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**Analytical Method for the Determination of Fenpropathrin Metabolites
CONH₂-Fenpropathrin, 4'-OH-Fenpropathrin, and TMPA
in Drinking Water by LC-MS/MS**

Method: GPL-MTH-085

Date: July 28, 2014

1. INTRODUCTION

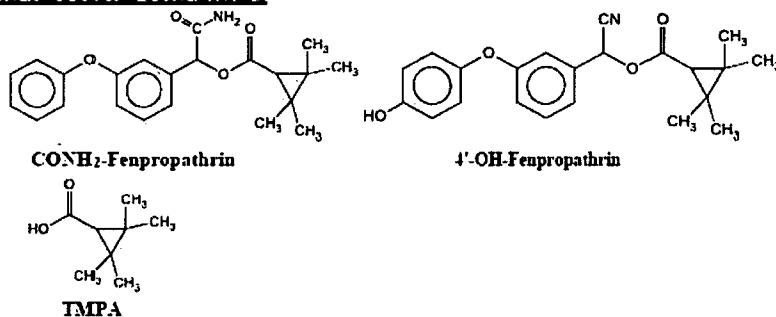
This method describes the determination of fenpropathrin metabolites CONH₂-fenpropathrin, 4'-OH-fenpropathrin, and TMPA in drinking water samples. The LOQ for all three analytes is 1 ppb (µg/L).

Briefly, for CONH₂-fenpropathrin and 4'-OH-fenpropathrin, the method involves the addition of methanol to the drinking water in a 1:1 ratio, followed by filtration and analysis by LC-MS/MS (API 4000).

For TMPA, the drinking water is combined with an aliquot of 100 mM phosphate buffer (aq.) (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. Following the methanol wash, the TMPA is eluted using 2% formic acid in methanol.

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



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CONH₂-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.

4'-OH-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.

TMFA 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.

CONH₂-Fenpropathrin/4'-OH-Fenpropathrin, 1.0/1.0 µg/mL solution.

Add 100 µL of the 1.0 mg/mL CONH₂-Fenpropathrin Stock solution and 100 µL of the 1.0 mg/mL 4'-OH-Fenpropathrin Stock solution into a 100-mL volumetric flask. Dilute to volume with methanol, mix, and store refrigerated.

CONH₂-Fenpropathrin/4'-OH-Fenpropathrin, 100/100 ng/mL solution.

Add 10 mL of the 1.0/1.0 µg/mL CONH₂-Fenpropathrin/4'-OH-Fenpropathrin solution into a 100-mL volumetric flask. Dilute to volume with methanol, mix, and store refrigerated.

CONH₂-Fenpropathrin/4'-OH-Fenpropathrin, 100/100 ng/mL solution (in methanol/water).

Add 10 mL of the 1.0/1.0 µg/mL CONH₂-Fenpropathrin/4'-OH-Fenpropathrin solution into a 100-mL volumetric flask. Dilute to volume with methanol/water (1:1, v/v), mix, and store refrigerated.

CONH₂-Fenpropathrin/4'-OH-Fenpropathrin, 10/10 ng/mL solution (in methanol/water).

Pipet 10 mL of the 100/100 ng/mL CONH₂-Fenpropathrin/4'-OH-Fenpropathrin Standard into a 100-mL volumetric flask and dilute to volume with methanol/water (1:1, v/v), mix, and store refrigerated.

CONH₂-Fenpropathrin/4'-OH-Fenpropathrin, 5/5 ng/mL solution (in methanol/water).

Pipet 5 mL of the 100/100 ng/mL CONH₂-Fenpropathrin/4'-OH-Fenpropathrin Standard into a 100-mL volumetric flask and dilute to volume with methanol/water (1:1, v/v), mix, and store refrigerated.

CONH₂-Fenpropathrin/4'-OH-Fenpropathrin, 2/2 ng/mL solution (in methanol/water).

Pipet 2 mL of the 100/100 ng/mL CONH₂-Fenpropathrin/4'-OH-Fenpropathrin into a 100-mL volumetric flask and dilute to volume with methanol/water (1:1, v/v), mix, and store refrigerated.

CONH₂-Fenpropathrin/4'-OH-Fenpropathrin, 1/1 ng/mL solution (in methanol/water).

Pipet 1 mL of the 100/100 ng/mL CONH₂-Fenpropathrin/4'-OH-Fenpropathrin Standard into a 100-mL volumetric flask and dilute to volume with methanol/water (1:1, v/v), mix, and store

refrigerated.

