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Identification of halogenated compounds produced by chlorination of humic acid in the presence of bromide

Ruud J.B. Peters[a,*], Ed W.B. de Leer[a], Johanna F.M. Verstegh[b]

[a]*TNO Institute of Environmental Sciences, Department of Analytical Chemistry, Schoemakerstraat 97, 2600 JA Delft, Netherlands*

[b]*National Institute of Public Health and Environmental Protection, Antonie van Leeuwenhoeklaan 9, 3720 BA Bilthoven, Netherlands*

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Abstract

The formation of halogenated compounds by chlorination of humic acid with and without bromide present was compared. The presence of bromide ion results in the production of many brominated and mixed bromo–chloro compounds. Trihalomethanes, halogenated aliphatic acids and diacids, and trihalomethane precursors are major products. The presence and identity of the halogenated compounds were also confirmed by the use of an atomic emission detector. The mutagenic activity was determined and shown to be 2–3 times higher when bromide is present during chlorination.

1. Introduction

Chlorine is often used for the disinfection of drinking water. Owing to its high reactivity, chlorine reacts very rapidly with many natural organic compounds, mainly humic materials, present in raw water. This results in the formation of numerous chlorination by-products and an increased mutagenic activity of chlorinated drinking water [1–3], indicating a potential risk for the consumer.

The type and relative amounts of the chlorination by-products varies not only with the organic content of the source water but also with the inorganic species present. If bromide is present then brominated compounds, such as bromoform and the other bromochloro trihalomethanes (THMs), are also produced [4]. During chlorination, bromide is oxidized by chlorine to bromine and chlorination and bromination become competitive reactions. In a study of the concentrations of THMs and dihaloacetonitriles (DHANs) in drinking water by Peters et al. [5], it was shown that the major part of the THMs and DHANs found in Dutch drinking water were brominated. Further, many of the volatile mutagens identified in chlorinated drinking water are brominated or mixed chloro–bromo compounds [6].

However, the volatile halogenated compounds represent only a minor part of the total halogenated products, and these compounds account for only a small part (5–10%) of the total mutagenic activity [6,7]. The major part of the halogenated products is found in the non-volatile

* Corresponding author.

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polar fraction, which is also responsible for most of the mutagenic activity.

Until now, most studies of the non-volatile polar fraction have focused on chlorinated products and little is known about brominated and mixed chloro–bromo products. The aim of this paper is to report the results of the aqueous chlorination of humic acid with and without bromide present. The chlorination products were identified and the mutagenic activity of the extracts of the reaction mixtures was determined.

2. Experimental

2.1. Materials

Analytical-reagent grade chemicals were used and all solutions were prepared with water purified with a Milli-Q system (Millipore). Humic acid was obtained from Fluka. A raw water sample was collected at a treatment plant and stored at 5°C. The dissolved organic carbon (DOC) of the raw water was 1.7 mg/l and the bromide content was ca. 0.2 mg/l. Standards of a number of identified products were purchased from Aldrich and Fluka and some others were synthesized in our laboratory according to procedures described in the literature.

2.2. Chlorination/bromination of raw water

The raw water sample was filtered through glass-fibre filters and a volume of ca. 900 ml was collected in three different bottles. To two bottles NaBr solution was added to achieve bromide contents of 0.5 and 1.5 mg/l. Next, NaOCl solution was added to achieve a chlorine concentration of 0.2 mg/l. Finally, the volume was adjusted to 1000 ml with raw water and the bottles were stored in the dark for 16 h. The extraction and analyses of the trihalomethanes using GC with electron-capture detection were performed as described previously [5].

2.3. Chlorination/bromination of aqueous humic acid

A 400-mg amount of humic acid was dissolved in 500 ml of 0.02 *M* NaOH solution and stirred overnight. After neutralizing the mixture with hydrochloric acid, it was filtered through glass-fibre filters. The resulting humic solution was mixed with 200 ml of 1.0 *M* phosphate buffer (pH 7), 0.05 *M* NaBr solution was added to achieve bromide to chlorine molar ratios of 0, 0.05 and 0.10 and the total volume was adjusted to about 900 ml with deionized water. Then a calculated amount of chlorine, in the form of 0.5 *M* NaOCl solution, was added to establish Cl_2/C molar ratios of 0.5 and 2.0, respectively. Finally, the volume was adjusted to 1000 ml with deionized water and the bottles were stored in the dark. After a reaction time of 16 h, any excess of chlorine was destroyed by the addition of solid sodium arsenite. The reaction mixture was saturated with sodium chloride and acidified to pH 0.5 with concentrated sulfuric acid. The mixture was extracted three times with 100 ml of glass-distilled diethyl ether. The combined ether extracts were stored overnight at −20°C to freeze out residual water and concentrated to 5 ml in a Kuderna–Danish apparatus. The extracts were then carefully purged with nitrogen until dryness. The residue was dissolved in 10.0 ml of ethyl acetate and divided into two portions to be used for the mutagenicity testing and GC–MS analysis. The samples were stored at −20°C.

