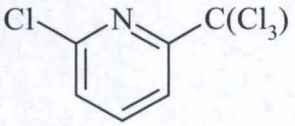
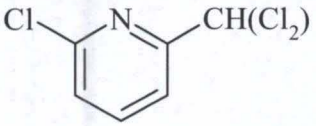
INTRODUCTION

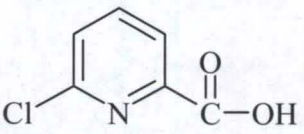
The use of nitrapyrin as a nitrification inhibitor on corn, sorghum, and wheat is a well-established fertilizer best management practice that results in improved fertilizer use efficiency in crop production (1). Nitrapyrin acts as a nitrogen stabilizer because of its highly selective action as a soil bactericide controlling *Nitrosomonas* spp., the bacteria which oxidize ammonium ions in the soil (2).



Nitrapyrin
CAS No. 1929-82-4



2-Cl-6-DCMP
CAS No. 78152-53-1



6-CPA
CAS No. 4684-94-0

Common and chemical names, molecular formulas, and the nominal masses for the above structures and related compounds are given in Table 1 of analytical method GRM 07.13 (Appendix A) and GRM 07.14 (Appendix B).

This study was primarily conducted to fulfill data requirements outlined in the U. S. EPA Ecological Effects Test Guidelines, OPPTS 850.7100 (3). The study also meets the requirements the European Commission Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 7 (4) and SANCO/3029/99 (5), and Pest Management Regulatory Agency, Health Canada, Residue Chemistry Guidelines as Regulatory Directive Dir98-02 (6).

Method GRM 07.13 (7) is applicable for the quantitative determination of residues of nitrapyrin and its 2-Cl-6-DCMP metabolite in soil, while method GRM 07.14 (8) is applicable for the quantitative determination of residues of the nitrapyrin metabolite, 6-CPA, in soil. The purpose of this study was to provide validation data to define the accuracy, precision, specificity, and ruggedness of the methods.

EXPERIMENTAL

Sample Origin, Numbering, Preparation and Storage

Untreated control soil samples were collected in association with the terrestrial field dissipation Study ARAP-07D-002 conducted by Ag Research Associates LLC (Dow AgroSciences LLC Study 090054 (9). Soil samples were prepared by Ag Research Associates by grinding with dry ice to a uniform particle size using a Hobart food cutter. The finely-ground samples were then shipped frozen to Dow AgroSciences LLC. Upon receipt at Dow AgroSciences LLC, all of the samples were inspected and found to be in good condition. All samples were tracked in the Dow AgroSciences LLC Regulatory Labs Information Management System (RLIMS) database. Unique sample numbers were assigned to the samples to track them during receipt, storage, and analysis. Complete source documentation is included in the study file for Study 090054. Soil characterization data for the soil samples used in the validation study are given in the table below.

Sample Number	Sample Description	USDA Textural Classification	pH	% Organic Carbon
001-0001 (Michigan)	Control (00-06")	Soil (Loam)	7.2	1.6
005-0002 (Michigan)	Control (06-12")	Soil (Sandy Loam)	7.1	1.7
003-0001 (Nebraska)	Control (00-06")	Soil (Silty Clay Loam)	6.8	2.3
018-0002 (Nebraska)	Control (06-12")	Soil (Silty Clay Loam)	5.6	1.7

During the course of the study, all samples were stored in temperature-monitored freezers at approximately -20 °C, except when removed for analysis.

Test Substances / Analytical Standards

The test substances and analytical standards used for the validation studies were the following:

Test Substance/ Analytical Standard	AGR/TSN Number	Percent Purity	Certification Date	Reference
nitrapyrin[a]	AGR023966	99.9	21-May-2007	FA&PC 073104
2-Cl-6-DCMP[b]	AGR235850	99.4	16-Aug-2006	FA&PC 063305
6-CPA[c]	AGR029021	99	22-Jan-2007	FA&PC 073016

[a] 2-chloro-6-(trichloromethyl)pyridine
[b] 2-chloro-6-(dichloromethyl)pyridine
[c] 6-chloropyridine-2-carboxylic acid

In addition, the following reagents were used as internal standards:

Internal Standard	Lot Number	Percent Purity	Certification Date	Reference
$^{13}C_2{}^{15}N$-2,3,5,6-TCP[a]	DE3-037089-33	- - -[c]	- - -	- - -
$^{13}C_3{}^{15}N$-6-CPA[b]	36891-75	- - -	- - -	- - -

[a] $^{13}C_2{}^{15}N$-2,3,5,6-tetrachloropyridine
[b] $^{13}C_3{}^{15}N$-6-chloropyridine-2-carboxylic acid
[c] '- - -' indicates that the information is not available

Preparation of Fortification Solutions and Calibration Standards

For method GRM 07.13, the nitrapyrin and 2-Cl-6-DCMP fortification solutions and calibration standards were prepared as described in Dow AgroSciences LLC Laboratory Notebook C 2173, pages 8-14, 32-35 and are cross-referenced to Section 7 of method GRM 07.13 (Appendix A).

For method GRM 07.14, the 6-CPA fortification solutions and calibration standards were prepared as described in Dow AgroSciences LLC Laboratory Notebook C 2173, pages 21-26, and are cross-referenced to Section 7 of method GRM 07.14 (Appendix B).

Fortification of Recovery Samples

For method GRM 07.13, the control soil samples were fortified as described in Dow AgroSciences LLC Laboratory Notebook C 2173, page 15, which is cross-referenced to Section 9.2.2 of method GRM 07.13 (Appendix A).

On the day of analysis, untreated control soil samples were freshly fortified with nitrapyrin and 2-Cl-6-DCMP over the concentration range of 0.010-10.0 µg/g for the generation of method validation recovery data. Reagent blank and untreated control samples — those that received no fortification — were also prepared for analysis along with the recovery samples to check for the presence of any background interferences. Untreated control soil samples were also fortified at 0.003 µg/g to demonstrate the method limit of detection.

For method GRM 07.14, the control soil samples were fortified as described in Dow AgroSciences LLC Laboratory Notebook C 2173, page 27, which is cross-referenced to Section 9.2.2 of method GRM 07.14 (Appendix B).

On the day of analysis, untreated control soil samples were freshly fortified with 6-CPA over the concentration range of 0.010-10.0 µg/g for the generation of method validation recovery data. Reagent blank and untreated control samples — those that received no fortification — were also prepared for analysis along with the recovery samples to check for the presence of any background interferences. Untreated control soil samples were also fortified at 0.003 µg/g to demonstrate the method limit of detection.

Sample Extraction and Analysis

For method GRM 07.13, the procedure used for the determination of nitrapyrin and 2-Cl-6-DCMP in soil is described in Dow AgroSciences LLC Laboratory Notebook C 2173, pages 15-16 and is cross-referenced to Section 9.2 of method GRM 07.13 (Appendix A).

Residues of nitrapyrin and its 2-Cl-6-DCMP metabolite were extracted from a 5-gram soil sample by sonication and shaking with an acetone/0.1 N hydrochloric acid (90:10) solution.

After adjusting to a final volume of 40.0 mL, a 4.0-mL aliquot of the extract was diluted with 25 mL of 0.1 N hydrochloric acid and then extracted with 1.0 mL of cyclohexane containing 100 ng/mL $^{13}C_2{}^{15}N$-2,3,5,6-tetrachloropyridine as an internal standard. A portion of the cyclohexane extract was subsequently analyzed by capillary gas chromatography with positive-ion electron-impact ionization mass spectrometry (GC/EI-MS). Analysis was performed using a J & W Scientific Durabond-5MS, 30 m x 0.25 mm i.d., 1.0-µm film thickness capillary column coupled to an Agilent Model 5973N GC/EI-MS system as described in Section 8 of method GRM 07.13 (Appendix A).

For method GRM 07.14, the procedure used for the determination of 6-CPA in soil is described in Dow AgroSciences LLC Laboratory Notebook C 2173, pages 27-29 and is cross-referenced to Section 9.2 of method GRM 07.14 (Appendix B).

Residues of 6-CPA were extracted from a 5-gram soil sample by sonication and shaking with an aqueous 0.5 N potassium hydroxide/10% potassium chloride solution. After adjusting to a final volume of 40.0 mL, a 1.0-mL aliquot of the extract was acidified with 1.5 mL of 0.5 N hydrochloric acid. The sample was shaken and centrifuged, and a 1.0-mL aliquot of the supernatant was purified using a Phenomenex Strata™-X polymeric solid-phase extraction (SPE) column. After elution from the SPE column with dichloromethane, an internal standard solution containing $^{13}C_3{}^{15}N$-6-chloropyridine-2-carboxylic acid ($^{13}C_3{}^{15}N$-6-CPA) was added to the column eluate, and the resulting solution was evaporated to dryness. The sample was then derivatized with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoracetamide (MTBSTFA) to form the *tert*-butyldimethylsilyl (TBDMS) derivatives of 6-CPA and $^{13}C_3{}^{15}N$-6-CPA, and subsequently analyzed by capillary gas chromatography with negative-ion chemical ionization mass spectrometry (GC/NCI-MS). Analysis was performed using a J & W Scientific Durabond-5MS, 30 m x 0.25 mm i.d., 1.0-µm film thickness capillary column coupled to an Agilent Model 5973N GC/NCI-MS system as described in Section 8 of method GRM 07.14 (Appendix B).

