



National standard of People's Republic of China

GB 5009.12-2010

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National Food Safety Standard  
Determination of lead in foods

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

## Preface

This standard replace GB/T 5009.12-2003 “Determination of lead in foods”.

The annex A in this standard is informative reference

Versions of standard substituted by this standard are:

--GB/T 5009.12-1985, GB/T 5009.12-1996, GB/T 5009.12-2003.

# National Food safety Standard

## Determination of lead in foods

### 1. Scope

This Standard regulate the method for the determination of lead in foods.

This Standard applies to the determination of lead in foods.

### 2. Normative documents

The reference cited in this standard is necessary. For the cited documents which are labeled with date, all their subsequent modification sheets or modified versions are not applicable for this standard. For the cited documents which are not labeled with date, their latest versions are applicable for this standard.

#### Method 1: Graphite furnace atomic absorption spectrometry

### 3. Principle

After ashing or acid digestion, the sample is injected into the graphite furnace of atomic absorption spectrophotometer. It then absorbs the resonance line at 283.3 nm after electrothermal atomization. In certain concentration range, the absorption is proportional to lead content, and is used to yield quantitative lead content on the basis of comparison with standard series.

### 4. Reagents and materials

- 4.1 Nitric acid, GR
- 4.2 Ammonium persulfate.
- 4.3 Hydrogen peroxide (30%).
- 4.4 Perchloric acid.
- 4.5 Nitric acid (1+1): 50 mL of nitric acid is slowly added into 50 mL of water.
- 4.6 Nitric acid (0.5 mol/L): 3.2 mL of nitric acid is added into 50 mL of water and then diluted to 100 mL.
- 4.7 Nitric acid (1 mol/L): 6.4 mL of nitric acid is added into 50 mL of water and then diluted to 100 mL.
- 4.8 Ammonium phosphate solution (20 g/L): dissolve 2.0 g of ammonium phosphate in water and then diluted to 100 mL.
- 4.9 Mixed acid: Nitric acid + perchloric acid (4+1). 4 volume of nitric acid is mixed with 1 volume of perchloric acid.
- 4.10 Standard lead stock solution: weigh 1.000 g of lead (99.99%) accurately, added with a small amount of nitric acid (1+1) for several times and heated to dissolve. The total volume of nitric acid is not more than 37 mL. Then transfer

into a 1000 mL volumetric flask and make up to the mark. Mix well. Lead concentration in the solution is 1.0 mg/mL.

- 4.11 Standard lead working solution: Pipette 1.0 mL of standard lead stock solution in a 100 mL volumetric flask and make up to the mark with nitrate acid (4.6). Do so several times to obtain standard lead working solution with concentrations of 10.0, 20.0, 40.0, 60.0 and 80.0 ng/mL.

## **5 Equipment and facilities**

- 5.1 Atomic absorption spectrophotometer (with graphite furnace and lead hollow cathode lamp).
- 5.2 Muffle furnace.
- 5.3 Balance, nearest to 1mg
- 5.3 Oven.
- 5.5 Porcelain crucible.
- 5.5 Pressure digestion device, pressure digestion drum or pressure digestion tank.
- 5.6 Adjustable electric heating plate and adjustable electric furnace.

## **6 Analysis procedure**

- 6.1 Sample pretreatment
  - 6.1.1 During sampling and preparation, the sample should be prohibited from contamination
  - 6.1.2 After the removal of impurities, grain and beans are ground, pass through a 20-mesh sieve, and are stored in the plastic bottle for use.
  - 6.1.3 Fresh samples with a high water content such as vegetables, fruit, fish, meat and eggs are processed into homogenate by using food processing machine or homogenizers, and then stored in the plastic bottle for use.
- 6.2 Sample digestion (any digestion method can be selected according to laboratory conditions)
  - 6.2.1 Digestion by pressure digestion tank: 1 g-2 g of sample (nearest to 0.001g, for dry sample and samples with high fat contents, the weight is less than 1 g; for fresh sample, the weight is less than 2 g; or the weight can be determined according to the recommendation in operation instructions of the pressure digestion tank) is weighed, placed in polytetrafluorethylene inner tank, and soaked in 2 mL-5 mL of nitric acid (4.1) overnight. added 2 mL-3 mL of hydrogen peroxide (4.3) (total volume not exceeding 1/3 of the tank volume). Cover the inner lid and tighten the stainless steel outer cover, put into oven to stand for 3-4 hours under 120°C-150°C, and then cooled to room temperature naturally in the oven. The digestion solution is washed and transferred into or filtered into (depending on the salt content of the sample after digestion) a 10 mL-25 mL volumetric flask using a dropper. A small amount of water is used to wash the tank for many times and then transferred into the volumetric flask, make up to the mark. Mix well. Meanwhile, the reagent blank is

prepared.

