NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.282-2020

National food safety standard - Determination of 1-methylimidazole, 2-methylimidazole and 4-methylimidazole in foods

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1 Scope

This standard specifies the method for the determination of 1-methylimidazole, 2-methylimidazole, and 4-methylimidazole in food by liquid chromatography-tandem mass spectrometry.

This standard applies to the determination of the content of 1-methylimidazole, 2-methylimidazole, and 4-methylimidazole in condensed milk, frozen drinks, jams, soy products, cocoa and chocolate products, candy, wheat flour products, biscuits, fillings and surface slurries, meat products, flavored syrups, condiments (soy sauce, vinegar, sauce and sauce products, cooking wine, compound seasoning), beverages, wine, jelly, and puffed food.

2 Principles

After adding the isotope internal standard to the sample, carry out the extraction with water or acid water, perform the purification and concentration by a solid-phase extraction column, determine by a liquid chromatography-tandem mass spectrometer, and use the isotope internal standard method for quantification.

3 Reagents and materials

Unless otherwise specified, the reagents used in this method are all analytical reagents, and the water is the grade I water specified in GB/T 6682.

3.2 Reagent preparation

3.2.1 2% formic acid aqueous solution. Measure out 20 mL of formic acid, add it to 980 mL of water, and mix well.

3.2.2 Ammonia methanol solution. Measure out 50 mL of ammonia water, add it to 950

mL of methanol, and mix well.

3.2.3 Ammonium acetate solution (5 mmol/L). Weigh 0.39 g of ammonium acetate, dissolve it in 1000 mL of water, and mix well.

3.2.4 Acetonitrile-ammonium acetate solution (9+1). Mix acetonitrile and ammonium

acetate solution (5 mmol/L) uniformly in a volume ratio of 9.1.

3.3 Standards

3.3.1 1-methylimidazole standard (C4H6N2, CAS number. 616-47-7). purity >=98%, or a standard substance certified by the country and granted a standard substance certificate.

3.3.2 2-methylimidazole standard (C4H6N2, CAS number. 693-98-1). purity >=98%, or a standard substance certified by the country and granted a standard substance certificate.

3.3.3 4-methylimidazole standard (C4H6N2, CAS number. 822-36-6). purity >=98%, or a standard substance certified by the country and granted a standard substance certificate.

3.3.4 Isotope internal standard 1-methylimidazole-D6 (C4H6N2, CAS number. 285978-

27-0). purity >=98%.

3.3.5 Isotopic internal standard 2-methylimidazole-D6 (C4H6N2, CAS number.

1173022-19-9). purity >=98%.

3.3.6 Isotope internal standard 4-methylimidazole-D6 (C4H6N2, CAS number. 1219804-

79-1). purity >=98%.

3.4.1 Standard stock solution (1 mg/mL). Accurately weigh 25 mg of 1-

methylimidazole standard (the weight shall be accurate to 0.01 mg), add 1 mL of water to dissolve it, and transfer it with acetonitrile to a 25 mL brown volumetric flask; make up to the

mark and shake well. Accurately weigh 25 mg of 2-methylimidazole standard and 4-methylimidazole standard respectively (the weight shall be accurate to 0.01 mg), dissolve them with acetonitrile, and transfer to 25mL brown volumetric flasks;

respectively dilute to the mark and shake well. Keep them sealed at -20 degrees C and away from light, then they are valid for 6 months.

3.4.2 Mixed standard working solution (2.0 ug/mL). Pipette 100 uL of each of the above

standard stock solutions into the same 50 mL brown volumetric flask, add acetonitrile to dilute to the mark, and shake well; then, the mixed standard solution of 2.0 ug/mL is obtained. Keep this solution sealed at 4 degrees C and away from light, then it is valid for 3 months.

3.4.3 Isotope internal standard stock solution (1 mg/mL). Accurately weigh 10 mg of

1-methylimidazole-D6, 2-methylimidazole-D6, and 4-methylimidazole-D6 respectively (the weight shall be accurate to 1 mg), dissolve them with acetonitrile, and transfer to

10 mL brown volumetric flasks; dilute to the mark and shake well. Keep them sealed at -20 degrees C and away from light, then they are valid for 6 months.

