# GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

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# National Food Safety Standard - Determination of Biogenic Amine in Food

食品安全国家标准

食品中生物胺的测定

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# National Food Safety Standard - Determination of Biogenic Amine in Food

# Method I Liquid Chromatography

## 1 Scope

This Standard specifies the determination method for tryptamine, β-phenylethylamine, putrescine, cadaverine, histamine, octopamine, tyramine, spermidine and spermine content in food.

This Standard is applicable to the determination of alcohol (wine, beer and yellow rice wine, etc.), condiments (vinegar and soy sauce), aquatic products (fish and its products; shrimp and its products), biogenic amine in meat.

## 2 Principle

Aquatic products (fish and its products; shrimp and its products) and meat: use 5% trichloroacetic acid to extract sample; use n-hexane to remove fat. After extraction and purification through trichloromethane-n-butanol (1 + 1) solution, use Dansyl chloride for derivation. Use $C_{18}$ chromatographic column for separation. Use high-performance liquid chromatography-UV detector for detection. Use internal standard method for quantification.

Alcohol (wine, beer and yellow rice wine, etc.) and sediments (vinegar and soy sauce): use Dansyl chloride for derivation. Use $C_{18}$ chromatographic column for separation. Use high-performance liquid chromatography-UV detector for detection. Use internal standard method for quantification.

## 3 Reagents and Materials

Unless it is otherwise stipulated, all reagents used in this Method are analytically pure; water is Grade-1 water stipulated in GB/T 6682.

### 3.1 Reagents

**3.1.1** Acetonitrile ($CH_3CN$): chromatographically pure.

**3.1.2** Acetone ($C_3H_6O$): chromatographically pure.

**3.1.3** Ether($C_4H_{10}O$): re-evaporated.

**3.2.7** Saturated sodium bicarbonate solution: weigh-take 15 g of sodium bicarbonate; add 100 mL of water to dissolve it; take the supernatant as the saturated solution.

**3.2.8** 50 mg/mL sodium glutamate solution: accurately weigh-take 5.0 g of sodium glutamate; use saturated sodium bicarbonate solution to dissolve it and reach a constant volume of 100 mL.

**3.2.9** 0.01 mol/L ammonium acetate solution that contains 1% acetic acid: weigh-take 0.77 g of ammonium acetate; dissolve it in water; then, transfer it into a 1,000 mL volumetric flask. Add 10 mL of formic acid; use water to reach a constant volume to the scale.

**3.2.10** Mobile phase A: measure-take 100 mL of 0.01 mol/L ammonium acetate solution that contains 1% acetic acid; add it to 900 mL of acetonitrile.

**3.2.11** Mobile phase B: measure-take 900 mL of 0.01 mol/L ammonium acetate solution that contains 1% acetic acid; add it to 100 mL of acetonitrile.

### 3.3 Standard Substances

**3.3.1** Histamine dihydrochloride ($C_5H_9N_3 \bullet 2HCl$, CAS No.: 56-92-8) standard substance (purity > 99%).

**3.3.2** β-phenylethylamine hydrochloride ($C_8H_{11}N \bullet HCl$, CAS No.: 64-04-0) standard substance (purity > 98%).

**3.3.3** Tyramine hydrochloride ($C_8H_{11}NO \bullet HCl$, CAS No.: 60-19-5) standard substance (purity > 98%).

**3.3.4** Putrescine dihydrochloride ($C_4H_{12}N_2 \bullet 2HCl$, CAS No.: 333-93-7) standard substance (purity > 98%).

**3.3.5** Cadaverine dihydrochloride ($C_5H_{14}N_2 \bullet 2HCl$, CAS No.: 1476-39-7) standard substance (purity > 98%).

**3.3.6** Tryptamine hydrochloride ($C_{10}H_{12}N_2 \bullet HCl$, CAS No.: 61-54-1) standard substance (purity > 99%).

**3.3.7** Spermine tetrahydrochloride ($C_{10}H_{26}N_4 \bullet 4HCl$, CAS No.: 306-67-2) standard substance (purity > 97%).

