GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

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National food safety standards - Determination of chlorate and perchlorate in food

食品安全国家标准　食品中氯酸盐和高氯酸盐的测定

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State Administration for Market Regulation.

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National food safety standards - Determination of chlorate and perchlorate in food

1 Scope

This Standard specifies the liquid chromatography-tandem mass spectrometry method for the determination of chlorate and perchlorate in food.

This Standard applies to the determination of chlorate and perchlorate in vegetables and their products, fruits and their products, cereals and their products, meat and meat products, aquatic products, eggs and egg products, milk and dairy products, condiments, beverages, infant formula foods, infant supplementary foods, and tea.

2 Principle

Use an acidified acetonitrile-water mixed solution to extract the chlorate and perchlorate in the specimen; use a solid-phase extraction column to purify; use high-performance liquid chromatography to separate; use tandem mass spectrometry to test; use the isotope internal standard method to quantify.

3 Reagents and materials

Unless otherwise stated, all reagents in this method are chromatographic grade; the water is grade 1 water specified in GB/T 6682.

3.1 Reagents

3.1.1 Acetonitrile (CH_3CN).

3.1.2 Methanol (CH_3OH).

3.1.3 Formic acid (HCOOH).

3.2 Reagent preparation

3.2.1 0.1% formic acid aqueous solution: Take 1 mL of formic acid; use water to dilute to 1000 mL and mix well.

3.2.2 Acetonitrile-formic acid aqueous solution: Take 60 mL of acetonitrile; use 0.1% formic acid aqueous solution to adjust the volume to 100 mL; mix well.

3.3 Standards

3.3.1 Sodium chlorate ($NaClO_3$, CAS number: 7775-09-9): Purity is >99%; or a standard certified by the state and granted the reference material certificate.

3.3.2 Sodium perchlorate ($NaClO_4$, CAS number: 7601-89-0): Purity is >99%; or a standard certified by the state and granted the reference material certificate.

3.3.3 $^{18}O_3$-chlorate (chlorate isotope internal standard): 100 μg/mL (calculated as $^{18}O_3$-chlorate).

3.3.4 $^{18}O_4$-perchlorate (perchlorate isotope internal standard): 100 μg/mL (calculated as $^{18}O_4$-perchlorate).

3.4 Preparation of standard solutions

3.4.1 Chlorate standard stock solution (1.0 mg/mL, calculated as chlorate): Accurately weigh 1.2801 g of sodium chlorate; use water to dissolve and dilute to 1000 mL; mix well. Store it at 2 °C~8 °C. The validity period is 1 year.

3.4.2 Perchlorate standard stock solution (1.0 mg/mL, calculated as perchlorate): Accurately weigh 1.2300 g of sodium perchlorate; use water to dissolve and dilute to 1000 mL; mix well. Store it at 2 °C~8 °C. The validity period is 1 year.

Note: The standard stock solutions of chlorate and perchlorate can use standards certified by the state and granted the reference material certificate.

3.4.3 Perchlorate standard stock intermediate solution (100 μg/mL): Accurately pipette 10.0 mL of perchlorate standard stock solution; place it in a 100 mL volumetric flask; use water to dilute and make up to volume; mix well; transfer the solution to a reagent bottle. Store it at 2 °C~8 °C. The validity period is 1 year.

3.4.4 Chlorate and perchlorate mixed standard intermediate solution: Respectively pipette 1.00 mL of chlorate standard stock solution AND 1.00 mL of perchlorate standard stock intermediate solution; place them in the same 100 mL volumetric flask. Use water to dilute and make up to volume; shake well; prepare a mixed standard intermediate solution with mass concentrations of chlorate and perchlorate of 10 μg/mL and 1.0 μg/mL respectively; transfer the solution to a reagent bottle. Store it at 2 °C~8 °C. The validity period is 3 months.

