IDENTIFICATION OF VOCs RESPONSIBLES FOR ODOURS IN WASTEWATER BY HS-SPME WITH GC-EI-Q-Orbitrap

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ABSTRACT

This study evaluates the capabilities of identification of unknown compounds and the optimisation of the extraction technique for odour-causing volatile organic compounds (VOCs) in wastewater samples based on headspace solid-phase microextraction (HS-SPME). The water samples were analysed by gas chromatography coupled to mass spectrometry using a Q-Orbitrap mass analyser. A total of 3 wastewater samples from 3 WWTPs (Castellón: influent; Benicasim: outlet of the primary settling tank; Villarreal: outlet of the biological reactor) were mixed and used as a model for the development and optimisation of the method. Additionally, a total number of 16 samples were collected every 15 days from the three WWTP. In order to optimise the HS-SPME method, a multivariate approach using the response surface method was used obtaining optimum values of 3 mL of sample, 45ºC as extraction temperature and 0.6 g of NaCl (20% in the sample). To ensure reliable identification, the parameters of comprehensive score greater than 80, RSI>700 and ΔRI≤50 were established as reference values. As a result, 67 compounds could be identified, from which 12 were responsible for odours.

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ABBREVIATIONS

WWTP Wastewater Treatment Plant

VOC Volatile Organic Compound

VSC Volatile Sulphur Compound

OAV Odour Activity Value

OTV Odour Threshold Value

OPM Odour Profile Method

GC Gas Chromatography

MS Mass Spectrometry

HRMS High-Resolution Mass Spectrometry

SPME Solid Phase Microextraction

P&T Purge and Trap Extraction

DI Direct Immersion

HS Headspace

APCI Atmospheric Pressure Chemical Ionisation

EI Electron Ionisation

PA Polyacrylate

PDMS Polydimethylsiloxane

DVB Divinylbenzene

CAR Carboxen

FWHM Full Width at Half Maximum

RSI Reversed Search Index

ΔRI Deviation of the Retention Index

1 INTRODUCTION

1.1 Environmental issues of odour-causing VOCs

Water is a fundamental element for sustaining life, and although 70% of the Earth' surface is covered with water, only 2.5% is freshwater compatible with terrestrial life. Nowadays, the demand for freshwater is worsened by more frequent droughts related to climate change, population growth and chemical/microbial pollution due to the intensive use of chemicals in everyday activities and unrestricted access to medicines. According to the European Environment Agency report, only about 40% of surface waters (rivers, lakes, transitional and coastal waters) are in good ecological status or potential, and 38% in good chemical status [1,2].

Over the last decades, pollution has increasingly become a crucial factor affecting the quality of life and health status of urban populations. Indeed, very harmful pollutants from industries and households can be drained into wastewater, leading to a deterioration of water quality and causing serious damage to human health, aquatic ecosystems, animals and the environment [3].

Wastewater treatment is well known and widespread in European countries. Different technologies or strategies can be used to treat urban wastewater, all with the inherent drawback of generating solid and gaseous residues during the applied treatments. Besides air and water pollution are reported to be the main cause of several diseases, such as cardiovascular dysfunction, inflammation, respiratory infections and cancer, resulting in millions of deaths worldwide each year. Gaseous streams are mainly responsible for air pollution, mainly in the form of odour, which can have a major impact on the population in the vicinity of wastewater treatment plants (WWTPs). These gaseous emissions are characterised by the presence of many volatile organic compounds (VOCs) at trace level, together with volatile sulphur compounds (VSCs), sulphur and ammonia, which are the main contributors to odour nuisance [4]. In fact, odours have recently been considered as air pollutants because they combine with nitrogen oxides in the atmosphere and with sunlight form ozone and other photochemical oxidants that are known to be harmful to vegetation and animal life [5].

Based on odour concentrations and odorant data in [4,6,7], the main odour generation stages along the wastewater process have been identified as wastewater collection, transfer and treatment. During these stages, as shown in **Figure 1**, many different compounds can be formed and emitted due to various reasons, such as: (i) the development of anoxic conditions in sewers leading to the formation and emission of hydrogen sulphide and sulphur-based organic compounds, (ii) turbulence generated in the WWTP receiving tank leading to the separation and volatilisation of compounds formed during wastewater transport, (iii) sludge treatment where anaerobic conditions can form new odours or (iv) settling tanks where still flows and large surface areas can promote the emission of compounds previously formed during wastewater treatment [4,6].

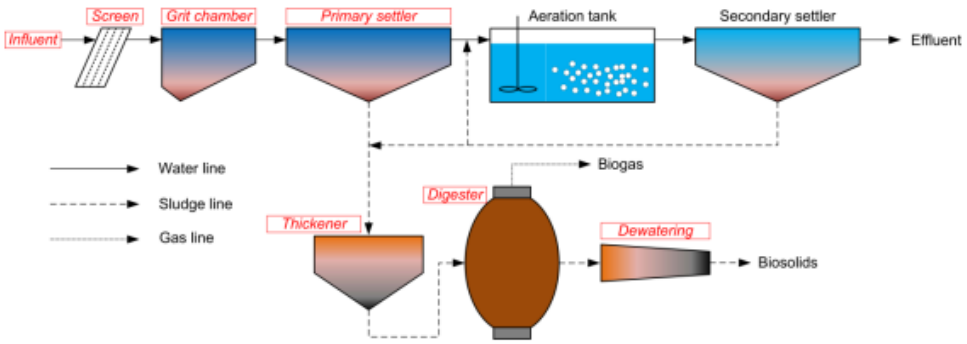

Figure 1. Flow diagram of a typical wastewater treatment plant, with the primary odour emission units labelled with red, italic text (directly reproduced from ref. [7])

In WWTPs, VOCs emissions can occur by diffusion or convection methods from the surface of the effluent ponds. Diffusion occurs when the surface concentrations are higher than in ambient air and the compounds try to reach equilibrium between the aqueous and gas phases. Convection is caused by air flow sweeping compounds from the surface into the air. Another mechanism is the off-gassing that occurs in ponds that are aerated. In gas stripping, the gas (usually air) is entrained in the wastewater and thus VOCs are transferred from the wastewater to the gas by mass transfer laws [8].

Furthermore, in many cases, these odorous emissions contain hundreds of compounds of which only a few are substantially responsible for the odour. The concentrations of these key compounds are often very low and vary by no more than a few ppm or ppb. However, odour thresholds, i.e. concentrations at which an average test person can no longer detect the odour, are in some cases several orders of magnitude lower [9].

These thresholds are usually measured in ppm (g m^{-3}). **Table 1** therefore shows some of the main odorants present in two WWTPs [10,11].

Table 1. Odour descriptor and threshold concentration of the main odorants present in emissions from WWTPs

Odorant	Odour descriptor	Odour threshold (g m^{-3})
S-Compounds		
H_2S	Rotten eggs	0.0005
Carbon disulfide	Disagreeable, sweet	0.007
N-Compounds		
Ammonia	Pungent	0.038
Indole	Faecal, nauseating	0.0001
Skatole	Faecal, nauseating	0.001
Volatile fatty acids		
Acetic acid	Vinegar	1.1
Propionic acid	Rancid, pungent	0.028
Ketones		
Butanone	Sweet, minty	0.25
Acetone	Fruity, pungent	20
Aldehydes		
Propionaldehyde	Sweet, ester	0.011
Valeraldehyde	Pungent	0.028
Hydrocarbons		
Toluene	Rubbery, mothballs	2.1
Benzene	Sweet, solventy	1.4
Phenol	Medicinal, sweet	46

To assess the contribution of each VOC in the odour produced, the odour activity value (OAV) of each compound has been calculated, which is the ratio between the concentration of the compound in air and its odour threshold value (OTV) based on literature data [12]. In this way, the most odorous compounds can be identified, as they produce the highest OAVs. The total odour was calculated as the total odour activity of the individual compounds (ΣOAV) [13,14].

Burlingame [15] initially developed the Odour Profile Method (OPM) to prioritise odour sources to control odour in a wastewater treatment plant based on odour character, intensity and duration. Character was defined using a wastewater odour wheel. Intensity was defined using a scale anchored to word descriptors. Duration was defined as the fraction of time that odours caused by a specific process were detected at the sewage treatment plant fence.

