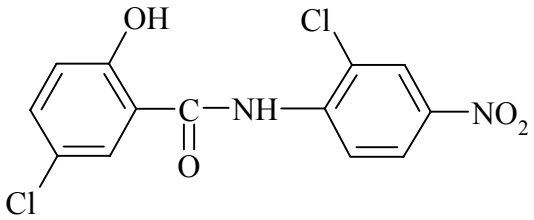
NICLOSAMIDE
599



ISO common name	Niclosamide
Chemical name	5-Chloro-2-hydroxy-*N*-(2-chloro-4-nitrophenyl)-benzamide (IUPAC); (CA; *1420-04-8*)
Empirical formula	$C_{13}H_8Cl_2N_2O_4$
RMM	327.1
m.p.	230 °C
v.p.	Less than 1×10^{-3} Pa at 20 °C
Solubility	In water: 5 - 8 mg/l at 20 °C; soluble in common organic solvents, 2-hydroxymethylammmonium salt: 230 mg/l in water
Description	Yellow powder
Stability	Stable under normal conditions
Formulations	Wettable powders and emulsifiable concentrates

NICLOSAMIDE TECHNICAL
*599/TC/M/-

1 Sampling. Take at least 100 g.

2 Identity tests

2.1 HPLC. Use the HPLC method below. The retention time of niclosamide peak of the sample solution should not deviate by more than 2 % from that of the calibration solution. The UV spectrum of this peak should match that of the one in the calibration solution.

2.2 Infrared. Grind approximately 2 mg of the sample with 300 mg potassium bromide. Prepare a clear disk and determine the infrared spectrum in the range from 4000 to 600 cm^{-1}. The spectrum obtained from the sample should not differ significantly from that of the standard (Fig. 23).

3 Niclosamide

SCOPE The method is intended for determination of niclosamide in the technical material as well as in niclosamide piperazine and ethanolamine salts.

OUTLINE OF METHOD Niclosamide is determined by reversed phase high performance liquid chromatography using UV detection at 236 nm and external standardisation.

REAGENTS

Water HPLC grade
Methanol HPLC grade
Niclosamide standard of known purity
Potassium dihydrogen phosphate
Ortho phosphoric acid 85 %
Mobile phase methanol - water - phosphoric acid, 700 + 300 + 1(v/v) with 1 g of potassium dihydrogen phosphate
Calibration solution. Weigh (to the nearest 0.1 mg) into a volumetric flask (100 ml) about 50 mg niclosamide standard (*s* mg). Add methanol (about 80 ml) and place the flask in an ultrasonic bath for 10 min. Allow to cool to room temperature and fill to the mark with methanol (solution C).

* CIPAC method 2000. Prepared by the German Committee (DAPA). Chairman W Dobrat. Based on a method supplied by Bayer AG, Germany.

APPARATUS

High performance liquid chromatograph equipped with a UV spectrometric detector capable of measuring at 236 nm and a 5 µl injection system
Column stainless steel, 150 × 3.9 mm (i.d.), packed with Waters Symmetry C_8, 5 µm, Inertsil RP_8, Nucleosil 100 C_{18}, 3 or 5 µm, or equivalent material of the same selectivity
Electronic integrator
Ultrasonic bath
Centrifuge

PROCEDURE

(a) Chromatographic conditions (typical):

Mobile phase	methanol - water - phosphoric acid, 700 + 300 + 1 (v/v) with 1 g of potassium dihydrogen phosphate
Column temperature	ambient or 40 °C
Flow rate	1.0 ml/min
Detector wavelength	236 nm
Injection volume	5 µl
Run time	approximately 20 min
Retention time	niclosamide: approximately 12 min

(b) Preparation of sample. Homogenise the sample. Weigh (to the nearest 0.1 mg) into a volumetric flask (100 ml) sufficient sample to contain about 50 mg niclosamide (*w* mg). Add methanol (about 80 ml) and place the flask in an ultrasonic bath for 10 min. Allow to cool to room temperature and fill to the mark with methanol (solution S).

