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Nonvolatile Organic Compounds in Treated Waters

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Over the past decade much information has been published on the analysis of organics extracted from treated water. Certain of these organics have been shown to be by-products of the chlorination disinfection process and to possess harmful effects at high concentrations. This has resulted in increased interest in alternative disinfection processes, particularly ozonation. The data on organics had been largely obtained by using gas chromatography-mass spectrometry, which is only capable of analyzing, at best, 20% of the organics present in treated water. Research in key areas such as mutagenicity testing of water and characterization of chlorination and ozonation by-products has emphasized the need for techniques suitable for analysis of the remaining nonvolatile organics.

Several methods for the isolation of nonvolatile organics have been evaluated and, of these, freeze-drying followed by methanol extraction appears the most suitable. Reverse-phase HPLC was used for separation of the methanol extract, but increased resolution for separation of the complex mixtures present is desirable. In this context, high resolution size exclusion chromatography shows promise. Characterization of separated nonvolatiles is possible by the application of state-of-the-art mass spectrometric techniques.

Results obtained by these techniques have shown that the nonvolatile organic fraction of chlorinated drinking water consists of many discrete compounds. Among these, some of the chlorinated compounds are almost certainly by-products of disinfection. Studies of the by-products of ozonation of fulvic and humic acids isolated from river waters have indicated a similar proportion of nonvolatile organics. Further, ozonation can result in the release of compounds that are trapped in the macromolecules.

Introduction

This paper presents a short review of research into nonvolatile organics in water, with particular emphasis on analytical methodology. In order to limit the size of the paper, the review is necessarily selective in the citation of research from the scientific literature. Results of recent work at the Water Research Centre (WRC) will be used to highlight important areas of research into nonvolatile organic compounds in treated water.

The need for methods of analysis of nonvolatile organics stems from the rapid growth of interest in organics in treated water over the past ten years (*1*, *2*). This interest arises from concern over the possible long-term health effects from ingestion of organics in treated waters, particularly halogenated species (*1*). The vast majority of identifications of organic material in treated waters have been generated by the use of gas chromatography-mass spectrometry (GC-MS) techniques, which provide a relatively rapid and straightforward method of analysis. It is now generally accepted that 80–90% of the organic matter present in both raw and treated waters is not amenable to this type of analysis due to insufficient volatility. Further, there is some evidence that a similar proportion of the halogenated organic matter produced by chlorination is also not amenable to GC-MS analysis (*3*). Methods for the characterization of the nonvolatile organics in treated waters are thus urgently needed to give information to complement that obtained from GC-MS. Undoubtedly, concern over possible health effects of the nonvolatile organics, which

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represent the bulk of the organic matter in water, is heightened by the paucity of information concerning them. The rapidly expanding application of short-term bioassay tests, such as the Ames test, to drinking waters is beginning to emphasize this need for qualitative techniques for nonvolatile compounds. Many of the mutagens detected do not seem to be identifiable by GC-MS methods (*4, 5*).

There are many diverse problems associated with the characterization of nonvolative organics in treated waters, but most of these are a result of the low levels often present, the wide range of compound types and extreme range of molecular weights. Currently there is no analytical method available for nonvolatiles that is comparable in sensitivity and ease of application to the GC-MS approach used for volatiles. It is unlikely that a single method will be sufficient and thus the various steps in the analysis of nonvolatiles are reviewed below.

Isolation Methods

Isolation of a specific compound from a very dilute aqueous solution of a complex mixture of components is a relatively easy process, since the extraction method can be tailored to the chemical and physical properties of that compound. However, isolation of the broadest possible fraction of a complex mixture of unknowns is a considerably more difficult problem. A large variety of methods have been examined for isolation of organics from water, and these have been recently reviewed (*6*). Most of these have been developed specifically for volatile compounds and are, therefore, outside the scope of this paper. Relatively few methods have been developed for nonvolatiles, but several of the methods used for volatiles have been applied, with or without modification.

Research specific to the isolation of nonvolatile organics from water has been mainly concerned with the humic substances. Methods used include adsorption, precipitation and liquid-liquid extraction, and these have been reviewed in some detail (*7, 8*). Recent research has focused on the use of XAD resin adsorption which has been shown to provide high recoveries (>80%) of humics from raw waters (*9, 10*). However, XAD is not recommended for the concentration of humic substances after chlorination, as these are very poorly adsorbed (*3*). Other techniques which have been applied to nonvolatile organics in water are carbon–chloroform extraction (*11*), reverse osmosis (*12*) and freeze-drying (*13, 14*) which suffers form the limitation of being a batch process. Result from these methods suggest that freeze-drying and reverse osmosis provide extracts containing the widest range of compounds. With freeze-drying, the recoveries obtained for particular compound types was found to be solvent dependent, but methanol provided the highest overall recoveries (*14*).

Separation Methods

Separation of nonvolatile organics ideally requires a method that avoids derivatization, does not chemically alter the organics during separation, and has high resolving power and high sensitivity of detection for separated compounds. While no method meets all of these criteria, high performance liquid chromatography (HPLC) approaches this ideal and is the method of choice for the separation of nonvolatile organics.

A variety of fractionation procedures have been used prior to HPLC, particularly where fractions are being prepared for mutagenicity testing. Thus, thin-layer chromatography, column liquid chromatography and chemical fractionation have all been applied for preseparation of nonvolatile organics, and these approaches have been recently reviewed (*5,15*). Modern HPLC often involves the use of several chromatographic methods and most of these have been used for separation of nonvolatile organics from treated waters. Size exclusion chromatography (SEC) has shown that the molecular weights of nonvolatiles in water range from a few hundred to >15,000 (*3,16,17*). One drawback in the characterization of nonvolatile organics according to molecular size by use of SEC is the competition between exclusion processes and nonexclusion processes such as adsorption. This is a particular problem for an unknown mixture of compounds of widely different polarity and can invalidate a molecular weight calibration produced using a mixture of known compounds.

Ion-exchange chromatography has provided high resolution separation of nonvolatiles from chlorinated and ozonated waters prior to identification and mutagenicity testing (*18,19*). Reverse-phase HPLC has been applied to the separation of nonvolatiles from treated water to provide fractions for mutagenicity testing (*20*) and mass spectometric characterization (*13,14*). Finally, adsorption HPLC on silica gel G was used to separate neutral organics from a drinking water concentrate prior to mass spectrometry (*21*).

