Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Prometryn

MRID #: 415464-01

Matrix: Soil

Analysis: LC/MS/MS

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If you have difficulties in downloading the method, or further questions concerning the methods, you may contact Elizabeth Flynt at 228-688-2410 or via e-mail at flynt.elizabeth@epa.gov.

Appendix IX

Enseco Thermospray LC/MS/MS Confirmations

Standard Operating Procedure:

"Determination of Prometryn, GS-11354 and GS-11526 in Soil by LC/MS/MS"

Mass Spectra of Prometryn and its metabolites by LC/MS and LC/MS/MS

Elanco

STANDARD OPERATING PROCEDURE

Subject or Title:
Determination of Prometryn, GS-11354 and GS-11526 in Soil by LC/MS/MS

SOP No.:	Revision No.:	Effective Date:
LM-CAL-3045	Original	April 23, 1990

Supersedes: None

1. PRINCIPLE

Prometryn and metabolites (GS-11354 and GS-11526) are extracted from soil using a 80:20 (v/v) acetonitrile-water solution. Sample extracts are centrifuged, reduced under nitrogen, and then analyzed by TSP-LC/MS/MS. The detection limit for this method is 10 ug/kg.

2. STANDARDS

2.1 Standards

2.1.1 Prepare a 1 mg/mL individual stock solutions of each analyte in HPLC grade methanol. Replace stock solution every four months or sooner.

2.1.2 Using the 1 mg/mL individual stock solutions from 2.1.1 prepare a 10 ug/mL mixed standard in methanol. Replace mixed analyte solution every four months or sooner.

2.1.3 Using the 10 ug/mL mixed analytical standard solution from 2.1.2 prepare 0.10 ug/mL, 1.0 ug/mL and 10 ug/mL fortification standards in methanol. Replace spiking standards every four months or sooner.

2.1.4 Using the 10 ug/mL mixed standard solution from 2.1.2 prepare calibration standards at 5 ng/mL, 25 ng/mL, 100 ng/mL, 250 ng/mL, and 500 ng/mL in 50/50 Meoh/H_2O. Replace calibration standards every four months or sooner.

Prepared By:	Date:
Frank Kenney	April 23, 1990
Management Approval:	Date:
Calvin K. Hazel	April 24, 1990
QA Officer Approval:	Date:

3. PROCEDURE

3.1 Extraction

3.1.1 Weigh 10.0 grams of soil into a 40-mL vial.

3.1.2 If applicable, prepare fortification samples by adding fortification standard.

3.1.2.1 Add 1.0 mL of the 0.10 ug/mL fortification standard to yield 10.0 ug/kg (ppb).

3.1.2.2 Add 1.0 mL of the 1.0 ug/mL fortification standard to yield 100.0 ug/kg (ppb).

3.1.2.3 Add 1.0 mL of the 10.0 ug/mL fortification standard to yield 1,000 ug/kg (ppb).

3.1.3 Add the acetonitrile-water solution (80:20 v/v).

3.1.3.1 Add 20.0 mL to the sample and method blank.

3.1.3.2 Add 19.0 mL to the fortified samples to give a total volume of 20.0 mL of the extracting solution.

3.1.4 Adjust the "pH" of the extracting solution to 7.2 - 7.8 with acetic acidic or NH_4OH.

3.1.5 Shake the extracts on the orbital shake for 3 hours at ca 240 rpm with the vials positioned horizontally.

3.1.6 Centrifuge the extracts up to 1000 rpm for about 5 minutes to obtain a clear supernatant.

3.1.7 Aliquot 5.0 mL of the supernatant and filter it through a 0.45 um syringe filter into an 8-mL test tube. Filter an additional 0.5 mL of 80:20 acetonitrile-water through the filter into the test tube.

3.1.8 Reduce under nitrogen to less than 1 mL to remove the acetonitrile.

3.1.9 Adjust the final volume to 1.0 mL with methanol.

3.1.10 Transfer the extract to a 4 mL vial. Final concentration of the sample is 2.5 g/1.0 mL.

3.1.11 Organize the sample vials into the vial box, and store it in the refrigerator at 2-6° C until analysis.

4. LIQUID CHROMATOGRAPH OPERATING PARAMETERS

4.1 The LC chromatography system parameters have been optimized for these analytes as follows:

Column: 1) HP Hypersil ODS 6 cm x 4.6 mm ID (3um)
No Guard or equivalent

Flow rate: 0.8 mL/min. + 0.4 mL/min.
Post column addition of Buffer

Mobile Phase: ACN/Water
(Water contains 0.1M ammonium acetate)

Gradient: 30/70 ACN/H_2O
Gradient to 65/35 at 2.5 minutes
to 100% ACN at 4 min.
Return to 30/70 at 6.5 minutes
or equivalent conditions which provide
baseline resolution of all analytes.

Retention Times: Prometryn 7:20 minutes (approx.)
GS-11354 2:40 minutes (approx.)
GS-11526 1:50 minutes (approx.)

5. TSP - MASS SPECTROMETER OPERATING PROCEDURES

5.1 Instrument Tuning. - The instrument must be capable of detecting 100 pg of each analyte, (20 uL of 5 ng/mL) with a signal/noise (S/N) ratio of 5:1.

5.1.1 If desired, a mixture of prometryn and the metabolites (1 mg/L) may be used as a tuning solution. This provides fine tuning of the TSP interface parameters.

