

Test Material: Sodium Bentazon

MRID: 49693501

Title: Validation of Analytical Method L0136/01 for the LC-MS/MS Determination of BAS 351 H (Bentazon) and its Metabolite BH 351-N-Me (Reg. No. 79520) in Soil and Sediment (including Amendment No. 1 and Amendment No. 2)

MRID: 49693502

Title: 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

EPA PC Code: 103901

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto

Signature:



Date: 11/4/15

Secondary Reviewer: Kathleen Ferguson

Signature:



Date: 11/4/15

QC/QA Manager: Joan Gaidos

Signature:



Date: 11/4/15

Analytical method for bentazon (BAS 351 H) and its metabolite, BH 351-N-Me, in soil and sediment

Reports: ECM: EPA MRID No. 49693501. Penning, H., M. Obermann. 2015. Validation of Analytical Method L0136/01 for the LC-MS/MS Determination of BAS 351 H (Bentazon) and its Metabolite BH 351-N-Me (Reg. No. 79520) in Soil and Sediment (including Amendment No. 1 and Amendment No. 2). BASF Study ID No.: 334544. BASF Registration Document No.: 2015/7001891. Report prepared; sponsored and submitted by BASF SE, BASF Agricultural Center Limburgerhof, Limburgerhof, Germany; 137 pages (Original report, 99 pages; Amendment 1, 7 pages; Amendment 2, 31 pages). Final original report issued October 27, 2009; Amendment No. 1 completion date, February 20, 2014; Amendment No. 2 completion date, July 13, 2015.

ILV: EPA MRID No. 49693502. Rodgers, C. 2015. 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. BASF Study No.: 433094. BASF Registration Document No.: 2015/7001576. Report prepared by ABC Laboratories, Inc., Columbia, Missouri, sponsored and submitted by BASF Crop Protection, Research Triangle Park, North Carolina; 234 pages. Final original report issued November 16, 2012; amendment (first) completion date, March 5, 2014; amendment (second) completion date, June 16, 2015; amendment (third) completion date, July 22, 2015.

Document No.: MRIDs 49693501 (ECM) & 49693502 (ILV)

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with OECD and German GLP, and with USEPA GLP which corresponds with those other GLP standards (p. 3 of MRID 49693501). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-5). Signatures pages for the Amendments were also included (pp. 106, 137).

ILV: The study was conducted in accordance with USEPA GLP (p. 3 of MRID 49693502). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-5). A signatures page for the Amended Final Report (July 22, 2015) was also included (p. 6).

Classification: This analytical method is classified as **ACCEPTABLE**. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. ILV linearity coefficients were <0.995 for three of the four calibration curves of BAS 351 H. Provided ECM chromatograms were not comprehensive for the test fortifications. The UPLC method was not validated by the ILV.

PC Code: 103901

Reviewer: He Zhong, Ph.D.
Biologist

Signature:
Date: 2-1-2016

Referenced page numbers for ECM MRID 49693501 refer to those listed in the bottom right-hand corner of the document.

Executive Summary

This analytical method, BASF Analytical Method L0136/01, is designed for the quantitative determination of bentazon (BAS 351 H) and its metabolite BH 351-N-Me in soil and sediment at the LOQ of 0.01 mg/kg (**10 ppb**). The LOQ is less than the lowest toxicological level of concern in soil/sediment **12.3 ppb** for both analytes. The method was validated by the ECM using loamy sand soil (4.8% clay; 1.89% organic carbon), sandy loam soil (13.0% clay; 1.31% organic carbon) and loamy sand sediment (5.5% clay; 2.42% organic carbon). The method was validated by the ILV with the first trial using clay loam soil (36% clay; 0.6% organic carbon); however, the acceptable results for BAS 351 H were achieved after re-injection for the LOQ level and after two re-injections for the 10×LOQ level. Only the HPLC/MS/MS method was validated by the ILV due to the fact that the UPLC/MS/MS method was not intended for enforcement purposes. ILV calibration data was insufficient to support the method. ECM chromatograms did not include all fortification levels.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Bentazon (BAS 351 H)	49693501	49693502		Soil ^{1,2}	27/10/2009 (Original)	BASF Corporation	HPLC/MS/MS or UPLC/MS/MS	0.01 mg/kg
BH 351-N- Me					20/02/2014 (Amendment 1)			

1 The ECM validated the method using loamy sand soil (4.8% clay; 1.89% organic carbon), sandy loam soil (13.0% clay; 1.31% organic carbon) and loamy sand sediment (5.5% clay; 2.42% organic carbon; USDA classification; p. 15; Appendices 5.1-5.3, pp. 50-52 of MRID 49693501).

