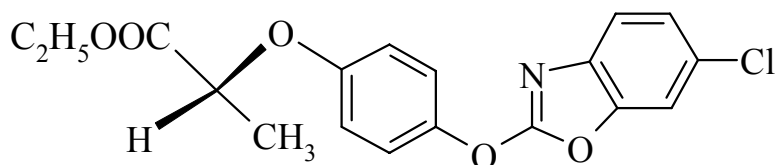


FENOXAPROP-P-ETHYL
484



<i>ISO common name</i>	Fenoxaprop-P-ethyl
<i>Chemical name</i>	Ethyl (<i>R</i>)-2-[4-(6-chloro-1,3-benzoxazol-2-yloxy)-phenoxy]propanoate (IUPAC); ethyl (<i>R</i>)-2-{4-[6-chloro-2-benzoxazol)yl oxy]phenoxy}propanoate (CA; 71283-80-2)
<i>Empirical formula</i>	C ₁₈ H ₁₆ ClNO ₅
<i>RMM</i>	361.8
<i>m.p.</i>	84 - 87 °C
<i>v.p.</i>	19.0 × 10 ⁻⁹ Pa at 20 °C
<i>Solubility</i>	In water 0.7 mg/l at 20 °C. Very soluble in most organic solvents
<i>Description</i>	White powder
<i>Formulations</i>	Oil in water emulsion

FENOXAPROP-P-ETHYL TECHNICAL 484/TC/M/-

1 Sampling. Take at least 100 g of the homogenised sample.

2 Identity tests

2.1 HPLC. Use the HPLC method 3.2 below. The retention time of fenoxaprop-P-ethyl for the sample solution should not deviate by more than 1% from that of the calibration solution.

2.2 TLC. Use the following conditions:

<i>TLC plate</i>	silicagel 60 F ₂₅₄ , 0.20 mm, 20 × 10 cm (Merck or equivalent)
<i>Eluting solvent</i>	toluene - ethyl acetate, 95 + 5 (v/v)
<i>Sample size</i>	1 µl of a methanolic solution containing about 4 mg/ml fenoxaprop-P-ethyl
<i>Visualisation</i>	UV at 254 nm
<i>R_F value</i>	approximately 0.3

The major spot from the sample should have the same R_F value as that from the standard.

3 Fenoxaprop-P-ethyl

OUTLINE OF METHOD Fenoxaprop-ethyl is a racemic mixture, containing equal amounts of fenoxaprop-P-ethyl (*R*-enantiomer) and fenoxaprop-M-ethyl (*S*-enantiomer). Hence two methods are used to determine the chemical and the enantiomeric purity.

The first analytical procedure determines the fenoxaprop content of the sample and the method determines the sum of both enantiomers. This method may also be used for the determination fenoxaprop racemic mixtures. The active ingredient is determined by normal phase HPLC using a silica stationary phase and gradient elution, UV detection and external standardisation.

The second part describes the determination of the enantiomeric purity. Fenoxaprop-P-ethyl is separated on a chiral phase (mobile phase isooctane: propan-2-ol-trifluoroacetic acid) from the other enantiomer and the enantiomeric ratio is determined using UV detection.

*3.1 Determination of the chemical purity

REAGENTS

Fenoxaprop-P-ethyl certified reference substance (Standard R1)

1,4 Dioxane HPLC quality (without water)

2-Ethylhexane (isooctane) HPLC quality

Water HPLC quality or distilled in glass

1,4 Dioxane with 0.15 % water HPLC quality (add 1.5 ml water to 1000 ml dioxane)

Eluent A isooctane - dioxane (with 0.15% water), 970 + 30 (v/v)

Eluent B isooctane - dioxane (with 0.15% water), 900 + 100 (v/v)

Calibration solution Weigh (to the nearest 0.1 mg) in duplicate into two volumetric flasks (100 ml) about 120 mg (s mg) fenoxaprop-P-ethyl (Standard R1). Dissolve in dioxane (10 ml) (10 min ultrasonic bath), add isooctane (80 ml), allow to cool to ambient temperature and make up to the mark with isooctane. Mix thoroughly. Pipette 10 ml of these solutions into separate volumetric flasks (100 ml) and make up to the mark with eluent B. Mix thoroughly. Filter the solutions through a 0.45 µm filter before injection.