2.4. Gas chromatographic analyses

Prior to analysis, the chlorinated humic acid extracts were methylated with diazomethane. To 2 ml of the extracts 2 ml of ethyl acetate and 0.2 ml of methanol were added to aid methylation. 1-Chlorododecane was added as an internal standard and the samples were methylated with diazomethane. Diazomethane gas was generated fresh from N-methyl-N-nitroso-*p*-toluenesulfonamide (Merck) and was stripped from the generation vessel into the sample vials with nitrogen gas.

The samples were analysed by GC–MS with an HP 5890 gas chromatograph interfaced with an HP 5970B mass-selective detector. The chromatograph was equipped with a 25 m × 0.2 mm I.D. HP-1 (film thickness 0.33 mm) fused-silica capillary column and helium was used as the carrier gas. The temperature settings were as follows; column oven, programmed from 50°C (5 min) to 300°C (10 min) at 8°C/min; and injector and transfer line, 280°C. For the identification of the chlorination products the mass spectrometer was operated in the scan mode (m/z = 40–400). The samples were also analysed by GC–atomic emission spectrometry (AES) with an HP 5890 gas chromatograph interfaced with an HP 5921A atomic emission detector. The chromatograph was equipped with a 50 m × 0.2 mm I.D. HP-1 (film thickness 0.33 mm) fused-silica capillary column and helium was used as the carrier gas. The temperature settings were the same as in GC–MS. With AES, four elements, carbon, hydrogen, chlorine and bromine, were monitored simultaneously at 495.7, 486.1, 479.5 and 478.6 nm, respectively. The plasma power was 50 W and an additional make-up flow of 40 ml/min of helium.

2.5. *Mutagenicity testing*

Mutagenicity test were performed according to Ames et al. [8] using *Salmonella typhimurium* strain TA 100 with and without metabolic activation (S9 mix). The mutagenic response was calculated as the slope of the linear part of the dose–response curves.

3. Results and discussion

Bromide is often present in raw water, from either natural or anthropogenic sources. In Dutch waters bromide concentrations vary from 0.1 to 0.5 mg/l. During chlorination of the raw water, bromide ion is oxidized to bromine. Bromine seems to be more effective as a halogen-substituting agent [9] and if bromine

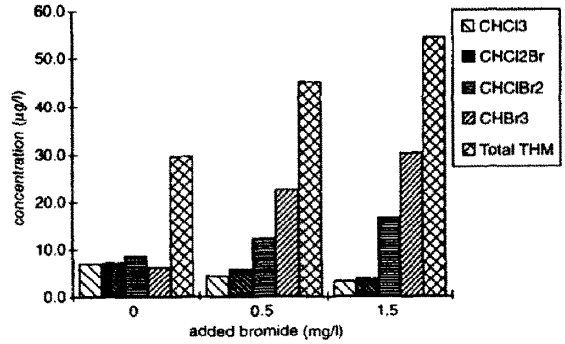

Fig. 1. Results of the chlorination (0.2 mg/l) of a raw water sample (DOC 1.8 mg/l) containing different amounts of bromide. The original raw water had a bromide content of approximately 0.2 mg/l.

acts as an oxidant it will be reduced to bromide ion, which may then be re-oxidized by chlorine. This results in a high bromine incorporation into the well known THMs, as is reflected by the results of the chlorination of raw water used to prepare drinking water (see Fig. 1). The bromide content of the raw water itself was not known exactly but was approximately 0.2 mg/l. Owing to this natural bromide background, chlorination of this raw water in the laboratory resulted in the production of all four THMs. However, if additional bromide was added to the samples the concentration of the higher brominated THMs increased, while that of chloroform and bromodichloromethane decreased. Finally, the total amount of THMs produced by chlorination also increased with high bromide concentrations. Ozone may be used as an alternative to chlorine but, like chlorine, ozone is also capable of oxidizing bromide to bromine, resulting in the production of brominated disinfection by-products [10].

