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Report Name: China Issues Draft Testing Method of Choline in Infants and Young Children Products

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Report Highlights:

On August 27, 2020 China issued Determination of Choline in Foods and Dairy Products for Infants and Young Children (Draft for comments). Comments can be sent via http://bz.cfsa.net.cn/cfsa_aiguo. The deadline for domestic comments submission is October 20, 2020. This report contains an unofficial translation of a domestic standard for comment. This standard has not yet been notified to the WTO SPS Committee.

BEGIN TRANSLATION

National Food Safety Standard

Determination of Choline in Foods and Dairy Products for Infants and Young Children

(Draft for Comments)

Preface

This standard replaces GB 5413.20-2013 "National Food Safety Standard-Determination of Choline in Foods and Dairy Products for Infant and Young Children"

Comparing with GB 5413.20-2013, the main changes in this standard are as follows:

-Delete Method II: Reinecke's salt spectrophotometry, and add ion chromatography as Method II

-Add liquid chromatography-tandem mass spectrometry as method III.

National Food Safety Standard

Determination of Choline in Foods and Dairy Products for Infants and Young Children

1 Scope

This standard specifies the determination method of choline in foods and dairy products for infants and young children.

This standard applies to the determination of choline in foods and dairy products for infants and young children, including method I: enzyme colorimetry, method II: ion chromatography, and method III: liquid chromatography-tandem mass spectrometry.

Method I: Enzyme Colorimetry

2 Principle

The choline in the sample is converted into free choline by acid hydrolysis, and then reacts with the chromogenic agent to produce colored substances after being catalyzed by enzymes. The color depth is proportional to the choline content within a certain concentration range.

3 Reagents and materials

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

3.1 Reagents

3.1.1 Trihydroxymethyl aminomethane [(CH2OH)3CNH2].

3.1.2 Phenol(C6H5OH).

3.1.3 Concentrated hydrochloric acid (HCl).

3.1.4 Sodium hydroxide (NaOH).

3.1.5 Choline oxidase: ≥10 units/mg, stored at -20℃.

3.1.6 Peroxidase: ≥250 units/mg, stored at 2℃~8℃.

3.1.7 4-aminoantipyrine(C11H13N3O).

3.1.8 Phospholipase D: ≥60 units/mg, stored at -20℃.

3.2 Reagent preparation

3.2.1 Hydrochloric acid solution (1 mol/L): Measure out 85mL concentrated hydrochloric acid and pour into about 900mL water, and then dilute to a constant volume of 1000 mL.

3.2.2 Hydrochloric acid solution (3 mol/L): Measure out 255mL concentrated hydrochloric acid and pour into about 600mL water, and then dilute to a constant volume of 1000 mL.

3.2.3 Trihydroxymethyl aminomethane buffer solution (Tris)(0.05mol/L): pH = 8.0±0.2. Accurately weigh 6.057g of trihydroxymethyl aminomethane and put into 500mL water, adjust pH value to 8.0±0.2 with hydrochloric acid solution (1 mol/L), and dilute to a constant volume of 1000mL with water. This solution can be stored in a refrigerator at 4℃ for one month.

3.2.4 Chromogenic reagent for enzyme reaction: take 100~120 activity units of choline oxidase, 250~280 activity units of peroxidase, 75~100 activity units of phospholipase D, 15mg 4-Aminoantipyrine and 50mg of phenol; add to a 100mL volumetric flask, dissolve with 0.05 mol/L Tris buffer solution and dilute to the mark. Prepare on site immediately before use.

3.2.5 Sodium hydroxide solution(500g/L): Weigh 500g of sodium hydroxide, dissolve in water and dilute to a constant volume of 1000 mL.

3.3 Standard sample

Choline bitartrate standard sample($C_9H_{19}NO_7$), CAS No.: 87-67-2, purity ≥99%, or a standard substance certified by the state and awarded with a standard substance certificate.

3.4 Preparation of standard solution

3.4.1 Choline(calculated as choline hydroxide, the same below) standard stock solution(2500mg/L): accurately weigh 0.523g choline bitartrate baked at 102℃±2℃ to constant weight(accurate to 0.1mg), dissolve in water and transfer to a 100mL volumetric flask, dilute to the mark, and mix well. Store at 4℃ in the dark, and the shelf life is three months.

3.4.2 Choline standard working solution(250mg/L): Draw 10.0mL choline standard stock solution and put into a 100mL volumetric flask, dilute to the mark with water, and store at 4℃ in the dark. The shelf life is one month.

3.5 Material

3.5.1 0.45μm water-based membrane syringe filter.

