Abstract

The objective of this validation study was to demonstrate the applicability and repeatability of BASF Analytical Method D1608/01 for the determination of BAS 450 I 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 using LC-MS/MS.

Principle of the method. Residues of BAS 450 I and its metabolites DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 in water samples are diluted with methanol and then analyzed by direct injection onto a high performance liquid chromatography (HPLC) column with detection by positive ion electrospray ionization tandem mass spectrometry (ESI-MS/MS) monitoring the following ion transitions: m/z 663→643 and 665→645 for BAS 450 I; m/z 545→525 and 547→527 for DC-DM-8007; m/z 559→539 and m/z 561→541 for DC-8007, m/z 649→242 and m/z 651→242 for DM-8007, and m/z 661→641 and m/z 661→621 for S(PFP-OH)-8007. The results are calculated by direct comparison of the sample peak responses to those of external standards.

Test conditions. For validation, untreated drinking (well) water and surface (lake) water samples were fortified with each analyte and analyzed according to the established method validation guidelines. The analytical sets for each water type typically consisted of a reagent blank, two controls, five replicates fortified with analyte at the method limit of quantitation, 5 ng/L (ppt) for BAS 450 I and 25 ng/L (ppt) for its metabolites, and five replicates fortified at a higher level, corresponding to 10X the limit of quantitation, 50 ng/L for BAS 450 I and 250 ng/L for its metabolites. For each analyte, the two mass transitions or confirmatory LC-MS/MS procedures described above were evaluated. In conjunction with the subject study, matrix- and solvent-matched standards were analyzed in a separate experiment to evaluate any potential matrix effects.

Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ of the method was set at 5 ng/L for BAS 450 I, which was lower than the lowest relevant eco-toxicology endpoint in water (NOEC is 6.3 ng/L) and was also defined as the lowest fortification level tested. The LOD for BAS 450 I was set at 1 ng/L, which was 20% of the defined LOQ. The LOQ for DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 was set at 25 ng/L for each analyte in water. The limit of detection for DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 was set at 5 ng/L for each analyte, 20% of the defined LOQ. The LOD was shown to be detectable as the absolute amount of analyte injected (0.0375 pg on column for BAS 450 I and 0.188 pg on column for the metabolites) into the LC-MS/MS when the lowest calibration standard was analyzed (0.0005 ng/mL for BAS 450 I; 0.0025 ng/mL for the metabolites) with acceptable signal to noise ratio (S/N) greater than 3:1.

Selectivity. The method determines BAS 450 I, DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 residues in water by LC-MS/MS. No interfering peaks were found at the retention times for these analytes. The multiple reaction monitoring (MRM) transitions used to identify each analyte were determined by product ion spectra. The experiment to evaluate any potential matrix effects showed that the matrix load in the samples from each water type had no significant influence on analysis (matrix effects <20%).

Linearity. Acceptable linearity was observed for the standard range tested for each analyte: The method-detector response, for the method validation sets, was linear over the 0.0005 to 0.01 ng/mL range for parent and 0.0025 to 0.05 range for the metabolites ($r \geq 0.9963$ for all analytes).

Standard stability. The stability of each analyte in standard solutions has been determined: In this study, DC-DM-8007 and DC-8007 were shown to be stable in the stock/fortifcation solutions prepared in acetonitrile for at least 92 days when stored under refrigeration. Each analyte was shown to be stable in calibration standard solutions prepared by serial dilution of the intermediate standards with 50/50 methanol/water and held under refrigeration for at least 33 days for parent and 33 days for DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007. Stability of parent, DM-8007, and S(PFP-OH)-8007 in acetonitrile stock/fortification solutions was determined in another study. A summary of the stability of each analyte in standard solutions is provided in Appendix E. During the course of this study, all solutions were held under refrigeration and all solutions were used within the demonstrated time period of stability.

