Cover Sheet for

Environmental Chemistry Method

Pesticide Name: Fenamidone (RPA 407213)

MRID#: 453858-17

Matrix: Soil

Analysis: LC/MS/MS

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Rhone-Poulenc Ag Company

Fenamidone (RPA 407213): Method of Analysis for RPA 407213 and Its Metabolites in Soil, Version 2

Environmental Chemistry

June 8, 1999

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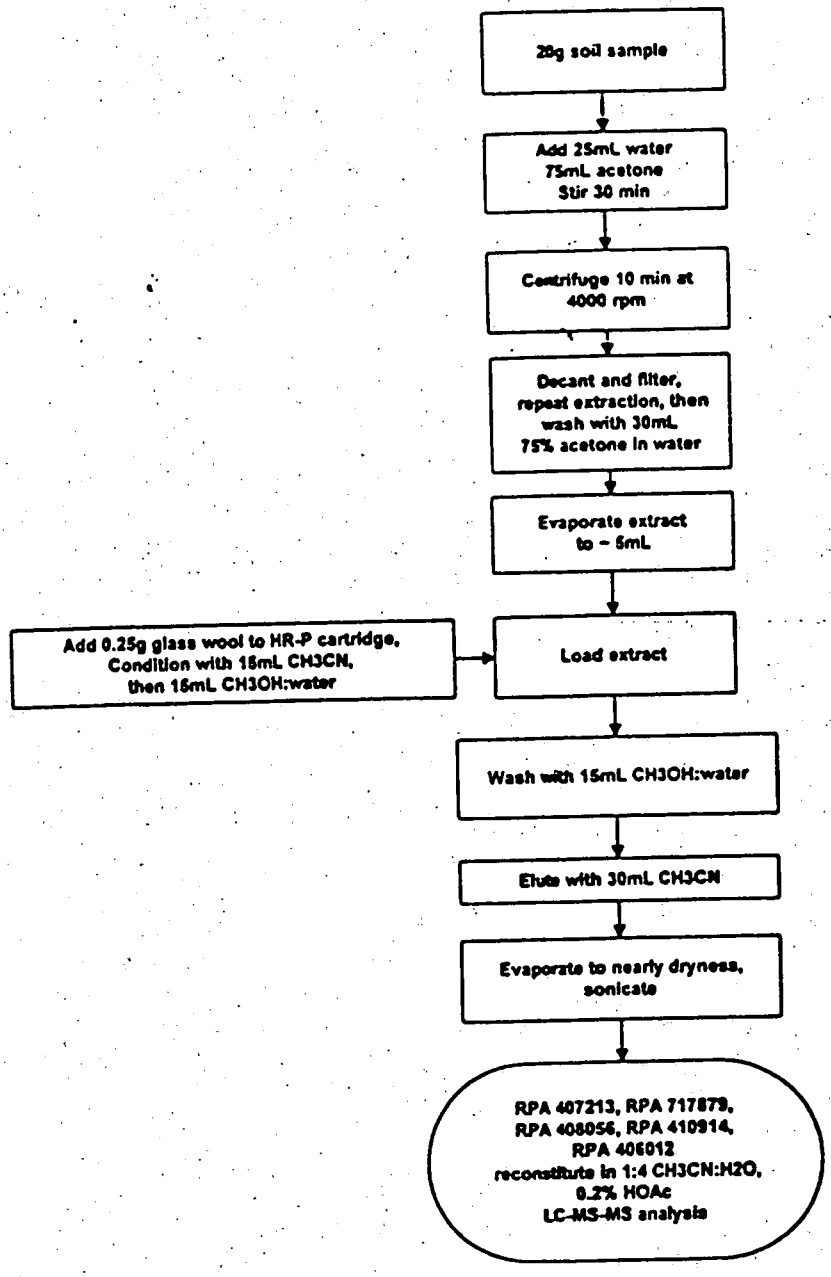
Summary Flowchart of Analytical Method



Fenamidone (RPA 407213) : Method of Analysis for RPA 407213 and Its Metabolites in Soil

I. INTRODUCTION

A. Scope

This method sets forth the procedure for determining the residues of RPA 407213 and its metabolites RPA 717879, RPA 408056, RPA 410914 and RPA 406012 in soil.

B. Principle

An analytical method is described for the determination of residues of RPA 407213 and its metabolites RPA 717879, RPA 408056, RPA 410914 and RPA 406012 in soil. Residues of RPA 407213, RPA 717879, RPA 408056, RPA 410914 and RPA 406012 are extracted from soil by stirring with acetone:water (3:1), and the extract centrifuged, decanted, and filtered. The extract is purified using a polystyrene-divinylbenzene polymer cartridge.

A reversed-phase C18 high performance liquid chromatography (HPLC) column is used to separate the compounds which are then quantified using the multiple reaction monitoring (MRM) mode. A turbo-ion interface is used to introduce the HPLC eluant into the mass spectrometer for analyte analysis. Quantitation is performed by daughter ion detection using liquid chromatography/mass spectrometry (LC/MS/MS) analysis. Quantification of results is based on a comparison of peak areas with those of known standards. The method has been verified at 10, 50 and 500 parts per billion (ppb) for RPA 407213, RPA 717879, RPA 408056, RPA 410914 and RPA 406012 by preparing and analyzing control and fortified samples of soil from Florida, California, North Dakota, and Washington.

C. Method Limits

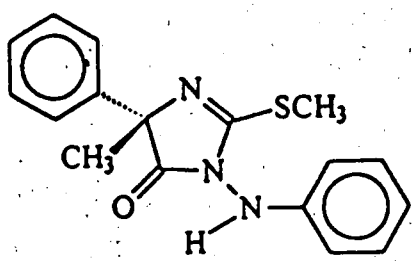
The minimum limits of detection (LOD) and limits of quantification (LOQ) for RPA 407213, RPA 717879, RPA 408056, RPA 410914 and RPA 406012 in each soil type have not been determined. This information will be obtained from the subsequent validation study. The target level for LOQ is 10 ppb for RPA 407213, RPA 717879, RPA 408056, RPA 410914 and RPA 406012.

Recoveries obtained during the field soil dissipation study were:

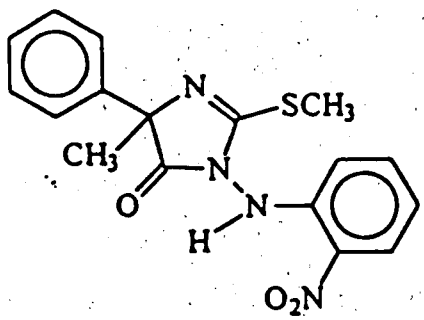
RPA 407213	86 %	RPA 410914	86 %
RPA 717879	110 %	RPA 406012	86 %
RPA 408056	100 %		

D. Chemical Structures

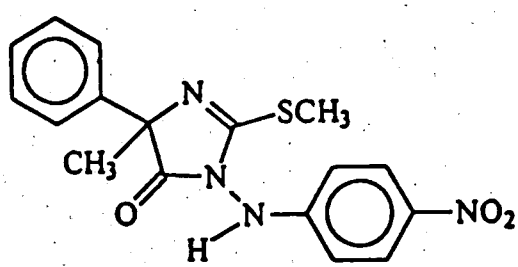
RPA 407213



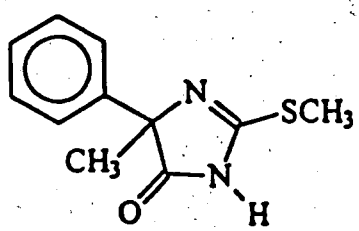
RPA 410914



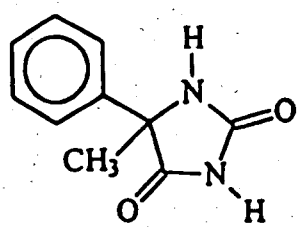
RPA 406012



RPA 408056



RPA 717879



II. MATERIALS

Unless otherwise noted, equivalent brands and/or suppliers can be used.

A. Reagents/Solvents

Acetic Acid Glacial (EM Science, Cat. No. AX0073-14)

Acetone Omni-Solv (EM Science, Cat. No. AX0116-1)

Acetonitrile Omni-Solv (EM Science, Cat. No. AX0142-1)

Methanol Omni-Solv (EM Science, Cat. No. MX0488-1)

Water (HPLC grade)

B. Equipment and Supplies

Adaptor, Bond Elut (Varian, Cat. No. 1213-1001)

Balance :
accuracy ± 0.1 mg (analytical standards) (Mettler AE 200 or equiv)
accuracy ± 0.1 g (samples and chemicals)(Mettler PC 4000 or equiv)

Bottles, amber, 4 oz. (Qorpak, No. 7919)

Bottles, wide mouth, polypropylene, 250mL (Nalge, Part No. 2105-0008)

Cartridges , Chromabond™ HR-P polystyrene-divinylbenzene (0.5 g)
(Machery Nagel, Cat. No. 730111, *no substitute, order from Bodman*)

Centrifuge (Marathon 10K)

Column, HPLC, Alltima C-18, 100mm x 4.6mm id., 5µm particle size
(Alltech, Cat. No. C-6000B, *custom order*)

Guard Column, HPLC, Alltima C18, 5 micron
(Alltech, Cat. No. 96361) optional

Centrifuge Tubes, Blue Max, disposable, polypropylene, 50mL
(Falcon, No. 2098)

Disposable pipettes

Filter paper, glass microfibre, grade GF/C, 7.0cm (Whatman, Cat. No. 1822 070)

Filter Adapter, neoprene crucible holder (VWR Cat. No. 24035-065)

Filter paper, grade 541, 70mm (Whatman, Cat. No. 1541 070)

Flasks, evaporation , flat-bottom, 500mL (Kimble, 25055-500)

Flasks, evaporation , flat-bottom, 125mL (Kimble, 25055-125)

Funnels, Buchner, 83mm (Coors, No. 60242)

Glass wool

Graduated cylinders

Hitachi AS2000 autosampler

Magnetic Stirrer (Corning, Model No. PC-410, Cat. No. 6795-410)

Perkin Elmer Sciex API III+ LC/MS/MS system coupled to an Hitachi L6200 HPLC pump via PE TurboIonSpray interface.

Perkin Elmer Sciex API 300 LC/MS/MS system coupled to an Hewlett Packard HP1100 HPLC via PE Turbo IonSpray interface.

Pipette bulb

Reservoir, empty, 70mL (Varian, Cat. No. 1213-1018)

Rotary vacuum evaporator (Buchi R-124)

SPE cartridge adaptors (University Research Glass, Part No. URG-2440-SPECA)

Septa, 8mm T/S, slit (Sun, 500-870)

Solvent jugs, 4 L brown glass

Stir bars, magnetic, 50.8 x 9.5mm (VWR, Cat. No. 58949-130)

Stopcocks, Luer Lock (Varian, 1213-1005)

Stoppers, glass, 24/40

Syringe, disposable, 3mL (Becton-Dickinson, Cat. No. BD309586)

Syringe filter, Nylon Acrodisc® 13mm, 0.45µm (Gelman, No. 4426)

Traps, rotary evaporator

Ultrasonic bath (Bransonic 52)

Vacuum manifold system for cartridge elution

Vacuum Gauges

Vacuum Pump, Duo Seal (Welch, Model No. 1400)

Varian Vac Elut SPS 24 Vacuum Manifold (Varian, Model 1223-4004)

Vials, clear, 1.5mL (Sun, 200-250)

Vial caps (Sun, 200-292)

Volumetric flasks

Volumetric pipettes

Vortex®-Genie Mixer (Scientific Industries, Model No. K-550-G)

C. Solutions

The following is a list of the solutions used in the analyses of soil. Example procedures for the preparation of each solution are also provided. *Note that the reagent water used in the preparations should be HPLC grade.*

1. Solution of 75 % Acetone in Water
   Using a 1 liter graduated cylinder, transfer ~750 mL of acetone and ~250 mL of water to a 4 L brown glass jug. Mix by shaking. Repeat until the desired quantity has been made.

2. Solution of 50% Methanol in Water
   Using a 1 liter graduated cylinder, transfer ~1000 mL of methanol and ~1000 mL of water to a 4 L brown glass jug. Mix by shaking. Repeat until the desired quantity has been made.

3. Solution of 0.2 % Acetic Acid in Water
   Using a 1 liter graduated cylinder, transfer ~998 mL of water to a 1 L HPLC solvent reservoir. Add ~2 mL acetic acid. Mix by shaking.

4. Solution of 20% Acetonitrile in Water, 0.2 % Acetic Acid
   Using a 1 liter graduated cylinder, transfer ~200 mL of acetonitrile and ~800 mL of 0.2 % acetic acid in water to a 4 L brown glass jug. Mix by shaking. Repeat until the desired quantity has been made.

5. Solution of 20% Acetonitrile in Water
   Using a 1 liter graduated cylinder, transfer ~200 mL of acetonitrile and ~800 mL of water to a 4 L brown glass jug. Mix by shaking. Repeat until the desired quantity has been made.

D. Analytical Standards

Common name/alias: fenamidone/ RPA 407213

Chemical name: (+)-(4S)-4-methyl-2-methylthio-4-phenyl-(1H)-1-phenylamino-2-imidazolin-5-one
(CAS No. 161326-34-7)

Solubility [1]:

acetone:	250 (unit : g/L)
acetonitrile:	86
dichloromethane:	330
methanol:	43
toluene:	40
water:	0.0078

Common name/alias: RPA 408056

Chemical name: 4-methyl-2-methylthio-4-phenyl-2-imidazolin-5-one

Common name/alias: RPA 717879

Chemical name: 4-methyl-4-phenylimidazolidin-2,5-dione

Common name/alias: RPA 410914

Chemical name: (4RS)-4-methyl-2-methylthio-(1H)-1-(2-nitrophenylamino)-4-phenyl-2-imidazolin-5-one

Common name/alias: RPA 406012

Chemical name: (4RS)-4-methyl-2-methylthio-(1H)-1-(4-nitrophenylamino)-4-phenyl-2-imidazolin-5-one

III. FORTIFICATION AND CALIBRATION STANDARD SOLUTIONS

A. Preparation

All the standard solutions must be stored in amber glass bottles, at or below 10°C when not in use. Solutions should be allowed to warm to room temperature prior to use. The following is an example of a procedure to follow in preparing standard solutions. Alternate or additional standards of appropriate weight and volume may be prepared as needed. The "~" symbol indicates approximately. RPA 717879 is kept as a solution separate from the other compounds.

1. Weigh ~0.0200g (corrected for purity) each of RPA 407213, RPA 408056, RPA 717879, RPA 410914, and RPA 406012 into separate 100-mL volumetric flasks and dilute to the marks with acetonitrile. Cap and mix by inversion. The concentration of these stock standards is ~200 µg/mL.

2. a. For the preparation of fortification standards of RPA 407213, RPA 408056, RPA 410914, and RPA 406012, transfer 10 mL of each of the ~200 µg/mL standard solutions, via volumetric class "A" pipettes, to one 100 mL volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~20 µg/ mL RPA 407213, RPA 408056, RPA 410914, and RPA 406012.

   b. For the preparation of the fortification standard for RPA 717879 transfer 10 mL of the ~200 µg/mL standard solution, via a volumetric class "A" pipette, to a 100 mL volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this standard is ~20 µg/ mL RPA 717879.

3. a. Using a class "A" volumetric pipette, transfer 10 mL of the mixed standard (step III.A.2a.) to a 100-mL volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this mixed standard is ~2 µg/mL RPA 407213, RPA 408056, RPA 410914, and RPA 406012.

   b. Using a class "A" volumetric pipette, transfer 10 mL of the standard (step III.A.2b.) to a 100-mL volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this standard is ~2 µg/mL RPA 717879.

4. a. Using a class "A" volumetric pipette, transfer 1 mL of the mixed standard (step III.A.3a.) to a 100-mL volumetric flask. Dilute to mark with 20% acetonitrile in water. Cap and mix by inversion. The concentration of this mixed standard is ~0.02 µg/mL RPA 407213, RPA 408056, RPA 410914, and RPA 406012.

   b. Using a class "A" volumetric pipette, transfer 5 mL of the standard (step III.A.3b.) to a 100-mL volumetric flask. Dilute to mark with 20% acetonitrile in water. Cap and mix by inversion. The concentration of this mixed standard is ~0.1 µg/mL RPA 717879.

5. a. For the preparation of calibration standards of RPA 407213, RPA 408056, RPA 410914, and RPA 406012 perform the following dilutions using the ~0.02 µg/mL mixed standard (step III.A.4a) :

mL of the ~0.02µg/mL mixed standard	Added to mL 20% acetonitrile in water	Concentration µg/mL
1.5	100	0.0003
3.0	100	0.0006
5.0	100	0.001
10	100	0.002
15	100	0.003

b. For the preparation of calibration standards of RPA 717879 perform the following dilutions using the ~0.1 µg/mL standard (step III.A.4b) :

mL of the ~0.1 µg/mL standard	Added to mL 20% acetonitrile in water	Concentration µg/mL
4.0	100	0.004
6.0	100	0.006
10	100	0.010
15	100	0.015

B. Stability

1. To evaluate the stability, the following formula has been used :

   percent stability = [1-(old std. soln. / new std. soln.)] x 100

   The old standard solution should give detector responses within 10% of those of the new standard solution in order for the given standard solution to be considered stable under the storage conditions.

2. Stock solutions: Each product prepared in acetonitrile and stored at 4°C ± 3°C was stable for up to 4 months[1].

3. The storage of solutions of less than 20 µg/mL should be no longer than 30 days. These solutions may be replaced earlier if deemed necessary.

IV. METHOD PROCEDURES

A. General Notes

A1. The "♦"symbol indicates an optional stopping point after completing the indicated step. Samples may be stored overnight in a refrigerator (at or below 10°C).

A2. The "~" symbol indicates 'approximately.'

A3. Conditioning of the cartridges in step B13 can be started earlier and does not have to be done after the completion of steps B1-B11. However, the cartridges should be used the day of conditioning.

A4. *A flow rate of ~2 mL/min is critical and should be maintained throughout the conditioning and elution process (unless otherwise specified). A faster flow rate will result in low recoveries. Cartridges should not be allowed to run dry.*

B. Soil

(Analysis for RPA 407213, RPA 408056, RPA 717879, RPA 410914, and RPA 406012)

♦ B1. Weigh ~20 g of sample into a 250 mL Nalgene® bottle. The sample may be stored in a refrigerator until needed.

B2. For recoveries, fortify the sample with the appropriate standard solution.

B3. Add ~100 mL of 75% acetone in water, add a stir bar and place on a magnetic stirrer for ~30 minutes.

B4. Remove the stir bar. Centrifuge at ~4000 rpm for ~10 minutes.

B5. Decant and vacuum filter the supernatant through a 541 filter (top) and two GF/C filters into a tared 500mL evaporation flask. Wet filter paper with water prior to filtering in order to hold it down.

B6. Add ~100 mL of 75% acetone in water to the sample bottle, add the stir bar and place on a magnetic stirrer for ~30 minutes.

B7. Remove the stir bar. Centrifuge at ~4000 rpm for ~10 minutes.

B8. Decant and vacuum filter the supernatant through a 541 filter (top) and two GF/C filters into a tared 500mL evaporation flask. Wet filter paper with water prior to filtering in order to hold it down.

B9. Scrape the soil from the bottom of the Nalgene® bottle with a spatula and transfer it to the filter. Wash the bottle twice with 15mL of 75% acetone in water and transfer it to the filter.

