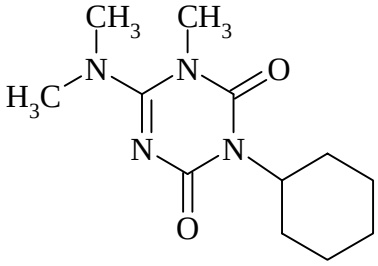
HEXAZINONE
374



ISO common name	Hexazinone
Chemical name	3-Cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione (IUPAC); 3-cyclo-hexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione (CA; *51235-04-2*)
Empirical formula	$C_{12}H_{20}N_4O_2$
RMM	252.3
m.p	115 - 117 °C
v.p.	1.30×10^{-5} Pa at 25 °C
Solubility	In water: 33 g/l at 25 °C; acetone: 790 g/kg; benzene: 940 g/kg; methanol: 2.65 kg/kg; hexane 3 g/kg; toluene: 386 g/kg; dimethylformamide: 836 g/kg; chloroform: 3.88 kg/kg; (all at 25 °C)
Description	White crystalline solid
Stability	Stable in aqueous solutions below 37 °C and pH 5 - 9
Formulations	Water dispersible granules, water soluble powders, soluble concentrates and water soluble granules

HEXAZINONE TECHNICAL
*374/TC/M/-

1 Sampling. Take at least 100 g.

2 Identity tests

2.1 HPLC. Use the HPLC method below. The retention time of hexazinone for the sample solution should not deviate by more than 5 % from that for the calibration solution.

2.2 Infrared. Prepare potassium bromide discs from the sample and from hexazinone standard using 1 to 2 mg material and 200 mg potassium bromide. Scan the discs from 400-4000 cm^{-1}. The absorbance maximum should be between 0.6 and 1.0 absorbance units. If the absorbance is outside this range, adjust the mixture accordingly. The spectrum from the sample should not differ significantly from that of the standard (Fig. 20).

3 Hexazinone

OUTLINE OF METHOD Hexazinone is determined by high performance liquid chromatography on a reversed phase column (C_8) using water (at pH 3) - acetonitrile 50 + 50 (v/v) as eluent and UV detection at 254 nm. The content of active ingredient is quantified using a calibration curve.

REAGENTS

Acetonitrile HPLC grade
Water HPLC grade
Hexazinone standard of known purity
Benzanilide internal standard
Phosphoric acid 85 %, HPLC grade
Mobile phase Adjust water (500 ml) to pH 3.0 with phosphoric acid using a pH meter standardised at pH 7.0 and pH 2.0. Add acetonitrile (500 ml) and mix. Mixing the mobile phase may also be accomplished using a binary solvent HPLC pump. Degas before use.
Sample solvent. Mix equal volumes of acetonitrile and water.
Internal standard solution. Weigh benzanilide (9.0 g) into a bottle (1 l) and add acetonitrile (500 ml). Place the bottle in an ultrasonic bath for approximately 10 min. Allow to cool to room temperature.

* CIPAC method 1999. Prepared by a committee chaired by P A Bloxham. Based on a method supplied by DuPont de Nemours, USA.

Calibration solution. Weigh (to the nearest 0.1 mg) 60, 80, and 100 mg (± 5 mg) of hexazinone standard into three separate bottles (150 ml). Add internal standard solution (10.0 ml) by pipette to each bottle. Add sample solvent (90 ml) to each bottle and place the bottles in an ultrasonic bath for 5 min. Label as solutions C_1, C_2, and C_3 respectively. Filter a portion of each calibration solution through a 0.2 µm filter prior to analysis.

APPARATUS

High performance liquid chromatograph equipped with a constant-temperature column compartment, a constant flow pump, a 5 µl loop injection valve, and a UV spectrophotometric detector capable of operating at 254 nm

Column stainless steel, 250 × 4.6 mm (i. d.) packed with Zorbax® RX-C_8 or Zorbax® SB-C_8, 5 µm particle size, with in-line filter with replaceable 0.5µm frit (Upchurch Scientific Inc model A-102X, A-318, or equivalent). Substitution of alternate columns must be accompanied by demonstrated equivalency and/or method revalidation.

Filtering apparatus disposable plastic syringes (3 ml) fitted with 0.2 µm filter (Acrodisc-CR or equivalent)

Integrator or *electronic data system*

Ultrasonic bath

PROCEDURE

(a) Operating conditions (typical):

Column	Zorbax® RX-C_8, 250 × 4.6 mm
Mobile phase	water at pH 3 - acetonitrile 50 + 50 (v/v)
Eluent flow rate	1.5 ml/min
Column temperature	40 °C
Injection volume	5 µl
Detection wavelength	254 nm (bandwidth 4 nm)
Reference wavelength	350 nm (bandwidth 80 nm)
Retention time	hexazinone: about 2.8 min
	benzanilide: about 4.4 min
Run time	8 min

(b) Sample preparation Mill or grind all samples prior to weighing. Weigh (to the nearest 0.1 mg) sufficient sample to contain 80 ± 5 mg of hexazinone (*w* mg) into a bottle (150 ml). Add internal standard solution (10.0 ml). Add sample solvent (90 ml), place the bottle in an ultrasonic bath for 5 min and mix. Filter a portion of the sample solution through a 0.2 µm filter prior to analysis.

