National Standard of the People's Republic of China

GB 5413.26—2010

National food safety standard
Determination of taurine in foods for infants and young children, milk and milk products

Issued at 2010-03-26 implemented at 2010-06-01

Issued by Ministry of Health of the People's Republic of China

Preface

The 2nd method of this standard equivalently adopts the AOAC Official Method 997.05 Taurine in powdered milk and powdered infant formulae.

This Standard is in substitution of GB/T 5413.26-1997 "Determination of taurine in Formula foods and milk powder for infants and young children".

Compared with GB/T 5413.26-1997, the main modifications of this Standard are as follows:

- The OPA post-column derivatization high performance liquid chromatography in the original standard is identified as the 1st method.
- The dansyl-Cl pre-column derivatization high performance liquid chromatography is provided as the 2nd method.
- The structure of the original standard is modified.
- The quantifying by external reference method is applied with the standard curve method.
- Appendix A of the liquid chromatography of guide specimen is added.

The Appendix A of this standard is an informative Appendix

All previous standards substituted by this standard are as follows:

- GB 5413-1985, GB/T 5413.11-1997

National food safety standard
Determination of taurine in foods for infants and young children, Milk and milk products

1. Scope

This Standard specifies the method for determination of taurine in foods for infants and young children, milk and milk products.

This standard applies to the determination of taurine in foods for infants and young children, milk and milk products.

2. Normative cited documents

The reference document of this standard is essential to the application. As for the dated references, only the dated editions are applicable to this Standard. As for the references that are not dated, their most updated editions (including all amendments) are applicable to this standard.

Method 1: OPA Post-column Derivatization Method

3. Principle

Sample is dissolved by metaphosphoric acid solution. After ultrasonic oscillation extraction, centrifugation and microporous filtering, the sample will have derivatization reaction with o-phthalaldehyde (OPA) during the separation of sodium ion chromatographic column and can be detected by fluorescence detector and quantified by external reference method.

4. Reagents and Materials

If not specified, all reagents used in this method are analytical reagents, and water used is the 1st grade Water specified in GB/T 6682.

4.1 Metaphosphoric acid（HPO_3）

4.2 Tri-sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)

4.3 Phenol (C_6H_6O)

4.4 Nitric Acid (HNO_3)

4.5 Methanol (CH_3OH)：ChromAR.

4.6 Boric acid (H_3BO_3)

4.7 Potassium hydroxide (KOH)

4.8 O-phthalaldehyde ($C_8H_6O_2$) (OPA)

4.9 2-mercaptoethanol (C_2H_6OS)

4.10 Polyoxyethylene lauric acid ether (Brij-35).

4.11 Taurine Standard: Purity ≥ 99%.

4.12.10g/L metaphosphoric acid solution: Weigh 10.0g metaphosphoric acid (see 4.1) and dissolve to1000mL with water.

4.13 Citric acid buffer solution: Weigh 19.6g Tri-sodium citrate (see 4.2), dissolve to 950mL with water, add 1mL phenol (see 4.3) and then adjust the pH value to 3.10 ~ 3.25 using HNO_3 (see 4.4). After the completion of the above procedures, filter the solution with 0.45μm microporous filtering film.

4.14 Post-column fluorescence derivatizing agent (o-phthalaldehyde solution)

4.14.1 0.5mol/L K_3BO_3 solution: Weigh 30.9g H_3BO_3 (see 4.6) and 26.3g KOH (see 4.7) and dissolve to 1000mL with water.

4.14.2 O-phthalaldehyde derivatizing reagent: Weigh 0.60g o-phthalaldehyde (see 4.8), dissolve with 10mL CH_3OH(see at 4.5), add 0.5mL C_2H_6OS (see 4.9) and 0.35g Brij-35 (see 4.10), and predetermine the volume to 1000mL with 0.5mol/L K_3BO_3 solution (see 4.14.1). After the completion of the above procedures, filter the solution with 0.45μm microporous filtering film. O-phthalaldehyde derivatizing reagent shall be prepared when it is required.

