

FENPYROXIMATE

PTRL Study Nos. 819W and 820W

Fenpyroximate Analytical Methods as Described in "Magnitude of the Residue of Fenpyroximate in/on Cotton Raw Agricultural Commodities and Processed Fractions of Cotton RACs" C. Hatton and E.Cotter, PTRL West, Inc, California, USA, PTRL Study Nos. P819W and P820W

This method is used for the determination of Fenpyroximate in or on cotton raw agricultural commodities and processed fractions of cotton raw agricultural commodities. This method has demonstrated a limit of quantitation (combined fenpyroximate plus M-1) of 0.02 ppm in undelinted cottonseed, and 0.05 ppm in Gin trash, Oil, Hulls and Meal.

MATERIALS AND METHODS

Equipment

Glassware and Miscellaneous Equipment

Balance, Mettler AT261

Balance, A&D FX 400 Toploading Electronic Balance

Balance, Fisher XL-5000 Toploading Electronic Balance

Beakers, 400 ml

Blender, Waring™ Commercial, with 1 quart cup

250 mL Bottle, centrifuge, polypropylene

Centrifuge, Mistral 3000E

Centrifuge tubes, 15 ml graduated, glass

Chromatography Column, 250 mL, 1.5 cm i.d., 25 cm length

Coffee Mill, Mr Coffee IDS-50

Dry Ice

Evaporator, N-evap, The Meyer Evap

Filter, Acrodisc® (13CR PTFE 0.45µm) Gelman Sciences, Ann Arbor, MI

Filter paper, Whatman #4

Flask, round bottom, 125 mL, 250 mL, 500, 1000 mL

Flask, vacuum side-arm, 500 mL

Flask, volumetric, various sizes

Funnel, Büchner

Funnel, glass, filling

Funnel, separatory, 500, ml

Glasswool

GPC Autoprep 1002A, ABC Labs, Columbia, MO
 Graduated cylinder, 250,500,1000 mL
 Pipettes, volumetric, various sizes
 Pipette, Pasteur
 Silica Gel SPE cartridges, 2g/12cc, Bond Elut, Varian
 Sonicator, Branson 2210
 Syringes, microliter, various sizes
 Syringes, 10cc, glass, luer lock
 Thermometer, mercury
 Vacuum evaporator, Rotovapor, Büchi Model RM-111 with RE461 temperature controlled bath, Brinkmann Instruments, Burlingame, CA
 Vials, GC, amber with crimp caps
 Vial, amber glass with Teflon[®]-lined lid, 8, 30, 60 mL
 Vials, (2 mL capacity) with Teflon[®]-lined crimp cap, Chromacol, Inc., Trumbull, CT
 Wrist action Shaker, Model 75, Burnell Scientific, Pittsburg, PA

Reagents and Standards

Standard Reference Substances

Nihon Nohyaku Company, Ltd. supplied reference compounds, Fenpyroximate, and M-1 with the following lot numbers (see Appendix B) and PTRL designations:

	<u>Lot No.</u>	<u>PTRL No.</u>	<u>Purity (%)</u>	<u>Date Analyzed</u>	<u>Expiration Date</u>
Fenpyroximate	6AA0011P	793W-003	99.7	7/24/96	11/4/99 ^a
M-1	7AA0109Q	803W-050A	98.6	5/24/99	5/25/01

(^a Fenpyroximate was recertified, 5/24/99, 100% purity and given a new expiration date of 8/24/02)

The neat reference standard compounds were stored under freezer conditions (<0°C). Separate stock solutions of Fenpyroximate and M-1 (1000 µg/mL) were prepared in hexane. All solutions were prepared using volumetric flasks, syringes and pipettes; all solvents were at least HPLC grade.

