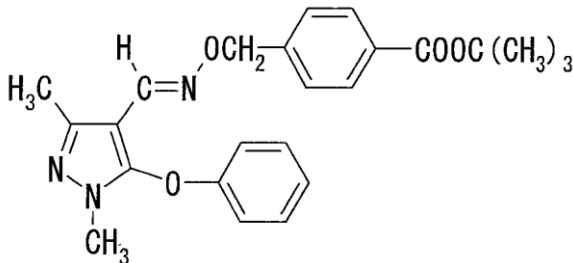


## 2 Reference Item Details

The following reference item was used in the study. The Certificate of Analysis is shown in Appendix A.

Common name	Fenpyroximate
IUPAC Name	<i>tert</i> -butyl(E)- $\alpha$ -(1,3-dimethyl-5-phenoxy-pyrazol-4-ylmethyleneamino-oxy)- <i>p</i> -toluate
CAS-Registry-No.	134098-61-6
Empirical formula	C <sub>24</sub> H <sub>27</sub> N <sub>3</sub> O <sub>4</sub>
Molar mass	421.5 g/mol
Structure	 <chem>CC1=CN(C)C(=C1Oc2ccccc2)C=NOCc3ccc(cc3)C(=O)OC(C)(C)C</chem>
ResChem Lot. No.	RAL 039/2015
Batch Identification	5AA0023P
Purity	99.4%
Expiry date	06 October 2021

### 3 Test System Details

#### 3.1 Specimen Origin

The validation was undertaken on LUFA Speyer soils type 2.2 (loamy sand) and 6S (clayey loam).

### 4 Analytical Methodology

The analytical method involves extraction of sub-samples of each soil type using sequential addition and shaking using methanol, acetone, methanol/water (1:1, v/v) and methanol/0.1M hydrochloric acid (1:1, v/v). The extracts are combined, the volume adjusted with deionised water followed by final determination by LC-MS/MS.

#### 4.1 Reagents Used

Reagent	Description	Supplier
Acetonitrile (for standard preparation)	AR Grade	Rathburns
Acetonitrile (for LC-MS mobile phase)	LC-MS Grade	VWR
Deionised Water (for sample extraction and standard preparation)	AR Grade	Rathburns
Deionised Water (for LC-MS mobile phase)	LC-MS Grade	VWR
Formic Acid (for LC-MS mobile phase)	Optima LC-MS Grade	Fisher Scientific
Methanol (for sample extraction)	HPLC Grade	Rathburns
Acetone (for sample extraction)	Glass distilled Grade	Rathburns
Hydrochloric acid	S.G. 1.18 AR Grade	Fisher Scientific

#### 4.2 Equipment and Apparatus used

Item	Description
Laboratory Balance's	A&D GR-202, Sartorius LP 4200
Ultrasonic Bath	GT Sonic, 10 L Capacity
Adjustable Pipettes	Gilson P100, P200, P1000, P10mL
Centrifuge	Hettich Rotanta 460
Orbital Shaker	Gerhardt Laboshake
General Laboratory Supplies	Volumetric Flasks, Pipette's, Beakers, Autosampler Vials, Centrifuge tubes, Measuring Cylinders, Nalgene jars etc.
LC-MS/MS	AB Sciex API 4000 Mass Spectrometer with an Agilent 1100 Binary HPLC Pump, Phenomenex DG-4400 Degasser, CTC Analytics HTS PAL Autosampler, Phenomenex Thermasphere TS-130 Column Oven and a Peak Scientific NM300DR Gas Generator
HPLC Column	Ascentis Express C18, 50 x 2.1 mm, 2.7 µm Particle Size, Sigma

### 4.3 Standard Preparation

Individual fenpyroximate stock solutions were prepared in acetonitrile with the aid of an ultrasonic bath, by dissolving 10 mg in 10 mL of solvent. The standards were allocated unique reference numbers i.e. FEN1-19.5.16 and FEN1-8.6.16.

The fenpyroximate stock solutions were further diluted for use as fortification standards in the procedural recovery process and for the preparation of intermediate standards for instrument set-up and matrix-matched standard preparation.

#### 4.3.1 Preparation of Fortification Solutions

Fortification standard solutions were prepared by serial dilution of the fenpyroximate stock solution using acetonitrile as listed below.

