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Aspartame-Sweetened Tap Water: Transformation Products and 2,6-Dichloro-1,4-Benzoquinone Formation

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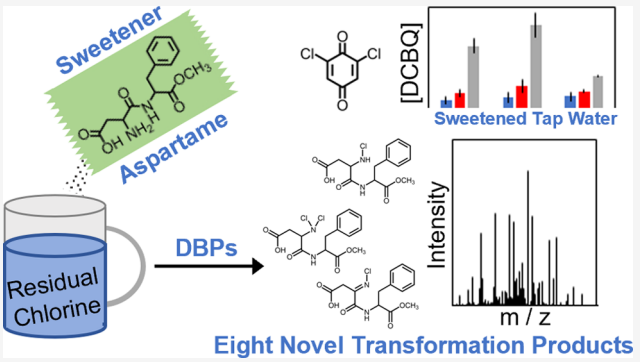
ABSTRACT: Aspartame (APM), a dipeptide of aspartic acid (ASP) and phenylalanine (PHE), is a widely used artificial sweetener in beverages. It is unclear whether residual chlorine in tap water can react with APM to form disinfection byproducts (DBPs). Therefore, we investigated the formation of DBPs from the reaction of APM with residual chlorine in authentic tap water. APM and a commercial sweetener (CS) packet containing APM were studied under authentic and simulated tap water conditions. Eight chlorinated products of APM were detected using solid-phase extraction (SPE) and high performance liquid chromatography quadrupole time-of-flight mass spectrometry (HPLC-QTOF-MS). These new chloro-products were tentatively identified based on accurate masses, isotopic patterns of $^{35,37}Cl$, and MS/MS spectra. Furthermore, we identified APM as a precursor to 2,6-dichloro-1,4-benzoquinone (DCBQ). DCBQ significantly increased to 2.3−12 ng/L with the addition of APM or CS in tap waters collected from different locations compared to 1.4−1.8 ng/L in the same tap water samples without sweetener. DCBQ and two of the chlorinated transformation products were identified in cold prepared tea containing APM. DCBQ formation was eliminated when the residual chlorine in tap water was reduced by ascorbic acid or boiling prior to the addition of APM or CS. This study found that eight new DBPs and DCBQ were produced by the reactions of residual chlorine with APM and CS. These findings show an unintended exposure source of emerging DBPs via APM sweetened beverages.



KEYWORDS: *aspartame, chlorination, chloramination, residual chlorine, disinfection byproducts (DBPs), halobenzoquinones (HBQs)*

1. INTRODUCTION

Routine disinfection of drinking water is essential to inactivate pathogenic microorganisms and prevents the transmission of waterborne disease. However, disinfection byproducts (DBPs) are unavoidably formed during the water treatment process through reactions between organic matter and disinfectants. Epidemiological evidence has shown a consistent association of chronic exposure to disinfected water with adverse human health effects.[1] However, exposure assessments typically focus on the presence of DBPs at the drinking water treatment plant (DWTP). Production or transformation of DBPs after the DWTP may alter the actual exposure of the population. Changes in water quality, including the formation and transformation of DBPs, can occur in the drinking water distribution system (DWDS).[2] A disinfectant residual, commonly free chlorine or monochloramine, is maintained within the DWDS to prevent microbial contamination of the treated water. This residual disinfectant can react with organic matter present in the DWDS, such as biofilms or debris, to produce DBPs.[3] Spatial variability of nitrosamines and halobenzoquinones (HBQs) concentration within the DWDS provides evidence that DBPs can be further transformed between the DWTP and consumers' taps.[4,5] Beyond the DWDS, residual disinfectants can react with organic matter in food and beverages, resulting in the formation of DBPs. Studies have identified emerging iodo-DBPs in simulated tap water containing iodized salt after boiling,[6] the formation of DBPs in brewed tea and coffee,[7,8] and during food processing.[9] To date, the formation of DBPs in beverages from the use of artificial sweeteners prepared with tap water has not been investigated.

Sweetened beverages are the largest source of beverage calories.[10] Consumer demand and regulatory pressure to reduce sugar consumption have led to an increased use of artificial sweeteners (AS). Aspartame (APM) is an intense AS that is approximately 200 times sweeter than sucrose and is found in a wide variety of products.[11] This includes general

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tabletop sweeteners (e.g., Equal and NutraSweet) and processed foodstuffs. Although APM use has declined relative to other artificial sweeteners, it remains popular; over 50% of American households purchased a product containing APM in 2018.[12] APM is a methyl ester of the dipeptide of aspartic acid (ASP) and phenylalanine (PHE). APM stability and hydrolysis products have been well studied.[13−15] When dissolved, APM is most stable between pH 3.4 and 5, and its major degradation product at typical tap water conditions (i.e., pH 6.5−8.5) is 5-benzyl-3,6-dioxo-2-piperazineacetic acid (DKP). APM can also break down into its corresponding amino acids (ASP, PHE, and PHE-methyl ester (PHE-ME)), especially at low pH values, and aspartyl-phenylalanine (ASP-PHE).

Amino acids and peptides can form various DBPs during water chlorination and chloramination.[16−18] Several studies have shown that the free amine group of amino acids and the N-terminal amino acid in peptides is rapidly chlorinated, which can be further oxidized to form smaller nitrogenous DBPs (N-DBPs).[19,20] The N-terminal amino acid of APM is ASP; other peptides with an N-terminus aspartyl residue have been shown to form dichloroacetonitrile (DCAN), *N*-chloro-2,2-dichloroacetamide (N−Cl-DCA), and dichloroacetic acid (DCAA).[21] APM also contains a phenylalanine residue; aromatic amino acids and peptides can form halobenzoquinones (HBQs) under chlorination and chloramination.[22,23] HBQs are a class of unregulated DBPs detected frequently in drinking water and are up to 1000× more cytotoxic than regulated DBPs.[24] 2,6-Dichloro-1,4-benzoquinone (DCBQ) typically has the highest occurrence and abundance of all HBQs in analyzed drinking water samples.[5,25]

Considering the common practice of preparing cold beverages in tap water, it remains unknown whether APM reacts with residual disinfectants (i.e., monochloramine or free chlorine) to form DBPs. In this study, we investigated APM reactions with residual chlorine in authentic tap water and under laboratory-controlled conditions. First, high resolution mass spectrometry (HRMS) was used to identify unknown transformation products of APM and a CS. Second, we examined DCBQ formation from APM and CS under simulated tap water conditions containing varying sodium hypochlorite and monochloramine (2−4 mg/L) doses at different pH (6.5−8.5), and after the addition of APM or CS in authentic tap water samples. Additionally, we analyzed samples of cold-brewed tea prepared in authentic tap water with APM and CS. Finally, we tested two methods of reducing residual chlorine in authentic tap water samples: the addition of ascorbic acid (AA) or boiling, to show how DCBQ exposure can be reduced. These results also provide further evidence of DCBQ formation due to reactions with residual chlorine. This study highlights the unintended exposure to DBPs via sweetening beverages with APM in disinfected tap water containing residual chlorine.

