Independent Lab Validation of BASF Analytical Method D1508/01: "Analytical Method for the Determination of residues of Pyraclostrobin metabolite, BF 500-3 (Reg. No. 340266) in Surface and Drinking Water by LC-MS/MS"

ABSTRACT

The purpose of the study was to demonstrate that BASF Analytical Method D1508/01 "Analytical Method for the Determination of residues of Pyraclostrobin metabolite, BF 500-3 (Reg. No. 340266) in Surface and Drinking Water by LC-MS/MS" could be performed successfully at an outside facility with no prior experience with the method. This method was originally developed and validated at BASF Crop Protection, RTP, NC (Reference 1).

Principle of the Method: The water samples (10 mL) were fortified with acetonitrile and thoroughly mixed. An aliquot of the resulting solution was analyzed to determine the residues of BF 500-3 using LC-MS/MS. The transitions at *m/z* 357.9 → *m/z* 164.1 and at *m/z* 357.9 → *m/z* 132.0 were monitored in positive mode for primary and confirmatory quantification, respectively.

Test Conditions: The method was validated at two fortification levels (0.03 and 0.3 µg/L) for BF 500-3 in surface and drinking water. For each fortification level, five replicates were analyzed. Additionally, at least two replicates of unfortified samples were examined.

Limit of Quantitation (LOQ) and Limit of Detection (LOD): The LOQ was defined as the lowest fortification level tested. The LOQ for BF 500-3 in surface water and drinking water was 0.03 µg/L. The LOD for BF 500-3 in surface water and drinking water was set at 0.006 µg/L, which was 20% of the defined LOQ. The LOD for BF 500-3 in surface water and drinking water was defined as the absolute amount of analyte injected (0.00006 ng) into the LC-MS/MS when the lowest calibration standard was analyzed (0.0060 ng/mL).

Selectivity: The method determines residues of BF 500-3 in water matrices. No interfering peaks were found at the retention time for BF 500-3. No matrix suppression or enhancement was found for BF 500-3.

Linearity: For both of the mass transitions of BF 500-3 in the standard calibration solutions, good linearity ($r^2 > 0.99$) was observed in the range of 0.0060 ng/mL to 0.15 ng/mL.

Standard Stability: Standard stability was not evaluated in this ILV study. However, it was determined in the validation of this method (Reference 1).

Extract Stability: Extract stability was not evaluated in this ILV study. However, it was determined in the validation of this method (Reference 1).

1. Introduction

1.1 Scope of the Method

BASF Method D1508/01 was developed to determine the residues of BF 500-3 in water using LC-MS/MS at BASF Crop Protection in Research Triangle Park, North Carolina. This method was validated at BASF Crop Protection in Research Triangle Park, North Carolina (Reference 1) and was independently validated at EPL Bio Analytical Services (EPL).

The independent lab validation was conducted using two fortification levels: limit of quantitation (0.03 µg/L) and ten times of limit of quantitation (0.3 µg/L) for both surface and drinking water. For each fortification level and matrix, five replicates were analyzed. Additionally, one reagent blank and two replicates of unfortified samples were examined.

1.2 Principle of the Method

The water samples (10 mL) were fortified with acetonitrile and thoroughly mixed. An aliquot of resulting solution was analyzed to determine the residues of BF 500-3 using LC-MS/MS. The transitions at *m/z* 357.9 → *m/z* 164.1 and at *m/z* 357.9 → *m/z* 132.0 were monitored in positive mode for primary and confirmatory quantification, respectively.

1.3 Specificity

To demonstrate the specificity of the analytical method, one additional mass transition (*m/z* 357.9 → *m/z* 132.0) was monitored simultaneous to the primary quantitation transition (*m/z* 357.9 → *m/z* 164.1) for analysis of BF 500-3. The method was able to accurately determine residues of BF 500-3 and no interference was observed at the retention time of the analyte peak. No matrix suppression or enhancement was found to affect the analyte.

