1. PRINCIPLE

The residues of Fenoxaprop P-Ethyl and its metabolites AE F088406, and AE F054014 in soil are determined by shaking a 20 gram soil sample twice with acetonitrile/water and once with water containing sodium chloride. An aliquot of the sample is diluted in methanol/water and analyzed by LC/MS/MS. Quantification is based on a comparison of peak areas with those of known standards. Two sets of MRM transitions are shown, one for quantitation and the second for confirmatory purposes.

The method limit of quantitation (LOQ) for Fenoxaprop-P-Ethyl and its metabolites AE F088406 and AE F065025 is 10ng/g in soil.

2. APPARATUS

Use as a guide; equivalent apparatus may be substituted.

VWR Pyrex® Brand volumetric pipets, glass class A (Assorted Volumes)
Eppendorf Reference Series 2000 pipettes (Cat. No.: 05-402-48 and 05-402-50)
VWR Pyrex® Brand volumetric flasks, glass class A (Assorted Volumes)
VWR Pyrex® Brand disposable Pasteur pipets (Cat. No.: 53283-910 & 53283-914)
National Scientific LC vials, Snap-Its (Cat. No.: C4011-5)
National Scientific LC vial Snap-It Seals, (Cat. No.: C4011-55)
Phenomenex® Luna C18(2)-HST 50 x 2.00 mm Column (Part No.: 00B-446-B0)
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 1.4.1 data collection software or higher version, or equivalent
Fisherbrand 125-mL glass jars (Cat. No. 02-911-455)
Centrifuge
Mechanical Shaker
Various general laboratory glassware and utensils

3. REAGENTS

Use as a guide; equivalents or different manufactures (brands) may be substituted.

Acetonitrile, (HPLC Grade)
Water (HPLC Grade or Millipore)
Methanol (HPLC Grade)
Sodium Chloride crystals (Mallinckrodt)
10 grams/liter sodium chloride aqueous solution. Dissolve 10g of sodium chloride in 1L of water (HPLC Grade or Millipore)
Methanol/water solution (70/30). Combine 700mL of methanol with 300mL of water and mix well
Acetonitrile/water solution (80/20). Combine 800mL of acetonitrile with 200mL of water and mix well
Certified analytical reference standards of Fenoxaprop-P-Ethyl, AE F088406 and AE F054014

4. PREPARATION OF ANALYTICAL STANDARDS

NOTE: The following procedure is an example description of how standard solutions may be prepared. Standards may be prepared as mixed solutions by dilution from individual stock solutions or prepared individually. 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 solutions should be stored in a refrigerator in amber glass bottles when not in use. Solutions should be allowed to warm to room temperature prior to use.

4.1 Primary Stock Standard Solutions

Prepare individual 100µg/mL stock solutions of Fenoxaprop-P-Ethyl, AE F088406, and AE F054014 by transferring 0.0100 grams of each analyte in separate 100mL volumetric flasks. Dilute to volume with acetonitrile and mix well. Store at <-10°C when not in use.

Prepare individual 20µg/mL solutions of Fenoxaprop-P-Ethyl, AE F088406, and AE F054014 by taking a 20.0mL aliquot of the 100µg/mL stock solutions and diluting to 10mL with acetonitrile. Store at <-10°C when not in use.

Prepare individual 2.0µg/mL solutions of Fenoxaprop-P-Ethyl, AE F088406, and AE F054014 by taking a 2.0mL aliquot of the 100µg/mL stock solutions and diluting to 100mL with acetonitrile. Store at <-10°C when not in use.

NOTE: Corrections for standard purities should be applied when expressing standard concentrations. AE F088406 and AE F054014 should be expressed as parent equivalent.

4.2 Fortification Standard Solutions

The individual 2.0µg/mL solutions of Fenoxaprop-P-Ethyl, AE F088406 and AE F054014 prepared in section 4.1 above may be used as the fortification solutions.

Further dilutions of this mixed fortification solution may be made as needed.

4.3 Calibration Standard Solutions

Prepare working calibration solutions consisting of 0.5, 1, 5, 10, 50, and 100ng/mL of Fenoxaprop-P-Ethyl, AE F088406, and AE F054014 as described in the following table and diluting all solutions to 100mL with 70:30v/ v methanol:water.

Concentration of Standard Solution used for dilution (µg/mL)	Aliquot Native mix Taken (mL)	Dilution Volume (mL)	Concentration of Calibration Solution (ng/mL)
2	0.025	100	0.5
2	0.05	100	1
2	0.25	100	5
2	0.5	100	10
20	0.25	100	50
20	0.5	100	100

Additional calibration solutions may be prepared as required. Refrigerate when not in use. All solutions are stable for approximately one week.

5. EXTRACTION

NOTE: The analytical targets in this method are subject to rapid degradation. It is important to complete the extraction in a timely manner.

