Application of large volume injection GC/MS to the picogram analysis of chlorinated and brominated anisoles in “earthy-musty” off-flavor water samples

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Abstract This paper summarizes the most commonly used methods to identify the origins of taste and odor episodes. An innovative analytical tool which allows the identification of odorous compounds at levels as low as 25 pg/L by injecting a large volume of extracts, is described. This large volume on-column technique was employed to investigate real “earthy-musty” cases which remained unsolved using traditional chromatographic methods. “Musty” odor causative agents could be identified in each sample studied, proving the efficiency of this new method. Estimated concentrations were well correlated with taste intensities. The relative importance of haloanisoles when dealing with musty odors was highlighted, and 2,4,6-tribromoanisole was detected for the first time in water samples. Finally, analysis of the corresponding halophenols for some of the samples studied gave insights concerning the origin of the odorous anisoles.

Keywords Drinking water; “earthy-musty” odor; large-volume on-column injection; picogram analysis; 2,4,6-tribromoanisole; 2,4,6-trichloroanisole

Introduction
Tastes and odors (T&O) in drinking waters together with water hardness and water turbidity represent one of the major causes of consumer complaints. Different surveys carried out in various French regional centers of the company Lyonnaise des Eaux indicate that taste and odor episodes are responsible for 30 to 50% of recorded complaints.

The European Drinking Water Directive stipulates a maximum taste and odor threshold (dilution ratio with a reference water beyond which no odor or taste is perceived) equal to 3. The recent World Health Organization (WHO) recommendations indicate that drinking waters must not present unpleasant tastes but do not include any guideline value. The above mentioned T&O guideline value will probably disappear from the revised drinking water directive. However, beyond these legal considerations, off-flavor episodes strongly contribute to the declining public image of water companies and lead to a worrying paradox: while the price of drinking water is gradually increasing under the influence of the enormous investments made necessary to comply with the more stringent quality guidelines, consumption of bottled water by the general public is also increasing. Consumers tend to believe that if their water tastes or smells bad, then it probably is not safe (McGuire, 1995).

Tastes, odors, mouth feels and nose feels from drinking waters have been classified according to a flavor wheel which comprises four groups for the basic tastes, one group for the mouth and nose feels, and eight odor groups (Mallevialle and Suffet, 1987). These odor groups have also been classified according to their frequency of occurrence (Mallevialle and Suffet, 1987). This classification may vary as a function of the type of water, in particular, descriptors from group 7 (medicinal–phenolic) may become much more common in waters treated with chlorine, due to the reaction between the latter disinfectant with iodides naturally present in many groundwaters or phenolic contaminants, a reaction which leads to the formation of iodinated trihalomethanes (Bruchet et al., 1989) or chlorophenols with characteristic medicinal odors. In general, the first two groups from the flavor wheel, earthy-musty-moldy and chlorinous-ozonous, respectively, indeed appear to be the most
common in France when examining consumer complaints. These two groups also appear to comprise the highest rate of unsolved episodes when the earthy-musty or chlorine descriptors cannot be attributed to the common algal metabolites geosmin and 2-methylisoborneol (MIB) or to residual chlorine. Therefore, new analytical tools still need to be developed to ultimately understand the origin of all T&O episodes.

This paper briefly reviews the most common traditional methods used to search for traces of odorous compounds, then focuses on the description of an innovative large-volume injection technique (LV GC-MS) that allows the detection of ultratraces of the highly odorous haloanisoles. Successful examples of application of this technique at various sites are presented.

Traditional analytical methods for trace odorous compounds

A wide range of analytical techniques have been developed to identify the volatile odorous compounds often present at trace levels. It should be kept in mind that odor thresholds of specific volatiles can be in the ng/L range or even below. Because of the wide array of possible preconcentration–extraction–separation and detection techniques, the analytical chemist faced with a T&O episode is compelled to make an a priori choice based on past experience and on the organoleptic characteristics of the sample. As an obvious example, a sample yielding an hydrocarbon odor will be searched for petroleum-based products by liquid–liquid extraction gas-chromatography or infrared absorption.