B10. Evaporate the extract to ~5 mL using a rotary evaporator with a <40°C water bath, then hold under a stream of Nitrogen for ~1 min. and then add 5 mL of water and sonicate for ~30 seconds. *The acetone must be removed completely.This is a critical step.*

B11. Transfer the extract to a 50mL centrifuge tube and centrifuge at ~2500 rpm for 10 minutes.

B12. Immediately set-up an HR-P cartridge and a stopcock on a purification system connected to a vacuum. Insert a plug of ~0.25 g of glass wool into the cartridge. Place a cartridge adaptor and a reservoir on top of the cartridge.

B13. Condition the cartridge with ~15 ml of acetonitrile followed by ~15 ml of 50% methanol in water. (~1 drop/2 sec, ~2 mL/min. Do not allow the cartridge to dry).

B14. Decant the extract onto the cartridge (~1 drop/2 sec, ~2mL/min). Elute (~1 drop/2 sec, ~2 mL/min. Do not allow the cartridge to dry) and discard the effluent.

B15. Add ~15mL of 50% methanol in water to the evaporation flask as a rinse and transfer the contents to the same centrifuge tube. Vortex or mix the centrifuge tube thoroughly. Centrifuge at ~2500 rpm for 10 minutes and decant onto the cartridge. Elute (~1 drop/2 sec, ~2 mL/min. Do not allow the cartridge to dry) and discard the effluent.

B16. Set-up a 125 ml evaporation flask. Add ~30 mL of acetonitrile to the evaporation flask as a rinse and transfer the contents onto the cartridge. Elute the compounds of interest from the cartridge (~1 drop/2 sec, ~2 mL/min. Do not allow the cartridge to dry).

B17. Rotary evaporate to nearly dryness using a water bath temperature of ~40°C and a vacuum of ~27 in. Hg. As solvent evaporates from the flask, adjust the level of the flask in the water bath so that only the solution is being heated.

♦ B18. Add an appropriate amount of 20% acetonitrile in water, 0.2% acetic acid to each flask to dissolve the residues and sonicate for ~ 2 minutes. Suggested dilution volumes are 20mL for analysis of RPA 717879 at the proposed LOQ of 10ppb. Filter a portion of the 20mL extract to be used for the analysis of RPA 717879 only through a Nylon Acrodisc® 13mm, 0.45 µm syringe filter. To analyze for the other compounds, a 1mL aliquot of the unfiltered 20mL extract is transferred to a 10mL volumetric flask and diluted to the mark with 20% acetonitrile in water, 0.2% acetic acid. The samples are ready for LC/MS/MS analysis.

V. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY-MASS SPECTROMETRY (LC/MS/MS)

A. Conditions

Instrument used: Perkin Elmer Sciex API III+ LC/MS/MS system
Hitachi L6200 HPLC pump
PE Turbo IonSpray Electrospray Interface.
Hitachi AS2000 autosampler

Ionization: Electrospray (TurboIonSpray), positive ion mode

Curtain gas flow: Nitrogen at ~1.2 L/min

Nebulizer pressure: 55 psi

Turbo IonSpray Settings: Heated air at ~4.75 L/min, 500°C

MS Mode: MS/MS with multiple reaction monitoring (MRM)

IonSpray / Orifice voltage: 5500V / 65 V

Collision gas: Argon at approximately 275 x 10^{13} atoms/cm^2

Collision energy (R2-R0): 13V - 30V= -17V

Mass Transitions:

717879:	191/120
408056:	221/120
407213:	312/236
406012:	357/120
410914:	357/120

Column: Alltech Altima C18 5u, 100x4.6mm

Mobile phase flow rate: 1.0 mL/min split to ~200µL/min

Mobile phase composition:

A=acetonitrile
B=1.0% acetic acid in HPLC grade water
Gradient program (dwell volume < ~1.5mL)

Time (min)	%A	%B
0.0	30.0	(100-A)
3.0	100.0	
3.5	100.0	
3.6	30.0	

~11 min between injections

Injection volume: 75 µL

B. Alternate Conditions

Instrument used: Perkin Elmer Sciex API 300 LC/MS/MS system
Hewlett Packard HP1100 QuatPump and Vacuum Degasser
PE Turbo IonSpray Electrospray Interface.
Hewlett Packard HP1100 Autosampler

Ionization: Electrospray (TurboIonSpray), positive ion mode

Curtain gas flow: Nitrogen at ~1.08 L/min

Nebulizer gas flow: Nitrogen at ~1.31 L/min

Turbo IonSpray Settings: Heated Nitrogen at ~7 L/min, 450°C

MS Mode: MS/MS with multiple reaction monitoring (MRM)

IonSpray / Orifice voltage: 5200V / 30 V

Collision gas: Nitrogen at approximately 0.82 L/min

Collision energy (R2-R0): 25V - 9V= -16V

Mass Transitions:

717879:	191/120
408056:	221/120
407213:	312/236
406012:	357/120
410914:	357/120

Column: Alltech Alltima C18 5u, 100x4.6mm
Guard Column : Alltech Alltima C18 5 micron

Mobile phase flow rate: 1.0 mL/min split to ~200µL/min
Mobile phase composition:

A=acetonitrile
B=0.2% acetic acid in HPLC grade water

Gradient program

Time (min)	%A	%B
0.0	30.0	(100-A)
1.0	30.0	
3.0	100.0	
4.0	100.0	
8.0	30.0	
9.0	STOP	

~11 min between injections

Injection volume: 100 µL

Note the indicated LC/MS/MS parameters are guidelines and should be optimized for the instrument and column actually used. Instrument parameters and mobile phase compositions may be adjusted to improve separation from interfering peaks.

APPROXIMATE RETENTION TIMES

RPA 717879	2.25 minutes
RPA 408056	3.14 minutes
RPA 406012	5.16 minutes
RPA 407213	5.21 minutes
RPA 410914	5.27 minutes

Retention times may vary from those presented above.

Example chromatograms are attached (see section X). Note that the retention times may vary from system to system.

C. Performance Criteria

First criterion:
Run a standard solution corresponding to a level at or below the estimated LOQ and obtain a signal to noise ratio of at least 9:1.

If this criterion cannot be met, optimize instrument operating parameters or change instrument method parameters such as split ratio or injection size until a signal to noise ratio of 9:1 is obtained.

If this criterion still cannot be met by changing operating parameters, run higher level standards until a signal to noise ratio of 9:1 is obtained. This will require adjusting the method final sample dilution such that this standard level corresponds to the required LOQ.

Second criterion:
Run a set of standards of four or more concentration levels, from at or below the LOQ, up to the highest concentration level to be included in the analysis. Generate a calibration curve for each analyte and obtain a linear regression with a correlation coefficient of at least 0.90 for each analyte. If this criterion is met, the samples may be run with standards interspersed. Do not use any sample run data if the combined regression for standards run immediately before, during and after the samples do not meet this criterion.

Note:
To stabilize the response of the instrument, it has been found useful to run at least one standard and three or more sample or untreated control solutions as "wake up" runs before the actual runs to be used in calculations are commenced.

VI. CALCULATIONS

Linear regression should be used to generate calibration curves for RPA 407213, RPA 717879, RPA 408056, RPA 406012 and RPA 410914. After the instrument performance criteria are met, a minimum of four standards over a range of concentration levels should be included with a set of samples. Standards should be interspersed with samples to compensate for any minor change in instrument response. Samples should be diluted such that any peak areas or heights are within the area or height range between the lowest and highest standards injected.

Linear regression coefficients should be calculated on standard concentration (ng/mL) versus peak area or height. The data from the analytical standards should then be fit to the linear model,

$$Y = A + BX.$$

The equation to be used to estimate the residues in the samples is:

$$E = \frac{(Y - A)}{B} \cdot \frac{C}{D}$$

where: Y = response of analyte of interest (peak area or height)
A = intercept from linear regression analysis (peak area or height)
B = slope from linear regression analysis (response per concentration)
C = final sample volume (mL)
D = starting weight in grams of sample in final volume (g)
E = concentration of analyte in sample in parts per billion (ppb or ng/mL)

VII. SAFETY

All available appropriate Material Safety Data Sheets should be available to the study personnel during the conduct of the study. General laboratory safety precautions should be taken. This method does not present any specific risks.

VIII. REFERENCES

1. "RPA 407213 and its metabolites: Analytical method for the determination of residues in soil" AR 138-96 F. Martial, B. Simonin, C. Venet, June 27, 1997.

IX. RECOVERY DATA

A. Verification Recovery Data

1. Florida Soil

Sample Identification	Fortification Level* (ppb)	Recovery (%)				
		RPA 407213	RPA 717879	RPA 408056	RPA 406012	RPA 410914
RA01541	UTC	ND	ND	ND	ND	ND
RA01541	UTC	ND	ND	ND	ND	ND
RA01541	10	84	104	100	80	88
RA01541	10	80	111	97	75	92
RA01541	50	88	109	102	85	82
RA01541	50	85	114	98	74	77
RA01541	500	87	100	105	84	93
RA01541	500	91	95	106	96	100

2. California Soil

Sample Identification	Fortification Level* (ppb)	Recovery (%)				
		RPA 407213	RPA 717879	RPA 408056	RPA 406012	RPA 410914
RA02130	UTC	ND	ND	ND	ND	ND
RA02130	UTC	ND	ND	ND	ND	ND
RA02130	10	83	120	88	78	77
RA02130	10	71	117	94	76	70
RA02130	50	91	117	109	100	94
RA02130	50	86	110	95	82	91
RA02130	500	87	120	111	100	95
RA02130	500	87	119	106	80	100

3. North Dakota Soil

Sample Identification	Fortification Level* (ppb)	Recovery (%)				
		RPA 407213	RPA 717879	RPA 408056	RPA 406012	RPA 410914
RT00223	UTC	ND	ND	ND	ND	ND
RT00223	UTC	ND	ND	ND	ND	ND
RT00223	10	79	95	87	92	76
RT00223	10	87	105	91	93	79
RT00223	50	94	108	90	93	91
RT00223	50	89	106	79	86	81
RT00223	500	98	115	85	97	101
RT00223	500	101	104	92	108	109

*RPA407213, RPA717879, RPA408056, RPA 406012 and RPA 410914

ND = Non Detect

4. Washington Soil

Sample Identification	Fortification Level* (ppb)	Recovery (%)				
		RPA 407213	RPA 717879	RPA 408056	RPA 406012	RPA 410914
RT00812	UTC	ND	ND	ND	ND	ND
RT00812	UTC	ND	ND	ND	ND	ND
RT00812	10	73	99	98	80	73
RT00812	10	76	124	99	79	75
RT00812	50	88	114	107	81	82
RT00812	50	81	113	112	83	78
RT00812	500	82	107	120	83	70
RT00812	500	90	108	118	85	80

*RPA407213, RPA717879, RPA408056, RPA 406012 and RPA 410914

ND = Non Detect

5. Summary Table

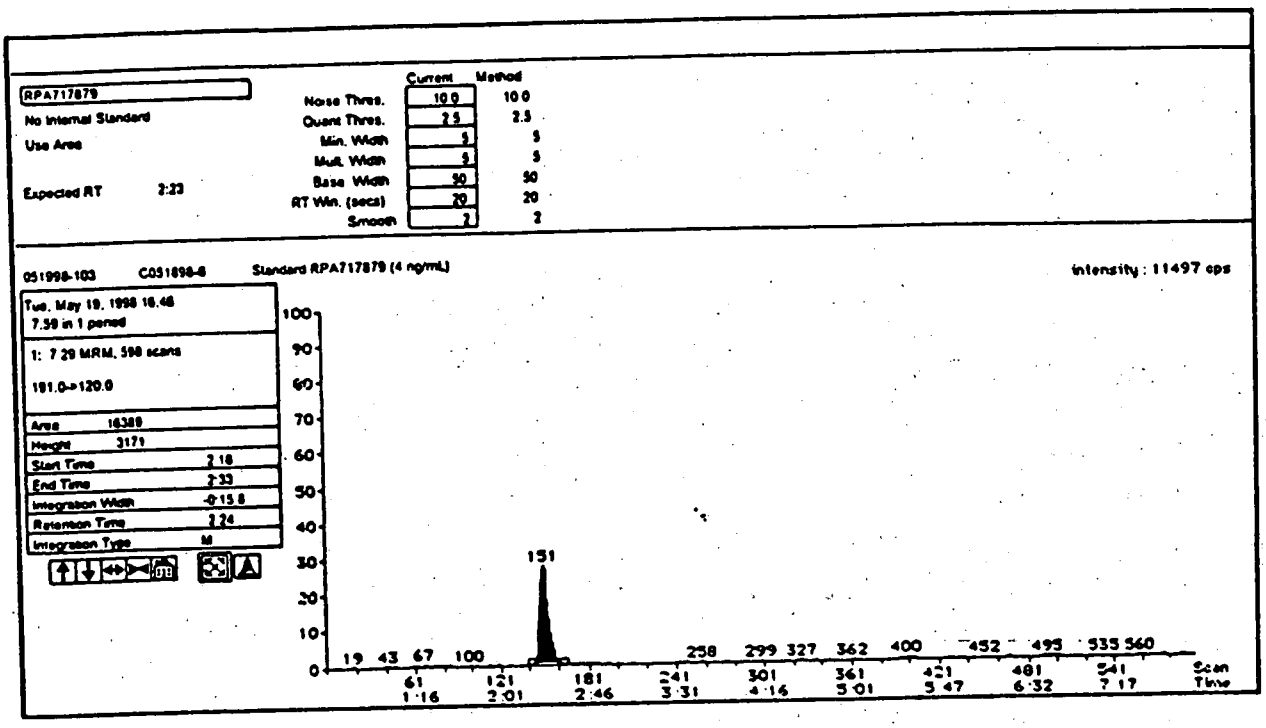
	RPA 407213	RPA 717879	RPA 408056	RPA 406012	RPA 410914
Mean LOQ Recovery (%)*	79	109	94	82	79
Standard Deviation at LOQ*	5.5	10.4	5.1	6.9	7.5
Mean Overall Recovery (%)**	86	110	100	86	86
Standard Deviation Overall**	7.1	7.9	10.5	9.0	10.8

* LOQ = 10 ppb level from Florida, California, North Dakota, and Washington.

** Overall = 10, 50, and 500 ppb levels from Florida, California, North Dakota, and Washington.

X. EXAMPLE CHROMATOGRAMS (P.E. Sciex API 300)

Figure 1. Standard: 4.0 and 6.0 ng/ml - RPA 717879



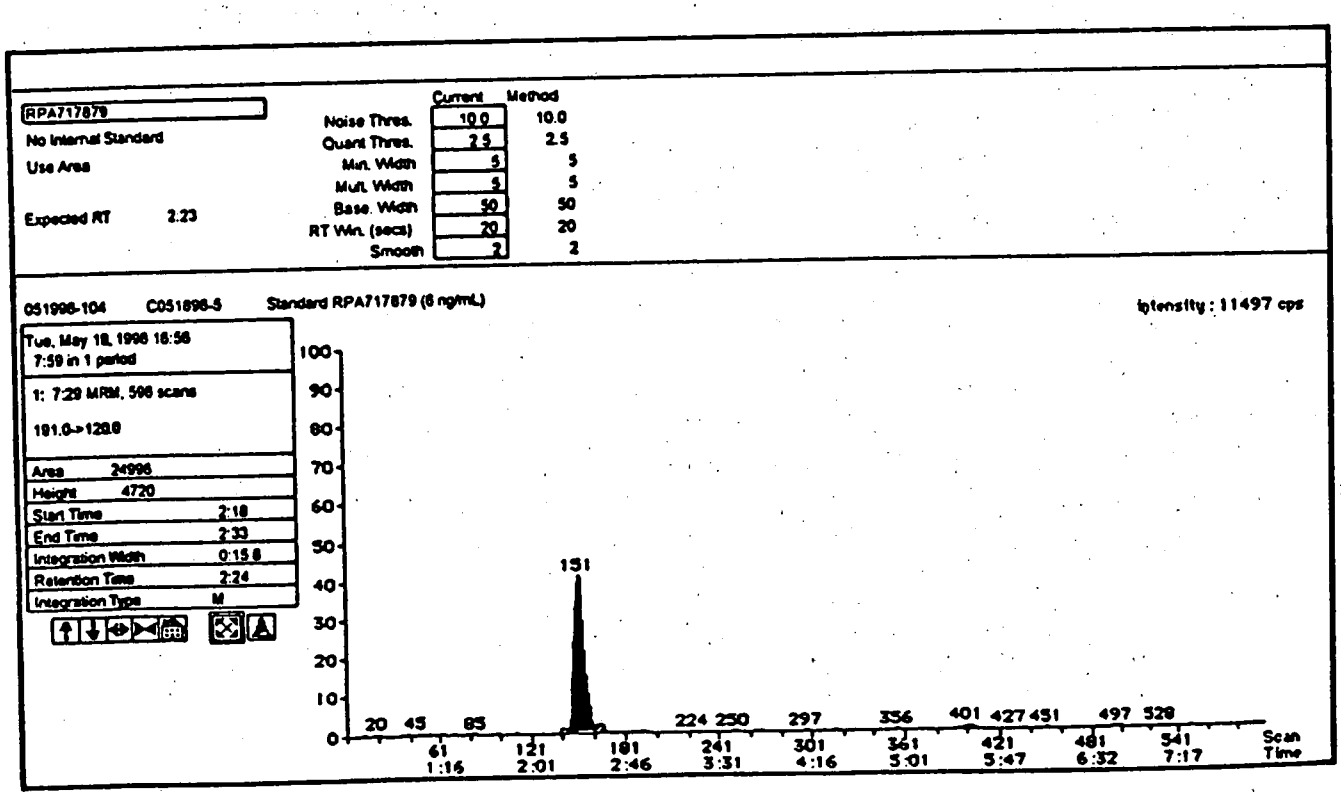
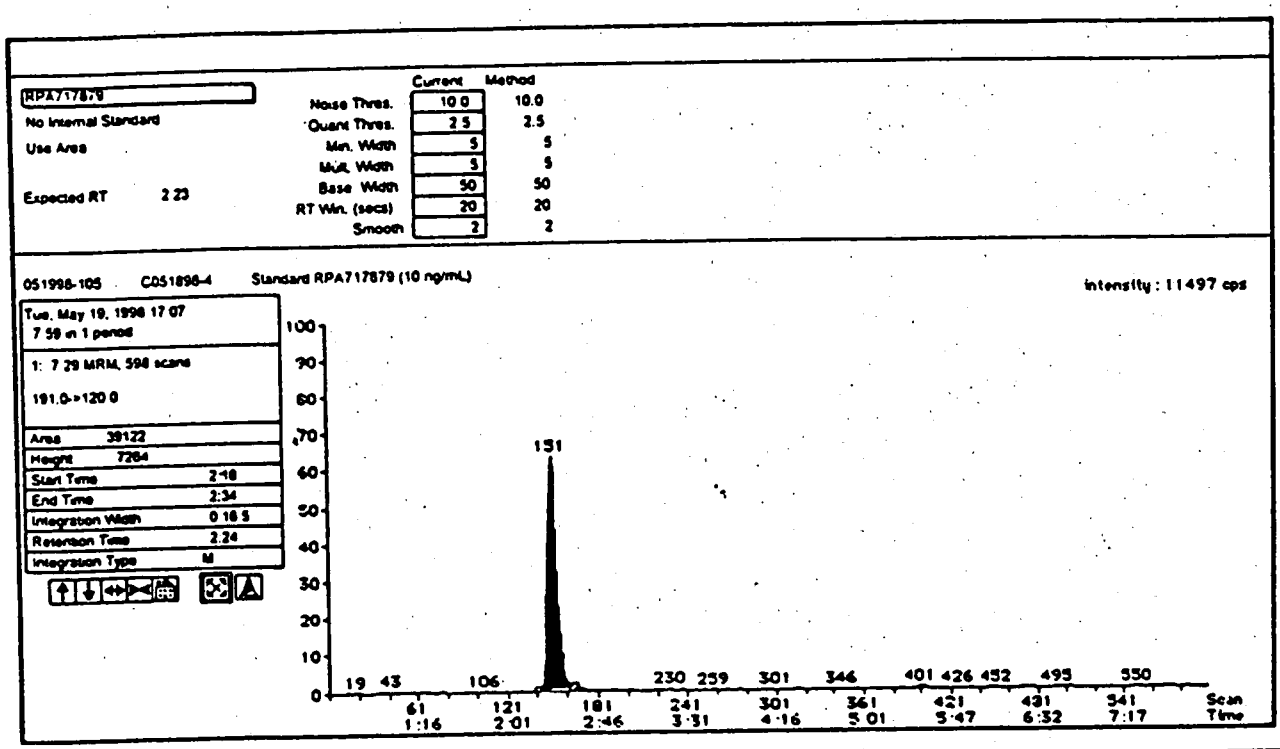


