Testing method of genistein purity by HPLC

1. Purpose

To establish the standard operating instruction for testing method of genistein purity by HPLC.

2. Range

Apply to the test method of genistein purity.

3. Comparison products

Genistein (standard product)

4. Reductant

4.1Acetonitrile (chromatographically pure)
4.2 Methyl alcohol (chromatographically pure)
4.3 Methyl alcohol(analytically pure)
4.4 Analytically pure(analytically pure)
4.5 Water (ultrapure water)

5. Instrument

5.1HPLC : Waters 2695 Infusion pump: Waters 2487 UV-detector :Empower

Chromatographic data working system.

5.2 Analytical balance: Sensitive quality.
5.3 USC instrument: Power: 250W Frequency 40KHz. Glass moving phase filter（0.45μm）
5.4 Needle micro hole filter membrance.
5.5 Volumetric glass:mearing cylinder (100ml), Volumetric flask(25、50ml).

6. Solution preparation.

6.1 Solution preparation of comparison products.
Get the genistein comparison products precisely about 10mg into the volumetric flask of 50ml, add the methyl alcohol about 40ml, after ultrasonic dissolving, place it under the indoor temperature, use methyl alcohol metered volume to the scale, shake up.
6.2 The preparation of sample solution.
Get the sample about 10mg into the volume falsk of 50mg precisely, add the methyl about 40ml, after ultrasonic vibrating extraction about 30min, place under the indoor temperature, use methyl metered volume to the scale, use the 0.45μm needle filter instrument to filter, then get the sample soution.

7.Chromatographic condition.

Chromatographic condition:Intersil ODS-C18 (150mm×4.6 mm ,5μm)
Wave length:262nm
Moving phase: methyl alcohol: acetonitrile: 0.1% phosphoric acid solution
Column temperature: indoor input sample 10μl
Sensitivity:2.000AUFS
The adaptable of system:According to the genistein number of theoretical plates can not less than 3000

8. Sample test:

Under the above chromatographic conditions, the comparison product solution and sample solution are absorbed precisely after the instrument is stabilized and the baseline is stabled
The retention time of genistein is about 6min. The purity of the sample is calculated by area external standard method.

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9. $$\text{Purity}(\%) = \frac{A1 \times W0 \times V1 \times K}{A_0 \times V_0 \times W_1} \times 100$$

A1: Peak area of genistein in sample solution.

A0: Peak area of genistein in comparison product solution

W1: Weighing sample weight mg W0: Weighing sample weight mg of comparison product

V1: Sample dimension ml

V0: Comparison products dimension ml

K: Comparison products purity

Sample purity results are take the average of two parallel sample purity.

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