column and a gradient mixture of water/formic acid (1000/1) and methanol/formic acid (1000/1) at a flow rate of 0.6 mL min^{-1}. Detection is accomplished in ESI positive mode at mass transitions 343→271 and 343→307 for quantification and confirmation.

Results and discussions
The mean recovery values of the validation experiment were between 70% and 110%. The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed results are given in the table below.

The standard stability of boscalid in methanol/water (80/20, v/v) was determined within BASF DocID *2000/1014856* (already EU-peer-reviewed study; refer to chapter 5.2.2), assessing stability of various analytes in solvents, including boscalid, in standard solutions. The standard solutions were stable over a time period of at least 4 weeks when stored under refrigerated conditions at 4 °C in the dark. The standard as well as extract stability was also determined in the method's ILV (BASF DocID 2016/1112645) which is reported below.

Table A 26: Recovery results from method validation of boscalid using analytical method L0127/1

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
groundwater	Boscalid (BAS 510 F)	0.03	89.9	3.8	Quantitation m/z 343 → 271
		0.3	87.6	8.4	
		0.03	90.8	5.6	Confirmation m/z 343 → 307
		0.3	89.6	6.9	
surface water	Boscalid (BAS 510 F)	0.03	90.1	4.5	Quantitation m/z 343 → 271
		0.3	95.2	4.5	
		0.03	86.9	3.2	Confirmation m/z 343 → 307
		0.3	95.1	6.9	

Table A 27: Characteristics for the analytical method used for validation of boscalid residues in groundwater and surface water

	Boscalid
Specificity	HPLC-MS/MS is a highly specific self-confirmatory technique. Two mass transitions were analyzed for quantitation and confirmation of boscalid. A mass spectrum is provided in BASF DocID 2016/1037754. As analytical conditions, such as the eluent, are of comparable composition, the mass spectrum obtained will be the same. The mass spectrum can also be found in the ILV (BASF DocID 2016/1112645). Significant interferences (> 30% of LOQ) were not observed at the retention time of boscalid in the untreated ground- and surface water control samples.
Calibration (type, number of data points)	The calculation of residue concentrations was based on linear regression. A linear correlation coefficient >0.999 was obtained. At least seven calibration standards prepared in methanol/water (80/20, v/v) were used.
Calibration range	0.025 ng mL ^{1} to 0.5 ng mL^{-1}
Assessment of matrix effects is presented	Yes. Matrix effects were tested by comparing solvent-based standard solutions with matrix-matched standards (quality control samples) at a concentration of 0.15 ng mL^{-1}. The findings showed a negligible influence of the matrix on the analysis of boscalid
Limit of determination/quantification	The method has a limit of quantification of 0.03 µg kg^{-1} boscalid in water, corresponding to the lowest validated concentration level with sufficient recovery and precision. The limit of detection (LOD) is 0.025 ng mL^{-1}, which is the lowest calibration standard tested.

Conclusion
The method L0127/01 for analysis of boscalid in groundwater and surface water fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine boscalid in water samples.

A 2.2.2.4.1.2 Independent laboratory validation

Comments of zRMS:	The method L0127/01 (471/0) was successfully independently validated for the determination of boscalid and two of its metabolites M510F47 (Reg.No. 107371) and M510F49 (Reg.No. 391572) in surface water and groundwater with the limit of quantification of 0.03 µg/L using LC-MS/MS with two mass transitions per analyte. The mean recovery values were between 70% and 100% in all matrices tested. The relative standard deviation (RSD) did not exceed 20% at any fortification level for either of the analytes. The study is acceptable.

Reference:	CP 5.2/16
Report	Report Amendment No. 1: ILV of the BASF method L0127 for the

	determination of Boscalid (BAS 510 F) and two of its metabolites M510F47 (Reg.No. 107371) and M510F49 (Reg.No. 391572) in surface water and groundwater, XXX M., 2016 Report No 776664 BASF DocID 2016/1112645 Authority registration No
Guideline(s):	SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010), EPA 850.6100 (2012), EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009
Deviations:	No
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)
Acceptability:	Yes

Remark: The revised final report does not contain any modified data; the report was issues as mere correction of typing and layout errors.

Remark: The objective of the study was to independently validate the determination of boscalid (BAS 510 F) and two of its metabolites M510F47 (Reg. No. 107371) and M510F49 (Reg. No. 391572) with LC-MS/MS in surface water and groundwater. Hence, version L0127/01 and L0127/02 were together independently validated in this study method.

Materials and methods
The study followed the analytical steps of method L0127/01 and L0127/02 for determining boscalid and its two metabolites M510F47 and M510F49, respectively.

Results and discussions
The results of the recovery experiments indicate that the independent laboratory validation was successfully completed. In both matrices tested, the mean recovery values were between 70% and 110%. The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed results are given in the table below.
Standard solutions of boscalid, M510F47 and M510F49 were stable for at least 35 days, when stored at 1 - 10 °C in the dark. Analytes boscalid and M510F49 in extracts prepared in methanol and methanol/water (80/20, v/v) and analyte M510F47 in extracts prepared in pure water were stable for at least 7 days, when stored at 1 - 10 °C.

