Ion Chromatographic Method with Post-Column Fuchsin Reaction for Measurement of Bromate in Chlorinated Water

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ABSTRACT

An ion chromatographic method that employs a post-column reaction with fuchsin and spectrophotometric detection was optimized for measuring bromate (BrO$_3^-$) in water. BrO$_3^-$ is converted to Br$_2$ by sodium metabisulfite and then reacted with acidic fuchsin to form a red-colored product that strongly absorbs at 530 nm. The reaction of BrO$_3^-$ and fuchsin reagent is optimum at pH 3.5 and 65 °C. The method has a limit of quantitation of 4.5 µg L$^{-1}$ and is linear up to 150 µg L$^{-1}$ BrO$_3^-$. Recoveries from spiked samples were high ranging from 95 to 102 % using external standard calibration and 87 to 103 % using standard addition method. Intra-batch and inter-batch reproducibility studies of the method resulted to RSD values ranging from 0.62 to 2.01 % and percent relative error of 0.12 to 2.94 % for BrO$_3^-$ concentrations of 10 µg L$^{-1}$ and 50 µg L$^{-1}$. This method is free of interferences from common inorganic anions at levels typically found in chlorinated tap drinking water without preconcentration. The optimized method can be applied to trace analysis of bromate in chlorinated tap drinking water samples.

Keywords: bromate, fuchsin, chlorinated water, post-column reaction, ion chromatography

INTRODUCTION

Bromate (BrO$_3^-$) may be present in various water types, including those intended for human consumption, either as a major disinfection by-product of the ozonation of water containing naturally occurring bromide ions or as a contaminant of hypochlorite disinfection (Fawell & Walker, 2006; Haag & Hoigné, 1983; Legube, et. al., 2004; von Gunten & Hoigné, 1994; Weinberg, et. al., 2003). Once generated and found in water, BrO$_3^-$ does not easily degrade.

Toxicological studies of BrO$_3^-$ in rats have provided evidence of its possible carcinogenicity (Fuji, et. al., 1984; Kurokawa, et. al., 1990). Acute exposure of rodents to BrO$_3^-$ has been shown to cause neuropathological disorders and induce tumors of the kidney, peritoneum and thyroid (De Borba, et. al., 2005; Kurokawa, et. al., 1990). The lifetime cancer risk determined for BrO$_3^-$ in drinking water for humans was 2×10$^{-5}$ per µg L$^{-1}$ assuming a 2-L daily water consumption (De Borba, et. al., 2005; Fawell & Walker, 2006). Lifetime risks of 10$^{-4}$, 10$^{-5}$ and 10$^{-6}$ were theoretically associated with exposures to BrO$_3^-$ concentrations of 5, 0.5 and 0.05 µg L$^{-1}$, respectively. The availability of analytical methods to monitor and determine BrO$_3^-$ in drinking water at sub-µg L$^{-1}$ levels is thus important.

A maximum admissible concentration (MAC) of 10 µg L$^{-1}$ in drinking water is recommended by the US Environmental Protection Agency (US EPA), the European Commission (EC) and the World Health Organization (WHO) (De Borba, et. al., 2005; Fawell & Walker, 2006; Guinamant & Ingrant, 2006; von Gunten & Hoigné, 1994; Weber, et. al., 1993). The availability of analytical methods to monitor and determine BrO$_3^-$ in drinking water at sub-µg L$^{-1}$ levels is thus important.
2000; Uraisin, et. al., 2006). This guideline was defined primarily on the basis of the detection capabilities of existing ion chromatographic methodologies. The Philippine National Standards for Drinking Water of 2007 has a maximum guideline level of 10 µg L⁻¹ BrO₃⁻ in drinking water based on the recent risk assessment of the WHO. The proposed detection limit of less than 2.5 µg L⁻¹ BrO₃⁻ by the EC has called for the development of more sensitive analytical methods and alternative techniques (Ingrant & Guinamant, 2002). A number of methods have been developed and adapted to meet the objectives of setting quality standards for BrO₃⁻ in water. Official methods for BrO₃⁻ determination include ion chromatography with conductivity detection. Recent studies have shown that the sensitivity of these analytical methods may be improved by coupling the separation using ion chromatography with a specific post-column reaction (Delcomyn, et. al., 2001; Uraisin, et. al., 2006). Three different post-column reaction techniques in ion chromatography have been compared. Post-column reactions with KI-(NH₄)₆MoO₄, NaBr-NaNO₂ and o-dianisidine showed low detection limits ranging from 0.17 to 0.24 µg L⁻¹ for BrO₃⁻ in water (Hautman, et. al., 2001; Salhi & von Gunten, 1999). Performance evaluation of the US EPA method 317.0, which employs both suppressed conductivity and spectrophotometric detection after post-column reaction with o-dianiside, demonstrated specificity and sensitivity for BrO₃⁻ with a detection limit of 0.042 µg L⁻¹ (Wagner, et. al., 2001).

