AgrEvo

Analytical Method

Department: Residue Chemistry

Date: July 21, 1997

RAM Number: BF/10/97

Title: An Analytical Method for the Determination of Residues of Buprofezin at Estimated Tolerance Levels in Almonds, Cottonseed, Citrus (Lemons), and Grapes by Gas Chromatography Using Nitrogen Phosphorus Detection

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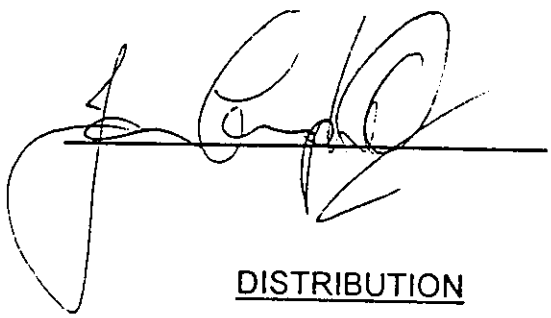
Signed: 

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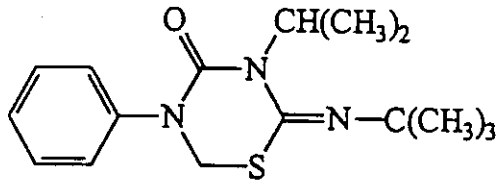
1. SCOPE

This method is suitable for the determination of the total extractable residues of buprofezin in Almonds, Cottonseed, Citrus (Lemons), and Grapes at estimated tolerance levels. The structure for buprofezin is shown below. The limit of quantitation for this procedure has been set at 0.05 ppm.



Common Name:	Buprofezin (BF1)
C.A. Name:	2-[(1,1-dimethylethyl)imino]tetrahydro-3-(1-methylethyl)-5-phenyl-4H-1,3,5-thiadiazin-4-one
IUPAC Name:	2-tert-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one
CAS Reg. No.:	69327-76-0

2. PRINCIPLE

Extractable residues of buprofezin are removed from the crop matrix by blending with acetone. After rotary evaporation to the aqueous phase, the extracts are transferred to separatory funnels with 1M hydrochloric acid. The acidic aqueous extracts are then cleaned up by partitioning with hexane. The hexane is back partitioned with additional 1M hydrochloric acid.

After the hexane partition is discarded, the combined acidic aqueous phase containing buprofezin is partitioned with dichloromethane. The dichloromethane extract is dried through a sodium sulfate pad, rotary evaporated to dryness, and redissolved in organic solvent to await cleanup by Florisil column chromatography. Following clean-up, buprofezin is quantified by gas chromatography using nitrogen phosphorous detection.

3. APPARATUS

Use as a guide; equivalent apparatus may be substituted.

- Autosampler vials, crimp top, Hewlett Packard
- Blender, Sorvall Omni-Mixer, Model 17105, Omni International
- Blender Blade Assembly, Omni International
- Blending jars (pint-size Mason jar)

- Boiling flasks (50-,125-, 250-, and 1000-mL)
- Büchi Rotovapor RE-124
- Büchi Waterbath B-481
- Büchner funnels (9 cm) and filter adapters (24/40)
- Chromatographic Columns with reservoir (12 mm x 240 mm, 250 mL reservoir), Krackler Scientific (Cat. No. 17810-11300)
- Class A volumetric pipettes (0.5-, 1.0-, 2.0-, 3.0-, 4.0-, 5.0-,6.0- and 10.0-mL)
- Fused silica megabore column, DB-1, 15 m x 0.53 mm i.d., 1.5-µm film thickness, J & W Scientific
- Glass fiber filter paper, Whatman 934-AH
- Glass powder funnels, long-stem, 75 mm
- Glass stoppers, 24/40, Pyrex
- Graduated cylinders, 50-mL, 100-mL, and 250-mL TD
- Hewlett-Packard 5890A gas chromatograph with capillary split/splitless inlet and nitrogen phosphorus detector equipped with a Model 7673A autosampler
- Micropipettes, 250-µL
- Separatory funnels, 250-mL, with stoppers
- Transfer Pasteur pipettes, flint glass, 5.75" and 9"
- Volumetric flasks (5-, 10-, 50-, and 100-mL)

4. REAGENTS

All solvents should be pesticide grade or better. Equivalents may be substituted.

- Acetone, pesticide grade
- Analytical standard of buprofezin (BF 01)
- Deionized water
- Dichloromethane, pesticide grade
- Ethyl acetate, pesticide grade
- Hexane, pesticide grade
- Florisil PR (60-100 mesh), Cat No.: 46382, Fluka Chemical
- Hydrochloric Acid, concentrated ACS reagent
- Sodium Sulfate, granular anhydrous, ACS reagent
- Toluene, pesticide grade

5. PROCEDURE

5.1 Extraction

1. Weigh a minimum of 20 grams of a finely ground representative crop sample into a blending jar. Fortify the recovery samples at the desired fortification level with buprofezin prepared in acetone (see Step 1 of Section 6.1).

   Note: Fortification experiments (see Section 6.3) are not required when analysis of samples is performed for tolerance enforcement. Fortification experiments are intended to be used for data collection methods or establishing & validating method efficiency when required.

2. Add 200 mL of acetone and blend for approximately 5 minutes. Ultrasonicate the sample for approximately 2 minutes.

3. Decant the acetone (leave the crop sample in the blending jar) and filter the extracts by suction through a Buchner funnel containing Whatman 934-AH filter paper and collect the extracts in a 1000 mL boiling flask.

4. Add an additional 100 mL of acetone to the crop sample contained in the blending jar and blend once again for approximately 5 minutes. Decant the acetone and the crop sample into the Buchner funnel and combine the extracts in the same 1000 mL boiling flask. Rinse the filter cake with acetone and collect the rinsings.

5. Rotary evaporate the extracts to the aqueous phase at 40 °C (± 5 °C) under reduced pressure. Transfer the extracts to a 250 mL separatory funnel. Rinse the boiling flask with a 1 x 50 mL portion of 1M hydrochloric acid and transfer the rinsings to the separatory funnel to await partitioning (Section 5.2).

5.2 Partitioning

Note: The hexane or dichloromethane partitioning volumes should be used to rinse the boiling flask which contained the acidic aqueous phase prior to addition to the separatory funnel.

1. Rinse the boiling flasks from Step 5 of Section 5.1 with a minimum of 120 mL of hexane and transfer to the separatory funnel. Shake for approximately one minute and allow the phases to separate. Drain the acidic aqueous phase back into the original boiling flask.

Partition the hexane with an additional 2 x 25 mL of 1M HCl and combine the acidic phases back into the original boiling flask. Discard the hexane partition.

2. Partition the acidic aqueous phase from Step 1 above three times with a minimum volume of 25 mL dichloromethane. Shake each partition for approximately one minute and allow the layers to separate. Drain each dichloromethane partition through a sodium sulfate pad (approximately 90 g of sodium sulfate in a powder funnel plugged with glass wool) and collect the extracts in a 250 mL boiling flask. Rinse the sulfate pad with 25 mL of dichloromethane. Discard the aqueous phase.

3. Rotary evaporate the combined dichloromethane extracts from Step 2 to dryness under reduced pressure at 40 °C (± 5 °C). Dissolve samples in 2.0 mL of hexane to await Florisil cleanup (Section 5.3).

   Note: If particulates form (mainly with cottonseed samples) after dissolving the residue in hexane, they may be dissolved by adding 100 µL of ethyl acetate to the extracts in Step 3 above prior to transferring them to the Florisil column.

5.3 Florisil Cleanup

Note: Adjustments in elution solvent strength or volume from the values reported in this method may be required depending on the lot or brand of Florisil used. No special treatment of the Florisil is needed but, each new lot of Florisil must be calibrated to ensure the complete elution of Buprofezin. The calibration pattern is checked by analyzing 10 mL fractions of the eluate after a 1.0 mL solution containing at least 5.0 µg of Buprofezin is loaded in hexane and eluted from the column as described below. The fractions are evaporated to dryness and then brought up to 5 mL in toluene. Recoveries should be calculated for each fraction and totaled. Adjustments in elution solvent strength or volume should be made as required and then the calibration rechecked to insure the complete elution of Buprofezin.

1. Prepare Florisil columns by slurry packing 5.0 grams of Florisil with 50 mL hexane into a chromatographic column and capping the columns with approximately 3/8" granular sodium sulfate.

