

I. INTRODUCTION

A. Purpose of the Study

The purpose of this study is to perform an Independent Laboratory Validation of Analytical Method Number L0141/01: "LC-MS/MS determination of BAS 351 H (Bentazon) in surface water and groundwater" and to demonstrate that the Method L0141/01 can be validated with acceptable recoveries (70-120%) at an outside facility without prior experience with the method.

B. Summary of the Results

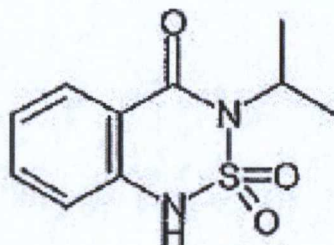
The independent laboratory validation of the BASF method L0141/01 was successfully validated in the first trial. Technical communication between the performing laboratory and the study monitor was not needed for successful completion of the method validation at the first trial.

II. SAMPLE HISTORY and REFERENCE SUBSTANCE

Homogenized control blank matrix (surface water) was provided by BASF. The sample was received on September 13, 2012. Upon receipt of this sample, ABC Laboratories did the inventory and stored the sample in the freezer at -20 °C. In addition, the aliquots taken for control and fortification purposes were also documented accordingly. The test system was received frozen and was stored under frozen conditions at all times when not in use. The characterization data for the control surface water is provided in [APPENDIX 5](#).

The BAS 351 H (Bentazon) reference substance was provided by the Sponsor. BAS 351 H (Bentazon lot number: 01196-1) was received on September 13, 2012 with a stated purity of 99.6%. The reference substance was stored at room temperature when not in use. The certificate of analysis is 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

III. PROCEDURE - METHOD SYNOPSIS

BASF Analytical Method L0141/01, version 01 (Dated July 14, 2009) is used as an ILV method to determine residues of BAS 351 H (Bentazon) in water as investigated for this validation study. 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 10 g aliquot of the water sample is adjusted to pH 2 and extracted by ENV solid phase extraction (SPE) cartridge using methanol. The methanol extracts are evaporated to dryness and the residues are re-dissolved in water-methanol (50:50, v:v). An aliquot of the final volume is measured for LC-MS/MS.

The final determination of BAS 351 H (Bentazon) was performed by LC-MS/MS in negative ion mode. One MRM parent-daughter ion (m/z 239.0 \rightarrow 132.0 and m/z 239.0 \rightarrow 197.0 (for confirmatory purposes) was monitored for quantitation. The ion transitions chosen for this ILV were those identified in Method L0141/01 (Reference [1](#)).

Due to hardware limitation, N-evaporation at ~ 40 °C was used instead of heated SPE column dryer as mentioned in the original method. In the BASF Analytical Method L0141/01, the HPLC system was coupled with a Sciex API 3000 mass spectrometer. The differences are discussed in detail in Section VI - Result and Discussion.

Method validation was accomplished by analyzing the analyte in a validation set consisting of 2 blank control specimens, 5 replicate specimens fortified at LOQ, and 5 replicate specimens fortified at 10x LOQ. The validated method achieved a limit of quantitation (LOQ) of 0.03 ppb ($\mu\text{g}/\text{kg}$) and a limit of detection (LOD) of 0.005 ppb ($\mu\text{g}/\text{kg}$).

A single analyst completed a sample set consisting of 13 samples (1 reagent blank, 2 matrix control samples, and 10 fortified samples) in approximately 14 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) in water is defined as the lowest fortification tested which is 0.03 ppb ($\mu\text{g}/\text{kg}$). The LOD is defined as the absolute amount (0.00075ng) of analyte injected into the LC-MS/MS parameters using the lowest calibration standard. This is equivalent to 17% of the LOQ, equivalent to 0.03 ppb ($\mu\text{g}/\text{kg}$). 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) were quantitated by external standards. A calibration curve for each analyte was generated by plotting the detector's response in peak area versus the amount (ng) 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 0.025 ng/mL to 0.5 ng/mL for the mass transition of BAS 351 H in mixed standard solutions.