5.2 TSP-LCMS Operation Parameters.

5.2.1 The following TSP-LCMS parameters must be optimized in order to achieve an optimum performance.

Vaporizer Control Point	- 75 °C - 120 °C
Aerosol Temp	- 250 °C - 320 °C
Repeller Voltage	- 40 V - 75 V

5.2.2 It has been determined that optimal sensitivity is achieved with filament on ionization mode, however, either mode is acceptable.

5.3 Selected Ion Monitoring.

5.3.1 Samples and standards products are analyzed by selected reaction monitoring (SRM) of products derived from the protonated molecular ions $(MH)^+$. DS - Atrazine is included as an internal standard for retention time verification and possible future quantitation.

5.3.2 The appropriate masses to be monitored are listed below:

Analyte	Precursor (MH^+)	Product Ion	Dwell Time
Prometryn	m/z 242	m/z 158 ± .3 amu	0.3 seconds/window
GS-11354	m/z 200	m/z 158 ± .3 amu	0.3 seconds/window
GS-11526	m/z 212	m/z 128 ± .3 amu	0.3 seconds/window
DS-Atrazine	m/z 221	m/z 179 ± .3 amu	0.3 seconds/window

The seconds/window time may be changed providing that at least 6 scans are acquired per peak.

5.4 Calibration and Sample Analysis.

5.4.1 During the course of analysis of a batch of samples each of the calibration standards (see Section 2.1.4) is run once. Between 1 and 6 samples is analyzed between these standards.

5.4.2 Calculate the calibration factor (CF) for each standard, defined as the ratio of the standard concentration to the response for each analyte at each standard concentration.

5.4.3 If the percent relative standard deviation is less than 20% over the working range, linearity can be assumed and the average calibration factor can be used in place of a calibration curve.

5.4.3.1 Average Calibration Factor = $\left|\frac{\text{Concentration (ng/mL)}}{\text{Total Area Counts}}\right|_n$

Where n = number of calibration injections.

5.4.4 Inject 20-50 uL of extract into the TSP-LCMS system for analysis.

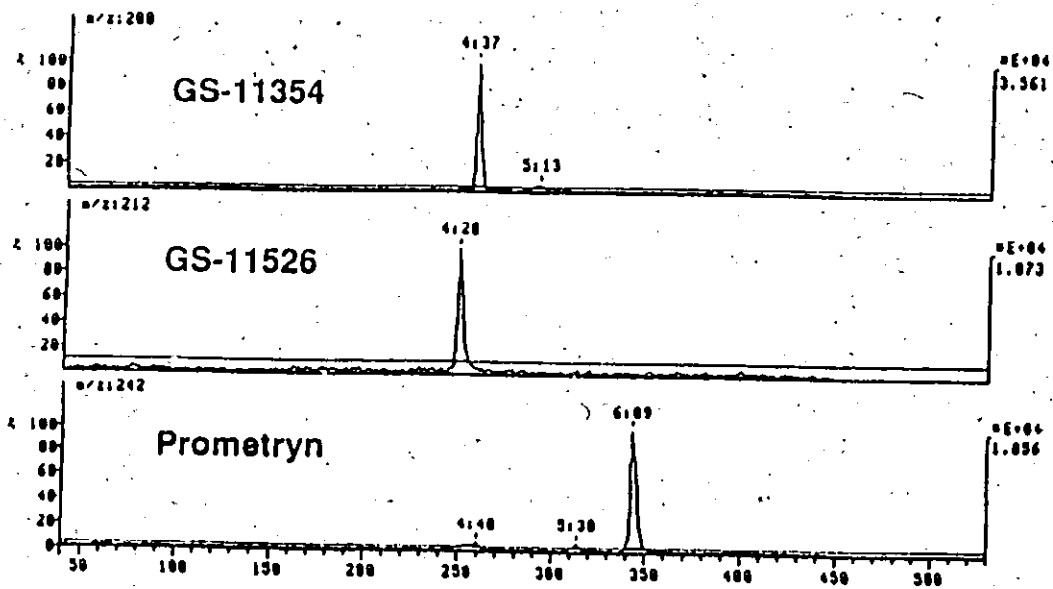
6. CALCULATIONS

6.1 The concentration of each analyte in a sample is calculated as follows:

$$\text{Concentration (ug/Kg, dry weight)} = \frac{(CF)\,(Vx)\,(Ax)}{(Ws)\,(Rw)\,(1000)}$$

