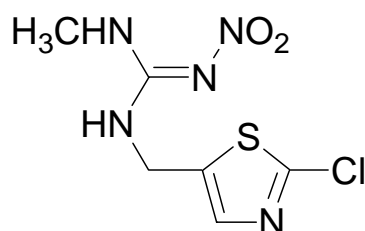


CLOTHIANIDIN

738



<i>ISO common name</i>	Clothianidin
<i>Chemical name</i>	(<i>E</i>)-1-(2-chloro-1,3-thiazol-5-ylmethyl)- 3-methyl-2-nitroguanidine (IUPAC); [<i>C(E)</i>]- <i>N</i> -[(2-chloro-5-thiazolyl)methyl]- <i>N'</i> -methyl- <i>N''</i> -nitroguanidine (CA)
<i>CAS No.</i>	210880-92-5
<i>Empirical formula</i>	C ₆ H ₈ ClN ₅ O ₂ S
<i>RMM</i>	249.7
<i>v.p.</i>	1.3 x 10 ⁻¹⁰ Pa (25°C)
<i>Solubility</i>	In water: 0.327 g/l; acetone: 15.2 g/l; methanol: 6.26 g/l, ethyl acetate: 2.03 g/l; xylene: 0.0128 g/l; n-heptane: <0.00104 g/l (water: at 20°C, other solvents: at 25°C)
<i>Description</i>	Off-white to pale yellow crystalline powder

CLOTHIANIDIN
738/TC/m/-

1 Sampling. Take at least 100 g.

2 Identity tests

2.1 HPLC. Use the HPLC method below. The retention time of clothianidin for the sample solution should not deviate by more than 0.2 min from that of the calibration solution.

2.2 Infrared. Prepare potassium bromide discs from the sample and pure clothianidin. Scan the discs from 4000 to 400 cm^{-1} . The spectrum produced from the sample should not differ significantly from that of the standard.

3 Clothianidin

OUTLINE OF METHOD Clothianidin is determined by reversed phase high-performance liquid chromatography using UV detection at 269 nm and external standardisation.

REAGENTS

Acetonitrile HPLC grade

Water HPLC grade

Phosphoric acid 85%

Clothianidin working standard technical product of certified purity. Store refrigerated.

Mobile phase acetonitrile - water - phosphoric acid, 200 + 800 + 1 (v/v/v)

Calibration solution. Prepare calibration solutions in duplicate. Weigh (to the nearest 0.1 mg) into volumetric flasks (100 ml) approximately 100 mg (*s* mg) of clothianidin working standard. Add acetonitrile (about 80 ml) and place the flasks in an ultrasonic bath for 10 min. Allow to cool to room temperature, dilute to volume with acetonitrile. Mix thoroughly. Transfer by pipette 10.0 ml of this solution into a volumetric flask (50 ml) and dilute to volume with acetonitrile. Mix thoroughly (Solutions C_A and C_B).

APPARATUS

High performance liquid chromatograph equipped with a detector suitable for operation at 269 nm, constant-temperature column compartment and an injector capable of delivering 10 μl .

Column 150 x 4.6 (i.d.) mm, stainless steel, packed with ZORBAX Eclipse XDB-C18 (5 μm), or equivalent.

Electric integrator or data system

Ultrasonic bath

PROCEDURE

(a) *Liquid chromatographic conditions* (typical):

<i>Column</i>	stainless steel, 150 x 4.6 (i.d.) mm, packed with ZORBAX Eclipse XDB-C18 (5 µm), or equivalent.
<i>Mobile phase</i>	acetonitrile - water - phosphoric acid, 200 + 800 + 1 (v/v/v)
<i>Temperature</i>	40°C
<i>Flow rate</i>	1.0 ml/min
<i>Detector wavelength</i>	269 nm
<i>Injection volume</i>	10 µl
<i>Retention times</i>	clothianidin: about 8 min

(b) *Linearity check.* Check the linearity of the detector response by injecting 10 µl of solutions with clothianidin concentrations 0.5, 1 and 2 times that of the calibration solution before conducting analysis.

(c) *System equilibration.* Prepare two calibration solutions. Inject 10 µl portions of the first one until the response factors obtained for two consecutive injections differ by less than 1.0%. Then inject a 10 µl portion of the second solution. The response factor for this solution should not deviate by more than 1.0% from that for the first calibration solution, otherwise prepare new calibration solutions.

