


Analytical method for mefentrifluconazole (BAS 750 F; Reg. No. 5834378) 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 water

- Reports:** ECM: EPA MRID No.: 49762550. Gooding R., and A.A. Guedez. 2017. Methods of Analysis of BAS 750 F and its Relevant Metabolites in Water with Limit of Determination (LOD) Calculation (L0359/01). BASF Study Nos: 836940 and 836940_1. BASF Registration Document No.: 2017/7008281. Report prepared by BASF Corporation, Crop Protection, Ecology and Environmental Analytics, Limburgerhof, Germany, and sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 285 pages. Final report issued May 25, 2017.
- ILV: EPA MRID No. 49762551. Stanislawski, T. 2017. Independent Laboratory Validation (ILV) of Method L0359/01 for the Determination of BAS 750 F (Reg. No.5834378) 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 and Surface Water by LC-MS/MS. BASF Study ID No.: 836906. PTRL Europe Study ID: P 4262 G. BASF Registration Document No.: 2017/1066522. Report prepared by EAG Laboratories, PTRL Europe GmbH, Ulm, Germany, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 185 pages. Final report issued May 2, 2017.
- Document No.:** MRIDs 49762550 & 49762551
- Guideline:** 850.6100
- Statements:** ECM: The study was not conducted in accordance with Good Laboratory Practice (GLP) standards since it was not a study (p. 3 of MRID 49762550). Signed and dated No Data Confidentiality and GLP statements were provided; Quality Assurance and Authenticity statements were not provided (pp. 2-3). The ECM was a compilation of the study reports for an analytical method and LOD determination (p. 5). These two study reports (BASF Study # 836940 and 836940_1) were conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160, as well as OECD and German GLP (Appendix A, pp. 10, 12; Appendix B, pp. 235, 237). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (Appendix A, pp. 9-12; Appendix B, pp. 234-237). Authenticity statements were not provided.
- ILV: The study was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160, as well as OECD and German GLP (pp. 3, 5 of MRID 49762551). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-5). Authenticity statement was not provided.

Classification: This analytical method is classified as supplemental. No documentation of the communication provided for review and clarification. The number of ILV trials required to validate the method was not specified.

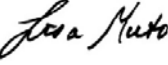
PC Code: 122000

EFED Final Reviewer: Dena Barrett

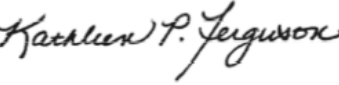
Signature: 
Date: 4/18/19

Senior Chemist

**CDM/CSS-
Dynamac JV
Reviewers:** Lisa Muto,
Environmental Scientist

Signature: 
Date: 3/6/18

Kathleen Ferguson, Ph.D.,
Environmental Scientist

Signature: 
Date: 3/6/18

Executive Summary

This analytical method, BASF Method L0359/01, is designed for the quantitative determination of mefentrifluconazole (BAS 750 F; Reg. No. 5834378), M750F003 (Reg. No. 5924326), M750F005 (Reg. No. 6003433), M750F006 (Reg. No. 5863469), M750F007 (Reg. No. 6003432) and M750F008 (Reg. No. 6010286) in water at the LOQ of 0.03 µg/L using LC/MS/MS. The LOQ for these methods should be less than the lowest toxicological level of concern in water for mefentrifluconazole and any metabolites that might be included in the Residues of Concern for drinking water exposure. This cannot be assessed currently since no risk assessment has yet been completed for this new chemical petition for registration. Although it was not specified, the reviewer assumed that the method was validated in the first trial with insignificant modifications to the analytical instrumentation; communication between the ECM and ILV was not detailed or clarified. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for all six analytes.

In a separate study, the method LOD of BASF Analytical Method No. L0359/01 was validated in accordance with the methodology set forth in 40 CFR Ch. 1 Part 136 Appendix B and determined to be 0.009 µg/L for the whole method.

Table 1. Analytical Method Summary – BASF Analytical Method L0359/01

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Mefentrifluconazole (BAS 750 F)	49762550 ¹	49762551		Water ^{2,3}	25/05/2017 ⁴	BASF Corporation	LC/MS/MS	0.03 µg/L
M750F003 (Reg. No. 5924326)								
M750F005 (Reg. No. 6003433)								
M750F006 (Reg. No. 5863469)								
M750F007 (Reg. No. 6003432)								
M750F008 (Reg. No. 6010286)								

1 A compilation of BASF Study #s 836940 and 836940_1.

2 In the ECM, ground water (pH 6.9, 1.1 mg/L total organic carbon, 17.2 mg/L calcium) obtained from Schifferstadt and surface water (pH 7.9, 12.2 mg/L total organic carbon, 32.8 mg/L calcium) obtained locally from Kelmetschweiher were used. Waters were characterized by BioChem agrar.

