

## Determination of Trace Levels of Bromate in Flour and Related Foods by Ion Chromatography

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In this paper, a method of determining trace levels of bromate in flour and related foods by ion chromatography with large volume injection has been proposed. The detection of bromate was performed with a suppressed conductivity detector after separation on an IonPac AS19 column with KOH as the gradient eluent. Parameters affecting the extraction efficiency of bromate, such as the flour-to-water ratio, extraction time, and temperature, were studied in detail. The optimized pretreatment process was then selected. By using the large volume injection technique, the solution detection limit was decreased to 0.5  $\mu\text{g/L}$ . The linear range of this method was from 5 to 1000  $\mu\text{g/L}$ , and the linear correlation coefficient was 0.9998. The method has been applied to the detection of bromate in flour and related foods, and different concentration levels of bromate were detected in various samples. The spiked recoveries ranged from 86 to 110%. The relative standard deviation (RSD) of the bromate peak height for the seven successive injections of the flour sample was 6.4%.

**KEYWORDS:** Bromate; flour; ion chromatography

### 1. INTRODUCTION

Wheat flour is one of the most widely consumed food materials in China, and it is usually used to make many daily foods such as bread, steamed bread, noodles, etc. Therefore, the quality and security of flour and related food are very important for our health. Potassium bromate, as an oxidant, can oxidize thiol groups to disulfide linkages, thus strengthening the protein network, so it is usually added to flour and related foods as an additive to make the flour stronger and more extensible. However, bromate is a kind of potential carcinogen, which has been proven by both the U.S. Environmental Protection Agency (EPA) and the World Health Organization (WHO) (1). Now, many countries have banned potassium bromate as a flour ingredient. In China, The Standard of Using Food Additives has also renewedly regulated the maximum level of use of potassium bromate up to 30 mg/kg in 1996, and the concentration of potassium bromate must be under the method detection limit in the food. However, in China it is very difficult to meet the regulation in The Standard of Using Food Additives because no highly sensitive standard method was developed for the determination of bromate levels at that time. Recently, the Ministry of Health in China mandated that potassium bromate be banned as a flour ingredient after July 1, 2005. Therefore, the need for a highly sensitive simple method for determining the level of bromate in flour and related foods is urgent.

In recent years, the determination of trace levels of bromate in drinking water has received considerable attention, and some novel approaches (2–5) had been developed for the detection of sub-microgram per liter levels of bromate in drinking water. However, the determination of levels of bromate in flour and related foods has not received considerable attention. Until now,

only a few methods (such as HPLC with UV detection, the spectrophotometric method, flow injection analysis, etc.) have been established for the determination of levels of bromate in flour or related foods (6–9). Himata et al. (8) have developed a HPLC method for determining levels of bromate in bread. In that paper, the bread sample was subjected to a series of simple pretreatment steps and then run on the HPLC system with postcolumn addition of *o*-dianisidine. This method is highly sensitive, the solution detection limit for bromate being 0.4 ng/mL; however, the instruments with the derivative reaction are highly complex, and the derivative condition is difficult to control. In addition, some postcolumn derivative reagents are harmful to the health of humans. The spectrophotometric method and flow injection analysis are both simple and rapid, but they also have disadvantages. For example, the sensitivity of the spectrophotometric method is not high, and the reaction in the flow injection analysis is unstable. To determine the levels of bromate, the method based on MS detection, which is highly sensitive, should be the best, but the instrumentation is very expensive.

In this paper, a very sensitive and simple ion chromatography (IC) method with conductivity is described for the determination of levels of bromate in flour and related foods. All samples are directly extracted with high-purity water and subjected to a series of simple pretreatment steps before they were run on the IC system. The method used a high-capacity and hydrophilic analytical IonPac AS19 column and a potassium hydroxide eluent in a linear gradient generated from EG40 on line, which contributed the minimal noise to the system thereby providing a superior sensitivity. By using the large volume (500  $\mu\text{L}$ ) injection technique, the low solution detection limit (0.5  $\mu\text{g/L}$ ) was obtained.

