

ABSTRACT

The objective of this validation study was to validate the analytical 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 (ground) and surface water by LC-MS/MS.

Principle of the Method

A 5 mL water aliquot sample is extracted by liquid-liquid extraction using 6 mL ethyl acetate. An aliquot of 4 mL of the organic phase is evaporated to dryness using a nitrogen evaporator (N-Vap) at 40°C and the obtained residues are reconstituted in 1 mL acetonitrile/water (50/50, v/v) prior to final determination by LC-MS/MS.

Test Conditions

The method was validated at two fortification levels (0.03 µg/L and 0.3 µg/L) for ground and surface water. For each fortification level and water type, five replicates were prepared and analysed. Additionally, at least two replicates of unfortified samples were analysed (untreated control samples). Two mass transitions were evaluated for quantification and confirmation of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008.

Limit of Quantification (LOQ) and Limit of Detection (LOD)

The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested, hence 0.03 µg/L. The limit of detection (LOD) is 0.009 µg/L, corresponding to the lowest calibration level used.

Selectivity

The method determined residues of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 in water. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered.

Specificity

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

Linearity

Good linearity ($r \geq 0.995$) was observed in the range of 0.03 ng/mL to 1 ng/mL for the mass transitions of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008. Six calibration levels, prepared in acetonitrile/water (50/50, v/v) and distributed over the tested concentration range, were used.

Stability in Working Solutions

Stability tests confirmed that BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 were stable for a maximum duration of 30 days in stock and calibration solutions, when stored refrigerated at approximately 4°C in the dark. Stock solutions were prepared in acetonitrile, while calibration solutions were prepared in acetonitrile/water (50/50, v/v).

Stability in Sample Extracts

The experiments demonstrate that BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 were stable in final water-sample extracts, prepared in acetonitrile/water, (50/50, v/v), over a time period of 7 days in case of surface water and 8 days in case of ground water, when stored refrigerated at approximately 4°C in the dark.

Matrix Effects

Solvent- as well as matrix-matched standards were analysed to assess potential matrix effects. As no significant matrix effects were identified, solvent standards, prepared in acetonitrile/water (50/50, v/v), were used for calibration and quantification of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008.

1 INTRODUCTION

1.1 Scope of the Study

The purpose of this study was to validate analytical method L0359/01 for the determination of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 in ground and surface water by LC-MS/MS according to the cited guidelines. Therefore, a residue analytical method for the determination of the test items in water with a limit of quantification (LOQ) of 0.03 µg/L was required.

This method was developed at BASF SE, located in Limburgerhof (Germany).

1.2 Principle of the Method

A 5 mL water aliquot sample was extracted by liquid-liquid extraction using 6 mL ethyl acetate. An aliquot of 4 mL of the organic phase was evaporated to dryness using a nitrogen evaporator (N-Vap) at 40°C and the obtained residues were reconstituted in 1 mL acetonitrile/water (50/50, v/v) prior to final determination by LC-MS/MS.

The limit of quantification (LOQ) is 0.03 µg/L for BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 in water. The limit of detection (LOD) is 0.009 µg/L.

1.3 Specificity

The method is highly specific for analysis of the test items (mass transitions from the positively charged molecule ions to typical fragment ions in MS/MS mode). The retention times of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 in extracts matched the retention times in calibration solutions. No peak interferences occurred at the retention times of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008. Since detection by MS/MS with two characteristic mass transitions is regarded to be highly specific, no further confirmatory method is required.

2 MATERIALS AND METHODS

2.1 Test Systems

The following test systems were considered in this validation study:

Test System 1: Ground Water (certificate of analysis see Figure A 135)

Test System 2: Surface Water (certificate of analysis see Figure A 136)

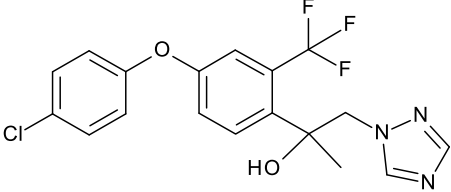
Test System 3: Acetonitrile for stability testing in stock solutions (section 3.1)

Test System 4: Acetonitrile/Water (50/50, v/v) for stability testing in calibration solutions (section 3.1) and final water samples extracts (section 3.2)

2.2 Test and Reference Items

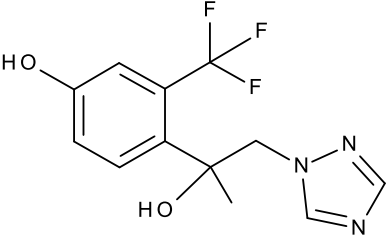
2.2.1 BAS 750 F (Reg.No.5834378)

The certificate of analysis of BAS 750 F is shown in Figure A 137.

