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APPLICATION NOTE 73051

# Determination of bromate in flour and bread by IC-MS


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**Keywords**

IonPac AS31 column, mass spectrometry, ISQ EC single quadrupole mass spectrometer, IC-MS, bromate, bread, flour


## Goal

To develop a method for determination of bromate in flour and flour products using IC coupled with single quadrupole MS detection

## Introduction

Potassium bromate is a commonly used food additive in the baking industry. It is often added to flour and flour products to make the flour stronger and more extensible. It acts by oxidizing thiol groups of the gluten protein in flour and forming disulfide bonds in the process. The overall effect is to make bread rise in the oven and increase loaf volume and texture.[1,2] However, potassium bromate is considered a carcinogenic and nephrotoxic substance.[3,4] Bromate has been listed as a potential carcinogen at low levels by the International Agency for Research on Cancer.[5,6] Due to its carcinogenic potential, many countries, including the United Kingdom, the European Union, Canada, Brazil, and China, have banned this use of potassium bromate. The US FDA restricts its use and allows up to 50 mg of potassium bromate to 1 kg of flour,[7] with the belief that the baking process converts potassium bromate to non-carcinogenic bromide. However, if too much potassium bromate is added, or the bread is not baked long enough or at a high enough temperature, then a residual amount will remain. Thus, it is important to carefully monitor the low levels of bromate in flour products. This analysis is challenging due to the complex sample matrices. The most commonly applied technology for bromate determination is IC with a combination of different detection modes (conductivity detection,[8] post-column derivatization followed by UV absorbance detection,[9,10] and ICP-MS[11,12]).

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In this application note, we developed a new method using ion chromatography coupled with single quadrupole mass spectrometry (IC-MS) for selective and sensitive determination of bromate in flour and flour products. The addition of a mass spectrometer detector, the Thermo Scientific™ ISQ™ EC Single Quadrupole Mass Spectrometer, to the IC system provides high detection specificity, confirmation, and quantification of analytes not chromatographically resolved. The ISQ EC mass spectrometer is easy-to-use and economical and allows seamless integration of IC with MS. This instrument features a heated electrospray ionization (ESI) probe (HESI-II probe), that improves the ESI interface by using high voltage and temperature to deliver better desolvation and enhanced sensitivity; thus, a make-up solvent is not needed. IC-MS takes advantage of the strengths of both techniques. IC separation with eluent generation and suppressed conductivity detection provides chromatographic selectivity and analytes in the ionic form. Heated ESI introduces the liquid IC stream (after suppression) as a fine spray into the MS source, where ions are selectively detected in selected ion monitoring (SIM) mode at *m/z* 126.9 ($^{79}BrO_3^-$) and 128.9 ($^{81}BrO_3^-$).

Flour samples were extracted with deionized water and subjected to a series of simple clean up steps before they were analyzed on the IC-MS system. We used a Thermo Scientific™ Dionex™ IonPac™ AS31 column to separate bromate from matrix anions. The Dionex IonPac AS31 column[13] is a high capacity column, which allows relatively large injection volumes, thus facilitating the determination of low bromate concentrations. In this work, six commercial flour and flour products, including homemade bread baked using bromated flour, were analyzed for their bromate content using IC-MS.

## Experimental

### Equipment

- Thermo Scientific™ Dionex™ ICS-6000 system, including:
  - DP Dual Pump (P/N 22181-60008)
  - EG Eluent Generator (P/N 22181-60020)
  - DC Detector/Chromatography Compartment (P/N 22181-60050)
  - CD Cell (P/N 061830)
- Thermo Scientific™ Dionex™ AS-AP Autosampler with tray temperature control option (P/N 074926)
- Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 600 trap column (P/N 088662)
- Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm) (P/N 088667)
- Thermo Scientific™ Dionex™ 6-port high-pressure valve (P/N 22153-60014)
- Thermo Scientific™ ISQ™ EC Single Quadrupole Mass Spectrometer (P/N ISQEC000IC)
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software was used for all data acquisition and processing.

