B97-7

RHÔNE-POULENC AG COMPANY

RTP, NC

Environmental Chemistry: Method Development

Title:	Insecticides, Fipronil: Analytical Method for the Determination of Fipronil and its Metabolites in Cotton and Potatoes

Analytes: Fipronil (MB 46030), MB 45950, MB 46136, MB 46513 and RPA 200766.

Substrates: Cotton and potato raw commodities and processed fractions, excluding cotton gin trash, as defined in Pesticide Assessment Guidelines, Subdivision O.

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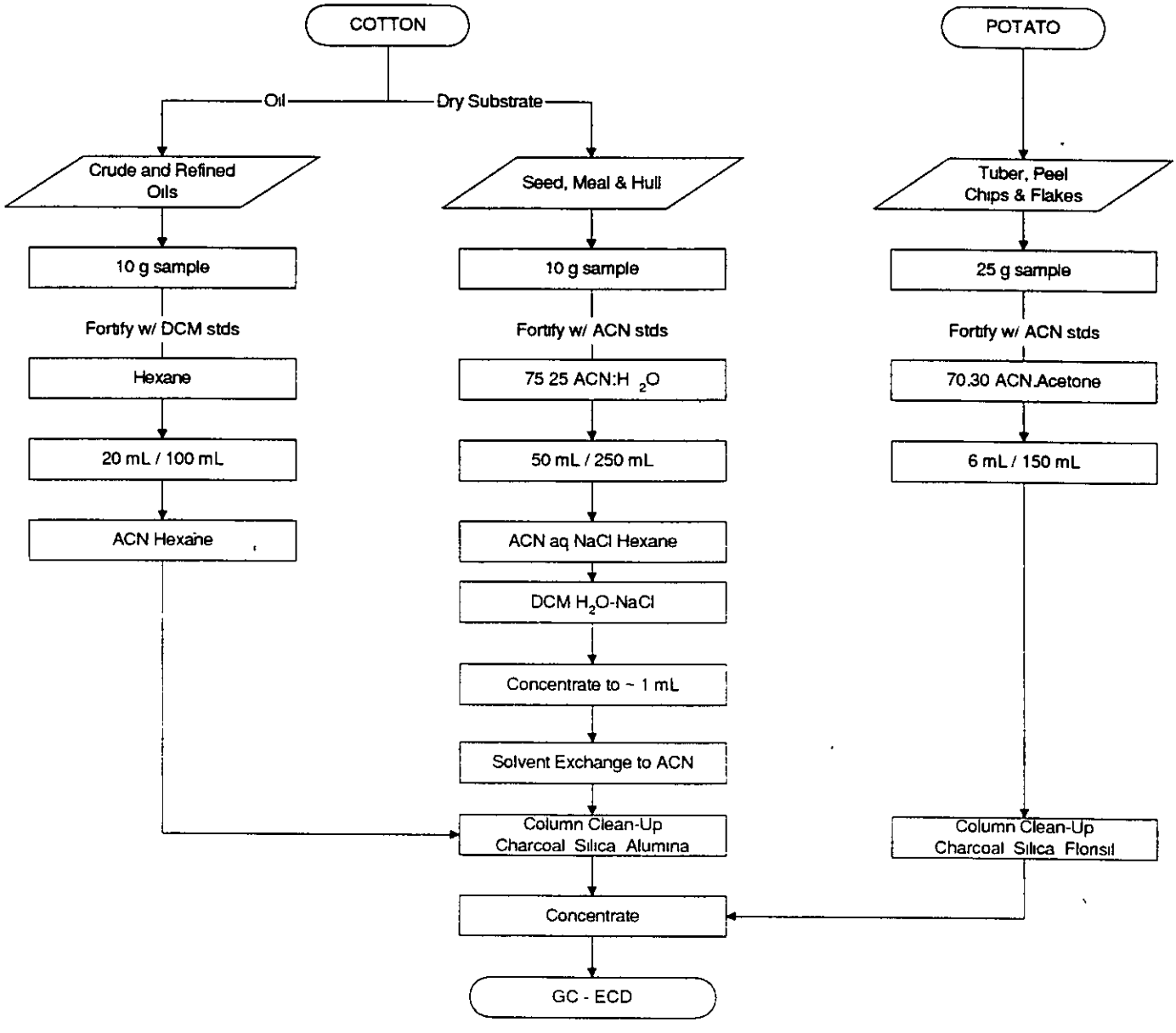
Summary Flowchart of Analytical Method



Insecticides, Fipronil: Analytical Method for the Determination of Fipronil and its Metabolites in Cotton and Potatoes

I. Introduction

A. Scope

An analytical method is described for the analysis of Fipronil and its metabolites in cotton and potato raw agricultutral commodities as well as in their respective processed fractions, excluding cotton gin trash, as defined in the Pesticide Assessment Guidelines, Subdivision O.

This method has been validated for fipronil (MB 46030) and its metabolites, MB 45950, MB 46136, MB 46513 and RPA 200766 in cotton seed, meal, hull, refined and crude oil and in potato tubers, wet peel, dry peel, flakes and chips. The method validation was conducted at Rhône-Poulenc Ag Company (RPAC) under study number EC-95-303.

Quantification of MB 45950, MB 46030, MB 46513 and RPA 200766 at 0.005 ppm each and of MB 46136 at 0.010 ppm was obtained in the cotton matrices. In the potato matrices the limit of quantitation was determined to be 0.003 ppm for all five analytes.

The fortification ranges validated included 0.005 to 0.500 ppm in the cotton substrates and 0.003 to 0.150 ppm in potatoes. All of the average recoveries fell between 73% and 124% with the exception of that for potato dry peel at the LOQ. Over the entire fortification range the mean recoveries for MB 46030, MB 45950, MB 46136, MB 46513 and RPA 200766 were 102%, 92%, 104%, 99% and 107% in cotton; and 92%, 85%, 104%, 94% and 99% in potatoes. Method precision was measured as the percent relative standard deviation of the mean recoveries of each analyte at the various fortification levels and ranged from 2.20 to 39.9%. Excluding cotton meal fortified at the 0.010 ppm level, the method precision is between 2.20 and 27.0%

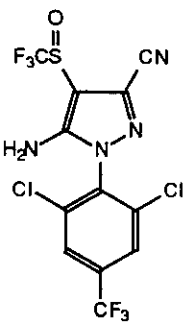
B. Principle

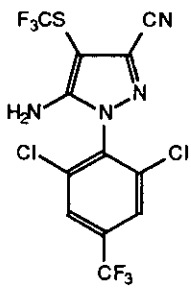
Residues are extracted from cotton seed, meal and hull with 75:25 (v/v) acetonitrile water and partitioned against hexane. After removal of the acetonitrile, the residues are partitioned into dichloromethane Residues are extracted from crude and refined oils after dilution with hexane. Column chromatography (charcoal, silica gel and alumina) is utilized for clean up

of all extracts. Quantification of fipronil and its metabolites is accomplished by gas chromatography using a Ni^{63} electron capture detector.

Residues are extracted from potato tubers, flakes, wet and dry peel and chips with 70:30 (v/v) acetonitrile:acetone. Following column chromatography (charcoal, silica gel and florisil), residues are quantified by gas chromatography using a Ni^{63} electron capture detector.

C. Structures


MB 46030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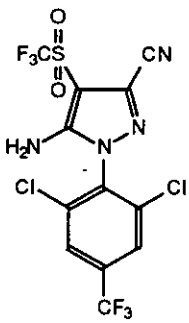
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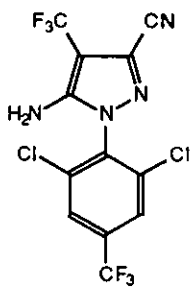
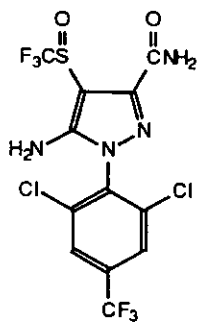

MB 46513


RPA 200766

II. Materials

Reagents and Solvents were used as received from supplier, unless otherwise noted. Equivalent reagents and equipment may be substituted where appropriate.

A. Reagents

1. Activated Carbon (Charcoal), Darco®, 20-40 mesh, granular, Aldrich, Cat. No 24,226-8. Purify by shaking charcoal with ~3 N HCl (~50 g to 100 mL) for ~25 minutes, filtering under vacuum, generously rinsing with distilled water and drying at ~130 °C for ~48 h.

2. Alumina A, Super I, particle size 63-150 µm, Selecto Scientific, Cat. No 129544 Used as received from the distributor

3. Florisil® 60/100, PR Grade, US Silica Company. Activate by heating overnight at ~130 °C

4. Silica Gel, 63-200 µm, 70-230 mesh, ICN Adsorbents, Cat No 04663 (available from Bodman). Dry by heating overnight at ~130 °C.