Figure 2. Standard: 10.0 and 15.0 ng/ml - RPA 717879



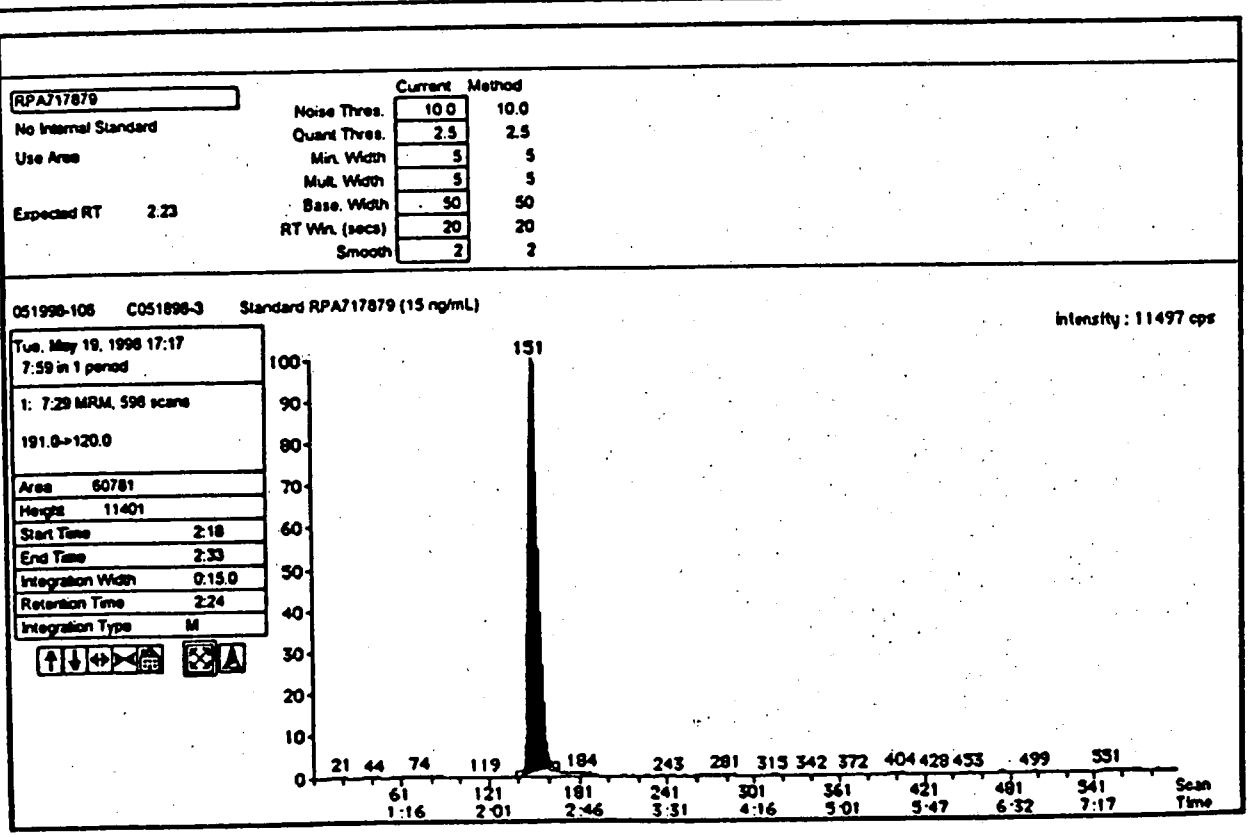
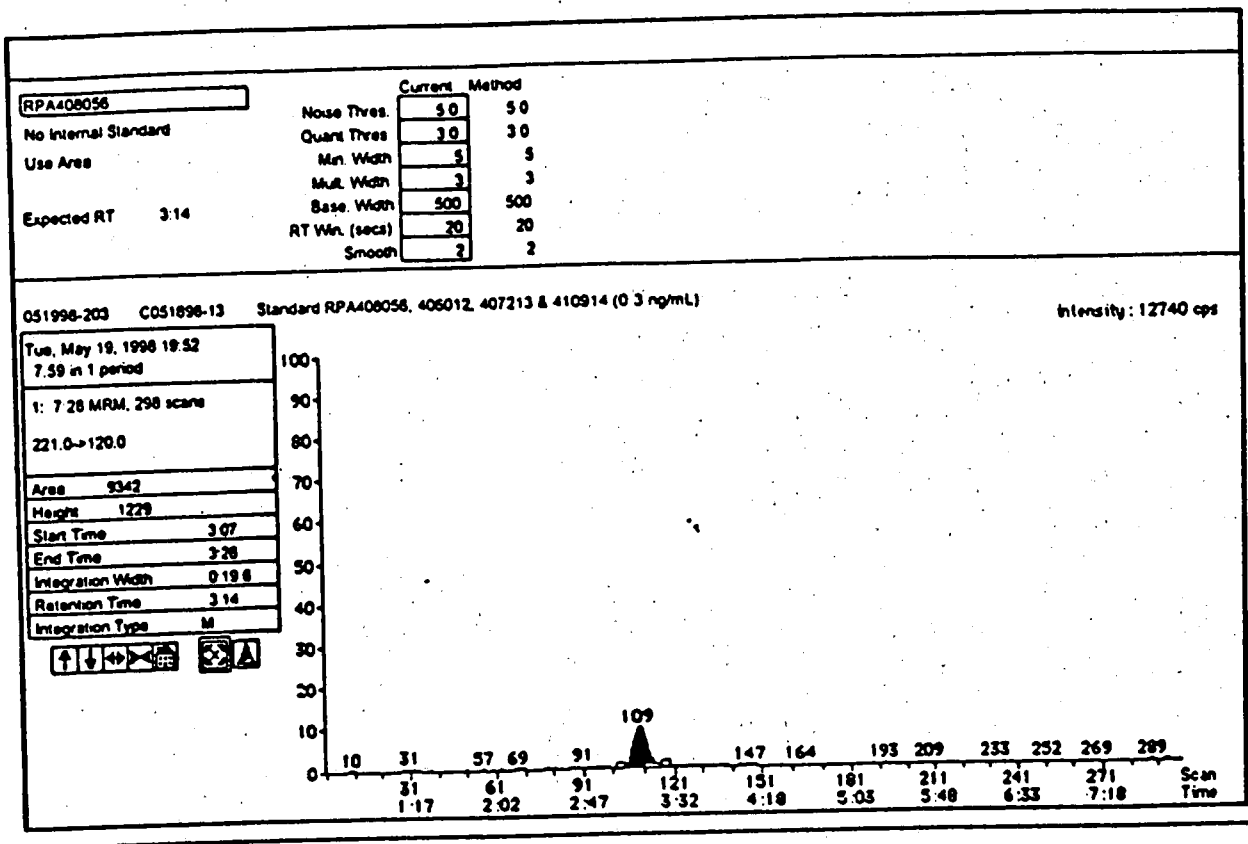


Figure 3. Standard: 0.3 and 0.6 ng/ml - RPA 408056



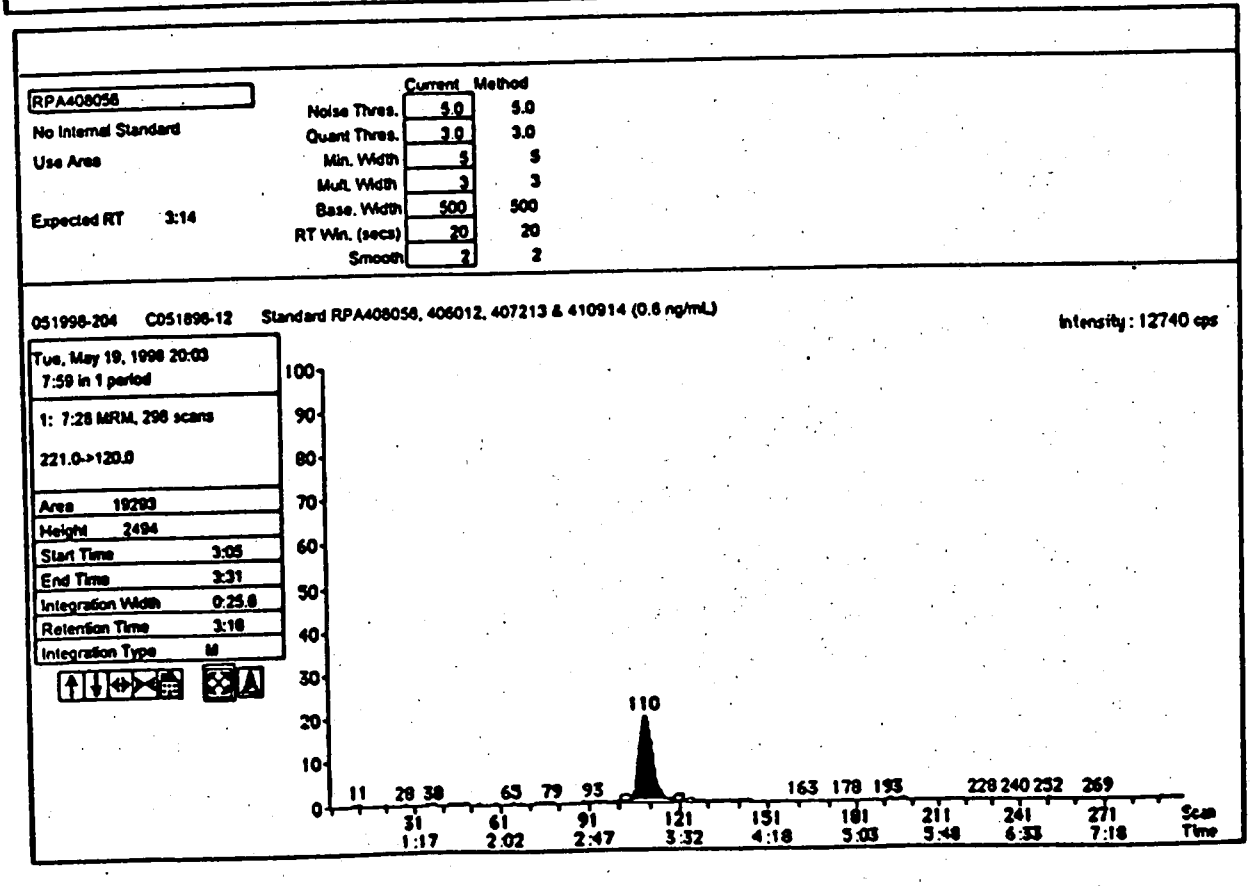
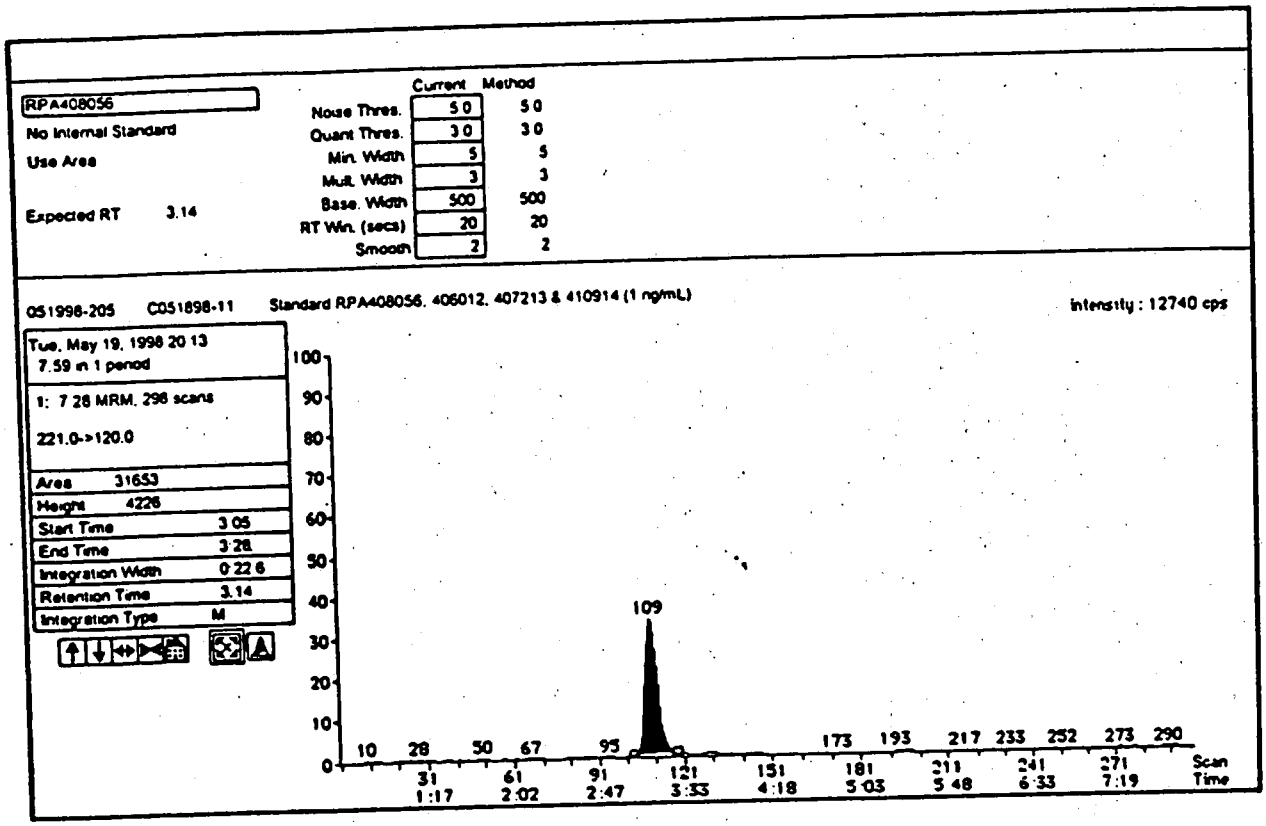


Figure 4. Standard: 1.0 and 2.0 ng/ml - RPA 408056



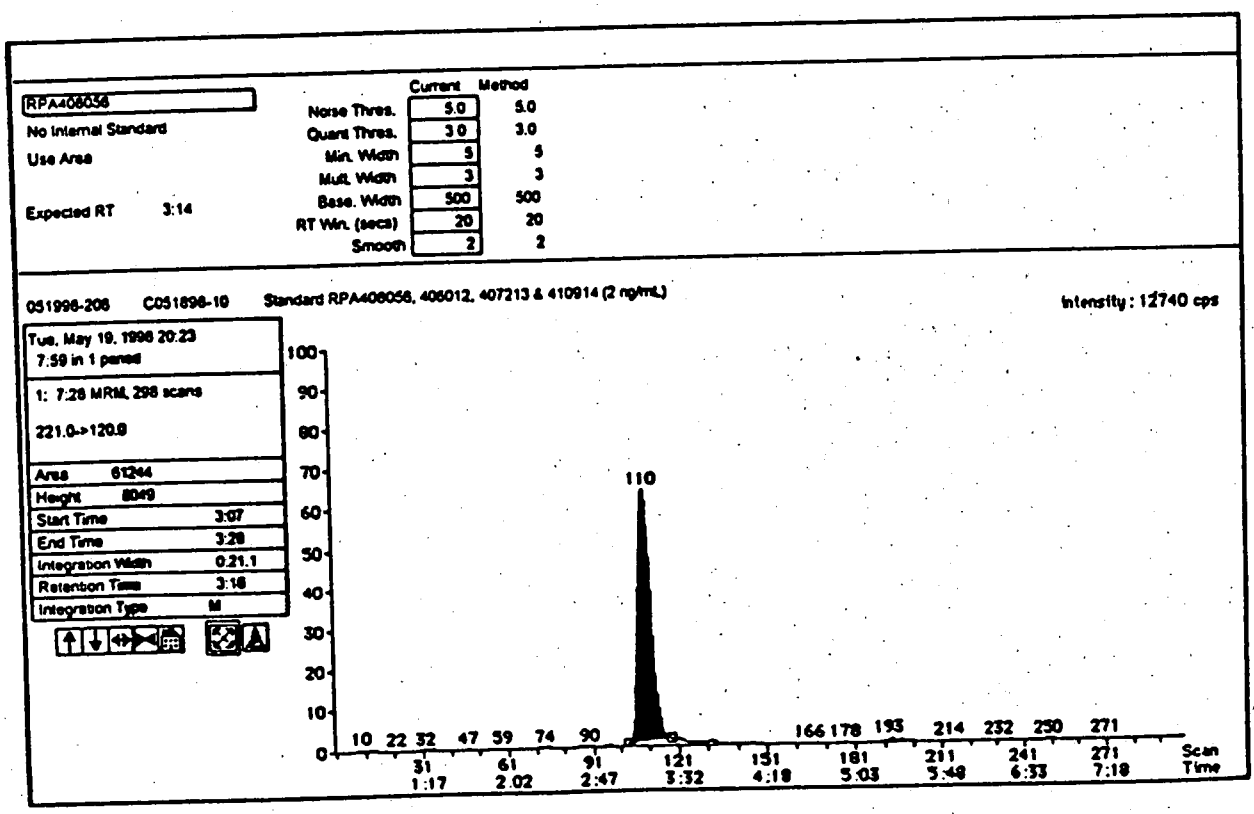
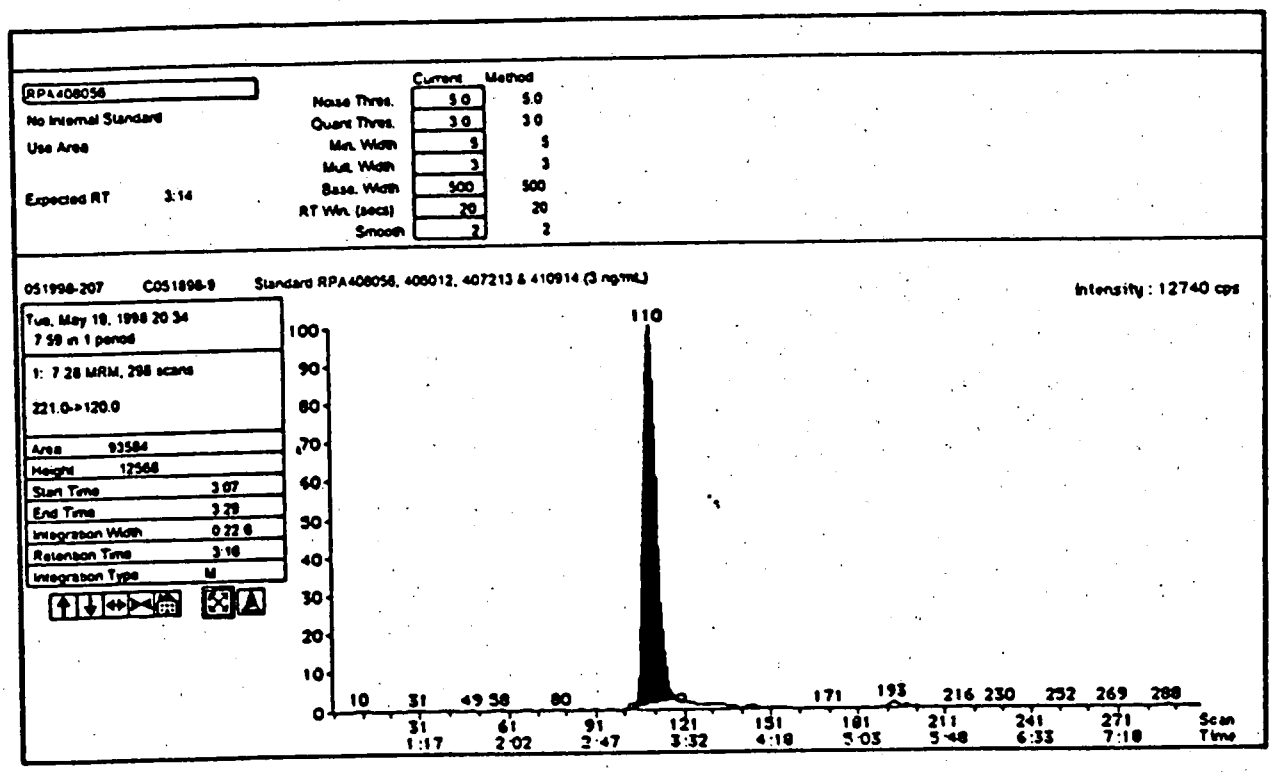


