Università degli Studi di Ferrara

DOTTORATO DI RICERCA IN
Scienze Chimiche

CICLO XXIII

COORDINATORE Prof. Bignozzi Carlo Alberto

DEVELOPMENT OF ADVANCED ANALYTICAL METHODS FOR THE DETERMINATION OF EMERGING POLLUTANTS IN ENVIRONMENTAL WATERS

Settore Scientifico Disciplinare CHIM/01

Dottoranda
Dott. Basaglia Giulia

Tutore
Prof. Pietrogrande Maria Chiara

Anni 2008/2010

Alla mia famiglia e agli amici

Contents

Acknowledgements

Durante questi anni ho avuto il piacere di collaborare con diverse persone che hanno contribuito alla mia crescita personale e professionale, vorrei esprimere a loro tutta la mia gratitudine.

Un ringraziamento speciale, innanzitutto, alla mia tutor in questo progetto, la Prof.ssa Maria Chiara Pietrogrande. Ringrazio la Dott.ssa Luisa Pasti per i preziosi consigli, il Prof. Francesco Dondi e tutto il gruppo di Chimica Analitica dell'Università di Ferrara, in particolar modo la Dott.ssa Valentina Costa, il dott. Dimitri Bacco e la Dott.ssa Marianna Nassi.

Un sentito grazie al gruppo GCQ dell'Università di Santiago di Compostela, Spagna, in particolare al Prof. Rafael Cela, alla Dott.ssa Rosario Rodil RodrÍguez e al Dott. José Benito Quintana.

Vorrei ringraziare tutto il gruppo Ambiente Beni Culturali Ricerca Giovani della Società Chimica Italiana per i continui e positivi stimoli e spunti di riflessioni.

Ringrazio tutti gli amici e parenti che hanno condiviso questi anni con me.

Un sentito e affettuoso ringraziamento va ai miei genitori, Rita e Nino.

List of Papers

- *Paper 1*: M.C. Pietrogrande, G. Basaglia, “GC-MS analytical methods for the determination of personal-care products in water matrices” – Trends in Analytical Chemistry, Vol.26, No. 11 (2007), 1086-1094.

- *Paper 2*: M.C. Pietrogrande, G. Basaglia, F.Dondi, “Signal processing to evaluate parameters affecting SPE for multi-residue analysis of personal care products” – Journal of Separation Science, Vol.32 (2009), 1249-1261.

- *Paper 3*: M.C. Pietrogrande, G. Basaglia, “Enantiomeric resolution of biomarkers in space analysis: Chemical derivatization and signal processing for gas chromatography-mass spectrometry analysis of chiral amino acids” – Journal of Chromatography A, Vol. 1217 (2010) 1126-1133.

- *Paper 4*: R.Rodil, J.B. Quintana, G. Basaglia, M.C. Pietrogrande, R. Cela, “Determination of synthetic phenolic antioxidants and their metabolites in water samples by downscaled solid-phase extraction, silylation and gas chromatography-mass spectrometry” – Journal of Chromatography A,Vol.1217 (2010) 6428-6435.

- *Paper 5:* G. Basaglia, L. Pasti, M.C. Pietrogrande, “Multi-residual GC-MS determination of Personal Care Products in waters using Solid Phase Micro Extraction” – Analytical and Bioanalytical Chemistry, 399 (2011) 2257-2265.

- *Paper 6:* G. Basaglia, M.C. Pietrogrande, “Optimization of multi-residual GC-MS determination of Pharmaceuticals and Personal Care Products in water using Solid Phase Micro Extraction with On-Fiber derivatization”, *submitted for publication*.

1 Introduction

This thesis work was focused on the analytical determination of emerging pollutants in environmental water matrix, concerning preparative step and analysis using Gas Chromatography coupled with Mass Spectrometry (GC-MS).

The emerging pollutants are so defined because they have been recognized only recently as pollutants and regulatory and monitoring plans are not yet implemented at Italian and European level.

Pharmaceuticals and Personal Care Products (PPCPs) are considered emerging contaminants, they are a diverse group of environmental chemicals that have captured the attention of scientists and the public; they describe a large class of chemical contaminants that can originate from human usage and excretions and veterinary applications.

There are a large number of different substances used as medicines. During and after treatment, humans and animals excrete a combination of intact and metabolised pharmaceuticals, many of which are generally soluble in water and have been discharged to the aquatic environment with little evaluation of possible risks or consequences for humans and environment. In addition, the chemicals that are components of Personal Care Products (PCPs) number in the thousands. The world's people consume enormous quantities of skin care products, dental care products, soaps, sunscreen agents, and hair styling products. PCPs continuously enter the wastewater after their regular use during showering or bathing. Recent studies indicate the potential widespread occurrence on low-level concentrations (ng-µg/L) of PPCPs in the aquatic environment.

Therefore, there's critical need for efficient and reliable analytical methods to address the occurrence concentrations, and fate of the PPCPs in environment. GC-MS has been the basic tool for environmental analyses of various organic pollutants and it has been the approach of this study choice because of its superior separation and identification capabilities.

The activity has been concentrated on the development of sample preparation procedures that could be fast, cost-effective and environment-friendly for the analysis of PPCPs. It's known that sample pre-treatment causes an analysis bottleneck that typically accounts for over 60% of the total analysis time. The work was focused on evaluation and optimization of different extraction techniques for treatment of water matrix.

There are fundamentally two kinds of approach for parameters optimization. One Factor At Time (OFAT) method involves the testing of factors, or causes, one at a time instead of all factors are changed at once. Even more people, prominent text books and academic papers currently favour design of experiments (DOE) approach; it's a statistically multivariate method for screening and/or optimization of different factors at the same time (multiple factors are changed at once). It shows several relevant advantages over OFAT approach: it requires less runs for the same (sometimes more) precision in effect estimation, it can estimate interactions and it provides a knowledge (and optimal settings of factors) in the whole experimental domain, where OFAT can miss them. For these reasons this approach was chosen (*Paper 4*, *5*, and *6*), using in particular Central Composite Design (CCD) or Box-Behnken Design (BBD).

Two different extraction procedures were studied: Solid Phase Extraction (SPE) and Solid Phase Micro Extraction (SPME).

The first technique was evaluated in order to optimize extraction step of several PCPs, including fragrances, PAHs, antioxidants, UV-filters, plasticizers, and pesticides, from water, using OFAT approach (*Paper 2*).

BBD was used for optimization of method determination of synthetic phenolic antioxidants and their metabolites in water samples by downscaled solid-phase extraction and silylation derivatization, with GC-MS analysis (*Paper 4*). This work was developed in collaboration with University of Santiago de Compostela (E).

SPME technique was investigated in order to extract a mixture of several PCPs (e.g. PAHs, antioxidants, fragrances, UV-filters, pesticides), evaluating extraction time and temperature and desorption time and temperature (*Paper 5*). A simple and sensitive method has been developed for the simultaneous GC-MS determination of various PCPs at trace levels in water.

The analysis was then extended to more polar compounds (with log Kow < 5), e.g. pharmaceuticals and antiseptics, with the necessity of derivatize the compounds before GC analysis (*Paper 6*). An on-fiber after extraction approach was chosen for SPME derivatization, using silylation agent.

The PhD project, in his totality, was a logic progressive work, expanding the study in term of kind of analytes studied, extraction techniques and optimization approach. A preliminary and careful study (*Paper 1*) was carried out in order to properly understand the current situation and the possible research developments of interest.

The study was initially focused on less polar analytes (PCPs), then extending to more polar ones (PPCPs). It was firstly taken in consideration the SPE technique, going to more innovative and preferable on several points of view SPME. The expanded range (in particular polarity range) of analytes makes necessary a derivatization step before GC-MS analysis, for these reason the subsequent step was the optimization of a method that includes SPME-derivatization. Also considering the optimization approach, the work started using OFAT optimization, this approach was left after the first work for the more accurate and precise DOE.
These works led to development of multiresidual analytical methods for the analysis of this kind of pollutants in water matrix. These methodologies may be the basis of water monitoring for temporal and spatial changes.

2 Emerging Pollutants

Emerging Pollutants are contaminants that have been only recently discovered in the environment. This term encompasses a wide range of compounds, they are chemicals or materials that are characterized by a perceived, potential or real threat to human or environment health, or a lack of published health regulations. A contaminant may also be “emerging” because of the ability for it to be detected be new test methods or by the discovery of a new source or a new pathway to humans.

Research is documented with increasing frequency that many chemical and microbial constituents that have not historically been considered as contaminants are present in the environment on a global scale.

During the past ten years or more there has been a lot of research and testing conducted by environmental world agency (e.g. EPA, USGS, EEA) in evaluating new pollutants of concern to the environment.

New analytical techniques that have become available recently have enabled scientists to detect very low levels of numerous chemicals in the environment. There’s mounting evidence that these low level emerging contaminants present in natural waters and sediments may affect wildlife, sometimes causing non-lethal but adverse ecological health effects.

These contaminants are commonly derived from municipal, agricultural, and industrial wastewater sources and pathways. They represent a shift in traditional thinking as many contaminants are produced industrially yet they are dispersed to the environment from domestic, commercial, and industrial uses.

New and emerging contaminants are unregulated and may be new contaminants (e.g. MTBE, now regulated in California) or those that may have been present but not detected (e.g. perchlorate). Also among the emerging pollutants are pharmaceuticals and personal care products (PPCPs), industrial chemicals present at low concentrations, and chemicals that may affect hormone status, referred to as “endocrine disruptors compounds” (EDCs).

2.1 PPCPs

PPCPs have been designated as emerging contaminants because they are disposed or discharged to the environment on a continual basis from domestic and industrial sewage including septic sewage, landfills, and wet weather runoff; and they lack published health standards.

PPCPs refers, in general, to any product used by individuals for personal health or cosmetic reasons, or used by agribusiness to enhance growth or health of livestock. They comprise a diverse collection of thousands of chemical substances, including prescription and over-the-counter therapeutic drugs, veterinary drugs, fragrances, and cosmetics.

Studies have shown that pharmaceuticals are present in world water bodies. Further research suggests that certain drugs may cause ecological harm. U.S. and European environmental agencies are committed to investigating this topic and developing strategies to help protect the health of both the environment and the public. For the most of the occurring emerging pollutants, risk assessment and ecotoxicological data are not available and therefore it's difficult to predict which health effects they may have on humans, terrestrial and aquatic organisms, and ecosystems.

It's reasonable to surmise that the occurrence of PPCPs in waters is not a new phenomenon. It has only become more widely evident in the last decade because continually improving chemical analysis methodologies have lowered the limits of detection for a wide array of xenobiotics in environmental matrices. PPCPs have probably been present in water and the environment for as long as humans have been using them.

The drugs that we take are not entirely absorbed by our bodies, during and after treatment, humans and animals excrete a combination of intact and metabolized pharmaceuticals, entering in wastewaters and receiving water bodies. In addition, fragrances, UV blockers, and preservatives are included in personal care formulations, for chemical and biological stabilization. Unlike pharmaceuticals, PCPs do not have to pass through the human body; they enter the wastewater after their regular use during showering or bathing. PPCPs in the environment illustrate the immediate connection of the actions/activities of individuals with the environment.

Based on published data [1], environmental concentrations and toxicity of PCPs have been largely overlooked in comparison to pharmaceutical compounds. Data developed thus far indicate most PCPs are relatively non-toxic to aquatic organisms at expected environmental concentrations. However, the primary concern is their potential cause estrogenic effects at relatively low concentrations. Preservatives and UV filters are knows as EDCs [2,3]; with Triclosan also suspected to cause endocrine effects [4]. Besides this, the other major concern is the PCPs potential do

bioaccumulate in aquatic organisms. UV filters, disinfectants, and fragrances have been shown to bioaccumulate in biota [5], and can potentially biomagnify in higher trophic levels.

The prime PPCPs anthropogenic sources are indicated in Fig. 1 [6] by the shaded boxes.

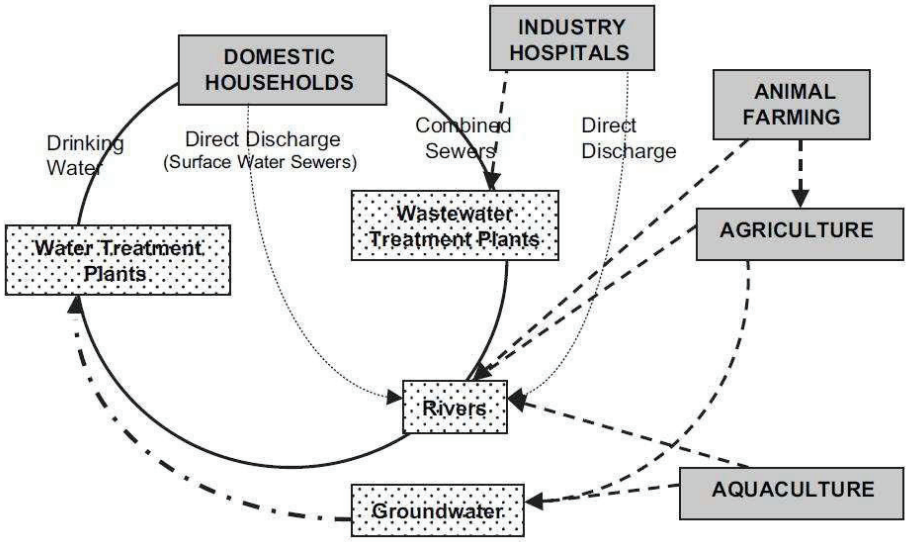


Fig. 1 Sources and pathways of PPCPs in the urban water cycle [6].

Releases direct to receiving waters from manufacture, processing and distribution are considered to be negligible. Losses from fish farms and agriculture pass directly to surface and groundwaters and are not subject to treatment within sewage works and discharges via surface water sewers also remain untreated. Thus the principal pathways (dashed lines in Fig. 1) of PPCPs into urban receiving waters are from individual household use or concentrated in hospital discharges and disposal of unwanted/outdated drugs and cosmetic products.

With advances in technology that improved the ability to detect and quantify these chemicals, we can identify what effects these chemicals have on human and environmental health [7].