2 In the ILV, clay loam soil (36% clay; 0.6% organic carbon) was used (USDA classification; Appendix 5, p. 232 of MRID 49693502).

I. Principle of the Method

Soil samples (5 g) were measured into a 150-mL glass vial with screw cap and fortified, as necessary, then extracted once with 50 mL of S1 (methanol:water; 50:50, v:v) via shaking for 60 minutes via a mechanical shaker at 225 rpm (p. 15; Appendix 5.5, pp. 92-94, 98 of MRID 49693501). A 5-mL aliquot was centrifuged (4000 rpm) for 5 minutes at 20°C. For determinations of BAS 351 H, the supernatant was diluted 20-fold with S1 (1000 mL final volume). For determinations of BH 351-N-Me, the supernatant was not diluted (50 mL final volume). The method advised to dilute higher concentrations further with S1, as necessary. An aliquot of the final extract was measured for analysis.

UPLC/MS/MS:

Samples were analyzed using an ACQUITY Binary Solvent Manager LC System coupled to an AB Sciex API 5000 Triple Stage Quadrupole Mass Spectrometer with Electrospray (ESI; Appendix 5.5, p. 94; Appendix 5.5, Amendment 2, p. 108 of MRID 49693501). The instrumental conditions consisted of an Acquity BEH C18 column (2.1 x 50 mm, 1.7- μ m; column temperature RT), a mobile phase gradient of (A) water:formic acid (1000:1, v:v) and (B) methanol:formic acid (1000:1, v:v) [percent A:B (v:v) at 0.0-0.2 min. 60:40, 0.9 min. 45:55, 0.91-1.3 min. 0:100, 1.31-1.7 min. 60:40] and MS/MS detection (MRM) in negative ion mode for BAS 351 H and positive ion mode for BH 351-N-Me. Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored: m/z 239 \rightarrow 132 (Q) and m/z 239 \rightarrow 197 (C) for bentazon (BAS 351 H) and m/z 255 \rightarrow 134 (Q) and m/z 255 \rightarrow 213 (C) for BH 351-N-Me. Approximate retention times were *ca.* 0.49 min. and *ca.* 0.85 min. for bentazon (BAS 351 H) and BH 351-N-Me, respectively. Injection volume was 5.0 μ L.

HPLC/MS/MS:

Samples were analyzed using an Agilent 1100 LC Binary Pump LC System coupled to an AB Sciex API 3000 Triple Stage Quadrupole Mass Spectrometer with Electrospray (ESI; Appendix 5.5, p. 95 of MRID 49693501). The instrumental conditions consisted of a Betasil C18 column (2.1 x 100 mm, 5- μ m; column temperature RT), a mobile phase gradient of (A) water:formic acid (1000:1, v:v) and (B) methanol:formic acid (1000:1, v:v) [percent A:B (v:v) at 0.0min. 50:50, 2.5-4.0 min. 35:65, 4.1-6.0 min. 0:100, 6.1-9.0 min. 50:50] and MS/MS detection (MRM) in negative ion mode for BAS 351 H and positive ion mode for BH 351-N-Me. Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored: m/z 239 \rightarrow 132 (Q) and m/z 239 \rightarrow 197 (C) for bentazon (BAS 351 H) and m/z 255 \rightarrow 134 (Q) and m/z 255 \rightarrow 213 (C) for BH 351-N-Me. Approximate retention times were *ca.* 2.7 min. and *ca.* 3.7 min. for bentazon (BAS 351 H) and BH 351-N-Me, respectively. Injection volume was 50 μ L.

Amendments to the original report did not include any changes to the extraction or analytical procedure; however, the correction of the UPLC/MS/MS mobile phase gradient was provided (Appendix 5.5, p. 94; Appendix 5.5, Amendment 2, p. 108 of MRID 49693501).

