ABSTRACT

The purpose of this study was to demonstrate that BASF Analytical Method D1608/01 “Method for the Determination of BAS 450 I (Reg. No. 5672774) and Its Metabolites DM-8007 (Reg. No. 5856361), DC-DM-8007 (Reg. No. 5936906), DC-8007 (Reg. No. 5936907) and S(PFP-OH)-8007 (Reg. No. 5959598) in Surface and Drinking Water by LC-MS/MS”, (reference 1) can be performed with acceptable recoveries at an outside facility.

Principle of the method. For the determination of the residues of BAS 450 I (Reg. No. 5672774) and its metabolites DM-8007 (Reg. No. 5856361), DC-DM-8007 (Reg. No. 5936906), DC-8007 (Reg. No. 5936907) and S(PFP-OH)-8007 (Reg. No. 5959598) in surface and drinking water, 10 mL methanol is added to a 10 gm water sample and mixed. After filtration, the sample is ready for analysis by LC-MS/MS.

Test conditions. For validation, untreated water samples were fortified with BAS 450 I, and its metabolites DM-8007, DC-DM-8007, DC-8007 and S(PFP-OH)-8007, and analyzed according to the established method validation guidelines. The analytical sets for each matrix typically consisted of a reagent blank, two control samples, five replicates fortified with each analyte at the method limit of quantitation of 5 ppt (LOQ) and five replicates fortified at 50 ppt (10×LOQ). The transitions for BAS 450 I at *m/z* 663 → 643 and at *m/z* 665 → 645 were monitored in positive mode for primary and confirmation quantification, respectively. The transitions for DC-DM-8007 at *m/z* 545 → 525 and at *m/z* 547 → 527 were monitored in positive mode for primary and confirmation quantification, respectively. The transitions for DC-8007 at *m/z* 559 → 539 and at *m/z* 561 → 541 were monitored in positive mode for primary and confirmation quantification, respectively. The transitions for DM-8007 at *m/z* 649 → 242 and at *m/z* 651 → 242 were monitored in positive mode for primary and confirmation quantification, respectively. The transitions for S(PFP-OH)-8007 at *m/z* 661 → 641 and at *m/z* 661 → 621 were monitored in positive mode for primary and confirmation quantification, respectively.

Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ of the method was set at 5 ng/L (5 ppt) in water for BAS 450 I and 25 ng/L (25 ppt) in water for the metabolites, which was lower than the lowest relevant endpoint in water ecotoxicology (LC50 > 4.8 μg/L) and also defined as the lowest fortification level for each analyte. The limit of detection (LOD) in water was set at 1 ng/L for BAS 450 I and 5 ng/L for the metabolites, which was 20% of the defined LOQ. The LOD for each analyte was shown to be detectable as the absolute amount of analyte injected (0.0000375 ng BAS 450 I and 0.0001875 ng of each metabolite on-column) with acceptable signal to noise ratio (S/N is > 3:1).

Selectivity. The method determines residues of BAS 450 I, and its metabolites DM-8007, DC-DM-8007, DC-8007 and S(PFP-OH)-8007 in water by LC-MS/MS using two transitions. No interfering peaks were found at the retention times for all analytes.

Linearity. Acceptable linearity was observed for the standard range and the two mass transitions tested. The method-detector response was linear over the 0.0005-0.01 ng/mL range for BAS 450 I and 0.0025-0.05 ng/mL range for metabolites ($r \geq 0.99$), in water analysis.

Standard Stability. Standards, stock and fortification solutions of BAS 450 I, DM-8007, DC- DM-8007, DC-8007, and S(PFP-OH)-8007 are prepared in acetonitrile, and calibration standard solutions were prepared by serial dilution of the intermediate standard solutions using 50/50 methanol/water (v:v). During the course of this study, the test/reference substance solutions were stored at temperature of 2°C - 6°C and all solutions were used within the demonstrated time period of stability.

All analytes have been shown to be stable in stock and fortifcation solutions prepared in acetonitrile for at least 92 days when stored under refrigeration. Each analyte has been shown to be stable in calibration

standard solutions prepared by serial dilution of the intermediate standard solutions with 50/50 methanol/water (v:v) and held under refrigeration for at least 30 days for all analytes.

Extract Stability. Surface and drinking water extracts have been shown to be stable, when stored under refrigeration, for at least 6 days for 450 I and 7 days for metabolites.

Recovery and Repeatability. The independent laboratory validation (ILV) was performed successfully for each water matrix and the LC-MS/MS ion transitions (primary and secondary) available for the method, using solvent-based standards.

Apparent residues of BAS 450 I and metabolites were below the method limit of detection in all of the control water samples.

1. INTRODUCTION

1.1 Scope of the Method

BASF Analytical Method No. D1608/01 was developed to determine the residues of BAS 450 I and its metabolites in surface and drinking water matrices by LC-MS/MS at BASF Crop Protection in Research Triangle Park, North Carolina. This method was validated at BASF Crop Protection in Research Triangle Park, North Carolina (Reference 1) and was independently validated at Primera Analytical Solution Corp.

The independent lab validation was conducted using two fortification levels limit of quantitation (LOQ) 5 ng/L (5 ppt) in water for BAS 450 I and 25 ng/L (25 ppt) in water for the metabolites and 10xLOQ.

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 samples (10 g) were weighed, fortified with the compounds and then extracted with 10 mL methanol. The extracts were then filtered and analyzed with LC-MS/MS.

1.3 Specificity

To demonstrate the specificity of the analytical method, one additional mass transition (*m/z* 665 → 645) was monitored simultaneously to the primary quantitation transition (*m/z* 663 → 643) for analysis of BAS 450 I. One additional mass transition (*m/z* 547 → 527) was monitored simultaneously to the primary quantitation transition (*m/z* 545 → 525) for analysis of DC-DM-8007. One additional mass transition (*m/z* 561 → 541) was monitored simultaneously to the primary quantitation transition (*m/z* 559 → 539) for analysis of DC-8007. One additional mass transition (*m/z* 651 → 242) was monitored simultaneously to the primary quantitation transition (*m/z* 649 → 242) for analysis of DM-8007. One additional mass transition (*m/z* 661 → 621) was monitored simultaneously to the primary quantitation transition (*m/z* 661 → 641) for analysis of S(PFP-OH)-8007. The method was able to accurately determine residues of BAS 450 I and all metabolites. No interference was observed at the retention times of the analyte peaks.