2. MATERIALS AND METHODS

2.1. Chemicals and Materials. Mass spectrometry grade formic acid (FA, 98%), sodium hypochlorite solution (NaOCl, reagent grade, 10−15% available chlorine), AA, DCBQ, and polyvinylidene difluoride (PVDF) syringe filters (0.45 μm) were obtained from Sigma-Aldrich (St. Louis, MO). Optima grade methanol, Optima grade water (ultra pure water), ammonium chloride, APM, anhydrous dibasic potassium phosphate, anhydrous monobasic potassium phosphate, and sodium bicarbonate were obtained from Thermo Fisher Scientific (Fair Lawn, NJ). Oasis HLB cartridges (6 mL, 200 mg) were obtained from Waters (Milford, MA). A commercial tabletop sweetener, containing APM, and green tea bags were purchased from a local grocery store (Text S1). Monochloramine (NH_2Cl) was freshly prepared according to a previously described method.[26] To briefly summarize the method, NaOCl was added dropwise to a NH_4Cl solution at pH 8.5 (0.7 Cl/N molar ratio) within a fume hood due to the exothermal reaction. Residual chlorine in authentic samples and the exact concentration of free chlorine in the NaOCl solution were determined with a chlorine amperometric titrator (Autocat 9000, HACH, London, ON). A pH meter (model 15, Accumet, Fisher Scientific, Nepean, ON) was used to monitor pH in all samples and reaction mixtures.

2.2. Preparation of Authentic Tap Water Samples. Authentic tap water samples (250 mL) were collected from taps or a municipal DWTP. The DWTP treats surface water sequentially through coagulation, flocculation, filtration, UV disinfection, and chlorination, followed by the addition of ammonium to form monochloramine before treated water enters the DWDS. A chlorine amperometric titrator, using the total chlorine—forward titration, measured the residual chlorine concentration of samples.

First, the water sample collected from the DWTP was prepared for nontargeted analysis of new products formed from APM. The DWTP water contained a chlorine residual of 2.1 ± 0.1 mg/L (as Cl_2). Duplicate sets of three different samples were prepared: Tap Water (TW), TW containing 15.8 mg of APM, and TW containing a 1 g packet of CS containing APM (15.8 mg) (Text S1). These samples were allowed to react for 15 min. Following which, the duplicate sets of each sample were split to test two different quenching conditions. One set of samples was quenched with only FA (0.25% v/v, final). The other set of samples and their corresponding controls were quenched with FA (0.25% v/v, final) and AA at a molar ratio of 1.2:1 AA to total chlorine residual. AA was added to determine if chlorine substitution occurred on the free amine group based on our previous study.[27] The quenched reaction solutions were SPE extracted and analyzed using HPLC-QTOF-MS nontargeted analysis. Experimental controls, consisting of reactants (APM and CS) dissolved in ultrapure water, were analyzed using the same procedure.

Second, for the analysis of DCBQ, tap water samples were collected from three locations (A, B, C) within the distribution system. Total chlorine (as Cl_2) was measured in the three tap water samples: (A) 1.59 ± 0.04 mg/L, (B) 2.11 ± 0.07 mg/L, and (C) 1.64 ± 0.03 mg/L. To mimic realistic household beverage preparation, each precursor (n = 3, APM or CS: 15.8 mg APM) was dissolved into 250 mL of authentic tap water (A, B or C) at 24 °C. Additionally, samples A, B, and C (n = 3) without the addition of precursors were analyzed. After 15 min reaction time in the dark at 24 °C, all samples were quenched and acidified with AA (1.2 molar ratio of AA to total chlorine residual) and FA (0.25% v/v, final). Experimental controls, consisting of reactants (APM and CS) dissolved in ultrapure water, were analyzed using the same procedure. The quenched reaction solutions and controls were SPE extracted and analyzed for HBQs using the HPLC-MS/MS targeted analysis method. Statistical differences between samples were determined using a paired t-test.

Two methods were used to reduce residual chlorine in freshly collected tap water prior to the addition of sweeteners (APM or CS, n = 3) to examine the effectiveness of reducing,

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or eliminating, DCBQ formation. The first method used AA to quench the residual chlorine in the tap water samples (250 mL; μM AA = 1.2 × measured μM total chlorine, as Cl_2). The second method used an electric kettle to boil (>90 °C) the tap water samples (250 mL) to reduce the residual chlorine concentration and then cool to room temperature (24 °C). The pH and residual chlorine concentration were determined in the authentic tap water before and after boiling. All samples were allowed to react for 15 min. Samples were SPE extracted and analyzed using the HPLC-MS/MS targeted analysis method.

Lastly, the effect of tea on the formation of transformation products of APM and DCBQ was investigated. Commonly used green tea bags were bought from a grocery store. The DWTP water contained a chlorine residual of 1.87 ± 0.04 mg/L (as Cl_2). Triplicate tea samples were prepared by steeping a green tea bag in 250 mL room temperature tap water for 3 min until they were removed. APM (15.8 mg) or CS containing APM 15.8 mg (n = 3) were added to the tea solutions (250 mL), and the tea solutions without APM or CS were used as the controls. The samples were allowed to react for 15 min and then quenched and acidified with FA (0.25% v/v, final). Additional experimental controls, consisting of reactants (tea with and without APM or CS) dissolved in ultrapure water, were analyzed using the same procedure. The quenched reaction solutions were SPE extracted and analyzed using the HPLC-QTOF-MS nontargeted analysis and the targeted HPLC-MS/MS method for HBQs.