Solvents

Acetone, Omnisolv, EM Science
 Acetonitrile, Omnisolv, EM Science

Cyclohexane, for HPLC/Residue analysis, Burdick/Jackson
 Dichloromethane, Omnisolv, EM Science
 Ethyl Acetate, Omnisolv, EM Science
 Hexane, Omnisolv, EM Science
 Methanol, Omnisolv, EM Science
 Toluene, Optima Grade, Fisher Scientific
 Water, Omnisolv, EM Science

Reagents

Nitrogen Gas
 Sodium Chloride, Fisher Certified A.C.S., Fisher Scientific
 Sodium Sulfate, anhydrous, ACS Grade, Fisher Scientific
 Silica Gel, (60-200 mesh), EM Science
 Bio-Rad BioBeads SX-3, 200-400 mesh

ANALYTICAL PROCEDURES

Preparation of Mixed Fortification Standards

Concentration (ea. analyte) of Fenpyroximate/M-1 mixed standard solutions	Dilutions (with toluene)
0.5 mg/mL Fenpyroximate and 0.5 mg/mL M-1	4.0 mL of 1.0 mg/mL Fenpyroximate plus 4.0 mL of 1.0 mg/mL M-1. (in hexane)
0.1 mg/mL Fenpyroximate and 0.1 mg/mL M-1	2.5 mL of 1.0 mg/mL Fenpyroximate plus 2.5 mL of 1.0 mg/mL M-1 diluted to 25 mL
10.0 µg/mL Fenpyroximate and 10.0 µg/mL M-1	1 mL of 0.5 mg/mL Fenpyroximate and 0.5 mg/mL M-1 mixed standard diluted to 50 mL
2.0 µg/mL Fenpyroximate and 2.0 µg/mL M-1	200 µL of 0.5 mg/mL Fenpyroximate and 0.5 mg/mL M-1 mixed standard diluted to 50 mL

All dilutions were made using volumetric pipettes, volumetric flasks and syringes. Fortification standards were stored at freezer temperatures.

Preparation of Linearity Standards

Linearity standards were prepared as follows:

Concentration (ea. analyte) of Fenpyroximate/M-1 mixed standard solutions	Dilutions (with toluene)
2 µg/mL	200 µL of 0.5 mg/mL Fenpyroximate and 0.5 mg/mL M-1 mixed standard diluted to 50 mL
1.7 µg/mL	170 µL of 0.5 mg/mL Fenpyroximate and 0.5 mg/mL M-1 mixed standard diluted to 50 mL
1.3 µg/mL	130 µL of 0.5 mg/mL Fenpyroximate and 0.5 mg/mL M-1 mixed standard diluted to 50 mL
1.0 µg/mL	100 µL of 0.5 mg/mL Fenpyroximate and 0.5 mg/mL M-1 mixed standard diluted to 50 mL
0.7 µg/mL	3.5 mL of 10 µg/mL Fenpyroximate and 10 µg /mL M-1 mixed standard diluted to 50 mL
0.5 µg/mL	2.5 mL of 10 µg/mL Fenpyroximate and 10 µg /mL M-1 mixed standard diluted to 50 mL
0.3 µg/mL	1.5 mL of 10 µg/mL Fenpyroximate and 10 µg /mL M-1 mixed standard diluted to 50 mL
0.1 µg/mL	0.5 mL of 10 µg/mL Fenpyroximate and 10 µg /mL M-1 mixed standard diluted to 50 mL
0.05 µg/mL	250 µL of 10 µg/mL Fenpyroximate and 10 µg /mL M-1 mixed standard diluted to 50 mL

All dilutions were prepared using microliter syringes and volumetric flasks. Linearity standards were stored at freezer temperatures except when in use.

Fortification Procedure

The matrices (10 g. portions for Gin Trash, Meal, Hulls and Undelinted Seeds, 20 g. portions for Cottonseed Oil) were fortified in triplicate by addition of the following quantities of appropriate fortification standard:

Matrix	Fenpyroximate plus M-1 Fortification Level (ppm)	Fortification Standard Added
Gin Trash	0.05	50 μ L of 10 μ g/mL Fenpyroximate and 10 μ g/mL M-1 mixed standard
	0.1	100 μ L of 10 μ g/mL Fenpyroximate and 10 μ g/mL M-1 mixed standard
	5.0	100 μ L of 0.5mg/mL Fenpyroximate and 0.5 mg/mL M-1 mixed standard
Undelinted Cottonseed	0.02	100 μ L of 2 μ g/mL Fenpyroximate and 2 μ g/mL M-1 mixed standard
	0.05	50 μ L of 10 μ g/mL Fenpyroximate and 10 μ g/mL M-1 mixed standard
	0.1	100 μ L of 10 μ g/mL Fenpyroximate and 10 μ g/mL M-1 mixed standard
	5.0	100 μ L of 0.5mg/mL Fenpyroximate and 0.5 mg/mL M-1 mixed standard
Cotton seed Oil	0.05	100 μ L of 10 μ g/mL Fenpyroximate and 10 μ g/mL M-1 mixed standard
	0.1	200 μ L of 10 μ g/mL Fenpyroximate and 10 μ g/mL M-1 mixed standard
	0.5	100 μ L of 0.1 mg/mL Fenpyroximate and 0.1 mg/mL M-1 mixed standard
Cottonseed Meal and Hulls	0.05	50 μ L of 10 μ g/mL Fenpyroximate and 10 μ g/mL M-1 mixed standard
	0.1	100 μ L of 10 μ g/mL Fenpyroximate and 10 μ g/mL M-1 mixed standard
	0.5	50 μ L of 0.1 mg/mL Fenpyroximate and 0.1 μ g/mL M-1 mixed standard

Extraction Method

The extraction and purification procedure is similar for gin trash, undelinted seed and cottonseed hulls but there are variations to the initial extraction procedure and purification methods for meal and cottonseed oil. Therefore specific details are provided for the initial extraction steps and the Silica gel clean up procedure.

1. A) *Gin Trash*

Weigh 10g sample into an appropriate volume centrifuge bottle, typically 250 mL. Fortify as necessary. Add 100 mL acetone:water (4:1, v:v). Using a wrist action shaker, shake sample for 10 minutes. Filter extract through Büchner funnel fitted with a 110 mm diameter Whatman #4 filter, using vacuum; collect filtrate in vacuum side arm flask (typically 500 mL). Return filter cake to bottle, add another 75 mL acetone:water (4:1, v:v) and repeat the shaking and filtration steps. Rinse bottle with 25 mL acetone:water (4:1, v:v). and use the rinsate to wash the filter cake. Repeat the rinse of the bottle and filter cake with further 25 mL acetone:water (4:1, v:v). Collect all in filter flask. Transfer entire volume of sample filtrate to a separatory funnel (typically 500mL). Rinse filter flask with 2 x ~10 mL acetone and combine rinses with filtrate in separatory funnel. Add an excess of sodium chloride (~20 g) to filtrate. Continue the procedure as in step 2.

1. B) *Undelinted Seed*

Weigh ~10.5 g sample and coarsely grind in a coffee mill (do not grind excessively into a powder). Weigh 10 g of the coarsely ground sample and transfer to a blender cup. Fortify sample as necessary. Add 100 mL acetone:water (4:1, v:v) and blend for 1-2 minutes. Filter extract through Büchner funnel fitted with a 110 mm diameter Whatman #4 filter, using vacuum; collect filtrate in vacuum side arm flask (typically 500 mL). Return filter cake to blender cup, and repeat extraction and filtration with an additional 75 mL acetone:water (4:1, v:v). Rinse blender cup with 5-10 mL acetone:water (4:1, v:v) and use the rinsate to wash the filter cake. Collect all in filter flask. Transfer entire volume of sample filtrate to a separatory funnel (typically 500mL). Rinse filter flask with 2 x ~10 mL acetone and combine rinses with filtrate in separatory funnel. Add an excess of sodium chloride (~20 g) to filtrate. Continue the procedure as in step 2.

1. C) *Cotton Seed Hulls and Meal*

Weigh 10 g cotton seed hulls or meal into a 400 mL beaker. Transfer sample to a blender cup and fortify as required. Extract by blending for ~2 minutes with 100 mL acetone:water (4:1, v:v). Filter extract through Büchner funnel fitted with a 110 mm diameter Whatman #4 filter, using vacuum; collect filtrate in vacuum side arm flask (typically 500 mL). Rinse blender cup with 50 mL acetone:water (4:1, v:v) then transfer to filter cake. Add 20 g of sodium chloride to filtrate and mix well. Let stand for ~10 minutes. Transfer entire volume of filtrate to 500 mL

separatory funnel. Rinse filter flask with 10 mL acetone and combine rinses with filtrate in separatory funnel.

