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Study Title:

Method for Determination of Residues of Acifluorfen and Metabolites in Soybean Grain by Gas Chromatography and Liquid Chromatography

Study No. 92161

Method No. D9205

Data Requirement:
Guideline 171-4 Residue Chemistry
Residue Analytical Method

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Study Completion Date:

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Performing Laboratory:

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PR 86-5 DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10 (d) (1) (A), (B), or (c).

COMPANY: BASF Corporation Agricultural Products

COMPANY AGENT: Karen R. Blundell **Date:** June 18, 1993

TITLE: Senior Registration Specialist

SIGNATURE: Karen R. Blundell

STATEMENT OF GLP COMPLIANCE

To the best of my knowledge and belief, this study meets the requirements for 40 CAR Part 160 except the following.

- Data on the stability of some of the test and reference substances in organic solvent is incomplete. All indications from handling the analytical standard solutions suggest these standards are quite stable. The instrument responses for the methyl ester compounds remained constant during the life-time of the solutions. Recoveries of acifluorfen through the analytical method are consistent within experimental error and independent of the length of storage of the analytical solution prior to fortification. Data on the stability of the neat standards is also incomplete, but since these standards are stored at <-5°C, and no instability has been observed in passing them through the methodology, no degradation is expected.

Submitter: Karen R Blundell

Sponsor: Karen R Blundell

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Method No. D9205 **Report Date: June 1993**

ABSTRACT:

Analytical Method No. D9205 was developed to determine the residues of sodium acifluorfen, the active ingredient in Blazer® Herbicide, and its metabolites in soybean grain. Method development and validation were carried out at BASF Corporation, Research Triangle Park, N.C., using representative samples from a magnitude of residue study. Sodium acifluorfen and its metabolites can be extracted by soaking in a basic aqueous solution and then blending with the addition of an acetic acid/acetonitrile solution. Acifluorfen (salt and acid forms) and acifluorfen methyl ester residues are quantitated by gas chromatography with electron capture detection, and the amine metabolite of acifluorfen and its methyl ester are quantitated by liquid chromatography with fluorescence detection. This study has shown that Analytical Method Number D9205 is suitable for measuring residues of sodium acifluorfen and its metabolites in soybean grain down to a quantitation limit of 0.02 ppm for each compound.

Pages of Report: 70 Experimental Dates:
Start: November 11, 1992
Termination: June 6, 1993

QAU STATEMENT

Method Number: D9205
BASF Study Number: 92161

Study Initiation Date: November 2, 1992

The quality assurance unit of the testing facility at the ARC has audited the protocol, the analytical portion including the raw data, and the report for this study and reported its findings to the study director and to management.

Date of Audit	Report to Study Director and to Management
October 27, 1992	October 27, 1992
December 10, 1992	December 10, 1992
May 21, 1993	May 21, 1993
June 18, 1993	June 18, 1993

JH Fensch
Signature of QAU

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1. INTRODUCTION AND SUMMARY

1.1 Scope and Source of the Method

1.1.1 Scope

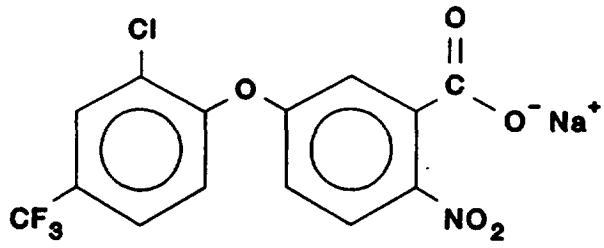
The method is used to determine the residues of acifluorfen (salt and acid forms) and metabolites in soybean grain.

1.1.2 Source

This method was developed at the BASF Agricultural Research Center in Research Triangle Park, North Carolina. This method was partially developed from Rhone Poulenc Method Number 160, "Enforcement Method for the Determination of Residues of Acifluorfen and Metabolites in/on Plant Tissue" (Reference 1). The control sample used for generation of the validation data is from the previous BASF crop field study number P9012, sample number 90072-5. An aliquot of this sample was reassigned number 92940-1 for this study.

1.2 Test and Reference Substances

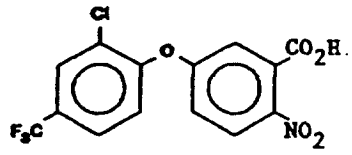
Common Name:	Sodium Acifluorfen
BAS Number:	BAS 9048 H
Chemical Name:	Sodium 5-[2-chloro-4(trifluoromethyl)phenoxy]-2-nitro benzoate
CAS Number:	62476-59-9
Structural Formula:	



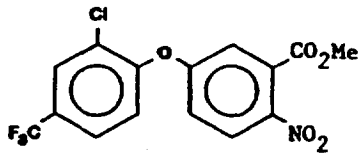
Empirical Formula:	$C_{14}H_6F_3ClNO_5Na$
Molecular Weight:	383.65 g/mole

Below are the acid form and the three regulated metabolites of sodium acifluorfen.

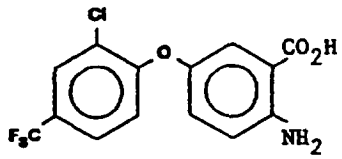
Common Name: Acifluorfen
Chemical Name: 5-[2-chloro-4-(trifluoromethyl) phenoxy]-2-nitro-benzoic acid
Code Name: BAS 9048 H (acid form)
Molecular Weight: 361.66



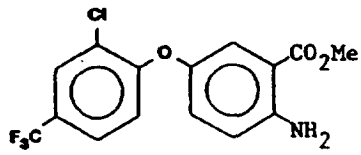
Common Name: Acifluorfen methyl ester
Chemical Name: Methyl 5-[2-chloro-4-(trifluoromethyl-phenoxy] -2-nitro-benzoate
Code Name: BH 9048-ME
Molecular Weight: 375.69



Common Name: Acifluorfen amine
Chemical Name: 2-amino-5-[2-chloro-4-(trifluoromethyl)phenoxy] benzoic acid
Code Name: BH 9048-A
Molecular Weight: 331.68



Common Name: Acifluorfen amine methyl ester
Chemical Name: Methyl 2-amino-5-[2-chloro-4(trifluoromethyl) phenoxy]benzoate
Code Name: BH 9048-AME
Molecular Weight: 345.71



1.3. Principle of the Method

The sample is soaked in a basic aqueous solution for one hour and then extracted with 1% acetic acid in acetonitrile. For gas chromatographic (GC) analysis, an aliquot is washed with heptane and acidic water and then methylated. The sample is purified further with a silica gel SPE column and, for final analysis, acifluorfen methyl ester is detected by GC with electron capture detection. For high performance liquid chromatographic (LC) analysis, an aliquot may be purified with an optional C_{18} SPE column, diluted for final analysis, and acifluorfen amine and acifluorfen amine methyl ester are detected by HPLC with fluorescence detection. The Limit of Quantitation (LOQ) for each analyte is 0.02 mg/kg.

2. MATERIALS AND METHODS

2.1 Equipment-Suggested Sizes/Manufacturer

Graduated Cylinder	500 mL
Buchner funnel	11 cm diameter
Bottles, Low density polyethylene	4 oz.
Filter Paper	Whatman No. 3, 11 cm i.d.
Phase Separation Filter Paper	Whatman 1PS, 15 cm i.d.
Vacuum Filtration Adapter, (Glass), 24/40	Kontes or equivalent
Flat Bottom Flask, 24/40	300 mL, 500 mL, 1 L
Rotary Evaporator	Buchi or equivalent
Temperature Bath	Buchi or equivalent
Separatory Funnel	125 mL, 500 mL
Glass Wool	
Ultrasonic Bath	Branson 1200 or equivalent
Pyrex centrifuge tube w/screw cap	15 mL, 50 mL
Autosampler Vials	Varian 12 x 32 mm
Vial Caps	Varian
Volumetric Flask	500 mL
Nitrogen Stream Evaporator	N-EVAP Organomation Associates Inc., or equivalent
Volumetric Pipette	1-10 mL, 100 mL
Blender and Blender Jar (1 qt.)	Waring
Balance (with at least tenth of a gram capability)	Mettler (or equivalent)
Spatula or small scoop	
Delivery Head	100 mL, 75 mL (Markson)
Vortex Mixer	Fisher Scientific or equivalent
Pasteur Pipets	23 cm long, disposable
Disposable Solid Phase Extraction Columns C_{18} and silica gel	J.T. Baker, 3 mL size 500 mg of packing
45 µm Uniprep Syringeless Filter	Genex
SPE Vacuum Collection assembly	Supelco or equivalent

Other general laboratory glassware and equipment as needed.

2.2. Reagents and Chemicals - Suggested Source/Preparation

Reagents and Chemicals	Source/Preparation
Acetone	Distilled, high purity (Burdick and Jackson)
Hydrochloric acid, conc.	Reagent grade
Celite filter aid Type 545,	Fisher or equivalent
Dichloromethane	Distilled, high purity (Burdick and Jackson)
Triethylamine (99%+ pure)	Aldrich Chemical Company
HPLC grade or "Ultra pure" water (18 Megohm-cm resistivity)	Millipore water purification system, or Fluka, Cat. No. 95305
Acetonitrile	Distilled, high purity (Burdick and Jackson)
Toluene	Distilled, high purity (Burdick and Jackson)
Acetic acid, glacial, reagent ACS	Fisher Scientific
(Trimethylsilyl) Diazomethane 2.0 M	Aldrich Chemical Company
Sodium Hydroxide (pellets)	Aldrich Chemical Company

2.2.1 Standard Substances

The lot numbers indicated were those used to generate the validation data. Other lots of at least 95% purity may be used. These substances were used as both test and reference substances during this study.

Abbreviation	Chemical Name	Lot Number	Purity
BAS 9048 H (acid form)	5-[2-chloro-4-(trifluoro-methyl)phenoxy]-2-nitro-benzoic acid	CH39/141-1	99.5%
BH 9048-ME	Methyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitro-benzoate	L47-287	99.1%
BH 9048-A	2-amino-5-[2-chloro-4-(trifluoromethyl)phenoxy] benzoic acid	41-160	98.9%
BH 9048-AME	Methyl 2-amino-5-[2-chloro--4-(trifluoromethyl)phenoxy] benzoate	CAN 429	97.3%

Store standards in a freezer. Store standard solutions in a refrigerator.

2.2.1 Standard Substances (continued)

Reference substances were maintained frozen (<-5°C) until their use in this study. The reference substances were characterized as required by 40 CFR part 160 FIFRA Good Laboratory Practices. Information on the synthesis and subsequent characterization of these substances is available to BASF and is located either at Landwirtschaftliche Versuchsstation der BASF, Limburgerhof, Germany or at BASF Corporation, Agricultural Research Center.

Reference substance solutions were refrigerated during their use in this study. For all compounds, stock solutions (1mg/mL) were made fresh every three months. For all compounds except acifluorfen amine, dilutions of the stock solution were made monthly. For acifluorfen amine, all solutions other than the stock solution were made daily. From all indications, these time frames for maintaining analytical standard solutions are appropriate. Instrument response and compound recovery through the analytical method remain constant within experimental error regardless of solution age within the above mentioned time frames.

2.2.2 Standard Solutions for Fortification

Low density polyethylene bottles should be used as storage containers with solutions of BH 9048-A. For other compounds, typical storage bottles such as amber bottles may be used. Any BH 9048-A solution with a concentration of 1 mg/mL or greater can be stored for a maximum of three months. Any BH 9048-A solution concentration between 1 µg/mL and 1 mg/mL can be stored for a maximum of one month. Any BH 9048-A solution concentration below 1 µg/mL should not be stored any longer than 48 hours. It is recommended for the other compounds that 1 mg/mL solutions or greater be stored for a maximum of three months and all other more dilute solutions be stored for a maximum of one month.

For BAS 9048 H, BH 9048-ME, BH 9048-A and BH 9048-AME solutions, the recommended concentrations are: 1000, 10, 4 and 0.4 µg/mL in acetonitrile.

Prepare a 1.0 mg/mL stock solution by weighing an appropriate amount into a volumetric flask. Dissolve with acetonitrile and dilute to the mark. For example, to prepare a 25 mL stock solution, dissolve 25.0 mg of the appropriate compound in a 25 mL volumetric flask.

Prepare a 10.0 µg/mL standard solution by transferring an appropriate amount of the 1.0 mg/mL stock solution with a volumetric pipet to a volumetric flask (typically 1 mL of the 1.0 mg/mL stock solution into a 100 mL volumetric flask). Dilute to the mark with acetonitrile.

Prepare 4.0 µg/mL and 0.4 µg/mL standard solutions by making sequential serial dilutions of the 10 µg/mL standard solution. Other concentrations may be used as appropriate.

2.2.3 Standard Solutions for GC Analysis

For the BH 9048-ME solutions, the recommended concentrations are: 1000, 10.0, 1.0, 0.03, 0.02, 0.01 and 0.005 µg/mL.

Prepare a 1.0 mg/mL BH 9048-ME stock solution by weighing an appropriate amount of BAS 9048 H into a volumetric flask. Dissolve with toluene and dilute to the mark. For example, to prepare a 25 mL stock solution, dissolve 25.0 mg of BAS 9048 H in a 25 mL volumetric flask.

Prepare a 10.0 µg/mL BH 9048-ME standard solution by transferring an appropriate amount of the 1.0 mg/mL stock solution with a volumetric pipet to a volumetric flask (typically 1 mL of the 1.0 mg/mL stock solution in a 100 mL volumetric flask). Dilute to the mark with toluene.

Prepare 1.0 µg/mL, 0.03 µg/mL, 0.02 µg/mL, 0.01 µg/mL and 0.005 µg/mL standard solutions by making sequential serial dilutions of the 10.0 µg/mL standard solution. Other concentrations may be used as appropriate.

2.2.4 Standard Solutions for HPLC Analysis

For BH 9048-A and BH 9048-AME solutions, the recommended concentrations are: 1000, 10 µg/mL in acetonitrile (ACN); 100, 2.0, 1.0 and 0.5 ng/mL in 85% (v/v) (1% acetic acid (HoAc) in ACN:water).

Prepare a 1.0 mg/mL stock solution by weighing an appropriate amount into a volumetric flask. Dissolve with acetonitrile and dilute to the mark. For example, to prepare a 25 mL stock solution, dissolve 25.0 mg of the appropriate compound in a 25 mL volumetric flask.

Prepare a 10.0 µg/mL stock solution by weighing an appropriate amount of the 1.0 µg/mL stock solution with a volumetric pipet to a 100 mL volumetric flask (typically 1 mL of the 1.0 mg/mL stock solution into a 100 mL volumetric flask). Dilute to the mark with acetonitrile.

Prepare 100 ng/mL, 2.0 ng/mL, 1.0 ng/mL and 0.5 ng/mL standard solution by making sequential serial dilutions of the 10.0 µg/mL standard solution and diluting with 85% (1% HoAc in ACN) in water (v/v). Other concentration may be used as appropriate.

2.3. Analytical Procedure

2.3.1 Preparation of Sample

Pulverize-homogenize samples of the crop by mechanical means (i.e. a blender or mill). Dry samples may be pulverized directly. For wet samples, the samples are best frozen with dry ice or liquid nitrogen while pulverizing. If dry ice is used, allow the dry ice to sublime before continuing with the method.

2.3.2 Extraction

a. Weigh 20.0 g of the sample into a one quart blender jar.

b. Add 75 mL of 0.1N NaOH and let sample soak for one hour.

c. Add 150 mL of 1% acetic acid in acetonitrile to the sample and blend for 5 minutes.

d. Vacuum filter the slurry through a Buchner funnel containing one sheet of Whatman Number 3 filter paper and approximately a 1-2 cm layer of celite and into a flat bottom flask attached with a vacuum adapter.

e. Rinse the marc with 3 x 50 mL of 1% acetic acid in acetonitrile; discard the marc.

f. Quantitatively transfer the filtrate to a 500 mL volumetric flask and dilute to the mark with 1% acetic acid in acetonitrile.

2.3.3 Preparation for Final Determination by GC

a. Heptane wash

1. Take a 100 mL aliquot from the 500 mL volumetric flask from step 2.3.2.f, wash with 1 x 100 mL of heptane in a separatory funnel, and collect the bottom acetonitrile layer. Save the heptane layer for the next step.

2. Extract the remaining heptane with an additional 1 x 50 mL of acetonitrile, and combine the acetonitrile layer with the previous in a flat-bottom flask.

3. Concentrate the acetonitrile solution from the heptane wash step to approximately 10 mL using a rotary evaporator at 45±5°C.

b. Dichloromethane Partition

1. Add 100 mL of dichloromethane (DCM) to the flask from 2.3.3.a.3 and quantitatively transfer the solution to a separatory funnel with DCM.

2. Wash the DCM layer with 1 x 100 mL of 1N HCl.

3. Pass the DCM layer through Whatman 1PS filter paper into a flat- bottom flask.

4. Concentrate the solution to approximately 10 mL using a rotary evaporator at 45±5°C and quantitatively transfer the residue to a 50 mL centrifuge tube with the aid of several acetone rinses.

5. Concentrate the sample to approximately but not greater than 1.0 mL with a flow of nitrogen.

c. Methylation by Trimethylsilyl(Diazomethane)

1. Add 1 mL of acetone to the residue remaining in the centrifuge tube, vortex and sonicate.

2. Add 10 mL of 0.04 M Trimethylsilyl(Diazomethane) solution in hexane, vortex, sonicate and let stand for at least 30 minutes.

2.3.3 Preparation for Final Determination by GC (continued)

d. Silica Gel Column Chromatography

1. Evaporate the methylated solution to approximately 1.0 mL with a flow of nitrogen, but not to dryness.

2. Add approximately 2.0 mL of hexane (total volume equal to 3.0 mL) and 1.0 mL of 1% triethylamine in DCM, vortex and sonicate.

3. Prewash a 3 mL silica gel SPE (Baker) column with 10 mL of DCM-hexane-triethylamine (30:69.8:0.2 v/v/v).

Note: Do not allow the column to reach dryness at any time in the cleanup except after the final eluate has been collected.

4. The sample is applied to the column and the eluate collected into a 15-50 mL centrifuge tube under vacuum with a flow rate of 1-3 mL/min.

5. The sample tube is rinsed with 5 mL of DCM-hexane-triethylamine (30:69.8:0.2, v/v/v) and this is applied to the column. Allow the column to run dry and combine the eluate with the eluate from the previous step.

6. Concentrate the sample to approximately 4.0 mL with a flow of nitrogen (N-EVAP or equivalent).

7. Add 8 mL of toluene to the centrifuge tube and concentrate the sample to approximately 4 mL with a flow of nitrogen (N-EVAP or equivalent).

8. Dilute the sample to the appropriate volume with toluene for GC analysis.

2.3.4 Preparation for Final Determination by HPLC

a. C18 Column Chromatography (optional)

Samples may be clean enough to inject without this cleanup, if so, proceed to 2.3.4.b.

1. Condition a 3 mL (500 mg) C18 SPE column with 10 mL of methanol and then 5 mL of acetonitrile. Do not allow the column to go to dryness.

2. Apply 10 mL of the sample extract from the 500 mL volumetric flask in step 2.3.2.e. to the column and allow the column to run dry under vacuum with a flow rate of 1-3 mL/min.

3. Collect the eluate in a graduated 15-50 mL centrifuge tube. Proceed to step 2.3.4.c.

2.3.4 Preparation for Final Determination by HPLC (continued)

b. Take a 1 to 2 mL aliquot from the 500 mL volumetric flask in step 2.3.2.e and pass through a 0.45 μm filter disc.

c. Do not concentrate, but dilute, if necessary, with 85% (1% acetic acid in acetonitrile) in water (v/v) to the appropriate volume for analysis by LC. Filter with a 0.45 μm microfilter if necessary.

2.4 GC Instrumentation

Different equipment and parameters than those listed below may be substituted into the method as long as interpretable chromatography results.

