

Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name: Isoxaflutole

MRID #: 448305-02

Matrix: Water

Analysis: LC/MS/MS

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RTP, NC

**Method of Analysis For the Quantification of Isoxaflutole
and Its Metabolites in Water Using Isotopically Labeled
Internal Standards - Revision 99.2**

Environmental Chemistry

May 18, 1999

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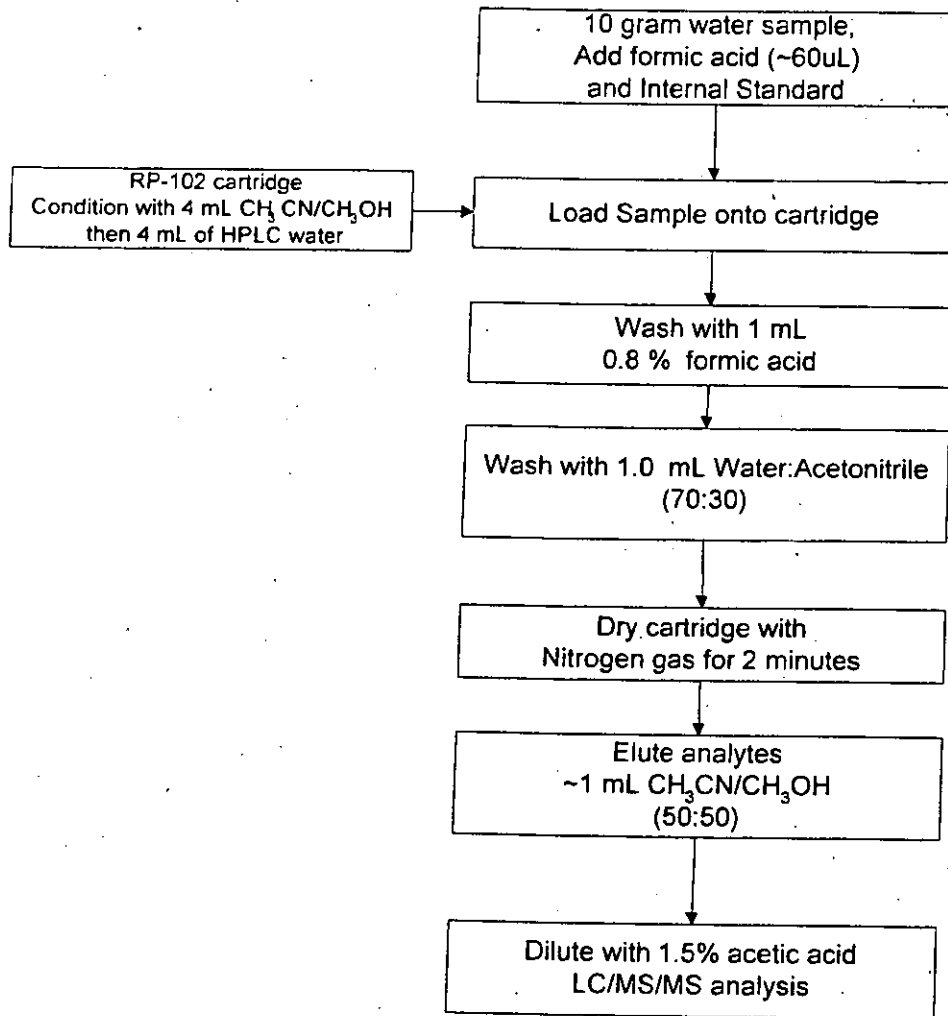
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Summary Flowchart of Analytical Method



**Method of Analysis For the Quantification of
Isoxaflutole and Its Metabolites in Water Using
Isotopically Labeled Internal Standards - Revision 99.2**

I. INTRODUCTION

A. Scope

This method sets forth the procedure for determining the residues of isoxaflutole and its metabolites RPA 202248 and RPA 203328 in ground and surface water.

B. Principle

An analytical method is described for the determination of residues of isoxaflutole and its metabolites (RPA 202248 and RPA 203328) in ground and surface water. Residues of isoxaflutole, RPA 202248 and RPA 203328 are extracted from water using a RP-102 resin cartridge, then removed with acetonitrile:methanol.

All residue analysis is accomplished by LC-MS-MS on a C8 column. Quantification of results is based on a comparison of the ratio of analyte response to internal standard response versus analyte response to internal standard response of known standards.

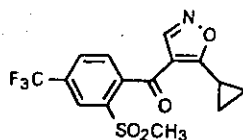
The use of isotopically labeled internal standards in the method causes sample recoveries to be relative and not absolute. The term recovery is better expressed as 'spiked sample accuracy' and as such reflects the accuracy of the method to correctly determine the level of analytes in a given sample. An estimate of the absolute recovery is obtained by comparison of the response to the internal standards in extracts of fortified samples with the response of the internal standard in calibration solutions.

C. Method Limits

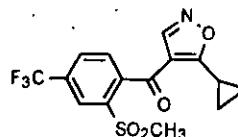
The limit of detection (LOD) was determined to be 1 parts per trillion (ppt, pg/g), for isoxaflutole, 1 ppt for RPA 202248, and 3 ppt for RPA 203328. The method has a validated LOQ of 10 ppt for isoxaflutole, RPA 202248, and RPA 203328.

D. Chemical Structures

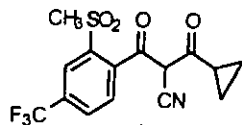
RPA 201772 ($^{12}\text{C}_6$)
m.w. 359.35



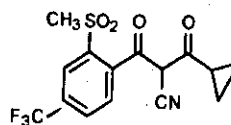
RPA 201772 ($^{13}\text{C}_6$)
m.w. 365.35



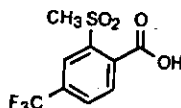
RPA 202248 ($^{12}\text{C}_6$)
m.w. 359.3



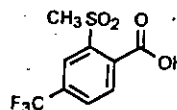
RPA 202248 ($^{13}\text{C}_6$)
m.w. 365.3



RPA 203328 ($^{12}\text{C}_6$)
m.w. 268.21



RPA 203328 ($^{13}\text{C}_6$)
m.w. 274.21

**II. MATERIALS**

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

A. Reagents/Solvents

Acetic Acid GR

(EM Science, Cat. No. AX0073-13)

Acetonitrile Omni-Solv

(EM Science, Cat. No. AX0142-1)

Formic Acid Suprapur

(EM Science, Cat. No. 11670-1)

Methanol Omni-Solv

(EM Science, Cat. No. MX0488-1)

Water

(HPLC grade)

B. Equipment and Supplies

Adapters (plastic), 1, 3 and 6 mL
(manual procedure) (Varian, 1213-1001)

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

Bottles, amber, 4 oz. (Qorpak)

Cartridges ,Spe-ed™ SPE, RP-102 Resin (200 mg/3mL)
(Applied Separations, Cat. No. 4208, *no substitute*)

Cartridge Adapters, SPE
(University Research Glass, Cat. No. URG-2440-SPECA)
(manual procedure)

Culture tube, disposable, 16 X 100mm
(automation procedure) (VWR, Cat No. 60825-425)

Disposable pipettes

Graduated cylinders

Column, HPLC, Columbus C8, 2.0 X 50mm, 5 μ m, 100A pore size
(Phenomenex; Cat. No. 00B-4187-B0, *no substitute*)

Pipette bulb

Precolumn HPLC Filter, Ultra Low Dead Volume, 0.5 μ m frit
(Upchurch, A-318)

Solvent jugs, 4 L brown glass

Stopcocks (plastic), Luer Lock
(manual procedure) (Varian, 1213-1005)

Volumetric flasks

Volumetric pipettes

Vials, chromatography, 2 or 4 ml, clear

Zymark Benchmate Series 1
(automation procedure)

C. Solutions

The following is a list of the solutions used in the analyses of ground and surface water. 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 ~0.8% Formic Acid in Water, pH 2.1
Calibrate pH meter prior to preparing solution. Place a 4 L brown glass jug onto a stir plate and add stir bar. Fill jug with ~3.0 L of H₂O. Add ~28mL of formic acid. Put the pH meter probe into the water and measure the pH of the water while it is stirring. Using a disposable pipette, add formic acid until a pH of 2.10 ± 0.02 is reached.
2. 90:10 Solution of ~0.8% Formic Acid in Water:Acetonitrile, pH 2.1
Using a 1000 mL graduated cylinder, transfer 900 mL of a solution of ~0.8% formic acid in H₂O, pH=2.1 and 100 mL CH₃CN to a 4 L brown glass solvent jug that is clean and dry or a jug which was previously used for this solution. Repeat until the desired quantity has been made.
3. Solution of 70:30 Water:Acetonitrile
Using a 1000 mL graduated cylinder, transfer 700 mL of H₂O and 300 mL CH₃CN to a 4 L brown glass solvent jug that is clean and dry or a jug which was previously used for this solution. Repeat until the desired quantity has been made.
4. Solution of 50:50 Acetonitrile:Methanol
Using a 1000 mL graduated cylinder, transfer 500 mL of CH₃OH and 500 mL CH₃CN to a 4 L brown glass solvent jug that is clean and dry or a jug which was previously used for this solution. Repeat until the desired quantity has been made.
5. Solution of ~1.5% Acetic Acid in Water

Pour ~950 mL of water into a 1 liter graduated cylinder. Using a volumetric pipette, add 15 mL of acetic acid. Make up to one liter with water. Transfer to a 4 L brown glass jug. Mix by shaking. Repeat until the desired quantity has been made.

D. Analytical Standards

Common name/alias: Isoxaflutole, RPA 201772
5-cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)isoxazole

Chemical name: Methanone, (5-cyclopropyl-4-isoxazolyl)
[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]
(CAS No. 141112-29-0)

*Solubility*¹:

acetone:	29.3(unit : g/100 ml)
acetonitrile:	23.3
hexane:	0.010
methanol:	1.38
drinking water (pH 5.5):	0.00062

Common name/alias: Isoxaflutole, RPA 201772 (¹³C₆) labeled

Chemical name: Methanone, (5-cyclopropyl-4-isoxazolyl)
[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl-¹³C₆]

Common name/alias: RPA 203328
2-methanesulphonyl-4-trifluoromethylbenzoic acid

Chemical name: Benzoic acid, 2-(methylsulfonyl)-4-
(trifluoromethyl)
(CAS No. 142994-06-7)

Common name/alias: RPA 203328 (¹³C₆) labeled

Chemical name: Benzoic acid-¹³C₆, 2-(methylsulfonyl)-4-
(trifluoromethyl)

Common name/alias: RPA 202248

2-cyclopropylcarbonyl-3-(2-methylsulphonyl-4-trifluoromethylphenyl)-3-oxopropanenitrile

Chemical name: Benzenepropanenitrile, α -(cyclopropylcarbonyl)-2-(methylsulfonyl)- β -oxo-4-(trifluoromethyl)
(CAS No. 143701-75-1)

Common name/alias: RPA 202248 ($^{13}\text{C}_6$) labeled

Chemical name: Benzene- $^{13}\text{C}_6$ -propanenitrile, α -(cyclopropylcarbonyl)-2-(methylsulfonyl)- β -oxo-4-(trifluoromethyl)

III. FORTIFICATION AND CALIBRATION STANDARD SOLUTIONS

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.

All reusable glassware should be baked in a muffle oven at ~450 °C for at least 2 hours to remove possible contamination before use.

A. Fortification Standards (non- $^{13}\text{C}_6$ labeled)

1. Weigh ~0.1000g (corrected for purity) each of isoxaflutole, RPA 202248 and RPA 203328 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 ~1000 $\mu\text{g/mL}$.
2. Transfer 10 mL each of the ~1000 $\mu\text{g/mL}$ of isoxaflutole, RPA 202248 and RPA 203328, via volumetric class "A" pipettes, to one 100 mL volumetric flask. Dilute to mark with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of this mixed standard is ~100 $\mu\text{g/mL}$ RPA 203328, isoxaflutole and RPA 202248.