2. Drain the excess hexane until the top of the column just begins to dry. (DO NOT ALLOW THE FLORISIL TO DRY.)

3. Transfer the hexane extract from Step 3 of Section 5.2 onto the Florisil column and drain the column until the top of the column just begins to dry. Rinse the boiling flask with 3 x 2 mL portions of hexane and load each rinse onto the column. Drain the column between each rinse until the column just begins to dry.

4. Elute the buprofezin metabolite with 40 mL of 20% (v/v) ethyl acetate/hexane and collect the extracts in a 250 mL boiling flask.

5. Rotary evaporate the extracts from Step 4 above to dryness under reduced pressure at 40 °C (± 5 °C) and dissolve the residue in 5.0 mL of toluene to await analysis by GC/NPD.

6. GC/NPD ANALYSIS

6.1 Preparation of Analytical Standard Solutions

1. Prepare a stock solution containing a nominal concentration of 1000 µg/mL of buprofezin in acetone. Make serial dilutions with acetone to yield fortification standards (when fortification experiments are required) of 100, 10, 1.0, and 0.10 µg/mL.

2. Prepare a standard solution containing a nominal concentration of 10 µg/mL of buprofezin in toluene. From this standard, make serial dilutions with toluene to yield GC calibration standards of 0.60, 0.40, 0.20, 0.10, 0.04, and 0.02 µg/mL.

6.2 Standardization and Detection of Sample Residues

1. Equilibrate the GC system under the conditions listed in Appendix I by making repeated (usually 4-5) 2 µL priming injections of the 0.60 µg/mL GC calibration standard solution until a consistent response (less than 10% variation for equivalent standards) is obtained.

2. Inject a 2 µL aliquot of each test sample (or fortified sample matrix) from Step 5 of Section 5.3 into the GC system under the conditions stated in Appendix I. An injection volume of 2 µL was found to give satisfactory results. Variations in equipment or sample characteristics may require different injection volumes or slight modifications in the chromatographic conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity. Make dilutions as necessary to maintain the response within the range of the standard curve.

3. Compare the peak height of the analyzed sample with the standard curve. Calculate the total residue concentration (R) using *Equation 1* as follows:

$$R\ (ppm) = \frac{(Y - b) / m}{C} \qquad \textit{(Equation 1)}$$

where: Y = peak height (or area) response (cts.)
b = Y-intercept of standard regression line (cts.)
m = slope of standard regression line (cts mL/µg)
C = crop/solvent ratio (g/mL)

The crop/solvent ratio *"C"* is defined by the concentration of sample in g/mL at injection using *Equation 2* for the crop matrix of interest. This factor incorporates all dilutions made to the sample during sample work-up. Further dilutions may be required to maintain the response within the range of the standard calibration curve.

$$C = \frac{W}{5\ mL} x\ D \qquad \textit{(Equation 2)}$$

where: W = sample weight in grams
D = dilution factor

The dilution factor D is defined by *Equation 3* below:

$$D = \frac{A}{V} \qquad \textit{(Equation 3)}$$

where: A = aliquot taken in mL at the final volume
V = total volume in mL of the dilution

6.3 Fortification Experiments

(Optional for Tolerance Enforcement)

Note: Fortification experiments are not required when analysis of samples is performed for tolerance enforcement. Fortification experiments are intended to be used for data collection methods or establishing & validating method efficiency when required.

With each sample set, analyze an untreated control sample and one or more fortified control samples. Calculate recoveries by *Equation 4* as follows:

$$\text{Recovery (\%)} = \frac{R - S}{T} \times 100 \qquad \textit{(Equation 4)}$$

where: R = ppm of target analyte found in fortified sample
S = ppm of target analyte found in control sample
T = theoretical ppm in fortified sample

Note: Fortification experiments are intended to be used for data collection methods or establishing & validating method efficiency when required. If fortification experiments are required, untreated control samples must be analyzed using the same analytical method described to verify that any endogenous substances present in the samples do not interfere with the final determination of buprofezin. See Figures 4, 7, 10, and 13 of Appendix III for typical control chromatograms of almonds, cottonseed, citrus (lemons), and grapes.

Method efficiency is monitored by calculating recoveries defined by *Equation 4*. Recoveries are determined by analyzing fortified control samples in conjunction with each sample set. Samples are fortified prior to extraction at the limit of quantitation of 0.01 ppm (µg/g) with buprofezin solutions prepared in acetone. Calculate the final residue R for the control and fortified control samples. Correct the results of the recovery samples by subtracting the final determined residue (real or apparent) detected in the control sample from the value determined for the fortified control sample. (See Figures 5, 6, 8, 9, 11, 12, 14, and 15 of Appendix III for typical fortified control chromatograms of almonds, cottonseed, citrus (lemons), and grapes.)

7. DISCUSSION

A set of 8-12 samples can be analyzed by one analyst in approximately 2 working days, assuming that all materials and equipment are available.

The most critical step in the method is the Florisil column calibration step. Unless the elution pattern is accurately established for the chromatographic column utilized, significant losses of analyte(s) could occur.

The limit of quantitation for this method is 0.05 ppm for. This is based on the recoveries achieved at that concentration. Typically, experimental recoveries for all matrices were within the range of 70% to 120% with standard deviations of about 10%. Results are presented in Table 1.

Recovery data listed in Table 1 were calculated using control samples from taken from various studies previously conducted for buprofezin. Lemon samples were purchased from a local grocer and assigned a unique sample number.

Table 1 Recovery Data for Buprofezin in Cottonseed, Almonds, Citrus (Lemons), and Grapes

Crop Matrix	Fortification Level (ppm)	Analytical Recovery (%) for Buprofezin[a]		
Cottonseed (135-030.01)	0.05	112	114	104
	0.50	101	94	97
	2.00	95	86	94
Almond Meats (149-113.01A)	0.05	98	107	105
	0.50	90	87	88
	2.00	83	83	73
Citrus (Lemons) (149-174.01)	0.05	88	89	86
	0.50	77	[illegible]	78
	2.00	85	71	81
Grapes (151-008.01)	0.05	84	94	90
	0.50	85	90	88
	2.00	85	83	90 (56)[b]
Number =			36	
Mean (%) =			90	
Std. Dev. =			± 10	

[a] Analytical recoveries corrected for apparent residue in control samples.

[b] 1st analysis (56%) excluded from statistics. This sample was repeated and the 2nd repeat analysis (90%) was reported and included in statistics.

8. REFERENCES

1. Anonymous, "Analytical Method of Buprofezin and ρ-Hydroxy Metabolite in Crops and Soil", Nihon Nohyaku Co. Ltd. (July, 1985). Reference: R30 (A-1005)

Appendix I Instrument Conditions

Gas Chromatography

GC/NPD CONDITIONS:

Instrument:	Hewlett-Packard 5890A gas chromatograph with capillary split/splitless inlet operated in the direct injection mode and containing a 6.3 mm o.d. x 78.5 mm cyclo uniliner. (Restek Corp. Cat. No. 20337)	
Column:	Fused silica megabore DB-1 bonded phase, 15 m x 0.53 mm i.d., 1.5-µm film thickness. (J & W Scientific)	
Carrier Gas:	Helium (Ultrapure 99.999%) Head pressure set to 4.5 psi @ 165 °C Column flow rate: 5 to 6 mL/min @ 165 °C	
Split Flow:	0 mL/min. @ 165 °C	
Septum Purge:	Approximately 3 mL/min	
Temperatures:	Injection Port:	275 °C
	Detector:	300 °C
	Oven:	Programmed
	Initial:	165 °C for 2.0 min
	Ramp:	20 °C/min to 185 °C; then hold for 2.0 min
	Ramp A:	25 °C/min to 300 °C; then hold for 2.0 min
Retention Times:	Buprofezin:	approximately 7.7 min

Appendix I (continued)

Injection

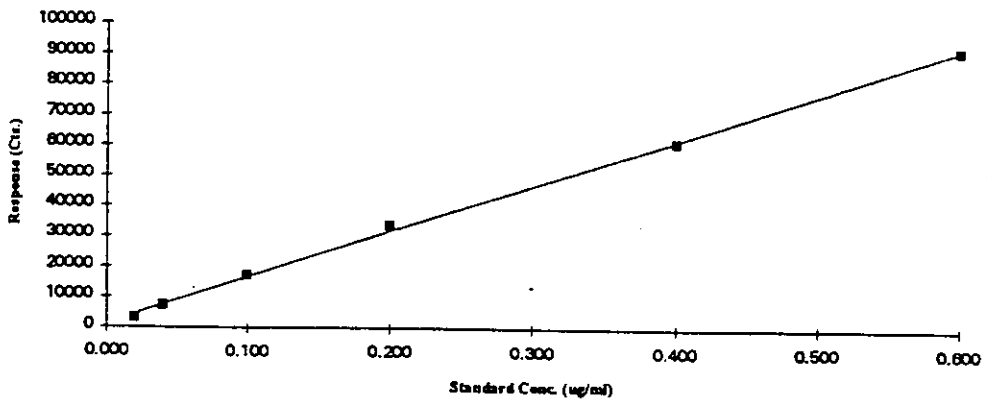
Autosampler:	Hewlett-Packard 7673A 2 µL injection volume, residence time of less than one second (fast injection)	
Detector:	Hewlett-Packard nitrogen phosphorus detector (NPD)	
Fuel Gases:	Hydrogen: Air:	3.0 - 3.5 mL/min 90 - 100 mL/min
Make-Up Gas:	Helium:	24 - 25 mL/min
Purge Value:	Initial value:	Off
Integration Parameters:	Data was collected on a PE Nelson Data system using Access*Chrom Software.	