Their presence in water and wastewater has been frequently reported after the early findings of Ternes (1998) and Daughton and Ternes (1999) [8-10]. These compounds are a source of concern because they are used and released in large quantities and their physical and chemical properties contribute to their widespread distribution into the environment. The presence of small concentration of PPCPs has been associated to chronic toxicity, endocrine disruption and the development of

pathogen resistance. The consequences are particularly worrying in aquatic organisms as they're subjected to multigenerational exposure. PPCPs represent a rising part of the trace organic micropollutants found in urban and domestic wastewaters that reach sewage treatment plants. Many of these substances escape to conventional wastewater treatments plants (WWTP) allowing them to reach surface water streams and distribute in the environment. WWTP effluents are so the major sources of environmentally relevant emerging contaminants, and secondarily terrestrial run-offs (roofs, pavement, roads, agricultural land) including atmospheric deposition. Characteristic of PPCPs is that they do not to need to be persistent in the environment to cause negative effects since their high transformation/removal rates is compensated by their continuous introduction into the environment.

In the European Union (EU), in 2003, about 3000 different substances are being used in medicines such as painkillers, antibiotics, contraceptives, beta-blockers, lipid regulators, tranquilizers, and impotence drugs [9]. In addition, the chemicals that compose personal care products also number in the thousands. The world's people consume enormous quantities of skin and dental care products, soaps, sunscreen agents, and hair styling products, to name just a few. In the early 1990s, annual production of these products exceeded 550,000 metric tons for Germany alone [10].

Daughton in 2004 [11] has suggested that there may be as many as 6 million PPCP substances commercially available worldwide and that the use of pharmaceuticals is increasing 3-4% by weight for annum. Hygienic products alone include at least 8000 preparations available within the EU market with some 140 000 tonnes of shampoo used annually in Germany. It's estimated that Germany also uses over 600 tonnes per year of antibiotics with some 300 tonnes per year used in France, Italy and Spain. A total of 170 pharmaceutical chemicals are estimated to be used in excess of 1 tonnes per year [12]. With increasing urbanization and associated commercial activities, and an increasing concern with personal care and health, the significance of PPCPs as a societal lifestyle cause of water pollution is likely to impose an increased risk.

2.2 Occurrence and fate

Interest in the presence of PPCPs in the environment has increased significantly over the last decades. The relevance of the world attention on PPCPs is to attribute to their worldwide increasing consumption and their frequent detection in the aquatic

and terrestrial environment, ranging from ng/L to µg/L [13-22]. Hundreds of tonnes of these compounds are dispensed in communities every year.

However relatively little research has been conducted to identify environmental concentration and potential toxicity, and possible new pollutants belonging PPCPs family. Most research project have been focused on the removal of PPCPs using different treatment processes, but data in terms of their degradation or transformation products during these processes and their fate in the environment are largely lacking.

From 1996 and 1998, a comprehensive Germany study investigated the occurrence of 55 pharmaceuticals, 6 hormones, 9 metabolites, 6 biocides and 1 flame retardant in the discharges from 49 WWTPs and in their respective receiving water bodies [23]. Concentrations at µg/L level of 32 pharmaceuticals, 4 hormones, 5 metabolites, and 5 biocides were detected in the WWTP outflow. The receiving water bodies contained concentrations of beta-blockers and anti-epileptic agents in excess of 1 µg/L.

In 1999 and 2000, a comprehensive U.S. study monitored an even broader range of organic pollutants, including pharmaceuticals, antioxidants, phytosteroids, biocides, and flame retardants [24]. Those researchers detected 82 of the 95 contaminants in at least one stream sample.

Hence, the situation in North America is similar to that in Europe. However, these comprehensive monitoring studies and the many subsequent individual studies [25-30] included only small subset (<15%) of the PPCPs predicted to potentially enter in the environment with consequent possible risks.

In 2007 United Kingdom researchers monitored for 10 months two contrasting Welsh rivers [13]. the River Taff, one of ten major rivers in the UK, which flows through industrial and urbanised areas, and the River Ely, a small and shallow river flowing through rural lowlands, for 56 target PPCPs. The range of these contaminants has not been investigated in the UK before this study, in particular the PCPs which are widely used but without any control on their usage. The results indicated similar patterns in rivers' contamination with PPCPs, although the River Ely was found to suffer from lower loads of PPCPs, which can be attributed mainly to the hider efficiency of treatment of discharged water effluent and characteristics of the catchment area. Most PPCPs were frequently found in both rivers at concentrations reaching µg/L. Treated wastewater effluent was found to be the main

cause of water contamination. Each collected sample was contaminated with the majority of PPCPs studied, although their concentration varied and depended mainly on the extent of water dilution resulting from rainfall. Several PPCPs were found to be both ubiquitous and persistent, e.g. codeine, carbamazepine, gabapentin). The calculated average daily loads of PPCPs indicated that in total almost 6 kg of studied compounds are discharged daily into the studied rivers. The most frequently detected ones represent the group of pharmaceuticals, in particular antibacterial drugs, anti-inflammatory/analgesic and antiepileptic drugs. Moreover illicit drugs were frequently found in rivers (concentration of ng/L). Their frequent occurrence in surface water is primarily associated with their high illegal usage and is strongly associated with the discharge of insufficiently treated wastewater effluent. Furthermore the results indicate that although PPCPs are not present at very high concentration, their frequent occurrence and possible synergic action is to concern and therefore the study of the presence and fate of multiple groups of active PPCPs is of significant importance. The authors suggest that, because some PPCPs were found in surface water with 100% frequency at the sampling points located below WWTPs, they can be used as chemical indicators of human fecal contamination; this approach was also propose by another research group in 2005 [31].

Following the discharge of treated sewage into the receiving water, residual PPCPs will be diluted and mixed with residuals derived from both direct surface water discharges and indirect groundwater seepage (Fig. 1). Conventional water treatment processes appear to be insufficient in removing raw water residuals and require additional oxidation, activated carbon or membrane filtration treatment to achieve non-detectable concentrations [32]. Without such additional treatment, PPCP residuals will be pseudo-persistent and liable to re-enter the urban water cycle.

PPCPs have also been proven to bioaccumulate in commercial shellfisheries downstream of wastewater treatment plants [33] whilst groundwater studies have indicated that some PPCPs (e.g. carbamazepina and primidone) can survive intact after travel times of 8-10 years through the surface [34].

Another relevant impact on human health from PPCPs is their possibility to be founded in drinking water, in addition there is quite limited information on the transformation of them upon drinking water disinfection. A recently study [35] demonstrates the transformation of 20 selected PPCPs to form nitrosamines during chloramine disinfection. This has become a significant issue for delivered drinking

water quality because of their potential carcinogenity, especially with the switch of secondary disinfectant from free chlorine to chloramine gaining much popularity in recent years. This study result have suggested that PPCPs with substituted amine groups can serve as potential nitrosamine precursors during chloramine disinfection. Due to their trace level in source waters, it is not likely that PPCPs will account for the majority of nitrosamine precursors in drinking water. However, this study proves the possible impact from different water matrices.

2.3 Consequences and regulation

In recent years, researchers have uncovered new environmental effects, such as feminilization or masculinization by hormones or structurally related compounds (xenoestrogens) that exhibited effects on fish down to 1 ng/L [36,37]. Studies were launched to investigate the effects of individual PPCPs on biota [38-40]. However, because of incomplete assessment data, researchers still lack a complete understanding of the environmental effects of most PPCPs. Thus, no one knows whether the relatively low environmental concentrations found for PPCPs produce adverse effects on aquatic and terrestrial biota or whether the toxicity of complex mixtures might be totally different from that of individual compounds [41]. Consequently, we expect that a reasonable and scientifically sound assessment of this complex set of compounds will take decades of research.

Some PCPs (e.g. UV-screens, insect repellents, and some synthetic musk fragrances) are known or have been suspected endocrine-disrupting compounds (EDCs), i.e. compounds that can mimic the natural hormones of animals [42-46].

Either we will have to wait until scientists can reasonably predict the risks caused by trace pollutants in complex mixtures or, for precautionary reasons, we can be proactive and reduce inputs of micropollutants to the environment as completely as possible through the introduction of cost-effective control options.

Different groups are currently working according to both of these philosophies. The EU and the U.S. have launched major research projects, such as the EU's Repharmawater and Poseidon, and U.S. EPA projects. The EU projects are designed to establish a basis for wastewater treatment that is appropriate for reducing these loads with relatively inexpensive approaches. In Project Poseidon, researchers elaborated on basic knowledge to understand the relevant processes in

WWTPs for removing human PPCPs in order to develop an appropriate strategy for removal measures.

Both the EU and the US environmental protection agency have identified a listing or priority pollutants and have developed water quality criteria for them. The list of 129 priority contaminants drawn up by the US EPA was developed with limited technical input and not externally peer-reviewed and it was recognized that the list did not represent a complete and whole or perhaps even an appropriate listing of the very wide variety of chemicals present in wastewaters and stormwater runoff that may be a threat to receiving waters. Within the context of the EU Water Framework Directive (WFD), a first list of 33 priority substances in the field of water policy has been identify under Directive 2000/60/EC, for the adoption of control measures over the next 20 years [45]. Member countries have additionally undertaken their own national reviews to identify emerging future contaminants.

Under the precautionary principle, the EU WFD priority substance list will be updated every 4 years and has identified future emerging priority candidates which include PPCPs of which diclofenac, ibuprofen, triclosan have been highlighted as of possible particular concern.

3 Analytical Method

It's clear that there is critical need for efficient and reliable analytical methods to address the occurrence, concentration, and fate of the PPCPs (present at trace levels) in real water samples. A preliminary and careful study (*Paper 1*) was carried out in order to properly understand the current situation and the possible research developments of interest.

Gas chromatography-mass spectrometry (GC-MS) has been the approach of choice because of its superior separation and identification capabilities. In addition, the development of faster, more cost-effective, more environment-friendly sample preparation procedures is a mandatory requirement, since tedious sample pre-treatment causes an analysis bottleneck that typically accounts for over 60% of the total analysis time. Anything we can do to make improvements in this area will translate into advances in time saving and convenience.

Moreover there is need for further improvements in order to develop analytical procedures that are versatile in simultaneous screening for a wide variety of compounds with large differences in physicochemical properties (e.g. log K_{ow}, water solubility, M_w).

A single method for the analysis of different classes of target analytes would be convenient, since it would reduce the overall analysis time, field sampling and costs. Even if most of the available methods are specifically devoted to a few contaminants or a single PPCP class, some multi-residue methods have been developed for determining organic pollutants in aqueous environment.

3.1 Sample extraction procedures

To analyze complex mixtures, such as water samples, a pretreatment procedure is required to provide a sample fraction enriched with all the target analytes and as free as possible from other matrix components [47].

A variety of sample preparation methods is available for extraction and concentration of contaminants in water: liquid-liquid extraction (LLE), solid-phase extraction (SPE), solid-phase microextraction (SPME), semi-permeable membrane devices (SPMDs) [48]. Until ten years ago many preparation practices were based on traditional technologies, such as LLE, which is time consuming, labour intensive, and also require the use of toxic solvents. The operating principle of any sample preparation method is to allow analytes to partition between sample matrix and an

extraction phase. Table 1 shows the main steps followed in different sample preparation techniques [49].

LLE	SPE	SPME
Addition of organic solvents to the sample	Conditioning of cartridges or membranes	Exposing SPME fibre to the sample
Addition of organic solvents to the sample	Sample elution	Exposing SPME fibre to the sample
Separation of aqueous and organic phases	Solvent elution to remove interferences and analyte desorption	
Removal of organic phase	Evaporation / concentration of the organic phase	
Evaporation/ concentration of the organic phase	Injection in the analytical instrument	
Injection in the analytical instrument		

Table 1 Protocols used in different sample preparation techniques: LLE, SPE, SPME [49].

As we can see, LLE is a multi-step procedure that often result in loss of analytes during the process, frequently making sample preparation the major source of errors in the analysis, and making it impeditive for integration with the rest of the analytical process. This PhD project was focused on the other two extraction techniques: SPE and SPME.

3.1.1 Solid Phase Extraction

SPE was developed in the 1980s, and has emerged as a powerful tool for chemical isolation and purification. From trace levels to industrial scale, SPE plays an important role in a broad range of applications.

SPE refers to the nonequilibrium, exhaustive removal chemical constituents from a flowing liquid sample via retention on a contained solid sorbent and subsequent recovery of selected constituents by elution from the sorbent. It is an increasingly useful sample preparation technique. With SPE, many of the problems associated with LLE can be prevented, such as incomplete phase separation, less-than-quantitative recoveries, use of expensive, breakable specialty glassware, and disposal of large quantities of organic solvents. SPE is more efficient than LLE,

yields quantitative extractions that are easy to perform, is rapid, and can be automated. Solvent use and lab time are reduced. SPE is often referred to a chromatographic technique, indicating the all-or-nothing extremes in the sorptive nature of the SPE sorbents, caused by the strong attraction for the analytes. Mathematically, a strong affinity equates to a large distribution coefficient, because the concentration in the sorbent extracting phase is large relative to the sample extracted.

The modern SPE era began in 1977 when Waters Corporation introduced commercially available, pre-packaged disposable cartridges/columns containing bonded silica sorbents. The most commonly cited benefits of SPE relative to LLE are reduced analysis time, reduced costs, and reduced labor, because it's faster and requires less manipulation than LLE (Table 1); reduced organic solvent consumption and disposal, which results in reduced analyst exposure to organic solvents, and waste productions. The potential for automation of SPE increased productivity because multiple simultaneous extractions can be accomplished. SPE provides higher concentration factors than LLE, and it is a multistage separation technique providing greater opportunity for selective isolation than LLE, such as fractionation of the sample into different compounds or groups of compounds.

SPE is the method of choice that is particularly well adapted to multi-residue analysis, including compounds with a wide range of polarity or characterized by various physico-chemical properties [50].

A typical SPE commercially available tube is shown in Fig. 2.

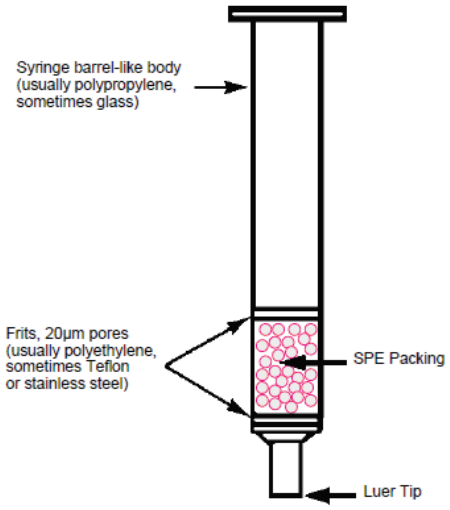


Fig. 2 Typical SPE tube [51].