Extract stability. As the method relies on the direct injection of the diluted water samples onto the HPLC column, there are no extracts. Instead, only stability in this “final volume” prepared for HPLC analysis was determined. The recoveries from stored solutions generated during extract stability experiments performed in conjunction with this study, which included tests on the HPLC final volume held under refrigeration, indicated that each analyte is stable in water extracts of for at least 6 days, sufficient to support the storage intervals and conditions incurred by extracts in future work using this method.

1 Introduction

1.1 Background and Purpose of Study

The objective of this validation study was to demonstrate the applicability and repeatability of BASF Analytical Method No. D1608/01, used for the determination of residues of BAS 450 I, DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 in water by LC-MS/MS.

2 Materials and Methods

2.1 Test Systems

The water samples used in this study were drinking (well) water and surface (lake) water samples, which were characterized by AGVISE Laboratories. The GLP water characterization reports are provided in Appendix K. The samples were refrigerated during the experimental period. Each analysis set was uniquely identified with a Master Sheet Number, which consisted of the study number plus a unique number (e.g., 725931-1). The test system samples were assigned unique numbers and these were recorded in each analytical set or "Master Sheet" (e.g., water fortification sample 725931-2-4 or CM16-016-LF1, from Master Sheet No. 725931-2 using control matrix sample CM16-016). The actual sample numbers used for the analysis were identified in the raw data and in this final report.

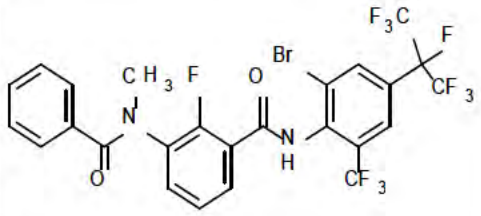
2.2 Test and Reference Substances

The test/reference standards, shown below, were synthesized by Mitsui Chemical Agro, Inc. (MCAG, Tokyo, Japan) and were maintained at room temperture until use in this study. Japan Analytical Chemistry Consultants Co., Ltd., on behalf of MCAG, determined characterization and purity prior to the substances being used in this study. Details of these determinations are available to BASF and are located at Japan Analytical Chemistry Consultants Co., Ltd. (Funado Itabashi, Tokyo, Japan). BASF has retained a reserve sample of these chemicals and has documentation at BASF Corporation, BASF Crop Protection (Research Triangle Park, North Carolina, USA).

The test/reference substances in solution were used in the study to generate data for both instrument and method performance. Quantitation of residues in all samples was achieved using calibration curves calculated by linear regression (no weighting) of instrument responses for the reference substances. The performance of the instrument was evaluated during each injection set.

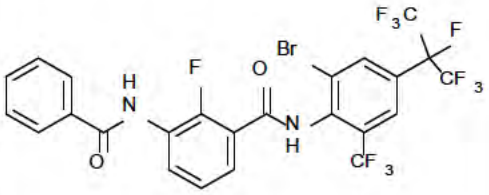
2.2.1 BAS 450 I

Common Name	Broflanilide
BAS Code Name	BAS 450 I
Chemical Name	*N*-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluoro-3-(N-methylbenzamido) benzamide
BASF Reg. No.	5672774
Molecular Formula	$C_{25}H_{14}BrF_{11}N_2O_2$
Molecular Weight	663.29
Lot No.	089-100112-1
Purity:	99.67%
Expiration Date	April 9, 2017



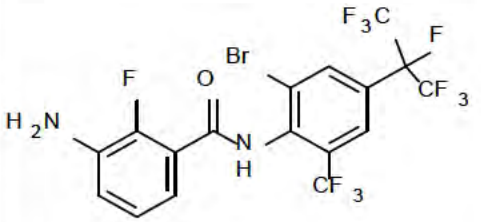
2.2.2 DM-8007

Common Name	DM-8007
Chemical Name	3-benzamido-*N*-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluorobenzamide
BASF Reg. No.	5856361
Molecular Formula	$C_{24}H_{12}$ $BrF_{11}N_2O_2$
Molecular Weight	649.3
Lot No.	296-007-81-1
Purity:	98.84%
Expiration Date	June 28, 2017



