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Sponsor:

Rhone-Poulenc Ag Company
Research Triangle Park, North Carolina

ANALYTICAL METHOD

Title:

DRAFT

EXP 30953B/Field Corn/Magnitude of Residue (US93702R): Analytical Method for the Determination of Residues of RPA 201772, RPA 202248, and RPA 203328 in Corn Forage, Silage, Grain, and Fodder

Author:

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Date:

December 30, 1994

Performing Laboratory:

Hazleton Wisconsin, Inc.
Madison, Wisconsin

Laboratory Project Identification:

HWI Study No. 6224-215
Rhone Poulenc Study No. US93702R

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Pan-Ag Study No. 94454
Rhône-Poulenc Project No. EC-94-292
RPAC File No. 44663

STUDY IDENTIFICATION

EXP 30953B/Field Corn/Magnitude of Residue (US93702R):
Analytical Method for the Determination of Residues of RPA 201772, RPA 202248, and RPA 203328 in Corn Forage, Silage, Grain, and Fodder

Test Substance	EXP 30953B
Sponsor	Rhone-Poulenc Ag Company 2 T.W. Alexander Drive Research Triangle Park, NC 27709
Study Director	James J. Cappy, PhD Rhone-Poulenc Ag Company 2 T. W. Alexander Drive Research Triangle Park, NC 27709 (919) 549-2560
Analytical Investigator	Ujjana B. Nandihalli, PhD Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, Wisconsin 53707 (608) 241-4471
Testing Facility	Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, Wisconsin 53707 (608) 241-4471

CONTENTS

RAC	Analyte	Spiking Level (ppm)	Mean Recovery (%)
Grain	RPA 201772	0.01	91.1
Grain	RPA 201772	0.05	85.2
Grain	RPA 202248	0.01	88.4
Grain	RPA 202248	0.05	84.8
Grain	RPA 203328	0.01	66.7
Grain	RPA 203328	0.05	64.2
Fodder	RPA 201772	0.01	96.8
Fodder	RPA 201772	0.05	92.3
Fodder	RPA 202248	0.01	89.6
Fodder	RPA 202248	0.05	82.4
Fodder	RPA 203328	0.01	87.3[a]
Fodder	RPA 203328	0.05	102

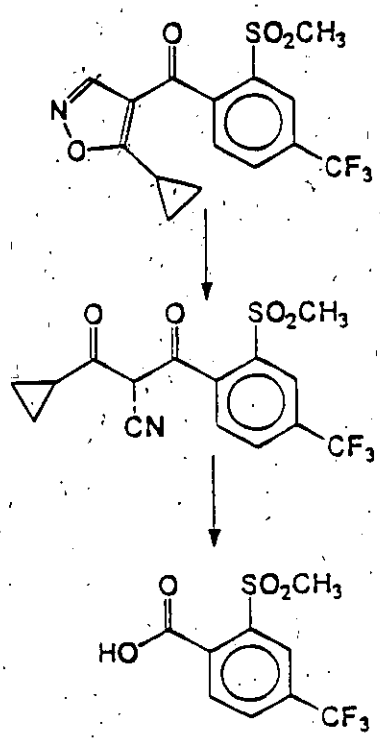
a n = 7

1. INTRODUCTION

The herbicide RPA 201772, 4-(2-methanesulphonyl-4-trifluoromethylbenzoyl)-5-cyclopropyl isoxazole is being developed by Rhone-Poulenc Agriculture Ltd., for use as a preplant incorporated and preemergence treatment for control of broad spectrum weeds in corn.

An analytical method is described for the analysis of RPA 201772 and its metabolites RPA 202248 and RPA 203328 (Figure 1) in corn forage, silage, grain, and fodder raw agricultural commodity (RAC) samples. The method described in this report is based on the method developed by Rhone-Poulenc Agriculture Ltd., in a study titled "Analytical Method for the Determination of Residues of RPA 201772, RPA 202248 and RPA 203328 in Maize and Fodder," J.D. Manley, Author [1].

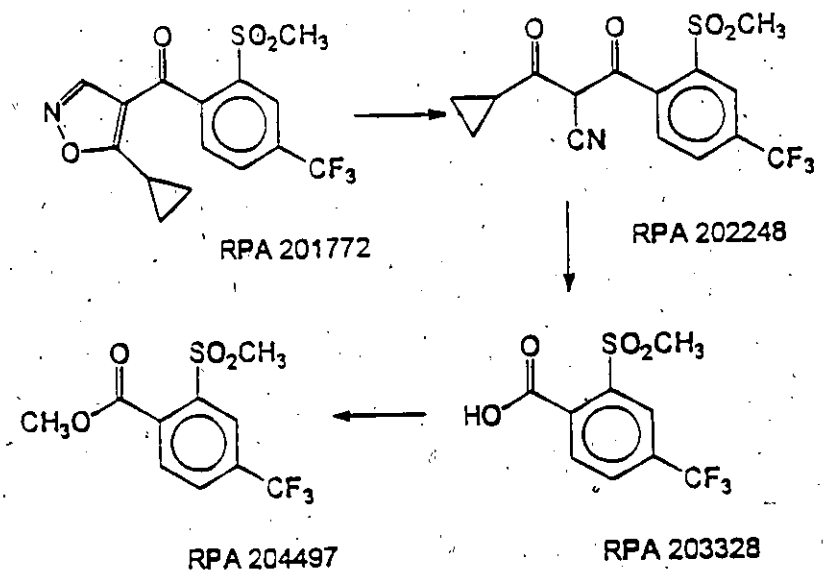
Figure 1: Metabolic Pathway of RPA 201772



2. PRINCIPLE

Residues of RPA 201772 and RPA 202248 are converted to RPA 203328. RPA 203328 is derivatized to form a methyl ester (RPA 204497) that is amenable to gas chromatography (Figure 2).

Figure 2



Residues of RPA 201772 and its metabolites (RPA 202248 and RPA 203328) are extracted by maceration with methanol. After addition of base RPA 201772 is readily hydrolyzed to RPA 202248. The methanol is evaporated and after liquid-liquid partition cleanup, the extract is acidified to allow liquid-liquid partition of both RPA 202248 and RPA203328 into dichloromethane. Residues of RPA202248 are hydrolyzed with strong base to RPA 203328 followed by liquid-liquid partition into dichloromethane. Combined RPA 203328 residues are then derivatized to give methyl ester (RPA 204497) for quantification by gas chromatography with mass selective detection.

Residues of the ester derivative are detected and quantified by gas chromatography with mass selective detection by selective ion monitoring of the two principle ions, m/z 251 and m/z 267. Ion m/z 251 is used for quantitation, with ion m/z 267 used as a qualifying ion.

Properties of RPA 201772, RPA 202248, RPA 203328 , and RPA 204497 are given below.

Compound	Mol Wt.	mp (°C)	Solubility in Water	LogP	pKa	CAS Registry
RPA 201772	359.32	141.2	3.5 mg/kg	2.19		141112-29-0
RPA 202248	359.32	137.3	326 mg/kg	-0.88	1.64	143701-75-1
RPA 203328	268.21	157.1				142994-06-7
RPA 204497	282.23					TBD

3. REAGENTS AND DISPOSABLE ITEMS

3.1 Chemicals

Chemicals	Source
RPA 201772 (99.7%), RPA 202248 (99.3%), RPA 203328 (97.4%), RPA 204497 (99.7%)	Rhone-Poulenc Ag Ltd.
Acetic acid (glacial), GR	EM Science
Hydrochloric acid, concentrated	Fisher Scientific
Potassium hydroxide, A.C.S	Fisher Scientific
Sodium chloride, A.C.S	Columbus Chemical Industries
Sodium hydroxide, AR	Mallinckrodt
Sodium sulfate (as the anhydrous salt), AR	Fisher Scientific
Diazald (N-methyl-N-nitroso-p-toluenesulphonamide) (99%)	Aldrich Chemical Company
Acetonitrile	Baxter, Burdick & Jackson (B&J Brand)
Dichloromethane	Baxter, Burdick & Jackson (B&J Brand)
Diethyl ether	Baxter, Burdick & Jackson (B&J Brand)
Methanol	Baxter, Burdick & Jackson (B&J Brand)
Petroleum ether (30-60°C fraction)	Baxter, Burdick & Jackson (B&J Brand)
Milli-Q® Water (double distilled, deionized ASTM II)	Millipore Corporation

3.2 Specialized Apparatus

Rotary film evaporator, Buchi Corporation
TurboVap® LV evaporator, Zymark Corporation
Aluminum heating block for hydrolysis, Pierce, Reacti-Therm III
Vortex mixer, VWR
Ultrasonic bath, Branson, Model 3200
Diazomethane preparation apparatus (see Section 4)
Gas chromatograph with mass selective detector (see Section 5), Hewlwtt-Packard

3.3 General Laboratory Apparatus

Waring blender cup (500 mL, Eberbach, Mnf.. No. 8470)
Filter funnel with fritted disc (40 to 60 µm porosity)
Round-bottom flask (500 and 1,000 mL)
TurboVap® tubes (16 x 125 mm and 15-mL Zymark® conical tubes with Teflon screw caps)
Separatory funnels (60 and 250 mL)
Volumetric flasks (100 and 200 mL)
Glass filter funnels
16 x 100 mm Pyrex® glass screw-cap test tubes (VWR, Cat. No. 60827-067)
2-mL glass crimp-cap GC vials
Pasteur pipettes
Glass pipettes

4. PREPARATION OF SOLUTIONS

4.1 Preparation of Standard Solutions

4.1.1 Fortifying Solutions (RPA 201772, RPA 202248 and RPA 203328)

4.1.1.1 Accurately weigh into a volumetric flask (200-mL capacity) a portion (0.0250 g) of pure analytical standard. Dissolve in acetonitrile and dilute to 200 mL.

4.1.1.2 Serially dilute accurately pipetted portions of this solution with acetonitrile to give solutions containing 1.25 and 0.25 µg/mL.

4.1.2 Standards for Gas Chromatography (RPA 204497)

4.1.2.1 Accurately weigh into a volumetric flask (200-mL capacity) a portion (0.0250 g) of pure analytical standard. Dissolve in dichloromethane and dilute to 200 mL.

4.1.2.2 Serially dilute accurately pipetted portions of this stock solution with dichloromethane to give solutions containing 0.01, 0.02, 0.05, 0.1, 0.2, and 0.5 µg/mL RPA 204497.

4.1.3 Storage and Stability

4.1.3.1 Store all solutions in a freezer when not in use.

4.1.3.2 Freshly prepare all stock solutions of RPA 201772 and dilutions therefrom each month. Do not use any solution more than 1 month after the preparation of the initial stock solution.

4.1.3.3 Freshly prepare all stock solutions of RPA 202248, RPA 203328, and RPA 204497 and dilutions therefrom at least every 3 months or when less than half full. Do not use any solution more than 3 months after the preparation of the initial stock solution.

4.2 Preparation of Reagents

4.2.1 2% sodium hydroxide solution

Dissolve 20.0 g of the solid material in deionized or glass-distilled water and dilute to 1,000 mL.

4.2.2 Saturated sodium chloride solution

Saturate 500 mL of deionized or glass-distilled water with sodium chloride.

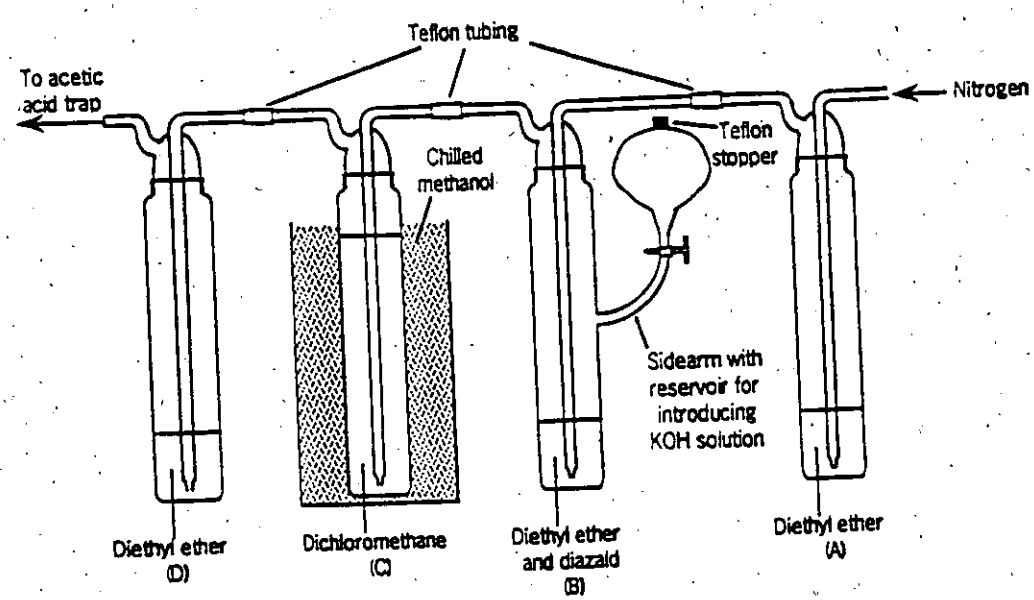
4.2.3 1M methanolic sodium hydroxide solution

Dissolve 4.0 g of the solid material in methanol and dilute to 100 mL.