**3.3.8** Spermidine trihydrochloride ($C_7H_{19}N_3 \bullet 3HCl$, CAS No.: 334-50-9) standard substance (purity > 97%).

**3.3.9** Octopamine hydrochloride ($C_8H_{11}NO_2 \bullet HCl$, CAS No.: 770-05-9) standard substance (purity > 97%).

**3.3.10** 1,7-Diaminoheptane ($C_7H_{18}N_2$, CAS No.: 646-19-5) internal standard substance

**5.1.3.1** Fat removal: transfer-take 10 mL of the above-mentioned sample extract into a 25 mL test tube with a plug. Add 0.5 g of sodium chloride; conduct vortex oscillation, till sodium chloride completely dissolves. Then, add 10 mL of n-hexane; conduct vortex oscillation for 5 min. Place it still for stratification; then, discard the upper layer of organic phase. Add 10 mL of n-hexane to the lower layer of sample solution to conduct fat removal one more time.

**5.1.3.2** Extraction: transfer-take 5 mL of the above-mentioned post-fat-removal sample solution into a 10 mL centrifuge tube with a plug. Use 5 mol/L sodium hydroxide solution (several drops) to adjust pH to around 12.0. Add 5 mL of N-butanol/trichloromethane (1 + 1) mixing solution. Conduct vortex oscillation for 5 min. At 5,000 r/min, conduct centrifugation for 5 min. Then, transfer the upper layer of organic phase into another 10 mL centrifuge tube with a plug. Re-extract the lower layer of the sample solution; combine the extract. Use N-butanol/trichloromethane (1 + 1) to dilute to the scale. Take 5 mL of the extract; add 200 μL of hydrochloric acid (1 mol/L); thoroughly mix it up. In 40 °C water bath, use nitrogen to blow it to dryness. Add 1 mL of hydrochloric acid (0.1 mol/L); conduct vortex oscillation. Thoroughly dissolve the residue. Reserve it for derivation.

**5.1.4 Derivation**

**5.1.4.1** Derivation of sample: successively add 1 mL of saturated sodium bicarbonate solution, 100 μL of sodium hydroxide solution (1 mol/L) and 1 mL of derivatization reagent to the above-mentioned sample solution, which is reserved for derivation. Conduct vortex and mixing for 1 min, then, place it in 60 °C constant-temperature water bath to conduct derivation for 15 min. Then, take it out; add 100 μL of sodium glutamate solution; conduct oscillation mixing. At 60 °C, conduct constant-temperature reaction for 15 min. Take it out, then, cool it down to room temperature. Add 1 mL of water to each centrifuge tube; conduct vortex mixing for 1 min. In 40 °C water bath, use nitrogen to blow it, so as to remove acetone (around 1 mL). Add 0.5 g of sodium chloride; conduct vortex oscillation, till sodium chloride completely dissolves. Then, add 5 mL of ether; then, conduct vortex oscillation for 2 min; place it still for stratification. Then, suck out the upper layer of organic phase (the ether layer); re-extract once. Combine the ether extract; in 40 °C water bath, use nitrogen to blow it to dryness. Add 1 mL of acetonitrile to conduct vortex oscillation, so that the residue can completely dissolve. Use 0.22 μm membrane needle filter to filter it into a small sample injection bottle. Reserve it for determination.

**5.1.4.2** Derivation of the standards: respectively transfer-take 1 mL of biogenic amine standard series solution; place it in a 10 mL test tube with a plug. Successively add 250 μL of internal standard working solution (100 mg/L). The following operation is the same as the steps of sample derivation.

**5.2 Alcohol and Condiments (vinegar and soy sauce)**

**5.2.1** Sample derivation: accurately measure-take 1.0 mL of sample; place it into a 15

# Method II Spectrophotometry

## 9 Scope

This Standard specifies the determination method for histamine content in aquatic products.

This Standard is applicable to the determination of histamine in aquatic products (fish and its products; shrimp and its products).