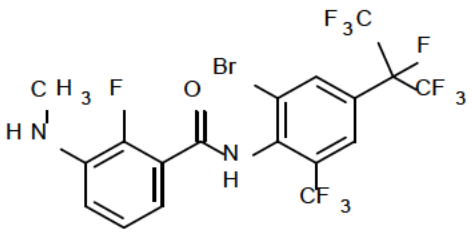
2.2.3 DC-DM-8007

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
Molecular Formula	$C_{17}H_8BrF_{11}N_2O$
Molecular Weight	545.1
Lot No.	296-009-094-2
Purity:	98.58%
Expiration Date	June 28, 2017



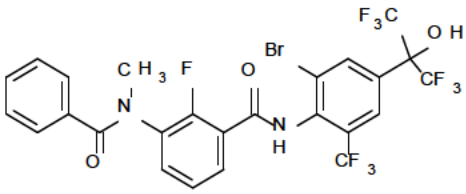
2.2.4 DC-8007

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
Molecular Formula	$C_{18}H_{10}BrF_{11}N_2O$
Molecular Weight	559.2
Lot No.	296-012-009-1
Purity:	99.07%
Expiration Date	June 28, 2017



2.2.5 S(PFP-OH)-8007

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
Molecular Formula	$C_{25}H_{15}BrF_{10}N_2O_3$
Molecular Weight	661.3
Lot No.	267-012-094-3
Purity:	99.06%
Expiration Date	June 28, 2017



Stock solutions of analytes were prepared in acetonitrile. The mixed intermediate/fortification solutions containing each analyte were prepared by combining aliquots of the stock solutions for each analyte and diluting with acetonitrile. The calibration standards were prepared by serial dilution of the intermediate standards using 50/50 methanol/water. The stability of the analytes in standard solutions (and stock/fortification solutions for DC-DM-8007 and DC-8007) was determined in conjunction with this study by analyzing aged standards containing each analyte against freshly prepared standard solutions. The stability of parent, DM-8007, and S(PFP-OH)-8007 in stock/fortification solutions has been determined in a related study (reference 2).

During the course of this study, the test/reference substance solutions were stored under refrigeration. Preparation and dilution data forms pertaining to the stock and working solutions are located in the analytical facility data and are archived periodically. Example standard dilution and use information, as performed in the subject study, are provided in Appendix L.

2.3 Route of Administration

In this method validation study, the test substances were applied to the test system as analytical standard solutions (in acetonitrile) by pipette to ensure precise delivery of a small amount of the test substances.

2.4 Analytical Method

2.4.1 Principle of the Method

Using BASF Analytical Method No. D1608/01, residues of BAS 450 I, DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 in water are quantified using LC-MS/MS. The method procedures validated in this study are provided in Appendix B. A description of the methodology follows: Briefly, residues in water samples (10 mL each) are diluted with 10 mL methanol and vortex mixed for approximately 1 min. Samples are filtered then analyzed by HPLC/MS/MS.

2.4.2 Specificity/Selectivity

Residues of BAS 450 I, DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 are determined by HPLC-MS/MS, in positive mode, monitoring the following ion transitions: m/z 663→643 and 665→645 for BAS 450 I; m/z 649→242 and 651→242 for DM-8007; m/z 545→525 and 547→527 for DC-DM-8007; m/z 559→539 and 561→541 for DC-8007; and m/z 661→641 and 661→621 for S(PFP-OH)-8007. The results are calculated by direct comparison of the sample peak responses to those of external standards. Two mass transitions are available for all analytes. Due to the high selectivity and specificity of LC-MS/MS. an additional confirmatory technique is not necessary. The multiple reaction monitoring (MRM) transitions used to identify each analyte were determined in a related study (reference 3).

