

Analytical method for mefentrifluconazole metabolites M750F002 (Reg.No. 6031465), M750F036 (Reg.No. 6055268) and M750F037 (Reg.No. 148502) in water

Reports: ECM: EPA MRID No.: 49762552. Gooding R. 2017. Method of Analysis of Additional Metabolites of BAS 750 F in Water with Limit of Determination (LOD) Calculation. BASF Study No.s: 788121 and 835160_1. BASF Registration Document No.: 2017/7008277. Report prepared, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 324 pages. Final report issued May 23, 2017.

ILV: EPA MRID No. 49762448. Perez, R., A. Ratliff. 2016. Independent Laboratory Validation of BASF Analytical Method D1605/01: "Method for the determination of M750F002 (Reg.No. 6031465), M750F036 (Reg.No. 6055268), and M750F037 (Reg.No. 148502) in Surface and Drinking Water by LC-MS/MS". BASF Study ID No.: 788120. BASF Registration Document No.: 2016/7006519. ADPEN Study No.: 16G0104. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 165 pages. Final report issued September 26, 2016.

Document No.: MRIDs 49762552 & 49762448



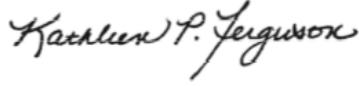
Guideline: 850.6100

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 49762552). 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 # 788121 and 835160_1) were conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (Appendix A, p. 10; Appendix B, p. 248). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (Appendix A, pp. 9-12; Appendix B, pp. 247-250).

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 49762448). 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. ILV validation did not fully satisfy the reproducibility of the method for M750F037 due to poor repeatability and specificity. The chemical purities of M750F036 and M750F037 were <90%. It could not be determined if the ILV matrices were adequately rigorous for the validation.

PC Code: 122000

**EFED Final
Reviewer:**Dena Barrett
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Date: 4/18/19**CDM/CSS-
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Date: 3/6/18Kathleen Ferguson, Ph.D.,
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Date: 3/6/18**Executive Summary**

This analytical method, BASF Method D1605/01, is designed for the quantitative determination of mefentrifluconazole metabolites M750F002 (Reg.No. 6031465), M750F036 (Reg.No. 6055268) and M750F037 (Reg.No. 148502) in water at the Limit of Quantification (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 at this time since no risk assessment has yet been completed for this new chemical petition for registration. The ECM and ILV validated the method with surface and ground water. However, it could not be determined if the ILV matrices were adequately rigorous for the validation since the ground water was the same as that of the ECM and the surface water was barely characterized. The method was validated in the first trial with modifications of the analytical method, including reduction of several HPLC injection volumes, changing the size of the analytical columns, and inversion of the mass transitions for M750F002. The ILV recommended that these modifications should be incorporated into the technical procedure; however, the reviewer could not recommend that the ECM be updated with the ILV modifications because no improvement was observed for the validations of M750F002 and M750F036 and less satisfactory data was presented by the ILV for the validation of M750F037, including unsatisfactory performance data in the surface water and unsatisfactory resolution in representative chromatograms. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for M750F002 and M750F036, and the ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for M750F037. However, the chemical purities of M750F036 and M750F037 were <90%. Overall, the ECM method was validated by the ILV for M750F002 and M750F036, but the ILV validation did not fully satisfy the reproducibility of the method for M750F037.

In a separate study, the method LOD of BASF Analytical Method No. D1605/01 was validated in accordance with the methodology set forth in 40 CFR Ch. 1 Part 136 Appendix B and determined to be 0.006 ng/mL for all analytes in water.

Table 1. Analytical Method Summary – BASF Analytical Method D1605/01

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						
M750F002 (Reg.No. 6031465)	49762552 ¹	49762448		Water ^{2,3}	23/05/2017 ⁴	BASF Corporation	LC/MS/MS	0.03 µg/L
M750F036 (Reg.No. 6055268)								
M750F037 (Reg.No. 148502)								

1 A compilation of BASF Study #s 788121 and 835160_1.

2 In the ECM, surface (lake) water (Golden Lake Water; pH 8.3, 14.4 ppm total organic carbon, hardness 693 mg equivalent CaCO₃/L) and ground water (CM15-030; pH 7.5, 0.8 ppm total organic carbon, hardness 116 mg equivalent CaCO₃/L) were used. Waters were characterized by Agvise Laboratories, Northwood, North Dakota; water sources were not further specified.

3 In the ILV, surface (lake) water (Golden Lake Water; 2015-11/SDBN418 Water; pH not reported, 15.1 ppm total organic carbon, hardness not reported) and ground water (CM15-030; pH 7.5, 0.8 ppm total organic carbon, hardness 116 mg equivalent CaCO₃/L) were used. Waters were characterized by Agvise Laboratories, Northwood, North Dakota, and supplied by the sponsor. The ground water of the ILV was the same as that of the ECM. Water sources were not further specified.

4 Method dates were April 20, 2017 for BASF Study # 788121 and May 2, 2017 for BASF Study # 835160_1.

All referenced page numbers for MRID 49762552 refer to those reported on the right-handed margin of the document pages.

I. Principle of the Method

Method D1605/01

M750F002, M750F036 and M750F037 residues in water samples are acidified with formic acid and then analyzed by direct injection onto a HPLC column with detection by MS/MS monitoring in the positive ionization mode ion transitions m/z 246→70 and 246→149 for M750F002, m/z 252→70 for M750F036, and m/z 126→70 and 126→99 for M750F037. In lieu of secondary ion transitions for M750F036, confirmatory analysis is performed using a different LC-MS/MS column. The results are calculated by direct comparison of the sample peak responses to those of external standards.

