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National Dairy Standard - Lead

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Report Highlights:
On November 20, 2009, China notified the WTO of "National Food Safety Standard of the People's Republic of China for the Determination of Lead in Foods" as SPS/N/CHN/180. The date for submission of final comments to the WTO is January 1, 2010. The proposed date of entry into force has not been specified.

Executive Summary:
On November 20, 2009, China notified the WTO of "National Food Safety Standard of the People's Republic of China for the Determination of Lead in Foods" as SPS/N/CHN/180. The date for submission of final comments to the WTO is January 1, 2010. The proposed date of entry into force has not been specified.

Thanks go to the consortium of industry and 3rd country Embassies in Beijing for their assistance in

translating and reviewing this standard.

This report contains an UNOFFICIAL translation of National Standard on Determination of Lead in Foods.

General Information:

BEGIN TRANSLATION

ICS 67.040
C

GB National Food Safety Standard
GB 5009.12-xxxx
In substitution of GB/T 5009.12-2003

Determination of lead in foods
(Draft for Comment)

Issued on xx-xx-xxxx Implemented on xx-xx-xxxx

Issued by the Ministry of Health

of the People's Republic of China

Preface

This standard is in substitution of GB/T 5009.12-2003 "Determination of lead in foods".

This standard is issued by and relevant to the Ministry of Health, People's Republic of China.

Versions of standard substituted by this standard are:
--GB/T 5009.12-1985, GB/T 5009.12-1996, GB/T 5009.12-2003.

Introduction

Lead is a harmful element that can accumulate inside the human body. The United Nations Food and Agriculture Organization/World Health Organization (FAO/WHO), the Codex Alimentarius Commission (CAC), and Joint FAO/WHO Expert Committee on Food Additives (JECFA) of 1993 recommend a provisional tolerable weekly intake (PTWI) of 25 μg/(kg bw) to lead. According to the

recommendation, a person with a weight of 60 kg can have an acceptable daily intake of 214 μg. The control of lead intake is categorized as an important monitoring item in the field of food supervision. It is prescribed by GB 14935-1994 “Hygienic standard of lead limit in foods” that the acceptable lead limit is not higher than 0.05 mg/kg for dairy products (fresh), and not higher than 0.2 mg/kg for eggs, vegetables and fruit. The sensitivities of flame atomic absorption spectrometry and disulfide hydrazone colorimetry listed in current national standard GB/T 5009.12 cannot meet the requirements of this hygienic standard. Graphite furnace atomic absorption spectrometry, on the other hand, has a high sensitivity. Nevertheless, its application is also restrained due to the high price of the instrument and the severe interference it generated in the determination of the matrix with a complex sample, which often results in negative effects on the accuracy of analysis results. To improve the method listed in the current national standard, experts have made amendments to the standard and proposed to determine lead in foods by hydride generation atomic fluorescence spectrometry, which relies on a domestic instrument and yields a high sensitivity, and thus can be easily popularized.

Determination of lead in foods

1 Scope

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

This Standard applies to the determination of lead in foods.

Detection limit of the present method: 5 μg/kg for graphite furnace atomic absorption spectrometry; 5 μg/kg for solid sample and 1 μg/kg for liquid sample in hydride generation atomic fluorescence spectrometry; 0.1 mg/kg for flame atomic absorption spectrometry; 0.25 mg/kg for colorimetry; and 0.085 mg/kg for single-sweep polarography.

Method 1: Graphite furnace atomic absorption spectrometry

2 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.

3 Reagents

3.1 Nitric acid.
3.2 Ammonium persulfate.
3.3 Hydrogen peroxide (30%).
3.4 Perchloric acid.
3.5 Nitric acid (1+1): 50 mL of nitric acid is slowly added into 50 mL of water.
3.6 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.

3.7 Nitric acid (1 mol/L): 6.4 mL of nitric acid is added into 50 mL of water and then diluted to 100 mL.
3.8 Ammonium phosphate solution (20 g/L): 2.0 g of ammonium phosphate is weighed, dissolved in water and then diluted to 100 mL.
3.9 Mixed acid: Nitric acid + perchloric acid (4+1). 4 equivalent of nitric acid is mixed with 1 equivalent of perchloric acid.
3.10 Standard lead stock solution: 1.000 g of lead (99.99%) is weighed 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 the solution is transferred into a 1000 mL volumetric flask, to which water is added to 1000 mL. After that the volumetric flask is shaken to allow the even mixing of the solution, which has a lead concentration of 1.0 mg/mL.
3.11 Standard lead working solution: 1.0 mL of standard lead stock solution is placed in a 100 mL volumetric flask, to which nitric acid (0.5 mol/L or 1 mol/L) is added to 100 mL. The standard lead working solution with concentrations of 10.0, 20.0, 40.0, 60.0 and 80.0 ng/mL is obtained by diluting the solution for many times.

