HPLC Determination of Chlorine in Air and Water Samples Following Precolumn Derivatization to 4-Bromoacetanilide

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Summary

Chlorine has been be determined in air and water samples by a rapid and sensitive method entailing precolumn derivatization to 4-bromoacetanilide. A mixed potassium bromide – acetanilide reagent was used as a trapping agent for chlorine in air, and for its derivatization. The 4-bromoacetanilide formed was determined by reversed-phase HPLC on an ODS column, using methanol - water, 65:35 (v/v) as mobile phase; detection was at 240 nm. A rectilinear calibration graph was obtained for the range 0.1–30 μg mL^{-1} chlorine; the limit of detection found to be 0.01 μg mL^{-1}.

The precolumn derivative has been found to have a shelf-life of at least 21 days; this enables the use of the method for samples transported from the field to the analytical laboratory, or the testing of a variety of conditions for chlorine scrubbing studies without the need for immediate analysis of samples. Humic substances do not cause any interference with the proposed method and the presence of nitrite does not lead to artificially high results and consequent misleading conclusions of the presence of high levels of chlorine.

Introduction

Chlorine is used on a large scale for disinfection of water and waste water, as a bleach in laundering, and in the pulp and textile industries [1,2]. Tap water usually contains 1 mg L^{-1} residual chlorine for sterilization. Chlorine is a toxic substance and its use has been linked with the formation of halogenated organic compounds many of which have been shown to be potentially carcinogenic [3,4].

Waste chlorine gas streams must be treated before their discharge to the atmosphere. One common method of treatment involves scrubbing with water in packed towers [5,6]. To determine the efficiency of the scrubbing operation, chlorine is continuously monitored in the air and water effluents. The emphasis which is laid on the analytical method used is that the method should be applicable to both air and water samples and that immediate analysis should not be required. The latter aspect means a long shelf life of the collected samples, which would enable a greater number of operating conditions to be tested each day and transportation of samples from field to the analytical laboratory.

Several methods have been reported for the determination of chlorine species; most are based on titrimetry, amperometry, or spectrophotometry [7,8]. The most widely accepted methods are based on the use of *N,N*-diethyl-1,4-phenylenediamine (DPD), as suggested by Palin [9,10]. Recent reports have described flow injection methods involving chemiluminescence detection after reaction with luminol [11], and spectrophotometric detection following the formation of a coloured semiquinoid free-radical of DPD [12] or an azo dye with 4-nitrophenylhydrazine and *N*-(1-naphthyl)-ethylenediamine [13].

Except for an ion chromatographic procedure which involves reduction of chlorine to more stable chloride which is subsequently determined [6], none of the analytical methods available embodies suitable shelf-life of the collected samples. The ion chromatographic procedure is, however, subject to interference from any chloride (or hydrochloric acid) already present in the sample; the reagents used should also be free from chloride impurities.

This communication proposes a new sensitive and specific HPLC method which involves precolumn reaction of chlorine with bromide and acetanilide: the

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sequence of reactions entails oxidation of bromide to bromine and electrophilic substitution reaction of acetanilide with bromine to produce 4-bromoacetanilide as a stable product. A large number of substances as present in air and water do not interfere with the method.

Experimental

Reagents

All solvents were HPLC grade and water was doubly distilled using all-glass apparatus. Acetanilide was recrystallized from hot water.

The mixed reagent solution was prepared by dissolving acetanilide (0.1 g) in glacial acetic acid (50 mL), mixing with a solution of potassium bromide (0.5 g) in water (10 mL) and diluting to 100 mL with water in a standard flask.

Standard hypochlorite solutions were freshly prepared by dilution of an aliquot (5 mL) of commercially available sodium hypochlorite solution (10-14 % available chlorine) to 100 mL followed by iodometric standardization [7]. A known volume of standard stock solution was further diluted to give 1000 µg mL^{-1} chlorine solution. Less concentrated working standards were prepared by rapid successive dilutions, standardized by the DPD method [10], and used within 1 h of preparation.

Apparatus

LC was performed with a system consisting of a Shimadzu LC-5A pump, a Rheodyne model 7010 10 µL loop injector, a 25 cm × 4.6 mm i.d. × 5 µm ODS-1 column (Anachem), a Shimadzu SPD-2A variable wavelength UV detector (operated at 240 nm), and a Shimadzu C-R2AX integrator fitted with a printer-plotter; peak area was used for quantitation. The mobile phase was methanol - water, 65:35 (v/v) at a flow rate of 1 mL min^{-1}

Procedures

Determination of Chlorine in Water

A known volume (200-1000 µL) of water sample was mixed with the mixed reagent (500 µL) in a 10 mL standard flask and diluted to volume with mobile phase. The solution was shaken for 1 min and a suitable volume (10 µL) injected on to the column.

