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ANALYSIS OF NON-VOLATILE ORGANIC COMPOUNDS IN WATER BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

An analytical scheme has been developed for the isolation, separation and identification of trace levels of non-volatile organic compounds from drinking water. The work described was carried out in two parts. In the first part, the methods used for the analysis of four selected non-volatile organics are detailed and the results of a limited survey for these compounds presented. The extension of this approach, using high-performance liquid chromatography (HPLC), to the analysis of a wider range of non-volatile organics is described in the second part of the work. Separation of extracts was carried out by reversed-phase HPLC both on analytical and preparative scale. Preparative HPLC fractions were then examined by electron impact and field ionisation mass spectrometry in order to attempt identification of separated components.

INTRODUCTION

In recent years concern has arisen over the potential health risks posed by trace levels of organic compounds in drinking water. This concern has developed from increasing knowledge of the importance of environmental chemicals in relation to chronic disease, especially cancer[1]. A particularly important factor in the growth of this concern has been the availability of suitable analytical techniques (especially gas chromatography–mass spectrometry, GC–MS) for the separation and identification of low levels of organic compounds in water.

While it is normal for drinking water supplies to contain several hundred milligrams per litre of inorganic constituents, the concentration of organic compounds rarely exceeds 20 mg/l, and is more typically in the range 5–10 mg/l for drinking water derived from surface water sources. This small quantity of organic material is a complex mixture of compounds with both synthetic and natural origin. The major source of organic compounds is probably domestic and industrial waste water, but other sources are agricultural and road run-off and from atmospheric fall-out. In addition all waters contain numerous organic compounds which are of natural origin.

Conventional water treatment systems are primarily designed for clarification,

by coagulation and filtration, and disinfection usually with chlorine. It is now realised however that a large number of organic compounds are not removed by this process. In addition, many organic compounds undergo substitution and/or oxidation reactions during the treatment of water with chlorine, producing a wide range of chlorinated and other organic compounds.

A large amount of work has now been carried out on the analysis and identification of organics in drinking water. For example in 1975 over 360 compounds had been identified in European potable waters[2] with many more now found in American drinking waters[3]. However this data was largely obtained by the use of GC–MS and was therefore heavily biased towards volatile substances. Since it has been estimated that only 10–20% of the organic matter in drinking water is amenable to analysis by GC–MS, little information exists on the bulk of organic matter present in potable supplies. The identification of the remaining 80–90% is therefore of prime interest. This was emphasised in a recent World Health Organisation report on the "Analysis of organic compounds in water to support health effects studies"[4], which gave a high priority to the need for the development of analytical techniques for organics not detectable by GC–MS.

High-performance liquid chromatography (HPLC) is ideally suited to the separation of mixtures of highly polar and non-volatile compounds. The pioneering work by Katz *et al.*[5] in identifying non-volatile compounds of biological origin in sewage effluent illustrated the potential of HPLC when applied to this problem. Fifty-eight compounds were identified in primary and secondary effluents and over 100 compounds were detected[6]. In another phase of the work over 60 chlorinated compounds were detected (17 tentatively identified) in municipal sewage after laboratory chlorination[7]. Separations were carried out on high-performance anion-exchange columns with detection by UV absorption, liquid scintillation counting after laboratory chlorination of samples with Cl^{36} (ref. 8) or by cerate oxidimetry[9]. Identification of the various compounds isolated was obtained using GC–MS (after suitable derivatisation). Derivatisation of organic compounds is a common method of increasing the number amenable to GC analysis, but its scope is limited since many non-volatile substances either do not derivatise or are still not volatile enough for GC analysis after derivatisation. Therefore a method is needed that is applicable to non-volatile organics in general[10].

This paper describes an approach to the problem of extraction, separation and identification of non-volatile organic compounds in drinking water. Initially our interest was centred on chlorinated non-volatile organics formed during water treatment since it has been reported that some of these are mutagenic[11]. A method was therefore developed for the analysis of four selected chlorine-containing compounds (*viz.*: 5-chlorouracil,5-chlorouridine, 4-chlororesorcinol and 5-chlorosalicylic acid) previously shown by Jolley and co-workers[7] to be formed during laboratory chlorination of lake, river and waste water samples. One of the reasons these particular compounds were chosen was that their non-chlorinated analogues represent classes of organic compound likely to be present in source waters used for potable supply. Further, each of the four compounds was amenable to derivatisation with silylating reagents and therefore their presence could be confirmed by glass capillary GC.

In the second part of the work the analytical scheme developed for monitoring the four selected chlorine-containing compounds was modified to include the

separation and identification of as wide a range as possible of non-volatiles present in drinking water.

EXPERIMENTAL

Materials and reagents

Methanol was generally used as received (Rathburn Chemicals, Walkerburn, Great Britain). When obtaining fractions for examination by field desorption mass spectrometry the methanol used was distilled in an all-glass apparatus with a PTFE-coated condenser, still-head and draw-off tube and then stored in PTFE bottles. The solvent reservoirs for the HPLC pump were also made of PTFE. The methanol purchased was found to contain trace levels of sodium and potassium ions which interfered with the desorption of organic compounds from the emitter during field desorption mass spectrometry. Distillation of the methanol in a PTFE lined apparatus was found necessary in order to reduce these levels. Water was obtained from an apparatus (Fisons, Cambridge, Great Britain) in which tap water is deionised and double distilled. Acetic acid was Aristar grade (BDH, Poole, Great Britain). Pyridine (silylation grade) and bis(trimethylsilyl)trifluoroacetamide were used as received (Pierce and Warriner, Chester, Great Britain). 5-Chlorouracil, 5-chlorouridine (Calbiochem, Bishops Stortford, Great Britain), 4-chlororesorcinol and 5-chlorosalicylic acid (Aldrich, Gillingham, Great Britain) were used as received.

Sampling

Samples were collected in clear glass bottles (5 l or 10 l capacity) with ground glass stoppers, previously washed with Decon® solution followed by rinsing with tap water, methanol and tap water again. Samples were taken of the finished water (*i.e.*, immediately before the water enters the distribution system) of conventional water treatment works. Samples were stored in the dark during transportation and extracted as soon as possible (usually the same day). When necessary, samples were stored in the dark at 4° while awaiting analysis.

Samples were spiked, when required, immediately before extraction. A standard solution (10 mg/l) of each of the four selected organics was made up in water and a microlitre syringe was used to dispense the desired amount.

Extraction

In the first stage of the work the four selected non-volatile compounds were isolated from water samples (1 l) by rotary evaporation to dryness *in vacuo* at 35°. The solid residue was extracted with methanol (3 × 25 ml). The methanol extract was then concentrated to *ca.* 2 ml by rotary evaporation and finally to 0.5 ml under a stream of nitrogen. For preparative-scale HPLC separations, water (0.5 ml) was added at this stage, and the extract then re-concentrated to 0.5 ml under a stream of nitrogen.

The second stage of the work used water samples (15–60 l) which were freeze-dried and the solid residue extracted with methanol (3 × 50 ml). This extraction was facilitated by holding the sample in an ultrasonic bath for *ca.* 5 min and then centrifuging. The methanol extracts were decanted, combined and concentrated to

ca. 5 ml by rotary evaporator; further concentration, if required, was carried out under a stream of nitrogen.