Standard Ref.	Standard Conc. ( $\mu\text{g/mL}$ )	Volume Used (mL)	Final Vol. (mL)	Final Conc. ( $\mu\text{g/mL}$ )	Standard Ref.
FEN1-19.5.16, FEN1-8.6.16	1000	1.0	10	100	FEN2-19.5.16, FEN2-8.6.16
FEN2-19.5.16, FEN2-8.6.16	100	1.0	10	10	FEN3-19.5.16, FEN3-8.6.16
FEN3-19.5.16, FEN3-8.6.16	10	1.0	10	1.0	FEN4-19.5.16, FEN4-8.6.16
FEN4-19.5.16, FEN4-8.6.16	1.0	1.0	10	0.1	FEN5-19.5.16, FEN5-8.6.16
FEN5-19.5.16	0.1	1.0	10	0.01	FEN6-19.5.16

### 4.3.2 Preparation of Intermediate Standard Solutions

Intermediate standard solutions were prepared by serial dilution using acetonitrile/water, 1/1, v/v as listed below

Standard Ref.	Standard Conc. (µg/mL)	Volume Used (mL)	Final Vol. (mL)	Final Conc. (µg/mL)	Standard Ref.
FEN4-19.5.16	1.0	1.0	10	0.1	FEN7-23.5.16
FEN4-19.5.16	1.0	0.4	10	0.04	FEN8-23.5.16
FEN4-19.5.16	1.0	0.2	10	0.02	FEN9-23.5.16
FEN7-23.5.16	0.1	1.0	10	0.01	FEN10-23.5.16
FEN7-23.5.16	0.1	0.4	10	0.004	FEN11-23.5.16
FEN10-23.5.16	0.01	1.0	10	0.001	FEN12-23.5.16
FEN3-19.5.16, FEN3-8.6.16	10	0.25	10	0.25	FEN13-26.5.16, FEN6-8.6.16
FEN4-19.5.16, FEN4-8.6.16	1.0	1.0	10	0.1	FEN14.26.5.16, FEN7-8.6.16
FEN4-19.5.16, FEN4-8.6.16	1.0	0.5	10	0.05	FEN15.26.5.16, FEN8-8.6.16
FEN4-19.5.16, FEN4-8.6.16	1.0	0.2	10	0.02	FEN16.26.5.16, FEN9-8.6.16
FEN14-19.5.16, FEN5-8.6.16	0.1	1.0	10	0.01	FEN17.26.5.16, FEN10-8.6.16
FEN14-19.5.16, FEN5-8.6.16	0.1	0.3	10	0.003	FEN18.26.5.16, FEN11-8.6.16

### 4.3.3 Preparation of Matrix-Matched Calibration Standard Solutions

Dilutions were performed in untreated clayey loam soil final extract as follows;

Standard Ref.	Standard Conc. (µg/mL)	Volume Used (mL)	Final Vol. (mL)	Final Conc. (µg/mL)	Standard Ref.
FEN13-26.5.16	0.25	0.05	1.0	0.0125	CLMM1-27.5.16
FEN14-26.5.16	0.1	0.05	1.0	0.005	CLMM2-27.5.16
FEN15-26.5.16	0.05	0.05	1.0	0.0025	CLMM3-27.5.16
FEN16-26.5.16	0.02	0.05	1.0	0.001	CLMM4-27.5.16
FEN17-26.5.16	0.01	0.05	1.0	0.0005	CLMM5-27.5.16
FEN18-26.5.16	0.003	0.05	1.0	0.00015	CLMM6-27.5.16

Dilutions were performed in untreated loamy sand soil final extract as follows;

Standard Ref.	Standard Conc. ( $\mu\text{g/mL}$ )	Volume Used (mL)	Final Vol. (mL)	Final Conc. ( $\mu\text{g/mL}$ )	Standard Ref.
FEN13-26.5.16	0.25	0.05	1.0	0.0125	LSMM1-31.5.16
FEN14-26.5.16	0.1	0.05	1.0	0.005	LSMM2-31.5.16
FEN15-26.5.16	0.05	0.05	1.0	0.0025	LSMM3-31.5.16
FEN16-26.5.16	0.02	0.05	1.0	0.001	LSMM4-31.5.16
FEN17-26.5.16	0.01	0.05	1.0	0.0005	LSMM5-31.5.16
FEN18-26.5.16	0.003	0.05	1.0	0.00015	LSMM6-31.5.16

#### 4.3.4 Preparation of Solvent Standard Solutions equivalent to the LOQ

Solutions were prepared for matrix effect and standard stability assessments (in methanol/acetone/water, 4/1/1, v/v/v) as follows;

Standard Ref.	Standard Conc. ( $\mu\text{g/mL}$ )	Volume Used (mL)	Final Vol. (mL)	Final Conc. ( $\mu\text{g/mL}$ )	Standard Ref.
FEN17-26.5.16	0.01	0.05	1.0	0.0005	SS1-31.5.16, SS1-27.5.16, SS1-26.5.16
FEN10-8.6.16	0.01	0.05	1.0	0.0005	SS1-8.6.16

#### 4.4 Extraction

1. An aliquot of soil (25 g) was dispensed into a polypropylene tube (25 mL).
2. Procedural recoveries were prepared by fortifying aliquots of untreated soil with the appropriate fenpyroximate fortification solution as detailed in the table below.

