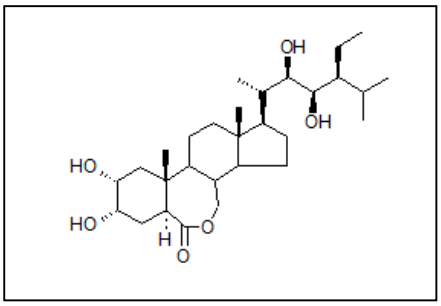
28-HOMOBRASSINOLIDE



Common name	28-Homobrassinolide
Chemical name	(5S,6R)-10-((2S,3R,4R,5S)-5-ethyl-3,4-dihydroxy-6-methylheptan-2-yl)-5,6-dihydroxy-7a,9a-dimethyltetradecahydro-1H-benzo[c]indeno[5,4-e]oxepin-3(12bH)-one
Empirical formula	$C_{29}H_{50}O_6$
RMM	494.8
m.p.	256~257°C
v.p.	1.01E-18mmHg at 25°C
Solubility	In water 5mg/l, acetonitrile 1.3g/l, ethanol 5.2g/l, methanol 2.7g/l at 20 °C
Description	White powder
Stability	Stable in neutral and weak alkaline condition but hydrolysed in acidic conditions
Formulation	Emulsifiable concentrate, soluble liquid

28-HOMOBRASSINOLIDE 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 28-Homobrassinolidein the sample solution should not deviate by more than 1.5% from that of calibration solution.

2.2 **Infrared.** Prepare potassium bromide discs for the 28-Homobrassinolide technical sample and reference substance. Scan the discs from 4000-400 cm-1. The spectrum produced from the sample should not differ significantly from that of the standard.

3. 28-Homobrassinolide

OUTLINE OF METHOD The derivative of 28-Homobrassinolide by Phenylboronic acid separated and determined by HPLC on ODS-C_{18} film stainless column with UV detector at 220 nm, quantified by external standard method.

REAGENTS
Methanol: HPLC grade
Acetonitrile: HPLC grade
Water: HPLC grade
28-Homobrassinolide reference standard of known purity: w≥ 97.0%
Phenylboronic acid:
Preparation of Phenylboronic acid solution: Weigh approximately (to the nearest 0.01g) 1500mg Phenylboronic acid into 250ml volumetric flask. Dissolve to the mark with methanol and mix thoroughly.

APPARATUS
High-performance liquid chromatography equipped with UV detector and quantitative sampling valve.
Chromatographic work station
Column stainless steel: 250mm X 4.6 mm (id), packed with ODS-C_{18}, or equivalent
Ultrasonic bath
Filter pore diameter: 0.45μm
Automatic sampler: 50μL
Thermostat

PROCEDURES
(a) LIQUID CHROMATOGRAPHIC CONDITIONS (typical)
Mobile phase: acetonitrile+ water = 80 + 20（v/v）
Flow rate: 1.0ml/min

Detector wavelength: 220 nm
Injection volume: 10μL
Column temperature: 25°C
Retention time:
28-Homobrassinolide: approximately 18.6min.

(b) Equilibration of the chromatographic system. Inject the calibration solution and repeat the injections until retention times and the response factors calculated from the peak areas vary by less than 1.5% for successive injections.

(c) Preparation of standard solution: prepare standard solution in duplicate. Weigh 0.01g (to the nearest 0.1mg) 28-Homobrassinolide standard into 25ml volumetric flask, dissolved by 15ml methanol. Add 4ml phenylboronic acid solution, react 30min in thermostat at 50°C. Allow the solution to cool to ambient temperature and fill to the mark with methanol. Mix thoroughly and place the flask in an ultrasonic bath for 5 min, then filter the solution through a 0.45μm filter membrane prior to analysis.
Preparation of sample solution: prepare sample solution in duplicate. Weigh 0.01g (to the nearest 0.1mg) sufficient sample to contain about 10mg 28-Homobrassinolide into 25ml volumetric flask, dissolved by 15ml methanol. Add 4ml phenylboronic acid solution, react 30min in thermostat at 50°C. Allow the solution to cool to ambient temperature and fill to the mark with methanol. Mix thoroughly and place the flask in an ultrasonic bath for 5 min, then filter the solution through a 0.45μm filter membrane prior to analysis. (Sample solutions S1 and S2)

(d)Determination: Inject in duplicate 10μL 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, etc.

