

2. INTRODUCTION

An independent laboratory validation for study RES-00059 is required to meet residue regulatory requirements.

3. OBJECTIVE

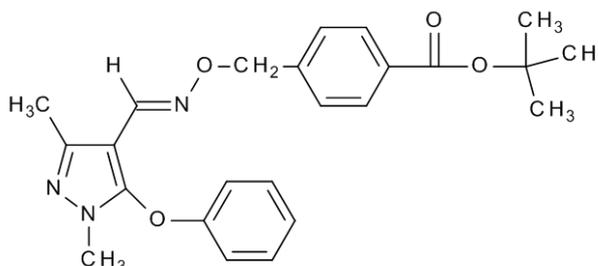
The study objective was to independently validate the reported method RES-00059 for the determination of fenpyroximate in surface water according to the EC guidance document SANCO/825/00 rev. 8.1 and EPA OCSPP 850.6100 (2012).

4. TEST ITEMS

The certificate of analyses for fenpyroximate is presented in Appendix 1.

4.1. Fenpyroximate

Product Name: Fenpyroximate Standard
Common Name: Fenpyroximate
Lot Number: 5AA0023P
Chemical Name: *tert*-Butyl (*E*)- α -(1,3-dimethyl-5-phenoxy-pyrazol-4-yl)methyleneamino-oxy)-*p*-toluate
Structural Formula:



Molecular Formula: C₂₄H₂₇N₃O₄
Molecular Weight: 421.50
CAS-Registry-No.: 134098-61-6
Purity: 99.4%
Storage Conditions: Refrigerated in the dark
Expiry Date: 06 October 2021

5. TEST SYSTEMS

Surface water samples were taken from Battelle validation control stock samples. See Appendix 2 for the characterisation report.

6. METHOD VALIDATION

The determination of fenpyroximate residues in surface water was performed using the method described in RES-00059 (Ref 1).

The control samples were fortified as described in the following table:

| Matrix | Analyte | Reagent Blank Replicates | Untreated Control Replicates | Replicates at LOQ Fortification Level | Replicates at LOQ × 10 Fortification Level | Replicates at LOQ × 100 Fortification Level |
|---------------|---------------|--------------------------|------------------------------|---------------------------------------|--|---|
| Surface Water | Fenpyroximate | 1 | 2 | 5 at 0.01 µg/L | 5 at 0.10 µg/L | 5 at 1.0 µg/L |

LOQ = Limit of Quantification

The method was validated in terms of linearity, selectivity, accuracy and precision, monitoring two ion mass transitions.

Matrix effects were investigated at the LOQ and LOQ×100 levels by comparing peak areas of solvent standard solutions to peak areas of matrix-matched standard solutions. Experiments assessed whether or not matrix effects were significant (i.e. >20% enhancement or suppression).

7. EXPERIMENTAL

7.1. Principle of the Method

Residues of fenpyroximate were extracted by partitioning with hexane followed by a solvent exchange. Final determination was by LC-MS/MS monitoring 2 ion mass transitions. The limit of quantification was 0.01 µg/L and the limit of detection was 0.002 µg/L.

The analytical flow chart is presented in Figure 1.

7.2. Equipment, Consumables and Reagents

Full details of all equipment and reagents are presented in Appendix 3.

7.3. Standards and Fortifications

7.3.1. Stock Solutions

Duplicate stock solutions were prepared by dissolving a known weight (approximately 10 mg) of fenpyroximate, correcting for purity and dissolving in acetonitrile to produce a final concentration of 1000 µg/mL. Full actual stock solution preparation and final concentrations are detailed in the following table:

| Analyte | Battelle (BUKL) Stock ID | Purity (%) | Actual Amount Weighed (mg) | Actual Volume Added (mL) | Concentration ($\mu\text{g/mL}$) |
|---------------|--------------------------|------------|----------------------------|--------------------------|------------------------------------|
| Fenpyroximate | BAT-6752 | 99.4 | 10.28 | 10.218 | 1000 |
| | BAT-6753 | 99.4 | 10.48 | 10.417 | 1000 |

One was used for calibration standard preparation and the other was used for recovery fortification preparation.

7.3.2. Fortification Solutions

Three fortification solutions, containing Fenpyroximate at concentrations of 0.01, 0.10 and 1.0 $\mu\text{g/mL}$, were prepared by diluting appropriate amounts of the stock solution with acetonitrile.