(c) Determination. Equilibrate the column by pumping the mobile phase through the column until a stable baseline has been obtained. Make duplicate injections of 5 µl of each calibration and sample solution. Peak response must be within the linear range of the detector. If necessary, perform additional dilutions after the addition of the internal standard to obtain appropriate detector response.
Calculate the hexazinone to benzanilide peak area ratio for each injection of the calibration and sample solutions. Prepare a calibration curve by plotting the average peak area ratio for each calibration solution (solutions C_1, C_2, and C_3) versus the corresponding hexazinone mass. Using the least-squares method calculate the line that best fits the experimental data. The correlation coefficient should be 0.999 or better. If not, repeat the calibration.

$$\text{Hexazinone content} = \frac{(R - b) \times P}{a \times w} \text{ g/kg}$$

where:
R = hexazinone to benzanilide peak area ratio of the sample solutions
a = slope of calibration curve
b = intercept of calibration curve
P = purity of the hexazinone standard (g/kg)
w = mass of sample taken (mg)

Repeatability r = 14 g/kg at 998 g/kg active ingredient content
Reproducibility R = 22 g/kg at 998 g/kg active ingredient content

HEXAZINONE WATER DISPERSIBLE GRANULES
[*]**374**/WG/M/-

1 Sampling. Take at least 500 g.

2 Identity tests
2.1 HPLC. As for **374**/TC/M/2.1.
2.2 Infrared. Extract the sample with acetonitrile, filter and evaporate the solvent in a stream of clean dry air. Continue as for **374**/TC/M/2.2.

[*] CIPAC method 1999. Prepared by a committee chaired by P A Bloxham. Based on a method supplied by DuPont de Nemours, USA.

3 Hexazinone. As for hexazinone **374**/TC/M/3.

Repeatability r = 13 g/kg at 749 g/kg active ingredient content
Reproducibility R = 21 g/kg at 749 g/kg active ingredient content

4 Suspensibility (Draft method)

REAGENTS AND APPARATUS As for **374**/TC/M/3 and MT 168.

PROCEDURE

(a) Preparation of suspension and determination of sedimentation. MT 168.

(b) Determination of hexazinone in the bottom 25 ml of suspension. After removal of the top 225 ml of suspension transfer the 25 ml remaining quantitatively to a volumetric flask (100 ml) and dilute to volume with acetonitrile. Place the volumetric flask in an ultrasonic bath for 5 min. Allow to cool to room temperature and take a suitable aliquot of the solution for the determination of hexazinone. Proceed as for **374**/TC/M/3.

(d) Calculation

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

where:
c = mass of active ingredient in sample taken
Q = mass of active ingredient in the 25 ml remaining in the cylinder

HEXAZINONE WATER SOLUBLE POWDERS
[*]**374**/SP/M/-

1 Sampling. Take at least 500 g.

2 Identity tests
2.1 HPLC. As for **374**/TC/M/2.1.
2.2 Infrared. Extract the sample with acetonitrile, filter and evaporate the solvent in a stream of clean dry air. Continue as for **374**/TC/M/2.2.

[*] CIPAC method 1999. Prepared by a committee chaired by P A Bloxham. Based on a method supplied by DuPont de Nemours, USA

3 Hexazinone. As for hexazinone **374**/TC/M/3.

Repeatability r = 27 g/kg at 913 g/kg active ingredient content
Reproducibility R = 36 g/kg at 913 g/kg active ingredient content

HEXAZINONE SOLUBLE CONCENTRATES
*374/SL/M/-

1 Sampling. Take at least 500 ml.

2 Identity tests
2.1 HPLC. As for **374**/TC/M/2.1.
2.2 Infrared. Evaporate sample to dryness in a stream of clean dry air. Extract the residue with acetonitrile, filter and evaporate the solvent in a stream of clean dry air. Continue as for **374**/TC/M/2.2.

3 Hexazinone. As for hexazinone **374**/TC/M/3, apart from the first sentence.

Repeatability r = 8 g/kg at 252 g/kg active ingredient content
Reproducibility R = 17 g/kg at 252 g/kg active ingredient content

HEXAZINONE WATER SOLUBLE GRANULES
*374/SG/M/-

1 Sampling. Take at least 500 g.

2 Identity tests
2.1 HPLC. As for **374**/TC/M/2.1.
2.2 Infrared. Extract the sample with acetonitrile, filter and evaporate the solvent in a stream of clean dry air. Continue as for **374**/TC/M/2.2.

3 Hexazinone. As for hexazinone **374**/TC/M/3.

Repeatability r = 24 g/kg at 744 g/kg active ingredient content
Reproducibility R = 38 g/kg at 744 g/kg active ingredient content

* CIPAC method 1999. Prepared by a committee chaired by P A Bloxham. Based on a method supplied by DuPont de Nemours, USA.