4.15 Taurine standard solution

4.15.1 1mg/mL taurine standard stock solution: Accurately weigh 0.1000g taurine standard (see 4.11), dissolve it to the volume of 100mL with water. The stock solution can be stored at 4℃ for 7 days.

4.15.2 Taurine standard working solution: Dilute the taurine standard stock solution (see 4.15.1) to prepare a series of standard solutions with the concentrations of 0μg/mL, 5μg/mL, 10μg/mL, 15μg/mL and 20μg/mL. It shall be prepared when it is required.

5. Equipments and devices

5.1 High-performance liquid chromatography (HPLC) with fluorescence detector.

5.2 Post-column reactor.

5.3 Fluorescence derivatizing reagent infusion pump.

5.4 Ultrasonic oscillator.

5.5 pH meter: with the accuracy of 0.01pH.

5.6 Centrifuge: rpm＞5000 r/min.

5.7 0.45μm microporous filtering film.

5.8 Analytic balance: with 1mg, 0.1mg sensitivity.

6. Analytical Procedure

6.1 Sample preparation

Accurately weigh 1.0 ~ 5.0g solid sample or 5g~20g liquid sample (accurate to 0.01g ,with the taurine content higher than 5μg), add 30mL metaphosphoric acid solution (see 4.12) for dissolving, shake fully and then put the mixture to a 100mL volumetric flask; put the flask into a ultrasonic oscillator for 10 ~ 15 min, and use water to predetermine the volume to the regulated scale after it cools down to the room temperature; then implement the centrifugation of the mixture for 10min under 5000 r/min; take the supernatant fluid and filter by the 0.45μm microporous filtering film(see 5.7), and then use the filtrate at the middle for specimen injection.

6.2 Determination

6.2.1 Reference Chromatographic Condition

Chromatographic column: sodium ion column specific for amino acid analysis (250mm × 4.6mm) or the equivalent function column.

Moving phase: Citric acid buffer solution (see 4.13).

Flow rate of mobile phase: 0.30 mL/min.

Flow rate of fluorescence derivatizing reagent: 0.30mL/min.

Column temperature: 55 ℃.

Detection wavelength: excitation wavelength: 338 nm; emission wavelength: 425 nm.

Injection volume: 20μL.

6.2.2 Standard Curve

Using the given recommended chromatographic condition to determine the taurine standard working solutions (see 4.15.2) which have been derivatizated respectively; record the chromatographic peak area, the Appendix A is Chromatogram for reference. Plot standard curve with the peak area as Y-axis and the concentration of taurine as X-axis.

6.2.3 Determination of sample solution

Determine the sample solution with the given recommended chromatographic condition.

The concentration of taurine in the sample solution can be obtained from the standard curve.

7. Result calculation and presentation

7.1 Result calculation

The content of taurine in the sample is calculated according to formula (1).

$$X = \frac{c \times V \times 100}{m \times 1000} \quad \cdots\cdots(1)$$

In this formula:

X ——the content of taurine in the sample, mg/100g;

c ——the concentration of the injected sample solution, μg/mL;

V——Constant volume of the sample solution, mL;

m——sample mass, g.

7.2 Result presentation

The calculated result is presented in arithmetic mean from two independent results of determination, and should display in 3 significant figures.

8. Precision

The absolute difference between the two independent determination results that are obtained under repeated conditions should not exceed 10% of the arithmetic mean.

Method 2: Dansyl Chloride Pre-column Derivatization Method

9. Principle

Dissolve the sample in the water and use the potassium ferrocyanide and the zinc acetate to precipitate protein. Take the supernatant fluid for the derivatization reaction with dansyl chloride, separate the derivative obtained by the C_{18} reversed-phase column, and determine the sample using external reference method by UV detector (wavelength of 254 nm) or fluorescence detector (excitation wavelength: 330 nm; emission wavelength: 530 nm) .

10. Reagents and Materials

Unless specified, the reagents used in the method are analytical reagents (AR), and the water used is the 1st grade Water specified in GB/T 6682.