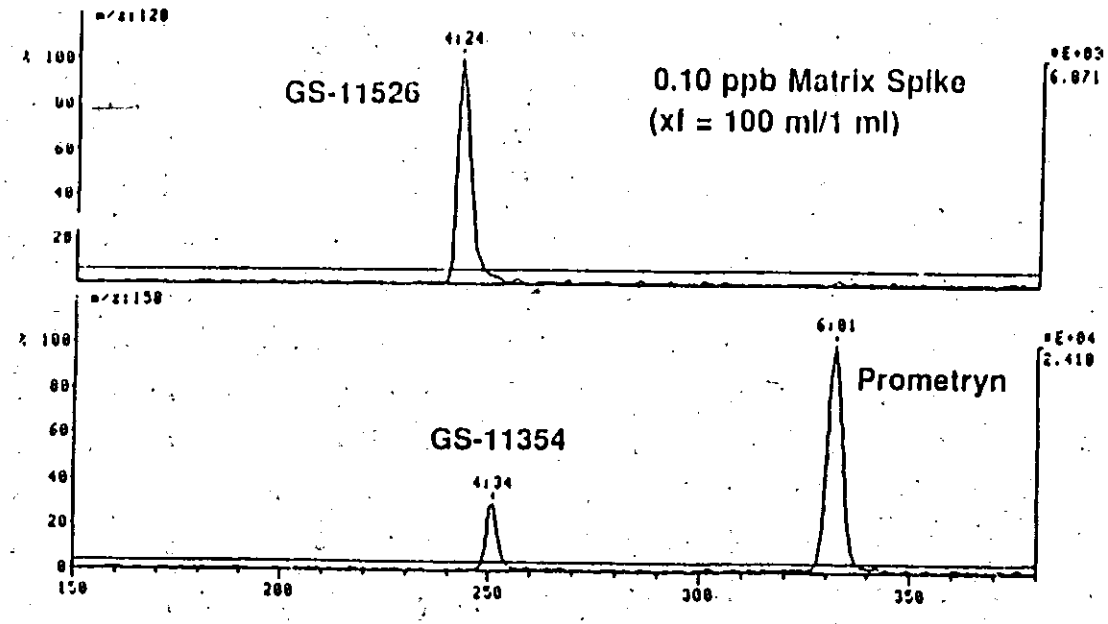
CF = Average Calibration factor (ng/mL)
Vx = Sample extract final volume (mL)
Rw = Dry Weight/Wet Weight Ratio
Ax = Sample Total Area Counts
Ws = Sample Wet Weight (Kg)

