Flupyradifurone

HPLC Method

CIPAC Collaboration Trial according to CIPAC Information Sheet No 308

by

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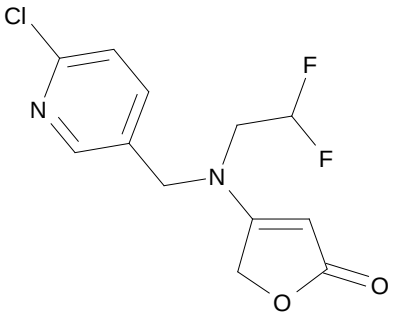
FLUPYRADIFURONE
987

Chemical name: 4-[[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino]furan-2(5H)-on
ISO common name: Flupyradifurone
CAS-No.: 951659-40-8

Structure:



Molecular mass: 288.7
Empirical formula: C_{12} H_{11} Cl F_2 N_2 O_2

m.p.: 69 °C
b.p.: Decomposition occurs at about 270°C before boiling point is reached
Solubility: 1,2-dichloromethane, ethylacetate, methanol > 250 g/L; toluene 3.7 g/L (all at 20°C)

Description: The technical product is a white to beige powder.

FLUPYRADIFURONE TECHNICAL
987/TC/M/-

1 Sampling. Take at least 100 g*. Grind the sample thoroughly in a mortar.
*(for this trial less amount is provided; please grind the entire sample)

2 Identity tests
2.1 HPLC. Use the HPLC method described below. The relative retention time of Flupyradifurone in the sample solution should not deviate by more than 2% from that of the calibration solution.

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

2.3 Infrared. Prepare by direct application pure flupyradifurone and the sample on an ATR unit. Scan from 4000 to 550 cm-1. The spectrum produced from the sample should not differ significantly from that of the standard. (Fig. 2)

3 Flupyradifurone

OUTLINE OF THE METHOD.
Flupyradifurone content is determined (g/kg) by reversed phase high performance liquid chromatography using UV detection at 280 nm and external standard calibration.

3.1 Determination of Flupyradifurone by reversed phase HPLC

REAGENTS
Flupyradifurone reference standard of known content
Acetonitrile (HPLC grade)
Phosphoric acid 85 % (puriss. p. a.)
Purified water (HPLC grade)
Eluent A: 10 mMol phosphoric acid in 1 L purified water
Eluent B: acetonitrile

APPARATUS
High performance liquid chromatograph equipped with an injection system capable to inject 5 µL and an UV spectrophotometric detector operated at 280 nm.
Liquid chromatography column, stainless steel, 50 x 4.6 (i.d.) mm, packed with Kinetex C 18; 2.6 µm or equivalent with the same selectivity.
Electronic integrator or data system
Ultrasonic bath

PROCEDURE
(a) Operating conditions (typical):

Flow rate: 2 mL/min
Column temperature: 40°C
Injection volume: 5 µL
Detector wavelength: 280 nm

Mobile phase:

Time [min]	10 mMol phosphoric acid in 1 L purified water [%v/v]	Acetonitrile [%v/v]
0.0	90	10
5.0	85	15
5.1	05	95
6.5	05	95
6.6	90	10
8.5	90	10

Retention time: approximately 4.1 min

(b) Equilibration of the system. Pump sufficient mobile phase through the column to equilibrate the system. Inject 5 µL portions of the calibration solution C1 and repeat the injections until retention times and peak areas deviate by less than ± 1 % from the mean for three successive injections.

(c) Calibration solution. Weigh in duplicate (to the nearest 0.1 mg) approximately 50 mg (*s* mg) of the Flupyradifurone reference standard into separate volumetric flasks (100 mL). Add 50 mL acetonitrile and place the flasks in an ultrasonic bath for 15 min. Make up the flasks with purified water to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with purified water and mix thoroughly (Calibration solutions C1, C2) (Fig. 1).

(d) Sample preparation. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about approximately 50 mg (*w* mg) of Flupyradifurone into separate volumetric flasks (100 mL). Add 50 mL acetonitrile and place the flasks in an ultrasonic bath for 15 min. Make up the flasks with purified water to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with purified water and mix thoroughly (Sample solutions S1, S2) (Fig. 2).

(e) Determination. Inject in duplicate each sample solution and bracket a series of sample solution injections by injections of the calibration solutions as follows: calibration solution 1, calibration solution 2, calibration solution 1, sample solution 1, sample solution 1, sample solution 2, sample solution 2, calibration solution 1, … (C1, C2, C1, S1, S1, S2, S2, C1, …).

Determine the peak areas of Flupyradifurone.

(f) Calculation
Calculate the response factors from the calibration solutions bracketing the injections of the sample solutions. Average the response factors of the calibration solutions preceding and following the sample solution injections. These must agree within ±1 % of the average otherwise repeat the determination. Calculate the content of the sample solutions.

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

$$\text{Flupyradifurone content (g/kg)} = \frac{H_W \times f}{w}$$

Where:

f_i = single response factor
f = average response factor
H_S = peak area of Flupyradifurone standard in the calibration solution
H_W = peak area of Flupyradifurone in the sample solution
s = weight of the Flupyradifurone standard in the calibration solution (mg)
w = weight of the sample (mg)
P = purity of the Flupyradifurone standard (g/kg)

Repeatability r = 17.75 g/kg at 960 g/kg active ingredient content
Reproducibility R = 21.74 g/kg at 960 g/kg active ingredient content

FLUPYRADIFURONE ANY OTHER LIQUID
987/AL/M/-

1 Sampling. Take at least 500 mL*. Shake the sample well prior to weighing.
*(for this trial less amount is provided)

2 Identity tests.
2.1 HPLC. As for Flupyradifurone 987/TC/M/-
2.2 UV spectrometry. As for Flupyradifurone 987/TC/M/-

3 Flupyradifurone.
Same approach as for Flupyradifurone 987/TC/M/-

3.1 Determination of Flupyradifurone by reversed phase HPLC
As for Flupyradifurone 987/TC/M/- except

Disposable PTFE syringe filter compatible with organic solvents and a 0.45 µm pore diameter or centrifuge.

