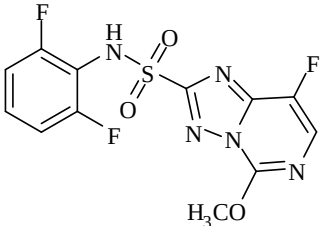
Florasulam

HPLC Method

Florasulam

Chemical name:	*N*-(2,6-difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo[1, 5-*c*]pyrimidine-2-sulfonamide
ISO common name:	Florasulam
CAS-No.:	145701-23-1
Structure:	
Molecular mass:	359.3
Empirical formula:	$C_{12}H_8F_3N_5O_3S$

FLORASULAM
/TC/

1 Sampling. Ensure that all samples taken for analysis are stored closed, with minimal headspace volume and minimal exposure to light until analysis.
Take at least 100 g. Grind the sample thoroughly in a mortar.

2 Identity tests

2.1 HPLC. Use the HPLC method described below. The relative retention time of Florasulam 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 Florasulam and the sample on an ATR unit. Scan from 4000 to 400 cm^{-1}. The spectrum produced by the sample should not differ significantly from that of the standard. (Fig. 2)

3 Florasulam

OUTLINE OF THE METHOD.

The content of Florasulam (g/kg) is determined by reversed phase high performance liquid chromatography using UV detection at 260 nm and external standard calibration.

3.1 Determination of Florasulam by reversed phase HPLC

Florasulam analytical standard of known purity
Acetonitrile (HPLC grade)
Phosphoric acid 85 % (HPLC grade)
Purified water (Resistivity>10 MΩ.cm)
Mobile phase A. Adjust purified water to pH2.1 with phosphoric acid.
Mobile phase B. Acetonitrile.
Calibration solutions C1, C2 and C3. Weigh (to the nearest 0.01 mg) 45mg, 50mg and 55mg (±1mg) of the Florasulam reference standard into separate volumetric flasks (100 mL). Add 40ml acetonitrile in the flask, place the flask in the ultrasonic water bath until the solids are dissolved. Make up the flasks with mobile phase A to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with mobile phase A and mix thoroughly (*Calibration solution C1, C2, C3,* chromatogram of C3 see Fig. 3*).*

APPARATUS

High performance liquid chromatograph equipped with an injection system capable to inject 5 µL and an UV spectrophotometric detector operated at 260 nm.
Column, stainless steel, 150×4.6 (i.d.) mm, packed with ZORBAX SB C18; 5 µm or equivalent with the same selectivity.
Electronic integrator or data system Ultrasonic bath

PROCEDURE

(a) Liquid chromatographic conditions (typical):

Column	Agilent 150 ×4.6 mm (i.d.), packed with ZORBAX SB C18, particle size 5 μm		
Mobile phase	Time (min)	Mobile phase A % (v/v)	Mobile phase B % (v/v)
	0	60	40
	8	60	40
	9	10	90
	11	10	90
	11.1	60	40
	16	60	40
Column temperature	25℃		
Flow rate	1.0 mL/min		
Inject volume	5μL		
Detection wavelength	260nm (bandwidth 4nm)		
Reference wavelength	360nm (reference bandwidth 80nm)		
Retention time	Approximately 6.4 minutes		
Run time	16 min		

(b) System equilibration. Pump sufficient mobile phase through the column to equilibrate the system. Inject 5 μL portions of the calibration solution *C2* and repeat the injections until retention times and peak areas deviate by less than ± 1 % from the mean for six successive injections.

(c) Preparation of sample solution. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.01 mg) sufficient sample (*w* in mg) (containing approximately 50 mg of Florasulam) into separate volumetric flasks (100 mL). Add 40ml acetonitrile in the flask, place the flask in the ultrasonic water bath until the solids are dissolved. Make up the flasks with mobile phase A to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with mobile phase A and mix thoroughly (sample solutions *S1, S2*, chromatogram of *S1* see Fig. 4)

(d) Determination. Equilibrate the column by pumping the mobile phase through the column until a stable baseline has been obtained. Then make duplicate injections of 5 μL of the calibration and the sample solutions (*C1*, *C1*, *C2*, *C2*, *C3*, *C3*, *S1, S1*, *S2*, *S2*).

Calculate the average florasulam peak area for each injection of each calibration and sample solution. Prepare a calibration curve by plotting the average peak area for each calibration solution versus the mass of florasulam in the calibration solutions. Using the method of least squares, calculate the equation for the straight line that best fits the experimental calibration data. Typically, the correlation coefficient should be 0.999 or better. If not, repeat the calibration. Determine the mass of florasulam in the sample solution using the equation of the calibration curve.

(e) Calculation.

Determine the mass of florasulam for each sample injecting using the following equation

$$\text{Florasulam content} = \frac{(y-b)\times P}{a\times W}\ \text{g/kg}$$

where:

y = Average florasulam peak area of the sample solution
b = Intercept of calibration curve
a = Slope of calibration curve
P = Purity of the florasulam standard (g/kg)
W = Mass of sample taken (mg)

FLORASULAM SUSPENSION CONCENTRATE /SC/

1 Sampling.

Ensure that all samples taken for analysis are stored closed, with minimal headspace volume and minimal exposure to light until analysis.
Take at least 500 mL. Shake the sample well before weighing.