High-performance liquid chromatography

The equipment used consisted of two solvent delivery systems (Waters Assoc., Milford, Mass., U.S.A.; Model 6000A), a gradient former (Waters Assoc., Model 660) and two UV absorption detectors operated in series (Cecil, Cambridge, Great Britain, Model CE 212; and a Laboratory Data Control, Stone, Great Britain, Model 1203). Syringe injections were made through a stop-flow septumless injection port (H.S. Chromatography Packings, Bourne End., Great Britain). Injection volumes ranged from 10 μl for analytical separations to 200 μl for preparative separations from which fractions were collected. Columns (15 or 20 cm $\times$ 7 mm I.D.) were packed in an upward manner[12] with Spherisorb ODS (particle size 5 μm; Phase Separations, Queensferry, Great Britain), by a slurry procedure under the following conditions: slurry liquid, acetone or isopropanol (AnalaR grade, BDH); slurry concentration, 10% (w/v); packing pressure, 350 bar using a pneumatic amplifier pump (Haskel Energy Systems, Sunderland, Great Britain, Model DST-122).

The column was then prepared for injection by removing 4 mm of packing from the top of the column, pressing in a stainless-steel mesh disc (diameter 7 mm) and re-filling with 100-μm glass beads[13]. Injections were then made onto the stainless-steel mesh disc. A linear gradient was established from two solvent mixtures consisting of 1% methanol in 0.1% aqueous acetic acid (A) and 90% methanol in 0.1% aqueous acetic acid (B). The gradient was run over 30 min from 0% to 100%. The flow-rate was maintained at 2.0 ml/min throughout.

Gas chromatography

Fractions collected from preparative HPLC of the four selected non-volatile compounds were silylated prior to GC analysis. The fractions (*ca.* 1 ml) were taken to dryness under a stream of dry nitrogen, bis(trimethylsilyl)trifluoroacetamide (500 μl) and pyridine (500 μl) added and the mixture heated (60° 1h). Aliquots (1 or 2 μl) of the silylated mixture were then analysed by glass capillary gas chromatography using a (50 m $\times$ 0.32 mm) wall-coated open tubular column coated with OV-1. The gas chromatograph (Finnegan, Sunnyvale, Calif., U.S.A., Model 9500) was fitted with a Grob type splitless injector used with a splitless time of 30 sec, during which time the column was kept at room temperature. The column was then heated to 100° and programmed to 255° at a rate of 8°/min. The carrier gas was helium at an inlet pressure of 13 p.s.i.g. (equivalent to a column flow-rate of 1 ml/min at 20°). The injector was maintained at a temperature of 250° and the flame ionisation detector at 200°.

Mass spectrometry

Preparative HPLC fractions were concentrated to a volume of 5–10 μl by evaporation to dryness under a stream of dry nitrogen and addition of the required volume of methanol. They were then submitted to mass spectrometric analysis by electron impact (EI) and, if sufficient material was available, field ionisation (FI).

EI-MS. EI mass spectra were recorded on a double beam, double focussing mass spectrometer (AEI, Manchester, Great Britain, Model MS-30), with an ion

source temperature of 200°, 20 eV electron voltage and 100 μA filament emission. Data acquisition and processing were performed by a Multispec computer system (VG-Data Systems, Manchester, Great Britain), using perfluorokerosene as mass calibrant. Samples were introduced via the direct insertion probe.

FI-MS. FI mass spectra were also obtained using a MS-30 mass spectrometer, fitted with a combined field desorption (FD)–field ionisation source in beam 2. Samples prepared as methanol solutions were introduced via the direct probe. The emitter anodes consisted of 10 μm tungsten wires, spot-welded on supporting posts and activated in an apparatus and manner similar to that described by Beckey *et al.*[14]. Spectra were obtained at a source temperature of 20°, resolution of 1000 and 4 kV accelerating voltage with −5 kV on the cathode. Data acquisition and processing were carried out as for the EI mass spectra.

RESULTS AND DISCUSSION

(a) Analysis of the four selected compounds

Extraction

Methanol was chosen as extraction solvent after recovery tests on vacuum-evaporated water samples spiked with each of the four selected compounds. These indicated that only water and methanol of the fourteen solvents examined yielded greater than 90% recovery for all four compounds. Methanol was finally selected because of its lower boiling point and because it dissolves less inorganic matter than water.

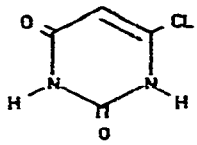
The recovery efficiency of the isolation and HPLC separation used in the first part of the work was checked by spiking potable waters containing negligible amounts of the four selected compounds. These waters were spiked at a level of 5 μg/l of each of the four compounds. The mean recoveries (%) were 62 ($\sigma = 13.5$) 60 ($\sigma = 11.0$), 32 ($\sigma = 21.6$) and 64 ($\sigma = 7.9$) for 5-chlorouracil, 5-chlorouridine, 4-chlororesorcinol and 5-chlorosalicylic acid respectively. Further experiments were carried out to ascertain the reasons for the anomalously low recoveries occasionally obtained for 4-chlororesorcinol. It was apparent from these subsequent results that this compound is unstable on storage in certain treated waters.

HPLC analysis

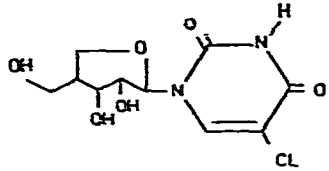
Separation of the four selected compounds. The structures of the four selected compounds are given in Fig. 1.

Initially, chromatography of the four compounds was attempted on both normal-phase and reversed-phase columns. The use of a normal-phase system was unsuccessful. With a non-polar eluent (*e.g.*, *n*-hexane saturated with water) the four compounds were retained on the column and although some separation was obtained with more polar eluents, such as dichloromethane–ethyl acetate, this was accompanied by severe peak tailing. Other workers[15] have noticed similar effects when chromatographing ionisable compounds on adsorption columns.

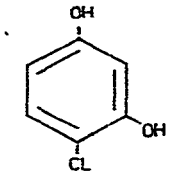
Reversed-phase columns were found to be much more suitable for this separation. 5-Chlorouracil and 5-chlorouridine were readily separated on a C_{18} bonded phase column using an isocratic elution with 8% methanol in water. 4-Chlororesorcinol was eluted with good peak shape using 25% methanol in water



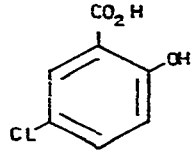
5 - Chlorouracil



5 - Chlorouridine



4-Chlororesorcinol



5- Chlorosalicylic acid

Fig. 1. Structures of the four specified non-volatile organic compounds.

However, under these conditions 5-chlorosalicylic acid, although retained (capacity ratio $k' \approx 1$) was eluted as a severely tailing peak. Previous research[16] suggests that it is necessary to buffer an ionisable compound with acid or base since changes in the degree of ionisation during elution cause peak asymmetry. Therefore initially the pH of the eluent was reduced to *ca.* 4.5 by replacing water with 0.05 *M* aqueous sodium dihydrogen orthophosphate. The peak shape was considerably sharper under these conditions (*i.e.*, 25% methanol in 0.05 *M* aqueous NaH_2PO_4) and the retention was increased ($k' \approx 4$). In addition, the chromatography of the three other compounds was not significantly affected.