(c) Calibration solution. Weigh in duplicate (to the nearest 0.1 mg) approximately 50 mg (*s* mg) of the Flupyradifurone reference standard into separate volumetric flasks (100 mL). Add 50 mL acetonitrile and place the flasks in an ultrasonic bath for 15 min. Make up the flasks with purified water to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with purified water and mix thoroughly. Transfer 8 mL of this solution into a separate volumetric flask (100 mL) and make up the flask with acetonitrile / purified water 50 / 50 %v/v to just the calibration mark and allow to cool to ambient temperature. Fill to the mark with acetonitrile / purified water 50 / 50 %v/v and mix thoroughly (Calibration solutions C3, C4) (Fig. 3).

(d) Sample preparation. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about approximately 2 mg (*w* mg) of Flupyradifurone into separate volumetric flasks (50 mL)*. Add 20 mL acetonitrile and place the flasks in an ultrasonic bath for 15 min. Make up the flasks with acetonitrile to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with acetonitrile and mix thoroughly. Filter the sample solution through a disposable filter or centrifuge the sample solution (Sample solutions S3, S4) (Fig. 4).

* This corresponds to a concentration of 4 mg in 100 mL

Repeatability r = 0.00180 g/kg at 0.08 g/kg active ingredient content
Reproducibility R = 0.00742 g/kg at 0.08 g/kg active ingredient content

FLUPYRADIFURONE EMULSIFIABLE CONCENTRATE 987/EC/M/-

1 Sampling. Take at least 500 mL*. Shake the sample well prior to weighing.
*(for this trial less amount is provided)

2 Identity tests.
2.1 HPLC. As for Flupyradifurone 987/TC/M/-
2.2 UV spectrometry. As for Flupyradifurone 987/TC/M/-

3 Flupyradifurone.
Same approach as for Flupyradifurone 987/TC/M/-

3.1 Determination of Flupyradifurone by reversed phase HPLC
As for Flupyradifurone 987/TC/M/- except

Disposable PTFE syringe filter compatible with organic solvents and a 0.45 µm pore diameter or centrifuge.

(d) Sample preparation. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about approximately 50 mg (*w* mg) of Flupyradifurone into separate volumetric flasks (100 mL). Dissolve in 20 mL purified water and add 50 mL acetonitrile and place the flasks in an ultrasonic bath for 15 min. Make up the flasks with purified water to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with purified water and mix thoroughly. Filter the sample solution through a disposable filter or centrifuge the sample solution (Sample solutions S5, S6) (Fig. 5).

Repeatability r = 0.94 g/kg at 64.7 g/kg active ingredient content
Reproducibility R = 1.32 g/kg at 64.7 g/kg active ingredient content

FLUPYRADIFURONE EMULSION, OIL IN WATER
987/EW/M/-

1 Sampling. Take at least 500 mL*. Shake the sample well prior to weighing.
*(for this trial less amount is provided)

2 Identity tests.
2.1 HPLC. As for Flupyradifurone 987/TC/M/-
2.2 UV spectrometry. As for Flupyradifurone 987/TC/M/-

3 Flupyradifurone.
Same approach as for Flupyradifurone 987/TC/M/-

3.1 Determination of Flupyradifurone by reversed phase HPLC
Same approach as for Flupyradifurone 987/EC/M/-
(Sample solutions S7, S8) (Fig. 6)

Repeatability r = 0.94 g/kg at 25 g/kg active ingredient content
Reproducibility R = 2.34 g/kg at 25 g/kg active ingredient content

FLUPYRADIFURONE FLOWABLE CONCENTRATE FOR SEED TREATMENT
987/FS/M/-

1 Sampling. Take at least 500 mL*. Shake the sample well prior to weighing.
*(for this trial less amount is provided)

2 Identity tests.
2.1 HPLC. As for Flupyradifurone 987/TC/M/-
2.2 UV spectrometry. As for Flupyradifurone 987/TC/M/-

3 Flupyradifurone.
Same approach as for Flupyradifurone 987/TC/M/-

3.1 Determination of Flupyradifurone by reversed phase HPLC
Same approach as for Flupyradifurone 987/EC/M/-
(Sample solutions S9, S10) (Fig. 7)

Repeatability r = 8.70 g/kg at 407 g/kg active ingredient content
Reproducibility R = 11.71 g/kg at 407 g/kg active ingredient content

4 Suspensibility

REAGENTS AND APPARATUS as for MT 184

PROCEDURE

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

(b) Determination of Flupyradifurone in the bottom 25 ml of suspension: After removal of the top 225 ml of suspension transfer approx. 250 mg* out of the remaining and well homogenized 25 ml to a 100 ml volumetric flask and add 20 ml purified water and 50 ml acetonitrile. To dissolve the substance, place the flask in an ultrasonic bath for 15 min and make up the flask with purified water to just below the calibration mark. Wait until the temperature has stabilized, then fill the flask up to the calibration mark with purified water. Determine the mass of flupyradifurone (Q g) by 987/TC/M/-).

* This is calculated for a 50% suspension, in case of other concentrations the volume has to be adjusted but it has to be ensured to work within the linear range.