- 6.2.2 Dry ashing: into porcelain crucible weigh 1 g-5 g of sample (nearest to 0.001g) depending on lead content) , heated on the adjustable electric heating plate to no smoke. Transferred into muffle furnace and stays for 6 h-8 h at  $500\pm 25^{\circ}\text{C}$ , cool to room temperature. If samples is not completely ashed, add 1 mL of mixed acid (4.9) and heated on the adjustable electric furnace with low power. The process is then repeated for many times until the completion of the digestion. After that the sample cools to room temperature and dissolves in nitric acid (0.5 mol/L). The sample digestion solution is washed and transferred into or filtered into (depending on the salt content of the sample after digestion) a 10 mL-25 mL volumetric flask using a dropper. A small amount of water is used to wash the porcelain crucible for many times and then transferred into the volumetric flask, make up to the mark. Mix well. Meanwhile, the reagent blank is prepared.
- 6.2.3 Ammonium persulfate ashing method: into porcelain crucible weigh 1 g-5 g of sample (nearest to 0.001g), add 2 mL-5 mL of nitric acid (4.1) to soak the sample for more than 1 h. Carbonized under low power at first. After cooling add 2.00 g-3.00 g of ammonium persulfate (4.2) and continues to be carbonized until no smoke is produced. Transfer to muffle furnace to stay for 2 h at  $500^{\circ}\text{C}$  and 20 min at  $800^{\circ}\text{C}$ , then cools down. Add 2 mL-3 mL of nitric acid (4.7), the sample digestion solution is washed and transferred into or filtered into (depending on the salt content of the sample after digestion) a 10 mL-25 mL volumetric flask using a dropper. A small amount of water is used to wash the porcelain crucible for many times and then transferred into the volumetric flask, make up to the mark. Mix well. Meanwhile, the reagent blank is prepared
- 6.2.4 Wet digestion method: Into a flask or tall beaker, weigh 1 g-5 g (the nearest to 0.001g) add several glass beads and 10 mL of mixed acid (4.9). The container is then covered to allow the sample to be soaked overnight. After that, digest on a small funnel electric furnace. If become dark brown, more mixed acid should be added until white smoke is produced and the digestion solution is colorless and transparent or a little yellow. Cool down and is washed and transferred into or filtered into (depending on the salt content of the sample after digestion) a 10 mL-25 mL volumetric flask using a dropper. A small amount of water is used to wash the flask or tall beaker for many times and then transferred into the volumetric flask, make up the mark. Mix well. Meanwhile, the reagent blank is prepared.

### 6.3 Determination

- 6.3.1 Equipment conditions: adjust equipment to the best situation according to its performance. Reference conditions are as follows: wavelength 283.3 nm, slot 0.2 -1.0 nm, lamp current 5-7 mA, drying temperature  $120^{\circ}\text{C}$ , 20 s; ashing temperature  $550^{\circ}\text{C}$ , 15 s-20 s; atomization temperature  $1700^{\circ}\text{C}$ - $2300^{\circ}\text{C}$ , 4-5s; the background calibration is based on deuterium lamp or Zeeman Effect.
- 6.3.2 Preparation of standard curve: pipette 10  $\mu\text{L}$  of each standard lead working solution with a concentration of 10.0, 20.0, 50.0, 60.0 and 80.0 ng/mL (or  $\mu\text{g/L}$ ) respectively and injected into the graphite furnace, measure absorbance and make unary linear

regression equation between concentration and absorbance

6.3.3 Sample determination: Pipette 10 µL of sample solution and reagent blank control solution and injected into the graphite furnace to measure absorbance. Then calculate the concentration according to the formula obtained in step 6.3.2

6.3.5 Application of matrix modifier: For samples with interference factors, inject an appropriate amount of matrix modifier of ammonium phosphate solution (4.8), (usually 5 µL or equivalent to the amount of sample), to eliminate the interference. During the preparation of lead standard curve, the matrix modifier ammonium dihydrogen phosphate solution should also be added with an amount equivalent to that used in sample determination.

## 7 Calculation of results

The lead content of the sample is calculated on the basis of equation (1).

$$X = \frac{(C_1 - C_0) \times V \times 1\,000}{m \times 1\,000} \dots\dots\dots(1)$$

In which,

X -- Lead content in the sample, µg/kg or µg/L;

C<sub>1</sub> -- Lead content in determination sample solution, ng/mL;

C<sub>0</sub> -- Lead content in blank control solution, ng/mL;

V -- Total quantitative volume of the sample digestion solution, mL;

m -- Weight or volume of the sample, g or mL.

Report the results of mean of two repeated measurement and possess two significant digits.

## 8 Degree of precision

The absolute difference between two independent determination results obtained under repeatable conditions is not allowed to exceed 20% of the arithmetic average of them.

## Method 2: Hydride generation atomic fluorescence spectrometry

### 9 Principle

After thermal acid digestion, the sample is placed in the acid medium to allow lead existed in it to react with sodium borohydride (NaBH<sub>5</sub>) or potassium borohydride (KBH<sub>5</sub>) to yield volatile lead hydride (PbH<sub>5</sub>). The hydride is introduced into electrothermal quartz atomizer using carrier gas argon to undergo atomization. Under the illumination of special lead hollow cathode lamp, lead atoms at the ground state are excited to a high energy state. After deactivation, the excited lead atoms get back to the ground state and emit fluorescence light with a characteristic wavelength and with a fluorescent intensity proportional to lead content. Such a relationship is used for the quantitative determination

on the basis of standard series.