APPARATUS

High performance liquid chromatograph fitted with a variable wavelength ultraviolet detector and an injector capable of delivering 20 µl

Liquid chromatographic column stainless steel, 125 × 4.0 mm (i.d.), packed with Hypersil Silica 3 µm

Column oven

Sample filtering device with a membrane filtration unit compatible with organic solvents and a 0.45 µm pore diameter

Electronic integrator or data system

Ultrasonic bath

Filter pore diameter 0.45 µm (Schleicher & Schuell, Spartan 30/B, Brown RIM L)

PROCEDURE

(a) *Operating conditions* (typical):

<i>Eluent composition</i>	A: isooctane - dioxane (with 0.15% water), 970 + 30 (v/v)
	B: isooctane - dioxane (with 0.15% water), 900 + 100 (v/v)

* CIPAC method 2000. Prepared by the German Committee (DAPA). Chairman: W Dobrat. Based on a method supplied by AgrEvo, FRG.

Time (min)	% A	% B	Eluent flow rate ml/min
0	100	0	1.3
10	100	0	1.3
12	0	100	2.0
18	0	100	2.0
20	100	0	1.3
25	100	0	1.3

<i>Temperature</i>	40 °C
<i>Injection volume</i>	20 µl
<i>Detector wavelength</i>	227 nm
<i>Retention time</i>	Fenoxaprop-ethyl: 4.6 min

(b) *Preparation of sample.* Weigh, in duplicate, (to the nearest 0.1 mg) into two volumetric flasks (100 ml) sufficient sample to contain about 120 mg of the fenoxaprop-P-ethyl (*w* mg). Dissolve in dioxane (10 ml) (10 min ultrasonic bath), add isooctane (80 ml), allow to cool to ambient temperature and make up to the mark with isooctane. Mix thoroughly. Pipette 10 ml of these solutions into separate volumetric flasks (100 ml) and make up to the mark with eluent B. Mix thoroughly. Filter the solutions through a 0.45 µm filter before injection.

(c) *Determination.* Inject a 20 µl portion of the first calibration solution into the liquid chromatograph. Inject the calibration solution at least three times, record the relevant peak areas and calculate the response factor. Repeat the injection until the response factors obtained for three successive injections differ by no more than $\pm 1\%$ of their mean. Then inject, in duplicate, 20 µl portions of each sample solution bracketing them by duplicate injections of the calibration solutions. Measure the relevant peak areas. Calculate the mean value of each pair of calibration factors bracketing the sample injections and use this value for evaluating the bracketed sample runs. Calculate for each sample the mean content.

(d) *Calculation*

$$f_i = \frac{s \times P}{H_s}$$

$$\text{Content of fenoxaprop-ethyl (enantiomeric mixture)} = \frac{H_w \times f}{w} \text{ g/kg (E)}$$

where:

f_i = single response factor

f = average response factor

H_s = area of fenoxaprop-ethyl peak in calibration solution

H_w = area of fenoxaprop-ethyl in the sample solution

s = mass of fenoxaprop-ethyl the calibration solution (mg)

w = mass of sample taken (mg)

P = purity of fenoxaprop-P-ethyl certified reference substance (g/kg)

Repeatability r = 18 g/kg at 950 g/kg active ingredient content

Reproducibility R = 28 g/kg at 950 g/kg active ingredient content

* 3.2 Determination of the enantiomeric purity

REAGENTS

Fenoxaprop-ethyl standard (racemate) of known purity (Standard R2)

2-Ethylhexane (isooctane) HPLC quality

Propan-2-ol HPLC quality

Trifluoroacetic acid e.g. Riedel-de-Haën

Eluent isooctane - propan-2-ol - trifluoroacetic acid, 995.5 + 2.5 + 2.0 (v/v).

Degassing of the mobile phase with helium over longer periods can change the composition.

Dilution solvent isooctane with 0.1% propan-2-ol

Control solution. Weigh into a volumetric flask (100 ml) about 10-15 mg of fenoxaprop-ethyl (Standard R2). Dissolve in diluting solvent (80 ml) by placing the flasks in an ultrasonic bath for 10 min. Allow to cool to ambient temperature and make up to the mark with diluting solvent. Mix thoroughly. Filter the solution through a 0.45 µm filter before injection.