Appendix II Standard Calibration Data and Example Calculations for Buprofezin

AgrEvo USA Company — Residue Chemistry Calculation Spreadsheet

Standard Soln. Ref. #	Standard Conc. (ug/ml)	Response (Cts.)	Calculated Line (Cts.)	Statistical Information Method BF/09/97 Validation Calculation Cotton (Set 4)	
BF/73/10	0.020	3175	4619		
BF/73/09	0.040	7388	7620	Slope (m)	1.5005E+05
BF/73/08	0.100	17257	16623	Y-Intercept (b)	1.6181E+03
BF/73/07	0.200	33572	31628		
BF/73/06	0.400	61002	61638	Coeff. of Determination	0.9989
BF/73/05	0.600	91381	91647		

STANDARD CALIBRATION CURVE (Buprofezin)



AgrEvo Sample #	Sample Weight (g)	Amount Analyte Added (ug)	Recovery Level (ppm)	Response (Cts.)	Det. Amt. of Analyte (ug/ml)	Crop / Solv. Ratio (g/ml)	Uncorrected Determined Res. (ppm)	Percent Recovery
Section 1: Control Samples								
135-030.01	20.0	0.000	0.00	0	N.D.	5.0000	N.D.	N/A
*Section 2: Recovery Samples (Corrected For Control Residues)**								
135-030.01 A	20.0	1.000	0.05	35186	0.2237	4.0000	0.0559	112%
135-030.01 B	20.0	1.000	0.05	35824	0.2280	4.0000	0.0570	114%
135-030.01 C	20.0	1.000	0.05	32905	0.2085	4.0000	0.0521	104%
135-030.01 D	20.0	10.00	0.50	31853	0.2015	0.4000	0.5038	101%
135-030.01 E	20.0	10.00	0.50	29947	0.1888	0.4000	0.4720	94%
135-030.01 F	20.0	10.00	0.50	30704	0.1938	0.4000	0.4846	97%
135-030.01 G	20.0	40.00	2.00	47092	0.3031	0.1600	1.8941	95%
135-030.01 H	20.0	40.00	2.00	42708	0.2738	0.1600	1.7115	86%
135-030.01 I	20.0	40.00	2.00	46919	0.3019	0.1600	1.8869	94%
Section 3: Treated Samples								
Reagent Blank	20.0	0.000	0.00	0	N.D.	4.0000	N.D.	N/A

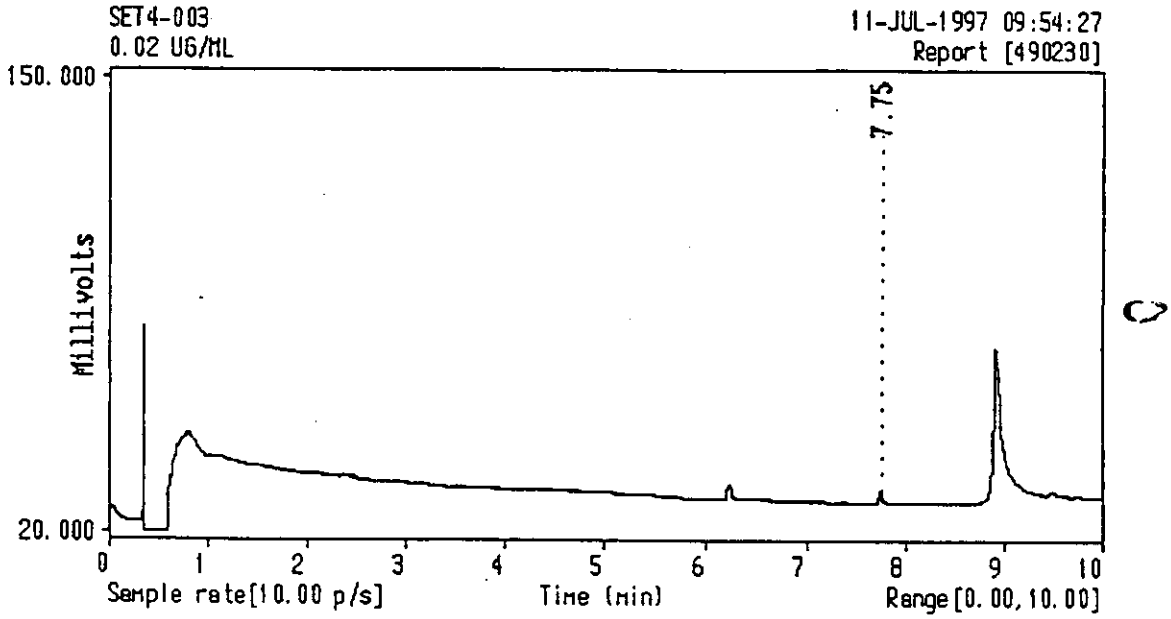
* Corrected for Residue Contained in Control
(+) Out of Standard Calibration curve. Requires dilution for accurate quantitation.
(-) Below Standard Calibration curve.

Appendix III Representative Chromatograms

Appendix III (continued)

Figure 1 0.02 µg/mL Calibration Standard

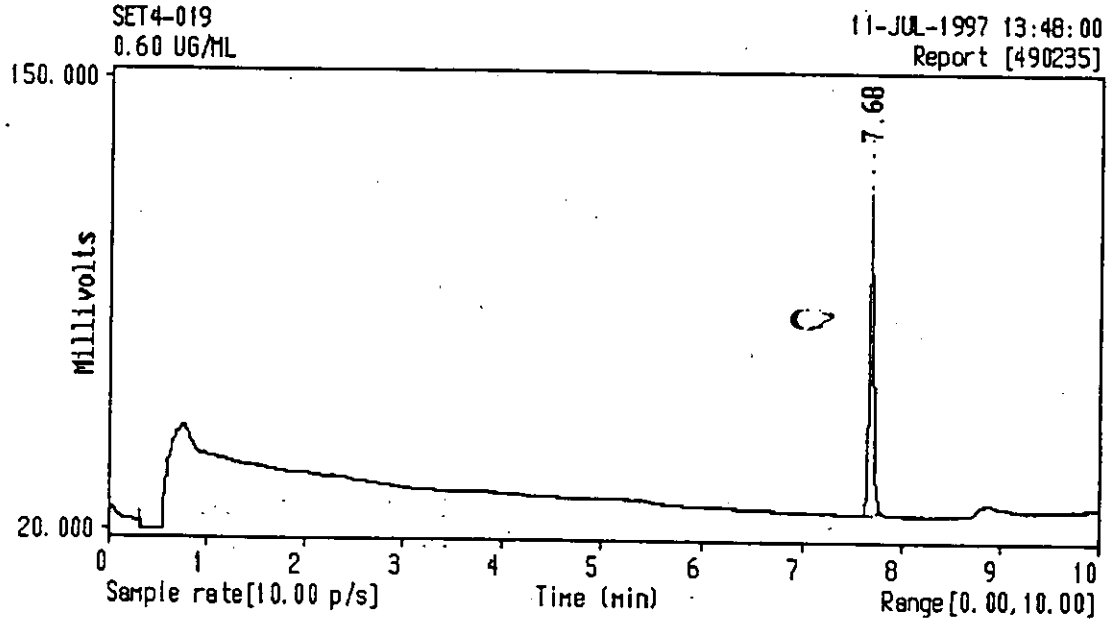
Sample Description:	BF/73/10	0.02 µg/mL Calibration Standard		
Retention Time (min)	**Analyte of Interest**	**Peak Height (Counts)**	**Determined Residue (ppm)**	**Analytical Recovery (%)**
7.75	Buprofezin	3175	N/A	N/A



Appendix III (continued)