Table A 28: Recovery results from independent laboratory validation of boscalid and its metabolites M510F47 and M510F49 using analytical method L0127/01 and L0127/02

Matrix	Analyte	Fortification level (~~mg/kg~~ µg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
groundwater	Boscalid (BAS 510 F)	0.03	108	4	Quantitation m/z 343 → 271
		0.3	89	3	
		0.03	108	4	Confirmation m/z 343 → 307
		0.3	89	4	
surface water		0.03	109	7	Quantitation m/z 343 → 271
		0.3	90	5	

Matrix	Analyte	Fortification level (~~mg/kg~~ µg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
		0.03	107	8	Confirmation m/z 343 → 307
		0.3	90	4	
groundwater	M510F47	0.03	101	1	Quantitation m/z 158 → 122
		0.3	96	4	
		0.03	100	1	Confirmation m/z 158 → 94
		0.3	96	5	
surface water		0.03	103	1	Quantitation m/z 158 → 122
		0.3	103	5	
		0.03	102	1	Confirmation m/z 158 → 94
		0.3	100	3	
groundwater	M510F49	0.03	89	6	Quantitation m/z 323 → 202
		0.3	91	4	
		0.03	92	8	Confirmation m/z 323 → 94
		0.3	93	3	
surface water		0.03	89	3	Quantitation m/z 323 → 202
		0.3	94	6	
		0.03	89	3	Confirmation m/z 323 → 94
		0.3	93	4	

Table A 29: Characteristics for the analytical method used for independent laboratory validation of residues of boscalid and its metabolites M510F47 and M510F49 in surface water and groundwater

	Boscalid	M510F47	M510F49
Specificity	HPLC-MS/MS is a highly specific self-confirmatory technique. Two mass transitions were analyzed for quantitation and confirmation of all three analytes. Mass spectra are provided for all analytes in the study report. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of boscalid, M510F47 and M510F49.		
Calibration (type, number of data points)	Good linearity (r > 0.995) was observed for the two mass transitions of boscalid. Six standard solution prepared in methanol/water (80/20, v/v) were used for the calibration.	Six standard concentrations prepared in matrix were used for the calibration. The calibration curves obtained for both ion mass transitions and both matrices were linear with r > 0.995.	Good linearity (r > 0.995) was observed for the two mass transitions of M510F49. Seven standard solution prepared in methanol/water (80/20, v/v) were used for the calibration.
Calibration range	0.025 – 0.50 ng/mL	0.35 – 10.0 ng/mL	0.010 – 1.0 ng/mL
Assessment of matrix effects is presented	Yes. Significant matrix effects (> 20%) on the detection of boscalid were not found.	Yes. Significant matrix effects were observed (mean > 20% for surface water and about 20% for groundwater).	Yes. Significant matrix effects (> 20%) on the detection of M510F49 were not found.
Limit of determination/ quantification	The limit of quantification (LOQ) of the method is 0.03 µg/L in surface and groundwater, resulting from the lowest fortification level successfully tested. The limit of detection was set at 0.005 µg/L, equivalent to a standard concentration of 0.025 ng/mL.	The limit of quantification (LOQ) of the method is 0.03 µg/L in surface and groundwater, resulting from the lowest fortification level successfully tested. The limit of detection was set at 0.009 µg/L, equivalent to a standard concentration of 0.45 ng/mL.	The limit of quantification (LOQ) of the method is 0.03 µg/L in surface and groundwater, resulting from the lowest fortification level successfully tested. The limit of detection was set at 0.009 µg/L, equivalent to a standard concentration of 0.045 ng/mL.

Conclusion

The results of the independent laboratory validation confirm the results of two validation studies (BASF DocIDs 2008/1086809 and 2015/1109588) and demonstrate that methods L0127/01 and L0127/02 are applicable to correctly determine residues of boscalid and its metabolites M510F47 and M510F49 in surface water and groundwater with a LOQ of 0.03 µg/L.

A 2.2.2.4.1.3 Confirmatory method

A confirmatory technique is not required as methods L0127/01 and L0127/02 uses two different mass transitions of boscalid for quantitation and confirmation.

A 2.2.2.4.1.4 Extraction efficiency

Water samples are extracted on a pre-conditioned C18 SPE column. After drying the column with a stream of N_2 at 30°C for 45 min, the column is prewashed with cyclohexane, which is discarded. Boscalid is eluted from the column with cyclohexane/ethyl acetate (1/1, v/v).
Extraction efficiency is not assessed as residues are dissolved in the matrix and do not form any kind of aged or incurred residues as they can be found in e.g. soil. Hence, fully suitability of the method has been shown by satisfactory recoveries during the validation experiments.

A 2.2.2.4.2 Method L0127/02: Determination of M510F47 (Reg.No. 107371) and M510F49 (Reg.No. 391572) in surface water and groundwater

A 2.2.2.4.2.1 Method validation

Comments of zRMS:	Analytical method L0127/02 has been successfully validated and fulfils the requirements with regard to specificity, linearity, limit of quantification, recoveries and repeatability for the analysis of boscalid metabolites M510F47 (Reg. No. 107371) and M510F49 (Reg. No. 391572) in groundwater and surface water with LOQ of 0.03 µg/L using LC-MS/MS. The present method L0127/02 is also accepted as confirmatory technique. The average recoveries were within the acceptable range of 70% to 110%. The relative standard deviations for all fortification levels were below 20%. The analytical method fulfils the requirements for residue analytical methods as outlined in SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1. The study is acceptable.