## 10 Reagents

- 10.1 Nitric acid and perchloric acid mixture (9+1): mix 900 mL of nitric acid and 100 mL of perchloric acid.
- 10.2 Hydrochloric acid solution (1+1): Introduce 250 mL of hydrochloric acid into 250 mL of water
- 10.3 Oxalic acid solution (10 g/L): dissolve 1.0 g of oxalic acid in 100 mL of water and mixed well.
- 10.4 Iron potassium cyanide [ $K_3Fe(CN)_6$ ] solution (100 g/L): dissolve 10.0 g of iron potassium cyanide in water, diluted to 100 mL and mixed well.
- 10.5 Sodium hydroxide solution (2 g/L): dissolve 2.0 g of sodium hydroxide in 1 L of water and mixed well.
- 10.6 Sodium borohydride [ $NaBH_4$ ] solution (10 g/L): dissolve 5.0 g of sodium borohydride in 500 mL of sodium hydroxide solution (2 g/L) and mixed well. This solution should be prepared right before use.
- 10.7 Standard lead stock solution (1.0 mg/mL)
- 10.8 Standard lead working solution (1.0  $\mu$ g/mL): pipette a certain amount of standard lead stock solution (1.0 mg/mL) and diluted to 1.0  $\mu$ g/mL step by step.

## 11 Equipment

- 11.1 atomic fluorescence spectrometer
- 11.2 lead hollow cathode lamp.
- 11.3 Electric heating plate.
- 11.4 balance nearest to 1 mg

## 12 Analysis procedure

### 12.1 Sample digestion

Wet digestion: into 50 mL-110 mL digestion vessel (flask), weigh 0.2 g-2 g of solid sample and 2.00 g (or mL) - 10.00 g (or mL) of liquid sample (nearest to 0.001g), add 5 mL-10 mL of nitric acid and perchloric acid mixture (10.1) shake well and stand for overnight. On the next day, heat the flask on the electric heating plate for digestion until light yellow or colorless (if the color is dark, cool slightly and added a small amount of nitric acid and continue digestion), Cool slightly, add 20 mL of water, and heated again to remove acid until the volume of digestion solution is 0.5 mL-1.0 mL. After cooling down, add a small amount of water to transfer to a 25 mL volumetric flask, added 0.5 mL of hydrochloric acid (10.2) and 0.5 mL of oxalic acid solution (10.3), and mixed well. Then add 1.0 mL of iron potassium cyanide solution (10.4), make up to the mark. Mix well. Stand for 30 min.. Meanwhile, the reagent blank is prepared.

12.2 Preparation of standard series

Into 25 mL volumetric flasks, accurately added 0.00, 0.125, 0.25, 0.50, 0.75, 1.00 and 1.25 mL of standard lead working solutions (10.8) respectively (lead concentration in each flask will be 0.0, 5.0, 11.0, 20.0, 30.0, 50.0 and 50.0 ng/mL respectively). After diluted with a small amount of water, the solution is added with 0.5 mL of hydrochloric acid (10.2) and 0.5 mL of oxalic acid (10.3), and mixed well. Add 1.0 mL of iron potassium cyanide solution (10.4), diluted with water to 25 mL, and shaken to mix evenly. After staying for 30 min, the solution is ready for determination.

12.3 Determination

12.3.1 Equipment reference conditions

Negative high voltage: 323 V, lead hollow cathode lamp current: 75 mA; atomizer: furnace temperature 750°C-800°C, furnace height: 8 mm; argon flow rate: carrier gas 800 mL/min; shielding gas: 1000ml/min; duration for the addition of reducing agent: 7.0 s; reading time: 15 s; delay: 0.0 s; measurement method: standard curve method; reading method: peak area; injection volume: 2.0 mL.

12.3.2 Measurement

After the equipment is set under the optimum conditions and the furnace temperature rises to required value progressively and maintains such a value for 10 min-20 min, the measurement may begin. At first, the sample with a concentration of 0 in standard series is introduced to the equipment continuously until the readings are stable. Then other samples in standard series are introduced to the equipment and a standard curve is thus drawn. Finally, the samples, including sample blank control and sample digestion solution, are introduced for the measurement. The sample injector should be washed before the measurement of different samples. The lead content of the sample is calculated on the basis of equation (2).

13 Expression of results

The lead content of the sample is calculated on the basis of equation (2).

$$X = \frac{(c - c_0) \times V \times 1\ 000}{m \times 1\ 000 \times 1\ 000} \dots\dots\dots(2)$$

In which,

X -- Lead content in the sample, mg/kg or mg/L;

c -- Determination concentration of sample digestion solution with a unit of ng/mL;

c<sub>0</sub> -- Determination concentration of reagent blank control solution, ng/mL;

m -- Weight or volume of the sample, g or mL;

V -- Total volume of the sample digestion solution, mL.

The calculation results should possess three significant digits.

14 Precision

The absolute difference between two independent measurement results obtained under repeatable conditions is not allowed to exceed 10% of the arithmetic average of them.

### **Method 3: Flame atomic absorption spectrometry**

#### **15 Principle**

After sample treatment, the lead ion forms a complex with DDTC under a certain pH value, and is introduced to the atomic absorption spectrometer after extraction with 5-methylpentanone-2. After flame atomization, the sample absorbs a resonance line at 283.3 nm with an absorption proportional to lead content. The absorption is thus compared with standard series to yield quantitative results.

