

OBJECTIVE

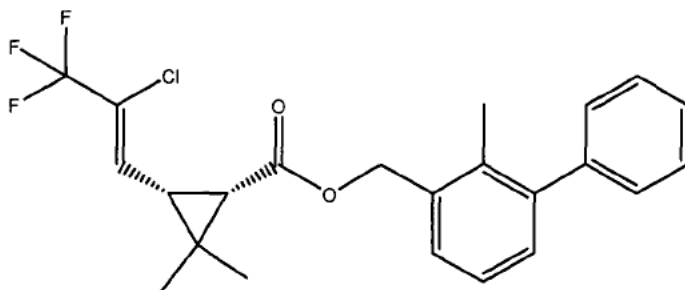
The purpose of this study was to perform an independent laboratory validation of the method of analysis for detection of Bifenthrin in sediment.

TEST SUBSTANCE

The Sponsor supplied the test substance, Bifenthrin. The test substance was received on April 12, 2013 and stored at ambient conditions. The Sponsor will supply written instructions regarding disposal or return of unused test substance. The Sponsor accepts responsibility for maintenance of retention sample of test substance per GLP requirements cited herein.

The standard and the stock solutions made from the test substance were stored in a refrigerator (~5-8 °C). Solubility and stability data for the reference substances, expiration and storage conditions were the responsibility of the Sponsor. Information concerning the test substance including purity is provided below.

- Bifenthrin





Common Name: Bifenthrin
Chemical Name: 2-methylbiphenyl-3-ylmethyl (Z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate
CAS No: 82657-04-3
Molecular Formula: $C_{23}H_{22}ClF_3O_2$
Lot No: PL09-0427
Purity: 98.1%
Expiration Date: September 01, 2014
Molecular Weight: 422.87 g/mole
Storage: Ambient

TEST SYSTEMS

The sediment used in the study was collected fresh from Wyoming on April 15, 2013 and received at Ricerca Biosciences on April 24, 2013. The sediment was stored in a refrigerator until used. The sediment was characterized prior to the start of the study. The GLP characterization of the sediment was given in Appendix A.

EQUIPMENTS

LC-MS/MS Instrument: Applied Biosystems API 4000 LC-MS/MS, SN: V03220311
Analytical Balance: Sartorius Analytical Balance AC 2105, SN: 40060024, Mettler AE163, SN: F38600
Analyst[®] software version 1.4.2
Pipettes: ThermoScientific Finnipipette FI, 10-100 μ L, SN: HH97660 and Class A volumetric pipettes
Centrifuge: Eppendorf 5810 R
Shaker: Burrell Wrist Action Shaker Model 75

REAGENTS

Water (Fisher, HPLC grade), hexane (Fisher, Optima grade), acetonitrile (Fisher, Optima HPLC grade), ethyl ether (Fisher, Laboratory Grade), and ammonium acetate (Fisher, HPLC grade) were used in this study.

PREPARATION OF REAGENTS

PREPARATION OF METHANOL:WATER (1:1, vv):

To an appropriately sized bottle, 500 mL of methanol and 500 mL of water were added using graduated cylinder. The solution was mixed by swirling and stored at ambient temperature when not in use.

PREPARATION OF HEXANE:ETHYL ETHER (9:1, vv):

To an appropriately sized bottle, 90 mL of hexane and 10 mL of ethyl ether were added. The solution was mixed by swirling and stored at ambient temperature when not in use.

PREPARATION OF 5 MM AMMONIUM ACETATE IN WATER

To an appropriately sized bottle, approximately 0.385 g of ammonium acetate was weighed and added 1L of water. The solution was mixed by inversion for uniform mixing and sonication for 5 minutes. The solution was stored at ambient temperature when not in use.

PREPARATION OF 5 MM AMMONIUM ACETATE IN ACETONITRILE : WATER (99:1, v:v)

To an appropriately sized bottle, approximately 0.385 g of ammonium acetate was weighed and added 990 mL of acetonitrile and 10 mL of water. The solution was mixed by inversion for uniform mixing and sonication for 5 minutes.

INSTRUMENT CONDITIONS

The LC-MS instrument is calibrated once per month with PPG calibration solution.

CHROMATOGRAPHIC CONDITIONS

Column: Varian MonoChrome C18, 3.0 µm, 30 x 2.0 mm (S/N: 415072)

Flow rate: 0.5 mL/min

Mobile Phase A: 5 mM Ammonium Acetate in HPLC grade water

Mobile Phase B: 5 mM ammonium acetate in acetonitrile:water (99:1, v:v)