For validation, untreated drinking (well) water and surface (lake) water samples were fortified with each analyte and analyzed according to the established method validation guidelines. The analytical sets for each water type typically consisted of a reagent blank, two controls, five replicates fortified with analyte at the method limit of quantitation, 30 ng/L (30 ppt), and five replicates fortified at a higher level, corresponding to 10X the limit of quantitation, 300 ng/L (300 ppt). For each analyte, the two mass transitions or confirmatory LC-MS/MS procedures 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 D1605/01 Used for the Quantitation of BAS 750 F Metabolite Residues in Drinking and Surface Water

Method ID	BASF method D1605/01	
Analyte(s)	M750F002, M750F036, and M750F037 residues in water	
Extraction Solvent/technique	None. M750F002, M750F036, and M750F037 residues in water samples (10 mL each) are acidified with formic acid and mixed.	
Cleanup Strategies	None	
Instrument	Analyses are performed using a Waters Aquity UPLC system equipped with an XSelect HSS T3 C18 column (150 x 3 mm, 2.5µm particle size) or, for confirmatory purposes for M750F036, an Xbridge BEH phenyl column (150 x 3 mm, 2.5µm particle size) using a mobile phase gradient of water:acetonitrile, each acidified with 0.1% formic acid (flow rate 600 µL/minute).	
Detector	AB Sciex API 6500 Mass Spectrometer	
Analyte	Quantitation (<i>m/z</i>)	Confirmation (<i>m/z</i>)
M750F002	246→70	246→149
M750F036	252→70	252→70*
M750F037	126→70	126→99
Ionization Mode	Positive	Positive
Standardization Method	Linear Regression, equal weighting	
Stability of Std Solutions	The stability of each analyte in standard solutions was determined in conjunction with the subject study. Stock and intermediate (fortification) standards of each analyte prepared in water and calibration standards prepared by dilution of the intermediate standards in acidified water were shown to be stable, when held under refrigeration, for at least 1 month (30 days).	
Expected Retention times (approximate minutes)	M750F002, 4.0; M750F036, 3.3; M750F037, 2.2; M750F036, 3.4 (using phenyl column)	

*In lieu of secondary (alternate) ion transitions for M750F036, confirmatory analysis was performed using a different LC-MS/MS column.

Instrument/Detector for Confirmatory Method: For M750F002 and M750F037, accurate quantitation is possible using one chromatographic method and two different mass transitions; therefore, no additional confirmatory techniques are required for these analytes. In lieu of secondary (alternate) ion transitions for M750F036, a confirmatory technique is available - and was validated in the subject study - using a different LC-MS/MS column (phenyl column).

A Method Flow Chart was provided (Appendix A, p. 50 of MRID 49762552).

ILV

The ILV performed the ECM method as written with modifications of the analytical method, including reduction of the injection volume for M750F036 using the XBridge BEH Phenyl LC column (100 µL to 40 µL) and for all analytes using the XSelect HSS T3 LC column (100 µL to 80 µL), changing the analytical columns, and inversion of the mass transitions for M750F002 (p. 23; Appendix A, p. 114 of MRID 49762448). The ILV recommended that these modifications should be incorporated into the technical procedure. The ILV parameters are reported in Table 3 below.

Table 3: Summary Parameters for the Analytical Method D1605/01 Used in the ILV for the Quantitation of BAS 750 F Metabolite Residues in Drinking and Surface Water

Method ID	BASF method D1605/01	
Analyte(s)	M750F002, M750F036, and M750F037 residues in water	
Extraction Solvent/technique	None. M750F002, M750F036, and M750F037 residues in water samples (10 mL each) are acidified with formic acid and mixed.	
Cleanup Strategies	None	
Instrument	Analyses were performed using an Agilent 1290 UPLC System equipped with an XSelect HSS T3 C18 column (150 x 2.1 mm, 2.5 µm particle size) or, for confirmatory purposes for M750F036, an Xbridge BEH phenyl column (100 x 2.1 mm, 2.5 µm particle size) using a mobile phase gradient of water:acetonitrile, each acidified with 0.1% formic acid (flow rate 800 uL/minute).	
Detector	AB Sciex 6500 QT Mass Spectrometer	
	Quantitation (m/z)	Confirmation (m/z)
Analyte		
M750F002	246→149	246→70
M750F036	252→70	252→70*
M750F037	126→70	126→99
Ionization Mode	Positive	Positive
Standardization Method	Linear Regression, 1/x weighting	
Stability of Std Solutions	The available storage stability data indicate that each analyte is stable in stock and intermediate (fortification) standards prepared in water and calibration standards prepared by dilution of the intermediate standards in acidified water, when held under refrigeration, for at least 1 month (30 days).	
Expected Retention times (approximate minutes)	M750F002, 2.6; M750F036, 2.0; M750F037, 1.0; M750F036, 1.5 (using phenyl column)	

*In lieu of secondary (alternate) ion transitions for M750F036, confirmatory analysis was performed using a different LC-MS/MS column.

Instrument/Detector for Confirmatory Method: For M750F002 and M750F037, accurate quantitation is possible using one chromatographic method and two different mass transitions; therefore, no additional confirmatory techniques are required for these analytes. In lieu of secondary (alternate) ion transitions for M750F036, a confirmatory technique is available - and was validated in the subject study - using a different LC-MS/MS column (phenyl column).

