

I. SUMMARY

The objective of this study was to perform an independent evaluation of the ruggedness, usability, and potential weaknesses of a method for the analysis of Fenoxaprop P-ethyl, AE F088406 and AE F054014 in surface water. The matrix used was surface water collected from Speed River in Guelph, ON. The method was performed as described in Bayer CropScience method number FP-003-W11-01 and evaluated following the EPA Test Guidelines OPPTS 850.7100 (d), and OPPTS 860.1340 (c)(6).

Primary Method

Fenoxaprop-P-Ethyl and its metabolites (AE F088406 and AE F054014) were determined by diluting a surface water aliquot with methanol and analyzing by LC/MS/MS.

The independent method validation trial consisted of one analytical set for surface water. This set consisted of one reagent blank, two (2) untreated control samples, five (5) replicate fortifications at the LOQ of 0.50 ng/mL (ppb) with Fenoxaprop P-Ethyl, AE F088406 and AE F054014, and five (5) replicate fortifications with Fenoxaprop P-ethyl, AE F088406 and AE F054014 at 5.0 ppb (10 X LOQ).

The validation results demonstrate that Bayer CropScience method FP-003-W11-01 is workable as written.

II. MATERIALS

A. Reference Substances and Chemical Structures

Fenoxaprop-P-Ethyl

Supplier: Bayer CropScience

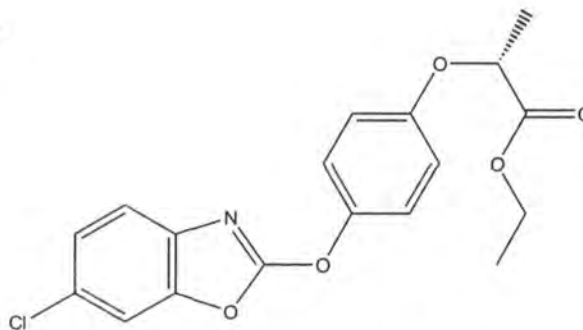
Purity: 99.5%

Appearance: White crystal

Lot #: K-1822

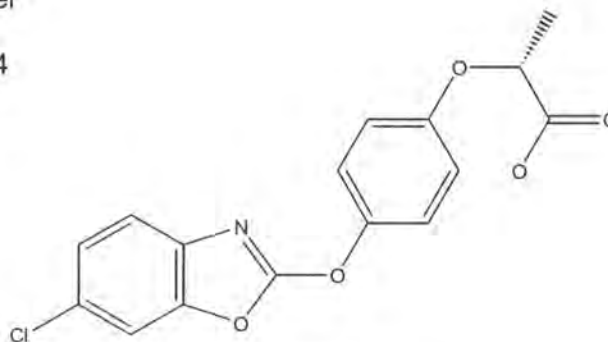
Expiry Date: 02-Oct-2012

Storage Temp.: <-10°C



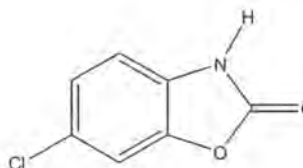
Fenoxaprop-P-Ethyl

Fenoxaprop-P (AE F088406)
Supplier: Bayer CropScience
Purity: 98.3%
Appearance: white powder
Lot #: K-1821
Expiry Date: 24-Aug-2014
Storage Temp.: <-10°C



AE F088406

6-Chloro-1,3-benzoxazolone (AE F054014)
Supplier: Bayer CropScience
Purity: 98.5%
Appearance: white crystal
Lot #: K-1957
Expiry Date: 09-Nov-2017
Storage Temp.: <-10°C



AE F054014

1. Primary Stock Standard Solutions Preparation

a. Fenoxaprop-P-Ethyl Stock Solution

A stock solution (99.6 µg/mL – corrected for % purity) of Fenoxaprop-P-Ethyl was prepared by quantitatively transferring 0.01001 g of the analytical standard into a 100 mL volumetric flask. The standard was dissolved and diluted to volume in acetonitrile. The solution was stored at <-10°C.

b. AE F088406 Stock Solution

A stock solution (104 µg/mL – corrected for % purity) of AE F088406 was prepared by quantitatively transferring 0.01060 g of the analytical standard into a 100 mL volumetric flask. The standard was dissolved and diluted to volume in acetonitrile. The solution was stored at <-10°C.

c. AE F054014 Stock Solution

A stock solution (106 µg/mL – corrected for % purity) of AE F054014 was prepared by quantitatively transferring 0.01072 g of the analytical standard into a 100 mL volumetric flask. The standard was dissolved and diluted to volume in acetonitrile. The solution was stored at <-10°C.

