ABSTRACT

The purpose of this study was to demonstrate that 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”, could be performed successfully at an outside facility with no prior experience with the method (Reference 1).

Principle of the method. The residues of M750F002, M750F036 and M750F037 are extracted from 10-mL of water by adding formic acid to achieve a 0.1% formic acid in water mixture. An aliquot of the resulting extract is then directly used for LC-MS/MS determination.

Test conditions. For validation, untreated water samples were fortified with all the analytes and analyzed according to the established method validation guidelines. The analytical sets for each matrix typically consisted of a reagent blank, two controls, five replicates fortified with each analyte at the method limit of quantitation (LOQ) and five replicates fortified at a higher level, corresponding to 10× the LOQ. The mass transitions evaluated are listed below.

	Quantitation (*m/z*)	**Confirmation (*m/z*)**
M750F002	*m/z* 245.99 → 149.00	*m/z* 245.99 → 70.00
M750F036*	*m/z* 252.05 → 70.00	*m/z* 252.05 → 70.00
M750F037	*m/z* 126.00 → 70.00	*m/z* 126.00 → 98.90

*Confirmation Ion Using Alternate Chromatographic Conditions.

Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ was defined as the lowest fortification level tested. The LOQ for M750F002, M750F036 and M750F037 in water matrices was 30 ppt. The LOD in water matrices was set at 6 ppt, which was 20% of the defined LOQ. The LOD is defined as the absolute amount of analyte injected (0.00048 ng) into the LC-MS/MS when the lowest calibration standard was analyzed (0.006 ng/mL) for all analytes except M750F036 using the alternate confirmatory method. The LOD is defined as the absolute amount of analyte injected (0.00024 ng) into the LC-MS/MS when the lowest calibration standard was analyzed (0.006 ng/mL) for M750F036 using alternate chromatographic conditions to confirm.

Selectivity. The method determines M750F002, M750F036 and M750F037 residues in water matrices by LC-MS/MS. No interfering peaks were found at the retention times for M750F002, M750F036 and M750F037. The experiment to evaluate any potential matrix effects showed that the matrix load in the samples from drinking water had significant influence on analysis (matrix effects >20%); therefore, the validation samples were analyzed using matrix-matched calibration standard solutions. The experiment to evaluate any potential matrix effects showed that the matrix load in the samples from surface water did not have significant influence on analysis (matrix effects <20%); therefore, the validation samples were analyzed using solvent-based calibration standard solutions.

Linearity. Acceptable linearity was observed for the standard range and the two mass transitions tested: The method-detector response was linear over the 0.006–1.0 ng/mL range (r = ≥0.990), for all water matrix analyses.

1. INTRODUCTION

1.1 Scope of the Method

BASF Analytical Method No. D1605/01 was developed to determine the residues of M750F002, M750F036 and M750F037 in water matrices using LC-MS/MS at BASF Crop Protection in Research Triangle Park, North Carolina. This method was independently validated at ADPEN Laboratories, Inc.

The independent lab validation was conducted using two fortification levels at the limit of quantitation (30 ppt) and ten times of limit of quantitation (300 ppt) for all water matrices. For each fortification level and matrix, five replicates were analyzed. Additionally, one reagent blank and two replicates of unfortified samples were examined.

1.2 Principle of the Method

The water samples (10 mL) were fortified with formic acid and thoroughly mixed. An aliquot of resulting solution was analyzed to determine the residues of M750F002, M750F036 and M750F037 using LC-MS/MS. The transitions for M750F002 and M750F037 were monitored in positive mode for primary and secondary quantification. The primary transition for M750F036 was monitored in positive mode for primary quantification. The quantification was confirmed using an alternate chromatographic method in absence of a confirmatory transition.

1.3 Specificity

To demonstrate the specificity of the analytical method, one additional mass transition was monitored simultaneous to the primary quantitation transition for analysis of M750F002 and M750F037. M750F036 was monitored using an alternate chromatographic method in absence of a secondary transition. Primary and secondary transitions for each analyte are listed below:

Analyte	Primary Transition Ion	Secondary Transition Ion
M750F002	*m/z* 245.99 → 149.00	*m/z* 245.99 → 70.00
M750F036*	*m/z* 252.05 → 70.00	*m/z* 252.05 → 70.00
M750F037	*m/z* 126.00 → 70.00	*m/z* 126.00 → 98.90

*Confirmation Ion Using Alternate Chromatographic Conditions.

The method was able to accurately determine residues of M750F002, M750F036 and M750F037 and no interference was observed at the retention time of the analyte peaks. No matrix suppression or enhancement was found to affect the analytes in surface water; however, matrix suppression or enhancement was found to affect the analytes in drinking water.