Figure 2 0.60 µg/mL Calibration Standard

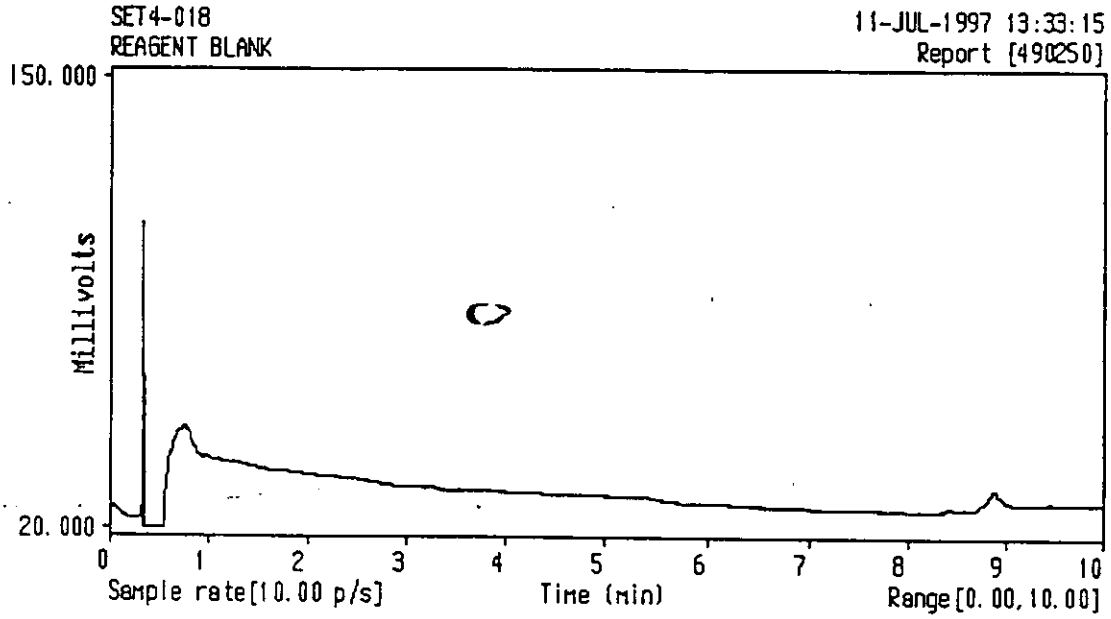
Sample Description:	BF/73/05	0.60 µg/mL Calibration Standard		
Retention Time (min)	**Analyte of Interest**	**Peak Height (Counts)**	**Determined Residue (ppm)**	**Analytical Recovery (%)**
7.68	Buprofezin	91381	N/A	N/A



Appendix III (continued)

Figure 3 Reagent Blank

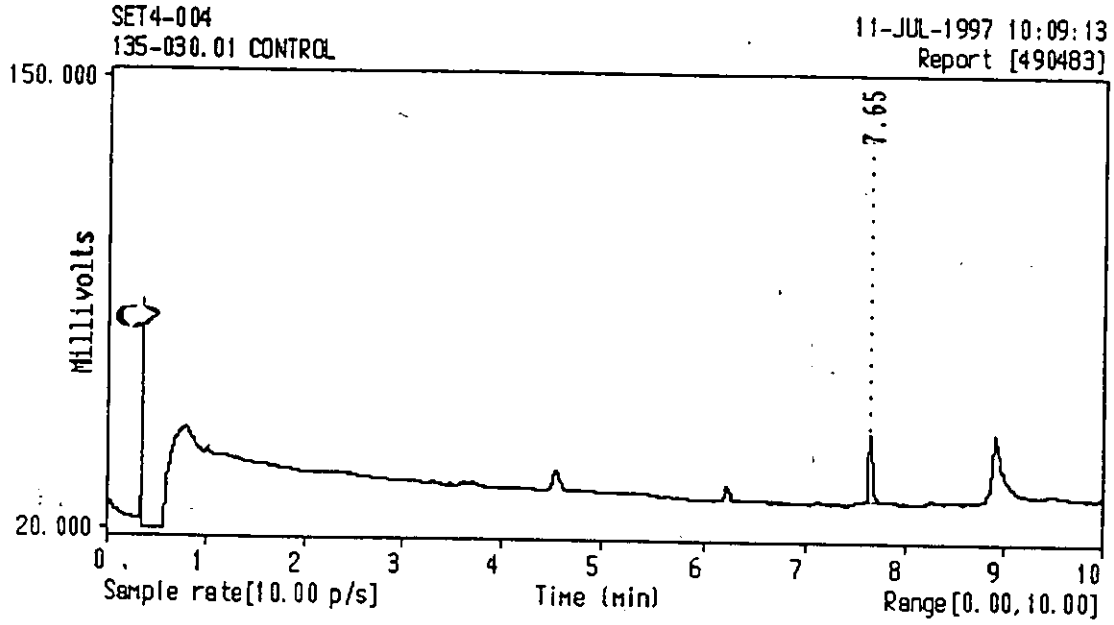
Sample Description:	Solvent	Reagent Blank		
Retention Time (min)	**Analyte of Interest**	**Peak Height (Counts)**	**Determined Residue (ppm)**	**Analytical Recovery (%)**
—	Buprofezin	N.D.	N.D.	N/A



Appendix III (continued)

Figure 4 Cottonseed Control

Sample Description:	135-030.01	Cottonseed Control		
Retention Time (min)	**Analyte of Interest**	**Peak Height (Counts)**	**Determined Residue (ppm)**	**Analytical Recovery (%)**
—	Buprofezin	N.D.	N.D.	N/A

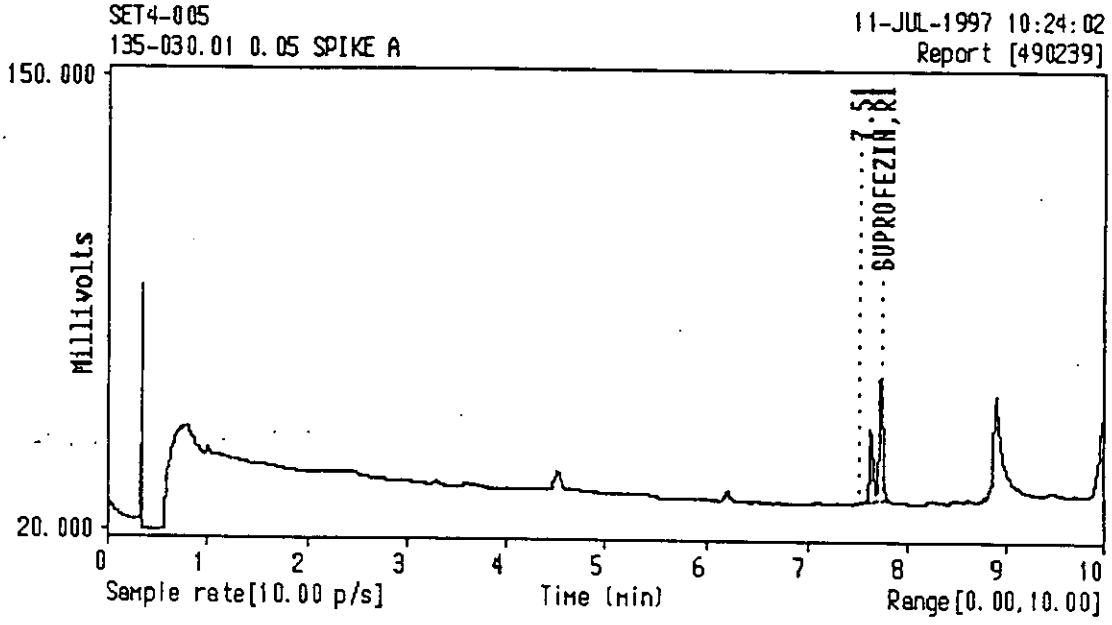


Appendix III (continued)

Figure 5 Cottonseed Control Fortified at 0.05 ppm

Sample Description:	135-030.01	Cottonseed Control Fortified at 0.05 ppm		
Retention Time (min)	**Analyte of Interest**	**Peak Height (Counts)**	**Determined Residue (ppm)**	**Analytical[a] Recovery (%)**
7.73	Buprofezin	35186	0.0559	112

[a] Corrected for residue detected in control sample.

