CPS Study ID Number: 14-CPS-015

Golden Pacific Laboratories
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Analytical Method for the Determination of Fenpropathrin Metabolites $CONH_2$-Fenpropathrin and TMPA in Soil by LC-MS/MS

Method: **GPL-MTH-084** Date: July 28, 2014

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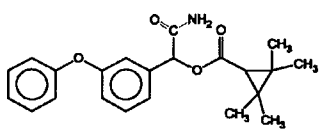
1. INTRODUCTION

This method describes the determination of fenpropathrin metabolites $CONH_2$-fenpropathrin and TMPA in soil samples. The LOQ (limit of quantitation) for both analytes is 0.01 ppm (µg/g). Briefly, the method involves addition of methanol/water (9:1, v/v) to a soil sample, shaking to extract residues, centrifuging and filtering the extract. After filtration, the methanol content of an aliquot of the sample extract is evaporated and the remaining water portion is combined with 100 mM phosphate buffer (pH = 7.2). The sample is then loaded into a preconditioned (with methanol, then water) mixed-mode anion exchange SPE cartridge. After sample loading, the column is rinsed with water and washed with 1% ammonium hydroxide (aq.) and methanol/water (1:1, v/v). The $CONH_2$-fenpropathrin is then eluted from the column using methanol. Following the elution of $CONH_2$-fenpropathrin, the TMPA is eluted using 2% formic acid in methanol.

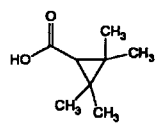
The eluted $CONH_2$-fenpropathrin is brought up to volume with water (final solution is methanol/water 1:1, v/v) and is further diluted with methanol/water (1:1, v/v). The sample is then analyzed by LC-MS/MS (API4000).

The eluted TMPA is brought up to volume with water (final solution is methanol/water/formic acid 50:50:1, v/v/v) and then analyzed by LC-MS/MS (API 5000).

2. ANALYTICAL STANDARDS



$CONH_2$-Fenpropathrin



TMPA

$CONH_2$-Fenpropathrin Stock Standard, 1.0 mg/mL solution.
Weigh 0.050 grams (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard) into a 50-mL volumetric flask. Dilute to volume with acetone, mix well, and store frozen.

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TMPA Stock Standard, 1.0 mg/mL solution.
Weigh 0.050 grams (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard) into a 50-mL volumetric flask. Dilute to volume with acetone, mix well, and store frozen.

$CONH_2$-Fenpropathrin, 10 µg/mL solution.
Add 1 mL of the 1.0 mg/mL $CONH_2$-Fenpropathrin Stock solution into a 100-mL volumetric flask. Dilute to volume with methanol, mix, and store refrigerated.

$CONH_2$-Fenpropathrin, 1.0 µg/mL solution.
Add 10 mL of the 10 µg/mL $CONH_2$-Fenpropathrin solution into a 100-mL volumetric flask. Dilute to volume with methanol, mix, and store refrigerated.

$CONH_2$-Fenpropathrin, 100 ng/mL solution (in methanol/water).
Add 10 mL of the 1.0 µg/mL $CONH_2$-Fenpropathrin into a 100-mL volumetric flask. Dilute to volume with methanol/water (1:1, v/v), mix, and store refrigerated.

$CONH_2$-Fenpropathrin, 10 ng/mL solution (in methanol/water).
Pipet 10 mL of the 100 ng/mL $CONH_2$-Fenpropathrin Standard into a 100-mL volumetric flask and dilute to volume with methanol/water (1:1, v/v), mix, and store refrigerated.

$CONH_2$-Fenpropathrin, 5 ng/mL solution (in methanol/water).
Pipet 5 mL of the 100 ng/mL $CONH_2$-Fenpropathrin Standard into a 100-mL volumetric flask and dilute to volume with methanol/water (1:1, v/v), mix, and store refrigerated.

$CONH_2$-Fenpropathrin, 2 ng/mL solution (in methanol/water).
Pipet 2 mL of the 100 ng/mL $CONH_2$-Fenpropathrin Standard into a 100-mL volumetric flask and dilute to volume with methanol/water (1:1, v/v), mix, and store refrigerated.

