

Optimization of a HS-SPME-GC/MS method for analysis of multi-class off-odor compounds in water

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Abstract

Noticeable drinking water problems often involve unusual odors characterized e.g. as rancid, fishy musty, moldy, grassy or swampy. Numerous biogenic or anthropogenic volatile organic compounds of various chemical classes that are present in source water or are introduced during water treatment or in the distribution system can be responsible for those incidents. Chemical classes include e.g. terpenoids, aldehydes, ketones, alcohols, pyrazines, amines, phenols or sulfurous compounds that vary widely in physicochemical and sensory properties. The aim of this study was to develop an optimized analytical procedure to detect, identify and quantitatively determine in one run a wide multi-class range of odor compounds in water using headspace solid-phase microextraction/gas chromatography–mass spectrometry (HS-SPME/GC–MS). The parameters studied include fiber type, salt addition, stirring rate (rpm), pre-incubation and extraction times, extraction temperature and desorption conditions. Design of experiments involved the screening of influential factors as well as response surface methodologies and desirability functions to determine optimum conditions for selected classes and for the whole multi-class range. Optimized conditions were further validated and protocols for class-targeted and multi-class non-targeted screening were developed. The method is applied as a diagnostic tool in source and drinking water samples and it is proved valuable as a diagnostic tool in cases of off-odor incidents.

Keywords: SPME, optimization, experimental design, water off-odors, GC/MS

1. Introduction

Water is known to be tasteless and odourless, however, taste and odour (T&O) is the most frequent source of consumer complaints with regards to drinking water. Numerous off-odour compounds can enter water supplies and make water completely unpalatable by consumers (WHO, 2011). When surface water is used as source for water supplies, a wide variety of T&O compounds can occur as metabolic or cell degradation products of aquatic plants or microorganisms commonly known as “algae” (including eukariotic and prokaryotic organisms). Those include e.g. alcohols, aldehydes, terpenes, ionones, amines, sulphurous compounds and various products through biotic or abiotic transformation in the environment (Watson, 2004). During water treatment, further compounds can be produced, some of them having intense odour, e.g.

halophenols during chlorine disinfection (Acero *et al.*, 2004). T&O compounds can migrate into water from materials used in water treatment plants, in the distribution network or in household water supplies (Tomboulou *et al.*, 2004). Further bacterial transformations in distribution system biofilms can lead to production of unpleasant odours (e.g. trichloroanisoles) (Nystrom *et al.*, 1992). Water T&O compounds vary widely with regards to chemical structure and physicochemical properties such as solubility, polarity, vapour pressure and Henry’s law constants. Some of them have extremely low odour threshold concentrations (OTCs), i.e. they can be detected by the human nose at very low concentrations, e.g. in the range of ng/L or below (Young *et al.*, 1996). Common odour descriptions include “musty”, “earthy”, “swampy”, “fishy”, “chemical”, “rancid”, “grassy”, among others. Sensory evaluation followed by chemical analysis is commonly applied by water utilities for diagnosis of T&O incidents. Flavor profile analysis (FPA), carried out by a trained sensory panel using the water “Taste and Odour Wheel” (APHA, 2012) for assessment, can give useful information and guidance about possible causes (APHA, 2012). Gas Chromatography – Mass Spectrometry (GC-MS) is a *sine qua non* technique for detection, identification and quantitation of T&O compounds in water. Extraction of T&O prior to GC-MS is usually carried out with static headspace (HS) sampling, dynamic-HS analysis (purge & trap), Stir-bar Sorptive Extraction (SBSE), Closed-Loop Stripping Analysis (CLSA) or HS-Solid Phase Microextraction (HS-SPME) (Kaloudis *et al.*, 2017). In recent years, HS-SPME is widely used for analysis of volatile compounds in water, due to method standardization (ISO), ease of use and availability of fully-automated samplers that can largely increase lab productivity (Lambropoulou *et al.*, 2007). However, HS-SPME performance is determined by several factors affecting the water-headspace distribution, adsorption and desorption processes, while optimum conditions are generally compound-specific (Pawliszyn, 1997). This is very important in forensic-type non-targeted analysis of T&O where the objective is to efficiently detect and identify the odour-causing compound(s) among thousands of candidates with essentially different properties. Thus, the objective of the present study was to determine optimal conditions for non-targeted screening of a widest range of T&O compounds in water with HS-SPME-GC/MS in one run.

