36. Steviol Glycoside

Stevioside

Rebaudioside A

Definition Steviol glycoside is obtained from *Stevia rebaudiana* Bertoni. The leaves are extracted with hot leaves and the aqueous extract is passed through an absorption resin and concentrate it. The product is recrystallized from methyl alcohol or ethyl alcohol and dried. Its major component is Steviol glucoside.

Compositional Specifications of Steviol Glycoside

Content When Steviol glycoside is dried and weighed, it should contain not less than 95.0% of whole Steviol glycoside.

Description Steviol glycoside is white to light yellow powder, flakes, or granules with strong sweet taste. It is odorless or having a slight characteristic odor.

Identification 0.5 g of Steviol glycoside is dissolved in 100 ml of water, test solution. 5 mg each of Stevioside for quantitative and Rebaudioside A is weighed and dissolved in 10 ml of water, standard solution. Liquid chromatography is carried out with test solution and standard solution under the operation conditions of assay. Retention time of the main peak of Test Solution is identical to the retention time of both peak of Stevioside and Rebaudioside or one peak of Standard Solution.

Purity (1) pH : pH of this aqueous solution (1→100) of Steviol glycoside should be 4.5~7.0 as determined by glass electrode method.

(2) Arsenic : 0.77 g of Steviol glycoside is placed in a platinum, quartz, or porcelain crucible. 10 ml of magnesium nitrate in ethyl alcohol(1→50) is added to the crucible and then alcohol is ignited. It is then reduced to ash by heating at 450~550℃. If carbonaceous substance persists, it is wetted with minute amount of nitric acid, which is further heat treated at 450~550℃. After cooling, 3 ml of hydrochloric acid is added to the residue, which is then dissolved by heating in a water bath. When test for arsenic is carried out with this test solution, it should be appropriate (not more than 1.3 ppm).

(3) Lead : Accurately weigh 10 g of Steviol glycoside and place in a platinum or quartz crucible. Add minute amount of sulfuric acid, wet, gradually heat and preliminarily heat-treat the solution at the temperature as low as possible. Again add 1 ml of sulfuric acid, gradually heat, ignite until it is heat-treated at 450~550℃. After heat-treating, add minute amount of nitric acid(1→150) to the residue, again, add nitric acid(1→150) to make 10 ml, test solution. When the test solution is tested by Atomic Absorption Spectrophotometry or Inductively Coupled Plasma

Emission Spectroscopy, its content should not be more than 1.0 ppm.

(4) Residual solvent : 2 g of Steviol glycoside is precisely weighed into a 300 ml round bottom flask, 200 ml of water is added, boiling chips and 1 ml of silicone resin are added and mixed well. Receiver containing is connected to this, 4 ml of internal standard solution is precisely weighed and added to a 100 ml flask. While caring for the bubbles not to overflow, distill the solution at the rate of 2~3 ml per 1 minute until the milky liquid becomes about 90 ml, and water is added to make 100 ml, test solution. However, tert-butyl alcohol (1→1,000) is used as internal standard solution. Separately, 0.5 g of methyl alcohol is precisely weighed and water is added to make 500 ml, again 2 ml of this solution and 4 ml of internal standard solution is weighed, water is added to make 100 ml, standard solution. 2μl of test solution and standard solution is taken respectively, and injected to gas chromatograph with the following operation condition. Then, ratio of methyl alcohol peak against tert-butyl alcohol peak in test Solution and standard solution, QT and QS, is calculated separately, and the content of methyl alcohol is calculated by following formula, the content should not be more than 200ppm.

$$\text{Content of methyl alcohol}(\%) = \frac{\text{Weight of methyl alcohol(g)}}{\text{Weight of sample(g)}} \times \frac{Q_T}{Q_S} \times \frac{2\times100}{500\times100} \times 100$$

QT : Ratio of methyl alcohol peak against tert-butyl alcohol peak in Test Solution

QS : Ratio of methyl alcohol peak against tert-butyl alcohol peak in standard solution

Operation Conditions

Column : PLOT Q or its equivalent

Detector : Hydrogen Flame Ionization Detector (FID)

Temperature at injection hole : 200℃

Column Temperature : 120℃

Detector temperature : 300℃

Carrier gas : Nitrogen or Helium

Ash When 1 g of Steviol glycoside is tested by Ash Limit Test, it should not be more than 1%.

Loss on Drying When 2 g of Steviol glycoside is dried for 2 hours at 105℃, the weight loss should not be more than 6%.

Assay Steviol glycoside is dried for 2 hours at 105℃, 60~120mg of steviosdie is precisely weighed, dissolved in mobile phase to make 100 ml, test solution. Separately,

stevioside and rebaudioside A standard are dried for 2 hours at 105℃, 50 mg of each is precisely weighed, dissolved in mobile phase to make 100 ml, standard solution. Each of test solution and standard solution is injected to liquid chromatograph with the following operation condition, and measure the content of whole steviol glycoside. Peak areas of dulcoside A , rubusoside, rebaudioside A, rebaudioside B, rebaudioside C, steviolvioside, stevioside of the sample are obtained. Also peak areas of dulcoside A, rubusoside, rebaudioside A, rebaudioside B, rebaudioside C, steviolvioside, stevioside of standard solution. The contents of the 7 components are obtained by the following formula. The sum of these contents is the content of steviolglycoside. When the peak of rebaudioside A is finished, the mobile phase is changed to 50 : 50 composition and the residuals in the column is washed out.

$$X\ \% = \frac{Ws}{W} \times \frac{Ax \times fx}{As} \times 100$$

Ws : Amount of stevioside in standard solution(mg)

W : Weight of sample in test solution (mg)

As : Peak area of stevioside in standard solution

Ax : Peak area of X in test solution

fx : Ratio of molecular weight of X against stevioside (stevioside 1.00, dulcosideA 0.98, rebaudioside A 1.20, rebaudioside C 1.18, rubusoside 0.80, steviolvioside 0.80, rebaudioside B 1.00)

When test proceed with following operation condition, retention time is as follows. Against rebaudiosideA(1.00), rubusoside 0.12~0.16, dulcoside A 0.25~0.30, steviolvioside 0.35~0.41, stevioside 0.45~0.48, rebaudioside C 0.63~0.69, rebaudioside B 0.73~0.79.

Operation Conditions

-Detector : UV 210nm

-Column : Supelcosil LC-NH_2 or its equivalent

-Column Temperature : 40℃

-Mobile phase : acetonitrile : water (80:20)

-Flow rate : adjust rebaudioside A is detected at about 21 minutes