(e)Calculation

$$f_i = \frac{S \times P}{H_S}$$

Content of 28-Homobrassinolide = $\frac{H_w \times f}{w}$ *g/kg*

where:

f_i=individual response factor

f= mean response factor

H_S=peak areas of 28-Homobrassinolide in the calibration solution

H_w=peak areas of 28-Homobrassinolide in the sample solution

s=mass of 28-Homobrassinolide standard (mg)

w=mass of sample taken(mg)

P=purity of 28-Homobrassinolide standard (g/kg)

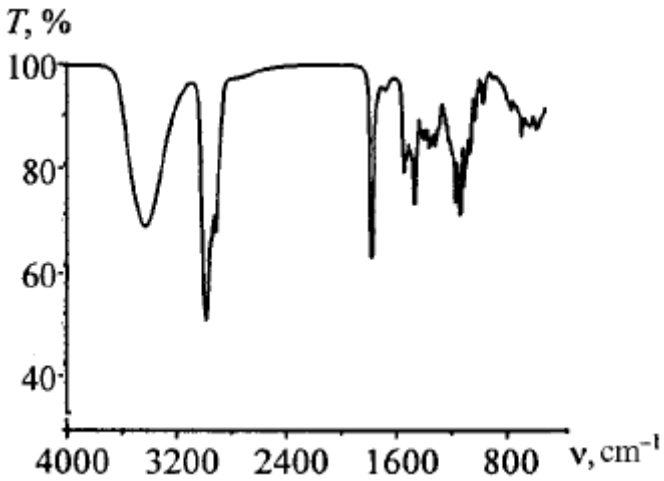


Fig 1 Infrared spectra of 28-Homobrassinolide

28-HOMOBRASSINOLIDE EMULSIFIABLE CONCENTRATE

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1. **Sampling.** Take at least 1l.

2. **Identity tests.** As for 28-Homobrassinolide technical ******

3. **28-Homobrassinolide.** As for 28-Homobrassinolide technical except:

(c) Preparation of standard solution: prepare standard solution in duplicate. Weigh 0.01g (to the nearest 0.1mg) 28-Homobrassinolide standard into 25ml volumetric flask, dissolved by 15ml methanol. Add 4ml phenylboronic acid solution, react 30min in thermostat at 50°C. Allow the solution to cool to ambient temperature and fill to the mark with methanol. Mix thoroughly and add 1ml solution into 10ml volumetric flask by pipette and fill to the mark with methanol. Mix thoroughly and place the flask in an ultrasonic bath for 5 min, then filter the solution through a 0.45μm filter membrane prior to analysis.

Preparation of sample solution: prepare sample solution in duplicate. Weigh 10g (to the nearest 0.1mg) sufficient sample to contain about 1mg 28-Homobrassinolide into 25ml volumetric flask, dissolved by 5ml methanol. Add 4ml phenylboronic acid solution, react 30min in thermostat at 50°C. Allow the solution to cool to ambient temperature and fill to the mark with methanol. Mix thoroughly and place the flask in an ultrasonic bath for 5 min, then filter the solution through a 0.45μm filter membrane prior to analysis. (Sample solutions S1 and S2)

28-HOMOBRASSINOLIDE SOLUBLE LIQUID

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1. **Sampling.** Take at least 1l.

2. **Identity tests.** As for 28-Homobrassinolide technical ******

3. **28-Homobrassinolide.** As for 28-Homobrassinolide technical except:

(c) Preparation of standard solution: prepare standard solution in duplicate. Weigh 0.01g (to the nearest 0.1mg) 28-Homobrassinolide standard into 25ml volumetric flask, dissolved by 15ml methanol. Add 4ml phenylboronic acid solution, react 30min in thermostat at 50°C. Allow the solution to cool to ambient temperature and fill to the mark with methanol. Mix thoroughly and add 1ml solution into 10ml volumetric flask by pipette and fill to the mark with methanol. Mix thoroughly and place the flask in an ultrasonic bath for 5 min, then filter the solution through a 0.45μm filter membrane prior to analysis. Preparation of sample solution: prepare sample solution in duplicate. Weigh 20g (to the nearest 0.1mg) sufficient sample to contain about 1mg 28-Homobrassinolide into 25ml volumetric flask. Add 4ml phenylboronic acid solution, react 30min in thermostat at 50°C. Allow the solution to cool to ambient temperature and fill to the mark with methanol. Mix thoroughly and place the flask in an ultrasonic bath for 5 min, then filter the solution through a 0.45μm filter membrane prior to analysis. (Sample solutions S1 and S2)