2. REFERENCE SUBSTANCE AND SAMPLING HISTORY

2.1 Test Systems

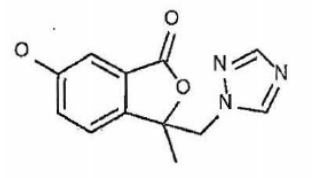
The test systems considered in this study were surface and drinking water.

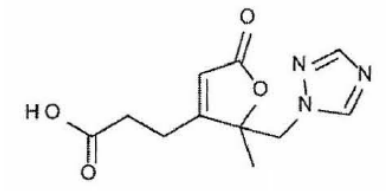
The control samples were provided by BASF. The water samples were received on June 28, 2016 and July 13, 2016 for drinking water and surface water, respectively. Upon arrival at the laboratory, the samples were opened, inspected, and checked against enclosed shipping forms. The test systems were received frozen and stored under frozen conditions at all times, unless necessary for laboratory analysis. The test systems were characterized at AGVISE Laboratories (604 Highway 15 West, Northwood, ND 58267). A copy of the characterization data for the sample is provided in Appendix E.

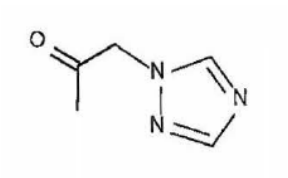
2.2 Test and Reference Substances

The standard substances were stored in a freezer (≤ -5°C) until use. BASF has retained a reserve sample of this chemical, and has documentation specifying the location of the synthesis and characterization information available at BASF Crop Protection, Research Triangle Park, North Carolina.

The M750F002, M750F036 and M750F037 reference substances were provided by the sponsor and received on July 14, 2016. The certificate of analysis for all substances is presented in Appendix B. A detailed summary of the reference substances is presented below.

BASF Code Name:	M750F002
Batch Number:	L85-138
BASF Registry Number:	6031465
IUPAC Name:	6-hydroxy-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one
Molecular Formula:	$C_{12}H_{11}N_3O_3$
Molecular Weight:	245.2 g/mol
Purity:	98.3%
Expiration Date:	August 1, 2017
Chemical Structure:	

BASF Code Name:	M750F036
Batch Number:	L85-190
BASF Registry Number:	6055268
IUPAC Name:	3-[2-methyl-5-oxo-2-(1H-1,2,4-triazol-1-ylmethyl)-2,5-dihydrofuran-3-yl]propanoic acid
Molecular Formula:	$C_{11}H_{13}N_3O_4$
Molecular Weight:	251.2 g/mol
Purity:	87.1%
Expiration Date:	May 1, 2018
Structural Formula:	

BASF Code Name:	M750F037
Batch Number:	L85-164
BASF Registry Number:	148502
IUPAC Name:	1-(1H-1,2,4-triazol-1-yl)propan-2-one
Molecular Formula:	$C_5H_7N_3O$
Molecular Weight:	125.1 g/mol
Purity:	86.7%
Expiration Date:	January 1, 2018
Structural Formula:	

2.3 Test System

Surface and drinking water samples were provided and homogenized by BASF. The surface water sample was sent from BASF Crop Protection, Inc. on July 12, 2016 and received by ADPEN Laboratories, Inc. on July 13, 2016. The drinking water sample was sent from BASF Crop Protection, Inc. on June 27, 2016 and received by ADPEN Laboratories, Inc. on June 28, 2016.

The Laboratory Information Management System (LIMS) provided a unique laboratory analysis code (e.g., 160713001-001) for each sample and is cross-referenced on the detailed reports to the assigned unique sample number.

3. ANALYTICAL METHOD

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" was used for the analysis of the samples.

The residues of M750F002, M750F036 and M750F037 are extracted from 10-mL of water by adding formic acid to achieve a 0.1% formic acid in water mixture. An aliquot is taken from the resulting extract and directly used for LC-MS/MS determination. Instrument parameters are described in Table 14. The primary (quantitative) and secondary (confirmatory) transition ions monitored are presented below:

Analyte	Transition (*m/z*)		Ionization Mode	Retention Time (min)
	Primary	Secondary		
M750F002	*m/z* 245.99 → 149.00	*m/z* 245.99 → 70.00	Positive	2.6
M750F036	*m/z* 252.05 → 70.00	--		2.0
M750F036*	--	*m/z* 252.05 → 70.00		1.5
M750F037	*m/z* 126.00 → 70.00	*m/z* 126.00 → 98.90		1.0

*Confirmation Ion Using Alternate Chromatographic Conditions.

5. SUMMARY OF METHOD

Summaries of the method parameters and characteristics are provided in Table 14.

