Characterization and Determination of Chloro- and Bromo-Benzoquinones as New Chlorination Disinfection Byproducts in Drinking Water

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We report the characterization and determination of 2,6-dichloro-1,4-benzoquinone and three new disinfection byproducts (DBPs): 2,6-dichloro-3-methyl-1,4-benzoquinone, 2,3,6-trichloro-1,4-benzoquinone, and 2,6-dibromo-1,4-benzoquinone. These haloquinones are suspected bladder carcinogens and are likely produced during drinking water disinfection treatment. However, detection of these haloquinones is challenging, and consequently, they have not been characterized as DBPs until recently. We have developed an electrospray ionization tandem mass spectrometry technique based on our observation of unique ionization processes. These chloro- and bromo-quinones were ionized through a reduction step to form [M + H]− under negative electrospray ionization. Tandem mass spectra and accurate mass measurements of these compounds showed major product ions, [M + H −HX]−, [M + H −HX −CO]−, [M + H −CO]−, and/or X− (where X represents Cl or Br). The addition of 0.25% formic acid to water samples was found to effectively stabilize the haloquinones in water and to improve the ionization for analysis. These improvements were rationalized from the estimates of pKₐ values (5.8−6.3) of these haloquinones. The method of tandem mass spectrometry detection, combined with sample preservation, solid phase extraction, and liquid chromatography separation, enabled the detection of haloquinones in chlorinated water samples collected from a drinking water treatment plant. The four haloquinones were detected only in drinking water after chlorination treatment, with concentrations ranging from 0.5 to 165 ng/L, but were not detectable in the untreated water. This method will be useful for future studies of occurrence, formation pathways, toxicity, and control of these new halogenated DBPs.

Disinfection of drinking water, one of the most effective public health measures of the 20th century, has saved millions of lives from infectious diseases such as cholera and typhoid.1,2 While disinfection is essential to inactivate microbial pathogens, disinfection byproducts (DBPs) are unintentionally produced from the reactions of disinfectants with organic matter naturally present in the water.3 Epidemiological studies show associations between human exposure to drinking water DBPs and increased risk of bladder cancer.2 There has been much less consistent evidence for colorectal, pancreatic, and brain cancer, as well as for adverse reproductive outcomes.4,4 Over 600 DBPs have been identified in drinking water treated with common disinfectants, usually strong oxidants, including chlorine, chloride dioxide, chloramines, and ozone.5 Some surrogate DBPs, including trihalomethanes (THMs), haloacetic acids (HAAs), chlorite, and bromate, have been regulated and are routinely monitored as a measure of safety and quality control of drinking water.6,7 A number of toxicological studies and risk assessments over the past three decades have indicated that the currently regulated DBPs are unlikely to account for the magnitude of the epidemiological estimates of increased risk of bladder cancer from consumption of chlorinated water.2,9,10 To date, it is still unclear which DBPs may be responsible for the observed bladder cancer risk.

A study modeling the reactions of disinfectants with substructures in natural organic matter (NOM) combined with quantitative structure and toxicity relationship analyses was conducted to predict the relative toxicological importance of DBPs as plausible bladder carcinogens.11 This study has predicted five classes of compounds as potential DBPs worth searching for in disinfectated drinking water.


drinking water.\textsuperscript{11} These compounds include haloquinones, halocyclopentaenoic acids, organic N-haloamines, nitrosamines, nitrosamides, halonitrides, and haloamides. The objective of the present research is to confirm whether or not haloquinones are produced during water disinfection treatments.

Our preliminary study found the presence of 2,6-dichloro-1,4-benzoquinone (DCBQ) in drinking water after chloramination treatment.\textsuperscript{12} Others have shown that various halomethanes (chloro-bromo-, and iodo-methanes) and haloacetic acids are produced during chlorination.\textsuperscript{3} We hypothesize that chloro- and bromo-haloquinones may also be produced in chlorinated drinking water. In order to test this hypothesis, a sensitive and selective analytical method is required because we anticipate that these DBPs in drinking water could be present at ng/L levels on the basis of our previous research on nitrosamines in drinking water.\textsuperscript{13,14}

This paper describes the development of a unique mass spectrometry technique combined with the preservation of the stability of the analytes, enrichment by solid phase extraction, and liquid chromatography separation. Our target analytes are four representative haloquinones, 2,6-dichloro-1,4-benzoquinone (DCBQ), 2,6-dichloro-3-methyl-1,4-benzoquinone (DCMBQ), 2,3,6-trichloro-1,4-benzoquinone (TCBQ), and 2,6-dibromo-1,4-benzoquinone (DBBQ). The ability to detect these suspected bladder carcinogens in drinking water will contribute to continuing efforts to optimize drinking water disinfection processes and assist public health protection.