Figure 5.Standard: 3.0 ng/ml - RPA 408056 and 0.3 ng/mL – RPA 407213



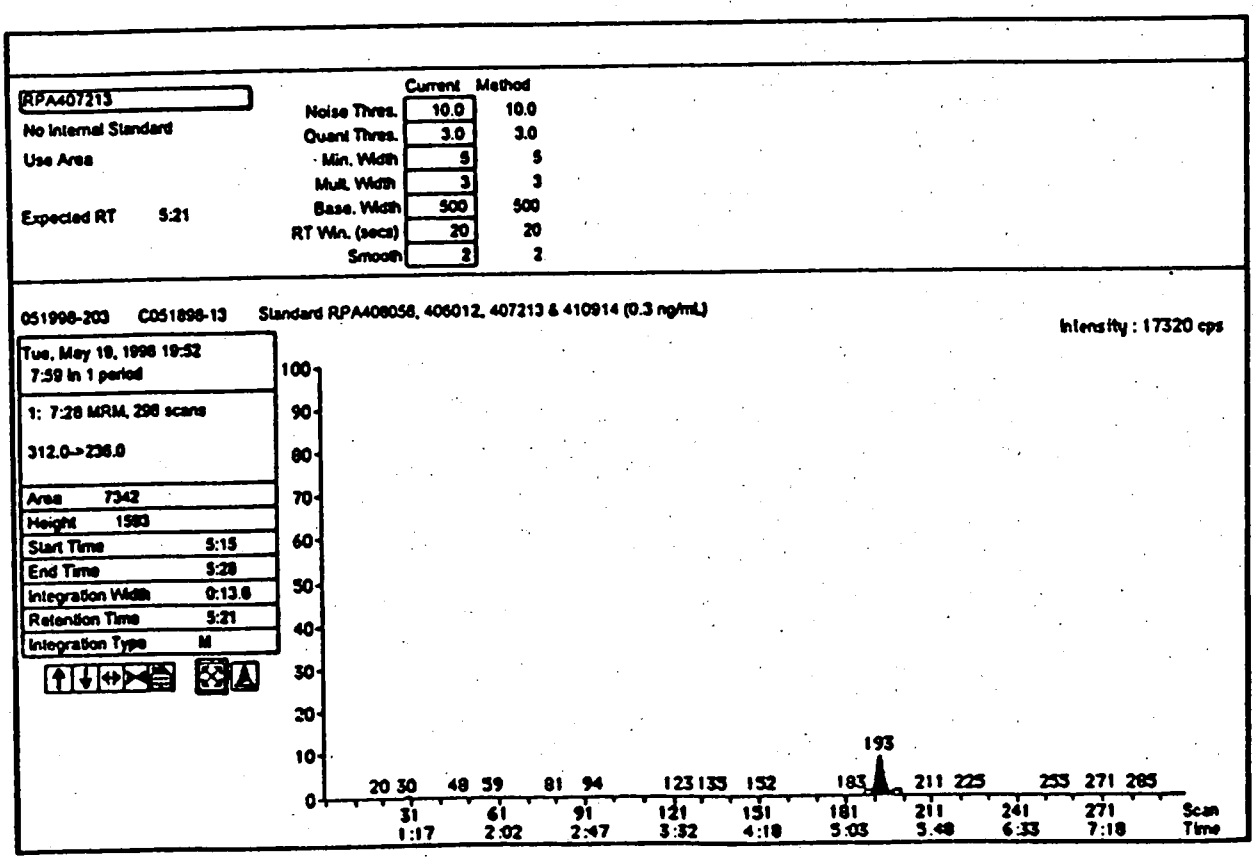
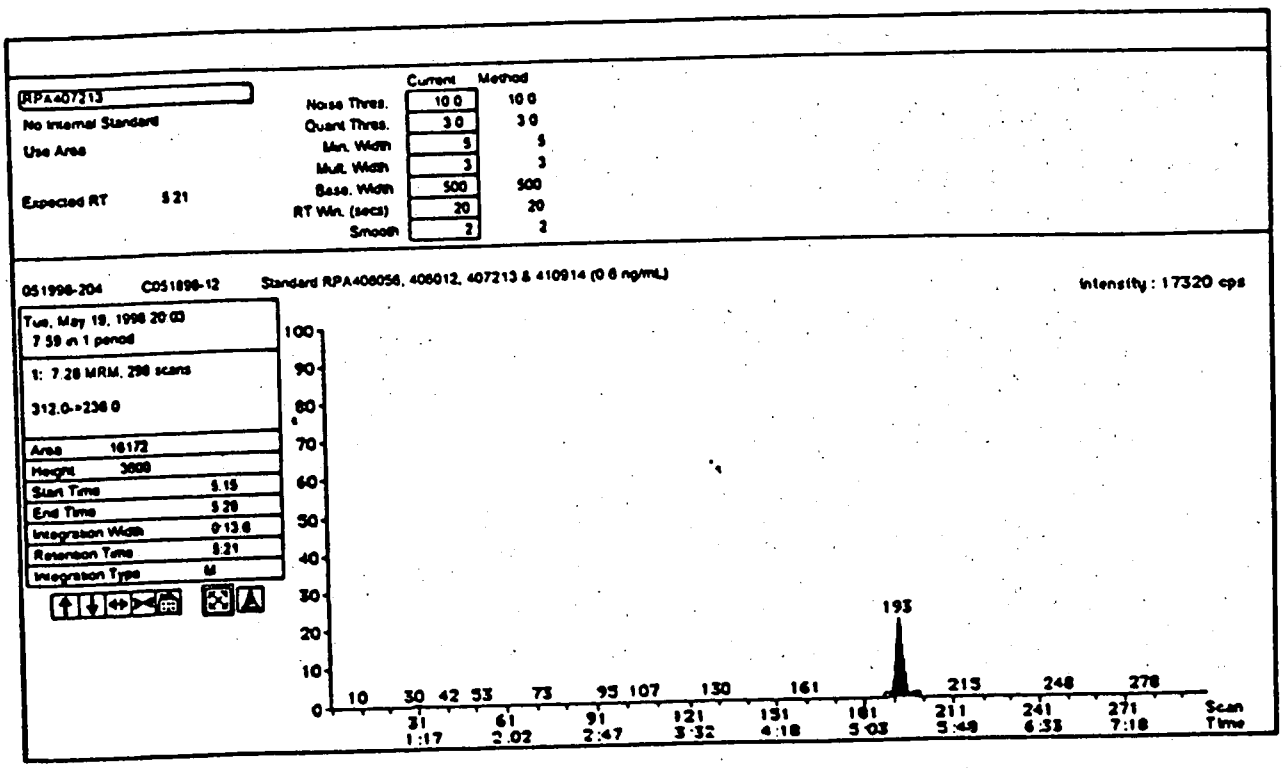


Figure 6.Standard: 0.6 and 1.0 ng/ml - RPA 407213



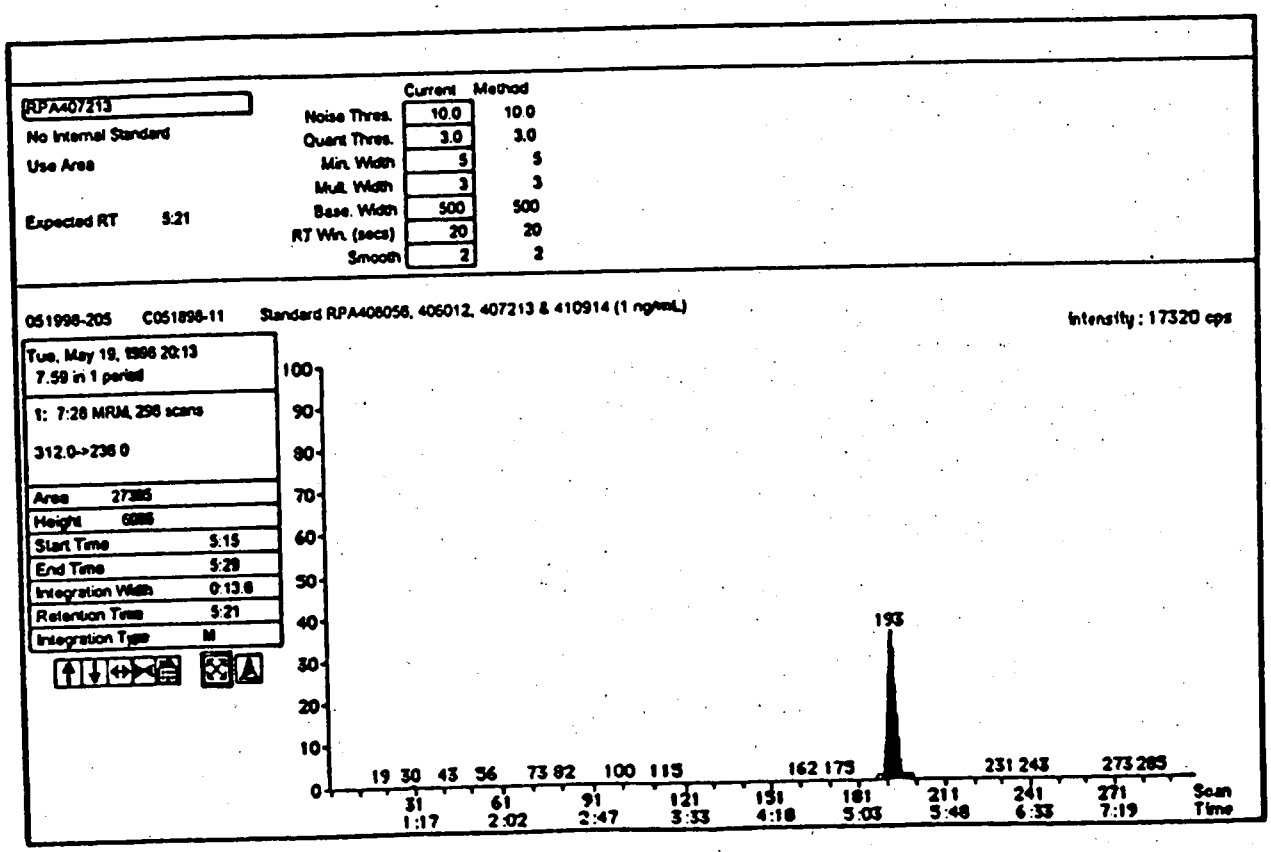
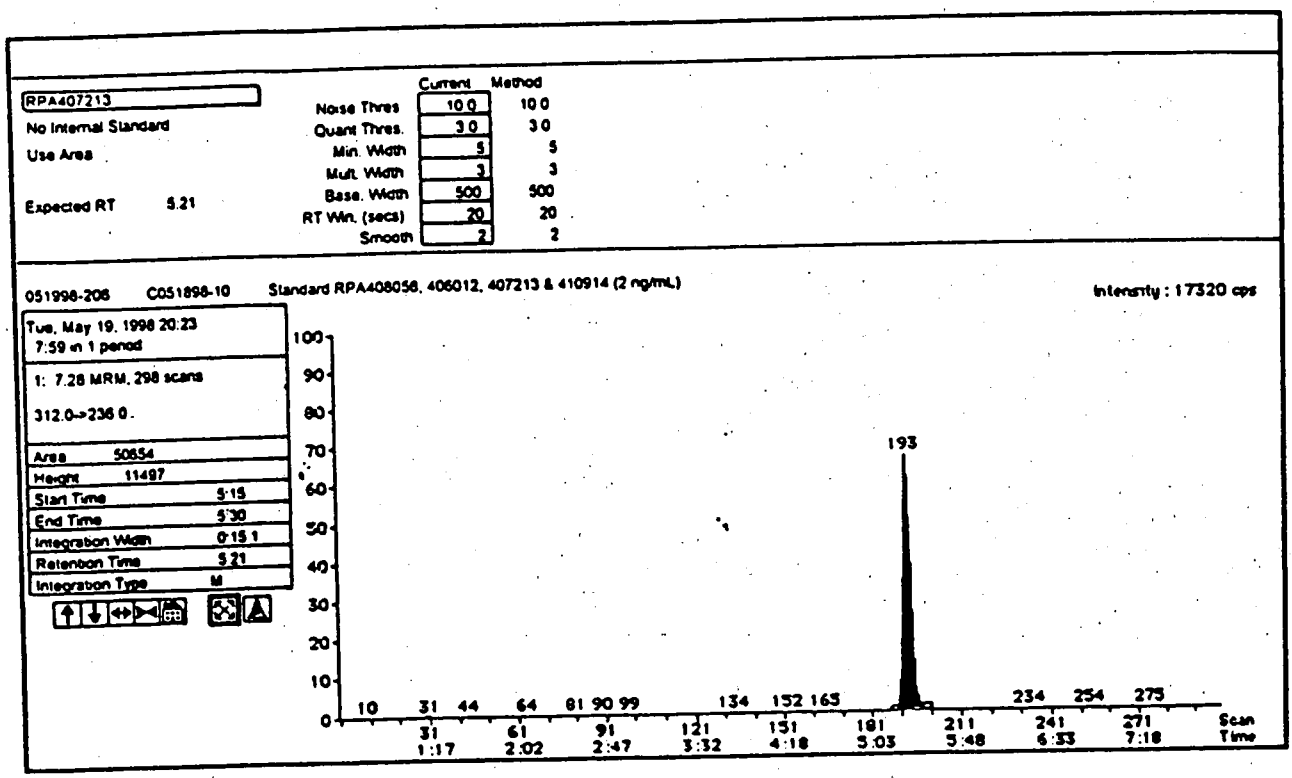


Figure 7.Standard: 2.0 and 3.0 ng/ml - RPA 407213



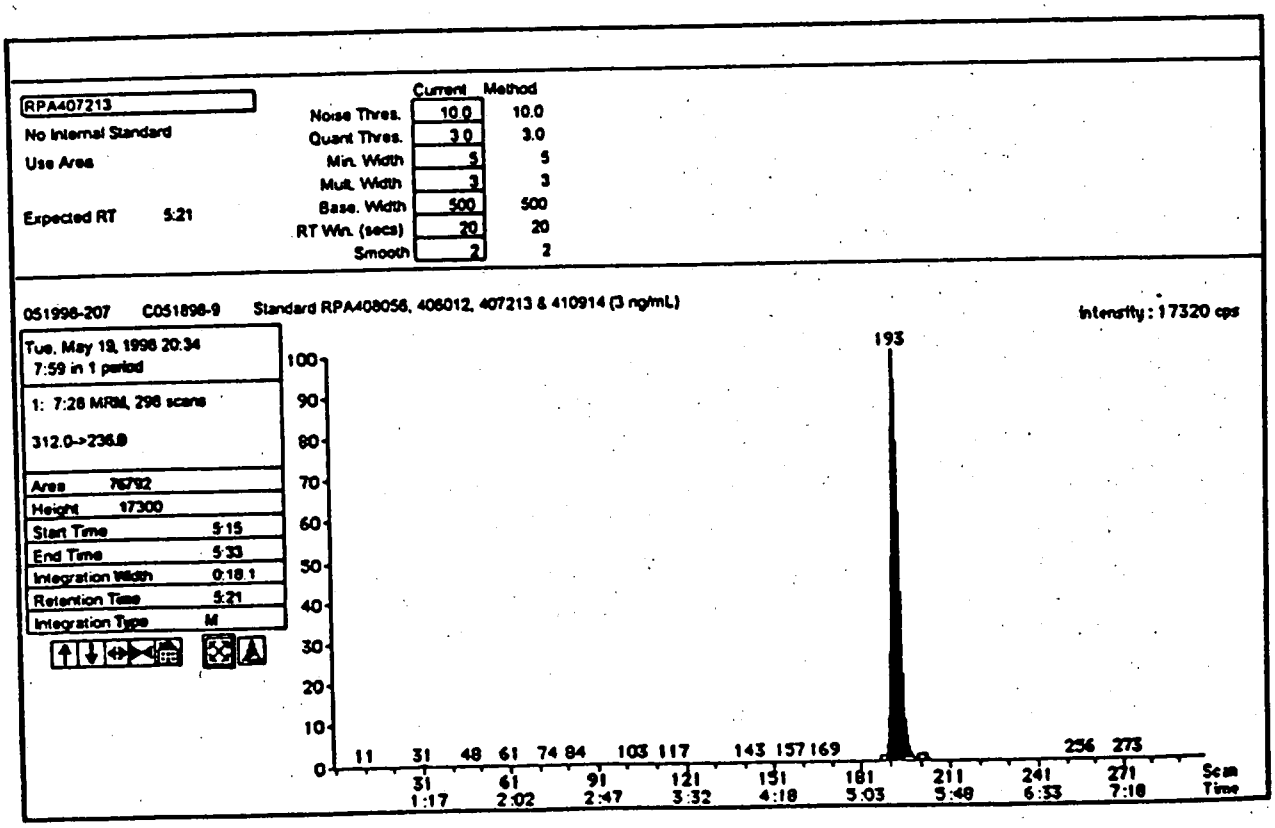
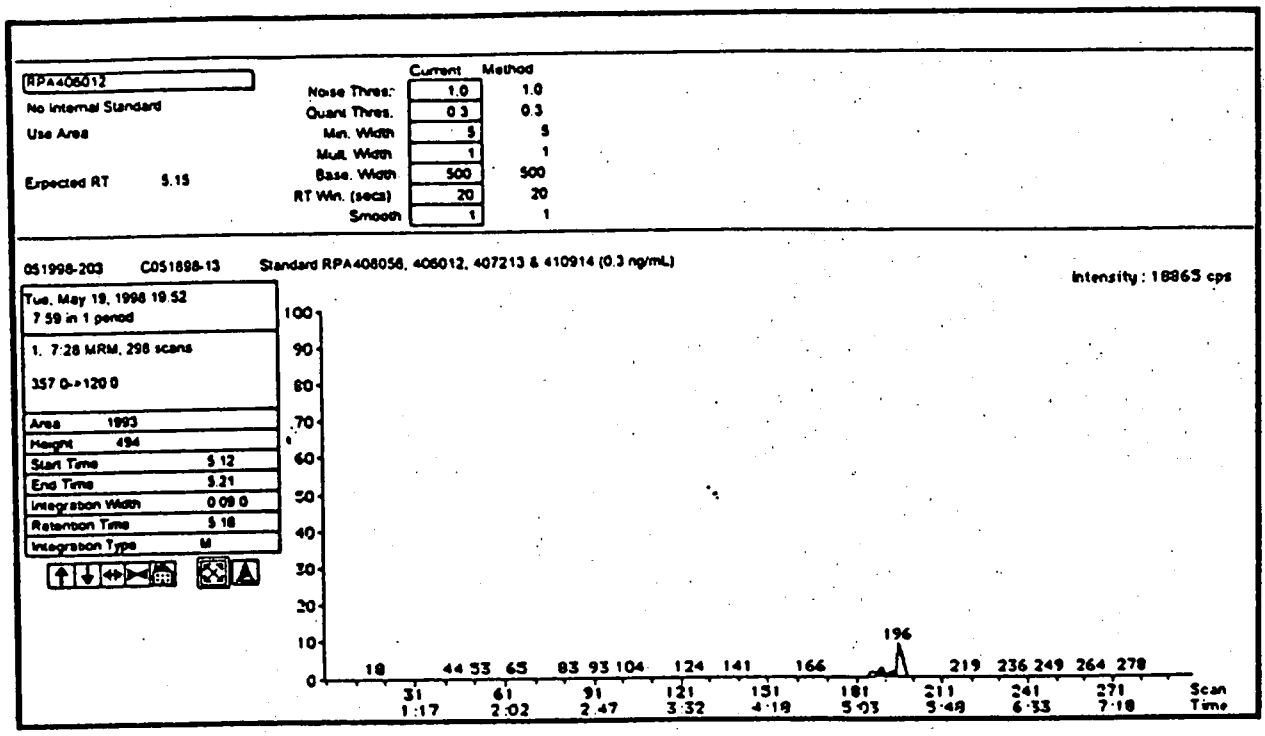