FLUPYRADIFURONE SUSPENSION CONCENTRATE 987/SL/M/-

1 Sampling. Take at least 500 mL*. Shake the sample well prior to weighing.
*(for this trial less amount is provided)

2 Identity tests.
2.1 HPLC. As for Flupyradifurone 987/TC/M/-
2.2 UV spectrometry. As for Flupyradifurone 987/TC/M/-

3 Flupyradifurone.
Same approach as for Flupyradifurone 987/TC/M/-

3.1 Determination of Flupyradifurone by reversed phase HPLC
Same approach as for Flupyradifurone 987/EC/M/-
(Sample solutions S11, S12) (Fig. 8)

Repeatability r = 3.20 g/kg at 171 g/kg active ingredient content
Reproducibility R = 3.29 g/kg at 171 g/kg active ingredient content

FLUPYRADIFURONE WATER DISPERSABLE GRANULE
987/WG/M/-

1 Sampling. Take at least 500 g*. Grind the sample thoroughly in a mortar.
*(for this trial less amount is provided; please grind the entire sample)

2 Identity tests.
2.1 HPLC. As for Flupyradifurone 987/TC/M/-
2.2 UV spectrometry. As for Flupyradifurone 987/TC/M/-

3 Flupyradifurone.
Same approach as for Flupyradifurone 987/TC/M/-

3.1 Determination of Flupyradifurone by reversed phase HPLC
Same approach as for Flupyradifurone 987/EC/M/-
(Sample solutions S13, S14) (Fig. 9)

Repeatability r = 2.91 g/kg at 120 g/kg active ingredient content
Reproducibility R = 3.56 g/kg at 120 g/kg active ingredient content

4 Suspensibility. As for Flupyradifurone 987/FS/M/- except
(b) Determination of Flupyradifurone in the bottom 25 ml of suspension. After removal of the top 225 ml of suspension transfer approx. 2700 mg* out of the remaining and well homogenized 25 ml to a 100 ml volumetric flask and add 20 ml purified water and 50 ml acetonitrile. To dissolve the substance, place the flask in an ultrasonic bath for 15 min and make up the flask with purified water to just below the calibration mark. Wait until the temperature has stabilized, then fill the flask up to the calibration mark with purified water. Determine the mass of flupyradifurone (Q g) by 987/AL/M/-)

* This is calculated for a 1.2% suspension, in case of other concentrations the volume has to be adjusted but it has to be ensured to work within the linear range.

