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ISOLATION OF OFF-FLAVOUR COMPOUNDS IN WATER BY CHROMATOGRAPHIC SNIFFING AND PREPARATIVE GAS CHROMATOGRAPHY

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SUMMARY

A procedure for the isolation and identification of off-flavour compounds in water was developed. Odorous volatile compounds were enriched by stripping and dichloromethane extraction. The extracts obtained were analysed by chromatographic sniffing and preparative gas chromatography, followed by sensory evaluation of different fractions dissolved in odourless water. The chromatographic sniffing technique was found to be sensitive enough to detect known odorous compounds in concentrations far below their threshold odour concentrations in water. However, in order to quantify the contribution of specific compounds to the off-flavour of the water, preparative gas chromatography and sensory evaluation of dissolved extracts were indispensable. One of the case studies performed showed that the origin of off-flavours in water can be very complex, with contributions from several so far unidentified compounds.

INTRODUCTION

Taste and odour problems in drinking water are primarily caused by naturally produced, volatile organic compounds. So far, research in this field has focused on a relatively small number of compounds. In particular, the occurrence of geosmin and 2-methylisoborneol (MIB) in water has been investigated in detail[1–6]. There is no doubt that these two compounds may cause serious off-flavour problems. However, several results indicate that the origin of objectionable tastes and odours in water involves a much larger group of compounds, many of them so far unidentified. By combining an efficient enrichment method for organics in water with chromatographic sniffing [high-resolution gas chromatography (GC) with sensory detection], one can easily detect 10–30 different odorous compounds in a surface water sample[7–11]. Results obtained by the so-called flavour profile analysis method give further evidence of the complexity of off-flavours in water. Trained panelists have been reported to perceive several different flavours in a single water sample[12].

The complex composition of off-flavours in water calls for a systematic method of establishing cause–effect relationships. A first step in this direction was taken by Lundgren *et al.*[11], who suggested a procedure based on enrichment of odorous organic

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compounds followed by fractionation by preparative GC. The contribution to the off-flavour of the water from different fractions of organic compounds was assessed by redissolving these fractions in odourless water and characterizing the resulting flavour of the water. In this study, this procedure was further developed and tested. Furthermore, the relevance of the chromatographic sniffing technique was evaluated by comparing the odour intensities of the redissolved extracts with those of chromatographic sniffing.

EXPERIMENTAL

Water samples

Water samples from two rivers used as raw water sources for drinking water production were analysed in detail: a moderately eutrophic river, only slightly affected by industrial pollution (Stångå River, Linköping, Sweden), and a eutrophic river affected by discharges from a pulp mill and several municipal sewage treatment plants (Motala River, Norrköping, Sweden). Sampling of the Stångå River was conducted in September 1988 and of the Motala River water in December 1988.

Concentration methods

Volatile organics in samples from the Stångå River and the Motala River were enriched by stripping. In addition, organics in samples from the Motala River were enriched by dichloromethane extraction.

Stripping was performed in an open system as described by Borén and co-workers[13,14]. The sample volume was 1 l, the stripping temperature 60°C and the stripping time 2 h. The carbon filter was extracted with distilled dichloromethane using an on-column syringe. The final volume of the extract was approximately 10 μl, thus giving a concentration factor of $1 \cdot 10^5$.

Enrichment of organics in water by dichloromethane extraction was performed as described by Wigilius *et al.*[15]. The sample volume was 7.5 l and the final volume of the concentrated extract was 75 μl, thus giving a concentration factor of $1 \cdot 10^5$.

Sensory evaluation of extracts redissolved in water

Sensory evaluation of extracts obtained by dichloromethane extraction or stripping was performed by dissolving the extracts in odourless water and characterizing the flavour created. More precisely, 2 μl of the extract were rapidly injected into 200 ml of MilliQ (Millipore) water in a graduated flask. The small droplets thus produced were left to dissolve slowly in the water without stirring. After 1 h the water sample was transferred to an erlenmeyer flask, covered with a watch-glass and heated at 60°C in a water-bath for approximately 15 min. The threshold odour number (TON) of the water with the dissolved extract was determined with triangle tests at 60°C[16]. Odour quality was characterized using the descriptors suggested by Mallevialle and Suffet[12].