2.4.1 Description of Equipment

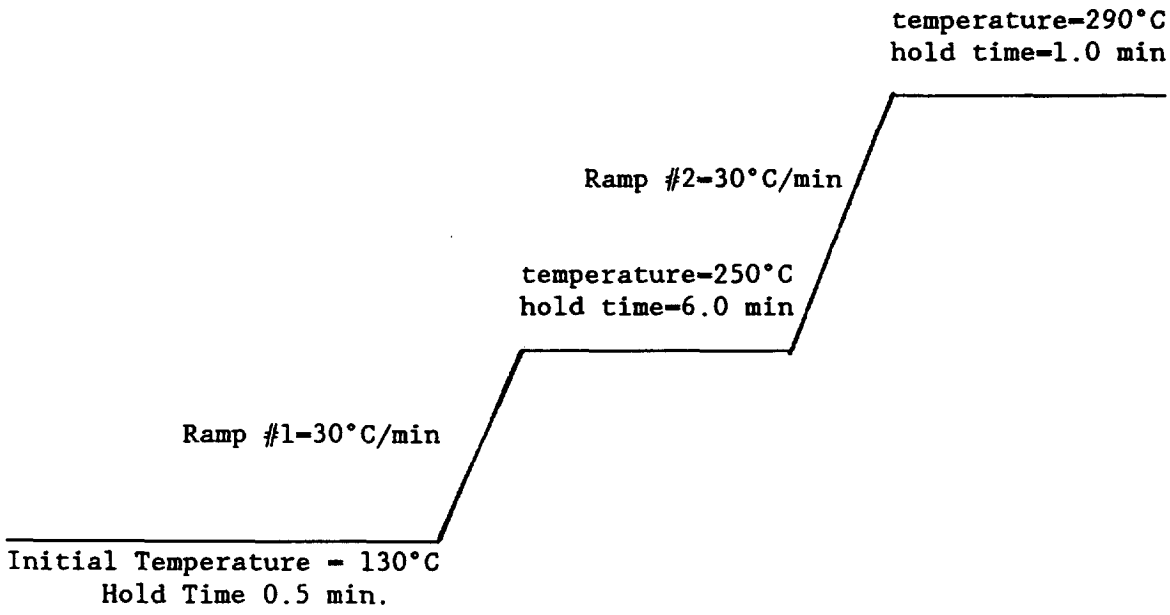
Gas Chromatography: Varian 3600 with Electron Capture Detector

Column: J & W Scientific DB-5 30 m x .32 mm i.d. x 1.0 μm film thickness or equivalent. The serial number of the GC column used to generate the validation data was 2548537A.

2.4.2 Typical Operating Conditions

Column Parameters

Column Head Pressure: 16.5 psig nitrogen
Column Temperature:



2.4.2 Typical Operating Conditions (continued)

Injector Parameters

Injector Temperature:	250°C
Septum Purge Flow:	2.0-3.0 mL/min
Splitless Injection:	0.5 min (purge on=0.5 min, purge off=0.0 min)

Detector Parameters

Detector Temperature:	300°C
Carrier Gas:	Helium @ 2.0-3.0 mL/min
Make Up Gas:	Nitrogen @ 30 mL/min
Range:	10
Attenuation:	32

Retention Time

BH 9048-ME = 10.4 min

2.4.3 System Conditioning

The peak height of acifluorfen methyl ester can vary depending on the conditions in the GC system. Using a new column or using a column that has been idle for a day or more can result in a very weak response for acifluorfen methyl ester. The result is that the response will increase during a run and produce unacceptable correlation coefficients for standard data.

The sensitivity of the column can be improved by saturating the column with a matrix extract. An extract of untreated soybean grain, which has been diluted for gas chromatography analysis at a low volume (i.e. 4.0 mL), has been shown to be an effective column conditioner. Any other matrix solution that has the same beneficial effect may be used.

The conditioner is alternately injected with standard solution until a stable response is achieved.

2.4.4 Calibration Procedure

Calculation of results is based on peak height measurements using a calibration curve. A standard curve is constructed for acifluorfen methyl ester. To obtain this standard curve, inject, for example 5, 10, 20 and 30 pg of acifluorfen methyl ester into the gas chromatograph. Plot peak height (signal counts) versus amount (pg) of standard injected.

2.4.5 Sample Analysis by GC

Inject 1.0 µL of each sample and 1.0 µL of each acifluorfen methyl ester standard into the gas chromatograph for analysis. For each set of samples, it is recommended that each standard be injected at least in duplicate, and that the sample injections are bracketed with standard injections. If necessary, acetone rinse injections can be used after each sample injection to maintain column stability and minimize sample carry over.

2.5 GC Interferences

2.5.1 Sample Matrices

If interfering peaks from the matrix occur in the chromatogram, alter the GC operating conditions. Other types of GC columns may be used.

2.5.2 Other Sources

Other Pesticides:	None have been observed to interfere to date. Lactofen and its metabolites are expected to interfere because of compound similarities.
Solvents:	None observed to date
Lab Ware:	None observed to date

2.6 Potential Problems

During the extraction, methylation or cleanup, a sample should not be allowed to go to dryness, as the compound of interest will adhere to the glassware. This is especially notable with acifluorfen methyl ester.

2.7 HPLC Instrumentation

Different equipment and parameters than those listed below may be substituted into the method as long as interpretable chromatography results.

2.7.1 Description of Equipment

Liquid Chromatograph	LDC Analytical Multiple Solvent System CM 4000
Autosampler	LDC Analytical Autosampler 713
Fluorescence Detector	Perkin Elmer LC 240
Column	250 mm x 4.6 mm, Phenomenex
Column Temperature	ambient
Stationary Phase	Nucleosil 5 C_{18}

The serial number of the HPLC column used to generate the validation data was 50203.

2.7.2 Mobile Phase Preparation

Mobile Phase: 75% Acetonitrile/25% (2.5% acetic acid in Millipore® water)

Prepare the mobile phase in a 1 L flask. Add 750 mL to the flask using a graduated cylinder. Then add 250 mL of 2.5% (v/v) acetic acid in Millipore® water.

For degassing purposes, the uncapped 1 L flask should be sonicated for 30 minutes. The alternative is to degas the mobile phase for 30 minutes using a slight stream of helium.

2.7.3 Typical Operating Conditions

Injection Volume:	100 µL	
Excitation Wavelength:	350 nm	
Emission Wavelength:	420 nm	
Flow Rate:	1 mL/min	
Retention Time:	BH 9048-A	5.5 min
	BH 9048-AME	10.7 mins
Run Time:	15.0 min	

2.7.4 System Conditioning

This HPLC system for analysis of BH 9048-A and BH 9048-AME is very stable. If initial response does not seem stable, several standards should be injected to stabilize the system or injections made until a stable response is achieved.

2.7.5 Calibration Procedures

As with gas chromatography, a calculation of results is based on peak height measurements using a calibration curve. One standard curve is constructed separately for acifluorfen amine and acifluorfen amine methyl ester. To obtain these standard curves, inject, for example a 50, 100 and 200 pg combined solution of acifluorfen amine and acifluorfen amine methyl ester into the HPLC. Plot peak heights (signal counts) versus amount (pg) of injected standard.

2.8 Sample Analysis by LC

Inject 100 µL of each sample and 100 µL of each acifluorfen amine/acifluorfen amine methyl ester standard into the liquid chromatograph for analysis. For each set of samples, it is recommended that each standard be injected at least in duplicate and that the sample injections are bracketed with standard injections. Also, one or more Millipore® water vials may be placed at the end of each set to clean the injection needle and prevent clogging from the matrix.

2.9 HPLC Interferences

2.9.1 Sample Matrices

If interfering peaks occur in the chromatogram, the HPLC conditions, may be altered.

2.9.2 Other Sources

Other Pesticides	None have been observed to interfere to date. Lactofen and its metabolites are expected to interfere because of compound similarities.
Solvents	None observed to date
Labware	None observed to date

2.10 Potential Problems

After extraction, the sample should never be concentrated, as the acifluorfen amine compound will adhere to the glassware.

2.11 Time Required for Analysis

For a set of 7 treated samples, 2 fortifications and 1 control, approximately 20 man hours, including the final determination by both GC and HPLC and data reduction, are required provided that no special problems arise.

2.12 Confirmatory Techniques

The final analyte for GC analysis, acifluorfen methyl ester, may be confirmed by GC-MS. The final analytes for LC analysis, acifluorfen amine and acifluorfen amine methyl ester, may be confirmed by LC-MS. If the residue determined by GC analysis must be distinguished between acifluorfen or acifluorfen methyl ester, the analysis can be repeated without the use of the methylating reagent.

3 METHODS OF CALCULATION

3.1 Calibration

Measure the peak heights of the standards. Construct a linear least squares working curve in the form $y = ax + b$ from the standards by plotting peak height versus amount of standard injected.

3.2 Analytes in Sample

3.2.1 Principle

Calculation of results is based on peak height measurements. The amount of acifluorfen methyl ester, acifluorfen amine and acifluorfen amine methyl ester in injected samples is determined from the calibration curve, and the equation described in 3.2.2 is utilized for the determination of residues (R). Calculation can also be made by a suitable computer program.

3.2. Analytes in Sample (continued)

At least one fortification and one untreated sample (= control) are run with each set of samples. The quantity of spiked standard for the fortification level should approximate the expected residue. The recovery is determined from the fortification experiments (see 3.2.3).

3.2.2 Calculation of Residues

R = Total Residue (ppm equivalents of BAS 9048 H, salt form)
A = ppm equivalent of acifluorfen methyl ester (GC)
B = ppm equivalent of acifluorfen amine (LC)
C = ppm equivalent of acifluorfen amine methyl ester (LC)

$$A = \frac{\text{Acifluorfen methyl ester found } (\mu g)}{\text{Sample Weight (g) x Aliquot}} \times \frac{\text{Final Volume (mL)}}{\text{Injection Volume (mL)}} \times MWCF_A$$

$$B = \frac{\text{Acifluorfen amine found } (\mu g)}{\text{Sample Weight (g) x Aliquot}} \times \frac{\text{Final Volume (mL)}}{\text{Injection Volume (mL)}} \times MWCF_B$$

$$C = \frac{\text{Acifluorfen amine methyl ester found } (\mu g)}{\text{Sample Weight (g) x Aliquot}} \times \frac{\text{Final Volume (mL)}}{\text{Injection Volume (mL)}} \times MWCF_C$$

MWCF = Molecular Weight Correction Factor

$$MWCF_A = \frac{\text{MW BAS 9048 H (=384)}}{MW_{ME} \ (=376)} = 1.02$$

$$MWCF_B = \frac{\text{MW BAS 9048 H (=384)}}{MW_{NH2} \ (=332)} = 1.16$$

$$MWCF_C = \frac{\text{MW BAS 9048 H (=384)}}{MW_{NH2\text{-}ME} \ (=346)} = 1.11$$

R = A + B + C

The molecular weight correction factor calculates the residues based on sodium acifluorfen equivalents.

3.2.3 Calculation of Recoveries

The fortification recovery for acifluorfen and acifluorfen methyl ester in % is calculated as follows:

$$\text{Recovery (\%)} = \left| \frac{W_F \times V_{EF}}{V_{IF}} - \frac{W_C \times V_{EC}}{V_{IC}} \right| \times \frac{MWCF}{A} \times \frac{100\%}{F_1 (\text{or } F_2)}$$

W_F (μg acifluorfen methyl ester found in fortified sample)
V_{EF} (Final volume of fortified sample)
V_{IF} (Injection volume of fortified sample)
W_C (μg of acifluorfen methyl ester found in control sample)
V_{EC} (Final volume of control sample)
V_{IC} (Injection volume of control sample)
MWCF (Molecular Weight Correction Factor for acifluorfen methyl ester to acifluorfen)
F_1 (μg of acifluorfen fortified)
F_2 (μg of acifluorfen methyl ester fortified)
A (aliquot)

The fortification recovery for acifluorfen amine and acifluorfen amine methyl ester in % is calculated from the recovery trials as follows:

$$\text{Recovery (\%)} = \left| \frac{W_{F1}(\text{or } W_{F2}) \times V_{EF}}{V_{IF}} - \frac{W_{C1}(W_{C2}) \times V_{EC}}{V_{IC}} \right| \times \frac{1}{A} \times \frac{100\%}{F_1 (\text{or } F_2)}$$

W_{F1} (μg acifluorfen amine found in fortified sample)
W_{F2} (μg acifluorfen amine methyl ester found in fortified sample)
V_{EF} (Final volume of fortified sample)
V_{IF} (Injection volume of fortified sample)
W_{C1} (μg of acifluorfen amine found in control sample)
W_{C2} (μg of acifluorfen amine methyl ester found in control sample)
V_{EC} (Final volume of control sample)
V_{IC} (Injection volume of control sample)
F_1 (μg of acifluorfen amine fortified)
F_2 (μg of acifluorfen amine methyl ester fortified)
A (aliquot)

4 RESULTS AND DISCUSSION

4.1 Accuracy and Precision of Validation Results

Subsamples of control soybean grain were fortified at levels of 0.02 and 0.2 ppm with acifluorfen, acifluorfen methyl ester, acifluorfen amine, and acifluorfen amine methyl ester, and were analyzed by Method D9205. A summary of the results is given in Table I and the individual results are given in Table II.

Quantitation of all samples was achieved using calibration curves calculated by linear regression of standard data of multiple levels. The standard data for each analysis set are summarized in Table III.

4 RESULTS AND DISCUSSION (continued)

4.2 Quantitation Limit

The total quantitation limit for sodium acifluorfen residues in soybean grain using Method D9205 is 0.10 ppm. This is the sum of the individual quantitation limits of 0.02 ppm for acifluorfen (salt and acid forms), acifluorfen methyl ester, acifluorfen amine, and acifluorfen amine methyl ester. At this level, control samples are relatively clean and good recoveries are obtainable. This is the lowest level which is proven by recovery data.

4.3 Ruggedness Testing

The method has been used successfully to analyze treated samples from a soybean magnitude of the residue study (Reference 2).

4.4 Limitations

None known to date.

5. CONCLUSIONS

This analytical procedure is applicable for measuring residues of acifluorfen in soybean grain down to a level of 0.02 ppm for each compound in the tolerance expression.

Statistical treatment of the validation data included determination of an average and standard deviation. Generally, good recoveries were obtained for all compounds fortified into soybean grain at the 0.02 and 0.2 ppm levels.

The raw data and final method pertaining to this study are maintained in the BASF Corporation Agricultural Research Center Archives.

6. PROTOCOL CHANGES

During the course of the study, the draft analytical method included in the protocol was modified several times. First, the method was amended to remove concentration of the sample prior to HPLC analysis as an option and to add the details for preparation of an HPLC standard at the 0.5 ng/µL level. Second, the method was modified to include an initial basic aqueous soaking period prior to the extraction. Also, the solvent for the HPLC standards and final dilution of samples was changed to 85% (1% acetic acid in acetonitrile) in water. These modifications were incorporated into the final method D9205.

7. REFERENCES

1. Norris F. Rhone-Poulenc Agrochemical Method No. 160. "Enforcement Method for the Determination of Residues of Acifluorfen and Metabolites in/on Plant Tissues. July 1982. Accession Number 71307.

2. Burkey, J. Magnitude of the Residue of Sodium Acifluorfen and Its Metabolites in Soybean Grain Raw Agricultural Commodity Samples. Report Number A9314. June 1993. Submitted to the EPA simultaneously with this method.

8. SIGNATURES

We, the undersigned, hereby declare that this study was performed under our supervision according to the procedures described herein, and that this progress report provides a true and accurate record of the results obtained.

Author: Steven F. Klaus Date: 6-18-93

Study Director: Jeffrey D. Burkey Date: June 18, 1993
Jeffrey D. Burkey
Agricultural Research Chemist

Approved By: Robert C. Paulick Date: June 17, 1993
Robert C. Paulick, Ph.D.
Group Leader, Analytical

Issued By: Nancy Cargile Date: June 18, 1993
Nancy Cargile, Ph.D.
Manager, Chemistry Section

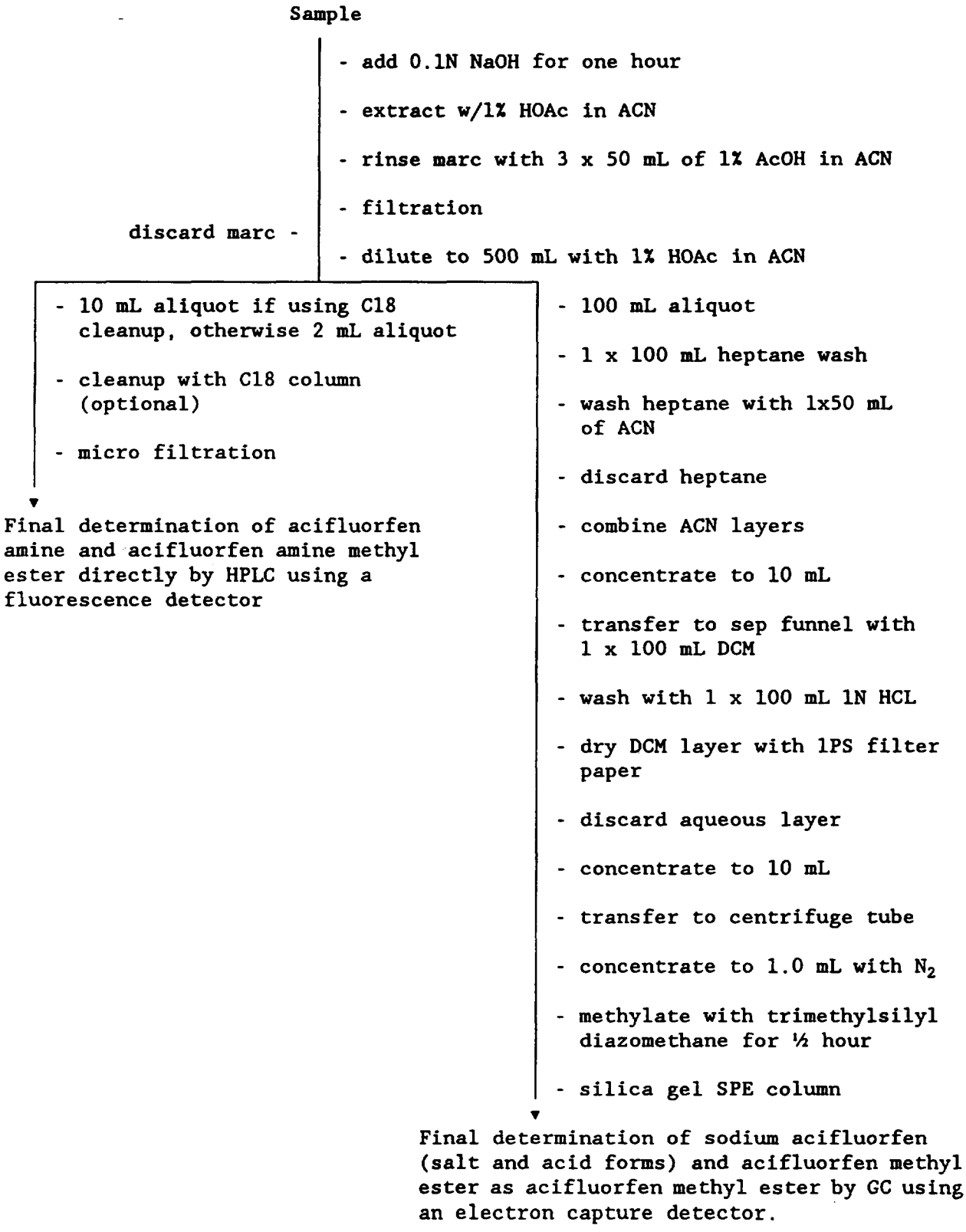


Figure 1. Flow Chart of Analytical Procedure

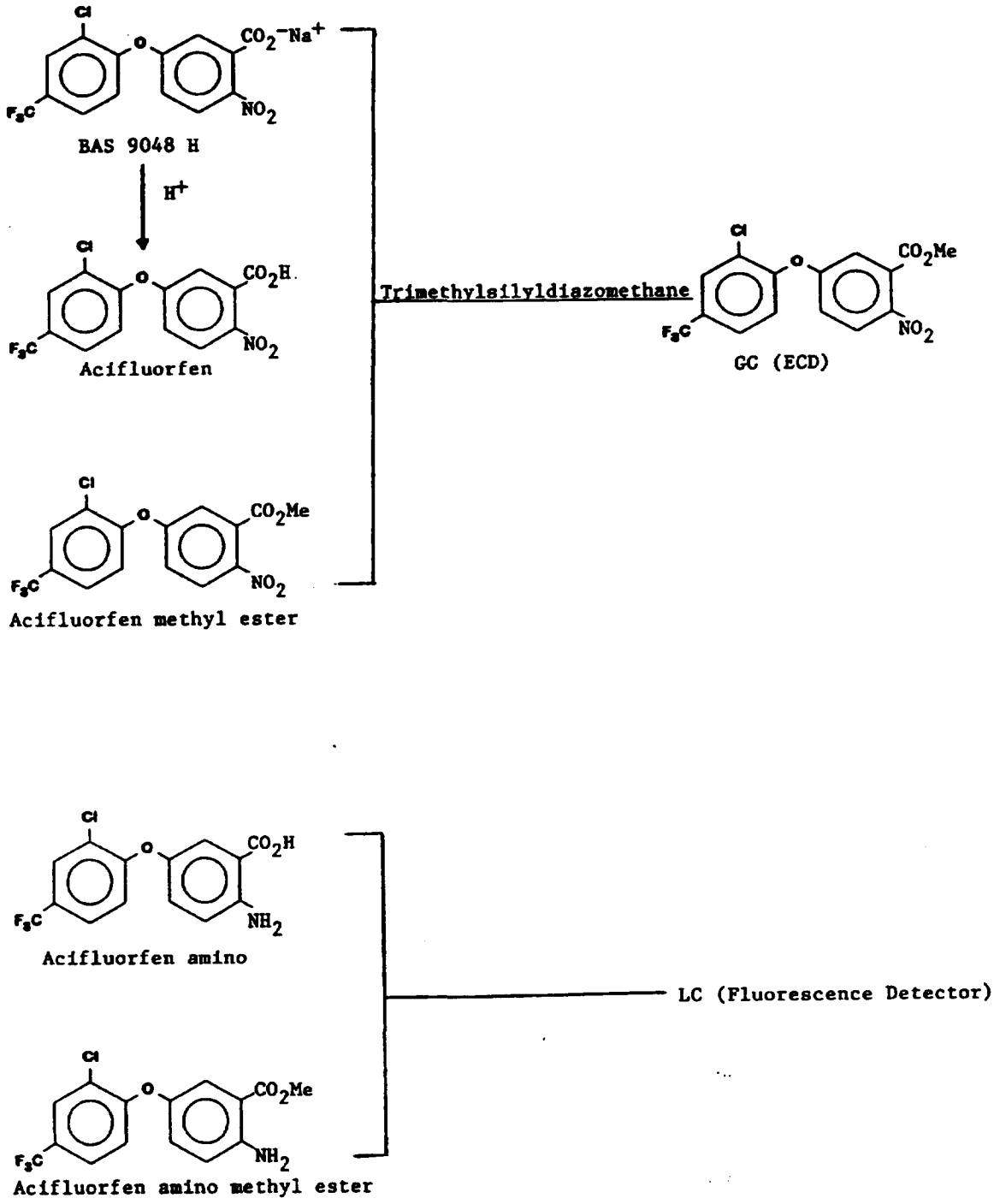


Figure 2. Structures of Detectable Compounds and Final Analytes of the Analytical Method.

