



TOKIWA PHYTOCHEMICAL CO.,LTD.

158,KINOKO, SAKURA-SHI, CHIBA 285-0801 JAPAN

TEL +81-43-498-0007

FAX +81-43-498-0561

Specification of Risego 100 mg White Tea Extract

Risego is extracted from *Camellia sinensis* leaves. It contains not less than 95.0 % of Total polyphenols and not less than 40.0 % of (-)Epigallocatechingallate.

Description Brown powder.

Identification test Weigh about 4 mg and dissolve in 100 mL of 50 % ethanol and add a few drops of ferric chloride solution: red-brown color is produced.

Purity test

- (1) Heavy metals Not more than 20 ppm (1.0 g, Method 2, Standard solution 2.0 mL).
- (2) Arsenic Not more than 4 ppm (0.5 g, Method 3, Equipment B).
- (3) Caffeine Not more than 0.5 %.

Weigh accurately about 20 mg of Risego and dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of caffeine, previously dried, dissolve in water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 μ L of the sample solution and the standard solution, and perform the test as directed under the Liquid Chromatography according to the following conditions. Determine the peak areas, A_T and A_S of caffeine in each solution, calculate the amounts of caffeine by the following equation.

Operating conditions -

Detector : UV Absorption 280 nm

Column : ODS Column

Mobile phase : water / methanol / trifluoroacetic acid = 850:150:1

Flow rate : Adjust the flow rate so that the retention time of (-)epigallocatechingallate is about 8 minutes.

Amount (mg) of caffeine

$$= \text{amount (mg) of caffeine for component determination} \times \frac{A_T}{A_S}$$

Water Not more than 8.0 % (by Karl-Fischer method).

Ash Not more than 1.0 % (1 g).

Content of (-) Epigallocatechingallate Not less than 40.0 %.

Weigh accurately about 20 mg of Risego and dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 2 mg of (-)epigallocatechingallate, previously dried, dissolve in water to make exactly 10 mL, and use this solution as the standard solution. Pipet 10 μ L of the sample solution and the standard solution, and perform the test as directed under the Liquid Chromatography according to the following conditions. Determine the peak areas, A_T and A_S of (-)epigallocatechingallate in each solution, calculate the amounts of (-)epigallocatechingallate by the following equation.

Operating conditions -

Detector : UV Absorption 280 nm

Column : ODS Column

Mobile phase : water / methanol / trifluoroacetic acid = 850:150:1

Flow rate : Adjust the flow rate so that the retention time of (-)epigallocatechingallate is about 8 minutes.

Amount (mg) of (-)epigallocatechingallate

$$= \text{amount (mg) of (-)epigallocatechingallate for component determination} \times \frac{A_T}{A_S} \times 5$$

Content of Total polyphenols Not less than 95.0 %.

(1) Reagent

- Ferrous tartrate reagent

Weigh accurately about 100 mg of ferrous sulfate 7-water and 500 mg of potassium sodium tartrate tetrahydrate and dissolve in water to make 100 mL.

- Sørensen buffer

Mixed with 1/15 M disodium hydrogenphosphate 12-water solution and 1/15 M potassium dihydrogenphosphate solution and adjust pH to 7.5

- Ethyl gallate standard solution

Weigh accurately about 5, 10, 15, 20, 25 mg of ethyl gallate (previously re-crystallized from water and dry at 100 °C 1 hour) and dissolve in water to make exactly 100 mL.

(2) The sample solution

Weigh accurately about 20 mg of Risego and dissolve in water to make exactly 100 mL.

(3) Making of calibration curve

Mixed with 5 mL of Ethyl gallate standard solution and 5 mL of Ferrous tartrate reagent, volume to 25 mL with Sørensen buffer (Coloring reaction is stop). Measure extinction of this solution at 540 nm, in 1 cm cell, with water as blank by spectrophotometer. Plot and make calibration curve. 1 mg of ethyl gallate is corresponding to 1.5 mg of polyphenols.

(4) Assay

Using the sample solution with same method of (3), and calculate amount of ethyl gallate of product using calibration curve.

Total polyphenol is amount of ethyl gallate by 1.5.

Microbiological test

- (1) Total aerobic microbial count Not more than 3000 cfu/g.
- (2) Escherichia coli Negative.

Test method is according to *Japan's Specifications and Standards for Food Additives*.