2 Identity tests.

2.1 HPLC. As for florasulam TC/

2.2 UV spectrometry. As for florasulam /TC/

3 Florasulam.

As for florasulam /TC/, except substitute *(c) Preparation of sample solution* for:

(b) Preparation of sample solution.

Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.01 mg) sufficient sample (*w* in mg) (containing approximately 50 mg of Florasulam) into separate volumetric flasks (100 mL). Add 40ml acetonitrile in the flask, place the flask in the ultrasonic water bath. Make up the flasks with mobile phase A to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with mobile phase A and mix thoroughly. Filter the sample solutions by 0.45μm filter prior to HPLC analysis (sample solutions *S3*, *S4*, chromatogram of *S3* see Fig. 5).

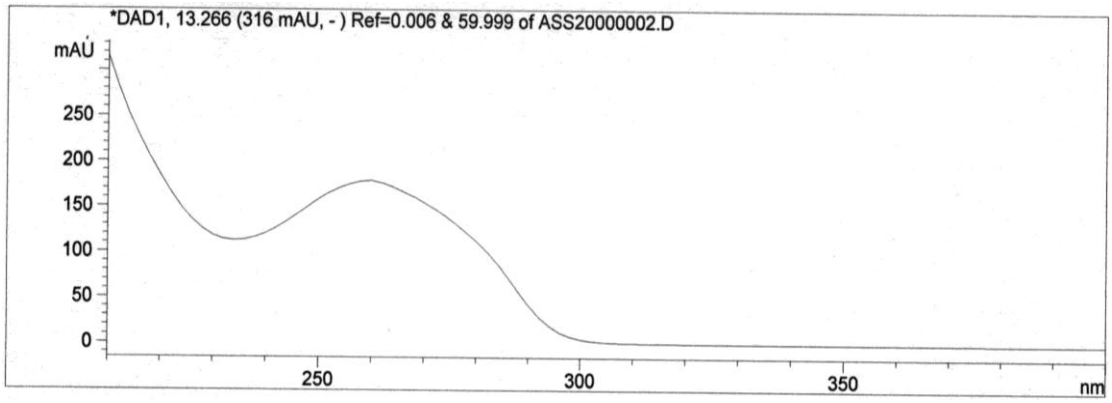


Fig. 1 UV Spectrum of florasulam

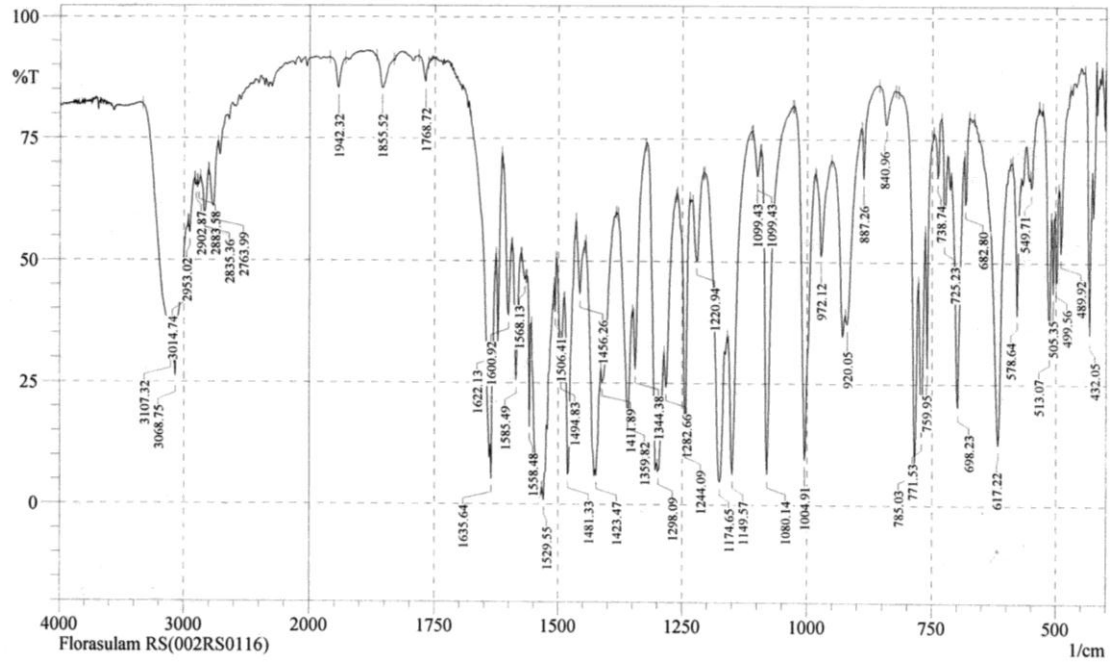


Fig. 2 Infrared Spectrum of florasulam

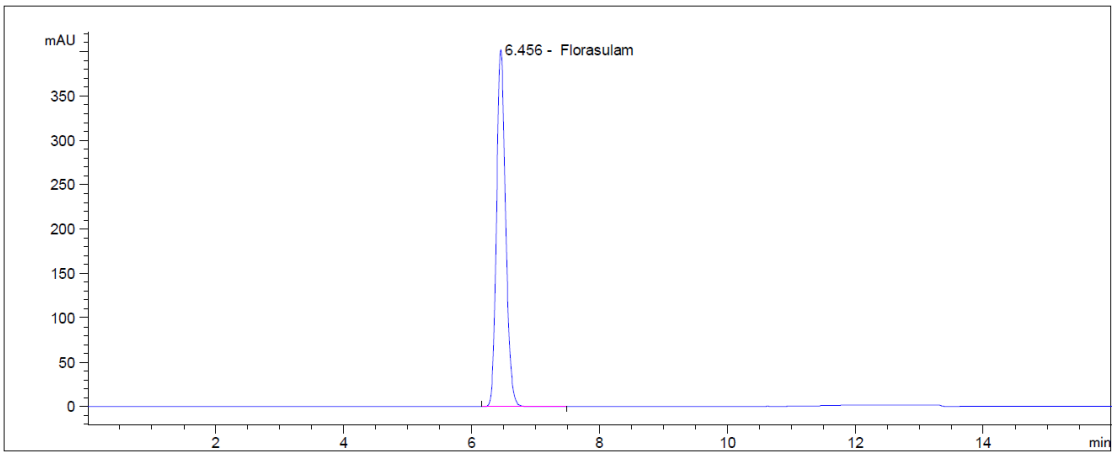


Fig. 3 Analytical Standard florasulam (*C2*)

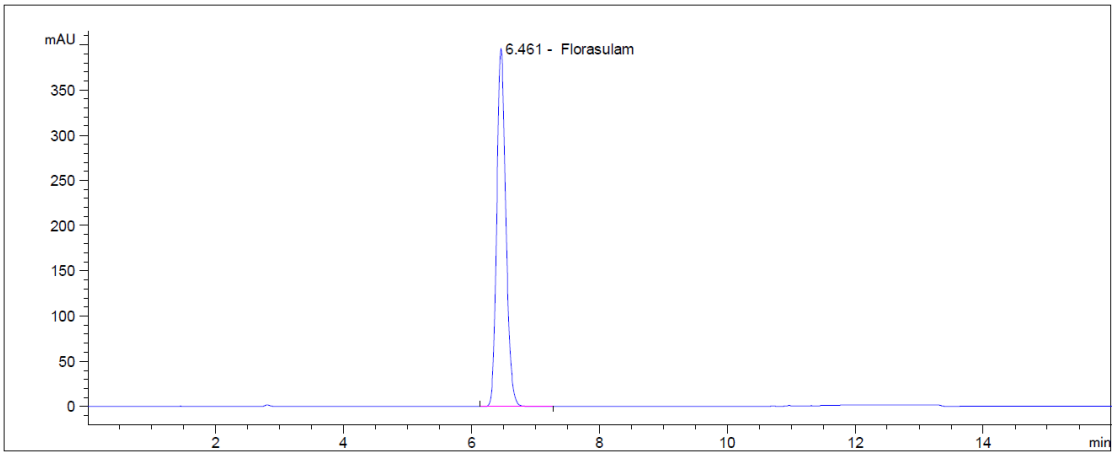


Fig. 4 Technical Material TC (*S1*)

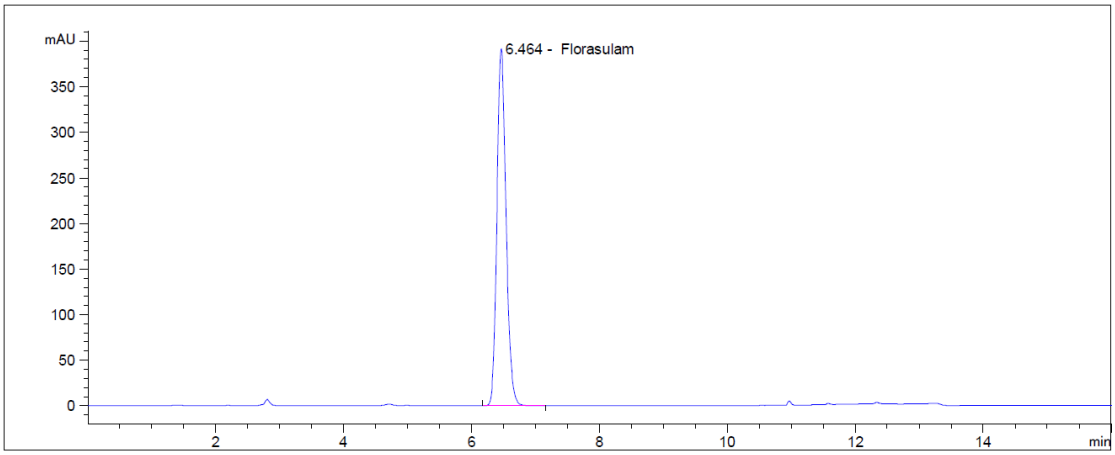


Fig. 5 Suspension Concentrate SC (*S3*)