Values below are typical for a field treated residue sample.

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acifluorfen methyl ester found = <5 pg (<5 x 10^{-6} μg)
acifluorfen amine found = <50 pg (<5 x 10^{-5} μg)
acifluorfen amine methyl ester found = <50 pg (<5 x 10^{-5} μg)
Injection Volume (GC) = 1 x 10^{-3} mL
Injection Volume (LC) = 0.1 mL
Sample weight = 20.0 g $MWCF_A$ = 1.02
Final volume (GC) = 4 mL $MWCF_B$ = 1.16
Final volume (LC) = 10 mL $MWCF_C$ = 1.11
Aliquot (GC) = 0.2
Aliquot (LC) = 0.02

R = A + B + C

A = ppm equivalent of acifluorfen methyl ester (GC)
B = ppm equivalent of acifluorfen amine (LC)
C = ppm equivalent of acifluorfen amine methyl ester (LC)

$$R = \left|\frac{\leq 5 \times 10^{-6}\ \mu g}{20.0\ g \times 0.2} \times \frac{4\ mL}{1 \times 10^{-3}\ mL} \times 1.02\right| + \left|\frac{\leq 5 \times 10^{-5}\ \mu g}{20.0\ g \times 0.02} \times \frac{10\ mL}{0.1\ mL} \times 1.16\right| +$$

$$\left|\frac{\leq 5 \times 10^{-5}\ \mu g}{20.0\ g \times 0.02} \times \frac{10\ mL}{0.1\ mL} \times 1.11\right|$$

R = <0.005 + <0.02 + <0.02 (All residues were <u>less than</u> the lowest standard on the standard curve.)

These results were calculated by assuming a residue <u>equal</u> to the lowest standard.

If the results were based on the limit of quantitation for each compound, then the total residue would be expressed as <0.10 ppm of BAS 9048 H equivalents (0.06 + <0.02 + <0.02 for A, B and C, respectively). The quantitation limit for A is the sum of the limits for sodium acifluorfen, acifluorfen, and acifluorfen methyl ester.

Figure 3. Typical Residue Calculation

a) Lab code: 108474, Control Soybean Grain + 0.02 ppm acifluorfen (acid form)
b) Lab code: 108476, Control Soybean Grain + 0.02 ppm acifluorfen methyl ester

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$$\text{Recovery (\%)} = \left[\frac{W_F \times V_{EF}}{V_{IF}} - \frac{W_C \times V_{EC}}{V_{IC}} \right] \times \frac{MWCF}{A} \times \frac{100\%}{F1 \text{ (or F2)}}$$

W_F (μg acifluorfen methyl ester found in fortified sample)
V_{EF} (Final volume of fortified sample)
V_{IF} (Injection volume of fortified sample)
W_C (μg of acifluorfen methyl ester found in control sample)
V_{EC} (Final volume of control sample)
V_{IC} (Injection volume of control sample)
MWCF (Molecular Weight Correction Factor for acifluorfen methyl ester to acifluorfen)
F_1 (μg of acifluorfen fortified)
F_2 (μg of acifluorfen methyl ester fortified)
A (aliquot)

a) Recovery of Acifluorfen (%) =

$$\left(\frac{18.5 \times 10^{-6} \ \mu g \times 4 \text{ mL}}{1 \times 10^{-3} \text{ mL}} - \text{No Quantifiable Residues} \right) \times \frac{0.963}{0.2} \times \frac{100\%}{0.4 \ \mu g} = 89\%$$

b) Recovery of Acifluorfen Methyl Ester (%) =

$$\left(\frac{17.8 \times 10^{-6} \ \mu g \times 4 \text{ mL}}{1 \times 10^{-3} \text{ mL}} - \text{No Quantifiable Residues} \right) \times \frac{1.0}{0.2} \times \frac{100\%}{0.4 \ \mu g} = 89\%$$

Figure 4. Typical Recovery Calculation from GC

a) Lab code: 108403, Control Soybean Grain + 0.02 ppm acifluorfen amine
b) Lab code: 108405, Control Soybean Grain + 0.02 ppm acifluorfen amine methyl ester

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$$\text{Recovery (\%)} = \left[\frac{W_{F1} \text{ (or } W_{F2}) \times V_{EF}}{V_{IF}} - \frac{W_{C1} \text{ (or } W_{C2}) \times V_{EC}}{V_{IC}} \right] \times \frac{100\%}{\text{F1 (or F2)}} \times \frac{1}{A}$$

W_{F1} (μg acifluorfen amine found in fortified sample)
W_{F2} (μg acifluorfen amine methyl ester found in fortified sample)
V_{EF} (Final volume of fortified sample)
V_{IF} (Injection volume of fortified sample)
W_{C1} (μg of acifluorfen amine found in control sample)
W_{C2} (μg of acifluorfen amine methyl ester found in control sample)
V_{EC} (Final volume of control sample)
V_{IC} (Injection volume of control sample)
F_1 (μg of acifluorfen amine fortified)
F_2 (μg of acifluorfen amine methyl ester fortified)
A (aliquot)

a) Recovery of Acifluorfen Amine (%) =

$$\left(\frac{62.8 \times 10^{-6} \ \mu g \times 10 \text{ mL}}{0.10 \text{ mL}} - \text{No Quantifiable Residues} \right) \times \frac{100\%}{0.4 \ \mu g} \times \frac{1}{0.02} = 79\%$$

b) Recovery Acifluorfen Amine Methyl Ester (%) =

$$\left(\frac{75.3 \times 10^{-6} \ \mu g \times 10 \text{ mL}}{0.10 \text{ mL}} - \text{No Quantifiable Residues} \right) \times \frac{100\%}{1.0 \ \mu g} \times \frac{1}{0.02} = 94\%$$

Figure 5. Typical Recovery Calculation from LC

TABLE I. Summary of Recovery Experiments

BAS 9048 H (acid form)

Fortification Level (ppm)	Average Recovery %	Standard Deviation ±%	Number of Analyses
0.02	69	6	4
0.20	79	7	4
Overall	74	8	8

BH 9048-ME

Fortification Level (ppm)	Average Recovery %	Standard Deviation ±%	Number of Analyses
0.02	83	8	4
0.20	89	5	4
Overall	86	7	8

BH 9048-A

Fortification Level (ppm)	Average Recovery %	Standard Deviation ±%	Number of Analyses
0.02	71	6	4
0.20	82	7	4
Overall	76	8	8

BH 9048-AME

Fortification Level (ppm)	Average Recovery %	Standard Deviation ±%	Number of Analyses
0.02	81	14	4
0.20	80	12	4
Overall	81	12	8

TABLE II.- Individual Recovery Data

Fortification Level (ppm)[1]	Lab Sample Code	Master Sheet Number (92161-)[2]	Peak Height (μV)[3]	Analyte Found (pg)[4]	Final Volume (mL)	Sample Weight Injected (mg)[5]	Net Residue (ppm)[6,7]	Recovery (%)[8]
Acifluorfen								
0.00	108192	7	ND	<5	4	1.0	<0.02	-
0.00	108467	8	ND	<5	4	1.0	<0.02	-
0.02	108194	7	10066	12.9	4	1.0	0.012	62
0.02	108195	7	12247	15.8	4	1.0	0.015	76
0.02	108463	8	9179	14.2	4	1.0	0.014	68
0.02	108470	8	9585	14.8	4	1.0	0.014	71
0.20	108199	7	11750	15.1	40	0.1	0.146	73
0.20	108200	7	12214	15.7	40	0.1	0.152	76
0.20	108473	8	10445	16.2	40	0.1	0.156	78
0.20	108474	8	11931	18.5	40	0.1	0.178	89
Acifluorfen Methyl Ester								
0.00	108192	7	ND	<5	4	1.0	<0.02	-
0.00	108467	8	ND	<5	4	1.0	<0.02	-
0.02	108196	7	13141	17.0	4	1.0	0.017	85
0.02	108198	7	11226	14.4	4	1.0	0.014	72
0.02	108471	8	10861	16.8	4	1.0	0.017	84
0.02	108472	8	11673	18.1	4	1.0	0.018	91
0.20	108201	7	14072	18.2	40	0.1	0.182	91
0.20	108202	7	12725	16.4	40	0.1	0.164	82
0.20	108475	8	12015	18.7	40	0.1	0.187	93
0.20	108476	8	11454	17.8	40	0.1	0.178	89
Acifluorfen Amine								
0.00	108175	7	ND	<50	10	4	<0.02	-
0.00	108400	8	ND	<50	10	4	<0.02	-
0.02	108177	7	18730	53.0	10	4	0.013	66
0.02	108178	7	20027	57.1	10	4	0.014	71
0.02	108402	8	19608	53.7	10	4	0.013	67
0.02	108403	8	22595	62.8	10	4	0.016	79
0.20	108181	7	40335	120.7	50	0.8	0.151	76
0.20	108182	7	41144	123.3	50	0.8	0.154	77
0.20	108406	8	47804	140.0	50	0.8	0.175	87
0.20	108407	8	47823	140.0	50	0.8	0.175	88
Acifluorfen Amine Methyl Ester								
0.00	108175	7	ND	<50	10	4	<0.02	-
0.00	108400	8	ND	<50	10	4	<0.02	-
0.02	108179	7	18181	52.8	10	4	0.013	66
0.02	108180	7	19909	58.3	10	4	0.015	73
0.02	108404	8	25454	74.4	10	4	0.019	93
0.02	108405	8	25740	75.3	10	4	0.019	94
0.20	108183	7	37716	115.0	50	0.8	0.144	72
0.20	108184	7	36470	111.1	50	0.8	0.139	69
0.20	108408	8	48632	149.0	50	0.8	0.186	93
0.20	108409	8	45862	140.0	50	0.8	0.175	88

TABLE II. Individual Recovery Data (continued)

FOOTNOTES

Residue sample number 92940-1 was used for all samples.

[1]Fortifications were added prior to extraction and were run concurrently with control samples.

[2]Samples for Master Sheet Number 92161-7 were extracted on 5-19-93, injected on the LC on 5-19-93 and injected on the GC on 5-21-93. Samples for Master Sheet Number 92161-8 were extracted on 6-2-93, injected on the LC on 6-3-93, and injected on the GC on 6-6-93.

[3]If no signal was detected by the computer, the value is listed as "ND".

[4]If no residue was detected, the value is listed as less than the level of the lowest standard.

[5]Sample Weight Injected (mg) = $\frac{\text{Sample Weight(g)}}{\text{Final Volume (mL)}}$ x Injection Volume (μL) x Aliquot

[6]Net Residue (ppm) = $\frac{\text{Analyte Found (pg)}}{\text{Sample Weight Injected (mg)}}$ x $\frac{\text{MWCF}}{1000}$

MWCF = 0.963 for acifluorfen and 1.0 for other compounds.

[7]Values for the control samples are listed as less than the quantitation limit.

[8]Recovery (%) = $\frac{\text{Net Residue (ppm) - Control Net Residue (ppm)}}{\text{Fortification Level (ppm)}}$ x 100%

- Sample size was 20.0 g.
- Injection Volume was 1.0 μL for GC analyses and 100 μL for LC analyses.
- Aliquots for the GC analyses were 0.2 and for the LC analyses were 0.02.

Values in this table have been rounded off for reporting purposes, but not for further calculations.

TABLE III: Summary of Standard Data

Master Sheet Number (92161-)	Analyte[1]	Signal (μV)[2]				Calibration Curve Data[3]	
		Level 1	Level 2	Level 3	Level 4	Slope	Intercept
7	A	3995 4223	7971 8150	15034 15362	24124 21943	0.1322E-02	-0.4121
7	B	17637 17792 17993	33286 33727 33958	65691 65651 65585	- - -	0.3134E-02	-5.677
7	C	17483 18334 16883	32154 32723 33103	65378 64020 63965	- - -	0.3188E-02	-5.209
8	A	3512 3610	5783 6608	12138 14309	18510 19395	0.1573E-02	-0.2425
8	B	18490 18400 17789	34643 34968 35426	67133 67985 66987	- - -	0.3057E-02	-6.214
8	C	17917 17373 17905	33841 33591 33573	63719 64789 64782	- - -	0.3216E-02	-7.518

[1]Analyte A = Acifluorfen Methyl Ester; Analyte B = Acifluorfen Amine; Analyte C = Acifluorfen Amine Methyl Ester. Residues for A are determined by GC and for B and C are determined by LC.

[2]For analyte A, levels 1, 2, 3 and 4 correspond to 5, 10, 20 and 30 pg, respectively. For analytes B and C, levels 1, 2 and 3 correspond to 50, 100 and 200 pg, respectively. No level 4 was used for analytes B and C.

[3]The formula for the calibration curve is:

pg Analyte Found = (slope x signal) + intercept

APPENDIX

Typical Raw Data For Analyses

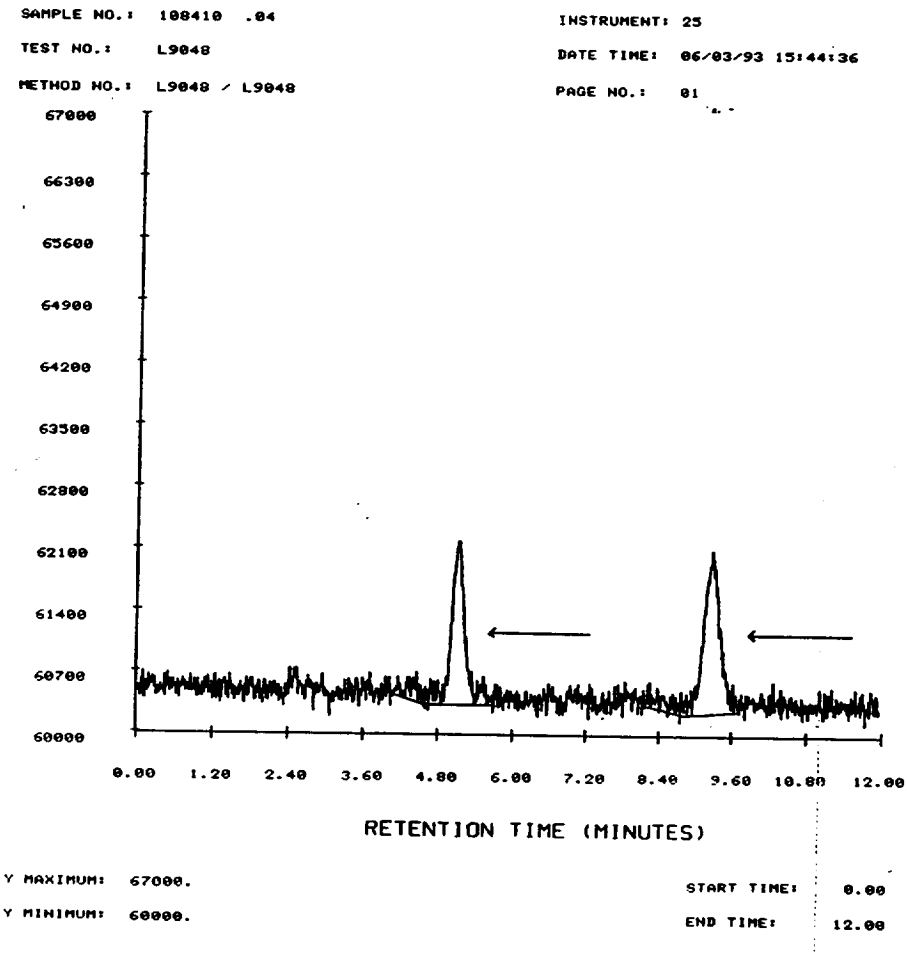
Figure	Description of Raw Data for LC Analysis
1	Typical chromatographic parameters for LC analyses.
2-4	Typical chromatographs of 50, 100 and 200 pg standards of BH 9048-A and BH 9048-AME.
5-6	Typical standard curves for 50, 100 and 200 pg amounts of BH 9048-A and BH 9048-AME.
7-8	Typical chromatograms of a control soybean grain sample.
9-12	Typical chromatograms of a control soybean grain sample fortified with 0.02 ppm and 0.20 ppm of BH 9048-A.
13-16	Typical chromatograms of a control soybean grain sample fortified with 0.02 ppm and 0.20 ppm of BH 9048-AME.
	Description of Raw Data for GC Analysis
17	Typical chromatographic parameters for GC analyses.
18-21	Typical chromatograms of 5, 10, 20 and 30 pg standards of BH 9048-ME.
22	Typical standard curve for 5, 10, 20 and 30 pg amounts of BH 9048-ME.
23-24	Typical chromatograms of a control soybean grain sample.
25-28	Typical chromatograms of a control soybean grain sample fortified with 0.02 ppm and 0.20 ppm of BAS 9048 H.
29-32	Typical chromatograms of a control soybean grain sample fortified with 0.02 ppm and 0.20 ppm of BH 9048-ME.

Note: All chromatograms except those shown in Figures 2-4 are printed with a relative y-scale. The y-scale is from 0 to 100% of the largest peak in the chromatogram. The absolute range in μV is given below the chromatogram as "Y minimum" and "Y maximum". The standard chromatograms in Figures 2-4 were plotted with an absolute scale to allow a visual comparison of the peak heights at different amounts of BH 9048-A and BH 9048-AME.

Figure 1. Typical chromatographic parameters for liquid chromatography analyses.

```
PE NO : 25/01            PUMP/CONTROLLER NO : 90          PUMP2 NO :
DETECTOR NO : 364          AUTOSAMPLER NO : 164          INJ VOL ul :  100
DATA SYST NO :           INIT FLOW ml/min :  1.00
INIT MOBILE PHASE : 75% ACN + 25% (2.5% AcOH IN MILLIPORE WATER)
COL 1 TYPE : NUCLEOSIL 5 C18 100A
COL 1 ID mm :  4.60                                  COL1 LENGTH mm : 250
     GRADIENT %/min :                          FINAL FLOW ml/min :
FINAL GRAD MOB PHASE :
COL SWITCHING WINDOW :
2ND MOBILE PHASE :
COL 2 TYPE :                                  COL 2 SERIAL NO :
FLOW 2 ml/min :          COL 2 ID mm :            COL 2 LENGTH mm :
DETECTOR WAVELENGTH : EX350EM420
COMMENTS 1 : COL 1 S/N 50203
COMMENTS 2 : PE FLUORESCENCE DET, MODEL # LC240 IN THE FLUOR. MODE
COMMENTS 3 :
```

Figure 2. Typical chromatogram of 50 pg standard of BH 9048-A and BH 9048-AME. Master sheet 92161-8.