ILV

In the ILV, the extraction procedure for both analytes was the same as the ECM, except that additional dilution of the final extracts prior to analysis was described (this additional dilution was allowed in the method; pp. 13, 15-16; Appendix 1, Appendix 5.5, pp.136-139; Appendix 2, p. 145 of MRID 49693502). The extracts were analyzed using HPLC/MS/MS only due to the fact that

UPLC/MS/MS equipment was not commonly available for the enforcement laboratory and was “intended to be used for data collection purposes” (p. 14). HPLC/MS/MS analysis was performed using an Agilent 1100 with a HTC PAL LC system coupled to a MDS Sciex API 4000 Mass Spectrometer (p. 16; Appendix 2, p. 145). The instrumental conditions were the same as those of the ECM, except that the column temperature was not reported and the injection volume was reduced to 30 μ L. The same two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored for each analyte. Approximate retention times were *ca.* 2.5 min. and *ca.* 3.4 min. for bentazon (BAS 351 H) and BH 351-N-Me, respectively.

LOQ/LOD

For bentazon (BAS 351 H) and BH 351-N-Me, the LOQ was reported as 0.01 mg/kg in both the ECM and ILV (p. 15; Table 5, pp. 22-45; Appendix 5.5, p. 98; Appendix 5.5, Amendment 1, p. 101 of MRID 49693501; pp. 7, 14; Appendix 3, p. 146 of MRID 49693502). The LOD was reported as 0.001 mg/kg (10% of the LOQ) and 0.002 mg/kg (20% of the LOQ), respectively, in the ECM and ILV.

II. Recovery Findings

ECM (MRID 49693501): All mean recoveries and relative standard deviations (RSD) were within guidelines (mean 70-120%; RSD \leq 20%) in loamy sand and sandy loam soils and loam sand sediment for bentazon (BAS 351 H) and BH 351-N-Me at the LOQ (0.01 mg/kg; 0.01 ppm) and 10 \times LOQ (0.1 mg/kg; 0.1 ppm; pp. 6-7; Tables 5-28, pp. 22-45). Mean recoveries and RSDs of the HPLC/MS/MS and UPLC/MS/MS methods were comparable, as were results of the quantitation and confirmation ions for each method. Calculations allowed for recoveries to be corrected for residues found in the controls; however, none were quantified (Tables 5-28, pp. 22-45; Appendix 5.5, p. 97). The soil/sediment matrices were fully characterized by BioChem (USDA soil characterization; p. 15; Appendices 5.1-5.3, pp. 50-52). Loamy sand soil (LUF 2.2; 85.6% sand, 9.6% silt, 4.8% clay; 1.89% organic carbon), sandy loam soil (LUF 5M; 56.6% sand, 30.4% silt, 13.0% clay; 1.31% organic carbon) and loamy sand sediment (RAGRA; Ranschgraben; 86.2% sand, 8.3% silt, 5.5% clay; 2.42% organic carbon) were used.

ILV (MRID 49693502): Mean recoveries and RSDs were within guidelines in clay loam soil for bentazon (BAS 351 H) and BH 351-N-Me at the LOQ (0.01 mg/kg; 0.01 ppm) and 10 \times LOQ (0.1 mg/kg; 0.1 ppm; Tables 1-2, pp. 18-22). Mean recoveries and RSDs of the quantitation and confirmation ions were comparable. The number of samples was five for all analyses, except those for BAS 351 H at 10 \times LOQ where n = 10. The results of the first and second re-injections were combined for the 10 \times LOQ samples of BAS 351 H. Only the HPLC/MS/MS method was validated due to the fact that the UPLC/MS/MS method was not intended for enforcement purposes (p. 14). Recoveries were not corrected for residues found in the controls (Figure 2, p. 25; Appendix 3, pp. 151-156). The method was validated with the first trial for both analytes in the test soil; however, the acceptable results for BAS 351 H were achieved after re-injection for the LOQ level and after two re-injections for the 10 \times LOQ level (pp. 7, 16). Due to initial unacceptable results for BAS 351 H at the LOQ and 10 \times LOQ (results not provided in the study report), the extracts were re-injected (p. 16; Table 1, p. 18; Appendix 3, pp. 153-156). The re-injection of the extracts yielded acceptable results at the LOQ; however, due to the unacceptable 10 \times LOQ results from this re-injection, the

first trial samples were extracted again and re-injected. This was considered the second injection of the first trial in the study report. The combined results of the first and second re-injections were acceptable. The soil matrix was fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; Appendix 5, p. 232). Clay loam soil (33% sand, 31% silt, 36% clay; 0.6% organic carbon) was used.

