Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS

INTRODUCTION

Scope

The objective of this study is to provide residue method validation data for the determination of clopyralid and picloram in various soil types utilizing SPE sample purification.

This method is applicable for the quantitative determination of residues of clopyralid and picloram in soil matrices (loamy sand, sandy clay loam, loam, and silt loam soil, per USDA Soil Class, equivalent to loamy sand, sandy clay, clay loam, and clay loam, respectively, per International Soil Class). The method was validated over the concentration range from the limit of quantitation (0.50 µg/kg) to 2000x the limit of quantitation (1000 µg/kg) with a limit of detection verification of 0.15 µg/kg. Common and chemical names, molecular formulas, and the nominal masses for the analytes are given in Table 1.

This study was conducted to fulfill data requirements outlined in the U. S. EPA Residue Chemistry Test Guidelines, OCSPP 850.6100 (1), the European Commission Guidance Document on Residue Analytical Methods, SANCO/3029/99 rev.4 (2) and SANCO/825/00 rev.8.1 (3), and PMRA Residue Chemistry Guidelines as Regulatory Directive Dir 98-02 (4).

Method Principle

Residues of clopyralid and picloram are extracted from soil samples by adding 25 mL of acetone:1N hydrochloric acid (90:10) then shaking and centrifuging, followed by 10 mL of additional acetone:1N hydrochloric acid (90:10) and further shaking and centrifuging. The acetone is then evaporated using nitrogen and brought to 8 mL final volume with 1N sodium hydroxide before vortexing and sonication. Approximately 8 mL of dichloromethane is added, with sonication, vortexing, and centrifuging to mix well, and the upper 6 mL extract layer is transferred to a clean glass tube and 6 mL of 1N hydrochloric acid is added. The sample is then passed through a pre-conditioned Waters HLB solid phase extraction (SPE) column. The sample bottle is then rinsed with 1N hydrochloric acid which is used to rinse the SPE column. The sample bottle is then rinsed with acetonitrile/1N formic acid (15:85) solution which is then used to rinse the SPE column, followed by drying under full vacuum. The SPE column is eluted with dichloromethane, which is evaporated to dryness using a gentle steam of nitrogen. The sample residue is reconstituted with a methanol/0.1% formic acid (10:90) solution filtered through a 0.2-µm PTFE syringe filter and then analyzed by liquid chromatography coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC-MS-MS).

Safety Precautions

Each analyst must be acquainted with the potential hazards of the equipment, reagents, products, solvents, and procedures used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: OPERATION MANUALS, MATERIAL SAFETY DATA

SHEETS, LITERATURE, AND OTHER RELATED DATA. Safety information should be obtained from the supplier. Disposal of waste materials, reagents, reactants, and solvents must be in compliance with applicable governmental requirements.

Acetone, acetonitrile, and methanol are flammable and should be used in well-ventilated areas away from ignition sources. Formic and hydrochloric acids, and sodium hydroxide are corrosive and can cause severe burns. Dichloromethane is an inhalation hazard and should be used with proper ventilation. It is imperative that proper eye and personal protection equipment be worn when handling these reagents.

Test Substances/Analytical Standard and Internal Standard

Test Substance	**TSN Number**	**Percent Purity**	**Certification Date**	**Reference**
Clopyralid	301194	99.5	12 Jul 2011	FAPC 11-000104
Picloram	029006-0001	99.7	17 Feb 2012	FAPC 12-000067

The above standards were obtained from the Test Substance Coordinator, Dow AgroSciences LLC, 9330 Zionsville Road, Building 304, Indianapolis, IN 46268-1054. The certificates of analysis were provided by Dow AgroSciences LLC, and are located in Appendix B.

Characterization of Control Matrices

The soil specimens were GLP characterized by Agvise Laboratories, Northwood, North Dakota; details of the characterization results are as follows:

PARAMETER	RESULTS			
ABC Designation	484	485	498	508
Geographic Location	Tift County , Georgia, USA	Willacy County, Texas, USA	Tehama County, California, USA	Boone County, Missouri, USA
Common Name	Tift	Raymondville	Tehama	Boone
Textural Class (USDA)	Loamy Sand	Sandy Clay Loam	Loam	Silt Loam
% Sand	87	53	39	23
% Silt	8	18	40	58
% Clay	5	29	21	19
Textural Class (International)	Loamy Sand	Sandy Clay	Clay Loam	Clay Loam
% Sand	91	63	57	37
% Silt	4	8	22	44
% Clay	5	29	21	19
Bulk Density (g/cc)	1.39	1.16	1.10	0.97
CEC (meq/100 g)	4.7	20.8	15.4	10.2
% Moisture at 0 bar (%)	32.4	67.1	57.5	70.7
% Moisture at 1/10 bar (%)	5.0	32.6	32.5	41.7
% Moisture at 1/3 bar (%)	3.8	20.9	21.3	34.3
% Moisture at 15 bar (%)	2.3	11.4	8.5	13.8
% Organic Matter (Walkley Black)	1.1	0.90	2.9	4.0
% Organic Carbon[b]	0.64	0.52	1.7	2.4
pH[a]	5.6	7.9	6.3	5.5
Nitrogen, Total (%)	0.05	0.07	0.18	0.21
Soluble Salts (mmhos/cm)	0.20	0.52	0.49	0.10
Calcium (ppm)	374	2880	1560	851
Magnesium (ppm)	49	326	491	143
Sodium (ppm)	13	93	54	18
Potassium (ppm)	102	441	236	64
Hydrogen (ppm)	21	22	26	46
Olsen Phosphorus (ppm)	23	12	48	9

Note: Soil characterization analyses were performed at Agvise Laboratories, Northwood, North Dakota.

[a] pH in 1:1 soil:water ratio

[b] Organic Carbon = Organic Matter / 1.72

Equipment, Glassware, and Materials

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 glassware and supplies are assumed to be readily available. Unless specified otherwise, Class A volumetric glassware is used to prepare analytical standards, fortification solutions, and calibration standards.

Laboratory Equipment

Balance, Model 1702MPB, Sartorius, Germany

Balance, Model XP205DR, Mettler

Balance, Model BB2440, Mettler

Centrifuge, Beckman GP Benchtop, Beckman Coulter

Centrifuge, Beckman Model GS 6R/HT, Beckman Coulter

Column, analytical, Accucore Phenyl-hexyl, 4.6 x 50 mm, 2.6 µm, Thermo Scientific

Evaporator, Meyer N-EVAP™ 112, Organomation Associates, Inc

Extraction vacuum manifold, Supelco Visiprep™ 24, Sigma-Aldrich

Liquid chromatograph, Leap Autosampler HTC Pal, Agilent 1100 Bin Pump, Agilent 1100 Degasser, Agilent 1100 Col. Comp., Valco Switching Valve

Mass spectrometer, Applied Biosystems/Sciex API 4000 MS/MS, Applied Biosystems Mass spectrometer data system, Model Analyst 1.5.1, Applied Biosystems

Pipet, Thermo Scientific* Finnpipette* F2 Adjustable-Volume Pipetter, 1-10mL (catalog no. 14-386-322), Fisher Scientific

Pipet, Gilson Microman® M25, 3-25 µL (part no. F148502), Gilson, Inc.

Pipet, Gilson Microman® M100, 10-100 µL (part no. F148504), Gilson, Inc.

Pipet, Gilson Microman® M250, 50-250 µL (part no. F148505), Gilson, Inc.

Pipet, Gilson Microman® M1000, 100-1000 µL (part no. F148506), Gilson, Inc. Glassware and Materials

Glass bottles, 4 oz (catalog no. 02-911-904), Fisher Scientific

Bottle caps, PTFE lined (catalog no. 16161-188), VWR

Column, Oasis HLB SPE, 6 mL, 200 mg (part no. WAT106202), Waters

Conical-bottom glass centrifuge tubes, Kimble* 50 mL (catalog no. 0553841A), Fisher Scientific

Disposable borosilicate glass tubes with threaded end (16 x 125mm), Fisherbrand* (catalog no. 14-959-35), Fisher Scientific

Syringe, BD (no. 309657) Vacutainer* Luer-Lok* Disposable Syringes with Luer-Lok* Tips (catalog no.14-823-435), Fisher Scientific

Syringe filters, 17 mm, 0.2 µm PTFE (catalog no. 42213PC), Fisher Scientific

Vial, autosampler, 2 mL (catalog no. C4011-5), National Scientific Company

Vial cap, for autosampler vial (catalog no. C4011-54), National Scientific Company