In contrast to the THMs there is only very little information about the information of non-volatile brominated compounds, and there has been no systematic study of these products. Recently, we reported that the major part of the haloacetic acids (HAAs), typical representatives of the non-volatile polar fraction, found in Dutch drinking waters are brominated [11]. Aqueous

chlorination of phenol in the presence of bromide also resulted in the formation of brominated and chloro/bromoacetic acids [12]. These findings suggest that brominated compounds may form a substantial part of the chlorination products if bromide is present in the source water.

The chlorination/bromination of humic acid at pH 7 was performed with chlorine to carbon molar ratios of 0.5 and 2.0 and bromide to chlorine molar ratios of 0.00, 0.05 and 0.10, respectively. Chromatograms of two of the high-chlorine dose experiments are given in Fig. 2a (Br^-/Cl_2 molar ratio = 0) and 2b (Br^-/Cl_2 molar ratio = 0.10), and show that many products were formed. Dominant products identified after chlorination of humic acid without bromide were chlorinated aliphatic acids and diacids, especially dichloro- and trichloroacetic acid, chloro- and dichloromaleic acid and dichlorosuccinic acid. Several chloroform precursors, previously identified by De Leer et al. [3], were also found. These are structures with a di- or trihalomethane group which is easily hydrolysed to trihalomethanes, and are found predominantly in the low-chlorine-dose experiments. Non-chlorinated products include aliphatic diacids and aromatic acids, in addition to small amounts of methyl-substituted aromatic acids and aromatic