1. Transfer 20±0.2 grams of soil into a 125ml glass jar.
2. Fortify the recovery samples at the desired fortification level using the individual standard solutions prepared in acetonitrile (see Section 4.2 Fortification Standard Solutions).
3. Add 30mL of acetonitrile/water solution (80/20). Cap and shake glass jar.
4. Place jar on a mechanical shaker for 20 minutes with the setting on high.
5. Remove the jar and centrifuge at approximately 2000 rpm for about 3 minutes. Because glass jars are used, the centrifuge speed should not exceed 2000 rpm because at higher rpm the glass jars could break.
6. After centrifugation, decant each sample into a 100mL stoppered measuring cylinder labeled with the sample ID number.
7. Add 30mL of acetonitrile/water solution (80/20). Shake vigorously by hand to break up the soil cake at the bottom of the jar before placing on the shaker.
8. Place jar on a mechanical shaker for 20 minutes with the setting on high.
9. Remove the jar and centrifuge at approximately 2000 rpm for about 3 minutes.
10. Decant the liquid into the same cylinder used in Step 5.6.

11. Add 20mL of the 10g/L sodium chloride aqueous solution. Shake vigorously by hand to break up the soil cake at the bottom of the jar before placing on the shaker.

12. Place jar on a mechanical shaker for 20 minutes with the setting on high.

13. Remove the jar and centrifuge at approximately 2000 rpm for about 3 minutes.

14. Decant the liquid into the same cylinder used in Step 5.6. Dilute the contents of the measuring cylinder to 80mL with acetonitrile/water solution (80/20). Stopper the cylinder and mix the contents.

15. Pipet a 0.75mL aliquot of the sample solution into a LC vial containing 0.75mL of 70:30v/v methanol:deionized water. Cap the vial and mix to await analysis by LC/MS/MS.

6. ANALYSIS

6.1 Sample Analysis

Step 1. Using the recommended procedures listed below; analyze an aliquot of the 0.5, 1, 5, 10, 50, and 100ng/mL standard solutions (these are calibration solution analyses).

Step 2. Analyze an aliquot of the analytical sample from Section 4.4 Step 15. Note: Up to 25 sample analyses can be made after the analysis of the standard solutions.

Step 3. Again analyze an aliquot of the 0.5, 1, 5, 10, 50, and 100ng/mL calibration standard solutions.

Step 4. When necessary, analyze additional samples and standard solutions. Always finish the procedure with the analysis of a set of standard solutions.

6.2 LC/MS/MS Standard Calibration and Residue Calculations

Standardize the LC/MS/MS response under the conditions outlined in Appendix 1 by injecting an aliquot of each LC/MS/MS calibration solution interspersed with samples.

The residues of Fenoxaprop-P-Ethyl and its metabolites AE F088406 and AE F054014 are quantified using external standard linear regression analysis. A separate calibration curve was produced for each set of samples analyzed on the LC/MS/MS. A calibration curve was generated by linear regression of the standard peak area versus the standard concentrations in ng/mL using Applied Biosystems Analyst Software (Version 1.4.1), a computer-programmed data capturing system. The Analyst Software uses the MS/MS standard responses to calculate the regression coefficients M and B, respectively called slope and intercept, for each analytical set.

The standards were fit to the linear equation: $Y = MX + B$

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

The calibration points are weighted 1/x to provide better fit near the limit of detection.

After regression coefficients were calculated, the residue in parts per billion was determined. The parts per billion (ppb) of fenoxaprop-p-ethyl in the soil was calculated using the following equation,

$$\text{Fenoxaprop-P-Ethyl found (ppb)} = \frac{(Y-B) \times D}{M}$$

$$\text{Where Dilution Factor (D)} = \frac{\text{Initial volume (V1)}}{\text{Initial sample wt. (W)}} \times \frac{\text{Final volume (V3)}}{\text{Aliquot (V2)}}$$

Where: W = 20g
V1 = 80mL
V2 = 0.75mL
V3 = 1.5mL

Analyst software was used to calculate the residues of Fenoxaprop-P-Ethyl in ppb for each sample and the percent recovery for the fortified samples. AE F088406 and AE F054014 residue concentrations are determined in a similar fashion.

6.3 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 & 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 0.01ug/g or other appropriate level with fortification solutions. Calculate the final residue R for the control (S) and fortified control (R) samples.

Appendix 1 Instrument Conditions For Fenoxaprop-P-Ethyl and its metabolites

Equipment with equivalent or better sensitivity and performance may be substituted.

LC/MS/MS Parameters

NOTE: Variations in equipment or sample characteristics may require slight modifications in the chromatographic or detector conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity. Therefore, the given LC/MS/MS parameters listed below are guidelines and may be modified. These parameters should be optimized for the instrument and column actually used. Also, instrument parameters and mobile phase may be adjusted to improve separation from any observed interfering peaks.

