



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAR 29 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ID #062719-EEG (CBTS #11610; Barcode #D189444).
Flumetsulam (DE-498) on Soybeans and Corn. Evaluation of
Method Trial Report dated 3/19/93.

FROM: Nancy Dodd, Chemist *Nancy Dodd*
Tolerance Petition Section II
Chemistry Branch I- Tolerance Support
Health Effects Division (H7509C)

THROUGH: Debra Edwards, Ph.D., Chief
Chemistry Branch I- Tolerance Support
Health Effects Division (H7509C)

TO: Joanne Miller, PM#23
Fungicide-Herbicide Branch
Registration Division (H7505C)

and

Albin Kocialski, Section Head
Registration Section
Chemical Coordination Branch
Health Effects Division (H7509C)

EPA's Analytical Chemistry Laboratory (ACS, ACB, BEAD) has informed us of the results of their method validation on flumetsulam (DE-498; N-(2,6-difluorophenyl)-5-methyl-1,2,4-triazolo-[1,5-a]-pyrimidine-2-sulfonamide) on soybeans and corn (Everett Greer, 3/19/93). The methods tested were DowElanco's methods ACR 91.6 on soybean grain and ACR 91.6.1S on corn grain and corn fodder.

Soybean grain, corn grain, and corn fodder were fortified at levels of 0.05 and 0.10 ppm. Recoveries were tabulated on page 4 of the ACS memo dated 3/19/93 (Attachment 1).

Comments from ACS are listed on pages 2 and 3 of Attachment 1.

Conclusions and Recommendations

CBTS cannot approve the enforcement methods for flumetsulam on soybeans and corn at this time. ACS's comments #1, 2, 3, 4, and 8



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contains at least 50% recycled fiber

in Attachment 1 should be addressed by the petitioner. Revised analytical methods should be submitted which incorporate ACS's comments #1, 2, 3, and 4. Concerning comment #8, the petitioner should assure that RTP has an uncontaminated deuterated DE-498 standard.

Attachment 1: ACS method validation memo (E. Greer, 3/19/93)

cc with Attachment 1: SF, N. Dodd (CBTS), PM#23, MTO File, PP#1G04006, PP#2F04036

cc without Attachment 1: RF, Circu, E. Haeberer (CBTS), M. Bradley, D. Marlow (ACB/BEAD), H. Hundley (ACB/BEAD)

RDI:E. Haeberer:3/23/93:R. Loranger:3/23/93
H7509C:CBTS:CM#2:Rm804F:305-5681:N.Dodd:nd:3/26/93



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Analytical Chemistry Section
Building 306, BARC-East
Beltsville, Maryland 20705

MAR 19 1993

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#9F3702. Tolerance Method Validation of DE-498
(Flumetsulam) on Soybeans and Corn

FROM: Everett S. Greer, Jr., Team Leader *ESG*
Alexander J. Krynitsky, Chemist *ajk*
Analytical Chemistry Section

THRU: Harvey K. Hundley, Head *HK*
Analytical Chemistry Section

THRU: Donald A. Marlow, Chief *DM*
Analytical Chemistry Branch

TO: Elizabeth Haeberer, Head
Tolerance Petition Section II
Chemistry Branch I-Tolerance Support
Health Effects Division

INTRODUCTION

The Analytical Chemistry Laboratory was requested by the Chemistry Branch I, Tolerance Support to conduct a method validation on the herbicide DE-498; flumetsulam (N-(2,6-difluorophenyl)-5-methyl[1,2,4]triazolo[1,5-a]pyrimidine-2-sulfonamide). The DowElanco analytical method, "Determination of Residues of DE-498 in Soybean By Capillary Gas chromatography-Mass Spectrometry", was validated for soybean grain fortified at the 0.05 ppm and 0.1 ppm levels. A supplement to this method (AC #: 91.6.S) was validated for corn grain and fodder fortified at the same levels. The methods are essentially identical except for a slightly modified extraction procedure for corn fodder.

METHOD SUMMARY

DE-498 is extracted from the crop material with a 90% acetone/10% 0.1 N HCl solution. The acetone is evaporated from an aliquot of the extract and the residue is diluted with 0.005 N HCl and washed with hexane. The sample is cleaned up on C₁₈ and

alumina SPE cartridges and derivatized with methyl iodide. The resulting N-methyl derivative is dissolved in N-d₃-methyl DE-498 internal standard solution and quantitated by gas chromatography/mass spectrometry.

COMMENTS

1. The method uses a deuterated internal standard and the response ratio between the analyte standard and the internal standard (m/z 134/145) is taken for quantitation. Analytical standards are derivatized along with each set of samples. During the course of the method validation it was noted that this ratio was consistently close to 3, but occasionally the ratio turned out to be approximately 2 or less. Standards that gave low response ratios would give high sample recoveries and therefore were not used. Another aliquot of the standard solution was derivatized and the sample set was reinjected into the GC/MS in order to obtain more accurate values. Changing the amount of derivatizing reagent from 10 microliters as stated in the method to 5 and 20 microliters did not seem to have an effect on the analytical standard response. The derivatized internal standard was prepared in sufficient quantity at the beginning of the validation and aliquots of this same solution were used throughout the analysis. An analyst should be aware of any changes in the m/z 134/145 response ratio so that corrections can be made in order to achieve reliable results.

2. The method states that an elution profile should be obtained for both the C₁₈ and alumina SPE columns, but no specific instructions are given for running these calibrations.

3. The method uses a recovery correction factor for calculating residue concentrations. This practice is precluded in a tolerance enforcement procedure.

4. The Supelco vacuum manifold used at ACL-Beltsville to elute the solid phase extraction columns was found to be a source of interference. A GC/MS peak eluting at the same retention time as the analyte for m/z 134 was detected each time the manifold was used. The plastic fittings between the SFC cartridge and the collection tubes were found to be the cause of this contamination. A B&J manifold equipped with Teflon fittings throughout was used to eliminate this problem. This was not a specific fault of the method, but we suggest that a note be included to use only Teflon or other inert fittings for manifold equipment.

4

5. A Hewlett Packard HP 5988 GC/MS was substituted for the MSD used in the method. Manual tuning of this instrument provided sufficient sensitivity for the residue levels requested for this validation study.

6. A set of six samples can be completed in 2 days.

7. Under the instrument parameters used for this TMV, the sensitivity of the method is estimated to be 0.01 ppm for all commodities.

