Independent Laboratory Validation of Analytical Method Number L0136/01:
LC-MS/MS determination of BAS 351 H (Bentazon) and its metabolite BH 351-N-Me (Reg. No. 79520) in soil and sediment

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ABSTRACT

The purpose of this study is to perform an Independent Laboratory Validation (ILV) of BASF Analytical Method Number L0136/01: "LC-MS/MS determination of BAS 351 H (Bentazon) and its metabolite BH 351-N-Me (Reg. No. 79520) in soil and sediment" and to demonstrate that the Method L0136/01 can be performed with acceptable recoveries at an outside facility with no prior experience with the method. The method was successfully validated at ABC Laboratories, Inc., on the first trial. Homogenized control soil was used for the ILV.

A 5 g soil sample is extracted with 50 mL methanol-water (50:50, v/v) by mechanical shaking for 60 min on high speed. A 5 mL aliquot of the extract is centrifuged for 5 min at 4000 rpm (20°C). The extract is taken directly or diluted with methanol-water (50:50, v/v) to the appropriate final volume and measured by HPLC-MS/MS. LC-MS/MS determination was conducted using Analyst 1.5 for primary and confirmatory quantitation. Ion transitions at *m/z* 239.0 → 132.0 and 239.0 → 197.0 for BAS 351 H (Bentazon), and *m/z* 255.0 → 134.0 and 255.0 → 213.0 for BH 351-N-Me were monitored for residue determination. The limit of quantitation (LOQ) of this method is 0.01 mg/kg. The limit of detection is set at 0.002 mg/kg, which is at 20% of LOQ.

For validation, untreated matrix samples were fortified with BAS 351 H (Bentazon) and BH 351-N-Me and analyzed according to the established method validation guidelines. The analytical sets each consisted of a reagent blank, two controls, five replicates fortified at the limit of quantitation (LOQ, 0.01 ppm) and five to ten replicates fortified at a higher level, corresponding to 10X the LOQ (0.1 ppm).

I. INTRODUCTION

A. Purpose of the Study

The purpose of this study is to perform an Independent Laboratory Validation (ILV) of BASF Analytical Method L0136/01: "LC-MS/MS determination of BAS 351 H (Bentazon) and its metabolite BH 351-N-Me (Reg. No. 79520) in soil and to demonstrate that the Method L0136/01 can be validated with acceptable average recoveries (70-120%) at an outside facility without prior experience with the method.

B. Summary of the Results

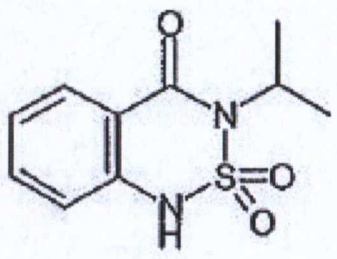
The independent laboratory validation of the BASF method L0136/01 was successfully validated in the first trial. Technical communication between the performing laboratory and the study monitor was not needed for successful completion of the method validation at the first trial.

II. SAMPLE HISTORY and REFERENCE SUBSTANCE

Homogenized control blank matrix was provided by BASF. The soil characterization classified the soil as clay loam and the characterization data is provided in APPENDIX 5. The Sponsor identified this was a soil that was harder to work with. The sample was received on September 13, 2012. Upon receipt of this sample, ABC Laboratories, Inc. did the inventory and stored the sample in the freezer at -20 ^{0}C. In addition, the aliquots taken for control and fortification purposes were also documented accordingly. The test system was received frozen and were stored under frozen conditions at all times.

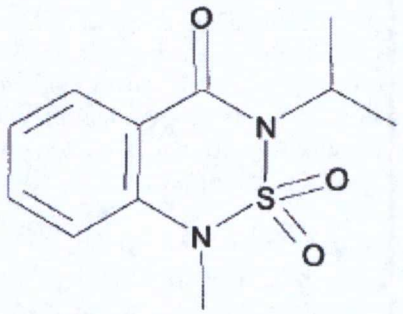
The BAS 351 H (Bentazon) and BH 351-N-Me reference substances were provided by the Sponsor. BAS 351 H (Bentazon, lot number: 01196-1) and BH 351-N-Me (lot number: L75-7) were received on September 13, 2012 and September 27, 2012, respectively, with a stated purity of 99.6% for both. The reference substances were stored at room temperature when not in use. The certificates of analysis are presented in Figure 1.

