Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS

INTRODUCTION

Scope

This method is applicable for the quantitative determination of residues of clopyralid and picloram in water matrices (ground water, drinking water, and surface water). The method was validated over the concentration range of 0.050-10 µg/L with a verification of the limit of detection of 0.015 µg/L. 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 water samples by passing 100 mL of water through a pre-conditioned Waters HLB solid phase extraction (SPE) column after adjusting the pH to below 2 with 1N HCl. The sample bottle is then rinsed with 1N HCl 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.

Acetonitrile and methanol are flammable and should be used in well-ventilated areas away from ignition sources. Formic and hydrochloric acids 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 water specimens were GLP characterized by ABC Laboratories; details of the characterization results are as follows:

Specimen (Date of Collection/ Characterization)	Conductivity (µS)	Alkalinity (mg/L)[a]	Total Hardness (mg/L)[a]	DO	pH	Dissolved Organic Carbon (ppm)	Total Organic Carbon (ppm)
Ground Water (06 Aug 12/09 Aug 12/17 Aug 12)	341	150	142	8.47	8.37	5.22	3.84
Drinking Water (06 Aug 12/09 Aug 12/17 Aug 12))	669	306	288	8.39	7.62	5.49	4.37
Surface Water (06 Aug 12/09 Aug 12/17 Aug 12))	111.1	30	30	8.33	9.30	14.32	13.23

[a]Calculated value of endpoint as $CaCO_3$.

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

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

Liquid chromatograph, Waters Acquity Column Manager, Waters Acquity Sample Manager, Waters Acquity Binary Solvent Manager, Waters Acquity Sample Organizer

Mass spectrometer, Applied Biosystems/Sciex API 5000 MS/MS, Applied Biosystems

Mass spectrometer data system, Model Analyst 1.5.1, Applied Biosystems

Pipet, Gilson 100-1000 µL, Microman, Gilson, Inc.

Pipet, Gilson 10-100 µL, Microman, Gilson, Inc.

Glassware and Materials

Glass bottles, 4 oz, Fisher Scientific catalog No. 02-911-904

Bottle caps, PTFE lined, VWR catalog No. 16161-188

Column, Oasis HLB SPE, 6 mL, 200 mg, Part number WAT106202, Waters

Syringe, 3 mL, BD Ref 309657

Syringe Filters, 0.2 µm PTFE, Fisher Scientific catalog No. 09-720-7

Vial, autosampler, 2 mL, catalog No. C4011-5, National Scientific Company

Vial cap, for autosampler vial, catalog number C4011-54, National Scientific Company

Reagents

Acetonitrile, Optima Grade, Fisher Scientific

Formic Acid, 99+%, Fisher Scientific

Concentrated Formic Acid, 88%, Sigma-Aldrich

Concentrated Formic Acid, >95%, Sigma-Aldrich

Concentrated HCl, ACS Grade, Fisher Scientific

Dichloromethane, Optima Grade, Fisher Scientific

Methanol, Optima Grade, Fisher Scientific

Water, HPLC/reagent grade, Fisher Scientific

Prepared Solutions (prepare to scale as necessary)

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

Combined 4 L each of acetonitrile, methanol and high purity water. Mix well.

Needle wash 1:1:2 Methanol/Acetonitrile/Water

Combined 1000 mL of acetonitrile, 1000 mL of methanol and 2000 mL of high purity water. Mix Well.

0.01% Formic Acid

Add 0.2 mL of formic acid (99+% Purity) to a bottle containing 2 L of high purity water. Cap and invert several times until mixed well. Transfer the solution to a properly labeled mobile phase container.

1 N Formic Acid

Place 40 mL of concentrated formic acid (88%, 25N) to a 1-L graduated cylinder and bring to volume with reagent water.

0.01% Formic Acid in Methanol

Add 0.2 mL of formic acid (99+% purity) to 2.0 L of methanol. Cap, and invert several times until mixed well.

60:40 Methanol/Acetonitrile 0.01% Formic acid

Place 2400 mL of methanol in a 4000-mL bottle and add 1600 mL of acetonitrile and 0.4 mL of concentrated formic acid.

