FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: BAS 768 00 F

Product name(s): Revytur

Chemical active substance(s):

Mefentrifluconazole, 25 g/L
Sulfur, 600 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: BASF

Submission date: March 2023

MS Finalisation date: 11/12/2023

Version history

When	What
03/2023	Initial dRR – BASF DocID 2023/2009308
04/2023	Dossier sent for evaluation
08/2023	zRMS evaluation of dRR
12/2023	Final version prepared by zRMS after Commenting period

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5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are ~~not~~ available for the active substance(s) and relevant impurities in the plant protection product.
Noticed data gaps are:

- none

The applicant's dRR was not rewritten by the zRMS. In the resulted RR comments/corrections/addons were placed on the grey background.
Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.
The analytical methods developed for mefentrifluconazole (BAS 750 F) and TDMs in plant and animal matrices were already submitted and evaluated in context of the previous process of Annex I Inclusion of mefentrifluconazole and TDMs EU evaluations. A new enforcement water method with its ILV was additionally submitted in the present application. A new method for body fluids was also submitted.
Since no MRLs are set, analytical methods for the determination of Sulphur residues in plants are not required.
In the context of the REVYTUR submission noticed data gaps are: none.

Commodity/crop	Supported/ Not supported
Cereals	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

The analytical method AFL1047/01 has been developed for the determination of the active ingredients Mefentrifluconazole (Reg.No.:5834378) and Sulfur (Reg.No.:240586) in BAS 768 00 F SC-formulation and in aqueous suspensions of BAS 768 00 F SC-formulation by HPLC.
The results of the validation report include data to confirm the linearity, the specificity, the identity, the precision, the accuracy and the stability of the analytical method AFL1047/01.
These investigations have shown that the analytical conditions employed in the respective method (AFL1047/01) are suitable for the quantification of Mefentrifluconazole (Reg.No.:5834378) and Sulfur (Reg.No.:240586) in BAS 768 00 F SC-formulation and in aqueous suspensions of BAS 768 00 F.

Reference:	CP 5.1.1/1
Report	Analytical method AFL1047/01: Quantitative Determination of the Active Ingredients Mefentrifluconazole and Sulfur in BAS 768 00 F and Aqueous Solutions of BAS 768 00 F by HPLC, Nemitz, A., 2021

	Report No
	BASF DocID 2021/2021957
	Authority registration No
Guideline(s):	no guidelines available
Deviations:	No
GLP:	No, not subject of GLP regulations
Acceptability:	Yes

Comments of zRMS:	Accepted

Materials and methods

The determination of the contents of the active ingredients Mefentrifluconazole (Reg.No.:5834378) and Sulfur (Reg.No.:240586) in BAS 768 00 F SC-formulation and in aqueous suspensions of BAS 768 00 F was performed by HPLC separation with UV-detection. The amounts of the active ingredients were calculated using external calibrations with authentic reference items by applying bracketing. The identity of the test item was confirmed by comparing retention times and the UV-spectra of the test item and the reference items.

Column	Nucleosil 100-5 C18 (4.6 mm x 150 mm; 5.0 µm) or equivalent
Column Temperature	40°C
Injection Volume	5 µL
Detection Wavelength	232 nm (Reg.No.:5834378) 263 nm (Reg.No.:240586)
Flow Rate	1 mL/min
Eluent	A: 500 mL Methanol + 500 mL Water B: 950 mL Methanol + 50 mL Wate
Gradient	Time [min] A [%] B [%] --- 0.00 50 50 5.00 0 100 10.00 0 100 10.10 50 50 15.00 50 50
Approx. Retention Times	4.8 min. Mefentrifluconazole (Reg.No.:5834378) 9.6 min. Sulfur (Reg.No.:240586)
Run Time	15 min.

Reference:	CP 5.1.1/2
Report	Validation of the Analytical Method AFL1047/01: Quantitative Determination of the Active Ingredients Mefentrifluconazole and Sulfur in BAS 768 00 F and Aqueous Solutions of BAS 768 00 F by HPLC, Nemitz, A., 2021 Report No 871257_1

BASF DocID 2021/2021956

Authority registration No

Guideline(s): 2004/10/EC, EC 1107/2009, US EPA OPPTS Harmonized Test Guideline 830.1000, US EPA OPPTS Harmonized Test Guideline 830.1800, SANCO/3030/99 rev. 5 (22 March 2019), SANCO 3029/99 Rev.4

Deviations: No

GLP: Yes, (certified by Landesamt fuer Umwelt, Mainz, Germany)

Acceptability: Yes

Comments of zRMS:	Accepted

Validation - Results and discussions

Table 5.2- 1: Methods suitable for the determination of active substances Mefentrifluconazole and sulphur in plant protection product BAS 768 00 F

	Mefentrifluconazole	**Sulphur**
Author(s), year	Nemitz A. ; 2021	
Principle of method	HPLC with UV-detection	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r) n=5	Range: 6.648 – 30.47 mg/mL (40 – 182% of nominal value) R: 1.0000 Slope: 0.2052 Intercept: 0.0118	Range: 160.4 – 726.7 mg/mL (40 – 181% of nominal value) R: 1.0000 Slope: 0.1130 Intercept: -0.0245
Precision – Repeatability Mean n = 5 (%RSD)	Mean: 1.933% w/w RSD: 0.64% Horrat value: 0.26	Mean: 45.59% w/w RSD: 0.81% Horrat value: 0.54
Accuracy (calculated via total recovery) n = 3 (% Recovery)	49.5% level (≙ 8.3 mg/mL): Recovery range: 100.9 – 101.7% Mean: 101.2% %RSD: 0.43% Recovery limit: 97 – 103 % 99.5% level (≙ 16.6 mg/mL): Recovery range: 100.2 – 100.7% Mean: 100.4% %RSD: 0.28% Recovery limit: 97 – 103 % 151.4% level (≙ 25.3 mg/mL): Recovery range: 100.0 – 100.8% Mean: 100.4% %RSD: 0.39% Recovery limit: 97 – 103 %	49.5% level (≙ 198.7 mg/mL): Recovery range: 100.0 – 100.4% Mean: 100.2% %RSD: 0.15% Recovery limit: 98 – 102 % 99.2% level (≙ 397.9 mg/mL): Recovery range: 100.1 – 100.4% Mean: 100.2% %RSD: 0.11% Recovery limit: 98 – 102 % 149.2% level (≙ 598.6 mg/mL): Recovery range: 100.9 – 101.4% Mean: 101.2% %RSD: 0.27% Recovery limit: 98 – 102 %
Suspensibility – Accuracy (calculated via total re-	4% level Recovery range: 97.0 – 100.1%	4% level Recovery range: 87.4 – 95.3%

	Mefentrifluconazole	Sulphur
covery) and Precision n = 5/5 (Accuracy range = 70 - 110% RSD range = ≤20%)	Mean: 98.4% %RSD: 1.57% 0.5% level Rcovery range: 100.0 – 101.6% Mean: 100.8% %RSD: 0.57%	Mean: 91.9% %RSD: 3.34% 0.5% level Recovery range: 99.7 – 102.8% Mean: 101.3% %RSD: 1.08%
Interference/ Specificity	No interference / specificity given	
Comment	Method suitable	

Conclusion

With respect to the conditions described for the analytical method AFL1047/01 all validation parameters (identity, specificity, accuracy, precision, intermediate precision and stability) are acceptable for Mefentrifluconazole (Reg.No.:5834378) and Sulfur (Reg.No.:240586) line with SANCO/3030/99 rev. 5.
Therefore, the method is valid without restriction in the tested concentration range and is suitable for the determination of Mefentrifluconazole (Reg.No.:5834378) and Sulfur (Reg.No.:240586) in BAS 768 00 F and in aqueous suspensions of BAS 768 00 F.

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

DETERMINATION OF THE RELEVANT IMPURITY DIMETHYLFORMAMIDE (DMF)

Mefentrifluconazole TGAI contains ≤ 0.5 g/kg N,N-dimethylformamide (DMF) which is a relevant impurity of toxicological concern (equivalent to 12.9 mg/L or 9.5 mg/kg DMF in the SC-formulation BAS 768 00 F.

The analytical method AFL1055/01 (GC-MS) has been developed for the determination of N,N-dimethylformamide (Reg. No. 159267) in SC-formulation BAS 768 00 F.
The results of the validation report include data to confirm the linearity, the specificity, the identity, the precision, the accuracy and the stability of the analytical method AFL1055/01.