Two trained panellists took part in all sensory evaluations of dissolved extracts. The main results were confirmed by a group of four panellists. Odour usually being the most important component of the flavour, the concepts of odour and flavour were used interchangeably.

Losses during dissolution and heating of extracts in water

Losses during dissolution and heating of extracts in water were evaluated with a mixture of fourteen organic compounds with different functional groups and boiling points from 174 to 274°C (see Table I). Aliquots of this mixture (2 μl of dichloromethane containing 5 ng/μl of each compound) were added to 200 ml of Milli-Q water in five different flasks. After dissolution and heating at 60°C in a water-bath for 30 min, the five water samples were combined and analysed by stripping analysis. In a parallel experiment, 10 μl of the test mixture were added directly to the stripping bottle.

GC parameters

The following conditions were used: gas chromatograph, Hewlett-Packard 5880; fused-silica column, DB-1 (0.25 μm), 60 m × 0.32 mm I.D. (J&W); carrier gas, helium at 40 cm/s; temperature programme, 40°C for 5 min, increased at 5°C/min to 230°C, held for 5 min; flame ionization detection (FID).

All extracts were analysed using on-column injection. Surface water extracts were also analysed using splitless injection with the split valve closed for 180 s.

The C_6, C_8, C_{10} and C_{12} 1-chloroalkanes were used as standards, and retention indices (I) were calculated according to

$$I = \frac{t_{R(\text{substance})} - t_{R(Z)}}{t_{R(Z+1)} - t_{R(Z)}} + Z$$

where $t_{R(Z)}$ and $t_{R(Z+1)}$ are retention times for the standards that bracket the substance of interest and $Z = 1$, 2 or 3.

Chromatographic sniffing

The GC capillary column was led through copper tubing to a sniffing funnel outside the chromatograph[9]. A trained observer recorded the retention time and assessed the perceived odour intensity and odour quality of each odorous compound in the column effluent. Assessments of odour intensity were made according to a 6-grade scale, with 1 = weak and 6 = strong.

The sensitivity of the chromatographic sniffing technique was evaluated by two different TON analyses of dichloromethane extracts of known odorous compounds. One of the TON values, TON_{water}, is the ordinary TON value at 60°C of a water sample obtained by dissolving 2 μl of the extract in 200 ml of odourless water (see below). The other, $TON_{GCsniff}$, denotes the largest dilution of the extract giving a detectable odour in chromatographic sniffing (on-column injection, 2 μl). For each of the compounds in Fig. 3, the concentration was adjusted to give a TON_{water} value of 8. The extracts thus obtained were then analysed by chromatographic sniffing at different dilutions with distilled dichloromethane. Perceived odour intensities in chromatographic sniffing were recorded and the $TON_{GCsniff}$ was determined.

When analysing extracts of surface water samples, the concentration factor was $2 \cdot 10^4$. The GC parameters were as above.

Preparative GC

Fractions of the GC effluent were cold-trapped in PTFE tubing (Habia Teknofluor; 20 cm × 1.2 mm I.D.). The device connecting the GC column to the PTFE tubing is shown in Fig. 1. From this device the PTFE tubing was led through the GC oven wall and, thereafter, through a small container of liquid nitrogen. This arrangement guaranteed that the entire GC column had the same temperature, thus eliminating retardation or cold-trapping at the end of the column. Further, this trapping device permitted the fast exchange of PTFE tubing during a chromatographic run.

In order to increase the trapping efficiency, the PTFE tubing was pre-treated with distilled dichloromethane. Several droplets of the solvent (total volume 3 μl) were applied inside the tubing. When the desired fraction had been collected, the tubing was extracted with dichloromethane, giving a final extract volume of 10 μl. The GC parameters were as above.

Instrumental evaluation of GC fractionation

The GC fractionation technique was evaluated instrumentally with the previously mentioned fourteen component mixture (see Tables 1 and 2). A 3-μl volume of this mixture (5 ng/μl of each compound in dichloromethane) was injected on-column. Suitable fractions of the GC effluent were collected as described above, and the recovery was determined by GC with FID. 1-Chloroundecane (50 ng/μl in dichloromethane) was used as an internal standard. The GC parameters were as above.

