ABSTRACT

Enviro-Test Laboratories performed an independent laboratory validation (ILV) of Syngenta Analytical Method No. 1200-03. This is a LC/MS/MS method for the analysis of mesotrione and its metabolites AMBA and MNBA in soil. Soil samples were fortified with all three analytes and analyzed according to the original method. The analysis set consisted of one reagent blank, two controls, five control samples fortified at the limit of quantitation (LOQ) of 2.0 ppb, and five control samples fortified at 10 times the LOQ (20.0 ppb).

The method was successfully validated for all analytes at their respective LOQ and 10X LOQ fortification levels. The mean recoveries, relative standard deviations (RSDs) and 95% confidence intervals were reported for each sample set. In addition, the statistics for the overall (LOQ and 10X LOQ) results for each analyte/matrix combination were reported.

For all analytes the individual and average recoveries were between 70 and 120 percent of theoretical with relative standard deviations of less than 20 percent.

1.0 INTRODUCTION

This report describes the independent laboratory validation (ILV) of Analytical Method No. 1200-03 as performed by Enviro-Test Laboratories for the determination of mesotrione and its metabolites AMBA and MNBA in soil using Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry.

This study was conducted to satisfy guideline requirements described in the US EPA FIFRA Pesticide Assessment Guidelines for Subdivisions N, E, and K, and addenda for Data Reporting Guideline for Environmental Methods [2]. It also satisfies the requirements outlined in the harmonized guidelines from the OPPTS, "Public Draft" - Data Reporting for Environmental Chemistry Methods, OPPTS 850.7100 [3].

2.0 STUDY PERSONNEL

The following personnel from Enviro-Test Laboratories participated in the conduct of this study.

Gary Bruns	Study Director
Susan Nelson	Lab Manager
Narinder Bains Connie Blenkinsop	Residue Analysts
Jillian Devine	Log-In and Sample Control

3.0 MATERIALS

3.1 Test and Reference Substances

Reference substances were shipped from Syngenta US to Enviro-Test Laboratories and were received February 6, 2001 and August 27, 2004 (see the Standard disposition sheets in the Raw Data package). The following substances were used:

Compound	Lot Number	Purity (%)	Expiration Date
Mesotrione	ASW01788-01R	99.6	Jan 2005
AMBA (NOA-422848)	DAH-XXIX-4	97.4	April 30, 2005
MNBA (NOA-437130)	DAH-XXIX-5	>99.9	Feb. 28, 2006

The reference substances were logged in and then kept stored in a freezer after arrival at ETL. Syngenta Crop Protection, Inc. maintains the characterization and stability data for the reference substances.

On October 25, 2004 stock standards were prepared from the neat reference substances for use in preparing instrument calibration and fortification solutions. All stock standards were prepared prior to the final signature of the protocol, but were done under GLP and as per the method. Fortification and working solutions were prepared from the stock standards on November 25, 2004. The stock standards were stored in a freezer and working solutions were kept stored in a refrigerator when not in use.

3.2 Control Soil

Control soil, supplied by Syngenta, was used to validate the method. The control soil sample was characterized by Agvise Laboratories in Northwood, ND.

Sand: 90%
Silt: 5%
Clay: 5%

USDA Soil Texture: Sand

Soil pH and organic matter analysis were performed for the 0-6" layer from three different areas of the test site. The results of the analysis are:

Boring ID	Depth Interval (feet bgs)	Organic Matter	Soil pH
GP-1	0.0-0.5	1.0%	5.8
GP-2	0.0-0.5	1.5%	5.8
GP-3	0.0-0.5	1.3%	5.3
Mean:	---	1.3%	5.6

The soil characterization data are from Syngenta Study number T000011-02. The original raw data for the soil characterization are stored in the Syngenta archives with report T000011-02.

3.3 Equipment and Reagents

The equipment and reagents used for the method validation were as outlined in Syngenta method 1200-03 (Section 2.0 Materials and Apparatus). Identical or equivalent apparatus and materials were used.

4.0 METHOD AND METHOD MODIFICATIONS

4.1 Modifications

No modifications were made to the extraction section of the method. It was performed exactly as written. As a result of the difference in LC/MS/MS systems the following specific modifications to the method are noted:

1. A PE Sciex API 4000 MS/MS system was used in place of the Micromass Ultima MS/MS and Waters Alliance Model 2695 LC System. The instrument specifications are listed in Table 1 and Table 2.