4.2.4 Preparation of Diazomethane

Diazomethane should be prepared immediately before use, because it is both carcinogenic and potentially explosive. It should be handled with care and in a well ventilated hood (preferably containing an open beaker of glacial acetic acid). Dissolve 6.0 g of diazald in diethyl ether (30 mL) and place in tube (B) (Figure 3). Immerse tube (B) in a beaker of water maintained at approximately 30°C covering the mixture. Stir the mixture while bubbling nitrogen (high purity, oxygen and moisture-free) slowly through it. Place chilled dichloromethane (120 mL) in tube (C) and place the tube in a chilled ice/methanol bath. Place 18 mL of potassium hydroxide solution (6.6 g dissolved in a final volume of 100 mL of methanol) into the reservoir connected to tube (B). After approximately five minutes of bubbling nitrogen through the diazald mixture, introduce KOH slowly into tube (B) with the aid of a pipette bulb fitted to the opening on top (in place of Teflon stopper) of the reservoir. Initially, the rate of addition of KOH should be 1 to 2 mL/min; the rate may be increased once bumping of reaction mixture is stopped. The diazomethane generated is trapped in chilled dichloromethane. Collect diazomethane until ethyl ether in tube (D) shows slight yellowish color. Destroy and remove excess diazomethane with glacial acetic acid.

Figure 3



5. GAS CHROMATOGRAPHIC CONDITIONS

Instrument:	HP 5890 series II plus with EPC, HP 7673 autosampler and HP 5972 mass selective detector (MSD)
Column:	HP 5, 5% phenyl methyl silicone, 30 m x 0.25 mm, 0.25-µm film thickness
Temperature program:	120°C for 3 minutes, 10°C/minute to 200°C, 45°C/minute to 275°C, hold 9.50 minutes
Injector temperature:	250°C
Detector interface temperature:	280°C, 310°C
Gas flows	Helium, pressure set at 15 psi (1.1 mL/min.). Vacuum compensation on.
Inlet pressure:	EPC 25 psi for 0.75 min, 99 psi/min to 13 psi.
MSD parameter:	Selective Ion Mode using Ions m/z 251 and m/z 267 for RPA 204497 Dwell 100 msec. Solvent delay 7.30 min.
Injection volume:	Auto, 1 µL, fast, splitless.
Purge valve on:	0.75 min

Typical retention time for RPA 204497 is 8.7 to 9.2 minutes.

The MSD is tuned prior to a run using the maximum sensitivity autotune procedure. During tune, the oven temperature is set at 150°C and gas pressure at 13 psi. For RPA 204497, both ions m/z 251 and m/z 267 are integrated and the areas are measured. Ion m/z 251 is used for quantitation. Ion m/z 267 is used as a qualifying ion; its area ratio with ion m/z 251 needs to be typically 14%±5 % to confirm the presence of RPA 204497. Since the ion ratio is dependent upon the MSD system and the concentration of the analyte, the ion ratio obtained with another system may be different from the range given above.

6. ANALYTICAL PROCEDURE

6.1 Extraction

6.1.1 Weigh 25 g of homogeneous sample into a 500-mL Waring blender cup. Add methanol (125 mL), cover with a lid, and macerate for 2 to 5 minutes.

6.1.2 Filter the sample through a filter funnel with fritted disc into a 1,000-mL round-bottom flask.

6.1.3 Remacerate sample with methanol (125 mL each time) two more times and filter. Combine filtrates from all three filtration.

6.2 Hydrolysis to RPA 202248

6.2.1 Add 2% sodium hydroxide solution (10 mL) to the combind filtrate and mix by shaking. Leave the extract for at least 60 minutes at ambient temperature to allow hydrolysis of any RPA 201772 present to RPA 202248.

6.2.2 Evaporate off methanol using rotary film evaporation at 40°C to leave a low-volume aqueous residue (15 to 30 mL depending upon the matrix).

6.3 Liquid-Liquid Partition Cleanup

6.3.1 Add saturated sodium chloride solution (10 mL) to a 250-mL separatory funnel.

6.3.2 Transfer the aqueous residue from step 6.2.2 to a 250-mL separatory funnel containing 10 mL of saturated NaCl. Wash round-bottom flask with Milli-Q® water (4 × 20 mL). Sonicate flask at least once during washing for 30 sec. Shake funnel to mix .

6.3.3 Wash extract with dichloromethane (2 x 35 mL) and discard dichloromethane. Wash extract with petroleum ether (30 to 60 °C fraction, 35 mL) and discard petroleum ether.

6.3.4 Add hydrochloric acid (approximately 2 mL , 12 M) to the extract, mix, and measure pH using a pH indicator paper. The pH of the extract should be close to 1.0. Add 50 mL of dichloromethane to the extract, shake for approximately one minute, and allow for phase separation. Drain dichloromethane layer into 500-mL round-bottom flask through anhydrous sodium sulfate (10 g) held in a glass funnel (Use silanized glass wool to hold sodium sulfate in the funnel). Repeat liquid-liquid partition two more times, using 50 mL dichloromethane each time. Rinse sodium sulfate with dichloromethane (2 x 10 mL).

The analysis was stopped after this step at the end of first day and the extracts were stored in a refrigirator overnight.

6.3.5 Evaporate off dichloromethane using a rotary film evaporator at 30°C to approximately 5 mL. Quantitatively transfer the contents of the round-bottom flask into a screw-cap 16 mm × 125 mm test tube with dichloromethane rinsings (sonicate flask once during transfer for 30 sec) and evaporate to dryness using a TurboVap LV evaporator at 40°C.

6.4 Hydrolysis to RPA 203328

The presence of any water will inhibit the hydrolysis; therefore, anhydrous condition must be attained.

6.4.1 Add 1M methanolic sodium hydroxide solution (3 mL) to the tube and contents from step 6.3.5, seal tube, vortex mix for 2 minutes, and heat on a heating block at 100°C for 1 hour.

6.4.2 Cool the tube, then add Milli-Q water (5 mL). Transfer extract to a 60-mL separatory funnel with water rinsings (4 x 5 mL). Sonicate at least once during transfer for 30 sec. Add concentrated hydrochloric acid (approximately 0.5 mL, 12 M), mix, and measure pH using a pH indicator paper. The pH of the extract should be close to 1.0. Extract the aqueous fraction with dichloromethane (3 x 5 mL). Drain dichloromethane layer into a 15-mL graduated conical tube through an anhydrous sodium sulfate (2 g) held in a glass funnel. Rinse sodium sulfate with dichloromethane (2 x 1 mL).

6.4.3 Evaporate to dryness using a TurboVap LV evaporator at 40°C.

6.5 Methylation of RPA 203328

6.5.1 Dissolve the dry residue in dichloromethane (1 mL). Add 4 mL of diazomethane solution prepared in dichloromethane. Cap the tube, shake, and sonicate for 30 sec. Allow to react for 1 hour at 30°C (water bath temperature) with periodic shaking.

6.5.2 Add acetic acid (50 µL) to destroy excess diazomethane. Adjust volume to 10 mL with dichloromethane. Sonicate for 30 sec if particulate matter is observed. Load 2-mL GC vial for analysis.

The analytical method (from step 6.1.1 through 6.5.2) required two days for completion.

7. QUANTIFICATION OF RESIDUES

7.1 Quantify any RPA 204497 by comparison with standard solutions of appropriate concentration injected on to the GC.

7.2 Whenever necessary, dilute the extracts so that peak height or area obtained is within the linear range of the detector.

7.3 Measure the peaks of RPA 204497 using the data capture system. The area or height of the peak is used to calculate the results.

7.4 Preferably inject approximately 10% of the samples twice. If the areas or heights of the two injections vary by more than 2% of their mean (relative standard deviation), this is indicative of poor reproducibility.

7.5 Calculate the analyte concentration (µg/mL) in the sample solution injected, either by bracketing the adjacent standard solutions or by using the calibration regression using the equation $Y = mX + c$. The regression can be calculated using standard regression analysis programs[2], spreadsheet software (Excel or Lotus 1-2-3) or graphically.

For bracketing the standard:

$$X = \frac{H_s \times S}{H_i}$$

For $Y = mX + c$:

$$X = \frac{H_s - c}{m}$$

Where:

X	=	Concentration of RPA 204497 in sample solution in (µg/mL)
H_s	=	Measured height/area of sample peak
H_i	=	Measured height/area of standard peak
S	=	Concentration of standard in µg/mL
m	=	Slope of calibration
c	=	Intercept of calibration

8. LINEARITY OF DETECTOR RESPONSE

Determine the linear range of the detector response to RPA 204497 by injections of known amounts of the compound. Use the detector within the linear range thus determined, diluting any sample solutions if necessary. With RPA 204497, the mass selective detector has shown a good linear range usually over two orders of magnitude (typically 0.01 to 0.5 µg/mL). An example standard curve is presented in Appendix I.

9. DETERMINATION OF RECOVERIES

Fortify portions of untreated control sample (25 g) at levels of 0.01 and 0.05 ppm, using 1.0 mL of an appropriate standard solution (see Section 4.1.2.2). Complete the analytical procedure (see Section 6).

Separate recovery determinations should be carried out with RPA 201772, RPA 202248, and RPA 203328. Since this is a common moeity method, all components are quantified as RPA 204497.

Therefore, the molecular weight conversion factors for each component need to be used to calculate the recovery efficiency.

Recovery efficiency $\frac{C \times D \times V \times MW}{W \times S}$

C = Concentration of RPA 204497 in sample (µg/mL)
D = Dilution factor
V = Volume of extract (mL) prior to dilution.
W = Weight of sample (g)
MW = Molecular weight conversion factor
S = Spiking level (µg/g)

Conversion factor for RPA 201772 = 1.273
Conversion factor for RPA 202248 = 1.273
Conversion factor for RPA 203328 = 0.950

10. CONFIRMATION OF RESIDUES

The use of a mass selective detector simultaneously allows the confirmation of residues of RPA 204497 with a high degree of confidence. As well as needing to have an identical retention time to analytical standards, the ratio of ions m/z 267 and m/z 251 needs to be typically 14% ± 5%. Since the ion ratio is dependent upon the MSD system and the concentration of the analyte, the ion ratio obtained with another system may be different from the value given above.

A typical mass fragmentation pattern is given in Appendix II.

Example chromatograms for standards and recovery samples are presented in Appendix III.

11. REFERENCES

1. Analytical Method for the Determination of Residues of RPA 201772, RPA 202248, and RPA 203328 in Maize Grain and Fodder (Interim Report), J.D. Manley, Rhone-Poulenc, Agriculture Ltd., 1994, p. 52.
2. Statistics for Analytical Chemistry, 2nd Edition, J.C. Miller, J.N.Miller, Published by Ellis Horwood Limited, 1988.