Reference:	CP 5.1.1/3
Report	Analytical Methode AFL1055/01: Determination of Reg.No.:159267 in BAS 768 00 F (SC - Formulation) by GC-MS, Schubring, M., 2021 Report No BASF DocID 2021/2045639 Authority registration No
Guideline(s):	None, no guidelines available
Deviations:	No
GLP:	No, not subject to GLP regulations

Acceptability: Yes/No/Supplementary

Comments of zRMS:	Accepted

Materials and methods

The analytical method AFL1055/01 is applicable to determine the content of the relevant impurity N,N-dimethylformamide (DMF, Reg. No. 159267) in SC-formulation BAS 768 00 F.

After preparation, the samples are analysed by headspace gas chromatography (GC) with mass spectrometric (MS) detection. Quantification is achieved by linearity using an authentic reference item with known amount. The chromatographic conditions are summarised below.

Chromatographic conditions:

Column	Rtx-200, 60 m x 0.32 mm, 1.5 µm; or equivalent			
Temperatures	Inlet: 250 °C MS source: 230 °C MS Quad: 150 °C Oven: 160 °C, hold for 5 minutes; to 250 °C at 30°C/min; hold for 4 minutes			
Carrier gas	Helium			
Detector	MSD (EI)			
Split ratio	50:1			
Column flow	1.5 mL/min (constant flow)			
Injection volume:	1.5 µL			
Retention times	Target compound	Retention time [min]	m/z (quantifier)	m/z (qualifier)
	DMF (Reg. No. 159567)	4	73	44

Reference: CP 5.1.1/4

Report: Validation of the Analytical Method AFL1055/01: "Determination of Reg.No.:159267 in BAS 768 00 F (SC - Formulation) by GC-MS",

Schubring, M., 2021

Report No 887831_1

BASF DocID 2021/2045581

Authority registration No

Guideline(s): 2004/10/EC, ABNT NBR 14029, CIPAC 3807, OPPTS 830.1800, US EPA OPPTS Harmonized Test Guideline 830.1000, SANCO/3030/99 rev. 5 (22 March 2019), EU Regulation 1107/2009

Deviations: No

GLP: Yes, (certified by Landesamt fuer Umwelt, Mainz, Germany)

Acceptability: Yes

Comments of zRMS:	Accepted. The method can be used for analyzing DMF in the PPP.

Validation - Results and discussions

Table 5.2- 2: Methods suitable for the determination of the relevant impurity Dimethylformamide (DMF) in plant protection product BAS 768 00 F

	Dimethylformamide (DMF) **max. 9.5 mg/kg (12.9 mg/L) or in BAS 768 00 F**
Author(s), year	Schubring M.,2021 (2021/2045581)
Principle of method	GC/MS
Linearity **(linear between** **mg/L)** **(correlation coefficient,** **expressed as R)** **n=6**	Range: 0.80 – 9.6 mg/mL (Equivalent to 5.0 mg/kg – 60 mg/kg of Reg.No.:159267 in BAS 768 00 F) R: 0.9996 Slope: 11328.3 Intercept: -5837.735
Precision – Repeatability Mean **n = 7** **(%RSD)**	Mean: 0.00085% (≙ 8.5mg/kg Reg.No.:159267 in BAS 768 00 F). RSD: 2.99% Horrat value: 0.4
Accuracy (calculation **based on total recovery)** **n = 5** **(% Recovery)**	0.00070% level (≙ 7.0 mg/kg): Recovery range: 83.3 –89.2% Mean: 85.8% %RSD: 3.52% Recovery limit: 70 - 130 % 0.00096% level (≙ 9.6 mg/kg): Recovery range: 85.3 –91.2% Mean: 88.2% %RSD: 2.99% Recovery limit: 70 - 130 % 0.0020% level (≙ 20.0 mg/kg): Recovery range: 87.6 –92.7% Mean: 90.6% %RSD: 2.94% Recovery limit: 70 - 130 %
Interference/ Specificity	No interference / specificity given
LOQ	7.0 mg/kg relevant impurity referred to formulation
Comment	Method suitable

Conclusion

With respect to the conditions described for the analytical method AFL1055/01 all validation parameters (linearity, precision, accuracy, identity, specificity and LoQ) are acceptable. Therefore, the method is valid without restriction in the tested concentration range and is suitable for the determination of Reg.No.:159267 in BAS 768 00 F according to SANCO 3030/99/rev.05.

DETERMINATION OF THE RELEVANT IMPURITY 1,2,4-(1H)-TRIAZOL

Mefentrifluconazole (BAS 750 F, Reg. No. 5834378) contains ≤ 1.0 g/kg 1,2,4-(1H)-Triazol which is

considered to be a relevant impurity (equivalent to 25.8 mg/L or 19.0 mg/kg 1,2,4-(1H)-Triazol in the formulation BAS 768 00 F (SC)).

The analytical method AFL1052/01 (LC-MS) has been developed for the determination of 1,2,4-(1H)-Triazol (Reg. No. 870084) in SC-formulation BAS 768 00 F.
The results of the validation report include data to confirm the linearity, the specificity, the identity, the precision, the accuracy and the LoQ of the analytical method AFL1052/01.

Reference:	CP 5.1.1/5
Report	Analytical Method AFL1052/01: Quantitative Determination of Reg.No.87084 in BAS 768 00 F by LC-MS, Nemitz, A., 2022 Report No BASF DocID 2021/2036361 Authority registration No
Guideline(s):	None, no guidelines available
Deviations:	No
GLP:	No, not subject to GLP regulations
Acceptability:	Yes

Comments of zRMS:	Accepted

Materials and methods

The analytical method APL1052/01 is applicable to determine the content of the relevant impurity 1,2,4-(1H)-Triazol (Reg. No. 87084) in the SC-formulation BAS 768 00 F.
After preparation, the samples are analysed by using a high-pressure liquid chromatographic system (HPLC) with mass spectrometric (MS/MS) detection. Quantification is achieved by linearity using an authentic reference item with known amount. The chromatographic conditions are summarised below.

Chromatographic conditions:

Column	Synergi Polar-RP 4µm, 150 mm x 4.6 mm (or equivalent type)
Mobile phase A	1000 ml water + 1mL formic acid (100%)
Mobile phase B	1000 ml acetonitrile + 1mL formic acid (100%)
Gradient	Time / A [%] / B [%] / Flow (constant) [mL/min]: 0.0 / 95 / 5 / 1.0; 5.0 / 95 / 5 / 1.0; 5.1 / 1 / 99 / 1.0; 10.0 / 1 / 99 / 1.0; 10.1 / 95 / 5 / 1.0; 15.0 / 95 / 5 / 1.0
Column temperature	40°C
Injection volume	10 µL
Detection	SIR-mode
MS Detection Signal	70 m/z (monoisotopic mass M + H*)
Running time	15 minutes
Retention time	approx. 2.2 minutes

Reference: CP 5.1.1/6

Report: Validation of the Analytical Method AFL1052/01: Quantitative Determination of Reg.No.87084 in BAS 768 00 F by LC-MS,

Nemitz, A., 2022

Report No 887832_1

BASF DocID 2021/2036360

Authority registration No

Guideline(s): CIPAC 3807, SANCO/3030/99 rev. 5 (22 March 2019), EU Regulation 1107/2009, US EPA OCSPP Harmonized Test Guideline 830.1000 and 830.1800

Deviations: No

GLP: Yes, (certified by Landesamt fuer Umwelt, Mainz, Germany)

Acceptability: Yes

Comments of zRMS:	Accepted

Validation - Results and discussions

Table 5.2- 3: Methods suitable for the determination of the relevant impurity 1,2,4-(1H)-Triazol in plant protection product BAS 768 00 F

	1,2,4-(1H)-Triazol max. 19.0 mg/kg (25.8 mg/L) in BAS 768 00 F (SC)
Author(s), year	Nemitz A., 2022 (2021/2036360)
Principle of method	LC with MS detection

	1,2,4-(1H)-Triazol **max. 19.0 mg/kg (25.8 mg/L) in BAS 768 00 F (SC)**
Linearity (linear between mg/L) (correlation coefficient, expressed as R) n=5	Range: 0.017 – 0.131 mg/mL (Equivalent to 4.25 mg/kg – 32.75 mg/kg of Reg.No.:87084 in BAS 768 00 F) R: 0.9998 Slope: 3211625
Precision – Repeatability Mean n = 5 (%RSD)	Mean: 0.00202% (≙ 20.2 mg/kg Reg.No.:87084 in BAS 768 00 F). RSD: 1.09% Horrat value: 0.16
Accuracy (calculation based on total recovery) n = 5/3/3 (% Recovery)	0.0014% level (≙ 14 mg/kg): Recovery range: 107.4 –111.5% Mean: 109.1% %RSD: 1.92% Recovery limit: 70 - 130 % 0.0019% level (≙ 19 mg/kg): Recovery range: 104.4 –107.3% Mean: 106.3% %RSD: 1.09% Recovery limit: 70 - 130 % 0.0023% level (≙ 23 mg/kg): Recovery range: 111.4 –116.7% Mean: 113.6% %RSD: 2.44% Recovery limit: 70 - 130 %
Interference/ Specificity	No interference / specificity given
LOQ	~~14 mg/kg Reg.No.:87084 related to BAS 768 00 F~~ 0.019% corresponding to 0.038 mg/L
Comment	Method suitable

Conclusion

With respect to the conditions described for the analytical method AFL1052/01 all validation parameters (linearity, precision, accuracy, intermediate precision, identity, specificity and LoQ) are acceptable. Therefore, the method is valid without restriction in the tested concentration range and is suitable for the determination of Reg.No.:87084 in BAS 768 00 F.