$CONH_2$-Fenpropathrin, 1 ng/mL solution (in methanol/water).
Pipet 1 mL of the 100 ng/mL $CONH_2$-Fenpropathrin Standard into a 100-mL volumetric flask and dilute to volume with methanol/water (1:1, v/v), mix, and store refrigerated.

$CONH_2$-Fenpropathrin, 0.50 ng/mL solution (in methanol/water).
Pipet 1 mL of the 100 ng/mL $CONH_2$-Fenpropathrin Standard into a 200-mL volumetric flask and dilute to volume with methanol/water (1:1, v/v), mix, and store refrigerated.

$CONH_2$-Fenpropathrin, 0.25 ng/mL solution (in methanol/water).
Pipet 0.5 mL of the 100 ng/mL $CONH_2$-Fenpropathrin Standard into a 200-mL volumetric flask and dilute to volume with methanol/water (1:1, v/v), mix, and store refrigerated.

TMPA, 10 µg/mL solution.
Add 1 mL of the 1.0 mg/mL TMPA solution into a 100-mL volumetric flask. Dilute to volume with methanol, mix, and store refrigerated.

TMPA, 1.0 µg/mL solution.
Add 10 mL of the 10 µg/mL TMPA solution into a 100-mL volumetric flask. Dilute to volume with methanol, mix, and store refrigerated.

TMPA, 1.0 µg/mL solution (in methanol/water/formic acid).
Add 10 mL of the 10 µg/mL TMPA solution into a 100-mL volumetric flask. Dilute to volume with methanol/water/formic acid (50:50:1, v/v/v), mix, and store refrigerated.

TMPA, 100 ng/mL solution (in methanol/water/formic acid).
Add 10 mL of the 1.0 µg/mL TMPA solution (in methanol/water/formic acid) into a 100-mL volumetric flask. Dilute to volume with methanol/water/formic acid (50:50:1, v/v/v), mix, and store refrigerated.

TMPA, 50 ng/mL solution (in methanol/water/formic acid).
Add 5 mL of the 1.0 µg/mL TMPA solution (in methanol/water/formic acid) into a 100-mL volumetric flask. Dilute to volume with methanol/water/formic acid (50:50:1, v/v/v), mix, and store refrigerated.

TMPA, 20 ng/mL solution (in methanol/water/formic acid).
Add 2 mL of the 1.0 µg/mL TMPA solution (in methanol/water/formic acid) into a 100-mL volumetric flask. Dilute to volume with methanol/water/formic acid (50:50:1, v/v/v), mix, and store refrigerated.

TMPA, 10 ng/mL solution (in methanol/water/formic acid).
Add 1 mL of the 1.0 µg/mL TMPA solution (in methanol/water/formic acid) into a 100-mL volumetric flask. Dilute to volume with methanol/water/formic acid (50:50:1, v/v/v), mix, and store refrigerated.

TMPA, 5.0 ng/mL solution (in methanol/water/formic acid).
Add 1 mL of the 1.0 µg/mL TMPA solution (in methanol/water/formic acid) into a 200-mL volumetric flask. Dilute to volume with methanol/water/formic acid (50:50:1, v/v/v), mix, and store refrigerated.

TMPA, 2.5 ng/mL solution (in methanol/water/formic acid).
Add 0.5 mL of the 1.0 µg/mL TMPA solution (in methanol/water/formic acid) into a 200-mL volumetric flask. Dilute to volume with methanol/water/formic acid (50:50:1, v/v/v), mix, and store refrigerated.

Similar dilutions may also be performed to generate appropriate standards.

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3. REAGENTS

Acetonitrile – HPLC grade or better

Ammonium Hydroxide – ACS grade

Formic Acid, 88% – ACS grade

Methanol – HPLC grade or better

Sodium Phosphate, Monobasic, Monohydrate – ACS Grade

Sodium Phosphate, Dibasic, Anhydrate – ACS Grade

Water – HPLC grade or better

4. REAGENT SOLUTIONS

Methanol/Water, 1:1 (v/v).
Combine 1 part methanol with 1 part water. For example, add 500 mL of methanol, 500 mL of water to a reagent bottle. Store at room temperature.

Methanol/Water/Formic Acid, 50:50:1 (v/v/v).
Combine 50 parts methanol and 50 parts water with 1 part formic acid (88%). For example, add 500 mL of methanol, 500 mL of water, and 1 mL of formic acid (88%) to a reagent bottle. Store at room temperature.