Due to the very low concentrations to be determined, an extraction–preconcentration step is an absolute prerequisite when searching for T&O compounds. The physico-chemical principles involved during this step include stripping, liquid–liquid partitioning and liquid–solid extraction. The use of stripping-based techniques is justified by the volatile character of most odorous compounds, which explains why the closed loop stripping analysis method (CLSA) (Mallevialle and Suffet, 1987) has become the primary choice of most analysts to search for ng/L levels. In particular, CLSA GC-MS allows the sub-threshold detection of odorous algal metabolites such as geosmin, 2-methylisoborneol, 2-isopropyl- or 2-isobutyl-3-methoxypyrazine, which yield intense earthy-musty odors at levels as low as 2–5 ng/L. The very low detection limits achieved with this method stem from the high concentration factor achieved (40,000 times) and the absence of a solvent concentration step.

Alternatively, purge and trap techniques that apply the same principles but tend to be less sensitive, can be very useful for the search of solvents above the 0.1 µg/L level. However, stripping techniques can prove inefficient for less volatile, more polar or more water-soluble compounds or compounds with low activity coefficients such as chlorophenols or iodinated trihalomethanes. In such cases, liquid–liquid extraction (LLE) will prove more useful. Finally, several authors have started to investigate the usefulness of liquid–solid phase-extraction (SPE) or solid phase microextraction (SPME) (Graham and Hayes, 1998).

From a chromatographic point of view, high resolution gas-chromatography coupled with mass spectrometry (HR GC-MS) is the sole method with enough separation and identification power to cope with the trace compounds involved during T&O episodes. High pressure liquid chromatography has only been occasionally applied to search for specific odorous products such as low molecular aldehydes after a derivatization step (Bruchet et al., 1992).

Use of LV GC-MS to solve “earthy-musty” taste and odor cases

Method

Samples. Three types of water samples were examined in this study:

- Raw water from the River Seine collected from a drinking water treatment plant upstream of Paris, after ultrafiltration (samples 1 and 2, collected in February 1999).
• Treated water (sample 3: water collected in December 1997 from a storage tank, which originated from a well and was treated only by chlorination, and sample 4: drinking water collected at a treatment plant in February 1998 originating from a well and treated only by chlorination).
• Tap water collected at CIRSEE (sample 5) in April 1999, and at refreshing fountains of the Technocentre Renault in January 1998 (samples 6 and 7). The CIRSEE tap water is supplied by a plant downstream of Paris, which treats a ground water artificially recharged from the River Seine water.

Sensory analysis. The samples were analyzed by two types of sensory analyses: the Threshold Taste Number (TTN) and the Flavor Profile Analysis (FPA) as described in Mallevialle and Suffet (1987).

Closed-loop stripping analysis (CLSA). Volatile “earthy-musty” compounds from one litre of water samples were adsorbed on an activated carbon trap by stripping the sample with nitrogen gas for two hours. The water bath was held at 45°C and the carbon filter at 55°C. The carbon filter was then extracted with 25 µL of carbon disulfide. Chloroalkanes were added as internal standards in the water samples (ClC6, ClC10, ClC12, ClC16), and on the carbon trap (ClC8, ClC14) to obtain a final concentration of 200 ng/L for each compound.

Large-volume injection gas chromatography mass spectrometry (LV GC-MS). The only way to deal with the very low detection limits required during musty odor episodes often caused by trace organic compounds is to apply the recently developed high volume injection GC-MS technique, preferably working in an on-column mode which avoids losses of volatiles. The principle of the injection technique is shown in Figure 1.

The sample extract (from 10 to 250 µL) is injected by an autosampler (AS 800) at a controlled speed through a cold on-column injector into a 15 m precolumn which comprises of 12 m of empty deactivated phase and a 3 m retaining column (UNCORET, proprietary design, Fisons Instruments, Milano, Italy). During a subsequent step, both the solvent and the most volatile solutes evaporate from the rear of the solvent injection band while heavier solutes remain throughout the precolumn (Magni et al., 1996). Under controlled pressure (DPFC 800) and temperature conditions the solvent vapours (90 to 95%) are vented through the solvent vapour exit (SVE) until a preselected amount of final sample volume is left inside the precolumn. The reconcentrated exact is then flushed into the analytical column. Both solvent and retention gap effects allow refocussing of the deposited analytes before the flushing step.

