National Standards of People's Republic of China

GB 5009.223-2014

National Food Safety Standard

Determination of Urethane in Foods

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Issued by National Health and Family Planning Commission

National Standard for Food Safety

Determination of Urethane in Foods

1. Scope

This standard specifies the determination of urethane content in beer, wine, rice wine, liquor and other alcoholic as well as in soy sauce by gas chromatography - mass spectrometry.

This standard applies to determination of urethane content in beer, wine, rice wine, liquor and other alcoholic as well as in soy sauce.

2. Principle

After the specimen is added with D5-urethane internal standard substance, it will be purified and eluted by alkaline diatomite SPE column. The elution solution will be calibrated by gas chromatography - mass spectrometry after it is being concentrated. Limit of qualification will be fixed by internal standard method.

3. Reagents and materials

Note: except otherwise specified, all reagents used in this method are analytical pure reagents and water are Class III water specified in GB/T6682.

3.1 Reagents

3.1.1 Anhydrous sodium sulfate (Na2SO4) .

3.1.2 Sodium chloride (NaCI).

3.1.3 Hexane (C6H14): chromatographic pure.

3.1.4 Ethyl acetate (C4H8O2): chromatographic pure.

3.1.5 Ether (C4H10O 2): chromatographic pure.

3.1.6 Methanol (CH4O): chromatographic pure.

3.1.7 Alkaline diatomite SPE column: packing of 4,000 mg, and column capacity of 12 ml.

3.2 Reagent compounding

3.2.1 Anhydrous sodium sulfate: baked for 4h at 450℃and stored in a dryer for standby application after it cools down.

3.2.2 5% ethyl acetate - ether solution: take 5 ml of ethyl acetate, dilute it with ether solution to 100 ml, and mix it well.

3.3 Standard products

3.3.1 Urethane standard product (C3H7O2N, CAS: 51-79-6): with a purity greater than 99.0%.

3.3.2 D5-urethane standard product (C3H2D5NO2, CAS: 73962-07-9): with a purity greater than 98.0%.

3.4 Formulation of standard solution

3.4.1 D5-urethane stock solution (1.00 mg/ml); weigh and take 0.01g (accurate to 0.0001 g) of D5-urethane standard product, dissolve it with methanol solution, dilute it to a constant volume of 10 ml, and keep it at 4℃

3.4.2 D5- urethane use solution (2.00 μg/ml): imbibe 0.10 ml of D5-urethane use solution (1.00 mg/ml), dilute it with methanol to 50 ml, and store it at 4℃or below.

3.4.3 Urethane stock solution (1.00 mg/ml): weigh and take 0.05g (accurate to 0.0001g) of urethane standard product, dissolve it with methanol and dilute it to constant volume of 50 ml, and store at 4℃or below. Shelf life lasts 3 months.

3.4.4 Urethane intermediate solution (10.0 μg/ml): imbibe and take 1.00 ml of urethane stock solution (1.00 mg/ml), dilute it with methanol to a constant volume of 100 ml, and store at 4℃or below. The shelf life lasts 1 month.

3.4.5 Urethane intermediate solution (0.50 μg/ml): imbibe and take 5.00 ml of urethane intermediate solution (10.0 μg/ml), and dilute it by methanol to a constant volume of 100 ml, which is to be prepared right away when needed.

3.4.6 Standard curve working solution: imbibe and take 20.0 μl, 50.0 μl, 100.0 μl, 200.0 μl, and 400.0 μl of urethane intermediate solution (0.50 μg /ml) as well as 40.0 μl and 100.0 μl of urethane intermediate solution (10.0μg /ml) respectively; transfer them into seven 1 ml volumetric flasks; add 100 μl of D5-urethane use solution with a concentration of 2.00 μg/ml respectively and dilute it by methanol to mark; standard curve solutions with a concentration of 10.0 ng/ml, 25.0 ng/ml, 50.0 ng/ml, 100 ng/ml, 200 ng/ml , 400 ng/ml, and l000 ng/ml result. It shall be prepared right away when needed.

4 Equipment and facilities

4.1 Gas chromatography - mass spectrometer, equipped with electron bombardment source (EI)

4.2 Vortex mixer

4.3 Nitrogen blowing instrument

4.4 Solid phase extraction device, equipped with vacuum pump

4.5 Ultrasonic cleaning machine

4.6 Muffle furnace

4.7 Balances: sensor volume of 0. 1 mg and 1mg.

5 Analytical procedures

5.1 Specimen preparation

Shake up the specimen; weigh and take 2 g (accurate to 0.001 g) of specimen (weigh and take 5min later after beer is ultrasonically degassed); add 100.0 μl of D5-urethane use solution with a concentration of 2.00 μg/ml as well as 0.3 g of sodium chloride (there is no need to add sodium chloride in case of soy sauce); dissolve it ultrasonically, mix it well and add specimen onto alkaline diatomite SPE column; let the specimen seep into the SPE column slowly under the vacuum condition and let it stand for 10 min. After being

sprinkled and washed by 10 ml of n-hexane, elute it by 10 ml of 5% ethyl acetate-ether solution at a flow rate of approx. 1 ml/min. After being dehydrated by a glass funnel containing 2 g of anhydrous sodium sulfate, the resulting elution solution will be loaded into a 10 ml graduated test tube; blow it slowly by nitrogen gas to 0.5 ml or so at room temperature, dilute it to a constant volume of 1.00 ml by methanol, thus getting assay solution prepared, which might be used for GC / MS analysis.