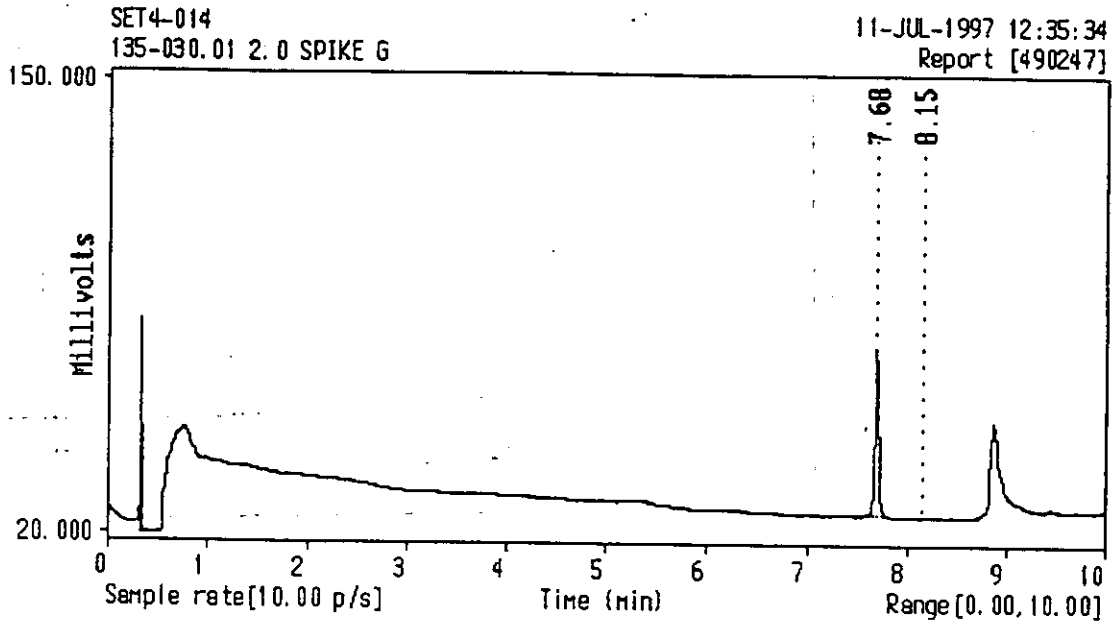


Appendix III (continued)

Figure 6 Cottonseed Control Fortified at 2.00 ppm

Sample Description:	135-030.01	Cottonseed Control Fortified at 2.00 ppm. Diluted 1-25 mL.		
Retention Time (min)	**Analyte of Interest**	**Peak Height (Counts)**	**Determined Residue (ppm)**	**Analytical[a] Recovery (%)**
7.68	Buprofezin	47092	1.894	95

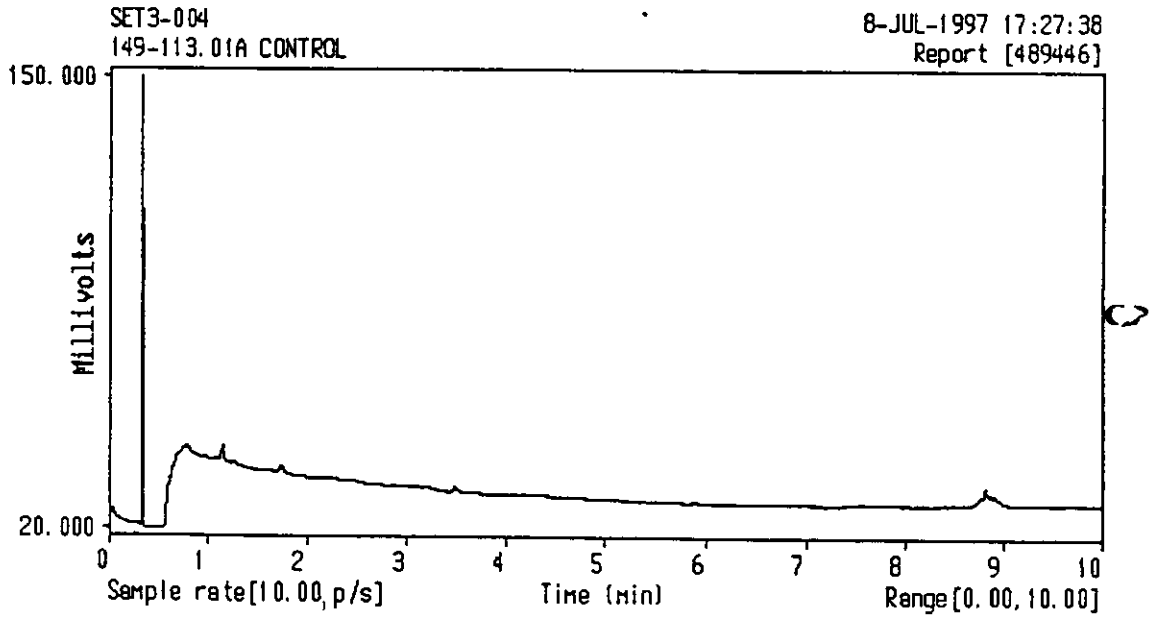
[a] Corrected for residue detected in control sample.



Appendix III (continued)

Figure 7 Almond Meat Control

Sample Description:	149-113.01A	Almond Meat Control		
Retention Time (min)	**Analyte of Interest**	**Peak Height (Counts)**	**Determined Residue (ppm)**	**Analytical Recovery (%)**
—	Buprofezin	N.D.	N.D.	N/A

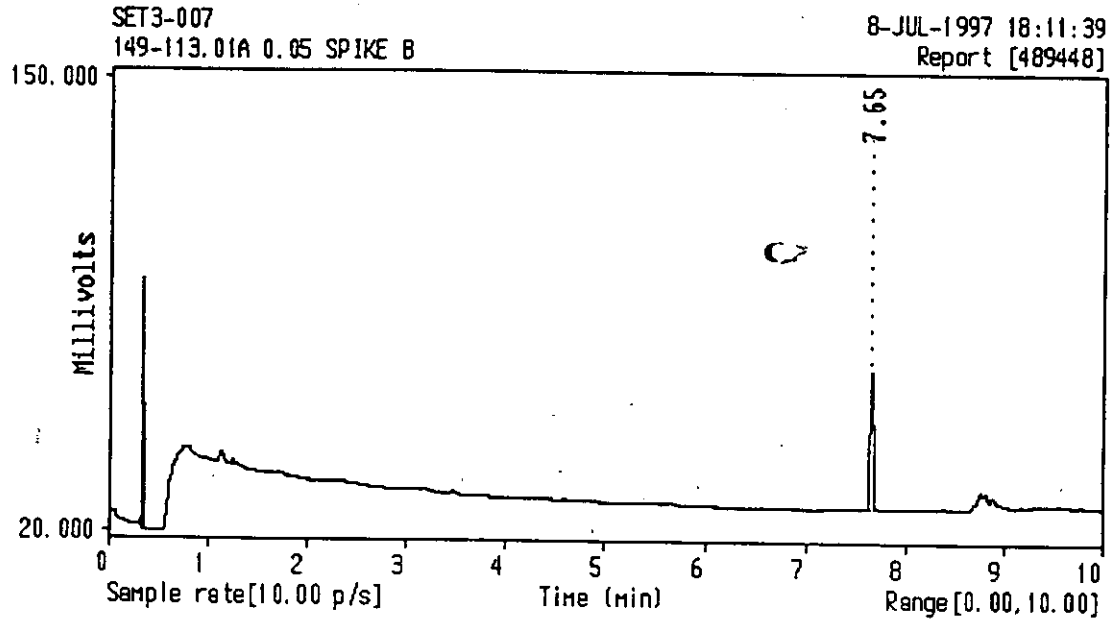


Appendix III (continued)

Figure 8 Almond Meat Control Fortified at 0.05 ppm

Sample Description:	149-113.01A	Almond Meat Control Fortified at 0.05 ppm		
Retention Time (min)	**Analyte of Interest**	**Peak Height (Counts)**	**Determined Residue (ppm)**	**Analytical[a] Recovery (%)**
7.65	Buprofezin	39063	0.05345	107

[a] Corrected for residue detected in control sample.