0.15 M Ammonium Hydroxide Solution (aq).
Add 1 mL of concentrated Ammonium Hydroxide (14.8 M) to approximately 30 mL of water in a 100-mL volumetric flask. Bring up to volume with water. Store at room temperature. (These amounts may be scaled as necessary).

2% *Formic Acid in Methanol.*
Add 2 mL of Formic Acid (88%) to approximately 30 mL of methanol in a 100-mL volumetric flask. Bring up to volume with methanol. Store at room temperature. (These amounts may be scaled as necessary).

Methanol/Water, 9:1 (v/v).
Combine 9 parts methanol with 1 part water. For example, add 900 mL of methanol, 100 mL of water to a reagent bottle. Store at room temperature.

0.2M Monobasic Sodium Phosphate (aq.).
Add 13.8 grams of monobasic sodium phosphate, monohydrate to approximately 300 mL of water in a 500-mL volumetric flask. Bring up to volume with water. Mix well (use a magnetic stir plate if necessary). Store at room temperature. (These amounts may be scaled as necessary).

0.2M Dibasic Sodium Phosphate (aq.).
Add 14.2 grams of dibasic sodium phosphate, anhydrate to approximately 300 mL of water in a 500-mL volumetric flask. Bring up to volume with water. Mix well (use a magnetic stir plate if necessary). Store at room temperature. (These amounts may be scaled as necessary).

100 mM Phosphate Buffer (aq.), pH = 7.2.
Add 28 mL of 0.2M Monobasic Sodium Phosphate (aq.) and 72 mL of 0.2M Dibasic Sodium Phosphate (aq.) into a 200-mL volumetric flask. Bring up to volume with water. Store at room temperature. (These amounts may be scaled as necessary).

Mobile Phase A for $CONH_2$-Fenpropathrin (Organic); 0.2% Formic Acid in Acetonitrile.
Add 2 mL of Formic Acid (88%) to approximately 800 mL of acetonitrile in a 1000-mL mixing cylinder. Bring up to volume with acetonitrile. Store at room temperature. (These amounts may be scaled as necessary).

Mobile Phase B for $CONH_2$-Fenpropathrin (Aqueous); 0.2% Formic Acid in Water.
Add 2 mL of Formic Acid (88%) to approximately 800 mL of water in a 1000-mL mixing cylinder. Bring up to volume with water. Store at room temperature. (These amounts may be scaled as necessary).

5. **EQUIPMENT**

Autosampler vials – 2 mL (or equivalent)

Balances, Analytical and Top Loading

Bottles, Nalgene (HDPE) – 125 or 250 mL

Centrifuge, Multipurpose

Centrifuge Tubes, Graduated, Polypropylene 15 mL

Evaporation System – TurboVap or equivalent

Graduated Cylinders – (1000, 250, 100, 50, 25 mL)

Micropipettes, Wiretrol (or equivalent) – (50, 100, 200 µL)

Pipettor, Automatic – capable of accurately dispensing volumes of 0.2 to 1.0 mL

Pipettes, Volumetric – 10, 5, 2, 1, and 0.5 mL

Reciprocating shaker – Eberbach or equivalent.

Refrigerator/Freezer

SPE Cartridges, MAX Oasis (60 mg, 3 cc). **Do not substitute.**

SPE manifold

Syringes, Plastic Disposable – 10 mL

Syringe Filters, PTFE 0.45µm

Volumetric Flasks – 50, 100, 200 and 500 mL

Vortex Mixer (optional)

Vials, Glass – approximately 16 mL

6. INSTRUMENTATION

Liquid Chromatograph/Mass Spectrometers (LC/MS-MS)

For $CONH_2$-Fenpropathrin:
Applied Biosystems API4000 Liquid Chromatograph/Mass Spectrometer system with Shimadzu LC-20AD HPLC Pumps, Shimadzu SCL-10A VP Controller, Shimadzu SIL-20AC HT Autosampler with Analyst Date System Version 1.5.2(or equivalent). *Conditions listed below are suggested.*