SPE is a four-step process, when the proper sorbent and tube was selected: conditioning, sample addition, washing where necessary, and elution (Fig.3). The conditioning step solvates the bonded phase so that it can readily accept the liquid sample load; the washing step removes possible interferences, and the elution step involves the use of a strong solvent to elute the analyte/analytes of interest in a small volume for direct injection into chromatographic column. Sometimes, the eluent is blown by solvent evaporation to further concentrate the analytes or to allow redissolution of the analytes in a solvent more compatible with the subsequent chromatographic technique.

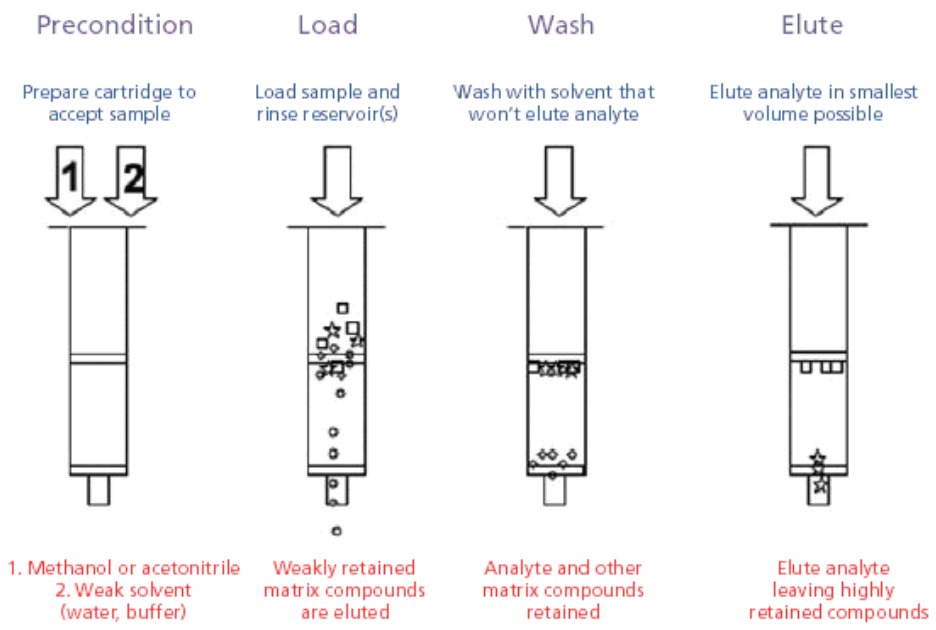


Fig. 3 Steps in the SPE process [52].

In the simplest form, SPE uses a packing material such as bonded silica or polymeric media packed into a plastic, medical grade syringe barrel. Similar to an HPLC columns, polymeric or metallic frits contain the packing in the cartridge format. Other popular SPE formats are disks, pipette tips, and 96-well plates. The stationary phases typically used are reversed-phase (C8, C18, polymeric sorbent such as Oasis® HLB from Waters Corporation or Strata^{TM}-X from Phenomenex), ion-exchange (strong anion and strong cation exchange), or normal-phase (silica, cyano, amino) packings. Appropriate SPE sorbent selection is critical to obtain efficient recovery of analytes, knowing the analyte structure is the clue to effective

isolation by SPE. Moreover most manufacturers provide such guidelines either in printed product literature or on websites.

3.1.2 Solid Phase Microextraction

SPME was introduced by Arthur and Pawliszyn in 1990 [53] and it's now widely accepted with constantly increasing numbers of new publications. Recently it was proposed as an alternative to SPE in the analysis of environmental samples: SPME is a unique sample preparation technique that require no solvents or complicated apparatus, it is a one-step extraction method based on the partition equilibrium of the analyte between the sample and a sorbent. Compared with SPE and LLE, SPME technique is less time consuming, and require less manipulation (Table 1). The extraction and enrichment of the analyte in SPME occurs by a thin layer of a suitable polymer at the surface of a fused silica fiber, on the inner wall of a stainless steel syringe or within a fused-silica capillary (Fig. 4).

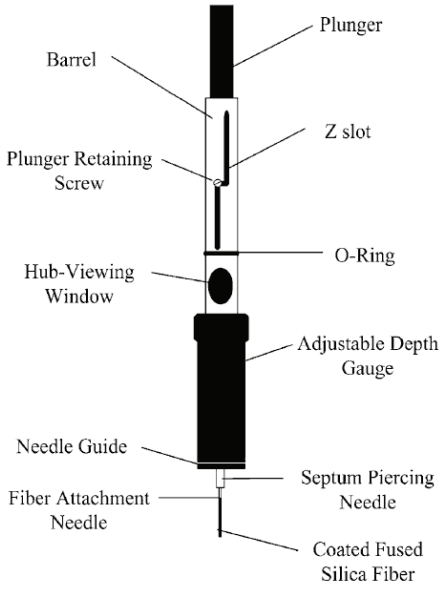


Fig. 4 Commercial SPME device (SPME fiber holder) [54].

One of the major advantages of SPME is its simplicity. It is a solventless sample preparation technique, the SPME fiber collects and concentrates the sample; it is straightforward procedure involving only sorption and desorption (Fig. 6), it is compatible with chromatographic analytical systems, and the process is easily automated. The SPME sampling device is portable, and it enables its use in field monitoring.

SPME has the advantages of high concentrating ability and selectivity. Conventional SPE exhaustively extracts most of the analyte (~ 80-90%) from a sample, but only 1 to 2 % of the sample is injected into the analytical instrument. SPME nonexhaustively extracts only a small portion of the analitye (~10-20%), whereas all of the sample is injected [55,56].

Several coatings are currently available, with different retention mechanisms (adsorbent or absorbent), different film thickness and polarity; the choice of a particular one is chemical structure dependent. Usually, when selecting an SPME sorbent, we can apply the general selection rule “like dissolves like”, so the polarity of the sorbent coating should match the polarity of the analyte of interest, and it would have a lack of affinity for interfering compounds.

The procedure for collecting the sample is shown in the top of the Fig. 6. When the plunger is depressed, the fiber extends, the polymer is exposed, and the sample is collected onto it by adsorption or absorption, depending on the type of coating. There are three modes of extraction using this technique (Fig. 5): the often applied direct-immersion extraction (DI-SPME) and headspace extraction (HS-SPME), and the rarely used membrane-protected SPME.

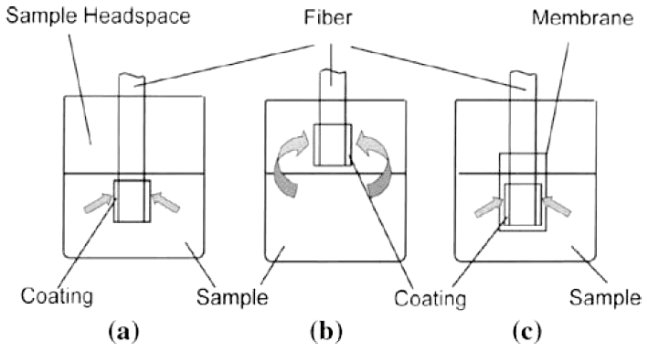


Fig. 5 SPME operation modes: (a) direct extraction, (b) headspace extraction, (c) membrane-protected [57].

After a suitable exposure time, the fiber is retracted. The bottom row of Fig. 6 illustrates the process for transferring the sample for analysis. The fiber is inserted into the GC injector or HPLC interface, and the plunger is depressed to expose the polymer, the heated port drives off the collected compounds, which then flow into the instrument for qualitative or quantitative analysis. Since the fiber has been cleaned by heating in the injector, it is ready to be reused.

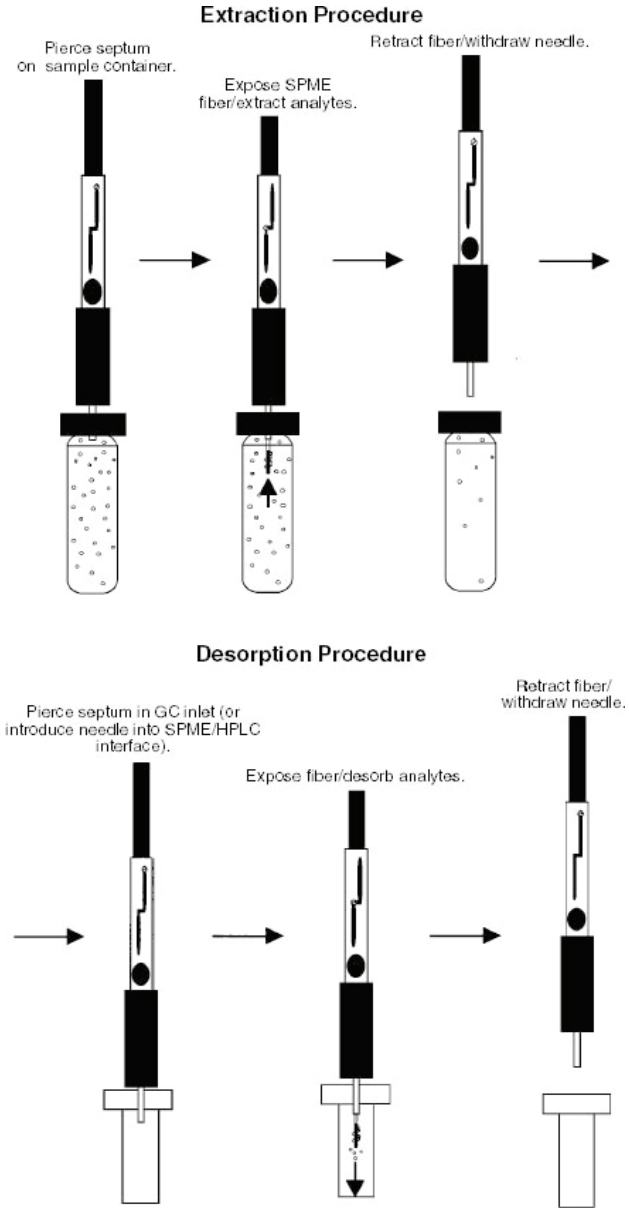


Fig. 6 SPME procedure for collecting and analyzing the sample [58].

One problem in the terminology applied today is that the SPME extraction phases are not always solids. Moreover, unlike SPE, SPME is an equilibrium and preequilibrium technique. Exhaustive extraction of analytes from sample matrix is not achieved, nor is it meant to occur. By SPME, samples are analyzed after equilibrium is reached or at a specified time prior to achieving equilibrium. Therefore, SPME operationally encompasses non exhaustive, equilibrium and preequilibrium, batch and flow-through microextraction techniques. Thus defined, SPME is distinctly different from SPE because it is exhaustive extraction procedure.

When equilibrium conditions are reached, the number of moles, n, of analyte extracted by the fiber coating is independent of increases in extraction time, such that:

$$n = \frac{K_{fs} V_f V_s C_0}{K_{fs} V_f + V_s}$$

where K_{fs} is the distribution constant between the coated fiber SPME sorbent and the aqueous sample matrix, V_f is the fiber coating volume, V_s the sample volume, and C_0 the initial concentration of a given analyte in the sample. K_{fs} is the ratio between the concentration of analyte in the fiber sorbent and the concentration of analyte in the aqueous sample phase, K_{fs} values are influenced by different conditions, e.g. temperature, salt, pH, and organic solvents. At the equilibrium the amount of analytes extracted onto the coating is linearly proportional to the analyte concentration in the sample. Although the amount of analyte extracted onto the fiber coating is maximum when the equilibrium is reached, a quantitative result may be achieved by careful control of experimental parameters even not in equilibrium conditions, so it is not necessary to continue an extraction by SPME. Extraction recovery can be optimized by changing sample conditions.

The combination of SPME and GC is particularly suitable for the determination of volatile and semivolatile, non-polar compounds. SPME has been widely used for analysis of environmental pollutants in air, water, soil, and sediment samples, in on-site or off-site analysis.

3.2 Derivatization for GC analysis

Derivatization is the process by which a compound is chemically modified, producing a new compound that has properties more amenable to a particular analytical method. Derivatization is a useful tool allowing the use of GC and GC-MS to be done on samples that would otherwise not be possible in various areas of chemistry such as medical, forensic, and environmental.

Some samples analyzed by GC require derivatization in order to make them suitable for analysis. Compounds that have poor volatility, poor thermal stability, or that can be adsorbed in the injector or GC column will exhibit non reproducible peak areas, heights, and shapes. For GC analysis, compounds containing functional groups with active hydrogens (e.g., -COOH, -OH, -NH and –SH) are the primary concern. The tendency of these functional groups to form intermolecular hydrogen bonds affects

the inherent volatility of compounds containing them, their tendency to interact deleteriously with column packing materials and their thermal stability. Other compounds that respond poorly on a specific detector (for example ECD) may need to be “tagged” with a different functional group to improve detection, for example the formation of trimehtylsilyl (TMS) derivatives to produce readily identifiable fragmentation patterns and mass ions form MS analysis.

The main derivatization advantage is that it converts polar analytes into their less polar analogues, so altering compounds with low volatilities to volatile derivatives, thus improving their gas chromatographic separation. The low volatility may result from the size of the molecule and the resultant large dispersion forces holding the molecule together. Smaller molecules may have a low volatility due to the strong intermolecular attractions between polar groups. In the latter case, masking the polar groups by derivatization can yield dramatic increases in volatility.

Derivatization can also be used to decrease volatility to allow analysis of very low molecular weight compounds, to minimize losses in manipulation and to help separate sample peaks from solvent peak. Some compounds, which can be volatilized, undergo partial thermal decomposition in the GC so they need to be made more stable.

Polar samples tend to adsorb on the active surfaces of the column walls and the solid support. Reduction of this adsorption can be accomplished by derivatization.

Also derivatization serves to accentuate the differences in the sample compounds to facilitate the chromatographic separation.

A good derivatizing reagent and procedure should produce the desired chemical modification of the compounds of interest, and be reproducible, efficient, and non hazardous.

For GC analysis, there are three basic type of derivatization reactions: silylation, acylation, and alkylation.

The term “silylation” usually is used to abbreviate trimethylsilylation [$Si(CH_3)_3$]. It is also used to designate the attachment of other silyl groups such as dimethylsilyl, t-butyldimethylsilyl and chloromethyldimethilsilyl. Silylating reagents react with compounds containing active hydrogens; these reagents are the most common type used for GC applications.

Acylating reagents react with highly polar functional groups such as amino acids or carbohydrates.

