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Rapid and selective determination of free chlorine in aqueous solution using electrophilic addition to styrene by gas chromatography/mass spectrometry

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ABSTRACT

We developed a rapid and selective method for determination of free chlorine in aqueous solution by gas chromatography/mass spectrometry for the first time. Free chlorine was converted to styrene chlorohydrin using electrophilic addition to styrene in sodium acetate buffer solution (pH 5). The chlorine derivative obtained was extracted with chloroform, and then analyzed by GC/MS. The calibration curve showed good linearity from 0.2–100 μg/mL (as available chlorine). The detection limit was 0.1 μg/mL, and the intra- and interday accuracy were measured at concentrations of 10, 50, and 75 μg/mL to be −1.3 to 6.9% (intraday) and 3.8–8.0% (interday) as % Bias. The precision was between 1.4 and 4.5% as % RSD. These results indicate that this method is a superior technique for the identification of free chlorine. This method was successfully applied to quantification in commercial samples and in samples of a criminal case.

1. Introduction

Because free chlorine is a strong oxidizing agent, it is widely used as a household cleaning agent and as a disinfectant for treatment of drinking water, swimming pools, and wastewater. Free chlorine in water exists in equilibrium among three species, molecular chlorine, hypochlorous acid, and hypochlorite ion [1]. The equilibrium is affected by solution pH, and the predominant species at pH values of 1, 5, and 9 are molecular chlorine, hypochlorous acid, and hypochlorite ion, respectively. Concentrated free chlorine solutions can cause respiratory problems, skin and throat irritation, abdominal pain, burning sensations, coughing, and vomiting [2]. Therefore, it is typically at concentrations between 10^{-5} and 10^{-2} mol/L in our daily life [1]. It is known to react with various organic compounds and produce chlorinated byproducts, which could be hazardous to human health. Therefore, the levels of chlorine in tap water need to be controlled and monitored frequently.

Free chlorine is a biologically important reactive oxygen species [3–5]. Endogenous free chlorine is essential to life, possesses important antibacterial properties, and plays an essential role in the prevention of microorganism invasion [6]. However, abnormal production of free chlorine can cause serious damage to biological systems, and even lead to atherosclerosis, arthritis, and cancer [6–8]. Therefore, selective and sensitive methods for detecting free chlorine are required.

As for forensic analysis, free chlorine is an important target. Because free chlorine is relatively inexpensive and readily available, it is frequently misused for criminal purposes. Free chlorine is typically misused to contaminate food and drink, to throw on people and/or property, to eliminate the foul smell of a corpse, and to remove bloodstains.

Many methods have been reported for the determination of free chlorine in aqueous media, including the normalized and well known iodometric titration [10], colorimetry [11–14], chemiluminescence [15,16], ion chromatography [17–20], liquid chromatography [21,22], flow injection analysis [2,23–25], electrochemical assay [26], and capillary electrophoresis [27]. Because most of these methods detect free chlorine on the basis of its absorbance and/or retention time, they sometimes lack specificity. By contrast, gas chromatography/mass spectrometry (GC/MS) is reliable method because it can identify substances based on both their retention times and mass spectra. Thus, most of guidelines for substance confirmation, such as forensic science and human performance testing [28], confirmation of drugs [29,30], workplace testing [31], and doping in sports [32], require mass-spectrometry-based identification. However, until now, GC/MS methods for free chlorine in aqueous solution have not been reported.

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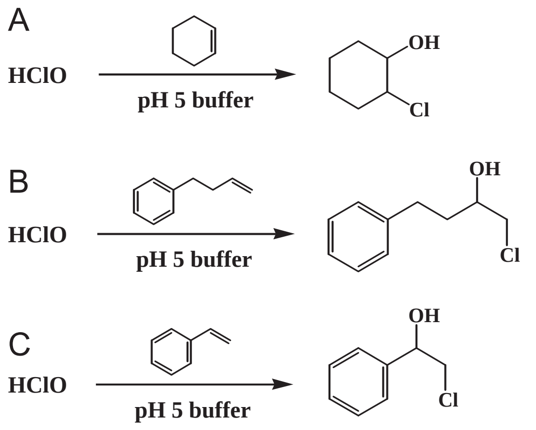

Fig. 1. Derivatization reactions of free chlorine with (A) cyclohexene, (B) 4-phenyl-1-butene, and (C) styrene.