5. Sodium Chloride GR, crystals, EM Science, Cat No. SX0420-1

6. Sodium Sulfate, anhydrous, granular, J.T. Baker, Cat. No. 3375-05

B. Solvents

1. Acetonitrile UV, B & J High Purity Solvents, Burdick & Jackson, Cat. No 015-4

2. Acetone, B & J Chrompure HPLC Solvent, Burdick & Jackson, Cat. No. CP80100-4

3. Cyclohexane, B & J High Purity Solvents, Burdick & Jackson, Cat. No. 053-4

4. Hexane UV, B & J High Purity Solvents, Burdick & Jackson, Cat. No. 216-4

5. Methanol, B & J Chrompure HPLC Solvent, Burdick & Jackson, Cat. No. CP80150-4

6. Methylene Chloride, B & J Chrompure HPLC Solvent, Burdick & Jackson, Cat. No. CP80175-4

7. Water, Purified by Millipore Milli-Q water purification system.

C. Solutions

1. 0.5 % Sodium Chloride Solution, dissolve ~5 g of sodium chloride in ~1 liter of purified water

2. 75:25 Acetonitrile:Water Solution, mix 750 mL of acetonitrile with 250 mL of purified water

3. 70:30 Acetonitrile:Acetone Solution, mix 700 mL of acetonitrile with 300 mL of purified water

4. 90:10 Acetonitrile:Methanol, mix 900 mL of acetonitrile with 100 mL of methanol

D. Equipment

1. Aluminum Crimp-Top Seal, 11 mm TFE/RUB Septum, Sun Brokers, Inc., Cat No 200-100

2. Analytical Balance

3. Autosampler Vials, 1 mL, clear, Wheaton, Cat. No. 223682

4. Blender, Waring, and Blender Jars (Small Size, ~500 mL, Eberbach, Cat. No. 8470, recommended but not required)

5. Büchner Funnels, appropriate sizes (~83 mm diameter, Coors, Cat. No. 60242, recommended but not required)

6. Chromatography Columns, 15 mm i.d., 250 mL Reservoir, Kontes

7. Disposable Pasteur Pipets

8. Filter Paper, Whatman No. 1, appropriate size to fit Büchner funnels

9 Glasswool

10. Graduated Cylinders, calibrated to deliver, appropriate sizes

11. Graduated Mixing Cylinders, calibrated to contain, 250 mL

12. Mechanical Shaker

13. Nalgene® 250 mL screw-capped bottles, Nalge Co., Cat No 2105-0008 (125 mL size optional, Cat. No. 2105-0004)

14 Separatory Funnels, 125 mL, 250 mL and 500 mL (1 L optional)

15 (a) TurboVap® II Concentration Workstation, Zymark, Cat. No. ZW8002, Concentrator Tubes, 200 mL with 1 mL endpoint, Zymark, Cat No ZW2038 and a Water Bath OR (b) Rotary Evaporator, Buchi, and Round Bottomed Flasks in appropriate sizes

16 Vacuum Adapter, At-Mar Glass, special order, Cat. No A005-31595 with following specifications: 3 cm drip top, ground glass joint on top is not required, bottom joint needs to ground glass §27 (or appropriated size to fit 250 mL graduated mixing cylinder) and there needs to be 6 cm beteween top of adaptor and top of inside taper

17 Volumetric Flasks, 10 mL & 100 mL, class A

18 Volumetric Pipets, appropriate sizes, class A

19. Hewlett Packard 5890 Series II GC equipped with ^{63}Ni detector (refer to Section V of this document for details)

20. Capillary Column, DB1701, 15 m X 0.32 mm i.d., 0.25 µm film thickness, J & W Scientific, Cat. No. 123-0712

21. Optional: Repeat-a-pet, appropriate sizes, Markson

22. Optional: Tongs or Forceps, Spatulas, Spoons

E. Analytical Standards

Analytical Standards available from Rhône-Poulenc Ag Company

1.	Fipronil (MB 46030):	5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-3-cyano-4-trifluoromethylsulfinylpyrazole
2	MB 45950:	5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-3-cyano-4-trifluoromethyl-thio-pyrazole
3.	MB 46136:	5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-3-cyano-4-trifluoromethylsulfonylpyrazole
4	MB 46513:	5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-3-cyano-4-trifluoromethylpyrazole
5.	RPA 200766,	5-amino-3-carbamoyl-1-(2,6-dichloro-4-trifluoromethyl-phenyl)-4-trifluoromethylsulfinylpyrazole

III. Standard Solution Preparation

A. General

1. The state concentrations of standard solutions should be adjusted to account for the purity of the neat solid standards.

2. After preparation, standards should be transferred from the volumetric flasks into screw-capped amber bottles to prevent possible photodegradation.

3 Store standard solutions in the refrigerator at or below 10 °C when not in use.

B. Fortification and Calibration Standard Solutions

The following is provided as an example of how standard solutions may be prepared. Other concentrations may be used as apropriate.

1. Weigh 0.1000 g (±0.1 mg) of each analytical standard individually into 100 mL volumetric flasks. Dissolve in acetonitrile and mix well. Dilute to final volume with acetonitrile. Concentration of each standard is 1000 µg / mL.

2. Withdraw a 10.0 mL aliquot from each of the 1000 µg / mL individual standards and add to a 100 mL volumetric flask. Dilute to volume with acetonitrile. The concentration of this standard is 100 µg / mL for each analyte in the acetonitrile solution. (Note: The analytical standards may be diluted and used separately for fortification and individual determinations.)

3. Withdraw a 10.0 mL aliquot from the 100 µg / mL mixed standard and add to a 100 mL volumetric flask. Dilute to volume with acetonitrile. The concentration of this standard is 10 µg / mL for each analyte in acetonitrile.

4. By further dilution of the 10 µg / mL mixed standard with acetonitrile, prepare a series of standards to serve as fortification standards and calibration standards.

5 Since the acetonitrile standards are immiscible with the cotton seed oils, prepare dichloromethane standards for fortification of these substrates. Withdraw a 10.0 mL aliquot from the 100 µg / mL mixed standard made in acetonitrile and add to a 100 mL volumetric flask. Dilute to volume with dichloromethane The concentration of this standard is 10 µg / mL for each analyte in dichloromethane. By further dilution of the 10 µg / mL mixed standard with dichloromethane, prepare a series of standards.

IV. Methods of Analysis

Stopping points in the method are left to the discretion of the analyst performing the method. The author has placed the diamond symbol (◆) throughout the method to indicate places that are convenient to stop. Overnight storage in a refrigerator is suitable, however, a freezer is recommended for longer time periods Samples should be allowed to warm / thaw prior to use

The tilde symbol (~) indicates 'approximately'.

A. Crude / Refined Cottonseed Oils

1. Sample Preparation

   Use oils as received from processor.

2. Extraction

   2.1 Weigh ~10 g of oil into a 100-mL volumetric flask.

   2.2 Fortify with methylene chloride standard solution, hand shake to mix and then let stand at least 30 minutes.

   2.3 Dilute to volume with hexane. Stopper the flask and mix well. Transfer to a labeled screw cap Nalgene® bottle. ♦

3. Partitioning

   3.1 Transfer a 20 mL portion (by class A TD graduated cylinder or volumetric pipet) of the hexane solution from step 2.3 into a 125-mL separatory funnel.

   3.2 Add ~20 mL of acetonitrile.

   3.3 Shake for ~30 seconds (vent frequently), then allow layers to separate

   3.4 Drain and collect the acetonitrile layer. Drain and discard the hexane layer.

   3.5 Return the acetonitrile layer to the separatory funnel. Add ~20 mL of hexane.

   3.6 Repeat steps 3.3 through 3.5.

   3.7 Repeat steps 3.3 and 3.4.

4. Column Chromatography

   4.1 Plug a 15 mm i.d. glass chromatography column with glass wool. Add approximately 2.0 g of acidic alumina activity grade I, ~2.0 g of 63-200 µm silica gel, followed by ~2.0 g of acid washed / oven dried 20-40 mesh activated carbon and finally ~6.0 g of anhydrous sodium sulfate. Tap the column after the addition of each compound to settle the packing. Wash the column with approximately 25 mL of methanol, eluting until the level of the solvent just reaches the top of the sodium sulfate. Repeat with ~25 mL acetone, followed by ~30 mL of acetonitrile. Discard the solvent washes.

4.2 Transfer the acetonitrile extract obtained in step 3.7 directly onto the column Rinse the glassware that contained the extract with a few mL of acetonitrile and transfer to column. Repeat. Load the sample onto the column When the level of the extract just reaches the top of the sodium sulfate, add approximately 75 mL of 90:10 acetonitrile:methanol. Collect all eluate in an Erlenmeyer or round bottomed flask, depending on method to be used for concentration (see step 4.3).

4.3 Evaporate just to dryness
(1) Place Erlenmeyer flask in a warm water bath (~37 °C) under a nitrogen stream (~45 min to 1 h). **OR**
(2) Attach round bottomed flask to a rotary evaporator (water bath ~45 °C, dry ice in cold finger).

4.4 Dilute the residue to desired volume (at least 4 mL to prevent excess contamination of injector port, refer to section V.C.6) with acetonitrile. Load samples into vials for analysis and seal. ♦ Analyze by gas chromatography.

B. Cotton Seed, Meal and Hull

1. Sample Preparation

Gin the cottonseed and then mill or grind. Meal and hull samples should be suitable for use as received from processor.

2 Extraction

2.1 Weigh ~10 g of substrate into a labeled 250 mL Nalgene® screw cap bottle

2.2 Fortify with acetonitrile standard solution, let stand at least 30 minutes.

2.3 Add ~100 mL of 75:25 (v/v) acetonitrile:water.

2.4 Shake for ~30 minutes on a mechanical shaker. (Optional: One to two revolutions of teflon tape may be added to the threads of the Nalgene bottle to help prevent leaks).