1. D) *Cotton Seed Oil*

Weigh 20 g cottonseed oil into a 250 mL centrifuge bottle. Fortify as necessary. Add 150 mL acetonitrile (ACN). Shake cottonseed oil by hand or wrist action shaker for about 2 minute. Centrifuge for 10 minutes @ 5000 rpm. To facilitate decanting of supernatant, samples may be submersed in an acetone/dry ice bath to solidify oil. Transfer supernatant to round bottom flask. Add a further 150 mL acetonitrile (ACN) to the centrifuge bottle and repeat the shaking and centrifugation steps. Combine supernatant with previously collected supernatant a 500 mL round bottom flask; swirl to mix. Continue the procedure as in step 4.

2. Add 100 mL dichloromethane (DCM) to separatory funnel and shake for about 2 minutes. Allow phases to separate completely (about 10 minutes) before discarding the lower, aqueous layer. Add 10g anhydrous sodium sulfate to separatory funnel and mix (~½ minute). Let stand for ~10 minutes (except for cotton seed).

3. To assure dryness of DCM fraction, pass extract through an approximately 1 inch bed of anhydrous sodium sulfate. Collect the dried DCM sample in an appropriate volume flask. Rinse the separatory funnel with 2 x 20 mL ethyl acetate and combine rinses with dried organic fraction by passing ethyl acetate rinses through sodium sulfate bed.

4. A) *For all matrices except Oil:* Concentrate sample to dryness by rotary evaporation under vacuum at ambient temperature at first then increase to ~35-45°C. (To facilitate reconstitution of sample in only 8 mL it is advised that the concentration proceeds in a step-wise fashion using a relatively small flask (typically 500 mL or less).)

B) *For Cottonseed Oil:* Concentrate sample to dryness by rotary evaporation under vacuum at ~30°C (Note: the presence of an oily residue may be observed.) Reconstitute sample residue in 5mL of ethyl acetate. Transfer reconstituted sample to a 15 mL graduated glass centrifuge tube using 2 x 3 mL ethyl acetate rinses of concentrating flask.

5. A) *For all matrices except Oil:* Reconstitute residue in 4 mL of ethyl acetate followed by 4 mL cyclohexane.

B) *For Cotton seed Oil:* Evaporate samples to less than 4 mL under a gentle stream of nitrogen in a ~35°C water bath. Adjust volume to 4 mL with ethyl acetate, and then add 4 mL cyclohexane for a final volume of 8 mL. Vortex to mix.

Gel Permeation Chromatography (GPC)

6. Load sample onto GPC using glass syringes fitted with a Gelman Acrodisc filter (13 CR PTFE 0.45 µm). It is important that the sample loop volume be exactly 5 mL and that the sample transfer line is not more than 2 mL in volume. (These criteria assure an on-column sample volume of 5 mL i.e. 5/8 of total sample volume.)

GPC parameters: *Column* : 63 cm x 2.5cm ID

Packing material: Bio-Rad Bio-Beads S-X3, 200-400 mesh (approximately 50g).

Mobile phase: Ethyl Acetate/Cyclohexane 1:1 (v:v).

Flow rate: ~5 mL/min.

The time of the Dump, Collect and Wash phases vary per column and must be ascertained after the GPC is calibrated using ¹⁴C fenpyroximate. (Typically the Dump phase has been from 19 to 23 minutes, and the Collect phase from 6 to 8 minutes. We routinely used a 10 minute Wash phase.)

7. Concentrate collected fraction to dryness by rotary evaporation at ~35°C
8. A) *For Cottonseed Oil and Meal*: Reconstitute sample in 5 mL dichloromethane:hexane (1:1, v:v). Sonicate for 5-10 seconds.
B) *For Undelinted Cottonseed, Hulls and Gin Trash*: Reconstitute sample in 3 mL hexane:acetone (8:2,v:v). Sonicate for 5-10 seconds.

Silica Gel Chromatographic Clean-up

9. A) *For Cottonseed Oil and Meal*:

1) *For Cottonseed oil*, condition a 2g/12cc silica gel SPE cartridge with one column volume of dichloromethane:hexane (1:1,v:v). Discard eluant. *For Cotton Meal*, condition a 2g/12cc silica gel SPE cartridge (layered with ~2 cm anhydrous sodium sulfate) with one column volume of hexane. Discard eluant.

2) For both cottonseed oil and meal, load sample onto preconditioned silica gel SPE cartridge then rinse cartridge with the following sequential order of solvent systems and discard eluants.