2. REFERENCE SUBSTANCE AND SAMPLING HISTORY

2.1 Test Systems

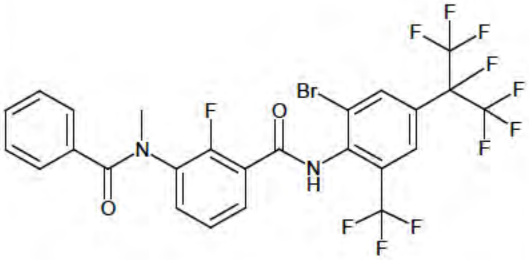
The test systems considered in this study were surface water and drinking water. The water matrices were characterized by AGVISE Laboratories. A copy of the characterization data is provided in Appendix E.

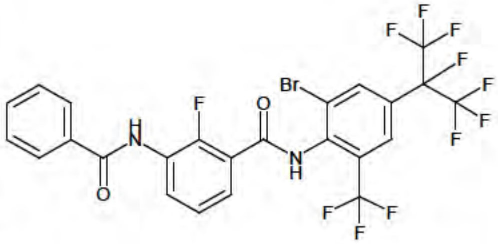
The control samples were provided by BASF. The samples were received on November 15, 2016. Upon arrival at the laboratory, the samples were opened, inspected, and checked against enclosed shipping forms and assigned a unique laboratory analysis code
(e.g.,161100-1).
The test systems were received frozen and were stored under frozen conditions at all times until sample analysis.

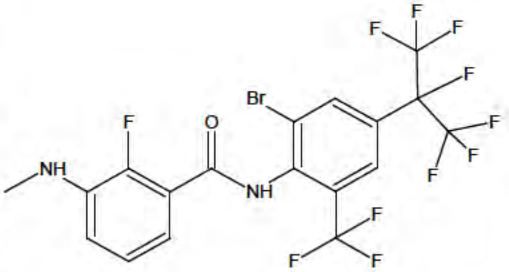
2.2 Test and Reference Substances

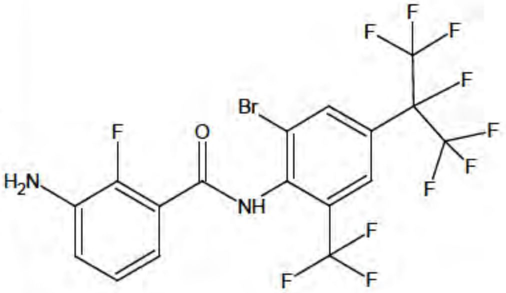
The standard substance was stored at room temperature. BASF has retained a reserve sample of this chemical, and has documentation specifying the location of the synthesis and characterization information available at BASF Crop Protection, Research Triangle Park, North Carolina.

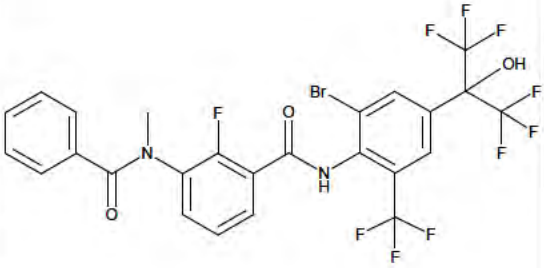
BAS 450 I (Lot No.089-100112-1), DM-8007 (Lot No. 296-007-81-1), DC-DM-8007 (Lot No. 296-009-094-2), DC-8007 (Lot No. 296-012-009-1) and S(PFP-OH)-8007 (Lot No. 267-012-094-3), reference substances were provided by the sponsor and received on August 02, 2016. The certificates of analysis of all substances are presented in Appendix B. A detailed summary of the reference substances is presented below.

BAS Code Name	BAS 450 I	
Name	Broflanilide	
Chemical Name	N-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluoro-3-(N-methylbenzamido)benzamide	
BASF Code No.	5672774	
CAS-No.	1207727-04-5	
Molecular Formula	$C_{25}H_{14}BrF_{11}N_2O_2$	
Molecular Weight	663.29	
Lot Number	089-100112-1	
Purity	99.67%	
Expiration Date	April 09, 2017	
Storage Condition	Room T, light-protection	

BAS Code Name	None	
Name	DM-8007	
Chemical Name	3-benzamido-*N*-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluorobenzamide	
BASF Code No.	5856361	
CAS-No.	None	
Molecular Formula	$C_{24}H_{12}BrF_{11}N_2O_2$	
Molecular Weight	649.3	
Lot Number	296-007-81-1	
Purity	98.84%	
Expiration Date	June 28, 2017	
Storage Condition	Room T, light-protection	

BAS-Code	None	
Common Name	DC-8007	
Chemical Name	N-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]- 2-fluoro-3-(methylamino)benzamide	
BASF Reg. No.	5936907	
CAS-No.	None	
Molecular Formula	$C_{18}H_{10}BrF_{11}N_2O$	
Molecular Weight	559.2	
Lot Number	296-012-009-1	
Purity	99.07%	
Expiration Date	June 28, 2017	
Storage Condition	Room T, light-protection	

BAS-Code	None	
Common Name	DC-DM-8007	
Chemical Name	3-amino-*N*-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2- fluorobenzamide	
BASF Reg. No.	5936906	
CAS-No.	None	
Molecular Formula	$C_{17}H_8BrF_{11}N_2O$	
Molecular Weight	545.1	
Lot Number	296-009-094-2	
Purity	98.58%	
Expiration Date	June 8, 2017	
Storage Condition	Room T, light-protection	

BAS Code Name	None	
Name	S(PFP-OH)-8007	
Chemical Name	*N*-[2-bromo-4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl-6-(trifluoromethyl)phenyl]-2-fluoro-3-(*N*-methlybenzamido)benzamide	
BASF Code No.	5959598	
CAS-No.	None	
Molecular Formula	$C_{25}H_{15}BrF_{10}N_2O_3$	
Molecular Weight	661.3	
Lot Number	267-012-094-3	
Purity	99.06%	
Expiration Date	June 28, 2017	
Storage Condition	Room T, light-protection	

3. ANALYTICAL METHOD

BASF Analytical Method D1608/01 of "Method for the Determination of BAS 450 I (Reg. No. 5672774) and Its Metabolites DM-8007 (Reg. No. 5856361), DC-DM-8007 (Reg. No. 5936906), DC-8007 (Reg. No. 5936907) and S(PFP-OH)-8007 (Reg. No. 5959598) in Surface and Drinking Water by LC-MS/MS" was used for sample analysis for this study.

