MRID: 50105409

FINAL REPORT

Study Title

Independent Laboratory Validation of BASF Analytical Method L0209/01: "Method for the Determination of BAS 720 H and its 2 Metabolites Reg. No. 4110603 and Reg. No. 4110542 in Water"

Guidelines Covered

OCSPP 850.6100,
SANCO/825/00 rev 8.1 (Nov. 16, 2010),
ENV/JM/MONO(2007)17

1.0 INTRODUCTION

1.1 Purpose of the Study

The purpose of the study was to demonstrate that BASF Analytical Method L0209/01, "Independent Laboratory Validation of BASF Analytical Method L0209/01: "Method for the Determination of BAS 720 H and its 2 Metabolites Reg. No. 4110603 and Reg. No. 4110542 in Water" could be performed successfully at an outside facility with no prior experience with the method.

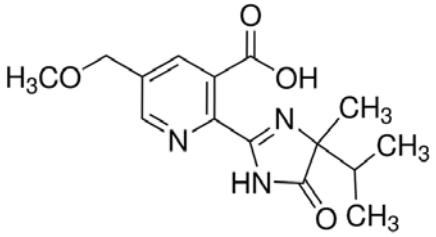
1.2 Summary of the Results

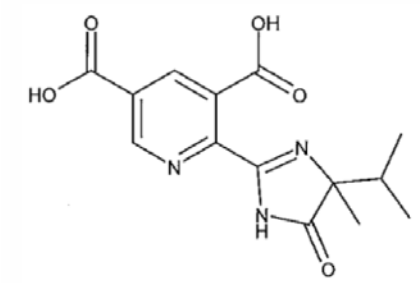
The independent laboratory validation of the BASF method was successfully completed in the first trial.

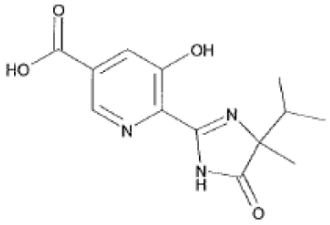
2.0 REFERENCE SUBSTANCE AND SAMPLING HISTORY

2.1 Reference Materials

The reference substances, imazamox, CL-No. 312622, and CL-No. 354825, was used for individual fortifications and LC-MS/MS calibration. Certificates of analysis are presented in Appendix A. Concentrated (stock), fortification, and calibration standards were prepared according to the analytical method. Example standard solution preparations are presented in Table 13.14. Standard solutions prepared for this study were stored in the refrigerator. A brief description of the reference standard used in this study is presented below.

BASF Code Name:	BAS 720 H
Common Name:	Imazamox
Batch No.:	AC12820-7
BASF Registry Number:	4096483
CAS Number:	114311-32-9
IUPAC Name:	(*RS*)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methoxymethylnicotinic acid
Molecular Formula:	$C_{15}H_{19}N_3O_4$
Molecular Weight:	305.3 g/mol
Purity:	99.5%
Expiration Date:	May 01, 2021
Chemical Structure:	

BASF Code Name:	CL-No. 312622
Batch No.:	L82-7
BASF Registry Number:	4110542
CAS Number:	146953-32-4
IUPAC Name:	2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-pyridine-3,5-dicarboxylic acid
Molecular Formula:	$C_{14}H_{15}N_3O_5$
Molecular Weight:	305.3 g/mol
Purity:	88.4%
Expiration Date:	November 01, 2013
Chemical Structure:	

BASF Code Name:	CL-No. 354825
Batch No.:	AC9918-101
BASF Registry Number:	4110603
CAS Number:	N/A
IUPAC Name:	5-hydroxy-6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid
Molecular Formula:	$C_{13}H_{15}N_3O_4$
Molecular Weight:	277.3 g/mol
Purity:	89.7%
Expiration Date:	August 01, 2015
Chemical Structure:	

2.2 Test System

Waters were sent from BASF Crop Protection, Inc. on October 8, 2013 and received by ADPEN Laboratories, Inc. on October 9, 2013. Upon receipt, the sample was stored in refrigerator E-57. The samples were weighed and extracted on October 10 and 11, 2013, respectively. The samples were transferred to freezer E-23, which had a temperature range of −17 to −9.5 °C during the course of the study. Water characterization reports are presented in Appendix B.

The Laboratory Information Management System (LIMS) provided a unique laboratory analysis code (e.g., 131009001-001) for the control samples and is cross-referenced on the detailed reports to the assigned unique sample number.