3. Using a class "A" volumetric pipette, transfer 1 mL of the ~100 $\mu\text{g}/\text{mL}$ mixed standard (step III.A.2.) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of this mixed standard is ~1 $\mu\text{g}/\text{mL}$ RPA 203328, isoxaflutole and RPA 202248.
4. Using a class "A" volumetric pipette, transfer 10 mL of the ~1 $\mu\text{g}/\text{mL}$ mixed standard (step III.A.3.) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of this mixed standard is ~0.10 $\mu\text{g}/\text{mL}$ RPA 203328, isoxaflutole and RPA 202248.
5. Using a class "A" volumetric pipette, transfer 10 mL of the ~0.10 $\mu\text{g}/\text{mL}$ mixed standard (step III.A.4.) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of this mixed standard is ~0.01 $\mu\text{g}/\text{mL}$ (~10.0 ng/ml) RPA 203328, isoxaflutole and RPA 202248.
6. Using a class "A" volumetric pipette, transfer 10 mL of the ~0.01 $\mu\text{g}/\text{mL}$ mixed standard (step III.A.5.) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of this mixed standard is ~0.001 $\mu\text{g}/\text{mL}$ (~1.0 ng/ml) RPA 203328, isoxaflutole and RPA 202248.

B. ¹³C₆ labeled Internal Standards

1. Weigh ~0.0100g (corrected for purity) each of ¹³C₆ isoxaflutole, ¹³C₆ RPA 202248 and ¹³C₆ RPA 203328 into separate 100-mL volumetric flasks and dilute to the marks with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of these stock standards is ~100 $\mu\text{g}/\text{mL}$.
2. Transfer 10 mL each of the ~100 $\mu\text{g}/\text{mL}$ of ¹³C₆ isoxaflutole, ¹³C₆ RPA 202248 and ¹³C₆ RPA 203328, via volumetric class "A" pipettes, to one 100 mL volumetric flask. Dilute to mark with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of this mixed standard is ~10 $\mu\text{g}/\text{mL}$ ¹³C₆ RPA 203328, ¹³C₆ isoxaflutole and ¹³C₆ RPA 202248.

3. Using a class "A" volumetric pipette, transfer 10 mL of the ~10 $\mu\text{g/mL}$ $^{13}\text{C}_6$ mixed standard (step III.B.2.) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of this mixed standard is ~1 $\mu\text{g/mL}$ $^{13}\text{C}_6$ RPA 203328, $^{13}\text{C}_6$ isoxaflutole and $^{13}\text{C}_6$ RPA 202248.
4. Using a class "A" volumetric pipette, transfer 10 mL of the ~1 $\mu\text{g/mL}$ $^{13}\text{C}_6$ mixed standard (step III.B.3.) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of this mixed standard is ~0.1 $\mu\text{g/mL}$ $^{13}\text{C}_6$ RPA 203328, $^{13}\text{C}_6$ isoxaflutole and $^{13}\text{C}_6$ RPA 202248.
5. Using a class "A" volumetric pipette, transfer 10 mL of the ~0.1 $\mu\text{g/mL}$ $^{13}\text{C}_6$ mixed standard (step III.B.4.) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of this mixed standard is ~0.01 $\mu\text{g/mL}$ (~10.0 ng/ml) $^{13}\text{C}_6$ RPA 203328, $^{13}\text{C}_6$ isoxaflutole and $^{13}\text{C}_6$ RPA 202248.
6. Using a class "A" volumetric pipette, transfer 10 mL of the ~0. $\mu\text{g/mL}$ (~10.0 ng/ml) $^{13}\text{C}_6$ mixed standard (step III.B.5) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of this mixed standard is ~0.001 $\mu\text{g/mL}$ (~1.0 ng/mL) $^{13}\text{C}_6$ RPA 203328, $^{13}\text{C}_6$ isoxaflutole and $^{13}\text{C}_6$ RPA 202248.

C. Calibration Standards

1. Using a class "A" volumetric pipette, transfer 10 mL of the ~1.0 ng/mL *fortification* mixed standard (step III.A.6) and 10 ml of the ~1.0 ng/mL $^{13}\text{C}_6$ labeled mixed standard (step III.B.6) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of this mixed standard is ~0.1 ng/mL RPA 203328, isoxaflutole and RPA 202248 and ~0.1 ng/mL $^{13}\text{C}_6$ RPA 203328, $^{13}\text{C}_6$ isoxaflutole and $^{13}\text{C}_6$ RPA 202248
2. Using a class "A" volumetric pipette, transfer 8 mL of the ~1.0 ng/mL *fortification* mixed standard (step III.A.6.) and 10 ml of the ~1.0

- ng/mL $^{13}\text{C}_6$ labeled mixed standard (step III.B.6) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of this mixed standard is ~0.08 ng/mL RPA 203328, isoxaflutole and RPA 202248 and ~0.1 ng/mL $^{13}\text{C}_6$ RPA 203328, $^{13}\text{C}_6$ isoxaflutole and $^{13}\text{C}_6$ RPA 202248
3. Using a class "A" volumetric pipette, transfer 5 mL of the ~1.0 ng/mL *fortification* mixed standard (step III.A.6.) and 10 ml of the ~1.0 ng/mL $^{13}\text{C}_6$ labeled mixed standard (step III.B.6) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of this mixed standard is ~0.05 ng/mL RPA 203328, isoxaflutole and RPA 202248 and ~0.1 ng/mL $^{13}\text{C}_6$ RPA 203328, $^{13}\text{C}_6$ isoxaflutole and $^{13}\text{C}_6$ RPA 202248
 4. Using a class "A" volumetric pipette, transfer 2 mL of the ~1.0 ng/mL *fortification* mixed standard (step III.A.6.) and 10 ml of the ~1.0 ng/mL $^{13}\text{C}_6$ labeled mixed standard (step III.B.6) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of this mixed standard is ~0.02 ng/mL RPA 203328, isoxaflutole and RPA 202248 and ~0.1 ng/mL $^{13}\text{C}_6$ RPA 203328, $^{13}\text{C}_6$ isoxaflutole and $^{13}\text{C}_6$ RPA 202248
 5. Using a class "A" volumetric pipette, transfer 1 mL of the ~1.0 ng/mL *fortification* mixed standard (step III.A.6) and 10 ml of the ~1.0 ng/mL $^{13}\text{C}_6$ labeled mixed standard (step III.B.6) to a 100-mL volumetric flask. Dilute to mark with a 90:10 solution of ~0.8% formic acid in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$, pH 2.1. Cap and mix by inversion. The concentration of this mixed standard is ~0.01 ng/mL RPA 203328, isoxaflutole and RPA 202248 and ~0.1 ng/mL $^{13}\text{C}_6$ RPA 203328, $^{13}\text{C}_6$ isoxaflutole and $^{13}\text{C}_6$ RPA 202248
 6. *Additional calibration standards.*
Higher concentration standards (0.1 ng/mL to 5 ng/mL) may be necessary for samples with residues above the previously described curve. The amount of internal standard should continue to be ~0.1 ng/ml for these higher concentrations.

D. Stability

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

$$\text{percent stability} = [1 - (\text{old std. soln.} / \text{new std. soln.})] \times 100$$

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 $4^{\circ}\text{C} \pm 3^{\circ}\text{C}$ was stable for up to 5.5 months².
3. 100 $\mu\text{g/L}$ standard solutions: A solution of isoxaflutole , RPA 202248, and RPA 203328 prepared in 80:20 water:acetonitrile was stable for up to 5.5 months²⁻⁴.
4. 100 $\mu\text{g/L}$ standard solutions: A solution of isoxaflutole , RPA 202248, and RPA 203328 prepared in 90:10 ~0.8% formic acid:acetonitrile (pH 2.1) was stable for at least one month.

IV. METHOD PROCEDURES

A. General Notes

- A1. 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 B5 can be started earlier and does not have to be done after the completion of steps C1-C3. However, the cartridges should be used the day of conditioning.
- A4. Throughout the conditioning and elution process (unless otherwise specified) cartridges should not be allowed to run dry.
- A5. The flow rate for loading the water sample on the cartridges (step B5) is faster than the conditioning and elution flow rate
- A6. Samples that are frozen may be thawed out at ambient temperature or overnight in a refrigerator.

- A7. The suggested volume used for fortification (both $^{12}\text{C}_6$ & $^{13}\text{C}_6$ standards) is ≤ 0.25 ml of standards per 10 gram sample. Enough internal standard needs to be fortified to insure that the final sample internal standard concentration is approximately equal to the concentration of internal standard in the calibration standards.

B. Reanalysis Criteria

(one or more of the following may apply)

- B1. Individual samples should be reanalyzed if any or all of the internal standard analytes are recovered at $\leq 40\%$ of the average of the corresponding internal standard analyte from the standard curve. The standards used to determine the average should be from the same data set as the individual sample. (Because the final sample volume is not volumetrically measured, internal standard recovery is an estimate and not absolute.)
- B2. Individual samples should be reanalyzed if the ratio of analyte response to internal standard response is larger than the ratio of analyte response to internal standard response of the highest calibration standard. For reanalysis a smaller sample size may be used. The smaller sample should be brought to a starting weight of ~ 10 gram with HPLC water. The internal standard fortification should be consistent with previous analysis. *[An alternative to reanalysis is the re-injection of high ratio samples using high level calibration standards that bracket the samples. (see section III.C.6)]*

C. Ground and Surface Waters

(Analysis for Isoxaflutole (RPA 201772), RPA 202248 and RPA 203328)

- C1. Weigh ~ 10 g of sample into an appropriately sized container. Acidify the sample with ~ 60 μL of formic acid. The sample may be stored in a refrigerator until needed.
- C2. For untreated controls and samples, fortify the sample with the appropriate amount of $^{13}\text{C}_6$ labeled internal standard. Immediately add ~ 60 μL of formic acid. Cap and mix. See example in step C3.

- C3. For 'sample accuracy', fortify the sample with the appropriate standard solutions, both non-labeled and ^{13}C labeled standards. Immediately add $\sim 60\ \mu\text{L}$ of formic acid. Cap and mix. *Do not allow fortified samples to sit at room temperature for more than ~ 2 hours or low recoveries of isoxaflutole may be obtained.*
Example fortification: 10 gram water sample spiked with 0.1 ng of analyte and 0.25 ng of internal standard. Final volume of 2.5 ml gives a final concentration of 0.04 ng/ml analyte and 0.1 ng/ml internal standard. The internal standard concentration now mirrors that of the calibration standards.
- C4. Immediately set-up a RP-102 cartridge (200 mg) on a purification system. A reservoir may need to be placed on top of the cartridge.
- C5. Condition the cartridge with ~ 4 ml of 50:50 acetonitrile/methanol followed by ~ 4 ml of HPLC water. (~ 2 mL/min. Do not allow the cartridge to dry).
- C6. Apply prepared sample to the cartridge (~ 1 drop/2 sec).
- C7. Add ~ 1.0 mL of a solution of 0.8 % formic acid in water to the cartridge. (~ 1 drop/2 sec. Do not allow the cartridge to dry). Elute and discard the effluent.
- C8. Add ~ 1.0 mL of a 30:70 solution of acetonitrile/water to the cartridge (~ 1 drop/2 sec. Do not allow the cartridge to dry). Elute and discard the effluent.
- C9. Dry the cartridge for ~ 2 minutes. Vacuum or positive nitrogen pressure may be used to dry the cartridge. If the samples are prepared on a automated system, 10-30 psi nitrogen pressure can be used. If the samples are prepared on a vacuum manifold system, then ~ 20 inches of mercury vacuum could be used.
- C10. Add ~ 1.0 mL of 50:50 acetonitrile/methanol to the cartridge. Apply positive pressure and push the solvent onto the cartridge. Take precautions to insure that no eluent is lost. *Positive pressure can be applied via a hand held nitrogen line.*
Manual method, vent the pressure and allow the cartridge to soak for 1-2 minutes. Reapply pressure and elute all solvent (~ 1 drop/second) into an appropriately sized volumetric flask or chromatography vial

Automated method, the entire 1 mL of acetonitrile/methanol should be *slowly* (~ 1 minute) eluted through the cartridge. Collect the eluent in an appropriately sized volumetric flask or chromatography vial.