2.5 Transfer sample to small blender jar. Rinse bottle with ~5 mL of acetonitrile.

2.6 Blend ~5 minutes.

2.7 Filter through Whatman #1 filter paper into a 250-mL graduated mixing

cylinder using a Büchner funnel under vacuum

2.8 Return at least 90% of filter cake to blender (tongs or forceps work well for transfer) Add ~100 mL of extraction solution (use this as an additional rinse of Nalgene bottle).

2.9 Blend ~2.5 minutes.

2.10 Repeat step 2.7 (filtering into same graduated cylinder)

2.11 Dilute filtrate to 250 mL with acetonitrile. Mix well. Transfer solution to Nalgene® bottle. ♦

3. Partitioning

3.1 Acetonitrile / Water / Hexane

3.1.1 Shake sample well. Transfer a 50 mL portion of the extract obtained in step 2.11 (by class A TD graduated cylinder or volumetric pipet) extract into a 250-mL separatory funnel containing 50 mL of acetonitrile and 25 mL of ~0.5% aqueous sodium chloride solution.

3.1.2 Add ~50 mL hexane. Shake for ~30 seconds Let layers separate.

3.1.3 Drain and collect aqueous acetonitrile layer. Drain and collect hexane layer. (Beakers may be used for the collection of the layers).

3.1.4 Return the aqueous acetonitrile layer to the separatory funnel.

3.1.5 Repeat steps 3.1.2 to 3.1.4 (Hexane layers combined).

3.1.6 Repeat 3.1.2

3.1.7 Drain aqueous acetonitrile layer into a TurboVap® tube or a round bottomed flask depending on method to be used for concentration (see step 3.1.8). Return hexane to separatory funnel. Rinse glassware with 5-10 mL of acetonitrile and add to separatory funnel, gently swirl to back-extract hexane Drain this acetonitrile layer into the same TurboVap® tube or round bottomed flask as above.

3.1.8 Evaporate acetonitrile from the extract. (Note: Incomplete removal of acetonitrile may afford lower recoveries).

(1) TurboVap®: temperature ~45 °C, pressure of ~1.1 bar (requires

approximately 1.5 h). Concentration to ~30 mL ensures removal of the acetonitrile **OR**

(2) Rotary Evaporator: dry ice in cold finger, water bath at ~45 °C (Note: Stop just after complete removal of acetonitrile. Removal of large portions of water will afford low/variable recoveries).

3.2 Water / NaCl / Dichloromethane

3.2.1 Add ~150 mL of water and ~5 g of sodium chloride to a 500 mL (1L also acceptable) separatory funnel and shake until the salt is dissolved.

3.2.2 Transfer the aqueous sample extract from step 3.1.8 to the separatory funnel. Rinse the TurboVap® tube or round bottomed flask with ~50 mL of dichloromethane then add to the separatory funnel. Shake for ~30 seconds (**Caution: significant pressure build up, vent frequently!**), then allow layers to separate.

3.2.4 Drain the dichloromethane layer into a clean TurboVap® tube or round bottomed flask. Leave the aqueous layer in the separatory funnel and extract two more times with ~50 mL portions of dichloromethane, rinsing the original turbo vap tube / round bottomed flask each time. (Dehydration of the collected dichloromethane is not necessary. The Na_2SO_4 in the column clean-up (step 4) is sufficient for this purpose.)

3.2.5 Concentrate the dichloromethane extract

(1) TurboVap® temperature of ~40 °C, pressure of ~0.9 bar. Evaporate to a volume of ~1 mL then add 10-15 mL of acetonitrile. Again concentrate to a volume of ~1 mL (temperature of ~45 °C, pressure of ~1.1 bar). **OR**

(2) Rotary Evaporate: bath temperature ~45 °C, dry ice in cold finger. Evaporate just to dryness then add ~1-2 mL acetonitrile.

4. Column Chromatography

4.1 Plug a 15 mm i.d. glass chromatography column with glass wool. Add approximately 2.0 g of acidic alumina activity grade I, ~2.0 g of 63-200 µm silica gel, followed by ~2.0 g of acid washed / oven dried 20-40 mesh activated carbon and finally ~6.0 g of anhydrous sodium sulfate. Tap the column after the addition of each compound to settle the packing. Wash the column with

approximately 25 mL of methanol, eluting until the level of the solvent just reaches the top of the sodium sulfate. Repeat with ~25 mL acetone, followed by ~30 mL of acetonitrile. Discard the solvent washes.

4.2 Transfer the concentrated extract obtained in step 3 2.5 directly onto the column (use of a disposable Pasteur pipet recommeded). Rinse the glassware that contained the extract with a few mL of acetonitrile and transfer to column. Repeat. Load sample onto column. When the level of the extract just reaches the top of the sodium sulfate, add approximately 75 mL of 90:10 (v/v) acetonitrile:methanol. Collect **all** eluate in an Erlenmeyer or round bottomed flask, depending on method to be used for concentration (see step 4.3).

4.3 Evaporate just to dryness.
(1) Place Erlenmeyer flask in a warm water bath (~37 °C) under a nitrogen stream (~45 min to 1 h). **OR**
(2) A round bottomed flask to a rotary evaporator (water bath ~45 °C, dry ice in cold finger)

4.4 Dilute the residue to desired volume with acetonitrile. Load samples into vials for analysis and seal. ♦ Analyze by gas chromatography.

C. Potato Tuber, Peel, Flakes and Chips

1. Sample Preparation

Tubers should be cut up and ground with dry ice in a food processor or blender. Chips should be crushed and treated similarly. Flakes should be pulverized into a powder in a blender. Wet and dry peel should be suitable for use from processor If necessary, the wet peel may be prepared like the tubers and the dry peel like the flakes

2. Extraction

2.1 Weigh ~25 g of substrate into a labeled 250 mL Nalgene® screw cap bottle.

2.2 Fortify with acetonitrile standard solution, let stand at least 30 minutes

2 3 Add ~100 mL of 70:30 acetonitrile:acetone (v/v).

2 4 Shake for ~15 minutes on a mechanical shaker.

2 5 For **flakes, dry peel and chips**: Proceed to step 2.6.

For **tuber and wet peel**: Add ~25 g of anydrous sodium sulfate. If using a blender, transfer sample to a small blender jar. Use 5-10 mL of acetonitrile as a rinse to obtain a complete transfer. If using an overhead homogenizer, the sample may be left in Nalgene® bottle Blend (homogenize) ~5 minutes. If the samples have a particularly high water content, let stand for ~30 min to an 1 h to allow sodium sulfate more time to dry sample.

2.6 Filter through Whatman #1 filter paper into a graduated mixing cylinder using a Büchner funnel under vacuum. Rinse blender jar with 15-20 mL of acetonitrile and pour over filter cake.

2.7 Dilute filtrate to 150 mL with acetonitrile. Transfer extract back to Nalgene® bottle. ♦

3. Partitioning - None

4. Column Chromatography

4.1 Plug a 15 mm i.d. glass chromatography column with glass wool. Add approximately 2.0 g of Florisil®, ~2.0 g of 63-200 µm silica gel, followed by ~2.0 g of acid washed / oven dried 20-40 mesh activated carbon (use ~**3.0 g** of charcoal for potato **chips**) and finally ~6.0 g of anhydrous sodium sulfate. Tap the column after the addition of each compound to settle the packing Wash the column with approximately 25 mL of methanol, eluting until the level of the solvent just reaches the top of the sodium sulfate. Repeat with ~25 mL acetone, followed by ~30 mL of acetonitrile Discard the solvent washes (The extra gram of charcoal is necessary for the clean up of chips due to the high fat content. A slight, but acceptable, decrease in recoveries is observed).

4.2 Pipet a 6 mL aliquot of the acetonitrile extract obtained in step 2.7 directly onto the column. Load sample onto column. When the level of the extract just reaches the top of the sodium sulfate, add approximately 75 mL of acetonitrile. Collect **all** eluate in an Erlenmeyer or round bottomed flask, depending on method to be used for concentration (see step 4.3).

4.3 Evaporate just to dryness

(1) Place Erlenmeyer flask in a warm water bath (~37 °C) under a nitrogen stream (~45 min to 1 h). **OR**

(2) A round bottomed flask to a rotary evaporator (water bath ~45 °C, dry ice in

cold finger).

4.4 Dilute the residue to desired volume with acetonitrile. Load samples into vials for analysis and seal. ♦ Analyze by gas chromatography.

D. General Method Notes / Potential Problems

1. If columns stop flowing due to air pockets in charcaol or sodium sulfate, tap on sides until air bubbles are released.

2. If significant carry over of residues becomes a problem in the untreated samples and reagent blanks, clean the blenders. **Totally disassemble** entire blade mechansim and soak the parts in acetone and/or acetonitrile for at least a couple of hours. Replace gaskets and washers as required.

3. Envi-Carb®, manufactured by Supelco, is not a suitable substitute for Darco activated carbon that has been acid washed and dried.

