Designation of Benzoic acid as a feed additive

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

Benzoic acid

Compositional specifications in general

The content of Benzoic acid in feed must be no more than 0.5%.

Specifications for method of manufacture of feed in general

Benzoic acid is allowed to be used as feed additives for pigs (no more than 70 kg of body weight) only.

Specifications for feed additives

Active Substance

Compositional specifications

Content: When this dried product is determined, it contains more than 99.5 % of Benzoic acid ($C_7H_6O_2$).

Physical and chemical properties

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

(2) Melting point: 121-123 °C

Confirmation test:

(1) Dissolve 1.0 g (0.95-1.04 g) of this product with 8 mL of 1 mol/L sodium hydroxide solution and add water to make 100 mL. When ferric chloride solution is added to this solution, light yellowish red precipitate is generated, and when dilute hydrochloric acid is added to this solution, white precipitate is generated.

Purity test:

(1) Lead:

2.0 g (1.95-2.04 g) of this product is weighed into a pot (platinum, quartz, or porcelain), and heat gradually until the sample begins to carbonize. Stop to heat, add 1 mL of sulfuric acid and heat gradually until the sample carbonizes and no white smoke of sulfuric acid is generated. If necessary, add more sulfuric acid and heat until the sample almost carbonizes. Cover the pot loosely, heat gradually and ignite at 450-600°C until the sample is incinerated. If any carbonized material remains, crush with a glass rod, moisten with 1 mL of sulfuric acid (1 in 4) and 1 mL of nitric acid, heat until no white smoke is

generated, and ignite to incinerate completely. To the residue add 10 mL of hydrochloric acid (1 in 4), heat on a water bath, and evaporate to dryness. To the residue add a small amount of nitric acid (1 in 100), and dissolve by warming. After cooling, add nitric acid (1 in 100) to make exactly 10 mL, and use this solution as the Sample solution. Pipet 0.4 mL of Standard Lead Solution into a 10-mL flask, add nitric acid (1 in 100) to the mark to make 10 mL, and use this solution as the Standard solution. Perform the test with the Sample solution and Standard solution as directed under Lead Tests (Atomic Absorption Spectrophotometry Method 1): the amount of lead shall be not more than 2 μg/g.

(2) Arsenic:

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) Phthalic acid:

To 0.10g (0.095-0.104 g) of this product, add 1 mL of water and 1 mL of resorcinol-sulfuric acid solution, heat in oil bath at 120-125°C to evaporate the water, and heat for 90 minutes. After cooling, dissolve the residue in 5 mL of water, to 1 mL of this solution, add 10 mL of sodium hydroxide solution (43 in 500) shake, and use this solution as the sample solution. Separately, dissolve 61 mg of potassium hydrogen phthalate (standard reagent) in water to make exactly 1000 mL. Pipet 1 mL of this solution, add 1 mL of resorcinol-sulfuric acid solution, proceed as directed for the sample solution, and use this solution as the control solution. When the sample solution and the control solution is irradiated with light of 470-490 nm: the green fluorescence of the sample solution shall not be more intense than that of the control solution (not more than 500 μg/g).

(4) Related substances:

Transfer 5.0g (4.95-5.04 g) of this product to a 25-mL volumetric flask, add 1 mL of propyl benzoate-dimethylformamide solution to dissolve, then add dimethylformamide to make 25 mL, and use this solution as the sample solution. Separately, weigh 40 mg each of propyl benzoate(NPB), biphenyl(BP), 2-methylbiphenyl(2MBP), 3-methylbiphenyl(3MBP), 4-methylbiphenyl(4MBP) and benzyl benzoate(BB), and 20 g of benzoic acid, transfer to a 100-mL volumetric flask, dissolve in about 50 mL of dimethylformamide, add dimethylformamide to make 100 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography according to the following conditions.

In the chromatogram of sample solution, identify the peaks of biphenyl(BP), 2-

methylbiphenyl(2MBP), 3-methylbiphenyl(3MBP), 4-methylbiphenyl(4MBP) and benzyl benzoate(BB) from the chromatogram of standard solution, and calculate the peak area Ac_1. For unknown peaks appearing between the peaks of 2-methylbiphenyl and benzoic acid, calculate sum of the peak areas Ac_1 as dimethylbiphenyl isomer. From Ac_1 and the peak area Ai_1 of propyl benzoate(NBP) calculated from the chromatogram of sample solution, the content of the related substances (biphenyl, 2-methylbiphenyl, 3-methylbiphenyl, 4-methylbiphenyl, benzyl benzoate and dimethylbiphenyl isomers) in the sample solution shall not exceed 100 µg/g when calculated by the following equation.

$$\text{Content of each component} = \frac{Ac_1 \times Mi_1 \times 1000}{RF_c \times Ai_1 \times Ms_1}$$

Ai1：peak area of NPB in the sample solution
Ac1：peak area of each component in the sample solution
Ms1：weight of sample in the sample solution（g）
Mi1：weight of NPB in the sample solution（mg）
RFc：Response factor of each component

Calculate the response factor (RFc) for each compound using the equation. For the calculation of dimethylbiphenyl isomers, use response factors of 3MBP.

$$\text{Response factor } (RF_c) = \frac{Ac_2 \times Mi_2}{Ai_2 \times Mc_2}$$

Ai2：the peak area of NPB in the standard solution
Ac2：the peak area of each component in the standard solution
Mc2：amount of each component in the standard solution（mg）
Mi2：amount of NPB in the standard solution（mg）

Operating condition:

Detector:

Hydrogen flame-ionization detector

Column:

A fused silica tube (inner diameter of 0.32 mm and length of 15 to 30 m) coated with polyethylene glycol modified with nitroterephthalic acid to a thickness of 1 µ m

Column temperature:

Hold at 100°C for 1 minute, then warm to 210°C at 3°C per minute.

Inlet temperature:

240°C (If the temperature programme can be set, the temperature rises to

270°C at 12°C per second at an initial temperature of 80°C.)

Detector temperature :

240-250° C

Carrier gas:

Helium

Flow rate:

Approx. 3 mL/min. (If pressure control is possible, hold at 47 kPa for 4.35 minutes, then boost to 58 kPa at 0.28 kPa/min.)

Injection method:

Split

Split ratio:

1:10

Loss on drying: ≤ 0.5 % (1 g, Drying with silica gel, 3 hours)

Ignition residue: ≤ 0.05 % (1 g)

Assay:

This product is dried and approximately 0.5 g of it is weighed to the digits of 0.001 g and the value is recorded. It is dissolved with 25 mL of neutralized ethanol and 25 mL of water, and titrate with 0.1 mol/L sodium hydroxide solution (indicator: 3 drops of indicator phenolphthalein test solution).

0.1 mol/L sodium hydroxide solution 1 mL = 12.21 mg $C_7H_6O_2$

Standard for method of manufacture

The products must be manufactured by air-oxidizing toluene.

Standard for method of storage

The products must be stored in airtight containers.

Product

Compositional specifications

Same as the Compositional specifications by Active Substance of Benzoic acid.

Standard for method of storage

Same as the standard for method of storage by Active Substance of Benzoic acid.