Independent Laboratory Validation of Dow AgroSciences Method GRM 01.32—Determination of Residues of Aminopyralid in Water by Liquid Chromatography with Tandem Mass Spectrometry

INTRODUCTION

An independent laboratory validation (ILV) study was conducted following both a draft and the final version of Dow AgroSciences LLC residue analytical method GRM 01.32, "Determination of Residues of Aminopyralid in Water by Liquid Chromatography with Tandem Mass Spectrometry Detection", dated November 7, 2003 (Appendix A) and December 9, 2003

(Appendix B), respectively. For the purpose of conducting the independent laboratory validation, the draft method is essentially the same as the final version with an effective date of December 9, 2003. The ILV study was conducted to fulfill the requirements described in U.S. EPA guidelines (References 1, 2, 3 and 4). Surface water, specifically river water, was arbitrarily selected as representative sample matrix for the study as no one water type seemed to be more difficult to analyze than another.

Method GRM 01.32 was developed and initially validated at Dow AgroSciences. The independent laboratory, the Study Director, and the analysts chosen to conduct the ILV were unfamiliar with the method, both in its development and in its subsequent use in analyzing field samples. The independent lab used its own equipment and supplies, so that there was no common link in equipment between Dow AgroSciences and the Study Director and/or the analysts. Throughout the conduct of the study, any communications between Dow AgroSciences and the Study Director and/or the analysts were logged for inclusion in the report. No one from Dow AgroSciences visited Morse Laboratories, Inc., during the ILV trial to observe, offer help, or to assist with the method evaluation. These steps successfully maintained the integrity of the ILV study.

ANALYTICAL

Sample Numbering, Preparation, and Storage

One water sample (~0.5 liter) was collected from the American River, near Sacramento, California by Morse Labs personnel for use in the study. Upon arrival at the laboratory, it was uniquely identified (1111A), then immediately placed in temperature-monitored refrigerated storage. The sample was stored refrigerated (1-8°C) except when removed for sample analysis.

Preparation of Solutions and Standards

All reagent solutions were prepared as described in Subsection 6.3. of method GRM 01.32 with the following exceptions:

Step 6.3.1. acetonitrile/pyridine:butanol (22:2:1) was prepared as follows:

Note: Only 500 mL of reagent was prepared rather than 1000 mL as specified in the method.

To a 500-mL graduated mixing cylinder, 440 mL of acetonitrile, 40 mL of pyridine and 20 mL of butanol were added. The contents were mixed well by multiple inversions of the cylinder. The resulting mixture was transfer to a 1-L solvent bottle.

The following analytical reference standards/test substances were utilized during the independent laboratory method validation:

Standards	AGR/TSN No.	Percent Purity	Certification Date	Reference
aminopyralid (XDE-750)	TSN102298	99.9	04-Oct-2002	FA&PC 023235
aminopyralid-1-^{15}N-2,6-^{13}C[a]	NA	NA	NA	E-1145-19

[a]Stable isotope (internal standard)

Standard solutions were prepared as described in Section 7. of method GRM 02.31 with the following exceptions:

1) Step 7.2.1. A stock solution of aminopyralid stable isotope was prepared at 76 µg/mL, rather than at 100 µg/mL as specified in the method. This was due to the smaller amount of analytical standard received from the Sponsor.

2) Step 7.2.2 In order to prepare a 1.0-μg/mL internal standard (stable isotope) solution, 6.579 mL of 76 μg/mL was diluted to a final volume of 500 mL.

3) Step 7.2.3. In order to prepare a 1.0-μg/mL internal standard (stable isotope) solution, 1.316 mL of the stock solution (76 μg/mL) was diluted to a final volume of 100 mL.

Fortification of Recovery Samples

The ILV trial was conducted on river water. The sample set composition was as follows:

One reagent blank
Two unfortified control samples
Five control samples fortified with aminopyralid at 0.05 μg/L (LOQ)
Five control samples fortified with aminopyralid at 0.50 μg/L (10 × LOQ)

Sample Extraction and Analysis

Residues of aminopyralid were extracted from an acidified water sample using a polymeric solid phase extraction cartridge (SPE). The SPE cartridge was washed with a water:methanol:formic acid solution (90:10:1) and eluted with a methyl-*tert*-butyl ether:methanol solution (90:10). An aliquot of an aminopyralid stable isotope solution was added to the sample, and the eluate was evaporated to dryness. The residue was reconstituted in an acetonitrile:pyridine:butanol (22:2:1) solution, and the sample and standards were derivatized with butyl chloroformate. After derivatization, both standard and sample mixtures were brought to a final volume of 1.0 mL with a methanol:water:acetic acid mobile phase (50:50:0.1) and analyzed by high performance liquid chromatography with positive-ion electrospray (ESI) tandem mass spectrometry (LC/MS/MS).

