Journal of Chromatography A, 1262 (2012) 219–222

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

# Sensitive and simple determination of bromate in foods disinfected with hypochlorite reagents using high performance liquid chromatography with post-column derivatization

Azusa Yokota[a], Hiroki Kubota[b], Satomi Komiya[b], Kyoko Sato[b], Hiroshi Akiyama[b], Ichiro Koshiishi[a,*]

[a] Graduate School of Health Sciences, Gunma University, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan
[b] Division of Food Additives, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

ARTICLE INFO

*Article history:*
Received 17 July 2012
Received in revised form 4 September 2012
Accepted 4 September 2012
Available online 10 September 2012

*Keywords:*
Carcinogenic
Fresh food
Drinking water
Disinfection
Tetramethylbenzidine
Strong anion-exchange

ABSTRACT

A novel analytical method for the quantification of bromate in fresh foods using high performance liquid chromatography (HPLC) with a post-column reaction has been developed. The fresh food sample solutions were pretreated with homogenization, centrifugal ultrafiltration and subsequent solid phase extraction using a strong anion-exchange resin. After separation on a strong anion-exchange chromatography column using a highly concentrated NaCl solution (0.3 M) as the eluent, the bromate was quantified by detection using a post-column reaction with a non-carcinogenic reagent (tetramethylbenzidine). The developed HPLC technique made it possible to quantify bromate in salt-rich fresh foods. The recoveries from fresh foods spiked with bromate at low levels (2 or 10 ng/g) satisfactorily ranged from 75.3 to 90.7%. The lowest quantification limit in fresh foods was estimated to be 0.6 ng/g as bromic acid. The method should be helpful for the quantification of bromate in fresh foods disinfected with hypochlorite solutions.

## 1. Introduction

Bromate is classified as possibly carcinogenic to humans (Group 2B) [1]. A decade ago, it was reported that bromate was present in the sodium hypochlorite solutions historically employed as disinfectants for drinking water [2]. Sodium hypochlorite is prepared from the reaction of chlorine and sodium hydroxide, which are produced from the electrolysis of brine which is a solution of sodium chloride. While, bromide ions are found to a very varying extent in the sodium chloride. Accordingly, the bromine in chlorine has been thought to be related to bromate production [2]. Therefore, a provisional guideline value of 10 ng/mL in drinking water is recommended by the WHO [3]. In general, sodium hypochlorite solutions have also been employed for the disinfection of fresh foods, including vegetables, meats and fishes, in many countries. However, residual bromate remaining in fresh foods treated with sodium hypochlorite solution remains obscure.

To date, several analytical methods for bromate detection have been reported [4–8]. Initially US Envirinmental Protection Agency (EPA) 300.1 method was established by using ion chromatography with conductivity detection. As more information concerning bromate toxicity became available, lower regulatory limits were imposed, resulting in demands for lower detection limits. This led to development of post-column derivatization and visible detection method EPA 317. In this method, bromate is specifically reacted with a post-column reagent, o-dianisidine, and detected by visible absorbance detection (450 nm). Separation is basically the same as in EPA 300.0. The ion chromatography method with a post-column reaction was reported for the quantification of residual bromate in bread [9] and announced as the official method by the Japanese Ministry of Health and Welfare (published in 1997). However, the use of o-dianisidine is a safety concern because of its carcinogenicity. In addition, the method requires time-consuming clean-up steps for the bread sample extract, including a C18 cartridge for defatting, a silver cartridge for removal of chloride ions, centrifugal ultrafiltration for deproteinization and a cation-exchange cartridge for removal of silver ions [7]. Furthermore, the method is difficult to apply to fresh foods, because excess amounts of byproducts derived from hypochlorite prevent the quantification of bromate. For the quantification of bromate in fresh foods using HPLC, we considered it necessary to develop a separation method and a detection system, as well as a pretreatment method that is closely linked to the separation and detection systems. In the present study, we report a novel analytical method for the quantification of bromate in fresh foods that is based on HPLC with post-column derivatization using tetramethylbenzidine (TMBz) [10,11].

---

* Corresponding author. Tel.: +81 27 220 8916; fax: +81 27 220 8916.
*E-mail address:* koshiishi_i@gunma-u.ac.jp (I. Koshiishi).

0021-9673/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2012.09.014

## 2. Materials and methods

### 2.1. Materials

TMBz was purchased from Tokyo Kasei (Tokyo, Japan). Potassium bromate and potassium bromide were purchased from Wako Pure Chemicals (Osaka, Japan). TSKgel SAX (particle size, 5 μm) was purchased from Tosoh Co. (Japan) and packed in a stainless empty column (4.6 mm i.d. × 50 mm). Amicon Ultra-15 Centrifugal Filter device (volume, 15 mL; MWCO, 30 kDa) was purchased from Millipore Co. Ltd. (USA). Muromac AG 1X8 (200–400 mesh, $Cl^-$ form) was purchased from Muromachi Chemical Inc. (Japan).

### 2.2. HPLC with post-column derivatization for bromate quantification

The HPLC assembly consisted of HPLC pumps (LC-10AD; Shimadzu), a sample injector (7725; Rheodyne), a column oven (CTO-10A; Shimadzu), a UV–vis detector (L-7485; Hitachi), a dry reaction bath (L-5050; Hitachi) and a chromatointegrator (Chromatocorder 21; SIC). Chromatographic conditions for the quantification of bromate were as follows: a separation column, TSKgel SAX (4.6 mm i.d. × 50 mm, particle size 5 μm); column temperature, 40 °C; eluent, 0.3 M NaCl (flow rate, 0.8 mL/min); TMBz reagent, 0.15 M sulfuric acid containing 10 g/L potassium bromide, 250 mg/L TMBz and 20% (v/v) methanol (flow rate, 0.4 mL/min); reaction temperature, 80 °C; cooling temperature, room temperature in water bath; reaction coil, Teflon tube (0.5 mm i.d. × 8 m); cooling coil, Teflon tube (0.5 mm i.d. × 2 m); detection, 450 nm and sample volume, 200 μL for quantification.