### Consumables

- Thermo Scientific™ Nalgene™ Syringe Filters, PES, 0.2 µm (Fisher Scientific, P/N 725-2520)
- Air-Tite™ All-Plastic Norm-Ject™ Syringes, 5 mL, sterile (Fisher Scientific, P/N 14-817-28)
- Thermo Scientific™ Dionex Vial Kit, 10 mL Polystyrene with Caps and Blue Septa (P/N 074228) or Thermo Scientific™ Dionex Vials, 1.5 mL Polypropylene, with caps and septa (P/N 079812)
- Thermo Scientific™ Dionex™ OnGuard™ II Ag/H Cartridges, 2.5 mL (P/N 057410)
- Thermo Scientific™ Dionex™ OnGuard™ II RP Cartridges, 2.5 mL (P/N 057084)

## Experimental conditions

| IC conditions | |
|---|---|
| Eluent: | 5 mM KOH (0–20 min);<br>70 mM KOH (20–25 min);<br>5 mM KOH (25–30 min) |
| Eluent source: | Dionex EGC 500 KOH cartridge with CR-ATC 600 |
| Flow rate: | 0.3 mL/min |
| Injection volume: | 62.5 µL in Push-Full mode |
| Column temp.: | 15 °C |
| **Detection conditions** | |
| Detection 1: | Suppressed conductivity |
| Suppressor: | Dionex ADRS 600 (2 mm) Suppressor, external water mode (flow 0.5 mL/min), 87 mA current |
| Detection/Suppressor compartment: | 15 °C |
| Background conductance: | 0.2–0.5 µS |
| System backpressure: | ~4050 psi |
| Noise: | 0.5–0.8 nS/cm |
| Run time: | 30 min |
| Detection 2: | Mass spectrometry (MS) |
| MS detector: | ISQ EC single quadrupole |
| Ionization interface: | Electrospray ionization (ESI), negative mode |
| Sheath gas pressure: | 45 psi |
| Aux gas pressure: | 4.5 psi |
| Sweep gas pressure: | 1 psi |
| Source voltage: | -2500 V |
| Vaporizer temp.: | 450 °C |
| Ion transfer tube temp.: | 200 °C |
| Chrom. filter peak width: | 32 s |

## Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better
- Potassium bromate ACS reagent, ≥99.8%, Sigma-Aldrich (P/N P6459)
- Potassium bromate (90–95% chemical purity) ($^{18}O_3$, 98%) 100 µg/mL in $^{18}O$-water (Cambridge Isotope Laboratories P/N OLM-8283-18O-1.2) standard used as an internal standard

## Instrument set up and installation

The Dionex ICS 6000 HPIC system is configured for conductivity detection, operating under high pressure conditions up to 5000 psi. To install this application, connect the Dionex AS-AP autosampler, Dionex ICS-6000 system modules, and ISQ-EC MS as shown in Figure 1. A six-port diverter valve is placed between the conductivity detector and mass spectrometer to prevent high concentrations of eluent during the wash step and/or sample matrix from going into the ESI source of mass spectrometer. The diverter valve (Figure 2) can be configured (in the script editor in the instrument method) in two positions, Position A (inject) and Position B (load). A small piece of red PEEK tubing called a “jumper” is installed in the IC diverter valve connecting port 2 to 4. Position A directs the eluent flow from CD to MS and external DI water to the suppressor Regen In. Position B directs the eluent flow from CD to suppressor Regen In and external DI water to the mass spectrometer.

Detailed instructions for configuring the IC-MS system are shown in Technical Note 72611.[14]

**Advanced scan mode**

| Scan name | Mass list (amu) | Scan time (s) | SIM width (amu) | Ion polarity | Source CID voltage |
|---|---|---|---|---|---|
| Bromate | 126.9 | 0.375 | 0.10 | Negative | 40 |
| | 128.9 | 0.375 | 0.10 | | |
| Bromate ISTD | 132.9 | 0.375 | 0.10 | Negative | 40 |
| | 134.9 | 0.375 | 0.10 | | |