3.5.2 Syringe: 5mL or equivalent.

4 Instruments and equipment

4.1 Balance: Sensitivity is 0.1 mg and 0.01 g.

4.2 Constant temperature water bath: The temperature can be controlled at 70℃±2℃ and 37℃±2℃.

4.3 pH meter: with an accuracy of 0.01.

4.4 Spectrophotometer.

5 Analysis steps

5.1 Pretreatment of sample

5.1.1 Liquid sample

Weigh 20g (accurate to 0.01g) of the well mixed sample, put it into a 100mL Erlenmeyer flask, and add 10mL hydrochloric acid solution (3 mol/L).

5.1.2 Semi-solid and solid sample

Weigh 5g (accurate to 0.01g) of the well-mixed sample, put it into a 100mL Erlenmeyer flask, and add 30mL hydrochloric acid solution (1 mol/L); put on the stopper and mix well.

5.1.3 Hydrolysis

Place the container with sample in a water bath of 70℃±2℃, hydrolyze for 3h (shaking every 30min), and cool. Adjust the pH to 3.5~4.0 with sodium hydroxide solution(500g/L), transfer to a 50mL volumetric flask, and dilute to the mark with water.

5.1.4 Filter

Filter the hydrolysate with filter paper, the filter should be clear; if it is not, filter again with a 0.45μm water-based membrane syringe filter. Place in a refrigerator at 4℃ for use later.

5.2 Determination

5.2.1 Production of standard curve

Draw 2mL, 4mL, 6mL, and 8mL choline standard working solution and put into a 10mL volumetric flask, dilute to the mark with water, and produce a series of standard working solutions with the concentrations of 50 mg/L, 100 mg/L, 150 mg/L, and 200 mg/L, which are numbered as dilution level 1, dilution level 2, dilution level 3, dilution level 4 and choline standard working solution(250mg/L). Take 6 test tubes, one is used as blank reagent (A), which is added with 0.100mL of water, and add 0.100mL of the same serial standard working solution into the other five test tubes numbered from 1 to 5, respectively. Then add 3.00mL of the chromogenic agent for enzyme reaction to the said 6 test tubes, cover the test tubes with sealing protective film, mix well, and place the test tubes into a 37℃±2℃ water bath for 15 min.

5.2.2 Determination of samples

Prepare 2 test tubes(B, C) for each sample, and add 0.100mL of the solution to be analyzed; add 3.00mL water to test tube B, and add 3.00mL of chromogenic reagent for enzyme reaction to test tube C. Cover the test tube with a sealed protective film, mix well, and put the test tubes into a water bath of 37℃±2℃ for 15 min.

5.2.3 Colorimetric determination

Take the sample and standard series solutions out of the water bath and cool to room temperature. At the wavelength of 505 nm, use water as the blank and measure the absorbance with a 1mL quartz cuvette. Take the concentration of the choline standard solution as the abscissa, and the absorbance of the standard solution minus the absorbance of the blank as the ordinate to make a standard curve.

6 Expression of analysis results

6.1 Calculation of Net Absorbance

Usually, the prepared reagent will show a slight color, and the filtrate also has its color due to hydrolysis. In order to remove these interference factors, the blank value (test tube A and test tube B) shall be subtracted from the total absorbance value.

The net absorbance of the sample can be calculated according to formula (1) as follows:

$$A = A_{tot} - A_{bl} - A_{ex} \quad (1)$$

Where:

A- net absorbance value;

Atot-total absorbance (test tube C);

Abl——Sample absorbance (test tube A);

Aex——Filtrate absorbance (test tube B);

Abl and Aex shall not be greater than 20% of the total absorbance. For the standard curve, Aex=0; if it is greater, it will be diluted appropriately, and the blank value (test tube A and test tube B) shall be diluted by the same multiple.

6.2 Calculation of choline content

Choline in the sample can be calculated according to formula (2) and expressed in milligrams per hundred grams(mg/100g):

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

Where:

X- The choline content in the sample in milligrams per hundred grams(mg/100g);

c- The concentration of choline found on the standard curve, in milligrams per liter(mg/L);

V-The volume of the hydrolysate to be diluted (usually 50mL), in milliliters(mL) ;

m- The mass of the sample, in grams(g)

100- conversion factor;

1000-conversion factor.

The calculation result shall be rounded up to integer.

7. Precision

The absolute difference between two independent determination results obtained under repeatable conditions shall not exceed 10% of the arithmetic value.

