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DEVELOPMENT OF ANALYTICAL METHODOLOGIES FOR THE ASSESSMENT OF ODOROUS AND FRAGRANCE COMPOUNDS IN WASTEWATER TREATMENT PLANTS

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Doctoral Thesis

DEVELOPMENT OF ANALYTICAL METHODOLOGIES FOR THE ASSESSMENT OF ODOROUS AND FRAGRANCE COMPOUNDS IN WASTEWATER TREATMENT PLANTS

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Supervised by Dr. Enriqueta Anticó Daró and Dr. Juan Manuel Sánchez Navarro

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Universitat de Girona

Dr. Enriqueta Anticó Daró and Dr. Juan Manuel Sánchez Navarro, of Universitat de Girona,

WE DECLARE:

That the thesis titles *Development of analytical methodologies for the assessment of odorous and fragrance compounds in wastewater treatment plants***,** presented by Anna Godayol Boix to obtain a doctoral degree, has been completed under our supervision.

For all intentions and purposes, we hereby sign this document.

Dr. Enriqueta Anticó Daró

Dr. Juan Manuel Sánchez Navarro

Girona, 10TH of February of 2014

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The research described in this thesis has result in the publication of three scientific papers and two more that are in preparation:

- Godayol, A., Alonso, M., Besalú, E., Sanchez, J.M., Anticó, E. **Odour-causing compounds in wastewater treatment plants: Evaluation of solid-phase microextraction as a concentration technique.** *Journal of Chromatography A*, 1218 (2011) 4863-4868.

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- Godayol, A., Alonso, M., Sanchez, J.M., Anticó, E. **Odour-causing compounds in air samples: Gas-liquid partition coefficients and determination using solid-phase microextraction and GC with mass spectrometric detection.** *Journal of Separation Science*, 36 (2013) 1045-1053.

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- Godayol, A., Marcé, R.M., Borrull, F., Anticó, E., Sanchez, J.M. **Development of a method for the monitoring of odor-causing compounds in atmospheres surrounding wastewater treatment plants.** *Journal of Separation Science*, 36 (2013) 1621-1628.

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  Times cited: 0 (17/12/2013)

- Godayol, A., Besalú, E., Anticó, E., Sanchez, J.M. **Monitoring of sixteen fragrance allergens and two polycyclic musks in wastewaters by solid phase microextraction coupled to gas chromatography.** *In preparation*.

- Godayol, A., Gonzalez-Olmos, R., Sanchez, J.M., Anticó, E. **UV and chlorination for the degradation of fragrances in water samples.** *In preparation*.

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LIST OF ABBREVIATIONS

BTEX	Benzene, toluene, ethylbenzene, and xylene isomers
CLLE	Continuous liquid-liquid extraction
CLSA	Closed loop stripping analysis
DLLME	Dispersive liquid-liquid microextraction
DMDS	Dimethyl disulphide
DoE	Design of experiments
FID	Flame ionization detection
FMOC	9-Fluorenyl-methylchloroformate
FPD	Flame photometric detection
GC	Gas chromatography
HS-SPME	Headspace-solid phase microextraction
LLE	Liquid liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
MALLE	Membrane-assisted liquid-liquid extraction
MEPS	Microextraction by packed sorbent
MTBSTFA	N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide
MS	Mass spectrometry
NPTFA	*p*-Nitrophenyl trifluoroacetate
OTC	Odour threshold concentration
OTNE	1-(1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone
PCP	Personal-care product
PFBAY	2,3,4,5,6-Pentafluorebenzylaldehyde
PFBHA	O-(2,3,4,5,6)-pentafluorobenzyl-hydroxylamine hydrochloride
PFBOA	O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine
PFPD	Pulsed flame photometric detection
PHWE	Pressurized hot water extraction
RSD	Relative standard deviation
SBME	Solvent bar microextraction
SBSE	Stir bar sorptive extraction
SCCS	Scientific Committee on Consumer Safety
SNPA	*N*-succinimidyl-*p*-nitrophenylacetate

SPDE	Solid phase dynamic extraction
SPE	Solid phase extraction
TD	Thermal desorption
TIC	Total ion chromatogram
TNT	2,4,6-Trinitrotoluene
UA	Ultrasound assisted
USAEME	Ultrasound-assisted emulsification-microextraction
US EPA	United States Environmental Protection Agency
UV	Ultraviolate
VFA	Volatile fatty acid
VOC	Volatile organic compound
VOSC	Volatile organic sulphur compound
WWTP	Wastewater treatment plant

Contents

Summary/resum/resumen

SUMMARY

In the last years, the generation of odours in wastewater treatment plants (WWTPs), as well as the incomplete elimination of several pollutants such as fragrances along the different wastewater treatments has become a subject of public concern. The research presented in this thesis is focused on the development of analytical methodologies for the determination of odorous and fragrance compounds in water and air samples from WWTPs.

Firstly, a method based on headspace solid-phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) was developed for the determination of a group of odorous compounds belonging to different chemical families (phenolic compounds, aldehydes, sulphur-containing compounds, nitrogen-containing compounds, and terpenes) in wastewater samples. Using a divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre, the SPME extraction parameters were evaluated applying an experimental design and the final optimised conditions were: 1 g of NaCl added, extraction time of 30 min and extraction temperature of 70^{o}C. After the validation of the proposed method, samples from the influent, the biological treatment effluent and the effluent of a WWTP were analysed and monitored. A decrease in the concentration of the target compounds was observed along the different treatments of the plant and some of the analytes were detected at concentrations above their odour threshold concentrations (OTCs) in some of the analysed samples.

As a consequence of the air-water partition equilibrium, some odour-causing compounds can be found in the atmospheres surrounding WWTPs. Thus, two different methods were developed for their quantification in WWTP air. The first method was based on SPME and GC-MS detection. Air samples were collected in 25 L Nalophan® bags and then transferred to a 0.5 L glass bulb for their SPME concentration using a DVB/CAR/PDMS fibre. The adsorption kinetics was studied and an extraction time of 10 min was found to be adequate to avoid the coating saturation. The method was validated and applied to the determination of the gas-liquid partition coefficients of the odorous compounds. The second method was based on active collection of odorous compounds in Tenax TA/Carbograph 1TD tubes followed by thermal desorption and GC-MS. After the evaluation of the effects of the desorption parameters (cold trap and tube desorption), the proposed method was validated. A breakthrough study was performed and a sample volume 1 L was found to be the most adequate to avoid losses of the target analytes. Both developed methods were applied to the analysis of air samples from different WWTPs and it was observed that while some of the analytes were not detected in

any sample, some of them were found to be present at concentrations above their OTCs. Moreover, those compounds with the higher partition coefficients were found to be present in the air samples at the highest levels.

The following part of this thesis was devoted to the development of an analytical methodology for the assessment of fragrance compounds in wastewaters. The proposed method consisted on HS-SPME concentration using a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibre followed by GC-MS detection. The SPME extraction parameters were optimised by means of a full factorial design and the optimum conditions for the simultaneous analysis of the target compounds were found to be 2.4 g of NaCl added, extraction time of 45 min and extraction temperature of 90^{o}C. The method was validated and applied to the analysis of samples from two different WWTPs. Only seven out of the 18 studied fragrances were detected in at least one of the samples. Four of the target analytes were found not to be eliminated during the wastewater treatment as they were detected at the effluent of both plants.

Finally, two conventional tertiary treatments (UV and chlorination) were evaluated under laboratory scale for the removal of the fragrances in waters. For that, the HS-SPME/GC-MS method developed for the monitoring of fragrances in wastewaters was applied. Elimination experiments showed that all target compounds were affected by at least one of the tested treatments, being the UV irradiation more effective than chlorination. However, these treatments were found not to be effective enough for the complete removal of the target fragrances from water. In a final stage, UV and chlorination transformations products were investigated. A total of 15 UV transformation products were detected and chemical structures were proposed for five of them, whereas in the chlorination experiments only by-products of two fragrances were detected.

RESUM

Durant els darrers anys, la generació d'olors a les estacions depuradores d'aigües residuals (EDARs), així com la incompleta eliminació d'un gran nombre de contaminants com ara les fragàncies, al llarg dels diferents tractaments de l'aigua residual, ha esdevingut un assumpte que preocupa a la població. Per aquests motius, la recerca presentada en aquesta tesi s'ha centrat en el desenvolupament de metodologies analítiques per a la determinació de compostos responsables de males olors i fragàncies en mostres d'aigua i aire procedents d'EDARs.

En primer lloc, es va desenvolupar un mètode basat en la microextracció en fase sòlida en espai de cap (HS-SPME) i la cromatografia de gasos acoblada a l'espectrometria de masses (GC-MS) per a l'anàlisi en mostres d'aigua residual d'un grup de compostos responsables de males olors i pertanyents a diferents famílies químiques (compostos fenòlics, aldehids, compostos de sofre, compostos de nitrogen i terpens). Utilitzant una fibra combinada de divinilbenzè/Carboxen/polidimetilsiloxà (DVB/CAR/PDMS), es van optimitzar els paràmetres de la extracció amb SPME mitjançant un disseny experimental i les condicions finals optimitzades van ser: addició de 1 g de NaCl, temps d'extracció de 30 min i temperatura d'extracció de 70^{o}C. Després de validar el mètode proposat, es van analitzar i monitoritzar mostres procedents de l'entrada, la sortida del tractament biològic i la sortida d'una EDAR. Es va observar una disminució de la concentració dels compostos estudiats al llarg dels diferents tractaments de l'aigua residual, així com la presència d'alguns dels anàlits a concentracions per sobre del seu llindar de percepció en algunes de les mostres.

Com a conseqüència de l'equilibri de partició aire-aigua, alguns compostos responsables de males olors poden trobar-se a les atmosferes que rodegen les EDARs. Per aquest motiu, es van desenvolupar dos mètodes per a la seva quantificació en aire procedent d'EDARs. El primer mètode es va basar en la SPME i detecció per GC-MS. Les mostres d'aire es van agafar en bosses de 25 L de Nalophan® i a continuació van ser transferides a un bulb de vidre de 0.5 L per la seva concentració mitjançant una fibra de SPME de DVB/CAR/PDMS. Es van estudiar les condicions de co-adsorció i es va trobar que un temps de 10 min era l'adequat per evitar la saturació de la fibra durant l'extracció. Es va validar el mètode i aquest es va utilitzar seguidament per a la determinació dels coeficients de partició gas-líquid dels compostos responsables de males olors. El segon mètode es va basar en l'adsorció dinàmica dels compostos olorosos en tubs de Tenax TA/Carbograph 1TD, la seva posterior desorció tèrmica i anàlisis per GC-MS. Després d'avaluar els paràmetres de desorció del tub i de la trampa freda,

es va validar el mètode proposat. Es va dur a terme l'avaluació del volum de ruptura dels adsorbents i es va observar que un volum de 1 L d'aire era el més adequat per evitar pèrdues dels anàlits. Ambdós mètodes es van aplicar a l'anàlisi de mostres d'aire procedents de diferents EDARs i es va observar que, mentre que alguns dels anàlits no van ser detectats a cap de les mostres, d'altres es van trobar a concentracions per sobre dels seus llindars de percepció. A més, els compostos amb els coeficients de partició més alts van resultar ser els que es trobaven a nivells més alts a les mostres d'aire.

La següent part de la tesi va consistir en el desenvolupament d'un mètode analític per a la determinació de fragàncies en aigües residuals. El mètode proposat estava basat en la concentració mitjançant HS-SPME utilitzant una fibra de polidimetilsiloxà/divinilbenzè (PDMS/DVB), seguida de la detecció amb GC-MS. Els paràmetres de l'extracció amb SPME es van optimitzar mitjançant un disseny factorial complet i les condicions òptimes per a l'anàlisi simultània dels anàlits van resultar ser l'addició de 2.4 g de NaCl, un temps d'extracció de 45 min i una temperatura d'extracció de 90^{o}C. Finalment, es va validar el mètode i es va aplicar a l'anàlisi de mostres de dues EDARs diferents. Només 7 de les 18 fragàncies estudiades es van detectar com a mínim en una de les mostres analitzades. No obstant, es va observar que 4 dels anàlits no van ser eliminats durant els tractaments de l'aigua residual, ja que van ser detectats a la sortida de les dues plantes.

Finalment, es van avaluar dos tractaments terciaris convencionals (radiació UV i cloració) a escala de laboratori per a l'eliminació de fragàncies en aigües. Per portar a terme el seguiment de la disminució en la concentració dels compostos, es va utilitzar el mètode basat en HS-SPME/GC-MS desenvolupat prèviament per al monitoratge de fragàncies en aigües residuals. Els experiments d'eliminació van mostrar que tots els compostos estudiats es veien afectats com a mínim per un dels tractaments avaluats, sent la radiació UV més efectiva que la cloració. Malgrat això, es va observar que aquests tractaments no són prou efectius per a la completa eliminació de les fragàncies estudiades en aigua. D'altra banda, es van investigar els productes de transformació generats a causa d'ambdós tractaments. Es van detectar un total de 15 productes de transformació a causa de la radiació UV i es van proposar estructures químiques per 5 d'ells, mentre que en els experiments de cloració tan sols es van detectar els productes de degradació de 2 fragàncies.

RESUMEN

Durante los últimos años, la generación de olores en las estaciones depuradoras de aguas residuales (EDARs), así como la incompleta eliminación de un gran número de contaminantes como las fragancias a lo largo de los distintos tratamientos del agua residual se ha convertido en un asunto que preocupa a la población. Por estos motivos, la investigación que se presenta en esta tesis se ha centrado en el desarrollo de metodologías analíticas para la determinación de compuestos responsables de malos olores y fragancias en muestras de agua y aire procedentes de EDARs.

En primer lugar, se desarrolló un método basado en la microextracción en fase sólida en espacio de cabeza (HS-SPME) y la cromatografía de gases acoplada a la espectrometría de masas (GC-MS) para el análisis de un grupo de compuestos responsables de malos olores y pertenecientes a diferentes familias químicas (compuestos fenólicos, aldehídos, compuestos de azufre, compuestos de nitrógeno y terpenos) en muestras de agua residual. Usando una fibra combinada de divinilbenceno/Carboxen/polidimetilsiloxano (DVB/CAR/PDMS), se optimizaron los parámetros de la extracción con SPME mediante un diseño experimental y las condiciones finales optimizadas fueron: adición de 1 g de NaCl, tiempo de extracción de 30 min y temperatura de extracción de 70^{o}C. Después de validar el método propuesto, se analizaron y monitorizaron muestras procedentes de la entrada, la salida del tratamiento biológico y la salida de una EDAR. Se observó una disminución de la concentración de los compuestos estudiados a lo largo de los diferentes tratamientos del agua residual, así como la presencia de algunos de los analitos a concentraciones por encima de su umbral de percepción en algunas de las muestras.

Como consecuencia del equilibrio de partición aire-agua, algunos compuestos responsables de malos olores pueden encontrarse en las atmósferas que rodean las EDARs. Por estos motivos, se desarrollaros dos métodos para su cuantificación en aire procedente de EDARs. El primer método se basó en la SPME y detección por GC-MS. Las muestras de aire se recogieron en bolsas de 25 L de Nalophan® y a continuación se transfirieron a un bulbo de vidrio de 0.5 L para su concentración mediante una fibra de SPME de DVB/CAR/PDMS. Se estudiaron las condiciones de co-adsorción y 10 min resultó ser el tiempo adecuado para evitar la saturación de la fibra durante la extracción. Se validó el método y a continuación éste se usó para la determinación de los coeficientes de partición gas-líquido de los compuestos responsables de malos olores. El segundo método se basó en la adsorción dinámica de los compuestos olorosos en tubos de Tenax TA/Carbograph 1TD, su posterior desorción térmica y análisis por GC-MS.

Después de evaluar los parámetros de desorción del tubo y de la trampa fría, se validó el método propuesto. Se llevó a cabo la evaluación del volumen de ruptura de los adsorbentes y se observó que un volumen de aire de 1 L era el más adecuado para evitar pérdidas de los analitos. Ambos métodos se aplicaron al análisis de muestras de aire procedentes de diferentes EDARs y se observó que, mientras que algunos de los analitos no se detectaron en ninguna de las muestras, otros se encontraron a concentraciones por encima de sus umbrales de percepción. Además, los compuestos con los coeficientes de partición mayores resultaron ser los que se encontraron a niveles más altos en las muestras de aire.

La siguiente parte de la tesis consistió en el desarrollo de un método analítico para la determinación de fragancias en aguas residuales. El método propuesto se basó en la concentración mediante HS-SPME utilizando una fibra de polidimetilsiloxano/divinilbenceno (PDMS/DVB), seguida de la detección con GC-MS. Se optimizaron los parámetros de la extracción con SPME mediante un diseño factorial completo, determinándose que las condiciones óptimas para el análisis simultáneo de los analitos fueron la adición de 2.4 g de NaCl, un tiempo de extracción de 45 min y una temperatura de extracción de 90^{o}C. Finalmente, se validó el método y se aplicó al análisis de muestras de dos EDARs diferentes. Tan solo 7 de las 18 fragancias estudiadas se detectaron como mínimo en una de las muestras analizadas. No obstante, se observó que 4 de los analitos no fueron eliminados durante los tratamientos del agua residual, ya que fueron detectados en la salida de las dos plantas.

Finalmente, se evaluaron dos tratamientos terciarios convencionales (radiación UV y cloración) a escala de laboratorio para la eliminación de fragancias en aguas. Para el seguimiento de la concentración de los analitos, se aplicó el método basado en HS-SPME/GC-MS desarrollado previamente para el monitoraje de fragancias en aguas residuales. Los experimentos de eliminación mostraron que todos los compuestos estudiados se veían afectados como mínimo por uno de los tratamientos evaluados, siendo la radiación UV más efectiva que la cloración. A pesar de esto, se observó que estos tratamientos no son suficientemente efectivos para la completa eliminación de las fragancias estudiadas en agua. Por otro lado, se investigaron los productos de transformación generados en ambos casos. Se detectaron un total de 15 productos de transformación generados a partir de la radiación UV y se propusieron estructuras químicas para 5 de ellos, mientras que en los experimentos de cloración tan solo se detectaron los productos de degradación de 2 fragancias.

CHAPTER 1:
General introduction

Water is an essential resource for life and good health. A lack of water to meet daily needs is a reality today for one in three people around the world. As cities and populations grow, and the needs for water increase in agriculture, industry and households, the importance of water reuse is straightforward. Water quality, on the other hand, is threatened for the continuous introduction of chemical pollutants and their bioactive metabolites into the environment. The way pollutants enter the environment depends on their pattern of usage and mode of application (e.g., industrial and agricultural wastes, municipal sewage, hospital effluents, and accidental spills). Because some pollutants are from human use, their emissions are an issue for some wastewater treatment plants (WWTPs) where it has been generally been assumed that they are eliminated through sewage treatment.

Besides conventional priority pollutants, a group of chemicals termed as emerging contaminants has acquired major relevance because they are continuously released in the environment and can accumulate in aquatic organisms with unpredictable consequences. In this group, pharmaceuticals, drugs of abuse, personal-care products (PCPs), steroids and hormones, surfactants and surfactant wastes, plasticizers and various industrial additives, and nanoparticles have gained relevance. These substances are not currently covered by existing water-quality regulations and are thought to be potential threats to environmental ecosystems and human health and safety. Moreover, their complete removal in sewage treatment plants cannot be assured by biological treatment methods [1-7]. The development of sensitive analytical methodologies for the quantification of these emerging pollutants and their metabolites, as well as the evaluation of different procedures for their elimination from waters, has become an issue of interest [8-11].

Another problem related to WWTPs is the generation of odours. WWTPs represent a common source of malodorous emissions that are a cause of concern for the population living nearby. Several studies have suggested these emissions affect the quality of life of people living in the vicinity of the plants, leading to symptoms such as nausea, sensory irritation, headache, lack of appetite, and insomnia [12-14]. As a consequence, the public concern has increased and the characterisation of odorous emissions from WWTPs has become one of the most important challenges to achieve [15-18]. Unfortunately, odours are difficult to quantify and so the identification of the compounds responsible for the odour problems in WWTPs is still under investigation.

In this context, this thesis has been focused on the development of analytical methodologies for the determination of odour-causing compounds and fragrances, which are common ingredients of PCPs, in water and air samples from WWTPs.

1.1 ODOUR-CAUSING COMPOUNDS

The generation of odours in WWTPs represents a problem that is a cause of concern for the population living in the vicinity of these plants. According to the United States Environmental Protection Agency (US EPA), the majority of the unit processes of WWTPs are potential sources of odour [19]. The greatest potential of odour is usually generated during sludge treatment, but there are also some other unit processes that generate odorous emissions in the wastewater treatment.

Volatile organic compounds (VOCs) are emitted from WWTPs' water and sludge to the atmosphere when a liquid-gas or solid-gas interface is present. Emissions occur as a consequence of different factors such as turbulences during the wastewater and sludge treatments, the treatment process itself, atmospheric conditions, and the physical characteristics of each individual substance [20].

The composition of odorous emissions from WWTPs is complex. They include organic and inorganic vapours and gases which are formed in the anaerobic decomposition of organic matter containing sulphur and nitrogen. In these emissions, hydrogen sulphide (H_2S), ammonia (NH_3), carbon dioxide (CO_2), and methane (CH_4) are found at high concentrations, being the first two powerfully malodorous. Moreover, other organic compounds that contribute to the malodorous perception such as mercaptans, organic sulphides, nitrogen-containing compounds, and oxygenated compounds can also be found at low concentrations (Table 1.1) [18,21,22].

Table 1.1. Compounds associated with the odorous emissions from WWTPs [23].

Class	Compound	Formula	Odour
Sulphurous	Hydrogen sulphide	H_2S	Rotten eggs
	Dimethyl sulphide	$(CH_3)_2S$	Decayed vegetables, garlic
	Diethyl sulphide	$(C_2H_5)_2S$	Nauseating, ether
	Diphenyl sulphide	$(C_6H_5)_2S$	Unpleasant, burnt rubber
	Diallyl sulphide	$(CH_2CHCH_2)_2S$	Garlic
	Carbon disulphide	CS_2	Decayed vegetables
	Dimethyl disulphide	$(CH_3)_2S_2$	Putrification
	Methyl mercaptan	CH_2SH	Decayed cabbage, garlic
	Ethyl mercaptan	$(CH_3)_2S_2$	Decayed cabbage
	Propyl mercaptan	C_3H_7SH	Unpleasant
	Butyl mercaptan	C_4H_6SH	Unpleasant
	tButyl mercaptan	$(CH_3)_3CSH$	Unpleasant
	Allyl mercaptan	CH_2CHCH_2SH	Garlic
	Crotyl mercaptan	$CH_3CHCHCH_2SH$	Skunk, rancid
	Benzyl mercaptan	$C_6H_5CH_2SH$	Unpleasant
	Thiocresol	C_7H_8S	Skunk, rancid
	Thiophenol	C_6H_5SH	Putrid, nauseating, decay
	Sulphur dioxide	SO_2	Sharp, pungent, irritating
Nitrogenous	Ammonia	NH_3	Sharp, pungent
	Methylamine	CH_3NH_2	Fishy
	Dimethylamine	$C_2H_5NH_2$	Fishy
	Trimethylamine	$(CH_3)_2NH$	Fishy, ammoniacal
	Ethylamine	$(CH_3)_3N$	Ammoniacal
	Diethylamine	$(CH_2H_5)_2NH_2$	
	Triethylamine	$(C_2H_5)_3N$	
	Diamines, e.g. Cadaverine	$NH_2(CH_2)_5NH_2$	Decomposing meat
	Pyridine	C_6H_5N	Disagreeable, irritating
	Indole	C_9H_8NH	Faecal, nauseating
	Skatole	C_8H_6NH	Faecal, nauseating
Acids	Acetic (ethanoic)	CH_3COOH	Vinegar
	Butyric (butanoic)	CH_3H_7COOH	Rancid, sweaty
	Valeric (pentanoic)	C_4H_9COOH	Sweaty
Aldehydes and ketones	Formaldehyde	CH_3CHO	Acrid, suffocating
	Acetaldehyde	CH_3CHO	Fruit, Apple
	Butyraldehyde	C_3H_7CHO	Rancid, sweaty
	Isobutyraldehyde	$(CH_3)_2CHCHO$	Fruit
	Isovaleraldehyde	$(CH_3)_2CHCH_2CHO$	Fruit, Apple
	Acetone	CH_3COCH_3	Fruit, sweet
	Butanone	$C_2H_5COCH_3$	Green apple

The origin of the substances present in WWTPs emissions is diverse. As can be seen in figure 1.1, odorous compounds can be emitted into the environment by three different ways: i) degradation of dissolved natural organic matter, ii) emission in industrial and agricultural processes and iii) use in daily products (e.g., cleaners and flavourings), which are then released into domestic waters.

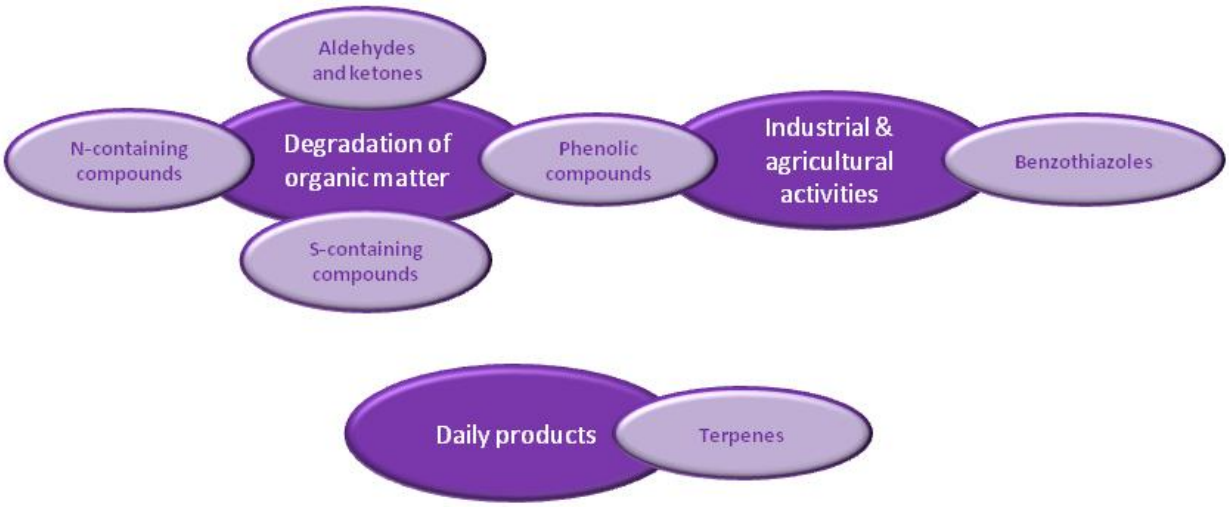


Figure 1.1. Main sources of odour-causing compounds.

1.1.1 DETERMINATION OF ODOUR-CAUSING COMPOUNDS IN AIR AND WATER

The analysis of air and water samples from WWTPs is complicated. Emissions from WWTPs usually contain a large number of substances but few compounds are responsible for the odour perception.

The odorous compounds present in air and water at high concentrations can be determined directly without a concentration step. H_2S has been determined in-situ with portable instruments [18,23,24]. Juarez-Galan et al. [25] performed a weighted average monitoring of several volatiles in air and described the use of an electrochemical detector for the determination of H_2S. NH_3 has been analysed by means of ion selective electrodes [25-27]. Specific techniques such as colorimetry have also been described for this purpose. Fang et al. [28] quantified NH_3 by means of colorimetric tubes, which indicate the measured concentration of the analyte by a change in their colour. Islam et al. [26] analysed NH_3 by means of a spectrophotometer at a wavelength of 630 nm. Trimethylamine has been determined in wastewaters by direct injection in a gas chromatograph [21,26], whereas primary and secondary amines have been analysed in wastewaters by means of reversed-

phase liquid chromatography with ultraviolet (UV) detection. Methods based on their derivatization using *N*-succinimidyl-*p*-nitrophenylacetate (SNPA) as a derivatizing agent can be found in the literature [21,26].

Whilst several compounds are present in the samples at high concentrations, the concentration of the majority of odour-causing compounds is usually very low (few $mg \cdot L^{-1}$ or $\mu g \cdot L^{-1}$ in water, and $\mu g \cdot m^{-3}$ in air) and their odour threshold concentrations (OTCs) are frequently some orders of magnitude below. To determine these compounds, gas chromatography with flame ionisation detection (GC-FID) and gas chromatography coupled to mass spectrometry (GC-MS) are commonly employed [24]. For sulphur-containing compounds, the use of GC with flame photometric detection (GC-FPD) or pulsed flame photometric detection (GC-PFPD) has also been described [21,26,28-33]. In some occasions, these separation techniques are complemented with a parallel olfactometry analysis in order to ascertain the contribution of the detected analytes in the odour perception [15,17,18,33-36]. For example, Kleeberg et al. [37] characterised odorous waste gas emissions from a fat refinery by means of GC-MS/olfactometry. Several odorous compounds were identified and the developed method was proposed for the evaluation of the treatment efficiency of waste gas treatment plants.

The separation techniques described above are sometimes not sensitive enough for the analysis of the odorous compounds present at low concentrations and a concentration step prior to their determination is therefore required.

1.1.1.1 Sampling and enrichment of air samples

As indicated in the previous section, odour-causing compounds are usually present in air at low concentration levels and, consequently, a concentration step is needed previous to the analysis. Figure 1.2 shows the common air sampling methodologies applied when odorous compounds are the target compounds to be analysed. As can be seen, the enrichment of the target compounds can be done in the laboratory after grab sampling and simultaneously with field sampling.

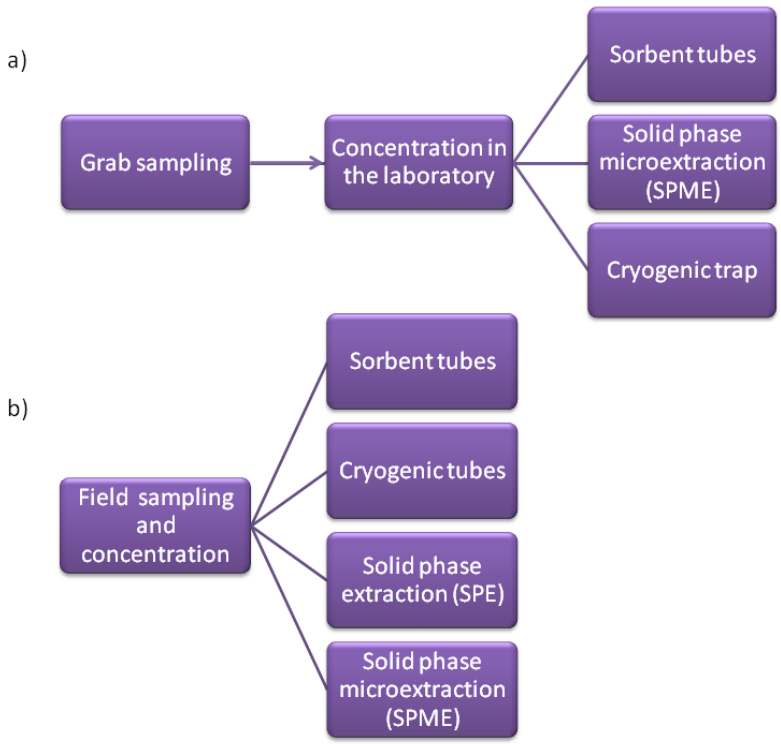


Figure 1.2. Sample enrichment methods in air. a) Concentration in the laboratory after grab sampling, b) simultaneous sampling and concentration on-site.

1.1.1.1.1 Air sampling devices

An adequate sampling device should be selected taking into account different factors such as the target compounds and the sample volume. In all cases, the materials used should be as inert as possible in order to minimize analyte losses and avoid possible reactions with the target compounds. In addition, the containers have to be conditioned prior to use.

Sampling of odour-causing compounds in air can be performed by different ways (Figure 1.3). Some authors have described the use of pre-conditioned and evacuated stainless steel canisters to collect a wide range of odorous compounds [32,38]. Glass sampling bulbs, which should be flushed approximately ten times the sampling volume with the sample in order to displace the air contained inside [39], have been employed to determine sulphur-containing compounds [40]. The use of restriction samplers to collect time integrated air samples has also been described [32], as well as the use of home-made sampling devices [41]. However, among all available sampling containers, sampling bags made of polymers are the most used to collect and transport odorous compounds. Tedlar® and Nalophan® are the most frequent selected materials for this purpose [15,16,18,28-30,35-37,42-46].

Figure 1.3. Examples of some types of containers used for sampling odour-causing compounds: a) Sampling bag, b) glass sampling bulb, c) canister.

1.1.1.1.2 Sample enrichment methods in air

As indicated in Figure 1.2, concentration of odorous compounds in air can be done simultaneously with field sampling. For this purpose, different methodologies such as sorbent tube capture, solid phase microextraction (SPME), cryogenic concentration, and solid phase extraction (SPE) can be found in the literature.

1.1.1.1.2.1 Sorbent tubes

Solid sorbent capture is a reference methodology for the sampling and concentration of air samples [47]. For odour-causing compounds determination, examples can be found in different sites: WWTPs [16,18,38,42,43,48-50], industrial areas [36,37,44,50-53], landfills [15,35], animal production environments [33,41], urban areas [29,51,54], indoor environments [55], and laboratories [51].

Concentration of the samples can be accomplished by active or passive sampling. In the active sampling the air is pumped at a constant fixed flow through a glass or metal tube containing a solid sorbent or a bed of sorbents for a predetermined period of time. Breakthrough studies should be performed in order to select the most appropriate sampling conditions (e.g., sorbents, flow rate and sampling time). In the passive sampling mode, sorbent tubes are

exposed to the air for a long period to obtain time-weighted averages of the target analytes. This methodology is simpler for field sampling as the movement of the analytes through the sorbent is done by diffusion and there is no need for external pumps to get a fixed air flow. As an example, Leach et al. [50] collected monthly average samples from an incinerator, a waste collector centre and a WWTP in order to investigate the occurrence and the temporal and spatial variation of a total of 148 analytes, including a large variety of odorous compounds.

There is a wide range of commercially available sorbents and the selection of the most suitable depends on the physical and chemical characteristics of the target analytes, the sampling time and the sample volume. Multi-sorbent beds are required in order to determine compounds belonging to many different chemical families [18,35,51-53,55,56]. Dincer et al. [35] used glass tubes containing Tenax TA and Carboxen 1000 for the assessment of odorous compounds in landfill air. More than 53 analytes including aldehydes, ketones, volatile fatty acids (VFAs), and compounds belonging to other chemical families were identified and quantified. Ribes et al. [51] reported the use of tubes filled with Carbotrap/Carbopack X and Carboxen-569 for the analysis of alcohols, aldehydes, ketones, terpenes, and other chemicals in industrial, urban and laboratory air. Multi-sorbent beds have also been employed for the determination of compounds belonging to the same chemical family. For instance, Ras et al. [48] developed a methodology based on the use of sorption tubes filled with Tenax TA and Unicarb for the quantification of a group of seven volatile organic sulphur compounds in WWTP air.

1.1.1.1.2.2 Solid phase microextraction

SPME is a solvent-free, simple and efficient procedure introduced by Pawliszyn and his research group in the early 1990s [57], which is based on the equilibrium partitioning of the analytes between an aqueous or gaseous sample and a stationary phase coated on a fused silica fibre (Figure 1.4). It integrates sampling, extraction, concentration, and sample introduction into a single step. As a result of its simplicity, SPME has become an accepted sample preparation technique for the determination of odour-causing compounds in air samples [29,30,37,40-42,45,46,54,58,59].

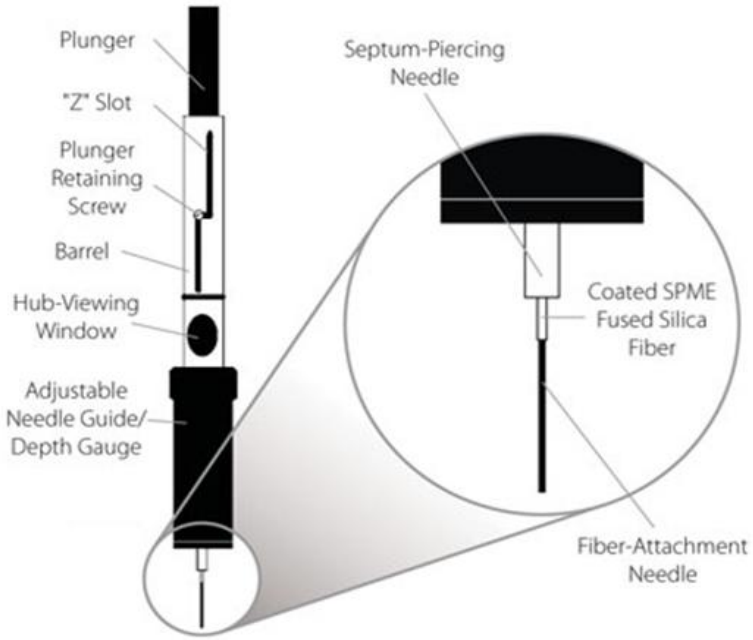


Figure 1.4. Scheme of a conventional SPME fibre [60].

In SPME enrichment, samples are usually collected using sampling containers and concentrated in the laboratory, although nowadays there is a commercial field sampler SPME device commercialised by Supelco [61]. Davoli et al. [45] collected air samples from a landfill using Nalophan® bags and concentrated them by means of SPME. The sampling bag was pierced with the needle of the SPME device and exposed in the bag for 30 min at room temperature. SPME concentration in combination with sampling in glass bulbs has also been described for the determination of a group of sulphur-containing compounds in air samples from a WWTP [40].

Among all the available coatings (Table 1.2), Carboxen/polydimehtylsiloxane (CAR/PDMS) has shown to be the most common for the analysis of odorous compounds in air samples [29,30,37,40-42,46]. Razote et al. [41] used this coating for the determination of a group of odorous compounds including VFAs, phenol and nitrogen-containing compounds in air from animal production environments. Lastremau et al. [30] tested three different coatings (polydimethylsiloxane –PDMS-, divinylbenzene/polydimethylsiloxane -DVB/PDMS- and carboxen/polydimethylsiloxane -CAR/PDMS-) and found that CAR/PDMS was the most suitable for the quantification of sulphur-containing compounds in industrial air. However, the determination of sulphur compounds using this type of SPME fibre has been found to be limited due to the formation of artefacts during the analyses [46]. Moreover, Davoli et al. [45]

demonstrated the efficacy of the DVB/CAR/PDMS fibre for the determination of a group of odour-causing compounds including sulphur compounds, VFAs, phenols, aldehydes, ketones, terpenes, and other chemicals in landfills air.

Table 1.2. Characteristics of the commercially available SPME fibre coatings [62,63]. PDMS: poly(dimethylsiloxane), PA: polyacrylate, DVB: divinylbenzene, CAR: carboxen, CW: carbowax, TR: templed resin.

Fibre coating	Film thickness (μm)	Applications
PDMS	100	Volatiles (MW 60-275)
	30	Non-polar semi-volatiles (MW 80-500)
	7	Non-polar high molecular weight compounds (MW 125-600)
PA	85	Polar semi-volatiles (MW 80-300)
PDMS/DVB	65	Volatiles, amines and nitro-aromatic compounds (MW 50-300)
	60	Amines and polar compounds (HPLC use only)
CAR/PDMS	75, 85	Gases and low molecular weight compounds (MW 30-225)
DVB/CAR/PDMS	50/30	Flavour compounds: volatiles and semi-volatiles, C3-C20 (MW 40-275)
CW	60	Alcohols and polar compounds (40-275)
CW/DVB	65	Polar organic compounds such as alcohols, ketones and nitroaromatics
CW/TR	50	Anionic surfactants and aromatic amines

Some methodologies based on analyte derivatization during the SPME procedure have also been described [58,59]. Derivatization is performed when the target analytes cannot be directly determined due to their chemical and physical characteristics (e.g., low volatility for GC analysis). The derivatizing agent can be anchored to the fibre before the extraction of the analytes from the sample or it can be directly exposed to the analytes adsorbed into the fibre after the sample extraction. For that, the fibre has to be exposed to the headspace of a high concentration of the derivatizing agent. Afterwards, the derivatised compounds are thermally desorbed in the injection port of the GC. As an example, Gómez Alvarez et al. [58] developed an on-fibre derivatization method for the determination of a group of carboniles in air matrices using O-(2,3,4,5,6)-pentafluorobenzyl-hydroxylamine hydrochloride (PFBHA) as a derivatizing agent.