Although HPLC shows inherent advantages for the separation of nonvolatile organics, it suffers from two limitations. There is no universal detector for HPLC which is equivalent in sensitivity to the flame ionization detector used in GC. Several highly sensitive detectors are available, but these are also highly selective and, therefore, inappropriate to the detection of a wide range of compounds. Detection of separated components is usually achieved by

ultraviolet absorption and is therefore limited to those compounds containing an ultraviolet chromophore. Secondly, since nonvolatile organic fractions isolated from water usually contain a large number of compounds of diverse chemical type, even high efficiency HPLC columns cannot provide complete separation into individual components. Thus, methods for characterization of HPLC fractions are required that are capable of handling a mixture of components, which is discussed below.

Characterization

Techniques for the characterization of nonvolatile organics can be divided into two broad categories on the basis of approximate molecular weight, each requiring different procedures.

High Molecular Weight Material (>2000). This category consists mostly of humic and fulvic acids—polydisperse polymeric materials, which are not amenable to mass spectral characterization as intact molecules. General methods for their characterization have been extensively reviewed (*8,22*) and only recent work which centers around the production of haloforms and other halogenated organics from chlorination of humic acid fractions (*23–25*) will be discussed.

Halogenated nonvolatile reaction products of humic acids have been characterized by total organohalogen measurement and size exclusion chromatography (*3,26*). The majority of structural studies on aquatic humic materials involve some form of degradation step to produce smaller, volatile compounds for analysis by GC-MS. Included in this approach are attempts to identify the volatile by-products from chlorination (*27*) and ozonation (*28*) of humic materials. The substituted aromatic compounds identified in degradation studies have similar structures to those obtained from soil humic material, suggesting that the two types of humics have similar compositions. Recently, attempts have been made to obtain mass spectrometric data on larger fragments of humic acid (*29*). This has involved field desorption mass spectrometric (FD-MS) analysis of fragments formed after chlorination or permethylation of humic material, but as yet no structural information has been reported.

Low Molecular Weight Compounds (<2000). As mentioned previously, any method used for characterization of nonvolatile organics in HPLC fractions must, in most cases, be capable of working with low levels of individual compounds in complex mixtures. Thus, most of the classical methods of characterization, e.g., nuclear magnetic resonance, infrared and ultraviolet spectrometry, are unsuitable, and necessarily some type of mass spectrometry must be employed. Conventional electron impact mass spectrometry using a direct insertion probe offers a considerable extension to the types of compound that can be handled by GC-MS. However, electron impact suffers from two limitations: the compound of interest has to be volatilized prior to ionization and the molecular ion is frequently of low intensity with respect to fragment ions. The first limitation can be overcome to some extent by the use of rapid heating and "in-beam" techniques, which have been recently reviewed (*30*). The second limitation, however, is difficult to overcome and results in extremely complex, overlapping mass spectra in the case of mixtures.

Ideally then, for mass spectrometry of mixtures of nonvolatiles, a method is required that can ionize compounds in the solid state with transfer of very little excess energy. This would provide a mass spectrum consisting of intense molecular ions with few, if any, interfering fragments ions. Several ionization methods which can achieve this are available, for example: direct chemical ionization (DCI) (*30*), plasma desorption (PD) (*31*), laser desorption (LD) (*32*), field desorption (FD) (*33*) and, most recently, fast atom bombardment (FAB) (*34*). All of these techniques provide intense molecular ions and only limited fragmentation for certain types of nonvolatile organics and are, to some extent, complementary. At the present time, FD-MS has received more extensive application than any of the other techniques and appears to be applicable to the widest range of compound types (*35*). Surprisingly, the use of FD-MS for the characterization of nonvolatile organics separated by HPLC has received only limited study. However, it has been used for analysis of herbicides and their by-products in surface waters (*36,37*) and organics in drinking water (*14,21*). While it has contributed to the identification of only few compounds so far (*13*), it has shown that a complex mixture of nonvolatile organics can be present in potable water but it is impossible to resolve this into individual components even by modern HPLC.

A limitation of FD-MS for the identification of nonvolatiles is the lack of structural information provided. Accurate mass measurement of molecular ions can provide empirical formulae, but a number of structures will still be possible. Recently, methods for obtaining structural data from selected ions, namely, collisional activation (CA) (*38*) coupled with either (B/E) linked scanning (*39*) or mass-analyzed ion kinetic energy spectrometry (MIKES) (*40,41*) have become more widely available. Useful fragmentation information similar to that obtained from electron impact mass spectrometry can be obtained for any ion (of sufficient intensity) in a mass spectrum, which makes the method advantageous for the analysis of mixtures. Reports of the

combined use of FD-MS and CA have only recently appeared, but the technique offers great promise for the identification of nonvolatile organics (*42*).

Chemical Studies

The broad spectrum approach to determining the nature and behavior of nonvolatile organics in water has been supplemented by laboratory studies on specific nonvolatile organics known or believed to be present in water. Much of this type of work relates to investigations of the organic by-products formed during chemical disinfection processes such as chlorination and ozonation (*3,18,27,29*). Most studies have been confined, due to a lack of suitable techniques, to products identifiable by GC-MS, i.e., volatile organics. Indirect evidence from such studies, such as results from TOC and TOCl measurement, suggests that a considerable proportion of the by-products are nonvolatile in nature. However, its characterization awaits the development and application of appropriate analytical methods.

The following sections of the paper are devoted to some relevant features of WRC research. In particular, an overall analytical procedure for identification of nonvolatile organics in water, which has been developed recently, is outlined in some detail. Two additional features related to "chemical studies" are included. One involves the laboratory chlorination of uracil. 5-Chlorouracil and other halogenated compounds were detected in treated water in earlier work (*13*). The work reported there was carried out to test the hypothesis that such a compound is formed from uracil (a constituent of sewage effluent) during water treatment chlorination. The other aspect, very much related to the subject of nonvolatiles in water, is the identification of by-products of aqueous ozonation of humic and fulvic acids and some preliminary findings from this work are presented.

Experimental

Isolation

The procedures used for isolation of nonvolatile organics from treated water have been reported in detail elsewhere (*13*). Basically, these involve the freeze-drying of water samples followed by methanol extraction of the recovered freeze-dried solids. Initially, adsorption by XAD was considered as a potential isolation method and in order to provide a comparison between freeze-drying and resin extraction, the following experiments were carried out. Small samples (5 l.) of a treated water were collected; one was acidified (to pH 3 by using ~4*N* HCl), one basified (to pH 10 by using ~4*N* NaOH) and the other left at neutral pH. Each of these samples and a blank consisting of double-distilled water were passed through a resin column (XAD-2), which was subsequently eluted with methanol (~100 ml). The methanol eluates were then concentrated and examined by HPLC as detailed below.