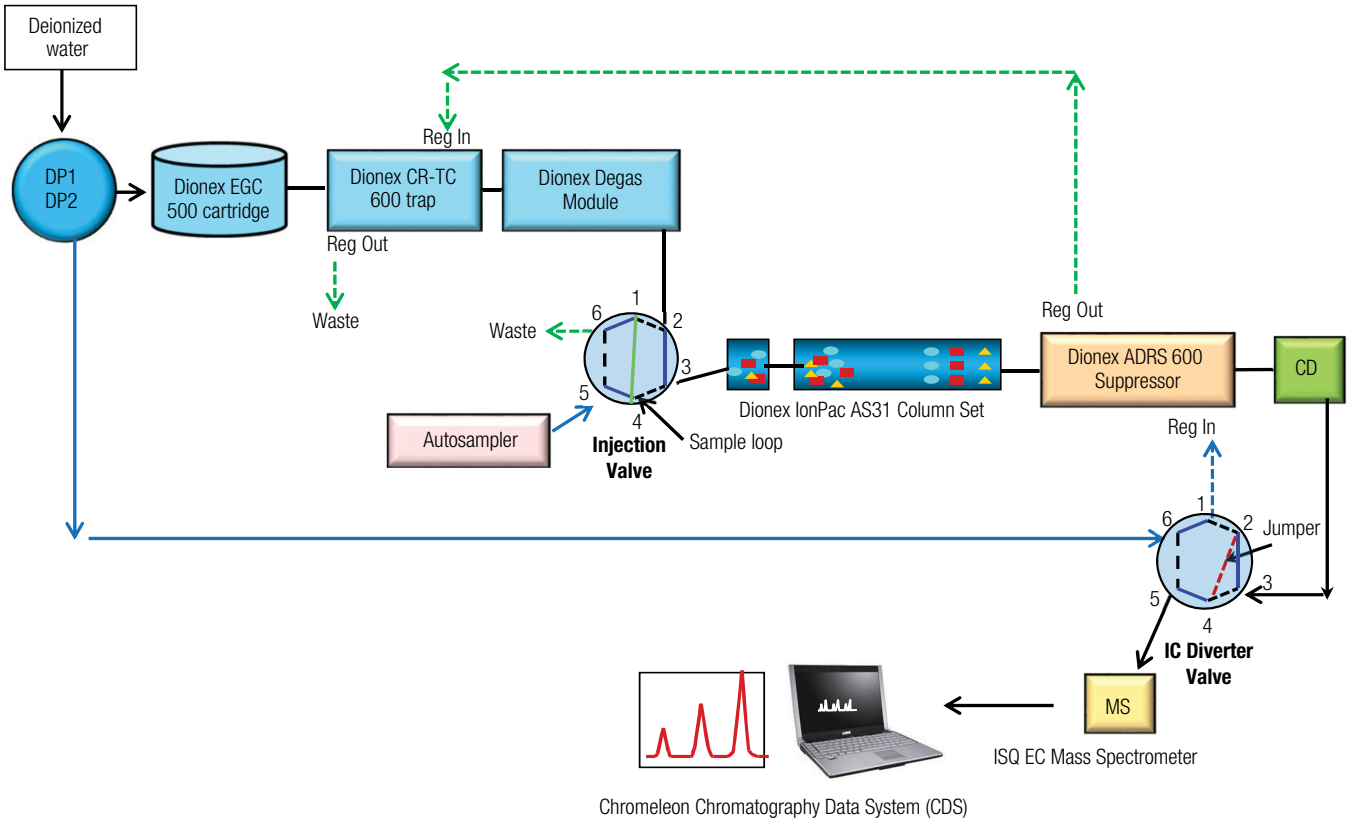


**Figure 1. Flow diagram for the IC-MS set up**

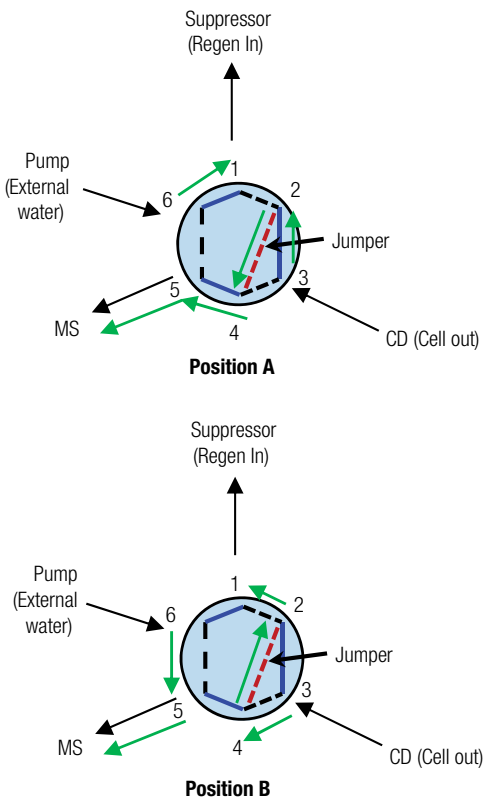


**Figure 2. Diverter valve positions**

## Preparation of solutions and reagents

### Stock standard solution

The stock standard solution (1000 mg/L) can be prepared by dissolving 1.3057 g of potassium bromate in 100 mL of DI water. The stock solution can be used for 6 months when stored in a refrigerator at 4 °C.

### Bromate internal standard solutions (ISTD)

The potassium bromate ($^{18}O_3$) standard from Cambridge Isotope Laboratories contains 77.4 mg/L of bromate $^{18}O$. Dilute to 1 mg/L bromate $^{18}O$ with DI water.

### Working standards and standards for method calibration

To prepare working standards, use a calibrated pipette to deliver the appropriate volume of 1000 mg/L stock standard into a volumetric flask and dilute to volume with DI water. For method linearity studies, the following standards of bromate were used: 500, 200, 100, 50, 20, 10, and 5 µg/L.

### Sample preparation

Mix flour samples thoroughly to ensure homogeneity, and place 500 g in the sample container as the stock sample. Slice the bread and bun samples, dry them overnight in an oven at 65 °C, and then crush and homogenize in a blender. Place the crushed and powdered sample in the sample container as the stock sample. Freeze samples to prevent deterioration and any change in composition. Follow this step-by-step procedure to prepare the sample for bromate determination:

Step 1: Weigh 5 g of ground and powdered sample, transfer it to a 250 mL volumetric flask, and add 250 mL DI water. Shake the mixture for 30 min at room temperature.