Here we proposed a rapid and selective GC/MS method to determine free chlorine in aqueous solution. We selectively converted hypochlorous acid to a stable compound based on electrophilic addition as shown in Fig. 1. The electrophilic addition of hypochlorous acid to alkenes is a well established reaction for chlorohydrin formation [33–37]. This reaction follows the Markovnikov rule, with the hydroxyl group adding to the more substituted carbon.

2. Materials and methods

2.1. Materials

A stock solution of free chlorine was prepared by diluting an aqueous 5% sodium hypochlorite ion solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan) with pure water to a concentration of 1000 micrograms of available chlorine per milliliter. This stock solution was standardized by iodometric method [9] and diluted to the various required concentrations with pure water. The following derivatization reagents were tested in this work: cyclohexene (97%) and 4-phenyl-1-butene (97%) (Wako Pure Chemical Industries, Ltd.); and styrene (99%) (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan). Diphenylmethane (99%, Wako Pure Chemical Industries, Ltd) was used as the internal standard (IS), and dissolved in chloroform at a concentration of 10 μg/mL. The water used in this study was purified by a Milli-Q SP RFG40 ICP-MS system (Merck Millipore, Billerica, MA, USA).

2.2. Derivatization and extraction

The derivatization reactions are shown in Fig. 1. One hundred microliters of sample containing free chlorine was put into a 1 mL glass tube with 300 μL of 0.5 mol/L sodium acetate/acetic acid buffer solution (pH 5). Twenty microliters of derivatization reagent was added to the mixture and the resulting solution was vortex mixed for 30 s at room temperature. Two hundred microliters of chloroform containing IS was added to the solution, and the mixture was vortex mixed for 30 s at room temperature to extract reaction products to organic phase, and then centrifuged at 2000 rpm for 3 min. The organic phase was placed in another glass tube, and 1 μL was injected into the GC/MS.

2.3. GC/MS conditions

GC/MS analysis was performed with a GCMS-QP2010 Plus (Shimadzu, Kyoto, Japan) equipped with a DB-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies, Santa Clara, CA, USA).

The GC oven temperature was held at 40 °C for 2 min; increased to 200 °C at 10 °C/min, and then increased to 250 °C at 30 °C/min (held for 1 min). The injection port temperature was 250 °C. The split injection mode was used (split ratio of 1:10), and the carrier gas was helium at a flow rate of 1.8 mL/min.

The MS interface temperature and ion source temperature were 250 and 250 °C, respectively. The MS was operated with a filament current of 150 μA and electron energy of 70 eV in electron ionization mode. The derivative was identified in full scan mode, and data were acquired from m/z 40 to 250. Quantification was performed in selected ion monitoring mode, and the ions at m/z 79, $\underline{107}$ and 156 for the chlorine derivative and m/z 91, 153 and $\underline{168}$ for the IS were monitored (the quantification ions are underlined).

3. Results and discussion

3.1. Optimization of the derivatization and extraction

3.1.1. Choice of derivatization reagent

Three different reagents for derivatization of free chlorine were investigated, including cyclohexene, 4-phenyl-1-butene, and styrene (Fig. 1). Chlorohydrin formations of these compounds were confirmed by mass spectrometry. As shown in Fig. 2, styrene gave chlorohydrin more selectively than the other two reagents. In addition, the styrene derivative was detected with the highest sensitivity as main peak, which will give a lower limit of quantification.

The reactivity of an olefin double bond to halogenating agent is determined by the electron-donating or -withdrawing character of local substituent groups. An electron-donating group near a double bond enhances the electron density of the double bond, and makes it susceptible to attack by electrophilic halogenating agents [38]. Styrene has a π-electron-rich phenyl group and this group enhances the electron density of the double bond. Therefore, derivatization with styrene gave good selectivity and sensitivity.