All trials were conducted exactly as described in method GRM 01.32 with the following exceptions:

Step 9.3.6. Evaporated the eluate in a 16 × 100 mm test tube using an N-Evap evaporator set at 40°C instead of a TurboVap evaporator.

Analytical Instrumentation

The following instruments were utilized and are considered equivalent to those described in Section 8. of method GRM 01.32:

Instrumentation:

Liquid Chromatograph/Tandem Mass Spectrometer

- Mass Spectrometer – PE Sciex API 2000 (MSD-29-02)
- Liquid Chromatograph – Two Shimadzu LC10ADvp pumps (PS-20-02, PS-21-02)
- Autosampler – Perkin Elmer Series 200 (AS-28-02)
- Solvent Degasser – Shimadzu DGU-14A (VB-05-02)
- System Controller – Shimadzu SCL10Avp (CN-15-02)
- Mass Spectrometer Data System – PE Sciex Analyst 1.1 data system
- Computer – Dell Optiplex, GX400 (CT-49-02)
- Printer - Laser jet HP4050 printer (CP-37-00)

Operating Parameters:

Liquid Chromatography

Column:	Diazem 3000, C18, 4.6 mm × 100 mm, 3 µm, Morse Labs column #511
Column Temperature:	35°C
Injection Volume:	100 µL
Run Time:	9.0 minutes
Mobile Phase:	A – water with 0.1% acetic acid B – methanol with 0.1% acetic acid

Flow Rate: 900 µL/min, flow diverted to source between 3.0 minutes and 4.5 minutes

Gradient:

Time, min	A, %	B, %
0.0	50	50
5.0-6.0	0	100
6.1-9.0	50	50

Mass Spectrometry (MS/MS)

API 2000:

Interface:	TIS (TurboIonSpray)
Scan Type:	MRM
Resolution:	Q1 – unit, Q3 – low
Curtain Gas (CUR):	40
Collision Gas (CAD):	12
Temperature (TEM):	425 °C
Ion Source Gas 1 (GS1):	45
Ion Source Gas 2 (GS2):	75
Period 1	
Time:	6 minutes
Polarity:	Positive
Ion Spray Voltage	5000

Compound	Ion, *m/z* Q1	Q3	Time, *ms*	CE, *v*
Aminopyralid butyl ester	263.2	134.1	150	49
Aminopyralid stable isotope butyl ester	268.2	139.1	150	49

Analytical Equipment and Materials

Equipment and materials were utilized in the conduct of the independent validation as described in method GRM 01.32 with the following exceptions (which are considered to be equivalent substitutions):

Equipment:

(1) Step 4.1.2. A Model 112 N-Evap evaporator was substituted for a TurboVap evaporator.

(2) Step 4.1.3. A Finnpipette 5-40 μL adjustable pipettor was substituted for an Eppendorf 5-100 μL adjustable pipettor.

(3) Step 4.1.4. A Finnpipette 40-200 μL adjustable pipettor was substituted for an Eppendorf 20-300 μL adjustable pipettor.

(4) Step 4.1.5. A Finnpipette 200-1000 μL adjustable pipettor was substituted for an Eppendorf 50-1000 μL adjustable pipettor.

(5) Step 4.1.8. A Varian Vac Elut SPS 24 manifold was substituted for a VacMaster-20 vacuum manifold.

(6) Step 4.1.9. A Thermolyne Maximix II vortex mixer was substituted for a Scientific Industries Model G-560 vortex mixer.