2. Materials and Methods

2.1 Test Systems

The test systems considered in this study were surface water (BASF Study 437860; RCN R130034) and drinking (tap) water (BASF reference number 22014).

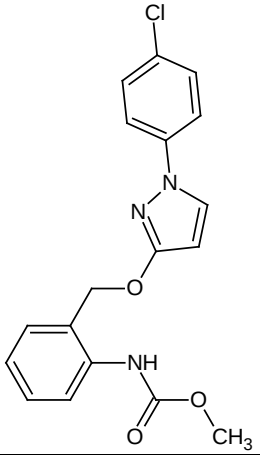
The control samples were provided by BASF. The water samples were received on November 4, 2014. Upon arrival at the laboratory, the samples were opened, inspected, and checked against enclosed shipping forms. The test systems were received frozen and were stored under frozen conditions at all times, unless necessary for laboratory analysis. The test systems were characterized at AGVISE Laboratories (604 Highway 15 West, Northwood, ND 58267). A copy of the characterization data for both water samples is provided in Appendix F.

2.2 Test and Reference Substances

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

The BF 500-3 (lot number L74-118) reference substance was provided by the sponsor and was received on July 22, 2015. The certificate of analysis is presented in Appendix C. A detailed summary of the reference substance is presented below.

BF 500-3

Code Name	BF 500-3 (500M07)
BASF Reg. No.	340266
CAS No.	512165-96-7
Molecular Formula	$C_{18}H_{16}ClN_3O_3$
Molecular Weight	357.8 g/mol
IUPAC Name	Methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) carbamate
Lot Number	L74-118
Purity	99.9 %
Storage	Refrigerated or frozen
Expiration Date	October 1, 2016
Chemical Structure	

2.3 Materials

2.3.1 Equipment

The equipment used in this study was documented in the raw data. Maintenance files and applicable Standard Operating Procedures (SOPs) for the equipment are retained at the testing facility.

Class A volumetric glassware
Laboratory glassware (beakers, graduated cylinders)
Volumetric pipettes, glass; various sizes
Analytical balance, capable of measuring to 0.01 mg
Air displacement pipette, various volumes with disposable tips
Vortex mixer
Sonicator
Pasteur pipettes (glass, disposable)
HPLC system: Agilent 1290

HPLC analytical column: XBridge C18 (2.1 x 50 mm, 2.5 µm)
Mass spectrometer: AB Sciex 6500 Q-Trap
HPLC autosampler vials with screw-top, pre-slit caps

2.3.2 Reagents

Chemicals

Chemical	Manufacturer/ Supplier	Lot Number(s)	Expiration Date(s)
Acetonitrile	Fisher	152967, 151811	6/30/2020
Methanol	EMD	54324	6/30/2020
Formic Acid	Fisher	141876	6/30/2019†, 6/30/2020†
HPLC Water	Burdick & Jackson	DL926-I	6/30/2019
DI Water	In House	N/A	N/A

†At EPL, expiration date assigned was based on receipt date. Study Director confirmed that multiple bottles of Lot 141876 were received on different dates and accordingly assigned expiration dates of 6/30/2019 and 6/30/2020.

Solutions and Solvent Mixtures

Description	Code	Composition
HPLC mobile phase A	LC1	0.1% Formic Acid in Water
HPLC mobile phase B	LC2	0.1% Formic Acid in Methanol

2.3.3 Standard Solutions

Stock Solution
The stock solution (1.0 mg/mL) was prepared by weighing an appropriate amount of the reference substance into a 10 mL volumetric flask and filling to volume with acetonitrile. The stock solution was sonicated and vortexed to ensure a complete homogeneous solution. Fortification and calibration standard solutions were prepared from different dilution series of a single stock solution.

Fortification Solutions

Standard solutions (10, 1.0, 0.1 and 0.01 µg/mL) for fortification were prepared with volumetric dilution of the stock standard solution in acetonitrile according to the scheme in the table below. The use of vortexing was also employed to ensure a complete homogeneous solution.