The odour wheels (**Figure 2**) consist of three rings: an inner ring segmented into general odour categories (e.g., rancid); a middle ring listing specific odour descriptors within each odour category (e.g., vinegar and rancid); and an outer virtual ring identifying chemical compounds associated with the categories and descriptors in the inner and middle rings (e.g., acetic acid and butyric acid) [16].

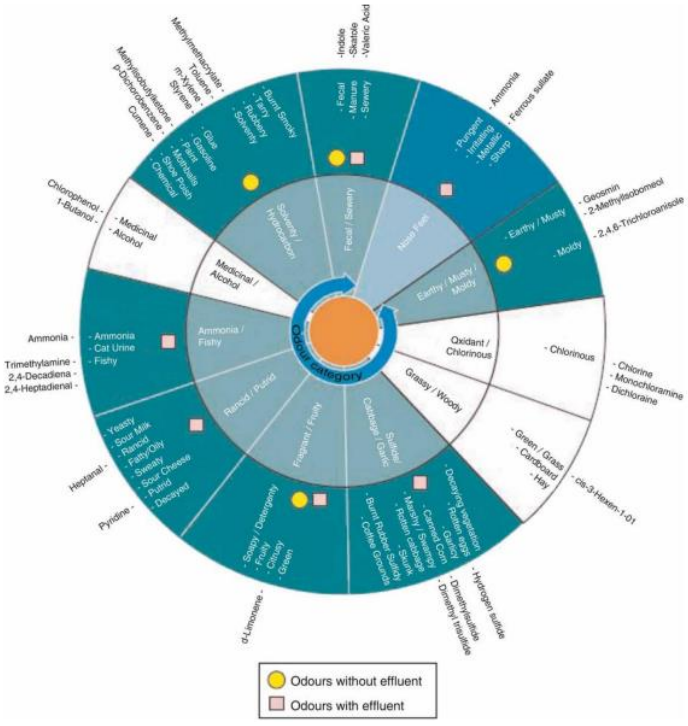

Figure 2. A wastewater odour wheel developed for sewers using data from both olfactory and chemical analysis (directly reproduced from ref. [17])

However, odour perception and emissions can be assessed using two approaches: chemical analysis and sensory analysis. Chemical analysis is widely used to determine the molecules present in the air and their chemical concentrations. This approach to environmental assessment has already been discussed and is considered a powerful and relevant methodology [18,19]. Nevertheless, chemical analysis faces several obstacles when applied to monitor odours in ambient air. First, chemical analysis does not provide data on the sensory properties of the analysed molecules. Second, the concentration levels at which some odours are smelled may be below instrumental detection limits. Thirdly, the effects of mixing on odour intensity and the nature of the odour are not considered in the chemical analysis. Finally, odours in the environment are emitted in puffs, which can be a challenge to analyse consistently [20].

Therefore, sensory analysis is used as a complementary tool. This approach relies on human assessors, called a panel, who smell and characterise the odour. Sensory analysis offers many advantages, such as providing organoleptic data and allowing odour quantification and qualification. It has been applied in many environmental domains, offering a less expensive technique relative to chemical analysis and is easier to implement in large areas [21]. However, sensory analysis is disputed in relation to the subjectivity of the human panel and psychological factors that could affect the analysis. Thus, objectivity in sensory analysis is a requirement for monitoring odours over long periods, comparing data from different panels and, ideally, establishing a relationship between sensory and chemical data, especially when considering a quantitative approach. This, together with the lack of a complete understanding of the effect of odour mixtures, constitutes the main obstacle when assessing odours in the environment [19].

For these reasons, it is absolutely necessary to develop methods to identify and quantify which volatile organic compounds are present in wastewater and which of them, in order to prevent their presence and spread through populated areas. In this way, a healthy, clean and high-quality environment is guaranteed, as well as the protection of the health of its inhabitants from the adverse effects of any type of pollution [22].

1.2 Analytical methodology

The most commonly used methodology for the analysis of volatile organic compounds responsible for odours in water samples is gas chromatography (GC) coupled to mass spectrometry, known as GC-MS, due to the low average polarity of the analytes and their high volatility. In the present study, a Q-Orbitrap mass spectrometer has been used as an instrument, which belongs to the high-resolution mass spectrometers (HRMS) with the aim of performing complete scans of the samples. Finally, the GC-HRMS combination allows a double separation: one based on the physical properties of the molecule, and the next one based on the charge/mass ratio, separating compounds that have not been separated by GC, or separating isomers that have the same molecular weight [7].

Sample preparation was also studied taking into account that the analytes are at trace level, so pre-concentration of the analytes is a key requirement, thus solid phase microextraction (SPME) was mainly considered.

1.2.1 Solid phase microextraction (SPME)

Most of the samples to be analysed are not in a suitable form to be injected into the gas chromatograph to determine VOCs at very low concentrations. Thus, a sample treatment needs to be developed and optimised. The sample treatment aims to extract, pre-concentrate and remove interferences (clean-up), in order to introduce the sample extract directly into the equipment to be analysed. This stage is very important in the analytical process because the success of the analysis depends on all its phases, and not only the final instrumental determination. In particular, some authors have reported that the techniques of choice in most cases are purge and trap extraction (P&T) and solid phase microextraction (SPME) [23,24].

In addition, SPME has recently become the preferred method for the determination of volatile and semi-volatile substances. It offers advantages such as absence of solvents, simplicity, economy and efficiency [24]. Thus, extraction of volatile analytes from the sample can be performed by direct immersion (DI) or by exposure of the fibre to the headspace (HS) of the sample. Although DI-SPME is the most commonly used technique for semi-volatile compounds in clean liquid samples, HS-SPME seems to be more appropriate for volatile compounds, especially when dealing with dirty or complex matrices [25]. To optimise the

HS-SPME procedure, the best fibre must be selected according to the nature of the analytes and then the experimental conditions such as the amount of salt to be added, the extraction temperature, the sample volume or the extraction time have to be chosen [26].

With HS-SPME (**Figure 3**), analytes are absorbed from the liquid or gaseous sample into an absorbent coated fused silica fibre, which is part of the syringe needle, for a fixed time and without being in contact with the sample. The number of molecules extracted by the fibre is proportional to their concentration in the sample, provided that thermodynamic equilibrium is reached. In case of short extraction times, manual stirring of the sample accelerates the extraction process. The fibre is then inserted directly into a GC injection port for thermal desorption [27].

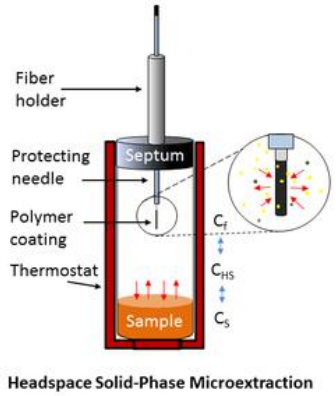

Figure 3. Headspace solid-phase microextraction (SPME) (directly reproduced from ref. [27])

1.2.2 Gas chromatography (GC)

Chromatography can be defined as a separation technique in which the mixture to be resolved is introduced into a system consisting of a fluid (mobile phase) that moves in close contact with a solid or liquid phase, which is immobile during the process (stationary phase). Depending on the characteristics of the mobile phase, chromatography can be divided into three types: liquid, gas and supercritical fluids. This section focuses on GC, where the mobile phase (gas) transports the analytes (sample vaporised in the injector) but does not interact with them, although the stationary phase (liquid) does. The most commonly used mobile phase is helium, although argon, hydrogen and nitrogen can also be used [28].

The analytes are separated according to thermodynamic (distribution-dependent, K_d) and kinetic (dispersion-dependent) parameters. Depending on the polarity and boiling point of the compounds in the sample in the stationary and mobile phases, these compounds will be retained and eluted at different times. A detector is needed to transform these chromatographic bands into more understandable information. Although there are many detectors, the most reliable for this type of analysis is high resolution mass spectrometry (HRMS). **Figure 4** shows the equipment used for the analysis of VOCs responsible for odours in the present work.

Figure 4. Gas chromatograph coupled to HRMS with autosampler used in the project

1.2.3 High resolution mass spectrometry (HRMS). Q-Orbitrap

Once separation by gas chromatography has been achieved, a detector is needed, as indicated before. Mass spectrometry (MS) is the detector of choice because of its great features: unrivalled sensitivity, selectivity, robustness, low detection limits, speed and diversity of applications. MS is a technique based on the generation of ions, which are then separated and quantitatively detected. Ions with different mass-to-charge ratios (*m/z*) have different trajectories when an electric and/or magnetic field is applied. The mass spectrometer consists of three main parts: an ionisation source, a mass analyser and a detector, all of which are kept under vacuum to allow transmission of the ions [29].