The primary (quantitative) and secondary (confirmatory) transition ions monitored are presented below:

Analyte	Transition (*m/z*)		Ionization Mode	Retention Time (min)
	Primary	Secondary		
BAS 450 I	663 →643	665 →645	Positive	4.19
DC-DM-8007	545 →525	547 →527	Positive	3.99
DC-8007	559 →539	561 →541	Positive	4.12
DM-8007	649 →242	651 →242	Positive	4.23
S(PFP-OH)-8007	661 → 641	661 → 621	Positive	3.92

STATISTICS AND DATA INTEGRITY

Statistical treatment of the data included simple descriptive statistics, such as determinations of averages, standard deviation and/or relative standard deviation (RSD) for the procedural recoveries and area counts and calculation of the calibration curve and correlation coefficient (r) by linear regression with 1/x weighting of the instrument responses for the reference standards. The statistical calculations throughout this report were performed using Microsoft Excel® and were rounded for presentation purposes.

Several measures were taken to ensure the quality of the study results. The quality assurance unit at Primera Analytical Solutions Corp. inspected the analytical procedures for compliance with Good Laboratory Practices that included adherence to the protocol. The dates inspected are detailed in the quality assurance unit statement. Study samples, test and reference items were maintained in a secured laboratory with limited access.

5. SUMMARY OF METHOD

Type of Method LC-MS/MS

Test Systems Surface water and drinking water

Selected mass transitions (m/z)

	Quantitation	Confirmation
BAS 450 I	663 → 643	665 → 645
DC-DM-8007	545 → 525	547 → 527
DC-8007	559 → 539	561 → 541
DM-8007	649 → 242	651 → 242
S(PFP-OH)-8007	661 → 641	661 → 621

Analytical Procedure BASF Analytical Method D1608/01, “Method for the Determination of BAS 450 I (Reg. No. 5672774) and Its Metabolites DM-8007 (Reg. No. 5856361), DC-DM-8007 (Reg. No. 5936906), DC-8007 (Reg. No. 5936907) and S(PFP-OH)-8007 (Reg. No. 5959598) in Surface and Drinking Water by LC-MS/MS”

Confirmatory Technique A secondary MRM transition for BAS 450 I at *m/z* 665 → 645, at *m/z* 547 → 527 for DC-DM-8007, at *m/z* 561 → 541 for DC-8007, at *m/z* 651 → 242 for DM-8007, at *m/z* 661 → 621 for S(PFP-OH)-8007 in positive mode monitored for confirmation quantification.

Method of Quantitation The quantitation is based on the monitoring of two mass transitions for BAS 450 I, DC-8007, DC-DM-8007, DM-8007 and S(PFP-OH)-8007. Recovery data was reported for each mass transition considered.

LOQ 5 ng/L (5 ppt) in water for BAS 450 I and 25 ng/L (25 ppt) in water for the metabolites

LOD 1 ng/L in water for BAS 450 I and 5 ng/L in water for the metabolites

Levels of Fortification	5 ppt and 50 ppt for BAS 450 I and 25 ppt and 250 ppt for metabolites in surface and drinking water
Time Required	A set of 13 samples requires approximately 8 hours of work (calculation of the results included).
Justification of Ions	The ions used to conduct the ILV were determined in the validation (Reference 1) and are shown in Appendix D.

7. RECOMMENDATIONS/ CONCLUSIONS FROM ILV

This independent laboratory validation was successfully completed on the first trial at Primera Analytical Solution Corp for all matrices with the exception of modified chromatographic conditions. Recovery results and statistical data demonstrate BASF Analytical Method D1608/01 can be performed successfully for quantitation of BAS 450 I (Reg. No. 5672774) and its metabolites DM-8007 (Reg. No. 5856361), DC-DM-8007 (Reg. No. 5936906), DC-8007 (Reg. No. 5936907) and S(PFP-OH)-8007 (Reg. No. 5959598) in surface and drinking water.

The method is well-written and contains a fair amount of comments to guide the analyst through the procedure for the first time. Recommendations for improvement of the analytical method are presented in Appendix A and it is recommended that they be incorporated into the method.

8. PROTOCOL, AMENDMENTS, AND DEVIATIONS

One amendment for the guidelines correction will be issued.

9. COMMUNICATION

Communications between the Study Director and the BASF study monitor and personnel are documented. At no time during the course of the study was anyone from BASF allowed to visit the testing facility.

The study monitor was informed of the successful completion of the study on Nov. 23, 2016.

10. REFERENCE

1. Delinsky, D. (2017): Validation of Method D1608/01: “Method for the Determination of BAS 450 I (Reg. No. 5672774) and Its Metabolites DM-8007 (Reg. No. 5856361), DC-DM-8007 (Reg. No. 5936906), DC-8007 (Reg. No. 5936907) and S(PFP-OH)-8007 (Reg. No. 5959598) in Surface and Drinking Water by LC-MS/MS” BASF Study Number 725931. BASF Reg. Doc. No. 2017/7000331.

2. JOSE, W. (2016): Validation of BASF Method D1417/01 for the determination of residues of BAS 450 I and its metabolites S(PFP-OH)-8007 and DM-8007 in wheat (grain), dry beans (seed), tomato (whole fruit), citrus (whole fruit) and coffee (grain) using LC-MS/MS. – GENCS. BASF Study Number 772495. BASF Reg. Doc. No. 2016/3004081.

Table 22 Instrument Conditions and Parameters

	Parameter[1]		
Chromatographic System	Waters Acquity		
Analytical-column	Acquity BEH C18 1.7um, 2.1x50mm		
Column Temperature	50°C		
Injection Volume	50 µL		
Mobile Phase A	Water / formic acid,	1000/1, v/v	
Mobile Phase B	Methanol / formic acid,	1000/1, v/v	
Flow Rate	800 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00	80	20
	0.25	80	20
	2.25	65	35
	4.50	5	95
	5.45	5	95
	5.50	80	20
	6.00	80	20
Detection System	Sciex 6500		
Ionisation	Electrospray (ESI)		
Ionisation Temperature[2]	600 °C		
Analyte	**Transitions (m/z)**	**Polarity**	**Expected Retention Time**
BAS 450 I Reg. No.5672774	663 → 643* 665 → 645	positive	approx. 4.19 min
DC-DM-8007 Reg. No. 5936906	545 → 525* 547 → 527	positive	approx. 3.99 min
DC-8007 Reg. No. 5936907	559 → 539* 561 → 541	positive	approx. 4.13 min
DM-8007 Reg. No. 5856361	649 → 242* 651 → 242	positive	approx. 4.24 min
S(PFP-OH)-8007 Reg. No. 5959598	661 --> 641* 661 --> 621	positive	approx. 3.93 min

1. The alternate method can be found in Appendix D, Section 4.2.1.

2. The method used "Sciex 5500" with the ionization temperature of 700°C. As we used "Sciex 6500", the ionisation temperature was changed to 600°C.