Summary of Method

Type of Method	LC-MS/MS
Test Systems	Surface and Drinking Water
Selected mass transitions (m/z)	M750F002 245.99 → 149.00[1] 245.99 → 70.00 M750F036 252.05 → 70.00[1] 252.05 → 70.00[2] M750F037 126.00 → 70.00[1] 126.00 → 98.90 [1]Primary quantification transition [2]Confirmation Transition Using Alternate Chromatographic Conditions.
Analytical Procedure	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" (Reference 1).
Confirmatory Technique	A secondary MRM transition for M750F002 and M750F037 was used for confirmation. A secondary or alternate chromatographic method for M750F036 was used for confirmation.

Method of Quantitation	The quantitation is based on the monitoring of five mass transitions for M750F002, M750F036 and M750F037. Recovery data was reported for each mass transition considered.
LOD	6 ppt
LOQ	30 ppt (lowest fortification level)
Levels of Fortification	30 ppt and 300 ppt
Time Required	A set of 13 samples requires approximately 4 hours of work.
Justification of Ions	The ions used to conduct the ILV were determined in this validation and are shown in Appendix F.

Table 13 Example Standard Solutions Preparation and Dilution Data

Stock Standard Solutions

Standard ID#	Analyte	Parent Standard ID#	Adjusted Net Weight (mg)	Dilution Volume (mL)[1]	Final Conc. (ng/µL)	Prep. Date
C8356	M750F002	P5562	9.99711	10	999.71	07/19/2016
C8357	M750F036	P5563	9.99037	10	999.04	
C8358	M750F037	P5564	21.675	25	867	

[1] Prepared in water

Intermediate Standard Solution

Standard ID#	Analyte	Parent Standard ID#	Parent Conc. (ng/µL)	Aliquot Volume (mL)	Dilution Volume (mL)[2]	Final Conc. (ng/µL)	Prep. Date
I8977	M750F002, M750F036, M750F037	C8356	999.71	1.00	10	100	08/09/2016
		C8357	999.04	1.00			
		C8358	867	1.15			

[2] Prepared in water

Calibration Standard Solutions

Standard ID#	Analyte	Parent Standard ID#	Parent Conc. (ppb)	Aliquot Volume (µL)	Dilution Volume (mL)[3]	Final Conc. (ng/mL)	Prep. Date
W13247	BAS 750 F (3-Mix)	W13218-3	10	100	1.0	1.0	8/17/2016
		W13218-3	10	50		0.5	
		W13218-3	10	20		0.2	
		W13247-1	1.0	60		0.06	
		W13247-2	0.5	60		0.03	
		W13247-3	0.2	75		0.015	
		W13247-4	0.06	100		0.006	

[3] Prepared in control drinking water

Table 14 Instrument Conditions and Parameters (All Analytes)

HPLC Conditions			
Chromatographic System:	Agilent 1290 UPLC System		
Column:	XSelect HSS T3; 2.5 µm, 2.1 × 150 mm; S/N 10533023154 03		
Temperature:	50 °C		
Flow rate (µL/min)	800		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.00	95.0	5.0
	0.25	95.0	5.0
	4.25	50.0	50.0
	5.50	1.0	99.0
	6.99	1.0	99.0
	7.00	95.0	5.0
	8.00	95.0	5.0
Mobile Phase A:	0.1% formic acid in water		
Mobile Phase B:	0.1% formic acid in acetonitrile		
Injection Volume:	80 µL		

MS/MS Conditions						
Detection System:	AB SCIEX 6500 QT					
Ionization:	Turbo Spray					
Polarity:	Positive					
Curtain gas (CUR):	20.00					
Internal Standard (IS):	5500.00					
Temperature (TEM):	700 °C					
Collision gas setting (CAD):	10.00					
GS1:	45.00					
GS2:	45.00					
Entrance potential (EP):	10.00					
Scan type:	MRM					
MRM Conditions	**Transition (m/z)**	**Time (min)**	**DP**	**CE**	**CXP**	**Retention Time (min)**
M750F002 Reg. No. 6031465	245.99 → 149.00	2.72	71.00	27.00	8.00	2.6
	245.99 → 70.00			21.00		
M750F036 Reg. No. 6055268	252.05 → 70.00	2.05	81.00	25.00	8.00	2.0
M750F037 Reg. No. 148502	126.00 → 70.00	1.10	81.00	23.00	8.00	1.0
	126.00 → 98.90			19.00	12.00	

Table 14 Instrument Conditions and Parameters (Secondary Column for M750F036 Using Alternate Chromatographic Conditions) (Continued)

HPLC Conditions			
Chromatographic System:	Agilent 1290 UPLC System		
Column:	XBridge BEH Phenyl; 2.5 µm, 2.1 × 100 mm; S/N 011634269156 08		
Temperature:	40 °C		
Flow rate (µL/min)	800		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.00	95.0	5.0
	0.25	95.0	5.0
	4.00	70.0	30.0
	6.50	1.0	99.0
	7.35	1.0	99.0
	7.40	95.0	5.0
	8.00	95.0	5.0
Mobile Phase A:	0.1% formic acid in water		
Mobile Phase B:	0.1% formic acid in acetonitrile		
Injection Volume:	40 µL		