Separation of all four compounds required the use of gradient elution. Thus a good separation was obtained using a linear gradient from 1% methanol in 0.05 *M* aqueous NaH_2PO_4 to 90% methanol in 0.05 *M* aqueous NaH_2PO_4 with the gradient terminated after 30 min.

Problems were encountered however in the trimethylsilylation of fractions collected from preparative scale liquid chromatography when using NaH_2PO_4 in the eluent. Thus an alternative system which did not interfere with the derivatisation had to be found.

The exact function of the sodium dihydrogen orthophosphate in the mobile phase is not clear. The main action however was thought to be a straightforward pH effect, *i.e.*, decreasing the pH decreases the degree of ionisation of the acid making it less polar and more amenable to analysis. Other compounds commonly used to reduce the pH of the mobile phase for the chromatography of acids are formic acid and acetic acid. Substituting 0.1% aqueous acetic acid (pH 3.5) for 0.05 *M* aqueous NaH_2PO_4 was found to give an adequate separation of the four compounds with reasonable peak shape and in addition did not cause any problems in the derivatisation of fractions. The separation achieved is shown in Fig. 2.

Fig. 3 shows the chromatograms from the analysis of a drinking water extract

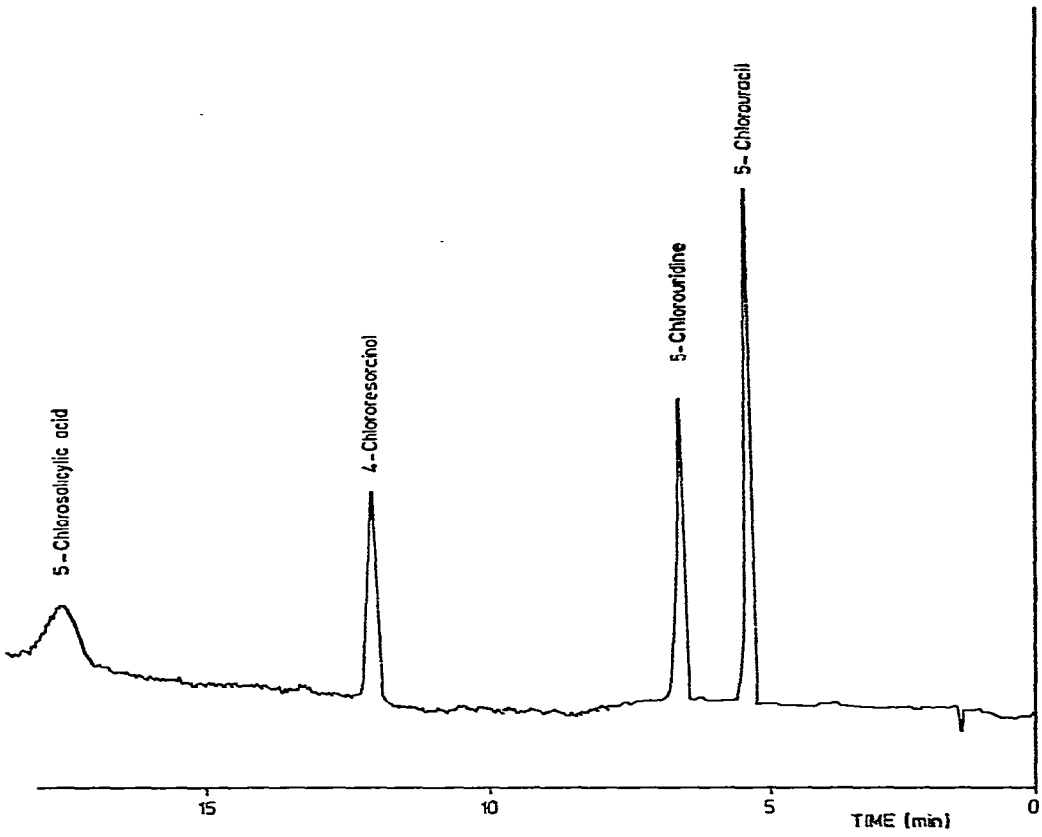


Fig. 2. Separation of a standard solution of the four specified non-volatile organic compounds. (200 ng each component). Column: 15 cm × 7 mm I.D.; packing, 5 μm Spherisorb ODS. Eluent: linear gradient from 1% methanol in 0.1% aqueous acetic acid to 90% methanol in 0.1% aqueous acetic acid over 30 min. Detection: UV absorption at 280 nm for 5-chlorouracil, 5-chlorouridine and 4-chlororesorcinol, and at 315 nm for 5-chlorosalicylic acid.

spiked at 5 μg/l with each of the four compounds. Fig. 4 shows the results from the analysis of an unspiked drinking water sample. The large peak at the start of each chromatogram is essentially unretained and is probably equivalent to an excluded peak containing much of the higher-molecular-weight material. A large peak in this position was always observed when analysing drinking water extracts.

Preparative scale liquid chromatography. In order to confirm the identity of a compound by a chromatographic technique it is advisable to carry out the analysis on two different systems. Identical retention times for sample and standard on both systems is accepted as reasonable proof of identity. As explained earlier, the four compounds were chosen so that they could also be analysed by GC (after derivatisation) and therefore provide confirmation of identity.

The method chosen for the analysis was an initial "analytical" separation by HPLC followed by preparative-scale separation and GC analysis of the fractions when appropriate. Preparative-scale HPLC can be carried out by either injecting a small volume of a concentrated solution or a larger volume of a dilute solution.

