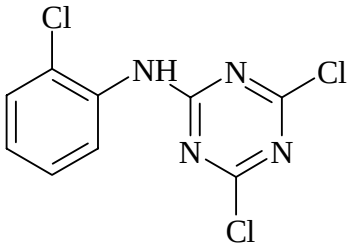
ANILAZINE
294



ISO common name	Anilazine
Chemical name	2-Chloro-N-(4,6-dichloro-1,3,5-triazin-2-yl) aniline (IUPAC); 4,6-dichloro-N-(2-chloro-phenyl)-1,3,5-triazine-2-amine(CA; *101-05-3*)
Empirical formula	$C_9H_5Cl_3N_4$
RMM	275.5
m.p.	159.0 °C
v.p.	8×10^{-7} Pa at 20 °C
Solubility	In water: 8 mg/l at 20 °C; in dichloromethane: 90 g/l; hexane: 2 g/l; 2-propanol: 6 g/l; toluene: 34 g/l, all at 20 °C.
Description	Colourless crystals
Stability	Stable in neutral and slightly acid media, but hydrolysed on heating with alkali.
Formulations	Wettable powders and suspension concentrates

ANILAZINE TECHNICAL
*294/TC/(M)/-

1 Sampling. Take at least 100 g.

2 Identity tests

2.1 Infrared. Prepare potassium bromide discs from the sample and pure anilazine using 1 mg material and 200 mg potassium bromide. Scan the discs from 4000 to 400 cm^{-1}. The spectrum produced from the sample disc should not differ significantly from that from
the standard.

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

3 Anilazine

OUTLINE OF METHOD Anilazine is determined by reversed phase high performance liquid chromatography using octanophenone as internal standard.

REAGENTS

Acetonitrile HPLC quality or distilled in glass
Water HPLC quality or distilled in glass
Mobile phase acetonitrile - water, 80 + 20 (v/v)
Octanophenone internal standard
Internal standard solution. Dissolve octanophenone (4 ml) in acetonitrile (250 ml)
Anilazine standard of known purity
Calibration solution. Weigh (to the nearest 0.1 mg) into a volumetric flask (100 ml) about 200 mg anilazine standard (s mg). Pipette internal standard solution (10.0 ml) into the flask, dilute to volume with acetonitrile, and mix well. Pipette of this solution 5.0 ml into a volumetric flask (100 ml), dilute to volume with acetonitrile, and mix well. Filter a portion of the final solution through a 0.45 µm filter.

APPARATUS

Liquid chromatograph able to generate more than 7 MPa of pressure and to measure the absorbence at 250 nm
Chromatographic column stainless steel, 250 × 4.6 (i.d.) mm packed with C18 bounded silicagel with a particle size of less than 10 µm (DuPont ODS or equivalent) and capable of resolving the bis-compound (6-chloro-*N*,*N'*-bis (2-chlorophenyl)-1,3,5-triazine-2,6-diamine) from anilazine.

* AOAC-CIPAC method 1990.

Chart recorder with at least 250 mm span, 10 mV range and a speed of 30 cm/h
Ultrasonic bath
Filter 0.45 µm porosity (Gelman Acrodisc-CR or equivalent)

PROCEDURE

(a) Operating conditions (typical):

Eluant flow rate	1.7 ml/min at 5.6 MPa
Column temperature	ambient
Injection volume	20 µl
Detector wavelength	250 nm
Detector sensitivity	0.32 absorbence unit full scale
Chart speed	0.5 cm/min
Retention times	Anilazine: about 2.5 min bis-compound: about 4.0 min octanophenone: about 6.6 min

(b) System equilibration. Pump the mobile phase through the column until the system is equilibrated (flat baseline). Allow 1 min after each elution of the internal standard before the next injection. Inject anilazine calibration solution and adjust the operating parameters so that anilazine elutes between 2.5 and 3.0 min. Adjust the injection volume and the attenuation to give the largest possible on-scale peaks. The bis-compound must be resolved from the anilazine and octanophenone peaks. If not, change or repack the column.

(c) Preparation of sample. Weigh (to the nearest 0.1 mg) into a volumetric flask (100 ml) enough sample to contain about 230 mg (w mg) of pure anilazine. Pipette internal standard solution (10.0 ml) into the flask, add acetonitrile (about 80 ml) and sonicate for 1 min. Allow to cool to room temperature, fill to the mark with acetonitrile, and mix well. Pipette of this solution 5.0 ml into a volumetric flask (100 ml), dilute to volume with acetonitrile, and mix well. Filter a portion of the final solution through a 0.45 µm filter and hold it for the chromtographic analysis.

(d) Determination. Using the same injection volume for all standards and calibration injections, make repetitive injections of calibration solution and calculate the response ratios by dividing the peak height of anilazine by that of the internal standard. The response ratios must agree within ± 1%. Average the duplicate response ratios obtained with the calibration solutions. Inject duplicate aliquots of each sample solution. Average the duplicate ratios for each sample solution. The response ratios must agree within µ 1%. If not, repeat the determination, starting with injections of the calibration solution.

Reinject the calibration solution twice. Average the response ratios of the calibration solutions immediately preceding and following the injections of the sample solution. They must agree within ± 1%. If not, repeat the determination.