MS/MS Conditions						
Detection System:	AB SCIEX 6500 QT					
Ionization:	Turbo Spray					
Polarity:	Positive					
Curtain gas (CUR):	20.00					
Internal Standard (IS):	5500.00					
Temperature (TEM):	700 °C					
Collision gas setting (CAD):	10.00					
GS1:	45.00					
GS2:	45.00					
Entrance potential (EP):	10.00					
Scan type:	MRM					
MRM Conditions	**Transition (m/z)**	**Dwell (msec)**	**DP**	**CE**	**CXP**	**Retention Time (min)**
M750F036 Reg. No. 6055268	252.05 → 70.00	50.00	81.00	25.00	8.00	1.5

Figure 28 Reside Calculations for Water Matrices

Peak integration and quantitation were performed within Analyst® 1.6.2 software; using the calibration curve equation to determine the amount of analyte found (ng) during sample analysis. Recovery results and additional sample concentrations were calculated for each set of samples within the Laboratory Information Management System (LIMS) and reported in Microsoft® Excel spreadsheet data reports, which are presented in Appendix C.

For the validation recoveries, the exact sample weight was used in calculating the final residues (ppt).

The following equations are used for residue and recovery calculations for M750F002, M750F036 and M750F037 in matrices of surface and drinking water.

a) Calibration curve: $y = mx + b$ Solving for x: $x = \frac{y-b}{m}$

Where, m = slope
b = y-intercept
x = Amount found (ng)
y = Peak area

b) Amount of sample injected (mL)= $\frac{\text{(injection size (mL) × sample vol. (mL))}}{\text{final sample volume (mL)}}$

c) Residue found (ppt) = $\frac{\text{Amount found (ng)}}{\text{Amount of sample injected (mL)}} \times 1{,}000{,}000$

d) Recovery (%) = $\frac{\text{Residue in sample (ppt)}}{\text{Amount fortified (ppt)}} \times 100$

As an example, calculations to obtain M750F002 (primary transition) recovery results using 16080503-Recovery1-2 from work order WO-16080503 (drinking water) are shown below:

a) Calibration curve: $y = (1.1e+007)x + 989$

Solving for x: $x = \frac{26767 - 989}{1.1e+007} = 0.002348$ ng

b) Amount of sample injected (mL) = $\frac{0.08 \text{ mL} \times 10.00 \text{ mL}}{10.0 \text{ mL}} = 0.08$ mL

c) Residue found (ppt) = $\frac{0.002348 \text{ ng}}{0.08 \text{ mL}} \times 1{,}000{,}000 = 29.3500$ ppt

d) Recovery (%) = $\frac{29.3500 \text{ ppt}}{30 \text{ ppt}} \times 100 = 98\%$

Statistical treatment of the data included calculation of means, standard deviations (SD), and percent relative standard deviations (%RSD). These calculations were performed using Microsoft® Excel and LIMS software. Results were rounded only for reporting purposes. No calculations were made with rounded numbers.

Appendix A. Recommendations/Findings for BASF Analytical Method D1605/01

The following recommendations should be incorporated into the technical procedure:

1. Section 4.2: Instrumental Analysis

   Some method modifications were done to successfully complete this ILV. Please see below.
   - i. For analysis of M750F036 using the XBridge BEH Phenyl LC column, an injection volume of 40 µL was used (versus 100 µL as stated in the method).
   - ii. For analysis of all analytes using the XSelect HSS T3 LC column, an injection volume of 80 µL was used (versus 100 µL as stated in the method).

There were no findings in this ILV. Method D1605/01 was run with the following changes:

1. The ILV used the transition *m/z* 245.99 → 149.00 for the quantitation transition and *m/z* 245.99 → 70.00 for the confirmation transition for M750F002. The transition's sensitivity was opposite of that in the validation and showed to be more sensitive and rugged with less interference. Both transitions have been validated and either can be used for data generation.
2. The following columns were used during the validation:
   - i. Xselect HSS T3, 150 × 3 mm, 2.5µm
   - ii. Xbridge BEH Phenyl, 150 × 3 mm, 2.5 µm
3. The following columns were used during the ILV:
   - i. XSelect HSS T3, 150 mm × 2.1mm, 2.5 µm
   - ii. XBridge BEH Phenyl, 100 × 2.1 mm, 2.5 µm

The difference in the column dimension had no adverse effect on recoveries, but did give different retention times for the analytes. The differences are captured in the tables below:

Primary Chromatographic Conditions for all Analytes (XSelect HSS T3)

MRM Conditions	Transition (m/z)	Retention Time (min) Validation	Retention Time (min) ILV
M750F002	245.99 → 149.00	4.75	2.6
	245.99 → 70.00		
M750F036	252.05 → 70.00	4.05	2.0
M750F037	126.00 → 70.00 126.00 → 98.90	2.90	1.0

Secondary Column for M750F036 Using Alternate Chromatographic Conditions (XBridge BEH Phenyl)

MRM Conditions	Transition (m/z)	Retention Time (min) Validation	Retention Time (min) ILV
M750F036	252.05 → 70.00	3.90	1.5

1 INTRODUCTION

BAS 750 F is a fungicide used against several diseases in various crops. The analytical method D1605/01 offers the possibility to determine residues of M750F002, M750F036, and M750F037 in water. Method D1605/01 was successfully tested during method development in surface and drinking water for all analytes.