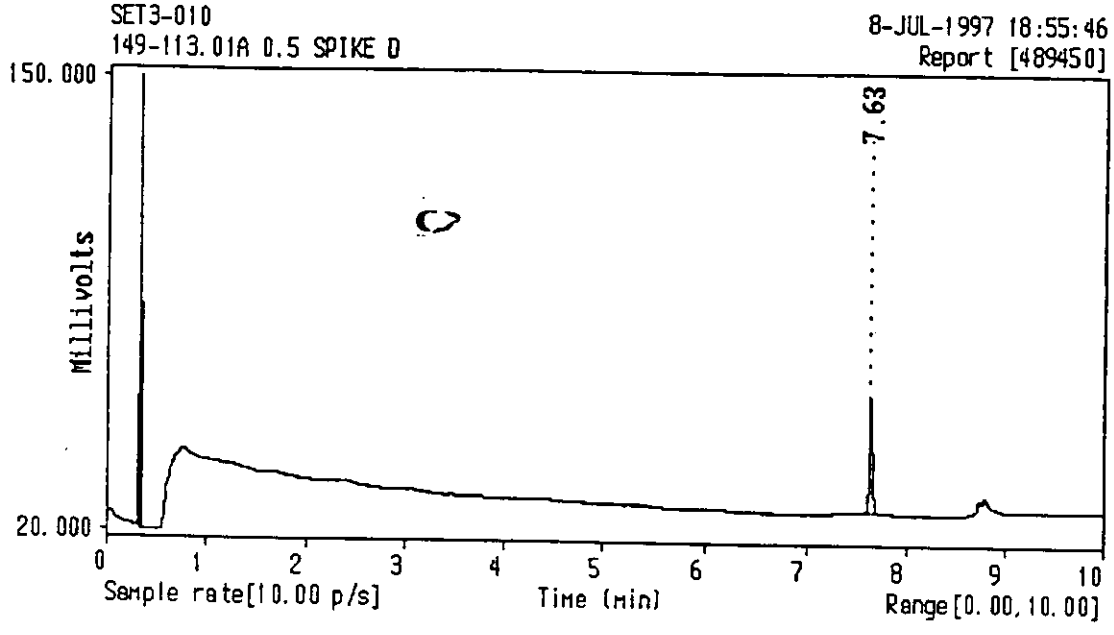


Appendix III (continued)

Figure 9 Almond Meat Control Fortified at 0.50 ppm

Sample Description:	149-113.01A	Almond Meat Control Fortified at 0.50 ppm. Diluted 1-10 mL.		
Retention Time (min)	**Analyte of Interest**	**Peak Height (Counts)**	**Determined Residue (ppm)**	**Analytical[a] Recovery (%)**
7.63	Buprofezin	32876	0.4491	90

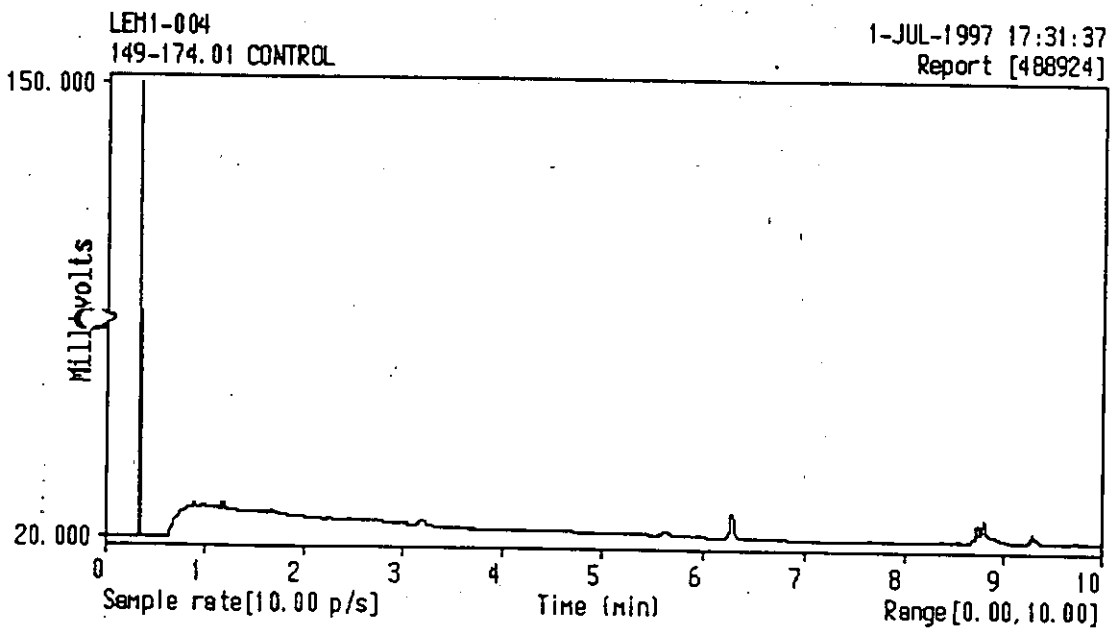
[a] Corrected for residue detected in control sample.



Appendix III (continued)

Figure 10 Citrus Control (Lemon)

Sample Description:	149-174.01	Citrus Control (Lemon)		
Retention Time (min)	**Analyte of Interest**	**Peak Height (Counts)**	**Determined Residue (ppm)**	**Analytical Recovery (%)**
—	Buprofezin	N.D.	N.D.	N/A

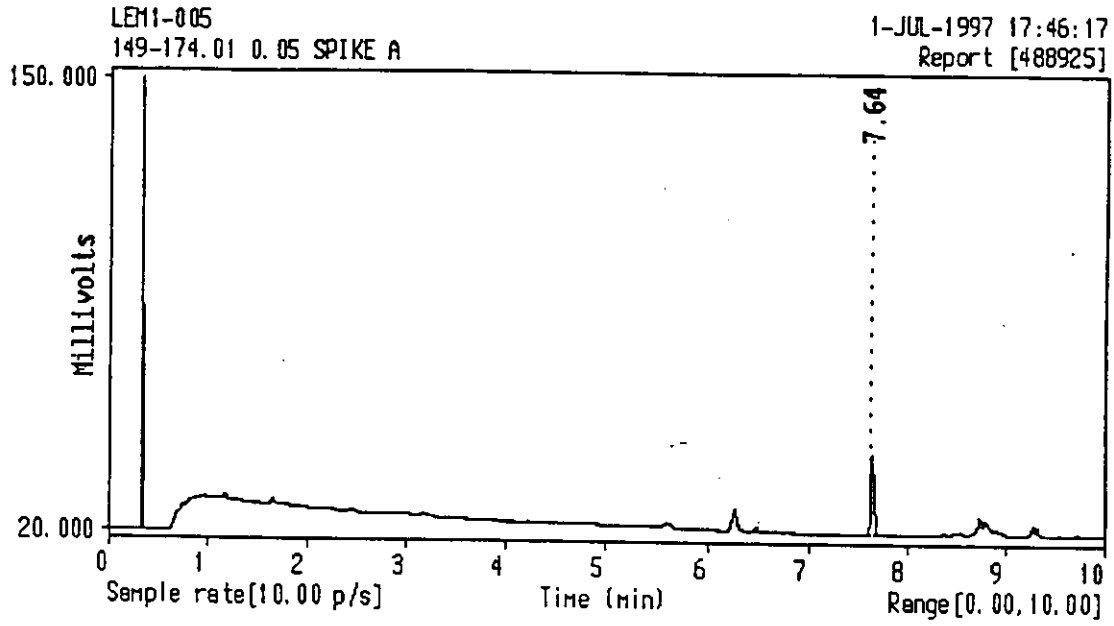


Appendix III (continued)

Figure 11 Citrus Control (Lemon) Fortified at 0.05 ppm

Sample Description:	149-174.01	Citrus Control (Lemon) Fortified at 0.05 ppm		
Retention Time (min)	**Analyte of Interest**	**Peak Height (Counts)**	**Determined Residue (ppm)**	**Analytical[a] Recovery (%)**
7.64	Buprofezin	22961	0.04396	88

[a] Corrected for residue detected in control sample.