Reagents

Acetone, HPLC Grade (catalog no. A949-4), Fisher Scientific

Acetonitrile, Optima Grade (catalog no. A996SK-4), Fisher Scientific

Dichloromethane, Optima Grade (catalog no. D151SK-4), Fisher Scientific

Formic acid (95%), (catalog no. F0507), Sigma Aldrich

Formic acid (99+%), Optima LC/MS Grade (catalog no. A117-50), Fisher Scientific

Formic acid (98%), ACS Grade (catalog no. MFX04406), EMD Chemicals

Hydrochloric acid (36.5-38.0 w/w%), ACS Grade Plus (catalog no. A144SI-212), Fisher Scientific

Methanol, Optima Grade (catalog no. A454SK-4), Fisher Scientific

Sodium hydroxide, Pellets/Certified ACS (98.9%) (catalog no. S318-500), Fisher Scientific

Water, HPLC grade (catalog no. W5-4), Fisher Scientific

Prepared Solutions (prepare to scale as necessary)

0.01% Formic Acid in Water

Add 0.4 mL of formic acid (99+% purity) to 4.0 L of water. Cap, and invert several times until mixed well.

0.1% Formic Acid in Water

Place 1000 mL of HPLC grade water in a 1 L bottle and add 1 mL of formic acid.

1 N Formic Acid

Place 40 mL of concentrated formic acid to a 1-L graduated cylinder containing approximately 900 mL of HPLC grade and bring to volume with HPLC grade water.

1N Hydrochloric Acid

Add 166 mL of concentrated hydrochloric acid to a 2-L bottle containing 1 L of HPLC grade water. Mix and add an additional 834 mL of HPLC grade water. Volumes may be adjusted proportionally to make greater or lesser amounts of the solution.

1.0 N Sodium Hydroxide (aq)

Weigh 40.2 g of sodium hydroxide (99.5%) into beaker. Quantitatively transfer to a 1000-mL graduated cylinder with water. Dilute to mark with HPLC grade water and mix well.

Acetone/1 N Hydrochloric Acid (90:10)

Place 100 mL of 1 N hydrochloric acid into a 1000-mL bottle containing 900 mL of acetone.

Acetonitrile/1 N Formic Acid (15:85)

Place 150 mL of acetonitrile in a 1000-mL graduated cylinder containing approximately 800 mL of 1 N formic acid, cool and bring to volume with 1 N formic acid.

Methanol/0.1% Formic Acid (10:90)

Place 100 mL of methanol in a 1000-mL bottle containing 900 mL of 0.1% formic acid. Mix well.

Methanol/Acetonitrile 0.01% Formic acid (60:40)

Add 2400 mL of methanol, 1600 mL of acetonitrile and 0.4 mL of formic acid (99+% purity) to a 4 L container and invert several times until mixed well. Volumes may be adjusted proportionally to make greater or lesser amounts of the solution.

Needle wash 1:1:1 Methanol/Acetonitrile/Water

Combined 4 L each of acetonitrile, methanol, and HPLC Grade water. Mix well.

EXPERIMENTAL

Instrumental Conditions

Typical Liquid Chromatography Operating Conditions

Instrumentation:	MDS SCIEX API 4000 LC-MS/MS System MDS SCIEX Analyst 1.5.1 data system Agilent Technologies 1100 Series
Column:	Accucore Phenyl-hexyl, 4.6 x 50 mm, 2.6-µm
Column Temperature:	35 °C
Injection Volume:	50 µL (may vary based on sensitivity)
Injection Wash Program:	Autosampler loop and needle washed with: 1:1:1 Acetonitrile/Methanol/Water
Run Time:	5.5 minutes
Mobile Phase:	A: water containing 0.01% formic acid B: methanol/acetonitrile (60:40) containing 0.01% formic acid
Flow Rate:	1000 µL/min, no split

Gradient:

Time, min	A, %	B, %
0.00	85	15
3.00	85	15
3.10	5	95
4.40	5	95
4.50	85	15
5.50	85	15

Note: The gradient may be adjusted to obtain satisfactory chromatography on a given system.