## 2. EXPERIMENTAL PROCEDURES

**2.1. Instruments and Chromatographic Conditions.** A Dionex (Sunnyvale, CA) model ICS 2000 ion chromatograph equipped with a dual-piston (in series) pump, a Dionex IonPac AS19 analytical column (4 mm × 250 mm), and an IonPac AG19 guard column (4 mm × 50 mm) was used throughout. The detection was performed with a Dionex DS6 conductivity detector. Suppression of the eluent was achieved with a Dionex anion ASRS electrolytic suppressor (4 mm) in the autosuppression external water mode. A 500  $\mu\text{L}$  sample loop was used for injection. All tubing in the chromatography path (from the outlet of the pump to the exit of the suppressor) was PEEK [0.005 in. (0.125 mm) inside diameter]. Both instrument control and data collection were performed with a personal computer equipped with a Dionex Chromeleon chromatography workstation.

**2.2. Reagents.** All reagents were analytical reagent-grade unless specified. All solutions were prepared with deionized water further purified with anEASYPure LF system (Barnstead) with a specific resistance of 18.3  $\text{M}\Omega\text{ cm}^{-1}$ . The eluent was potassium hydroxide that was generated from Dionex EG40 on line, which were run in a linear gradient at a flow rate of 1.0 mL/min. All determinations were performed at 30  $^{\circ}\text{C}$ .

**2.3. Preparation of the Standard Solution.** To prepare the stock solution of bromate, 200 mL of deionized water was placed in a volumetric flask; 1.3057 g of potassium bromate was then added (ChemService). The bottle was then shaken to thoroughly dissolve the sample. Deionized water was added to bring the final volume to 1 L and the sample mixed thoroughly. The 1.0 g/L stock solution was obtained. The stock solutions were stored in a refrigerator at 4  $^{\circ}\text{C}$ .

A series of standard solutions of bromate were prepared by diluting the stock solution with deionized water daily.

**2.4. Pretreatment of Flour and Related Food Samples.** All flour samples were purchased from market. The pretreatment processes were as follows. First, the sample was mixed thoroughly, and then 500 g was placed in the sample sack as the stock sample. For bread, steamed bread, and noodle samples, they were sliced and dried in an oven at 50  $^{\circ}\text{C}$ , and then the samples were milled into 80 mesh using mortar before weighing. All samples were homogenized in a blender. Finally, flour and related food samples were frozen to prevent deterioration and any change in composition.

Five grams of flour or related samples, which have been ground into flour, was accurately weighed in a 250 mL conical flask; 250 mL of water was added, and the sample was mixed thoroughly before sonication for 30 min at room temperature. The samples were centrifuged for 10 min at 3000 rpm, and the supernatant solution was removed.

The supernatant solution (15 mL) was passed through a 0.22  $\mu\text{m}$  Nylon membrane and OnGuard II RP (and OnGuard II Ag and H column if the chloride concentration in the sample solution is  $>50\text{ mg/L}$ ) successively. The first 3 or 6 mL was discarded on the basis of the size of the OnGuard column (1.0 or 2.5 mL), and the following solution was collected for injection.

## 3. RESULTS AND DISCUSSION

**3.1. Optimization of Chromatographic Parameters.** The IonPac AS19 column is a new advancement in Dionex polymer bonding technology and uses a high-capacity resin (350  $\mu\text{equiv/}$  column) with optimized selectivity for bromate. It is also a highly hydrophilic, hydroxide-selective anion-exchange column. It has been used as a separation column for the determination of levels of bromate in drinking water (3, 5). In this paper, the IonPac AS19 column was selected. The eluent potassium hydroxide was obtained from the EG40 eluent generator, and at the same time, the CR-ATC trap column installed between the EG40 and the Degass assembly will minimize the background shift during the gradient due to carbonate. In this way, the performance of ion chromatography methods can be improved.