2. Serial dilutions performed were 10.0 ppm, 1.00 ppm and 0.100 ppm instead of the listed concentrations of 5 ppm, 0.5 ppm and 0.02 ppm.

3. Change in Q1/Q3 transition for AMBA (213 to 155).

4. Change in injection volume for AMBA and MNBA from 10 µL to 50 µL.

4.2 Sample Preparation, Fortification, and Extraction

The validation trial consisted of one analytical set. This set consisted of 13 samples: one reagent blank, two matrix blanks, five matrix blanks fortified at the LOQ (2.0 ppb) and five matrix blanks fortified at 10X LOQ (20.0 ppb).

Twelve 10g weighings of soil were used as samples. Samples designated as spikes were fortified with either 200 µL of a 100 µg/L mixed standard fortification solution (for LOQ fortifications) or 200 µL of 1000 µg/L (for 10X LOQ fortifications). See detailed method below:

Extraction:

1. Weighed representative amounts of soil (10 ± 0.1 g) into separate 50 mL disposable plastic centrifuge tubes.

2. Fortified appropriate samples with either 200 µL of the 100 ppb solution mix or 200 µL of the 1000 ppb solution mix. The samples were capped and mixed. Allowed fortified control samples to equilibrate for at least 5 minutes before proceeding with the extraction.

3. Added 20 mL of 0.05 M NH_4OH, capped and shaken flat (horizontally) on a mechanical shaker at a speed that visibly agitated the sample for a minimum of 30 minutes.

4. Centrifuged samples at 6000 rpm for about 5 minutes. Decanted the supernatant liquid into a separate plastic 125 mL screw-cap bottle.

5. Added 20 mL of 50:50 0.05M NH_4OH:acetone to the pellet. Capped and shaken by hand or vortex to mix. Shaken flat on a mechanical shaker for a minimum of 30 minutes.

6. Centrifuged samples at 6000 rpm for about 5 minutes. Decanted the supernatant liquid into the separate plastic 125 mL screw-cap bottle containing the first extraction.

7. Added 20 mL of acetone to the pellet. Capped and shaken by hand or vortex to mix. Shaken flat on a mechanical shaker for a minimum of 30 minutes.

8. Centrifuged samples at 6000 rpm for about 5 minutes. Decanted the supernatant liquid into the separate plastic 125 mL screw-cap bottle containing the first two extractions. Capped and shaken to mix.

9. Centrifuged the combined extracts at 6000 rpm for about 5 minutes.

10. Removed a 6 mL aliquot to a 15 mL disposable, graduated centrifuge tube. Evaporate off the organic solvent until only aqueous remained at a temperature of about 40°C. Reduced to a volume of about 2.5 mL.

11. Brought to a final volume of 10 mL using 2% formic acid. Capped and shaken well to mix. Sonicated for 3-5 minutes, if needed. Centrifuged at 4500 rpm for 5 minutes.

12. Transferred an aliquot of sample to a glass autosampler vial for analysis by LC with triple quadrupole mass spectrometric detection (LC/MS/MS).

4.3 LC/MS/MS Instrumentation

All samples were analyzed using an Applied Biosystems API-4000 Triple Quadrupole Mass Spectrometer with Turbo Ion Spray Interface. The following components completed the system:

HPLC: Two Perkin Elmer Series 200 Micropumps
Autoinjector: CTC HTS PAL
Column Heater: Waters Temp. Control module Millipore
Data System: Dell Precision 360, Intel(R) Pentium Computer, 4 CPU 3.00 GHz running Microsoft Windows 2000 Version 5 and Analyst Version 1.4

The HPLC operating parameters are shown in Table 1. The API 4000 MS/MS operating parameters are shown in Table 2.

4.4 Data Acquisition and Reporting

Peak integration and quantitation were performed by using Analyst, Version 1.4 (Applied Biosystems). Analytes were quantitated by external calibration. The MS detector response (peak area) versus the standard concentration was used to generate calibration curves for the analytes. Best-fit weighted 1/x linear regression equation for the curves were derived and these equations were used to calculate the concentration of analytes in the samples. Recovery results were computed for each sample. The equations used for quantitation are presented in Table 5.

Statistical treatment of the data includes calculation of averages, standard deviations, relative standard deviations and confidence limits. The calculations were performed using Excel 97 SR-2. Results were rounded off for reporting purposes but not during calculations.