The determination of environmental contaminants necessitates the continuous development of detection strategies. This places stringent demands on the level of reliability to identify contaminants, which in turn depends on the large and continuously increasing number of chemicals, some of which are unequivocally known to be hazardous. Although unit mass resolution analysers such as quadrupole (GC-MS) can be used for contaminant

detection using nominal spectral libraries, high resolution mass spectrometry (HRMS) is a superior technique for detection purposes, especially when dealing with unknown compounds. In a wide range of analytical contexts, HRMS has been identified as the method of choice due to its ability to measure accurate mass and, more importantly, structural information. This makes it suitable for applications focused on the identification of unknown structures or the detection of non-target contaminants. In recent years, atmospheric pressure chemical ionisation (APCI) has been implemented in GC-HRMS instruments and offers attractive features for detection. However, electron ionisation (EI) is the most widely applied technique due to its robustness, reproducibility and the existence of standardised commercial libraries [30]. Multiclass detection methods using GC-EI-TOF-MS have been successfully applied for the investigation of organic contaminants in environmental samples using different search strategies based on libraries of nominal spectra or libraries of accurate mass spectra. On the other hand, GC-EI-Q-Orbitrap-MS is a new technology that became commercially available for the first time in 2015. It has the advantage of high resolving power (up to 120 K at m/z 200) and mass accuracy below 1 ppm, but its potential for the detection of non-target contaminants in the environment has not been sufficiently explored yet [31–33].

The way this analyser works is that ions injected into the Orbitrap are trapped in an electrostatic field and each ion oscillates axially with a frequency that is proportional to its mass, reproduced from **Figure 5** [34]. A current image of these oscillations is measured using a split outer electrode and this image is converted into a mass spectrum using the Fourier transform, which is a commonly used analysis method to transform the current signal into a mass spectrum. In addition, the sensitivity in full scan mode using GC-EI-Q-Orbitrap-MS can reach or even exceed that of selected reaction monitoring with a QqQ-MS [35].

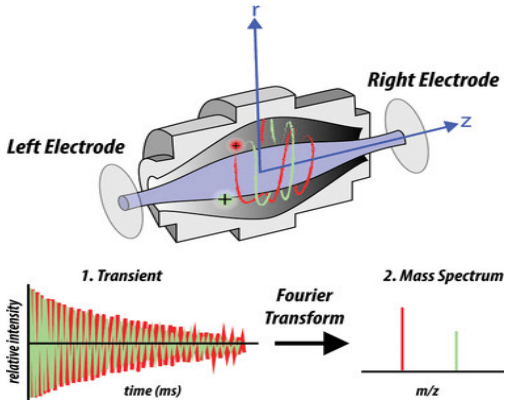

Figure 5. Orbitrap mass analyser (directly reproduced from ref. [34])

2 OBJECTIVES

The aim of this project is to develop and optimise an analytical method for the identification of volatile organic compounds (VOCs) responsible for odours in water samples derived from wastewater treatment plants. The method is based on gas chromatography (GC) coupled to mass spectrometry (MS) using a Q-Orbitrap mass analyser and headspace solid phase microextraction (HS-SPME) as sample treatment.

The main objective can be divided into the following parts:

- Development and optimization of HS-SPME procedure using a multivariate approach
- Development of a MS screening method for the identification of unknown VOCs
- Quantitate detected analytes and relate with odour sources

3 EXPERIMENTAL

3.1 Reagents and chemicals

Hexane (GC-MS grade) and sodium chloride were purchased from Scharlau (Scharlab, Barcelona, Spain). In addition, the mixed solution of n-alkanes C_7-C_{30} was obtained from Sigma-Aldrich (St. Louis, MO, USA).

3.2 Sample collection

The experimental sites were three large-scale urban wastewater treatment plants located in the province of Castellón. A map with the distribution of the three WWTPs is presented in **Figure 6**. The one for the city of Castellón currently manages an average daily flow of 39,143 m^3 to serve a population equivalent of 150,782 inhabitants. The next most important is the Villarreal WWTP, which treats a daily flow of 22,486 m^3, and lastly, the Benicasim WWTP, with a daily flow of 18,000 m^3 [36,37].

Figure 6. Location of the three WWTPs in the province of Castellón

Samples were collected every two weeks between May and June 2022. Samples were collected from 5 treatment points in each WWTP, except in Castellón where 6 points were considered. All treatment points are listed in **Table 2**. All samples were collected once

per point in 500 mL amber glass bottles with minimal headspace. Samples were delivered to the laboratory on the same day of collection and stored at 4°C until analysis.

Table 2. Treatment units monitored during the study

Treatment unit	WWTP Castellón	WWTP Benicasim	WWTP Villarreal
Plant inlet	X	X	X
Primary decanter outlet	X	X	X
Homogenisation tank	X		X
Physico-chemical sludge		X	
Biological reactor outlet	X	X	X
Desanding-degreasing outlet	X		
Plant outlet	X	X	X

3.3 Solid phase microextraction (SPME)

An automated SPME holder was used with three different types of fibre purchased from Thermo Fisher Scientific (Bremen, Germany). Before their first use, the new fibres were conditioned in the injection port of the GC according to the manufacturer's instructions. **Table 3** shows some properties of the SPME fibres.

Table 3. Properties of the tested SPME fibers

Fiber type	Coating	Mechanism	Film thickness, length	Desorption temperature (ºC)
PA	Polyacrylate	Absorption	85 µm, 1 cm	270
PDMS	Polydimethylsiloxane	Absorption	100 µm, 1 cm	250
DVB/CAR/ PDMS	Divinylbenzene/carboxen /polydimethylsiloxane	Adsorption	80 µm, 1 cm	280

Then, using a synthetic sample prepared as a pool of 3 wastewater samples, the different fibres, extraction times and temperatures, sample volumes and salt addition were evaluated to achieve maximum extraction efficiency and high reproducibility of the results using a multivariate approach by applying response surfaces. The HS-SPME procedure was the selected extraction mode. To ensure a faster extraction, the vial was kept in agitation during the extraction period. SPME was automatically performed directly on the TriPlus RSH autosampler (Thermo Fisher Scientific (Bremen, Germany).

Samples were prepared as follows: 3 mL of pool wastewater sample was placed in a 20 mL glass vial. Then 0.6 g NaCl (20% in the sample) was added. The vials were immediately closed with a magnetic cap equipped with a PTFE-silicone septum. The DVB/CAR/PDMS fibre was exposed to the headspace in the glass vial for extraction of the target compounds at 45°C for 15 min. Once this was done, the samples were automatically desorbed into a split/splitless injector, where desorption of the analytes occurs at 280°C for 5 min. After desorption, the fibre remained in the injector port for another 5 min.

3.4 GC-full scan HRMS analysis

The analyses were carried out on a Trace 1310 GC, equipped with a TriPlus RSH autosampler and coupled to a Q-Exactive Orbitrap mass analyser. All devices were from Thermo Fisher Scientific (Bremen, Germany).

A DB-WAX column (Agilent J&W, Santa Clara, CA) of 30 mx 0.25 mm i.d. x 0.25 µm film thickness was used. Helium (99.9999 % purity) was used as carrier gas at a constant flow rate of 1 mL min^{-1}. The injector temperature was set at 280 °C. The split flow rate was 50 mL min^{-1} and the split-free time was 3.0 min. The column oven was maintained at 40°C for 5 min and the temperature was increased to 260°C at a rate of 10°C min^{-1} and finally, this temperature was maintained for 3 min. The total run time was 30 min.

MS was performed in positive electron ionisation (EI) at 70 eV, operating in full scan mode with a resolution of 60,000 full width at half maximum (FWHM). The scanned range was 40 to 600 m/z with an automatic gain control target value of 3 x 10^6. Ion source and transfer line temperatures were set at 270 and 290°C. In addition, the C_7-C_{30} alkane series was used for the external non-isothermal retention index (IR).