Alkylating reagents target active hydrogens on amines and acidic hydroxyl groups. It is in general used as the first step to further derivatizations or as a method of protection of certain active hydrogens. In general, the products of alkylation are less polar than the starting materials because an active hydrogen has been replaced by an alkyl group. Although TMS derivatives of carboxylic acids are easily formed, these compounds suffer from limited stability. The alkyl esters, on the other hand, afford excellent stability and can be isolated and stored for extended periods if necessary.

Multiple derivatizing reagents may be necessary for compounds containing several different functional groups. In these multi-step derivatization procedures the use of other types of reagents, such as oxime, hydrazone, methylation, and cyclic derivatives, may be necessary.

Several derivatization techniques were deeply studied in our laboratory during last years, focused on chiral amino acids analysis (*Paper 3*). The studies were applied to space analysis, because GC-MS provides to be the best analytical technique for *in situ* search for organic molecules in extraterrestrial environments [59]. The performance of different derivatization reactions were investigated and optimized in order to develop a rapid, reproducible, trace level quantitative method for the simple and automatic GC enantioselective separation of amino acids. These knowledges were also used in this PhD work for choose an appropriate derivatization reaction for analysis of PPCPs with GC-MS, and could be a good start point for development of analytical method for analysis of chiral contaminants in environment.

3.2.1 Silylation

Silylation is a common derivatization technique used to derivatize polar compounds prior to GC-MS analysis. During the silylation reaction, all the hydroxyl groups are converted into their corresponding trimethylsilyl derivatives via a substitution reaction (nucleophilic attack) which yields one main product for each compound and with high conversion efficiency (Fig.7).

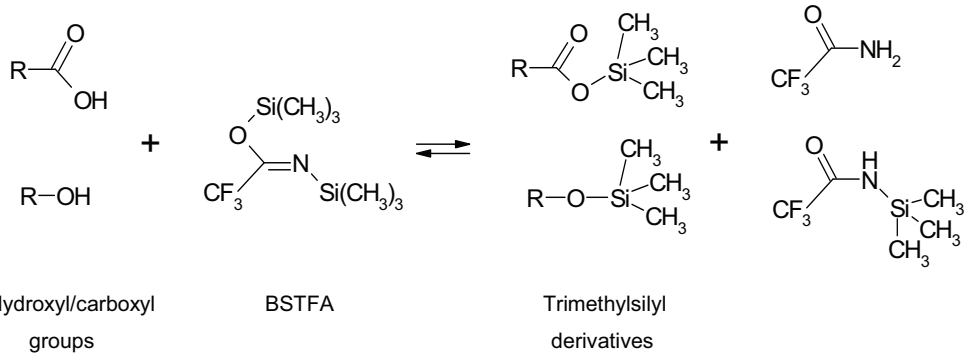


Fig. 7 General silylation derivatization procedure.

The silyl derivatives are more volatile, less stable, and more thermally stable.

The usual reagents are trimethylchlorosilane (TMCS), N-methyl-trimethylsilyltrifluoroacetamide (MSTFA), N,O-bis-(trimethylsilyl)trifluoro acetamide (BSTFA) and N-(t-butyldimethylsilyl)-Nmethyltrifluoroacetamide (MTBSTFA). A wide range of reagents are available for the introduction of the TMS group, these differ in their reactivity, selectivity, side reactions and the character of the reaction byproducts from the silylation reagent. Considerable literature is available to assist the researcher in the selection of the most suitable in reagent for the particular compounds or systems in question.

The reaction is low moisture sensitive and requires mild conditions to complete the derivatization needed to achieve GC-MS detection at very low concentrations. In opposition to alkylation, silylation normally does not require a purification step and the derivatives can be injected directly into the GC system. However, it presents some drawbacks, such as the fact that the silylation reagent is dangerous and some artefacts can be produced in the reaction

Both silylation reagents and trimethylsilyl derivatives are hydrolytically unstable and must be protected from moisture. The hydrolysis rates of various reagents and derivatives are different. Reagents that introduce a t-butyldimethylsilyl (TBS) group in place of the trimethylsilyl group were developed to impart greater hydrolytic stability to the derivatives. These TBS derivatives not only improve stability against hydrolysis, but they also have the added advantage of distinctive fragmentation patterns, which makes them useful in a variety of GC-MS applications.

Most TMS and TBS derivatives have excellent thermal stability and are amenable to a wide range of injection and column conditions.

One of the most advantages of using TMS derivatives is their thermal stability. They are routinely used at column and injector temperatures of 300℃, but temperatures of 350℃ and above have been used successfully. The TMS reagents themselves are also quite thermally stable; however, the more reactive silyl donors such as BSTFA will decompose at elevated temperature, especially in the presence of metals. Care must be used when temperatures above 75℃ are needed for a derivatization procedure using these reagents because decomposition of the reagents can be significant at these temperatures.

Nonpolar organic solvents (hexane, ether, benzene, toluene) are excellent solvents for the reagents and the reaction products, but they don't accelerate the rate of reaction. More polar solvents, such ad pyridine, dimethylformamide (DMF), dimethylsulfoxide (DMSO), tetrahydrofuran (THF) and acetonitrile are used more often because they tend to facilitate the reaction.

Silylation has several advantages: ability to silylate a wide variety of compounds, large number of silylating reagents available, easily prepared. On the other hand the disadvantages are that silylation reagents are moisture sensitive, there is need to use aprotic (no protons available) organic solvents.

Derivatization process optimization involves five variables, like derivatization agent, derivatization solvent, reaction temperature, duration of reaction, and the conditions under which the derivatization solution is dried subsequent to the reaction (prior to GC analysis).

3.2.2 Derivatization on SPME

The detectability problem of polar species using SPME and GC analysis can be overcome with SPME-derivatization approach. It can be performed in three ways: direct derivatization in the sample matrix, doping the fibre coating with the derivatization reagent, and derivatization in the GC injection port [60,61].

The first one requires the derivatizing reagents to be reactive toward target analytes in sample matrices and not with the water, and produce stable reaction products. It is quite challenging, since most of derivatizing reagents require the presence of organic media, as they will be hydrolyzed when they're used directly in aqueous solution; for obvious reasons, this strategy is not suitable for moisture-sensitive reagents.

In the in-port derivatization, polar analytes, with acid-base properties, are extracted in the SPME fibre as ion pairs which are further decomposed, at the high

temperatures of the GC injection port, to produce volatile by-products and the alkyl derivatives of the target compounds.

Derivatization of analytes directly in the polymeric coating of the SPME device can be performed in two ways. The first one is to perform the reaction and extraction simultaneously by exposing the fibre containing the derivatizing reagent to the matrix. During the partition, polar analytes are extracted and derivatized into less polar and less volatile derivatives which remain in the fiber coating rather than going into the sample matrix. The other way is to perform postderivatization following SPME of target analytes from sample matrices.

4 Design of Experiments

Experimental design ad optimization are tools that are used to systematically examine different types of problems that arise within, e.g., research, development and production. Unfortunately, nowadays experimental design is not as known and applied as it should be. The real advantage of the design of experiments (DOE) approach is to reduce experimental effort and to increase quality of information that can be obtained [62,63,64].

The screening or optimization performed one variable at a time (OVAT) does not guarantee at all that the real optimum will be hit. This because this approach would be valid only if the variables in consideration would be totally independent from each other. It is evident that this is an unreal simplification.

Another extremely difference between the two approaches is that with OVAT, only a local knowledge was obtained, this meaning that only the results of the experiments performed could be known, each of them with an incertitude corresponding to the experimental error. Instead, from the results that a researcher obtains with a DOE approach a simple mathematical model could be obtained, relating the response with the experimental conditions:

$$Y = b_0 + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 + b_{11} x_1^{\,2} + b_{22} x_2^{\,2}$$

After having estimated the constant and the coefficients of the linear terms, of the interaction and of the quadratic terms, by simply replacing variables x_1 and x_2 with actual values it will be possible to predict the response for any possible point of the experimental domain, even for those experiments that have not been actually performed. Moreover we know the variance of the estimate of the response in that point, using the leverage plot of the experimental design. This can be computed in every point of the experimental domain and it depends on the experimental matrix and on the postulated model and not on the outcome of the experiments. This means that, since it can be computed before starting to do the experiments, knowing the experimental variance, it is possible to know in advance whether the precision of the estimate will be acceptable or not.

Moreover the rotatability of a DOE ensures that the variance depends only on the distance from the design center and not on the direction. Indeed, in a rotatable design, the variance of the predicted values of the response is a function of the distance of a point from the center of the design and is not a function of the direction

the point lies from the center. Before a study begins, little or no knowledge may exist about the region that contains the optimum response. Therefore, the DOE matrix should not bias an investigation in any direction. The value of making rotatability (or near-rotatability) a design goal becomes clear when you consider that the experimenter doesn't know the location of the optimum point within the region of interest before the experiments are conducted, so it is desirable that all points a given distance from the center point in any direction have the same magnitude of prediction error.

Therefore, we can observe that the DOE takes into account the interaction among the variables, while OVAT does not; the DOE provides a global knowledge, while OVAT gives a local knowledge; in each point of the experimental domain the quality of the information obtained by DOE approach is considerably higher than the information obtained by OVAT; and the number of experiments required by DOE is smaller than the number performed with OVAT.

A common experimental design is one with all input factors set at two levels each. These levels are called "high" and "low" or "+1" and "-1" respectively. A design with all possible high/low combinations of all the input factors is called a full Factorial Design (FD) in two levels (Fig. 8).

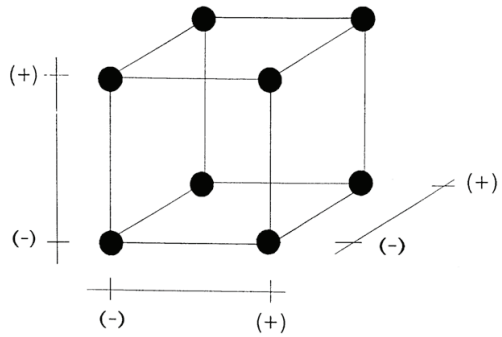


Fig. 8 Two-levels full Factorial Design example for three factors

If the experimenters want to consider k factors, each at two levels, a FD involves 2^k experiments. When the number of factors is five or greater, a FD requires a large number of runs, and it is not always efficient and possible to apply. In this case the solution is to use only a fraction of the runs specified by the complete FD, a Fractional Factorial Design (FFD) it could be overcome the problem. A FFD is a factorial design in which only an adequately chosen fraction of the treatment combinations required for the complete FD experiment is selected to be run.

Generally the number of experiments of a FFD is a fraction, e.g. ½, ¼, of the runs called for by the FD.

In Factorial Designs, multiple factors are investigated simultaneously during the test. The objective of these designs is to identify the factors that have a significant effect on the response/responses, as well as investigate the effect of the interactions, depending on the experiment design used.

The experimental designs in general identify factors that affect the response (or responses). Once the important factors have been identified, the next step is to determine the settings for these factors that result in the optimum value of the response/responses. It may either be a maximum value or a minimum value, depending upon the product or process in question. Methodologies that help the experimenter reach the goal of optimum response are referred to as Response Surface Methods (RSM). These are exclusively used to examine the “surface” or the relationship between the response and the factors affecting the response. The experimenter needs to fit a model between the response and the factors. The fitted model is used to arrive at the best operating conditions. If a number of responses may have to be optimized at the same time, a balanced setting has to be found that gives the most appropriate values for all the responses.

RSM explores the relationships between the evaluated factors and one or more response variable. RSM can be defined as a statistical method that uses quantitative data from appropriate experiments to determine and simultaneously solve multivariate equations. It may involve just main effects and interaction or it may also have quadratic (and possibly cubic) terms to account for curvature. The two most common designs generally used in RSM are Central Composite Designs and Box-Behnken designs (see 4.1 Central Composite Design, 4.2 Box-Behnken Design)

In DOE approach, it is often recommended that experiments be performed in a random sequence, to minimize uncontrolled effects on the estimated effects. The centre point or one/several design experiments can be replicated to enable estimation of the experimental error.

4.1 Central Composite Design

A Box-Wilson Central Composite Design, commonly called central composite design (CCD) is the most commonly used RSM design. Since introduced by Box and Wilson in 1951, it has been studied and used by many researchers.

It contains an imbedded factorial of fractional factorial design with center points that is augmented with a group of “star points” that allow estimation of curvature (Fig. 9). If the distance from the center of the design space to a factorial point is ± 1 unit for each factor, the distance from the center to a star point is ± α, with $|\alpha|>1$. The precise value of α depends on certain properties desired for the design and on the numbers of factors involved.

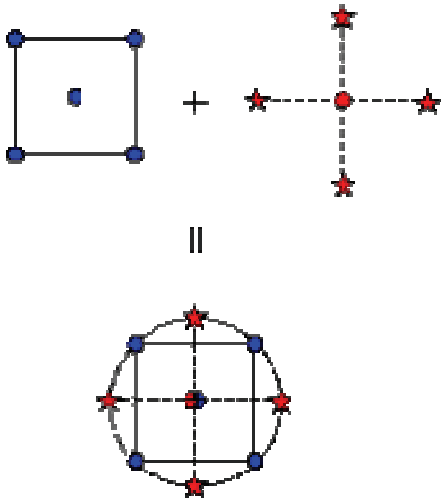


Fig. 9 CCD scheme.

The factorial points represent a variance optimal design for a first-order model or a first-order plus two-factor interaction type model. The center points clearly provide information about the existence of curvature in the system and a measure of process stability and inherent variability.

These designs allow the estimation of all the regression parameters required to fit a second order model to a given response. The number of experiments required is $2^k + 2k + N$, where k is the number of factors and N is the number of replicates of the center point. With a CCD it is possible to define a model with also quadratic terms (and cubic), that is not possible using a FD or FFD.

There are three varieties of CCD, illustrated in Fig. 10.

* CCC is the Circumscribed Central Composite design, it is the original form of the CCD. The star points are at distance α from the center, they establish new extremes for the low and high settings for all factors. CCC design have circular, spherical, or hyperspherical symmetry and require five levels for each factor.

* CCI is the Inscribed Central Composite design. It is chosen when the limits specified for factor settings are truly experimental limits. The CCI design uses the factor settings as the star points and creates a factorial of fractional factorial design within those limits. This design also requires five levels of each factor.