Reagents:

Section 6.1: The following substitutions (which are considered to be equivalent to what was used in the method) were made regarding reagents:

1. Burdick and Jackson HPLC grade acetonitrile was substituted for Mallinckrodt Baker brand
2. Burdick and Jackson, HPLC grade methanol was substituted for Mallinckrodt HPLC grade

Additional equipment used:

Analytical Balance: Mettler AB104 (B-20-97)

Top-Loading Balance: Mettler PE 1600 (B-02-86)

Pipettor: Finnpipette, adjustable, 5-40 μL, Fisher Scientific (AP-13-94)

Pipettor: Finnpipette, adjustable, 40-200 μL, Fisher Scientific (AP-05-89)

Pipettor: Finnpipette, adjustable, 200-1000 μL, Fisher Scientific (AP-30-96)

Vacuum pump: Welch Gem 1.0 vacuum pump (P-50-93)

Vacuum manifold: Vac Elut SPS 24 manifold, Varian (MC-26-93)

Vortex mixer: Thermolyne Maximix II (M-11-88)

Evaporator: N-Evap, Model 112, Organomation Associates, Inc. (SB-07-04)

Culture tubes: 16 × 100 mm, VWR Scientific

Computer: Dell Optiplex, GX400 (CT-49-02)

Microsoft Excel '97 for Windows, Microsoft Corporation

Calculations

The percent recovery of aminopyralid from river water was calculated as described in Section 10. of method GRM 01.32. Calibration standards for aminopyralid (0.0001 μg/mL, 0.0005 μg/mL, 0.001 μg/mL, 0.005 μg/mL, 0.01 μg/mL, 0.02 μg/mL, 0.035 μg/mL, each containing 0.025 μg/mL of $^{13}C_2{}^{15}N$-aminopyralid stable isotope internal standard) were analyzed with the sample set. Also, a 0.025-μg/mL aminopyralid stable isotope crossover standard (containing 0.025 μg/mL of $^{13}C_2{}^{15}N$-aminopyralid stable isotope internal standard only) was included in the sample set for isotopic crossover determination. The calculation process involved two steps: 1) determination of isotopic crossover, and 2) calculation of residue found after correction for isotopic crossover.

1. Determination of Isotopic Crossover

In this assay, the analyte and its stable isotope internal standard were quantified using MS/MS transitions characteristic of each compound. When using stable-isotope labeled internal standards, there is a possibility that isotopic contributions will occur between the transitions used for quantitation of the unlabeled and labeled compounds. As discussed in the method, the only isotopic overlap of consequence is the crossover of the $^{13}C_2{}^{15}N$-aminopyralid stable isotope internal standard to aminopyralid (ISTD→analyte). A 0.025 μg/mL aminopyralid stable isotope crossover standard was analyzed to determine a "crossover factor", which is an internal standard quantitation ratio with respect to isotopic contribution of the $^{13}C_2{}^{15}N$-aminopyralid internal standard to the

aminopyralid. It was subsequently used to correct quantitation ratios used to construct the analyte standard curve and residues in the samples. The transitions measured were: *m/z* 263→134 for aminopyralid and *m/z* 268→139 for $^{13}C_2{}^{15}N$-aminopyralid. The crossover factor was calculated as follows using the data derived from the stable isotope crossover standard:

$$\text{Crossover Factor (ISTD} \rightarrow \text{analyte)} = \frac{\text{peak area at } m/z\ 263 \rightarrow 134}{\text{peak area at } m/z\ 268 \rightarrow 139}$$

Example Calculation of Crossover Factor:

The crossover factor used for analytical set #2, river water, was calculated as follows:

Stable isotope crossover standard (ISTD→analyte):
peak area *m/z* 263→134 = 210
peak area *m/z* 268→139 = 77600

$$\therefore \text{Crossover Factor (ISTD} \rightarrow \text{analyte)} = \frac{210}{77600} = 0.002706$$

The resulting crossover factor was used to correct the quantitation ratios discussed in the next section.

2. Calculation of Residue Found

A validated software application (GraphPad Prism®, version 3.03) was used to generate a standard curve for aminopyralid from a set of standard concentrations (in µg/mL) versus their respective quantitation ratios. A quantitation ratio for each standard was determined by dividing the peak area for aminopyralid transition (m/z 263→134) by the peak area for the stable isotope internal standard transition (m/z 268→139).

$$\text{Quantitation ratio (uncorrected)} = \frac{\text{peak area of aminopyralid (m/z } 263 \rightarrow 134)}{\text{peak area of stable isotope internal standard (m/z } 268 \rightarrow 139)}$$