Separation: High Performance Liquid Chromatography

The equipment used consisted of two solvent delivery systems (Waters Associates, Model 6000A), a gradient former (Waters Associates, Model 660) and two ultraviolet absorption detectors operated in series (Cecil Instruments, Model CE 212; LDC, Model 1203), at 280 nm and 254 nm. Syringe injections were made through a stop-flow septumless injection port for analytical separations (up to 10 μl injected) and via an injection valve (Rheodyne Inc., Model 7125) for preparative separations (up to 200 μl injected). Columns (20 cm × 7 mm ID) were packed in our laboratory (*13*) with 5 μm particle size Spherisorb-ODS (Phase Separations Ltd.) and generally had an efficiency of about 18,000 theoretical plates (HETP, 0.01 mm; reduced plate height, 2.2) as measured from a standard mixture of phenols (*43*).

A linear gradient was established from two solvent mixtures consisting of 1% methanol in 0.1% aqeous acetic acid (A) and 90% methanol in 0.1% aqueous acetic acid (B). The gradient was run over 30 min from 0% to 100% B. The flow rate was maintained at 2.0 ml/min.

Size exclusion chromatography (SEC) was carried out on a TSK 3000 SW (Toyo Soda Co.) column (30 cm × 7 mm I.D.) with the use of either water or an aqueous solution of potassium chloride and sodium dihydrogen orthophosphate (0.1*M*) as eluent (1 ml/min). Injection and detection were performed as for reverse-phase chromatography.

Characterization

Preparative HPLC fractions were selected on the basis of a relatively high ultraviolet absorption for mass spectrometric analysis on a VG Micromass ZAB lF instrument. Electron impact was carried out at 70 eV electron voltage, 200 μA trap current, 8 kV accelerating voltage, source temperature of 200°C and a resolution of ~4000. Samples were introduced into the source via an independently heated direct insertion probe. Field desorption mass spectra were obtained on the same instrument, also at 8 kV accelerating voltage and with a total extraction voltage of 10 kV. The FD mass spectra were obtained at a resolution of ~1500, using 10 μm

tungsten wires with carbon microneedles (average length ~40 μm) grown in the usual manner (*44*). All mass spectra were acquired and processed on a VG Datasystem computer.

Accurate mass measurements were made on selected peaks using either data system acquisition or peak matching. Collisional activation (CA) studies were performed by admitting the collision gas (helium) into a collision cell (in the first field free region) to a pressure sufficient to reduce the intensity of the parent ion beam by 60%. The CA mass spectrum was then obtained by scanning the mass spectrometer at a fixed ratio of B/E and recorded oscillographically.

Chemical Studies

Chlorination of Uracil. A series of laboratory experiments was carried out on the aqueous chlorination of uracil. Distilled water (2 l.), buffered (pH 7.5) with borate, was spiked with uracil (1 and 10 mg/l.) and aqueous hypochlorite added to produce a chlorine residual (12 and 1.2 mg/l.). The solutions were stirred continuously and maintained at ambient temperature (20°C) in a water bath. The experiments were carried out in the dark, and samples were withdrawn at regular intervals for determination of pH, free and total chlorine residuals (DPD method) and 5-chlorouracil production. The latter was monitored by freeze-drying the sample (10 ml), extracting the residue with methanol (3 × 5 ml) and submitting the extract to HPLC. One sample from the experiment carried out at high chlorine residual was extracted with *n*-pentane (2 × 5 ml) and analyzed for haloforms by GC-MS. System blanks were carried out in an identical manner to that above, but without addition of uracil.

Ozonation of Humic and Fulvic Acids. Ozone was prepared from dry oxygen (typically ~15 mg/min at a flow rate of 180 ml/min), using a Gallenkamp GE-150 generator. The output was monitored before each experiment by determining the amount of free iodine liberated from KI solution (*45*). Reactions were performed in a twin-necked round-bottomed flask (500 ml) fitted with a bubbler.

Solutions of fulvic and humic acids (200 mg) extracted from river water as previously described (*46*) were prepared in aqueous 1*M* NaOH (25 ml) and diluted with double-distilled water (175 ml). Solution pH was adjusted to ~6 by addition of aqueous HCl prior to ozonation (1 hr, typically 5 mg O_3/mg fulvic or humic acid). By means of a technique based upon adsorption onto XAD-2 resin (*47*), unozonized and ozonized solutions were extracted after acidification to pH 1 with aqueous HCl. After concentration under reduced pressure (~ 2 ml), the aqueous eluate was examined by HPLC.

The XAD-2 resin column was sequentially eluted with ether (25 ml) and methanol (30 ml). The ether fraction was concentrated (to ~1 ml) by evaporation prior to GC and GC-MS analysis, and the methanol fraction was concentrated (to ~2 ml) under reduced pressure prior to HPLC analysis.

The XAD-2 resin was regenerated using a modified procedure to reduce the amount of organic contaminants released from the resin (*48*). Blank experiments were carried out for all of the ozonations and resulted in chromatograms which were essentially devoid of peaks.

GC was performed on a chromatograph equipped with flame ionization detector (Carlo Erba Fractovap 2150), Grob splitless injector, and a wall-coated OV-1 Pyrex capillary column (25 m). GC-MS was carried out in the electron impact mode (Finnigan 4000) with computerized data acquisition and processing (INCOS 2300). A wall-coated silicone-fluid fused silica capillary column (20 m) was used with a Grob splitless injector.

HPLC equipment used was similar to that outlined earlier in the experimental section consisting of two-solvent delivery systems (Waters Associates, Model 6000 A), a gradient programmer (Water Associates, Model 660), and an ultraviolet absorption detector (LDC Spectromonitor II) operating at 240 nm. A reverse-phase column (25 cm × 4.6 mm I.D.) of C_{18}-bonded silica (DuPont Zorbax ODS) was used with a linear gradient of water/methanol (0% to 100%) containing an ion-pairing reagent (tetrabutyl ammonium bromide: 0.01*M*).

Results and Discussion

Isolation

As mentioned above, our studies with model compounds have indicated that freeze-drying and methanol extraction is a very efficient method for isolation of a wide range of nonvolatile organics from drinking water. However, it does suffer from two limitations: (1) only a fixed sample size can be processed in one batch and (2) a proportion of the inorganics in the sample is dissolved in the methanol extract. These problems led us to explore the possibility of using XAD-2 resin as a method for obtaining a nonvolatile organic concentrate that was relatively low in inorganics from a large volume of water sample.

