Bayer CropScience

# Mefenpyr-diethyl

# HPLC Method

# XXX

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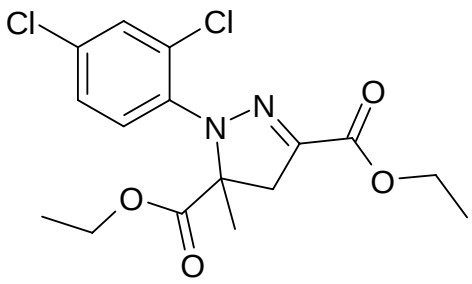
# Mefenpyr-diethyl
# XXX




| | |
|---|---|
| *ISO common name* | Mefenpyr-diethyl |
| *Chemical name* | Diethyl (*R,S*)-1-(2,4-dichlorophenyl)-5-methyl-2-pyrazoline-3,5-dicarboxylate (IUPAC)<br>(CA; 135590-91-9) |
| *Empirical formula* | $C_{16}H_{18}Cl_2N_2O_4$ |
| *RMM* | 373.26 |
| *Description* | The technical product is a brown, non corrosive solidified melt with weak non-characteristic odor. May cristallise on occasion |
| *m.p.* | 50 - 52°C (substance pure) |
| *v.p.* | 1.4 x $10^{-5}$ Pa at 25°C |
| *Solubility* | Water 20 mg/L at 20°C. Soluble in acetone (>500 g/L), dichloromethane (>500 g/L), toluene (>400 g/L), n-hexane (35 g/L), methanol (>400 g/L), iso-propanol (151 g/L), ethylacetate (>400 g/L) and dimetylsulfoxide (>500 g/L). |
| *Formulations* | Emulsifiable concentrate, emulsion oil in water, water dispersible granule, oil dispersion and in mixtures with other pesticides |

## MEFENPYR-DIETHYL TECHNICAL

***XXX/TC/M/-**

**1. Sampling.** Take at least 100 g. Unless the material is a completely crystalline powder, heat the sample for approximately 0.5 h at 80°C to ensure the sample is homogenous. Shake the melted sample well prior to weighing.

**2. Identity tests.**

**2.1 HPLC**. Use the HPLC method described below. The relative retention time of mefenpyr-diethyl in the sample solutions should not deviate by more than 10 s from that of the calibration solution.

**2.2 UV spectrometry**. Record the UV spectrum during the HPLC determination. The characteristic spectrum obtained from the sample should not differ significantly from that of the standard (Fig. 1-2).

## 3. Mefenpyr-diethyl.

**OUT LINE OF THE METHOD.** The TC sample is dissolved in a mixture of acetonitrile and 0.01 mol/L phosphoric acid. The mefenpyr-diethyl content is determined by reversed phase high performance liquid chromatography using external standardisation with UV detection at 300 nm.

REAGENTS
*Acetonitrile* (HPLC grade)
*Phosphoric acid 85 %* (puriss. p. a.)
*High purity water* (HPLC grade)
*Eluent* 50% acetonitrile/50% water (v/v)

APPARATUS
*High performance liquid chromatograph* equipped with an automatic loop injector (20 µl) and a UV spectrophotometric detector operated at 300 nm.
*Column,* stainless steel, 125 x 4.0 (i.d.) mm, packed with Hypersil ODS, C18; 5 µm or equivalent.
*Electronic integrator* or *data system*

PROCEDURE
*(a) Operating conditions (typical):*

| | |
|---|---|
| Column | stainless steel 125 x 4.0 (i.d.) mm, packed with Hypersil ODS, C 18, 5 µm |
| Mobile phase | 50% acetonitrile/ 50% water (v/v) |
| Flow rate | 1.5 ml/min |
| Column temperature | 30°C |
| Injection volume | 20 µl |
| Detector wavelength | 300 nm |
| Retention time | 7 to 9 min |

*(b) Calibration solution.* Weigh (to the nearest 0.1mg) in duplicate approximately 100 mg (*s* mg) of the mefenpyr-diethyl analytical standard into separate volumetric flasks (100 ml). Dissolve in acetonitrile and dilute to the mark with acetonitrile. Mix well. Transfer by pipette 5 ml of each solution into separate volumetric flasks (100 ml), dilute with 45 ml of acetonitrile and add approximately 45 ml of 0.01 mol/L phosphoric acid. Mix well. Equilibration to room temperature and correction of the volume with 0.01 mol/L phosphoric acid are necessary prior to injection (Calibration solutions C1, C2) (Fig. 8).

*(c) Preparation of sample.* Weigh from a homogenized sample (to the nearest 0.1mg) in duplicate approximately 100 mg (*w* mg) into separate volumetric flasks (100 ml). Dissolve in acetonitrile and dilute to the mark with acetonitrile. Mix well. Transfer by pipette 5 ml of each solution into separate volumetric flasks (100 ml), dilute with 45 ml of acetonitrile and add approximately 45 ml of 0.01 mol/L phosphoric acid. Mix well. Equilibration to room temperature and correction of the volume with 0.01 mol/L phosphoric acid are necessary prior to injection (Sample solutions S1, S2) (Fig 9).

*(d) Determination.* Inject each sample solution in duplicate and bracket a series of sample solution injections by injections of the calibration solutions as follows: calibration solution 1 (double injection), calibration solution 2 (double injection), sample solution 1 (double injection), sample solution 2 (double injection), calibration solution 1 (single injection), calibration solution 2 (single injection), (C1, C2, S1, S2, C1, C2). Measure the relevant peak areas.

Calculate the mean response factors for each set of calibration solutions bracketing a set of two samples and use this value for calculating the mefenpyr-diethyl contents in the bracketed sample solution.