- C11. Dilute with ~1.5% acetic acid in H₂O. Mix. Samples are ready for LC-MS-MS analysis. Suggested final dilution volumes are ~2.5 mL for samples containing expected residues near the LOQ level of 10ppt.

ZYMARK BENCHMATE (Automated Sample Preparation)

A. Conditions

Flow Rates:

Aspirate:	0.75 ml/sec
Dispense	1.00 ml/sec
Internal Standard	0.10 ml/sec
Mix	1.25 ml/sec
Air Push	0.25 ml/sec

SPE Parameters:

Condition flow:	0.15 ml/sec
Load flow	0.08 ml/sec
Rinse flow	0.15 ml/sec
Elute flow	0.02 ml/sec
Push delay	5 seconds
Air factor	1.5

Method Procedure:

Step 1:	<u>Add internal standard</u>
Step 2:	Mix by cycling 10 ml in tube 5 times
Step 3:	Condition column with 4 ml of 50:50 ACN/MeOH
Step 4:	Condition column with 4 ml of HPLC water
Step 5:	Load sample onto column
Step 6:	Rinse column with 1.0 ml of 0.8% formic
Step 7:	Rinse column with 1.0 ml of 30:70 ACN/water
Step 8:	Dry column with gas for 120 seconds
Step 9:	Collect 1.0 ml fraction into next tube using 50:50 ACN/MeOH
Step 10:	Add 1.5 ml of ~1.5% acetic acid

Step 11: Mix by cycling 3 ml in tube 5 times
Step 12: Wash syringe with 10 ml of 50:50 ACN/MeOH
Step 13: Wash syringe with 10 ml of 0.8% formic
Step 14: END

Note the indicated benchmate parameters are guidelines and should be optimized for the instrument used. Instrument parameters may be adjusted to improve sample analysis.

It has been found useful to run at least one blank solution as a "wake up" before the actual sample runs

B. Performance Criteria

Run a set of ten 0.8% formic acid sample, surrogate fortified, blanks through the method. Set the benchmate to deliver enough internal standard per sample so that the final internal standard concentration is approximately equal to the calibration standards. Determine the standard deviation for each of the $^{13}\text{C}_6$ analytes peak area or height. If the relative standard deviation is greater than 20% for any of the analytes, optimize the instrument.

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

A. Conditions

Instrument used: Perkin Elmer Sciex API 3000 LC/MS/MS System
PE Sciex Turbo IonSpray Electrospray Interface.
Shimadzu LC-10AD VP HPLC Pumps (2) with
250 μL High Pressure Mixer and SCL-10A VP
Pump Controller
Perkin Elmer Series 200 Autosampler

Ionization and MS Mode: Electrospray (TurboIonSpray) - negative ion mode
MS/MS with multiple reaction monitoring (MRM)

IonSpray / Orifice Voltage: -4800V / -59V

Nebulizer Setting: 15 (Air)

Curtain Gas Setting: 9 (Nitrogen)

Turbo IonSpray Settings: Heated air at ~8.5L/min, 500°C

Collision Gas Setting: 8 (Nitrogen)

Collision Energy (R02-Q0): (36-10)V = 26V
(See data for complete list of instrument dependent state file parameters)

Mass Transitions (Dwell times in milli seconds):

Period 1:

RPA203328: 267/159 (375 ms)

¹³C₆ RPA203328: 273/165 (275)

Period 2:

RPA202248 and IFT: 358/79 (375)

¹³C₆ RPA202248 and ¹³C₆ IFT: 364/79 (275)

Column: Phenomenex, Columbus C8, 2.0 x 50 mm,
5µm particle size, 100A pore size

Note: Other brands tested have not retained RPA 202248.
Note: The column needs to be reconditioned after about 12 hours of use or whenever the RPA202248 peak has shifted to a retention time greater than about 6 minutes. To recondition, the column should be flushed with 100% acetonitrile for 15 minutes and then stored in that solvent for about 8 hours before re-use. Storing columns in mobile phase will result in extremely long retention time and a tailing peak for RPA202248. After the columns are stored they will have to be conditioned with the mobile phase again for approximately 30 minutes or until the RPA202248 peak is fully separated from the RPA201772 peak.

Mobile phase flow rate: 0.400 mL/min no split

Mobile phase: 48% Acetonitrile / 52% (1.5% Acetic acid in HPLC water)

Injection volume: 95 µL

Retention times: See chromatograms and data reports

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.

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.

VII. CALCULATIONS

Linear regression should be used to generate calibration curves for RPA 201772, RPA 202248, and RPA 203328. 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 or bracket samples to compensate for any minor change in instrument response. Samples should be diluted such that any peak areas or heights of the internal standards are approximately equal ($\pm 60\%$) to the internal standard peak responses in the calibration standards.

Linear regression coefficients should be calculated for the ratio of analyte to internal standard area or height plotted versus the ratio of analyte to internal standard concentration. 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:

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

where: Y = ratio of analyte response (area or height) to internal standard response (area or height)

A = intercept from linear regression analysis

B = slope from linear regression analysis (area ratio per concentration ratio)

X = ratio of analyte concentration in standards to internal standard concentration.

D = weight of internal standard (ng) added to sample divided by sample weight (g).

E = concentration of analyte in sample in parts per billion (ppb or ng/g)

B = slope from linear regression

VIII. 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.

IX. REFERENCES

1. Study number 93-129 part C R&D/CRLD/AN/9316923
2. "Isoxaflutole and/or metabolites: an analytical method for the determination of residues in drinking water" AR 112-95 I. Le Gren, July 27, 1995.
3. "Isoxaflutole and/or metabolites: Analytical method for the determination of residues in soil" AR 106-94(E) I. C. Simonin, Jan 17, 1995.
4. "Isoxaflutole and/or metabolites: Analytical method for the determination of residues in animal products" AR 109-95(E) I. Le Gren, April 12, 1995.

X SPIKED SAMPLE ACCURACY

Ground Water Accuracy Data

Procedure	Sample ID	Fortification level (ppt)	Accuracy (%)		
			RPA 203328	RPA 202248	RPA 201772
automation	324AP2-1	0	na	na	0
automation	324AP2-2	10	198	100	95
automation	324AP2-3	10	105	105	100
automation	324AP2-6	100	98	100	103
automation	324AP2-7	100	98	100	113

Surface Water Accuracy Data

Procedure	Sample ID	Fortification level (ppt)	Accuracy(%)		
			RPA 203328	RPA 202248	RPA 201772
automation	3181676-BM1	0	na	< 1ppt	na
automation	3181676-BM2	10	98	100	88
automation	4021676-4BM	100	104	98	94
manual	329-1826-1	0	na	< 1ppt	1ppt
manual	329-1826-2	10	93	100	110
manual	329-1826-5	100	98	95	110