CONH₂-Fenpropathrin/4'-OH-Fenpropathrin, 0.50/0.50 ng/mL solution (in methanol/water).

Pipet 1 mL of the 100/100 ng/mL CONH₂-Fenpropathrin/4'-OH-Fenpropathrin Standard into a 200-mL volumetric flask and dilute to volume with methanol/water (1:1, v/v), mix, and store refrigerated.

CONH₂-Fenpropathrin/4'-OH-Fenpropathrin, 0.25/0.25 ng/mL solution (in methanol/water).

Pipet 0.5 mL of the 100/100 ng/mL CONH₂-Fenpropathrin/4'-OH-Fenpropathrin Standard into a 200-mL volumetric flask and dilute to volume with methanol/water (1:1, v/v), mix, and store refrigerated.

TMPA, 2.0 µg/mL solution.

Add 200 µL of the 1.0 mg/mL TMPA Stock solution into a 100-mL volumetric flask. Dilute to volume with methanol, mix, and store refrigerated.

TMPA, 2.0 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, 200 ng/mL solution.

Add 10 mL of the 2.0 µg/mL TMPA solution into a 100-mL volumetric flask. Dilute to volume with methanol, mix, and store refrigerated.

TMPA, 100 ng/mL solution (in methanol/water/formic acid).

Add 5 mL of the 2.0 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 2.0 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, 20 ng/mL solution (in methanol/water/formic acid).

Add 1 mL of the 2.0 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 0.5 mL of the 2.0 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 0.5 mL of the 2.0 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 250 μ L of the 2.0 μ g/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.

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).

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 CONH₂-Fenpropathrin/4'-OH-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 CONH₂-Fenpropathrin/4'-OH-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

Centrifuge Tubes, Graduated, Polypropylene 15 mL

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

Micropipettes, *Wetrol* (or equivalent) – (50, 100, 200 μ L)

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

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

Refrigerator/Freezer

CPS Study ID Number: 14-CPS-014

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

SPE manifold

Syringes, Plastic Disposable – 5 or 10 mL

Syringe Filters, PTFE 0.45µm

Volumetric Flasks – 50, 100, 200 and 500 mL

Vials, Glass – approximately 8, 16, and 22 mL or larger

6. INSTRUMENTATIONLiquid Chromatograph/Mass Spectrometers (LC/MS-MS)**For CONH₂-Fenpropathrin and 4'-OH-Fenpropathrin:**

Applied Biosystems API4000 Liquid Chromatograph/Mass Spectrometer system with Shimadzu LC-20AD HPLC Pumps, Shimadzu SCL-10A VP Controller, Shimadzu SIL-20A HT Autosampler with Analyst Data 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

CPS Study ID Number: 14-CPS-014

Retention Time (CONH₂-Fenpropathrin): ~2.5 minutes, see Figures 1-4

Retention Time (4'-OH-Fenpropathrin): ~2.7 minutes, see Figures 5-8

MS/MS Conditions:

MS Sample Introduction:	Electrospray Ionization
Acquisition Time:	0 to 5 minutes
Scan Type:	MRM

Period 1 Conditions (0.00- 2.60 minutes) for CONH₂-Fenpropathrin

Polarity:	Positive (Unit/Unit Resolution)
Primary Ion Pair (Q1/Q3 Mass):	368.0/125.0, Dwell = 150, CE = 19
Confirmatory Ion Pair (Q1/Q3 Mass):	368.0/97.0, Dwell = 150, CE = 45

Source Conditions:	CUR = 25, GS1 = 40, GS2 = 40, IS = 5500, TEM = 100, CXP = 10, CAD = 5, EP = 6, DP = 50
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Period 2 Conditions (2.60-5.00 minutes) for 4'-OH-Fenpropathrin

Polarity:	Negative (Unit/Unit Resolution)
Primary Ion Pair (Q1/Q3 Mass):	364.2/141.0, Dwell = 150, CE = -24
Confirmatory Ion Pair (Q1/Q3 Mass):	364.2/213.0, Dwell = 150, CE = -21

Source Conditions:	CUR = 25, GS1 = 40, GS2 = 40, IS = -2000, TEM = 100, CXP = -10, CAD = 5, EP = -6, DP = -80
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Note: The retention times of CONH₂-fenpropathrin and 4'-OH-fenpropathrin are similar. The length of each MS/MS period may need to be adjusted prior to each chromatographic run.

