

ABSTRACT

The purpose of this study was to demonstrate that BASF Analytical Method D1603/01 “Method for the determination of 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 Soil by LC-MS/MS (at LOQ of 1ppb)” could be performed successfully at an outside facility with no prior experience with the method. The analytical method was originally developed and validated at BASF Crop Protection.

Principle of the method. The residues of BAS 450 I and its metabolites DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 are extracted from 5 grams of soil by adding 10 mL of methanol, mixing by vortex, then shaking for 30 minutes on a mechanical shaker. After centrifugation, the supernatant is decanted into a 50-mL plastic centrifuge tube. Ten mL of methanol:water (70:30,v/v) is added to the soil marc and the sample is mixed by vortex before shaking for another 30 minutes. After centrifugation, the supernatants are combined into the same 50-mL plastic centrifuge tube and the sample is mixed well before being diluted five times in methanol:water (50:50, v/v). Samples known to exceed the highest calibration level were diluted an additional 10 times. The sample extracts are filtered through a 0.45 PTFE syringe filter and then analyzed by HPLC-MS/MS.

Test conditions. For validation, untreated soil 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 set 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. Solvent-based calibration standards ranging from 0.01 ng/mL to 0.2 ng/mL were used to establish calibration curves. Two mass transitions were evaluated for the parent and its metabolites and are listed below:

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

Limit of Quantitation (LOQ) and Limit of Detection (LOD): The LOQ of the method was set at 0.001 mg/kg in soil for BAS 450 I, DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007; which was lower than the lowest relevant endpoint in soil ecotoxicology ($LC_{50} > 1000$ mg/kg of active ingredient in dry soil). The LOQ is also defined as the lowest fortification level for each analyte. The LOD for BAS 450 I, DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 in soil was set at 0.0002 mg/kg, which was 20% of the defined LOQ. The LOD for each analyte was shown to be detectable as the absolute amount of analyte injected (0.001 ng on-column) with acceptable signal to noise ratio ($S/N > 3:1$).

Selectivity: The method determines BAS 450 I, DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 residues in soil by LC-MS/MS. No interfering peaks were found at the retention times for BAS 450 I and DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007.

Linearity: Acceptable linearity was observed for the standard range: The method-detector response was linear over the 0.01–0.2 ng/mL range ($r^2 = \geq 0.980$) for all analytes, except for the

secondary mass transition (m/z 661→621) used originally in the method for S(PFP-OH)-8007. Therefore, mass transition (m/z 663→643) was used in the ILV study.

Standard Stability: Stock and fortification solutions of BAS 450 I, DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 were prepared in acetonitrile. Calibration standard solutions were prepared by serial dilution of the intermediate standard solutions using methanol water (50:50, v:v). During the course of this study, the test/reference substance solutions were stored under refrigerated conditions and all solutions were used within the demonstrated time period of stability.

All analytes have been shown to be stable in stock and fortification 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 methanol water (50:50, v:v) and held under refrigeration for at least 30 days for all analytes.

Extract Stability: Extract stability was established in the validation study. The initial extracts stored under refrigeration and HPLC final volume held under refrigeration indicated that each analyte is stable in soil for at least 7 days.

Recovery and Repeatability: The independent laboratory validation (ILV) was performed successfully in the first trial for soil for all LC-MS/MS ion transitions (primary and secondary) available for the method except for the secondary mass transition for S(PFP-OH)-8007 (m/z 661→621) using solvent-based standards. A different secondary mass transition for S(PFP-OH)-8007 (m/z 663→643) was identified at the request of the Study Monitor. A second trial was performed successfully for the alternate secondary mass transition.

Residues of BAS 450 I, DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 were not detected in any of the control soil samples.

1. INTRODUCTION

1.1 Scope of the Method

BASF Analytical Method No. D1603/01 was developed to determine the residues of BAS 450 I and its metabolites DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 in soil using LC-MS/MS at BASF Crop Protection in Research Triangle Park, North Carolina (Reference 1). This report represents the validation by an independent laboratory, Critical Path Services, LLC, in Garnet Valley, Pennsylvania.

The ILV was conducted using two fortification levels, the LOQ (0.001 mg/kg) and ten times the LOQ (0.01 mg/kg) for soil. Five replicates were analyzed for each fortification level. Additionally, one reagent blank and two replicates of unfortified samples were examined.