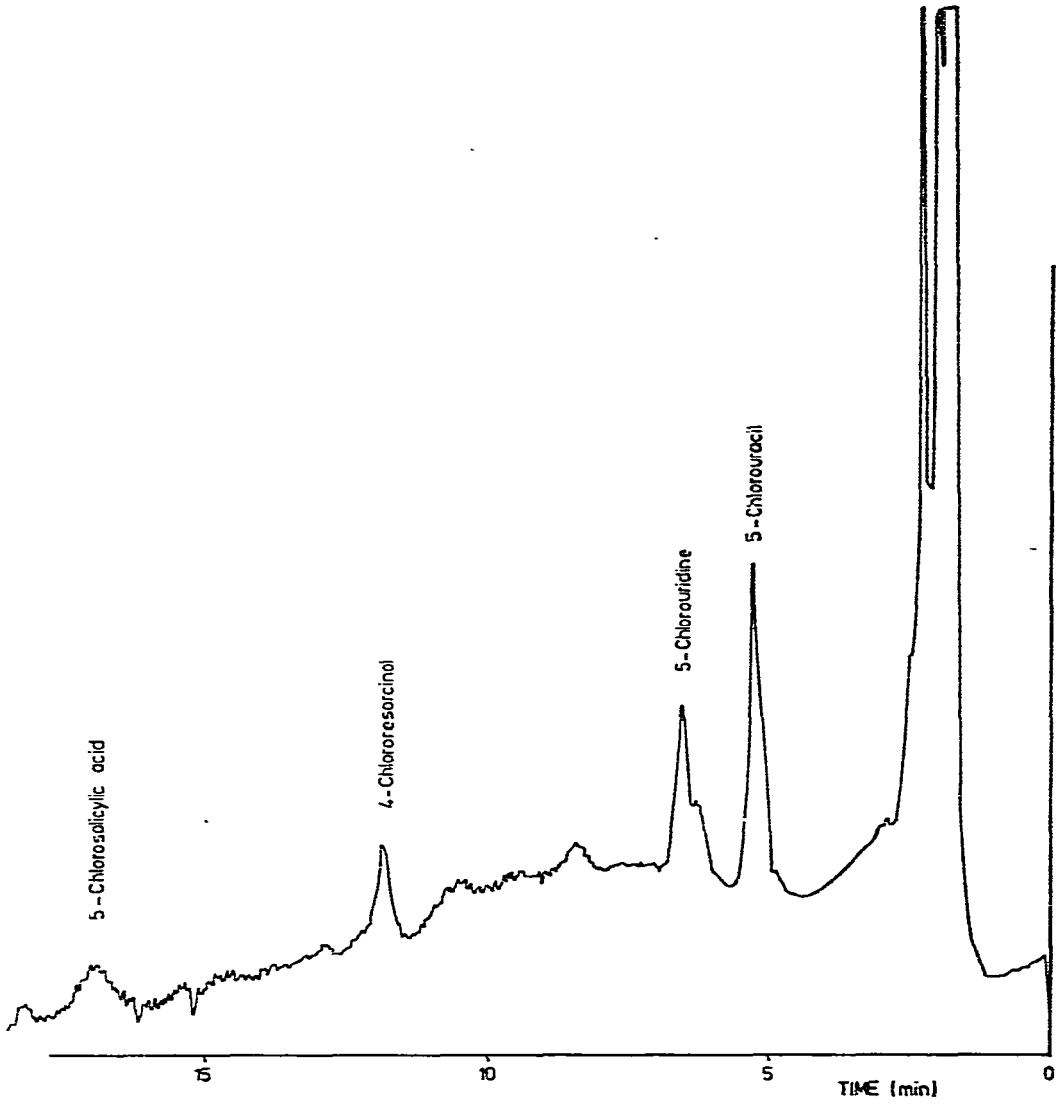


Fig. 3. Separation of an extract from a drinking water sample spiked at 5 μg/l with the four specified compounds. Conditions as for Fig. 2.

Overloading of the column, resulting in reduced efficiency, can occur with both methods.

All chromatographic materials can only accept a certain load of sample before overloading occurs resulting in deterioration of peak shape and resolution. Recent work by Done[17] showed that for efficient columns (height equivalent to a theoretical plate, HETP $\approx$ 0.02 mm) this can occur at very low solute loads and in order to retain maximum efficiency he recommended that loading should not exceed 10 μg of solute per gram of packing. Separations carried out under these conditions are generally termed "analytical". It is common practice to carry out preparative work

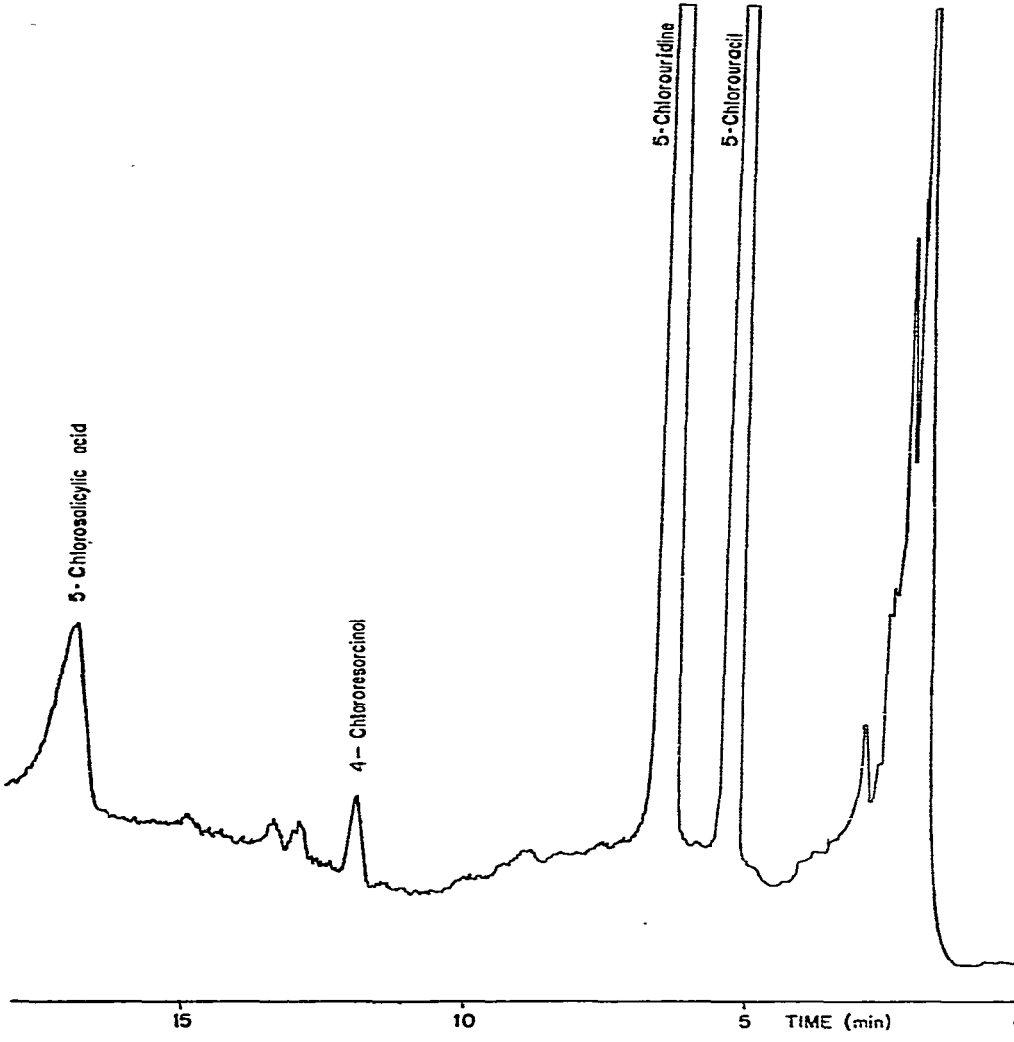


Fig. 4. Chromatogram from the analysis of a drinking water extract for the four specified compounds. Conditions as for Fig. 2.

with the column operated in an overloaded condition. Thus when preparative work is being carried out the operator must compromise between column efficiency and sample loading. A large injection volume causes peaks to broaden by increasing the retention of the rear of the peak while the front of the peak remains at constant retention[18]. Bristow[19] has recently shown that injection volumes of up to 500 μl can give reasonable results when injected onto 8 mm I.D. columns.