8.0 TABLES

Table 1. HPLC System

(Mesotrione)

Analytical Column:	PLRP-S, 4.6 x 50 mm, 5 µm, Serial No. RPSI-1298-32
Guard Column:	Polymer Laboratories PLRP-S (5 x 3 mm), Cat. No.: 1612-1801
Mobile Phase Flow Rate:	Gradient Profile Below
Mobile Phase A:	0.1% acetic acid in water
Mobile Phase B:	0.1% acetic acid in acetonitrile
Run time:	8.5 minutes
Injection Volume:	20 µL

Mobile Phase Program:

Duration (min.)	%A	%B	Flow (mL/min.)
0.0	85	15	0.8
1.0	85	15	0.8
3.5	5	95	0.8
6.0	5	95	0.8
6.5	85	15	1.0
8.5	85	15	1.0

Analyte Retention times:

Analyte	Min.
Mesotrione	3.61

Table 2. HPLC System

(AMBA and MNBA)

Analytical Column:	SYNERGI 4μ Fusion – RP 80A, 4.6 x 75 mm, 4 μm, Serial No. 264360-12
Guard Column:	Fusion - RP guard column (4 x 3.0 mm, 5 μm), Part No.: AJ0-7557
Mobile Phase Flow Rate:	800 μL/min
Mobile Phase A:	0.1% Acetic acid in water
Mobile Phase B:	0.1% Acetic acid in acetonitrile
Run time:	12.5 minutes
Injection Volume:	50 μL

Mobile Phase Gradient Program:

Duration (min.)	%A	%B
0.0	95	5
1.0	95	5
5.0	5	95
8.0	5	95
9.5	95	5
12.5	95	5

Analyte Retention times:

Analyte	Min.
AMBA (NOA-422848)	4.20
MNBA (NOA-437130)	6.93

Table 3. LC/MS/MS Operating Parameters

AMBA and MNBA (Period 1 Experiment 1) was analyzed using negative ion detection. The MRM (multiple reaction monitoring) scan mode was used for the signal acquisition.

	AMBA	MNBA
CUR	10.00	10.00
Q1 Mass	213.80	243.70
Q3 Mass	155.10	199.80
Dwell time (msec)	500	500
Resolution Q1	UNIT	UNIT
Resolution Q3	UNIT	UNIT
Declustering potential (DP)	-25	-30
Entrance potential (EP)	-6	-6
Collision energy (CE)	-28	-10
Collision cell exit potential (CXP)	-1	-11

Mesotrione was analyzed using negative ion detection. The MRM scan mode was used for the signal acquisition.

	Mesotrione
CUR	10.00
Q1 Mass	338.2
Q3 Mass	291.00
Dwell time (msec)	200
Resolution Q1	UNIT
Resolution Q3	UNIT
Declustering potential (DP)	-55
Entrance potential (EP)	-10
Collision energy (CE)	-14
Collision cell exit potential (CXP)	-7.0

Table 7. Calculations

Peak areas and external calibrations were used for data analysis. The Analyst Version 1.4 quantitation software package was used to calculate a best fit, 1/x weighted line of the standards. Extract concentration found was determined from the analyte peak area versus the calibration.

- Calculation of Analyte Concentration:

$$\text{Calc.Conc.(ppb)} = \frac{(x - b)}{m} \times \text{D.F.}$$

Where:
x = Peak Area of the analyte
b = Intercept from weighted 1/x regression analysis (Peak Area)
m= Slope from weighted 1/x regression analysis (response per concentration)
D.F. = Dilution Factor

$$\text{D.F.} = \frac{\text{Final Volume (mL)}}{\text{Sample Weight (g)}} \times \frac{\text{Extraction Volume (mL)}}{\text{Aliquot Volume (mL)}} = \frac{10.0\,\text{mL}}{10\,\text{g}} \times \frac{60.0\,\text{mL}}{6.0\,\text{mL}} = 10.0$$

The Analyst data processing software generates both the slope and intercept.

The calculation of averages, standard deviations, relative standard deviations and 95% confidence limits were performed in Excel.

The report percent recoveries shown on Table 4 to 6 may not exactly match the corresponding recoveries on the Analyst Result tables shown in Appendix 3. This is because Analyst uses a large string of un-rounded numbers to calculate the percent recoveries.

- Calculation of Percent Recovery (Fortification Only)

$$\%\,\text{Recovery} = \frac{\text{ppb Found in Sample Extract}}{\text{Fortification Level (ppb)}} \times 100$$