Figure 1 compares the reverse phase HPLC chromatograms derived from a drinking water sample at neutral pH using the freeze-drying/methanol extraction and XAD-2 resin/methanol extraction techniques. The XAD-2 extract exhibits a more pronounced "hump" of unresolved compounds and fewer discrete peaks than the freeze-dried extract.

Furthermore, a larger proportion of the XAD-2 extract (eight times) had to be used to obtain a similar ultraviolet response on the chromatograms. The XAD-2 extracts obtained at acidic and basic pH gave chromatograms similar to the neutral pH extract but with fewer discrete peaks. From this comparison of XAD-2 extraction with freeze-drying, it is possible to make the following point with respect to ultraviolet-absorbing compounds present in treated water. XAD-2 resin is less efficient at concentrating nonvolatile organics than freeze-drying and results in an HPLC chromatogram with fewer discrete peaks.

It is probable that this lower efficiency of XAD-2 is to some extent sample-dependent, since the water sample used to compare the different extraction methods was a chlorinated drinking water. It has been suggested (*3*) that the adsorption efficiency of XAD-2 resin for humics is significantly lower for water that has been chlorinated, as compared to the source water.

Freeze-drying and methanol extraction appears to offer the best overall recoveries for a wide range of organics. The major problem with this method remains the significant quantity of inorganics which is dissolved by the methanol. These elute as broad peaks on reverse-phase HPLC and SEC and can interfere with mass spectrometric characterization, which is discussed below. We have evaluated several methods for desalting methanol extracts (*14*), but none of these have been successful in reducing the level of inorganics without adversely affecting the organics. It is possible that the problem will be alleviated by the use of SEC as a further HPLC separation step.

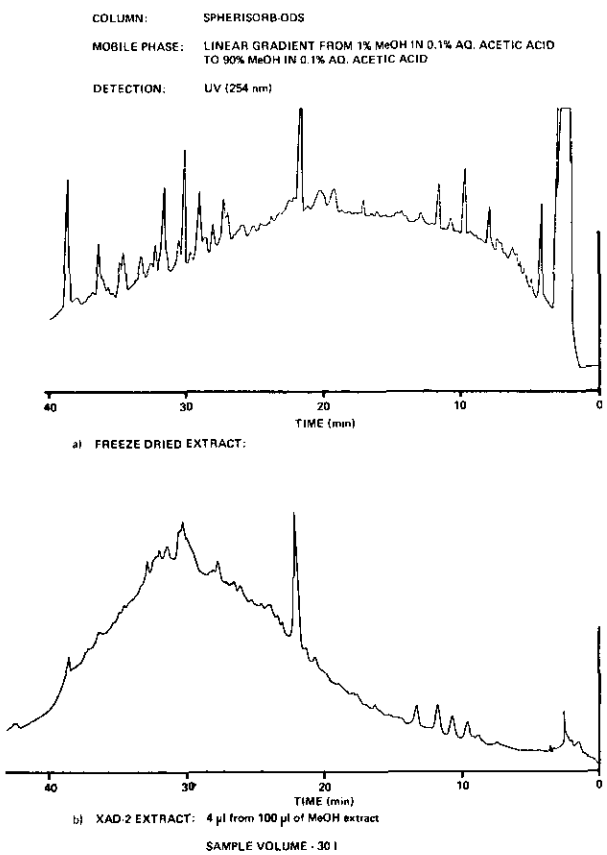

FIGURE 1. HPLC profiles of methanol extracts of treated water samples concentrated by (*a*) freeze-drying and (*b*) resin adsorption. Eight times more sample was needed to produce chromatogram (*b*).

Separation

Reverse-phase HPLC is the most suitable method for the separation of complex mixtures of nonvolatile organics isolated from treated water. HPLC chromatograms of treated water extracts consist of a large early eluting peak and many discrete peaks superimposed on a "hump", presumably consisting of unresolved ultraviolet-absorbing compounds (e.g., Fig. 1*a*). Using preparative reverse-phase HPLC, it is possible to separate an organic extract into a large number of fractions for characterization by mass spectrometry. Unfortunately, it is not practicable to examine all of these using the newer methods of MS characterization and it is essential to develop criteria for selection of particular HPLC fractions. Previously, we have selected fractions according to either relatively high ultraviolet absorption and/or relatively high total organohalogen (TOX) values (*14*). However, recent MS evidence (see below) has indicated that the use of TOX levels as a criterion is severely handicapped by the presence of inorganic chlorides. Preparative reverse-phase HPLC fractions that are selected on the basis of high ultraviolet absorption are always found to consist of mixtures of compounds by subsequent MS analysis and only rarely consist of one major compound. This complexity has encouraged us to seek a further separation step in an attempt to reduce the complexity of the mixtures prior to MS characterization. Further separation according to molecular size was an attractive possibility and a high performance size exclusion column was evaluated for this purpose.

The retention volumes and molecular weights of a series of proteins, used to provide a molecular weight calibration, and some model compounds are shown in Table 1. These were obtained on an SEC column using both water (very low ionic strength) and 0.1*M* KCl/NaH_2PO_4 (high ionic strength) as the mobile phase. With water as the mobile phase, an elution order based on molecular size was not obtained. One of the proteins, albumin, was irreversibly adsorbed on the column and the largest

Table 1. Retention volumes on SEC under different elution conditions.

Solute	Mol. wt.	Retention volume, ml[a]	
		H_2O	0.1*M* KCl/NaH_2PO_4
Ferritin	444000	7.0	6.8
Catalase	232000	5.0	8.0
Albumin	67000	NE	8.8
Ribonuclease	13700	ND	10.5
Adenosine diphosphate	550	4.9	11.1
Chlorouridine	278	5.8	11.5
Chlorouracil	146	6.0	11.7
Uracil	112	7.7	12.0
Anisole	108	17.0	ND

[a]ND = not determined; NE = not eluted.

protein, ferritin, eluted after a smaller one, catalase. Further evidence of the occurrence of ionic effects was provided by the small retention volumes of four of the model compounds and the exceptionally high retention volume for the smallest model compound, anisole. Changing the mobile phase to one of higher ionic strength resulted in the expected elution order for the proteins and model compounds based purely on molecular size. The inherently low peak capacity of SEC columns is also demonstrated in that a retention volume of only ~5 ml is available for separation of compounds with a molecular weight spread of ~100 to 500,000. Thus, these columns are appropriate for a crude separation of nonvolatile organics, and are best applied to fractions obtained from reverse-phase HPLC rather than to a total drinking water extract. In order to give some insight into the resolving power of SEC columns for nonvolatile organics of drinking water, a broad reverse-phase HPLC fraction was chromatographed on a SEC column. The chromatogram (Fig. 2) shows two major peaks with some fine structure superimposed suggesting that the extract contains nonvolatile organics of two broad molecular size ranges. It is not possible to determine the molecular size range from this chromatogram, since this is dependent on the nature of the compounds used for calibration of the column which cannot be extrapolated to a complex mixture of unknown composition.