8. The standard used for this TMV was supplied to ACL by the RTP repository. ACL derivatized an In-House deuterated DE-498 standard as per method because the deuterated standard provided by RTP appeared to have been contaminated. The commodities were supplied by the petitioner.

9. The corn fodder, provided by the petitioner, was wetter than normal and needed to have the moisture determined in order to correct for recovery. By not correcting for moisture the recoveries would be biased lower by approximately 3%.

10. This method meets the requirements in the Subdivision O guidelines for a tolerance enforcement procedure provided the petitioner incorporates solid phase extraction cartridge calibration instructions (Comment 2) into the method and deletes references to recovery correction factors from the method.

5

<u>Commodity</u>	<u>ppm Added</u>	<u>ppm Found</u>	<u>% Recovery</u>
Soybean	Control	N.D. ¹	-
	Control	N.D.	-
	0.05	0.0425	85.0
	0.05	0.0349	70.0
	0.10	0.0821	82.1
	0.10	0.0509	50.9
	0.10	0.0577	57.7
	0.10	0.0532	53.2
	0.10	0.0682	68.2
	0.10	0.0760	76.0 ²
Corn Fodder	Control	N.D. ¹	-
	Control	N.D.	-
	0.05	0.0373	74.6
	0.05	0.0402	80.4
	0.10	0.0797	79.7
	0.10	0.0688	68.8
Corn Grain	Control	N.D. ¹	-
	Control	N.D.	-
	0.05	0.0327	65.4
	0.05	0.0376	75.1
	0.10	0.0793	79.3
	0.10	0.0748	74.8

1. N.D. - Not detected above 0.01 pm for DE498.

2. This number is the average of three analyses of the same sample extract carried through the entire method starting with step 9.h. The recoveries were 46.3%, 99.2% and 88.5%.

Modifications to method (Major or Minor):

See comments section of report.

Special precautions to be taken:

See comments section of report.

If derivatized is used, give source:

Derivatized standard is prepared as part of the method.

Instrument for quantitation:

GC/MS HP model number HP5988

Instrument for confirmation:

N/A

If instrument parameters differ from those in given in method, list parameters used:

An HP 5988 GC/MS was substituted for the MSD used in the method. All GC parameters used were as decried in the method.

Commercial sources for any special chemicals of apparatus:

N/A

Additional comments:

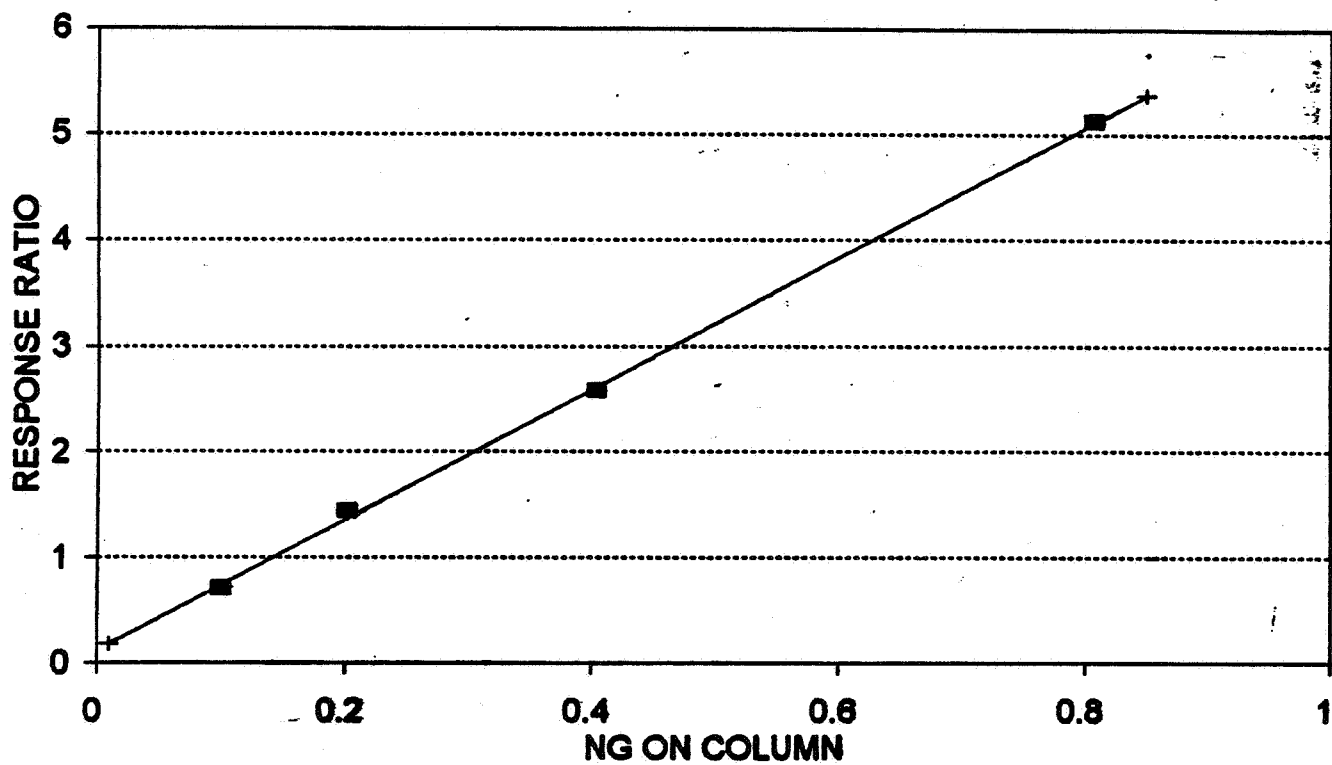
See report.