(c) System equilibration. Pump sufficient eluent through the column to equilibrate the system. Inject 5 µl portions of the calibration solution and repeat the injections until retention times and peak areas vary by less than 0.5 % of the mean of three successive injections.

(d) Determination. Inject 5 µl portions of the calibration solution and sample solution in the following sequence: C_1, S_1, S_2, C_2…etc.
Determine the peak area of the niclosamide peaks and calculate the mean response factor *(f)* of the calibration solution injections bracketing the injections of the sample solutions.

(e) Calculation

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

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

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

Repeatability r = 19 g/kg at 980 g/kg active ingredient content
Reproducibility R = 28 g/kg at 980 g/kg active ingredient content

NICLOSAMIDE AMINE SALTS TECHNICAL

[*]**599.110**/TC/M/-
[*]**599.115**/TC/M/-

1 Sampling. Take at least 100 g.

2 Identity tests
2.1 HPLC. As for niclosamide technical **599**/TC/M/2.1.
2.2 Infrared. As for niclosamide technical **599**/TC/M/2.2.

[*] CIPAC method 2000. Prepared by the German Committee (DAPA). Chairman W Dobrat. Based on a method supplied by Bayer AG, Germany.

3 Niclosamide. As for niclosamide technical **599**/TC/M/3.

Repeatability r (piperazine salt)	=	38 g/kg at 862 g/kg active ingredient content
Reproducibility R (piperazine salt)	=	38 g/kg at 862 g/kg active ingredient content
Repeatability r (ethanol amine salt)	=	26 g/kg at 836 g/kg active ingredient content
Reproducibility R (ethanol amine salt)	=	32 g/kg at 836 g/kg active ingredient content

NICLOSAMIDE WETTABLE POWDERS
*599/WP/M/-

1 Sampling. Take at least 500 g.

2 Identity tests
2.1 HPLC. As for niclosamide technical **599**/TC/M/2.1.
2.2 Infrared. As for niclosamide technical **599**/TC/M/2.2.

3 Niclosamide. As for niclosamide technical **599**/TC/M/3, except:

(b) Preparation of sample. Weigh (to the nearest 0.1 mg) into a volumetric flask (100 ml) sufficient sample to contain about 80 mg niclosamide (*w* mg). Add solvent mixture (about 80 ml) and place the flask in an ultrasonic bath for 10 min. Shake the flask several times, allow to cool to room temperature and fill to the mark with solvent mixture (solution S). If necessary, centrifuge or filter the solution to obtain a clear solution.

Repeatability r	= 13 g/kg at 693 g/kg active ingredient content
Reproducibility R	= 40 g/kg at 693 g/kg active ingredient content

* CIPAC method 2000. Prepared by the German Committee (DAPA). Chairman W Dobrat. Based on a method supplied by Bayer AG, Germany.

4 Suspensibility (Draft method)

REAGENTS AND APPARATUS As for **599**/TC/M/3 and MT 15.

PROCEDURE

(a) Preparation of suspension. MT 15.1(*i*).

(b) Determination of sedimentation. MT 15.1(*ii*).

(c) Determination of niclosamide in the bottom 25 ml of suspension. After removal of the top 225 ml of suspension, transfer the rest into a 200 ml volumetric flask. Rinse the measuring cylinder in which the suspension was prepared three times with 20 ml tetrahydrofuran and transfer the rinsings to the graduated flask. Fill the up to the graduation with methanol.

Determine the mass of niclosamide (Q g) by **599**/TC/M/3 except:

Calibration solution. Weigh (to the nearest 0.1 mg) into a volumetric flask (200 ml) about 120 mg niclosamide standard (s mg). Add 60 ml tetrahydrofuran and 115 ml methanol and place the flask in an ultrasonic bath for 10 min. Allow to cool to room temperature and fill to the mark with water.

