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Method of Identification for Bromate in Foods

1. Scope

This method is applicable to the determination of bromate in wheat flour and its products.

2. Method

After extraction and purification, bromate is determined by liquid chromatograph/tandem mass spectrometry (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: electrospray ionization, ESI.

2.1.1.2. Column: ACQUITY UPLC BEH Amide, 1.7 μm, 2.1 mm i.d. × 10 cm, or an equivalent product.

2.1.2. Centrifuge: centrifugal force ≥ 5000 ×g, temperature control < 5°C.

2.1.3. Ultrasonicator.

2.1.4. Solid phase extraction vacuum manifolds.

2.2. Chemicals

Acetonitrile, HPLC grade;
Ammonium formate, reagent grade;
Deionized water, resistivity ≥ 18 MΩ • cm (at 25°C);
Potassium bromate, reference standard.

2.3. Apparatus

2.3.1. Volumetric flask: 5 mL and 10 mL.

2.3.2. Centrifuge tube: 50 mL, PP.

2.3.3. Solid phase extraction cartridge: Sep-Pak® C18, 200 mg, 3 mL, or an equivalent product.

2.3.4. Membrane filter: 0.22 μm, PTFE.

2.4. Reagents

2.4.1. 70% acetonitrile

Dilute 70 mL of acetonitrile with deionized water to 100 mL.

2.5. Mobile phase

2.5.1. Solvent A

Dissolve and dilute 3.153 g of ammonium formate with deionized water to 1000 mL, and filter with a membrane filter.

2.5.2. Solvent B

Acetonitrile.

2.6. Standard solution preparation

Accurately weigh equivalent to 10 mg of bromate reference standard into a 10 mL volumetric flask, dissolve and dilute with 70% acetonitrile to volume as the standard stock solution. Store in a refrigerator. When to use, mix appropriate volume of standard stock solution and dilute with 70% acetonitrile to 5 ng/mL as the standard solution.

2.7. Sample solution preparation

2.7.1. Extraction

Transfer about 1 g of the homogenized sample accurately weighed into a centrifuge tube, and add 9.5 mL of 70% acetonitrile. Ultrasonicate for 15 min, and centrifuge at 5000 ×g for 30 min at 5°C. Collect the supernatant for further purification.

2.7.2. Purification

Transfer the solution for purification from section 2.7.1. into a cartridge prerinsed with 5 mL of methanol and 5 mL of deionized water. Collect the eluent and bring the volume into 10 mL with 70% acetonitrile, filter with a membrane filter as the sample solution.

2.8. Identification

Accurately inject 2 μL of the sample solution and the standard solution into LC-MS/MS separately, and operate according to the following conditions. Identify bromate based on the retention time and the relative ion intensities(note1).

LC-MS/MS operating conditions(note2)

Column: ACQUITY UPLC BEH Amide, 1.7 μm, 2.1 mm i.d. × 10 cm.

Mobile phase: a gradient program of solvent A and solvent B is as follows:

Time (min)	A (%)	B (%)
0.0 → 3.0	10 → 10	90 → 90
3.0 → 5.0	10 → 40	90 → 60
5.0 → 8.0	40 → 40	60 → 60
8.0 → 9.0	40 → 10	60 → 90
9.0 → 15.0	10 → 10	90 → 90

Flow rate: 0.3 mL/min.

Injection volume: 2 μL.

Capillary voltage: 1.5 kV.

Ionization mode: ESI^-.

Ion source temperature: 150°C.

Desolvation temperature: 450°C.

Cone gas flow rate: 150 L/hr.

Desolvation flow rate: 950 L/hr.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, declustering potential and collision energy are as follows.

Analyte	Ion pair Precursor ion (*m/z*) > product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)
Bromate	127 > 111	40	20
	129 > 113	40	20

Note 1: Relative ion intensities are calculated by peak areas of 2 detection ions (≤ 100%). Maximum permitted tolerances for relative ion intensities by LC-MS/MS are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20～50	± 25
> 10～20	± 30
≤ 10	± 50

Note 2: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

Remark

1. Limit of detection (LOD) for bromate is 0.05 mg/kg.
2. Further validation should be performed when interference compounds appear in samples.

References

1. Himata, K., Noda, M., Ando, S. and Yamada, Y. 2000. Measurement of bromate in bread by liquid chromatography with post-column flow reactor detection. J. AOAC Int. 83: 347-355.
2. Kosaka, K., Asami, M., Takei, K. and Akiba, M. 2011. Analysis of bromate in drinking water using liquid chromatography–tandem mass spectrometry without sample pretreatment. Anal. Sci. 27: 1091-1095.

Reference chromatogram

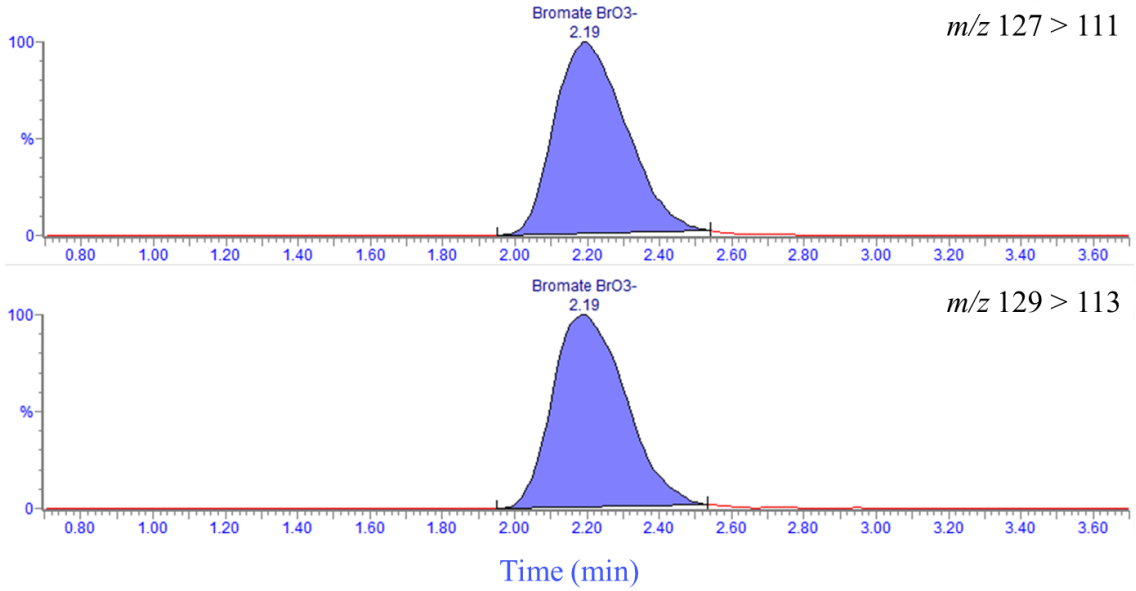

Figure. MRM chromatograms of bromate standard analyzed by LC-MS/MS.