Table 2. Initial Validation Method Recoveries for Bentazon (BAS 351 H) and BH 351-N-Me in Two Soils and One Sediment^{1,2}

Analyte	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
LUFA 2.2 Soil						
Quantitative ion						
HPLC Mode						
Bentazon (BAS 351 H)	0.01 (LOQ)	5	100.4-101.8	101.2	0.6	0.6
	0.1	5	98.6-100.6	99.9	0.8	0.8
	1.0	5	99.6-100.8	100.2	0.5	0.5
BH 351-N-Me	0.01 (LOQ)	5	94.0-97.8	96.2	1.4	1.5
	0.1	5	95.0-98.5	96.2	1.5	1.5
	1.0	5	95.9-99.2	97.6	1.4	1.4
UPLC Mode						
Bentazon (BAS 351 H)	0.01 (LOQ)	5	89.0-94.8	92.8	2.5	2.7
	0.1	5	91.4-100.4	96.4	3.4	3.6
	1.0	5	94.8-102.4	98.2	3.4	3.5
BH 351-N-Me	0.01 (LOQ)	5	96.9-104.0	99.2	2.8	2.8
	0.1	5	94.0-102.0	97.2	3.9	4.0
	1.0	5	94.8-101.0	97.4	2.7	2.7
Confirmatory ion						
HPLC Mode						
Bentazon (BAS 351 H)	0.01 (LOQ)	5	99.2-102.4	101.0	1.3	1.2
	0.1	5	98.2-101.6	100.2	1.3	1.3
	1.0	5	99.2-102.2	100.7	1.3	1.3
BH 351-N-Me	0.01 (LOQ)	5	94.5-98.1	96.3	1.3	1.3
	0.1	5	97.0-98.8	98.2	0.8	0.8
	1.0	5	97.6-99.7	98.8	0.8	0.8
UPLC Mode						
Bentazon (BAS 351 H)	0.01 (LOQ)	5	90.4-100.4	94.7	3.7	3.9
	0.1	5	92.8-98.2	96.6	2.2	2.3
	1.0	5	93.6-103.6	98.5	4.1	4.2
BH 351-N-Me	0.01 (LOQ)	5	95.0-99.8	97.5	1.7	1.8
	0.1	5	94.2-101.0	98.3	2.8	2.9

	1.0	5	95.7-98.7	97.2	1.3	1.3
LUFA 5M Soil						
Quantitative ion						
HPLC Mode						
Bentazon (BAS 351 H)	0.01 (LOQ)	5	97.4-103.6	101.9	2.5	2.5
	0.1	5	100.0-101.4	100.6	0.5	0.5
	1.0	5	97.0-102.2	99.1	2.4	2.4
BH 351-N-Me	0.01 (LOQ)	5	93.4-95.9	94.7	1.0	1.0
	0.1	5	97.6-99.9	98.8	0.9	0.9
	1.0	5	98.2-101.0	99.2	1.1	1.1
UPLC Mode						
Bentazon (BAS 351 H)	0.01 (LOQ)	5	86.6-97.4	91.4	4.3	4.7
	0.1	5	95.0-99.2	96.6	1.8	1.8
	1.0	5	91.6-97.6	95.4	2.4	2.5
BH 351-N-Me	0.01 (LOQ)	5	96.2-101.0	97.6	2.0	2.0
	0.1	5	98.4-104.0	100.6	2.4	2.4
	1.0	5	95.2-100.0	98.2	2.0	2.0
Confirmatory ion						
HPLC Mode						
Bentazon (BAS 351 H)	0.01 (LOQ)	5	100.2-103.2	101.8	1.4	1.3
	0.1	5	99.6-103.6	101.1	1.7	1.6
	1.0	5	97.8-104.2	100.4	2.4	2.3
BH 351-N-Me	0.01 (LOQ)	5	93.2-94.5	93.8	0.5	0.5
	0.1	5	96.7-99.5	98.3	1.1	1.1
	1.0	5	97.6-99.3	98.4	0.7	0.7
UPLC Mode						
Bentazon (BAS 351 H)	0.01 (LOQ)	5	88.0-96.8	92.4	4.1	4.4
	0.1	5	94.6-99.6	97.1	1.8	1.8
	1.0	5	90.6-100.2	95.0	3.5	3.7
BH 351-N-Me	0.01 (LOQ)	5	93.7-97.6	96.4	1.6	1.6
	0.1	5	95.5-103.0	100.1	3.1	3.1
	1.0	5	94.5-102.0	97.9	3.0	3.0
Ranschgraben Sediment						
Quantitative ion						
HPLC Mode						
Bentazon (BAS 351 H)	0.01 (LOQ)	5	97.8-104.4	101.8	2.5	2.4
	0.1	5	96.8-98.8	97.8	0.8	0.9
	1.0	5	95.0-97.8	96.0	1.2	1.3
BH 351-N-Me	0.01 (LOQ)	5	87.0-87.7	87.4	0.3	0.4
	0.1	5	88.5-94.1	92.5	2.4	2.6
	1.0	5	93.0-97.1	95.1	1.5	1.5