1.1.1.1.2.3 Cryogenic concentration

In cryogenic concentration methods, the enrichment of the sample is accomplished by circulating an air flow through a tube, usually filled with glass beads, cooled in order to condense the target compounds. This technique has been applied to the determination of odorous compounds in a wide range of air matrices such as wastewater [38,43], landfill [15], industrial [44,53] and urban air [31], and animal production environments [32].

Cryogenic concentration has been used in combination with canister sampling. Wu et al. [38] collected WWTP air in stainless steel canisters and determined a wide range of substances including odorous compounds such as oxygenated and sulphur compounds. The proposed method provided limits of detection (LODs) in the low $\mu g \cdot m^{-3}$ level for most of the analytes. Trabue et al. [32] developed a canister field sampling and analysis method that allowed the quantification of sulphur-volatile compounds at $\mu g \cdot m^{-3}$ levels in animal production environments.

The combination of cryogenic concentration with sampling in polymeric bags has also been reported. Zarra et al. [43] described a cryogenic concentration method for the odour monitoring of a WWTP. Sampling was done in the field with Nalophan® bags and the samples were concentrated in the laboratory using a Tenax TA. Thirty-nine substances, including sulphur-containing compounds, terpenes, VFAs, aldehydes, ketones, and other chemicals, were detected at maximum concentrations in the low $mg \cdot m^{-3}$. Capelli et al. [15] developed an analytical method based on sampling in Nalophan® bags for the quantification of landfill odorous emissions. The proposed method allowed the quantification of different chemical families such as oxygenated, nitrogen-containing, and sulphur-containing compounds.

Sampling and concentration can sometimes take place in a single step by using collector tubes immersed in a freezing liquid (e.g. nitrogen) during the sampling process. These tubes are then heated using a warm bath in order to volatilise the analytes and transfer them into the instrument port. Filipy et al. [53] used cryogenic tubes to collect a group of 39 volatiles, including alcohols, amines, sulphur-containing compounds, and others from a dairy. Campos et al. [31] proposed a cryogenic capture based method for the determination of reduced sulphur compounds in air samples. The air was pumped through cryogenic collectors which were immersed in a freezing liquid inside a sampling mobile unit (Figure 1.5). They optimised the sampling parameters, validated the proposed method and successfully applied it to the analysis of urban areas.

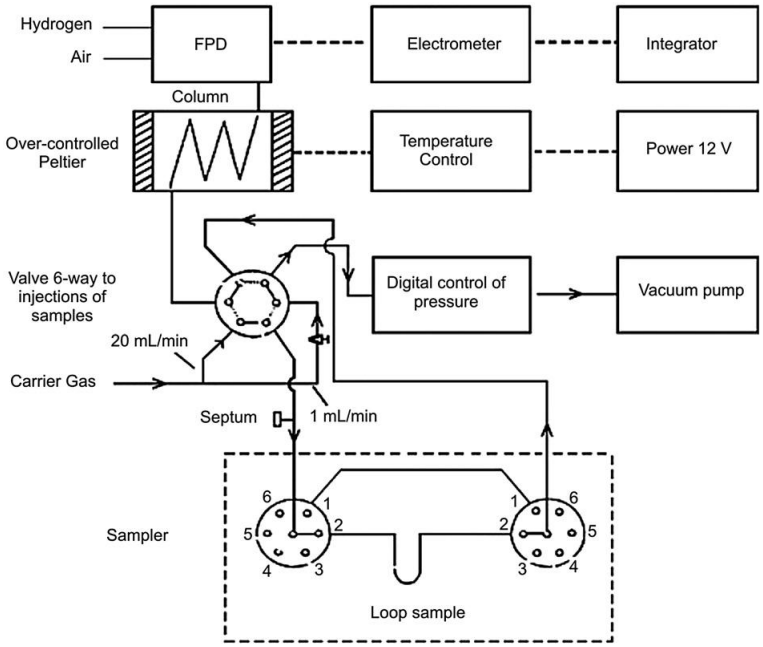


Figure 1.5. Schematic representation of the chromatographic system used by Campos et al. [31]. The sampling mobile unit containing the collector tubes can be observed at the bottom.

Although cryogenic concentration has been applied to the determination of odorous compounds in air samples, this technique is not recommended as the concentration of analytes is performed at very low temperatures (usually between -100°C and -180°C) and substantial amounts of water can be trapped (around 18 μl of water for 1 L of sample at 70% relative humidity at 25°C), which can cause interferences, degrade performance of detectors and block the system.

1.1.1.1.2.4 Solid phase extraction

SPE is a concentration technique commonly used in the analysis of semi-volatile organic compounds in aqueous samples [2,64,65]. Only few applications are found in the literature for the enrichment of odour-causing compounds in air. For that, the air sample is pumped through a cartridge containing an adequate packaging for a short period of time and analytes are transferred from the sample to the sorbent. As a final step, the sorbent is treated with a suitable organic solvent to elude the target compounds. Cháfer-Pericás et al. [66] developed a methodology for the determination of trimethylamine in air using C_{18} cartridges. They obtained a LOD of 0.22 $mg \cdot m^{-3}$, which was found to be comparable with those reported in the literature [67], but the sample volume required was significantly reduced. The sampling

efficiency of C_{18} SPE cartridges has been also evaluated for a mixture of primary amines [68]. The authors obtained LODs in the range of $mg \cdot m^{-3}$ and proposed the use of SPE cartridges as a rapid and simply alternative for air sampling and concentration.

1.1.1.2 Sample enrichment methods in water

As in air samples, the presence of odorous compounds at low concentrations makes necessary a concentration of liquid samples prior to their analysis. Only few studies have been reported dealing with the determination of nitrogen-containing compounds by direct injection into a gas chromatograph port [21,26]. Looking at the literature, microextraction based methods are the most commonly used for the enrichment of odour-causing compounds in water samples.

1.1.1.2.1 Liquid-liquid extraction (LLE)

LLE is a traditional extraction and concentration technique based on the transfer of analytes from an aqueous sample to a water immiscible organic solvent. Despite the drawbacks of this technique (time consuming, use of a high amount of organic solvents and possible losses of analytes), some studies can still be found in the literature dealing with its use for the analysis of odorous compounds. Nawrocki et al. [69] developed a method based on the derivatization of carbonyl compounds from bottled water. They used O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBOA) as a derivatizing agent in order to form oximes and extracted them with hexane. Ventura et al. [70] applied a method based on an extraction with dichloromethane to determine a wide variety of odour-causing compounds in river water. Another application of LLE is the one reported by Hwang et al. [21], who effectively extracted indole and skatole from influent and primary effluent wastewater samples using *n*-hexane.

1.1.1.2.2 Closed loop stripping analysis (CLSA)

In CLSA, the solution to be analysed is bubbled with air or nitrogen gas and the analytes transferred into the air stream are captured on a carbon trap. The air is recirculated through the solution in a closed air loop and, after a period of time, the carbon trap is removed and extracted with an adequate solvent. Escalas et al. [71] applied a CLSA method to the monitoring of a group of 47 compounds belonging to four different chemical families (aromatic compounds, terpenes, sulphur-containing compounds, and chlorinated compounds) in

wastewater. Most of the determined compounds were detected in the samples at levels of $\mu g \cdot L^{-1}$. Ginzburg et al. [34] identified oligosulphide compounds in water samples from a lake. The developed method also allowed the quantification of other odorous compounds such as aldehydes, terpenes, VFAs, and nitrogen-containing compounds at $ng \cdot L^{-1}$. Espadaler et al. [72] applied a CLSA method for the identification and quantification of hydrocarbons and aldehydes in river samples. The proposed method allowed the detection of the target compounds at concentrations of few $\mu g \cdot L^{-1}$. Ventura et al. [70] compared the LLE method described in the previous section with a CLSA method for the determination of a group of 45 odour-causing compounds in river samples. They found that the LLE method was more suitable for the individual characterisation of the analytes.

1.1.1.2.3 Microextraction based techniques

SPME methods have been applied to the analysis of a wide range of odour-causing compounds in water matrices [73-76]. Ábalos et al. [73] developed a SPME method for the quantification of five alkyl sulphides in water samples. After the method was optimised and validated, they applied it to the analysis of wastewaters and detected the target compounds at levels of $\mu g \cdot L^{-1}$. Pan et al. [74] determined a group of seven primary amines in wastewaters using a method based on 2,3,4,5,6-pentafluorebenzylaldehyde (PFBAY) derivatization coupled to SPME. They observed that the obtained LODs, which were in the low $\mu g \cdot L^{-1}$ to the high $ng \cdot L^{-1}$ range, were significantly lower than those obtained without the derivatization step. Herráez-Hernández et al. [75] applied an SPME method with on-fibre derivatization using 9-fluorenyl-methylchloroformate (FMOC) as a derivatizing agent to determine methylamine in wastewaters. They compared the proposed method with a solid support-assisted derivatization method with C_{18} SPE cartridges which had previously been applied to determine short-chain aliphatic amines [77] and found that both of them provided comparable accuracy and precision, but the sample handing was significantly reduced with the SPME method. Two analytical HS-SPME methods to determine VFAs in WWTP water samples have also been reported [78,79], both of them with LODs at $\mu g \cdot L^{-1}$ levels.

Next to these SPME methods, alternative techniques have been developed in recent years. Solvent bar microextraction (SBME), i.e. extraction using an organic solvent sandwiched in a hollow fibre, has been successfully applied to the quantification of trace levels of aliphatic amines in well water samples [80]. Solid phase dynamic extraction (SPDE), which is based on the extraction of VOCs from gaseous or liquid samples using a sorbent coated in the internal wall of a steel needle (Figure 1.6), has been employed for the analysis of a group of aldehydes and other volatiles in melted snow water [81]. All the extraction and desorption parameters were optimised and LODs in the lower $ng \cdot L^{-1}$ level were obtained.

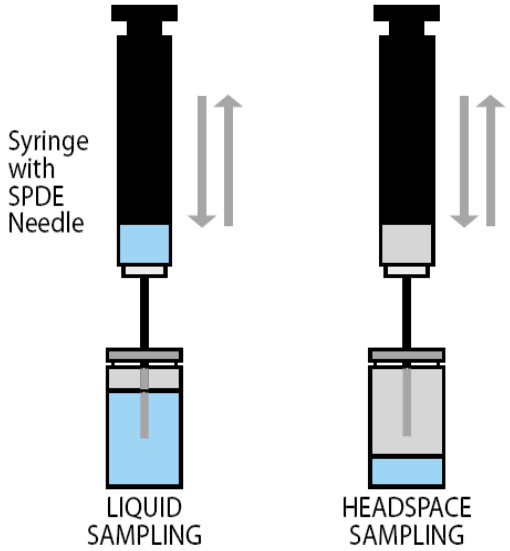
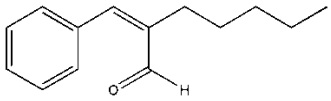
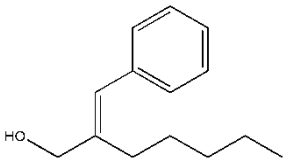
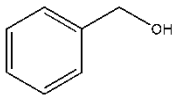
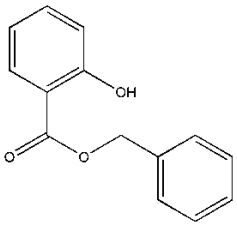
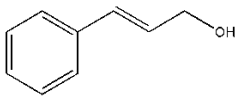
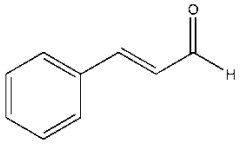
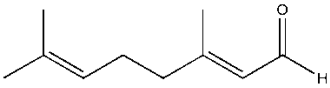
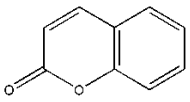
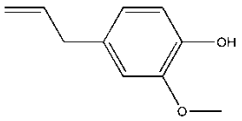


Figure 1.6. Scheme of the SPDE procedure [82]. The analytes are concentrated onto the phase by repeatedly moving the plunger up and down.

1.2 FRAGRANCES

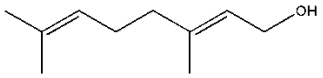
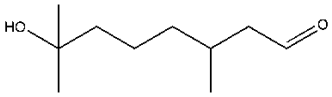
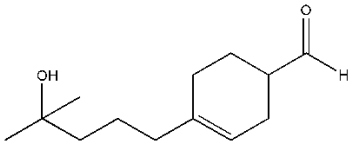
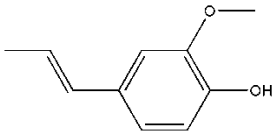
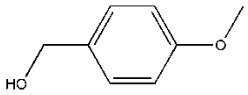
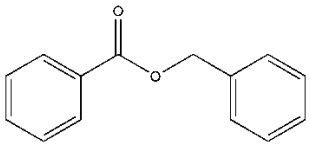
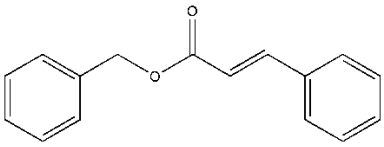
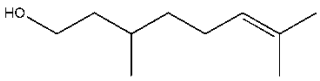
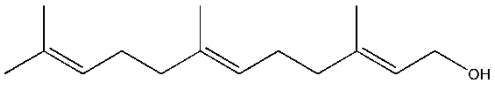
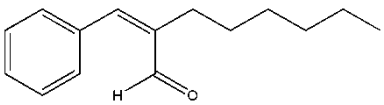
Fragrances are a common ingredient of PCPs, usually added to soaps, detergents, cosmetics, and other consumer products in order to mask the odour of other chemical ingredients and give the consumer a pleasing sensation. The Scientific Committee on Consumer Safety (SCCS) [83] has elaborated a list of 26 fragrance compounds that have been identified as likely to cause contact allergies. As can be seen in Table 1.3, this group of compounds include alcohols, aldehydes, terpenes, and esters. Moreover, two natural extracts (oak and tree moss, not depicted in Table 1.3) can also be found in the SCCS list.

Table 1.3. Fragrances included in the SCCS list [83], with their chemical structures and main properties.

Compound	IUPAC name	Structure	M.W. ($g \cdot mol^{-1}$)	Henry's law constant[a] ($atm \cdot m^3 \cdot mol^{-1}$)
Fragrance chemicals, which according to existing knowledge, are most frequently reported and well-recognised consumer allergens				
Amyl cinnamal	2-phenylmethylene-heptanal		202	$1.0 \cdot 10^{-5}$
Amyl cinnamyl alcohol	2-phenylmethylene-1-heptanol		204	$7.7 \cdot 10^{-7}$
Benzyl alcohol	Benzene methanol		108	$2.2 \cdot 10^{-7}$
Benzyl salicylate	2-hydroxy-phenylmethyl ester benzoic acid		228	$3.7 \cdot 10^{-7}$
Cinnamyl alcohol	3-phenyl-2-propen-1-ol		134	$1.6 \cdot 10^{-7}$
t-Cinnamaldehyde	3-phenyl-2-propenal		132	$1.6 \cdot 10^{-6}$
Citral	3,7-dimethylocta-2,6-dienal		152	$3.8 \cdot 10^{-4}$
Coumarin	2H-1-benzopyran-2-one		146	$6.9 \cdot 10^{-6}$
Eugenol	2-methoxy-4-prop-2-enyl-phenol		164	$4.8 \cdot 10^{-8}$

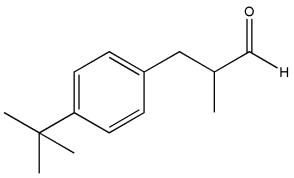
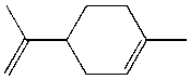
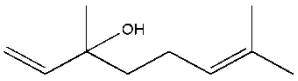
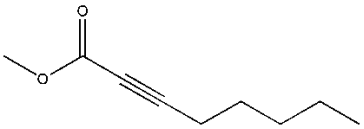
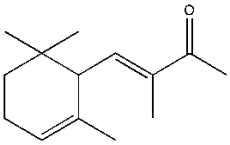
[a] Calculated with the EPI Suite™ v4.11 computer program [84].

Table 1.3 ***(continued)*****.** Fragrances included in the SCCS list [83], with their chemical structures and main properties.

Compound	IUPAC name	Structure	M.W. ($g \cdot mol^{-1}$)	Henry's law constant[a] ($atm \cdot m^3 \cdot mol^{-1}$)
Geraniol	3,7-dimethyl-2,6-octadien-1-ol		154	$5.9 \cdot 10^{-5}$
Hydroxycitronellal	7-hydroxy-3,7-dimethyloctanal		172	$2.4 \cdot 10^{-8}$
Lyral	4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde		210	$2.6 \cdot 10^{-8}$
Isoeugenol	2-methoxy-4-(1-propenyl)-phenol		164	$2.7 \cdot 10^{-8}$
Fragrance chemicals which are less frequently reported and thus less documented as consumer allergens				
Anisyl alcohol	4-methoxy-benzene methanol		138	$1.3 \cdot 10^{-8}$
Benzyl benzoate	2-hydroxy-phenylmethyl ester benzoic acid		212	$2.8 \cdot 10^{-6}$
Benzyl cinnamate	3-phenyl phenylmethyl ester-2-propenoic acid		238	$3.3 \cdot 10^{-7}$
β-Citronellol	3,7-dimethyloct-6-en-1-ol		156	$5.7 \cdot 10^{-5}$
Farnesol	3,7,11-trimethyldodeca-2,6,10-trien-1-ol		222	$2.5 \cdot 10^{-4}$
Hexyl cinnamaldehyde	2-phenylmethylene-octanal		216	$1.0 \cdot 10^{-5}$

[a] Calculated with the EPI Suite™ v4.11 computer program [84].

Table 1.3 ***(continued)*****.** Fragrances included in the SCCS list [83], with their chemical structures and main properties.

Compound	IUPAC name	Structure	M.W. ($g \cdot mol^{-1}$)	Henry's law constant[a] ($atm \cdot m^3 \cdot mol^{-1}$)
Fragrance chemicals which are less frequently reported and thus less documented as consumer allergens				
Lilial	3-(4-tert-butylphenyl)-2-methylpropanal		204	$2.5 \cdot 10^{-5}$
d-Limonene	1-methyl-4-prop-1-en-2-yl-cyclohexene		136	$3.8 \cdot 10^{-1}$
Linalool	3,7-dimethylocta-1,6-dien-3-ol		154	$4.2 \cdot 10^{-5}$
Methyl-2-octynoate	methyl ester 2-octynoic acid		154	$1.0 \cdot 10^{-4}$
Ionone	4,-(2,6,6-trimethyl 2-cyclohexen-1-yl)-3-methyl-3-buten-2-one		206	$2.8 \cdot 10^{-4}$

[a] Calculated with the EPI Suite™ v4.11 computer program [84].

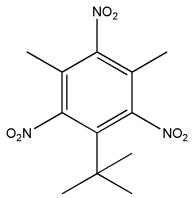
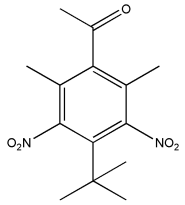
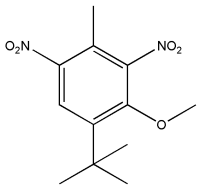
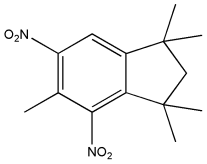
The presence of these compounds in cosmetic products must be indicated on the label of the final product if a limit of 0.01 % for rinse-off and 0.001 % for leave-on products is exceeded (Regulation EC No 1223/2009). Some of these substances have a direct impact on the skin, eyes and mucous membranes. Moreover, the natural barrier of the skin can be broken by other detergents present in consumer products, allowing allergens and other chemicals to penetrate it. Some fragrances have also been related to other effects on human health. Benzyl salicylate, benzyl benzoate and lilial have been reported to possess oestrogenic activity [85]. Furthermore, some nervous and brain system effects have been attributed to coumarin [86].

With regards to musk fragrances, they comprise a broad range of different compounds, including polycyclic, nitro and macrocyclic musks (Table 1.4). The first nitro musk compound

(call *Musk Baur*) was prepared by Albert Baur in 1888 when he synthesised the t-butyl derivative of the explosive compound 2,4,6-trinitrotoluene (TNT) in an attempt to produce a more effective form of this explosive [87].

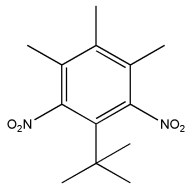
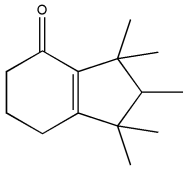
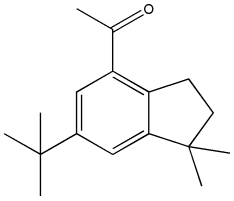
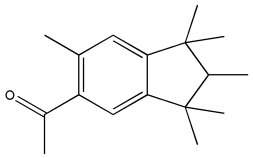
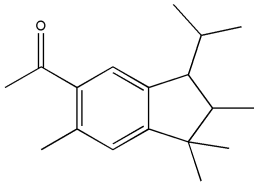
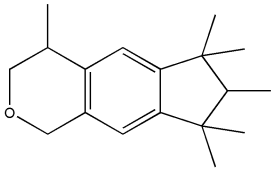
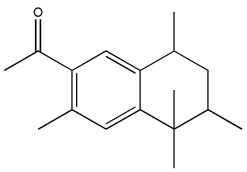
Some toxicological problems related to the presence of a nitroaromatic compound in the structure of nitro musks [88] and their bioaccumulation potential [89,90] have led to a decrease in their use. As a consequence, another group of musk fragrances, called polycyclic musk, was developed in the 1950s and 60s. Since then, this group of musks has become the most commonly used, especially galaxolide and tonalide, which have been included on the Environmental Protection Agency's (EPA) high production list [91]. Macrocyclic musks are not as widely used as policyclyc musks because of their high synthesis cost.

Table 1.4. Chemical structure and main properties of musk fragrances.

Compound	IUPAC name	Structure	M.W. ($g \cdot mol^{-1}$)	Henry's law constant[a] ($atm \cdot m^3 \cdot mol^{-1}$)
Nitro musk fragrances				
Musk xylene	2,4,6-trinitro-1,3-dimethyl-5-tertbutylbenzene		297	$1.0 \cdot 10^{-9}$
Musk ketone	4-aceto-3,5-dimethyl-2,6-dinitro-tertbutylbenzene		294	$4.8 \cdot 10^{-10}$
Musk ambrette	1-tert-butyl-2-methoxy-4-methyl-3,5,-dinitrobenzene		268	$1.4 \cdot 10^{-8}$
Musk moskene	1,1,3,3,5-pentamethyl-4,6-dinitroindan		278	$2.1 \cdot 10^{-7}$

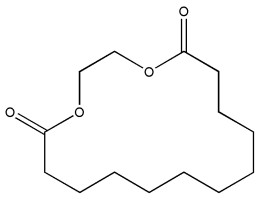
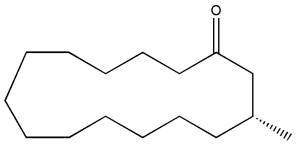
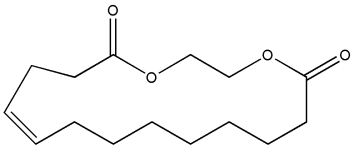
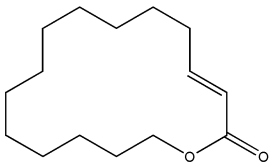
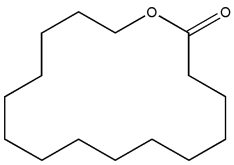
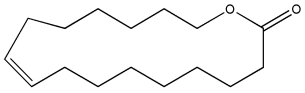
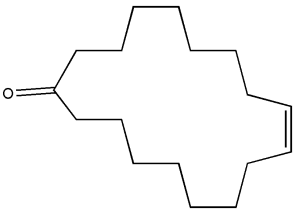
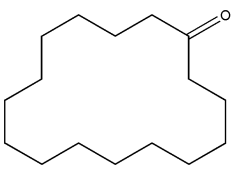
[a] Calculated with the EPI Suite™ v4.11 computer program [84].

Table 1.4 ***(continued)*****.** Chemical structure and main properties of musk fragrances.

Compound	IUPAC name	Structure	M.W. ($g \cdot mol^{-1}$)	Henry's law constant[a] ($atm \cdot m^3 \cdot mol^{-1}$)
Polycyclic musk fragrances				
Musk tibetene	1-tert-butyl-3,4,5-trimethyl-2,6-dinitrobenzene		266	$2.9 \cdot 10^{-7}$
Cashmeran	6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone		206	$1.4 \cdot 10^{-4}$
Celestolide	4-acetyl-1,1-dimethyl-6-tertbutylindane		244	$3.2 \cdot 10^{-5}$
Phantolide	6-acetyl-1,1,2,3,3,5-hexamethylindane		244	$3.2 \cdot 10^{-5}$
Traesolide	5-acetyl-1,1,2,6-tetramethyl-3-isopropyl-indane		258	$4.2 \cdot 10^{-5}$
Galaxolide	1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(γ)-2-benzopyran		258	$1.3 \cdot 10^{-4}$
Tonalide	7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphtalene		258	$4.22 \cdot 10^{-5}$

[a] Calculated with the EPI Suite™ v4.11 computer program [84].

Table 1.4 ***(continued).*** Chemical structure and main properties of musk fragrances.

Compound	IUPAC name	Structure	M.W. ($g \cdot mol^{-1}$)	Henry's law constant[a] ($atm \cdot m^3 \cdot mol^{-1}$)
		Macrocyclic musk fragrances		
Musk MC4	Ethylenedodecanedioate		255	$2.4 \cdot 10^{-6}$
Muscone	3-Methylcyclopentadecanone		238	$8.7 \cdot 10^{-4}$
Musk-NN	Ethylenetridecanedioate		270	$3.1 \cdot 10^{-6}$
Habanolide	Oxacyclohexadecen-2-one		238	$2.3 \cdot 10^{-3}$
Exaltolide	Oxacyclohexadecan-2-one		240	$2.3 \cdot 10^{-3}$
Ambrettolide	Oxacycloheptadec-8-en-2-one		252	$2.7 \cdot 10^{-3}$
Civetone	9-Cycloheptadecen-1-one		250	$1.0 \cdot 10^{-3}$
Exaltone	Cyclopentadecanone		224	$6.6 \cdot 10^{-4}$

[a] Calculated with the EPI Suite™ v4.11 computer program [84].

Due to their wide use in daily products, fragrances are continuously introduced into the environment, mainly via WWTP effluents, where they have been detected at concentration levels ranging from several ng L^{-1} to µg L^{-1} [1,3,5,11,71,92-103]. They have also been found in rivers [1,3,95-98,100,103,104], lakes [1,97] and reservoirs [1]. In the case of the polycyclic musks, due their high lipophilicity and slow biodegradation rates, they are also present in sediments [105], sludge [8,106] and muscle tissue [107].

1.2.1 DETERMINATION OF FRAGRANCES IN WATER SAMPLES

As indicated in the previous section, fragrances are usually detected in water samples at very low concentration levels. Thus, the use of sensitive methodologies is required for their determination. While their analysis is mainly performed by GC-MS [102,108], many different methodologies have been described for their concentration, being SPME the most used technique [95,102,104,109-114].

1.2.1.1 Solid phase microextracion

SPME has been applied for fragrance analysis in wastewater [95,102,109-113], baby bathwater [102,114], surface [95,104,109], and swimming pool water [102]. In SPME, the selection of an adequate coating is fundamental. Lamas et al. [114] tested five different SPME coatings (PDMS, PDMS/DVB, DVB/CAR/PDMS, CAR/PDMS, and PA) for the extraction of 15 common fragrance allergens from baby bathwater. They found that PDMS/DVB and DVB/CAR/PMDS were the coatings which gave the best results, but the latter was not adequate for the extraction of limonene and coumarin. PDMS/DVB was therefore selected for the simultaneous determination of the target compounds. The use of the PDMS/DVB coating in fragrance analysis has also been reported by other authors [95,102,104,109,110,112]. Winkler et al. [104] compared four different fibres (DMS/DVB, PA, CAR, and PDMS) and observed again that PDMS/DVB was the one which gave better recoveries in the analysis of musk fragrances. Using this fibre, they optimised and validated the method and finally applied it to the determination of the studied musk compounds in river water samples. Becerril et al. [102] also used PDMS/DVB coating for the determination of 24 suspected allergens in bathwater, swimming pool water and wastewater samples. The developed method allowed the quantification of the analytes at levels of few µg·L^{-1}. In the determination of musk compounds, other coatings have also been used. Basaglia et al. [113] employed a PA fibre for the simultaneous determination

of pharmaceuticals and PCPs, including tonalide and galaxolide, in wastewaters. García-Jares et al. [111] compared four different fibres (PDMS, PDMS/DVB, CAR/PDMS, and CW/DVB) for the quantification of musk fragrances in wastewaters and found that both CAR/PDMS and PDMS/DVB coatings were adequate for the analysis, but a slight matrix effect was observed for the latter.

1.2.1.2 Stir bar sorptive extraction (SBSE)

SBSE has been proposed as an alternative to SPME since the concentration capacity is enhanced as the amount of sorbent used in SBSE is higher. This technique has mainly been applied to the determination of musk fragrances. Silva et al. [96] used a SBSE method to quantify four musk compounds in different types of water matrices (river, sea, tap, and wastewater) at $ng \cdot L^{-1}$ levels. Ramirez et al. [92] developed a method based on SBSE coupled with thermal desorption (TD)-GC-MS for the quantification of nine synthetic musks in wastewaters and river water. The proposed method, with a limited manipulation of the sample, provided LODs at low $ng \cdot L^{-1}$ level. Pintado-Herrera et al. [115] developed a multi-residue method based on the combination of SBSE and pressurized hot water extraction (PHWE) for the analysis of pharmaceuticals and some PCPs, including galaxolide, in wastewater, sea and pore water. The authors proposed the developed methodology as more environmentally friendly than other traditional and widely used techniques such as Soxhlet extraction and SPE. Santiago-Morales et al. [116] applied a SBSE method to the study of the photochemical and oxidative degradation of tonalide and galaxolide in wastewaters. Moreover, studies based on the use of SBSE for the simultaneous determination of suspected allergens and musks in wastewaters can also be found in the literature [117].

1.2.1.3 Solid phase extraction

SPE methods have mostly been used for the simultaneous analysis of fragrances and other PCPs. As an example, Lee et al. [100] applied a SPE method to evaluate the occurrence of a wide variety of organic compounds (e.g., musk fragrances, flame retardants, herbicides, and others) in river water samples. Most of the studied analytes were found to be present in the samples at $\mu g \cdot L^{-1}$ level. Furthermore, some SPE based methods have been described for the removal study of these compounds from WWTPs [118-120].

Different types of SPE cartridges have been employed. The use of polymeric SPE cartridges was reported by Reyes-Contreras et al. [8], who evaluated the removal of a group of pharmaceuticals, musks and other PCPs from wastewaters. Chase et al. [1] compared a SPE method based on the use of C_{18} SPE disks and a SBSE method in the determination of musk compounds in different water matrices. Limits of quantification (LOQs) obtained for the SPE method were found to be lower than those obtained for the SBSE method.

1.2.1.4 Liquid-liquid extraction

The use of LLE has been described for the analysis of musk compounds in different water matrices. Lee et al. [98] developed a LLE method based on an extraction with dichloromethane to determine musk fragrances in wastewaters and surface waters. Teijon et al. [5] extracted musk compounds and other water contaminants from treated wastewater and groundwater using hexane. Bester et al. [121] investigated the presence and transformation of galaxolide and tonalide along a river. Samples were extracted with toluene and musks at $ng \cdot L^{-1}$ levels were detected. Stackelberg et al. [3] reported the application of a continuous liquid-liquid extraction (CLLE) method to the evaluation of the persistence of several wastewater-related compounds including fragrances, insecticides, flame retardants, and other chemicals in a conventional WWTP.

1.2.1.5 Closed loop striping analysis

Although CLSA is not commonly used to concentrate fragrances in water samples, some studies can be found in the literature. Mitjans et al. [122] applied a CLSA based method for the determination of musks in wastewaters, tap water, surface water, and rivers. The proposed method was found to be suitable for the analysis of musks at trace levels, as LODs of $ng \cdot L^{-1}$ were obtained. Furthermore, Romero et al. [123] determined a group of compounds including surfactants, antioxidants, musks, and other classes of chemicals in wastewaters by means of a CLSA method which allowed the quantification of the target analytes at $ng \cdot L^{-1}$ levels.

1.2.1.6 New sample enrichment methods

Recently, new extraction procedures have been applied to the analysis of fragrances in water samples in order to simplify the sample treatments, making them less tedious and avoiding the use of high quantities of organic solvents. This group of novel techniques include dispersive liquid-liquid microextraction (DLLME), ultrasound-assisted emulsification-microextraction (USAEME) and microextraction by packed sorbent (MEPS) [101,124-127].

1.2.1.6.1 Dispersive liquid-liquid microextraction

DLLME is based on the dispersion of tiny droplets of the extraction liquid within an aqueous solution containing the target analytes (Figure 1.7). This extraction technique has been applied to the determination of both suspected allergens and musk fragrances. Tsiallou et al. [124] developed a DLLME based method and applied it to the determination of 21 suspected allergens in tap water, baby bathwater, wastewater, and water from a recreational and public washing place. The proposed methodology gave adequate relative standard deviation (RSD) values (4-16%) and the obtained LODs were found to be in the low $\mu g \cdot L^{-1}$ level. Yang et al. [125] determined musk fragrances in surface waters and WWTP effluents by means of an ultrasound assisted (UA)-DLLME concentration. The authors obtained LODs at the $ng \cdot L^{-1}$ level and proposed the developed method as a good alternative extraction method for the assessment of organic compounds in water samples.

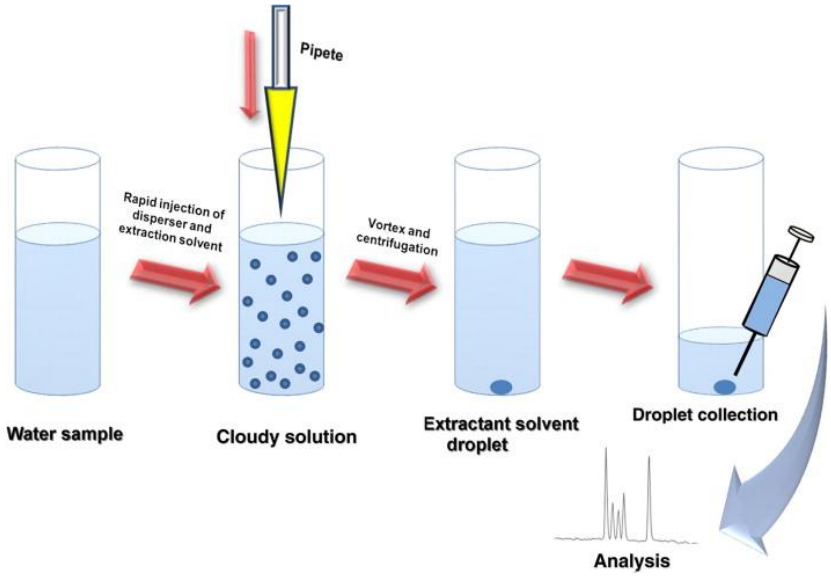


Figure 1.7. Scheme of the DLLME procedure [128].

1.2.1.6.2 Ultrasound-assisted emulsification-microextraction

USAEME is based in the same principle than DLLME but, in USAEME, the dispersion of the extraction liquid and the consequent formation of a cloudy solution are performed by the application of ultrasonic radiation. The only application of this technique found in the literature is the one reported by Becerril-Bravo et al. [101], who optimised the USAEME process (solvent, time, temperature and the NaCl percentage) by a multivariate study. The authors validated the optimised method and finally applied it to the quantification of 25 fragrance allergens at $\mu g \cdot L^{-1}$ level in baby bathwater, swimming pool and spa water, public clothes washing place water, and wastewater.

1.2.1.6.3 Microextraction by packed sorbent

In the MEPS procedure, the extraction and concentration of the analytes is performed in a sorbent cartridge integrated into a microlitre syringe (Figure 1.8). Moeder et al. [126] applied this technique to the determination of UV filter and polycyclic musk compounds in wastewaters and obtained LODs in the medium $ng \cdot L^{-1}$ to low $\mu g \cdot L^{-1}$ range. Cavalheiro et al. [127] quantified musk compounds in environmental waters and the obtained LODs were found to be in the same range.

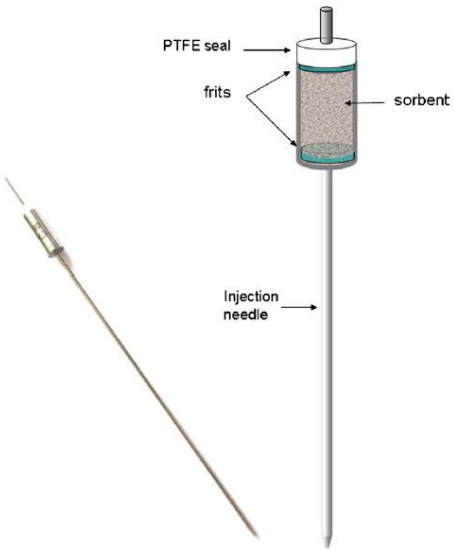


Figure 1.8. Scheme and picture of a MEPS syringe [126].

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CHAPTER 2: Objectives

One of the major problems of WWTPs is the generation of odours, which has been a cause of concern for the population living near these plants during the last decades. Moreover, the presence of some pollutants such as fragrances in wastewaters, as well as their incomplete elimination and subsequent accumulation in the environment, is also a topic of increasing interest. Taking into account these considerations, the main objective of this thesis is **to develop analytical methodologies for the assessment of odorous and fragrance compounds in water and air samples from WWTPs.** This objective can be divided in more specific and detailed aims:

1. To develop a HS-SPME method for the determination of a group of odour-causing compounds belonging to different chemical families in water samples from WWTPs.

2. To develop different methods for the assessment of a group of odour-causing compounds belonging to different chemical families in air samples from WWTPs and determine their gas-liquid partition coefficients to explain their presence in the air samples:

    - Development of a method based on SPME as a concentration technique.
    - Development of a method based on the concentration by means of active sampling on multibed sorbent tubes.

3. To develop a HS-SPME method for the determination of fragrances in water samples, aimed to the monitoring of the target fragrances in WWTPs, and evaluate the efficacy of two conventional WWTP tertiary treatments (UV and chlorination) for the fragrance removal from water.

CHAPTER 3:

Odour-causing compounds in wastewater treatment plants: Evaluation of solid-phase microextractinon as a concentration technique

3.1 INTRODUCTION

Odorous emissions from wastewater collection systems and treatment facilities represent a problem that has affected citizens for decades [1,2]. Odour emissions affect quality of life, leading to psychological stress and symptoms such as insomnia, loss of appetite and irrational behaviour [3]. As a consequence of the poor public image of WWTPs, public concern and complaints have been increasing in recent years.

Solid sorbent capture followed by GC determination is commonly the technique of choice when VOCs are investigated in air samples [4-8]. Traps with more than one sorbent material are used to facilitate quantitative retention and desorption of VOCs over a wide range of compounds. Dincer et al. [2] collected samples from the headspace of tanks located in WWTP units and sludge management areas with a multi-bed trap packed with Tenax TA and Carboxen 1000. They identified 29 compounds belonging to four different types of chemicals (sulphur-containing compounds, aldehydes, monoaromatics and halogenated compounds). A method for the determination of volatile organic sulphur compounds (VOSCs) in air from sewage management plants in Tarragona and Reus (Spain) has also been developed [7]. A trap of Tenax TA and Unicarb was used and seven VOSCs (ethyl mercaptan, dimethyl sulphide, carbon disulphide, propyl mercaptan, butyl mercaptan, dimethyl disulphide, and 1-pentantehiol) were detected and quantified.