SVE control

Figure 1 Principle of on-column large-volume injector
During the present study, because the classical GC-MS injection conditions (1 µL) did not allow the identification of any odorous compounds, the remaining carbon disulfide extracts were diluted to 50 µL, and 40 µL aliquots were then analyzed by LV GC-MS. LV GC-MS analyses were carried out on a GC 8000/MD 800 instrument (CE Instruments). The conditions of injection were the following: inlet temperature 52ºC, inlet pressure 140 kPa, solvent vapour exit delay time 19 s. The GC conditions were 52ºC for 15 min, 3ºC/min up to 130ºC, then 7ºC/min up to 250ºC for 10 min. The analytical column was a 50 m × 0.32 mm inside diameter, CPSil 19 CB (Chrompack). The detection was performed in full scan mode in the range from 80 to 400 amu (0.45 s/scan).

**Identification and quantification.** Target compounds that have to be searched during “earthy-musty” off-flavor episodes, together with their Threshold Odor Concentration (TOC) and their characteristic ions are listed in Table 1.

Hence the full scan chromatograms of the CLSA extracts obtained in this work were searched for the specific ions of these target compounds. “Earthy-musty” compounds detected in samples were identified by the presence of their specific ions reported in Table 1, with the correct isotopic ratios, at the corresponding linear retention index (calculated relative to the chloroalkane internal standards).

**Results and discussion**

**Results of sensory analyses**

The results from the sensory analyses (TTN and FPA) are reported in Table 2. All the episodes studied were characterized by an intense musty descriptor. Whenever flavor profile analysis was used, an intensity of 6 to 10 (strong) on the 1 (threshold) to 12

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(very strong) scale of this method, was found by the trained panelists. Episodes when the threshold taste number test (TTN) was used revealed values ranging between 1 and ≥ 5. It should be recalled that the European Directive for drinking waters stipulates a maximum TTN of 3 at 25ºC. Therefore samples 3, 4, 6 and 7 exceeded this guideline. Samples 2 (TTN = 3) and sample 5 (odor and taste intensities = 6 and 8) also showed musty odor or taste intensities easily detectable by consumers.

Identification and quantification of the “musty” odor causative agent(s)

GC-MS chromatograms of the CLSA extracts acquired under classical conditions (1 µL injected) did not reveal any compound known to impact “musty” taste like MIB, geosmin or trichloroanisoles to drinking water. On the other hand, an illustration of the power of on-column LV GC-MS in terms of increased sensitivity is depicted in Figure 2 which compares the response obtained by injecting a classical volume of 1 µL (Figure 2a) and a large 40 mL volume (Figure 2b), respectively, of the CLSA solvent extract from sample 4 (chlorinated ground water) and acquiring in the full scan mode. In this case, the characteristic fragments of the musty-smelling 2,4,6-trichloroanisole were detected with a signal to noise ratio equal to 141, 85 and 50, respectively, for the ions m/z = 195, 210 and 167 with LV GC-MS, while this compound was not detected using classical GC-MS.

The application of LV GC-MS and the specific search for the presence of the odorous compounds listed in Table 1 could establish the origin of the odor in the seven “earthy-musty” cases studied. The causative agents could be identified and their concentrations were evaluated for each sample. The results are presented in Table 3.

The reported concentrations have to be compared with the threshold odor concentration of each compound (comparison of Tables 1 and 3). It can be noticed that at least one of the compounds detected in each sample was at a level superior to its threshold odor concentration. Thus the characteristic “musty” note of these samples could be explained.

Classical algae metabolites like geosmin or MIB were not detected in any sample, thus highlighting that a wide range of “musty” compounds have to be searched during such “musty” cases and demonstrating also the interest of full scan mass spectrometric detection when searching for the causative agent of an odor. Moreover, this work demonstrates the need for very sensitive analytical methods because the main causing taste agents which have been identified in this study were 2,4,6-trichloroanisole and 2,4,6-tribromoanisole which both have an extremely low odor threshold around 30 pg/L (UKWIR, 1996).