Methodology to Evaluate MDL and LOD

Matrix/Medium: Surface (lake) water samples, which were characterized (a GLP water characterization report was attached to the final report). Matrix effects were evaluated in the validation of method D1605/01. While no significant matrix effects were shown ($\geq 20\%$), surface water did show consistently higher matrix effects when compared to drinking water. Therefore, surface water was chosen as the water to use when conducting the MDL and LOD evaluation.

Evaluation of LOD of BASF Analytical Method No. D1605/01 required the experimental determination of MDL as defined by 40 CFR Ch.1 Part 136 appendix B. Within the scope of the method to determine the residues of M750F002, M750F036, and M750F037, two independent injections were used for analysis. A brief description of the methodology to determine MDL is as follows:

1. Injections of standards containing all analytes were injected using LC-MS/MS parameters from D1605/01. All transitions were monitored, including secondary chromatographic methods. The least sensitive transition of the least sensitive analyte for each independent injection of the method was determined qualitatively through visual inspection of factors such as peak height, relative background level, area count, etc. Once the appropriate analyte and transition was selected, an estimation was made to what level a sample in matrix would produce a S/N of 2-10.
2. Using BASF Analytical Method No. D1605/01, seven control samples (10 g) were analyzed by adding 10 μ L of formic acid to each sample. For analysis, the extract was fortified with a spiking solution (water, 0.01 mL) 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 neat standards for quantitation.
3. Using the standard curve to calculate the concentrations of the seven matrix-spiked samples, the results are put into the equation below:

$$\text{MDL} = S \times t_{(N-1, 1-\alpha=.99)}$$

MDL = Method detection limit

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

$t_{(N-1, 1-\alpha=.99)}$ = 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.

If either of the above to criteria were not met, the experiment had to be repeated at a higher or lower spiking concentration, respectively, until all criteria are met. Reinjection of the samples with lower or higher injection volumes could be done in place of preparing new matrix-spiked samples as it changes the amount (pg) injected on the column the same.

A matrix-spiked sample at the calculated MDL was injected on the LC-MS/MS (no standard curve is required) to verify the MDL can be seen at ≥ 2 S/N.

LOQ/LOD

The LOQ was defined by the lowest fortification level successfully tested. The validated LOQ for M750F002, M750F036, and M750F037 residues in water is 0.03 μ g/L. The limit of determination is set to be 0.006 μ g/L (20% of the LOQ) for each analyte in water.

II. Recovery Findings

Method D1605/01

ECM (MRID 49762552): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD $\leq 20\%$) for analysis of mefentrifluconazole metabolites M750F002 (Reg.No. 6031465), M750F036 (Reg.No. 6055268) and M750F037

(Reg.No. 148502) in two water matrices at fortification levels of 0.03 µg/L (LOQ) and 0.3 µg/L (10×LOQ; Appendix A, pp. 16-17). Recoveries in both matrices were calculated using solvent-based and matrix-matched calibration curves. Analytes were identified using two ion transitions. Performance data (recovery results) from primary and confirmatory analyses were acceptable and comparable, except in the case of M750F036 in surface water for which matrix-matched standards were required. Surface (lake) water (Golden Lake Water; 2015-13 SDBN420; pH 8.3, 14.4 ppm total organic carbon, hardness 693 mg equivalent CaCO₃/L) and ground water (CM15-030; pH 7.5, 0.8 ppm total organic carbon, hardness 116 mg equivalent CaCO₃/L) were used (Appendix A p. 21; Appendix A, Appendix K, pp. 237-238). Waters were characterized by Agvise Laboratories, Northwood, North Dakota; water sources were not further specified.

ILV (MRID 49762448): Mean recoveries and RSDs were within guideline requirements for analysis of mefentrifluconazole metabolites M750F002 (Reg.No. 6031465), M750F036 (Reg.No. 6055268) and M750F037 (Reg.No. 148502) in two water matrices at fortification levels of 0.03 µg/L (LOQ) and 0.3 µg/L (10×LOQ), except mean recoveries of M750F037 at the LOQ in surface water (61-62%; pp. 9-10). Recoveries in the surface and ground water matrices were calculated using solvent-based and matrix-matched calibration curves, respectively. Analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable, except for M750F037 at 10×LOQ in surface water. Surface (lake) water (Golden Lake Water; 2015-11/SDBN418 Water; pH not reported, 15.1 ppm total organic carbon, hardness not reported) and ground water (CM15-030; pH 7.5, 0.8 ppm total organic carbon, hardness 116 mg equivalent CaCO₃/L) were used (pp. 16-17; Appendix E, pp. 152-155). Waters were characterized by Agvise Laboratories, Northwood, North Dakota, and supplied by the sponsor. The ground water of the ILV was the same as that of the ECM. Water sources were not further specified. The method was validated in the first trial with modifications of the analytical method, including reduction of the injection volume for M750F036 using the XBridge BEH Phenyl LC column (100 µL to 40 µL) and for all analytes using the XSelect HSS T3 LC column (100 µL to 80 µL), changing the size of the analytical columns, and inversion of the mass transitions for M750F002 (p. 23; Appendix A, p. 114).