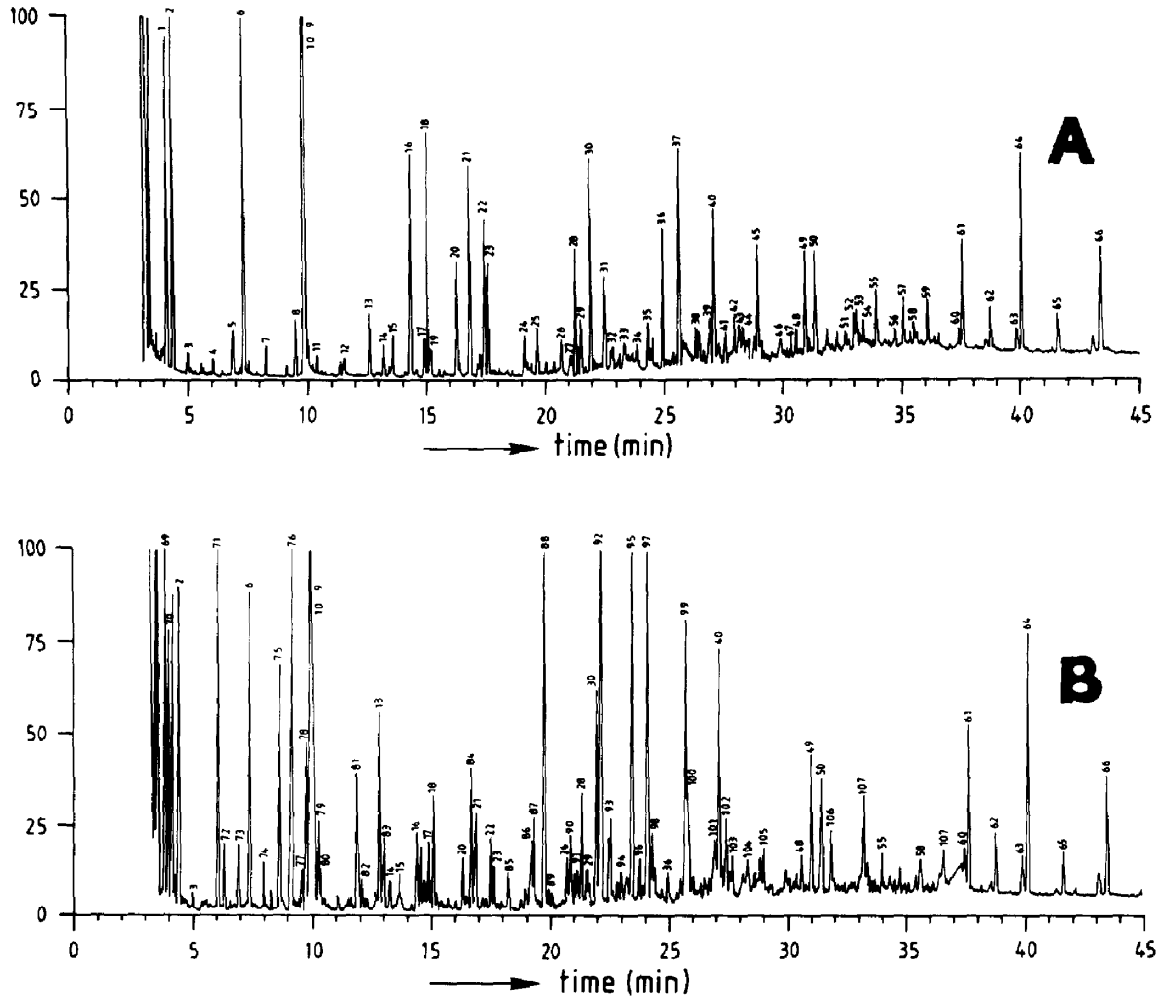

Fig. 2. Chromatograms of two of the high-chlorine-dose experiments (A) without, and (B) with bromide present during aqueous chlorination of humic acid. For peak identification, see Table 1.

glyoxylic acids. Generally, these results agree well with those of earlier studies [1–3].

If bromide was present during chlorination, the chlorinated products were present in smaller amounts, and in addition many brominated products with similar structures were identified. Although dichloro- and trichloroacetic acid are still major products, brominated and mixed bromo/chloroacetic acids are also important products. The same is true for the halogenated diacids. A noticeable difference is the presence of several halogenated alkanes. These include not only the THMs, but also dibromoethane and chlorobromo- and dibromopropane. The latter compounds have also been identified in mutagenic extracts of chlorinated drinking water and shown to be active mutagens [6]. Interesting also is the tentative identification of several THM precursors. The structures of these compounds are similar to the chloroform precursors that were identified by De Leer et al. [3], and they show major peaks in the chromatograms of the reaction mixtures. The non-chlorinated products that were identified were the same as in the chlorination experiment without bromide.

All products that were identified are given in Table 1. The compound numbers correspond with those in the chromatograms in Figs. 1 and 2. The identifications in Table 1 are differentiated into standard confirmed (SC), confident (C) and tentative (T) identifications. SC means that a standard of the identified compound was available, thus allowing confirmation on the same GC–MS system. C means that a reference mass spectrum of the identified compound was available. T means that no standard or reference mass spectrum was available and that the identity was determined from the mass spectrum. If a dash appears in the ID column the compound was not identified at all. However, it was possible to determine the number of chlorine/bromine atoms in the molecule from the isotopic clusters in the mass spectrum. In other instances the mass spectrum allowed the identification of part of the compound. In such an event, the known part of the structure is given in Table 1 and the unknown part is depicted by the letter X.

The reaction products were identified using GC–MS but additional information concerning the elemental composition of the chlorination products was obtained with the use of AES. AES is an element-specific detection method and hence is able to discriminate between chlorinated, brominated and mixed chloro/bromo compounds. In GC–AES, the GC effluent enters a high-temperature microwave-induced helium plasma. The organic compounds are decomposed into their elemental constituents, and the atoms are excited to a higher energy level. If a sufficient number of atoms are exited, the atomic emission signals can be recorded at element specific wavelengths. In this work the emissions of bromine, chlorine, carbon and hydrogen were recorded simultaneously. A multi-element chromatogram of the high-chlorine-dose experiment with bromide is given in Fig. 3. The peak heights of trichloro- and dibromoacetic acid and the internal standard *n*-chlorododecane were used to calculate the relative elemental response factors and thus the empirical formulae of the compounds. The results of the GC–AES analysis confirmed the MS interpretations.

The mutagenic activity of the samples was also determined to see if the addition of bromide resulted in an increased TA-100 activity. In general, the results in the Ames test without metabolic activation showed that the samples in the high-chlorine-dose experiments were twice as mutagenic as those in the low-chlorine-dose experiments. In both instances, however, the addition of bromide during chlorination led to a 2–3 times higher mutagenic activity of the samples. When the Ames test was performed with metabolic activation (S9 mix) the mutagenic activity was substantially lower. The bacterial mutagenicity assay, known as the Ames test, provides a rapid and relatively simple method for detecting mutagenic activity. *Salmonella typhimurium* strain TA-100, containing well characterized mutations in certain genes limiting the growth of colonies, is exposed to a part of the final ethyl acetate extract. If mutagenic compounds are present, reverse mutations will occur, and hence the number of colonies will increase. In order to simulate the metabolism in

