

### Determination of Chlorogenic acid contents

- 1 Instrument: High performance liquid chromatography (SP880) equipped with UV detector (SP100).
- 2 Reagents: Methanol: HPLC grade; Water: Ultra-pure water; Formic acid: HPLC grade
- 3 HPLC parameters:
  - a. Column: C18, 250 mm×4.6 mm(i.d.), 5 μm;
  - b. Mobile phase: Methanol: Water: Formic acid =30:70:1
  - c. Flow rate: 1.0 mL/min;
  - d. Column temperature: Ambient temperature;
  - e. Wave length: 330 nm;
- 4 Reference solution: accurately weigh 1 mg reference material into a 10 mL volumetric flask, dissolve with methanol and ultrasonic vibrations 10 mins then dilute to the scale with methanol.
- 5 Sample solution: accurately weigh 20 mg sample into a 50 mL volumetric flask, dissolved with methanol and ultrasonic vibrations 10 mins then dilute to the scale with methanol.
- 6 Calculation

$$\text{Content} = A_x/A_s \cdot C_s/C_x \cdot 100\%$$

Cx: concentration of Sample solution

Cs: concentration of Reference solution

Ax: Peak area of Sample

As: Peak area of Reference