1.0 INTRODUCTION

This report describes the independent laboratory validation (ILV) of Analytical Method No. 200747 as performed by ALS Laboratory Group, Environmental Division for the determination of O-Desmethyl MKH (Metabolite of MKH 6562) in soil using High Performance Liquid Chromatography with Triple Quadrapole Mass Spectrometric Detection (LC-MS/MS).

2.0 STUDY PERSONNEL

The following personnel from ALS Laboratory Group, Environmental Division participated in the conduct of this study.

Susan Nelson	Study Director
Narinder Bains	Residue Analyst
Elzbieta Grzegorzewska	Laboratory Assistant
Jillian Devine	Log-In and Sample Control

3.0 MATERIALS

3.1 Test and Reference Substances

Reference substances were shipped from Arysta Life Science North America Corporation (formerly Arvesta Corporation) to ALS Laboratory Group, Environmental Division and were received on October 4, 2005. The following substances were used:

Compound	Lot Number	Purity (%)	Expiration Date
O-Desmethyl MKH 6562	K-661	98.8*	Jun. 02/2008
O-Desmethyl MKH 6562 -d_3	K-779	100**	Sep. 14/2006
Flucarbazone Methylcarbamate (N-Methylcarbamate)	97B96-137 (K-789)	96.2*	Jun. 02/2008
d_3-Flucarbazone Methylcarbamate (N-Methylcarbamate-d_3)	97B96-140 (K-790)	98.6*	Jun. 02/2008

* These standards were received by ALS Laboratory Group, Environmental Division and had expired prior to the start of the study. Therefore they were re-certified by ALS Laboratory Group, Environmental Division before analysis was conducted.

** No purity value was supplied on the Certificate of Analysis, therefore a purity of 100% was assumed for O-Desmethyl MKH 6562-d_3.

The reference substances were logged in and then kept stored in a freezer after arrival at ALS Laboratory Group, Environmental Division. Arysta Life Science North America Corporation maintains the characterization and stability data for the reference substances.

On July 14 & 17, 2006, stock standards were prepared from the neat reference substances. On July 14 & 17, 2006, fortification standards were prepared from stock standards. On July 18 & August 3, 2006 instrument calibration standards were prepared from intermediate standards. All standards were prepared as per the method with the exception of the native N-methylcarbamate and N-methylcarbamate-d_3 internal standard, which was prepared by weighing a nominal 10 mg into a 100 mL volumetric flask. This was done in place of weighing 5 mg into a 50 mL volumetric flask due to the analytical precision of the balance in use at ALS Laboratory Group, Environmental Division. The stock standards, calibration, and fortification standards were kept stored in a freezer or refrigerator when not in use.

3.2 Control Soil

Control soil obtained from Arysta Life Science North America Corporation was used to validate the method. The control soil sample was characterized by Agvise Laboratories, Inc. The sample I.D. number was MSL-PF 0-6" and the trial I.D. was 11-1-05. The date received at Agvise was November 2/05. A copy of the characterization report can be found in Appendix 2.

The soil site was located in New York, USA.

Soil Characterization **Sample I.D.: MSL-PF 0-6"**	
Percent Sand	63%
Percent Silt	18%
Percent Clay	19%
Bulk Density (disturbed) (gm/cc)	0.98
CEC (meg/100 g)	17.9
% moisture at 1/3 bar	23.0
% OM	3.2
pH	6.6
Ca (percent/ppm)	60.5%/ 2170 ppm
Mg (percent /ppm)	22.5%/ 484 ppm
K (percent /ppm)	3.6%/ 253 ppm
Na (percent /ppm)	0.8%/ 34 ppm
H (percent /ppm)	12.5%/ 22 ppm
USDA Textural Class	Sandy Loam

3.3 Equipment and Reagents

The equipment and reagents used for the method validation were as outlined in Method 200747 (Section 3.0, Experimental, see Appendix 4). Identical or equivalent apparatus and materials were used.

4.0 METHOD AND METHOD MODIFICATIONS

4.1 Modifications

No modifications were made to any sections of the method. They were 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 3000 MS/MS system was used in place of the TSQ 700 LC/Tandem Mass Spectrometer. The instrument specifications are listed in Table 1 and Table 2.
2. A weighted 1/x linear regression curve was used for quantitation in place of a quadratic weighted $1/x^2$ as stated in the method.

4.2 Sample Preparation, Fortification, and Extraction

The validation trial consisted of one analytical set. This set consisted of 12 samples: two matrix blanks, five matrix blanks fortified at the LOQ (0.5 ppb) and five matrix blanks fortified at 10X LOQ (5.0 ppb).

Twelve 25 g portions of soil were used as samples. Samples designated as spikes were fortified with either 125 µL of a 100 µg/L standard fortification solution (for LOQ fortifications) or 125 µL of 1000 µg/L (for 10X LOQ fortifications). Spikes were mixed and left standing for 10 minutes. See detailed method below:

Extraction:

1. Screw an end cap onto the end of a 33 mL extractor body which is closest to the Dionex Logo (This is designated as the bottom of the extractor cell).

2. Insert a disposable cellulose filter in the bottom of the 33 mL extractor cell with an insertion tool.

3. Weigh 2-3 g of Hydromatrix and load it into the 33 mL extractor cell with a funnel.

4. Weigh 25 ± 0.5 g (wet weight) of sample and load it into the 33 mL extractor cell with a funnel.

5. Add 50 µL of the 1 µg/mL O-Desmethyl MKH 6562-phenyl-3,4,6-d_3 internal standard into the 25 g soil. (Note: Perform for sample analysis only, not for method validation.)