To ensure the determination of levels of bromate and elute strong retention ions from the column, the eluent was generated

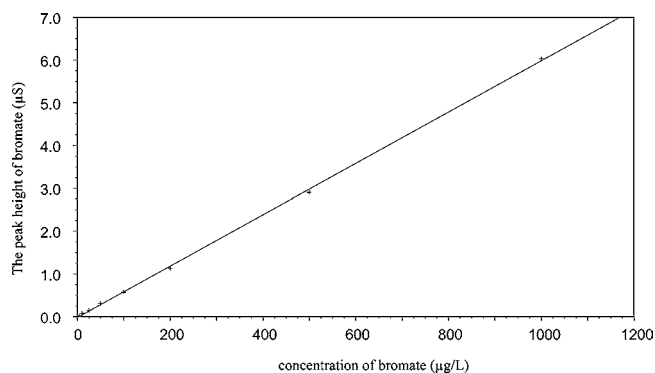
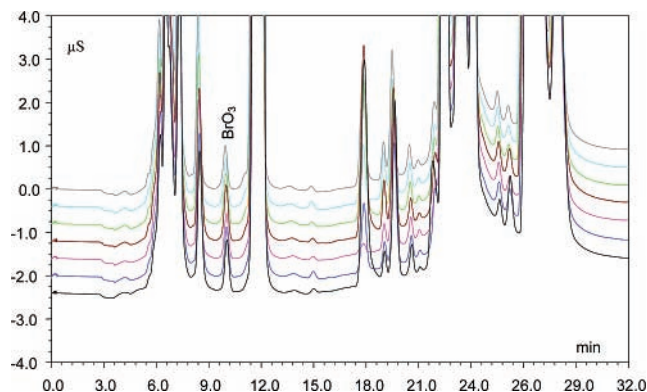


Figure 1. Calibration graph.

in a linear gradient. At first, the potassium hydroxide eluent was maintained at 8 mmol/L for 10 min, then changed to 35 mmol/L within 10 min in a linear mode, maintained at 35 mmol/L for 10 min, and finally changed to 8 mmol/L at 30.1 min to equilibrate the system. Using this linear gradient eluent, fluoride, bromate, and chloride could be separated in a baseline fashion and strong retention ions could be eluted easily.

**3.2. Study of Factors Affecting Extraction Efficiency.** In this paper, the factors (such as extraction time, temperature, and mass ratio of flour to water) affecting extraction efficiency were studied. The effects of extraction time (here extraction time is termed sonication time) showed that the peak height of bromate changed with sonication time. The peak height of bromate was the highest when the sonication time was 30 min. In this paper, 30 min was selected as the extraction time. The temperature effect was investigated at 25, 35, 50, 70, and 90  $^{\circ}\text{C}$ , and no immediate effect on extraction efficiency was observed. In addition, the experimental result showed that the extraction mixture solution was very difficult to pass through the 0.22  $\mu\text{m}$  Nylon membrane when the extraction temperature was higher than 70  $^{\circ}\text{C}$ . As a result, the extraction experiment was carried out at ambient temperature. The best mass ratio of flour to water was 5:250, which was determined by studying the extraction efficiency at different ratios (such as 1:250, 3:250, 5:250, 6:250, 7:250, 5:100, 5:50, 5:200, etc.). The result showed that the extraction efficiency slightly increased when the flour-to-water ratio decreased. When the flour-to-water ratio was equal to or less than 5:250, the best extraction efficiency was obtained. However, if the flour weight is less than 5 g, it is difficult to represent the whole quality of the sample. In this paper, a ratio of 5:250 was selected as the flour-to-water ratio.

**3.3. Calibration, Precision, and Detection Limit.** To validate the method for the analysis of bromate at microgram per liter levels, the calibration graphs were generated using a 500  $\mu\text{L}$  loop. The solution detection limit and the linear ranges of the bromate are 0.5  $\mu\text{g/L}$  (signal-to-noise ratio of 3) and 5–1000  $\mu\text{g/L}$ , respectively. A series of concentration levels of bromate (5, 10, 25, 50, 100, 200, 500, and 1000  $\mu\text{g/L}$ ) were added to the flour sample to which bromate was not added as an additive. The result showed a good linear relationship between the peak height of bromate and the concentration of bromate added into the flour, and the linear correlation coefficient was 0.9998. The calibration graph is shown in Figure 1. The RSD of the peak height for the seven successive injections of the flour sample extraction solution was 6.4%. Figure 2 is the overlay of the chromatograms of seven successive injections for a flour sample extraction solution. The described method here could yield a similar sensitivity with HPLC proposed by Himata (8). In this method, the use of the EG40 eluent generator system, which can generate high-purity

