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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 5009.223-2014

**National Food Safety Standard -- Determination of ethyl amino
acid ethyl ester in food**

食品安全国家标准 食品中氨基甲酸乙酯的测定
(English Translation)

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Table of Contents

1 Scope
3 Reagents and materials
3.1 Reagent
3.2 Reagent preparation
3.3 Standard
3.4 Standard Preparation
4 Instruments and equipment
5 Step analysis
5.1 Sample Preparation
5.2 Instrument reference conditions
5.3 Qualitative determination
6 Expression analysis results
7 Precision
8 Other

1 Scope

This standard specifies the beer, wine, rice wine, soy sauce, white wine and other alcoholic and ethyl carbamate content by gas chromatography - mass spectrometry Determination.

This standard applies to the determination of soy sauce and a urethane content of beer, wine, rice wine, wine and other alcoholic beverages.

Principle 2

After the sample was added D5- urethane internal standard, solid-phase through celite alkaline extraction cartridge eluate was concentrated by gas chromatography Spectrum - mass spectrometer measurement, the amount of internal standard.

3 Reagents and materials

NOTE: Unless otherwise indicated, the reagents used were of analytical grade standard, water as three water GB/T 6682 provisions.

3.1 Reagent

3.1.1

anhydrous sodium sulfate (Na_2SO_4).

3.1.2

Sodium chloride (NaCl).

3.1.3

hexane (C_6H_{14}). chromatography.

3.1.4

ethyl acetate ($\text{C}_4\text{H}_8\text{O}_2$). chromatography.

3.1.5

ether ($\text{C}_4\text{H}_{10}\text{O}$). chromatography.

3.1.6

methanol (CH_4O). chromatography.

3.1.7

basic Celite SPE. packing 4000mg, column capacity 12mL.

3.2 Reagent preparation

3.2.1

anhydrous sodium sulphate. 450 °C baking 4h, cooled and then stored in a desiccator.

3.2.2 5% ethyl acetate - ether solution. Take 5mL of ethyl acetate, diluted with ether to 100mL, and mix until use.

3.3 Standard

3.3.1

urethane standards ($\text{C}_3\text{H}_7\text{O}_2\text{N}$, CAS. 51-79-6). purity greater than 99.0%.

3.3.2

D5- urethane standards ($\text{C}_3\text{H}_2\text{D}_5\text{NO}_2$, CAS. 73962-07-9). purity greater than 98.0%.

3.4 Standard Preparation

3.4.1

D5- urethane stock solution (1.00mg/mL). 0.01g accurately weighed (accurate to 0.0001g)

D5- urethane standard Standard products, dissolved in methanol, the volume to less 10mL,

4 °C stored.

3.4.2

D5- using a liquid urethane (2.00µg/mL). Accurately suck D5-urethane stock solution (1.00mg/mL) 0.10mL, with methanol to 50mL, at 4 °C or less.

3.4.3

urethane stock solution (1.00mg/mL). 0.05 g of accurately weighed (accurate to 0.0001g) urethane standards, Dissolved in methanol, the volume to 50mL, 4 °C below Save to 3 months.

3.4.4

urethane intermediate liquid (10.0µg/mL). Accurately suck urethane stock solution (1.00mg/mL) 1.00mL, with Methanol to 100mL, 4 °C below Save to 1 month.

3.4.5

urethane intermediate solution (0.50µg/mL). Accurately suck liquid urethane intermediate (10.0µg/mL) 5.00mL, with A Alcohol volume to 100mL, now with the current.

3.4.6

standard curve working solutions. imbibe liquid urethane intermediate (0.50µg/mL) 20.0µL, 50.0µL, 100.0µL, 200.0µL, 400.0µL and urethane intermediate standard solution (10.0µg/mL) 40.0µL, 100.0µL 1mL volumetric flask to 7 , Each plus 2.00µg/mL D5- urethane used was 100 L, with methanol to volume, to give 10.0ng/mL, 25.0ng/mL, 50.0ng/mL, 100ng/mL, 200ng/mL, 400ng/mL, 1000ng/mL working solution of the standard curve, now Service is used.

4 Instruments and equipment

4.1

Gas chromatography - mass spectrometer with electron impact source (EI) source.

4.2

vortex shaker.

4.3

Nitrogen blowing instrument.

4.4

SPE with a vacuum pump.

4.5

ultrasonic cleaning machine.

4.6

muffle furnace.

4.7

Balance. a sense of 0.1mg and 1mg.

5.1 Sample Preparation

Shake the sample, 2g weighed (accurate to 0.001 g of) the sample (after weighing degassed beer ultrasonic 5min), plus 100.0μL 2.00μg/mL After D5- urethane working solution, 0.3 g of sodium chloride (if the sauce is not sodium chloride), sonicate to dissolve, mix, loaded onto celite alkaline solid Phase extraction column under vacuum, the sample solution was slowly penetrate into the extraction column, and allowed to stand 10min. After rinsing 10mL hexane, Solution in diethyl ether/min flow rate to about 1mL eluted, eluant containing 2g anhydrous sodium glass - with 10mL 5% ethyl acetate After dehydration funnel, collected in a 10mL graduated test tube, gently blowing with nitrogen at room temperature and about 0.5 mL, diluted with methanol to 1.00mL made it was measured for GC/MS analysis.

5.2 Instrument reference conditions

Gas chromatography - mass spectrometer reference conditions.

- capillary column. DB-INNOWAX, 30m x 0.25mm (internal diameter) x 0.25μm (film thickness) column or equivalent;
- Inlet temperature. 220 °C;
- Column temperature. initial temperature 50 °C, 1min, to then 8 °C/min was raised to 180 °C, the program finishes running, 240 °C for 5min;
- Carrier gas. Helium, purity ≥99.999%, flow rate 1mL/min;
- Ionization mode. Electron ionization (EI), energy of 70 eV;
- quadrupole temperature. 150 °C;
- Ion source temperature. 230 °C;
- Transfer line temperature. 250 °C;
- Solvent delay. 11min;
- Injection mode. splitless;
- injection volume. 1μL ~ 2μL;
- Detection method. selected ion monitoring (the SIM);