TSP LC/MS of Prometryn and Two Degradation Products



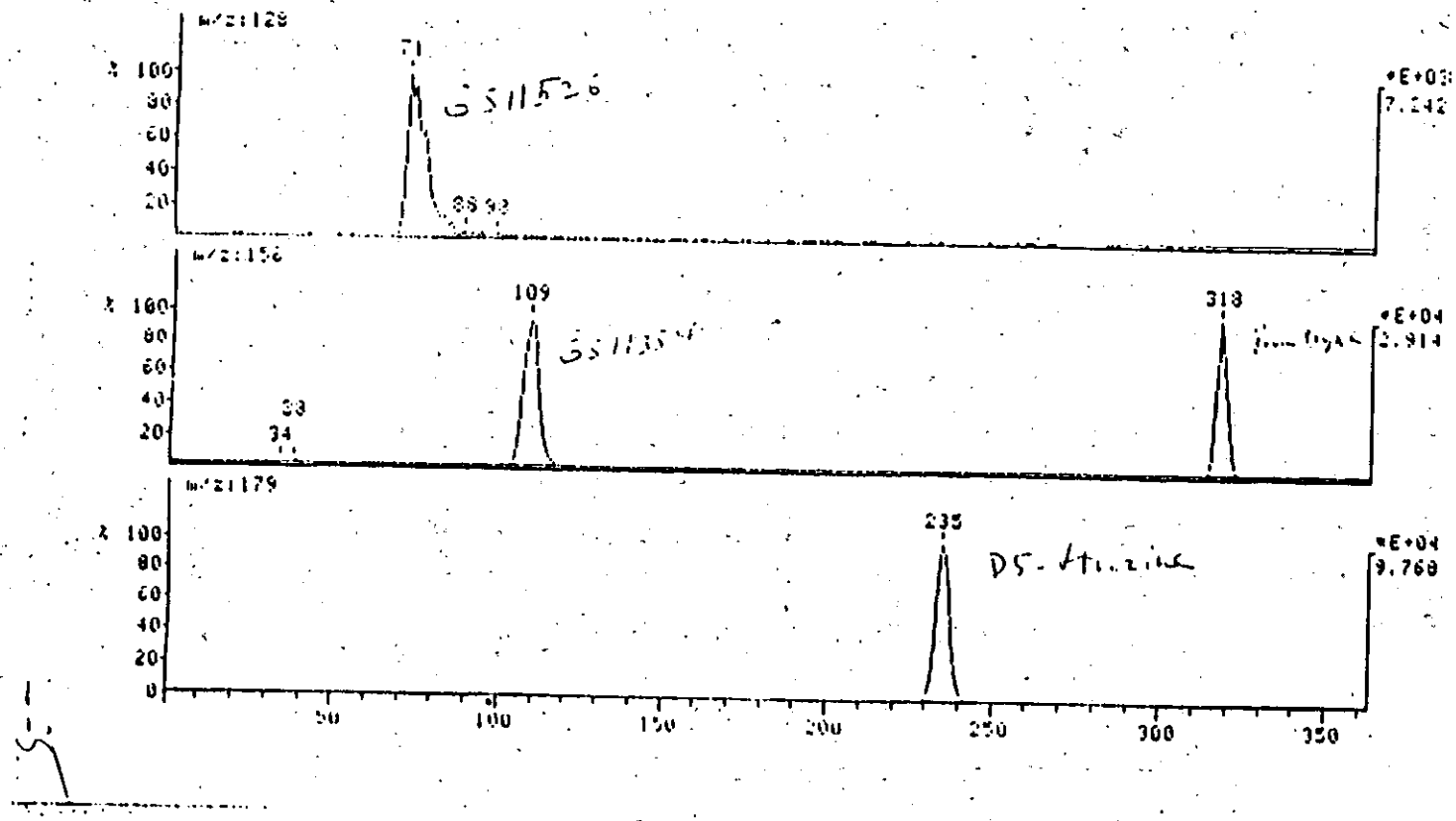
20 ul of 0.5 ppb Spike

TSP LC/MS/MS of Prometryn and Two Degradation Products



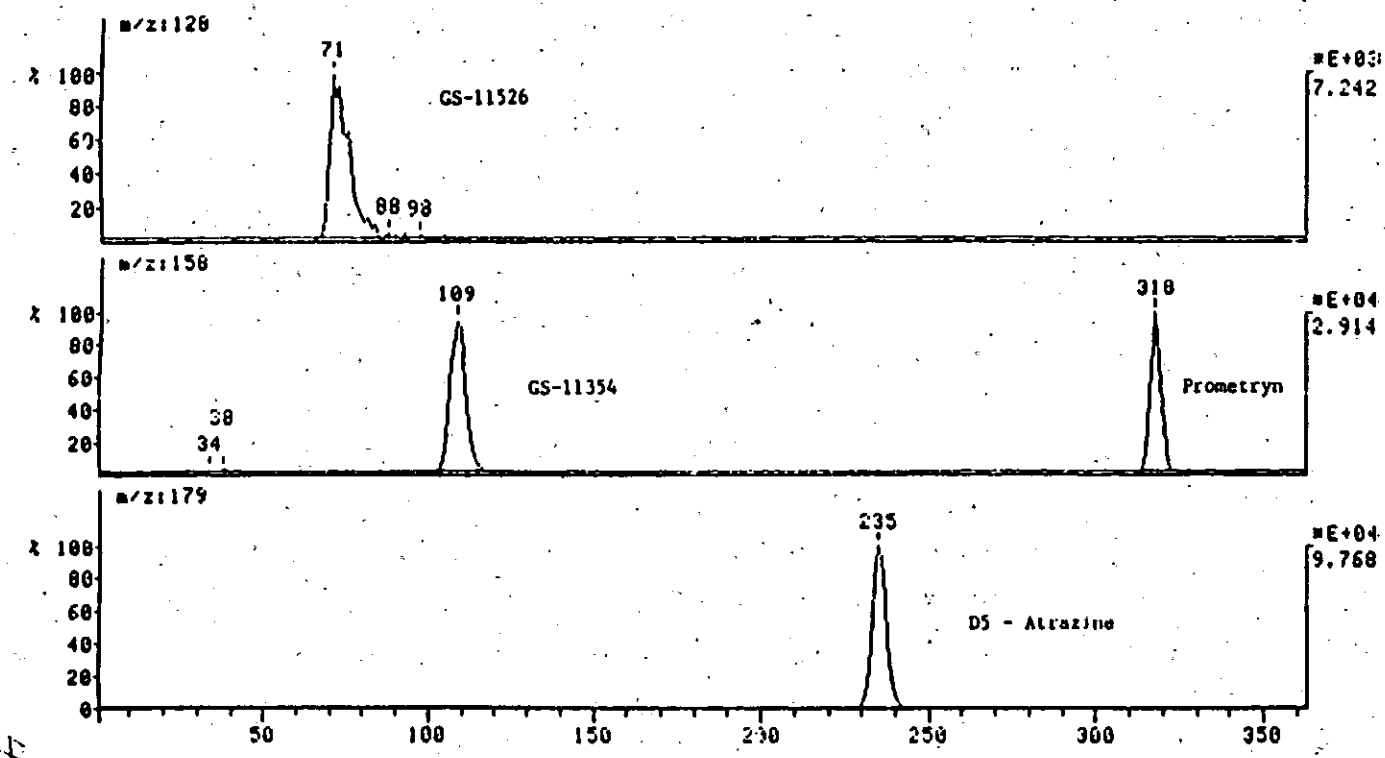
CHRO: PD9003281 ver 2 on UIC 002002 28-MAR-90 Elapse: 00:05:40.9 241
Samp: SS-900310B 10NG/ML Start : 12:49:20 363
Comm: 20UL ACDAUFRON V=90 S=230 EM=1200X8 FILOFF CID=2.0MTORR
Mode: TSP +DAU LNR SYNTH GAS UP LR
Oper: FHK
Peak: 1000.00 amu Label wndw: 1 > 363
Area: 0, 4.00 Baseline : 15, 3
Inlet :
Masses: 128 > 179
Label : -2, 10.00

PROMETRYN + METAB
BY LC/MS/MS
SIM TRACES



CHRO: PD900328J ver 2 on UIC 002002 28-MAR-90 Elapse: 00:05:40.9 241
Samp: SS-900310B 1PNG/ML Start : 12:49:20 363
Comm: 20UL ACDAUPROM V=90 S=200 EM=1200X0 FILOFF CID=2.0MTORR
Mode: TSP +DAU LMR SYNTH GAS UP LR
Oper: FHK Inlet :
Peak: 1000.00 amu Label wndw: 1 > 363 Masses: 128 > 179
Area: 0, 4.00 Baseline : 15, 3 Label : -2, 10.00