1.2 Principle of the Method

The residues of BAS 450 I and its metabolites DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 are extracted from 5 grams of soil by adding 10 mL of methanol, mixing by vortex, then shaking for 30 minutes on a mechanical shaker. After centrifugation the supernatant is decanted into a 50-mL plastic centrifuge tube. Ten mL of methanol:water (70:30, v/v) is added to the soil marc and the sample is mixed by vortex before shaking for another 30 minutes. After centrifugation the supernatants are combined into the same 50-mL plastic centrifuge tube and the sample is mixed well before being diluted five times in methanol:water (50:50, v/v). Samples known to exceed the highest calibration level were diluted an additional 10 times. The sample extracts are filtered through a 0.45 PTFE syringe filter and then analyzed by HPLC-MS/MS to determine the residues of BAS 450 I and its metabolites DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007. 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 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 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 S(PFP-OH)-8007 at m/z 661→641 and at m/z 663→643 were monitored in positive mode for primary and confirmation quantification, respectively.

1.3 Specificity

To demonstrate the specificity of the analytical method, one additional mass transition for each BAS 450 I and its metabolites DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 was monitored simultaneous to the primary quantitation transition. The method was able to accurately determine residues of BAS 450 I and its metabolites DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 and no interference was observed at the retention time of the analyte peaks.

2. REFERENCE SUBSTANCE AND SAMPLING HISTORY

2.1 Test Systems

The test system in this study was soil.

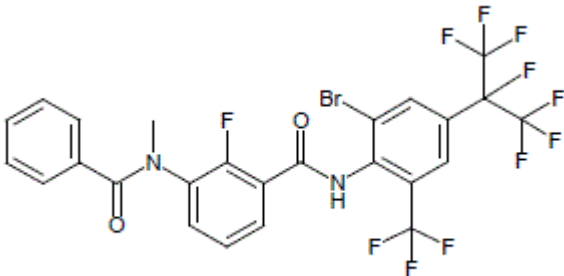
The control sample was provided by BASF. The soil sample was received on March 28, 2017. Upon arrival at the laboratory, the sample was opened, inspected, and checked against enclosed shipping forms. The test system was received frozen and was stored under frozen conditions at all times, unless necessary for laboratory analysis.

2.2 Test and Reference Substances

The standard substances were stored in a desiccator at room temperature until use. BASF has retained reserve samples of these chemicals, and has documentation specifying the location of the synthesis and characterization information available at BASF Crop Protection, Research Triangle Park, North Carolina.

The 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 March 29, 2017. The certificates of analysis for both substances are presented in Appendix B. A summary of the reference substances is presented below.

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.	1207727-04-5
Lot No.	089-100112-1
Purity (Certificate)	99.67%
Expiry	March 23, 2020
Molecular Formula	C ₂₅ H ₁₄ BrF ₁₁ N ₂ O ₂
Molecular Weight	663.29

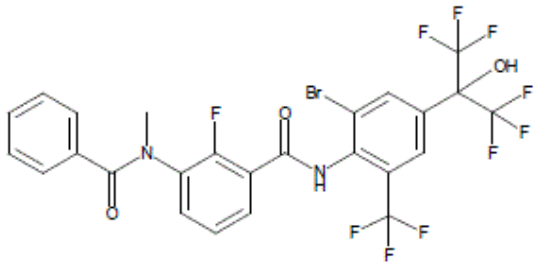


The chemical structure of Broflanilide is shown. It consists of a central benzamide core. The benzamide ring is substituted with a bromine atom at the 2-position, a trifluoromethyl group at the 4-position, and a perfluoropropan-2-yl group at the 6-position. The amide nitrogen is substituted with a methyl group. The benzamide carbonyl group is attached to a phenyl ring.

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	
Lot No.	296-007-81-1	
Purity (Certificate)	98.84%	
Expiry	June 28, 2017	
Molecular Formula	C ₂₄ H ₁₂ BrF ₁₁ N ₂ O ₂	
Molecular Weight	649.3	

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	
Lot No.	296-009-094-2	
Purity (Certificate)	98.58%	
Expiry	June 28, 2017	
Molecular Formula	C ₁₇ H ₈ BrF ₁₁ N ₂ O	
Molecular Weight	545.1	

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	
Lot No.	296-012-009-1	
Purity (Certificate)	99.07%	
Expiry	June 28, 2017	
Molecular Formula	C ₁₈ H ₁₀ BrF ₁₁ N ₂ O	
Molecular Weight	559.2	