Determination of Chlorine in Air

Air was sampled for chlorine by passing a known volume of air through a midget impinger containing a mixture of mixed reagent (5 mL) and water (15 mL). The trapping solution was carefully transferred into a 100 mL standard flask and the impinger washed with mobile phase. The solution was diluted to volume with mobile phase and a suitable aliquot (10 µL) used for liquid chromatography.

Results and Discussion

The precolumn derivatization of chlorine utilizes the oxidizing property of chlorine to convert bromide into bromine, and electrophilic substitution of acetanilide with bromine to form 4-bromoacetanilide which may be analyzed by HPLC.

Selection of the Precolumn Reaction of Chlorine

Because chlorine as such is not suitable for determination by HPLC, the best way of analyzing the gas appeared to be to utilize its redox property and analyze one of the stable products of reaction as a measure of the chlorine present. Reduction of chlorine to chloride with sodium sulphite and ion chromatography of chloride was proposed earlier [6], but the method has the serious drawback of interference by chloride. Chlorine can undergoes a wide variety of reactions (addition, substitution, and oxidation) with many compounds which are present in effluents and coexist with the chloride which is a by-product of many such reactions. Formation of a stable substitution compound by reaction of chlorine with an aromatic compound, and determination of the halo-organic by reversed-phase HPLC with UV detection appeared to us to be an interesting approach to the determination of chlorine, and such a procedure seemed likely to be free from interference from chloride.

Whereas the redox reactions of chlorine occur rapidly, the element's substitution and addition reactions are usually slow, and thus unsuitable for quantitative work. Since bromination of most phenols, aromatic amines, and anilides is rapid and quantitative, it was decided that a redox reaction of chlorine with bromide to give an equivalent amount of bromine could be performed with simultaneous scavenging of the liberated bromine in an aromatic substitution reaction by means of a precolumn derivatization technique.

Also desired was the formation of only one isomer of the bromo-aromatic compound in order to prevent the appearance of more than one peak for the analyte in the chromatogram. This was achieved by selection of a suitably substituted organic compound with only one position available for electrophilic bromination. Derivatives of aniline and phenol which have substituents already present at both the *ortho* positions appeared to be good choice since in such compounds the *para* position alone would be available for bromination. Unfortunately, nuclear substituted derivatives of aniline are susceptible to oxidation by either chlorine or bromine, and halogenated phenols are toxic; both properties limit their prospective use as precolumn reagents. Acetanilide (*N*-acetylaniline, *N*-phenylacetamide) proved to be the best choice for the precolumn derivatization reaction. The acetylamino group in acetanilide is big enough to impede any *ortho* bromination, thus *para* substitution alone occurs.

The Chemistry of Derivatization

The sequence of reactions which occur during the precolumn derivatization of chlorine with bromide and acetanilide to give 4-bromoacetanilide is given in Figure 1. The reactions occur over a wide range of pH, in solutions containing strong mineral acids to pH 5. Acetic acid was used as a cosolvent in the mixed reagent since it served to maintain acidity and prevent any precipitation of acetanilide or its derivative.

Validation of Derivatization

The chromatographic peak for 4-bromoacetanilide was identified by comparison of its retention time with that of the authentic substance under the same chromatographic conditions, and by UV and IR spectroscopy of peaks of corresponding retention time when the agreement was excellent. As is evident from the reactions in Figure 1 chlorine produces an equal molar mass of 4-bromoacetanilide on precolumn derivatization; equal molar masses of authentic 4-bromoacetanilide and that formed by a precolumn derivatization reaction of the corresponding molar mass of chlorine produced peaks of equal areas (± 2 % agreement), thus confirming that the derivatization reaction was quantitative.

Chromatographic Conditions

Preliminary studies indicated that the chromatographic separation of acetanilide and 4-bromoacetanilide can be achieved on a reversed-phase octadecylsilane (ODS) column using methanol - water or acetonitrile - water as mobile phase. Acetanilide and its substituted derivatives give a strong UV absorption band at 240 nm, making this wavelength suitable for their detection. Best chromatographic separation was obtained on a 25 cm x 4.6 mm i.d. x 5 μm particle size ODS column using methanol - water, 65:35 (v/v) as eluent at a flow rate of 1 mL min^{-1}. Under these conditions, separation was complete within 6 min (Figure 2).