Figure 8. Standard: 0.3 and 0.6 ng/ml - RPA 406012



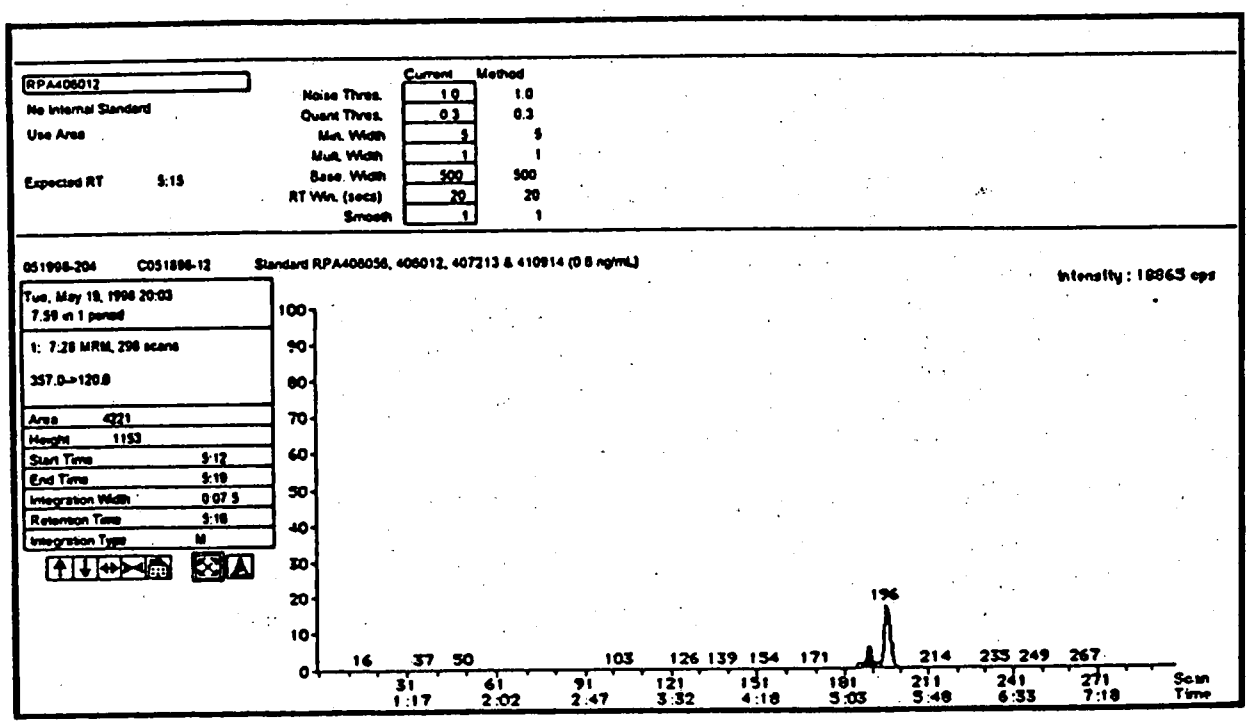


Figure 9. Standard: 1.0 and 2.0 ng/ml - RPA 406012

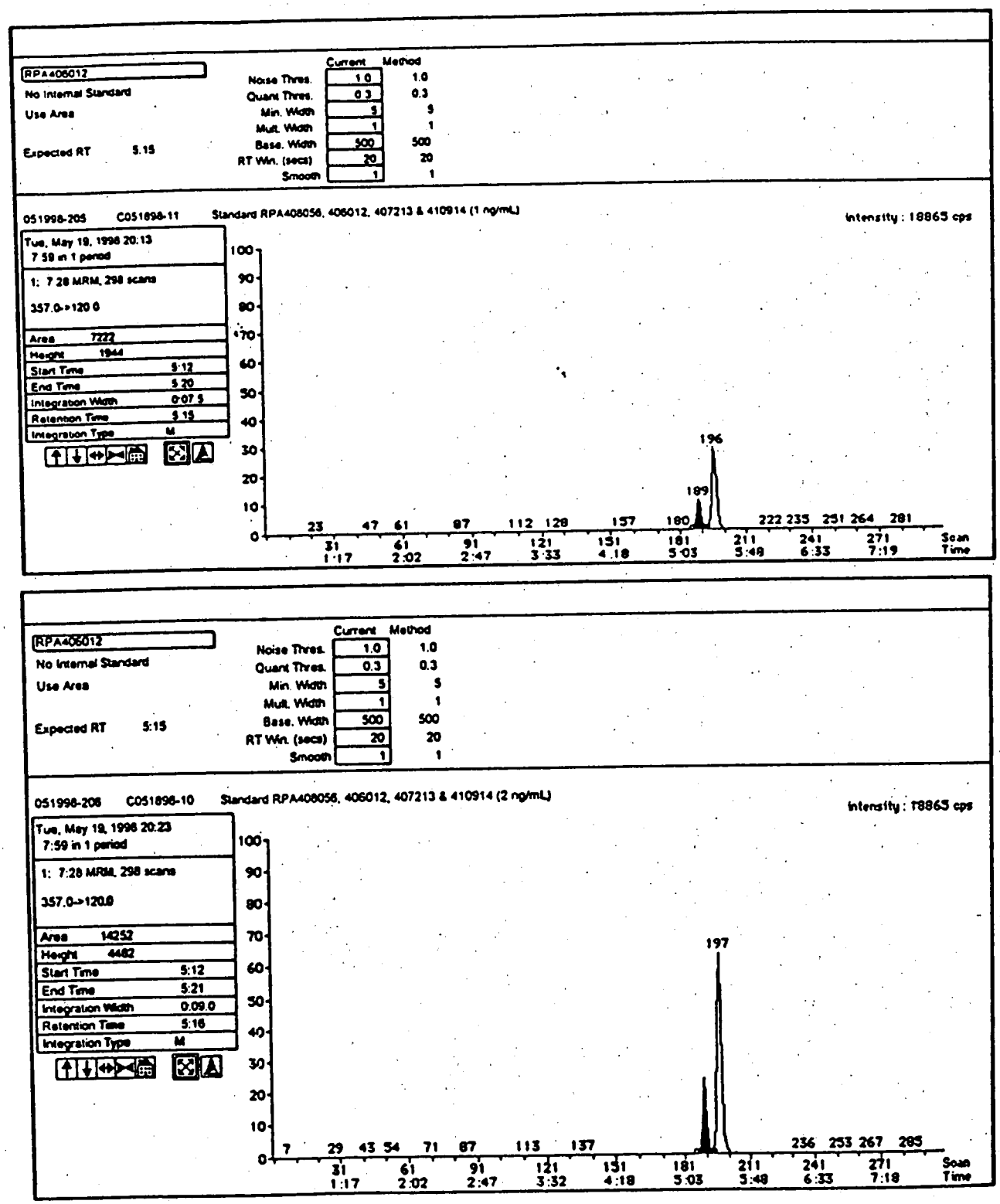
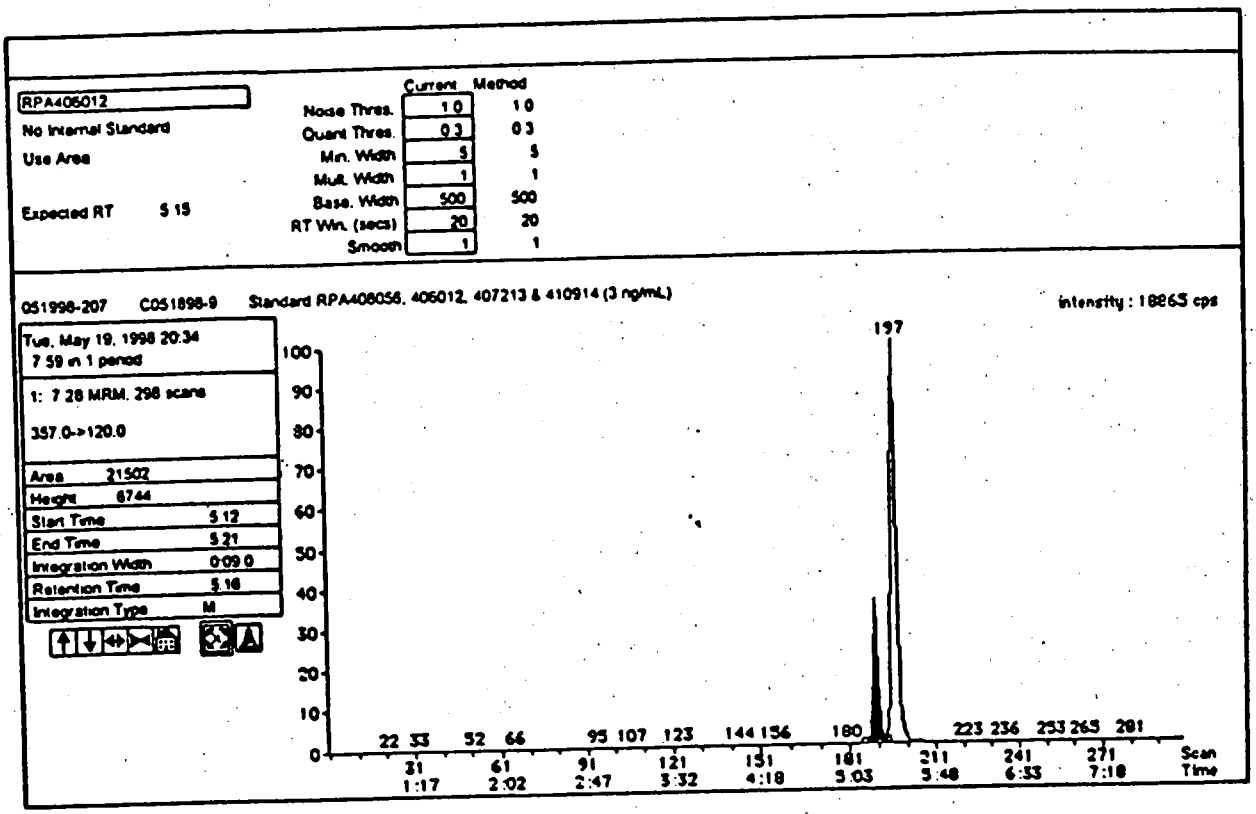


Figure 10. Standard: 3.0 ng/ml - RPA 406012 and 0.3 ng/mL - RPA 410914



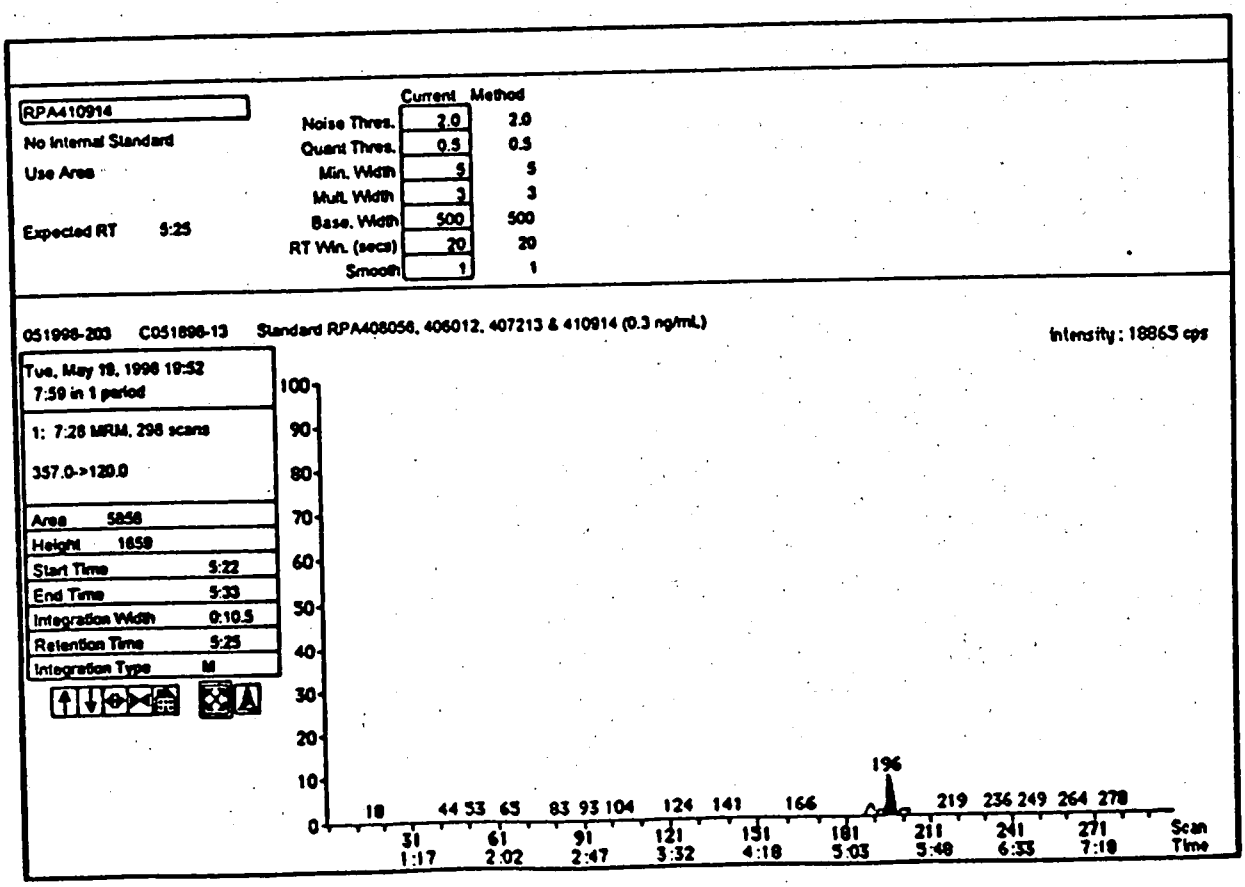
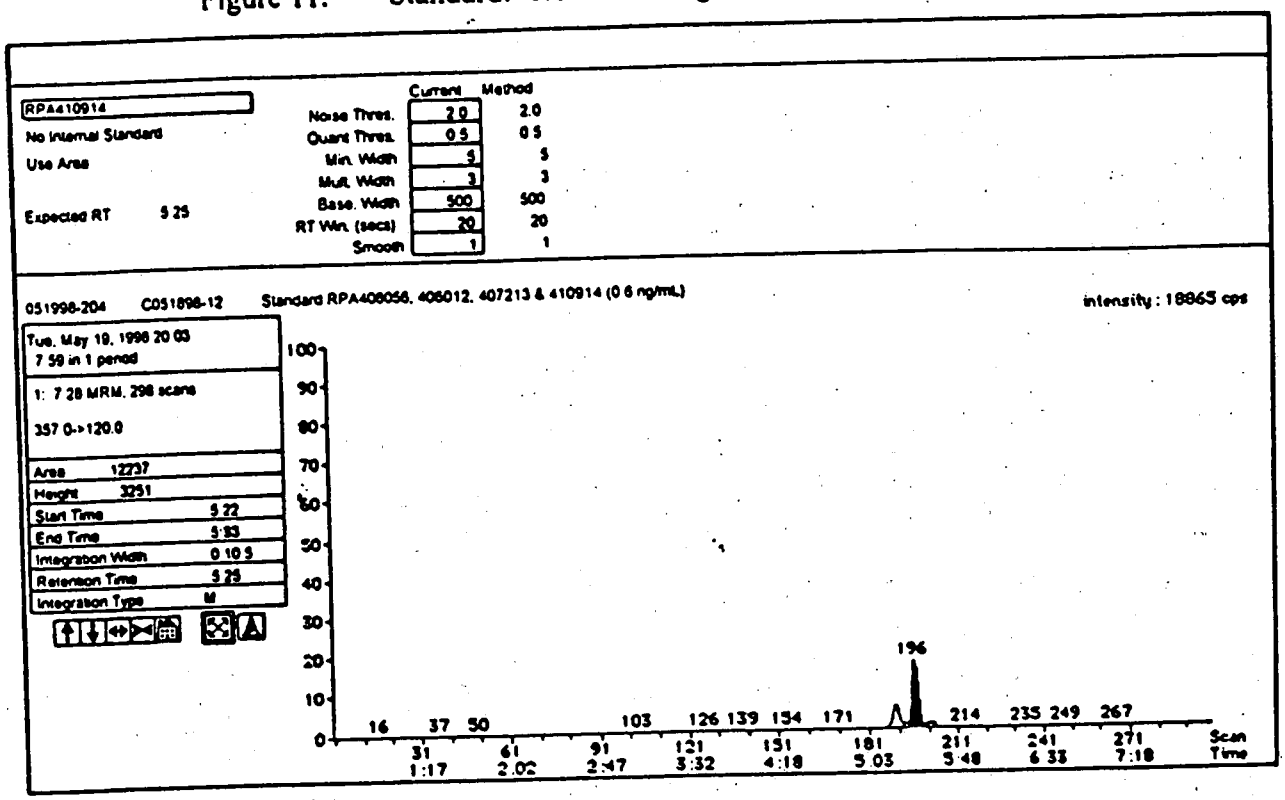