*(e) Calculation*

$$f_i = \frac{H_s}{s \; x \; P}$$

$$\text{Mefenpyr-diethyl content (g/kg)} = \frac{H_w}{w \; x \; f}$$

Where:

$f_i$ = single response
$f$ = average response factor
$H_s$ = peak area of mefenpyr-diethyl standard in the calibration solution
$H_w$ = peak area of mefenpyr-diethyl in the sample solution
$s$ = weight of the mefenpyr-diethyl standard in the calibration solution (mg)
$w$ = weight of the sample (mg)
$P$ = purity of the mefenpyr-diethyl standard (g/kg)

**Repeatability r** = 16.2 g/kg at 956.3 g/kg
**Reproducebility R** = 20.5 g/kg at 956.3 g/kg

# MEFENPYR-DIETHYL WATER DISPERSIBLE GRANULES

***XXX/WG/M/-**

**1. Sampling.** Take at least 500 g. Mix the sample well prior to weighing.

**2. Identity tests.**

**2.1** **HPLC**. Use the HPLC method described below. The relative retention time of mefenpyr-diethyl in the sample solutions should not deviate by more than 10 s from that of the calibration solution.

**2.2** **UV spectrometry**. Record the UV spectrum during the HPLC determination. The characteristic spectrum obtained from the sample should not differ significantly from that of the standard (Fig. 3-4).

**3. Mefenpyr-diethyl.**

**OUT LINE OF METHOD** The sample is dissolved in a mixture of 1.4-dioxane and 2.2.4-trimethylpentane (iso-octane). The mefenpyr-diethyl content is determined by normal phase high performance liquid chromatography using external standardisation and UV detection at 227 nm (Fig. 3). Prior to injection it is advisable to filter the sample solutions through disposable syringe-filters. Safety precautions should be taken while working with 1.4-dioxan according to the material safety data sheet. Slight variations in the retention time may be observed due to different water contents of the formulation.

REAGENTS
*2.2.4-trimethylpentane (iso-octane),* (HPLC grade)
*1.4-dioxane (HPLC grade)*
*High purity water* (HPLC grade)
*Pre-mix of 1.4-dioxane mixed with 0.15 %* water

APPARATUS
*High performance liquid chromatograph* equipped with an automatic loop injector (20 µl) and a UV spectrophotometric detector operated at 227 nm.
*Column,* stainless steel, 125 x 4.0 (i.d.) mm, packed with Hypersil Silica or Prontosil Hypersorb SI, 3 µm or equivalent
*Electronic integrator* or *data system*
*Disposable PTFE filter,* e.g. Schleicher & Schuell; Rezist 30/0.45 RC 0.45 µm TE green rim or equivalent

PROCEDURE
*(a) Operating conditions (typical):*

| | |
|---|---|
| Column | stainless steel, 125 x 4.0 (i.d.) mm, packed with Hypersil Silica, 3 µm |

Mobile phase:

| Time (min) | 2.2.4-trimethylpentane: 1.4-dioxane (+ 0.15 % v/v water) 97 : 3 (% v/v) | 2.2.4-trimethylpentane: 1.4-dioxane (+ 0.15 % v/v water) 90 : 10 (% v/v) |
|---|---|---|
| 0 | 100 | 0 |
| 10 | 100 | 0 |
| 12 | 0 | 100 |
| 18 | 0 | 100 |
| 20 | 100 | 0 |
| 25 | 100 | 0 |

| | |
|---|---|
| Flow rate | 1.3 ml/min |
| Column temperature | 40°C |
| Injection volume | 20 µl |
| Detector wavelength | 227 nm |
| Retention time | 5 to 7 min |

*(b) Calibration solution.* Weigh (to the nearest 0.1mg) in duplicate approximately 35 mg (*s* mg) of the mefenpyr-diethyl analytical standard into separate volumetric flasks (100 ml) and dissolve in 10 ml 1.4-dioxane. Place the flask in an ultrasonic bath for at least 10 minutes to ensure complete dissolution of the active ingredient. Add approximately 80 ml of 2.2.4-trimethylpentane, equilibrate to ambient temperature and dilute to the mark with 2.2.4-trimethylpentane. Mix well. Transfer by pipette 10 ml of each solution into separate volumetric flasks (100 ml) and dilute to volume with 2.2.4-trimethylpentane/1.4-dioxane 90/10 % v/v. Mix well (Calibration solutions C1, C2).

*(c) Preparation of sample.* Weigh from the homogenized sample (to the nearest 0.1mg) in duplicate a sufficient amount of the sample to contain an equivalent of approx. 35 mg (*w* mg) of mefenpyr-diethyl into separate volumetric flasks (100 ml). Add 0.5 ml of water and dissolve in 10 ml 1.4-dioxane. Place the flask in an ultrasonic bath for at least 10 minutes to ensure complete dissolution of the active ingredient. Add approximately 80 ml of 2.2.4-trimethylpentane, equilibrate to ambient temperature and dilute to the mark with 2.2.4-trimethylpentane. Mix well. Transfer by pipette 10 ml of each solution into separate volumetric flasks (100 ml) and dilute to volume with 2.2.4-trimethylpentane/1.4-dioxane 90/10 % v/v. Filter the sample solution through a disposable filter (Sample solutions S1, S2). The concentration of mefenpyr-diethyl in the final sample solutions is equivalent to the respective concentration in the final calibration solutions (Fig 10).

*(d) Determination.* Inject each sample solution in duplicate and bracket a series of sample solution injections by injections of the calibration solutions as follows: calibration solution 1 (double injection), calibration solution 2 (double injection), sample solution 1 (double injection), sample solution 2 (double injection), sample solution 3 (double injection), sample solution 4 (double injection), calibration solution 1 (single injection), calibration solution 2 (single injection), sample solution 5 (double injection), sample solution 6 (double injection), sample solution 7 (double injection), sample solution 8 (double injection), calibration solution 1

(single injection), calibration solution 2 (single injection) (C1, C2, S1, S2, S3, S4, C1, C2, S5, S6, S7, S8, C1, C2). Measure the relevant peak areas.
Calculate the mean response factors for each set of calibration solutions bracketing a set of four samples and use this value for calculating the mefenpyr-diethyl contents in the bracketed sample solution.

