Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Tepraloxydim

MRID #: 444672-45

Matrix: Soil

Analysis: LC/MS/MS

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PERFORMING LABORATORY

BASF CORPORATION
AGRICULTURAL PRODUCTS GROUP
Agricultural Products Center
Post Office Box 13528
Research Triangle Park, NC 27709

STUDY TITLE

BASF Method Number D96115: Analytical Method for the Determination of Caloxydim Metabolite BH 620-FP in Soil Using LC/MS/MS.

GUIDELINE REFERENCE

Subdivision N, #164-1

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Validation Work by:	Jeannine Jordan

PROTOCOL:	97018 (Laboratory ID No.)
REPORT:	97/5089

ABSTRACT:

The method, developed at BASF, determines residues of caloxydim metabolite BH 620-FP in soil using LC/MS/MS. BH 620-FP is a metabolite of the active ingredient caloxydim that was found in significant quantities during environmental fate studies [1]. In this method a soil sample is extracted with methanol/water, the extract is concentrated and filtered, and analyzed directly by LC/MS/MS. The limit of quantitation is 0.01 ppm for the analyte. A validation was done by fortifying two sets of control soils at 0.01, 0.10, and 1.0 ppm of BH 620-FP. Recovery results for 0.01 ppm, 0.1 ppm, and 1.0 ppm respectively are 99%, 84%, and 91%.

Study Experimental Start Date: January 15, 1997
Experimental Completion Date: March 7, 1997
Study End Date: May 28, 1997
Pages of Report: 24

STUDY COMPLETION DATE

May 29, 1997

TOTAL PAGES

24

Rec'd ACB 6/3/99

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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 E. Warkentien

Title: Senior Registration Specialist

Signature: _____

Date: 8 DEC 97

GOOD LABORATORY PRACTICE STATEMENT

BASF Method Number D96115: Analytical Method for the Determination of Caloxydim Metabolite BH 620-FP in Soil Using LC/MS/MS

Compliance with Good Laboratory Practice Standards

The study described in this report was conducted in compliance with the Good Laboratory Practice Standards as described in the United States Environmental Protection Agency, Title 40 Code of Federal Regulations Part 160, Federal Register, issued 17 August 1989.

Jeannine Jordan 12/8/97

Jeannine Jordan

Study Director

8 DEC 97

Karen E. Warkentien
Senior Registration Specialist
BASF Corporation
Sponsor/Submitter

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Statement of the BASF Quality Assurance Unit

Report Number: 97/5089
Protocol Number: 97018

Name/Number of Test Substance: BH -620-FP

Type of Study: BASF Method Number D96115: Analytical Method for the Determination of Caloxydim Metabolite BH-620-FP in Soil Using LC/MS/MS.

The BASF Quality Assurance Unit of the testing facility at the APC has inspected/audited the protocol, study, raw data and final report of this study and reported any findings to the study director and management.

Date of Inspection	Reported to Study Director and Management
January 14, 1997	January 14, 1997
January 20, 1997	January 20, 1997
May 9, 1997	May 9, 1997

Judy Fesch
Signature of QAU

1.0 INTRODUCTION AND SUMMARY

1.1 SCOPE AND SOURCE OF THE METHOD

1.1.1 Purpose of Study and Scope

Caloxydim, a herbicide jointly developed by BASF and NISSO, is effective in controlling annual and perennial grasses. The purpose of this study is to validate and report this method which determines the residues of caloxydim metabolite BH 620-FP in soil using LC/MS/MS. BH 620-FP is a metabolite of the active ingredient caloxydim that was found in significant quantities during environmental fate studies [1].

1.1.2 Source

The method was developed at BASF

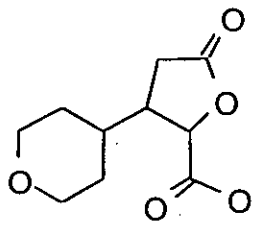
1.2 TEST AND REFERENCE SUBSTANCES

Fortification and HPLC Standards

BASF code: BH 620-FP

Chemical name: 3-(tetrahydropyran-4-yl)-5-oxotetrahydrofuran-2-carboxylic acid

Structural formula:



Empirical formula: $C_{10}H_{14}O_5$

Molecular weight: 214.22 g/mol

Appearance: white solid

1.3 PRINCIPLE OF THE METHOD

The soil is extracted, the extract concentrated and filtered, and analyzed by LC/MS/MS. The limit of quantitation (LOQ) is 0.01 ppm.

2.0 MATERIALS AND METHODS

2.1 EQUIPMENT — SUGGESTED SIZES/MANUFACTURER

Volumetric flasks:	10, 25, 50, 100 and 250 mL
Volumetric pipettes:	5, 10, 25 and 50 mL
Adjustable pipettes:	100 µL and 1000 µL, Gilson
Erlenmeyer flask:	1000 mL
Graduated cylinders:	100 mL , 250 mL, and 1000 mL
Autoinjector vials:	1.5 mL Perkin Elmer
Centrifuge filters:	0.1 µm , Ultrafree-MC, Non-Sterile, Millipore
Pasteur Pipettes:	5 3/4"
Positive displacement pipettes:	50 µl Fisherbrand

Other general laboratory glassware and supplies.

Note: Equivalent equipment may be used.

2.2 REAGENTS AND CHEMICALS — SUGGESTED SOURCE/PREPARATION

Methanol, CAS 67-56-1	Baxter Healthcare Corporation, B&J Brand
Water, CAS 7732-18-5	Millipore water system
Ammonium Formate	Fluka

Note: Equivalent reagents and chemicals may be substituted.

