Environmental Chemistry Method (ECM) and Independent Laboratory Validation (ILV) for Determination of Bentazon Residues in Soil and Sediment

Reports: ECM: 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

ILV: 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

Document No.:   [MRIDs 48970701 & 49141202]

Guideline: 850.6100 [U.S.], (Soil and Sediment);

Statements: This study was conducted in accordance with the OECD Principles of Good Laboratory Practice and the GLP Principles of the German "Chemikaliengesetz" (Chemicals Act) and meets the United States Environmental Protection Agency Good Laboratory Practice Standards [40 CFR Part 160 (FIFRA) and Part 792 (TSCA)], with the exception that recognized differences exist between the GLP Principles/Standards of OECD and the Principles/Standards of FIFRA and TSCA.

Classification: The ECM and ILV methods are classified as **Supplemental** for monitoring bentazon parent compound in water. Both methods are upgradable upon provision of the correction of the following deficiencies.

Deficiencies:
1). The limit of detection (LOD) was not reported in the ECM
2). The levels of the calibration were not reported in the ECM.
3). The mass spectrum images were not included in the ECM and ILV methods.
4). The ILV has poor individual sample recovery even though its mean % recovery is acceptable. Several individual sample % recoveries did not meet the % recovery criteria (70-120%).
5). The ILV failed to disclose the content of the homogenized soil matrix samples. A clarification of the soil source sample is needed.

PC Code: 103901

Reviewer: He Zhong, Ph.D.   Signature:
                   Biologist   Date: 11/13/2013

Executive Summary

This analytical method (ECM MRID 48970701) is for the quantitative determination of BAS 351 H (parent bentazon) and BH 351-N-Me (metabolite) in soil and sediment using LC-MS/MS. The method limit of detection (LOD) is not reported in the ECM and limit of quantification (LOQ) is 0.01 ppm (mg/Kg) (Table 1). The ILV (MRID 49141202) has reported an estimated LOD as 0.001 ppm (mg/Kg) and confirmed ECM's LOQ limits as 0.01 ppm. The ILV did not report the ECM study deficiency. The LOQ (0.01 ppm) is below the level of concerns (LOCs) for honey bee (*Apis mellifera L.*), (48-hr LD_{50} > 0.1 ppm/bee, MRID 00161395) and rat (*Rattus norvegicus*), (NOAEL = 15 mg/kg/day, MRID 41054902).

Table 1. Analytical Method Summary

Analyte(s)	MRID		Matrix	Method Date (m/d/y)	Registrant	Analysis	Limit of Detection[1] (mg/Kg)	Limit of Quantitation (mg/Kg)
	ECM	ILV						
BAS 351 H (Bentazon)	48970701	49141202	Soil/Sediment	11/16/2012	BASF	LC-MS/MS	0.001	0.01
BH 351-N-Me	48970701	49141202	Soil/Sediment	11/16/2012	BASF	LC-MS/MS	0.001	0.01

[1]The limit of detection (LOD) is estimated in Independent Laboratory Validation (ILV MRID 49141202) as 10% of limit of quantitation (LOQ) but not reported in Environmental Chemistry Method (ECM MRID 48970701)

I. Principle of the Method

A 5 g soil sample is extracted with 50 ml methanol/water (50/50, v/v) by mechanical shaking for 60 min at 225 rpm. 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 Ultra Performance Liquid Chromatography (UPLC)-MS/MS or HPLC-MS/MS. The method has a limit of quantitation of 0.01 mg/kg in soil and sediment.

II. Recovery Findings

The mean recoveries and the relative standard deviations (RSD) of bentazon (BAS 351 H) and metabolite (BH 351-N-Me) were within guideline requirements (mean 70-120%; RSD ≤20%) for ECM (**Tables 2.1 – 2.2**) and ILV (**Table 3**). There are data deficiencies in ILV e.g.: some of individual recoveries in ILV were lower than 70% for 0.1 ppm fortification level and exceed 120% for 0.01 ppm fortification level.