Typical Mass Spectrometry Operating Conditions

Interface:	ESI
Polarity:	Negative
Scan Type:	MRM (MRM)
Resolution:	Q1 – unit, Q3 – unit
Curtain Gas (CUR):	40
Collision Gas (CAD):	8.0
Temperature (TEM):	500 °C
Ion Source Gas 1 (GS1):	50
Ion Source Gas 2 (GS2):	60

Period 1

Pre-acquisition Delay:	0.0 min
Acquisition Time	3.3 min
IonSpray Voltage (IS):	-2000 volts
Entrance Potential (EP):	-10 volts

Analytes:	Precursor Ion Q1	Product Ion Q3	Dwell Time (ms)	Collision Energy (CE)	Declustering Potential (DP)	Cell Exit Potential (CXP)
Clopyralid (quantification)	189.9	145.9	125	-16 V	-35 V	-15 V
Clopyralid (confirmation)	191.9	147.9	125	-16 V	-35 V	-15 V
Picloram (quantification)	240.9	196.9	125	-16 V	-35 V	-15 V
Picloram (confirmation)	238.9	194.9	125	-16 V	-35 V	-15 V

See Figure 1 and Figure 2 for mass spectra of the analytes.

Preparation of Standard Solutions

Weigh 0.0250 g (adjusted for purity) of each analyte into separate 50-mL volumetric flasks. Dilute to volume with methanol to obtain a stock solutions containing 0.50 mg/mL of each analyte. Volumes may be adjusted proportionally to make different amounts or concentrations of the standard solutions.

Preparation of Fortification Solutions

Pipet 2.0 mL of each of the 0.50-mg/mL standard solutions prepared above into a single 50-mL volumetric flask. Dilute to volume with methanol to obtain a mixed 20.0-µg/mL fortification stock solution; mix well.

Pipet 1.0 mL of the 20.0-µg/mL standard solution prepared above into a 100-mL volumetric flask. Dilute to volume using a methanol to obtain a 0.20-µg/mL fortification stock solution.

Pipet 3.0 mL of the 0.20-µg/mL standard solution prepared above into a 10-mL volumetric flask. Dilute to volume using methanol to obtain a 0.060-µg/mL fortification solution.

Preparation of Calibration Standards for Samples

Prepare calibration standards by diluting the appropriate calibration standard stock solutions using a methanol/0.1% formic acid (10:90) solution according to the following table:

The concentrations of the calibration standards are as follows:

Concentration of Stock Soln.	Aliquot of Stock Soln.	Final Soln. Volume	Calibration Std. Final Conc.	Equivalent Sample Conc.[a]
	mL	mL	ng/mL	µg/kg
20.0 µg/mL	5.0	100	1000	NA
1000 ng/mL	5.0	100	50.0	13
1000 ng/mL	1.25	50	25.0	6.7
1000 ng/mL	1.0	100	10.0	2.7
10.0 ng/mL	5.0	50	1.00	0.27
10.0 ng/mL	4.0	100	0.40	0.11

[a] The equivalent sample concentrations are based on taking a 6.0-mL initial aliquot of the 8-mL sample extract and purifying on an SPE cartridge and reconstituting the eluate to a final volume of 1.0 mL using a methanol/0.1% formic acid (10:90) solution (equivalent to 3.75 g of soil per mL of final sample as prepared for assay).

Sample Origin, Numbering, Preparation and Storage

Untreated control samples of soil were obtained from commercial or local sources. All samples were tracked by ABC Laboratories, Inc. Complete source documentation is included in the raw data.

Soil samples were stored frozen prior to analysis.

Sample Analysis

1. Weigh 5.0 ± 0.05 g of each sample into 50-mL glass tubes equipped with caps.

   Note: all post-extraction steps in the procedure should be carried out in glass containers.

2. For recovery samples, add appropriate aliquots of spiking solution to obtain concentrations ranging from 0.15-1000 µg/kg for each analyte. (A reagent blank contains no matrix.) Refer to table below for example fortification levels to obtain this concentration range.

Sample Description	Spiking Volume µL	Spiking Solution µg/mL	Fortification Level µg/kg[a]
CONTROL	---	---	---
LOD	12.5	0.06	0.15[b]
LOQ	12.5	0.20	0.5
HIGH (2000 x LOQ)	250	20.0	1000

[a] Based on a 5.0-g initial sample.
[b] Used for qualitative view of LOD only.

3. Sample Extraction:

   a. Add 25 mL of acetone/1N hydrochloric acid (90:10) solution to each sample, cap the tubes, and shake for approximately 2 hours on a reciprocating shaker.

   b. Centrifuge at 3000 rpm for approximately 5 min and decant the solvent into clean 50-mL glass tubes.

c. Add additional 10 mL of acetone/1N hydrochloric acid (90:10) solution to original samples and shake for another 30 minutes on a reciprocating shaker.

d. Centrifuge at approximately 3000 rpm for approximately 5 min and decant the solvent into the same 50-mL glass tubes.

e. Remove acetone under a gentle stream of nitrogen at room temperature (approximately 2 mL will remain in glass tubes).

f. Bring samples to final volume of 8 mL with 1N sodium hydroxide. Vortex and sonicate.

