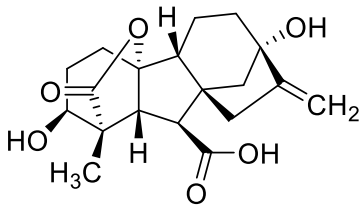
GIBBERELLIC ACID

307



ISO Common name	Gibberellic acid
Chemical name	(1α,2β,4aα,4bβ,10β)-2,4a,7-trihydroxy-1-methyl-8-methylenegibb-3-ene-1,10-dicarboxylic acid 1,4a-lactone
CAS No.	77-06-5
Empirical formula	$C_{19}H_{22}O_6$
RMM	346.4
m.p.	223-225 ℃
v.p.	0.001 mPa at 25 ℃
Solubility	In water 4.6 g/l, acetone 30.8 g/l, chloroform 0.028 g/l (all at 20-25 ℃)
Description	Crystalline solid
Stability	Dry gibberellic acid is stable at room temperature, but slowly undergoes hydrolysis in aqueous or aqueous-alcoholic solutions.

GIBBERELLIC ACID TECHNICAL

307/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 gibberellic acid 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 gibberellic acid 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. Gibberellic acid

OUTLINE OF METHOD

Gibberellic acid is determined by high performance liquid chromatography on a reversed phase column (C18) with UV detection at 210 nm and external standardization.

REAGENTS

Gibberellic acid reference standard of known purity.

Methanol HPLC grade.

Water Ultrapure quality or distilled in glass.

Phosphoric acid AR grade.

0.05% Phosphoric acid aqueous solution Dilute 1ml phosphoric acid into 2000 ml water.

Mobile Phase solution: Mix 330ml methanol and 670 ml phosphoric acid aqueous solution, thoroughly degassed.

Calibration solution. Weigh in duplicate (to the nearest 0.1 mg) 100 mg

of gibberellic acid reference standard (*s* mg) into separate volumetric flasks (100ml). Add about 5 ml methanol and shake well. Make up to volume with mobile phase. Mix thoroughly and filter the solution through a 0.45 μm filter membrane prior to analysis (calibration solutions C_A and C_B).

APPARATUS

High performance liquid chromatograph equipped with a UV detector capable for operation at 210 nm, a constant-temperature column compartment and an injection system capable of injecting 5μl.

Column stainless steel 150 ×4.6 mm (i.d), packed with SB-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

PROCEDURE

(a) Liquid chromatographic conditions (typical):

Column	stainless steel, 150 ×4.6 mm (i.d), packed with Agilent SB-C_{18} 5.0 μm, or equivalent with the same selectivity
Mobile phase	methanol: 0.05% phosphoric acid aqueous solution, 33:67 (v/v)
Column temperature	33℃
Flow rate	1.0 ml/min
Detector wavelength	210 nm
Injection volume	5 μl
Retention time	approximately 7.1 min

Run time 15 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 100 mg of gibberellic acid into a volumetric flask (100 ml). Add about 5 ml methanol and shake well. Make up to volume with mobile phase. Mix thoroughly and filter the solution through a 0.45 µm filter membrane prior to analysis (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 gibberellic acid contents of the bracketed sample injections.

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

$$Gibberellic\ acid\ content\ (g/kg) = \frac{H_w \times f}{W}$$

where:

f_i = individual response factor
f = mean response factor
H_s = peak area of gibberellic acid in the standard solution
H_w = peak area of gibberellic acid in the sample solution
S = mass of gibberellic acid in the standard solution (mg)
W = mass of sample taken (mg)
P = purity of gibberellic acid 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

