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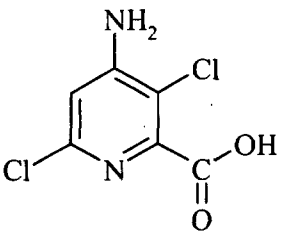
Determination of Residues of Aminopyralid in Drinking Water, Ground Water, and Surface Water by Liquid Chromatography with Tandem Mass Spectrometric Detection

B. M. Wendelburg and E. L. Olberding

1. SCOPE

This method is applicable for the quantitative determination of residues of aminopyralid (4-amino-3,6-dichloro-2-pyridinecarboxylic acid) in drinking water, ground water, and surface water. The method was validated over the concentration range of 0.05-5.00 µg/L with a validated limit of quantitation of 0.05 µg/L.



Aminopyralid
CAS No. 150114-71-9

Common and chemical names, molecular formulas, and the nominal masses for the above structure and related compounds are given in Table 1.

2. PRINCIPLE

Residues of aminopyralid are extracted from an acidified water sample using a reverse phase polymeric solid-phase extraction (SPE) cartridge. After elution from the SPE cartridge with a methyl *tert*-butyl ether/methanol (90:10) solution, a stable-isotope labeled internal standard ($^{13}C_2{}^{2}H^{15}N$-aminopyralid) is added, and the eluate is then evaporated to dryness. The residue is reconstituted in an acetonitrile/pyridine/1-butanol (22:2:1) solution, and derivatized with butyl chloroformate to form the 1-butyl esters (1-BE) of the analyte and internal standard. After derivatization, the mixture is diluted with a methanol/water (40:60) solution containing 0.05% formic acid and 5 mM ammonium formate and then analyzed by liquid chromatography with positive-ion electrospray tandem mass spectrometry (LC/MS/MS).

3. SAFETY PRECAUTIONS

3.1. Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Safety information on non Dow AgroSciences LLC products should be obtained from the container label or from the supplier. Disposal of reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.

3.2. Acetonitrile, 1-butanol, butyl chloroformate, methanol, methyl *tert*-butyl ether, and pyridine are flammable and should be used in well-ventilated areas away from ignition sources.

3.3. Butyl chloroformate, formic acid, and sulfuric acid are corrosive and toxic and can cause severe burns. It is imperative that proper eye and personal protection equipment be worn when handling these reagents.

3.4. Pyridine is a possible carcinogen. It is imperative that proper eye and personal protection equipment be worn when handling this reagent.

4. EQUIPMENT (Note 12.1.)

4.1. Laboratory Equipment

4.1.1. Balance, analytical, Model AE100, Mettler-Toledo, Inc., Columbus, OH 43240.

4.1.2. Balance, pan, Model PG2002, Mettler-Toledo, Inc.

4.1.3. Evaporator, N-Evap, Model 111, Organomation Associates, Inc., South Berlin, MA 01503.

4.1.4. Pipet, electronic, Eppendorf, 100-5000 µL, catalog number 022461346, Brinkmann Instruments, Inc., Westbury, NY 11590. (Note 12.2.)

4.1.5. Pipet, positive displacement, Pos-D, 200-1000 µL, catalog number MR-1000, Gilson, Inc., Middleton, WI 53562.

4.1.6. Pipet, positive displacement, Pos-D, 10-100 µL, catalog number MR-100, Gilson, Inc.

4.1.7. Pipet, positive displacement, Pos-D, 0.5-10 µL, catalog number MR-10, Gilson, Inc.

4.1.8. Vacuum manifold, Model spe-12G, Mallinckrodt Baker, Inc., Phillipsburg, NJ 08865.

4.1.9. Vortex mixer, Model G-560, Scientific Industries, Inc., Bohemia, NY 11716.

4.2. Chromatographic System

4.2.1. Column, analytical, Zorbax SB-C8, 4.6 x 75 mm, 3.5-µm particle size, catalog number 7995208-344, Agilent Technologies, Wilmington, DE 19808.

