Section I. Example ECM/ILV Review

Analytical method for Pethoxamid in soil

Reports:	ECM MRID 49813426 "Terrestrial Field Dissipation of Pethoxamid at Four Sites, USA" ILV: MRID 49813421 Independent Laboratory Validation of Analytical Method M-80127, "Analytical Method for the Determination of Pethoxamid and MET-42 in Soil via LC/MS/MS
Document No.:	MRIDs 49813426 & 49813421
Guideline:	850.6100 Terrestrial Field Dissipation Studies at Four sites in U.S. - Environmental Chemistry Methods (ECM) and the Independent Laboratory Validation (ILV)
Statements:	The method validations were conducted in compliance with FIFRA GLP standards and whether signed and dated Data Confidentiality, GLP Compliance, Quality Assurance, and Authenticity Certification statements were provided for the method and its ILV.
Classification:	This analytical method is classified as supplemental. Although, different study author for the ECM and ILV in soil; the laboratory performing the analysis of the samples was the same. Additionally, there was significant communication between the two study authors before, during and after the completion of the ILV. However, the independent laboratory validated LOQ for pethoxamid and its degradate MET-42 is the same in the ECM and ILV (10 µg/kg).
PC Code:	090208
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Executive Summary

The procedure for the analysis of pethoxamid and its degradation product, MET-42, in soil Samples is described below. This method was based on ABC Method No. M-80127, "Analytical Method for the Determination of pethoxamid and MET-42 in Soil via LC/MS/MS" MRID 49813521.

The LOQ and LOD in soil for both the ECM and ILV was 10 µg/kg and 3 µg/kg for pethoxamid and MET-42, respectively. Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of pethoxamid in soil.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Pethoxamid	49813426	49813421		Soil	09/25/2015	Cheminova, Inc	LC/MS/MS	10 µg/kg
MET-42	49813426	49813421		Soil	09/25/2015	Cheminova, Inc	LC/MS/MS	10 µg/kg

I. Principle of the Method

Soil Extraction Procedure

Soil samples, 5.0 ± 0.05 grams of soil were weighed into 50-mL centrifuge tubes. Samples were fortified as appropriate. The samples were fortified using Pethoxamid and MET-42 spiking solutions in Injection solvent at concentrations of 10 and 100 ppb. Steel balls and 15 mL of acetonitrile (ACN) were added to the sample container. The samples were shaken for 10 minutes at high speed on a shaker then centrifuged at ~3000 RPM for five minutes. The extract was decanted into the same 50-mL centrifuge tube as the earlier extract. The samples were extracted a third time with fifteen milliliters of extraction solution (50:50 ACN/water). Samples were capped and vortexed until the pellet was broken up. The sample was placed on a Geno/grinder® set to 1200 shakes per minute for two minutes then centrifuged at approximately 3000 RPM for five minutes. The extract was decanted into the same 50-mL centrifuge tube as the earlier extract. Volume was adjusted to 50 mL with 0.1% formic acid (aqueous), the tube was capped, and vortexed to mix. The sample was diluted with 0.1% formic acid in 30:70 ACN/water and analyzed via LC-MS/MS. This method is compatible with MRID 49813521 (ILV).

II. Recovery Findings

Average recoveries for pethoxamid in soil ranged from 81-102% with the highest standard deviation of 3.4% and its transformation product, MET-42, ranged from 84-111% with a standard deviation of 4.1%. The reproducibility was within acceptable range for all samples tested (70-120%). The calibration curves showed linearity in response to a wide range of concentrations (0.05 to 10 ng/mL) for both the parent and MET-42. Pethoxamid and the degradation product, MET-42, had an LOQ of 10 µg/kg and a LOD of 3 µg/kg. Two different ion transitions were studied in the ILV: 296.2/131.1 and 296.2/250.2 for pethoxamid and 340.0/120.7 and 340/79.9 for MET-42.

The correlation coefficient values (r) for all ion transitions of pethoxamid were 1.000 and ≥ 0.999 for the MET-42 curves from the ILV therefore, the linearity was acceptable.