3.1.2. Effect of pH on the derivatization

The effect of the buffer pH on the derivatization of free chlorine was studied from pH 1 to 9. The highest derivatization yield was obtained at pH 5 (Fig. 3), while the yield decreased steeply at more basic and acidic conditions. At pH 5, all chlorine was in the form of hypochlorous acid, while at lower or higher pHs, molecular chlorine or hypochlorite ion exists in equilibrium with hypochlorous acid, respectively [1]. It was reported that hypochlorous acid rather than hypochlorite ion is the reactive species on the reaction of unsaturated fatty acid [39]. It is well known that chlorohydrin readily loses hydrogen chloride at basic pH to form the corresponding epoxide [40]. Therefore, the poor derivatization yields observed at pH 7 and 9 can be explained by the low content of the reactive hypochlorous acid and the instability of chlorohydrin.

On the other hand, formation of a dichloro derivative is the reason for the poor derivatization yields at lower pH values (1 and 3). Because molecular chlorine is the predominant species at pH 1 and 3, molecular chlorine reacted with styrene to form a dichloro derivative (data not shown). The formation of a dichloro derivative was also reported for chlorination of α-terpineol under acidic conditions [41].

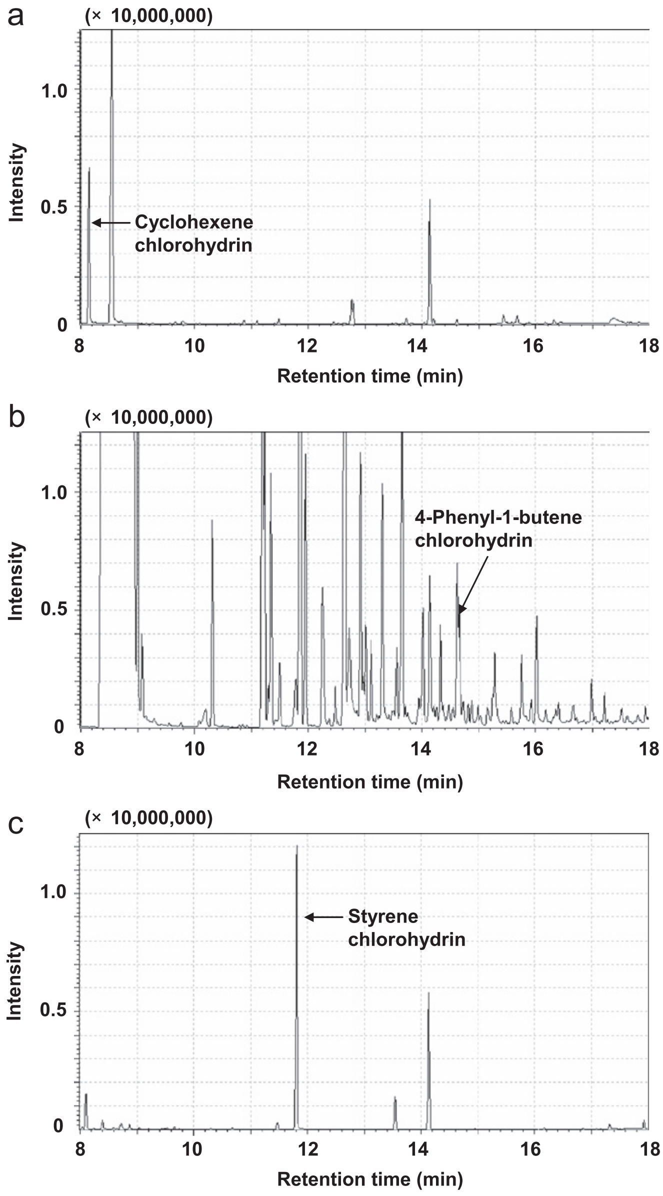

Fig. 2. Total ion chromatograms of the extracted products after derivatization with (A) cyclohexene, (B) 4-phenyl-1-butene, and (C) styrene. Chlorine concentration: 50 μg/mL.