Chromatograms:

Copies attached

LINEAR REGRESSION

FLUMETSULAM TMV B93-11/12



■ DATA POINTS + CALC. POINTS

FLUMETSULAM TMV
 LINEAR REGRESSION
 B93-11/12

STD	STD	AVG	INT	STD	INT	STD	AVG	RESPONSE	CALC
CONC	VOL	INJ	STD	INJ	AREA 1	AREA 2	AREA	RATIO	AREA
ng/ul	ul	ng							
0.0505	2.0	0.1010	50031	51190	50611	67955	73407	70681	0.7160
0.1009	2.0	0.2018	85672	104167	94920	57936	73555	65746	1.4437
0.2018	2.0	0.4036	158978	177731	168355	61769	68541	65155	2.5839
0.4036	2.0	0.8072	347485	414817	381151	66051	82787	74419	5.1217
		0.0100							0.1827
		0.8500							5.3715

Regression Output:

Constant 0.1289
 Std Err of Y Est 0.0613
 R Squared 0.9993
 No. of Observations 4.0000
 Degrees of Freedom 2.0000

X Coefficient(s 6.1772
 Std Err of Coef 0.1133

Data file: DATA:SOY_A01A.D
File type: GC / MS DATA FILE

Sample Name: 2UL INJ 0.2018 UG/ML DE498
Misc Info: B93-11/12, EM @ 2336
Operator: ESG

SA DE-498

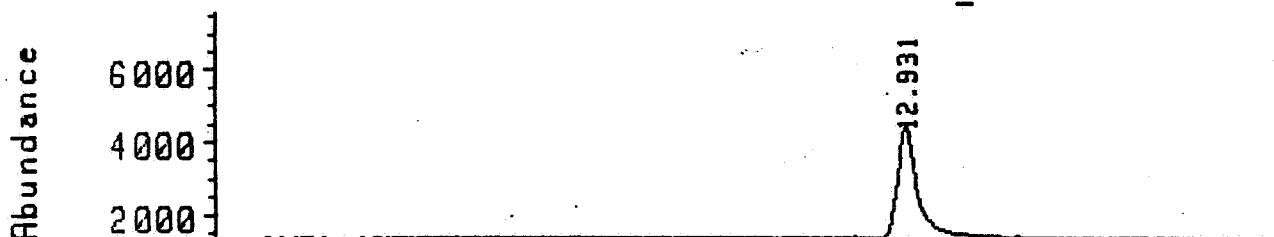
Date: 25 Feb 93 3:35 pm
Instrument: MS_5988
Inlet: GC

Sequence index: 1
ALS bottle num: 1
Replicate num: 1

Ion 134.00 amu. from DATA:SOY_A01A.D



Ion 142.00 amu. from DATA:SOY_A01A.D



Ion 145.00 amu. from DATA:SOY_A01A.D



Ion 134.00 amu. from DATA:SOY_A01A.D 1 integration peaks found

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.930	VV	0.059	246165	12.818	13.136

Ion 142.00 amu. from DATA:SOY_A01A.D 1 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.931	VV	0.061	131352	12.793	13.140

Ion 145.00 amu. from DATA:SOY_A01A.D 1 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.912	VV	0.064	77001	12.830	13.143

1000000 CORNER 0.1PPM B93-11/12: EM C 2336

Operator: ESG

Date: 1 Mar 93 1:03 pm

Instrument: MS_5988

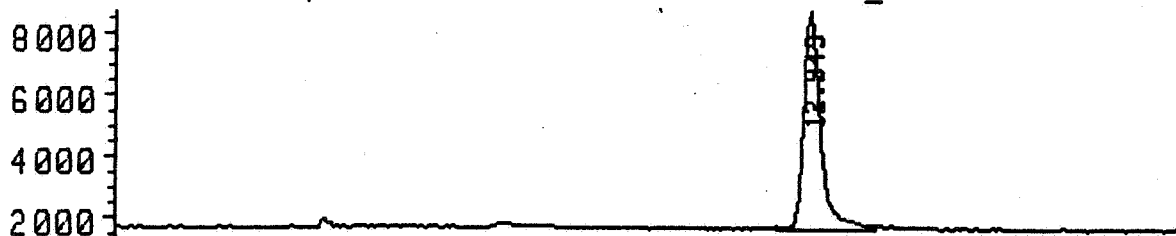
Inlet: GC

Sequence index: 1

Als bottle num: 8

Replicate num: 1

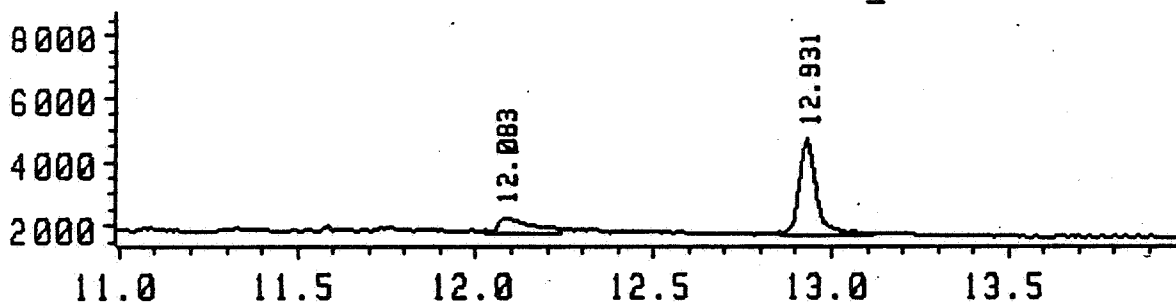
Ion 134.00 amu. from DATA2:COG_A08A.D



Ion 142.00 amu. from DATA2:COG_A08A.D



Ion 145.00 amu. from DATA2:COG_A08A.D



Ion 134.00 amu. from DATA2:COG_A08A.D 1 integration peaks found

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.949	UV	0.051	239126	12.848	13.125

Ion 142.00 amu. from DATA2:COG_A08A.D 1 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.951	UV	0.054	124048	12.857	13.127

Ion 145.00 amu. from DATA2:COG_A08A.D 2 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.083	UV	0.093	33276	12.029	12.239
2	12.931	UV	0.054	106096	12.857	13.114

Data file: DATA2:COG_A05B.D
File type: GC / MS DATA FILE

Sample Name: CG.05-A 2G/1ML 2UL INJ
Misc Info: CORN GR 0.05 PPM B93-11/12; EM @ 2336
Operator: ESG

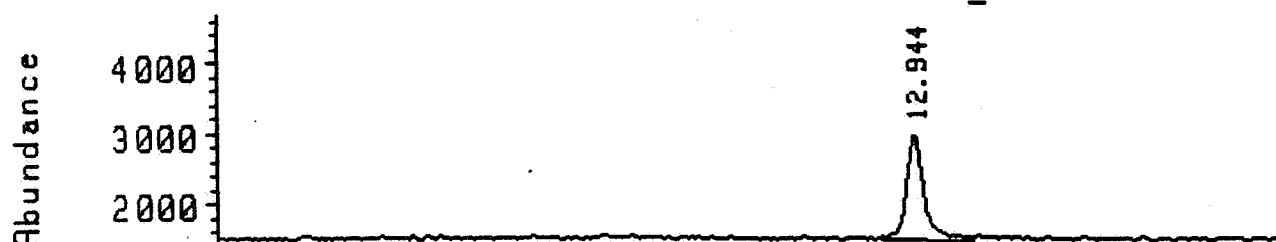
Date: 1 Mar 93 11:05 am
Instrument: MS_5988
Inlet: GC