Table 23 Typical Analytical Standards Dilutions and Use Record

Solution ID	Analyte[1]	Standard (Lot #) / Parent Solution ID	Amount Weighed / Volume	Final Dilution Vol. (mL)	Final Conc.[2]	Solvent[3]	Prep. Date[4]
Stock solutions							
S0826201606	BAS 450 I	089-100112-1	10.020 mg	10	0.9987 mg/ml	Acetonitrile	08/26/16
S0826201605	S(PFP-OH)-8007	267-012-094-3	10.340 mg	10	1.024 mg/ml	Acetonitrile	08/26/16
S0826201602	DM-8007	296-007-81-1	10.040 mg	10	0.9923 mg/ml	Acetonitrile	08/26/16
S0826201603	DC-DM-8007	296-009-094-2	10.070 mg	10	0.9927 mg/ml	Acetonitrile	08/26/16
S0826201604	DC-8007	296-012-009-1	10.150 mg	10	1.005 mg/ml	Acetonitrile	08/26/16
Serial dilutions							
F20161121-1	Mix	S0826201606 S0826201605 S0826201602 S0826201603 S0826201604	100 uL 488 uL 504 uL 504 uL 497 uL	10	10/50 µg/mL	Acetonitrile	11/21/16
F20161121-2	Mix	F20161121-1	50 uL	50	0.010/0.050 µg/mL	Acetonitrile	11/21/16
F20161121-3	Mix	F20161121-2	5000 uL	50	0.001/0.005 µg/mL	Acetonitrile	11/21/16
Calibration							
C20161121-1	Mix	F20161121-3	5.0	50	0.10/0.50 ng/mL	FV1	11/21/16
C20161121-2	Mix	C20161121-1	10.0	100	0.01/0.05 ng/mL	FV1	11/21/16
C20161121-3	Mix	C20161121-2	25.0	50	0.005/0.025 ng/mL	FV1	11/21/16
C20161121-4	Mix	C20161121-3	12.5	50	0.0025/0.0125 ng/mL	FV1	11/21/16
C20161121-5	Mix	C20161121-4	5.0	50	0.001/0.005 ng/mL	FV1	11/21/16
C20161121-6	Mix	C20161121-5	2.5	50	0.0005/0.0025 ng/mL	FV1	11/21/16

Fortification

Sample Data		Fortification Data					Final Volume (mL)
Surface water sample	**Wt. (g)**	**ppt Fortified**	**Analyte**	**Vol. of Standard Used**	**Standard Conc.**	**Standard Number**	
CM16-016	10	Control	None	NA	NA	NA	20
CM16-016	10	5/25	BAS 450 I/metabolites	50 µL	1.0/5.0 ng/mL	F20161121-3	20
CM16-016	10	50/250	BAS 450 I/metabolites	50 µL	10/50 ng/mL	F20161121-2	20

1. Mix= BAS 450 I+ S(PFP-OH)-8007+DM-8007+DC-DM-8007+DC-8007
2. Where two concentrations are listed in a cell, the concentration to the left is for BAS 450 I and the concentration to the right is for the metabolites.
3. FV1 = 0.1% Formic Acid in 50% Acetonitrile and 50% water.
4. Stock solutions are stored for up to 3 months. Fortification and Calibration solutions are stored for up to 4 weeks.

Figure 81 Calculation Formula and Example.

Sample Description: surface water LOQ-1-Fortified surface water (Results File:20161122-Set 1.rdb)

$$\text{Concentration of Analyte } (\mathrm{C_A})(\mathrm{ng/mL}) = \frac{\text{peak area} - \text{intercept}}{\text{slope}}$$

$$\text{Concentration of Analyte } (\mathrm{C_B})(\mathrm{ppt}) = \mathrm{C_A} * \text{dilution factor} * 1000$$ (dilution factor = 2)

Analyte	**BAS 450 I**	**DM-8007**	**S(PFP-OH)-8007**	**DC-DM-8007**	**DC-8007**
Peak Area =	4480	10600	18100	40000	49400
Intercept =	143	6.76	-520	-128	20.6
Slope =	1860000	832000	1690000	4090000	4280000
C_A (ng/mL) =	0.00233	0.0127	0.0110	0.00981	0.0115
C_A (ppt) =	4.66	25.4	22.2	19.6	23.0

NOTE: Slight rounding differences with analyst® data may be noted when using a hand calculator. Full computer/calculator precision was used for any intermediate calculations. Only the final value was rounded.

Appendix A. Recommendations for BASF Method Number D1608/01

It is recommended to use the following instrument conditions (Sciex 5500, 700ºC).

	Parameter		
Chromatographic System	Waters Acquity		
Analytical-column	XBridge BEH C18 1.7um, 2.1x50mm		
Column Temperature	50°C		
Injection Volume	50 µL		
Mobile Phase A Mobile Phase B	Water / formic acid, Methanol / formic acid,	1000/1, v/v 1000/1, v/v	
Flow Rate	800 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00	80	20
	0.25	80	20
	2.25	65	35
	4.50	5	95
	5.45	5	95
	5.50	80	20
	6.00	80	20
Detection System	Sciex 5500		
Ionisation	Electrospray (ESI)		
Ionisation Temperature	700 °C		
Analyte	**Transitions (m/z)**	**Polarity**	**Expected Retention Time**
BAS 450 I (Reg. No.5672774)	663 → 643* 665 → 645	positive	approx. 5.83 min
DC-DM-8007 Reg. No. 5936906	545 → 525* 547 → 527	positive	approx. 5.33 min
DC-8007 Reg. No. 5936907	559 → 539* 561 → 541	positive	approx. 5.65 min
DM-8007 Reg. No. 5856361	649 → 242* 651 → 242	positive	approx. 5.84 min
S(PFP-OH)-8007 Reg. No. 5959598	661 --> 641* 661 --> 621	positive	approx. 5.51 min

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

Working Procedure:

Method for the Determination of BAS 450 I (Reg. No. 5672774) and Its Metabolites DM-8007 (Reg. No. 5856361), DC-DM-8007 (Reg. No. 5936906), DC-8007 (Reg. No. 5936907) and S(PFP-OH)-8007 (Reg. No. 5959598) in Surface and Drinking Water by LC-MS/MS

BASF Method Number D1608/01

Final

Author
David Delinsky

Date
August 29, 2017

Test Facility
BASF Crop Protection
26 Davis Drive
PO Box 13528
Research Triangle Park, NC 27709
USA

Number of Pages
18

ABSTRACT

BASF Method D1608/01 is developed to determine the residues of BAS 450 I (Reg. No. 5672774) and its metabolites DM-8007 (Reg. No. 5856361), DC-DM-8007 (Reg. No. 5936906), DC-8007 (Reg. No. 5936907) and S(PFP-OH)-8007 (Reg. No. 5959598) in surface and drinking water using LC-MS/MS at BASF Crop Protection, Research Triangle Park, N.C.