Reference:	CP 5.2/17
Report	Validation of analytical method L0127/02 for the determination of M510F47 (Reg.No. 107371) and M510F49 (Reg.No. 391572) in surface and groundwater, XXX T., XXX S., 2015 Report No 770846 BASF DocID 2015/1109588 Authority registration No
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.6100, OECD-ENV/JM/MONO/(2007)17 (OECD No. 39), 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

The enrichment and clean-up of both analytes is accomplished using solid-phase extraction (SPE). For analysis of M510F47, a 50 mL water sample is concentrated using OASIS HLB SPE-cartridges. The analyte is eluted from the column using methanol, with the eluate being evaporated to dryness and re-constituted in 1 mL pure water. Prior to sample concentration over the SPE-column, the natural water sample needs to be acidified to a pH $\leq$ 2 by addition of 6 M HCl. For analysis of M510F49, a 10 mL water sample is concentrated using Octadecyl (C_{18}) Bakerbond™ SPE columns. The analyte is eluted from the column using methanol. The eluate is evaporated to dryness and re-constituted in 2 mL of a mixture of methanol/pure water (80/20, v/v). Analysis is accomplished by LC-MS/MS using a XSelect HSST3 column (M510F47) or Betasil C_{18} column (M510F49) using a gradient mixture of water (0.1% formic acid) and methanol (0.1% formic acid). Detection is accomplished in ESI positive mode for M510F47 and in ESI negative mode for M510F49 at mass transitions 158→122 (M510F47) and 323→202 (M510F49) for

quantification and 158→94 (M510F47) and 323→94 (M510F49) for confirmation.

Results and discussions

The mean recovery values of the validation experiment were between 70% and 110%. The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed results are given in the table below.

The storage stability of standard solutions of M510F47 (prepared in pure water) and M510F49 (prepared in methanol/water (80/20, v/v)), was investigated. The results demonstrate stability of both analytes for a maximum duration of 29 days when stored refrigerated at 4 ± 2 °C in the dark. Analytes M510F47 and M510F49 were considered stable in methanol extracts of surface and groundwater samples as well as in the final extracts of surface and groundwater samples over a time period of 7 days, when stored refrigerated at 4 ± 2 °C in the dark. M510F47 extracts were prepared in pure water and M510F49 extracts were prepared in methanol/pure water (80/20, v/v). An independent laboratory validation has been successfully conducted and is reported above (BASF DocID 2016/1112645).

Table A 30: Recovery results from method validation of boscalid metabolites M510F47 and M510F49 using analytical method L0127/2

Matrix	Analyte	Fortification level (~~mg/kg~~ µg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
groundwater	M510F47	0.03	88.3	2.8	Quantitation m/z 158 → 122
		0.3	89.1	2.7	
		0.03	89.6	1.7	Confirmation m/z 158 → 94
		0.3	88.1	1.7	
surface water		0.03	94.0	2.1	Quantitation m/z 158 → 122
		0.3	89.1	4.4	
		0.03	90.8	6.0	Confirmation m/z 158 → 94
		0.3	86.9	3.0	
groundwater	M510F49	0.03	78.1	4.8	Quantitation m/z 323 → 202
		0.3	73.6	9.1	
		0.03	77.6	6.9	Confirmation m/z 323 → 94
		0.3	73.0	9.6	
surface water		0.03	79.3	3.8	Quantitation m/z 323 → 202
		0.3	77.7	4.4	
		0.03	79.9	3.7	Confirmation m/z 323 → 94
		0.3	78.3	5.1	

Table A 31: Characteristics for the analytical method used for validation of M510F47 and M510F49 residues in groundwater and surface water

	M510F47	M510F49
Specificity	HPLC-MS/MS is a highly specific self-confirmatory technique. Two mass transitions of M510F47 and M510F49 were analyzed for quantitation and confirmation. Mass spectra of both analytes are provided in the study report. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of both analytes.	
Calibration (type, number of data points)	Good linearity of r > 0.9989 was observed in the standard range tested. At least seven matrix-matched standards were used for the calibration.	Good linearity of r > 0.998 was observed in the standard range tested. Standards were prepared in methanol/pure water (80/20, v/v). At least seven calibration points were used.
Calibration range	0.35 ng/mL - 10 ng/mL	0.01 ng/mL - 1.0 ng/mL
Assessment of matrix effects is presented	Yes. The results obtained from surface water samples showed deviations greater than 20%, indicating a matrix effect.	Yes. Significant matrix effects (differences >20%) were not observed.
Limit of determination/quantification	The method has a limit of quantification of 0.03 µg/L M510F47 in water, corresponding to the lowest validated concentration level. The limit of detection (LOD) is 0.007 µg/L, which is the lowest calibration standard tested.	The method has a limit of quantification of 0.03 µg/L M510F49 in water, corresponding to the lowest validated concentration level. The limit of detection (LOD) is 0.002 µg/L, which is the lowest calibration standard tested.