All analyses were performed at the Dow AgroSciences LLC—Regulatory Laboratories in Indianapolis, Indiana.

Calculation of Percent Recovery

For method GRM 07.13, the calculation of percent recovery for fortified samples was performed as described in Section 10.2, while for method GRM 07.14, the calculation of percent recovery for fortified samples was performed as described in Section 10.3.

Statistical Treatment of Data

Statistical treatment of data for methods GRM 07.13 and GRM 07.14 included the calculation of the power regression equations, the coefficients of determination (r^2) describing the linearity of the calibration curves, the calculation of the means, standard deviations, and the relative standard deviations of the results from the fortified recovery samples.

GRM: 07.13
EFFECTIVE: 06-Jan-2015
SUPERSEDES: New

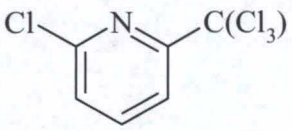
Dow AgroSciences

Determination of Residues of Nitrapyrin and its 2-Chloro-6-(dichloromethyl)pyridine Metabolite in Soil by Gas Chromatography with Positive-Ion Electron-Impact Ionization Mass Spectrometry Detection

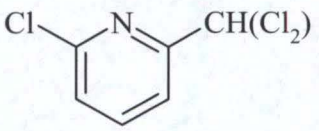
E. L. Olberding and G. E. Dial, Jr.

1. SCOPE

This method is applicable for the quantitative determination of residues of nitrapyrin and its 2-chloro-6-(dichloromethyl)pyridine (2-Cl-6-DCMP) metabolite in soil. The method was validated over the concentration range of 0.010-10.0 µg/g with a validated limit of quantitation of 0.010 µg/g.



Nitrapyrin
CAS No. 1929-82-4



2-Cl-6-DCMP
CAS No. 78152-53-1

The common and chemical names, the molecular formulas, and the nominal masses for the above structures and related compounds are given in Table 1.

2. PRINCIPLE

Residues of nitrapyrin and its 2-Cl-6-DCMP metabolite are extracted from a 5-gram soil sample by sonication and shaking with an acetone/0.1 N hydrochloric acid (90:10) solution. After adjusting to a final volume of 40.0 mL, a 4.0-mL aliquot of the extract is diluted with 25 mL of 0.1 N hydrochloric acid and then extracted with 1.0 mL of cyclohexane containing 100 ng/mL $^{13}C_2{}^{15}N$-2,3,5,6-tetrachloropyridine as an internal standard. A portion of the cyclohexane extract is subsequently analyzed by capillary gas chromatography with positive-ion electron-impact ionization mass spectrometry (GC/EI-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. Acetone and cyclohexane are flammable and should be used in well-ventilated areas away from ignition sources.

3.3. Hydrochloric acid is corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be worn when handling this reagent.

3.4. Dry ice and liquid nitrogen can cause severe burns or pose a suffocation hazard. It is imperative that proper eye and personal protection equipment be worn when handling these chemicals.

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 PM600, Mettler-Toledo Inc.

4.1.3. Centrifuge, with rotor to accommodate 45-mL vials, Model Centra-GP8, Thermo International Equipment Company, Needham Heights, MA 02194.

4.1.4. Desiccator, 250-mm i.d., catalog number 08-615B, Fisher Scientific, Pittsburgh, PA 15275.

4.1.5. Hammer mill, with 1/8- and 3/16-inch screens, Model 2001, AGVISE Laboratories, Inc., Northwood, ND 58267.

4.1.6. Oven, Model OV-490A-2, Blue M Electric Company, Blue Island, IL 60406.

4.1.7. Pipet, positive-displacement, 10-100 µL capacity, catalog number M100, Gilson Inc., Middleton, WI 53562.

4.1.8. Pipet, positive-displacement, 200-1000 µL capacity, catalog number M1000, Gilson Inc.

4.1.9. Shaker, variable speed reciprocating with box carrier, Model 6000, Eberbach Corporation, Ann Arbor, MI 48106.

4.1.10. Ultrasonic cleaner, Model 1200, Branson Cleaning Equipment Company, Shelton, CT 06484.

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

4.2. Chromatographic System

4.2.1. Column, capillary gas chromatography, Durabond-5MS liquid phase, 30 m x 0.25 mm i.d., 1.0-µm film thickness, catalog number 122-5533, Agilent Technologies, Santa Clara, CA 95051.

4.2.2. Gas chromatograph, Model 6890A, Agilent Technologies.

4.2.3. Gas purifier, catalog number OT3-2, Agilent Technologies. (Note 12.2.)

4.2.4. Injector, automatic, Model 7683, Agilent Technologies.

4.2.5. Inlet liner, PTV, multi-baffled, catalog number 5183-2037, Agilent Technologies.

4.2.6. Mass spectrometer, Model 5973N, Agilent Technologies.

4.2.7. Mass spectrometer data system, Model G1701DA, Agilent Technologies.

5. GLASSWARE AND MATERIALS (Note 12.1.)

5.1. Cylinder, graduated mixing, 50-mL, catalog number 3002-50, Corning Inc., Acton, MA 01720.

5.2. Dish, aluminum weighing, catalog number 08-732, Fisher Scientific.

5.3. Flask, volumetric, 50-mL, catalog number 5640-50, Corning Inc.

5.4. Flask, volumetric, 100-mL, catalog number 5640-100, Corning Inc.

5.5. Flask, volumetric, 200-mL, catalog number 5640-200, Corning Inc.

5.6 Flask, volumetric, 1000-mL, catalog number 5640-1L, Corning Inc.

5.7. Flask, volumetric, 2000-mL, catalog number 5640-2L, Corning Inc.

5.8. Pipet, serological, 5-mL, catalog number 7077-5N, Corning Inc.

5.9. Pipet, serological, 25-mL, catalog number 7077B-25N, Corning Inc.

5.10. Pipet, volumetric, 1.0-mL, catalog number 13-650-3B, Fisher Scientific.

5.11. Pipet, volumetric, 4.0-mL, catalog number 13-650-3E, Fisher Scientific.

5.12. Pipet, volumetric, 10-mL, catalog number 13-650-3L, Fisher Scientific.

5.13. Pipet, volumetric, 20-mL, catalog number 13-650-3N, Fisher Scientific.

5.14. Pipet, volumetric, 25-mL, catalog number 13-650-3P, Fisher Scientific.

5.15. Pipet, volumetric, 30-mL, catalog number 13-650-3Q, Fisher Scientific.

5.16. Pipet, volumetric, 50-mL, catalog number 13-650-3S, Fisher Scientific.

5.17. Pipet, volumetric, 200-mL, catalog number 13-650-3W, Fisher Scientific.

5.18. Pipet tip, positive-displacement, 100-µL capacity, catalog number CP100, Gilson Inc.

5.19. Pipet tip, positive-displacement, 1000-µL capacity, catalog number CP1000, Gilson Inc.

5.20. Vial, 2-mL, autosampler, catalog number C4000-1W, National Scientific Company, Rockwood, TN 37854.

5.21. Vial, 11-mL, with PTFE-lined screw cap, catalog number 2504T, Qorpak, Bridgeville, PA 15017.

5.22. Vial, 45-mL, catalog number 60958A-11, Kimble Glass Co., Vineland, NJ 08360.

5.23. Vial cap, PTFE-lined, for 45-mL vial, catalog number 5205, Qorpak.

5.24. Vial cap, for autosampler vial, catalog number C4000-54B, National Scientific Company.

5.25. Vial insert, limited-volume, for autosampler vial, catalog number C4011-631, National Scientific Company.

6. REAGENTS, STANDARDS, AND PREPARED SOLUTIONS

6.1. Reagents

6.1.1. Acetone, OmniSolv grade, catalog number AX0116-1, EMD Chemicals Inc., Gibbstown, NJ 08027.

6.1.2. Cyclohexane, OmniSolv grade, catalog number CX2286-1, EMD Chemicals Inc.

6.1.3. Desiccant, Drierite adsorbent, catalog number 24001, W. A. Hammond Drierite Company, Xenia, OH 45385.

6.1.4. Helium, gas, 99.995% purity, BOC Gases, New Providence, NJ 07974.

6.1.5. Hydrochloric acid, 0.1 N, certified concentration, catalog number SA54-1, Fisher Scientific.

6.1.6. Nitrogen, refrigerated liquid, catalog number LQNI, BOC Gases, New Providence, NJ 07974. (Note 12.3.)

6.1.7. Peanut oil, cooking grade, Planters Company, East Hanover, NJ 07936.

6.1.8. Water, OmniSolv grade, catalog number WX0004-1, EMD Chemicals Inc.

6.2. Standards

6.2.1. nitrapyrin, 2-chloro-6-(trichloromethyl)pyridine

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

6.2.2. 2-chloro-6-(dichloromethyl)pyridine

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

6.2.3. $^{13}C_2{}^{15}N$-2,3,5,6-tetrachloropyridine

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

6.3. Prepared Solutions

6.3.1. acetone/0.1 N hydrochloric acid (90:10 v/v)

Pipet 200 mL of 0.1 N hydrochloric acid into a 2000-mL volumetric flask containing approximately 1500 mL of acetone. Swirl the flask and allow to equilibrate to room temperature. Dilute to volume with acetone.

6.3.2. cyclohexane containing 250 µg/mL of peanut oil (Note 12.4.)

