Test Material: Bentazon

MRID: 49338403

Title: Validation of Analytical Method L0141/01 for the LC-MS/MS Determination of BAS 351 H (Bentazon) in Surface Water and Groundwater (Including Amendment No. 1)

MRID: 49338404

Title: AMENDED FINAL REPORT - Independent Laboratory Validation of Analytical Method Number L0141/01: LC-MS/MS determination of BAS 351 H (Bentazon) in surface water and groundwater

EPA PC Code: 275200

OCSPP Guideline: 850.6100

**For CDM Smith**

**Primary Reviewer:** Lynne Binari

**Signature:** Lynne Binari

**Date:** 8/26/14

**Secondary Reviewer:** Lisa Muto

**Signature:** Lisa Muto

**Date:** 8/26/14

**QC/QA Manager:** Joan Gaidos

**Signature:**

**Date:** 8/26/14

## Analytical method for bentazon (BAS 351 H) in water

**Reports:** ECM: EPA MRID No.: 49338403. Penning, H. and M. Obermann. 2014. Validation of Analytical Method L0141/01 for the LC-MS/MS Determination of BAS 351 H (Bentazon) in Surface Water and Groundwater (Including Amendment No. 1). BASF Study No.: 334546 and Registration Document No.: 2014/7000540. Report prepared by BASF SE, Limburgerhof, Germany, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 55 pages. Final report issued July 14, 2009. Final report with Amendment No. 1 issued February 20, 2014.
ILV: EPA MRID No. 49338404. Grant, J. 2014. AMENDED FINAL REPORT - Independent Laboratory Validation of Analytical Method Number L0141/01: LC-MS/MS determination of BAS 351 H (Bentazon) in surface water and groundwater. ABC Laboratories Study No.: 69118. BASF Study No.: 433095 and Registration Document No.: 2014/7000479. Report prepared by ABC Laboratories, Inc., Columbia, Missouri, sponsored and submitted by BASF Crop Protection, Research Triangle Park, North Carolina; 140 pages, plus pages 58a, 59a, and 60a. Final report issued November 16, 21012. Amended Final Report issued March 5, 2014.

**Document No.:** MRIDs 49338403 & 49338404

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in accordance with OECD Principles of Good Laboratory Practice (GLP) and the GLP Principles of the German "Chemikaliengesetz" (Chemicals Act, p. 3 of MRID 49338403). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity Certification statements were provided (pp. 2-5).
ILV: The study was conducted in accordance with USEPA GLP standards (p. 3 of MRID 49338404). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity Certification statements were provided (pp. 2-5).

**Classification:** This analytical method is classified as **SUPPLEMENTAL**. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. LODs differed in the ECM and ILV. The ECM method should be amended to include an alternative drying procedure for the SPE column. The water used in the ILV was not characterized. The study can be used for analysing the parent bentazon, but can not used for monitoring the degradates in water.

**PC Code:** 275200

**Reviewer:** He Zhong, Ph.D.
Biologist, EPA/OPP/EFED

**Signature:**

Digitally signed by HE ZHONG
DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=HE ZHONG, dnQualifier=0000051219
Date: 2015.01.29 13:33:11 -05'00'

**Date:** 1-29-2015

**Page citations refer to the page numbers located in the upper right corner of MRID 49338404 (ILV, since this report contains the ECM), unless noted otherwise.**

## Executive Summary

This analytical method, BASF Method L0141/01, is designed for the quantitative determination of bentazon in water using HPLC/MS/MS. The method is quantitative for bentazon at the stated LOQ of 0.03 µg/L. The LOQ is less than the lowest toxicological level of concern (LOC) in water for Molluscs, Pacific oyster (*Crassostrea gigas*), (NOAEC = 7.04 ppb, ECOTOX Ref# 111918) and aquatic algae, Marine diatom (*Chaetoceros gracilis*), (EC50 = 60 ppb, ECOTOX Ref# 114118). The independent laboratory validated the method after one trial. LODs were set at 0.005 µg/L in the ECM and 0.006 µg/L in the ILV. No major modifications were made by the independent laboratory; however, the ECM method should be amended to include an alternative drying procedure for the SPE column. The water used in the ILV was not characterized.