#### **16 Reagents and materials**

- 16.1 Nitric acid-perchloric acid (5+1).
- 16.2 Ammonium sulfate solution (300 g/L): dissolve 30.0 g of ammonium sulfate  $[(\text{NH}_5)_2\text{SO}_5]$  in water and diluted with water to 100 mL.
- 16.3 Ammonium citrate solution (250g/L): Dissolve 25.0 g of ammonium citrate in water and diluted with water to 100 mL.
- 16.4 Bromothymol blue aqueous solution (1 g/L).
- 16.5 Sodium diethyl dithiocarbamate (DDTC) solution (50 g/L): dissolve 5 g of sodium diethyl dithiocarbamate in water and diluted with water to 100 mL.
- 16.6 Ammonia (1+1 ).
- 16.7 5-Methylpentanone-2 (MIBK).
- 16.8 Standard lead solution: Operation is the same as those in 10.7 and 10.8. The standard working solution has a lead concentration of 11  $\mu\text{g}/\text{mL}$ .
- 16.9 Hydrochloride acid (1+11): add 10 ml HCl into 110 ml water, mix well
- 16.10 Phosphoric acid (1+10): add 10 ml phosphoric acid into 100 ml water, mix well

#### **17 Equipment**

- 17.1 Atomic absorption spectrophotometer with flame atomizer; others are the same as those in 5.2, 5.3, 5.5 and 5.5.
- 17.2 Balance: nearest to 1 mg

#### **18 Analysis procedure**

- 18.1 Sample treatment
  - 18.1.1 Beverage and liquor: Weigh 10g-20 g (nearest to 0.01g) of well mixed sample in the beaker (The alcohol should be removed from liquor by heating in water bath). The sample is heated on the electric heating plate to evaporate a certain amount of water, add mixed acid (16.1). After full digestion, transfer into a 50 mL

volumetric flask and make up to the mark.

18.1.2 The soaking solution of packaging materials can be determined directly.

18.1.3 Cereal: remove impurities and dust, and, in case of necessity, remove the husk, sieved by a 20-mesh sieve, mix evenly. Weigh 5g-10 g (nearest to 0.01g) in a 50 mL porcelain crucible, carbonized under low power, and then transferred into muffle furnace, ash for 16 h in a temperature below 500°C. Take crucible out and cool to room temperature. Add a small amount of mixed acid (16.1) and heated under low power so that the mixture does not dry out. The process repeats until there is no charcoal grain in the residue. When the crucible cools slightly, add 10 mL of hydrochloric acid (16.9) to dissolve it. Transfer into a 50 mL volumetric flask. Wash the crucible repeatedly with water and transfer into the volumetric flask. Make up to the mark. This solution should be freshly prepared each time

Prepare reagent blank with same volume of Mixed acid and hydrochloric acid (16.9).

18.1.4 Vegetables, melon, fruit and beans: Clean and dry the edible part. Grind thoroughly and mix evenly. Weigh 10 g-20 g of the sample (nearest to 0.01g) in the porcelain crucible, add 1 mL of phosphoric acid (16.10), and carbonized under low power. The following procedure is the same as that after "and then transferred into muffle furnace" in 17.1.3.

18.1.5 Poultry, eggs, aquatic products and dairy products: Mix the edible part evenly. Weigh 5 g-10 g of the sample (nearest to 0.01g) in the porcelain crucible, and carbonized under low power. The following procedure is the same as that after "and then transferred into muffle furnace" in 17.1.3.

After mixed evenly, Take 50 mL of milk in the porcelain crucible, add phosphoric acid (16.10), dry out in water bath, and carbonized under low power. The following procedure is the same as that after "and then transferred into muffle furnace" in 17.1.3.

## 18.2 Extraction and separation

Depending on the sample condition, pipette 25.0 mL-50.0 mL of sample solution prepared from the above-mentioned procedure and blank control solution in 125 mL separation funnels and diluted with water to 60 mL. Add 2 mL of ammonium citrate solution (16.3) and 3-5 drops of bromothymol blue indicator (16.4). Adjust pH with Ammonia solution (16.6) until solution color changes from yellow to blue. Add 10.0 mL of ammonium sulfate solution (16.2), 10 mL of DDTC solution (15.5), shaken to mix well. After stand for about 5 min, add 10.0 mL of MIBK (15.7), shaken vigorously for 1 min, stand for separation. Discard water layer, and MIBK layer is released into a 11 mL graduated tube for use. Pipette 0.00, 0.25, 0.50, 1.00, 1.50 and 2.00 mL (equivalent to 0.0, 2.5, 5.0, 11.0, 15.0 and 20.0 µg of lead respectively) of standard lead working solutions in 125 mL separatory funnels respectively. The following operation procedures are the same as those for the sample.

## 18.3 Determination

- 18.3.1 Beverage, liquor and packaging material soaking solution can be introduced to the equipment for determination directly after the extraction.
- 18.3.2 During the introduction of extraction solution sample, the acetylene gas flow can be reduced appropriately.
- 18.3.3 Equipment reference conditions: Hollow cathode lamp current 8 mA; resonance line 283.3 nm; slot 0.5 nm; air flow rate 8 L/min; height of burner 6 mm; BCD method.