Common 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-methylbenzamido)benzamide	
BASF Reg. No.	5959598	
CAS No.	None	
Lot No.	267-012-094-3	
Purity (Certificate)	99.06%	
Expiry	June 28, 2017	
Molecular Formula	C ₂₅ H ₁₅ BrF ₁₀ N ₂ O ₃	
Molecular Weight	661.3	

2.3 Test System

Control soil was provided and homogenized by BASF. The soil was sent from BASF Crop Protection, Inc., on March 27, 2017 and received by Critical Path Services, LLC on March 28, 2017. A characterization report of the soil used in the study is attached in Appendix E.

The control sample was logged in to CPS records and given a unique sample number (GS-17-50-1).

3. ANALYTICAL METHOD

BASF Analytical Method D1603/01 "Method for the Determination of 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 Soil by LC-MS/MS (at LOQ of 1ppb)" was used for the analysis of the samples.

The residues of BAS 450 I and its metabolites DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 are extracted from 5 grams of soil by adding 10 mL of methanol, mixing by vortex, then shaking for 30 minutes on a mechanical shaker. After centrifugation, the supernatant is decanted into a 50-mL plastic centrifuge tube. Ten mL of methanol:water (70:30, v/v) is added to the soil marc and the sample is mixed by vortex before shaking for another 30 minutes. After centrifugation the supernatants are combined into the same 50-mL plastic centrifuge tube and the sample is mixed well before being diluted five times in methanol:water (50:50, v/v). Samples known to exceed the highest calibration level were diluted an additional 10 times. The sample extracts are filtered through a 0.45 PTFE syringe filter and then analyzed by HPLC-MS/MS. Instrument parameters are described in Table 12.

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

Analyte	Transition (<i>m/z</i>)		Polarity	RT (min)
	Primary	Secondary		
BAS 450 I	663→643	665→645	Positive	5.6
DM-8007	649→242	651→242		5.6
DC-DM-8007	545→525	547→527		5.2
DC-8007	559→539	561→541		5.4
S(PFP-OH)-8007	661→641	663→643		5.3

Summary of Method

Type of Method	LC-MS/MS
Test System	Soil
Selected mass transitions (m/z)	BAS 450 I <i>m/z 663→m/z 643*</i> <i>m/z 665→m/z 645</i> DM-8007 <i>m/z 649→m/z 242*</i> <i>m/z 651→m/z 242</i> DC-DM-8007 <i>m/z 545→m/z 525*</i> <i>m/z 547→m/z 527</i> DC-8007 <i>m/z 559→m/z 539*</i> <i>m/z 561→m/z 541</i> S(PFP-OH)-8007 <i>m/z 661→m/z 641*</i> <i>m/z 663→m/z 643</i> *Primary quantification transition
Analytical Procedure	BASF Analytical Method D1603/01 "Method for the determination of 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 Soil by LC-MS/MS (at LOQ of 1ppb)"
Confirmatory Technique	A secondary MRM transition was used for confirmation.
Method of Quantitation	The quantitation is based on the monitoring of two mass transitions for BAS 450 I and its metabolites DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007. Recovery data were reported for each mass transition considered.
LOD	0.0002 mg/kg
LOQ	0.001 mg/kg (lowest fortification level)
Levels of Fortification	0.001 mg/kg and 0.01 mg/kg
Time Required	A set of 13 samples requires approximately 8 hours of work (calculation of the results included).

7. RECOMMENDATIONS/CONCLUSIONS FROM ILV

This ILV was successfully completed in the first two trials at CPS. Recovery results and statistical data demonstrate BASF Analytical Method D1603/01 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 soil.

Due to low signal for the secondary transition for S(PFP-OH)-8007 (m/z 661→621) from the analytical method, it was not suitable for quantitation. A product ion scan was performed to identify an alternative mass transition (for confirmation only). The product ion scan can be found in Appendix F.

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

In trial 1 the confirmatory mass transition (m/z 661→621) failed to meet the specification for linearity ($r^2 \geq 0.98$). A protocol amendment was issued to produce product ion scans for S(PFP-OH)-8007. Based on these product ion scans, an alternative confirmatory mass transition (m/z 663→643) was selected. Trial 2 was performed using only the alternative confirmatory transition for S(PFP-OH)-8007.