XI. EXAMPLE CHROMATOGRAMS

A. Calibration Data

Figure 1. Standard: 0.01 ng/ml - RPA 202248, RPA 201772, RPA 203328

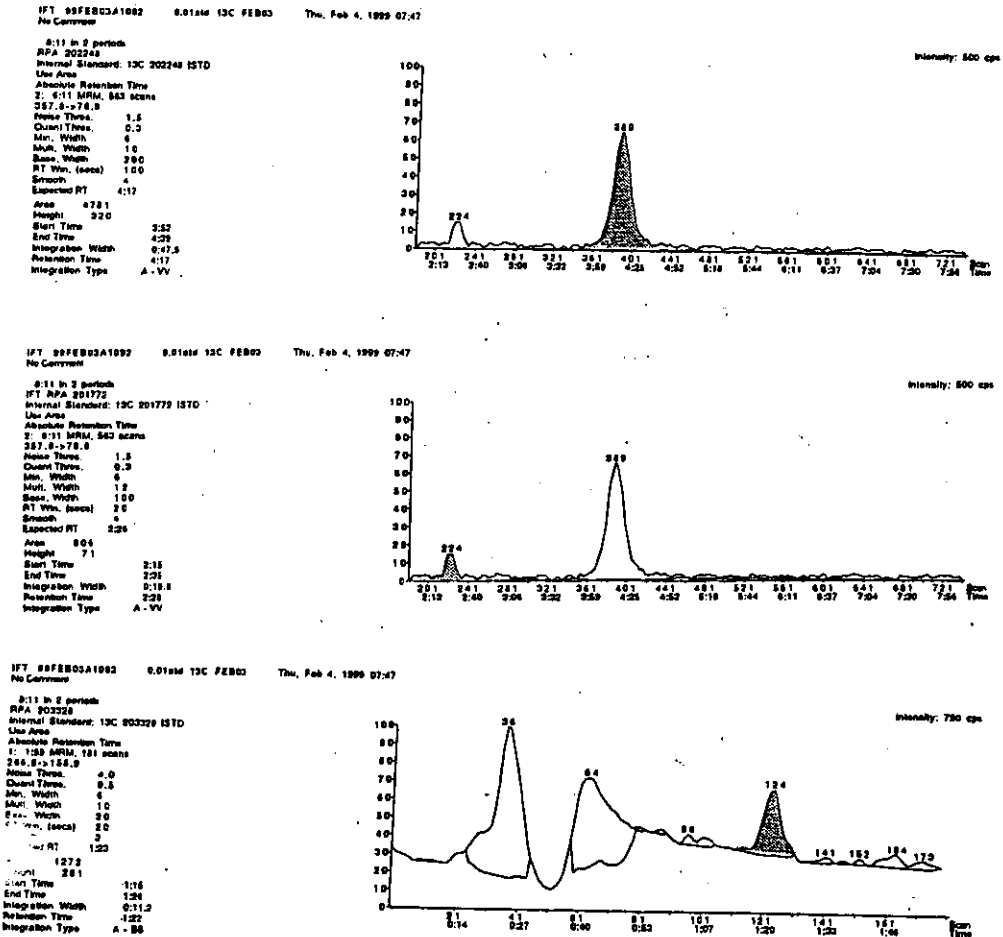


Figure 2. Standard: 0.02 ng/ml - RPA 202248, RPA 201772, RPA 203328

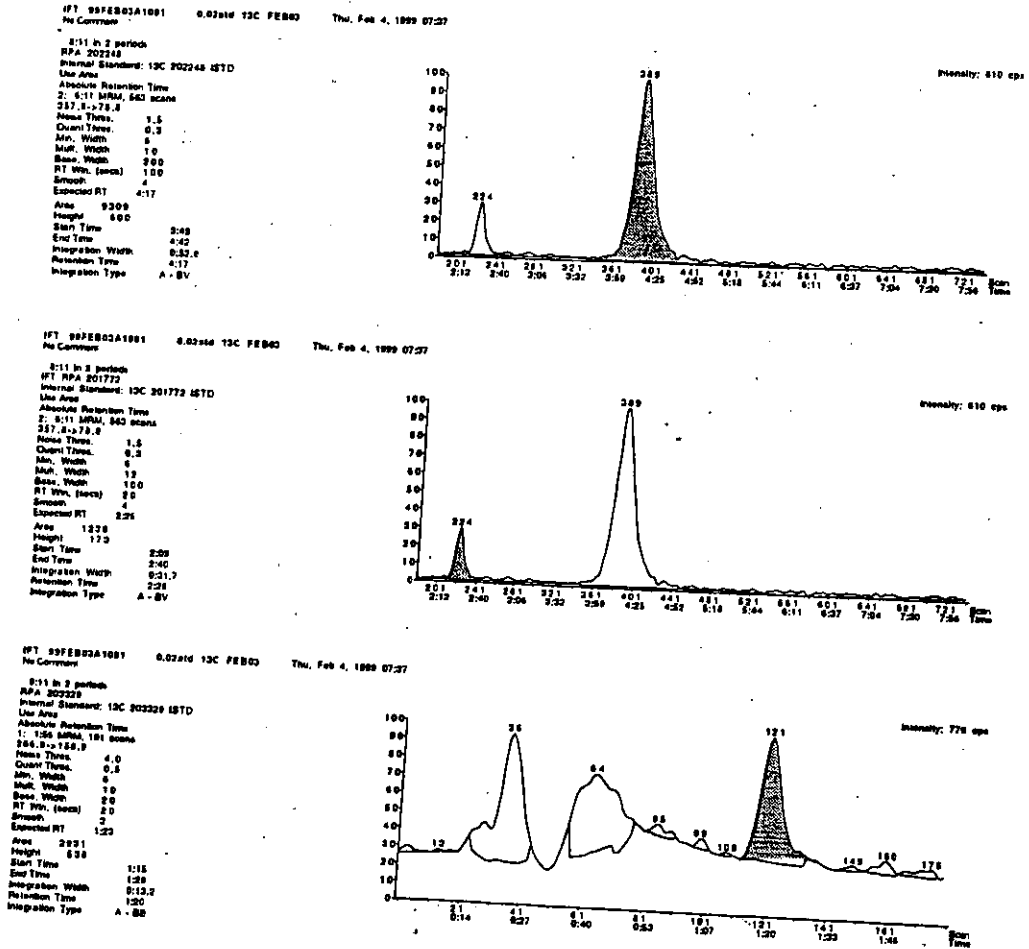


Figure 3. Standard: 0.05 ng/ml - RPA 202248, RPA 201772, RPA 203328

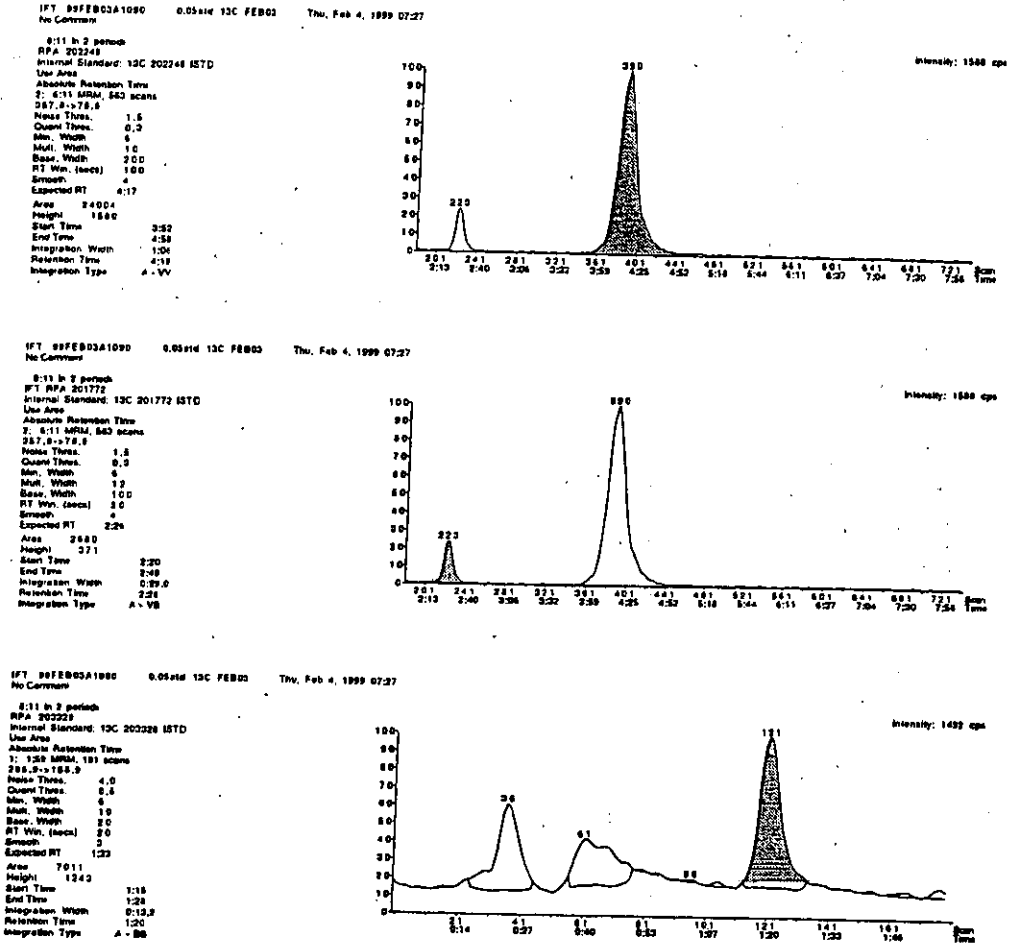


Figure 4. Standard: 0.08 ng/ml - RPA 202248, RPA 201772, RPA 203328

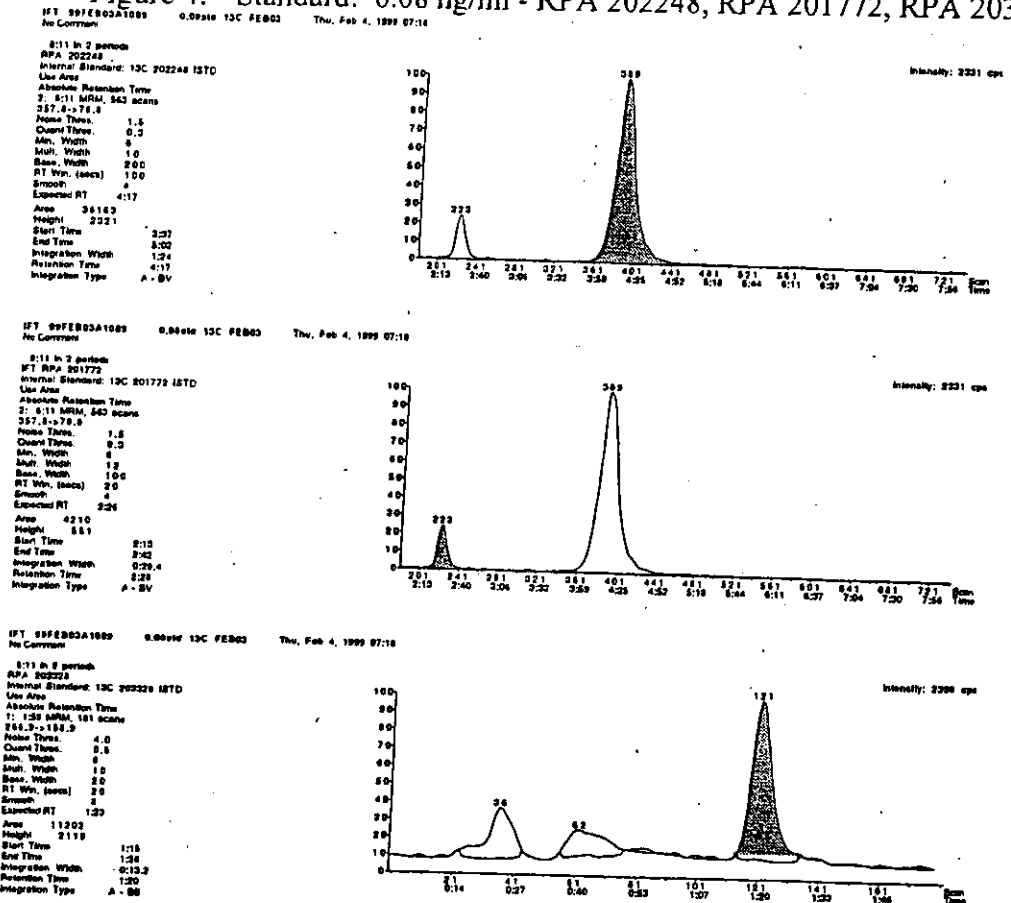
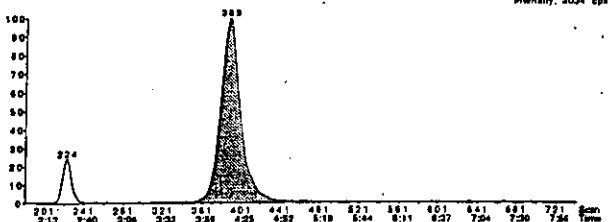


Figure 5. Standard: 0.10 ng/ml - RPA 202248, RPA 201772, RPA 203328

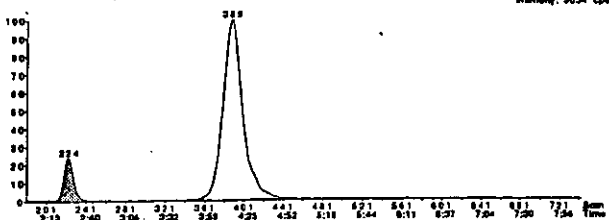
IFT 09FEB03A1088 0.1016 13C FEB03 Thu, Feb 4, 1999 07:00

0:11 In 2 periods
 RPA 202248
 Internal Standard: 13C 202248 ISTD
 Use Area
 Absolute Retention Time
 2: 6:11 MRM, 642 acans
 287.8-278.8
 Noise Thres. 1.5
 Quant Thres. 0.3
 Min. Width 6
 Mult. Width 1.0
 Base. Width 200
 RT Wn. (secs) 100
 Smooth 4
 Expected RT 4:17
 Area 47918
 Height 3022
 Start Time 3:48
 End Time 6:11
 Integration Width 1:20
 Retention Time 4:17
 Integration Type A - VV



IFT 09FEB03A1088 0.1016 13C FEB03 Thu, Feb 4, 1999 07:00

0:11 In 2 periods
 RPA 201772
 Internal Standard: 13C 201772 ISTD
 Use Area
 Absolute Retention Time
 2: 6:11 MRM, 563 acans
 287.4-278.6
 Noise Thres. 1.5
 Quant Thres. 0.3
 Min. Width 6
 Mult. Width 1.2
 Base. Width 100
 RT Wn. (secs) 20
 Smooth 4
 Expected RT 2:28
 Area 6513
 Height 728
 Start Time 2:16
 End Time 2:51
 Integration Width 0:35.6
 Retention Time 2:28
 Integration Type A - VV



IFT 09FEB03A1088 0.1016 13C FEB03 Thu, Feb 4, 1999 07:00

0:11 In 2 periods
 RPA 203328
 Internal Standard: 13C 203328 ISTD
 Use Area
 Absolute Retention Time
 1: 1:29 MRM, 161 acans
 288.5-278.8
 Noise Thres. 4.0
 Quant Thres. 0.5
 Min. Width 6
 Mult. Width 1.0
 Base. Width 28
 RT Wn. (secs) 28
 Smooth 3
 Expected RT 1:22
 Area 14645
 Height 2678
 Start Time 1:18
 End Time 1:26
 Integration Width 0:12.2
 Retention Time 1:20
 Integration Type A - BS

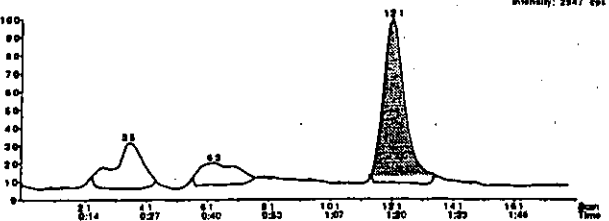
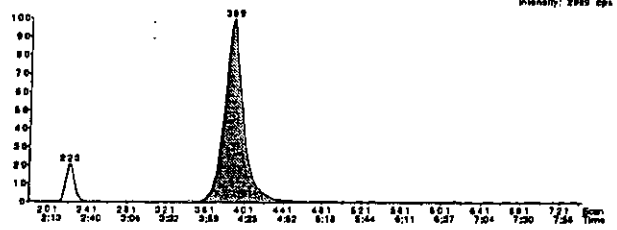