Figure 11. Standard: 0.6 and 1.0 ng/ml - RPA 410914



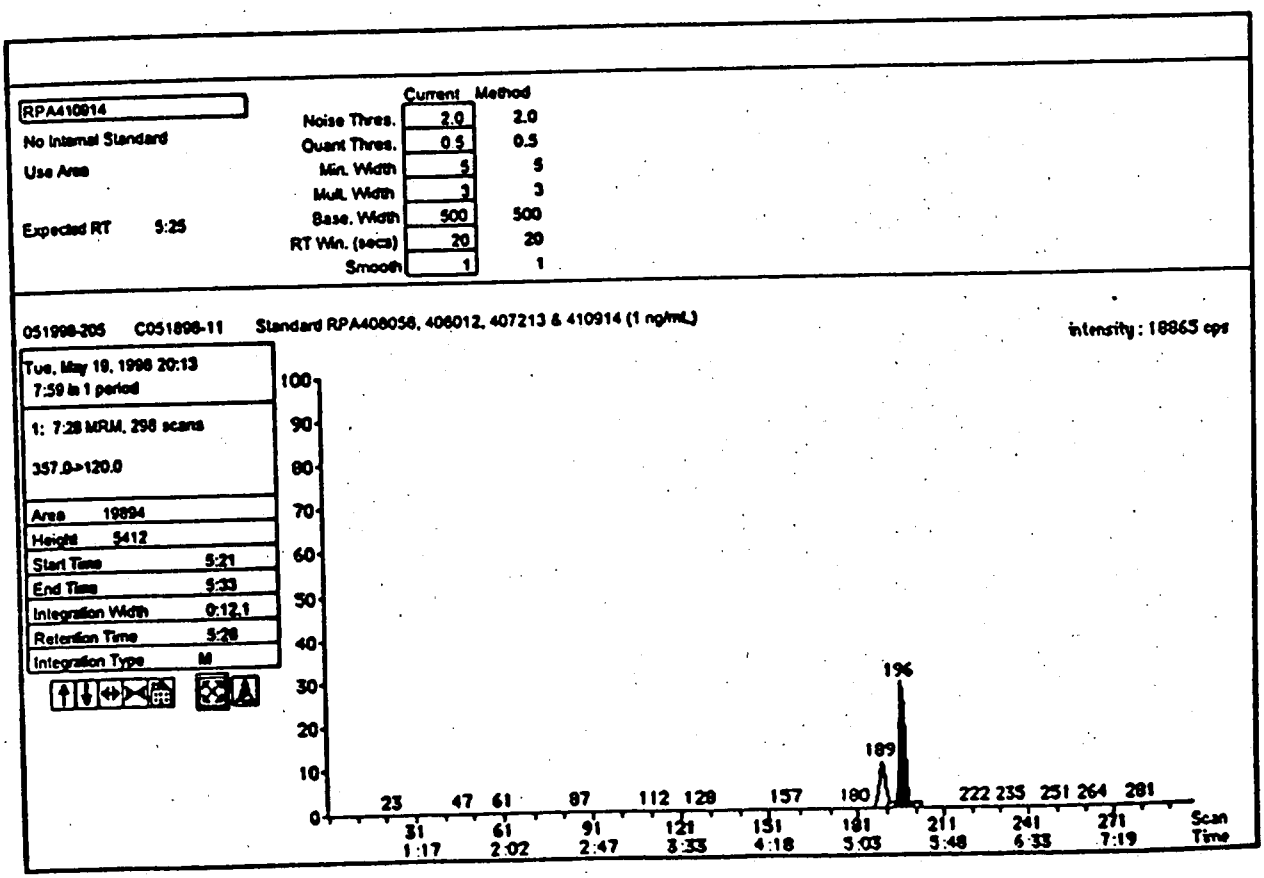
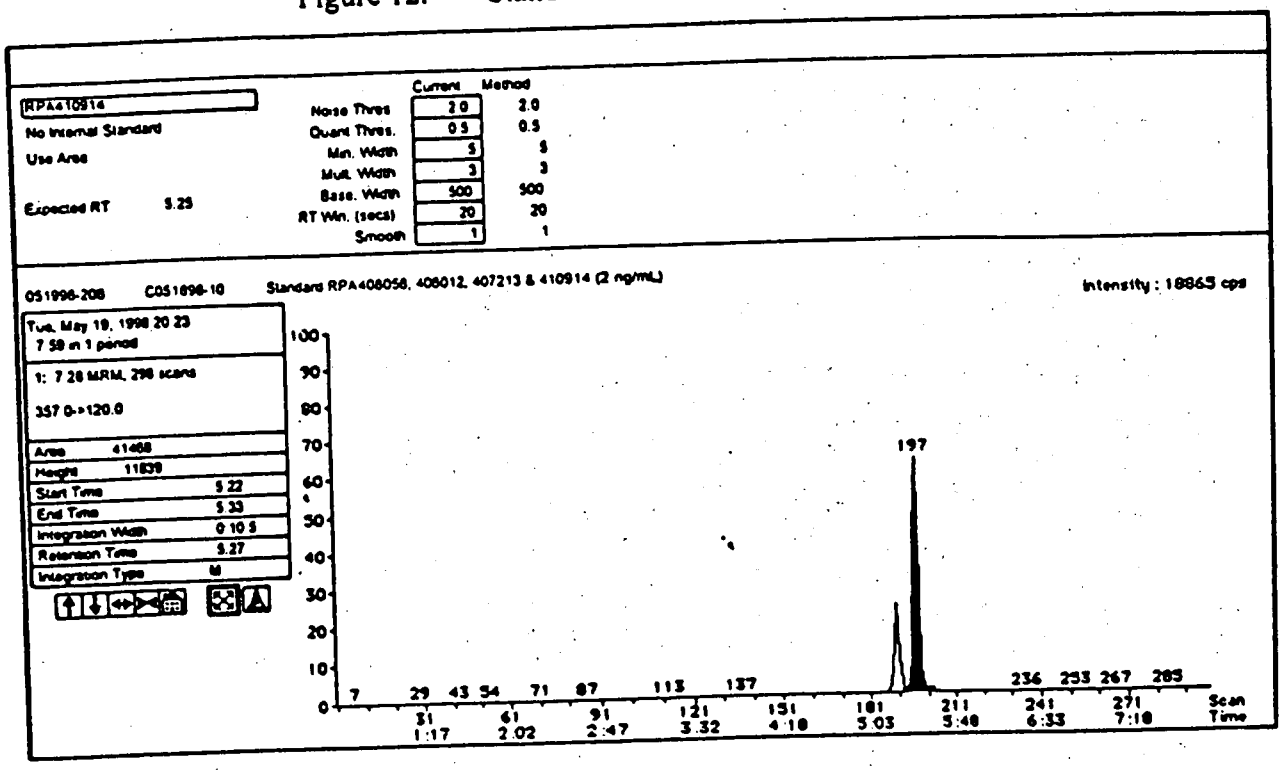


Figure 12. Standard: 2.0 and 3.0 ng/ml - RPA 410914



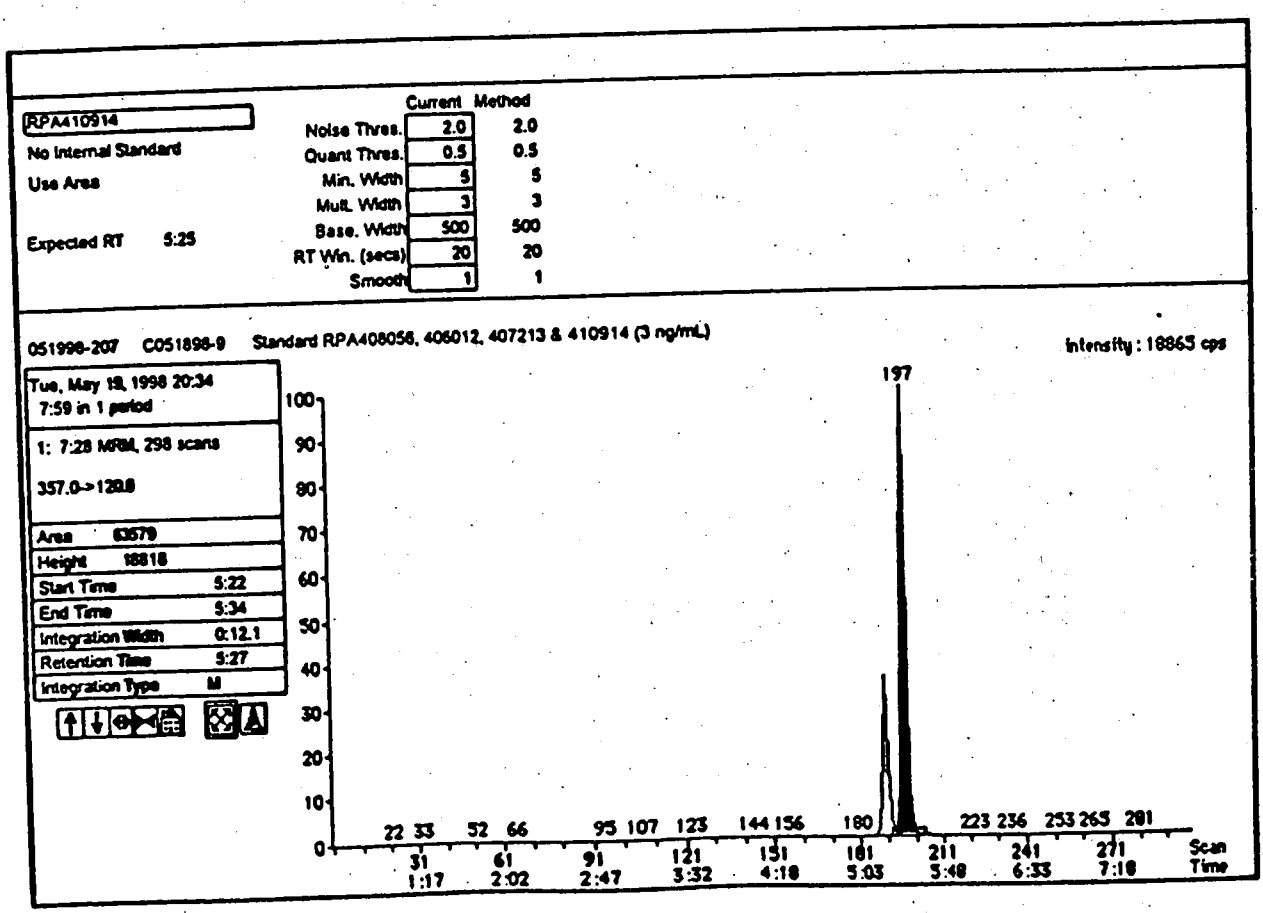
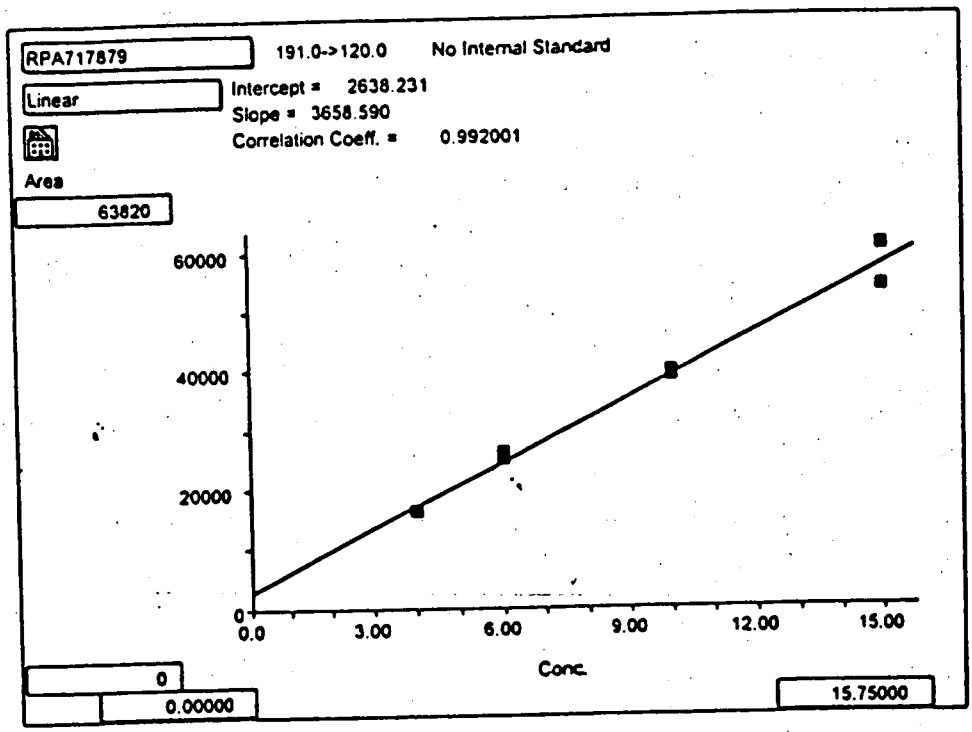


Figure 13. Standard Calibration Curve* for RPA 717879 and RPA 408056
*Conc. = ng/mL vs Peak Area.



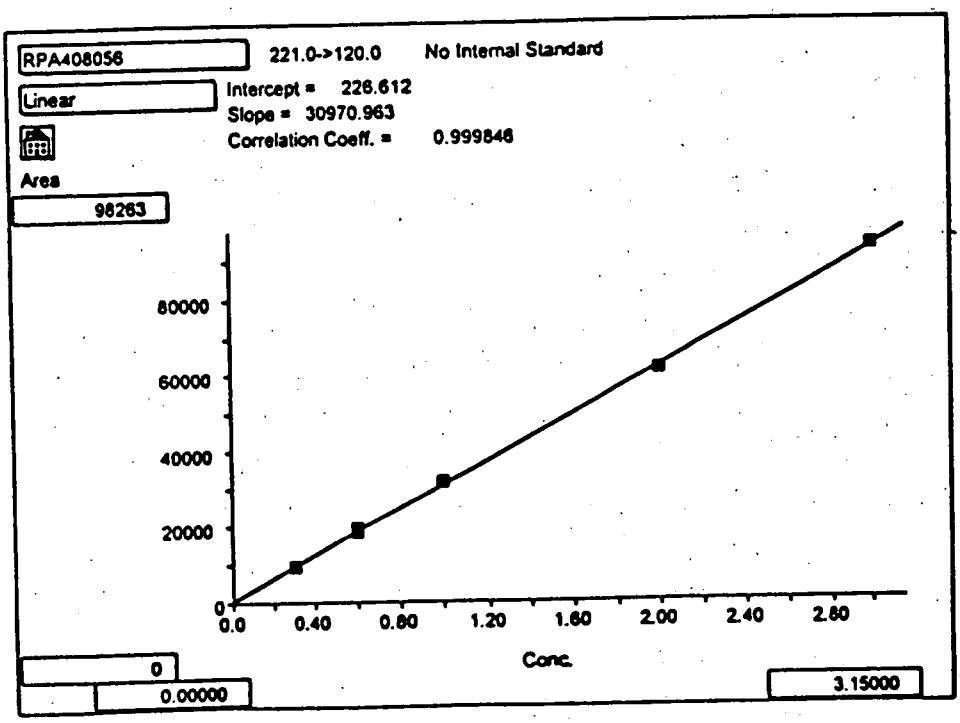
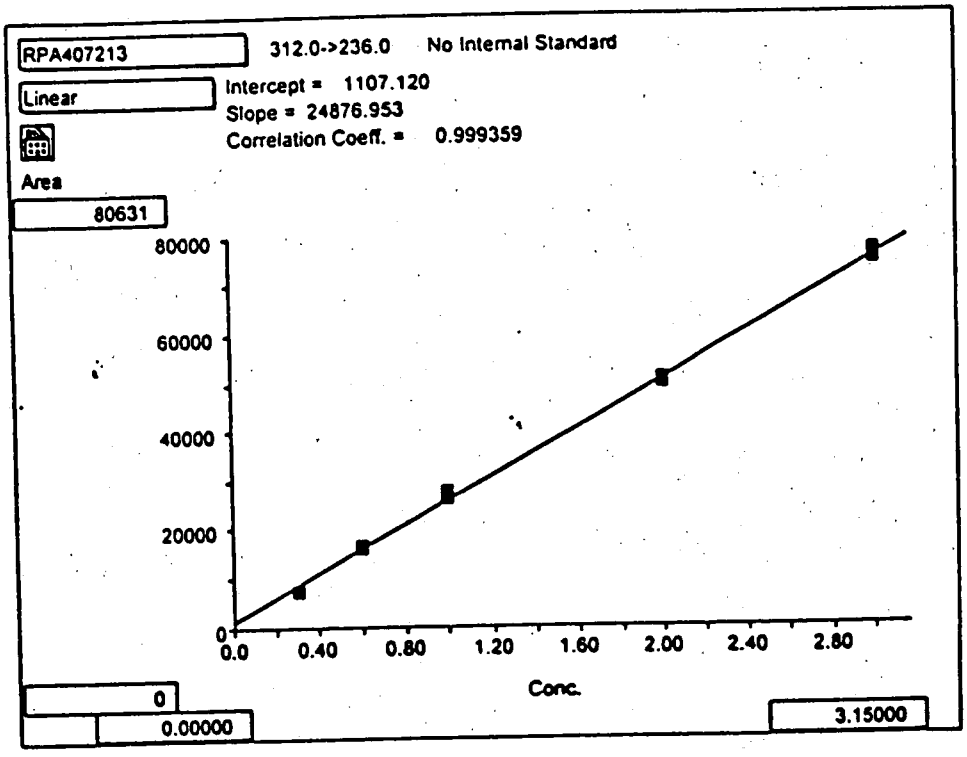