Deviation 1 was issued to document two GLP deviations that occurred during the study. They are listed on the GLP compliance statement in this report (page 3).

9. COMMUNICATION

Communications between the Study Director and the BASF study monitor and personnel are documented in the study file. 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, for all analytes except the confirmation ion transition ion (m/z 661→ m/z 621) for S(PFP-OH)-8007, after the completion of the first trial on June 7, 2017. The study monitor was informed of the successful completion of the study, for the alternative confirmation ion transition ion (m/z 663→ m/z 643) for S(PFP-OH)-8007, after the completion of the second trial on June 29, 2017.

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.
3. Jacob, M., et al., (Expected, 2017). Terrestrial Field Dissipation of the Insecticide BAS 450 I Following Broadcast Applications of BAS 450 00 I (SC). WEI Study Number: 227.32. BASF Study Code No. 710464. BASF Reg. Doc. No. 2017/7008695.
4. Delinsky, D. "Method for the Determination of 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 Soil by LC-MS/MS (at LOQ of 1 ppb)"; BASF Study Number 815843; BASF Registration Document Number: 2017/7001823.

Table 11 Example Standard Solutions Preparation and Dilution Data

Stock Standard Solutions

Standard ID#	Analyte	Parent Standard ID#	Net Weight (mg)	Dilution Volume (mL) ^a	Final Conc. (µg/mL)	Prep. Date
SS-0293-01	BAS 450 I	17-CPS-Mar29-01	10.25	10.0	1025	10 May 2017
SS-0293-02	BAS 450 I	17-CPS-Mar29-01	10.06	10.0	1006	10 May 2017
SS-0293-03	DM-8007	17-CPS-Mar29-02	10.10	10.0	1010	10 May 2017
SS-0293-04	DM-8007	17-CPS-Mar29-02	10.04	10.0	1004	10 May 2017
SS-0293-05	DC-DM-8007	17-CPS-Mar29-03	10.09	10.0	1009	10 May 2017
SS-0293-06	DC-DM-8007	17-CPS-Mar29-03	10.37	10.0	1037	10 May 2017
SS-0293-07	DC-8007	17-CPS-Mar29-04	10.03	10.0	1003	10 May 2017
SS-0293-08	DC-8007	17-CPS-Mar29-04	10.25	10.0	1025	10 May 2017
SS-0293-09	S(PFP-OH)-8007	17-CPS-Mar29-05	10.16	10.0	1016	10 May 2017
SS-0293-10	S(PFP-OH)-8007	17-CPS-Mar29-05	10.09	10.0	1009	10 May 2017

^a Prepared in acetonitrile

Fortification Standard Solution

Standard ID#	Analyte	Parent Standard ID#	Parent Conc. (µg/mL)	Aliquot Volume (mL)	Dilution Volume (mL) ^b	Final Conc. (µg/mL)	Prep. Date
FS-0293-04	BAS 450 I	17-CPS-Mar29-01	1006	0.497	20.0	10.0	10 May 2017
	DM-8007	17-CPS-Mar29-02	1004	0.498			
	DC-DM-8007	17-CPS-Mar29-03	1037	0.482			
	DC-8007	17-CPS-Mar29-04	1025	0.488			
	S(PFP-OH)-8007	17-CPS-Mar29-05	1009	0.496			

^b Prepared in acetonitrile

Calibration Standard Solutions

Standard ID#	Analytes	Parent Standard ID#	Parent Conc. (ng/mL)	Aliquot Volume (mL)	Dilution Volume (mL) ^c	Final Conc. (ng/mL)	Prep. Date
CS-0293-01	BAS 450 I DM-8007 DC-DM-8007 DC-8007 S(PFP-OH)-8007	FS-0293-06	100	0.5	50	1.00	10 May 2017
CS-0293-02		CS-0293-01	1.00	20	100	0.200	
CS-0293-03		CS-0293-02	0.200	25	50	0.100	
CS-0293-04		CS-0293-02	0.200	12.5	50	0.0500	
CS-0293-05		CS-0293-02	0.200	5.0	50	0.0200	
CS-0293-06		CS-0293-02	0.200	2.5	50	0.0100	

^c Prepared in methanol:water (50:50, v/v)

