

Standard operating procedure (SOP) for measuring the steviol glycoside content of Reb-M samples.

1. **Determine the percentages of the individual steviol glycosides by HPLC under following conditions.**

Reagents

Acetonitrile: more than 95% transmittance at 210 nm.

Standards

Stevioside: more than 99.0% purity on the dried basis.

Rebaudioside A: more than 99.0% purity on the dried basis.

Rebaudioside M: more than 95% purity on the dried basis.

Standard solution

Accurately weigh 20-30 mg of stevioside, rebaudioside A and rebaudioside M standard into each of 100-ml volumetric flasks. Dissolve and make up to volume with water-acetonitrile (7:3).

Sample solution

Accurately weigh 20-30 mg of sample into a 100-ml volumetric flask. Dissolve and make up to volume with water-acetonitrile (7:3).

Procedure

Inject 5 µl of sample solution under the following conditions.

Column: Luna 5µ C18(2) 100A (Phenomenex) (length: 250 mm; inner diameter: 4.6 mm, particle size: 5µm)

Mobile phase: 32:68 mixture of acetonitrile and 10 mmol/L sodium phosphate buffer (pH 2.6)

Flow rate: 1.0 ml/min

Detector: UV at 210 nm

Column temperature: 40°

Record the chromatogram for about 30 min.

Identification of the peaks and Calculation

Identify the peaks from the sample solution by comparing the retention time with the peaks from the steviol glycosides standard solution. Measure the peak areas for the steviol glycosides from the sample solution. Measure the peak area for stevioside, rebaudioside A and Rebaudioside M from their standard solutions.

2. Moisture measurement:

Accurately weigh 20-30 mg (W_b) of stevioside, rebaudioside A and rebaudioside M standard and samples into each of weighting bottle. Open the lid of each weighting bottle and bake these samples in 95° oven for 2 hours. Close the lid of each weighting bottle and take them out from oven. After cooling down, weigh each sample (W_a) again and calculate the percentages of moisture.

$$\text{Moisture (\%)} = \frac{W_b - W_a}{W_b} \times 100$$

3. Calculate contents of steviol glycosides in sample:

Calculate the percentage of steviol glycoside in samples based on the standard.

For example:

Calculate the concentration of Reb M (S_m) in samples solution from the formula:

$$S_m = \frac{\frac{P_s}{P_m}}{P_{Um} \cdot (1 - M_m) \cdot C_m}$$

P_s : peak area of Reb M in sample solution

P_m : peak area of Reb M standard

P_{Um} : purity of Reb M standard

M_m : moisture of Reb M standard

C_m : concentration of Reb M standard solution

Calculate the purity of Reb M in samples from the formula:

$$\text{Purity (\%)} = \frac{S_m}{C_s \cdot (1 - M_s)} \times 100$$

S_m : concentration of Reb M in sample solution

C_s : concentration of sample solution

M_s : moisture of sample