10:90, Methanol/0.1% Formic Acid

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

0.1% Formic Acid in Water

Place 1000 mL of water in a 1-L bottle and add 1 mL of concentrated formic acid.

0.01% Formic Acid in Water

Add 0.40 mL of formic acid to 4 L of HPLC-grade water.

1N HCl

Add 332 mL of concentrated HCl to a 4-L graduated cylinder containing 2 L of HPLC-grade water. Mix and add an additional 1668 mL of HPLC-grade water.

Acetonitrile/1 N Formic Acid (15:85)

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

EXPERIMENTAL

Instrumental Conditions

Typical Liquid Chromatography Operating Conditions

Instrumentation:	MDS SCIEX API 5000 LC-MS/MS System MDS SCIEX Analyst 1.5.1 data system
Column:	Accucore Phenyl-hexyl, 4.6 x 50 mm, 2.6-µm
Column Temperature:	30 °C
Injection Volume:	15 µL
Injection Wash Program:	Autosampler loop and needle washed with: 1:1:1 Acetonitrile/Methanol/Water, followed by 1:1:2 Acetonitrile/Methanol/Water
Run Time:	5.6 minutes
Mobile Phase:	A: water containing 0.01% formic acid B: methanol:acetonitrile (60:40) containing 0.01% formic acid
Flow Rate:	1.00 mL/min, no split

Gradient:

Time, min	A, %	B, %
0.00	79	21
2.00	79	21
2.10	5	95
3.50	5	95
3.60	79	21
5.60	79	21

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):	10
Collision Gas (CAD):	6.0
Temperature (TEM):	500°C
Ion Source Gas 1 (GS1):	50
Ion Source Gas 2 (GS2):	50

Period 1

Pre-acquisition Delay:	0.0 min
Acquisition Time	3.5 min
IonSpray Voltage (IS):	-4500 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)	190.0	146.0	100	-12 V	-35 V	-15 V
Clopyralid (confirmation)	191.9	147.9	100	-12 V	-35 V	-15 V
Picloram (quantification)	241.0	196.8	100	-14 V	-35 V	-15 V
Picloram (confirmation)	239.0	194.9	100	-16 V	-35 V	-15 V

Preparation of Standard Solutions

Weigh 0.0250 g (adjusted for purity) of each analyte into separate 25-mL volumetric flasks. Dilute to volume with methanol to obtain a stock solutions containing 1.00 mg/mL of analyte.

Preparation of Fortification Solutions

Pipet 2.0 mL of each of the 1.00-mg/mL standard solutions prepared above into a single 100-mL volumetric flask. Dilute to volume with methanol to obtain a mixed 20.0-µg/mL fortification stock solution; mix well.

Pipet 5.0 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 1.00-µg/mL fortification stock solution.

Pipet 5.0 mL of the 1.00-µg/mL standard solution prepared above into a 50-mL volumetric flask. Dilute to volume using methanol to obtain a 0.100-µg/mL fortification solution.

Pipet 3.0 mL of the 1.00-µg/mL standard solution prepared above into a 100-mL volumetric flask. Dilute to volume using methanol to obtain a 0.0300-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/L
20.0 µg/mL	2.5	50	1000	10.0
1000 ng/mL	2.5	50	50.0	0.50
1000 ng/mL	1.25	50	25.0	0.25
1000 ng/mL	0.5	50	10.0	0.10
25.0 ng/mL	4.0	50	2.00	0.020
10.0 ng/mL	5.0	50	1.00	0.010

[a] The equivalent sample concentration of analyte is based on taking a 100-mL initial sample volume 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 100 mL of water per mL of final sample as prepared for assay).

Sample Origin, Numbering, Preparation and Storage

Untreated control samples of ground water, drinking water, and surface water were obtained from local sources. All samples were tracked by ABC Laboratories, Inc. Complete source documentation is included in the raw data.

Water samples were stored refrigerated prior to analysis.