For the work described here it was decided to use columns of 7 mm I.D. and 15 cm or 20 cm length. This meant that both the analytical and preparative separations could be carried out on the same column. In fact it was generally found throughout the work that 7 mm I.D. columns were somewhat more efficient for analytical separations (based on a comparison of plate height, number and reduced

plate height as measured from a standard mixture of phenols[20]) than the more conventional 4.6 mm I.D. columns of the same length.

In order to test a column (15 cm × 7 mm I.D.) for preparative work, 1 mg of each of the four selected compounds was injected and fractions collected at the appropriate retention time. Peak shapes, although noticeably broader when compared to analytical separations, were acceptable. Re-injection of an aliquot of each fraction indicated that *ca.* 90% of the solute injected was recovered in each case.

A series of large volume injections were made in order to determine what volume could be injected onto a 15 cm × 7 mm I.D. column without significantly impairing column performance. The results were in broad agreement with previous findings[19] and showed that 100 μl could be injected without significantly affecting peak shape. Injections of 200 μl caused some peak broadening and loss of resolution but 500 μl injections considerably reduced performance.

From this work it was concluded that preparative separations could be carried out by injecting between 100 μl and 200 μl with each injection loading 1–10 mg onto the column.

When carrying out preparative scale analyses, the methanol used to extract the sample had to be removed and replaced with water (see Experimental), *i.e.*, closer to the composition of the mobile phase in which the sample was to be injected. Otherwise injection of a large volume of methanol onto a column in which the mobile phase is essentially water produces a non-equilibrium condition. This results in the solute band being extended down the column by the methanol rather than remaining in a small volume at the top of the column, producing peak tailing and, in extreme cases, single components eluting as a split peak.

Detection limits. Monitoring the four selected compounds was carried out using UV detection at two wavelengths. The absolute detection limit is defined here as the amount injected onto the column which results in a signal to noise ratio of 2:1. These are shown in Table I together with the detection limit with respect to the water sample. The latter is calculated from a 1 l sample, with a final extract volume of 0.5 ml and injection volume of 10 μl.

TABLE I

DETECTION LIMITS FOR THE FOUR SPECIFIED COMPOUNDS

Compound	$\lambda_{max.}$ *(nm)*	*Detection limit on column (ng)*	*Detection limit in sample (μg/l)*
5-Chlorouracil	280	2.0	0.1
5-Chlorouridine	280	4.0	0.2
4-Chlororesorcinol	280	10.0	0.5
5-Chlorosalicylic acid	315	20.0	1.0

GC analysis

The efficiency of the silylation step was examined by comparing concentrations of the four selected organics calculated from HPLC chromatograms with those calculated from GC chromatograms. This was done by splitting a spiked extract, analysing one half by HPLC, silylating the remainder and then analysing by capillary GC. Peak area calculations were carried out to obtain values for the concentrations of

the four organics. This showed that 5-chlorouracil, 4-chlororesorcinol and 5-chlorosalicylic acid were all fully silylated in virtually 100% yield, but 5-chlorouridine gave, at best, 50% yield of the tetrakis(trimethylsilyl) derivative under the conditions used.

Derivatisation of fractions collected by preparative HPLC proved to be more difficult than derivatisation of the same amounts of pure substance. Previous research[21] suggested that this may be due to the presence of phosphate buffer, since this has been shown to react with silylation reagents. This problem was solved when the mobile phase composition was changed from 0.05 *M* aqueous NaH_2PO_4 to 0.1% aqueous acetic acid. The silyl derivatives of the four selected compounds were quite amenable to capillary GC, giving well separated peaks. The retention data obtained from capillary GC was used in conjunction with that from HPLC to provide identification of the four compounds in extracts isolated from a series of treated waters.

Results of a limited survey for the four selected compounds

Samples (5 l) were taken of the finished water from various water treatment works. One litre of this sample was then extracted and analysed as described previously. A further 1 l of the same sample was spiked with 5 μg of each of the four selected compounds and analysed as described. In this way approximate recovery figures for each of the four compounds were obtained. The results of the survey are given in Table II.

TABLE II

RESULTS OF THE SURVEY OF DRINKING WATER SAMPLES FOR THE FOUR SPECIFIED COMPOUNDS

All figures shown are in μg/l; — indicates not detected (*i.e.*, below detection limit, see Table I).

Sample site	*5-Chlorouracil*	*5-Chlorouridine*	*4-Chlororesorcinol*	*5-Chlorosalicylic acid*
A	0.3	0.7	—	—
B	14.1*	25.7*	4.7*	12.5*
B (repeat)	0.8	1.2	—	—
C	—	—	—	—
D	—	—	—	—
E	0.1	—	—	—
F	—	—	1.6	2.3
G	—	—	—	—
H	—	—	—	—

* Presence confirmed by retention time on glass capillary GC.

Discussion

A method has been described which is suitable for the analysis of four non-volatile organic compounds in drinking water. Results, presented in Table II, show that these compounds are present in certain drinking water samples. The levels quoted in Table II are based upon the recoveries given previously. The reason for the high variability of these recoveries became apparent during the course of the work.

The main problem is that these four compounds decompose when stored in some treated waters. This problem first became apparent from the analysis of water from sample site B. The analysis of an unspiked sample showed that fairly high levels

of the four compounds were present (see Table II). Analysis of the spiked sample however gave results much lower than expected (*i.e.*, 5-chlorouracil, 4.5 μg/l; 5-chlorouridine, 5.5 μg/l; 4-chlororesorcinol, 2.0 μg/l; 5-chlorosalicylic acid, 2.5 μg/l). This sample had been stored for 2 days (in the dark, at 4°) after spiking with the four compounds and before carrying out the analysis. Further evidence for decomposition during storage was provided when another 1 l of the unspiked water from sample site B was analysed (storage time, 3 days). Results showed a significant decrease in the levels of each of the four compounds (*i.e.*, 5-chlorouracil, 0.5 μg/l; 5-chlorouridine, 0.1 μg/l; 4-chlororesorcinol and 5-chlorosalicylic acid were undetected).

The samples used to produce recovery figures were therefore all analysed within 1 day of spiking. The results showed that although the recovery of 4-chlororesorcinol is still variable under these conditions, recoveries of the other three compounds are fairly consistent. However difficulties may still be encountered for all four compounds if a longer delay takes place between spiking and analysis.

Further study is needed in order to clarify the behaviour of these compounds in drinking water.

Another problem became evident when water from site B was re-sampled and analysed. The results, shown in Table II, indicated the presence of much lower levels than were found in the earlier sample even though sampling and analysis took place on the same day. The reason for this was not self-evident although one possibility was that the nature of the raw water supply had changed.

(b) Identification of other non-volatiles

Extraction

In the second part of the work the method was extended to include the separation and identification of non-volatiles generally. Larger sample sizes were used, requiring the use of large-scale concentration equipment which was not available in-house. However access to a large scale freeze drier was available and this method of concentration was used for this stage of the work.

Total organic carbon measurements (TOC; Tocsin, Phase Separations) carried out on treated water samples before and after freeze drying indicated a high recovery of the total organic carbon. Similar TOC measurements on blanks consisting of double distilled deionised water with added inorganic salts showed that there was a negligible increase of TOC as a result of the freeze drying process. No experiments have so far been carried out to ascertain the recovery efficiency of the methanol extraction during this stage of the work.