2.3. Preparation of Laboratory-Controlled Reactions. First, we performed chlorination of APM in laboratory experiments which mimicked tap water conditions for nontargeted analysis. Reaction solutions consisting of 15.8 mg (214.7 μM) of APM or 1 package of CS were prepared in 10 mM phosphate buffer (pH 7.5, 250 mL). Then, 2.0 mg/L (as Cl_2) of NaOCl was added. Experimental controls, consisting of each reactant (APM, CS, and NaOCl) prepared separately in 10 mM phosphate buffer (pH 7.5, 250 mL), were analyzed using the same procedures. After 15 min, one set of samples and controls were quenched with only FA (0.25% v/v, final). The other set of samples and controls were quenched with AA at a molar ratio of 1.2:1 AA to chlorine (as Cl_2) and FA (0.25% v/v, final). Ascorbic acid was used to differentiate the sites of the chlorine substitution based on a previous study.[27] The quenched reaction solutions and controls were SPE extracted and analyzed using the HPLC-QTOF-MS nontargeted analysis method.

Second, we studied the effect of pH under conditions mimicking the maximum residual chlorine in tap water. Reaction samples (250 mL) in phosphate buffer at pH 6.5, 7.5, and 8.5 were used to encompass the U.S. EPA Secondary Drinking Water Standards guideline range of 6.5−8.5 for tap water.[30] The concentration of NaOCl or NH_2Cl was 80 μM based on the maximum levels of residual chlorine in tap water in North America. The molar ratio of NaOCl or NH_2Cl to the precursor (APM or CS, 10 μM) was 8:1, which is similar to the optimal molar ratio for the formation of DCBQ from chlorination or chloramination of phenol (10:1, chlorine:phenol).[25] After 24 h in the dark at 24 °C, samples were quenched with AA (100 μM) at a molar ratio of 1.2:1 AA to NaOCl or NH_2Cl (as Cl_2) to ensure that any remaining reactive chlorine was completely removed from the reaction solution. FA was then added (0.25% v/v, final) to stabilize DCBQ prior to sample preparation and analysis. Samples were SPE extracted and analyzed using the HPLC-MS/MS targeted analysis method.

Lastly, the effect of residual chlorine concentration was studied under simulated tap water conditions. These reactions contained a realistic dose of the precursor: 15.8 mg of APM or a 1 g packet of CS containing APM (15.8 mg) in 10 mM phosphate buffer (pH 7.5, 250 mL). Varying concentrations (1, 2, or 4 mg/L) of NaOCl or NH_2Cl (as Cl_2) were added. Reactions were quenched with excess AA at a molar ratio of 1.2:1 AA to NaOCl or NH_2Cl (as Cl_2) after 15 min in the dark at 24 °C. Finally, FA was added (0.25% v/v, final). Experimental controls, consisting of each reactant (APM, CS, NaOCl, and NH_2Cl) prepared separately, were analyzed using the same procedures. The quenched reaction solutions and controls were SPE extracted and analyzed using the HPLC-MS/MS targeted analysis method.

2.4. Solid Phase Extraction. Samples (250 mL) were extracted and concentrated using the solid-phase extraction (SPE) method previously reported.[28] A HLB cartridge mounted in a VISIPREP SPE manifold (Supelco, Bellefonte, PA) was activated with 12 mL of methanol (0.25% FA, v/v), then rinsed with 12 mL portions of ultrapure water (0.25% FA, v/v). The quenched reaction solution was drawn through the cartridge under vacuum at a flow rate of ∼2 mL/min. Next, the cartridge was washed with 12 mL portions of ultrapure water (0.25% FA, v/v), and the analytes were finally eluted with 10 mL of methanol (0.25% FA, v/v). The eluate was evaporated down to 100 μL under a gentle (<5 psi) nitrogen stream (TurboVap LV Concentration Workstation, Caliper Life Sciences, Waltham, MA). Finally, the sample was reconstituted with ultrapure water (0.25% FA, v/v) to a final volume of 500 μL resulting in a water/methanol solution (v/v, 4/1). Finally, the reconstituted samples were filtered with PVDF syringe filters (0.45 μm).

2.5. Nontargeted HPLC-QTOF-MS Method. A quadrupole time-of-flight mass spectrometer (QTOF; Sciex x500R) was coupled to an Agilent 1290 series high-performance liquid chromatography (HPLC) system for nontargeted analysis of APM transformation products. The Supporting Information (Text S2) provides details for the HPLC-QTOF-MS analysis with information dependent acquisition (IDA) for nontargeted analysis, including instrument parameters, system control, data collection, and data analysis. SciexOS was used for the analysis of the nontargeted MS data.

2.6. Targeted HPLC-MS/MS Methods. A triple quadrupole ion trap tandem mass spectrometer (MS/MS; Sciex QTRAP 5500) was coupled to an Agilent 1290 series HPLC to determine APM hydrolysis products. To understand the differences between solutions containing APM and CS, we analyzed the hydrolysis products of APM when APM and CS were separately dissolved in ultrapure water and authentic tap water at 5, 15, and 25 min. The details of the HPLC-MS/MS method for hydrolysis products, including instrument parameters, are described in Text S3 and Table S1.

DCBQ was determined in prepared samples using a triple quadrupole ion trap tandem mass spectrometer (MS/MS; Sciex QTRAP 5500) coupled to an Agilent 1290 series HPLC. The HPLC-MS/MS with the multiple reaction monitoring (MRM) mode was adapted from previously reported studies.[28] The method can detect four HBQs, however, DCBQ was the only product detected in all samples. The Supporting Information (Text S4 and Table S2) provides the details of the HPLC−MS/MS methods for the analysis of DCBQ,

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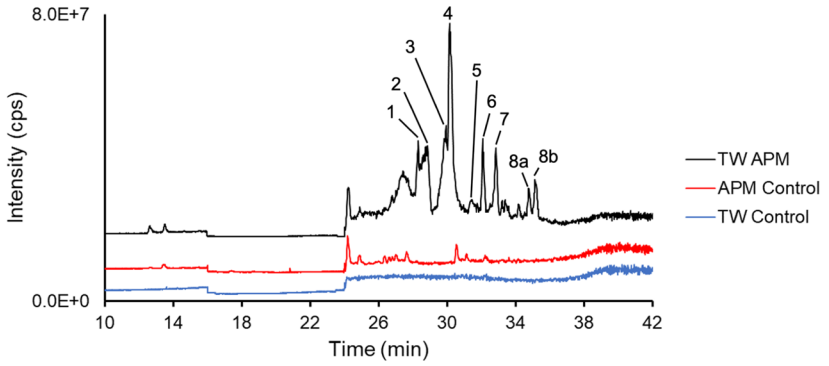

Figure 1. Total Ion Chromatogram (TIC) of extracted tap water containing 15.8 mg of aspartame (TW APM, black trace), APM (15.8 mg) in ultrapure water (red trace), and TW (blue trace) without AA treatment. The tap water contained residual chlorine (2.1 ± 0.1 mg/L). From 16−24 min, the LC flow was diverted to waste to avoid APM contamination of the MS due to the extremely high concentration of APM in the samples.

including instrument parameters, system control, data collection, and MRM ion-pair transitions. DCBQ was quantified using standard addition, as described in Text S5.

