mL of water, warm in a water bath for 5 minutes with occasional shaking, and filter. Cool the filtrate, and add 2 to 3 drops of gelatin TS: a white turbidity or precipitate is produced.

(2) Shake 0.1 g of Powdered Gambir with 20 mL of dilute ethanol for 2 minutes, and filter. Mix 1 mL of the filtrate with 9 mL of dilute ethanol, and to the solution add 1 mL of vanillin-hydrochloric acid TS: a light red to red-brown color develops.

Total ash Not more than 6.0%.

Acid-insoluble ash Not more than 1.5%.

Extract content Dilute ethanol-soluble extract: not less than 70.0%.

β-Galactosidase (Aspergillus)

Aspergillus Galactosidase

β-ガラクトシダーゼ(アスペルギルス)

[*9031-11-2*]

β-Galactosidase (Aspergillus) contains an enzyme produced by *Aspergillus oryzae*. It is an enzyme drug having lactose decomposition activity, and contains 8000 to 12000 units per g. Usually, it is diluted with a mixture of maltose and dextrin, maltose and D-mannitol, or maltose, dextrin and D-mannitol.

Description β-Galactosidase (Aspergillus) occurs as a white to light yellow powder.

It is slightly soluble in water with a turbidity, and practically insoluble in ethanol (95) and in diethyl ether.

Identification (1) Dissolve 0.025 g of β-Galactosidase (Aspergillus) in 100 mL of water, then to 1 mL of this solution add 9 mL of lactose substrate TS, and stand at 30°C for 10 minutes. To 1 mL of this solution add 6 mL of glucose detection TS, and stand at 30°C for 10 minutes: a red to red-purple color develops.

(2) Dissolve 0.1 g of β-Galactosidase (Asperigillus) in 100 mL of water, and filter the solution if necessary. Determine the absorption spectrum of the solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Odor—β-Galactosidase (Aspergillus) has no any rancid odor.

(2) Heavy metals—Proceed with 1.0 g of β-Galactosidase (Aspergillus) according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of β-Galactosidase (Aspergillus) according to Method 3, and perform the test using Apparatus B (not more than 2 ppm).

Loss on drying Not more than 9.0% (0.5 g, in vacuum, 80°C, 4 hours).

Residue on ignition Not more than 3.0% (0.5 g).

Nitrogen content Weigh accurately about 0.07 g of β-Galactosidase (Aspergillus), and perform the test as directed under the Nitrogen Determination: the amount of nitrogen (N: 14.007) is between 0.5% and 5.0%, calculated on the dried basis.

Assay (i) Substrate solution: Dissolve 0.172 g of 2-nitrophenyl-β-D-galactopyranoside in disodium hydrogenphosphate-citric acid buffer solution, pH 4.5 to make 100 mL.

(ii) Procedure: Weigh accurately about 0.025 g of β-Galactosidase (Aspergillus), dissolve in water to make exactly 100 mL, then pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the sample solution. Take exactly 3.5 mL of the substrate solution, stand at 30 ± 0.1°C for 5 minutes, add exactly 0.5 mL of the sample solution, immediately mix, and stand at 30 ± 0.1°C for exactly 10 minutes, then add exactly 1 mL of sodium carbonate TS and mix immediately. Determine the absorbance, A_1, of this solution at 420 nm using water as the control. Separately, take exactly 3.5 mL of the substrate solution, add exactly 1 mL of sodium carbonate TS and mix, then add exactly 0.5 mL of the sample solution and mix. Determine the absorbance, A_2, of this solution in the same manner as above.

$$\text{Units per g of β-Galactosidase (Aspergillus)} = \frac{A_1 - A_2}{0.917} \times \frac{1}{0.5} \times \frac{1}{10} \times \frac{1}{W}$$

0.917: Absorbance of 1 μmol/ 5 mL of *o*-nitrophenol

W: Amount (g) of the sample in the sample solution per mL

Unit: One unit indicates an amount of the enzyme which decomposes 1 μmol of 2-nitrophenyl-β-D-galactopyranoside in 1 minute under the above conditions.

Containers and storage Containers—Tight containers.

Storage—In a cold place.

β-Galactosidase (Penicillium)

β-ガラクトシダーゼ(ペニシリウム)

[*9031-11-2*]

β-Galactosidase (Penicillium) contains an enzyme, having lactose decomposition activity, produced by *Penicillium multicolor*. It contains not less than 8500 units and not more than 11,500 units in each g. Usually, it is diluted with D-mannitol.

Description β-Galactosidase (Penicillium) occurs as a white to pale yellowish white, crystalline powder or powder.

It is soluble in water with a turbidity, and practically insoluble in ethanol (95).

It is hygroscopic.

Identification (1) Dissolve 0.05 g of β-Galactosidase (Penicillium) in 100 mL of water, then to 0.2 mL of this solution add 0.2 mL of lactose substrate TS, and allow to stand at 30°C for 10 minutes. To this solution add 3 mL of glucose detection TS, and allow to stand at 30°C for 10 minutes: a red to red-purple color develops.