ANALYTICAL METHOD SUMMARY

Analytical Method for Analysis of RPA 201772, RPA 202248 and RPA 203328 in corn forage, silage, grain, and fodder

25 g of corn RAC
Add 125 mL of methanol, macerate 2-5 minutes, filter
Reextract with methanol (125 mL), filter
Reextract with methanol (125 mL), filter
Add 2% sodium hydroxide (10 mL) and leave 60 minutes
Rotary evaporate off methanol
Add saturated sodium chloride solution (10 mL)
Add 80 mL of water (4 x 20 mL)
Wash with dichloromethane (2 x 35 mL)
Wash with petroleum ether (35 mL)
Add concentrated hydrochloric acid (Approximately 2 mL)
Extract with dichloromethane (3 x 50 mL)
Dry dichloromethane over anhydrous sodium sulfate (10 g)
Evaporate off dichloromethane using TurboVap
Dissolve in 1 M methanolic sodium hydroxide (3 mL)
Heat at 100°C for 1 hour
Cool, add water (5 mL x 5 mL) and concentrated hydrochloric acid (approximately 0.5 mL)
Extract with dichloromethane (3 x 5 mL)
Dry dichloromethane over anhydrous sodium sulfate (2 g)
Evaporate to dryness
Dissolve residue in dichloromethane (1 mL)
Add 4 mL of diazomethane solution prepared in dichloromethane
Shake and leave 60 minutes (30°C water bath)
Destroy excess diazomethane with acetic acid (50 µL)
Dilute upto 10 mL
Transfer sample to a GC vial
GC using MSD with SIM (ions m/z 251 & m/z 267)

SIGNATURE

Ujjana B. Nandihalli, PhD
Principal Analytical Investigator
Hazleton Wisconsin, Inc.

Date

APPENDIX I

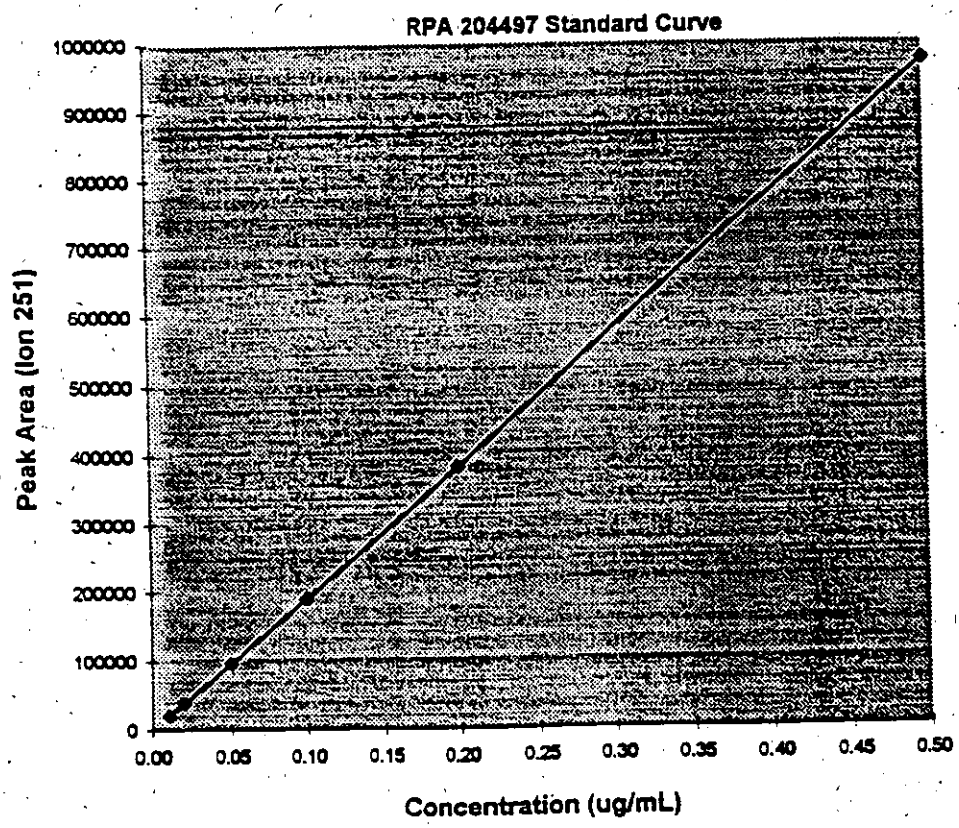
RPA 204497 Standard Curve

Concentration (ug/mL)	Peak Area (Ion 251)
0.01	20737
0.02	38354
0.05	96575
0.10	190773
0.20	382266
0.50	972210

Linear Regression

y = mx + c

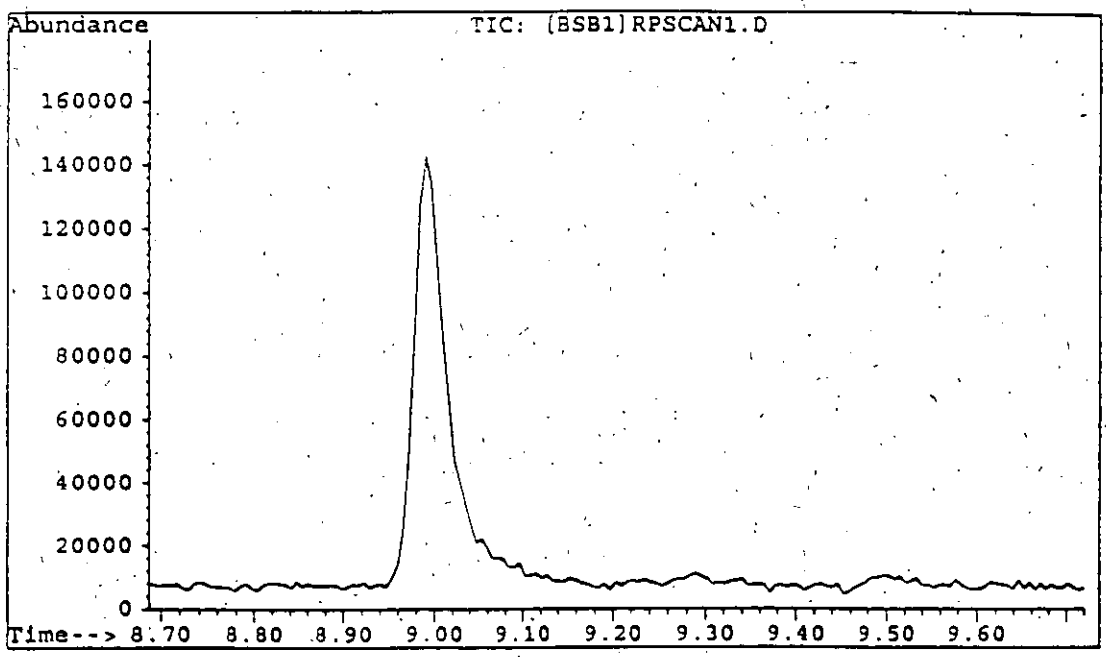
Slope (m) =	1943107.51
y-intercept (c) =	-1503.268
Correlation Coefficient (r) =	1.0000

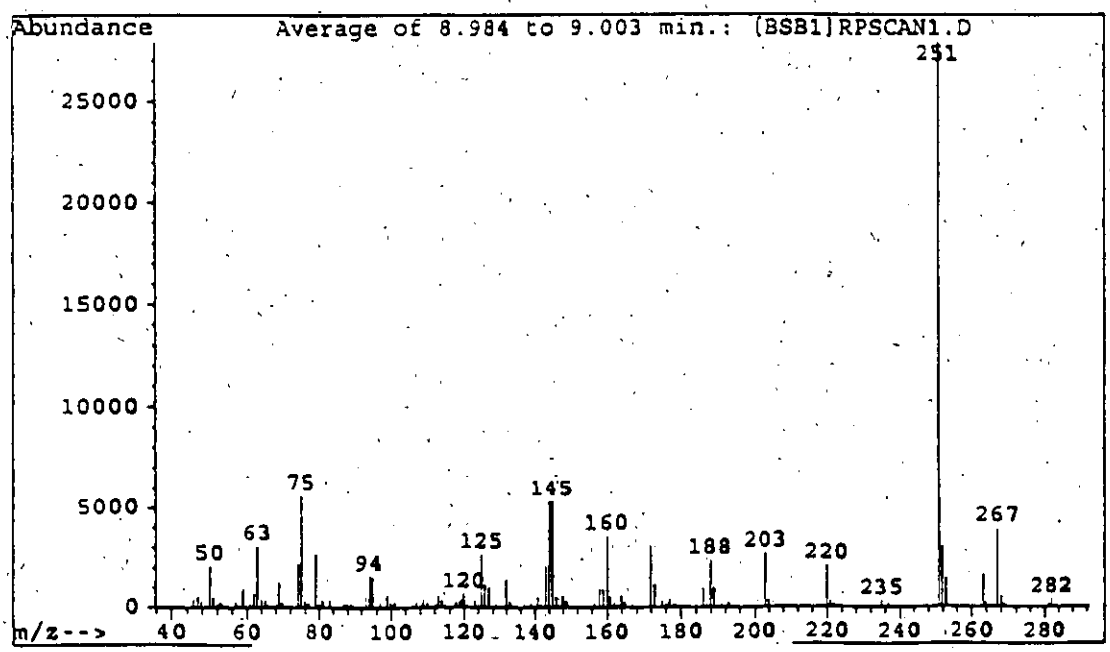


APPENDIX II

Mass Fragmentation of RPA 204497

File : C:\HPCHEM\1\DATA\BSB\RPSCAN1.D
Operator : [BSB1]
Acquired : 30 Aug 94 8:36 am using AcqMethod RPSCAN
Instrument : 5972-GC-M
Sample Name: 0.5 ug/mL standard
Misc Info : Mass Fragmentation of RPA 204497
Vial Number: 1

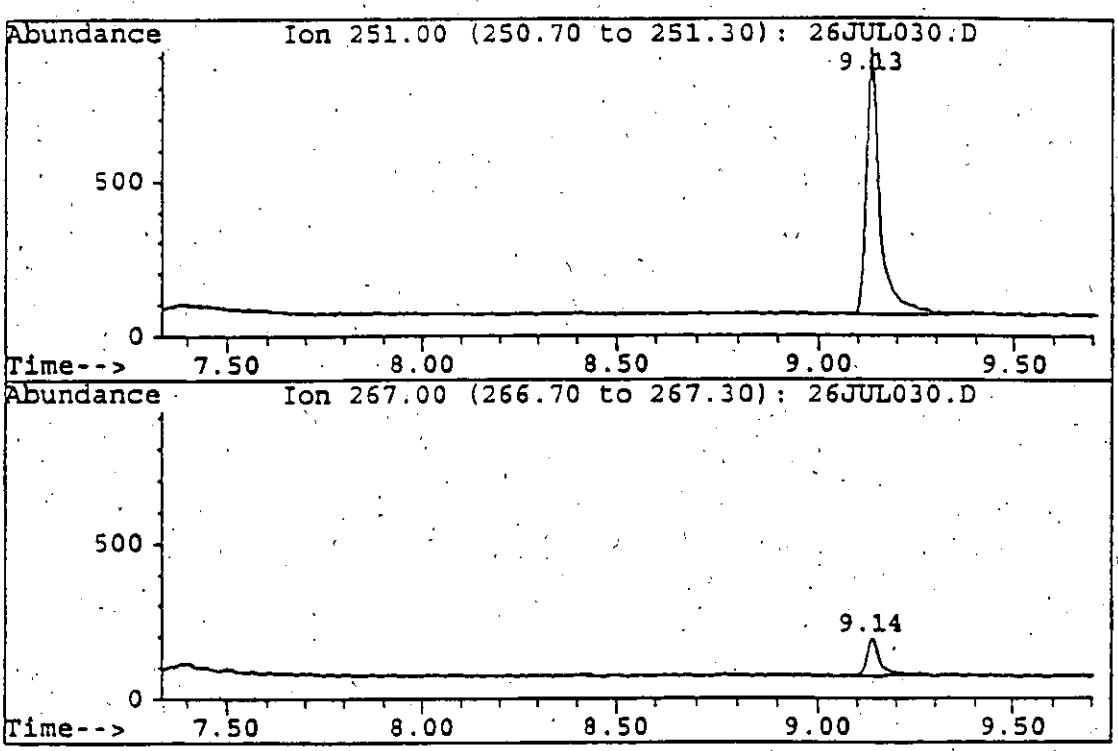




APPENDIX III

Standard: 0.01 µg/mL RPA 204497

File: C:\HPCHEM\1\DATA\26JUL94\26JUL030.D
Operator: UJJANA NANDIHALLI
Date Acquired: 27 Jul 94 5:30 am
Instrument: 5972-GC-M
Method File: RPASIM12
Sample Name: STD 0.0100
Misc Info: RPA-204497-0.0100 UG/ML HWI 6224-215
Vial Number : 18



Ion 251.00 (250.70 to 251.30): 26JUL030.D
STD 0.0100

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.132	PV	0.037	20737	9.081	9.329