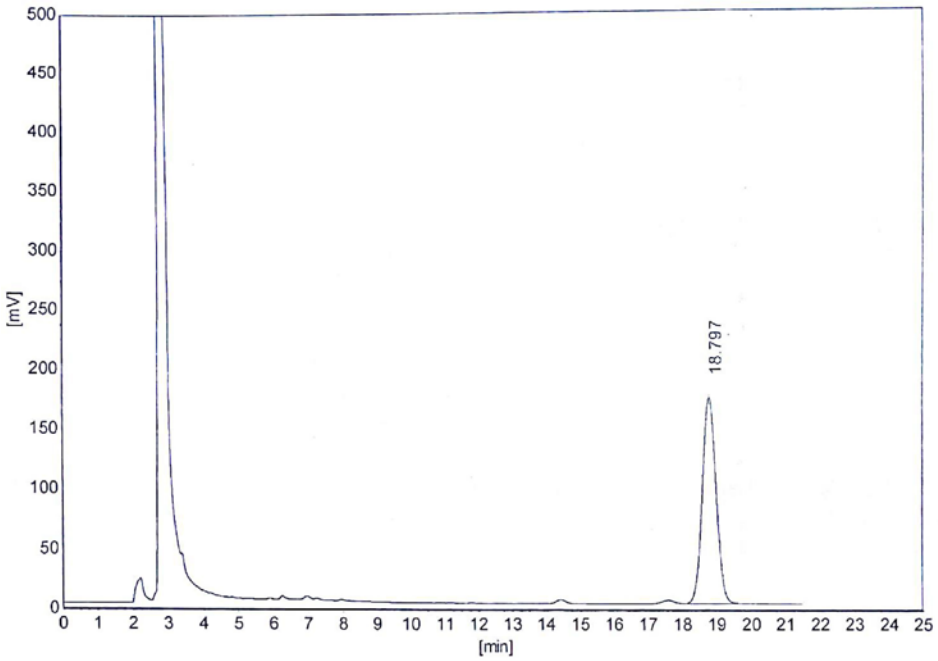

Fig 2 Chromatogram of 28-Homobrassinolide standard

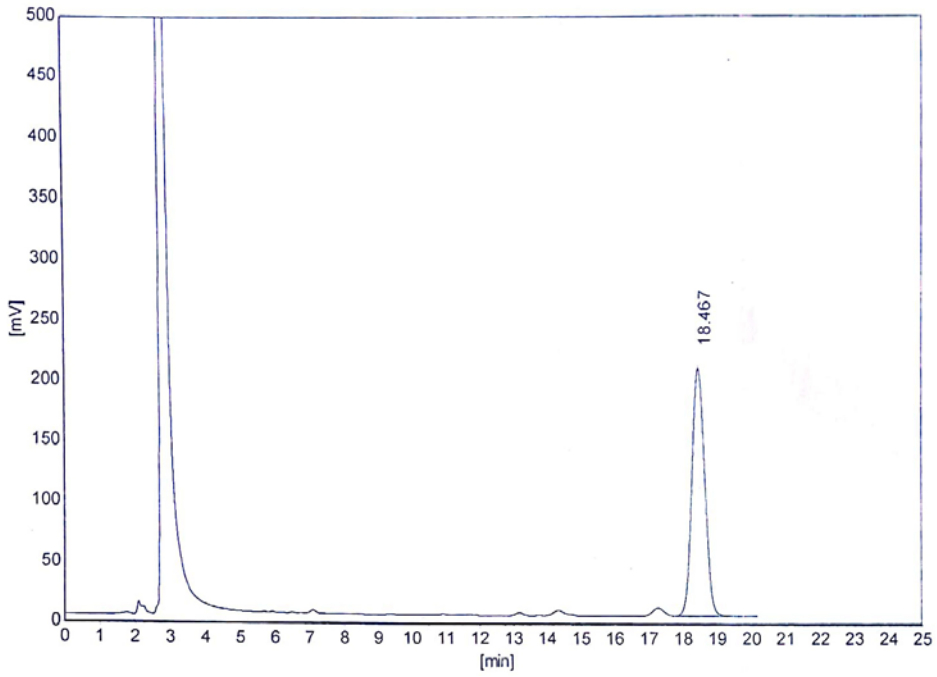

Fig 3 Chromatogram of 28-Homobrassinolide TC sample