BAS-Code	BAS 750 F	
Reg.No.	5834378	
Common Name	Mefentrifluconazole	
IUPAC Name	(2RS)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol	
CAS-No.	1417782-03-6	
Molecular Formula	C ₁₈ H ₁₅ ClF ₃ N ₃ O ₂	
Molecular Weight	397.8 g/mol	
Batch No.	L85-124	
Purity	99.7%	
Expiration Date	01.07.2017	

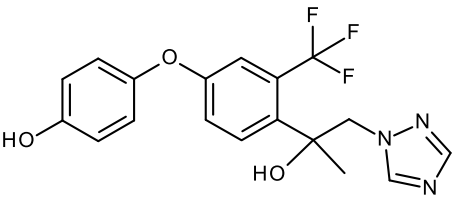
2.2.2 M750F003 (Reg.No.5924326)

The certificate of analysis of M750F003 is shown in Figure A 138.

Metabolite-Code	M750F003	
Reg.No.	5924326	
IUPAC Name	4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenol	
CAS-No.	-/-	
Molecular Formula	C ₁₂ H ₁₂ F ₃ N ₃ O ₂	
Molecular Weight	287.2 g/mol	
Batch No.	L84-250	
Purity	99.6%	
Expiration Date	01.05.2017	

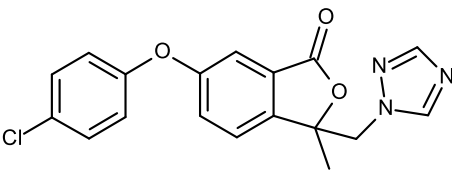
2.2.3 M750F005 (Reg.No.6003433)

The certificate of analysis of M750F005 is shown in Figure A 139.

Metabolite-Code	M750F005	
Reg.No.	6003433	
IUPAC Name	4-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenol	
CAS-No.	-/-	
Molecular Formula	C ₁₈ H ₁₆ F ₃ N ₃ O ₃	
Molecular Weight	379.3 g/mol	
Batch No.	L87-34	
Purity	99.4%	
Expiration Date	01.10.2018	

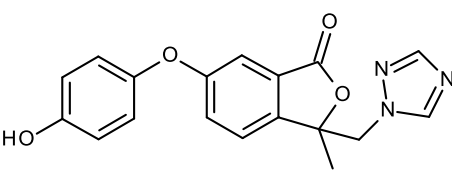
2.2.4 M750F006 (Reg.No.5863469)

The certificate of analysis of M750F006 is shown in Figure A 140.

Metabolite-Code	M750F006	
Reg.No.	5863469	
IUPAC Name	6-(4-chlorophenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one	
CAS-No.	-/-	
Molecular Formula	C ₁₈ H ₁₄ ClN ₃ O ₃	
Molecular Weight	355.8 g/mol	
Batch No.	L85-170	
Purity	95.6%	
Expiration Date	01.02.2018	

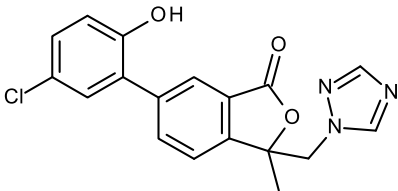
2.2.5 M750F007 (Reg.No.6003432)

The certificate of analysis of M750F007 is shown in Figure A 141.

Metabolite-Code	M750F007	
Reg.No.	6003432	
IUPAC Name	6-(4-hydroxyphenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one	
CAS-No.	-/-	
Molecular Formula	C ₁₈ H ₁₅ N ₃ O ₄	
Molecular Weight	337.3 g/mol	
Batch No.	L87-32-1	
Purity	94.4%	
Expiration Date	01.01.2019	

2.2.6 M750F008 (Reg.No.6010286)

The certificate of analysis of M750F008 is shown in Figure A 142.