Brief description of the method:

10 mL methanol is added to a 10 mL water sample and mixed. After filtration, the sample is ready for analysis by LC-MS/MS.

The method has a limit of quantitation (LOQ) of 5 ng/L (5 ppt) in water for BAS 450 I and 25 ng/L (25 ppt) in water for the metabolites. The limit of detection (LOD) in water is 1 ng/L for BAS 450 I and 5 ng/L for the metabolites.

DEFINITIONS AND ACRONYMS

Sample Set:	A group of samples that are extracted and cleaned up at the same time using the same method represented.
Untreated Sample:	A sample that has not been treated with the test substance.
Control Sample:	Usually an untreated sample used for fortification experiments (can be acquired from same study or from a different source).
Unknown Sample:	The samples with unknown residues.
Treated Sample:	A sample that has been treated with the test substance.
Blank:	Solvent, solution or mobile phase injected together with a sample set.
Reagent Blank:	A complete analysis conducted using solvents and reagents only in absence of any sample. Also known as blank of reagents or procedural blank. This sample is analyzed within the sample set in order to evaluate possible contamination on chemicals/reagents.
Procedural Recovery:	A control sample to which a known amount of analyte has been added before sample work up. This sample is then carried through the method and analyzed with the unknown samples in order to determine the reliability of the method.
Instrument Recovery:	A control sample which is carried through the method and to which a known amount of analyte has been added before injection. This sample is analyzed within the sample set in order to evaluate the matrix effect in the instrument.
Analytical Run:	A group of samples that undergo a determinative measurement on an analytical instrument (such as GC, HPLC, CE, GC/MS, or LC/MS/MS) in a defined and continuous sequence under identical instrumental conditions.
Limit of Quantitation (LOQ):	Lowest tested concentration of the analyte in a sample that can be determined with acceptable accuracy and precision according to the method.
Limit of Detection (LOD):	Concentration of analyte equivalent to a defined percentage of the limit of quantitation of the method (e.g 20% of LOQ). At this concentration, the analyte must be qualitatively detectable in sample matrix (analyte peak height at least 3-5 x baseline noise).

1 INTRODUCTION

BAS 450 I is a new insecticide that will be used for various crops. The analytical method D1608/01 offers the possibility to determine residues of BAS 450 I (Reg. No. 5672774) and its metabolites DM-8007 (Reg. No. 5856361), DC-DM-8007 (Reg. No. 5936906), DC-8007 (Reg. No. 5936907) and S(PFP-OH)-8007 (Reg. No. 5959598) in water. Method D1608/01 was successfully validated in surface and drinking water for all analytes.

This method was developed at BASF Crop Protection, Research Triangle Park, NC.

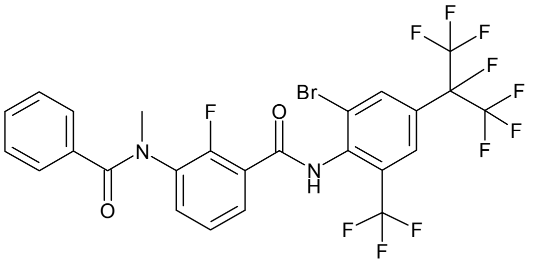
2 MATERIALS

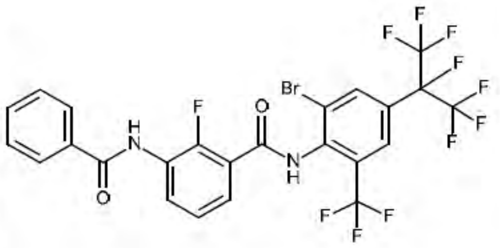
2.1 Safety

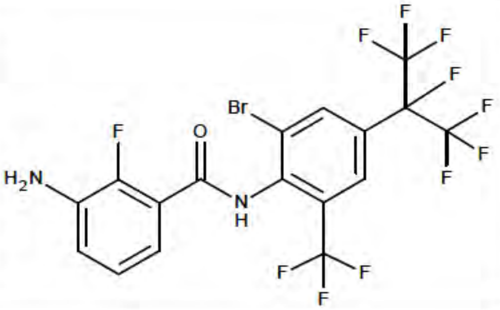
The test and reference items, as well as the chemicals required for this analysis, should be handled in accordance with good industrial hygiene and safety practice. Avoid contact with the skin, eyes and clothing. Wearing of closed work clothing is recommended. Remove contaminated clothing. Ensure work clothing is stored 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 Materials Safety Data Sheets (MSDS) 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.

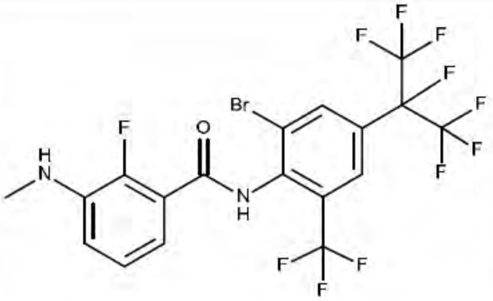
2.2 Test and Reference Items

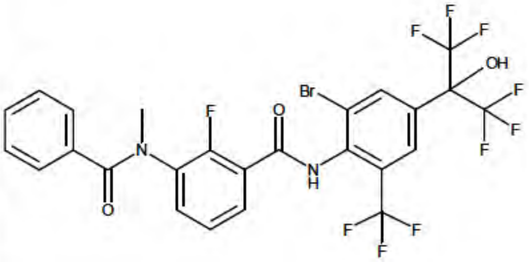
Test and reference items should be stored according to the information provided in the certificate of analysis.