2.5 Validation of Method

For validation, untreated drinking (well) water and surface (lake) water samples were fortified with each analyte and analyzed according to the established method validation guidelines. To test the repeatability of the method, the analytical sets consisted of a reagent blank, and for each matrix, two controls, five replicates fortified with each analyte at the method limit of quantitation, 5 ng/L for BAS 450 I and 25 ng/L for metabolites, and five replicates fortified at a higher level, corresponding to 10X the limit of quantitation,50 ng/L for BAS 450 I and 250 ng/L for metabolites. For each analyte, the two mass transitions described above were evaluated.

2.6 Influence of Matrix Effects on Analysis

In conjunction with the subject study, matrix-matched standards and solvent-based standards were analyzed in a separate experiment to evaluate any potential matrix effects on LC/MS/MS analysis. This involved comparing calibration standards prepared in control matrix against calibration standard solutions prepared with 50/50 methanol/water (v/v). The matrix-matched standards were prepared by diluting mixed standards containing each analyte with control drinking or surface water to 0.00125 ng/mL, 0.0025 ng/mL, and 0.005 ng/mL for BAS 450 I and 0.00625, 0.0125, and 0.025 ng/mL for the metabolites. These standards were then compared to solvent-based calibration standards at the same concentrations. Each set of matrix-matched standards (for each water type) was bracketed by a block of solvent-based calibration standards and included additional single injections of the tested standard levels during the run.

The data generated were evaluated by comparing the average area response of the standards for three or more injections of each type (with and without matrix) for the three standard concentration levels. Acceptability (i.e., matrices had no significant influence on the analysis) requires a difference in area of <20%, calculated as the "Mean Area Change (%)". For each matrix, an overall average "Mean Area Change (%)" across the two tested concentrations was calculated to make a general assessment of acceptability with respect to matrix effects.

2.7 Stability of Extracts

As the method does not consist of a typical “extraction”, the water samples are diluted and analyzed, “extracts” and “final volume” are used interchangeably in this report. The stability of each analyte in stored “extract” solutions was determined in conjunction with the subject method validation study. The stability in the final volume, the solution prepared for LC-MS/MS injection, was established for each matrix by reanalyzing a control and five recovery samples which had been stored under refrigeration at the final volume stage. Quantification of the analytes in the stored samples for this experiment was performed for the primary mass transitions.

Extracts. The method validation fortification sample extracts were analyzed within 2 days of extraction. The generally acceptable method recoveries obtained during analyses demonstrate the storage stability of residues of BAS 450 I, DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 in the extracts in the brief period prior to analysis. In addition, the recoveries from stored solutions generated during extract stability experiments performed in conjunction with this study, which included tests on the HPLC final volume held under refrigeration, indicated that each analyte, for both water types tested (surface and drinking water) is stable in extracts for at least the time period tested, 6 days for BAS 450 I in surface and drinking water and 7 days and 8 days for metabolites in both drinking and surface water, respectively, as shown in Appendix D.

4 CALCULATIONS AND RAW DATA

An example calculation is included in Appendix C. Detailed analytical data such as supporting raw data necessary for re-calculations, standards and calibration curve data are provided in Appendix F. Example standard curves are provided in Appendix H. Example chromatograms are provided in Appendix I.

5 STATISTICS AND DATA INTEGRITY

Statistical treatment of the data included simple descriptive statistics, such as determinations of averages, standard deviation and/or RSD for the procedural recoveries and area counts and calculation of the calibration curve and correlation coefficient (r) by linear regression of the instrument responses for the reference standards. The statistical calculations throughout this report were performed using an automated computer spreadsheet (Microsoft Excel®) and were rounded for presentation purposes. Slight differences may be noted in hand calculations using the recoveries presented in the tables. These are due to rounding and have no effect on the scientific conclusions presented in this report. The detailed analytical data may be consulted for confirmation of the calculated results.