3.0 TECHNICAL PROCEDURE

BASF Analytical Method L0209/01, "Method for the Determination of BAS 720 H and its 2 Metabolites Reg. No. 4110603 and Reg. No. 4110542 in Water" for the analysis of BAS 720 H in ground and drinking water. A flow chart is presented in Table 13.1.

3.1 Standard Solutions

Stock Solutions

Prepare a 1.0 mg/mL stock solution individually by weighing an appropriate amount of each analyte into a flask and add the required volume.

For example, to prepare 10 mL of 1.0 mg/mL stock solution of imazamox in methanol, weigh 10 mg imazamox into a 10 mL volumetric flask. Dissolve and dilute to mark with methanol. Ensure a complete homogeneous solution (e.g., by sonication or vortexing). The stock solutions for all other analytes are made in a similar fashion.

A correction for purity is done if the purity is ≤ 95%. If the purity is > 95 % correction is optional.

Fortification Solutions

Prepare mixed standard solutions for fortification by combining stock solutions of each analyte (see above) in a flask. 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 methanol to a final volume of (mL)	Concentration (µg/mL)
100	1	10	10
10	1	10	1.0
1.0	2	10	0.2
0.2	1	10	0.02
0.02	1	10	0.002

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 as long as the proportions are not modified.

Calibration Standard Solutions

Prepare mixed standard calibration solutions for LC-MS/MS analysis by using the solutions that were prepared in Section "stock solutions" or "standard fortification solutions" in flasks. Dilute volumetrically with solvent S2 (0.1% formic acid in water/methanol (80:20, v/v) 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 (µg/mL)	Volume (mL)	Dilute with S2 to a final volume of (mL)	Concentration (ng/mL)
0.2	0.125	10	2.5
0.2	0.100	10	2.0
0.02	0.500	10	1.0
0.02	0.250	10	0.5
0.02	0.125	10	0.25
0.002	0.500	10	0.1
0.002	0.400	10	0.08
0.002	0.250	10	0.05
0.0001	0.100	1	0.01

* In case matrix-matched standards (instrument recovery samples) are needed for successful analysis, calibration standard solutions 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 as long as the proportions are not modified.

Use amber bottles with Teflon®-lined screw caps as storage containers for all standard solutions.

3.1.1 Stability of Standard Solutions

Results during method development demonstrated that fortification and calibration solution were stable (less than 10% decline) for four weeks when stored refrigerated.

3.2 Sample Preparation

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

3.3 Sample Storage

Until analysis, water samples are stored at approximately −20 °C.

3.4 Weighing and Fortification

For treated samples and control samples, weigh 10 g of sample into a 15-mL centrifuge tube.

For fortified samples, weigh at this stage 10 g of control sample into a centrifuge tube and add fortification solutions on the matrix.

The following scheme may be used:

Sample Type	Sample Weight	Concentration of Spiking Solution	Volume of Spiking Solution	Level of Fortification
Control	10 g	-	-	-
Fortification (LOQ)	10 g	2.012 ng/mL	0.125 mL	0.025 µg/L *
Fortification (10x LOQ)	10 g	20.12 ng/mL	0.125 mL	0.25 µg/L
Treated	10 g	-	-	-

* limit of quantification

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

3.5 Extraction of Sample Material

Add 10 g of the water to a 15-mL centrifuge tube followed by the addition of 0.5 mL of

2 N HCl. For fortification experiments specimens are fortified at this step.

3.6 Conditioning of C_{18} SPE Columns

Attach a Varian Bond Elut C_{18} column on to a vacuum manifold by washing the column subsequently with 3 mL of each of the following solutions: hexane, DCM, methanol, water, and 0.01 N HCl.

In a separate vacuum manifold, prepare the SCX SPE column by eluting 3 mL of methanol through the column. Fill the column with 2 mL of methanol and set aside.

3.7 C_{18} SPE Column Only

Load the whole acidified specimen extract from step 3.5 to the C_{18} column carefully. Wash the column with 3 mL of water followed by 3 mL of hexane. Stop the column when the liquid level passes just below the frit. Discard the wash.

Elute the above C_{18} column with 3 mL of DCM and collect into a 15-mL glass centrifuge vial. Evaporate the DCM eluent at 40 °C with a gentle nitrogen stream. Save the glass vial.

