

FINAL REPORT

Study Title

**Independent Laboratory Validation of BASF Analytical Method D0304:
“Method for the Determination of Residues of BAS 715 H and its Major Metabolite
CL 354825 in Ground Water Utilizing LC-MS/MS”**

Guidelines

U.S. EPA Ecological Effects Test Guideline, OCSPP 850.6100 Environmental Chemistry
Methods and Associated ILV

SANCO/825/00 rev. 8.1 (Nov 16, 2010)

Final Report Completion Date

20 December 2016

1. INTRODUCTION

1.1 Scope of the Method

BASF Analytical Method No. D0304 was developed to determine the residues of BAS 715 H and its metabolite CL 354825 in water matrices using LC-MS/MS at BASF Crop Protection in Research Triangle Park, North Carolina. This method was independently validated at ADPEN Laboratories, Inc.

The independent lab validation was conducted using two fortification levels at the limit of quantitation (0.05 ppb) and ten times of limit of quantitation (0.50 ppb) for all water matrices. 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 and thoroughly mixed. An aliquot of resulting solution was analyzed to determine the residues of BAS 715 H and its metabolite using LC-MS/MS. The transitions for BAS 715 H and its metabolite were monitored in positive mode for primary and secondary quantification. CL 354825 was also monitored in positive mode for tertiary quantification in surface water only.

1.3 Specificity

To demonstrate the specificity of the analytical method, one additional mass transition was monitored simultaneous to the primary quantitation transition for analysis of both analytes. Primary and secondary transitions for each analyte are listed below:

Analyte	Primary Transition Ion	Secondary Transition Ion
BAS 715 H	m/z 276.1 \rightarrow 231.0	m/z 276.1 \rightarrow 163.0
CL 354825	m/z 278.1 \rightarrow 233.0	m/z 278.1 \rightarrow 236.1 Tertiary: (m/z 278.1 \rightarrow 165.0)

The method was able to accurately determine residues of BAS 715 H and its metabolite. No interference was observed at the retention time of BAS 715 H, but was observed at the retention time for the confirmatory (secondary) transition of CL 354825, which yielded the further analysis of a tertiary transition in surface water only. No matrix effects were found to affect the analytes in drinking water; however, matrix effects were found to affect the analytes in surface water.

2. REFERENCE SUBSTANCE AND SAMPLING HISTORY

2.1 Test Systems

The test systems considered in this study were surface and drinking water.

The control samples were provided by BASF. The water samples were received on June 28, 2016. Upon arrival at the laboratory, the samples were opened, inspected, and checked against enclosed shipping forms. The test systems were received frozen with dry ice and

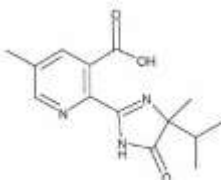
stored under frozen conditions at all times, unless necessary for laboratory analysis. The test systems were characterized at AGVISE Laboratories (604 Highway 15 West, Northwood, ND 58267). A copy of the characterization data for the samples is provided in Appendix E.

2.2 Test and Reference Substances

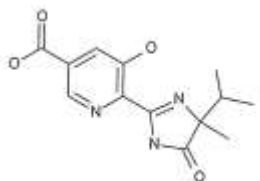
The standard substances were stored in a freezer ($\leq -5^{\circ}\text{C}$) until use. BASF has retained a reserve sample of this chemical, and has documentation specifying the location of the synthesis and characterization information available at BASF Crop Protection, Research Triangle Park, North Carolina.

The BAS 715 H and CL 354825 reference substances were provided by the sponsor and received on July 20, 2016. The certificate of analysis for all substances is presented in Appendix B. A detailed summary of the reference substances is presented below.

BASF Code Name: BAS 715 H
Common Name: Imazapic
Batch Number: AC10606-119
BASF Registry Number: 4095755
CAS Number: 104098-48-8
IUPAC Name: (RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methylnicotinic acid
Molecular Formula: $\text{C}_{14}\text{H}_{17}\text{N}_3\text{O}_3$
Molecular Weight: 275.3 g/mol
Purity: 99.7%
Expiration Date: June 1, 2021
Chemical Structure:



BASF Code Name: CL 354825
Batch Number: L67-144
BASF Registry Number: 4110603
IUPAC Name: 5-hydroxy-6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)nicotinic acid
Molecular Formula: $\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}_4$
Molecular Weight: 277.3 g/mol
Purity: 98.2%
Expiration Date: July 1, 2020
Structural Formula:



2.3 Test System

Surface and drinking water samples were provided and homogenized by BASF. The water samples were sent from BASF Crop Protection, Inc. on June 27, 2016 and received by ADPEN Laboratories, Inc. on June 28, 2016.

The Laboratory Information Management System (LIMS) provided a unique laboratory analysis code (e.g., 160629003-001) for each sample and is cross-referenced on the detailed reports to the assigned unique sample number.