The presence of odour compounds can be investigated directly in water and wastewater samples. In such cases, purge and trap and closed-loop stripping methods have been applied to concentrate VOCs [3,8,9]. Since the introduction by Pawliszyn and his research group of SPME as a sample preparation technique [10], it has become an accepted method for the determination of volatile and semi-volatile substances in wastewater and air samples. Kleeberg et al. [11] analysed waste gas from a fat refinery using SPME. The fibre was exposed to the sample, collected in a sampling bag at ambient temperature and a total of 56 substances including aldehydes, terpenes and esters were identified. A procedure based on the application of CAR/PDMS fibre for the extraction and concentration of a group of seven VOSCs (ethyl mercaptans, dimethyl sulphide, carbon disulphide, propyl mercaptans, butyl mercaptans, dimethyl disulphide, and 1-pentanethiol) in air samples from a sewage treatment plant has also been developed [12]. In this case, target analytes were extracted in glass bulbs used for field sampling of air. Pan et al. [13] determined amines in air and water using derivatisation combined with SPME, being *p*-nitrophenyl trifluoroacetate (NPTFA) and PFBAY the derivatising reagents. As for aqueous samples, Tsai et al. [14] applied a method based on

HS-SPME using on-fibre derivatisation with PFBHA for the analysis of aldehydes in water. Ábalos et al. [15] developed a method based on HS-SPME for the determination of volatile sulphides and disulphides in wastewaters. Huang et al. [16] analysed amines in wastewater samples by means of HS-SPME technique using a PDMS fibre. Furthermore, an analytical procedure to determine free VFAs in wastewater samples has also been reported [17].

Most of the published works using HS-SPME as an extraction technique for VOCs in aqueous matrices determine groups of compounds belonging to the same chemical family (e.g. aldehydes, sulphides and mercaptans, amines, and VFAs). In this work, a method based on HS-SPME and using GC-MS is described for the characterisation of a list of compounds belonging to different chemical families in wastewater matrices. Several variables affecting the chromatographic behaviour of the target compounds (e.g. splitless time) were considered and experimental conditions affecting their extraction using HS-SPME (e.g. type of sorbent, time and extraction temperature) were investigated according to the design of experiments (DoE) methodology. Finally, the developed method was applied to the analysis of aqueous samples from a WWTP.

3.2 EXPERIMENTAL

3.2.1 CHEMICALS

Dimethyl disulphide (DMDS, 99%), octanal (99%), (R)-(+)-limonene (99%), *m*-cresol (99.7%), nonanal (95%), (-)-carvone (99%), butyric acid (99.5%), indole (99%), and skatole (98%) were obtained from Sigma-Aldrich (Steinheim, Germany). Phenol (99.5%) was obtained from Dr. Ehrenstorfer (Augsburg, Germany). Sodium chloride (99.9%) and HPLC-gradient grade methanol were from Carlo-Erba Reagents (Milan, Italy). Milli-Q water from a Milli-Q Plus water purification system (Millipore Iberica, Barcelona, Spain) was used.

Stock standard solutions were prepared by weight in methanol and stored them at 4^{o}C for up to a week. Working solutions were made daily by diluting the standard solutions to the required concentration with Milli-Q water.

Primary treatment effluent, secondary treatment and effluent water samples were obtained from a WWTP located in Castell-Platja d'Aro (Girona, Spain) and stored in glass bottles at -16^{o}C. Some of these samples were used for validation purposes as indicated in Section 3.3.3.

3.2.2 HEADSPACE- SOLID PHASE MICROEXTRACTION PROCEDURE

SPME experiments were performed with a manual fibre holder. We tested two different commercially available fibre coatings: a 75 µm CAR/PDMS and a 50/30 µm DVB/CAR/PDMS. The fibre holder and coatings were supplied by Supelco (Bellefonte, PA, USA). Before use, each fibre was conditioned according to the manufacturer's instructions to remove contaminants and stabilise the solid phase.

Sample solution (5 mL) was introduced into a 15 mL screw-cap glass vial, NaCl was added, the vial was closed, and it was put over a magnetic stirrer (Variomag®, Germany) in a water-thermostated bath. Magnetic stirring (medium speed) was applied during the extraction using a PTFE-coated stir bar and the fibre was exposed to the headspace above the aqueous solution. The final extraction conditions were: 1 g of NaCl added, extraction time 30 min, and extraction temperature 70^{o}C. After completion of sampling, the fibre was pulled into the needle and the SPME device was removed from the vial and inserted into the injection port of the GC for thermal desorption and analysis. After each chromatographic run the fibre was reinserted into the injection port of the GC during 15 min to ensure that no compounds remained in the coating.

3.2.3 EXPERIMENTAL DESIGN

A full factorial design was performed to evaluate the influence of the parameters on the extraction of odorous compounds from an aqueous solution. This allowed us to determine the influence of all the experimental variables studied and also to ascertain the interactions between them.

For each analyte, three variable factors that can affect the extraction yield were considered: ionic strength quantified as NaCl concentration (c), temperature (T) and extraction time (t). Then we selected a 2^3 full factorial design. Table 3.1 shows the experimental range for each factor. The central point (0.5 g, 50^{o}C, 20 min) was also measured and considered as an experiment.

Table 3.1. Factor levels considered in the experimental design optimisation.

Variable	Low level (–)	Medium level (0)	High level (+)
c (g)	0	0.5	1
T (^{o}C)	30	50	70
t (min)	10	20	30

All the experiments were performed in triplicate and in random order. The Minitab v14 computer program was used for data manipulation and calculations [18].

3.2.4 EQUIPMENT AND CHROMATOGRAPHIC CONDITIONS

Gas chromatographic analyses were performed with a Trace GC 2000 coupled to a PolarisQ ion trap mass spectrometer detector (Thermo Scientific, Waltham, MA, USA). Analytes were separated with a TRB-5 MS capillary column (Teknokroma, Spain) (30m × 0.25 mm i.d.; 0.25 µm film thickness). The split/splitless injection port was equipped with a 0.75 mm ID SPME liner and operated at 250^{o}C. The carrier gas was helium at a constant inlet flow rate of 1 mL·min^{-1}.

The oven temperature program was: initial temperature 35^{o}C, held for 10 min; then increasing by 5^{o}C·min^{-1} up to 150^{o}C and by 15^{o}C·min^{-1} up to 250^{o}C, and held for 2 min; total run 42 min. MS analyses were conducted in full-scan mode and monitored masses between 40 and 300 amu. Ionisation was carried out in the electron impact mode at 70eV. The transfer line temperature was maintained at 280^{o}C and the ion source temperature was 225^{o}C. The acquisition of chromatographic data was performed using Xcalibur 1.4 software (Thermo Scientific). Table 3.2 shows the list of the target compounds, their respective odour threshold concentrations and details of the GC-MS analysis.

Table 3.2. OTCs, retention times and m/z ratios of the target compounds. Values in bold are the quantifier ions. n.a.: not available.

Compound	OTC* (μg·L^{-1})	Retention time (min)	m/z
DMDS	0.3, 1.0	5.21	45, 79, **94**
Phenol	n.a.	18.81	66, **94**
Octanal	0.7, 1.4	19.44	69, 84, **95**
Limonene	200, 1000	20.33	68, **93**
m-cresol	800	22.19	79, **107**, **108**
Nonanal	1, 2.5	23.09	81, 98, **143**
Carvone	10	27.42	82, 108, **151**
Indole	370	28.82	90, **117**
Skatole	1.2	31.34	**130**, 131

(*) Compendium data from [16], [19] and [20].

3.3 RESULTS AND DISCUSSION

In this study, a list of odorous compounds belonging to different chemical families was selected for their determination in wastewaters by HS-SPME (Table 3.2); phenolic compounds, aldehydes, sulphur-containing compounds, nitrogen-containing compounds, and terpenes were included. All of them had previously been reported as present in wastewaters and in the atmosphere [2,3,8,12,15,21]. Although H_2S, NH_3 and amines are some of the most important contributors to the malodorous emissions from WWTPs, they were discarded after considering the specific chromatographic conditions required for their analysis.

Preliminary experiments were performed to assay the possibility of adding VFAs to the list of target compounds. On-fibre silylation with N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide (MTBSTFA) was required to analyse these compounds [22]. Losses of other target analytes were observed during the derivatisation step. For this reason, VFAs were not included in the study.

3.3.1 SELECTING FIBRE COATINGS AND SPLITLESS TIME

Due to the different volatility of molecules studied, two fibre coatings (CAR/PDMS and DVB/CAR/PDMS) were selected for evaluation. CAR/PDMS fibre has previously been used to characterise odorous waste gas emissions [10] and to determine volatile alkyl sulphides [15] and benzene, toluene, ethylbenzene, and xylene isomers (BTEX) [23] in wastewaters. High efficiency is usually obtained with this fibre coating for small polar analytes that can be rapidly

desorbed at temperatures around 270-280°C. On the other hand, Larreta et al. have observed that DVB/CAR/PDMS fibre showed the best extraction/desorption yields for the determination of phenols and indoles in cow slurry [24]. DVB-based coatings have also been used for the analysis of a large variety of taste and odour compounds in water samples [25,26].

In this work a clear difference between the two coatings has been observed in terms of peak shape. As can be seen in Figure 3.1, for some selected analytes CAR/PDMS gave increased peak tailing especially in the case of limonene and *m*-cresol. This can be attributed to the presence of carbon in the coating composition causing a strong interaction with polar compounds that are not easily released from the fibre. Peak shape is improved when using DVB/CAR/PDMS coating and for this reason it was selected for further experiments.

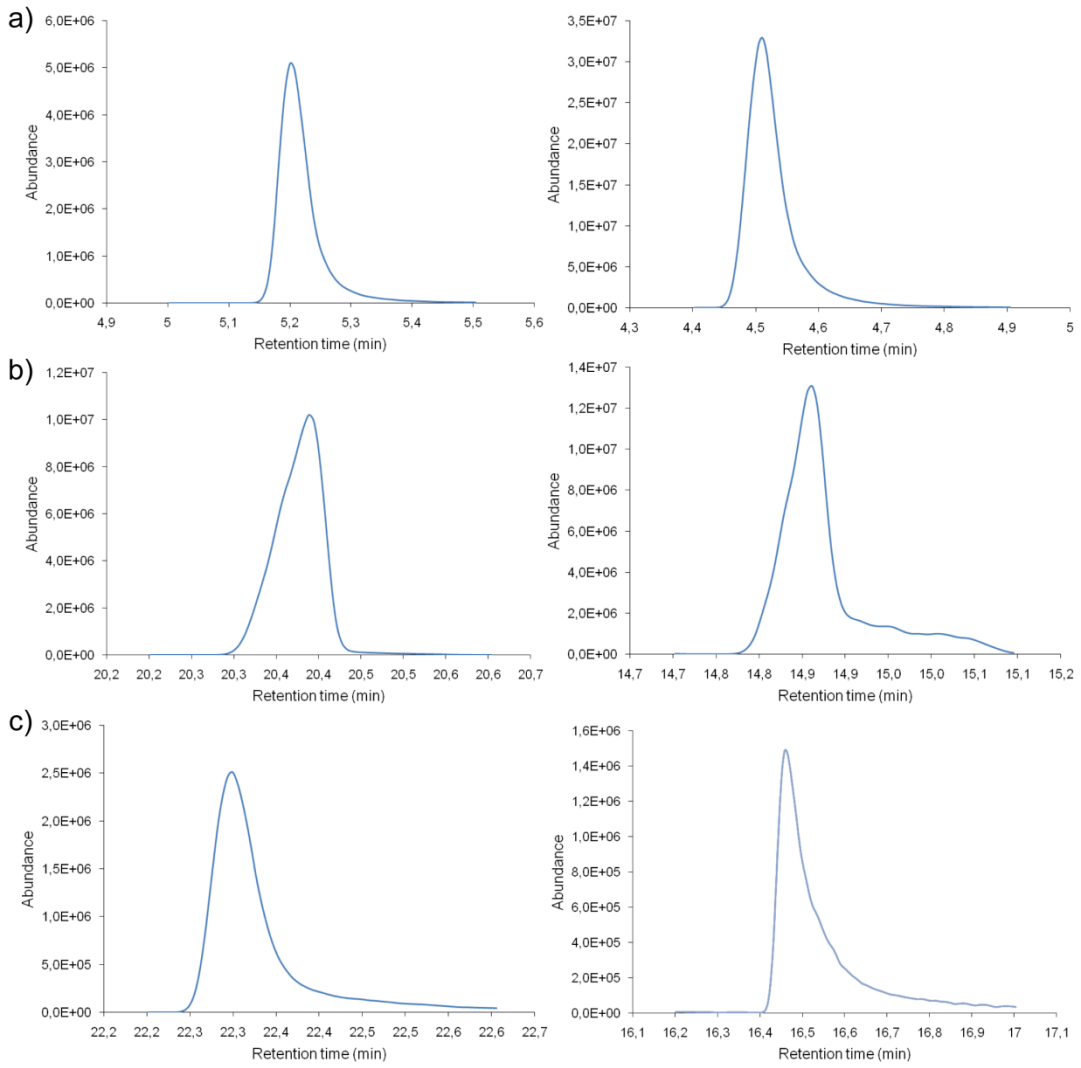


Figure 3.1. Chromatographic peaks for some selected compounds (0.1 µg·L^{-1} of each compound) obtained with the two fibre coatings: on the left, with DVB/CAR/PDMS fibre; on the right, with CAR/PDMS fibre. Extraction conditions: 30 min at 50°C and 1.2 g of NaCl added to the sample. a) DMDS (m/z = 94), b) Limonene (m/z = 93), c) *m*-Cresol (m/z = 107, 108).

In SPME, splitless injection using narrow-bore glass liners is required to produce a high linear flow rate of the carrier gas around the fibre and facilitate the rapid removal of desorbed analytes from the injector [27]. Selecting the most appropriate splitless conditions, good chromatographic peak shape and widths can be obtained as long as the GC oven temperature is held at a minimum of 50^{o}C below the boiling point of the most volatile compounds when 0.25 µm film thickness columns are used [28]. In the case of very volatile compounds, short desorption times (less than 1 min) are expected to be sufficient for the quantitative transfer of the extracted analytes [23]. On the contrary, splitless times from 1 to 5 min are usual for semi-volatiles. In this study splitless times of 30 s, 1 and 2 min were considered with DVB/CAR/PDMS fibre and for each analyte several factors were evaluated, for example peak shape, peak area and carryover. When desorption was performed for only 30 s, the peak areas values obtained were 50% lower than those obtained when desorption was performed during 1 min. One and two minutes gave statistically comparable results without affecting the peak shape. The only exceptions were carvone and nonanal, which resulted in higher peak area values when 2 min of splitless time was considered. The possible carryover was evaluated for these two compounds at 1 min splitless time by acquiring a new chromatogram after the analysis of a sample. No peaks corresponding to these analytes were identified at the corresponding retention times. These findings let us to select 1 min as the most appropriate desorption time for all the analytes.

3.3.2 STUDY OF THE SAMPLING CONDITIONS

An experimental domain was defined to ascertain the influence of temperature, time of extraction and salt content on the extraction of odorous compounds from aqueous solutions (Table 3.1). A full two-level factorial design was carried out to check for the presence of double interactions and evidence of curvature effects that could not be detected using a classic procedure based on the evaluation of each variable individually. Absolute peak areas were analysed and the results obtained are summarised in Table 3.3, where the significances (*p*-values) are given. The sign beside each variable name indicates the optimal level to maximise the response. Results showed that for all compounds no statistically relevant interactions occurred between the variables evaluated (the corresponding *p*-values for single interactions are much smaller than those for double and triple interactions). Additionally, there were no statistically relevant effects for limonene.

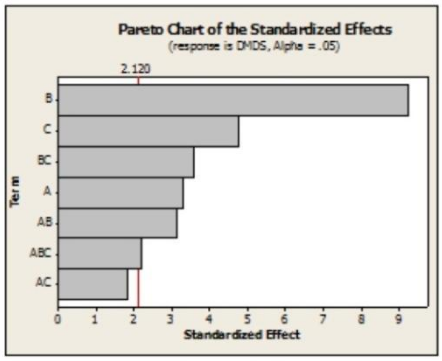
Table 3.3. Statistical results for the experimental design. Significance *p*-values are given for main effects, double and triple interactions and for curvature evidence. Most relevant single and double variable terms effects are also shown in decreasing order of importance.

Analyte	Single variable effects		Double variable effects		Triple variable effects	*p*-value for curvature evidence
	p-value	Significant terms	*p*-value	Significant terms	*p*-value	
DMDS	0.000	-T +c +t	0.001	-Tc	0.043	0.496
Phenol	0.000	+T +c +t	0.000		0.009	0.226
Octanal	0.000	+t +T +c	0.265		0.008	0.019
Limonene	0.453		0.931		0.100	0.470
m-cresol	0.000	+T +c +t	0.000	+c -tT	0.000	0.005
Nonanal	0.000	+t +T	0.011		0.057	0.063
Carvone	0.000	+T +c	0.497		0.419	0.989
Indole	0.000	+T +c +t	0.000		0.000	0.083
Skatole	0.000	+T +c +t	0.000		0.015	0.070

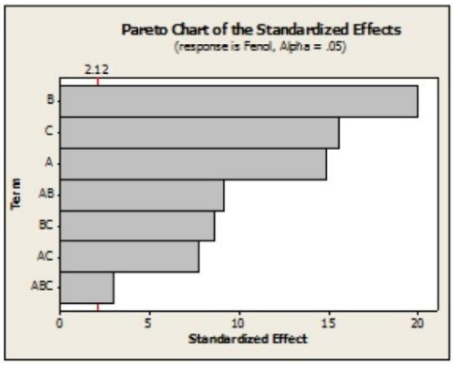
As can be seen in Table 3.3, temperature was a crucial variable as it had a noticeable influence on six analytes (DMDS, phenol, *m*-cresol, carvone, indole, and skatole) and the response was maximised when temperature was set at the highest level. Extraction yields can be enhanced when an optimum temperature is applied during sampling. In general, the amount of extracted analyte increased at higher temperatures that facilitate the transport of the analytes from the solution to the headspace phase. In the case of the most volatile target compound (DMDS), the extraction yield was not enhanced when the temperature was set at the highest level due to competition with the thermal desorption process. Thus, low temperatures might be used to avoid losses of this analyte. Taking into account the response for all compounds, the sampling temperature was set at 70^{o}C.

Extraction times with SPME usually vary from a few minutes to an hour or more, depending on the matrix, analytes, fibre phase and the desired sensitivity. In the case of sulphur-containing compounds, it has been found that small extraction times are required to reach equilibrium (less than 15 min) [29,30]. On the contrary, for semi-volatile compounds longer extraction times are necessary, even longer than 60 min [27,31]. Due to the range in volatility of the substances evaluated in this work, extraction times between 10 and 30 min were evaluated to find the best conditions for the majority of the target analytes. Extraction times longer than 30 min were not considered to avoid extending the total analysis time for each sample. As can be seen in Table 3.3, extraction time had a clear influence on octanal and nonanal extraction, and must be kept at the highest level. For this reason an extraction time of 30 min was selected.

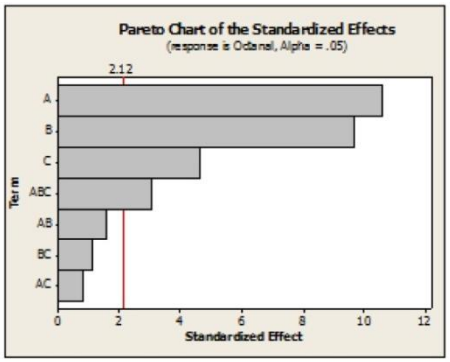
When studying the NaCl content, it is expected as a general trend that increasing the ionic strength of the sample makes organic substances less soluble, increasing the partition coefficients [27]. This effect depends on the polarity of the analyte, the concentration of salt and the sample matrix. For the compounds evaluated in this study, the addition of salt enhanced the extraction. Therefore, sampling was carried out at the highest salt level (1 g NaCl). These main conclusions are better visualised in Pareto graphs (Figure 3.2).



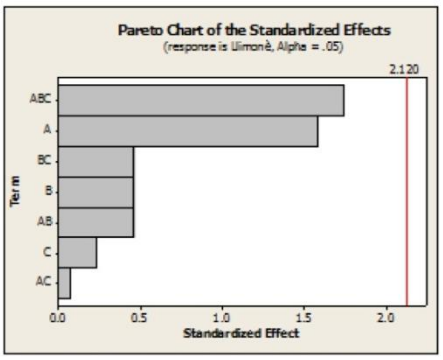
Pareto graph of DMDS.



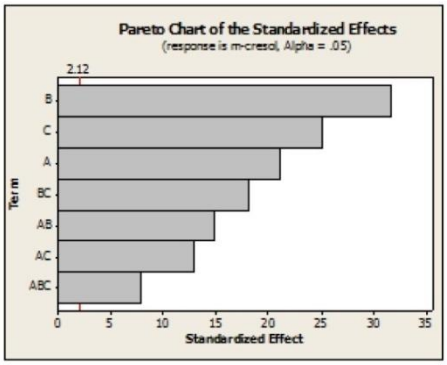
Pareto graph of phenol.



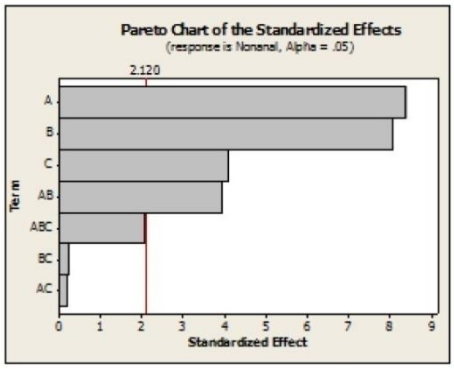
Pareto graph of octanal.



Pareto graph of limonene.

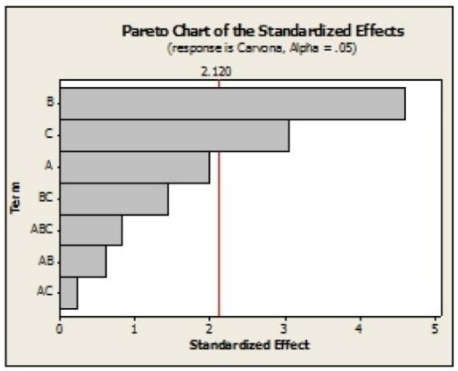


Pareto graph of *m*-cresol.

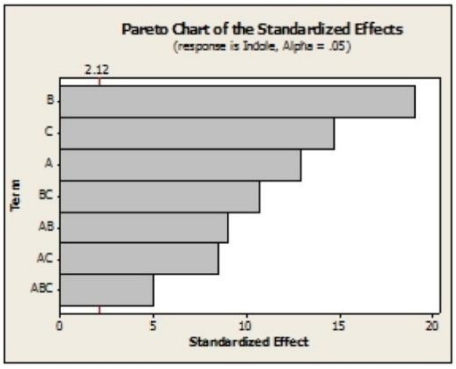


Pareto graph of nonanal.

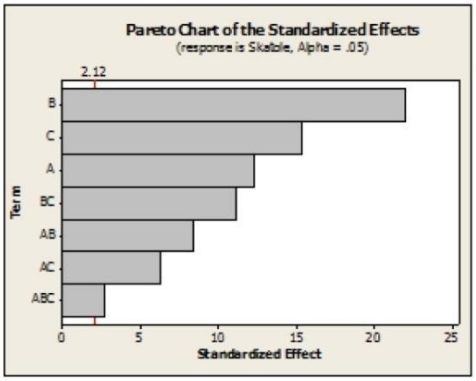
Figure 3.2. Pareto graphs obtained as a result of the experiments developed according to the design of experiments methodology proposed. A: extraction time (min), B: extraction temperature (^{o}C), C: NaCl concentration (g).



Pareto graph of carvone.



Pareto graph of indole.



Pareto graph of skatole.

Figure 3.2 ***(continued)*****.** Pareto graphs obtained as a result of the experiments developed according to the design of experiments methodology proposed. A: extraction time (min), B: extraction temperature (^{o}C), C: NaCl concentration (g).

3.3.3 QUALITY PARAMETERS

The linearity of the HS-SPME method was tested in the ranges shown in Table 3.4. Each concentration level was analysed in triplicate. For all compounds, residual plots confirmed linearity in the range evaluated, with a determination coefficient (r^2) greater than 0.97. Samples were analysed (n=7) at reduced concentrations to experimentally determine the LODs and the LOQs, and took the calculated standard deviation for each compound as the standard deviation of the blank. IUPAC 3σ and 10 σ criteria were used to determine LODs and LOQs, respectively, which are summarised in Table 3.4.

Table 3.4. Quality parameters obtained in standard solutions analysis. Standard deviations are showed in parenthesis. a: intercept, S_a :standard deviation of the intercept, b: slope, $S_{b:}$ standard deviation of the slope.

Compound	Working range ($\mu g \cdot L^{-1}$)	a (S_a) ($\times 10^5$)	b (S_b) ($\times 10^5$)	r^2	LOD ($\mu g \cdot L^{-1}$)	LOQ ($\mu g \cdot L^{-1}$)
DMDS	0.1 – 100	4.7 (7.2)	1.8 (0.2)	0.9719	0.03	0.10
Phenol	1.4 – 250	2.4 (2.7)	0.5 (0.2)	0.9939	0.4	1.4
Octanal	1.9 – 15	0.2 (2.7)	0.61 (0.03)	0.9958	0.6	1.9
Limonene	1.1 – 10	3.7 (4.7)	8 (1)	0.9853	0.3	1.1
m-cresol	0.5 – 150	8.6 (7.2)	1.92 (0.09)	0.9940	0.2	0.5
Nonanal	1.9 – 10	3.4 (1.6)	5.0 (0.3)	0.9913	0.6	1.9
Carvone	0.1 – 10	2.9 (3.9)	6.3 (0.6)	0.9723	0.03	0.10
Indole	0.7 – 225	1.6 (3.9)	0.74 (0.04)	0.9926	0.2	0.7
Skatole	0.2 – 20	7.9 (9.9)	10 (1)	0.9780	0.06	0.20

As can be observed, the developed method allows the quantification of odorous substances present in water samples well below their odour threshold concentration. Furthermore, LODs and LOQs were also evaluated using spiked samples prepared using water from the secondary treatment unit. No effect from the matrix was observed and equivalent limits were obtained.

Recoveries and intra-day precision (n=5) of the method were evaluated at the concentration levels indicated in Table 3.5. Spiked samples (Milli-Q water as well as water samples obtained at the primary treatment effluent of the WWTP) were used and prepared just before analysis to evaluate these parameters. Concentrations of those compounds initially present were subtracted from the spiked values. Recoveries ranging from 72 to 120% (Milli-Q water) and from 72 to 96% (WWTP water) were obtained for all compounds. These values are in agreement with the "single laboratory validation guidelines" of AOAC [32], which set an acceptable recovery range of between 70 and 120% at these concentration levels. Only the recovery for octanal in spiked wastewater was not quantitative (< 70%) which might be attributed to a rapid degradation of this compound in the WWTP sample, probably due to microbial activity.

Table 3.5. Concentrations, recoveries and intra-day precision values (n=5) obtained in spiked milli-Q water solution and real sample analysis. Standard deviations are shown in parenthesis.

Compound	Concentration ($\mu g \cdot L^{-1}$)	Recovery (%)		Intra-day precision (% RSD)	
		Spiked milli-Q water	Primary effluent water samples	Spiked milli-Q water	Primary effluent water samples
DMDS	50	72 (4)	86 (3)	5	14
Phenol	150	79 (9)	96 (4)	12	9
Octanal	5	79 (6)	49 (7)	6	15
Limonene	7.5	75 (8)	82 (1)	10	20
m-cresol	100	84 (9)	92 (15)	12	7
Nonanal	5	90 (10)	96 (2)	10	13
Carvone	7.5	90 (4)	94 (8)	5	11
Indole	90	90 (15)	73 (20)	16	18
Skatole	10	120 (20)	72 (30)	16	15

3.3.4 ANALYSIS OF WASTEWATER SAMPLES

The proposed method was applied to the analysis of samples obtained from a WWTP in Castell-Platja d'Aro (Girona, Spain). Samples from the primary treatment effluent, the biologic treatment effluent and the plant effluent (after UV treatment) were obtained. Figure 3.3 illustrates the extracted chromatograms of samples taken at the three sampled points of the WWTP (day 1). The method also allowed the semi-quantitative determination of BTEX which were also present in two of these samples (primary treatment and biologic treatment effluents).

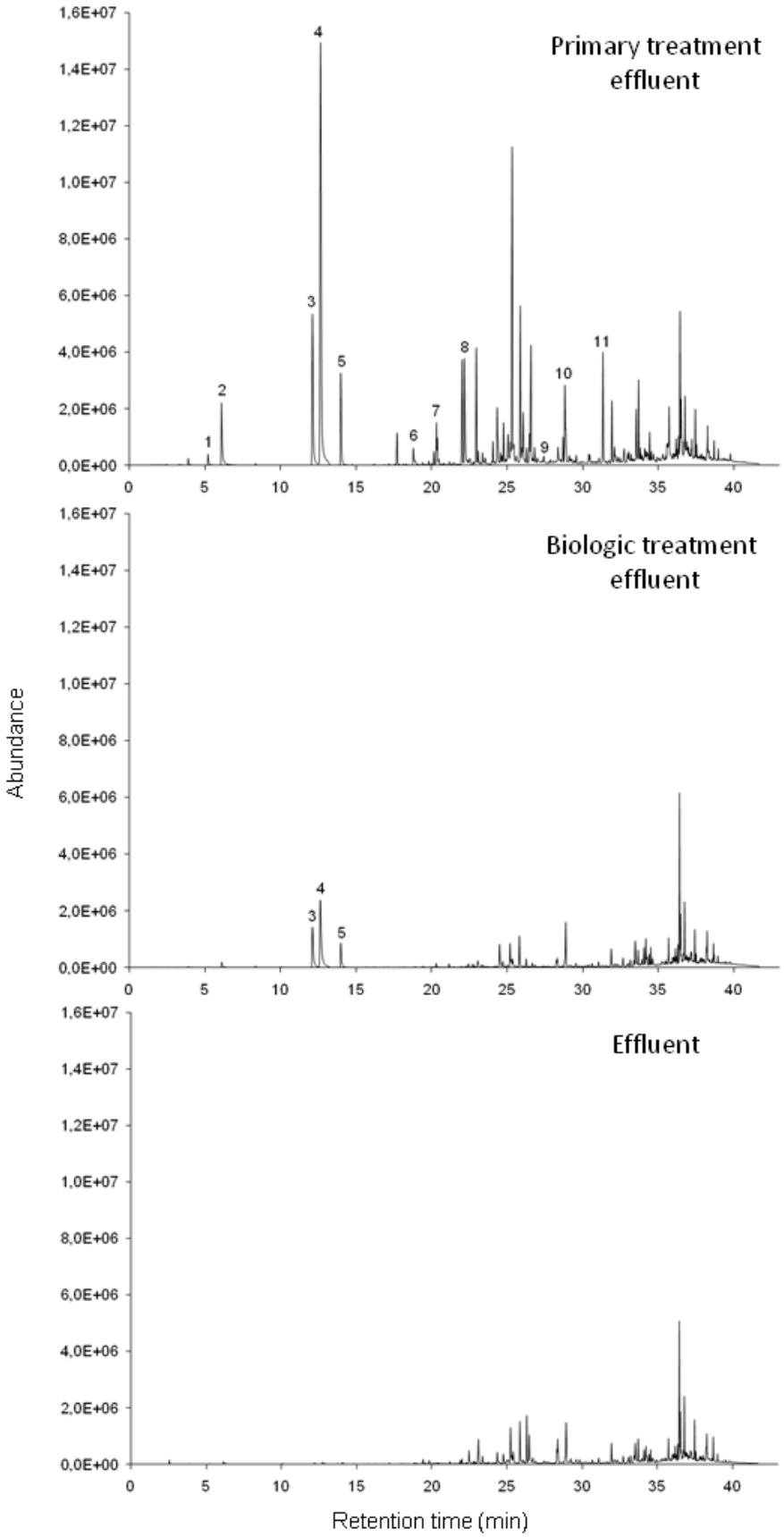


Figure 3.3. Extracted chromatograms (m/z ratios showed in bold in Table 3.2) of samples taken at the three sampling points of the WWTP (day 1) using optimised experimental conditions. 1. DMDS, 2. Toluene, 3. Ethyl benzene, 4. *p*-Xylene, 5. *o*-Xylene, 6. Phenol, 7. Limonene, 8. *m*-Cresol 9. Carvone, 10. Indole, 11. Skatole.

Figure 3.3, as well as the results summarised in Table 3.6, show a decrease in the concentration of the target compounds along the different treatments. All compounds were usually detected in primary effluent samples, and *m*-cresol, indole, phenol, and skatole were present at higher concentrations. Octanal was detected (but not quantified) in 55% of the wastewater samples analysed, which indicates that this compound was present at concentrations above its odour threshold value. Skatole and DMDS gave concentrations above their respective odour threshold values only in primary effluent samples (Table 3.2). Moreover, carvone was determined in samples from the plant effluent.

Our results are in agreement with those published in other studies. Islam et al. [19] detected DMDS in samples from the individual package treatment at concentrations between 0.08 and 7.49 $\mu g \cdot L^{-1}$. Additionally, they detected indole and skatole in samples from the sludge treatment process. Indole was found at concentrations between 6 and 61.8 $\mu g \cdot L^{-1}$ and skatole was found at 4.83 $\mu g \cdot L^{-1}$. Hwang et al. [1] detected DMDS in primary effluent samples at concentrations between 3 and 27 $\mu g \cdot L^{-1}$.and indole at 570 $\mu g \cdot L^{-1}$. However, they also detected DMDS in samples from the plant effluent. Octanal was detected in snow samples by Sieg et al. [33] at concentrations between 0.324 and 0.594 $\mu g \cdot L^{-1}$.

Table 3.6. Results obtained in WWTP samples analysis. Concentrations in $\mu g \cdot L^{-1}$. Standard deviations are showed in parenthesis. n.d.: not detected. (n=3)

	Primary treatment effluent			**Biologic treatment effluent**			**Plant effluent (after U.V. treatment)**		
Compound	**Day 1**	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**
DMDS	5 (1)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phenol	38 (5)	27 (2)	39.3 (0.8)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Octanal	n.d.	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ	n.d.	n.d.
Limonene	1.14 (0.09)	<LOQ	1.28 (0.09)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
m-Cresol	80 (10)	100 (15)	151 (7)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Nonanal	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.
Carvone	0.70 (0.04)	1.00 (0.08)	1.26 (0.06)	n.d.	0.500 (0.007)	0.516 (0.002)	n.d.	0.520 (0.003)	0.50 (0.01)
Indole	90 (7)	47 (8)	66 (5)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Skatole	10 (1)	10 (2)	13.5 (0.7)	n.d.	0.90 (0.06)	n.d.	n.d.	n.d.	n.d.

3.4 CONCLUSIONS

An HS-SPME method followed by GC-MS has been developed and successfully applied to analyse odorous volatiles from aqueous samples from wastewater treatment plants. The method has been optimised for a list of compounds belonging to different chemical families, including volatiles sulphides, aldehydes, phenols, indole, skatole, and some terpenes. DVB/CAR/PDMS coating showed better performance in the microextraction process and experimental conditions were fixed as: 1 g of NaCl added, extraction time 30 min, and extraction temperature 70^{o}C. The optimised method was validated using spiked Milli-Q water and real water samples: good detection limits (between 0.03 and 0.6 $\mu g \cdot L^{-1}$) as well as good intra-day precision values (RSD ranging from 7 to 20%, n = 5) were found. From the analysis of water samples from WWTPs, the presence of almost all the target compounds was found. Some of these compounds appeared in concentrations above their odour threshold value.

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CHAPTER 4:

Odour-causing compounds in air samples: Gas-liquid partition coefficients and determination using solid-phase microextraction and GC with mass spectrometric detection

4.1 INTRODUCTION

Odorous emissions from WWTPs are one of the major environmental problems that have affected populations for decades [1,2]. The source of the odorous compounds in WWTP environments is diverse. Volatile sulphur compounds originate from the fermentation of organic sulphur compounds and sulphur-bearing amino acids in proteinaceous material [3]. Some nitrogen-containing compounds such as indole and skatole are the breakdown products of tryptophan, which is present in faeces [4]. Phenolic compounds are present in water environments due to industrial and agricultural activities, and they also originate from the degradation of some natural substances [5]. Aldehydes can be formed through the photodegradation of dissolved natural organic matter. They have also been identified as the major products of gas phase reactions involving oleic acid and linoleic acid with ozone [6]. Benzothiazoles are used and emitted in a large variety of industrial processes [7]. Finally, terpenes can also have different origins. For example, limonene and carvone are present in wastewater, the former owing to its use as a cleaning agent and degreaser [4,8] and the latter because it is employed as a flavouring, a fragrance and also in alternative medicine [9].

VOCs are emitted from WWTP water and sludge to the atmosphere when a liquid-gas or solid-gas interface is present. Their emission depends on different factors such as the turbulences that take place during the wastewater and sludge treatments, the treatment process itself, the atmospheric conditions and the physical characteristics of each particular substance. Some researchers have evaluated the partition coefficient as an indicator of the tendency of the compounds present in aqueous matrices and oils to be exchanged with the air phase. Some studies have addressed the determination of partition coefficients for a few compounds of environmental concern, targeting chlorinated hydrocarbons, aromatic compounds and other VOCs such as ethanol [10-15].

VOCs are usually determined by gas chromatography with FID detection or with mass spectrometric detection [16-19,20]. In gaseous samples, the sampling and preconcentration can be performed in different ways depending on the characteristics of the volatile compounds and the purpose of the survey. For example, continuous and automated on-line instruments are used for anthropogenic volatile halocarbon monitoring since these compounds are monitored on a global scale in different sampling sites due to their role in global change [21]. Off-line methods are preferable in grab sampling campaigns when long-term concentration trends are assessed. In this case, samples are collected using plastic bags or metal canisters, transported to the laboratory and analysed using GC, by direct injection or

more frequently in combination with a concentration step [21-23]. Adsorption enrichment on solid adsorbing materials is a technique commonly used to obtain time weighted average concentrations. For that, tubes filled with one or more sorbents and solid-phase extraction cartridges have been employed [24,25].

SPME is an alternative technique for the concentration of VOCs in air samples [26]. It has been widely used for the analysis of air samples in industrial chimneys, schools, biogas-production plants, homes and waste treatment plants [20,27-30]. Larroque et al. [27] developed a method based on SPME for the determination of VOCs in school environments. Static sampling was performed in glass bulbs and about 20 compounds belonging to four different types of chemicals (alkanes, aromatics, oxygenated and terpenes) were identified. Domeño et al. [28] determined volatile organic pollutants emitted by an industrial stack using a pilot plant connected to an industrial chimney. Davoli et al. [20] applied a method based on SPME for the characterisation of odorant emissions from landfills. Samples were collected in Nalophan® bags and concentrated using a DVB/CAR/PDMS fibre. A large number of odorous compounds were identified and quantified. Volatile organic sulphur compounds have also been investigated by different authors using SPME [29,31,32]. Furthermore, SPME-based analytical procedures to determine amines in air and water [33] and to determine carbonyls in complex air matrices [34] have also been reported.

In chapter 3, a SPME method has been evaluated to determine a group of odour-causing substances belonging to different chemical families in wastewaters. In the present study, a method based on SPME with GC-MS is described for the air monitoring of odour-causing compounds. Simultaneous extraction of all target compounds was performed to define a convenient extraction time without fibre saturation. The method was validated and applied to the analysis of air samples from a WWTP located in the northeast of Catalonia (Spain). Determination of partition coefficients for the target compounds was also performed in separate experiments using the developed method.

4.2 EXPERIMENTAL

4.2.1 CHEMICALS AND MATERIALS

Dimethyl disulphide (DMDS, 99%), octanal (99%), (R)-(+)-limonene (99%), *m*-cresol (99.7%), nonanal (95%), benzothiazole (90%), (-)-carvone (99%), indole (99%), and skatole (98%) were obtained from Sigma-Aldrich (Steinheim, Germany). Phenol (99.5%) was obtained from Dr. Ehrenstorfer (Augsburg, Germany). HPLC-gradient grade methanol and hexane were from Carlo-Erba Reagents (Milan, Italy). Nitrogen 5.0 (99.9990% purity) was used to clean the glass sampling bulbs and generate standard gas mixtures. Water from a Milli-Q Plus water purification system (Millipore Iberica, Barcelona, Spain) was used.

