GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

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National Food Safety Standard - Determination of Heterocyclic Amine Substances in High-temperature Cooked Foods

食品安全国家标准

高温烹调食品中杂环胺类物质的测定

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1 Scope

This Standard specifies the liquid chromatography-mass spectrometry/mass spectrometry for the determination of heterocyclic amines in high-temperature cooked foods such as 2-amino-3,4-dimethylimidazo [4,5-f] quinoline (MeIQ), 2-amino-3,8-dimethylimidazo [4,5-f] quinoline (MeIQx), 2-amino-3,4,8-trimethylimidazo [4,5-f] quinoline (4,8-DiMeIQx), 2-amino-3,7,8-trimethylimidazo [4,5-f] quinoline (7,8-DiMeIQx), 2-amino-1-methyl-6-phenyl-imidazo [4,5-b] pyridine (PhIP).

This Standard applies to the determination of MeIQ, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, PhIP in grilled fish, barbecue, and their products.

2 Principles

The sample is extracted using sodium hydroxide/methanol solution, purified by solid-phase extraction column, detected by liquid chromatography-tandem mass spectrometry, and quantified by internal standard method.

3 Reagents and materials

Unless otherwise specified, the reagents used are analytically pure; and the water is Grade 1 water specified in GB/T 6682.

3.1 Reagents

3.1.1 Sodium hydroxide.

3.1.2 Ammonium acetate: Purity≥98%.

3.1.3 Methanol: chromatographically pure.

3.1.4 Ethanol: chromatographically pure.

3.1.5 N-hexane: chromatographically pure.

3.1.6 Dichloromethane: chromatographically pure.

3.1.7 Acetonitrile: chromatographically pure.

3.1.8 Glacial acetic acid: chromatographically pure.

3.2 Reagents preparation

3.2.1 Sodium hydroxide solution (40 g/L): WEIGH 40.0 g of sodium hydroxide; USE water to dissolve and dilute to 1 L.

3.2.2 Sodium hydroxide solution (4 g/L): MEASURE and TAKE 50 mL of 40 g/L sodium hydroxide solution (3.2.1); ADD 450 mL of water; MIX well.

3.2.3 40 g/L sodium hydroxide-methanol mixed solution (70+30, volume fraction): MEASURE and TAKE 70 mL of 40 g/L sodium hydroxide solution (3.2.1); ADD 30 mL of methanol; MIX well.

3.2.4 4 g/L sodium hydroxide-methanol mixed solution (45+55, volume fraction): MEASURE and TAKE 45 mL of 4 g/L sodium hydroxide solution (3.2.2); ADD 55 mL of methanol; MIX well.

3.2.5 Ethanol-dichloromethane mixed solution (10+90, volume fraction): MEASURE and TAKE 10 mL of ethanol; ADD 90 mL of dichloromethane; MIX well.

3.2.6 Acetonitrile-water solution (5+95, volume fraction): MEASURE and TAKE 5 mL of acetonitrile; ADD 95 mL of water; MIX well.

3.2.7 Acetic acid-ammonium acetate buffer solution: WEIGH 1.155 g of ammonium acetate; USE 450 mL of water to dissolve; USE acetic acid to adjust the pH to 5.0±0.5; ADD water to dilute to 500 mL.

3.2.8 Acetic acid buffer solution-acetonitrile mixed solution (50+50, volume fraction): MEASURE and TAKE 50 mL of acetic acid-ammonium acetate buffer solution (3.2.7); ADD 50 mL of acetonitrile; MIX well.

3.3 Standards

3.3.1 Heterocyclic amine standard substances: MeIQ ($C_{12}H_{12}N_4$, 77094-11-2), MeIQx ($C_{11}H_{11}N_5$, 77500-04-0), 4,8-DiMeIQx ($C_{12}H_{13}N_5$, 95896-78-9), 7,8-DiMeIQx ($C_{12}H_{13}N_5$, 92180-79-5), PhIP ($C_{13}H_{12}N_4$, 105650-23-5). The purity is greater than 99%.

3.3.2 Internal standard standard substance 4,7,8-TriMeIQx ($C_{13}H_{15}N_5$, 132898-07-8). The purity is greater than 99%.

5 Analytical procedures

5.1 Sample preparation

The edible portion from grilled fish, barbecue, and their products is taken, mashed, and mixed; and after being labelled, stored frozen at -18 °C.

5.2 Sample processing

5.2.1 Extraction: WEIGH 2 g (accurate to 0.01 g) of sample into a 50 mL centrifuge tube; ADD 200 μL of internal standard working solution (3.4.4); and then ADD 9.8 mL of 40 g/L sodium hydroxide-methanol mixed solution (3.2.3); homogenize for 1 min. The homogenizer cutter head is washed twice using 5.0 mL of 40 g/L sodium hydroxide-methanol mixed solution (3.2.3). The washing liquid is combined into a sample extraction centrifuge tube. The sample is centrifuged at 10000 r/min for 10 min to be purified.

5.2.2 Purification: The solid-phase extraction column (3.5.2) is activated in advance subsequently using 2 mL of methanol and 3 mL of 4 g/L sodium hydroxide solution (3.2.2). MEASURE and TAKE 10 mL of extract into the solid-phase extraction column. After discarding the effluent, subsequently USE 3 mL of 4 g/L sodium hydroxide-methanol mixed solution (3.2.4) and 2 mL of n-hexane to rinse. After each rinse, the rinse solution in the column shall be drained. Finally, USE 1.5 mL of ethanol-dichloromethane solution (3.2.5) to elute. The elution flow rate shall be less than 1 mL/min. After the eluent is nitrogen-concentrated in a water bath at 35 °C to near dryness, 1.0 mL of acetic acid buffer solution-acetonitrile mixed solution (3.2.8) is added; vortex-mixed; and filtered through the microporous membrane (3.5.1) to the injection vial, for assay determination on the machine.

5.3 Determination

5.3.1 Liquid chromatographic conditions

5.3.1.1 Chromatographic column: C_{18} column (2.5 μm, 100 mm×2.1 mm) or equivalent.

5.3.1.2 Mobile phase: A is acetic acid-ammonium acetate buffer solution (3.2.7). B is acetonitrile. For the gradient elution procedures, see Appendix A, A.1.

5.3.1.3 Flow rate: 0.3 mL/min.

5.3.1.4 Column temperature: 40 °C.

5.3.1.5 Injection volume: 5 μL.

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