Table 4: Method Validation Recoveries for BAS 750 F Water Metabolites in Fortified Control Water Samples¹ Using BASF Analytical Method D1605/01

Matrix	Fortification Levels (µg/L)	n	Recovery (%)	Average Recovery (%)	Standard Deviation	%RSD ^a
Surface Water (Solvent-Based Standards)	M750F002: Primary Quantitation (m/z 246→ m/z 70)					
	0.030	5	88, 91, 91, 81, 86	87	4	5
	0.300	5	89, 85, 95, 83, 94	89	5	6
	Overall	10	Range, 81 - 95	88	5	5
	M750F002: Confirmatory Quantitation (m/z 246→ m/z 149)					
	0.030	5	80, 89, 86, 80, 89	85	5	5
	0.300	5	82, 86, 88, 82, 91	86	4	5
	Overall	10	Range, 80 - 91	85	4	5
	M750F036: Primary Quantitation (m/z 252→ m/z 70)					
	0.030	5	102, 112, 104, 95, 105	104	6	6
	0.300	5	103, 105, 101, 100, 109	104	4	3
	Overall	10	Range, 95 - 112	104	5	5
	M750F036: Confirmatory Quantitation (m/z 252→ m/z 70)					
	Matrix-matched standards were required (refer to subsequent pages) ^b					
	M750F037: Primary Quantitation (m/z 126→ m/z 70)					
	0.030	5	81, 75, 84, 75, 79	79	4	5
	0.300	5	76, 78, 82, 73, 77	77	3	4
	Overall	10	Range, 73 - 84	78	3	4
	M750F037: Confirmatory Quantitation (m/z 126 → m/z 99)					
	0.030	5	77, 86, 80, 75, 91	82	7	8
0.300	5	71, 86, 75, 78, 68	76	7	9	
Overall	10	Range, 68 - 91	79	7	9	
Drinking Water (Solvent-Based Standards)	M750F002: Primary Quantitation (m/z 246→ m/z 70)					
	0.030	5	96, 95, 91, 88, 87	91	4	4
	0.300	5	99, 100, 96, 98, 97	98	2	2
	Overall	10	Range, 87 - 100	95	5	5
	M750F002: Confirmatory Quantitation (m/z 246→ m/z 149)					
	0.030	5	96, 92, 91, 92, 90	92	2	2
	0.300	5	93, 95, 98, 96, 97	96	2	2
	Overall	10	Range, 90 - 98	94	3	3
	M750F036: Primary Quantitation (m/z 252→ m/z 70)					
	0.030	5	90, 90, 87, 90, 84	88	3	3
	0.300	5	93, 97, 97, 92, 94	95	2	2
	Overall	10	Range, 84 - 97	91	4	5
	M750F036: Confirmatory Quantitation (m/z 252→ m/z 70)					
	0.030	5	93, 94, 90, 89, 83	90	4	5
	0.300	5	93, 94, 99, 92, 88	93	4	4
	Overall	10	Range, 83 - 99	92	4	5
	M750F037: Primary Quantitation (m/z 126→ m/z 70)					
	0.030	5	87, 87, 89, 85, 83	86	2	3
	0.300	5	87, 86, 89, 89, 92	89	2	3
	Overall	10	Range, 83 - 92	87	3	3
M750F037: Confirmatory Quantitation (m/z 126 → m/z 99)						
0.030	5	94, 100, 89, 84, 80	89	8	9	
0.300	5	82, 87, 89, 94, 86	88	4	5	
Overall	10	Range, 80 - 100	89	6	7	

^aRelative Standard Deviation = (Standard Deviation ÷ Average Recovery) × 100

^bMatrix-related effects necessitated the use of recovery data generated using matrix-matched standards.

Table 4: Method Validation Recoveries for BAS 750 F Water Metabolites in Fortified Control Water Samples¹ Using BASF Analytical Method D1605/01 (continued)

Matrix	Fortification Levels (µg/L)	n	Recovery (%)	Average Recovery (%)	Standard Deviation	%RSD ^a
Surface Water (Matrix-Matched Standards)	M750F002: Primary Quantitation (m/z 246 → m/z 70)					
	0.030	5	86, 98, 92, 84, 92	90	6	6
	0.300	5	92, 95, 101, 93, 98	96	4	4
	Overall	10	Range, 84 - 101	93	5	6
	M750F002: Confirmatory Quantitation (m/z 246 → m/z 149)					
	0.030	5	88, 96, 97, 86, 95	92	5	5
	0.300	5	92, 92, 98, 90, 99	94	4	4
	Overall	10	Range, 86 - 99	93	4	5
	M750F036: Primary Quantitation (m/z 252 → m/z 70)					
	0.030	5	90, 93, 99, 86, 97	93	5	6
	0.300	5	92, 94, 97, 92, 101	95	4	4
	Overall	10	Range, 86 - 101	94	4	5
	M750F036: Confirmatory Quantitation (m/z 252 → m/z 70)					
	0.030	5	87, 92, 98, 83, 94	91	6	6
	0.300	5	98, 90, 94, 91, 95	94	3	3
	Overall	10	Range, 83 - 98	92	5	5
	M750F037: Primary Quantitation (m/z 126 → m/z 70)					
	0.030	5	92, 96, 99, 87, 92	93	5	5
	0.300	5	87, 87, 100, 91, 98	93	6	7
	Overall	10	Range, 87 - 100	93	5	5
	M750F037: Confirmatory Quantitation (m/z 126 → m/z 99)					
	0.030	5	93, 91, 104, 86, 82	91	8	9
	0.300	5	98, 104, 100, 103, 102	101	2	2
	Overall	10	Range, 82 - 104	96	8	8
Drinking Water (Matrix-Matched Standards)	M750F002: Primary Quantitation (m/z 246 → m/z 70)					
	0.030	5	91, 91, 89, 88, 87	89	2	2
	0.300	5	92, 89, 94, 93, 96	93	3	3
	Overall	10	Range, 87 - 96	91	3	3
	M750F002: Confirmatory Quantitation (m/z 246 → m/z 149)					
	0.030	5	94, 91, 90, 88, 88	90	2	3
	0.300	5	91, 92, 95, 93, 92	93	2	2
	Overall	10	Range, 88 - 95	91	2	3
	M750F036: Primary Quantitation (m/z 252 → m/z 70)					
	0.030	5	94, 91, 94, 90, 85	91	4	4
	0.300	5	95, 92, 97, 94, 94	94	2	2
	Overall	10	Range, 85 - 97	93	3	4
	M750F036: Confirmatory Quantitation (m/z 252 → m/z 70)					
	0.030	5	90, 95, 89, 90, 85	90	4	4
	0.300	5	90, 94, 95, 96, 94	94	2	2
	Overall	10	Range, 85 - 96	92	4	4
	M750F037: Primary Quantitation (m/z 126 → m/z 70)					
	0.030	5	93, 95, 94, 96, 89	93	3	3
	0.300	5	88, 94, 96, 96, 96	94	3	4
	Overall	10	Range, 88 - 96	94	3	3
	M750F037: Confirmatory Quantitation (m/z 126 → m/z 99)					
	0.030	5	98, 95, 96, 95, 87	94	4	4
	0.300	5	88, 99, 98, 96, 89	94	5	5
	Overall	10	Range, 87 - 99	94	4	5