3 In the ILV, drinking water (pH 7.42, 0.58 mg/L total organic carbon, 124.0 mg/L calcium) obtained locally from Labor Alpha and surface (river) water (pH 7.93, 3.00 mg/L total organic carbon, 102.0 mg/L calcium) obtained locally from river Brenz, Herbrechtingen were used; characterization was completed by Institut Alpha.

4 Method date was May 12, 2017 for BASF Study # 836940 and 836940_1.

All referenced page numbers for MRID 49762550 refer to those reported on the right-handed margin of the document pages.

I. Principle of the Method

Method L0359/01

BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 in surface and in drinking water are extracted by shaking with ethyl acetate. Residues in an aliquot of the organic phase are evaporated to dryness, re-dissolved in acetonitrile/water (1/1, v/v), and analyzed by liquid chromatography using tandem mass spectrometric detection (LC-MS/MS), monitoring two mass transitions, one proposed for quantification and one for confirmation for each analyte: m/z 398→70 and 400→70 for parent BAS 750 F; m/z 288→70 and 288→43 for M750F003; m/z 380→70 and 380→109 for M750F005; m/z 356→259 and 356→217 for M750F006; m/z 338→241 and 338→269 for M750F007 and m/z 356→259 and 356→241 for M750F008. The results are calculated by direct comparison of the sample peak responses to those of external standards.

For validation, untreated drinking water and surface water samples were fortified with each analyte and analyzed according to the established method validation guidelines. The analytical sets for each water type consisted of two controls, five replicates fortified with analyte at the method limit of quantitation, 0.03 µg/L (0.030 ppb), and five replicates fortified at a higher level,

corresponding to 10X the limit of quantitation, 0.3 µg/L (0.3 ppb). For each analyte, the two mass transitions described above were evaluated. In conjunction with the subject study, matrix- and solvent-matched standards were analyzed in a separate experiment to evaluate any potential matrix effects.

Summary parameters for the analytical method are listed in the table shown below (Table 2).

Table 2: Summary Parameters for the Analytical Method L0359/01 Used for the Quantitation of BAS 750 F and Metabolite Residues in Drinking and Surface Water

Method ID	BASF method L0359/01		
Analyte(s)	Residues of BAS 750 F and M750F003, M750F005, M750F006, M750F007, and M750F008 in water		
Extraction Solvent/technique	Residues of BAS 750 F in water samples (5 mL each) are extracted by shaking with ethyl acetate. The residues in an aliquot of the organic phase are then evaporated to dryness, and re-dissolved in acetonitrile/water (1/1, v/v) prior to analysis.		
Cleanup Strategies	None		
Instrument	Waters Acquity LC chromatographic system with a Waters XBridge C18 column (50 x 2.1 mm, 2.5µm particle size) was used with a mobile phase gradient of acidified water and acidified acetonitrile (95:5 to 1:99, v/v, over ~3.5 minutes, flow rate 800 µL/minute).		
Detector	PE Sciex API 6500 Mass Spectrometer		
	Analyte	Quantitation (m/z)	Confirmation (m/z)
	BAS 750 F	398→70	400→70
	M750F003	288→70	288→43
	M750F005	380→70	380→109
	M750F006	356→259	356→217
	M750F007	338→241	338→269
	M750F008	356→259	356→241
Ionization Mode	Positive		
Standardization Method	Linear Regression, no weighting		
Stability of Std Solutions	The available storage stability data indicate that stock and fortification solutions of each analyte prepared in acetonitrile are stable held under refrigeration for at least 3 months. In addition, fortification and calibration standards prepared in acetonitrile/water (1/1, v/v) were shown to be stable for at least 1 month (30 days) when held under refrigeration.		
Retention times (approximate minutes) observed	BAS 750 F, 2.3; M750F003, 1.4; M750F005, 1.9; M750F006, 2.1; M750F007, 1.6; and M750F008, 1.9		

Instrument/Detector for Confirmatory Method: For each analyte, accurate quantitation is possible using one chromatographic method and two different mass transitions; therefore, no additional confirmatory techniques are required for these analytes.

A Method Flow Chart was provided (Appendix A, Figure A 19, p. 109 of MRID 49762550).

ILV

The ILV performed the ECM method as written with insignificant modifications of the analytical method (pp. 68, 71 of MRID 49762551). The ILV parameters are reported in Table 3 below.