GC fractionation of extracts of water samples

In order to isolate the fractions giving the largest contribution to the off-flavour of the water, the original extract was split into fractions step by step. Each fraction was dissolved in odourless water, and the fractions creating the highest TON values were further split into smaller fractions. The results of the chromatographic sniffing were used as a guide for the selection of suitable retention time intervals in the fractionation procedure.

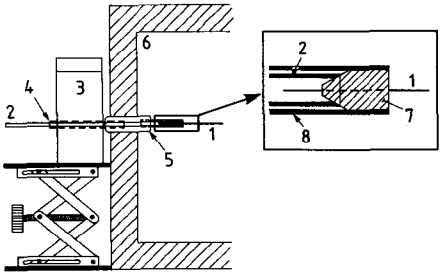

Fig. 1. Device for cold trapping of different fractions of the GC effluent. 1 = GC column; 2 = PTFE tubing for fraction collection; 3 = liquid nitrogen container; 4 = metal tubing through the liquid nitrogen container; 5 = connecting PTFE block; 6 = GC oven; 7 = PTFE plug; 8 = PTFE tubing.

RESULTS

Losses during dissolution and heating of extracts in water

In the standard procedure for dissolving extracts in water, the extract was left to dissolve in cold water for 1 h and then heated for about 15 min at 60°C in a water-bath before sensory evaluation was begun. Losses of different compounds in the fourteen-component mixture after 30 min at 60°C in a water-bath are shown in Table I. For compounds with permanent dipoles, losses from water were normally negligible. Substances with a long hydrocarbon chain, such as methyldecanoate, were exceptions. As expected, the largest losses were obtained for non-polar, volatile compounds. Sensory evaluations of extracts containing such compounds should therefore be performed as quickly as possible.

The loss of dichloromethane during dissolution and heating was determined by dibutyl ether extraction and GC analysis with FID. The results obtained showed that the dichloromethane concentration decreased only slowly at 60°C. After 30 min about 50% of the solvent remained, and after 60 min about 35% remained. The extract-to-water ratio ($1:10^5$) in the standard procedure for dissolving extracts in water was close to the highest ratio permitting sensory evaluation of the extracts without interference from the smell of the solvent.

Instrumental evaluation of the GC fractionation technique

When the solvent elutes from the column, it condenses in the cold trap, thus creating a solvent film on the inside wall of the PTFE tubing. This solvent film, which could also be created by pre-treating the PTFE tubing with small droplets of dichloromethane, proved to have a crucial effect on the recovery of many compounds. The results in Table II show that pretreated tubing gave a recovery of at least 80% for

TABLE I

RELATIVE LOSSES FROM WATER DURING DISSOLUTION AND HEATING (60°C, 30 min) OF 2 μl OF A DICHLOROMETHANE EXTRACT (5 ng/μl OF EACH COMPOUND) IN 200 ml OF WATER

Compound	*b.p. (°C)*	*Relative loss (%)*
n-Decane	174	90
Acetophenone	202	0
1-Chlorooctane	180	75
1-Octanol	194	0
Benzyl acetate	215	0
Naphthalene	218	25
2,6-Dichloroanisole	220	8
3-Phenyl-1-propanol	236	[a]
1,2,3,5-Tetrachlorobenzene	246	43
Methyl decanoate	224	58
2,3,6-Trichloroanisole	240	0
Diphenyl ether	258	0
2-Methoxynaphthalene	274	0
Ethyl cinnamate	272	[a]

[a] Not possible to analyse by stripping analysis.

TABLE II

RECOVERIES OF MODEL COMPOUNDS (5 ng/μl) OF EACH COMPOUND IN DICHLOROMETHANE) AFTER ON-COLUMN INJECTION (3 μl), COLD-TRAPPING IN PTFE TUBING PRETREATED WITH 3 μl OF DICHLOROMETHANE AND EXTRACTION OF THE TUBING WITH 7 μl OF DICHLOROMETHANE

Mean values and standard deviations (n = 5).