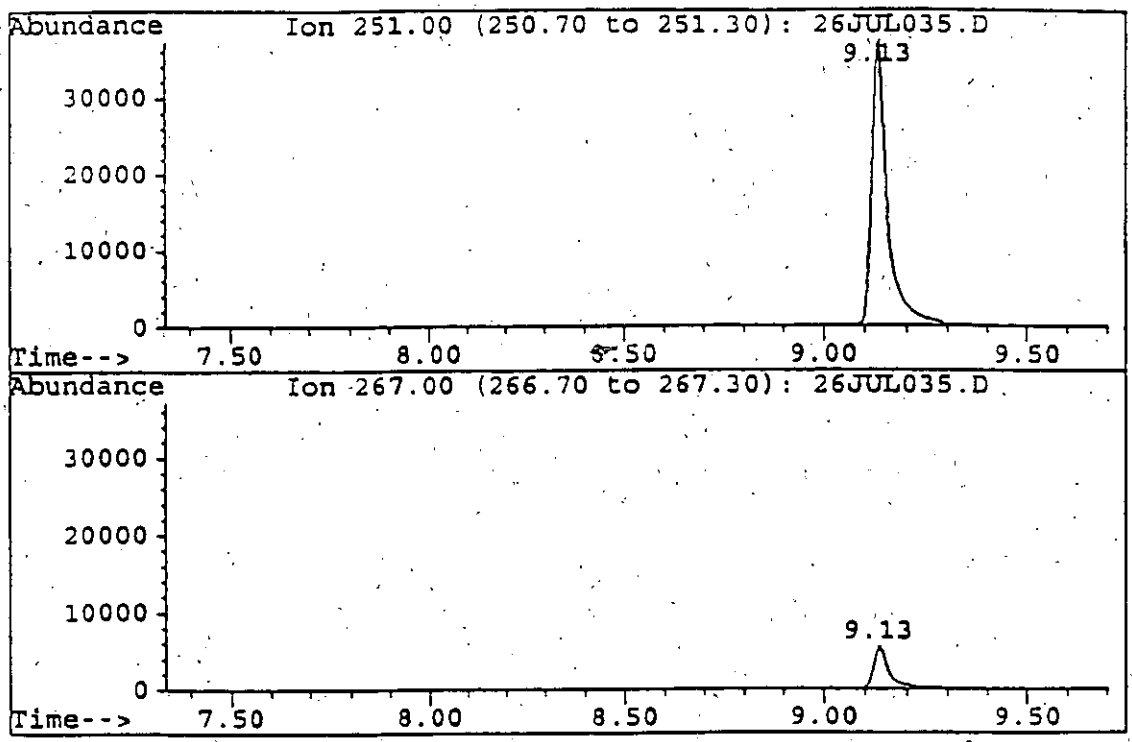
Ion 267.00 (266.70 to 267.30): 26JUL030.D
STD 0.0100

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.136	PV	0.035	2870	9.088	9.248

APPENDIX III

Standard: 0.500 µg/mL RPA 204497

File: C:\HPCHEM\1\DATA\26JUL94\26JUL035.D
Operator: UJJANA NANDIHALLI
Date Acquired: 27 Jul 94 7:43 am
Instrument: 5972-GC-M
Method File: RPASIM12
Sample Name: STD 0.500
Misc Info: RPA-204497-0.500 UG/ML HWI 6224-215
Vial Number : 23



Ion 251.00 (250.70 to 251.30): 26JUL035.D
STD 0.500

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.129	BV	0.038	972210	9.067	9.513

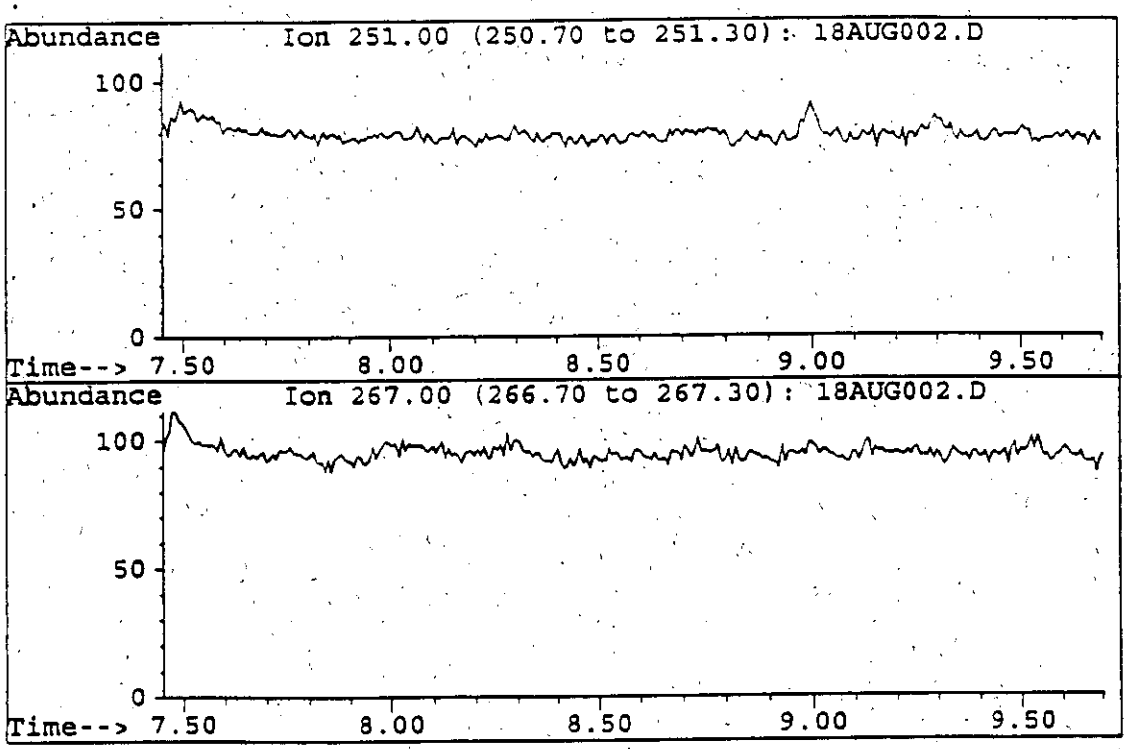
Ion 267.00 (266.70 to 267.30): 26JUL035.D
STD 0.500

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.133	PV	0.038	138918	9.073	9.358

APPENDIX III

Forage Untreated Control

File: C:\HPCHEM\1\DATA\18AUG94\18AUG002.D
Operator: UJJANA NANDIHALLI
Date Acquired: 18 Aug 94 7:27 pm
Instrument: 5972-GC-M
Method File: RPASIM12
Sample Name: MV-2 FORAGE UTC
Misc Info: HWI 6224-215
Vial Number : 2



Ion 251.00 (250.70 to 251.30): 18AUG002.D
MV-2 FORAGE UTC

Peak#	Ret Time	Type	Width	Area	Start Time	End Time

No peaks detected

Ion 267.00 (266.70 to 267.30): 18AUG002.D
MV-2 FORAGE UTC

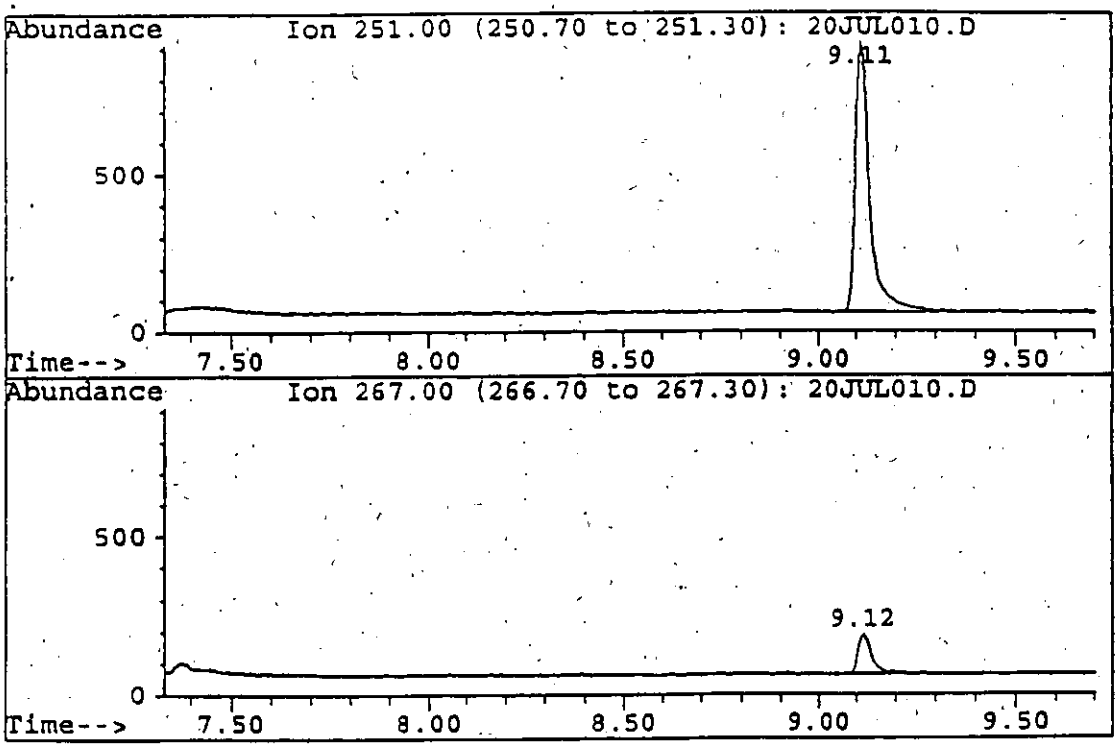
Peak#	Ret Time	Type	Width	Area	Start Time	End Time

No peaks detected

APPENDIX III

Forage Spiked with 0.01 ppm RPA 201772

File: C:\HPCHEM\1\DATA\20JUL94\20JUL010.D
Operator: UJJANA NANDIHALLI
Date Acquired: 20 Jul 94 9:50 pm
Instrument: 5972-GC-M
Method File: RPASIM12
Sample Name: MV-3A FORAGE 0.0100 UG/G RECOVERY
Misc Info: RPA-201772-0.250 UG/ML HWI 6224-215
Vial Number : 8



Ion 251.00 (250.70 to 251.30): 20JUL010.D
MV-3A FORAGE 0.0100 UG/G RECOVERY

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.112	BV	0.038	21194	9.022	9.300

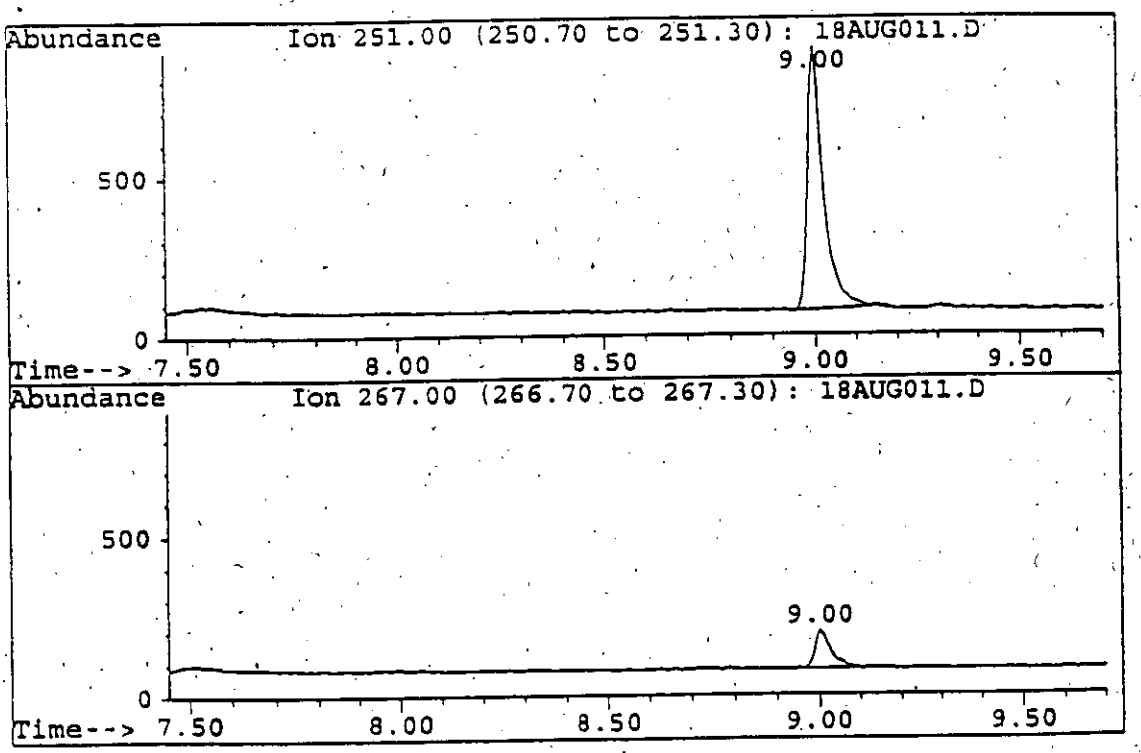
Ion 267.00 (266.70 to 267.30): 20JUL010.D
MV-3A FORAGE 0.0100 UG/G RECOVERY