Stock solutions for each analyte of about 200–300 $mg \cdot L^{-1}$ were prepared in methanol. Mixed standard solutions in a concentration range between 1 and 50 $mg \cdot L^{-1}$ were obtained by dilution also using methanol as solvent. Both stock and mixed standard solutions were kept at -18°C. Standard gas mixtures were generated in 500 mL glass sampling bulbs (Supelco, Bellefonte, PA, USA) by injecting 1 µL of the standard solution with a syringe through the septum into a clean bulb previously filled with high purity nitrogen. Stock solutions in methanol were also used for the preparation of spiked water samples in the determination of partition coefficients.

To determine the extracted amount of the analytes by the SPME fibre, standard solutions were prepared in hexane. The concentrations ranged from 0.5 to 50 $mg \cdot L^{-1}$. One-microlitre injections were made in duplicate for each point of the calibration curves.

4.2.2 SAMPLING AND PREPARATION OF THE SAMPLES

Sampling was performed in open air at a WWTP located in Castell-Platja d'Aro (Girona, Spain). Samples were collected in laboratory-made 25 L Nalophan® bags (Olfatec GmbH, Germany), which were filled on site using an air sampling pump (KNF Neuberger GmbH, Freiburg, Germany). The sampling rate was 15 $L \cdot min^{-1}$. The samples were transported to the laboratory and analysed within 3 hours. To do that, air samples were transferred to a glass bulb, which was flushed approximately 10 times the sampling volume with the sample to ensure the displacement of the air contained inside the glass bulb [35].

Relative humidity was controlled during the sampling and values between 25.9 and 55.0 % were obtained. The effect of relative humidity was not studied in our work since water vapour was reported not to affect adsorption of compounds having high affinity for the sorbent [36].

4.2.3 SOLID PHASE MICROEXTRACTION PROCEDURE

SPME experiments were performed with a manual fibre holder. Two different commercially available fibre coatings were used: a 75 µm CAR/PDMS and a 50/30 µm DVB/CAR/PDMS fibre, which were previously conditioned according to the manufacturer's instructions to stabilise the solid phase and remove contaminants. The fibre holder and coatings were obtained from Supelco (Bellefonte, PA, USA).

Extraction was performed at 22 ± 1°C in the glass bulb. After the bulb was flushed with the sample, the stopcocks were closed to perform SPME in static mode. The fibre was inserted through the septum and exposed to the analytes for 10 min. After sampling was completed, the fibre was pulled into the needle and removed the SPME device from the glass bulb. Then, it was inserted into the injection port of the GC for thermal desorption and analysis.

For the determination of the partition coefficients, 125 mL of a synthetic aqueous solution were introduced into a 250 mL glass bottle with a screw cap and PTFE septum. After an equilibration time of five hours, the fibre was inserted through the septum and exposed to the gas phase for 10 min at 22 ± 1°C. Then, the SPME device was inserted into the injection port of the GC for thermal desorption and analysis. Concentrations of the target compounds in the gas phase were obtained after interpolation of the chromatographic peak areas in the calibration graph obtained for the glass bulb, taking into account the different volume of the gas phase.

4.2.4 EQUIPMENT AND CHROMATOGRAPHIC CONDITIONS

Gas chromatographic analysis was performed using a Trace GC 2000 coupled to a PolarisQ ion trap mass spectrometer detector (Thermo Scientific, Waltham, MA, USA). A Trace GC 2000 equipped with a FID was also used for some preliminary experiments. The separation was performed using a BPX5 capillary column (SGE Europe, UK) (30 m × 0.25 mm i.d.; 0.25 µm film thickness).

The injection port operated at 250°C and was equipped with a 0.75 mm ID SPME liner. The oven temperature programme started at 35°C, held for 10 min; ramped at 5°C·min^{-1} to 150°C and then ramped at 15°C·min^{-1} to 250°C, and held for 2 min; total run 42 min. Helium carrier gas was used at a constant inlet flow rate of 1 mL·min^{-1}. MS analyses were conducted in full-scan mode and masses between 40 and 300 amu were monitored. Ionisation was carried out

in the electron impact mode at 70 eV. The ion source temperature was maintained at 225°C and the transfer line temperature was 280°C.

Chromatographic data was acquired by means of Xcalibur 1.4 software (Thermo Scientific). The list of the target compounds, their respective odour threshold concentrations and details of the GC-MS analysis are summarised in Table 4.1.

Table 4.1. Volatile compounds evaluated in the present study, with their OTCs, retention times and m/z ratios. n.a.: not available.

Compound	OTC in air ($\mu g \cdot m^{-3}$)[a]	OTC in water ($\mu g \cdot L^{-1}$)[a]	Retention time (min)	m/z [b]
DMDS	303	0.3, 1.0	5.47	45, 79, **94**
Phenol	39, 46	n.a.	19.20	66, **94**
Octanal	21, 7.21	0.7,1.4	19.95	69, 84, **95**
Limonene	55000	200, 1000	20.74	68, **93**
m-Cresol	0.57	800	22.76	79, **107**, **108**
Nonanal	13.3	1, 2.5	23.61	81, 98, **143**
Benzothiazole	n.a.	80	27.77	108, **135**
Carvone	85, 150	10	28.14	82, 108, **151**
Indole	7.1	370	29.73	90, **117**
Skatole	0.35, 0.5	1.2	32.26	**130**, 131

[a] Compendium data from [37,39,40]
[b] Quantifier ions in bold

4.3 RESULTS AND DISCUSSION

In the previous chapter, a method was developed to determine malodorous compounds (DMDS, phenol, octanal, limonene, *m*-cresol, nonanal, benzothiazole, carvone, indole, and skatole) in water samples from the WWTP located in Castell-Platja d'Aro. Most of them were found to be present in the analysed samples during the period from July 2010 to July 2011. In an attempt to go one step further, a new study was undertaken to investigate their presence in the atmosphere surrounding the plant. The analytical method selected for that purpose was SPME to preconcentrate the target analytes and GC-MS for their separation and quantification. The chromatographic conditions chosen (see Section 4.2) allowed us to determine the different compounds with an adequate resolution.

4.3.1 SELECTION OF THE FIBRE COATING

From the large amount of commercially available SPME coatings, most authors have chosen a CAR/PDMS fibre for the analysis of volatile compounds. Larroque et al. [27] used this fibre for the analysis of some VOCs in indoor air. CAR/PDMS fibre has also been used for the analysis of sulphur compounds [29,31,32] and BTEX [41] in air. Other authors describe the use of DVB-based coatings. For example, Davoli et al. [20] analysed odorant emissions from landfills with a DVB/CAR/PDMS fibre. Additionally, some papers have reported the use of this coating for the analysis of fragrances in indoor air [42,43]. Consequently, CAR/PDMS and DVB/CAR/PDMS coatings were evaluated.

DVB/CAR/PDMS fibre showed the best performance for all compounds except for DMDS (Figure 4.1). This analyte gave increased peak area when the CAR/PDMS fibre was used as high efficiency is usually obtained with this fibre coating for small polar analytes that can be rapidly desorbed at temperatures around 270–280°C [32]. As a result, the DVB/CAR/PDMS coating was selected for further experiments. These results were in accordance with those obtained in the previous chapter dealing with aqueous samples.

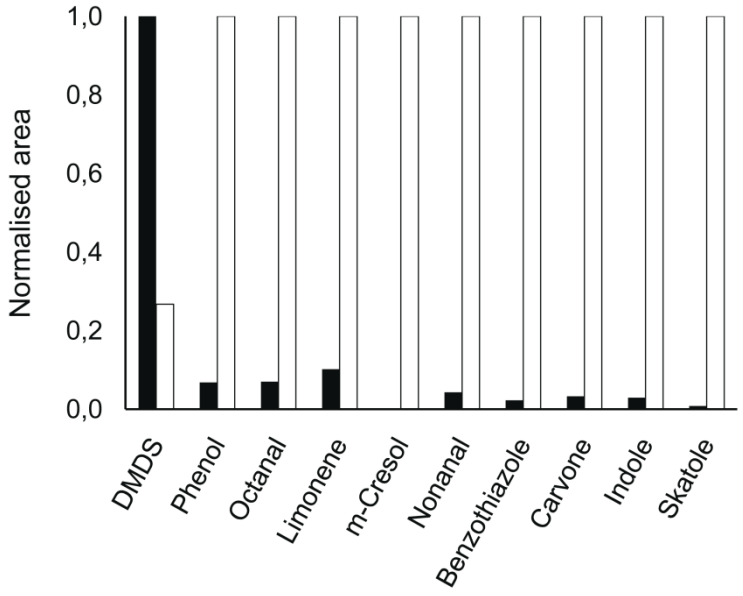

Figure 4.1. Peak areas (normalised to the highest value) obtained using the two fibre coatings in the analysis of a mixture of target analytes. Concentrations: 400 $\mu g \cdot m^{-3}$ except for DMDS (20 $\mu g \cdot m^{-3}$). Extraction conditions: 10 min at 22 ± 1°C. Black bars: CAR/PDMS coating; white bars: DVB/CAR/PDMS coating.

4.3.2 SELECTION OF THE EXTRACTION TIME

The aim of SPME extraction is to reach equilibrium when no further increase in the amount extracted by the fibre occurs within experimental error of analysis [44]. However, in some cases short extraction times are needed depending on the nature of the fibre. SPME extractions using Carboxen solid phases involve adsorption onto the coating surface and, as a consequence, competitive adsorption can take place since sorption sites are limited. Then, molecules having high affinity for the adsorbent can displace molecules with lower affinity. For this reason, some authors have used short exposure times to avoid analyte discrimination and saturation of the coating [32,45,46].

Adsorption kinetics was studied and, for that, different extraction times were evaluated for the extraction of model gaseous mixtures containing about 200 µg·m^{-3} of DMDS, *m*-cresol and skatole, which have different volatilities. It was observed that the analytes displayed linear adsorption at short extraction times (<30 min), when co-adsorption of all odorous compounds occurred (Figure 4.2). At longer extraction times, a decrease in the adsorption rate is observed for DMDS due to the competition of the molecules for the sorbent sites.

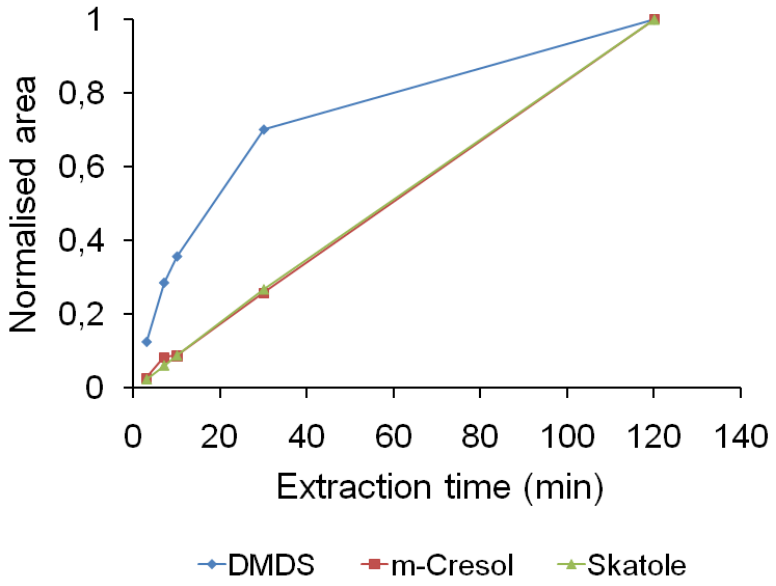

Figure 4.2. Variation of area (normalised to the highest value) versus extraction time (min) obtained for DMDS, *m*-cresol and skatole in the analysis of mixtures containing about 200 µg·m^{-3} of each compound.

For more complex mixtures and different concentration values other authors have developed a theoretical approach based on Fick's diffusion law [32,45-47]. Briefly, considering the SPME coating as a passive sampler, concentrations in the gas sample can be determined from Fick's first law of diffusion:

$$m = D \cdot \frac{A}{l} \cdot \int (C_a - C_{sorb}) dt \quad (4.1)$$

where m is the amount of analyte that is extracted by the fibre (µg), D is the diffusion coefficient of the analyte in air or water ($cm^2 \cdot min^{-1}$), A is the diffusion surface (cm^2), l is the length of the diffusion zone (cm), C_a is the analyte concentration in the sample ($\mu g \cdot m^{-3}$), C_{sorb} is the analyte concentration above sorbent surface ($\mu g \cdot m^{-3}$), and t is the exposure time (min).

When sampling times are short, C_{sorb} can be considered negligible compared to C_a. Then, the adsorbent acts as a perfect sink and equation (4.1) can be rewritten as:

$$m = D \cdot \frac{A}{l} \cdot C_a \cdot t \quad (4.2)$$

Considering the ratio $D \cdot A/l$, or uptake rate (U), constant for a given temperature (depends only on the compound), equation (4.2) can be simplified as:

$$m = U \cdot C_a \cdot t \quad (4.3)$$

It can be assumed that if a linear relationship according to equation (4.3) is obtained for target compounds, quantification by external calibration can be performed accurately. Otherwise standard addition should be used.

In this study four sampling times within the co-adsorption range were tested: 3, 7, 10, and 20 min. Standard gas mixtures containing target compounds at concentrations of 50 and 100 $\mu g \cdot m^{-3}$ were analysed. In Figure 4.3, the extracted amount of the analytes by the SPME fibre is plotted vs. the product $C_a \cdot t$ (equation 4.3). As can be seen in Figure 4.3, for all the odorous compounds significant correlations were obtained until 20 min. Consequently, external calibration with an extraction time of 10 min was selected to avoid extending the total analysis time for each sample.

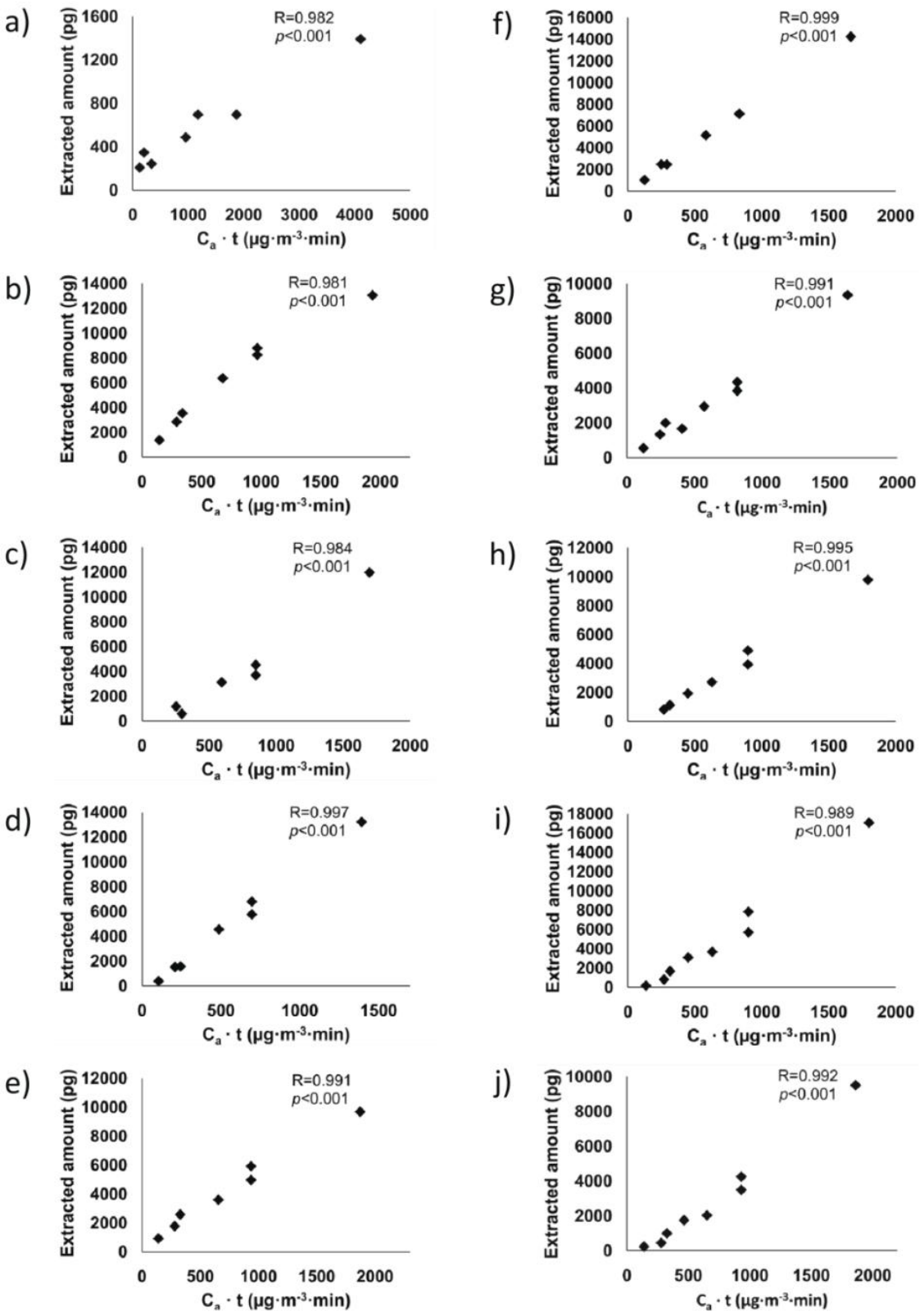

Figure 4.3. Correlation curves obtained by applying Fick's law-based model for the studied compounds: (a) DMDS, (b) Phenol, (c) Octanal, (d) Limonene, (e) *m*-Cresol, (f) Nonanal, (g) Benzothiazole, (h) Carvone, (i) Indole, (j) Skatole.

4.3.3 METHOD VALIDATION

Linearity ranges and LODs were evaluated for the SPME method. Linearity was confirmed for all compounds from the residual plots (data not shown) in the ranges shown in Table 4.2, with r^2 values greater than 0.98. Each concentration level was analysed in triplicate. LODs were determined applying the $3s_{blank}$ criteria. Standards at reduced concentrations (between LOD and LOQ values) were measured five times and the standard deviation obtained was chosen as s_{blank}. The obtained values, as well as the LOQ values ($10s_{blank}$ criteria), are shown in Table 4.2. As can be seen, LODs for most of the compounds were below their odour threshold concentration, even for the aldehydes octanal and nonanal, that could only be detected at a concentration greater than 20 $\mu g \cdot m^{-3}$. LOD for aldehydes can be improved by using SPME together with in-fibre derivatisation with PFBHA [34], but this makes the experimental procedure more complicated and usually introduces an important source of error for the rest of the compounds. For this reason, we keep using the method proposed here which is simpler and still allows the investigation of these compounds at a concentration level close to their OTCs.

Inter-day precision of the method was also tested (n=5). Gaseous standards containing about 4 and 20 $\mu g \cdot m^{-3}$ of each compound were used, except for octanal and nonanal, whose concentration was 85 $\mu g \cdot m^{-3}$. Values of between 12% and 24% were obtained (Table 4.2), which can be considered as acceptable according to the AOAC International recommendations and Horwitz function [48]. Trueness of the method was also assessed by calculating the relative bias (%), i.e. the difference between the measured and the theoretical concentration of gaseous standards prepared in the laboratory. For that, standards containing 4 and 30 $\mu g \cdot m^{-3}$ of each compound (85 $\mu g \cdot m^{-3}$ for octanal and nonanal) were analysed (n=5). The obtained bias values, which mostly ranged from 0.1 to 10%, are showed in Table 4.2. As can be seen, appropriate biases were obtained for all compounds at the two concentrations evaluated. DMDS, *m*-cresol and benzothiazole were the compounds which gave the highest biases (between 13 and 22.2%) and that were obtained when the lowest concentration level was assessed.

Table 4.2. Quality parameters obtained in the analysis of gaseous standards. (-): not determined.

							Inter-day precision (RSD, %)		Relative bias (%)	
Compound	**Working range (μg·m^{-3})**	**a (s_a) (×10^4)**	**b (s_b) (×10^4)**	**r^2**	**LOD (μg·m^{-3})**	**LOQ (μg·m^{-3})**	**4 μg·m^{-3}**	**20 μg·m^{-3}**	**4 μg·m^{-3}**	**30 μg·m^{-3}**
DMDS	0.4 – 40	1.9 (0.8)	1.09 (0.03)	0.9959	0.1	0.4	16	15	-22. 2	-0.1
Phenol	0.6 – 40	4 (2)	1.82 (0.07)	0.9955	0.2	0.6	24	16	9.4	-1.5
Octanal	70 – 100	0.04 (0.12)	0.041 (0.002)	0.9879	20	70	-	13	-	-2.6
Limonene	2.2 – 40	0.8 (0.4)	1.6 (0.02)	0.9993	0.7	2.2	20	16	8.9	-1.9
m-Cresol	1.3 – 40	6 (2)	1.75 (0.08)	0.9932	0.4	1.3	17	15	13.0	-1.1
Nonanal	70 – 100	0.1 (0.7)	0.31 (0.01)	0.9934	20	70	-	17	-	5.2
Benzothiazole	2.6 – 40	0.8 (1.6)	1.7 (0.1)	0.9905	0.8	2.6	12	13	-14.5	4.8
Carvone	2.9 – 40	2.0 (0.9)	0.49 (0.04)	0.9842	0.9	2.9	12	19	4.9	5.2
Indole	0.8 – 40	4 (3)	2.1 (0.1)	0.9917	0.2	0.8	16	16	-5.9	6.9
Skatole	1.0 – 40	2 (2)	2.04 (0.09)	0.9920	0.3	1.0	14	18	3.9	-2.2

4.3.4 DETERMINATION OF THE PARTITION COEFFICIENTS

Partition coefficients can be used as indicators of the tendency of the compounds present in aqueous matrices and oils to be exchanged with the air phase. The partition coefficient (p_c, dimensionless) can be defined as the concentration of VOC at equilibrium at the interface between air and water:

$$p_c = \frac{C_g^*}{C_L^*} \quad (4.4)$$

where C_g* is the gaseous VOC concentration at equilibrium and at a constant water temperature and C_L^* is the equilibrium VOC concentration in the aqueous phase [14].

Several reports have stated that partition coefficients are affected by parameters associated with the aqueous VOC solution, such as temperature, salinity and initial aqueous concentration [10-12,14,15,49], with the effect of temperature on p_c being the most evaluated factor. Sample size is another factor that can affect equilibrium [45] and, consequently, some authors have evaluated different volume ratios of gas to liquid phases. For example, Cheng et al. [14] compared three volume ratios and the statistical analyses showed that differences were non-significant. In other cases, a constant volume ratio of gas to liquid phases is employed [15,50]. In the present study, partition coefficients were determined for a single volume ratio and temperature (22 ± 1°C). We used a 250 mL glass bottles containing a volume (V_L) of 125 mL spiked solution prepared in Milli-Q water (volume ratio 1:1). The initial aqueous concentration (C_L) was also maintained constant and the selected values are summarised in Table 4.3. As can be seen in the table, the values were similar to the concentration levels found in influent water samples from three different WWTPs located in Girona (Spain), except for octanal, nonanal, benzothiazole and carvone. In the case of the two aldehydes, benzothiazole and carvone, the chosen concentrations were higher than the values found in real samples to ensure their detection in the gas phase.

Table 4.3. Concentrations ($\mu g \cdot L^{-1}$) for the studied compounds found in wastewater samples at different WWTPs. Standard deviations are showed in parenthesis. n.d.: not detected. Last column shows the selected values for the spiked water sample used in the determination of partition coefficients.

Compound	Palamós	Blanes	Castell	Spiked water
DMDS	4.9 (0.3)	0.47 (0.09)	1.898 (0.002)	3
Phenol	28.9 (0.8)	37 (3)	17 (1)	30
Octanal	n.d.	n.d.	n.d.	3
Limonene	2.6 (0.1)	4.3 (0.3)	11.8 (0.7)	3
m-Cresol	> 350	> 350	54 (2)	350
Nonanal	n.d.	0.84 (0.06)	n.d.	3
Benzothiazole	n.d.	n.d.	n.d.	6
Carvone	0.5 (0.1)	0.73 (0.03)	0.75 (0.02)	6
Indole	70 (20)	49 (7)	8.7 (0.4)	65
Skatole	100 (20)	23 (4)	5.6 (0.1)	100

From equation (4.4) and using the corresponding mass balance, the following expression can be derived:

$$p_c = \frac{C_g^*}{\left(\frac{V_L C_L - C_g^* V_g}{V_L}\right)} = \frac{C_g^* V_L}{V_L C_L - C_g^* V_g} \quad (4.5)$$

Preliminary experiments were carried out to determine the partition equilibrium time (n=3). As can be observed in Figure 4.4, for all of the compounds equilibrium was reached after 1 hour. An equilibrium time of 5 h was used to calculate the gas-liquid partition coefficients and values obtained are depicted in Table 4.4. As can be seen, the highest values were for limonene and DMDS (0.13 and 0.09, respectively) while phenol and indole presented the lowest ones (0.0004). No reference values for the target compounds were found in the literature. From the data made available in studies undertaken by Dewulf and co-workers [12], we can conclude that the obtained coefficients are much lower than those for benzene and toluene (0.194 and 0.224, respectively). However, Cheng et al. determined partition coefficients around 0.002-0.001 for more polar compounds such as methyl ethyl ketone or *iso*-butanol [14].

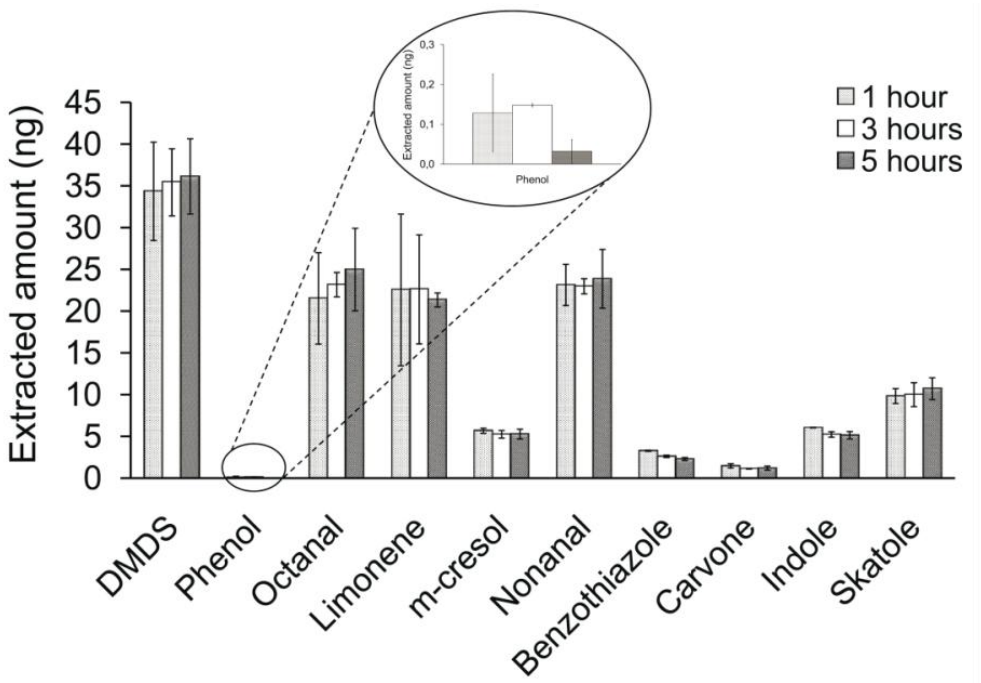

Figure 4.4. Extracted amount of the analytes using different partition equilibrium times in the analysis of synthetic aqueous solutions (n=3).

Table 4.4. Partition coefficient (p_c) values obtained for the target compounds applying the proposed method. Three independent samples were analysed by SPME (n=2).

Compound	p_c	SEM [a]
DMDS	0.09	0.01
Phenol	0.0004	0.0002
Octanal	0.043	0.04
Limonene	0.13	0.05
m-Cresol	0.00020	0.00004
Nonanal	0.070	0.006
Benzothiazole	0.0022	0.0008
Carvone	0.0018	0.0001
Indole	0.00046	0.00009
Skatole	0.0008	0.0001

[a] Standard error of the mean.

In Table 4.4 it can also be observed that for octanal and limonene the precision is poor. As it was found in the previous chapter, this can be attributed to the presence of these compounds in the ambient air and in the water used to prepare the spiked samples. This fact introduces a source of variation which is reflected in the results.

4.3.5 ANALYSIS OF AIR SAMPLES FROM A WWTP

The developed method was applied to the analysis of samples obtained from a WWTP in Castell-Platja d'Aro (Girona, Spain). Gaseous samples were taken close to the plant influent and the primary effluent as well as at the sludge pre-treatment area, during the period from July 2011 to July 2012. Figure 4.5 illustrates the extracted chromatograms of air samples taken at the three sampling points of the WWTP.

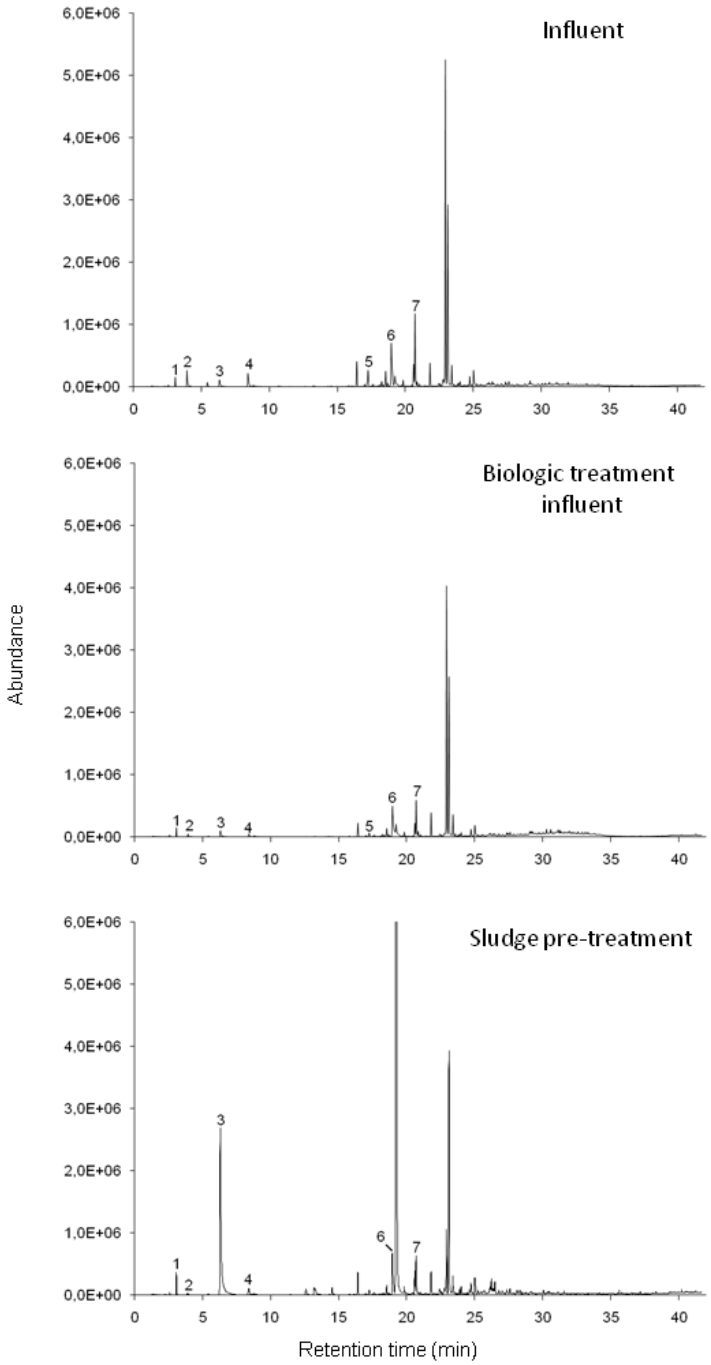

Figure 4.5. Extracted chromatogram (m/z ratios showed in bold in Table 4.1) of air samples taken at the three sampling points of the WWTP, analysed using the selected experimental conditions. 1. Carbon tetrachloride; 2. DMDS; 3. Toluene; 4. Tetrachoroethene; 5. Camphene; 6. Phenol; 7. Limonene.

In Table 4.5, the concentrations found for the target compounds are summarised. DMDS, phenol and limonene were the only compounds detected in the air samples. The maximum value for DMDS in the samples evaluated was 16.6 $\mu g \cdot m^{-3}$ (in the sludge pre-treatment zone), while concentrations around 200 $\mu g \cdot m^{-3}$ were reported in other works [2,33]. The lower values obtained in the present study may be explained by the characteristics of the monitored WWTP, which only receives domestic wastewaters (no industrial inputs) from an equivalent population of 175,000 habitants. Limonene was found at a concentration similar to the values reported previously [33]. With respect to phenol, we found a maximum concentration of 18.5 $\mu g \cdot m^{-3}$ in the biologic treatment influent. Values ranging between 3 and 5 $\mu g \cdot L^{-1}$ were reported in gas cow slurries of intensive production farms [51], which indicates the biological origin of this compound. Moreover, it should be emphasised that the concentration values found for DMDS, limonene and phenol do not surpass their corresponding odour threshold values.

Table 4.5. Results obtained in the analysis of WWTP gas samples. Concentrations in $\mu g \cdot m^{-3}$. n.d.: not detected.

Compound	Influent (n=4)	Biologic treatment influent (n=4)	Sludge pre-treatment (n=3)
DMDS	7.6 – 12.7	<LOQ – 8.7	4.8 – 16.6
Phenol	n.d. – 11.4	<LOQ – 18.5	2.1
Octanal	n.d.	n.d.	n.d.
Limonene	7.8 – 41.0	<LOQ – 19.0	n.d. – 17.0
m-Cresol	n.d.	n.d.	n.d.
Nonanal	n.d.	n.d.	n.d.
Benzothiazole	n.d.	n.d.	n.d.
Carvone	n.d.	n.d.	n.d.
Indole	n.d.	n.d.	n.d.
Skatole	n.d.	n.d.	n.d.

m-Cresol, octanal, nonanal, benzothiazole, indole, skatole and carvone were not detected in any air sample, despite being found in the wastewater samples taken at the same sampling points. This finding can be related with the low partition coefficients calculated for these compounds, which indicate a higher affinity for the liquid phase. Furthermore, it must be considered that most of the samples were taken from open spaces and concentration levels are influenced by the dispersion of emission gases into the atmosphere. The air sample corresponding to the pre-treatment sludge area, which is a confined space, did not show a different pattern when compared to the other samples taken in the open air zones. The malodorous perception in this zone can be assigned to the high concentrations of H_2S (compound routinely measured by the personnel working in the plant).

Taking advantage of the capability of the SPME-GC/MS method, screening of other analytes was performed and the results are summarised in Table 4.6. A total of 26 substances including sulphur-containing compounds, amines, aldehydes, aromatic compounds, and acetic acid were identified in the air samples. Those compounds which were also found in the blank chromatogram and as a consequence their provenance is not 100% certain are indicated in the table. As discussed before, the presence of background contamination (mainly some organic solvents or compounds originated from the vegetation in the surrounding zone) was detected. Our results are in agreement with data obtained by other studies. Dincer et al. [2] identified 29 compounds belonging to four different types of chemicals (sulphur-containing compounds, aldehydes, monoaromatics and halogenated compounds) in odorous emissions from a large urban WWTP. Fourteen of these compounds were also found to be present in the air samples analysed in our study. Zarra et al. [52] characterised the odour sources and the volatile substances that cause annoyance in small WWTPs. They identified and quantified a total of 39 substances including sulphur-containing compounds, aldehydes and ketones, aromatics, terpenes, alcohols, hydrocarbons, and volatile fatty acids. Seven out of these 39 compounds were also identified in our samples. Finally, we identified dimethyl sulphide and carbon disulphide, which have also been detected in the air from some sewage treatment plants [31,53].

Table 4.6. Compounds identified in the air samples with their retention times and Kovats retention indexes. Theoretical Kovats indexes obtained from NIST MS Seach 2.0 library are showed in parenthesis. (-): not determined.

Compound	Retention time (min)	Kovats retention index	Influent	Biologic treatment influent	Sludge pre-treatment
Methyl mercaptan[b]	1.44	-	✓	✓	✓
Trimetilamine[b]	1.56	-	✓		
Dimethyl sulfide[a]	1.73	523.3 (526)	✓	✓	✓
Methylene chloride[b,*]	1.81	-			
Carbon disulfide[b]	1.84	-	✓	✓	✓
Acetic acid[b]	2.32	-	✓	✓	✓
1,1-Dicloroethene[b]	2.40	-	✓	✓	✓
Chloroform[a]	2.59	616.7 (615)	✓	✓	✓
Carbon tetrachloride[b,*]	3.09	-			
Trichloroethene[b]	3.95	-	✓		
Toluene[a,*]	6.33	768.2 (762)			
Tetrachloroethene[b]	8.43	-	✓	✓	✓
Ethylbenzene[a]	12.63	867.4 (855.5)	✓	✓	✓
m,p-Xylene[a]	13.22	876.4 (861.7)	✓	✓	✓
o-Xylene[a]	14.54	891.1 (888)	✓	✓	✓
N,N-Dimethylacetamide[b]	13.72	-			✓
Heptanal[a]	15.41	901.5 (898)	✓	✓	✓
Camphene[a]	17.26	961.0 (954)	✓	✓	✓

Table 4.6 ***(continued).*** Compounds identified in the air samples with their retention times and Kovats retention indexes. Theoretical Kovats indexes obtained from NIST MS Seach 2.0 library are showed in parenthesis. (-): not determined.

Compound	Retention time (min)	Kovats retention index	Influent	Biologic treatment influent	Sludge pre-treatment
1-Ethyl-2-methylbenzene[b]	18.35	-	✓	✓	✓
1,3,5-Trimethylbenzene[b]	18.71	-	✓	✓	✓
1,2,4-Trimethylbenzene[b]	19.39	-	✓	✓	✓
3-Carene[b]	19.83	-	✓	✓	✓
1,2-Diclorobenzene[a]	20.41	1044.3 (1045.72)	✓	✓	✓
Acetophenone[a]	22.43	1071.7 (1066)	✓	✓	✓
(1-Methoxy-1-methylethyl)-benzene[b]	22.93	-	✓	✓	✓
α,α-Dimethylbenzylalcohol[b]	23.11	-	✓	✓	✓

[a] Identification based on the retention time and Kovats retention index.
[b] Identification based on the comparison of mass spectra with mass spectra from the library (NIST MS Search 2.0).
* Compounds found in the blank chromatogram.

4.4 CONCLUSIONS

A SPME/GC-MS approach has been developed to simultaneously determine compounds belonging to different chemical families in air samples. The co-adsorption conditions have been studied for nine compounds, including volatile sulphides, phenols, aldehydes, terpenes, and amines (indole and skatole). An extraction time of 10 min has been selected in order to avoid analyte discrimination and saturation of the coating. Performance of the present method has been demonstrated in terms of linearity and precision (RSD ranging from 12% to 24%). Appropriate detection limits, ranging between 0.1 and 0.9 $\mu g \cdot m^{-3}$, were found for the target compounds, excluding octanal and nonanal which presented higher values. The proposed method has been applied to the determination of gas-liquid partition coefficients of the target compounds. In air samples from WWTPs only DMDS, phenol and limonene were found, which were present at concentrations under their odour threshold value. Moreover, the developed method allowed the identification of a total of 26 compounds.

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CHAPTER 5:

Development of a method for the monitoring of odour-causing compounds in atmospheres surrounding wastewater treatment plants

5.1 INTRODUCTION

Interest in the characterization of individual VOCs in air has increased over the last decades as their emissions have been associated with environmental effects [1,2] and several adverse effects on human health [3,4]. Malodour pollution associated to industrial or animal activities has become an important issue because human tolerance to offensive smells is gradually decreasing [5]. Some VOCs have been related to the perception of odours in environments such as the vicinity of WWTPs [6-8] or animal facilities [9].

Gaseous emissions from WWTPs are complex mixtures which include many different volatile compounds. The complexity of these emissions and the low odour threshold limits of many odorous compounds points to the need for highly sensitive analytical techniques for the determination of target VOCs. Sampling and preconcentration have been performed in different ways: active or passive sampling using adsorption tubes followed by TD-GC [6-8,10-17], grab sampling followed by SPME as the preconcentration technique [18,19,20], and other recently developed methodologies such as micro sorbent-traps [21].

