



## Detection Method of 4-hydroxyphenylacetic acid

### A. 1 Reagents and materials

A. 1.1 Acetonitrile: chromatographic purity.

A. 1.2 Phosphoric acid: analytical purity.

A. 1.3 2% Phosphoric acid solution.

### A. 2 Instruments and equipment.

High performance liquid chromatograph: UV detector or other equivalent detector shall be equipped according to Chapter 5 of GB / t27579-2011.

### A. 3 Reference chromatographic conditions.

A. 3.1 Chromatographic column: C18 liquid chromatographic column, 250mm long, 4.6mm inner diameter and 5.5mm particle size  $\mu\text{m}$ ; Or other equivalent chromatographic column.

A. 3.2 Mobile phase: 1000ml 50% methanol plus 1ml glacial acetic acid.

A. 3.3 Column temperature: 35  $^{\circ}\text{C}$ .

A. 3.4 Pump: isocratic pump.

A. 3.5 Flow rate: 1.0ml/min.

A. 3.6 Injection volume: 10  $\mu\text{L}$ .

A. 3.7 Detection wavelength: 277nm.

### A. 4 Analysis steps

#### A. 4.1 Preparation of reference solution

Accurately weigh 0.015g of sample water, dissolve it, add 2 drops of methanol, and shake it for standby

#### A. 4.2 Preparation of sample solution

Accurately weigh 0.015g of sample, dissolve it with mobile phase, transfer it into a 100ml volumetric flask, and add mobile phase to constant volume.

100mL. 20 $\mu\text{L}$  for the resulting solution filter the filtrate by membrane and reserve it.

#### A. 4.3 Determination

Under the reference chromatographic conditions of A.3, measure the reference solution and sample solution respectively, and record the main peak area.

### A. 5 Result calculation

The mass fraction  $W_1$  of 4-hydroxyphenylacetic acid content is calculated according to formula (A.1):

$$W_1 = \frac{A_1 \times m_2 \times w_2}{A_2 \times m_1} \times 100\% \quad \dots\dots\dots(A.1)$$

A1——North Peak Peak;

m2——The mass of the reference substance, in grams (g);

Address: D-1205, Haibo plaza, 9th Fengcheng, Xi'an,

Shaanxi Province, China

Tel: 0086-029-86680917

Web: [www.sxgreenfine.com](http://www.sxgreenfine.com)



陕西绿清生物工程有限公司

Shaanxi Green Bio-Engineering Co.,Ltd

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w2—The quality of the content in the reference substance, %;

A2—The peak top value of the main peak of the reference substance solution vertically upward;

m1—The mass of the student, in grams (g)