Matrix	Sample Weight (g)	Standard Reference	Standard Conc. ( $\mu\text{g/mL}$ )	Volume added (mL)	Fortification Level (mg/kg)
Soil	25.0	FEN4-19.5.16	1.0	0.25	0.01
	25.0	FEN3-19.5.16	10	0.25	0.1

3. Methanol (50 mL) was added and the soil extracted by shaking on an orbital shaker (150 orbits/min, 20 mins).
4. Tubes were then placed in a centrifuge (1500 rpm, 2 mins).
5. Extracts were filtered through cotton wool and the residual soil re-extracted sequentially with methanol (2x 50 mL), acetone (50 mL), methanol/water (1/1, v/v, 50 mL) and methanol/0.1M hydrochloric acid (1/1, v/v, 50 mL) using the conditions detailed in sections 3-4.
6. All extracts were combined, mixed and the final volume adjusted to 500 mL using de-ionised water.
7. An aliquot of the final extract was transferred to an autosampler vial prior to quantitation of fenpyroximate by LC-MS/MS.

#### 4.5 LC-MS/MS Conditions

An AB Sciex API 4000 Mass Spectrometer with an Agilent 1100 Binary HPLC Pump, Phenomenex DG-4400 Degasser, CTC Analytics HTS PAL Autosampler, Phenomenex Thermasphere TS-130 Column Oven and a Peak Scientific NM300DR Gas Generator was used for quantitation.

##### 4.5.1 Chromatography Parameters for Fenpyroximate

Parameter	Description		
HPLC Column	Ascentis Express C18, 50 x 2.1 mm, 2.7 µm Particle Size, Sigma		
Column Temperature	45 °C		
Injection Volume	10 µL		
Retention Time	2.6 minutes (approx.)		
Mobile Phase	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Acetonitrile		
Flow Rate	0.5 mL/min		
Gradient	Time (minutes)	A (%)	B (%)
	0.0	40	60
	3.5	40	60

##### 4.5.2 Mass Spectrometry Parameters for Fenpyroximate

Parameter	Description			
Ionisation Mode	Turbospray (Electrospray)			
Probe Position	5 Horizontal, 5 Vertical			
Polarity	Positive			
Curtain Gas	40			
CAD Gas	8			
Gas 1	45			
Gas 2	40			
Source Temperature	550 °C			
Spray Voltage	5500			
Declustering Potential	70			
Entrance Potential	12			
Mass Transitions	Ions monitored (m/z)	Collision Energy	Cell Exit Potential	Primary / Confirmatory
	422 → 366	37	10	Primary
	422 → 135	45	6	Confirmatory

#### 4.6 Quantitation

The quantitative determination of all analytical samples was carried out by external standardisation using calibration standards in matrix.

A linear calibration curve was constructed using the method of least squares (1/x weighting) using 6 different concentrations of fenpyroximate ranging from 0.00015 to 0.0125 µg/mL.

The calibration curves were calculated from the area of the calibration solutions with their corresponding concentrations using the following equation:

$$Y = A \times C + B$$

where:

Y = Peak area (integration units)

A = slope of the linear least squares fit of the calibration curve

C = concentration determined from the standard curve (µg/mL)

B = Y-intercept of the linear least squares fit of the calibration curve

The amount of fenpyroximate in each specimen was calculated from the generated calibration curve using Analyst® software.

The concentration (C) of fenpyroximate determined from the standard curve is

$$C = (Y - B) / A$$

The residue of fenpyroximate in each test specimen is calculated as follows;

$$\text{Residue, mg/kg} = \frac{V_f \times n \times C}{W_1}$$

Where:  $V_f$  = Final extraction volume (500 mL)

$W_f$  = Final weight (25 g)

n = dilution factor (if applicable)

Percent recovery from fortified samples was calculated as described below:

$$\text{Recovery (\%)} = \frac{(R_{\text{fortified}})}{F} \times 100$$

where:

$R_{\text{fortified}}$  = Residue determined in fortified sample (mg/kg)

F = Fortification rate (mg/kg)

#### 4.7 Example Calculations

For a 0.01 mg/kg fortified soil sample (sample no. 1287 (422>366 m/z)) the percent recovery found was calculated as follows:

$$\begin{aligned} \text{Recovery (\%)} &= (0.009901 \text{ mg/kg} / 0.01 \text{ mg/kg}) \times 100 \\ &= 99.0 \% \end{aligned}$$



#### 4.8 Method Flow Chart