Compound	*Recovery (%)*	
	Mean	*Standard deviation*
n-Decane	81	3
Acetophenone	81	5
1-Chlorooctane	83	6
1-Octanol	82	6
Benzyl acetate	82	4
Naphthalene	81	4
2,6-Dichloroanisole	83	4
3-Phenyl-1-propanol	82	5
1,2,3,5-Tetrachlorobenzene	68	8
Methyl decanoate	74	5
2,3,6-Trichloroanisole	74	6
Diphenyl ether	75	5
2-Methoxynaphthalene	69	7
Ethyl cinnamate	73	7

the most volatile compounds in the test mixture. Without pretreatment the recovery of these compounds was less than 50%. The small decrease in the recovery of very high-boiling compounds in Table II may also be related to the solvent film. High-boiling compounds are probably adsorbed on the walls of the PTFE tubing before they reach the solvent film, and may therefore be more difficult to extract.

The average recovery of the fourteen model compounds in Table II was 78%. Additional experiments showed that losses were primarily caused by incomplete recovery of solvent from the PTFE tubing. When this factor was eliminated by calculating recoveries with respect to an internal standard added directly to the solvent in the PTFE tubing, the average recovery for the model compounds increased to 96%.

The feasibility of collecting very small fractions is illustrated by the chromatograms in Fig. 2. The recovery of the selected compound was 66%, and there were no traces of surrounding compounds. The retention time distances to preceding and subsequent peaks were 28 and 29 s, respectively.

Sensitivity of chromatographic sniffing

The results in Fig. 3 demonstrate the high sensitivity of the chromatographic sniffing technique. Let TOC_{water} denote the threshold odour concentration of a certain compound in water (as determined by triangle tests at 60°C) and assume that this compound is concentrated by a factor of 10^5 by the enrichment method used. The lowest concentration in water that can be detected by chromatographic sniffing, $TOC_{GCsniff}$, is then

$$TOC_{GCsniff} = TOC_{water} \cdot TON_{water}/TON_{GCsniff}$$

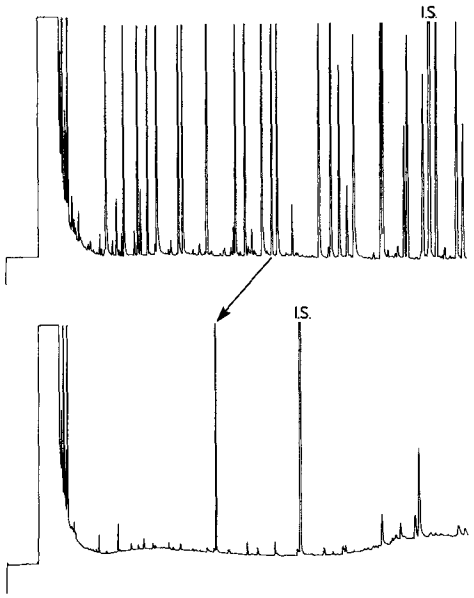

Fig. 2. Cold trapping of one selected compound (1-chlorooctane) in a mixture of organic compounds (5 ng/μl in dichloromethane). On-column injection. PTFE tubing pretreated with 3 μl of dichloromethane. Internal standard added to the extract, 1-chloroundecane.

For all compounds tested, this concentration was at least ten times lower than the TOC_{water}. For compounds such as MIB and 2,4,6-trichloroanisole it was more than 300 times lower.

Artefacts in GC fractionation

Two different injection techniques were used in the GC fractionation. For one of the surface water extracts (obtained by dichloromethane extraction of water from the

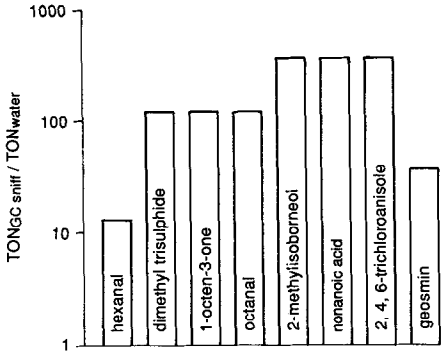

Fig. 3. Ratios of $TON_{GCsniff}$ and TON_{water} for dichloromethane extracts of known odorous compounds. $TON_{GCsniff}$ determined by on-column injection of 2 μl of the extract; TON_{water} determined at 60°C after dissolving 2 μl of the extract in 200 ml odourless water.

