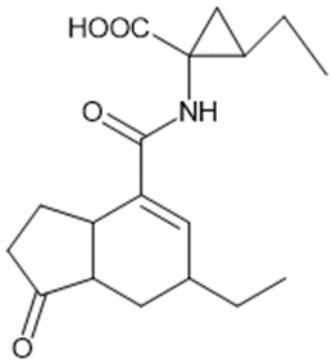
CORONATINE

XXX



ISO Common Name	Coronatine
Chemical Name	2-Ethyl-1-{[(6-ethyl-1-oxo-2,3,3a,6,7,7a-hexahydro-1H-inden-4-yl)(hydroxy)methylidene] amino} cyclopropane-1-carboxylic acid
CAS Number	73366-39-9
Empirical formula	$C_{18}H_{25}NO_4$
Molecular mass	319.4
m.p.	163.2 ºC~165.9 ºC
b.p.	209.5 °C at 0.1 Pa
v.p	2.45×10^{-7} Pa at 20 ºC 5.84×10^{-7} Pa at 25 ºC
d^{20}	1.56
Solubility	In water 1.177 g/l; in methanol >250 g/l; acetone 160-200 g/l; ethyl acetate 50-57g/l； n-hexane <48.74 mg/l; toluene 294.87 mg/l; 1,2-dichloroethane 50-57g/l (all at 20ºC)
Stability	Stable in acidic and neutral conditions, stable at room temperature

Description	White powder
Formulations	Soluble concentrate

CORONATINE TECHNICAL

*XXX/TC/(M)/-

1 Sampling. Take at least 100 g.

2 Identity tests

2.1 HPLC. Use the HPLC method below. The relative retention time of the coronatine peak in the sample solution should not deviate by more than 1.5% from that of the calibration solution.

2.2 Infrared. Prepare potassium bromide discs for the technical sample and coronatine reference substance. Scan the discs from 4000 to 400 cm^{-1}. The spectrum from the sample should not differ significantly from that of the reference substance.

3 Coronatine

OUTLINE OF METHOD

Coronatine is determined by reversed phase high performance liquid chromatography using UV detection at 220 nm and external standardization.

REAGENTS

Coronatine: reference standard of known purity

Methanol: HPLC grade

Acetonitrile: HPLC grade

Water: Ultrapure water

Phosphoric acid: analytical grade, minimum 85%

0.1% Phosphoric acid aqueous solution: Dissolve 1 ml phosphoric acid into 1000 ml water.

Calibration solution. Weigh in duplicate (to the nearest 0.1 mg) 20 mg of coronatine reference standard (*s* mg) into separate volumetric flasks (50 ml). Dissolve with 30 ml mobile phase and place the flask in an ultrasonic bath for 3 minutes. Allow to cool to ambient temperature and fill to the mark with mobile

phase. Mix thoroughly. Filter the solution through a 0.45 µm filter before injection (calibration solutions C_A and C_B).

APPARATUS

High performance liquid chromatograph equipped with a UV detector capable for operation at 220 nm, a constant-temperature column compartment and an injection system capable of injecting 5µl.

Column stainless steel 250 ×4.6 mm (i.d), packed with C_{18} 5.0 µm, or equivalent with the same selectivity.

Filtering apparatus disposable plastic syringes (or equivalent) fitted with 0.45 µm filters.

Electronic integrator or data system

Ultrasonic bath

PROCEDURE

(a) Liquid chromatographic conditions (typical):

Column	stainless steel, 250 ×4.6 mm (i.d), packed with Agilent C_{18} 5.0 µm, or equivalent
Mobile phase	acetonitrile: 0.1% phosphoric acid aqueous solution, 30:70 (v/v)
Column temperature	35°C
Flow rate	1.0 ml/min
Detector wavelength	220 nm
Injection volume	5 µl
Retention time	approximately 21.5 min
Run time	28 min

(b) System equilibration. Inject 5 µl portions of calibration solution C_A until the response factors (*fi*) obtained for two consecutive injections differ by less than

1.5%. Then inject 5 µl portions of calibration solution C_B. The response factor (*fi*), for two consecutive injections should not deviate by more than 1.5% from that of solution C_A, otherwise prepare new calibration solutions.

(c) Sample preparation. Prepare solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about 20 mg of coronatine into a volumetric flask (50 ml). Dissolve with 30 ml mobile phase and place the flask in an ultrasonic bath for 3 minutes. Allow to cool to ambient temperature and fill to the mark with mobile phase. Mix thoroughly. Filter the solution through a 0.45 µm filter before injection (sample solutions S_1 and S_2).

(d) Determination. Inject in duplicate 5 µl portions of each sample solution bracketing them by injections of the calibration solutions as follows:

C_A, S_1, S_1, C_B, S_2, S_2, C_A, ...

