# NATIONAL STANDARD OF THE

# PEOPLE'S REPUBLIC OF CHINA



# GB 5009.189-2023

## National food safety standard - Determination of bongkrekic acid in foods

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## 1 Scope

This Standard specifies the methods for the determination of bongkrekic acid in foods.

In this Standard, Method 1 is applicable to the determination of bongkrekic acid in tremella fungus and its products, fermented rice noodles and its products.

In this Standard, Method 2 is applicable to the determination of bongkrekic acid in tremella fungus and its products, fungus and its products, cereals and its products.

Method I - Liquid Chromatography

## 2 Principle

The bongkrekic acid in the specimen is extracted by solvent, purified and concentrated by a mixed strong anion exchange column or liquid-liquid extraction method. Adopt a high performance liquid chromatograph for analysis and the external standard method for quantitative determination.

## 3 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are all analytically pure, and the water is Grade-1 water specified in GB/T 6682.



### 3.2.1 Phosphoric acid solution (45.4%). measure-take 45.4 mL of phosphoric acid, place it in a

100 mL volumetric flask, use water to dilute to a constant volume to the scale, and shake it well.

### 3.2.2 Sodium bicarbonate solution (40 g/L). weigh-take 40 g of sodium bicarbonate, add water

to dissolve it, transfer to a 1,000 mL volumetric flask, reach a constant volume to the scale, and shake it well.

### 3.2.3 Hydrochloric acid solution (6 mol/L). measure-take 50 mL of hydrochloric acid, place it

in a 100 mL volumetric flask, add water to dilute to a constant volume to the scale, and shake it well.

### 3.2.4 Ammonia water-methanol solution. measure-take 80 mL of methanol, add 1.0 mL of

ammonia water, add water to reach a constant volume of 100 mL, and shake it well.

### 3.2.5 Formic acid-methanol solution (2%). draw-take 2.0 mL of formic acid, add methanol to

100 mL, and shake it well.

### 3.2.6 Formic acid aqueous solution (pH 2.5). use formic acid to adjust the pH of water to 2.5 ?

0.1, and shake it well.

### 3.3 Reference Material

Bongkrekic acid (C28H38O7, CAS. 11076-19-0). purity ? 95%, or a standard substance certified by the state and awarded a reference material certificate.

NOTE. the reference material may use commercial standard solutions that satisfy the traceability requirements.

### 3.4.1 Bongkrekic acid standard stock solution (0.1 mg/mL). accurately weigh-take 10.0 mg

(accurate to 0.01 mg) of bongkrekic acid reference material, use methanol to dissolve it, transfer to a 100 mL volumetric flask, and use methanol to reach a constant volume to the scale. Store it in a ?20 ?C refrigerator away from light. It shall remain valid for 6 months.

### 3.4.2 Bongkrekic acid standard series of working solutions. respectively and accurately draw-

take bongkrekic acid standard stock solution and use methanol to dilute it to a constant volume.

Thus, standard working solutions respectively with a mass concentration of bongkrekic acid of Use a medium-speed filter paper to filter it, transfer the filtrate to the same 100 mL volumetric flask, and use ammonia water-methanol solution to reach a constant volume to the scale, and evenly mix it. Accurately measure-take 50.0 mL of the extracting solution, place it in 80 ?C water bath and concentrate to about 3 mL by rotary evaporation, and reserve it for purification.

5.2.2 Specimen purification Transfer all the concentrated specimen to the activated solid phase extraction column, successively use 5 mL of water and 5 mL of methanol to rinse it and discard the effluent. Then, use 6 mL of formic acid-methanol solution (2%) to elute it, collect the eluent; in 40 ?C water bath, use nitrogen to blow it to dryness. Accurately add 0.5 mL of methanol, vortex to dissolve it, evenly mix it, filter it through a 0.45 ?m microporous organic filter membrane, and reserve it for later use.

5.3 Liquid-liquid Extraction Method

5.3.1 Specimen extraction Weigh-take 20 g (accurate to 0.01 g) of specimen and place it in a conical flask. Add about 20 mL of methanol and soak it at room temperature in the dark for 1 hour. Then, add about 70 mL of chloroform and 0.2 mL of phosphoric acid solution (45.4%), oscillate for 30 min, filter it, transfer the filtrate to a 100 mL volumetric flask, and use chloroform to reach a constant volume to the scale, evenly mix it. Accurately measure-take 50.0 mL of the filtrate and reserve it for extraction.

5.3.2 Specimen extraction Transfer the above-mentioned filtrate into a 150 mL separatory

funnel, add sodium bicarbonate solution (40 g/L) that has an equivalent volume with the filtrate, shake it for 2 minutes, and let it stand for stratification. Then, take out the lower layer and place it in another separatory funnel.

Use 10 mL of sodium bicarbonate solution (40 g/L) to repeat the extraction twice, and gently shake it. Combine the sodium bicarbonate solutions extracted in three times, add 25 mL of chloroform, shake for 2 minutes and let it stand for stratification. Then, discard the chloroform and slowly add hydrochloric acid solution (6 mol/L) into the separatory funnel, adjust the pH of the solution to 2 ~ 3, add 50 mL of petroleum ether, shake it for 3 minutes, let it stand for stratification, and take out the petroleum ether layer and place it in a rotary evaporator. Then, respectively use 30 mL and 20 mL of petroleum ether to perform the extraction once and combine the petroleum ether layer into the same bottle. In 40 ?C water bath, evaporate and concentrate it to dryness; use a small amount of methanol to dissolve the extract in the rotary evaporation bottle in several times and transfer it to a 5.00 mL glass centrifuge tube or concentration bottle. At 40 ?C, blow nitrogen to concentrate it to dryness; accurately add 0.5 mL of methanol, vortex to dissolve it, evenly mix it, filter it through a 0.45 ?m microporous organic filter membrane and reserve it for later use.

5.4 Reference Conditions of Instrument

5.4.1 Chromatographic column. C18 chromatographic column (column length. 250 mm, inner V2---the volume of the specimen solution taken for purification, expressed in (mL).

The calculation results shall retain 3 significant figures.

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 The detection limit of this Method is 5 ?g/kg, and the quantitation limit is 15 ?g/kg.

Method II - Liquid Chromatography - Mass Spectrometry / Mass Spectrometry

9 Principle The bongkrekic acid in the specimen is extracted with ammoniated methanol and purified by a mixed strong anion exchange column. Adopt liquid chromatograph - tandem mass spectrometer for detection, and the external standard method for quantitative determination.

10 Reagents and Materials Unless it is otherwise specified, the reagents used in this Method are all analytically pure, and the water is Grade-1 water specified in GB/T 6682.

10.1 Reagents

10.1.1 Acetonitrile (CH3CN). chromatographically pure.

10.1.2 Formic acid (CH2O2). chromatographically pure.

10.1.3 Methanol (CH3OH).

10.1.4 Ammonia water (NH3 ? H2O). 25% ~ 28%.