Figure 14. Standard Calibration Curve* for RPA 407213 and RPA 406012



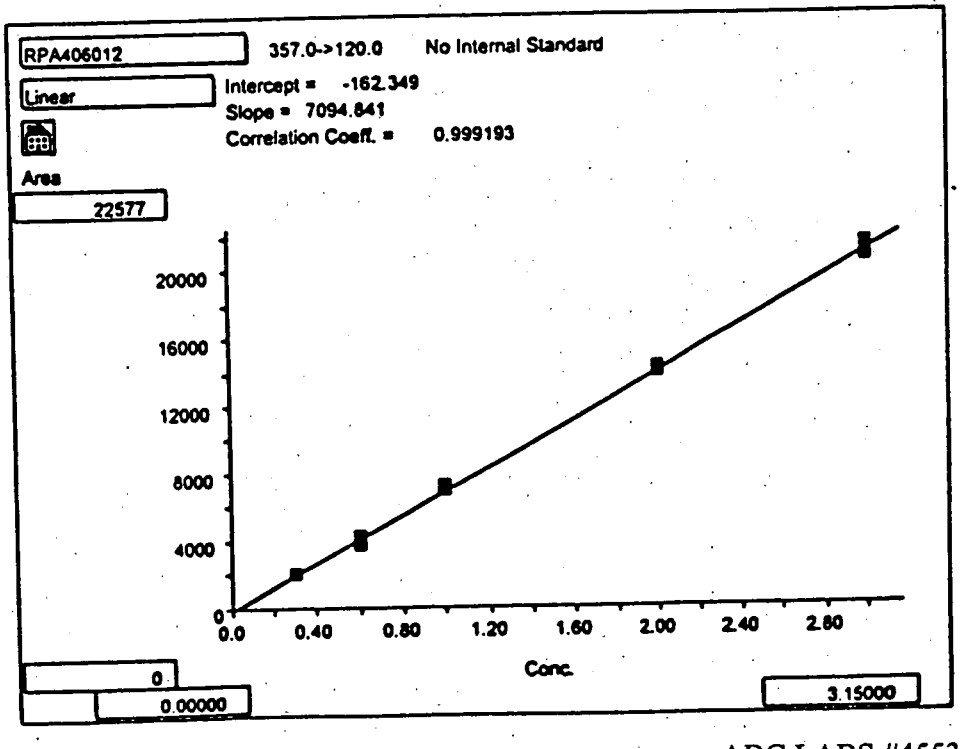
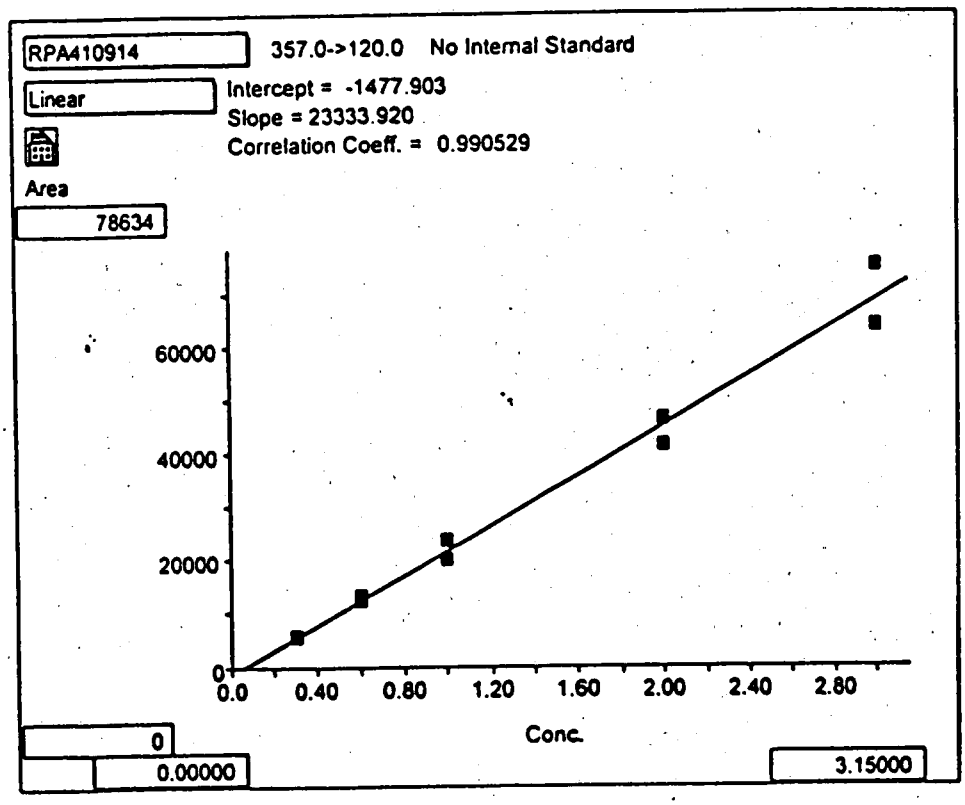


Figure 15. Standard Calibration Curve* for RPA 410914



B. Results Tables

Table 1. RPA 717879 Results

RPA717879
No Internal Standard
191.0->120.0

Linear
Intercept = 2638.231
Slope = 3658.59
Correlation Coeff. = 0.992001
Use Area

Filename	Filetype	Sample Name	Accuracy	Conc.	Calc. Conc.	Area	Height	R.T.
051998-101	QC	C051898-3	104.1	15	15.621	59790	10764	2:25
051998-102	QC	C051898-6	97.2	4	3.888	16863	3089	2:24
051998-103	Standard	C051898-6	94	4	3.758	16389	3171	2:24
051998-104	Standard	C051898-5	101.9	6	6.111	24996	4720	2:24
051998-105	Standard	C051898-4	99.7	10	9.972	39122	7264	2:24
051998-106	Standard	C051898-3	105.9	15	15.892	60781	11401	2:24
051998-107	Sample	982071 Blk A1	n/a	n/a	n/a	n/a	n/a	n/a
051998-108	Sample	982071 Blk B1	n/a	n/a	n/a	n/a	n/a	n/a
051998-109	Standard	C051898-6	93.7	4	3.748	16350	2903	2:24
051998-110	QC	982071 Spk A1	103.8	10	10.376	40599	7818	2:24
051998-111	QC	982071 Spk B1	111.4	10	11.139	43390	8400	2:24
051998-112	Standard	C051898-5	106.6	6	6.397	26042	4845	2:24
051998-113	QC	982071 Spk C1	108.7	10	10.872	42414	7654	2:24
051998-114	QC	982071 Spk D1	114.1	10	11.415	44400	8040	2:24
051998-115	Standard	C051898-4	102	10	10.2	39955	7403	2:24
051998-116	QC	982071 Spk E1	100.2	10	10.016	39283	7207	2:24
051998-117	QC	982071 Spk F1	95.2	10	9.516	37455	6616	2:24
051998-118	Standard	C051898-3	92.8	15	13.922	53572	9544	2:24

Table 2. RPA 408056 Results

RPA408056
No Internal Standard
221.0->120.0

Linear
Intercept = 226.612
Slope = 30970.963
Correlation Coeff. = 0.999846
Use Area

Filename	Filetype	Sample Name	Accuracy	Conc.	Calc. Conc.	Area	Height	R.T.
051998-201	QC	C051898-9	101.2	3	3.036	94265	12503	3:14
051998-202	QC	C051898-13	97	0.3	0.291	9242	1217	3:14
051998-203	Standard	C051898-13	98.1	0.3	0.294	9342	1229	3:14
051998-204	Standard	C051898-12	102.6	0.6	0.616	19293	2494	3:16
051998-205	Standard	C051898-11	101.5	1	1.015	31653	4226	3:14
051998-206	Standard	C051898-10	98.5	2	1.97	61244	8049	3:16
051998-207	Standard	C051898-9	100.5	3	3.014	93584	12568	3:16
051998-208	Sample	982071 Blk A1	n/a	n/a	n/a	n/a	n/a	n/a
051998-209	Sample	982071 Blk B1	n/a	n/a	-0.007	0	0	0:00.0
051998-210	Standard	C051898-12	97.4	0.6	0.584	18326	2538	3:16
051998-211	QC	982071 Spk A1	100.4	1	1.004	31312	4119	3:16
051998-212	QC	982071 Spk B1	96.8	1	0.968	30202	3935	3:16
051998-213	Standard	C051898-11	101.8	1	1.018	31751	4323	3:16
051998-214	QC	982071 Spk C1	101.8	1	1.018	31769	4088	3:16
051998-215	QC	982071 Spk D1	98	1	0.98	30563	4098	3:16
051998-216	Standard	C051898-10	99	2	1.98	61546	8290	3:16
051998-217	QC	982071 Spk E1	105.2	1	1.052	32797	4368	3:16
051998-218	QC	982071 Spk F1	106.2	1	1.062	33116	4338	3:16
051998-219	Standard	C051898-9	100.3	3	3.009	93407	12426	3:16

Table 3. RPA 407213 Results

RPA407213
No Internal Standard
312.0->236.0

Linear
Intercept = 1107.12
Slope = 24876.953
Correlation Coeff. = 0.999359
Use Area

Filename	Filetype	Sample Name	Accuracy	Conc.	Calc. Conc.	Area	Height	R.T.
051998-201	QC	C051898-9	105.1	3	3.154	79576	17728	5:21
051998-202	QC	C051898-13	87.4	0.3	0.262	7630	1657	5:21
051998-203	Standard	C051898-13	83.5	0.3	0.251	7342	1593	5:21
051998-204	Standard	C051898-12	100.9	0.6	0.606	16172	3600	5:21
051998-205	Standard	C051898-11	105.6	1	1.056	27385	6066	5:21
051998-206	Standard	C051898-10	99.6	2	1.992	50654	11497	5:21
051998-207	Standard	C051898-9	101.4	3	3.042	76792	17300	5:21
051998-208	Sample	982071 Blk A1	n/a	n/a	n/a	n/a	n/a	n/a
051998-209	Sample	982071 Blk B1	n/a	n/a	n/a	n/a	n/a	n/a
051998-210	Standard	C051898-12	102.5	0.6	0.615	16413	3765	5:21
051998-211	QC	982071 Spk A1	84	1	0.84	22009	4861	5:21
051998-212	QC	982071 Spk B1	79.8	1	0.798	20963	4650	5:21
051998-213	Standard	C051898-11	101.4	1	1.014	26323	5793	5:21
051998-214	QC	982071 Spk C1	87.6	1	0.876	22902	5094	5:21
051998-215	QC	982071 Spk D1	85.2	1	0.852	22312	4887	5:21
051998-216	Standard	C051898-10	97.6	2	1.952	49661	11122	5:21
051998-217	QC	982071 Spk E1	87.3	1	0.873	22835	4942	5:21
051998-218	QC	982071 Spk F1	90.7	1	0.907	23679	5215	5:21
051998-219	Standard	C051898-9	99.1	3	2.973	75062	16498	5:21

Table 4. RPA 406012 Results

RPA406012
No Internal Standard
357.0->120.0

Linear
Intercept = -162.349
Slope = 7094.641
Correlation Coeff. = 0.999193
Use Area

Filename	Filetype	Sample Name	Accuracy	Conc.	Calc. Conc.	Area	Height	R.T.
051998-201	QC	C051898-9	95	3	2.849	20049	5066	5:16
051998-202	QC	C051898-13	103.7	0.3	0.311	2045	544	5:15
051998-203	Standard	C051898-13	101.3	0.3	0.304	1993	494	5:16
051998-204	Standard	C051898-12	103	0.6	0.618	4221	1153	5:16
051998-205	Standard	C051898-11	104.1	1	1.041	7222	1944	5:15
051998-206	Standard	C051898-10	101.6	2	2.032	14252	4482	5:16
051998-207	Standard	C051898-9	101.8	3	3.053	21502	6744	5:16
051998-208	Sample	982071 Blk A1	n/a	n/a	n/a	n/a	n/a	n/a
051998-209	Sample	982071 Blk B1	n/a	n/a	n/a	n/a	n/a	n/a
051998-210	Standard	C051898-12	90.7	0.6	0.544	3699	1167	5:16
051998-211	QC	982071 Spk A1	79.9	1	0.799	5507	1771	5:16
051998-212	QC	982071 Spk B1	75.5	1	0.755	5194	1626	5:16
051998-213	Standard	C051898-11	99.2	1	0.992	6878	2159	5:16
051998-214	QC	982071 Spk C1	85.1	1	0.851	5873	1852	5:16
051998-215	QC	982071 Spk D1	73.6	1	0.736	5061	1594	5:16
051998-216	Standard	C051898-10	99.2	2	1.983	13907	4360	5:16
051998-217	QC	982071 Spk E1	84	1	0.84	5798	1785	5:16
051998-218	QC	982071 Spk F1	96.2	1	0.962	6666	2125	5:16
051998-219	Standard	C051898-9	97.8	3	2.933	20646	6523	5:16

Table 5. RPA 410914 Results

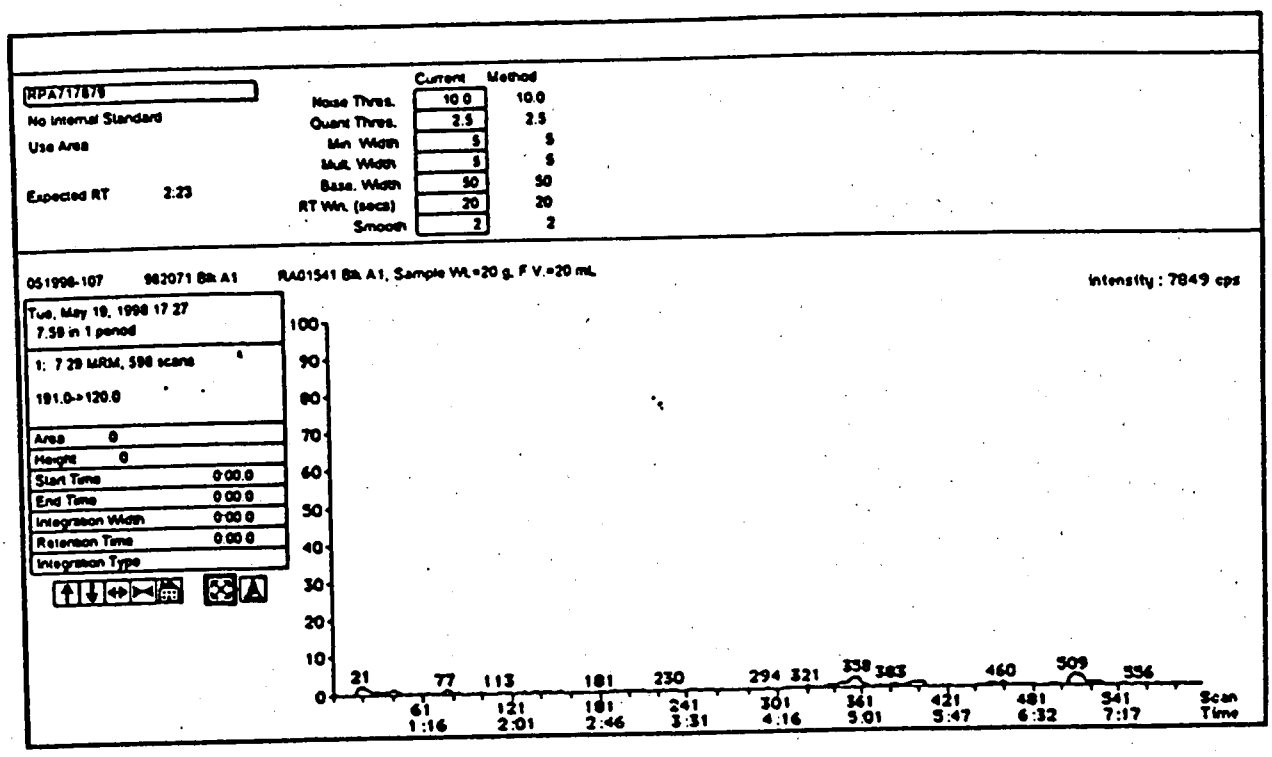
RPA410914
No Internal Standard
357.0->120.0

Linear
Intercept = -1477.903
Slope = 23333.92
Correlation Coeff. = 0.990529
Use Area

Filename	Filetype	Sample Name	Accuracy	Conc.	Calc. Conc.	Area	Height	R.T.
051998-201	QC	C051898-9	81.4	3	2.441	55487	13631	5:27
051998-202	QC	C051898-13	97.9	0.3	0.294	5375	1389	5:25
051998-203	Standard	C051898-13	104.8	0.3	0.314	5856	1659	5:25
051998-204	Standard	C051898-12	98	0.6	0.588	12237	3251	5:25
051998-205	Standard	C051898-11	91.6	1	0.916	19894	5412	5:26
051998-206	Standard	C051898-10	92	2	1.841	41468	11839	5:27
051998-207	Standard	C051898-9	92.9	3	2.788	63579	18818	5:27
051998-208	Sample	982071 Blk A1	n/a	n/a	n/a	n/a	n/a	n/a
051998-209	Sample	982071 Blk B1	n/a	n/a	n/a	n/a	n/a	n/a
051998-210	Standard	C051898-12	105.9	0.6	0.635	13350	3524	5:27
051998-211	QC	982071 Spk A1	87.8	1	0.878	19002	5820	5:27
051998-212	QC	982071 Spk B1	92.4	1	0.924	20091	6153	5:27
051998-213	Standard	C051898-11	108.8	1	1.088	23912	7211	5:27
051998-214	QC	982071 Spk C1	81.7	1	0.817	17575	5137	5:27
051998-215	QC	982071 Spk D1	76.8	1	0.768	16452	4941	5:27
051998-216	Standard	C051898-10	102.9	2	2.057	46521	13800	5:27
051998-217	QC	982071 Spk E1	93.1	1	0.931	20236	6122	5:27
051998-218	QC	982071 Spk F1	100.4	1	1.004	21940	6570	5:27
051998-219	Standard	C051898-9	109.1	3	3.273	74890	22323	5:27

C. Chromatograms of Samples

Figure 16. Soil (Florida) Untreated Control
RA01541 UTC : RPA717879 and RPA408056