Weigh 0.50 gram of peanut oil and quantitatively transfer to a 2000-mL volumetric flask containing approximately 1500 mL of cyclohexane. Swirl the flask, and dilute to volume with cyclohexane.

7. PREPARATION OF STANDARDS

7.1. Preparation of Nitrapyrin and 2-Chloro-6-(dichloromethyl)pyridine Fortification Solutions

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

7.1.2. Weigh 0.1000 g of 2-chloro-6-(dichloromethyl)pyridine analytical standard and quantitatively transfer to a 100-mL volumetric flask with acetone. Dilute to volume with acetone to obtain a 1000 µg/mL stock solution.

7.1.3. Pipet 10.0 mL of the 1000-µg/mL solution from Section 7.1.1 and 10.0 mL of the 1000-µg/mL solution from Section 7.1.2 into a single 200-mL volumetric flask and dilute to volume with acetone to obtain a stock solution containing 50.0 µg/mL of each compound.

7.1.4. Pipet 20.0 mL of the 50.0-µg/mL solution from Section 7.1.3 into a 200-mL volumetric flask and dilute to volume with acetone to obtain a solution containing 5.00 µg/mL of each compound.

7.1.5. Pipet 20.0 mL of the 5.00-µg/mL solution from Section 7.1.4 into a 200-mL volumetric flask and dilute to volume with acetone to obtain a solution containing 0.500 µg/mL of each compound.

7.1.6. Prepare solutions for spiking samples by diluting the above stock solutions from Sections 7.1.3-7.1.5 with acetone as follows:

Concentration of Stock Soln.	Aliquot of Stock Soln.	Final Soln. Volume	Spiking Soln. Final Conc.	Equivalent Sample Conc.[a]
µg/mL	mL	mL	µg/mL	µg/g
0.500	30.0	100	0.150	0.003
0.500	50.0	100	0.250	0.005
0.500	- - -	- - -	0.500	0.010
5.00	25.0	100	1.250	0.025
5.00	50.0	100	2.50	0.050
5.00	- - -	- - -	5.00	0.100
50.0	25.0	100	12.5	0.250
50.0	50.0	100	25.0	0.500
50.0	- - -	- - -	50.0	1.00

[a] The equivalent sample concentration is based on fortifying a 5.0-gram soil sample with 100 µL of spiking solution. Samples can be fortified using up to 1.0 mL of spiking solution.

7.2. Preparation of the $^{13}C_2{}^{15}N$-2,3,5,6-Tetrachloropyridine Internal Standard Solution

7.2.1. Weigh 0.0050 g of the $^{13}C_2{}^{15}N$-2,3,5,6-tetrachloropyridine ($^{13}C_2{}^{15}N$-2,3,5,6-TCP) reference standard and quantitatively transfer to a 50-mL volumetric flask with acetone. Dilute to volume with acetone to obtain a 100.0-µg/mL stock solution.

7.2.2. Pipet 1.0 mL of the 100.0-µg/mL solution from Section 7.2.1 into a 1000-mL volumetric flask and dilute to volume with cyclohexane containing 250 µg/mL of peanut oil. This solution contains 100.0 ng/mL of $^{13}C_2{}^{15}N$-2,3,5,6-TCP.

7.3. Preparation of Calibration Standards for the Quantitation of Nitrapyrin and 2-Chloro-6-(dichloromethyl)pyridine

7.3.1. Prepare calibration standards by dispensing 100 µL of the 0.150-50.0-µg/mL fortification solutions from Section 7.1.6 into a series of 11-mL vials containing 10.0 mL of the internal standard solution from Section 7.2.2 and firmly seal with a cap. Vortex mix the standards for 1-2 seconds. The concentrations of the calibration standards are as follows:

Concentration of Spiking Soln. µg/mL	Aliquot of Spkg. Soln. µL	Final Soln. Volume mL	Calib Soln. Final Conc. ng/mL	Equivalent Sample Conc.[a] µg/g
- - -	- - -	10.0	0.00	0.000
0.150	100	10.0	1.50	0.003
0.250	100	10.0	2.50	0.005
0.500	100	10.0	5.00	0.010
1.250	100	10.0	12.5	0.025
2.50	100	10.0	25.0	0.050
5.00	100	10.0	50.0	0.100
12.5	100	10.0	125.0	0.250
25.0	100	10.0	250.0	0.500
50.0	100	10.0	500.0	1.00

[a] The equivalent sample concentration is based on using a 5.0-gram soil sample and a final sample volume of 1.0 mL.

8. INSTRUMENTAL CONDITIONS

8.1. Column

Install the PTV column inlet liner and the capillary column in the PTV injection port of the gas chromatograph following the manufacturer's recommended procedures.

8.2. Typical Gas Chromatography Operating Conditions (Note 12.5.)

Instrumentation:	Agilent Model 6890A gas chromatograph Agilent Model 7683 autoinjector Agilent Model 5973N mass spectrometer Agilent Model G1701DA data system
Column:	J & W fused silica capillary Durabond-5MS liquid phase 30 m x 0.25 mm i.d. 1.0-µm film thickness

Carrier Gas Method:	helium
Constant Flow	1.0 mL/min
Vacuum Compensation	on
Initial Head Pressure	~60 kPa
Linear Velocity	~37 cm/s
Oven Method:	
Initial Temperature	70 °C
Initial Time	1.17 min
Program Rate	20 °C/min
Final Temperature	320 °C
Final Time	1.33 min
Transfer Line	280 °C
Injection Method:	programmable temperature vaporizer - splitless
Initial Temperature	30 °C
Initial Time	0.10 min
Program Rate	500 °C/min
Final Temperature	280 °C
Final Time	5.0 min
Purge Delay	1.07 min
Splitter Flow	50 mL/min
Septum Purge	on
Injection Volume	1 µL

8.3. Typical Mass Spectrometry Operating Conditions (Note 12.5.)

Detector Mode:	positive-ion electron-impact ionization
Source Temperature	230 °C
Quad Temperature	150 °C
Calibration Program	electron-impact ionization autotune
Electron Multiplier	1750 volts (~ 450 volts above autotune)
SIM Resolution	high
Dwell Time	50 msec

Ions Monitored:

nitrapyrin	
quantitation	*m/z* 194
confirmation (primary)	*m/z* 196
confirmation (secondary)	*m/z* 198
2-Cl-6-DCMP	
quantitation	*m/z* 160
confirmation (primary)	*m/z* 162
confirmation (secondary)	*m/z* 195
$^{13}C_2{}^{15}N$-2,3,5,6-TCP (ISTD)	
quantitation	*m/z* 220

8.4. Mass Spectra

Full-scan mass spectra of nitrapyrin, 2-chloro-6-(dichloromethyl)pyridine, and the $^{13}C_2{}^{15}N$-2,3,5,6-tetrachloropyridine internal standard are illustrated in Figure 1.

8.5. Typical Calibration Curves and Chromatograms

For the determination of nitrapyrin in soil using the quantitation ion *m/z* 194, a typical calibration curve and representative chromatograms of a standard, a control sample, and a 0.010-µg/g (LOQ) recovery sample are illustrated in Figures 2-5.

For the determination of nitrapyrin in soil using the confirmation ion *m/z* 196, a typical calibration curve and representative chromatograms of a standard, a control sample, and a 0.010-µg/g (LOQ) recovery sample are illustrated in Figures 6-9.

For the determination of nitrapyrin in soil using the confirmation ion *m/z* 198, a typical calibration curve and representative chromatograms of a standard, a control sample, and a 0.010-µg/g (LOQ) recovery sample are illustrated in Figures 10-13.

For the determination of 2-Cl-6-DCMP in soil using the quantitation ion *m/z* 160, a typical calibration curve and representative chromatograms of a standard, a control sample, and a 0.010-µg/g (LOQ) recovery sample are illustrated in Figures 14-17.

For the determination of 2-Cl-6-DCMP in soil using the confirmation ion *m/z* 162, a typical calibration curve and representative chromatograms of a standard, a control sample, and a 0.010-µg/g (LOQ) recovery sample are illustrated in Figures 18-21.

For the determination of 2-Cl-6-DCMP in soil using the confirmation ion *m/z* 195, a typical calibration curve and representative chromatograms of a standard, a control sample, and a 0.010-µg/g (LOQ) recovery sample are illustrated in Figures 22-25.

9. DETERMINATION OF RECOVERY OF NITRAPYRIN AND 2-CHLORO-6-(DICHLOROMETHYL)PYRIDINE FROM SOIL

9.1. Sample Preparation

Prepare soil samples for analysis by freezing with dry ice or liquid nitrogen and then grinding or chopping using a hammer mill with a 1/8- or 3/16-inch screen size. Prepared soil samples should be stored frozen at approximately -10 to -20 °C until analysis.

9.2. Sample Analysis

9.2.1. Weigh 5.0-g portions of the prepared soil sample into a series of 45-mL glass vials.

9.2.2. For preparing fortified samples, add 100-µL aliquots of the appropriate spiking solutions (Section 7.1.6) to encompass the necessary concentration range.

9.2.3. Add 25 mL of the acetone/0.1 N hydrochloric acid (90:10) extraction solution to the sample vial.

9.2.4. Cap the vial with a PTFE-lined cap, and sonicate the sample for approximately 10 minutes.

9.2.5. Shake the sample vial for a minimum of 30 minutes on a reciprocating shaker at approximately 180 excursions/minute.