Table 3: Summary Parameters for the Analytical Method L0359/01 Used in the ILV for the Quantitation of BAS 750 F and Metabolite Residues in Drinking and Surface Water

Method ID	BASF method L0359/01	
Analyte(s)	Residues of BAS 750 F and M750F003, M750F005, M750F006, M750F007, and M750F008 in water	
Extraction Solvent/technique	Residues of BAS 750 F in water samples (5 mL each) are extracted by shaking with ethyl acetate. The residues in an aliquot of the organic phase are then evaporated to dryness, and re-dissolved in acetonitrile/water (1/1, v/v) prior to analysis.	
Cleanup Strategies	None	
Instrument	Agilent 1290 infinity series chromatographic system with a Waters XBridge C18 column (50 x 2.1 mm, 2.5µm particle size) was used with a mobile phase gradient of acidified water and acidified acetonitrile (95:5 to 1:99, v/v, over ~3.5 minutes, flow rate 800 µL/minute).	
Detector	Applied Biosystems MDS Sciex API 6500 triple quadrupole LC-MS/MS system with TurboIonSpray (ESI) source	
Analyte	Quantitation (m/z)	Confirmation (m/z)
BAS 750 F	398→70	400→70
M750F003	288→70	288→43
M750F005	380→70	380→109
M750F006	356→259	356→217
M750F007	338→241	338→269
M750F008	356→259	356→241
Ionization Mode	Positive	Positive
Standardization Method	Linear Regression, no weighting	
Stability of Std Solutions	The available storage stability data indicate that stock and fortification solutions of each analyte prepared in acetonitrile are stable held under refrigeration for at least 3 months. In addition, fortification and calibration standards prepared in acetonitrile/water (1/1, v/v) were shown to be stable for at least 16 days when held under refrigeration.	
Retention times (approximate minutes) observed in the ILV	BAS 750 F, 2.4; M750F003, 1.5; M750F005, 2.0; M750F006, 2.2; M750F007, 1.8; and M750F008, 2.1	

The available storage stability data indicate that each analyte is stable in stock solutions prepared in acetonitrile stored under refrigeration for at least 3 months. In conjunction with the subject study, it was shown that each analyte is stable in stock solutions prepared in acetonitrile, and intermediate (fortification) and calibration standards prepared by dilution of the stock solutions in acetonitrile/water (1/1, v/v), when held under refrigeration in the dark for at least 16 days, the longest interval tested.

Instrument/Detector for Confirmatory Method: For each analyte, accurate quantitation is possible using one chromatographic method and two different mass transitions; therefore, no additional

confirmatory techniques are required for these analytes.

A Method Flow Chart was provided (Figure 7, p. 80 of MRID 49762551).

Methodology to Evaluate MDL and LOD

In conjunction with the method validation study for method L0359/01 (BASF Study No. 836940, BASF Reg. Doc. No. 2017/1066523), the least sensitive analyte/transition was determined by the injection of solvent standards. Based on this information, the appropriate analyte and transition was selected, and an estimation was made to what level a sample in matrix would produce a S/N ratio of 2-10 for the identified, worst-case analyte and transition. All analytes and mass transitions were monitored; however, only the results for the the least sensitive analyte/transition identified for the MDL determination was evaluated and was reported.

1. Using BASF Analytical Method L0359/01, seven control sample aliquots (5 mL, matrix surface water) were extracted by liquid/liquid extraction using ethyl acetate, an aliquot of the organic phase was evaporated to dryness and the residues reconstituted with acetonitrile/water (50/50, v/v).

For analysis, an aliquot (0.49 mL) from this solution was diluted with a calibration standard (acetonitrile/water 50/50 v/v; 0.01 mL of 1 ng/mL solution) to an appropriate spiking level for analysis by LC-MS/MS.

These seven matrix spiked samples were injected on the LC-MS/MS with bracketing solvent standards for quantitation.

2. Using the standard curve to calculate the concentrations of the seven matrix-spiked samples, the results were put into the equation shown below:

$$\text{MDL} = S \times t_{(N-1, 1-\alpha=0.99)}$$

MDL = Method detection limit

S = Standard deviation of the matrix-spiked sample set concentrations

$t_{(N-1, 1-\alpha=0.99)}$ = Critical t value from a student t-test table at 99% confidence

The acceptance criteria for the MDL calculation were:

- a. The calculated MDL must be able to be seen on the instrument with S/N of ≥ 2 .
 - b. The concentration of the matrix-spiked samples must be no greater than 10X the calculated MDL.
3. A matrix-spiked sample at the calculated MDL was injected on the LC-MS/MS (no standard curve was required) to verify the MDL could be observed at ≥ 2 S/N.

LOQ/LOD

The LOQ was defined by the lowest fortification level successfully tested. The validated LOQ for residues of BAS 750 F and its metabolites in water is 0.03 µg/L for each analyte. The limit of determination is set to be 0.009 µg/L for each analyte in water.

II. Recovery Findings

Method L0359/01

ECM (MRID 49762550): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of mefentrifluconazole (BAS 750 F; Reg. No. 5834378), M750F003 (Reg. No. 5924326), M750F005 (Reg. No. 6003433), M750F006 (Reg. No. 5863469), M750F007 (Reg. No. 6003432) and M750F008 (Reg. No. 6010286) in two water matrices at fortification levels of 0.03 µg/L (LOQ) and 0.3 µg/L (10×LOQ; Appendix A, pp. 14-16). Analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. Ground water (pH 6.9, 1.1 mg/L total organic carbon, 17.2 mg/L calcium) obtained from Schifferstadt and surface water (pH 7.9, 12.2 mg/L total organic carbon, 32.8 mg/L calcium) obtained locally from Kelmetschweiher were used (Appendix A, p. 33; Appendix A, Figures A 135-136, pp. 224-225). Waters were characterized by BioChem agrar.

