陕西绿清生物工程有限公司

Shaanxi Green Bio-Engineering Co.,Ltd

High performance liquid chromatography detection method of Kaempferol

1. Purpose

Establish HPLC standard inspection procedures for the determination of kaempferol content.

2. Range

Suitable for kaempferol content measurement

3. Reference substance

Kaempferol(reference substance)

4. Reagents:

4.1 Acetonitrile (chromatographically pure)

4.2 Methanol (chromatographically pure)

4.3 Methanol (analytical grade)

4.4 Water (ultra pure water)

5. Apparatus:

5.1 High performance liquid chromatograph: Waters 2695 infusion pump; Waters 2487 UV detector; Empower chromatography

Data working system

5.2 Analytical balance: Sensitivity 0.00001g

5.3 Ultrasonic cleaner: power 250W, frequency 40KHz

5.4 Glass mobile phase filter (0.45μm)

5.5 Needle type microporous membrane (0.45μm)

5.6 Glass measuring device: measuring cylinder (100ml), volumetric flask (25, 50ml), etc.

6. Preparation of the solution:

6.1 Preparation of reference solution:

Accurately weigh about 10mg of the kaempferol reference substance into a 50ml volumetric flask, add about 40ml of methanol, and dissolve by ultrasound.

After that, place it to room temperature, dilute to volume with methanol, shake well, and get it.

6.2 Preparation of sample solution:

Accurately weigh about 10mg of the sample into a 50ml volumetric flask, add about 40ml of methanol, and extract with ultrasonic shaking for about 30 minutes.

After that, place it at room temperature, dilute to the mark with methanol, and filter with a 0.45 μm syringe filter to obtain a sample solution.

7. Chromatographic conditions:

Column: Intersil ODS-C18 (150mm×4.6 mm ,5μm)

Detection wavelength: 260nm
Mobile phase: methanol: water (60:40 v/v)
Flow rate: 0.8
Column temperature: room temperature Injection volume: 10μl
Sensitivity: 2.000AUFS
System adaptability: The number of theoretical plates based on the kaempferol peak shall not be less than 3000.
8. Sample determination:
Under the above chromatographic conditions, after the instrument is stable and the baseline is stable, accurately draw the reference solution and the sample solution 10μl, each sample was injected for determination, the retention time of kaempferol was about 10 min, and the area external standard method was used to calculate the sample content.

9. Content calculation

$$\text{Content \%} = \frac{A1 \times W0 \times V1 \times K}{A_0 \times V_0 \times W_1} \times 100$$

A1：The peak area of genistein in the sample solution
A0：The peak area of genistein in the reference solution

W1：Sample weighing mg W0：Reference substance weighing mg

V1：Sample volume ml V0：Reference substance volume ml

K： Reference substance content

The sample content result is the average of the content of two parallel samples