2. Materials and methods

A set of known T&O compounds of different chemical classes/properties was selected, in addition to fluorobenzene which is used as a common internal standard in HS-SPME. Standard compounds were purchased from Sigma Aldrich. Standard mixtures were prepared in methanol (>99,9%, Sigma Aldrich). NaCl (99%, Fluka) was baked at 400 °C for 30 min before use. An DVB/CAR/PDMS SPME Fiber Assembly, 50/30um, Stableflex (Supelco) that was conditioned according to manufacturer's instructions was used in the study. A GC 456 (Bruker) with a CombiPAL autosampler operating in HS-SPME mode and a TQ triple quadrupole MS (Bruker) operating in Full Scan mode (35-350 amu) was used for T&O analysis. The chromatographic column was a Rxi®-624Sil MS, 60 m, 0.32mm ID, 1.8 µm df (Restek) and a fast temperature ramp (35 to 250°C at 10°C/min) was applied. Chromatographic data processing was carried out with MSWS software (Bruker). Table 1 lists the compounds used in this study and their respective GC-MS data. Design of experiments using factorial, response surface and Taguchi designs as well as analysis and optimization was carried out with Minitab.

Table 1. T&O compounds

No	Compound	RT (min)	Quant Ion
1	Dimethyl disulfide	13,11	94
2	2-Heptanone	14,87	58
3	Dimethyl trisulfide	15,97	126
4	(R)-(+)-Limonene	16,14	93
5	Eucalyptol	16,33	108
6	γ-Terpinene	16,37	136
7	Indane	16,49	117
8	2-Isopropyl-3-methoxypyrazine	16,66	137
9	Indene	16,70	116
10	2-Isobutyl-3-methoxypyrazine	17,56	124
11	trans, 2-Nonenal	17,74	70
12	Camphor	18,02	95
13	2-MIB	18,33	107
14	β-Cyclocitral	18,64	192
15	trans,trans-2,4-Decadienal	19,63	81
16	2,4,6 Trichloroanisole	19,84	195
17	α-Ionone	21,33	121
18	(±) Geosmin	21,47	112
19	β-Ionone	22,29	177

3. Results and discussion

Several factors affecting HS-SPME performance were studied: equilibration time, equilibration temperature, extraction time, extraction temperature, NaCl concentration, methanol concentration, agitation, desorption temperature, desorption time. Extraction performance was evaluated by the peak areas of the extracted-quantitation ions of the compounds as responses. Multivariate experimental designs were used for assessing the effects and for optimization of responses. Preliminary Plackett-Burman factorial designs (Plackett & Burman, 1946) were used to assess significant factors that affect extraction efficiency. Subsequently, Box-Behnken (Box & Behnken, 1960) and Central Composite Designs (Box & Wilson, 1951) response surface designs were used to

assess main effects, interactions and to optimize responses. Taguchi (Roy, 2001) orthogonal arrays (Roy, 2001) were further used for robust parameter design. Optimization was based on desirability functions that reflect the objectives for maximum sensitivity screening of the whole range or of certain classes/groups of compounds. An example of optimization using a Box-Behnken design with 3 factors having a significant effect in extraction (extraction temperature, extraction time and NaCl concentration) is presented. Peak areas of the compounds under study were the responses, with the objective of maximization. Table 2 shows the BB3 experimental design.

Table 2. Randomized run-order BB3 design

Std Order	Run Order	PtType	Extr. Temp. (°C)	Extr. Time (min)	NaCl g/10ml
8	1	2	75	10	3
10	2	2	55	15	0
11	3	2	55	5	3
15	4	0	55	10	1,5
1	5	2	35	5	1,5
4	6	2	75	15	1,5
2	7	2	75	5	1,5
3	8	2	35	15	1,5
14	9	0	55	10	1,5
5	10	2	35	10	0
12	11	2	55	15	3
9	12	2	55	5	0
13	13	0	55	10	1,5
7	14	2	35	10	3
6	15	2	75	10	0