3.4.4 Isotope internal standard mixed working solution (1.0 ug/mL). Pipette 100 uL of

each of the above isotope internal standard stock solutions into the same 100 mL brown volumetric flask, dilute to the mark with acetonitrile, and shake well; then, the isotopic internal standard mixed working solution of 1.0 ug/mL is obtained. Keep this solution sealed at 4 degrees C and away from light, then it is valid for 3 months.

3.4.5 Standard series solutions. Pipette 25 uL, 75 uL, 150 uL, 300 uL, 600 uL, and

1000 uL of mixed standard working solutions into 5 mL brown volumetric flasks, respectively, add 250 uL of isotope internal standard mixed working solution, and use acetonitrile- ammonium acetate solution (9+1) to dilute the mixed solution to the scale;

then, the mixed standard series solution of 1-methylimidazole, 2-methylimidazole and 4-methylimidazole are prepared, and the mass concentration is 10 ng/mL, 30 ng/mL,

60 ng/mL, 120 ng/mL, 240 ng/mL, 400 ng/mL respectively. In the actual sample determination, the added amount and dilution multiple of the isotope internal standard mixed working solution can be adjusted according to the sample concentration.

3.5.1 Mixed cation exchange solid phase extraction column. N-vinylpyrrolidone-

divinylbenzene copolymer matrix-sulfonic acid group, 150 mg, 6 mL, or equivalent.

Before use, activate it with 5 mL methanol first, and then rinse it with 5 mL water to balance.

3.5.3 Organic filter membrane. The pore size shall be 0.22 um.

volume to 20 mL, and shake well; put it in a centrifuge tube, and centrifuge at 10,000 r/min for 5 min; the supernatant is to be purified later.

5.1.2.2 Semi-fluid samples (such as jam, oyster sauce, flavored syrup, frozen drinks)

and gum-based candy, tapioca balls Weigh 2 g of uniformly mixed sample (the weight shall be accurate to 0.001 g), and put it in a 50 mL centrifuge tube; add 100 uL of 1.0 ug/mL isotope internal standard mixed working solution, and mix well; add 20 mL of water accurately, vortex for 1 min, and heat it in a water bath at 70 °C for 10 min. After it cools to room temperature, perform the ultrasonic extraction for 20 min, and centrifuge at the speed of 10,000 r/min and the temperature of 4 degrees C for 5 min; the supernatant is to be purified later.

5.1.2.3 Jelly Weigh 2 g of uniformly mixed sample (the weight shall be accurate to 0.001g), and put it in a 50 mL centrifuge tube; add 100 uL of 1.0 ug/mL isotope internal standard mixed working solution, and mix well; add 2 g of sodium chloride, accurately add 20 mL of water, vortex for 1 min, and heat it in a 70 degrees C water bath for 10 min. After it cools to room temperature, perform the ultrasonic extraction for 20 min, and centrifuge at the speed of 10,000 r/min and the temperature of 4 degrees C for 5 min; the supernatant is to be purified later.

5.1.2.4 Condensed milk, cocoa and chocolate products Weigh 2 g of uniformly mixed sample (the weight shall be accurate to 0.001 g), and put it in a 50 mL centrifuge tube; add 100 uL of 1.0 ug/mL isotope internal standard mixed working solution, and mix well; accurately add 20 mL of 2% formic acid aqueous solution, and heat it in a 60 °C water bath for 5 min; add 2 homogeneous particles, vortex for 5 min, and centrifuge at 10,000 r/min for 5 min; the supernatant is to be purified later.

5.1.2.5 Other samples such as bean products, sauces and sauce products Weigh 2 g of uniformly mixed sample (the weight shall be accurate to 0.001 g), and put it in a 50 mL centrifuge tube; add 100 uL of 1.0 ug/mL isotope internal standard mixed working solution, and mix well; add 20 mL of water accurately, homogenize for 1 min, and centrifuge at the speed of 10,000 r/min and the temperature of 4 °C for 5 min; the supernatant is to be purified later.

5.1.3 Sample purification

5.1.3.1 Soy sauce, vinegar, beverages, wine, condensed milk, cocoa and chocolate