BAS-Code	BAS 450 I	
Common Name	Broflanilide	
Chemical Name	N-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluoro-3-(N-methylbenzamido)benzamide	
BASF Reg. No.	5672774	
CAS-No.	None	
Molecular Formula	$C_{25}H_{14}BrF_{11}N_2O_2$	
Molecular Weight	663.29	

BAS-Code	None	
Common Name	DM-8007	
Chemical Name	3-benzamido-*N*-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluorobenzamide	
BASF Reg. No.	5856361	
CAS-No.	None	
Molecular Formula	$C_{24}H_{12}BrF_{11}N_2O_2$	
Molecular Weight	649.25	

BAS-Code	None	
Common Name	DC-DM-8007	
Chemical Name	3-amino-*N*-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluorobenzamide	
BASF Reg. No.	5936906	
CAS-No.	None	
Molecular Formula	$C_{17}H_8BrF_{11}N_2O$	
Molecular Weight	545.15	

BAS-Code	None	
Common Name	DC-8007	
Chemical Name	N-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluoro-3-(methylamino)benzamide	
BASF Reg. No.	5936907	
CAS-No.	None	
Molecular Formula	$C_{18}H_{10}BrF_{11}N_2O$	
Molecular Weight	559.17	

BAS-Code	None	
Common Name	S(PFP-OH)-8007	
Chemical Name	*N*-[2-bromo-4-(1,1,1,3,3,3-hexafluorofluoro-2-hydroxypropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluoro-3-(*N*-methlybenzamido)benzamide	
BASF Reg. No.	5959598	
CAS-No.	None	
Molecular Formula	$C_{25}H_{15}BrF_{10}N_2O_3$	
Molecular Weight	661.3	

2.3 Equipment

Equipment	Size, Description	Manufacturer	Catalog No.
Amber Bottles	60 mL, Boston Round bottle with PTFE-faced PE lined cap attached	VWR	89042-908
Balance, Top-Load	150 g, CP153	Sartorius	
Beakers	Various Sizes	PYREX Brand, VWR Scientific Products	13922-029
Centrifuge Tubes (disposable)	50 mL	VWR	89039-660
Filters, Syringe Tip	13mm Syringe Filter, 0.45 µm PTFE membrane	PALL Life Sciences	4555
Graduated Cylinder	10 mL, PYREX	VWR	89090-636
HPLC Column	XBridge BEH Phenyl, 2.5 µm, 2.1x100 mm	Waters	186006067
LC-MS/MS injection vials	1.5 mL, Target DP	VWR, Thermo Scientific	00162506
Microman Pipettes	1000 µL 250 µL 50 µL	Gilson	M1000 M250 M50
Microman Pipette tips	1000 µL tips 250 µL tips 50 µL tips	Gilson	CP1000 CP250 CP50
Pasteur Pipettes, disposable	2 mL, 14.6 cm Borosilicate Glass	VWR	14673-010
Scintillation Vials	20 mL	VWR	66022-060
Shaker	KS501 digital	IKA Labortechnik	0002526401
Spatula		Various	
Syringes, Disposable	1 mL	Thermo Scientific	S7510-1
Volumetric Flasks	10 mL, 50 mL, 100 mL	Various	
Volumetric Pipettes	Various, class-A	Various	
Vortex Mixer	Genie 2	Fisher Scientific Co	12-812
Vortexer	Multi-tube vortexer, VX-2500	VWR	444-7063

Note: The equipment and instrumentation listed above may be substituted by that of similar specifications. The applicability 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 Grade	EMD	AX0145P-1
Methanol	HPLC Grade	EMD	MX0475P-1
Water	HPLC Grade	BDH ARISTAR PLUS	87003-652
Formic Acid	≥95%	Sigma-Aldrich	F0507

Note: Equivalent reagents and chemicals from other suppliers may be substituted.

2.4.2 Solutions and Solvent Mixtures

Description	Code	Composition
Final Volume Solvent	FV1	**Methanol-water, 50:50, v/v** Add 500 mL of methanol and 500 mL of water into a 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 Methanol** Add 1000 mL of Methanol and 1 mL of concentrated formic acid into a, e.g., 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.

Note: If necessary, the solutions may also be prepared in different volumes as long as the proportions of solvents are not modified.

2.4.3 Standard Solutions

Stock Solutions

Prepare a 1.0 mg/mL stock solution individually by weighing an appropriate amount of the analyte into a flask and add the required volume.
For example, to prepare 10 mL of 1.0 mg/mL stock solution of BAS 450 I in acetonitrile, weigh 10 mg of BAS 450 I into a 10 mL volumetric flask. Dissolve and dilute to mark with acetonitrile. Ensure a complete homogeneous solution (e.g. by sonication or vortexing). The stock solutions for all other analytes are made in a similar fashion.

Independence of standard calibration and fortification solutions should initially be confirmed to show correct preparation of the solutions. This can be achieved for example using one of the following approaches:

- Two stock solutions are independently prepared. One is used for preparation of fortification solutions, the other for calibration standard solutions.
- 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 standard solutions for fortification by dilution of the above stock solution. Dilute volumetrically 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

Take solution (µg/mL)†	Volume (mL)	Dilute with acetonitrile to a final volume of (mL)	Concentration (µg/mL)†
1000	0.1 (BAS 450 I)	10	10 / 50
	0.5 (metabolites)		
10 / 50	0.05	50	0.010 / 0.050
0.010 / 0.050	5	50	0.001 / 0.005

† Where two concentrations are listed in a cell, the concentration to the left is for BAS 450 I and the concentration to the right is for the metabolites.

Note: A different concentration scheme may be used, if other fortification levels are needed for the analysis.
If necessary, the volume of solution prepared may be changed.

Calibration Standard Solutions

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

Preparation of standard solutions for calibration

Take solution (ng/mL)†	Volume (mL)	Dilute with FV1* to a final volume of (mL)	Concentration (ng/mL)†
1.0 / 5.0	5.0	50	0.10 / 0.50 ‡
0.10 / 0.50	10	100	0.01 / 0.05
0.01 / 0.05	25	50	0.005 / 0.025
0.01 / 0.05	12.5	50	0.0025 / 0.0125
0.01 / 0.05	5.0	50	0.001 / 0.005
0.01 / 0.05	2.5	50	0.0005 / 0.0025

† Where two concentrations are listed in a cell, the concentration to the left is for BAS 450 I and the concentration to the right is for the metabolites.

‡ Not intended to be a calibration standard but needed to prepare subsequent calibration standards.

* In case matrix-matched standards (= instrument recovery samples) are needed for successful analysis, calibration standard solution are prepared in matrix solution, i.e., final volume of a control 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. In addition the matrix load should be the same in all calibration standard solutions.

