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# DETERMINATION OF FLUMICLORAC PENTYL ESTER AND ITS DEGRADATE, IMCA, IN WATER

**METHOD RM-29W-4** **DATE: November 14, 2011**

## INTRODUCTION

This method determines residues of flumiclorac pentyl ester and IMCA in water. This method is a revision of RM-29W-3. (Reference 1) The solvent volumes have been reduced and derivatization and column cleanup steps have been excluded as the samples are analyzed by LC/MS/MS.

Briefly, the residues are extracted from acidified water samples using ethyl acetate. The residues are evaporated and analyzed by triple quadrupole LC/MS/MS.

## REAGENTS

Acetone - pesticide quality or equivalent.

Ethyl acetate - 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.

Water - HPLC grade and deionized.

## REAGENT SOLUTIONS

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.

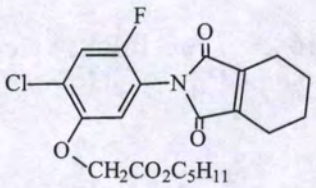
Formic acid in water, 0.05% (v/v) - Add 0.5 mL of formic acid to 1 liter of HPLC grade water. Stopper and mix. Store at room temperature.

Hydrochloric acid, 1 *N* - carefully add 10 ml of concentrated acid to 100 mL of deionized water. Dilute to 120 mL, then 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 of 0.05% formic acid in methanol and 100 mL of 0.05% formic acid in water sequentially to a reagent bottle. Store at room temperature.

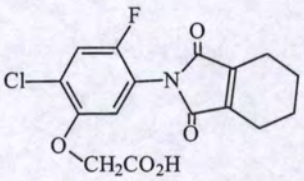
## REFERENCE STANDARDS

**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 a 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 containing flumiclorac pentyl ester and IMCA into a 100-mL volumetric flask, and dilute to volume with acetone. Store in a refrigerator when not in use.

Calibration Standard Solutions - Prepare a 50 µg/L calibration standard by diluting the 1 µg/mL fortifying standard solution containing flumiclorac pentyl ester and IMCA with 0.05% formic acid in methanol:0.05% formic acid in water (1:1, v/v). Use the 50 µg/L calibration standard to further dilute to concentrations of 10, 5, 1, and 0.5 µg/L with 0.05% formic acid in methanol:0.05% formic acid in water (1:1, v/v). The 5 µg/L 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.

Graduated Cylinders – various sizes.

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 an equivalent system).

Pasteur pipets - 9".

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

Round-bottom flasks – 50 mL.

Volumetric flasks – 100 ml and 10 mL.

Volumetric pipettes – various sizes

Ultrasonic cleaner – Branson 3200 or equivalent.

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

## ANALYTICAL PROCEDURE

### 1. Extraction

Place 20 mL of each water 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 1 mL of 1 *N* HCl to centrifuge tube containing the sample and shake briefly to mix. Add 10 mL of ethyl acetate, cap securely, and shake for 1 minute. Pipette the upper layer of ethyl acetate extract into a 50-mL round-bottom flask.

Re-extract the water sample with an additional 10 mL of ethyl acetate as described above and transfer, combining this ethyl acetate extract with the first in the 50-mL round-bottom flask. Evaporate the combined ethyl acetate extracts to dryness using a rotary vacuum evaporator equipped with a water bath set at $\leq 40^{\circ}C$.

### 2. Final Volume

Add 5 mL of 0.05% formic acid in methanol to the 50-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 50-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 into 20-mL screw cap vials (or equivalent) for storage.

### 3. LC/MS/MS Conditions

Condition the instrument with sample extracts. Analyze a range of calibration standards within the analytical sequence. The continuing calibration standards (a mid-range calibration standard) 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 4x dilution of each sample extract by adding 250 µL of the sample and 750 µL of 0.05% formic acid in methanol:0.05% formic acid in water (1:1, v/v) to an autosampler vial. 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: Turb 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.

## 4. 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 calibrations 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 calibration standard concentration. For example:

| Standard Concentration (µg/L) | Number of Entries in Data Set |
|---|---|
| 50 | 1 |
| 10 | 5 |
| 5 | 10 |
| 1 | 50 |
| 0.5 | 100 |

Example:

For calibration standard area responses of:

| µg/L | Area |
|---|---|
| 50 | 1,841,690 |
| 10 | 381,182 |
| 5 | 188,817 |
| 1 | 37,603 |
| 0.5 | 19,018 |

The resulting equation from the Excel spreadsheet is as follows:

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

A = 5.528 E-13
B = 2.612 E-05
C = 8.721 E-03

To ensure that the equation is appropriate, the areas of the calibration standards are entered into the equation of the 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 5 µg/L standard, which has an area of 188,817. The calculated concentration would be 4.96 µg/L, which is 99% of the known concentration.

A sample extract with an area response of 39,485 would have a concentration as follows:

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

$$\mu g/L = (5.528\ E\text{-}13 \times 39{,}485 \times 39{,}485) + (2.612\ E\text{-}05 \times 39{,}485) + 8.721\ E\text{-}03$$
$$\mu g/L = 1.041$$

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

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

where:

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

FV = final volume of extract. (10 mL)

DF = dilution factor. (4, or greater)

W = sample volume analyzed. (20 mL)

Example: From the above example, the concentration in a water sample (with a calculated extract concentration of 1.041 µg/L) would be calculated as follows:

$$ppb = \frac{(1.041\,ug/L)x(10\,mL)x(4)}{20\,mL}$$

$$ppb = 2.08$$

The recoveries for fortified samples are calculated using the formula:

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

For the fortification sample Ft C (fortified at 2 ppb), the following values were utilized to calculate the amount of flumiclorac pentyl ester in the sample:

ppb found in fortified sample = 2.08
ppb found in untreated control sample = 0.0000

$$\text{Percent recovery (\%)} = \frac{2.08 - 0.0000}{2.0} \times 100\% = 104\%$$

## LIMITS OF QUANTITATION AND DETECTION

The validated limit of quantitation (LOQ) of flumiclorac pentyl ester and IMCA analyzed by this method is 2 ppb. The estimated limit of detection (LOD) is 1 ppb. This LOD is calculated from the lowest concentration calibration standard (0.5 µg/L) and the dilution of the matrix in the sample extracts:

$$LOD = [0.5\ \mu g/L] \times [10\ mL/20\ mL \times 4] = 1\ ppb$$

## ANALYSIS TIME

A trained analyst, familiar with this method, can complete the analysis of a set of twelve samples in approximately 4 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 standards analyzed 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. Green, Charles, *Determination of Flumiclorac Pentyl Ester And Its Degradate, IMCA, In Water,* Valent Method RM-29W-3, Valent USA Corporation, February 27, 1996

## METHOD APPROVAL

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