ILV (MRID 49762551): Mean recoveries and RSDs were within guideline requirements for analysis of mefentrifluconazole (BAS 750 F; Reg. No. 5834378), M750F003 (Reg. No. 5924326), M750F005 (Reg. No. 6003433), M750F006 (Reg. No. 5863469), M750F007 (Reg. No. 6003432) and M750F008 (Reg. No. 6010286) in two water matrices at fortification levels of 0.03 µg/L (LOQ) and 0.3 µg/L (10×LOQ; pp. 9-10). Analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. Drinking water (pH 7.42, 0.58 mg/L total organic carbon, 124.0 mg/L calcium) obtained locally from Labor Alpha and surface (river) water (pH 7.93, 3.00 mg/L total organic carbon, 102.0 mg/L calcium) obtained locally from river Brenz, Herbrechtingen were used; characterization was completed by Institut Alpha (p. 30; Figures 128-129, pp. 184-185). Although it was not specified, the reviewer assumed that the method was validated in the first trial with insignificant modifications to the analytical instrumentation (pp. 8, 68, 71).

Table 1: Method Validation Recoveries for BAS 750 F and Metabolites in Fortified Control Water Samples Using BASF Analytical Method L0359/01¹

Matrix Water	Mass Transition	Fortification Level [µg/L]	Range (N)	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]		
BAS 750 F									
Ground Water	398 → 70	0.03	92-97 (5)	95	2.1	97	2.6		
		0.3	96-101 (5)	98	2.0				
	400 → 70	0.03	92-98 (5)	96	2.7	97	2.8		
		0.3	94-101 (5)	98	2.8				
Surface Water	398 → 70	0.03	102-105 (5)	103	1.3	102	1.5		
		0.3	100-104 (5)	102	1.5				
	400 → 70	0.03	98-106 (5)	101	3.2	100	3.2		
		0.3	94-101 (5)	98	2.7				
		M750F003							
		Ground Water	288 → 70	0.03	81-85 (5)	83	1.9	85	3.2
0.3	84-90 (5)			87	2.9				
288 → 43	0.03		81-97 (5)	87	7.6	88	6.0		
	0.3		84-91 (5)	89	4.3				
Surface Water	288 → 70	0.03	85-90 (5)	87	2.5	89	2.9		
		0.3	89-92 (5)	91	1.4				
	288 → 43	0.03	79-102 (5)	89	9.4	91	8.0		
		0.3	85-102 (5)	92	7.3				
M750F005									
Ground Water	380 → 70	0.03	85-91 (5)	88	2.7	90	3.6		
		0.3	89-96 (5)	93	3.0				
	380 → 109	0.03	83-91 (5)	88	3.5	89	4.9		
		0.3	85-99 (5)	91	5.6				
Surface Water	380 → 70	0.03	89-94 (5)	91	2.0	92	2.4		
		0.3	89-95 (5)	93	2.7				
	380 → 109	0.03	84-108 (5)	93	9.4	90	7.7		
		0.3	82-92 (5)	87	4.3				

Table 4: Method Validation Recoveries for BAS 750 F and Metabolites in Fortified Control Water Samples Using BASF Analytical Method L0359/01¹ (continued)

Matrix Water	Mass Transition	Fortification Level [µg/L]	Range (N)	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
M750F006							
Ground Water	356 → 259	0.03	93-102 (5)	96	3.9	98	3.6
		0.3	97-103 (5)	100	2.4		
	356 → 217	0.03	89-101 (5)	96	4.5	99	4.4
		0.3	99-105 (5)	101	2.1		
Surface Water	356 → 259	0.03	99-104 (5)	102	2.1	101	2.2
		0.3	98-104 (5)	100	2.3		
	356 → 217	0.03	99-107 (5)	101	3.7	101	3.7
		0.3	94-105 (5)	101	4.2		
M750F007							
Ground Water	338 → 241	0.03	79-87 (5)	84	3.7	85	4.6
		0.3	81-92 (5)	86	5.3		
	338 → 269	0.03	83-85 (5)	84	0.9	85	3.1
		0.3	82-90 (5)	86	3.8		
Surface Water	338 → 241	0.03	85-91 (5)	88	2.8	88	2.7
		0.3	86-92 (5)	89	3.0		
	338 → 269	0.03	79-85 (5)	83	2.7	83	2.3
		0.3	82-86 (5)	84	1.6		
M750F008							
Ground Water	356 → 259	0.03	87-95 (5)	91	3.2	92	3.8
		0.3	87-97 (5)	93	3.9		
	356 → 241	0.03	87-92 (5)	89	2.7	91	3.5
		0.3	89-96 (5)	93	2.7		
Surface Water	356 → 259	0.03	88-97 (5)	93	3.9	93	2.7
		0.3	92-94 (5)	93	1.0		
	356 → 241	0.03	88-95 (5)	92	3.8	93	3.5
		0.3	91-98 (5)	95	3.1		

Data (uncorrected recovery results, Appendix B, pp. 236-237 and Tables 10-21, pp. 246-251) were obtained from Appendix B, pp. 213 of MRID 49762550.