**EXPERIMENTAL SECTION**

**Reagents and Chemicals.** DCBQ and DBBQ were purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and Indofine Chemical Company (Hillsborough, NJ), respectively. DCMBQ and TCBQ were synthesized by Shanghai Acana Pharmtech (Shanghai, China). Optima grade water and HPLC grade methanol were purchased from Fisher Scientific (Fair Lawn, NJ). Analytical grade formic acid (FA) was obtained from Sigma (St. Louis, MO).

**Sample Collection.** Raw and treated water samples were collected from a water treatment plant using chlorination as disinfection treatment. In September 2009, two treated water and two source water samples (4 L/sample) were collected; one was acidified to contain 0.25% FA (v/v, pH 2.6–2.8), and the other was not acidified. Samples were collected in 4 L amber glass bottles that were precleaned with methanol and water. A field blank sample (1 L of optima water acidified with 0.25% FA) was included in sampling and shipped back to the laboratory with the samples. The samples were transported in coolers with ice packs overnight and then stored at 4 °C prior to analysis. Samples were analyzed within a day after collection.

**Solid Phase Extraction of Samples.** Samples were concentrated using solid phase extraction with Waters Oasis HLB cartridges (6 mL, 200 mg per cartridge; Milford, MA) mounted in a VISIPREP SPE manifold (Supelco, Bellefonte, PA) with flow control lines. Prior to sample loading, each HLB cartridge was prepared with one rinse of 6 mL of methanol containing 0.25% FA followed by two washes of 6 mL of acidified water with 0.25% FA. Each sample (500 mL) was forced through the cartridges at a flow rate of approximately 8 mL/min. After sample loading, the cartridges were washed with 6 mL of acidified water (0.25% FA) and 6 mL of methanol/water (v/v 50/50 with 0.25% FA) and then dried for 10 min under vacuum. The analytes on the cartridge were eluted with 6 mL of methanol (0.25% FA), and the methanol extract was evaporated down to 100 μL under a gentle nitrogen stream and then reconstituted with water (0.25% FA) to a final volume of 500 μL water/methanol (v/v 80/20, with 0.25% FA). It is important not to completely dry the extract during evaporation to avoid loss of the analytes.

**Liquid Chromatography.** An Agilent 1100 series LC system consisting of a binary pump and an autosampler (Agilent, Waldbronn, Germany) was used for LC separation with a Luna C18(2) column (100 × 2.0 mm i.d., 3 μm; Phenomenex, Torrance, CA) at room temperature (22 °C). A 10-port 2-position switching valve (Rheodyne, Rohnert Park, CA) was used between the HPLC column outlet and the inlet of the MS for directing the LC effluent to the MS detector: the LC effluent was diverted to waste for the initial 10 min, then directly sprayed to the MS during the elution of 10–25 min, and finally diverted to waste during the elution time of 25–40 min. The flow rate of the mobile phase was 150 μL/min, and the injection volume was 20 μL.

The initial mobile phase gradient for the SPE optimization consisted of solvent (A) water containing 0.25% FA and solvent (B) methanol containing 0.25% FA. The initial gradient was 0–7 min, 50% B; increased to 90% B in 0.1 min; 7.1–12 min, 90% B; decreased to 50% B in 0.1 min; 12.1–30 min, 50% B. After the SPE optimization, the gradient program for sample analysis was further optimized to begin with 20% methanol containing 0.25% FA (B); linearly increased B to 90% in 20 min, and kept for 5 min; decreased B to 20% at 25 min, and kept for 15 min.

**Mass Spectrometry.** A hybrid quadrupole time-of-flight mass spectrometer (API Q-STAR Pulsar I; ABSciex, Concord, ON, Canada) with a standard ion-spray source was used to obtain accurate mass measurements of the parent ions and product ions of the haloquinones. The conditions used in this study were negative TOF-MS mode, ion source voltage of −4500 V, declustering potential (DP1) of −55 V, focusing potential of −300 V, declustering potential (DP2) of −15 V, ion source gas (N₂) of 50 arbitrary units, curtain gas (N₂) of 25 arbitrary units, accumulation time of 1 s, and scan range (m/z) of 50–300 amu.