Compound: BAS 351 H (Bentazon)
Chemical Structure:



Purity: 99.6%
Batch Number: 01196-1
Date Received: September 13, 2012
Expiration Date: November 1, 2014
Storage Conditions: Room Temperature

Compound: BH 351-N-Me (Reg. No. 79520)
Chemical Structure:



Purity: 99.6%
Batch Number: L75-7
Date Received: September 27, 2012
Expiration Date: September 1, 2014
Storage Conditions: Room Temperature

III. PROCEDURE - METHOD SYNOPSIS

BASF Analytical Method L0136/01 (dated February, 2009) is used as an ILV method to determine residues of BAS 351 H (Bentazon) and BH 351-N-Me in soil as investigated for this validation study (Reference 1). The detailed analytical method (Technical Procedure) is described in Appendix 1. The method was revised by the sponsor subsequent to the performance of the ILV (Reference 2).

The following is a brief summary of the validation procedures:

A 5 g soil sample is extracted with 50 mL methanol-water (50:50, v/v) by mechanical shaking for 60 min on high speed. A 5 mL aliquot of the extract is centrifuged for 5 min at 4000 rpm (20°C). The extract is taken directly or diluted with methanol-water (50:50, v/v) and measured by HPLC-MS/MS. For BAS 351 H (Bentazon), samples are diluted by either 20-fold (controls and LOQ fortifications) or 200-fold (10 times LOQ fortifications) prior to injection. For BH 351-N-Me, no additional dilution is performed on the control or LOQ fortification and a 10-fold dilution is performed on the 10 times LOQ fortification.

The final determination of BAS 351 H (Bentazon) was performed by LC-MS/MS in negative ion mode and BH 351-N-Me was performed by LC-MS/MS in positive ion mode. For each analyte, one MRM parent-daughter ion (*m/z* 239.0 → 132.0 and *m/z* 255.0 → 134.0 for parent and the metabolite respectively) was monitored for primary quantitation. A secondary MRM transitions

(*m/z* 239.0 → 197.0 and *m/z* 255.0 → 213.0 for parent and the metabolite, respectively) was used for confirmation purposes. The ion transitions chosen for this ILV were those identified in Method L0136/01. Although both HPLC and UPLC methods were validated in the method validation study, only the HPLC method was chosen to conduct the validation in the ILV study. A UPLC was not so commonly available for the enforcement lab; it was intended to be used for data collection purposes. Therefore, the ILV was conducted only with HPLC-MS/MS methodology to be used for enforcement purposes.

Because of hardware limitation, different brands of HPLC columns and a different mass spectrometer platform were used in the ILV validation. In the BASF Analytical Method L0136/01, a CTC PAL autosampler, a Thermo Betasil C18 100mm x 2.1 mm, 5µm column and an API Sciex 3000 were utilized. The Mass spectrometer system was replaced with a Sciex API 4000. The differences are discussed in detail in Section VI - Result and Discussion.

In the ILV study, validation was accomplished by analyzing each of the two analytes in a validation set consisting of 2 blank control specimens, 5 replicate specimens fortified at LOQ, and 5 to 10 replicate specimens fortified at 10xLOQ. The limit of quantitation (LOQ) is defined as the lowest fortification tested which is 0.01 ppm (mg/kg) and the limit of detection (LOD) is set at 10% of the LOQ which is 0.001 ppm.

A single analyst completed a sample set consisting of 13 samples (1 reagent blank, 2 matrix control samples, and 10 fortified samples) in approximately 8 hours plus additional HPLC-MS/MS determination time.

IV. LIMIT OF QUANTITATION AND DETECTION

The limit of quantitation (LOQ) for residues of BAS 351 H (Bentazon) and BH 351-N-Me in soil is defined as the lowest fortification tested which is 0.01 ppm (mg/kg) for each analyte. The LOD is defined as the absolute amount (0.0003 and 0.006 ng for BAS 351 H and BH 351-N-Me, respectively) of analyte injected into the LC-MS/MS parameters using lowest calibration standard. This is equivalent to 20% of the LOQ for both analytes, equivalent to 0.002 ppm (mg/kg). This percentage is the ratio of the concentration of the low standard to the final analyte concentration of the fortified control sample at LOQ.