Table 2.1. Validation of Analytical Method (ECM) Recoveries for Bentazon and Metabolite in Soil and Sediment (HPLC-MS/MS)

Matrix	Analyte	Transition (m/z)	Fortification Level (mg/Kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	% RSD
LUFA 2.2 Soil	BAS 351 H (Bentazon)	239/132	0.01	5	100.4-101.8	101.2	0.6
			0.1	5	98.6-100.6	99.9	0.8
			1	5	99.6-100.8	100.2	0.5
		239/197	0.01	5	99.2-102.4	101.0	1.2
			0.1	5	98.2-101.6	100.2	1.3
			1	5	99.2-102.2	100.7	1.3
	BH 351-N-Me (Metabolite)	239/132	0.01	5	94-97.8	96.2	1.5
			0.1	5	95-98.5	96.2	1.5
			1	5	95.9-99.2	97.6	1.4
		239/197	0.01	5	94.5-98.1	96.3	1.3
			0.1	5	97-98.8	98.2	0.8
			1	5	97.6-99.7	98.8	0.8
LUFA 5M Soil	BAS 351 H (Bentazon)	239/132	0.01	5	97.4-103.6	101.9	2.5
			0.1	5	100-101.4	100.6	0.5
			1	5	97-102.2	991	2.4
		239/197	0.01	5	100.2-103.2	101.8	1.3
			0.1	5	99.6-103.6	101.1	1.6
			1	5	97.8-104.2	100.4	2.3
	BH 351-N-Me (Metabolite)	239/132	0.01	5	93.4-95.9	94.7	1.0
			0.1	5	97.6-99.9	98.8	0.9
			1	5	98.2-101	99.2	1.1
		239/197	0.01	5	93.2-94.5	93.8	0.5
			0.1	5	96.7-99.5	98.3	1.1
			1	5	97.6-99.3	98.4	0.7
Ranschgraben Sediment	BAS 351 H (Bentazon)	239/132	0.01	5	97.8-104.4	101.8	2.4
			0.1	5	96.8-98.8	97.8	0.9
			1	5	95-97.8	96.0	1.3
		239/197	0.01	5	99.2-106.8	102.5	2.9
			0.1	5	96.8-99.2	98.1	1.0
			1	5	95-97.8	96.0	1.2
	BH 351-N-Me (Metabolite)	239/132	0.01	5	87-87.6	87.4	0.4
			0.1	5	88.5-94.1	92.5	2.6
			1	5	93-97.1	95.1	1.5
		239/197	0.01	5	86-89.6	87.9	1.6
			0.1	5	90.9-93.2	92.4	1.0
			1	5	94-96.8	95.7	1.1

Table 2.2. Validation of Analytical Method (ECM) Recoveries for Bentazon and Metabolite in Soil and Sediment (UPLC-MS/MS)

Matrix	Analyte	Transition (m/z)	Fortification Level (mg/Kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	% RSD
LUFA 2.2 Soil	BAS 351 H (Bentazon)	239/132	0.01	5	89.0-94.8	92.8	2.7
			0.1	5	91.4-100.4	96.4	3.6
			1	5	94.8-102.4	98.2	3.5
		239/197	0.01	5	90.4-100.4	94.7	3.9
			0.1	5	92.8-98.2	96.6	2.3
			1	5	93.6-103.6	98.5	4.2
	BH 351-N-Me (Metabolite)	239/132	0.01	5	96.9-104	99.2	2.8
			0.1	5	94-102	97.2	4.0
			1	5	94.8-101	97.4	2.7
		239/197	0.01	5	97.3-99.8	97.5	1.8
			0.1	5	94.2-101	98.3	2.9
			1	5	95.7-98.7	97.2	1.3
LUFA 5M Soil	BAS 351 H (Bentazon)	239/132	0.01	5	94-97.4	91.4	4.7
			0.1	5	95-99.2	96.6	1.8
			1	5	91.6-97.6	95.4	2.5
		239/197	0.01	5	88-96.8	92.4	4.4
			0.1	5	97-99.6	97.1	1.8
			1	5	90.6-100.2	95.0	3.7
	BH 351-N-Me (Metabolite)	239/132	0.01	5	96.2-101	97.6	2.0
			0.1	5	98.4-104	100.6	2.4
			1	5	95.2-100	98.2	2.0
		239/197	0.01	5	93.7-97.6	96.4	1.6
			0.1	5	95.5-103	100.1	3.1
			1	5	94.5-102	97.9	3.0
Ranschgraben Sediment	BAS 351 H (Bentazon)	239/132	0.01	5	95.8-100.4	97.6	2.0
			0.1	5	93-100.2	96.1	2.9
			1	5	92.4-100.2	95.8	3.0
		239/197	0.01	5	94.2-99.6	96.8	2.0
			0.1	5	92-99	96.6	2.8
			1	5	90.6-99	95.8	3.4
	BH 351-N-Me (Metabolite)	239/132	0.01	5	95.3-96.9	95.8	1.2
			0.1	5	90.8-98.5	94.9	2.9
			1	5	94.4-98.4	96.7	2.0
		239/197	0.01	5	97.7-104	101.1	2.4
			0.1	5	96.4-101	98.6	1.9
			1	5	92.9-98.6	95.7	2.6