Several measures were taken to ensure the quality of the study results. The quality assurance unit at BASF 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 and test and reference items were maintained in secured (i.e. pad-locked) storage with limited access. Freezer temperatures were continuously monitored by electronic means.

6 SUMMARY OF METHOD

Summaries of the method parameters and characteristics are provided in Appendix B.

7 INDEPENDENT LABORATORY VALIDATION

Primera provided the following comments on the method:

This independent laboratory validation was successfully completed on the first trial at Primera Analytical Solution Corp for all matrices. 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.

9 CONCLUSIONS

The results of this method validation study demonstrate that BASF Analytical Method No D1608/01 fulfils the requirements with regard to specificity, repeatability, limit of quantification, and recoveries and is, therefore, applicable to correctly determine BAS 450 I, DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 residues in water.

10 PROTOCOL, AMENDMENTS, AND DEVIATIONS

The study was conducted according to a study protocol. All protocol deviations that occurred during the conduct of this study were reported and reviewed by the Study Director. A list of all protocol amendments and deviations is provided in Appendix . None of the changes had an impact on the validity of the study.

11 REFERENCES

1. Xu, A. (2017). Amended Report for 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”. BASF Study Number: 776692. BASF Registration Document Number 2017/7015840.
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. Delinsky, D. “Validation of 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 1 ppb)”; BASF Study Number 815843; BASF Registration Document Number: 2017/7001823.

Table 2. Summary Parameters for the Analytical Method Used for the Quantitation of Residues of BAS 450 I, DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007 in Water

Method ID	BASF Analytical Method No. D1608/01
Analyte(s)	Residues of BAS 450 I, DM-8007, DC-DM-8007, DC-8007, S(PFP-OH)-8007 in water
Extraction solvent/technique	None. Residues of BAS 450 I, DM-8007, DC-DM-8007, DC-8007, S(PFP-OH)-8007 in water samples (10 mL each) are diluted with methanol and mixed.
Cleanup strategies	None
Instrument/Detector	High performance liquid chromatography (HPLC) column with detection by positive ion electrospray ionization tandem mass spectrometry (ESI-MS/MS) monitoring the following ion transitions: : m/z 663→643 and 665→645 for BAS 450 I; m/z 649→242 and 651→242 for DM-8007; m/z 545→525 and 547→527 for DC-DM-8007; m/z 559→539 and 561→541 for DC-8007; and m/z 661→641 and 661→621 for S(PFP-OH)-8007 All analyses are performed using a Waters Aquity UPLC system equipped with an XBridge BEH Phenyl column (100 x 2.1 mm, 2.5µm particle size using a mobile phase gradient of water:methanol, each acidified with 0.1% formic acid (flow rate 600 uL/minute). Detection is obtained with a AB Sciex API 5500 Mass Spectrometer.
Standardization method	Direct comparison of the sample peak responses to those of external standards
Stability of std solutions	The stability of each analyte in standard solutions has been determined: In this study, DC-DM-8007 and DC-8007 were shown to be stable in the stock/fortifcation solutions prepared in acetonitrile for at least 92 days when stored under refrigeration. Each analyte was shown to be stable in calibration standard solutions prepared by serial dilution of the intermediate standards with methanol-water (50:50, v:v) and held under refrigeration for at least 33 days for all analytes. Stability of parent, DM-8007, and S(PFP-OH)-8007 in acetonitrile stock/fortification solutions was determined in another study (Reference 2). A summary of the stability of each analyte in standard solutions is provided in Appendix E. During the course of this study, all solutions were held under refrigeration and all solutions were used within the demonstrated time period of stability.
Retention times	See Appendix B. for typical retention times

Table 3. Characteristics for the Analytical Method Used for the Quantitation of Residues of BAS 450 I, DM-8007, DC-DM-8007, DC-8007, S(PFP-OH)-8007 in Water Matrices