**Table 1. Analytical Method Summary**

| Analyte(s) by Pesticide | MRID | | Matrix | Method Date (dd/mm/yyyy) | Registrant | Analysis | Limit of Quantitation (LOQ) |
|---|---|---|---|---|---|---|---|
| | Environmental Chemistry Method | Independent Laboratory Validation | | | | | |
| Bentazon | 49338403 | 49338404 | Water | 14/07/2009<br>Amended Final Report<br>20/02/2014 | BASF | HPLC/MS/MS | 0.03 µg/L |

## I. Principle of the Method

Samples (10 g) of water were acidified to pH 2 with 30 µL of 6M HCl, then loaded onto a Bond Elut-ENV (200 mg, 3 mL) solid phase extraction (SPE) column preconditioned with methanol and pH 2.0 water (Appendix 1, pp. 73-75, 79). The loaded column was air-dried under vacuum (400 mbar) for 5 minutes, then under nitrogen flow at 40°C for 30-45 minutes using a SPE column dryer. Bentazon was eluted with methanol (3 x 3 mL). The eluate was taken to dryness (TurboVap LV, 40°C), with the resulting residues reconstituted in methanol:water (50:50, v:v) for LC/MS/MS analysis.

Samples were analyzed for bentazon (BAS 351 H) by HPLC (Betasil C18, 2.1 mm x 100 mm, 5 µm column) using a mobile phase of (A) water:formic acid (1000:1, v:v) and (B) methanol:formic acid (1000:1, v:v) [percent A:B at 0.0 min. 50:50, 2.5-4.0 min. 35:65, 4.1-6.0 min. 0:100, 6.1-9.0 min. 50:50] with MS/MS-ESI (electrospray ionization, negative ion mode) detection and multiple reaction monitoring (MRM; Appendix 1, p. 76). Injection volume was 50 µL. Bentazon was identified and quantified using two ion transitions. Ion transitions monitored were as follows: *m/z* 239→132 and *m/z* 239→197.

The ILV was performed by drying the loaded SPE column using either N-evaporation at *ca*. 40°C or air-drying under vacuum for 10 minutes, rather than by SPE column dryer, due to hardware limitation; there was conflicting information as to which procedure was used (pp. 14-15; Appendix

4, p. 126). Additionally, LC injection volume was 5 µL, and a MDS Sciex API 4000 LC/MS/MS was used, rather than a Sciex API 3000 (pp. 14-15; Appendix 2, p. 84). The modifications are not considered substantial changes to the ECM.

The LOQ for bentazon was the same in the ECM and ILV at 0.03 µg/kg (ppb; p. 13; Appendix 1, p. 55). The LOD was reported as 0.005 µg/kg (17% of LOQ) in the ECM and 0.006 µg/kg (20% of LOQ) in the ILV.

## II. Recovery Findings

ECM (MRID 49338403): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of bentazon in groundwater and surface water at fortification levels of 0.03 µg/kg (LOQ) and 0.3 µg/kg (10x LOQ), with the exception of one 0.03 µg/kg fortified groundwater sample that was "unintentionally" double fortified and excluded from the statistical calculations (Appendix 1, pp. 51-54 of MRID 49338404). Bentazon was identified and quantified using two ion transitions. The two water matrices were fully characterized (Appendix 1, pp. 57, 58a, 59a, 60a).

ILV (MRID 49338404): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of bentazon in surface water at fortification levels of 0.03 µg/kg (LOQ) and 0.3 µg/kg (10x LOQ; Table 1, p. 17). Bentazon was identified and quantified using two ion transitions. The method was validated at both fortification levels after one trial, with minor method and instrument parameter modifications (pp. 14-15; Appendix 1, p. 76; Appendix 2, p. 84; Appendix 4, p. 126). The water matrix was not characterized, but was reported as supplied by BASF (p. 12).