Table 12 Instrument Conditions and Parameters

UPLC Conditions			
Chromatographic System:	Agilent 1290 UPLC		
Column:	XBridge BEH Phenyl; 2.5 µm, 2.1 x 100 mm		
Temperature:	50 °C		
Flow rate (µL/min):	600		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.00	70	30
	0.10	70	30
	4.00	40	60
	6.00	5	95
	7.20	5	95
	7.25	70	30
	8.00	70	30
Mobile Phase A:	0.1% formic acid in water		
Mobile Phase B:	0.1% formic acid in methanol		
Injection Volume:	5 µL		

MS/MS Conditions						
Detection System:	Agilent 6410 Triple Quad					
Ionization:	ESI					
Polarity:	Positive					
Gas Temp:	250 °C					
Gas Flow:	11 l/min					
Nebulizer:	35 psi					
Capillary:	5000 V					
MRM Conditions	Transition (m/z)	Dwell (msec)	Frag	CE	Cell Acc	Retention Time (min)
BAS 450 I (Reg. No.5672774)	663→643	30	180	25	4	5.6
	665→645			25	4	
DC-DM-8007 (Reg. No. 5936906)	545→525	30	132	25	4	5.2
	547→527			25	4	
DC-8007 (Reg. No. 5936907)	559→539	30	156	29	4	5.4
	561→541			29	4	
DM-8007 (Reg. No. 5856361)	649→242	30	240	21	4	5.6
	651→242			21	4	
S(PFP-OH)-8007 (Reg. No. 5959598)	661→641	30	240	20	4	5.3
	663→643			20	4	

Appendix A. Recommendations for BASF Analytical Method D1603/01

The following recommendations should be incorporated into the technical procedure:

Section 4.2.1: Instrumentation and Conditions for BAS 450 I and its Metabolites

This section should replace the mass transition m/z 661→621 for S(PFP-OH)-8007 with m/z 663→643. Due to low signal for m/z 661→621 in trial 1, it was not suitable for quantitation. A product ion scan was performed to identify an alternative mass transition (for confirmation only). The product ion scan can be found in Appendix F.



Working Procedure:

Method for the determination of 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 Soil by LC-MS/MS (at LOQ of 1ppb)

BASF Method Number D1603/01

Final

Authors

David Scott Malinsky
David Delinsky

Date

August 15, 2017

Test Facility

BASF Crop Protection
26 Davis Drive
Research Triangle Park, NC 27709
USA

Number of Pages

21

ABSTRACT

BASF Method D1603/01 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 soil using LC-MS/MS at BASF Crop Protection, Research Triangle Park, N.C.

A 5 g soil sample aliquot is extracted by shaking with methanol followed by methanol-water (70:30, v/v). An aliquot of the combined extract is diluted with methanol-water (50:50, v/v), filtered and the residues are analyzed by LC-MS/MS.

The limit of quantitation of BAS 450 I and all metabolites is 0.001 mg/kg in all matrices. The limit of detection for each analyte is set to 20% of LOQ, equivalent to 0.0002 mg/kg.

DEFINITIONS AND ACRONYMS

<u>Sample Set:</u>	A group of samples that are extracted and cleaned up at the same time using the same method represented.
<u>Untreated Sample:</u>	A sample that has not been treated with the test substance.
<u>Control Sample:</u>	Usually an untreated sample used for fortification experiments (can be acquired from same study or from a different source).
<u>Unknown Sample:</u>	The samples with unknown residues.
<u>Treated Sample:</u>	A sample that has been treated with the test substance.
<u>Blank:</u>	Solvent, solution or mobile phase injected together with a sample set.
<u>Reagent Blank:</u>	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.
<u>Procedural Recovery:</u>	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.
<u>Instrument Recovery:</u>	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.
<u>Analytical Run:</u>	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.
<u>Limit of Quantitation (LOQ):</u>	Lowest tested concentration of the analyte in a sample that can be determined with acceptable accuracy and precision according to the method.
<u>Limit of Detection (LOD):</u>	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 D1603/01 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 soil.

Method D1603/01 was successfully validated in different soil types (Reference 1). The method has a limit of quantitation of 0.001 mg/kg for 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 soil matrices.

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

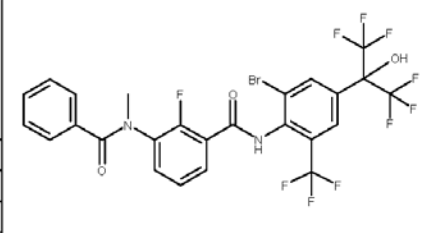
Test and reference items should be stored according to the information provided in the certificate of analysis.

