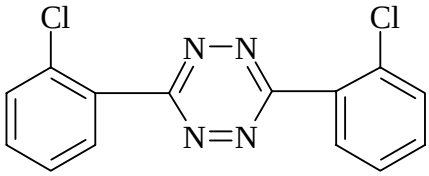
CLOFENTEZINE
418



ISO common name	Clofentezine
Chemical name	3,6-Bis(2-chlorophenyl)-1,2,4,5-tetrazine (IUPAC and CA; *74115-24-5*)
Empirical formula	$C_{14}H_8Cl_2N_4$
RMM	303.1
m.p.	182-183 °C
v.p.	1.3×10^{-7} Pa at 25°C
Solubility	In water: less than 1 mg/l at 20°C; acetone: 9.3 g/l; dichloromethane: 37 g/l; ethanol: 0.5 g/l; xylene: 5 g/l all at 25°C
Stability	Hydrolyses in alkaline conditions (half life: 4.3 hours at 22°C and pH 9.2)
Formulations	Suspension concentrates (200 and 500g/l)

CLOFENTEZINE TECHNICAL
*418/TC/M/-

1 Sampling. Take at least 100 g.

2 Identity tests

2.1 HPLC. Use the method outlined below. The relative retention time of clofentezine with respect to the internal standard in the sample solution should not deviate by more than 1% from that of the calibration solution.

2.2 Infra-red. Prepare potassium chloride discs from the sample and pure clofentezine using 1 mg material and 250 mg potassium chloride. Scan the discs from 4000 to 600 cm^{-1}. The spectrum produced from the sample disc should not differ significantly from that of the standard.

3 Clofentezine

OUTLINE OF METHOD Clofentezine is separated from other components by high performance liquid chromatography on a reversed phase column and quantified using benzyl butyl phthalate as internal standard.

REAGENTS

Clofentezine certified reference substance
Benzyl butyl phthalate internal standard, free from significant response at the retention time of clofentezine
Acetone
Acetonitrile HPLC grade
Water deionised
Orthophosphoric acid
Mobile phase acetonitrile-water 650 + 350 (v/v); degas before use
Diluting solvent. Dissolve orthophosphoric acid (2 g) in acetone (2 l), mix thoroughly.
Internal standard solution. Dissolve benzyl butyl phthalate (4.0 g) in diluting solvent (500 ml).

* CIPAC method 1995. Prepared by the Clofentezine Panel of PAC-UK. Chairman: D Wooldridge. Based on a method supplied by ArgEvo UK, England.

Calibration solution. Weigh (to the nearest 0.1 mg) between 95 and 105 mg (*s* mg) clofentezine reference substance into a stoppered conical flask (100 ml). Add by pipette internal standard solution (20.0 ml) and diluting solvent (60 ml). Sonicate to dissolve.

APPARATUS

High performance liquid chromatograph including variable wavelength UV detector and an injector capable of delivering 5 µl
Column 250 × 4.6 (i.d.) mm, stainless steel, packed with 5 µm Spherisorb ODS-2, or equivalent
Integrator/data system
Ultrasonic bath

PROCEDURE

(a) Operating conditions (typical):

Flow rate	1.4 ml/min
Detector wavelength	235 nm
Injection volume	5 µl
Retention times	clofentezine: 6.3 min
	benzyl butyl phthalate: 7.8 min

(b) Preparation of sample solution. Weigh (to the nearest 0.1mg) about 100 mg (*w* mg) clofentezine into a stoppered conical flask (100 ml). Add by pipette internal standard solution (20.0 ml) and diluting solvent (60 ml). Sonicate to dissolve.

(c) Determination. Inject onto the liquid chromatograph 5 µl portions of calibration solution until the response factors for three successive injections differ by no more than ± 1% of mean. Then make duplicate 5 µl injections of the sample solution, followed by another injection of the calibration solution. Average the response factors of the injections following and preceding the sample injection and record the relevant peak areas of the sample peaks.