^aRelative Standard Deviation = (Standard Deviation ÷ Average Recovery) × 100

Data (uncorrected recovery results, Appendix A, Appendix C, p. 53; Appendix A, Appendix F, pp. 65-87) were obtained from Appendix A, pp. 16-17 of MRID 49762552.

1 Surface (lake) water (Golden Lake Water; pH 8.3, 14.4 ppm total organic carbon, hardness 693 mg equivalent CaCO₃/L) and ground water (CM15-030; pH 7.5, 0.8 ppm total organic carbon, hardness 116 mg equivalent

CaCO₃/L) were used (Appendix A p. 21; Appendix A, Appendix K, pp. 237-238). Waters were characterized by Agvise Laboratories, Northwood, North Dakota; water sources were not further specified.

Table 5: Independent Laboratory Validation Summary Results of Method D1605/01: BAS 750 F Water Metabolite Residues in Drinking and Surface Water*

Matrix	Fortification Levels (ppt)	n	Recovery (%) ¹	Average Recovery (%)	Standard Deviation	%RSD ²
Surface Water (Solvent-based standards)	M750F002					
	Primary Ion (m/z 245.99 → 149.00) using LC-MS/MS, AB Sciex 6500					
	30	5	91, 89, 90, 91, 91	90	0.8	0.9
	300	5	102, 100, 100, 99, 99	100	1.1	1.1
	Overall	10	Range: 89–102	95	5.2	5.5
	Secondary Ion (m/z 245.99 → 70.00) using LC-MS/MS, AB Sciex 6500					
	30	5	92, 95, 91, 88, 90	91	2.6	2.9
	300	5	101, 102, 97, 97, 99	99	2.3	2.4
	Overall	10	Range: 88–102	95	4.9	5.1
	M750F036					
	Primary Ion (m/z 252.05 → 70.00) using LC-MS/MS, AB Sciex 6500					
	30	5	94, 99, 92, 97, 91	94	3.1	3.2
	300	5	106, 107, 105, 103, 103	105	1.8	1.8
	Overall	10	Range: 91–107	100	6.0	6.0
	Primary Ion (m/z 252.05 → 70.00) using LC-MS/MS, AB Sciex 6500 (Alternate Chromatographic Conditions) – phenyl column					
	30	5	92, 91, 89, 85, 87	89	2.9	3.2
	300	5	109, 110, 108, 105, 107	108	1.9	1.8
	Overall	10	Range: 85–110	98	10.2	10.4
	M750F037					
	Primary Ion (m/z 126.00 → 70.00) using LC-MS/MS, AB Sciex 6500					
	30	5	(61), (62), (60), (61), (66)	62	2.4	3.9
300	5	96, 96, 98, 95, 95	96	1.2	1.2	
Overall	10	Range: 60–98	79	18.2	23.0	
Secondary Ion (m/z 126.00 → 98.90) using LC-MS/MS, AB Sciex 6500						
30	5	(66), (60), (60), (57), (60)	61	3.5	5.8	
300	5	80, 70, 71, 77, (67)	73	5.2	7.1	
Overall	10	Range: 57–80	67	7.7	11.6	

¹Individual recoveries outside of the acceptable range 70–110% are shown in parentheses.

²Relative Standard Deviation = (Standard Deviation ÷ Average Recovery) × 100

Table 5: Independent Laboratory Validation Summary Results of Method D1605/01: BAS 750 F Water Metabolite Residues in Drinking and Surface Water* (continued)