(d) *Preparation of sample solution.* Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) into a volumetric flask (100 ml) sufficient sample to contain about 100 mg (*w* mg) of clothianidin. Add acetonitrile (about 80 ml) and place the flasks in an ultrasonic bath for 10 min. Allow to cool to room temperature, dilute to volume with acetonitrile. Mix thoroughly. Transfer by pipette 10.0 ml of this solution into a volumetric flask (50 ml) and dilute to volume with acetonitrile. Mix thoroughly (Solutions S_A and S_B).

(e) *Determination.* Inject in duplicate 10 µl portions of each sample solution bracketing them by injections of the calibration solutions as follows; calibration solution C_A, sample solution S_A, sample solution S_A, calibration solution C_B, sample solution S_B, sample solution S_B, calibration solution C_A, and so on. Measure the relevant peak areas.

(f) *Calculation.* Calculate the mean value of each pair of response factors bracketing the two injections of a sample and use this value for calculating the clothianidin contents of the bracketed sample injections.

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

$$\text{Clothianidin content} = \frac{f \times H_w}{w} \text{ (g/kg)}$$

where:

f_i = individual response factor

f = mean response factor

H_s = peak area of clothianidin in the calibration solution

H_w = peak area of clothianidin in the sample solution

s = mass of clothianidin working standard in the calibration solution
(mg)

w = mass of sample taken (mg)

P = purity of clothianidin working standard (g/kg)

Repeatability r = 17 g/kg at 990 g/kg active ingredient content
= 17 g/kg at 986 g/kg active ingredient content

Reproducibility R = 16 g/kg at 990 g/kg active ingredient content
= 17 g/kg at 986 g/kg active ingredient content

CLOTHIANIDIN WATER DISPERSIBLE GRANULE 738/WG/m/-

1 Sampling. Take at least 100 g.

2 Identity tests

2.1 HPLC. As for 738/TC/m/2.1

2.2 Infrared. Extract the sample with a suitable solvent, filter and evaporate the solvent with a stream of clean dry air. Proceed as for 738/TC/m/2.2

3 CLOTHIANIDIN. As for 738/TC/m/3 except:
change ' APPARATUS' as follows:

Column 150 x 4.6 (i.d.) mm, stainless steel, packed with ZORBAX Eclipse XDB-C18 (5 μ m), or equivalent. Confirm the peaks of formulants do not interfere the determination.

add 'PROCEDURE (d) Preparation of sample solution.' as follows:
Filter a portion of each sample solution through a 0.45 μ m filter prior to analysis (Solutions S_A and S_B).

- Repeatability r** = 21 g/kg at 504 g/kg active ingredient content
= 12 g/kg at 502 g/kg active ingredient content
= 11 g/kg at 503 g/kg active ingredient content
- Reproducibility R** = 24 g/kg at 504 g/kg active ingredient content
= 14 g/kg at 502 g/kg active ingredient content
= 12 g/kg at 503 g/kg active ingredient content