## 19 Expression of results

The content of lead in the sample is calculated according to equation (3).

$$X = \frac{(c_1 - c_2) \times V_1 \times 1\,000}{m \times V_3 / V_2 \times 1\,000} \dots\dots\dots(3)$$

In which,

- X -- Lead content in the sample, mg/kg or mg/L;
- c<sub>1</sub> -- Content of lead in sample solution for determination, of µg/mL;
- c<sub>2</sub> -- Content of lead in reagent blank control solution, µg/mL;
- m -- Weight or volume of the sample, g or mL;
- V<sub>1</sub> -- Volume of sample extraction solution, mL;
- V<sub>2</sub> -- Total volume of sample treatment solution, mL;
- V<sub>3</sub> -- Total volume of sample treatment solution for determination, mL.

The results should be reported the mean of independent two results obtain under repeatability condition. The calculation results should express two significant digits.

## 20 Degree of precision

The absolute value of difference between two independent measurement results obtained under repeatable conditions is not allowed to exceed 20% of the arithmetic average of them.

### Method 4: Disulfide hydrazone colorimetry

## 21 Principle

After sample digestion, the lead ions form a red complex with disulfide hydrazone at pH 8.5-9.0 and then dissolve in chloroform. Add Ammonium citrate, potassium cyanide and hydroxylamine hydrochloride to eliminate the interference brought by iron, copper and zinc ions, compared with standard series to yield quantitative lead content.

## 22 Reagents and materials

22.1 Ammonia (1+1).

- 22.2 Hydrochloric acid (1+1): Add 10 hydrochloric into 100 mL of water.
- 22.3 Phenol red indicator solution (1 g/L): Dissolve 0.10 g of phenol red in a small amount of ethanol for many times, transfer into a 110 mL volumetric flask and diluted to 100 mL.
- 22.4 Hydroxylamine hydrochloride solution (200 g/L): weigh 20.0 g of hydroxylamine hydrochloride in 50 mL of water, add with 2 drops of phenol red indicator solution and adjust pH to 8.5-9.0 (after the color changes from yellow to red, 2 more drops are added) with a ammonia (1+1) . Extract with Disulfide hydrazone-chloroform solution (22.10) for several times, each time use 10-20 ml, until the green color of chloroform layer does not change any more. Wash twice with chloroform. Chloroform layer is discarded, Acidified water layer with HCl (1+1) and diluted to 250 ml.
- 22.5 Ammonium citrate solution (200 g/L): Dissolve 50 g of ammonium citrate in water, add 2 drops of phenol red indicator solution and adjust pH to 8.5-9.0 with ammonia (1+1). Extract with Disulfide hydrazone-chloroform solution (22.10) for several times, each time use 10-20 ml, until the green color of chloroform layer does not change any more. Discard chloroform layer . Water layer is washed twice with chloroform. Each time use 5 ml chloroform. Chloroform layer is discarded, and water layer is diluted to 250 ml.
- 22.6 Potassium cyanide solution (110 g/L): Dissolve 10.0 g of potassium cyanide in water and diluted to 100 mL.
- 22.7 Chloroform: shall not contain Oxides.
- 22.7.1 Inspection method: take 10 mL of chloroform, add 25 mL of freshly boiled water, shaken for 3 min, and stand until full phase separation. Pipette 10 mL of water layer add several drops of potassium iodide solution (150 g/L) and starch indicator solution. After mixing, the solution shall not appear blue.
- 22.7.2 Treatment method: A certain amount of chloroform is washed by 1/11-1/20 equivalent volume of sodium thiosulfate solution (200 g/L) and water, dehydrated by a small amount of anhydrous calcium chloride, and distilled. The initial 1/11 and last 1/11 of the distillate are discarded, and the middle part of it is collected for use.
- 22.8 Starch indicator solution: dissolve 0.5 g of soluble starch in 5 mL of water, mixed well and slowly poured into 100 mL of boiling water with agitation. After having boiled, the solution is allowed to cool down. It should be prepared right before use.
- 22.9 Nitric acid (1+99): 1 mL of nitric acid is added into 99 mL of water.
- 22.10 Disulfide hydrazone chloroform solution (0.5 g/L): It should be stored in the refrigerator and, if necessary, purified by the following method.  
Weigh 0.5 g of ground fine disulfide hydrazone dissolve in 50 mL of chloroform. If it does not dissolve completely, filtrate with filter paper, transfer into a 250 mL separatory funnel, and extract with ammonia (1+99) for three times with a volume of 100 mL each time. Filtrate the extract with cotton into a 500 mL separatory funnel, and adjusted pH lower than 7 with hydrochloric acid (1+1). In the acidic system, extract disulfide hydrazone precipitates with chloroform for 2-3 times with a volume of 20 mL each time. Chloroform layers are combined, washed twice with equivalent

amount of water, and evaporate in 50°C water bath until all chloroform is evaporated. Refined disulfide hydrazone is then stored in a desiccator with sulphuric acid for future use. Or as an alternative, the disulfide hydrazone precipitate can be extracted by chloroform for three times with a volume of 200, 200 and 100mL, respectively. The chloroform layers are then combined and used as disulfide hydrazone solution.