V. CALIBRATION, CALCULATIONS AND STATISTICS

Residues of BAS 351 H (Bentazon) and BH 351-N-Me were quantitated by external standards. A calibration curve for each analyte was generated by plotting the detector's response in peak area versus the concentration (ng/mL) of standard injected. The data system derived an equation for the fit of the standard curve and this equation was used to calculate intercept and slope of the linear regression curve. Good linearity ($r > 0.990$) was observed in the range of 0.010 ng/mL to 0.25 ng/mL and 0.20 ng/mL to 5.0 ng/mL, respectively, for the mass transitions of BAS 351 H (Bentazon) and BH 351-N-Me in mixed standard solutions.

The calibration curve was obtained by direct injection of 30 µL of the mixed BAS 351 H (Bentazon) and BH 351-N-Me standards into LC-MS/MS in the range of 0.010 ng/mL to 0.25 ng/mL and 0.20 ng/mL to 5.0 ng/mL, respectively. In a given injection run, the same injection volume was used for all samples and standards. Example calibration curves and chromatograms of standards are shown in Figure 3 and Figure 4, respectively.

Peak integration and quantitation were performed using Applied Biosystem Analyst software version 1.5. Ppm calculations and recovery results were computed for each set of samples by Microsoft Excel® and reported in a spreadsheet data report. Equations used for quantitation are presented in Figure 2.

VI. RESULTS AND DISCUSSION

The objective of this study was to validate the BASF analytical method L0136/01 for the determination of BAS 351 H (Bentazon) and its metabolite BH 351-N-Me in soil at a limit of quantitation (LOQ) of 0.01 mg/kg (per analyte), using LC-MS/MS for quantitation and confirmation. The analytes were extracted by methanol-water (50:50, v/v) by mechanical shaking. 5 mL of the extract was aliquotted and centrifuged. The final determination of BAS 351 H (Bentazon) was performed by LC-MS/MS using negative mode. The metabolite, BH 351-N-Me, was performed by LC-MS/MS using positive mode.

The validation set contained one solvent blank, two untreated control matrix samples, 5 samples fortified at the LOQ, 5 to 10 samples fortified at 10xLOQ, and one set of standards. Each standard set contained five concentration levels. The initial evaluation of the instrument recovery sample shows that there was no need for matrix matched standards. Samples were both bracketed by injection standards and had standards dispersed in the analytical run.

A. Equipment/Reagents

Balances:	Mettler Model XP205DR and Mettler Model BB2440 (Mettler Toledo)
Centrifuge:	Sorvall RC-5B (Thermo Fisher Scientific)
Pipettes:	Various sizes, Gilson Brand
Formic Acid:	Fisher Scientific
Methanol:	Fisher Scientific
Water:	Fisher Scientific

B. Solutions/Standards

Solution and standard preparation was performed as described in the method.

C. Sample Analysis

LC-MS/MS monitored one parent-daughter ion (MRM) for each analyte (*m/z* 239.0 → 132.0 and *m/z* 255.0 → 134.0 for parent and the metabolite, respectively) for quantitation and one parent-daughter ion (MRM) for each analyte (*m/z* 239.0 → 197.0 and *m/z* 255.0 → 213.0 for parent and the metabolite respectively) for quantitative confirmation. The summary of the average recovery results is provided in Table 1. The average recovery, standard deviation, and RSD from both primary and confirmatory quantitation met acceptance criteria of between 70-120%. The detailed analytical data sheets can be found Appendix 3. Example chromatograms of reagent blank, control, and fortified samples are shown in Figure 5.

BASF Analytical Method L0136/01 was submitted to ABC Laboratories, Inc. for an independent laboratory method validation using soil as the experimental matrix. Homogenized control soil samples were used in this ILV study. Technical communication between the performing laboratory and the study monitor was not needed for successful completion of the method validation on the first trial. See Appendix 4 for communications during the study.