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/09/93 09:27:20

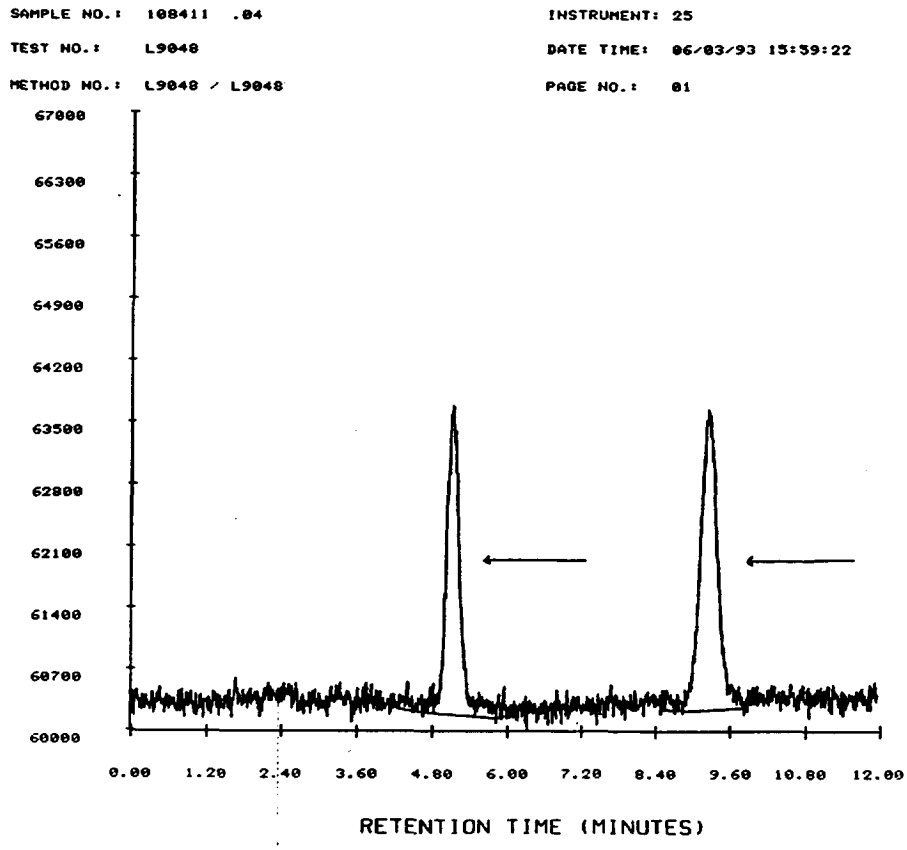
MULTILEVEL EXTERNAL STANDARD

SAMPLE: 108410 .04 INST:25 VIAL: 0 SEQ NUMBER:001
TEST : L9048 DATE-TIME COLLECTED : 06/03/93 15:44:36
COLLECTION TIME : 11.96 DATE-TIME PROCESSED : 06/03/93 15:58:34
METHOD: L9048 / L9048 ANALYST: U25517 SAMP RATE: 3.13
50PG STND

SAMPLE WT : 1.0000 STANDARD WT : 50.0000 DILUTION FACTOR : 1.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	PEAK BL	PEAK HEIGHT	HEIGHT RESULT
004	5.105	M	ACIF MM2	5.105		T	18490	
010	9.248	M	ACIF MM2 ME	9.248			17917	
							36407	

Figure 3. Typical chromatogram of 100 pg standard of BH 9048-A and BH 9048-AME. Master sheet 92161-8.



PERKIN-ELMER LIMS 2000/8 B A S F RTP, NC 06/03/93 22:16:26

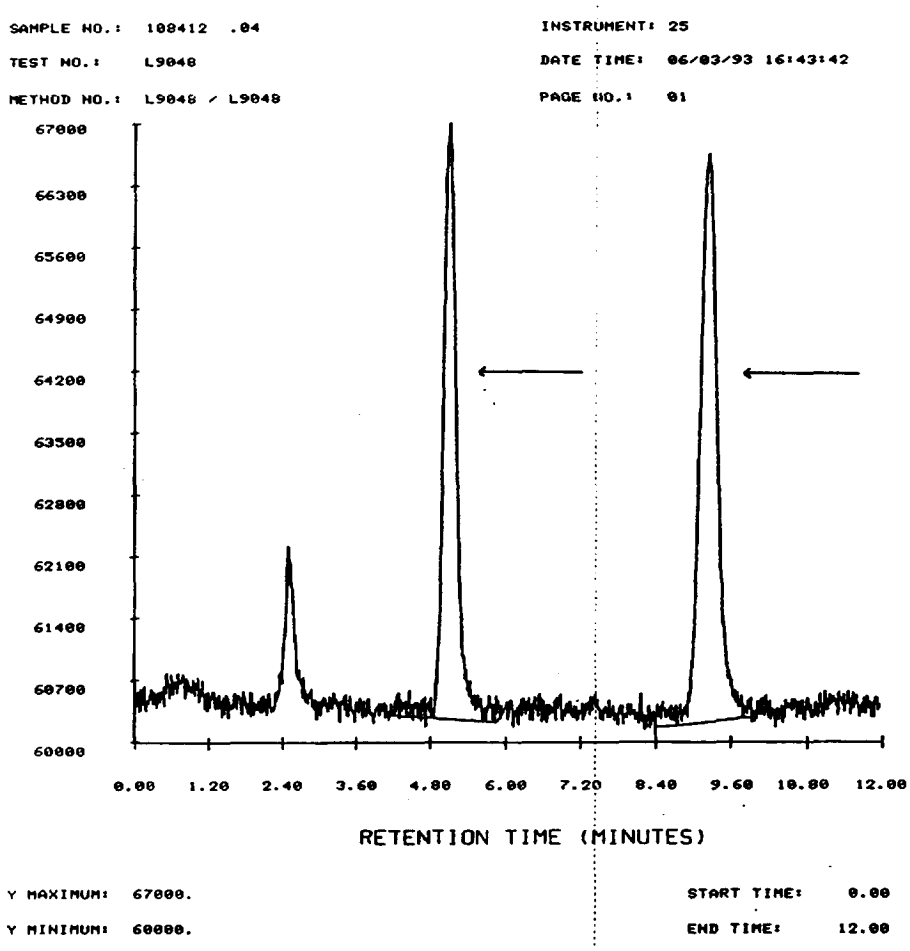
MULTILEVEL EXTERNAL STANDARD

SAMPLE: 108411 .04 INST:25 VIAL: 0 SEQ NUMBER:002
TEST : L9048 DATE-TIME COLLECTED : 06/03/93 15:59:22
COLLECTION TIME : 11.96 DATE-TIME PROCESSED : 06/03/93 16:13:30
METHOD: L9048 / L9048 ANALYST: U25517 SAMP RATE: 3.13
100PG STND

SAMPLE WT : 1.0000 STANDARD WT :100.0000 DILUTION FACTOR : 1.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT RESULT
004	5.101	M	ACIF NH2	5.101		T	34643	
010	9.224	M	ACIF NH2 ME	9.224			33841	
							68484	

Figure 4. Typical chromatogram of 200 pg standard of BH 9048-A and BH 9048-AME. Master sheet 92161-8.



PERKIN-ELMER LIMS 2000/B B A S F RTP, NC 06/03/93 22:18:51

MULTILEVEL EXTERNAL STANDARD

SAMPLE: 108412 .04 INST:25 VIAL: 0 SEQ NUMBER:005
TEST : L9048 DATE-TIME COLLECTED : 06/03/93 16:43:42
COLLECTION TIME : 11.96 DATE-TIME PROCESSED : 06/03/93 16:58:13
METHOD: L9048 / L9048 ANALYST: U25517 SAMP RATE: 3.13
200PG STND

SAMPLE WT : 1.0000 STANDARD WT :200.0000 DILUTION FACTOR : 1.0000

PK NO.	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	PEAK HEIGHT	HEIGHT RESULT
004	5.094	M	ACIF NH2	5.094		T 67133	
009	9.237	M	ACIF NH2 ME	9.237		V 63719	
						130852	

Figure 5. Typical standard curve for 50, 100 and 200 pg amounts of BH 9048-A. Master sheet 92161-8.

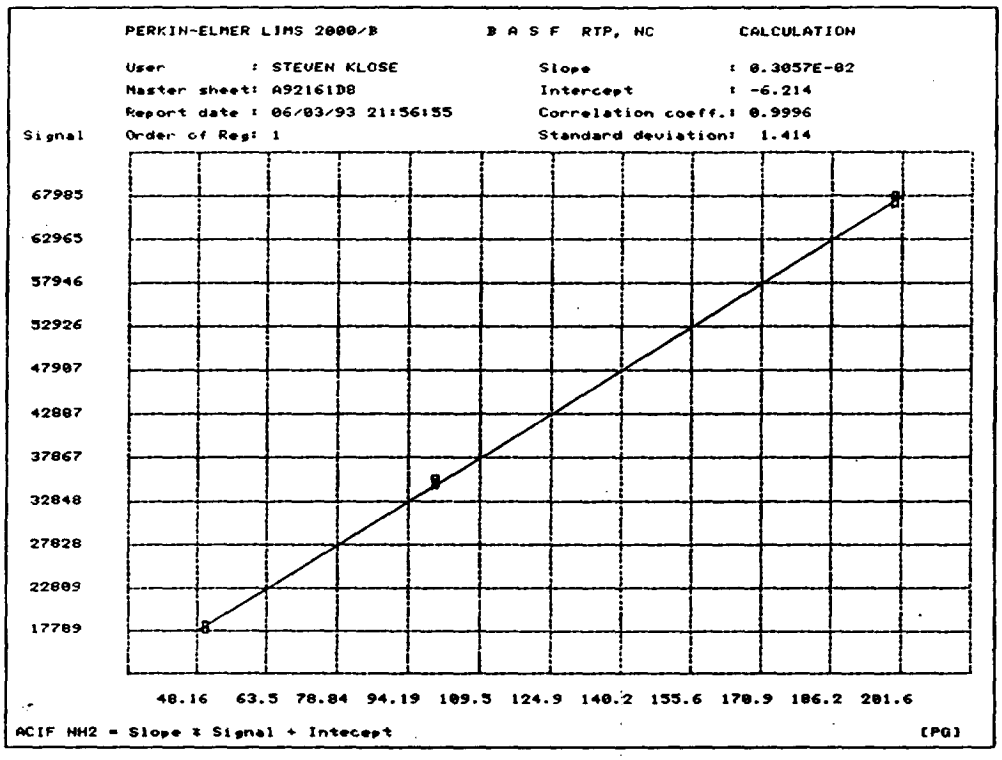


Figure 6.- Typical standard curve for 50, 100 and 200 pg amounts of BH 9048-AME.

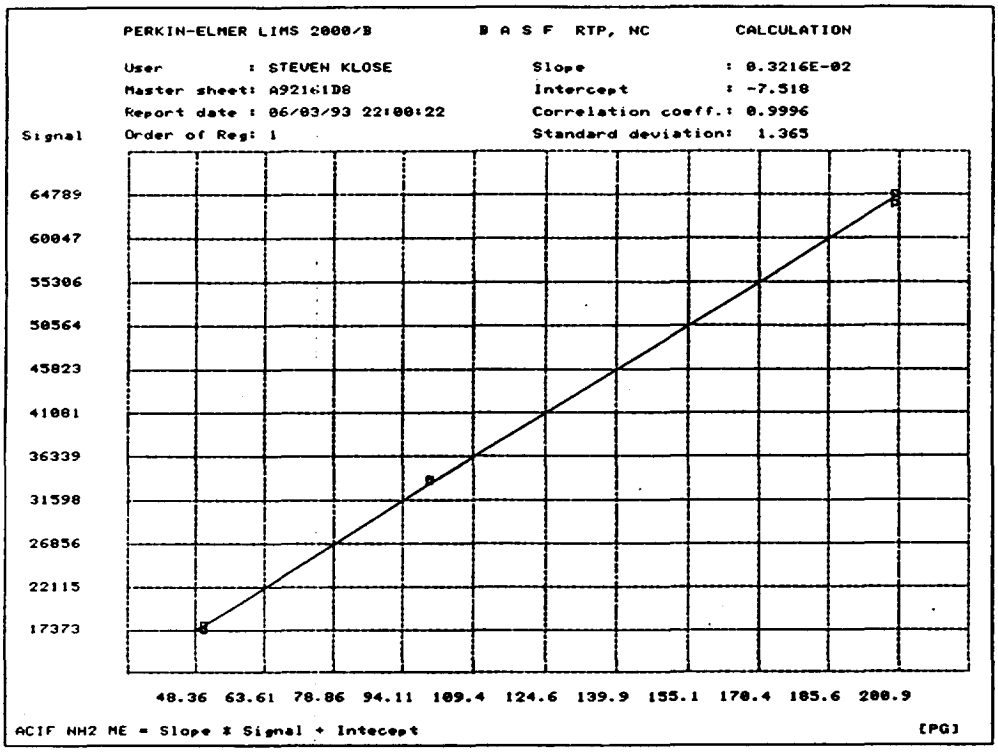
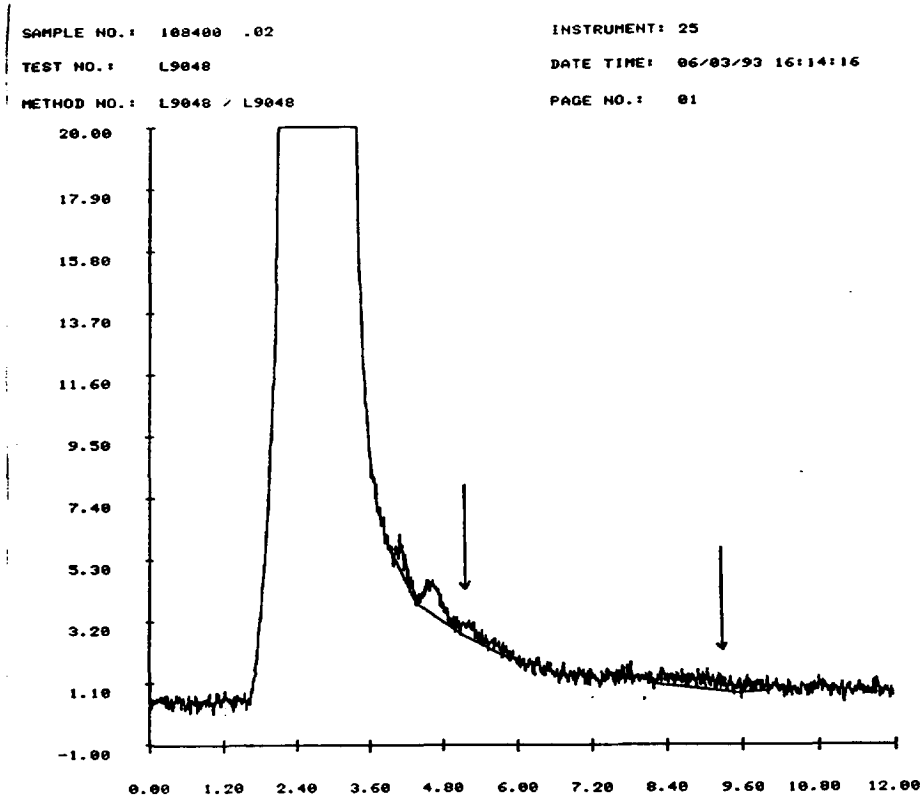


Figure 7. Typical chromatogram of a control soybean grain sample. Sample number 92940-1, lab code 108400. The sample weight injected was 4.0 mg. The sample contained <0.02 ppm equivalents of BH 9048-A and BH 9048-AME.



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/03/93 22:04:54

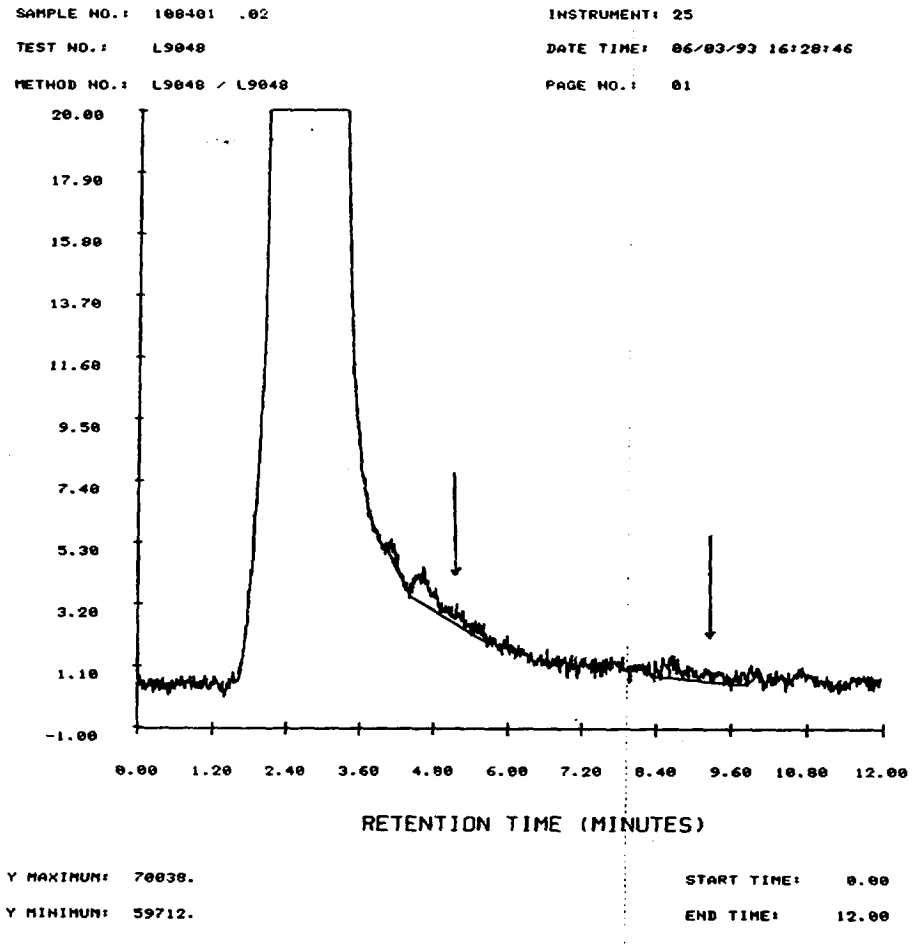
EXTERNAL STANDARD

SAMPLE: 108400 .02 INST:25 VIAL: 0 SEQ NUMBER:003
TEST : L9048 DATE-TIME COLLECTED : 06/03/93 16:14:16
COLLECTION TIME : 11.96 DATE-TIME PROCESSED : 06/03/93 17:01:00
METHOD: L9048 / L9048 ANALYST: U25517 SAMP RATE: 3.13
CONTROL:1

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 10.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT RESULT
	5.100	M	ACIF NH2	5.100	1.00000			
	9.300	M	ACIF NH2 ME	9.300	1.00000			
							0	0.000

Figure 8.- Typical chromatogram of a control soybean grain sample. Sample number 92940-1, lab code 108401. The sample weight injected was 4.0 mg. The sample contained <0.02 ppm equivalents of BH 9048-A and BH 9048-AME.