Two similar studies based on the hyphenation of an ion chromatographic system and post-column fuchsin reaction with visible absorbance detection showed the pH and temperature dependence of the BrO₃⁻-fuchsin reaction (Archilli, et. al., 1999; Valsecchi, et. al., 1999). Using a standard carbonate-bicarbonate mobile phase, a linear range of 0.1 to 100 µg L⁻¹ and a detection limit of 0.1 µg L⁻¹ BrO₃⁻ were reported by Archilli, et. al. (1999). Valsecchi, et. al. (1999), on the other hand, used a tetraborate mobile phase and obtained a linear range of 0.5 to 10 µg L⁻¹ and detection limit of 0.4 µg L⁻¹ BrO₃⁻. Their methods were successfully used in quantifying trace levels of BrO₃⁻ in actual drinking water samples.

This study aimed to optimize an ion chromatographic method involving post-column fuchsin reaction followed by spectrophotometric detection for trace BrO₃⁻ in chlorinated water as well as provide an alternative analytical technique useful in the strict compliance of water quality standards in the Philippines.

**MATERIALS AND METHODS**

**Reagents and Standard Solutions**

All chemicals used were analytical reagent grade. The standards and blank solutions were prepared using ultrapure water at resistance of 18.0 MΩ (Nanopure® Barnstead, USA). Bromate standard solutions were made by dilution of a 1000 µg L⁻¹ stock solution of potassium bromate (Merck, Germany). The fuchsin stock solution (FSS) was prepared by dissolving 100 mg basic fuchsin (C₁₉H₁₈N₃Cl, Beijing Chemical Works, China) in 100 mL of ultrapure water. Stored at 4 °C in a glass amber bottle, this solution is stable for several months. The color developing solution which acts as post-column reagent was prepared by acidifying 10 mL of FSS with 0.5 mL of 12 M HCl (Merck, Germany) followed by the addition of 350 mg of sodium metabisulfite (Mallinckrodt, USA). The solution was made up to 100 mL with ultrapure water in a glass flask and was left to stand overnight for complete decoloration.

The mobile phase solutions were prepared from 2.5 mM phthalic acid (Merck, Germany) and 2.4 mM tris(hydroxymethyl)aminoethane (Merck, Germany) solutions buffered at pH 3.5. These solutions were filtered through 47 mm×0.2 µm cellulose nitrate membrane filters (Whatman, England) using a filtering apparatus attached to a vacuum source and degassed for several minutes by sonication (Branson Model 8510 Ultrasonic cleaner, USA) prior to use. For the interference studies, inorganic anions (HCO₃⁻, Cl⁻, SO₄²⁻, Br⁻, F⁻, NO₂⁻, NO₃⁻, PO₄³⁻ and ClO₄⁻) of different concentrations (1, 5, 50 and 100 mg L⁻¹) were prepared by dissolving appropriate amounts of the anions in their potassium and sodium salts.