2.7. Quality Control and Quality Assurance. The QTOF-MS was tuned before running each sample batch and every three samples using standard calibration solutions to ensure the accuracy (2 ppm). All reaction solutions (simulated and authentic tap water) were analyzed in triplicate for quantification of DCBQ. Each sample was quantified using standard addition for the determination of DCBQ concentration (mean ± SD) (Text S5). For HPLC−MS/MS (MRM) analysis, confirmation of the identity of DCBQ was based on the MRM ion pair ratio and matching retention times using an authentic standard. We also investigated the possible interference of APM with DCBQ determination (Text S6 and Table S3). The recovery of DCBQ in ultrapure water, in the presence of CS, and in the presence of APM was 89, 73, and 61%, respectively (Text S7). Experiments confirmed that AA or AA/FA did not affect the DCBQ signal (Text S8 and Figure S1).

3. RESULTS AND DISCUSSION

3.1. Nontargeted Analysis of APM Transformation Products in Authentic Tap Water Samples. APM has many reactive sites for chlorine substitution; however, the free amine of the aspartyl residue of APM is the most reactive, leading to formation of *N*-chloro (*N*−Cl) products. A natural bond orbital (NBO) analysis of APM was calculated to illustrate that many carbon atoms may act as substitution positions leading to *C*-chloro (*C*−Cl) products in addition to $-NH_2$ group (Text S9 and Figure S2). Samples were analyzed using nontargeted high-resolution HPLC-QTOF-MS with IDA. The characteristic isotopic patterns of $^{35,37}Cl$ in the MS spectra of the parent ions and fragment ions having intensities higher than 10% in the MS/MS spectrum, were used for structural analysis. Figure 1 shows the eight chlorinated products detected when APM (15.8 mg) was dissolved in authentic tap water (residual Cl: 2.1 ± 0.1 mg/L). These new products were not found in the available MS databases, and their standards are not commercially available. Therefore, we manually interpreted the MS and MS/MS spectra of these new products and compared them to theoretical spectra, details are available in Supporting Information. The eight chlorinated products of APM were consistently found in authentic tap water and laboratory samples containing either APM or CS. Table 1 summarizes the accurate mass (*m*/*z* values), retention time, predicted molecular formula with corresponding mass error, putative structure, and level of confidence according to the Schymanski scale for the eight chlorinated products.[29] In addition to these chlorinated transformation products, a hydrolysis product of APM, PHE-ME, was also detected in both the APM control and the TW APM reaction solution. Identification of PHE-ME was confirmed with a standard (Figure S3). Based on these products and previous studies of the chlorination of peptides containing ASP and PHE, we proposed potential chlorination pathways for APM (Figure S4).[21,30−35]

Unknowns 1 and 5 are discussed first because of their MS spectral similarity. Figure 2 shows the tentative identification of Unknowns 1 and 5, including the extracted ion chromatogram (XIC), mass spectrum (MS), and tandem mass spectrum (MS/MS). The detected accurate mass of *m*/*z* 329.0906 of Unknown 1 matched that of monochlorinated APM (Cl-APM, $[C_{14}H_{17}ClN_2O_5 + H]^+$, *m*/*z* 329.0899) with a mass error of 2.4 ppm. Figure 2A shows the XIC of *m*/*z* 329.0906 in the TW APM solution, as well as the MS and MS/MS spectra. The isotopic pattern of the parent ion at *m*/*z* 329.0906 matched the theoretical MS of $[C_{14}H_{17}ClN_2O_5 + H]^+$ (shown in red). All fragments in the MS/MS spectrum of *m*/*z* 329.0906 matched those (Table S4) of the putative structure, Cl-APM, depicted in Figure 2A. The major fragment ions of Unknown 1 can be attributed to the fragmentation of the peptide bond ($C_4H_5NO_3$), followed by the loss of the methyl ester group ($C_2H_4O_2$) or by the direct loss of the methyl ester group ($C_2H_4O_2$). The major fragmentation pattern of Unknown 1 is consistent with fragmentation of APM, supported by the MS/MS spectrum of APM (Figure S5). These results support the tentative identification of Unknown 1 as *N*−Cl-APM. Additional evidence for the Cl-substitution on the free amino group was provided by analyzing tap water samples containing APM with and without AA treatment. We used the specific reactivity of AA toward chlorine substitution on the free amine group to support the identification of Unknown 1.[27] The peak at *m*/*z* 329.0906 was no longer detectable after AA treatment, providing additional evidence for the Cl-substitution on the free amine group (Figure S6).

Using the same approach for Unknown 5 as Unknown 1, we tentatively identified Unknown 5 as *N*,*N*−Cl_2-APM. Figure 2B shows the XIC, MS, and MS/MS of Unknown 5 (*m*/*z* 363.0519). The accurate mass and $^{35,37}Cl$ isotopic pattern at *m*/*z* 363.0519 matched those of dichlorinated APM (Cl_2-APM, $[C_{14}H_{16}Cl_2N_2O_5 + H]^+$, *m*/*z* 363.0505) with a mass error of

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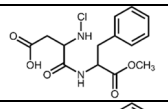
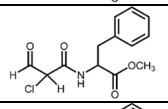
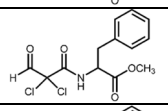
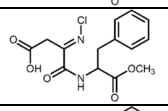
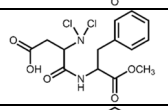
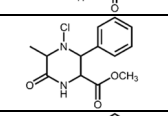
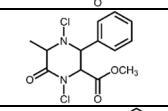
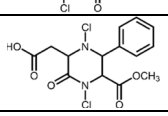
Table 1. New Chlorinated Transformation Products Detected by Nontargeted Analysis of APM Dissolved in Authentic Tap Water.