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/03/93 22:05:48

MULTILEVEL EXTERNAL STANDARD

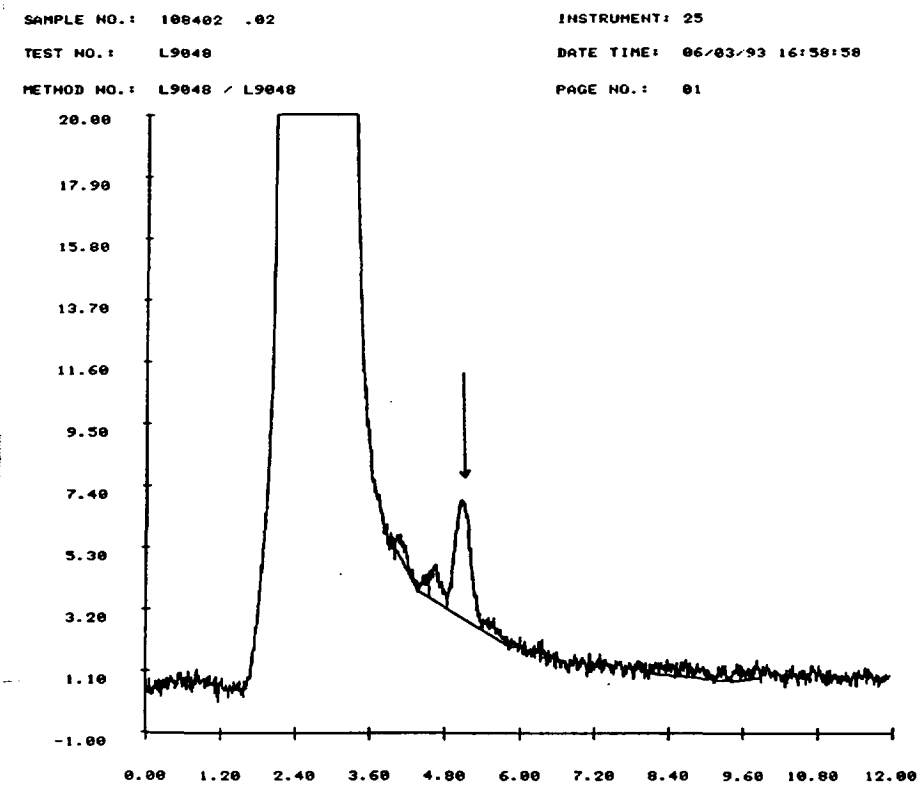
SAMPLE: 108401 .02 INST:25 VIAL: 0 SEQ NUMBER:004
TEST : L9048 DATE-TIME COLLECTED : 06/03/93 16:28:46
COLLECTION TIME : 11.96 DATE-TIME PROCESSED : 06/03/93 16:42:52
METHOD: L9048 / L9048 ANALYST: U25517 SAMP RATE: 3.13
CONTROL02

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 10.0000

PK NO	RT . MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT RESULT
002	4.630	M	ACIF NH2	4.630		V	6069	
	9.300	M	ACIF NH2 ME	9.300				
							6069	

Figure 9.- Typical chromatogram of a control soybean grain sample fortified with 0.02 ppm of BH 9048-A (the quantitation limit). Sample number 92940-1, lab code 108402. The sample weight injected was 4.0 mg. The sample contained 53.7 pg of BH 9048-A which is equivalent to 0.013 ppm of BH 9048-A.

Recovery of BH 9048-A: 67%



PERKIN-ELMER LIMS 2000/B B A S F RTP, NC 06/03/93 22:06:57

MULTILEVEL EXTERNAL STANDARD

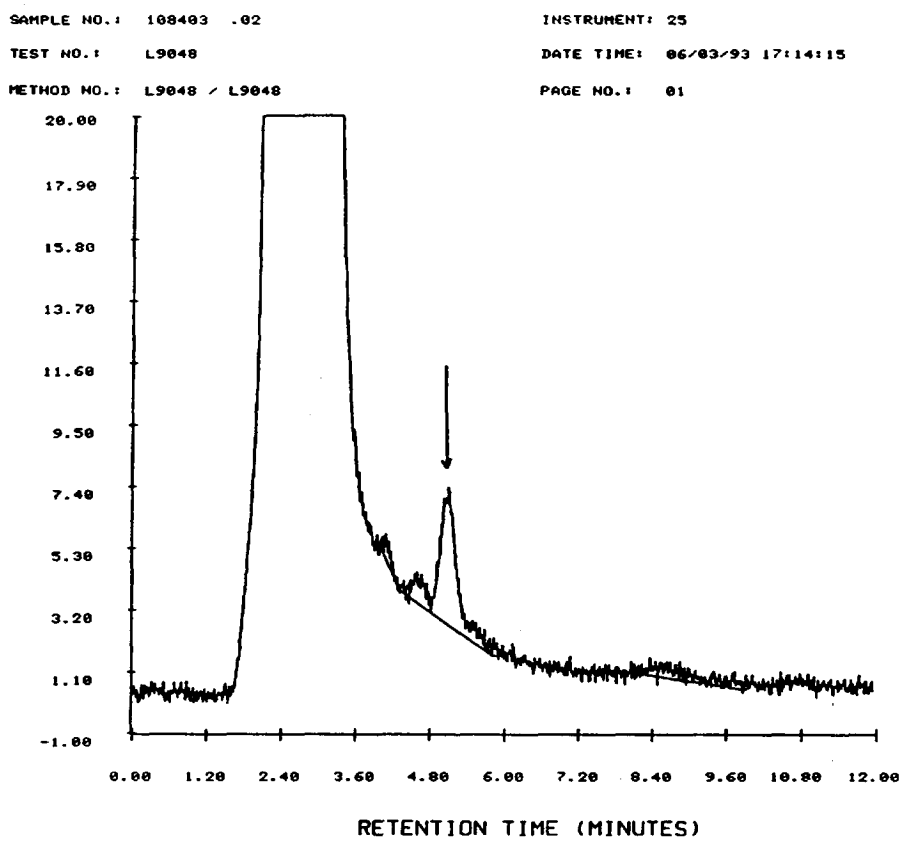
SAMPLE: 108402 .02 INST:25 VIAL: 0 SEQ NUMBER:006
TEST : L9048 DATE-TIME COLLECTED : 06/03/93 16:58:58
COLLECTION TIME : 11.96 DATE-TIME PROCESSED : 06/03/93 17:13:25
METHOD: L9048 / L9048 ANALYST: U25517 SAMP RATE: 3.13
.02PPM ACIF-NH2#1

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 10.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT RESULT
003	4.653			4.653	1.0000E+0	T	6413	3206.563
004	5.050	M	ACIF NH2	5.050		T	19608	
	9.300	M	ACIF NH2 ME	9.300				
							26021	3.207E+3

Figure 10. Typical chromatogram of a control soybean grain sample fortified with 0.02 ppm of BH 9048-A (the quantitation limit). Sample number 92940-1, lab code 108403. The sample weight injected was 4.0 mg. The sample contained 62.8 pg of BH 9048-A which is equivalent to 0.016 ppm of BH 9048-A.

Recovery of BH 9048-A: 79%



PERKIN-ELMER LINS 2000/3 B A S F RTP, NC 06/03/93 22:08:08

MULTILEVEL EXTERNAL STANDARD

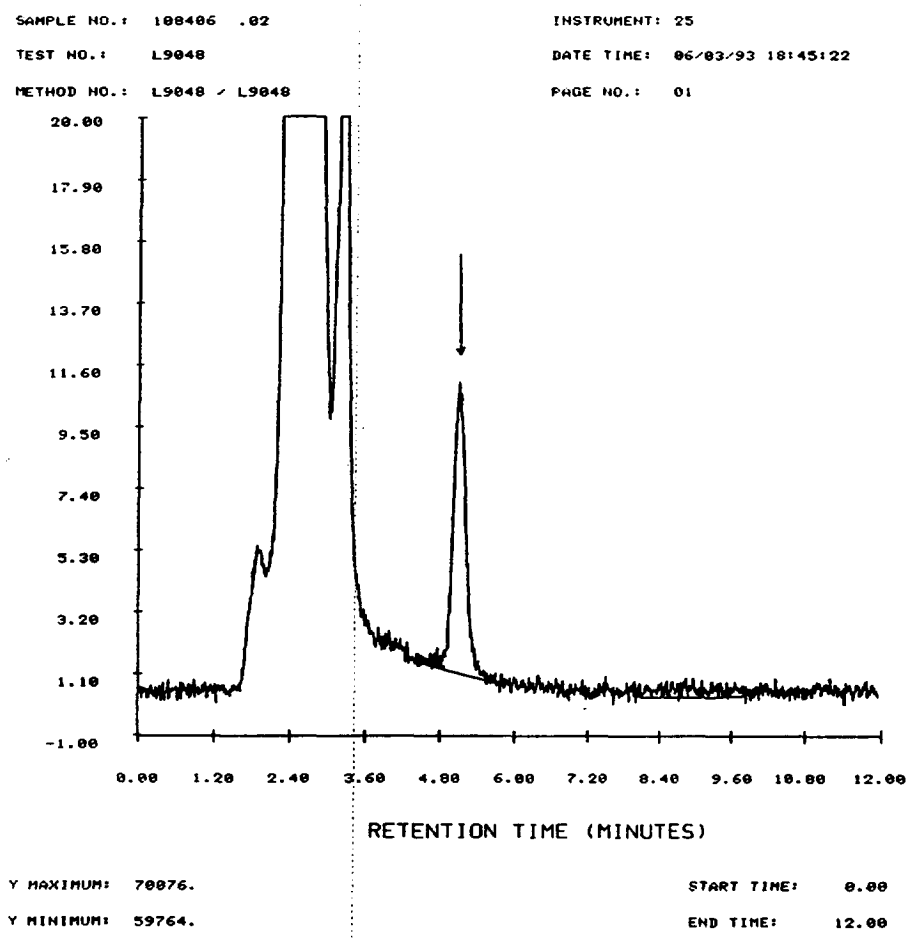
SAMPLE: 108403 .02 INST:25 VIAL: 0 SEQ NUMBER:007
TEST : L9048 DATE-TIME COLLECTED : 06/03/93 17:14:15
COLLECTION TIME : 11.96 DATE-TIME PROCESSED : 06/03/93 17:28:01
METHOD: L9048 / L9048 ANALYST: U25517 SAMP RATE: 3.13
.02PPM ACIF-NH2#2

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 10.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT RESULT
003	5.090	M	ACIF NH2	5.090		T	22595	
	9.300	M	ACIF NH2 ME	9.300				
							22595	

Figure 11. Typical chromatogram of a control soybean grain sample fortified with 0.20 ppm of BH 9048-A. Sample number 92940-1, lab code 108406. The sample weight injected was 0.8 mg. The sample contained 139.9 pg of BH 9048-A which is equivalent to 0.175 ppm of BH 9048-A.

Recovery of BH 9048-A: 87%



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/03/93 22:10:20

MULTILEVEL EXTERNAL STANDARD

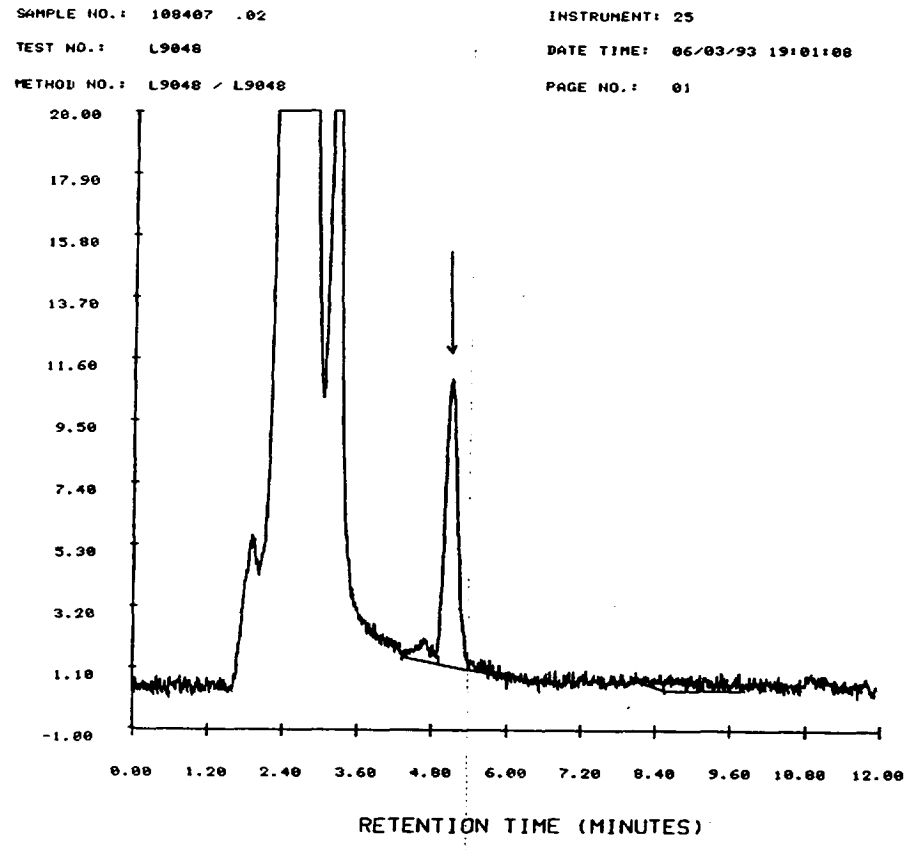
SAMPLE: 108406 .02 INST:25 VIAL: 0 SEQ NUMBER:013
TEST : L9048 DATE-TIME COLLECTED : 06/03/93 18:45:22
COLLECTION TIME : 11.96 DATE-TIME PROCESSED : 06/03/93 19:00:19
METHOD: L9048 / L9048 ANALYST: U25517 SAMP RATE: 3.13
.2PPM ACIF-NH201

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 50.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT RESULT
003	5.082	M	ACIF NH2	5.082			47804	
	9.389	M	ACIF NH2 ME	9.390				
							47804	

Figure 12. Typical chromatogram of a control soybean grain sample fortified with 0.20 ppm of BH 9048-A. Sample number 92940-1, lab code 108407. The sample weight injected was 0.8 mg. The sample contained 140.0 pg of BH 9048-A which is equivalent to 0.175 ppm of BH 9048-A.

Recovery of BH 9048-A: 88%



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/03/93 22:11:05

MULTILEVEL EXTERNAL STANDARD

SAMPLE: 108407 .02
TEST : L9048
COLLECTION TIME : 11.96
METHOD: L9048 / L9048

INST:25 VIAL: 8 SEQ NUMBER:814
DATE-TIME COLLECTED : 06/03/93 19:01:08
DATE-TIME PROCESSED : 06/03/93 19:16:20
ANALYST: U25517 SAMP RATE: 3.13
.2PPM ACIF-NH2#2

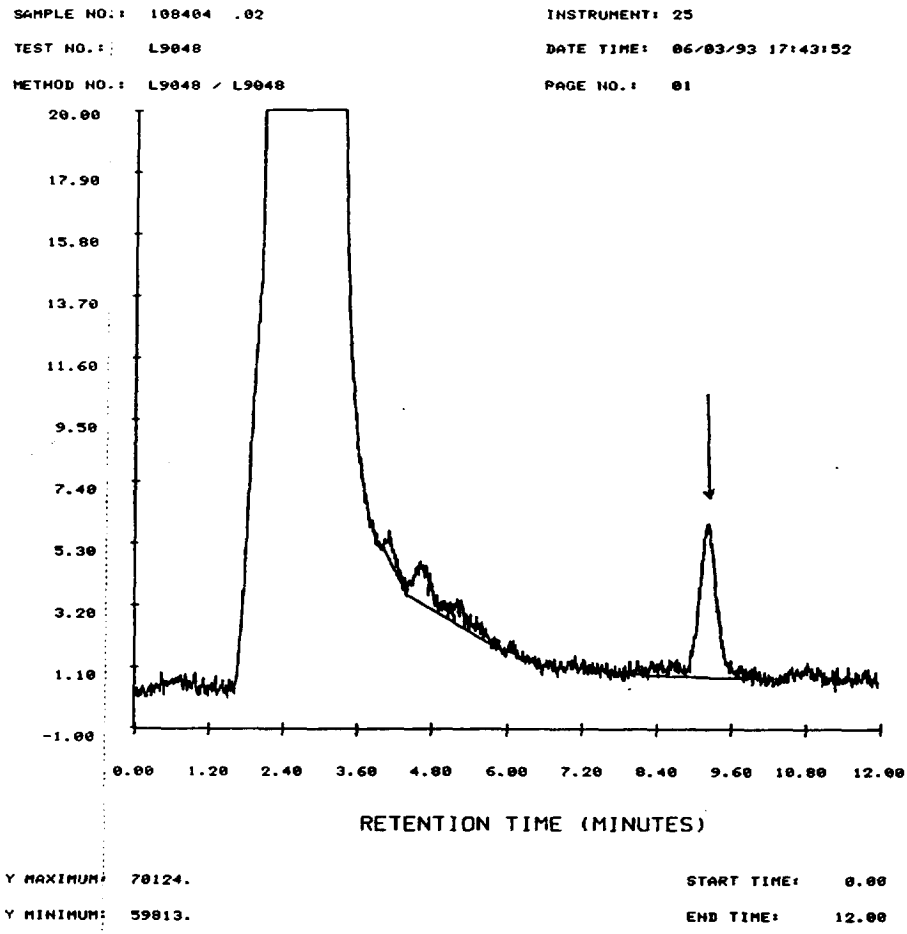
SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 50.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	PEAK BL	HEIGHT	HEIGHT RESULT
003	5.098	M	ACIF NH2	5.098		T	47823	
	9.300	M	ACIF NH2 ME	9.300				

47823

Figure 13. Typical chromatogram of a control soybean grain sample fortified with 0.02 ppm of BH 9048-AME. Sample number 92940-1, lab code 108404. The sample weight injected was 4.0 mg. The sample contained 74.4 pg of BH 9048-AME which is equivalent to 0.019 ppm of BH 9048-AME.

Recovery of BH 9048-AME: 79%



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/03/93 22:08:52

MULTILEVEL EXTERNAL STANDARD

SAMPLE: 108404 .02
TEST : L9048
COLLECTION TIME : 11.96
METHOD: L9048 / L9048

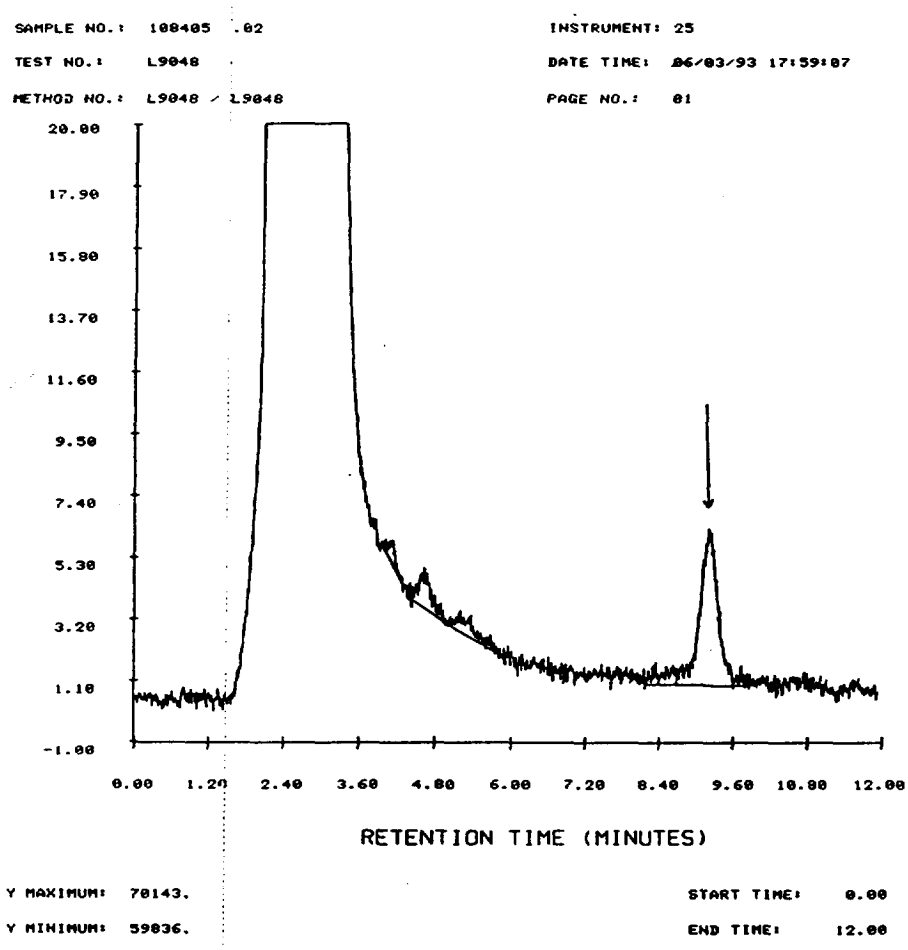
INST:25 VIAL: 0 SEQ NUMBER:009
DATE-TIME COLLECTED : 06/03/93 17:43:52
DATE-TIME PROCESSED : 06/03/93 17:58:19
ANALYST: U25517 SAMP RATE: 3.13
.02PPM ACIF-NH2-ME0

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 10.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT RESULT
002	4.616	M	ACIF NH2	4.616		T	6421	
007	9.287	M	ACIF NH2 ME	9.287		T	25454	
							31875	

Figure 14: Typical chromatogram of a control soybean grain sample fortified with 0.02 ppm of BH 9048-AME. Sample number 92940-1, lab code 108405. The sample weight injected was 4.0 ng. The sample contained 75.3 pg of BH 9048-AME which is equivalent to 0.019 ppm of BH 9048-AME.