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.116	BV	0.038	3094	9.048	9.242

APPENDIX III

Forage Spiked with 0.01 ppm RPA 202248

File: C:\HPCHEM\1\DATA\18AUG94\18AUG011.D
Operator: UJJANA NANDIHALLI
Date Acquired: 18 Aug 94 11:28 pm
Instrument: 5972-GC-M
Method File: RPASIM12
Sample Name: MV-9 FORAGE 0.01 UG/G 202248
Misc Info: HWI 6224-215
Vial Number : 9



Ion 251.00 (250.70 to 251.30): 18AUG011.D
MV-9 FORAGE 0.01 UG/G 202248

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.001	BV	0.040	21343	8.944	9.138

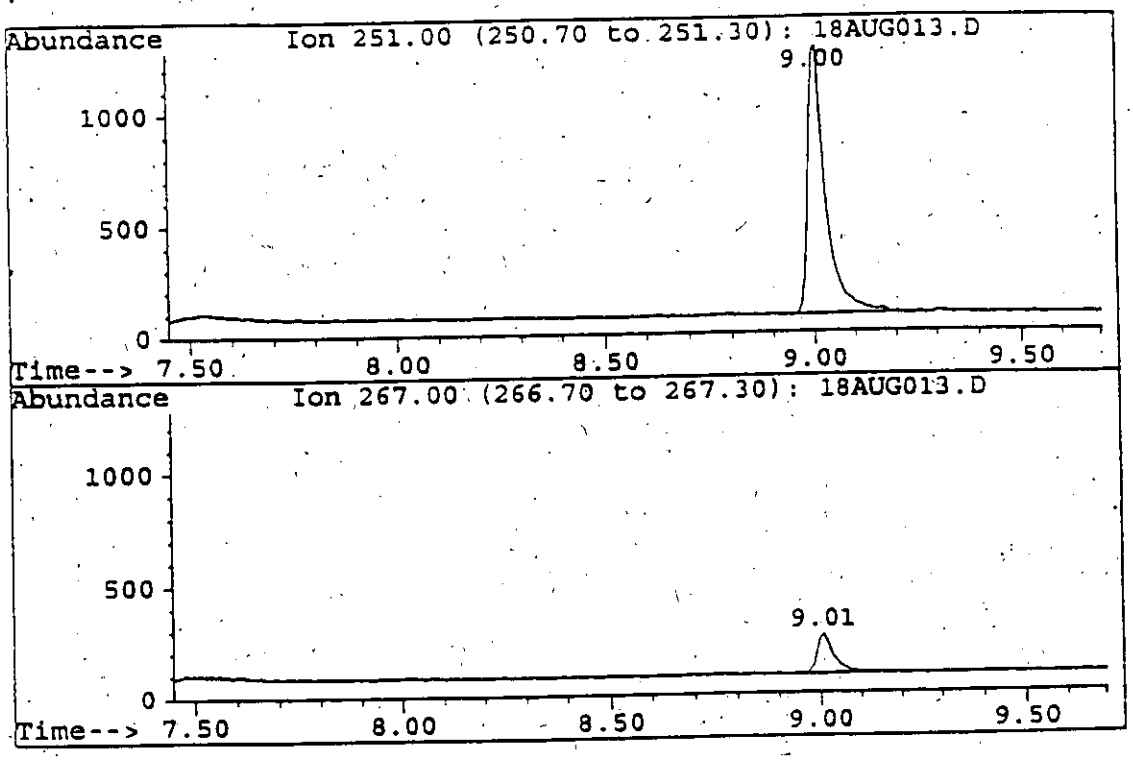
Ion 267.00 (266.70 to 267.30): 18AUG011.D
MV-9 FORAGE 0.01 UG/G 202248

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.004	BV	0.040	3055	8.931	9.109

APPENDIX III

Forage Spiked with 0.01 ppm RPA 203328

File: C:\HPCHEM\1\DATA\18AUG94\18AUG013.D
Operator: UJJANA NANDIHALLI
Date Acquired: 19 Aug 94 12:21 am
Instrument: 5972-GC-M
Method File: RPASIM12
Sample Name: MV-16 FORAGE 0.01 UG/G 203328
Misc Info: HWI 6224-215
Vial Number : 10



Ion 251.00 (250.70 to 251.30): 18AUG013.D
MV-16 FORAGE 0.01 UG/G 203328

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.005	BBA	0.043	35149	8.912	9.200

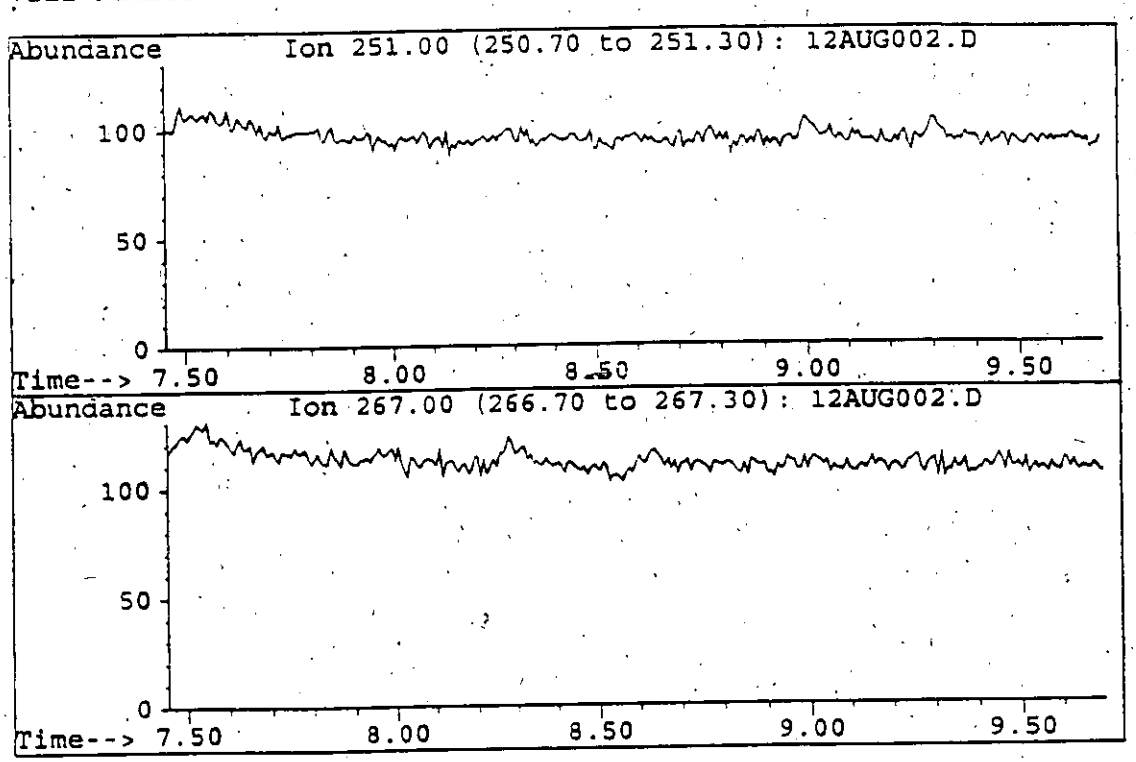
Ion 267.00 (266.70 to 267.30): 18AUG013.D
MV-16 FORAGE 0.01 UG/G 203328

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.008	BV	0.043	5099	8.918	9.139

APPENDIX III

Silage Untreated Control

```
File:           C:\HPCHEM\1\DATA\12AUG94\12AUG002.D
Operator:       UJJANA NANDIHALLI
Date Acquired:  12 Aug 94    5:01 pm
Instrument:     5972-GC-M
Method File:    RPASIM12
Sample Name:                      MV-173 SILAGE UTC
Misc Info:                        HWI 6224-215
Vial Number   : 2
```



Ion 251.00 (250.70 to 251.30): 12AUG002.D
MV-173 SILAGE UTC

Peak#	Ret Time	Type	Width	Area	Start Time	End Time

No peaks detected

Ion 267.00 (266.70 to 267.30): 12AUG002.D
MV-173 SILAGE UTC

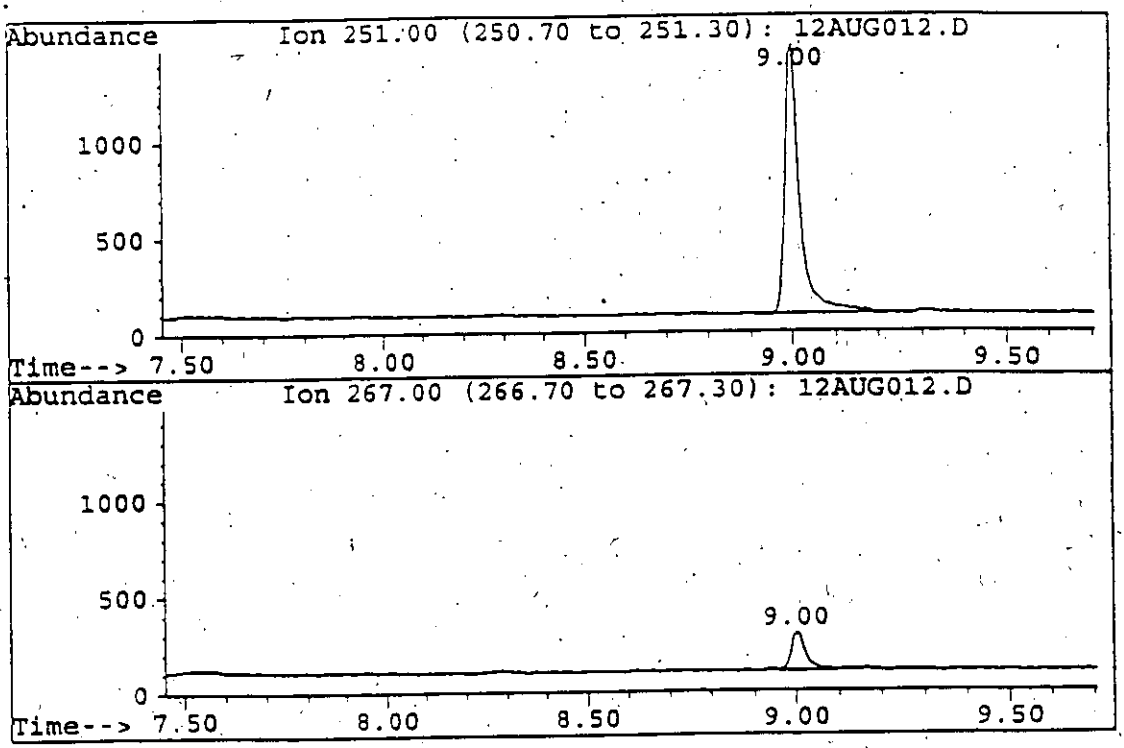
Peak#	Ret Time	Type	Width	Area	Start Time	End Time

No peaks detected

APPENDIX III

Silage Spiked with 0.01 ppm RPA 201772

File: C:\HPCHEM\1\DATA\12AUG94\12AUG012.D
Operator: UJJANA NANDIHALLI
Date Acquired: 12 Aug 94 9:28 pm
Instrument: 5972-GC-M
Method File: RPASIM12
Sample Name: MV-174A SILAGE 0.01 UG/G 201772
Misc Info: HWI 6224-215
Vial Number : 9



Ion 251.00 (250.70 to 251.30): 12AUG012.D
MV-174A SILAGE 0.01 UG/G 201772

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	8.998	BBA	0.037	33891	8.905	9.200