4 Equipment

All glassware should be soaked in nitric acid (1+5) overnight, washed by water repeatedly and finally rinsed with de-ionized water.
4.1 Atomic absorption spectrophotometer (with graphite furnace and lead hollow cathode lamp).
4.2 Muffle furnace.
4.3 Constant temperature drying oven.
4.4 Porcelain crucible.
4.5 Pressure digestion device, pressure digestion drum or pressure digestion tank.
4.6 Adjustable electric heating plate and adjustable electric furnace.

5 Analysis procedure

5.1 Sample pretreatment

5.1.1 During sampling and preparation, the sample should be prohibited from being polluted.

5.1.2 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.

5.1.3 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.

5.2 Sample digestion (any digestion method can be selected according to laboratory conditions)

5.2.1 Digestion by pressure digestion tank: 1.00 g-2.00 g of sample (for dry sample and samples with high fat contents, the weight is less than 1.00 g; for fresh sample, the weight is less than 2.0 g; or the weight can be determined in light of operation instructions of the pressure digestion tank) is weighed, placed in polytetrafluorethylene inner tank, and soaked in 2 mL-4 mL of nitric acid overnight. Into the system is added 2 mL-3 mL of hydrogen peroxide (30%) (total volume not exceeding 1/3 of the tank volume). After the inner lid is covered and the stainless steel outer cover is tightened, the tank is then placed in the constant temperature drying oven for 3 h-4 h, which is kept at 120^{o}C-140^{o}C, and then cooled to room temperature naturally in the oven. The digestion solution is

washed and transferred into or filtered and transferred 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, to which water is added to the full scale. After that the volumetric flask is shaken to allow the solution to mix evenly. Meanwhile, the reagent is used to provide blank control.

5.2.2 Dry ashing: 1.00 g-5.00 g of sample (depending on lead content) is weighed and placed in the porcelain crucible, which is heated on the adjustable electric heating plate with low power until the carbonization produces no smoke. After that, it is transferred into muffle furnace and stays for 6 h-8 h at 500^{o}C, and then cools to room temperature. A few samples whose ashing process is not complete are added with 1 mL of mixed acid 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 and transferred 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, to which water is added to the full scale. After that the volumetric flask is shaken to allow the even mixing of the solution. Meanwhile, the reagent is used to provide blank control.

5.2.3 Ammonium persulfate ashing method: 1.00 g-5.00 g of sample is weighed and placed in the porcelain crucible, to which 2 mL-4 mL of nitric acid is added to soak the sample for more than 1 h. The sample is carbonized under low power at first. After cooling down, it is covered with 2.00 g-3.00 g of ammonium persulfate and continues to be carbonized until no smoke is produced. The sample is transferred to muffle furnace to stay for 2 h at 500^{o}C and 20 min at 800^{o}C, and then cools down. After the addition of 2 mL-3 mL of nitric acid (1.0 mol/L), the sample digestion solution is washed and transferred into or filtered and transferred 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, to which water is added to the full scale. After that the volumetric flask is shaken to allow the solution to mix evenly. Meanwhile, the reagent is used to provide blank control.

5.2.4 Wet digestion method: 1.00 g-5.00 g of sample is weighed and placed in a flask or tall beaker, to which several glass beads and 10 mL of mixed acid are added. The container is then covered to allow the sample to be soaked overnight. After that, it is placed on a small funnel electric furnace so that the digestion can occur. If the system becomes 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. The sample digestion solution then cools down and is washed and transferred into or filtered and transferred 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, to which water is added to the full scale. After that the volumetric flask is shaken to allow the solution to mix evenly. Meanwhile, the reagent is used to provide blank control.

5.3 Determination

5.3.1 Equipment conditions: Each piece of equipment is adjusted to the best state according to its performance. Reference conditions are as follows: wavelength 283.3 nm, slot 0.2 nm-1.0 nm, lamp current 5 mA-7 mA, drying temperature 120^{o}C, 20 s; ashing temperature 450^{o}C, 15 s-20 s; atomization temperature 1700^{o}C-2300^{o}C, 4 s-5 s; the background calibration is based on deuterium lamp or Zeeman Effect.

5.3.2 Preparation of standard curve: 10 μL of each standard lead working solution with a concentration of 10.0, 20.0, 40.0, 60.0 and 80.0 ng/mL (or μg/L) respectively is drawn and injected into the graphite furnace, from which the optical density is measured. The unary linear regression equation correlating optical density and concentration is thus obtained, which serves as the standard curve.