Example Preparation of Fortification solutions

Take solution (µg/mL)	Volume (mL)	Dilute with acetonitrile to a final volume of (mL)	Concentration (µg/mL)
1000	0.5	50	10.0
10.0	5.0	50	1.0
1.0	5.0	50	0.10
0.10	5.0	50	0.01

Calibration Standard Solutions

Standard calibration solutions for LC-MS/MS analysis were prepared using the solution which was prepared in the previous section "Stock Solution," and by diluting it with acetonitrile as exemplified in the table above. Calibration standard solutions were prepared in a separate dilution series from fortification solutions. The use of vortexing was also used to ensure a complete homogeneous solution.

Example Preparation of Standard Solutions for Calibration

Take solution (ng/mL)	Volume (mL)	Dilute with HPLC water final volume of (mL)	Concentration (ng/mL)
100.0	0.5	50	1.0†
1.00	7.5	50	0.15
0.150	20	50	0.060
0.060	25	50	0.030
0.030	25	50	0.015
0.015	20	50	0.006

†This solution was not included in the standard curve, but was needed to prepare the subsequent calibration solutions.

The solution stability information can be found in the method validation study (Reference 1). Stock and fortification solution of BF 500-3 exhibited stability up to 42 days in acetonitrile. Additionally, the calibration solutions showed stability up to 31 days in water.

3. Analytical Procedure

For the analysis of BF 500-3, an aliquot of water sample was measured into a 10 mL volumetric flask and fortified with 0.030 mL of acetonitrile to determine the residues of BF 500-3 with LC-MS/MS.

3.1 Weighing and Fortification

For control samples, 10 mL of water was measured into a 10 mL volumetric flask. For fortified samples, 10 mL of the matrix was also measured into a 10 mL volumetric flask. Then, fortification solutions were added to the matrix as shown in the following table:

Sample Type	Sample Volume	Concentration of Spiking Solution	Volume of Spiking Solution	Level of Fortification
Control	10 mL	-	-	0.00
Fortification (LOQ*)	10 mL	0.01 µg/mL	0.030 mL	0.03 µg/L
Fortification (10× LOQ*)	10 mL	0.1 µg/mL	0.030 mL	0.3 µg/L

* Limit of quantification (LOQ)

3.2 Extraction of Sample Material

Acetonitrile (0.030 mL) was added to each control sample to ensure that all samples had the same solution proportions as the fortified samples. Samples were capped with glass stoppers and vortexed to thoroughly mix.

3.3 Preparation for Measurement

An aliquot (~ 1 mL) of the sample solution was pipetted (with a glass Pasteur pipette) into an autosampler vial for LC-MS/MS determination. High fortification samples were diluted with DI water as needed to fit into the calibration curve and then pipetted (with a glass Pasteur pipette) into an autosampler vial for LC-MS/MS determination.

3.4 Method Modifications

No method modifications were necessary for the implementation of method D1508/01 during ILV.

4. Instrumentation and Conditions

	Parameter		
Chromatographic System	Agilent 1290		
Analytical-column	XBridge C18 ; 2.5 µm, 2.1 x 50 mm		
Column Temperature	50 °C		
Injection Volume	10 µL		
Mobile Phase	A = 0.1% Formic Acid in Water B = 0.1% Formic Acid in Methanol		
Flow Rate	600 µL/minute		
Steps (including wash and equilibration)	Time (min)	Composition	
		%A	%B
	0.00	85	15
	0.25	85	15
	3.75	1	99
	4.45	1	99
	4.50	85	15
	6.00	85	15
Detection System	AB Sciex Instruments 6500 Q-Trap		
Ionization	Turbo Ion Spray		
Analyte	**Transitions (*m/z*) Positive mode**		**Expected Retention Time**
	Primary	**Secondary**	
BF 500-3	357.9→164.1	357.9→132.0	~3.3 min