This method was developed at BASF Crop Protection, RTP, NC.

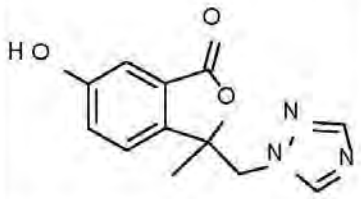
2 MATERIALS

2.1 Safety

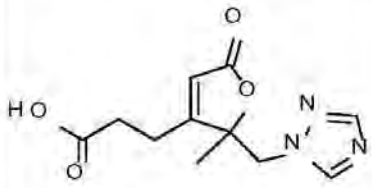
The test and reference items, as well as the chemicals required for this analysis, should be handled in accordance with good industrial hygiene and safety practice. Avoid contact with the skin, eyes and clothing. Wearing of closed work clothing is recommended. Remove contaminated clothing. Store work clothing separately. Keep away from food, drink and animal feed stuffs. No eating, drinking, smoking or tobacco use at the place of work. Hands and/or face should be washed before breaks and at the end of the shift. Details are given in the Safety Data Sheets (SDS) of the individual substances. All procedures involving organic solvents should be performed in a well-ventilated hood.
Disposal of samples and chemicals must be done in compliance with on-site safety policies and procedures.

2.2 Test and Reference Items

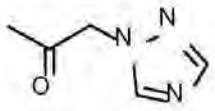
Test and reference items should be stored according to the information provided in the certificate of analysis.

Internal-Code	M750F002	
Common Name	-----	
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	$C_{12}H_{11}N_3O_3$	
Molecular Weight	245.2	

Internal-Code	M750F036
Common Name	----
IUPAC Name	3-[2-methyl-5-oxo-2-(1 H-1,2,4-triazol-1-ylmethyl)-2,5-dihydrofuran-3-yl)propanoic acid
BASF Reg. No.	6055268
CAS-No.	-----
Molecular Formula	$C_{11}H_{13}N_3O_4$
Molecular Weight	251.2



Internal-Code	M750F037
Common Name	-----
IUPAC Name	1 -(1 H-1,2,4-triazol-1-yl)propan-2-one
BASF Reg. No.	148502
CAS-No.	64882-52-6
Molecular Formula	$C_5H_7N_3O$
Molecular Weight	125.1



2.3 Equipment:

Equipment	Size, Description	Manufacturer	Catalog No.
Balance, Analytical	Model AT100	Mettler	----
Beakers	Various Sizes	PYREX Brand, VWR Scientific Products	13922-029
Amber HDPE Bottles	25 mL	VWR	414004-116
Centrifuge Tubes, Polypropylene	15 mL	VWR	89039-666
Cylinder, Graduated	Various sizes	Various	----
HPLC Column : Xselect HSS T3 C18	150 x 3 mm, 2.5 µm particle size	Waters	186006737
HPLC Column : Xbridge BEH Phenyl	150 x 3 mm, 2.5 µm particle size	Waters	186006719
LC	Acquity UPLC	Waters	---
LC Vials	2 mL injection vials	National Scientific	C400-79
MicroMan pipettes	10-1000 µL	Gilson	M-25, M-50, M-250, M-1000
MS/MS	API 6500	AB Sciex	---
Mechanical shaker	KS501 digital	IKA Labortechnik	---
Ultrasonic Bath	Model FS 7652H	Fisher Scientific	---
Various Flask, Volumetric	100, 50, 25 ,10 and 5 mL	Various	---
Volumetric pipettes	Various sizes	VWR	---
Vortex mixer	Genie 2	VWR	58816-121

Note: The equipment and instrumentation listed above represents typical laboratory equipment and can be substituted by equipment of similar technical specifications. Suitability of the entire set of equipment is confirmed if the recoveries of the fortification experiments are in the expected concentration range.

2.4 Reagents

2.4.1 Chemicals:

Chemical	Grade	Manufacturer/Supplier	Catalog No.
Acetonitrile	HPLC	EMD	AX0145P-1
Formic acid	98%	EMD	Fx0440-6
Methanol	HPLC Grade	EMD	MX0475P-1
Water, e.g. Baker® or Millipore®	Gradient Grade	BDH ARISTAR PLUS	87003-652

Note: Equivalent reagents and chemicals from other suppliers may be used. If not stated otherwise, common laboratory grade chemicals are used.