Conclusion
The method L0127/02 for analysis of boscalid metabolites M510F47 and M510F49 in groundwater and surface water fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine the two boscalid metabolites in water samples.

A 2.2.2.4.2.2 Independent laboratory validation

The independent laboratory validation study is described above (A 2.2.2.4.1.2) together with analytical method L0127/01 for determination of boscalid in water samples.

A 2.2.2.4.2.3 Confirmatory method

A confirmatory technique is not required as method L0127/02 uses two different mass transitions of M510F47 and M510F49 for quantitation and confirmation.

A 2.2.2.4.2.4 Extraction efficiency

Water samples are extracted with solid-phase extraction (SPE). For analysis of M510F47 a OASIS HLB SPE-cartridge is used; for analysis of M510F49 a Octadecyl (C18) Bakerbond™ SPE column is used. The analytes are eluted from the column using methanol.
Extraction efficiency is not assessed as residues are dissolved in the matrix and do not form any kind of aged or incurred residues as they can be found in e.g. soil. Hence, fully suitability of the method has been shown by satisfactory recoveries during the validation experiments.

A 2.2.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

A 2.2.2.5.1 Method L0336/01: Determination of boscalid in air

A 2.2.2.5.1.1 Method validation

Comments of zRMS:	Analytical method L0336/01 has been successfully validated and fulfils the requirements with regard to specificity, linearity, limit of quantification, recoveries and repeatability for the analysis of boscalid in air with LOQ of 0.0012 µg/m³ using HPLC-MS/MS. The present method L0336/01 is also accepted as confirmatory technique. The average recoveries were within the acceptable range of 70% to 110%. The relative standard deviations for all fortification levels were below 10%. The analytical method fulfils the requirements for residue analytical methods as outlined in SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1. The study is acceptable.

Reference:	CP 5.2/18
Report	Validation of analytical method L0336/01: Determination of BAS 510 F (Boscalid) in Air, XXX M., 2016 Report No 799838 BASF DocID 2016/1037754 Authority registration No
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 860.1340 (1996)
Deviations:	No
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)
Acceptability:	Yes

Materials and methods

The analyte boscalid is spiked onto the front filter of an adsorbent tube. The tubes are then placed in an acclimatized chamber without light and air is sucked with a flow rate of 1 L min^{-1} for 8 hours through the tubes at 35 ± 2°C and a relative humidity of ≥ 80%. After sucking air through the tube, the test item is extracted from the adsorbent material with acetone. The determination of boscalid is performed by LC-MS/MS using a Phenomenex Luna 5µ C_{18} column and applying a water/methanol gradient with formic acid as modifier. Detection is accomplished in ESI positive mode at mass transitions 343→307 for quantification and 343→140 for confirmation.

Results and discussions

The mean recovery values of the validation experiment were between 70% and 110%. The relative standard deviations (RSD, %) for all fortification levels were below 10%. The detailed results are given in the table below. Under the sampling conditions described, no breakthrough of boscalid to the rear end of the bed of

the tubes was observed. Breakthrough must therefore be greater than a concentration of 833 µg/m³ of boscalid in air at a flow rate of 480 L within 8 hours.
The calibration solutions of boscalid in acetone/water (1 /1, v/v) were stable up to 16 days of storage in a refrigerator (between 95% and 103% of freshly diluted solution). The stability of boscalid in tubes and extracts was assessed over a period of 9 days. Storage of tubes (at ambient temperature, in a refrigerator and in a freezer) and extracts (in a refrigerator and in a freezer) was possible for up to 9 days without any significant loss of analyte.

Table A 32: Recovery results from method validation of boscalid using analytical method L0336/01

Matrix	Analyte	Fortification level (µg/m³) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
air	Boscalid (BAS 510 F)	0.0012	94	7	Quantitation m/z 343 → 307
		0.012	79	3	
		0.0012	96	5	Confirmation m/z 343 → 140
		0.012	80	3	

Table A 33: Characteristics for the analytical method used for validation of boscalid in air

	Boscalid
Specificity	HPLC-MS/MS is a highly specific, self-confirmatory technique. Two different mass transitions of boscalid were analyzed for quantitation and confirmation. Mass spectra of boscalid are provided in the study report. The retention times of the test item in extracts matched the retention times in solvent. No peak interferences occurred at the retention times of BAS 510 F.
Calibration (type, number of data points)	The detector response for both ion transitions of boscalid was linear within the used calibration range from 0.015 ng/ml to 5.0 ng/ml with r > 0.998. Standards were prepared in acetone/water (1/1, v/v). 9 or 10 calibration points were used.
Calibration range	A calibration range from 0.015 ng/ml to 5.0 ng/ml was used. This range corresponds to a fortification level of 0.0003125 µg/m³ to 0.1042 µg/m³ and thus covers the range from no more than 30% of the LOQ and at least + 20% of the highest analyte concentration detected in a sample.
Assessment of matrix effects is presented	Yes. Calibration standard solutions were compared against their respective calibration standards prepared in blank matrix extracts/water (1/1, v/v). No significant matrix effects were observed (< 20%).
Limit of determination/quantification	The method has a limit of quantification of 0.0012 µg/m³ in air, corresponding to the lowest validated concentration level. The limit of detection (LOD) in air is 0.00036 µg/m³, corresponding to a concentration of 0.0346 ng/mL in the extract.