Step 2: Centrifuge a portion of the solution from step 1 at 3000 RPM. Aspirate the supernatant and filter through a 0.22 µm syringe filter.

Step 3: Prepare a Dionex OnGuard II RP, 2.5 cc cartridge by flushing it with 10 mL methanol, then connect the Dionex OnGuard II Ag/H cartridge in series such that OnGuard II Ag/H cartridge sits on the top of the Dionex OnGuard II RP cartridge. Flush with 15 mL of DI water at a flow rate of less than 2 mL/min, then discard the effluent. Load 8 mL of sample and discard the first 6 mL into a waste container. Collect the next 2 mL for analysis.

Note: The Dionex OnGuard II RP cartridge removes hydrophobic substances such as aromatics, hydrocarbons, etc., from samples. The Dionex OnGuard II Ag/H cartridge layers the resins from both Dionex OnGuard II Ag and OnGuard II H cartridges. The Dionex OnGuard II Ag cartridge removes chloride, bromide, and iodide from samples. The Dionex OnGuard II H cartridge traps silver and other cations leached from the silver cartridge.[15]

## Results and discussion

### Optimization of chromatographic parameters

A good separation is required for the determination of low bromate concentrations in complex matrices such as flour and flour products to avoid overestimation of the content as a result of analyte co-elution. A Dionex IonPac AS31 column was used to separate bromate from matrix anions.[13] The Dionex IonPac AS31 column is a high capacity column that allows relatively large injection volumes, thus facilitating the determination of low bromate concentrations. It is a low bleed column designed to be used for IC separations coupled with mass spectrometry (IC/MS) or (IC/MS/MS).