Prometryn and Metabolites
by LC/MS/MS
Simazine Traces



QUAN: TEMP.09 ver 1 on UIC 002002 Entries: 4

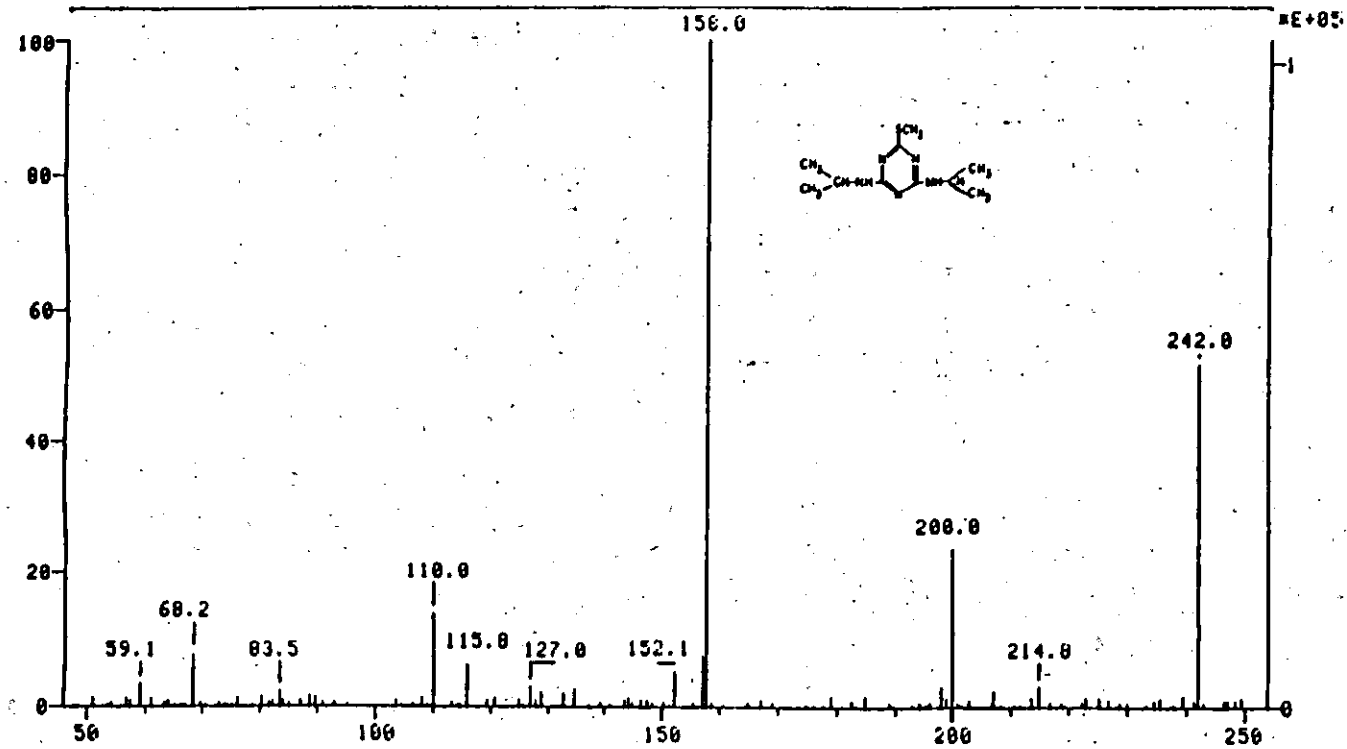
Data file(s) used
File: PD900328J ver 2 on UIC 002CG2 28-MAR-90
Samp: SS-9003108 10NG/ML Start : 12:49:20 338
Comm: 20UL ACDAUPROM V=90 S=280 EM=1200X8 FILOFF CID=2.0MTORR
Mode: TSP +DAU LMR SYNTH GAS UP LR
Oper: FHK Inlet :
Disp: SEAR CHRO
Amount = (area * ref amnt) / (ref area * response factor)
Response factors from quantitation library

#	Compound Name	Time	MH+	Area	Amount
1.	D5-ATRAZINE	5:32	179	686525.	50.00
2	PROMETRYNE	7:28	158	149523.	10.00
3	GS11526	1:43	128	72230.	10.00
4	GS11354	2:36	158	226703.	10.00

#	Compound Name	RF	Scan	Pred Time	Delta Time	Height	S/N
1	D5-ATRAZINE	0.000073	235	5:32	0.0	97765.	*****
2	PROMETRYNE	0.000067	318	7:28	0.0	29101.	653.8
3	GS11526	0.000138	71	1:43	0.0	7283.	228.0
4	GS11354	0.000044	109	2:36	0.0	27640.	391.3

