

US EPA ARCHIVE DOCUMENT



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

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

JUL 23 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#5G04484 & PP#6F4664. Report on Method Validation of Isoxaflutole in/on Corn Fodder, Forage and Grain.

FROM: Everett S. Greer, Jr., Team Leader
Dallas P. Wright, Jr., Chemist
Analytical Chemistry Section

THRU: Harvey K. Hundley, Head
Analytical Chemistry Section

THRU: Donald A. Marlow, Chief
Analytical Chemistry Branch

To: E. Zager, Acting Chief,
Chemistry Branch I, Tolerance Support
Health Effects Division (7509c)

INTRODUCTION

The Analytical Chemistry Laboratory was requested by the Chemistry Branch I, Tolerance Support to conduct a method trial on the herbicide 4-(methanesulphonyl-4-trifluoromethylbenzoyl)-5-cyclopropyl isoxazole (RPA 201772) and its metabolites RPA 202248 and RPA 203328 on corn fodder, forage and grain. ACL used the method entitled "EXP 30953B/Field Corn/Magnitude of Residue (US93702R): Analytical Method for the Determination of Residues of RPA 201722, RPA 202248, and RPA 203328 in Corn Forage, Silage, Grain, and Fodder" for the following corn commodity/analyte levels:

Table with 3 columns: Commodity, Analyte, Level. Rows include Fodder, Forage, Grain with associated RPA numbers and detection levels in ppm.

METHOD SUMMARY

RPA 201772 and its metabolites are extracted with methanol, and RPA 201772 is hydrolyzed to RPA 202248 with sodium hydroxide. The methanol is evaporated, and the extract is cleaned up by liquid-liquid partitioning, acidified with HCl and extracted with dichloromethane to allow partitioning of RPA 202248 and RPA 203328. Residues of RPA 202248 are hydrolyzed with methanolic sodium hydroxide to RPA 203328. After partitioning with dichloromethane the combined RPA residues are derivatized with diazomethane to the methyl ester (RPA 204497) for quantification by gas chromatography with mass selective detection.

COMMENTS

1. In a conversation between ACL personnel and George Kramer of CBTS, it was decided that it would be unnecessary to validate this common moiety method for the metabolites of RPA 201772 at the 0.2 ppm and 0.4 ppm levels as was originally requested if ACL can demonstrate that the method performs well for the these compounds at the 0.01 ppm level.

2. An ethereal diazomethane solution prepared with an Aldrich kit was used in place of the dichloromethane solution used in the method for the derivatization step. ACL made this substitution because the glassware for preparing the dichloromethane solution was not well described. The ILV report stated that the independent validation laboratory also used an ethereal solution. Sources and catalog numbers should be included in the method for the apparatus needed to prepare this reagent.

3. The standards for this method trial were acquired from Rhône-Poulenc Ag. The EPA repository has the isoxaflutole metabolites, but not the parent compound in its inventory. At this time the availability of the GC standard is not known. ACL has requested this information but has not received a response from RTP.

4. The Limits of detection and quantitation were estimated at 0.3 ppb and 1 ppb respectively.

5. A set of six samples can be extracted, cleaned up and injected into the MSD system in three working days.

6. This method meets the requirements for a tolerance enforcement method as described in the Pesticide Assessments Guidelines, Subdivision O, Section 171-4 (b) provided the comment concerning diazomethane preparation and the comments noted in the method pre-review are addressed.

<u>Commodity</u>	<u>Chemical Added</u>	<u>PPM Added</u>	<u>PPM Found</u>	<u>Percent Recovery</u>	
Corn Fodder	Isoxaflutole	Control	N.D.*	-	
		Control	N.D.	-	
		0.01	0.00944	94.4	
		0.01	0.0110	110	
		0.2	0.141	70.5	
		0.2	0.129	64.5	
		0.4	0.362	90.5	
Corn Forage	Isoxaflutole	Control	N.D.	-	
		Control	N.D.	-	
		0.01	0.00817	81.7	
		0.01	0.00959	95.9	
		0.2	0.195	97.5	
		0.2	0.195	97.5	
		0.4	0.364	91.0	
Grain	Isoxaflutole	Control	N.D.	-	
		Control	N.D.	-	
		0.01	0.00912	91.2	
		0.01	0.0101	101	
		0.2	0.167	83.5	
		0.2	0.164	82.0	
		0.4	0.291	72.8	
		0.4	0.303	75.8	
		RPA 203328	0.00985	0.00833	84.6
			0.00985	0.00931	94.5
RPA 202248	0.0102	0.00731	71.7		
	0.0102	0.00969	95.0		

* N.D. = Less than the estimated LOD of 0.3 ppb

Modifications to method (major or minor):

See Comments section of report.

Special precautions to be taken:

None

Source of analytical standards:

Rhône-Poulenc Ag

If derivatized standard is used, give source:

Rhône-Poulenc Ag

Instrumentation for quantitation:

GC/MSD

Instrumentation for confirmation:

N/A

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

All parameters as per method except initial inlet pressure was reduced from 25 psi to 20 psi to prevent auto shutdown.

Commercial sources for any special chemicals or apparatus:

N/A

Additional comments:

See report.

Chromatograms

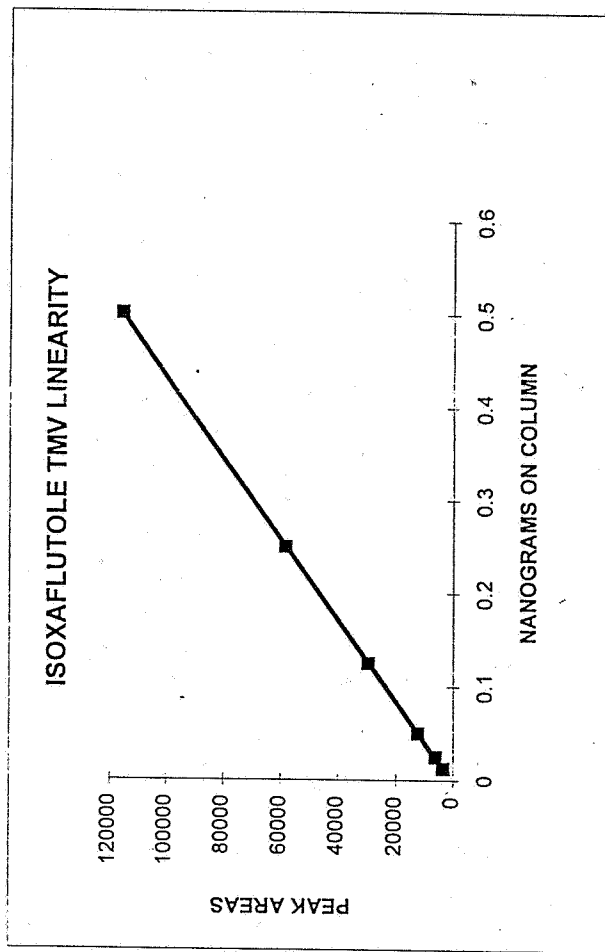
Copies attached

ISOXAFLOTOLE TMV LINEARITY

ISOXAFLOTOLE TMV B96-20,21,22,23
LINEARITY FOR RPA204497 5/3/96

NANOGRAMS

N COLUMN	AREA 1	AREA2	AVERAGE
0.0125	3505	3176	3340.5
0.025	5779	6132	5955.5
0.0501	12149	12095	12122
0.125	29858	29323	29590.5
0.25	59044	57608	58326
0.501	118331	113075	115703