Both weakly retained ions (fluoride, bromate, chloride, etc.) as well as strongly retained ions (sulfate, phosphate, etc.) are present in the flour and flour products. To obtain a separation of bromate from other analytes, gradient elution must be used. With the gradient program, the weakly retained components can be effectively separated at low eluent concentration and the strongly retained components can be removed with high eluent concentration. Different gradient conditions were tried. After the optimization of gradient conditions, the best gradient eluent program was selected. Bromate was separated on a 2 × 250 mm Dionex IonPac AS31 column at a 0.3 mL/min flow rate with 5 mM KOH produced by the Dionex EGC 500 KOH eluent generator cartridge. Eluent concentration was increased to 70 mM KOH to remove sulfate, phosphate, and other strongly retained anions. A large volume injection (62.5 µL) was used to obtain the lowest detection limit without overloading the column. After the separation, bromate was detected in sequence by suppressed conductivity (CD) and MS. A Dionex ADRS 600 Anion Dynamically Regenerated Suppressor was placed before the CD detector to convert the KOH eluent to water, thus lowering the background signal and increasing CD sensitivity. This also prevents KOH from entering the mass spectrometer. Electrospray ionization in the negative polarity mode by the HESI II microprobe was used to introduce the suppressed eluent and analytes into the single quadrupole mass spectrometer where the ions were analyzed in selected ion monitoring (SIM) mode. Bromate has two major isotopes of molecular weight of 126.92 g/mol and 128.91 g/mol, thus signal was detected at SIM channels of *m/z* 127 ($^{79}BrO_3^-$) and 129 ($^{81}BrO_3^-$). Figure 3 shows chromatography of a 1 µg/L bromate standard. The top chromatogram displays the CD profile and bottom chromatogram displays the MS profile. The retention time for bromate (CD profile) is ~19.98 min. A delay time of 0.18 min is applied to the MS profile. When two or more detectors are connected in series, the time required for analytes to travel from the cell of the first detector to the second detector is known as the delay time. In this method, analytes travel through the CD cell before going into the mass spectrometer, thus a delay time is applied to the MS profile so that retention times match the CD profile. In comparison to CD detection, bromate is detected at lower levels by MS. The MS signal-to-noise (S/N) ratio is ~40, almost eight times higher than CD S/N ratio.

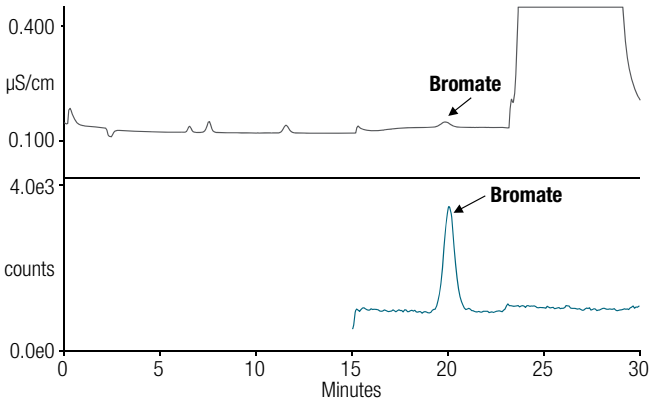


**Figure 3. Chromatogram of a 1 µg/L bromate standard.** Top: CD profile; Bottom: MS profile

## Calibration and quantification

Calibration standards for bromate were prepared in DI water. Nine calibration standards ranging from 0.5 to 500 µg/L were used. For CD detection, the external calibration method was used. For MS, the internal standard calibration method was employed. A calibration curve was created using a series of standard solutions for the analyte that are spiked with the labeled internal standard and the response factor (% ISTD; Instrument Response for analyte/instrument response for internal standard) is determined. This is done automatically in Chromeleon software when internal standard calibration is selected. Table 1 summarizes the calibration results.

**Table 1. Results of calibration, LOD, and LOQ for bromate by CD and MS detections**

| | CD detection | MS detection |
|---|---|---|
| Calibration range (µg/L) | 5–500 | 0.5–500 |
| $r^2$ | >0.999 | >0.999 |
| LOD (µg/L) | 0.75 | 0.10 |
| LOQ (µg/L) | 2.5 | 0.34 |

The sensitivity of the IC-MS method was assessed by estimating the limit of detection (LOD) and limit of quantitation (LOQ). To determine the LOD and LOQ, the baseline noise was first determined by measuring the peak-to-peak noise in a representative one-minute segment of the baseline where no peaks elute, but close to the peak of interest. The signal was determined from the average peak height of three injections of 1 µg/L and 0.25 µg/L bromate standard respectively for CD and MS detection. The estimated LODs and LOQs of bromate with the two detection methods are summarized in Table 1. In comparison to CD detection, LODs and LOQs are ~7- to 8-fold lower with MS detection.