9.2.6. Centrifuge the sample vial for 5 minutes at 2000 rpm.

9.2.7. Transfer the acetone/0.1 N hydrochloric acid extraction solution into a clean 50-mL graduated mixing cylinder.

9.2.8. Repeat Steps 9.2.3-9.2.6 with 14 mL of the acetone/0.1 N hydrochloric acid (90:10) extraction solution.

9.2.9. Combine the acetone/0.1 N hydrochloric acid extraction solution from Step 9.2.8 with the 25 mL from Step 9.2.7.

9.2.10. Adjust the volume in the graduated mixing cylinder to 40.0 mL with additional acetone/0.1 N hydrochloric acid (90:10) extraction solution. Stopper the cylinder and mix thoroughly.

9.2.11. Pipet 4.0 mL of the sample solution from Step 9.2.10 into a clean 45-mL vial.

9.2.12. Add 25.0 mL of 0.1 N hydrochloric acid, 5 grams of sodium chloride, and 1.0 mL of cyclohexane containing the internal standard (Section 7.2.2) to the sample vial.

9.2.13. Cap the sample vial with a PTFE-lined cap, and shake the sample for a minimum of 10 minutes on a reciprocating shaker at approximately 180 excursions/minute.

9.2.14. Centrifuge the sample vial for 5 minutes at 2000 rpm.

9.2.15. Transfer a portion of the cyclohexane (top) layer to a 2-mL autosampler vial containing a limited-volume insert and seal the vial with a cap.

9.2.16. Analyze the calibration standards (Section 7.3) and samples by capillary gas chromatography with electron-impact ionization mass spectrometry as described in Sections 8.2 and 8.3. Determine the suitability of the chromatographic system using the following performance criteria:

a. Standard curve linearity: Determine that the coefficient of determination (r^2) equals or exceeds 0.990 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 2-25 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 5.00-ng/mL calibration standard.

9.2.17. If the sample extracts contain analyte concentrations that exceed the linear range of the standard calibration curve, dilute those samples with an appropriate amount of the cyclohexane internal standard solution from Section 7.2.2 to obtain responses within the range of the calibration curve.

Alternatively, another aliquot of the sample solution from Step 9.2.11 can be taken, and extracted as described in Steps 9.2.12-9.2.14 using a larger volume of cyclohexane containing the internal standard.

10. CALCULATIONS

10.1. Calculation of Standard Calibration Curve

10.1.1. Inject the series of calibration standards described in Section 7.3.1 and determine the peak areas for the analytes and internal standard as indicated below.

nitrapyrin	*m/z* 194 (quantitation) *m/z* 196 (primary confirmation) *m/z* 198 (secondary confirmation)
2-Cl-6-DCMP	*m/z* 160 (quantitation) *m/z* 162 (primary confirmation) *m/z* 195 (secondary confirmation)
$^{13}C_2{}^{15}N$-2,3,5,6-TCP	*m/z* 220 (quantitation)

10.1.2. For each standard, calculate each analyte's quantitation ratio.

For example, using the nitrapyrin *m/z* 194 data from Figure 3:

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

$$\text{Quantitation Ratio} = \frac{\text{nitrapyrin peak area at } m/z \text{ 194}}{^{13}C_2{}^{15}N\text{-2,3,5,6-TCP peak area at } m/z \text{ 220}}$$

$$\text{Quantitation Ratio} = \frac{3556}{82557} = 0.04307$$

10.1.3. Prepare a standard curve by plotting the analyte concentration on the x-axis and the respective quantitation ratio on the y-axis as shown in Figure 2. Using regression analysis, determine the equation for the curve with respect to the abscissa.

For example, using power regression (13.1) with the nitrapyrin *m/z* 194 data from Figure 2:

$$Y = \text{constant} \times X^{(\text{exponent})}$$

$$X = \left(\frac{Y}{\text{constant}}\right)^{1/\text{exponent}}$$

$$\text{nitrapyrin (ng/mL)} = \left(\frac{\text{nitrapyrin quantitation ratio}}{\text{constant}}\right)^{1/1.01891}$$

$$\text{nitrapyrin (ng/mL)} = \left(\frac{\text{nitrapyrin quantitation ratio}}{0.00834}\right)^{1/1.01891}$$

10.2. Calculation of Percent Recovery

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

For example, using the data for the nitrapyrin *m/z* 194 ion from Figure 5:

$$\text{nitrapyrin (gross ng/mL)} = \left(\frac{\text{nitrapyrin quantitation ratio}}{0.00834}\right)^{1/1.01891}$$

$$\text{nitrapyrin (gross ng/mL)} = \left(\frac{0.04427}{0.00834}\right)^{1/1.01891}$$

$$\text{nitrapyrin (gross)} = 5.147 \text{ ng/mL}$$

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

$$\text{nitrapyrin (gross µg/g)} = \text{nitrapyrin (gross ng/mL)} \times \left(\frac{\text{extraction volume}}{\text{aliquot volume}} \times \frac{\text{final volume}}{\text{sample weight}} \times \frac{1.00 \text{ µg}}{1000 \text{ ng}}\right)$$

$$\text{nitrapyrin (gross µg/g)} = \text{nitrapyrin (gross ng/mL)} \times \left(\frac{40.0 \text{ mL}}{4.00 \text{ mL}} \times \frac{1.00 \text{ mL}}{5 \text{ gram}} \times \frac{1.00 \text{ µg}}{1000 \text{ ng}}\right)$$

$$\text{nitrapyrin (gross µg/g)} = 5.147 \text{ ng/mL} \times 0.002 \frac{\text{mL} \times \text{µg}}{\text{g} \times \text{ng}}$$

$$\text{nitrapyrin (gross)} = 0.0103 \text{ µg/g}$$

10.2.3. Determine the net concentration in each recovery sample by subtracting any contribution found at the expected retention time of the analyte in the control sample from that of the gross analyte concentration in the recovery sample.

For example, using the data for the nitrapyrin *m/z* 194 ion from Figures 4 and 5:

$$\text{nitrapyrin (net µg/g)} = \text{nitrapyrin (gross µg/g)} - \text{nitrapyrin (control µg/g)}$$

$$\text{nitrapyrin (net µg/g)} = 0.0103 \text{ µg/g} - 0.0000 \text{ µg/g}$$

$$\text{nitrapyrin (net)} = 0.0103 \text{ µg/g}$$

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

$$\text{Recovery} = \frac{\text{concentration found}}{\text{concentration added}} \times 100\%$$

$$\text{Recovery} = \frac{0.0103\ \mu g/g}{0.0100\ \mu g/g} \times 100\%$$

$$\text{Recovery} = 103\%$$

10.3. Determination of Nitrapyrin and 2-Chloro-6-(dichloromethyl)pyridine in Soil

10.3.1. Determine the gross concentration of the analyte in the treated sample by substituting the quantitation ratio obtained into the equation for the standard calibration curve and calculating the uncorrected residue result as described in Sections 10.2.1-10.2.2.

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

For example, using the data for the nitrapyrin *m/z* 194 ion from Figure 5 and the average recovery for the samples analyzed on 15-Dec-2008 from Table 2:

$$\text{nitrapyrin (corrected } \mu g/g) = \text{nitrapyrin (net } \mu g/g) \times \left(\frac{100}{\%\ \text{Recovery}}\right)$$

$$\text{nitrapyrin (corrected } \mu g/g) = 0.0103\ \mu g/g \times \frac{100}{105}$$

$$\text{nitrapyrin (corrected)} = 0.0098\ \mu g/g$$

10.4. Determination of Soil Moisture (Field Samples Only)

10.4.1. Accurately weigh a 10-g portion of soil into a tared aluminum weighing dish.

10.4.2. Place the sample in an oven at 110 °C and allow to dry for a minimum of 16 hours.

10.4.3. Remove the sample from the oven and place in a desiccator containing Drierite® adsorbent. Re-weigh the sample when it has cooled to room temperature.

10.4.4. Calculate the percent moisture (dry weight basis) as follows:

$$\text{percent moisture (dry weight basis)} = \frac{\text{water, g}}{\text{dry soil, g}} \times 100$$

$$\text{percent moisture (dry weight basis)} = \frac{\left(\text{sample weight before drying, g}\right) - \left(\text{sample weight after drying, g}\right)}{\text{sample weight after drying, g}} \times 100$$

10.5. Determination of Dry Weight Concentrations of Nitrapyrin and 2-Chloro-6-(dichloromethyl)pyridine in Soil

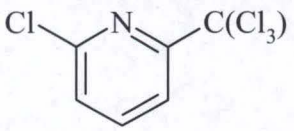
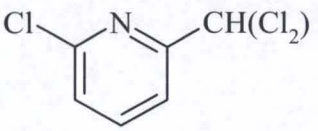
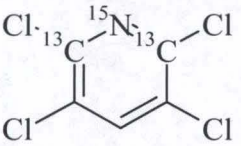
10.5.1. Determine the analyte concentration in the sample as described in Section 10.3.