8. Others

When the weight of solid and semi-solid sample is 5g, the detection limit of the method is 1mg/100g, and the quantification limit is 3mg/100g. When the liquid sample is about 20 g, the detection limit of the method is 0.25mg/100g, and the quantification limit is 0.75mg/100g.

Method II Ion Chromatography

9 Principle

After the sample is hydrolyzed with hydrochloric acid to extract choline, it is purified by a purification column, and separated by a cation analysis column; detected by a conductivity detector and quantified by an external standard method.

10 Reagents and materials

The reagents used in this method are in accordance with the requirements of the method, and the water is the primary water specified in GB/T 6682.

10.1 Reagents

10.1.1 Concentrated hydrochloric acid (HCl): Guaranteed reagent.

10.1.2 Methanesulfonic acid (CH4O3S): chromatographic pure.

10.2 Reagent preparation

10.2.1 Hydrochloric acid solution (1 mol/L): Measure 85mL concentrated hydrochloric acid and pour into about 900mL water, and then dilute to a constant volume of 1000mL.

10.2.2 Hydrochloric acid solution (1.7 mol/L): Measure 144.5mL concentrated hydrochloric acid and pour into about 800mL of water, and then dilute to a constant volume of 1000mL.

10.2.3 Methanesulfonic acid solution (6 mmol/L): Pipette 0.390mL methanesulfonic acid and dilute to a constant volume of 1000mL with water.

10.2.4 Methanesulfonic acid solution (15 mmol/L): Pipette 0.974mL methanesulfonic acid and dilute to a constant volume of 1000mL with water.

10.2.5 Methanesulfonic acid solution (25 mmol/L): Pipette 1.62mL of methanesulfonic acid and dilute to a constant volume of 1000mL with water.

10.3 Standard sample

Choline bitartrate standard sample (C9H19NO7), CAS No.: 87-67-2, purity ≥99%, or a standard substance certified by the state and awarded with a standard substance certificate.

10.4 Preparation of standard solution

10.4.1 Choline(calculated as choline hydroxide, the same below) standard stock solution(2500mg/L): accurately weigh 0.523g choline bitartrate baked at 102℃±2℃ to constant weight(accurate to 0.1mg), dissolve in water and transfer to a 100mL volumetric flask, dilute to the mark, and mix. Store at 4℃ in the dark, and the shelf life is three months.

10.4.2 Choline standard working solution(100mg/L): Pipette 2.0mL choline standard stock solution(2500mg/L) into a 50mL volumetric flask, and dilute with methane sulfuric acid solution(15 mmol/L) to the mark of constant volume; store at 4℃ in the dark. The shelf life is one month.

10.4.3 Choline standard series working solution: draw 0.2mL, 0.5mL, 1.0mL, 2.5mL, and 5.0mL of choline standard working solution(100mg/L) and put into a set of 100mL volumetric flasks, and dilute with methane sulfuric acid solution(15 mmol/L) to the mark and mix well. The standard series of working solutions with choline concentration of 0.2mg/L, 0.5mg/L, 1.0mg/L, 2.5mg/L, and 5.0mg/L are thus obtained. It shall be prepared on site immediately before use.

Note: The range of the standard series can be adjusted according to the choline concentration in the sample.

10.5 Materials

10.5.1 0.45μm water-based membrane syringe filter.

10.5.2 Purification column: 1.0mL C18 solid phase extraction cartridges of or equivalent.

10.5.3 Syringe: 5mL or equivalent.

11 Instruments and equipment

11.1 Ion chromatograph (IC): with conductivity detector, high capacity cation exchange column.

11.2 Analytical balance: the sensitivity is 0.1 mg and 0.01g, respectively.

11.3 Electric heating constant temperature water bath: the temperature can be controlled at 70℃±2℃.

11.4 Vortex mixer.

12 Analysis steps

12.1 Sample pretreatment

12.1.1 Sample extraction

12.1.1.1 Liquid sample

Accurately weigh 10g(accurate to 0.01g) of the well-mixed liquid sample into a 50mL graduated glass test tube with stopper, and add 15mL hydrochloric acid solution(1.7mol/L); cover, mix well and put into water bath at 70℃± 2℃ for 3h(shaking once every 30min). Cool the hydrolysis product to room temperature, dilute to the mark of constant volume with water, and mix well. This solution can be stored at 4℃ for testing.

12.1.1.2 Semi-solid and solid sample

Accurately weigh 2.5g (accurate to 0.01g) of semi-solid or solid sample and put into a 50mL graduated glass test tube with stopper; add 25mL of hydrochloric acid solution (1mol/L), cover, and rotate until there is no agglomeration in the sample solution After it is well mixed, add it to a 70℃±2℃ water bath to hydrolyze for 3h (shaking once every 30min). Cool the hydrolyzed product to room temperature, dilute to the mark with water, and mix well. This solution can be stored at 4℃ for testing.