2.3 STANDARD SUBSTANCES AND SOLUTIONS

Compound	Code	Lot Number	Purity
3-(tetrahydropyran-4-yl)-5-oxotetrahydrofuran-2-carboxylic acid	BH 620-FP	31-6477-A0	96.1%

Standards supplied by:

Dr. Rita Roscher

BASF Aktiengesellschaft, APS/UP

Landwirtschaftliche Versuchsstation

D-67114 Limburgerhof, Germany

Telephone: 06236/68/27103

Solid BH 620-FP was maintained frozen (< -5°C) until use in this study. This substance was characterized as required by 40 CFR part 160, FIFRA Good Laboratory Practices. Information on the synthesis and subsequent characterization of this substance is available from BASF at Landwirtschaftliche Versuchsstation, Limburgerhof, Germany.

2.3.1 Standard Solutions for Fortifications

BH 620-FP, 1.00 mg/mL, 100.0 µg/mL and 10.0 µg/mL in methanol.

These concentrations are suggested. Different preparation and concentration schemes may be used and additional standard concentrations may be prepared and used as needed.

Stock Solution Preparation

Note: Be sure the solutions are at room temperature and sonicated for about 5 minutes prior to taking aliquots for dilution.

Prepare a 1.00 mg/mL BH 620-FP stock solution by weighing an appropriate amount of BH 620-FP into an appropriate volumetric flask. Dissolve in methanol with adequate vortexing and dilute to the mark. For example, to prepare a 10 mL stock solution of BH 620-FP weigh 10.0 mg of BH 620-FP into a 10 mL volumetric flask. Mix well before preparing further dilutions using this stock solution.

Prepare a 100.0 µg/mL standard solution of BH 620-FP by transferring an appropriate amount of the 1.00 mg/mL stock solution with a volumetric pipette into a volumetric flask. For example, transfer 5 mL of the 1.00 mg/mL solution of BH 620-FP into a 50 mL volumetric flask. Add some methanol, vortex well and dilute to the mark with methanol. Mix well before preparing further dilutions using this standard.

Prepare a 10.0 µg/mL standard solution of BH 620-FP by transferring an appropriate amount of the 100.0 µg/mL standard solution with a volumetric pipette into a volumetric flask (typically 5 mL of the 100.0 µg/mL stock solution into a 50 mL volumetric flask). Dilute to the mark with methanol. Mix well before preparing further dilutions using this standard.

Transfer each stock and standard solution to an amber glass bottle with a Teflon-lined screw cap and store either in the refrigerator or freezer. Replace stock solutions every 3 months after preparation and standard fortification solutions 30 days after preparation.

2.3.2 Calibration Curve Standard Solutions for LC/MS/MS analysis:

50.0 ng /mL, 100.0 ng /mL, 250.0 ng /mL and 500.0 ng /mL BH 620-FP in a 1:1 mixture of the HPLC mobile phases A and B.

Prepare a 1.0 µg/mL standard solution of BH 620-FP by transferring an appropriate amount of the 10.0 µg/mL standard solution with a volumetric pipette into a volumetric flask (typically 5 mL of the 10.0 µg/mL stock solution into a 50 mL volumetric flask). Dilute to the mark with methanol. Mix well before preparing further dilutions using this standard.

Prepare a 50.0 ng/mL standard solution of BH 620-FP by transferring an appropriate amount of the 1.0 µg/mL standard solution with a volumetric pipette into a volumetric flask (typically 5 mL of the 1.0 µg/mL stock solution into a 100 mL volumetric flask). Dilute to the mark with the 1:1 mixture of the HPLC solvents A and B and mix well.

Prepare a 100.0 ng/mL standard solution of BH 620-FP by transferring an appropriate amount of the 1.0 µg/mL standard solution with a volumetric pipette into a volumetric flask (typically 10 mL of the 1.0 µg/mL stock solution into a 100 mL volumetric flask). Dilute to the mark with the 1:1 mixture of the HPLC solvents A and B and mix well.

Prepare a 250.0 ng/mL standard solution of BH 620-FP by transferring an appropriate amount of the 1.0 µg/mL standard solution with a volumetric pipette into a volumetric flask (typically 25 mL of the 1.0 µg/mL stock solution into a 100 mL volumetric flask). Dilute to the mark with the 1:1 mixture of the HPLC solvents A and B and mix well.

Prepare a 500.0 ng/mL standard solution of BH 620-FP by transferring an appropriate amount of the 1.0 µg/mL standard solution with a volumetric pipette into a volumetric flask (typically 50 mL of the 1.0 µg/mL stock solution into a 100 mL volumetric flask). Dilute to the mark with the 1:1 mixture of the HPLC solvents A and B and mix well.

These concentrations are suggested. Different preparation and concentration schemes may be used and additional standard concentrations may be prepared and used as needed.

Note:

The compound BH 620-FP is not stable in acidic solutions over a longer period. Store the standard solutions in refrigerator and replace solutions no longer than 30 days after preparation.

3.0 ANALYTICAL PROCEDURE

3.1 METABOLITE ISOLATION

3.1.1 Sample Preparation

The bulk soil samples received from the field are homogenized by a sample preparation procedure prior to analysis. Homogenized samples are stored frozen (< -5°C) and allowed to thaw to room temperature before analysis. Weigh a 50 g (± 0.1 g) to the nearest tenth of a gram aliquot of the soil sample and add into a 150 mL centrifuge bottle.