Motala River), the choice of injection technique had a marked effect on the sensory properties of the collected GC effluent. When dissolved in water, the effluent from splitless injection created a pungent smell. Using on-column injection, however, the original odour of the river water was recreated. Chromatographic sniffing and GC fractionation confirmed these results. During then first 10 min of the GC run, chromatographic sniffing gave several detections that were much stronger for splitless injection than for on-column injection. Sensory evaluation of GC fractions dissolved in water gave similar results. This strongly indicated that new odorous compounds were formed in the splitless injector, which had a temperature of 250°C.

Case study I: Stångå River

The water from the Stångå River had a TON value of 64. A strong earthy/musty odour indicated, even prior to the GC fractionation, that MIB or geosmin might be present in the water.

When the stripping extract was dissolved in odourless water, the original earthy/musty odour of the river water was recreated with a TON value of 32. This indicated that the compounds making the largest contribution to the off-flavour of the Stångå River water could be enriched by stripping. Further, these compounds were apparently gas chromatographable. A 3-μl volume of the stripping extract was injected, and the whole GC effluent (retention time interval 0–35 min) was collected. When redissolved in water, the extract with this effluent created a flavour of the same intensity and quality as the original stripping extract.

Prior to GC fractionation, the stripping extract was analysed by chromatographic sniffing. At a concentration factor of $2 \cdot 10^4$, twelve odorous compounds, including MIB and geosmin, were detected. The sniffing chromatogram is shown in Fig. 4.

In an attempt to confirm that MIB and geosmin were the two most important off-flavour compounds in the stripping extract, the GC effluent was split into three fractions: a small fraction with MIB, a small fraction with geosmin and a large fraction

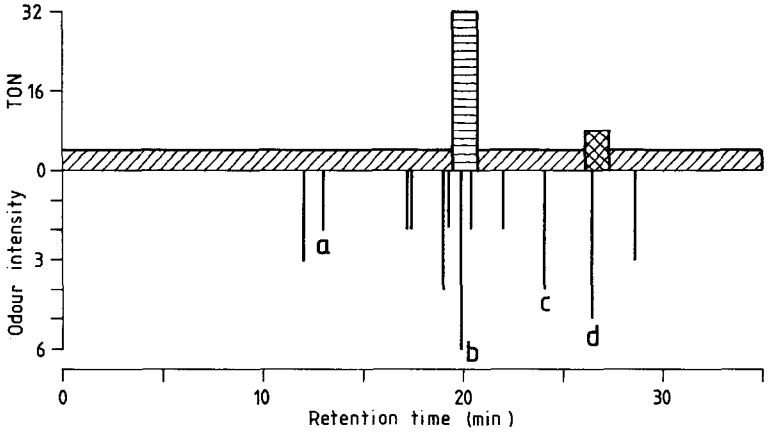

Fig. 4. GC fractionation of a stripping extract of a surface water sample from the Stångå River. TON values of different fractions, dissolved in odourless water, and sniffing chromatogram. a, 1-Octen-3-one; b, 2-methylisoborneol; c, 2,4,6-trichloroanisole; d, geosmin (see text).

covering the remaining parts of the chromatogram (Fig. 4). When dissolved in water, the MIB fraction created the same odour quality and TON value (32) as the original stripping extract. The geosmin fraction gave a TON value of only 8. The third fraction gave nine detections in chromatographic sniffing. However, when dissolved in water, this fraction gave a TON value of only 4. It was concluded that MIB was the most important off-flavour compound in the water from the Stångå River.

Case study II: Motala River

The water from the Motala River had a TON value of 32. The odour quality was described as being more marshy/swampy than earthy/musty.

The off-flavour compounds in the Motala River water were not satisfactorily enriched by stripping. When dissolved in odourless water, the stripping extract of this water created just a faint flavour (TON = 4). Dichloromethane extraction proved to be a more efficient concentration method. The extract obtained by this method was able to recreate a flavour that was difficult to distinguish from that of the original water sample. Provided that on-column injection was used (see above), this odour could also be recreated by the GC effluent of the dichloromethane extract. Actually, the TON value (32) of the water with the dissolved GC effluent was the same as that for the original river water sample. When analysing the dichloromethane extract by chromatographic sniffing, more than twenty detections were made (Fig. 6). The stepwise GC fractionation of this extract and the TON value of each fraction, dissolved in water, are shown in Fig. 5.