3.8 C_{18} Column Couples with SCX SPE Column

Attach the C_{18} column to the top of the SCX SPE column (still containing the 2 mL of methanol from step 3.6).

Add additional 3 mL of methanol to the top of C_{18} column and eluate. Detach and discard the C_{18} column from the SCX SPE column.

3.9 SCX SPE Column Only

Wash the SCX SPE column with 3 mL of methanol and discard the wash.

Elute the SCX SPE column with 6 mL of water/methanol (20:80, v/v) into the same glass vial used to collect the eluent from the C_{18} column. Swirl the solvent to combine and mix with a vortex.

3.10 Sample Preparation for Analysis

Evaporate the combined eluent from step 3.9 using a nitrogen evaporator with the water bath set to 70 °C until dryness.

Reconstitute once completely dry, all specimens in 0.1 % formic acid in water/methanol (80:20, v/v). Vortex to obtain a homogeneous solution. The samples are ready for analyses.

3.11 Instrumentation and Conditions

Agilent 1200 HPLC Conditions			
Column:	Zorbax Eclipse, 1.8 µm, 4.6 mm × 50 mm		
Temperature:	40 °C		
Flow (µL/min)	600		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.00	80.0	20.0
	0.50	80.0	20.0
	3.00	50.0	50.0
	7.00	5.0	95.0
	7.10	5.0	95.0
	10.00	5.0	95.0
	10.10	80.0	20.0
	15.00	80.0	20.0
Mobile Phase A:	0.1% formic acid in water		
Mobile Phase B:	0.1% formic acid in methanol		
Injection Vol.:	20 µL		

MS/MS Conditions	
Interface	AB SCIEX API 4000 TurboIonSpray®
Polarity	Positive
Curtain gas (CUR)	20.0
Temperature (TEM)	550 °C
Collision gas setting (CAD)	Medium
GS1	30.0
GS2	30.0
Entrance potential (EP)	10.0
Dwell Time (msec)	100.00
Scan type	MRM

Analyte	Q1 / Q3 (*m/z*)	Declustering potential (DP)	Collision energy (CE)	Collision cell exit potential (CXP)	Retention Time (min)
BAS 720 H (Primary)[1]	306.0 / 261.0	60.00	31.00	18.00	~4.8
BAS 720 H (Confirmation)	306.0 / 86.0	60.00	40.00	5.00	
CL-No. 312622 (Primary)	306.0 / 261.0	60.00	31.00	18.00	~4.3
CL-No. 312622 (Confirmation)	306.0 / 264.0	75.00	28.00	19.00	
CL-No. 354825 (Primary)	278.0 / 233.0	70.00	29.00	16.00	~6.7
CL-No. 354825 (Confirmation)	278.0 / 165.0	70.00	41.00	10.00	

[1] The instrument conditions for BAS 720 H (primary) and CL-No. 312622 (primary) are identical.

4.0 LIMITS OF QUANTITATION AND DETECTION

The LOQ and LOD for imazamox and its metabolites in water are 0.025 and 0.005 ppb, respectively.

5.0 CALIBRATION, CALCULATIONS, AND STATISTICS

Quantitation of residues in all samples was achieved using an external calibration curve calculated by linear regression of instrument responses for the reference substance at multiple concentrations.

Individual standard curve was prepared for using mixed-standards of imazamox and its metabolites by injecting standard solutions at appropriate concentrations. Calibration standard concentrations ranged from 0.01–2.5 ng/mL (0.0002–0.05 ng injected). A calibration standard was interspersed with sample injections. Analyst® 1.5.2 software created the standard curve based on linear regression using 1/x weighting. The regression functions were used to calculate the best-fit line by plotting the analyte found (ng) on the x-axis versus the detector's peak response (peak area) on the y-axis.

The following equations are used for residue and recovery calculations for water;

a) Calibration curve: $y = mx + b$ Solving for x: **Error! Bookmark not defined.**$x = \frac{y-b}{m}$

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

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

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

d) $\text{Recovery (\%)} = \frac{\text{Residue in sample (ppb)} - \text{Residue in control sample (ppb)}}{\text{ppb added}} \times 100$

As an example, calculations to obtain imazamox (primary mass transition, 306.0 → 261.0 *m/z*) recovery results using 13100904-Recovery1-5 from work order WO-13100904 are shown below:

a) Calibration curve: y = (4.64e+006)x + 342

successfully completed on the first trial for ground and drinking water and the Study Monitor was informed of the successful completion on October 22, 2013.