We found that derivatization reaction was rapidly completed in 30 s at pH 5 (Fig. S1). We compared three extracting solvents (chloroform, toluene, and hexane) for styrene chlorohydrin. Chloroform, which had the highest polarity among the solvents tested, provided the best extraction efficiency (Fig. S2). Additionally, extraction time profile of the chlorine derivative was studied, and are shown in Fig. S3. The chlorine derivative reached equilibrium after 30 s. Hereafter, we conducted derivatization reaction for 30 s at pH 5, and then extracted styrene chlorohydrin using chloroform for 30 s.

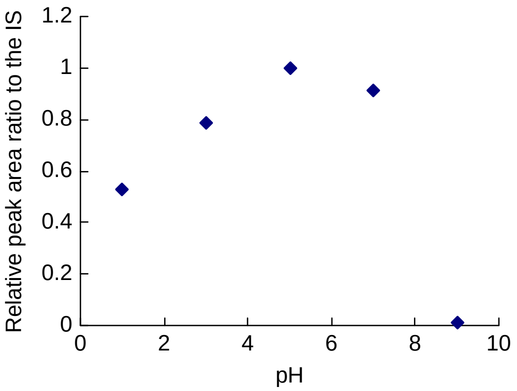

Fig. 3. The effect of buffer pH on the derivatization. The derivatization with styrene were carried out in 0.5 mol/L potassium chloride/hydrochloric acid buffer (pH 1), 0.5 mol/L sodium formate/formic acid buffer (pH 3), 0.5 mol/L sodium acetate/acetic acid buffer (pH 5), 0.5 mol/L sodium hydrogen phosphate/sodium dihydrogen phosphate (pH 7), or 0.5 mol/L sodium carbonate/sodium hydrogen carbonate (pH 9). Chlorine concentration: 50 μg/mL. Each value represents the mean of three measurements.

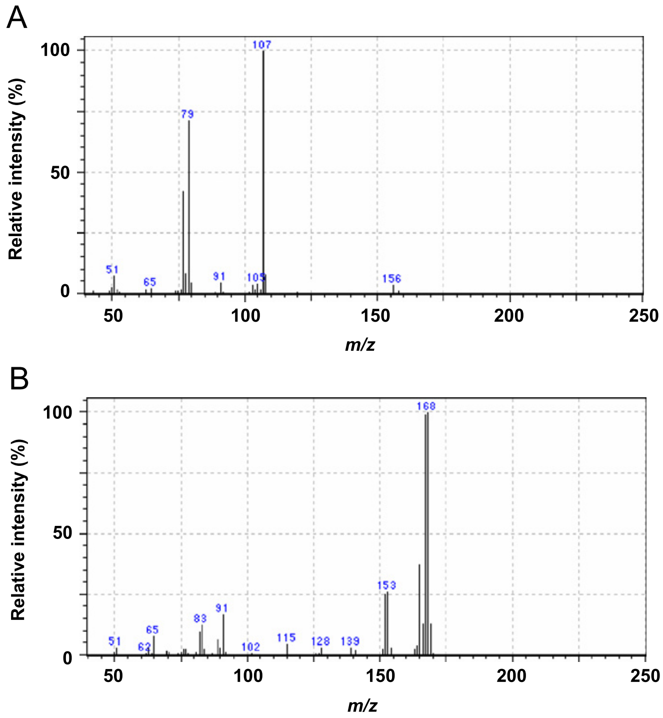

Fig. 4. Mass spectra of (A) the chlorine derivative and (B) the internal standard.