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 ₂ O 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 ₂ O 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 ₈ BrF ₁₁ N ₂ O	
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 ₂ O	
Molecular Weight	559.17	

BAS-Code	None	
Common Name	S(PFP-OH)-8007	
Chemical Name	<i>N</i> -[2-bromo-4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluoro-3-(<i>N</i> -methylbenzamido)benzamide	
BASF Reg. No.	5959598	
CAS-No.	None	
Molecular Formula	C ₂₅ H ₁₅ BrF ₁₀ N ₂ O 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	Allegra 6R	Beckman	
Centrifuge Tubes (disposable)	50 mL	VWR	89039-660
Culture Tubes (glass)	16x100mm, Lime glass disposable	VWR	60825-425
Culture Tube caps	Safe-T-Flex Caps	VWR	60828-768
Filters, Syringe Tip	13mm Syringe Filter, 0.45 µm PTFE membrane	PALL Life Sciences	4555
Graduated Cylinder	10 mL, PYREX	VWR	89090-636
LC-MS/MS injection vials	1.5 mL, Target DP	VWR, Thermo Scientific	00162506
LC Column	XBridge BEH Phenyl, 2.5 µm, 2.1x100 mm	Waters	186006067
LC System	Acquity	Waters	
Mass Spectrometer	API 5500	Sciex	
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	BD	BD301025
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
Extraction Solvent	S1	Methanol-water, 70:30, v/v Add 700 mL of methanol and 300 mL of water into a 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
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 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 $\leq 95\%$. 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 ($\mu\text{g/mL}$)	Volume (mL)	Dilute with acetonitrile to a final volume of (mL)	Concentration ($\mu\text{g/mL}$)
1000	0.5	50	10
10	5	50	1.0
1.0	5	50	0.10

Note: A different concentration scheme may be used and other concentration levels may be prepared as needed.

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)
100	0.5	50	1.0
1.0	20.0	100	0.20
0.2	25	50	0.10
0.2	12.5	50	0.05
0.2	5.0	50	0.02
0.2	2.5	50	0.01

* 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.

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

Samples must be sufficiently homogenized beforehand, in order to assure that the aliquot taken for residue analysis is representative for the whole sample.

3.2 Sample Storage

Soil Samples are to be kept frozen until analysis.

3.3 Weighing and Fortification

For treated samples and control samples, weigh 5 g of soil sample into a 50 mL plastic centrifuge tube. For fortification samples, volumetrically add an appropriate volume of standard solution to the respective control. For example, pipette 50 µL of the 0.1 µg/mL solution for a 1 ppb fortification.

The following scheme may be used:

Sample Type	Sample Weight	Concentration of Spiking Solution	Volume of Spiking Solution	Level of Fortification
Control	5 g	-	-	0.00 µg/kg
Fortification (LOQ*)	5 g	100 ng/mL	0.05 mL	1 µg/kg
Fortification (10xLOQ)	5 g	1.0 µg/mL	0.05 mL	10 µg/kg
Treated	5 g	-	-	-

* limit of quantification

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

3.4 Extraction of Sample Material

Weigh 5 g of soil into a 50 mL plastic centrifuge tube, add exactly 10 mL of methanol, vortex to mix, shake for 30 minutes at 300 rpm on a mechanical shaker and then centrifuge at 3000 for 5 minutes. Decant supernatant into a 50 mL plastic centrifuge tube. Add exactly 10 mL of S1 to soil marc, vortex to mix, shake for 30 minutes at 300 rpm on a mechanical shaker and then centrifuge at 3000 for 5 minutes. Combine both supernatants into the same 50 mL plastic centrifuge tube. Mix well.

3.5 Sample Clean-up

No sample clean-up necessary proceed to section 3.6.

3.6 Preparation for Measurement

All samples – Remove an aliquot from the sample and perform a 1:5 dilution using FV1 (methanol-water, 50:50, v/v). For example, transfer 1 mL aliquot from extract (Section 3.4) to a culture tube and add 4 mL of FV1, cap and vortex to mix. 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.

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.

3.7 Influence of matrix effects on analysis

It has been demonstrated that the matrix load in the samples from the soil 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). If matrix effects are shown to be significant, matrix matched standards can be used for analysis.