Table 1
Compounds identified after chlorination/bromination of humic acid. Peak numbers refer to peak numbers in the chromatograms in Figs. 2 and 3

Peak No.	Compound	ID[a]	RRT[b]
1	Ethyl propionate	SC	0.188
2	1-Butanol	SC	0.200
3	Chloroethanoic acid	SC	0.227
4	2-Chloropropenoic acid	C	0.277
5	Propanedioic acid	SC	0.314
6	Dichloroethanoic acid	SC[c]	0.336
7	Trichloroethanal	SC[c]	0.378
8	1,1,1-Trichloro-2,3-epoxypropane	C	0.434
9	Trichloroethanoic acid	SC[c]	0.449
10	Trimethyl phosphate	SC	0.458
11	3,3-Dichloropropenoic acid	C	0.476
12	2-Hydroxypropanoic acid	C	0.529
13	Butenedioic acid	SC	0.576
14	Chloropropanedioic acid	SC	0.604
15	3,3,2-Trichloropropenoic acid	T[c]	0.614
16	Chlorobutenedioic acid	SC[c]	0.656
17	Chlorobutanedioic acid	SC	0.682
18	Dichloropropanedioic acid	SC[c]	0.688
19	3,3,3-Trichloro-2-hydroxypropanoic acid	T	0.693
20	2,3-Dichloro-4-oxopentenoic acid	T[c]	0.744
21	2,2-Dichlorobutanedioic acid	SC[c]	0.767
22	2,3-Dichlorobutenedioic acid	SC[c]	0.798
23	Isomer of 22	SC	0.803
24	Dichlorinated compound	–	0.874
25	2,2-Dichloropentaneoic acid	C	0.898
26	Methylfurandicarboxylic acid	C	0.944
27	Benzenedicarboxylic acid	SC	0.965
28	2,3-Dimethoxybutenedioic acid	T	0.971
29	2-Chloro-3-dichloromethylbutenedioic acid	SC	0.981
30	1-Chlorododecane	SC[c]	1.000
31	Isomer of 27	SC	1.027
32	2,3,4,4-Tetrachloro-2-pentenedioic acid	T	1.041
33	4-Oxoheptanoic acid	T	1.068
34	Trichlorinated compound	–	1.089
35	2-Carboxy-3,5,5,5-tetrachloro-4-oxopentanoic acid	T	1.100
36	2,3,3,5,5,5-Hexachloro-4-hydroxypentanoic acid	T	1.137
37	Isomer of 35	T	1.169
38	Trichlorinated compound	–	1.207
39	2,3-Dicarboxybutenedioic acid	T	1.229
40	Benzenetricarboxylic acid	SC	1.235
41	Phthalate	T	1.257
42	Isomer of 40	–	1.275
43	Trichlorinated compound	–	1.284
44	Methylbenzenetricarboxylic acid	C	1.291
45	Trichlorinated compound	–	1.321
46–53	Benzenetetracarboxylic acid and glyoxylic acid isomers of 46	C	
54–58	Benzenepentacarboxylic acid and glyoxylic acid isomers of 54	C	
59–68	Fatty acids and alkanes	C	
69	Dibromometane	SC[c]	0.174
70	Bromodichlorometane	SC[c]	0.179
71	Chlorodibromomethane	SC[c]	0.274

Table 1 (*continued*)

Peak No.	Compound	ID[a]	RRT[b]
72	1,2-Dibromoethane	SC[c]	0.287
73	Bromoethanoic acid	SC[c]	0.314
74	1,3-Bromochloropropane	T[c]	0.363
75	Tribromomethane	SC[c]	0.393
76	2-Chlorobutenoic acid	T[c]	0.416
77	Isomer of 76	T[c]	0.437
78	Bromochloroethanoic acid	SC[c]	0.443
79	Monochlorinated acid	–	0.468
80	1,2-Dibromopropane	T[c]	0.471
81	Dibromoethanoic acid	SC[c]	0.540
82	1,2-Bromochloropropanoic acid	T	0.550
83	Butanedioic acid	SC	0.588
84	Tribromoethanoic acid	SC[c]	0.759
85	Dibromopropanedioic acid	T	0.831
86	Bromochlorobutenedioic acid	T	0.876
87	Isomer of 86	T	0.879
88	2,4-Dichloro-2-bromo-3-oxobutanoic acid	T[c]	0.899
89	2-Carboxy-3-bromobutanoic acid	T	0.909
90	Dibromobutenedioic acid	T	0.951
91	2-Chloro-3-bromobutanedioic acid	T	0.965
92	2,5-Dichloro-4-bromo-3-oxopentanoic acid	T[c]	1.008
93	2-Chloro-4-bromo-3-oxopentanedioic acid	T	1.025
94	Non-halogenated compound	–	1.028
95	4,5-Dibromo-2-chloro-3-oxopentanoic acid	T[c]	1.067
96	Non-halogenated acid	–	1.082
97	2,5-Dibromo-6-chloro-3-oxopentanoic acid	T	1.097
98	Halogenated acid	–	1.107
99	X-COCHBrCH$_2$COOH	–	1.171
100	X-COCHClCOOH	–	1.174
101	X-COCHBrCH$_2$COOH	–	1.226
102	X-COCHBrCH$_2$CH$_2$COOH	–	1.249
103	Dibrominated compound	–	1.262
104	Phthalate	T	1.313
105	Halogenated acid	–	1.321
106	X-COCHClCOOH	–	1.449
107	X-COCHClCOOH	–	1.513