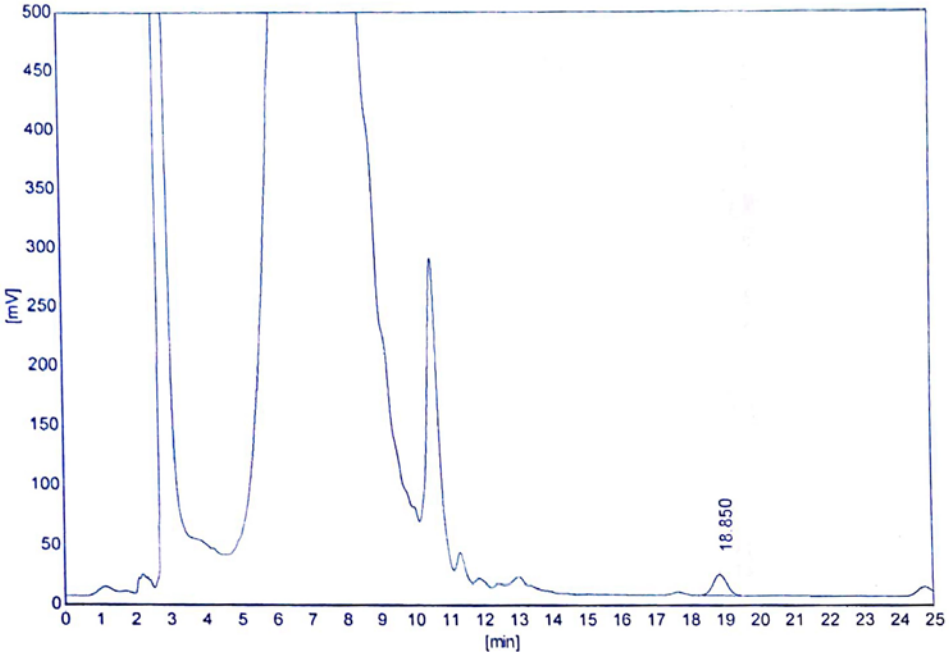

Fig 4 Chromatogram of 28-Homobrassinolide 0.01%EC sample

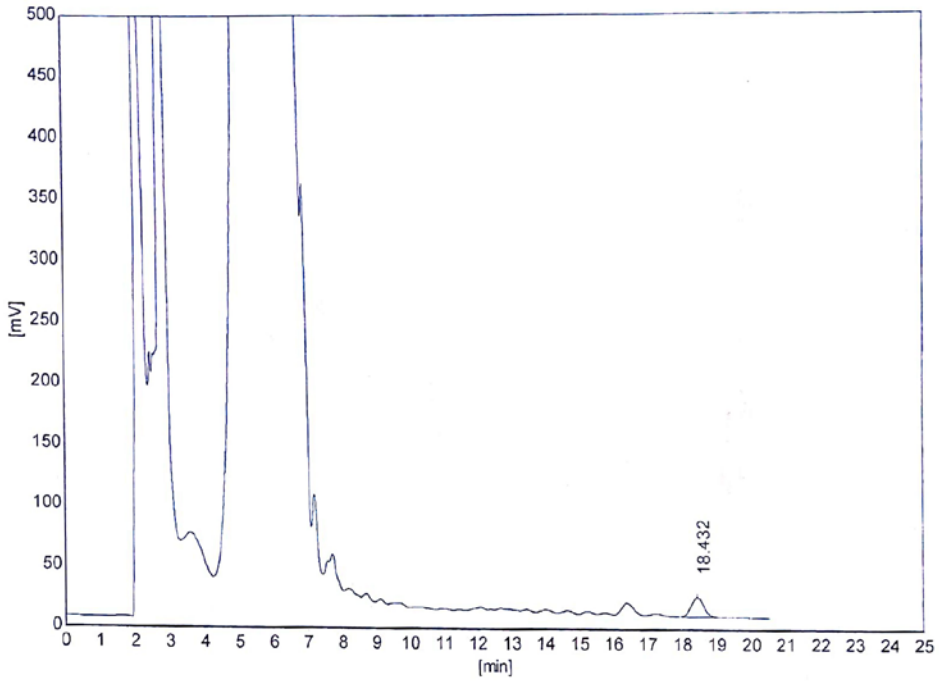

Fig 5 Chromatogram of 28-Homobrassinolide 0.004%SL sample