3.1.2 Fortification of Procedural Recovery Sample

The recommendation is that each analysis set include one untreated sample and at least two procedural recovery samples. The procedural recovery samples should typically be run at the limit of quantitation and at a level comparable to the expected residue levels. For each procedural recovery sample transfer the appropriate amount of the standard solution by volumetric or positive displacement pipette to a weighed amount of the control soil. For example:

A transfer of 0.050 mL of the 100.0 ug/mL standard to 50.0 g of soil results in a fortification level of 1 ppm.

A transfer of 0.050 mL of the 10.0 µg/mL standard to 50.0 g of soil results in a fortification level of 0.1 ppm.

A transfer of 0.050 mL of the 1.0 µg/mL standard to 50.0 g of soil results in a fortification level of 0.01 ppm.

The precision and accuracy of pipettes used to transfer volumes of less than 0.5 mL must be addressed in a standard operating procedure which includes routine calibration. Prior to the use of pipettes to transfer volumes of less than 0.5 mL in the study, the accuracy and precision of the transfer of similar volumes of the solvent used for the administration of the test substance must be established. For example, the transfer of 10 aliquots of the solvent to be used with a mean accuracy of > 95 % and a range of variability of < 5 % of weight transferred at the 100 uL range would be acceptable. The conduct and results of the test for precision and accuracy of the pipettes used to deliver volumes of less than 0.5 mL must be included in the raw data for the study.

3.1.3 Extraction

Add 80 mL of a mixture of methanol / water (4:1) to the centrifuge bottle containing the soil (50 g) and shake at 300 rpm for 20 minutes.

The mixture of methanol / water is prepared by measuring 800 mL of methanol and 200 mL of water in two separate graduated cylinders and combining the two solvents in a 1000 mL Erlenmeyer flask. Shake the mixture well before using.

Centrifuge at 3000 rpm for 10 minutes. Decant the supernatant into a 250 mL flat bottom flask. Add 80 mL of the mixture of methanol / water (4:1) to the soil marc, vortex to loosen the soil and allow to mix for consistency. Shake at 300 rpm for 20 minutes. Centrifuge at 3000 rpm for 10 min. Decant the supernatant into the above 250 mL flat bottom flask. Concentrate the combined extract to less than 2 mL (but not to dryness) using a rotary evaporator with the water bath set at 40°C. Remove the liquid as fast as possible without having the solution frothing or bumping (this should take less than 60 minutes).

3.1.4 Preparation of the Sample for Analysis

Transfer the concentrated extract to a 10 mL graduated centrifuge glass and fill up to 2.5 mL with water. Rinse the 250 mL flat bottom flask 2 times with 1 mL of the following methanolic solution and transfer to the centrifuge glass too. Shake well and fill up to 5 mL with the following methanolic solution and shake well again.

Methanolic solution: Methanol, 0.2% Formic Acid, 8 mM Ammonium formate

At this point of the extraction scheme the appropriate amount for further injections or dilutions has to be filtered by using a 0.1 µm centrifugal filter.

The extract of the fortification level of 0.01 ppm can be injected without further dilution. This solutions contains 100 ng/mL BH 620-FP.

The extract of the fortification level of 0.1 ppm should be diluted by factor 10 with a 1:1 mixture of the HPLC solvents A and B. For example take 0.2 mL and add 1.8 mL of a 1:1 mixture of the HPLC solvents A and B. This results in a solution with 100 ng/mL BH 620-FP.

The extract of the fortification level of 1 ppm should be diluted by factor 40 with a 1:1 mixture of the HPLC solvents A and B. For example take 0.04 mL and add 1.560 mL of a 1:1 mixture of the HPLC solvents A and B. This results in a solution with 250 ng/mL BH 620-FP.

If samples need to be diluted after the first injection, the dilution solvent should be a 1:1 mixture of HPLC solvent A and B.

3.2 INSTRUMENTATION

3.2.1 Description of Instrumentation

HPLC Pump:	Varian 9010
Autoinjector:	Perkin Elmer Series 200
Data System:	Macintosh 8500/120 Power Macintosh
Column:	Prism RP (100 x 3.2 mm)
	Keystone Scientific, Bellefonte, PA
	P/N 103-321-3

Note: Other equivalent hardware may be used. The use of a guard column is optional.

Mass Spectrometer: PE SCIEX API 300

Turbo Ionspray was used to enhance sensitivity.

This method could conceivably be validated on another instrument manufacturer's platform. If this is the case, instrument parameters and flow splits will be different, however, the basic principles of analysis by LC/MS/MS will remain the same.

3.2.2 Typical Operating Conditions

HPLC Operating Conditions:

Mobile Phase A: Water with 0.1% Formic Acid and 4 mM Ammonium formate

Mobile Phase B: Methanol with 0.1% Formic Acid and 4 mM Ammonium formate

Flow: 0.2 mL/min

Isocratic: (A/B) 40% HPLC Solvent A

60% HPLC Solvent B

Injection Volume: 10 µL into 200 µL loop

To make the mobile phase Use 999 mL of Water or MeOH, add 1 mL of Formic acid and 252 mg of Ammonium Formate.

MS Operating Conditions:

Mode: Negative ion mode for the analysis of BH 620-FP.

Turbo Ionspray is used to enhance sensitivity but is not required if adequate sensitivity can be achieved without the use of Turbo Ionspray.

Note: The following instrument parameter were found to be optimal for the instrument used for the method development. The exact values used must be optimized for each instrument. It is recommended to optimize the instrument for the analyses of BH 620-FP with the 50 ng/mL standard solution. A peak to noise ratio of at least 5:1 should be achieved.