¹ Ground water (pH 6.9, 1.1 mg/L total organic carbon, 17.2 mg/L calcium) obtained from Schifferstadt and surface water (pH 7.9, 12.2 mg/L total organic carbon, 32.8 mg/L calcium) obtained locally from Kelmetschweiher were used (Appendix A, p. 33; Appendix A, Figures A 135-136, pp. 224-225). Waters were characterized by BioChem agrar.

Table 5: Independent Laboratory Validation Summary Results of Method L0359/01: Residues of BAS 750 F in Drinking and Surface Water¹

Matrix	Mass Transition	Fortification Level [µg/L]	Range (N)	Mean [%]	RSD [%]	Mean [%]	RSD [%]
BAS 750 F							
Drinking Water	Quant. Ion 398 m/z -> 70 m/z	0.030	82.7-94.3 (5)	87.7	5.6	95.0	11
		0.30	86.2-113 (5)	102	10		
	Qual. Ion 400 m/z -> 70 m/z	0.030	88.5-94.3 (5)	92.2	2.6	99.9	9.8
		0.30	93.6-113 (5)	108	7.4		
Surface Water	Quant. Ion 398 m/z -> 70 m/z	0.030	99.0-120 (5)	108	7.8	108	7.6
		0.30	99.0-122 (5)	108	8.3		
	Qual. Ion 400 m/z -> 70 m/z	0.030	107-114 (5)	110	2.8	109	2.6
		0.30	104-110 (5)	108	2.3		
M750F003							
Drinking Water	Quant. Ion 288 m/z -> 70 m/z	0.030	80.2-93.6 (5)	87.2	6.8	95.2	11
		0.30	89.7-110 (5)	103	8.3		
	Qual. Ion 288 m/z -> 43 m/z	0.030	80.5-97.2 (5)	88.3	7.2	97.4	11
		0.30	105-110 (5)	107	4.0		
Surface Water	Quant. Ion 288 m/z -> 70 m/z	0.030	97.6-107 (5)	102	3.3	102	3.4
		0.30	97.6-107 (5)	101	3.9		
	Qual. Ion 288 m/z -> 43 m/z	0.030	92.5-112 (5)	106	7.4	100	9.1
		0.30	89.1-108 (5)	94.9	7.7		
M750F005							
Drinking Water	Quant. Ion 380 m/z -> 70 m/z	0.030	81.2-96.7 (5)	90.8	7.2	97.5	11
		0.30	89.6-113 (5)	104	8.9		
	Qual. Ion 380 m/z -> 109 m/z	0.030	87.1-105 (5)	94.6	7.8	98.0	8.2
		0.30	91.9-109 (5)	101	7.7		
Surface Water	Quant. Ion 380 m/z -> 70 m/z	0.030	102-107 (5)	105	1.8	103	3.5
		0.30	97.0-109 (5)	102	4.4		
	Qual. Ion 380 m/z -> 109 m/z	0.030	103-111 (5)	107	2.9	103	8.1
		0.30	82.1-110 (5)	99.7	11		

Table 5: Independent Laboratory Validation Summary Results of Method L0359/01: Residues of BAS 750 F in Drinking and Surface Water¹ (continued)