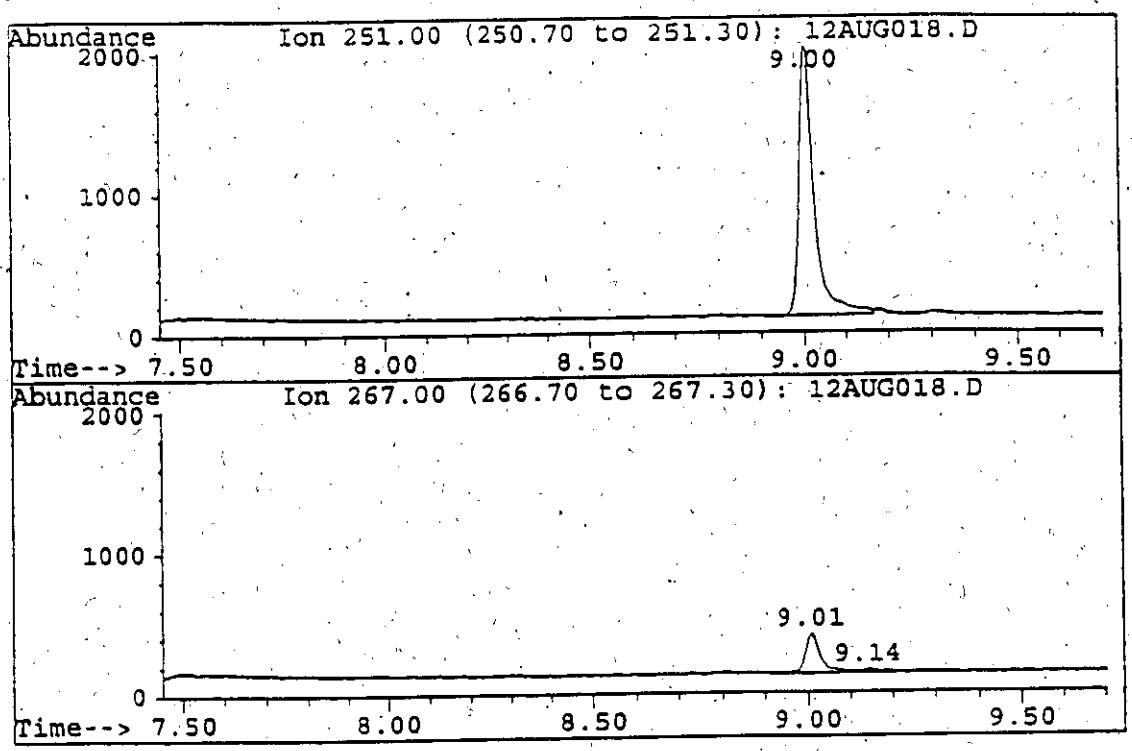
Ion 267.00 (266.70 to 267.30): 12AUG012.D
MV-174A SILAGE 0.01 UG/G 201772

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.001	VV	0.037	4748	8.955	9.086

APPENDIX III

Silage Spiked with 0.01 ppm RPA 202248

File: C:\HPCHEM\1\DATA\12AUG94\12AUG018.D
Operator: UJJANA NANDIHALLI
Date Acquired: 13 Aug 94 12:08 am
Instrument: 5972-GC-M
Method File: RPASIM12
Sample Name: MV-183 SILAGE 0.01 UG/G 202248
Misc Info: HWI 6224-215
Vial Number : 14



Ion 251.00 (250.70 to 251.30): 12AUG018.D
MV-183 SILAGE 0.01 UG/G 202248

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.004	BV	0.037	47865	8.912	9.164

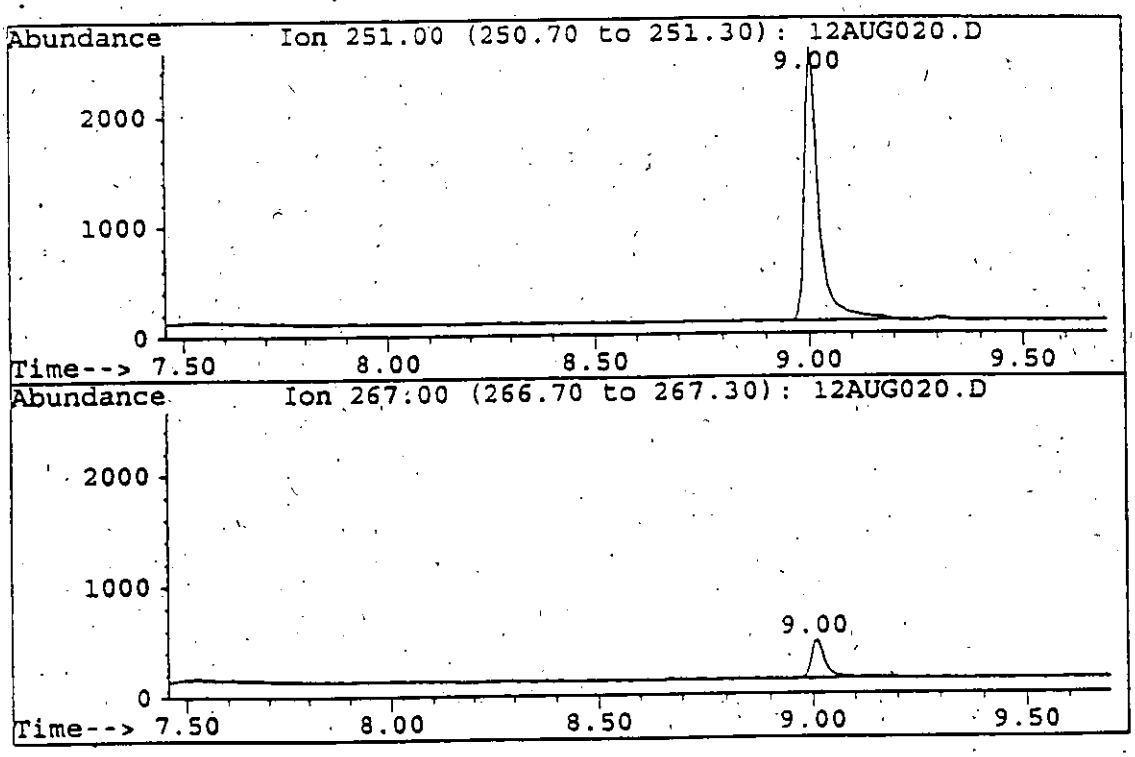
Ion 267.00 (266.70 to 267.30): 12AUG018.D
MV-183 SILAGE 0.01 UG/G 202248

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.008	BV	0.038	6973	8.918	9.115
2	9.142	VBA	0.042	536.82	9.115	9.203

APPENDIX III

Silage Spiked with 0.01 ppm RPA 203328

File: C:\HPCHEM\1\DATA\12AUG94\12AUG020.D
Operator: UJJANA NANDIHALLI
Date Acquired: 13 Aug 94 1:01 am
Instrument: 5972-GC-M
Method File: RPASIM12
Sample Name: MV-187 SILAGE 0.01 UG/G 203328
Misc Info: HWI 6224-215
Vial Number: 16



Ion 251.00 (250.70 to 251.30): 12AUG020.D
MV-187 SILAGE 0.01 UG/G 203328

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.002	BBA	0.036	57901	8.905	9.200

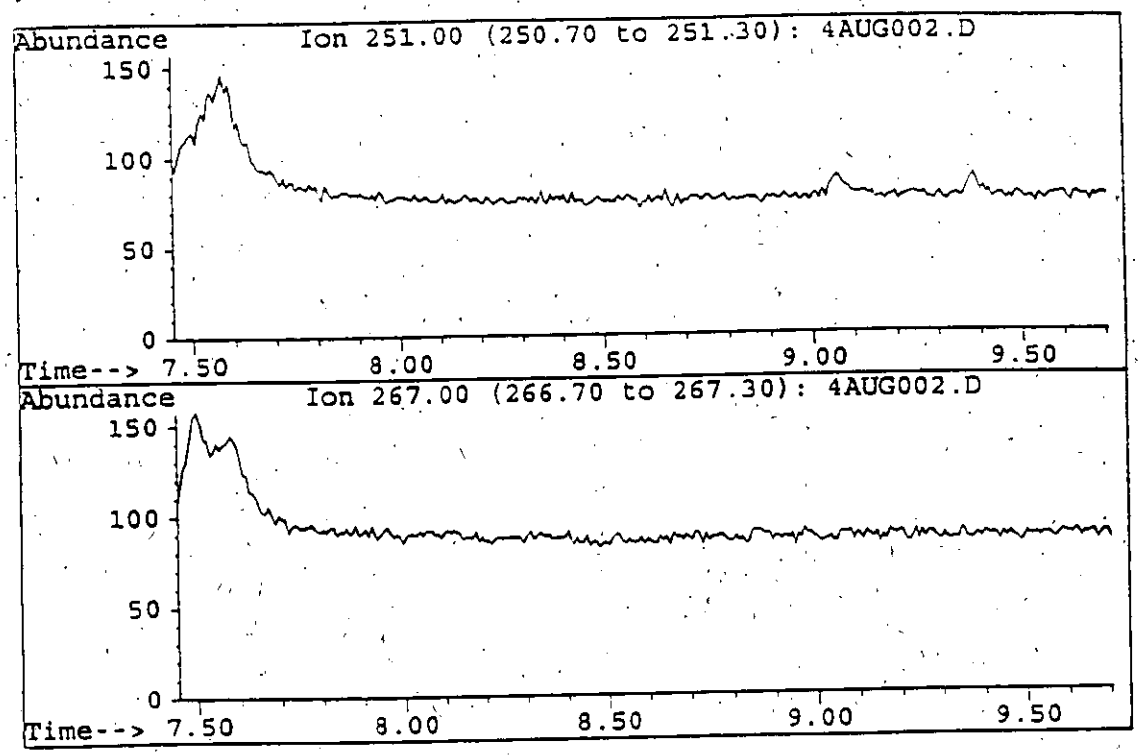
Ion 267.00 (266.70 to 267.30): 12AUG020.D
MV-187 SILAGE 0.01 UG/G 203328

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.005	BV	0.034	7718	8.905	9.105

APPENDIX III

Grain Untreated Control

```
File:           C:\HPCHEM\1\DATA\4AUG94\4AUG002.D
Operator:       UJJANA NANDIHALLI
Date Acquired:  4 Aug 94    6:02 pm
Instrument:     5972-GC-M
Method File:    RPASIM12
Sample Name:                    MV-142 GRAIN UNTREATED CONTROL
Misc Info:                      HWI 6224-215
Vial Number   : 1
```



Ion 251.00 (250.70 to 251.30): 4AUG002.D
MV-142 GRAIN UNTREATED CONTROL

Peak#	Ret Time	Type	Width	Area	Start Time	End Time

No peaks detected

Ion 267.00 (266.70 to 267.30): 4AUG002.D
MV-142 GRAIN UNTREATED CONTROL

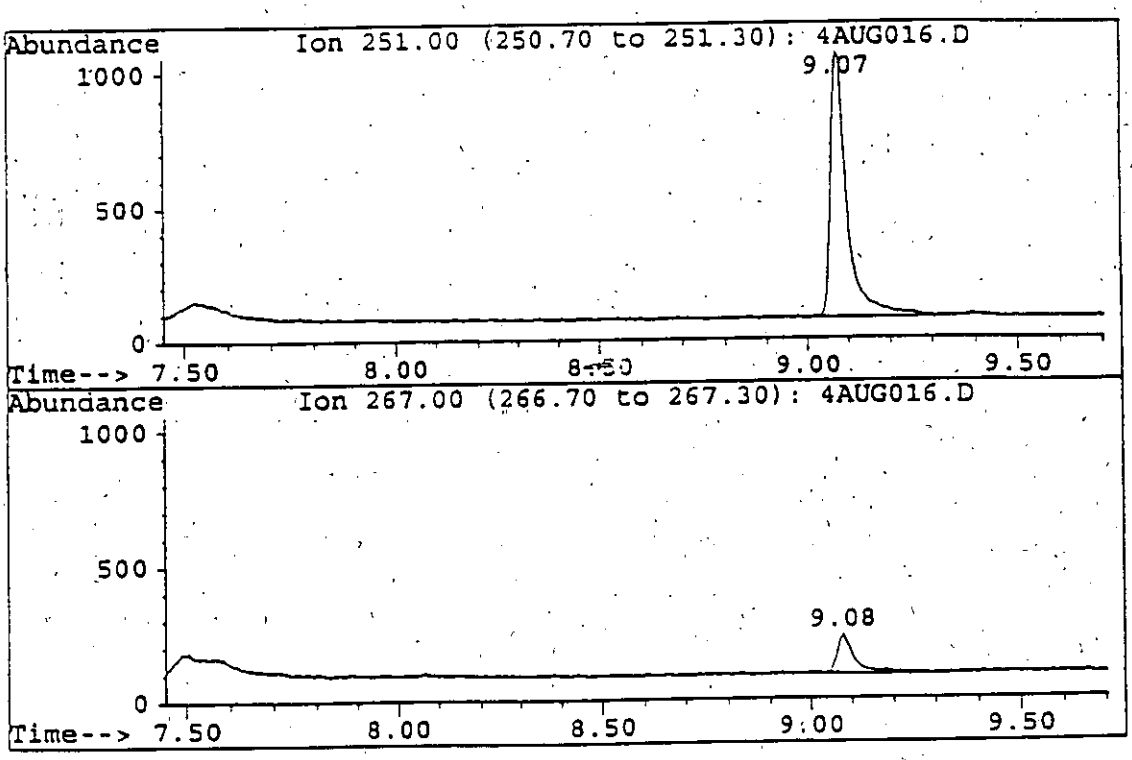
Peak#	Ret Time	Type	Width	Area	Start Time	End Time