Turbo Temperature: 250°C (not applicable without Turbo)

Typical Retention Times:

Analyte	Transition	Retention Time (approx.)
BH 620-FP	213.0 / 185.0	5.04 minutes
BH 620-FP	213.0 / 169.0	5.04 minutes

3.2.3 Calibration Procedures

The standard curve is derived using the area response of the analytes (y) versus the concentration of the native compounds (x) from standards injected with the analysis set. A weighted linear regression (1/x) standard curve is used for quantitation of all samples.

A five point calibration curve is used for quantitation of sample extracts. At lease two standards at each concentration must be injected in an analysis set.

Instrument analysis must begin and end with the injection of a standard. No more than three samples may be injected between standard injections.

Acceptance of each sample set will be made by evaluation of the correlation coefficient for each analyte. Correlation coefficients must be >0.98.

3.2.4 Sample Analysis

Inject 10 µL of the calibration standards and samples. Depending on the instrument sensitivity the method may be validated with smaller or larger injection volumes.

Directly compare the response (peak area) of unknown samples injected with the standard curve to obtain the weight of BH 620-FP in the sample.

Bracket samples with standards to check for shifts in sensitivity. It is recommended that two standards are injected at the beginning and two at the end of the set to ensure the bracketing of samples. If the peak area of the unknown is larger than the highest standard, dilute the unknown appropriately and reinject.

Standards and extracts are analyzed using a reverse phase liquid chromatography column interfaced to a triple stage mass spectrometer using Ionspray (ISV) atmospheric pressure ionization (API). The analysis is performed by isocratic elution. It is recommended to use Turbo Ionspray in order to enhance sensitivity.

Note:
This method could conceivably be validated on another instrument manufacturer's platform. If this is the case, instrument parameters and flow splits will be different, however, the basic principles of analysis by LC/MS/MS will remain the same.

3.3 INTERFERENCES

3.3.1 Sample Matrices

Cleaning the chromatographic system and column periodically by injecting solvent and running a gradient is desirable.

3.3.2 Other Sources

Other Pesticides:	None known to date
Solvents:	None known to date
Labware:	None known to date

3.4 CONFIRMATORY TECHNIQUES

The structural identity of the analytes can be confirmed by LC/MS.

3.5 TIME REQUIRED FOR ANALYSIS

Analysis of a set of 8 soil samples requires 2 working days including data analysis.

3.6 POTENTIAL PROBLEMS

BH 620-FP is susceptible to heat. In Section 3.1.3 be sure the water bath is at 40°C. After centrifuging in Step 3.1.3, the soil marc may have formed a pellet. Be sure to vortex the sample to be sure the soil is thoroughly mixed in the next extraction step. Sensitivity of the LC/MS/MS must be optimized to be sure of a signal to noise of 3 to 1 on the lowest standard.

4.0 METHOD OF CALCULATION

4.1 CALIBRATION

Construct a linear least squares working curve (weighted 1/x) in the form y =bx + c from the standards by plotting peak area versus concentration of standard injected.

4.2 ANALYTE IN SAMPLE

Calculate results based on the peak area measurements. Using the peak area measurements for BH 620-FP in the samples, determine the amount of BH 620-FP in the samples from the appropriate least squares calibration curve.

Calculate ppm values by the equation below.

$$\text{ppm} = \frac{(A) \times (B)}{1000}$$

where A = ppb value interpolated from standard curve

B = Dilution Factor = (final volume / original volume)

The "final dilution volume" includes any dilutions which have been made.

4.3 CALCULATION OF PROCEDURAL RECOVERIES

Correct results in the procedural fortifications for residues found in the control sample as follows:

ppm (corrected) = ppm in fortified control - ppm in control

Determine percent recovery from the fortification experiments as follows:

$$\%\ \text{Recovery} = \frac{\text{ppm} \times 100}{\text{ppm BH 620-FP added}}$$

Do not correct treated sample results for either control residues or procedural recoveries.

Procedural recoveries for the validation are statistically averaged and a standard deviation obtained.

5.0 VALIDATION

The validity of this method was established by fortifying two sets of control soil at levels of 0.01 ppm, 0.1 ppm, and 1.0 ppm with BH 620-FP. The soil used was from an on-going soil dissipation study for BAS 620 H from RCNs 95006 and 95007. The validation results are shown in Table 1.

6.0 RESULTS AND DISCUSSION

6.1 GENERAL

Representative chromatograms of residue analyses of selected control and fortified samples are shown in Figures 3 and 4. Standard curves were generated from standard solutions containing BH 620-FP injected concurrently with the analysis set. Standard injections bracketed the sample injections. Typical standard chromatograms are shown in Figure 1. Typical standard curves are presented in Figure2.

6.2 ACCURACY AND PRECISION

Accuracy and precision of the method is represented in the statistical interpretation of the validation data. The mean of the eleven fortified samples is 91.3 ± 15.5. The relative standard deviation is 12.5%.

6.3 DETERMINATION LIMIT

For this method, the limit of quantitation for BH 620-FP residues in soil was not statistically calculated. The limit of quantitation was determined as 0.01 µg/g (or 0.01 ppm) as it is the lowest fortification used that resulted in acceptable method recoveries.

6.4 RUGGEDNESS TESTING

Two different soil types were used for the validation of this method to establish ruggedness. A third type was used for the development stage. Approximately 5 sets was performed for the method development and two for the validation. These sets show an acceptable ruggedness for the method.