2. Mixed Fortification Standard Solutions Preparation

a. A mixed standard solution containing 1.00 µg/mL Fenoxaprop-P-Ethyl, AE F088406 and AE F054014 was prepared by transferring concentration adjusted volumes of stock standard solutions of Fenoxaprop-P-Ethyl (1.005 mL), AE F088406 (0.962 mL), and AE F054014 (0.944 mL) to a 100 mL volumetric flask. The solution was diluted to volume with acetonitrile and stored at <-10°C.

b. A mixed standard solution containing 0.100 µg/mL Fenoxaprop-P-Ethyl, AE F088406 and AE F054014 was prepared by transferring 10.0 mL of the mixed standard solution containing 1.00 µg/mL of Fenoxaprop-P-Ethyl, AE F088406 and AE F054014 to a 100 mL volumetric flask. The solution was diluted to volume with acetonitrile and stored at <-10°C.

3. Calibration Standard Solutions Preparation

Six calibration standards suitable for LC/MS/MS analysis were prepared by serial dilution in 70:30 methanol/water. The solutions were stored at <-10°C. The dilution scheme was as follows:

Mixed Standard Concentration (ppb)	Aliquot of Mixed Standard (mL)	Final Volume (mL)	Final Calibration Standard Concentration (ppb)
0.100	0.0500	100	0.0500
0.100	0.100	100	0.100
1.00	0.0500	100	0.500
1.00	0.100	100	1.00
1.00	0.500	100	5.00
1.00	1.00	100	10.0

B. Reagents/Solvents

Acetonitrile - EMD
Ammonium formate - J.T. Baker
Formic acid, concentrated - EMD
HPLC Water - EMD
Methanol - Fisher Scientific

- 70:30 methanol/water -1400 mL of methanol was mixed well with 600 mL of HPLC water.
- 90:10 water/methanol with 10mM ammonium formate and 120 µL/L formic acid - Dissolve 12.6 mg of ammonium formate into 1800 mL of HPLC water. Measure and add 200 mL of methanol and 240 µL of formic acid.
- 90:10 methanol/water with 10mM ammonium formate and 120 µL/L formic acid - Dissolve 12.6 mg of ammonium formate into 200 mL of HPLC water. Measure and add 1800 mL of methanol and 240 µL of formic acid.

C. Laboratory Materials and Supplies

Balance (analytical) - Mettler XS 205DU (#33)
 Glass autosampler vials and caps, 2 mL - National Scientific
 Graduated Cylinders - VWR
 Microman pipettors and tips
 Volumetric Flasks, class A - Kimax

D. Instrumentation, Conditions and Parameters

1. Instrumentation for LC/MS/MS analysis

Mass Spectrometer: AB Sciex 4000 LC/MS/MS system
 HPLC: Perkin Elmer 200 series controller and pumps
 Autoinjector: CTC HTS-PAL
 Software: Analyst 1.4.2

2. HPLC operating conditions for Fenoxaprop-P-Ethyl, AE F088406 and AE F054014

Column - Phenomenex® Luna C₁₈ (2)-HST 50 x 2.00 mm
 Column temperature - 50°C
 Mobile phase A - 90/10 water/methanol with 10mM ammonium formate and 120 µL/L formic acid
 Mobile phase B - 90/10 methanol/water with 10mM ammonium formate and 120 µL/L formic acid
 Injection volume - 25 µL
 Gradient Conditions:

Time (min.)	% A	% B	Flow Rate (µL/min)
0.0	95.0	5.0	500
2.5	5.0	95.0	500
6.0	5.0	95.0	500
7.0	5.0	95.0	500
7.1	95.0	5.0	500
8.0	95.0	5.0	500

3. MS acquisition conditions for Fenoxprop-p-ethyl, AE F088406 and AE F054014

Period 1 Experiment 1:

Analyte	MRM transition	Polarity	RT (min:sec)	Dwell (msec)	DP	CE	CXP
AE F054014	168.0 → 131.8	Negative	2.57	250	-65.0	-26.0	-7.0
AE F054014 confirmatory	168.0 → 76.0	Negative	2.57	250	-70.0	-34.0	-5.0

Parameter	Negative Mode
Gas 1 (GS1) (N ₂)	35
Gas 2 (GS2) (N ₂)	40
Curtain gas (N ₂)	30
CAD gas (N ₂)	6.00
Temperature (°C)	500
Interface Heater	On
Ion Spray Voltage	-4500
EP	-10.0

Period 2 Experiment 1:

Analyte	MRM transition	Polarity	RT (min:sec)	Dwell (msec)	CE
Fenoxaprop-P-Ethyl	362.3 → 288.2	Positive	3.48	600	26.00
Fenoxaprop-P-Ethyl confirmatory	362.3 → 77.0	Positive	3.48	600	83.00

Parameter	Positive Mode
Gas 1 (GS1) (N ₂)	35
Gas 2 (GS2) (N ₂)	40
Curtain gas (N ₂)	30
CAD gas (N ₂)	6.00
Temperature (°C)	500
Interface Heater	On
Ion Spray Voltage	4500
EP	11.0
DP	85.0
CXP	8.00

Period 2 Experiment 2:

Analyte	MRM transition	Polarity	RT (min:sec)	Dwell (msec)	DP	CE	CXP
AE F088406	332.1 → 259.9	Negative	3.46	250	-70.0	-18.0	-7.0
AE F088406 confirmatory	332.1 → 152.0	Negative	3.46	250	-65.0	-30.0	-11.0

Parameter	Negative Mode
Gas 1 (GS1) (N ₂)	35
Gas 2 (GS2) (N ₂)	40
Curtain gas (N ₂)	30
CAD gas (N ₂)	6.00
Temperature (°C)	500
Interface Heater	On
Ion Spray Voltage	-4500
EP	-10.0

III. CALCULATION

Calibration standards and samples were analyzed using LC/MS/MS. Calibration curves and residue values were calculated using Analyst 1.4.2 data handling software using linear regression with $1/x^2$ weighing.

The standards were fit to the linear equation:

$$y = mx + b$$

where: x = concentration of the reference standard/sample

m = calibration line slope

b = calibration line intercept

y = peak area

Example Calculation:

Sample ID: L2447-1+1 (water control)

Analyte: Fenoxaprop-p-ethyl (primary transition)

Fortification level: 0.500 ng/mL

$$\text{Dilution Factor} = \frac{\text{Final Volume}}{\text{Sample Value}} = \frac{100 \text{ mL}}{30 \text{ mL}} = 3.33$$

$$X \text{ ng/mL(ppb)} = \frac{37600 - (-74.3) \times 3.33}{2.58 \times 10^5} = 0.486 \text{ ng/mL(ppb)}$$

Recovery Calculation:

The percent recovery was calculated for fortified samples by subtracting the corresponding control residue from the residue of the fortified control.

Fort level: 0.500 ppb

Corresponding control ID: L2447-1-1 (water control)

Corresponding control residue: 0.00 ppb

Percent Recovery:

$$\frac{(0.486 \text{ ppb} - 0.00 \text{ ppb})}{0.500 \text{ ppb}} \times 100 = 97\%$$

IV. ANALYTICAL PROCEDURE

The analytical method (FP-003-W11-01) used for the ILV was received from Bayer CropScience. A copy of the method is included in Appendix III.

The procedure was followed exactly, with slight modifications of instrument operating conditions after optimizing sensitivity.

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1. PRINCIPLE

The residues of Fenoxaprop P-Ethyl and its metabolites AE F088406 and AE F054014 in water are determined by diluting a sample aliquot with methanol and analyzing the diluted sample by LC/MS/MS. Quantification is based on a comparison of peak areas with those of known standards.

The method limit of quantitation (LOQ) for Fenoxaprop-P-Ethyl and its metabolites AE F088406 and AE F054014 is 0.5ng/mL in water.