An API 5000 mass spectrometer (ABSciex, Concord, ON, Canada) coupled with the Agilent HPLC were used for LC/MS analysis of haloquinones. The MS instrumental parameters were optimized as follows: ionspray voltage, −4500 V; source temperature, 475 °C; gas I, 50 arbitrary units; gas II, 60 arbitrary units; curtain gas, 30 arbitrary units. The MS detection was operated with negative electrospray ionization (ESI) mode and multiple reaction monitoring (MRM). Specific MRM ion transitions for DCBQ, DCMBQ, TCBQ, and DBBQ as well as their optimized compound-dependent parameters including declustering potential (DP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP) are presented in Table 1.
Quantification of Haloquinones. The standard addition method was used to build calibration curves for quantification of the analytes. From the 500 µL reconstituted SPE extract, an aliquot of the extract (120 µL) was directly analyzed with LC-MS/MS to estimate the concentration levels of the haloquinones, then appropriate levels of the standards were added to the samples prior to SPE. Four 90 µL aliquots were used to prepare a set of solutions to establish a calibration curve by adding 10 µL of mix standards at various concentrations depending on their estimated levels. DCBQ was spiked at 10, 50, and 100 ng/L, and DCMBQ, TCBQ, and DBBQ were spiked at 1, 5, and 10 ng/L. The peak areas of the spiked samples determined with LC-MS/MS (MRM) were used to calibrate against the spiked concentrations. The four-point calibration curves offered linear coefficients of 0.993–0.999, and they were used to determine the concentrations of the haloquinones in water samples. The standard addition method was used to reduce the matrix effects on quantification.

Quality Assurance/Quality Control. Field blank samples were included in the sampling, and triplicate analyses were performed along with the samples to examine the contamination of sample collection. Method blank samples consisting of optima pure water with 0.25% FA were also analyzed in parallel to control for any contamination during analysis. For each water sample, triplicate extractions and triplicate runs of each extract were performed and the average concentration and standard error were reported. Recoveries of individual haloquinones after SPE from the spiked water samples were determined.

RESEARCH AND DISCUSSION

This research focuses on four representative haloquinones, DCBQ, DCMBQ, TCBQ, and DBBQ, as the target analytes because (1) they are predicted to be plausible bladder carcinogens; (2) they are speculated to be possible DBPs, but there is lack of experimental evidence; and (3) the standards of these compounds are available to allow for analytical characterization. The main analytical challenges include (1) previously experienced difficulties in generating reproducible and strong mass spectral signals from electrospray ionization; (2) instability of these compounds, requiring appropriate conditions for preservation; (3) trace levels of these compounds potentially present in disinfected drinking water; and (4) potential interference from the sample matrices after preconcentration of the samples. We have carefully considered each of these issues and have developed a method involving acidification of water samples, solid phase extraction (SPE), liquid chroma-
Figure 1. Accurate mass measurements of the product ions of the \([\text{M} + \text{H}]^–\) of DCBQ, DCMBQ, TCBQ, and DBBQ. The experimental data (in black) are in agreement with the theoretical values (in red).

Figure 2. MRM chromatograms of DCBQ, DCMBQ, TCBQ, and DBBQ obtained from the extracts when the SPE was performed with varying washing solvents: (A) without washing, (B) washing with 30% MeOH in 0.25% aqueous FA, and (C) washing with 50% MeOH in 0.25% aqueous FA.
Followed by rapid deprotonation to produce \([\text{M} - \text{Br}]^+\) of these compounds using quadrupole time-of-flight. Figure 1 shows their tandem MS spectra, isotope patterns of \(^{35/37}\text{Cl}\) and \(^{79/81}\text{Br}\), and accurate mass measurements of the parent and product ions. The explanation is consistent with the observation of \([\text{M} - \text{Br}]^+\) ions as the most abundant ions from negative ESI of the four haloquinones in acidic solutions (pH 2.6–2.8 with 0.25% FA).

To confirm the identity of the \([\text{M} + \text{H}]^+\) species, we obtained accurate mass measurements of the parent and product ions of these compounds using quadrupole time-of-flight. Figure 1 shows their tandem MS spectra, isotope patterns of \(^{35/37}\text{Cl}\) and \(^{79/81}\text{Br}\), and accurate mass measurements (in black) compared with the theoretical values (in red). Under the collision-induced dissociation mode, the precursor \([\text{M} + \text{H}]^+\) of the four haloquinones formed \([\text{M} + \text{H} - \text{HX}]^+\), \([\text{M} + \text{H} - \text{HX} - \text{CO}]^-\), \([\text{M} + \text{H} - \text{CO}]^-\), and/or \(\text{X}^-\) product ions. The \([\text{M} + \text{H} - \text{HCl}]^-\) and \([\text{M} + \text{H} - \text{HCl} - \text{CO}]^-\) were the most abundant for DCBQ, DCMBQ and TCBQ, whereas \([\text{M} + \text{H} - \text{HBr} - \text{CO}]^-\) and \(\text{Br}^-\) were predominant for DBBQ. The accurate mass measurements of \([\text{M} + \text{H}]^+\) and fragment ions were consistent with their theoretical values. The results support that the parent \([\text{M} + \text{H}]^+\) ions are produced in negative ESI instead of the traditional \([\text{M} - \text{H}]^-\) ions.