10.1 Acetonitrile(CH_3CN): chromAR.

10.2 Glacial acetic acid（CH_3COOH）.

10.3 Hydrochloric acid

10.4 Anhydrous sodium carbonate（Na_2CO_3）

10.5 Potassium ferrocyanide[$K_4Fe(CN)_6$]

10.6 Zinc acetate [$Zn(CH_3COO)_2$]

10.7 Sodium acetate [$Na(CH_3COO)$]

10.8 Methylamine hydrochloride （$CH_3NH_2 \cdot HCl$）

10.9 Dansyl chloride (5-dimethylamino-naphthalene -1 -sulfonyl chloride): chromAR

Note: dansyl chloride is instable with light and humidity..

10.10 Taurine Standard: Purity ≥ 99%.

10.11 1mol/L hydrochloric acid solution: take 9mL hydrochloric acid (see at 10.3), dilute with water and predetermine the volume to 100mL.

10.12 Precipitator

10.12.1 Precipitator I: Weigh 15.0g [$K_4 Fe(CN)_6$] (see at 10.5), dissolve with water, and predetermine the volume to 100mL. The precipitator is stable within 3 months at room temperature.

10.12.2 Precipitator II: Weigh 30.0g [$Zn(CH_3 COO)_2$] (see at 10.6), dissolve with water, and predetermine the volume to 100mL. The precipitator is stable within 3 months at room temperature

10.13 80mmol/L sodium carbonate buffer solution (pH 9.5): Weigh 0.424g anhydrous sodium carbonate (see 10.4), dissolve with 40mL water, and adjust the pH value to 9.5 with 1mol/L hydrochloric acid solution (see 10.11); predetermine the volume to 50mL with water. The solution is stable within 3 months at room temperature.

10.14 1.5mg/mL dansyl chloride solution: Weigh 0.015g dansyl chloride (see at 10.9) and predetermine the volume to 100mL with acetonitrile (see at 10.1). It shall be prepared when it is required.

10.15 20mg/mL methylamine hydrochloride solution: Weigh 2.0g methylamine hydrochloride (see at 10.8) and dissolve and predetermine the volume to 100mL with water. The solution is stable within 3 months at room temperature.

10.16 10mmol/L sodium acetate buffer solution (pH 4.2): Weigh 0.820g [$Na(CH_3COO)$] (see at 10.7), dissolve to 800mL with water, and adjust the pH value to 4.2 with glacial acetic acid (see at 10.2); predetermine the volume to 1000mL with water and filter the solution with 0.45μm microporous filtering film.

10.17 Taurine standard solution

10.17.1 1mg/mL taurine standard stock solution: Accurately weighe 0.1000g taurine standard (see at 10.10), dissolve in water and predetermine the volume to100mL. The stock solution can be stored at 4℃ for 7 days.

10.17.2 Taurine standard working solution（for ultraviolet detection）: Diluted the taurine standard stock solution (see at 10.17.1) with water to prepare a series of standard solutions with the concentration of 0μg/mL, 5μg/mL,10μg/mL,15μg/mL and 20μg/mL. It shall be prepared when it is required.

10.17.3 Taurine standard working solution（for fluorescence detection）: Diluted the taurine standard stock solution (see at 10.17.1) with water to prepare a series of standard solutions with the concentration of 0μg/mL, 5μg/mL,10μg/mL,15μg/mL and 20μg/mL. It shall be prepared when it is required.

11. Equipments and devices

11.1 High-performance liquid chromatography (HPLC): with UV detector or diode array detector or fluorescence detector.

11.2 pH meter: with the accuracy of 0.01.