3.2. *Gas chromatography/mass spectrometry*

The mass spectrum showed a molecular ion peak for the chlorine derivative at *m*/*z* 156 (Fig. 4A). This peak showed the characteristic isotope distribution pattern indicative of the presence of Cl (^{35}Cl:^{37}Cl isotope ratio of approximately 3:1). A base peak observed at *m*/*z* 107 corresponds to the compound lost CH_2Cl from styrene chlorohydrin. The molecular ion peak of the IS appeared as a base peak at *m*/*z* 168 (Fig. 4B).

Mass chromatograms of the derivatized extract obtained from the water spiked with chlorine at 50 μg/mL are shown in Fig. 5. Sharp and symmetrical peaks were observed for the chlorine derivative and the IS at 11.8 and 14.1 min, respectively.

3.3. *Ability of the proposed method*

The linearity of the signal versus concentration plot was evaluated from a calibration curve of a series of solutions with various chlorine concentrations (0.2–100 μg/mL). The peak area ratio of the base peak of the chlorine derivative at *m*/*z* 107 to that of IS at *m*/*z* 168 was plotted against the chlorine concentration. Linear regression analysis was used with a weighting factor of $1/\chi$, and this provided better accuracy for quantification than not using a weighting factor. The correlation coefficient was 0.998 and indicated good linearity.

Intra- and interday precision and accuracy were evaluated by analyzing sets of five spiked waters samples at three concentration levels (10, 50, and 75 μg/mL) on three separate days. Accuracy (%Bias) was defined as mean relative error between the measured and nominal concentrations: ((measured concentration − nominal concentration)/nominal concentration) × 100). Precision (%RSD) was defined as the percentage of standard deviation of the measured concentrations divided by their mean concentrations: ((standard deviation/mean concentration) × 100). The results are presented in Table 1, indicating that our method is very stable and has high reproducibility.

The limit of detection based on a signal-to-noise ratio of 3 was determined to be 0.1 μg/mL. The limit of quantification based on a signal-to-noise ratio of 10 was calculated to be 0.2 μg/mL. To evaluate the stability of the chlorine derivative after extracting into chloroform, the sample solution was kept in the auto sampling system at room temperature and analyzed at intervals 0–24 h after preparation. No detectable loss of styrene chlorohydrin for 24 h-storage was observed, indicating high stability of the compound in chloroform.

3.4. Interference investigation

The influence of some possible interfering substances was investigated. The substances and their additive amounts shown in Table 2 were selected for investigation of the interference against the proposed method. Each mixed solution containing free chlorine at a constant concentration of 100 μg/mL and 0.1 equivalent of substance was prepared. Recoveries were determined by comparing the signal obtained for the mixed solution with that obtained for the chlorine alone solution. Among them, magnesium chloride, sucrose, fumaric acid, benzaldehyde, and citric acid did not interfere, but ammonium chloride, alanine, and ascorbic acid did negatively (Table 2). Free chlorine reacts with ammonia/ammonium to give a complex series of reactions, the initial product of the reaction being monochloramine [42]. In the case of α-amino acid such as alanine, a decarboxylation and a desamination follows initial chloramination step which leads to a carbonyl compound, ammonia and a nitrile [43]. Oxidation–reduction reaction between free chlorine and ascorbic acid is very fast, and yields dehydroascorbate and chloride [44]. It was reported in other free chlorine methods that decreases in signal are observed when the interfering substances, which react with free chlorine, coexist [1,42]. Therefore, in this study, the low recoveries can be explained by the low content of free chlorine, which resulted from reactions with these interfering substances.

3.5. Practical application

3.5.1. Chlorine-containing commercial samples

In order to test the reliability of our method, we analyzed a set of five commercial samples such as bleaches and disinfectants including high concentration of free chlorine. These samples contain potential interferents such as surfactant, sodium hydroxide, and stabilizer. The samples were also analyzed by iodometric method [10], which is certified as a standard method for the chlorine quantification by Japanese Industrial Standards. As summarized in Table 3, these two methods provides almost same values for each sample and the correlation coefficient between the two methods was 0.999, showing that our method is highly reliable for chlorine determination. The advantage of our method over the iodometric method is the specificity to chlorine. The iodide can react with other oxidants, resulting in false positive signal [10].