5.3.3 Sample determination: 10 μL of sample solution and reagent blank control solution are drawn and injected into the graphite furnace to yield their optical densities. After the substitution of optical densities in the unary linear regression equation, lead content in the sample solution can be obtained.

5.3.4 Application of matrix modifier: For samples with interference factors, an appropriate amount, usually 5 μL or equivalent to the amount of sample, of matrix modifier ammonium dihydrogen phosphate solution (20 g/L) is injected 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.

6 Calculation of results

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

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

In which,

X -- Lead content in the sample with a unit of μg/kg or μg/L;

C_1 -- Lead content in determination sample solution with a unit of ng/mL;

C_0 -- Lead content in blank control solution with a unit of ng/mL;

V -- Total quantitative volume of the sample digestion solution with a unit of mL;

m -- Weight or volume of the sample with a unit of g or mL.

The calculation results should possess two significant digits.

7 Degree of precision

The absolute value of 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

8 Principle

After thermal acid digestion, the sample is placed in the acid medium to allow lead existed in it to react with sodium borohydride ($NaBH_4$) or potassium borohydride (KBH_4) to yield volatile lead hydride (PbH_4). 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.

9 Reagents

9.1 Nitric acid and perchloric acid mixture (4+1): 400 mL of nitric acid and 100 mL of perchloric acid are drawn and mixed evenly.
9.2 Hydrochloric acid solution (1+1): 250 mL of hydrochloric acid is poured into 250 mL of water and the mixture is then mixed evenly.
9.3 Oxalic acid solution (10 g/L): 1.0 g of oxalic acid is weighed, dissolved in 100 mL of water and then mixed evenly.
9.4 Iron potassium cyanide [$K_3Fe(CN)_6$] solution (100 g/L): 10.0 g of iron potassium cyanide is weighed, dissolved in water, diluted to 100 mL and then mixed evenly.
9.5 Sodium hydroxide solution (2 g/L): 2.0 g of sodium hydroxide is weighed, dissolved in 1 L of water and mixed evenly.
9.6 Sodium borohydride [$NaBH_4$] solution (10 g/L): 5.0 g of sodium borohydride is weighed, dissolved in 500 mL of sodium hydroxide solution (2 g/L) and mixed evenly. This solution should be prepared right before use.
9.7 Standard lead stock solution (1.0 mg/mL)[1].
9.8 Standard lead working solution (1.0 μg/mL): A certain amount of standard lead stock solution (1.0 mg/mL) is accurately drawn and diluted to 1.0 μg/mL step by step.

10 Equipment

10.1 Double-channel atomic fluorescence spectrometer or similar equipment.
10.2 Computer system and code lead hollow cathode lamp.
10.3 Electric heating plate.

11 Analysis procedure

11.1 Sample digestion
Wet digestion: 0.20 g-2.00 g of solid sample and 2.00 g (or mL) - 10.00 g (or mL) of liquid sample are placed in a 50 mL-100 mL digestion vessel (flask), to which 5 mL-10 mL of nitric acid and perchloric acid mixture (4+1) are added. The system is shaken to mix well and stays overnight. On the next day, the flask is placed on the electric heating plate to allow the thermal digestion. When the digestion solution appears light yellow or colorless (if the color is dark, the digestion solution should be allowed to cool slightly and be added with a small amount of nitric acid to let the digestion continue), it is then allowed to cool slightly, added with 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, it is added with a small amount of water, transferred to a 25 mL volumetric flask, added with 0.5 mL of hydrochloric acid (1+1) and 0.5 mL of oxalic acid solution (10 g/L), and mixed evenly. Then it is added with 1.0 mL of iron potassium cyanide solution (100g/L), diluted to 25 mL with water, and mixed evenly. After staying for 30 min, the solution is ready for determination. Meanwhile, the reagent is used to provide blank control.

11.2 Preparation of standard series

[1] Standard lead stock solution (1.0 mg/mL) is a product provided by National Research Center for Certified Reference Materials. Herein the provision of this information is for the convenience of users of this standard, but not an indication of recognition of this product. Other products, if capable of producing the same effect, can also be used.

Into 7 25 mL volumetric flasks are accurately added 0.00, 0.125, 0.25, 0.50, 0.75, 1.00 and 1.25 mL of standard lead working solutions (1.00 μg/mL), respectively (lead concentration in each flask will be 0.0, 5.0, 10.0, 20.0, 30.0, 40.0 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 (1+1) and 0.5 mL of oxalic acid (10 g/L), and mixed well. And then the solution is added with 1.0 mL of iron potassium cyanide solution (100 g/L), diluted with water to 25 mL, and shaken to mix evenly. After staying for 30 min, the solution is ready for determination.

11.3 Determination

11.3.1 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: 1000 mL/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; volume of sample introduction: 2.0 mL.