8.0 RECOMMENDATION

The following recommendation should be incorporated into BASF Method L0209/01: if matrix effects are observed dilute samples (1:5) to minimize matrix effects (BASF Method L0209/01; Section 3.9, Sample Preparation for Analysis).

9.0 PROTOCOL CHANGES

This study was conducted according to study protocol 715771 which was approved on October 8, 2013 and no protocol changes were documented for the study.

TABLE 13.1 Flow Diagram of the Analytical Procedure

10 g sample material are weighed in 15ml centrifuge tube

Add 0.5 mL 2 N HCl (aq.)

Clean up C_{18} Column

Condition the C_{18} column as follows:
Add 3 mL hexane
Add 3 mL dichloromethane
Add 3 mL methanol
Add 3 mL water
Add 3 mL 0.01 N HCl (aq.)

Load acidified extract to the column
Wash with 3 mL water followed by 3 mL hexane
Discard all washings
Elute with 3 mL dichloromethane in a 15-mL glass centrifuge tube
Evaporate to dryness at 40 °C

Clean up SCX SPE Column

Condition the SCX SPE column as follows:
Add 3 mL methanol
Fill column with 2 mL, stop flow

Attach C_{18} column to the filled SCX SPE column
Add 3 mL methanol
(elute metabolites on SCX SPE column with 6 mL methanol/water (80:20, v/v)
Wash with 3 mL methanol
Discard all washings
Combine with 6 mL water/methanol (20:80, v/v)
Add residue from C_{18} clean up, vortex
Evaporate to dryness with nitrogen evaporator (70 °C)
Reconstitute in 0.1% formic acid in water/methanol (80:20, v/v)
Vortex and sonicate

↓

LC-MS/MS Determination

TABLE 13.14 Example Standard Solutions Preparation and Dilution Data

Typical Analytical Standards Dilutions and Use Records for Imazamox

Conc. Standard	Analyte[1]	Analytical Standard	Amount Weighed (mg)[2]	Final Dil. Vol. (mL)	Final Conc. (ng/µL)	Prep. Date
C6877	Imazamox	P5012	9.95	10.0	995	10/09/2013
C6878	CL-No. 312622	P5013	9.99	10.0	999	
C6879	CL-No. 354825	P5014	10.01	10.0	1001	

Int. Standard	Analyte[1]	Parent Conc. Std. No.	Parent Conc. (ng/µL)	Aliquot Vol. (mL)	Dil. Vol. (mL)	Final Conc. (ng/µL)	Prep. Date
I7579	Imazamox, CL-No. 312622, CL-No. 354825	C6877, C6878, C6879	995	1.0	10.0	100	10/10/2013
			999				
			1001				

Working Standard	Analyte[1]	Parent Int. Std. No.	Parent Conc. (ng/µL)	Aliquot Vol. (mL)	Dilution Vol. (mL)	Final Conc. (ng/µL)	Prep. Date
W10204-1	Imazamox, CL-No. 312622, CL-No. 354825	I7579	100	1	10	10	10/10/2013
W10204-2		W10204-1	10	1	10	1.0	
W10204-3		W10204-2	1.0	1	10	0.2	
W10204-4		W10204-3	0.2	1	10	0.02	
W10204-5		W10204-4	0.02	1	10	0.002	

Working Standard	Analyte[3]	Parent Standard No.	Conc. (ng/µL)	Aliquot Vol. (mL)	Dilution Vol. (mL)	Final Conc. (ng/mL)	Prep. Date
W10205-1	Imazamox, CL-No. 312622, CL-No. 354825	W10204-3	0.2	0.125	10	2.5	10/10/2013
W10205-2		W10204-3	0.2	0.100	10	2.0	
W10205-3		W10204-4	0.02	0.500	10	1.0	
W10205-4		W10204-4	0.02	0.250	10	0.5	
W10205-5		W10204-4	0.02	0.125	10	0.25	
W10205-6		W10204-5	0.002	0.500	10	0.1	
W10205-7		W10204-5	0.002	0.400	10	0.08	
W10205-8		W10204-5	0.002	0.250	10	0.05	
W10205-9		W10205-6	0.1	0.100	1.0	0.01	

[1] Diluted in methanol.
[2] Corrected for purity (%).
[3] Diluted in 0.1% formic acid in water/methanol (80:20, v/v).