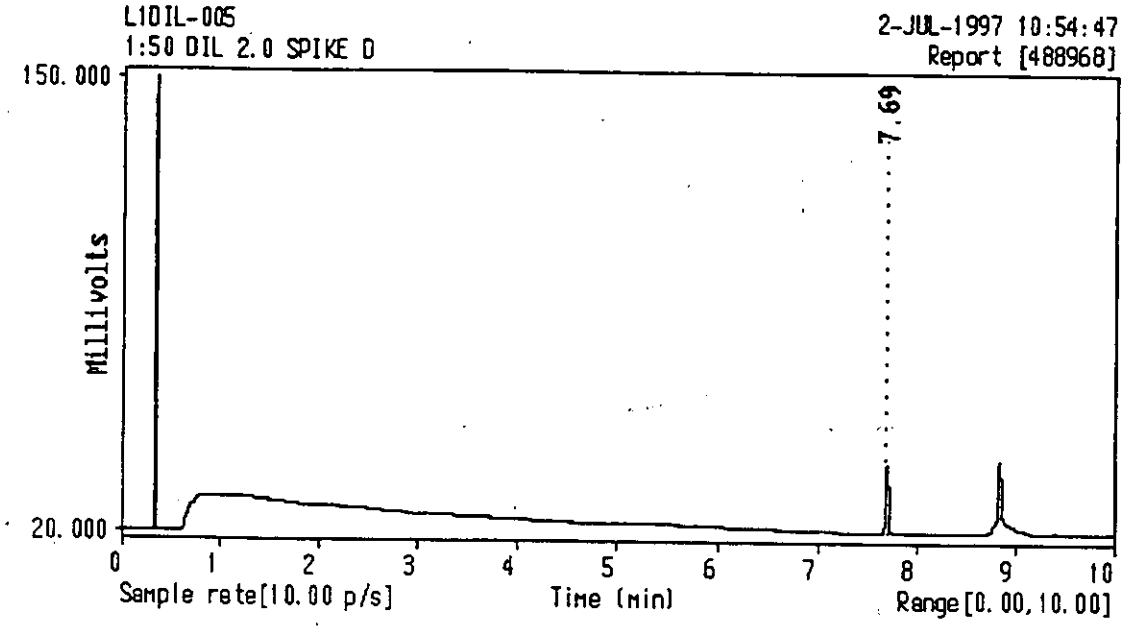


Appendix III (continued)

Figure 12 Citrus Control (Lemon) Fortified at 2.00 ppm

Sample Description:	149-174.01	Citrus Control (Lemon) Fortified at 2.00 ppm. Diluted 1-50 mL		
Retention Time (min)	**Analyte of Interest**	**Peak Height (Counts)**	**Determined Residue (ppm)**	**Analytical[a] Recovery (%)**
7.69	Buprofezin	19152	1.695	85

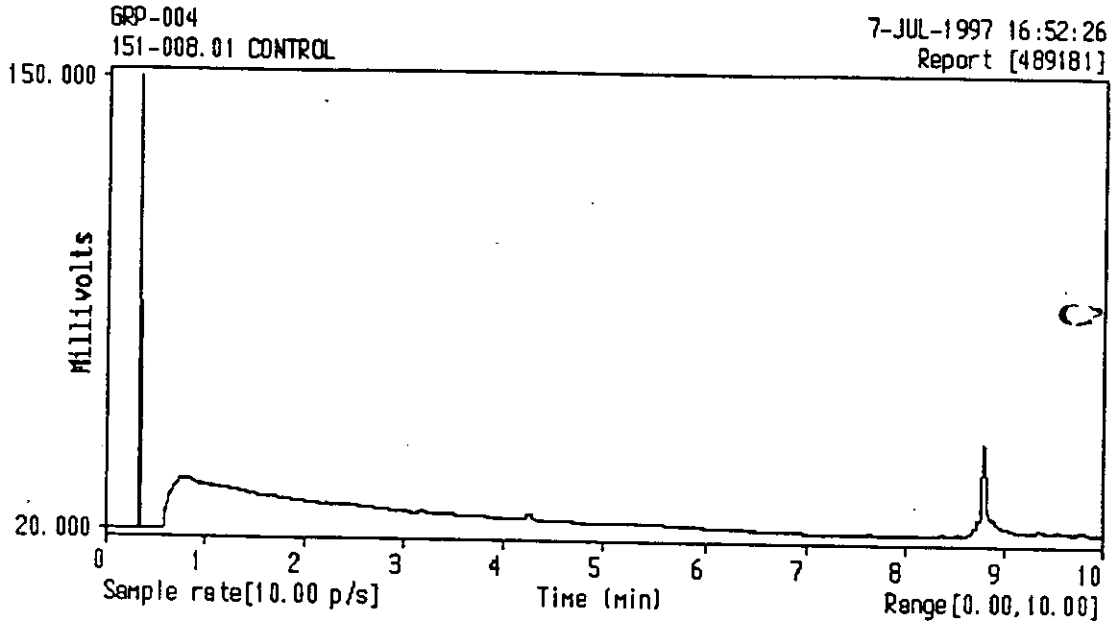
[a] Corrected for residue detected in control sample.



Appendix III (continued)

Figure 13 Grapes Control

Sample Description:	151-008.01	Grapes Control		
Retention Time (min)	**Analyte of Interest**	**Peak Height (Counts)**	**Determined Residue (ppm)**	**Analytical Recovery (%)**
—	Buprofezin	N.D.	N.D.	N/A

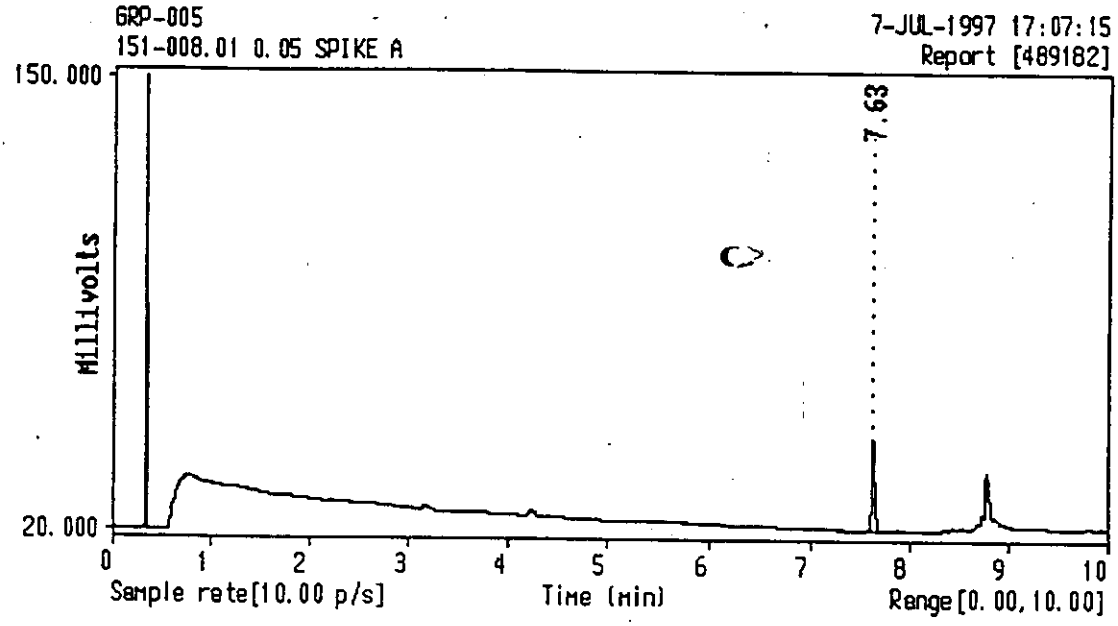


Appendix III (continued)

Figure 14 Grape Control Fortified at 0.05 ppm

Sample Description:	151-008.01	Grape Control Fortified at 0.05 ppm		
Retention Time (min)	**Analyte of Interest**	**Peak Height (Counts)**	**Determined Residue (ppm)**	**Analytical[a] Recovery (%)**
7.63	Buprofezin	25661	0.04202	84

[a] Corrected for residue detected in control sample.

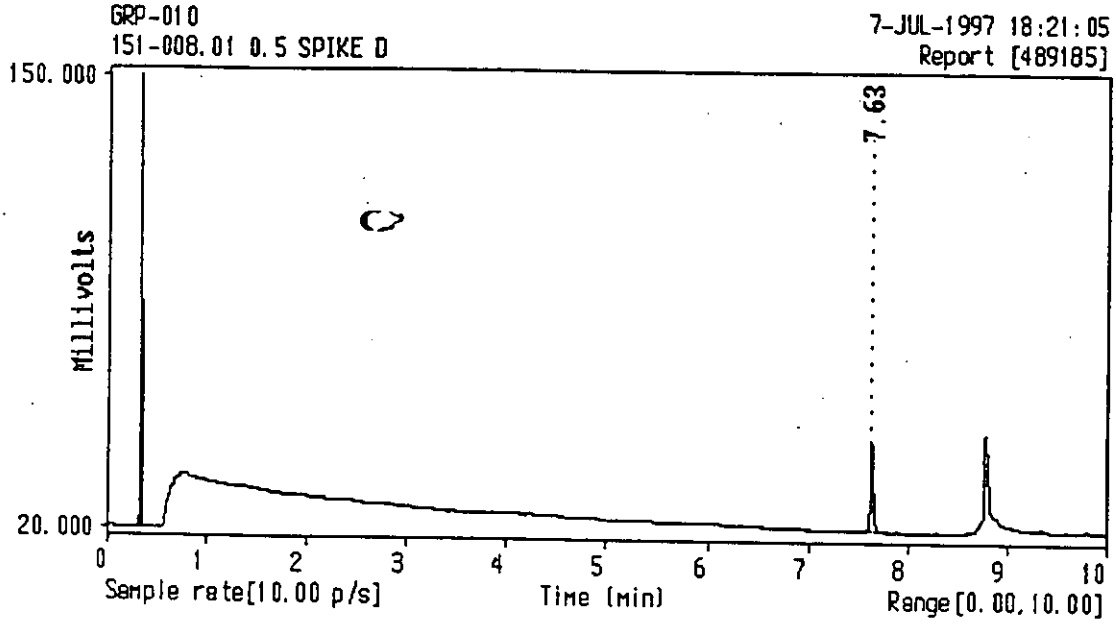


Appendix III (continued)