11.3.2 Measurement of concentrations

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).

11.3.3 Measurement of automatic calculation results of equipment

The equipment is set under the optimum conditions. On the sample parameter screen, the following parameters are input: Weight or volume of the sample (g or mL), diluting volume (mL) and concentration unit of the result. The furnace temperature then rises to required value progressively. After it stays stable, 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. Before the sample is introduced to the equipment for the measurement, the equipment is set for the measurement of blank control value. The blank control digestion solution sample is then introduced to the equipment and yields an average measurement value which is used as the blank control value to be deducted. After that, the sample solution can be determined one by one. After the determination is finished, the determination results can be automatically printed out when the “Print report” button is clicked.

12 Calculation of results

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

$$X = \frac{(c - c_0) \times V \times 1\,000}{m \times 1\,000 \times 1\,000} \quad \text{……(2)}$$

In which,

X -- Lead content in the sample with a unit of mg/kg or mg/L;

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

c_0 -- Determination concentration of reagent blank control solution with a unit of ng/mL;
m -- Weight or volume of the sample with a unit of g or mL;
V -- Total volume of the sample digestion solution with a unit of mL.
The calculation results should possess three significant digits.

13 Degree of precision

The absolute value of 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

14 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 4-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.

15 Reagents

15.1 Nitric acid-perchloric acid (4+1).
15.2 Ammonium sulfate solution (300 g/L): 30.0 g of ammonium sulfate [$(NH_4)_2SO_4$] is weighed, dissolved in water and diluted with water to 100 mL.
15.3 Ammonium citrate solution (250g/L): 25.0 g of ammonium citrate is weighed, dissolved in water and diluted with water to 100 mL.
15.4 Bromothymol blue aqueous solution (1 g/L).
15.5 Sodium diethyl dithiocarbamate (DDTC) solution (50 g/L): 5 g of sodium diethyl dithiocarbamate is weighed, dissolved in water and diluted with water to 100 mL.
15.6 Ammonia (1+1).
15.7 4-Methylpentanone-2 (MIBK).
15.8 Standard lead solution: Operation is the same as those in 3.10 and 3.11. The standard working solution has a lead concentration of 10 μg/mL.

16 Equipment

Atomic absorption spectrophotometer with flame atomizer; others are the same as those in 4.2, 4.3, 4.4 and 4.5.

17 Analysis procedure

17.1 Sample treatment
17.1.1 Beverage and alcohol: 10.0 g-20.0 g of well mixed sample is placed in the beaker. The ethanol should be removed from alcohol by heating in water bath before use. The sample is heated on the electric heating plate to allow a certain amount of water evaporates, and then it is added with nitric acid-perchloric acid (4+1). After full digestion, the solution is transferred into a 50 mL volumetric flask and diluted to 50 mL.

17.1.2 The soaking solution of packaging materials can be drawn and determined directly.

17.1.3 Cereal: After the removal of impurities and dust, and, in case of necessity, the shell, cereal is crushed, sifted by a 20-mesh sieve, and mixed evenly. 5.0 g-10.0 g of the product is weighed, placed in a 50 mL porcelain crucible, carbonized under low power, and then transferred into muffle furnace, in which the sample undergoes ashing for 16 h in a temperature below 500^{o}C. Then the crucible is taken out and allowed to cool to room temperature. The sample is added with a small amount of mixed acid and heated under low power so that the mixture does not dry out. When necessary, a small amount of mixed acid is added again. The process repeats until there is no charcoal grain in the residue. When the crucible cools slightly, l0 mL of hydrochloric acid (1+11) is added into the residue to dissolve it. The solution is transferred into a 50 mL volumetric flask. Water is then used to wash the crucible repeatedly and transferred into the volumetric flask. The solution in the flask is then diluted to 50 mL and mixed evenly for use.

Mixed acid and hydrochloric acid (1+11) with an amount equivalent to that of the sample are drawn and used for reagent blank control test with the same operation method.

17.1.4 Vegetables, melon, fruit and beans: The edible part is cleaned and dried, chopped thoroughly and mixed evenly. 10.00 g-20.00 g of the sample is weighed, placed in the porcelain crucible, added with 1 mL of phosphoric acid (1+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.

17.1.5 Poultry, eggs, aquatic products and dairy products: The edible part is taken out and mixed evenly. 5.00 g-10.00 g of the sample is weighed, placed 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, 50 mL of milk is placed in the porcelain crucible, added with phosphoric acid (1+10), heated to 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.

