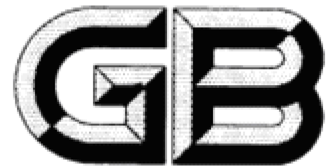

National Food Safety Standard of the People's Republic of China

GB5009.93-2010

National Food Safety Standard
Determination of Selenium in Foods

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

Preface

The Standard substitutes GB/T 5009.93-2003 “Determination of selenium in foods”.
The replaced former editions are:

- GB/T 5009.93-2003.
- GB/T 12399-1996.
- GB/T 13105-1991.

National Food Safety Standard

Determination of Selenium in Foods

1. Scope

The Standard stipulates the determination method of selenium in foods with hydride generation-atomic fluorescence spectrometry (HG-AFS) and fluorescence method.

The Standard is applicable to the determination of selenium in foods.

2. Normalized References

The documents referred in the standard are requisite for the application of the standard.

For dated references, only the version dated is applicable to the standard.

For undated references, the latest version including all modification notices are applicable to the standard.

Methoed I: Hydride generation-atomic fluorescence spectrometry (HG-AFS)

3. Principle

After the sample has been heated and digested by acid, in the 6mol/l HCl medium, selenium of +6 valence is reduced to +4 valence. Take $NaBH_4$ or KBH_4 as reducing agent, selenium of +4 valence is reduced to SeH_2 in the HCl medium, it is brought into an atomizer with carrier gas Ar for atomization, under the illumination of selenium specially made hollow cathode lamp, the ground state selenium atom is excited to high energy state, when it is deactivated and goes back to the ground state, it emits fluorescence with characteristic wavelength, and the fluorescence intensity is proportional to the selenium content. Compare the result with standard series to make quantitation.

4. Reagents

4.1 Nitric acid (GR).

4.2 Perchloric acid (GR).

4.3 Hydrochloric acid (GR).

4.4 Mixed acid: Nitric acid+ perchloric acid (4+1)

4.5 Sodium hydroxide (GR).

4.6 Sodium borohydride (8g/l): weigh 8.0g sodium borohydride and solve it in sodium hydroxide solution (5g/l), set to the marked volume 1000ml.

4.7 Red potassium prussiate (100g/l): weigh 10.0g red potassium prussiate and solve it in 100ml water, mix adequately.

4.8 Selenium standard stock solution: accurately weigh 100.0g selenium (spectroscopically pure), solve it in little nitric acid, add 2ml perchloric acid, put it in the boiling water bath and heat for 3h~4h, cool down and add 8.4ml hydrochloric acid, put it in the boiling water bath again and heat for 2min, accurately dilute to 1000ml, the concentration of hydrochloric acid is 0.1mol/l, the concentration of the stock solution is equivalent to 100μg/ml selenium.

4.9 Selenium standard application solution: Take the 100μg/ml selenium standard stock solution 1.0ml, set to the marked volume 100ml, the concentration of the application solution is 1μg/ml.

4.10 HCl (6mol/l): measure 50ml HCl (4.3), then add into to 40ml water slowly, cool down and set to the marked volume 100ml.

4.11 H2O2 (30%)

5. Instruments
5.1 Atomic fluorescence spectrophotometer.
5.2 Electric heating plate.
5.3 Microwave digestion system
5.4 Scale: 1mg
5.5 Pulverizer
5.6 Oven

6. Analytical Procedures
6.1 Sample preparation
6.1.1 Grain: wash the sample with water for three times, bake at 60□, crush and store in a plastic bottle for use.
6.1.2 Vegetable and other plant food: Take the edible part, wash with water and suck the water drop with gauze, crush to homogeneous thick liquid for use.
6.1.3 Other solid sample: crush and blending for use.
6.1.4 Liquid sample: blending for use.
6.1.5 Sample digestion
6.1.5.1 Digestion with electric heating plate: weigh 0.5~2g sample (accuracy at 0.001g), suck1.00ml~10.00ml for liquid sample, then put into a digestion bottle, and add 10.0ml mixed acid and several glass beads, cover the watch glass, cold digest overnight.
Heat it on the electric heating plate on the next day, and supplement mixed acid in time. When the solution becomes clear and colorless together with white smoke, continue heating until the remaining volume is 2ml or so, do not evaporate to dry. Cool down, add 5ml hydrochloric acid(4.10), continue heating until the solution becomes clear and colorless together with white smoke, selenium of +6 valence is reduced to +4 valence completely. Cool down, transfer to a 50ml volumetric flask and set to the marked volume. Do the blank test at the same time.
6.1.4.2 Microwave digestion: weigh 0.5-2g sample (accuracy at 0.001g) into a digestion tube, add 10ml HCl and 2 ml H_2O_2, then put it in the digestion oven to digest after blending equably.
The recommended conditions for digestion are in Table1:

Table1 Recommended conditions of microwave digestion

STAGE	POWER		RAMP	° C	HOLD
1	1600W	100%	6:00	120° C	1:00
2	1600W	100%	3:00	150° C	5:00
3	1600W	100%	5:00	200° C	10:00

Cool down and transfer to triangular flask, add several glass beads, continue heating on the electric heating plate, do not evaporate to dry. And add 5ml hydrochloric acid(4.10) until the solution becomes clear and colorless together with white smoke, selenium of +6 valence is reduced to +4 valence completely. Cool down, transfer to a 50ml volumetric flask and set to the marked volume. Do the blank test at the same time.
Suck 10ml sample of digestive solution to a 15ml centrifuge tube, add 2ml concentrated hydrochloric acid and 1ml red potassium prussiate solution, and mix adequately for determination.