V. Gas Chromatography

A. Instrumentation

1	Gas Chromatograph:	Hewlett-Packard 5890 Series II GC, 7673 Autosampler, 18594B Sampler Controller, 3396A Integrator, Split/Splitless Injector, or an equivalent system
2	Detector.	Ni^{63} - Electron Capture Hewlett-Packard Model G1223A, G1224A, or equivalent
3	Data Acquisition.	Waters® 860 Data Capture System, or equivalent
4	Column:	J & W Scientific DB1701 15 m X 0.32 mm i.d., 0.25 µm film thickness

B. GC Conditions

1	Detector Make-Up Gas	5% Methane in Argon, ~50-60 mL/min
2	Carrier Gas	Helium, ~2-3 mL/min

3. Inlet Liner: 4-mm i.d. nominal volume 900 µl, borosilicate glass with silanized glass wool plug (HP part #5062-3587)

4. Injector Temperature: 280 °C

5. Detector Temperature: 300 °C

6. Oven Temperatures: Initial: 50 °C, hold 1 minute
Ramp 30 °C / min to 200 °C, hold 20 minutes
Ramp 30 °C / min to 230 °C, hold 10 minutes
Ramp 30 °C / min to 250 °C, hold 12 minutes

7. Injection Volume: 1.0 µL

8. Splitless injection with split vent off for 30 seconds.

C. General Chromatography Notes / Potential Problems

1. Gas flows set while oven at 50 °C.

2. Several standards should be injected prior to actual analysis using a new column or after the GC has set idle for any considerable length of time to condition and/or to remove any contaminants.

3. A gold plated seal is used at the interface of the glass liner and column Hewlett-Packard Part No. 18740-20885

4. Giga bore liner installed in detector make-up gas adapter. Hewlett-Packard Part No. 19233-20625.

5 The GC parameters are guidelines and can be optimized for the instrument and column actually used Record the actual GC conditions used for data acquisition and include in report.

6 During the analysis of the cotton seed oils and potato chips, frequent cleaning of the injector with methanol is necessary, as well as changing of the septum, inlet liner, O-ring, gold seal and washer washer supporting the inlet sleeve beneath the injector (HP 5890 GC) Since oils/fats have such high boiling points, residue can build up on the injector port of the GC after a series of injections High recoveries are obtained for RPA 200766 and MB 46136 since the response for these compounds is higher in the

samples than in the standard solutions. Poor determination coefficients (R^2) may also be obtained when the injector is dirty.

7. During the analysis of substrates with high a water content, potato tubers and wet peel, frequent changing of the inlet liner is warranted. A decrease in the response of RPA 200766 is indicative of the need to do this.

8. Typical values for the coefficient of determination (R^2) for a standard curve derived from five standards of concentrations of 0.001, 0.0025, 0.005, 0.008 and 0.010 µg/mL are:

MB 46513	0.999, 0.989
MB 45950	0.993, 0.997
MB 46030	0.999, 0.996
RPA 200766	0.998, 0.997
MB 46136	0.997, 0.972 (When less than 0.97, refer to notes 6 and 7 above)

9. Residues of MB 45950, MB 46030, MB 46136, MB 46513 and RPA 200766 may be qualitatively confirmed by gas chromatography using a DB-5MS column with electron capture detection. The following operating parameters are given as guidelines and may need to be optimized for the instrument and column actually used. Carrier gas: helium at ~0.5 mL/min (set at 50 °C); septum purge: helium at ~2.5 mL/min; split vent: helium at ~60 mL/min; injector temperature: 280 C, detector temperature: 300 °C; detector make-up gas: 5% methane in argon at ~ 50 mL/min (anode purge 5-6 mL/min), temperature program: initial temperature 50 °C with 1 minute hold, ramp at 30 °C/min to 200 °C, hold for ~20 minutes, ramp at 30 °C/min to 230 °C, hold for ~15 minutes, ramp at 30 °C/min to 240 °C, hold ~ 20 minutes, injection volume: one microliter.

VI. Quantification of Residues

A. Calibration Curves

1. Linear regression should be used to generate calibration curves for all analytes. At least five different standard concentrations should be run with each set of samples. Standards should be interspersed with samples to compensate for any minor change in instrument response. Extracts should be diluted such that the peak areas obtained are within the area range between the lowest and highest standards injected.

2. Linear regression coefficients should be calculated from 'peak area' (or 'peak height') versus 'nanogram / mL injected'. Data from the analytical standards should be fit to the linear equation, $y = a + bx$.

where: y = peak area or height
a = calibration line intercept
b = calibration line slope
x = conc of analyte in inj soln

B. Quantification of Residues

1. Fipronil and its metabolites should be quantified by comparision to the standard curves obtained from a linear regression analysis of the data.

2. Equations

2.1 Concentration of analyte in sample in ppb (parts per billion).

$$z = (y - a) / b \times c / d$$

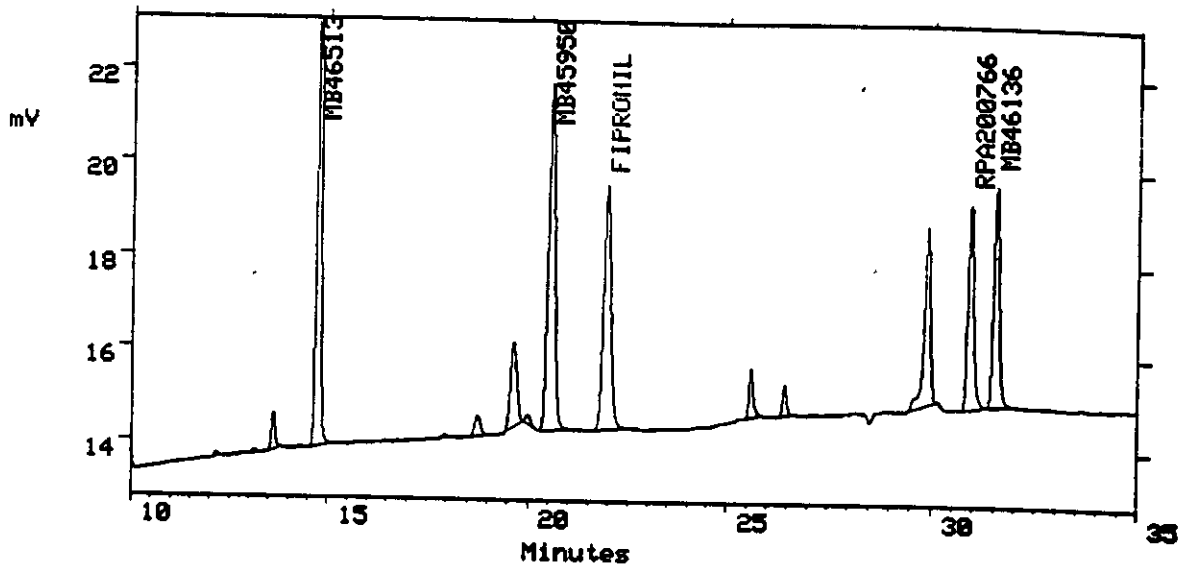
where: y = peak area (or height), response of analyte of interest
a = intercept of calibration line from linear regression (area or height)
b = slope of calibration curve from linear regression (response per ng/mL)
c = final volume of sample (mL)
d = sample weight (g)
z = conc of analyte in sample (ppb)

2.2 Percent recovery

$$\% \text{ recovery} = \frac{(\text{ppb found in fortified sample} - \text{ppb found in UTC})}{\text{actual fortification level in ppb}} \times 100\%$$

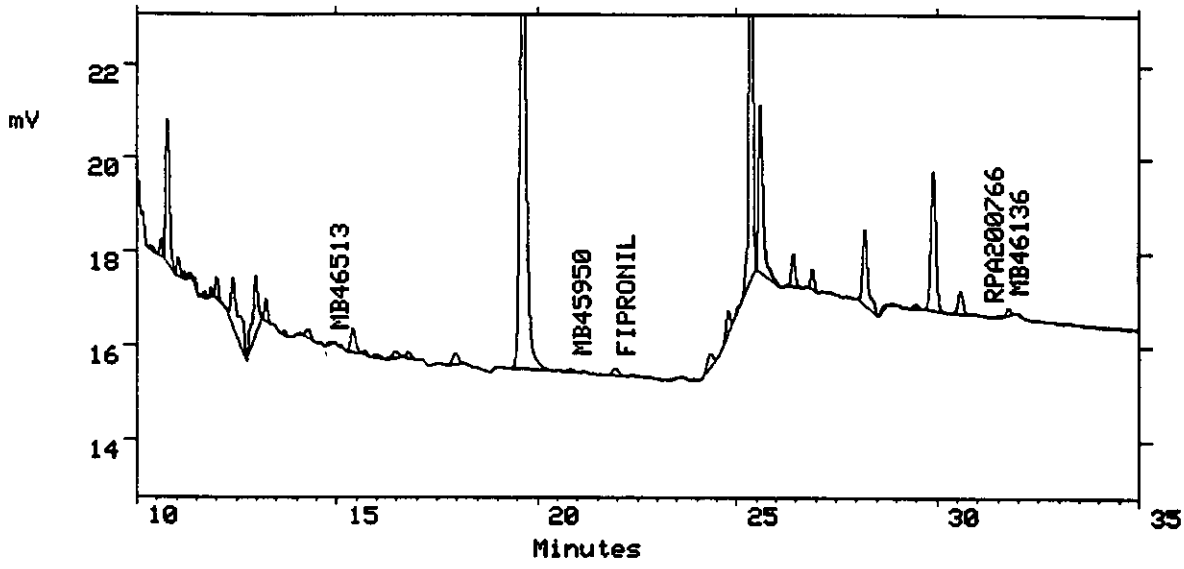
VII. Example Chromatograms (Waters 860 Data Capture)