2.4.2 Solutions and Solvent Mixtures

Description	Code	Composition
Final Volume	S1	0.1% Formic Acid in Water Add 1000 mL of water and 1 mL of concentrated formic acid into a, e.g., 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
HPLC mobile phase A	LC1	0.1% Formic Acid in Water Add 1000 mL of water and 1 mL of concentrated formic acid into a, e.g., 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
HPLC mobile phase B	LC2	0.1% Formic Acid in Acetonitrile Add 1000 mL of Acetonitrile and 1 mL of concentrated formic acid into a, e.g., 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.

Note: The total volume of solutions / mixtures prepared can vary depending on the required total amounts; however, mixture ratios have to be kept as described. If necessary, the solutions may also be prepared in different volumes as long as the proportions are not modified.

2.4.3 Standard Solutions

Stock Solution

Prepare a 1.0 mg/mL stock solution individually by weighing an appropriate amount of each analyte into a flask and add the required volume of water.

For example, weigh 10 mg of M750F002 into a 10-mL volumetric flask. Dissolve and dilute to mark with water. This creates a solution containing 1 mg/mL of M750F002. Ensure a complete homogeneous solution (e.g., by sonication and/or vortexing).

Note: Sonication is required for the dissolution of M750F002, and the sample should be repeatedly sonicated in 3 minute intervals until fully dissolved.

Standard calibration and fortification solutions should initially be confirmed to show correct preparation of the solutions. This can be achieved by using one of the following approaches:

1. Two stock solutions are independently prepared. One is used for preparation of fortification solutions, the other for calibration standard solutions.
2. Fortification and calibration standard solutions should be prepared from one stock solution in separate dilution series.

For subsequent preparations of solutions, freshly prepared solutions can be compared directly to previous standard solutions.

A correction for purity is done if the purity is ≤ 95%. If the purity is > 95 % correction is optional.

Fortification Solutions

Prepare mixed standard solutions for fortification with appropriate solvents as exemplified in the table below and ensure a complete homogeneous solution (e.g. by sonication or vortexing).

Preparation of mixed Fortification solutions for M750F002, M750F036, and M750F037.

Take solution (µg/mL)	Aliquot Volume (mL)	Dilute with water to a final volume of (mL)	Final Concentration (µg/mL)
1000	0.1 (of each solution)	10	10
10	1	100	0.1
0.1	1	10	0.01

Note: Different concentration schemes can be used, if different fortification levels are required.

Calibration Standard Solutions

Prepare mixed standard calibration solutions for LC-MS/MS analysis by using the solutions that were prepared in Section "stock solutions" or "fortification solutions" in flasks. Prepare the calibration standards as exemplified in the table below and ensure a complete homogeneous solution (e.g. by sonication or vortexing).

Preparation of standard solutions for calibration for M750F002, M750F036, and M750F037.

Initial Concentration (ng/mL)	Aliquot Volume (mL)	Dilute with S1 to a final volume of (mL)	Final Concentration (ng/mL)
*100	1.5	50	3*
10	2	100	0.2
0.2	15	50	0.06
0.2	7.5	50	0.03
0.2	7.5	100	0.015
0.2	3	100	0.006

* Not intended to be a calibration standard but needed to prepare subsequent calibration solutions.

Note: Different concentration schemes can be used, if different fortification levels are required.

Matrix-matched standards are required for this method when determining residues in surface and drinking water matrices. Standard solutions may be used for evaluation of matrix-effects.

Depending on the matrix, significant matrix effects may interfere with the analysis of the samples. If significant matrix-effects occur, matrix-matched standards may be utilized. Matrix-matched calibration standards are used for quantitation when signal suppression or enhancement is >20% compared to the response for standards prepared in calibration solution alone. In this case, calibration standard solution are prepared in matrix solution, i.e., using a final volume mixture from multiple control samples or using a large batch of sample, carried through the analytical procedure. Matrix-matched standards should be prepared in a way that the matrix load is at least 90% of the matrix load in the unknown samples. See section 3.5 for details on matrix-matched standards preparation.

2.4.4 Stability of Standard Solutions

BASF recommends that stock solutions (1 mg/mL) in water are prepared freshly every 3 months. Dilutions of stock solutions should be stored refrigerated no longer than one month or according to their established storage stability in the particular solvent.

The stability of M750F002, M750F036, and M750F037 in stock, fortification and calibration solutions will be established during the method validation.

3 ANALYTICAL PROCEDURE

3.1 Sample Preparation

Sample homogenization is not needed for water samples.

3.2 Sample Storage

Water samples are stored frozen in clean amber Nalgene (HDPE) bottles.