(d) Calculation

$$\mathrm{Q} = \frac{H_w \times f}{1000000}\ \mathrm{g}$$

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

where:

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

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

NICLOSAMIDE EMULSIFIABLE CONCENTRATES
*599/EC/M/-

1 Sampling. Take at least 500 ml.

2 Identity tests

2.1 HPLC. As for niclosamide technical **599**/TC/M/2.1.

2.2 Infrared. Add approximately 10 ml acetone to approximately 1 g of the sample, then add 5 ml diluted hydrochloric acid. The active ingredient forms a voluminous precipitate. Suck off the solvent with a G4 glass filter frit and wash thoroughly with diluted hydrochloric acid. Dry the residue on a clay plate. Mix approximately 2 mg of the residue with 300 mg of KBr and determine the infrared spectrum in the range from 4000 to 600 cm-1. The spectrum must comply with niclosamide, modification 1, beta form (Fig. 24).

3 Niclosamide. As for niclosamide wettable powders **599**/WP/M/3,

Repeatability r = 7.9 g/kg at 253 g/kg active ingredient content
Reproducibility R = 10 g/kg at 253 g/kg active ingredient content

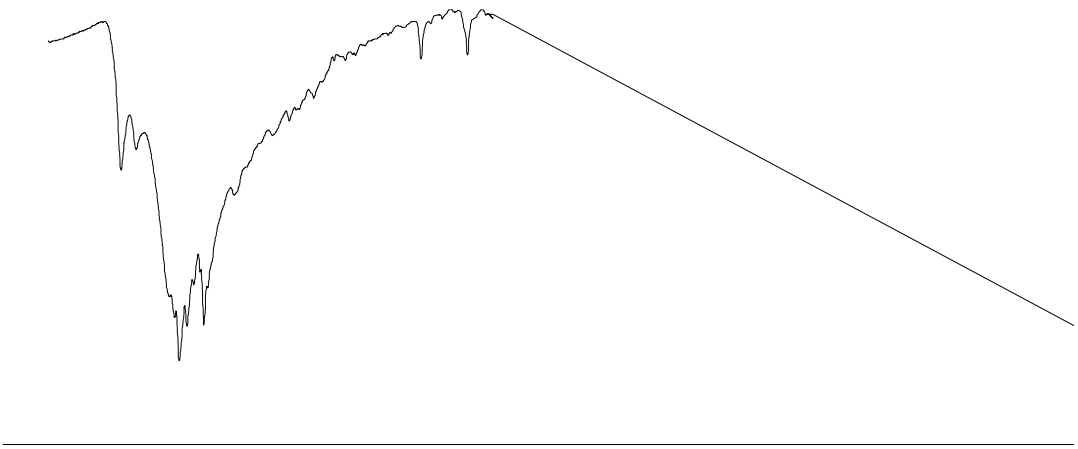


Fig. 23 IR spectrum of niclosamide

* CIPAC method 2000. Prepared by the German Committee (DAPA). Chairman W Dobrat. Based on a method supplied by Bayer AG, Germany.