Sequence index: 1
Als bottle num: 5
Replicate num: 2

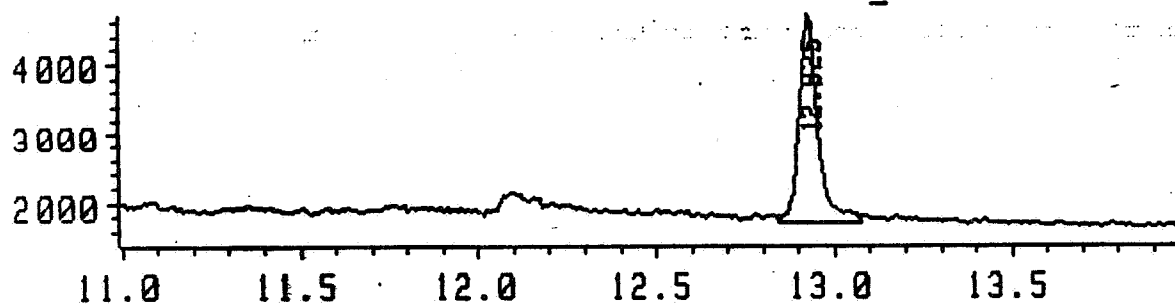
Ion 134.00 amu. from DATA2:COG_A05B.D



Ion 142.00 amu. from DATA2:COG_A05B.D



Ion 145.00 amu. from DATA2:COG_A05B.D



Ion 134.00 amu. from DATA2:COG_A05B.D 1 integration peaks found

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.944	UU	0.052	99691	12.829	13.063

Ion 142.00 amu. from DATA2:COG_A05B.D 1 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.944	UU	0.053	53561	12.857	13.107

Ion 145.00 amu. from DATA2:COG_A05B.D 1 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.925	UU	0.054	104066	12.842	13.073

Data File: DATA2:COG_A02A.D
File type: GC / MS DATA FILE

Sample Name: CG-A 2G/1ML 2UL INJ
Misc Info: CORN GRN CONTROL B93-11/12 EM @ 2336
Operator : ESG

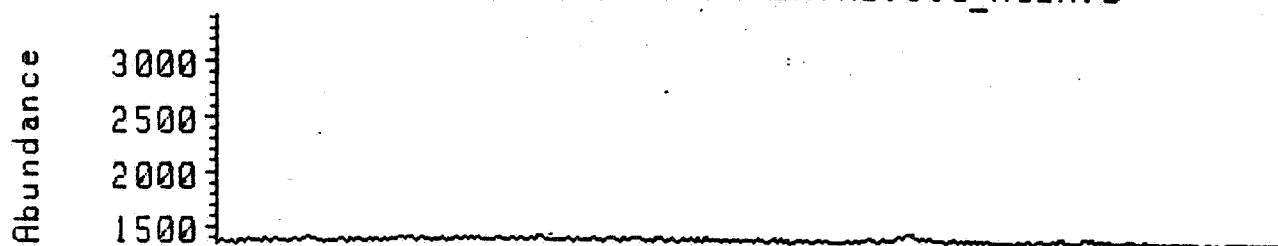
Date : 1 Mar 93 8:50 am
Instrument: MS_5988
Inlet : GC

Sequence index : 1
Als bottle num : 2
Replicate num : 1

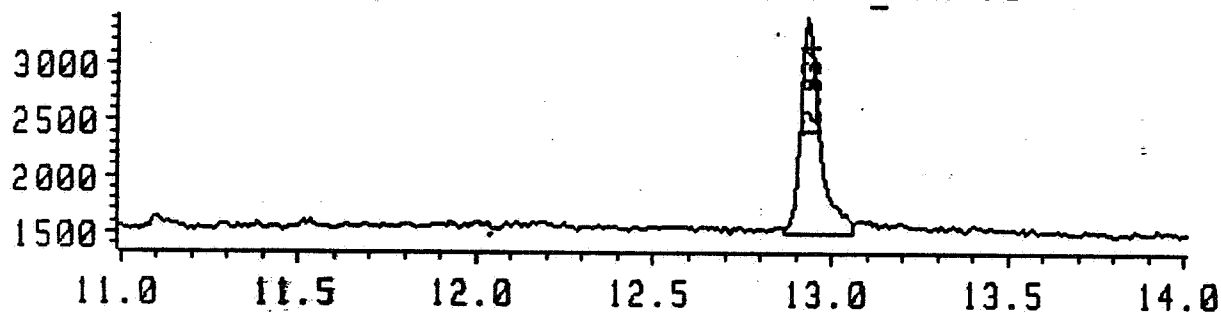
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Ion 142.00 amu. from DATA2:COG_A02A.D



Ion 145.00 amu. from DATA2:COG_A02A.D



Ion 134.00 amu. from DATA2:COG_A02A.D 0 integration peaks found.

Page 1

Ion 142.00 amu. from DATA2:COG_A02A.D 0 integration peaks found.

Page 1

Ion 145.00 amu. from DATA2:COG_A02A.D 1 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.934	UV	0.056	73210	12.867	13.063