ISOXAFLUOLE TMV REGRESSION

ISOXAFLUOLE TMV B96-20,21,22,23

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.999985
R Square	0.99997
Adjusted R Square	0.999962
Standard Error	265.904
Observations	6

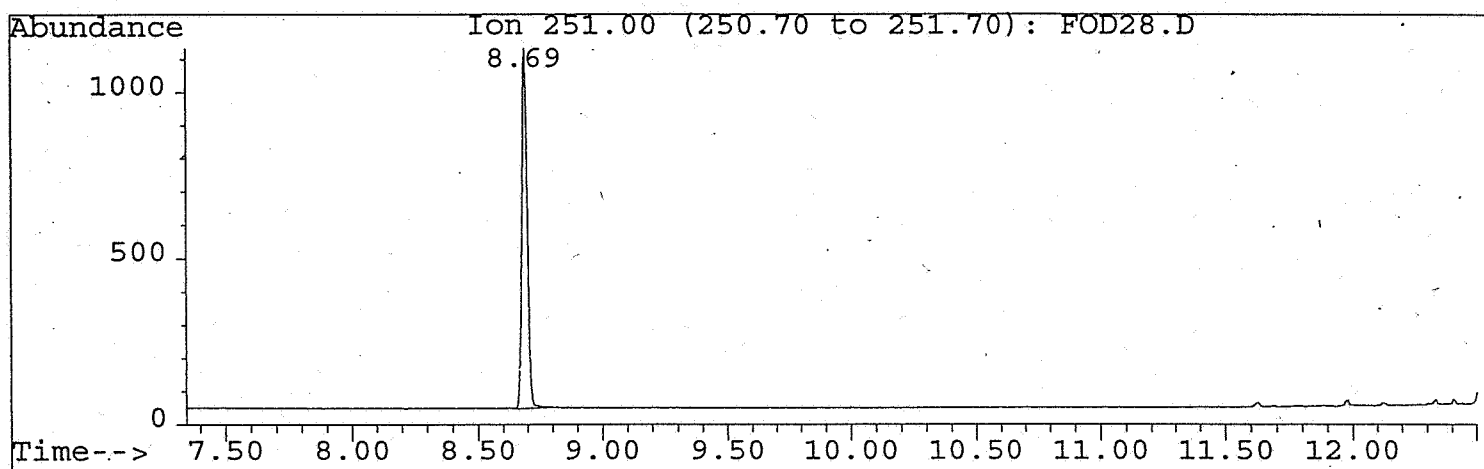
ANOVA					
	df	SS	MS	F	Significance F
Regression	1	9.42E+09	9.42E+09	133196.9	3.38E-10
Residual	4	282819.7	70704.93		
Total	5	9.42E+09			

	Coefficient	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0000%	Upper 95.0000%
Intercept	530.3417	148.4884	3.571604	0.023342	118.071	942.6123	118.071	942.6123
X Variable 1	230236	630.8502	364.9615	3.38E-10	228484.5	231987.6	228484.5	231987.6

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\FOD28.D
Operator :
Acquired : 10 Jun 96 3:56 pm using AcqMethod ISOX
Sample Name: FOD0.4-B 0.4 PPM SPIKE-201772
Misc Info : FODDER 12.5G/10ML 1UL INJ.
Vial Number: 7
CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M



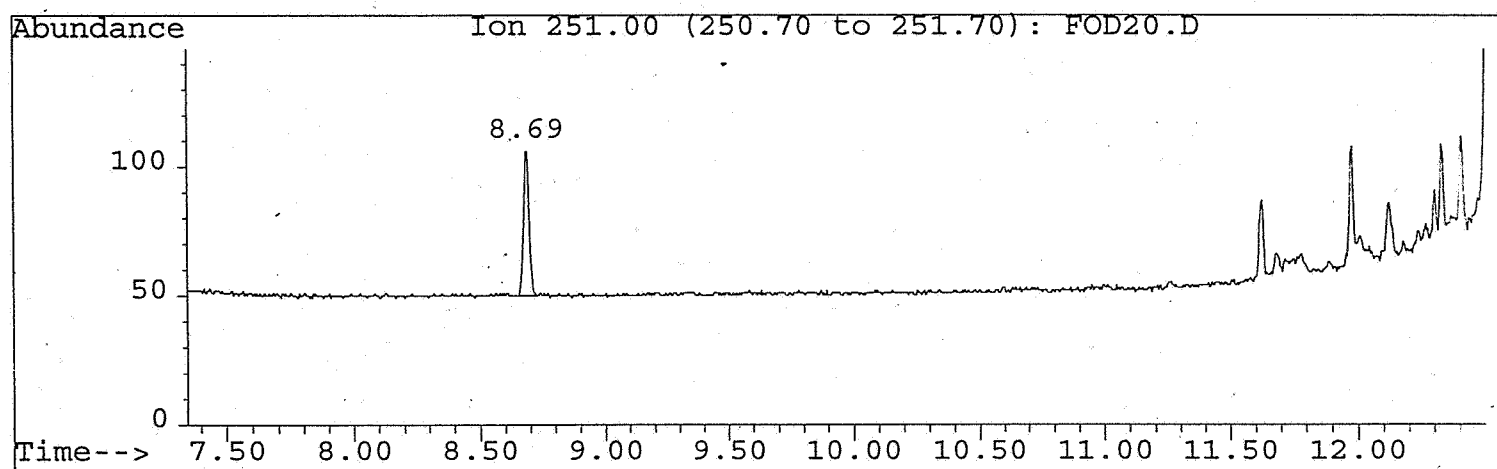
Ret Time	Signal Descr	Area	% Pk	%LPk
8.693	251.00 amu	16747	100.000	100.000

7

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\FOD20.D
Operator :
Acquired : 10 Jun 96 1:32 pm using AcqMethod ISOX
Sample Name: FOD0.01-B 0.01PPM SPIKE 201772
Misc Info : 25G/10ML 1UL INJ.
Vial Number: 3
CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M