*(e) Calculation.* The calculation may be done by the data system or by the following equations.

$$f_i = \frac{H_s}{s \; x \; P}$$

$$\text{Mefenpyr-diethyl content} = \frac{H_w}{w \; x \; f} \text{ g/kg}$$

where:

$f_i$ = single response
$f$ = average response factor
$H_s$ = peak area of mefenpyr-diethyl in the calibration solution
$H_w$ = peak area of mefenpyr-diethyl in the sample solution
$s$ = mass of the mefenpyr-diethyl in the calibration solution (mg)
$w$ = mass of the sample taken (mg)
$P$ = purity of the mefenpyr-diethyl standard (g/kg)

**Repeatability r** = 5.8 g/kg at 13.2 g/kg
**Reproducebility R** = 9.4 g/kg at 13.2 g/kg

## 4. Suspensibility

REAGENTS AND APPARATUS as for **XXX**/TC/M/- and MT 184.

PROCEDURE
*(a) Preparation of suspension and determination of sedimentation.* MT 184.

(b) Determination of mefenpyr-diethyl in the bottom 25 ml of suspension. After removal of the top 225 ml of suspension transfer the remaining 25 ml to a 50 ml volumetric flask and dilute to the mark with acetonitrile / 0.01 mol/L phosphoric acid 50 / 50 %v/v. Mix well. Analyse by HPLC as mefenpyr-diethyl TC XXX/TC/M/-.

*(d) Calculation*

$$\text{Suspensibility} = \frac{111(c - Q)}{c} \%$$

where:

$c$ = mass of mefenpyr-diethyl in sample taken for the preparation of the suspension (g)

$Q$ = mass of mefenpyr-diethyl in the bottom 25 ml of suspension

## MEFENPYR-DIETHYL OIL DISPERSION

***XXX/OD/M/-**

**1. Sampling.** Take at least 500 g. Shake the sample well prior to weighing.

**2 Identity tests.**
**2.1 HPLC**. As for mefenpyr-diethyl XXX/WG/M/-
**2.2 UV spectrometry**. As for mefenpyr-diethyl XXX/WG/M/-(Fig. 5)

**3 Mefenpyr-diethyl.** As for mefenpyr-diethyl WG except for the following:
*(c) Preparation of sample.* Weigh from the homogenized sample (to the nearest 0.1mg) in duplicate a sufficient amount of the sample to contain an equivalent of approx. 3.5 mg (*w* mg) of mefenpyr-diethyl into separate volumetric flasks (100 ml) and dissolve in 10 ml 1.4-dioxane. Place the flask in an ultrasonic bath for at least 10 minutes to ensure complete dissolution of the active ingredient. Add approximately 80 ml of 2.2.4-trimethylpentane, equilibrate to ambient temperature and dilute to the mark with 2.2.4-trimethylpentane. Mix well. Filter the sample solution through a disposable filter. (Sample solutions S3, S4, Fig. 11).

**Repeatability r** = 1.6 g/kg at 24.6 g/kg
**Reproducebility R** = 2.2 g/kg at 24.6 g/kg

## MEFENPYR-DIETHYL EMULSION OIL IN WATER

***XXX/EW/M/-**

**1. Sampling.** Take at least 500 g. Shake the sample well prior to weighing.

**2 Identity tests.**
**2.1 HPLC**. As for mefenpyr-diethyl XXX/WG/M/-
**2.2 UV spectrometry**. As for mefenpyr-diethyl XXX/WG/M/-(Fig. 6)

**3 Mefenpyr-diethyl.** As for mefenpyr-diethyl Oil Dispersion OD (Sample solutions S5, S6, Fig.12)

**Repeatability r** = 4.7 g/kg at 70.9 g/kg
**Reproducebility R** = 4.7 g/kg at 70.9 g/kg

# MEFENPYR-DIETHYL EMULSIFIABLE CONCENTRATE

***XXX/EC/M/-**

**1. Sampling.** Take at least 500 g. Shake the sample well prior to weighing.

**2 Identity tests.**
**2.1 HPLC**. As for mefenpyr-diethyl XXX/WG/M/-
**2.2 UV spectrometry**. As for mefenpyr-diethyl XXX/WG/M/-(Fig. 7)