Data file: DATA2:COG_A02B.D
File type: GC / MS DATA FILE

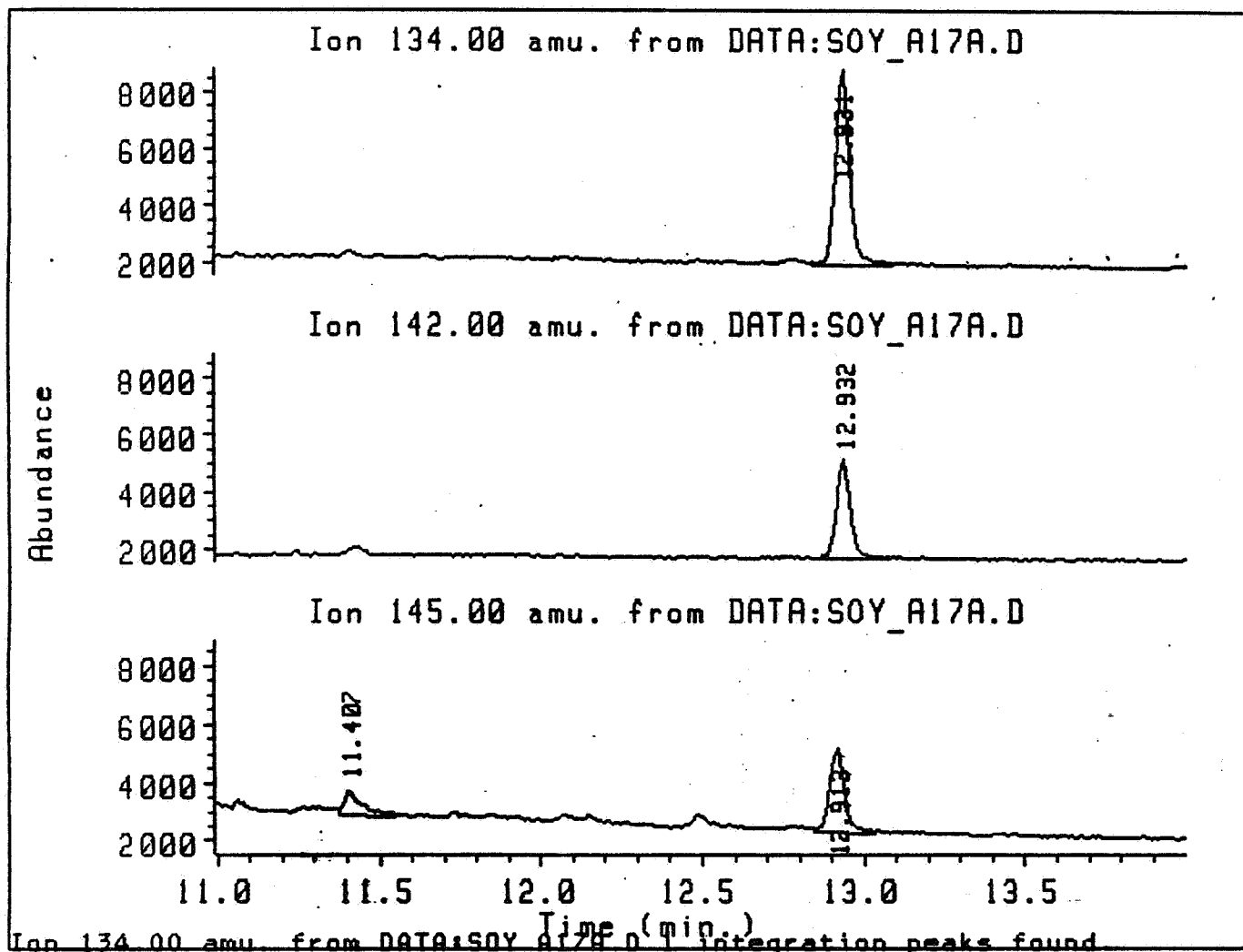
Sample Name: CG-A 2G/1ML 2UL INJ
Misc Info: CORN GRN CONTROL B93-11/12 EM @ 2336
Operator : ESG

Data file: DATA:SOY_A17A.D
File type: GC / MS DATA FILE

Sample Name: CF0.1-C 2G/ML 2 UL INJ
Misc Info: CORN FODDER 0.1PPM B93-11/12 EM 2336
Operator : ESG

Date : 26 Feb 93 1:23 am
Instrument: MS_5988
Inlet : GC

Sequence index : 1
Als bottle num : 17
Replicate num : 1



Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.931	PV	0.046	208063	12.840	13.084

Ion 142.00 amu. from DATA:SOY_A17A.D 1 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.932	VV	0.047	106416	12.866	13.080

Ion 145.00 amu. from DATA:SOY_A17A.D 2 integration peaks found.

Page 1

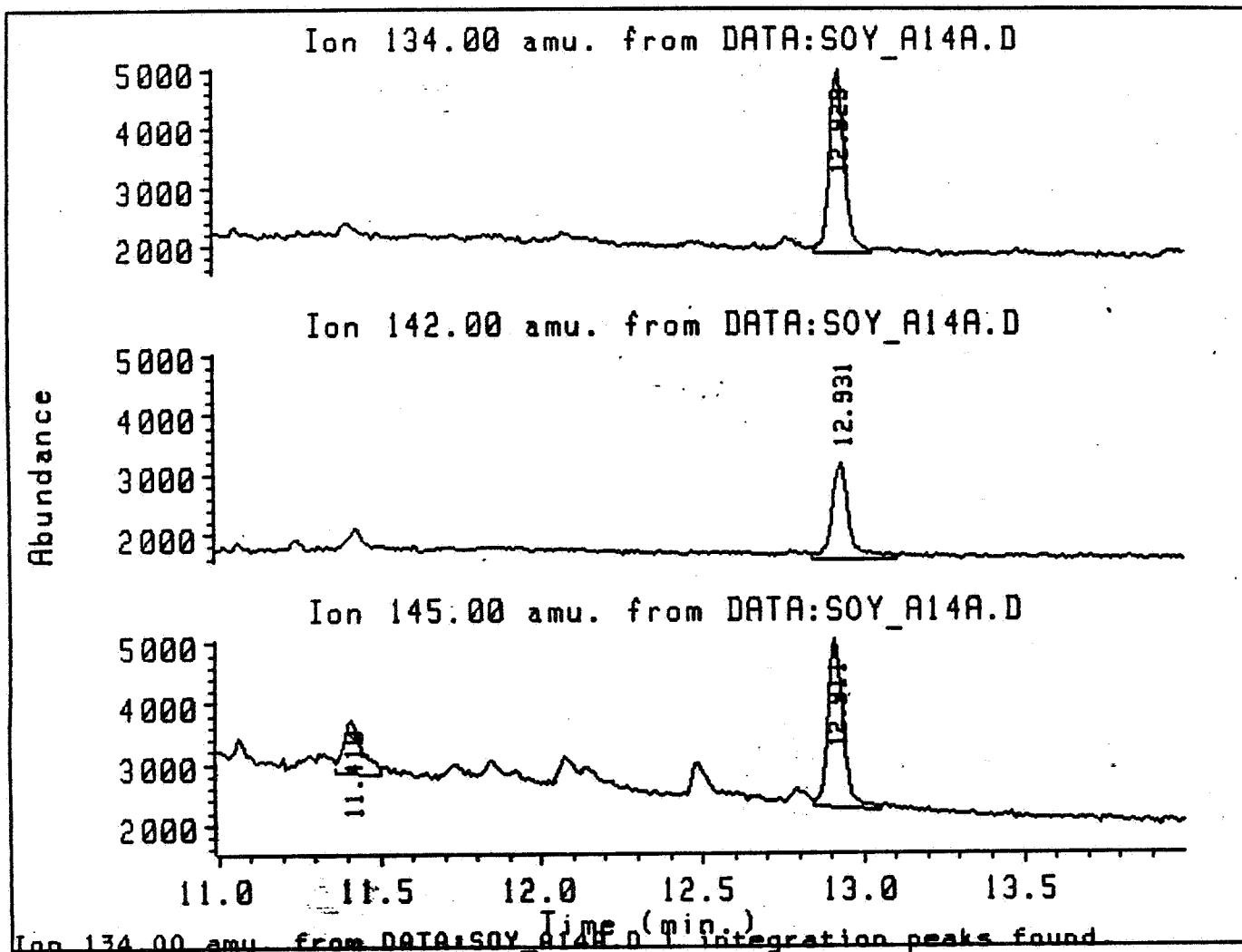
Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	11.407	VV	0.056	31847	11.374	11.543
2	12.913	VV	0.047	87491	12.846	13.030