4.2.2. Liquid chromatograph autosampler, Model 1100, Agilent Technologies.

4.2.3. Liquid chromatograph binary pump, Model 1100, Agilent Technologies.

4.2.4. Liquid chromatograph column oven, Model 1100, Agilent Technologies.

4.2.5. Liquid chromatograph vacuum degasser, Model 1100, Agilent Technologies.

4.2.6. Mass spectrometer, Model API 3000, Applied Biosystems, Foster City, CA 94404.

4.2.7. Mass spectrometer data system, Model Analyst 1.4.1, Applied Biosystems.

5. GLASSWARE AND MATERIALS (Note 12.1.)

5.1. Cap, 96-well piercable sealing, catalog number 121-5204, Argonaut Technologies, Redwood City, CA 94063.

5.2. Cartridges, SPE, Strata-X, 3-mL, 60-mg packing, catalog number 8B-S100-UBJ, Phenomenex Inc.

5.3. Collection plate, polypropylene 96-well, 2 mL/well, catalog number AH0-7194, Phenomenex, Inc., Torrance, CA 90501.

5.4. Cylinder, graduated mixing, 250-mL, catalog number 08-531E, Fisher Scientific, Pittsburgh, PA 15275.

5.5. Cylinder, graduated mixing, 2000-mL, catalog number 08-531H, Fisher Scientific.

5.6. Flask, volumetric, 10-mL, catalog number 10-209A, Fisher Scientific.

5.7. Flask, volumetric, 100-mL, catalog number 10-209D, Fisher Scientific.

5.8. Flask, volumetric, 200-mL, catalog number 10-209E, Fisher Scientific.

5.9. Flask, volumetric, 250-mL, catalog number 10-209F, Fisher Scientific.

5.10. Pipet tips, Eppendorf, 100-5000 µL, catalog number 022491989, Brinkmann Instruments, Inc.

5.11. Pipet tips, Pos-D, 200-1000 µL, catalog number CP-10, Gilson, Inc.

5.12. Pipet tips, Pos-D, 10-100 µL, catalog number CP-100, Gilson, Inc.

5.13. Pipet tips, Pos-D, 0.5-10 µL, catalog number CP-1000, Gilson, Inc.

5.14. Pipet, transfer, 5.75-in, catalog number 13-678-20B, Fisher Scientific.

5.15. Pipet, volumetric, 1.0-mL, catalog number 13-650-2B, Fisher Scientific.

5.16. Pipet, volumetric, 2.0-mL, catalog number 13-650-2C, Fisher Scientific.

5.17. Pipet, volumetric, 10-mL, catalog number 13-650-2L, Fisher Scientific.

5.18. Pipet, volumetric, 20-mL, catalog number 13-650-2N, Fisher Scientific.

5.19. Pipet, volumetric, 30-mL, catalog number 13-650-2Q, Fisher Scientific.

5.20. Pipet, volumetric, 80-mL, catalog number 13-650-2S, Fisher Scientific.

5.21. Reservoir, 10-mL SPE, catalog number 120-1004-G, International Sorbent Technology Ltd, Hengoed, Mid Glamorgan UK and distributed by Biotage, Foxboro, MA 02035.

5.22. Vial, 8-mL, with PTFE-lined screw cap, catalog number 03-340-60B, Fisher Scientific.

5.23. Vial, 40-mL, with PTFE-lined screw cap, catalog number 03-971-7G, Fisher Scientific.

6. REAGENTS, STANDARDS, AND PREPARED SOLUTIONS (Note 12.1.)

6.1. Reagents

6.1.1. Acetonitrile, Chrom AR HPLC grade, catalog number 2856, Mallinckrodt Baker, Inc.

6.1.2. Ammonium formate, HPLC grade, catalog number A666-500, Fisher Scientific.

6.1.3. 1-Butanol, 99.8% purity, catalog number 28,154-9, Sigma-Aldrich, St. Louis, MO 63178.

6.1.4. Butyl chloroformate, 98% purity, catalog number 18,446-2, Sigma-Aldrich.

6.1.5. Formic acid, 95%, ACS reagent grade, catalog number F0507-100ML, Sigma-Aldrich.

6.1.6. Methanol, ChromAR HPLC grade, catalog number 3041, Mallinckrodt Baker Inc.

6.1.7. Methyl *tert*-butyl ether, HPLC grade, catalog number E127-4, Fisher Scientific.

6.1.8. Nitrogen, refrigerated liquid, catalog number LQNI, BOC Gases, New Providence, NJ 07974.

6.1.9. Pyridine, 99.9+% purity, catalog number 27,040-7, Sigma-Aldrich.

6.1.10. Sulfuric acid, concentrated, certified ACS plus grade, catalog number A300-500, Fisher Scientific.

6.1.11. Water, HPLC grade, catalog number WX0004-1, EMD Chemicals, Gibbstown, NJ 08027.

6.2. Standards

6.2.1. aminopyralid (4-amino-3,6-dichloro-2-pyridinecarboxylic acid)

Obtain from Test Substance Coordinator, Dow AgroSciences LLC, 9330 Zionsville Road, Building 304, Indianapolis, IN 46268-1054.

6.2.2. $^{13}C_2{}^{2}H^{15}N$-aminopyralid (4-amino-3,6-dichloropicolinic acid-1-^{15}N-2,6-^{13}C-5-*d*)

Obtain from Specialty Synthesis Group, Dow AgroSciences LLC, 9330 Zionsville Road, Building 306, Indianapolis, IN 46268-1054.

6.3. Prepared Solutions

6.3.1. derivatization coupling reagent — acetonitrile/pyridine/1-butanol (22:2:1) (v/v/v)

Pipet 10.0 mL of 1-butanol and 20.0 mL of pyridine into a 250-mL volumetric flask containing approximately 150 mL of acetonitrile. Swirl the flask, and allow to equilibrate to room temperature. Dilute to volume with acetonitrile.