Matrix	Fortification Levels (ppt)	n	Recovery (%) ¹	Average Recovery (%)	Standard Deviation	%RSD ²
Drinking Water (Matrix-matched standards)	M750F002					
	Primary Ion (m/z 245.99 → 149.00) using LC-MS/MS, AB Sciex 6500					
	30	5	107, 98, 97, 100, 96	99	4.4	4.4
	300	5	(118), 102, 99, 101, 100	104	8.0	7.7
	Overall	10	Range: 96–118	102	6.5	6.4
	Secondary Ion (m/z 245.99 → 70.00) using LC-MS/MS, AB Sciex 6500					
	30	5	107, 103, 100, 103, 96	102	4.1	4.1
	300	5	(114), 104, 99, 100, 99	103	6.6	6.4
	Overall	10	Range: 96–114	102	5.2	5.1
	M750F036					
	Primary Ion (m/z 252.05 → 70.00) using LC-MS/MS, AB Sciex 6500					
	30	5	102, 105, 95, 94, 94	98	5.1	5.2
	300	5	107, 102, 104, 110, 107	106	3.2	3.0
	Overall	10	Range: 94–110	102	5.9	5.8
	Primary Ion (m/z 252.05 → 70.00) using LC-MS/MS, AB Sciex 6500 (Alternate Chromatographic Conditions) – phenyl column					
	30	5	(111), 92, 98, 104, 103	102	6.9	6.8
	300	5	109, 103, 102, 96, 100	102	4.6	4.5
	Overall	10	Range: 92–111	102	5.5	5.4
	M750F037					
	Primary Ion (m/z 126.00 → 70.00) using LC-MS/MS, AB Sciex 6500					
30	5	(112), 106, 108, 106, 102	107	3.3	3.1	
300	5	103, 100, 102, 100, 101	101	1.3	1.2	
Overall	10	Range: 100–112	104	3.9	3.7	
Secondary Ion (m/z 126.00 → 98.90) using LC-MS/MS, AB Sciex 6500						
30	5	102, 108, 97, 96, 108	102	5.5	5.4	
300	5	102, 100, 110, 105, 110	105	4.3	4.0	
Overall	10	Range: 96–110	104	5.0	4.8	

¹Individual recoveries outside of the acceptable range 70–110% are shown in parentheses.

²Relative Standard Deviation = (Standard Deviation ÷ Average Recovery) × 100

Data (uncorrected recovery results) were obtained from pp. 9-10 of MRID 49762448

* Surface (lake) water (Golden Lake Water; 2015-11/SDBN418 Water; pH not reported, 15.1 ppm total organic carbon, hardness not reported) and ground water (CM15-030; pH 7.5, 0.8 ppm total organic carbon, hardness 116 mg equivalent CaCO₃/L) were used (pp. 16-17; Appendix E, pp. 152-155). Waters were characterized by Agvise Laboratories, Northwood, North Dakota, and supplied by the sponsor. The ground water of the ILV was the same as that of the ECM. Water sources were not further specified.

III. Method Characteristics

The LOQ was defined by the lowest fortification level successfully tested. The validated LOQ for residues of M750F002, M750F036, and M750F037 in water is 30 ng/L (30 ppt), for each analyte. In a separate study, the LOD of BASF Analytical Method No. D1605/01 was tested and evaluated in accordance with the methodology set forth in 40 CFR Ch. 1 Part 136 Appendix B. The method detection limit (MDL) was proven to be 0.383 pg on-column. From this value the LOD was set at 0.6 pg on-column, i.e. 6 pg/mL (0.006 ng/mL) injected at 0.1 mL. The LOD value on-column corresponds to a LOD of 6 ng/L (ppt) for all analytes in water (based on the workup of the method, e.g. aliquot actor, final volume, etc).

Within the scope of the BASF analytical method D1605/01, two independent injections were used for analysis. Determination of the least sensitive analyte was conducted on both independent injections. An MDL calculation and subsequent LOD evaluation was conducted on the method monitoring the least sensitive analyte's transition.

To determine the least sensitive analyte and transition to use, neat standards were injected using all validated analytical LC-MS/MS runs from method D1605/01. It was determined qualitatively that the MS/MS ion transition for M750F037 (m/z 126 \rightarrow m/z 99) was the least sensitive transition and, therefore, the best candidate to conduct the LOD evaluation for this injection.

The MDL was determined to be 0.383 pg on-column for this injection. To determine this value, the seven matrix-spiked samples were spiked at 10 pg/mL and 0.1 mL (100 μ L) and each sample was injected on the LC-MS/MS using the confirmatory chromatographic method in D1605/01 for M750F037. The calculated MDL was verified by injecting the same matrix-spiked samples with an injection volume of 0.04 mL (40 μ L) on LC-MS/MS. This was confirmed to be a peak at \sim 2.9 S/N. Representative chromatograms were provided in the final report.

Based on this calculated MDL, the LOD for this injection was set at 0.6 pg on-column (i.e. 6 pg/mL injected at 0.1 mL [100 μ L]). This increase from the MDL the LOD is to account for variability in the residue method, natural drift of the LC-MS/MS instrumentation, potential contamination issues, untested matrix effects, and potential unseen background interferences. If an incurred residue sample at 6 ng/L (ppt) were brought through the analytical method (extracted, aliquoted, cleaned up, and brought to final volume) the resulting concentration of the sample would be 6 pg/mL (0.006 ng/mL); it would be at the LOD. The results for this injection are shown below.

Table 1: Calculation of MDL for M750F037 (m/z 126 \rightarrow m/z 99) for Method D1605/01, using the Methodology Set Forth in 40 CFR Ch 1 Part 136 Appendix B

0.01 ng/mL Matrix-Spike Replicate	Calculated Concentration (ng/mL)
1	0.00732
2	0.00920
3	0.00752
4	0.00620
5	0.00915
6	0.00659
7	0.00664
Standard Deviation (S) =	0.001218
N-1 =	6
Critical t value (t) =	3.143
Injection Volume (μ L)	100
MDL (ng/mL)=	0.00383
MDL (ng on-column)=	0.0003828
MDL (pg on-column)=	0.383

$$\text{MDL on-column (pg)} = \text{MDL (ng/mL)} \times \text{Injection Volume (\mu L)}$$