* CCF is the Face Centered Central Composite design; in this DOE the star points are at the center of each face of the factorial space, so α=±1. This variety requires three levels of each factor. The CCF design can be used when the region of operability encompasses the region of interest as defined by the variable bounds. It is a non rotatable design.

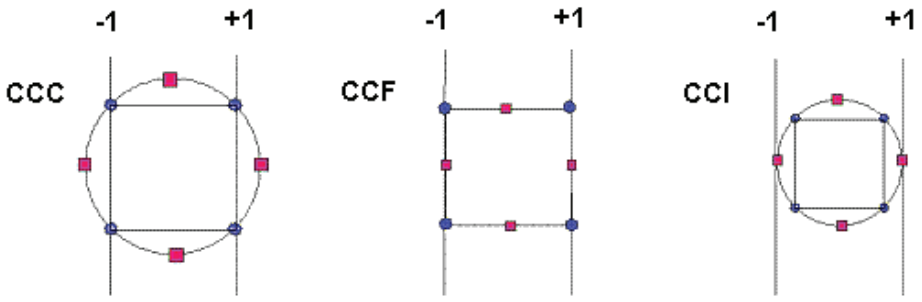


Fig. 10 Comparison of the three typologies of CCDs.

To successfully carry out any designed experiment, the region of operability must encompass the region of interest. The first step in selecting the variety of CCD is to compare the region of interest to the region of operability. CCC explores the largest process space and CCI the smallest one.

CCDs are very efficient, providing several information on experiment variable effects and overall experimental error in a minimum number of required runs. They are very flexible, the availability of several varieties of CCDs enables their use under different experimental regions of interest and operability.

4.2 Box-Behnken Design

The Box-Behnken Design (BBD) is an independent quadratic design, it does not contain an embedded factorial or fractional design. In this DOE the treatment combinations are at the midpoints of the edges of the process space and at the center. It can fit a full quadratic model, and this design use three levels of each factor. This make it appealing when the factors are quantitative but the set of achievable values is small.

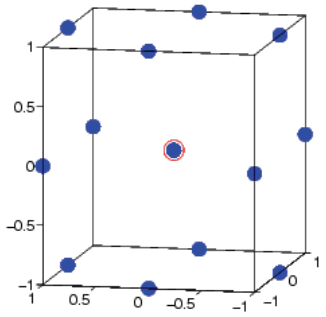


Fig. 11 Geometry of a BBD for three factors.

The geometry of this design (Fig. 11) suggests a sphere within the process space such that the surface of the sphere protrudes through each face with the surface of the sphere tangential to the midpoint of each edge of the space.

BBD is a class of rotatable or nearly rotatable second-order design based on three-level incomplete FD. The number of experiments required for the development of BBD corresponds to $2k(k-1)+N$, where k is the number of factors and N of the central points.

The designs have limited capability for orthogonal blocking compared to the CCD. Thus, if there is a need to separate runs into blocks for BBD, then designs are available that allow block to be used in such a way that the estimation of the regression parameters for the factor effects are not affected by the blocks. In other words, in these designs the block effects are orthogonal to the other factor effects.

The advantages of BBD include the fact that it is a spherical design and require factors to be run at only three levels.

5 Results and discussion

With the growing attention to PPCPs as emerging contaminants in the environment, there is an increasing demand for analytical procedures to extract and to identify a sufficiently broad variety of substances (*Paper 1*). Sample preparation is one of the most critical steps; in addition to the most popular SPE, SPME and membrane extraction are becoming alternatives in the analysis of environmental water samples. There are developments towards on-line techniques for more automated and rapid analyses, and large-volume injection for lower LODs.

In addition to methods specifically devoted to a single class of PPCPs, multi-residue methods are being developed for the determination of several PPCPs with one extraction and limited sample preparation.

In multi-residue methods, there is the need for accurate optimization of the extraction procedure – chemical composition of the sorbent, eluent type, extraction conditions – in order to achieve acceptable recovery values for all the target PPCPs.

GC-MS and GC-MS^2 provide the sensitivity and the selectivity necessary for identification and quantification of GC-amenable PCPs at trace levels. These techniques may be extended to highly polar compounds by using an efficient derivatization.

The present PhD project focused on optimization of methods for the analysis of PPCPs present in trace in waters. The first technique studied was SPE, applied on extraction of target PCPs and synthetic phenolic antioxidants and their metabolites.

5.1 SPE

5.1.1 SPE applied to PCPs extraction

In the first study (*Paper 2*) the focus was the simultaneous extraction of 25 less polar compounds, belonging PCPs general class (including fragrances, PAHs, antioxidants, UV-filters, plasticizers, and pesticides). The results confirm that the effect of the eluent properties, i.e., chemical composition and elution volume, on the SPE performance is very complex and cannot be predicted in detail when a wide range of compounds are to be analyzed. Therefore, in this case an experimental investigation is needed to optimize the operative parameters. This search is particularly challenging for multi-residue methods, such as the present case of a large variety of PPCPs, since they require the joint extraction and determination of many compounds displaying a broad range of polarities.

The optimized parameters were different: SPE sorbent, type and volume of eluent, elution rate, and evaporation procedure. The better recovery yield considering the totality of PCPs were found extracting with a Strata-X SPE cartridge, using a volume of 15 mL of Ethyl Acetate as solvent, operating with slow flow rate, and evaporating at 40℃ of temperature. Under these conditions the procedure achieves a recovery higher than 70% for most PCPs investigated (with LOD ranged from 5 to 10 pg injected on GC), this result makes the developed method suitable for comprehensive chemical profiling of PCPs in various aqueous matrices.

It is clear that higher recovery values can be achieved only for selected PCPs using specific target-compound methods. It was done for method optimization for determination of two synthetic phenolic antioxidants and their five main metabolites in water.

5.1.2 SPE applied to antioxidants analysis

This work (*Paper 4*) represents the first published method dedicated solely to the determination of this chemical class in water samples. The methodology was developed using SPE approach with derivatization before GC-MS analysis. Extraction on 10 mg Oasis HLB cartridges provides a satisfactory enrichment factor for environmental samples avoiding the need of solvent evaporation and reducing SPE costs and organic solvent wastes. After extraction, polar metabolites are derivatized with MTBSTFA to produce stable, less polar analytes that are determined by GC-MS at low levels. The usage of two surrogate internal standards results in a method providing good accuracy, with relative recoveries between 80 and 110%, and limits of detection (2-44 ng/L). The application of the method to wastewater and river samples showed BHT and BHT-Q as the compounds in higher concentrations in wastewater (up to 800 ng/L) and the metabolites BHT-CHO and BHT-COOH as the most resistant to water treatment, being at the 10–100 ng/L in sewage and river samples. Possible losses of analytes during the extract evaporation step were observed in both the SPE studies, this has been found as a critical step, according with previous work results.

The optimization was done with an experimental design approach. The results of this and the subsequent works confirm that optimization using DOE and RSM is an extremely efficient tool for fast, complete optimization of the parameters affecting the extraction efficiency throughout the analytical procedure.

5.2 SPME

Another sample preparation procedure considered for this PhD project is SPME technique. As a solvent-free method for sample preparation, SPME offers the benefits of high sensitivity and high sample throughput, thus making it a good alternative to conventional LLE and SPE for complex environmental matrices. These properties are particularly relevant for multi-residue methods, such as the present case of a large variety of PPCPs, since they require the joint extraction and determination of many compounds displaying a broad range of polarities. Moreover, using SPME methodology we overcame the extract evaporation step, with loss of analytes.

5.2.1 SPME applied to PCPs extraction

A simple SPME method has been developed (*Paper 5*) for the simultaneous GC-MS determination of 23 PCPs (i.e. antioxidants, PAHs, UV-filters, pesticides, fragrances, plasticizers, that display log Kow values > 3.2) at trace levels in water.

From the literature and from the screening results, four factors were identified as able to influence extraction recovery: extraction temperature and time, and desorption temperature and time. A CCI design approach was employed, the factor were investigated inside the operative limits imposed by experimental requirements. A direct immersion mode was used for extraction from a fixed sample volume. Samples were immersed in a thermostatic bath at a given temperature. The optimum SPME operating conditions have been defined as: extraction time of 90 min at a temperature of 80℃, desorption time of 11 min at 260℃. Under these conditions the procedure provides low detection limits (≤4 ppb) and satisfactory reproducibility (RSD%≤1%) for most of the PCPs investigated.

5.2.2 SPME applied to PPCPs analysis

In the subsequent study the analysis was extended to extraction of more polar compounds (i.e. antiseptic, antinflammatory drugs, estrogens, UV-filters), for a total of 21 PPCPs that display a range of log Kow between 1.2 and 6.4.

A precise, sensitive, and solvent free method for the determination of a wide range of PPCPs at trace levels in water samples has been developed (*Paper 6*). The on-fiber after extraction silylation of the oxydrilic compounds was chosen because of the extraction was done in water, this make impossible a direct silylation in sample,

or on-fiber derivatization with extraction simultaneously by exposing the fibre containing the silylating reagent to the matrix.

The derivatization can be performed in only 30.5 minutes with a very small consumption of silylation reagent and without need of high temperature. The reaction is performed using the derivatizing agent vapour, rather than the pure liquid or a solution; this should favour desirable kinetics and regioselectivity. Also, steps involving the removal of the derivatizing agent are eliminated, reducing a likely source of sample loss, error in the method and time.

This extraction, derivatization, and analysis procedure is very straightforward, although optimization of both extraction and derivatization for the analytes will be required. It's noted that the SPME fibers hold as well throughout the process, as over 80 complete analysis have been performed, with no apparent fiber degradation.

Under the optimized conditions of extraction, derivatization and desorption, the procedure provides low detection limits (≤ 1 ppb for the non polar analytes and ≤ 35 ppb for the derivatized ones) and satisfactory reproducibility (RSD% ≤ 10% or 20%) for most of the PPCPs investigated: this result makes the developed method suitable for comprehensive chemical profiling of PPCPs in various aqueous matrices. The developed can easily be extended to non-target compounds with similar physico-chemical characteristics. Finally, the method should be amenable to automation.

The developed methods may be the basis of wastewater monitoring for temporal and spatial changes of both target and non target compounds.

Bibliography

[1] J.M. Brausch, G.M. Rand, Chemosphere 2011, 82: 1518-1532

[2] P.Y. Kunz, K. Fent, Aquat. Toxicol. 2006, 79:305-324

[3] P.D. Darbre, J.R. Byford, L.E.Shaw, R.A. Horton, G.S. Pope, S. Hall, N.G. Coldham, G.S. Pope, M.J. Sauer, J. Appl. Toxicol. 2003, 23: 43-51

[4] H. Ishibashi, N. Matsumura, M. Hirano, M. Matsuoka, H. Shiratsuchi, Y. Ishibashi, Y. Takao, K. Arizono, Aquat. Toxicol. 2004, 67: 167-179

[5] M.A. Coogan, R.E. Edziyie, T.W. La Point, B.J. Venables, Chemosphere, 2007, 67: 1911-1918

[6] J.B. Ellis, Environ. Pollution 2006, 144, 184-189

[7] www.epa.gov/ppcp

[8] T.A. Ternes, Water Res. 1998, 32, 3245-3260

[9] T.A. Ternes, A. Joss, H. Siegrist, Environmental Science & Technology, October 15 2004, 393A-399A

[10] C.G. Daughton, T.A. Ternes, Environ. Health Perspect. 1999, 107, 907-938

[11] C.G. Daughton, T.A. Ternes, Environ. Impact Assessment Review 2004, 24 (7/8), 711-732

[12] S. Webb, Risk assessment for pharmaceuticals. In: Proceedings of International Seminar “Pharmaceuticals in the Environment”, 9 March 2000, Technological Institute, Brussels.

[13] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Water Res., 2008, 42, 3498-3518

[14] D. Calamari, E. Zuccato, S. Castiglioni, R. Bagnati, R. Fanelli, Environ. Sci. Technol. 2003, 37, 1241-1248

[15] J.M. Conley, S.J. Symes, M.S. Schorr, S.M. Richards, Chemosphere, 2008, 73, 1178-1187

[16] E. Godfrey, W.W. Woessner, M. Benotti, Ground Water 2007, 45 (3), 263-271

[17] S.J. Jasim, A. Irabelli, P. Yang, S. Ahmed, L. Schweitzer, Ozone Sci. Eng. 2006, 28,415-423

[18] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, Environ. Sci. Technol. 2002, 36, 1202-1211

[19] D.W. Kolpin, M. Skopec, M.T. Meyer, E.T. Furlong, S.D. Zaugg, Sci. Total Environ. 2004, 328, 119-130

[20] C.D. Metcalfe, X.S. Miao, B.G. Koenig, J. Struger, Environ. Toxicol. Chem. 2003, 22 (12), 2881-2889

[21] M.R. Servos, M. Smith, R. McInnis, B.K. Burnison, B.H. Lee, P. Seto, S. Backus, Water Qual. Res. J. Can. 2007, 42 (2), 130-137

[22] E. Zuccato, S. Castiglioni, R. Fanelli, J. Hazard. Mater. 2005, 122, 205-209.

[23] T.A. Ternes, Water Res. 1998, 32, 3245-3260

[24] D.W. Kolpin et al., Environ. Sci. Technol. 2002, 36, 1202-1211.