Table 2. Summary of the % Recoveries Results of Pethoxamid from Texas soil (ILV)

Ion Transition (Da)	Nominal Fortification Level (ppb)	Recovery (%)	Mean Recovery (%)	SD	% RSD
296.2/131.1	10	81 81 80 82 81	81	0.6	0.7
	100	99 99 98 99 99	99	0.7	0.8
		Overall	90		10.4
296.2/250.2	10	101 100 101 100 99	100	0.8	0.8
	100	97 97 98 99 99	98	0.8	0.8
		Overall	99		1.4

Table 3. Recoveries of Pethoxamid (ILV) from Iowa soil

Ion Transition (Da)	Nominal Fortification Level (ppb)	Recovery (%)	Mean Recovery (%)	SD	% RSD
296.2/131.1	10	82 84 84 83 81	83	1.1	1.3
	100	100 102 101 101 99	100	1.3	1.3
		Overall	92		10.3
296.2/250.2	10	105 103 101 104 96	102	3.4	3.3
	100	100 104 100 101 97	100	2.5	2.5
		Overall	101		2.9

Table 4. Summary of the % Recoveries Results of MET-42 in Texas (ILV)

Ion Transition (Da)	Nominal Fortification Level (ppb)	Recovery (%)	Mean Recovery (%)	SD	% RSD
340.0/120.7	10	95	96	4.1	4.3
		94			
		91			
		101			
		100			
	100	107	108	2.7	2.5
		109			
		106			
		113			
		106			
		Overall	102		7.0
340.0/79.9	10	95	95	3.7	3.9
		90			
		92			
		100			
		96			
	100	111	111	2.8	2.5
		111			
		108			
		115			
		109			
		Overall	103		8.9

Table 5. Summary of the % Recovery Results for MET-42 in Iowa (ILV)

Ion Transition (Da)	Nominal Fortification Level (ppb)	Recovery (%)	Mean Recovery (%)	SD	% RSD
340.0/120.7	10	82	88	3.6	4.1
		88			
		89			
		91			
		91			
	100	94	95	2.1	2.2
		96			
		96			
		97			
		91			
		Overall	92		4.8
340.0/79.9	10	80	84	3.3	3.9
		84			
		83			
		89			
		86			
	100	96	97	1.1	1.1
		98			
		98			
		97			
		96			
		Overall	91		7.8

Table 6. Environmental Chemistry Method Validation Recoveries for Pethoxamid in Soil

Percent Recovery in Soil Samples		
	Pethoxamid	**MET-42**
Iowa		
Average Recovery [%]	101	94

Number of Samples	50	50
Standard Deviation [%]	10	13
New York		
Average Recovery [%]	99	93
Number of Samples	30	30
Standard Deviation [%]	7	11
Texas		
Average Recovery [%]	104	94
Number of Samples	48	48
Standard Deviation [%]	12	15
California		
Average Recovery [%]	101	92
Number of Samples	50	50
Standard Deviation [%]	10	13

The LOQ and LOD were 10 and 3.0 ppb, respectively.

III. Method Characteristics

Table 4 describe all the method characteristics. The LOQ for the ECM and ILV was the same: 10 µg/kg for both pethoxamid and MET-42. Similarly, the LOD for both, ECM and ILV was 3 µg/kg for the two analytes. The reproducibility is satisfactory as recoveries were within the acceptable range (70-120%) and RSDs are ≤20%. Its linearity is satisfactory when $r^2 \geq 0.995$.[1]

Table 4. Method Characteristics

	Pethoxamid	MET-42
Limit of Quantitation (LOQ)	10 µg/kg	10 µg/kg
Limit of Detection (LOD)	3 µg/kg	3 µg/kg
Linearity (calibration curve r^2 and concentration range)	$r^2 = 1.000$ 0.05 –50 ng/mL	$r^2 \geq 0.999$ 0.05 –50 ng/mL
Repeatable	Yes	Yes
Reproducible	Yes	Yes
Specific	Yes	Yes

IV. Method Deficiencies and Reviewer's Comments

1. Although, the ECM and ILV study authors are different, the performing laboratories were the same. In addition, there were communication between the study authors before, during and after the ILV was completed.

[1] This criterion is consistent with Superfund analytical methods for inorganic analytes at http://www.epa.gov/superfund/programs/clp/download/ism/ism1nfg.pdf (accessed Nov. 7, 2012).