A. Mixed Standard, Each Analyte ~0.010 ug/mL in Acetonitrile

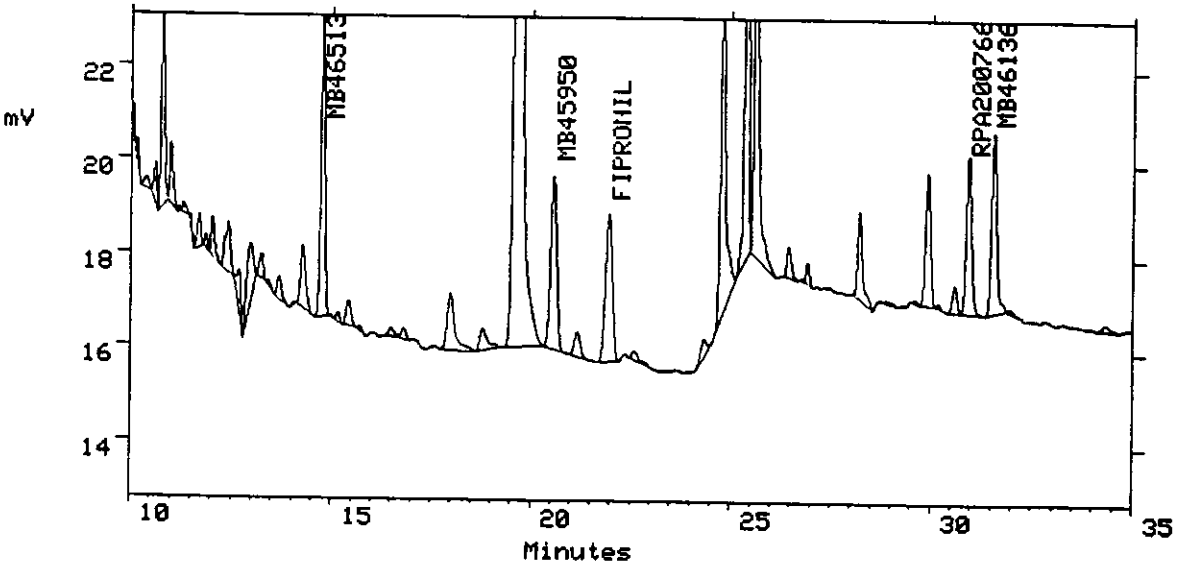


B. Cotton Samples

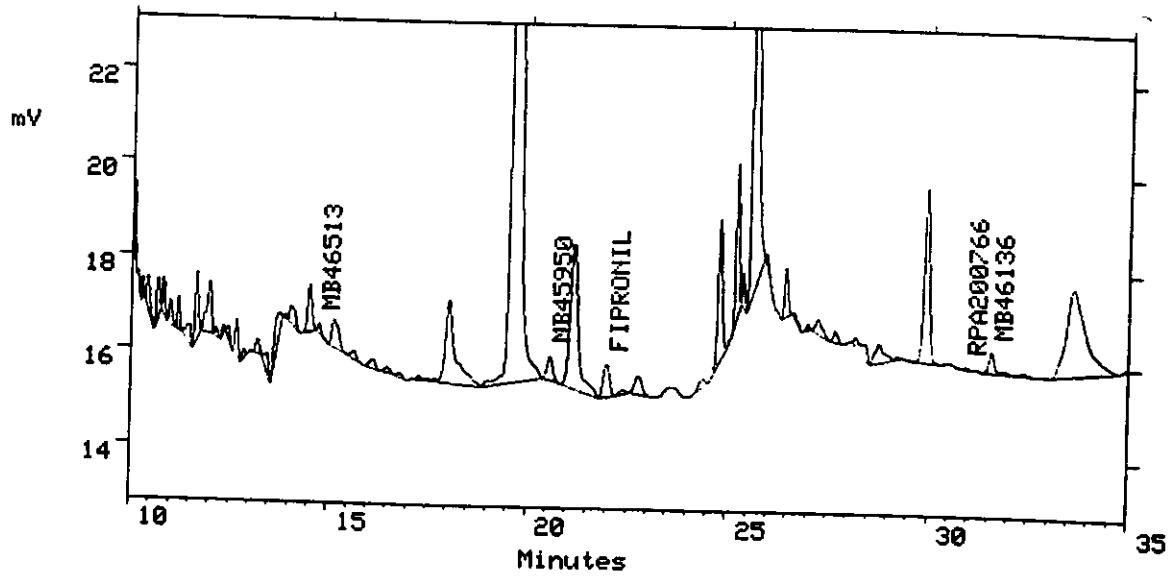
1. Crude Cottonseed Oil, Untreated Control



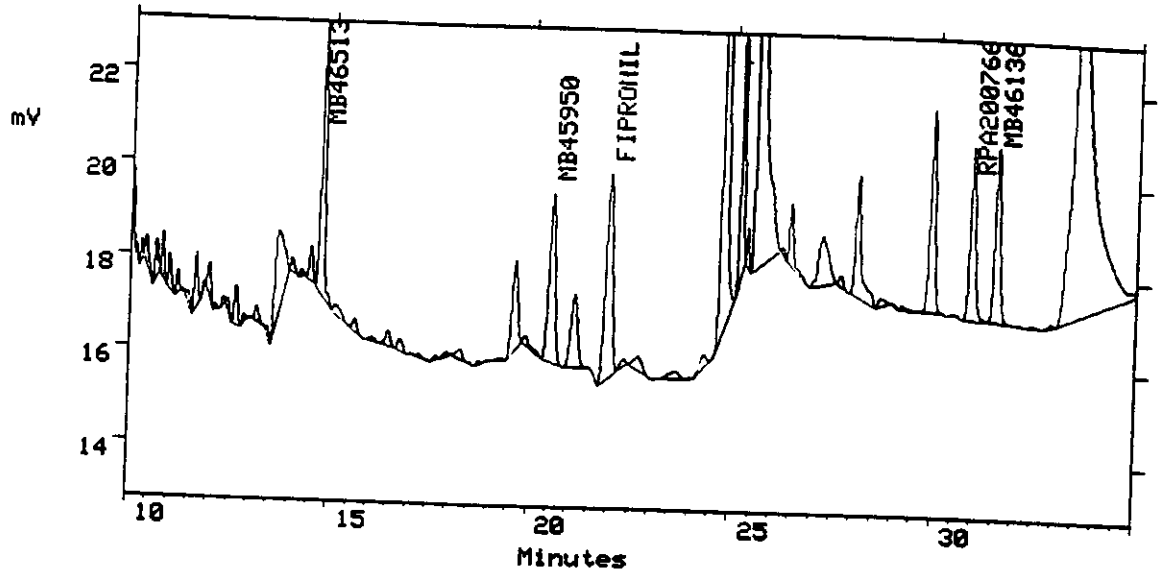
2 Crude Cottonseed Oil, Fortified at 5 ppb Level