6.3.2. methanol with 0.05% formic acid and 5 mM ammonium formate

Weigh 0.63 g of ammonium formate into a 40-mL vial and quantitatively transfer with 100 mL methanol to a 2-L graduated mixing cylinder containing approximately 1800 mL of methanol. Add 1.0 mL of formic acid, dilute to volume with methanol, and mix well.

6.3.3. methyl *tert*-butyl ether/methanol (90:10)

Combine 225 mL of methyl *tert*-butyl ether and 25 mL of methanol in a 250-mL graduated mixing cylinder. Place a glass stopper on the cylinder and invert several times to mix. Allow the solution to equilibrate to room temperature before use.

6.3.4. water with 0.05% formic acid and 5 mM ammonium formate

Weigh 0.63 g of ammonium formate into a 40-mL vial and quantitatively transfer with 100 mL HPLC water to a 2-L graduated mixing cylinder containing approximately 1800 mL of water. Add 1.0 mL of formic acid, dilute to volume with water, and mix well.

6.3.5. water/methanol (60:40) with 0.05% formic acid and 5 mM ammonium formate

Pipet 80 mL of methanol with 0.05% formic acid and 5 mM ammonium formate (Section 6.3.2.) into a 200-mL volumetric flask containing approximately 80 mL of water with 0.05% formic acid and 5 mM ammonium formate (Section 6.3.4.). Swirl the flask, and allow to equilibrate to room temperature. Dilute to volume with water with 0.05% formic acid and 5 mM ammonium formate (Section 6.3.4.).

6.3.6. water/methanol (90:10) with 1% formic acid

Combine 180 mL of HPLC grade water and 20 mL of methanol in a 250-mL graduated mixing cylinder. Pipet 2.0 mL of formic acid into the solvent mixture. Place a glass stopper on the cylinder and invert several times to mix. Allow the solution to equilibrate to room temperature before use.

6.3.7. water/conc. sulfuric acid (99.50:0.5)

Carefully pipet 1.0 mL of conc. sulfuric acid into a 250-mL graduated mixing cylinder containing approximately 180 mL of HPLC water. Dilute to 200 mL with HPLC water. Place a glass stopper on the cylinder and invert several times to mix. Allow the solution to equilibrate to room temperature before use.

7. PREPARATION OF STANDARDS

7.1. Preparation of Aminopyralid Spiking Solutions for Samples

7.1.1. Weigh 0.1000 g of aminopyralid analytical standard and quantitatively transfer to a 100-mL volumetric flask with acetonitrile. Dilute to volume with acetonitrile to obtain a 1000-µg/mL stock solution.

7.1.2. Prepare solutions for spiking samples by diluting the solution from Section 7.1.1 with acetonitrile as follows:

Concentration of Stock Solution	Aliquot Of Stock Solution	Final Solution Volume	Concentration of Spiking Solution	Equivalent Sample Concentration[a]
µg/mL	mL	mL	µg/mL	µg/L
1000	10.0	100	100.0	- - -
100	10.0	100	10.0	- - -
10.0	10.0	100	1.00	5.00
1.00	10.0	100	0.10	0.50
0.10	10.0	100	0.01	0.05
0.01	30.0	100	0.003	0.015

[a] The equivalent sample concentration is based on fortifying a 10.0-mL sample with 50 µL of spiking solution.

7.2. Preparation of the $^{13}C_2{}^{2}H^{15}N$-Aminopyralid Internal Standard Solution

7.2.1. Accurately weigh 0.0025 g of $^{13}C_2{}^{2}H^{15}N$-aminopyralid standard and quantitatively transfer to a 100-mL volumetric flask with acetonitrile. Dilute to volume with acetonitrile to obtain a 25.0-µg/mL stock solution.

7.2.2. Using a volumetric pipet, dispense 1.0 mL of the 25.0-µg/mL solution in Section 7.2.1 into a 200-mL volumetric flask and dilute to volume with acetonitrile to obtain a 0.125-µg/mL (125-ng/mL) stock solution.

7.3. Preparation of Aminopyralid 1-Butyl Ester Calibration Standards for Quantitation

7.3.1. Using a positive displacement pipet, dispense aliquots of the 0.01-10.0-µg/mL spiking solutions from Section 7.1.2 into a series of 10-mL volumetric flasks and dilute to volume with derivatization coupling reagent solution (Section 6.3.1.). The concentration of the calibration solutions are as shown in the fourth column in the following table:

Concentration of Spiking Solution	Aliquot Of Spiking Solution	Final Solution Volume	Concentration of Calibration Solution	Concentration of Derivatized Calib. Standard	Equivalent Sample Concentration
µg/mL	mL	mL	ng/mL	a.e. ng/mL	µg/L
0.01	0.150	10.0	0.150	0.030	0.015
0.01	0.500	10.0	0.500	0.100	0.050
0.10	0.125	10.0	1.25	0.250	0.125
0.10	0.250	10.0	2.50	0.500	0.250
0.10	0.500	10.0	5.00	1.00	0.500
1.00	0.125	10.0	12.5	2.50	1.25
1.00	0.250	10.0	25.0	5.00	2.50
1.00	0.500	10.0	50.0	10.0	5.00
1.00	1.000	10.0	100.0	20.0	10.00
10.0	0.125	10.0	125.0	25.0	12.50

7.3.2. Prepare calibration standards with each sample set by dispensing 80 µL of the internal standard solution containing 125 ng/mL of $^{13}C_2{}^2H^{15}N$-aminopyralid (Section 7.2.2.) into a series of 12-mL vials.