**3 Mefenpyr-diethyl.** As for mefenpyr-diethyl Oil Dispersion OD (Sample solutions S7, S8, Fig. 13).

**Repeatability r** = 1.6 g/kg at 34.1 g/kg
**Reproducebility R** = 2.0 g/kg at 34.1 g/kg

**Identity and Typical Chromatograms of mefenpyr-diethyl**

**Fig. 1** UV Analytical Standard mefenpyr-diethyl

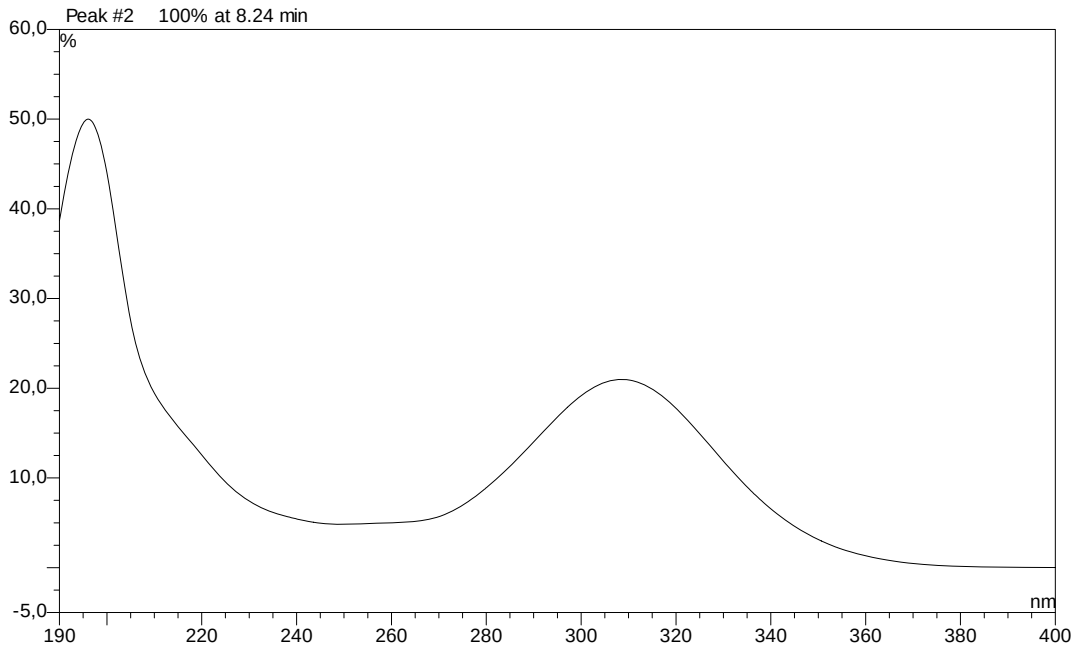


**Fig. 2** UV mefenpyr-diethyl Technical Material TC

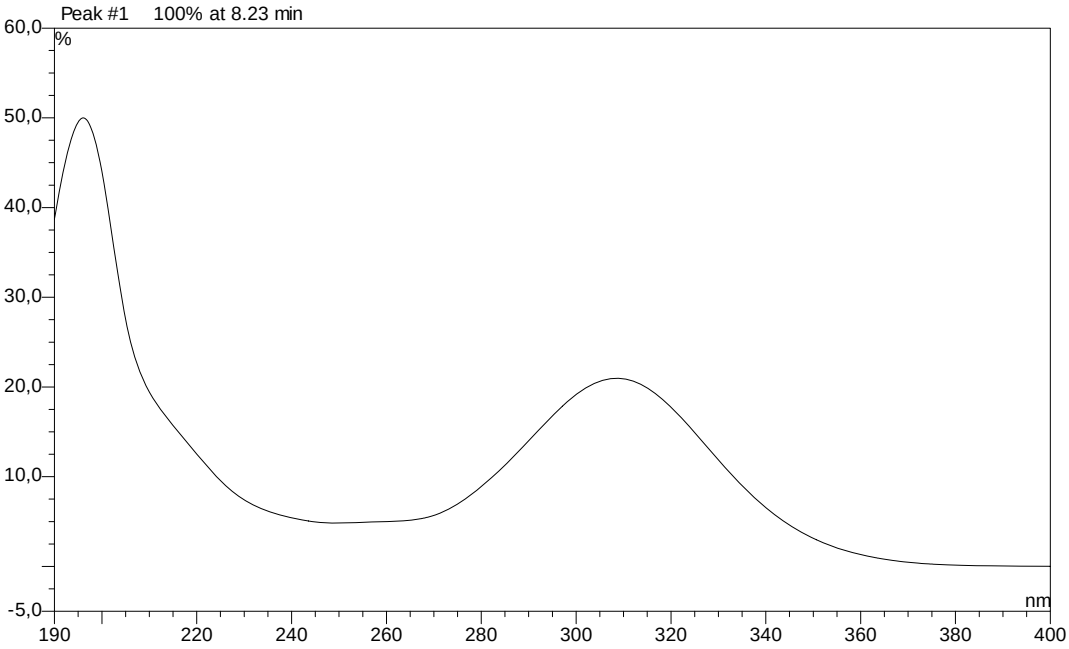



**Fig. 3** UV Analytical Standard mefenpyr-diethyl

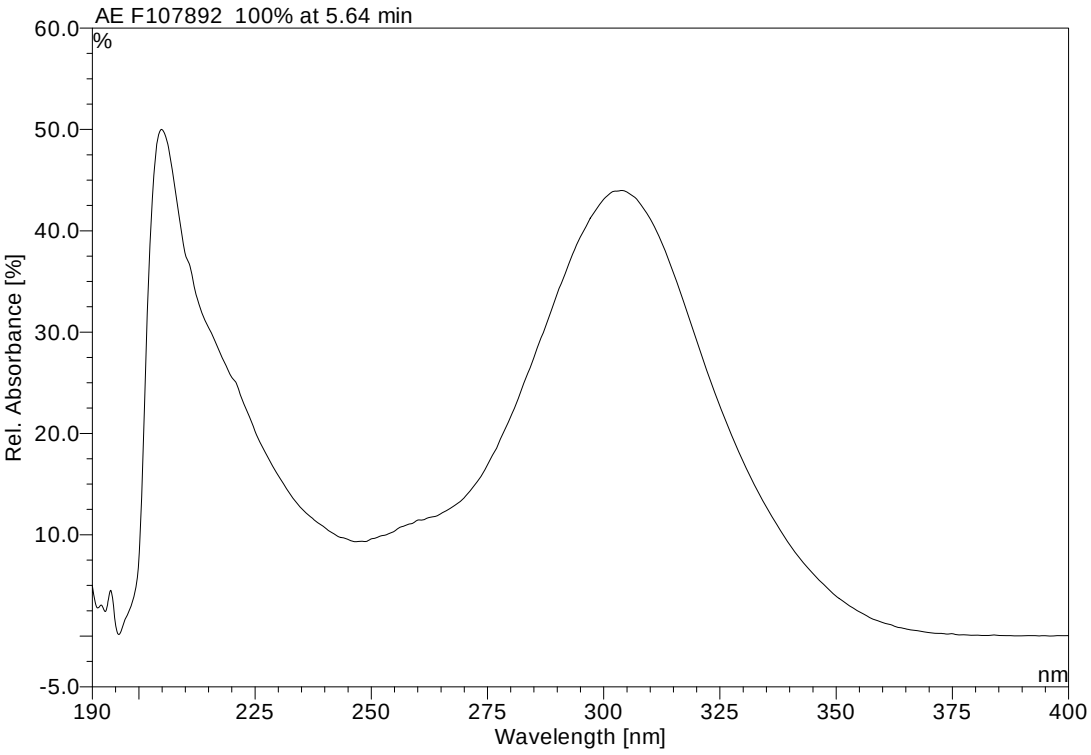


**Fig. 4** UV mefenpyr-diethyl in Sekator new – AE F075032 08 