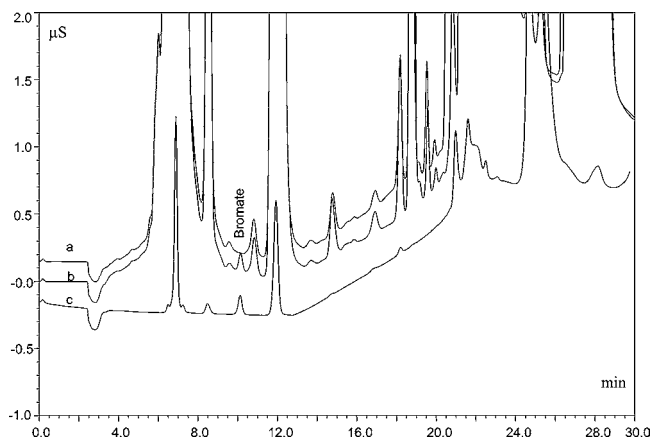


**Figure 2.** Chromatograms of seven successive injections of the flour sample extraction solution.

**Table 1.** Results of the Determination of Levels of Bromate in Flour Samples and Spiked Standards ( $n = 3$ )

sample	found ( $\mu\text{g/L}$ )	spiked ( $\mu\text{g/L}$ )	found ( $\mu\text{g/L}$ )	recovery (%)
flour #1	202.3	200.0	373.0	86.5
flour #2	140.1	100.0	251.2	109.1
flour #3	ND <sup>a</sup>	25.0	22.0	88.0
steamed bread	ND <sup>a</sup>	20.0	18.33	91.7
bread	84.0	80.0	163.4	99.3
noodles	34.4	30.0	62.8	94.7

<sup>a</sup> Not determined.



**Figure 3.** Chromatogram of bromate in ordinary flour, spiked with 25  $\mu\text{g/L}$  bromate and 25  $\mu\text{g/L}$  bromate in deionized water: (a) flour sample, (b) flour sample spiked 25  $\mu\text{g/L}$  bromate, and (c) flour sample spiked with 25  $\mu\text{g/L}$  bromate in deionized water.

and contaminant-free potassium hydroxide with different concentrations on line successively utilizing only deionized water as the carrier, could eliminate some problems associated with manually preparing the eluent off line and save time. Therefore, this method became simpler and safer than HPLC in which postcolumn reaction was necessary.

**3.4. Analysis of Levels of Bromate Ion in Flour and Related Foods.** The method described herein has been used in the determination of levels of bromate in flour and related foods in China. The sample was treated with a series of steps described in section 2.4 and then run on the IC system. On-Guard RP was used to remove lipids in the flour and flour products, which can contaminate the analytical column. On-Guard Ag was used to remove chloride to ensure the accuracy of the determination of levels of bromate. Further treatment of the extract with On-Guard H was used to remove the silver that leached from On-

Guard Ag. The analysis showed that no bromate was found in the ordinary flour sample. Especially in treated flour and related foods (such as bread or steamed bread and noodles), bromate was found at different concentrations. Recovery experiments were carried out by spiking with different concentrations (20–200  $\mu\text{g/L}$ ) of bromate in terms of the concentration of bromate in the original sample. The method showed a good recovery, ranging from 86 to 110% (Table 1). Chromatograms of the flour sample, the flour sample spiked with 25  $\mu\text{g/L}$  bromate, and the 25  $\mu\text{g/L}$  standard solution in deionized water are depicted in Figure 3.

**3.5. Conclusion.** In this paper, a simple and sensitive ion chromatographic method for the determination of trace levels of bromate in flour and related foods was developed. This method involved the use of a high-capacity separation column and the advanced eluent generation technique. By using the eluent generator, we can generate high-purity KOH eluent and thus reduce the noise background and increase the sensitivity. With a high-capacity column and large volume injection, the solution detection limit could be reduced to 0.5  $\mu\text{g/L}$  and the linear range is from 5 to 1000  $\mu\text{g/L}$ . This method requires only simple sample pretreatment and was successfully applied in the detection of bromate in flour and related foods in China. The results showed that in some flour samples different concentrations of bromate were detected. This method can be used to detect bromate in flour and related food samples.

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