(d) Calculation

$$f = \frac{s \times I_r}{H_s} 1$$

$$\text{Content of clofentezine} = \frac{f_m \times H_w \times P}{I_q \times w} \text{ g/kg}$$

where:

f_m = average response factor
H_s = area of clofentezine peak in the calibration solution
H_w = area of clofentezine peak in the sample solution
I_r = area of internal standard peak in the calibration solution
I_q = area of internal standard peak in the sample solution
s = mass of clofentezine in the calibration solution (mg)
w = mass of sample taken (mg)
P = purity of clofentezine reference substance (g/kg)

Repeatability r = 13 g/kg at 1000 g/kg active ingredient content
Reproducibility R = 22 g/kg at 1000 g/kg active ingredient content

CLOFENTEZINE SUSPENSION CONCENTRATES
*__418__/SC/M/-

1 Sampling. Take at least 500 g.

2 Identity tests

2.1 HPLC. As for clofentezine technical **418**/TC/M/2.1.

2.2 Infra-red. To about 10 mg sample in a vial (20 ml) add water (1 ml) and sonicate to disperse. Add dichloromethane (5 ml) and shake well. If an emulsion forms, add up to 100 mg anhydrous calcium chloride. Allow to settle and remove the upper aqueous layer. Add 2 to 3 g anhydrous sodium sulphate, then evaporate to dryness a 1 ml portion of the dichloromethane solution. Use this to produce a potassium chloride disc and proceed as for **418**/TC/M2.2.

* CIPAC method 1995. Prepared by the Clofentezine Panel of PAC-UK. Chairman: D Wooldridge. Based on a method supplied by AgrEvo UK, England.

3 Clofentezine. As for clofentezine technical **418**/TC/M/3 except:
(b) Preparation of sample solution. Weigh (to the nearest 0.1 mg) enough sample to contain 95 to 105 mg clofentezine (*w* mg) into a stoppered conical flask (100 ml). Add deionised water (5 ml) and sonicate to disperse. Add, by pipette, internal standard solution (20.0 ml), using the same pipette as for the preparation of the calibration solution. Then add diluting solvent (55 ml). Sonicate to dissolve. Filter a portion through a suitable micro-filter.

Repeatability r	= 5.9 g/kg at 187 g/kg active ingredient content
	= 13 g/kg at 420 g/kg active ingredient content
Reproducibility R	= 9.7 g/kg at 187 g/kg active ingredient content
	= 24 g/kg at 420 g/kg active ingredient content

4 Suspensibility (Draft method)
REAGENTS AND APPARATUS As for MT 161 and **418**/TC/M/3 except:
Internal standard solution. Dissolve benzyl butyl phthalate (0.8 g) in diluting solvent.
Calibration solution. Weigh (to the nearest 0.1 mg) into a volumetric flask (100 ml) 95-105 mg clofentezine reference substance (*s* mg). Add diluting solvent (about 90 ml) and sonicate to dissolve. Dilute to volume with diluting solvent, shake to mix, then pipette 20.0 ml into a 100 ml stoppered conical flask. Add, by pipette, internal standard solution (25.0 ml). Then add diluting solvent (35 ml) and CIPAC water D (25 ml) and mix well.

PROCEDURE

(a) Preparation of suspension. MT 161.

(b) Determination of sedimentation. MT 161.

(c) Determination of clofentezine in residual 25 ml. Add by pipette internal standard solution (25.0 ml) and diluting solution (55 ml) and mix well. Proceed as for **418**/TC/M/3*(c).* Record the relevant peak areas and calculate the mass of clofentezine present in the 25 ml of suspension (*Q*).

(d) Calculation

$$f = \frac{s \_ I_r}{H_s} 2$$

$$Q = \frac{f \_ H_w \_ P}{I_q \_ 10} 3$$

$$\text{Suspensibility} = \frac{111(C - Q)}{C} 4\ \%$$

where:

f	=	response factor
s	=	mass of clofentezine in calibration solution (mg)
I_r	=	area of internal standard peak in calibration solution
H_s	=	area of clofentezine peak in calibration solution
H_w	=	area of clofentezine peak in sample solution
I_q	=	area of internal standard peak in sample solution
P	=	purity of clofentezine reference substance (g/kg)
C	=	mass of clofentezine in the sample taken for preparation of the suspension (g)
Q	=	mass of clofentezine in residual 25 ml (g)