## Sample pretreatment

The samples were treated with a series of clean up steps described in the sample preparation section and then run on the IC-MS system. A Dionex OnGuard II RP cartridge was used to remove lipids in the flour and flour products. Lipids can contaminate the analytical column. A Dionex OnGuard II Ag/H cartridge (a layered cartridge containing both Dionex OnGuard II Ag and Dionex OnGuard II H resins) was used to remove excess chloride and other halide ions from the extraction solution that could potentially interfere with bromate detection by CD. These clean up steps are important when only CD is used. For MS detection these clean up steps are not necessary as MS is a mass selective technique and detects ions based on their *m/z* value. We demonstrated this effect by preparing flour samples and bread samples as described in the *Sample preparation* section but without treatment with Dionex OnGuard II RP and Ag/H cartridges. Figure 4 displays the chromatogram of one of the flour samples FS3, with and without treatment with Dionex OnGuard cartridges. No difference was observed in the MS profile. In the CD profile, a more isolated and baseline-resolved bromate peak was observed for treated sample compared to untreated sample. Our preliminary experiments suggest it is possible to eliminate the Dionex OnGuard cartridges treatment if only using MS. Not removing chloride from the sample will create a sample of higher ionic strength, which will limit the ability to increase injection volume and for some samples lead to column overload.

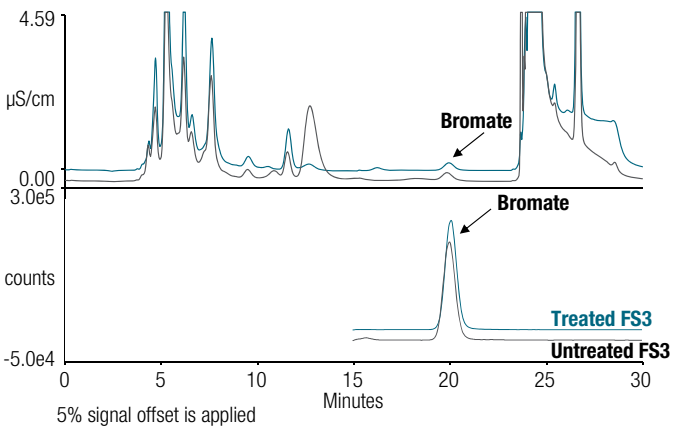


**Figure 4. Chromatogram of flour sample 3 with and without Dionex OnGuard cartridge treatment.** Top: CD profile; Bottom: MS profile

## Sample analysis

Bromate concentration was determined in a broad range of commercial flour and flour products including all-purpose flour, bromated flours, white bread, hamburger buns, and bread baked using bromated flour. All flour samples were bought from Sunnyvale, California, markets except bromated flour samples, which were bought online (https://www.bakersauthority.com). Table 2 lists the commercial flour and flour products analyzed for their bromate content. Figure 5 shows the chromatograms of three flour samples along with a 10 µg/L bromate standard. Out of three flour samples, two were bromated flour samples and were found to contain ~15 and ~9 mg/kg bromate each. No bromate was detected in the all-purpose flour sample (FS1). Figure 6 displays the chromatograms of three baked samples: two commercial baked goods (sliced white bread and hamburger bun) and homemade bread baked with flour containing 65 mg/kg potassium bromate. The CD profile shows that bromate is not very well resolved in any of the three bread samples. Because of the coelution, CD detection was not used for the quantification of these samples. MS detection can resolve co-eluting peaks using their mass-to-charge ratios and was used for the quantification. No bromate was detected in bread samples BS1 and BS2. In homemade bread sample BS3, bromate was detected at 1.2 µg/L, corresponding to 60 µg/kg bromate in bread which is ~0.1% of the bromate originally added in the flour. All bromate determination results are presented in Table 2. The results show that the proposed method can be used for quality control of flour and flour products.