10.5.2. Determine the soil moisture as described in Section 10.4.

10.5.3. Determine the dry weight analyte concentration in the sample as follows:

$$\text{nitrapyrin (dry weight } \mu\text{g/g)} = \text{nitrapyrin } (\mu\text{g/g}) \times \left(1 + \frac{\%\ \text{Moisture}}{100}\right)$$

Table 1. Identity and Structure of Nitrapyrin, 2-Chloro-6-(dichloromethyl)pyridine, and Internal Standard

Common Name of Compound	Structural Formula and Chemical Name
nitrapyrin Molecular Formula: $C_6H_3Cl_4N$ Molecular Weight: 230.907 Monoisotopic Mass: 229 CAS Number 1929-82-4	 2-chloro-6-(trichloromethyl)pyridine
2-chloro-6-(dichloromethyl)pyridine Molecular Formula: $C_6H_4Cl_3N$ Molecular Weight: 196.463 Monoisotopic Mass: 195 CAS Number 78152-53-1	 2-chloro-6-(dichloromethyl)pyridine
[2,6-$^{13}C^{15}N$]-2,3,5,6-tetrachloropyridine Molecular Formula: $^{13}C_2C_3HCl_4{}^{15}N$ Molecular Weight: 219.859 Monoisotopic Mass: 218 CAS Number not available	 [2,6-$^{13}C^{15}N$]-2,3,5,6-tetrachloropyridine

GRM: 07.14
EFFECTIVE: 06-Jan-2015
SUPERSEDES: New

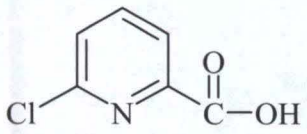
Dow AgroSciences

Determination of Residues of 6-Chloropyridine-2-carboxylic Acid in Soil by Gas Chromatography with Negative-Ion Chemical Ionization Mass Spectrometry Detection

E. L. Olberding and G. E. Dial, Jr.

1. SCOPE

This method is applicable for the quantitative determination of residues of the nitrapyrin metabolite, 6-chloropyridine-2-carboxylic acid (6-CPA), in soil. The method was validated over the concentration range of 0.010-10.0 µg/g with a validated limit of quantitation of 0.010 µg/g.



6-CPA
CAS No. 4684-94-0

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

2. PRINCIPLE

Residues of 6-CPA are extracted from a 5-gram soil sample by sonication and shaking with an aqueous 0.5 N potassium hydroxide/10% potassium chloride solution. After adjusting to a final volume of 40.0 mL, a 1.0-mL aliquot of the extract is acidified with 1.5 mL of 0.5 N hydrochloric acid. The sample is shaken and centrifuged, and a 1.0-mL aliquot of the supernatant is purified using a polymeric solid-phase extraction (SPE) column. After elution from the SPE column with dichloromethane, an internal standard solution containing $^{13}C_3{}^{15}N$-6-chloropyridine-2-carboxylic acid ($^{13}C_3{}^{15}N$-6-CPA) is added to the column eluate, and the resulting solution is evaporated to dryness. The sample is then derivatized with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoracetamide (MTBSTFA) to form the *tert*-butyldimethylsilyl (TBDMS) derivatives of 6-CPA and $^{13}C_3{}^{15}N$-6-CPA, and subsequently analyzed by capillary gas chromatography with negative-ion chemical ionization mass spectrometry (GC/NCI-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. Acetone, cyclohexane, methanol, and MTBSTFA are flammable and should be used in well-ventilated areas away from ignition sources.

3.3. Hydrochloric acid and potassium hydroxide are corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be worn when handling these reagents.

3.4. Dry ice and liquid nitrogen can cause severe burns or pose a suffocation hazard. It is imperative that proper eye and personal protection equipment be worn when handling these chemicals.

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 PM600, Mettler-Toledo Inc.

4.1.3. Bath, water, catalog number 15-460-20, Fisher Scientific, Pittsburgh, PA 15275.

4.1.4. Centrifuge, with rotor to accommodate 8- and 45-mL vials, Model Centra-GP8, Thermo International Equipment Company, Needham Heights, MA 02194.

4.1.5. Desiccator, 250-mm i.d., catalog number 08-615B, Fisher Scientific.

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

4.1.7. Hammer mill, with 1/8- and 3/16-inch screens, Model 2001, AGVISE Laboratories, Inc., Northwood, ND 58267.

4.1.8. Oven, Model OV-490A-2, Blue M Electric Company, Blue Island, IL 60406.

4.1.9. Pipet, positive-displacement, 1-10 µL capacity, catalog number M10, Gilson Inc., Middleton, WI 53562.

4.1.10. Pipet, positive-displacement, 10-100 µL capacity, catalog number M100, Gilson Inc.

4.1.11. Pipet, positive-displacement, 50-250 µL capacity, catalog number M250, Gilson Inc.

4.1.12. Pipet, positive-displacement, 200-1000 µL capacity, catalog number M1000, Gilson Inc.

4.1.13. Shaker, variable speed reciprocating with box carrier, Model 6000, Eberbach Corporation, Ann Arbor, MI 48106.

4.1.14. Ultrasonic cleaner, Model 1200, Branson Cleaning Equipment Company, Shelton, CT 06484.

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

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

4.2. Chromatographic System

4.2.1. Column, capillary gas chromatography, Durabond-5MS liquid phase, 30 m x 0.25 mm i.d., 1.0-µm film thickness, catalog number 122-5533, Agilent Technologies, Santa Clara, CA 95051.

4.2.2. Gas chromatograph, Model 6890A, Agilent Technologies, Wilmington, DE 19808.

4.2.3. Gas purifier, catalog number OT3-2, Agilent Technologies. (Note 12.2.)

4.2.4. Gas purifier, catalog number G1999-80410, Agilent Technologies. (Note 12.3.)

4.2.5. Injector, automatic, Model 7683, Agilent Technologies.

4.2.6. Inlet liner, PTV, multi-baffled, catalog number 5183-2037, Agilent Technologies.

4.2.7. Mass spectrometer, Model 5973N, Agilent Technologies.

4.2.8. Mass spectrometer data system, Model G1701DA, Agilent Technologies.

5. GLASSWARE AND MATERIALS (Note 12.1.)

5.1. Column, Strata-X polymeric SPE, 30-mg sorbent, 1-mL reservoir, catalog number 8B-S100-TAK, Phenomenex, Torrance, CA 90501.

5.2. Cylinder, graduated mixing, 50-mL, catalog number 3002-50, Corning Inc., Acton, MA 01720.

5.3. Dish, aluminum weighing, catalog number 08-732, Fisher Scientific.

5.4. Flask, volumetric, 50-mL, catalog number 5640-50, Corning Inc.

5.5. Flask, volumetric, 100-mL, catalog number 5640-100, Corning Inc.

5.6. Flask, volumetric, 200-mL, catalog number 5640-200, Corning Inc.

5.7. Flask, volumetric, 2000-mL, catalog number 5640-2L, Corning Inc.

5.8. Pipet, serological, 2-mL, catalog number 7077-2N, Corning Inc.

5.9. Pipet, serological, 25-mL, catalog number 7077B-25N, Corning Inc.

5.10. Pipet, volumetric, 1.0-mL, catalog number 13-650-3B, Fisher Scientific.

5.11. Pipet, volumetric, 10-mL, catalog number 13-650-3L, Fisher Scientific.

5.12. Pipet, volumetric, 20-mL, catalog number 13-650-3N, Fisher Scientific.

5.13. Pipet, volumetric, 25-mL, catalog number 13-650-3P, Fisher Scientific.

5.14. Pipet, volumetric, 30-mL, catalog number 13-650-3Q, Fisher Scientific.

5.15. Pipet, volumetric, 50-mL, catalog number 13-650-3S, Fisher Scientific.

5.16. Pipet tip, positive-displacement, 10-µL capacity, catalog number CP10, Gilson Inc.

5.17. Pipet tip, positive-displacement, 100-µL capacity, catalog number CP100, Gilson Inc.

5.18. Pipet tip, positive-displacement, 250-µL capacity, catalog number CP250, Gilson Inc.

5.19. Pipet tip, positive-displacement, 1000-µL capacity, catalog number CP1000, Gilson Inc.

5.20. Vial, 2-mL, autosampler, catalog number C4000-1W, National Scientific Company, Rockwood, TN 37854.

5.21. Vial, 8-mL, with PTFE-lined screw cap, catalog number 2503T, Qorpak, Bridgeville, PA 15017.

5.22. Vial, 45-mL, catalog number 60958A-11, Kimble Glass Co., Vineland, NJ 08360.

5.23. Vial cap, PTFE-lined, for 45-mL vial, catalog number 5205, Qorpak.

5.24. Vial cap, for autosampler vial, catalog number C4000-54B, National Scientific Company.

6. REAGENTS, STANDARDS, AND PREPARED SOLUTIONS

6.1. Reagents

6.1.1. Acetone, OmniSolv grade, catalog number AX0116-1, EMD Chemicals Inc., Gibbstown, NJ 08027.

6.1.2. Cyclohexane, OmniSolv grade, catalog number HX0296-1, EMD Chemicals Inc.

6.1.3. Desiccant, Drierite adsorbent, catalog number 24001, W. A. Hammond Drierite Company, Xenia, OH 45385.

6.1.4. Dichloromethane, OmniSolv grade, catalog number DX0831-1, EMD Chemicals Inc.

6.1.5. Helium, gas, 99.995% purity, BOC Gases, New Providence, NJ 07974.

6.1.6. Hydrochloric acid, 0.1 N, certified concentration, catalog number SA54-1, Fisher Scientific.

6.1.7. Hydrochloric acid, 0.5 N, certified concentration, catalog number SA50-1, Fisher Scientific.

6.1.8. MTBSTFA (*N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide), catalog number 48920, Pierce Chemical Company, Rockford, IL 61105.

