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

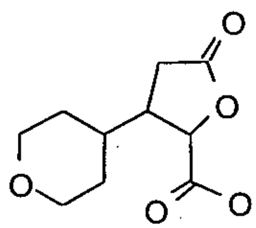
1.2 TEST AND REFERENCE SUBSTANCES

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

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

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:

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

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.