3.4.5 Chlorate and perchlorate mixed standard use solution I: Pipette 1.00 mL of chlorate and perchlorate mixed standard intermediate solution; place it in a 10 mL volumetric flask. Use water to dilute and make up to volume; shake well; prepare a mixed standard use solution with mass concentrations of chlorate and perchlorate of 1000 ng/mL and 100 ng/mL respectively; transfer the solution to a reagent bottle. Store it at 2 °C~8 °C. The validity period is 1 month.

3.4.6 Chlorate and perchlorate mixed standard use solution II: Pipette 1.00 mL of chlorate and perchlorate mixed standard use solution I; place it in a 10 mL volumetric flask. Use water to dilute and make up to volume; shake well; prepare a mixed standard use solution with mass concentrations of chlorate and perchlorate of 100 ng/mL and 10 ng/mL respectively; transfer the solution to a reagent bottle. Store it at 2 °C~8 °C. The validity period is 1 month.

3.4.7 Chlorate and perchlorate isotope internal-standard mixed standard intermediate solution: Pipette 0.50 mL of chlorate isotope internal standard AND 50 μL of perchlorate isotope internal standard; place them in the same 10 mL volumetric flask. Use water to dilute and make up to volume; shake well; prepare a mixed standard intermediate solution with mass concentrations of chlorate internal standard and perchlorate internal standard of 5.0 μg/mL and 0.50 μg/mL, respectively. Store it at 2 °C~8 °C. The validity period is 3 months.

3.4.8 Chlorate and perchlorate isotope internal-standard mixed standard use solution: Pipette 2.00 mL of chlorate and perchlorate isotope internal-standard mixed standard intermediate solution; place it in a 10 mL volumetric flask. Use water to dilute and make up to volume; shake well; prepare a mixed standard use solution with mass concentrations of chlorate internal standard and perchlorate internal standard of 1.0 μg/mL and 0.10 μg/mL, respectively. Store it at 2 °C~8 °C. The validity period is 3 months.

3.4.9 Chlorate and perchlorate standard series solutions: Respectively pipette 0 μL, 250 μL, and 500 μL of chlorate and perchlorate mixed standard solution II; 100 μL, 500 μL, 1000 μL, and 5000 μL of chlorate and perchlorate mixed standard use solution I; AND 200 μL of isotope internal-standard mixed standard use solution. Use water to dilute and adjust the volume to 10 mL. The mass concentrations of chlorate are 0 ng/mL, 2.5 ng/mL, 5.0 ng/mL, 10 ng/mL, 50 ng/mL, 100 ng/mL, and 500 ng/mL, respectively. The mass concentrations of perchlorate are 0 ng/mL, 0.25 ng/mL, 0.50 ng/mL, 1.0 ng/mL, 5.0 ng/mL, 10 ng/mL, and 50 ng/mL, respectively. The mass concentration of the chlorate isotope internal standard solution in the standard working solution is 20 ng/mL; the concentration of the perchlorate isotope internal standard solution is 2.0 ng/mL. Prepare at the time of use.

3.5 Materials

3.5.1 Solid-phase extraction column: Graphitized carbon black column (500 mg, 6 mL) or one with equivalent performance. Before use, use 6 mL of methanol for activation; use 6 mL of acetonitrile-formic acid aqueous solution to elute and balance. Before loading the sample, drain the remaining solution in the column.

3.5.2 Ag/H column (1 g, 2.5 mL) or one with equivalent performance. Before use, use 10 mL of water to rinse; let stand and activate for 30 min.

Weigh 1 g of specimen (accurate to 0.01 g); add 20.0 μL of chlorate and perchlorate isotope internal-standard mixed standard use solution; vortex for 10 s; use a liquid chromatograph-tandem mass spectrometer for determination.