6.1.9. Methane, gas, 99.995% purity, BOC Gases.

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

6.1.11. Nitrogen, refrigerated liquid, catalog number LQNI, BOC Gases, New Providence, NJ 07974. (Note 12.4.)

6.1.12. Potassium chloride, certified ACS, catalog number P217-500, Fisher Scientific.

6.1.13. Potassium hydroxide, pellets, certified ACS, catalog number P250-500, Fisher Scientific.

6.1.14. Water, OmniSolv grade, catalog number WX0004-1, EMD Chemicals Inc.

6.2. Standards

6.2.1. 6-chloropyridine-2-carboxylic acid

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

6.2.2. $^{13}C_3{}^{15}N$-6-chloropyridine-2-carboxylic acid

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

6.3. Prepared Solutions

6.3.1. 0.5 N potassium hydroxide/10% potassium chloride

Weigh 56.11 grams of potassium hydroxide (corrected for purity) and 200 grams of potassium chloride into a 2000-mL volumetric flask. Add water and swirl the flask until the solids are dissolved. Allow the solution to equilibrate to room temperature and dilute to volume with water.

7. PREPARATION OF STANDARDS

7.1. Preparation of 6-Chloropyridine-2-carboxylic Acid Fortification Solutions

7.1.1. Weigh 0.1000 g of the 6-CPA analytical standard and quantitatively transfer to a 100-mL volumetric flask with methanol. Dilute to volume with methanol to obtain a 1000-µg/mL stock solution.

7.1.2. Pipet 10.0 mL of the 1000-µg/mL solution from Section 7.1.1 into a 200-mL volumetric flask and dilute to volume with methanol to obtain a 50.0-µg/mL stock solution.

7.1.3. Pipet 20.0 mL of the 50.0-µg/mL solution from Section 7.1.2 into a 200-mL volumetric flask and dilute to volume with methanol to obtain a 5.00-µg/mL stock solution.

7.1.4. Pipet 20.0 mL of the 5.00-µg/mL solution from Section 7.1.3 into a 200-mL volumetric flask and dilute to volume with methanol to obtain a 0.500-µg/mL stock solution.

7.1.5. Pipet 20.0 mL of the 0.500-µg/mL solution from Section 7.1.4 into a 200-mL volumetric flask and dilute to volume with methanol to obtain a 0.050-µg/mL stock solution.

7.1.6. Prepare solutions for spiking samples by diluting the above stock solutions from Sections 7.1.3-7.1.5 with methanol as follows:

Concentration of Stock Soln.	Aliquot of Stock Soln.	Final Soln. Volume	Spiking Soln. Final Conc.	Equivalent Sample Conc.[a]
µg/mL	mL	mL	µg/mL	µg/g
0.050	30.0	100	0.015	0.003
0.050	50.0	100	0.025	0.005
0.050	- - -	- - -	0.050	0.010
0.500	25.0	100	0.125	0.025
0.500	50.0	100	0.250	0.050
0.500	- - -	- - -	0.500	0.100
5.00	25.0	100	1.25	0.250
5.00	50.0	100	2.50	0.500
5.00	- - -	- - -	5.00	1.00
50.0	- - -	- - -	50.0	10.0

[a] The equivalent sample concentration is based on fortifying a 5.0-gram soil sample with 1.00 mL of spiking solution.

7.2. Preparation of the $^{13}C_3{}^{15}N$-6-Chloropyridine-2-carboxylic Acid Internal Standard Solution

7.2.1. Weigh 0.0050 g of the $^{13}C_3{}^{15}N$-6-chloropyridine-2-carboxylic acid reference standard and quantitatively transfer to a 50-mL volumetric flask with methanol. Dilute to volume with methanol to obtain a 100-µg/mL stock solution.

7.2.2. Dispense 250 µL of the 100-µg/mL solution from Section 7.2.1 into a 50-mL volumetric flask and dilute to volume with methanol. This solution contains 0.50 µg/mL (0.50 ng/µL) of $^{13}C_3{}^{15}N$-6-CPA.

7.3. Preparation of Calibration Standards for the Quantitation of 6-Chloropyridine-2-carboxylic Acid

7.3.1. Prepare calibration standards with each sample set by dispensing 10.0 µL of the 0.015-5.00-µg/mL fortification solutions from Section 7.1.6 into a series of 8-mL vials containing 100 µL of the internal standard solution (Section 7.2.2) as follows:

Concentration of Spiking Soln.	Aliquot of Spkg. Soln.	Final Soln. Volume	Calib Soln. Final Conc.	Equivalent Sample Conc.[a]
µg/mL	µL	mL	ng/mL	µg/g
- - -	- - -	2.0	0.000	0.000
0.015	10.0	2.0	0.075	0.003
0.025	10.0	2.0	0.125	0.005
0.050	10.0	2.0	0.250	0.010
0.125	10.0	2.0	0.625	0.025
0.250	10.0	2.0	1.25	0.050
0.500	10.0	2.0	2.50	0.100
1.25	10.0	2.0	6.25	0.250
2.50	10.0	2.0	12.50	0.500
5.00	10.0	2.0	25.0	1.00

[a] The equivalent sample concentration is based on using a 5.0-gram soil sample and a final volume of 2.0 mL.

7.3.2. Evaporate the solution to dryness using an N-Evap evaporator set at 35 °C and a nitrogen flow rate of approximately 500 mL/min.

7.3.3. Add 500 µL of acetone and 100 µL of the MTBSTFA derivatizing reagent to the sample vial and firmly seal with a PTFE-lined cap. Vortex mix the sample for 1-2 seconds, and then sonicate the sample for 1-2 seconds.

7.3.4. Place the sample vial in an oven at 60 °C and allow the mixture to react for 60 minutes.

7.3.5. Remove the sample vial from the oven and allow the reaction mixture to cool to room temperature.

7.3.6. Using a serological pipet, adjust the volume in the sample vial to 2.0 mL with cyclohexane and firmly seal with a PTFE-lined cap. Vortex the sample for 1-2 seconds, and then sonicate the sample for 1-2 seconds.

7.3.7. Transfer a portion of the sample to a 2-mL autosampler vial and seal the vial with a cap.

7.4. Preparation of the 6-Chloropyridine-2-carboxylic Acid TBDMS Standard to Determine Isotopic Crossover

7.4.1. Using a 100-µL syringe or positive-displacement pipet, dispense 100 µL of the 0.50-µg/mL 6-CPA solution in Section 7.1.6 into an 8-mL vial and derivatize according to the procedure described in Section 7.3.2-7.3.7. The resulting solution contains 6-CPA-TBDMS equivalent to 25.0 ng/mL of 6-CPA.

7.5. Preparation of the $^{13}C_3{}^{15}N$-6-Chloropyridine-2-carboxylic Acid TBDMS Standard to Determine Isotopic Crossover

7.5.1. Using a 100-µL syringe or positive-displacement pipet, dispense 100 µL of the 0.50-µg/mL $^{13}C_3{}^{15}N$-6-CPA solution in Section 7.2.2 into an 8-mL vial and derivatize according to the procedure described in Section 7.3.2-7.3.7. The resulting solution contains $^{13}C_3{}^{15}N$-6-CPA-TBDMS equivalent to 25.0 ng/mL of $^{13}C_3{}^{15}N$-6-CPA.

8. INSTRUMENTAL CONDITIONS

8.1. Column

Install the PTV column inlet liner and the capillary column in the PTV injection port of the gas chromatograph following the manufacturer's recommended procedures.

8.2. Typical Gas Chromatography Operating Conditions (Notes 12.5. and 12.6.)

Instrumentation:	Agilent Model 6890A gas chromatograph Agilent Model 7683 autoinjector Agilent Model 5973N mass spectrometer Agilent Model G1701DA data system
Column:	J & W fused silica capillary Durabond-5MS liquid phase 30 m x 0.25 mm i.d. 1.0-µm film thickness
Carrier Gas Method:	helium
Constant Flow	1.0 mL/min
Vacuum Compensation	on
Initial Head Pressure	~48 kPa
Linear Velocity	~37 cm/s

Oven Method:

Initial Temperature	40 °C
Initial Time	1.32 min
Program Rate	20 °C/min
Final Temperature	320 °C
Final Time	0.68 min
Transfer Line	280 °C

Injection Method: programmable temperature vaporizer - splitless

Initial Temperature	30 °C
Initial Time	0.10 min
Program Rate	500 °C/min
Final Temperature	280 °C
Final Time	5.0 min
Purge Delay	1.22 min
Splitter Flow	50 mL/min
Septum Purge	on
Injection Volume	1 µL

8.3. Typical Mass Spectrometry Operating Conditions (Note 12.5.)

Detector Mode: negative-ion chemical ionization

Source Temperature	150 °C
Quad Temperature	106 °C
Reagent Gas	methane
Flow Setting	40%
Pressure	2.0×10^{-4} torr
Calibration Program	negative-ion chemical ionization autotune
Electron Multiplier	1400 volts (same as autotune)
SIM Resolution	high
Dwell Time	50 msec

Ions Monitored:

6-CPA-TBDMS	
quantitation	*m/z* 271
confirmation	*m/z* 273
$^{13}C_3{}^{15}N$-6-CPA-TBDMS (ISTD)	
quantitation	*m/z* 277

8.4. Mass Spectra

Full-scan mass spectra of the TBDMS derivatives of 6-chloropyridine-2-carboxylic acid and the $^{13}C_3{}^{15}N$-6-chloropyridine-2-carboxylic acid internal standard are illustrated in Figure 1.