7.3.3. Evaporate the internal standard solution to dryness using an N-Evap evaporator set at 40 °C and a nitrogen flow rate of approximately 500 mL/min.

7.3.4. Using a positive displacement pipet, dispense 200 µL of the 0.15-125.0-ng/mL calibration solutions from Section 7.3.1 into the series of vials in Step 7.3.3.

7.3.5. Derivatize the calibration standards (Section 7.3.4.) by pipetting 10 µL of butyl chloroformate derivatizing reagent into the vials.

7.3.6. Vortex the sample vials and allow the mixture to react for 5 minutes.

7.3.7. Add 790 µL of the methanol/water (40:60) solution with 0.05% formic acid and 5 mM ammonium formate to the sample vials. Vortex mix for approximately 10 seconds, transfer to a 96-well plate, and firmly seal with a cap. The final concentration range of these calibration standards is from 0.030-25.0 ng/mL, as shown in the fifth column in the table above.

7.4. Preparation of the Aminopyralid 1-Butyl Ester Standard to Determine Isotopic Crossover

7.4.1. Using a positive displacement pipet, dispense 100 µL of the 0.10-µg/mL aminopyralid solution in Section 7.1.2 into an 8-mL vial and derivatize according to the procedure described in Section 9.3.6-9.3.10. The resulting solution contains aminopyralid 1-butyl ester equivalent to 10.0 ng/mL of aminopyralid.

7.5. Preparation of the $^{13}C_2{}^2H^{15}N$-Aminopyralid 1-Butyl Ester Standard to Determine Isotopic Crossover

7.5.1. Using a positive displacement pipet, dispense 80 µL of the 125-ng/mL $^{13}C_2{}^2H^{15}N$-aminopyralid solution in Section 7.2.2 into an 8-mL vial and derivatize according to the procedure described in Section 9.3.6-9.3.10. The resulting solution contains $^{13}C_2{}^2H^{15}N$-aminopyralid 1-butyl ester equivalent to 10.0 ng/mL of $^{13}C_2{}^2H^{15}N$-aminopyralid.

8. INSTRUMENTAL CONDITIONS

8.1. Typical HPLC Operating Conditions (Note 12.3.)

Instrumentation:	Agilent Model 1100 autosampler Agilent Model 1100 binary pump Agilent Model 1100 degasser MDS/Sciex API 3000 LC/MS/MS System MDS/Sciex Analyst 1.4.1 data system
Column:	Zorbax SB-C8 4.6 x 75 mm, 3.5-µm
Column Temperature:	35 °C
Injection Volume:	35 µL
Run Time:	8.0 minutes
Mobile Phase:	A – methanol with 0.05% formic acid and 5 mM ammonium formate B – water with 0.05% formic acid and 5 mM ammonium formate
Flow Rate:	900 µL/min (approx 200 µL/min split to source)

Gradient:

Time, min	Solvent A, %	Solvent B, %
0.0	40	60
1.0	40	60
6.0	100	0
8.0	100	0

Flow Diverter Program:	1) 0.0 to 4.5 min: flow to waste 2) 4.5 to 6.5 min: flow to source 3) 6.5 to 7.0 min: flow to waste
Equilibration Time:	3.0 minutes

8.2. Typical Mass Spectrometry Operating Conditions (Note 12.3.)

Interface:	Electrospray
Scan Type:	MRM
Resolution:	Q1 – unit, Q3 – unit
Nebulizer Gas (NEB):	13
Curtain Gas (CUR):	9
Collision Gas (CAD):	7

Temperature (TEM):	425 °C
Ion Source Gas 1 (GS1):	8 psi
Ion Source Gas 2 (GS2):	7000 mL/min
Declustering Potential:	34 volts
Focusing Potential:	170 volts
Entrance Potential:	9 volts
IonSpray Voltage (IS):	1300 volts
Pre-acquisition Delay:	0.0 minutes
Acquisition Time:	7.0 minutes
Polarity:	Positive

Analytes:	Precursor Ion Q1	Product Ion Q3	Collision Time, ms	Cell Exit Potential	Collision Energy
aminopyralid BE (quantitation)	263.1	189.0	150	14	25
aminopyralid BE (confirmation 1)	263.1	161.1	150	12	39
aminopyralid BE (confirmation 2)	263.1	134.1	150	10	57
$^{13}C_2{}^2H^{15}N$-aminopyralid BE	269.1	194.9	150	14	27

8.3. Mass Spectra

Full-scan and product-ion mass spectra of aminopyralid 1-butyl ester showing the $(M+H)^+$ at *m/z* 263 and the product ions at *m/z* 189, *m/z* 161, and *m/z* 134 are illustrated in Figure 1. Full-scan and product-ion mass spectra of $^{13}C_2{}^2H^{15}N$-aminopyralid 1-butyl ester showing the $(M+H)^+$+2 at *m/z* 269 and the product ion at *m/z* 195 are illustrated in Figure 2.