UPLC Mode						
Bentazon (BAS 351 H)	0.01 (LOQ)	5	95.8-100.4	97.6	1.9	2.0
	0.1	5	93.0-100.2	96.1	2.8	2.9
	1.0	5	92.4-100.2	95.8	2.9	3.0
BH 351-N-Me	0.01 (LOQ)	5	94.0-96.9	95.8	1.2	1.2
	0.1	5	90.8-98.5	94.9	2.8	2.9
	1.0	5	94.4-98.4	96.7	2.0	2.0
Confirmatory ion						
HPLC Mode						
Bentazon (BAS 351 H)	0.01 (LOQ)	5	99.2-106.8	102.5	2.9	2.9
	0.1	5	96.8-99.2	98.1	1.0	1.0
	1.0	5	95.0-97.8	96.0	1.1	1.2
BH 351-N-Me	0.01 (LOQ)	5	86.0-89.6	87.9	1.4	1.6
	0.1	5	90.9-93.2	92.4	0.9	1.0
	1.0	5	94.0-96.8	95.7	1.1	1.1
UPLC Mode						
Bentazon (BAS 351 H)	0.01 (LOQ)	5	94.2-99.6	96.8	2.0	2.0
	0.1	5	92.0-99.0	96.6	2.7	2.8
	1.0	5	90.6-99.0	95.8	3.3	3.4
BH 351-N-Me	0.01 (LOQ)	5	97.7-104.0	101.1	2.4	2.4
	0.1	5	96.4-101.0	98.6	1.9	1.9
	1.0	5	92.9-98.6	95.7	2.5	2.6

Data (uncorrected recovery results; Appendix 5.5, p. 97) were obtained from pp. 6-7; Tables 5-28, pp. 22-45 of MRID 49693501.

1 The soil/sediment matrices were fully characterized by BioChem (USDA soil characterization; p. 15; Appendices 5.1-5.3, pp. 50-52). Loamy sand soil (LUFA 2.2; 85.6% sand, 9.6% silt, 4.8% clay; 1.89% organic carbon), sandy loam soil (LUFA 5M; 56.6% sand, 30.4% silt, 13.0% clay; 1.31% organic carbon) and loamy sand sediment (RAGRA; Ranschgraben; 86.2% sand, 8.3% silt, 5.5% clay; 2.42% organic carbon) were used.

2 Ion transitions monitored were as follows (quantitative ion and confirmatory ion, respectively): m/z 239 \rightarrow 132 and m/z 239 \rightarrow 197 for bentazon (BAS 351 H) and m/z 255 \rightarrow 134 and m/z 255 \rightarrow 213 for BH 351-N-Me (Appendix 5.5, pp. 94-95).