17.2 Extraction and separation

Depending on the sample condition, 25.0 mL-50.0 mL of sample solution prepared from the above-mentioned procedure and blank control solution are respectively placed in 125 mL separator funnels and diluted with water to 60 mL. Into the separator funnel are added 2 mL of ammonium citrate solution and 3-5 drops of bromothymol blue indicator. Ammonia (1+1) is used to adjust the pH until solution color changes from yellow to blue. The mixture is then added with 10.0 mL of ammonium sulfate solution and 10 mL of DDTC solution, and shaken to mix well. After staying for about 5 min, the mixture is added with 10.0 mL of MIBK, shaken vigorously for 1 min, and kept still to allow the full phase separation. Water layer is discarded, and MIBK layer is released into a 10 mL graduated tube with a septum for use. 0.00, 0.25, 0.50, 1.00, 1.50 and 2.00 mL (equivalent to 0.0, 2.5, 5.0, 10.0, 15.0 and 20.0 μg of lead respectively) of standard lead working solutions are drawn and placed in 125 mL separator funnels respectively. The following operation procedures are the same as those for the sample.

17.3 Determination

17.3.1 Beverage, alcohol and packaging material soaking solution can be introduced to the equipment for determination directly after the extraction.

17.3.2 During the introduction of extraction solution sample, the acetylene gas flow can be reduced appropriately.

17.3.3 Equipment reference conditions: Hollow cathode lamp current 8 mA; resonance line 283.3 nm; slot 0.4 nm; air flow rate 8 L/min; height of burner 6 mm; BCD method.

18 Calculation of results

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

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

In which,
X -- Lead content in the sample with a unit of mg/kg or mg/L;
c_1 -- Content of lead in sample solution for determination with a unit of μg/mL;
c_2 -- Content of lead in reagent blank control solution with a unit of μg/mL;
m -- Weight or volume of the sample with a unit of g or mL;
V_1 -- Volume of sample extraction solution with a unit of mL;
V_2 -- Total volume of sample treatment solution with a unit of mL;
V_3 -- Total volume of sample treatment solution for determination with a unit of mL.
The calculation results should possess two significant digits.

19 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

20 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. Ammonium citrate, potassium cyanide and hydroxylamine hydrochloride are added into the solution to eliminate the interference brought by iron, copper and zinc ions. The results are compared with standard series to yield quantitative lead content.

21 Reagents

21.1 Ammonia (1+1).
21.2 Hydrochloric acid (1+1): 100 mL of hydrochloric acid is added into 100 mL of water.
21.3 Phenol red indicator solution (1 g/L): 0.10 g of phenol red is weighed, dissolved by a small amount of ethanol for many times, transferred into a 100 mL volumetric flask and diluted to 100 mL.
21.4 Hydroxylamine hydrochloride solution (200 g/L): 20.0 g of hydroxylamine hydrochloride is weighed, dissolved by 50 mL of water, added with 2 drops of phenol red indicator solution and a certain amount of ammonia (1+1) to adjust pH to 8.5-9.0 (after the color changes from yellow to red, 2 more drops are added). Disulfide hydrazone-chloroform solution is used to extract the solution until the green color of chloroform layer does not change any more. Then water layer is washed twice with chloroform. Chloroform layer is discarded, and water layer is added with hydrochloric acid (1+1) until pH is lower than 7, and diluted with water to 100 mL.
21.5 Ammonium citrate solution (200 g/L): 50 g of ammonium citrate is weighed, dissolved by 100 mL of water, added with 2 drops of phenol red indicator solution and a certain amount of ammonia (1+1) to adjust pH to 8.5-9.0. Disulfide hydrazone-chloroform solution is used to extract the solution

for several times with a volume of 10 mL-20 mL each time until the green color of chloroform layer does not change any more. Then chloroform is used to wash the water layer twice with a volume of 5 mL each time. The chloroform layer is discarded, and water layer is diluted with water to 250 mL.
21.6 Potassium cyanide solution (100 g/L): 10.0 g of potassium cyanide is weighed, dissolved by water and diluted to 100 mL.
21.7 Chloroform: Oxides shall not be included.

21.7.1 Inspection method: 10 mL of chloroform is drawn, added with 25 mL of freshly boiled water, shaken for 3 min, and allowed to stay until phase separation occurs fully. Then 10 mL of aqueous solution is drawn, added with several drops of potassium iodide solution (150 g/L) and starch indicator solution. After mixing, the solution shall not appear blue.

21.7.2 Treatment method: A certain amount of chloroform is washed by 1/10-1/20 equivalent volume of sodium thiosulfate solution (200 g/L) and water, dehydrated by a small amount of anhydrous calcium chloride, and distilled. The initial 1/10 and last 1/10 of the distillate are discarded, and the middle part of it is collected for use.