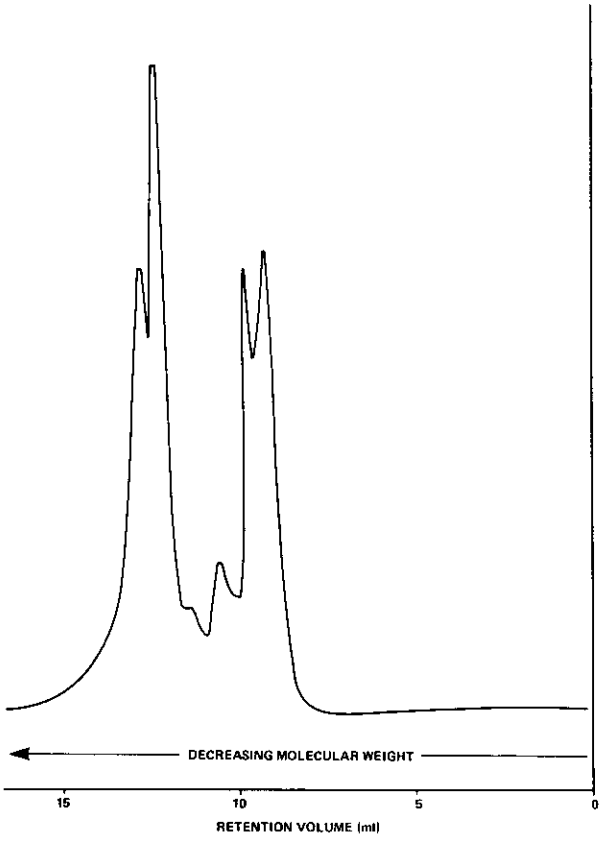

FIGURE 2. Analysis of a reverse-phase HPLC fraction from a drinking water extract using size exclusion chromatography.

Characterization

The results obtained using electron impact (EI) and field desorption (FD) mass spectrometry both show that reverse-phase HPLC fractions of drinking water extracts contain mixtures of compounds with a wide range of molecular weights in agreement with other reports (*21*). An example of a FD-MS of a reverse-phase HPLC fraction of a treated water extract is shown in Figure 3. This represents one of the more complex fractions obtained but illustrates the wide range of compounds present, since the majority of ions can be considered to be molecular ions under the FD-MS conditions used. Identification of these compounds presents many problems and the identifications reported to date (*13*) generally constitute cases where HPLC fractions have contained only one or two major compounds. Halogenated compounds are present, as illustrated by the FD-MS of another HPLC fraction of a treated water extract (Fig. 4). This shows

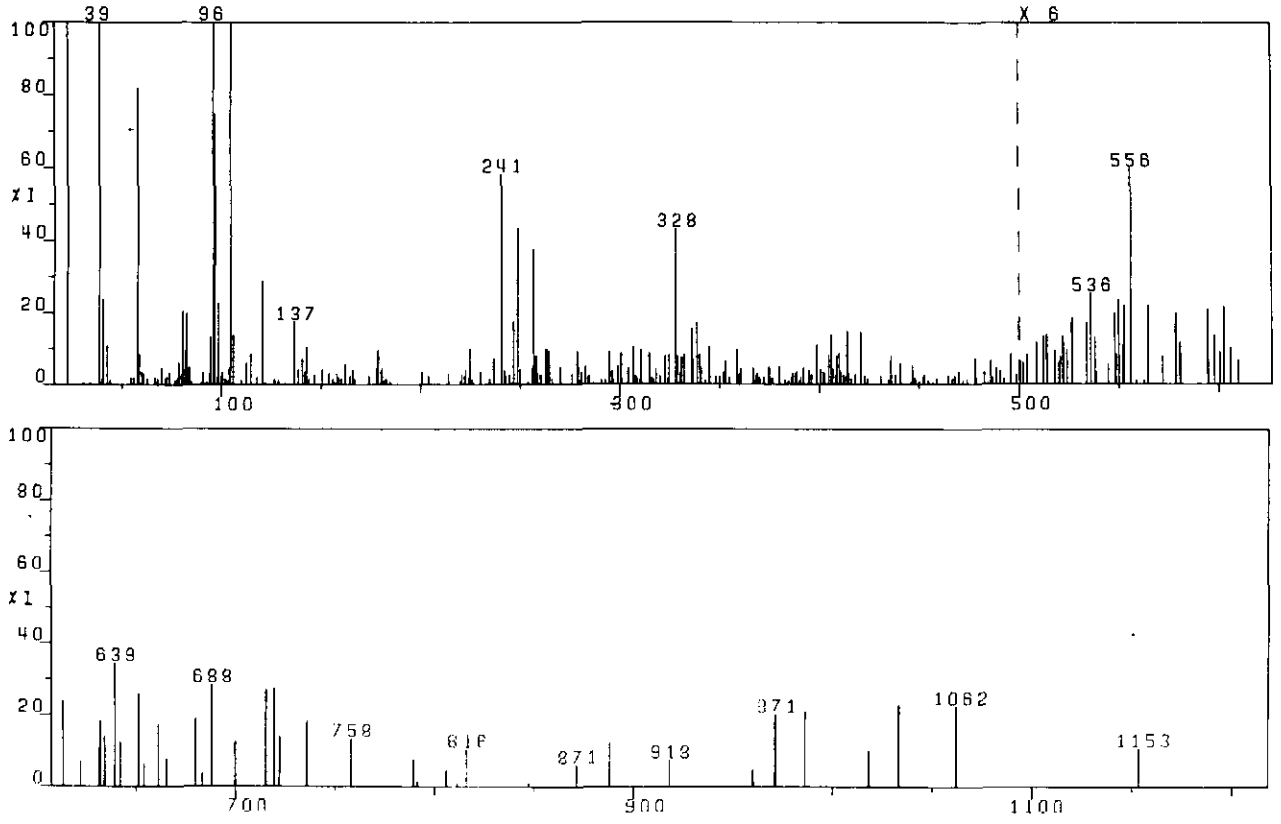

FIGURE 3. FDMS of a reverse-phase HPLC fraction from a treated water extract.