12.1.2 Sample purification

After diluting the extracted solution 50 times with water[a], take about 15mL of the diluted solution and pass it through a 0.45μm water based membrane syringe filter and a C18 solid phase extraction cartridge [b] (1.0mL); Discard the first 3mL, and collect the eluent solution for testing.

Note a: The dilution multiple can be adjusted appropriately according to the concentration of choline in the sample, and it shall not be lower than 10 times.

Note b: The C18 solid phase extraction cartridge (1.0mL) shall be passed through with 10mL methanol and 15mL water in sequence before use, and it is allowed to stand and activate for 30 min.

12.2 Instrument reference conditions

12.2.1 Instrument reference conditions 1

a) Ion chromatographic column parameters: a high-capacity cation exchange column with carboxyl, such as Ion Pac CS12A 4 mm×250 mm (with Ion Pac CG12A guard column 4 mm×50 mm), or an equivalent column.

b) Isocratic eluting: isocratic eluting with methanesulfonic acid solution (15 mmol/L), with a collection time of 25min.

c) Flow rate: 1.0mL/min.

d) Conductivity detector: equipped with suppressor or equivalent suppression device.

e) Sampling volume: 100 μL (can be adjusted according to the measured ion content in the sample).

12.2.2 Instrument reference conditions 2

a) Ion chromatographic column parameters: a high-capacity cation exchange column with carboxyl, such as Ion Pac CS19 4 mm×250 mm (with Ion Pac CG19 guard column 4 mm×50 mm), or an equivalent column.

b) Isocratic eluting: isocratic eluting with methanesulfonic acid solution (6 mmol/L), with a collection time of 25min.

c) Flow rate: 1.0mL/min.

d) Conductivity detector: equipped with suppressor or equivalent suppression device.

e) Sampling volume: 100 μL (can be adjusted according to the measured ion content in the sample).

12.3 Preparation of standard curve

Inject the standard series of working solution into the ion chromatograph and measure the signal of peak area or peak height of the corresponding conductivity. Use the concentration of the standard series of working solution as the abscissa and the conductivity peak area or peak height signal as the ordinate to draw a standard curve.

See Appendix A for the ion chromatogram of the choline standard solution.

12.4 Determination of sample solution

Inject the sample solution into the ion chromatograph to obtain the peak area or peak height signal of corresponding conductivity and obtain the concentration of choline in the solution to be tested according to the standard curve.

13 Expression of analysis results

The content of choline in the sample is calculated according to formula (3):

$$X = \frac{c \times V \times f \times 100}{m \times 1000} \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (3)$$

Where:

X—— Choline content in the sample, in milligrams per hundred grams(mg/100g);

c——The concentration of choline in the sample solution obtained from the standard curve, in milligrams per liter(mg/L);

V——Volume of constant volume, in milliliters(mL);

m——Sampling amount, in grams(g);

f——Dilution factor;

100——Conversion factor;

1000——Conversion factor.

The calculation result shall correct to three significant figures.

14 Precision

The absolute difference between two independent determination results obtained under repeatable conditions shall not exceed 10% of the arithmetic mean.

15 Other

When the solid and semi-solid sample is about 2.5g, the detection limit of the method is 2 mg/100g, and the quantification limit is 6 mg/100g. When the liquid sample is about 10g, the detection limit of the method is 0.5 mg/100g, and the quantification limit is 1.5 mg/100g.

Method III Liquid Chromatography-Tandem Mass Spectrometry

16 Principle

After the choline in the sample is hydrolyzed by hydrochloric acid and extracted, the pH value is adjusted, and it is filtered through a microporous membrane; then it is determined by liquid chromatography-tandem mass spectrometry, and quantified by the isotope internal standard method.

17 Samples and materials

Unless otherwise specified, the reagents used in this method are of analytical reagent, and the water is the primary water specified in GB/T 6682.

17.1 Reagents

17.1.1 Formic acid (HCOOH): chromatographic pure.

17.1.2 Acetonitrile (CH3CN): chromatographic pure.

17.1.3 Ammonium formate (HCOONH4): purity ≥99.9%.

17.1.4 Concentrated hydrochloric acid (HCl).

17.1.5 Sodium hydroxide (NaOH): purity ≥99.9%.

17.2 Reagent preparation

17.2.1 Ammonium formate aqueous solution (10 mmol/L): Weigh 0.63g(accurate to 0.01g) of ammonium formate, and dissolve in water and transfer to a 1000mL volumetric flask; adjust the pH to 5.0±0.1 with formic acid, dilute to the mark with water, and perform ultrasonic mixing.