21.8 Starch indicator solution: 0.5 g of soluble starch is weighed, added with 5 mL of water, mixed homogeneously, 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.
21.9 Nitric acid (1+99): 1 mL of nitric acid is added into 99 mL of water.
21.10 Disulfide hydrazone chloroform solution (0.5 g/L): It should be stored in the refrigerator and, if necessary, purified by the following method.

0.5 g of ground fine disulfide hydrazone is weighed, dissolved by 50 mL of chloroform. If it does not dissolve completely, the system can be filtered using filter paper, transferred into a 250 mL separator funnel, and exacted with ammonia (1+99) for three times with a volume of 100 mL each time. The extract is then filtered by cotton, transferred into a 500 mL separator funnel, and adjusted by hydrochloric acid (1+1) to pH lower than 7. In the acidic system, disulfide hydrazone precipitates and is extracted by 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 distilled in 50^{o}C water bath until all chloroform has evaporated. Refined disulfide hydrazone is then stored in a sulphuric acid desiccator 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.
21.11 Disulfide hydrazone working solution: 1.0 mL of disulfide hydrazone solution is diluted with chloroform to 10 mL and mixed evenly. 1 cm cuvette is used for the determination, and chloroform is used for the zero point adjustment. The absorbance (A) is measured at a wavelength of 510 nm and used in equation (4) to 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%.

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

21.12 Nitric acid-sulphuric acid mixed solution (4 +1).
21.13 Standard lead solution: 0.1598 g of lead nitrate is precisely weighed and added with 10 mL of nitric acid (1+99). After the solid fully dissolves, the solution is transferred into a 100 mL volumetric flask and diluted with water to 100 mL. The solution has a lead concentration of 1.0 mg/mL.
21.14 Standard lead working solution: 1.0 mL of standard lead solution is drawn, placed in a 100 mL volumetric flask, and diluted with water to 100 mL. The solution has a lead concentration of 10.0 μg/mL.

22 Equipment

All glassware should be soaked in nitric acid (10%-20%) for more than 24 h, washed by tap water repeatedly and finally rinsed with de-ionized water.
Spectrophotometer.

23 Analysis procedure

23.1 Sample pretreatment
The same as the operation in 5.1.
23.2 Sample digestion
23.2.1 Nitric acid-sulfuric acid method
23.2.1.1 Grain, bean vermicelli, bean noodle, bean dry product, pastry, tea and other solid food with low moisture content: 5.00 g-10.00 g of crushed sample is weighed, placed in a 250 mL-500 mL nitrogen determination flask, added with a little water to dampen it, and then added with several glass beads and 10 mL-15 mL of nitric acid, allowed to stay for a moment and then heated slowly under low power. After the reaction slows down, the flask is allowed to cool down naturally. Along the glass wall is introduced 5 mL or l0 mL of sulphuric acid, and the mixture is heated again. After the liquid in the flask turns brown, nitric acid is introduced 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. It is then allowed to cool down. (In the following operations, be careful to avoid explosive boiling and explosion) The solution is added with 20 mL of water and heated until it boils to remove remaining nitric acid until white smoke is generated. After this process is repeated again, the solution is allowed to cool down. The solution is then transferred into a 50 mL or 100 mL volumetric flask. Water is used to wash the nitrogen determination flask and transferred into the volumetric flask. The solution in the volumetric flask is allowed to cool down, diluted with water to the full scale, and mixed evenly. In the final solution, the sample concentration is 0.1 g/mL and sulphuric acid concentration is 10% (v/v). Nitric acid and sulphuric acid with an amount equivalent to the amount of digestion sample are drawn and used for the reagent blank control test with the same method.
23.2.1.2 Vegetable and fruit: 25.00 g or 50.00 g of clean, homogenate sample is weighed, placed in a 250 mL-500 mL nitrogen determination flask, and added with several glass beads and 10 mL-15 mL of nitric acid. The following procedure is the same as that after “allowed to stay for a moment” in 23.2.1.1. But in the final solution here, the sample concentration is 0.5 g/mL, and sulphuric acid concentration is 10% (v/v).
23.2.1.3 Sauce, soy sauce, vinegar, cold drink, tofu, fermented bean curd and sauce preserved vegetable: 10.00g or 20.00 g of sample is weighed (or 10.0 mL or 20.0 mL of liquid sample is drawn), placed in a 250 mL-500 mL nitrogen determination flask, added with several glass beads and 5 mL-15 mL of nitric acid. The following procedure is the same as that after “allowed to stay for a moment” in 23.2.1.1. But in the final solution here, the sample concentration is 0.2 g/mL or 20% (v/v).
23.2.1.4 Alcohol beverage or carbon dioxide beverage: 10.00 mL or 20.00 mL of sample is drawn, placed in a 250 mL-500 mL nitrogen determination flask, added with several glass beads, heated under low power to remove ethanol or carbon dioxide, then added with 5 mL-10 mL of nitric acid, and mixed evenly. The following procedure is the same as that after “allowed to stay for a

moment" in 23.2.1.1. But in the final solution here, the sample concentration is 20% (v/v).