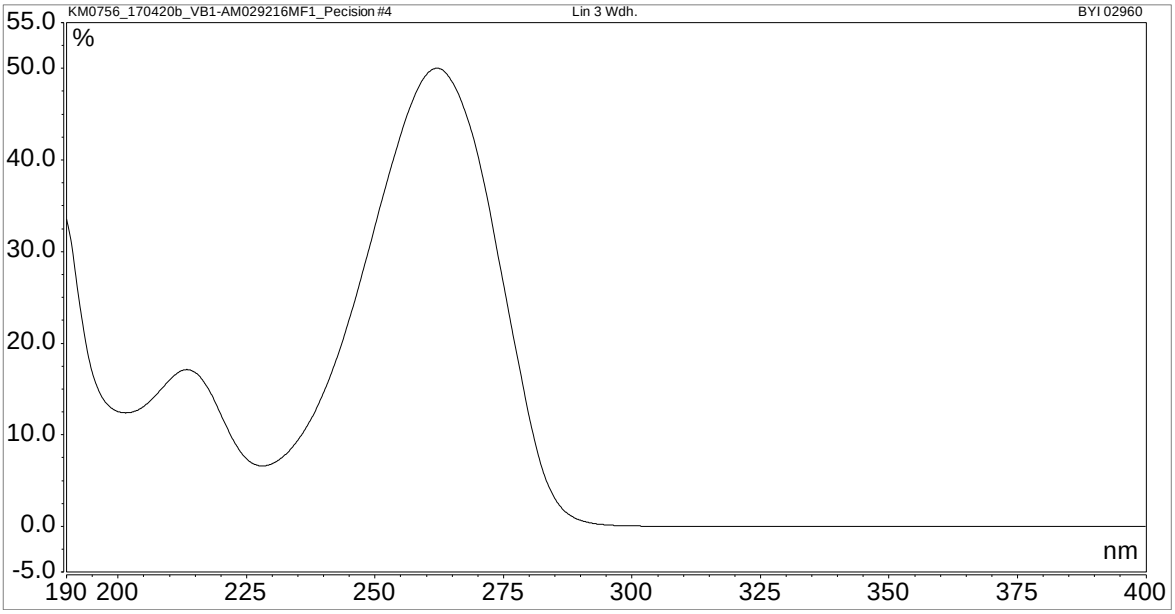


Fig. 1 UV-Spectrum of Flupyradifurone

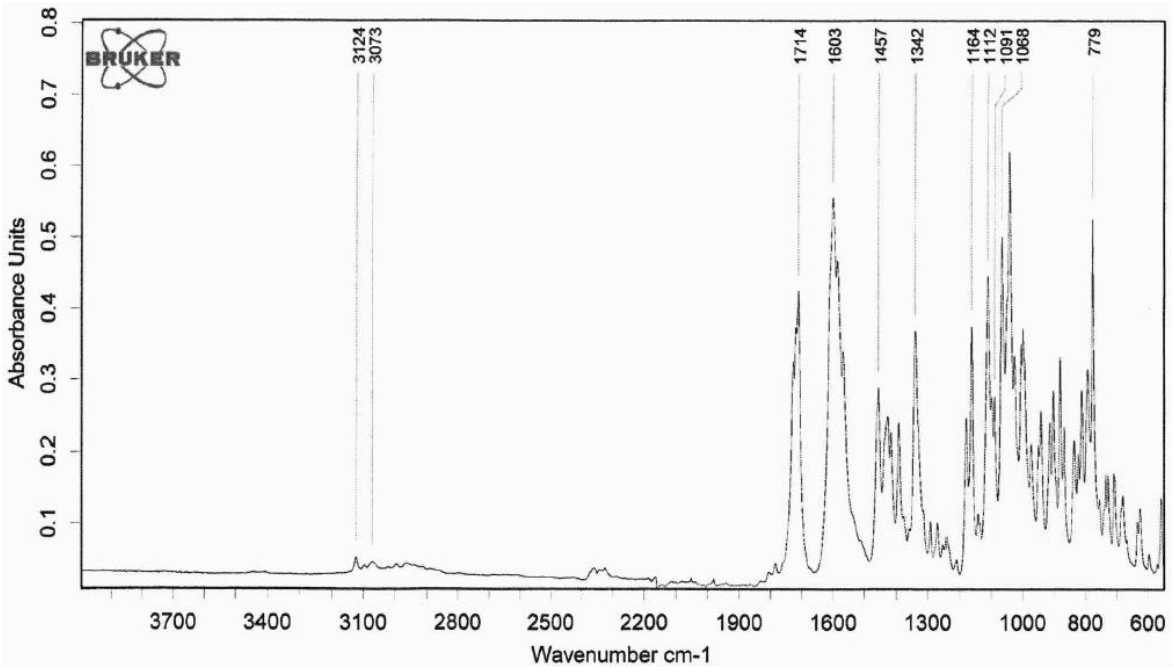


Fig. 2 Infrared Spectrum of Flupyradifurone

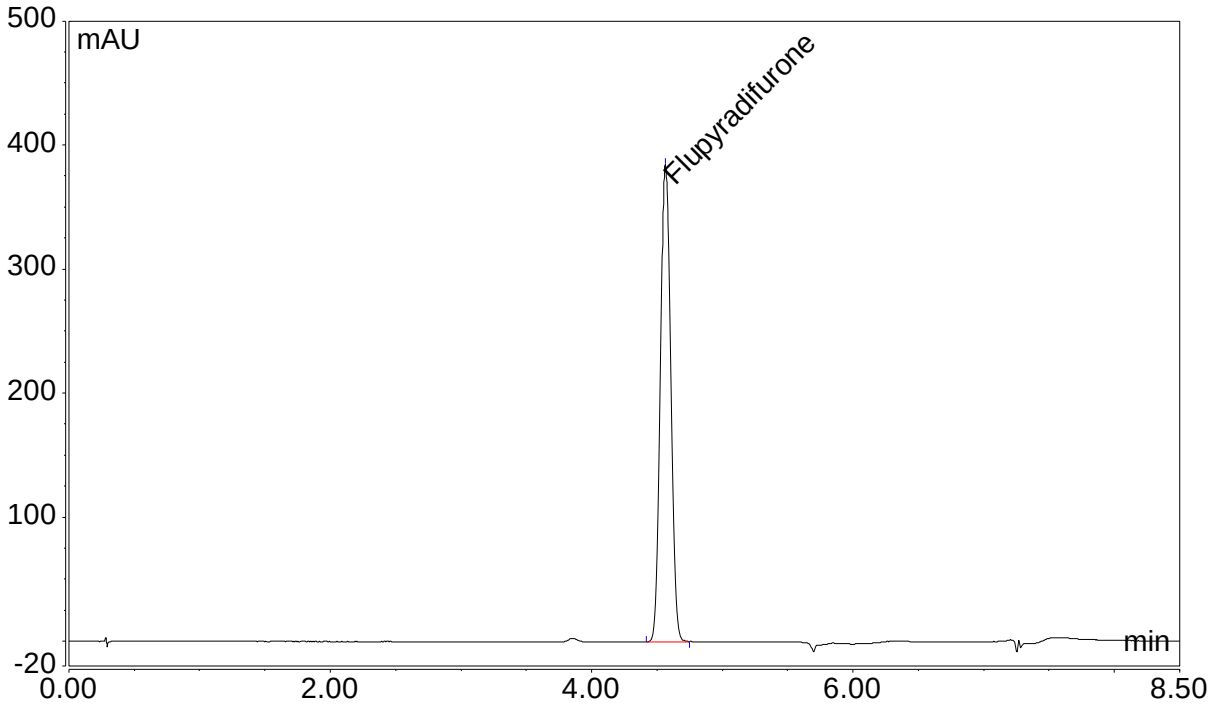


Fig. 3 Chromatogram of Flupyradifurone (Standard)