Data file: DATA:SOY_A14A.D
File type: GC / MS DATA FILE

Sample Name: CF0.05-C 2G/ML 2 UL INJ
Misc Info: CORN FODDER 0.05 PPM B93-11/12 EM 2336
Operator: ESG

Date: 25 Feb 93 11:33 pm
Instrument: MS_5988
Inlet: GC

Sequence index: 1
Als bottle num: 14
Replicate num: 1



Ion 134.00 amu. from DATA:SOY_A14A.D 1 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.929	VV	0.048	96426	12.857	13.032

Ion 142.00 amu. from DATA:SOY_A14A.D 1 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.931	VV	0.054	58055	12.842	13.104

Ion 145.00 amu. from DATA:SOY_A14A.D 2 integration peaks found.

Page 1

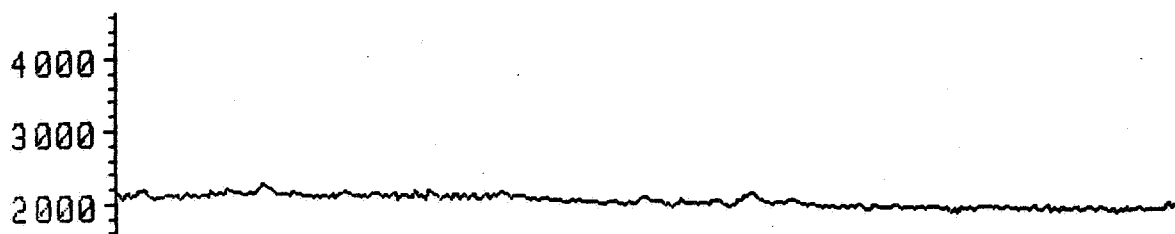
Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	11.410	VV	0.054	34461	11.365	11.505
2	12.911	VV	0.046	85172	12.844	13.049

File type: GC / MS DATA FILE
 Sample Name: CF-D 2G/ML 2UL INJ
 Misc Info: CORN FODDER CONTROL B93-11/12 EM 2336
 Operator: ESG

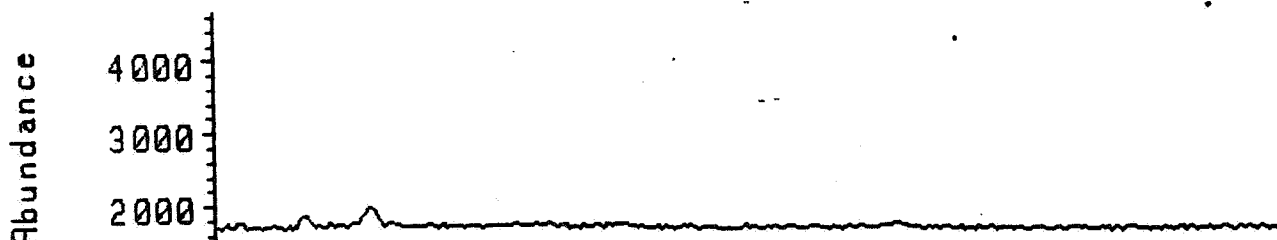
Date: 25 Feb 93 10:38 pm
 Instrument: MS_5988
 Inlet: GC

Sequence index: 1
 Als bottle num: 12
 Replicate num: 2

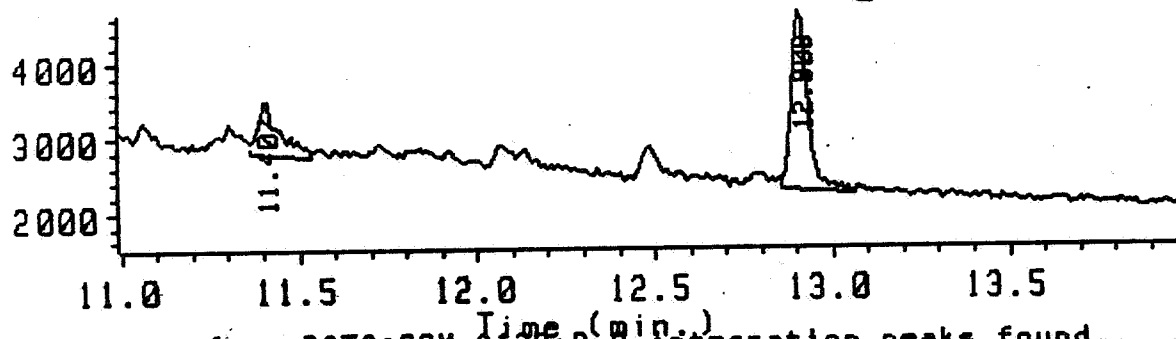
Ion 134.00 amu. from DATA:SOY_A12B.D



Ion 142.00 amu. from DATA:SOY_A12B.D



Ion 145.00 amu. from DATA:SOY_A12B.D



Ion 134.00 amu. from DATA:SOY_A12B.D 0 integration peaks found.

Page 1

Ion 142.00 amu. from DATA:SOY_A12B.D 0 integration peaks found.