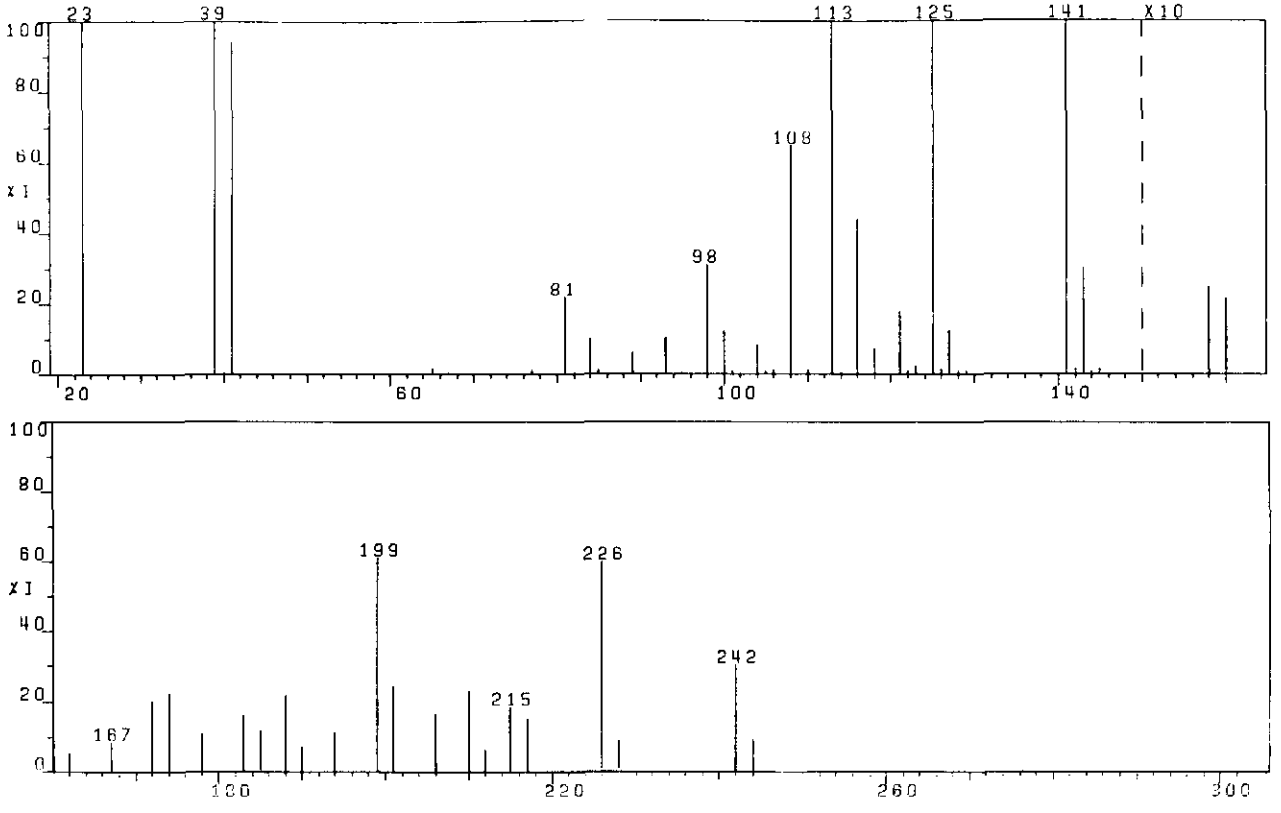

FIGURE 4. FDMS of a reverse-phase HPLC fraction from a treated water extract indicating halogen-containing compounds.

several ions with isotope ratios indicative of compounds which contain chlorine and/or bromine. The methodology we are applying to attempt identification of the nonvolatile organics is shown schematically (Fig. 5).

First, low resolution FD-MS is obtained on a fraction to provide nominal mass data for the organics present and information on the emitter current at which they desorb. Then accurate mass data and hence empirical formulae are obtained on each FD-MS peak of interest by using suitable reference compounds. Either a calibration table is created by the Datasystem which then automatically performs the accurate mass determination or the peak is manually matched against a reference ion and its accurate mass calculated. Structural information is then obtained for each compound by the use of collisional activation and B/E linked scanning. This technique produces the equivalent of a normal electron impact mass spectrum for any molecular ion of sufficient intensity generated by FD-MS.

An example of a FD-CA mass spectrum obtained by linked scanning is shown in Figure 6. The compound used to produce this spectrum was tetrabutylammonium chloride and the FD-CA spectrum was obtained on the tetrabutyl ammonium ion, *m/z* 242. The structure of this ion is clearly indicated by the elimination of methane (*m/z* 226), ethane (*m/z* 212), propane (*m/z* 198) and butane (*m/z* 184). The formation of the tributylammonium ion (*m/z* 185) and similar eliminations from this (e.g., *m/z* 169, elimination of methane) provide further structural information. The combination of this data with the empirical formula information would allow relatively easy identification of this compound.

Application of this approach is still in its early stages, but it is possible to comment on some of the problems that have arisen. FD-MS is a relatively sensitive technique, requiring only a few nanograms of compound, but produces low intensity fluctuating ion beams as compared to EI-MS. In order to carry out collisional activation and linked scanning on a FD ion beam, a minimum of 0.5μ/g of each compound must be deposited on the emitter. Not only does this mean that there is a relatively high (for mass spectrometry) minimum sample requirement, but also if there is a very complex mixture, it may only be possible to examine one or two of the compounds present before exhausting all of the sample. The presence of large amounts of inorganics in methanol extracts of treated water and in some derived HPLC fractions can interfere with FD-MS in several ways; (1) they limit the absolute amount of a fraction that can be deposited on a FD emitter, (2) they can give rise to high molecular weight cluster ions for polar molecules (*49*) and (3) they can yield, by formation of cluster ions, FD-MS spectra with ions at similar molecular weights to compounds of interest, which can complicate interpretation of the mass spectrum (*33*). However, it is likely that the problems due to inorganic constituents will be overcome by either improved HPLC resolution and/or FD-MS handling techniques. Even if these problems cannot be solved, FD-MS with collisional activation and linked scanning is by far the most promising technique available for the identification of nonvolatile organics isolated from water samples.