17.2.2 Hydrochloric acid solution (1 mol/L): Measure 85mL of concentrated hydrochloric acid and pour into about 900mL of water, and then dilute to a constant volume of 1000mL with water.

17.2.3 Sodium hydroxide solution (1 mol/L): Weigh 2.0g (accurate to 0.01g) of sodium hydroxide and dissolve with water and transfer to a 50mL volumetric flask; dilute to the mark with water and shake well for use later.

17.2.4 80% acetonitrile aqueous solution: Take 80mL of acetonitrile and dilute to 100mL with water.

17.3 Standard sample

17.3.1 Choline bitartrate standard sample ($C_9H_{19}NO_7$), CAS No.: 87-67-2, purity ≥99%, or a standard substance certified by the state and awarded with a standard substance certificate.

17.3.2 Stable isotope internal standard: Choline chloride- -d_4($C_5H_{10}NOD_4Cl$), CAS No.: 285979-70-6, purity ≥99.8%, or equivalent.

17.4 Preparation of standard solution

17.4.1 Choline (calculated as choline hydroxide) standard stock solution(100mg/L): accurately weigh 20.92 mg (accurate to 0.01 mg) choline bitartrate, dissolve it with 80%

acetonitrile aqueous solution and dilute to 100mL. After mixing, transfer the solution to a brown glass bottle and store at 4℃ in the dark. The shelf life is one month.

17.4.2 Choline standard working solution (1.0mg/L): Draw 1.00mL choline standard stock solution(100mg/L) into a 100mL volumetric flask and dilute to the mark with ammonium formate aqueous solution (10 mmol/L). It shall be prepared on site when use.

17.4.3 Choline-d_4 internal standard stock solution(1000mg/L): accurately weigh 13.3 mg(accurate to 0.01mg) of choline chloride-d_4, and dissolve it with 80% acetonitrile aqueous solution; transfer to a 10mL brown volumetric flask, dilute to the mark, and store at 4℃ in the dark. The shelf life is three months.

17.4.4 Choline-d_4 internal standard working solution(1.0mg/L): Pipette 0.10mL choline-d_4 internal standard stock solution(1000mg/L) and put into a 100mL volumetric flask; dilute with ammonium formate aqueous solution(10 mmol/L) to the mark, and store at 4℃ in the dark. The shelf life is one month.

17.4.5 Standard series of working solutions: accurately draw 0.1mL, 0.2mL, 0.5mL, 1.0mL, 1.5mL, and 2.0mL choline standard working solution(1.0mg/L) and put into to 10mL volumetric flasks, respectively, and add 500 μL Choline-d_4 internal standard working solution(1.0mg/L); Dilute with ammonium formate aqueous solution (10 mmol/L) and mix well. The choline concentrations of this standard series are 10 μg/L, 20 μg/L, 50 μg/L, 100 μg/L, 150 μg/L, and 200 μg/L, respectively. It shall be prepared on site immediately before use.

17.5 Materials

17.5.1 0.22 μm water-based membrane syringe filter.

17.5.2 Syringe: 5mL or equivalent.

18 Apparatus and equipment

18.1 Liquid chromatography-tandem mass spectrograph: with electrospray ionization source (ESI).

18.2 Balance: with sensitivity of 0.01 mg and 0.01g, respectively.

18.3 Constant temperature water bath: the temperature can be controlled at 70℃±2℃.

18.4 pH meter: with an accuracy of 0.01.

18.5 Ultrasonic cleaner.

19 Analysis steps

19.1 Sample pretreatment

19.1.1 Liquid and semi-solid samples

Accurately weigh 2.5g~10g (accurate to 0.01g) sample, dissolve it in warm water, and transfer it to a 25mL volumetric flask; cool to room temperature, dilute to the mark with water and shake it evenly. Draw 1mL of the sample solution and put in a 50mL graduated glass test tube with stopper and set aside.

19.1.2 Solid samples

Accurately weigh 1g~5g (accurate to 0.01g) sample, dissolve it in warm water, and transfer it to a 100mL volumetric flask; cool to room temperature, and dilute to the mark with water. Draw 1mL of the sample solution and put in a 50mL graduated glass test tube with stopper and set aside.

