NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5413.20-2022

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

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Foreword

This Standard replaced GB 5413.20-2013 National Food Safety Standard - Determination of

Choline in Infant Foods and Dairy Products.

Compared with GB 5413.20-2013, the major changes of this Standard are as follows.

--- Delete the second method of Ray's salt spectrophotometry; and add ion chromatography

as the second method;

--- Add liquid chromatography-tandem mass spectrometry as the third method.

National Food Safety Standard - Determination of Choline in

Foods, Milk and Milk Products for Infants and Young Children

1 Scope

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

This Standard applies to the determination of choline in food, milk and milk products for infants

and young children.

The First Method - Enzymic Colorimetric Method

2 Principle

The specimen is hydrolyzed by acid; and then reacts with the color developer to form colored

substances after enzymatic action. The alkali content is proportional to the external standard

method. Within a certain concentration range, the color depth is proportional to the choline content, and the external standard method is used for quantification.

3 Reagents and Materials

Unless otherwise specified, the reagents used in this method are all analytically pure; and the

water is Class-3 water specified in GB/T 6682.

3.1.6 Peroxidase. >=250U/mg; store at 2 degrees C~8 degrees C.

3.1.7 4-Aminoantipyrine (C11H13N3O).

3.3 Standard product

Choline bitartrate standard product (C9H19NO7, relative molecular mass. 253.25, CAS number.

87-67-2). purity >= 99%, or the reference material certified by the state and awarded the

reference material certificate.

5.1.1 Sample pretreatment

Accurately take 20g (accurate to 0.001g) of the mixed liquid specimen into a 100mL conical flask; add 10mL of 3mol/L hydrochloric acid solution; stopper it and mix well.

Accurately take 5g (accurate to 0.001g) of the mixed semi-solid or solid specimen into a 100mL

conical flask; add 30mL of 1mol/L hydrochloric acid solution; stopper it and mix well.

5.1.3 Filtration

Filter the hydrolyzate with filter paper; if the filtrate is not clear; filter again with a 0.45μm aqueous filter membrane syringe filter. The filtrate is collected for testing.

5.2.1 Drawing of standard curve

Respectively pipette 2.00mL, 4.00mL, 6.00mL, 8.00mL, and 10.00mL of choline standard working solution (250mg/L) into a 10mL volumetric flask; make constant volume to the mark with water; mix well; and prepare the standard series working solutions with concentrations of

50.0 mg/L, 100 mg/L, 150 mg/L, 200 mg/L and 250 mg/L, respectively. Prepare 6 colorimetric

tubes; one colorimetric tube is used as reagent blank (A); add 100μL of water; add 100μL of standard series working solutions to the other 5 colorimetric tubes, respectively; and then add

3.00 mL of color developer, respectively; mix well; the colorimetric tube is placed in a water

bath at 37°C+/-2°C for 15 min for thermal-insulation reaction.

6.1 Calculation of net absorbance value

Usually formulated reagents shall produce a slight color; and the filtrate is not colorless due to

hydrolysis. In order to remove these interference factors, The respective blank values (colorimetric tubes A and B) must be subtracted from the total absorbance value.

The net absorbance value of the specimen is calculated according to Formula (1).

6.2 Calculation of choline content

The choline content (by choline hydroxide) in the specimen shall be calculated according to

Formula (2).

7 Precision

The absolute difference between the results of two independent determinations obtained under

repeatability conditions shall not exceed 10% of the arithmetic mean.

8 Others

When the solid or semi-solid specimen weighs 5g, the detection limit of the method is

1mg/100g, and the limit of quantification is 3mg/100g.

9 Principle

The specimen is hydrolyzed by acid, purified by solid phase extraction column, then separated

by ion chromatography; detected by conductivity detector, and quantified by external standard

method.

10 Reagents and Materials

Unless otherwise specified, the reagents used in this method are all analytically pure; and the

water is the Class-1 water specified in GB/T 6682.

10.2.1 Hydrochloric acid solution (1 mol/L). Pipette 85 mL of concentrated hydrochloric acid

and inject it into about 900 mL of water; and dilute to 1000mL.

10.2.2 Hydrochloric acid solution (1.7 mol/L). Pipette 145 mL of concentrated hydrochloric

acid and inject it into about 800 mL of water; and dilute to 1000mL.