Page 1

Ion 145.00 amu. from DATA:SOY_A12B.D 2 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	11.407	UU	0.056	30154	11.365	11.536
2	12.908	UU	0.048	<u>73974</u>	12.857	13.060

Data file: DATA:SOY_A13A.D
 File type: GC / MS DATA FILE

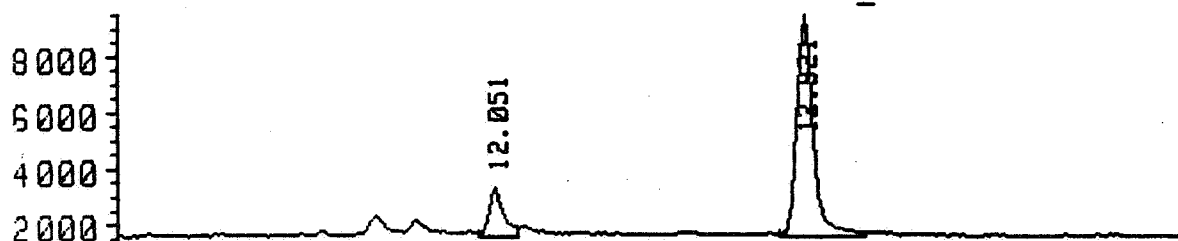
Sample Name: 2UL INJ 0.2018 UG/ML DE498
 Misc Info: B93-11/12: EM @ 2336
 Operator: ESG

Sample Name: S0.1-C 2G/1ML 2UL INJ
 Misc Info: SOYBEAN 0.1PPM B93-11/12; EM @ 2336
 Operator: ESG

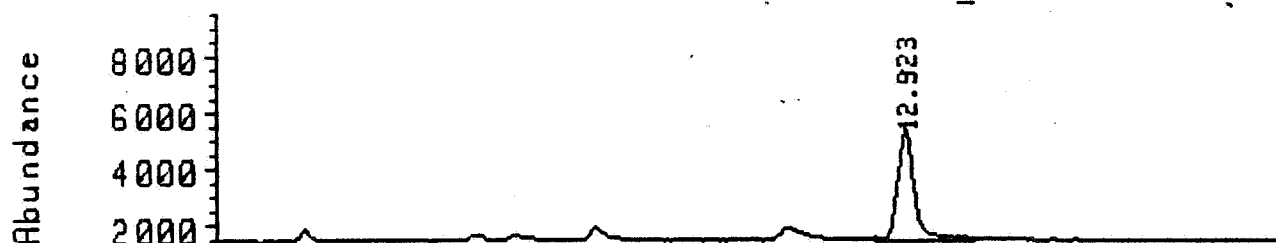
Date: 25 Feb 93 8:12 pm
 Instrument: MS_5988
 Inlet: GC

Sequence index: 1
 Als bottle num: 8
 Replicate num: 2

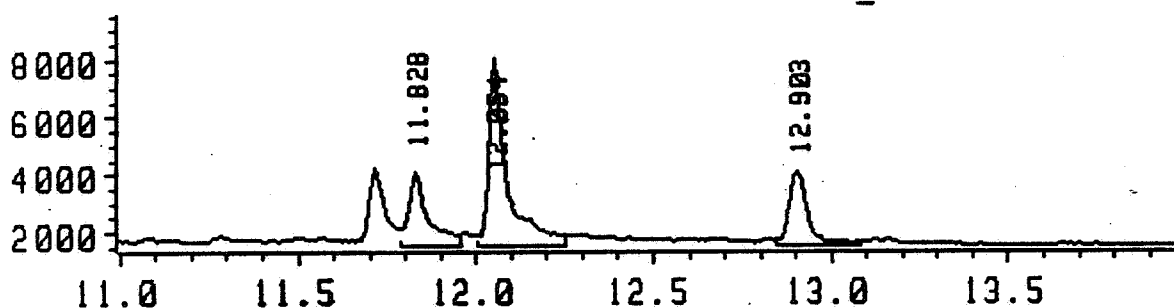
Ion 134.00 amu. from DATA:SOY_A08B.D



Ion 142.00 amu. from DATA:SOY_A08B.D



Ion 145.00 amu. from DATA:SOY_A08B.D



Ion 134.00 amu. from DATA:SOY_A08B.D 2 integration peaks found

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.051	VV	0.042	47520	12.006	12.114
2	12.921	VV	0.047	245374	12.850	13.092

Ion 142.00 amu. from DATA:SOY_A08B.D 1 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.923	VV	0.049	130092	12.839	13.115

Ion 145.00 amu. from DATA:SOY_A08B.D 3 integration peaks found.

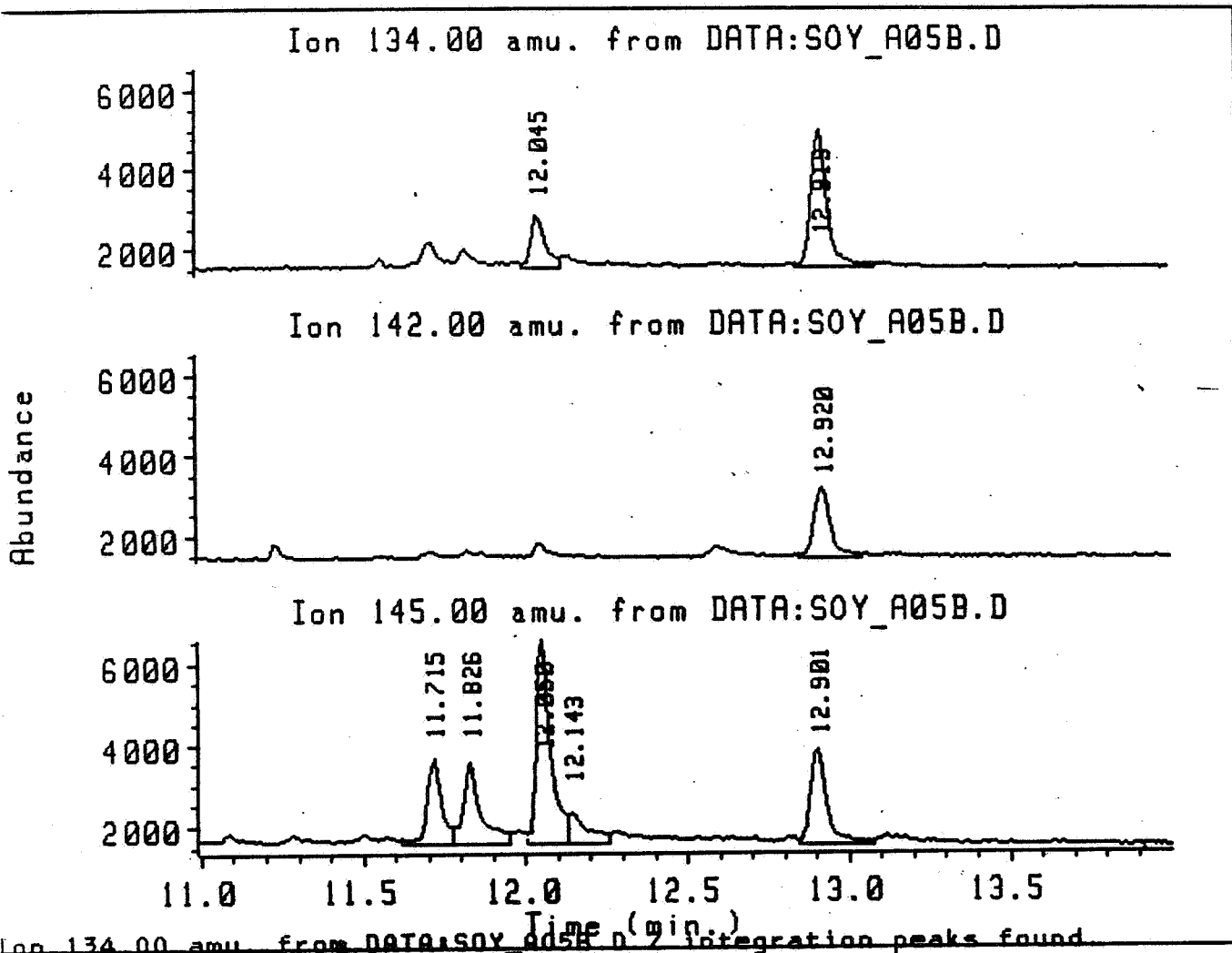
Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	11.828	VV	0.056	101983	11.784	11.958
2	12.054	VV	0.051	232493	12.003	12.255
3	12.903	VV	0.056	94377	12.842	13.080