4.1 Calibration Procedures

Calculation of results was based on peak area measurements using a linear calibration curve. The calibration curves for BF 500-3 were obtained by direct injection of the calibration standards containing known amounts of analyte in the range of 0.0060 ng/mL to 0.15 ng/mL. Standard curves (linear regression, weighted 1/x) were created using Analyst® 1.6. The regression functions were used to calculate the best-fit line by plotting the standard concentrations (ng/mL) on the x-axis versus the detector's peak response (peak area) on the y-axis. Typical calibration curves and representative chromatograms for calibration standards for BF 500-3 are presented in Appendices A and B.

4.2 Rounding Numbers

Numerical values in this report are frequently rounded to a smaller degree of precision (number of digits) than were used in the actual calculation to increase readability and to indicate the approximate precision of the reported results. Minor differences in the results obtained with such “rounded” values in comparison to those obtained with higher precision values are well within the limits of the experimental accuracy and therefore of no practical concern.

4.3 Statistical Analysis of Data

Mean recoveries were calculated on the data generated where appropriate. Full computer/calculator precision was used in any intermediate calculations, and only the final value was rounded. Slight differences may be noted in hand calculations versus calculations in the

individual data tables presented in this report due to rounding and significant figures presented in calibration curve data provided by the mass spectroscopy instrumentation. Simple descriptive statistics were performed on the data (average and/or standard deviation), as considered appropriate. Statistical treatment of the data included simple descriptive statistics, such as determinations of averages for the procedural recoveries, area counts, and calculation of the calibration curve correlation coefficient (r^2) by linear regression of the instrument responses for the reference standards.

4.4 Calculation of Residues and Recoveries

Peak integration and quantitation were performed within Analyst® 1.6 software using the calibration curve equation to determine sample concentrations of the analyte found during sample analysis. The data processing was completed in MultiQuant, which is a companion software program accessed via Analyst. Recovery results and additional sample concentrations were calculated for each set of samples within Microsoft Office Excel 2007 and reported in spreadsheet data reports, which are presented in Appendix D. Typical residue and recovery calculations are presented in Appendix H.

The calculations were performed in the following way:

Relative Error Accuracy (%) =

$$\frac{\text{(Calculated Standard Concentration (ng/mL) – Nominal Standard Concentration (ng/mL))}}{\text{Nominal Standard Concentration (ng/mL)}} * 100$$

µg/L Found =

$$\frac{\text{Amount Found (ng/mL) * Final Vol. (mL) * Dilution Factor}}{\text{Sample Volume (L) * 1000 ng/µg}}$$

Fortification Level (µg/L) =

$$\frac{\text{Volume Spiking Solution (mL) * Conc. of Spiking Solution (µg/mL)}}{\text{Sample Volume (L)}}$$

Corrected Recovery (%) =

$$\frac{\text{[µg/L Found in Fortified sample – Average µg/L Found in Control] * 100}}{\text{Nominal Fortification Level (µg/L)}}$$

5.2 Summary of Method

Type of Method	LC-MS/MS
Test Systems	Surface Water and Drinking Water
Selected mass transitions (m/z)	BF 500-3 *m/z* 357.9 → *m/z* 164.1* *m/z* 357.9 → *m/z* 132.0 *Primary quantification transition
Analytical Procedure	BASF Analytical Method D1508/01: "Analytical Method for the Determination of residues of Pyraclostrobin metabolite, BF 500-3 (Reg. No. 340266) in Surface and Drinking Water by LC-MS/MS" (Reference 1)
Confirmatory Technique	A secondary MRM transition (m/z 357.9 → *m/z* 132.0) was used for confirmation.
Method of Quantitation	The quantitation is based on the monitoring of two mass transitions for BF 500-3. Recovery data was reported for each mass transition considered, as shown in Appendices A-B.
LOD	0.006 µg/L
LOQ	0.03 µg/L (lowest fortification level)