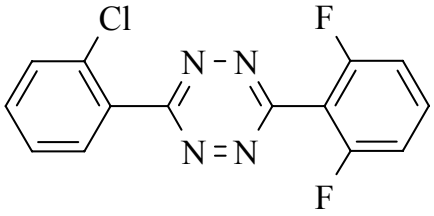
DIFLOVIDAZIN
734



ISO common name	Diflovidazin (proposed)
Other names	Flufenzine, SZI 121
Chemical name	3-(2-Chlorophenyl)-6-(2,6-difluorophenyl)-1,2,4,5-tetrazine (IUPAC); 1,2,4,5-tetrazine, 3-(2-chlorophenyl)-6-(2,6-difluorophenyl) (CA; *162320-67-4*)
Empirical formula	$C_{14}H_7ClF_2N_4$
RMM	304.7
m.p.	187-189 ºC
v.p.	Less than 10^{-5} Pa at 25 ºC
Solubility	In water: 0.2 mg/l; acetone: 14.4 g/l; benzene: 3.2 g/l; ethanol: 1.8 g/l; hexane: 0.3 g/l; chloroform: 50 g/l; acetonitrile: 13.7 g/l; ethyl acetate: 18 g/l; acetic acid: 6.3 g/l; tetrahydrofuran: 13.1 g/l; dimethylformamide: 81.6 g/l; methanol: 3.7 g/l; dichloromethane: 7.2 g/l (all at 25 ºC)
Description	Purple, microcrystalline powder
Stability	Stable at 180 ºC; stable at pH <7, but rapidly hydrolysed at pH >7 (DT_{50}: about 60 h at 25 ºC and pH 9)
Formulations	Suspension concentrate

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DIFLOVIDAZIN TECHNICAL
*734/TC/M/-

1 Sampling. Take at least 100 g.

2 Identity tests
2.1 HPLC. Use the HPLC method below. The retention time of the diflovidazin peak in the sample solution should not deviate by more than 2 % from that of the calibration solution.
2.2 Infrared. Prepare potassium bromide discs from the technical sample and from diflovidazin standard. The spectrum obtained from the sample should not differ significantly from that of the standard. (Fig. 24)

3 Diflovidazin

OUTLINE OF METHOD. Diflovidazin is determined by reversed phase high performance liquid chromatography using a Spherisorb C-18 column, UV detection at 270 nm and external standardisation.

REAGENTS

Acetonitrile HPLC grade
Water HPLC grade
Diflovidazin standard of known purity. Stable at ambient temperature if kept in a well-closed container (available from AGRO-CHEMIE, Hungary).
Mobile phase acetonitrile + water, 55 + 45 (v/v). Filter through a 0.45 µm filter and degas in an ultrasonic bath for 10 min or by purging with helium, before use. Stable for 1 month.
Calibration solution. Prepare calibration solutions in duplicate (C_A and C_B). Weigh (to the nearest 0.1 mg) about 20.0 mg diflovidazin standard (*s* mg) into a volumetric flask (25 ml). Dissolve in acetonitrile (20 ml) and place the flask in an ultrasonic bath for 10 min. Allow to cool to room temperature and make up to volume with acetonitrile. Filter through a 0.45 µm filter into sample vials before injection (stable for 24 h).

APPARATUS

Liquid chromatograph equipped with a constant flow pump, a sample injector capable of injecting 10 µl aliquots and a variable-wavelength UV detector operated at 270 nm.

* CIPAC method 2004. Prepared by the Hungarian committee. Based on a method supplied by Agro- Chemie Ltd, Hungary.

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Column stainless steel, 250 × 4.6 mm (i.d.) packed with Spherisorb 5 ODS-1 or equivalent. (The number of theoretical plates must be at least 7000 per 25 cm calculated for the diflovidazin peak).
Guard column Nova-Pak 4 µm 60 Å C-18 Guard-Pak insert or equivalent
Integrator or *computing data system*
Ultrasonic bath
Filtering apparatus disposable plastic syringes (3 ml) fitted to a 0.45 µm Millex®-AA Syringe driven filter unit or equivalent.