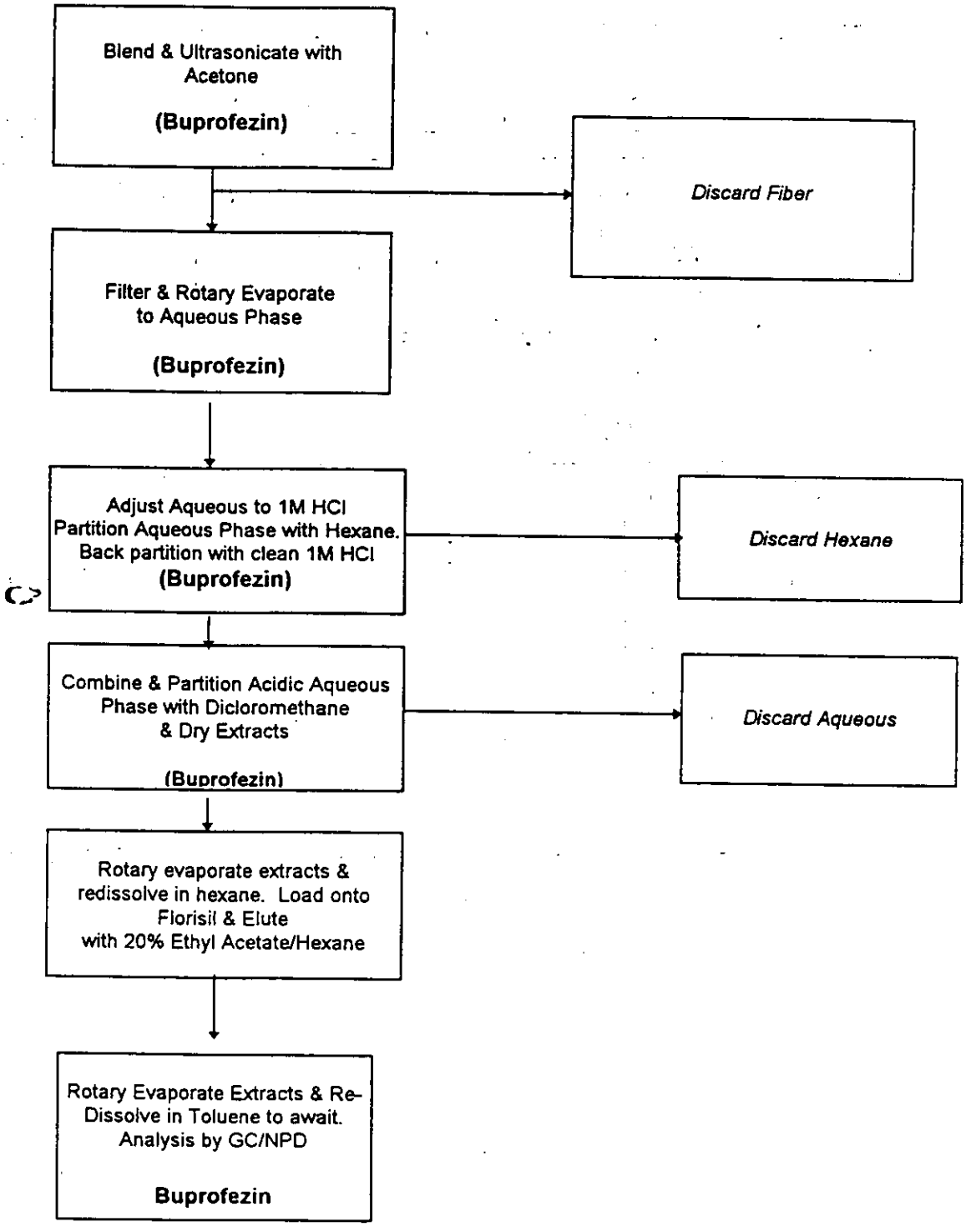
Figure 15 Grape Control Fortified at 0.50 ppm

Sample Description:	151-008.01	Grape Control Fortified at 0.50 ppm. Diluted 1-10 mL.		
Retention Time (min)	**Analyte of Interest**	**Peak Height (Counts)**	**Determined Residue (ppm)**	**Analytical[a] Recovery (%)**
7.63	Buprofezin	25970	0.4254	85

[a] Corrected for residue detected in control sample.



Appendix IV Flow Diagram of Analytical Method BF/09/97



EPA ADDENDUM

1) ACB substituted equivalent equipment and materials.

A) ACB used a Hewlett Packard 6890 GC with nitrogen - phosphorus detector with the following parameters:

Column: DB-1, 15 m x 0.53 mm id, 1.5 um film thickness
Inlet Temperature: 275°
Detector temperature: 300° C
Oven Program: Initial 165° C, hold for 2.0 min.
20° C/min. to 185° C, hold for 2.0 min.
25° C/min. To 300° C, hold for 6.0 min.
Gas Flows:
Carrier: 6.9 ml/min. Helium
Hydrogen: 3.0 ml/min.
Air: 60 ml/min.
Injection Volume: 2 ul

B) For milk, the ACB modified the above program to separate an interference from the analyte.

Oven Program: Initial 165° C, hold for 2.0 min.
20° C/min. to 185° C, hold for 2.0 min.
5° C/min. To 230° C, hold for 3.0 min.
30° C/min. To 300° C, hold for 11.0 min.

C) The ACB used an IKA Works T25 Tissumizer in place of the Omni-Mixer for all commodities except raisins. When using the Tissumizer, the ACB shortened the time to approximately 3 minutes for the first blending, and 2 min. for the second. For raisins, the ACB used a VirTis 23.

D) After checking the elution pattern of Florisil, the ACB increased from the stated 40 ml to 50 ml of 20% e.a./hexane.

E) For the meat and milk method, the ACB collected the eluant from the SPE aminopropyl columns in test tubes. The extracts were evaporated to dryness with a nitrogen evaporator.

2) The ACB suggests changes and clarification to the method as stated below are needed. These should be attached to the current method when distributed and included in any revised method.

A) The petitioner's instructions for calculations do not meet the Residue Chemistry Test Guidelines, OPPTS 860.1340 with respect to analyte found in controls. It is a Residue Chemistry Test Guideline requirement that control values are not subtracted from fortified or unknown samples, but to report the amount found in each sample.

B) The ACB found that sonication aided to dissolve dried extract residues into hexane prior to Florisil cleanup.

C) The ACB obtained clarification from J. Neal, Aventis, concerning the packing of Florisil columns. As per phone conversation (5/3/01), 50 ml of hexane is added to the glass column and 5 gram of dry Florisil is then poured into the glass column.

D) The ACB experienced severe emulsion problems with cottonseed and almond hulls resulting in low recoveries. Using a Tissumizer may have extracted more efficiently, increasing the amount of suspended particles present in the partitioning step. The ACB contacted J. Neal, Aventis, to determine if the petitioner had encountered these problems and discuss any possible solutions. During the third trial of cottonseed, the ACB found that gently rocking the separatory funnel was sufficient during the hexane and acidic aqueous step to achieve partitioning. During the second trial, almond hull solutions still emulsified, even with gentle rocking. The ACB drained the aqueous and emulsion layers together for further partitioning with methylene chloride.

Extraction by a Tissumizer or similar blenders using generators instead of blades, may result in more emulsions and higher background. The beef liver was the only commodity that showed significantly higher background levels in chromatograms than either the petitioner or the ILV.

3) The ACB prepared liver extracts by following the method as written without the hexane/aqueous partitioning and also with the hexane/aqueous partitioning. The ACB also ran a procedural blank with the additional partitioning step, and found that while some background could be attributed to the reagents, much originated from the liver. The ACB found the final extracts to be cleaner and able to be quantitated using GC/NPD in place of GC/MSD.