DETERMINATION OF THE RELEVANT IMPURITY TOLUENE:

Mefentrifluconazole (BAS 750 F, Reg. No. 5834378) contains ≤ 1g/kg toluene which is considered to be a relevant impurity (equivalent to 25.8 mg/L or 18.9 mg/kg Toluene in the formulation BAS 768 00 F (SC)). The analytical method AFL1046/01 (GC-MSD) has been developed for quantitative determination of toluene (Reg. No. 4005250) in the SC formulation BAS 768 00 F.

Reference: CP 5.1.1/7

Report Analytical Method AFL1046/01: Determination of Toluene in BAS

768 00 F (SC - Formulation) by GC-MS,

Schubring, M., 2021

Report No

2021/2019123

Authority registration No

Guideline(s): None, no guidelines available

Deviations: No

GLP: No, not subject to GLP regulations

Acceptability: Yes

Comments of zRMS:	Accepted

Materials and methods

This method (GC-MSD) is applicable to the determination of the content of toluene (Reg.No. 4005250) in formulations containing mefentrifluconazole (BAS 750 F).
The analyte was extracted in acetonitrile and separated from other components by gas chromatography using an RTX-200 capillary column. The analyte was detected using a MS detector and quantified with external standard by applying linear regression. The specific response ratio of the sample was compared with those of the standard of known quality.

Column	RTX-200; 30m x 0.32 mm; 1.5µm		
Injector temperature	250 °C		
MS transferline temperature	250 °C		
Oven temperature	Rate [°C/min]	Value [°C]	Hold Time [min]
	-	100	4
	20	250	4
Carrier gas	Helium		
Detector	MSD		
Split ratio	10:1		
Column flow	1.5 mL/min (constant flow)		
Injection volume	1.5 µL		
Analysis time	15.5 min		
Source temperature	230 °C		
Quad temperature	150 °C		
Solvent delay	Approx.3.3 min		
MS off	After 5 min		

Target compound	**Retention time [min]**	**m/z [quantifier]**	**m/z [qualifier]**
Reg.No.:4005250	Approx.3.8	91	92

Reference:	CP 5.1.1/8
Report	Validation of the Analytical Method AFL1046/01: Determination of Toluene in BAS 768 00 F (SC - Formulation) by GC-MS, Schubring, M., 2021 Report No 887830_1 BASF DocID 2021/2019122 Authority registration No
Guideline(s):	2004/10/EC, ABNT NBR 14029, CIPAC 3807, EC 1107/2009, 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

Comments of zRMS:	Accepted

Validation - Results and discussions

Table 5.2- 4: Methods suitable for the determination of the relevant impurity Toluene in plant protection product BAS 768 00 F

	Toluene (Reg. No. 4005250) max. 19.0 mg/kg (25.8 mg/L) in BAS 768 00 F (SC)
Author(s), year	Schubring M., 2021 (2021/2019123)
Principle of method	GC/MS
Linearity (linear between mg/L) (correlation coefficient, expressed as R)	Range: 0.246 – 0.673 mg/mL (Equivalent to 11.0 mg/kg – 30.0 mg/kg of Toluene in BAS 768 00 F) R: 0.9992 Slope: 69493.557 Intercept: -2266.358
Precision – Repeatability Mean n = 5 (%RSD)	Mean: 0.00153% (≙ 15.3 mg/kg Toluene in BAS 768 00 F). RSD: 1.16% Horrat value: 0.16
Accuracy (calculation based on total recovery) n = 5/3/3	<u>0.00140% level (≙ 14.0 mg/kg):</u> Recovery range: 107.7 –110.5% Mean: 109.1% %RSD: 1.16% Recovery limit: 70 - 130 % <u>0.00190% level (≙ 19.0 mg/kg):</u> Recovery range: 108.9 –110.7% Mean: 109.9% %RSD: 0.81% Recovery limit: 70 - 130 %

	Toluene (Reg. No. 4005250) **max. 19.0 mg/kg (25.8 mg/L) in BAS 768 00 F (SC)**
	0.00230% level (≙ 23.0 mg/kg): Recovery range: 106.1 –110.1% Mean: 108.3% %RSD: 1.86% Recovery limit: 70 - 130 %
Interference/ Specificity	No interference / specificity given
LOQ	14.0 mg/kg relevant impurity referred to formulation (corresponding to 0.343 mg/L)
Comment	Method suitable

Conclusion

With respect to the conditions described for the analytical method AFL1046/01 all validation parameters (linearity, precision, accuracy, intermediate precision, identity, specificity, and LoQ) are acceptable. Therefore, the method is valid without restriction in the tested concentration range and is suitable for the determination of Reg.No.:4005250 in BAS 768 00 F.

5.2.1.3 Description of 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)

To date, there is no CIPAC method available for the analysis of mefentrifluconazole in technical or formulated material.

CIPAC method 18 is a titration method whereby the sulphur is convered by refluxing with sodium sulphite to sodium thiosulphate. The thiosulphate is then titrated with standard iodine solution.

There is no CIPAC method available for the simultaneous determination of mefentrifluconazole and sulphur in the SC formulations as BAS 768 00 F.

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 for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.2- 5: 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 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 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 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- 6: Validated methods for the generation of pre-authorization data for mefentrifluconazole in plant and animal matrices-Triazole derivative metabolites

Components of interest: 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 method	Different confirmatory method(s) are available. Monitoring a confirmatory mass transition (157→88 m/z, positive mode for triazole alanine), 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 derivatization 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 Metaboite Group to CRD. Therfore 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- 7: 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)*	Studenreoth 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- 8: Validated methods for the generation of pre-authorization data for triazole in soil matrices

Component of residue definition: 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 BAS 555 F and metabolite Reg.n0 5924326)*	Geschke S., 2014 BASF DocID 2013/1377001 Method L0203/01 EU agreed (metconazole Dossier)

Table 5.2- 9: Validated methods for the generation of pre-authorization data for mefentrifluconazole and metabolites in surface water and sediment matrices

Component of residue definition: mefentrifluconazole + M750F001 (1,2,4-triazole) + M750F003 + M750F005 +M750F006 + M750F007 + M750F008				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Surface, drinking water (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	30 ng/L	LC-(ESI)-MS/MS *(except 1,2,4-triazole)*	Malinsky D.S., 2016 BASF DocID 2015/7001125 Report Amendment DocID: 2016/7010048 Method D1506/01 EU agreed
Surface, drinking water (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two columns used for confirmation)	0.05 ng/L	HPLC-MS/MS *(1,2,4-triazole only)*	Penning H. et a1., 2013 BASF DocID 2012/1297158 Method L0199/01 EU agreed

Table 5.2- 10: Validated methods for the generation of pre-authorization data for mefentrifluconazole in air

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

zRMS accepts.