Solid sorbent capture has become a reference methodology for the sampling of VOCs in air samples [13]. There is a wide variety of commercially available sorbents and the selection of the most appropriate one depends on the physical and chemical characteristics of the target analytes, the time of sampling, the sampling volume, and the presence of other interfering compounds (matrix effect). Dincer et al. [6] collected air samples from WWTPs and sludge management areas using a Tenax TA/Carboxen 1000 trap and identified 29 compounds belonging to different chemical families. A group of seven volatile organic sulphur compounds was determined in air samples from two WWTPs using a dual-bed of Tenax TA/Unicarb [7]. Ambient air samples from different locations of Delhi were analysed for the determination of toxic organic compounds using an activated charcoal sorbent tube and a total of 77 VOCs were identified [15], many of them appearing in the US EPA list of hazardous air pollutants. Another method was developed for the determination of VOCs in an urban airborne environment close to a municipal incinerator, a waste collection centre and a wastewater treatment plant [8]. Tubes containing Chromosorb-106 were used and a total of 148 VOCs were identified. Air-quality and malodorous episodes in urban, rural and industrial environments were evaluated with a method using multisorbent tubes containing Carbotrap/Carbopack X and Carboxen 569 [11]. 48 VOCs were analysed in air samples collected near to an industrial complex and a petroleum refinery in Singapore using Tenax/Carbopack X sorbent tubes [22]. High concentrations of toluene, ethylbenzene, xylene isomers, 2-butanone, and hexane were

constantly detected. Three types of sorbent tubes were evaluated for the analysis of 12 VOCs considered as representative of emissions from sewer networks [17]. The compounds that showed the largest levels were trichloromethane, toluene, xylene isomers, and limonene. Active sampling on solid sorbents presents good stability of the target analytes during transport and storage (several months [23]) and allows on-site concentration of the compounds.

In an attempt to go one step further, a new study has been undertaken to evaluate solid sorbent capture as a preconcentration technique for odorous VOCs in the air of WWTPs. A method based on active adsorption in multi-bed sorbent tubes, thermal desorption with cryofocusing in a cold trap, and GC-MS analysis has been evaluated for the determination of a group of 16 VOCs including odour-causing compounds belonging to different chemical families and ozone precursors, whose determination is recommended by European legislation regarding ozone in ambient air (EU Directive 2002/3/CE). The thermal desorption conditions have been evaluated and the developed method has been validated. The proposed method has then been applied to the analysis of air samples from two wastewater treatment plants.

5.2 EXPERIMENTAL

5.2.1 CHEMICALS

The standards contained a mixture of BTEX at 2000 $mg \cdot L^{-1}$ (BTEX Mix in Methanol, Supelco, Bellefonte, PA, USA) and the individual standards of 1,4-dioxane (99.8%), dimethyl disulphide (DMDS, 99%), 1,2,3-trimethylbenzene (90%), (R)-(+)-limonene (99%), 1,4-diethylbenzene (96%), *m*-cresol (99.7%), nonanal (95%), benzothiazole (90%), (-)-carvone (99%), indole (99%), and skatole (98%). All of these compounds were obtained from Sigma-Aldrich (Steinheim, Germany). Phenol (99.5%) was obtained from Dr. Ehrenstorfer (Augsburg, Germany).

Stock solutions in methanol for gas chromatography with purity >99.9% (SDS, Peypin, France) were prepared and stored at 4^{o}C for up to a week. Working solutions were prepared at the moment of calibration.

5.2.2 SORBENT TUBES

Commercially available stainless-steel tubes from Markes International Limited (Llantrisant, UK) (89 mm length × 6.4 mm o.d. × 5 mm i.d.) were used. Two sorbent configurations were

evaluated: firstly, a single sorbent trap with 150 mg of Tenax TA; and, secondly, a two-sorbent bed containing 350 mg of Tenax TA/Carbograph 1TD. Before use, tubes were activated and conditioned by passing 99.9990% pure nitrogen gas (Carburos Metálicos, Barcelona, Spain) at a flow of 100 mL·min^{-1}. The two-sorbent bed cartridges were conditioned at 100, 200, 300 and 335^{o}C for 1h each, while Tenax TA cartridges were activated at 320^{o}C for 2h followed by 30 min at 335^{o}C, in accordance with the supplier's recommendations.

5.2.3 SAMPLING

Air samples were obtained from two WWTPs located in Reus (Tarragona, Spain) and Castell-Platja d'Aro (Girona, Spain). A Sidekick air sampling pump (SKC Ltd., Dorset, UK) was used, which was calibrated using an ADM 3000 digital flow-meter (Agilent Technologies, Palo Alto, CA, USA). Air samples were pumped through preconditioned tubes at a flow rate of 35 mL·min^{-1} to collect one litre air volume. After sampling, tubes were immediately sealed with end caps fitted with PTFE ferrules and stored at 4^{o}C in glass jars. They were then transported to the laboratory, stored in a refrigerator at 4^{o}C, and analysed before 24h.

5.2.4. TD-GC-MS EQUIPMENT AND CONDITIONS

Desorption was carried out in a UNITY thermal desorber (Markes). The sorbent tube was loaded from an ULTRA automatic sampler (Markes). For tube desorption, the sorbent tube was heated to 275^{o}C for 10 min with a helium flow rate of 30 mL·min^{-1}. The desorbed compounds were refocused into a general purpose hydrophobic cold trap filled with Tenax TA/Carbograph 1TD (Markes) at –10^{o}C using splitless mode. The next step consisted on the cold trap desorption, which was carried out at 300^{o}C for 10 min using a split flow of 10 mL·min^{-1} (split ratio 1:10).

Analytes were separated and detected in a 6890N gas chromatograph and a 5973 inert mass spectrometer (Agilent Technologies). A ZB-5 capillary column (60 m × 0.32 mm i.d., 1 µm film thickness) from Micron Phenomenex (Torrance, CA, USA) was used with 99.9990% pure helium (Carburos Metalicos) as the carrier gas at a constant inlet flow rate of 1 mL·min^{-1}. The oven temperature was initially held at 40^{o}C for 5 min; ramped at 5^{o}C·min^{-1} to 150^{o}C and then ramped at 15^{o}C·min^{-1} to 250^{o}C, and held for 5 min: the whole run lasted 38 min.

The GC-MS interface was set at 280°C. The mass spectrometer acquired data in scan mode with an *m/z* interval from 40 to 300 amu and an electron impact energy of 70eV. Chromatographic data was acquired by means of MSD ChemStation software (Agilent Technologies). Compounds were quantified by a target ion and identified by qualifier ions and retention times. The target compounds are shown in Table 5.1 with their respective OTCs and details of the GC-MS analysis.

Table 5.1. Target compounds in chromatographic elution order with their retention times, OTCs and *m/z* ratios. n.a.: not available.

Compound	OTC ($\mu g \cdot m^{-3}$) [a]	Retention time (min)	m/z [b]	Boiling point (°C)
1,4-Dioxane	n.a.	13.2	57, 58, **88**	101
DMDS	303	14.9	45, 79, **94**	110
Toluene	80	16.0	**91**, 92	111
Ethylbenzene	400	20.3	**91**, 106	136
m-Xylene	850	20.7	**91**, 105, 106	139
p-Xylene	570	20.7	**91**, 105, 106	138
o-Xylene	850	21.7	**91**, 105, 106	144
Phenol	39, 46	25.1	66, **94**	182
1,2,3-Trimethylbenzene	n.a.	26.8	**105**, 120	175
Limonene	55000	26.9	68, **93**	178
1,4-Diethylbenzene	n.a.	27.8	105, **119**	183
m-Cresol	0.57	28.3	79, **107**, 108	203
Nonanal	13.3	28.9	81, 98, **143**	195
Benzothiazole	n.a.	32.1	108, **135**	227
Carvone *(2-methyl-5-(1-methylethenyl)-2-cyclohexenone)*	85, 150	32.2	82, 108, **151**	231
Indole	7.1	33.1	90, **117**	253
Skatole *(3-methylindole)*	0.35, 0.5	34.4	**130**, 131	265

[a] Compendium data from [6] and [24].
[b] Quantifier ion in bold.

For calibration purposes, liquid standards were loaded into sorbent tubes using a Calibration Solution Loading Ring (Agilent Technologies). A conventional GC syringe was used to inject volumes of between 1 and 5 µL of each standard while a flow of 100 $mL \cdot min^{-1}$ of 99.9990% pure helium passed through the tube flowing in the direction of the injection. After the injection, the syringe needle was maintained within the loading ring for 20 seconds to achieve complete evaporation of the target analytes, as recommended by the manufacturer. The tube was immediately desorbed.

5.3 RESULTS AND DISCUSSION

5.3.1 METHOD DEVELOPMENT

In this chapter, a list of compounds belonging to different chemical families, presenting different volatilities and chromatographic behaviour, was selected. The compounds included aromatic compounds, aldehydes, phenolic compounds, sulphur-containing compounds, and terpenes. Chromatographic conditions were evaluated in order to obtain a good separation of all the target compounds within a reasonable analysis time (Figure 5.1). The final temperature programme was set as described in Section 5.2.4.

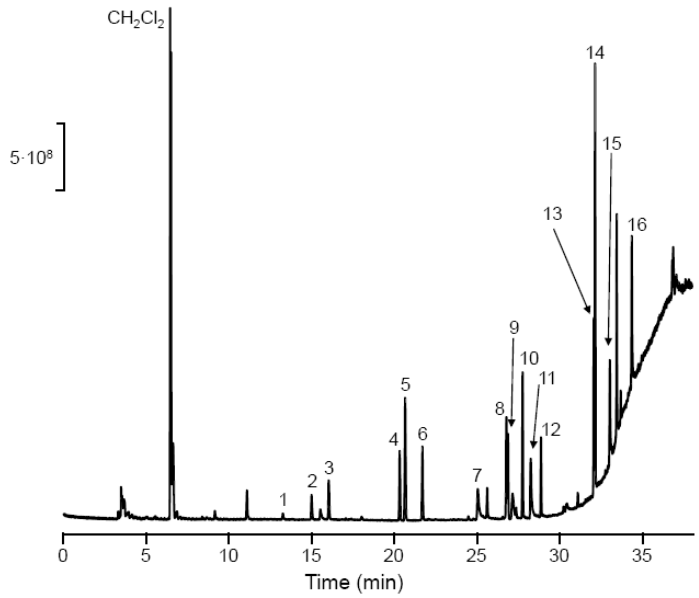

Figure 5.1. Total ion chromatogram (TIC) of an standard sample analysed with the GC conditions indicated in Section 5.2.4. The main peak at 6.5 min correspond to dichloromethane, the solvent used for the preparation of the stock solutions. 1. 1,4-Dioxane; 2. DMDS; 3. Toluene; 4. Ethylbenzene; 5. *m-,p*-Xylene; 6. *o*-Xylene; 7. Phenol; 8. 1,2,3-Trimethylbenzene; 9. Limonene; 10. 1,4-Diethylbenzene; 11. *m*-Cresol; 12. Nonanal; 13. Benzothiazole; 14. Carvone; 15. Indole; 16. Skatole.

Due to the different characteristics of the studied analytes, special attention was given to the evaluation of memory effects since semi-volatile compounds can undergo partial desorption and accumulation in the transfer lines. For this reason, blank chromatograms were acquired after each analysis. The amount of the standards introduced in the sampling tubes was also controlled in order to avoid overloading.

5.3.1.1 Selection of sorbent tube

In order to check the best sorbent tube for the retention of the studied compounds, two tubes were tested, one containing Tenax TA and the other containing Tenax TA/Carbograph 1TD. Tenax TA is a weak strength and hydrophobic sorbent. It has been used in the determination of non-polar VOCs, slightly polar VOCs, terpenes, aldehydes>C_5, and acids<C_3 [25]. Volatile organic sulphur compounds have also been analysed using cartridges containing this sorbent [7]. It has been found that Tenax TA retains quantitatively, without showing significant breakthrough, VOCs with boiling points above 100^{o}C [11]. Carbograph 1TD is a hydrophobic, medium strength sorbent and is commonly used in the analysis of different VOC groups, such as a wide range of aromatic compounds and chlorinated compounds [12,26,27].

For thermal desorption, the initial conditions were set within the range of conditions recommended by EPA method TO-17 [13]. Tube desorption was carried out at 275^{o}C for 15 min using a flow of 30 mL·min^{-1} in splitless mode. Cold trap desorption was performed at 300^{o}C for 10 min with a split flow of 15 mL·min^{-1}. 25 ng of the studied compounds were loaded in both tubes (n=3 for each tube). No significant differences (p>0.05, t-test) were obtained for most compounds using the two types of sorbent tubes except for 1,2,3-trimethylbenzene (p=0.047), 1,4-diethylbenzene (p=0.035) and carvone (p=0.042) (Figure 5.2). In those cases showing differences, responses were higher when the two-bed tube was used. Wang et al. [17] compared the same two sorbents for a group of 12 VOCs and found no differences between the two sorbents for the compounds evaluated, except for decane that gave higher responses with the dual bed trap.

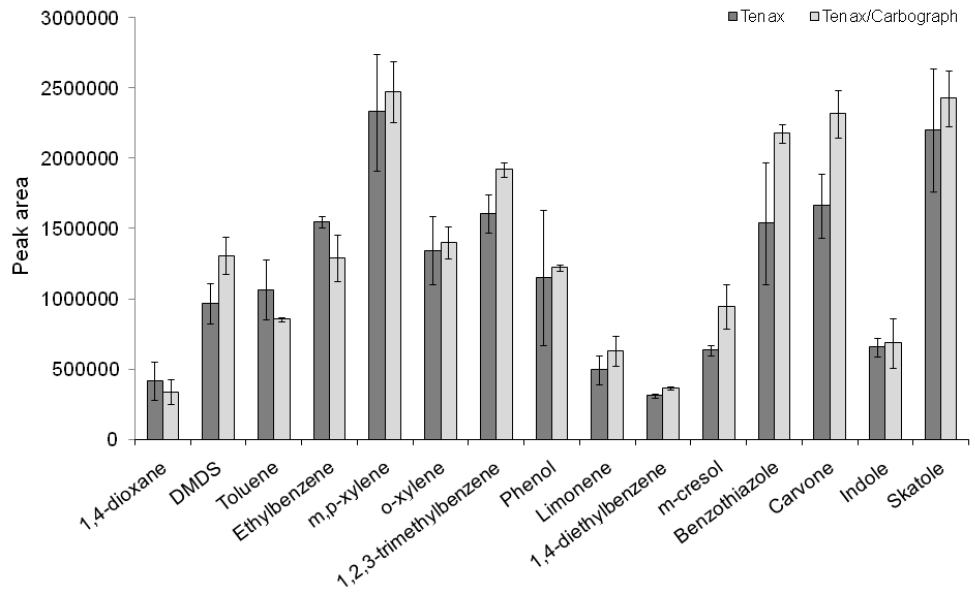

Figure 5.2. Peak areas obtained in the analysis of 25 ng of the studied compounds using the two tested traps and working with thermal desorption conditions within the ranges recommended by EPA method TO-17 (n=3).

Blank (carryover) analyses were also compared and no significant differences were observed between the two types of sorbent tubes. As a result, the dual bed Tenax TA/Carbograph 1TD tube was selected for further experiments.

5.3.1.2 Selection of the thermal desorption parameters

The sorbent tube and cold trap desorption parameters were evaluated to ensure the best desorption conditions. Tubes containing the dual bed Tenax TA/Carbograph 1TD and loaded with 25 ng of the target compounds were analysed (n=3 in each case).

5.3.1.2.1 Cold trap desorption

The cold trap conditions were first evaluated to ensure that quantitative desorption from the cold trap is achieved and to avoid memory effects. Temperatures of between 250 and 320^{o}C and times of 3 and 10 min were tested, maintaining the initial conditions for the tube desorption. Blank (carryover) analyses were evaluated after each sample to check whether the compounds were quantitatively desorbed from the cold trap.

For desorption temperature, no differences (p>0.05, ANOVA test) were observed for most analytes at the temperatures evaluated (Figure 5.3). The only exception was phenol (p=0.02), which showed a decrease when the cold trap desorption temperature was set at 320°C. In the case of the less volatile compound evaluated (1,4-dioxane), the variability obtained in the results at the lowest temperature (250°C) was significantly greater than at the other temperatures.

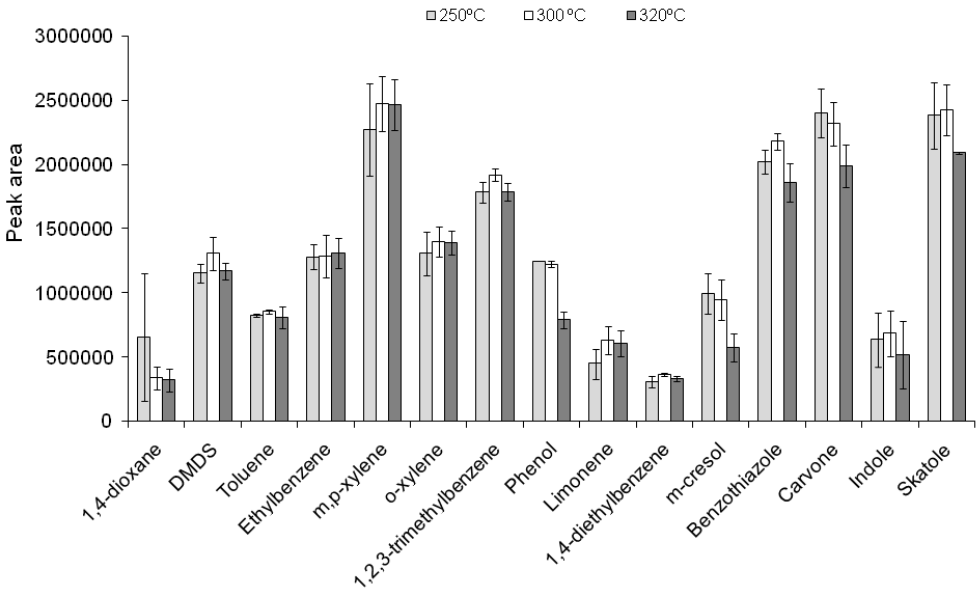

Figure 5.3. Effect of the cold trap desorption temperature on the performance of the method (n=3). Experimental: 25 ng of the studied compounds; tube desorption at 275°C for 15 min at 30 mL·min^{-1} (splitless mode); cold trap desorption for 10 min with a split flow of 15 mL·min^{-1}.

No differences (p>0.05, t-test) were observed for the analytes at the different desorption times evaluated (Figure 5.4), except for benzothiazole (p=0.01) and skatole (p=0.02). Cold trap desorption at 300°C for 10 min was selected in order to ensure the quantitative desorption of analytes and no carryover.

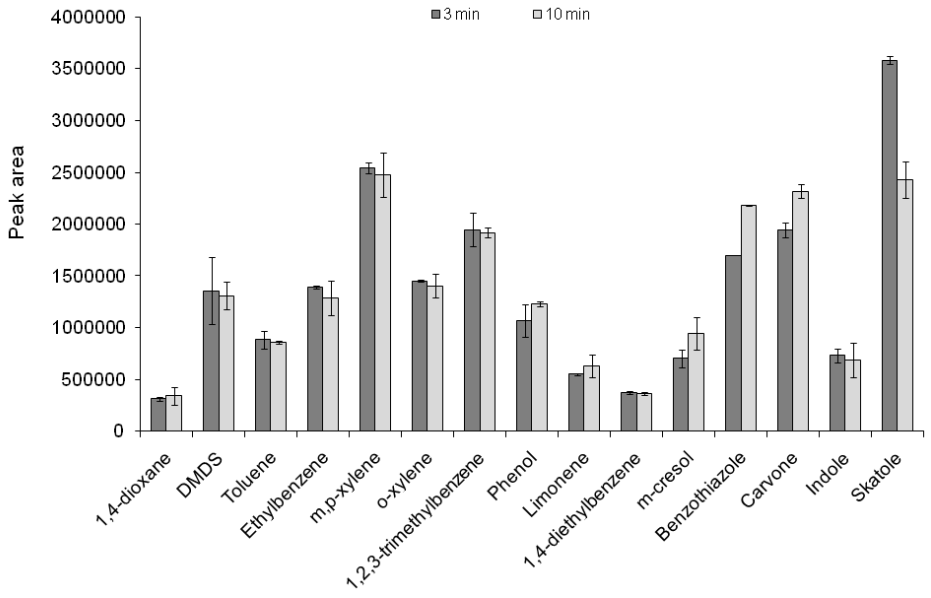

Figure 5.4. Peak areas obtained in the analysis of 25 ng of the studied compounds at different cold trap desorption times. Tube desorption conditions: 15 min at 275°C and a flow of 30 mL·min^{-1}. Cold trap desorption conditions: 300°C and a split flow of 15 mL·min^{-1}.

Different split flow rates were also assessed to evaluate the response level of the analytes. Split flows of 5 and 10 mL·min^{-1} were evaluated and a small decrease in the response of all analytes was observed when the split flow was increased. Despite this reduction in sensitivity, a split flow of 10 mL·min^{-1} was selected in order to prevent the saturation of the capillary column in the analysis of samples containing high amounts of the studied compounds.

5.3.1.2.2 Sorbent tube desorption

The tube desorption conditions were evaluated in order to obtain the highest responses. The cold trap desorption parameters were set at the values selected in the previous section.

Tube desorption times of 5, 10 and 15 min at 275°C were evaluated. Peak areas obtained from standard chromatograms increased for the majority of the studied compounds when desorption time was increased from 5 to 10 min, and did not show significant differences between 10 and 15 min. Blank (carryover) analyses performed after each analysis at the different desorption times tested revealed phenol to be the only compound present in blank chromatograms with relatively large peak areas, which increased with desorption time. This fact could be attributed to the degradation of the sorbents into a wide variety of carbon-

containing compounds [28], which increases with desorption time. A tube desorption time of 10 min was selected to avoid excessive phenol peaks in blank analyses.

Different tube desorption temperatures were also evaluated. Analyses at 275, 300 and 320°C were carried out and no significant differences were obtained in terms of peak area (Figure 5.5). Blank (carryover) analyses were also performed at each desorption temperature and, as previously observed with tube desorption times, only phenol presented peak areas higher than those obtained in the analysis of blank samples, which increased with the tube desorption temperature applied. Taking into account these results, a temperature of 275°C was chosen for tube desorption in order to obtain the best blank chromatograms.

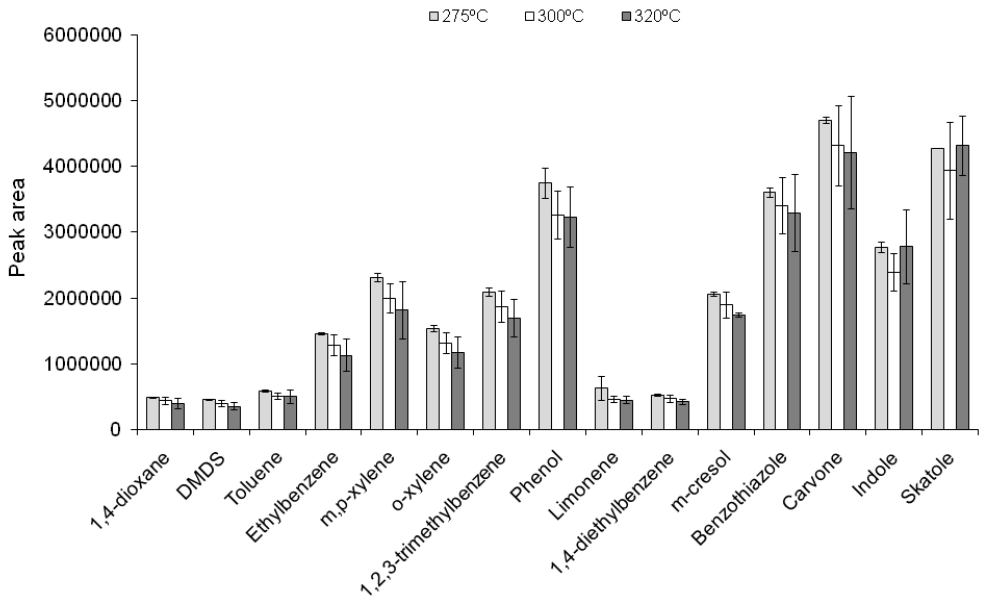

Figure 5.5. Effect of the sorbent tube desorption temperature on the performance of the method (n=3). Experimental: 25 ng of the studied compounds; tube desorption for 10 min at 30 mL·min^{-1} (splitless mode); cold trap desorption at 300°C for 10 min with a split flow of 10 mL·min^{-1}.

Finally, the tube desorption flow was evaluated. Analyses were performed using flows of 30 and 50 mL·min^{-1}. A significant decrease in the signals was observed when the desorption flow was set at 50 mL·min^{-1} for all compounds except for indole and skatole, the least volatile compounds evaluated. This fact indicates that some target analytes are not efficiently retained by the cold trap during primary desorption at high flows and so the desorption flow through the sorbent tube was set at 30 mL·min^{-1}.

5.3.2 BREAKTHROUGH EVALUATION

Breakthrough data are available for many individual sorbent materials but these data has to be used with care as correspond to synthetic samples with no presence of interferences. Safe sampling volume was evaluated to ensure that no breakthrough takes place. Air from the inlet of a WWTP, at a height ~1 m above the water level, was pumped through two sorption tubes filled with Tenax TA/Carbograph 1TD connected in series. Back tubes were analysed to check for target compounds as a means of investigating whether analytes were quantitatively retained in the front tube. Air volumes up to 3 L were sampled at a flow rate of 35 $mL \cdot min^{-1}$.

This sampling point was chosen as high levels of VOCs and large relative humidity are expected in this area. Although the two sorbents used are hydrophobic, the effect of the relative humidity was taken into account as a competition for the adsorbent active surface can occur between water and the target compounds, which reduces the sorption capacity of the sorbent [29].

DMDS, toluene, ethylbenzene, xylenes, phenol, and limonene were detected in the back tube in quantities between 8 and 16% (with respect of a total amount determined from the sum of amounts found in the front and the back tubes) when 3 L of air was sampled. These percentages are excessive as typical VOCs recommended breakthrough values are <5% [13,30]. When 1 L of air was sampled, analytes were quantitatively retained in the front tube. A previous study analysing emissions from two sewer sites in Sydney did not find breakthrough for 2 L samples [17]. A sample volume of 1 L was selected in the present study to prevent breakthrough.

The LODs of the method obtained (Table 5.2) are below the range of concentrations usually found in contaminated atmospheres, such as WWTPs, petrochemical complexes and industrial areas [6-8,16,26,31]. For non-contaminated atmospheres the LODs may not allow the quantification of some of the target compounds [32,33]. However, in this situation, breakthrough volumes will increase significantly and large volumes of samples can be taken, which will lead to a decrease in the LODs. When less contaminated environmental samples have been checked, indoor and outdoor air in the city of Barcelona, the use of a Tenax TA tube showed breakthrough for >10 L sampling volumes [11].

Table 5.2. Quality parameters obtained in standard analysis. (-): not determined.

Compound	Working range [1] ($\mu g \cdot m^{-3}$)	a (S_a)($\cdot 10^5$)	b (S_b)($\cdot 10^5$)	r^2	LOD ($\mu g \cdot m^{-3}$) [2]	Intra-day precision[3] (RSD,%)	Inter-day precision[3] (RSD,%)
1,4-Dioxane	4 - 100	0.6 (0.4)	0.098 (0.005)	0.994	1	3	9
DMDS	1.0 - 100	0.06 (0.12)	0.037 (0.002)	0.995	0.3	3	11
Toluene	1.3 - 300	6 (5)	1.479 (0.008)	0.992	0.4	4	6
Ethylbenzene	3 - 100	5 (5)	1.858 (0.008)	0.995	0.9	1	5
m,p-Xylene	1.0 - 100	8 (9)	3.09 (0.01)	0.995	0.3	3	5
o-Xylene	1.0 - 100	2 (4)	1.507 (0.006)	0.995	0.3	3	7
1,2,3-Trimethylbenzene	0.7 - 100	6 (5)	1.865 (0.005)	0.997	0.2	3	8
Phenol	7 - 100	0.6 (3.4)	1.062 (0.004)	0.994	2	6	15
Limonene	1.0 - 300	0.04 (1.27)	0.42 (0.02)	0.994	0.3	12	19
1,4-Diethylbenzene	1.0 - 100	2 (5)	1.62 (0.08)	0.991	0.3	3	10
m-Cresol	7 - 100	5 (3)	0.83 (0.03)	0.996	2	2	7
Nonanal	67 - 300	1.3 (0.1)	0.028 (0.001)	0.996	20	-	-
Benzothiazole	1.7 - 100	3 (5)	1.51 (0.06)	0.994	0.5	2	11
Carvone	1.0 - 100	0.9 (8.8)	2.2 (0.1)	0.990	0.3	1	6
Indole	1.0 - 100	7 (8)	1.73 (0.09)	0.992	0.3	3	12
Skatole	1.0 - 100	7 (8)	2.76 (0.09)	0.996	0.3	11	18

[1] The lowest calibration level was fixed at the determined LOQs.
[2] Method LODs determined for a sample volume of 1 L.
[3] n=3, 25 ng.

5.3.3 METHOD VALIDATION

External calibration in the ranges shown in Table 5.2 was performed. Each concentration level was analysed three times. Linearity was confirmed for all compounds from the evaluation of the residual plots, with r^2 values greater than 0.99. Standards at reduced concentrations were analysed (n=5) to determine the LODs and LOQs of the method, which are summarised in Table 5.2. The calculated standard deviation for each compound was taken as the standard deviation of the blank, and IUPAC 3σ and 10σ criteria were used to determine LODs and LOQs, respectively. LODs ranged between 0.2 and 2 $\mu g \cdot m^{-3}$ (for a sample volume of 1 L), except for nonanal (LOD=20 $\mu g \cdot m^{-3}$).

For the determination of the precision of the method, desorption analyses (n=3) of 25 ng of the target compounds were performed within the same day (intra-day precision) and between days (inter-day precision). RSDs of between 1 and 12% were obtained for intra-day repeatability and values of between 5 and 19% for inter-day precision (Table 5.2), which were found to meet EPA standards [13].

The stability of the sorption tubes loaded with all target compounds was evaluated after 24 h of storage. The results obtained confirm that there were no significant losses of the target analytes (recoveries >80%). These results agree with a previous study that evaluated stability during storage of 90 VOCs in Tenax TA/Carbograph 1TD tubes [34] in which it was found that there was no significant loss of analytes after 3 and 7 days of storage. All samples evaluated in the present study have been analysed within 24 h of sampling.

5.3.4 ANALYSIS OF AIR SAMPLES

Samples were obtained from the entrance of each plant, the biological treatment inlet and the sludge pre-treatment area. Figure 5.6 illustrates the total ion chromatogram of an air sample taken at the biological treatment inlet of Castell-Platja d'Aro WWTP. It must be taken into account that samples were taken in open areas and that concentration levels in the air are influenced by the dispersion of emission gases into the atmosphere.

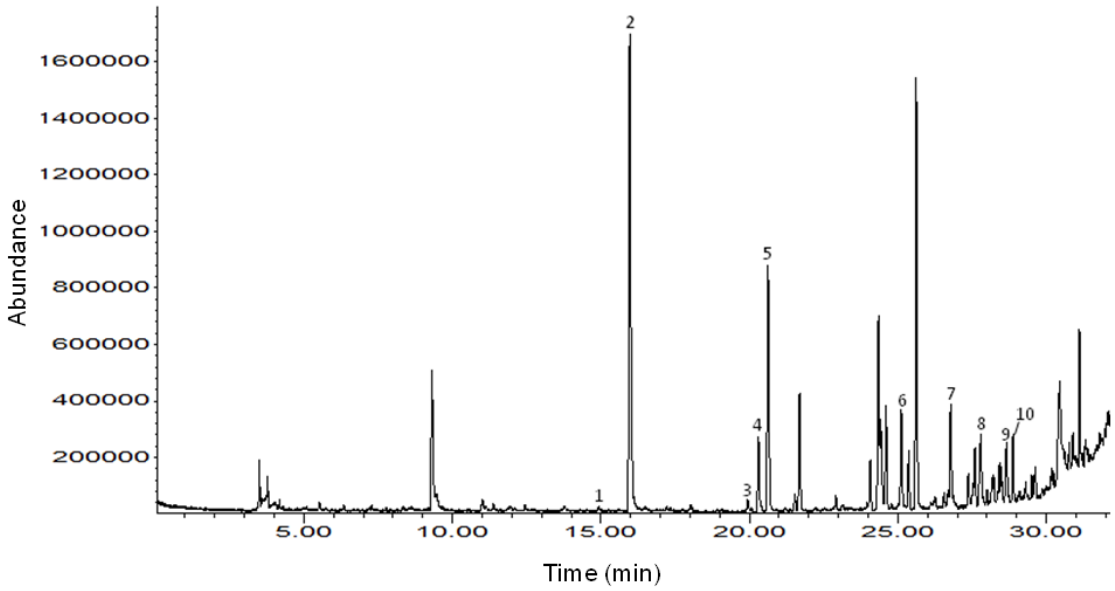

Figure 5.6. TIC of an air sample taken at the biological treatment inlet of Castell D'Aro WWTP. 1. DMDS; 2. Toluene; 3. Ethylbenzene; 4. *m,p*-Xylene; 5. *o*-Xylene; 6. Phenol; 7. Limonene; 8. 1,4-Diethylbenzene; 9. *m*-Cresol; 10. Nonanal.

Eleven of the target VOCs were detected in samples from WWTPs. Table 5.3 shows the concentrations found for the target compounds at each sampling point. Toluene, limonene and nonanal were the compounds found at higher levels, with maximum values of 437.9, 232.6 and 382.4 $\mu g \cdot m^{-3}$, respectively. These values agree with previous studies where WWTPs and sludge management areas were evaluated (Table 5.4).

1,4-dioxane, benzothiazole, carvone, indole, and skatole were not detected in the samples analysed. Other studies confirm that 1,4-dioxane is not usually detected in atmospheres from WWTPs. This compound has only been detected in the air of a petrochemical complex but at a maximum level of 0.9 $\mu g \cdot m^{-3}$ [16], which is below the LOD of the method proposed here. The other four compounds that were not detected were evaluated as they are usually found in water samples from the inlet of the WWTPs evaluated in chapter 3. However, the determination of their gas-liquid partition coefficients indicates the limited partitioning of these compounds to the air surrounding the plants (chapter 4). Therefore, although indole and skatole have previously been detected in air from some sewage treatment plants [8], calculations made taking into account the levels detected in water samples and their calculated partitioning coefficients confirm that air levels well below the LODs are to be expected in the air surrounding the WWTPs.

Table 5.3. Minimum and maximum concentrations found (μg·m^{-3}) at sampled sections from Castell-Platja d'Aro and Reus WWTPs. <LOQ: below limit of quantification, n.d.: not detected.

Compound	Influent		Biologic treatment influent		Sludge pre-treatment	
	Castell-Platja d'Aro (n=3)	Reus (n=3)	Castell-Platja d'Aro (n=1)	Reus (n=3)	Castell-Plaja d'Aro (n=3)	Reus (n=1)
1,4-dioxane	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DMDS	1.4 – 17.8	<LOQ - 1.7	1.9	3.5 - 5.1	3.2 – 11.2	4.2
Toluene	60.2 – 308.8	8.3 - 26.5	213.3	19.7 - 21.8	134.9 – 437.9	9.3
Ethylbenzene	8.1 – 19.4	3.1 - 5.0	25.4	3.4 - 3.6	<LOQ –3.6	4.6
m,p-xylene	11.6 – 31.0	4.0 - 6.8	44.4	4.2 - 4.9	3.7 – 5.1	4.0
o-xylene	6.2 – 16.1	2.7 - 5.4	37.5	5.3 - 6.3	2.3 – 3.4	2.5
Phenol	n.d. – <LOQ	n.d. - 18.9	<LOQ	<LOQ	n.d. – <LOQ	6.1
1,2,3-trimethylbenzene	11.7 – 18.9	4.4 - 6.4	27.5	4.5 - 5.1	3.8 – 4.3	3.8
Limonene	42.2– 232.6	11.9 - 34.8	2.9	2.9 - 5.1	<LOQ – 5.7	1.6
1,4-diethylbenzene	2.0 – 3.5	1.4 - 3.8	4.6	2.0 - 2.3	n.d.	1.4
m-cresol	n.d. – 11.9	n.d. - 8.7	<LOQ	12.0 - 13.8	10.7 – 13.3	8.5
Nonanal	<LOQ – 132.5	<LOQ - 382.4	n.d.	<LOQ - 74.3	n.d. – 78.8	<LOQ
Benzothiazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Carvone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Indole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Skatole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 5.4. Comparison of concentrations found (μg·m^{-3}) in different studies. <LOQ: below limit of quantification, n.d.: not detected. The number in brackets for each column corresponds to the reference number.

	WWTPs and sludge management areas					**Urban areas**			**Present study**
	[6]	[7]	[8]	[17]	[33] [b]	[22]	[12]	[16]	
DMDS	0.6 - 119.6	1.0 - 857.8	2.0 - 39.3	--	212.6	--	--	--	<LOQ - 17.8
Toluene	10.0 – 1356.9		24.7 - 191.8	61.1 -111.3	509.2	3.8 - 90.5	0.4 - 45.2	2.2 - 12.1	8.3 - 437.9
Ethylbenzene	1.2 - 409.2		11.5 - 26.7	--	14.7	0.2 - 28.3	0.1 - 7.4	1.4 - 13.6	<LOQ - 19.4
m,p-Xylene	2.6 - 1254.7		45.6 -169.7 [a]	105.7 - 183.7	47.2	0.2 - 20.0	0.2 - 25.6	0.8 - 3.3	3.7 - 44.4
o-Xylene	2.6 - 935.4			45.7 - 70.6	--	0.2 - 13.9	n.d. - 5.6	0.4 - 1.7	2.5 - 37.5
Phenol	--		--	--	--	n.d. - 2.6	--	--	n.d. - 18.9
1,2,3-Trimethylbenzene	--		--	--	--	0.1 - 3.3	--	n.d. - 0.8	3.8 - 27.5
Limonene	--		--	110.0 - 191.1	114.6	--	--	--	<LOQ - 232.6
1,4-Diethylbenzene	--		--	--	--	--	--	n.d. - 0.3	n.d. - 4.6
m-Cresol	--		n.d. - 24.5	--	--	--	--	--	n.d. - 13.3
Nonanal	--		--	--	19.8	n.d. - 5.6	--	--	n.d. - 382.4

[a] All xylene isomers determined together.
[b] Only maximum values detected are given.

As can be seen from Table 5.3, the presence of toluene, ethylbenzene and xylene isomers in urban areas is significantly smaller than in areas closed to WWTPs, with maximum values being at least one order of magnitude higher in areas closer to WWTPs. Moreover, the contents of these compounds tend to be larger in the vicinities of sludge management areas.

The odorous compounds evaluated in this study are not usually detected or found at levels well below their OTC values in urban areas. However, in the case of WWTPs, some of these compounds are detected at levels above their respective OTCs. In the present study, toluene, *m*-cresol and nonanal gave concentrations above their OTCs in some samples. *m*-Cresol and nonanal were present at concentrations above their odour threshold concentrations in the majority of the samples from the two WWTPs. Other studies (Table 5.4) also showed levels of toluene, *m*-cresol and nonanal above their OTCs in some WWTP samples. Moreover, a study [6] showed odorous levels of ethylbenzene and xylene isomers in the sludge management area of a WWTP.

5.4 CONCLUSIONS

This study shows that thermal desorption followed by GC-MS analysis is an effective procedure for the determination of 16 volatile organic compounds including odour-causing compounds belonging to four different chemical families and ozone precursors. A two-sorbent bed containing Tenax TA/Carbograph 1TD showed the best performance in the concentration of the analytes. Tube and cold trap desorption parameters have been evaluated and the developed method has been validated. Appropriate method detection limits, ranging between 0.2 and 2.0 $\mu g \cdot m^{-3}$, were obtained for the target compounds, excluding nonanal which presented a higher value. The developed method has been applied to the analysis of air samples from two WWTPs. Toluene, limonene and nonanal were the compounds found at the highest concentrations, while 1,4-dioxane, benzothiazole, carvone, indole, and skatole were not detected at all. Only toluene, *m*-cresol and nonanal were detected at concentration levels above their respective odour threshold concentrations in some samples.