4. Dichloromethane Partition Cleanup:

   a. Add approximately 8 mL of dichloromethane, sonicate, and vortex for 30 seconds to mix well.

   b. Centrifuge samples for approximately 5 min at approximately 3000 rpm.

   c. Transfer 6.0 mL of the upper extract layer to clean 15-mL glass tubes and add 6 mL of 1N hydrochloric acid.

5. Purify samples using the following HLB procedure:

   a. Condition 0.2 g Waters HLB columns with 5 mL of methanol followed by 5 mL of 1N hydrochloric acid.

   b. Transfer the sample solutions onto the HLB columns at a rate of approximately 2 mL/min.

   c. Rinse the sample tubes with 1 mL of 1N hydrochloric acid. Wash the HLB columns with the rinse.

   d. Rinse the sample tubes with 5 mL of, acetonitrile/1N formic acid (15:85) solution. Wash the HLB columns with the rinse, and then pull dry for at least 30 minutes under full vacuum.

   e. Elute the sample from the columns with 14 mL of dichloromethane, collect in clean 15-mL glass tubes.

   Note: Ensure that each SPE cartridge is drawing adequate vacuum to dry the adsorbent material in each cartridge. If recoveries drop below acceptance criteria, the HLB columns should be profiled in the presence of matrix to determine quantitative analyte recovery with this load/wash/elute pattern.

6. Evaporate the dichloromethane to dryness at approximately 40°C using a gentle stream of nitrogen.

7. Reconstitute the samples in 1.0 mL of methanol/0.1% formic acid (10:90) solution with sonication and vortexing. This step is critical in dissolving all residues from the sides of the tube and should be done individually by hand and repeated three times alternating vortexing and sonication.

   Note: This step of sample dissolution into the 1 mL of, methanol/0.1% formic acid (10:90) solution is critical. Good technique is important, be sure to roll tube so that the solution

comes in contact with the entire internal surface area in between each vortexing and sonication step.

8. Appropriately dilute high fortifications and residues to within the curve. For example: transfer a 0.10-mL aliquot of the 1000 µg/kg fortification sample final solution into a 15 mL glass tube and add 9.90 mL methanol/0.1% formic acid (10:90) solution (DF=100). Cap and vortex.
9. Filter final extracts through 0.2 µm PTFE syringe filters.
10. Analyze the calibration standards and samples by negative-ion ESI LC-MS/MS (see instrument parameters below), injecting the calibration standards interspersed with the samples throughout the run.
11. Calculate the percent recovery found for each analyte.
12. Determine the suitability of the chromatographic system using the following criteria:
    a. Standard curve linearity: Determine that the correlation coefficient (r) equals or exceeds 0.990 for the least squares equation which describes the detector response as a function of standard curve concentration. Weighting of 1/x may be necessary for accurate concentration determinations at the lower end of calibration curve.
    b. Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in the final method with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 3:1 has been attained for the 0.4-ng/mL calibration standard (approximately equivalent to 0.15 µg/kg of clopyralid/picloram in the sample).
13. Dilute any samples that have a concentration above 80% of the highest calibration standard for re-analysis using a methanol/0.1% formic acid (10:90) solution. Samples should be within the range of the standard curve.

Calculations

Calculations for instrumental analysis were conducted using a validated software application (Applied BioSystems/MDS Sciex Analyst, version 1.5.1) to create a standard curve based on linear regression. The regression functions were used to calculate a best-fit line (from a set of standard concentrations in ng/mL versus peak area response) and to determine concentrations of the analyte found during sample analysis from the calculated best-fit line. For each analytical batch, five levels of calibration standards were injected over the range 0.40 ng/mL to 50 ng/mL. All standards injected and their corresponding peak responses were entered into the program to create the standard curve. Weighting (1/x) was used. With no weighting, the slope of the line (curve) tends to be dominated by the highest point. When weighting of 1/concentration (1/x) is used, the slope more closely approximates the majority of the points used to construct it.