WG19 A3 – Batch: 2006-000195

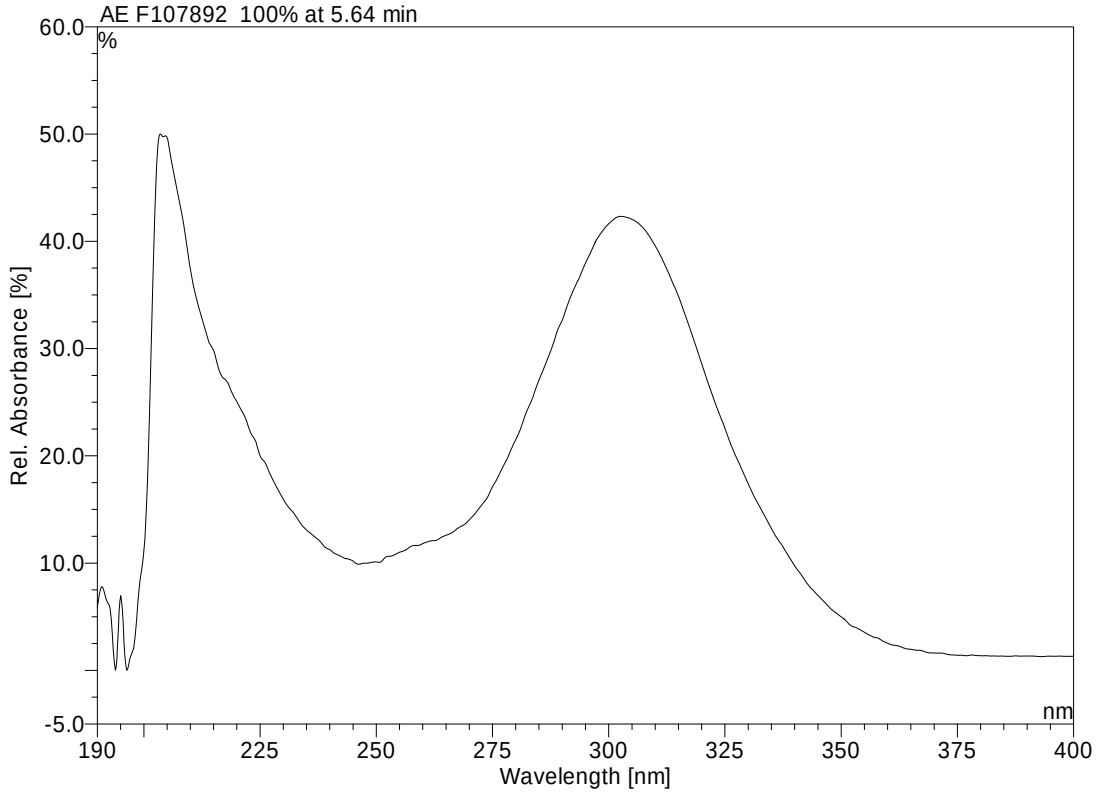



**Fig 5** UV mefenpyr-diethyl in OD Hussar OF – AE F046360 52 1L09 B1 – Batch: EFKM001297

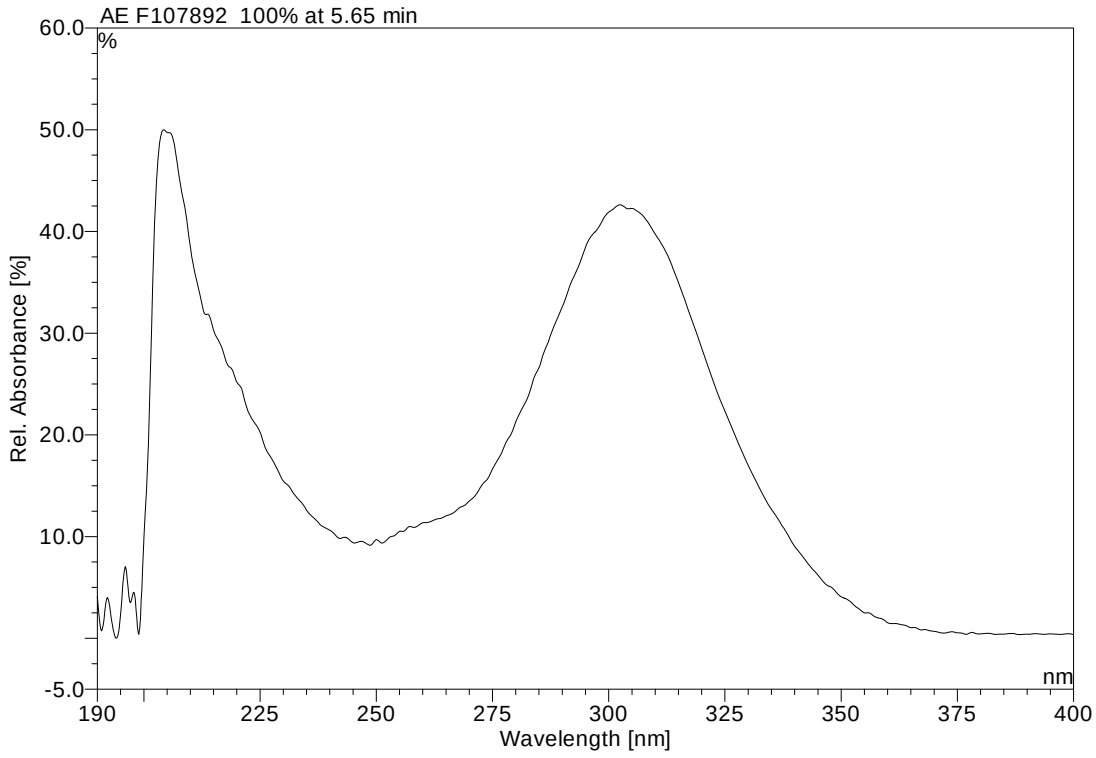


**Fig 6** UV mefenpyr-diethyl in Ralon Super EW – Batch: EFKM001272

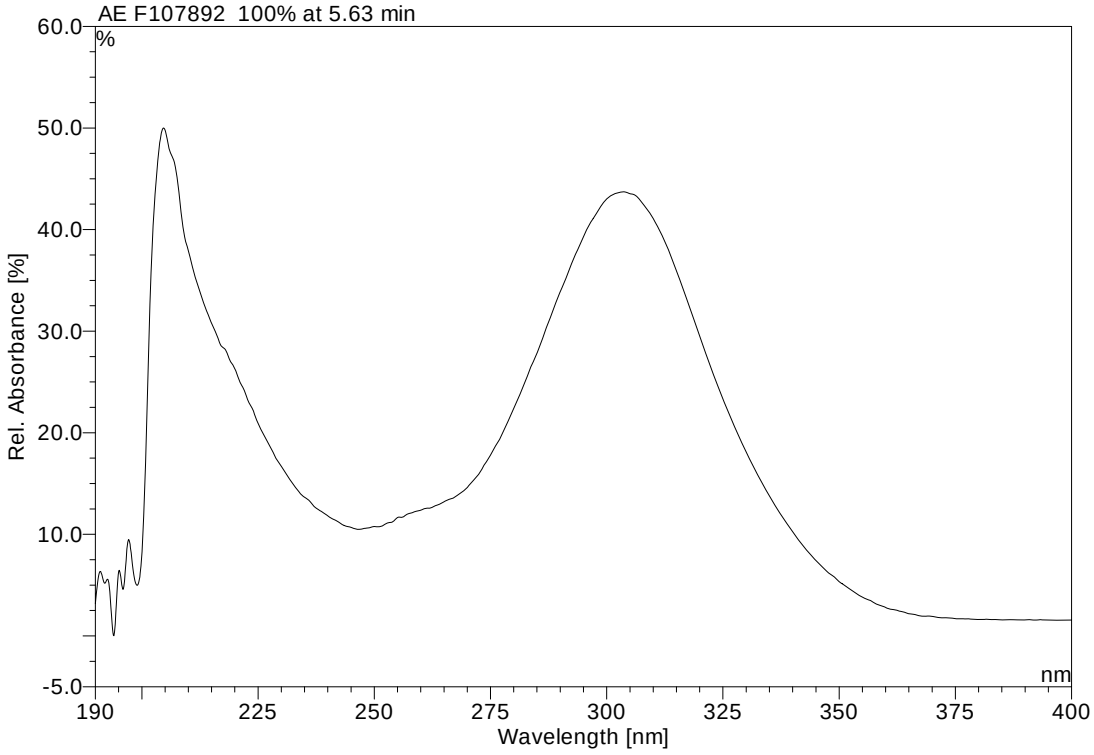