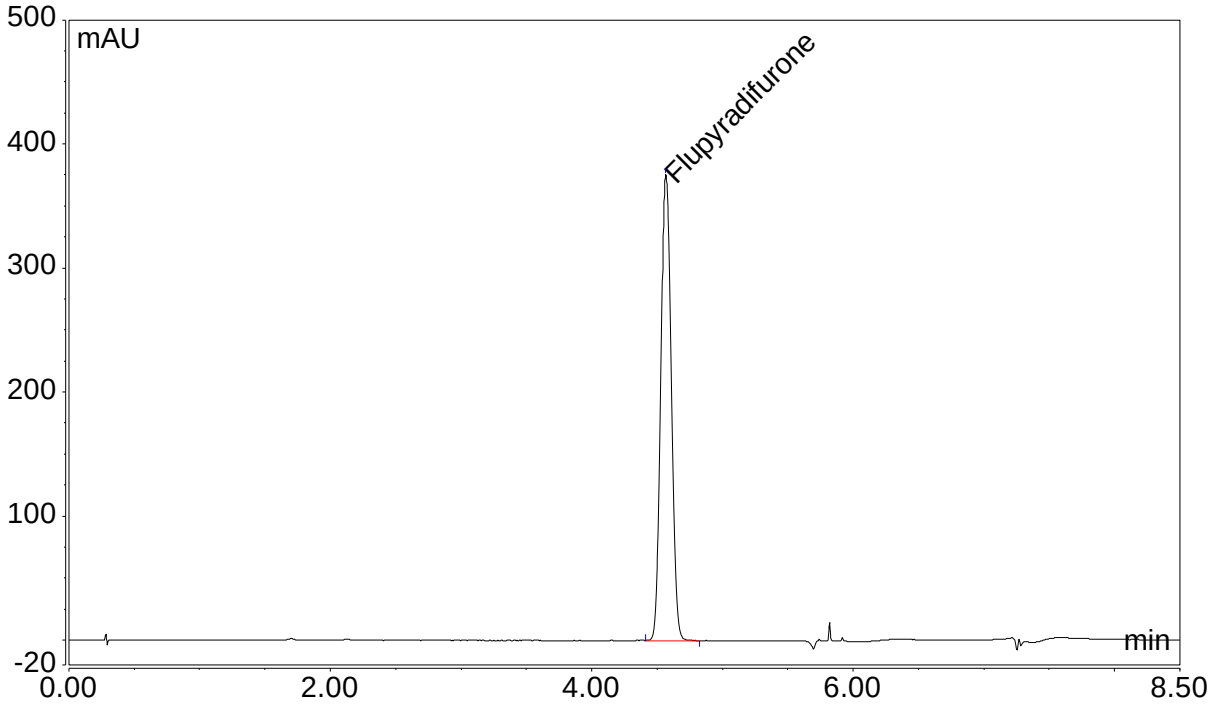


Fig. 4 Chromatogram of Flupyradifurone TC

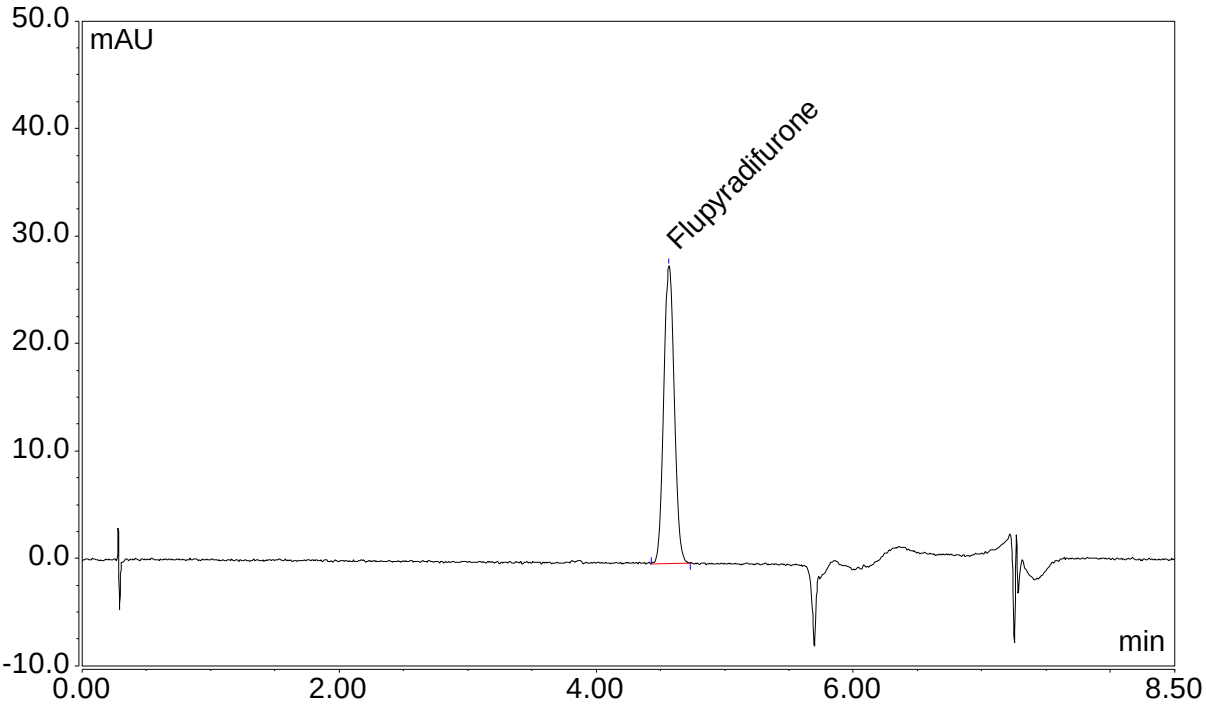


Fig. 5 Chromatogram of Flupyradifurone (Standard for AL formulation)