Analyte	Residues of BAS 450 I, DM-8007, DC-DM-8007, DC-8007, S(PFP-OH)-8007 in water
Equipment ID	Waters Aquity UPLC system equipped with an XBridge BEH Phenyl column (100 x 2.1 mm, 2.5µm particle size using a mobile phase gradient of water:methanol, each acidified with 0.1% formic acid (flow rate 600 uL/minute). Detection is obtained with a AB Sciex API 5500 Mass Spectrometer.
Limit of quantitation (LOQ)	The validated LOQ for residues in water is 5 ng/L for BAS 450 I and 25 ng/L for its metabolites, which corresponds to a concentration in the final volume of 0.0025 ng/mL for BAS 450 I and 0.0125 ng/mL for its metabolites.
Limit of detection (LOD)	The limit of detection was set at 20% of the LOQ, equivalent to 1 ng/L for BAS 450 I and 5 ng/L for DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007). The lowest standard for each analyte in the calibration curve has good detectability (signal to noise ratio greater than 3:1).

We create chemistry

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

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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.

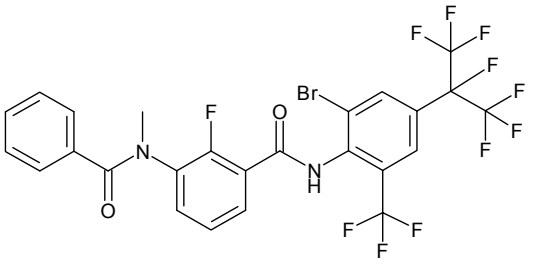
2 MATERIALS

2.1 Safety

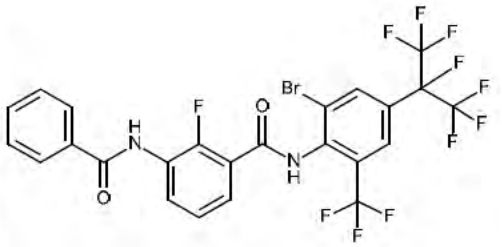
The test and reference items, as well as the chemicals required for this analysis, should be handled in accordance with good industrial hygiene and safety practice. Avoid contact with the skin, eyes and clothing. Wearing of closed work clothing is recommended. Remove contaminated clothing. Ensure work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

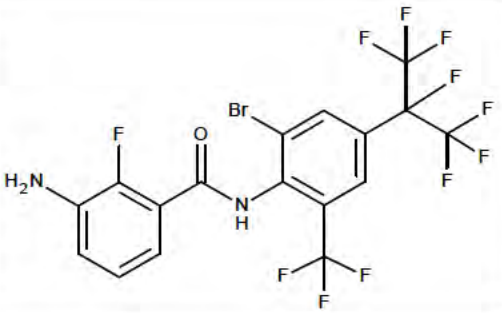
Test and reference items should be stored according to the information provided in the certificate of analysis.

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	

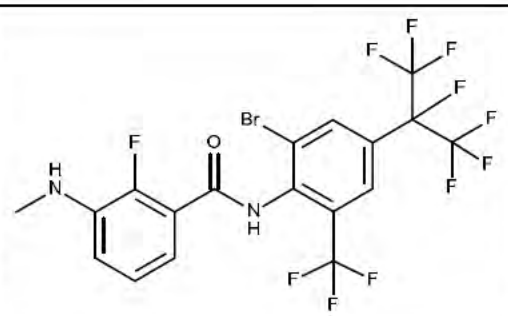
BAS-Code	None
Common Name	DM-8007
Chemical Name	3-benzamido-*N*-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluorobenzamide
BASF Reg. No.	5856361
CAS-No.	None
Molecular Formula	$C_{24}H_{12}BrF_{11}N_2O_2$
Molecular Weight	649.25