Samples were fortified according to the following table:

| Matrix | Sample Volume (mL) | Fortification Standard Concentration ($\mu\text{g/mL}$) | Fortification Volume (μL) | Fortification Level ($\mu\text{g/L}$) |
|---------------|--------------------|---|--|---|
| Surface Water | 20 | 0.01 | 20 | 0.01 |
| | 20 | 0.10 | 20 | 0.10 |
| | 20 | 1.0 | 20 | 1.0 |

7.3.3. Calibration Solutions

Intermediate solutions containing Fenpyroximate were prepared at concentrations of 1000 and 5.00 ng/mL by diluting appropriate amounts of the stock solution with acetonitrile: water (1:1, v: v). The intermediate solutions were then used to prepare calibration solutions.

Calibration solutions in acetonitrile: water (1:1 v:v) were prepared at concentrations of 5.00, 2.50, 2.00, 0.500, 0.300, 0.200, 0.0600 and 0.0500 ng/mL by diluting appropriate amounts of the intermediate solutions.

All standard solutions were stored in the refrigerator when not in use.

7.4. Extraction Procedure

Aliquots of 20 mL of surface water were placed into 50 mL centrifuge tubes. For recovery efficiency tests, the control matrices were fortified with the appropriate spiking solutions at the LOQ, 10xLOQ and 100xLOQ levels and were shaken to mix.

For all samples, 2.5 mL of hexane was added to each tube followed by a 30 second vortex mix. Each tube was then centrifuged (3000 rpm) for 5 minutes. The upper hexane layer was removed using a Pasteur pipette and placed into a 15 mL centrifuge tube. The partition was repeated with a further 2.5 mL hexane. After centrifugation, the two hexane layers were combined and dried under a stream of air at 50 °C. Samples were reconstituted in 1.0 mL acetonitrile: water (1:1 v: v) using an ultrasonic bath and the vortex mixer.

For the 100xLOQ samples, a 0.1 mL aliquot of each final extract was then transferred into a vial containing 0.9 mL of acetonitrile: water (1:1 v: v). For all other samples, an aliquot of the final extract was transferred directly into a vial. Residue levels were then determined by LC-MS/MS.

The extraction method is presented in the form of a flow chart in Figure 1.

7.5. LC-MS/MS Analysis

All samples were analysed by liquid chromatography coupled with a tandem mass spectrometer (LC-MS/MS), monitoring two ion mass transitions.

A summary of these conditions are presented below:

| Mass Spectrometer and General Instrument Conditions | | | | |
|---|---|------------------|------------------|---------------------|
| Instrument | API 5500 Triple Quadrupole Mass Spectrometer fitted with Turbo ion spray ion source | | | |
| Ion Source | Positive Electrospray (ESI+) | | | |
| Run Time | 3.5 minutes (Approximate retention time of 2.6 minutes) | | | |
| LC-MS/MS Mass Spectrometric Conditions | | | | |
| Analyte | Dwell Time (msec) | Transition (m/z) | Collision Energy | Cell Exit Potential |
| Fenpyroximate | 100 | 422/366 | 10 | 10 |
| | 100 | 422/135 | 49 | 4 |
| Curtain Gas | 40 | | | |
| CAD Gas | -2 (Medium) | | | |
| Gas 1 | 45 | | | |
| Gas 2 | 40 | | | |
| Spray Voltage | 5500 V | | | |
| Source Temperature | 550°C | | | |
| Declustering Potential | 21 | | | |
| Entrance Potential | 10 | | | |

| HPLC Conditions | | | |
|-------------------------|---|----|----|
| Columns | Sigma, Ascentis Express C18, 50 x 2.1 mm, 2.7 μ m | | |
| Column Oven Temperature | 45 °C | | |
| Mobile Phase A | Water + 0.1% Formic acid | | |
| Mobile Phase B | Acetonitrile + 0.1% Formic acid | | |
| Method | Time | %A | %B |
| | 0 | 40 | 60 |
| | 3.5 | 40 | 60 |
| Flow Rate | 0.5 mL/min | | |
| Injection Volume | 5 μ L | | |

8. CALIBRATION AND CALCULATIONS

A multi-point calibration curve was obtained from injections of calibration solutions by plotting peak areas of fenpyroximate versus the concentration in ng/mL. The curves were calculated by the method of least squares linear regression. 1/x weighting factor was applied to the curves to improve the accuracy.