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)
- 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 (Applied Biosystems), or equivalent.
- Fisherbrand 125-mL glass jars (Cat. No. 02-911-455)
- Various general laboratory glassware and utensils

3. REAGENTS

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

- Water (HPLC Grade; Fischer Scientific, Cat. No 113542.)
- Acetic Acid, glacial (Fischer Scientific, Cat. No. 012251)
- Acetonitrile (ACN; HPLC Grade; Fischer Scientific, Cat. No. 114505)
- Methanol (Fischer Scientific, Cat. No. 113436)
- Formic acid (Fischer Scientific)
- Ammonium Formate (Fischer Scientific, Cat. No. 030113)
- Methanol/water solution (70/30). Mix 700mL of methanol with 300mL of water and mix well
- 90/10 water/methanol with 10mM ammonium formate and 120uL/L formic acid. Mix 900mL of water and 100mL of methanol. Add 6.306mg of ammonium formate and add to the 90/10 mixture. Add 120uL of formic acid and mix well.
- 90/10 methanol/water with 10mM ammonium formate and 120uL/L formic acid. Mix 900mL of methanol and 100mL of water. Add 6.306mg of ammonium formate and add to the 90/10 mixture. Add 120uL of formic acid and mix well.
- Certified analytical reference standards of Fenoxaprop-P-Ethyl, AE F088406 and AE F054014

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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 100 mL volumetric flasks. Dilute to volume with acetonitrile and mix well. Store at <-10°C when not in use.

Prepare individual 1.0µg/mL solutions of Fenoxaprop-P-Ethyl, AE F088406, and AE F054014 by taking a 1.0mL aliquot of the 100µg/mL stock solutions and diluting to 100mL with acetonitrile. Store at <-10°C when not in use.

Prepare individual 0.1µg/mL solutions of Fenoxaprop-P-Ethyl, AE F088406, and AE F054014 by taking a 10.0mL aliquot of the 1.0µg/mL stock solutions and diluting to 10mL with acetonitrile. Store at <-10°C when not in use.

NOTE: Corrections for standard purities should be applied when expressing standard concentrations.

4.2 Fortification Standard Solutions

The individual 1.0µg/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 the individual fortification solutions may be made as needed.

4.3 Calibration Standard Solutions

Prepare working calibration solutions consisting of 0.05, 0.1, 0.5, 1.0, 5.0 and 10.0ng/mL of Fenoxaprop-P-Ethyl, AE F088406, and AE F054014 by diluting to 100mL with 70:30v/v methanol:deionized water.

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Concentration of Standard Solution used for dilution (ng/mL)	Aliquot Native mix Taken (mL)	Dilution Volume (mL)	Concentration of Calibration Solution (ng/mL)
100	0.05	100	0.05
100	0.1	100	0.1
1000	0.05	100	0.5
1000	0.10	100	1.0
1000	0.50	100	5.0
1000	1.0	100	10.0

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

5. EXTRACTION

1. Transfer 30 ± 0.1 mL of water into a stoppered 100 mL measuring cylinder.
2. Fortify the recovery samples at the desired fortification level using the individual standard solutions prepared in acetonitrile (see Section 4.2 Fortification Stock Solutions, for fortifications at the LOQ, fortify with 15 μ L of each of the three 1.0 μ g/mL fortification solutions).
3. Dilute to 100 mL with methanol. Cap and shake the measuring cylinder.
4. Transfer an aliquot of the sample solution into a LC vial, 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 0.05, 0.1, 0.5, 1.0, 5.0 and 10.0 ng/mL standard solutions (these are calibration solution analyses).
- Step 2. Analyze an aliquot of the analytical samples. Note: Up to 25 sample analyses can be made after the analysis of the standard solutions.
- Step 3. Again analyze an aliquot of the 0.05, 0.1, 0.5, 1.0, 5.0 and 10.0 ng/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.

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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 water was calculated using the following equation,

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

$$\text{Where Dilution Factor (D)} = \frac{\text{Final Volume (V2)}}{\text{Aliquot (V1)}} = 3.333$$

Where: V1 = 30mL
V2 = 100mL

Analyst software was used to calculate the amount of Fenoxaprop-P-Ethyl in ppb for each sample and the percent recovery for the fortified samples.

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.

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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.

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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 API 4000 LC/MS/MS system.