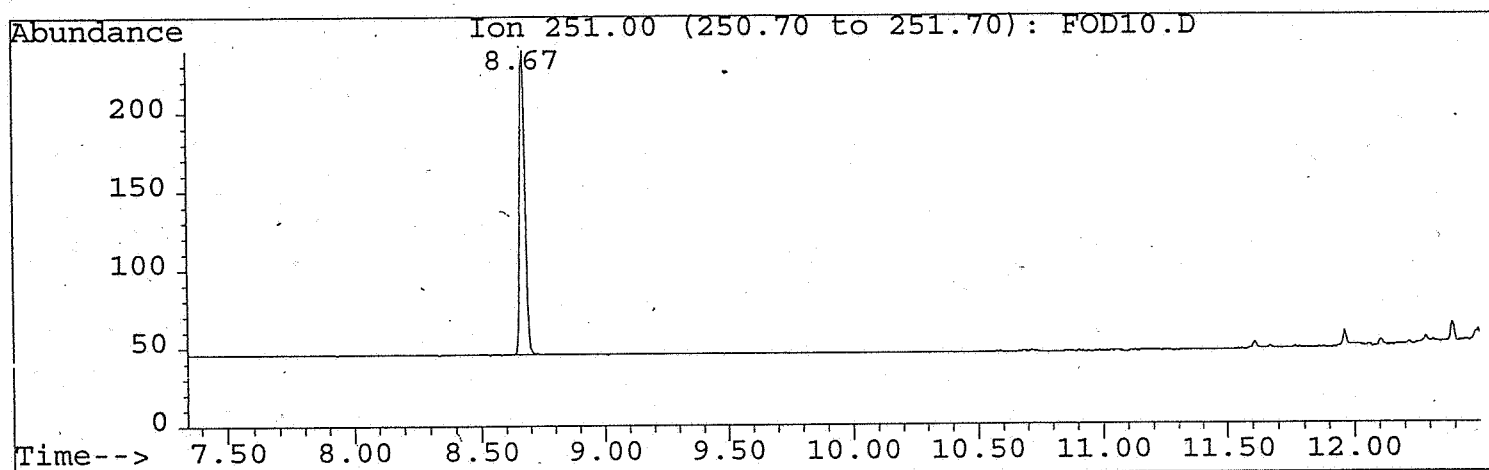
Ret Time	Signal Descr	Area	% Pk	%LPk
8.689	251.00 amu	873	100.000	100.000

8

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\FOD10.D
Operator :
Acquired : 30 May 96 1:18 pm using AcqMethod ISOX
Sample Name: FOD0.2-A 0.2 PPM SPIKE-201772
Misc Info : FODDER 25G/10ML 1UL INJ.
Vial Number: 5
CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M



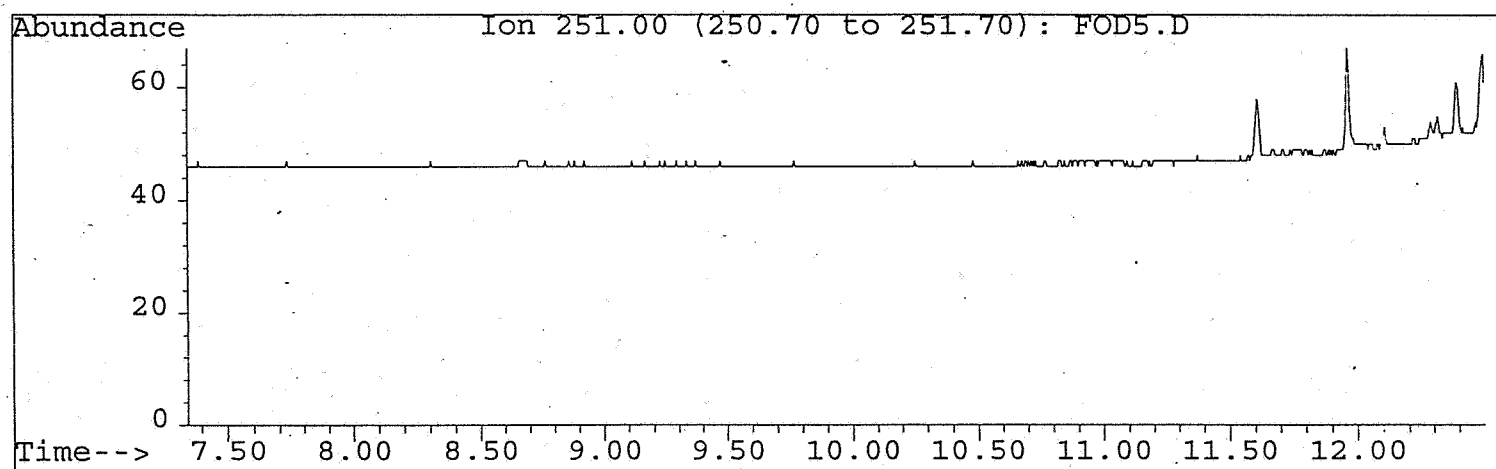
Ret Time	Signal Descr	Area	% Pk	%LPk
8.673	251.00 amu	2931	100.000	100.000

9

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\FOD5.D
Operator :
Acquired : 30 May 96 11:49 am using AcqMethod ISOX
Sample Name: FODC-2 FODDER CONTROL
Misc Info : 25G/10ML 1UL INJ.
Vial Number: 3
CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M



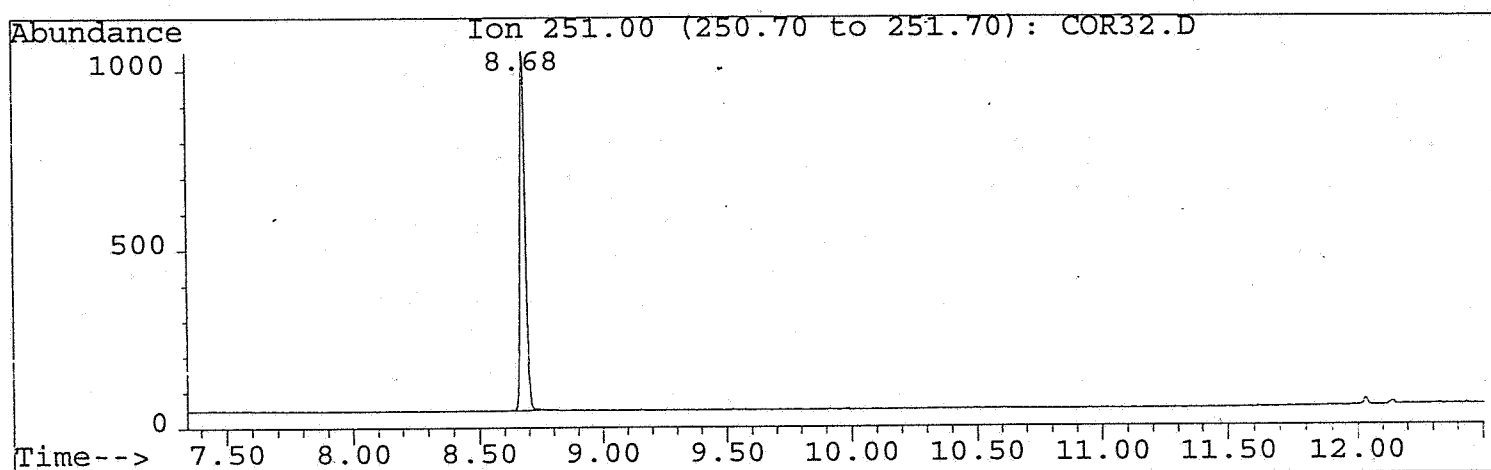
Ret Time	Signal Descr	Area	% Pk	%LPk
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6