Metabolite-Code	M750F008	
Reg.No.	6010286	
IUPAC Name	6-(5-chloro-2-hydroxyphenyl)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one	
CAS-No.	-/-	
Molecular Formula	C ₁₈ H ₁₄ ClN ₃ O ₃	
Molecular Weight	355.8 g/mol	
Batch No.	L85-94	
Purity	90.4%	
Expiration Date	01.01.2019	

2.3 Materials and Methods

2.3.1 Equipment

Equipment	Size, Description	Manufacturer
Analytical Balance	XP 205	Mettler (Germany)
Pipettes	Various volumes	Gilson Medical Electronics S.A. (France)
Measuring pipets	5 mL	Hirschmann (Germany)
Tubes	12 mL	Schott Glaswerke (Germany)
Autosampler vials	0.2 mL, 1.5 mL N11 with integrated insert	Macherey-Nagel GmbH (Germany)
Snap caps	Snap Ring Caps N11 with cross-slit	Macherey-Nagel GmbH (Germany)
Nitrogen dryer	1-VIS (N-Vap)	VLM (Germany)
Amber bottles	15 mL	Sigma-Aldrich/Supelco (Germany)
Regular laboratory equipment	--	--

2.3.2 Reagents

2.3.2.1 Chemicals

Chemical	Grade
Purified water of low conductivity	Gradient grade
Acetonitrile	Gradient grade
Formic acid	Gradient grade
Ethyl acetate	Gradient grade

2.3.2.2 Solutions and Solvent Mixtures

Description	Code	Composition
Solution 1	S1	Pure water of low conductivity
Solution 2	S2	Ethyl acetate
Solution 3	S3	Acetonitrile
Solution 4	S4	Acetonitrile/water (50/50, v/v)
HPLC mobile phase A	LC1	Water / Formic acid (1000/1, v/v)
HPLC mobile phase B	LC2	Acetonitrile / Formic acid (1000/1, v/v)

2.3.3 Working Solutions

2.3.3.1 Stock Solutions

Individual stock solutions with a concentration of 1 mg/mL were prepared for each of the six analytes by weighing an appropriate amount of each analyte into a flask and adding the required volume of acetonitrile, e.g. 5.74 mg AS + 5.74 mL acetonitrile. As the purity of analytes M750F007 and M750F008 was below 95% (see section 2.2), a correction of the weigh-in for purity was done for all analytes.

2.3.3.2 Fortification Solutions

Mixed fortification solutions were prepared by adding 1 mL of each individual prepared stock solution of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 in a flask and diluting with acetonitrile (S3) to a mixed fortification solution with a concentration of 100 µg/mL. Then, 1 mL of the mixed fortification solution was taken and diluted with acetonitrile (S3) to a mixed fortification solution with a concentration of 10 µg/mL. Further dilution series were prepared by using acetonitrile/water (50/50, v/v, S4). Homogenous solutions were achieved by vortexing of the solutions.

Dilution series for mixed fortification solutions were prepared as exemplarily described for analysis packages L002 and L003 in the table below.

Preparation of Fortification Solutions (exemplified for Analysis Packages L002 and L003)

Take Solution [µg/mL]	Volume [mL]	Solution Identifier	Dilute to a Volume of S3 [mL]	Concentration (mixed) [µg/mL]	Solution Identifier
1000 (BAS 750 F)	1.0	SsoL171097	10	100	SsoL171103
1000 (M750F003)	1.0	SsoL171098			
1000 (M750F005)	1.0	SsoL171099			
1000 (M750F006)	1.0	SsoL171100			
1000 (M750F007)	1.0	SsoL171126			
1000 (M750F008)	1.0	SsoL171127			
100	1.0	SsoL171103	10	10	SsoL171104
Take Solution (mixed) [µg/mL]	Volume [mL]	Solution Identifier	Dilute to a Volume of S4 [mL]	Concentration (mixed) [µg/mL]	Solution Identifier
10	1.0	SsoL171104	10	1.0	SsoL171105
1.0	1.0	SsoL171105	10	0.1	SsoL171106
0.1	3.0	SsoL171106	10	0.03 ¹⁾	SsoL171107
0.03	1.0	SsoL171107	10	0.003 ²⁾	SsoL171108

¹⁾ Used as fortification solution at 10x LOQ level

²⁾ Used as fortification solution at LOQ level

2.3.3.3 Calibration Solutions

Mixed calibration solutions were prepared by adding 1 mL of each individual prepared stock solution of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 in a flask and diluting with acetonitrile (S3) to a mixed calibration solution with a concentration of 100 µg/mL. Then, 1 mL of the mixed calibration solution was taken and diluted with acetonitrile (S3) to a mixed calibration solution of 10 µg/mL. Further dilution series were prepared by using acetonitrile/water (50/50, v/v, S4). Homogenous solutions were achieved by vortexing of the solutions.