#	Exact Mass	R.T. (min)	Predicted Molecular Formula	Mass Error (ppm)	Putative Structure	Level of Confidence[31]
1	329.0906	28.30	$[C_{14}H_{17}ClN_2O_5 + H]^+$	2.4		Level 3
2	284.0690	28.88	$[C_{13}H_{14}ClNO_4 + H]^+$	4.6		Level 3
3	318.0302	29.94	$[C_{13}H_{13}Cl_2NO_4 + H]^+$	3.8		Level 3
4	327.0751	30.19	$[C_{14}H_{15}ClN_2O_5 + H]^+$	2.4		Level 3
5	363.0519	31.34	$[C_{14}H_{16}Cl_2N_2O_5 + H]^+$	3.8		Level 3
6	283.0846	32.10	$[C_{13}H_{15}ClN_2O_3 + H]^+$	0.5		Level 3
7	317.0459	32.86	$[C_{13}H_{14}Cl_2N_2O_3 + H]^+$	2.3		Level 3
8a, 8b	361.0362	34.78 and 35.18	$[C_{14}H_{14}Cl_2N_2O_5 + H]^+$	2.5		Level 3

3.8 ppm. The MS/MS fragmentation pattern at m/z 363.0519 was similar to those of Unknown 1 (Cl-APM), and all fragments matched those (Table S5) of the putative structure, Cl_2-APM, depicted in Figure 2B. Additionally, the XIC peak at m/z 363.0519 is no longer detectable after AA treatment, suggesting that Cl-substitution occurred at the free amine leading to *N,N*−Cl_2-APM (Figure S6). The formation of Unknown 1 and Unknown 5 are consistent with previous studies of chlorination of dipeptides that produced products containing Cl substitutions on free amine groups.[27]

The accurate mass and ^{35,37}Cl isotopic patterns of m/z 284.0690, Unknown 2, in Figure S7A provided evidence for a monochlorinated compound with the molecular formula $[C_{13}H_{14}ClNO_4 + H]^+$ (m/z 284.0677, mass error 4.6 ppm). A major fragment in the MS/MS spectrum (Figure S7a) is m/z 120.0812 that was found in the MS/MS spectrum of the APM standard (Figure S5). The fragmentation losses of $C_2H_4O_2$, CO, and C_2HClO suggest that Unknown 2 may be chloroaldehyde or chloroketone, as shown in Figure S7a. The fragment ions at m/z 284.0690, Unknown 2, matched those (Table S6) of the putative structure, $[C_{13}H_{14}ClNO_4 + H]^+$, with a mass error of less than 5 ppm.

The MS/MS spectrum of Unknown 3 (Figure S7b) is similar to Unknown 2. Additionally, the accurate mass and ^{35,37}Cl isotopic patterns of Unknown 3 (m/z 318.0302) in Figure S7b provided evidence for a dichlorinated compound with the molecular formula $[C_{13}H_{13}Cl_2NO_4 + H]^+$ (m/z 318.0290, mass error 3.8 ppm). Therefore, Unknown 3 is likely a dichlorinated form of Unknown 2. The MS/MS spectra of Unknown 3 matched with those (Table S7) of the putative structure $[C_{13}H_{13}Cl_2NO_4]$, as shown in Figure S7B.

Figure S8 shows the XIC of Unknown 4 in the reaction solution at 30.0 min, MS, and the MS/MS spectrum. The accurate mass and ^{35,37}Cl isotopic patterns of Unknown 4 at m/z 327.0751 matched $[C_{14}H_{15}ClN_2O_5 + H]^+$ (m/z 327.0743) with a mass error of 2.4 ppm. The MS/MS spectrum of Unknown 4 had some similarity to the fragmentation patterns of APM (Figure S5). The fragment ions of Unknown 4 (m/z 327.0751) matched those of the putative structure $[C_{14}H_{15}ClN_2O_5 + H]^+$, an *N*-chloroaldimine (Table S8). Formation of an *N*-chloroaldimine may result from the dehydrohalogenation of *N,N*−Cl_2-APM, consistent with a previous study showing chlorination of peptides containing an N-terminal aspartyl residue.[21] The small peak at 29.5 min in Figure S8 had identical MS and similar MS/MS spectra to those of the peak at 30.0 min, suggesting the presence of an isomer. This isomer could be explained by the putative structure forming a piperazine-containing structure through intramolecular cyclization.

Figure S9 shows the XIC of both Unknown 6 and 7, along with their MS and MS/MS spectrum. Unknown 6 (Figure S9a) at m/z 283.0846 corresponds to the mono-chlorinated product $[C_{13}H_{15}ClN_2O_3 + H]^+$. The accurate mass and ^{35,37}Cl isotopic patterns at m/z 317.0459 for Unknown 7 (Figure S9b) support a dichlorinated compound with the molecular formula $[C_{13}H_{14}Cl_2N_2O_3 + H]^+$ (m/z 317.0452, mass error 2.3 ppm). The MS/MS spectrum of Unknown 6 was similar to that of Unknown 7, supporting the idea that Unknowns 6 and 7 share a similar structure. Both MS/MS spectra show