8.4. Typical Calibration Curve

A typical calibration curve for the determination of aminopyralid in water is shown in Figure 3.

8.5. Typical Chromatograms

Typical chromatograms of a standard, a control sample, a 0.05-µg/L (LOQ) recovery sample, and a 5.00-µg/L recovery sample for the determination of aminopyralid in water are illustrated in Figures 4-13.

9. DETERMINATION OF RECOVERY OF AMINOPYRALID FROM WATER

9.1. Method Validation

Validate the analytical procedure given in Section 9.3 by analyzing the following with each sample set:

At least one reagent blank.
At least one unfortified control.
At least one control fortified at the limit of detection.
At least two controls fortified at the limit of quantitation.
At least two controls fortified at the expected residue concentration in the samples.

9.2. Sample Preparation

No sample preparation is required for water samples. Samples should be stored refrigerated or deep frozen prior to analysis.

9.3. Sample Analysis

9.3.1. Pipet a 10.0-mL aliquot (or weigh a 10.0 ± 0.1-g aliquot) of the water sample into a 40-mL vial.

9.3.2. For recovery samples, add 50-µL aliquots of the 0.003-, 0.01-, 0.10-, and 1.0-µg/mL fortification solutions (Section 7.1.2.) to control water samples to obtain concentrations ranging from 0.1-µg/mL to 5.0 µg/L.

9.3.3. Using a positive displacement pipet, carefully add 50 µL of concentrated sulfuric acid to the sample vial. Cap the vial and mix.

9.3.4. Purify the sample using the following SPE procedure (Section 11.4.):

a. Attach a 10-mL reservoir to a 60-mg (3-mL) Phenomenex Strata-X SPE cartridge.

b. Condition the SPE cartridge with 3 mL of methanol followed by 3 mL of water/concentrated sulfuric acid (99.5:0.5). Dry the cartridge under full vacuum (approximately -10 inches Hg) for 5 seconds between solvents.

c. Transfer the acidified water sample from Step 9.3.3 to the SPE cartridge. Pull the water through the cartridge at approximately 2 mL/min, discarding the eluate. Dry the cartridge under full vacuum (approximately -10 inches Hg) for 30 seconds.

d. Rinse the 40-mL vial with 2 mL of water/methanol (90:10) containing 1% formic acid and apply the rinse to the SPE cartridge.

e. Dry the SPE cartridge for 20 minutes at full vacuum (approximately -10 inches Hg).

f. Elute the aminopyralid from the SPE cartridge at approximately 1 mL/min with two 2.0-mL aliquots of a methyl *tert*-butyl ether/methanol (90:10) solution, collecting the eluate in 12-mL vials.

9.3.5. Add 400 µL of the internal standard solution containing 125 ng/mL of $^{13}C_2{}^{2}H^{15}N$-aminopyralid (Section 7.2.2.) to the vials containing the SPE eluate. (See Section 7.3.2 for concurrent calibration standard preparation)

9.3.6. Evaporate the sample eluate to incipient dryness using an N-Evap evaporator set at approximately 40 °C with a nitrogen gas flow rate of approximately 500 mL/min.

9.3.7. Add 200 µL of the derivatization coupling reagent (Section 6.3.1.) to the sample vials.

9.3.8. Derivatize the sample (Section 9.3.7.), the calibration standards (Section 7.3.5.), and the crossover standards (Sections 7.4.1 and 7.5.1, if needed) by pipetting 10 µL of butyl chloroformate derivatizing reagent into the vials.

9.3.9. Vortex the sample vials and allow the mixture to react for 5 minutes.

9.3.10. Add 4790 µL of the methanol/water (40:60) solution containing 0.05% formic acid and 5 mM ammonium formate to the sample vials. Calibration standards should have only 790 µL added as noted in Section 7.3.7. Vortex mix for approximately 10 seconds, transfer to a 96-well plate, and firmly seal with a cap.

9.3.11. Analyze the crossover standards (Sections 7.4.1 and 7.5.1, if needed), calibration standards (Section 7.3.7.), and samples by HPLC with positive-ion electrospray tandem mass spectrometry as described in Section 8. Determine the suitability of the chromatographic system using the following performance criteria:

a. Standard curve linearity: Determine that the correlation coefficient equals or exceeds 0.995 for the least squares equation which describes the detector response as a function of standard curve concentration. If power regression is used, the power exponent should be between 0.90-1.10.

b. Peak resolution: Visually determine that sufficient resolution has been achieved for the analyte and internal standard relative to background interferences.

c. Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in Figures 4-13 with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 10:1 has been attained for the analyte in the 0.10-ng/mL calibration standard.