[25] T. Heberer, Toxicol.Lett. 2002, 131, 5-17

[26] C.S. McArdell et al. Environ. Sci. Technol. 2003, 37, 5479-5486

[27] C.H. Huang, D.L. Sedlak, Environ. Toxicol. Chem. 2001, 20, 133-139

[28] C.D. Metcalfe et al., Environ. Toxicol. Chem. 2003, 22, 2881-2889

[29] A.B.A. Boxall et al, Rev. Environ. Contam. Toxicol. 2004, 180, 1-91

[30] O.A.H- Jones, N. Voulvoulis, J.N. Lester, Environ. Technol, 2001, 22, 1383-1394

[31] S.T. Glassmeyer, E.T. Furlong, D.W. Kolpin, J.D. Cahill, S.D. Zaugg, S.L. Werner, M.T. Meyer, D.D. Kryak, Environ. Sci. Technol. 2005, 39, 5157-5169

[32] T.A. Ternes, M. Meisenheimer, D. McDowell, F. Sacher, H.-J. Brauch, B. Haste-Gulde, G. Preuss, U. Wilme, N. Zulei-Seibert, Environ. Sci. Technol. 2002, 36, 3855-3863

[33] B. Bridges, Flavour & Fragrance Journal 2002, 17 (5), 361-371

[34] J.E. Drewes, R. Arnold, P. Fox, Fate of pharmaceutical chemicals during groundwater recharge using reclaimed water. In: Procedings of Conference on Emerging Issues. National Groundwater Association, Westerville, Ohio, US, 2000

[35] S. Ruqiao, S.A. Andrews, Water Res. 2011, 45, 944-952

[36] R. Länge et al., Environ. Toxicol. Chem. 2001, 20, 1216-1227

[37] E.J. Routledge et al., Environ. Sci. Technol. 1998, 32, 1559-1565

[38] D.B. Hugget et al. Environ. COntam. Toxicol. 2002, 43, 229-235

[39] B. Ferrari et al. Ecotoxicol. Environ. Saf. 2003, 55, 359-370

[40] L. Wollenberger, B. Halling-Sorensen, K. Kusk, Chemoshere 2000, 40, 723-730

[41] R. Altenburger et al. Environ. Toxicol. Chem. 2000, 19, 2341-2347

[42] C.G. Daughton, T.L. Jones-Lepp. Pharmaceuticals and Personal Care Products in the Environment, ACS Symposium Series 791, Oxford University Press. Washington. USA. 2001

[43] N.Y. Yoon, P. Westerhoff, S.A. Snyder, E.C. Wert, J. Membr. Sci. 2006, 270, 88
[44] P. Westerhoff, Y. Yoon S. Snyder, E. Wert, Environ. Sci. Technol. 2005, 39, 6649.
[45] R.A. Trenholm, B.J. Vanderford, J.C. Holady, D.J. Rexing, S.A. Snyder, Chemosphere 2006, 65, 1990
[46] H.B. Lee, T.E. Peart, M.L. Svoboda, J. Chromatogr. A, 2005, 1094, 122
[47] A.M. Peck, Anal. Bioanal. Chem. 2006, 386, 907
[48] S. Mitra, Sample Preparation Techniques in Analytical Chemistry, Wiley, New York, 2003.
[49] M.F. Alpendurada, J. Chromatogr. A, 2000, 889, 3-14
[50] V. Pichon, J. Chromatogr. A, 2000, 885, 195
[51] www.sigmaaldrich.com
[52] R.E. Majors, LCGC 2007, 25 (1), 16
[53] C.L. Arthur and J. Pawliszyn, Anal. Chem. 1990, 62, 2145
[54] Z. Mester, R. Sturgeon, J. Pawliszyn, Spectrochim Acta B, 2001, 56, 233
[55] J.S. Fritz, M.Macka, J. Chromatogr. A, 2000, 902: 137
[56] J.S. Fritz, Analytical Solid-Phase Extraction, Wiley-VCH, New York, 1999.
[57] H. Lord, J. Pawliszyn, J. Chromatogr. A, 2000, 885:153
[58] Bulletin 923A, Supelco 1999
[59] M.G. Zampolli, G. Basaglia, F. Dondi, R. Sternberg, C Szopa, M.C. Pietrogrande, J. Chromatogr. A 2007, 1150: 162.
[60] L.Pan, J. Pawliszyn, Anal. Chem. 1997, 69: 196
[61] J.B. Quintana, I. RodrÍguez, Anal. Bioanal. Chem. 2006, 384: 1447-1461
[62] R. Leardi, Anal. Chimica Acta 2009, 652: 161-172
[63] B. Dejaegher, Y. Vander Heyden, Acta Chromatographica 2009, 21 (2): 161-201
[64] D.L. Massart, B.G.M Vandeginste, S.N. Deming, Y. Minchotte, L. Kaufman, Chemometrics: a texbook – volume 2, Elsevier, New York, 1988

Papers

Paper 1

M.C. Pietrogrande, G. Basaglia, “GC-MS analytical methods for the determination of personal-care products in water matrices” – Trends in Analytical Chemistry, Vol.26, No. 11 (2007), 1086-1094

GC-MS analytical methods for the determination of personal-care products in water matrices

Maria Chiara Pietrogrande, Giulia Basaglia

This article discusses the more recent methods combining gas chromatography and mass spectrometry (GC-MS) for analysis of personal-care products (PCPs) in water matrices. We describe different procedures for sample extraction and preparation as well as different instrumental methods commonly used for these compounds. GC-MS and GC-tandem MS (GC-MS^2), which are complementary to liquid chromatography combined with MS (LC-MS), allow identification and quantification of PCPs belonging to different classes with the sensitivity and the selectivity necessary for environmental monitoring. The compounds investigated include fragrances (e.g., nitro and polycyclic musks), antimicrobial compounds (e.g., triclosan), ultraviolet blockers (e.g., methylbenzylidene camphor), antioxidants and preservatives (e.g., phenols and p-hydroxybenzoic acid (parabens)) and insect repellents (e.g., N,N-diethyl-m-toluamide (DEET)). We critically review data in the literature by focusing attention on analytical methods devoted to simultaneous detection and quantification of structurally diverse pharmaceuticals and PCPs.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Antimicrobial; Antioxidant; Emerging contaminant; Gas chromatography; GC-MS; Insect repellent; Mass spectrometry; Nitro musk; PCP; Personal-care product

Maria Chiara Pietrogrande*
University of Ferrara, Department of Chemistry, Via L. Borsari, 46, 44100 Ferrara, Italy

Giulia Basaglia
L.A.R.A., Land Network Laboratory for Waters of Emilia Romagna Region, University of Ferrara, Department of Chemistry, Via L. Borsari, 46, Ferrara, 44100, Italy

*Tel.: +39 532 455 152;
Fax: +30 532 240 709;
E-mail:
chiara.pietrogrande@unife.it

1. Introduction

Personal-care products (PCPs) are synthetic organic chemicals derived from usage by individuals in soaps, lotions, toothpaste, cosmetics and other PCPs. Together with various pharmaceuticals, they constitute the class of pharmaceuticals and PCPs (PPCPs) that form a wide variety of important ''unrecognized'' or ''emerging'' pollutants in everyday urban activities [1–4]. Following the precautionary principle, the EU Water Framework Directive has identified some PPCPs as future emerging priority candidates for monitoring and regulation [5,6].

The principal pathway by which PPCPs enter the environment is disposal in urban receiving waters from individual households, after showering and bathing. A variety of PPCPs have been detected everywhere at the nL-concentration level in effluents of wastewater-treatment plants (WWTPs), since conventional water-treatment processes do not seem to be sufficient to remove PPCPs from sewage water (30–90% efficiency) [2,3,7–12].

The occurrence of PPCPs in municipal sewage effluent and other environmental samples could negatively impact the health of the ecosystem and the health of humans, due to persistent, long-term chronic exposure of aquatic organisms to concentrations of PPCPs [1,2]. Moreover, there is some evidence of potential interactive effects of PPCPs, so that low doses may lead to cumulative stress and synergic toxicity effects in exposed organisms [1,2,13]. Some PCPs (e.g., ultraviolet (UV) screens, insect repellents (e.g., N,N-diethyl-m-toluamide (DEET)), p-hydroxybenzoic acid (parabens), and some synthetic musk fragrances) have been suspected endocrine-disrupting compounds (EDCs) (i.e. compounds that can mimic the natural hormones of animals) [1,9,11,12,14,15].

The issue of emerging contaminants is closely related to analytical capabilities of monitoring their occurrence in the various environmental compartments. With the development of sophisticated and sensitive analytical procedures – more efficient extraction techniques and better detectors – more and more PPCPs can be detected at trace levels in the environment [1,16]. Consequently, a number of new or previously ignored and/or unrecognized contaminants have been brought under scrutiny.

There is therefore the need for further improvements to develop quick and sensitive analytical procedures, in particular in two directions:

0165-9936/$ - see front matter © 2007 Elsevier Ltd. All rights reserved. doi:10.1016/j.trac.2007.09.013

1. high sensitivity at trace levels (up to ng/L); and,
2. versatility in simultaneous screening for a wide variety of compounds with large differences in physicochemical properties (e.g., log K_{ow}, water solubility, pK_a, M_w).

A single method for the analysis of different classes of target analytes would be convenient, since it would reduce the overall analysis time, field sampling and costs. Moreover, comprehensive information about multiple classes of PPCPs coinciding in an environmental sample is required for contaminant-monitoring planning and risk-assessment studies, since chemicals may interact to yield synergic toxicity effects on exposed organisms [1,13]. Even if most of the available methods are specifically devoted to a few contaminants or a single PPCP class, some multi-residue methods have been developed for determining organic pollutants in aqueous environment.

This article discusses the more recent methods combining gas chromatography and mass spectrometry (GC-MS) for the analysis of several GC-amenable compounds (e.g., potential PCPs in various water matrices); in particular, we critically review data in the literature by focusing attention on analytical methods for the simultaneous detection and quantification of structurally diverse PPCPs as representative molecular markers of water pollution.

2. PCP classes

PCP compounds in this article belong to the following chemical classes:

- fragrances (e.g., nitro and polycyclic musks);
- antimicrobial compounds (e.g., triclosan);
- UV blockers (e.g., methylbenzylidene camphor);
- antioxidants and preservatives (e.g., phenols and parabens); and,
- insect repellents (e.g., DEET).

These compounds were selected from the large number of chemical possibilities based upon usage, toxicity, potential hormonal activity, and persistence in the environment.

2.1. Synthetic musk fragrances

Two types of synthetic musk fragrances are widely used in Europe and North America: polycyclic and nitro musks (PNMs). They can be found in almost all consumer products (e.g., perfumes, deodorants, cosmetics and soaps) and are released into wastewater after use of the consumer products, so they are present in the environment due to wastewater discharges and land application of biosolids [1,2,17–20].

2.2. Antimicrobial compounds

Triclosan (5-chloro-2-[2,4-dichloro-phenoxy]-phenol, TCS) is one of the antimicrobial compounds used most in many consumer products (e.g., toilet soaps, toothpaste, detergents, deodorants, and sports clothing). It has been detected in surface waters and sewage plants (at a concentration level $\approx$1 μg/L) in various countries, and it has been found to be acutely and chronically toxic to aquatic organisms. A TCS derivative, methyl-triclosan (M-TCS), is a more lipophilic and environmentally persistent metabolite than the parent compound [1,7,9,21,22].

2.3. Sunscreen agents

Sunscreen agents are increasingly added (in relative amounts of 0.1–10%) to cosmetics and lotions as protection against harmful UV radiation. Though the high hydrophobicity of many of these compounds ($\log K_{ow} = 5 - 8$) indicates the potential for bioaccumulation, relatively little is known about the occurrence and the fate of UV filters in the environment. Several of these compounds show estrogenic activity [23–25].

2.4. Insect repellents

DEET and the more recent Bayrepel (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl), 1-methylpropyl ester) are the insect repellents used most [11,26]. They have been widely detected in aquatic systems; from limited toxicity data, it can be inferred that DEET is slightly toxic to aquatic invertebrates, fish, and birds [9,14].

2.5. Preservatives

Parabens are the most common preservatives used in PCPs, pharmaceuticals and food products. Methylparaben and propylparaben are the most widely used and are normally used together due to their synergistic preservative effects [1,15,27]. Parabens exhibit estrogenic behavior [27].

3. Analytical methods

To analyze complex mixtures, such as water samples, a pretreatment procedure is required to provide a sample fraction enriched with all the target analytes and as free as possible from other matrix components [16]. Fig. 1 shows the general analytical procedure for analysis of PCPs in aqueous samples.

A variety of sample preparation methods is available for extraction and concentration of contaminants in water: liquid–liquid extraction (LLE); solid-phase extraction (SPE); solid-phase microextraction (SPME); and, semi-permeable membrane devices (SPMDs) [16,28].

Detection and quantification are based on GC paired with MS (Pathway A, Fig. 1), high-performance liquid chromatography (HPLC) coupled with MS, immunoassays, or a combination of techniques [16].

Choosing between GC and HPLC is generally based on the physiochemical qualities of the target analyte. HPLC-MS is usually used to determine more polar and less

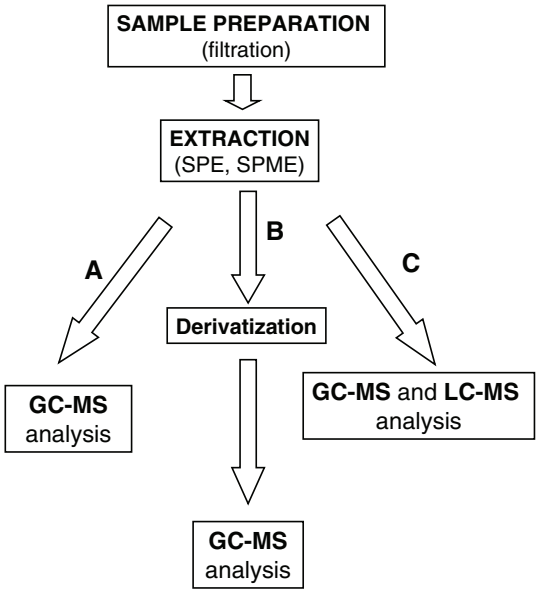


Figure 1. Scheme of the general analytical procedure for analysis of PCPs in aqueous samples; Pathway A: the extracted sample is directly submitted to GC-MS analysis; Pathway B: the extracted sample is submitted to derivatization reaction prior to GC-MS analysis; Pathway C: the extracted sample is directly submitted to a combination of GC-MS and LC-MS analysis.

volatile compounds, while GC-MS is used to identify and quantify volatile or volatizable compounds and metabolites [16–29], in particular when resolution is essential to separate isomers or congeners [30]. Combining of LC and GC is a very powerful approach to developing multi-residue analytical methods for wide-range screening of microcontaminants (Pathway C, Fig. 1) [12–14,23,31,32].

3.1. Sample extraction and preparation methods

We review the following techniques that are the most accepted in the practice of modern analytical chemistry.

3.1.1. SPE. SPE is the method of choice that is particularly well adapted to multi-residue analysis, including compounds with a wide range of polarity or characterized by various physico–chemical properties [28]. Table 1 summarizes the most recent applications in PCP analysis (the multi-residue methods are highlighted in italic font).

Octadecyl and octyl bonded silicas have been universal extraction sorbents for many years: the use of C_{18} silica is really appropriate for trace enrichment of compounds characterized by log K_{ow} values higher than 2, as most PCPs are [16,28]. C_{18} cartridges have been used in SPE procedures for the analysis of different PCP classes: synthetic musk fragrance; triclosan; UV filters; and, insect repellents [16,23,24]. To achieve the best recovery for the target analytes, different eluents are selected according to solvent eluting strength and solute polarity: acetone, dichloromethane and methanol, or mixtures of them.