Recovery of BH 9048-AME: 93%



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/03/93 22:09:36

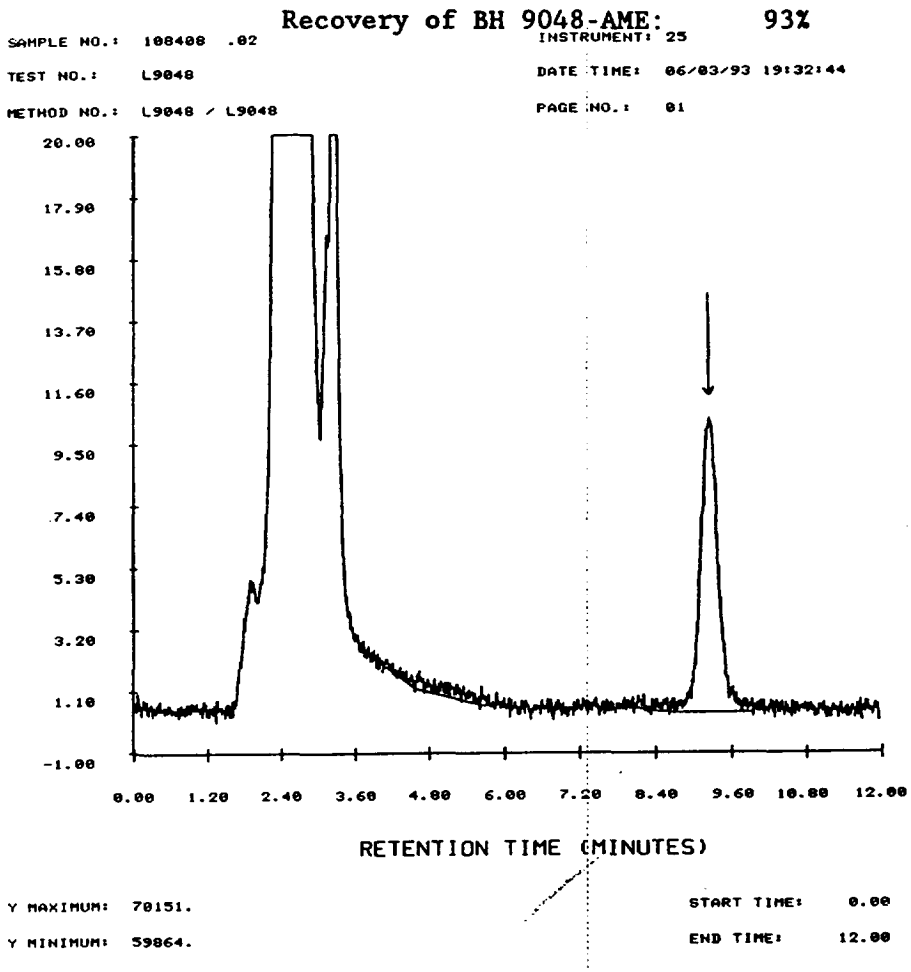
MULTILEVEL EXTERNAL STANDARD

SAMPLE: 108405 .02 INST:25 VIAL: 0 SEQ NUMBER:010
TEST : L9048 DATE-TIME COLLECTED : 06/03/93 17:59:07
COLLECTION TIME : 11.96 DATE-TIME PROCESSED : 06/03/93 18:13:29
METHOD: L9048 / L9048 ANALYST: U25517 SAMP RATE: 3.13
.02PPM ACIF-NH2-ME

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 10.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT RESULT
002	4.639	M	ACIF NH2	4.639		V	6084	
008	9.287	M	ACIF NH2 ME	9.287		T	25740	
							31824	

Figure 15. Typical chromatogram of a control soybean grain sample fortified with 0.20 ppm of BH 9048-AME. Sample number 92940-1, lab code 108408. The sample weight injected was 0.8 mg. The sample contained 149.0 pg of BH 9048-AME which is equivalent to 0.186 ppm of BAS 9048-AME.



PERKIN-ELMER LIMS 2000/B B A S F RTP, NC 06/03/93 22:11:49

MULTILEVEL EXTERNAL STANDARD

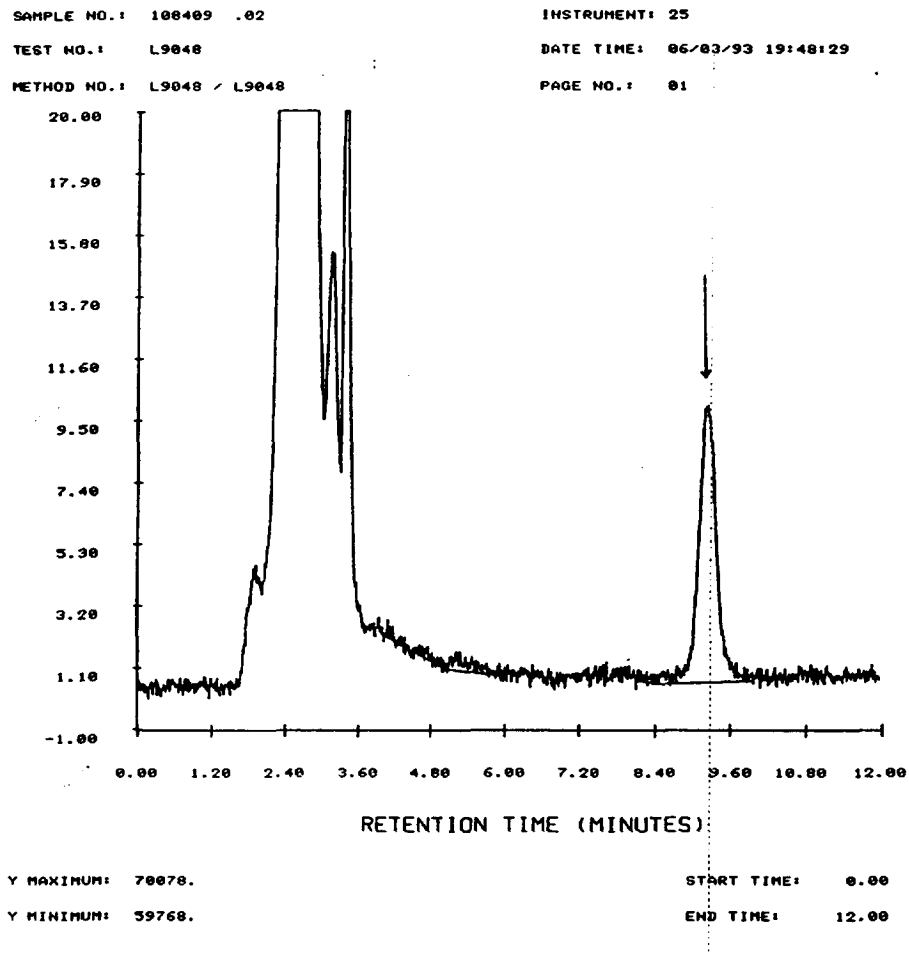
SAMPLE: 108408 .02 INST:25 VIAL: 0 SEQ NUMBER:016
TEST : L9048 DATE-TIME COLLECTED : 06/03/93 19:32:44
COLLECTION TIME : 11.96 DATE-TIME PROCESSED : 06/03/93 19:47:36
METHOD: L9048 / L9048 ANALYST: U25517 SAMP RATE: 3.13
.2PPM ACIF-NH2-ME01

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 50.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT RESULT
	5.100	M	ACIF NH2	5.100				
011	9.225	M	ACIF NH2 ME	9.225		T	48632	
								48632

Figure 16. Typical chromatogram of a control soybean grain sample fortified with 0.20 ppm of BH 9048-AME. Sample number 92940-1, lab code 108409. The sample weight injected was 0.8 mg. The sample contained 140.0 pg of BH 9048-AME which is equivalent to 0.175 ppm of BAS 9048-AME.

Recovery of BH 9048-AME: 88%



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/03/93 22:12:34

MULTILEVEL EXTERNAL STANDARD

SAMPLE: 108409 .02 INST:25 VIAL: 0 SEQ NUMBER:017
TEST : L9048 DATE-TIME COLLECTED : 06/03/93 19:48:29
COLLECTION TIME : 11.96 DATE-TIME PROCESSED : 06/03/93 20:03:54
METHOD: L9048 / L9048 ANALYST: U25517 SAMP RATE: 3.13
.2PPM ACIF-NH2-ME#2

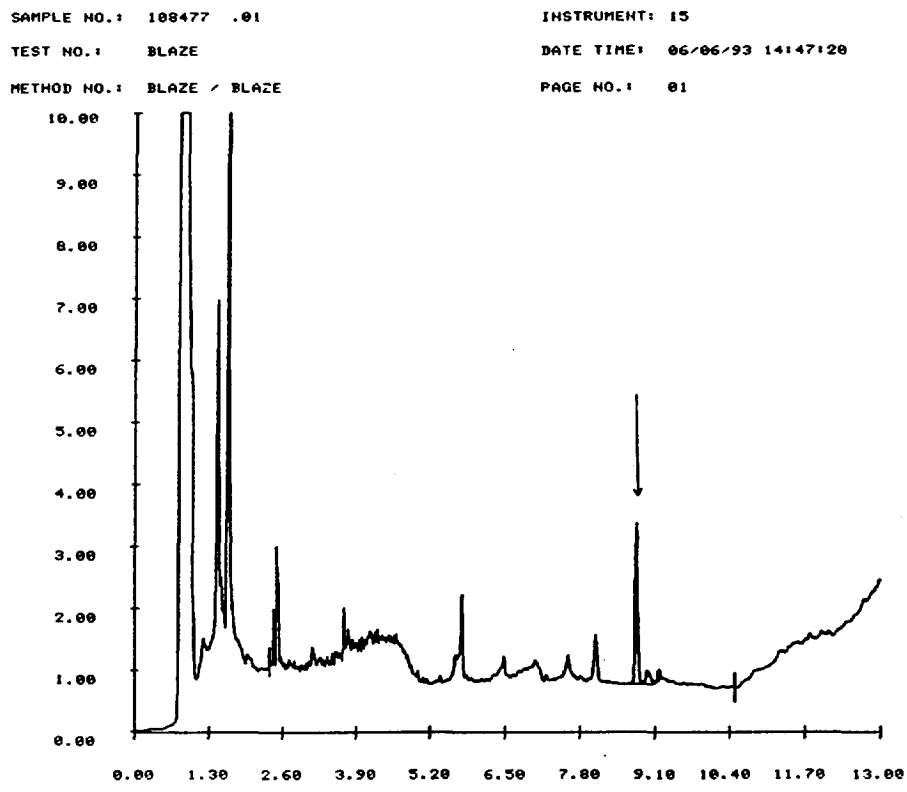
SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 50.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT RESULT
	5.100	M	ACIF NH2	5.100				
009	9.228	M	ACIF NH2 ME	9.228			45862	
							45862	

Figure 17. Typical chromatographic parameters for gas chromatography analysis.

```
PE NO : 15          BASF NO : 0017          APPARATUS : VARIAN 3600 GC W/ ECD
COL PHASE : DB-5                                    COL SERIAL NO : 2548537A
ID mm : 0.32        LENGTH m :  30.0                FILM um :  1.00
SUPPORT :                                           MESH :
CARRIER ID : HE                CARRIER PSI :            FLOW ml/min : 3.1
SPLIT OFF min :                              SPLIT RATIO : NONE
INLET C : 250                AUXILIARY C :              INJ VOL ul :  1.0
                             OVEN INIT C : 130          INIT HOLD TIME :   0.5
RATE 2 C/min : 30.0             OVEN 2 C : 250          HOLD TIME 2 :  6.0
RATE 3 C/min : 30.0             OVEN 3 C : 290          HOLD TIME 3 :  1.0
RATE 4 C/min :                  OVEN 4 C :              HOLD TIME 4 :
DETECTOR : ECD                                          DETECTOR C : 300
MAKE-UP ml/min : 27                          AIR ml/min :
HYDROGEN ml/min :                            OXYGEN ml/min :
COMMENTS1 : ATTENUATION=32   RANGE=10   AUTO ZERO ON
COMMENTS2 : SPLITLESS INJECTION
```

Figure 18. Typical chromatogram of 5 pg standard of BH 9048-ME. Master sheet 92161-8.



PERKIN-ELMER LIMS 2000/8 B A S F RTP, NC 06/07/93 11:04:21

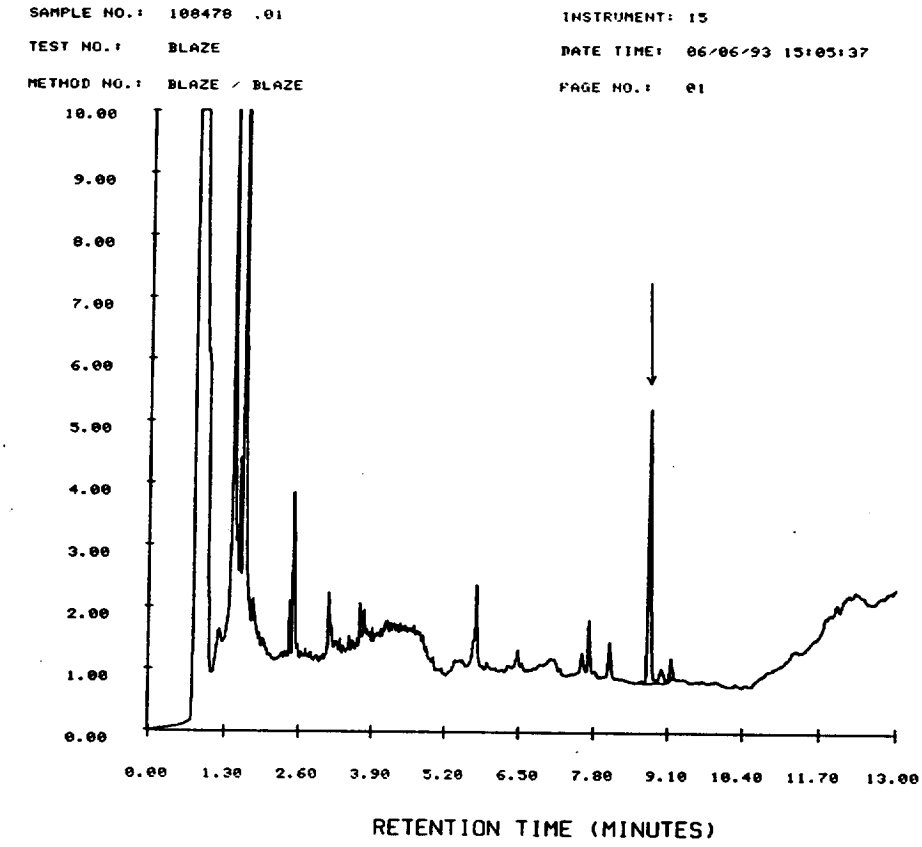
MULTILEVEL EXTERNAL STANDARD

SAMPLE: 108477 .01 INST:15 VIAL: 0 SEQ NUMBER:001
TEST : BLAZE DATE-TIME COLLECTED : 06/06/93 14:47:20
COLLECTION TIME : 13.00 DATE-TIME PROCESSED : 06/06/93 15:00:34
METHOD: BLAZE / BLAZE ANALYST: U25517 SAMP RATE: 12.50
SPG STND

SAMPLE WT : 1.0000 STANDARD WT : 5.0000 DILUTION FACTOR : 1.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	PEAK BL	HEIGHT	HEIGHT PG
003	8.767	M	ACIFLUORFEN-METHYL	8.767		T	3512	
							3512	

Figure 19. Typical chromatogram of 10 pg standard of BH 9048-ME. Master sheet 92161-8.



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/07/93 11:05:51

MULTILEVEL EXTERNAL STANDARD

SAMPLE: 108478 .01 INST:15 VIAL: 0 SEQ NUMBER:002
TEST : BLAZE DATE-TIME COLLECTED : 06/06/93 15:05:37
COLLECTION TIME : 13.00 DATE-TIME PROCESSED : 06/06/93 15:18:58
METHOD: BLAZE / BLAZE ANALYST: U25517 SAMP RATE: 12.50
10PG STND

SAMPLE WT : 1.0000 STANDARD WT : 10.0000 DILUTION FACTOR : 1.0000

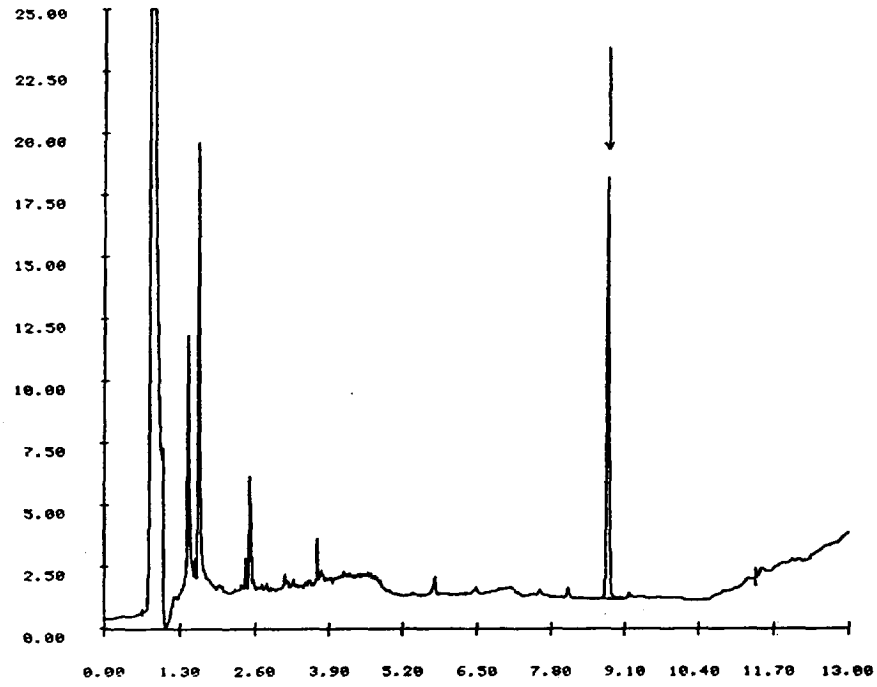
PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT PG
001	8.771	M	ACIFLUORFEN-METHYL	8.771		T	5783	8.995
							5783	8.995

Figure 20. Typical chromatogram of 20 pg standard of BH 9048-ME. Master sheet 92161-8.