Conclusion

It could be demonstrated that method L0336/01 fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine boscalid in air.

A 2.2.2.5.1.2 Confirmatory method

A confirmatory technique is not required as method L0336/01 uses two different mass transitions of boscalid for quantitation and confirmation.

A 2.2.2.5.1.3 Extraction efficiency

Boscalid in air samples is extracted from air with the help of adsorbent tubes (ORBO-402 Tenax tubes). Air is passed through the filter material of the tubes for 8 hours. Then boscalid is extracted from the adsorbent material with acetone. Extraction efficiency is not assessed as fully removal of fresh and stored sampling devises has been shown by the fortification and extraction experiments.

A 2.2.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

A 2.2.2.6.1 Analytical method L0342/01 for the determination of BAS 510 F (boscalid) and its metabolite M510F01 in body fluids

A 2.2.2.6.1.1 Method validation

Comments of zRMS:	The analytical method L0342/01 ((based on multi-residue method QuEChERS) has been satisfactorily validated for the determination of residues of BAS 510 F (Boscalid) and its metabolite M510F01 in body fluids (blood and urine) using LC-MS/MS for quantitation and confirmation in accordance with the guidance documents SANCO/825/00, rev. 8.1 and SANCO/3029/99, rev. 4 with a LOQ of 0.01 mg/L. The LOD was set at 0.003 mg/L. the mean recovery values were between 70% and 110% of the nominal value for both mass transitions for each analyte in all matrices tested. The relative standard deviations (RSD, %) for all fortification levels were below 5%. The study is acceptable.

Reference:	CP 5.2/19
Report	Validation of BASF analytical method L0342/01 for the determination of BAS 510 F (Boscalid) and its metabolite M510F01 in body fluids, XXX S., XXX S., 2016 Report No 798888 BASF DocID 2016/1193046 Authority registration No
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07, EPA 860.1340 (1996)
Deviations:	No
GLP:	yes (certified by Freie und Hansestadt Hamburg, Behoerde fuer Gesundheit und Verbraucherschutz, Hamburg, Germany)
Acceptability:	Yes

Materials and methods

BASF analytical method L0342/01 based on multi-residue method QuEChERS. Blood (bovine) and urine (porcine) specimens are thoroughly homogenized by stirring. Blood is stabilized with 5 g/L Na-EDTA. 10 mL aliquots of the homogenized samples are extracted with acetonitrile and if necessary, after addition of water (blood samples). A salt mixture containing magnesium sulphate, sodium chloride and sodium citrate is added and the extract is shaken. After centrifugation an aliquot of the acetonitrile phase is cleaned by adding primary secondary amine (PSA). The final determination of BAS 510 F and M510F01 is performed by HPLC-MS/MS using a 1200 Binary Rapid Resolution LC System equipped with an API 5000 detector system, and applying a methanol/water gradient with 0.1% formic acid as modifier. The analytical column used is a Nucleosil 100-5 C_{18} column. Detection is accomplished by electrospray ionization in positive mode at two mass transitions for each analyte. The results are calculated by direct comparison of the sample peak responses to those of external standards.

Results and discussions

Blood and urine samples are fortified with the analytes at the limit of quantification of 0.01 mg/L and 10 times higher (0.1 mg/L). Mean recovery values (mean of five replicates per fortification level and analyte) are between 86% and 103% for BAS 510 F and between 89% and 97% for M510F01 (see table below). The relative standard deviations (RSD, %) for all fortification levels are below 10%.

Table A 34: Recovery results from method validation of boscalid and its metabolite M510F01 using the analytical method L0342/01

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Blood	Boscalid (BAS 510 F)	0.01 (n=5)	103	2.7	Quantitation m/z 343→307
		0.1 (n=5)	100	1.8	
		0.01 (n=5)	101	6.4	Confirmation m/z 343→271
		0.1 (n=5)	100	1.7	
	M510F01	0.01 (n=5)	97	5.5	Quantitation m/z 359→323
		0.1 (n=5)	96	1.7	
		0.01 (n=5)	97	4.1	Confirmation m/z 359→140
		0.1 (n=5)	95	1.1	
Urine	Boscalid (BAS 510 F)	0.01 (n=5)	90	3.0	Quantitation m/z 343→307
		0.1 (n=5)	91	2.6	
		0.01 (n=5)	86	5.6	Confirmation m/z 343→271
		0.1 (n=5)	88	2.8	
	M510F01	0.01 (n=5)	89	3.2	Quantitation m/z 359→323
		0.1 (n=5)	93	1.9	
		0.01 (n=5)	90	2.4	Confirmation m/z 359→140
		0.1 (n=5)	92	2.2	

