653. Cyazofamid

HPLC method

CIPAC Full Scale Collaborative Trial

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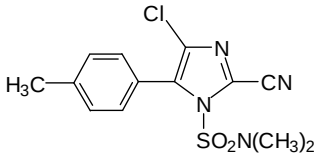
CYAZOFAMID
653



ISO common name:	Cyazofamid
Chemical name:	4-chloro-2cyano-*N,N*-dimethyl-5-*P*-tolylimidazole -1- sulfonamide (IUPAC) 4-chloro-2cyano-*N,N*-dimethyl-5-(4-methylphenyl)-1*H*- imidazole- 1-sulfonamide (CA)
CAS-Number:	120116-88-3
RMM:	324.8
Empirical formula:	$C_{13}H_{13}ClN_4O_2S$
m.p.	152.7 °C
V.p.	<1.33x10 $^{-5}$ Pa at 35 °C
Solubility	In water at 20 °C: 0.107-0.121 mg/l at pH 5, 7 and 9 acetone: 41.92 g/l at 20 °C dichloromethane: 101.84 g/l at 20 °C ethyl acetate: 15.63 g/l at 20 °C hexane: 0.03 g/l at 20 °C methanol: 1.54 g/l at 20 °C n-octanol: 0.25 g/l at 20 °C toluene: 5.28 g/l at 20 °C acetonitrile: 29.42 g/l at 20 °C propan-2-ol: 0.39 g/l at 20 °C
Stability	Keep frozen (< -18 °C) when not in use and avoid exposure to light.
Hydrolysis	DT_{50} at 20 °C: 24.6 days (pH 4), 27.2 days (pH 5), 24.8 days (pH 7) and 24.8 days (pH 9)
Description	White powder
Formulation	Suspension concentrates

CYAZOFAMID TECHNICAL
653/TC/M/-

1 Sampling. Take at least 20 g.

2 Identity test

2.1 HPLC. Use the reversed phase HPLC method described below. The relative retention time of the cyazofamid peak in the sample solution should not deviate by more than 2% from that of the calibration solution. The UV spectrum measured from this peak should match that obtained from the calibration substance.

3 Cyazofamid

OUTLINE OF METHOD. Cyazofamid is determined by reversed phase high performance liquid chromatography using UV detection at 280 nm and external standard calibration.

REAGENTS

Cyazofamid reference standard with known content
Water HPLC grade
Acetic acid analytical reagent grade
Acetonitrile HPLC grade
Methanol HPLC grade
Mobile phase: water (pH 4 with acetic acid) – acetonitrile – methanol (43-32-25 v/v)
Solvent: acetonitrile
Calibration solutions. Weigh in duplicate (to the nearest 0.1 mg) 85 mg of cyazofamid reference standard (*s* mg) into separate volumetric flasks (50 ml). Add acetonitrile (about 40 ml) and sonicate for 5 minutes until complete dissolution. Allow the solutions to cool to ambient temperature and fill to the mark with acetonitrile (calibration solutions C_A and C_B). Mix well.

APPARATUS

High performance liquid chromatograph equipped with a detector suitable for operation at 280 nm (UV-detection) and an injection system capable of injecting 5 µl
Liquid chromatographic column stainless steel, 250 x 4.6 mm (i.d), packed with C_{18}, 5 µm, Phenomenex SphereClone ODS-2 or equivalent with the same selectivity
Electronic integrator or data system
Ultrasonic bath
pHmeter

PROCEDURE

(a) Chromatographic conditions (typical)

Column temperature	40°C
Flow rate	1.0 ml/min
Detector wavelength	280 nm
Injection volume	5 µl
Mobile phase	water (pH 4 with acetic acid) – acetonitrile – methanol (43-32-25 v/v)
Elution	isocratic
Run time	45 min
Retention time	approximately 23 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 C_A and repeat the injections until retention times and peak areas deviate by less than ± 0.5 % from the mean for three successive injections.

(c) Sample preparation. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about 85 mg cyazofamid into a volumetric flask (50 ml). Add acetonitrile (about 40 ml) and sonicate for 5 minutes until complete dissolution. Allow the solutions to cool to ambient temperature and fill to the mark with acetonitrile (sample solutions S_1 and S_2). Mix thoroughly.