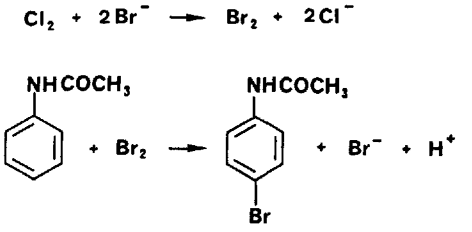

Figure 1

Reaction scheme for the derivatization of chlorine to 4-bromoacetanilide.

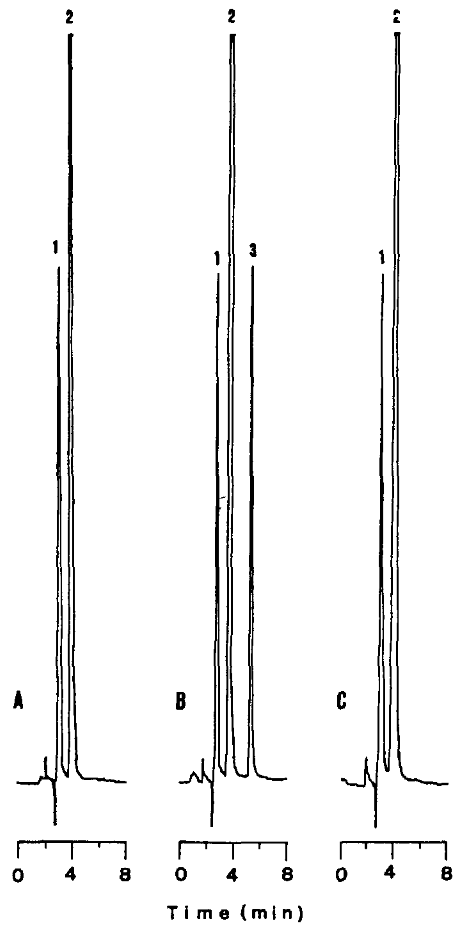

Figure 2

Chromatograms obtained from (A) mixed reagent blank, (B) chlorine (5 μg mL^{-1}) derivatized on precolumn with mixed reagent, and (C) nitrite (2000 μg mL^{-1}) treated with mixed reagents: mobile phase, methanol - water, 65:35 (v/v); flow rate, 1 mL min^{-1}; column, 25 cm x 4.5 mm i.d. x 5 μm particle size ODS; detection at 240 nm; sensitivity, 0.08 a.u.f.s.; 1, unknown; 2, acetanilide; 3, 4-bromoacetanilide.

Stability of Precolumn Derivative

A 5 μg mL^{-1} solution of chlorine was precolumn derivatized in triplicate and chromatographed daily over a period of 30 days during which time the solutions were stored at 18 °C. The chromatograms were found to be identical during this period of storage. The area of the 4-bromoacetanilide peak was reproducible within ± 1 % up to 21 days; thereafter there was a decrease of 5 % after 30 days shelf-life.

Calibration Graph and Detection Limit

A limit of detection (signal-to-noise ratio 3:1) of the method was found to be 0.01 μg mL^{-1} chlorine. A calibration plot of peak area of the precolumn derivative against amount of chlorine was found to be linear over the range 0.1-30 μg mL^{-1}; the regression coefficient (r) was 0.9989 (n = 20).

In routine laboratory work the chlorine standard solutions should be freshly prepared using distilled deionized water with zero oxygen demand, and should usually be used within 1 h of preparation. Protection from light and heat is also necessary. The observation in this work was that the precolumn derivatization of chlorine was quantitative and that solutions of 4-bromoacetanilide in mobile phase are stable for long periods, it was thus decided to use 4-bromoacetanilide as a standard in chlorine determination. Standard solutions containing 3.0-90.4 μg mL^{-1} 4-bromoacetanilide (equivalent to 0.1-30 μg μL^{-1} chlorine in respect of 4-bromoacetanilide formed by precolumn derivatization) were chromatographed without subjecting them to any precolumn reaction and a calibration graph was constructed of 4-bromoacetanilide concentration against peak area. The peak areas obtained by the present method from chlorine test samples were referred to this graph, the 4-bromoacetanilide to chlorine conversion factor was 0.3318. The results obtained for unknown test solutions using chlorine and 4-bromoacetanilide calibration standards were in agreement within ± 1.5 %.