3.3 Weighing and Preparation of Fortified /Treated Samples

For treated samples and control samples, measure 10 ±0.1 g (or 10 mL) of water sample into a HDPE Nalgene bottle.

For fortified samples, measure 10 ±0.1 g (or 10 mL) of water sample into a HDPE Nalgene bottle. Fortify the solution with analyte(s) and shake for approximately 15 minutes to ensure homogenization.

The following scheme may be used:

Sample Type	Sample Weight	Concentration of Spiking Solution	Volume of Spiking Solution	Level of Fortification
Control	10 g (or mL)	-	-	0.00 ng/L
Fortification (**LOQ**)	10 g (or mL)	10 ng/mL	0.03 mL	30 ng/L * (30 ppt)
Fortification (**10xLOQ**)	10 g (or mL)	100 ng/mL	0.03 mL	300 ng/L (300 ppt)
Treated	10 g (or mL)	-	-	-

* limit of quantification

Note: Different concentration schemes can be used, if different fortification levels are required.

Total volume of solutions prepared can be changed if overall ratios are maintained. Volume of spiking solution added should not exceed 1% of sample volume.

3.4 Preparation for Measurement

Add 10 µL of formic acid to the water sample and shake/vortex for approximately 1 minute to ensure homogenization. Transfer an aliquot to LC vial for analysis. High fortification and high residue treated samples may need to be diluted further with control matrix (see section 3.5).

3.5 Influence of Matrix Effects on Analysis

Depending on the matrix, significant matrix effects may interfere with the analysis of the samples. If significant matrix-effects occur, matrix-matched standards may be utilized.

a) Prepare precursor standard solutions for matrix-matched calibration standards according to the following table:

Preparation of Precursor Solutions for Matrix-Matched Standards for M750F002, M750F036, and M750F037.

Initial Concentration (ng/mL)	Aliquot Volume (mL)	Dilute with S1 to a final volume of (mL)	Final Concentration (ng/mL)
100	10	50	20
100	3	50	6
100	1.5	50	3
10	7.5	50	1.5
10	3	50	0.6

b) When preparing 5 matrix-matched standards, prepare at least two extra control samples by completing all steps through 3.4.
c) Combine all samples prepared according to 3.5 [b] above into one sample container and vortex to ensure homogeneity.
d) Prepare matrix-matched calibration standards according to the table below using precursor standard solutions prepared in 3.5 [a] and control matrix in 3.5 [c]:

Preparation of Matrix-Matched Standards for M750F002, M750F036, and M750F037.

Take Precursor Solution (ng/mL)	Volume of Precursor Solution (mL)	Dilute with Control Matrix to a final volume of (mL)	Final Concentration (ng/mL)
20	0.01	1	0.2
6	0.01	1	0.06
3	0.01	1	0.03
1.5	0.01	1	0.015
0.6	0.01	1	0.006

e) Dilute high residue samples to an appropriate concentration with control matrix prepared in 3.5 [c].

3.6 Stability in Sample Matrix

Stability in surface and well water will be tested during the method validation.

4 QUANTIFICATION AND CALCULATION

4.1 Set-up of the analytical run

A sequence for measurement generally consists of:

- Calibration standards
- Control samples
- Procedural recovery samples
- Unknown samples
- Instrument recovery sample

Reagent blanks or blanks can also be injected if considered necessary. Each injection set should begin and end with an injection of a calibration standard. Standards should be interspersed with samples. Each calibration standard should at least be injected twice. At least 5 calibration levels are needed.

4.2 Instrumental analysis

4.2.1 Instrumentation and Conditions

Reg. No.'s 6031465, 6055268 and 148502	Parameter		
Chromatographic System	Waters Acquity UPLC System		
Analytical-column	Xselect HSS T3 C18, 150 x 3 mm, 2.5µm particle size		
Column Temperature	50°C		
Injection Volume	100 µL		
Mobile Phase A Mobile Phase B	Water / formic acid, 1000/1, v/v Acetonitrile / formic acid, 1000/1, v/v		
Flow Rate	800 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00	95	5
	0.25	95	5
	4.25	50	50
	5.50	1	99
	6.99	1	99
	7.00	95	5
	8.00	95	5
Detection System	AB Sciex API 6500 Mass Spectrometer		
Ionisation	Turbo Spray (ESI) Source Temp.: 700°C		
Analyte	**Transitions**	**Polarity**	**Expected Retention Time**
Reg. No. 6031465 **M750F002**	246 --> 70* 246 --> 149	positive	Approx 4.75 min
Reg. No. 6055268 **M750F036**	252 --> 70*	positive	approx. 4.05 min.
Reg. No. 148502 **M750F037**	126 --> 70* 127 --> 99	positive	approx. 2.90 min.