The quantification of fenpyroximate in the samples was made by comparison to the calibration curve of the form $y = mx + c$. The amount of analyte in a given sample was calculated as follows:

$$\text{Compound } [\mu\text{g/L}] = \frac{(A - c) \times F \times D}{m \times V}$$

Where:

- A = Area of analyte peak
- m = slope of the calibration curve
- c = intercept of the calibration curve
- F = final volume (mL)
- V = initial sample volume (mL)
- D = dilution factor (if applicable)

The recovery efficiency in the fortified samples was calculated as follows:

$$\text{Recovery efficiency } [\%] = \frac{\text{Amount found } (\mu\text{g/L})}{\text{Amount spiked } (\mu\text{g/L})} \times 100$$

Example LC-MS/MS chromatograms of calibration solutions, control samples and fortified samples are presented in Figure 4 to Figure 11.

Examples of calibration curves are presented in Figure 3 and response factors are presented in Figure 12.

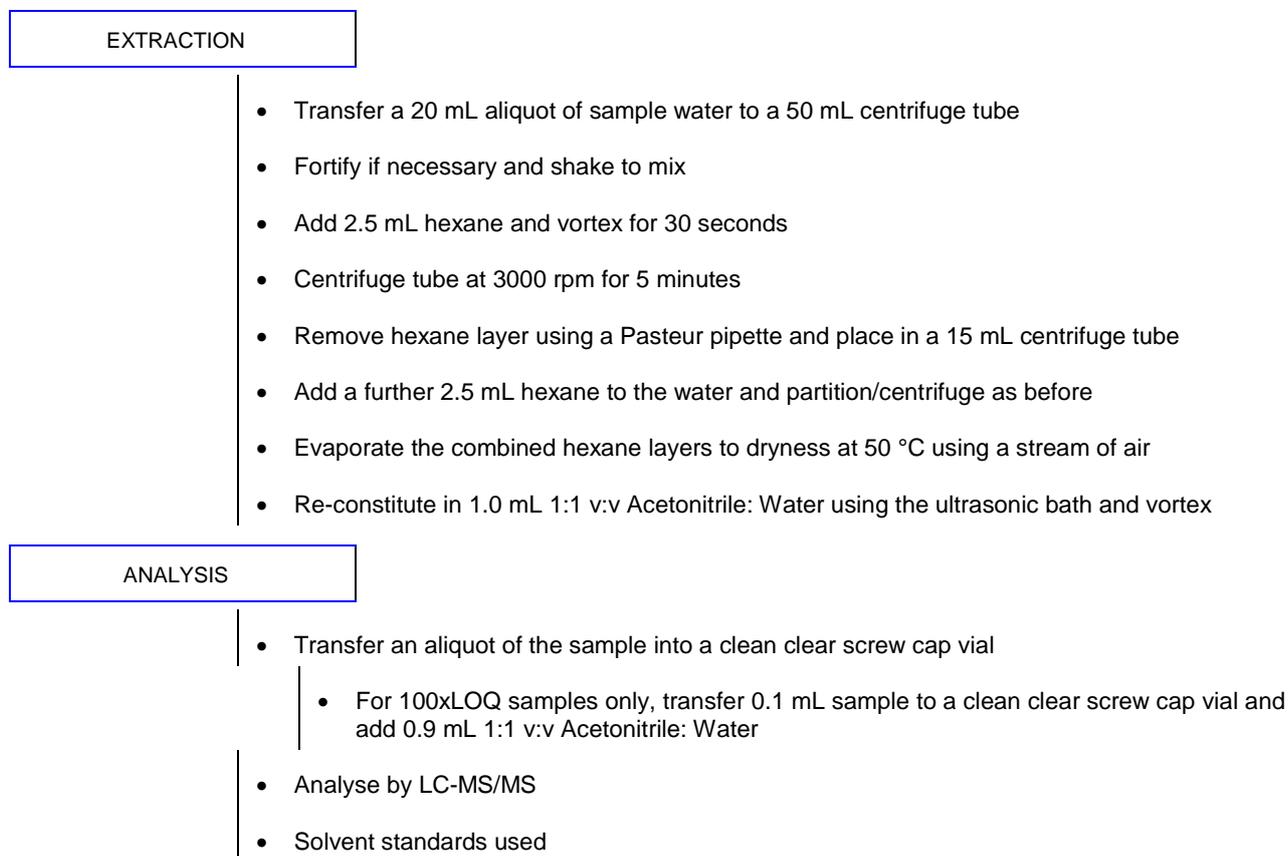
The response factor was calculated as follows:

$$\text{Response Factor} = \frac{\text{Response area (counts)}}{\text{Nominal concentration (ng/mL)}^*}$$

* Prior to any concentration corrections.

16. FIGURES

Figure 1: Analytical Flow Chart



The LOQ of the validated method was: 0.01 µg/L