Column:	Luna C18 (Phenomenex), 30 mm x 2 mm, 3 µm
	Phenomenex Part Number: 00A-4114-B0
Temperature:	Ambient (approximately 20°C)
Column Flow:	500 µL/minute
Injection Volume:	10 µL
Mobile Phase A:	0.2% Formic Acid in Acetonitrile
Mobile Phase B:	0.2% Formic Acid in Water

HPLC Gradient:

Total Time (min)	% Mobile Phase A	% Mobile Phase B
0.0	40	60
2.0	70	30
3.5	70	30
3.6	90	10
4.6	90	10
4.7	40	60
6.5	40	60

Retention Time: ~2.4 minutes (*Figures 1-4*)

MS/MS Conditions:

MS Sample Introduction:	Electrospray Ionization
Acquisition Time:	0 to 5 minutes
Scan Type:	MRM
Polarity:	Positive (Unit/Unit Resolution)
Primary Ion Pair (Q1/Q3 Mass):	368.0/125.0, Dwell = 250, CE = 19
Confirmatory Ion Pair (Q1/Q3 Mass):	368.0/97.0, Dwell = 50, CE = 45

Source Conditions: CUR = 25, GS1 = 40, GS2 = 40, IS = 5500, TEM = 100, CXP = 10, CAD = 5, EP = 6, DP = 50

For TMPA:
Applied Biosystems API5000 Liquid Chromatograph/Mass Spectrometer system with Shimadzu LC-20AD XR HPLC Pumps, Shimadzu CBM-20A Controller, Shimadzu SIL-20AC XR Autosampler with Analyst Date System Version 1.5.2(or equivalent). *Conditions listed below are suggested. TMPA can be difficult to see during MS/MS parameter optimization (infusion). If TMPA cannot be differentiated from background noise levels, increase the TMPA concentration to find Q1.*

Column:	Luna C18 (Phenomenex), 30 mm x 2 mm, 3 µm
	Phenomenex Part Number: 00A-4114-B0
Temperature:	Ambient (approximately 20°C)
Column Flow:	500 µL/minute
Injection Volume:	50 µL

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Mobile Phase A: 100% Acetonitrile

Mobile Phase B: 100% Water

HPLC Gradient:

Total Time (min)	% Mobile Phase A	% Mobile Phase B
0.0	10	90
3.0	60	40
4.0	60	40
4.1	90	10
4.5	90	10
4.6	10	90
6.5	10	90

Retention Time: ~2.8 minutes (*Figure 5-8*)

MS/MS Conditions:

MS Sample Introduction:	Electrospray Ionization
Acquisition Time:	0 to 5 minutes
Scan Type:	MRM
Polarity:	Negative (Unit/Unit Resolution)
Primary Ion Pair (Q1/Q3 Mass):	141.0/106.9, Dwell = 250, CE = -27
Confirmatory Ion Pair (Q1/Q3 Mass):	141.0/97.0, Dwell = 50, CE = -16

Source Conditions: CUR = 30, GS1 = 40, GS2 = 40, IS = -4500, TEM = 500, CXP = -5, CAD = 2, EP = -2, DP = -90

Note: The confirmation ion pair can be used to confirm identity of the chromatographic peak for > LOQ levels. The confirmation ion pair cannot be used for quantitation for < 10x LOQ levels.

7. ANALYTICAL PROCEDURES

1. Sample Setup

Weigh 10.0 g (±0.1 g) of each homogenized soil sample into 125 or 250 mL HDPE Nalgene bottle. At this point, if required by the testing facility, a control sample to be used for method recoveries may be fortified with the analytes. (*See Note 1*).

2. Extraction with Methanol/Water (9:1, v/v)

Add 50 mL of methanol/water (9:1, v/v) into the Nalgene bottle. Cap the bottle, and place bottle on a mechanical shaker for 45 minutes (at ~ 200 rpm). Centrifuge the sample in a centrifuge set to 3 minutes at 3000 rpm. Syringe filter approximately 20 mL of soil extract through a 0.45-µm PTFE filter.

3. Concentration though Evaporation

Aliquot 10 mL of filtered soil extract into a glass tube. Evaporate the aliquot with a gentle stream of nitrogen in an evaporation system with a water bath set to 40 °C. (This may take as long as two hours depending on the set-up of the evaporation system. The pressure of nitrogen can be carefully increased as the total volume of extract is reduced).