(e) Calculation

$$\text{Anilazine content} = \frac{R \times s \times P}{R' \times w} \text{ g/kg}$$

where:

R	=	average peak height ratio of anilazine to internal standard for the sample solution
R'	=	average peak height ratio of anilazine to internal standard for the calibration solution
s	=	mass of anilazine in the calibration solution (mg)
w	=	mass of anilazine in the sample solution (mg)
P	=	purity of the standard anilazine (g/kg)

ANILAZINE TECHNICAL CONCENTRATES
*294/TK/(M)/-

1 Sampling. Take at least 500 g.

2 Identity tests

2.1 Infrared. Treat approximately 100 mg of the sample in a 10 ml vial with 5 ml dichloromethane and mix thoroughly. Draw up part of the suspension into a 2 ml disposable syringe and expel through a 0.45 µm disposable membrane filter (Millex 0.45 µm filter unit, Millipore Cat. no SLHV 025 NB, or equivalent), into a clean 10 ml sample vial. Repeat the procedure with a second 2 ml portion of the suspension and expel into the same 10 ml sample vial. Evaporate the clear solution to dryness under a stream of dry nitrogen, preferably on a water bath. Use the solid residue to prepare a potassium bromide disc as described under anilazine technical 294/TC/(M)/2.1.

2.2 HPLC. As for anilazine technical **294**/TC/(M)/2.2.

* AOAC-CIPAC method 1990.

3 Anilazine. As for anilazine technical **294**/TC/(M)/3.

Repeatability r = 10.4 g/kg at 785 g/kg active ingredient content

Reproducibility R = 37.0 g/kg at 785 g/kg active ingredient content

ANILAZINE WETTABLE POWDERS
*294/WP/(M)/-

1 Sampling. Take at least 500 g.

2 Identity tests. As for anilazine technical concentrates **294**/TK/(M)/2.

3 Anilazine. As for anilazine technical **294**/TC/(M)/3.

Repeatability r = 25.5 g/kg at 706 g/kg active ingredient content and 20.4 g/kg at 493 g/kg active ingredient content

Reproducibility R = 24.1 g/kg at 706 g/kg active ingredient content and 6.2 g/kg at 492 g/kg active ingredient content

4 Suspensibility (Draft method)
(a) Preparation of suspension MT 15.1 (*i*)
(b) Determination of sedimentation MT 15.1 (*ii*)
(c) Determination of anilazine in the bottom 25 ml of suspension. After removal of the top 225 ml suspension, quantitatively transfer the 25 ml suspension remaining in the cylinder to a centrifuge tube rinsing the cylinder with 10 ml portions of water to remove any residue remaining in the bottom 25 ml of the cylinder. Add the rinsings to the centrifuge tube and centrifuge to give a clear aqueous layer. Decant off this layer. Take up the residue in acetonitrile and transfer to a volumetric flask. Choose the volumetric flask and/or the dilutions with acetonitrile to obtain a final concentration of about 0.1 mg/ml anilazine and before filling the volumetric flask to the mark add the required volume of internal standard solution to give a final concentration of 0.5 ml per 100 ml final acetonitrile solution. Use this final solution for the chromatographic analysis according to the method as described under anilazine technical **294**/TC/(M)/3.

* AOAC-CIPAC method 1990.

(d) Calculation of suspensibility

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

where:

c = mass of anilazine in the sample taken for the preparation of the suspension (g)

Q = mass of anilazine in the bottom 25 ml of suspension (g)

ANILAZINE SUSPENSION CONCENTRATES
*294/SC/(M)/-

1 Sampling. Take at least 1 kg.

2 Identity tests.

2.1 Infrared. Transfer approximately 1 ml of the sample to a volumetric flask (50 ml), make up to the mark with water and homogenize. Pipette 5 ml of the dilute suspension into a separating funnel (25 ml), add dichloromethane (5 ml) and extract for 1 min. After the phases have separated run off the lower (organic) phase into a test tube (10 ml) containing approximately 1 g anhydrous sodium sulphate. Shake well and after standing, decant off the clear solution from the drying agent into a 5 ml sample vial. Evaporate this solution to dryness under a stream of dry nitrogen, preferably on a water bath. Use the solid residue to prepare a potassium bromide disc as described under anilazine technical **294**/TC/(M)/2.1.

2.2 HPLC. As for anilazine technical **294**/TC/(M)/2.2.

3 Anilazine. As for anilazine technical **294**/TC/(M)/3 except:

(*c) Preparation of sample*. Weigh (to the nearest 0.1 mg) into a volumetric flask (100 ml) enough sample to contain about 230 (*w* mg) of pure anilazine. Add water (5 ml) and swirl until the sample is thouroughly dispersed. Pipette internal standard solution (10.0 ml) into the flask, add acetonitrile (about 75 ml) and sonicate for 1 min. Allow to cool to room temperature, fill to the mark with acetonitrile, and mix well. Pipette of this solution 5.0 ml into a volumetric flask (100 ml), dilute to volume with acetonitrile, and mix well. Filter a portion of the final solution through a 0.45 µm filter and hold it for the chromatographic analysis.

* AOAC-CIPAC method 1990.

Repeatability r = 3.1 g/kg at 383 g/kg active ingredient content

Reproducibility R = 20.2 g/kg at 383 g/kg active ingredient content

4 Suspensibility

(a) Preparation of suspension and determination of sedimentation MT 161

(b) Determination of anilazine in the bottom 25 ml of suspension. As for anilazine wettable powders **294**/WP/(M)/4 (c).