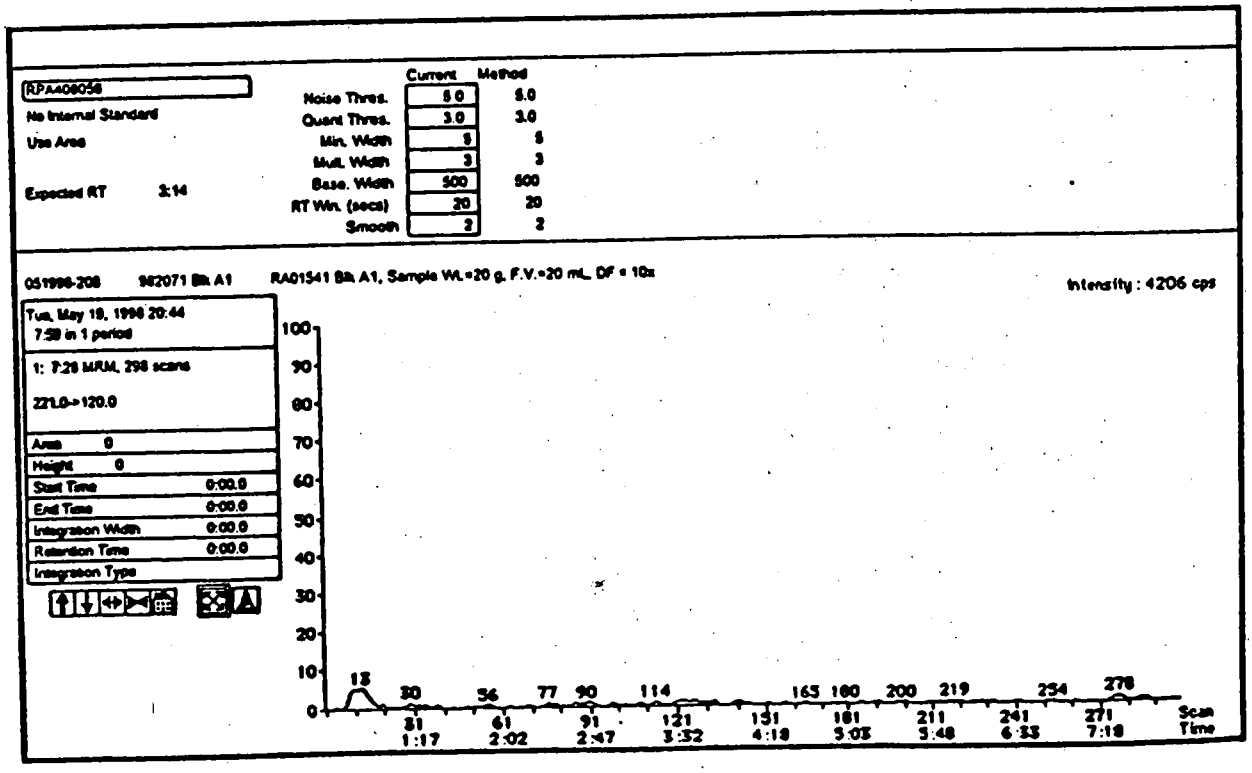
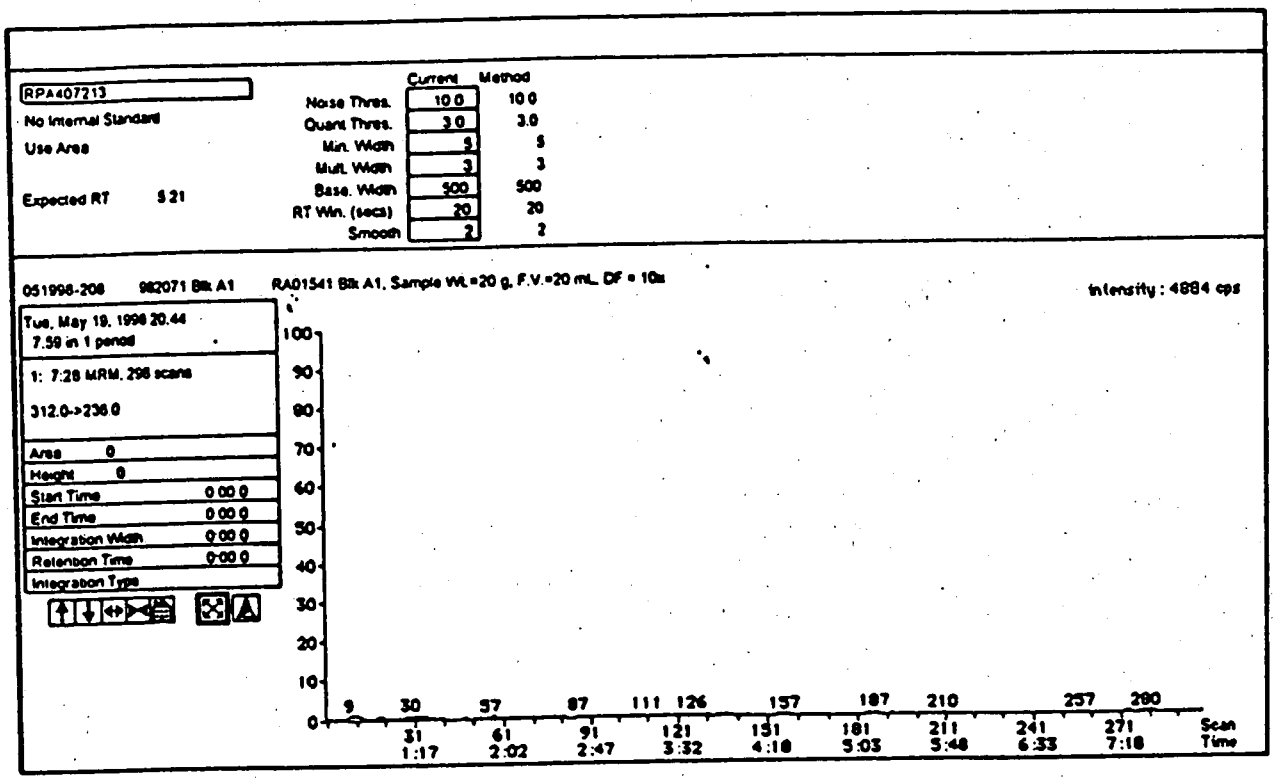


Figure 17. Soil (Florida) Untreated Control
RA01541 UTC : RPA407213 and RPA406012



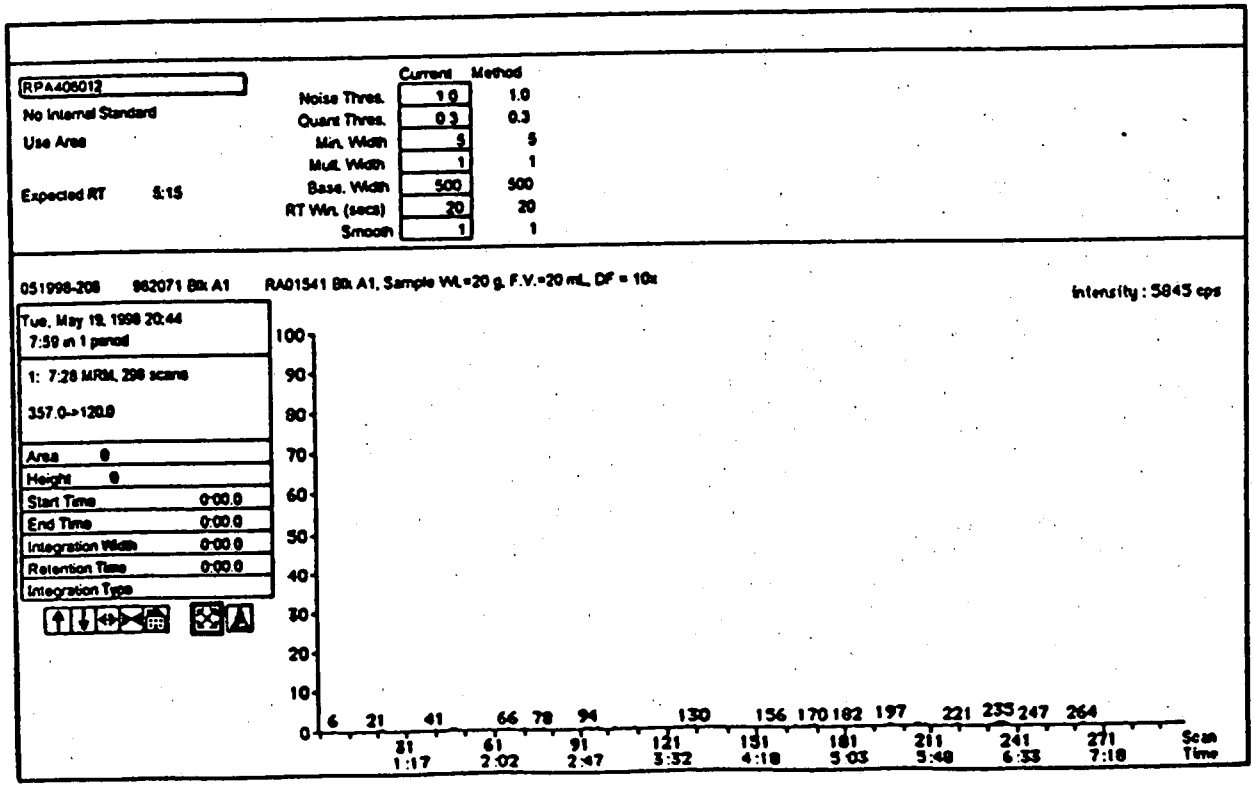


Figure 18. Soil (Florida) Untreated Control
RA01541 UTC : RPA410914

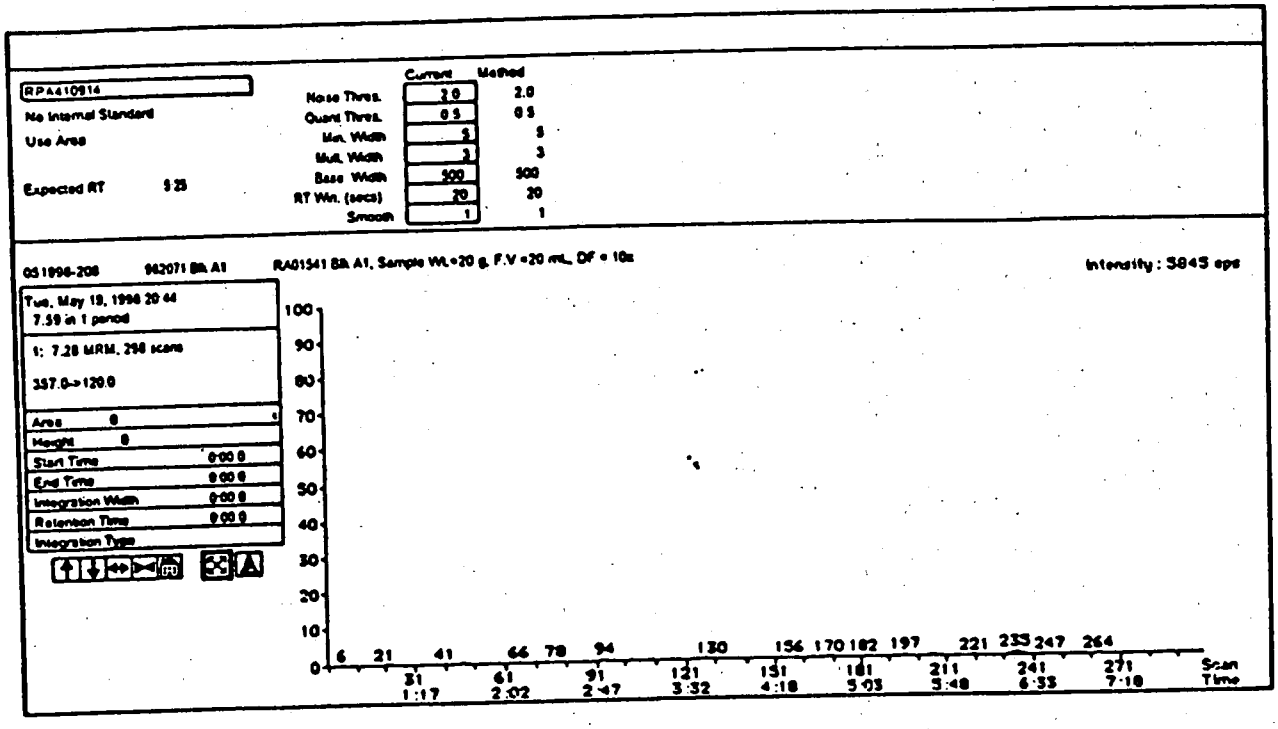


Figure 19. Soil (Florida) 10 ppb RA01541 LOQ : RPA717879

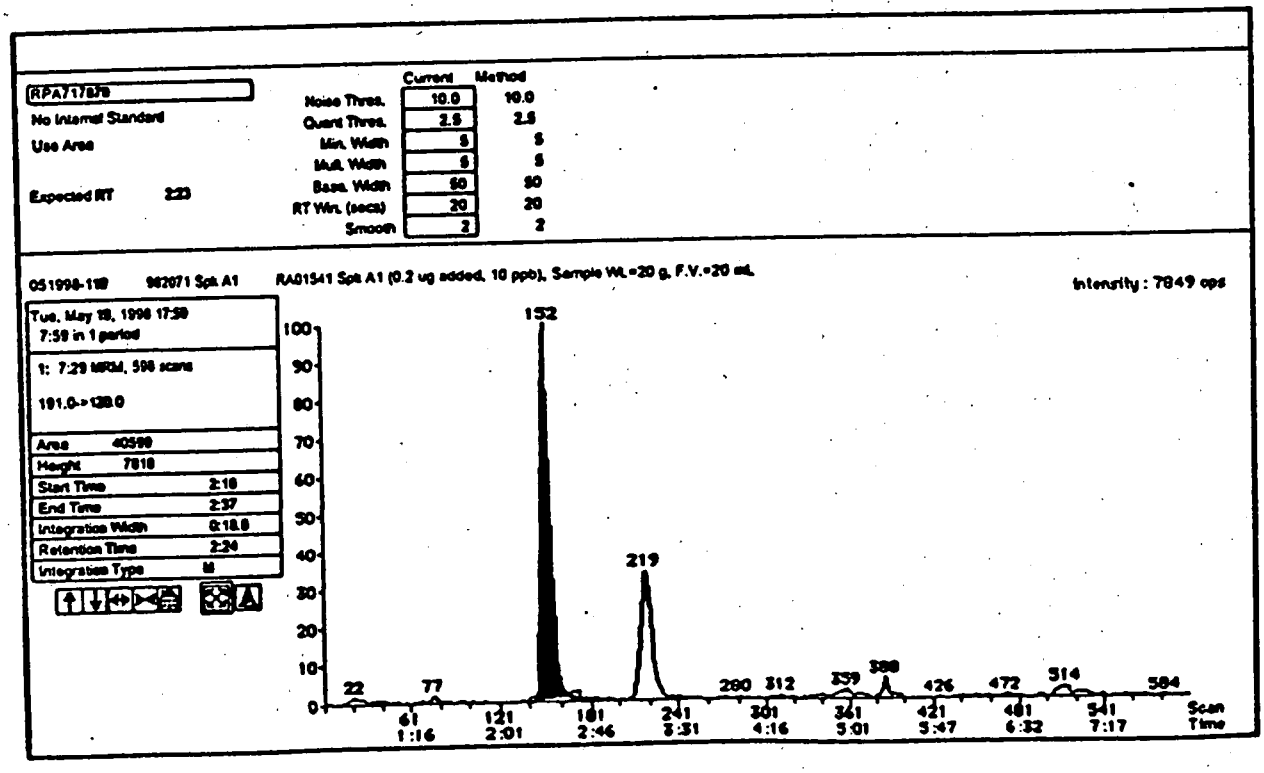
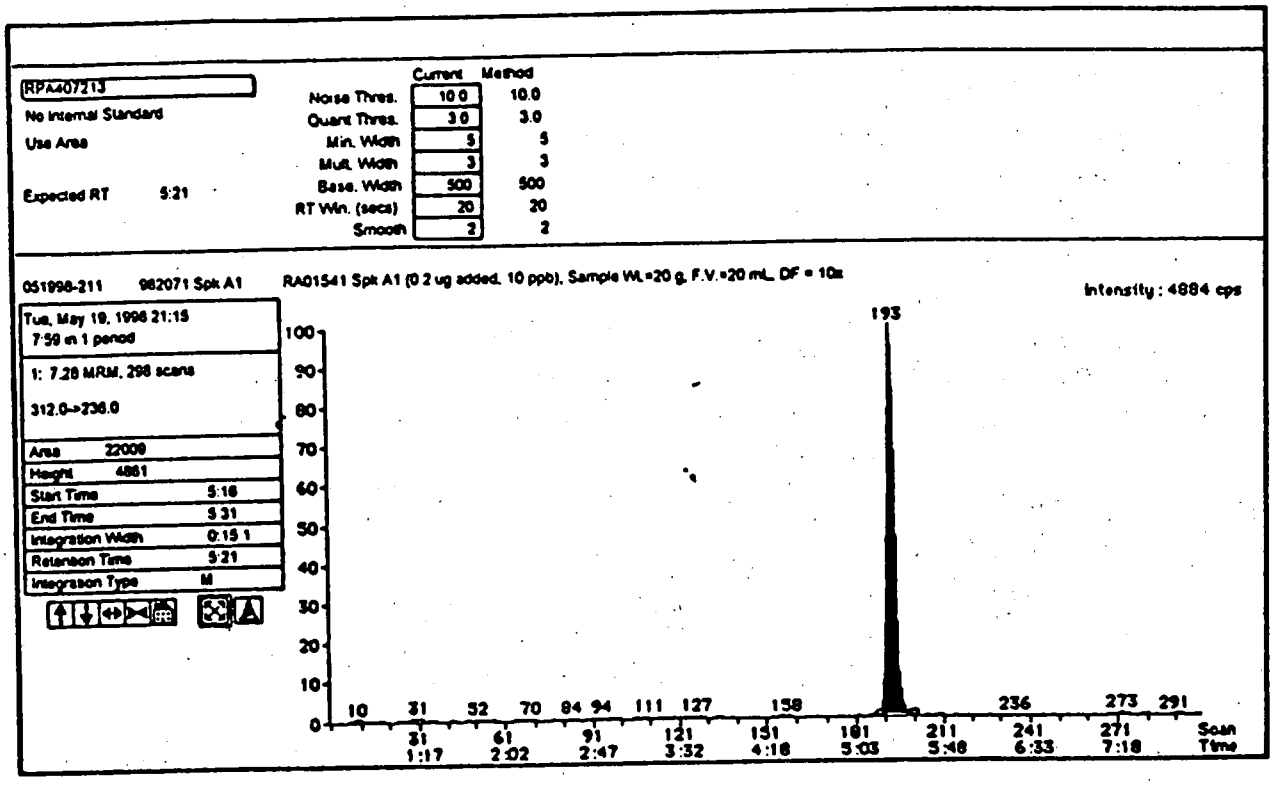


Figure 20. Soil (Florida) 10 ppb RA01541 LOQ : RPA407213 and RPA 408056



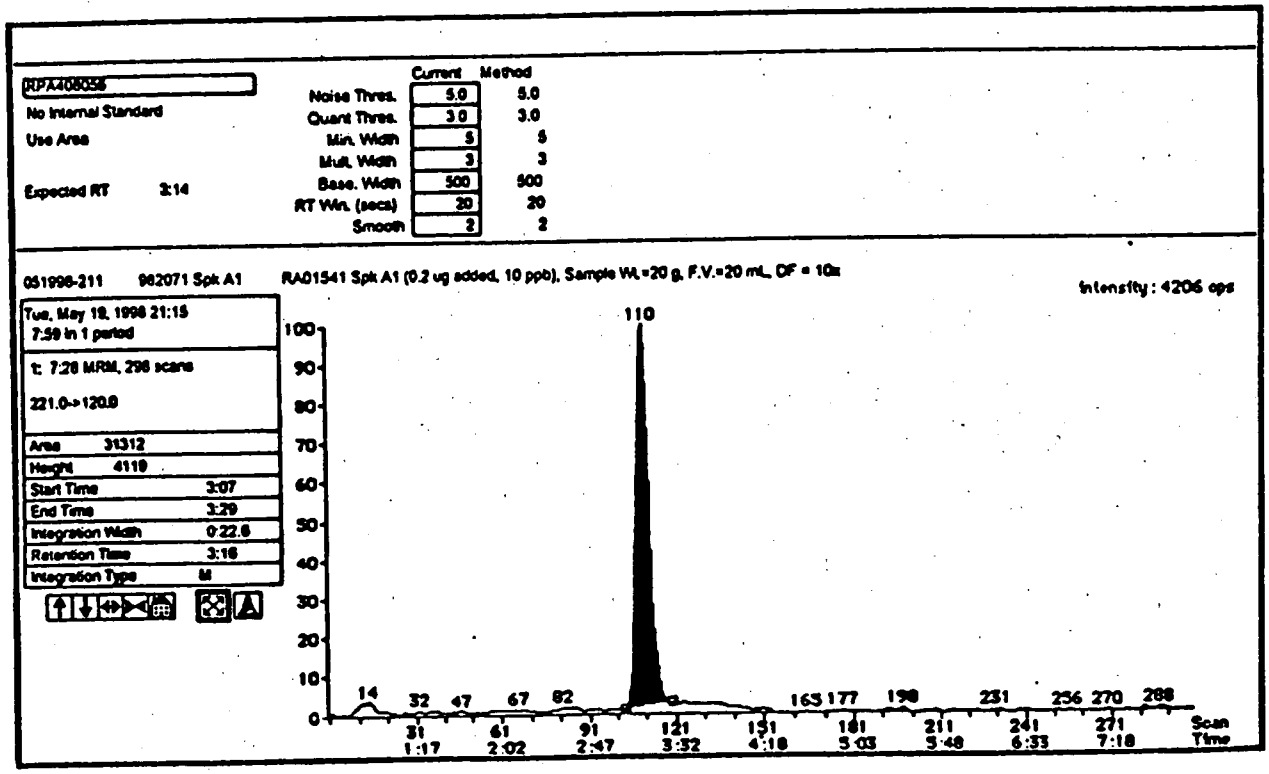


Figure 21. Soil (Florida) 10 ppb RA01541 LOQ : RPA406012 and RPA410914