6.5 LIMITATIONS

BH 620-FP breaks into eight various components in the MS/MS. The method uses the monitoring of only one of these fragment ions. The LC/MS/MS needs to be fully optimized for the highest sensitivity . The signal to noise for the lowest standard should be 3 to 1 for the method to be running properly. None known to date.

7.0 STANDARD STABILITY

Standard stability was tested by injecting a set of standards on the LC/MS/MS in 1, 4, and 6 week intervals. Criteria for stability is that the relative standard deviation between old and new standards does not exceed 15%. The BH 620-FP standards have been shown to be stable for 38 days in refrigeration at -5°C.

8.0 CONCLUSION

This method is valid for the determination of BH 620-FP in soil.

9.0 QUALITY ASSURANCE PROCEDURE

The raw data and final copy of the method will be stored in the BASF archives at:

BASF Corporation

Agricultural Products Center

26 Davis Drive

Research Triangle Park, NC 27709-3528

9.0 REFERENCES

Shiotani, Hironori, "BAS 620H - Photodegradation on Soil", NISSO Report No. EC-518, Study in Progress.

10.0 SAFETY AND HEALTH CONSIDERATIONS

10.1 GENERAL

Use personal protective equipment such as lab coats, safety glasses and gloves (nitrile/latex gloves are recommended) when performing the operations described in this method. Dispose of hazardous wastes in an environmentally acceptable manner, in compliance with applicable laws and regulations.

10.2 SOLVENTS, REAGENTS AND STANDARDS

Review of the Material Safety Data Sheets (MSDSs) for all solvents and reagents used in this method is recommended prior to initiation of analysis.

Note that formic acid is easily absorbed through the skin and causes severe burns to the skin. Safe handling precautions in using this compound include avoidance of contact with skin and eyes, using only in an adequately ventilated area, and using gloves and proper clothing .

SIGNATURES

Jeannine Jordan - Study Director — 5-29-97 Date

Dr. Arno Krotzky - BASF Management — 5/29/97 Date

Table 1. BH 620-FP Method Validation Results

Description	Sample Number	File Name	Peak Area	Recovery
Set 1				
control	1154-4-12	C970127013	---	---
0.01 ppm A	1154-4-13	C970127015	6197	106.8
0.01 ppm B	1154-4-14	C970127017	*	*
0.10 ppm A	1154-4-15	C970127019	4330	76.7
0.10 ppm B	1154-4-16	C970127021	6816	116.8
1.0 ppm A	1154-4-17	C970127023	5188	90.6
1.0 ppm B	1154-4-18	C970127024	4539	80.1
Set 2				
control	1154-11-11	C970129006	---	---
0.01 ppm A	1154-11-12	C970129008	6352	107.4
0.01 ppm B	1154-11-13	C970129009	5477	93.7
0.10 ppm A	1154-11-14	C970129011	4025	71.1
0.10 ppm B	1154-11-15	C970129013	4008	70.8
1.0 ppm A	1154-11-16	C970129015	5920	100.6
1.0 ppm B	1154-11-17	C970129016	5231	89.9

* integration of sample is unacceptable therefore sample not used.

Mean = 91.3

SD = 15.5

RSD = 12.5

Table 2. Set 1154-4 Method Validation Set 1 Standard Curve Information

Intercept = -433.163

Slope = 6.208

Correlation Coefficient = 0.999

Description	Standard Number	Filename	Peak Area
50 ng/mL	1154-7-8	C970127008	2466
100 ng/mL	1154-7-10	C970127009	5963
250 ng/mL	1154-7-11	C970127010	15693
500 ng/mL	1154-7-12	C970127011	30890
50 ng/mL	1154-7-8	C970127014	2526
100 ng/mL	1154-7-10	C970127018	5887
250 ng/mL	1154-7-11	C970127020	15830
500 ng/mL	1154-7-12	C970127022	30354
50 ng/mL	1154-7-8	C970127025	2446
100 ng/mL	1154-7-10	C970127026	5141
250 ng/mL	1154-7-11	C970127027	15298
500 ng/mL	1154-7-12	C970127028	29911

Figure 1. Standard Curve for Set 1154-4 Method Validation Set 1

MacQuan, version 1.5b3 Page 1 of 1
Printed: Tue, Jan 28, 1997 08:08
Calibration File: Cali012797 Path: API 300 #2 :MS DATA:GLP DATA:1997:01/97:012797:
Comments: BH 620-FP SOIL METHOD VALIDATION SET #1 STUDY NUMBER 97018 J.JORDAN.

99 1/27/97

FP-185 213.0->185.0 No Internal Standard
Linear
Intercept = -433.163
Slope = 6.208
Correlation Coeff. = 0.999

