# 1.0 INTRODUCTION

This report describes the independent laboratory validation (ILV) of Analytical Method No. 200748 as performed by ALS Laboratory Group, Environmental Division for the determination of the MKH 6562 and its metabolites NODT, sulfonic acid, and sulfonamide 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 |
| Jillian Devine | Log-In and Sample Control |
| Brent Finnestad | 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 |
|---|---|---|---|
| Flucarbazone (MKH 5730) MKH 6562 acid | 0909200501 (K-1536) | 95.5 | Aug. 19/2010 |
| Flucarbazone-methyl-d3 | 0621200502 | 99.8 | Jan. 20/2015 |
| NODT | K-751 | 86.7* | June 02/2008 |
| NODT-d3 | 96B0330129 (K-704) | 99.2 | Mar. 25/2008 |
| MKH 6562 Sulfonic acid (sodium salt) | 0621200503 | 95.5 | Aug. 11/2015 |
| MKH 6562 Sulfonic acid -d3 (ammonium salt) | 0909200504 (K-1545) | 100 | Aug. 12/2015 |
| MKH 6562 Sulfonamide | 95R-31-86C (K-826) | 99.7* | June 02/2008 |
| MKH 6562 Sulfonamide-d3 | 0909200503 (K-1537) | 99.8 | Aug. 23/2015 |

* 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.

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 December 2, 2005, stock standards were prepared from the neat reference substances. On December 8, 2005 and December 19, 2005, instrument fortification and calibration standards were prepared from stock standards. All standards were prepared as per the method. The stock standards, calibration, and fortification standards were kept stored in a refrigerator when not in use. Fortification standards and internal standards prepared December 8, 2005 were used for sample extraction. Calibration standards prepared December 19, 2005 were not used, since some analytes were pending re-certification (see page 10).

On June 3, 2006, stock standards were prepared from the neat reference substances. On June 3, 2006 and June 6, 2006, instrument fortification and calibration standards were prepared from stock standards. All standards were prepared as per the method with the exception of the native sulfonic acid, which was made up in ACN only. The stock standards, calibration, and fortification standards were kept stored in a refrigerator when not in use. Calibration standards were prepared from neat, re-certified standards.

## 3.2 Control Soil

Control soil obtained from Arysta Life Science North America Corp. 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**<br>**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 200748 (Section 3.0, Materials and Apparatus, 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 the extraction or cleanup 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 PE Sciex API-III+ Mass Spectrometer. The instrument specifications are listed in Table 1 and Table 2.

### 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.

Twelve (10) g portions of soil were used as samples. Samples designated as spikes were fortified with either 500 µL of a 10.0 µg/L a mixed standard fortification solution (for LOQ fortifications) or 500 µL of 100 µg/L (for 10X LOQ fortifications). Spikes were mixed and left standing for 10 minutes. See detailed method below:

Extraction:

1. Weigh twelve representative amounts of soil (10 ± 0.05 g) into separate 50 mL glass vial. Five control samples fortified with known amounts of MKH 6562 and its three metabolites (NODT, sulfonic acid, and sulfonamide) in acetonitrile at 0.500 ppb (LOQ), and five at 5.00 ppb (10X LOQ).

2. Add 20 mL of extraction solvent [ACN/0.2 M $NH_4OAc$ with 1% HCl (4:1, v/v)] into the glass vial.

3. Shake the sample for about an hour in a mechanical shaker at ~ 130 cycles/min at ambient temperature.

4. Remove the glass vial from the shaker and centrifuge it at about 2300 rpm for about 10-15 minutes.

5. Pipette 8 mL of the extract into disposable 15 mL culture tube.

6. Add 100 µL of 0.1 µg/mL of mixed internal standard into the same culture tube.

7. Vortex the culture tube for ~ 15 seconds.

8. With Turbo Vap, evaporate the extract aliquot to ~1 mL at water bath temperature of ~40$^0$C. Add ~ 2 mL of methanol and then continue to dryness.

9. Reconstitute the residue with 1 mL of 95:5 water/100 mM ammonium acetate in methanol. Vortex for ~15 seconds and then sonicate for ~2 minutes.