For each standard concentration, the uncorrected quantitation ratio was corrected for the for the isotopic contribution of the $^{13}C_2{}^{15}N$-aminopyralid internal standard to the aminopyralid as follows:

$$\text{Quantitation Ratio (corrected)} = \text{Quantitation Ratio (uncorrected)} - \text{Crossover Factor}$$

A quadratic non-linear regression equation was used to determine concentrations of the analyte found in the sample. It was the most appropriate equation for use in this study that best defined the relationship between concentration and quantitation ratio with the detector used over the range of fortification levels evaluated. The equation is:

$$y = a + bx + cx^2$$

where:

y	=	corrected quantitation ratio
x	=	µg/mL found for peak of interest
a. b, c	=	factors derived from calculation of standard curve

Note: A standard curve was generated by plotting the standard concentration (in µg/mL) on the x-axis and the respective corrected quantitation ratio on the y-axis.

Using the standard curve generated to determine µg/mL of aminopyralid found, the concentration (in µg/L) of residue in the sample was determined using the following equation:

$$\text{aminopyralid (gross } \mu\text{g/L)} = \text{aminopyralid } (\mu\text{g/mL}) \times \left(\frac{1000}{10.0 \text{ mL}} \times 1.0 \text{ mL} \right)$$

where:

aminopyralid (µg/mL)	=	µg/mL analyte found from standard curve
1000	=	converts mL to L
10.0 mL	=	volume of water sample taken through procedure
1.0 mL	=	final volume of extract submitted to HPLC analysis

3. Calculation of Percent Recovery

The following equations were used:

$$\text{net } \mu\text{g/L in fortified control} = (\mu\text{g/L fort. cont.}) - \left[\frac{(\mu\text{g/L cont. 1}) + (\mu\text{g/L cont. 2})}{2} \right]$$

$$\%\ \text{Recovery} = \frac{\text{net } \mu\text{g/L in fort. cont.}}{\text{fortification level } (\mu\text{g/L})} \times 100$$

Example Calculations

1. Crossover factor, River water, Set 2 (Figure 8):

Stable isotope crossover standard (ISTD→analyte):
peak area m/z 263→134 = 210
peak area m/z 268→139 = 77600

$$\text{Crossover Factor (ISTD} \rightarrow \text{analyte)} = \frac{210}{77600} = 0.002706$$

2. River water, 1111A, Control 2, Set 2, dil. factor = 1 (Figure 10):

Peak area aminopyralid (m/z 263→134): 202
Peak response, internal standard (m/z 217): 60000

$$\text{Quantitation ratio (uncorrected)} = \frac{202}{60000} = 0.003367$$

$$\text{Quantitation ratio (corrected)} = 0.003367 - 0.002706 = 0.000660$$

0.000660 from standard curve = 0.0000378864 µg/mL

$$\text{gross µg/L} = 0.0000378864 \text{ µg/mL} \times \left(\frac{1000}{10.0 \text{ mL}} \times 1.0 \text{ mL} \right)$$

$$= 0.00378864$$

Reported µg/L (gross) = (0.00379)

3. River water, 1111A, Fortified Control 2 @ 0.05 µg/L, Set 2, dil. factor = 1 (Figure 11):

Peak area aminopyralid (*m/z* 263→134): 3090
Peak response, internal standard (*m/z* 217): 59100

$$\text{Quantitation ratio (uncorrected)} = \frac{3090}{59100} = 0.052284$$

$$\text{Quantitation ratio (corrected)} = 0.052284 - 0.002706 = 0.049578$$

0.049578 from standard curve = 0.0005619659 µg/mL

$$\text{gross } \mu g/L = 0.0005619659\ \mu g/mL \times \left(\frac{1000}{10.0\ mL} \times 1.0\ mL \right)$$

$$= 0.05619659$$

$$\text{Reported } \mu g/L \text{ (gross)} = 0.0562$$

$$\text{net } \mu g/L \text{ in fortified control} = 0.0562\ \mu g/L - \left[\frac{(0.00378864\ \mu g/L) + (0.000\ \mu g/L)}{2} \right]$$

$$\text{net } \mu g/L = 0.0543$$

$$\%\ \text{Recovery} = \frac{0.0543\ \mu g/L}{0.05\ \mu g/L} \times 100$$

$$\text{Recovery} = 109\%$$

Statistical Treatment of Data

Statistical methods used were limited to calculation of the means, standard deviations and relative standard deviations. A validated software program, Microsoft Excel '97, was employed to develop all statistical data.