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CHAPTER 6:

Monitoring of sixteen fragrance allergens and two polycyclic musks in wastewaters by solid phase microextraction coupled to gas chromatography

6.1 INTRODUCTION

PCPs are a large and diverse class of substances that are used as active ingredients or preservatives in cosmetics, antimicrobials, fragrances and many others. These compounds are grouped into functional classes depending on their use, being the fragrances one of the most extensively used. Fragrances are commonly added to soaps, detergents, cosmetics, and other consumer products in order to give a scent to a product or mask the malodour of other chemical ingredients. The Scientific SCCS has identified 26 of these compounds as likely to cause contact allergies, which are called fragrance allergens [1]. In Europe, their presence in cosmetic products must be indicated on the product label if a limit of 0.01% for rinse-off and 0.001% for leave-on products is exceeded [2].

Fragrances are often found in domestic waters due to their widespread use in everyday products and levels ranging from $ng \cdot L^{-1}$ to $\mu g \cdot L^{-1}$ have been detected in WWTPs [3-19]. However, the main part of these studies are only devoted to the analysis of musk fragrances and found that galaxolide and tonalide (two polycyclic musks) are the most abundant detected in WWTPS. It has been found that although the concentrations of these polycyclic musks decrease along the different treatments, they are not completely eliminated by conventional WWTPs [3-5,7,9,11,12,14,15]. For this reason, these compounds have also been detected in rivers [3,6-8,9,11,13,19,20], lakes [3,6], and reservoirs [3].

Different analytical methods have been proposed for the determination of fragrances, but they are particularly focusing on synthetic musks [3-15,21]. Several procedures have been used for their extraction and concentration. These include LLE [7,13,14,22], SPE [6,8,23,24], SBSE [3,10,11], CLSA [16], USAEME [17], membrane-assisted liquid-liquid extraction (MALLE) [25], and DLLME [25,26]. The suitability of SPME for the analysis of fragrances in waters has been recently verified by Becerril et al. [18]. They applied SPME preconcentration to analyse 24 suspected allergens in bathwater, swimming pool water, and wastewater samples and found the presence of allergens in all sample types. An analytical procedure based on SPME and GC-MS to determine polycyclic musks and earthy-musty compounds in different environmental waters (groundwater, surface water and wastewater effluent) has also been reported [9].

In our knowledge, few studies have been devoted to the specific evaluation of fragrance allergens in WWTPs. Only two studies included a list of 24 [18] and 25 allergens [17] but a very limited number of WWTP samples were evaluated (n=9 and 1, respectively). Other studies only

evaluate a short list of allergens: one specific allergen, such as benzyl salicylate [6,12,19] and limonene [8]; or a set of three, such as limonene, linalool and β-citronellol [16].

In this chapter 16 common fragrance allergens and two polycyclic musks were monitored in a large set of wastewater samples (n=58) over a three-month period. A method based on SPME and GC-MS was optimised and validated before being applied to the monitoring of the target compounds in water samples from two different WWTPs located in Girona (north-east Spain).

6.2 EXPERIMENTAL

6.2.1 CHEMICALS

The 16 fragrance allergens, d-limonene (99%), linalool (95%), β-citronellol (99%), citral cis and trans mixture (96%), geraniol (99%), trans-cinnamaldehyde (98%), hydroxycitronellal (95%), cinnamyl alcohol (98%), eugenol (99.8%), coumarin (99%), lilial (90%), lyral (97%), benzyl benzoate (98%), benzyl salicylate (98%), and benzyl cinnamate (99%), were obtained from Sigma-Aldrich (Steinheim, Germany). The two polycyclic musks, galaxolide and tonalide, were from LGC Standards (Teddington, UK). Sodium chloride (99.9%) and HPLC-gradient grade methanol were supplied by Carlo-Erba Reagents (Milan, Italy). Sodium tiosulphate 5-hydrate (99.5%) was obtained from Panreac Química S.L.U. (Barcelona, Spain). Milli-Q water from a Milli-Q Plus water purification system (Millipore Ibérica, Barcelona, Spain) was used.

Stock solutions containing about 500 mg L^{-1} were prepared for each analyte by weight in methanol and stored at 4^{o}C for up to a week. Mixed working solutions were made daily by diluting the stock solutions to the required concentration with Milli-Q water.

6.2.2 HEADSPACE SOLID-PHASE MICROEXTRACTION PROCEDURE

SPME experiments were performed with a SPME Triplus autosampler (Thermo Scientific, Waltham, MA, USA). Two different commercially available fibre coatings were tested: a 65 µm PDMS/DVB fibre and a 50/30 µm DVB/CAR/PDMS fibre from Supelco (Bellefonte, PA, USA). Before use, each fibre was conditioned according to the manufacturer's instructions to remove contaminants and stabilise the solid phase.

Ten mL of sample solution were placed in 20 mL glass vials containing 2.4 g of NaCl. The vials were then closed with aluminium caps furnished with Teflon-faced septa. Samples were

introduced in the oven and the fibre was exposed at 90°C for 45 min to the headspace above the aqueous solution (final conditions). Constant stirring was applied during the extraction process. After the completion of sampling, the fibre was pulled into the needle and the SPME device was removed from the vial and inserted into the injection port of the GC for thermal desorption and analysis.

6.2.3 EXPERIMENTAL DESIGN

A full factorial design was performed to assess the influence of the experimental parameters on the extraction of the fragrance compounds from aqueous solutions. This allowed us to determine the influence of the experimental variables studied and also to ascertain their interactions.

A 2^3 full factorial design was used and three variable factors that can affect the extraction yield of the analytes were considered: ionic strength (variable c, quantified as NaCl content, ranging from 1.2 to 2.4 g), temperature (T, 50-90°C) and extraction time (t, 15-45 min). The central point (1.6 g, 70°C, 30 min) was also measured and considered as an experiment. All the experiments were performed in triplicate and in random order. The Minitab v14 computer program was used for data manipulation and calculations [27].

6.2.4 EQUIPMENT AND CHROMATOGRAPHIC CONDITIONS

Gas chromatographic analyses were performed with a Trace GC 2000 coupled to a PolarisQ ion trap mass spectrometer detector (Thermo Scientific). A TG-5SIL MS capillary column (30m × 0.25 mm i.d.; 0.25 µm film thickness) (Thermo Scientific) was used and the carrier gas was 99.9990 % pure helium (Carburos Metálicos, Barcelona, Spain) at a constant inlet flow rate of 1 $mL \cdot min^{-1}$. The split/splitless injection port was equipped with a 0.75 mm ID SPME liner and operated in splitless mode (maintained for 2 min) at 220°C.

The oven temperature program started at 45°C, held for 2 min; then ramped up to 233°C at 8°C/min, and held for 2 min; total run 27.50 min. MS analyses were conducted in scan mode with an *m/z* interval from 39 to 400 amu. Ionisation was performed in the electron impact mode at 70 eV. The transfer line temperature was set at 280°C and the ion source temperature at 225°C. The acquisition of chromatographic data was performed using Xcalibur 1.4 software

(Thermo Scientific). Table 6.1 shows the list of the target compounds, their retention times and their qualifier and quantifier ions.

Table 6.1. Fragrance compounds monitored with their retention times and *m/z* ratios.

Compound	t_R (min)	m/z[a]	Henry law constant[b] ($atm \cdot m^3 \cdot mol^{-1}$)
Limonene	8.84	68,**93**	$3.8 \cdot 10^{-1}$
Linalool	10.20	43,55,**71**,93	$4.2 \cdot 10^{-5}$
β-Citronellol	12.58	41,67,**69**,81	$5.7 \cdot 10^{-5}$
Citral A*	12.80	39,41,**69**,84	$3.8 \cdot 10^{-4}$
Citral B*	13.32	39,41,**69**,84	$3.8 \cdot 10^{-4}$
Geraniol	13.02	41,69,93,**123**	$5.9 \cdot 10^{-5}$
t-Cinnamaldehyde	13.44	51,77,103,**131**	$1.6 \cdot 10^{-6}$
Hydroxycitronellal	13.65	41,43,59,**71**	$2.4 \cdot 10^{-8}$
Cinnamyl alcohol	14.02	78,91,92,**134**	$1.6 \cdot 10^{-7}$
Eugenol	14.81	39,77,103,**164**	$4.8 \cdot 10^{-8}$
Coumarin	17.26	63,90,118,**146**	$6.9 \cdot 10^{-6}$
Lilial	17.60	131,147,**189**	$2.5 \cdot 10^{-5}$
Lyral	19.64	79,91,93,**136**	$2.6 \cdot 10^{-8}$
Benzyl benzoate	21.08	77,91,105,**194**	$2.8 \cdot 10^{-6}$
Benzyl salicylate	22.45	39,65,**91**	$1.3 \cdot 10^{-4}$
Benzyl cinnamate	25.20	91,**131**,192	$4.2 \cdot 10^{-5}$
Galaxolide	22.08	213,243,**258**	$3.7 \cdot 10^{-7}$
Tonalide	22.18	159,243,**258**	$3.3 \cdot 10^{-7}$

[a] Values in bold are the quantifier ions.
[b] Calculated with the EPI Suite™ v4.11 computer program [28].
* A and B are used for cis and trans isomers, respectively.

6.2.5 SAMPLING OF WASTEWATERS

This study was focused on the evaluation of the target fragrances in two WWTPs located in Castell-Platja d'Aro and Girona. Both WWTPs are situated near the Mediterranean coast in the north of Catalonia (Spain).

Sampling was performed from January to March 2013. Samples from the primary effluent and the biological treatment effluent of both plants were obtained. In the case of the Castell-Platja d'Aro WWTP, a tertiary treatment (UV followed by chlorination) is applied when required (e.g., for agricultural purposes), so tertiary effluent samples were also taken when this process was running. 0.5 L amber glass bottles were used for sampling and water samples were stored at 4^{o}C and analysed before 24 h.

6.2.6 STATISTICAL DATA TREATMENT

Statistical analyses were performed using SPSS for Windows version 15.0. Significance was set at 0.05.

6.3 RESULTS AND DISCUSSION

6.3.1 SELECTION OF SPME EXTRACTION CONDITIONS

Some authors have identified the PDMS/DVB fibre as the best sorbent phase for the determination of fragrances in different matrices [18,29-31]. On the other hand, the DVB/CAR/PDMS fibre has been widely used in the analysis of volatile compounds in water and air samples [32]. Consequently, a preliminary study for the selection of the best coating was performed.

As can be seen in Figure 6.1, no significant differences (p>0.05, t-test) were observed for the studied compounds between the two fibres, except for t-cinnamaldehyde (p=0.01) and cinammyl alcohol (p=0.007), which showed lower response at the concentrations tested when the DVB/CAR/PDMS coating was used. As a result, the PDMS/DVB fibre was selected for further experiments.

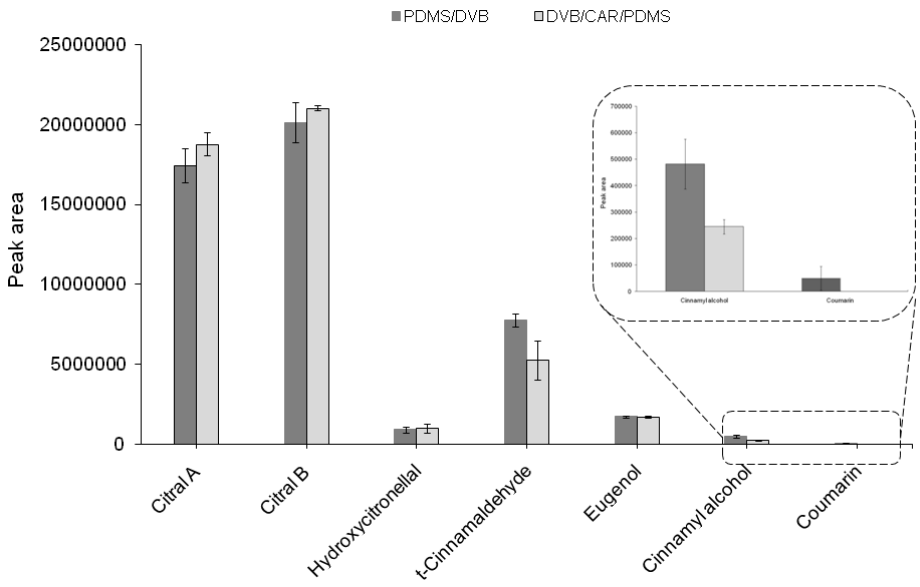


Figure 6.1. Peak areas obtained in the analysis of an aqueous solution containing some selected compounds using the two fibre coatings (n=3). Concentrations: between 75 and 450 µg·L^{-1}, depending on the compound. Extraction conditions: 30 min at 70°C and 1.2g NaCl added.

A splitless time of 2 minutes is frequently used in the analysis of fragrances [18,29,33,34]. Preliminary experiments evaluating splitless times ranging from 2 to 10 min showed that there were no significant differences (p>0.05, ANOVA test) between the times evaluated (Figure 6.2) and so a splitless time of 2 minutes was selected.

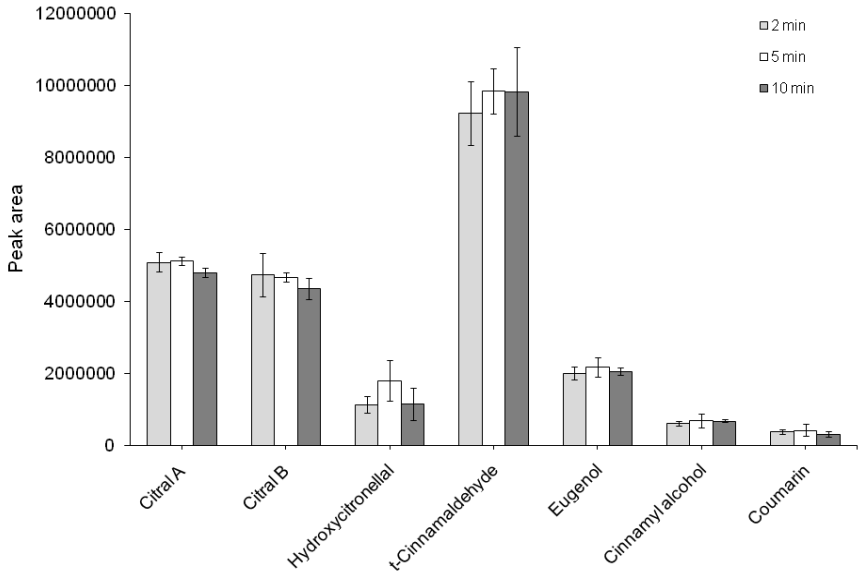


Figure 6.2. Peak areas obtained in the analysis of an aqueous solution containing some selected compounds using different splitless times (n=3). Concentrations: between 75 and 450 µg·L^{-1}, depending on the compound. Extraction conditions: 30 min at 70°C and 1.2g NaCl added.

An experimental domain was defined to evaluate the influence of temperature, time of extraction and salt content on the extraction of the target fragrances from aqueous solutions. A full two-level factorial design was used in order to study not only each variable individually, but also the presence of interactions and evidence of curvature effects.

Absolute peak areas were analysed and the results obtained are summarised in Table 6.2, where the significances (*p*-values) are given. Temperature has the highest influence on all the target analytes and the optimal temperature level is seen to depend on the partition of the compounds towards the gas volatility. For the most volatile compounds (limonene and linalool), the best extraction conditions are obtained at the lowest temperature due to a competitive desorption process of these analytes that takes places at high temperatures. For intermediate volatility compounds (β-citronellol, both citrals and geraniol), there is a double interaction between time and temperature showing that using short times, higher temperatures yield better extraction whereas at long times the competitive desorption becomes significant and low temperatures are preferred. For most of the target compounds, 12 out of 18, there is an increase in the response when temperature is set at the highest level. Similar results were reported in previous studies [18,33], where different behaviours were also observed depending on the compound.

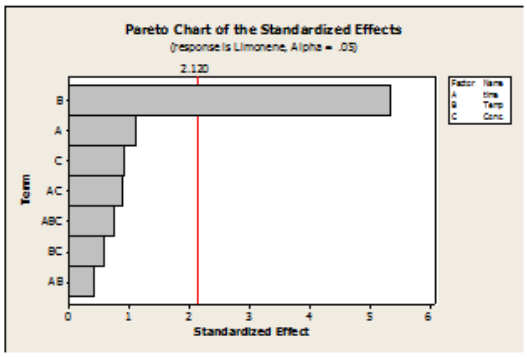
With regards to the extraction time, only eight of the target compounds were influenced. Lilial, galaxolide and tonalide presented better responses when the time was set at the highest level. For β-citronellol, both citrals, geraniol, and t-cinnamaldehyde, the previously mentioned interactions between time and temperature were observed, yielding better extractions when the combination of these two variables decreased (e.g., high T and low t or low T and high t).

Finally, in the study of the NaCl content it was observed that the addition of salt improved the extraction of β-citronellol, both citrals, geraniol, t-cinnamaldehyde, eugenol, and lilial, with no significant effects on the other compounds. This can be explained by the fact that as a general trend increased ionic strength results in organic substances being less soluble and an increase in the partition coefficients [35]. These results are in agreement with those reported by Lamas et al. [33], where the influence of salt addition was found to be positive for most of the compounds they studied.

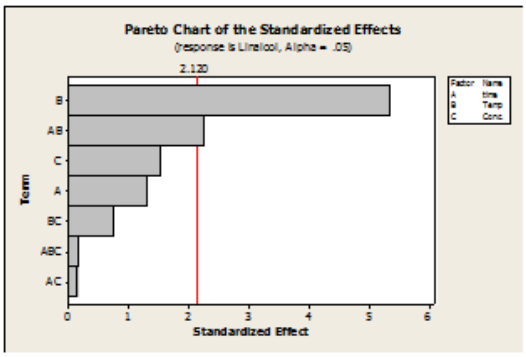
Table 6.2. Statistical results obtained for the experimental design. *p*-values are given for main effects, double and triple interactions and for curvature evidence. If two or more significant terms are found, these are shown in decreasing order of importance. The sign beside each variable name indicates the optimal level to maximise the response.

Compound	Single variable effects		Double variable effects		Triple variable effects	*p*-value for curvature evidence
	p-value	Significant terms	*p*-value	Significant terms	*p*-value	
Limonene	0.001	-T	0.743		0.475	0.814
Linalool	0.000	-T	0.171		0.878	0.470
β-Citronellol	0.006	+c	0.005	-tT	0.800	0.013
Citral A	0.001	+c	0.001	-tT	0.837	0.016
Citral B	0.001	+c	0.000	-tT	0.854	0.006
Geraniol	0.005	+c	0.004	-tT	0.214	0.006
t-Cinnamaldehyde	0.000	+c +T	0.001	-tT	0.146	0.000
Hydroxycitronellal	0.000	+T	0.003		0.962	0.072
Cinnamyl alcohol	0.000	+T	0.000		0.595	0.000
Eugenol	0.000	+T +c	0.010		0.264	0.016
Coumarin	0.000	+T	0.000		0.007	0.523
Lilial	0.000	+T +t +c	0.275		0.637	0.132
Lyral	0.000	+T	0.000		0.004	0.446
Benzyl benzoate	0.000	+T	0.000		0.392	0.992
Galaxolide	0.000	+T +t	0.515		0.932	0.164
Tonalide	0.000	+T +t	0.207		0.509	0.101
Benzyl salicylate	0.000	+T	0.001		0.758	0.991
Benzyl cinnamate	0.000	+T	0.000		0.046	0.321

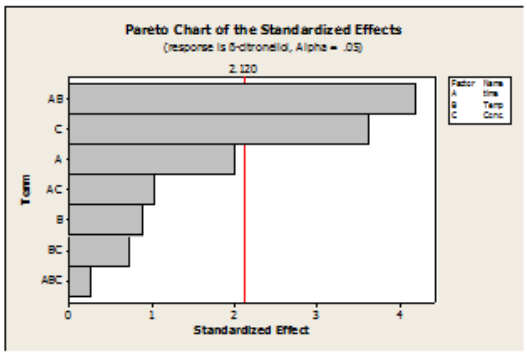
Taking into account the whole list of target analytes, it can be concluded that the most favourable conditions for SPME extraction are at the highest values for each factor: a temperature of 90°C, a time of 45 min, and 2.4 g of NaCl. Although these conditions are not the optimum for limonene, linalool, β-citronellol, the two citrals, and geraniol, the selected method allows their quantification with good sensitivity. These main conclusions are best visualised in Pareto graphs (Figure 6.3).



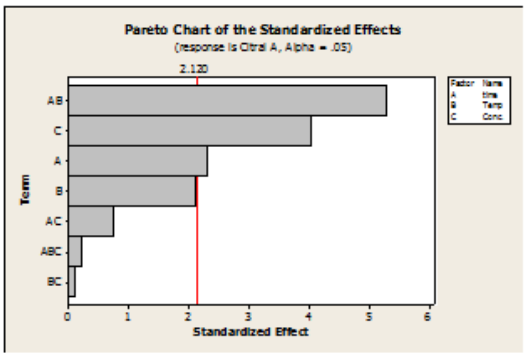
Pareto graph of limonene.



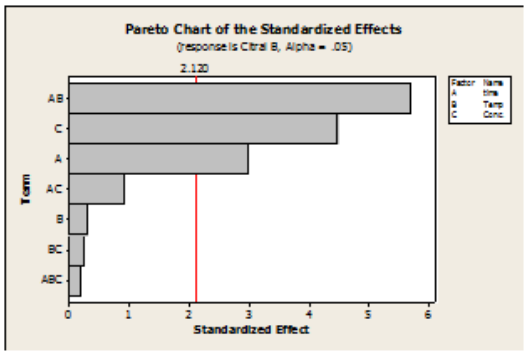
Pareto graph of linalool.



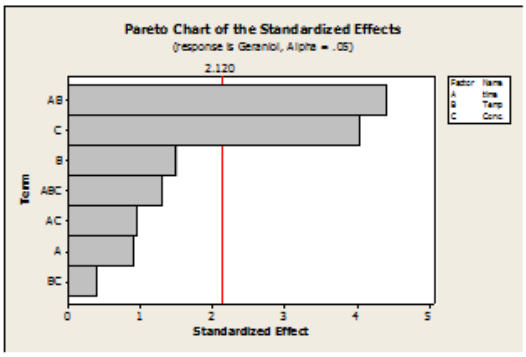
Pareto graph of β-citronellol.



Pareto graph of citral A.

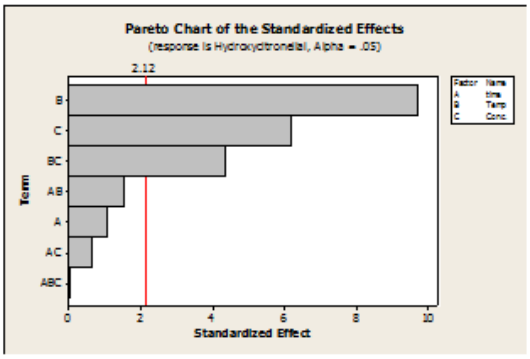


Pareto graph of citral B.

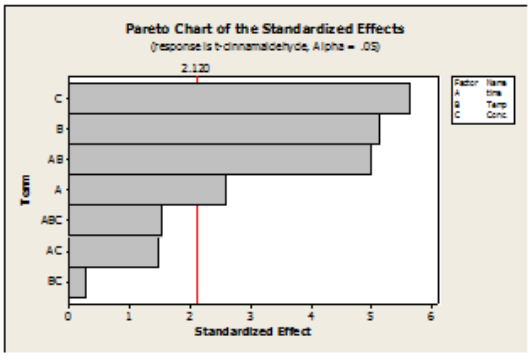


Pareto graph of geraniol.

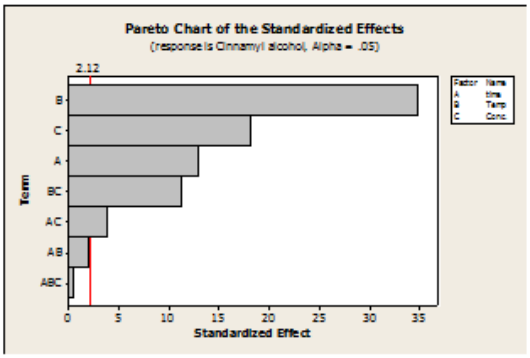
Figure 6.3. Pareto graphs obtained for the studied fragrances as a result of the experiments developed according to the design of experiments methodology proposed. A: extraction time (min), B: extraction temperature (°C), C: NaCl concentration (g).



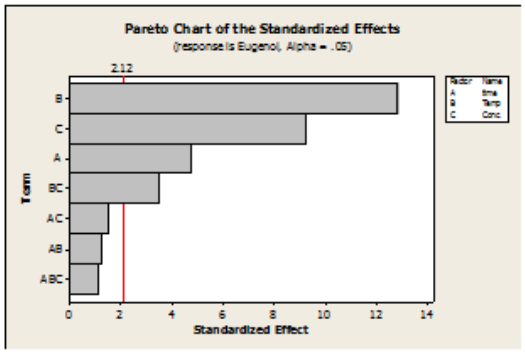
Pareto graph of hydroxycitronellal.



Pareto graph of t-cinnamaldehyde.



Pareto graph of cinnamyl alcohol.



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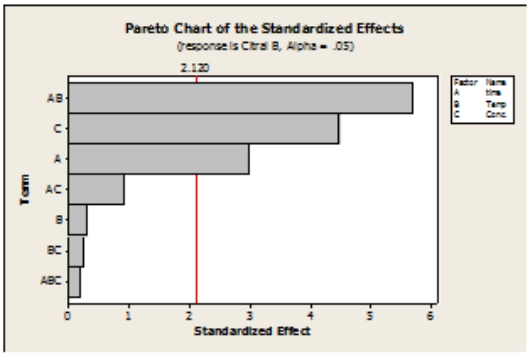

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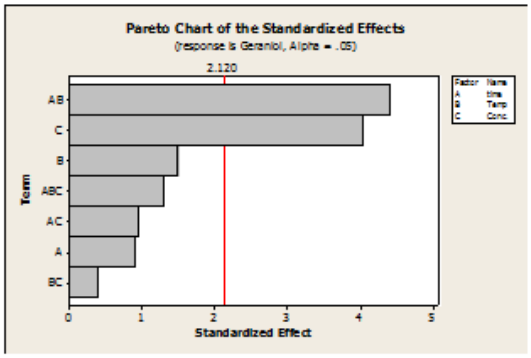
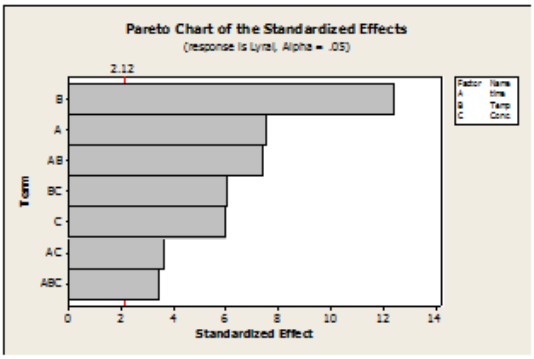
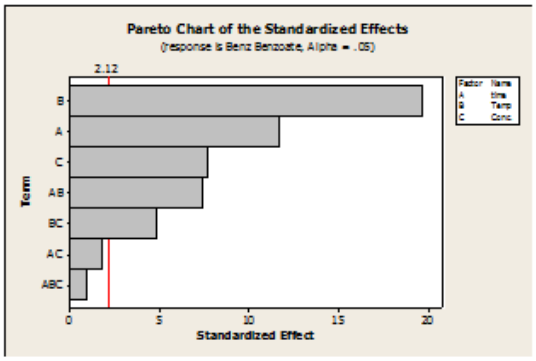


Pareto graph of lilial.

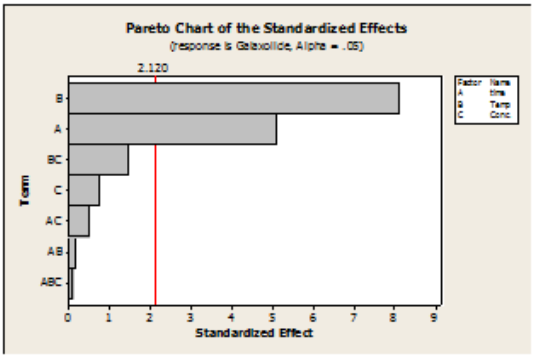
Figure 6.3 ***(continued)*****.** Pareto graphs obtained for the studied fragrances as a result of the experiments developed according to the design of experiments methodology proposed. A: extraction time (min), B: extraction temperature (^{o}C), C: NaCl concentration (g).



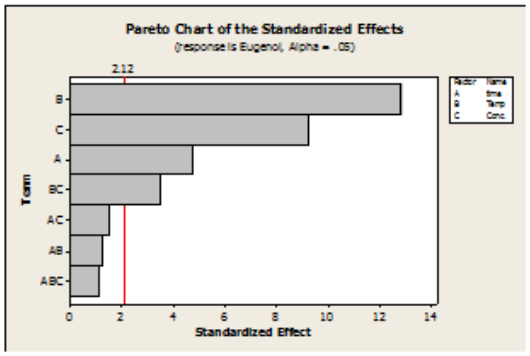
Pareto graph of lyral.



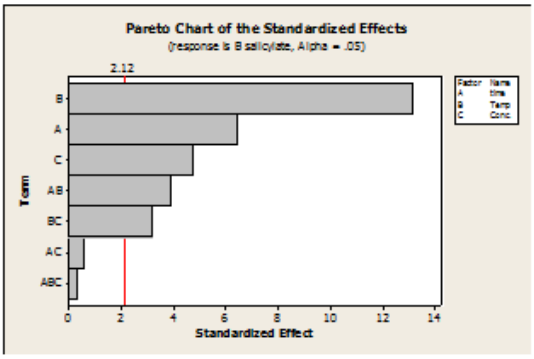
Pareto graph of benzyl benzoate.



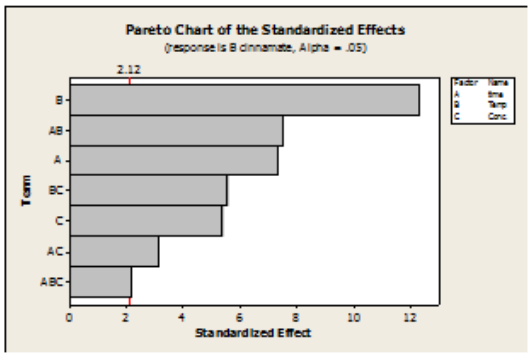
Pareto graph of galaxolide.



Pareto graph of tonalide.



Pareto graph of benzyl salicylate.



Pareto graph of benzyl cinnamate.

Figure 6.3 ***(continued)*****.** Pareto graphs obtained for the studied fragrances as a result of the experiments developed according to the design of experiments methodology proposed. A: extraction time (min), B: extraction temperature (^{o}C), C: NaCl concentration (g).

6.3.2 METHOD VALIDATION

External calibration was performed (Table 6.3). Each concentration level was analysed in triplicate. Linearity was confirmed for all compounds from evaluation of the distribution of residuals, with r^2 values greater than 0.985. The LODs and the LOQs of the method were calculated as three and ten times the signal-to-noise ratio, respectively. The obtained values, which ranged from 0.01 to 1.7 μg L^{-1}, are shown in Table 6.3. Lamas et al. [33] obtained LODs in the same range for most of the fragrances applying an SPME/GC-MS method. Equivalent LODs were also obtained in studies where other microextraction methods, like USAEME [17] and DLLME [25], were used.

Table 6.3. Quality parameters of the developed method. Standard concentrations close to LOQ (low level) and close to the highest calibration level (high level) were used for precision evaluation (n=5).

Compound	Working range[a] (μg·L^{-1})	a (s_a) (x10^5)	b (s_b) (x10^5)	r^2	LOD (μg·L^{-1})
Limonene	1.0 – 90	4 (2)	0.67 (0.04)	0.9878	0.3
Linalool	1.0 – 50	0.2 (0.8)	0.47 (0.03)	0.9902	0.3
β-Citronellol	2.0 – 160	1 (2)	0.56 (0.02)	0.9937	0.6
Citral A	2.7 – 30	0.02 (0.02)	0.021 (0.009)	0.9945	0.8
Citral B	1.8 – 30	0.3 (0.3)	0.54 (0.02)	0.9974	0.5
Geraniol	0.4 - 110	0.05 (0.04)	0.054 (0.008)	0.9989	0.1
Hydroxycitronellal	5.7 – 160	0.2 (0.4)	0.060 (0.004)	0.9851	1.7
t-Cinnamaldehyde	0.23 – 14	1.46 (0.8)	2.1 (0.1)	0.9934	0.07
Cinnamyl alcohol	1.4 – 90	0.003 (0.007)	0.0062 (0.0002)	0.9968	0.4
Eugenol	0.07 – 7	0.3 (0.6)	3.4 (0.2)	0.9936	0.02
Coumarin	0.4 – 65	0.5 (0.2)	0.330 (0.009)	0.9980	0.1
Lilial	0.17 – 4	6 (2)	12.4 (0.8)	0.9865	0.05
Lyral	1.4 – 140	1.4 (0.9)	0.28 (0.01)	0.9923	0.4
Benzyl benzoate	0.03 – 6	2 (2)	14.9 (0.6)	0.9963	0.01
Galaxolide	0.13 – 7	0.7 (1.4)	11.8 (0.6)	0.9930	0.04
Tonalide	0.13– 4	0.7 (1.3)	15.6 (0.5)	0.9967	0.04
Benzyl salicylate	0.03 – 3	5.5 (12.5)	155 (6)	0.9969	0.01
Benzyl cinnamate	0.11 – 7	3 (2)	15.6 (0.6)	0.9953	0.03

[a] LOQs were fixed as the lowest calibration level.

Inter-day precision and recoveries of the method were also tested (Table 6.4). Water samples obtained from the biological treatment effluent of the Castell-Platja d'Aro WWTP were divided in two portions and one was spiked (n=5 for each type of sample). Spiking solutions at concentrations around the LOQ (low level) and close to the highest calibration concentration (high level) were used. For those analytes presenting a short working range only the high concentration level was evaluated. In the case of lyral, experiments using WWTP water were assessed only at the high level due to the presence of an unknown compound at the same retention time, which did not allow the quantification of this analyte at low concentrations.

Table 6.4. Recoveries obtained in the analysis of spiked wastewater samples from the biological effluent of Castell-Platja d'Aro WWTP (n=5). Standard concentrations close to LOQ (low level) and close to the highest calibration level (high level) were used. Standard deviations are shown in parenthesis. (-): not determined.

Compound	Inter-day precision (RSD, %)		Recoveries (%)			
	Low level	High level	Low level	High level	Low level ($Na_2S_2O_3$ addition)	High level ($Na_2S_2O_3$ addition)
Limonene	33	28	60 (20)	90 (30)	80 (30)	80 (20)
Linalool	22	14	78 (8)	57 (8)	80 (10)	50 (10)
β-Citronellol	17	9	50 (10)	67 (6)	40 (10)	73 (9)
Citral A	-	13	-	60 (10)	-	56 (6)
Citral B	-	8	-	60 (10)	-	72 (9)
Geraniol	10	11	60 (10)	57 (6)	61 (9)	70 (5)
Hydroxycitronellal	21	9	20 (20)	38 (8)	70 (20)	70 (10)
t-Cinnamaldehyde	-	11	-	90 (5)	-	80 (10)
Cinnamyl alcohol	20	16	70 (8)	57 (6)	80 (10)	80 (10)
Eugenol	-	14	-	47 (9)	-	70 (10)
Coumarin	23	18	80 (10)	80 (10)	100 (10)	100 (10)
Lilial	-	16	-	30 (20)	-	45 (7)
Lyral	-	7	-	20 (10)	-	79 (7)
Benzyl benzoate	-	8	-	64 (6)	-	89 (5)
Galaxolide	-	7	-	30 (6)	-	40 (10)
Tonalide	-	4	-	71 (7)	-	84 (1)
Benzyl salicylate	-	23	-	25 (7)	-	42 (3)
Benzyl cinnamate	-	17	-	20 (20)	-	60 (10)

The RSD values obtained, which can be considered as acceptable [36], ranged from 4 to 23% (Table 6.4). The worst results were obtained for limonene, whose RSD values were >25% in all concentration levels tested.

As can be seen in Table 6.4, recoveries in WWTP water were <50% for six analytes (hydroxycitronellal, lilial, lyral, galaxolide, benzyl salicylate, and benzyl cinnamate). Similar results were obtained by Lamas et al. [33] in spiked bath and swimming pool waters. They evaluated the capacity of the addition of sodium tiosulphate to samples to avoid the oxidation of some analytes due to the presence of oxidants in the sample matrix during storage and extraction of the samples and found that final recoveries increased to >80 % for most compounds. We treated the WWTP samples with 0.1 μg L^{-1} of sodium tiosulphate to prevent this matrix effect and the resulting recoveries increased significantly for all the compounds and were satisfactory for most of them (Table 6.4).

6.3.3 OCCURRENCE OF FRAGRANCES

The developed HS-SPME/GC-MS method was applied to the monitoring of 18 fragrances (16 allergens and two polycyclic musks) in two WWTPs located at Castell-Platja d'Aro and Girona (Girona province, Spain) over a three-month period.

The Girona WWTP was designed for an equivalent population of 206,250 habitants, with a design flow of 55,000 $m^3 \cdot day^{-1}$. Its average working flow during the sampling period was 35,000 and 40,000 $m^3 \cdot day^{-1}$. The Castell-Platja d'Aro plant was designed for an equivalent population of 175,000 habitants (this plant gives service to a touristic area which had a significantly reduced population during the sampling period), with a design flow of 35,000 $m^3 \cdot day^{-1}$. Its average working flow during the period monitored was around 15,000 $m^3 \cdot day^{-1}$. Secondary digestion in both plants consists of activated sludge processes.

Table 6.5 shows the concentrations of the fragrances detected. Only seven of the target analytes (limonene, linalool, eugenol, lilial, galaxolide, tonalide, and benzyl salicylate) were detected in at least one of the samples. The results obtained are in agreement with those published by other studies [5,6,12,17-19].

Table 6.5. Concentrations found (μg·L^{-1}) at the sampling points from Castell-Platja d'Aro and Girona WWTPs. (-): not determined, n.d.: not detected, sd: standard deviation. Numbers shown in parentheses are the number of samples in which the compound was not detected.

		Castell-Platja d'Aro							Girona					
Compound		**n**	**Minimum**	**Maximum**	**Median**	**Mean**	**sd**		**n**	**Minimum**	**Maximum**	**Median**	**Mean**	**sd**
Limonene	Primary	13	n.d. (6)	3.02	0.31	0.80	0.88	Primary	12	n.d. (10)	1.39	0.19	0.32	0.35
	Secondary	14	n.d.	n.d.	n.d.	n.d.	-	Secondary	12	n.d.	n.d.	n.d.	n.d.	-
	Tertiary	7	n.d.	n.d.	n.d.	n.d.	-							
Linalool	Primary	13	1.54	20.22	7.65	8.54	5.73	Primary	12	n.d. (1)	11.62	5	5.37	3.08
	Secondary	14	n.d.	n.d.	n.d.	n.d.	-	Secondary	12	n.d.	n.d.	n.d.	n.d.	-
	Tertiary	7	n.d.	n.d.	n.d.	n.d.	-							
Eugenol	Primary	13	n.d. (2)	1.8	0.52	0.55	0.48	Primary	12	n.d. (1)	1.00	0.68	0.56	0.3
	Secondary	14	n.d.	n.d.	n.d.	n.d.	-	Secondary	12	n.d.	n.d.	n.d.	n.d.	-
	Tertiary	7	n.d.	n.d.	n.d.	n.d.	-							
Lilial	Primary	13	n.d. (2)	0.89	0.27	0.29	0.26	Primary	12	n.d. (4)	1.58	0.13	0.37	0.47
	Secondary	14	n.d.(3)	1.18	0.27	0.42	0.38	Secondary	12	n.d. (9)	0.23	0.03	0.06	0.06
	Tertiary	7	n.d. (1)	0.81	0.57	0.40	0.31							
Galaxolide	Primary	13	2.31	3.49	2.92	2.92	0.30	Primary	12	1.9	3.43	2.37	2.50	0.53
	Secondary	14	2.87	5.21	3.97	3.94	0.74	Secondary	12	2.5	4.23	3.32	3.36	0.48
	Tertiary	7	2.67	4.58	3.72	3.56	0.78							
Tonalide	Primary	13	n.d. (2)	0.36	0.22	0.19	0.13	Primary	12	n.d. (5)	0.48	0.12	0.14	0.15
	Secondary	14	n.d. (1)	0.94	0.50	0.48	0.31	Secondary	12	n.d. (2)	1.24	0.28	0.37	0.35
	Tertiary	7	n.d. (2)	0.91	0.22	0.29	0.32							
Benzyl salicylate	Primary	13	n.d. (4)	0.86	0.49	0.38	0.32	Primary	12	n.d. (3)	0.96	0.24	0.28	0.28
	Secondary	14	n.d.(10)	0.62	0.01	0.07	0.16	Secondary	12	n.d.	n.d.	n.d.	n.d.	-
	Tertiary	7	n.d. (6)	0.14	0.01	0.03	0.05							

Statistical calculations were used to discuss about the presence of these compounds in the two WWTPs. A value of LOD/√2 was used as the concentration when a compound was not detected. β-Citronellol, both citrals, geraniol, t-cinnamaldehyde, cinnamyl alcohol, coumarin, lyral, benzyl benzoate, and benzyl cinnamate are not included in the calculations since they were not detected in any of the samples analysed.