Figure 6. Internal Standard: 0.10 ng/ml - ¹³C₆ RPA 202248,
¹³C₆ RPA 201772, ¹³C₆ RPA 203328

IFT 99FEB03A1088 0.1000 13C FEB03 Thu, Feb 4, 1999 07:00
 No Comment

8:11 in 2 periods
 13C 202248 ISID
 use as Internal Standard

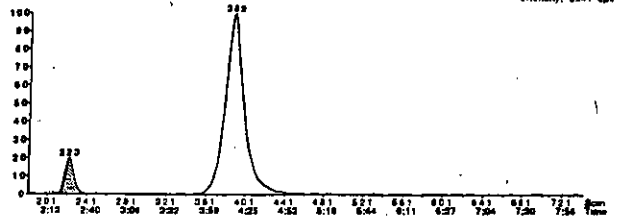
2: 8:11 MRM, 843 scans
 262.8->78.8
 Noise Thresh. 5.0
 Quant Thresh. 0.3
 Min. Width 4
 Mult. Width 10
 Base. Width 200
 RT Wn. (secs) 100
 Smooth 2
 Expected RT 8:15
 Area 43883
 Height 2583
 Start Time 8:30
 End Time 4:56
 Integration Width 100
 Retention Time 4:17
 Integration Type A - BV



IFT 99FEB03A1088 0.1000 13C FEB03 Thu, Feb 4, 1999 07:00
 No Comment

8:11 in 2 periods
 13C 201772 ISID
 use as Internal Standard

2: 8:11 MRM, 843 scans
 262.8->78.8
 Noise Thresh. 4.8
 Quant Thresh. 0.3
 Min. Width 5
 Mult. Width 10
 Base. Width 100
 RT Wn. (secs) 20
 Smooth 4
 Expected RT 2:24
 Area 4323
 Height 525
 Start Time 2:17
 End Time 2:34
 Integration Width 0:26.8
 Retention Time 2:21
 Integration Type A - BV



IFT 99FEB03A1088 0.1000 13C FEB03 Thu, Feb 4, 1999 07:00
 No Comment

8:11 in 2 periods
 13C 203328 ISID
 use as Internal Standard

1: 1:20 MRM, 181 scans
 273.9->184.9
 Noise Thresh. 20.0
 Quant Thresh. 0.5
 Min. Width 5
 Mult. Width 10
 Base. Width 20
 RT Wn. (secs) 20
 Smooth 3
 Expected RT 1:23
 Area 13885
 Height 2383
 Start Time 1:12
 End Time 1:29
 Integration Width 0:22.1
 Retention Time 1:19
 Integration Type A - BV

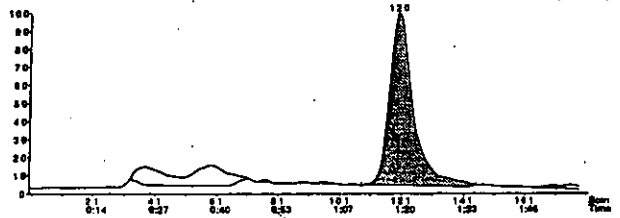


Figure 7. Standard Calibration Curve for RPA 203328

RPA 203328 266.9->158.9 Internal Standard: 13C 203328 ISTD

Linear

Intercept = 0.001

Slope = 9.960

Correlation Coeff. = 0.9992

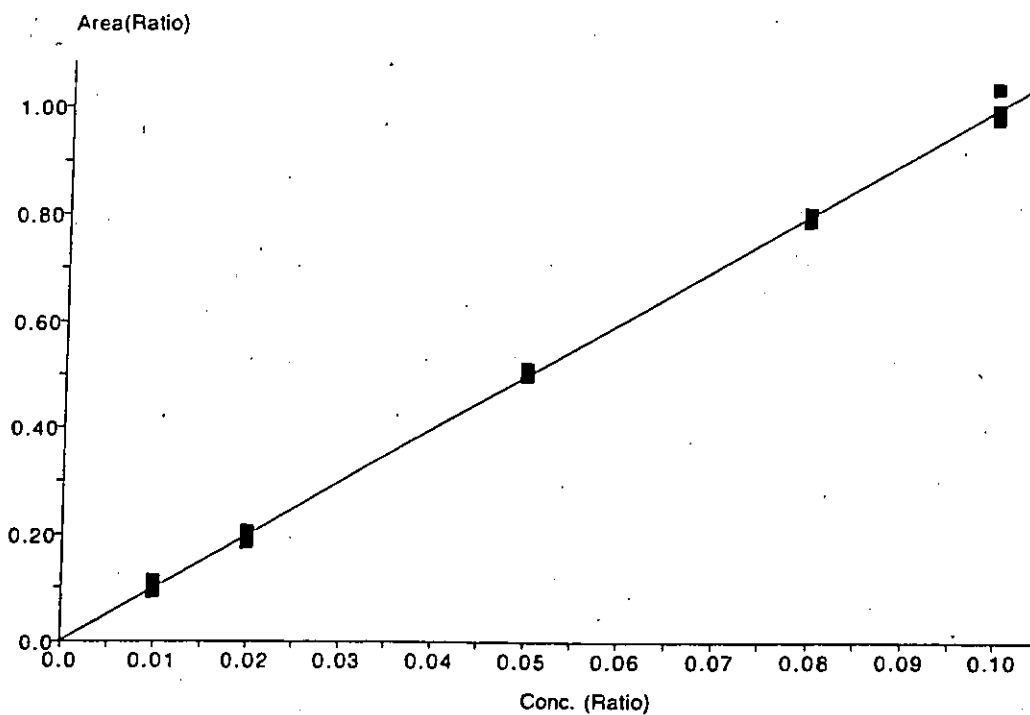


Figure 8. Standard Calibration Curve for RPA 202248

RPA 202248 357.8->78.8 Internal Standard: 13C 202248 ISTD
Linear
Intercept = 0.014
Slope = 10.324
Correlation Coeff. = 0.9991

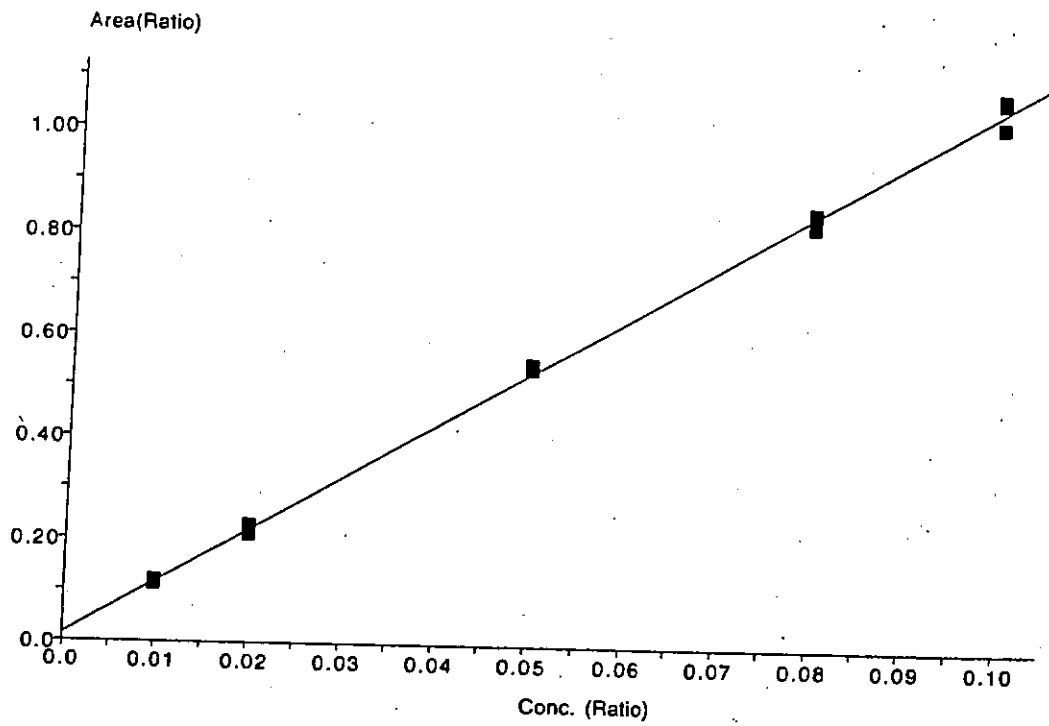
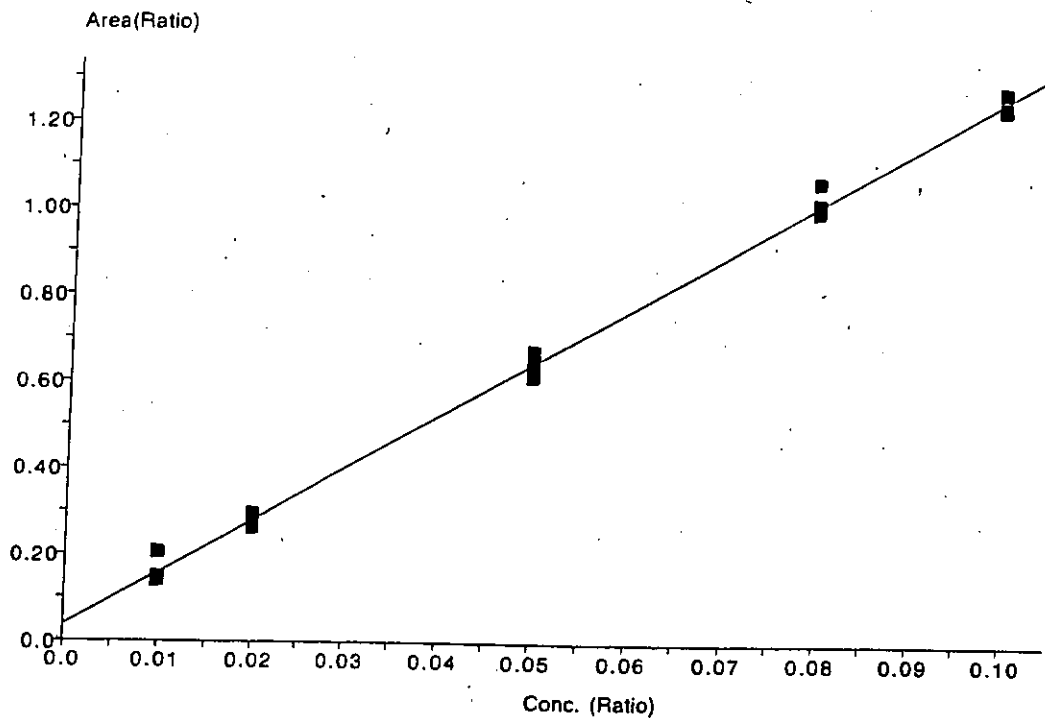


Figure 9. Standard Calibration Curve for RPA 201772

IFT RPA 201772 357.8->78.8 Internal Standard: 13C 201772 ISTD
Linear

Intercept = 0.036
Slope = 12.182
Correlation Coeff. = 0.9983



B. Results Tables

RPAC 203328 Results Table

RPA 203328
 Internal Standard: 13C 203328 ISTD
 266.9->155.9
 Linear
 Intercept = 0.001
 Slope = 9.960
 Correlation Coeff. = 0.9992
 Use Area