- a) 1 mL hexane
- b) 6 mL toluene:hexane (35:65, v:v)
- c) 6 mL toluene
- d) 6 mL acetone:toluene (5:95,v:v)
- e) 2 mL acetone:toluene (20:80,v:v) except for Meal

3) Elute analyte(s) from cartridge with an additional 4 mL (6 mL for Meal) acetone:toluene(20:80,v:v). Collect eluant.

4) Concentrate sample to dryness under a gentle stream of nitrogen gas at ambient temperature.

9.B) For *Cottonseed Hulls, Undelinted seed and Gin Trash*:

1) Prepare a silica gel chromatographic column (250 mL, 15mm ID) with 10 grams (11 grams for Gin trash) activated silica gel (60-200 mesh) topped with ~1 cm anhydrous sodium sulfate. Activate silica gel by removing water (4 hours at ~130°C). It is **IMPORTANT** to wet-pack column slowly by adding silica slurry while continuously tapping column to assure homogeneous packing. Discard excess solvent to top of packing before loading sample.

2) Transfer sample to silica gel column. Allow sample to migrate into silica to top of packing before proceeding. Rinse concentration flask with hexane:acetone (8:2, v:v), 3 x 3 mL and add to column. Discard eluant.

3) Add 10 ml (15 mL for Gin Trash) hexane:acetone (8:2,v:v). Discard eluant.

4) Elute analyte(s) with an additional 40 mL (35 mL for Gin Trash) hexane:acetone (8:2,v:v). Collect in a 125 mL concentration flask.

5) Concentrate to ~1-2 mL by rotary evaporation at ~35°C. Quantitatively transfer concentrate to test tube with hexane:acetone(8:2,v:v) rinses of 125 mL concentrating flask, then continue concentrating to dryness under a gentle stream of nitrogen gas.

10. Reconstitute sample in 1 mL toluene (dilute as needed). Analyze by GC/NPD.

Instrumentation - Gas Chromatography

Instrumentation: Model No. 5890 Hewlett Packard Gas Chromatograph (GC) equipped with NP Detector (NPD)

Column: Chrompack CP-Sil-5 CB-MS Low Bleed Capillary Column
30m x 0.25mm i.d. x 0.25 µm film thickness or
30m x 0.32mm i.d. x 0.25 µm film thickness

Flow Rate: Carrier Gas = 3.0 mL/minute: Helium

Detector gases: Air = ~100 ml/minute

Hydrogen= ~3 ml/minute

Injector Temperature: 240°C and 230°C (Cotton Oil)

Detector Temperature: 300°C

Injection Volume: 1 μ L (2 μ L for Meal); by Hewlett Packard 7673A Autosampler

Oven Temperatures:

For all matrices except Cotton Oil:

Initial Temperature: 200°C
Ramp: 200°C to 250°C at 10°C/minute
250°C to 270°C at 1.5°C/minute
270°C to 300°C at 30°C hold for 4 minutes
Total run time = 23.33 minutes

Retention Time: ~ 10.5 minutes for M-1 and
~ 11.6 minutes for Fenpyroximate

Cotton Oil matrix:

Initial Temperature: 175°C
Ramp: 175°C to 300°C at 5°C hold for 5 minutes
Total run time = 33 minutes

Retention Time: ~ 20.9 minutes for M-1 and
~ 21.9 minutes for Fenpyroximate

Separation of the analyte was achieved by capillary gas chromatography. The analyte was identified by the coincidence of its retention time with the calibration standard and quantitated by integration of the peak areas relative to the calibration standard linearity curve. A typical injection sequence for Fenpyroximate/M-1 samples in all matrices required a series of 'priming' injections to condition the system (typically this was full set of linearity standards or a series of 6-8 injections of one of the high standards) followed by linearity standards interspersed between samples (e.g. 0.05 μ g/mL mixed standard, 0.1 μ g/mL mixed standard, reagent blank, control, 0.3 μ g/mL mixed standard, fortified control sample, fortified control sample, fortified control sample, 0.5 μ g/mL mixed standard, etc.). A mid range calibration standard was re-injected at the end of each sample set as a quality control sample. Where appropriate, dilutions of high fortified controls were made to bring the expected concentration within the range of linearity.