Note: A different concentration scheme may be used and additional standards may be prepared as needed.
If necessary, the volume of solution prepared may be changed.

Additional Information:

- Use amber bottles with PTFE-faced PE lined screw caps as storage containers for all standard solutions.

3 ANALYTICAL PROCEDURE

3.1 Sample Preparation

Sample homogenization is not needed for water samples.

3.2 Sample Storage

Water samples are to be kept frozen until analysis.

3.3 Weighing and Fortification

For treated samples and control samples, measure 10 ±0.1 g (or 10 mL) of water sample into a disposable tube (such as 50 mL plastic centrifuge tube).

For fortification samples, measure 10 ±0.1 g (or 10 mL) of water sample into a disposable tube (such as 50 mL plastic centrifuge tube). Fortify the solution with analytes and shake/vortex for approximately 1 minute to ensure sample homogeneity.

The following scheme may be used:

Sample Type	Sample Weight	Concentration of Spiking Solution†	Volume of Spiking Solution	Level of Fortification†
Control	0.010 L	-	-	0.00 ng/L
Fortification (LOQ*)	0.010 L	1.0 / 5.0 ng/mL	0.05 mL	5 / 25 ng/L (ppt)
Fortification (10xLOQ)	0.010 L	10 / 50 ng/mL	0.05 mL	50 / 250 ng/L (ppt)
Treated	0.010 L	-	-	-

* limit of quantification

† Where two concentrations are listed in a cell, the concentration to the left is for BAS 450 I and the concentration to the right is for the metabolites.

Note: Volume of spiking solution added to generate the fortified sample should not exceed 10% of sample weight or volume.

3.4 Preparation for Measurement

Add 10 mL methanol to all samples and shake/vortex for approximately 1 minute to ensure homogeneity. Syringe filter all samples using 0.45µm PTFE syringe filters directly into HPLC injection vials, passing the first approximately 0.2 – 0.3 mL to waste. Samples are ready for injection.

High fortification and high residue samples - further dilute with **FV1** (methanol-water, 50:50, v/v) as necessary, to fit in the calibration curve.

3.5 Influence of matrix effects on analysis

During method validation, it was demonstrated that the matrix load in the samples from the water matrices had no significant influence on the analysis (i.e., matrix effects < 20). Therefore, samples can be analyzed using calibration standard solutions prepared in solvent FV1 (see 2.4.3).

3.6 Stability of Extracts / Final Volumes

Each analyte has been shown to be stable in extracts for at least the time period tested, 6 days for BAS 450 I and 7 days for metabolites in both drinking and surface water.

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 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 be at least injected twice. At least 5 calibration levels need to be injected.

4.2 Instrumental analysis

4.2.1 Instrumentation and Conditions for BAS 450 I and Its Metabolites

<table>
<tr><th></th><th colspan="3">Parameter</th></tr>
<tr><td>Chromatographic System</td><td colspan="3">Waters Acquity</td></tr>
<tr><td>Analytical-column</td><td colspan="3">XBridge BEH Phenyl 2.5um, 2.1x100mm</td></tr>
<tr><td>Column Temperature</td><td colspan="3">50°C</td></tr>
<tr><td>Injection Volume</td><td colspan="3">75 µL</td></tr>
<tr><td>Mobile Phase A
Mobile Phase B</td><td colspan="3">Water / formic acid, 1000/1, v/v
Methanol / formic acid, 1000/1, v/v</td></tr>
<tr><td>Flow Rate</td><td colspan="3">600 µL/min</td></tr>
<tr><td rowspan="8">Gradient (including wash and equilibration)</td><td>Time (min)</td><td>Phase A</td><td>Phase B</td></tr>
<tr><td>0.00</td><td>70</td><td>30</td></tr>
<tr><td>0.10</td><td>70</td><td>30</td></tr>
<tr><td>4.00</td><td>40</td><td>60</td></tr>
<tr><td>6.00</td><td>5</td><td>95</td></tr>
<tr><td>7.20</td><td>5</td><td>95</td></tr>
<tr><td>7.25</td><td>70</td><td>30</td></tr>
<tr><td>8.00</td><td>70</td><td>30</td></tr>
<tr><td>Detection System</td><td colspan="3">Sciex 5500</td></tr>
<tr><td>Ionisation</td><td colspan="3">Electrospray (ESI)</td></tr>
<tr><td>Ionisation Temperature</td><td colspan="3">700 °C</td></tr>
<tr><td>Analyte</td><td>Transitions (m/z)</td><td>Polarity</td><td>Expected Retention Time</td></tr>
<tr><td>BAS 450 I
(Reg. No.5672774)</td><td>663 → 643*
665 → 645</td><td>positive</td><td>approx. 5.83 min</td></tr>
<tr><td>DC-DM-8007
Reg. No. 5936906</td><td>545 → 525*
547 → 527</td><td>positive</td><td>approx. 5.33 min</td></tr>
<tr><td>DC-8007
Reg. No. 5936907</td><td>559 → 539*
561 → 541</td><td>positive</td><td>approx. 5.65 min</td></tr>
<tr><td>DM-8007
Reg. No. 5856361</td><td>649 → 242*
651 → 242</td><td>positive</td><td>approx. 5.84 min</td></tr>
<tr><td>S(PFP-OH)-8007
Reg. No. 5959598</td><td>661 --> 641*
661 --> 621</td><td>positive</td><td>approx. 5.51 min</td></tr>
</table>

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

4.2.2 Instrumentation and Conditions for BAS 450 I and Its Metabolites (used for ILV)

	Parameter		
Chromatographic System	Waters Acquity		
Analytical-column	XBridge BEH C18 1.7um, 2.1x50mm		
Column Temperature	50°C		
Injection Volume	50 µL		
Mobile Phase A	Water / formic acid,	1000/1, v/v	
Mobile Phase B	Methanol / formic acid,	1000/1, v/v	
Flow Rate	800 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00	80	20
	0.25	80	20
	2.25	65	35
	4.50	5	95
	5.45	5	95
	5.50	80	20
	6.00	80	20
Detection System	Sciex 5500		
Ionisation	Electrospray (ESI)		
Ionisation Temperature	700 °C		
Analyte	**Transitions (m/z)**	**Polarity**	**Expected Retention Time**
BAS 450 I (Reg. No.5672774)	663 → 643* 665 → 645	positive	approx. 5.83 min
DC-DM-8007 Reg. No. 5936906	545 → 525* 547 → 527	positive	approx. 5.33 min
DC-8007 Reg. No. 5936907	559 → 539* 561 → 541	positive	approx. 5.65 min
DM-8007 Reg. No. 5856361	649 → 242* 651 → 242	positive	approx. 5.84 min
S(PFP-OH)-8007 Reg. No. 5959598	661 --> 641* 661 --> 621	positive	approx. 5.51 min

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

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.