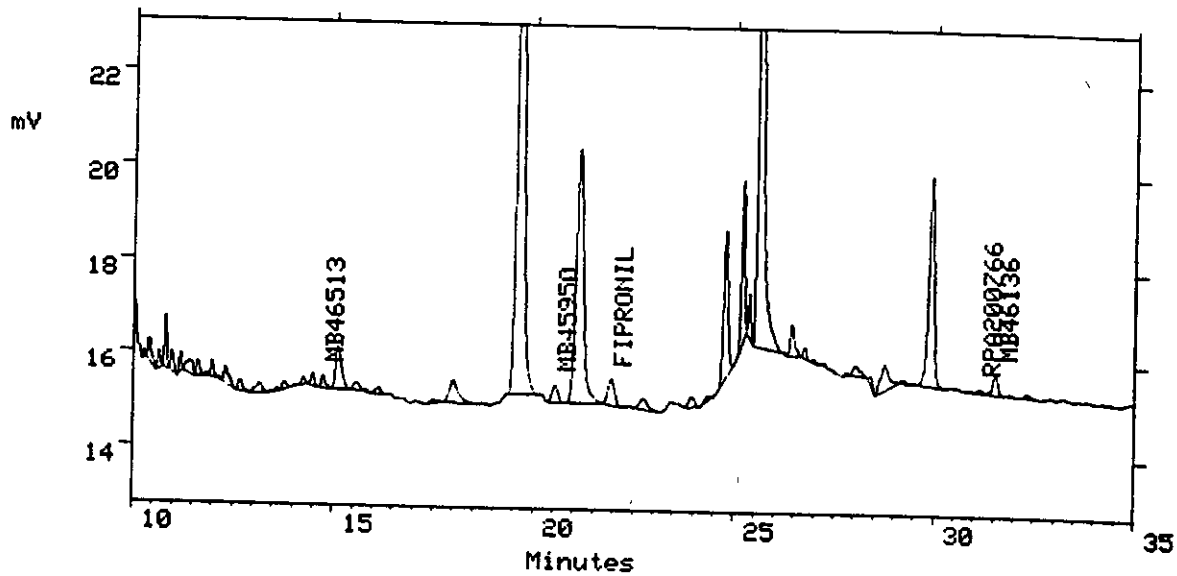
3. Cottonseed, Untreated Control



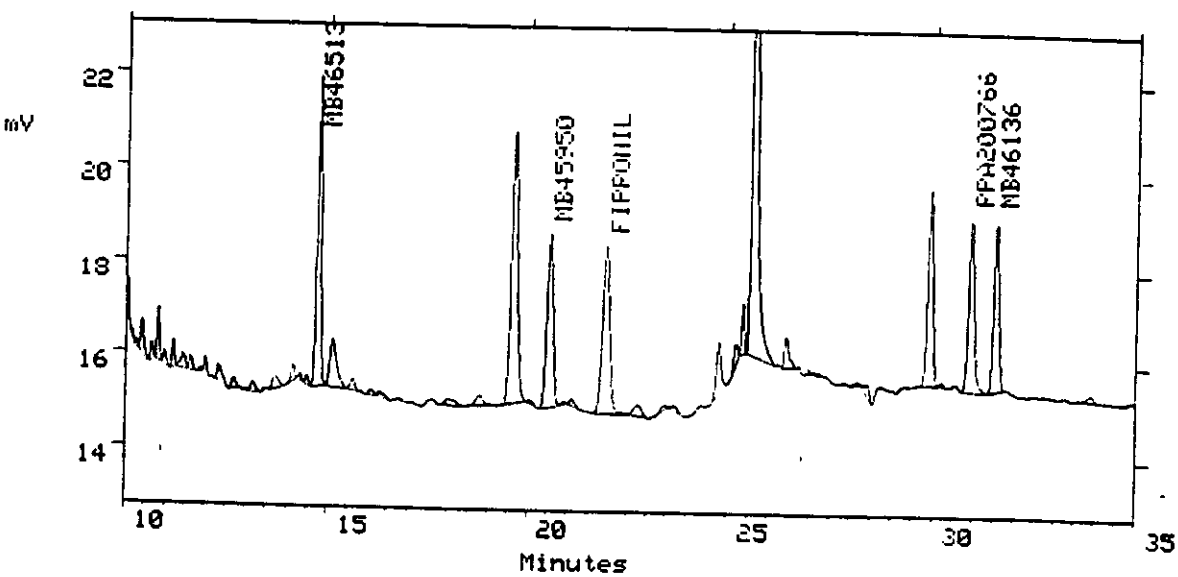
4 Cottonseed, Fortified at 5 ppb Level



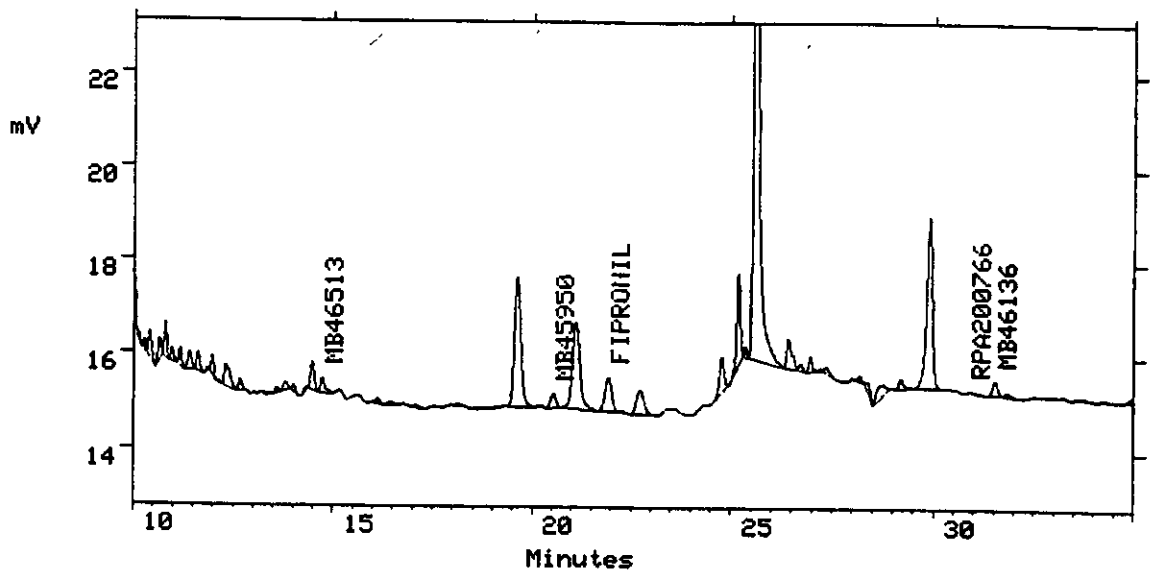
5. Cottonseed Meal, Untreated Control



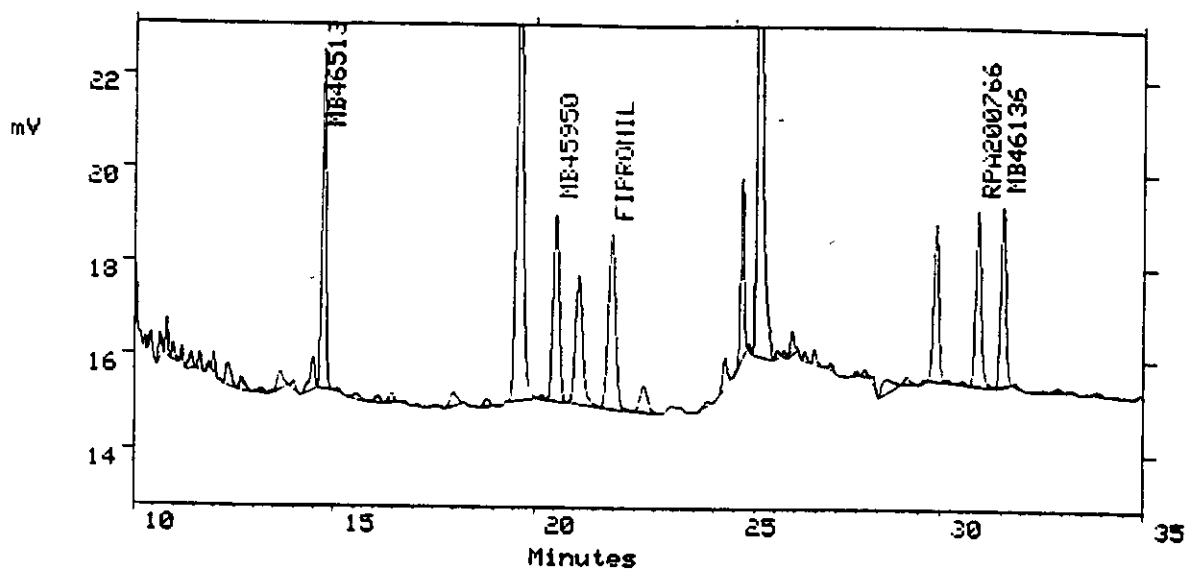
6 Cottonseed Meal, Fortified at 5 ppb Level