4. Mixed-Mode Anion Exchange SPE

After the evaporation step, add 5 mL of 100 mM phosphate buffer (pH = 7.2) to the concentrated soil extract and hand shake the sample for approximately 5 seconds. (If necessary, samples may be further mixed using a vortex mixer). *(See Note 2).*

Attach a 60 mg, 3 cc MAX Oasis SPE to a SPE manifold. All the SPE steps should be allowed to drain until the level of the liquid is at the top of the frit of the SPE cartridge and can be performed using no vacuum unless otherwise stated. Condition the column with 3 mL of methanol followed by 3 mL of water. Load the sample onto the column. Wash the column with 3 mL of water followed by 3 mL of 0.15 M ammonium hydroxide (aq.) followed by 3 mL methanol/water (1:1, v/v). Discard all eluent before the following step.

Elute the $CONH_2$-fenpropathrin using 5 mL of methanol collecting the eluate in a 15-mL graduated polypropylene centrifuge tube.

Elute the TMPA using 2 mL of 2% formic acid in methanol into a separate 15-mL graduated polypropylene centrifuge tube, pulling the liquid completely through. Apply high vacuum to collect the remainder of the liquid. Bring the eluate up to 4 mL using water. Vial for LC-MS/MS analysis. If necessary, additional dilutions must be made using methanol/water/formic acid (50:50:1, v/v/v).

Bring the $CONH_2$-fenpropathrin to 10 mL with water and dilute 1+3 (DF = 4) using methanol/water (1:1, v/v). *Alternatively a dilution of 0.2+0.8 (or equivalent dilution) can be made directly into an HPLC vial.* Vial for LC-MS/MS analysis. If necessary, additional dilutions must be made using methanol/water (1:1, v/v).

5. LC/MS-MS Analysis

Instrument calibration is performed using a linear regression with 1/x weighting where the line is not forced through the intercept. The calibration is performed with calibration standards that are distributed within each analytical sequence.

Condition the instrument, typically with at least six injections of a calibration standard prior to starting the analytical sequence. Analyze at least five calibration standard concentrations *within the analytical sequence* to generate the linear curve. A typical set of calibration standards for $CONH_2$-fenpropathrin would include concentrations of 0.250, 0.500, 1.00, 2.00, 5.00, and 10.0 ng/mL (with an injection volume of 10 µL). A typical set of calibration standards for TMPA would include concentrations of 2.50, 5.00, 10.0, 20.0, 50.0, and 100 ng/mL (with an injection volume of 50 µL).

The coefficient of determination (r^2) is calculated from the calibration standards, and this value must be greater than 0.99 for the instrument response to be considered acceptable over the range of concentrations. In addition, the concentration calculated from the peak area using the linear regression curve with 1/x weighting must be within 15% of the corresponding standard concentrations, unless approved by the supervising chemist responsible for the analysis.

An analytical set should be constructed so that a continuing calibration standard (mid-range calibration standard) is analysed at the beginning, middle, and at the end of the sequence, making a minimum of three (3) continuing calibration standards within the analytical sequence. The continuing calibration standard should be calculated as an unknown sample in order to verify the calibration curve is valid throughout the analytical run. There should be a minimum of five calibration standards, interspersed within the analytical sequence, that bracket the concentration range of interest (with the lowest standard corresponding to approximately 50% of the LOQ).

The coefficient of variation of the continuing calibration standard responses must be 15% or less for the analysis set to be acceptable.

If the peak area observed for a sample is greater than the peak area of the highest calibration standard, the sample extract must be diluted and the diluted extract analyzed. The sample extract must be diluted with the dilution solvent listed at the end of the analytical procedure such that the peaks obtained are within the documented linear response range of the LC/MS-MS.

6. Calculations

The aliquot factor is determined as follows:

$$Aliquot\ Factor = \frac{Extraction\ Volume\ (50\ mL)}{Aliquot\ Volume\ (10\ mL)}$$

The sample concentration is calculated as follows:

$$Sample\ Concentration\ (\mu g/g) = \frac{Extract\ Concentration\ (ng/mL) \times Aliquot\ Factor \times Final\ Volume\ (mL) \times 1\ \mu g}{Sample\ Amount\ (g) \times 1000\ ng}$$