11.3 Vortex mixer

11.4 Ultrasonic oscillator

11.5 Centrifuge: rpm＞5000r/min

11.6 0.45μm microporous filtering film

11.7 Analytical balance: with 1mg, 0.1mg sensitivity

12. Analytical Procedure

12.1 Sample preparation

12.1.1 Sample solution extraction

Weigh 1g~5g solid sample or 5~30g liquid sample (accurate to 0.01g; for UV detector taurine content should be ＞1μg; for fluorescence detector taurine content should be＞50μg) , put the sample into a 100mL volumetric flask, add 80mL warm water (50 ~ 60 ℃) for dissolving, shake fully and then put the flask into a ultrasonic oscillator for 10 min, after it cools down to the room temperature, add 1.0mL precipitator I (see at 10.12.1) and implement the vortex mixing, add the 1.0 mL precipitant II(10.12.2) and mix the solution with the vortex; predetermine the volume to the regulated scale with water and mix the sample solution fully. Centrifuge the sample solution with 5000r/min for 10 min, take the supernatant fluid for follow-up application. The supernatant fluid is stable within 24 hours at 4℃ in a dark place.

12.1.2 Derivatization of sample solution

Accurately take 1.00mL supernatant fluid to a 10mL glass test tube with plug and add 1.00mL sodium carbonate buffer solution (see at 10.13) and 1.00mL dansyl chloride solution (see at 10.14) for full mixing. Conduct derivatization reaction 2 hours in cool and dark place (Shake after 1 hour). Add 0.10mL methylamine hydrochloride solution (see at 10.15) and mix with vortex to terminate the reaction, wait till the precipitation is fully completed. Filter the supernatant fluid with 0.45μm microporous filtering film (see at 11.6) and take the filtrate for follow-up application. The derivatives can be kept within 48 hours at 4℃ and in a dark place.

Take 1.00 ml taurine standard working solution see at 10.17.2) for derivatization given above.

12.2 Determination

12.2.1 Reference Chromatographic Condition

Chromatographic column: C_{18} reversed-phase column, (Dp5μm, 250mm × 4.6mm) or the equivalent function column.

Mobile phase: 10mmol/L sodium acetate buffer solution (see at 10.16)-acetonitrile (see at 10.1) = 70+30.

Flow rate of mobile phase:1.00mL/min.

Column temperature: room temperature.

Detection wavelength:

UV detector or diode array detector: 254 nm

Or fluorescence detector: excitation wavelength: 330 nm; emission wavelength: 530 nm.

Injection volume: 20μL.

12.2.2 Standard Curve

Using the given recommended chromatographic condition to determine the taurine standard working solutions which have been derivatizated respectively (for UV detection see at 10.17.2; for fluorescence detection see at 10.17.3); record the chromatographic peak area, Appendix A is the Chromatogram for reference. Plot the standard curve with the peak area as Y-axis and the concentration of taurine as X-axis.

12.2.3 Determination of sample solution

Determine the sample solution with given recommended chromatographic condition.

The taurine concentration of the sample solution is obtained according to the standard curve.

13. Result calculation and presentation

The content of taurine in the sample is calculated according to formula (2).

$$X = \frac{c \times V \times 100}{m \times 1000} \quad \cdots\cdots(2)$$

Where:

X - The content of taurine in the sample, mg/100g;

c –the concentration of the injected sample solution, μg/mL;

V - Constant volume of the sample solution, mL;

m - sample mass, g.

The calculated result is presented in arithmetic mean from two independent results of determination, and should display in 3 significant figures.

14. Precision

The absolute difference between the two independent determination results that are obtained under repeated conditions should not exceed 10% of the arithmetic mean

15. Others

The quantitative limit of this standard: when taking 10.00g sample, the Method 1 is 0.5mg/100mg; the Method 2 is 5mg/100mg for UV detection, 0.1mg/100mg for fluorescence detection.