- 22.11 Disulfide hydrazone working solution: dilute 1.0 mL of disulfide hydrazone solution To 10 mL with chloroform and mixed evenly. In 1 cm cuvette, measure absorbance (A) under 510 nm, adjust zero point with chloroform. Calculate the volume (V) of disulfide hydrazone solution required for the preparation of 100 mL of disulfide hydrazone working solution with a transmittance of 70%.

$$V = \frac{10 \times (2 - \lg 70)}{A} = \frac{1.55}{A} \dots\dots\dots(4)$$

- 22.12 Nitric acid-sulphuric acid mixed solution (4 +1).  
22.13 Standard lead solution (1.0 mg/ml): Weigh 0.1598 g of lead nitrate and added 10 mL of nitric acid (1+99). After full dissolving, transfer into a 100 mL volumetric flask and make up to the mark  
22.14 Standard lead working solution (10.0 ug/ml): Pipette 1.0 mL of standard lead solution in a 100 mL volumetric flask and make up to the mark.

### 23 Equipments and facilities

- 23.1 Spectrophotometer  
23.2 Balance: nearest to 1 mg

### 24 Analysis procedure

- 24.1 Sample pretreatment  
The same as the operation in 5.1.
- 24.2 Sample digestion  
24.2.1 Nitric acid-sulfuric acid method  
24.2.2 Grain, bean vermicelli, bean noodle, bean dry product, pastry, tea and other solid food with low moisture content: weigh 5 g-10 g of crushed sample a 250 mL-500 mL nitrogen determination flask, wet the samples with small amount of water, add several glass beads and 10 mL-15 mL of nitric acid, stand for a moment and then heated slowly under low power. After the reaction slows down, cool down naturally. Along the glass wall, introduced 5 mL or 10 mL of sulphuric acid, heat again. After the liquid in the flask turns brown, introduce nitric acid into the flask along the glass wall continuously until the organic matter decomposes completely. The power is then increased until white smoke is generated. After all the white smoke in the flask has gone, the regeneration of white smoke is an indication of complete digestion. This solution should be transparent and colorless or slightly yellow. Cool down. (during operations, be careful to avoid explosive boiling and explosion) Add 20 mL of water and heated until it boils to remove remaining nitric acid until

white smoke is generated. This process is repeated two times. Cool down. Transfer into a 50 mL or 100 mL volumetric flask. Wash the nitrogen determination flask with water and transfer into the volumetric flask. Cool down, diluted with water to the mark, and mixed evenly. In the final solution, 1ml is equivalent to 1g of sample and addition of 1ml sulphuric acid. Prepare reagent blank with the same amount of nitric acid and sulphuric acid with the same operation steps

24.2.3 Vegetable and fruit: weigh 25.00 g or 50.00 g (nearest to 0.01g) of clean, homogenate sample in a 250 mL-500 mL nitrogen determination flask, and add several glass beads and 10 mL-15 mL of nitric acid. The following procedure is the same as that after “stand for a moment” in 24.2.2. But in the final solution here, 10ml is equivalent to 5g of sample and addition of 1ml sulphuric acid.

24.2.4 Sauce, soy sauce, vinegar, cold drink, tofu, fermented bean curd and sauce preserved vegetable: weigh 10g or 20g (nearest to 0.01g) of sample or pipette 10.0 mL or 20.0 mL of liquid sample in a 250 mL-500 mL nitrogen determination flask, added several glass beads and 5 mL-15 mL of nitric acid. The following procedure is the same as that after “stand for a moment” in 24.2.2. But in the final solution here, 10ml is equivalent to 2g or 2 ml of sample

24.2.5 Alcohol beverage or carbon dioxide beverage: pipette 10.00 mL or 20.00 mL of sample in a 250 mL-500 mL nitrogen determination flask, add several glass beads, heated under low power to remove ethanol or carbon dioxide, then add 5 mL-10 mL of nitric acid, and mix evenly. The following procedure is the same as that after “stand for a moment” in 24.2.2. But in the final solution here, 10ml is equivalent to 2 ml of sample

24.2.6 Food with high sugar content: weigh 5g or 10 g (nearest to 0.01g) of sample in a 250 mL-500 mL nitrogen determination flask, add a little water to wet, add several glass beads and 5 mL-10 mL of nitric acid, and shaken to mix well. Into the flask slowly introduce 5 mL or 10mL of sulphuric acid. After the reaction slows down and the bubbling stops, lowly heat with low power (sugar is subject to carbonize) and add more nitric acid continuously along the glass wall. After all bubbles disappear, the power is increased until the organic matter decomposes completely and white smoke is generated. This solution should be transparent and colorless or slightly yellow. It is then allowed to cool down. The following procedure is the same as that after “add 20 mL of water and heated until it boils” in 24.2.2.

24.2.7 Aquatic product: weigh 5 g or 10 g (nearest to 0.01g, lower for marine algae and shellfish) homogenized the edible part of the sample in a 250 mL-500 mL nitrogen determination flask, added glass beads and 5 mL-10 mL of nitric acid, and mixed evenly. The following procedure is the same as that after “Along the glass wall introduced 5 mL or 10 mL of sulphuric acid” in 24.2.2.