Table A 35: Characteristics for the analytical method used for validation of boscalid and M510F01 residues in body fluids

	Boscalid and M510F01
Specificity	No significant interferences above 30% of the LOQ were detected in any of the control specimen extracts of each matrix (blood, urine), so that a high level of selectivity was demonstrated. As LC-MS/MS is regarded as a highly-specific detection method when two ion transitions have been validated, an additional confirmatory technique is not necessary.
Calibration (type, number of data points)	Calibration standards were prepared in acetonitrile / 0.1% formic acid (1:1, v/v). Ten calibration points were used and individual calibration data was presented. Linear correlations with coefficients (R^2) >0.99 were obtained for boscalid and its metabolite M510F01.
Calibration range	Calibration points distributed over a concentration range of 0.15 to 50 ng/mL were used (corresponding to approximately 0.003 mg/L to 1.0 mg/L in blood and urine).
Assessment of matrix effects is presented	The effect of matrix on the LC-MS/MS response was assessed by comparing peak areas of matrix-matched standards (90% matrix amount) with solvent standards at equivalent concentrations. Matrix effects on the detection of BAS 510 F and its metabolite M510F01 in extracts of blood and urine were found to be insignificant (<20%).
Limit of determination/quantification	The limit of quantification (LOQ) is the lowest validated fortification level for BAS 510 F and its metabolite M510F01 and was thus successfully established at 0.01 mg/L in blood and urine matrices for the two mass transitions monitored. The limit of detection (LOD) was set at 0.003 mg/L for all matrices, which is 30% of the LOQ.
Stability of standard and stock solutions	Analytical standards of BAS 510 F and its metabolite M510F01 prepared in acetonitrile / 0.1% formic acid (1:1, v/v) were found to be stable for at least 13 days when stored refrigerated (1 - 10 °C) in the dark. The stability values for BAS 510 F and M510F01 were 81% to 95% and 84% to 98% for both mass transitions in solvent standards, respectively. Both analytes were found to be stable in stock solutions when stored refrigerated (1 - 10 °C) in the dark: BAS 510 F is stable for at least 164 days when prepared in acetonitrile; M510F01 is stable for at least 259 days when prepared in methanol.
Extract Stability	BAS 510 F and its metabolite M510F01 were found to be stable in the final extracts and the raw extracts of both animal matrices (blood and urine) for at least 16 days when stored refrigerated (1 - 10 °C) in the dark. The stability values for BAS 510 F were between 101% and 115% (final extracts) and 93% and 109% (raw extracts) for both mass transitions and matrices. The stability values for M510F01 were between 96% and 118% (final extracts) and 101% and 115% (raw extracts) for both mass transitions and matrices.

Conclusion

BASF analytical method L0342/01 for analysis of BAS 510 F (boscalid) and its metabolite M510F01 in animal matrices (urine, blood) uses LC-MS/MS for final determination, with a limit of quantification of 0.01 mg/L. It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification, recoveries and linearity and is therefore applicable to correctly determine residues of BAS 510 F and its metabolite M510F01 in the animal matrices urine and blood.

A 2.2.2.6.1.2 Confirmatory method

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

A 2.2.2.6.1.3 Extraction efficiency

The extraction efficiency of QuEChERS methods is confirmed by a vast number of validated methods available on the EURL (European Reference Laboratories for Residues of Pesticides) Data Pool.

A 2.2.2.7 Other Studies/ Information

A 2.2.2.7.1 Standard stability of BAS 510 F in methanol/acetate buffer solution

Comments of zRMS:	The standard stability of boscalid in methanol/acetate buffer solution (80/20, v/v) at concentration levels of 0.1 µg/mL and 1.0 ng/mL was determined. The standard solutions were stable over a time period of at least 30 days when stored under refrigerated conditions at 4°C in the dark. The study is accceptable.

Reference:	CP 5.2/20
Report	Standard stability of BAS 510 F in methanol / acetate buffer solution, XXX H., 2010 Report No 546429_2 BASF DocID 2010/1046613 Authority registration No
Guideline(s):	not cited (information on storage stability assessed as required according to SANCO/825/00 rev. 8.1 16/11/2010)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Materials and methods

Standard solutions were prepared according to BASF method L0096/01 (see appendix section A 2.2.2.3.1). Briefly, a stock solution of 1 mg L^{-1} boscalid in methanol was prepared. The stock solution was then further diluted with methanol/acetate buffer solution (80/20, v/v; pH 4.65). The stability of boscalid was tested in standard solutions of 0.1 µg mL^{-1} and 1.0 ng mL^{-1}. Standard solutions were stored at 4°C in the dark and measured at day 0 and after 7, 14 and 30 days against a calibration curve of freshly prepared standards.
The standard solutions in methanol/acetate buffer solution (80/20, v/v; pH 4.65) were analyzed for boscalid with BASF method L0096/01. No extraction was necessary, standard solutions were only diluted and analyzed by HPLC-MS/MS.

