GIBBERELLIC ACID

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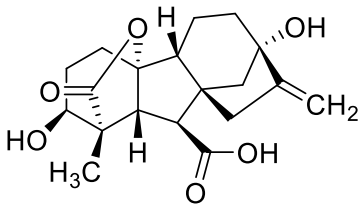

ISO Common Name	Gibberellic acid
Chemical Name	(1α,2β,4aα,4bβ,10β)-2,4a,7-trihydroxy-1-methyl-8-methylenegibb-3-ene-1,10-dicarboxylic acid 1,4a-lactone
CAS Number	77-06-5
Empirical formula	$C_{19}H_{22}O_6$
Molecular mass	346.4
m.p.	223-225 °C
v.p	0.001 mPa at 25°C
Solubility	In water 4.6 g/l, acetone 30.8 g/l, chloroform 0.028 g/l (all at 20-25°C)
Stability	Dry gibberellic acid is stable at room temperature, but slowly undergoes hydrolysis in aqueous or aqueous-alcoholic solutions
Description	Crystalline solid

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 reversed phase high performance liquid chromatography using UV detection at 210 nm and external standardization.

REAGENTS

Gibberellic acid: reference standard of known purity

Methanol: HPLC grade

Water: Ultrapure water

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	150 ×4.6 mm (i.d), Agilent SB-C_{18} 5.0 μm, or equivalent
Mobile phase	methanol: 0.05% phosphoric acid aqueous solution, 33:67 (v/v)
Column temperature	33°C
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.

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

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

where:

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

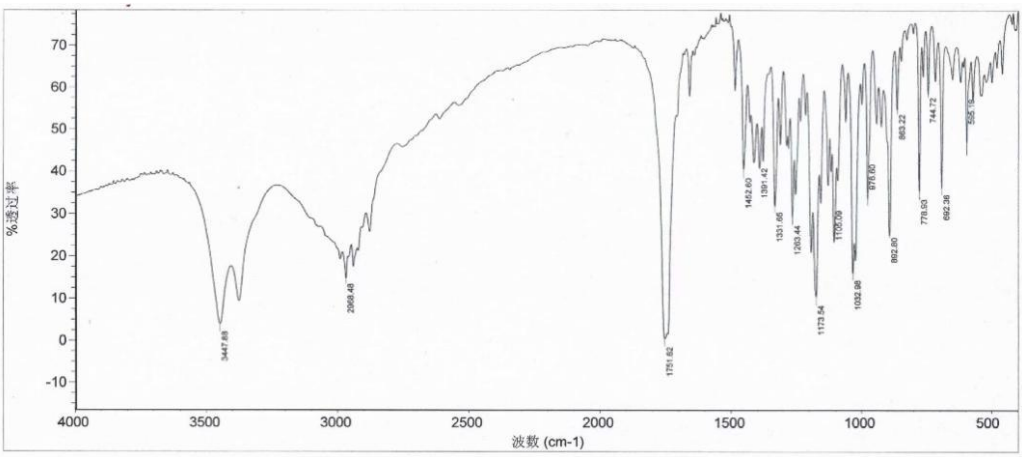

Fig. 1 FTIR spectrum of Gibberellic acid standard

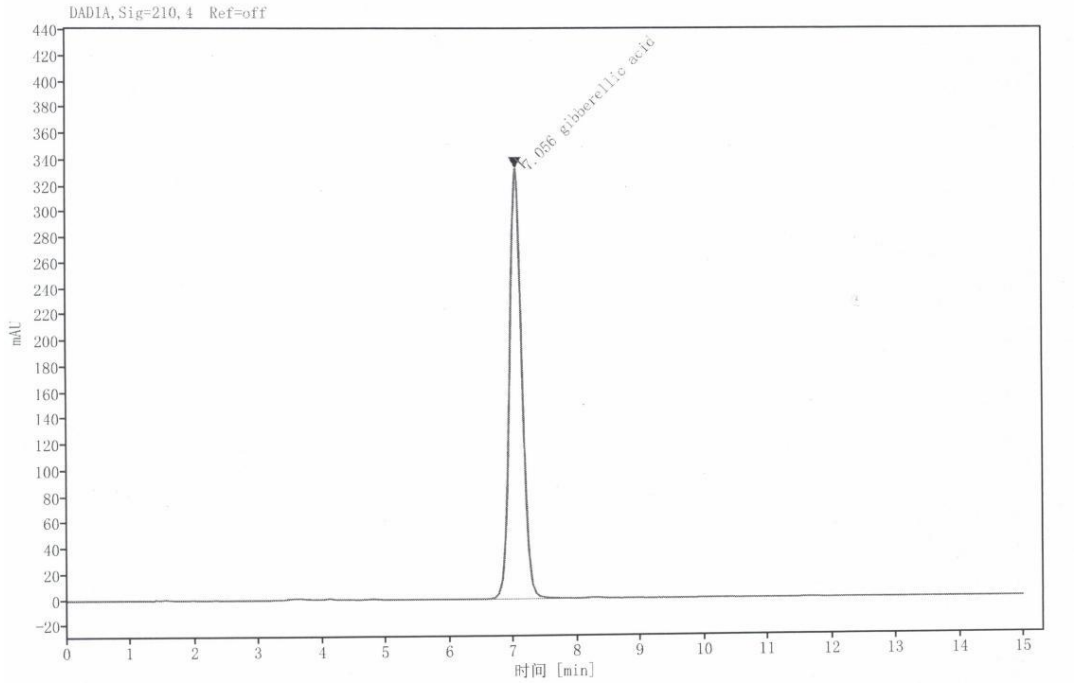

Fig. 2 HPLC Chromatogram of gibberellic acid standard

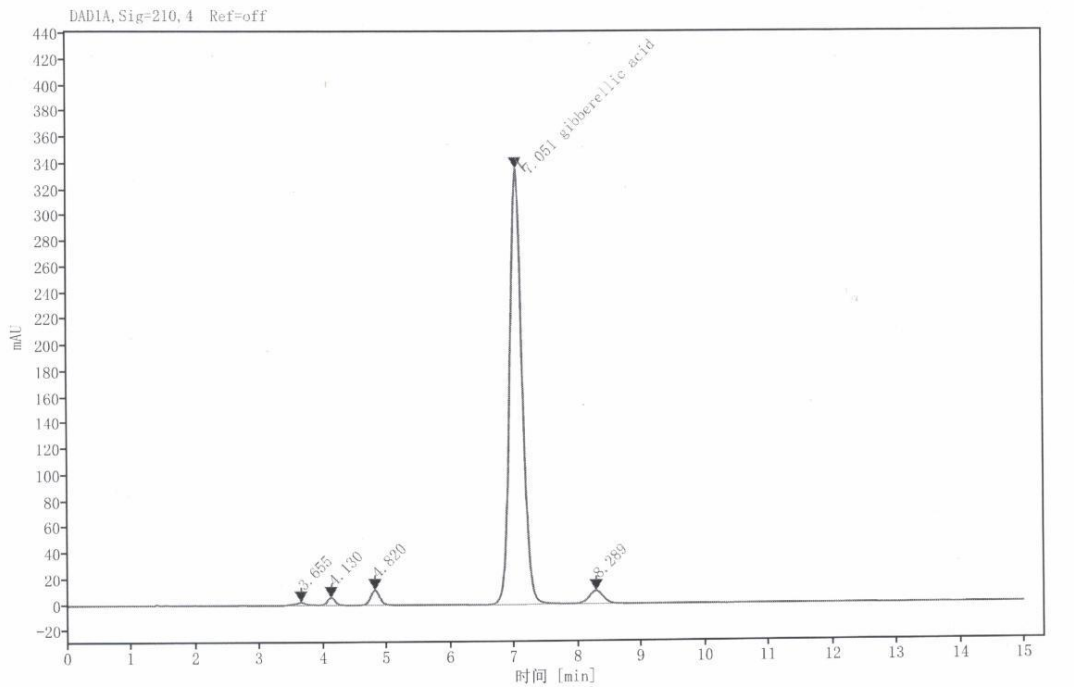

Fig. 3 HPLC Chromatogram of gibberellic acid TC