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

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

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Mefentrifluconazole	0.01 mg/kg	Annex I inclusion
Plant, high acid content		0.01 mg/kg	Annex I inclusion
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Annex I inclusion
Plant, high oil content		0.01 mg/kg	Annex I inclusion
Plant, difficult matrices (hops, spices, tea)		0.01 mg/kg	Annex I inclusion
Muscle	Mefentrifluconazole	0.01 mg/kg	Annex I inclusion
Milk		0.01 mg/kg	Annex I inclusion
Eggs		0.01 mg/kg	Annex I inclusion
Fat		0.01 mg/kg	Annex I inclusion
Liver, kidney		0.01 mg/kg	Annex I inclusion
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} inhalation (NOAEL sys: 25 mg/kg bw/d
Tissue (meat or liver)	Mefentrifluconazole	0.01 mg/kg	Not classified as T / T+
Body fluids		0.07 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 for analysis of mefentrifluconazole in plant matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

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

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	Primary (VAL) Confirmatory method not necessary (two mass transitions used for conrmation)	0.01 mg/kg	LC-MS/MS	Klimmek S. et al., 2015 BASF DocID 2015/1106708 Method L0295/01 (QuEChERS) EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for conrmation)	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	Primary (VAL) Confirmatory method not necessary (two mass transitions used for conrmation)	0.01 mg/kg	LC-MS/MS	Klimmek S. et al., 2015 BASF DocID 2015/1106708 Method L0295/01 (QuEChERS) EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for conrmation)	0.01 mg/kg	LC-MS/MS	Richter S., Schmiedt S., 2015 BASF DocID 2015/1240004 Method L0295/01 (QuEChERS) EU agreed
High oil content	Primary (VAL) Confirmatory method not necessary (two mass transitions used for conrmation)	0.01 mg/kg	LC-MS/MS	Klimmek S. et al., 2015 BASF DocID 2015/1106708 Method L0295/01 (QuEChERS) EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for conrmation)	0.01 mg/kg	LC-MS/MS	Richter S., Schmiedt S., 2015 BASF DocID 2015/1240004 Method L0295/01 (QuEChERS) EU agreed

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

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Extraction efficiency of data generation method (L0076/09) and multi-methods (QuEChERS, DFG S 9, 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).

zRMS accepts.

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. No new studies were conducted.

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 conrmation)	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 conrmation)	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 conrmation)	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 conrmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Richter S., Djedovic S., 2015 BASF DocID 2015/1240005 Method L0272/01 EU agreed
Muscle	Primary (VAL) Confirmatory method not necessary (two mass transitions used for conrmation)	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 conrmation)	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	0.01 mg/kg	LC-MS/MS *(parent only)*	Devine C., 2015 BASF DocID 2015/1106707 Method L0272/01 EU agreed

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

zRMS accepts.

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 was 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%).

zRMS accepts.

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

An overview on the acceptable methods and possible data gaps for analysis of mefentrifluconazole in soil is given in the following tables. No new studies were conducted.

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 conrmation)	0.002 mg/kg	LC-MS/MS *(also Reg. No. 5924326, 1,2,4-triazole and M750F003)*	Studenroth S., Lueer D., 2015 BASF DocID 2015/1039006 Report Amendment 1: 2016/1030227 Report Amendment 2: 2016/1215646 Method L0214/01 EU agreed

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

zRMS accepts.

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

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

Component of residue definition: mefentrifluconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary (VAL) Confirmatory method not necessary (two mass transitions used for conrmation)	30 ng/L	LC-MS/MS	Obermann M., 2017 BASF DocID 2017/1066523 Method L0359/01 New study, not peer reviewed
	ILV Confirmatory method not necessary (two mass transitions used for conrmation)	30 ng/L	LC-MS/MS	Stanislowski T., 2017 BASF DocID 2017/1066522 Method L0359/01 New study, not peer reviewed
Surface water	Primary (VAL) Confirmatory	30 ng/L	LC-MS/MS	Obermann M., 2017 BASF DocID 2017/1066523

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**
	method not necessary (two mass transitions used for conrmation)			Method L0359/01 New study, not peer reviewed
	ILV Confirmatory method not necessary (two mass transitions used for conrmation)	30 ng/L	LC-MS/MS	Stanislowski T., 2017 BASF DocID 2017/1066522 Method L0359/01 New study, not peer reviewed
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

zRMS accepts.

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

An overview on the acceptable methods and possible data gaps for analysis of mefentrifluconazole in air is given in the following tables. No new studies were conducted.

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 conrmation)	0.01 ng/L	LC-MS/MS	Obermann M., Studenroth S., 2015 BASF DocID 2015/1111330 Method L0327/01 EU agreed

zRMS accepts.

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			
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 conrmation)	0.01 mg/L	LC-MS/MS	Wiesner F., Breyer N., 2016 BASF DocID 2016/1148911 Method L0339/01 EU agreed
Primary (VAL) Confirmatory method not necessary (two mass transitions)	0.01 mg/L	LC-MS/MS	**New study** KCP 5.2/3, not peer-reviewed Homazava N. 2019 BASF DocID 2019/1046404 Method L0339/02

zRMS accepts.

5.3.2.8 Other studies/ information

No further studies submitted.

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

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

Table 5.3-10: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	**Analytical endpoints/methods for residues (Annex IIA, point 4.2) EU agreed method [EFSA Scientific Report (2008) 221, Sulfur]**
Food of plant origin	Analytical methods using HPLC/UV (ILV: Mollerfeld J. (2002); Garofani J. (2005)) for the determination of sulphur residues in plants with high water content, high acid content and dry plants have already been provided by the notifiers in the DAR (August 2007) and was considered as validated with LOQ = 5.0 mg/kg in grape fruits, LOQ = 50 mg/kg in hops, LOQ = 0.5mg/kg in apples, cucumbers and wheat grain. Since no MRLs are set, analytical methods for the determination of Sulphur residues in plants are not required. No further data required.
Food of animal origin	Sulphur is an essential element for mammalians and humans, naturally present in food. Since no MRLs are set, analytical methods for the determination of

	Sulphur residues in foods of animal origin are not required.
Soil	An analytical method (Elzner J. (1992)) using HPLC/UV for the determination of Sulphur residues in soil has already been submitted at EU level in the DAR (August 2007) but not validated (no raw data provided and no confirmatory method). According to EFSA, Sulphur is a component of the environment with low toxicity for organisms. Since it is not necessary to propose a residue definition for monitoring, no analytical method is required.
Surface water, drinking/groundwater	According to EFSA, Sulphur is naturally occurring in water with no effect on aquatic organisms at the level of solubility. Since it was not necessary to propose a residue definition for monitoring, no method of analysis is required.
Air	According to the EFSA Conclusion, sulfur is a naturally occurring element, the vapor pressure is low and the use pattern will not substantially increase levels above the background level, therefore no analytical methods are required for air.

zRMS accepts.

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

No analytical methods for the determination of residues of sulfur in food and feed commodities are required according to guideline SANCO 825/00 rev. 8.1. Sulfur is a naturally occurring element and the corresponding agreement as laid down in the EFSA Scientific Report (2008) 221 neither to propose an ADI and ARfD nor a residue definition and corresponding MRLs for sulfur. Nevertheless, analytical methods in plants are available.

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Matrix	**Analytical endpoints/methods for residues (Annex IIA, point 4.2) EU agreed method [EFSA Scientific Report (2008) 221, Sulfur]**
Food of plant origin	Analytical methods using HPLC/UV (ILV: Mollerfeld J. (2002); Garofani J. (2005)) for the determination of sulphur residues in Plants with high water content, high acid content and dry plants have already been provided by the notifiers in the DAR (August 2007) and was considered as validated with LOQ = 5.0 mg/kg in grape fruits, LOQ = 50 mg/kg in hops, LOQ = 0.5mg/kg in apples, cucumbers and wheat grain. Since no MRLs are set, analytical methods for the determination of Sulphur residues in plants are not required. No further data required.

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

No analytical methods for the determination of residues of sulfur in food and feed commodities are required according to guideline SANCO 825/00 rev. 8.1. Sulfur is a naturally occurring element and the corresponding agreement as laid down in the EFSA Scientific Report (2008) 221 neither to propose an ADI and ARfD nor a residue definition and corresponding MRLs for sulfur.

Matrix	Analytical endpoints/methods for residues (Annex IIA, point 4.2) EU agreed method [EFSA Scientific Report (2008) 221, Sulfur]
Food of animal origin	Sulphur is an essential element for mammalians and humans, naturally present in food. Since no MRLs are set, analytical methods for the determination of Sulphur residues in foods of animal origin are not required.

zRMS accepts.

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

Matrix	Analytical endpoints/methods for residues (Annex IIA, point 4.2) EU agreed method [EFSA Scientific Report (2008) 221, Sulfur]
Soil	An analytical method (Elzner J. (1992)) using HPLC/UV for the determination of Sulphur residues in soil has already been submitted at EU level in the DAR (August 2007) but not validated (no raw data provided and no confirmatory method). According to EFSA, Sulphur is a component of the environment with low toxicity for organisms. Since it is not necessary to propose a residue definition for monitoring, no analytical method is required.