5.2.2 Other samples

For solid samples or condiments, weigh 1 g (accurate to 0.01 g). For liquid or semi-solid samples (except condiments), weigh 2 g (accurate to 0.01 g). Place in a 50 mL centrifuge tube; add 40.0 μL of chlorate and perchlorate isotope internal-standard mixed standard intermediate solution; mix by shaking and let stand for 30 min. Add 4.0 mL of 0.1% formic acid aqueous solution; shake and mix after plugging; vortex and mix for 30 s; ultrasonic extraction for 15 min; add 6.0 mL of acetonitrile; ultrasonic extraction for 30 min; centrifuge at 10000 r/min for 5 min; take the supernatant for purification.

5.3 Specimen purification

5.3.1 High-salt samples (soy sauce, oyster sauce, spiced salt, etc.)

Pipette 2.0 mL of the supernatant through the graphitized carbon black solid-phase extraction column; discard the effluent; then pipette 5.0 mL of the supernatant through the graphitized carbon black solid-phase extraction column; use a centrifuge tube to collect the effluent. Then use an Ag/H cartridge to purify; discard the first 3 mL; collect the subsequent effluent; pass it through a regenerated cellulose filter membrane; use a liquid chromatograph-tandem mass spectrometer for determination.

5.3.2 Other samples

Pipette 2.0 mL of the supernatant through the graphitized carbon black solid-phase extraction column; discard the effluent. Then pipette 2.0 mL of the supernatant through the graphitized carbon black solid-phase extraction column; pass the effluent through the regenerated cellulose filter membrane; use a liquid chromatograph-tandem mass spectrometer for determination.

5.4 Reference conditions for liquid chromatography-tandem mass spectrometry

5.4.1 Liquid chromatography conditions are as follows:

a) Chromatographic column: Pentafluorophenyl column with positive charge on the packing surface (column length is 100 mm; inner diameter is 2.1 mm; packing particle size is 1.7 μm); or one with equivalent performance.

b) Mobile phase: Phase A is acetonitrile; phase B is 0.1% formic acid aqueous solution. The gradient elution procedure is shown in Table A.1 in Appendix A.

c) Flow rate: 0.3 mL/min.

d) Column temperature: 35 °C.

e) Injection volume: 3 μL.

5.4.2 Mass spectrometry conditions are as follows:

a) Ionization mode: Electrospray ionization negative ion mode (ESI^-).

b) Scanning method: Multiple reaction monitoring (MRM) scan.

c) Mass spectrometry tuning parameters AND qualitative and quantitative ions are shown in Table A.2 in Appendix A.

d) The multiple reaction monitoring (MRM) ion channel diagrams of chlorate and perchlorate are shown in Figure B.1 in Appendix B.

5.5 Preparation of standard curve

Use liquid chromatography-tandem mass spectrometry to determine the chlorate and perchlorate standard series solutions; obtain the corresponding peak area. Use the concentration of chlorate and perchlorate in the standard series working solution as the abscissa; use the peak area ratio as the ordinate; draw a standard curve.

5.6 Qualitative determination

Select two groups of ions for the component to be measured. Each group of ions contains a parent ion and a product ion. Under the same test conditions, the retention time of the substance to be tested in the sample AND the corresponding retention time in the standard solution deviate within 2.5%. The signal-to-noise ratio of the monitored ion pairs must be greater than or equal to 3 (S/N≥3). And the relative abundance of the qualitative ion of the measured component in the sample IS compared with the relative abundance of the corresponding qualitative ion in the standard solution with a similar concentration. If the deviation does not exceed the range specified in Table 1, it can be determined that there is a corresponding analyte in the sample.

Table 1 -- Maximum allowable deviation of relative ion abundance in qualitative confirmation

Relative ion abundance (K)/%	$K>50$	$20<K\leqslant50$	$10<K\leqslant20$	$K\leqslant10$
Maximum allowable deviation/%	±20	±25	±30	±50

5.7 Quantitative determination

The specimen solution obtained by the treatment is analyzed by liquid chromatograph-tandem mass spectrometer; to obtain the ratio OF the peak area of chlorate and perchlorate TO the peak area of the corresponding isotope internal standard. According to the standard curve, obtain the contents of chlorate and perchlorate in the liquid to be tested.

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