ORYZA POLYAMINE-LC(BG30)

This product is a water solution of *Oryza sativa* Linné (*Gramineae*) germ extract with citric acid solution adding 1,3-Butylene Glycol solution. It contains not less than 0.004% of total polyamine (total amount of putrescine: $C_4H_{12}N_2$ 88.15, spermidine: $C_7H_{19}N_3$ 145.25 and spermine: $C_{10}H_{26}N_4$ 202.34).

Manufacturing method

After extracting *Oryza sativa* Linné (*Gramineae*) germ with citric acid solution, adding purified water solved sodium citrate to filtrate, furthermore, adding 1,3-butylene glycol, mix and filter as the product.

Raw material: Rice Germ 1kg ⟶ Product: about 5 to 6 kg

Description

This product is a pale yellow liquid, having slightly characteristic odor.

Purity

- Heavy metals

  Take 1.0g of this product, determine heavy metals according to method 2: the limit is not more than 10ppm. Use 1.0mL of standard lead solution as the control solution.

- Arsenic

  Take 1.0g of this product, prepare the test solution according to method 3, and perform the test: the limit is not more than 1ppm.

Assay

- Total Polyamines

  Use experimental apparatus made of polypropylene when handle polyamine directly, as Polyamine is easy to unite glass material. Prepare 120 μM each of putrescine (Wako Chemical, 1st class), spermidine (Wako Chemical, Bio-chemical class) and spermine (Wako Chemical, reagent class) with 0.1mol/L of hydrochloride [K8180, special class]. Mix equal amount of putrescine, spermidine and spermine solution, and use this solution as the polyamine standard solution. (The final concentration of each putrescine, spermidine and spermine solution, is 40 μM.) Weigh accurately 2g of this product add water to make exactly 5mL, and use this solution as the test solution. Dissolve 0.013g of 1,7-heptanediamine (Wako Chemical, Bio-chemical class) with 0.1mol/L of hydrochloride, prepare 0.05mM concentration, and use this solution as the internal standard solution. Add about 125g of sodium carbonate decahydrate [K8624, special class] to 50mL of water and dissolve at 60℃, and use this solution as the saturated sodium carbonate solution. Add 0.03g of dansyl chloride (Wako Chemical, Bio-chemical class) to 3mL of acetone [K8034, special class] and use this solution as 1w/v% of dansyl chloride solution. Add 1.0g of L-Proline [K9107, special class] to 10mL of water and use this solution as 10w/v% of proline solution.

  Mix 360 μL of water, 20 μL of the polyamine standard solution or the test solution, 20 μL of the internal standard solution, and 200 μL of dansyl chloride solution for 5 minutes. Upon warming this solution at 60℃ (±1℃) for 1 hour (±3minutes) while avoid light. After warming and allow to stand for

1 hour, and cool at room temperature for 10 to 12 minutes while the light-shielding. After cooling, add 200 μL of proline solution, and mix well while the light-shielding. Upon warming this solution at 60℃ (±1℃) for 30 minutes (±2minutes) while avoid light, cool at room temperature for 10 to 12 minutes, and use this solution as the labeled solution.

Warm the labeled solution at 30℃, and remove acetone while flowing the nitrogen gas. After removing the acetone, add 600 μL of toluene [K8680, special class] and mix well for 20 minutes. Centrifuge at room temperature (22 to 24℃) for 1 minutes, at 10,000 r.p.m, and take gently toluene layer in an upper layer. Warm toluene layer at 30℃ while the light shielding, and remove perfectly acetone while flowing nitrogen gas for 30 to 40 minutes. Add 300 μL of methanol [K8891, special class] to the solution and mix and dissolve well.

Perform the test with 10 μL each of polyamine standard solution and the test solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and calculate the mass percentage of each polyamine, using the mass of the sample solution obtained from the height of the peaks obtained from the sample solution and standard solution: the total amount of these polyamine is not less than 0.004%.