Filename	Filetype	Sample Name	Accuracy	Calc. Conc.	Conc.	Area	IS Area	R.T.	S/N
IFT 99FEB03A1086	Standard	0.1std 13C FEB03	103.6	0.10364	0.10000	14443	13993	1:20	154.531
IFT 99FEB03A1089	Standard	0.06std 13C FEB03	100.3	0.06028	0.06000	11202	13999	1:20	123.646
IFT 99FEB03A1090	Standard	0.05std 13C FEB03	99.7	0.04984	0.05000	7011	14106	1:20	67.848
IFT 99FEB03A1081	Standard	0.02std 13C FEB03	102.7	0.02054	0.02000	2931	14284	1:20	34.752
IFT 99FEB03A1092	Standard	0.01std 13C FEB03	91.3	0.00912	0.01000	1272	13893	1:22	16.938
IFT 99FEB03A1093	CC	blk	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1094	CC	blk	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1095	CC	0.8 FORMIC BLANK-1	n/a	n/a	0.0	792	n/a	1:21	11.485
IFT 99FEB03A1096	CC	0.8 FORMIC BLANK-3	n/a	n/a	0.0	926	n/a	1:21	8.901
IFT 99FEB03A1097	CC	0.8 FORMIC BLANK-5	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1098	CC	0.8 FORMIC BLANK-7	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1099	CC	0.8 FORMIC-10PPT-1	113.3	0.05666	0.05000	6361	11258	1:22	91.671
IFT 99FEB03A1100	CC	0.8 FORMIC-10PPT-3	107.0	0.05350	0.05000	6090	11416	1:21	58.350
IFT 99FEB03A1101	CC	0.8 FORMIC-10PPT-5	97.2	0.04859	0.05000	5302	10641	1:22	59.100
IFT 99FEB03A1102	CC	0.8 FORMIC-10PPT-7	106.8	0.05339	0.05000	5699	10704	1:21	73.444
IFT 99FEB03A1103	CC	0.8 FORMIC-10PPT-9	103.7	0.05183	0.05000	6309	12207	1:22	61.898
IFT 99FEB03A1104	CC	1826-10PPT-1	111.2	0.05582	0.05000	5352	9662	1:21	63.758
IFT 99FEB03A1105	CC	1826-10PPT-3	106.9	0.05344	0.05000	5783	10851	1:22	73.664
IFT 99FEB03A1106	CC	1826-10PPT-5	100.3	0.05016	0.05000	6282	10558	1:21	60.860
IFT 99FEB03A1107	CC	1826-10PPT-7	105.7	0.05287	0.05000	5979	11340	1:21	81.518
IFT 99FEB03A1108	CC	1826-10PPT-9	114.8	0.05739	0.05000	6242	10919	1:23	73.780
IFT 99FEB03A1109	Standard	0.1std 13C FEB03	98.0	0.09797	0.10000	14227	14871	1:20	136.221
IFT 99FEB03A1110	Standard	0.06std 13C FEB03	99.2	0.07934	0.06000	11434	14457	1:20	135.739
IFT 99FEB03A1111	Standard	0.05std 13C FEB03	98.4	0.04971	0.05000	7107	14337	1:19	75.999
IFT 99FEB03A1112	Standard	0.02std 13C FEB03	92.7	0.01853	0.02000	2733	14758	1:19	34.766
IFT 99FEB03A1113	Standard	0.01std 13C FEB03	111.9	0.01119	0.01000	1874	14935	1:21	18.195
IFT 99FEB03A1114	CC	blk	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1115	CC	blk	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1116	CC	1826-20PPT-1	106.3	0.10627	0.10000	11284	10655	1:20	107.951
IFT 99FEB03A1117	CC	1826-20PPT-2	110.7	0.11072	0.10000	12156	11017	1:20	111.189
IFT 99FEB03A1118	CC	1826-20PPT-3	109.8	0.10981	0.10000	10131	9257	1:20	95.373
IFT 99FEB03A1119	CC	1826-20PPT-4	99.6	0.09957	0.10000	9148	9219	1:19	104.959
IFT 99FEB03A1120	CC	1826-20PPT-5	96.2	0.09624	0.10000	9992	10416	1:20	73.595
IFT 99FEB03A1121	CC	0.8 FORMIC BLANK-7	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1122	CC	0.8 FORMIC BLANK-8	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1123	CC	0.8 FORMIC BLANK-9	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1124	CC	1826-BLANK-1	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1125	CC	1826-BLANK-2	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1126	CC	1826-BLANK-3	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1127	CC	M-RB	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1128	CC	M-UTC	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1129	CC	M-0.8 FORMIC-10PPT-1	86.9	0.04345	0.05000	3731	8613	1:18	40.394
IFT 99FEB03A1130	CC	M-0.8 FORMIC-10PPT-2	86.5	0.04825	0.05000	4329	8885	1:18	41.235
IFT 99FEB03A1131	CC	M-0.8 FORMIC-10PPT-1	93.3	0.04667	0.05000	3943	8471	1:18	33.961
IFT 99FEB03A1132	CC	M-0.8 FORMIC-10PPT-2	n/a	n/a	0.05000	n/a	n/a	n/a	n/a
IFT 99FEB03A1133	CC	M-1826-20PPT-1	102.7	0.10268	0.10000	8437	8244	1:18	104.640
IFT 99FEB03A1134	Standard	0.1std 13C FEB03	99.4	0.09945	0.10000	14478	14608	1:19	182.389
IFT 99FEB03A1135	Standard	0.06std 13C FEB03	98.6	0.07884	0.06000	11393	14487	1:19	134.838
IFT 99FEB03A1136	Standard	0.05std 13C FEB03	102.3	0.05114	0.05000	7488	14885	1:19	70.990
IFT 99FEB03A1137	Standard	0.02std 13C FEB03	97.3	0.01948	0.02000	2665	14748	1:20	28.476
IFT 99FEB03A1138	Standard	0.01std 13C FEB03	109.6	0.01095	0.01000	1590	14492	1:22	14.166

RPA 202248 Results Table

RPA 202248
 Internal Standard: 13C 202248 ISTD
 157.6->76.E
 Lines: 1
 Intercept = 0.014
 Slope = 10.324
 Correlation Coeff. = 0.9991
 Use Area

Filename	Filetype	Sample Name	Accuracy	Calc. Conc.	Conc.	Area	IS Area	R.I.	S/N
IFT 99FEB03A1088	Standard	0.1std 13C FEB03	101.7	0.10169	0.10000	47214	44363	4:17	267.210
IFT 99FEB03A1089	Standard	0.08std 13C FEB03	97.2	0.07775	0.08000	36163	44249	4:17	223.710
IFT 99FEB03A1090	Standard	0.05std 13C FEB03	102.2	0.05110	0.05000	24004	44286	4:18	155.019
IFT 99FEB03A1091	Standard	0.02std 13C FEB03	94.2	0.01884	0.02000	9309	44530	4:17	62.569
IFT 99FEB03A1092	Standard	0.01std 13C FEB03	93.7	0.00937	0.01000	4781	42965	4:17	30.793
IFT 99FEB03A1093	CC	blk	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1094	CC	blk	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1095	CC	0.8 FORMIC BLANK-1	n/a	n/a	0.0	1562	n/a	4:10	11.306
IFT 99FEB03A1096	CC	0.2 FORMIC BLANK-2	n/a	n/a	0.0	1361	n/a	4:08	7.135
IFT 99FEB03A1097	CC	0.2 FORMIC BLANK-5	n/a	n/a	0.0	1175	n/a	4:13	8.571
IFT 99FEB03A1098	CC	0.8 FORMIC BLANK-7	n/a	n/a	0.0	959	n/a	4:11	5.780
IFT 99FEB03A1099	CC	0.2 FORMIC-10PPT-1	101.0	0.05050	0.05000	23220	43330	4:16	149.165
IFT 99FEB03A1100	CC	0.2 FORMIC-10PPT-3	101.2	0.05060	0.05000	23633	44037	4:15	155.573
IFT 99FEB03A1101	CC	0.2 FORMIC-10PPT-5	100.0	0.05000	0.05000	23548	44371	4:17	146.222
IFT 99FEB03A1102	CC	0.2 FORMIC-10PPT-7	100.6	0.05030	0.05000	23955	44877	4:15	142.967
IFT 99FEB03A1103	CC	0.2 FORMIC-10PPT-9	102.9	0.05147	0.05000	23598	44011	4:17	149.459
IFT 99FEB03A1104	CC	1826-10PPT-1	100.4	0.05019	0.05000	23444	44111	4:16	148.975
IFT 99FEB03A1105	CC	1826-10PPT-3	96.6	0.04829	0.05000	23192	45204	4:17	164.804
IFT 99FEB03A1106	CC	1826-10PPT-5	102.7	0.05136	0.05000	24277	44582	4:17	162.605
IFT 99FEB03A1107	CC	1826-10PPT-7	101.5	0.05073	0.05000	23398	44111	4:16	141.174
IFT 99FEB03A1108	CC	1826-10PPT-9	101.2	0.05058	0.05000	24330	45335	4:19	145.917
IFT 99FEB03A1109	Standard	0.1std 13C FEB03	97.2	0.08715	0.10000	44491	43723	4:16	283.041
IFT 99FEB03A1110	Standard	0.08std 13C FEB03	98.0	0.07842	0.08000	35951	43620	4:14	208.312
IFT 99FEB03A1111	Standard	0.05std 13C FEB03	101.0	0.05052	0.05000	24075	44812	4:12	158.560
IFT 99FEB03A1112	Standard	0.02std 13C FEB03	101.8	0.02036	0.02000	10205	45407	4:10	65.612
IFT 99FEB03A1113	Standard	0.01std 13C FEB03	97.7	0.00977	0.01000	5435	47117	4:19	36.306
IFT 99FEB03A1114	CC	blk	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1115	CC	blk	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1116	CC	1826-20PPT-1	102.6	0.10259	0.10000	45934	42782	4:05	311.170
IFT 99FEB03A1117	CC	1826-20PPT-2	94.1	0.08414	0.10000	47313	47962	4:09	294.422
IFT 99FEB03A1118	CC	1826-20PPT-3	101.3	0.10133	0.10000	46409	43734	4:11	266.062
IFT 99FEB03A1119	CC	1826-20PPT-4	99.2	0.09923	0.10000	45769	44053	4:10	275.097
IFT 99FEB03A1120	CC	1826-20PPT-5	101.2	0.10121	0.10000	44912	42931	4:11	272.892
IFT 99FEB03A1121	CC	0.2 FORMIC BLANK-7	n/a	n/a	0.0	1722	n/a	4:15	9.351
IFT 99FEB03A1122	CC	0.2 FORMIC BLANK-8	n/a	n/a	0.0	888	n/a	4:13	6.887
IFT 99FEB03A1123	CC	0.2 FORMIC BLANK-9	n/a	n/a	0.0	1020	n/a	4:13	5.781
IFT 99FEB03A1124	CC	1826-BLANK-1	n/a	n/a	0.0	960	n/a	4:15	6.473
IFT 99FEB03A1125	CC	1826-BLANK-2	n/a	n/a	0.0	795	n/a	4:13	4.839
IFT 99FEB03A1126	CC	1826-BLANK-3	n/a	n/a	0.0	888	n/a	4:13	5.547
IFT 99FEB03A1127	CC	M-RB	n/a	n/a	0.0	738	n/a	4:06	4.340
IFT 99FEB03A1128	CC	M-LTC	n/a	n/a	0.0	738	n/a	4:06	4.896
IFT 99FEB03A1129	CC	M-0.8 FORMIC-10PPT-1	102.0	0.08102	0.05000	23589	43584	4:07	135.113
IFT 99FEB03A1130	CC	M-0.8 FORMIC-10PPT-2	101.4	0.05088	0.05000	24085	44789	4:04	130.190
IFT 99FEB03A1131	CC	M-0.8 FORMIC-10PPT-1	99.0	0.04892	0.05000	30532	58076	4:02	164.785
IFT 99FEB03A1132	CC	M-0.8 FORMIC-10PPT-2	101.7	0.05085	0.05000	22484	41878	4:03	127.611
IFT 99FEB03A1133	CC	M-1826-20PPT-1	102.6	0.10262	0.10000	46112	42936	4:04	270.543
IFT 99FEB03A1134	Standard	0.1std 13C FEB03	102.4	0.10240	0.10000	46777	43645	4:13	286.844
IFT 99FEB03A1135	Standard	0.08std 13C FEB03	100.6	0.08051	0.08000	37386	44207	4:14	248.893
IFT 99FEB03A1136	Standard	0.05std 13C FEB03	102.9	0.05144	0.05000	24686	45249	4:16	160.223
IFT 99FEB03A1137	Standard	0.02std 13C FEB03	102.9	0.02058	0.02000	10192	44809	4:17	66.147
IFT 99FEB03A1138	Standard	0.01std 13C FEB03	100.9	0.01009	0.01000	5291	44590	4:23	33.776