19.1.3 Sample extraction

Add 50 μL choline- d_4 internal standard stock solution(1000mg/L) in the above said sample solution, and add 10mL of hydrochloric acid solution(1 mol/L); Shake well, and ultrasound mixing for 5 min. Heat the sample in a water bath at 70℃±2℃ for 3h(shake every 30 min), after cooling to room temperature, adjust the pH to 5.0~5.3 with sodium hydroxide solution(1 mol/L); Transfer to a 100mL volumetric flask, and dilute to the mark with water. Then it was diluted by 10 times with ammonium formate aqueous solution(10mmol/L) and filtered through a 0.22 μm hydrophilic membrane into a sample bottle for testing later.

19.2 Instrument reference conditions

19.2.1 Reference conditions for liquid chromatography

a) Chromatographic column: Amide chromatographic column (1.7μm, 2.1mm×100mm), or equivalent.

b) Mobile phase A: ammonium formate aqueous solution (10 mmol/L) (pH=5.0); mobile phase B: acetonitrile.

c) Flow rate: 0.3mL/min.

d) Column temperature: 40˚C.

e) Injection volume: 2 μL.

f) See Table 1 for gradient elution conditions.

Table 1 Gradient elution conditions

Time(min)	Mobile phase A (%)	Mobile phase B (%)
0 ~ 1.0	10	90
1.0 ~ 3.0	40	60
3.0 ~ 4.0	40	60
4.0 ~ 5.0	10	90
5.0 ~ 8.0	10	90

19.2.2 Mass spectrometry reference conditions

a) Ionization mode: ESI+;

b) Detection method: multi-ion reaction monitoring (MRM);

c) Capillary voltage: 3.1 kV;

d) Ion source temperature: 150℃;

e) Auxiliary gas temperature: 350℃;

f) Auxiliary air flow: 600 L/h;

g) Foramen purge air flow: 150 L/h;

h) LM2 RF lens voltage: 2.75 V;

i) HM2 RF lens voltage: 14.99 V.

Table 2 Main mass spectrum parameters

Compound	Precursor ion(m/z)	Cone voltage(V)	Product ion(m/z)	Collision energy(eV)
Choline	104.0	25	60.0*/45.0	15
Choline chloride - d_4	108.0	25	60.0*/49.0	15

Note: * is the quantitative product ion

19.3 Preparation of standard curve

Inject the choline standard series working solutions into the liquid chromatography-tandem mass spectrometer from low to high concentrations. Take the choline concentrations as the abscissa, and the product of the internal standard peak area ratio of choline to choline chloride-d4 ($A_{Choline}/A_{choline\ chloride\text{-}d4}$) and the mass concentration of the isotope as the ordinate, the standard curve of choline is drawn.

19.4 Determination of sample solution

19.4.1 Qualitative determination

The response of choline and choline chloride-d4 in the sample solution shall be within the detection linear range of the instrument. The ratio of the relative abundance of ions in the sample solution to that of the standard solution shall meet the requirements in Table 3. The qualitative reference precursor ion is 104.0 m/z, the reference product ion 1 is 60.0 m/z, and the reference product ion 2 is 45.0 m/z (see Appendix B, Figure B.1). The reference retention time of the choline scan peak is 3.31 min (see Appendix B, Figure B.2).

Table 3 The range of allowable deviation of the relative abundance of ions in the sample solution

Relative abundance (%)	> 50	> 20-50	> 10-20	≤10
Allowable deviation (%)	± 20	± 25	± 30	± 50

19.4.2 Quantitative determination

The sample solution is injected into the liquid chromatography-tandem mass spectrometer, and the concentration of choline in the solution to be tested is obtained according to the standard curve.

20 Expression of analysis results

The content of choline in the sample is calculated according to formula (4):

$$X = \frac{c \times V \times f \times 100}{m \times 1\,000\,000} \quad \text{.....} \quad (4)$$

Where:

X —— the content of choline in the sample, in milligrams per hundred grams (mg/100g);

c —— The concentration of choline in the injection solution calculated according to the standard curve, in nanograms per milliliter(ng/mL);

V —— The constant volume of solution after the sample being dissolved in warm water, in milliliter(mL);

m ——mass of the sample, in grams(g);

f ——Dilution factor, with a value of 1000

100-conversion factor;

1000000 —— Conversion factor; the calculation result shall correct to three significant figures.

21 Precision

The absolute difference between two independent determination results obtained under repeatable conditions shall not exceed 10% of the arithmetic mean.