5.2 Reference conditions for equipment

- Reference conditions of gas chromatography - mass spectrometer analysis:
- Capillary column: DB-INNOWAX, 30 m × 0.25 mm (inner diameter) × 0.25 μm (film thickness) or equivalent capillary column
- Injection port temperature: 220℃
- Column temperature: initial temperature of 50℃, holding for 1min and rising to 180℃ at a rate of 8℃/min; after program running finishes, run 5 min after the temperature reaches 220℃;
- Carrier gas: helium gas, purity≥99.999%, a flow rate of 1ml/min;.
- Ionization mode: electron bombardment source (EI), with an energy of 70 eV;
- Quadrupole temperature: 150℃
- Ion source temperature: 230℃
- Transfer line temperature: 250℃
- Solvent delay: 11min
- Injection mode: splitless
- Injection volume: 1 μl ~ 2 μl
- Detection mode: selected ion monitoring (SIM)
- Urethane selected ion monitoring (m/z):44, 62, 74, 89, quantitative ion 62;
- D5-urethane selected ion monitoring (m/z) 64, 76, quantitative ion 64.

5.3 Qualitative determination

Determine the standard working solution and specimen as per the conditions applicable to the method; constant volume may be reduced when specimen of low concentration is to be characterized. Allowable tolerance between mass chromatographic peak retention time of the specimen and that of the reference substance shall be less than ± 2.5%; allowable tolerance between the relative abundance and concentration of qualitative ion pairs vs. that of the standard working solution shall not exceed that specified in Table 1.

Table 1 Maximum allowable tolerance of relative ion abundance when quantitation is confirmed

Relative ion abundance /%	> 50	> 20~50	> 10~20	≤10
Maximum allowable tolerance /%	±50	±25	±30	±50

5.4 Quantitative determination

5.4.1 Standard curve mapping

Conduct gas chromatography - mass spectrometer determination over urethane standard working curve solutions of 10.0 ng/ml, 25.0 ng/ml, 50.0 ng/ml, 100 ng/ml, 200 ng/ml, 400 ng/ml, and l000 ng/ml (containing 200 ng/ml), with urethane concentration being taken as the horizontal axis, and peak area ratio between urethane and D5-urethane in the standard curve working solution as the vertical axis to map the standard curve.

5.4.2 Specimen determination

Determine the specimen solution against the standard curve working solution; calculate urethane content in the specimen as per that in the determination solution of urethane. When the specimen has a low concentration of urethane, it is advised to take the standard curve working solution of 10.0 ng/ml, 25.0 ng/ml, 50.0 ng/ml, 100 ng/ml, and 200 ng/ml map the standard curve.

Please refer to Annex A for mass spectrum of the standard solution.

6 Statement of analysis result

Urethane content in the specimen is to be calculated as per Formula (1):

$$X = \frac{c \times V \times 1\,000}{m \times 1\,000} \qquad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (1)$$

In the formula:

X- Urethane content in the specimen, in microgram per kilogram (μg/kg)

c- urethane content in determination solution, in nanogram per milliliter (ng/ml)

V- constant volume of specimen determination solution, in milliliter (ml)

m- specimen mass, in gram (g)

1000 - conversion coefficient.

The calculated results shall be represented by the arithmetic mean of two independent measurement results obtained under repeatability conditions and three-digit valid number shall be retained.

7 Precision

As regards the relative deviation of two independent determination results obtained under repeatability conditions, when the content is ≤ 50 μg/kg, it shall not exceed 15% of the arithmetic mean; when the content is ＞50 μg/kg, it shall not 10% of the arithmetic mean.

8 Others

When 2 g of specimen is taken, the detection limit of urethane in this method shall be 2 μg/kg and the limit of quantification be 5.0 μg/kg.

Appendix A

Mass spectrum of the standard solution

A.1 Total ion of urethane and D5- urethane is shown in Figure A.1.