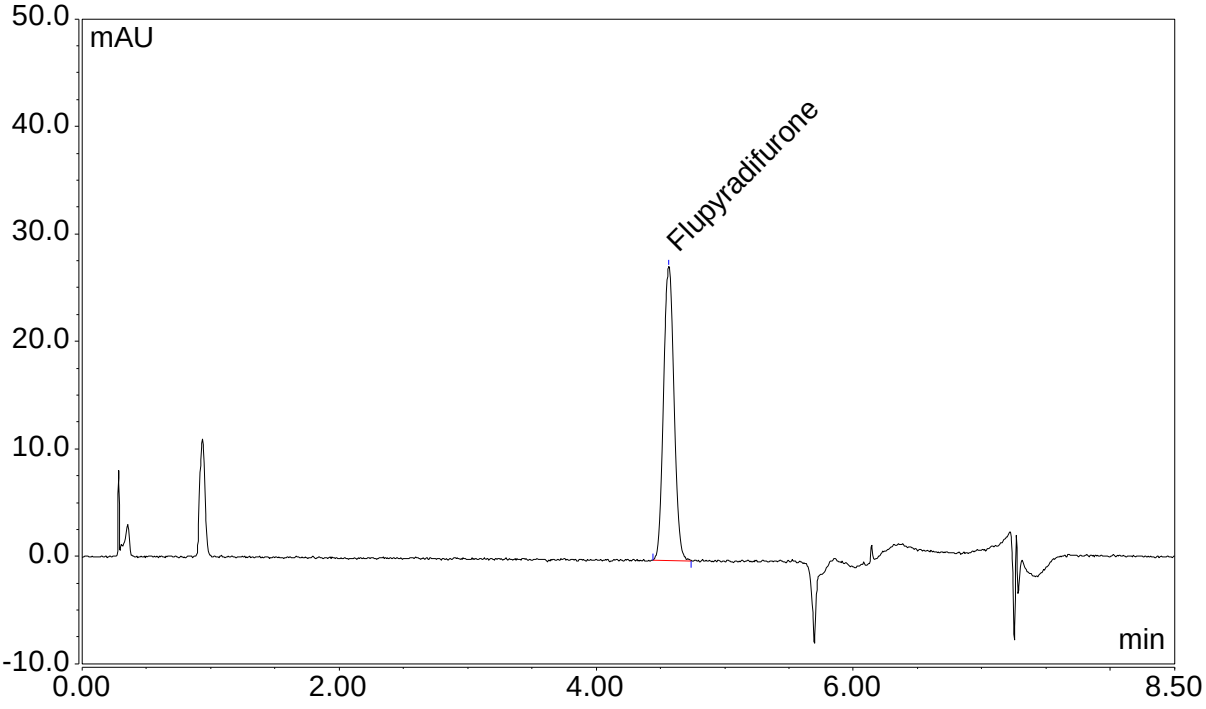


Fig. 6 Chromatogram of Flupyradifurone AL

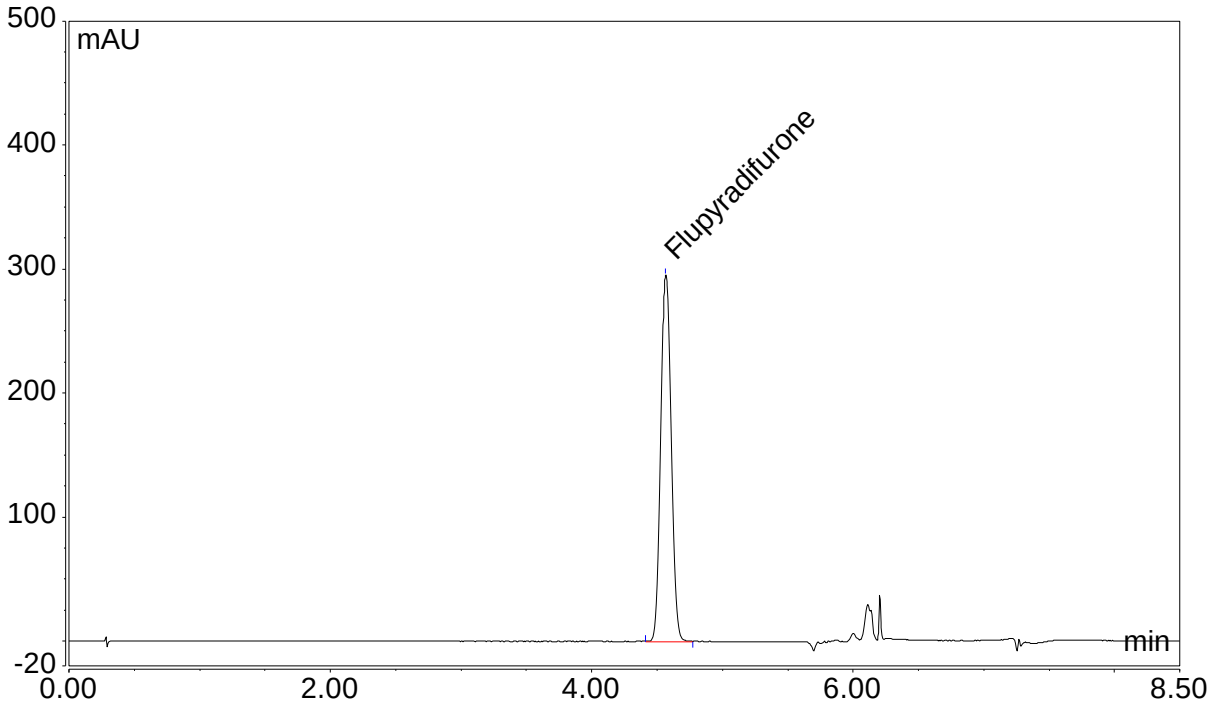


Fig. 7 Chromatogram of Flupyradifurone EC