7. Cottonseed Hull, Untreated Control

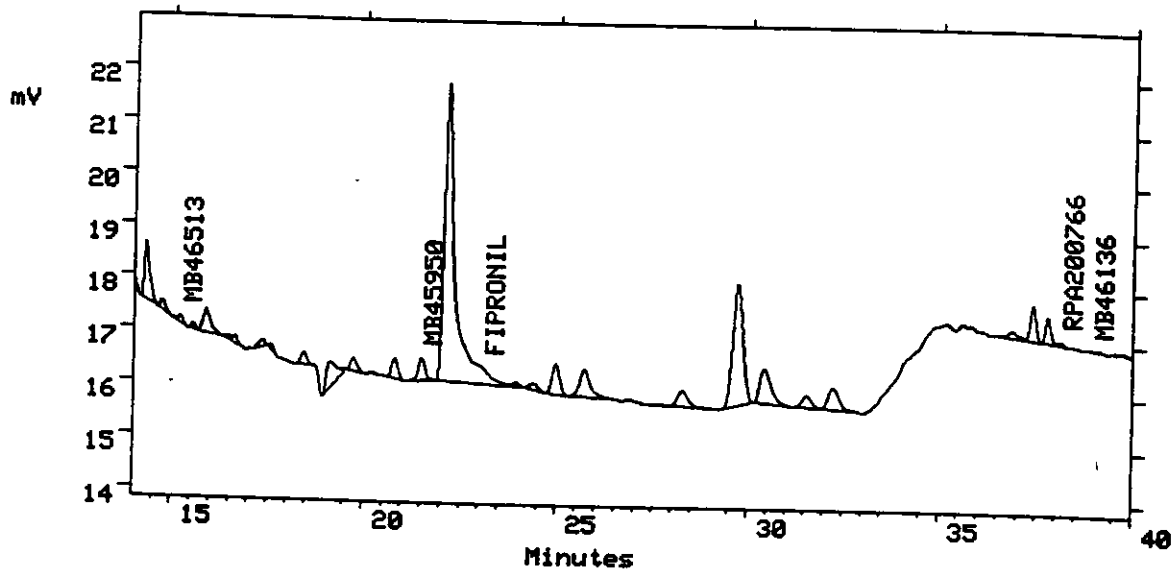


8. Cottonseed Hull, Fortified at 5 ppb Level

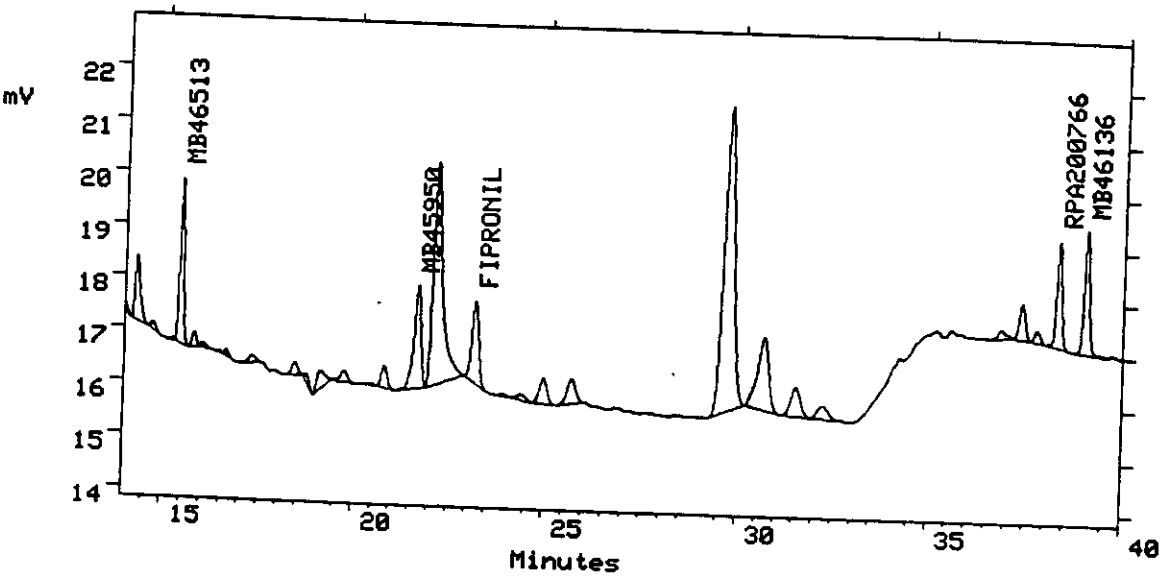


C. Potato Samples

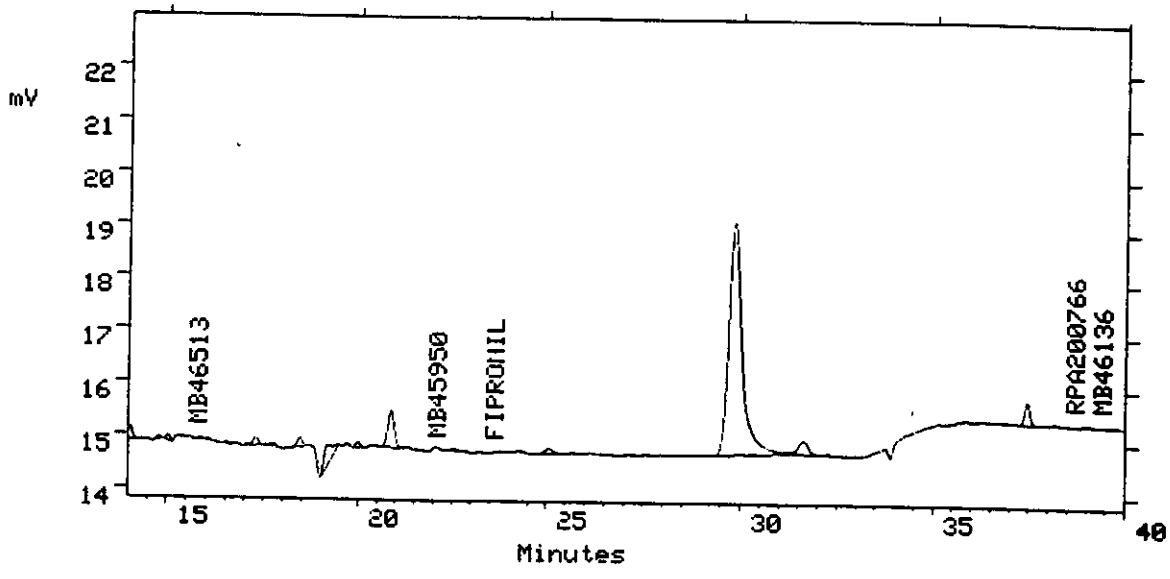
1 Potato Tuber, Untreated Control



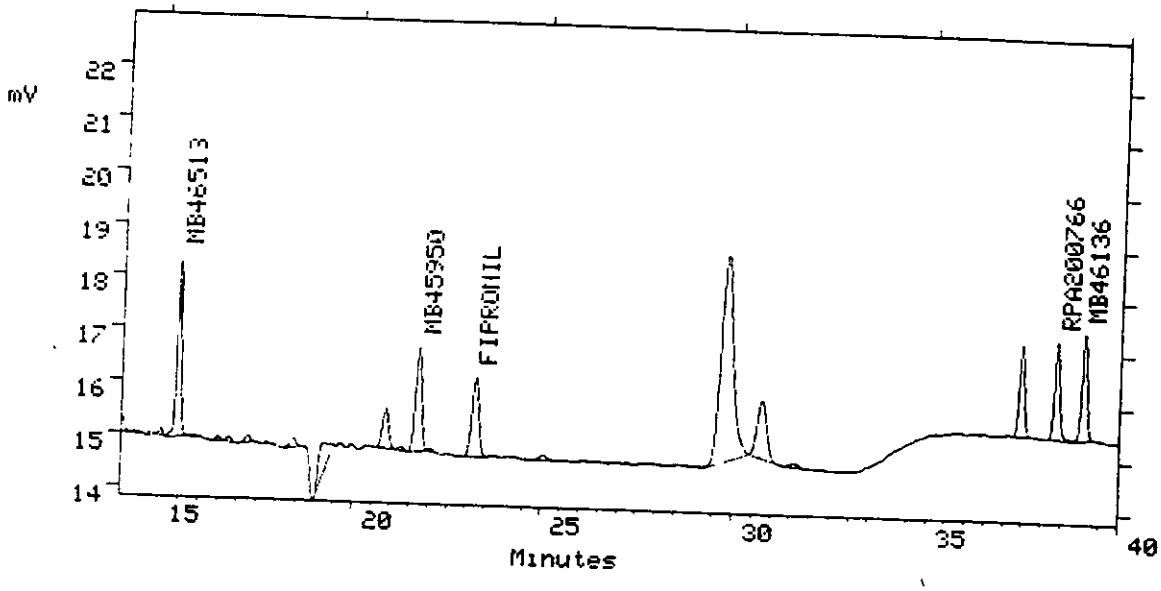
2. Potato Tuber, Fortified at 3 ppb Level