Table 3. Independent Validation Method Recoveries for Bentazon (BAS 351 H) and BH 351-N-Me in One Soil^{1,2}

Analyte	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil						
Primary Quantification - HPLC mode						
Bentazon (BAS 351 H)	0.01 (LOQ)	5 ³	105-122	115	8	7
	0.1	10 ^{3,4}	57-95	72	12	17
BH 351-N-Me	0.01 (LOQ)	5	76-79	78	1	2
	0.1	5	87-120	100	14	14
Confirmatory Quantification - HPLC mode						
Bentazon (BAS 351 H)	0.01 (LOQ)	5 ³	102-125	111	9	8
	0.1	10 ^{3,4}	51-94	70	13	18
BH 351-N-Me	0.01 (LOQ)	5	77-81	79	2	2
	0.1	5	85-116	98	13	14

Data (uncorrected recovery results, Figure 2, p. 25; Appendix 3, pp. 151-156) were obtained from Tables 1-2, pp. 18-22 of MRID 49693502.

- 1 The soil matrix was fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; Appendix 5, p. 232). Clay loam soil (33% sand, 31% silt, 36% clay; 0.6% organic carbon) was used.
- 2 Ion transitions monitored were as follows (quantitative ion and confirmatory ion, respectively): m/z 239 → 132 and m/z 239 → 197 for bentazon (BAS 351 H) and m/z 255 → 134 and m/z 255 → 213 for BH 351-N-Me (Table 1, p. 18).
- 3 Re-injection of first trial extracts (n = 5; p. 16; Table 1, p. 18).
- 4 Re-extraction and injection of first trial, considered the second injection in the study report (n = 5; p. 16; Table 1, p. 18).

III. Method Characteristics

For bentazon (BAS 351 H) and BH 351-N-Me, the LOQ was reported as 0.01 mg/kg in both the ECM and ILV (p. 15; Tables 5-28, pp. 22-45; Appendix 5.5, p. 98; Appendix 5.5, Amendment 1, p. 101 of MRID 49693501; pp. 7, 14; Appendix 3, p. 146 of MRID 49693502). In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested. The LOD was reported as 0.001 mg/kg (10% of the LOQ) and 0.002 mg/kg (20% of the LOQ), respectively, in the ECM and ILV. It was defined as the absolute amount of analyte injected into the LC/MS/MS instrument using the lowest standard of the calibration curve. In the ECM, the LOD corresponded to 0.25 pg for BAS 351 H and 5.0 pg for BH 351-N-Me. In the ILV, the LOD corresponded to 0.3 pg for BAS 351 H and 6 pg for BH 351-N-Me. No calculations or comparison to background levels were provided for the LOQ or LOD.

Table 4. Method Characteristics

		Bentazon (BAS 351 H)	BH 351-N-Me
Limit of Quantitation (LOQ)		0.01 ppm (0.01 mg/kg)	
Limit of Detection (LOD)	ECM	0.001 ppm (0.001 mg/kg) (10% of the LOQ)	
		corresponded to 0.25 pg	corresponded to 5.0 pg
	ILV	0.002 ppm (0.002 mg/kg) (20% of the LOQ)	
		corresponded to 0.3 pg	corresponded to 6 pg
Linearity (calibration curve r^2 and concentration range)	ECM ¹	HPLC	
		$r^2 = 0.9990$ (Q) $r^2 = 0.9990$ (C)	$r^2 = 0.9998$ (Q) $r^2 = 0.9998$ (C)
		UPLC	
		$r^2 = 0.9990$ (Q) $r^2 = 0.9974$ (C) (0.005-0.25 ng/mL)	$r^2 = 0.9996$ (Q) $r^2 = 0.9996$ (C) (0.1-5.0 ng/mL)
	ILV (HPLC) ²	$r^2 = 0.9946$ -0.9968 (Q) $r^2 = 0.9932$ -0.9936 (C) (0.010-0.25 ng/mL)	$r^2 = 0.9990$ (Q) $r^2 = 0.9978$ (C) (0.20-5.0 ng/mL) or (0.010-0.25 ng/mL) ³
Repeatable	ECM ⁴	Yes at LOQ, 10×LOQ and 100×LOQ; (n = 5).	
		HPLC and UPLC performed.	
	ILV ⁵	Yes at LOQ (n = 5). Yes at 10×LOQ (n = 10). ⁶	Yes at LOQ and 10×LOQ; (n = 5).
		Only HPLC performed.	
Reproducible	ECM	Yes at LOQ and 10×LOQ (HPLC).	
	ILV		
Specific	ECM	Matrix interferences at analyte retention times were <LOD in HPLC and UPLC chromatograms.	Matrix interferences at analyte retention times were <LOD. HPLC chromatograms generally showed a cleaner baseline than the UPLC chromatograms.
		Only chromatograms of the LOQ, control and one calibration standard were provided; chromatograms were provided for each matrix.	
	ILV	Matrix interferences at analyte retention times were <LOD. Some peak integration was not standard (BAS 351 H, LOQ; confirmation ion; Figure 5, p. 38).	