Divinylbenzene/N-vinylpyrrolidone copolymer Oasis HLB is a commercial adsorbent for extracting organics from water samples. Oasis HLB has been the most commonly used SPE sorbent for the analysis of triclosan [13,14,16,32]. Oasis HLB has been found suitable for multi-residue methods with a proper selection of the eluent. Methanol was used for quantitatively eluting different PPCPs, including fragrances, contained in the influent and effluent of municipal WWTPs [13,32,33].

Sequential elution was performed to extract selected PPCPs, including triclosan and insect repellents, from sewage samples: hexane was used to remove lipophilic interferences followed by ethyl acetate and methanol to elute acidic compounds [31].

An ethinylbenzene-divinylbenzene copolymer (LiChrolut EN) was the sorbent used in an SPE procedure for the analysis of some EDCs eluting with acetone followed by methanol [16].

A mixed-mode sorbent with reversed-phase and anion-exchange functionality – Oasis MAX cartridge – extends the extraction procedure to acidic compounds. Selective elution of 21 PPCPs into two fractions could be achieved: the least acidic phenols were eluted with pure methanol and the most acidic compounds, including triclosan and parabens, were removed by methanol acidified with formic acid [15].

Bio Beads SM-2 was used to extract some UV filters – octyl methyoxycinnamate, 4-methylbenzylidene camphor, benzophenone-3, octocrylene, and avobenzone – from surface-water and wastewater samples [25,30]. The same sorbent was also suitable for extracting polycyclic musks [16,18] from surface-water samples using methanol and dichloromethane as eluents.

Polystyrene-divinylbenzene resins (PS–DVB) (e.g., Amberlite XAD) proved very useful in multi-residue extraction for trapping the polar compounds that are not extracted on C_{18} silica [28]. Further advantages offered by this sorbent over C_{18} silica are its stability when percolating samples in the 1–14 pH range and the suitability for passing large volumes of water when great amounts of sorbents of large particle size are used in custom-made cartridges [34,35].

XAD-2 resin has been used to concentrate synthetic musk fragrances and their amino-metabolite samples, eluting with hexane/acetone [19].

XAD-4 resin was suitable for the extraction step in wide-range screening of microcontaminants in surface waters, including fragrances, plasticizers and triclosan; 150 target compounds were detected in the low-ng/L and low-μg/L ranges [34].

PS-DVB resin SDB-1 was used as sorbent in a device specially designed and constructed to extract a large volume of water (10*L*) for the non-target screening for

Table 1. Analytical methods of personal-care products (PCPs) in water using solid-phase extraction (SPE); the multi-residue methods are highlighted in italic font

Matrix	Analytes	Extraction and preparation	Analytical method	Ref.
WWTP influent and effluent	Synthetic musks	SPE (C18); eluent: DCM	GC-EIMS	[16]
Groundwater	Synthetic musks	1. SPE (C18); eluent: Acet/Hex 2. Silica purification	GC-EIMS	[16]
Surface water and wastewater	*Triclosan (and acidic pharmaceuticals)*	1. Acidified to pH < 3 2. SPE (C18); eluent: Ac 3. Derivatized with MCF	*GC*-EIMS	[16]
Water	Sunscreen agents	SPE (C18 extraction disks) SPME on PDMS-DVB	GC-EIMS	[24]
Swimming-pool water	Sunscreen agents	SPE (C18); eluent: EtAc/DCM	HPLC-UV/DAD GC-EIMS	[23]
Surface water	Sunscreen agents	SPE (C18, Empore disk); eluent: DCM	GC-FID GC-EIMS	[16]
Surface water	*6 PCPs, including DEET*	*SPE (Empore disk, C18); eluent: ACN*	GC-EIMS	[16]
Surface water	Synthetic musks	SPE (C18); eluent: DCM	GC-EIMS	[16]
Surface water	Synthetic musk fragrances	SPE (XAD-2 resin); eluent: Ac/Hex	GC-EIMS	[19]
Surface water and wastewater	Polycyclic musks	SPE (Bio Beads SM-2); eluents: MeOH, DCM (sequential)	GC-EIMS/SIM	[16]
Wastewater	*18 antiseptics, including triclosan*	1. Acidified to pH 2 2. SPE (Phenomenex Strata X); eluent: ACN 3. Derivatized with PFBBr	*GC-EIMS*	[7]
Surface water, treated water and storm water	*9 PCPs, including triclosan*	1. Acidified to pH 2 2. SPE (SDB-XC Empore disk); eluents: MeOH, DCM, MeOH (sequential) 3. Silica purification 4. Derivatized with BSTFA	*GC-EIMS*	[38]
Surface water	*150 industrial, agrochemical and household chemicals*	*SPE (XAD-4 and XAD-8); eluent: Ac* *Compared to SPMD: eluents: Hex-EtAc- Ac*	*GC-AEDMS*	[34]
Municipal WWTP effluent	*Acid PPCPs, including polycyclic musks and triclosan*	1. Acidified to pH 2 2. SPE (Oasis HLB); eluent: MeOH 3. Derivatized with BSTFA + TMCF	*GC-EIMS*	[33]
Wastewater	*Organic toxicants*	1. SPE (Oasis HLB); eluent: MeOH 2. Sample fractionation by HPLC – bioassay testing	*GC-EIMS, LCMS*^{2}	[13]
Surface water and WWTP effluent	Triclosan	1. Acidified to pH 3 2. SPE (Oasis HLB); eluent: EtAc/Ac 3. Derivatized with diazomethane	GC-EIMS	[16]
Surface and drinking water	*Polar PPCPs*	1. pH adjusted to 7 2. SPE (LiChrolut EN); eluents: Ac, MeOH (sequential) 3. Derivatized with PFBCl	*GC-NCIMS/SIM*	[16]

(*continued on next page*)

Table 1 (*continued*)

Matrix	Analytes	Extraction and preparation	Analytical method	Ref.
Wastewater and surface water	*58 EDCs and PPCPs, including triclosan and DEET*	*SPE (Oasis HLB)* 1. Eluents: MeOH and MeOH/MTBE for LC 2. Eluent: DCM for GC	*GC-EIMS*^{2} *LC-ESIMS*^{2}	[14]
Surface water and wastewater	Triclosan and methyl triclosan	1. Acidified to pH 2 2. SPE (Bio Beads SM-2); eluent: MeOH/DCM. 3. Derivatized with diazomethane and diazoethane SPMD using cypen	GC-EIMS/SIM	[18]
Surface water	*Pesticides, industrial chemicals, pharmaceuticals*	1. Filtration 2. SPE (SDB-1, glass fiber filter candles); eluent: Hex/EtAc.	*GC*-EIMS	[35]
Surface water and wastewater	*Neutral and Acid PPCPs and pesticides*	1. SPE (Oasis HLB); eluent: EtAc/Ac. 2. Acid PPCPs derivatized with diazemethane	*GC-EIMS/SIM GC-MS*^{2}	[40]
Surface water and wastewater	Sunscreen agents	1. SPE (Biobeads SM-2); eluent: MeOH/DCM 2. SPMD using cypen/DCM	GC-EIMS GC-EIMS/SIM	[25]
Wastewater	*96 organics: fragrances, insecticides, preservatives*	1. POCIS 2. SPE (Oasis HLB); eluents: MeOH, acidified MeOH (sequential)	*LC-MS* *GC*-EIMS	[32]
Influent and effluent of drinking water treatment processes	*62 EDC and PPCPs organics: fragrances, insecticides, preservatives*	*SPE (Oasis HLB); eluent: MeOH/MTBE*	*GC*-EIMS *GC-MS*^{2} *LC-MS*^{2}	[11]
Surface water	Sunscreen agents	1. SPE (Biobeads SM-2); eluents: MeOH, DCM (sequential) 2. Silica purification; eluent: EtAc. SPMD using cypen	*GC-EIMS*	[30]
Swimming pool water	Sunscreen agents	SPE (SDB disk)	HPLC-UV/DAD GC-EIMS	[23]
Sewage influent and effluent	*21 phenols and acids, including triclosan and parabens*	1. Filtered water 2. Acidified to pH 2 3. SPE (Oasis MAX); eluents: MeOH, 2% formic acid in MeOH (sequential) 4. Derivatization with PFPA	*GC-EIMS/SIM* *GC-CIMS/SIM*	[15]
Surface and drinking water	*Polar PPCPs*	1. pH adjusted to 7 2. SPE (LiChrolut EN); eluents: Ac, MeOH (sequential) 3. Derivatized with PFBCl	*GC-NCIMS/SIM*	[16]
Wastewater and sewage water and seawater	*15 pharmaceuticals and antibacterial, including DEET and triclosan*	1. pH adjusted to 7 2. SPE (Oasis HLB); eluents: Hex, EtAc, MeOH (sequential) 3. Derivatization with methyl chloromethanoate	*GC*-EIMS *LC-MS*^{2}	[31]

Table 1 (*continued*)

Matrix	Analytes	Extraction and preparation	Analytical method	Ref.
Surface water and WWTP influent and effluent	Bayrepel and Bayrepel acid	1. Acidifed to pH 2 2. SPE (LiChrolut EN/Isolut C18); eluents: Ac/EtAc, MeOH (sequential) 3. derivatized with diazomethane	GC-EIMS LC-ESIMS LC-ESI/TOFMS	[26]

DCM, Dichloromethane; Hex, Hexane; Pent, Pentane; Tol, Toluene; Cyhex, Cyclohexane; Cypen, Cyclopentane; EtAc, Ethyl acetate; Ac, Acetone; MTBE, Methyl-ter-butyl ether; ACN, Acetonitrile; iPrOH, Isopropanol; DEET, N,N-diethyl-m-toluamide; EDC, Endocrine-disrupting compound; PCPs, Personal-care products; PPCPs, Pharmaceuticals and personal-care products; WWTP, Wastewater-treatment plant.

organic contaminants covering wide ranges of polarity and chemical classes, including several pharmaceuticals, pesticides and industrial chemicals. The possibility of extracting large volumes enabled large enrichment factors and thus very low limits of detection (LODs) (at concentrations even in the pg/L range) [35].

For various analytes, the extraction-concentration procedure is critical and strongly affects the results obtained; significant differences can be displayed by results obtained with different types of extraction (SPE versus continuous LLE) as well as differing types of sample pretreatment (filtered versus whole water) [16].

3.1.2. SPME. Recently, SPME was proposed as an alternative to SPE in the analysis of environmental water samples: it is a solvent-free, one-step extraction method based on the partition equilibrium of the analyte between the sample and a sorbent (i.e. a solid-phase coated on a silica-fiber support) [36].

The combination of SPME and GC is particularly suitable for the determination of volatile and semi-volatile, non-polar compounds; numerous papers have described methods using different coating materials on fibers to extract different classes of PCPs from water matrices (summarized in Table 2) [16,20,24,27,37]. Nowadays, the SPME procedure may display some limitations in comparison with SPE, mainly related to the reduced possibility of method manipulation and the limited choice of sorbent coatings on the market to obtain selective adsorption; the results may yield poor extraction efficiencies and high LOD values ill-suited to trace analysis [36]. Each SPME procedure therefore requires the various experimental parameters to be optimized to achieve effective, efficient extraction.

As an example, four fibers (PDMS, PDMS-DVB, polyacrylate (PA) and carboxen) and different experimental set-ups (i.e. extraction time, temperature, and stirring velocity) were compared to optimize direct SPME (DI-SPME) of synthetic musk fragrances in water. The best recoveries (nearly 70%) were achieved with PDMS-DVB fibers and extraction times of 45 min at 30°C [20].

SPME was used for UV filters (e.g., benzophenone-3 and its metabolites) to provide adequate recoveries similar to those provided by SPE using C_{18} [24]. An SPME fiber was used to extract and concentrate five esters of parabens from river-water and sewage-water samples. The extraction procedure was optimized by investigating the influence of different factors (i.e. chemical structure of coated fibers, sample pH and ionic strength): limits of quantification (LOQs) at the low-ng/L level were achieved, with acceptable precision and free of matrix effects [27].

3.1.3. Semi-permeable-membrane devices (SPMDs). As an alternative to traditional procedures based on a periodic collection of water sample, a new strategy is

Table 2. Analytical methods of personal-care products (PCPs) in water using solid-phase microextraction (SPME)

Matrix	Analytes	Extraction and preparation	Analytical method	Ref.
Surface water	Polycyclic musks	DS-SPME (PDMS)	GC-EIMS	[16]
WWTP effluent	Polycyclic musks	DS-SPME (PDMS, PDMS-DVB, CAR-PDMS, CW-DVB)	GC-EIMS	[16]
Surface water	Synthetic musk fragrances	DS-SPME (PDMS, PDMS-DVB, PA)	GC-EIMS	[20]
Water	Sunscreen agents	SPE (C18 extraction disks) SPME on PDMS-DVB	GC-EIMS	[24]
Surface water	Sunscreen agents	DI-SPME (PDMS 100 μm or PA 85 μm)	GC-FID GC-EIMS	[16]
Surface water	Triclosan and metabolites	SPME on PA On-line derivatization with MTBSTFA	GC-EIMS	[37]
Surface and sewage water	Parabens	SPME on PA On-line derivatization with MTBSTFA	GC-MS^2	[27]

PDMS, Polydimethylsiloxane; PDMS-DVB, Polydimethylsiloxane-divinylbenzene; PA, Polyacrylate; CAR-PDMS, Carboxen fiber-polydimethylsiloxane; CW-DVB: Carbowax-polydimethylsiloxane; WWTP, Wastewater-treatment plant.

emerging employing passive, in situ, sampling devices that can be deployed over extended periods (days or week) and provide time-weighted average concentrations to estimate the potential exposure of aquatic organisms to waterborne contaminants. Passive sampling devices use SPMDs to ensure continuous diffusion of the pollutant from the bulk water phase to the receiving phase in order to sample and to concentrate trace levels of hydrophilic organics [16,30,34].

Different procedures have been developed to extract target PCPs belonging to specific classes from surface water: UV filters [25]; triclosan; and, methyl triclosan [21].

A polar organic chemical integrative sampler (POCIS) was used for sampling different organic contaminants, including musk fragrances and triclosan; it was designed to mimic the respiratory exposure of aquatic organisms to dissolved chemicals [32].