The calibration curve was obtained by direct injection of 5 μ L of BAS 351 H (Bentazon) standards into LC-MS/MS in the range of 0.025 ng/mL to 0.5 ng/mL. 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. Ppb 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 L0141/01 for the determination of BAS 351 H (Bentazon), in water at a limit of quantitation (LOQ) of 0.03 ppb (μ g/kg), using LC-MS/MS for quantitation and confirmation. The analyte was extracted from the sample by decreasing the pH (pH2) and then cleaned-up via a SPE procedure. The final determination of BAS 351 H (Bentazon) was performed by LC-MS/MS using negative mode.

The validation set contained one solvent blank, two untreated control matrix samples, 5 samples fortified at the LOQ, 5 samples fortified at 10xLOQ, and one set of standards. Each standard set contained five concentrations. Samples were both bracketed by injection standards and had standards dispersed in the analytical run.

LC-MS/MS monitored one parent-daughter ion (MRM) at (m/z 239.0 \rightarrow 132.0 and m/z 239.0 \rightarrow 197.0 for BAS 351 H (Bentazon) for quantitation and quantitative confirmation. The summary of the average recovery results is provided in [Table 1](#). The recovery, standard deviation, and RSD from both primary and confirmatory quantitations 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 L0141/01 was submitted to ABC Laboratories, Inc. for an independent laboratory method validation using water as the experimental matrix. Homogenized control surface water was 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 completed on October 18, 2012. Acceptable recoveries between 70-120% were obtained for BAS 351 H (Bentazon). A summary of the individual recoveries for BAS 351 H (Bentazon), 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 3000.

Due to hardware limitation, N-evaporation at ~40 °C was used instead of heated SPE column dryer as mentioned in the original method. In the BASF Analytical Method L0141/01, the HPLC system was coupled with a Sciex API 3000 mass spectrometer.

LC-MS/MS parameters for primary and confirmatory chromatographic methods can be found in [Appendix 2](#).

Adherence to the method instructions and notes is critical in achieving acceptable recoveries of BAS 351 H (Bentazon). No recommendations or suggestions are needed for this method.

In summary, the ILV was completed successfully at the first trial; therefore, BASF Analytical Method L0141/01 is suitable for determining the residues of BAS 351 H (Bentazon), in water down to a level of 0.03 ppb ($\mu\text{g}/\text{kg}$).

VII. COMMUNICATION / CONTACT

The independent laboratory method validation of BASF Method L0141/01 was successfully completed with communication for clarifications of protocol and report preparation, minor method modifications, fortification scheme, receipt of test substance and control matrix, and instrument optimization, including after trial initiation communications concerning recovery results with the study monitor. The study monitor was informed of the successful completion of the study after the first trial on October 22, 2012.

VIII. PROTOCOL CHANGES

Two protocol changes were needed for the validation. The first protocol change contained one substitution and one correction. A 10 minute drying time via vacuum was substituted for the heated SPE column dryer apparatus. The last paragraph in Section 5 Experimental Design was corrected to remove language that conflicted with the method in regards to the preparation of the quality control sample (QCS).

The second protocol change described the deviation to the specified LOD in Section 3, Analytical Method. The LOD was set to be 0.005 ppb ($\mu\text{g}/\text{kg}$) or 17%

of the LOQ to reflect the experimental determination of the LOD as defined in the method.

IX. ARCHIVING

The study protocol, raw data and original final report will be stored in the archives belonging to the test facility (BASF Agricultural Research Center, 26 Davis Drive, Research Triangle Park, North Carolina) at least for the period of time specified in the GLP regulations. Electronic copies of the report and raw data will be maintained at ABC Laboratories, Inc. archives.

Raw data on the calibration or inspection of technical equipment used for the conduct of field part of the study at GLP compliant facilities ("facility-maintained" raw data) will be archived at the facilities where they have been generated. In no case will raw data be discarded without the consent of the sponsor.

X. REFERENCES

1. Penning, H.; BASF Analytical Method L0141/01: "LC-MS/MS determination of BAS 351 H (Bentazon) in surface water and groundwater", BASF Registration Document Number 2009/1076476.
2. Report Amendment zu 334546 "Validation of Analytical Method L0141/01 for the LC-MS/MS determination of BAS 351 H (Bentazon) in surface water and groundwater", BASF Registration Document Number 2014/1083366.