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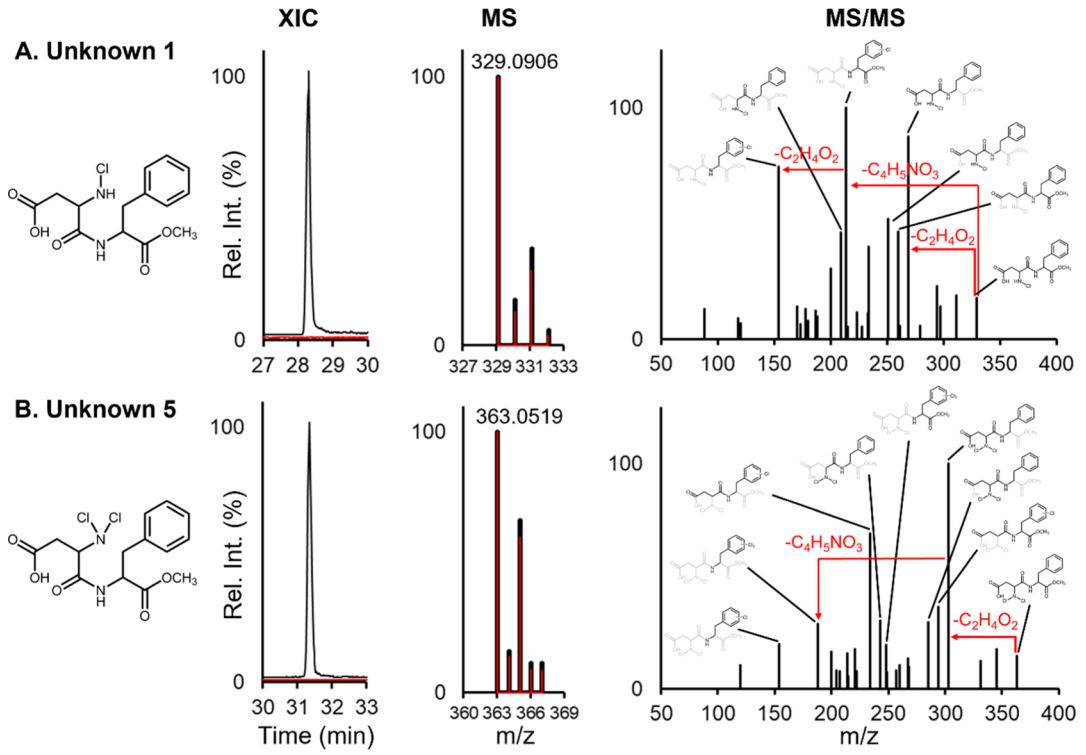

Figure 2. HRMS detection of Unknown 1 (m/z 329.0906) and Unknown 5 (m/z 363.0519), showing XIC, MS, and MS/MS. The XIC shows the authentic sample (black trace) and APM control (red trace). The MS spectrum shows the authentic sample (black trace) as well as the theoretical isotopic distribution of the putatively identified compound (red trace). The fragmentation pathways of the molecular ion are shown in the MS/MS and its correlating matching fragments of the putatively identified structure.

fragments corresponding to $C_7H_7^+$ and losses of CO, which were not found in the MS/MS spectra of APM or chlorinated forms of APM (Unknowns 1 and 5). Unknown 4 and Unknown 6 share a common major fragment (m/z 223.0638), suggesting a similar structure. All fragments at m/z 283.0846 (Table S9) and m/z 317.0459 (Table S10) matched the theoretical fragments of the putative structure, $[C_{13}H_{15}ClN_2O_3]$ and $[C_{13}H_{14}Cl_2N_2O_3]$, as shown in Figure S9, respectively.

The characteristic fragment ions of Unknown 8 were observed to be similar to those of Unknowns 6 and 7 (Figure S10). The XIC of Unknown 8 with a m/z 361.0362 showed two peaks, Unknowns 8a and 8b, that have the identical isotopic patterns in their MS spectrum and identical MS/MS spectra. The parent ion m/z 361.0362 matched the molecular formula of $[C_{14}H_{17}ClN_2O_5 + H]^+$ with a mass error of 2.5 ppm (Figure S10). Unknown 8 shares a major fragment ion (m/z 257.0246) with Unknown 7 as well as similar fragmentation pathways to Unknown 4, 6, and 7. Like Unknowns 4, 6, and 7, a piperazine containing structure is likely formed through intermolecular cyclization. Unknown 8a and 8b, matched those (Table S11) of the putative structure, as shown in Figure S10. Like Unknown 5, the presence of an isomer could be explained by the noncyclic structure, as shown in Figure S10.

These new chlorinated transformation products were also detected when the CS was dissolved in tap water, consistent with APM dissolved in tap water (Figure S11). Peak intensities of the putatively identified transformation products are similar after the addition of either APM or CS and were not found in authentic tap water without addition of either precursor. These eight transformation products of APM were also detected when APM was dissolved in ultrapure water containing 2 mg/L of NaOCl (Figure S12). The accurate mass, MS, and MS/MS spectra of the 8 transformation products in simulated tap water were identical to those produced in authentic tap water. These results support the formation of eight major transformation products of APM produced by residual chlorine in tap water. Identification of these Unknowns in APM sweetened tap water demonstrates that DBPs could form from the reactions of residual chlorine in tap water with APM, suggesting potentially unintended exposure to DBPs.

3.2. Formation of DCBQ from APM. APM and its major products contain aromatic structures, which led us to investigate whether DCBQ can be formed from chlorination and chloramination of APM. Table S12 presents the concentration of DCBQ detected after 24 h of laboratory chlorination or chloramination of APM and CS (each containing 10 μM APM) at typical drinking water pH 6.5–8.5. Chlorination of APM and CS at pH 6.5 produced DCBQ at 13 ± 2 ng/L and 15 ± 7 ng/L, respectively. At pH 7.5, chlorination of APM and CS produced DCBQ at 4 ± 3 and 3.9 ± 0.8 ng/L, respectively. In comparison, chloramination of APM and CS produced DCBQ at 0.9 ± 1.4 and 0.3 ± 0.6 ng/L at pH 6.5, respectively. APM and CS formed similar concentrations of DCBQ at 1.3 ± 0.7 and 0.4 ± 0.2 ng/L under chloramination at pH 7.5. At pH 8.5, DCBQ was not detectable, consistent with previous reports that DCBQ stability decreases dramatically at high pH.[5,25] Chlorination produced a higher concentration of DCBQ than chloramination. Under the simulated chlorination and chloramination conditions, CS (containing 10 μM APM) produced a similar concentration of DCBQ to that from the 10 μM pure APM solution.

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The formation of APM and the CS was also examined under simulated drinking water conditions. These reactions had a realistic dose of the precursor (APM: 15.8 mg, equivalent to 1 sweetener pack) and varying concentrations (1, 2, 4 mg/L) of NaOCl or NH_2Cl to encompass typical values in authentic drinking water. Table S13 presents the concentration of DCBQ detected after 15 min of chlorination or chloramination of APM and CS under these conditions at a typical drinking water pH 7.5. Overall, the concentration of DCBQ under these conditions was lower than the values found under the ideal laboratory reaction conditions with most being close to the detection limit of the HPLC-MS/MS method. Generally, chlorination produced a higher concentration of DCBQ than chloramination.