First, normality tests were performed and normal distributions were found for all target compounds (Shapiro-Wilk test, p>0.05), except for those analytes which were not detected in >30 % of samples (limonene and benzyl salicylate in Castell-Platja d'Aro, and limonene, lilial, tonalide, and benzyl salicylate in Girona).

Linalool and galaxolide were the fragrances found at the highest concentrations in both WWTPs, with maximum values of 20.22 and 5.21 $\mu g \cdot L^{-1}$ respectively in Castell-Platja d'Aro, and 11.62 and 4.23 $\mu g \cdot L^{-1}$ in Girona. Linalool was also detected as the allergenic fragrance with the highest content at the primary effluent (mean concentration of 6.4 $\mu g \cdot L^{-1}$, n=16) in another study evaluating three allergens at a treatment plant close to the ones evaluated in the present study [16]. In the case of polycyclic musk, levels of galaxolide ranging from 1-20 $\mu g \cdot L^{-1}$ have also been reported in many studies [5,12].

Limonene, linalool and eugenol were only detected at the primary effluent of the two monitored WWTPs, which confirms their quantitative elimination during the secondary treatments. Escalas et al. [16] also found the quantitative elimination of linalool in a WWTP.

Lilial was found to be the most persistent allergen and was detected at all sampling points of the two plants, which agrees with the results obtained by Becerril et al. [18]. For Castell-Platja d'Aro, statistical analysis showed no significant differences between the values obtained in the three sampling points (ANOVA test, p=0.591), while significant differences were observed between the primary and secondary effluents of the Girona WWTP (t-test, p=0.04) (Figure 6.4).

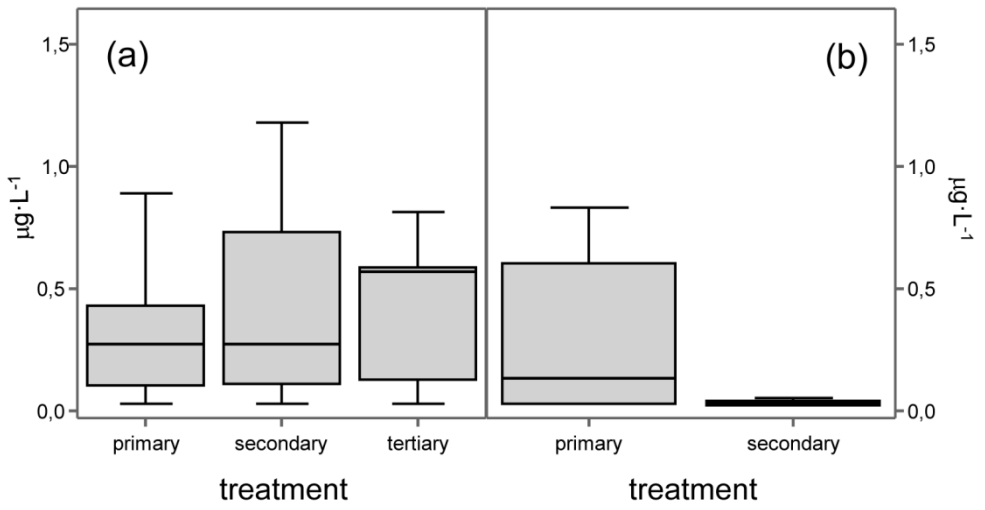


Figure 6.4. Box plots of data obtained for lilial in (a) Castell-Platja d'Aro and (b) Girona. The bottom and top of the box are 25th and 75th percentiles, the line inside the box is the median (50th percentile) and the whiskers indicate the lowest and highest data within 1.5 inter-quartile range.

With regards to benzyl salicylate, statistical tests revealed that there were significant differences between the concentrations found at the primary and secondary effluents for both plants (ANOVA test, p=0.001 for Castell-Platja d'Aro; t-test, p=0.007 for Girona). This analyte was detected at the primary effluent of both WWTPs (69% of the primary effluent samples in Castell d'Aro and 75% in Girona) and was not detected at the secondary effluent of the Girona WWTP, while at the Castell d'Aro plant was detected in four secondary samples (28.6%).

The two polycyclic musks, galaxolide and tonalide, were also detected at all sampling points of the two plants (Figure 6.5). In the case of Castell-Platja d'Aro, significant differences between the three sampling points were obtained for both compounds (ANOVA test, p=0.001 for galaxolide and p=0.021 for tonalide) and musk concentrations were always significantly higher at the secondary rather than the primary effluent. Data obtained from the Girona WWTP show the same behaviour for galaxolide (t-test, p=0.044), whereas no significant differences were observed for tonalide between the two treatments performed at this plant (t-test, p=0.058). Similar behaviour, with an increase of some PCPs during the treatment process, has also been described in other studies [12,37]. Simonich et al. [12] evaluated 17 different treatment plants in UK and USA and found that in the case of activated sludge plants the average relative profile in secondary effluent is enriched in the non-biodegradable, sorptive musks, such as galaxolide and tonalide.

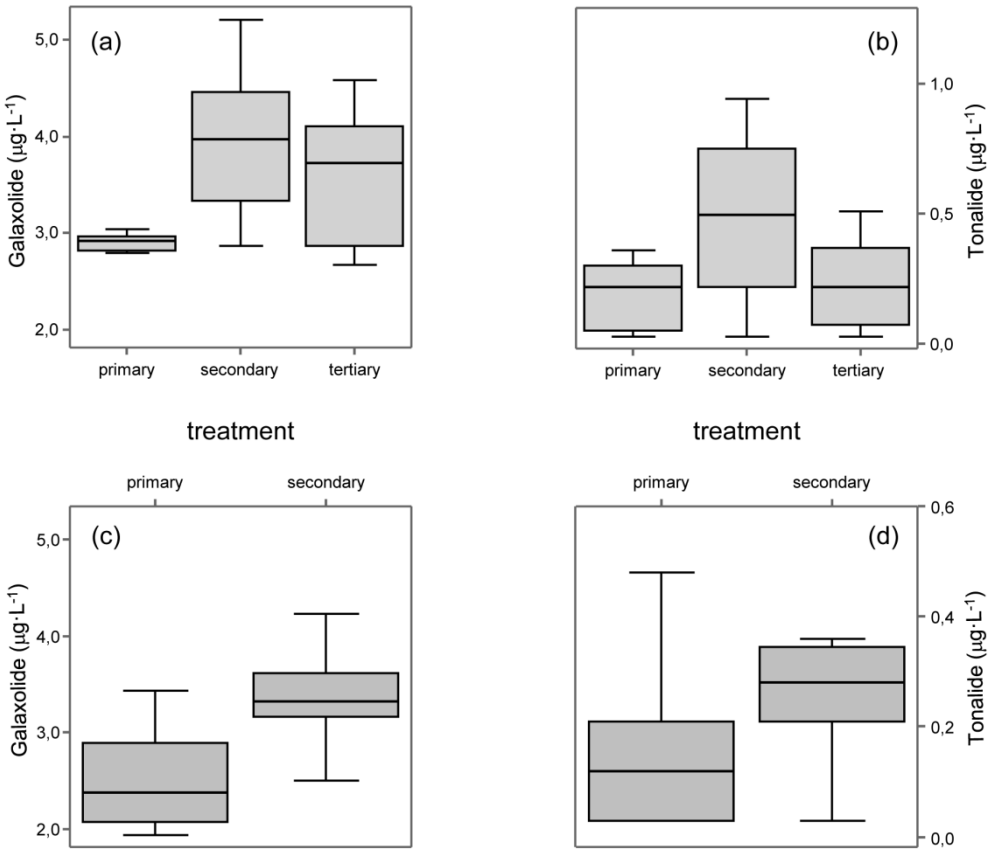


Figure 6.5. Box plots of data obtained for (a) galaxolide (Castell-Platja d'Aro), (b) tonalide (Castell-Platja d'Aro), (c) galaxolide (Girona) and (d) tonalide (Girona). The bottom and top of the box are 25th and 75th percentiles, the line inside the box is the median (50th percentile) and the whiskers indicate the lowest and highest data within 1.5 inter-quartile range.

When comparing the values obtained between the two WWTPs, no significant differences were observed between their primary effluents for limonene, linalool, eugenol, lilial, tonalide, and benzyl salicylate (t-test, p>0.05). Galaxolide concentrations were found to be higher at Castell-Platja d'Aro primary effluent (t-test, p=0.048). No significant differences were observed between the secondary effluents of the monitored WWTPs for galaxolide (t-test, p=0.051), tonalide (p=0.477) and benzyl salicylate (p=0.191). In the case of lilial, higher concentrations were found at Castell-Platja d'Aro secondary effluent (t-test, p=0.009).

In general, few differences were found for the target fragrances between the two studied WWTPs. It should be mentioned that the occurrence of four of the target analytes (lilial, benzyl salicylate, tonalide, and galaxolide) at the effluent of the plants highlights the need for more advanced processes in water and sludge treatments in order to avoid their accumulation in the environment.

6.4 CONCLUSIONS

A method based on HS-SPME was successfully developed for the simultaneous determination of 16 fragrance allergens and two polycyclic musks in aqueous samples from WWTPs. The microextraction procedure was optimised and the final conditions were fixed at 2.4 g of NaCl, 45 min extraction time, and 90^{o}C extraction temperature. The optimised method gives adequate detection limits for all target compounds (between 0.01 and 1.7 µg·L^{-1}) as well as good inter-day precision values (RSD ranging from 4 to 23 % in WWTP water, n=5). The proposed method was applied to the monitoring of the target fragrances in two WWTPs located in Girona (Spain). Seven of the analytes were detected at the influent of the evaluated plants and limonene, linalool and eugenol were found to be eliminated during their treatments. Lilial, benzyl salicylate, galaxolide, and tonalide were detected at the effluent samples, where galaxolide was the compound detected at the highest concentrations (from 2.50 to 4.58 µg·L^{-1}). More advanced treatments should therefore be used for a quantitative removal of these analytes.

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CHAPTER 7:
UV and chlorination treatments for the degradation of fragrances in water samples

7.1 INTRODUCTION

The effluent discharge from WWTPs is one of the major sources of emerging contaminants in the aquatic environment. A high amount of organic compounds such as pharmaceuticals, pesticides and fragrances are present in domestic waters and continuously released into WWTPs where they are subjected to different treatment processes. However, as many of them are only partially removed in conventional municipal sewage systems, they are commonly released into effluents and surface waters [1-3].

Nowadays some additional strategies are being used in order to remove or decrease the concentration of emerging contaminants in wastewater effluents, such as membrane bioreactors [4] and advanced oxidation treatments [5,6]. At laboratory scale, the efficacy of chlorination [7-9], UV radiation [10-12] and advanced oxidation treatments [12,13] has also been assessed. Chlorination of pharmaceuticals and some metabolites in different types of water (drinking water, surface water and wastewater) have been investigated by many authors [8,14-17]. UV treatment for the degradation of emerging contaminants including pharmaceuticals, corrosion inhibitors, biocides, and pesticides has also been studied as an effective alternative for the removal of these micropollutants [10,18-21].

Fragrances are substances commonly added to many consumer products to mask the odour of other chemicals and give the consumer a pleasing sensation. They are permitted in unlimited quantities in cosmetic products under the EU cosmetics directive 76/768/EEC. Moreover the SCCS has identified 26 fragrance allergens which are nowadays regulated. Some of the fragrance compounds are also suspected to have oestrogenic activity and as such they have been linked to adverse health effects [22]. These fragrance allergens are not included in the group of emerging contaminants, and probably for this reason, its occurrence in the environment has been poorly investigated. Its presence in bath waters and swimming pools has been documented. Some fragrance compounds -i.e. tonalide, galaxolide , musk xylene, musks ketone, and 1-(1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone (OTNE)- have been detected in treated wastewater, in surface waters, in fish, and in sediments [1,23,24]. Despite their presence at the effluent of wastewaters treatment plants after conventional treatments, their removal upon chlorination or UV treatment has scarcely been investigated. As an example, Gibs et al. [7] evaluated the persistence of 98 organic compounds, including limonene, tonalide and galaxolide, in chlorinated drinking water as a function of time. They found that 50 out of the 98 studied compounds, including the musk compounds, were not degraded in the presence of chlorine during the evaluated time.

Since surface waters are often used as source for drinking water production, and treated wastewaters are utilised to recharge groundwater aquifers in regions affected by freshwater scarcity, and for agricultural purposes especially during hot seasons, increasing attention has been given to the formation of micropollutant transformation products and of potentially harmful by-products during wastewater treatment. Within the group of fragrances, Sanchez-Prado et al. [25] studied the photo degradation of polycyclic musk compounds and proposed chemical structures for their transformation products, as well as some possible intermediates. Matamoros et al. [26] reviewed different analytical methods for determining degradation intermediates of PCPs including stimulants, fragrances, sunscreens, antimicrobials, and insect repellents in environmental matrixes. Furthermore, Janzen et al. [13] studied and identified the transformation products of tonalide and galaxolide when advanced wastewater treatments with ozone are applied. To the best of our knowledge, no much more work has been performed in the field of fragrance compounds removal.

Some years ago our group undertook a monitoring campaign in WWTPs located in Catalonia (NE, Spain). The presence of emerging contaminants and some fragrances in treated water was emphasized. The aim of this chapter is to go one step further and shed light about the efficiency of UV irradiation and chlorination in the elimination of the micropollutants which are present in the effluent water. Thus, chlorination and UV irradiation were applied to synthetic samples containing 16 allergens and two polycyclic musks, in a laboratory scale experiment, and the efficiency and transformation products were evaluated.

7.2 EXPERIMENTAL

7.2.1 CHEMICALS

The 16 fragrance allergens: d-limonene (99%), linalool (95%), β-citronellol (99%), citral cis and trans mixture (96%), geraniol (99%), trans-cinnamaldehyde (98%), hydroxycitronellal (95%), cinnamyl alcohol (98%), eugenol (99.8%), coumarin (99%), lilial (90%), lyral (97%), benzyl benzoate (98%), benzyl salicylate (98%), and benzyl cinnamate (99%), were obtained from Sigma-Aldrich (Steinheim, Germany). The two polycyclic musks, galaxolide and tonalide, were from LGC Standards (Teddington, UK). Sodium chloride (99.9%) and HPLC-gradient grade methanol were supplied by Carlo-Erba Reagents (Milan, Italy). Sodium tiosulphate 5-hydrate (99.5%) was obtained from Panreac Química S.L.U. (Barcelona, Spain). Sodium hypochlorite

with a 14.1 % of free chlorine was from Sigma-Aldrich. Milli-Q water from a Milli-Q Plus water purification system (Millipore, Ibérica, Barcelona, Spain) was used.

Stock solutions containing about 500 $mg \cdot L^{-1}$ were prepared for each analyte by weight in methanol and stored at $4^{o}C$ for up to a week. Mixed working solutions were daily made by diluting the stock solutions to the required concentration with Milli-Q water. For the evaluation of the transformation products, individual solutions containing about 50 $mg \cdot L^{-1}$ of each fragrance were prepared by diluting the stock solutions with Milli-Q water.

7.2.2 EXPERIMENTAL SET UP AND PROCEDURE

UV experiments were performed in a cylindrical water-jacketed metal reactor equipped with a magnetic agitator. The reactor temperature was maintained at 20 ± $1^{o}C$ by a thermostatic water bath. A 15 W low-pressure UV lamp (Herans, Germany) isolated by a quartz tube (92% of transmittance) was used. The light source had a monochromatic emission, predominantly at 253.4nm with irradiation at the sample position of ≈0.04 $W \cdot cm^{-2}$. 25 mL sample solution aliquots were placed in 30 mL quartz tubes, closed and irradiated by the light emitted from the lamp, which was immersed in the middle of the reactor. Then, samples were taken at prescribed intervals which ranged from 5 to 120 min and analysed immediately as is described in Sections 7.2.3 and 7.2.4.

Chlorination experiments were performed in similar conditions than UV tests. For that, a home-made reactor equipped with a magnetic agitator and a water bath maintained at 20 ± $1^{o}C$ was used. 25 mL sample solution aliquots were introduced in 30 mL quartz tubes, sodium hypochlorite was added in order to obtain chlorine concentrations of 1 and 5 $mg \cdot L^{-1}$ and the tube was closed. Under these conditions, the pH of the aqueous solution was 7. The chlorination reaction was stopped at prescribed intervals which ranged from 5 min to 24 hours by adding an excess of Na_2SO_3. Then, samples were taken and analysed as is described in Sections 7.2.3 and 7.2.4.

7.2.3 HEADSPACE-SOLID PHASE MICROEXTRACTION PROCEDURE

SPME experiments were performed with a SPME Triplus autosampler (Thermo Scientific, Waltham, MA, USA). A 65 µm PDMS/DVB fibre coating from Supelco (Bellefonte, PA, USA) was

used, which was conditioned before use according to the manufacturer's instructions in order to stabilise the solid phase and remove contaminants.

The SPME conditions used in this chapter were optimised in chapter 6. Ten mL of sample solution were placed in 20 mL glass vials containing 2.4 g of NaCl. Then, the vials were closed with aluminium caps furnished with Teflon-faced septa. Samples were introduced in the oven and the fibre was exposed for 45 min to the headspace above the aqueous solution at 90^{o}C. Constant stirring was applied during the extraction process. Afterwards, the fibre was pulled into the needle, the SPME device was removed from the vial and inserted into the injection port of the GC for thermal desorption and analysis.

7.2.4 EQUIPMENT AND CHROMATOGRAPHIC CONDITIONS

Analytes were separated and detected in a Trace GC 2000 gas chromatograph coupled to a PolarisQ ion trap mass spectrometer detector (Thermo Scientific). A TG-5SIL MS capillary column (30m × 0.25 mm i.d.; 0.25 µm film thickness) (Thermo Scientific) was used and the carrier gas was 99.9990 % pure helium (Carburos Metálicos, Barcelona, Spain) at a constant inlet flow rate of 1 mL·min^{-1}. The split/splitless injection port was equipped with a 0.75 mm ID SPME liner and operated in splitless mode (maintained for 2 min) at 220^{o}C.

The oven temperature was initially held at 45^{o}C for 2 min; ramped at 8^{o}C·min^{-1} to 233^{o}C, and held for 2 min; total run 27.50 min. MS analyses were conducted in full-scan mode and monitored masses were between 39 and 400 amu. Ionisation was performed in the electron impact mode at 70 eV. The transfer line temperature was 280^{o}C and the ion source temperature was maintained at 225^{o}C. Chromatographic data was acquired by means of Xcalibur 1.4 software (Thermo Scientific). Table 7.1 shows the list of the target compounds and details of the GC-MS analysis.

Table 7.1. Fragrance compounds studied in the present work in chromatographic elution order with their retention times, *m/z* ratios, LODs, and concentrations used in the removal study.

Compound	t_R (min)	m/z[a]	LOD (μg·L^{-1})	Working concentration (μg·L^{-1})
Limonene	8.84	68,**93**	0.3	3.7
Linalool	10.20	43,55,**71**,93	0.3	4.0
β-Citronellol	12.58	41,67,**69**,81	0.6	4.2
Citral A*	12.80	39,41,**69**,84	0.8	4.8
Citral B*	13.32	39,41,**69**,84	0.5	4.8
Geraniol	13.02	41,69,93,**123**	0.1	3.9
t-Cinnamaldehyde	13.44	51,77,103,**131**	0.07	56
Cinnamyl alcohol	14.02	78,91,92,**134**	0.4	60
Eugenol	14.81	39,77,103,**164**	0.02	4.8
Coumarin	17.26	63,90,118,**146**	0.1	38
Lilial	17.60	131,147,**189**	0.05	3.2
Lyral	19.64	79,91,93,**136**	0.4	46
Benzyl benzoate	21.08	77,91,105,**194**	0.01	4.7
Galaxolide	22.08	213,243,**258**	0.04	3.2
Tonalide	22.18	159,243,**258**	0.04	1.7
Benzyl salicylate	22.45	39,65,**91**	0.01	2.1
Benzyl cinnamate	25.20	91,**131**,192	0.03	4.4

[a]Values in bold are the quantifier ions.

(*): the terminology A and B is used for cis and trans isomers, respectively.

7.3 RESULTS AND DISCUSSION

Our group is involved in a research project dealing with fragrance compounds monitoring in WWTPs. In chapter 6, a HS-SPME/GC-MS method was developed to determine fragrances and monitored them in two different WWTPs from Girona (Spain). Lilial, tonalide, galaxolide, and benzyl salicylate were detected in samples taken after a tertiary treatment involving chlorination and UV, at maximum concentrations of 0.89, 3.49, 0.36 and 0.86 μg·L^{-1}, respectively. Thus, in order to evaluate these tertiary treatments, a new study focused on the elimination and transformation of 17 fragrances including allergens and polycyclic musks has been undertaken.

7.3.1 STUDY OF THE FRAGRANCES REMOVAL

Two different tertiary treatments usually applied in WWTPs were tested for the removal of the fragrances. For that, mixed Milli-Q water solutions containing the target compounds at concentrations between 1.7 and 60 $\mu g \cdot L^{-1}$ depending on the analyte (see Table 7.1) were prepared and analysed in duplicate (chlorination experiments) or triplicate (UV experiments). In the case of t-cinnamaldehyde, UV experiments were performed using an individual milli-Q water solution to avoid confused results since cinnamyl alcohol oxidation can produce the corresponding aldehyde.

Firstly, a stability study was performed to check that there was no degradation of the analytes due to external factors. The spiked Milli-Q water solutions were kept in the darkness and samples were taken for their analysis (n=2) at prescribed intervals which ranged from 5 to 120 min. No degradation was observed for any of the target compounds during the evaluated period (p>0.05, ANOVA test).

7.3.1.1. UV

UV treatment is a very popular method for disinfecting potable water and it has also been applied for the removal of micropollutants, alone or combined with H_2O_2. We essayed UV treatment for the removal of fragrance compounds in synthetic waters, and quantitative elimination (final concentration of the treated sample <LOD) was achieved for 13 of the analytes (limonene, linalool, β-citronellol, both citrals, geraniol, t-cinnamaldehyde, cinnamyl alcohol, eugenol, lyral, benzyl benzoate, tonalide, and benzyl salicylate) after being irradiated during 120 min (Table 7.2).

Table 7.2. Removal % of the studied fragrances after applying the UV treatment during 5 and 120 min (n=3). Standard deviations are showed in parenthesis. (-): not determined.

Compound	5 min	120 min
Limonene	97 (4)	100 (0)
Linalool	6 (8)	95 (9)
β-Citronellol	4 (4)	100 (0)
Citral A	100 (0)	100 (0)
Citral B	100 (0)	100 (0)
Geraniol	0 (0)	97 (5)
t-Cinnamaldehyde	-	100 (0)
Cinnamyl alcohol	100 (0)	100 (0)
Eugenol	54 (7)	100 (0)
Coumarin	20 (10)	57 (7)
Lilial	10 (20)	60 (10)
Lyral	20 (10)	80 (20)
Benzyl benzoate	20 (20)	90 (10)
Galaxolide	20 (10)	60 (20)
Tonalide	100 (0)	97 (4)
Benzyl salicylate	80 (20)	97 (4)
Benzyl cinnamate	75 (6)	70 (10)

The evaluation of the transformation process showed that limonene, both citrals, cinnamyl alcohol, tonalide, and benzyl salicylate were quantitatively eliminated after being irradiated for 5 min (Table 7.2), while the quantitative removal of linalool, β-citronellol, geraniol, eugenol, and lyral was achieved after a treatment time of about 20 min. On the other hand, four of the target fragrances (lilial, coumarin, benzyl cinnamate, and galaxolide) were found to be only partially eliminated after an irradiation time of 120 min (Table 7.2). This is important from an engineering point of view because it means that even at a high UV exposure (120 min) these compounds cannot be completed removed. In conventional WWTPs, equipped with UV treatments, typically the exposure time used to be in the range of 10-60 seconds. That means that in principle, those WWTPs equipped with low pressure lamps for disinfection purposes, could remove just those compounds that present good removal efficiencies at shorter times (5 min). So it can be expected that UV treatment can be highly ineffective to remove fragrances if the current exposure or UV doses are not increased.

In the case of tonalide and galaxolide, our results are in agreement with previous one published by Sanchez-Prado et al. [25], who studied the photo degradation of polycyclic musks using an UV lamp at 254 nm. They observed that, after being irradiated during 30 min, the removal percentages of tonalide and galaxolide were ~100% and ~60%, respectively. They also observed that tonalide was removed in few minutes, whereas after 120 min galaxolide was still not completely eliminated (removal of ~90%).

Under real operation conditions in a WWTP, biodegradation, volatilization and sorption can be considered the main mechanisms responsible for the removal of organic pollutants mechanisms. However, as it was reported in chapter 6, some of the fragrances studied in this work were still found in the effluent of WWTP. That was the case for lilial, tonalide, galaxolide, and benzyl salicylate which were present in WWTP samples taken after a tertiary treatment consisting of UV irradiation followed by chlorination in Castell-Platja d'Aro. According to the results presented here, the conditions of UV irradiation in the treatment plant have to be optimized to achieve at least the complete removal of tonalide and benzyl salicylate, and thus improving the quality of treated water.

7.3.1.2 Chlorination

Chlorination is also a common treatment process applied in drinking-water-treatment plants for the disinfection of drinking water. In WWTPs chlorination is applied especially when effluents are used for agricultural purposes. The effectiveness of chlorination was evaluated for the removal of the studied fragrances and diverse behaviours could be observed depending on the compound and the concentration of chlorine. As can be seen in Table 7.3, β-citronellol, both citrals, geraniol, cinnamyl alcohol, eugenol, and lyral were quantitatively eliminated after only 5 min treatment at the two chlorine concentrations tested.

Table 7.3. Removal % of the studied fragrances after applying the chlorination during 5 and 120 min (n=2). Standard deviations are showed in parenthesis.

	1 ppm Cl_2		5 ppm Cl_2	
Compound	**5 min**	**120 min**	**5 min**	**120 min**
Limonene	15 (3)	50 (7)	8 (10)	10 (20)
Linalool	60 (20)	77.1 (0.1)	30 (40)	42 (5)
β-Citronellol	100 (0)	100 (0)	100 (0)	100 (0)
Citral A	100 (0)	100 (0)	100 (0)	100 (0)
Citral B	100 (0)	100 (0)	100 (0)	100 (0)
Geraniol	100 (0)	100 (0)	100 (0)	100 (0)
t-Cinnamaldehyde	35 (8)	45 (4)	94 (5)	96 (5)
Cinnamyl alcohol	100 (0)	100 (0)	100 (0)	100 (0)
Eugenol	100 (0)	100 (0)	100 (0)	100 (0)
Coumarin	10 (20)	31 (3)	4 (2)	20 (10)
Lilial	20 (10)	20.8 (0.8)	21 (1)	9 (10)
Lyral	98 (1)	98 (2)	100 (0)	100 (0)
Benzyl benzoate	10 (6)	17 (2)	0.6 (0.9)	5 (1)
Galaxolide	20 (8)	12 (7)	61 (9)	60 (20)
Tonalide	30 (10)	23 (7)	20 (10)	29.3 (0.4)
Benzyl salicylate	5.8 (0.1)	23 (1)	96.8 (0.5)	100 (0)
Benzyl cinnamate	0 (0)	0 (0)	78 (3)	90 (2)

Table 7.3 also shows that limonene, coumarin, lilial, benzyl benzoate, and tonalide were slightly affected by the presence of chlorine since removals of ≤50% were observed for them after being treated during 120 min irrespectively of the chlorine concentrations. For t-cinnamaldehyde, galaxolide, benzyl salicylate, and benzyl cinnamate, a significant improvement on their elimination was observed when the chlorine concentration was increased up to 5 $mg \cdot L^{-1}$. In contrast, for linalool and benzyl benzoate, a better removal was achieved at the lower concentration of chlorine.

Our results agree with those reported by Gibs et al. [7]. They evaluated the persistence of several organic compounds, including limonene, galaxolide and tonalide, in drinking water containing 1.2 $mg \cdot L^{-1}$ of free chlorine as a function of time. They found that the removal percentage of limonene after one day was 53 %, whereas for galaxolide and tonalide, after one day treatment, the removal was also not quantitative (25 % for each musk).

7.3.2. REACTION KINETICS STUDY

For those fragrances exhibiting pseudo-first order dependence on the concentration of the compound, the following equation holds:

$$\ln C = \ln C_o - kt \qquad (1)$$

where C_o is the initial concentration of the analyte (μg·L^{-1}), C is the concentration of the analyte (μg·L^{-1}) at a time t (s) and k (s^{-1}) is the first order rate constant.

For the UV treatment, reaction kinetics were evaluated for the different compounds and linear response was only obtained for coumarin and benzyl benzoate when plotting ln (C/C_o) against time (Figure 7.1).

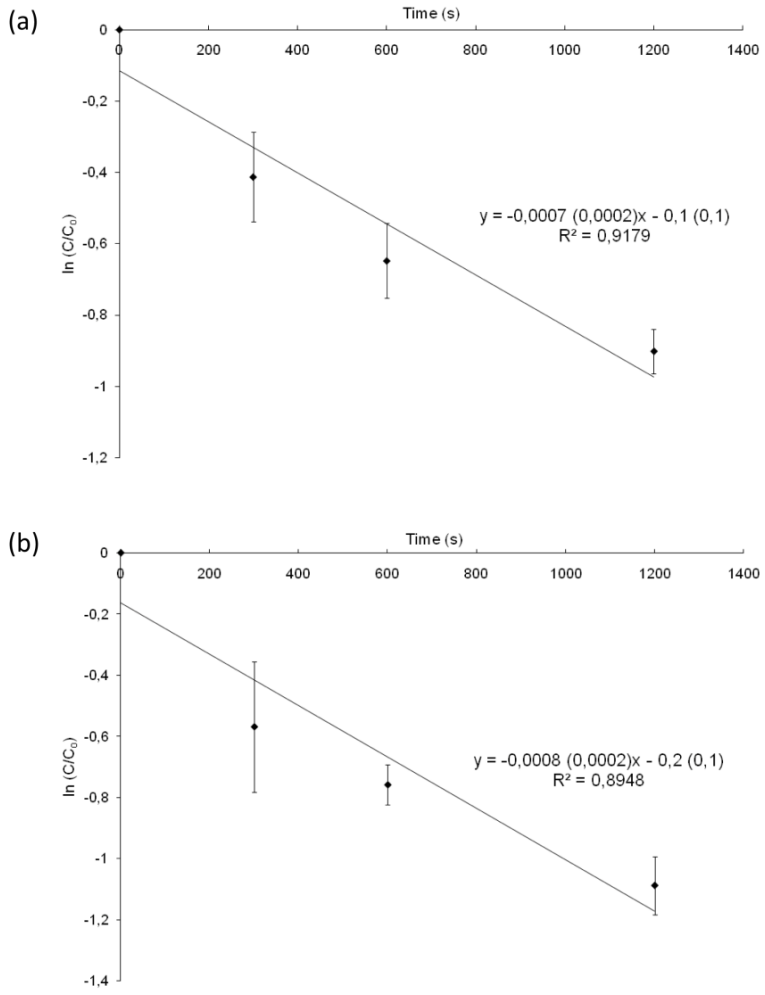

Figure 7.1. First order reaction plot for the UV removal of (a) coumarin and (b) benzyl benzoate. Standard deviations are showed in parenthesis.

Limonene, both citrals, cinnamyl alcohol, tonalide, and benzyl salicylate were quantitatively eliminated after being irradiated for 5 min. For the rest of the evaluated fragrances, no linear relationship was obtained according to Eq. (1). It was observed that lilial and lyral presented similar degradation profiles (Figure 7.2), in which the decrease in the concentration of the analytes started once the samples had been irradiated for 10 min.

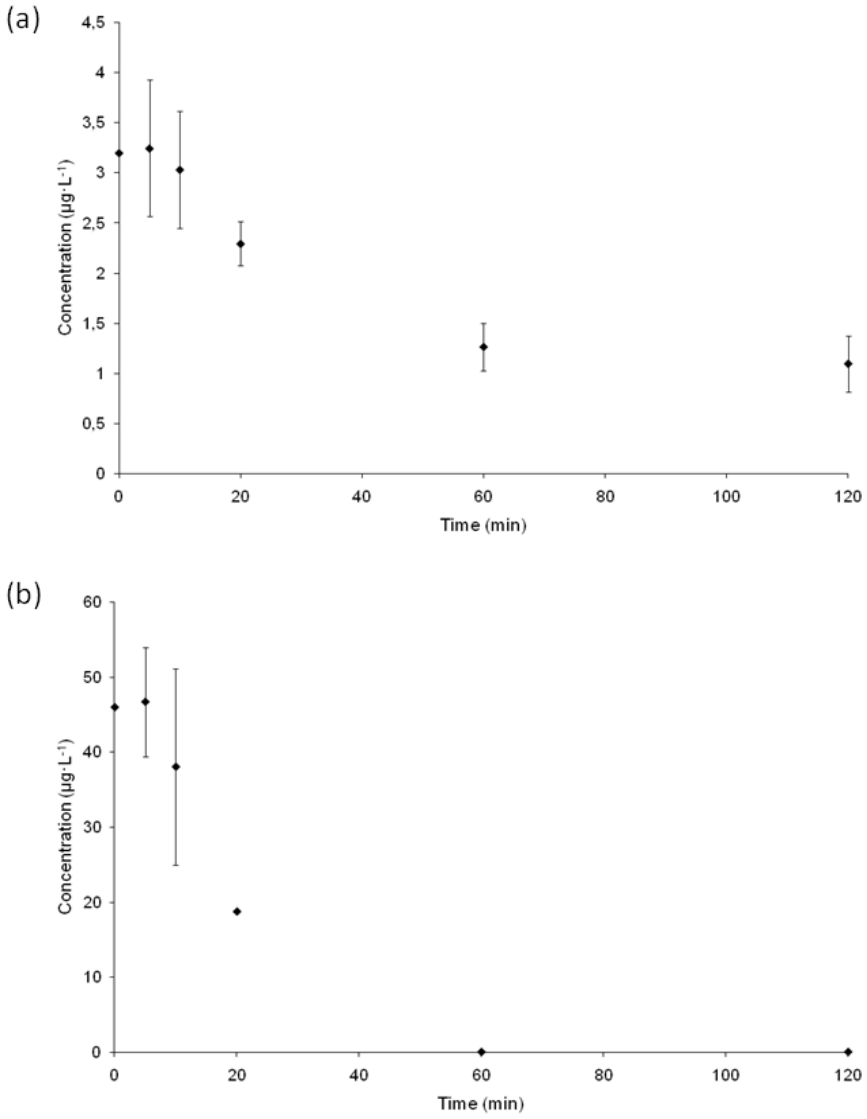

Figure 7.2. UV degradation profile (n=3) for (a) lilial and (b) lyral (n=3).

The calculated first order rate constants and their corresponding half-life time ($t_{1/2}$), were found to be $7 \cdot 10^{-4}$ s^{1} and 990 s, respectively, for coumarin, and $8 \cdot 10^{-4}$ s^{-1} and 866 s, respectively for benzyl benzoate. Kim et al. [11] evaluated the UV first order rate constant for a group of 30 pharmaceuticals and PCPs and obtained values which ranged from $6 \cdot 10^{-5}$ s^{-1} to $2.4 \cdot 10^{-2}$ s^{-1}.

For the chlorination experiments, the corresponding rate constants could not be calculated because the compounds were found to be either quantitatively eliminated after being treated for 5 min (β-citronellol, both citrals, geraniol, cinnamyl alcohol, eugenol, and lyral), or their removal remained invariable after the first 5 min treatment (Table 7.3). Similar trends were observed irrespectively of the chloride concentration used.

7.3.3 TRANSFORMATION PRODUCTS OF FRAGRANCES

For the target compounds partially or totally eliminated in the removal experiment described in Section 7.3.1, transformation products were investigated. For that, individual spiked Milli-Q water solutions containing 50 $\mu g \cdot L^{-1}$ of each analyte were prepared and analysed by duplicate. Blank analyses (irradiated and chlorinated milli-Q water) were performed (n=2) to confirm that the transformation products we found did not come from the treated milli-Q water.

7.3.3.1 UV transformation products

Several studies have shown that organic compounds can undergo degradation by direct UV photolysis [10,11,27]. The light induces electronic excitation of the organic substrate in a first step, followed by photooxidation or homolysis. In the present study, transformation products of nine of the target compounds (both citrals, geraniol, cinnamyl alcohol, lilial, coumarin, tonalide, benzyl benzoate, and benzyl cinnamate) were detected. Chromatographic and mass spectrometric data of all detected transformation products are listed in Table 7.4.

Table 7.4. Retention times, Kovats retention indexes and mass spectra for the transformation products found after the UV treatment of individual standards containing 50 µg·L^{-1} of each analyte. Information about the proposed transformation products is showed in cursive. (-).: not determined, n.a.: not available.

Transformation product	t_R (min)	Kovats RI	Mass fragments (relative abundance)
Citral by-product 1, Geraniol by-product 1	11.18	1138	81 (100), 79 (64), 67 (64), 123 (61), 95 (47), 91 (41)
Cinnamyl alcohol by-product 1	11.42	1146	91 (100), 92 (79), 77 (28), 78 (27), 51 (22),65 (17)
Benzenepropanal		*1160*	*91 (100), 92 (82), 78 (52), 77 (36), 51 (28), 65 (22)*
Lilial by-product 1	14.13	1316	147 (100), 91 (89), 119 (35), 148 (10), 77 (10), 115 (10)
Lilial by-product 2	15.74	1415	119 (100), 91 (89), 147 (80), 175 (60), 189 (58), 131 (48)
Lilial by-product 3	18.13	1365	189 (100), 204 (33), 147 (229), 73 (19), 91 (11)
Coumarin by-product 1	14.81	1359	174 (100), 145 (91), 146 (67), 132 (26), 173 (24), 196 (24)
Tonalide by-product 1	21.87	2157	229 (100), 173 (61), 43 (45), 145 (25), 187 (24), 230 (18)
Tonalide by-product[a]		*n.a.*	*229 (100), 173 (36), 187 (19), 230 (19), 231 (19)*
Tonalide by-product 2	22.98	2321	245 (100), 43 (36), 189 (27), 203 (24), 246 (18), 260 (14)
Tonalide by-product[a]		*n.a.*	*245 (100),243 (57), 260 (41), 259 (19), 246 (17), 203 (15)*
Tonalide by-product 3	24.83	2475	229 (100), 43 (42), 173 (39), 272 (35), 230 (28), 187 (19)
Tonalide derivative[b]		*n.a.*	*229 (100), 272 (64), 187 (36), 173 (29)*
Tonalide by-product[c]		*n.a.*	*229 (100), 272 (51), 173 (41) 239 (36), 187 (29)*
Tonalide by-product 4	25.85	-	257 (100), 201 (93), 215 (69), 197 (48), 171 (23), 258 (17)
Benzyl cinnamate by-product 1	23.42	2359	194 (100), 152 (80), 195 (15), 58 (11), 193 (10)
Benzyl benzoate by-product 1	22.29	2227	194 (100), 152 (97), 166 (94), 236 (28), 87 (14), 153 (11)
Benzyl benzoate by-product 2	22.41	2246	192 (100), 91 (97), 131 (63), 193 (61), 103 (49)
Benzyl benzoate by-product 3	22.62	2281	194 (100), 166 (98), 152 (69), 91 (15), 236 (11), 195 (11)
Benzyl benzoate by-product 4	22.92	2316	194 (100), 152 (84), 166 (30), 100 (18), 193 (11)

[a]Sanchez-Prado et al. [25] by-products, [b]Valdersnes et al. [28] derivative, [c]Janzen et al. [13] by-product.