From another point of view, the identification of the 2,4,6-tribromoanisole musty compound in three of the seven samples examined proved the interest of including this analyte in the list of target compounds which have to be searched during “musty” off-flavor episodes. Subsequently, the chromatograms of samples 6 and 7 acquired by classical GC-

Figure 2 Chromatograms from the CLSA extract of sample 4 (chlorinated well water): (a) acquired by classical GC-MS; (b) acquired by LV GC-MS (2,4,6 or 2,3,6-TCA = 2,4,6 or 2,3,6-trichloroanisole)
MS were reexamined by specifically extracting the signal of the characteristic ions \( m/z = 303, 331, 346 \) or 2,4,6-tribromoanisole. This verification showed that in these two cases the signal to noise ratio obtained for these ions (signal to noise ratio in the range of 25 to 130 depending on the fragment and the sample) was sufficient and the mass spectra well resolved to ensure that 2,4,6-tribromoanisole was present. However the peak corresponding to 2,4,6-tribromoanisole could not have been detected by processing the full scan GC-MS chromatogram. In fact, the odor threshold of 2,4,6-tribromoanisole had been published in the literature (UKWIR, 1996), but this compound had never been reported in drinking water samples, explaining why it was not searched during routine analysis of “musty” smelling compounds. Conversely, 2,3,4,6-tetrachloroanisole which was also included in the list of “musty” target compounds during this work based on literature odor threshold data, was not detected in any of the seven samples investigated.

**Correlation between sensory and chemical results**

Concerning sample 4, the odorous intensity of 6 to 8 detected by FPA, suggested that the concentration(s) of 2,4,6- and/or 2,3,6-trichloroanisoles must be significantly higher than their odor threshold concentrations, respectively equal to 0.05 and 7 ng/L (see table 1). This later remark is in concordance with the 1 ng/L level of concentration of 2,4,6-trichloroanisole, estimated by the CLSA-LVGC/MS method. Therefore the presence of this compound at a level equal to 20 times its odor threshold concentration explained the high intensity of the “earthy-musty” odor.

For sample 3, 6 and 7 the taste threshold numbers were equal or superior to 5. Hence the only correlation between chemical and analytical results which could be inferred, was that the concentration of at least one of the musty compounds detected in those samples must be superior or equal to five times its threshold odor concentration. That means that the concentration of 2,4,6-tribromoanisole must be equal or superior to 0.15 ng/L (= 0.03 × 5) in samples 3, 6 and 7. A comparison between the latter mentioned concentrations and those reported in Table 2 demonstrates a good agreement between the concentrations estimated from sensory analyses and from chemical analyses for these three samples.

In the same way, the taste threshold number of 1 and 3 samples 1 and 2, respectively, indicates that the concentration of 2,4,6-trichloroanisole must be close to 0.05 ng/L in sample 1 and to 0.150 ng/L (= 3 × 0.05) in sample 2. Again both values are well correlated with those reported in Table 2.

**Preliminary investigations and hypotheses concerning the origin of the musty compounds**

It is now well established that haloanisoles are metabolized from their corresponding halophenols by microbiological methylation (Nyström et al., 1992; Whitfield et al., 1997).
Their halophenolic precursors are reported to result from the chlorination of phenol, humic substances, or other precursors present in the raw water before chlorine disinfection of drinking water. It is also reported that enzyme-mediated formation of reactive chlorine could yield chlorinated by-products and could thus be a natural source of 2,4,6-trichlorophenol (Karlson et al., 1995).

To determine the source of 2,4,6-trihaloanisoles present in samples 1, 2 and 3, a sample of water collected at the same point as sample 3 and ultra-filtrated River Seine water (same origin as sample 1 and 2) were concentrated by liquid–liquid extraction and analyzed by LV GC–MS and by screening the chromatograms obtained for phenol and halophenols.

Hence 2,4,6-tribromophenol at a level of about 100 ng/L was detected in the water sample collected at the same point as sample 3, and 2,4,6-trichlorophenol at a level of about 10 ng/L was measured in the River Seine after ultra-filtration.

This seems to imply that 2,4,6-trichlorophenol is already present in the River Seine, arising from natural sources or more likely from pollution by urban or industrial wastewaters, and that 2,4,6-trichloroanisole is formed by biological activity in the River Seine itself. Because the cut-off of the ultra-filtration membrane used at the ultra-filtration plant investigated is too high (only compounds of atomic weight superior to 100,000 daltons are retained), both compounds were detected in the River Seine water after ultra-filtration. The concentrations of about 10 ng/L of 2,4,6-trichlorophenol with a concentration of 0.15 ng/L of 2,4,6-trichloroanisole determined for the River Seine are in line with results obtained by Karlson et al. (1995) in seemingly unpol-luted surface waters (raw water of the Norrköping treatment plant was found to contain about 9 ng/L of 2,4,6-trichlorophenol and 0.1 ng/L of 2,4,6-trichloroanisole). In both cases, the conversion rate between the halophenol and the haloanisole is of the order of 1% (Montiel et al., 1987).