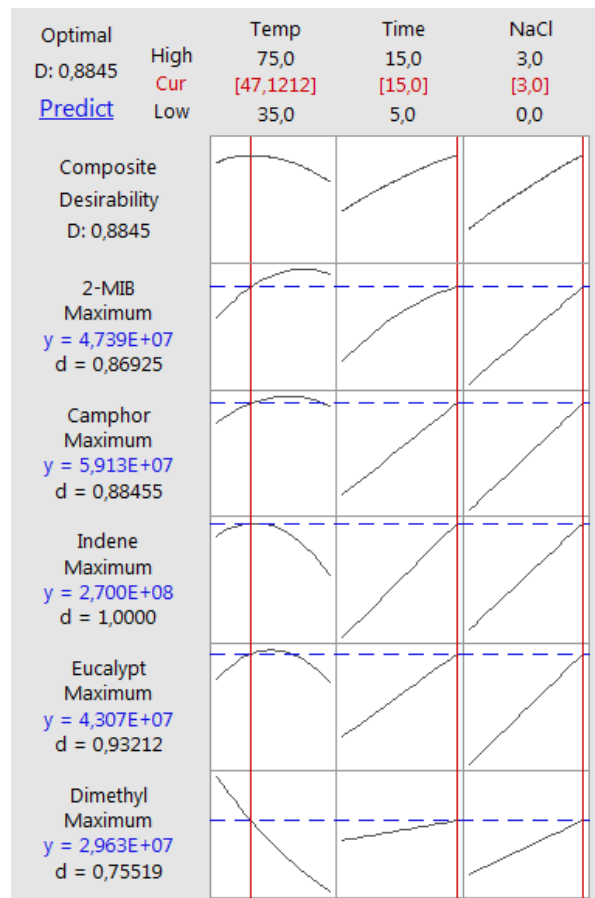


Figure 1: Illustrated example of multivariate optimization using a response surface design (BB3) in Minitab.

Main effects and interactions were assessed by their significance ($p < 0.05$). The resulting models were reduced to contain only significant effects and further reduced by backward elimination ($\alpha = 0.1$). Effects (1st-order, 2nd-order and interactions) are shown in Table 3. The table shows that for early-eluting compounds quadratic effects of extraction temperature are generally observed that produce curvatures in responses, while for late-eluting compounds interaction effects between factors are also significant. The models produced can be used for multivariate optimization according to the objectives of the laboratory. For example, if a general screening of T&O compounds is needed without any focus on specific compounds or classes of compounds, a set of optimized conditions [Extr. Temp.: 56 °C, Extr. Time : 15 min, NaCl: 3g] was predicted. Predicted conditions were further experimentally confirmed. In some cases, the laboratory may need to focus on specific compounds or classes of compounds. For example, it is known that certain cyanobacteria species (e.g. *Microcystis*) produce β -cyclocitral and ionones in addition to sulphurous compounds (Jüttner, 1984). If

Microcystis is suspected to occur in a sample, the laboratory may wish to focus on those compounds that serve as markers for the presence of this cyanobacteria species. An optimized extraction temperature of 39°C is predicted by the model with a composite desirability of 0,9253, when the above compounds are targeted and the weights and importance of the dimethyl sulfides are increased (set at 10) in the desirability function. *Ad hoc* optimized conditions can be predicted by defining the weighing and importance parameters in the desirability function. A graphic illustration of the response optimization process is shown in Figure 1 (produced with Minitab). Example results presented in this short paper show that HS-SPME-GC/MS is a powerful technique for targeted and non-targeted screening of a wide range of T&O compounds. However, the diverse properties and behavior of T&O as well as their low odor thresholds require strategies for optimization towards maximum sensitivity of detection. Factorial, response surface and Taguchi experimental designs can be used efficiently to provide reliable predictions of optimized conditions.

Table 3. Significant effects in the model produced by the BB3 design.

	Temp	Time	NaCl	Temp x Time	Temp x NaCl	Time x NaCl
Fluorobenzene (IS)	2nd order	1st order	2nd order	No effect	No effect	No effect
Dimethyl disulfide	2nd order	1st order	1st order	No effect	No effect	No effect
2-Heptanone	2nd order	1st order	1st order	No effect	No effect	No effect
Dimethyl trisulfide	2nd order	1st order	1st order	No effect	No effect	No effect
(R)-(+)-Limonene	2nd order	1st order	1st order	No effect	No effect	No effect
Eucalyptol	2nd order	1st order	1st order	No effect	No effect	No effect
γ -Terpinene	2nd order	1st order	1st order	No effect	No effect	No effect
Indane	2nd order	1st order	1st order	No effect	No effect	No effect
2-Isopropyl-3-methoxypyrazine	2nd order	1st order	1st order	No effect	No effect	No effect
Indene	2nd order	1st order	1st order	No effect	No effect	No effect
2-Isobutyl-3-methoxypyrazine	2nd order	1st order	1st order	No effect	No effect	No effect
<i>trans</i> , 2-Nonenal	2nd order	1st order	1st order	No effect	No effect	No effect
Camphor	2nd order	1st order	1st order	No effect	No effect	No effect
2-MIB	2nd order	1st order	1st order	No effect	No effect	No effect
β -Cyclocitral	2nd order	1st order	1st order	No effect	No effect	No effect
2,4-Decadienal	2nd order	1st order	1st order	No effect	No effect	No effect
2,4,6 Trichloroanisole	2nd order	1st order	1st order	No effect	No effect	No effect
α -Ionone	2nd order	1st order	1st order	No effect	No effect	No effect
(\pm) Geosmin	2nd order	1st order	1st order	No effect	No effect	No effect
β -Ionone	2nd order	1st order	1st order	No effect	No effect	No effect

Effects: 2nd order 1st order No effect

Acknowledgements

The authors would like to acknowledge CYANOCOST-COST Action ES1105, www.cyanocost.net for networking and sharing of knowledge.

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