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\COR32.D
Operator :
Acquired : 28 Jun 96 3:48 pm using AcqMethod ISOX
Sample Name: COR0.4-A 0.4 PPM SPIKE 201772
Misc Info : ~~FORAGE~~ 12.5G/10ML 1 UL INJ.
Vial Number: 5 *Gram 8.1*
CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M

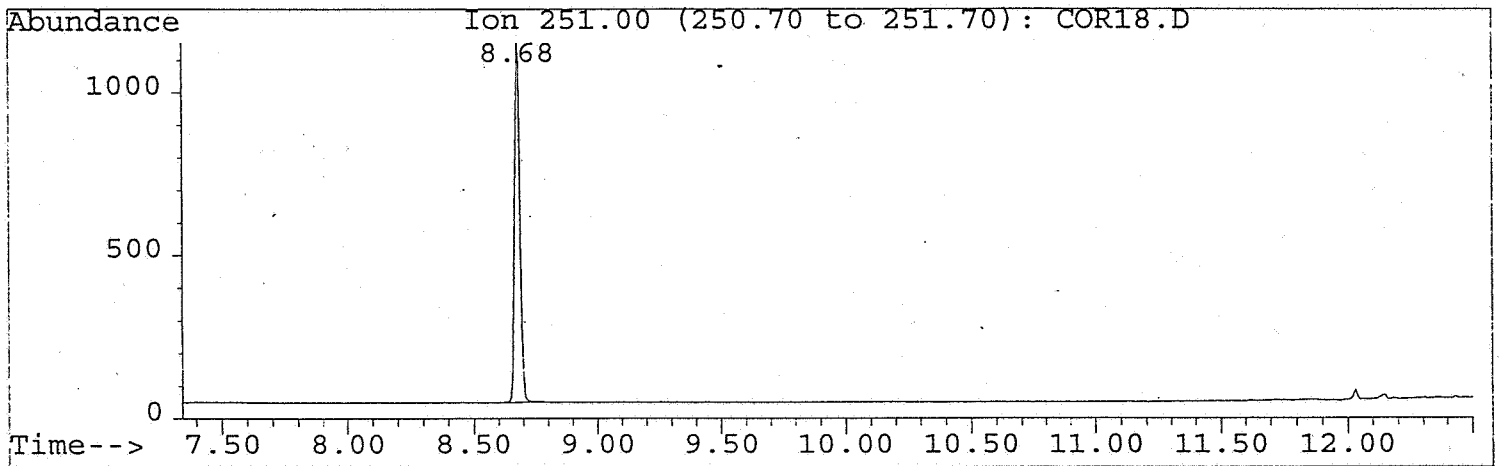


Ret Time	Signal Descr	Area	% Pk	%LPk
8.682	251.00 amu	14901	100.000	100.000

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\COR18.D
Operator :
Acquired : 27 Jun 96 3:07 pm using AcqMethod ISOX
Sample Name: COR0.2-A 0.2 PPM SPIKE 201772
Misc Info : ~~FORAGE~~ 25G/10ML 1 UL INJ.
Vial Number: 2 *Gram 25*
CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M



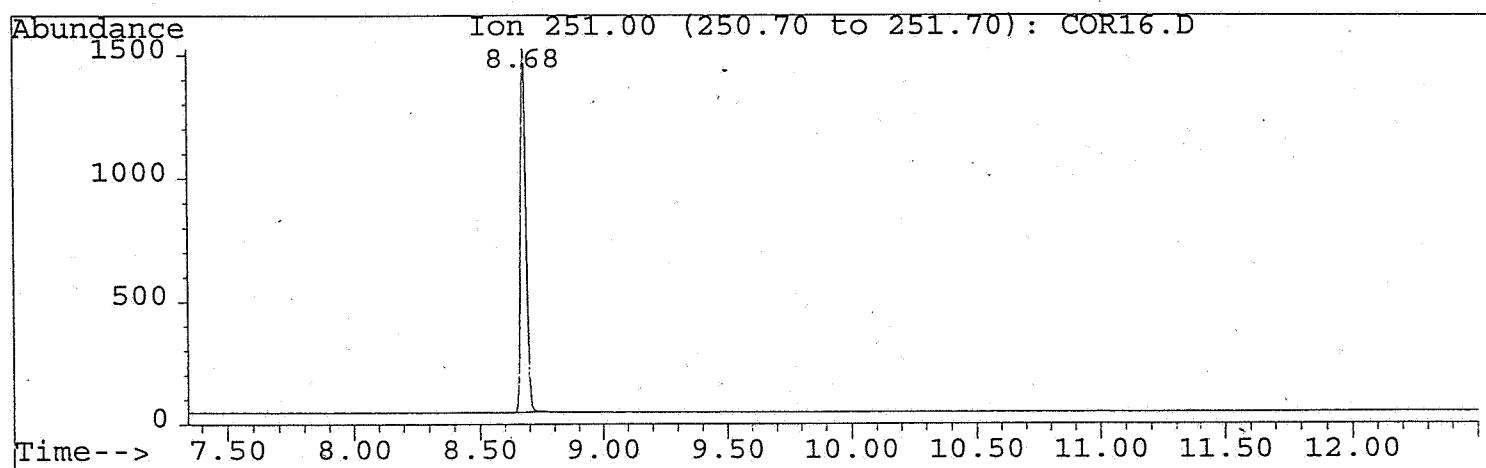
Ret Time	Signal Descr	Area	% Pk	%LPk
8.681	251.00 amu	16281	100.000	100.000

12

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\COR16.D
Operator :
Acquired : 27 Jun 96 2:31 pm using AcqMethod ISOX
Sample Name: STD RPA204497
Misc Info : 0.0.501 NG/UL 1UL INJ .
Vial Number: 1
CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M



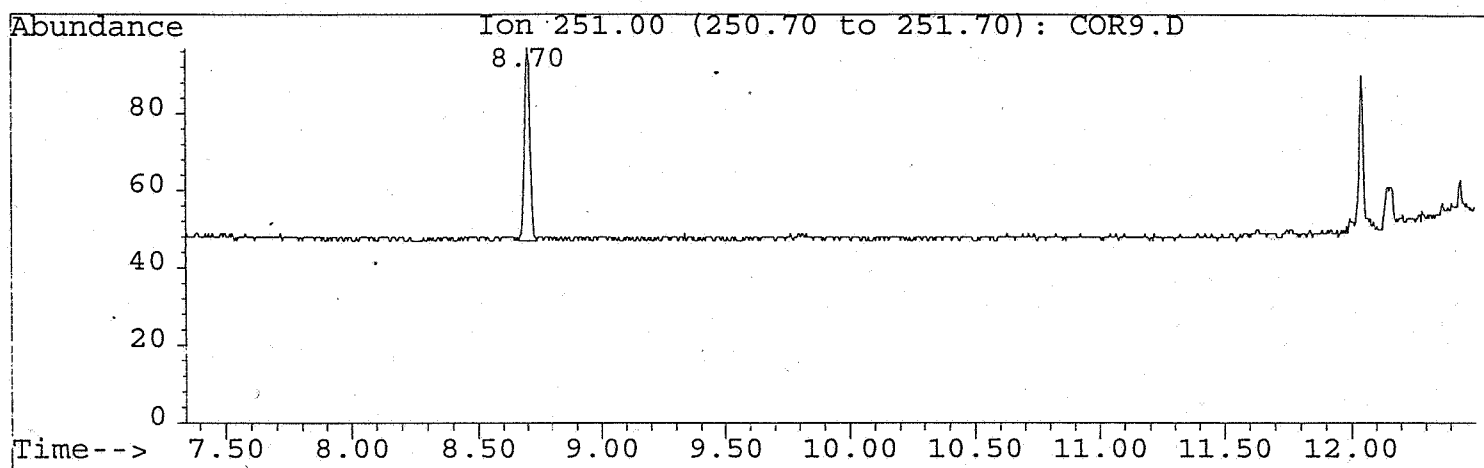
Ret Time	Signal Descr	Area	% Pk	%LPk
8.682	251.00 amu	22631	100.000	100.000