23.2.1.5 Food with high sugar content: 5.00 g or 10.0 g of sample is weighed, placed in a 250 mL-500 mL nitrogen determination flask, added with a little water to dampen it at first, then added with several glass beads and 5 mL-10 mL of nitric acid, and shaken to mix well. Into the flask is slowly introduced 5 mL or 10mL of sulphuric acid. After the reaction slows down and the bubbling stops, the system is slowly heated with low power (sugar is apt to carbonize) and more nitric acid is continuously added into the system 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 "The solution is added with 20 mL of water and heated until it boils" in 23.2.1.1.

23.2.1.6 Aquatic product: The edible part of the sample is processed into homogenate, 5.00 g or 10.0 g (lower for marine algae and shellfish) of which is weighed, placed in a 250 mL-500 mL nitrogen determination flask, added with several 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 is introduced 5 mL or l0 mL of sulphuric acid" in 23.2.1.1.

23.2.2 Ashing

23.2.2.1 Grain and other foods with low moisture content: 5.00 g of the sample is weighed, placed in a quartz or porcelain crucible, heated until it is carbonized, transferred into muffle furnace to allow 3 h of ashing at 500^{o}C. After cooling down, the crucible is taken out, added with nitric acid (1+1) to dampen the ash content, heated with low power to evaporate water, burnt for 1 h at 500^{o}C, and then allowed to cool down. Then the crucible is taken out, added with 1 mL of nitric acid (1+1) and heated to make the ash content dissolve. The solution is transferred into a 50 mL volumetric flask. Water is used to wash the crucible and then incorporated into the volumetric flask. The solution in the flask is diluted with water to 50 mL, and mixed evenly for use.

23.2.2.2 Food with high moisture content or liquid sample: 5.00 g or 5.00 mL of the sample is placed in an evaporating dish, which is heated in water bath to evaporate water. The following procedure is the same as that after "heated until it is carbonized" in 23.2.2.1.

23.3 Determination

10.0 mL of solution after digestion and 10 mL of reagent blank control solution are drawn, placed in 125 mL separator funnels and diluted with water to 20 mL respectively.

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 are separately drawn, placed in 125 mL separator funnels, and diluted with nitric acid (1+99) to 20 mL. Into the sample digestion solution, reagent blank control solution and standard lead solution are added 2.0 mL of ammonium citrate solution (200 g/L), 1.0 mL of hydroxylamine hydrochloride solution (200 g/L) and 2 drops of phenol red indicator solution. They are adjusted by ammonia (1+1) until the color turns red. Each solution is added with 2.0 mL of potassium cyanide solution (100 g/L), mixed evenly, then added with 5.0 mL of disulfide hydrazone working solution, and shaken vigorously for 1 min. The system then stays still to allow full phase separation. After filtration with degreased cotton, a certain amount of sample drawn from chloroform layer is introduced into a 1 cm cuvette. Chloroform is used for the zero point adjustment and absorbance is measured at a wavelength of 510 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.

24 Calculation of results

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

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

In which,
X -- Lead content in the sample with a unit of mg/kg or mg/L;
m_1 -- Weight of lead in sample solution for determination with a unit of μg;
m_2 -- Weight of lead in reagent blank control solution with a unit of μg;
m_3 -- Weight or volume of the sample with a unit of g or mL;
V_1 -- Total volume of sample treatment solution with a unit of mL;
V_2 -- Total volume of sample treatment solution for determination with a unit of mL.

The calculation results should possess two significant digits.

25 Degree of precision

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

Method 5: Single-sweep polarography

26 Principle

After sample digestion, lead exists in the form of ions (pb^{2+}) and forms PbI_4^{2-} complex ions with I^- in acidic medium. 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.

27 Reagents

27.1 Base solution: 5.0 g of potassium iodide, 8.0 g of potassium sodium tartrate and 0.5 g of ascorbic acid are weighed, placed in a 500 mL beaker, dissolved by 300 mL of water, added with 10 mL of hydrochloric acid, transferred into a 500 mL volumetric flask and diluted with water to 500 mL (it is stored in refrigerator and can be preserved for 2 months).
27.2 Standard lead stock solution: 0.1000 g of lead (purity 99.99%) is accurately weighed, placed in a beaker, added with 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. Lead concentration in the solution is 1.0 mg/mL.
27.3 Standard lead working solution: Prior to the use of lead solution, 1.00 mL of standard lead stock solution is drawn, placed in a 100 mL volumetric flask, diluted with water to 100 mL and mixed evenly. Lead concentration in the solution is 10.0 μg/mL.
27.4 Mixed acid: Nitric acid-perchloric acid (4+1). 80 mL of nitric acid is added with 20 mL of perchloric acid and mixed evenly.