Data were obtained from pp. 6-7, 15; Tables 5-28, pp. 22-45; Appendix 5.4, pp. 53-84; Appendix 5.5, pp. 93, 98; Appendix 5.5, Amendment 1, p. 101 of MRID 49693501; pp. 7, 14, 16; Tables 1-2, pp. 18-22; Figures 3-5, pp. 26-43; Appendix 3, pp. 146-156 of MRID 49693502. Q = quantitation ion; C = confirmation ion.

1 ECM r^2 values are reviewer-generated from reported r values of 0.9987- 0.9995 for BAS 351 H and 0.9998-0.9999 for BH 351-N-Me (HPLC and UPLC methods and ions combined; Appendix 5.4, pp. 81-84 of MRID 49693501; DER Attachment 2).

2 ILV r^2 values are reviewer-generated from reported r values of 0.9966-0.9984 for BAS 351 H and 0.9989-0.9995 for BH 351-N-Me (ions combined; Figure 3, pp. 26-29; Appendix 3, pp. 151-156 of MRID 49693502; DER Attachment 2).

3 Based on information provided in Figure 3, pp. 26-29 and Appendix 3, pp. 151-156 of MRID 49693502.

4 The ECM validated the method using loamy sand soil (LUF 2.2; 85.6% sand, 9.6% silt, 4.8% clay; 1.89% organic carbon), sandy loam soil (LUF 5M; 56.6% sand, 30.4% silt, 13.0% clay; 1.31% organic carbon) and loamy sand

sediment (Ranschgraben; 86.2% sand, 8.3% silt, 5.5% clay; 2.42% organic carbon; USDA classification; sources not further specified; p. 15; Appendices 5.1-5.3, pp. 50-52 of MRID 49693501).

5 In the ILV, clay loam soil (33% sand, 31% silt, 36% clay; 0.6% organic carbon) was used (USDA classification; source not specified; Appendix 5, p. 232 of MRID 49693502).

6 The method was validated with the first trial for both analytes in the test soil; however, the acceptable results for BAS 351 H were achieved after re-injection for the LOQ level and after two re-injections for the 10×LOQ level (pp. 7, 16 of MRID 49693502). Due to initial unacceptable results for BAS 351 H at the LOQ and 10×LOQ (results not provided in the study report), the extracts were re-injected (p. 16; Table 1, p. 18; Appendix 3, pp. 153-156). The re-injection of the extracts yielded acceptable results at the LOQ; however, due to the unacceptable 10×LOQ results from this re-injection, the first trial samples were extracted again and re-injected. This was considered the second injection of the first trial in the study report. The combined results of the first and second re-injections were acceptable.

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. The ECM MRID 49693501 was a combination of BASF Reg. Doc# 2009/1091211 (EPA MRID 48970701), 2014/1083365, & 2015/1111336; ECM MRID 49693501 replaced MRID 48970701 (p. 1 of MRID 49693501). The ILV referenced BASF Reg. Doc# 2009/1091211 (MRID 48970701) and BASF Reg. Doc# 2014/1083365, but not the updated MRID 49693501 or BASF Reg. Doc# 2015/1111336 (p. 17 of MRID 49693502).

The changes to the amended final reports of the ILV were listed in Appendix 6 (p. 234) of MRID 49693502). Among the changes, the ILV BASF Reg. Doc# changed from 2014/7000478 (EPA MRID 49338402) to 2015/7001109 to 2015/7001576.

2. The estimations of the LOQ and LOD in the ECM were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested (p. 15; Tables 5-28, pp. 22-45; Appendix 5.5, p. 98; Appendix 5.5, Amendment 1, p. 101 of MRID 49693501; pp. 7, 14; Appendix 3, p. 146 of MRID 49693502). The LOD was defined as the absolute amount of analyte injected into the LC/MS/MS instrument using the lowest standard of the calibration curve. No calculations or comparison to background levels were provided for the LOQ or LOD. The LOD of the ECM differed from that of the ILV.