SAMPLE NO.: 108479 .02 INSTRUMENT: 15

TEST NO.: BLAZE DATE TIME: 06/06/93 19:57:15

METHOD NO.: BLAZE / BLAZE PAGE NO.: 01



Y MAXIMUM: 52039. START TIME: 0.00

Y MINIMUM: 49933. END TIME: 13.00

PERKIN-ELMER LIMS 2000/B B A S F RTP, NC 06/07/93 11:07:40

EXTERNAL STANDARD

SAMPLE: 108479 .02 INST:15 VIAL: 0 SEQ NUMBER:017
TEST : BLAZE DATE-TIME COLLECTED : 06/06/93 19:57:15
COLLECTION TIME : 13.00 DATE-TIME PROCESSED : 06/07/93 10:27:42
METHOD: BLAZE / BLAZE ANALYST: U25517 SAMP RATE: 12.50
20PG STND

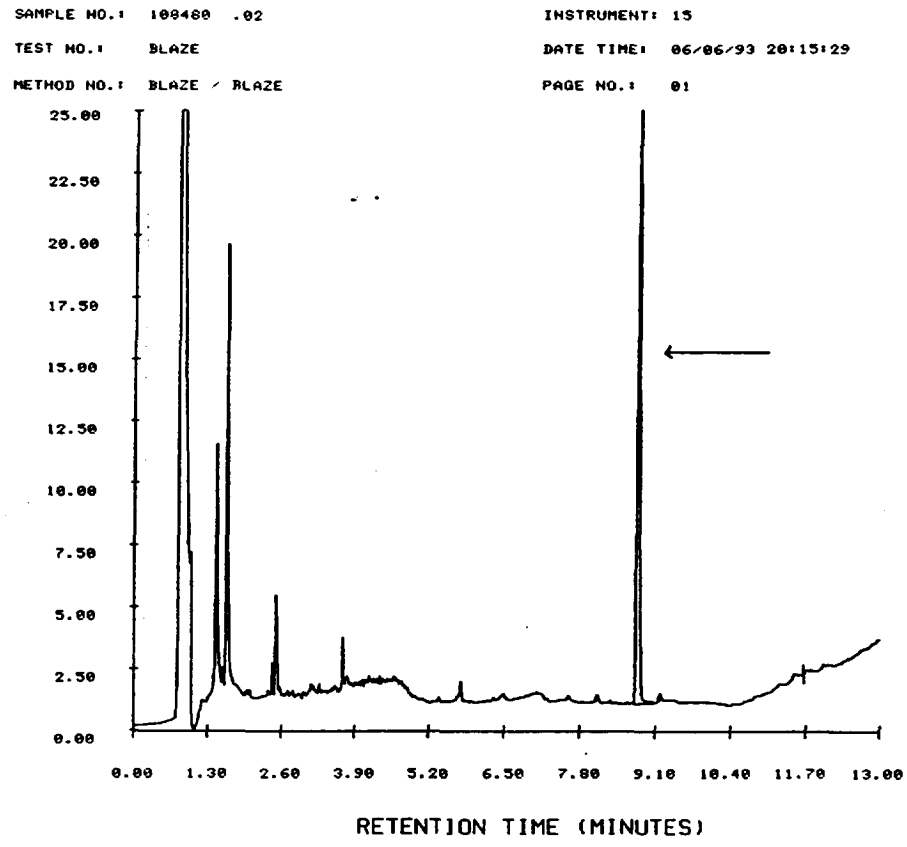
SAMPLE WT : 1.0000 STANDARD WT : 20.0000 DILUTION FACTOR : 1.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT PG
003	8.791	M	ACIFLUORFEN-METHYL	8.791	1.0000E+0	T	14309	2.862E+5
							14309	2.8619E+5

GROUP REPORT - 108479 .02

GP#	GROUP NAME	HEIGHT PG
M		286190.100

Figure 21. Typical chromatogram of 30 pg standard of BH 9048-ME. Master sheet 92161-8.



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/07/93 11:08:37

EXTERNAL STANDARD

SAMPLE: 100480 .02 INST:15 VIAL: 0 SEQ NUMBER:018
TEST : BLAZE DATE-TIME COLLECTED : 06/06/93 20:15:29
COLLECTION TIME : 13.00 DATE-TIME PROCESSED : 06/07/93 10:29:29
METHOD: BLAZE / BLAZE ANALYST: U25517 SAMP RATE: 12.50
30PG STND

SAMPLE WT : 1.0000 STANDARD WT : 30.0000 DILUTION FACTOR : 1.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT PG
004	8.787	M	ACIFLUORFEN-METHYL	8.787	1.0000E+0	T	19395	5.0184E+5
							19395	5.0184E+5

GROUP REPORT - 100480 .02

GP#	GROUP NAME	HEIGHT PG
M		501841.100

Figure 22. Typical standard curve for 5, 10, 20 and 30 pg amounts of BH 9048-ME. Master sheet 92161-8.

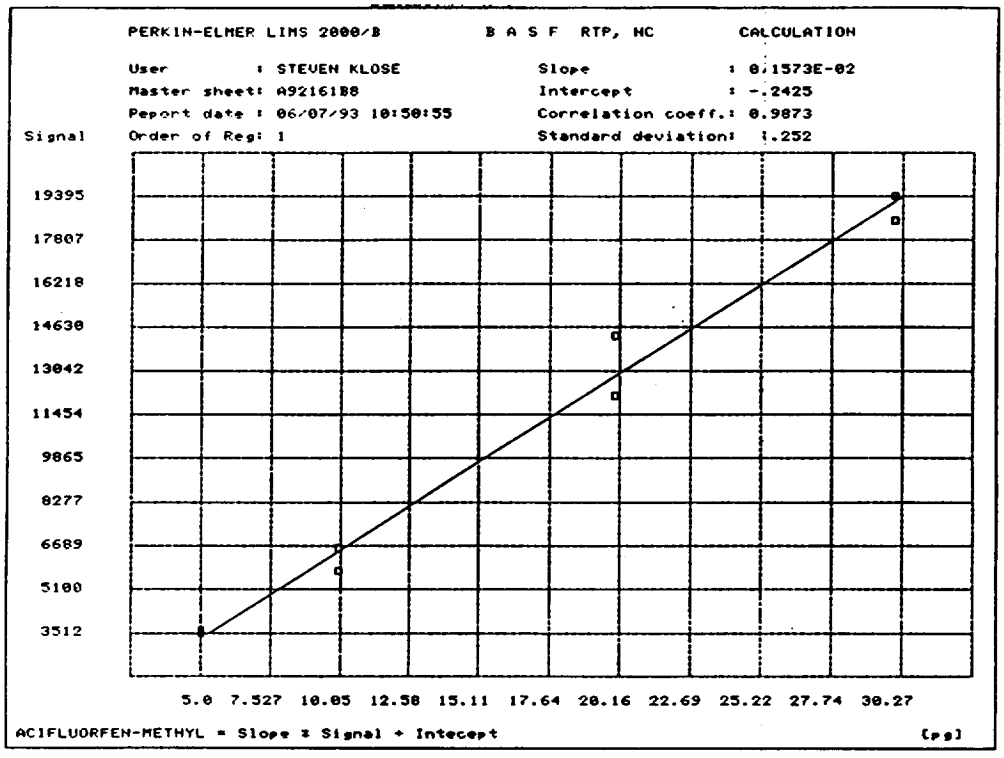
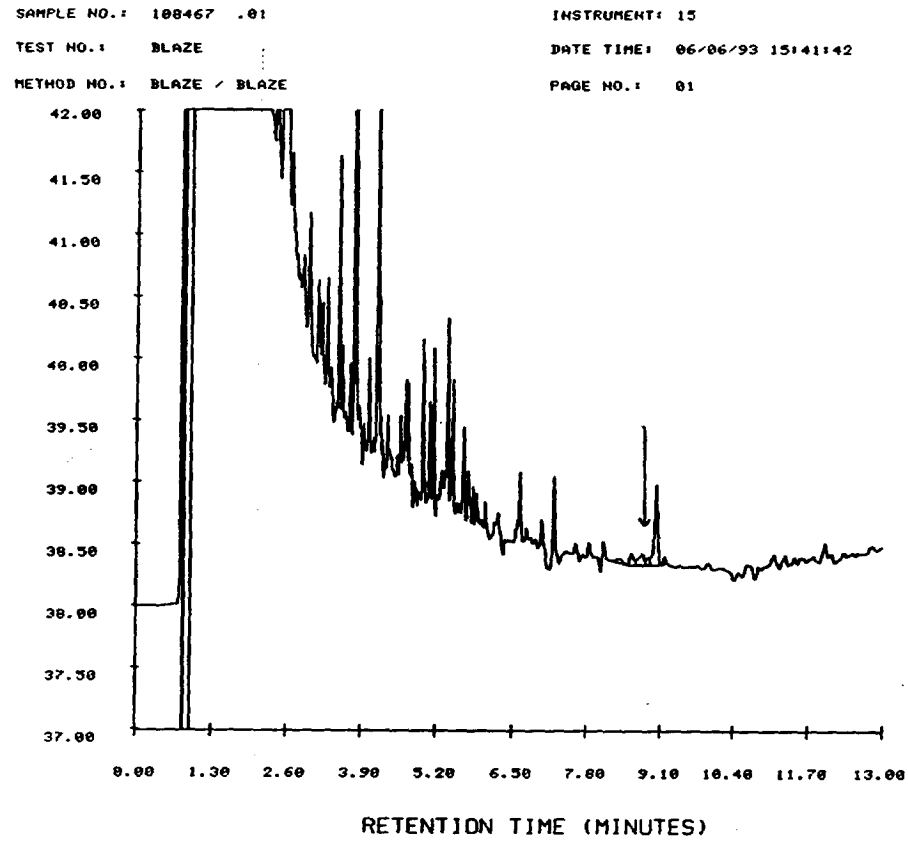


Figure 23. Typical chromatogram of a control soybean grain sample. Sample number 92940-1, lab code 108467. The sample weight injected was 1.0 mg. The sample contained <0.02 ppm equivalents of BH 9048-ME.



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/07/93 11:19:01

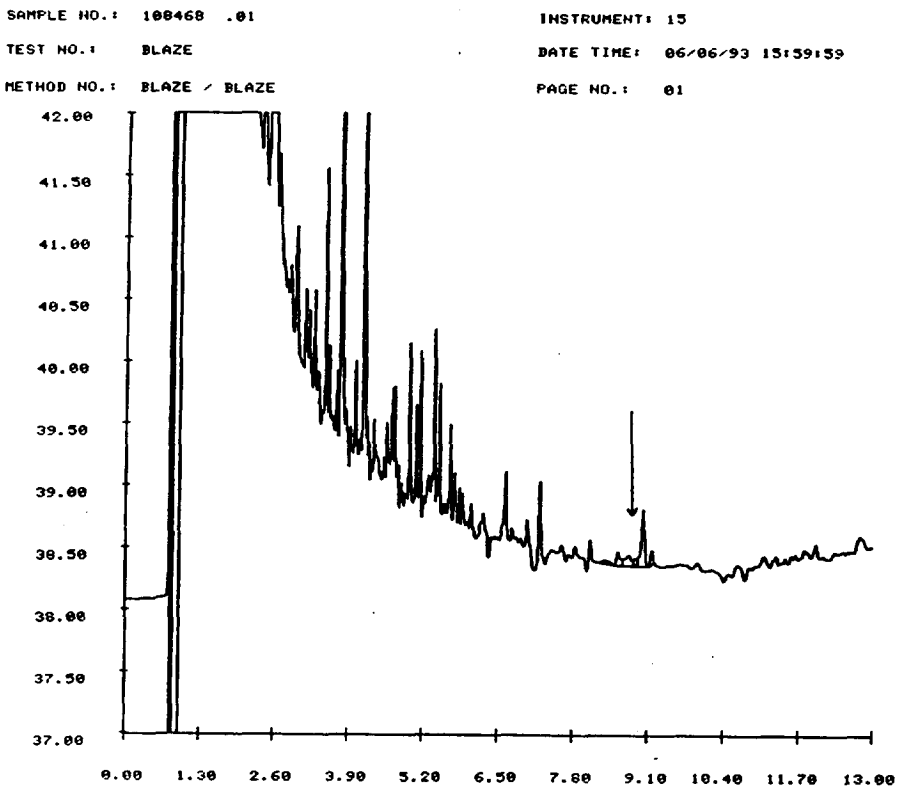
EXTERNAL STANDARD

SAMPLE: 108467 .01 INST:15 VIAL: 0 SEQ NUMBER:003
TEST : BLAZE DATE-TIME COLLECTED : 06/06/93 15:41:42
COLLECTION TIME : 13.00 DATE-TIME PROCESSED : 06/07/93 10:34:53
METHOD: BLAZE / BLAZE ANALYST: U25517 SAMP RATE: 12.50
CONTROL01

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 4.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT PG
	8.750	M	ACIFLUORFEN-METHYL	8.750	1.0000E+0			
006	9.018			9.018	1.0000E+0	V	8436	1687.125
							8436	1687.125

Figure 24. Typical chromatogram of a control soybean grain sample. Sample number 92940-1, lab code 108468. The sample weight injected was 1.0 mg. The sample contained <0.02 ppm equivalents of BH 9048-ME.



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/07/93 11:19:31

EXTERNAL STANDARD

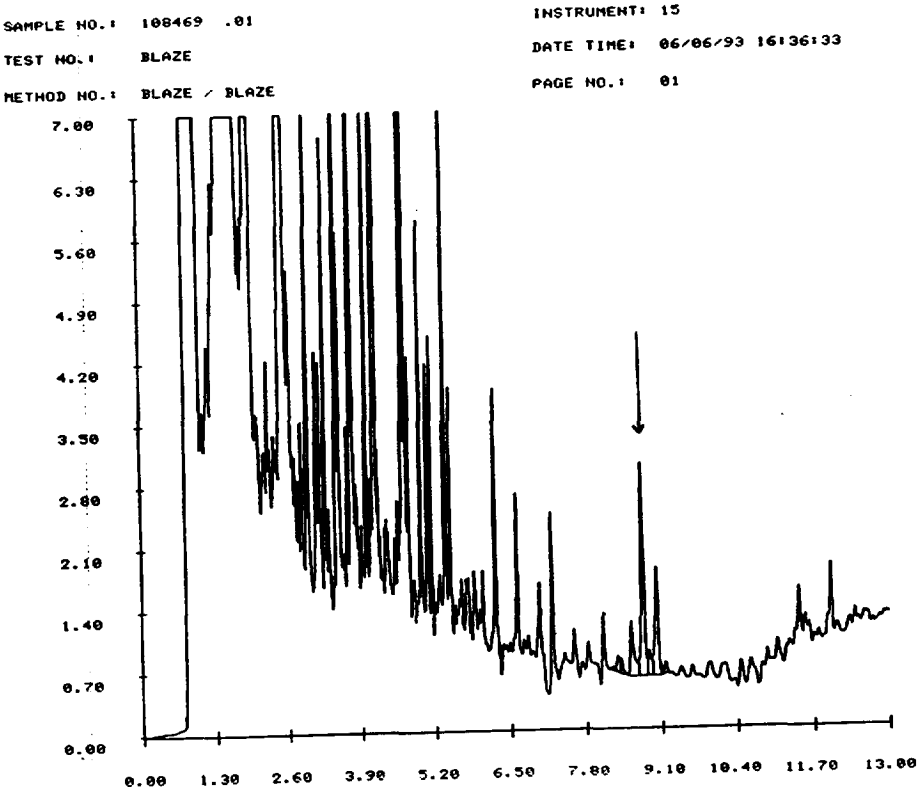
SAMPLE: 108468 .01 INST:15 VIAL: 0 SEQ NUMBER:004
TEST : BLAZE DATE-TIME COLLECTED : 06/06/93 15:59:59
COLLECTION TIME : 13.00 DATE-TIME PROCESSED : 06/07/93 10:36:16
METHOD: BLAZE / BLAZE ANALYST: U25517 SAMP RATE: 12.50
CONTROL#2

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 4.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT PG
003	8.582			8.582	1.0000E+0	T	1589	301.859
	8.750	M	ACIFLUORFEN-METHYL	8.750	1.0000E+0			
006	9.013			9.013	1.0000E+0	V	6073	1214.609
							7582	1516.469

Figure 25. Typical chromatogram of a control soybean grain sample fortified with 0.02 ppm of BAS 9048 H (the quantitation limit). Sample number 92940-1, lab code 108469. The sample weight injected was 1.0 mg. The sample contained 14.2 pg of BH 9048-ME which is equivalent to 0.014 ppm of BAS 9048 H.

Recovery of BAS 9048 H: 68%



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/07/93 11:27:21

MULTILEVEL EXTERNAL STANDARD

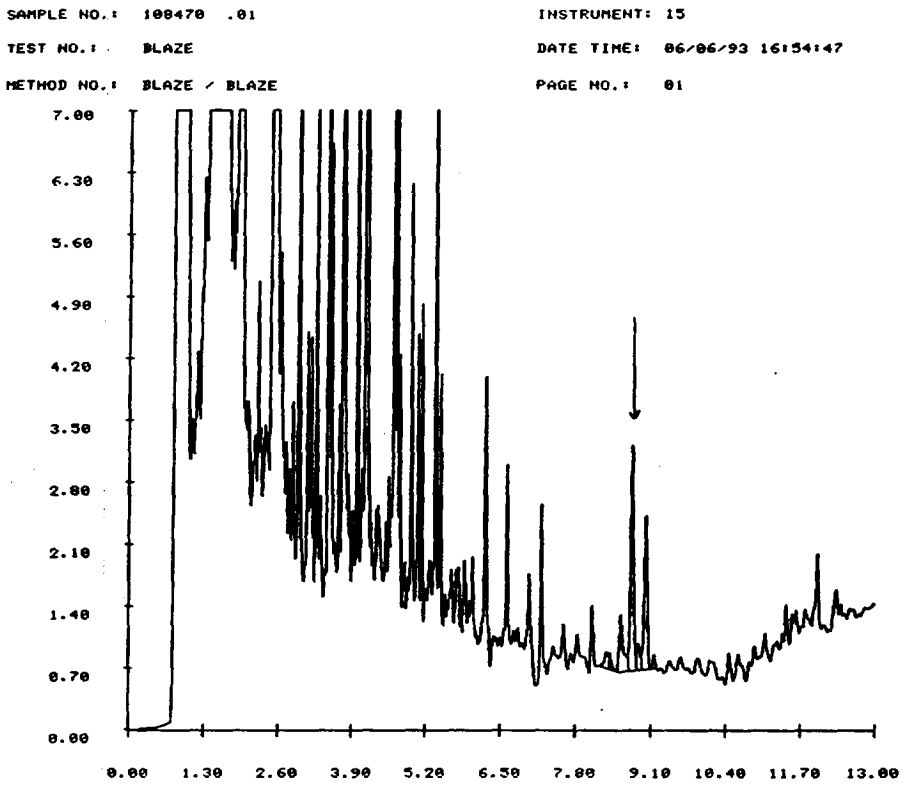
SAMPLE: 108469 .01 INST:15 VIAL: 0 SEQ NUMBER:006
TEST : BLAZE DATE-TIME COLLECTED : 06/06/93 16:36:33
COLLECTION TIME : 13.00 DATE-TIME PROCESSED : 06/06/93 16:49:46
METHOD: BLAZE / BLAZE ANALYST: U25517 SAMP RATE: 12.50
.02PPM ACIF01

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 4.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT PG
004	8.579			8.579	1.0000E+0	T	2372	474.492
005	8.774	M	ACIFLUORFEN-METHYL	8.774		T	9179	1.271
006	8.875			8.875	1.0000E+0	T	1082	216.430
007	9.009			9.009	1.0000E+0	V	4659	931.734
							17292	1.624E+3

Figure 26: Typical chromatogram of a control soybean grain sample fortified with 0.02 ppm of BAS 9048 H (the quantitation limit). Sample number 92940-1, lab code 108470. The sample weight injected was 1.0 mg. The sample contained 14.8 pg of BH 9048-ME which is equivalent to 0.014 ppm of BAS 9048 H.

Recovery of BAS 9048 H: 71%



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/07/93 11:27:58

MULTILEVEL EXTERNAL STANDARD

SAMPLE: 108470 .01
TEST : BLAZE
COLLECTION TIME : 13.00
METHOD: BLAZE / BLAZE

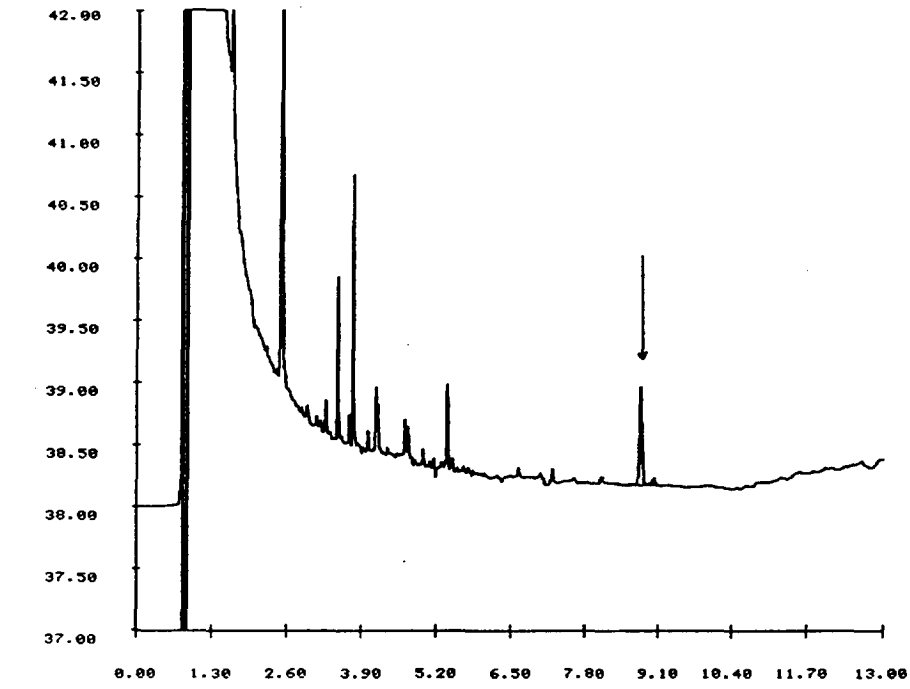
INST:15 VIAL: 0 SEQ NUMBER:007
DATE-TIME COLLECTED : 06/06/93 16:54:47
DATE-TIME PROCESSED : 06/06/93 17:00:01
ANALYST: U25517 SAMP RATE: 12.50
.02PPM ACIF02

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 4.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT PG
003	8.577			8.577	1.0000E+0	T	2463	492.602
004	8.773	M	ACIFLUORFEN-METHYL	8.773		T	9585	1.401
005	8.871			8.871	1.0000E+0	T	1147	229.344
006	9.013			9.013	1.0000E+0	V	6556	1311.258
							19751	2.035E+3

Figure 27. Typical chromatogram of a control soybean grain sample fortified with 0.20 ppm of BAS 9048 H. Sample number 92940-1, lab code 108473. The sample weight injected was 0.1 mg. The sample contained 16.2 pg of BH 9048-ME which is equivalent to 0.156 ppm of BAS 9048 H.