3. ANALYTICAL METHOD

BASF Analytical Method D0304, "Method for the Determination of Residues of BAS 715 H and its Major Metabolite CL 354825 in Groundwater Utilizing LC-MS/MS" was used for the analysis of the samples.

The residues of BAS 715 H and its metabolite CL 354825 are extracted from 10-mL of water by adding 0.1 mL of fortification solution. An aliquot is taken from the resulting extract and directly used for LC-MS/MS determination. Instrument parameters are described in Table 11. The primary (quantitative), secondary (confirmatory) and tertiary (confirmatory) transition ions monitored are presented below:

Analyte	Transition (<i>m/z</i>)		Ionization Mode	Surface Water Retention Time (min)	Drinking Water Retention Time (min)
	Primary	Secondary			
BAS 715 H	<i>m/z</i> 276.1 → 231.0	<i>m/z</i> 276.1 → 163.0	Positive	5.1	4.1
CL 354825	<i>m/z</i> 278.1 → 233.0	<i>m/z</i> 278.1 → 236.1		5.5	4.6
		Tertiary: (<i>m/z</i> 278.1 → 165.0)		4.2	

STATISTICS AND DATA INTEGRITY

Statistical treatment of the data included simple descriptive statistics, such as determinations of averages, standard deviation and/or relative standard deviation (RSD) for the procedural recoveries and area counts and calculation of the calibration curve and correlation coefficient (r) by linear regression of the instrument responses for the reference standards. The statistical calculations throughout this report were performed using a Laboratory Information Management System (LIMS) and reported 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 ADPEN Laboratories, Inc. 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 a secured laboratory with limited access.

5. SUMMARY OF METHOD

Summaries of the method parameters and characteristics are provided in Table 11.

Summary of Method

Type of Method	LC-MS/MS
Test Systems	Surface and Drinking Water
Selected mass transitions (m/z)	BAS 715 H 276.1 → 231.0* 276.1 → 163.0
	CL 354825 278.1 → 233.0* 278.1 → 236.1 278.1 → 165.0 (Tertiary)
	*Primary quantification transition

Analytical Procedure	BASF Analytical Method D0304, "Method for the Determination of Residues of BAS 715 H and its Major Metabolite CL 354825 in Groundwater Utilizing LC-MS/MS" (Reference 1)
Confirmatory Technique	A secondary MRM transition for BAS 715 H and CL 354825 was used for confirmation. A tertiary MRM transition for CL 354825 was also used for confirmation in surface water only.
Method of Quantitation	The quantitation is based on the monitoring of two mass transitions for BAS 715 H, two mass transitions for CL 354825 in drinking water and three mass transitions for CL 354825 in surface water only. Recovery data was reported for each mass transition considered.
LOD	0.01 ppb
LOQ	0.05 ppb (lowest fortification level)
Levels of Fortification	0.05 ppb and 0.50 ppb
Time Required	A set of 13 samples requires approximately 13 hours of work.
Justification of Ions	The ions used to conduct the ILV were determined in this validation and are shown in Appendix F.

6. DISCUSSION

Linearity

Acceptable linearity was observed for the standard range and the two mass transitions tested: The method-detector response was linear over the 0.02–2 ng/mL range ($r = \geq 0.9991$), for all water matrix analyses.

Specificity

Method D0304 determines residues of BAS 715 H and CL 354825 in water matrices. No interfering peaks were found at the retention time for BAS 715 H; however, an interference peak that was only partially resolved was seen at the retention time for the confirmatory (secondary)

transition of CL 354825, which yielded the further analysis of a tertiary transition in surface water only.

Limit of Quantification and Limit of Detection

The LOQ was defined as the lowest fortification level tested. The LOQ for BAS 715 H and its metabolite in water matrices was 0.05 ppb. The LOD for all analytes in water matrices was set at 0.01 ppb, which was 20% of the defined LOQ. The LOD for each analyte in water was shown to be detectable as the absolute amount of analyte injected (0.00080 ng on column) into the LC-MS/MS when the lowest calibration standard was analyzed (0.02 ng/mL) with acceptable signal to noise ratio (S/N is >3:1).

Repeatability

The overall relative standard deviation (RSD, %) for all fortification levels were below 20%.

It was demonstrated that the method D0304 fulfills the requirements with regards to specificity, repeatability, limit of quantification, limit of detection, linearity and recoveries and is therefore applicable to correctly determine residues of BAS 715 H and CL 354825 in surface and drinking water matrices.

8. PROTOCOL, AMENDMENTS, AND DEVIATIONS

No protocol amendments were documented for the validation study.