Appendix A

(Informative appendix)

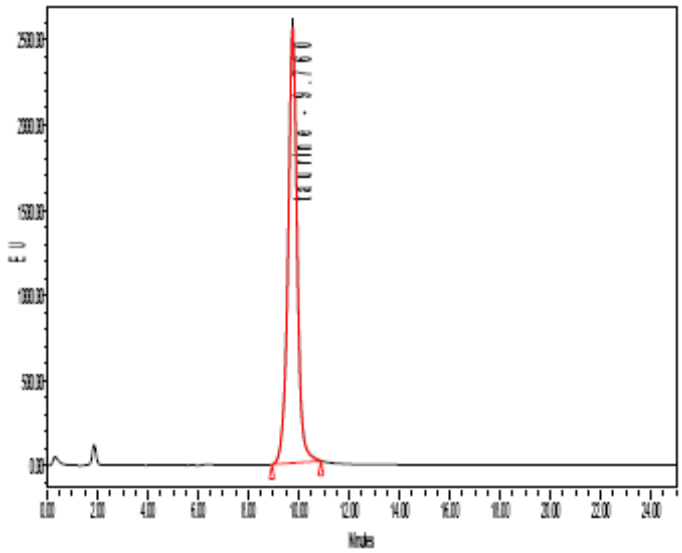
Standard solution liquid chromatogram

A.1 standard solution liquid chromatogram

The OPA post-column derivatizing high performance liquid chromatogram is referred to Figure A.1

The dansyl chloride pre-column high performance liquid chromatogram (for UV detection) is referred to Figure A.2

The dansyl chloride pre-column high performance liquid chromatogram (for fluorescence detection) is referred to Figure A.3



FigureA.1 The OPA post-column derivatization high performance liquid chromatogram

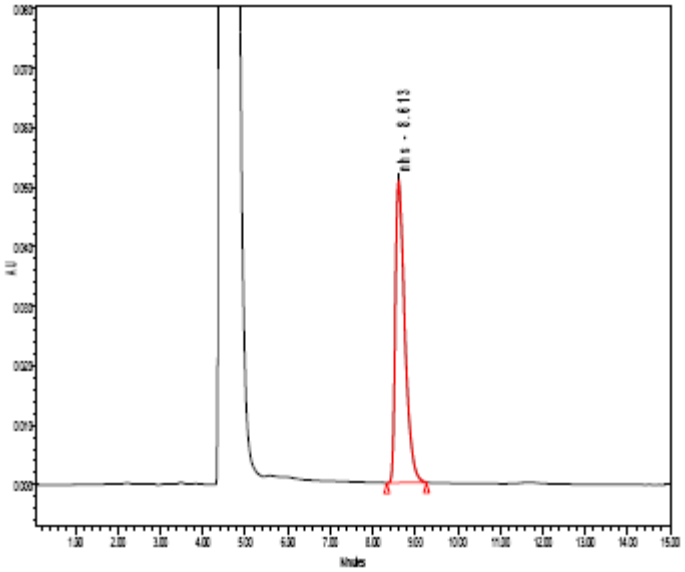


Figure A.2 The dansyl chloride pre-column high performance liquid chromatogram (for UV detection)

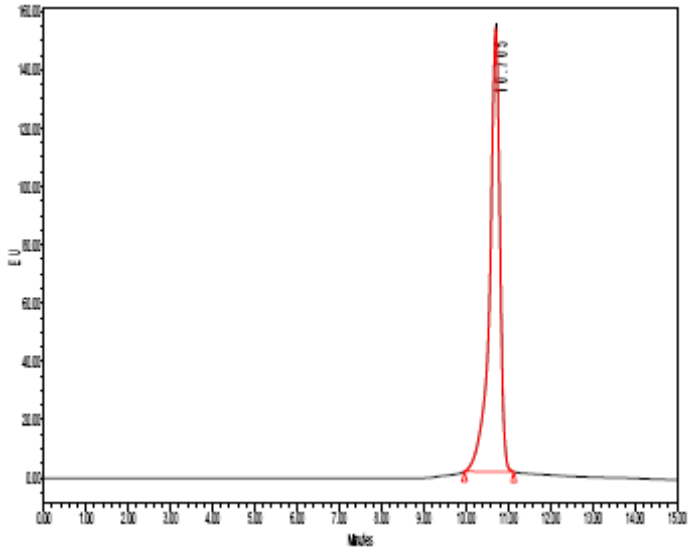


Figure A.3 The dansyl chloride pre-column high performance liquid chromatogram (for UV detection)