Bayer Method ET-001-S13-02

An Analytical Method for the Determination of Residues of Ethephon in Soil and Sediment Using LC/MS/MS

1.0 SUMMARY

An analytical method was developed to determine the residues of Ethephon in soil and sediment and is based on Bayer method 00899.[1]

Residues of ethephon are extracted from soil and sediment using water with phosphoric acid with microwave extraction. An isotopic internal standard is added to the sample and an aliquot is centrifuged. The supernatant is placed into a vial for analysis by LC/MS/MS with quantification based on a comparison of peak areas with those of known standards.

The method limit of quantitation (LOQ) in all sample matrices for ethephon is 5 ng/g.

2.0 BACKGROUND

The analytical method presented in this report is designed to measure residues of ethephon in soil and sediment using isotopically labeled internal standards and LC/MS/MS detection.

3.0 APPARATUS

(Functional equivalents may be substituted)

- Various general laboratory glassware and utensils
- MicroMan pipettors and tips
- Eppendorf Centrifuge 5810
- Milestone Ethos E Microwave Labstation, equipped with a Terminal 640 Touch Screen Controller and automatic temperature control with fiber optic sensor
- TurboVap
- Phenomenex Aqua C18, 150 mm X 4.6 mm, 3 µm particle size, (Part No: 00F-4311-E0)
- ABSciex API 4000 chromatograph/mass spectrometer (LC-MS/MS) equipped with electrospray ionization (ESI) interface, Shimadzu HPLC pumps and a CTC PAL autosampler, and Analyst 1.6.1 data collection software (ABSciex)

4.0 REAGENTS AND CONSUMABLES

(Functional equivalents may be substituted)

- Acetonitrile (Optima Grade, Fisher Part No. A996-4)
- Formic Acid 99% (Acros, Part no. 14793-0010)
- Water (Optima Grade; Fisher Part No. W7-4)
- Methanol (optima Grade, Fisher Part No. A456-4)
- Phosphoric acid 85% (Acros Part no. 295700010)

- 0.1% formic acid in water. Add 1 mL formic acid to 1000 mL water. Mix well.
- 0.7% phosphoric acid in water. Add 7 mL of phosphoric acid to 1000 mL water. Mix well.
- Fisherbrand 125mL 4oz glass jars (Part No. 02-911-455)
- Fisherbrand 2 mL microcentrifuge tube (Part No. 02-681-266)
- HPLC vials and caps (2-mL, National Scientific, Part Nos. C4011-5W and C4011-55)
- Disposable stir bars, 1 x 5/16 (Fisher Part No. 1451394)

5.0 PREPARATION OF STANDARD SOLUTIONS

Ethephon analytical standards and the isotopic internal standards ethephon-d_4 are needed. These standards may be obtained from Bayer CropScience, 2 T.W. Alexander Drive, Research Triangle Park, North Carolina, 27709. Additional details about these chemicals are given in Appendix 1.

The toxicities of these chemicals have not been precisely determined. Thus, each chemical must be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest reasonable level.

NOTE: The following procedure is an example description of how these standard solutions may be prepared. Alternate or additional standards of appropriate weight and volume may be prepared as needed.

Class "A" volumetric glassware or calibrated pipets should be used in the preparation of all analytical standards. All standard stock solutions should be stored in a freezer when not in use and fortification and calibration standard solutions should be stored in a refrigerator when not in use. Solutions should be allowed to warm to room temperature prior to use. Corrections for standard purities should be applied when expressing standard concentrations.

5.1 Primary Stock Standard Solution

100 µg/mL solution of Ethephon

Standards used to prepare initial stock solutions should be weighed on an analytical balance capable of accurately weighing samples to ± 0.01 mg. Standards are typically provided in 9.0 to 12.0 mg aliquots. The standards are quantitatively transferred to a 100 mL volumetric flask using 0.7% phosphoric acid in water and diluted to volume.

5.2 Fortification Standard Solutions

10 µg/mL solution of Ethephon

Prepare a 10 µg/mL solution of ethephon by taking an appropriate volume (~10.0 mL) of the ethephon primary stock solution and diluting to 100 mL with 0.7% phosphoric acid in water.

1 µg/mL solution of Ethephon

Transfer 10 mL of the 10 µg/mL ethephon standard solution into a 100 mL volumetric flask. Dilute to volume with 0.7% phosphoric acid in water. Mix well.

5.3 Isotopic Internal Standard Solutions

100 µg/mL solution of Ethephon-d_4

Standards used to prepare initial stock solutions should be weighed on an analytical balance capable of accurately weighing samples to ± 0.01 mg. Standards are typically provided in 2.0 to 5.0 mg aliquots. The standards are quantitatively transferred to a 50 mL volumetric flask using 0.7% phosphoric acid in water and diluted to volume.

