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DETERMINATION OF FLUMICLORAC PENTYL ESTER AND ITS DEGRADATE, IMCA, IN/ON SOIL

METHOD RM-29S-1 **DATE: November 21, 2011**

INTRODUCTION

This method determines residues of flumiclorac pentyl ester and IMCA in soil. This method is a revision of RM-29S.(Reference 1) The sample size and solvent volumes have been reduced and new LC/MS/MS parameters allow for both compounds to be analyzed in a single run.

Briefly, the residues are extracted from soil using acetone and 0.1 *N* HCl. The residues are partitioned into dichloromethane, evaporated, and analyzed by triple quadrupole LC/MS/MS.

REAGENTS

Acetone- pesticide quality or equivalent.

Dichloromethane - pesticide quality or equivalent.

Formic acid - reagent grade or equivalent.

Hydrochloric acid - 36.5-38.0%, Baker-Analyzed, JT Baker Cat.#9530-00, or equivalent.

Methanol - pesticide quality or equivalent.

Sodium chloride - reagent grade or equivalent.

Sodium sulfate - anhydrous, granular, AR grade or equivalent.

Water- HPLC grade and deionized.

REAGENT SOLUTIONS

Acetone:0.1 *N* HCl (4:1, v/v) - Combine 4 parts acetone with 1 part 0.1 *N* HCl. For example, add 400 mL acetone and 100 mL 0.1 *N* HCL water sequentially to a reagent bottle. Store at room temperature.

Formic acid in methanol, 0.05% (v/v) - Add 0.5 mL of formic acid to 1 liter of methanol. Stopper and mix. Store at room temperature.

Formic acid in water, 0.05% (v/v) - Add 0.5 mL of formic acid to 1 liter of HPLC-grade water. Stopper and mix. Store at room temperature.

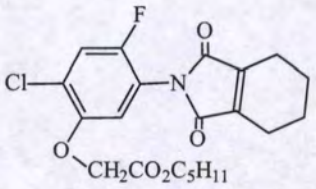
Hydrochloric acid, 0.1 *N* - carefully add 10 ml of concentrated acid to 1 liter of deionized water. Stopper and mix. Store at room temperature.

0.05% formic acid in methanol:0.05% formic acid in water (1:1, v/v) - Combine 1 part 0.05% formic acid in methanol with 1 part 0.05% formic acid in water. For example, add 100 mL 0.05% formic acid in methanol and 100 mL 0.05% formic acid in water sequentially to a reagent bottle. Store at room temperature.

Sodium chloride:water- 5% (w/v) - add 50 grams of sodium chloride to 1 L of deionized water and shake until dissolved.

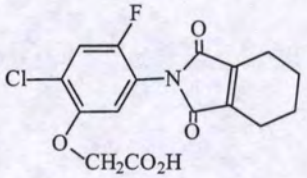
REFERENCE STANDARDS

Flumiclorac Pentyl Ester, [pentyl 2-chloro-4-fluoro-5-(3,4,5,6-tetrahodrophthalimido) phenoxyacetate] - analytical standard of known purity.



Prepare a stock solution containing 1.0 mg/mL in acetone. Store in refrigerator when not in use.

IMCA, [2-chloro-4-fluoro-5-(3,4,5,6-tetrahydrophthalimido)phenoxyacetate acid] - analytical standard of known purity.



Prepare a stock solution containing 1.0 mg/mL in acetone. Store in refrigerator when not in use.

STANDARD SOLUTIONS

Fortifying Standard Solution - 10 µg/mL - Transfer 1.0 mL of each 1.0 mg/mL stock solution of flumiclorac pentyl ester and IMCA into a single 100-mL volumetric flask and dilute to volume with acetone. Store in a refrigerator when not in use.

Fortifying Standard Solution - 1 µg/mL - Transfer 10.0 mL of the 10 µg/mL fortifying standard solution in to a 100-mL volumetric flask and dilute to volume with acetone. Store in a refrigerator when not in use.

Calibration Standard Solutions - Prepare a 0.05 µg/mL calibration standard by diluting the 1 µg/mL fortifying standard solution with 0.05% formic acid in methanol:0.05% formic acid in water (1:1, v/v). Use the 0.05 µg/mL calibration standard to further dilute to concentrations of 0.01, 0.005, 0.001, and 0.0005 µg/mL with 0.05% formic acid in methanol:0.05% formic acid in water (1:1, v/v). The 0.005 µg/mL calibration standard will also be used as the continuing calibrating standard. (See Note 1) All standard solutions should be kept refrigerated when not in use.

EQUIPMENT

Centrifuge Tubes, Polypropylene Free Standing, 50 mL, VWR part # 21008-951 or equivalent.

Filter funnel - approximately 10 cm in diameter.

Glass wool - Pyrex® (or equivalent).

Graduated cylinders – various sizes with stoppers.