Water from three sites (see Table III) has been extracted in the way described above and submitted for analysis by HPLC and MS.

HPLC analysis

HPLC analyses carried out during the second stage of the work used the same conditions as described for the analysis of the four selected compounds *i.e.*, the column (20 cm × 7 mm I.D.) was packed with Spherisorb ODS (particle size 5 μm) and a mobile phase of a linear gradient from 1% methanol in aqueous 0.1% acetic acid to 90% methanol in aqueous 0.1% acetic acid over 30 min was used. Analytical and preparative separations were carried out on the extracts obtained from freeze drying water samples.

Extracts were monitored at three wavelengths, 254 nm, 280 nm and 315 nm. None of the extracts showed any extra peaks at 315 nm compared to 254 nm and 280 nm, only a general reduction in all peak size was observed. Fig. 5 shows the chromatograms obtained from the analysis of sample E. The numbers on the chromatograms in Fig. 5 correspond to the major components in the extract which were examined further by mass spectrometry (see Table III). Also included in Table III are details of the analysis of the two other samples examined in the second stage of the work.

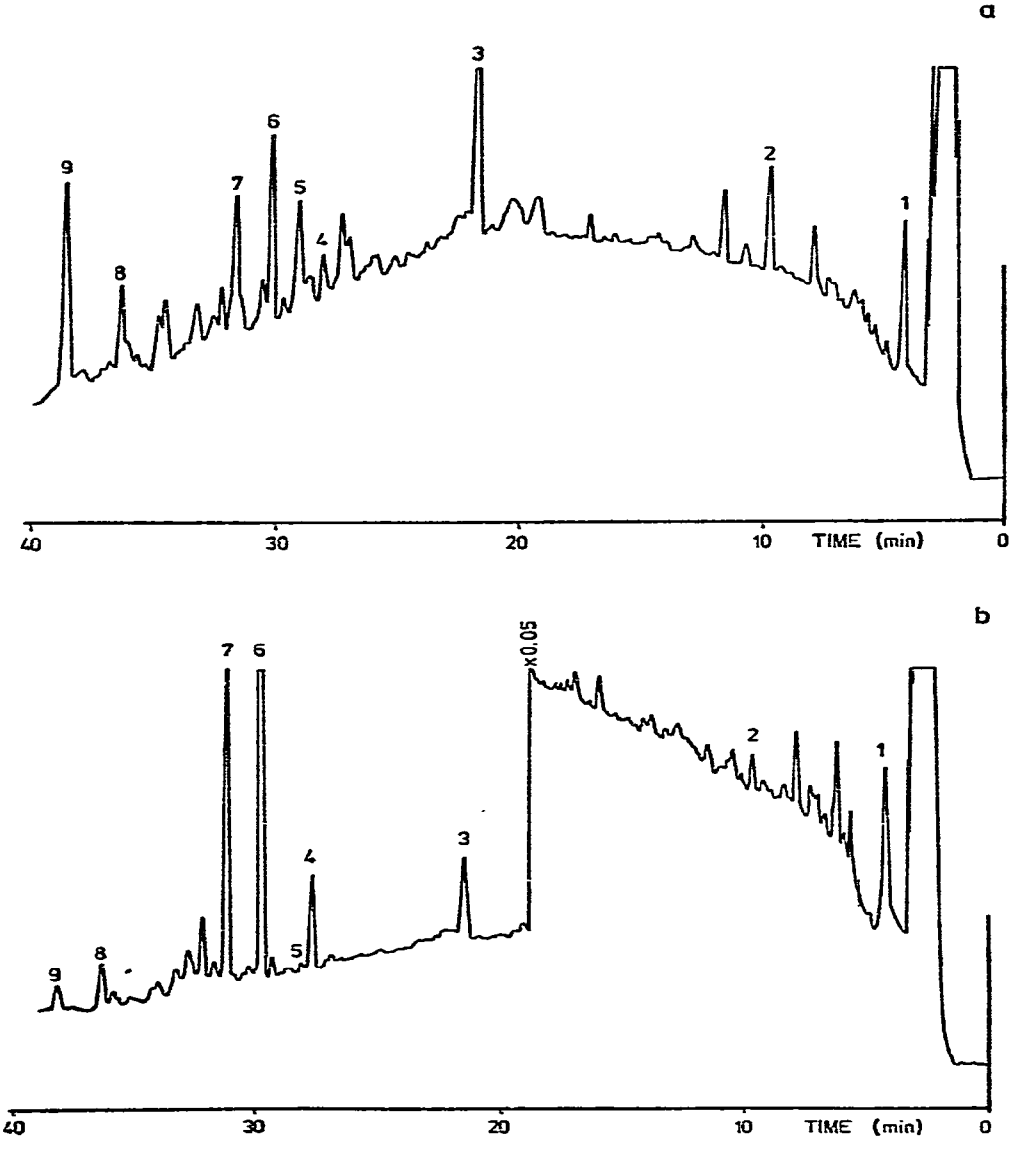


Fig. 5. Separation of a drinking water extract (sample site E) monitored at 254 nm (a) and 280 nm (b). Column: 20 cm × 7 mm I.D.; packing and eluent as for Fig. 2.

TABLE III

MAJOR COMPONENTS PRESENT IN TREATED WATER EXTRACTS AS INDICATED BY UV ABSORPTION ON HPLC

Initial identities from MS data. Some confirmed by comparison of MS and HPLC data with those of authentic compounds. Approximate levels were based on UV absorption from HPLC chromatogram by comparison with authentic compounds.

Fraction	*HPLC retention time (min)*	*Sample site*	*Identity*	*Identification*	*Confirmation*	*Approximate level (μg/l)*
1	4.2	E, I	Multi-halogenated	EI-MS	–	–
2	9.5	E, I	–		–	–
3	21.5	B, E, I	2-Hydroxybenzothiazole	EI-MS	HPLC, EI-MS, FI-MS	5–25
4	27.5	B, E, I	Partly 2,4,6-trichlorophenol	EI-MS	HPLC	<1
5	28.5	E	–		–	–
6	29.5	B, E, I	Dimethylacridan	EI-MS	–	–
7	34.0	B, E, I	*p*-Isopropyldiphenylamine	EI-MS	HPLC, EI-MS	10–15
8	35.8	E, I	–		–	–
9	38.0	B, E, I	di-*n*-Octyl phthalate	EI-MS, FI-MS	HPLC, EI-MS, FI-MS	10

Initial identification of components was made by mass spectrometry but wherever possible, authentic compounds were obtained and retention time on HPLC was used to confirm identity. Concentrations are also given in Table III. These were estimated by peak height comparison of standards and sample but it must be emphasised however that as yet no attempt has been made to accurately quantify the extraction stage and therefore the levels reported are approximate.

Mass spectrometric analysis

The major HPLC fractions collected from the three treated waters examined are shown in Table III together with their identities, where these have been elucidated.