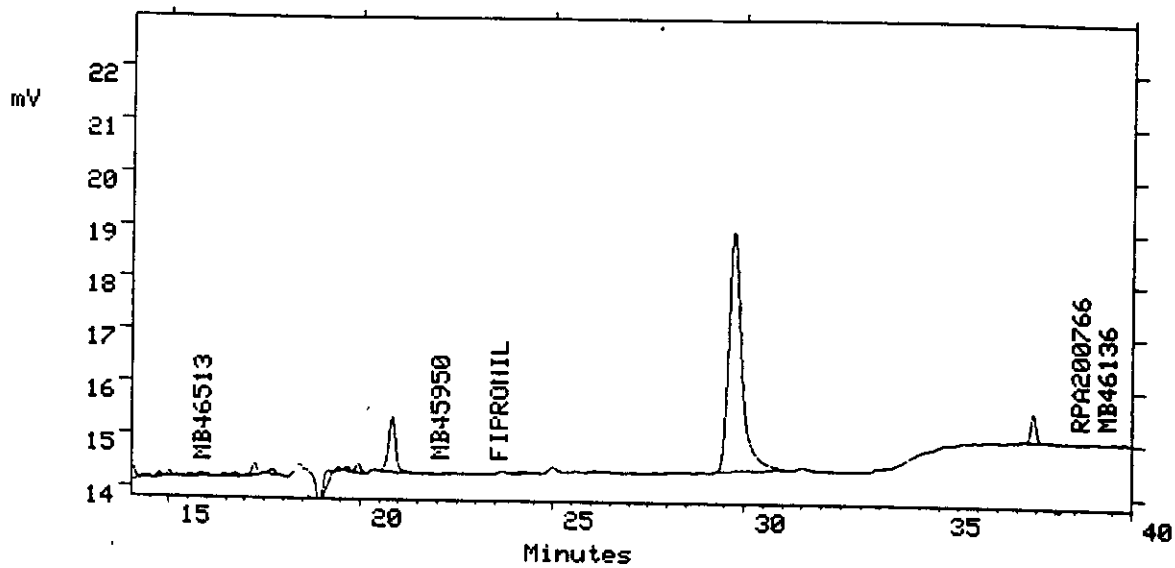
3. Wet Peel, Untreated Control



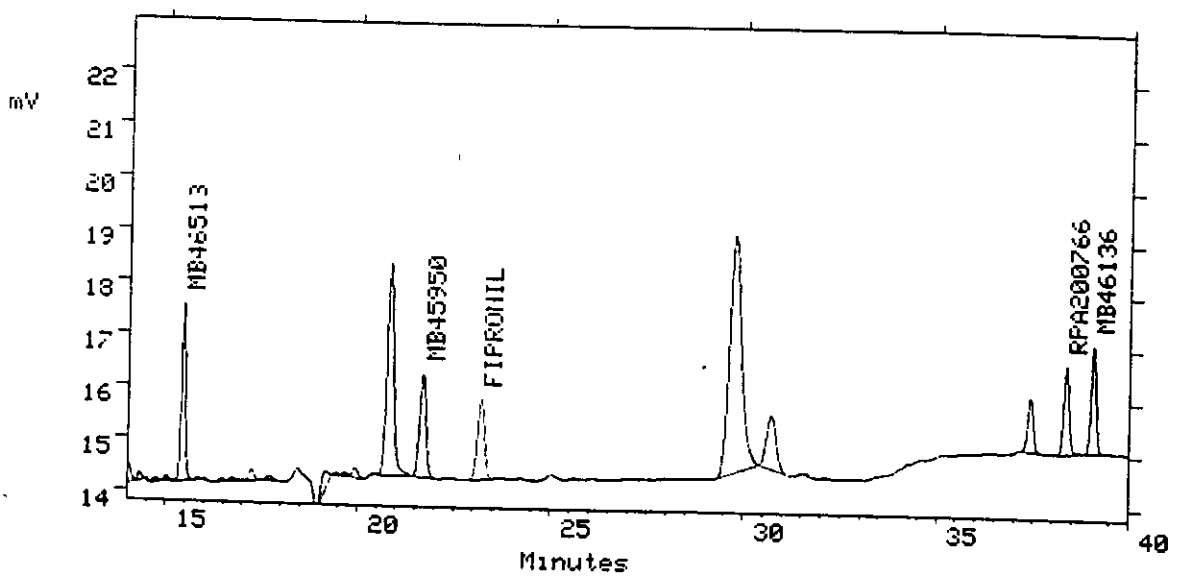
4. Wet Peel, Fortified at 3 ppb Level



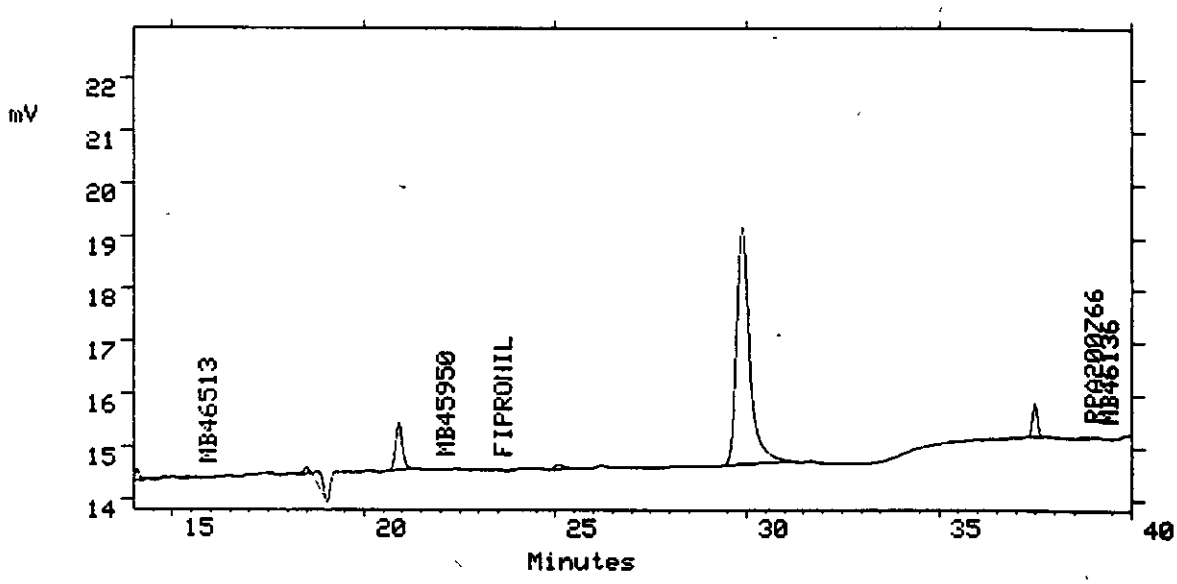
5. Dry Peel, Untreated Control



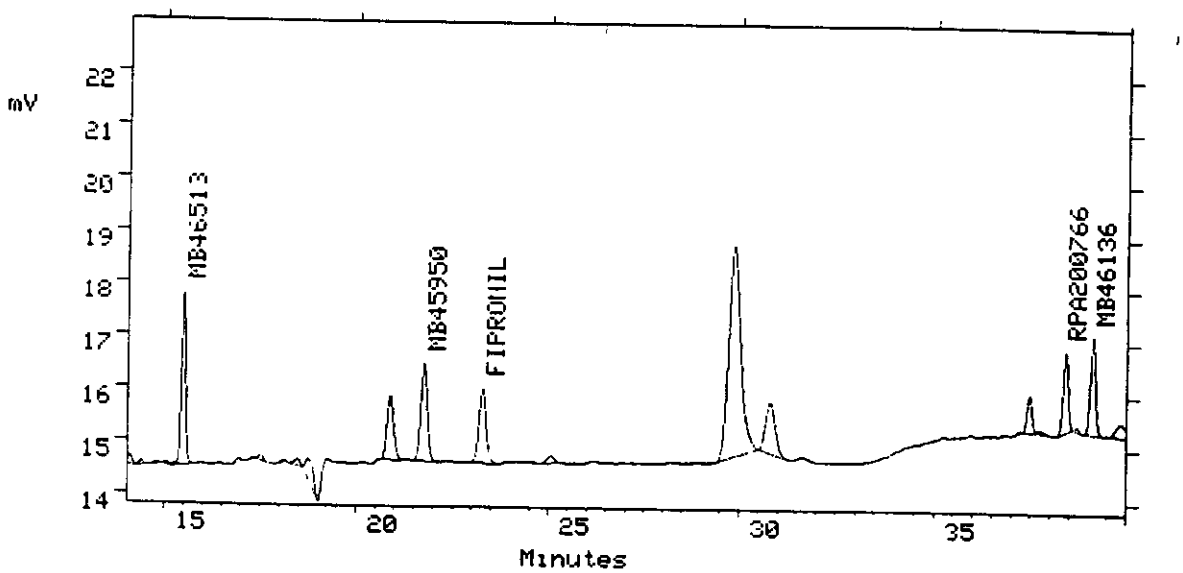
6. Dry Peel, Fortified at 3 ppb Level



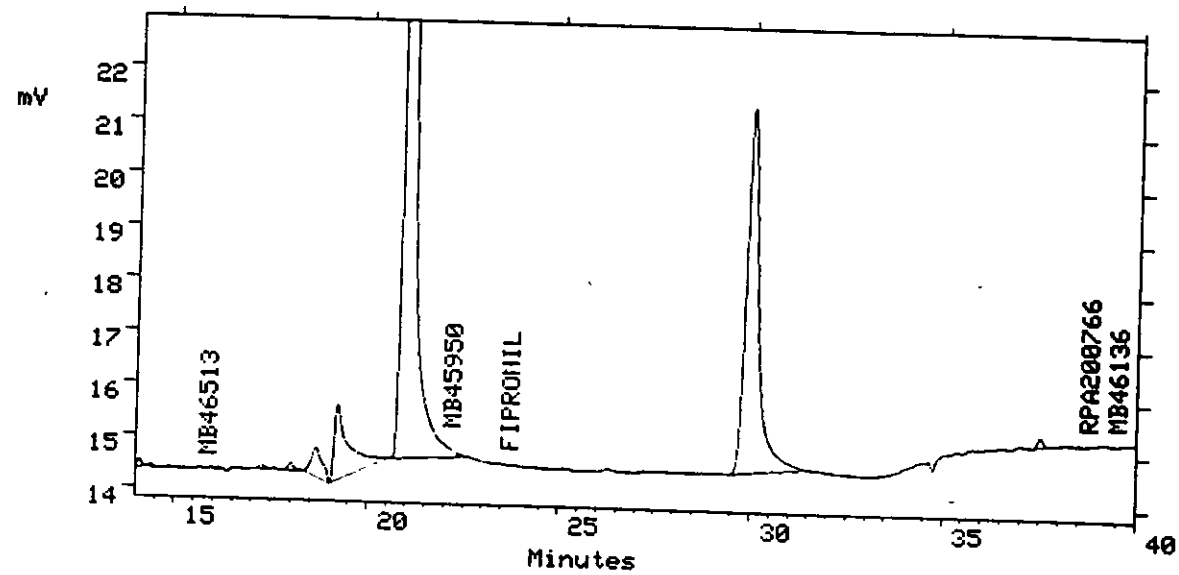
7. Potato Chips, Untreated Control



8. Potato Chips, Fortified at 3 ppb Level



9. Potato Flakes, Untreated Control



10. Potato Flakes, Fortified at 3 ppb Level