After detecting DCBQ formation from APM under controlled conditions, DCBQ formation in authentic water samples sweetened with APM was investigated. Figure S13 shows the concentration of DCBQ detected in the three different tap water samples with and without the addition of CS or APM. Without addition of any APM or CS, DCBQ concentrations in the tap water samples A, B, and C were 1.4 ± 0.5, 1.7 ± 0.7, and 1.9 ± 0.6 ng/L, respectively. This concentration of DCBQ is consistent with previous results reported from the same DWDS.[5,25] After addition of APM (15.8 mg) to the three tap water samples (A, B, and C), DCBQ increased to 2.3 ± 0.5, 3.4 ± 0.9, and 2.6 ± 0.1 ng/L, respectively. The addition of one pack of CS (APM: 15.8 mg, 214.7 μM) led to a statistically significant ($p < 0.05$) increase in DCBQ to 8.8 ± 0.9, 12 ± 2, and 4.7 ± 0.1 ng/L in all the tap water samples (A, B, and C, respectively).

Other HBQs, including 2,6-dibromo- and 2,6-diiodo-1,4-benzoquinone (DBBQ and DIBQ), were not detected in any of the samples. This is likely due to the low concentrations of Br^- and I^- in the tap water being too low to form detectable levels of bromo- or iodo-HBQs, which is consistent with previous studies.[5,25] The exact reasons are unclear for the difference in the DCBQ formation after the addition of CS compared to APM. There is a difference in the recovery of DCBQ in the CS matrix compared to that of APM (Text S7). The pH values of the APM and CS sweetened samples were between 7.5 and 7.7, similar to the pH 7.6−7.7 of tap water samples (Table S14); this suggests that pH is not a factor. Furthermore, we analyzed and compared the formation of common APM hydrolysis products when the APM and CS were dissolved in ultrapure water and tap water using a HPLC-MS/MS method (Section S3 and Table S1). Figure S14 shows that the concentrations of DKP, PHE, PHE-ME, and ASP-PHE were greater in the CS samples compared to APM samples in both ultrapure water and tap water. Specifically, the differences between CS TW and APM TW for PHE, PHE-ME, DKP, and ASP-PHE at 15 min is 2.5, 0.9, 31.7, and 37.0 μg/L, respectively. The concentrations (μg/L) of the hydrolysis products were an order of magnitude greater than that of DCBQ at a few ng/L. The only hydrolysis product that had a change in concentration over 25 min was DKP when APM or CS was dissolved in tap water. The higher amount of aromatic hydrolysis products in the CS samples likely contributed to the increased formation of DCBQ.

3.3. Prevention of DCBQ Formation after Reducing Residual Chlorine. To provide further evidence for the formation of DCBQ from reactions with residual chlorine, we evaluated DCBQ formation after the removal of residual chlorine by two different methods. In the first experiment, we quenched tap water samples with AA at a molar ratio of 1.2:1 AA to total chlorine residual prior to the addition of APM or CS. In the second experiment, we boiled the tap water to reduce residual chlorine and after cooling added APM or CS to the boiled tap water.

Figures 3A,B compare the DCBQ formation from APM and CS before and after residual chlorine was reduced by AA.

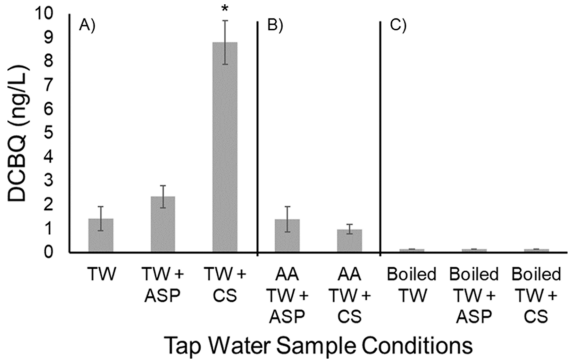

Figure 3. Concentration of DCBQ detected after 15 min when 15.8 mg APM or one CS packet was dissolved in 250 mL of authentic tap water (TW) (A), TW quenched with AA (μM AA = 1.2 × μM total chlorine) (B), and TW that was boiled and cooled for 1 h (C) ($n = 3$, $^*p < 0.05$). N.D.: not detected; the concentration is lower than the detection limit.

Without quenching the residual chlorine with AA, the DCBQ concentration was 2.3 ± 0.5 and 8.8 ± 0.9 ng/L after addition of APM or CS in the tap water, respectively (Figure 3A). When the tap water was prequenched with AA (μM AA = 1.2 × measured μM total chlorine) before the addition of APM or CS, the concentration of DCBQ was 1.4 ± 0.5 and 1.0 ± 0.2 ng/L, respectively (Figure 3B). After the tap water was boiled in an electric kettle and allowed to cool for 1 h, the total chlorine content in the tap water was reduced from 1.7 to 0.8 mg/L (Table S15). After the boiling process, DCBQ was undetectable in the boiled and cooled tap water (Figure 3C). The concentration of DCBQ after the addition of APM or CS to the boiled and cooled tap water was also at undetectable levels (Figure 3C). Therefore, using AA or boiling to reduce the residual chlorine concentration in the tap water effectively eliminated the formation of DCBQ. These results also support the hypothesis that APM reacts with the residual chlorine in tap water to produce DCBQ.