Table 11 Instrument Conditions and Parameters for BAS 715 H and CL 354825 in Surface and Drinking Water

Chromatographic System	Agilent 1290 UPLC			
Analytical Column	TSK-gel Super ODS, 2 × 50 mm, 2 μm (S/N S0006-81PM)			
Column Temperature	30 °C			
Injection Volume	40 μL			
Mobile Phase A	1% Acetic Acid in Water			
Mobile Phase B	1% Acetic Acid in Methanol			
Flow Rate:	0.30 mL/min			
Gradient:	Time (min)	Flow (mL/min)	A (%)	B (%)
	0	0.30	99	1
	4	0.30	0	100
	5.5	0.30	0	100
	5.6	0.30	99	1
	8	0.30	99	1

Detection System	Agilent 6490 Triple Quad	
Interface:	ElectroSpray Ionization (ESI)	
Gas Flow:	14 L/min	
Temperature:	150 °C	
Capillary (V):	3000	
V Charging:	1600	
Nebulizer (psi):	45	
Sheath gas heater:	300	
Sheath gas flow:	12	
Polarity:	Positive	
MRM Conditions	BAS 715 H (Primary)	CL 354825 (Primary)
MS1:	276.14	278.12
MS2:	231.00	233.00
Dwell time:	50	20
Frag (V):	380	380
Collision Energy (V):	25	18
Cell Acc (V):	7	7
MRM Conditions	BAS 715 H (Secondary)	CL 354825 (Secondary)
MS1:	276.14	278.12
MS2:	163.00	236.10
Dwell time:	50	20
Frag (V):	380	380
Collision Energy (V):	18	18
Cell Acc (V):	7	7

Table 11 Instrument Conditions and Parameters for CL 354825 (Tertiary Transition) in Surface Water Only (Continued)

Chromatographic System	Agilent 1290 UPLC			
Analytical Column	TSK-gel Super ODS, 2 × 50 mm, 2 µm (S/N S0006-81PM)			
Column Temperature	30 °C			
Injection Volume	40 µL			
Mobile Phase A	1% Acetic Acid in Water			
Mobile Phase B	1% Acetic Acid in Methanol			
Flow Rate:	0.35 mL/min			
Gradient:	Time (min)	Flow (mL/min)	A (%)	B (%)
	0	0.35	99	1
	4	0.35	1	99
	5.5	0.35	1	99
	5.6	0.35	99	1
	8	0.35	99	1

Detection System	Agilent 6490 Triple Quad
Interface:	ElectroSpray Ionization (ESI)
Gas Flow:	14 L/min
Temperature:	150 °C
Capillary (V):	3000
V Charging:	1600
Nebulizer (psi):	45
Sheath gas heater:	300
Sheath gas flow:	12
Polarity:	Positive
MRM Conditions	CL 354825 (Tertiary)
MS1:	278.12
MS2:	165.00
Dwell time:	20
Frag (V):	380
Collision Energy (V):	25
Cell Acc (V):	7

Figure 21 Residue Calculations for Water Matrices

Peak integration and quantitation were performed within Agilent MassHunter software; using the calibration curve equation to determine the amount of analyte found (ng) during sample analysis. Recovery results and additional sample concentrations were calculated for each set of samples within the Laboratory Information Management System (LIMS) and reported in Microsoft® Excel spreadsheet data reports, which are presented in Appendix C.

For the validation recoveries, the exact sample weight was used in calculating the final residues (ppb).

The following equations are used for residue and recovery calculations for BAS 715 H and its metabolite in matrices of surface and drinking water.

a) Calibration curve: $y = mx + b$ Solving for x: $x = \frac{y-b}{m}$

Where, m = slope
 b = y-intercept
 x = Amount found (ng)
 y = Peak area

b) Amount of sample injected (mL) = $\frac{(\text{injection size (mL)} \times \text{sample vol. (mL)})}{\text{final sample volume (mL)}}$

c) Residue found (ppb) = $\frac{\text{Amount found (ng)}}{\text{Amount of sample injected (mL)}}$

d) Recovery (%) = $\frac{\text{Residue in sample (ppb)}}{\text{Amount fortified (ppb)}} \times 100$

As an example, calculations to obtain BAS 715 H (primary transition) recovery results using 16070803-Recovery1-1 from work order WO-16070803 (drinking water) are shown below:

a) Calibration curve: $y = (226875.696160)x - 10.513516$

Solving for x: $x = \frac{419 + 10.513516}{226875.696160} = 0.001893 \text{ ng}$

b) Amount of sample injected (mL) = $\frac{0.04 \text{ mL} \times 10.00 \text{ mL}}{10.0 \text{ mL}} = 0.0400 \text{ mL}$

c) Residue found (ppb) = $\frac{0.001893 \text{ ng}}{0.0400 \text{ mL}} = 0.0473 \text{ ppb}$

d) Recovery (%) = $\frac{0.0473 \text{ ppb}}{0.05 \text{ ppb}} \times 100 = 95\%$

Statistical treatment of the data included calculation of means, standard deviations (SD), and percent relative standard deviations (%RSD). These calculations were performed using Microsoft® Excel and LIMS software. Results were rounded only for reporting purposes. No calculations were made with rounded numbers.