Fraction 1 exhibited an EI mass spectrum with a base peak at *m/e* 406 and an isotope pattern indicative of a mixed chlorine- and bromine-containing compound. Further work is in progress to try to identify this compound. Fraction 2 exhibited an indeterminate EI mass spectrum with a large number of low mass fragments. FI failed to yield any significant molecular ion species and further work using FD-MS is in progress in order to identify this compound. Fraction 3 was identified as 2-hydroxybenzothiazole by comparison of its EI and FI mass spectra (Figs. 6a and 7a) and HPLC retention time with those of an authentic standard (Figs. 6b and 7b). Fraction 4 yielded an EI mass spectrum containing a multiplicity of intense low mass fragment ions and lower intensity ions up to *m/e ca.* 300. However, a cluster of ions at *m/e* 196, 198 and 200 showed isotope ratios indicative of a trichlorophenol. The identity was confirmed as 2,4,6-trichlorophenol by comparison with the EI-MS and HPLC retention time of an authentic compound. However, this fraction is undoubtedly a mixture and 2,4,6-trichlorophenol represents only one, possibly minor, component.

No useful EI mass spectral data were obtained on fraction 5 and further work is being carried out in an attempt to identify the compound.

Fraction 6 exhibited an EI mass spectrum with the base peak at *m/e* 194 and only one other major ion, probably the molecular ion, at *m/e* 209. There are several possibilities for the structure of this compound, but most were ruled out by comparison of the HPLC retention time and EI-MS data with those of the authentic compounds. The most likely identity of this compound was dimethylacridan and a source of this compound is currently being sought. Fraction 7 showed an EI mass spectrum with a molecular ion at *m/e* 211 and a base peak at *m/e* 196. Comparison with literature spectra indicated *p*-isopropyl-diphenylamine and this was confirmed after synthesising the authentic compound[22].

Fraction 8 yielded no useful EI-MS data and is receiving further investigation.

Fraction 9 was positively identified as di-*n*-octyl phthalate by comparison of its EI-MS, FI-MS and HPLC retention data with those of an authentic standard.

Discussion

The complexity of the extracts obtained from the drinking water samples studied is illustrated by the two chromatograms shown in Fig. 5. Moreover these chromatograms only demonstrate those compounds which absorb at 254 and 280 nm so the extract undoubtedly is of greater complexity than indicated. This has been confirmed by MS analysis of LC fractions, which generally indicates the presence of

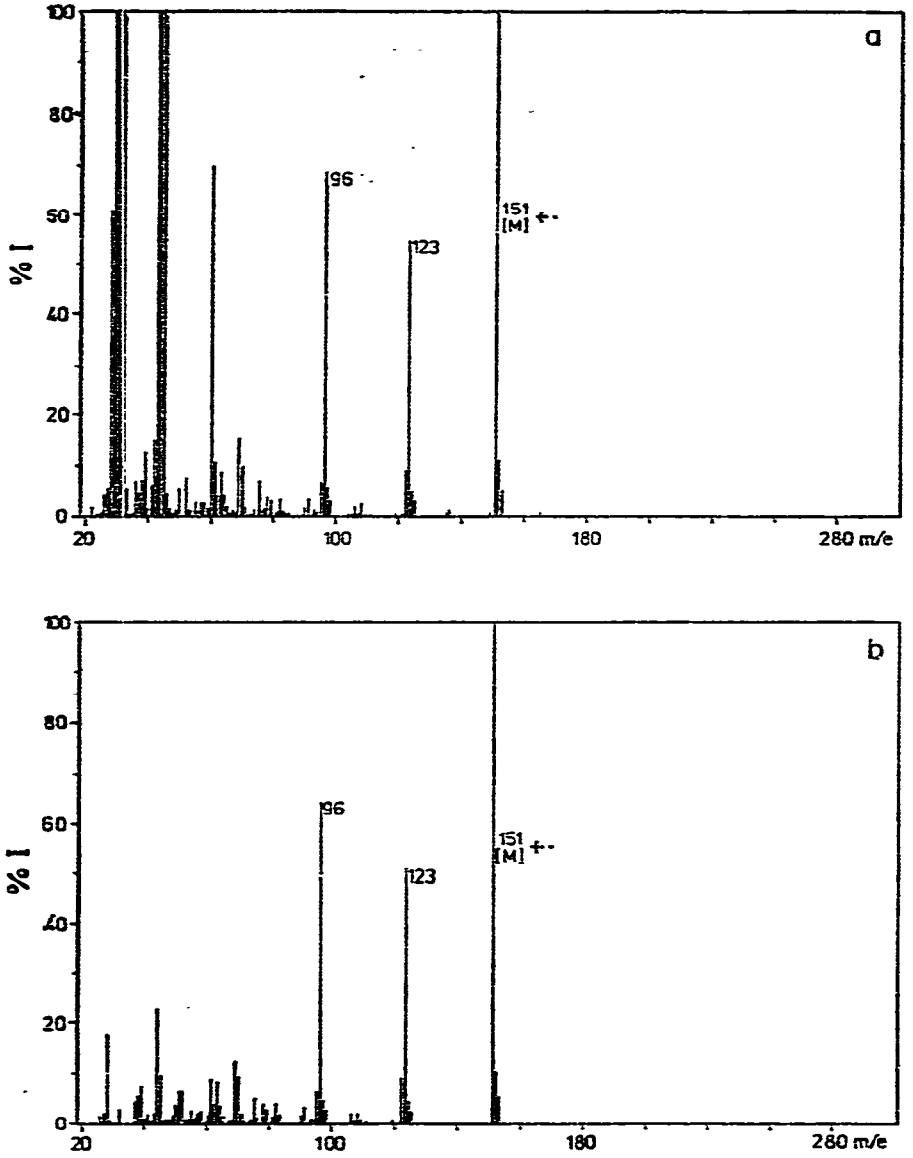


Fig. 6. Electron impact mass spectra of fraction 3 from drinking water sample E (see Fig. 5) (a) and of an authentic sample of 2-hydroxybenzothiazole (b).

a mixture of components when only a single peak is shown by UV absorption. The large peak at the start of the chromatogram is always observed when analysing drinking water extracts. This peak is essentially unretained and probably is comprised of the high-molecular-weight organic compounds which are not partitioned and are eluted as an excluded peak.