8.5. Typical Calibration Curve

A typical calibration curve for the determination of 6-chloropyridine-2-carboxylic acid in soil using the quantitation ion *m/z* 271 is shown in is illustrated in Figure 2.

A typical calibration curve for the determination of 6-chloropyridine-2-carboxylic acid in soil using the confirmation ion *m/z* 273 is shown in is illustrated in Figure 3.

8.6. Typical Chromatograms

Typical chromatograms of a 25.0-ng/mL 6-CPA TBDMS standard and a 25.0-ng/mL $^{13}C_3{}^{15}N$-6-CPA TBDMS standard used to determine isotopic crossover are illustrated in Figures 4-5, respectively.

Typical chromatograms of a standard, a control sample, a 0.010-µg/g (LOQ) recovery sample, and a 10.0-µg/g recovery sample for the determination of 6-chloropyridine-2-carboxylic acid in soil are illustrated in Figures 6-9.

9. DETERMINATION OF RECOVERY OF 6-CHLOROPYRIDINE-2-CARBOXYLIC ACID FROM SOIL

9.1. Sample Preparation

Prepare soil samples for analysis by freezing with dry ice or liquid nitrogen and then grinding or chopping using a hammer mill with a 1/8- or 3/16-inch screen size. Prepared soil samples should be stored frozen at approximately -10 to -20 °C until analysis.

9.2. Sample Analysis

9.2.1. Weigh 5.0-g portions of the prepared soil sample into a series of 45-mL glass vials.

9.2.2. For preparing fortified samples, add 1.00-mL aliquots of the appropriate spiking solutions (Section 7.1.6) to encompass the necessary concentration range.

9.2.3. Add 25 mL of the aqueous 0.5 N potassium hydroxide/10% potassium chloride extraction solution to the sample vial.

9.2.4. Cap the vial with a PTFE-lined cap, and place the sample in a water bath for 30 minutes at 70 °C.

9.2.5. After extraction, allow the sample vial to cool to room temperature.

9.2.6. Shake the sample vial for a minimum of 15 minutes on a reciprocating shaker at approximately 180 excursions/minute.

9.2.7. Centrifuge the sample vial for 5 minutes at 2000 rpm.

9.2.8. Transfer the extraction solution into a clean 50-mL graduated mixing cylinder.

9.2.9. Repeat Step 9.2.3 and Steps 9.2.6-9.2.7 with 14 mL of the aqueous 0.5 N potassium hydroxide/10% potassium chloride extraction solution.

9.2.10. Combine the extraction solution from Step 9.2.9 with the 25 mL from Step 9.2.8.

9.2.11. Adjust the volume in the graduated mixing cylinder to 40.0 mL with additional aqueous 0.5 N potassium hydroxide/10% potassium chloride extraction solution. Stopper the cylinder and mix thoroughly.

9.2.12. Pipet 1.0 mL of the sample solution from Step 9.2.11 into a clean 8-mL vial and add 1.5 mL of 0.5 N hydrochloric acid to the vial. Cap the vial with a PTFE-lined cap, and mix thoroughly. Allow the vial to set for 15 minutes.

9.2.13. If there appear to be suspended particles in the solution, or if the solution is cloudy, centrifuge the sample vial for 5 minutes at 2000 rpm.

9.2.14. Purify the sample using the following reversed-phase SPE procedure:

a. Place a Phenomenex Strata-X SPE column on the vacuum manifold.

b. Condition the SPE column with 1 mL of methanol followed by 1 mL of 0.1 N hydrochloric acid. Dry the SPE column under full vacuum (~-15 in Hg) for 5 seconds between solvents.

c. Transfer 1.0 mL of the sample solution from Step 9.2.13 to the SPE column. Draw the sample through the column at a flow rate of approximately 1 mL/min, discarding the eluate.

d. Rinse the SPE column with three 1.0-mL aliquots of 0.1 N hydrochloric acid. Draw the solvent through the column at a flow rate of approximately 1 mL/min, discarding the eluate. Dry the SPE column under full vacuum (~-15 in Hg) for approximately thirty minutes. (Note 12.7.)

e. Elute the 6-CPA from the SPE column with two 750-µL aliquots of dichloromethane, collecting the eluate in an 8-mL vial.

9.2.15. Add 100 µL of the internal standard solution (Section 7.2.2) to the sample eluate and mix thoroughly. Evaporate the sample eluate to dryness using an N-Evap evaporator set at 35 °C and a nitrogen flow rate of approximately 500 mL/min.

9.2.16. Add 500 µL of acetone and 100 µL of the MTBSTFA derivatizing reagent to the sample vial and firmly seal with a PTFE-lined cap. Vortex mix the sample for 1-2 seconds, and then sonicate the sample for 1-2 seconds.

9.2.17. Place the sample vial in an oven set at 60 °C and allow the mixture to react for 60 minutes.

9.2.18. Remove the sample vial from the oven and allow the reaction mixture to cool to room temperature.

9.2.19. Using a serological pipet, adjust the volume in the sample vial to 2.0 mL with cyclohexane and firmly seal with a PTFE-lined cap. Vortex the sample for 1-2 seconds, and then sonicate the sample for 1-2 seconds.

9.2.20. Transfer a portion of the sample to a 2-mL autosampler vial and seal the vial with a cap.

9.2.21. Analyze the crossover standards (Sections 7.4.1 and 7.5.1), calibration standards (Section 7.3) and samples by capillary gas chromatography with negative-ion chemical ionization mass spectrometry as described in Sections 8.2 and 8.3. Determine the suitability of the chromatographic system using the following performance criteria:

a. Standard curve linearity: Determine that the coefficient of determination (r^2) equals or exceeds 0.990 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 6-9 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 0.25-ng/mL calibration standard.

10. CALCULATIONS

10.1. Determination of Isotopic Crossover

In this assay, the analyte and internal standard are quantitated using major fragment ions for each compound. When using stable-isotope labeled internal standards there is a possibility that isotopic contributions will occur between the ions 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 6-CPA and $^{13}C_3{}^{15}N$-6-CPA, inject the 25.0-ng/mL 6-CPA-TBDMS crossover standard (Section 7.4.1) and the 25.0-ng/mL $^{13}C_3{}^{15}N$-6-CPA-TBDMS internal standard solution (Section 7.5.1) and determine the peak areas for the analyte and internal standard as indicated below.

6-CPA	*m/z* 271 (quantitation) *m/z* 273 (confirmation)
$^{13}C_3{}^{15}N$-6-CPA	*m/z* 277 (quantitation)

For example, to determine the contribution of the 6-CPA to the $^{13}C_3{}^{15}N$-6-CPA internal standard using the 6-CPA data from Figure 4:

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

$$\text{Crossover Factor (}m/z\text{ 271}\rightarrow\text{ISTD)} = \frac{\text{peak area at } m/z \text{ 277}}{\text{peak area at } m/z \text{ 271}} = \frac{0}{684519} = 0.0000$$

In a similar manner, to determine the contribution of the $^{13}C_3{}^{15}N$-6-CPA internal standard to the 6-CPA using the $^{13}C_3{}^{15}N$-6-CPA data from Figure 5:

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

$$\text{Crossover Factor (ISTD}\rightarrow m/z\text{ 271)} = \frac{\text{peak area at } m/z \text{ 271}}{\text{peak area at } m/z \text{ 277}} = \frac{0}{223163} = 0.0000$$

During method development, there was no isotopic crossover 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 ratio corrected for accurate determination of concentrations (13.1, 13.2).

10.2. Calculation of Standard Calibration Curve

10.2.1. Inject the series of calibration standards described in Section 7.3.1 and determine the peak areas for the analyte and internal standard as indicated below.

6-CPA	*m/z* 271 (quantitation) *m/z* 273 (confirmation)
$^{13}C_2{}^{15}N$-6-CPA	*m/z* 277 (quantitation)

10.2.2. For each standard, calculate the 6-CPA quantitation ratio.

For example, using the data for the 6-CPA *m/z* 271 ion from Figure 6:

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

$$\text{Quantitation Ratio} = \frac{\text{6-CPA peak area at } m/z \text{ 271}}{^{13}C_3\,^{15}N\text{-6-CPA peak area at } m/z \text{ 277}}$$

$$\text{Quantitation Ratio} = \frac{6839}{235563} = 0.02903$$

10.2.3. Prepare a standard curve by plotting the analyte concentration on the x-axis and the respective quantitation ratio on the y-axis as shown in Figure 2. Using regression analysis, determine the equation for the curve with respect to the abscissa.