(2) Dissolve 0.15 g of β-Galactosidase (Penicillium) in 100 mL of water, filter if necessary, and determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 278 nm and 282 nm.

Purity (1) Odor—β-Galactosidase (Penicillium) has no any rancid odor.

(2) Heavy metals—Proceed with 1.0 g of β-Galactosidase (Penicillium) according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of β-Galactosidase (Penicillium) according to Method 3, and perform the test using Apparatus B (not more than 2 ppm).

(4) Nitrogen—Weigh accurately about 0.1 g of β-Galactosidase (Penicillium), and perform the test as directed under the Nitrogen Determination: not more than 3 mg of nitrogen (N: 14.007) is found for each labeled 1000 Units.

(5) Protein contaminants—Dissolve 0.15 g of β-Galactosidase (Penicillium) in 4 mL of water, and use this solution as the sample solution. Perform the test with 15 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak having retention time of about 19 minutes is not more than 75% of the total area of all peaks, and the areas of peaks other than the peaks having retention times of about 3, 16 and 19 minutes are not more than 15% of the total area of all peaks.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column about 7.5 mm in inside diameter and about 75 mm in length, packed with strongly acidic ion-exchange resin for liquid chromatography of sulfopropyl group-binding hydrophilic polymer (10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A solution obtained by dissolving 2.83 g of sodium acetate in 1000 mL of water, and adjusting to pH 4.5 with acetic acid (100) (mobile phase A), and a solution obtained by dissolving 29.2 g of sodium chloride in 1000 mL of mobile phase A (mobile phase B).

Flow system: Adjust a linear concentration gradient from the mobile phase A to the mobile phase B immediately after injection of the sample so that the retention times of non-retaining protein and the enzyme protein are about 3 minutes and 19 minutes, respectively, when the flow runs 0.8 mL per minute, and then continue the running of the mobile phase B.

Selection of column: Dissolve 15 mg of β-lactoglobulin in 4.5 mL of water, add 0.5 mL of a solution of cytosine (1 in 5000), and use this solution as the column-selecting solution. Proceed with 15 μL of the column-selecting solution under the above operating conditions, and calculate the resolution. Use a column giving elution of cytosine and β-lactoglobulin in this order with the resolution between these peaks being not less than 4.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of β-lactoglobulin from 15 μL of the column-selecting solution is between 5 cm and 14 cm.

Time span of measurement: About 1.4 times as long as the retention time of β-lactoglobulin.

Loss on drying Not more than 5.0% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition Not more than 2.0% (1 g).

Assay (i) Substrate solution—Dissolve 0.603 g of 2-nitrophenyl-β-D-galactopyranoside in disodium hydrogenphosphate-citric acid buffer solution, pH 4.5 to make 100 mL.

(ii) Procedure—Weigh accurately about 0.15 g of β-Galactosidase (Penicillium), dissolve in water with thorough shaking to make exactly 100 mL, and allow to stand at room temperature for an hour. Pipet 2 mL of this solution, add disodium hydrogenphosphate-citric acid buffer solution, pH 4.5 to make exactly 100 mL, and use this solution as the sample solution. Transfer exactly 0.5 mL of the sample solution to a test tube, stand at 30 ± 0.1°C for 10 minutes, add exactly 0.5 mL of the substrate solution previously kept at 30 ± 0.1°C, then mix immediately, and stand at 30 ± 0.1°C for exactly 10 minutes. Then add exactly 1 mL of sodium carbonate TS, mix immediately to stop the reaction. To this solution add exactly 8 mL of water, mix, and use as the colored sample solution. Separately, pipet 0.5 mL of disodium hydrogenphosphate-citric acid buffer solution, pH 4.5, then proceed in the same manner as the sample solution, and use the solution so obtained as the colored blank solution. Perform the test with the colored sample solution and the colored blank solution as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank, and determine the absorbances, A_T and A_B, at 420 nm.

$$\text{Units per g of } \beta\text{-Galactosidase (Penicillium)} = \frac{A_T - A_B}{0.459} \times \frac{1}{10} \times \frac{1}{W}$$

0.459: Absorbance of 1 μmol/10 mL of *o*-nitrophenol

W: Amount (g) of the sample in 0.5 mL of the sample solution.

Unit: One unit indicates an amount of the enzyme which decomposes 1 μmol of 2-nitrophenyl-β-D-galactopyranoside in 1 minute under the above conditions.

Containers and storage Containers—Tight containers.

Gardenia Fruit

Gardeniae Fructus

サンシシ

Gardenia Fruit is the fruit of *Gardenia jasminoides* Ellis (*Rubiaceae*).

Description Nearly long ovoid to ovoid fruit, 1 - 5 cm in length, 1 - 1.5 cm in width; usually having 6, rarely 5 or 7, markedly raised ridges; calyx or its scar at one end, and sometimes peduncle at the other end; inner surface of pericarp yellow-brown, smooth and lustrous; internally divided into two loculi, containing a mass of seeds in yellow-red to dark red placenta; seed nearly circular, flat, about 0.5 cm in major axis, blackish brown or yellow-red. Odor, slight; taste, bitter.