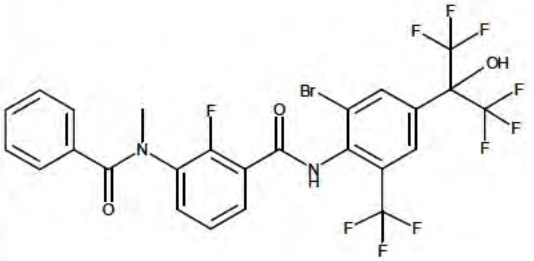
BAS-Code	None
Common Name	DC-DM-8007
Chemical Name	3-amino-*N*-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluorobenzamide
BASF Reg. No.	5936906
CAS-No.	None
Molecular Formula	$C_{17}H_8BrF_{11}N_2O$
Molecular Weight	545.15



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:
- o Calibration standards
- o Control samples
- o Procedural recovery samples
- o Unknown samples
- o 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	75 µL		
Mobile Phase A Mobile Phase B	Water / formic acid, 1000/1, v/v 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. 5.83 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

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 Mobile Phase B	Water / formic acid, 1000/1, v/v 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]** $= \dfrac{\text{Response} - \mathit{Intercept}}{\mathit{Slope}} = C_A$

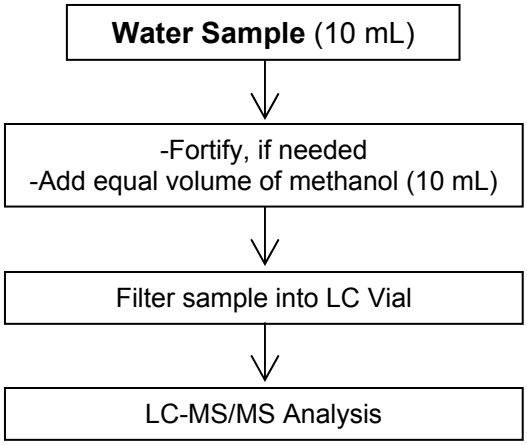
II. **Residue [ng/L]** $= \dfrac{V_{end} \times C_A}{G \times A_F}$

V_{end} = Final volume of the extract after all dilution steps [mL]
C_A = Concentration of analyte as read from the calibration curve [ng/mL]
G = Volume of 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 %** $= \dfrac{(\text{Residue in fortified sample} - \text{Residue in control}) \times 100}{\text{Amount of analyte fortified}}$

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.

Typical Recovery Calculation for LC/MS/MS Quantitation

Sample No. CM16-016-LF1. Control surface water sample fortified at the LOQ with BAS 450 I, DM-8007, DC-DM-8007, DC-8007, and S(PFP-OH)-8007, Master Sheet No. 725931-4.

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

	BAS 450 I
Peak Area =	14663
Intercept =	1684
Slope =	5410000
Conc. (ng/mL) =	0.0024

The concentration of analyte in ng/kg (ng/L) is calculated as shown in equation:

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

Where:

V_{end} = Final volume [mL]
C_A = Concentration of analyte as read from the calibration curve [ng/mL]
G = Weight of the sample extracted
A_F = Aliquotation factor

	BAS 450 I
V_{end} =	20 mL
A_F =	100%
G =	10.0
Conc. (ng/mL) =	0.0024
Residue (ng/L) =	4.80

Net residue (ng/L of analyte) = Residue (ng/L of analyte) - Residue in Control (ng/L)

$$\text{Recovery of analyte (\%)} = \frac{\text{Residue (ng/L of analyte) - Residue in Control (ng/L)}}{\text{Amount Fortified (ng/L)}} \times 100$$

	BAS 450 I
Amount fortified (ng/L) =	5
Residue (ng/L) =	4.80
Residue in control =	0.0000
%Recovery	96%

Use full calculator precision in any intermediate calculations. Round only the final value.