PROCEDURE

(a) Operating conditions (typical):

Column	stainless steel, 250 × 4.6 mm (i.d.) packed with Spherisorb 5 ODS-1
Mobile phase	acetonitrile + water, 55 + 45 (v/v)
Column temperature	ambient (typically 22 ± 3 ºC)
Flow rate	1.4 ml/min
Injection volume	10 µl
Detector wavelength	270 nm
Run time	15 min
Retention time	diflovidazin: about 8.3 min

(b) System check and equilibration. Equilibrate the column prior to the analysis by pumping mobile phase through the system at 1.4 ml/min for 20-30 min. After the system has stabilised, resulting in a flat baseline, make several injections of the calibration solution C_A. The retention times should not vary by more than 2 %.
Inject in duplicate calibration solutions C_A, C_B and record the corresponding peak areas. Calculate the calibration factors of the four standards. The individual calibration factors and the calculated mean calibration factor of the four standard solutions should not differ by more than 1.5 %.

(c) Preparation of sample. Weigh (to the nearest 0.1 mg) sufficient sample to contain 20 mg diflovidazin (*w* g) into a volumetric flask (25 ml). Dissolve in acetonitrile (approximately 20 ml) and place the flask in an ultrasonic bath for 10 min. Allow to cool to room temperature and fill to the mark with acetonitrile. Filter the solution through a 0.45 µm filter into sample vials before injection. Prepare two solutions (solutions S_1, S_2) for each sample (stable for 24 h).

(d) Determination. Inject 10 µl portions of the calibration and sample solutions in the following order:

C_A, C_A, C_B, C_B, S_1, S_1, S_2, S_2, C_A, C_A, S_3, S_3, S_4, S_4, C_B, C_B

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Record the areas of the diflovidazin peaks. Calculate the average response factor from calibration injections. Response factors should agree within 1.5 %. Calculate the diflovidazin content using the average response factor of the calibration solutions preceding and following each series of sample solution injections.

(e) Calculation

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

$$\text{Diflovidazin content} = \frac{H_w \times f}{w} \text{ g/kg}$$

where:
f_i = individual response factor
f = mean response factor
H_s = peak area of diflovidazin in the calibration solution
H_w = peak area of diflovidazin in the sample solution
s = mass of diflovidazin in the calibration solution (mg)
w = mass of sample (mg)
P = purity of diflovidazin standard (g/kg)

The content of diflovidazin in the sample is the mean value of the results of two sample solutions.

Repeatability r = 22.5 g/kg at 988 g/kg active ingredient content
Reproducibility R = 23.5 g/kg at 988 g/kg active ingredient content

DIFLOVIDAZIN SUSPENSION CONCENTRATES
*734/SC/M/-

1 Sampling. Take at least 500 g.

2 Identity tests
2.1 HPLC. As for technical **734**/TC/M/2.1.
2.2 Ultraviolet spectrum. Record the UV spectrum of the diflovidazin peak during the HPLC determination. The spectrum obtained from the sample should not differ significantly from that of the standard.

* CIPAC method 2004. Prepared by the Hungarian committee. Based on a method supplied by Agro- Chemie Ltd, Hungary.

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3 Diflovidazin. As for diflovidazin technical **734**/TC/M/3 except:
(c) Preparation of sample solutions. Homogenise the sample before use in the following way: swing the bottle in a horizontally circular movement (about 20 cm) for one minute. Then immediately weigh (to the nearest 0.1 mg) sufficient sample to contain 20 mg diflovidazin (*w* g) into a volumetric flask (25 ml). Dissolve in acetonitrile (approximately 20 ml) and place the flask in an ultrasonic bath for 10 min. Allow to cool to room temperature and fill to the mark with acetonitrile. Filter the solution through a 0.45 µm filter into sample vials before injection. Prepare two solutions (solutions S_1, S_2,) for each sample (stable for 24 h).

Repeatability r = 4.1 g/kg at 186 g/kg active ingredient content
Reproducibility R = 12 g/kg at 186 g/kg active ingredient content

3. Suspensibility (Draft method)

REAGENTS AND APPARATUS as for **734**/TC/M/3 and MT 184.

PROCEDURE

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

(b) Determination of diflovidazin in the bottom 25 ml of suspension. After removal of the top 225 ml of suspension transfer the remaining 25 ml suspension quantitatively into a volumetric flask (50 ml), add acetonitrile (about 20 ml),and dissolve using an ultrasonic bath for 10 min. Allow to cool to room temperature and fill up to mark with acetonitrile. Determine the mass of diflovidazin (*Q* g) by **734**/TC/M/3, except:
Calibration solution. Weigh (to the nearest 0.1 mg) about 25 mg of diflovidazin standard into a volumetric flask (10 ml). Dissolve in acetonitrile (8 ml) using an ultrasonic bath for 10 min. Allow to cool to room temperature and fill to mark with acetonitrile. Add by pipette exactly 1.0 ml from this solution to a volumetric flask (50 ml) and fill to the mark with acetonitrile.