RPA 201772 Results Table

IFT RPA 201772
 Internal Standard: 13C 201772 ISTD
 357.8->78.8
 Linear
 Intercept = 0.036
 Slope = 12.182
 Correlation Coeff. = 0.9983
 Use Area

Filename	Filetype	Sample Name	Accuracy	Calc. Conc.	Conc.	Area	IS Area	RI	S/N
IFT 99FEB03A1082	Standard	0.1std 13C FEB03	101.5	0.10153	0.10000	5515	4333	2:28	69.145
IFT 99FEB03A1089	Standard	0.06std 13C FEB03	105.3	0.06420	0.08000	4210	3965	2:28	53.063
IFT 99FEB03A1090	Standard	0.05std 13C FEB03	99.2	0.04859	0.05000	2660	4185	2:28	36.400
IFT 99FEB03A1091	Standard	0.02std 13C FEB03	105.7	0.02114	0.02000	1232	4216	2:28	18.065
IFT 99FEB03A1092	Standard	0.01std 13C FEB03	90.2	0.00802	0.01000	60E	4153	2:28	6.796
IFT 99FEB03A1093	CC	blk	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1094	CC	blk	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1095	CC	0.8 FORMIC BLANK-1	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1096	CC	0.8 FORMIC BLANK-3	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1097	CC	0.8 FORMIC BLANK-5	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1098	CC	0.8 FORMIC BLANK-7	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1099	CC	0.8 FORMIC-10PPT-1	106.3	0.06313	0.05000	233E	3418	2:27	32.089
IFT 99FEB03A1100	CC	0.8 FORMIC-10PPT-3	99.0	0.04848	0.05000	247E	3680	2:26	32.460
IFT 99FEB03A1101	CC	0.8 FORMIC-10PPT-5	106.6	0.05301	0.05000	2717	3953	2:27	32.151
IFT 99FEB03A1102	CC	0.8 FORMIC-10PPT-7	106.5	0.05327	0.05000	2689	3925	2:27	34.477
IFT 99FEB03A1103	CC	0.8 FORMIC-10PPT-9	101.5	0.05076	0.05000	2562	3914	2:28	32.358
IFT 99FEB03A1104	CC	1826-10PPT-1	91.0	0.04552	0.05000	2204	3731	2:28	29.831
IFT 99FEB03A1105	CC	1826-10PPT-3	100.8	0.05041	0.05000	249E	3837	2:28	33.625
IFT 99FEB03A1106	CC	1826-10PPT-5	115.8	0.05789	0.05000	2692	3632	2:27	30.403
IFT 99FEB03A1107	CC	1826-10PPT-7	93.7	0.04884	0.05000	2334	3647	2:27	29.999
IFT 99FEB03A1108	CC	1826-10PPT-9	88.9	0.04443	0.05000	2220	384E	2:28	26.954
IFT 99FEB03A1109	Standard	0.1std 13C FEB03	99.6	0.09863	0.10000	548E	4436	2:27	73.899
IFT 99FEB03A1110	Standard	0.06std 13C FEB03	100.1	0.08010	0.08000	426E	4208	2:26	61.535
IFT 99FEB03A1111	Standard	0.05std 13C FEB03	94.6	0.04732	0.05000	2751	4491	2:25	36.897
IFT 99FEB03A1112	Standard	0.02std 13C FEB03	92.5	0.01850	0.02000	1184	4528	2:24	14.184
IFT 99FEB03A1113	Standard	0.01std 13C FEB03	83.5	0.00835	0.01000	64E	4608	2:26	9.466
IFT 99FEB03A1114	CC	blk	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1115	CC	blk	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1116	CC	1826-20PPT-1	88.4	0.08841	0.10000	4218	3789	2:24	60.859
IFT 99FEB03A1117	CC	1826-20PPT-2	94.3	0.09428	0.10000	4650	3825	2:25	63.377
IFT 99FEB03A1118	CC	1826-20PPT-3	106.0	0.10602	0.10000	4754	3681	2:26	50.107
IFT 99FEB03A1119	CC	1826-20PPT-4	105.3	0.10533	0.10000	5224	3960	2:26	66.324
IFT 99FEB03A1120	CC	1826-20PPT-5	99.7	0.09865	0.10000	4839	3871	2:26	62.248
IFT 99FEB03A1121	CC	0.8 FORMIC BLANK-7	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1122	CC	0.8 FORMIC BLANK-9	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1123	CC	0.8 FORMIC BLANK-8	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1124	CC	1826-BLANK-1	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1125	CC	1826-BLANK-2	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1126	CC	1826-BLANK-3	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1127	CC	M-RB	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1128	CC	M-UTC	n/a	n/a	0.0	n/a	n/a	n/a	n/a
IFT 99FEB03A1129	CC	M-0.8 FORMIC-10PPT-1	114.0	0.05700	0.05000	3133	4289	2:24	41.119
IFT 99FEB03A1130	CC	M-0.8 FORMIC-10PPT-2	92.5	0.04824	0.05000	2857	476E	2:24	36.507
IFT 99FEB03A1131	CC	M-0.8 FORMIC-10PPT-1	88.4	0.04421	0.05000	3137	545E	2:23	42.795
IFT 99FEB03A1132	CC	M-0.8 FORMIC-10PPT-2	103.0	0.05150	0.05000	2613	3938	2:24	32.838
IFT 99FEB03A1133	CC	M-1826-20PPT-1	107.9	0.10794	0.10000	5352	3981	2:24	74.656
IFT 99FEB03A1134	Standard	0.1std 13C FEB03	98.2	0.09821	0.10000	5318	4314	2:26	70.873
IFT 99FEB03A1135	Standard	0.06std 13C FEB03	98.2	0.07854	0.08000	4244	4275	2:26	57.007
IFT 99FEB03A1136	Standard	0.05std 13C FEB03	104.3	0.05214	0.05000	2806	4180	2:27	34.680
IFT 99FEB03A1137	Standard	0.02std 13C FEB03	94.8	0.01896	0.02000	1117	4183	2:26	16.182
IFT 99FEB03A1138	Standard	0.01std 13C FEB03	137.8	0.01378	0.01000	844	4138	2:28	9.273

C. Chromatograms of Samples

Figure 10. Surface water (1826 Ferriday, La.)

Untreated Control

(ISTD Final Conc. = 0.1 ng/ml ¹³C₆ RPA 202248, ¹³C₆ RPA 201772 and ¹³C₆ RPA 203328)

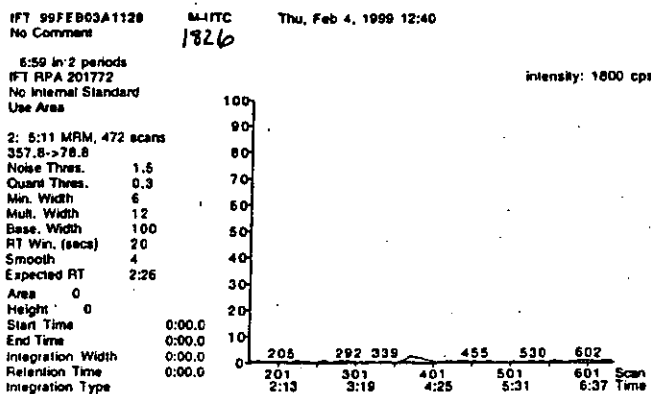
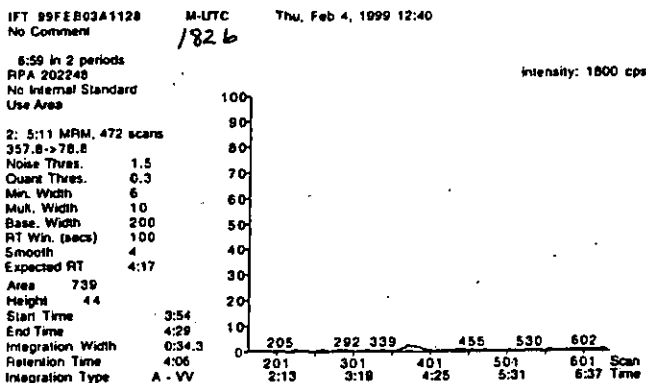
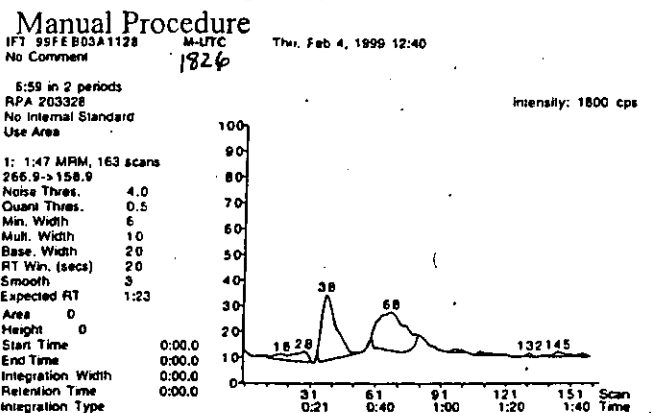


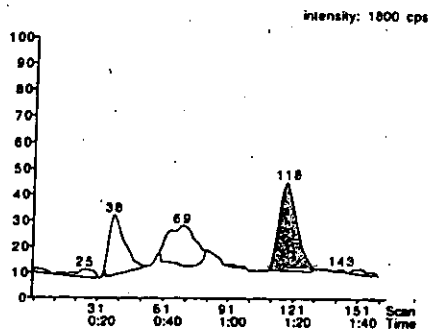
Figure 11. Surface water (1826 Ferriday, La.)
 10ppt RPA 202248, RPA 201772, RPA 203328
 (ISTD Final Conc. = 0.1 ng/ml ¹³C₆ RPA 202248, ¹³C₆ RPA 201772 and ¹³C₆ RPA 203328)

Manual Procedure

IFT 99FEB03A1131 M-0.8 FORMIC-10PPT-1 Thu, Feb 4, 1999 13:05
 No Comment 1826

6:59 in 2 periods
 RPA 203328
 No Internal Standard
 Use Area

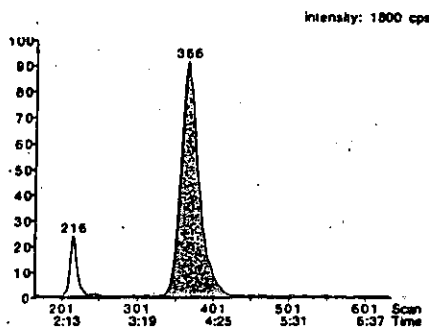
1: 1:47 MRM, 163 scans
 266.9->158.9
 Noise Thres. 4.0
 Quant Thres. 0.5
 Min. Width 6
 Mult. Width 10
 Base. Width 20
 RT Win. (secs) 20
 Smooth 3
 Expected RT 1:23
 Area 3943
 Height 612
 Start Time 1:13
 End Time 1:26
 Integration Width 0:13.2
 Retention Time 1:18
 Integration Type A - BB