(d) Determination. Inject 5 µl portions of the second calibration solution (C_B) for two successive injections. The mean response factor for this solution should deviate by no more than 1% from that for the first calibration solution (C_A) (see paragraph (b) Equilibration of the system), otherwise the calibration solutions should be prepared again.

Inject in duplicate 5 µl portions of each sample solution (S_1, S_2, …,etc.) bracketing them by single injections of calibration solutions (C_A and C_B) using the following sequence:

$C_A, S_1, S_1, S_2, S_2, C_B, S_3, S_3, S_4, S_4, C_A$…

(e) Calculation. Determine the peak area of cyazofamid and calculate the mean value of response factors from the calibration solutions bracketing the injections of the sample solutions and use this value for calculating the cyazofamid content of the bracketed sample solutions. The cyazofamid content is the mean value of two sample solutions.

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

$$\text{cyazofamid content} = \frac{f \times H_w}{w} \quad \text{[g/kg] } (M)$$

where:

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

CYAZOFAMID SUSPENSION CONCENTRATES

653/SC/M/-

1 Sampling. Take at least 100 g.

2 Identity test

2.1 HPLC. As for cyazofamid technical 653/TC/M/2.1

3 Cyazofamid

As for cyazofamid technical 653/TC/M/3 except

(c) Sample preparation. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about 85 mg cyazofamid into a volumetric flask (50 ml). Add acetonitrile (about 40 ml) and sonicate for 15 minutes until complete dissolution. Allow the solutions to cool to ambient temperature and fill to the mark with acetonitrile (sample solutions S_1 and S_2). Mix thoroughly. Filter an aliquot of each prepared solution through a 0.45 µm PTFE filter prior to analysis.