Table 3. Independent Lab Validation (ILV) Method Recoveries for Bentazon in Homogenized Soil

Analyte	Matrix	Fortification Level (µg/Kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	CV (%)
Bentazon Quantitation m/z 239/132	Homogenized Soil	0.01	5	105-122	115	7
		0.1	10	57-95	72	17
Bentazon Confirmation m/z 239/197	Homogenized Soil	0.01	5	102-125	111	8
		0.1	10	51-94	70	18
BH 351-N-Me Quantitation m/z 239/132	Homogenized Soil	0.01	5	76-79	78	2
		0.1	5	87-120	100	14
BH 351-N-Me Confirmation m/z 239/197	Homogenized Soil	0.01	5	77-81	79	2
		0.1	5	85-106	98	14

III. Method Characteristics

The ECM method characteristics and ILV confirmation are listed in Tables 4.1, 4.2 and 5.

Table 4.1. Bentazon ECM Method Characteristics and ILV Method Confirmation

	Bentazon (ECM)	Bentazon (ILV)
Limit of Quantitation (LOQ)	0.01 mg/Kg	0.01 mg/Kg
Limit of Detection (LOD)	N/A	[1]0.001 mg/Kg
Linearity ([2]calibration curve r^2 and concentration range)	$r^2 = 0.999$ 0.005 – 0.25 µg/L	$r^2 = 0.995$ 0.01 – 0.25 µg/L
Repeatable	Yes	Yes
Reproducible	Yes	Yes
Specific	Yes	Yes

[1]Estimated value reported at 10% of LOQ
[2]Calibration curve is based on linear regression (y=a+bx) and r-values are reported in ECM/ILV method and r^2-values are calculated based on the r-values.

Table 4.2. BH 351-N-Me ECM Method Characteristics and ILV Method Confirmation

	BH 351-N-Me (ECM)	BH 351-N-Me (ILV)
Limit of Quantitation (LOQ)	0.01 mg/Kg	0.01 mg/Kg
Limit of Detection (LOD)	N/A	[1]0.001 mg/Kg
Linearity ([2]calibration curve r^2 and concentration range)	$r^2 = 0.999$ 0.01 – 5.0 µg/L	$r^2 = 0.999$ 0.02 – 5.0 µg/L
Repeatable	Yes	Yes
Reproducible	Yes	Yes
Specific	Yes	Yes

[1]Estimated value reported at 10% of LOQ
[2]Calibration curve is based on linear regression (y=a+bx) and r-values are reported in ECM/ILV method and r^2-values are calculated based on the r-values.

Linearity is established in the calibration (y=a+bx) using external standards. The correlation coefficient of the calibration curves was above 0.999. The **limit of quantification** (LOQ) is 0.01 mg/Kg for both parent bentazon and metabolite BH 351-N-Me. The method in general satisfies the **repeatability** criteria with mean recoveries are in the range of 70-120% and RSDs are ≤20%. **Reproducibility** is satisfactory with the independent validation confirmed the LOQ(s) established by the initial validation. This method using LCMS/MS demonstrated excellent **specificity** by selecting the following daughter and parent ions (Table 5). However, both ECM and ILV method did not include the Mass Spectrum Graph to confirm the method specificity for the parent and daughter ions.

Table 5. Method Specificity—LC-MS/MS Parent and Daughter ions

Analyte	Parent ion	Daughter ion
Bentazon	132	197
BH 351-N-Me	134	213

IV. Method Deficiencies and Reviewer's Comments

ECM:
The limit of detection (LOD) is not reported. The calibration levels for the calibration curve were not reported. The images for mass spectrum were not included for the visual view of the primary and secondary ions.

ILV:
The ILV failed to disclose the content of the homogenized soil matrix samples. Even though the average recovery is within the criteria limit (70-120%), some of the individual recoveries fell outside of the EFED criteria limit. The images for mass spectrum were not included for the visual view of the primary and secondary ions.

V. References

Rodgers,C. 2012. 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 MRID 49141202

Penning, H. 2009. 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 MRID 48970701