Table 7. Method Characteristics - Analytical Method D1605/01

Analyte		M750F002 (Reg.No. 6031465)	M750F036 (Reg.No. 6055268)	M750F037 (Reg.No. 148502)	
Limit of Quantitation (LOQ)	ECM	0.030 µg/mL (0.030 ppb)			
	ILV				
Limit of Detection (LOD)	ECM	0.006 µg/L (30% of the LOQ)			
	ILV				
Linearity (calibration curve r^2 and concentration range) ¹	ECM	Solvent	$r^2 = 0.9984$ (Q) $r^2 = 0.9990$ (C)	$r^2 = 0.9992$ (Q) $r^2 = 0.9998$ (C)	$r^2 = 0.9926$ (Q) $r^2 = 0.9906$ (C)
		Surface	$r^2 = 0.9992$ (Q) $r^2 = 0.9994$ (C)	$r^2 = 0.9994$ (Q) $r^2 = 0.9990$ (C)	$r^2 = 0.9996$ (Q) $r^2 = 0.9994$ (C)
		Ground	$r^2 = 0.9998$ (Q & C)	$r^2 = 0.9998$ (Q & C)	$r^2 = 0.9996$ (Q) $r^2 = 0.9994$ (C)
	Concentration Range		0.006-0.2 ng/mL		
	ILV	Solvent	$r^2 = 0.9996$ (Q & C)	$r^2 = 0.9974$ (Q) $r^2 = 0.9952$ (C)	$r^2 = 0.9994$ (Q) $r^2 = 0.9958$ (C)
		Ground	$r^2 = 0.9980$ (Q) $r^2 = 0.9986$ (C)	$r^2 = 0.9996$ (Q) $r^2 = 0.9990$ (C)	$r^2 = 0.9982$ (Q) $r^2 = 0.9994$ (C)
	Concentration Range		0.006-1.0 ng/mL		
Repeatable	ECM ^{2,3}	Yes at LOQ and 10×LOQ (two characterized water matrices)			
	ILV ^{4,5,6}	Yes at LOQ and 10×LOQ (two characterized water matrices)		Yes at LOQ and 10×LOQ in ground water. No at LOQ in surface water; Yes at 10×LOQ.	
Reproducible		Yes at LOQ and 10×LOQ (two matrices)		Yes at LOQ and 10×LOQ (one matrix)	
Specific	ECM ⁷	Yes, no matrix interferences were observed. The baseline interfered with peak attenuation in some chromatograms.		Yes, no matrix interferences were observed; however, analyte peak was small at LOQ in surface water. The baseline noise interfered with peak attenuation in most chromatograms.	
	ILV ⁸	Yes, matrix interferences were <5% of the LOQ (based on peak area).	Yes, matrix interferences were <5% of the LOQ (based on peak area). Analyte peak was small at LOQ compared to baseline noise (C). ⁹	No , matrix interferences were <20% of the LOQ (based on peak area); however, analyte peak was small at LOQ in both matrices and poorly resolved from the baseline in the drinking water. ¹⁰ The analyte peak in C surface water chromatogram was unresolved from the baseline at LOQ ⁹ .	

Data were obtained from Appendix A of MRID 49762552; MRID 49762448. 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 and matrix-based calibration standards were used in the ECM and ILV.
 - 2 In the ECM, surface (lake) water (Golden Lake Water; pH 8.3, 14.4 ppm total organic carbon, hardness 693 mg equivalent CaCO_3/L) and ground water (CM15-030; pH 7.5, 0.8 ppm total organic carbon, hardness 116 mg equivalent CaCO_3/L) were used (Appendix A p. 21; Appendix A, Appendix K, pp. 237-238 of MRID 49762552). Waters were characterized by Agvise Laboratories, Northwood, North Dakota; water sources were not further specified.
 - 3 Recoveries in both matrices were calculated using solvent-based and matrix-matched calibration curves. Analytes were identified using two ion transitions. Performance data (recovery results) from primary and confirmatory analyses were acceptable and comparable, except in the case of M750F036 in surface water for which matrix-matched standards were required.
 - 4 In the ILV, surface (lake) water (Golden Lake Water; 2015-11/SDBN418 Water; pH not reported, 15.1 ppm total organic carbon, hardness not reported) and ground water (CM15-030; pH 7.5, 0.8 ppm total organic carbon, hardness 116 mg equivalent CaCO_3/L) were used (pp. 16-17; Appendix E, pp. 152-155 of MRID 49762448). Waters were characterized by Agvise Laboratories, Northwood, North Dakota, and supplied by the sponsor. The ground water of the ILV was the same as that of the ECM. Water sources were not further specified.
 - 5 The method was validated in the first trial with modifications of the analytical method, including reduction of the injection volume for M750F036 using the XBridge BEH Phenyl LC column (100 μL to 40 μL) and for all analytes using the XSelect HSS T3 LC column (100 μL to 80 μL), changing the size of the analytical columns, and inversion of the mass transitions for M750F002 (p. 23; Appendix A, p. 114 of MRID 49762448).
 - 6 Recoveries in the surface and ground water matrices were calculated using solvent-based and matrix-matched calibration curves, respectively.
 - 7 Based on Appendix A, Appendix I, Figures 31-50, pp. 170-230 of MRID 49762552.
 - 8 Based on Figures 7-14, pp. 63-75 (Surface) and Figures 21-27, pp. 100-111 (Ground) of MRID 49762448.
 - 9 A confirmation method is not usually required when LC/MS or GC/MS is the primary method to generate study data.
 - 10 Based on Figures 14 and 27, pp. 74 and 110 of MRID 49762448.
- Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. The ILV validation did not fully satisfy the reproducibility of the method for M750F037. The method was validated by the ILV in the first trial with modifications of the analytical method, including reduction of the injection volume for M750F036 using the XBridge BEH Phenyl LC column (100 μL to 40 μL) and for all analytes using the XSelect HSS T3 LC column (100 μL to 80 μL), changing the size of the analytical columns, and inversion of the mass transitions for M750F002 (p. 23; Appendix A, p. 114 of MRID 49762448). The ILV recommended that these modifications should be incorporated into the technical procedure. The ECM study report noted the ILV recommendations; however, the ECM did not incorporate these ILV recommendations (pp. 25-26 of MRID 49762552).