The first trial was extracted and injected on October 24, 2012. Acceptable average recoveries between 70-120% were obtained for the metabolite BH-351-N-Me for both transitions. BAS 351 H (Bentazon) did not have acceptable average recoveries at either the LOQ or 10x LOQ for the October 24, 2012 injection. The first trial was injected again on October 25, 2012 to verify the recoveries. Acceptable average recoveries between 70-120% were obtained for BAS 351 H (Bentazon) for both transitions at the LOQ for the second injection. A second extraction and injection on November 01, 2012 of the first trial was performed at 10X LOQ for BAS 351 H (Bentazon). The second extraction was performed because the recovery results of the first extraction at 10 x LOQ were unacceptable. The second extraction confirmed the results of the first extraction (second injection) although the average of the recoveries at 10 x LOQ was then acceptable. Acceptable average recoveries between 70-120% were obtained from the first extraction/second injection and the second extraction at 10X LOQ for BAS 351 H (Bentazon) for both transitions. A summary of the individual recoveries for BAS 351 H (Bentazon) and its metabolite, BH 351-N-Me, obtained in the ILV trial are shown in Table 1, and detailed residue results are shown in Table 2. The analytical method was run exactly as written except noted as follows:

A Sciex API 4000 was used instead of a Sciex API 5000 or Sciex API 3000.

LC-MS/MS parameters for primary and confirmatory chromatographic methods can be found in Appendix 2.

The method is well written, easy to follow, and contains one deficiency. No stability data is given for the preparation of stock standards in methanol. Adherence to the method instructions and notes is critical in achieving acceptable recoveries of BAS 351 H (Bentazon) and its metabolite, BH 351-N-Me. The addition of stability information for the stock standard in

methanol is recommended to be included in this method. No other recommendations or suggestions are needed for this method.

In summary, the ILV was completed successfully at the first trial; therefore, BASF Analytical Method L0136/01 is suitable for determining the residues of BAS 351 H (Bentazon) and its metabolite, BH 351-N-Me, in soil down to a level of 0.01 ppm.

VII. COMMUNICATION / CONTACT

The independent laboratory method validation of BASF Method L0136/01 was successfully completed with communication for clarifications of protocol preparation, guideline requirements, analytical series requirements, preparation of stock solutions, selecting HPLC-MS/MS over UPLC-MS/MS, and instrument optimization with the study monitor. The study monitor was informed of the successful completion of the study after the first trial on November 02, 2012

VIII. PROTOCOL CHANGES

One protocol change was needed for the validation.

The protocol change described the deviation to the specified LOD in protocol Section 3, Analytical Method. The LOD was set to be 0.002 ppm or 20% of the LOQ as defined in the method and reflected by the experimental determination.

Primary Quantitation

Instrument:	MDS Sciex API 4000		
Inlet [HPLC System]:	Agilent 1100 with a HTC PAL		
Software Version:	Analyst 1.5		
Column:	Thermo Betasil C18 100mm x 2.1 mm, 5µm		
Injection:	30 µL		
Mobile Phase:	A: 0.1% formic acid (aq) B: 0.1% formic acid in methanol Needle Rinse: 1:1 Methanol:Water 0.1% formic acid		
Gradient	Total Time (min)	Mobile Phase	
		A%	B%
	0.00	50	50
	2.50	35	65
	4.00	35	65
	4.10	0	100
	6.00	0	100
	6.10	50	50
	9.00	50	50
Flow Rate:	500 µL/minute		
Analytes	Expected Retention Times (minutes)	Transitions (m/z) :	
		Quantitation ion	Secondary ion*
BAS 351 H (Bentazon)	2.5	239.0 → 132.0	239.0 → 197.0
BH 351-N-Me	3.4	255.0 → 134.0	255.0 → 213.0
Ionization Mode:	Negative ion (BAS 351 H), Positive ion (BH 351-N-Me); Turbospray (500°C)		

*The quantitation ion listed was validated. The secondary ion data was monitored but not used because of poor sensitivity at the low end of the curve.

(**NOTE**: Suggested HPLC-MS/MS operating conditions can be modified, if necessary)