6.2 Preparation of standard curve
Take 0.00ml, 0.10ml, 0.20ml, 0.30ml, 0.40ml, 0.5ml standard application solution respectively to 15ml centrifuge tube, set to the marked volume of 10ml with deionized water, then add 2ml concentrated hydrochloric(4.3) acid and 1.0ml red potassium prussiate solution(4.7) respectively,

mix adequately, make the standard working curve.

6.3 Determination

6.3.1 Instrument reference condition: negative high voltage: 340V; lamp current: 100mA; atomization temperature: 800□; oven height: 8mm; carrier gas speed: 500ml/min; shield gas speed: 1000ml/min; determination method: standard curve method; reading mode: peak area; delay time: 1s; read time: 15s; solution feed time: 8s; sample feed volume: 2ml.

6.3.2 Determination: set the optimum condition of the instrument, rise the oven temperature to the desired temperature step by step, keep steady for 10min~20min and begin to determine. Continuously feed sample with zero-tube of the standard series, wait for the number becomes steady and begin the standard series determination, make the standard curve. Begin to determine the sample, determine sample blank and sample digestive juice respectively, wash the sample injector before each determination of different sample. The sample determination result is calculated as per 7.

7. Representation of analytical results

per Formula (1) to calculate selenium content in the sample:

$$X = \frac{(c - c_0) \times V \times 1\,000}{m \times 1\,000 \times 1\,000} \qquad \text{..........}(1)$$

Where,

X-selenium content in the sample, with the unit mg/kg or mg/l;

c-determined concentration of sample digestive juice, with the unit ng/ml;

c_0-determined concentration of sample blank digestive juice, with the unit ng/ml;

m-sample volume, with the unit g or ml;

V-total volume of sample digestive juice, with the unit ml.

two independent determination results under the same condition

The result is represented by the mean arithmetical value from two independent determination results under the same condition, and keeps three digits.

8. Precision

The absolute difference of two independent determination results under the same condition shall not exceed 10% of the arithmetical mean.

Method II: Fluorescence Method

9. Principle

After the sample has been digested by mixed acid, selenium compound is oxidized to inorganic Se^{4+}. In acid condition, Se^{4+} reacts with 2,3-diaminonaphthalene (DAN) and produces 4,5-benzo piaselenol, then extract it with cyclohexane. Determine the fluorescence intensity with the exciting light wavelength of 376nm and the emitting light wavelength of 520nm, and then calculate the content of selenium in the sample.

10. Reagent

10.1 Selenium standard solution

Accurately weigh 100.0mg element selenium (spectroscopically pure), solve it in little concentrated nitric acid, add 2ml perchloric acid (70~72%), heat in the boiling water bath for

3~4h, cool down and add 8.4ml hydrochloric acid (concentration of 0.1mol/l). Boil it in the boiling water bath for 2min. Dilute accurately to 1000ml, and this is the stock solution (selenium content: 100μg/ml). Dilute the stock solution to 0.5μg/ml selenium with the 0.1mol/l hydrochloric acid before applied. Store it in a refrigerator, and it's valid within 2 years.

10.2 DAN (1g/l) reagent

The reagent is prepared in dark room. Weigh 200mg DAN (purity of 95~98%) in a conical flask with cover, add 200ml 0.1mol/l hydrochloric acid, agitate for 15min to solve it completely. Add about 40ml cyclohexane, continue agitating for 5min. Pour the solution into a separating funnel plugged with glass cotton (or absorbent cotton), filter the cyclohexane layer after stratification, collect DAN solution, repeatedly purify DAN with cyclohexane until fluorescence in cyclohexane reduces to the minimum (purify about 5~6 times). Store the purified DAN solution in a brown bottle, add about 1cm thick cyclohexane to cover the surface, store in a refrigerator. Purify it once more before use when necessary.

Warning: This reagent has some toxicity, the personnel who use this reagent should have working experience in standard laboratory. It is the user's responsibility to take appropriate safety and health measures and ensure to conform to relative national regulations.

10.3 Mixed acid

Mix nitric acid with perchloric acid with the volume ratio of 9+1.

10.4 Non-selenium sulfuric acid

Add 200ml concentrated sulfuric acid to 200ml water, then add 30ml 48% hydrobromic acid, mix adequately, heat on the sand bath until dense white smoke appears, the volume should be 200ml at that time.