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

Matrix	Analytical endpoints/methods for residues (Annex IIA, point 4.2) EU agreed method [EFSA Scientific Report (2008) 221, Sulfur]
Surface water, drinking/groundwater	According to EFSA, Sulphur is naturally occurring in water with no effect on aquatic organisms at the level of solubility. Since it was not necessary to propose a residue definition for monitoring, no method of analysis is required.

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

Matrix	Analytical methods for residues (Annex IIA, point 4.2) EU agreed method [EFSA Scientific Report (2008) 221, Sulfur]
Air	According to the EFSA Conclusion, sulfur is a naturally occurring element, the vapour pressure is low and the use pattern will not substantially increase levels above the background levels, no analytical methods are required for air.

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

Matrix	Analytical methods for residues (Annex IIA, point 4.2) EU agreed method [EFSA Scientific Report (2008) 221, Sulfur]
Body fluids and tissues	According to the EFSA Conclusion, Sulfur is a naturally occurring element, the vapour pressure is low and the use pattern will not substantially increase levels above the background levels. Sulfur is not classified as toxic or highly toxic. Thus, no analytical methods are required for body fluids and tissues.

5.3.3.8 Other studies/ information

Not required.

zRMS accepts.

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 5.1.1/1	Nemitz, A.	2021	Analytical method AFL1047/01: Quantitative Determination of the Active Ingredients Mefentrifluconazole and Sulfur in BAS 768 00 F and Aqueous Solutions of BAS 768 00 F by HPLC 2021/2021957 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 5.1.1/2	Nemitz, A.	2021	Validation of the Analytical Method AFL1047/01: Quantitative Determination of the Active Ingredients Mefentrifluconazole and Sulfur in BAS 768 00 F and Aqueous Solutions of BAS 768 00 F by HPLC 2021/2021956 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/3	Schubring, M.	2021	Analytical Methode AFL1055/01: Determination of Reg.No.:159267 in BAS 768 00 F (SC - Formulation) by GC-MS 2021/2045639 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1/4	Schubring, M.	2021	Validation of the Analytical Method AFL1055/01: "Determination of Reg.No.:159267 in BAS 768 00 F (SC - Formulation) by GC-MS" 2021/2045581 BASF SE yes Unpublished	No	BASF
KCP 5.1.1/5	Nemitz, A.	2022	Analytical Method AFL1052/01: Quantitative Determination of Reg.No.87084 in BAS 768 00 F by LC-MS 2021/2036361 BASF SE no Unpublished	No	BASF
KCP 5.1.1/6	Nemitz, A.	2022	Validation of the Analytical Method AFL1052/01: Quantitative Determination of Reg.No.87084 in BAS 768 00 F by LC-MS 2021/2036360 BASF SE yes Unpublished	No	BASF
KCP 5.1.1/7	Schubring, M.	2021	Analytical Method AFL1046/01: Determination of Toluene in BAS 768 00 F (SC - Formulation) by GC-MS 2021/2019123 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1/8	Schubring, M.	2021	Validation of the Analytical Method AFL1046/01: Determination of Toluene in BAS 768 00 F (SC - Formulation) by GC-MS 2021/2019122 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/1	Obermann, M.	2017	Validation of analytical method L0359/01 for the determination of BAS 750 F and its metabolites M750F003, M750F005, M750F006 (Reg.No.5863469), M750F007 (Reg.No.6003432) and M750F008 (Reg.No.6010286) in drinking and surface water by LC-MS/MS 2017/1066523 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/2	Stanislowski, T.	2017	Independent laboratory validation (IVL) of method L0359/01 for the determination of BAS 750 F and its metabolites M750F005, M750F006, M750F007 and M750F008 in drinking water and surface water by LC-MS/MS 2017/1066522 EAG Laboratories PTRL Europe, Ulm, Germany Fed.Rep. yes Unpublished	No	BASF

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 5.2/3	Homazava, N.	2019	Validation of BASF Analytical Method L0339/02 for the determination of M750F015, M750F016 and M750F017 in body fluids 2019/1046404 IES - Innovative Environmental Services Ltd., Witterswil, Switzerland yes Unpublished	No	BASF

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

Please refer to the reference list.

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)

No new or additional studies have been submitted

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

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

No new or additional studies have been submitted.

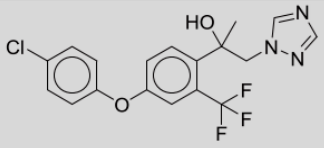
A 2.1.2.2 Description of Methods for the Analysis of Soil (KCP 5.2)

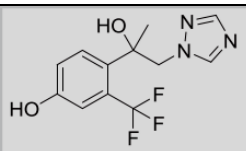
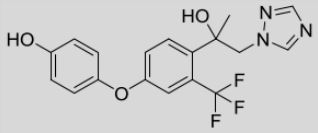
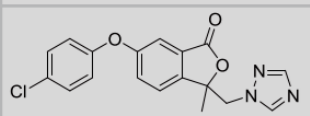
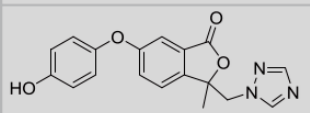
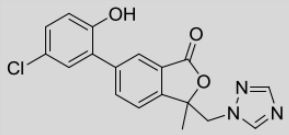
No new or additional studies have been submitted.

A 2.1.2.3 Description of Methods for the Analysis of Water (KCP 5.2)

A 2.1.2.3.1.1 Method validation 1

Comments of zRMS:	The validation has been accepted. The objective was to validate the analytical method no. L0359/01 for the determination of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 in drinking (ground) and surface water by LC-MS/MS. The mentioned compounds have the following chemical names and structures: (EFSA Journal 2018;16(7):5379)

Code/trivial name[a]	Chemical name/SMILES notation[b]	Structural formula[b]
Mefentrifluconazole BAS 750 F	(2*RS*)-2-[4-(4-chlorophenoxy)-α,α,α-trifluoro-*o*-tolyl]-1-(1*H*-1,2,4-triazol-1-yl)propan-2-ol CC(CN1C=NC=N1)(c2c(C(F)(F)F)cc(Oc3ccc(Cl)cc3)cc2)O JERZEQUMJNCPRJ-UHFFFAOYSA-N	

M750F003 4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenol	4-[2-hydroxy-1-(1*H*-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenol OC1=CC=C(C(C)(O)CN2N=CN=C2)C(C(F)(F)F)=C1 WZNDGSSEDNFWRU-UHFFFAOYSA-N	
M750F005 4-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenol	4-{4-[2-hydroxy-1-(1*H*-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenol OC1=CC=C(OC2=CC=C(C(C)(O)CN3N=CN=C3)C(C(F)(F)F)=C2)C=C1 XXTGZVUVWIHRHS-UHFFFAOYSA-N	
M750F006 6-(4-chlorophenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one	6-(4-chlorophenoxy)-3-methyl-3-[(1*H*-1,2,4-triazol-1-yl)methyl]-2-benzofuran-1(3*H*)-one O=C1OC(CN2N=CN=C2)(C)C3=CC=C(OC4=CC=C(Cl)C=C4)C=C13 PUYHXMAKCXYUBF-UHFFFAOYSA-N	
M750F007 6-(4-hydroxyphenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one	6-(4-hydroxyphenoxy)-3-methyl-3-[(1*H*-1,2,4-triazol-1-yl)methyl]-2-benzofuran-1(3*H*)-one O=C1OC(CN2N=CN=C2)(C)C3=CC=C(OC4=CC=C(O)C=C4)C=C13 CVTZVGKIZRMGMX-UHFFFAOYSA-N	
M750F008 6-(5-chloro-2-hydroxyphenyl)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one	6-(5-chloro-2-hydroxyphenyl)-3-methyl-3-[(1*H*-1,2,4-triazol-1-yl)methyl]-2-benzofuran-1(3H)-one O=C1OC(CN2N=CN=C2)(C)C3=CC=C(C4=CC(Cl)=CC=C4O)C=C13 VNJWOMIREKKDGP-UHFFFAOYSA-N	

The principle of the method was a classic sample extraction, an obtained aliquot evaporation to dryness using nitrogen and a sample re-dissolving in acetonitrile/water system prior to final determination by LC-MS/MS.
The method was validated at two fortification levels (0.03 µg/L and 0.3 µg/L) for ground and surface water. For each fortification level and water type, five replicates were prepared and analysed. Additionally, at least two replicates of unfortified samples were analysed (untreated control samples). Two mass transitions were evaluated for quantification and confirmation of BAS 750 F and its metabolites.