Xcalibur 4.1 and TraceFinder 4.0 software (Thermo Scientific) were used for data processing. This tool allowed peak detection with spectral deconvolution and tentative identification of compounds against the NIST library. To study and process the EI-Q-Orbitrap spectra, the NIST mass spectral library and search software (NIST 2014/EPA/NIH), which contains more than 276,000 spectra, was used.

3.5 Data analysis and workflow of non-targeted analysis method

The workflow of the non-targeted analysis method is as follows (**Figure 7**). First, the volatiles released from the samples under specific headspace conditions were analysed by GC-MS (Q-Orbitrap). After deconvolution of the peaks, using the manufacturer sotware TraceFinder, and deduction of the blanks, the main peaks (all peaks with an area above 1,000,000) with high intensity in each sample were analysed. Secondly, a reliable qualitative analysis of unknown substances was carried out according to four identification steps. The single step is based on the comprehensive score (reversed search index [RSI], etc.) and the retention index. The RSI value represents the positive match rate between the measured spectrum and the standard spectrum. Therefore, higher RSI values indicate that the results have a higher reliability. Prior to sample analysis, the mixed solution of C_7-C_{30} n-alkanes was analysed by the same separation method to determine the retention time of each n-alkane, which was used to calculate the retention rate of unknown substances. Finally, the deviation of the retention rate (ΔRI) was calculated by comparing it with the retention rate of the compounds included in the NIST library. A smaller ΔRI means that the result is more reliable. Thirdly, all volatile components of the samples were classified and analysed. Finally, a list of volatile odour-causing substances in the wastewater was compiled based on the detection rate and response intensity of the identified substances. In order to carry out the statistical visualization data were imported into R running under RStudio environment.

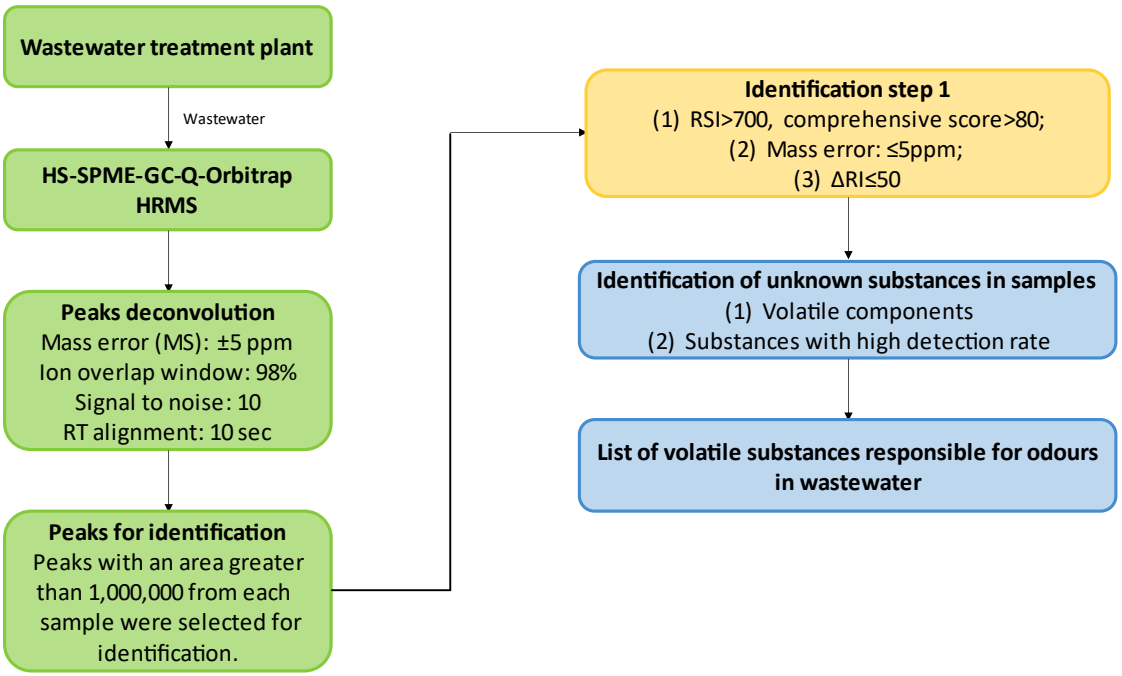

Figure 7. Workflow for non-targeted analysis of unknown substances in WWTPs

4 RESULTS AND DISCUSSION

4.1 Solid phase microextraction optimisation

With a pool obtained by mixing 3 samples from the above mentioned wastewater treatment plants (Castellón: influent; Benicasim: outlet of the primary settling tank; Villarreal: outlet of the biological reactor) the following parameters, normally considered in a SPME optimisation, were studied: fibre type, sample volume, extraction temperature and salt addition.

4.1.1 Selecting the optimum SPME fibre coating

First, three types of fibres were tested to select the one that was able to adsorb the highest number of compounds: polyacrylate (PA) 85 µm, polydimethylsiloxane (PDMS) 100 µm and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 80 µm. A generic SPME procedure was applied using 3 mL of the sample pool, extracted at 60°C for 15 min in a 20 mL vial with shaking. The results obtained for the fibres are shown in **Figure 8**.

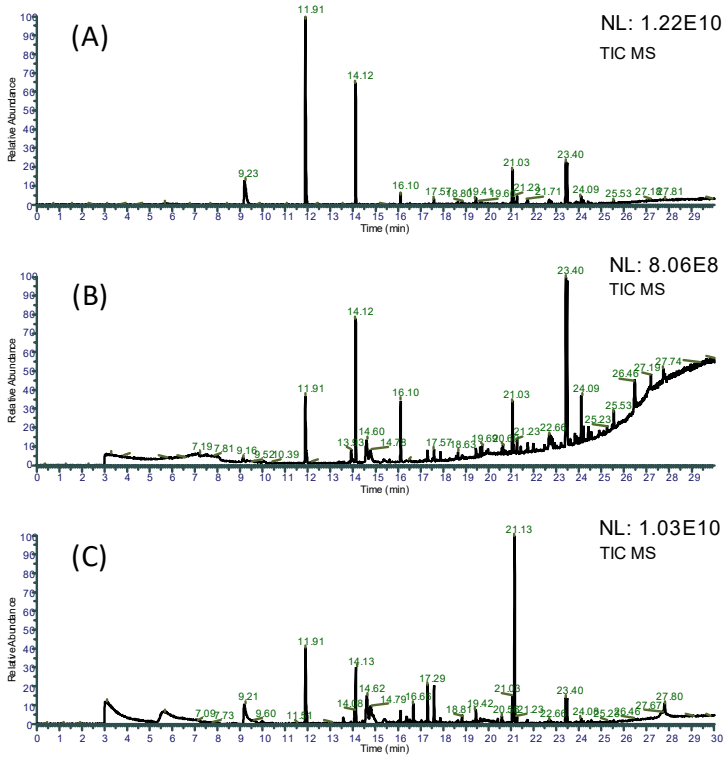

Figure 8. TIC MS chromatograms of the different fibres tested: (A) PA 85 µm, (B) PDMS 100 µm and (C) DVB/CAR/PDMS 80 µm

In view of the chromatograms, higher peak intensities were obtained with the PA and DVB/CAR/PDMS fibres compared to the PDMS fibre for all peaks. But more peaks are shown for the last two fibres (PDMS and DVB/CAR/PDMS). Thus, a fibre blank is made for each of them and it is decided to process only the peaks with areas higher than 1,000,000 for both blank and sample chromatogram and shown in **Table 4**.

Table 4. Peaks detected in the sample and the blank with an area greater than 1,000,000

	Sample	Blank	Difference
PA	152	73	79
PDMS	177	13	164
DVB/CAR/PDMS	285	42	243

Therefore, the three-phase DVB/CAR/PDMS fibre was considered the most suitable for this study and was selected for further experiments because it shows the most peaks with an area greater than 1,000,000, making the difference between the blank and the sample.

4.1.2 Non-target analysis of unknown substances

In a second step, a non-target analysis was performed after a deconvolution process to generate peak-clean mass spectra. All search results were comprehensively classified according to the RSI values in the spectral library. The retention index is also an important parameter for qualitative analysis. ΔRI lower than 50 can greatly assures the qualitative identification. However, some compounds do not have retention index data in the NIST library; therefore, their ΔRI cannot be obtained. Theoretically, the reliability of the identified results is high when the comprehensive score is>80, RSI>700 and ΔRI≤50.