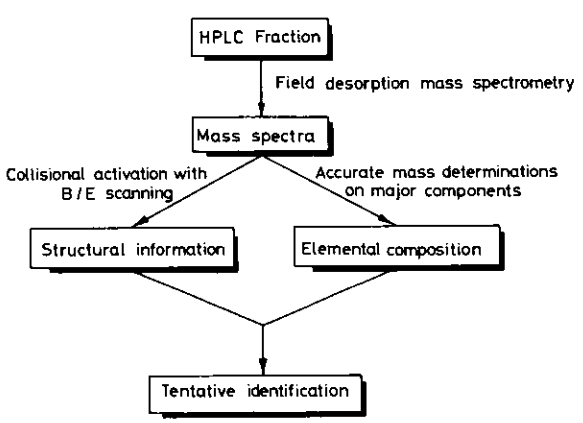

FIGURE 5. Scheme for mass spectrometric analysis.

Chemical Studies

Chlorination. The results obtained from laboratory chlorination of uracil are shown in Table 2. A negligible drop in free chlorine residual of the system blanks was observed during the experiments. GC-MS analysis of a low chlorine residual experiment showed negligible production of haloforms. Several points emerge from study of these results, albeit from a limited number of experiments: (1) the experiment using a high chlorine residual demonstrates that formation of 5-chlorouracil from chlorine and uracil is rapid; (2) 5-chlorouracil is appar-

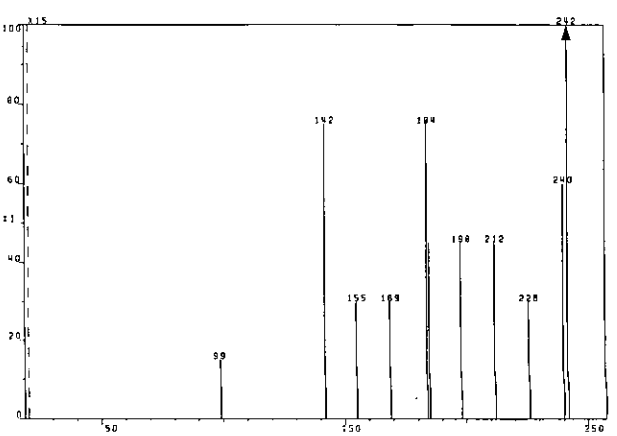

FIGURE 6. FD-CA mass spectrum of tetrabutylammonium chloride.

ently not the ultimate product of the chlorination reaction; and (3) although the ultimate product(s) were not identified, they are not haloforms, at least under the conditions used.

The method used to monitor the course of the reaction did not indicate the appearance of any products other than 5-chlorouracil, hence the end product(s) either does not absorb in the ultraviolet region at 254 or 280 nm or is sufficiently volatile to be lost during freeze-drying or is not extracted into methanol.

Ozonation. The compound types identified by GC-MS of the XAD-2/ether extracts before and after ozonation of humic and fulvic acids are shown in Table 3. Identification is limited to the functional groups that can be recognized from the mass spectral fragmentations, and does not exclude the presence of further oxygenated functionalities in each compound. Full details of the structural assignments will be published at a later date. A typical GC-MS chromatogram of an ether extract of ozonized fulvic acid is shown (Fig. 7) to indicate the complexity of the mixture of volatile organic products.

These results show that the volatile products due to ozonation of humic acid are mainly aromatic, while those of fulvic acid are chiefly aliphatic. Such findings are in agreement with previously published results (*8*). Ozonation of humic acid does not seem to liberate any volatile compounds resulting from fragmentation of the polymer skeleton, although changes are apparent among some of the components detectable after XAD-2 resin extraction of unozonized material. With fulvic acid, ozonation leads to the appearance of certain aromatic acids and normal, branched and possibly cyclic alkanes. While the aromatic acids found in the humic acid extract only after ozonation may result from oxidative cleavage of the macromolecular structure, the alkanes found in the fulvic acid extract only after ozonation were presumably trapped within the polymeric matrix. However, the release of alkanes and DDT and related compounds which are extracted by XAD from humic acid prior to ozonation may be due to a function of the macromolecular structure, which is known to be more "open" at the low pH involved in the XAD extraction procedure (*22*). Estimation of the proportion of volatile organics extracted before and after ozonation suggests that there is little difference for humic acid (<1%) and only a slight increase (~8% before and ~12%

Table 2. Laboratory chlorination of uracil at high and low chlorine residual.

	Time, min	Uracil, concn, μg/l	Cl-uracil concn, μg/l	Chlorine concn, mg/l	
				Free	Total
High Cl	0	1000	0	12	
	0.5	5	50	11	
	30	5	20	10	
	60	0	0	10	
Low Cl	0	10	0	1.2	1.2
	0.5	5	0.5	1.1	1.1
	15	5	0.5	0.75	0.75
	30	4	0.5	0.55	0.55
	45	5	0.5	0.4	0.4
	50	4	1.0	0.35	0.35

Table 3. Compound types present in XAD-2 ether extracts of ozonized and unozonized humic and fulvic acid.

Humic acid	Fulvic acid
Alkyl benzenes (chiefly methyl and ethyl)[a]	Hydroxybenzoic acids
Naphthalene and methylnaphthalenes[a]	Aliphatic alcohols (up to ~ C_{20})
Hydroindenes[a]	Aliphatic diols (up to ~ C_{10})[c]
Aromatic acids (1,2,3-ring)	Aliphatic acids (mainly branched up to ~ C_{20})
Aromatic carbonyl compounds[b]	
Substituted phenols (CHO, OMe, COMe, COOH groups)	Aliphatic diacids (C_{2-10})
Heterocyclics (chiefly furans and benzofurans)	Possibly ethers and ketones (aliphatic and mixed aliphatic-aromatic)
Aliphatic acids (n-C_{5-18})	
DDT and derived compounds	Alkanes (normal, branched, ~C_{20-30} and possibly cyclic)[b]
n-Alkanes (n-C_{23-32})	

[a] Present only in unozonized ether extracts.
[b] Present only in ozonized ether extracts.
[c] Probably also includes hydroxy acids, more predominant in ozonized sample.