Table III shows the compounds so far identified in this stage of the work. The source of these compounds in potable waters is not clear although some speculation

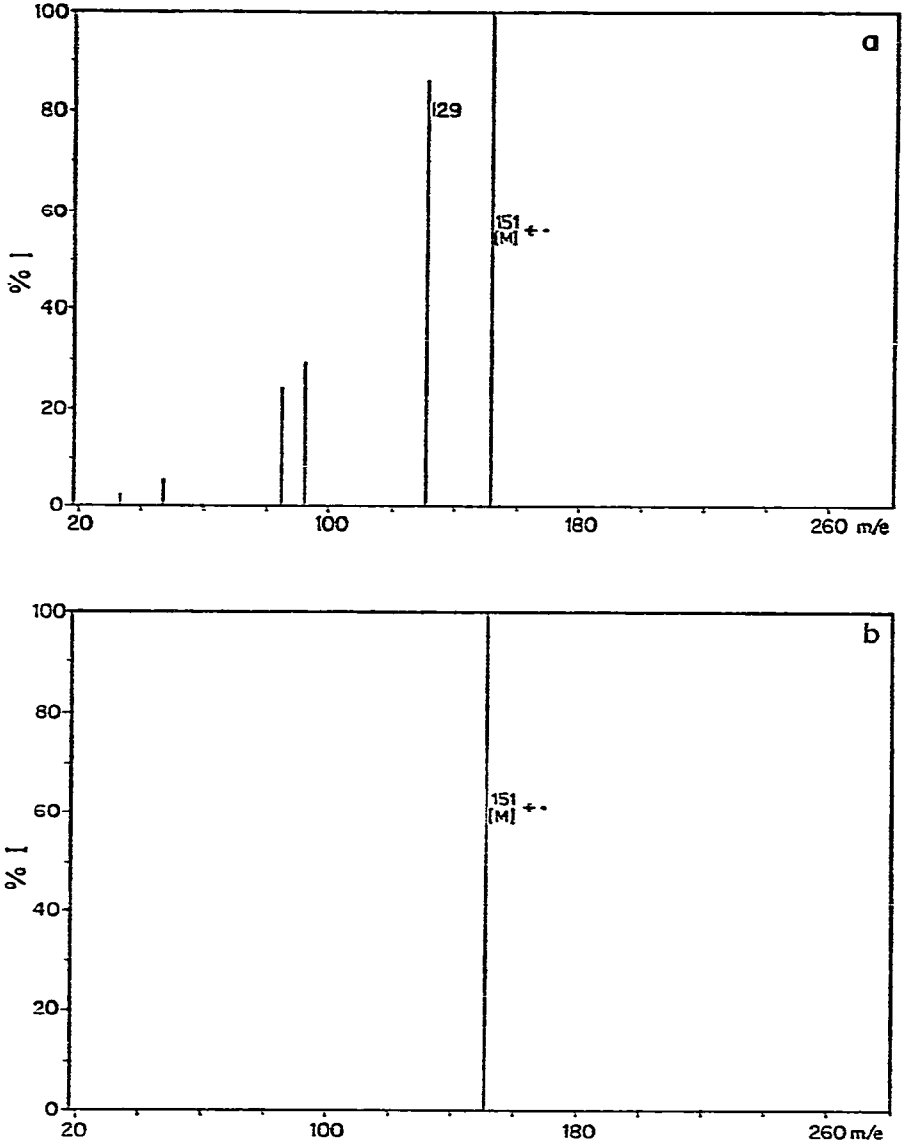


Fig. 7. Field ionisation mass spectra of fraction 3 from drinking water sample E (see Fig. 5) (a) and of an authentic sample of 2-hydroxybenzothiazole (b).

on this subject is possible. *p*-Isopropyldiphenylamine and dimethylacridan have been reported to be present in waste water from tyre manufacturers[23] and also to be commonly used in tyre manufacture as anti-oxidants[24]. There are no large scale tyre manufacturers discharging into the river waters from which the drinking water samples were derived and therefore the main source of these two compounds may be run-off. 2-Hydroxybenzothiazole may be formed during treatment from 2-mercaptobenzothiazole which is commonly used as a vulcanisation accelerator and has been

detected in waste water from tyre manufacturers[23]. 2,4,6-Trichlorophenol is probably of pharmaceutical origin. The origin of di-*n*-octyl phthalate however is less obvious since, unlike some other dialkyl phthalates, it is not commonly used as a plasticiser. It has previously been found in waste water from a chemical plant and a sewage works[2] although its presence in drinking water has not previously been reported.

LC RETENTION DATA ON STANDARDS

Table IV gives the retention times of various organic compounds chromatographed by HPLC using the same conditions as those detailed previously. The compounds were analysed in order to gain information on the retention characteristics of various classes of organic compounds on the HPLC system used for the analysis of drinking water extracts. Each standard (*ca.* 1 $\mu g/\mu l$ in methanol) was generally injected individually.

TABLE IV

RETENTION DATA FOR VARIOUS ORGANIC COMPOUNDS

HPLC conditions as specified for Fig. 5.

Compound	*Retention time (min)*	*Compound*	*Retention time (min)*
Uracil	5.5	4-Chlorobenzoic acid	24.5
Uridine	6.6	Atrazine	25.2
5-Chlorouracil	7.6	5-Chlorosalicylic acid	25.5
5-Methyluridine	8.7	2,3,6-Trichlorophenol	26.0
5-Chlorouridine	9.1	2,4,6-Trichlorophenol	27.5
Phthalic acid	10.2	2,3,4-Trichlorophenol	27.5
4-Chlororesorcinol	13.8	Linuron	28.1
6-Chloroguanine	15.0	Diisopropyl phthalate	28.2
3-Chlorobenzoic acid	17.0	2,4,5-Trichlorophenol	28.5
3-Chloro-4-hydroxybenzoic acid	17.0	2,3,5-Trichlorophenol	28.7
Salicylic acid	18.5	3,4,5-Trichlorophenol	29.5
Fenuron	18.6	Di-*n*-propyl phthalate	29.6
2-Hydroxy-3-methoxycinnamic acid	19.0	Chloroxuron	30.1
Benzoic acid	19.3	Diisobutyl phthalate	31.0
Pyrazon	19.7	2,6-Di-t-butyl-4-methylphenol	32.0
Dimethyl phthalate	20.5	Di-*n*-pentyl phthalate	32.2
2-Hydroxybenzothiazole	21.5	Diisopropyldiphenylamine	34.0
Diethyl phthalate	21.5	4-Chlorophenylacetic acid	34.2
2-Hydroxybenzimidazole	22.0	Di-*n*-octyl phthalate	38.0
2-Chlorobenzoic acid	23.5	Di-*n*-nonyl phthalate	40.4
Simazine	23.6	Diethylhexyl phthalate	41.2
Monolinuron	24.1	Diisodecyl phthalate	44.0

CONCLUSIONS

The isolation and concentration of non-volatile organic matter from drinking water can be successfully carried out by either vacuum evaporation or by freeze-drying. The latter can, if required, process large samples (up to 60 l).

HPLC has been shown to be ideally suited to the separation of the extracted

non-volatile matter. A reversed-phase system using a broad linear gradient was found to be most suitable. The analysis of standards under the same conditions has shown that it is possible to separate a wide range of organic compounds by this method. The use of preparative HPLC with collection of appropriate fractions enabled mass spectrometric data to be obtained on many components. Several of these compounds have now been identified in drinking water extracts using this technique.

This paper has reported the initial results obtained from a long-term study on the identification of non-volatiles in potable waters. A large amount of work is still required in order to identify a large number of compounds of this type.

Future work will involve refinement of the extraction technique in order to obtain more of the organic matter in a suitable form for analysis. Modifications to the HPLC separation will need to be made in order to prepare purer fractions for analysis by mass spectrometry. One of the major restrictions at present is the limitation of LC detection to that of UV absorption. More versatility in this direction would enable a larger number of components to be examined.

Positive identification of the non-volatiles detected is still, however, a major obstacle but currently field desorption MS techniques are under investigation in the WRC laboratories in an effort to identify non-volatiles in drinking water.

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