Typical analytical standards dilution and use records for Analytes

Standard Number	Analyte	Standard (Lot # used)	Amount Weighed / Volume	Final Dilution Vol. (mL)	Final Conc. [1]	Solvent[2]	Prep. Date	Expiry Date
Stock solutions								
ERS16-1367	BAS 450 I	089-100112-1	10.0 mg	10	1 mg/mL	Acetonitrile	13-Sep-16	13-Dec-16
ERS16-1368	DM-8007	296-007-81-1	10.0 mg	10	1 mg/mL	Acetonitrile	13-Sep-16	13-Dec-16
ERS16-1418	DC-DM-8007	296-009-094-2	10.0 mg	10	1 mg/mL	Acetonitrile	19-Sep-16	19-Dec-16
ERS16-1419	DC-8007	296-012-009-1	10.0 mg	10	1 mg/mL	Acetonitrile	19-Sep-16	19-Dec-16
ERS16-1420	S(PFP-OH)-8007	267-012-094-3	10.0 mg	10	1 mg/mL	Acetonitrile	19-Sep-16	19-Dec-16
Serial dilutions								
ERS16-1570	All	ERS16-1367 ERS16-1368 ERS16-1418 ERS16-1419 ERS16-1420	0.1 mL 0.5 mL 0.5 mL 0.5 mL 0.5 mL	10	10 µg/mL/50 µg/mL	Acetonitrile	30-Sep-16	30-Oct-16
ERS16-1571	All	ERS16-1570	0.05 mL	50	0.01 µg/mL/0.05 µg/mL	Acetonitrile	30-Sep-16	30-Oct-16
ERS16-1572	All	ERS16-1571	5 mL	50	0.001 µg/mL/0.005 µg/mL	Acetonitrile	30-Sep-16	30-Oct-16
Calibration Standards								
ERS16-1573	All	ERS16-1572	5 mL	50	0.1 ng/mL/0.5 ng/mL	Mixture1	30-Sep-16	30-Oct-16
ERS16-1574	All	ERS16-1573	10 mL	100	0.01 ng/mL/0.05 ng/mL	Mixture1	30-Sep-16	30-Oct-16
ERS16-1575	All	ERS16-1574	25 mL	50	0.005 ng/mL/0.025 ng/mL	Mixture1	30-Sep-16	30-Oct-16
ERS16-1576	All	ERS16-1574	12.5 mL	50	0.0025 ng/mL/0.0125 ng/mL	Mixture1	30-Sep-16	30-Oct-16
ERS16-1577	All	ERS16-1574	5 mL	50	0.001 ng/mL/0.005 ng/mL	Mixture1	30-Sep-16	30-Oct-16
ERS16-1578	All	ERS16-1574	2.5 mL	50	0.0005 ng/mL/0.0025 ng/mL	Mixture1	30-Sep-16	30-Oct-16

1. The concentration for each analyte shown in column 2. In the case of two concentrations, the first number is the concentration of BAS 450 I, and the second number is the concentration of the metabolites.
2. Mixture1 = 50/50 Methanol/Water

Example fortification scheme used for analysis (Master Sheet No. 725931-4)

SAMPLE DATA	FORTIFICATION DATA						FINAL VOL (mL)
	Volume (mL)	PPB Fortified	COMPOUND	VOLUME (mL)	STD CONC	STD NO.	
725931-04-1	10	0	Reagent Blank	NA	NA	NA	20
CM16-016a	10	0	Control	NA	NA	NA	20
CM16-016-LF1	10	0.005/ 0.025	All 5 analytes	0.05	1 ng/mL/ 5 ng/mL	ERS16-1569	20
CM16-016-HF1	10	0.05/ 0.25	All 5 analytes	0.05	10 ng/mL/ 50 ng/mL	ERS16-1568	200

1) The analytical method was updated to change the LC gradient conditions to reduce observed matrix-effects during development.

2) Correction of a typographical error referencing OCSPP 850.6100 in the Guideline section of the original study protocol. Chacnged the acceptable range for recoveries from 70-110% to 70-120%.

3) Correction of a typographical error to reference the use of a micropipette rather than a volumetric pipette for the fortification of samples.

These changes did not have an adverse effect on the outcome of this study.