The LOQ - the lowest fortification level successfully tested, hence 0.03 µg/L. The LOD is 0.009 µg/L, corresponding to the lowest calibration level used.
Selectivity: The method determined residues of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 in water. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered.

Specificity: The method allows the specific determination of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 in water using LC-MS/MS. Detection is accomplished by high selective MS/MS detection using two mass transitions.

Linearity: Good ($r \geq 0.995$) was observed in the range of 0.03 ng/mL to 1 ng/mL for the mass transitions of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008. 6 calibration levels were used.

Stability in Working Solutions: Stability tests confirmed that BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 were stable for a maximum duration of 30 days in stock and calibration solutions, when stored at approximately 4°C in the dark.
Stability in Sample Extracts: The experiments demonstrate that BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 were stable in final extracts over a time period of 7 days in case of surface water and 8 days in case of ground water, when stored at approximately 4°C in the dark.

Matrix Effects: As no significant matrix effects were identified, solvent standards were used for calibration and quantification of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008.

Recovery and Repeatability: The analytical method L0359/01 is suitable to determine residues of BAS 750 F and its metabolites in ground and surface water. The mean recovery values ranged between 83% and 103%. The RSD for all analytes and both fortification levels were below 10%.

Recoveries of BAS 750 F (Reg.No.5834378) in Water

Matrix Water	Mass Transition	Fortification Level [µg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Ground Water	398 → 70	0.03	5	95	2.1	97	2.6
		0.3	5	98	2.0		
	400 → 70	0.03	5	96	2.7	97	2.8
		0.3	5	98	2.8		
Surface Water	398 → 70	0.03	5	103	1.3	102	1.5
		0.3	5	102	1.5		
	400 → 70	0.03	5	101	3.2	100	3.2
		0.3	5	98	2.7		

Recoveries of M750F003 (Reg.No.5924326) in Water

Matrix Water	Mass Transition	Fortification Level [µg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Ground Water	288 → 70	0.03	5	83	1.9	85	3.2
		0.3	5	87	2.9		
	288 → 43	0.03	5	87	7.6	88	6.0
		0.3	5	89	4.3		
Surface Water	288 → 70	0.03	5	87	2.5	89	2.9
		0.3	5	91	1.4		
	288 → 43	0.03	5	89	9.4	91	8.0
		0.3	5	92	7.3		

Recoveries of M750F005 (Reg.No.6003433) in Water

Matrix Water	Mass Transition	Fortification Level [µg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Ground Water	380 → 70	0.03	5	88	2.7	90	3.6
		0.3	5	93	3.0		
	380 → 109	0.03	5	88	3.5	89	4.9
		0.3	5	91	5.6		
Surface Water	380 → 70	0.03	5	91	2.0	92	2.4
		0.3	5	93	2.7		
	380 → 109	0.03	5	93	9.4	90	7.7
		0.3	5	87	4.3		

Recoveries of M750F006 (Reg.No.5863469) in Water

Matrix Water	Mass Transition	Fortification Level [µg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Ground Water	356 → 259	0.03	5	96	3.9	98	3.6
		0.3	5	100	2.4		
	356 → 217	0.03	5	96	4.5	99	4.4
		0.3	5	101	2.1		
Surface Water	356 → 259	0.03	5	102	2.1	101	2.2
		0.3	5	100	2.3		
	356 → 217	0.03	5	101	3.7	101	3.7
		0.3	5	101	4.2		

Recoveries of M750F007 (Reg.No.6003432) in Water

Matrix Water	Mass Transition	Fortification Level [µg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Ground Water	338 → 241	0.03	5	84	3.7	85	4.6
		0.3	5	86	5.3		
	338 → 269	0.03	5	84	0.9	85	3.1
		0.3	5	86	3.8		
Surface Water	338 → 241	0.03	5	88	2.8	88	2.7
		0.3	5	89	3.0		
	338 → 269	0.03	5	83	2.7	83	2.3
		0.3	5	84	1.6		

Recoveries of M750F008 (Reg.No.6010286) in Water

Matrix Water	Mass Transition	Fortification Level [µg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Ground Water	356 → 259	0.03	5	91	3.2	92	3.8
		0.3	5	93	3.9		
	356 → 241	0.03	5	89	2.7	91	3.5
		0.3	5	93	2.7		
Surface Water	356 → 259	0.03	5	93	3.9	93	2.7
		0.3	5	93	1.0		
	356 → 241	0.03	5	92	3.8	93	3.5
		0.3	5	95	3.1		

It could be demonstrated that analytical method L0359/01 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 (Reg.No. 5834378) and its metabo-

	lites M750F003 (Reg.No. 5924326), M750F005 (Reg.No. 6003433), M750F006 (Reg.No. 5863469), M750F007 (Reg.No. 6003432) and M750F008 (Reg.No. 6010286) in ground and surface water.

Reference: CP 5.2/1

Report: Validation, analytical method L0359/01, BAS 750 F (Reg.No.5834378) and metabolites M750F003 (Reg.No.5924326), M750F005 (Reg.No.6003433), M750F006 (Reg.No.5863469), M750F007 (Reg.No.6003432) and M750F008 (Reg.No.6010286) in drinking and surface water by LC-MS/MS

Obermann, M., 2017

report No 836940

BASF DocID 2017/1066523

Guideline(s): EPA 850.6100 (2012), SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010)

Deviations: No

GLP: yes

(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Acceptability: Yes

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

Materials and methods

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

Results and discussions

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

Table A 1: 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

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
		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 2: 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 2.1.2.3.1.2 Independent laboratory validation 1

Comments of zRMS:	The validation has been accepted. The objective of this study was to independently validate an analytical method L0359/01 for the determination of mefentrifluconazole (BAS 750 F) and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 in surface and in drinking water. The target and achieved LOQ is 0.03 µg/L per analyte, using LC-MS/MS with two mass transitions. The principle of the method was a classic sample extraction, an obtained aliquot evaporation to dryness using nitrogen and a sample re-dissolving in acetonitrile/water system prior to final determination by LC-MS/MS. The method was validated at two fortification levels (0.03 µg/L and 0.3 µg/L) for ground and surface water. For each fortification level and water type, five replicates were prepared and analysed. Additionally, at least two replicates of unfortified samples were analysed (untreated control samples). Two mass transitions were evaluated for quantification and confirmation of BAS 750 F and its metabolites. The LOQ - the lowest fortification level successfully tested, hence 0.03 µg/L. The LOD is 0.009 µg/L, corresponding to the lowest calibration level used. Selectivity: The highly selective and sensitive LC-MS/MS method was used for determination of all analytes with monitoring of 2 characteristic mass transitions per analyte. Consequently, no further confirmatory method is required. The interferences/residues of the analyte measured in the control samples were below 20 % of the LOQ for each matrix and each mass transition. Linearity: Linear calibration curves in the range of 0.03 to 1.0 ng/mL were calculated and plotted by regression analysis. Correlation coefficients (r) were always ≥ 0.993. Standard Stability: BAS 750 F and its five metabolites indicated sufficient stability (less than 10 % difference) in stock solution for 16 days as well as solutions used for fortification and calibration (less than 20 % difference for BAS 750 F or less than 10 % difference for the five metabolites) when stored refrigerated in the dark. Extract stability: Final sample extracts were re-injected after 7, 11 or 15 days of storage under refrigerated conditions. No significant decrease (80.4 to 98.6 % of initial value) or increase (101 to 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 7, 11 or 15 days under refrigerated storage conditions. Recovery and Repeatability: The method L0359/01 is suitable for the determination of BAS 750 F and its metabolites in drinking water and surface water at a limit of quantification of 0.03 µg/L. The mean recovery values were between 87.2 % and 110 % of the nominal values. The RSD for all fortification levels were at or below 13 %. Matrix Effect: The matrix effect was tested for each matrix and analyte. No significant matrix effect was observed. Thus the method for the determination of residues of BAS 750 F, M750F003, M750F005, M750F006, M750F007 and M750F008 in drinking and surface water by LC-MS/MS was successfully independently validated, demonstrating the LOQ of 0.03 µg/L. The LOD of the method was demonstrated to be 0.009 µg/L. It is concluded that this method fulfils the reproducibility requirements as defined in EC Guidance document on residue analytical methods (SANCO/825/00 rev. 8.1) as well as in OCSPP 860.1340 (August 1996), OCSPP 850.6100 and OECD ENV/JM/MONO (2007) 17 and is, therefore, applicable as residue and enforcement method.