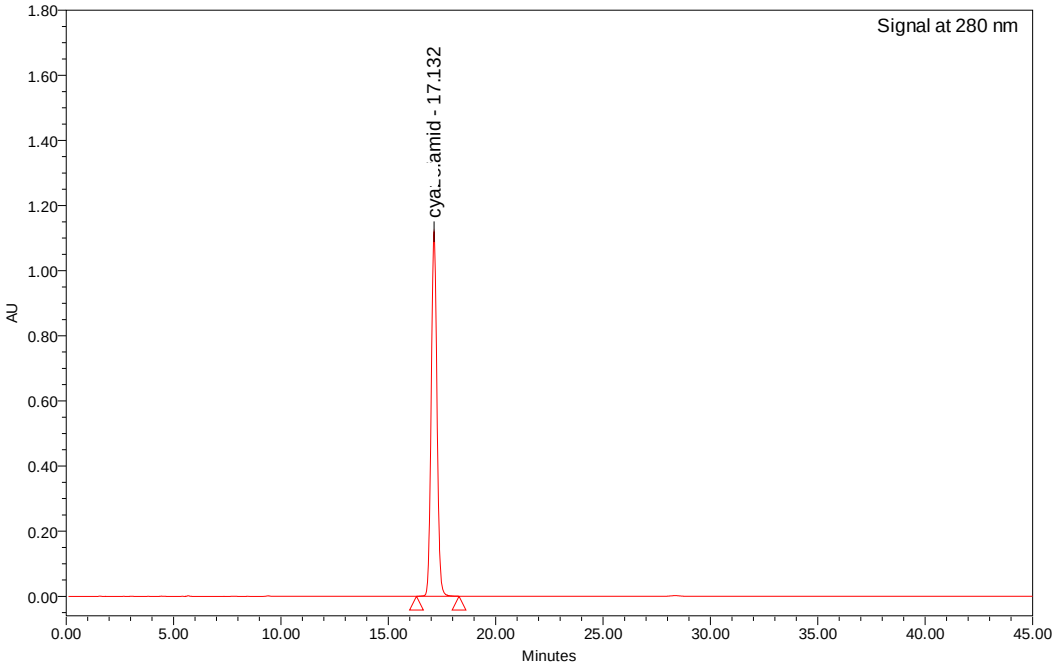


Fig 1 Typical HPLC-chromatogram of calibration solution

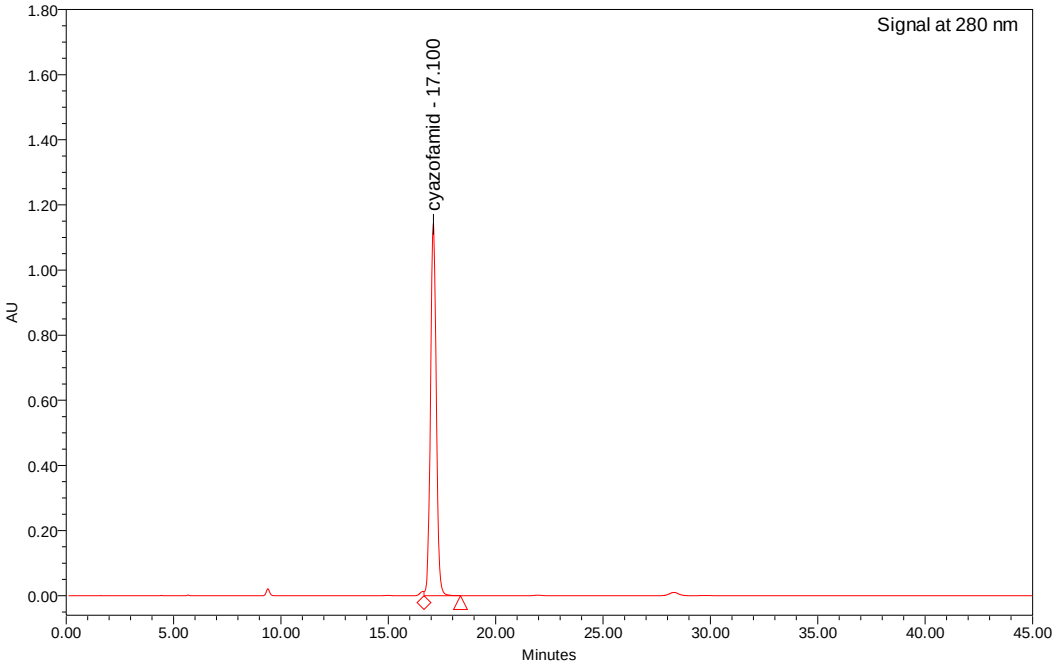


Fig 2 Typical HPLC-chromatogram of Cyazofamid technical material

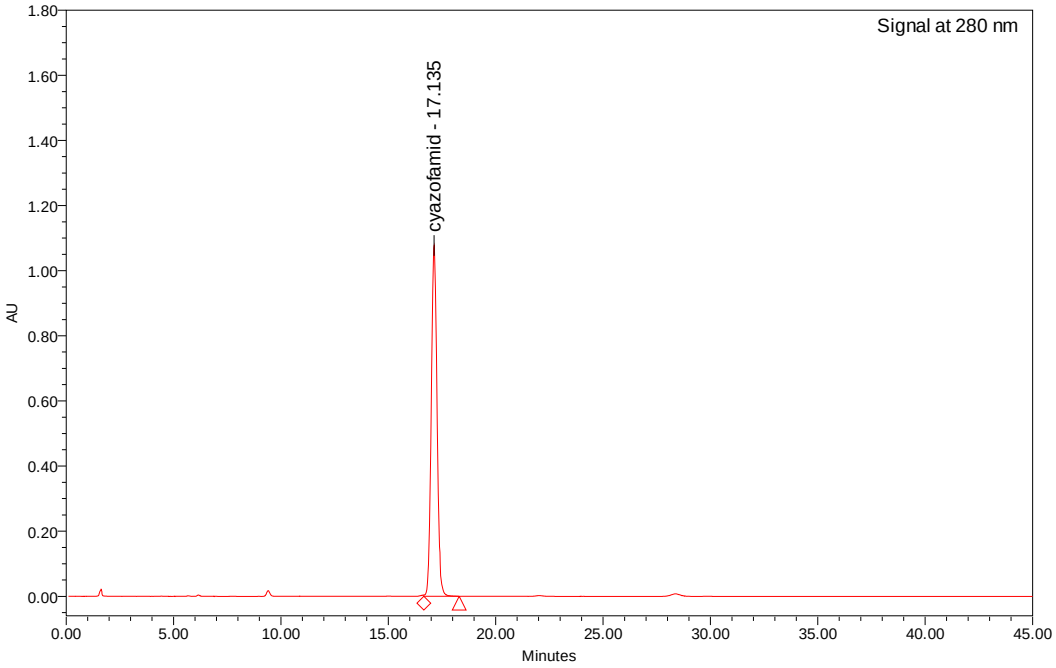


Fig 3 Typical HPLC-chromatogram of Cyazofamid formulation 400 SC