High Pressure Liquid Chromatograph with MS/MS detector – Hewlett Packard 1200 Quaternary Pump HPLC system with an autosampler coupled to a Applied Biosystems API 4000 MS/MS triple quadrupole mass spectrometer with an electrospray ionization interface (or equivalent system).

Pasteur pipets - 9".

Reciprocating shaker – Eberbach or equivalent.

Rotary evaporator - Büchi or equivalent, equipped with a temperature controlled water bath.

Round-bottom flasks – 250 mL with ℸ 24/40 ground glass joints.

Separatory funnels – 250 mL.

Volumetric flasks – 10 mL.

Ultrasonic cleaner – Branson 3200 or equivalent.

Vials - 20 mL, with polyethylene-lined screw caps or equivalent.

ANALYTICAL PROCEDURE

1. Extraction

Weigh 2.5 grams (± 0.1 grams) of each soil sample into a centrifuge tube. At this point, if required by the testing facility, control samples for method recovery should be fortified with the analytes (See Note 2). Add 25 mL of acetone:0.1 *N* HCl (4:1,v/v), cap securely, place the centrifuge tube on its side on a reciprocating shaker, and shake for 10 minutes.

Centrifuge the sample for 5 minutes at about 2000 rpm. Decant sample extract through a glass funnel containing glass wool into a 250-mL separatory funnel. Add 25 mL of acetone:0.1 *N* HCl (4:1,v/v) to sample in centrifuge tube. Break up sample, if needed, and shake for 10 minutes. Centrifuge the sample for 5 minutes and decant sample extract through glass funnel into the 250-mL separatory funnel containing the first extract.

2. Water/Dichloromethane Partition

Add 75 mL of 5% aqueous sodium chloride solution to the 250-mL separatory funnel. Add 50 mL of dichloromethane to the separatory funnel. Shake for approximately 1 minute.

Filter the dichloromethane extract through a 10-cm filter funnel containing approximately 50 grams of sodium sulfate (suspended on a plug of glass wool), collecting the extract in a 250-mL round-bottom flask.

Repeat the partition and filtration steps with an additional 50-mL portion of dichloromethane. Rinse the sodium sulfate cake with 20 mL of dichloromethane. Evaporate the combined dichloromethane extracts to dryness using a rotary vacuum evaporator equipped with a water bath set at $\leq 40^{\circ}C$.

3. Final Volume

Add 5 mL of 0.05% formic acid in methanol to the 250-mL round-bottom flask, sonicate for approximately 15 seconds, and transfer to a 10-mL volumetric flask. Add approximately 3 mL of 0.05% formic acid in water to the 250-mL round-bottom flask, sonicate for approximately 15 seconds, and transfer to the 10-mL volumetric flask. The methanol extract MUST be transferred to the volumetric flask before addition of water to the round-bottom flask. Bring the sample extract up to volume by adding 0.05% formic acid in water. Samples may be transferred to 20-mL screw cap vials (or equivalent) for storage.

4. LC/MS/MS Conditions

Condition the instrument with sample extract. Analyze a range of calibration standards within the analytical sequence. The continuing calibration standards (a mid-range calibration) should be interspersed with the samples in the run sequence, and each sequence must begin and end with a continuing calibration standard. The recommended sequence of samples and standards for analysis is: continuing calibration standard, sample, calibration standard, sample, sample, continuing calibration standard, etc.

Make a 5x dilution of the sample extract by adding 200 µL of the sample and 800 µL of 0.05% formic acid in methanol:0.05% formic acid in water(1:1, v/v) to an autosampler vial and analyze, along with the calibration standards and continuing calibration standards, using the following operating conditions:

Applied Biosystems API 4000 LC/MS/MS System, using Analyst software and an Agilent 1200 LC system.

Mass Spectrometer Method Properties:	Flumiclorac Pentyl Ester	IMCA
Scan Type	MRM	MRM
Polarity:	Positive	Positive
Resolution Q1:	Unit	Unit
Resolution Q3:	Unit	Unit
Precursor Ion (amu):	424.0	354.0
Product Ion (amu):	308.2	308.1
Dwell time (msec):	150	150

Probe/Source: Turbo Ion Spray (Electrospray)

MS/Parameters:		
Ion Source voltage:	4000	5500
Temperature:	450	450
Declustering Potential:	71	86
Collision Energy:	21	19

All other mass spectrometer properties will vary with each analytical instrument and must be optimized by tuning with flumiclorac pentyl ester and IMCA prior to the initiation of analysis.

Liquid Chromatograph Method Properties:

Column:	YMC ODS-AM, 3µm, 100 mm x 3.0 mm (Waters No. AM 125031003WT)
Column Oven Temperature:	30°C
Injection Volume (µL):	25

Mobile Phase Flow:	500 µL/min.
Solvent A:	0.05% Formic Acid in HPLC water
Solvent B:	0.05% Formic Acid in Methanol

Gradient Program:

Step	Time	A (%)	B (%)
0	0.0	50	50
1	1.0	50	50
2	6.0	10	90
3	10.0	10	90
4	10.5	50	50
5	15.0	50	50

The instrument parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography, to resolve matrix interferences, or to utilize other types of LC/MS instruments. Each set of chromatograms must be clearly labeled with the LC/MS/MS parameters used.

