Designation of L-Isoleucine as a feed additive

Ministry of Agriculture, Forestry and Fisheries (MAFF) will designate L-Isoleucine as a feed additive and establish its standards and specifications in the ministerial ordinance. Outline of standards and specifications is as follows.

L-Isoleucine

Specifications for feed additives

Active Substance

Compositional specifications

Content: When this product is determined following a 4-hour drying at 105°C, it contains more than 90.0 % of L-Isoleucine ($C_6H_{13}NO_2$).

Physical and chemical properties

(1) It comes in white crystals or crystalline powder.

(2) pH of aqueous solution or aqueous suspension (1 part solid/100 parts solution) is 4.5 to 7.0.

Confirmation test:

(1) To 5 mL of a solution of it (1 part solid/1000 parts solution) add 1 mL of a ninhydrin solution (1 part solute/1000 parts solution), and heat for 3 minutes: a purple color develops.

Purity test:

(1) Specific rotation:

Weigh about 2 g of this product, previously dried at 105°C for 4 hours, and record its weight to the digit of 0.01 g. Dissolve it in 6 mol/L hydrochloric acid solution to make 50 mL, and filter if necessary: the specific rotation [α]20D of this solution is between +38.0° and +41.5°.

(2) Ammonium salt:

0.5 g(0.45-0.54 g) of this product is weighed, prepare the sample solution according to Method 3 of the Arsenic Test, and perform the test for arsenic according to the method using Equipment A: the color of the absorbing solution shall not be more intense than the standard color (not more than 3 μg/g).

(3) Lead:

Weigh 0.5 g (0.45 to 0.54 g) of this product, and perform the test for lead as directed in the Lead Test (Atomic Absorption Spectrophotometry Method 1): the amount of lead is not more than 2 μg/g. Pipet 0.5 mL of the Standard Lead Solution and add nitric acid (1 in 150) to make exactly 50 mL, and use this solution as the standard solution.

(4) Arsenic:

Place 1.0 g (0.95 to 1.04 g) of this product in a decomposition flask, add 10 mL of nitric acid and 5 mL of sulfuric acid, and heat gently. If the solution is brown in color, allow to cool, add 1 to 2 mL of nitric acid, heat, and repeat this procedure until the solution is colorless to pale yellow. After cooling, add 0.5 mL of perchloric acid, and heat until white fumes are evolved. After cooling, add 15 mL of a saturated solution of ammonium oxalate, and heat until white fumes are evolved again. After cooling, add water to make about 10 mL, and use this solution as the sample solution. Perform the test with the sample solution according to the method with Apparatus A. Observe the color of the absorbing solution: the color produced is not more intense than the standard color (not more than 2 μg/g).

Loss on drying: ≤ 2.0 % (5 g, Drying at 105 °C, 4 hours)

Ignition residue: ≤ 1.0 % (1 g)

Assay:

Weigh about 0.25 g of this product, previously dried at 105°C for 4 hours, record its weight to 4 significant figures, add water to dissolve and make exactly 250 mL. Use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography according to the following conditions. Determine the peak area of L-isoleucine in the chromatogram, calculate the concentration of L-isoleucine based on the calibration curve separately determined, and calculate the content.

Operating conditions

Apparatus: Use an apparatus consisting of 2 pumps for sending a mobile phase and coloring solution, sample injection port, column, reaction vessel, detector, and recorder. The column and reaction vessel can be kept at a constant temperature.

Detector: A fluorometric detector (excitation wavelength of 338 nm, fluorescence wavelength of 425 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 150 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: 60°C

Reaction vessel (reaction coil): A column about 0.25 mm in inside diameter and about 3.0 m in length.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate and 1.62 g of sodium 1-octane sulfonate in water to make 1000 mL. To this solution, add 266 mL of methanol, and adjust the pH to 2.5 with phosphoric acid.

Reaction solution: Dissolve 13.25 g of potassium hydroxide, 15.00 g of boric acid,

0.35 g of o-phthalaldehyde, 1 mL of 2-mercaptoethanol, 5 mL of methanol, and 1.25 mL of 3.5% polyoxyethylene (23) lauryl ether in water to make 1000 mL.

Flow rate of the mobile phase: 1.0 mL per minute

Flow rate of the reaction solution: 0.5 mL per minute

Reaction temperature: 60°C

Creation of calibration curve

Weigh about 0.25 g of L-isoleucine for assay and record its weight to the digit of 0.001 g, add water to dissolve and make exactly 50 mL. Use this solution as the standard stock solution. (1 mL of this solution contains 5 mg of L-isoleucine [$C_6H_{13}NO_2$]). Prior to use, dilute exactly a certain amount of the standard stock solution with water so that each mL of the solution contains 0.5 mg, 1.0 mg, and 1.5 mg. Where necessary, filter each solution through a membrane filter with a pore size of 0.45 μm, and use the filtrate as the standard solution. Perform the test with 5 μL each of the standard solutions as directed under Liquid Chromatography in the same manner as the sample solution. Determine the peak area of L-isoleucine in the chromatogram and prepare a calibration curve.

Standard for method of manufacture

Culture Corynebacterium glutamicum KCCM 80189 strain, filter the culture broth to remove bacteria after completion of the culture, and isolate the crude L-isoleucine crystal fraction. Furthermore, purify the crude crystals and dry the solid substances obtained.

Standard for method of storage

The products must be stored in well-closed containers..

Product

Compositional specifications

Same as the Compositional specifications by Active Substance of L-Isoleucine.

Standard for method of storage

Same as the standard for method of storage by Active Substance of L-Isoleucine.