Instrument Used: Applied Biosystems API 4000 LC/MS/MS System
 Interface: PE Sciex Turbo Ion Spray Electrospray
 Synchronization Mode: LC Sync
 AutoEquilibration: Off
 Acquisition Duration: 7 min. 0 sec.
 Periods in File: 1
 Acquisition Module: Acquisition Method
 Software Version: Analyst 1.5.1

Period 1 Experiment 1:

Scan Type: MRM (MRM)
 Polarity: Negative
 Scan Mode: N/A
 Ion Source: Turbo Spray
 Resolution Q1: Unit
 Resolution Q3: Low
 Intensity Thres.: 0.00 cps

Analyte (~5.1 Min.)	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	Parameter	Start
AE F088406	332.1	259.9	100	DP	-70
				EP	-10
				CE	-18
				CXP	-7
Analyte (~5.1 Min.)	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	Parameter	Start
AE F088406 Confirmatory	332.1	152	100	DP	-65
				EP	-10
				CE	-30
				CXP	-11

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Appendix 1 (Continued)

Analyte (~3.4 Min.)	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	Parameter	Start
AE F054014	168.0	131.8	100	DP	-65
				EP	-10
				CE	-26
				CXP	-7

Analyte (~3.4 Min.)	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	Parameter	Start
AE F054014 Confirmatory	168.0	76	100	DP	-70
				EP	-10
				CE	-34
				CXP	-5

Parameter Table	CUR:	30
	GS1:	30
	GS2:	50
	IS:	-4500
	TEM:	500
	CAD:	6

Period 1 Experiment 2:

Scan Type:	MRM (MRM)
Polarity:	Positive
Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	Unit
Intensity Thres.:	0.00 cps
Settling Time:	700.0000 msec
MR Pause:	5.0070 msec
MCA:	No

Analyte (~5.2 Min.)	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	Parameter	Start
Fenoxaprop-P-Ethyl	362.3	288.2	600	DP	76
				EP	10
				CE	27
				CXP	10

Analyte (~5.2 Min.)	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	Parameter	Start
Fenoxaprop-P-Ethyl Confirmatory	362.3	77	600	DP	76
				EP	10
				CE	83
				CXP	4

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Appendix I (continued)

Parameter Table CUR: 30
 GS1: 30
 GS2: 50
 IS: 4500
 TEM: 500
 CAD: 6

CTC PAL Autosampler Properties

Inject Details

Syringe Size (µl): 100
Injection Volume (µl): 12µL (Adjust for LC/MS/MS system being used)

HPLC Parameters

Pumps Used: Two Shimadzu LC-10ADVP (High Pressure Mixer) pumps with a Shimadzu SCL-10 controller
Column Temperature: 50°C
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:

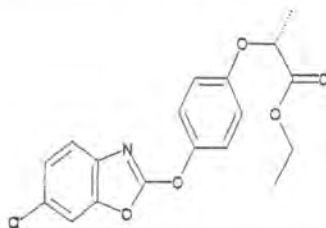
<u>Time (min.)</u>	<u>Module</u>	<u>Flow Rate (mL/min)</u>	<u>A(%)</u>	<u>B(%)</u>
0.5	Pumps	0.50	95	5
2.5	Pumps	0.50	5	95
6.0	Pumps	0.50	5	95
7.0	Pumps	0.50	5	95
7.1	Pumps	0.50	95	5
8.0	System Controller	Stop		

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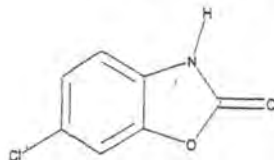
Appendix 2 Structures

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: C₁₈ H₁₆ Cl N O₅
Molecular Weight: 361.7763

Chemical Name: AE F054014
(Metabolite)



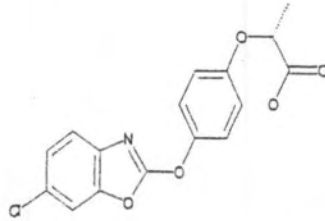
CAS Name: 6-Chloro-2(3H)-benzoxazolone
CAS Number: 19932-84-4
Molecular Formula: C₇ H₄ Cl N O₂
Molecular Weight: 169.5652

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Appendix 2 (continued)

Chemical Name: AE F088406
(Metabolite)



CAS Name: (2R)-2-[4-[(6-Chloro-2-benzoxazolyl)oxy]phenoxy]propanoic acid
CAS Number: 113158-40-0
Molecular Formula: C₁₆ H₁₂ Cl N O₅
Molecular Weight: 333.7232