3.4. Formation of DCBQ and Transformation Products and DCBQ in Tea Samples. APM is often used to sweeten tea or coffee. We investigated the formation of the transformation products and DCBQ in tea prepared with tap water and APM or CS along with the control tea samples without sweetener. Nontargeted analysis of the samples detected two peaks at the same retention times as Unknown 2 and Unknown 3 in the APM and CS sweetened tea samples (Figure S15). Figure 4 shows the XIC, MS, and MS/MS spectra of the two peaks that matched Unknown 2 (m/z 284.0679, mass error 1.8 ppm) and Unknown 3 (m/z 318.0296, mass error 0.5 ppm). The accurate mass, $^{35,37}Cl$ isotopic patterns, and fragmentation patterns of the two peaks in the sweetened tea samples (Figure 4) were consistent with those of Unknown 2 and 3 described above (Figure S7). These compounds were not found in any of the controls including tea prepared in tap water without APM or CS. Interestingly, the

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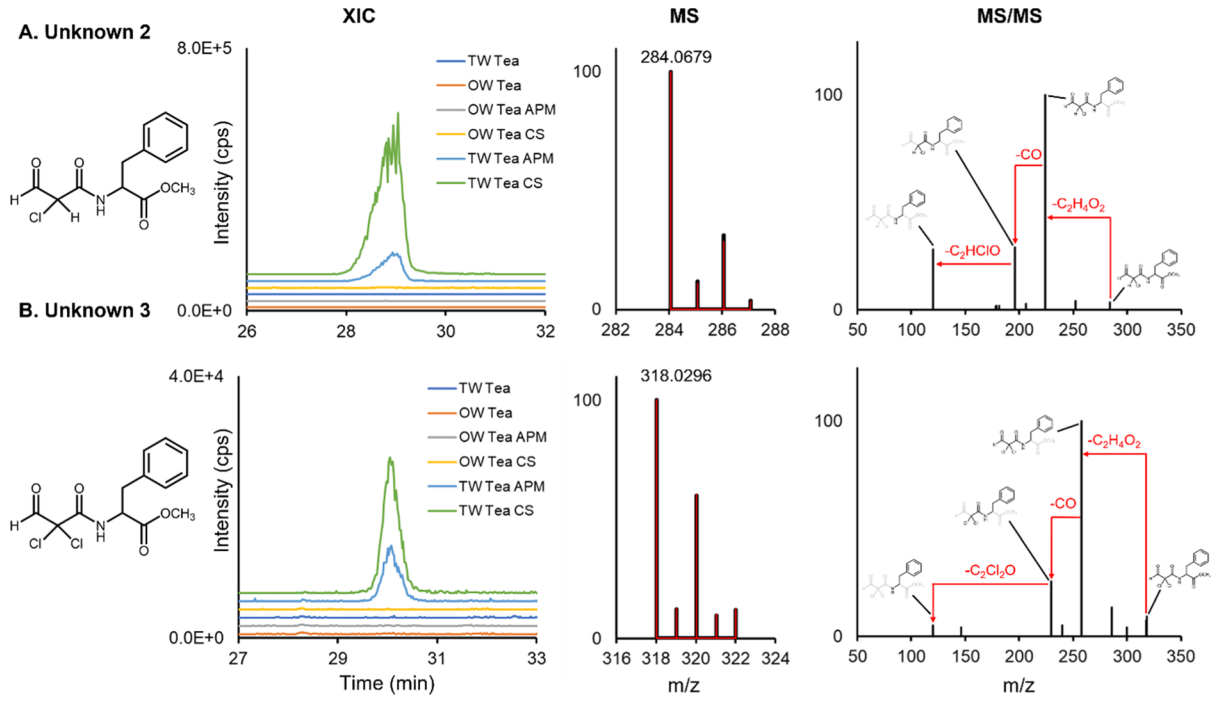

Figure 4. HRMS detection of Unknown 2 with m/z 284.0690 and Unknown 3 with m/z 318.0302 showing XIC, MS, and MS/MS. The XIC shows ultrapure water (OW) or tap water (TW) samples containing Tea, 15.8 mg aspartame APM), or commercial sweetener (CS). The MS spectrum shows the authentic sample (black trace) as well as the theoretical isotopic distribution of the putatively identified compounds $[C_{13}H_{14}ClNO_4 + H]^+$ and $[C_{13}H_{13}Cl_2NO_4 + H]^+$ for Unknowns 2 and 3, respectively (red trace). MS and MS/MS spectra are from TW Tea CS sample. The fragmentation pathways of the molecular ion are shown in the MS/MS and its correlating matching fragments of the putatively identified structure.

peak area of both compounds was greater in the tea samples containing CS. These results support that chlorinated products of APM can be produced in tea prepared with cold tap water.

Figure S16 shows the concentration of DCBQ detected in the tap water samples containing tea with and without the addition of CS or APM. Without addition of any APM or CS, DCBQ concentrations in the tap water sample was 4 ± 3 ng/L. After addition of APM or CS to the tap water samples, DCBQ increased to 6 ± 1 and 9 ± 1 ng/L, respectively, but the difference was not statistically significant.

3.5. Implications. This study demonstrates the first evidence for the formation of new DBPs and DCBQ in tap water and tea sweetened with APM at authentic residual chlorine levels. The identification of novel APM transformation products is significant because APM is used in large quantity.[11,12] The transformation products and hydrolysis products of APM may serve as precursors to produce other DBPs, such as DCBQ, during chlorination and chloramination. The detection of new transformation products of APM and DCBQ in sweetened tea highlights unintentional exposure to new DBPs resulting from residual chlorine reacting with APM. This study demonstrated simple approaches to minimize DBP formation and exposure resulting from APM, which is consistent with other studies.[6,7,36]

A variety of artificial sweeteners are widely used in food, beverages, and medications, resulting in their widespread occurrence in wastewater and the environment.[37] However, the formation of DBPs from chlorination of sweeteners is not well studied. This study is the first to report on the formation of new transformation products and potentially toxic DBPs, such as DCBQ, from APM sweetener, even under the typical tap water residual chlorine dose. This study demonstrates the unintentional exposure to new DBPs of artificial sweeteners, highlighting the need to study the formation and toxicity of new DBPs resulting from widely used sweeteners.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c07156.

Ingredients of a commercial tabletop sweetener packet containing APM; HPLC-QTOF-MS method for non-targeted analysis; analysis of hydrolysis products of APM using HPLC-MS/MS (MRM) method; HPLC-MS/MS (MRM) method for determination of DCBQ; standard addition for quantification of DCBQ; investigating interference of aspartame on DCBQ detection; recovery of DCBQ in the presence of APM and CS using the HLB cartridge; evaluation of AA and FA on DCBQ stability; NBO analysis of aspartame; MRM parameters for the HPLC-MS/MS analysis; ratios of peak areas of DCBQ in ultrapure water; MS/MS fragments of the precursor ions; concentration of DCBQ; pH; relative DCBQ signal; calculated NBO charge distribution; HRMS detection; proposed chlorination pathways; HRMS detection; HRMS analysis; DCBQ detected after 15 min; and peak area of hydrolysis products (PDF)

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Notes

The authors declare no competing financial interest.

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