*proposed as quantification transition. Any of these transitions could be used for quantitation in case interference is observed at the same retention time

The system is a UPLC instrument. However, the method operates under HPLC conditions (<400 bar).

4.2.2 Confirmatory Instrumentation and Conditions (for Reg. No. 6055268)

Reg. No.'s 6055268 and 148502	Parameter		
Chromatographic System	Waters Acquity UPLC System		
Analytical-column	Xbridge BEH Phenyl, 150 x 3 mm, 2.5 µm particle size		
Column Temperature	50°C		
Injection Volume	100 µL		
Mobile Phase A Mobile Phase B	Water / formic acid, 1000/1, v/v Acetonitrile / formic acid, 1000/1, v/v		
Flow Rate	800 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00	95	5
	0.25	95	5
	4.00	70	30
	6.50	1	99
	7.35	1	99
	7.40	95	5
	8.00	95	5
Detection System	AB Sciex API 6500 Mass Spectrometer		
Ionisation	Turbo Spray (ESI) Source Temp.: 700°C		
Analyte	**Transitions**	**Polarity**	**Expected Retention Time**
Reg. No. 6055268 **M750F036**	252 --> 70	positive	approx. 3.90 min

[1] The system is a UPLC instrument. However, the method operates under HPLC conditions (<400 bar).

Note: Instruments with similar specifications may substitute the equipment listed above. The instruments used are applicable for analysis if the recoveries of the fortification experiments are in the acceptable range.

A divert valve can be used to reduce the matrix load on the detection system.

Instrument conditions, e.g. injection volume, column, gradient steps may be modified, however changes have to be documented in the raw data. Changes are acceptable, if the recoveries of the fortification experiments are in the acceptable range of the required guidelines.

If the use of different analytical columns (different stationary phase) is required, then methodology has to be validated by analyzing at least five replicates of fortified samples prepared at e.g. LOQ and 10xLOQ. Assessment of matrix impact by preparation of at least one concentration level of a matrix matched standard is also required.

The same applies to different mass transitions used. Validation of the methodology is required as described above (fortification and assessment of matrix effect).

Other parameters, such as ion source gas flows and voltages, are highly specific of the equipment used and therefore not listed. Those parameters may need to be adapted to the actual instrument.

4.2.3 Calibration procedures

Calculation of results is based on peak area measurements using a calibration curve. At least 5 calibration levels need to be injected (e.g., required for enforcement). The calibration curve is obtained by direct injection of standards for LC-MS/MS in the range of 0.2 ng/mL to 0.006 ng/mL. In a given injection run, the same injection volume is used for all samples and standards.

Linear calibration functions are preferred for evaluation. If other functions are used (e.g. quadratic), this should be fully justified.

4.2.4 Calculation of Residues and Recoveries

Calculation of results is based on area measurements.
For the procedural recoveries, a sample volume of 10 g (or 10 mL) will be considered in the final calculation of residues [ng/L]. This approach requires that the sample volume has to be within a measuring precision of 10 ± 0.1 g (or mL) for fortification samples (matrix). The recovery is the percentage of the fortified amount of the analyte (µg or ng), which is recovered after the entire sample work-up steps.

Calculation is described by the equation given below:

The residues of BAS 750 F in ng/L are calculated as shown in equations I and II:

I. **Concentration [ng/mL]** $= \frac{\text{Response} - \textit{Intercept}}{\textit{Slope}} = C_A$

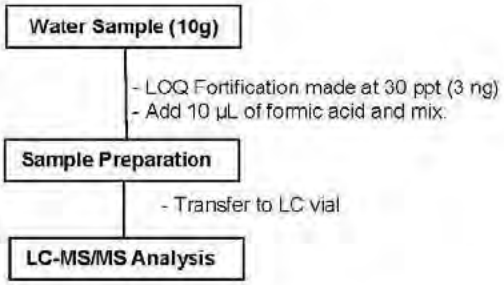
II. **Residue [ng/L]** $= \frac{V_{end} \times C_A}{G \times A_F}$

V_{end} = Final volume of the extract after all dilution steps [mL]
C_A = Concentration of analyte as read from the calibration curve [ng/mL]
G = Volume of sample extracted in L
A_F = Aliquot factor (1 for this method)

The recoveries of spiked compounds are calculated according to equation III:

III. **Recovery %** $= \frac{(\text{Residue in fortified sample} - \text{Residue in control}) \times 100}{\text{Amount of analyte fortified}}$

5 FLOWCHART



6 METHOD MANAGEMENT AND TIME REQUIREMENTS

The analysis of one series of samples (= 13 total samples, 1 reagent blank, 2 controls, 5 fortified samples at LOQ and 5 fortified samples at 10x LOQ) requires 1 working day (8 hours) to complete. This time includes the calculation of the results, the preparation of the equipment as well as the reporting of all raw data under GLP.