5. Calculations

The concentration of flumiclorac pentyl ester and IMCA in each sample extract is calculated on the basis of peak area using a second-order polynomial equation. The equation is automatically generated through the use of the graphing functions of an Excel spreadsheet. (See Note 3). The data is presented graphically as concentration verses the peak area of the calibration standards which results in the following equation:

$$Y = Ax^2 + Bx + C$$

The data is weighted relative (or proportional) to the concentration of the highest standard concentration. For example:

Standard Concentration (µg/mL)	Number of Entries in Data Set
0.05	1
0.01	5
0.005	10
0.001	50
0.0005	100

Example:

For calibration standard area responses of:

µg/mL	Area
0.05	775,593
0.01	147,620
0.005	72,227
0.001	14,606
0.0005	7,247

The resulting equation from the Excel spreadsheet is as follows:

$$Y = Ax^2 + Bx + C$$

$$A = -5.754\ E\text{-}15$$
$$B = 6.892\ E\text{-}08$$
$$C = 5.365\ E\text{-}07$$

To ensure that the equation is appropriate, the areas of the calibration standards are entered into the equation of the polynomial curve and the concentrations are calculated. Each calculated standard concentration must be within 15% of its known concentration unless approved by the chemist responsible for the analysis. An example of this from the above data is the 0.005 µg/mL standard, which has an area of 72,227. The calculated concentration would be 0.0049 µg/mL, which is 99% of the known concentration.

A sample extract with an area response of 13,592 would have a concentration as follows:

$$\mu g/mL = Ax^2 + Bx + C$$

$$\mu g/mL = (-5.754\ E\text{-}15 \times 13{,}592 \times 13{,}592) + (6.892\ E\text{-}08 \times 13{,}592) + 5.365\ E\text{-}07$$
$$\mu g/mL = 0.00094$$

The amount of flumiclorac pentyl ester or IMCA found in each sample is calculated using the following formula:

$$ppm = \frac{CxFVxDF}{W}$$

where:

C = concentration of extract. (µg/mL from equation)

FV = final volume of extract. (10 mL)

DF = dilution factor. (5, or greater)

W = sample weight analyzed. (2.5 g)

Example:

From the above example, the concentration in a soil sample (with a calculated extract concentration of 0.00094 µg/mL) would be calculated as follows:

$$ppm = \frac{(0.00094\ ug / mL)x(10\ mL)x(5)}{2.5\ g}$$

ppm = 0.0187

The recoveries for fortified samples are calculated using the formula:

$$\text{Percent recovery (\%)} = \frac{\text{ppm in fortified sample - ppm in control sample}}{\text{fortification level, ppm}} \times 100\%$$

For the fortification sample fortified at 0.02 ppm, the following values were utilized to calculate the amount of IMCA in the sample:

ppm found in fortified sample = 0.0187
ppm found in untreated control sample = 0.0000

$$\text{Percent recovery (\%)} = \frac{0.0187 - 0.0000}{0.02} \times 100\% = 94\%$$

LIMITS OF QUANTITATION AND DETECTION

The validated limit of quantitation (LOQ) of flumiclorac pentyl ester and IMCA analyzed by this method is 0.02 ppm. The estimated limit of detection (LOD) is 0.01 ppm. This LOD is calculated from the lowest concentration calibration standard (0.0005 µg/mL) and the dilution of the matrix in the sample extracts:

LOD = [0.0005 µg/mL] x [10 mL/2.5 g x 5] = 0.01 ppm

ANALYSIS TIME

A trained analyst, familiar with this method, can complete the analysis of a set of twelve samples in approximately 8 hours. The results are available within 24 hours of initiating the analysis.

NOTES

1. For flumiclorac pentyl ester and IMCA , reproducibility of an analytical run is determined by calculating the CV from the peak areas obtained for the continuing calibration analyzed

standards during the run. For a run to be acceptable, these CV's must be ≤ 15% unless approved by the chemist responsible for the analysis.

2. At Valent, a standard operating procedure requires that a fortified control sample be analyzed with each set of samples. If the testing facility does not require concurrent analysis of fortified control samples, or if a UTC sample is not available, this method requirement may be waived. The level of fortification is generally at the LOQ of the method and/or ten times the LOQ. Method recoveries must be 70% to 120% to be acceptable unless approved by the chemist responsible for the analysis.

3. There are other programs that can calculate a weighted regression graph, such as Curve Expert 1.3 (Hyams Development, Starkville,MS).

REFERENCE

1. Kowalsky, J., *Method Determination of Flumiclorac Pentyl Ester and its Degradate, IMCA, in/on Soil,* Valent Method RM-29S, Valent USA Corporation, July 29, 2004

METHOD APPROVAL

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