9.3.12. If the sample concentrations exceed the range of the standard calibration curve, dilute the sample with derivatized $^{13}C_2{}^{2}H^{15}N$-aminopyralid internal standard solution (Section 7.5.1.) to obtain responses within the range of the calibration curve.

10. CALCULATIONS

10.1. Determination of Isotopic Crossover

In this assay, the analyte and internal standard are quantitated 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. This isotopic overlap between the analyte and the internal standard is determined empirically by analyzing standard solutions of each compound and should be addressed for accurate determination of concentrations.

10.1.1. To determine the isotopic crossover for aminopyralid and $^{13}C_2{}^2H^{15}N$-aminopyralid, inject the derivatized crossover standards described in Sections 7.4.1 and 7.5.1, and determine the peak areas for the analyte and internal standard as indicated below.

aminopyralid 1-BE	Q1/Q3 *m/z* 263/189
$^{13}C_2{}^2H^{15}N$-aminopyralid 1-BE	Q1/Q3 *m/z* 269/195

For example, to determine the contribution of the unlabeled aminopyralid to the labeled $^{13}C_2{}^2H^{15}N$-aminopyralid internal standard:

$$\text{Crossover Factor (analyte}\rightarrow\text{ISTD)} = \frac{\text{peak area of internal standard transition}}{\text{peak area of analyte transition}}$$

$$\text{Crossover Factor (analyte}\rightarrow\text{ISTD)} = \frac{\text{peak area at } m/z \text{ 269/195}}{\text{peak area at } m/z \text{ 263/189}}$$

In a similar manner, to determine the contribution of the labeled $^{13}C_2{}^2H^{15}N$-aminopyralid internal standard to the unlabeled aminopyralid:

$$\text{Crossover Factor (ISTD}\rightarrow\text{ analyte)} = \frac{\text{peak area of analyte transition}}{\text{peak area of internal standard transition}}$$

$$\text{Crossover Factor (ISTD}\rightarrow\text{ analyte)} = \frac{\text{peak area at } m/z \text{ 263/189}}{\text{peak area at } m/z \text{ 269/195}}$$

During method development, no mass spectral isotopic crossover was observed and therefore no correction of the measured quantitation ratio was performed. If isotopic crossover is encountered it should be assessed and the respective quantitation ratios corrected for accurate determination of concentrations (13.1, 13.2).

10.2. Calculation of Standard Calibration Curve (Note 12.4)

10.2.1. Inject the series of calibration standards as described in Sections 7.3.2-7.3.7 and determine the peak areas for the analyte as indicated below.

Aminopyralid	Q1/Q3 *m/z* 263/189 (quantitation) Q1/Q3 *m/z* 263/161 (confirmation 1) Q1/Q3 *m/z* 263/134 (confirmation 2)
$^{13}C_2{}^2H^{15}N$-aminopyralid	Q1/Q3 *m/z* 269/195

10.2.2. For each standard, calculate the aminopyralid quantitation ratio.

For example, using the data for the aminopyralid 0.1-ng/mL standard from Figures 3 and 4:

$$\text{Quantitation Ratio} = \frac{\text{peak area of quantitation ion}}{\text{peak area of internal standard ion}}$$

$$\text{Quantitation Ratio} = \frac{\text{aminopyralid peak area at Q1/Q3 } m/z \text{ 263/189}}{\text{ISTD peak area at Q1/Q3 } m/z \text{ 269/195}}$$

$$\text{Quantitation Ratio} = \frac{6868}{458485}$$

$$\text{Quantitation Ratio} = 0.015$$

10.2.3. Prepare a standard curve by plotting the concentration of the analytes on the abscissa (x-axis) and the respective quantitation ratio on the ordinate (y-axis), as shown in Figure 3. Using linear regression analysis (13.3.) with a 1/x weighting (13.4.), determine the equation for the curve with respect to the abscissa.

For example, using the aminopyralid data from Figure 3:

$$X = \left(\frac{Y - \text{intercept}}{\text{slope}}\right)$$

$$\text{aminopyralid conc. (ng/mL)} = \left(\frac{\text{aminopyralid quantitation ratio} - \text{intercept}}{\text{slope}}\right)$$

$$\text{aminopyralid conc. (ng/mL)} = \left(\frac{\text{aminopyralid quantitation ratio} - (-0.0002)}{0.1371}\right)$$

10.3. Calculation of Percent Recovery

10.3.1. Determine the gross concentration in each recovery sample extract by substituting the quantitation ratio obtained into the above equation and solving for the concentration.