Matrix	Mass Transition	Fortification Level [µg/L]	Range (N)	Mean [%]	RSD [%]	Mean [%]	RSD [%]
M750F006							
Drinking Water	Quant. Ion 356 m/z -> 259 m/z	0.030	87.1-103 (5)	95.5	6.5	102	8.7
		0.30	96.6-114 (5)	108	6.1		
	Qual. Ion 356 m/z -> 217 m/z	0.030	78.6-105 (5)	95.2	11	101	10.1
		0.30	96.6-110 (5)	107	5.5		
Surface Water	Quant. Ion 356 m/z -> 259 m/z	0.030	92.0-102 (6)	98.3	3.4	96.5	4.3
		0.30	88.6-101 (6)	94.7	4.6		
	Qual. Ion 356 m/z -> 217 m/z	0.030	89.7-110 (6)	102	7.9	101	6.6
		0.30	92.9-107 (6)	98.9	5.1		
M750F007							
Drinking Water	Quant. Ion 338 m/z -> 241 m/z	0.030	79.9-96.4 (5)	88.4	6.8	97.3	11
		0.30	95.8-111 (5)	106	5.7		
	Qual. Ion 338 m/z -> 269 m/z	0.030	80.5-100 (5)	88.7	8.8	97.2	13
		0.30	84.8-115 (5)	106	11		
Surface Water	Quant. Ion 338 m/z -> 241 m/z	0.030	96.2-102 (5)	98.8	3.0	99.3	5.6
		0.30	93.2-113 (5)	99.8	7.8		
	Qual. Ion 338 m/z -> 269 m/z	0.030	94.9-113 (5)	104	6.9	102	6.7
		0.30	91.6-105 (5)	98.7	5.7		
M750F008							
Drinking Water	Quant. Ion 356 m/z -> 259 m/z	0.030	77.4-95.9 (5)	86.8	9.1	92.4	11
		0.30	83.4-107 (5)	98.0	8.9		
	Qual. Ion 356 m/z -> 241 m/z	0.030	83.1-102 (5)	90.1	8.6	94.9	11
		0.30	81.2-108 (5)	99.7	11		
Surface Water	Quant. Ion 356 m/z -> 259 m/z	0.030	94.9-106 (5)	102	4.1	103	4.3
		0.30	99.9-110 (5)	104	4.5		
	Qual. Ion 356 m/z -> 241 m/z	0.030	80.3-107 (5)	96.5	11	99.4	9.2
		0.30	94.1-111 (5)	102	7.6		

Data (uncorrected recovery results) were obtained from p. 22 of MRID 49762551

¹ Drinking water (pH 7.42, 0.58 mg/L total organic carbon, 124.0 mg/L calcium) obtained locally from Labor Alpha and surface (river) water (pH 7.93, 3.00 mg/L total organic carbon, 102.0 mg/L calcium) obtained locally from river Brenz, Herbrechtingen were used; characterization was completed by Institut Alpha (p. 30; Figures 128-129, pp. 184-185).

III. Method Characteristics

The LOQ was defined as the lowest fortification level tested. The LOQ for residues of BAS 750 F (including parent compound and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008) in water was 0.030 µg/mL (0.030 ppb). The method LOD was defined as the lowest analyte concentration injected as a calibration solution, resulting in an LOD of 0.009 µg/L (30% of the LOQ).

The least sensitive analyte/transition for Method L0359/01 was determined by the injection of solvent standards. By comparison of the chromatograms of calibration solutions used, it was determined qualitatively that the MS/MS ion transition for M750F003 (m/z 288→43) was the least sensitive and, therefore, the best candidate to conduct the MDL/LOD evaluation.

Analyte	Mass Transition	Signal/Noise Ratio
BAS 750 F	398 → 70	346:1
	400 → 70	100:1
M750F006	356 → 259	118:1
	356 → 217	61:1
M750F003	288 → 70	22:1
	288 → 43	7:1
M750F007	338 → 241	40:1
	338 → 269	22:1
M750F005	380 → 70	172:1
	380 → 109	9:1
M750F008	356 → 259	15:1
	356 → 241	31:1

The MDL was determined to be 0.02 ng/mL based on sample measurement level. To determine this value, the seven matrix-spiked samples (matrix surface water) were spiked at 0.1 ng/mL and each sample was injected on the LC-MS/MS instrument using method L0359/01 for quantification. The calculated MDL was verified by injecting the same matrix-spiked samples with an injection volume of 10 µL on LC-MS/MS. Representative chromatograms were provided in the report.

This calculated MDL of 0.02 ng/mL based on sample measurement level, supports the LOD of 0.03 ng/mL (smallest calibration standard) originally defined for Method L0359/01, which gives a LOD of 0.009 µg/L for the whole method. The increase from the MDL to the stated LOD enables variability in the residue method, natural drift of the LC-MS/MS instrumentation, potential contamination issues, untested matrix effects, and potential unseen background interferences to be accounted for. For confirmation of the calculated MDL value, a matrix-spiked sample, spiked at the MDL level of 0.02 ng/mL, showed an acceptable signal to noise ratio of 2.6.

Table 6: Calculation of MDL for M750F003 (m/z 288→43), the Least Sensitive Analyte/Ion Transition for Method L0359/01, using the Methodology Set Forth in 40 CFR Ch 1 Part 136 Appendix B

0.1 ng/mL Matrix-Spike Replicate	Measured Concentration (ng/mL)
1	0.101
2	0.108
3	0.092
4	0.092
5	0.095
6	0.094
7	0.104
Standard Deviation (S) =	0.006
N-1 =	6
Critical t value (t) =	3.143
Injection Volume (μL)	10
MDL (ng/mL)=	0.020