No peaks detected

APPENDIX III

Grain Spiked with 0.01 ppm RPA 201772

```
File:           C:\HPCHEM\1\DATA\4AUG94\4AUG016.D
Operator:       UJJANA NANDIHALLI
Date Acquired:   5 Aug 94   12:15 am
Instrument:     5972-GC-M
Method File:    RPASIM12
Sample Name:                    MV-143 GRAIN 0.01 UG/G 201772
Misc Info:                      HWI 6224-215
Vial Number   : 13
```



Ion 251.00 (250.70 to 251.30): 4AUG016.D
MV-143 GRAIN 0.01 UG/G 201772

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.074	BV	0.041	27162	8.951	9.307

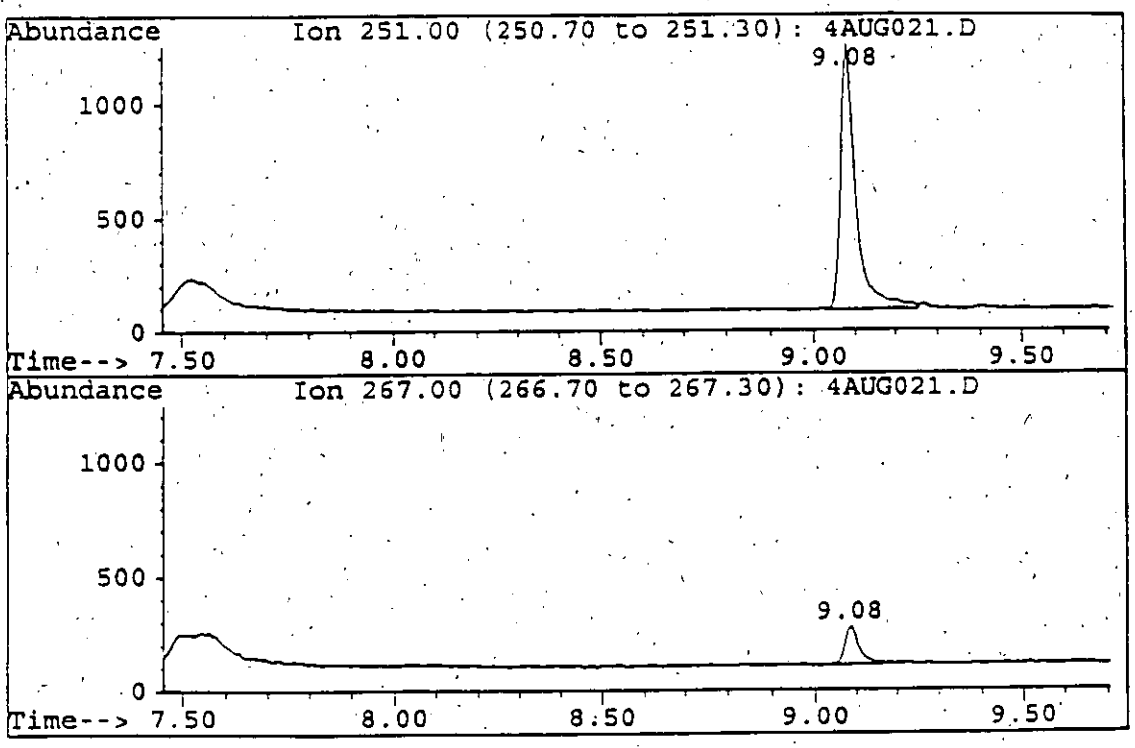
Ion 267.00 (266.70 to 267.30): 4AUG016.D
MV-143 GRAIN 0.01 UG/G 201772

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.078	BV	0.043	4190	8.905	9.289

APPENDIX III

Grain Spiked with 0.01 ppm RPA 202248

```
File:           C:\HPCHEM\1\DATA\4AUG94\4AUG021.D
Operator:       UJJANA NANDIHALLI
Date Acquired:  5 Aug 94    2:29 am
Instrument:     5972-GC-M
Method File:    RPASIM12
Sample Name:                    MV-150 GRAIN 0.01 UG/G 202248
Misc Info:                      HWI 6224-215
Vial Number   : 18
```



Ion 251.00 (250.70 to 251.30): 4AUG021.D
MV-150 GRAIN 0.01 UG/G 202248

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.080	VV	0.039	29756	9.030	9.255

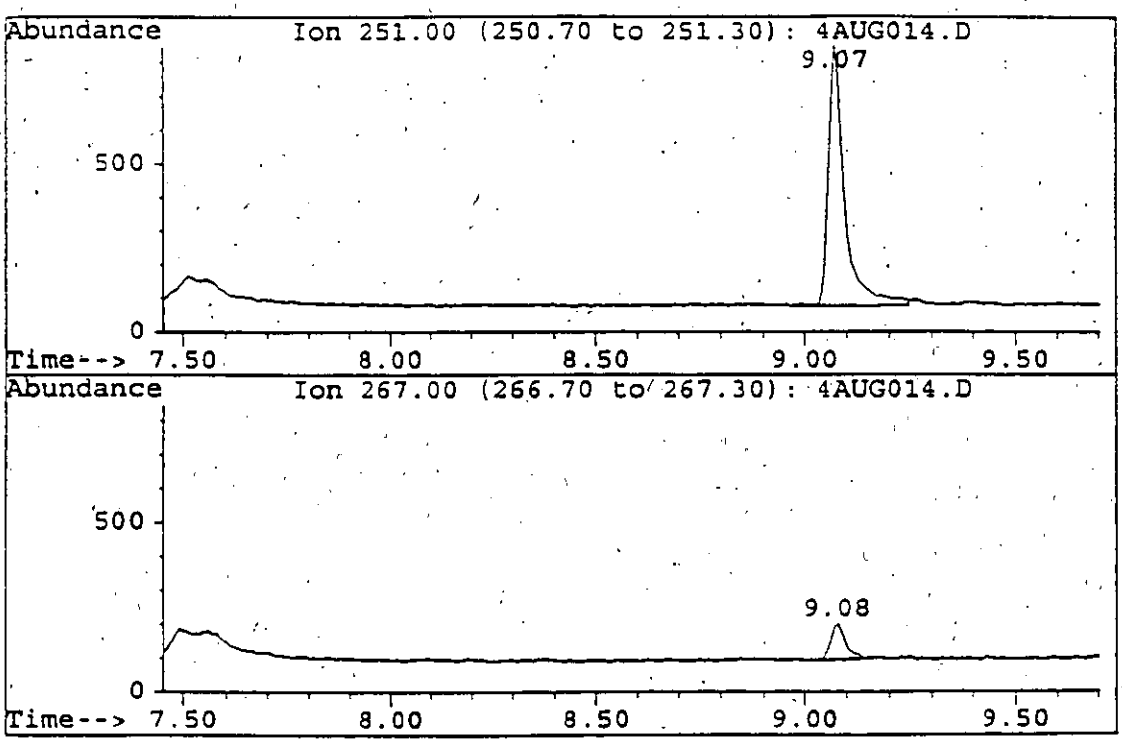
Ion 267.00 (266.70 to 267.30): 4AUG021.D
MV-150 GRAIN 0.01 UG/G 202248

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.084	PV	0.037	4008	8.985	9.192

APPENDIX III

Grain Spiked with 0.01 ppm RPA 203328

```
File:           C:\HPCHEM\1\DATA\4AUG94\4AUG014.D
Operator:       UJJANA NANDIHALLI
Date Acquired:   4 Aug 94   11:22 pm
Instrument:     5972-GC-M
Method File:    RPASIM12
Sample Name:                    MV-157 GRAIN 0.01 UG/G 203328
Misc Info:                      HWI 6224-215
Vial Number   : 12
```



Ion 251.00 (250.70 to 251.30): 4AUG014.D
MV-157 GRAIN 0.01 UG/G 203328

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.072	PV	0.040	20707	8.964	9.245

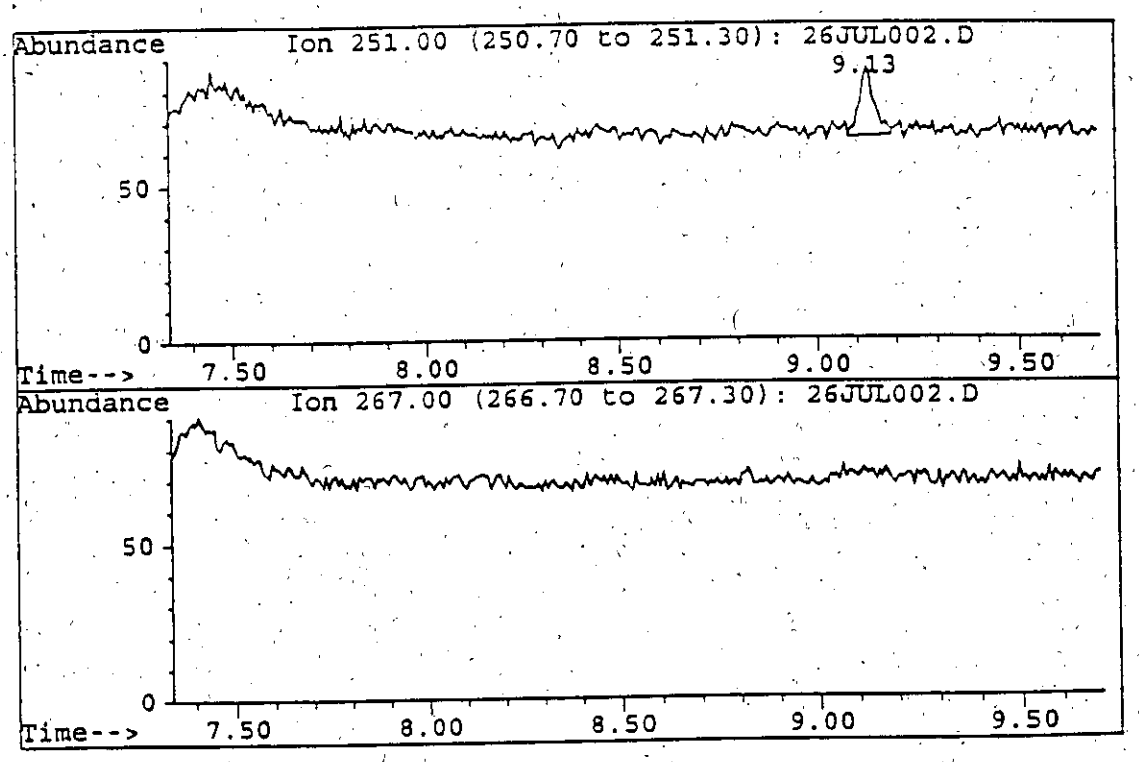
Ion 267.00 (266.70 to 267.30): 4AUG014.D
MV-157 GRAIN 0.01 UG/G 203328

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.076	PV	0.037	2467	9.002	9.158