For example, using the data for aminopyralid recovery in tap water sample 023-0001 + 0.05 µg/L A10 from Figure 6:

$$\text{aminopyralid gross (ng/mL)} = \left(\frac{\text{aminopyralid quantitation ratio} - (-0.0002)}{0.1371}\right)$$

$$\text{aminopyralid gross (ng/mL)} = \left(\frac{0.0122 + 0.0002}{0.1371}\right)$$

$$\text{aminopyralid (gross)} = 0.0904 \text{ ng/mL}$$

10.3.2. Convert the concentration (ng/mL) of the analyte found in the prepared extract to the concentration (µg/L) of the analyte found in the original sample as follows

$$\text{aminopyralid (gross µg/L)} = 0.0904 \text{ ng/mL} \times \left(\frac{\text{(Final Vol)}}{\text{(Sample Vol)}}\right)$$

$$\text{aminopyralid (gross µg/L)} = 0.0904 \text{ ng/mL} \times \left(\frac{5.0 \text{ mL}}{10.0 \text{ mL}}\right) \times \frac{1.00 \text{ µg}}{1000 \text{ ng}} \times \frac{1000 \text{ mL}}{1.00 \text{ L}}$$

$$\text{aminopyralid (gross)} = 0.0452 \text{ µg/L}$$

10.3.3. Determine the net concentration in each recovery sample by subtracting any signal found in the control sample from that of the gross analyte concentration.

For example, using the data for control tap water 023-0001 A9 in Figure 5, and continuing with data for tap water recovery sample 023-0001 + 0.05 µg/L A10 in Figure 6:

$$\text{aminopyralid net (µg/L)} = \text{aminopyralid (gross µg/L)} - \text{aminopyralid (control µg/L)}$$

$$\text{aminopyralid net (µg/L)} = 0.0452 - 0.0000$$

$$\text{aminopyralid (net)} = 0.0452\ \mu g/L$$

10.3.4. Determine the percent recovery by dividing the net concentration of each recovery sample by the theoretical concentration added.

$$\text{Recovery} = \frac{\text{Concentration Found}}{\text{Concentration Added}} \times 100\%$$

$$\text{Recovery} = \frac{0.0452\,\mu g/L}{0.0500\,\mu g/L} \times 100\%$$

$$\text{Recovery} = 90\%$$

Note that the precision in these calculations is reduced by rounding at each step as was shown above for demonstration purposes.

10.4. Determination of Aminopyralid in Water Samples

10.4.1. Determine the gross concentration of the analyte in each treated sample by substituting the respective quantitation ratio into the equation for the standard calibration curve and calculating the uncorrected residue result as described in Sections 10.3.1-10.3.2.

10.4.2. For those analyses that require correction for method recovery, use the average recovery of all the recovery samples for that matrix fortified at or above the limit of quantitation from a given sample set to correct for method efficiency.

For example, using the data for aminopyralid from Figure 6 and Table 2 for the tap water recovery samples analyzed with set 071121 S10 on 17-Jul-2007:

$$\text{aminopyralid (corrected µg/L)} = \text{aminopyralid (gross µg/L)} \times \left(\frac{100}{\text{Avg \% Recovery}}\right)$$

$$\text{aminopyralid (corrected µg/L)} = 0.0452\ \mu g/L \times \frac{100}{95}$$

$$\text{aminopyralid (corrected)} = 0.0476\ \mu g/L$$

11.4. Standardization of Phenomenex Strata-X SPE Elution Profile

Variation in the Phenomenex Strata-X SPE cartridge may influence the elution profile of the aminopyralid. If it is necessary to obtain an elution profile for the SPE plates used to optimize recovery and clean-up efficiency, the following procedure can be used:

11.4.1. Pipet a 10-mL aliquot of HPLC grade water into a 40-mL vial.

11.4.2. Pipet 50 µL of the 1.0 µg/mL fortification solution (Section 7.1.2.) into the HPLC grade water.

11.4.3. Carefully add 50 µL of concentrated sulfuric acid to the sample vial. Cap the vial and mix.

11.4.4. Profile the SPE cartridge using the following procedure:

a. Attach a 10-mL reservoir to a 60-mg (3-mL) Phenomenex Strata-X SPE cartridge.

b. Condition the SPE cartridge with 3 mL of methanol followed by 3 mL of water/concentrated sulfuric acid (99.5:0.5). Dry the cartridge under full vacuum (approximately -10 inches Hg) for 5 seconds between solvents.

c. Transfer the acidified water sample from Step 11.4.3 to the SPE cartridge. Pull the water through the cartridge at approximately 2 mL/min, discarding the eluate. Dry the cartridge under full vacuum (approximately -10 inches Hg) for 30 seconds.

d. Rinse the 40-mL vial with 2 mL of water/methanol (90:10) containing 1% formic acid and apply the rinse to the SPE cartridge.

e. Dry the SPE cartridge for 20 minutes at full vacuum (approximately -10 inches Hg).

f. Elute the aminopyralid from the SPE cartridge at approximately 1 mL/min with three 2.0-mL aliquots of a methyl *tert*-butyl ether/methanol (90:10) solution collecting the eluate in separate 12-mL vials.