The following conditions were used on an ABSciex API 4000 LC/MS/MS system.

HPLC Parameters

Pumps Used:	Two Shimadzu LC-10ADVP (High Pressure Mixer) pumps with a Shimadzu SCL-10 controller	
Autosampler	CTC PAL	
Column Temperature:	50°C	
Injection Volume:	10uL	
Column:	Manufacturer:	Phenomenex®
	Type:	Luna C18 (2)-HST
	Particle Size:	2.5 µm
	Diameter:	2.0 mm
	Length:	50 mm
Mobile Phase A:	Water/Methanol 90:10 (v/v) with 10mmol/L ammonium formate and 120µL formic acid/L	
Mobile Phase B:	Methanol/Water 90:10 (v/v) with 10mmol/L ammonium formate and 120µL formic acid/L	

HPLC gradient program:

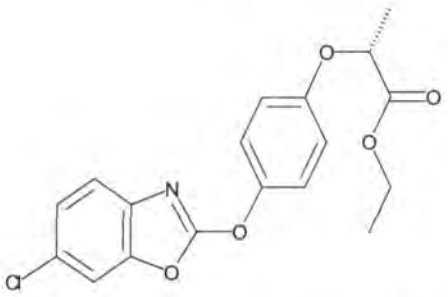
Time (min.)	Module	Flow Rate (mL/min)	A(%)	B(%)
0.0	Pumps	0.50	95	5
0.5	Pumps	0.50	95	5
2.5	Pumps	0.50	5	95
6.0	Pumps	0.50	5	95
7.0	Pumps	0.50	5	95
7.1	Pumps	0.50	95	5
8.0	System Controller	Stop		

Mass Spectrometer Instrument Conditions

Component:	Fenoxaprop-P-ethyl	Fenoxaprop-P-ethyl	AE F088406	AE F088406	AE F054014	AE F054014
Retention Time	5.2 minutes	5.2 minutes	5.1 minutes	5.1 minutes	3.4 minutes	3.4 minutes
Transition	MRM1	MRM2	MRM1	MRM2	MRM1	MRM2
Parent Ion	363	363	332	332	168	168
Product Ion	288	77	260	152	132	76
Ionization Mode	ESI	ESI	ESI	ESI	ESI	ESI
Polarity	+	+	-	-	-	-
Dwell Time (ms)	600	600	100	100	100	100
Declustering Potential (DP)	76	76	-70	-65	-65	-70
Entrance Potential (EP)	10	10	-10	-10	-10	-10
Collision Energy (CE:	27	83	-18	-30	-26	-34
Collision Cell Exit Potential (CXP)	10	4	-7	-11	-7	-5
Curtain Gas (CUR)	30	30	30	30	30	30
Collision Gas (CAD)	6	6	6	6	6	6
Ion Source Gas 1 (GS1)	30	30	30	30	30	30
Ion Source Gas 2 (GS2)	50	50	50	50	50	50
Source Temp (TEM)	500	500	500	500	500	500
Interface Heater (IHE)	On	On	On	On	On	On
Ion Transfer Voltage (IS)	4500	4500	-4500	-4500	-4500	-4500

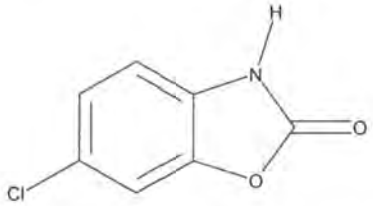
Appendix 2 Structures

Chemical Name: Fenoxaprop-P-Ethyl
(Parent Molecule)



CAS Name: Ethyl (2R)-2-[4-[(6-Chloro-2-benzoxazolyl)oxy]phenoxy]propanoate
CAS Number 71283-80-2
Molecular Formula: $C_{18}H_{16}ClNO_5$
Molecular Weight: 361.7763

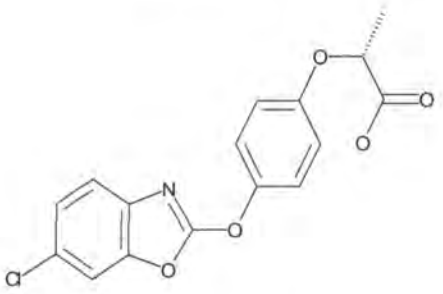
Chemical Name: AE F054014
(Metabolite)



CAS Name: 6-Chloro-2(3H)-benzoxazolone
CAS Number 19932-84-4
Molecular Formula: $C_7H_4ClNO_2$
Molecular Weight: 169.5652

Appendix 2 (continued)

Chemical Name: AE F088406
(Metabolite)



CAS Name: (2*R*)-2-[4-[(6-Chloro-2-benzoxazolyl)oxy]phenoxy]propanoic acid
CAS Number 113158-40-0
Molecular Formula: $C_{16}H_{12}ClNO_5$
Molecular Weight: 333.7232