In general a divert valve is used to reduce the matrix load on the detection system.

Instrument conditions, e.g. injection volumes, columns, gradient steps or mass transitions may be modified, but any changes must be recorded in the raw data. Changes are acceptable, when the recoveries of the fortification experiments are in the acceptable range.

Other parameters like gas flows and voltages are depended of the equipment used and therefore not listed. Those parameters may need to be adapted for the instrument used.

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 (in the range of 0.01 ng/mL to 0.0005 ng/mL for BAS 450 I and 0.05 ng/mL to 0.0025 ng/mL for the metabolites) for LC-MS/MS. 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, the 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.

The residues of BAS 450 I in mg/kg are calculated as shown in equations **I** and **II**:

I. **Concentration [ng/mL]**

$$= \frac{\text{Response} - \mathit{Intercept}}{\mathit{Slope}} = \mathrm{C}_A$$

II. **Residue [ng/L]**

$$= \frac{\mathrm{V}_{\mathrm{end}} \times \mathrm{C}_A}{G \times A_F}$$

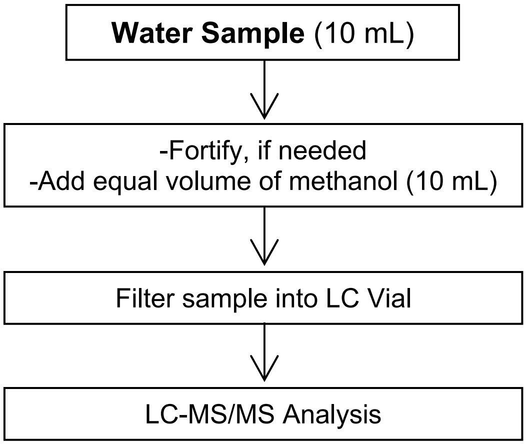
V_{end} = Final volume of the extract after all dilution steps [mL]
C_A = Concentration of analyte as read from the calibration curve [ng/mL]
G = Volume of the sample extracted in L
A_F = Aliquot factor (1 for this method)

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 unknown samples, 2 fortified samples for recovery experiments, 1 blank sample) requires 0.5 working day (4 hours) per laboratory assistant. This time includes the calculation of the results, the preparation of the equipment as well as the reporting of all raw data under GLP.

7 CONCLUSION AND METHOD CAPABILITIES

Limit of Quantification (LOQ) and Limit of Detection (LOD)
The limit of quantification is defined as the lowest fortification level successfully tested. The limit of quantification is 5 ng/L (5 ppt) for BAS 450 I and 25 ng/L (25 ppt) for the metabolites. The limit of detection was estimated at 20% of the limit of quantification, equivalent to 1 ng/L for BAS 450 I and 5 ng/L for the metabolites. The lowest standard for each analyte in the calibration curve has good detectability (signal to noise ratio greater than 3:1).

Selectivity
The tested untreated water samples showed no significant interferences (< 20 or 30 %) at the retention time of the analytes.

Confirmatory Techniques
The LC-MS/MS final determination for BAS 450 I is a highly selective detection technique. For every compound the quantitation is possible at two different transitions. Therefore, no additional confirmatory technique is required.

Potential Problems
A PVDF filter is not suitable for use with this method, however, GHP and nylon filters may be found to be acceptable.

The glassware used for the method should be thoroughly rinsed with methanol followed by acetone to prevent contamination.

It has been found that BAS 450 I in water samples has the potential to adhere to container walls. As a result, any water samples to be analyzed must be transferred to a new container (while measuring sample volume). An equal volume of methanol should be added to the original container and shaken. The methanol should then be transferred to the new container that is holding the sample. The mixture should be shaken/vortexed for approximately 1 minute to ensure homogeneity. (Be sure the new container used has adequate capacity to contain both the sample and the methanol to be added as well as allow adequate mixing.) The diluted sample should then be filtered and analyzed as specified earlier in this document.

Interference peaks have been observed to be extracted into final volumes from polypropylene syringes used to filter samples. Care should be taken to avoid interferences.

Following changes are made to the Amended report of Study 776692:

This amended report is replacing the original report with BASF Registration Document Number 2017/7000325 which was amended to include: 1) Correction of title with Method umber (Page 1, Section 6 - Discussion, Section 7 - Recommendations/Conclusions from ILV); 2) New BASF Reg. Doc. No. (Page 1, and Report Header); Correction of guideline (page 1 and 16); Updated TOC . The details are listed here

1. **Change of the title from**
   "Independent Laboratory Validation of "Method for the Determination of BAS 450 I (Reg. No. 5672774) and Its Metabolites DM-8007 (Reg. No. 5856361), DC-DM-8007 (Reg. No.5936906), DC-8007 (Reg. No. 5936907) and S(PFP-OH)-8007 (Reg. No. 5959598) in Surface and Drinking Water by LC-MS/MS"
   to,
   "Independent Laboratory Validation of Method D1608/01: "Method for the Determination of BAS 450 I (Reg. No. 5672774) and Its Metabolites DM-8007 (Reg. No. 5856361), DC-DM-8007 (Reg. No. 5936906), DC-8007 (Reg. No. 5936907) and S(PFP-OH)-8007 (Reg. No. 5959598) in Surface and Drinking Water by LC-MS/MS"

2. **Change of the guideline from**
   "U.S. EPA Residue Chemistry Test Guideline, OPPTS 860.1340, Residue Analytical Method; SANCO/825/00 rev.8.1 (Nov 16, 2010);
   OECD ENV/JM/MONO (2007) 17 - Guidance Document on Pesticide Residue Analytical Methods"
   to
   "US Environmental Protection Agency (EPA) Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation; SANCO/825/00 rev.8.1 (Nov 16, 2010);"

3. Correction of title with Method umber (Page 1, Section 6 - Discussion, Section 7 - Recommendations/Conclusions from ILV);

4. Incorporation of New BASF Reg. Doc. No. (Page 1, and Report Header);

5. Correction of guideline (page 1 and 16);

6. Replaced working procedure (Appendix D), dated August 14, 2017 with an updated version, dated August 29, 2017, correcting typoes including solution stability information.

7. Update of TOC