Dilution series for mixed calibration solutions were prepared as exemplarily described for analysis packages L001 to L006 in the table below.

Preparation of Mixed Calibration Solutions (exemplified for Analysis Packages L001 to L006)

Take Solution [µg/mL]	Volume [mL]	Solution Identifier	Dilute to a Volume of S3 [mL]	Concentration (mixed) [µg/mL]	Solution Identifier
1000 (BAS 750 F)	1.0	SsoL171109	10	100	SsoL171115
1000 (M750F003)	1.0	SsoL171110			
1000 (M750F005)	1.0	SsoL171111			
1000 (M750F006)	1.0	SsoL171112			
1000 (M750F007)	1.0	SsoL171113			
1000 (M750F008)	1.0	SsoL171114			
100	1.0	SsoL171115	10	10	SsoL171116
Take Solution (mixed) [ng/mL]	Volume [mL]	Solution Identifier	Dilute to a Volume of S4 [mL]	Concentration (mixed) [ng/mL]	Solution Identifier
10000	1.0	SsoL171116	10	1000	SsoL171117
1000	1.0	SsoL171117	10	100	SsoL171118
100	1.0	SsoL171118	10	10	SsoL171119
10	1.0	SsoL171119	10	1.0 ¹⁾	SsoL171120
10	0.5	SsoL171119	10	0.5 ¹⁾	SsoL171121
10	0.25	SsoL171119	10	0.25 ¹⁾	SsoL171122
10	0.1	SsoL171119	10	0.1 ¹⁾	SsoL171123
10	0.05	SsoL171119	10	0.05 ¹⁾	SsoL171124
10	0.03	SsoL171119	10	0.03 ¹⁾	SsoL171125

¹⁾ Used as calibration level

2.3.3.4 Matrix-matched Calibration Solutions

Mixed matrix-matched standards of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 were prepared to investigate the influence of the matrix load after sample preparation on the analysis (for details see section 3.4). For this purpose, mixed matrix-matched standard solutions were prepared, by reconstitution of the residues after evaporation of the ethyl acetate extract of a control sample, by using adequate standard solutions instead of the pure acetonitrile/water mixture (50/50, v/v, S4). Therefore the matrix-load in the prepared matrix-matched standards was 100% of the possible matrix level for all matrix matched standards. Corresponding to the solvents standards, six mixed matrix-matched standards for each water type were prepared at concentration levels 0.03, 0.05, 0.1, 0.25, 0.5 and 1.0 ng/mL.

2.3.4 Set-up of the Analytical Run

Each analytical set began with a calibration standard and ended with the injection of a calibration standard. In general, calibration standards were interspersed with samples (e.g. fortified, control and quality control samples and blanks). Within each analytical series, each calibration standard was measured at least 2 times. Six calibration levels with concentrations ranging between 0.03 ng/mL and 1 ng/mL were injected.

2.4 Instrumental Analysis

2.4.1 Analytical Procedure

2.4.1.1 Weighing and Fortification

For the preparation of control samples, an aliquot of 5 mL of the water sample was transferred into a centrifuge tube with screw cap.

For the preparation of fortified samples, an aliquot of 5 mL of the water sample was transferred into a centrifuge tube with screw cap and spiked with the respective fortification solution. The principle of fortification is described in the table below:

Sample Type	Sample Volume [mL]	Concentration of Spiking Solution [$\mu\text{g/mL}$]	Volume of Spiking Solution [mL]	Level of Fortification [$\mu\text{g/L}$]
Control	5	--	--	0.0
Fortification (LOQ)	5	0.003	0.05	0.03 ¹⁾
Fortification (10x LOQ)	5	0.03	0.05	0.3

¹⁾ Limit of quantification

2.4.1.2 Extraction of Sample Material

A 5 mL aliquot of the water sample was transferred into a sealable 12 mL centrifuge tube.

After adding 6 mL of ethyl acetate, the centrifuge tube was shaken on a vortexer at 3000 rpm for 10 sec, and subsequently shaken on a mechanical shaker at 225 rpm for 1 min.

After phase separation, 4 mL of the ethyl acetate phase was transferred into a 12 mL glass test tube. The solvent was evaporated to drying by applying a gentle stream of nitrogen at 40°C (N-Vap).