APPENDIX III

Fodder Untreated Control

File: C:\HPCHEM\1\DATA\26JUL94\26JUL002.D
Operator: UJJANA NANDIHALLI
Date Acquired: 26 Jul 94 5:03 pm
Instrument: 5972-GC-M
Method File: RPASIM12
Sample Name: MV-22B FODDER UNTREATED CONTROL
Misc Info: HWI 6224-215
Vial Number : 1



Ion 251.00 (250.70 to 251.30): 26JUL002.D
MV-22B FODDER UNTREATED CONTROL

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.126	VV	0.035	509.58	9.081	9.191

Ion 267.00 (266.70 to 267.30): 26JUL002.D
MV-22B FODDER UNTREATED CONTROL

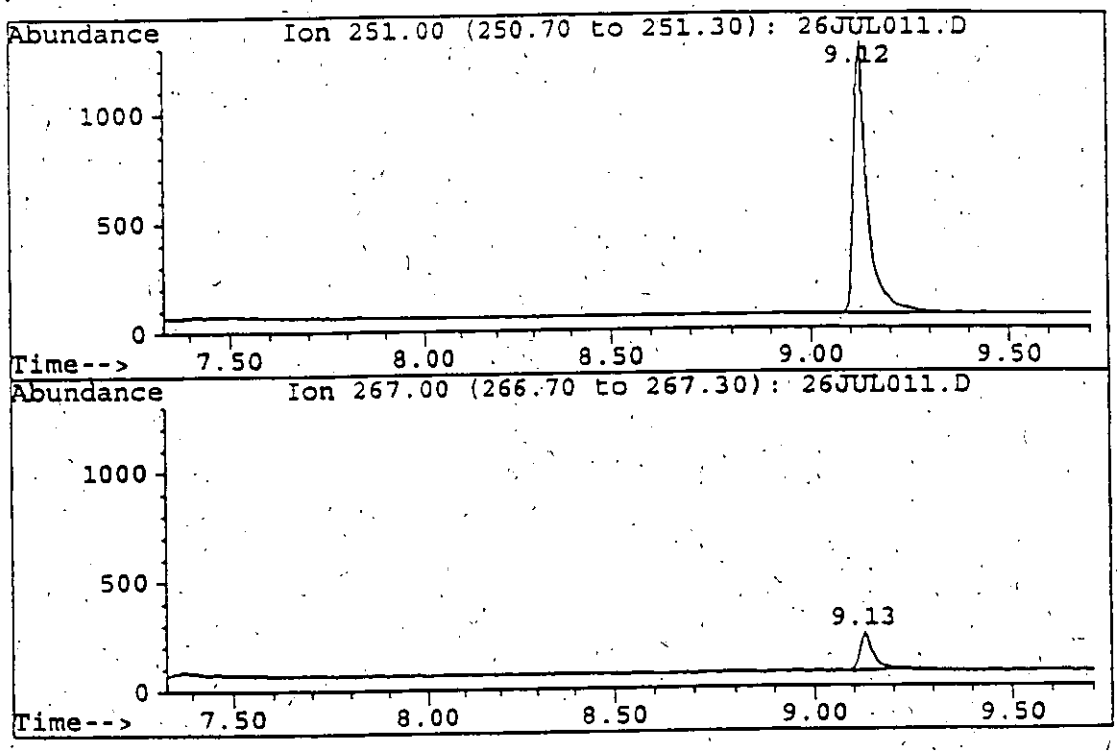
Peak#	Ret Time	Type	Width	Area	Start Time	End Time

No peaks detected

APPENDIX III

Fodder Spiked with 0.01 ppm RPA 201772

File: C:\HPCHEM\1\DATA\26JUL94\26JUL011.D
Operator: UJJANA NANDIHALLI
Date Acquired: 26 Jul 94 9:03 pm
Instrument: 5972-GC-M
Method File: RPASIM12
Sample Name: MV-23A FODDER 0.01 UG/G 201772
Misc Info: HWI 6224-215
Vial Number : 9



Ion 251.00 (250.70 to 251.30): 26JUL011.D
MV-23A FODDER 0.01 UG/G 201772

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.123	BV	0.036	29903	9.022	9.321

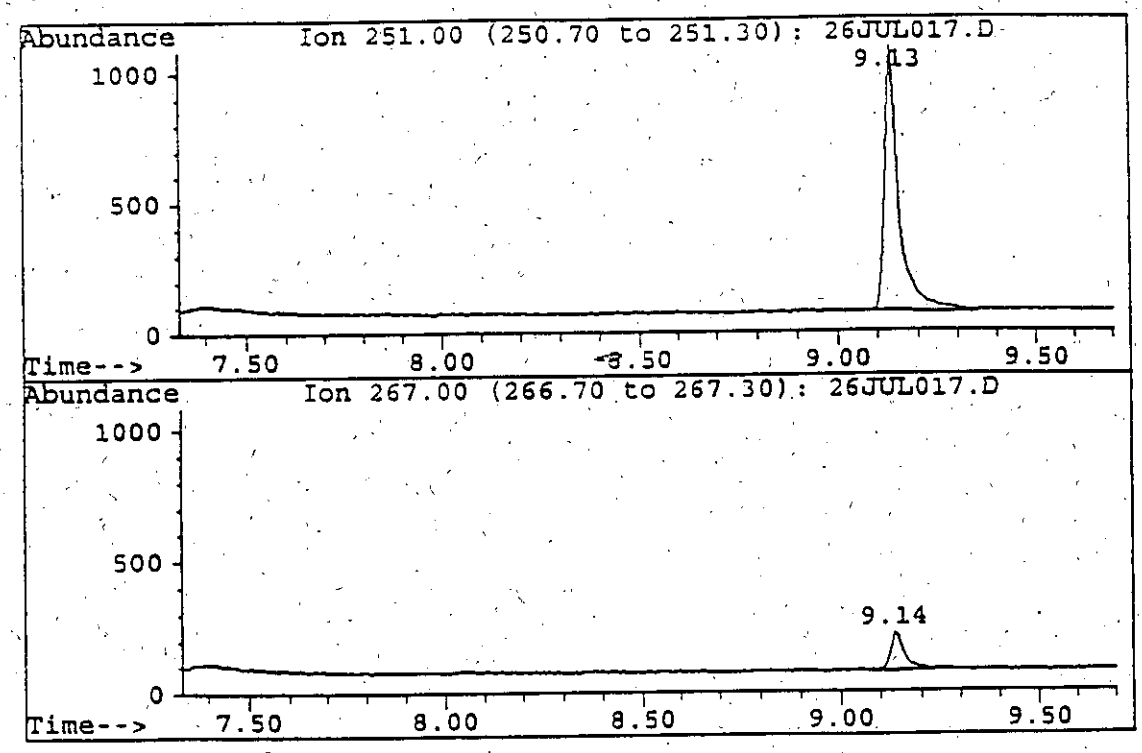
Ion 267.00 (266.70 to 267.30): 26JUL011.D
MV-23A FODDER 0.01 UG/G 201772

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.127	BV	0.034	3870	9.073	9.216

APPENDIX III

Fodder Spiked with 0.01 ppm RPA 202248

File: C:\HPCHEM\1\DATA\26JUL94\26JUL017.D
Operator: UJJANA NANDIHALLI
Date Acquired: 26 Jul 94 11:43 pm
Instrument: 5972-GC-M
Method File: RPASIM12
Sample Name: MV-29A FODDER 0.01 UG/G 202248
Misc Info: HWI 6224-215
Vial Number : 12



Ion 251.00 (250.70 to 251.30): 26JUL017.D
MV-29A FODDER 0.01 UG/G 202248

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.132	BV	0.037	24934	9.022	9.346

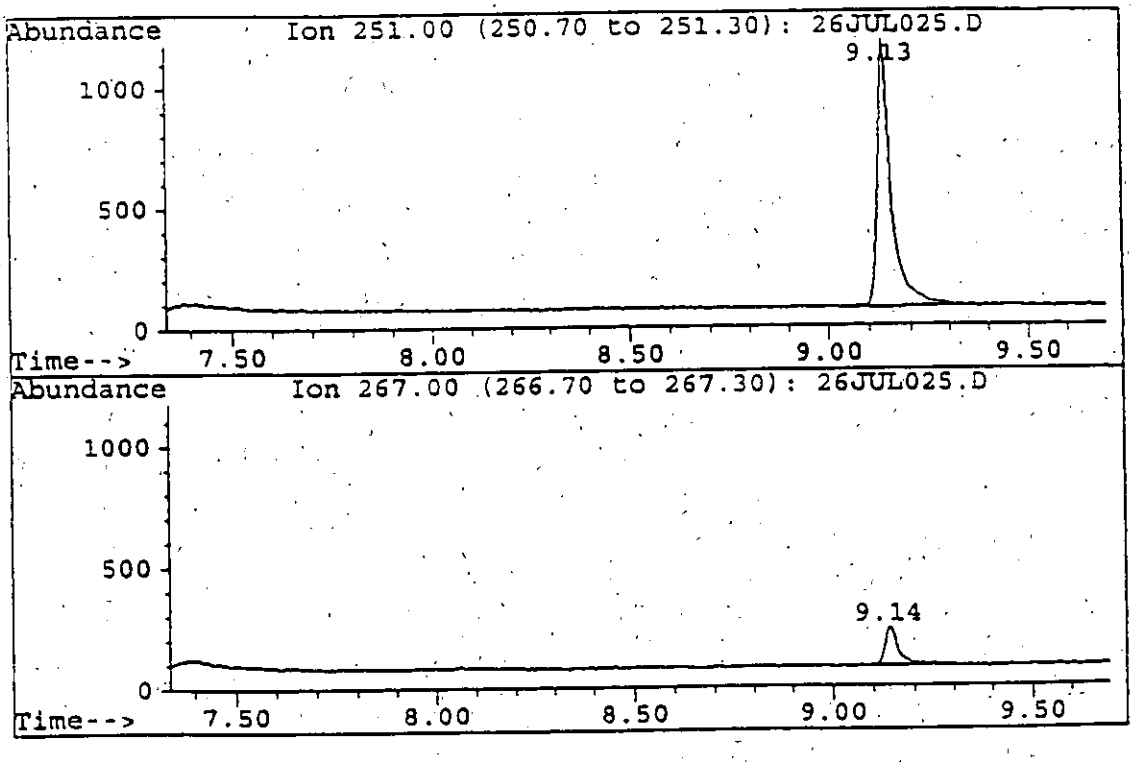
Ion 267.00 (266.70 to 267.30): 26JUL017.D
MV-29A FODDER 0.01 UG/G 202248

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.135	BV	0.035	3342	9.067	9.227

APPENDIX III

Fodder Spiked with 0.01 RPA 203328

File: C:\HPCHEM\1\DATA\26JUL94\26JUL025.D
Operator: UJJANA NANDIHALLI
Date Acquired: 27 Jul 94 3:16 am
Instrument: 5972-GC-M
Method File: RPASIM12
Sample Name: MV-36A FODDER 0.01 UG/G 203328
Misc Info: HWI 6224-215
Vial Number : 16



Ion 251.00 (250.70 to 251.30): 26JUL025.D
MV-36A FODDER 0.01 UG/G 203328

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.134	BV	0.037	27035	9.028	9.339

Ion 267.00 (266.70 to 267.30): 26JUL025.D
MV-36A FODDER 0.01 UG/G 203328

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.137	PV	0.036	3629	9.084	9.236

1. An analytical batch for method verification portion constituted one reagent blank, one untreated control and 18 fortified samples.

2. 16-18 hours each of two analysts (for 20 samples).

3. One reagent blank per batch. Analysis of reagent blank prior to sample analysis is recommended.

4. Equivalent reagents/suplies may be used. Reagents used in the preparation of diazomethane should be free of water and preservatives. Store bought distilled water can be used.

5A. Single tapered liner prepacked with glass wool (Hewlett-Packard Cat. NO. 5062-3587). With a new liner, three to four injections (from the same vial) of fortified matrix sample (matrix that is going to be injected that day) must be injected prior to sample analysis. Make sure the response is stabilized after the last injection.

5B. Depends upon the matrix. Normally, six to eight weeks. Best way is to wait until the chromatography is unacceptable.

6. Sample extracts were normally analyzed immediately after extraction. Based on the stability of RPA 204497 (3 months), it appears that the extracts can be stored in a freezer (-10 to -20 C) for up to 3 months (however, the maximum time we stored prior to analysis was 3 weeks).