Fig. 5 A shows that the first half of the chromatogram (0–21.70 min), including MIB, made only a small contribution (TON = 8) to the odour intensity of the whole

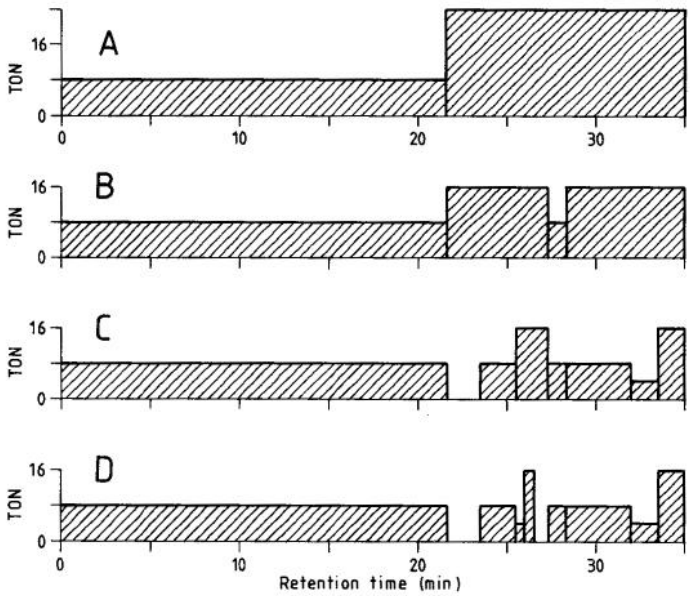

Fig. 5. Stepwise GC fractionation of a surface water sample from the Motala River. Enrichment by dichloromethane extraction. TON values of different fractions dissolved in odourless water.

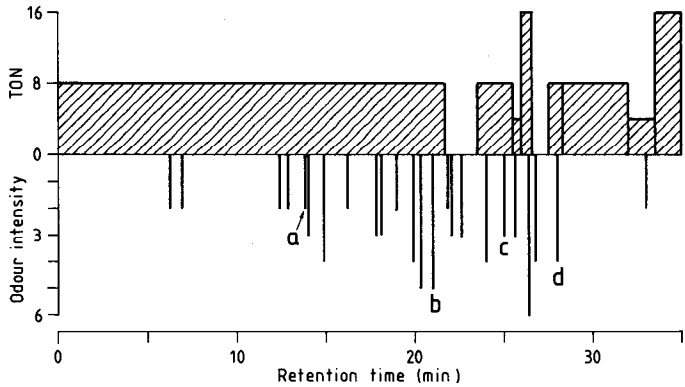

Fig. 6. TON values of different fractions obtained by GC fractionation and sniffing chromatogram. Enrichment by dichloromethane extraction. Water sample from the Motala River. a, 1-Octen-3-one; b, 2-methylisoborneol; c, 2,4,6-trichloroanisole; d, geosmin.

GC effluent. The most important off-flavour compounds were obviously present in the second half of the chromatogram (21.70–35.00 min), which gave a TON value of 16–32. Fractionation of the second half of the chromatogram (Fig. 5B) showed that geosmin, which had a retention time of approximately 28 min, was surrounded by important off-flavour compounds. However, the geosmin fraction alone created a weak flavour (TON = 8), indicating that neither geosmin nor MIB belonged to the more prominent off-flavours in the Motala River water.

On splitting the fraction from 21.70 to 27.25 min into three subfractions, two entirely different flavours were revealed. The last subfraction (25.50–27.25 min) had a musty flavour with TON = 16, while the middle fraction (23.75–25.50 min) had a fragrant flavour with TON = 8 (Fig. 5C). On splitting the fraction from 28.30 to 35.00 min in a similar way, another three flavours, with TON values of 8, 4 and 16, were revealed. The first was described as fragrant and the other two as earthy/musty.

The first fraction with TON = 16 (Fig. 5C) was further split into three subfractions (Fig. 5D). Sensory evaluation of these subfractions, dissolved in odourless water, strongly indicated that the retention time interval 26.00–26.60 min (retention index 3.40–3.51) contained one of the compounds contributing the most to the off-flavour of the Motala River water.

The agreement between the TON values of different fractions and the results of the chromatographic sniffing was partially good and partially less satisfactory (Fig. 6). One of the two fractions having the highest TON value (16) contained the compound having the highest odour intensity in chromatographic sniffing. However, in the other fraction, giving a TON value of 16, chromatographic sniffing gave no detections at all. Further, it is worth noting that, for some fractions that gave no flavour when dissolved in water, column sniffing resulted in several detections.