The residue was dissolved with an appropriate volume of acetonitrile/water (50/50, v/v, S4).

2.4.1.3 Preparation for Measurement

For residues around the LOQ and control samples, an aliquot ($V_{\text{end}} = \text{final volume} = 1 \text{ mL}$) was transferred into a LC vial and analysed by LC-MS/MS without further dilution.

For residues >9x LOQ, samples were diluted with acetonitrile/water (50/50, v/v) into the calibration range prior to LC-MS/MS analysis.

2.4.2 Instrumentation and Conditions

The chromatographic system and conditions used for the analysis of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 are shown in the table below.

		Parameter		
Chromatographic System	Waters Acquity LC system			
Analytical Column	Waters Xbridge C18, 50 x 2.1 mm, 2.5 µm particle size			
Column Temperature	30°C			
Injection Volume	10 µL Partial loop with needle overfill; load ahead; loop offline - disabled			
Injection Procedure	Strong wash with acetonitrile (200 µL), weak wash with acetonitrile/water (50/50, v/v) (600 µL)			
Mobile Phase A	Water / formic acid, 1000/1, v/v			
Mobile Phase B	Acetonitrile / formic acid, 1000/1, v/v			
Flow Rate	800 µL/min			
Gradient (including wash and equilibration, equilibration time)	Time [min]	Phase A [%]	Phase B [%]	
	0.00	95	5	
	0.25	95	5	
	1.50	60	40	
	2.50	1	99	
	3.45	1	99	
	3.50	95	5	
4.00	95	5		
Divert Valve	yes			
Time to waste	0.5 min to 3.5 min to MS; remaining elution time to waste			
Detection System	PE Sciex API 6500 Mass Spectrometer			
Ionisation	Electrospray (ESI)			
Analyte	Transitions	Polarity	Retention Time	
BAS 750 F (Reg.No. 5834378)	398 → 70 ¹⁾ 400 → 70	Positive	approx. 2.3 min	
M750F003 (Reg.No. 5924326)	288 → 70 ¹⁾ 288 → 43	Positive	approx. 1.4 min	
M750F005 (Reg.No. 6003433)	380 → 70 ¹⁾ 380 → 109	Positive	approx. 1.9 min	
M750F006 (Reg.No. 5863469)	356 → 259 ¹⁾ 356 → 217	Positive	approx. 2.1 min	
M750F007 (Reg.No. 6003432)	338 → 241 ¹⁾ 338 → 269	Positive	approx. 1.6 min	
M750F008 (Reg.No. 6010286)	356 → 259 ¹⁾ 356 → 241	Positive	approx. 1.9 min	

¹⁾ Proposed as quantification transition

2.4.3 Calibration Procedure

The calculation of the results was based on the peak area measured using a calibration curve. Six calibration levels were injected. Calibration curves were obtained by direct injection of solvent standards covering a concentration range of 0.03 ng/mL to 1 ng/mL. The same volume (10 µL) was injected for all samples and standard solutions.

2.4.4 Influence of Matrix Effects on the Analysis

In order to assess the influence of the matrix effects on the analysis, the response of the analytes in the presence of the water matrices was compared to calibration standards prepared in acetonitrile/water (50/50, v/v) (see Table A 1 to Table A 24). For preparation of matrix-matched standards, refer to section 2.3.3.4.

2.4.5 Quality Control Samples

Quality control samples were prepared to check the instrument performance during the analytical series for recovery experiments. For this purpose, a calibration solution with a concentration of 1.0 ng/mL was diluted by a factor of 10 with an untreated control sample extract, resulting in a concentration of 0.1 ng/mL. Therefore, the matrix load in the quality control samples was at least 90%.

2.4.6 Untreated Control Samples and Reagent Blank

The untreated control samples were prepared in an identical manner as the fortified water samples, except that no test items had been applied prior to extraction. Furthermore, reagent blanks were analysed, which were prepared by taking only the solutions and solvents, without any matrix or analytes, through the entire extraction and workup procedure.

2.4.7 Calculation of Residues and Recoveries

For the procedural recoveries, a sample volume of 5 mL will be considered in the final calculation of residues [$\mu\text{g/L}$]. The recovery is the percentage of the fortified amount of the analyte, which is recovered after the entire sample work-up steps.