Table 5 lists the compounds tentatively identified in the sample pool, together with their retention times (R_T), exact masses and mass errors for the molecular ions. The overall score, RSI and ΔRI values for all compounds are also shown.

Table 5. Non-target compounds tentatively identified in wastewaters

Score	Compound	Molecular formula	*m/z* (mass error, ppm)	R_T	RSI	ΔRI
92.6	Ethylbenzene	C_8H_{10}	106.077728 (0.245)	8.451	910	4
93.0	1,3-dimethylbenzene (m-xylene)	C_8H_{10}	106.077728 (0.245)	8.734	915	4
94.7	Phenylurea	$C_7H_8N_2O$	*	9.476	936	**
92.2	o-xylene	C_8H_{10}	106.077728 (0.245)	9.629	906	3
84.6	Eucalyptol	$C_{10}H_{18}O$	154.135132 (-0.551)	9.995	814	2
87.7	1-ethyl-4-methyl-benzene (toluene)	C_9H_{12}	120.093338 (-0.117)	10.391	851	6
88.7	o-cymene	$C_{10}H_{14}$	134.109085 (0.619)	11.191	863	4
82.1	1,2,4-trimethylbenzene	C_9H_{12}	120.093338 (-0.117)	11.382	785	7
87.2	2-methylpentanal (valeraldehyde)	$C_6H_{12}O$	*	11.433	845	13
85.7	5-methyl-1-phenyl-1-hexanone	$C_{13}H_{18}O$	*	12.287	827	**
86.3	trans-rose oxide	$C_{10}H_{18}O$	154.135132 (-0.551)	12.492	835	6
86.1	2,3,6-trimethyl-pyridine	$C_8H_{11}N$	121.088539 (-0.512)	12.678	832	10
87.8	Indane	C_9H_{10}	118.077812 (0.932)	12.820	853	15
84.0	Dimethyl trisulfide	$C_2H_6S_3$	125.962677 (0.500)	12.998	807	18
83.9	2-nonanone	$C_9H_{18}O$	142.135147 (-0.492)	13.040	806	7
86.7	3,7-dimethyl-3-octanol	$C_{10}H_{22}O$	*	13.578	840	36
85.2	2,6-dimethyl-2-octanol	$C_{10}H_{22}O$	*	13.810	822	21
89.0	Acetic acid	$C_2H_4O_2$	60.020599 (0.300)	13.934	867	13
88.9	Phtocitral A	$C_{10}H_{16}O$	*	14.079	866	43
80.6	p-menthone	$C_{10}H_{18}O$	154.135132 (-0.551)	14.143	767	3
80.8	β-patchoulene	$C_{15}H_{24}$	204.187103 (-0.730)	14.426	769	10
90.8	1,2-dichlorobenzene	$C_6H_4Cl_2$	145.968521 (0.438)	14.503	889	11
87.6	(+)-2-bornanone	$C_{10}H_{16}O$	152.119492 (-0.493)	14.889	850	7
91.6	3-(tert-butylsulfanyl)-5-methylpyridine	$C_{10}H_{15}NS$	*	15.269	898	**
83.4	Hexadecane	$C_{16}H_{34}$	*	15.729	800	1

Score	Compound	Molecular formula	*m/z* (mass error, ppm)	R_T	RSI	ΔRI
80.7	α-guaiene	$C_{15}H_{24}$	204.187103 (-0.730)	15.793	756	6
86.2	Menthol	$C_{10}H_{20}O$	*	16.371	833	16
81.1	Germacrene D	$C_{15}H_{24}$	*	16.488	773	12
84.7	3,7-dimethyl-1-octanol	$C_{10}H_{22}O$	*	16.664	815	11
80.4	Isoborneol	$C_{10}H_{18}O$	*	16.791	764	28
87.3	α-terpineol	$C_{10}H_{18}O$	*	17.023	847	10
80.3	4-hidroxyindole-3-carboxilic acid	$C_9H_7NO_3$	*	17.294	763	**
89.2	N-formyl aniline	C_7H_7NO	*	17.388	868	**
85.7	2-methyl-1-hexanol	$C_7H_{16}O$	*	17.703	828	**
81.5	δ-cadinene	$C_{15}H_{24}$	204.187103 (-0.730)	17.779	729	16
86.5	5-methyl-2-(1-methylethyl)hexanol	$C_{10}H_{22}O$	*	17.806	837	**
97.5	Benzenemethanesulfonyl fluoride	$C_7H_7FO_2S$	*	17.988	969	**
91.7	(4-nitrophenyl)-2,3,4-trifluorobenzoate	$C_{13}H_6F_3NO_4$	*	18.093	899	**
87.3	4-hydroxybenzamide	$C_7H_7NO_2$	*	18.384	847	**
91.2	2-formyl-4,6-dichlorophenyl ester-2-trifluoromethylbenzoic acid	$C_{15}H_7Cl_2F_3O_3$	*	18.530	894	**
86.1	(1-pentylhexyl)benzene	$C_{17}H_{28}$	232.218460 (-0.396)	18.553	832	24
90.0	(1-butylheptyl)benzene	$C_{17}H_{28}$	232.218460 (-0.396)	18.631	879	23
82.1	trans-calamenene	$C_{15}H_{22}$	202.171539 (-0.312)	18.653	814	27
81.9	α-isomethyl ionone	$C_{14}H_{22}O$	206.166382 (-0.655)	18.813	722	7
93.2	2-formyl-4,6-dichlorophenyl ester-6-fluoro-2-trifluoromethylbenzoic acid	$C_{15}H_6Cl_2F_4O_3$	*	19.031	918	**
86.7	Dimethylcyanamide	$C_3H_6N_2$	*	19.258	840	**

Score	Compound	Molecular formula	*m/z* (mass error, ppm)	R_T	RSI	ΔRI
92.7	1-nitrosoadamantane	$C_{10}H_{15}NO$	*	19.280	912	**
88.5	(1-pentylheptyl)benzene	$C_{18}H_{30}$	246.234085 (-0.479)	19.603	861	25
83.5	β-methyl-benzenepropanol	$C_{10}H_{14}O$	150.103851 (-0.440)	20.195	801	**
90.6	Biphenyl	$C_{12}H_{10}$	154.077652 (-0.325)	20.334	886	28
88.2	Phenol	C_6H_6O	94.041283 (-0.351)	20.380	899	18
87.5	Diphenyl ether	$C_{12}H_{10}O$	170.072418 (-1.170)	20.561	849	20
84.3	(1-butylnonyl)benzene	$C_{19}H_{32}$	260.249817 (-0.138)	20.715	811	27
90.6	Amberonne	$C_{16}H_{26}O$	*	21.032	886	**
90.7	3-methylphenol (m-cresol)	C_7H_8O	108.056717 (-2.304)	21.128	888	5
88.3	Thymol	$C_{10}H_{14}O$	150.103851 (-0.440)	22.067	859	4
80.7	Patchouli alcohol	$C_{15}H_{26}O$	222.197830 (0.059)	22.226	768	40
92.7	2-ethoxy-napthalene	$C_{12}H_{12}O$	172.088226 (-0.238)	22.547	912	**
82.9	3,5-bis(1,1-dimethylethyl)-phenol	$C_{14}H_{22}O$	206.166382 (-0.655)	23.184	794	2
90.9	2-hydroxy-2-methylbutyl ester-benzoic acid	$C_{12}H_{16}O_3$	*	23.220	890	**
80.9	Tonalid	$C_{18}H_{26}O$	258.197815 (-0.008)	23.397	758	32
92.9	7-hydroxycadalene	$C_{15}H_{18}O$	*	23.605	914	**
87.7	1-hexyl-1-nitrocyclohexane	$C_{12}H_{23}NO_2$	*	23.808	852	**
92.1	Indole	C_8H_7N	117.057335 (0.290)	24.535	904	29
91.5	3-methylindolizine	C_9H_9N	131.072952 (0.008)	24.922	897	**
86.2	N-methyl-1-hidroxycarbozole	$C_{13}H_{11}NO$	*	25.537	833	**
89.3	Phenanthrene	$C_{14}H_{10}$	178.077606 (-0.539)	26.570	871	6

* Molecular ion not found due to excessive fragmentation of the molecule

** Not calculated

From the 67 tentatively identified compounds, an attempt is made to find out from the literature which of them are responsible for odours. Thus, 12 are related to odours [7,11,38–40] and are shown in **Table 6** together with their threshold value and their characteristic odour.