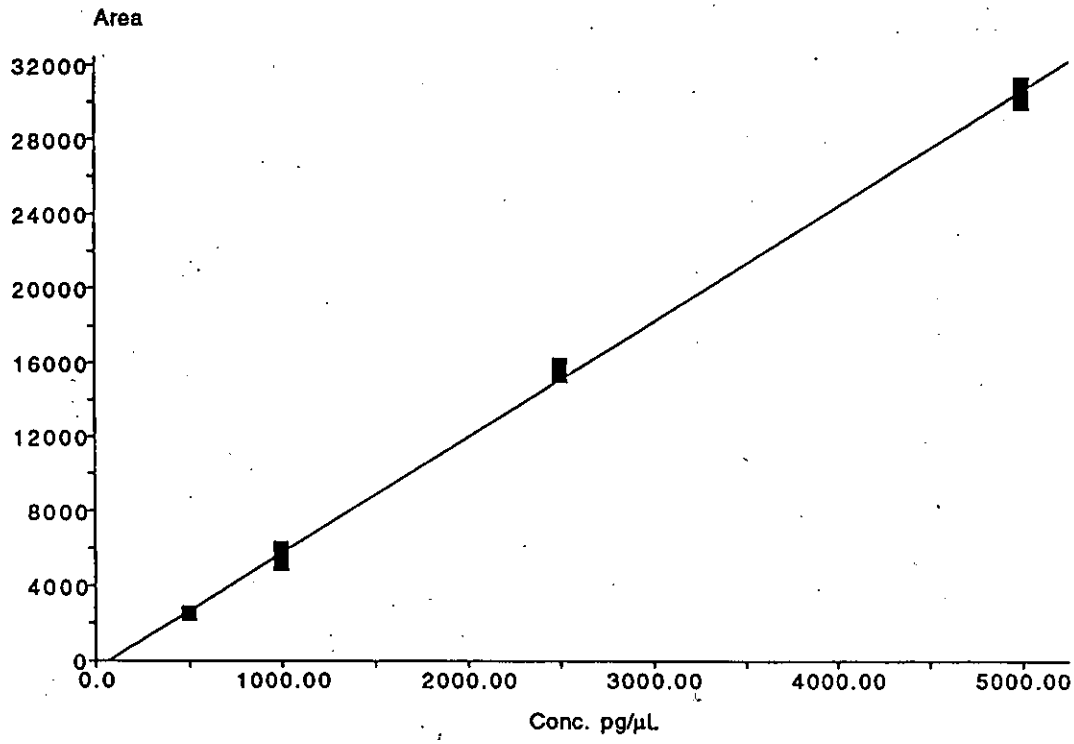
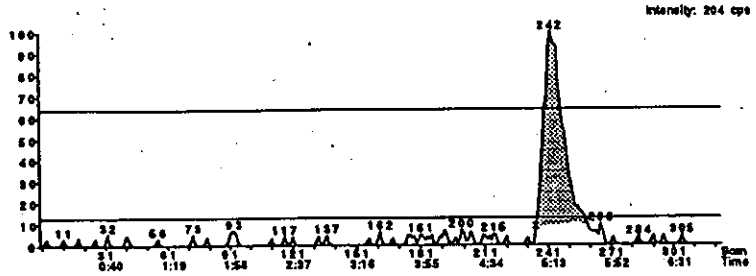


Figure 2. Standards 50 pg/uL, 100 pg/uL, 250 pg/mL, 500 pg/uL BH 620-FP

C970127006 1154-7-8 Mon, Jan 27, 1997 16:54
BASF784 ND MRM -ve10µl MS01 1154-7-8 Std 50 pg/µl

6:59 in 1 period
FP-185
No Internal Standard
Use Area

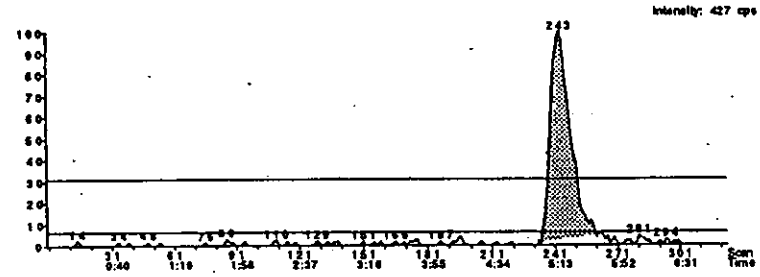
1: 6:59 MRM, 323 scans
213.0->185.0
Noise Thres. 10.0
Quant Thres. 2.0
Min. Width 5
Mult. Width 5
Base. Width 100
RT Win. (secs) 20
Smooth 1
Expected RT 6:15
Area 2468
Height 185
Start Time 5:56
End Time 6:36
Integration Width 0:39.8
Retention Time 6:15
Integration Type A - BB



C970127008 1154-7-10 Mon, Jan 27, 1997 17:02
BASF784 ND MRM -ve10µl MS01 FP 1154-7-10 Std 100 pg/µl

6:59 in 1 period
FP-185
No Internal Standard
Use Area

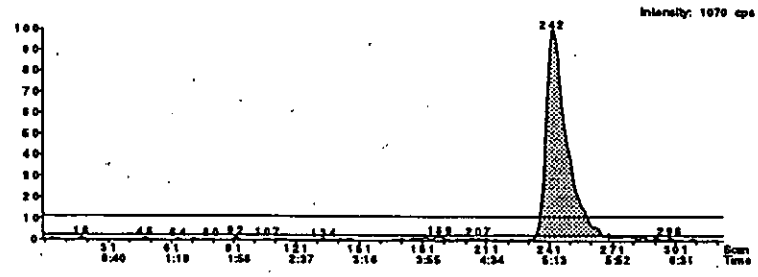
1: 6:59 MRM, 323 scans
213.0->185.0
Noise Thres. 10.0
Quant Thres. 2.0
Min. Width 5
Mult. Width 5
Base. Width 100
RT Win. (secs) 20
Smooth 1
Expected RT 6:15
Area 5863
Height 414
Start Time 6:04
End Time 6:38
Integration Width 0:33.8
Retention Time 6:16
Integration Type A - BB



C970127010 1154-7-11 Mon, Jan 27, 1997 17:10
BASF784 ND MRM -ve10µl MS 1 FP 1154-7-11 Std 250 pg/µl