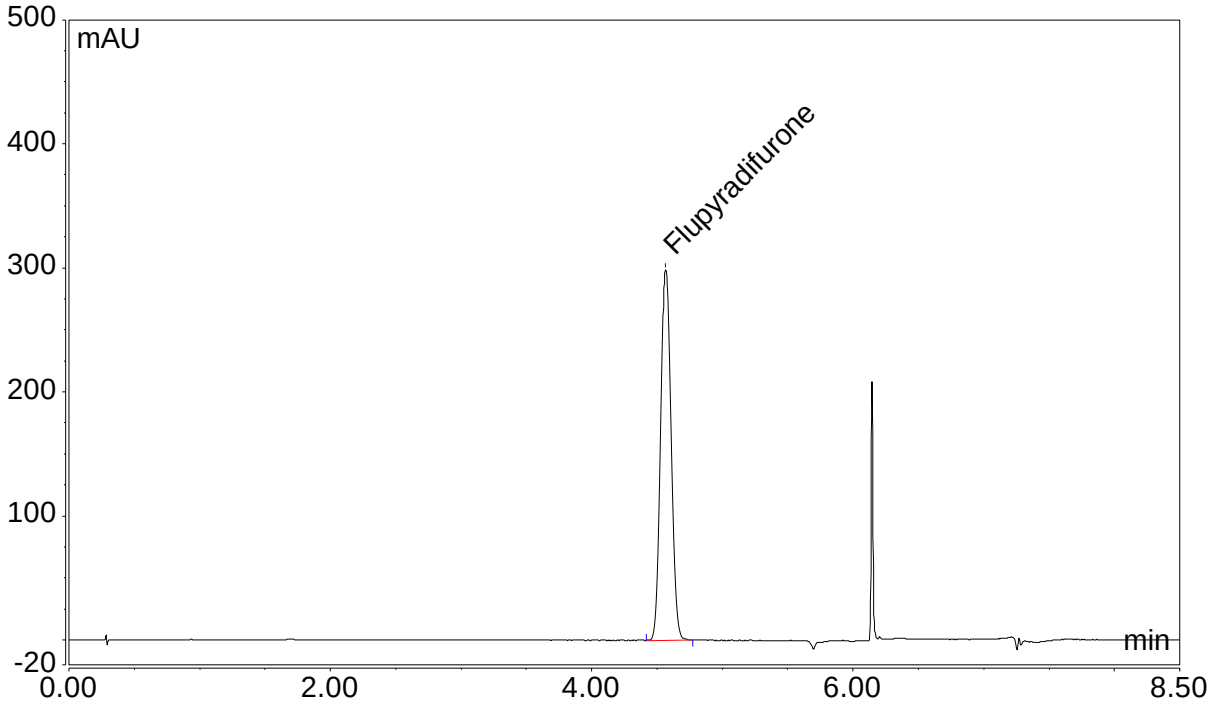


Fig. 8 Chromatogram of Flupyradifurone EW

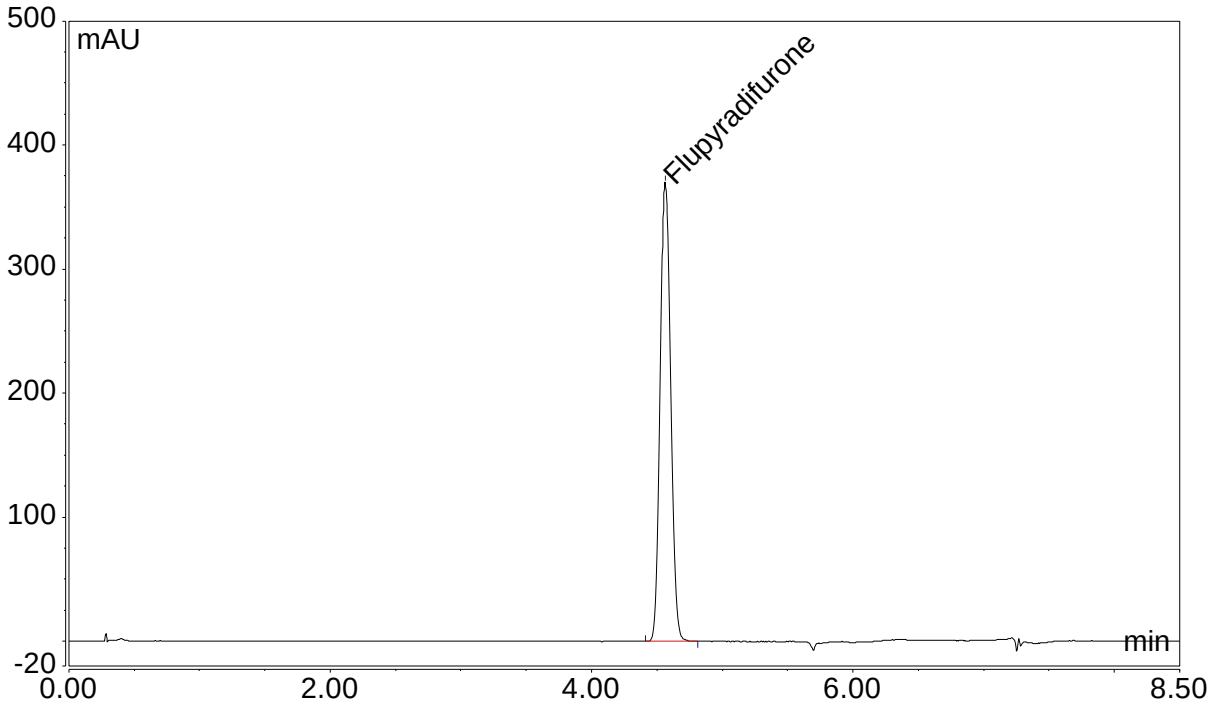


Fig. 9 Chromatogram of Flupyradifurone FS