IFT 99FEB03A1131 M-0.8 FORMIC-10PPT-1 Thu, Feb 4, 1999 13:05
 No Comment 1826

6:59 in 2 periods
 RPA 202248
 No Internal Standard
 Use Area

2: 5:11 MRM, 472 scans
 357.8->78.8
 Noise Thres. 1.5
 Quant Thres. 0.3
 Min. Width 6
 Mult. Width 10
 Base. Width 200
 RT Win. (secs) 100
 Smooth 4
 Expected RT 4:17
 Area 30532
 Height 1645
 Start Time 3:32
 End Time 5:03
 Integration Width 1:31
 Retention Time 4:02
 Integration Type A - BV



IFT 99FEB03A1131 M-0.8 FORMIC-10PPT-1 Thu, Feb 4, 1999 13:05
 No Comment 1826

6:59 in 2 periods
 IFT RPA 201772
 No Internal Standard
 Use Area

2: 5:11 MRM, 472 scans
 357.8->78.8
 Noise Thres. 1.5
 Quant Thres. 0.3
 Min. Width 6
 Mult. Width 12
 Base. Width 100
 RT Win. (secs) 20
 Smooth 4
 Expected RT 2:26
 Area 3137
 Height 427
 Start Time 2:12
 End Time 2:36
 Integration Width 0:23.1
 Retention Time 2:23
 Integration Type A - BV

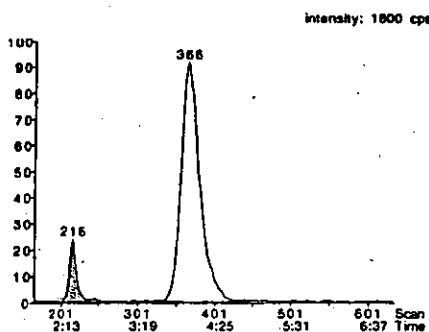
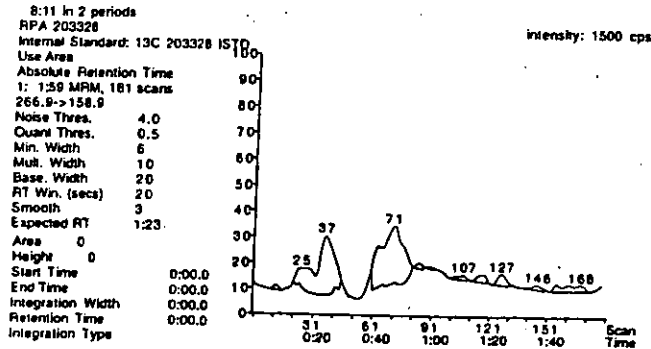


Figure 12. Ground water (Clovis, Ca)-
Untreated Control

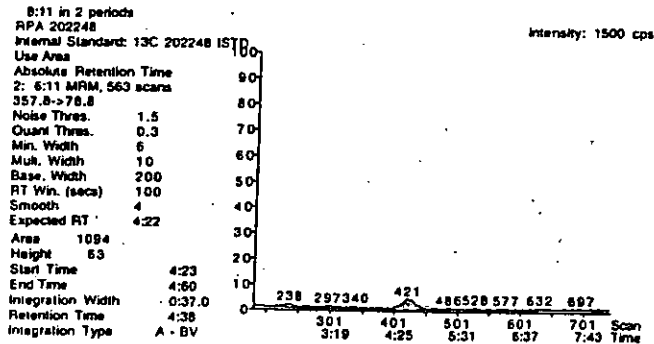
(ISTD Final Conc. = 0.1 ng/ml ¹³C₆, RPA 202248, ¹³C₆, RPA 201772 and ¹³C₆, RPA 203328)

Automation Procedure

IFT89JAN 29A1065 CLOVIS GW CON A Sat, Jan 30, 1999 02:16
No Comment



IFT89JAN 29A1065 CLOVIS GW CON A Sat, Jan 30, 1999 02:16
No Comment



IFT89JAN 29A1065 CLOVIS GW CON A Sat, Jan 30, 1999 02:16
No Comment

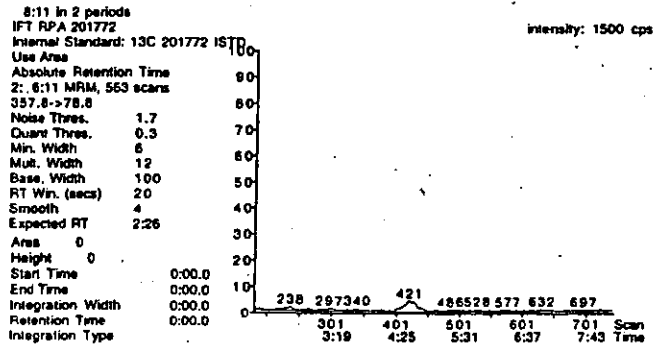
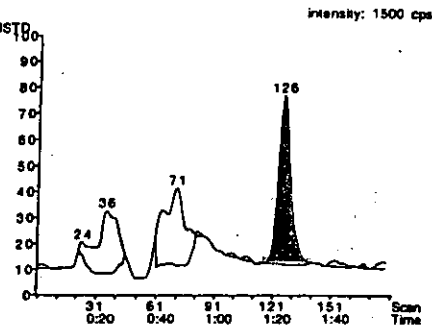


Figure 13. Ground water (Clovis, Ca)-
 10ppt RPA 202248, RPA 201772, RPA 203328
 (ISTD Final Conc. = 0.1 ng/ml ¹³C₆, RPA 202248, ¹³C₆, RPA 201772 and ¹³C₆, RPA 203328)

Automation Procedure

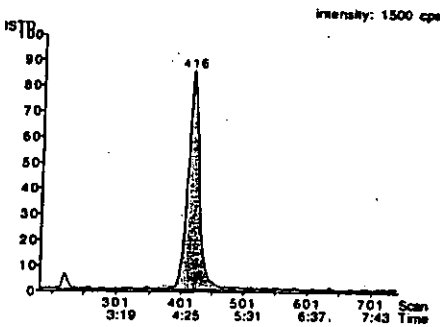
IFT99JAN 29A1068 CLOVIS GW SPK B Sat, Jan 30, 1999 02:46
 No Comment

8:11 in 2 periods
 RPA 203328
 Internal Standard: 13C 203328 ISTD
 Use Area
 Absolute Retention Time
 1: 1:59 MRM, 181 scans
 266.9->158.9
 Noise Thres. 4.0
 Quant Thres. 0.5
 Min. Width 6
 Mult. Width 10
 Base. Width 20
 RT Win. (secs) 20
 Smooth 3
 Expected RT 1:23
 Area 4906
 Height 975.
 Start Time 1:18
 End Time 1:31
 Integration Width 0:13.2
 Retention Time 1:23
 Integration Type A - BB



IFT99JAN 29A1068 CLOVIS GW SPK B Sat, Jan 30, 1999 02:46
 No Comment

8:11 in 2 periods
 RPA 202248
 Internal Standard: 13C 202248 ISTD
 Use Area
 Absolute Retention Time
 2: 6:11 MRM, 563 scans
 357.8->78.8
 Noise Thres. 1.5
 Quant Thres. 0.3
 Min. Width 6
 Mult. Width 10
 Base. Width 200
 RT Win. (secs) 100
 Smooth 4
 Expected RT 4:22
 Area 18705
 Height 1276
 Start Time 4:11
 End Time 5:09
 Integration Width 0:58.1
 Retention Time 4:35
 Integration Type A - VV



IFT99JAN 29A1068 CLOVIS GW SPK B Sat, Jan 30, 1999 02:46
 No Comment

8:11 in 2 periods
 RPA 201772
 Internal Standard: 13C 201772 ISTD
 Use Area
 Absolute Retention Time
 2: 6:11 MRM, 563 scans
 357.8->78.8
 Noise Thres. 1.5
 Quant Thres. 0.3
 Min. Width 6
 Mult. Width 12
 Base. Width 100
 RT Win. (secs) 20
 Smooth 4
 Expected RT 2:26
 Area 595
 Height 93
 Start Time 2:19
 End Time 2:34
 Integration Width 0:15.2
 Retention Time 2:26
 Integration Type M

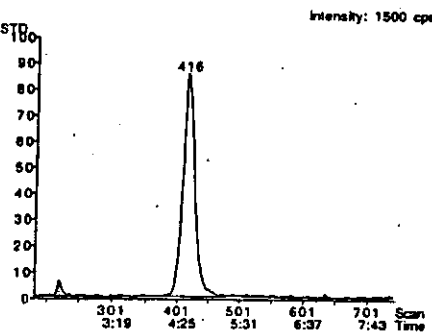
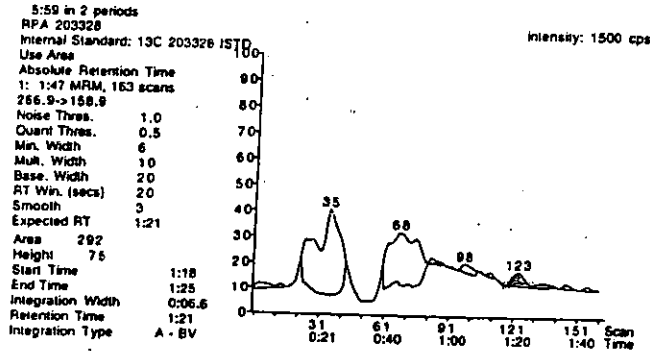


Figure 14. Surface water (1806 Ontario, Or.)
 Untreated Control

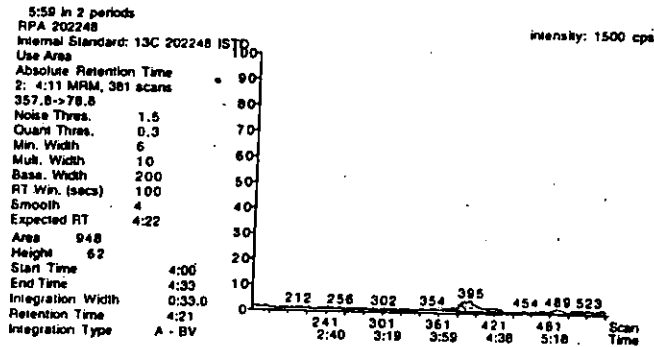
(ISTD Final Conc. = 0.1 ng/ml ¹³C₆ RPA 202248, ¹³C₆ RPA 201772 and ¹³C₆ RPA 203328)

Automation Procedure

IFT99JAN 29A1021 1806 CON A Fri, Jan 29, 1999 19:46
 No Comment



IFT99JAN 29A1021 1806 CON A Fri, Jan 29, 1999 19:46
 No Comment



IFT99JAN 29A1021 1806 CON A Fri, Jan 29, 1999 19:46
 No Comment

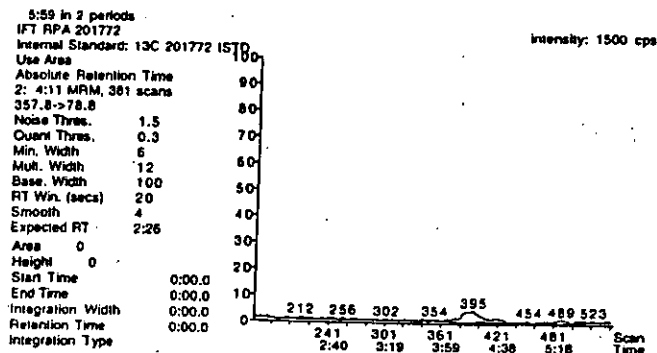


Figure 15. Surface water (1806 Ontario, Or.)
 10ppt RPA 202248, RPA 201772, RPA 203328
 (ISTD Final Conc. = 0.1 ng/ml ¹³C₆ RPA 202248, ¹³C₆ RPA 201772 and ¹³C₆ RPA 203328)
 Automation Procedure

