

2. INTRODUCTION

An independent laboratory validation for study RES-00061 is required to meet residue regulatory requirements.

3. OBJECTIVE

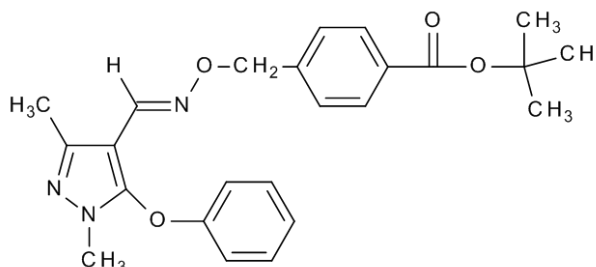
The study objective was to independently validate the method reported RES-00061 for the determination of fenpyroximate in soil according to the EC guidance document SANCO/825/00 rev. 8.1, ENV/JM/MONO(2007)17 and EPA OCSPP 850.6100 (2012).

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

Sandy loam and clayey loam soil samples were taken from Battelle validation control stock samples. See Appendix 3 and Appendix 4 for the characterisation reports. The soil moisture for each soil type was determined prior to analysis.

6. METHOD VALIDATION

The determination of fenpyroximate residues in soil was performed using the method described in RES-00061 (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
Sandy Loam	Fenpyroximate	1	2	5 at 0.01 mg/kg	5 at 0.10 mg/kg
Clayey Loam	Fenpyroximate	1	2	5 at 0.01 mg/kg	5 at 0.10 mg/kg

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×10 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 subsequent extractions with methanol, acetone, methanol/water and methanol/hydrochloric acid. Final determination was by LC-MS/MS monitoring two ion mass transitions. The limit of quantification (LOQ) was 0.01 mg/kg and the limit of detection (LOD) was 0.0002 mg/kg (clayey loam) and 0.0004 mg/kg (sandy loam).

The analytical flow chart is presented in Figure 1.

7.2. Equipment, Consumables and Reagents

Full details of all equipment, consumables and reagents are presented within the analytical methods presented in Appendix 2.

7.3. Standards and Fortifications

7.3.1. Stock Solutions

Duplicate stock solutions were prepared by dissolving a known weight (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

Two fortification solutions, containing fenpyroximate at concentrations of 1.0 and 10 $\mu\text{g/mL}$, were prepared by diluting appropriate amounts of the stock solution with acetonitrile.

Recovery efficiency samples were fortified according to the following table:

Matrix	Sample Weight* (g)	Fortification Standard Concentration ($\mu\text{g/mL}$)	Fortification Volume (μL)	Fortification Level (mg/kg)
Sandy and Clayey Loam Soil	25	1.0	250	0.01
	25	10	250	0.10

* Dry weight of soil

7.3.3. Calibration Solutions

An intermediate solution containing fenpyroximate at a concentration of 1000 ng/mL was prepared by diluting an appropriate amount of the stock solution with acetonitrile: water (1:1 v : v). Further intermediate solutions containing Fenpyroximate were prepared at concentrations of 250, 200, 100, 50, 20, 10, 3.0 and 2.5 ng/mL by diluting appropriate amounts of the 1000 ng/mL intermediate solution with acetonitrile: water (1:1, v: v). The intermediate solutions were then used to prepare matrix matched calibration solutions.

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

7.4. Soil Moisture Determination

Approximately 60 g (3 x 20 g aliquots) of each soil were weighed and left to dry in an oven at 105 °C. The samples were initially weighed after approximately 3 hours of drying. The samples were returned to the oven and re-weighed again after 17 hours. After comparison of the two weights, the difference was deemed negligible and the soil samples were determined to be dry. The final dry weight for each aliquot was used to calculate the moisture of the initial sample

The moisture determination in each soil sample was calculated according to the following equation:

$$\text{Moisture Content [\%]} = \frac{\text{Initial wet weight (g)} - \text{Final dry weight (g)}}{\text{Initial wet weight (g)}} \times 100$$

The mean moisture content for each soil type was calculated by averaging the three individual moistures for each soil type.

7.5. Extraction Procedure

Aliquots of 25 g (dry weight) of soil were placed into 125 mL extraction bottles. For recovery efficiency tests, the control matrices were fortified with the appropriate spiking solutions at the LOQ and 10xLOQ.