Table 6. List of odorous compounds

Compound	Odour	Odour threshold value (mg m^{-3})
Ethylbenzene	Sweet, solventy	0.01 – 78.3
m-xylene	Rubbery	0.052 - 86
o-xylene	Rubbery	0.77 – 23.6
Toluene	Rubbery, mothballs, tarry	0.4 - 590
o-cymene	Lemon, fruity, fuel-like, sweet, herbal, spicy	0.004 – 7.2
1,2,4-trimethylbenzene	Sweet, solventy	0.14 - 12
Valeraldehyde	Pungent	0.0025 – 17.5
Dimethyl trisulfide	Rotten cabbage	0.00006 – 0.014
Acetic acid	Pungent/vinegar-like	0.025 - 25
Phenol	Medicinal, sweet, phenolic plastic rubber	0.022 - 20
m-cresol	Medicinal, phenolic	0.00057 – 0.011
Indole	Manure, faecal, nauseating	0.000033 – 0.0071

4.1.3 Multifactorial approach to the optimisation of SPME variables

Once the 67 compounds have been identified, a GC-MS quantitative method is created by monitoring the target ion of each one with the TraceFinder software in order to optimise the SPME variables by applying a Central Composite Design with three variables, as shown in **Table 7**, by processing data with this new method. The following variables were evaluated: sample volume (1.5 and 4 mL), extraction temperature (40 and 60ºC) and NaCl addition (8 and 30%).

Table 7. List of experiments for the multifactorial approach

Number of tests	Sample volume (mL)	Extraction temperature (ºC)	NaCl (%)
1	2.75	50	19
2	2.75	50	19
3	1.5	60	8
4	4	60	8
5	4	40	30
6	4	60	30
7	2.75	50	19
8	2.75	50	19
9	1.5	40	8
10	1.5	60	30
11	1.5	40	30
12	4	40	8
13	2.75	50	19
14	2.75	50	19
15	0.65	50	19
16	4.85	50	19
17	2.75	33	19
18	2.75	67	19
19	2.75	50	1
20	2.75	50	37

After extracting and injecting the 20 experiments, the samples were processed with the quantitative method in order to obtain the areas of the target ion and analyse the response surface model in the RStudio software to get the optimum values for the three variables of the study. In this way, a script was created (see Annex I) in which the area of the target ion of each of the 67 compounds is taken into account for the 20 experiments proposed. **Table 8** shows the stationary (optimum) value of each of the three variables for each compound.

Table 8. Stationary (optimal) values of each compound when performing the response surface

Compound	Sample vol. (mL)	Extract. Temp. (ºC)	NaCl (%)
Ethylbenzene	**	**	**
1,3-dimethylbenzene (m-xylene)	**	**	**
Phenylurea	**	**	**
o-xylene	4.26	110.34 *	28.43
Eucalyptol	1.40	32.27 *	42.62 *
1-ethyl-4-methyl-benzene (toluene)	2.39	41.36	21.80
o-cymene	4.83	35.36	30.24
1,2,4-trimethylbenzene	3.46	46.59	21.03
2-methylpentanal (valeraldehyde)	**	**	**
5-methyl-1-phenyl-1-hexanone	3.44	48.13	19.53
trans-rose oxide	-0.58 *	7.44 *	9.32
2,3,6-trimethyl-pyridine	2.79	58.00	3.22
Indane	3.85	45.45	20.61
Dimethyl trisulfide	2.73	50.01	18.96
2-nonanone	**	**	**
3,7-dimethyl-3-octanol	0.85	44.34	30.64
2,6-dimethyl-2-octanol	**	**	**
Acetic acid	**	**	**
Phtocitral A	3.20	30.59 *	22.21
p-menthone	**	**	**
β-patchoulene	**	**	**
1,2-dichlorobenzene	3.61	49.09	19.09
(+)-2-bornanone	2.21	41.73	10.54
3-(tert-butylsulfanyl)-5-methylpyridine	3.18	53.79	21.56
Hexadecane	1.81	35.07	24.79
α-guaiene	1.75	16.29 *	38.19 *
Menthol	1.92	45.52	11.59
Germacrene D	**	**	**
3,7-dimethyl-1-octanol	**	**	**
Isoborneol	**	**	**

Compound	Sample vol. (mL)	Extract. Temp. (ºC)	NaCl (%)
α-terpineol	**	**	**
4-hidroxyindole-3-carboxilic acid	**	**	**
N-formyl aniline	**	**	**
2-methyl-1-hexanol	**	**	**
δ-cadinene	**	**	**
5-methyl-2-(1-methylethyl)hexanol	**	**	**
Benzenemethanesulfonyl fluoride	**	**	**
(4-nitrophenyl)-2,3,4-trifluorobenzoate	4.32	98.39 *	65.01 *
4-hydroxybenzamide	**	**	**
2-formyl-4,6-dichlorophenyl ester-2-trifluoromethylbenzoic acid	4.81	102.57 *	-24.13 *
(1-pentylhexyl)benzene	1.74	33.41	27.67
(1-butylheptyl)benzene	1.63	35.30	28.61
trans-calamenene	7.51	147.64 *	82.06 *
α-isomethyl ionone	3.26	36.11	19.74
2-formyl-4,6-dichlorophenyl ester-6-fluoro-2-trifluoromethylbenzoic acid	3.21	35.40	23.72
Dimethylcyanamide	**	**	**
1-nitrosoadamantane	**	**	**
(1-pentylheptyl)benzene	0.91	35.04	27.47
β-methyl-benzenepropanol	**	**	**
Biphenyl	-182.46 *	9091.11 *	-344.34 *
Phenol	2.72	37.75	21.00
Diphenyl ether	3.32	36.19	20.14
(1-butylnonyl)benzene	1.44	32.61 *	28.04
Amberonne	3.31	15.04 *	23.00
3-methylphenol (m-cresol)	1.58	44.44	8.64
Thymol	2.92	38.06	22.94
Patchouli alcohol	2.66	42.21	8.27
2-ethoxy-napthalene	3.37	35.62	19.66

Compound	Sample vol. (mL)	Extract. Temp. (ºC)	NaCl (%)
3,5-bis(1,1-dimethylethyl)-phenol	2.82	33.35	18.39
2-hydroxy-2-methylbutyl ester-benzoic acid	4.46	64.74	6.17
Tonalid	0.98	23.95 *	27.70
7-hydroxycadalene	3.13	22.75 *	32.39
1-hexyl-1-nitrocyclohexane	2.51	42.68	19.88
Indole	-2.54 *	69.37 *	-20.01 *
3-methylindolizine	**	**	**
N-methyl-1-hidroxycarbozole	2.40	40.00	14.46
Phenanthrene	**	**	**

* Values outside the limits of the multivariate approach

** They have no optimal value because in some of the 20 experiments the peak area has not been obtained and they have been discarded.

As an example, the optimisation graphical outputs generated for two model compounds (dimethyl trisulfide and indole) for which the optimum value for three variables is located inside the experimental limits or outside the limits, respectively, are shown in **Figure 9**.

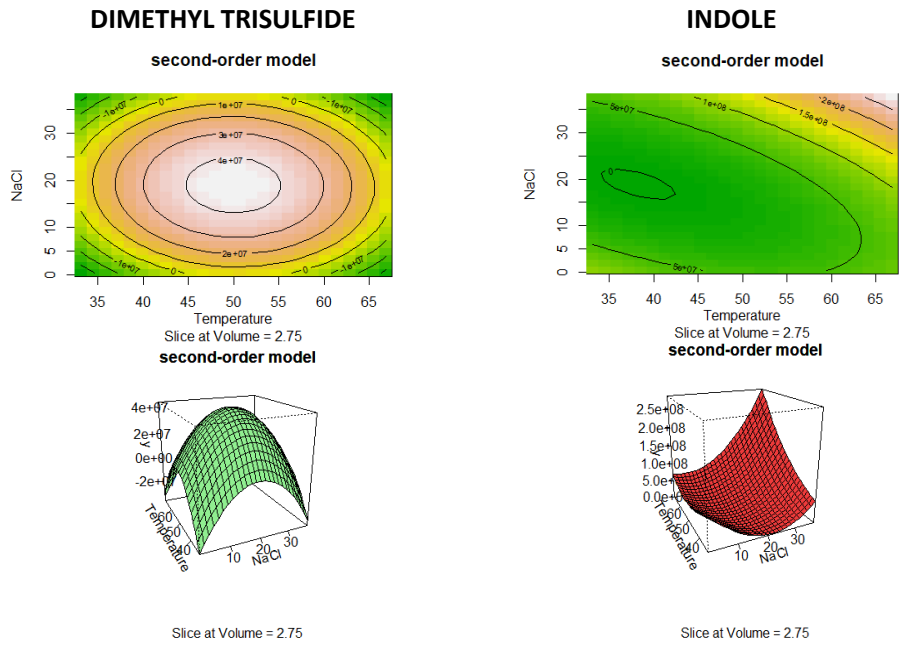

Figure 9. Graphical outputs (response surface) of the optimisation for dimethyl trisulfide and indole

To optimise these 3 variables, all values are averaged excluding those with data outside the limits established in the multifactor approach. Finally, the optimal values selected and with which the rest of the analyses will be carried out are: 3 mL of sample volume, 45ºC as extraction temperature and 0.6 g of NaCl (20% in the sample).