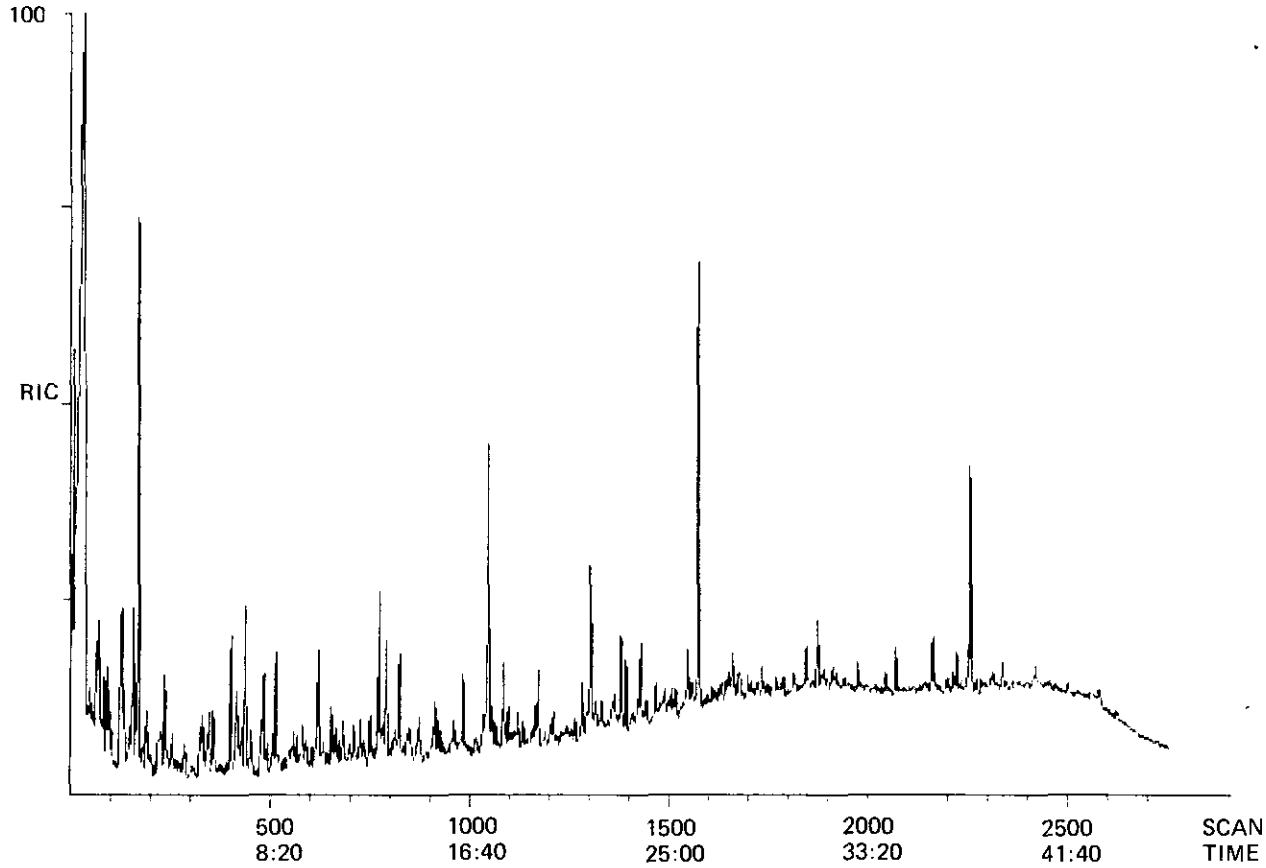

FIGURE 7. GC-MS chromatogram of an XAD-2 ether extract from ozonized fulvic acid.

after) for fulvic acid. Thus, despite the severe conditions of ozonation (~10:1 w/w ozone to humic acid), only a relatively small amount of the humic and fulvic acids was converted to volatile organic compounds.

Ozonation of both humic and fulvic acid resulted in the disappearance of most of the color of the starting solutions, which could suggest that a major structural change occurred. Since no major change was detected in the volatile components, it seems that such changes should be detectable in the nonvolatile components. The reverse-phase ion pair HPLC chromatograms of the XAD-2/methanol extract both before and after ozonation (Fig. 8) of humic acid show similar features, i.e., three major peaks. A method is, therefore, required to provide information on the structural changes undergone by the polymeric matrix of the humic and fulvic acids. One appropriate method is FD-MS (see above), which will be applied to the nonvolatile ozonation products in the near future.

The results of TOC measurements of the aqueous solutions which are applied to the XAD column and the aqueous eluate indicate that the majority of the organic matter is not adsorbed onto the resin column. These water-soluble organics are also nonvolatile in nature and exhibit similar chromatographic behavior to those in the methanol extract of the XAD column. Investigations of these water-soluble organics will be reported at a later date.

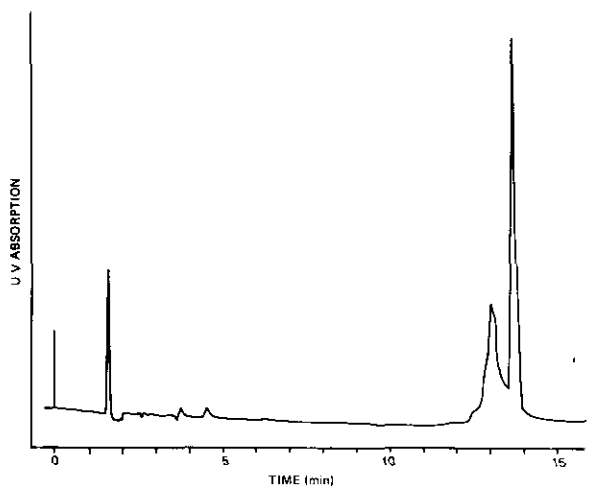

FIGURE 8. Reverse-phase ion pair HPLC chromatogram of an XAD-2/methanol extract of humic acid after ozonation.

Conclusions

Nonvolatile organic compounds account for most of the organic matter present in both raw and treated water. Many of the industrial, agricultural and pharmaceutical compounds in widespread use are nonvolatile and undoubtedly some proportion of them find their way into the aquatic environment. Studies of the organic by-products of chlorination and ozonation of water have shown that a significant proportion of these are nonvolatile. The increasing application of short-term bioassay tests to drinking water has shown that nonvolatile organics could be responsible for some of the mutagenic response.

Until recently there have been no really suitable techniques available for the isolation, separation and characterization of nonvolatile organics from water. Consequently, few data are available on the types and amounts of nonvolatile organics present in drinking water.

Recently there have been significant analytical developments, particularly in mass spectrometry, of direct relevance to nonvolatile organics. New techniques have emerged for the characterization of nonvolatile organics and the approach using freeze-drying, HPLC separation and FD-CA mass spectrometry appears to offer great promise in this respect. Without doubt, the identification of nonvolatile organics in water would benefit considerably from a more widespread application of new techniques such as these.

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