3.5.2. Samples in a criminal case

Samples were obtained from a case in Japan where four people who drank bottled water in the dining room of nursing home for the elderly became ill. These people suffered from nausea,

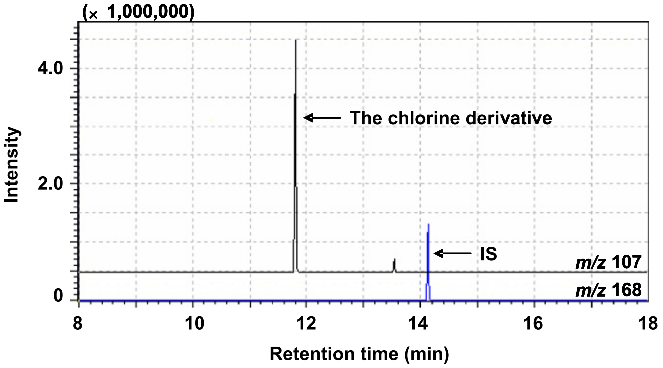

Fig. 5. Mass chromatograms of the derivatized extract obtained from the water spiked with chlorine at 50 μg/mL.

Table 2
Effect of some potentially interfering substances on the determination of free chlorine [a].

Species added	Recovery[b](%)
Magnesium chloride	100.5 ± 1.0
Sucrose	100.0 ± 1.7
Fumaric acid	100.8 ± 1.4
Benzaldehyde	100.2 ± 0.8
Citric acid	99.9 ± 0.8
Ammonium chloride	43.3 ± 0.2
Alanine	55.5 ± 0.2
Ascorbic acid	84.6 ± 0.4

[a] Chlorine concentration: 100 μg/mL. Additive amount: 0.1 mol equivalent to chlorine.

[b] Mean ± standard deviation ($n=3$).

Table 1
Intra- and inter-day precision and accuracy.

Nominal concentration (μg/mL)	Intraday ($n=5$)			Interday ($n=3$)		
	Mean ± SD[a]	Precision (%RSD[b])	Accuracy (% Bias)	Mean ± SD	Precision (% RSD)	Accuracy (% Bias)
10	10.7 ± 0.2	1.4	6.9	10.7 ± 0.3	2.5	6.9
50	52.4 ± 1.1	2.1	4.8	54.0 ± 1.7	3.1	8.0
75	74.0 ± 1.1	1.5	− 1.3	77.8 ± 3.5	4.5	3.8

[a] Standard deviation.

[b] Relative standard deviation.

Table 3
Quantification of chlorine in commercial samples.

Commercial samples	Iodometric method[a] (× 10^3 μg/mL)	Proposed method[b] (× 10^3 μg/mL)
Bleach A	55.5	57.7 ± 1.2
Bleach B	19.7	19.8 ± 0.7
Disinfectant A	28.7	28.7 ± 1.2
Disinfectant B	26.9	27.0 ± 0.4
Disinfectant C	11.1	11.4 ± 0.2

[a] Values determined by iodometric method according to Ref. [10].
[b] Mean ± standard deviation ($n=3$).

sweating, and headaches, and were sent to a hospital. The water had been stored in a refrigerator, and a bottle of unknown liquid that was suspected to have been used to spike the bottle water was found in the dining room. Samples of the bottled water and unknown liquid were analyzed by our method. The samples were diluted with pure water before derivatization to adjust the concentrations to within the linear detection range of the MS. The concentrations of free chlorine in the samples were 0.20×10^3 (bottled water) and 61.0×10^3 μg/mL (unknown liquid), respectively. Thus, it was concluded that the high concentration of free chlorine in the bottled water caused the above-mentioned symptoms for the four people.

4. Conclusions

In this study, we developed a rapid and selective method for analysis of free chlorine in aqueous solution by GC/MS for the first time, and applied it to commercial samples and samples obtained in an actual criminal case. Because this method has high specificity and good reliability, it will be very useful in forensic science and aquatic science.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.10.011.

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