Recoveries for BAS 750 F in Drinking and Surface Water

Matrix	Mass Transition	Fortification Level [µg/L]	Number of Replicates	Mean [%]	RSD [%]	Mean [%]	RSD [%]
Drinking Water	Quant. Ion 398 m/z -> 70 m/z	0.030	5	87.7	5.6	95.0	11
		0.30	5	102	10		
	Qual. Ion 400 m/z -> 70 m/z	0.030	5	92.2	2.6	99.9	9.8
		0.30	5	108	7.4		
Surface Water	Quant. Ion 398 m/z -> 70 m/z	0.030	5	108	7.8	108	7.6
		0.30	5	108	8.3		
	Qual. Ion 400 m/z -> 70 m/z	0.030	5	110	2.8	109	2.6
		0.30	5	108	2.3		

Recoveries for M750F003 in Drinking and Surface Water

Matrix	Mass Transition	Fortification Level [µg/L]	Number of Replicates	Mean [%]	RSD [%]	Mean [%]	RSD [%]
Drinking Water	Quant. Ion 288 m/z -> 70 m/z	0.030	5	87.2	6.8	95.2	11
		0.30	5	103	8.3		
	Qual. Ion 288 m/z -> 43 m/z	0.030	5	88.3	7.2	97.4	11
		0.30	5	107	4.0		
Surface Water	Quant. Ion 288 m/z -> 70 m/z	0.030	5	102	3.3	102	3.4
		0.30	5	101	3.9		
	Qual. Ion 288 m/z -> 43 m/z	0.030	5	106	7.4	100	9.1
		0.30	5	94.9	7.7		

Recoveries for M750F005 in Drinking and Surface Water

Matrix	Mass Transition	Fortification Level [µg/L]	Number of Replicates	Mean [%]	RSD [%]	Mean [%]	RSD [%]
Drinking Water	Quant. Ion 380 m/z -> 70 m/z	0.030	5	90.8	7.2	97.5	11
		0.30	5	104	8.9		
	Qual. Ion 380 m/z -> 109 m/z	0.030	5	94.6	7.8	98.0	8.2
		0.30	5	101	7.7		
Surface Water	Quant. Ion 380 m/z -> 70 m/z	0.030	5	105	1.8	103	3.5
		0.30	5	102	4.4		
	Qual. Ion 380 m/z -> 109 m/z	0.030	5	107	2.9	103	8.1
		0.30	5	99.7	11		

Recoveries for M750F006 in Drinking and Surface Water

Matrix	Mass Transition	Fortification Level [µg/L]	Number of Replicates	Mean [%]	RSD [%]	Mean [%]	RSD [%]
Drinking Water	Quant. Ion 356 m/z -> 259 m/z	0.030	5	95.5	6.5	102	8.7
		0.30	5	108	6.1		
	Qual. Ion 356 m/z -> 217 m/z	0.030	5	95.2	11	101	10.1
		0.30	5	107	5.5		
Surface Water	Quant. Ion 356 m/z -> 259 m/z	0.030	6	98.3	3.4	96.5	4.3
		0.30	6	94.7	4.6		
	Qual. Ion 356 m/z -> 217 m/z	0.030	6	102	7.9	101	6.6
		0.30	6	98.9	5.1		

Recoveries for M750F007 in Drinking and Surface Water

Matrix	Mass Transition	Fortification Level [µg/L]	Number of Replicates	Mean [%]	RSD [%]	Mean [%]	RSD [%]
Drinking Water	Quant. Ion 338 m/z -> 241 m/z	0.030	5	88.4	6.8	97.3	11
		0.30	5	106	5.7		
	Qual. Ion 338 m/z -> 269 m/z	0.030	5	88.7	8.8	97.2	13
		0.30	5	106	11		
Surface Water	Quant. Ion 338 m/z -> 241 m/z	0.030	5	98.8	3.0	99.3	5.6
		0.30	5	99.8	7.8		
	Qual. Ion 338 m/z -> 269 m/z	0.030	5	104	6.9	102	6.7
		0.30	5	98.7	5.7		

Recoveries for M750F008 in Drinking and Surface Water

Matrix	Mass Transition	Fortification Level [µg/L]	Number of Replicates	Mean [%]	RSD [%]	Mean [%]	RSD [%]
Drinking Water	Quant. Ion 356 m/z -> 259 m/z	0.030	5	86.8	9.1	92.4	11
		0.30	5	98.0	8.9		
	Qual. Ion 356 m/z -> 241 m/z	0.030	5	90.1	8.6	94.9	11
		0.30	5	99.7	11		
Surface Water	Quant. Ion 356 m/z -> 259 m/z	0.030	5	102	4.1	103	4.3
		0.30	5	104	4.5		
	Qual. Ion 356 m/z -> 241 m/z	0.030	5	96.5	11	99.4	9.2
		0.30	5	102	7.6		

Reference: CP 5.2/2

Report: Independent laboratory validation (IVL) of method L0359/01 for the determination of BAS 750 F and its metabolites M750F005, M750F006, M750F007 and M750F008 in drinking water and surface water by LC-MS/MS

Stanislowski, T., 2017

report No EU-836906, P 4262 G

BASF DocID 2017/1066522

Guideline(s): EPA 850.6100, EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 8.1 (16 November 2010)

Deviations: No

GLP: yes

(certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Acceptability: Yes

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

Materials and methods

There were no significant deviations from the primary method.

Results and discussions

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

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

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

Table A 4: 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

	Mefentrifluconazole
	refrigerated in the dark. Final sample extracts in acetonitrile/water (1/1, v/v) were re-injected after 11 (for surface water) or 15 days (for drinking water) of storage under refrigerated conditions. No significant decrease (80.4-98.6% of initial value) or increase (101-114% of initial value) in recovery in the stored final extracts was observed when the results were evaluated with freshly prepared calibration solutions in solvent. Thus, stability of final extracts is considered sufficiently proven for at least 11 or 15 days under refrigerated storage conditions.

Conclusion

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

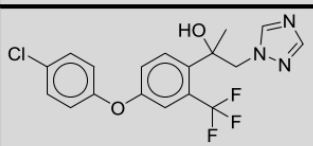
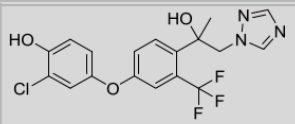
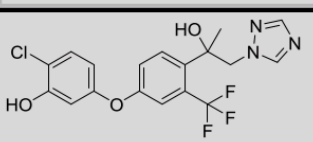
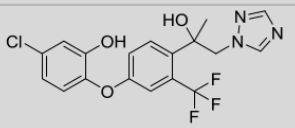
A 2.1.2.4 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

A 2.1.2.5 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

Comments of zRMS:

The validation has been accepted.
The purpose of the study was to validate the analytical BASF Method L0339/02 for the determination of mefentrifluconazole metabolites M750F015, M750F016 and M750F017 in body fluids. The final determination of M750F015, M750F016 and M750F017 was performed by LC-MS/MS with two parent-daughter ion transitions for each compound, one for quantification and one for confirmation. The LOQ of the method for all analytes is 0.010 mg/L.
The compounds tested have the following chemical names and structures:
(EFSA Journal 2018;16(7):5379)

Code/trivial name[a]	Chemical name/SMILES notation[b]	Structural formula[b]
Mefentrifluconazole BAS 750 F	(2*RS*)-2-[4-(4-chlorophenoxy)-α,α,α-trifluoro-*o*-tolyl]-1-(1*H*-1,2,4-triazol-1-yl)propan-2-ol CC(CN1C=NC=N1)(c2c(C(F)(F)F)cc(Oc3ccc(Cl)cc3)cc2)O JERZEQUMJNCPRJ-UHFFFAOYSA-N	
M750F015 2-chloro-4-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl) phenoxy}phenol	2-chloro-4-{4-[2-hydroxy-1-(1*H*-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenol OC1=CC=C(OC2=CC=C(C(C)(O)CN3N=CN=C3)C(C(F)(F)F)=C2)C=C1Cl BPZYSFCSGCSWRX-UHFFFAOYSA-N	
M750F016 2-chloro-5-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl) phenoxy}phenol	2-chloro-5-{4-[2-hydroxy-1-(1*H*-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenol OC1=CC(OC2=CC=C(C(C)(O)CN3N=CN=C3)C(C(F)(F)F)=C2)=CC=C1Cl WEJBGHCFVNQQDI-UHFFFAOYSA-N	
M750F017 5-chloro-2-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl) phenoxy}phenol	5-chloro-2-{4-[2-hydroxy-1-(1*H*-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenol OC1=CC(Cl)=CC=C1OC2=CC=C(C(C)(O)CN3N=CN=C3)C(C(F)(F)F)=C2 FQLCIFALHFSMGH-UHFFFAOYSA-N	