**Table 2. Initial Validation Method Recoveries for Bentazon in Water[1]**

| Analyte | Fortification Level (µg/kg) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---|---|---|---|---|---|---|
| | **Groundwater (Well) Water** | | | | | |
| Bentazon (BAS 351 H) | *m/z* 239→132 | | | | | |
| | 0.03 (LOQ) | 4[2] | 89.3-104.0 | 94.0 | 6.8 | 7.3 |
| | 0.3 | 5 | 80.0-84.0 | 82.1 | 1.6 | 1.9 |
| | *m/z* 239→197 | | | | | |
| | 0.03 (LOQ) | 4[2] | 90.7-104.7 | 94.8 | 6.6 | 7.0 |
| | 0.3 | 5 | 81.3-85.3 | 82.8 | 1.5 | 1.8 |
| | **Surface (Pond) Water** | | | | | |
| Bentazon | *m/z* 239→132 | | | | | |
| | 0.03 (LOQ) | 5 | 70.7-82.0 | 77.5 | 5.1 | 6.6 |
| | 0.3 | 5 | 83.3-86.7 | 85.7 | 1.5 | 1.7 |
| | *m/z* 239→197 | | | | | |
| | 0.03 (LOQ) | 5 | 70.0-80.7 | 76.1 | 5.0 | 6.6 |
| | 0.3 | 5 | 82.7-87.3 | 85.3 | 1.7 | 2.0 |

Data (uncorrected recovery results) were obtained from Appendix 1, 51-54.

1 Source and characterization of the two water matrices were provided (Appendix 1, pp. 57, 58a, 59a, and 60a).

2 Five replicate fortifications were performed; however, one replicate (218.7% *m/z* 239→132, 218.0% *m/z* 239→197) was "unintentionally spiked twice... and not included in statistical calculations" (Appendix 1, pp. 51-52).

## Table 3. Independent Validation Method Recoveries for Bentazon in Surface Water[1]

| Analyte | Fortification Level (µg/kg) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---|---|---|---|---|---|---|
| Bentazon<br>(BAS 351 H) | *m/z* 239→132 (primary quantitation) | | | | | |
| | 0.03 (LOQ) | 5 | 74-102 | 90 | 11.1 | 12.2 |
| | 0.3 | 5 | 77-107 | 94 | 12.6 | 13.4 |
| | *m/z* 239→197 (secondary quantitation) | | | | | |
| | 0.03 (LOQ) | 5 | 72-95 | 84 | 10.2 | 12.1 |
| | 0.3 | 5 | 70-97 | 88 | 11.1 | 12.6 |

Data were obtained from Table 1, p. 17.

1 The surface water was not characterized, but was reported as supplied by BASF (p. 12).

## III. Method Characteristics

In the ECM and ILV, the LOQ for bentazon in water was 0.03 µg/kg (p. 13; Appendix 1, p. 55). The LOD was reported as 0.005 µg/kg (17% of LOQ) in the ECM and 0.006 µg/kg (20% of LOQ) in the ILV. The ECM defined the LOQ as the lowest fortification level successfully tested (Appendix 1, pp. 42, 55, 80). The ECM defined the LOD as the absolute amount of analyte injected into the LC/MS/MS instrument using the lowest standard of the calibration curve; 1.25 pg for bentazon, equivalent to 0.005 µg/kg (Appendix 1, pp. 55, 80).

## Table 4. Method Characteristics

| | Bentazon |
|---|---|
| Limit of Quantitation (LOQ) | 0.03 µg/kg |
| Limit of Detection (LOD) | 0.006 µg/kg[1] |
| Linearity (calibration curve $r^2$ and concentration range)[2] | $r^2$ = **0.9913**, **0.9920**<br>(0.01-0.25 ng/mL) |
| Repeatable | Yes |
| Reproducible | Yes |
| Specific[3] | Yes |

Data were obtained from p. 14; Figure 3, pp. 26-29; and DER Attachment 2.

1 LOD was set at 0.005 µg/kg in ECM, based on the lowest calibration standard (Appendix 1, pp. 55, 80).

2 Reviewer-calculated $r^2$ values for the provided ILV calibration curves (DER Attachment 2). The ILV reported r values of 0.9956 and 0.9960 (Figure 3, pp. 22-23). Linearity of ECM calibration curves were not verified by the reviewer as individual calibration curve data were not provided. Reported r values were 0.9996 and 0.9997 for 0.025-1 ng/mL bentazon (Appendix 5.3, p. 30; Amendment 1, p. 53 of MRID 49338403), with resultant reviewer-calculated $r^2$ values of 0.9992 and 0.9994, respectively.