(c) Calculation

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

where:
c = mass of diflovidazin in the sample taken for the preparation of the suspension (g)
Q = mass of diflovidazin in the bottom 25 ml of suspension (g)

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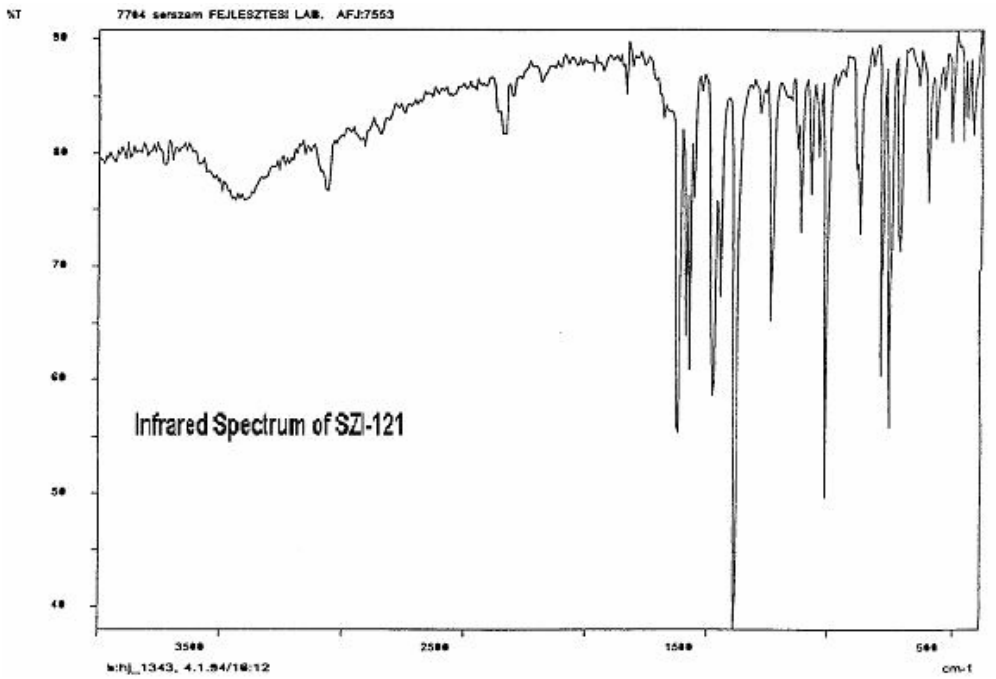

Fig. 24 IR spectrum of diflovidazin

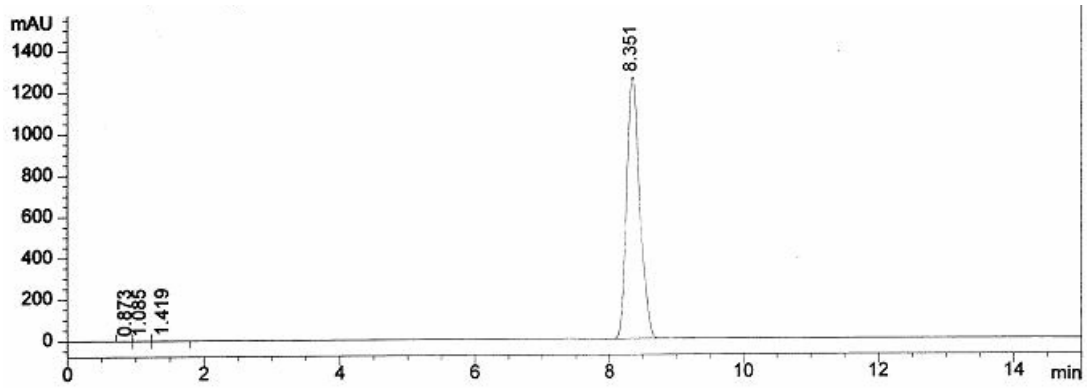

Fig. 25 Typical chromatogram of diflovidazin analytical standard

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