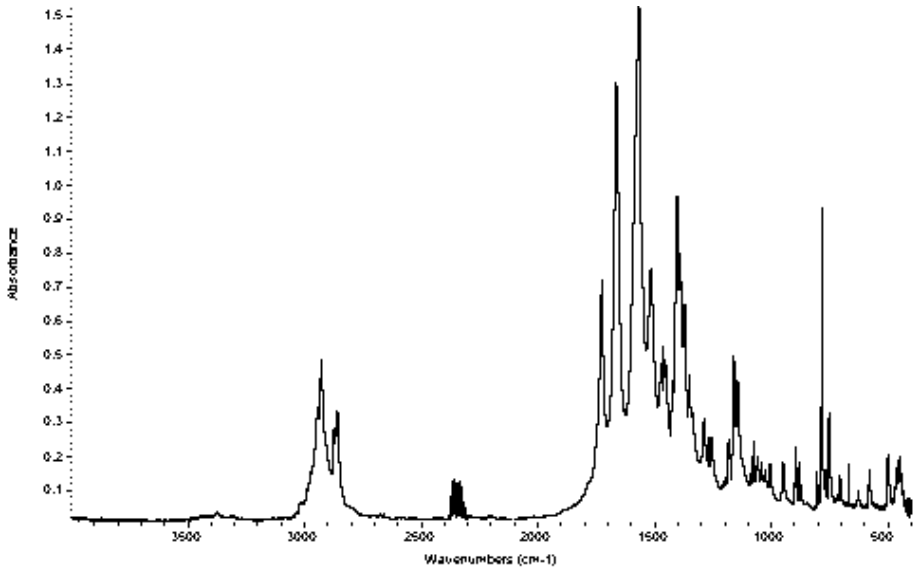


Fig. 20 IR spectrum of hexazinone standard

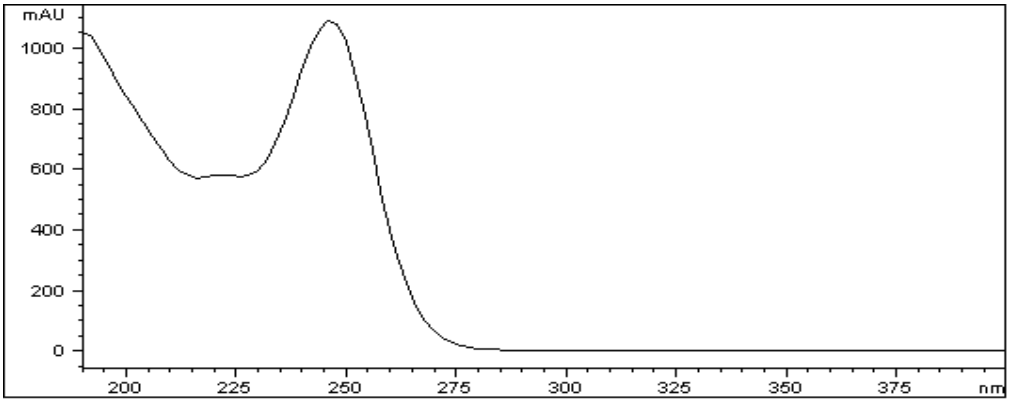


Fig. 21 UV spectrum of hexazinone standard

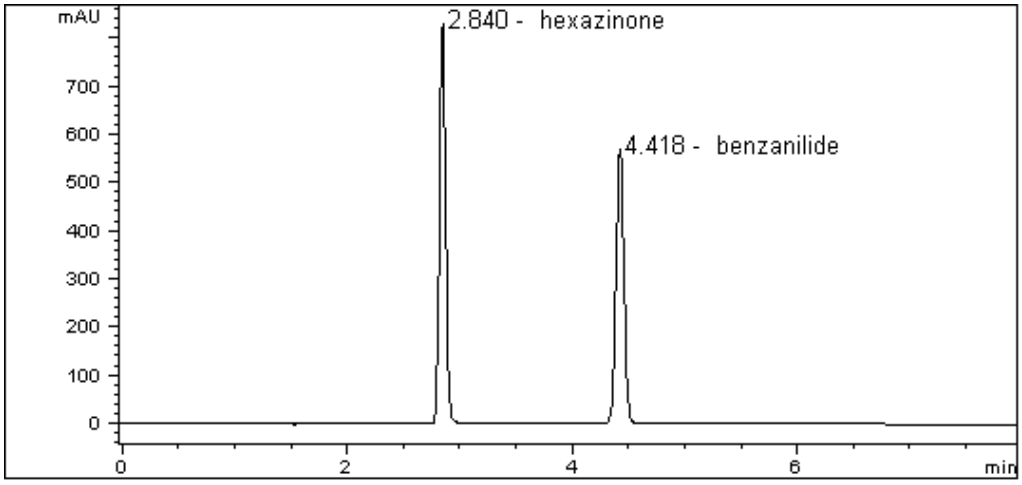


Fig. 22 Chomatogram of hexazinone standard