Time Required for analysis

A validation set typically consists of 1 control, 1 reagent blank, and 3 sets (of 3 replicates) of fortified controls (fortified at low, medium and high levels). The extraction time is about 22

hours and the instrument analysis time is approximately 14.0 hours. This routinely requires about 3 calendar days.

Method of Calculations

Preparation of Stock Standards

$$\text{Volume of solvent (mL)} = \frac{(W) \times (P)}{(FC)}$$

where W = Milligrams of neat standard
P = Chemical purity of neat standard
FC = final concentration (mg/mL)

Recoveries

For calibrants, the equation of the line based on the sum of the peak areas for Fenpyroximate and M-1 versus $\mu\text{g/mL}$ (each analyte) injected was generated by least squares linear regression with Microsoft Excel[®]. The correlation coefficient (r^2) calculated for each set of standards could not be less than 0.97 for the data to be considered acceptable.

Concentration of Fenpyroximate and M-1 was calculated as follows:

$$\mu\text{g/mL Fenpyroximate} = \frac{y - b}{m}$$

where y = Total peak area response for both analytes
b = Calibration intercept
m = slope

$$\text{ppm Fenpyroximate} = \frac{(\mu\text{g/mL calculated conc.} \times \text{final volume (mL)} \times \text{GPC correction factor} \times \text{Dilution Factor})}{\text{Sample weight (g)}}$$

(where GPC correction factor = (Total sample volume (8 mL) \div Sample loop volume (5 mL))

$$\text{Percent Recovery} = \frac{(\text{ppm Total residue of Fortified Sample} - \text{ppm Total residue of Control})}{\text{Fortification Level (each analyte)}} \times 100$$

Validity of the analytical method was established by acceptable recovery (70-120%) from the fortified control matrix samples. Control Cotton matrices were fortified as summarized in the Fortification Procedure.

Rep.....

An example calculation for the recovery of Fenpyroximate and M-1 (0.05 ppm) from undelinted cottonseed is shown below:

Linear regression analysis of the mixed Fenpyroximate and M-1 standards gave the summed linearity formula of $y = 3,782,887x + 156,619$ ($r^2 = 0.9882$). The combined concentration of Fenpyroximate/M-1 in the 0.05 ppm fortified sample (F1B) was calculated from this curve.

$$(1,287,383 - 156,619) \div 3,782,887 = 0.299 \text{ } \mu\text{g/mL Fenpyroximate/M-1 (combined)}$$

Calculation of the residue (ppm) is calculated as follows:

$$[(0.299 \text{ } \mu\text{g/mL} \times 1\text{mL} \times (8/5) \times 1) \div 10 \text{ g}] = 0.048 \text{ } \mu\text{g/g (ppm)}$$

The percent recovery in the sample is:

$$\left(\frac{0.048 \mu\text{g/g} - 0.000 \mu\text{g/g}}{0.05} \right) \times 100 = 96\%$$

where 0.000 $\mu\text{g/g}$ was the total summed apparent residue in the control undelinted cottonseed. Note that the numbers in the sample calculations may differ from those calculated by Excel™ due to hidden digits carried through in the computer program. Therefore, all spread sheets were calculated with the "precision as displayed" feature in effect.

Nihon Nohyaku America, Inc.

Linden Hill Park, Suite 501
4550 New Linden Hill Road
Wilmington, DE 19808
Tel: 302-636-9001 Fax: 302-636-9122
E-Mail: mariemaks@compuserve.com

October 10, 2000

Dr. Johannes Corley
Associate Coordinator
IR-4 Project
681 U.S. Highway #1, South
New Brunswick, NJ 08902-3390

Subject: Residue Method for Analysis of Fenpyroximate in Hops

Dear Dr. Corley:

Per your request, attached is a residue method for the analysis of fenpyroximate on cotton that can be adapted to hops. The title of the method is the following:

Fenpyroximate Analytical Methods as Described in "Magnitude of Residue of Fenpyroximate in/on Cotton Raw Agricultural Commodities and Processed Fractions of Cotton RACs"
C. Hatton and E. Cotter
PTRL West, Inc.
PTRL Study Nos. P819W and P820W

If you have any questions, you can reach me on (302) 636-9001.

Sincerely,



Marie A. Maks
Manager, Regulatory Affairs

cc: Mr. Kenneth Chisholm Nihon Nohyaku America Inc.