Concerning the high amount of 2,4,6-tribromoanisole found in the water collected at the same point as sample 3, other experimental data and further discussion have already been reported (Malleret and Bruchet, 1999) and will not be detailed here. In summary, 2,4,6-tribromophenol was directly leaching from the tank coating and then transformed in the mass of the coating into 2,4,6-tribromoanisole by biological activity.

For other samples, complementary experiments were not carried out. However additional observations can be made. Firstly, at the treatment plant where sample 4 was collected, the musty taste of the water disappeared due to a disinfection by chlorine dioxide instead of chlorine. It is then probable that 2,4,6-trichlorophenol (precursor of 2,4,6-trichloroanisole) was formed during the disinfection by chlorine, further explaining the medicinal taste detected in sample 4. In general, disinfection by chlorine dioxide decreased or avoided the formation of disinfection by-products such as 2,4,6-trichlorophenol.

Secondly, sample 6 and 7 originated from cooling fountains, which often favor stagnation phenomena which increases the development of microorganisms and then biological activity. Intense biological activity could justify high levels of 2,4,6-tribromoanisole determined in these samples. The origin of the 2,4,6-tribromoanisole precursor and its nature (even if it was probably 2,4,6-tribromophenol) were not elucidated. Finally, it is interesting to note that sample 5 corresponded to water treated by the Ouest Yvelines Regional Center of Lyonnaise des Eaux. This center is also in charge of the treatment plant from where sample 3 was collect-ed. The presence of 2,4,6-tribromoanisole in sample 5 might then be correlated with the presence of this compound in sample 3 because of connection between distribution systems.

From a global point of view, samples 1, 2, 3 and 5, which contain 2,4,6-trichloroanisole or tribromoanisole (and their phenolic homologues in samples 1, 2 and 3), are representative of two major water resources for the Paris area.
Conclusion
As already discussed above, T&O episodes are most often due to infinitesimal traces of compounds present in a complex mixture. For this reason, high resolution gas-chromatography coupled with MS has been the separation and identification method of choice to deal with the complex multicomponent extracts obtained during taste and odor episodes. Detecting 1 ng/L of an odorous compound recovered at a 50% yield from 1 litre of water in a 25 µL CLSA extract is equivalent to injecting 20 pg/mL into the chromatographic column. This is about the current detection limit of modern benchtop quadrupole or ion-trap GC-MS instruments working in the full-scan mode. Although impressive, this detection limit remains too high in some instances. As an example, a human nose often detects odors during GC-sniffing. Furthermore, some of the background earthy-musty odors which dominate most natural waters cannot be related to the presence of the well known MIB or geosmin algal metabolites. As shown in this paper, such background odors often find their origin in compounds present at sub-nanogram per litre levels.

The method of choice to deal with such levels is to apply the recently developed high volume injection GC technique described in this paper. The advantage of this technique is to provide extremely low detection limits (around 25 pg/L) while retaining the full-scan GC-MS mode which allows unambiguous identification of the analytes. Furthermore this innovative approach involving large volume on-column injection is no more time-consuming and costly than classical CISA/GCMS, but provides detection limits 40 times lower, therefore opening a new area of research in the taste and odor field. Even if the instrument does represent an investment, the loss of earnings for laboratories caused by the incapability to identify the cause of taste and odor problems, and for water companies due to the use of inappropriate treatment process, must nevertheless be borne in mind. In particular, this new analytical tool has allowed us to solve most earthy-musty episodes submitted at CIRSEE during the period 1998–1999 and has significantly increased our ability to find the origin of yet unsolved episodes. This technique also hints at the widespread occurrence of traces of haloanisoles in drinking waters probably due to the biological transformation of the corresponding halophenols, which themselves arise from the chlorination of low molecular weight phenolic compounds which are widely distributed in natural waters. In particular, two of the sites where these compounds were identified represent major drinking water resources located both upstream and downstream of Paris.

In conclusion, a wide range of analytical methods is now available to help solve T&O episodes. It can be estimated that these methods allow the identification of the odorous compounds in 60% to 70% of such episodes. Development of new techniques will remain necessary in the future to better understand specific T&O groups or mouth feels which often remain unsolved.

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