To summarize, the fractionation performed showed that the Motala River water contained at least six different compounds that were able to give the water a TON value of 8 or higher. Two fractions, each having a TON value of 16, were pointed out as particularly important for the flavour of the water. The compounds causing these off-flavours were present in concentrations too low to permit identification by GC–mass spectrometry.

DISCUSSION

The use of chromatographic sniffing and sensory evaluation of GC fractions dissolved in odourless water has several aspects worth discussing, including technical measures to guarantee optimum performance, relevance of chromatographic sniffing data and application areas of the methods proposed.

Stripping enrichment has been used successfully in several studies of off-flavours in water[2,5,9]. Recently, Lundgren *et al.*[11] observed that certain off-flavours were more efficiently enriched by other concentration methods, *e.g.*, dichloromethane extraction. GC fractionation of extracts of the Motala River water confirmed this observation. However, it also showed that the extracts obtained by solvent extraction were not ideal for GC fractionation. When on-column injection was used, the life of the GC column was markedly shortened by the high-molecular-weight substances in the extract. When splitless injection was used, odorous compounds formed in the injector interfered with the sensory analysis of odorous compounds in the water. Therefore, as long as the flavour of the water sample can be recreated by dissolving the stripping extract in odourless water, stripping is the enrichment technique to be preferred.

Preparative GC has previously been used in several areas[17–19]. This work emphasized the development of a simple device, giving high recoveries of compounds over a fairly wide range of boiling points. Cold trapping in PTFE tubing produced very satisfactory results, provided that the tubing was pretreated with a few droplets of dichloromethane. The last step in the proposed procedure, dissolution of GC fractions in odourless water, worked properly, provided that the extracts were given sufficient time to dissolve in cold water.

Sävenhed *et al.*[9] have previously shown that, for some well known odorous compounds, chromatographic sniffing gives a lower detection limit than FID. This study demonstrated both the strength and the weakness of the chromatographic sniffing technique. The odorous model compounds in Fig. 3 could all be detected by this technique at concentrations far below their threshold odour concentrations in water. However, these results also showed that TON values, as determined by chromatographic sniffing, are of limited value in predicting the contribution of specific compounds to the off-flavour of a water sample. This was further demonstrated by the Motala River case study.

It seems reasonable to assume that, for less volatile compounds, chromatographic sniffing (in combination with an efficient enrichment method) implies higher exposure than direct sensory evaluation of the water. This would explain why compounds such as MIB and 2,4,6-trichloroanisole gave a higher $TON_{GCsniff}$-to-TON_{water} ratio than more volatile compounds such as hexanal. However, there were also indications of high-boiling compounds being trapped in the sniffing device. The sensitivity to geosmin in chromatographic sniffing was surprisingly low, and the last GC fractions from the Motala River extract made a considerable contribution to the flavour of the water, even though these fractions gave few or no detections in chromatographic sniffing.

In summary, this study has shown that chromatographic sniffing is very useful for listing a number of potentially important off-flavour compounds. It can also provide guidance in the selection of suitable retention-time intervals in GC fractionation. However, the relevance of perceived odour intensities in chromatographic

sniffing is difficult to judge, unless this technique is complemented by GC fractionation and sensory evaluation of different fractions dissolved in water.

Studies of the origin of off-flavours in water may have two objectives: to connect the off-flavour to certain activities, processes or organisms, and to identify the substances causing the off-flavour. In both instances, the combined use of chromatographic sniffing and sensory evaluation of GC fractions redissolved in odourless water is a powerful technique. The chromatogram of a surface water sample, analysed by stripping enrichment, high-resolution GC and FID, is normally very complex. The list of candidates of off-flavour substances thus produced may exceed 100 compounds. Still, it is uncertain whether this list will include the compounds making the largest contribution to the off-flavour of the water[9]. Chromatographic sniffing may reduce the list of candidates to 10–30 compounds and increase the probability that the important compounds are included. However, in order to quantify the contribution of specific compounds to the off-flavour of the water, preparative GC and sensory analysis of extracts dissolved in odourless water are indispensable.

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