The following transitions were monitored:

Analyte	M750F015
Quantification transition	*m/z* 414 → 70
Confirmation transition	*m/z* 414 → 143
Analyte	M750F016
Quantification transition	*m/z* 414 → 70
Confirmation transition	*m/z* 414 → 182
Analyte	M750F017
Quantification transition	*m/z* 414 → 70
Confirmation transition	*m/z* 414 → 143

The method validation was performed with 1 reagent blank; 2 untreated control samples; 5 untreated samples fortified at LOQ and 5 fortified at 10 x LOQ for all matrices. Bovine plasma and human urine were used. To estimate the matrix influence, the response of calibration standards in solvent was compared to calibration standards prepared in the presence of matrix for both primary and confirmatory

transitions.
LC-MS/MS method used for analysis is highly sensitive and selective. Untreated control samples were free from interference and residues above 30% of the LOQ for each analyte and each mass transition. The linearity of the LC-MS/MS detector response was confirmed by injecting 7 solvent standard solutions, covering a working range of 0.1 ng/mL to 10 ng/mL. The LC-MS/MS detector response for each transition was linear within the range tested, with correlation coefficients (r) ≥ 0.99. Raw extracts shown to be stable for 8 days when stored at 2 – 8 °C in the dark for all body fluid matrices tested. Stability tests showed that M750F015, M750F016 and M750F017 stock, fortification and calibration solutions were stable for 11 days when stored at 2 – 8 °C in the dark. No significant matrix effects (i.e > ±20% signal suppression or signal enhancement) were observed for M750F015, M750F016 and M750F017 in any of bovine plasma and human urine samples tested. Therefore, solvent calibration standards were used for the quantification for all matrices.
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 RSD for all fortification levels were below 20%. The method L0339/02 is suitable to determine M750F015, M750F016 and M750F017 in body fluids at a LOQ of 0.010 mg/L. The validation fulfils the requirements of SANCO/825/00 rev. 8.1, as well as SANCO/3029/99 rev. 4. with regard to specificity, repeatability, limit of quantification, recoveries and linearity.

Recoveries for M750F015 in Body Fluids

Matrix	Mass Transition	Fortification Level [mg/L]	Number of Replicates	Mean [%]	RSD [%]	Mean [%]	RSD [%]
Plasma	Quant. Ion 414 → 70	0.010	5	94.7	1.7	92.2	3.2
		0.10	5	89.8	1.6		
Plasma	Qual. Ion 414 → 182	0.010	5	95.4	1.9	92.7	3.6
		0.10	5	90.0	2.1		
Urine	Quant. Ion 414 → 70	0.010	5	99.5	2.8	97.1	3.5
		0.10	5	94.7	2.0		
Urine	Qual. Ion 414 → 182	0.010	5	102	3.1	98.2	4.7
		0.10	5	94.6	2.5		

Recoveries for M750F016 in Body Fluids

Matrix	Mass Transition	Fortification Level [mg/L]	Number of Replicates	Mean [%]	RSD[1)] [%]	Mean [%]	RSD[1)] [%]
Plasma	Quant. Ion 414 → 70	0.010	5	87.4	3.2	85.4	3.9
		0.10	5	83.4	3.3		
Plasma	Qual. Ion 414 → 182	0.010	5	86.8	2.7	85.2	3.5
		0.10	5	83.7	3.5		
Urine	Quant. Ion 414 → 70	0.010	5	97.6	2.0	95.3	3.0
		0.10	5	93.1	1.7		
Urine	Qual. Ion 414 → 182	0.010	5	100	3.9	96.2	5.0
		0.10	5	92.5	1.9		

Recoveries for M750F017 in Body Fluids

Matrix	Mass Transition	Fortification Level [mg/L]	Number of Replicates	Mean [%]	RSD[1)] [%]	Mean [%]	RSD[1)] [%]
Plasma	Quant. Ion 414 → 70	0.010	5	91.5	1.3	89.7	2.8
		0.10	5	87.9	2.6		
Plasma	Qual. Ion 414 → 143	0.010	5	90.5	1.8	88.1	3.2
		0.10	5	85.8	1.6		
Urine	Quant. Ion 414 → 70	0.010	5	98.8	2.0	95.9	3.6
		0.10	5	93.0	1.6		
Urine	Qual. Ion 414 → 143	0.010	5	99.1	2.4	95.2	4.9
		0.10	5	91.3	2.4		

Reference: CP 5.2/3

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

Homazava, N., 2019

report No EU-20180309,EU-867704,20180309

BASF DocID 2019/1046404

Authority registration No

Guideline(s): EPA 860.1340 (1996), OECD-ENV/JM/MONO/(2007)17, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010)

Deviations: No

GLP: yes

(certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Acceptability: Yes

Materials and methods

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

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

Results and discussions

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

Table A 5: Results of the method validation for the determination of M750F015, M750F016 and M750F017 in body fluids

Analyte	Matrix	m/z	Fortification level [mg metabolite/L]	Number of replicates	Mean recovery [%]	RSD [%]	Overall recovery [%]	Overall RSD [%]
M750F015	Plasma	414→70	0.010	5	94.7	1.7	92.2	3.2
			0.10	5	89.8	1.6		
		414→182	0.010	5	95.4	1.9	92.7	3.6
			0.10	5	90.0	2.1		
	Urine	414→70	0.010	5	99.5	2.8	97.1	3.6
			0.10	5	94.7	2.0		
		414→182	0.010	5	102	3.1	98.2	4.7
			0.10	5	94.6	2.5		

Table A 5: Results of the method validation for the determination of M750F015, M750F016 and M750F017 in body fluids

Analyte	Matrix	m/z	Fortification level [mg metabolite/L]	Number of replicates	Mean recovery [%]	RSD [%]	Overall recovery [%]	Overall RSD [%]
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 6: 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. The following calibration curves were received: M750F015 $y= 7.48 \cdot 10^5x+1.7710^4$ (transition 414 → 70), R=0.9996 $y=5.79 \cdot 10^4x+980$ (transition 414 → 143), R=0.9996 M750F016: $y=7.37 \cdot 10^5x+1.97 \cdot 10^4$ (transition 414 → 70), R=0.9996 $y=8.15 \cdot 10^4x+2.32 \cdot 10^3$ (transition 414 → 182), R=0.9994 M750F017: $y=7.9 \cdot 10^5x+1.81 \cdot 10^4$ (transition 414 → 70), R=0.9997 $y=5.54 \cdot 10^4x+1.55 \cdot 10^3$ (transition 414 → 143), R=0.9996
Calibration range	Calibration points distributed over a concentration range of 0.10 ng/mL to 10 ng/mL were used. This covers the tested concentration range.
Assessment of matrix effects is presented	No significant matrix effects (i.e > ±20% signal suppression or signal enhancement) were observed for M750F015, M750F016 and M750F017 in any of the body fluid matrices tested. Therefore, solvent calibration standards were used for the quantification for all matrices.

	M750F015, M750F016, M750F017
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.01 mg/L, corresponding to the lowest fortification level successfully tested. The limit of detection (LOD) is 0.10 ng/mL, corresponding to the lowest calibration standard.
Standard stability	Stability tests showed that M750F015, M750F016 and M750F017 stock, fortification and calibration solutions in acetonitrile and acetonitrile/water (1/1, v/v) were stable for 11 days, when stored refrigerated (2 – 8°C) in the dark. Raw extracts and final volume samples fortified at LOQ and 10x LOQ were shown to be stable for 8 days when stored refrigerated (2 – 8°C) in the dark for all body fluid matrices tested. Final volume samples were re-injected after 8 days of storage and raw extracts were carried through the complete work-up procedure and injected after 8 days of storage.

Conclusion

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

A 2.1.2.6 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted

A 2.2 Analytical methods for Sulfur

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

A 2.3 Analytical methods for the active substance 3

Not relevant