For example, using power regression (13.3) with the 6-CPA *m/z* 271 data from Figure 2:

$$Y = \text{constant} \times X^{(\text{exponent})}$$

$$X = \left(\frac{Y}{\text{constant}}\right)^{1/\text{exponent}}$$

$$\text{6-CPA (ng/mL)} = \left(\frac{\text{6-CPA quantitation ratio}}{\text{constant}}\right)^{1/\text{exponent}}$$

$$\text{6-CPA (ng/mL)} = \left(\frac{\text{6-CPA quantitation ratio}}{0.11960}\right)^{1/0.99762}$$

10.3. Calculation of Percent Recovery

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

For example, using the data for the 6-CPA *m/z* 271 ion from Figure 8:

$$\text{6-CPA (gross ng/mL)} = \left(\frac{\text{6-CPA quantitation ratio}}{0.11960}\right)^{1/0.99762}$$

$$\text{6-CPA (gross ng/mL)} = \left(\frac{0.03112}{0.11960}\right)^{1/0.99762}$$

$$\text{6-CPA (gross)} = 0.2594 \text{ ng/mL}$$

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

$$\text{6-CPA (gross µg/g)} = \text{6-CPA (gross ng/mL)} \times \left(\frac{\text{extraction volume}}{\text{aliquot volume}} \times \frac{\text{pre-SPE volume}}{\text{SPE load volume}} \times \frac{\text{final volume}}{\text{sample weight}} \times \frac{1.00\ \mu g}{1000\ ng}\right)$$

$$\text{6-CPA (gross µg/g)} = \text{6-CPA (gross ng/mL)} \times \left(\frac{40.0\ mL}{1.00\ mL} \times \frac{2.50\ mL}{1.00\ mL} \times \frac{2.00\ mL}{5\ gram} \times \frac{1.00\ \mu g}{1000\ ng}\right)$$

$$\text{6-CPA (gross µg/g)} = 0.2594 \text{ ng/mL} \times 0.040 \frac{mL \times \mu g}{g \times ng}$$

$$\text{6-CPA (gross)} = 0.0104 \text{ µg/g}$$

10.3.3. Determine the net concentration in each recovery sample by subtracting any contribution found at the expected retention time of the analyte in the control sample from that of the gross analyte concentration in the recovery sample.

For example, using the data for the 6-CPA *m/z* 271 ion from Figures 7 and 8:

$$\text{6-CPA (net µg/g)} = \text{6-CPA (gross µg/g)} - \text{6-CPA (control µg/g)}$$

$$\text{6-CPA (net µg/g)} = 0.0104 \text{ µg/g} - 0.0011 \text{ µg/g}$$

$$\text{6-CPA (net)} = 0.0093 \text{ µg/g}$$

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

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

$$\text{Recovery} = \frac{0.0093\ \mu g/g}{0.0100\ \mu g/g} \times 100\%$$

$$\text{Recovery} = 93\%$$

10.4. Determination of 6-Chloropyridine-2-carboxylic Acid in Soil

10.4.1. Determine the gross concentration of the analyte in the treated sample by substituting the quantitation ratio obtained 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 fortified at or above the limit of quantitation from a given sample set to correct for method efficiency.

For example, using the data for the 6-CPA *m/z* 271 ion from Figure 8 and the average recovery for the samples analyzed on 11-Dec-2008 from Table 2:

$$\text{6-CPA (corrected } \mu g/g) = \text{6-CPA (net } \mu g/g) \times \left(\frac{100}{\%\ \text{Recovery}}\right)$$

$$\text{6-CPA (corrected } \mu g/g) = 0.0093\ \mu g/g \times \frac{100}{95}$$

$$\text{6-CPA (corrected)} = 0.0098\ \mu g/g$$

10.5. Determination of Soil Moisture (Field Samples Only)

10.5.1. Accurately weigh a 10-g portion of soil into a tared aluminum weighing dish.

10.5.2. Place the sample in an oven at 110 °C and allow to dry for a minimum of 16 hours.

10.5.3. Remove the sample from the oven and place in a desiccator containing Drierite® adsorbent. Re-weigh the sample when it has cooled to room temperature.

10.5.4. Calculate the percent moisture (dry weight basis) as follows:

$$\begin{matrix}\text{percent moisture} \\ \text{(dry weight basis)}\end{matrix} = \frac{\text{water, g}}{\text{dry soil, g}} \times 100$$

$$\begin{matrix}\text{percent moisture} \\ \text{(dry weight basis)}\end{matrix} = \frac{\begin{pmatrix}\text{sample weight} \\ \text{before drying, g}\end{pmatrix} - \begin{pmatrix}\text{sample weight} \\ \text{after drying, g}\end{pmatrix}}{\text{sample weight after drying, g}} \times 100$$

10.6. Determination of Dry Weight Concentrations of 6-Chloropyridine-2-carboxylic Acid in Soil

10.6.1. Determine the analyte concentration in the sample as described in Section 10.4.

10.6.2. Determine the soil moisture as described in Section 10.5.

10.6.3. Determine the dry weight analyte concentration in the sample as follows:

$$\begin{matrix}\text{6-CPA} \\ \text{(dry weight } \mu\text{g/g)}\end{matrix} = \begin{matrix}\text{6-CPA} \\ (\mu\text{g/g})\end{matrix} \times \left(1 + \frac{\%\ \text{Moisture}}{100}\right)$$

11.4. Assay Time

A typical analytical run would consist of a minimum of nine standards encompassing the expected range of sample concentrations, a reagent blank, a control (a non-fortified sample), a minimum of three fortified controls (two of which must be at the LOQ), and 15 samples. This typical analytical run can be prepared in approximately 6 hours, followed by the chromatographic analysis.

There are several acceptable "stopping points" in the method, where sample preparation (Section 9) may be suspended, upon completion of a step, without deleterious effects on the sample analysis. These are indicated below:

a. Step 9.2.11.

b. Step 9.2.13.

c. Step 9.2.14.e.

d. Step 9.2.18.

e. Step 9.2.19.

If samples are to be stored overnight, the vials should be capped with PTFE-lined caps.

11.5. Standardization of Phenomenex Strata-X Solid-Phase Column Elution Profile

There is a possibility that variation in the Phenomenex Strata-X SPE column may influence the elution profile of 6-CPA. If it is necessary to obtain an elution profile for the SPE columns used to optimize recovery and clean-up efficiency, the following procedure can be used:

11.5.1. Dispense 50.0 µL of the 5.00-µg/mL fortification solution from Section 7.1.6 into an 8-mL vial containing 5.0 mL of 0.1 N hydrochloric acid.

11.5.2. Cap the sample vial with a PTFE-lined cap and then vortex mix the sample for 1-2 seconds.

11.5.3. Purify the sample using the following SPE procedure:

a. Place a Phenomenex Strata-X (30-mg) SPE column on the vacuum manifold.

b. Condition the SPE column with 1 mL of methanol followed by 1 mL of 0.1 N hydrochloric acid. Dry the SPE column under full vacuum (~-15 in Hg) for 5 seconds between solvents.

c. Transfer 1.0 mL of the sample solution from Step 11.5.2 to the SPE column. Draw the sample through the column at a flow rate of approximately 1.0 mL/min, discarding the eluate.

d. Rinse the SPE column with three 1.0-mL aliquots of 0.1 N hydrochloric acid. Draw the solvent through the column at a flow rate of approximately 1 mL/min, discarding the eluate. Dry the SPE column under full vacuum (~-15 in Hg) for approximately thirty minutes. (Note 12.7.)

e. Elute the 6-CPA from the SPE column with 3.0 mL of dichloromethane, collecting 750-µL aliquots of the eluate in separate 8-mL vials.

11.5.4. For each fraction collected, proceed as described in Sections 9.2.15 through 9.2.19.

11.5.5. Calculate the percent recovery as described in Section 10.3.

A typical elution profile for the Phenomenex Strata-X SPE column for the determination of 6-chloropyridine-2-carboxylic acid in soil is illustrated in Figure 10.

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. The gas purifier is used in the carrier gas supply line to purify the helium entering the gas chromatograph.

12.3. The gas purifier is used in the reagent gas supply line to purify the methane entering the mass spectrometer.

12.4. The liquid nitrogen is used for sample preparation (if needed) and for cooling the programmable temperature vaporizer injection port of the gas chromatograph.

12.5 The TBDMS derivatives of 6-CPA and $^{13}C_3{}^{15}N$-6-CPA are thermally labile and may degrade if using a conventional split/splitless injection port. Therefore, a programmable temperature vaporizer (PTV) or cool on-column injection port should be used for this analysis.

12.6. The GC/NCI-MS operating conditions may be modified to obtain optimal chromatographic separation and mass spectrometric performance.

12.7. It is important that the SPE column be completely dry before eluting the 6-CPA with dichloromethane. The observation of salt or water in the sample vial following Step 9.2.15 may indicate that the SPE column requires further drying.

13. REFERENCES

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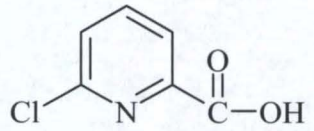
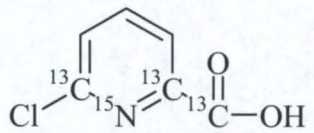
Table 1. Identity and Structure of 6-Chloropyridine-2-carboxylic Acid and $^{13}C_3{}^{15}N$-6-Chloropyridine-2-carboxylic Acid Internal Standard

Common Name of Compound	Structural Formula and Chemical Name
6-chloropyridine-2-carboxylic acid Molecular Formula: $C_6H_4ClNO_2$ Formula Weight: 157.56 Monoisotopic Mass: 157 CAS Number 4684-94-0	 6-chloropyridine-2-carboxylic acid
$^{13}C_3{}^{15}N$-6-chloropyridine-2-carboxylic acid Molecular Formula: $^{13}C_3C_3H_4Cl^{15}NO_2$ Formula Weight: 161.53 Monoisotopic Mass: 161 CAS Number not available	 $^{13}C_3{}^{15}N$-6-chloropyridine-2-carboxylic acid