10 µg/mL solution of Ethephon-d_4

Prepare a 10 µg/mL solution of ethephon-d_4 by taking an appropriate volume (~10.0 mL) of the 100 µg/mL ethephon-d_4 internal standard solution and diluting to 100 mL with 0.7% phosphoric acid in water.

1 µg/mL solution of Ethephon-d_4

Transfer 10 mL of the 10 µg/mL ethephon-d_4 internal standard solution into a 100 mL volumetric flask. Dilute to volume with 0.7% phosphoric acid in water. Mix well.

5.4 Calibration Standard Solutions

Prepare working calibration solutions consisting of 0.8, 2, 5, 10, 50 and 100 ng/mL of ethephon diluted to 50 mL with 0.7% phosphoric acid in water. Before bringing the calibration solutions to volume, add by pipet 0.5 mL of the 1 µg/mL ethephon-d_4 internal standard solution to each of the calibration solutions.

Concentration of Standard Solution used for dilution (µg/mL)	Concentration of Internal Standard Solution used for dilution (µg /mL)	Aliquot Native mix Taken (mL)	Aliquot Internal Standard Taken (mL)	Dilution Volume (mL)	Concentration of Calibration Solution (ng/mL)	Concentration of Internal Standard (ng/mL)
10	1	0.5	0.5	50	100	10
10	1	0.25	0.5	50	50	10
1	1	0.5	0.5	50	10	10
1	1	0.25	0.5	50	5	10
1	1	0.1	0.5	50	2	10
1	1	0.04	0.5	50	0.8	10

Further calibration solutions may be prepared as needed. Depending on the analytical range for the soil samples, at least six calibration standards are needed.

6.0 PROCEDURE

6.1 Sample extraction for Ethephon

Appendix 2 shows the analytical scheme for the extraction of ethephon in soil. The detailed stepwise procedure is as follows:

1. Weigh 20 ± 0.05 grams of soil/sediment into a 125 mL glass jar containing a magnetic stirbar.
2. Fortify the recovery samples at the desired fortification level with the appropriate standard solution.
3. Add 40 mL of 0.7% phosphoric acid in water to each sample.
4. Place jars with soil-solvent mixture into the microwave extractor.
5. Switch on the magnetic stirrer.
6. Extract for three minutes at 250 W.
7. Add 0.40 mL of the 1 µg/mL ethephon-d_4 internal standard solution. Mix well.
8. Transfer about 1.5 mL of the extract into a centrifuge tube. Centrifuge for 5 minutes at 10,000 rpm to remove fine particles of soil.
9. Transfer an aliquot to a vial for LC/MS/MS analysis.

7.0 ANALYSIS BY LC-MS/MS

7.1 Analytical Procedure

Step 1. Using the recommended procedures listed below, analyze an aliquot of each of the calibration standard solutions (if necessary, additional standard solutions may be added).

Step 2. Analyze an aliquot of each of the analytical samples.

Note: Up to 20 sample analyses can be made after the analysis of the standard solutions. In the case of over 20 samples, extra standard solutions could be added between sample analyses.

Step 3. Again, analyze an aliquot of each of the calibration standard solutions (and, if necessary, additional standard solutions).

7.2 HPLC Conditions

Note: The analyst should optimize chromatographic conditions to obtain satisfactory chromatography. The following recommended conditions were used on an ABSciex API 4000 instrument.

Mobile Phase A: Water containing 0.1% formic acid
Mobile Phase B: Acetonitrile

HPLC column: Phenomenex Aqua C18, 150 mm X 4.6 mm 3 µm particle size
Column Temperature: 60°C
Injection volume: 25 µL
Retention Time: ~2.7 minutes

Time (min)	Mobile Phase %B	Flow rate (A &B) µL/min
0.1	5	1000
3.0	5	1000
3.5	95	1000
4.5	95	1000
5.0	5	1000
8.0	5	1000

7.3 Mass Spectrometer Conditions

Note: The analyst should optimize the mass spectrometer conditions to obtain satisfactory system response. The following conditions were used on a ABSciex API 4000 instrument.

Negative ion mode

CUR: Curtain Gas	30
CAD: Collision Gas	12
GS1: Ion Source Gas 1	60
GS2: Ion Source Gas 2	70
TEM: Source Temp.	600°C
IHE: Interface Heater	OFF
IS: Ion Transfer Voltage	-4500

7.4 Mass Spectrometer Data Collection

Note: The analyst should optimize the mass spectrometer data collection to obtain satisfactory system response. As the HPLC column ages, the retention times of the analytes will change. A standard solution should be analyzed before each set of samples to confirm the data collection parameters.