13

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\COR9.D
 Operator :
 Acquired : 20 Jun 96 5:43 pm using AcqMethod ISOX
 Sample Name: COR0.01-A 0.01 PPM SPIKE 201772
 Misc Info : ~~FORAGE~~ 25G/10ML 1 UL INJ.
 Vial Number: 5 *Grani*
 CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M



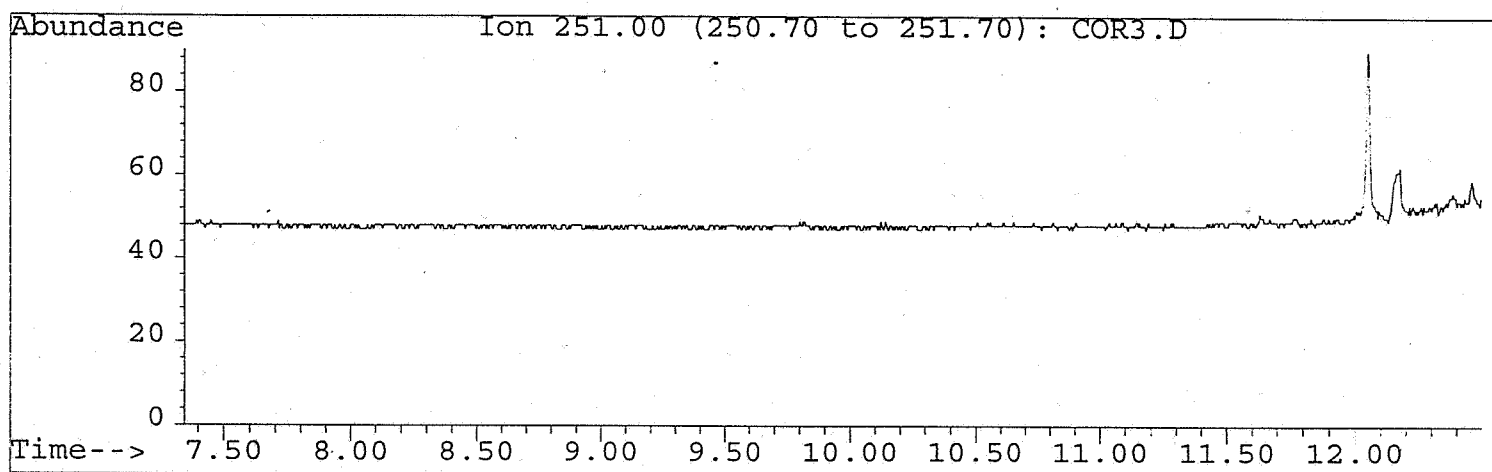
Ret Time	Signal Descr	Area	% Pk	%LPk
8.705	251.00 amu	750	100.000	100.000

14

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\COR3.D
Operator :
Acquired : 20 Jun 96 3:55 pm using AcqMethod ISOX
Sample Name: CORC-1 CORN CONTROL
Misc Info : 25G/10ML 1UL INJ. Gram ~~8A~~
Vial Number: 2
CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M



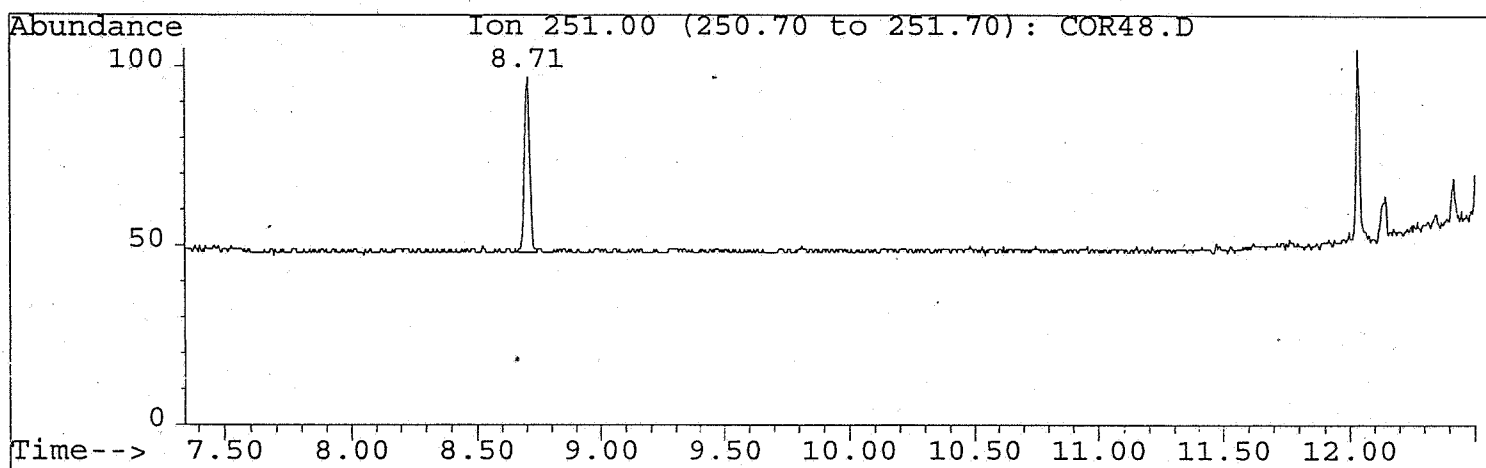
Ret Time	Signal Descr	Area	% Pk	%LPk
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15

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\COR48.D
Operator :
Acquired : 8 Jul 96 11:23 am using AcqMethod ISOX
Sample Name: COR40.01-A 0.01 PPM SPIKE 202248
Misc Info : GRAIN 25G/10ML 1 UL INJ.
Vial Number: 5
CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M