The residues of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 in $\mu\text{g/L}$ are calculated as shown in equations I and II:

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

$$\text{II. Residues in Water } [\mu\text{g/L}] = \frac{V_{\text{end}} \times C_A}{A \times A_F}$$

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]
A	=	Amount of the sample extracted [mL]
A_F	=	Aliquotation factor

Recovery is the percentage of the fortified amount of the analyte, which is recovered through the method. The recoveries of spiked compounds are calculated according to equation III:

$$\text{III. Recovery [\%]} = \frac{\text{Residue in fortified sample} \times 100}{\text{Amount of analyte fortified}}$$

$$\text{Recovery corrected [\%]} = \frac{(\text{Residue in fortified sample} - \text{Residue in control}) \times 100}{\text{Amount of analyte fortified}}$$

Example of Calculation:

BAS 750 F, mass transition 398→70 in ground water fortified at 0.03 µg/L:

The following values were used in this calculation:

Worklist no.	2017vwt0004
Peak area of fortified sample (ForL0003)	164124
Peak area of control sample ¹⁾ (ConL0001 and ConL0002)	0.0
Slope	1700000
Intercept	2930
Sample Aliquot (A)	5 mL
Final Volume (V _{end})	1 mL
Aliquotation Factor (A _F)	0.666667 (= 66.6667%)

¹⁾ Area of two control samples in the same worklist

$$\text{Concentration of fortified sample [ng/mL]} = \frac{(164124 - 2930)}{1700000} = 0.0948 \text{ ng/mL}$$

$$\text{Residue fortified sample [µg/L]} = \frac{1 \text{ mL} \times 0.0948 \text{ ng/mL}}{5 \text{ mL} \times 0.666667} = 0.0284 \text{ ng/mL} \equiv \mu\text{g/L}$$

$$\text{Recovery [\%]} = \frac{0.0284 \mu\text{g/L}}{0.03 \mu\text{g/L}} \times 100 \% = 95 \%$$

$$\text{Recovery corrected [\%]} = \frac{(0.0284 \mu\text{g/L} - 0.0 \mu\text{g/L}) \times 100 \%}{0.03 \mu\text{g/L}} = 95 \%$$

Since the control value (untreated sample) is less than the limit of detection, the corrected recovery is only calculated exemplarily here.

Remark: Calculations in this example were performed with rounded numerical values.

3.5 Summary of Method

Type of Method: LC-MS/MS

Test Systems: Ground and Surface Water

Analyte and selected Mass Transitions:

BAS 750 F	398 → 70
(Reg.No. 5834378)	400 → 70
M750F003	288 → 70
(Reg.No. 5924326)	288 → 43
M750F005	380 → 70
(Reg.No. 6003433)	380 → 109
M750F006	356 → 259
(Reg.No. 5863469)	356 → 217
M750F007	338 → 241
(Reg.No. 6003432)	338 → 269
M750F008	356 → 259
(Reg.No. 6010286)	356 → 241

Analytical Procedure: Ethyl acetate extraction of the analytes, after evaporation and reconstitution of the residues in ACN/water final determination by LC-MS/MS measurement.

Confirmatory Technique: Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary.

The quantification of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 is based on the monitoring of two mass transitions. Recovery data are reported for each mass transition and matrix considered.

Matrix Effects: It was demonstrated that the matrix-load in the tested matrix-matched standards had no significant influence on the detection of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008. Therefore, no matrix-matched standards are required for quantification.

Limit of Detection (LOD): The limit of detection (LOD) is 0.009 µg/L (field sample level), corresponding to the lowest calibration level of 0.03 ng/mL (measurement sample level).

<u>Limit of Quantification (LOQ):</u>	The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. The LOQ is 0.03 µg/L (field sample level), corresponding to a concentration of 0.1 ng/mL (measurement sample level) in the water sample extract after work-up.
<u>Levels of Fortification:</u>	0.03 µg/L and 0.3 µg/L (LOQ and 10x LOQ).
<u>Time required:</u>	A set of 13 samples requires about 12 hours of work (calculation of the results, preparation of equipment and
<u>Linearity:</u>	Good linearity ($r \geq 0.995$) was observed in the range of 0.03 ng/mL to 1 ng/mL for the mass transitions of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008.
<u>Specificity:</u>	The method L0359/01 determines residues of BAS 750 F and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008 in ground and surface water. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of the analytes.
<u>Reproducibility:</u>	Reproducibility of the method was not determined within this validation study, but an ILV has been conducted by an independent laboratory [Ref. 4].

6.3 Additional Information on the Method

Figure A 19: Method Flowchart