3.8 Stability of Extracts and Final Volumes

All analytes have been shown to be stable in extracts and final volumes for at least 7 days (Reference 1).

3.9 Moisture Determination

The procedural recoveries will not be corrected for moisture content of the sample. Results of soil analysis are reported on a "dry weight" basis for residue determination. Therefore field treated soil sample weights must be corrected for moisture content by any method the laboratory customarily uses. The moisture determination will be conducted for the treated samples with residue value above LOD. An example of a moisture determination procedure is provided below:

The percent moisture is determined using an automated moisture determination equipment (Mettler Toledo HR83) using the formula below:

$$\text{Moisture content [\%]} = ((\text{Weight moist soil} - \text{Weight dry soil}) / \text{Weight moist soil}) \times 100$$

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

		Parameter		
Chromatographic System	Waters Acquity			
Analytical-column	XBridge BEH Phenyl 2.5um, 2.1x100mm			
Column Temperature	50°C			
Injection Volume	20 µL			
Mobile Phase A	Water / formic acid,		1000/1, v/v	
Mobile Phase B	Methanol / formic acid,		1000/1, v/v	
Flow Rate	600 µL/min			
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B	
	0.00	70	30	
	0.10	70	30	
	4.00	40	60	
	6.00	5	95	
	7.20	5	95	
	7.25	70	30	
	8.00	70	30	
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. 5.6 min	
DC-DM-8007 (Reg. No. 5936906)	545 → 525* 547 → 527	positive	approx. 5.2 min	
DC-8007 (Reg. No. 5936907)	559 → 539* 561 → 541	positive	approx. 5.5 min	
DM-8007 (Reg. No. 5856361)	649 → 242* 651 → 242	positive	approx. 5.7 min	
S(PFP-OH)-8007 (Reg. No. 5959598)	661 --> 641* 661 --> 621	positive	approx. 5.3 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 used instrument.

4.2.2 Instrumentation and Conditions for BAS 450 I and Its Metabolites used for Independent Laboratory Validation

		Parameter		
Chromatographic System	Waters Acquity			
Analytical-column	XBridge BEH Phenyl 2.5um, 2.1x100mm			
Column Temperature	50°C			
Injection Volume	20 µL			
Mobile Phase A	Water / formic acid,		1000/1, v/v	
Mobile Phase B	Methanol / formic acid,		1000/1, v/v	
Flow Rate	600 µL/min			
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B	
	0.00	70	30	
	0.10	70	30	
	4.00	40	60	
	6.00	5	95	
	7.20	5	95	
	7.25	70	30	
	8.00	70	30	
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. 5.6 min	
DC-DM-8007 (Reg. No. 5936906)	545 → 525* 547 → 527	positive	approx. 5.2 min	
DC-8007 (Reg. No. 5936907)	559 → 539* 561 → 541	positive	approx. 5.5 min	
DM-8007 (Reg. No. 5856361)	649 → 242* 651 → 242	positive	approx. 5.7 min	
S(PFP-OH)-8007 (Reg. No. 5959598)	661 --> 641* 663 --> 643	positive	approx. 5.3 min	

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

4.2.3 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 BAS 450 I/metabolites standards for LC-MS/MS in the range of 0.2 ng/mL to 0.01 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, the sample weight will be considered 5 g in the final calculation of residues [mg/kg]. The method requires that the sample weight to be 5 ± 0.1 g for fortification samples. The recovery is the percentage of the fortified amount (μg or ng), which is recovered through the method and the weights cancels out, as shown in the equation below, during the final calculation step.

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

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

$$\text{II. Residue [mg/kg]} = \frac{V_{\text{end}} \times C_A}{G \times A_F \times 1000}$$

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 = Weight of the sample extracted [g]
 A_F = Aliquotation factor
1000 = Factor remaining after all unit conversions