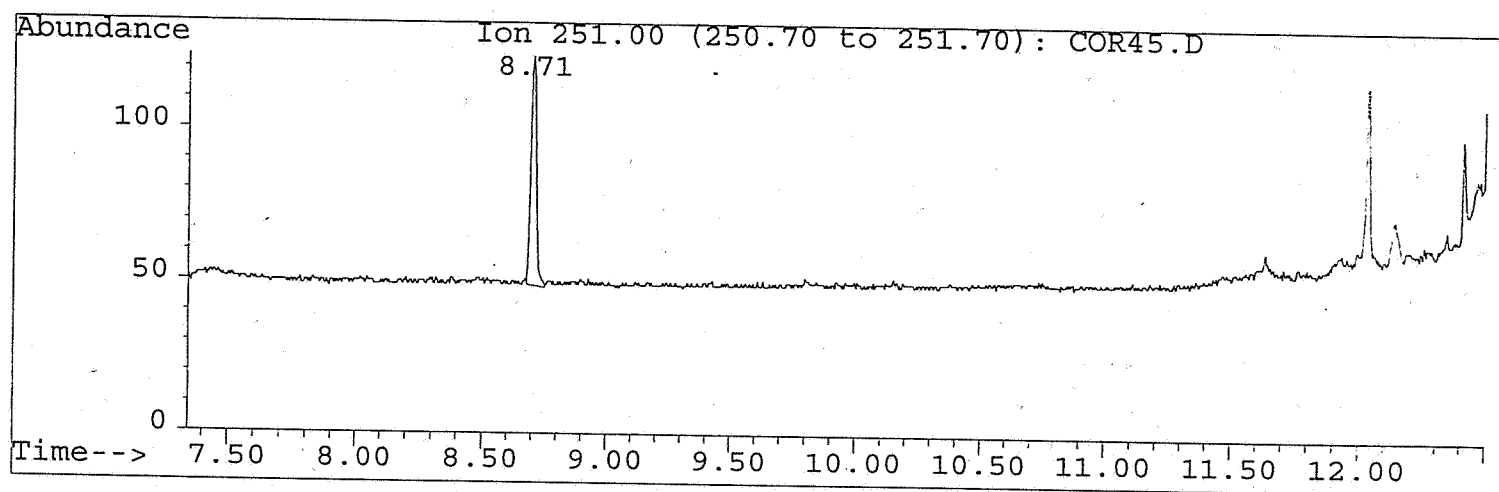
Ret Time	Signal Descr	Area	% Pk	%LPk
8.707	251.00 amu	698	100.000	100.000

16

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\COR45.D
Operator :
Acquired : 8 Jul 96 10:30 am using AcqMethod ISOX
Sample Name: COR20.01-B 0.01 PPM SPIKE 203328
Misc Info : GRAIN 25G/10ML 1UL INJ.
Vial Number: 3
CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M



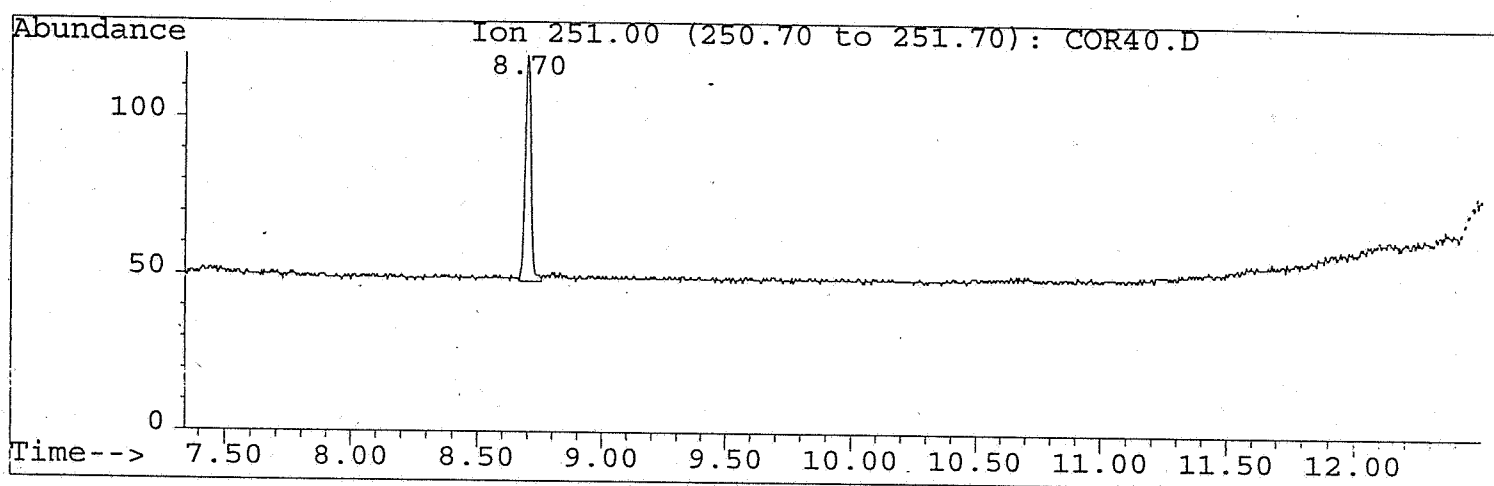
Ret Time	Signal Descr	Area	% Pk	%LPk
8.708	251.00 amu	1117	100.000	100.000

17

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\COR40.D
Operator :
Acquired : 8 Jul 96 9:00 am using AcqMethod ISOX
Sample Name: STD RPA204497
Misc Info : 0.0.025 NG/UL 1UL INJ .
Vial Number: 1
CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M



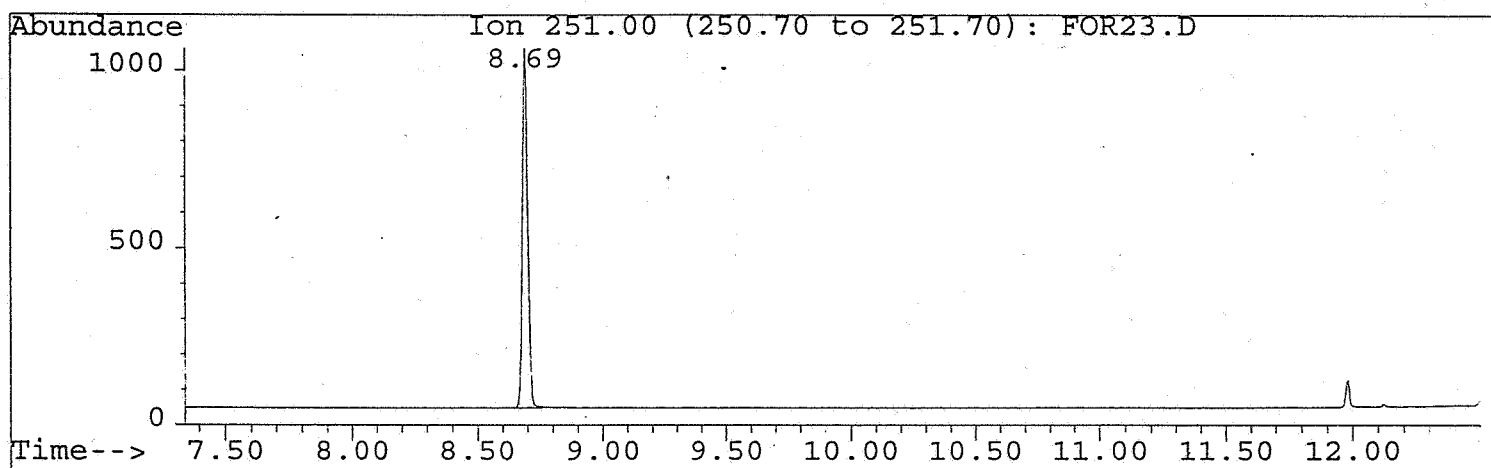
Ret Time	Signal Descr	Area	% Pk	%LPk
8.702	251.00 amu	1116	100.000	100.000