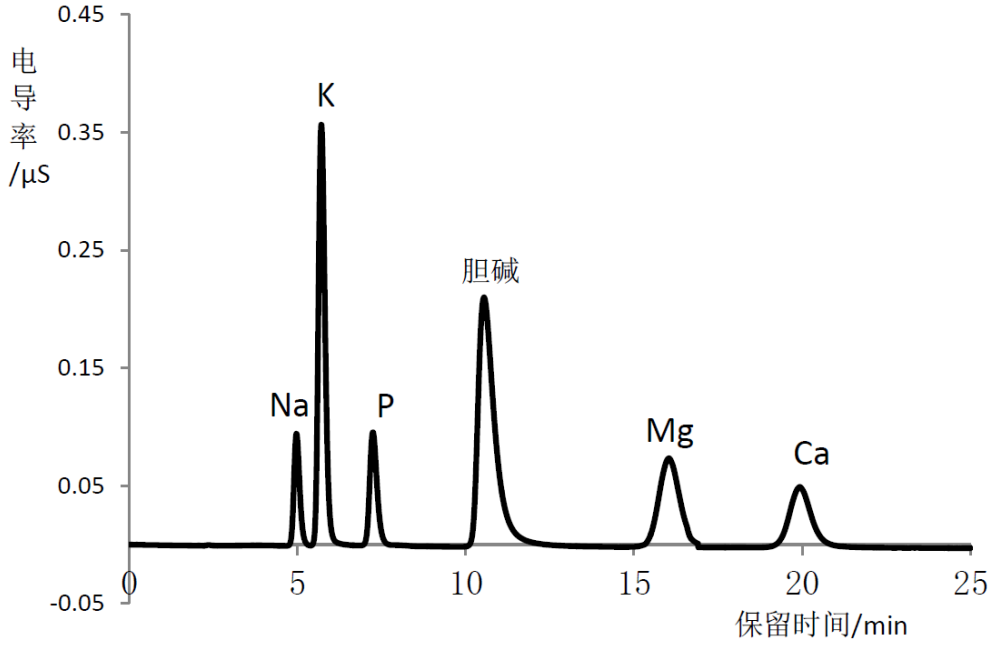
22 Other

When weighing 2.5g of liquid and semi-solid samples, the detection limit of this method is 0.24 mg/100g, and the limit of quantification is 0.8 mg/100 g; when weighing 1g of solid samples, the detection limit of this method is 2mg/100g, and the limit of quantification is 8mg/100g.

Appendix A

Ion Chromatogram of Standard Choline Solution

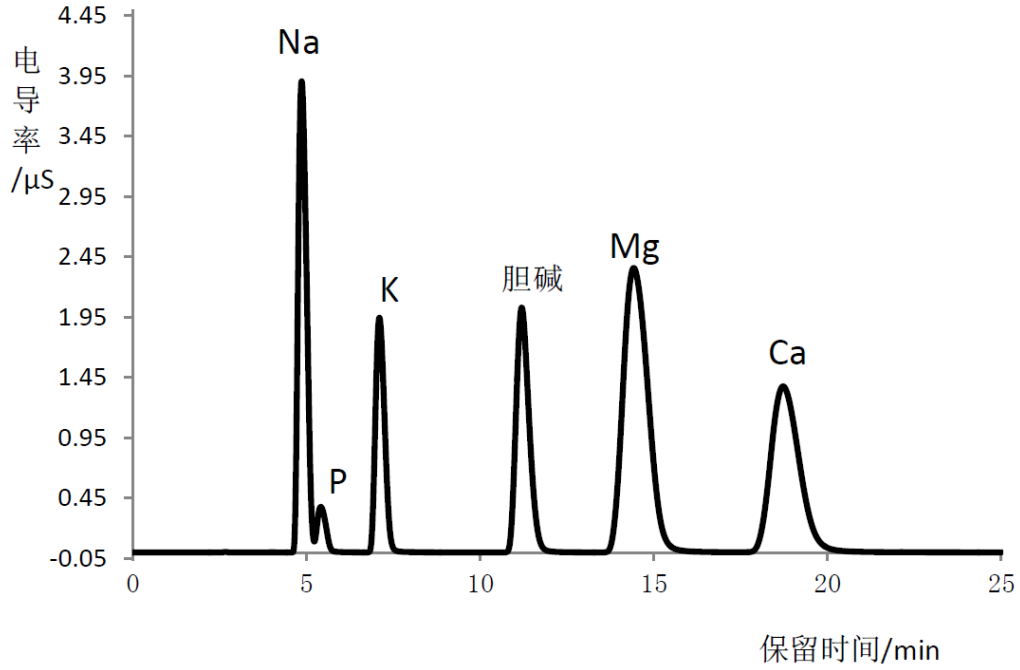
Refer to Figure A.1 for reference condition-ion chromatogram (concentration 1mg/L).