The equation used for the least squares fit is:

$$Y = \text{slope} \times X + \text{intercept}$$

Y = detector response (peak area) for each analyte

$$X = \frac{Y - \text{intercept}}{\text{Slope}} = \text{ng/mL}$$

The standard (calibration) curve generated for each analytical set was used for the quantitation of clopyralid or picloram in the samples from the set. The correlation coefficient (r) should be greater than 0.990 (r^2 equal to or greater than 0.98).

For the determination of clopyralid or picloram in terms of µg/kg, the following equation is used:

$$\text{Found (µg/kg)} = \frac{\text{[ng/mL found] x Final Vol. (mL) x Dilution Factor x Extract Volume (mL)}}{\text{Sample Weight (g) x Aliquot (mL)}}$$

Example: clopyralid recovery of a Tift soil sample fortified at 0.50 µg/kg (68931-069). See Figure 23.

The concentration determined from the standard curve is = 1.5104 ng/mL (as per Analyst 1.5.1)

The residue of clopyralid in the final solution is calculated as follows:

$$\text{Clopyralid (µg/kg)} = \frac{1.5104\ \text{ng/mL x } 1.0\ \text{mL x 1 x } 8.0\ \text{mL)}}{5.0\ \text{g x } 6.0\ \text{mL}} = 0.4028\ \text{ng/g} = 0.4028\ \text{µg/kg}$$

Procedural recovery data from fortified samples are calculated via the following equation:

$$\text{Percentage Recovery} = \frac{\text{µg/kg found}}{\text{µg/kg added}} \times 100$$

$$\text{Percentage Recovery} = \frac{0.4028\ \text{µg/kg}}{0.50\ \text{µg/kg}} \times 100 = 81\%\ \text{clopyralid recovery}$$

Confirmation of Residue Identity

The method is specific for the determination of clopyralid and picloram by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, one additional MS/MS ion transition is monitored. A series of calibration standards are injected as described above and the peak areas are determined for the analytes as indicated below.

clopyralid	*m/z* Q1/Q3 190/146 (quantitation) *m/z* Q1/Q3 192/148 (confirmation)
picloram	*m/z* Q1/Q3 241/197 (quantitation) *m/z* Q1/Q3 239/195 (confirmation)

For each standard, confirmation ratios are calculated to confirm the presence of the analyte in the soil samples. Confirmation ratio differences are calculated as a percent difference relative to the average confirmation ratio found for the standards.

$$\text{Confirmation Ratio} = \frac{\text{peak area of quantitation ion transition}}{\text{peak area of confirmation ion transition}}$$

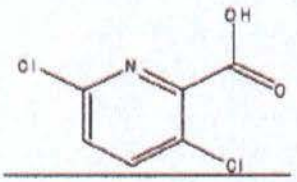
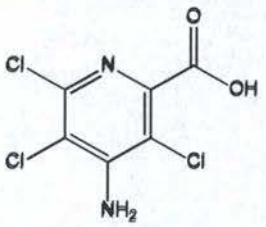
$$\text{Conf. Ratio Difference}(\%) = \frac{\text{Avg. Standard Conf. Ratio - Sample Conf. Ratio}}{\text{Avg. Standard Conf. Ratio}} \text{ X } 100$$

Confirmation of the presence of the analyte is indicated when the retention time of the samples matches that of the standards and the confirmation ratio is in the range of ±20% of the average found for the standards.

Statistical Treatment of Data

The mean recoveries for the fortified samples were calculated using the "AVERAGE" function of the Microsoft Excel spreadsheet computer program, which divides the sum of the selected cells by the number of determinations. The standard deviation of the µg/L found and recoveries for each fortification level and overall recoveries for each matrix type was calculated using the "STDEV" function of the same spreadsheet program, which sums the squares of the individual deviations from the mean, divides by the number of degrees of freedom (n-1), and extracts the square root of the quotient. Percent relative standard deviation, % RSD, was calculated by dividing the standard deviation by the mean, and then multiplying by 100.

Table 1. Identity and Structure of Clopyralid and Picloram

Common Name of Compound	Structural Formula and Chemical Name
Clopyralid Molecular Formula: $C_6H_3Cl_2NO_2$ Formula Weight: 192.00 Nominal Mass: 191 CAS Number 1702-17-6	 3,6-dichloropyridine-2-carboxylic acid or 3,6-dichloropicolinic acid
Picloram Molecular Formula: $C_6H_3Cl_3N_2O_2$ Formula Weight: 241.46 Nominal Mass: 240 CAS Number 1918-02-1	 4-Amino-3,5,6-trichloropyridine-2-carboxylic acid