10.5 EDTA mixed liquid

10.5.1 0.2mol/l EDTA: weigh disodium EDTA 37g, add water and heat to solve completely, cool down, dilute to 500ml.

10.5.2 100g/l hydroxylamine hydrochloride solution: weigh 10g hydroxylamine hydrochloride and solve it into water, dilute to 100ml.

10.5.3 0.2g/l cresol red indicator: weigh 50mg cresol red and solve it into little water, add a drop of ammonia water (1+1), add water to dilute it to 250ml after it is completely solved.

10.5.4 Take EDTA(10.5.1) and hydroxylamine hydrochloride solution(10.5.2) 50ml respectively, add cresol red indicator solution(10.5.3) 5ml, add water to dilute it to 1L, mix adequately.

10.6 Ammonia water (ammonia water +water=1+1).

10.7 hydrochloric acid

10.8 Cyclohexane

Test the newly bought cyclohexane for fluorescence impurity, rerun before use when necessary, and the used cyclohexane can be recycled.

10.9 hydrochloric acid solution(1+9).

11. Instruments

11.1 Fluorospectrophotometer.

11.2 scale: 1mg

11.3: Oven

11.4 Pulverizer

11.5 Electric heating plate

11.6: water bath pot

12. Analytical Procedures

12.1 Sample preparation

12.1.1 Grain

Wash the sample with water for three times, bake at 60 to evaporate the surface water, crush with stainless steel mill, store in a plastic bottle, put a small package of camphor, fasten the bottle cork, store for use.

12.1.2 Vegetable and other plant food

Take the edible part, wash with distilled water for three times and suck the water drop with gauze, cut up with stainless steel knife, take some sample and dry it at 60 in an air-circulating oven, weigh, calculate the water content. Mill to powder, and then store for use.

Convert into fresh sample quantity for calculation.

12.2 Digestion of sample

Weigh grain, vegetable0.01ug~0.5ug or animal sample 0.5~2.0g (accuracy at 0.001g) or suck 1.00ml~10.0ml for liquid sample in a ground glass stoppered conical flask, add 10ml 5% non-selenium sulfuric acid, after the sample is wet, add 20ml mixed acid, stay overnight, heat slowly on a sand bath the next day. After a violent reaction, the solution becomes colorless, continue heating until white smoke is produced, the solution slowly becomes light yellow at this time, and it is the end. Some vegetable sample becomes cloudy after digestion, and it is hard to confirm the end, take off the conical flask as soon as there is heavy white smoke, cool down the solution, and it will become colorless again. Some vegetable has high selenium content, so there is much more Se^{6+}, it needs 10ml more 10% hydrochloric acid after digestion, continue heating until the reaction is finished, and the Se^{6+} is completely reduced to Se^{4+}, otherwise, the result will be lower.

12.3 Determination

Add 20ml EDTA mixed liquid into the above digested sample solution, then add ammonia water (1+1) and hydrochloric acid until the solution becomes light reddish orange (pH1.5~2.0). The following procedures are proceed in dark room: Add DAN reagent 3ml, mix, heat in the boiling water bath for 5min, take out and cool down, add cyclohexane 3.0ml, shake for 4min, transfer all the solution into a separating funnel, discard the water layer after stratification, pour the cyclohexane layer from the back cut of the separating funnel to a tube with cover carefully, ensure that there is no water in the cyclohexane. Determine the fluorescence intensity of the piaselenol on the fluorospectrophotometer with the exciting light wavelength of 376nm and the emitting light wavelength of 520nm.

10.4 Plotting of selenium standard curve

Accurately measure 0.00ml, 0.20ml, 1.00ml, 2.00ml and 4.00ml standard selenium solution (0.05µg/ml) (equivalent to 0.00µg, 0.01µg, 0.05µg, 0.10µg and 0.20µg selenium), add water to 5ml, determine at the same time with sample and as the same procedure as the sample determination.

Fluorescence intensity keeps the linear relationship with selenium content when the selenium content is below 0.5µg. In the regular sample determination, it is only necessary to do the reagent blank standard tube which is close to the sample in selenium content (duplicate).

10.5 Representation of analytical results

per Formula (2) to calculate selenium content in the sample:

$$X = \frac{m_1}{F_1 - F_0} \times \frac{F_2 - F_0}{m} \quad \cdots\cdots (2)$$

Where,

X-selenium content in the sample, with the unit μg/g orμg/ml.

m_1-selenium quantity in the standard tube, with the unit μg.

F_1-fluorescence reading number of standard selenium.

F_2-fluorescence reading number of sample.

F_0-fluorescence reading number of blank tube.

m-sample quantity, with the unit g.

The result is represented by the mean arithmetical value from two independent determination results under the same condition, and keeps three digits.

13 Precision

The absolute difference of two independent determination results under the same condition shall not exceed 10% of the arithmetical mean.