

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

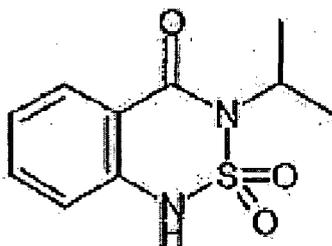
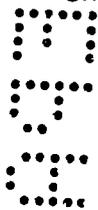
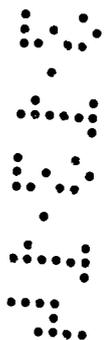
The independent laboratory validation of the BASF method L0141/01 was successfully validated in the first trial. Technical communication between the performing laboratory and the study monitor was not needed for successful completion of the method validation at the first trial.

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 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 $\mu\text{g/L}$ and a limit of detection (LOD) of 0.006 $\mu\text{g/L}$.

IV. LIMIT OF QUANTITATION AND DETECTION

The limit of quantitation (LOQ) for residues of BAS 351 H (Bentazon) in water is 0.03 ppb. The limit of detection (LOD) is 20% of the LOQ, equivalent to 0.006 ppb.

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.

The calibration curve was obtained by direct injection of 5 μL 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](#).

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 $\mu\text{g/L}$, 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.

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 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.

VII. COMMUNICATION / CONTACT

The independent laboratory method validation of BASF Method L0141/01 was successfully completed without technical communication 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

One protocol change was needed for the validation. The 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).

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.

FIGURE 2 Typical Calculations for the Quantitation of Residues in Water

For the calculation of residues the following formula was used:

$$R \text{ [ppb]} = (C_{\text{End}} \times V_{\text{End}} \times \text{DF}) / G$$

Where:

R: Analyte residue in ppb ($\mu\text{g}/\text{kg}$).

C_{End} : Final concentration of analyte in extract in ng/mL calculated by Analyst v1.5.
(where multiple injections were evaluated: mean).

V_{End} : Volume of final extract used for LC/MS/MS: 2 mL.

G: Water mass: 10 g.

DF: Dilution Factor of sample (e.g. DF=1, if no dilution is prepared).

Recoveries (Rec.) were calculated for the fortified specimens as follows:

$$\text{Rec.} = (R / R \text{ fortified}) \times 100 \%$$

Example: 10 x LOQ, 69118-MV10 (DF=5)

$$\begin{aligned} R \text{ [ppb]} &= C_{\text{End}} \times V_{\text{End}} \times \text{DF} / G \\ &= (0.29299 \text{ ng/mL} \times 2 \text{ mL} \times 5) / 10 \text{ g} \\ &= 0.2930 \text{ ppb} \end{aligned}$$

R: Analyte residue in ppb ($\mu\text{g}/\text{kg}$).

C_{End} : 0.2930 ng/mL

V_{End} : 2 mL

G: 10 g

Recoveries (Rec.) were calculated for the fortified specimens as follows:

$$\text{Rec.} = (0.2930 \text{ ppb} / 0.3 \text{ ppb}) \times 100 \% = 98\%$$