Conductivity/μS
Choline
Retention time/min

Figure A.1 Ion Pac CS12A isocratic elution chromatogram (concentration 1mg/L)

The reference condition 2- ion chromatogram (concentration 5mg/L) is shown in Figure A.2.



Conductivity/μS
Choline
Retention time/min

Figure A.2 Ion Pac CS19 isocratic elution chromatogram (concentration 5mg/L)

Appendix B

Mass Spectrogram of Choline Standard Sample and Multiple Reaction Monitoring (MRM) Chart of Standard Solution and Internal Standard Solution

Mass Spectrogram of Choline Standard Sample is as shown in Figure B.1.

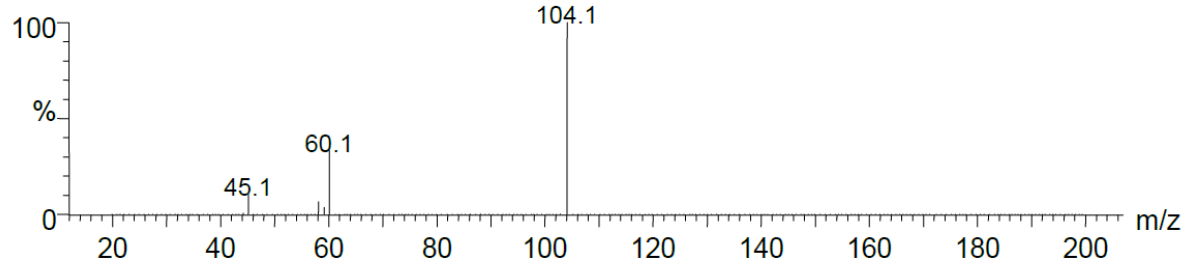
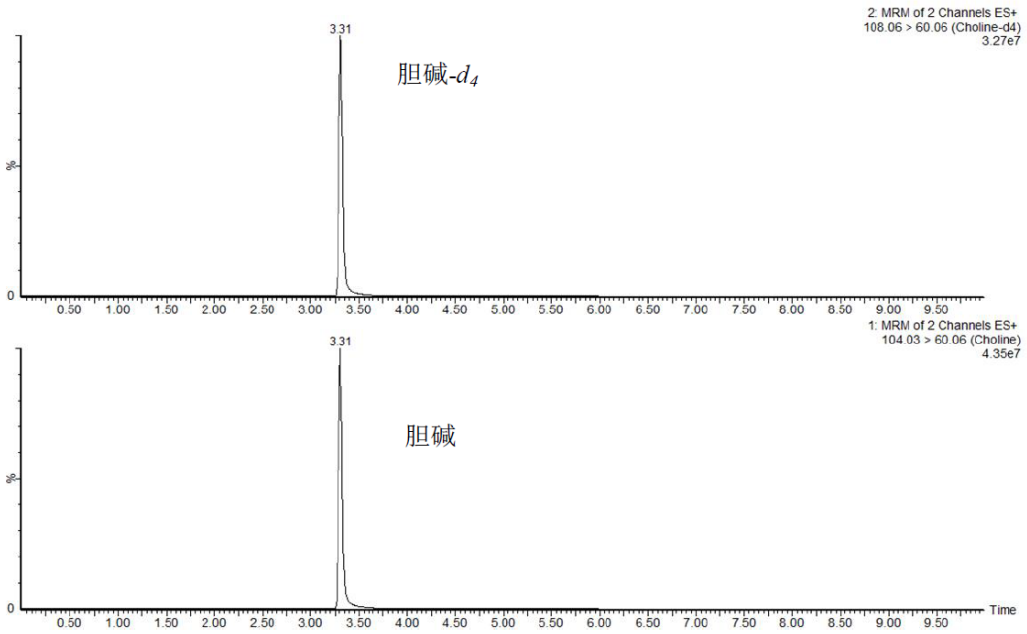


Figure B.1 Mass Spectrogram of Choline Standard Sample (100μg/L)

(104.1 m/z in the figure is the reference precursor ion, 60.1 m/z is the reference product ion 1, and 45.1 m/z is the reference product ion 2)

The multiple reaction monitoring (MRM) chart of choline standard solution and its internal standard sample is as shown in Figure B.2



Choline-d_4
choline

Figure B.2 MRM chart of choline standard solution (50 μg/L) and its internal standard

END TRANSLATION

Attachments:

No Attachments.