6:59 in 1 period
FP-185
No Internal Standard
Use Area

1: 6:59 MRM, 323 scans
213.0->185.0
Noise Thres. 10.0
Quant Thres. 2.0
Min. Width 5
Mult. Width 5
Base. Width 100
RT Win. (secs) 20
Smooth 1
Expected RT 6:15
Area 15693
Height 1054
Start Time 6:04
End Time 6:48
Integration Width 0:44.2
Retention Time 6:15
Integration Type A - BB



C970127011 1154-7-12 Mon, Jan 27, 1997 17:18
BASF784 ND MRM -ve10µl MS01 FP 1154-7-12 Std 500 pg/µl

6:59 in 1 period
FP-185
No Internal Standard
Use Area

1: 6:59 MRM, 323 scans
213.0->185.0
Noise Thres. 10.0
Quant Thres. 2.0
Min. Width 5
Mult. Width 5
Base. Width 100
RT Win. (secs) 20
Smooth 1
Expected RT 6:15
Area 30690
Height 2171
Start Time 6:03
End Time 6:58
Integration Width 0:54.6
Retention Time 6:16
Integration Type A - BB

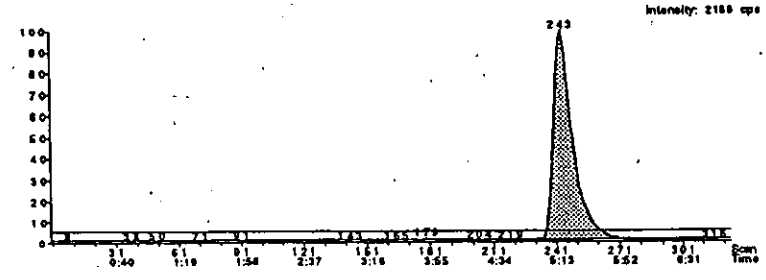
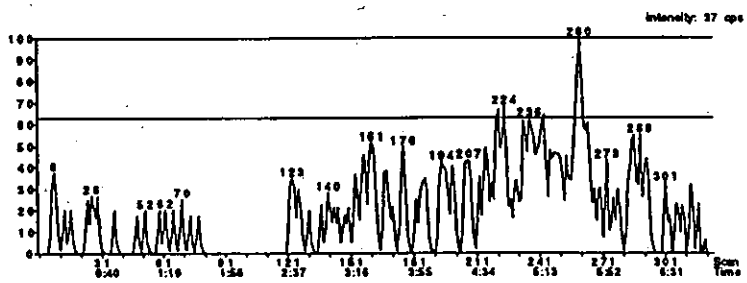


Figure 3. Control, Fortification of BH FP at 0.01 ppm Fortification Level

C970127013 1154-4-12 Mon, Jan 27, 1997 17:34
BASF784 ND MRM -ve10µl MS#1 1154-4-12 CONTROL

6:59 in 1 period
FP-185
No Internal Standard
Use Area

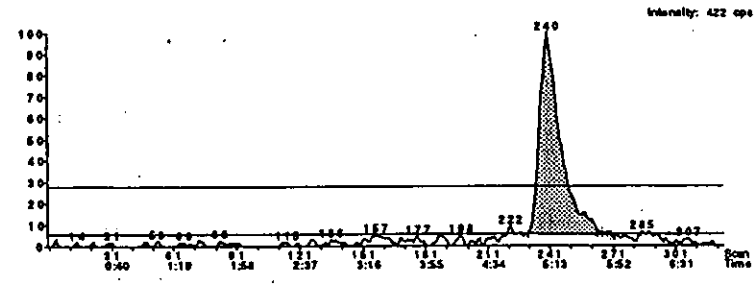
1: 6:59 MRM, 323 scans
213.0->185.0
Noise Thres. 10.0
Quant Thres. 2.0
Min. Width 5
Mult. Width 5
Base. Width 100
RT Win. (secs) 20
Smooth 1
Expected RT 5:15
Area 0
Height 0
Start Time 0:00.0
End Time 0:00.0
Integration Width 0:00.0
Retention Time 0:00.0
Integration Type



C970127015 1154-4-13 Mon, Jan 27, 1997 17:51
BASF784 ND MRM -ve10µl MS#1 1154-4-13 0.01PPM FORT

6:59 in 1 period
FP-185
No Internal Standard
Use Area

1: 6:59 MRM, 323 scans
213.0->185.0
Noise Thres. 10.0
Quant Thres. 2.0
Min. Width 5
Mult. Width 5
Base. Width 100
RT Win. (secs) 20
Smooth 1
Expected RT 5:15
Area 6107
Height 401
Start Time 4:59
End Time 5:51
Integration Width 0:52.0
Retention Time 5:12
Integration Type A - BB



C970127017 1154-4-14 Mon, Jan 27, 1997 18:07
BASF784 ND MRM -ve10µl MS#1 1154-4-14 0.01PPM FORT

6:59 in 1 period
FP-185
No Internal Standard
Use Area

1: 6:59 MRM, 323 scans
213.0->185.0
Noise Thres. 10.0
Quant Thres. 2.0
Min. Width 5
Mult. Width 5
Base. Width 100
RT Win. (secs) 20
Smooth 1
Expected RT 5:15
Area 5464
Height 357
Start Time 4:56
End Time 5:47
Integration Width 0:49.4
Retention Time 5:12
Integration Type A - BB