Gradient Table

Time (minutes)	% A	% B
0.1	80	20
2	5	95
5	5	95
5.1	80	20
6.0	80	20

AUTOSAMPLER PROPERTIES

Rinsing Volume (µL)	200
Injection Volume (µL)	20
Sampling Speed (µL/sec)	5

MASS SPECTROMETER METHOD PROPERTIES

Scan Type	MRM
Polarity	Positive
Ion Source	Turbo Spray
Ion Source Gas 1 (GS1)	40 psi
Ion Source Gas 2 (GS2)	10 psi
Collision Gas (CAD)	10 psi
Curtain Gas (CUR)	10 psi
Ion Spray Voltage (IS)	5500 V
Temperature (TEM)	150 °C
Entrance Potential (EP)	10 V

MASS TRANSITIONS AND VOLTAGES

Analyte	Q1 Mass (amu)	Q3 Mass (amu)	Dwell Time (msec.)	Declusterin g Potential (DP)	Collision Energy (CE)	Collision Gas Exit Potential (CXP)
Bifenthrin	440.2	181.1	300	46 V	17 V	14 V

VALIDATION PROCEDURES

The independent laboratory validation was conducted on May 2 to May 7, 2013 at Ricerca Biosciences, LLC, Concord, Ohio.

STANDARD SOLUTION PREPARATION

Stock Standard Solution Preparation:

25.9 mg of Bifenthrin standard (98.1% purity) was weighed into a 25 mL Class A volumetric flask and filled to the mark with methanol. The flask was shaken to dissolve the analyte completely to obtain a solution with 1.02 mg/mL concentration. 100 µL of this stock solution was transferred into a Class A 10 mL volumetric flask and filled to the mark with methanol to obtain a stock solution with 10.2 µg/mL concentration. 100 µL of this solution was further transferred to a 10 mL Class A volumetric flask and filled to the mark with methanol to obtain a concentration of 102 ng/mL concentration. The 102 ng/mL solution was used as a stock solution for the calibration standards and also used as a fortification solution in the 10X LOQ samples.

Calibration Standard Solution Preparation:

A series of calibration standard solutions were prepared starting from the dilution of 102 ng/mL concentration of Bifenthrin stock solution, and the aliquot volume and the final concentrations were given in Table 1.

RESIDUE SAMPLE PREPARATION:

Fortification of Sediment Samples:

Approximately 10 g of sediment were measured into twelve 50-mL centrifuge tubes. The tubes were labeled as Control 1 to 2, LOQ 1 to 5, and 10X LOQ 1 to 5. The sediment samples were fortified with Bifenthrin standard solutions prepared as above. The samples were then shaken and mixed. An empty centrifuge tube without soil was used for the reagent blank and was not fortified. The concentration and volume of the fortification solution were given in Table 2.

ANALYTICAL METHODOLOGY

Extraction and sample clean-up procedures were conducted according to the method PASC 058-0612A [2].

EXTRACTION

1. To each 50-mL centrifuge bottle containing Bifenthrin fortified sediment samples 15 mL of methanol: water (1:1, v:v) and 10 mL of hexane were added using graduated cylinders.
2. The samples were shaken for approximately 60 minutes in Burrell wrist-action shaker.
3. After shaking, the samples were centrifuged at 4,000 rpm for 5 minutes.
4. The top hexane layer was transferred into 15-mL centrifuge tubes using disposable pipettes, and the volume was measured and recorded.
5. The hexane extracts were evaporated to dryness under nitrogen.
6. The residues in the centrifuge tubes were reconstituted with 2 mL of hexane with sonication.

CLEAN-UP

1. Agilent Silica Bond Elut SPE cartridges (500 mg, 3 mL) were used to clean up the concentrated hexane extracts.
2. The SPE cartridges were placed on the manifold and conditioned with 3 mL of hexane and drawn through under vacuum to the level of the top frit at a flow rate of 2 mL per minute. The cartridges were not allowed to go to dryness. The eluates were discarded.
3. The samples were loaded on to the cartridges and allowed to penetrate through under gravity or low vacuum (~ 200 mbar). The eluates were discarded.
4. Then 1 mL of hexane was added to the cartridge and allowed to penetrate through under gravity or low vacuum. The eluates were discarded.
5. Appropriately labeled, clean, 15-mL polypropylene, centrifuge tubes were placed under the SPE cartridge.
6. 6 mL of hexane:ethyl ether (9:1, v:v) was then added to the cartridges. The solvent was drawn through under gravity or low vacuum at a rate of 2 mL per minute. The eluate was collected in the tubes.
7. The eluates were evaporated to dryness under nitrogen. The residue in the centrifuge tubes were reconstituted with 2 mL of methanol and sonicated for 5 minutes.



8. Samples were placed in HPLC vials and stored refrigerated until LC-MS/MS analysis. The extraction and clean-up procedures were summarized in Table 3.

INJECTION SEQUENCE

Five replicate samples at two fortification levels were used to evaluate the method efficiency. Calibration standards were injected within the analysis set to ensure detector linearity and stable response.

The validation set contained one reagent blank, two unfortified matrix controls, five matrix control samples fortified at 0.102 ppb for sediment as LOQ and five matrix control sample fortified at 1.02 ppb for sediment as 10X LOQ level. The injection sequences are given in Table 4.