Citral and geraniol gave a transformation product at a retention time of 11.18 min (Figures 7.3 and 7.4, respectively), whose Kovats retention index (Table 7.4) (i.e. system-independent constants calculated by normalising the retention time of the target compounds to the retention times of adjacently eluting n-alkanes) show resemblance to photocitral A [29]. This compound has previously been identified as citral UV transformation product by other authors [30,31].

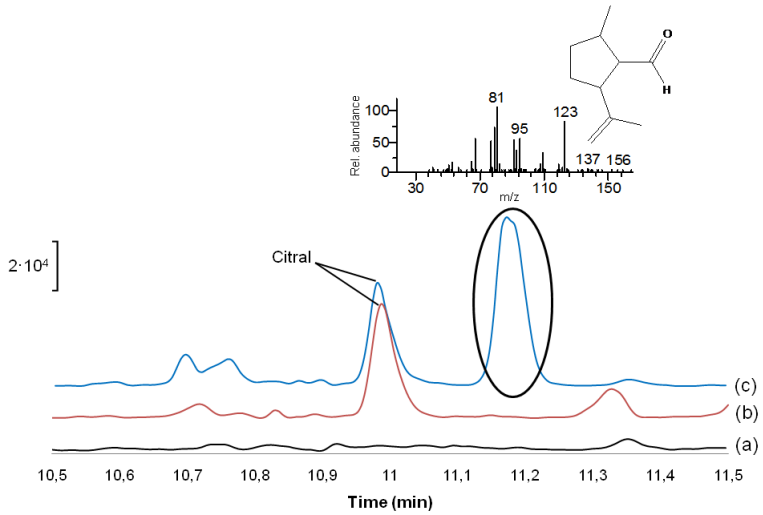

Figure 7.3. Comparison of extracted chromatograms (m/z=69) between (a) blank, (b) untreated 50 µg·L^{-1} citral standard and (c) 50 µg·L^{-1} citral standard after 5 min of irradiation.

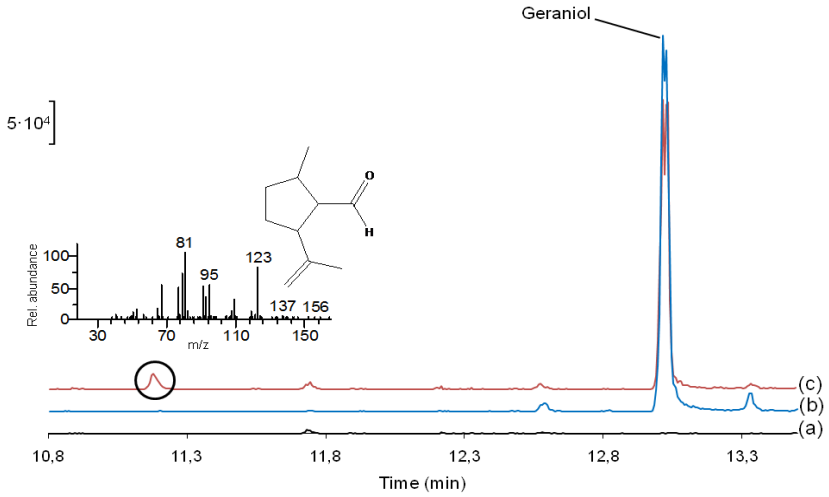

Figure 7.4. Comparison of extracted chromatograms (m/z=123) between (a) blank, (b) untreated 50 µg·L^{-1} geraniol standard and (c) 50 µg·L^{-1} geraniol standard after 15 min of irradiation.

In Figure 7.5, the extracted chromatogram of (b) untreated and (c) irradiated cinnamyl alcohol standards are compared with a blank chromatogram (a), and the formation of a by-product at the retention time of 11.42 min (cinnamyl alcohol by-product 1) can be observed. As it can be seen in Table 7.4, the mass spectrum of the formed by-product is similar to benzenepropanal. Moreover, their Kovats retention indexes are also comparable.

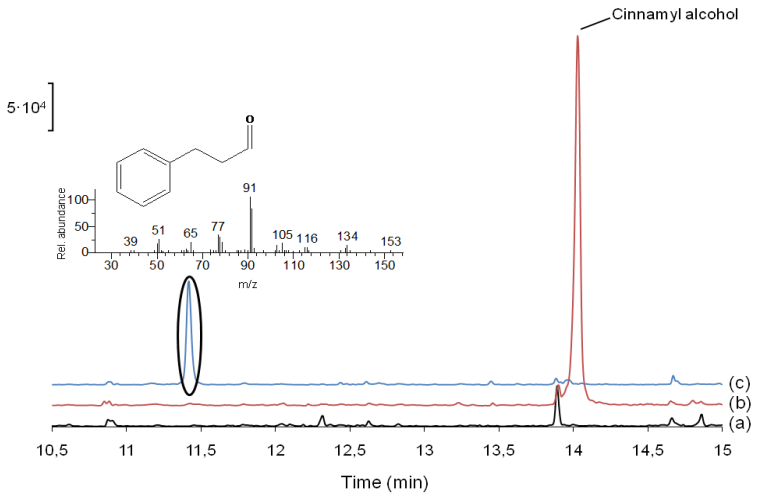

Figure 7.5. Comparison of extracted chromatograms (m/z=134) between (a) blank, (b) untreated 50 µg·L^{-1} cinnamyl alcohol standard and (c) 50 µg·L^{-1} cinnamyl alcohol standard after 5 min of irradiation.

Taking into account the structural information obtained from mass spectra of the product formed, a transformation pathway consisting on the migration of a hydrogen atom, accompanied by a switch of a single bond and adjacent double bond giving the keto form is proposed in Figure 7.6.

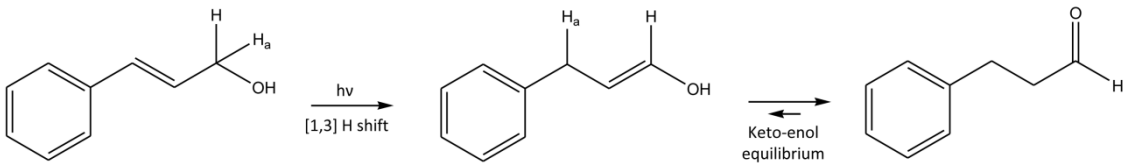

Figure 7.6. Proposed degradation pathway for the UV transformation of cinnamyl alcohol into benzenepropanal.

Four tonalide transformation products were also detected (Table 7.4) and chemical structures were proposed for three of them (Figures 7.7 to 7.9). The tonalide by-products at the retention times of 21.87 min (tonalide by-product 1) and 22.98 min (tonalide by-product 2) show great similarities to 6-ethyl-1,1,2,4,4,7-hexamethyltetralin and 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethylnaphtalen-2-yl)ethanol, respectively, which were also identified as UV transformation products by Sanchez-Prado et al. [25]. Significant similarities were also found between the tonalide by-product at 24.83 min (tonalide by-product 3) and 3-acetyl-5,6,7,8-tetrahydro-5,5,7,8,8-pentamethyl-2-naphthalenecarbaldehyde, which is a tonalide derivative synthesised by Valdersnes et al. [28] and identified as an ozonation by-product by Janzen et al. [13].

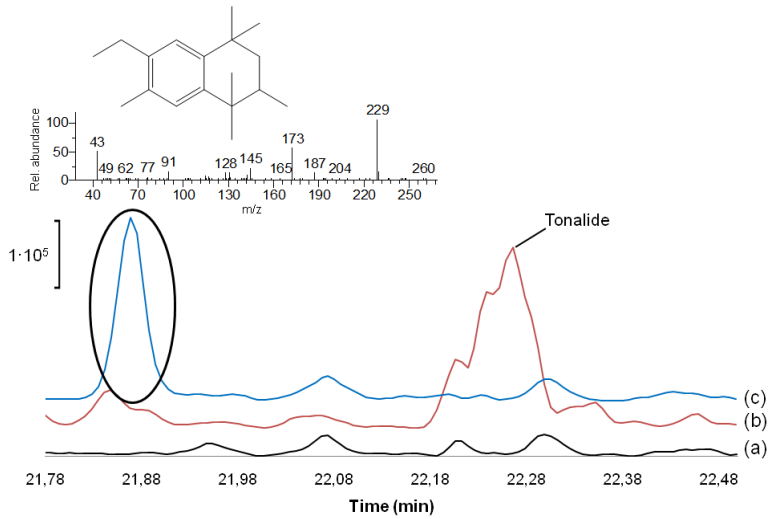

Figure 7.7. Comparison of extracted chromatograms (m/z=229) between (a) blank, (b) untreated 50 µg·L^{-1} tonalide standard and (c) 50 µg·L^{-1} tonalide standard after 5 min of irradiation.

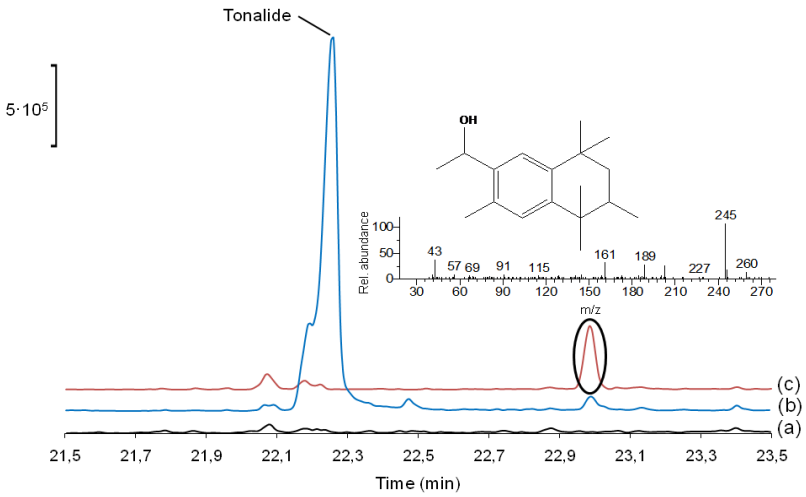

Figure 7.8. Comparison of extracted chromatograms (m/z=245,260) between (a) blank, (b) untreated 50 µg·L^{-1} tonalide standard and (c) 50 µg·L^{-1} tonalide standard after 5 min of irradiation.

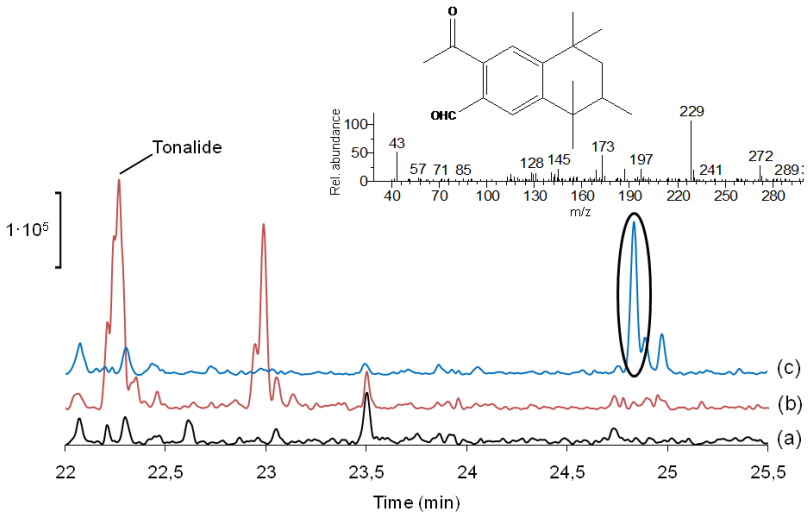

Figure 7.9. Comparison of extracted chromatograms (m/z=229) between (a) blank, (b) untreated 50 µg·L^{-1} tonalide standard and (c) 50 µg·L^{-1} tonalide standard after 5 min of irradiation.

The rest of transformation products could not be identified since no similarities were found between their mass spectra and the mass spectra of the compounds included in the library (NIST MS Search 2.0).

Although eugenol, galaxolide and benzyl salicylate were also affected by the UV treatment (section 7.3.1.1), their transformation products did not appear under the chromatographic conditions used in our study.

7.3.3.2 Chlorination transformation products

Gaseous chlorine and hypochlorite are normally used in chlorination treatments of WWTPs. Among the different aqueous chlorine species, the main reactive forms during the water treatment at pH=7 (pH used in the chlorination experiments of this study) are ClO^- and HOCl, being the last specie the one which predominates [17,32]. The oxidizing power of hypoclorous acid and the polarization of its Cl-OH bond allow this species to react with organic compounds by means of three different ways: (i) oxidation reactions, (ii) addition reactions to unsaturated bonds and (iii) electrophilic substitution reactions at nucleophilic sites [32].

During chlorination of olefins, HOCl addition reactions are expected to happen [32]. However, hypochlorous acid reactions with unsaturated bonds are generally too slow to be appreciated under water treatment conditions. In aldehydes or ketones chlorination, substitution reactions on the α-carbon to the carbonyl group can take place [9,32]. In alcohol chlorination, both

primary and secondary alcohols can be oxidized to carbonyl compounds through a very slow reaction after being dehydrogenated [32]. Chlorination of aromatic compounds usually results in electrophilic substitutions and the formation of C-OH, C=C and C-Cl bonds [8,16,32].

In the present study, it was observed that chlorination of benzyl cinnamate resulted in one transformation product, whereas chlorination of galaxolide resulted in the formation of seven by-products. Their retention times, Kovats retention indexes and mass spectra are showed in Table 7.5.

Table 7.5. Retention times, Kovats retention indexes and mass spectra for the transformation products found after the chlorination treatment of individual standards containing 50 $\mu g \cdot L^{-1}$ of galaxolide and benzyl cinnamate. (-): not determined.

Product	t_R (min)	Kovats RI	Mass fragments (relative abundance)
Galaxolide by-product 1	23.35	2353	243 (100), 201 (70), 187 (52), 183 (44), 258 (31)
Galaxolide by-product 2	23.65	2378	215 (100), 295 (42), 216 (23), 297 (18), 170 (18)
Galaxolide by-product 3	24.68	2463	257 (100), 239 (58), 272 (39), 197 (34), 183 (26)
Galaxolide by-product 4	25.33	-	257 (100), 239(57), 197 (42), 272 (30), 258 (17)
Galaxolide by-product 5	25.52	-	243 (100), 225 (23), 157 (21), 171 (19), 197 (18)
Galaxolide by-product 6	26.06	-	257 (100), 201 (18), 258 (17), 239 (17), 197 (16)
Galaxolide by-product 7	26.54	-	257 (100), 201(33), 197 (28), 171 (21), 183 (20)
Benzyl cinnamate by-product 1	25.84	-	219 (100), 91 (70), 191 (31), 102 (23), 159 (18)

As can be seen in the table, the mass spectra of one of the galaxolide by-products (galaxolide by-product 2) present fragments at 295 and 297, with relative abundances of 42 and 18 %, respectively, which is a kind of distribution typical of molecules containing a chlorine atom. Taking into account the structural information obtained from its mass spectra, a transformation pathway consisting on a radical chain reaction of chlorine in presence of light to give a chloride derivative can explain the formation of this by-product [33].

The detected transformation products could not be identified as no matches were found within the compounds included in the library (NIST MS Search 2.0). However, Table 7.5 shows that all transformation products retain masses of their respective precursor (see Table 7.1) in their mass spectra, corroborating that they are originated when benzyl cinnamate and galaxolide are treated with chlorine.

Although more fragrance compounds were affected by the presence of chlorine in the removal study described in Section 7.3.1.2, their transformation products did not appear under the chromatographic conditions used in our study.

7.4 CONCLUSIONS

This study has shown that chlorination and UV treatments are not completely effective for the removal of the target fragrances from water. In general, only seven analytes (β-citronellol, both citrals, geraniol, cinnamyl alcohol, eugenol, and lyral) were quantitatively removed by the two treatments tested. The rest of the target compounds showed different behavior depending on the treatment applied, being the UV irradiation more effective than the chlorination. More advanced technologies should therefore be used for the removal of all analytes.

The UV and chlorination reaction kinetics have been studied for the target compounds. UV first order rate constants of $7 \cdot 10^{-4}$ s^{1} and $8 \cdot 10^{-4}$ s^{-1} have been found for coumarin and benzyl benzoate, respectively, whereas the corresponding chlorination rate constants could not be calculated.

UV transformation products have been found for nine analytes and chemical structures have been proposed for cinnamyl alcohol, citral, geraniol, and tonalide by-products. In chlorination experiments, transformation products for benzyl cinnamate and galaxolide were detected.

Finally, it should be emphasized that the stability and toxicity of the degradation products found in the present study should be further investigated in order to evaluate their impact on the environment.

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CHAPTER 8: Global discussion

This chapter includes a global discussion of the results summarized in chapters 3-7. As the analytes studied in this thesis belong to two different groups of compounds, the results obtained will be discussed separately in two parts. The first part is focused on the development of analytical methodologies for the analysis of odour-causing compounds in WWTP water and air. The second part is devoted to the development of an analytical methodology for the quantification of fragrances in wastewater and the study of their elimination and transformation products in water samples.

In this thesis, sampling of WWTP water and air samples was performed in different sampling campaigns and in three different plants, where elimination of organic compounds takes place by conventional treatment systems with activated sludge. In chapters 3 and 4, samples from a plant located at Castell-Platja d'Aro (Girona province, Spain) were analysed. In chapter 5, air sampling was performed in Castell-Platja d'Aro and Reus (Tarragona province, Spain) WWTPs. In chapters 6 and 7, Castell-Platja d'Aro and Girona (Girona province, Spain) WWTPs were monitored.

8.1 DEVELOPMENT OF ANALYTICAL METHODS FOR THE ANALYSIS OF ODOROUS COMPOUNDS IN WATER AND AIR SAMPLES FROM WWTPS

The determination of odour-causing compounds in aqueous samples using HS-SPME as the extraction technique is usually devoted to compounds belonging to the same chemical family (e.g., aldehydes, sulphides, amines, and VFAs). In chapter 3, a HS-SPME method was developed for the determination of a group of odorous compounds belonging to different chemical families (phenolic compounds, aldehydes, sulphur-containing compounds, nitrogen-containing compounds, and terpenes) in wastewater samples.

The selection of an adequate coating is essential in SPME. Thus, two different coatings previously reported for the determination of odour-causing compounds in environmental samples (CAR/PDMS and DVB/CAR/PDMS) were evaluated. A clear difference was observed between them in terms of peak shape, being the DVB/CAR/PDMS fibre the one giving the best results.

The SPME extraction conditions (salt content, extraction time and temperature) were investigated by means of a 2^3 full factorial design in order to ascertain their influence on the extraction of the odorous compounds from aqueous solutions. Using a sample volume of 5 mL,

1 g of NaCl added, an extraction time of 30 min and an extraction temperature of 70°C were found to be the best conditions for the simultaneous analysis of the target compounds.

The optimised method provided LODs ranging from 0.03 and 0.6 $\mu g \cdot L^{-1}$, which allowed the quantification of the odorous compounds well below their respective OTCs. Recoveries and intra-day precision of the method were evaluated using milli-Q water and WWTP water and were found to meet the AOAC values (recoveries between 70 and 120%). The only exception was octanal, whose recovery in wastewater was 49%. This fact was attributed to the rapid degradation of this compound in the sample probably as a consequence of microbial activity.

The developed method was applied to the analysis of samples obtained from the primary treatment effluent, the biologic treatment effluent and the effluent (after UV treatment) of the Castell-Platja d'Aro WWTP. As it can be seen in Table 3.6 (chapter 3), all compounds were usually detected in primary samples, being *m*-cresol, phenol, indole, and skatole the compounds present at higher concentrations. A decrease in the concentration of the target compounds was also observed along the different treatments of the WWTP. In addition, some of the compounds were detected at concentrations above their OTCs in some of the analysed samples. Octanal was found above its OTC in at least one sample of each sampling point. Skatole and DMDS were found at concentrations above their OTCs in all primary effluent samples in which they were detected (3 out of 3 and 1 out of 3, respectively). This fact indicates that these compounds may have a great influence on the odorous perception in WWTP environments.

A study focused on the determination of the odour causing compounds in atmospheres surrounding the WWTPs was undertaken in chapter 4. As SPME is a simple and accepted method for the enrichment of volatile and semi-volatile substances in air, this technique was selected for the concentration of the odorous compounds in air samples from a WWTP.

Sampling was performed in open air at the Castell-Platja d'Aro WWTP using laboratory-made 25 L Nalophan® bags, which were filled on-site with the help of an air sampling pump. The collected whole air samples were transported to the laboratory and analysed within 3 hours. To do that, samples were transferred to a 0.5 L glass sampling bulb, where the SPME extraction was performed at 22 ± 1°C. Again, the DVB/CAR/PDMS coating was the one which provided the best results for the simultaneous extraction of the target compounds. The co-adsorption conditions of the odorous compounds were afterwards evaluated. First, the adsorption kinetics was evaluated for three target compounds presenting different volatilities

(DMDS, *m*-cresol and skatole), and it was observed that the co-adsorption range was up to 30 min. Then, a theoretical approach based on Fick's diffusion law was applied to determine the extraction time for a gas mixture containing all target compounds. As some authors had previously observed, short exposure times were found to be adequate to avoid analyte discrimination and saturation of the coating. According to our results, an extraction time of 10 minutes was therefore selected.

The obtained LODs ranged between 0.1 and 0.9 $\mu g \cdot m^{-3}$, except for octanal and nonanal that could only be detected at concentrations greater than 20 $\mu g \cdot m^{-3}$. These LODs were found to be satisfactory since they allowed the determination of the target compounds at concentrations below their OTCs. The performance of the method was also demonstrated in terms of precision and trueness, with RSD values ranging from 12 to 24 % and relative biases between 0.1 and 10 %.

The validated method was thereafter applied to the determination of the partition coefficients of the odour-causing compounds. Partition coefficients, which can be defined as the concentration of VOCs at equilibrium at the interface between air and water, can be used as indicators of the tendency of the compounds present in water to be exchanged with the air phase. Using a partition equilibrium time of 5 h, gas-liquid partition coefficients between 0.00020 and 0.13 were obtained in chapter 4 (Table 8.1).

Table 8.1. Partition coefficient (p_c) of the target odour-causing compounds and results obtained in the analysis of Castell-Platja d'Aro WWTP air samples. n.d.; not detected

		Concentration in air ($\mu g \cdot m^{-3}$)		
Compound	**p_c (SEM[a])**	**Influent (n=4)**	**Biologic treatment influent (n=4)**	**Sludge pre-treatment (n=3)**
DMDS	0.09 (0.01)	7.6 – 12.7	<LOQ – 8.7	4.8 – 16.6
Phenol	0.0004 (0.0002)	n.d. – 11.4	<LOQ – 18.5	2.1
Octanal	0.043 (0.04)	n.d.	n.d.	n.d.
Limonene	0.13 (0.05)	7.8 – 41.0	<LOQ – 19.0	n.d. – 17.0
m-Cresol	0.00020 (0.00004)	n.d.	n.d.	n.d.
Nonanal	0.070 (0.006)	n.d.	n.d.	n.d.
Benzothiazole	0.0022 (0.0008)	n.d.	n.d.	n.d.
Carvone	0.0018 (0.0001)	n.d.	n.d.	n.d.
Indole	0.00046 (0.00009)	n.d.	n.d.	n.d.
Skatole	0.0008 (0.0001)	n.d.	n.d.	n.d.

[a] Standard error of the mean.

No reference values for the target compounds were found in the literature, but the obtained values were found to be satisfactory as they were much lower than those for most volatile compounds, such as benzene and toluene (0.194 and 0.224, respectively). Limonene and DMDS were found to be the compounds which presented the highest values, while phenol and indole presented the lowest ones. As can be seen in Table 8.1, the partition coefficients obtained were in agreement with the experimental concentrations obtained in the analysis of air samples from the Castell-Platja d'Aro WWTP, where DMDS and limonene were detected in all air samples. For phenol, although its partition coefficient was low (p_c=0.00020), a maximum concentration of 18.5 $\mu g \cdot m^{-3}$ was found in the biologic treatment influent. Its presence in the air samples was then attributed to a biogenic origin, as $\mu g \cdot L^{-1}$ levels had also been reported in gas cow slurries of intensive production farms.

Even though adequate LODs were obtained with the SPME method developed in chapter 4, a new study focused on the use of another technique for the determination of the odorous compounds was undertaken in chapter 5 in order to improve the obtained values. Solid sorbent capture, which is another technique usually applied for the sampling and enrichment of air samples, was then tested for the concentration of a group of VOCs including odour-causing compounds and ozone precursors in WWTP air samples (Table 5.1). The list of compounds determined in this study included substances belonging to different chemical families, presenting different volatilities and chromatographic behaviours. Two types of sorbents (Tenax TA and Tenax TA/Carbograph 1TD) were evaluated in order to check the best one for the simultaneous retention of the studied analytes. The dual-bed trap was selected since responses for 1,2,3-trimethylbenzene, 1,4-diethylbenzene and carvone were higher when this trap was used. Then, the TD parameters (cold trap and tube desorption) were evaluated to ensure the best desorption conditions. In the final TD method, the tube desorption was performed at 275^{o}C for 10 min with a helium flow rate of 30 $mL \cdot min^{-1}$. The desorbed compounds were refocused into a Tenax TA/Carbotrap 1TD cold trap at -10^{o}C using splitless mode, which was then desorbed at 300^{o}C for 10 min a split flow of 10 $mL \cdot min^{-1}$.

A breakthrough evaluation was performed using air from the inlet of a WWTP, where high levels of VOCs and large relative humidity were expected. A volume of 1 L was selected as it allowed the quantitative retention of all the analytes in the sorbent tube without breakthrough. In the method validation, LODs ranging between 0.2 and 2 $\mu g \cdot m^{-3}$ were obtained (sample volume of 1 L). The only exception was nonanal, whose LOD was 20 $\mu g \cdot m^{-3}$.

As can be seen in Table 8.2, for most analytes the obtained LODs were found to be in the same order than those obtained with the SPME method developed in chapter 4.

Table 8.2. LODs ($\mu g \cdot m^{-3}$) obtained with the two methods developed for the determination of the odorous compounds in WWTP air samples. (-): not determined.

Compound	SPME method	TD method
DMDS	0.1	0.3
Phenol	0.2	2
Octanal	20	-
Limonene	0.7	0.3
m-Cresol	0.4	2
Nonanal	20	20
Benzothiazole	0.8	0.5
Carvone	0.9	0.3
Indole	0.2	0.3
Skatole	0.3	0.3

The proposed method provided RSD values which ranged from 1 to 12 % (intra-day precision) and from 5 to 19 % (inter-day precision), which were found to be satisfactory. The stability of the sorption tubes loaded with the target compounds was evaluated after 24 h of storage and no significant losses were found for the analytes, which is an important advantage respect SPME concentration: samples can be stored in the tubes for several hours whereas in SPME enrichment samples should be analysed immediately after the extraction. Then, larger sampling campaigns can be performed when sorbent tubes are used.

The TD-GC-MS method was applied to the analysis of air samples from the plants located in Reus and Castell-Platja d'Aro. Eleven of the target compounds were detected in samples of the monitored WWTPs. As can be seen in table 5.3 (chapter 5), toluene, limonene and nonanal were the compounds found at the highest concentrations, while 1,4-dioxane, benzothiazole, carvone, indole, and skatole were not detected at all. In addition, toluene, *m*-cresol and nonanal were detected at concentrations above their OTCs in some of the analysed samples, which indicate that these substances may have an important contribution to the odour generation in the evaluated WWTPs.

The results obtained in chapters 4 and 5 show that both SPME and TD methods can be successfully applied to the analysis of the odorous compounds in air samples. The obtained LODs, which were found to be in the same order for both methods, allowed the detection of most of the target compounds at concentrations above their OTCs. The worst results were

obtained for the two aldehydes, octanal and nonanal, whose LODs were 20 $\mu g \cdot m^{-3}$. These values can be improved by including a derivatisation step, but this complicates the experimental procedure and introduces an important source of error for the rest of compounds. An advantage of the TD method proposed in chapter 5 was that it permitted the determination of the odorous compounds in a larger concentration range than the corresponding SPME method (chapter 4). In addition, with the TD method the enrichment of the samples can be done in a single step in the field, whereas with the SPME method samples have to be collected in sampling bags, transported to the laboratory, transferred to the glass bulb, and extracted as soon as possible. Under these conditions, analyte losses and contamination can occur due to the diffusion of some compounds through the polymer bag. However, while a special chromatographic equipment (equipped with a desorption unit) is required for the solid sorbent capture based method, with SPME concentration analyses can be performed with a conventional GC.

As can be seen in Table 8.3, although most of the target compounds were found to be present in primary effluent wastewaters in chapter 3, nonanal, carvone, indole, and skatole were not detected in the air samples from the same sampling points (but different sampling campaigns) analysed in chapters 4 and 5. This fact can be explained by the low gas-liquid coefficients calculated for these compounds (chapter 4), which indicates that these analytes have a high affinity towards the liquid phase.

Table 8.3. Concentrations found for the odour-causing compounds in the analysis of wastewaters and air from the primary treatment effluent of Castell-Platja d'Aro WWTP. n.d.; not detected, (-): not determined.

		Concentration in air ($\mu g \cdot m^{-3}$)	
Compound	**Concentration in water[a] ($\mu g \cdot L^{-1}$)**	**SPME method[b]**	**TD method[c]**
DMDS	5	8.7	1.9
Phenol	39.3	18.5	<LOQ
Octanal	<LOQ	n.d.	-
Limonene	1.28	19.0	2.9
m-Cresol	151	n.d.	<LOQ
Nonanal	<LOQ	n.d.	n.d.
Carvone	1.26	n.d.	n.d.
Indole	90	n.d.	n.d.
Skatole	13.5	n.d.	n.d.

[a] n=3, [b] n=4, [c] n=1.

Limonene was one of the compounds detected at higher concentrations in air, with maximum values of 41.0 and 232.6 $\mu g \cdot m^{-3}$ (influent samples) in chapters 4 and 5, respectively. However, it has to be considered that this analyte presents a high OTC in air (55000 $\mu g \cdot m^{-3}$) and there was no contribution of this analyte to the malodorous perception in the evaluated WWTPs. On the other hand, the occurrence of some of the target compounds at concentration levels above their OTCs in the WWTP water (DMDS, octanal and skatole) and air samples (toluene, *m*-cresol and nonanal) analysed in the present thesis suggests the fact that this class of substances should be considered as responsible for the odour generation in WWTP environments. It should be taken into account that other odorous VOCs not determined in this thesis such as H_2S, which is routinely measured by the personnel working in the evaluated plants, may have the most important contribution to the malodorous perception in WWTPs.

8.2 DEVELOPMENT OF A METHOD FOR THE ANALYSIS OF FRAGRANCES: OCCURRENCE IN WASTEWATER AND REMOVAL STUDY

Some fragrance compounds have been identified as suspected to cause allergies and several studies dealing with their determination in bath water and swimming pool water samples have been reported in the last years. However, few studies have been addressed to their monitoring in WWTPs. Only polycyclic musks has been routinely analysed in previous works and they have been found not to be completely eliminated along the different WWTP treatments.

Among all the techniques that have been reported for the enrichment of fragrances in water samples (e.g. LLE, SPE, SBSE, and SPME), SPME was selected in chapter 6 as the concentration technique due to its simplicity and the satisfactory results obtained in chapters 3 and 4. A method based on HS-SPME and GC-MS determination was developed for the simultaneous monitoring of allergens and polycyclic musks in wastewaters. A preliminary evaluation of two different fibres (PDMS/DVB and DVB/CAR/PDMS) showed that PDMS/DVB was the most suitable coating for the extraction of the target compounds. Afterwards, a 2^3 full factorial design was applied to investigate the effects of the SPME extraction parameters. For 10 mL of sample, the optimum conditions for the simultaneous analysis of the target compounds were found to be 2.4 g of NaCl added, an extraction time of 45 min and an extraction temperature of 90^{o}C. These final conditions were not found to be the optimum for limonene, linalool, β-citronellol, the two citrals, and geraniol, as the extraction temperature selected was the highest one, in which a competitive desorption process of these analytes from the fibre takes places.

The developed method provided adequate LODs, which ranged between 0.01 and 1.7 $\mu g \cdot L^{-1}$. These LODs were found to be in the same range than those obtained previously in studies where SPME and other microextraction methods (e.g., USAEMA and DLLME) were used. The obtained RSD values ranged from 4 and 20 % in WWTP water. Limonene was the analyte presenting the worst RSD value, which was >25 % at the two concentration levels tested. Limonene was also determined in chapter 3, where the developed HS-SPME method provided the same LOD but better inter-day precision (RSD=20 %). This fact is probably attributed to the extraction temperatures used in both developed methods: while extraction takes place at 70°C in chapter 3, in chapter 6 the temperature was 90°C, which was found not to be the most adequate for the most volatile compounds, such as limonene. Recoveries from spiked WWTP samples were also evaluated and results obtained were <50 % for hydroxycitronellal, lilial, lyral, galaxolide, benzyl salicylate, and benzyl cinnamate. These low recovery results were attributed to an oxidation of these analytes in the presence of oxidants or to the microbiological activity in wastewaters. Good recovery results were achieved when samples were treated with sodium tiosulphate to prevent any oxidation.

The validated HS-SPME/GC-MS method was applied to the monitoring of the target fragrances in the WWTPs located at Castell-Platja d'Aro and Girona. An important difference between these plants is that a tertiary treatment involving UV and chlorination is applied in the case of Castell-Platja d'Aro WWTP, whereas in the plant located at Girona there is no tertiary treatment. As can be observed in Table 6.5 (chapter 6), only seven of the target analytes (limonene, linalool, eugenol, lilial, galaxolide, tonalide, and benzyl salicylate) were detected in at least one of the samples, being linalool and galaxolide the fragrances found at the highest concentrations in both WWTPs. Limonene, linalool and eugenol were only detected at the primary effluent of the two monitored WWTPs, which indicated their quantitative elimination during the secondary treatments. In the case of limonene, the obtained results were in agreement with those obtained in chapter 3, where it was also determined in wastewater from the Castell-Platja d'Aro plant (Table 8.4).

Table 8.4. Maximum concentrations ($\mu g \cdot L^{-1}$) found for limonene in chapters 3 and 6. n.d.: not detected.

	Chapter 3 (n=3)	**Chapter 6[a]**
Primary treatment effluent	1.28	3.02
Biologic treatment effluent	n.d.	n.d.
Plant effluent	n.d.	n.d.

[a] n=13, n=14 and n=7 for primary treatment effluent, biologic treatment effluent and plant effluent, respectively.

However, four of the studied fragrances (lilial, tonalide, galaxolide, and benzyl salicylate) were found not to be eliminated during all wastewater treatments as they were detected at the effluent of both plants. In the case of Castell-Platja d'Aro WWTP, which is the one that presents a tertiary treatment consisting of UV irradiation followed by chlorination, these results suggest that these treatments may not be effective enough for the elimination of the four fragrances detected at the effluent of the plant.

As some target fragrances were detected in samples taken after a tertiary treatment involving UV and chlorination, a new study focused on the use of these treatments for the degradation of 17 fragrances was undertaken in chapter 7. UV experiments were performed in a cylindrical water-jacketed metal reactor equipped with a magnetic agitator at 20 ± 1^{o}C. A 15 W low-pressure UV lamp with a monochromatic emission predominantly at 253.4 nm was used. Chlorination experiments were performed in a home-made reactor equipped with a magnetic agitator and a water bath maintained at 20 ± 1^{o}C. Chlorine concentrations of 1 and 5 mg·L^{-1} were tested. In both cases, samples were taken at prescribed intervals and analysed immediately by means of the HS-SPME/GC-MS method developed in chapter 6.

In general, the UV treatment was found to be more effective in the removal of the target compounds than the chlorination. Thirteen out of the 17 target fragrances were quantitatively removed after 120 min of UV irradiation, while only seven and nine compounds were eliminated when chlorine concentration was 1 and 5 mg·L^{-1}, respectively. These results show that even at a high UV exposure (120 min) some analytes cannot be completed removed. It is important to take into account that WWTPs equipped with low pressure lamps for disinfection purposes have exposure times in the range of 10-60 s. That means they could remove just only those compounds that present good removal efficiencies at short times (5 min).

UV first order rate constants of 7·10^{-4} s^{1} and 8·10^{-4} s^{-1} were found for coumarin and benzyl benzoate, respectively, whereas the corresponding chlorination rate constants could not be calculated.

The second part of chapter 7 consisted on the evaluation of the transformation products of those analytes that had been partially or totally degraded by the two tested treatments. UV transformation products were detected for citral, geraniol, cinnamyl alcohol, lilial, coumarin, tonalide, benzyl cinnamate, and benzyl benzoate. Taking into account the structural information obtained from mass spectra of the by-products formed and their Kovats retention indexes, chemical structures were proposed for five of them: photocitral A as citral and

geraniol by-product, benzenepropanal as cinnamyl alcohol by-product, and 6-ethyl-1,1,2,4,4,7-hexamethyltetralin, 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethylnaphtalen-2-yl)ethanol and 3-acetyl-5,6,7,8-tetrahydro-5,5,7,8,8-pentamethyl-2-naphthalenecarbaldehyde as UV by-products of tonalide. It should be emphasized that the transformation products identified in this study have previously been identified by other authors. The investigation of chlorination by-products was also done and it was observed that chlorination of benzyl cinnamate resulted in one transformation product, whereas for galaxolide resulted in the formation of seven by-products. However, these transformation products were not identified.

Results obtained in chapters 6 and 7 show that conventional chlorination and UV treatments are not effective for the quantitative removal of some of the studied fragrances from wastewaters, as they were detected at the monitored plants' effluents (chapter 6) and not eliminated in the experiments performed at laboratory scale (chapter 7). Therefore, more advanced technologies in WWTPs are needed in order to achieve a complete removal of these compounds.

CHAPTER 9: Conclusions

In this thesis, different analytical methodologies have been developed for the assessment of odorous and fragrance compounds in air and water samples from WWTPs. Although specific conclusions have been included at the end of each chapter, the general main conclusions are summarised here:

1. A HS-SPME method has been developed and applied to the determination of a group of odour-causing compounds belonging to different chemical families in water samples from a WWTP. A decrease in the concentration of the target compounds was observed along the different treatments of the WWTP. Some of the target compounds were detected at concentrations above their OTCs in a few of the analysed samples.

2. Two different methods have been developed for the determination of a group of odour-causing compounds belonging to different chemical families in air samples from WWTPs:

    a. An SPME method has been applied to the assessment of the odorous compounds in air from a WWTP and to the determination of their gas-liquid partition coefficients. The calculated partition coefficients were found to be in agreement with the experimental concentrations obtained in the analysis of air and water samples from a WWTP.

    b. A method based on the concentration of analytes on multibed sorbent tubes using active sampling has been applied to the determination of a group of VOCs including odour-causing compounds and ozone precursors in WWTP air samples. This method has been found to be a good alternative to SPME as it allows the determination of the target compounds in a large concentration range, is field portable and provides adequate LODs with a sample volume of 1 L.

3. A HS-SPME method has been developed for the simultaneous determination of 16 allergens and two polycyclic musks in water samples. The proposed method has been applied to monitor the target compounds in two different WWTPs. Seven of the analytes have been detected at the effluent of the primary treatment of the evaluated plants. Four of them (lilial, tonalide, galaxolide, and benzyl salicylate) have been found not to be removed during the whole wastewater treatments.

4. The efficacy of two conventional WWTP tertiary treatments (UV and chlorination) for the fragrance removal from water has been evaluated at laboratory scale. Different behaviors have been observed depending on the analyte and the treatment applied, and the UV irradiation has been found more effective than the chlorination.

5. UV transformation products have been observed for nine analytes and chemical structures have been proposed for five by-products. In chlorination experiments, transformation products for benzyl cinnamate and galaxolide have been detected.

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