The daughter ions used in this method were chosen due to their optimum sensitivity on the ABSciex API 4000 instrument used for this study. The following recommended ion transitions and conditions were example conditions used on an ABSciex API 4000 instrument:

Analyte Name	Polarity	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	DP	EP	CE	CXP
Ethephon	-	142.9	106.8	100	-51	-10	-12	-7
Ethephon IS	-	146.9	111.0	100	-51	-10	-12	-7
Ethephon Confirmatory	-	106.8	78.8	100	-51	-10	-12	-7

8.0 CALCULATION OF RESULTS

The example calculation displayed below was used by the laboratory developing this method. Alternate calculation procedures appropriate to the reporting requirements may be substituted.

Residue concentrations were determined using calibration curves which were generated after each analysis using Analyst software (version 1.6) using linear regression with 1/x weighting.

The standards were fit to the linear equation:

$$Y = MX + B \text{ with } 1/x \text{ weighting.}$$

where: X is the concentration of the reference standard in ng/mL
M is the calibration line slope
B is the calibration line intercept
Y is the native peak area: isotopic peak area ratio

After regression coefficients were calculated, the residue in ng/g was determined using the following equation,

$$\text{Residue found (ng/g)} = \frac{(Y-B) \times D}{M}$$

$$\text{Where Dilution Factor (D)} = \frac{\text{Initial volume } (V_1)}{\text{Initial sample wt. (W)}} \times \frac{\text{Final dilution volume } (V_3)}{\text{Aliquot taken } (V_2)}$$

	Ethephon
W=	20 g
V_1=	40 mL
V_2=	1 mL
V_3=	1 mL
D=	2

Analyst software was used to calculate the amount of ethephon in ng/g for each sample and the percent recovery for the spiked samples.

8.1 Fortification Experiments

Note: Fortification experiments may be performed as needed to monitor method efficiency and reproducibility, but are not required when analysis of samples is performed for tolerance enforcement. Fortification experiments are intended to be used for data collection methods or establishing and validating method efficiency.

With each sample set, analyze an untreated control sample and one or more fortified control samples. Calculate recoveries using the following equation:

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

Where: R = ppb of target analyte found in fortified sample
S = ppb of target analyte found in control sample, real or apparent
T = theoretical ppb in fortified sample

Recoveries are determined by analyzing fortified control samples alone or in conjunction with a sample set. Samples may be fortified prior to extraction at the LOQ of 5 ng/g or other appropriate level with fortification solutions. Calculate the final residue for the control (S) and fortified control (R) samples.

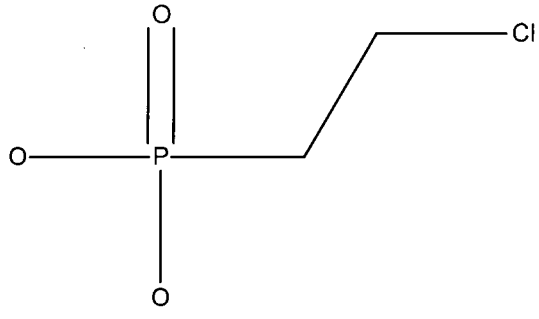
9.0 REFERENCES

1. Brumhard, B. Enforcement Method 00899 for the Determination of Residues of Ethephon in Soil by HPLC-MS/MS. 2004

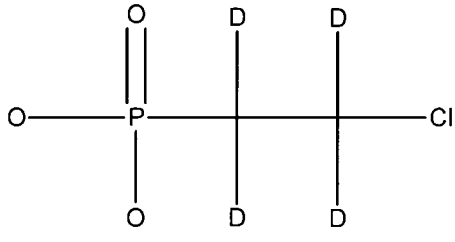
Appendix 1 Test and Reference Substances

The toxicities of these chemicals have not been precisely determined. Thus, each chemical must be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest reasonable level.

Code Name:	Ethephon
Molecular Formula:	$C_2 H_6 Cl O_3 P$
Molecular Weight:	144.5 g/mol



Code Name:	Ethephon-d_4
Molecular Formula:	$C_2 H_2 Cl D_4 O_3 P$
Molecular Weight:	148.5 g/mol



Appendix 2 Extraction Scheme for Ethephon in Soil/Sediment Samples

Weigh an aliquot of soil/sediment into a 125 mL glass jar

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Add ~40 mL of 0.7% phosphoric acid in water

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Microwave extraction. 250W for 3 minutes

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Add 0.4 mL of the 1 µg/mL ethephon internal standard solution

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Centrifuge an aliquot for 5 minutes at ~10,000 rpm

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Transfer an aliquot to a vial for LC/MS/MS analysis