Table 7. Method Characteristics - Analytical Method L0359/01

Analyte		Mefentrifluconazole (BAS 750 F; Reg. No. 5834378)	M750F003 (Reg. No. 5924326)	M750F005 (Reg. No. 6003433)	M750F006 (Reg. No. 5863469)	M750F007 (Reg. No. 6003432)	M750F008 (Reg. No. 6010286)
Limit of Quantitation (LOQ)	ECM	0.030 µg/mL (0.030 ppb)					
	ILV						
Limit of Detection (LOD)	ECM	0.009 µg/L (30% of the LOQ)					
	ILV						
Linearity (calibration curve r^2 and concentration range) ¹	ECM	$r^2 = 0.9998$ (Q & C)	$r^2 = 0.9990$ (Q) $r^2 = 0.9986$ (C)	$r^2 = 0.9998$ (Q) $r^2 = 0.9980$ (C)	$r^2 = 0.9998$ (Q & C)	$r^2 = 0.9994$ (Q) $r^2 = 0.9990$ (C)	$r^2 = 0.9972$ (Q) $r^2 = 0.9992$ (C)
	ILV	$r^2 = 0.9986$ (Q) $r^2 = 0.9988$ (C)	$r^2 = 0.9996$ (Q) $r^2 = 0.9962$ (C)	$r^2 = 0.9996$ (Q) $r^2 = 0.9970$ (C)	$r^2 = 0.9994$ (Q) $r^2 = 0.9988$ (C)	$r^2 = 0.9996$ (Q) $r^2 = 0.9982$ (C)	$r^2 = 0.9984$ (Q) $r^2 = 0.9986$ (C)
	Concentration Range	0.03-1 ng/mL					
Repeatable	ECM ²	Yes at LOQ and 10×LOQ (two characterized water matrices)					
	ILV ^{3,4}	Yes at LOQ and 10×LOQ (two characterized water matrices)					
Reproducible	Yes at LOQ and 10×LOQ						
Specific	ECM ⁵	Yes, no matrix interferences were observed.		Yes, no matrix interferences were observed. Analyte peak was small at LOQ compared to baseline noise (C). ⁶	Yes, no matrix interferences were observed.		
	ILV ⁷	Yes, no matrix interferences were observed. Minor baseline noise interfered with analyte peak attenuation and integration.	Yes, no matrix interferences were observed. Analyte peak was small at LOQ compared to baseline noise.		Yes, no matrix interferences were observed. Minor baseline noise interfered with analyte peak attenuation and integration.	Yes, no matrix interferences were observed. Analyte peak was small at LOQ compared to baseline noise (C). ⁶	

Data were obtained from Appendix A of MRID 49762550; MRID 49762551. Q = Quantitation ion transition or primary method; C = Confirmation ion transition or method. NA = Not applicable.

1 Correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (see DER Attachment 2). Solvent-based calibration standards were used in the ECM and ILV.

2 In the ECM, ground water (pH 6.9, 1.1 mg/L total organic carbon, 17.2 mg/L calcium) obtained from Schifferstadt and surface water (pH 7.9, 12.2 mg/L total organic carbon, 32.8 mg/L calcium) obtained locally from Kelmetschweiher were used (Appendix A, p. 33; Appendix A, Figures A 135-136, pp. 224-225 of MRID 49762550). Waters were characterized by BioChem agrar.

- 3 In the ILV, drinking water (pH 7.42, 0.58 mg/L total organic carbon, 124.0 mg/L calcium) obtained locally from Labor Alpha and surface (river) water (pH 7.93, 3.00 mg/L total organic carbon, 102.0 mg/L calcium) obtained locally from river Brenz, Herbrechtingen were used; characterization was completed by Institut Alpha (p. 30; Figures 128-129, pp. 184-185 of MRID 49762551).
- 4 Although it was not specified, the reviewer assumed that the method was validated in the first trial with insignificant modifications to the analytical instrumentation (pp. 8, 68, 71 of MRID 49762551).
- 5 Based on Appendix A, Figures A 44-127, pp. 123-164 of MRID 49762550.
- 6 A confirmation method is not usually required when LC/MS or GC/MS is the primary method to generate study data.
- 7 Based on Figures 10-115, pp. 83-135 of MRID 49762551.

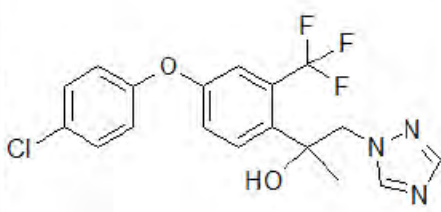
IV. Method Deficiencies and Reviewer's Comments