**Ion Chromatographic Analysis**

A Perkin Elmer Lambda 40 UV-Vis spectrophotometer was used for visible spectrum scanning. The Shimadzu HIC-6A ion...
The chromatograph system used in this study is composed of the following modules: LP-6A delivery pump unit (LC-6A liquid pump with a high-sensitivity noise filter), SPD-6AV UV-Vis spectrophotometric detector, CTO-6AS column oven, SCL-6B system controller, LC-9A liquid pump used for post-column reagent, SIL-6A auto sample injector, and C-R4A integrator and printer/plotter for data processing. Three stainless steel columns (Shimadzu, Japan) were used in the chromatographic system: Shim-pack IC-PC1 precolumn, Shim-pack IC-GA1 guard column and Shim-pack IC-A1 analytical column. The injected samples passed through the preheater before entering the guard column and analytical column inside an oven set at 65 °C. The 4.6 mm (i.d.) × 10 cm (length) × 12.5 μm (particle size) analytical column was packed with an anion exchange resin on a polymethacrylate support incorporating a quaternary ammonium base which is a strong anion-exchange functional group. The ions that elute from the column mix with the fuchsin post-column reagent in the mixing tee. BrO$_3^-$ and fuchsin react completely in the 204-cm reaction coil to form the red-colored product that is directed to the UV-Vis detector. Measurement of BrO$_3^-$ concentration was carried out using external standard calibration and standard addition methods.

RESULTS AND DISCUSSION

Bromate, upon reaction with fuchsin reagent, is detected in the visible region (Espino & Cimatu, 2003; Romele & Achilli, 1998). In this study, a maximum absorbance was obtained at 530 nm when a 10 µg L$^{-1}$ BrO$_3^-$ and acidified fuchsin solution was scanned in the visible range of 400 to 635 nm. The 530 nm wavelength also gave a maximum peak response for the same BrO$_3^-$-fuchsin solution when determined by ion chromatography using different wavelength settings from 520 to 535 nm. The 530 nm wavelength was then used in the subsequent ion chromatographic analyses.

Previous studies revealed that the reaction of BrO$_3^-$ with fuchsin reagent occurs in the acidic range (Achilli & Romele, 1999; Romele & Achilli, 1998; Valsecchi, et. al., 1999). In this study, the optimum pH for the BrO$_3^-$-fuchsin reaction was investigated by varying the pH of the mobile phase from 2.0 to 7.0. Figure 1 shows that a maximum peak response is obtained when the pH of the mobile phase was maintained at 3.5.

Similar to pH, temperature also affects the BrO$_3^-$-fuchsin reaction. Previous ion chromatographic studies found that the BrO$_3^-$ peak response increases as the temperature is raised from ambient to an optimum reaction temperature, e.g., 65 or 80 °C (Achilli & Romele, 1999; Valsecchi, et. al., 1999). In the present study, the optimum temperature is 65 °C as shown in Figure 2.

A blank measurement experiment was performed to validate the use of ultrapure water as solvent in preparing calibration or sample solutions. No peak response was observed in the chromatographic measurements of seven replicate blank solutions consisting only of ultrapure water and fuchsin reagent. This confirms that ultrapure water does not contain BrO$_3^-$.

![Figure 1](image1.png)  
Figure 1. Average peak areas (n=3) for 10 µg L$^{-1}$ BrO$_3^-$ at varying pH of the mobile phase.

![Figure 2](image2.png)  
Figure 2. Average peak areas (n=3) and retention times (n=3) for 10 µg L$^{-1}$ BrO$_3^-$ at varying oven temperatures in °C.
water spiked with 10 µg L\(^{-1}\) BrO\(^3\); the second set of seven solutions consisted of bromate-free drinking water spiked with 10 µg L\(^{-1}\) BrO\(^3\). The average peak areas and % RSD of the two sets of data are in good agreement as shown in Table 1. This further validates the use of ultrapure water in procedural blanks and sample analysis using ion chromatography with post-column fuchsin reaction.

**Table 1.** Comparison of the average peak areas and percent relative standard deviations of spiked blanks using ultrapure water and bromate-free drinking water as solvents.

<table>
<thead>
<tr>
<th>Spiked blanks</th>
<th>Average peak area(^<em>(n=7)</em>)</th>
<th>SD</th>
<th>RSD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank using ultrapure water +10 µg L(^{-1}) BrO(^3)</td>
<td>38212</td>
<td>708</td>
<td>1.85</td>
</tr>
<tr>
<td>Blank using bromate-free bottled water(^*)+10 µg L(^{-1}) BrO(^3)</td>
<td>38010</td>
<td>729</td>
<td>1.92</td>
</tr>
</tbody>
</table>

\(\text{Average peak area}^*\) (n=7) = Seven replicates with three measurements per replicate.

\(^{\text{b}}\) Bottled distilled drinking water (brand: Absolute).