For all samples, 50 mL of methanol was added to each bottle and mechanically shaken for 20 minutes. After centrifugation (1500 rpm for 2 minutes), the sample was filtered through cotton wool. The extraction process was repeated 5 more times with the following solvents;

1. 50 mL methanol
2. 50 mL methanol
3. 50 mL acetone
4. 50 mL methanol: water (1:1 v:v)
5. 50 mL methanol: 0.1 M hydrochloric acid (aq, 1:1 v:v)

After combining all extracts, the final volume was adjusted to 500 mL with water and an aliquot transferred to a glass HPLC 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.

7.6. 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				
Instruments	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 2.5 minutes)			
Analyte	Dwell Time (msec)	Transition (m/z)	Collision Energy	Cell Exit Potential
Fenpyroximate	100	422/366	10	10
	100	422/135	45	10
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			

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Run Time	3.5 minutes (Approximate retention time of 2.5 minutes)			
Analyte	Dwell Time (msec)	Transition (m/z)	Collision Energy	Cell Exit Potential
Fenpyroximate	100	422/366	17	16
	100	422/135	43	8
Curtain Gas	40			
CAD Gas	-2 (Medium)			
Gas 1	45			
Gas 2	40			
Spray Voltage	5500 V			
Source Temperature	550°C			
Declustering Potential	1			
Entrance Potential	10			

HPLC Conditions			
Columns	Sigma, Ascentis Express C18, 50 x 2.1 mm, 2.7 µm		
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	10 µL		

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:

$$\text{Compound [mg/kg]} = \frac{(A - c) \times F}{m \times W \times 1000}$$

Where:

- A = Area of analyte peak
- m = slope of the calibration curve
- c = intercept of the calibration curve
- F = final volume (mL)
- W = initial sample weight (g)

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

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

Example LC-MS/MS chromatograms of calibration solutions, control samples and fortified samples are presented in Figure 5 to Figure 18.

Examples of calibration curves are presented in Figure 3 to Figure 4 and response factors are presented in Figure 19 to Figure 20.

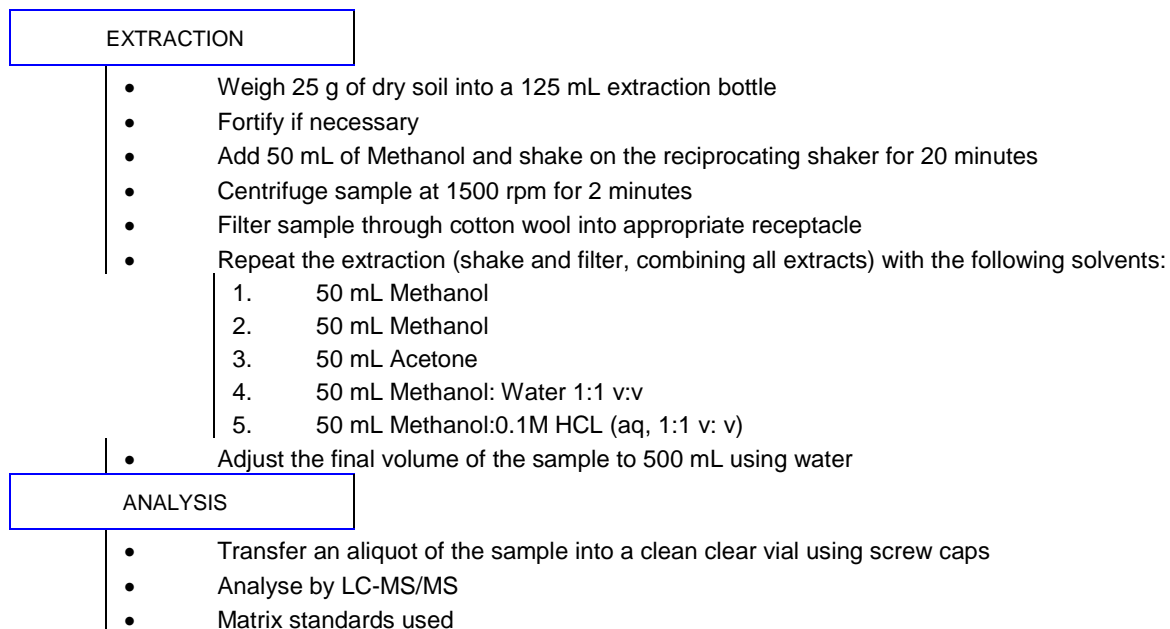
The response factor was calculated as follows:

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

* Prior to any concentration corrections.

15. FIGURES

Figure 1: Analytical Flow Chart



The LOQ of the validated method was: 0.01 mg/kg