11.4.5. Pipet 400 µL of the 125-ng/mL stable isotope standard solution (Section 7.2.2.) into the 12-mL vials containing the elution solvent. (See Section 7.3.2 for concurrent calibration standard preparation.)

11.4.6. Evaporate the elution solvent to dryness in a N-Evap evaporator set at 40 °C (approximately 500 mL/min nitrogen gas flow).

11.4.7. Pipet 200 µL of the derivatization coupling reagent (Section 6.3.1.) into the sample vials.

11.4.8. Derivatize the sample fractions (Section 11.4.7.) and the calibration standards (Section 7.3.5.) by pipetting 10 µL of butyl chloroformate derivatizing reagent into the vials.

11.4.9. Vortex mix the sample vials and allow the mixture to react for 5 minutes.

11.4.10. Add 4790 µL of the methanol/water (40:60) solution with 0.05% formic acid and 5 mM ammonium formate to the sample vials. Calibration standards should have only 790 µL added as noted in Section 7.3.7. Vortex mix for approximately 10 seconds, transfer to a 96-well plate, and firmly seal with a cap

11.4.11. Analyze the elution fractions and the calibration standards using LC/MS/MS conditions listed in Section 8.

11.4.12. Calculate the percent recovery according to the procedure outlined in Section 10.3. If the elution profile differs from that illustrated in Figure 14, adjust the volume of the methyl *tert*-butyl ether/methanol (90:10) solution to be used for elution in Step 9.3.4.f.

12. NOTES

12.1. Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests. Common laboratory supplies are assumed to be readily available and are, therefore, not listed.

12.2 Electronic pipets are used only for pipetting aqueous solutions. If they are used for pipetting non-aqueous solutions, the pipets should be calibrated following the manufacturer's instruction manual and Standard Operating Procedures. (13.6)

12.3. The LC/MS/MS operating conditions may be modified to obtain optimal chromatographic separation and mass spectrometric performance. Additionally, the assignment of quantitation and confirmation transitions may be exchanged if needed.

12.4. The type of regression model can be chosen to give the best fit for the data.

13. REFERENCES

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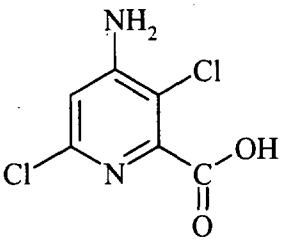
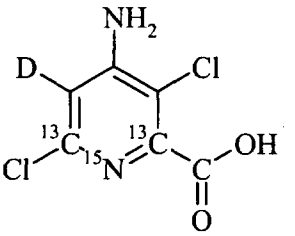
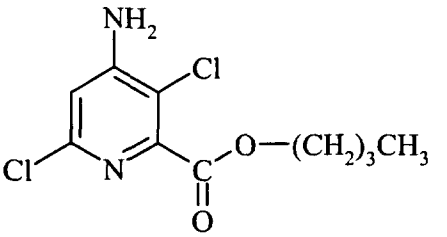
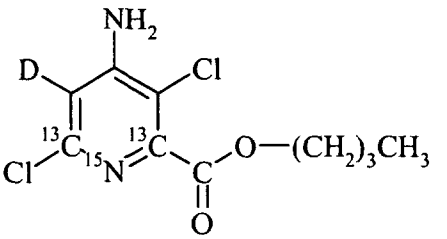
Table 1. Identity and Structures of Aminopyralid and Related Compounds

Common Name of Compound	Structure and CAS Name
Aminopyralid Molecular Formula: $C_6H_4Cl_2N_2O_2$ Formula Weight 207.02 Nominal Mass: 206 CAS Number: 150114-71-9	 4-amino-3,6-dichloro-2-pyridinecarboxylic acid
$^{13}C_2{}^2H^{15}N$-Aminopyralid Molecular Formula: $^{13}C_2C_4{}^2HH_3Cl_2{}^{15}NNO_2$ Formula Weight 211.00 Nominal Mass: 210 CAS Number: not available	 4-amino-3,6-dichloro-2-pyridinecarboxylic acid-1-^{15}N-2,6-^{13}C-5-*d*
Aminopyralid 1-Butyl Ester Molecular Formula: $C_{10}H_{12}Cl_2N_2O_2$ Formula Weight 263.12 Nominal Mass: 262 CAS Number: not available	 4-amino-3,6-dichloro-2-pyridinecarboxylic acid, 1-butyl ester
$^{13}C_2{}^2H^{15}N$-Aminopyralid 1-Butyl Ester Molecular Formula: $^{13}C_2C_8{}^2HH_{11}Cl_2{}^{15}NNO_2$ Formula Weight 267.11 Nominal Mass: 266 CAS Number: not available	 4-amino-3,6-dichloro-2-pyridinecarboxylic acid-1-^{15}N-2,6-^{13}C-5-*d*, 1-butyl ester