Recovery of BAS 9048 H: 78%



PERKIN-ELMER LIMS 2000/2 B A S F RTP, NC 06/07/93 11:19:54

MULTILEVEL EXTERNAL STANDARD

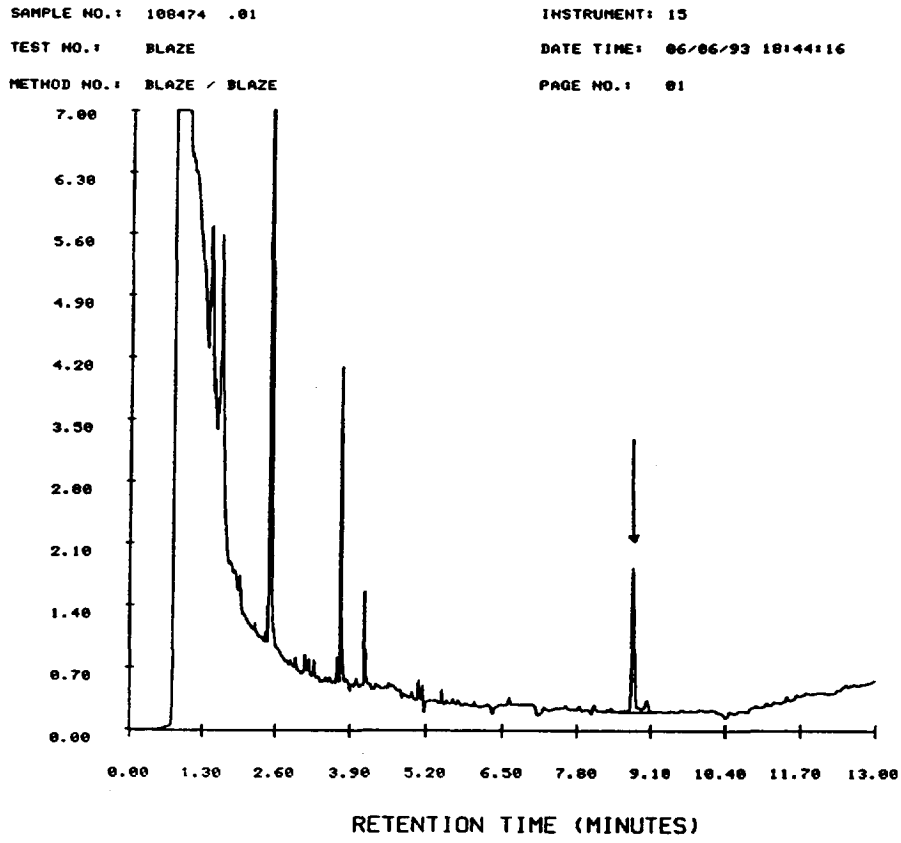
SAMPLE: 108473 .01 INST:15 VIAL: 0 SEQ NUMBER:012
TEST : BLAZE DATE-TIME COLLECTED : 06/06/93 18:26:01
COLLECTION TIME : 13.00 DATE-TIME PROCESSED : 06/06/93 18:39:16
METHOD: BLAZE / BLAZE ANALYST: U25517 SAMP RATE: 12.50
.20PPM ACIF01

SAMPLE WT.: 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 40.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT PG
004	8.777	M	ACIFLUORFEN-METHYL	8.777		T	10445	16.773
							10445	16.773

Figure 28. Typical chromatogram of a control soybean grain sample fortified with 0.20 ppm of BAS 9048 H. Sample number 92940-1, 108474. The sample weight injected was 0.1 mg. The sample contained 18.5 pg of BH 9048-ME which is equivalent to 0.178 ppm of BAS 9048 H.

Recovery of BAS 9048 H: 89%



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/07/93 11:29:35

MULTILEVEL EXTERNAL STANDARD

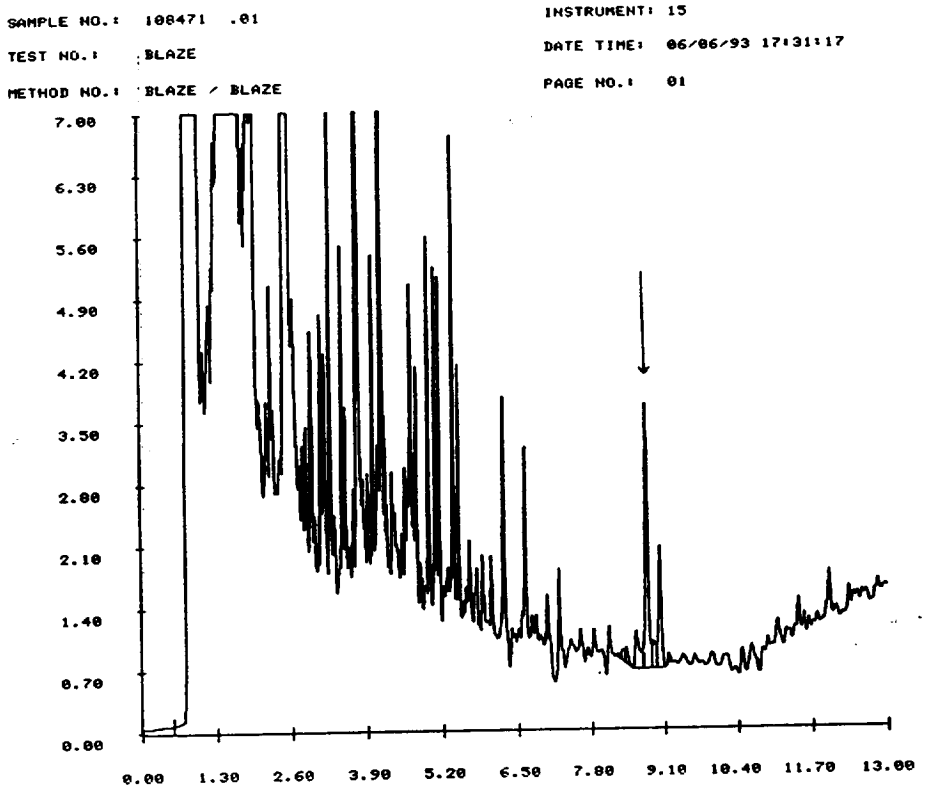
SAMPLE: 108474 .01 INST:15 VIAL: 0 SEQ NUMBER:013
TEST : BLAZE DATE-TIME COLLECTED : 06/06/93 18:44:16
COLLECTION TIME : 13.00 DATE-TIME PROCESSED : 06/06/93 19:57:31
METHOD: BLAZE / BLAZE ANALYST: U25517 SAMP RATE: 12.50
.20PPM ACIF#2

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 40.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT PG
004	8.780	M	ACIFLUORFEN-METHYL	8.780		T	11931	21.546
005	9.024			9.024	1.0000E+0		1002	2003.828
							12933	2.026E+3

Figure 29.- Typical chromatogram of a control soybean grain sample fortified with 0.02 ppm of BH 9048-ME (the quantitation limit). Sample number 92940-1, lab code 108471. The sample weight injected was 1.0 mg. The sample contained 16.8 pg of BH 9048-ME which is equivalent to 0.017 ppm of BH 9048-ME.

Recovery of BH 9048-ME: 84%



PERKIN-ELMER LIMS 2000/B B A S F RTP, NC 06/07/93 11:28:35

MULTILEVEL EXTERNAL STANDARD

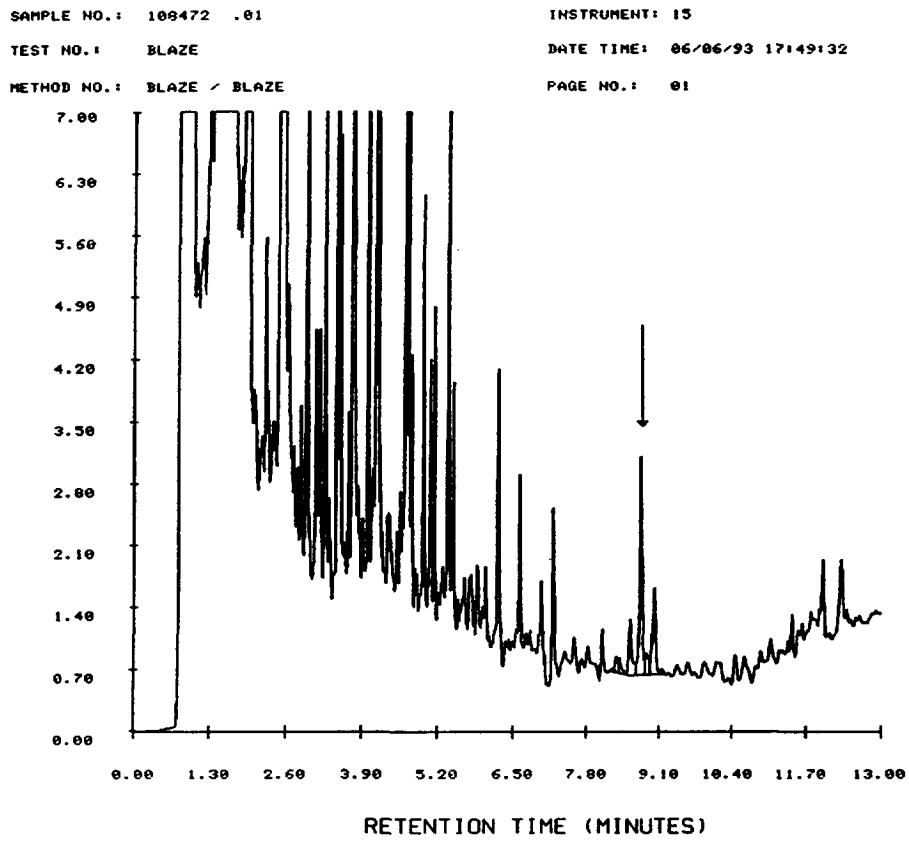
SAMPLE: 108471 .01 INST:15 VIAL: 0 SEQ NUMBER:009
TEST : BLAZE DATE-TIME COLLECTED : 06/06/93 17:31:17
COLLECTION TIME : 13.00 DATE-TIME PROCESSED : 06/06/93 17:44:31
METHOD: BLAZE / BLAZE ANALYST: U25517 SAMP RATE: 12.50
.02PPM ACIF-ME01

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 4.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT PG
004	8.581			8.581	1.0000E+0	T	1527	305.313
005	8.773	M	ACIFLUORFEN-METHYL	8.773		T	10861	1.811
006	8.874			8.874	1.0000E+0	T	1134	226.836
007	9.015			9.015	1.0000E+0	V	4966	993.125
							18488	1.527E+3

Figure 30. Typical chromatogram of a control soybean grain sample fortified with 0.02 ppm of BH 9048-ME (the quantitation limit). Sample number 92940-1, lab code 108472. The sample weight injected was 1.0 mg. The sample contained 18.1 pg of BH 9048-ME which is equivalent to 0.018 ppm of BH 9048-ME.

Recovery of BH 9048-ME: 91%



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/07/93 11:29:11

MULTILEVEL EXTERNAL STANDARD

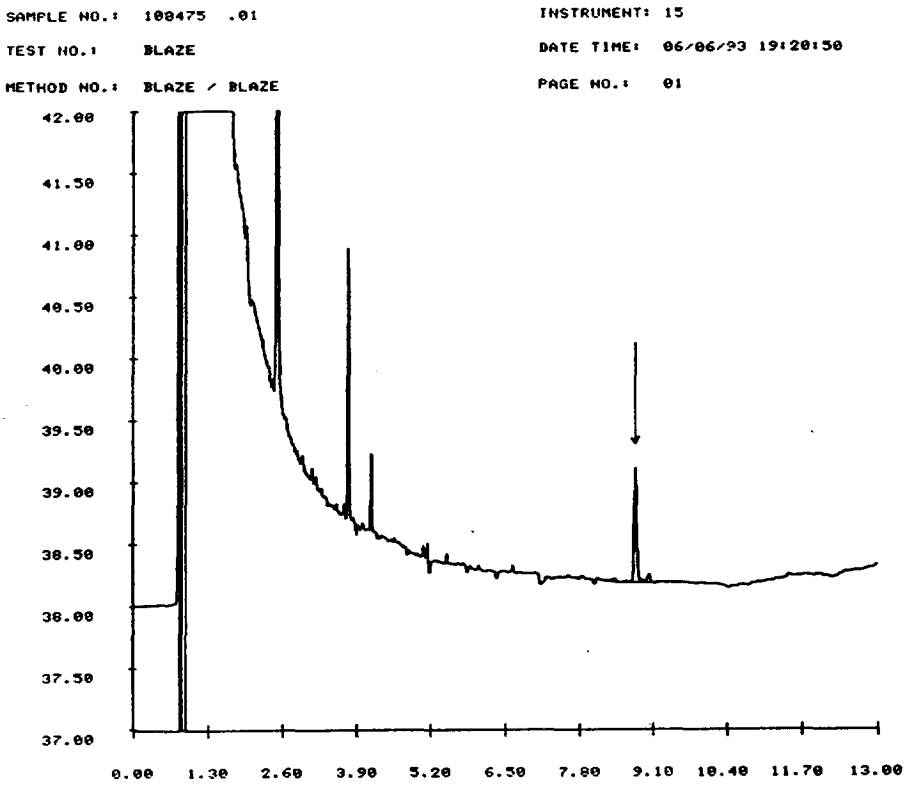
SAMPLE: 108472 .01 INST:15 VIAL: 0 SEQ NUMBER:010
TEST : BLAZE DATE-TIME COLLECTED : 06/06/93 17:49:32
COLLECTION TIME : 13.00 DATE-TIME PROCESSED : 06/06/93 18:02:46
METHOD: BLAZE / BLAZE ANALYST: U25517 SAMP RATE: 12.50
.02PPM ACIF-ME02

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 4.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT PG
004	8.579			8.579	1.0000E+0	T	2995	599.039
005	8.775	M	ACIFLUORFEN-METHYL	8.775		T	11673	2.072
006	8.877			8.877	1.0000E+0	T	1156	231.189
007	9.019			9.019	1.0000E+0	T	4617	923.453
							20441	1.756E+3

Figure 31. Typical chromatogram of a control soybean grain sample fortified with 0.20 ppm of BH 9048-ME. Sample number 92940-1, lab code 108475. The sample weight injected was 0.1 mg. The sample contained 18.7 pg of BH 9048-ME which is equivalent to 0.187 ppm of BH 9048-ME.

Recovery of BH 9048-ME: 93%



PERKIN-ELMER LIMS 2000/3 B A S F RTP, NC 06/07/93 11:20:16

EXTERNAL STANDARD

SAMPLE: 108475 .01 INST:15 VIAL: 0 SEQ NUMBER:015
TEST : BLAZE DATE-TIME COLLECTED : 06/06/93 19:20:50
COLLECTION TIME : 13.00 DATE-TIME PROCESSED : 06/07/93 10:47:01
METHOD: BLAZE / BLAZE ANALYST: U25517 SAMP RATE: 12.50
.20PPM ACIF-ME01

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 40.0000

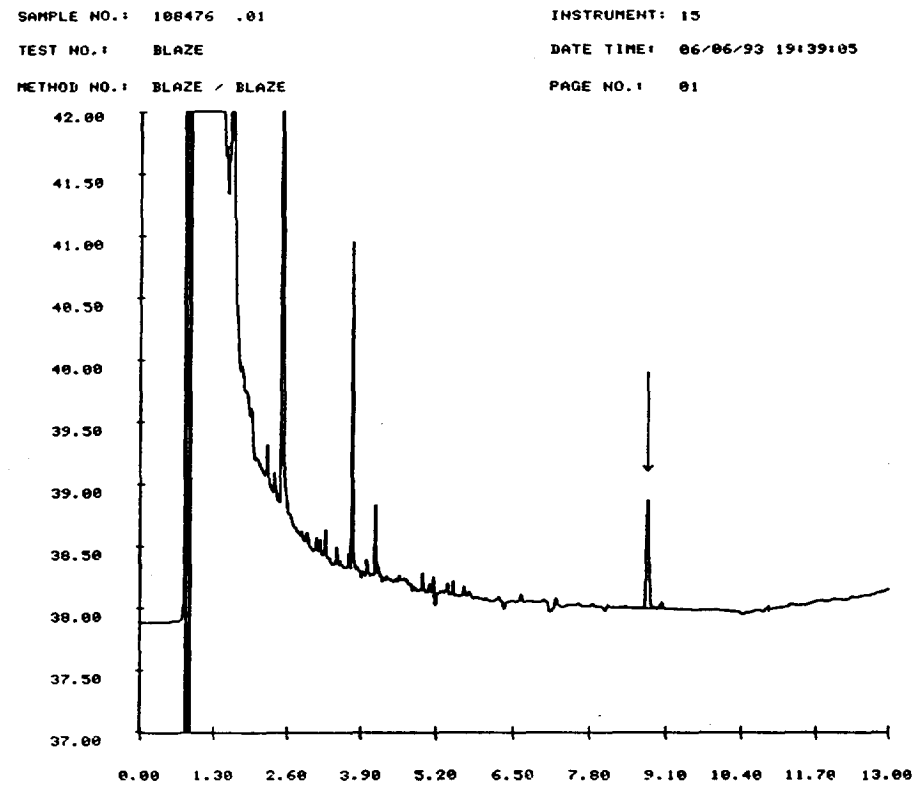
PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT PG
003	8.787	M	ACIFLUORFEN-METHYL	8.787	1.0000E+0	T	12015	2.403E+4
004	9.023			9.023	1.0000E+0	V	1015	2038.859
							13030	26068.160

GROUP REPORT - 108475 .01

GP#	GROUP NAME	HEIGHT PG
M		24029.320

Figure 32. Typical chromatogram of a control soybean grain sample fortified with 0.20 ppm of BH 9048-ME. Sample number 92940-1, lab code 108476. The sample weight injected was 0.1 mg. The sample contained 17.8 pg of BH 9048-ME which is equivalent to 0.178 ppm of BH 9048-ME.

Recovery of BH 9048-ME: 89%



PERKIN-ELMER LINS 2000/3 B A S F RTP, NC 06/07/93 11:20:39

EXTERNAL STANDARD

SAMPLE: 108476 .01 INST:15 VIAL: 0 SEQ NUMBER:016
TEST : BLAZE DATE-TIME COLLECTED : 06/06/93 19:39:05
COLLECTION TIME : 13.00 DATE-TIME PROCESSED : 06/07/93 10:48:19
METHOD: BLAZE / BLAZE ANALYST: U25517 SAMP RATE: 12.50
.20PPM ACIF-ME02

SAMPLE WT : 20.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 40.0000

PK NO	RT MINUTES	GR NO	COMPONENT NAME	RRT MINUTES	RESPONSE FACTOR	BL	PEAK HEIGHT	HEIGHT PG
003	8.785	M	ACIFLUORFEN-METHYL	8.785	1.0000E+0	T	11454	2.291E+4
							11454	22908.670

GROUP REPORT - 108476 .01

GP#	GROUP NAME	HEIGHT PG
M		22908.690