6. Label the corresponding 60 mL collection vial and place into the vial tray.

7. Operate the Accelerated Solvent Extractor (ASE) under the following conditions:

   (Due to the instability of the O-Desmethyl MKH 6562, extractions must be done within three hours after fortification. Therefore, samples may need to be put on the ASE in two or more groups.)

Solvent: methanol:water (9:1, v/v)

(The above is used for both extraction and derivatization of O-Desmethyl MKH 6562 to MKH 6562 N-methylcarbamate.)

Pressure:	1500 psi
Temperature:	80°C
Static Time:	5 min
Cycle:	1
Flush Volume:	50%
Purge Time:	60 s

8. After extraction, remove the I-Chem vials from the vial tray and place them in a Turbo Vap LV.

9. Evaporate the extract to dryness under nitrogen in a Turbo Vap LV (equipped with ASE compatible rack number 60911) at 55 60 °C.

10. Reconstitute the extract with 1.0 mL of HPLC mobile phase (i.e., 20% MeOH and 80% 5 mM ammonium acetate in water).

11. With a disposable syringe, filter the extract (about 2-3 mL) from the I-Chem vial into an HPLC autosampler vial through a 0.45 µm nylon Acrodisc®.

12. Store the extracts in a freezer until ready for LC-MS/MS analysis.

4.3 LC-MS/MS Instrumentation

All samples were analyzed using an Applied Biosystems API 3000 Triple Quadrapole Mass Spectrometer with Turbo Ion Spray Interface. The following components completed the system:

HPLC: Two Perkin Elmer Series 200 Micropumps
Autoinjector: Perkin Elmer 200
Column Heater: Waters Temp. Control module Millipore
Data System Version: Mac Quan 1.7.1

The HPLC operating parameters are shown in Table 1. The API 3000 MS/MS operating parameters are shown in Table 2.

4.4 Data Acquisition and Reporting

Peak integration and quantitation were performed by using Mac Quan, Version 1.7.1 (Apple). Quantitation of native analyte was based on five point calibration curve with a concentration range from 0.2 to 10 ppb. The peak area ratio of native to internal standard of each

compound was plotted with its standard concentration. The slope and intercept from a weighted (1/X) linear regression curve was used for quantitation of O-Desmethyl MKH 6562.

$$\text{Conc.}(ppb) = \frac{\text{Native Area} \times \text{Conc. Internal Std.}}{\text{Average R.F.} \times \text{Area Internal Std. in Sample}}$$

Recovery in Spiked Validation Samples

$$\%\,\text{Recovery} = \frac{\text{Conc.}_{NAT}}{\text{Spiked Level}} \times 100$$

Recovery results were computed for each sample and are presented in Table 3.

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

8.0 TABLES

Table 1. HPLC System

Analytical Column:	Phenomenex- Luna C18 column, 150 x 4.6 mm, 5 µm Part No. 00F-4041-E0
Mobile Phase Flow Rate:	800 µL/min
Mobile Phase A:	5 mM ammonium acetate in water
Mobile Phase B:	5 mM ammonium acetate in methanol
Run time:	15 minutes
Injection Volume:	50 µL

Mobile Phase Gradient Program:

Time (min.)	%A	%B
0	80	20
1.0	10	90
7.0	10	90
8.0	80	20
9.0	80	20

Analyte Retention Times:

Analyte	Min.
O-Desmethyl MKH 6562	7:28

Table 2. LC-MS/MS Operating Parameters

N-methylcarbamate MKH6572 and N-methylcarbamate-d3 using Positive Ion detection:

	N-Methylcarbamate	N-Methylcarbamate-d3
Q1 Mass	298	301
Q3 Mass	85	85

The MRM (multiple reaction monitoring) scan mode was used for the signal acquisition.

Interface:	Turbo Ion-Spray	
Polarity:	Positive	
Nebuliser Gas (GS1):	12	
Curtain Gas (CUR):	13 (arbitrary units)	
Temperature (TEM):	450	
Ion-Spray voltage:	3500	
Collision gas (CAD):	Nitrogen 8 (arbitrary units)	
Scan type:	MRM	
Dwell time (msec)		100
OR		-20
RNG		-130
Q0		15
IQ1		15.2
Electron multiplier setting (CEM)		2400

Table 4. Clarifications, Communication, and Recommendations to Perform Analytical Method No. 200747

Clarifications, Communication and Recommendations:

The Independent Laboratory Validation of Analytical Method 200747 did not require communication regarding the method with the Sponsor and the Study Director at ALS Laboratory Group, Environmental Division. Communications with the sponsor dealt with standard re-certification requirements and personnel/location changes.

Table 5. Calculations

Peak areas and external calibrations were used for data analysis. The Mac Quan Version 1.7.1 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.

a) Calculated Concentration in Samples:

Concentration Internal Standard = 1.0

$$\text{R.F.} = \frac{\text{Native Area} \times \text{Conc. Internal Std.}}{\text{Std. Conc.} \times \text{Internal Area of Std.}}$$

Where:
R.F. = Response Factor

$$\text{Conc. (ppb)} = \frac{\text{Native Area} \times \text{Conc. Internal Std.}}{\text{Average R.F.} \times \text{Area Internal Std. in Sample}}$$

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 3 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.