5 FUTURE WORK

After what has been done during this work, there are several lines of research open to continue studying the effect of volatile organic pollutants responsible for odours derived from WWTPs.

Firstly, after optimising three variables of the extraction method, in this case SPME, the effect of the extraction time will be studied, considering 6 analyses in which the extraction times of 5, 10, 15, 30, 60 and 90 minutes will be evaluated. With the quantitative method designed throughout this work, the areas of the target ion provided for each time will be obtained and the equilibrium time is estimated, both graphically and mathematically (using an exponential equation).

With all the variables optimised, the method will be applied to the study of the stored real samples. For each sample, a target and a non-target method will be applied. The target method will be applied after having made the library of the 67 compounds and the non-target method because it is of interest to know if there are new compounds in these samples.

Finally, it will be of interest to quantify the VOCs responsible for odours, so the corresponding standards of these compounds will be searched for in the laboratory and the validation of the method will be designed to subsequently study the odour profile, the odour activity value and quantify them.

6 CONCLUSIONS

The applicability of an analytical methodology based on headspace solid-phase microextraction and the use of GC-HRMS for the identification of unknown compounds using accurate mass measurements has been investigated. Wastewater from 3 wastewater treatment plants in the province of Castellón was used for this study.

The determination of the non-target compounds was performed in a single chromatographic run of 30 minutes desorbing the SPME fibre. This required an adequate optimisation of the fibre, in which multivariate studies were used to achieve this objective, although there were certain compounds that did not work properly and it was necessary to choose compromise values.

On the other hand, the applicability of the method as a whole was evaluated, highlighting the high sensitivity achieved by combining HS-SPME and the Q-Orbitrap, achieving a very powerful method that allowed the identification of 67 VOCs, of which 12 of them were odorous compounds. To ensure this identification, it was necessary to establish different parameters with limiting values, such as the comprehensive score, the RSI and the deviation of the retention index.

In conclusion, the methodology has proven to be a viable option for the reliable identification of volatile organic compounds, although the time required for experimental studies does not always allow complete results to be obtained, especially in new studies such as the one described above.

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ANNEX I

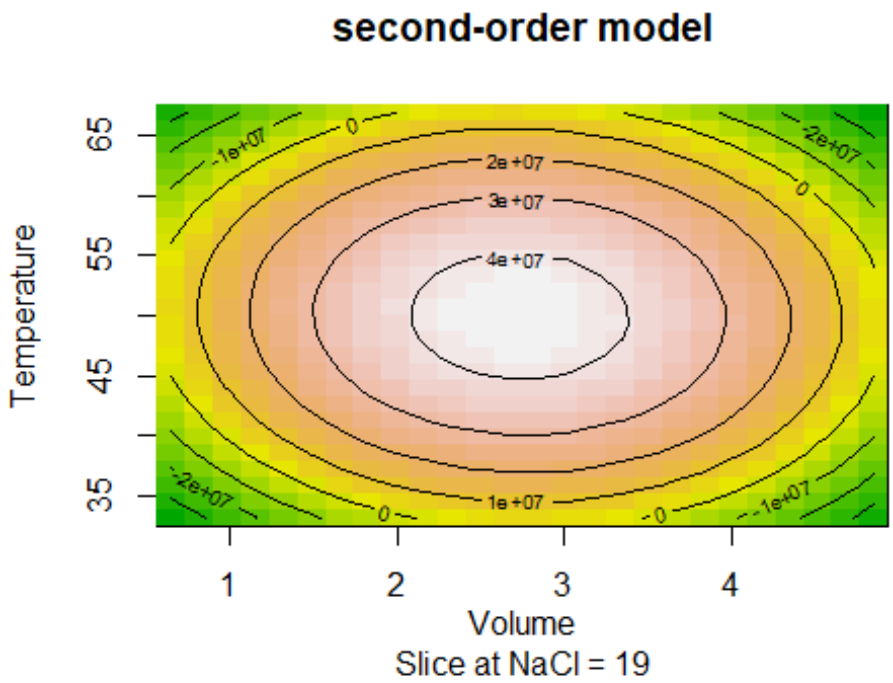
The data shown below refer to the RStudio software script for RSM optimisation of the dimethyl trisulphide compound, including R output.

```
library (DoE.base)

## Loading required package: grid

## Loading required package: conf.design

## Registered S3 method overwritten by 'DoE.base':
##   method           from
##   factorize.factor conf.design

##
## Attaching package: 'DoE.base'

## The following objects are masked from 'package:stats':
##
##     aov, lm

## The following object is masked from 'package:graphics':
##
##     plot.design

## The following object is masked from 'package:base':
##
##     lengths

library(FrF2)
library (DoE.wrapper)

## Loading required package: rsm

rsm.base<-fac.design(
  nlevels = 2,
  factor.names = list(
    Volume = c(1.5,4),
    Temperature = c(40,60),
    NaCl=c(8,30)),
  replications = 1,
  repeat.only = FALSE,
  randomize = TRUE,
  seed = 1313
)

## creating full factorial with 8 runs ...

Design.RSM <- ccd.augment( rsm.base , alpha= "rotatable" , ncenter=c(
6,0 ) ,randomize= FALSE)
Dimethyltrisulfide=c(5141112,
      2358205,
      6965658,
      2176403,
```