The estimated \(p_K_a\) and ionization characteristics also explain the instability of these compounds in water, a major problem that has hampered the investigation of these DBPs in drinking water. We have previously reported that, in the neutral pH conditions of drinking water, spiked standard DCBQ degraded to half of the original concentration after 6 to 7 h. To preserve haloquinones for analysis, we examined various conditions and found that acidification of water samples with 0.25% FA effectively prevented these analytes from degradation. The four analytes were successfully detected in water samples acidified with 0.25% FA, whereas without acidification the analytes in the same set of samples were barely detectable. Therefore, 0.25% FA was used in the preparation of solutions and for preservation of the analytes in authentic water samples.

**SPE.** To achieve ng/L level quantification of these DBPs, it was necessary to concentrate the analytes from a large volume of water samples for the LC-MS/MS analysis. We used the SPE procedure to selectively remove interference from sample matrices as well as to provide 1000-fold preconcentration of haloquinones. This was obtained through the concentration of the haloquinones from 500 mL of water samples down to 500 μL of the final extracts. We optimized the SPE method through a series of testing of tap water samples containing the four haloquinones at 30 ng/L each. We found the composition of washing solvents important for efficiently retaining the analytes on the cartridge while removing sample matrices. Figure 2 illustrates the chromatograms of the extracts when the washing step was performed with varying solvent compositions: (1) no wash; (2) 30% methanol in 0.25% aqueous FA; (3) 50% methanol in 0.25% aqueous FA. Figure 2A shows that without washing, the analytes in the extract are barely detectable due to severe matrix interference. Using 30% methanol in 0.25% aqueous FA to wash the SPE cartridge dramatically improved the separation and detection of the haloquinones in the extract (Figure 2B), but the background interference was still severe. Increasing the methanol to 50% in 0.25% aqueous FA as the washing solvent dramatically reduced the background peaks and enhanced the detection of the haloquinones (Figure 2C). Further increasing the methanol content in the washing solvent also resulted in loss of the analytes. A series of optimization experiments showed that the minimum interference and maximum recovery of the analytes were achieved with methanol/water (v/v 50/50 with 0.25% FA) as the washing solvent and methanol with 0.25% FA as the elution solvent.

![Figure 2](image-url)
We then optimized the separation of the four haloquinones using four LC columns with C18, C8, activated graphic carbon, and phenyl stationary phases. The C18 column provided better separation of these compounds than the other columns. Optimization of mobile phase compositions showed that 0.25% aqueous FA as solvent A and methanol with 0.25% FA instead of acetonitrile with 0.25% FA as solvent B resulted in lower background and better separation. After a thorough optimization, the optimal gradient began with 20% methanol with 0.25% FA (B); linearly increased B to 90% in 20 min, and kept steady for 5 min; decreased B to 20% at 25 min, and held for 15 min. Figure 3 shows the typical chromatograms of the four haloquinones obtained under the optimized gradient LC separation and MS detection conditions. All four peaks are in symmetric shape. The elution order of DCBQ, DBBQ, DCMBQ, and TCBQ is according to the strength of hydrophobic interaction of the analytes with the C18 stationary phase. As shown in Table 3, the limits of quantification (LOQs) (at 10 times of the signal-to-noise ratios, S/N) of the LC-MS/MS (MRM) method for DCBQ, DCMBQ, TCBQ, and DBBQ were 1.0, 1.9, 1.7, and 0.5 ng/mL, respectively.

Combination of the SPE with the LC-MS/MS method enabled the determination of the four haloquinones at ng/L levels. We validated the SPE-LC-MS/MS method through the analysis of a series of treated water samples spiked with the standards at 1, 5, 50, and 100 ng/L. Table 3 presents the recovery, the LOQ, and precision of the SPE-LC-MS/MS method. With the presence of sample matrices, the limits of quantification of the SPE-LC-MS/MS method (at S/N of 10) were as low as 1–6 ng/L, and the recoveries of DCBQ, DCMBQ, TCBQ, and DBBQ from the chlorinated water samples were 84 ± 1%, 69 ± 3%, 59 ± 9%, and 78 ± 3%, respectively. The precision (relative standard deviation) of the method was 1–15%.