Applications

The method was used to determine chlorine in air and water samples; the results obtained are given in Table I. Chlorine test solutions were standardized by iodometry [7] or by the DPD method [10] and the results obtained by the present method were compared with standard values. The relative standard deviation was in the range 0.5-2.5 % (n = 6). For analysis in air, hypochlorite standards and mixed reagent were taken in two separate midget impingers connected with a short length of PTFE tubing. The hypochlorite solution was acidified with 2M sulphuric acid and air was drawn through the system to sweep chlorine into the trapping solution.

Table I. Determination of chlorine in air and water and comparison of results with those produced by standard iodometric [7] and DPD [10] procedures.

Sample	μg mL^{-1} chlorine		
	Iodometry (n = 3)	DPD (n = 3)	Present method (% RSD) (n = 6)
Air		0.1	0.101 (2.5)
Water		0.1	0.099 (2.2)
Air		0.2	0.197 (2.6)
Water		0.2	0.199 (2.0)
Water		0.5	0.503 (1.9)
Air		1.0	1.03 (0.7)
Water		2.0	2.02 (0.5)
Water		3.0	2.95 (0.6)
Air	5.0		4.95 (0.6)
Water	5.0		4.98 (0.7)
Water	7.0		6.92 (0.8)
Water	10.0		10.21 (1.1)
Air	10.0		9.96 (0.9)
Water	15.0		15.25 (1.0)
Water	20.0		19.80 (1.1)
Air	20.0		20.31 (1.3)
Air	25.0		24.74 (1.5)
Water	30.0		30.51 (1.5)

Table II. Determination of chlorine in swimming pool water and paper bleaching effluents.

Sample	μg mL^{-1} chlorine		
	Iodometry (n = 3)	DPD (n = 3)	Present method (% RSD) (n = 6)
SPW*		1.12	1.30 (0.6)
SPW		0.82	0.85 (0.7)
PBE**	18.6		18.3 (1.3)
PBE	20.5		20.1 (1.2)

*SPW: swimming pool water

**PBE: paper bleaching effluent

Two water samples from a swimming pool and bleaching effluents of a paper mill were analyzed by the method; the results are given in Table II. Whereas the DPD method could be applied to swimming pool water, both the DPD and iodometric comparison methods were difficult to use for the analysis of coloured bleaching effluents. In such cases instead of using the conventional starch - iodine end-point detection, best results were obtained by adding few millilitres of dichloromethane to the titration solution and observing the complete decolorization of the iodine in the lower organic layer as the end point.

Interferences

In contrast with spectrophotometric methods this chromatographic method is free from interference by coloured and humic substances. Should there be any interference of unknown peaks with the 4-bromoacetanilide peak, chromatographic resolution may be improved by changing the conditions, *e.g.* the composition of the mobile phase.

Some substances which may occur in air or water samples were tested as potential interferences. Solutions of standard hypochlorite and each tested species were mixed, and the apparent chlorine concentrations in these samples were measured by the method. The tolerated interferent:chlorine ratios (m/m) in the determination of 5 μg mL^{-1} of chlorine were: Cl^-, ClO_4^-, NO_3^-, PO_4^{3-}, SO_4^{2-}, Zn^{2+}, Cd^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, citrate, tartrate, 500; IO_3^-, Fe^{3+}, Cu^{2+}, and Hg^{2+} and Pb^{2+} (both masked with EDTA), 100; MnO_4^-, $Cr_2O_7^{2-}$, 10; and BrO_3^-, 1. Many reducing substances such as hydrogen sulfide, sulfur dioxide, thiosulfate, nitrite, and thiocyanate are readily oxidized by chlorine and do not therefore coexist with chlorine in real samples. Nitrite is a stubborn species which even in the absence of any chlorine gives a positive reaction with many spectrophotometric reagents [9,10,12-15] leading to the false conclusion of the presence of high levels of chlorine. A test sample containing 2000 μg mL^{-1} nitrite and no chlorine was analyzed by the present method and the chromatogram obtained

(Figure 2C) found to be devoid of any peak in the region of the 4-bromoacetanilide peak, indicating that no misleading conclusions are produced by nitrite when the proposed method is used.

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