**Fig 7** UV mefenpyr-diethyl in Puma Wheat EC – Batch: AAKI00885

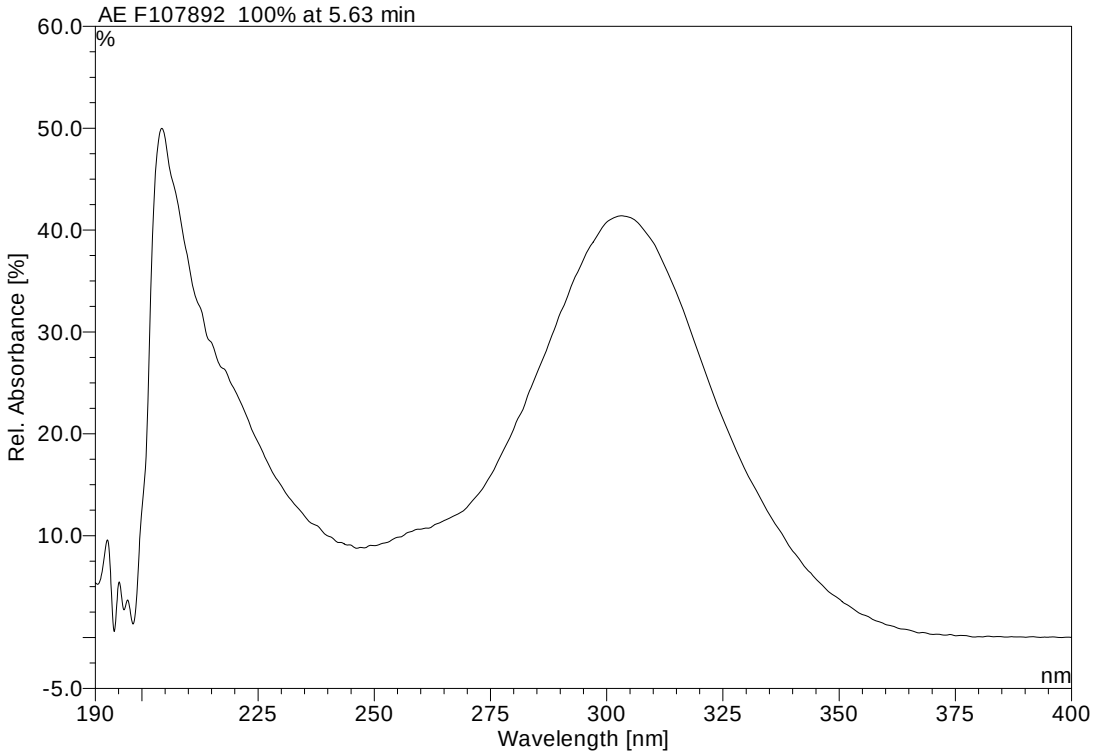


**Fig. 8 HPLC** Analytical Standard mefenpyr-diethyl

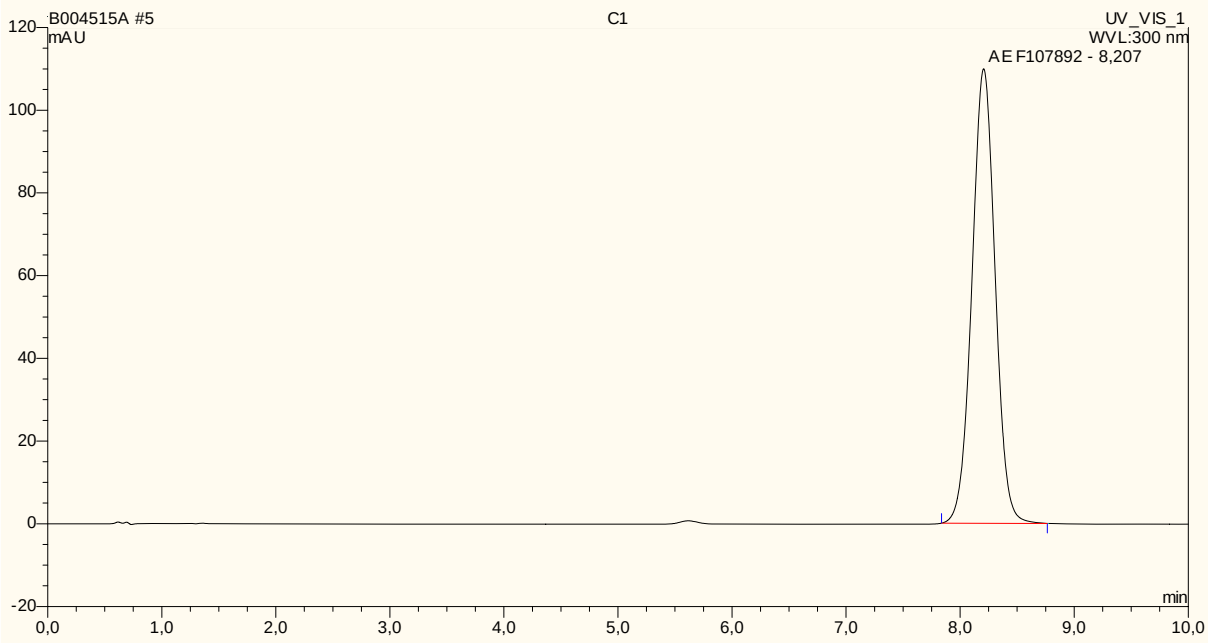



**Fig 9.** HPLC Technical Material TC

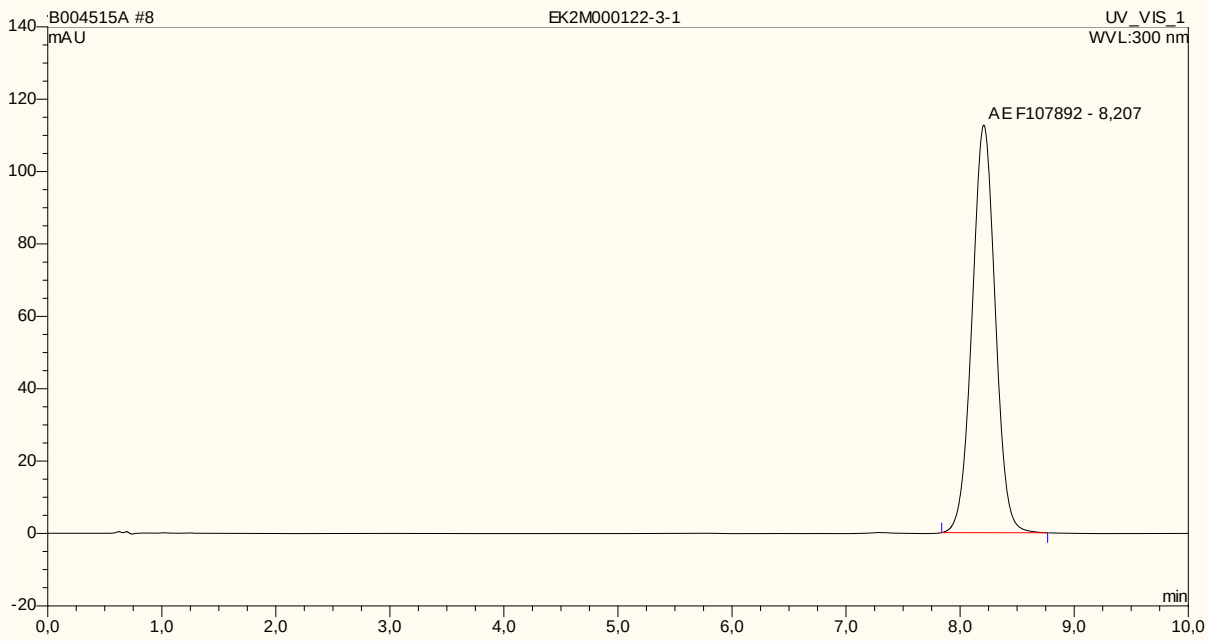