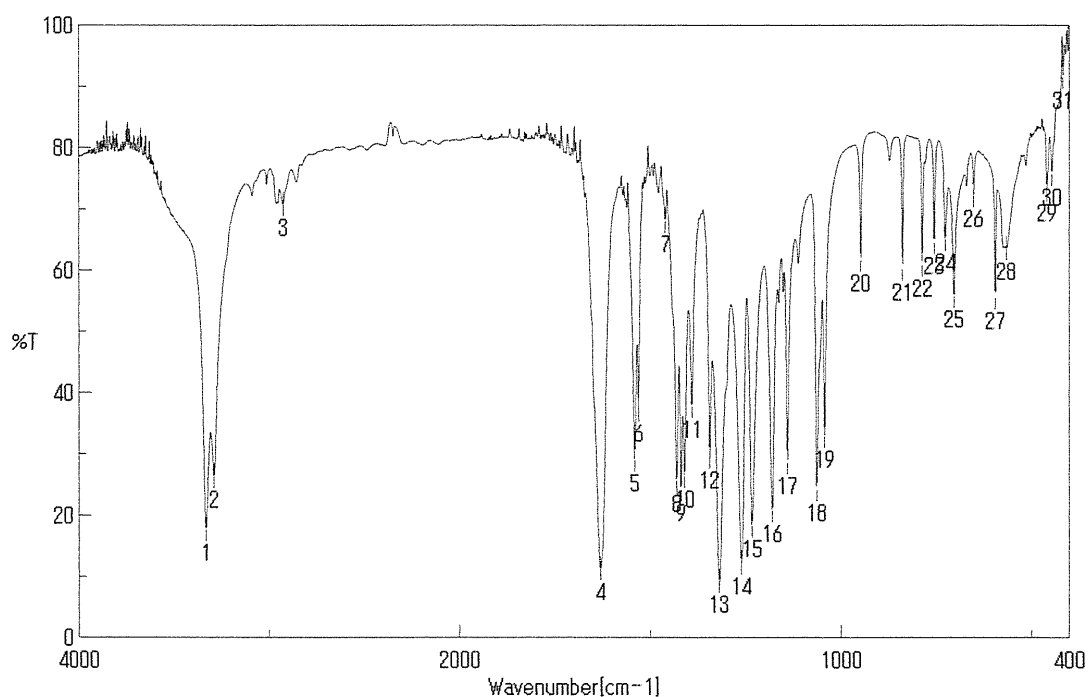


Fig.1 Infrared Spectrum of Clothianidin

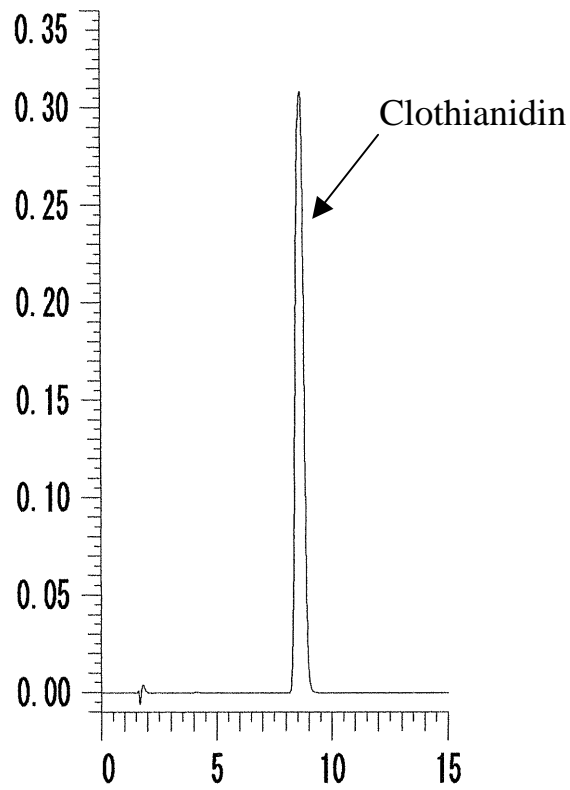


Fig.2 Example of Liquid Chromatogram of Clothianidin TC

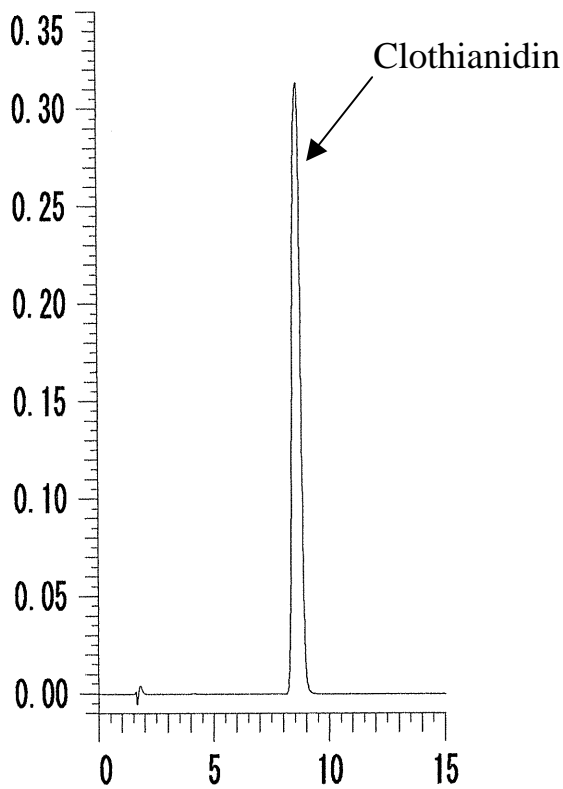


Fig.3 Example of Liquid Chromatogram of Clothianidin WG