APPARATUS

High performance liquid chromatograph fitted with a variable wavelength ultraviolet detector and an injector capable of delivering 20 µl

Liquid chromatographic column stainless steel column 250 × 4 mm (i.d.), packed with Nucleosil Chiral, 3.5 µm, Macherey-Nagel

* Provisional CIPAC method 1999. Prepared by the German Committee (DAPA). Chairman: W Dobrat. Based on a method supplied by AgrEvo, FRG.

Column thermostat

Sample filtering device with a membrane filtration unit compatible with organic solvents and a 0.45 µm pore diameter

Electronic integrator or data system

Ultrasonic bath

PROCEDURE

(a) Operating conditions (typical):

<i>Mobile phase</i>	isooctane + 2-propanol trifluoroacetic acid, 995.5 + 2.5 + 2.0 v/v
<i>Flow</i>	1.5 ml/min
<i>Temperature</i>	between 15 °C (better separation) and 25 °C
<i>Injection volume</i>	20 µl
<i>Detector wavelength</i>	237 nm
<i>Retention time</i>	Fenoxaprop-M-ethyl: about 17 min (at 15 °C) Fenoxaprop-P-ethyl: about 19 min (at 15 °C)

(b) *System equilibration and check.* Pump the mobile phase through the column for about 2 h until the system is equilibrated and a flat base line is obtained.

Inject 20 µl of the control solution. The chromatogram should show the two enantiomers, fenoxaprop-P-ethyl and fenoxaprop-M-ethyl in a ratio of 50 : 50 (area %) with a deviation of less than 1%. If not, calculate a correction factor (see section (e)). A column temperature of 15 °C is usually the best to obtain good separation. At higher temperatures, up to 25 °C, the separation may deteriorate. Temperatures higher than 25 °C must be avoided. If the chromatogram does not show the expected separation, rinse the column for about ½ h with isooctane with 10 % propan-2-ol as eluent and repeat the system equilibration.

(c) *Preparation of sample.* Weigh in duplicate (to the nearest 0.1 mg) into two volumetric flasks (100 ml) sufficient sample to contain 10-15 mg of fenoxaprop-P-ethyl. Dissolve in dilution solvent by placing the flasks in an ultrasonic bath for 10 min. Allow to cool to ambient temperature and make up to the mark with dilution solvent. Mix thoroughly. Filter the solutions through a 0.45 µm filter before injection.

(d) *Determination.* Inject in duplicate 20 µl portions of the control solution into the liquid chromatograph. The individual results for both enantiomers should not deviate from the mean by more than ± 1%, otherwise repeat the calibration. Then inject in duplicate 20 µl portions of the control solution and of each sample solution. After the injection of three samples repeat the duplicate injections of the control solution. Finish the injection sequence with duplicate injections of the control solution.

Measure the peak areas for both enantiomers. If the control solution deviates from 50 % by more than $\pm 1\%$, calculate the correction factor with the four injections of the control solution bracketing the sample solutions (see section (e)).

(e) *Calculation.* The enantiomeric ratio can be calculated directly from the peak areas.

$$R_i = \frac{H_P \times 100}{H_P + H_M}$$

$$\text{Enantiomeric ratio (R)} = R_P : R_M$$

where:

H_P = peak area of fenoxaprop-P-ethyl

H_M = peak area of fenoxaprop-M-ethyl

R_P = enantiomer percentage of fenoxaprop-P-ethyl

R_M = enantiomer percentage of fenoxaprop-M-ethyl

If the control measurement of the racemate fenoxaprop-ethyl shows a result outside the range $50 \pm 1\%$ for the fenoxaprop-P-ethyl and the fenoxaprop-M-ethyl enantiomer correct the enantiomeric ratio as follows:

$$F = \frac{H_P}{H_M}$$

$$R_P = \frac{H_P \times 100}{H_P + (H_M \times F)}$$

where:

F = correction factor

Repeatability r = 1.4 % $R/(R+S)$ at 95% enantiomeric purity

Reproducibility R = 1.6 % $R/(R+S)$ at 95% enantiomeric purity

3.3 Calculation of the fenoxaprop-P-ethyl content

$$\text{Content of fenoxaprop-P-ethyl} = \frac{E \times R_P}{100} \text{ g/kg}$$

where:

E = fenoxaprop-ethyl content (see section 3.1(d))

R_P = enantiomer percentage of fenoxaprop-P-ethyl

FENOXAPROP-ETHYL OIL IN WATER FORMULATIONS 484/EW/M/-

1 Sampling. Take at least 500 ml of the homogenised sample.

2 Identity tests

2.1 HPLC. As for 484/TC/M/2.1.

2.2 TLC. As for 484/TC/M/2.2.

3 Fenoxaprop-P-ethyl

*** 3.1. Determination of the chemical purity.** As for 484/TC/M/3.1 except:

(a) *Operating conditions* (typical):

Retention times

The chromatograms of some oil in water formulations show additional peaks due to the presence of formulants or other active ingredients which are not quantified as part of this determination.

(b) *Preparation of sample.* Weigh (to the nearest 0.1 mg) in duplicate into two volumetric flasks (100 ml) sufficient sample to contain about 12 mg of fenoxaprop-P-ethyl (*w* mg). Add dioxane (10 ml) and mix thoroughly for 10 min in the ultrasonic bath. Add isooctane (80 ml), allow to cool to ambient temperature and make up to the mark with isooctane (the solutions can be slightly turbid). Filter the solutions through a 0.45 µm filter before injection.

(e) *Calculation*

$$\text{Content of fenoxaprop-ethyl (enantiomeric mixture)} = \frac{H_w \times f \times V}{w} \text{ g/kg}$$

where:

V = dilution factor 0.1

Repeatability r	=	3.6 g/kg at 120 g/kg active ingredient content
	=	1.4 g/kg at 75 g/kg active ingredient content
	=	2.5 g/kg at 75 g/kg active ingredient content
Reproducibility R	=	5.4 g/kg at 120 g/kg active ingredient content
	=	3.4 g/kg at 75 g/kg active ingredient content
	=	4.0 g/kg at 75 g/kg active ingredient content

* CIPAC method 2000. Prepared by the German Committee (DAPA). Chairman: W Dobrat. Based on a method supplied by AgrEvo, FRG

*** 3.2 Determination of the enantiomeric purity.** As for **484/TC/(M)/3.2** except:

(d) Preparation of sample. Weigh in duplicate (to the nearest 0.1 mg) into two volumetric flasks (100 ml) sufficient sample to contain 10 to 15 mg of fenoxaprop-P-ethyl. Evaporate the water from the formulation using a stream of nitrogen and an infrared lamp for about 30 min (or place the flasks in an oven at 80° C for about 2 hours). Residual water in the sample will cause poor separation of the enantiomers. After drying, dissolve the residual material in dilution solvent (about 80 ml) by placing the flasks in an ultrasonic bath for 10 min. Allow to cool to ambient temperature and make up to the mark with diluting solvent (the solutions can be slightly turbid). Mix thoroughly. Filter the solutions through a 0.45 µm filter before injection.

Repeatability r	=	1.6 % $R/(R+S)$) at 95% enantiomeric purity and 120 g/kg active ingredient
	=	1.6 % $R/(R+S)$) at 95% enantiomeric purity and 75 g/kg active ingredient content
	=	1.9 % $R/(R+S)$) at 95% enantiomeric purity and 75 g/kg active ingredient content
Reproducibility R	=	2.0 % $R/(R+S)$) at 95% enantiomeric purity and 120 g/kg active ingredient content
	=	1.9 % $R/(R+S)$) at 95% enantiomeric purity and 75 g/kg active ingredient content
	=	2.2 % $R/(R+S)$) at 95% enantiomeric purity and 75 g/kg active ingredient content

3.3 Calculation of the fenoxaprop-P-ethyl content. As for **484/TC/(M)/3.3**.

* Provisional CIPAC method 1999. Prepared by the German Committee (DAPA). Chairman: W Dobrat. Based on a method supplied by AgrEvo, FRG