Inorganic anion interferences in the ion chromatographic determination of BrO\(^3\) were also investigated. Figure 3 shows the effect of adding common inorganic anions (HCO\(_3\), Cl\(^-\), NO\(_3\), SO\(_4^{2-}\), Br\(^-\), F\(^-\), NO\(_2\), PO\(_4^{3-}\), and ClO\(_3^\cdot\)) in four different concentrations to 10 µg L\(^{-1}\) BrO\(^3\) solutions prepared in ultrapure water. A paired t-test was performed to determine whether or not the inorganic anions were interfering at different concentrations. The mean peak area differences of solutions of 10 µg L\(^{-1}\) BrO\(^3\) in ultrapure water were not significantly greater than zero before and after the same solutions were spiked with the inorganic anions at four different concentrations. Student’s t-test gave test statistic t values for inorganic anions (HCO\(_3\) = -0.0392, Cl\(^-\) = -3.07, NO\(_3\) = 0.556, SO\(_4^{2-}\) = 1.09, Br\(^-\) = 1.55, F\(^-\) = -3.93, NO\(_2\) = -2.32, PO\(_4^{3-}\) = 1.00, and ClO\(_3^\cdot\) = 1.14) which were lower than the critical t value of 3.18 at 95 % confidence interval from the t-distribution table. These statistical tests proved that the inorganic anions do not interfere in the determination of BrO\(^3\). Further, low RSD values ranging from 0.24 to 2.5 % were obtained for the 10 µg L\(^{-1}\) BrO\(^3\) solutions spiked with different concentrations of these anions. It was also observed that in the absence of BrO\(^3\), the red-colored product that absorbs at 530 nm was not formed in solutions spiked with the anions. Hence, these anions do not react with the fuchsin reagent which appeared to be BrO\(^3\)-specific.

Using the optimum conditions, eleven BrO\(^3\) standard solutions ranging from 2 to 1500 µg L\(^{-1}\) were prepared to get the range of concentration from the estimated lowest detectable concentration to a concentration where a departure from linearity will be observed. A linear response was obtained and the limits of the linear dynamic range are presented in Figure 4.

Based on the results of the analysis in wide concentration range (2 to 1500 µg L\(^{-1}\)), low-bromate (2 to 150 µg L\(^{-1}\)) and high-bromate (150 to 2000 µg L\(^{-1}\)) calibration solutions were then measured and compared. Table 2 summarizes the regression and residual analyses for low- and high-bromate concentration calibration curves. Linear (first-order polynomial) and quadratic (second-order polynomial) least square (LS) regression models
were used to assess the degree of goodness of fit of
the experimental calibration data. The correlation
coefficients and residual values were also derived
using these models. The linear and quadratic least
squares are polynomials described by a set of
coefficients in the following equations:

\[
y = a_o + a_1 x \quad (linear, \text{Rdf}=2) \quad (1)
\]
\[
y = a_o + a_1 x + a_2 x^2 \quad (quadratic, \text{Rdf}=3) \quad (2)
\]

where \( y \) is the peak area, \( a_0 \), \( a_1 \) and \( a_2 \) are the
coefficients of the polynomial, \( x \) is the concentration
in \( \mu g L^{-1} \), and Rdf is the required degrees of
freedom. The results of the regression and residual
analysis showed good agreement between the two
LS regression models. This means that the values of
\( x \) from the linear Equation 1 and quadratic Equation
2 will almost be the same. In this study, the simpler
linear LS regression was used to calculate BrO\(_3\)–
concentration.

**Table 2. Comparison between low-BrO\(_3\)– and high-BrO\(_3\)– calibration data in terms of regression and residual analyses.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Low-bromate concentration range 2 to 150 ( \mu g L^{-1} ) (n=3)</th>
<th>High-bromate concentration range 150 to 2000 ( \mu g L^{-1} ) (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression equation</td>
<td>( Y=10628 + 2724x )</td>
<td>( Y=45267 + 258x - 0.0855x^2 )</td>
</tr>
<tr>
<td>Correlation Coefficient, ( r )</td>
<td>0.9994</td>
<td>0.9704</td>
</tr>
<tr>
<td>Residuals (absorbance units)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-7.5152</td>
<td>2907.06</td>
<td>-56522</td>
</tr>
<tr>
<td>460.991</td>
<td>2629.89</td>
<td>-8339.4</td>
</tr>
<tr>
<td>-483.49</td>
<td>514.680</td>
<td>29972</td>
</tr>
<tr>
<td>-6824.1</td>
<td>-7897.1</td>
<td>30753</td>
</tr>
<tr>
<td>4575.93</td>
<td>-549.0</td>
<td>33831</td>
</tr>
<tr>
<td>8630.05</td>
<td>3958.0</td>
<td>4694.8</td>
</tr>
<tr>
<td>-6351.8</td>
<td>-1563.4</td>
<td>-34389</td>
</tr>
</tbody>
</table>