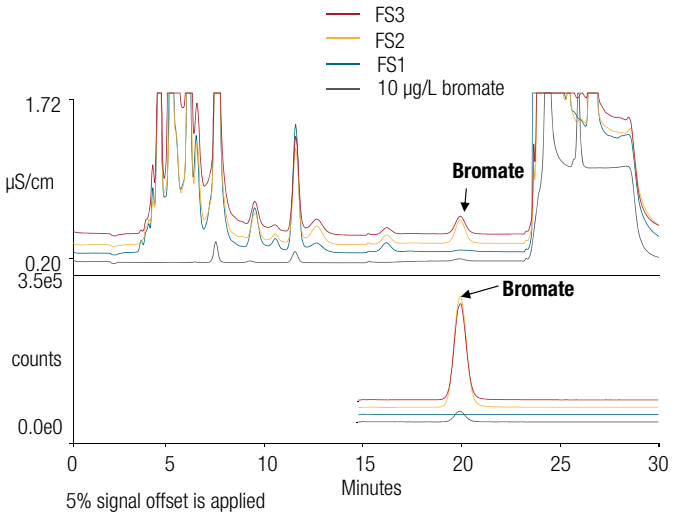


**Figure 5. Chromatograms of flour samples and a 10 µg/L bromate standard.** Top: CD profile; Bottom: MS profile

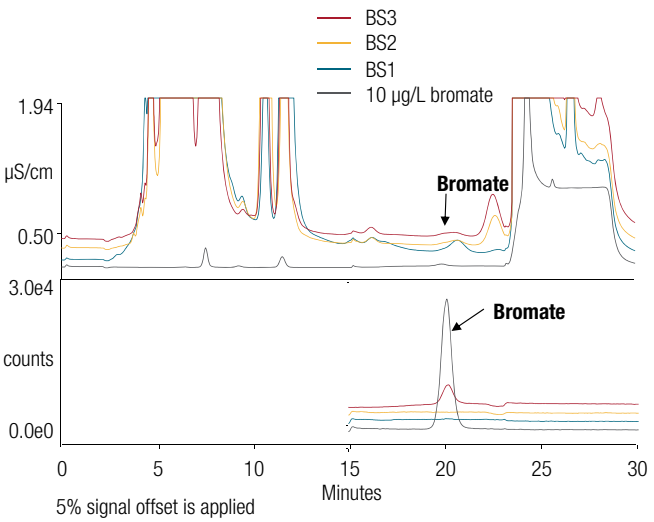


**Figure 6. Chromatograms of bread samples and a 10 µg/L bromate standard.** Top: CD profile; Bottom: MS profile

**Table 2. Bromate determination results in flour and flour products**

| # | Sample | Bromate (mg/kg) |
|---|---|---|
| FS1 | All purpose flour | Not found |
| FS2 | Brand A Full-strength bromated flour | 15 |
| FS3 | Brand B Hi gluten bromated flour | 9 |
| BS1 | Commercial white bread | Not found |
| BS2 | Fast food hamburger bun | Not found |
| BS3 | Bread baked using bromated flour | 0.06 |

## Accuracy and precision

To evaluate the method accuracy, recovery experiments were carried out by spiking with different concentrations (0.5–100 µg/L) of bromate in terms of the concentration of bromate in the original sample. The recovery percentages were calculated using the formula shown below:

$$Recovery\ \% = \frac{C\ spiked\ sample - C\ unspiked\ sample}{C\ analyte\ added} \times 100$$

Figure 7 shows the chromatograms of flour samples FS2 and FS2 spiked with 100 µg/L bromate. Figure 8 shows the chromatograms of the bread sample BS3, BS3 spiked with 0.5 and 1 µg/L bromate, and the 1 µg/L standard solution. Due to the interfering peaks in the bread sample, bromate could not be detected by CD, and thus only MS detection was used for the calculation of bromate recoveries in bread samples (Table 3).