**Fig.10** HPLC Water Dispersible Granule WG

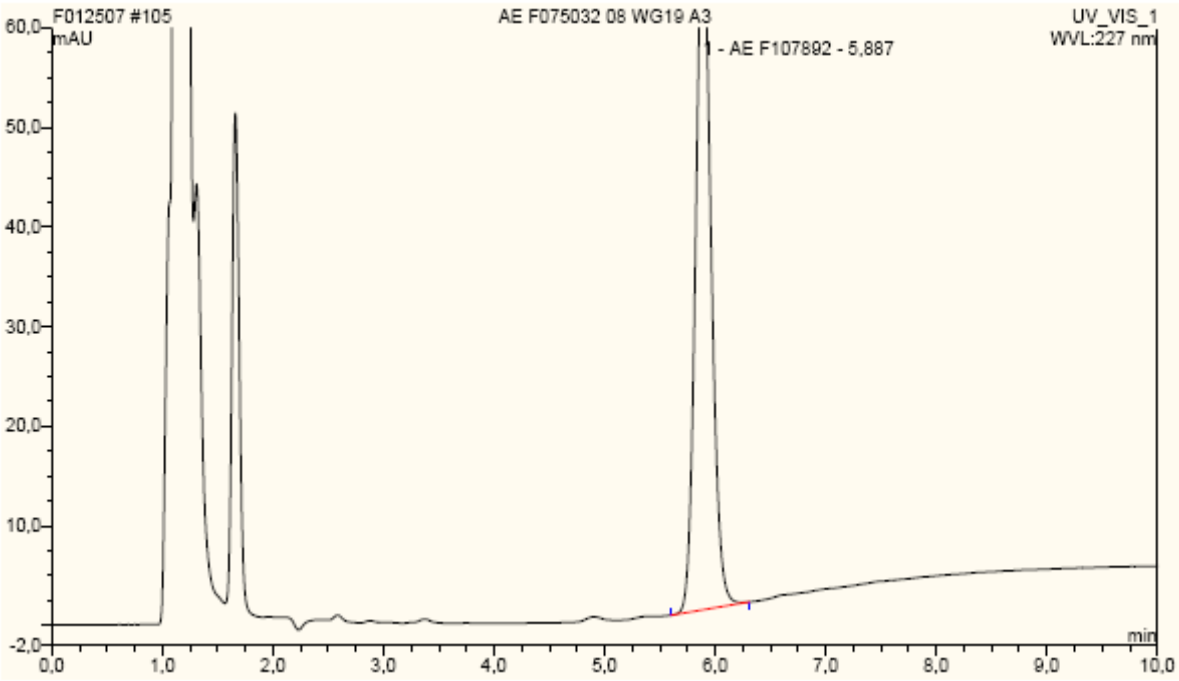



**Fig 11.** HPLC Oil Dispersion OD

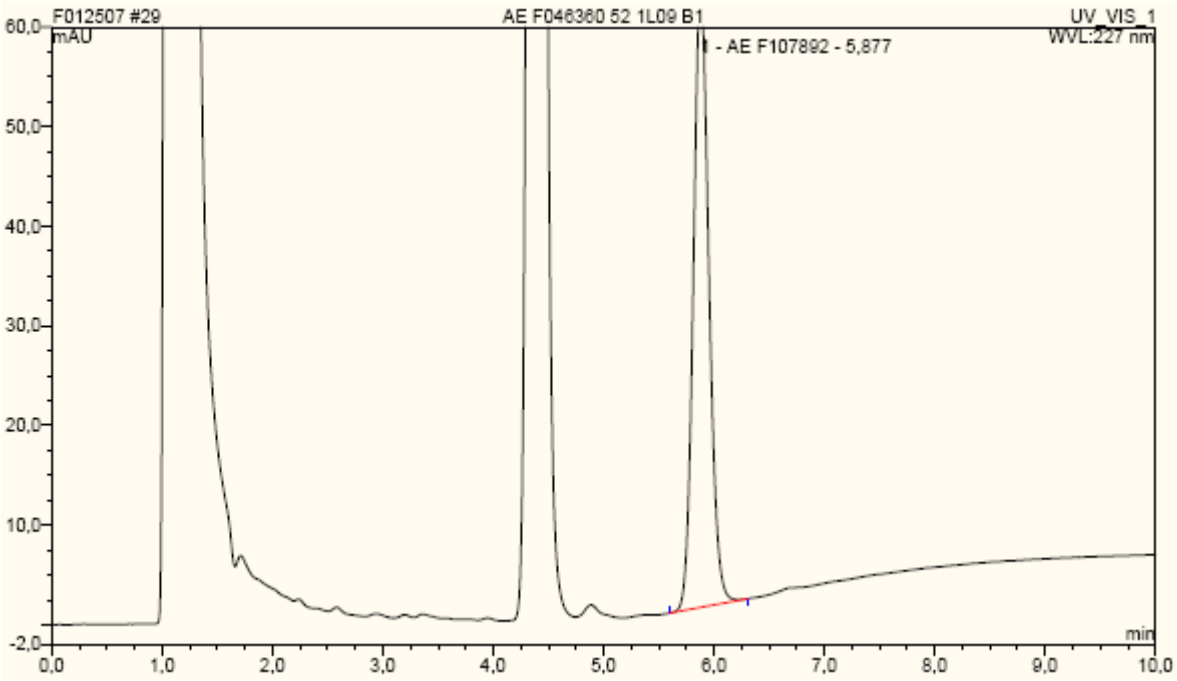


**Fig. 12** HPLC Emulsion Oil in Water EW

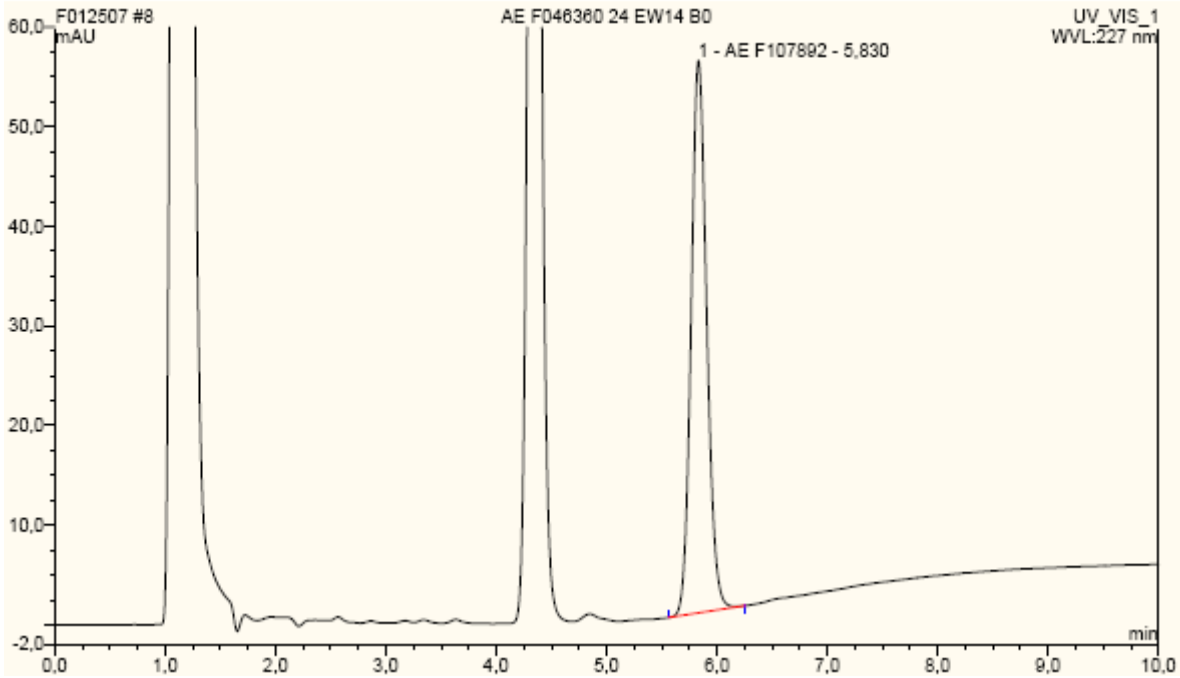



**Fig. 13 HPLC** Emulsifiable Concentrate EC