Independent Laboratory Validation of a Dow AgroSciences Method for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS

INTRODUCTION

Scope

The objective of this study was to assess and to independently validate Dow AgroSciences Method 120612 for the determination of clopyralid and picloram in soil. The method was developed on behalf of Dow AgroSciences LLC at ABC Laboratories, Inc. as study number 68931, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS" [1]. The independent laboratory validation demonstrated that the method can be considered applicable for use in the determination of residues of clopyralid and picloram in soil matrices. The methodology was successfully independently validated over the concentration range of 0.50 – 1000.0 µg/kg with an independently validated lower limit of quantification of 0.50 µg/kg.

The soil matrix was represented by a fully characterized Lufa Speyer 2.2 soil sample.

The chemical names, molecular structures, molecular formulas and molecular weights for the analytes are given in Table 1.

This study was conducted to fulfill data requirements outlined in Commission Regulation (EU) No 283/2013 setting out the data requirements, in accordance with Regulation (EC) No 1107/2009 [2] and Guidance Documents SANCO/825/00 rev.8.1. [3] and EPA Guideline OCSPP 850.6100 [4].

Method Principle

Residues of clopyralid and picloram are extracted from soil samples by adding 25 mL of acetone/1N hydrochloric acid (90:10) solution followed by shaking and centrifugation. The solvent is decanted before an additional 10 mL of acetone/1N hydrochloric acid (90:10) solution is added to the sample followed by further shaking and centrifugation. The two solvent extracts are combined, and the acetone is evaporated using nitrogen before being brought to a final volume of 8 mL using a 1N sodium hydroxide solution. The sample is vortex mixed and sonicated. Approximately 8 mL of dichloromethane is added and the sample is mixed well using vortex mixing and sonication. The sample is centrifuged before a 6 mL aliquot of the upper extract layer is transferred to a new glass tube and 6 mL of 1N hydrochloric acid is added to the upper extract layer. The acidified upper extract layer is then passed through a pre-conditioned Waters HLB SPE cartridge, and the sample tube is rinsed with 1N HCl which is then transferred to and passed through the SPE cartridge. This is followed by rinsing the sample tube with acetonitrile/1N formic acid (15:85) and passing this rinse through the SPE cartridge also. The cartridge is dried under full vacuum for 30 minutes before elution of the analytes with dichloromethane. The sample is evaporated to dryness using nitrogen and reconstituted in

1.0 mL of methanol/0.1% formic acid (10:90) solution before being filtered through a 0.2 µm PTFE syringe filter. The final sample is analysed for clopyralid and picloram by liquid chromatography coupled with negative-ion electrospray tandem mass spectrometry (LC-MS/MS).

Test Substances/Analytical Standards

Analytical Standard[a]	TSN Number	Percent Purity	Re-Certification Date	Reference
Clopyralid	TSN301194	99.9	01 July 2015	FAPC13-000100
Picloram	TSN 029006-0001	99.7	06 June 2014	FAPC12-000067

[a]The molecular formula and structure for both compounds are given in Table 1. The certificates of analysis are given in Figure 1 and Figure 2.

EXPERIMENTAL

Sample Origin, Preparation and Storage

The analytical method was independently validated using a fully characterised Lufa Speyer 2.2 soil sample, obtained from Battelle UK stocks of control samples. The soil samples were characterised by Agvise, Northwood, ND, 58267 and full characterisation details are given in Appendix 2. The soil samples were stored refrigerated, and no homogenisation was necessary prior to analysis. Unique sample numbers were assigned to the samples to track them during storage and analysis.

Calculation of Standard Calibration Curve

Calculation of a standard curve begins with the injection of a series of calibration standards described in Appendix 1 and acquisition of peak areas for the following analytes:

Clopyralid	*m/z* Q1/Q3 190/146 (quantitative) *m/z* Q1/Q3 192/148 (confirmatory)
Picloram	*m/z* Q1/Q3 241/197 (quantitative) *m/z* Q1/Q3 239/195 (confirmatory)

In order to generate a standard curve, plot the analyte concentration on the abscissa (x-axis) and the respective analyte peak area on the ordinate (y-axis) in Analyst. Using regression analysis,

determine the equation for the curve with respect to the abscissa. Refer to Figure 3 through Figure 6 for example calibration plots and to Figure 7 and Figure 8 for example calculations.

Confirmation of Residue Identity

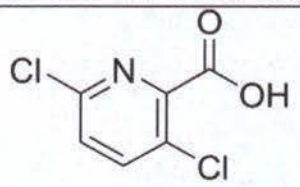
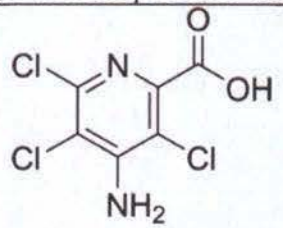
The method is selective for the determination of clopyralid and picloram by virtue of the chromatographic separation and MS/MS detection. To demonstrate further confirmation, an additional MS/MS ion transition is monitored for each analyte.
Inject the series of calibration standards and acquire peak areas for the following analytes:

Clopyralid	*m/z* Q1/Q3 190/146 (quantitative) *m/z* Q1/Q3 192/148 (confirmatory)
Picloram	*m/z* Q1/Q3 241/197 (quantitative) *m/z* Q1/Q3 239/195 (confirmatory)

Statistical Treatment of Data

Statistical treatment of data included but was not limited to the calculation of regression equations, correlation coefficients (r) for describing the linearity of calibration curves, and means, standard deviations, and relative standard deviations of the results for the fortified recovery samples.

Table 1 Identities and Structures of Clopyralid and Picloram

Common Name	Structural Formula and Chemical Name
Clopyralid Molecular Formula: $C_6H_3Cl_2NO_2$ Molecular Weight: 192.00 CAS Number: 1702-17-6	 3,6-dichloropicolinic acid
Picloram Molecular Formula: $C_6H_3Cl_3N_2O_2$ Molecular Weight: 241.46 CAS Number: 1918-02-01	 4-amino-3,5,6-trichloro-2-pyrinecarboxylic acid