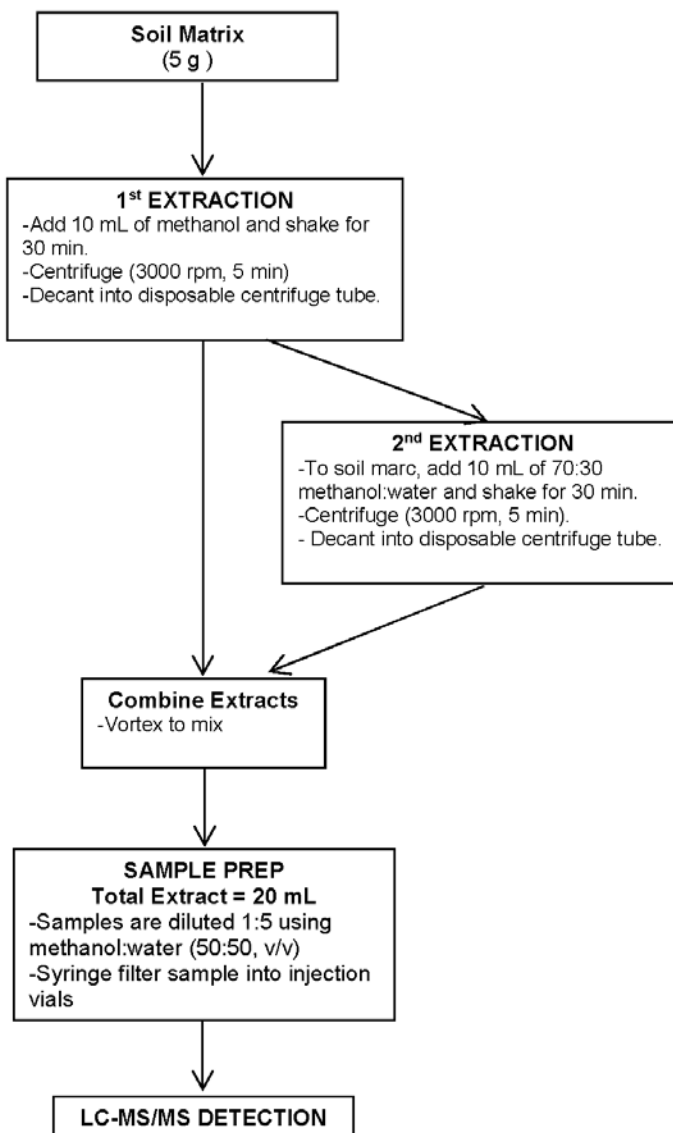
The recoveries of spiked compounds are calculated according to equation III:

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

IV. Soil residues based on soil dry weight

$$\text{Residue [mg/kg] (Dry residue)} = \frac{\text{Wet Sample Residue [mg/kg]} \times 100}{(100 - \text{"moisture content [\%]"})}$$

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 1 working day (8 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 0.001 mg/kg for all analytes. The limit of detection was estimated at 20% of the limit of quantification, equivalent to 0.0002 mg/kg for BAS 450 I/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 soil 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.

8 REFERENCES

1. Delinsky, D. "Method for the Determination of 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 Soil by LC-MS/MS (at LOQ of 1 ppb)"; BASF Study Number 815843; BASF Registration Document Number: 2017/7001823
2. Veiga, A. and Jose, W. (2017) "Validation of BASF Method Number 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), soybean (seed) and coffee (grain) using LC-MS/MS"; BASF Study Number, 772495; BASF Registration Document Number: 2016/3004081
3. D. Delinsky, 2017: Validation of BASF 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.

9 APPENDIX

9.1 Example of Calculation

Example: BAS 450 I; soil sample fortified at 0.001 mg/kg:

Concentration in the final volume [ng/mL]

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

Residue in the sample [mg/kg]

$$\text{Residue [mg/kg]} = \frac{V_{\text{end}} \times C_A}{G \times A_p \times 1000}$$

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

The following values were used in this calculation:

Response of fortified sample	1366
Response of control sample	56
Slope:	2.81e4
Intercept:	54.4
Sample Weight (G):	5 g
Final Volume (V _{end}):	5 mL
Aliquotation factor A _F :	0.05 (= 5%)
Conversion factor ng → µg:	1000

$$\text{Concentration (ng/mL)} = \frac{1366 - 54.4}{2.81e4} = 0.0467 \text{ ng/ml}$$

$$\begin{aligned} \text{Residue (mg/kg)} \\ = \frac{5 \text{ ml} \times 0.0467 \text{ ng/ml}}{5 \text{ g} \times 0.05 \times 1000} &= 0.000934 \text{ µg/g} = 0.000934 \text{ mg/kg} \end{aligned}$$

$$\text{Recovery \%} = \frac{(0.000934 \text{ mg/kg} - 0.00000 \text{ mg/kg}) \times 100}{0.0010 \text{ mg/kg}} = 93.4\%$$