28 Equipment

All glassware should be soaked in 10% nitric acid solution overnight, washed by water repeatedly, rinsed by distilled water, and dried for use.
28.1 Polarographic analyzer.
28.2 Universal electric furnace with an electronic regulator.

29 Analysis procedure

29.1 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.
29.2 Preparation of standard curve
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 μg of lead) of standard lead solutions are accurately drawn, separately placed in 6 10 mL colorimetric tubes, diluted with base solution to l0.0 mL and mixed evenly. The tubes are transferred into the electrolytic cell one after another and placed in a three-electrode system. The determination can be carried out under the above-mentioned reference conditions of polarographic analysis. The peak current of each lead sample is taken. The peak current is then plotted against lead content to yield the standard curve.
29.3 Sample treatment
The sample with low moisture content such as grain and bean should be ground and sifted by a 20-mesh sieve after impurities are removed. The fresh sample with high moisture content such as vegetable, fruit, fish and meat should be processed into homogenate using a homogenizer, and stored in the plastic bottle.

29.3.1 Sample treatment (including grain, bean, pastry, tea and meat, except for salt and white sugar): 1.0 g-2.0 g of sample is weighed, placed in a 50 mL flask, added with 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, added with an appropriate amount of nitric acid, and heated again to continue digestion. When the color of the solution no longer darkens, 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.

29.3.2 Salt and white sugar: 2.0 g of the sample is weighed and placed in a beaker for use.

29.3.3 Liquid sample: 2.0 g of sample is weighed, placed in a 50 mL flask (the sample containing ethanol or carbon dioxide should be heated in 80^{o}C water bath to remove them), added with 1 mL-l0 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 29.3.1. The sample is ready for the determination.

29.4 Sample determination
10.0 mL of base solution is added into the above-mentioned sample bottle for determination and reagent blank control bottle, respectively, to dissolve the residue. The solution is then transferred into the electrolytic cell. The following procedure is the same as that after “Preparation of standard curve”

in 29.2. The peak currents of sample and reagent blank control are recorded respectively, and are used to yield lead content in the sample through the comparison with standard curve.

30 Calculation of results

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

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

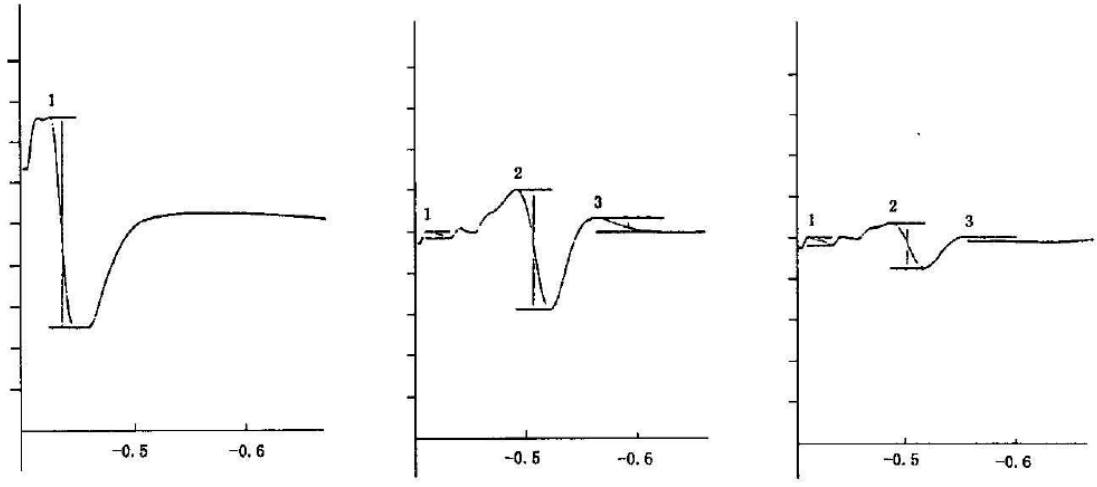
In which,
X -- Lead content in the sample with a unit of mg/kg or mg/L;
A -- Weight of lead in the sample solution read from standard curve with a unit of μg;
A_0 -- Weight of lead in the reagent blank control solution read from standard curve with a unit of μg;
m -- Weight or volume of the sample with a unit of g or mL;

31 Degree of precision

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

32 Polarograms of lead in reagent blank control, standard lead solution and tea

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



a) b) c)

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