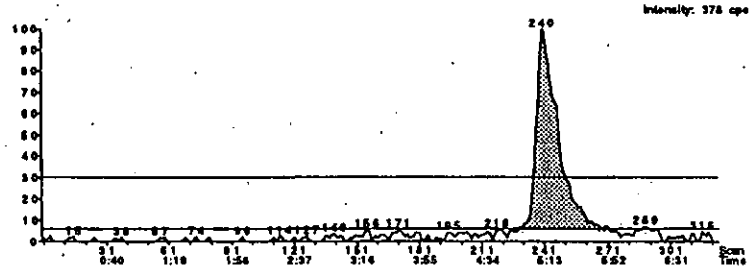


Figure 4. Fortification s of BH 620-FP at 0.1 ppm and 1.0 ppm Fortification Level

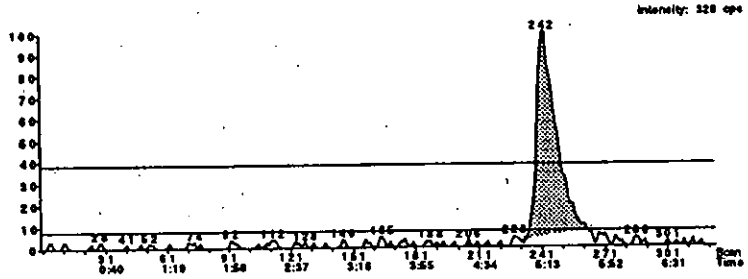
5. 1.0 ppm fortifications

C970127018 1154-4-15 Mon, Jan 27, 1997 18:23
BASF784 ND MRM -ve10µl MS01 1154-4-15 0.1PPM FORT

6:59 In 1 period
FP-185
No Internal Standard
Use Area

1: 6:50 MRM, 323 scans
213.0->185.0
Noise Thres. 10.0
Quant Thres. 2.0
Min. Width 6
Mult. Width 6
Base. Width 100
RT Win. (secs) 20
Smooth 1
Expected RT 6:15

Area	4330
Height	311
Start Time	6:04
End Time	6:41
Integration Width	0:36.4
Retention Time	6:15
Integration Type	A - BB

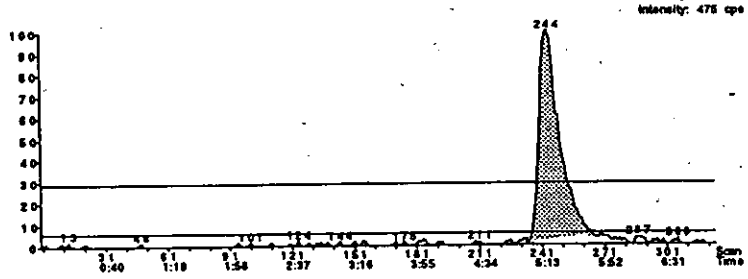


C970127021 1154-4-16 Mon, Jan 27, 1997 18:38
BASF784 ND MRM -ve10µl MS01 1154-4-16 0.1PPM FORT

6:59 In 1 period
FP-185
No Internal Standard
Use Area

1: 6:50 MRM, 323 scans
213.0->185.0
Noise Thres. 10.0
Quant Thres. 2.0
Min. Width 6
Mult. Width 6
Base. Width 100
RT Win. (secs) 20
Smooth 1
Expected RT 6:15

Area	6618
Height	462
Start Time	6:06
End Time	6:43
Integration Width	0:37.7
Retention Time	6:17
Integration Type	A - BB

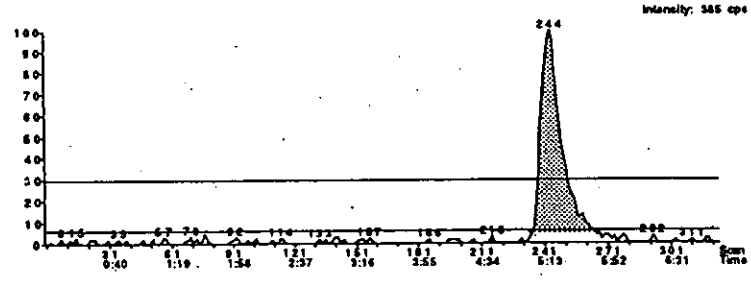


C970127023 1154-4-17 Mon, Jan 27, 1997 18:56
BASF784 ND MRM -ve10µl MS01 1154-4-17 1.0 PPM FORT

6:59 In 1 period
FP-185
No Internal Standard
Use Area

1: 6:50 MRM, 323 scans
213.0->185.0
Noise Thres. 10.0
Quant Thres. 2.0
Min. Width 6
Mult. Width 6
Base. Width 100
RT Win. (secs) 20
Smooth 1
Expected RT 6:15

Area	5166
Height	368
Start Time	6:06
End Time	6:45
Integration Width	0:39.0
Retention Time	6:17
Integration Type	A - BB



C970127024 1154-4-18 Mon, Jan 27, 1997 19:04
BASF784 ND MRM -ve10µl MS01 1154-4-18 1.0 PPM FORT

6:59 In 1 period
FP-185
No Internal Standard
Use Area

1: 6:50 MRM, 323 scans
213.0->185.0
Noise Thres. 10.0
Quant Thres. 2.0
Min. Width 6
Mult. Width 6
Base. Width 100
RT Win. (secs) 20
Smooth 1
Expected RT 6:15

Area	4539
Height	297
Start Time	6:06
End Time	6:43
Integration Width	0:37.7
Retention Time	6:17
Integration Type	A - BB