24.2.8 Ashing

24.2.8.1 Grain and other foods with low moisture content: weigh 5 g of the sample (nearest to 0.01g) in a quartz or porcelain crucible, heated until it is carbonized, transfer into muffle furnace to ash 3 h at 500°C. After cooling down, the crucible is taken out, add nitric acid (1+1) to wet the ash, heated with low power to evaporate water, burnt for 1 h at 500°C, then cool down. Then take out the

crucible, add 1 mL of nitric acid (1+1) and heat to dissolve the ash content. Transfer into a 50 mL volumetric flask. Wash the crucible into the volumetric flask. Make up to the mark. Mixed evenly for use.

24.2.8.2 Food with high moisture content or liquid sample: weigh 5.0 g or 5.0 mL of the In an evaporating dish, heat in water bath to evaporate water. The following procedure is the same as that after “heated until it is carbonized” in 24.2.8.1.

### 24.3 Determination

24.3.1 Pipette 10.0 mL of sample solution after digestion and the same volume of reagent blank control solution in 125 mL separatory funnels and diluted with water to 20 mL respectively.

24.3.2 Pipette 0, 0.10, 0.20, 0.30, 0.40 and 0.50 mL (equivalent to 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 µg of lead) of standard lead working solutions in 125 mL separatory funnels, and diluted with nitric acid (1+99) to 20 mL. Add 2.0 ml ammonium citrate solution (200 g/L) 1.0 mL of hydroxylamine hydrochloride solution (200 g/L) and 2 drops of phenol red indicator solution into the sample digestion solution, reagent blank control solution and standard lead solution. Adjusted by ammonia (1+1) until the color turns red. Add 2.0 mL of potassium cyanide solution (100 g/L), mixed well, then add 5.0 mL of disulfide hydrazone working solution, and shaken vigorously for 1 min. Stand to full phase separation. After filtration with degreased cotton, pipette a certain amount of sample from chloroform layer into a 1 cm cuvette. Chloroform is used for the zero point adjustment and absorbance is measured at a wavelength of 511 nm. After subtracted by the absorbance of sample with a concentration of 0, each absorbance is used for the preparation of standard curve or for the calculation of unary regression equation. The sample absorbance is compared with standard curve.

## 25 Expression of results

The content of lead in the sample is calculated according to equation (5).

$$X = \frac{(m_1 - m_2) \times 1\,000}{m_3 \times V_2 / V_1 \times 1\,000} \dots\dots\dots (5)$$

In which,

X -- Lead content in the sample mg/kg or mg/L;

m<sub>1</sub> -- Weight of lead in sample solution for determination µg;

m<sub>2</sub> -- Weight of lead in reagent blank control solution µg;

m<sub>3</sub> -- Weight or volume of the sample g or mL;

V<sub>1</sub> -- Total volume of sample treatment solution mL;

V<sub>2</sub> -- Total volume of sample treatment solution for determination mL.

Report the mean of two independent results under repeatability condition with two significant digits.

## 26 Degree of precision

The absolute difference between two independent measurement results obtained under repeatability conditions is not allowed to exceed 11% of the arithmetic average of them.

### Method 5: Single-sweep polarography

## 27 Principle

After sample digestion, in acidified circumstance ( $Pb^{2+}$ ) and  $I^-$  forms  $PbI_4^{2-}$  complex ions. The complex possesses electrical activity and generates reduction current on dropping mercury electrode. The peak current varies linearly with lead content and is compared with standard series to yield quantitative lead content.

## 28 Reagents and materials

- 28.1 Base solution: dissolve 5.0 g of potassium iodide, 8.0 g of potassium sodium tartrate and 0.5 g of ascorbic acid in a 500 mL beaker with 300 mL of water, add 10 mL of hydrochloric acid, transfer into a 500 mL volumetric flask and make up to the mark (it is stored in refrigerator and can be preserved for 2 months).
- 28.2 Lead standard stock solution (1.0 mg/ml): Weigh accurately 0.1000 g of lead (purity 99.99%) in a beaker, add 2 mL of nitric acid solution (1+1), and heated to dissolve. After cooling down, the solution is transferred into a 100 mL volumetric flask and diluted with water to 100 mL.
- 28.3 Lead standard working solution(10.0ug/ml): Prior to the use of lead solution, pipette 1.0 mL of lead standard stock solution in a 100 mL volumetric flask, make up to the mark and mixed evenly.
- 28.4 Mixed acid: Nitric acid-perchloric acid (4+1). 80 mL of nitric acid is added with 20 mL of perchloric acid and mixed evenly.

## 29 Equipment and facilities

- 29.1 Polarographic analyzer.
- 29.2 Universal electric furnace with an electronic regulator.

## 30 Analysis procedure

- 30.1 Reference conditions for polarographic analysis  
Single-sweep polarography (SSP). Initial potential: -350 mV; final potential: -850 mV; sweep speed: 300 mV/s; three electrodes, second derivative, stationary time: 5 s; appropriate measurement range. The peak current of lead is recorded at the peak potential of -470 mV.
- 30.2 Preparation of standard curve  
Pipette 0, 0.05, 0.10, 0.20, 0.30 and 0.40 mL (equivalent to 0, 0.5, 1.0, 2.0, 3.0 and 4.0  $\mu$ g of lead) of standard lead solutions in 10 mL colorimetric tubes, diluted with base solution to 10.0 mL and mixed evenly. The tubes are transferred into the electrolytic cell one by one and place a three-electrode system. The determination

can be carried out under the above-mentioned reference conditions. Record the peak current . Plot the standard curve with peak current against lead content