[a] SC = standard confirmed; C = confident; T = tentative.
[b] Retention time relative to the internal standard 1-chlorododecane.
[c] Elemental composition confirmed by GC–AES.

higher organisms, a rat liver homogenate (S9 mix) can be incorporated in the test. Compounds that are not mutagenic may be metabolically activated and vice versa.

The most important mutagen identified in chlorinated drinking water so far is 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone, referred to as Mutant X or MX [13]. The identification of many brominated compounds in finished drinking water and in model studies suggests that brominated MX analogues (BMX) may also be present in finished drinking waters. Although we have already confirmed the formation of BMX in model experiments their presence in drinking water is still unknown [14,15]. If BMX is present in drinking water this may explain the part of the mutagenicity not accounted for by MX.

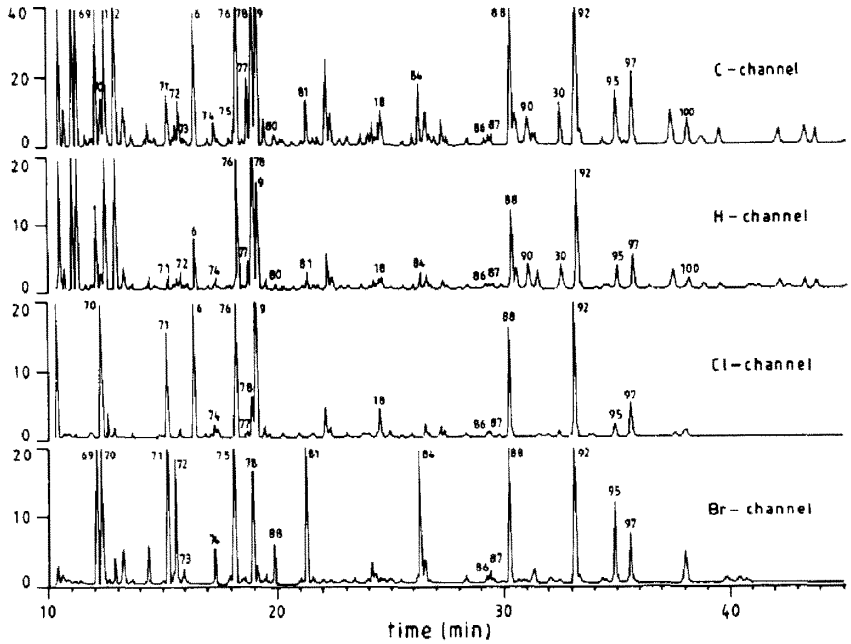

Fig. 3. Multi-element chromatogram of the GC–AES analysis of a sample of chlorinated/brominated humic acid. The emissions of carbon, hydrogen, chlorine and bromine were recorded simultaneously and are shown in that order. Compounds that were identified using GC–AES are indicated in Table 1.

4. Conclusions

The chlorination of humic acid in the presence of bromide resulted in the production of many brominated and mixed bromo–chloro compounds. The type of compounds formed is similar to previously identified chlorinated products. Trihalomethanes, halogenated aliphatic acids and diacids and trihalomethane precursors were major chlorination products. The presence and elemental composition of several of the brominated compounds were also confirmed by the use of an atomic emission detector. If bromide was present during chlorination, the mutagenic activity increased significantly. The results of this study indicate that brominated organohalogen compounds are important disinfection by-products and that brominated MX analogues may explain that part of the mutagenic activity of chlorinated drinking water that is not accounted for to date.

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