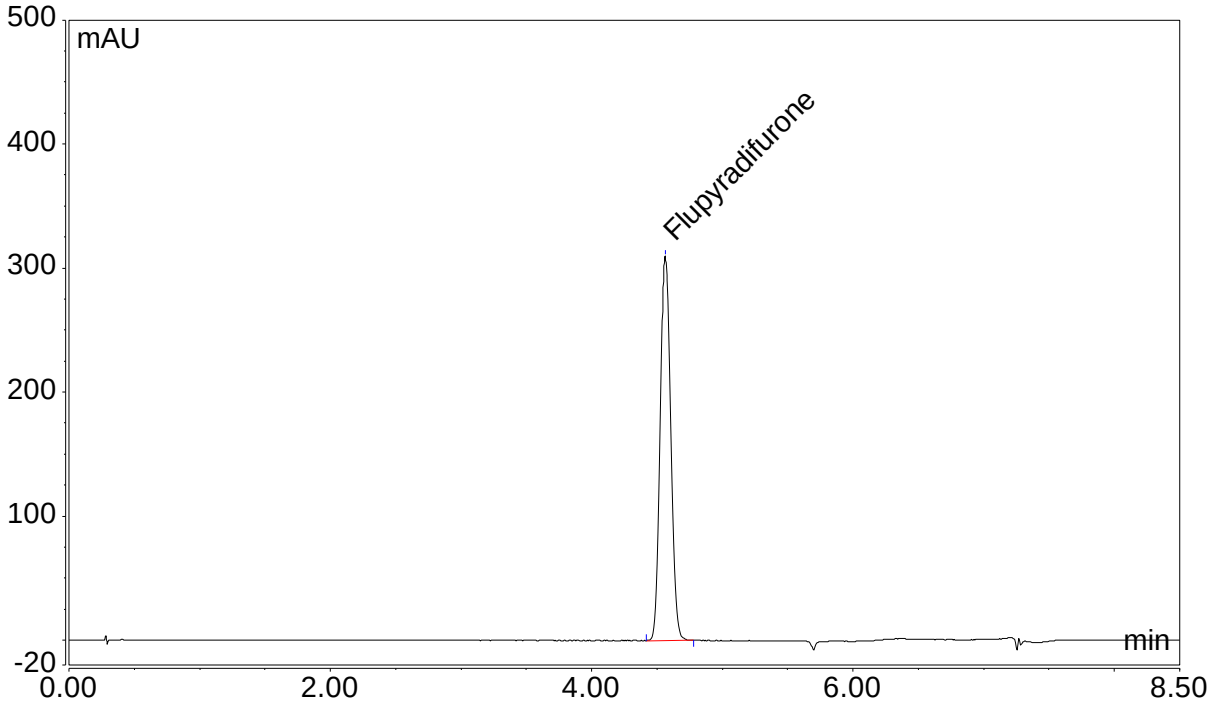


Fig. 10 Chromatogram of Flupyradifurone SL

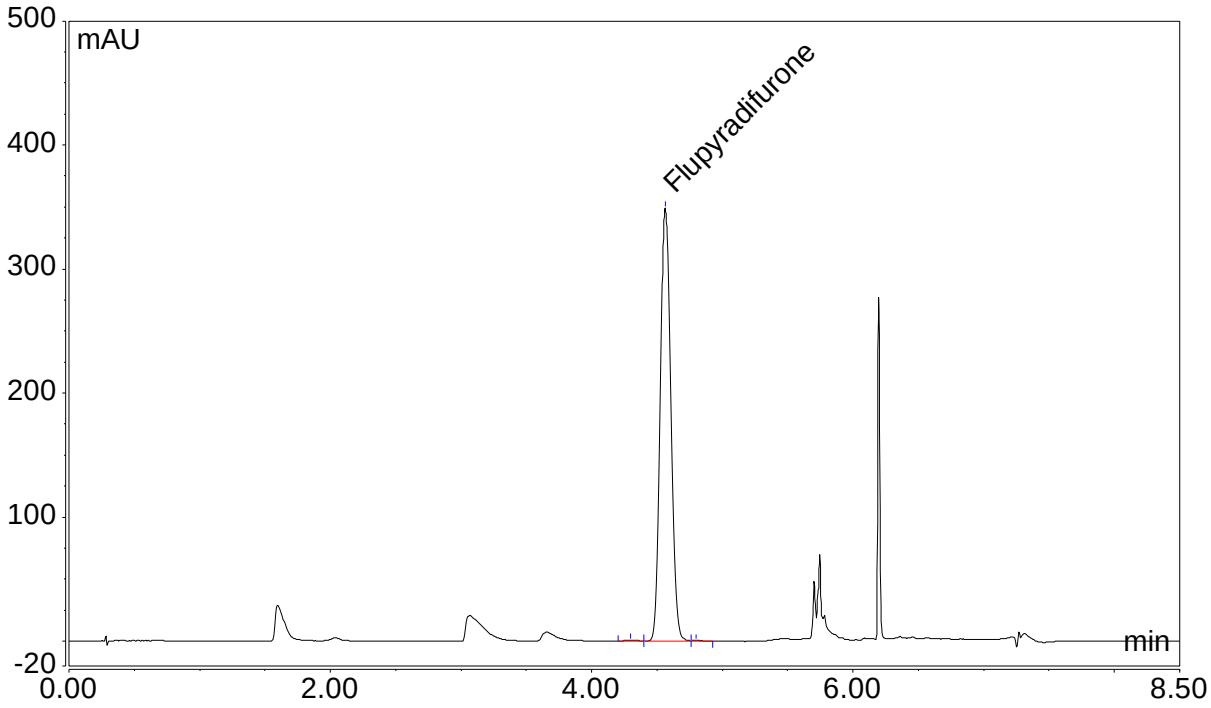


Fig. 11 Chromatogram of Flupyradifurone WG