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

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

$$\text{Content of Coronatine} = \frac{H_W \times f}{W} \text{ (g/kg)}$$

where:

fi = individual response factor
f = mean response factor
H_s = peak area of coronatine in the calibration solution
H_w = peak area of coronatine in the sample solution
s = mass of coronatine reference standard in the calibration solution (mg)
w = mass of sample taken (mg)
P = purity of the coronatine reference standard (g/kg)

Repeatability r = g/kg at an active ingredient content of g/kg
Reproducibility R = g/kg at an active ingredient content of g/kg

CORONATINE SOLUBLE CONCENTRATE

*XXX/SL/(M)/-

1 Sampling. Take at least 200 ml.

2 Identity tests.

2.1 HPLC. As for coronatine technical **XXX**/TC/(M)/2.1

2.2 Infrared. As for coronatine technical **XXX** /TC/(M)/2.2

3 Coronatine. As for coronatine technical **XXX** /TC/(M)/3 except:

REAGENTS

Calibration solution. Weigh in duplicate (to the nearest 0.1 mg) 20 mg of coronatine reference standard (*s* mg) into separate volumetric flasks (25 ml). Dissolve with 5 ml methanol and place the flask in an ultrasonic bath until the sample has been dissolved completely. Allow to cool to ambient temperature and fill to the mark with methanol and mix thoroughly as stock solution. Then transfer by pipette 2.0 ml of each stock solution into separate 25 ml volumetric flask, dilute to volume with mobile phase and mix thoroughly. Filter the solution through a 0.45 µm filter before injection (calibration solutions C_A and C_B).

PROCEDURE

(a) Liquid chromatographic conditions (typical):

Injection volume 20 µl

(c) Sample preparation. Prepare solutions in duplicate for each sample. Add to the mark of each 25 ml volumetric flask with coronatine sample, accurately weigh the sample mass (to the nearest 0.1 mg). Filter the solution through a 0.45 µm filter before injection (solutions S_1 and S_2).

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

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

a

$$Content\ of\ Coronatine\ = \frac{H_w \times f}{W \times 12.5}\ (g/kg)$$

where:

f_i = individual response factor
f = mean response factor
H_s = peak area of coronatine in the calibration solution
H_w = peak area of coronatine in the sample solution
s = mass of coronatine reference standard in the calibration solution (mg)
W = mass of sample taken (mg)
P = purity of coronatine reference standard (g/kg)
12.5 = dilution ratio of the standard relative to the sample

Repeatability r = g/kg at an active ingredient content of g/kg
Reproducibility R = g/kg at an active ingredient content of g/kg