18

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\FOR23.D
Operator :
Acquired : 17 Jun 96 4:04 pm using AcqMethod ISOX
Sample Name: FOR0.4-A 0.4 PPM SPIKE-201772
Misc Info : FORAGE 12.5G/10ML 1 UL INJ.
Vial Number: 12
CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M



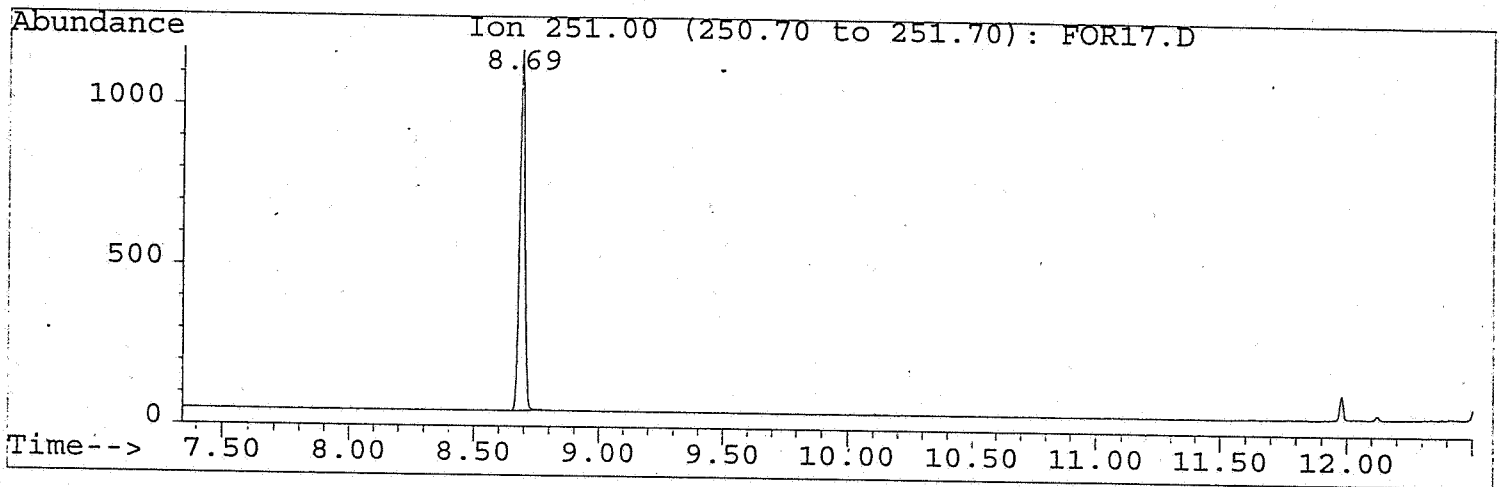
Ret Time	Signal Descr	Area	% Pk	%LPk
8.693	251.00 amu	15043	100.000	100.000

19

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\FOR17.D
Operator :
Acquired : 17 Jun 96 2:16 pm using AcqMethod ISOX
Sample Name: FOR0.2-A 0.2 PPM SPIKE-201772
Misc Info : FORAGE 25G/10ML 1 UL INJ.
Vial Number: 9
CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M



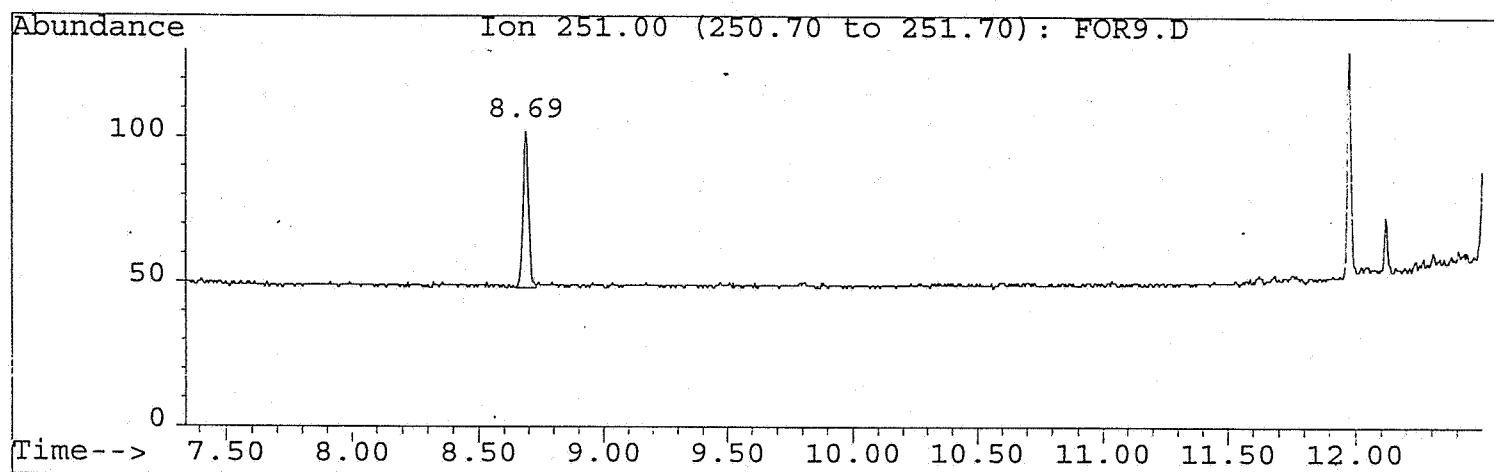
Ret Time	Signal Descr	Area	% Pk	%LPk
8.693	251.00 amu	16812	100.000	100.000

20

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\FOR9.D
Operator :
Acquired : 17 Jun 96 11:52 am using AcqMethod ISOX
Sample Name: FOR0.01-A 0.01 PPM SPIKE-201772
Misc Info : FORAGE 25G/10ML 1 UL INJ.
Vial Number: 5
CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M



