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# NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

# GB 5009.283-2021

## National Food Safety Standard Determination of Azoformamide in Food

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## 1 Scope

This standard specifies the liquid chromatography method for the determination of azoformamide in food.

This standard applies to the determination of azoformamide in wheat flour.

## 2 Principle

The sample is extracted with N,N-dimethylformamide solution, centrifuged, and derivatized with triphenylphosphine. The derivative is determined by liquid chromatography, external standard method Quantitative.

## 3 Reagents and materials

Warning---Triphenylphosphine and N,N-dimethylformamide should avoid contact with skin and eyes during operation, and operate in a ventilated environment.

Unless otherwise stated, all reagents in this method are analytically pure, and the water is the first grade water specified in GB/T 6682.



### 3.2 Reagent preparation

Triphenylphosphine derivative solution. accurately weigh 100mg of triphenylphosphine, dissolve it with N,N-dimethylformamide and dilute to 10mL, prepare The derivative solution with a concentration of 10.0mg/mL should be stored in a dark place for later use. Prepare before use.

### 3.3 Standard products

Azoformamide standard product (C2H4N4O2, CAS. 123-77-3), purity >97%. Or certified by the country and awarded a certificate of standard material Standard product.

### 3.4 Preparation of standard solution

3.4.1 Standard stock solution. accurately weigh 50 mg of azoformamide standard (accurate to 0.1 mg) and dissolve it with N,N-dimethylformamide And dilute to 500mL, mix well, prepare a standard stock solution with a concentration of 100ug/mL, transfer the solution to a brown glass container, Store in the dark at -20 degrees C for later use, and the validity period is 20 days.

3.4.2 Blank matrix extraction solution. Use blank wheat flour to process the sample in accordance with 5.2 method to obtain a blank matrix extraction solution. blank The content of azoformamide in wheat flour should be lower than the detection limit of this method.

3.4.3 Standard series working solution. draw 100ug/mL standard stock solution 50.0uL, 100uL,.200uL, 500uL, 1000uL in a set of 10mL volumetric flasks, dilute the volume to the mark with the blank matrix extraction solution, mix well, and prepare the concentration to be 0.500ug/mL, 1.00ug/mL, 2.00ug/mL, 5.00ug/mL, 10.0ug/mL standard series of working solutions. Prepare before use.

### 3.5 Materials

Organic microporous membrane. 0.22um.

### 5.1 Sample preparation

Weigh.200 g of a representative wheat flour sample, mix it evenly, and store it in a brown wide-mouth bottle for later use.

### 5.2 Sample processing

Weigh 2.50g sample, place it in a 50mL centrifuge tube with stopper, add 1g anhydrous magnesium sulfate, mix well, add 25.0mL accurately N,N-Dimethylformamide, mix well on a vortex shaker, shake and extract for 20min, let stand, centrifuge at 10000r/min for 5min, take it The clear liquid is ready to be derived.



### 5.3.1 Derivatization of the sample solution

Aspirate 1.00 mL of filtrate, place it in a 10 mL stoppered centrifuge tube, add 100 uL of triphenylphosphine derivative solution, and place on a vortex mixer Mix thoroughly, and keep it in the dark for more than 12h at an ambient temperature of 20 degrees C~30 degrees C (if the ambient temperature does not meet the requirements, it can be at 20 degrees C~ The derivatization reaction is carried out in a temperature controllable device at 30°C). After the derivative solution is passed through a 0.22um organic microporous membrane, it is used for liquid chromatograph measurement (the test solution is in Measured within 60h at an ambient temperature of 20 degrees C~30 degrees C).

### 5.3.2 Derivatization of standard solutions

Draw 1.00 mL of standard series of working solutions of different concentrations, follow the operation steps of 5.3.1, and derive synchronously with the sample solution to prepare Standard solution working curve.

### 5.4 Reference conditions for liquid chromatography

a) Chromatographic column. C18 chromatographic column (4.6mmx250mm, 5um) or equivalent;

b) Mobile phase. A is water, B is methanol, and the gradient elution program is shown in Table 1;

c) Flow rate. 1.0mL/min;

d) Column temperature. 30 degrees C;

e) Detection wavelength. 230nm;

f) Injection volume. 10uL.

### 5.5 Preparation of standard curve

The derivatized standard series of working solutions were injected into the high performance liquid chromatograph, and the peak areas of the corresponding derivatives were measured.

The concentration of azoformamide in the working solution is the abscissa, and the response value of the derivative peak area is the ordinate to draw a standard curve. Azomethine Refer to Figure A.1 in Appendix A for the chromatogram of amide standard solution derivatives.

### 5.6 Determination of sample solution

Inject the sample derivative solution into the high performance liquid chromatograph to obtain the chromatographic peak area of the azoformamide derivative.

The concentration of azoformamide in the sample solution. The concentration of azoformamide should be within the linear range of the standard curve, and it should be used if it exceeds the linear range.

Dilute the sample extraction solution with N,N-dimethylformamide solution, dilute the blank matrix extraction solution synchronously, prepare a standard curve, and synchronously derive Health, and then analyze.

## 6 Expression of analysis results

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

X= cxVx1000 mx1000 (1)

Where.

X --- The content of azoformamide in the sample, in milligrams per kilogram (mg/kg);

c ---The concentration of azoformamide in the sample solution derived from the standard curve, in micrograms per milliliter (ug/mL);

V ---The volume of the sample extraction solution, in milliliter (mL);

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

1000---unit conversion factor.

The calculation result retains two significant digits.

## 7 Precision