However, the reviewer could not recommend that the ECM be updated with the ILV modifications because no improvement was observed for the validations of M750F002 and M750F036 and less satisfactory data was presented by the ILV for the validation of M750F037, including the following deficiencies:

Performance data was unsatisfactory for M750F037 at the LOQ in surface water (means 61-62%; pp. 9-10 of MRID 49762448). OCSPP guidelines state that mean recoveries and relative standard deviations (RSDs) should be 70-120% and $\leq 20\%$, respectively.

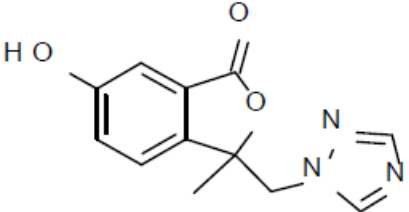
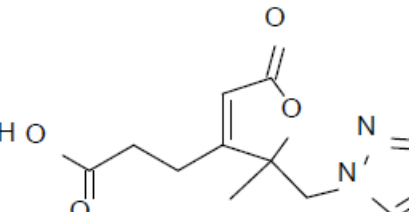
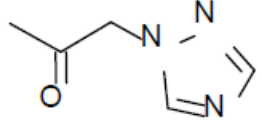
The specificity of the method was not supported for M750F037 based on the ILV representative chromatograms because the analyte peak was small at LOQ in both matrices and poorly resolved from the baseline in the drinking water (Figures 14 and 27, pp. 74 and 110 of MRID 49762448). Additionally, analyte peak in C surface water chromatogram was unresolved from the baseline at LOQ.

2. The reviewer noted that the chemical purities of M750F036 and M750F037 were <90% (86.7-87.1%) in the ECM and ILV (p. 22 of MRID 49762552; p. 17 of MRID 49762448).
3. It could not be determined if the ILV matrices were adequately rigorous for the validation. The ILV surface water matrix was barely characterized (pp. 16-17; Appendix E, pp. 152-155 of MRID 49762448). Also, the ground water of the ILV was the same as that of the ECM. Water sources were not further specified.
4. ECM linearity was not satisfactory for M750F037 with solvent-based calibration standards ($r^2 = 0.9926$ (Q); $r^2 = 0.9906$ (C); see DER Attachment 2). Linearity is satisfactory when $r^2 \geq 0.995$.
5. The reviewer noted that ILV recoveries in the surface and ground water matrices were calculated using solvent-based and matrix-matched calibration curves, respectively (pp. 9-10, 16 of MRID 49762448). No matrix effects data was provided in the study report. The reviewer believed that matrix-matched calibration standards may have improved that performance data for M750F037 in surface water.
6. No documentation of the communication provided for review. The ILV study report only stated that no one from BASF was allowed to visit ADPEN during the course of the study and that the Study Monitor was informed of the successful ILV trial (p. 23 of MRID 49762448). The identity of the Study Monitor was reported as Carlan Downs of BASF; this Study Monitor was not listed in the ECM personnel (p. 6).
7. In the ECM, it was reported that the stability of each analyte in standard solutions was determined in conjunction with the subject study (Tier II Summary for MRID 49762552). Stock and intermediate (fortification) standards of each analyte prepared in water and calibration standards prepared by dilution of the intermediate standards in acidified water were shown to be stable, when held under refrigeration, for at least 1 month (30 days). During the course of the study, the test/reference substance solutions were stored in a refrigerator and all solutions were used within the demonstrated time period of stability. As the method relies on the direct injection of the water samples onto the HPLC column, there are no extracts. Instead, only stability in this "final volume" prepared for HPLC analysis was determined. The recoveries from stored solutions generated during extract stability experiments performed in conjunction with this study, which included tests on the HPLC final volume held under refrigeration, indicated that each analyte is stable in extracts for at least the time period tested, 6 days for drinking water and 7 days for surface water.
8. It was reported for the ILV that one sample set of 13 samples required *ca.* 4 hours of work (p. 22 of MRID 49762448).

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

Internal-Code	M750F002	
IUPAC Name	6-hydroxy-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one	
BASF Reg. No.	6031465	
CAS-No.	-----	
Molecular Formula	C ₁₂ H ₁₁ N ₃ O ₃	
Molecular Weight	245.2	
Lot Number	L85-138	
Purity	96.8%	
Expiration Date	March 1, 2018	
Internal-Code	M750F036	
IUPAC Name	3-[2-methyl-5-oxo-2-(1H-1,2,4-triazol-1-ylmethyl)-2,5-dihydrofuran-3-yl]propanoic acid	
BASF Reg. No.	6055268	
CAS-No.	-----	
Molecular Formula	C ₁₁ H ₁₃ N ₃ O ₄	
Molecular Weight	251.2	
Lot Number	L85-190	
Purity	87.1%	
Expiration Date	May 1, 2018	
Internal-Code	M750F037	
IUPAC Name	1-(1H-1,2,4-triazol-1-yl)propan-2-one	
BASF Reg. No.	148502	
CAS-No.	64882-52-6	
Molecular Formula	C ₅ H ₇ N ₃ O	
Molecular Weight	125.1	
Lot Number	L85-164	
Purity	86.7%	
Expiration Date	January 1, 2018	