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/sediment was not reported. An LOQ above toxicological level of concern results in an unacceptable method classification.

3. In the ILV, the linearity coefficients were <0.995 for three of the four calibration curves of BAS 351 H (Figure 3, pp. 26-29; Appendix 3, pp. 151-156 of MRID 49693502; DER Attachment 2).
4. In the ECM, only chromatograms of the LOQ, control and one calibration standard were provided for each matrix (Appendix 5.4, pp. 53-80 of MRID 49693501). Chromatograms of the reagent blank and 10×LOQ and 100×LOQ fortifications were not included.

Representative chromatograms should be provided for all calibration standards, matrices and fortification levels, as well as for controls and reagent blanks.

5. The UPLC method was not validated by the ILV (p. 14 of MRID 49693502). The extracts were analyzed using HPLC/MS/MS only due to the fact that UPLC/MS/MS equipment was not commonly available for the enforcement laboratory and was “intended to be used for data collection purposes” (p. 14). The decision to only perform the HPLC analysis was initiated by the sponsor representative based on method acceptance criteria (Appendix 4, p. 183).
6. The ILV study report noted that the only recommendation or suggestion for the ECM was to include stability information for the stock standards in methanol (pp. 16-17 of MRID 49693502). The stability information provided in ECM MRID 49693501 addressed the stability of the methanol:water (50:50, v:v) fortification and standard solutions (pp. 19-20 of MRID 49693501).
7. In the ILV, the communications between the ILV study author and study sponsor/ECM study authors was provided (pp. 12, 16-17; Appendix 4, pp. 157-230 of MRID 49693502). No technical guidance was required. Communication regarded “clarifications of protocol preparation, guideline requirements, analytical series requirements, preparation of stock solutions, selecting of HPLC-MS/MS over UPLC-MS/MS, and instrument optimization with the study monitor” (p. 17).

In the communications, the reviewer noted that the ILV questioned the acceptability of “Nalgene (PP)” vessels for the extraction, instead of using glass vials (Appendix 4, p. 159 of MRID 49693502). The ILV noted that no problem of use of plastic vessels was noted in the method. The ECM study monitor responded that only glass vessels should be used, “in case there is[are] problems due to adherence of analyte in plastic type” (Appendix 4, p. 159). The reviewer noted that the ECM method materials section specified that “if the use of supplies other than stated is intended, applicability to this method must be confirmed prior to method validation” (Appendix 5.5, p. 91 of MRID 49693501).

The reviewer also noted that the ECM correction of the UPLC mobile phase gradient (Amendment 2, p. 108 of MRID 49693501) was provided to the ILV in communications (Appendix 4, p. 186 of MRID 49693502).

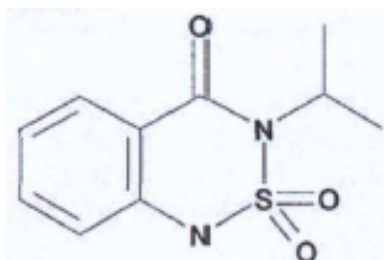
8. The ECM study authors monitored the stability of the analytes in the fortification and standard solutions (pp. 19-20; Appendix 5.5, p. 92 of MRID 49693501). The solutions were stable up to 35 days of storage under refrigeration (4°C) in the dark.
9. In the ILV, it was reported that a set consisting of 13 samples required approximately 8 work hours, plus additional HPLC-MS/MS determination time (p. 14 of MRID 49693502).
10. The ILV listed one protocol change: the change of the LOD from 0.001 ppm to 0.002 ppm (p. 17 of MRID 49693502).

11. The reviewer noted that subsection enumeration was erroneous in the 5.4 Quantitation section of the Analytical Method, where often the “5” was replaced with “3” (Appendix 5.5, pp. 94-96 of MRID 49693501).

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; Reg. No. 51929)****IUPAC Name:** 3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide.**CAS Name:** Not reported**CAS Number:** 25057-89-0**SMILES String:** Not found**BH 351-N-Me (Reg. No. 79520)****IUPAC Name:** 3-Isopropyl-1-methyl-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide.**CAS Name:** Not reported**CAS Number:** 61592-45-8**SMILES String:** Not found