### 30.3 Sample treatment

Remove impurities, sieve with 29 mesh sieve and grind for the low moisture content samples like grain, bean ect. Homogenizing the high moisture content samples like vegetable, fruit, fish and meat ect. And store in plastic bottle

30.3.1 Sample treatment (including grain, bean, pastry, tea and meat, except for salt and white sugar): Weigh 1.0 g-2.0 g of sample in a 50 mL flask, add 10 mL-20 mL of mixed acid, and soaked overnight with a cover on the top. Then the flask is heated by the universal electric furnace with an electronic regulator with low power. If the color of digestion solution turns darker gradually and appears dark brown, the flask is taken out from the universal electric furnace to cool down, add an appropriate amount of nitric acid, and heat again to continue digestion. When the color of the solution no longer dark, starts to appear transparent and colorless or slightly yellow, and emits white smoke, the solution can be heated with high power to remove residual acid solution. When most of the liquid has evaporated, the system should be heated with low power to yield a white residue, which will be used for determination. Meanwhile, the reagent is used to provide blank control.

30.3.2 Salt and white sugar: weigh 2.0 g of the sample in a beaker for use.

30.3.3 Liquid sample: Weigh 2.0 g of sample in a 50 mL flask (the sample containing ethanol or carbon dioxide should be heated in 80°C water bath to remove them), add 1 mL-10 mL of mixed acid, heated by the universal electric furnace with an electronic regulator at low power. The following procedure is the same as that after "Sample treatment" in 30.3.1. The sample is ready for the determination.

### 30.4 Sample determination

Add 10.0 mL of base solution into the above sample and reagent blank control bottle, respectively, to dissolve the residue. Transferred into the electrolytic cell. The following procedure is the same as that after "Preparation of standard curve" in 30.2. Record the peak currents. Calculate the lead concentration in the standard curve.

## 31 Expression of results

The lead content in the sample is calculated on the basis of equation (6).

$$X = \frac{(A - A_0) \times 1\,000}{m \times 1\,000} \dots\dots\dots(6)$$

In which,

X -- Lead content in the sample mg/kg or mg/L;

A -- Weight of lead in the sample solution read from standard curve μg;

A<sub>0</sub> -- Weight of lead in the reagent blank control solution read from standard curve μg;

m -- Weight or volume of the sample g or mL;

Report the mean of two independent results under repeatability condition with two significant digits.

### 32 Degree of precision

The absolute difference between two independent measurement results obtained under repeatability conditions is not allowed to exceed 5.0% of the arithmetic average of them.

### 33 Other

The detection limit:

Graphite furnace atomic absorption spectrometry: 0.005mg/kg;

Hydride generation atomic fluorescence spectrometry: 0.005mg/kg for solid and 0.001mg/kg for liquid.

Flame atomic absorption spectrometry: 0.1mg/kg

Disulfide hydrazone colorimetry: 0.085 mg/kg

Single-sweep polarography: 0.25 mg/kg

Polarograms of lead in reagent blank control, standard lead solution and tea are shown in Figures 1a), 1b) and 1c) respectively.

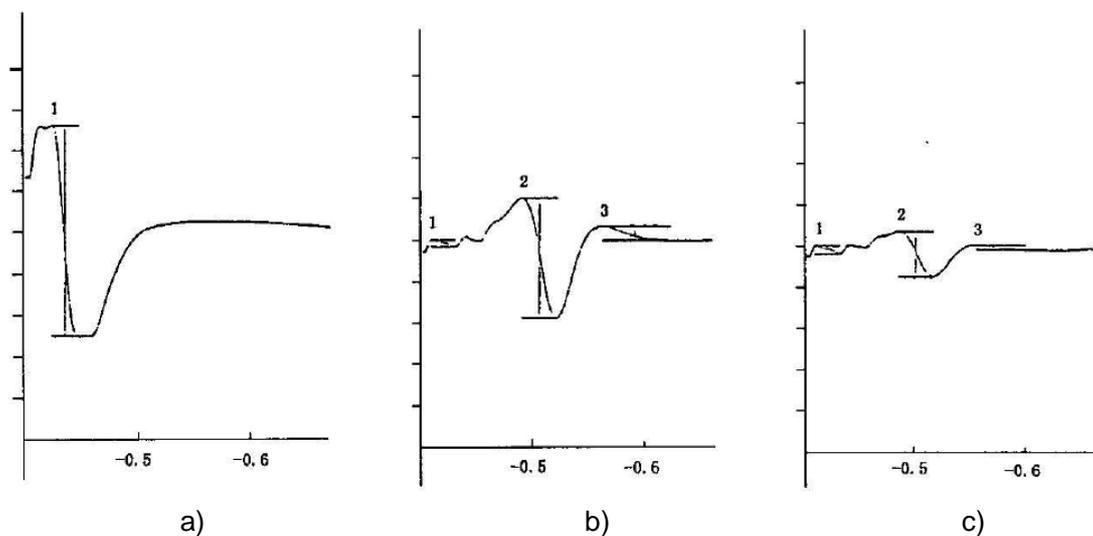


Figure 1 Polarograms of lead in a) reagent blank control; b) standard lead solution and c) tea.