3 For the ILV, there were no significant interferences (≤11% of LOQ) in the reagent blanks and matrix control samples at the retention time of the analyte (Table 2, p. 18; Figure 5, pp. 30-31; Appendix 3, pp. 86-87). In the ECM, there were no significant interferences (<30% of LOQ) in the matrix control samples; results from reagent blanks were not reported (Appendix 1, pp. 50, 62-65, 80).

## IV. Method Deficiencies and Reviewer's Comments

1. The ECM was revised by BASF subsequent to performance of the ILV (pp. 13, 16 of MRID 49338404). The ECM amendment included addition of information regarding the use of mass per mass unit in the report Principle of the Method section, LOD in the report Discussion section, calibration standard range for the calibration curves and a product ion scan (Amendment No. 1, pp. 50-55 of MRID 49338403).

2. The determination of the LOQ and LOD were not based on scientifically acceptable procedures. In the ECM and ILV, the LOQ for bentazon in water was 0.03 µg/kg (p. 13; Appendix 1, p. 55). The LOD was reported as 0.005 µg/kg (17% of LOQ) in the ECM and 0.006 µg/kg (20% of LOQ) in the ILV. The ECM defined the LOQ as the lowest fortification level successfully tested (Appendix 1, pp. 42, 55, 80). The ECM defined the LOD as the absolute amount of analyte injected into the LC/MS/MS instrument using the lowest standard of the calibration curve; 1.25 pg for bentazon, equivalent to 0.005 µg/kg (Appendix 1, pp. 55, 80). The ILV provided no justification for setting the LOD at 0.006 µg/kg.

   Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in soil was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

3. The independent laboratory did not have the SPE column dryer ($N_2$) and modified the method to dry the loaded SPE column using either N-evaporation at *ca*. 40°C or air-drying under vacuum for 10 minutes; however, there was conflicting information as to which procedure was used (pp. 14-15; Appendix 4, p. 126). The drying procedure used should be clarified by the independent laboratory. The modification is not a substantial change to the ECM; however, the ECM method should be amended to include an alternative drying procedure for the SPE column.

4. The water used in the ILV was not characterized. The water matrix was reported as supplied by BASF (p. 12).

5. For the ECM, standard curves were provided, but chromatograms were only provided of one calibration standard concentration (0.15 ng/mL bentazon; Appendix 1, pp. 61, 66). The reviewer could not verify calibration curve linearity because individual calibration data were not reported. Chromatograms of 10x LOQ fortified samples were not provided. Chromatograms of reagent blank samples were not provided for the ECM or ILV.

6. For the ILV, linearity ($r^2$) of the calibration standards was not ≥0.995 (see Table 4 above). BASF considered $r^2$ ≥0.980 acceptable (Appendix 4, p. 114).

7. Communications between the ILV study director and BASF study monitor were extensively documented (p. 9; Appendix 4, pp. 88-140). The ILV study director/author reported that the ILV of "BASF Method L0141/01 was successfully completed without technical communication with the study monitor." (p. 15). Pre-trial communications concerned protocol and report preparation, minor method modifications, fortification scheme, receipt of

test substance and control matrix, and instrument optimization (Appendix 4, pp. 89-127). After trial initiation, communications concerned recovery results (Appendix 4, pp. 128-140).

8. The ILV did not report the time required to complete a sample set (typically thirteen samples consisting of one reagent blank, two matrix control samples, and ten fortified samples). It was reported in the ECM that analysis of a sample set consisting of twenty-two samples (seventeen unknown samples, two fortified samples for recovery experiments, one reagent blank, and two matrix control samples) required 1.5 working days (12 hours) per analyst; this time included calculation of results, preparation of equipment, and reporting of all raw data under GLP (Appendix 1, p. 55).

## V. References

U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

## Attachment 1: Chemical Names and Structures

### Bentazon (BAS 351 H; p. 11 of MRID 49338403)

| | |
|---|---|
| **IUPAC Name:** | 3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide |
| **CAS Name:** | |
| **CAS Number:** | 25057-89-0 |
| **SMILES String:** | |