Sample Analysis

1. Measure 100 mL of each sample into individual glass bottles equipped with caps. Note: all 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 0.015-10 µg/L for each analyte. (A reagent blank contains no water.) 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/L[a]
CONTROL	---	---	---
LOD	50	0.030	0.015
LOQ	50	0.10	0.050
HIGH (200 x LOQ)	50	20.0	10.0

[a] Based on a 100-mL initial sample.

3. Extract/purify samples using the following SPE procedure:

    a. Add 5 mL of 1N HCl to each sample. Check the pH to be sure it is below pH 2. Adjust with additional 1N HCl if necessary.

    b. Condition 0.2 g Waters HLB SPE columns with 5 mL of methanol followed by 5 mL of 1N HCl. Pull dry for approximately 10 seconds.

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

    d. Rinse the sample bottles with 1 mL of 1N HCl. Wash the SPE columns with the rinse.

    e. Rinse the sample bottles with 5 mL of an acetonitrile/1N formic acid (15:85) solution. Wash the SPE columns with the rinse then pull dry for at least 30 minutes under full vacuum.

    f. Elute the SPE columns with 14 mL of dichloromethane, collecting the eluate in glass test tubes.

    Note: The SPE columns must be profiled in the presence of matrix to determine quantitative clopyralid and picloram recovery with this load/wash/elute pattern.

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

5. Reconstitute the samples in 1.0 mL (25.0 mL for the 10 µg/L spike) of methanol/0.1% formic acid (10:90) solutions 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 2-3 times alternating vortexing and sonication.

6. Filter final extracts through 0.2-µm PTFE syringe filters.

7. Analyze the calibration standards and samples by negative-ion ESI LC-MS/MS (see below), injecting the calibration standards interspersed with the samples throughout the run.

8. Calculate the percent recovery found for each analyte.

9. 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 (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 1.0-ng/mL calibration standard (equivalent to 0.010 µg/L of clopyralid/picloram in the sample).

10. Samples should be within the range of the standard curve. Dilute any samples that have a concentration above 80% of the highest standard in the calibration curve for re-analysis using a methanol /0.1% formic acid (10:90) solution.

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 1.0 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. For this study, the correlation coefficient (r) for each calibration curve was greater than 0.990 (r^2 equal to or greater than 0.98).

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

$$\text{Found } (\mu g/L) = \frac{[\text{ng/mL found}] \text{ x Final Vol.(mL)}}{\text{Sample Volume(mL)}}$$

Example: clopyralid recovery of a drinking water sample fortified at 0.050 µg/L (68631-011). See Figure 31.

The concentration determined from the standard curve is = 4.4901 ng/mL (as per Analyst 1.5.1)

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

$$\text{Clopyralid } (\mu g/L) = \frac{4.4901 \text{ ng/mL x } 1.0 \text{ mL}}{100 \text{ mL}} = 0.0449 \text{ µg/L}$$

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

$$\text{Percentage Recovery} = \frac{\mu g/L \text{ found}}{\mu g/L \text{ added}} \times 100$$

$$\text{Percentage Recovery} = \frac{0.0449 \mu g/L}{0.050 \ \mu g/L} \times 100 = 90\% \text{ clopyralid recovery}$$

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 (see Figure 1 and Figure 2). 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 water samples. Confirmation ratio differences are calculated as a percent difference relative to the average confirmation ratio found for the standards.

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

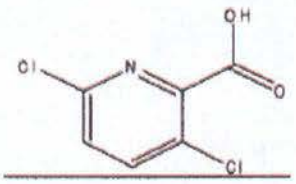
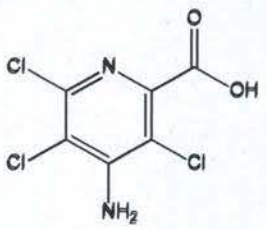
$$\text{Conf. Ratio Difference}(\%) = \frac{\text{Avg. Standard Conf. Ratio - Sample Conf. Ratio}}{\text{Avg. Standard Conf. Ratio}} \text{ X } 100$$

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