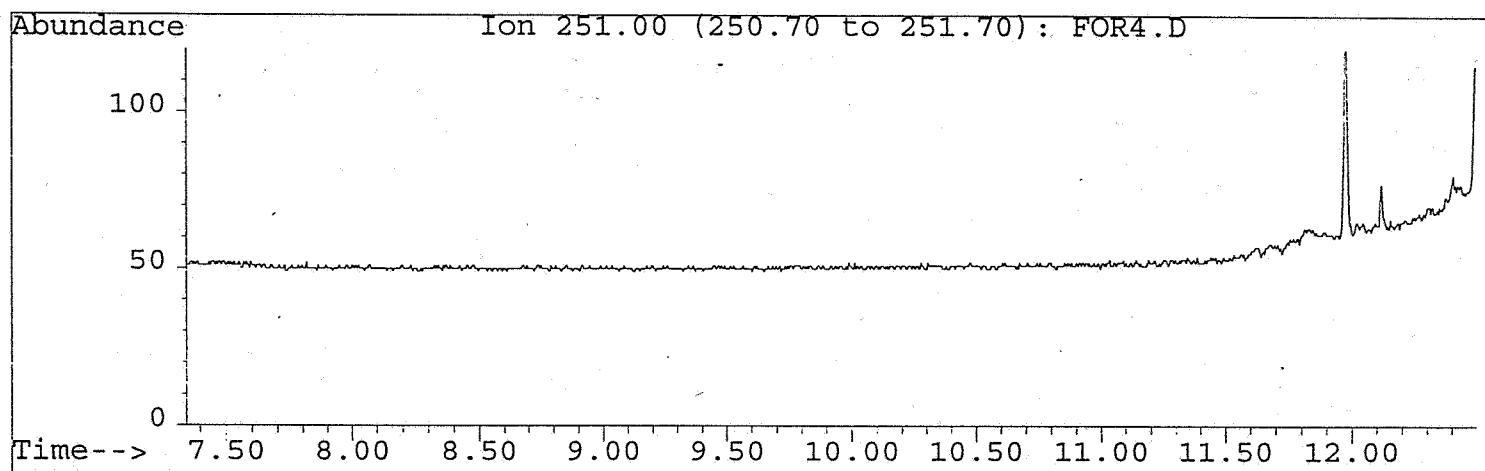
Ret Time	Signal Descr	Area	% Pk	%LPk
8.691	251.00 amu	805	100.000	100.000

21

Area Percent Report -- Sorted by Retention Time

Information from Data File:

File : C:\HPCHEM\1\DATA\DPWESG\FOR4.D
Operator :
Acquired : 17 Jun 96 10:22 am using AcqMethod ISOX
Sample Name: FORC-1 FORAGE CONTROL
Misc Info : 25G/10ML 1UL INJ.
Vial Number: 2
CurrentMeth: C:\HPCHEM\1\METHODS\ISOX.M



Ret Time	Signal Descr	Area	% Pk	%LPk
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22

TMV Pre-review of Isoxaflutole
in Corn

Reviewed by: Everett S. Greer, Jr. *EG*

Date: 3-11-96

Laboratory assignment number: B96-20,21,22,23

Analytes: Isoxaflutole, RPA 202248 and RPA 203328

Commodities: Corn grain, fodder and forage

Petitioner: Rhône-Poulenc

Independent validation laboratory: ABC Laboratories, Inc., Pan-Ag
Division

Method: Analytical Method for the Determination of Residues of
RPA 201772, RPA 202248, and RPA 203328 in Corn Grain and
Fodder

4.2 Preparation of reagents

4.2.4 Preparation of diazomethane

The procedure for stirring the mixture in tube (B) and the assembly of the acetic acid trap in Figure 3 should be described.

5. Gas chromatographic conditions

This section lists only one qualifying ion to be monitored by the MSD. A tolerance enforcement method should include at least two confirmatory ions.

Additional reviewer's comments

1. This method uses diazomethane for derivatizing the hydrolyzed analytes. Due to safety concerns it is the agency's policy that analytical methods submitted in support of registration must use an alternate derivatizing reagent unless the registrant can show through submitted data that a feasible alternative is not available. There is no discussion of the registrant's attempt at trying alternative derivatizing reagents in any of the documents sent to ACL from CBTS.

2. The registrant has provided mean recovery data with the method for corn grain, forage and fodder fortified with the three requested analytes at the 0.01 ppm and 0.05 ppm levels. No individual recovery data are included. Representative chromatograms are included for the three analytes at the lowest level requested by CBTS.

3. Satisfactory recovery data were reported in the independent laboratory report for corn grain fortified at the 0.05 ppm, 0.1 ppm and 0.5 ppm levels. Representative chromatograms were also included in the report.

4. The ILV found that GC/MS linearity and reproducibility were affected when silanized glass wool was not used in the GC injection liner.

ANALYTICAL CHEMISTRY BRANCH
SCREEN FOR RESIDUE METHODS FOR TMV

1. LABORATORY ASSIGNMENT NUMBER: B 96-20, 21, 22, 23
2. PP#: 5604484
CF04664
3. TECHNICAL REVIEWER: Everett S. Greer, Jr.
4. DATE: 6-11-96
5. ANALYTES/LEVEL: Isoxaflutole, RPA 202248, RPA 203328 / 0.01, 0.10, 0.20, 0.40 ppm
6. COMMODITIES: Corn grain, Fodder, forage
7. METHOD: RPA 201772, RPA 202248, and RPA 203328 in Corn Grain and Fodder
EXP 30953B / Analytical Method for the Determination of Residues of

The Analytical Chemistry Section has been asked to screen the residue chemistry methods submitted by the registrant in order to determine if they contain the essential requirements identified in the Residue Chemistry Guidelines. Full scientific review and laboratory evaluation of those methods will take place after the initial screen. The following items need to be resolved before the analytical method can be evaluated.

	<u>YES</u>	<u>NO</u>
1. Does the method use exotic equipment and/or supplies that are not commercially available in the U.S.?	_____	_____ ✓
2. Does the method require any new equipment before the laboratory work begins?	_____	_____ ✓
3. Are chromatograms included?	_____ ✓	_____
a. Is (are) peak(s) of interest sufficiently resolved from other peaks?	_____ ✓	_____
b. Has registrant included chromatograms of analyses at or below tolerance on all crop types for which tolerance is requested by HED?	_____ ✓	_____
c. Do the control samples have reasonably low levels of the analyte in relation to the proposed tolerance?	_____ ✓	_____
d. Is the method sufficiently sensitive and specific to measure and identify the residues at levels specified by HED in the TMV request?	_____ ✓	_____

YES NO

- 4. Has recovery data been provided to ACL for the residues that are specified in the TMV request? See narrative review
- 5. Are recovery values between 70% and 120% at all levels and for all commodity types? ✓
- 6. Are all procedures clearly written with no ambiguities so that the method can be run without communication with the registrant? ✓
- 7. Does the method require correction for a sample of the untreated commodities or a blank? ✓
- 8. Does the method require the use of an internal or procedural standard to compensate for lost analyte during analysis? ✓
- 9. Are 2nd laboratory validation data provided with the method? ✓
- 10. Are there any deficiencies other than those covered above that would prevent ACS from conducting a method trial? ✓
- 11. Is this method suitable for validation testing? ✓

Any deficiencies/problems noted for any above items should be addressed in the full scientific review of this method to be attached as an addendum.

Robert S. Sheehy
Signature

3-11-96
Date

The following is to be completed by the analyst performing the TMV.

- 12. Can a set of 6 samples be run within 24 hours? ✓
- 13. a. Are standards available at RTP repository? See report
- b. Are derivatized analytical reference standards available? ✓