Sample Name: SO.05-C 2G/1ML 2UL INJ
 Misc Info: SOYBEAN 0.05 PPM B93-11/12: EM @ 2336
 Operator: ESG

Date: 25 Feb 93 6:21 pm
 Instrument: MS_5988
 Inlet: GC

Sequence index: 1
 Als bottle num: 5
 Replicate num: 2



Ion 134.00 amu. from DATA:SOY_A05B.D 2 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.045	VV	0.045	41182	11.999	12.118
2	12.919	VV	0.052	119359	12.845	13.088

Ion 142.00 amu. from DATA:SOY_A05B.D 1 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.920	VV	0.052	60274	12.851	13.042

Ion 145.00 amu. from DATA:SOY_A05B.D 5 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	11.715	VV	0.045	62459	11.616	11.776
2	11.826	VV	0.052	73987	11.776	11.947
3	12.050	VV	0.044	147226	12.004	12.132
4	12.143	VV	0.070	31645	12.132	12.260
5	12.901	VV	0.052	80087	12.843	13.073

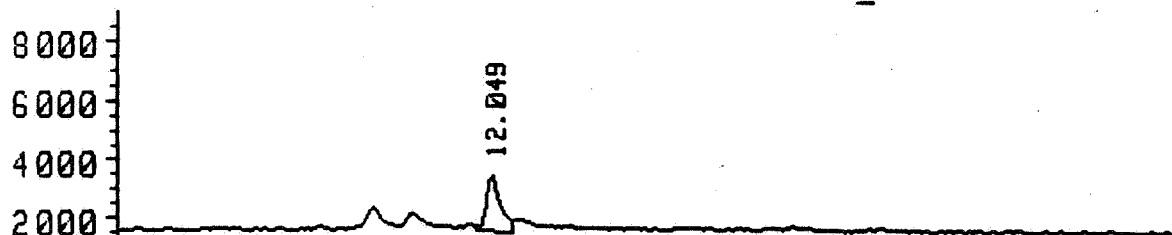
Data File: DATA:SOY_A02A.D
 File type: GC / MS DATA FILE

Sample Name: SC-C 2G/1ML 2UL INJ
 Misc Info: SOYBEEN CONTROL B93-11/12 EM @ 2336
 Operator: ESG

Date: 25 Feb 93 4:12 pm
 Instrument: MS_5988
 Inlet: GC

Sequence index: 1
 Als bottle num: 2
 Replicate num: 1

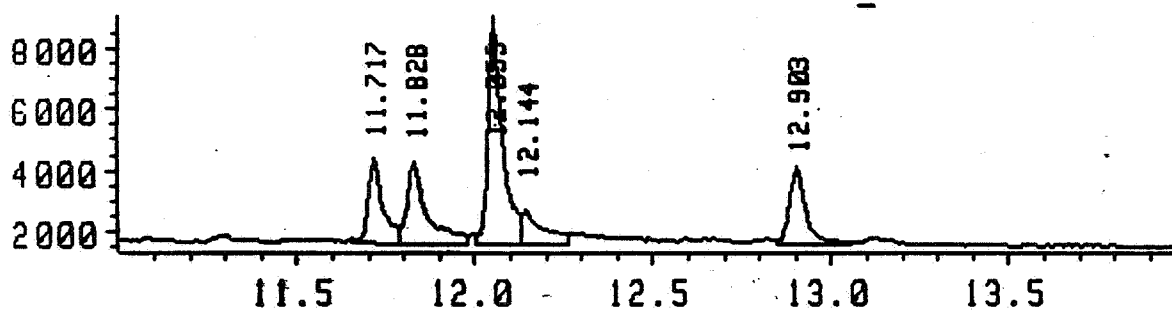
Ion 134.00 amu. from DATA:SOY_A02A.D



Ion 142.00 amu. from DATA:SOY_A02A.D



Ion 145.00 amu. from DATA:SOY_A02A.D



Ion 134.00 amu. from DATA:SOY_A02A.D 1 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	12.049	VV	0.042	53367	12.003	12.106

Ion 142.00 amu. from DATA:SOY_A02A.D 0 integration peaks found.

Page 1

Ion 145.00 amu. from DATA:SOY_A02A.D 5 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	11.717	VV	0.043	81590	11.652	11.790

Ion 145.00 amu. from DATA:SOY_A02A.D 5 integration peaks found.

Page 2

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
2	11.828	VV	0.052	97900	11.790	11.978
3	12.053	VV	0.042	204310	12.005	12.131
4	12.144	VV	0.070	45934	12.131	12.265