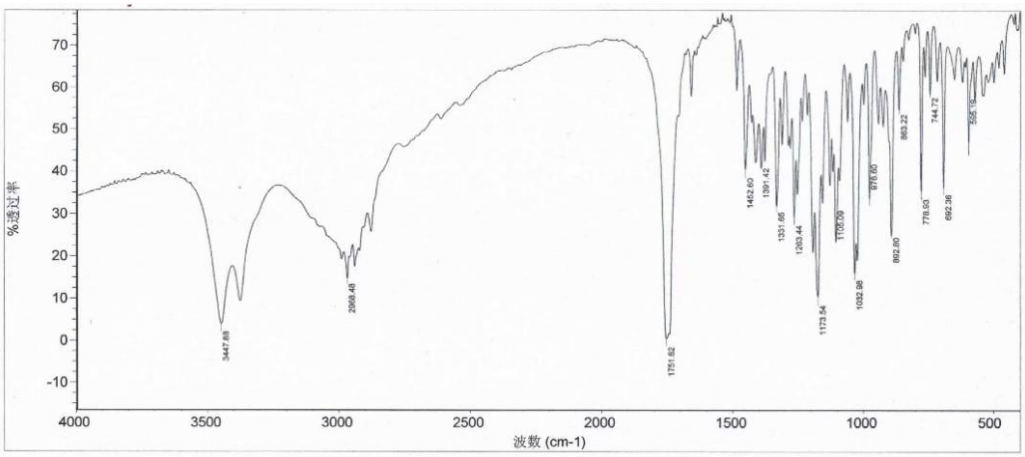


Fig. 1 FTIR spectrum of gibberellic acid standard

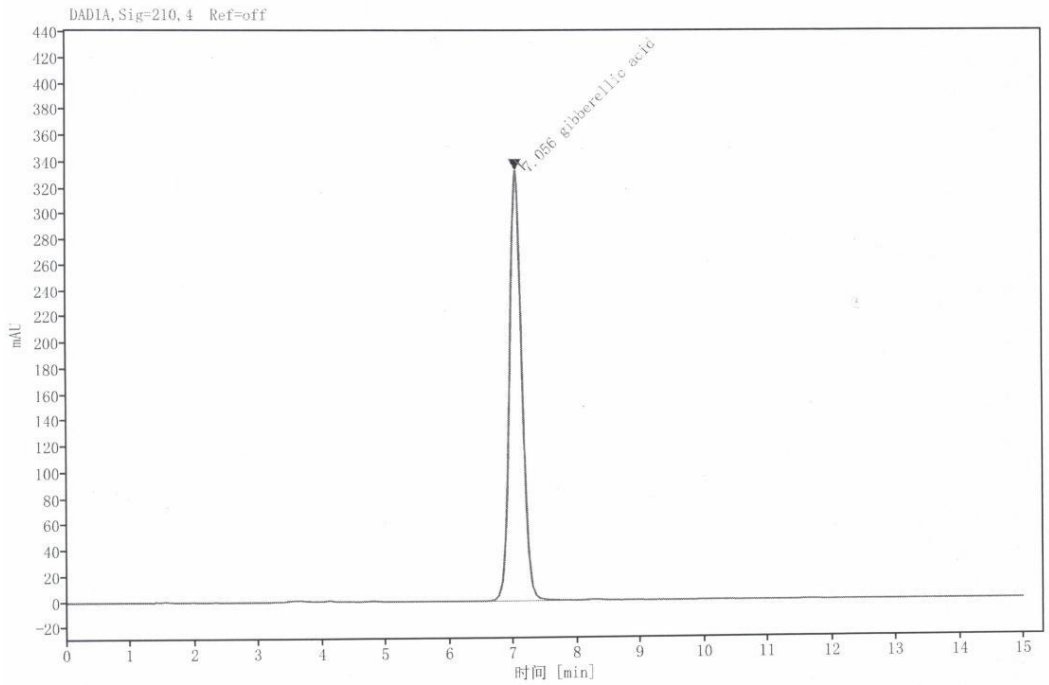


Fig. 2 HPLC Chromatogram of gibberellic acid standard

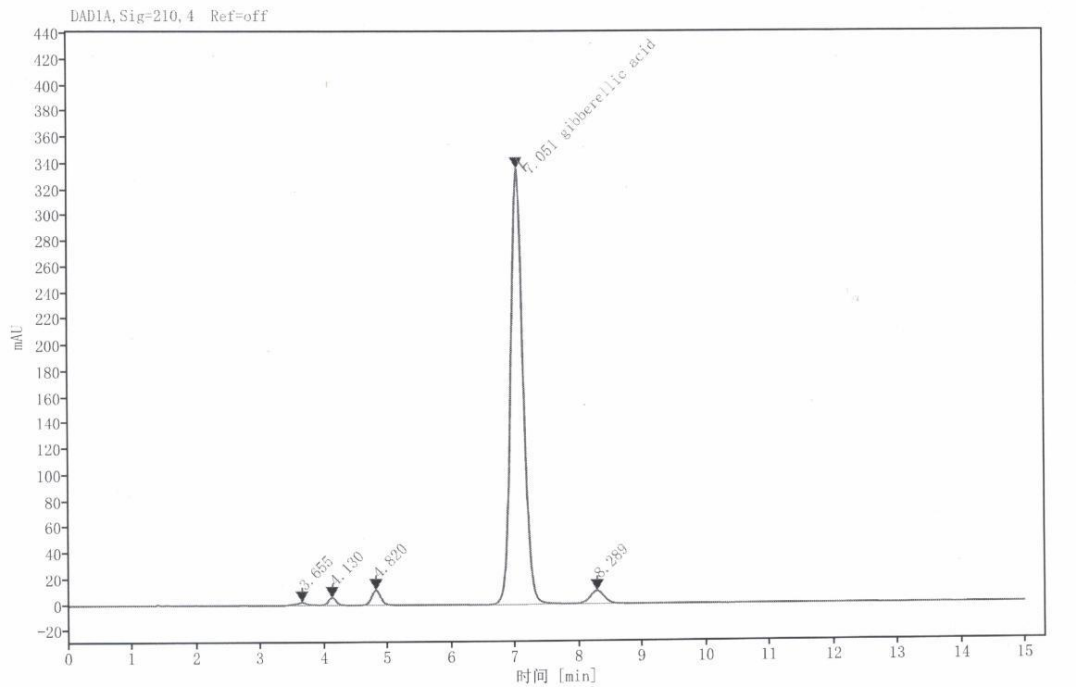


Fig. 3 HPLC Chromatogram of gibberellic acid TC