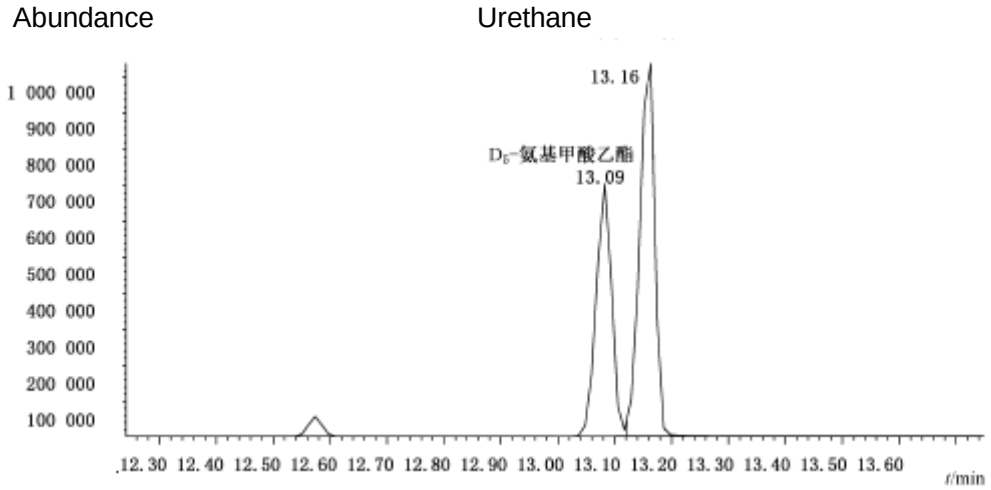

Fig. A.1 Total ion of urethane and D5-urethane

A.2 Urethane mass spectrum is shown in Figure A.2.

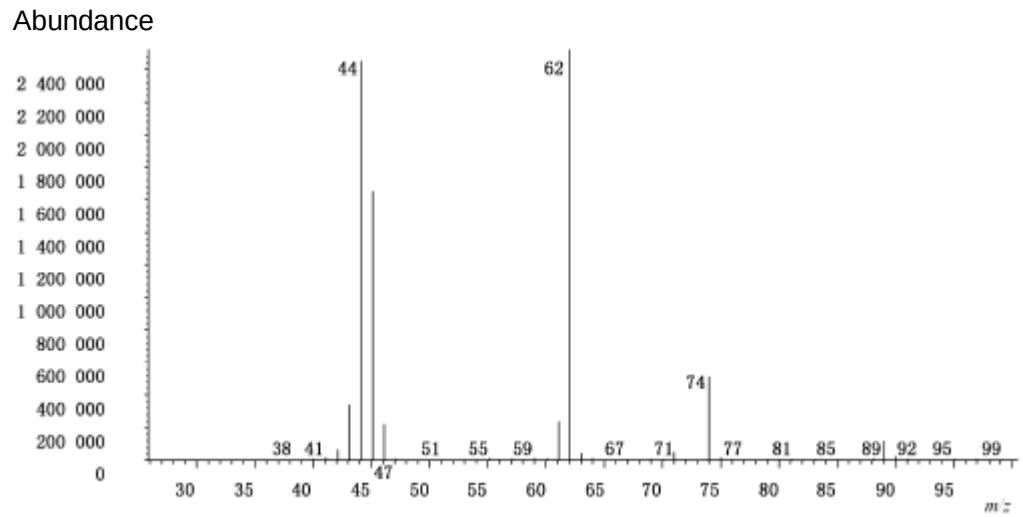

Fig. A.2 Urethane mass spectrum

A.3. D5-urethane mass spectrum is shown in Figure A.3.

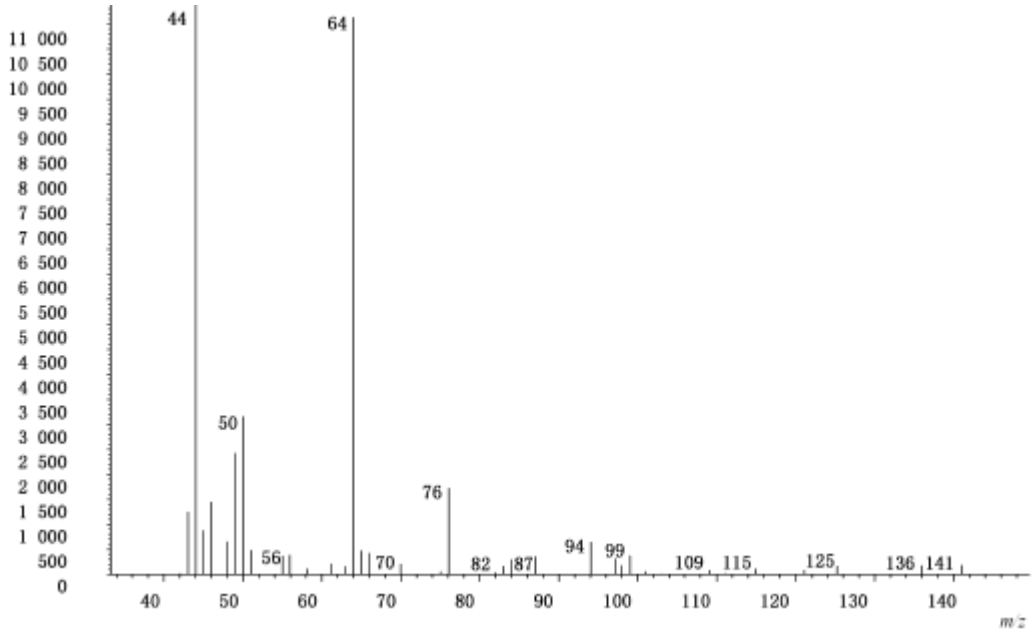

Fig. A.3 D5-urethane mass spectrum