



Appendix A

Mensuration of Naringenin Assay

A.1 Reagents and Materials

A.1.1 Acetonitrile: LC (Liquid chromatography)

A.1.2 Methanoic Acid: LC (Liquid chromatography)

A.1.3 Reference Materials of Naringenin: The purity is equal or greater than 98%

A.1.4 Water: First grade water with GB/T6682

A.2 Instruments and Equipment

High performance liquid chromatograph: Equipped with UV detector or other equivalent detectors accord with the rules in chapter 5 of GB/T27579—2011.

A.3 Reference Chromatographic Condition

A.3.1 Chromatographic column: C18 liquid chromatographic column, 150mm in length, 4.6mm in internal diameter, 5 μ m in particle size; or other equivalent chromatographic columns.

A.3.2 Mobile phase: Consisting of acetonitrile with a volume fraction of 40% and 60% formic acid aqueous solution (volume fraction of 0.5%).

A.3.3 Column temperature: 30 $^{\circ}$ C。

A.3.4 Pump: isocratic pump

A.3.5 Flow velocity: 1.0mL/min

A.3.6 Injection volume: 10 μ L

A.3.7 Detection wavelength: 285nm

A.3.8 Runtime: About 12min

A.4 Analytical procedure

A.4.1 Preparation of reference solution



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Weigh and finely 0.01g of Naringenin reference material, which accurate to 0.0001g and dissolved by mobile phase, then move them into the 100ml volumetric flask, make metered volume to 100ml by adding mobile phase. The resulting solution is reserved which has been filtered with 0.45μm filter membrane .

A.4.2 Preparation of specimen solution

Weigh and finely 0.01g specimen which accurate to 0.0001g and dissolved by mobile phase, then move them into the 100ml volumetric flask, make metered volume to 100ml by adding mobile phase. The resulting solution is reserved which has been filtered with 0.45μm filter membrane .

A.4.3 Mensuration

According to the A.3 chromatographic condition, mensurate the reference solution and specimen solution separately , record the peak area.

A.5 Result calculation

Calculate the mass fraction w_1 of naringenin assay with formula(A.1).

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

Formula A.1

A.1——The peak area of principal peak in specimen solution chromatogram map;

m_2 ——The mass of reference material, unit for gram;

w_2 ——The mass fraction w_1 of naringenin assay,%;

A2——The peak area of principal peak in reference solution chromatogram map;

m_1 ——The mass of specimen, unit for gram;