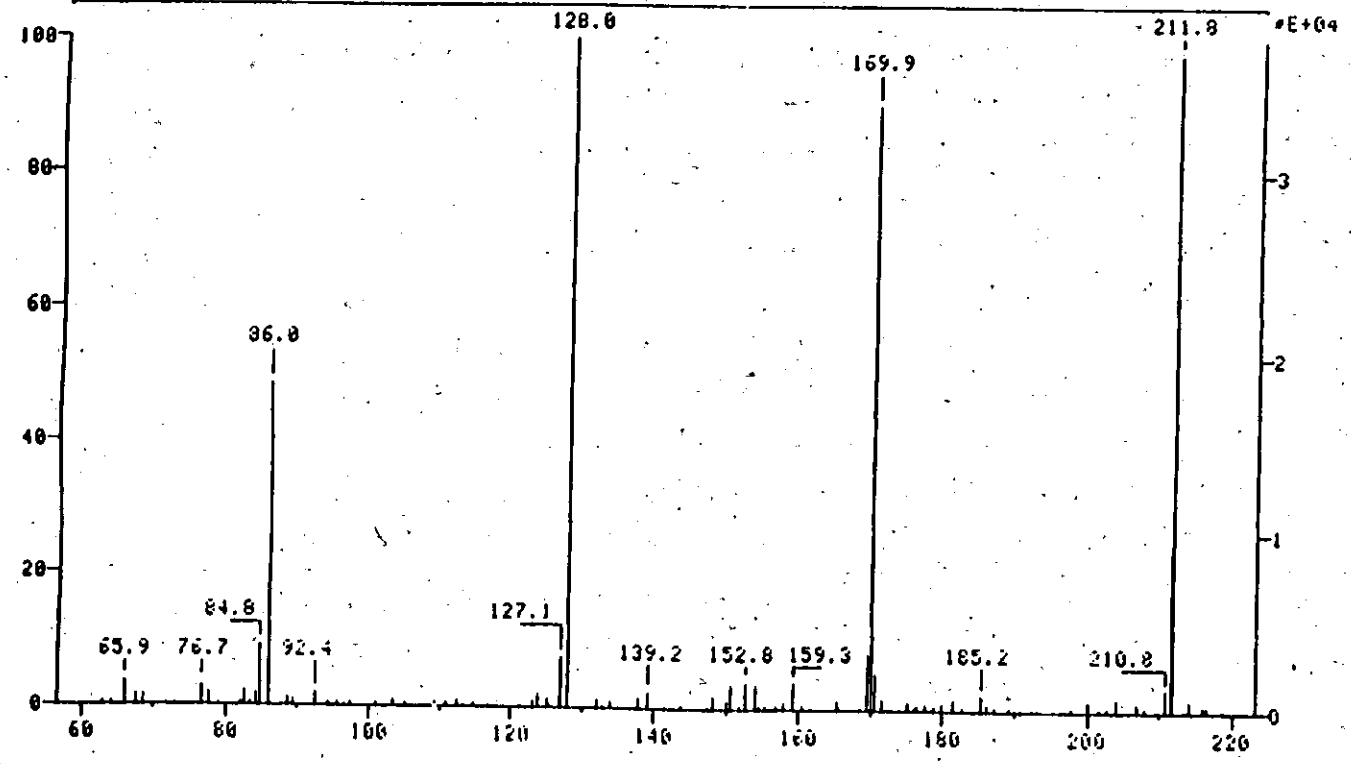
SPEC: TS890228B ver 2 on UIC 2 5 28-FEB-89 DERIVED SPECTRUM 9
Samp: PROMETRYNE Start : 09:05:44 10
Comm: 1ML OF 10PPB COFF=-20, CID=0.7
Mode: EI +DAU 242.2 @ -20eV LMR SYNTH GAS UP LR
Oper: ACQDAU619 Inlet :
Base: 158.0 Inten : 103711 Masses: 50 > 250
Norm: 158.0 RIC : 324186 # peaks: 123
Peak: 1000.00 amu
Data: + 60 > 71 - 58

Daughter Spectrum of Mass 242
(Prometryn)



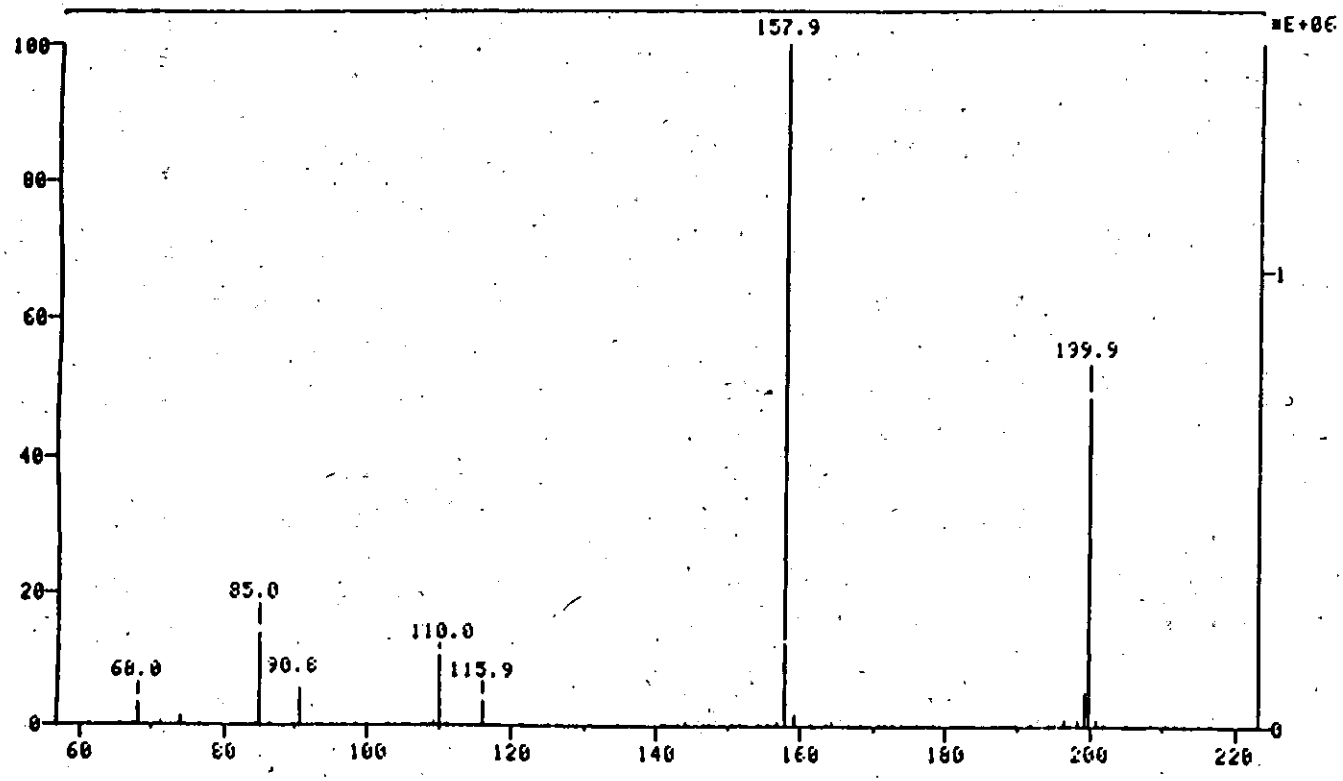
SPEC: PROM88 ver 4 on UIC 002005 1-APR-88 DERIVED SPECTRUM 9
Samp: GS11526 Start : 17:53:19 10
Comm: 100HG WITH 55% MEOH FULL SCAN DAU V=95
Mode: EI +DAU 212.1 0 -20eV LMR SYNTH GAS UP LR
Oper: ACQ3TSP Inlet :
Base: 128.0 Inten : 37636 Masses: 60 > 220
Norm: 128.0 RIC : 185114 # peaks: 116
Peak: 1000.00 amu
Data: / 12 > 15 - 5