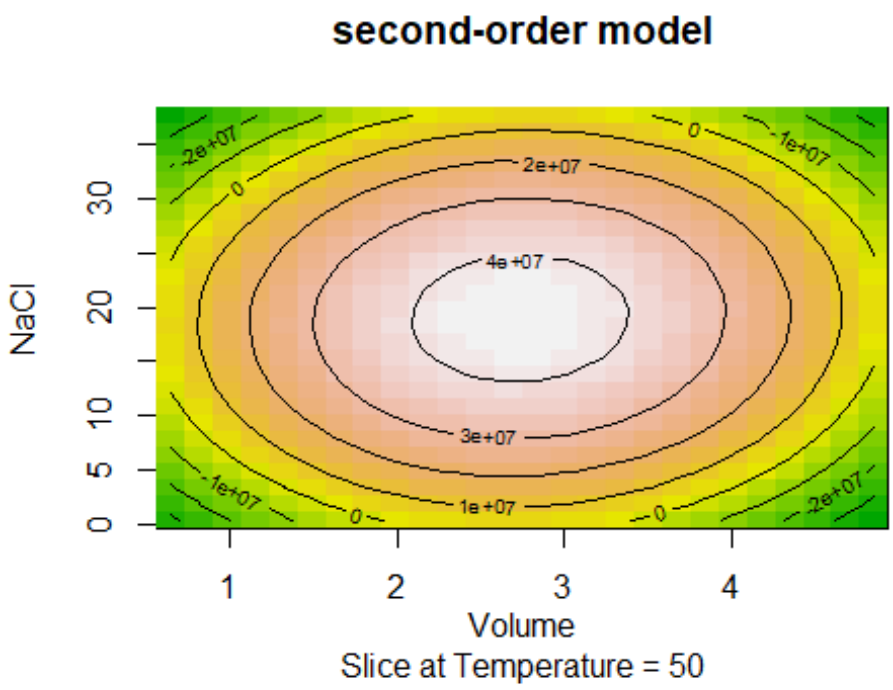
```
      5294726,
      2054749,
      3945336,
      248732254,
      4056048,
      3147848,
      2983810,
      1961659,
      2329135,
      1625407,
      1055097,
      1214558,
      1816452,
      1829490,
      2200909,
      2597522
)
Design.RSM.resp <- add.response(design = Design.RSM, response = Dimeth
yltrisulfide)
rsm1 <- rsm(Dimethyltrisulfide ~ SO(Volume,Temperature,NaCl), data = D
esign.RSM.resp)
summary(rsm1)

##
## Call:
## rsm(formula = Dimethyltrisulfide ~ SO(Volume, Temperature, NaCl),
##     data = Design.RSM.resp)
##
##                        Estimate  Std. Error t value Pr(>|t|)
## (Intercept)        -4.2648e+08  6.0881e+08 -0.7005   0.4996
## Volume              5.1471e+07  1.2585e+08  0.4090   0.6912
## Temperature         1.4302e+07  2.0059e+07  0.7130   0.4921
## NaCl                4.4758e+06  1.3883e+07  0.3224   0.7538
## Volume:Temperature -6.0989e+04  2.0072e+06 -0.0304   0.9764
## Volume:NaCl         7.3650e+04  1.8247e+06  0.0404   0.9686
## Temperature:NaCl   -7.0458e+03  2.2809e+05 -0.0309   0.9760
## Volume^2           -9.1159e+06  1.1964e+07 -0.7620   0.4637
## Temperature^2      -1.4000e+05  1.8693e+05 -0.7489   0.4711
## NaCl^2             -1.1402e+05  1.5449e+05 -0.7380   0.4774
##
## Multiple R-squared:  0.1236, Adjusted R-squared:  -0.6651
## F-statistic: 0.1568 on 9 and 10 DF,  p-value: 0.9949
##
## Analysis of Variance Table
##
## Response: Dimethyltrisulfide
##                                  Df     Sum Sq    Mean Sq F value Pr(
>F)
## FO(Volume, Temperature, NaCl)   3 2.2086e+12 7.3621e+11  0.0001 1.0
000
## TWI(Volume, Temperature, NaCl)  3 1.7659e+13 5.8864e+12  0.0012 0.9
999
## PQ(Volume, Temperature, NaCl)   3 7.0850e+15 2.3617e+15  0.4690 0.7
105
```

```
## Residuals                         10 5.0359e+16 5.0359e+15
## Lack of fit                        5 6.2925e+13 1.2585e+13  0.0013 1.0
000
## Pure error                         5 5.0296e+16 1.0059e+16
##
## Stationary point of response surface:
##      Volume Temperature        NaCl
##    2.732493   50.006543   18.964614
##
## Eigenanalysis:
## eigen() decomposition
## $values
## [1]  -113368.7  -140400.5 -9116124.1
##
## $vectors
##                      [,1]         [,2]         [,3]
## Volume       0.004513719 -0.002806963  0.999985874
## Temperature -0.136205277  0.990674817  0.003395628
## NaCl         0.990670353  0.136218680 -0.004089304
```

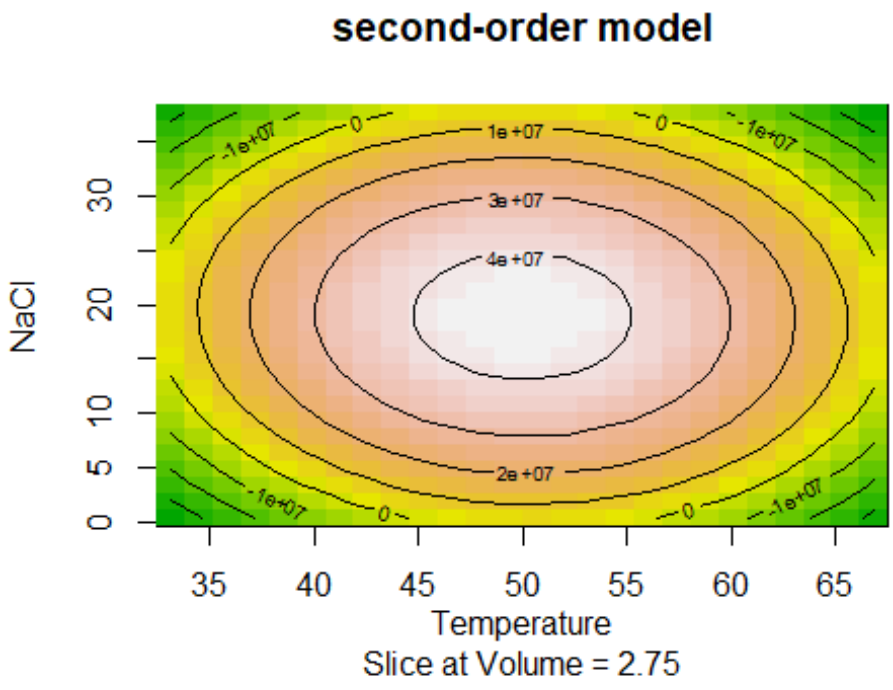
```
contour(rsm1, ~ Volume + Temperature, image = TRUE, main="second-order
model")
```



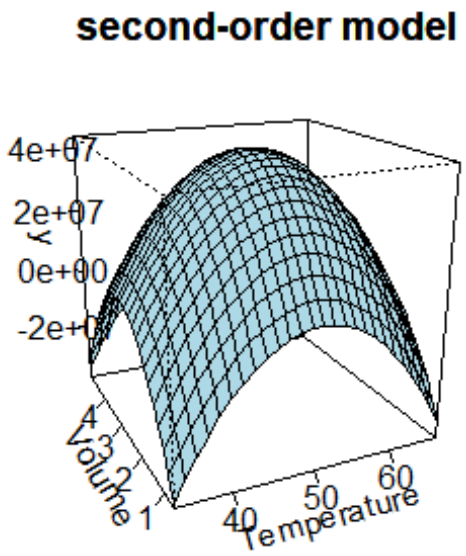

```
contour(rsm1, ~ Volume + NaCl, image = TRUE, main="second-order model"
)
```




```
contour(rsm1, ~ Temperature + NaCl, image = TRUE, main="second-order m
odel")
```

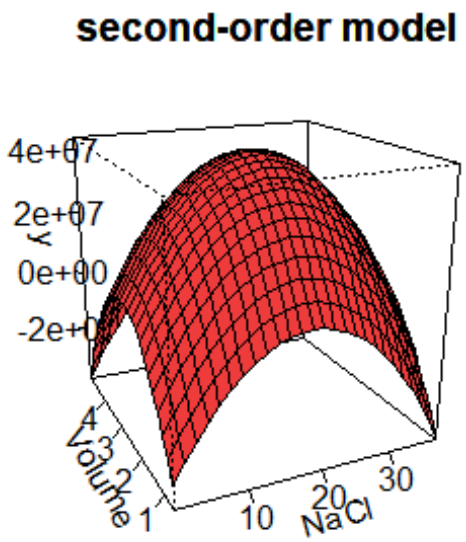



```
persp(rsm1, Volume ~ Temperature, zlab = "y", main="second-order model
",col = "lightblue")
```



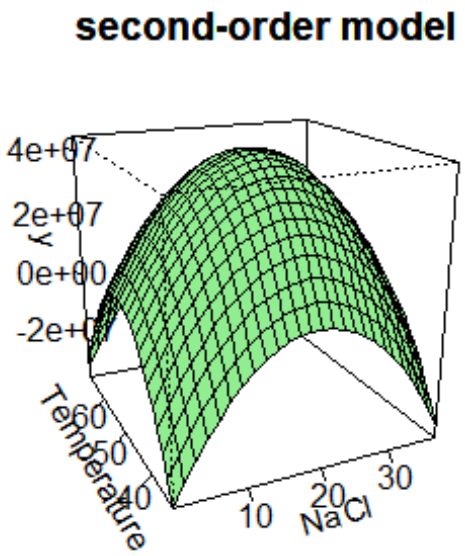
Slice at NaCl = 19

```
persp(rsm1, Volume ~ NaCl, zlab = "y", main="second-order model",col =
"brown2")
```



Slice at Temperature = 50

```
persp(rsm1, Temperature ~ NaCl, zlab = "y", main="second-order model",
col = "palegreen2")
```


second-order model
4e+07
2e+07
0e+00
-2e+07
y
Temperature
60
50
40
NaCl
10
20
30
Slice at Volume = 2.75