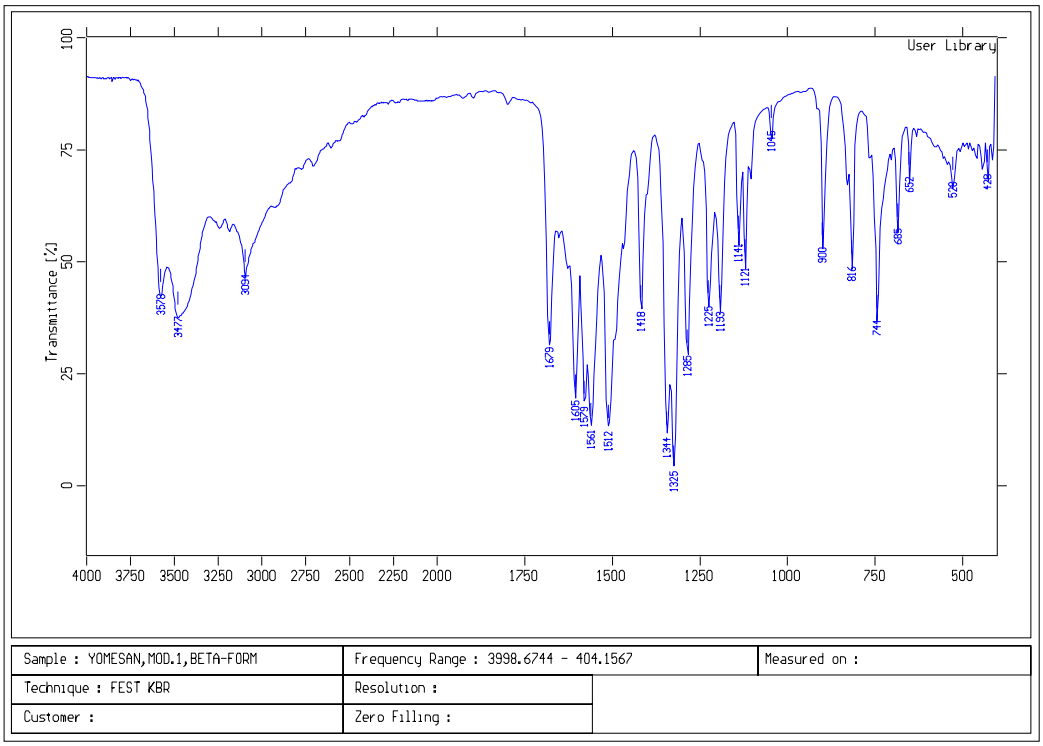


Fig. 24 IR spectrum of niclosamide (modification 1, beta form)

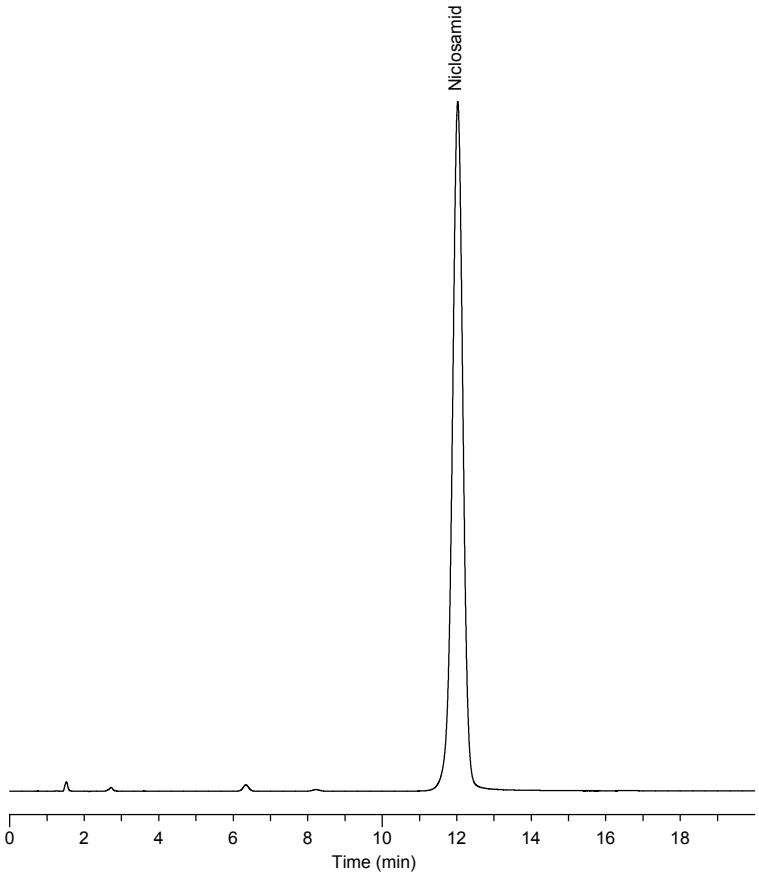


Fig. 25 Chromatogram of niclosamide