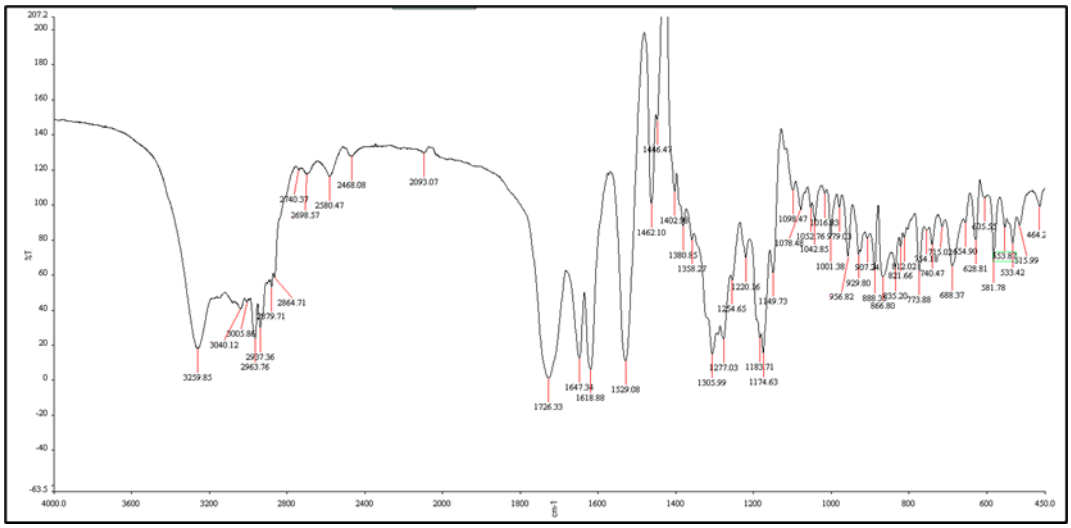


Fig. 1 FTIR spectrum of coronatine standard

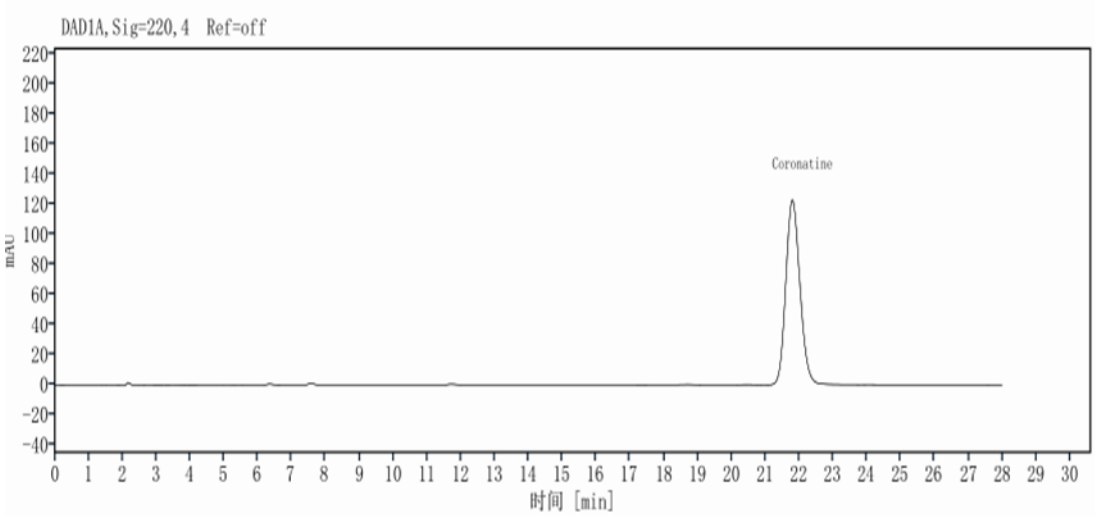


Fig. 2 HPLC Chromatogram of coronatine standard

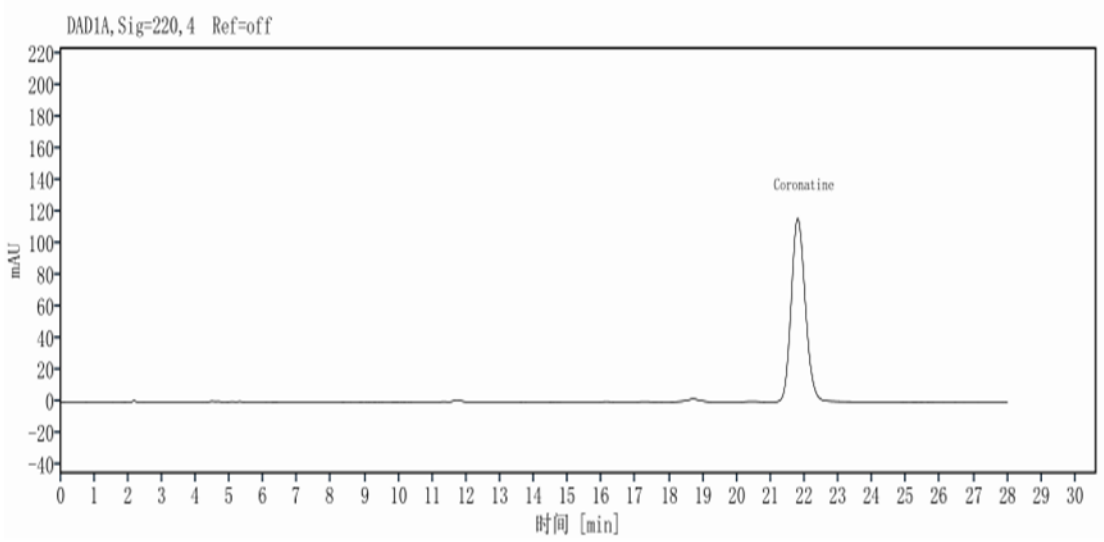


Fig. 3 HPLC Chromatogram of coronatine TC

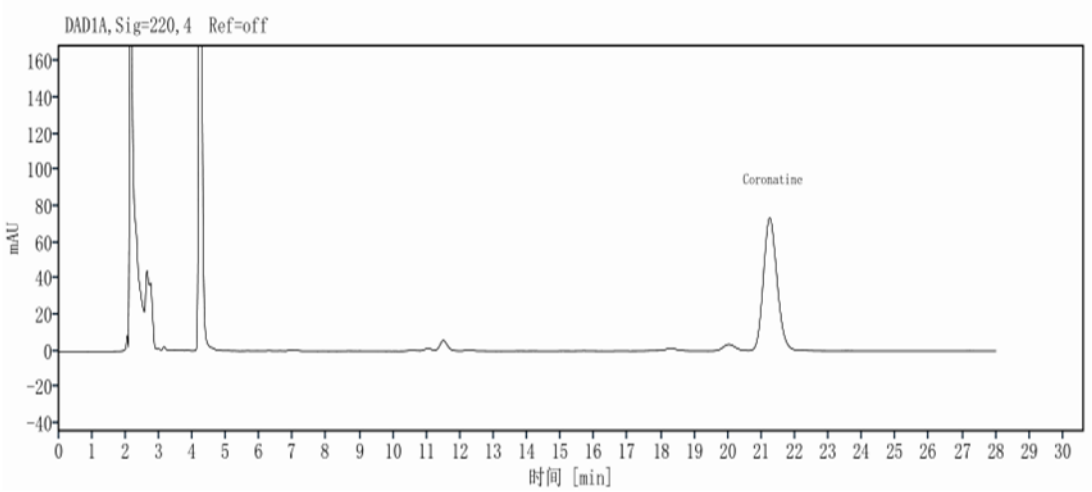


Fig. 4 HPLC Chromatogram of coronatine SL