Daughter Spectrum of Mass 212
(GS-11526)



SPEC: PROM81 ver 1 on UIC 002005 1-APR-88 DERIVED SPECTRUM 9
Samp: GS11354 Start : 17:50:31 10
Comm: 100NG WITH 55% MEOH FULL SCAN DAU V=95
Mode: EI +DAU 200.1 0 -20eV LMR SYNTH GAS UP LR
Oper: ACQ3TSP Inlet :
Base: 157.9 Inten : 1500078 Masses: 60 > 220
Norm: 157.9 RIC : 3312322 # peaks: 179
Peak: 1000.00 mmu
Data: + 9 > 13 - 4

Daughter Spectrum of Mass 200
(GS-11354)



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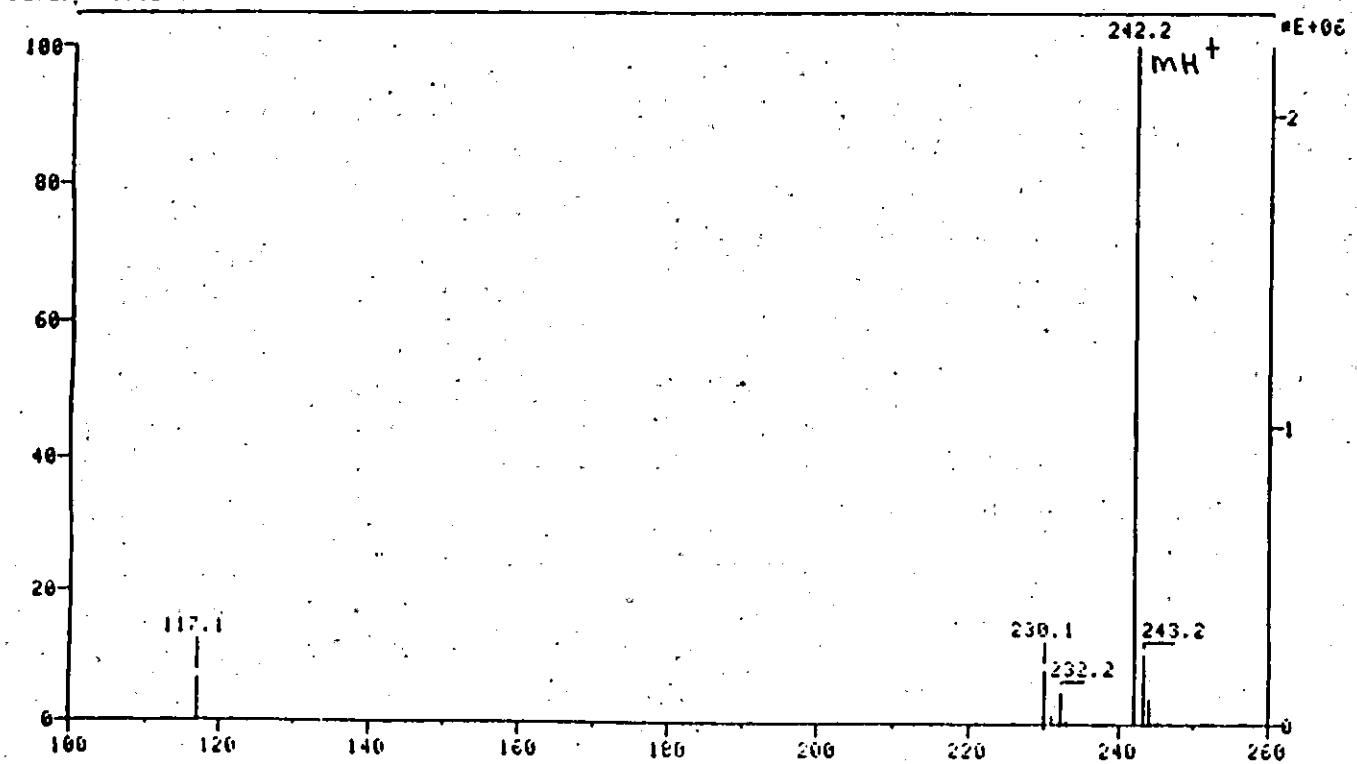
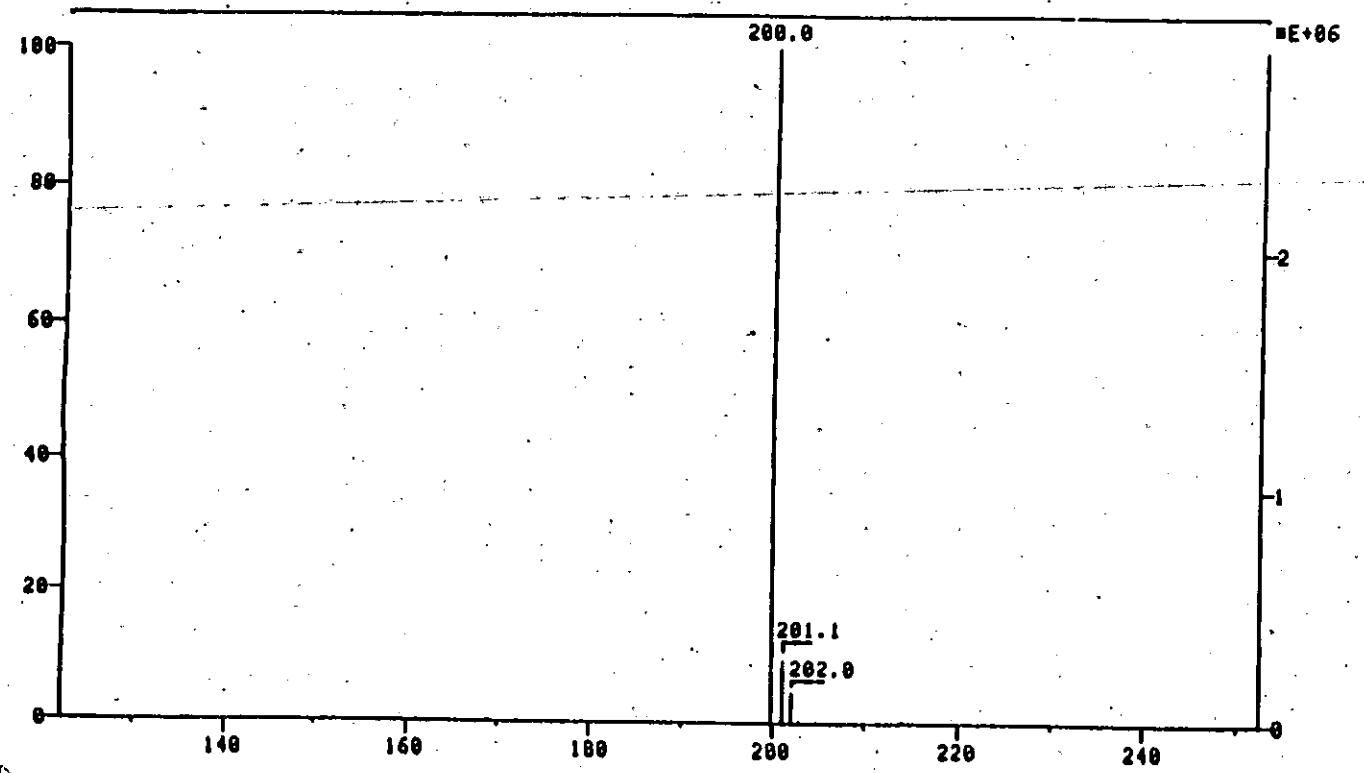
SPEC: PROMETRI ver 3 on UIC 002005 1-FEB-89 DERIVED SPECTRUM 9
Samp: PROMETRYNE Start : 19:33:29 10
Comm: 20UL OF 10UG/ML, .05M, FILAMENT OFF, JET=250, VAP=230
Mode: EI +Q3MS LMR UP LR
Oper: ACQTSPLP/R Inlet :