**Confirmation and Determination of Haloquinones as New Chlorination DBPs.** Having established optimum conditions for sample preservation, SPE concentration, and LC-MS/MS (MRM) determination, chlorinated drinking water samples collected from a chlorination water treatment plant were analyzed. The four haloquinones in the treated water were identified on the basis of matching (1) retention time, (2) MRM transitions, and (3) isotopic (35,37Cl or 79,81Br) patterns of transition ions with those of the standards. Figure 4 shows that several ion transitions for MRM monitoring of the four chloro- and bromoquinones detected in the samples are in agreement with those of the standards, supporting their identification in the samples. The most intense MRM of DCBQ (m/z 177 > 113), DCMBQ (m/z 191 > 127), TCBQ (m/z 247 > 171), and DBBQ (m/z 261 > 193) transitions were used for quantification.

**Figure 4.** Relative intensities of the MRM ion transitions (m/z) of DCBQ, DCMBQ, TCBQ, and DBBQ detected in a treated water sample (peaks) compared with those of the standards (solid lines).

LC-MS/MS. We then optimized the separation of the four haloquinones using four LC columns with C18, C8, activated graphic carbon, and phenyl stationary phases. The C18 column provided better separation of these compounds than the other columns. Optimization of mobile phase compositions showed that 0.25% aqueous FA as solvent A and methanol with 0.25% FA instead of acetonitrile with 0.25% FA as solvent B resulted in lower background and better separation. After a thorough optimization, the optimal gradient began with 20% methanol with 0.25% FA (B); linearly increased B to 90% in 20 min, and kept steady for 5 min; decreased B to 20% at 25 min, and held for 15 min. Figure 3 shows the typical chromatograms of the four haloquinones obtained under the optimized gradient LC separation and MS detection conditions. All four peaks are in symmetric shape. The elution order of DCBQ, DBBQ, DCMBQ, and TCBQ is according to the strength of hydrophobic interaction of the analytes with the C18 stationary phase. As shown in Table 3, the limits of quantification (LOQs) (at 10 times of the signal-to-noise ratios, S/N) of the LC-MS/MS (MRM) method for DCBQ, DCMBQ, TCBQ, and DBBQ were 1.0, 1.9, 1.7, and 0.5 ng/mL, respectively.

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**Figure 5.** MRM chromatograms of the DCBQ, DCMBQ, TCBQ, and DBBQ obtained from the extracts of treated water compared with those of source water and the field blank sample. The peak areas were used for quantification.

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(m/z 211 > 175), and DBBQ (m/z 267 > 79) were used to quantify their concentrations in treated water samples, as shown in Figure 5. The concentrations of DCBQ, DCMBQ, TCBQ, and DBBQ in the chlorinated water samples were 165.1 ± 9.1, 1.3 ± 0.2, 9.1 ± 0.6, and 0.5 ± 0.1 ng/L, respectively. No haloquinones were detected in the source water and field blank samples, as shown in Figure 5. DCBQ and TCBQ in the treated water samples were detected above the LOQs, whereas DCMBQ and DBBQ levels were lower than the LOQs but clearly detectable. To confirm the identification of DCMBQ and DBBQ in treated water, we compared isotopic patterns in the MRM transitions m/z 191 > 127 and m/z 193 > 127/129 obtained from the sample with those of the standard DCMBQ. Similarly, we compared transition ions m/z 267 > 79/81 plus m/z 265 > 79 and m/z 269 > 81 detected in the sample with those of the standard DBBQ (Figure 4). These isotopic patterns detected in the samples matched those of the standards. The detection of DCBQ, DCMBQ, TCBQ, and DBBQ in the chlorinated water but not in the source water supports that they are produced in drinking water during the chlorination disinfection process.

**CONCLUSIONS**

An SPE-LC-MS/MS method was developed for the characterization and determination of chloro- and bromo-benzoquinones in drinking water. Using this method, we were able to determine DCBQ, DCMBQ, TCBQ, and DBBQ in chlorinated drinking water, which has never been achieved before. The observation of 165.1–0.5 ng/L of the four chloro- and bromo-benzoquinones in chlorinated drinking water demonstrates that haloquinones are likely present as a class of DBPs in some drinking water systems. Previous work has shown that several chloro- and bromo-methanes were produced during chlorination. The capability of our SPE-LC-MS/MS method extends studies of drinking water disinfection byproducts to include haloquinones, a group of DBPs more toxic than the regulated halomethanes. The potential toxic effects of these compounds warrant further investigations into the occurrence, human exposure, and management of haloquinones in drinking water.

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