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 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
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CPS Study ID Number: 14-CPS-014

Temperature: Ambient (approximately 20°C)
Column Flow: 500 µL/minute
Injection Volume: 50 µL
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, see Figures 9-12

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**A. Procedure for CONH₂-Fenpropathrin and 4'-OH-Fenpropathrin****1. Sample Setup**

Measure 10 mL of a pre-mixed drinking water sample into a 22-mL (or larger) glass vial. 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

Add 10 mL of methanol to the vial. Cap the vial and hand shake for approximately 5 seconds. Syringe filter approximately 1.5 mL of sample extract through a 0.45- μ m PTFE filter into an HPLC vial. If necessary, additional dilutions must be made using methanol/water (1:1, v/v).

B. Procedure for TMPA**1. Sample Setup**

Measure 20 mL of a pre-mixed drinking water sample into a 22-mL (or larger) glass vial. 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. Mixed-Mode Anion Exchange SPE

Add 1 mL of 100 mM phosphate buffer (pH = 7.2) to the drinking water sample and hand shake the sample for approximately 5 seconds. (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 Solution (aq.) followed by 3 mL methanol. Discard all eluent before the following step.

Elute the TMPA using 2 mL of 2% formic acid in methanol into a 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).

3. 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 mixed calibration standards for CONH₂-fenpropathrin/4'-OH-fenpropathrin would include concentrations of 0.250/0.250, 0.500/0.500, 1.00/1.00, 2.00/2.00, 5.00/5.00, and 10.0/10.0 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.

4. Calculations

The sample concentration for CONH₂-fenpropathrin and 4'-OH-fenpropathrin is calculated as follows:

$$\text{Sample Concentration (ppb)} = \text{Extract Concentration (ng/mL)} \times \text{Final Dilution Factor}$$

The sample concentration for TMPA is calculated as follows:

$$\text{Sample Concentration (ppb)} = \frac{\text{Extract Concentration (ng/mL)} \times \text{Final Volume (mL)}}{\text{Sample Amount (mL)}}$$

Additionally, the standard deviation of the concentrations is calculated from at least three continuing calibration standards, and from this, the coefficient of variation (CV) is calculated. The CV for the continuing calibration standards must be less than 15%, unless approved by the supervising chemist responsible for the analysis.

8. LIMIT OF DETECTION

The limit of detection (LOD) for all three analytes of this method is 0.5 ppb ($\mu\text{g/L}$). For CONH_2 -fenpropathrin and 4'-OH-fenpropathrin the LOD is based on a sample size of 10 mL and a final dilution factor of 2 and 0.25 ng/mL calibration standard as the lowest standard.

For CONH_2 -fenpropathrin and 4'-OH-fenpropathrin:

$$\text{LOD (ppb)} = 0.25 \text{ ng/mL} \times 2$$

For TMPA the LOD is based on a sample size of 20 mL, a final volume of 4 mL and a 2.5 ng/mL (TMPA) calibration standard as the lowest standard.

For TMPA:

$$\text{LOD (ppb)} = \frac{2.5 \text{ ng/mL} \times 4 \text{ mL}}{20 \text{ mL}}$$

10. NOTES

1. Fortified control samples are typically analyzed with each set of samples. If the testing facility does not require concurrent analysis of fortified control samples, or if an untreated control (UTC) sample is not available, this method requirement may be waived.

Typically, samples are fortified at 1 ppb and/or 10 ppb. For example, for CONH₂-fenpropathrin and 4'-OH-fenpropathrin determination, add 100 µL of the 100/100 ng/mL of the CONH₂-fenpropathrin/4'-OH-fenpropathrin standard solution (1 ppb) or 100 µL of the 1.0/1.0 µg/mL CONH₂-fenpropathrin/4'-OH-fenpropathrin standard solution (10 ppb), to a 10 mL drinking water control sample. Further, for TMPA, add 100 µL of the 200 ng/mL TMPA standard solution (in methanol) (1ppb), or 100 µL of 2.00 µg/mL TMPA Standard solution (in methanol) (10 ppb), to a 20 mL drinking water control sample. Method recoveries must be 70 to 120% to be acceptable, unless approved by the supervising chemist responsible for the analysis.

2. If low recoveries for CONH₂-fenpropathrin or 4'-OH-fenpropathrin are observed accompanied by high recoveries for TMPA, the amount or strength of the buffer solution may need to be increased (as CONH₂-fenpropathrin and 4'-OH-fenpropathrin can degrade into TMPA at high pH). Alternatively, if low recoveries of TMPA are discovered, additional buffer may be required so that the TMPA is retained on the SPE cartridge. The optimum pH of the sample extract, when loading the sample on the SPE column, is approximately pH 7.