Base: 242.2 Inten : 2225331 Masses: 100) 300
Norm: 242.2 RIC : 3866532 # peaks: 38
Peak: 1000.00 mmu
Data: / 9)12-4)8

1st Stage Spectrum Prometryn

mw = 241.14



SPEC: GS11354 ver 1 on VIC 002002 24-APR-90 DERIVED SPECTRUM 9
Samp: GS-11354 Start : 14:42:54 10 Dealkalated Metabolite GS-11354
Comm: 20UL OF 10UG/ML
Mode: EI +03MS LMR UP LR
Oper: CKT/FHK Inlet :
Base: 200.0 Inten : 2034742 Masses: 125 > 250
Norm: 200.0 RIC : 3200969 # peaks: 36
Peak: 1000.00 amu
Data: 1 56



SPEC: GS11526A ver 1 on UIC 002002 24-APR-90 DERIVED SPECTRUM 9
Start : 14:41:24 10 Hydroxy Prometryn GS-11526
Samp: GS-11526
Conc: 20UL OF 10UG/ML
Mode: EI +Q3MS LMR UP LR
Oper: CKT/FHK
Base: 212.0 Inten : 4764559
Norm: 212.0 RIC : 5338829
Peak: 1000.00 amu
Data: 1 44

Inlet :
Masses: 125 > 250
peaks: 71