The average \% RSD (n=3) of the peak areas in the
low-bromate concentration curve was 1.47 \%, while
in high-bromate concentration it was 0.97 \%. As
expected, the precision improved in high-bromate
concentration due to less deviation in replicate
analysis that is almost always associated with
measurements in higher concentrations.
Table 3. Intra-batch and inter-batch reproducibility studies for precision and accuracy of the ion chromatographic method.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Measured Concentration (SD)</th>
<th>RSD, %</th>
<th>Rel. Error, %</th>
<th>Concentration</th>
<th>Measured Concentration (SD)</th>
<th>RSD, %</th>
<th>Rel. Error, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.00</td>
<td>9.988(0.19)</td>
<td>1.93</td>
<td>0.12</td>
<td>10.00</td>
<td>9.900(0.13)</td>
<td>2.01</td>
<td>1.00</td>
</tr>
<tr>
<td>50.00</td>
<td>51.47(0.42)</td>
<td>0.81</td>
<td>2.94</td>
<td>50.00</td>
<td>50.88(1.16)</td>
<td>0.62</td>
<td>1.76</td>
</tr>
</tbody>
</table>

SLOQ = Sblank + 10sblank

\[ x_{LOQ} = \left( \frac{S_{LOQ} - S_{blank}}{m} \right) \]  

Percent recoveries of BrO\textsuperscript{3} - spiked in tap drinking water samples in three different concentrations (low, 5.0 µg L\textsuperscript{-1}; medium, 50.0 µg L\textsuperscript{-1}; and high, 100.0 µg L\textsuperscript{-1}) were determined to test the reliability and accuracy of the method using an actual sample matrix. Table 4 gives the % recoveries using external standard calibration and standard addition quantitation methods. Using the external standard calibration method, % recoveries were generally acceptable based on the recommended 80 to 120 % as reported by Lesnik (1992). Low % RSD values were found across the three BrO\textsuperscript{3} - concentrations. Notably, better recoveries were obtained for medium and high concentrations when using standard addition method.

Table 4. Percent recovery data for low, medium and high bromate concentrations in tap drinking water\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Concentration Method</th>
<th>Low 50 µg L\textsuperscript{-1}</th>
<th>Medium 50.0 µg L\textsuperscript{-1}</th>
<th>High 100.0 µg L\textsuperscript{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>External standard Calibration</td>
<td>99 (1.84)</td>
<td>101 (0.89)</td>
<td>102 (0.77)</td>
</tr>
<tr>
<td>Standard addition</td>
<td>89 (1.07)</td>
<td>101 (1.37)</td>
<td>99 (1.08)</td>
</tr>
<tr>
<td>(using peak height)</td>
<td>97 (1.54)</td>
<td>95 (0.86)</td>
<td>96 (1.40)</td>
</tr>
<tr>
<td>External standard calibration</td>
<td>87 (1.13)</td>
<td>98 (1.60)</td>
<td>103 (0.77)</td>
</tr>
<tr>
<td>Standard addition</td>
<td>97 (1.45)</td>
<td>95 (1.37)</td>
<td>102 (1.08)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Tap water sample taken from the Faculty Lounge of the Institute of Chemistry, University of the Philippines. 
\textsuperscript{b}Mean for seven replicates with three measurements per replicate.

The ion chromatographic method described in this study is reproducible, accurate, sensitive and suitable for the analysis of BrO\textsuperscript{3} - in chlorinated tap drinking water at trace levels. The method can be used for monitoring BrO\textsuperscript{3} - in chlorinated water in the treatment plants as well as in the distribution...
lines. This is an available method that can be used to ensure strict compliance of the 10 µg L$^{-1}$ BrO$_3^-$ stipulated in the Philippine National Standards for Drinking Water.

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