## 3. Results and discussion

### 3.1. Post-column detection for bromate quantification

TMBz has generally been used as a chromogen for horseradish peroxidase in enzyme immunoassays, and is also widely used as an Ames test negative reagent [10]. Therefore, this non-mutagenic chromogen was used for bromate detection. When a potassium bromate solution was mixed with an acidic solution containing potassium bromide and TMBz, the TMBz was oxidized to a yellow product with a λmax at 450 nm. In this reaction, hydrochloric and sulfuric acids were found to be appropriate as acidic reagents for colour development, whereas nitric acid was not because it caused precipitation of TMBz (data not shown).

### 3.2. Chromatographic separation of bromate

Inorganic anions are generally separated by ion chromatography, in which both a special column with low capacity and detection by a conductivity detector are employed. Therefore, the concentration of salts in the eluent should be lowered to enable detection. As a result, the ion concentration in the sample solution is also required to be as low as possible. On the other hand, the solid phase extraction of bromate in sample solutions using an ion-exchange resin in the pretreatment steps requires a high concentration of the salt for the elution of the bound bromate on the ion-exchange resin. To solve these problems, the use of a high capacity, strong anion-exchange resin for HPLC, TSKgel SAX (exchange capacity, 1.0 equiv./L; particle size, 5 μm), was investigated. Using this column, a comparatively high concentration of sodium chloride (0.3 M) could be used in the eluent to separate the bromate.

To achieve the lowest quantification limit of 1.0 ng/mL as bromic acid, a high injection volume of up to 200 μL was required. Because a sample solution containing a high concentration of salt was subjected to ion-exchange HPLC with a high injection volume, it was presumed that the bromate might be eluted as a broad peak. Theoretically, the concentration of the salts in the sample solution should be similar to that in the eluent. As shown in Fig. 1A, when the concentration of salt in the sample solution is significantly higher than that in the eluent, the correlation between the peak height and injection volume is not linear. Therefore, the concentration of NaCl in the sample solution was adjusted to 0.3 M, which is identical to the concentration of NaCl in the eluent. Consequently, linearity was observed for the correlation between the peak height and injection volume (Fig. 1B). A chromatogram of bromate in 0.3 M NaCl and a calibration curve for the quantification of bromate in the range from 0.64 to 12.9 ng/mL (as bromic acid) are shown in Fig. 2. The lowest quantification limit (S/N = 5) was estimated to be 0.6 ng/mL as bromic acid. The relative standard deviation at 0.64 ng/mL bromate (approximately the lowest quantification limit) was 10.3% (*N* = 3).

Hypochlorite solutions (100 μg/mL as chlorite) were prepared from a sodium hypochlorite feedstock solution and dry calcium hypochlorite disinfectant by diluting with or dissolving in water and then subjected to HPLC (data not shown). There is no peak interfering with the quantification of bromate in the hypochlorite solution.

### 3.3. Pretreatment of homogenates prepared from fresh foods

Fresh foods including vegetables, meats and fish were homogenized to 10% (w/v) homogenates in water. The homogenate of cabbage, to which potassium bromate was spiked (final content was 10 ng/mL), was centrifuged at 5000 × *g* for 15 min at 4 °C, and then the supernatant was subjected to centrifugal ultrafiltration using an Amicon Ultra-15 Centrifugal Filter device (volume, 15 mL; MWCO, 30 kDa; 5000 × *g* for 90 min). When the filtrate was subjected to HPLC, the peak for bromate was detected and the recovery was estimated to be 102% (data not shown). This result demonstrates that the present HPLC method requires no special pretreatment techniques, except centrifugal ultrafiltration.

For the solid phase-extraction of the bromate in the filtrate, anion-exchange chromatography using Muromac AG 1X8 (Muromachi Chemical Inc.; 200–400 mesh, Cl− form) was examined. A Pasteur pipette (5 mm i.d.) was used as a disposal column tube, to which 120 μL of Muromac AG 1X8 was packed (5 mm i.d. × 6 mm). Defatted cotton was used as a filter to prevent outflow of the resin. After equilibration with water, 10 mL of the filtrate prepared from the cabbage homogenate spiked with potassium bromate to 1.28 ng/mL was passed through the column. The column was washed with 5 mL of water, and then the bound bromate was eluted by 0.3 M NaCl. An initial 0.15 mL corresponding to the dead volume of the column was discarded, and then the eluent was fractionated into 0.5 mL aliquots. The bromate concentration in each fraction was quantified using HPLC (data not shown). The bromate was eluted in a total of 1 mL of 0.3 M NaCl; therefore, the bromate in 10 mL of ultrafiltrate obtained from a 10% homogenate was concentrated to 1 mL of a 0.3-M NaCl solution.

The recoveries for bromate from cabbage, poultry and horse mackerel were then examined. Bromate was spiked into each homogenate at a concentration of 2 or 10 ng/mL, and the homogenates were analysed after pretreatment of the sample solution. The results are shown in Table 1. The recoveries ranged from 75.3% to 90.7%. Chromatograms of the non-spiked sample solutions prepared from cabbage, poultry and horse mackerel are shown in Fig. 3. Interestingly, although residual bromate was not detected in the non-spiked sample solutions of cabbage and horse mackerel, a trace amount of bromate was detected in the non-spiked sample solutions prepared from commercially available poultry. It is, however, difficult to confirm whether this poultry was disinfected