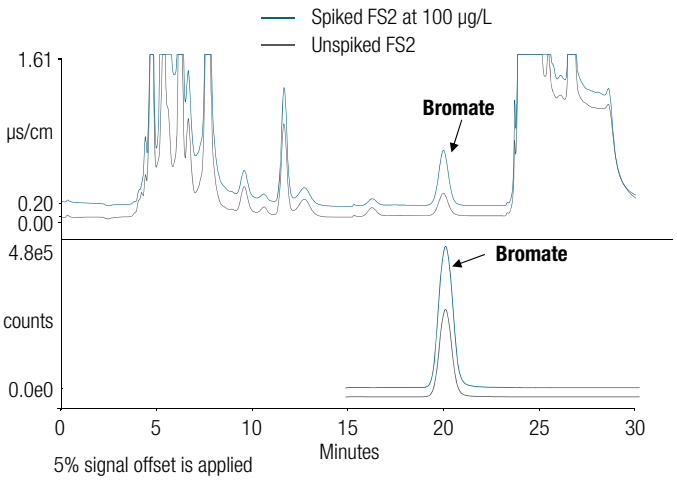


**Figure 7. Chromatograms of flour sample FS2 and FS2 spiked at 100 µg/L bromate.** Top: CD profile; Bottom: MS profile

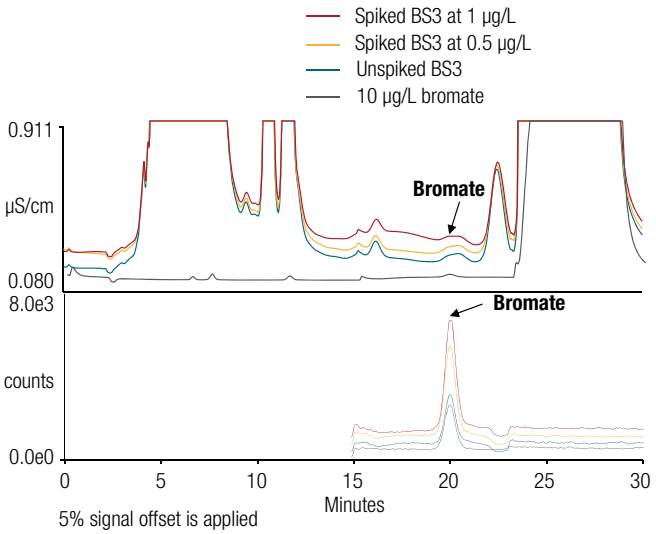


**Figure 8. Chromatograms of bread samples BS3 and BS3 spiked at 0.5 and 1 µg/L bromate along with a 1 µg/L bromate standard.** Top: CD profile; Bottom: MS profile

The precision of the method was evaluated by duplicate injections of bromated flour sample (FS2) prepared and run on four separate days and calculating the relative standard deviation (RSD) across all eight injections. The peak area RSDs and retention time RSDs were 4.29% and 0.19%, respectively, for CD detection and 2.86% and 0.15%, respectively, for MS detection.

**Table 3. Recovery of bromate calculated by CD and MS detection**

| # | CD detection | | | | MS detection | | | |
|---|---|---|---|---|---|---|---|---|
| | Found (µg/L) | Spiked (µg/L) | Recovered (µg/L) | Recovery (%) | Found (µg/L) | Spiked (µg/L) | Recovered (µg/L) | Recovery (%) |
| FS1 | Not found | 10 | 8.56 | 85.6 | Not found | 10 | 9.20 | 92.0 |
| | | 100 | 101 | 101 | | 100 | 102 | 102 |
| FS2 | 295 | 100 | 382 | 87.0 | 304 | 100 | 395 | 91.0 |
| FS3 | 181 | 100 | 284 | 103 | 183 | 100 | 281 | 98.0 |
| BS1 | – * | | | | Not found | 10 | 8.90 | 89.0 |
| | | | | | | 100 | 92.6 | 92.6 |
| BS2 | – * | | | | Not found | 10 | 8.58 | 85.8 |
| | | | | | | 100 | 104 | 104 |
| BS3 | – * | | | | 1.20 | 0.5 | 1.75 | 108 |
| | | | | | | 1 | 2.31 | 110 |
| | | | | | | 10 | 11.2 | 99.7 |
| | | | | | | 100 | 91.8 | 90.5 |

* CD detection not used for these samples

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## Conclusion

Using the high capacity anion exchange Dionex IonPac AS31 column and a large volume injection, low concentrations of bromate were determined in flour and bread samples. The addition of an MS detector to the IC system provided high detection specificity and quantification of those samples with an unresolved bromate peak. Six commercial flour and flour products, including homemade bread baked using flour containing potassium bromate, were analyzed. The method showed good precision with RSDs <0.2%, and <5% (n=8), for retention time and peak area, respectively. Bromate recoveries from flour samples ranged from 86% to 110%. The limits of detection and quantitation of bromate in the prepared solution were 0.10 µg/L and 0.34 µg/L, respectively, which corresponded to 5 µg/kg and 17 µg/kg in bread.

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