Calculation method-

(ⅰ) Calculate the area coefficient of putrescine, spermidine and spermine in polyamine standard solution using following formula (n=3).

Area coefficient of putrescine = $(RS_1/RC_1)/(IRS/IRC)$

Area coefficient of spermidine = $(RS_2/RC_2)/(IRS/IRC)$

Area coefficient of spermine = $(RS_3/RC_3)/(IRS/IRC)$

RS_1: Area of putrescine in polyamine standard solution

RS_2: Area of spermidine in polyamine standard solution

RS_3: Area of spermine in polyamine standard solution

IRS: Area of internal standard solution

RC_1: Concentration of putrescine in polyamine standard solution (nmol/mL)

RC_2: Concentration of spermidine in polyamine standard solution (nmol/mL)

RC_3: Concentration of spermine in polyamine standard solution (nmol/mL)

IRC: Concentration of internal standard solution in polyamine standard solution (nmol/mL)

(ⅱ) Calculate the area ratio of putrescine, spermidine and spermine in TS using following formula.

Area ratio of putrescine = TS_1/ITS

Area ratio of spermidine = TS_2/ITS

Area ratio of spermine = TS_3/ITS

TS_1: Area of putrescine in TS

TS_2: Area of spermidine in TS

TS_3: Area of spermine in TS

ITS: Area of internal standard solution in TS

(ⅲ) Calculate the Molar concentration (nmol/mL) of putrescine, spermidine and spermine in TS using following formula.

Molar concentration of putrescine (nmol/mL) = $B_1 \times A_1 \times C$

Molar concentration of spermidine (nmol/mL) = $B_2 \times A_2 \times C$

Molar concentration of spermine (nmol/mL) = $B_3 \times A_3 \times C$

A_1: Area coefficient of putrescine

A_2: Area coefficient of spermidine

A_3: Area coefficient of spermine

B_1: Area ratio of putrescine

B_2: Area ratio of spermidine

B_3: Area ratio of spermine

C: Concentration of internal standard solution in TS (nmol/mL)

(ⅳ) Calculate the mass percentage of total polyamines in TS using following formula.

(Molecular weigt;Putrescine:88.15, Spermidine:145.25, Spermine:202.34)

Total polyamines content (%)

$= M_R \times [(D_1 \times 88.15) + (D_2 \times 145.25) + (D_3 \times 202.34)]/M_S \times 10^{-6} \times 100$

M_R: Amount (g) of TS

M_S: Amount (g) of this product for assay

D_1: Molar concentration of putrescine (nmol/mL)

D_2: Molar concentration of spermidine (nmol/mL)

D_3: Molar concentration of spermine (nmol/mL)

Operating conditions-

Detector : Fluorometric detector (excitation wavelength: 365nm, detection wavelength 510nm)

Column : A stainless steel column 4.6mm in inside diameter and 25cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Colum temp. : 40℃

Mobile phase : A solution: Water, B solution: Methanol

Gradient condition

Time (minutes)	0.00	23.00	23.01	33.00	33.01	43.00
A solution (%)	40	5	0	0	40	40
B solution (%)	60	95	100	100	60	60

Flow rate : 1.5mL/min.

Bacterial Count

Take 5g of this solution, make 50mL test solution with diluent and perform the bacterial count test according to Hygiene Test Method; the limit is not more than 1×10^2 cfu /g.

Mold Count

Take 5g of this solution, make 50mL test solution with diluent and perform the mold count test according to Hygiene Test Method; the limit is not more than 1×10^2 cfu /g.

Coli form

Take 1mL of the solution which prepare the bacterial count test, and perform the coli form test according to Hygiene Test Method; Negative / Not observe any colony.

These standards and test method are referred to General Notices and General Tests, Processes and Apparatus of The Japanese Standards of Quasi-drug Ingredients, unless otherwise specified.

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