Results and discussions

Concentrations of boscalid after 0, 7, 14 and 30 days of storage are given in Table A 36 and Table A 37 for the solutions of 0.1 µg mL^{-1} and 1.0 ng mL^{-1}, respectively. Quantification was done for both mass transitions of boscalid validated in BASF method L0096/01.
Recoveries at both concentration levels at all time points ranged between 93% and 102% of applied. For evaluation of the stability test of boscalid, an analysis was performed assuming first order degradation kinetics (details given in study report). Degradation was found to be 2.5% and -5% of applied after 30 days for 0.1 µg mL^{-1} and 1.0 ng mL^{-1}, respectively. This is within the analytical uncertainty of the analytical method and cannot be assigned to degradation.

Table A 36: Standard Stability of boscalid in methanol/acetate buffer (80/20, v/v; pH 4.65) at 0.1 µg mL^{-1} after storage at 4°C in the dark

Days of storage	Transition *m/z* 343 → 307		Transition *m/z* 343 → 271	
	[µg mL^{-1}]	Recovery of 0.1 µg mL [%]	[µg mL^{-1}]	Recovery of 0.1 µg mL^{-1}
0	0.104		0.099	
	0.099		0.088	
	0.102	**101.7**	**0.093**	**93.4**
7	0.095		0.103	
	0.097		0.097	
	0.096	**96.2**	**0.100**	**100.2**
14	0.095		0.094	
	0.095		0.094	
	0.095	**95.0**	**0.094**	**93.8**
30	0.099		0.098	
	0.097		0.095	
	0.098	**97.9**	**0.097**	**96.7**

Table A 37: Standard Stability of boscalid in methanol/acetate buffer (80/20, v/v; pH 4.65) at 1.0 ng mL^{-1} after storage at 4°C in the dark

Days of storage	Transition *m/z* 343 → 307		Transition *m/z* 343 → 271	
	[ng mL^{-1}]	Recovery of 1.0 ng mL^{-1} [%]	[ng mL^{-1}]	Recovery of 1.0 ng mL^{-1}
0	0.884		0.984	
	0.978		1.040	
	0.931	**93.1**	**1.012**	**101.2**
7	1.010		1.000	
	0.873		0.995	
	0.942	**94.2**	**0.998**	**99.8**
14	0.941		0.935	
	0.928		0.914	
	0.935	**93.5**	**0.925**	**92.5**
30	0.992		1.000	
	0.966		0.942	
	0.979	**97.9**	**0.971**	**97.1**

Conclusion

Stability of boscalid at 0.1 µg mL^{-1} and 1.0 ng mL^{-1} in methanol/acetate buffer solution (80/20, v/v; pH 4.65) is given at 4°C in the dark for at least 30 days.

A 2.2.2.7.2 Comparative analysis of extraction procedures on boscalid originating from a field accumulation and dissipation study in Northern Italy

Comments of zRMS:	The results of the residue analytical method L0096/01 (extraction procedure 1 - set to 100% reference) showed good comparability with the extraction efficiencies of the aerobic and anaerobic soil metabolism studies (extraction procedures 2, 3 and 4). The results ranged from 95.2% to 100.8% for field sample L1503380002 and from 101.5% to 103.2% for field sample L1503380003. Overall, the results for BAS 510 F (Reg. No. 300355) obtained with the 3 different extraction schemes of the different soil metabolism studies ranged from 95.2% to 103.2%. Additional Information on Residue Analytical Method L0096/01: The extract stability was not determined within the validation study of XXX M., 2009 – „*Validation of analytical method L0096/01: Determination of Boscalid Reg. No. 300355 in soil using HPLC/MS-MS*" (2008/1084832), but it was assessed in DocID 2015/1109589. Soil extracts are stable samples over a time period of 6 weeks, when stored refrigerated at 4°C in methanol/acetate buffer pH 4.65 (80/20, v/v). The study is acceptable.

Reference:	CP 5.2/21
Report	Comparative analysis of extraction procedures on Boscalid (BAS 510 F) originating from a field accumulation and dissipation study in Northern Italy, XXX T., XXX S., 2015 Report No 772971 BASF DocID 2015/1109589 Authority registration No
Guideline(s):	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

The extraction efficiency of residue analytical method L0096/01 using methanol/acetate buffer pH 4.65 (80/20, v/v) as extraction solvent was compared with the extraction procedures of two aerobic and one anaerobic soil metabolism studies applying sequential extraction schemes using methanol and a mixture of methanol/pure water (50/50, v/v) at different ratios of soil to extraction solvent. Selected field soil samples generated during a field accumulation and dissipation study [BASF DocID 2015/1178191] performed with boscalid (BAS 510 F) were extracted with each of the four extraction procedures. The analysis of boscalid in the different soil extracts obtained by applying the four different extraction procedures was accomplished by LC-MS/MS, based on the principles described in residue analytical method L0096/01. The results of residue analytical method L0096/01 (set to 100% reference) were compared to the extraction procedures used in the aerobic and anaerobic soil metabolism studies.