1. No documentation of the communication provided for review. The ILV study author briefly summarized that communications between the ILV laboratory and ECM study sponsor (Study Monitor) asking for clarification of details concerning the method procedure. The communication regarded the first provided version of the Technical Procedure which contained inconsistencies regarding extraction volumes and fortification and calibration solutions (p. 68 of MRID 49762551). The identity of the Study Monitor was not reported in the ILV study report.
2. The number of ILV trials required to validate the method was not specified; the reviewer assumed that the method was validated in the first trial with insignificant modifications to the analytical instrumentation (pp. 8, 68, 71 of MRID 49762551).
3. The reviewer noted that the ILV referenced an ECM (Version of February 2017) which appeared to be an earlier version of the ECM provided in MRID 49762550 (dated May 12, 2017; p. 8 of MRID 49762550; pp. 6, 71-72 of MRID 49762551).
4. In the ECM, it was reported that the available storage stability data indicate that each analyte is stable in stock solutions prepared in acetonitrile stored under refrigeration for at least 3 months (Tier II Summary for MRID 49762550). In conjunction with the subject study, it was shown that each analyte is stable in stock solutions prepared in acetonitrile, and intermediate (fortification) and calibration standards prepared by dilution of the stock solutions in acetonitrile/water (1/1, v/v), when held under refrigeration in the dark, for at least 16 days, the longest interval tested. The stability of each analyte in water extracts was also determined in this study. Residues of BAS 750 F and its metabolites were shown to be stable in the final sample extracts of acetonitrile/water (1/1, v/v) for 1-2 weeks under refrigerated storage conditions. Also, the generally acceptable method validation recoveries obtained with each set demonstrated the stability of each analyte in the water samples stored prior to analysis.
5. It was reported for the ILV that one sample set of 12 samples required *ca.* 10 hours, including calculation of results (p. 70 of MRID 49762551).

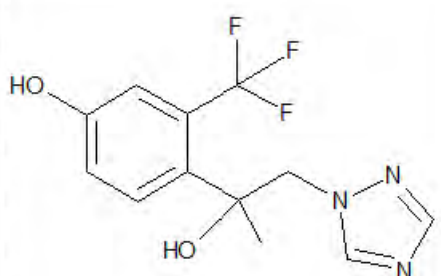
V. References

- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

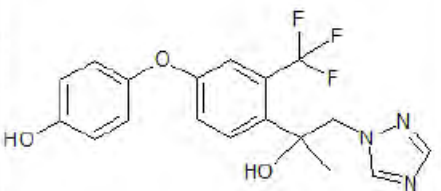
Attachment 1: Chemical Names and Structures**2.2.1 Reg.No.5834378**

BAS-Code	750 F	
Metabolite-Code	M750F000	
IUPAC Name	(2RS)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol	
BASF Reg.No.	5834378	
Batch No.	L85-124	
CAS-No.	1417782-03-6	
Molecular Formula	C ₁₈ H ₁₅ ClF ₃ N ₃ O ₂	
Molecular Weight	397.8	

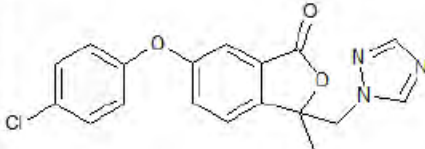
2.2.2 Reg.No.5924326

Metabolite-Code	M750F003	
IUPAC Name	4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenol	
BASF Reg.No.	5924326	
Batch No.	L84-250	
CAS-No.	–	
Molecular Formula	C ₁₂ H ₁₂ F ₃ N ₃ O ₂	
Molecular Weight	287.2	

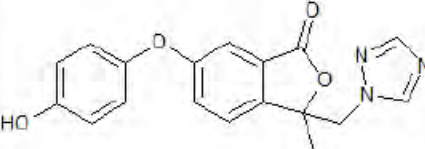
2.2.3 Reg.No.6003433

Metabolite-Code	M750F005	
IUPAC Name	4-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenol	
BASF Reg.No.	6003433	
Batch No.	L87-34	
CAS-No.	–	
Molecular Formula	C ₁₈ H ₁₅ F ₃ N ₃ O ₃	
Molecular Weight	379.3	

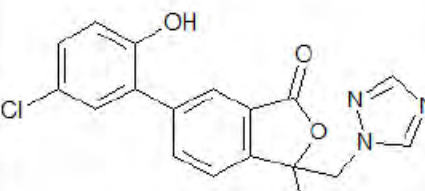
2.2.4 Reg.No.5863469

Metabolite-Code	M750F006	
IUPAC Name	6-(4-chlorophenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one	
BASF Reg.No.	5863469	
Batch No.	L85-170	
CAS-No.	–	
Molecular Formula	C ₁₈ H ₁₄ ClN ₃ O ₃	
Molecular Weight	355.8	

2.2.5 Reg.No.6003432

Metabolite-Code	M750F007	
IUPAC Name	6-(4-hydroxyphenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one	
BASF Reg.No.	6003432	
Batch No.	L87-32-1	
CAS-No.	–	
Molecular Formula	C ₁₈ H ₁₅ N ₃ O ₄	
Molecular Weight	337.3	

2.2.6 Reg.No.6010286

Metabolite-Code	M750F008	
IUPAC Name	6-(5-chloro-2-hydroxyphenyl)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one	
BASF Reg.No.	6010286	
Batch No.	L85-94	
CAS-No.	–	
Molecular Formula	C ₁₈ H ₁₄ ClN ₃ O ₃	
Molecular Weight	355.8	