10. Filter the extract with 0.45-µm filter (Nylon Acrodisc, 13 mm and 0.45 µm Gelman) into a HPLC vial.

11. Store these in a freezer until ready for LC-MS/MS.

## 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 0.25 to 25 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 MKH 6562, NODT, MKH 6562 sulfonamide and MKH 6562 sulfonic acid.

$$\text{Conc.(ppb)} = \frac{\frac{\text{Native Area}}{\text{Internal Standard Area}} - \text{Intercept}}{\text{Slope}} \times \text{Dilution Factor}$$

Recovery in Spiked Validation Samples

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

## 8.0 TABLES

### Table 1. HPLC System

Analytical Column: Phenomenex-Synergi Max-RP column, 75 x 4.6 mm, 4µm, 80 °A, Part No. 00C-4337-EO

Mobile Phase Flow Rate: 400 µL/min
Mobile Phase A: 95:5 water/100 mM ammonium acetate in methanol
Mobile Phase B: 5 mM ammonium acetate in methanol
Run time: 9.5 minutes
Injection Volume: 20 µL

Mobile Phase Gradient Program:

| Duration (min.) | %A | %B |
|---|---|---|
| 0 | 90 | 10 |
| 1.0 | 90 | 10 |
| 8.0 | 10 | 90 |
| 1.0 | 10 | 90 |
| 1.0 | 90 | 10 |
| 4.0 | 90 | 10 |

Analyte Retention Times:

| Analyte | Min. |
|---|---|
| NODT | 4.48 |
| MKH 6562 sulfonic acid | 6.44 |
| MKH 6562 | 7.35 |
| MKH 6562 sulfonamide | 8.12 |

**Table 2. LC-MS/MS Operating Parameters**

NODT and NODT-d3 using Positive Ion detection:

| | NODT | NODT-d3 |
|---|---|---|
| Q1 Mass | 130 | 133 |
| Q3 Mass | 14.5 | 114.5 |

The samples were analyzed using positive ion detection for NODT and NODT-d3. The MRM (multiple reaction monitoring) scan mode was used for the signal acquisition.

| | |
|---|---|
| Interface: | Turbo Ion-Spray |
| Polarity: | Positive |
| Nebuliser Gas (GS1): | 10 |
| Turbo Gas (GS2): | 60 |
| Curtain Gas (CUR): | 12 (arbitrary units) |
| Temperature (TEM): | 300 |
| Ion-Spray voltage: | 3000 |
| Collision gas (CAD): | Nitrogen 6 (arbitrary units) |
| Scan type: | MRM |
| Dwell time (msec) | 200 |
| OR | 46 |
| RNG | 300 |
| Q0 | -10 |
| IQ1 | -11 |
| Electron multiplier setting (CEM) | 2400 |

MKH 6562 acid, MKH 6562 acid-d3, MKH 6562, MKH 6562-d3, MKH 6562 amide, and MKH 6562-d3 using Negative Ion detection.

| | MKH 6562 acid | MKH 6562 acid-d3 |
|---|---|---|
| Q1 Mass | 241 | 244 |
| Q3 Mass | 85 | 85 |

| | MKH 6562 | MKH 6562-D3 |
|---|---|---|
| Q1 Mass | 395 | 398 |
| Q3 Mass | 127.6 | 130.6 |

| | MKH 6562 amide | MKH 6562 amide-d3 |
|---|---|---|
| Q1 Mass | 240 | 243 |
| Q3 Mass | 85 | 85 |

The samples were analyzed using negative ion detection for MKH 6562 acid, MKH 6562 acid-d3, MKH 6562, MKH 6562-d3, MKH 6562 amide, and MKH 6562-d3. The MRM (multiple reaction monitoring) scan mode was used for the signal acquisition.

| | |
|---|---|
| Interface: | Turbo Ion-Spray |
| Polarity: | Positive |
| Nebuliser Gas (GS1): | 10 |
| Turbo Gas (GS2): | 60 |
| Curtain Gas (CUR): | 12 (arbitrary units) |
| Temperature (TEM): | 300 |
| Ion-Spray voltage: | 3000 |
| Collision gas (CAD): | Nitrogen 6 (arbitrary units) |
| Scan type: | MRM |
| Dwell time (msec) | 200 |
| OR | -20 |
| RNG | -140 |
| Q0 | 10 |
| IQ1 | 11 |
| Electron multiplier setting (CEM): | 2400 |

## Table 4. Clarifications, Communication, and Recommendations to perform Analytical Method No. 200748

**Clarifications, Communication and Recommendations:**

The Independent Laboratory Validation of Analytical Method 200748 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:

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

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

$$\text{D.F.} = \frac{\text{Extraction Volume(mL)}}{\text{Aliquot Volume(mL)}} \times \frac{\text{Final Volume(mL)}}{\text{Sample Weight (g)}} = \frac{20\,\text{mL}}{8\,\text{mL}} \times \frac{1.0\,\text{mL}}{10.0\,\text{g}} = 0.250$$

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.