Extraction Procedure 1: Extraction Procedure of Residue Analytical Method L0096/01 [*BASF DocID 2008/1084832 and 2015/1174527*]

5 g of a wet soil sample were extracted with 50 mL of a mixture of methanol and acetate buffer pH 4.65 (80/20, v/v). The sample was shaken for 60 min at 225 rpm. Phase separation was accomplished by centrifugation for 5 min at 4000 rpm and 20°C. A final volume of 50 mL of the decanted supernatant, which corresponds to soil control matrix in methanol/acetate buffer pH 4.65 (80/20, v/v) with boscalid, was adequate for residue levels between 0.01 mg kg^{-1} (limit of quantification; LOQ) to 0.05 mg kg^{-1} (5xLOQ). Soil extracts were diluted with appropriate amounts of methanol/acetate buffer pH 4.65 (80/20, v/v) at higher residue levels.

Extraction Procedure 2: based on Aerobic Soil Metabolism Study [*BASF DocID 1999/11807*]

5 g of a wet soil sample were sequentially extracted 3 times with 9 mL pure methanol, followed by 3 times 9 mL methanol/pure water (50/50, v/v). The sample was shaken for 30 min at 225 rpm. After each extraction, the samples were centrifuged; the methanol extracts were centrifuged for 5 min at 3000 rpm and 20°C and methanol/pure water extracts were centrifuged for 10 min at 3000 rpm and 20°C. All individual extracts were combined in a 100 mL flask by filtration over cotton wool and filled up to the mark with methanol/pure water (75/25, v/v). Prior to quantitative determination by LC-MS/MS, an aliquot of 5 mL was taken and centrifuged for 5 min at 4000 rpm and 20°C. Further dilutions were made as appropriate.

Extraction Procedure 3: based on Anaerobic Soil Metabolism Study [*BASF DocID 2000/1014990*]

10 g of a wet soil sample was suspended with 5 mL pure H_2O, then 6 mL pure methanol were added. The samples were shaken for 40 min at 225 rpm. Phase separation was accomplished by centrifugation for 10 min at 3000 rpm and 20°C. Then, the soil remnant was further sequentially extracted twice with 10 mL pure methanol and then 3 times with 10 mL of a mixture of methanol/pure water (50/50, v/v). Phase separation was accomplished as described above. All individual extracts were combined in a 100 mL flask by filtration over cotton wool and filled up to the mark with methanol/pure water (75/25, v/v). Prior to quantitative determination by LC-MS/MS, an aliquot of 5 mL was taken and centrifuged for 5 min at 4000 rpm and 20°C. Further dilutions were made as appropriate.

Extraction Procedure 4: based on Aerobic Soil Metabolism Study [*BASF DocID 2002/5002772*]

5 g of a wet soil sample was sequentially extracted 3 times with 12.5 mL pure methanol, followed by 3 times 12.5 mL methanol/pure water (50/50 v/v). Extraction was accomplished by shaking for 45 min at 225 rpm. After each extraction, the samples were centrifuged; the methanol extracts were centrifuged for 5 min at 3000 rpm and 20°C and the methanol/pure water (50/50, v/v) extracts were centrifuged for 10 min at 3000 rpm and 20°C. All individual extracts were combined in a 100 mL flask by filtration over cotton wool and filled up to the mark with methanol/pure water (75/25, v/v). Prior to quantitative determination by LC-MS/MS, an aliquot of 5 mL was taken and centrifuged for 5 min at 4000 rpm and 20°C. Further dilutions were made as appropriate.

Results and discussions

The results of the residue analytical method L0096/01 (extraction procedure 1 - set as 100% reference) showed good comparability with the extraction efficiencies of the aerobic and anaerobic soil metabolism studies (extraction procedures 2, 3 and 4). The results ranged from 95.2% to 100.8% for field sample

L1503380002 and from 101.5% to 103.2% for field sample L1503380003. A summary of the residue results of the extraction procedures is given in Table A 38.

Table A 38: Summary of residue results of boscalid

Sample No.	Extraction procedure	residues of boscalid [mg/kg]	% of residue analytical method L0096/01
L1503380002	1	2.00	
	2	2.01	100.8
	3	1.90	95.2
	4	1.97	98.8
L1503380003	1	1.58	
	2	1.63	103.2
	3	1.62	102.3
	4	1.61	101.5

The stability of boscalid in field soil extracts, prepared according to residue analytical method L0096/01, was investigated. Boscalid was stable in the final extracts of fortified and treated field soil samples over a time period of 6 weeks, when stored refrigerated at 4°C in methanol/acetate buffer pH 4.65 (80/20, v/v). Extract stability was conducted to obtain additional information for the validation of method L0096/01.

Matrix effects were assessed for all extraction solvents used in addition to the one of the residue analytical method already evaluated during validation of method L0096/01, by comparison of solvent standards prepared in methanol/acetate buffer pH 4.65 (80/20, v/v) against standards prepared in soil control matrix according to residue analytical method L0096/01. The matrix load in the tested soil samples had negligible influence on the analysis of boscalid; therefore, no matrix matched standards are needed.

Conclusion

The results of the reference residue analytical method L0096/01 (set as 100% reference) showed good comparability with the extraction efficiencies of the aerobic and anaerobic soil metabolism studies. Overall, the results for boscalid ranged from 95.2% to 103.2%, hence the proposed residue analytical method removes incurred residue to a satisfactory high extent.