## 10 Principle

Take trichloroacetic acid as the extraction solution; conduct oscillation extraction. Through extraction and purification with N-pentanol, histamine and azo reagent trigger chromogenic reaction. Then, use spectrophotometer for detection; use external standard method for quantification.

## 11 Reagents and Materials

Unless it is otherwise stipulated, all reagents used in this Method are analytically pure; water is Grade-1 water stipulated in GB/T 6682.

### 11.1 Standards and Reagents

**11.1.1** Histamine phosphate ($C_5H_9N_3 \bullet 2H_3PO_4$).

**11.1.2** N-pentanol ($C_5H_{12}O$).

**11.1.3** Trichloroacetic acid ($C_2HCl_3O_2$).

**11.1.4** Sodium carbonate ($Na_2CO_3$).

**11.1.5** Sodium hydroxide (NaOH).

**11.1.6** Hydrochloric acid (HCl, 37%).

**11.1.7** P-nitroaniline ($C_6H_6N_2O_2$).

**11.1.8** Sodium nitrite ($NaNO_2$).

### 11.2 Preparation of Reagents

**11.2.1** Histamine standard stock solution: at 100 °C (± 5 °C), dry histamine phosphate standard for 2 h. Then, weigh-take 0.2767 g (accurate to 0.001 g) of it, place it in a 50 mL beaker. Use a proper amount of water to completely dissolve it, then, transfer it into

mark them. Store them at –20 °C.

### 12.2 Sample Analysis

#### 12.2.1 Sample extraction

Accurately weigh-take 10 g (accurate to 0.01 g) of sample, which is already minced and homogenized. Place it into a 100 mL conical flask with a plug. Add 20 mL of 10% trichloroacetic acid solution to soak it for 2 h ~ 3 h. Conduct oscillation for 2 min, then, mix it up. Use filter paper to filter it. Accurately absorb 2.0 mL of the filtrate, then, place it in a separating funnel. Dropwise add sodium hydroxide solution to adjust pH between 10 ~ 12. Add 3 mL of N-pentanol, then, conduct oscillation extraction for 5 min. Place it still for stratification. Transfer the N-pentanol extract (the upper layer) to 10 mL scale test tube. Extract N-pentanol for 3 times; combine the extract; use N-pentanol to dilute to the scale. Absorb 2.0 mL of N-pentanol extract into separating funnel; add 3 mL of hydrochloric acid; conduct oscillation extraction. Place it still for stratification. Transfer the hydrochloric acid extract (the lower layer) to 10 mL scale test tube. Conduct extraction for 3 times; then, combine the extract; use hydrochloric acid solution to dilute it to the scale.

#### 12.2.2 Determination

Respectively absorb 0, 0.20, 0.40, 0.60, 0.80 and 1.0 mL of histamine standard working solution (equivalent to 0, 4.0, 8.0, 12, 16 and 20 μg of histamine) and 2.0 mL of sample extract into a 10 mL colorimetric tube. Add water to 1 mL, then, add 1 mL of hydrochloric acid solution; mix it up. Add 3 mL of sodium carbonate solution and 3 mL of azo reagent. Add water to the scale, mix it up; place it for 10 min. Transfer “0” tube solution into a 1 cm cuvette; adjust the wavelength of the spectrophotometer to 480 nm; adjust the absorbance to “0”. Then, successively test the absorbance of the series standard solution and the sample solution. Take absorbance A as the vertical axis; take the mass of histamine as the horizontal axis; draw a standard curve.

## 13 Result Calculation

The content of histamine in the sample shall be calculated in accordance with Formula (2):

$$X = \frac{m_1 V_1 \times 10 \times 10}{m_2 \times 2 \times 2 \times 2} \times \frac{100}{1\,000} \qquad \cdots\cdots (2)$$

Where:

$X$---the content of histamine in the sample, expressed in (mg/100 g);

$m_1$---the mass of histamine which is corresponding with the absorbance value of histamine in the sample, expressed in (μg);

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