SPMD extraction was used to extract a wide range of microcontaminants from river water. Its performance was compared to SPE (XAD-4 eluted with acetone): SPE displayed higher efficiency for more hydrophilic contaminants ($\log K_{ow} \approx 0-4$) while SPME for the more lipophilic ($\log K_{ow} \approx 3-7$) [34].

3.2. Derivatization

Some of the PPCPs of interest (i.e. parabens, DEET and some UV filters) are highly-polar, thermally-fragile compounds that require transformation into more volatile compounds to make them suitable for GC analysis (Pathway B, Fig. 1) [16,29]. The best derivatization reaction:

(1) permits detection of the compounds containing polar function groups with adequate signal-to-noise (S/N) ratio;
(2) encourages complete derivatization (>90%); and,
(3) is time efficient.

Many variables are involved in optimizing derivatization: the derivatizing agent; derivatization solvent; reaction temperature; and, duration of reaction. In developing multi-residue methods, the efficiency of the derivatization reaction of individual PCPs has to be investigated and compared with that of a mixture of them. Derivatization broadens the applicability of GC-MS analysis to more polar PCPs (e.g., phenols). Usually, GC-MS analysis after derivatization is an efficient alternative to LC-MS and decreases the LODs of the GC-MS methods to achieve sensitivity comparable to that of LC-MS [2,4].

Silyl reagents are the most commonly applied for PCPs, due to their rapid, quantitative reactions that yield stable products with good chromatographic properties; a variety of reagents is used to give different ether derivatives. Different silyl compounds can derivatize triclosan (e.g., N-t-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) [37], and MTBSTFA with 1% t-butyldimethylchlorosilane (TBDMSCl)) [15].

N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) is suitable for multi-residue methods: it has been applied to derivatize acid compounds, including PCPs and EDCs [38], as well as acidic drugs and polycyclic musks prior to GC-MS analysis to reach an LOQ of 5ng/L [33].

Four different derivatizing agents were investigated and compared for GC-MS detection of diverse PPCPs, including polar compounds, such as triclosan: it was found that the reagents MSTFA + 1% trimethylchlorosilane (TMCS) yielded the most effective reaction [39].

Diazomethane is another reagent used to transform polar PCPs into methyl derivatives (i.e. DEET and triclosan) [21,22,26,40]. The main drawback of this reagent is that it is poisonous, carcinogenic, and explosive, so that great care is needed to manipulate it.

Methyl chloromethanoate can also be used in multi-residue methods to form methyl esters; it was applied for the analysis of acid PPCPs, including DDET and triclosan [31].

Pentafluoropropionic acid anhydride (PFPA) has been used to form pentafluoropropionyl derivatives of 21 endocrine-disrupting phenols and acids, including triclosan and parabens [15]. The same PCPs can be also derivatized using pentafluorobenzyl bromide (PFBBr) [7]. The main advantage of pentafluoro reagents compared to silylation reagents is that they turn the analytes into highly electrophilic derivatives by introducing 5 or 10 fluorine atoms to improve sensitivity and selectivity in MS detection.

An on-fiber derivatization reaction has been developed by combining on-line the derivatization reaction with SPME; it broadens the applicability of GC to polar species, which are not easily derivatized in the presence of water. Five parabens have been extracted and converted into their tert-butyldimethylsilyl derivatives that are suitable for GC-MS^2 analysis. The performance of the on-fiber derivatization step can be optimized by a proper selection of different factors (i.e. temperature, time and volume of MTBSTFA) [27,37].

For multi-residual procedures, it has been found that harsh chemicals or high temperature of the derivatization procedure may result in thermal breakdown or transformation of underivatizable parent compounds [39], so it has been proposed to split the sample into two fractions prior to GC-MS analysis: one half is submitted to derivatization for analysis of polar target compounds, the other is directly analyzed by GC.

4. Detection and quantification

Most of the published methods for PCP analysis report GC-MS identification based on electron-impact (EI) ionization and single-quadrupole MS as the preferred GC detection system. This is the most appropriate technique if the analysis is focused on the enforcement of

maximum residue levels, simultaneous identification and quantification of a very large number of target analytes. Advantages of EI ionization are the small influence of molecular structure on response and the large number of characteristic fragments. The full-scan mass spectra are obtained over a proper m/z range and the total ion current (TIC) signal is the basis for compound identification. Positive identification of a compound requires elution within the expected retention-time window; in addition, sample spectra and ion-abundance ratios are required to match those of the reference standard compounds [16,29].

Chemical ionization (CI), as a soft ionization mode, provides information on the fragmentation pattern useful for structure identification of PCPs and their metabolites. CI modes (positive CI (PCI) and negative CI (NCI)) were used for the detection of nitro and polycyclic musks, with methane as reagent. Using quadrupole MS, the performances of EI, PCI and NCI were investigated and compared for musks and their amino metabolites: NCI was found to be the most sensitive method, followed by EI and PCI. NCI detection increases sensitivity to achieve an LOD comparable to ion-trap MS^2 detection (i.e. as little as 2pg for most of the musks on the column) [17].

In many methods, MS detection has been optimized by developing a selected ion monitoring (SIM) program using the most prominent masses (i.e. charge ratio (m/z) values associated with each target compound, determined by running the GC-MS analysis in scan mode). SIM detection improves sensitivity for the quantitative analyses of target compounds, with no attempt to report data for non-target compounds [15,18,21,25,30].

GC-MS^2 analysis allows suppression of matrix background to achieve excellent selectivity and sensitivity. This is very advantageous in quantifying very low levels of pollutants present in matrices with high levels of background interference. It combines the high selectivity provided by MS^2 and the high sensitivity obtained by using large-volume injections [12,14,17,27]. MS^2 experiments can be performed using ion-trap and triple-quadrupole mass analyzers. The precursor ions are selected and the MS^2 conditions (e.g., isolation (wideband application, isolation time) and fragmentation (resonance excitation voltage, fragmentation voltage)) are optimized to obtain a balance between maximum sensitivity, minimum spectral interferences and enough structural information for unequivocal identification [27]. Nowadays, in addition to the standard EI ionization mode, many ion-trap instruments offer the possibility of performing CI in combination with MS^2 as a soft ionization mode for the efficient production of molecular ions. When ion-trap MS is used and multiple reaction monitoring (MRM) in positive electron impact mode is applied, high selectivity and sensitivity at the ng/L level necessary for analysis at environmentally relevant levels can be achieved for many PCPs [14].

After qualitative criteria are met, quantification of compound concentrations is performed by calculation from 5–8-point calibration curves. Correct quantification can be achieved only if reference compounds are available; isotopically-labeled compounds are best as internal and surrogate standards, since environmental samples are complex and unknown mixtures. As isotopically-labeled PCP standards (^{13}C, D) are rare and costly, external calibration is used to quantify injected masses. Spike recovery experiments by adding labeled surrogates to the sample have been performed to quantify the injected masses and evaluate the method performance (recovery, variability matrix interference) [7,9,12,14,18,21,22,25,38].

The high resolution of GC retention allows separation of isomers or congeners; this is the case for some UV filters that undergo isomerization under the influence of light. The (E)- and (Z)-isomers of MBC (4-methylbenzylidene camphor) and EHMC (ethylhexyl methoxycinnamate) can be identified and quantified (at the μg/L level) in surface-water extracts from the GC-MS TIC chromatograms [25,30].

5. Conclusions

With the growing attention to PPCPs as emerging contaminants in the environment, there is an increasing demand for analytical procedures to extract and to identify a sufficiently broad variety of substances. In addition to methods specifically devoted to a single class of PCPs, multi-residue methods are being developed for the determination of several PPCPs with one extraction and limited sample preparation.

Sample preparation is one of the most critical steps; in addition to the most popular SPE, SPME and membrane extraction are becoming alternatives in the analysis of environmental water samples. There are developments towards on-line techniques for more automated and rapid analyses, and large-volume injection for lower LODs.

In multi-residue methods, there is the need for accurate optimization of the extraction procedure – chemical composition of the sorbent, eluent type, extraction conditions – in order to achieve acceptable recovery values for all the target PCPs.

A promising alternative to conventional analytical techniques may be the combination with bioassays on toxicity effects: components with toxic activity can be selectively isolated from the complex sample and then the compounds responsible of the observed activity identified by chromatography.

GC-MS and GC-MS^2 provide the sensitivity and the selectivity necessary for identification and quantification of GC-amenable PCPs at trace levels. These techniques may be extended to highly polar compounds by using an efficient derivatization.

At present, a combination of the LC-MS and GC-MS techniques seems the most powerful and comprehensive approach for multi-residue procedures, since the application of the two complementary methodologies increases confidence in structural assignment and quantification of target and non-target analytes.

References

[1] C.G. Daughton, T.L. Jones-Lepp, Pharmaceuticals and Personal Care Products in the Environment, ACS Symposium Series 791, Oxford University Press, Washington, USA, 2001.
[2] C.G. Daughton, Environ. Impact Assess. Rev. 24 (2004) 711.
[3] J.B. Ellis, Environ. Pollut. 144 (2006) 184.
[4] D. Barcelo, M. Petrovic, Anal. Bioanal. Chem. 387 (2007) 1141.
[5] http://www.epa.gov/esd/chemistry/pharma/about.htm.
[6] http://ec.europa.eu/environment/water/.
[7] J.T. Yu, E.J. Bouwer, M. Coelhan, Agric. Water Manage. 86 (2006) 72.
[8] T.A. Ternes, A. Joss, H. Siegrist, Environ. Sci. Technol. 38 (2004) 393A.
[9] N.Y. Yoon, P. Westerhoff, S.A. Snyder, E.C. Wert, J. Membr. Sci. 270 (2006) 88.
[10] M. Carballa, F. Omil, J.M. Lema, M. Llompart, C. Garcia-Jares, I. Rodriguez, M. Gomez, T. Ternes, Water Res. 38 (2004) 2918.
[11] P. Westerhoff, Y. Yoon, S. Snyder, E. Wert, Environ. Sci. Technol. 39 (2005) 6649.
[12] S.A. Snyder, S. Adham, A.M. Redding, F.S. Cannon, J. DeCarolis, J. Oppenheimer, E.C. Wert, Y. Yoon, Desalination 202 (2007) 156.
[13] M. Grung, R. Lichtenthaler, M. Ahel, K.E. Tollefsen, K. Langford, K.V. Thomas, Chemosphere 67 (2007) 108.
[14] R.A. Trenholm, B.J. Vanderford, J.C. Holady, D.J. Rexing, S.A. Snyder, Chemosphere 65 (2006) 1990.
[15] H.B. Lee, T.E. Peart, M.L. Svoboda, J. Chromatogr., A 1094 (2005) 122.
[16] A.M. Peck, Anal. Bioanal. Chem. 386 (2006) 907.
[17] D. Herren, J.D. Berset, Chemosphere 40 (2000) 565.
[18] I.J. Buerge, H.-R. Buser, M.D. Müller, T. Poiger, Environ. Sci. Technol. 37 (2003) 5636.
[19] A.M. Peck, K.C. Hornbuckle, Environ. Sci. Technol. 38 (2004) 367.
[20] M. Winkler, J.V. Headley, K.M. Peru, J. Chromatogr., A 903 (2000) 203.
[21] A. Lindstrom, I.J. Buerge, T. Poiger, P.A. Bergqvist, M.D. Muller, H.R. Buser, Environ. Sci. Technol. 36 (2002) 2322.
[22] M.E. Balmer, T. Poiger, C. Droz, K. Romanin, P.A. Bergqvist, M.D. Muller, H.R. Buser, Environ. Sci Technol. 38 (2004) 390.
[23] V.A. Sakkas, D.L. Giokas, D.A. Lambropoulou, T.A. Albanis, J. Chromatogr., A. 1016 (2003) 211.
[24] T. Felix, B.J. Hall, J.S. Brodbelt, Anal. Chim. Acta 371 (1998) 195.
[25] M.E. Balmer, H.R. Buser, M.D. Muller, T. Poiger, Environ. Sci. Technol. 39 (2005) 953.
[26] T.P. Knepper, J. Chromatogr., A 1046 (2004) 159.
[27] P. Canosa, I. Rodriguez, E. Rubi, M.H. Bollain, R. Cela, J. Chromatogr., A 1124 (2006) 3.
[28] V. Pichon, J. Chromatogr., A 885 (2000) 195.
[29] N.S.D. Richardson, Anal. Chem. 78 (2006) 4021.
[30] T. Poiger, H.R. Buser, M.E. Balmer, P.A. Bergqvist, M.D. Muller, Chemosphere 55 (2004) 951.
[31] S. Weigel, U. Berger, E. Jensen, R. Kallenborn, H. Thoresen, H. Huhnerfuss, Chemosphere 56 (2004) 583.
[32] D.A. Alvarez, P.E. Stackelberg, J.D. Petty, J.N. Huckins, E.T. Furlong, S.D. Zaugg, M.T. Meyer, Chemosphere 61 (2005) 610.
[33] L. Lishman, S.A. Smyth, K. Sarafin, S. Kleywegt, J. Toito, T. Peart, L. Lee, M. Servos, M. Beland, P. Seto, Sci. Total Environ. 367 (2006) 544.
[34] L.L.P. van Stee, P.E.G. Leonards, W.M.G.M. van Loon, A.J. Hendriks, J.L. Maas, J. Struijs, U.A.T. Brinkman, Water Res. 36 (2002) 4455.
[35] S. Weigel, K. Bester, H. Huhnerfuss, J. Chromatogr., A 912 (2001) 151.
[36] J. Pawliszyn, Solid Phase Microextraction - Theory and Practice, Wiley-VCH, New York, USA, 1997.
[37] P. Canosa, I. Rodriguez, E. Rubi, M.H. Bollain, R. Cela, Anal. Chem. 79 (2007) 1675.
[38] G.R. Boyd, J.M. Palmeri, S.Y. Zhang, D.A. Grimm, Sci. Total Environ. 333 (2004) 137.
[39] S.L. Rice, S. Mitra, Anal. Chim Acta 589 (2007) 125.
[40] S. Ollers, H.P. Singer, F. Fassler, S.R. Muller, J. Chromatogr., A 911 (2001) 225.

Paper 2

M.C. Pietrogrande, G. Basaglia, F.Dondi, “Signal processing to evaluate parameters affecting SPE for multi-residue analysis of personal care products” – Journal of Separation Science, Vol.32 (2009), 1249-1261

Maria Chiara Pietrogrande
Giulia Basaglia
Francesco Dondi

Department of Chemistry, University of Ferrara, Ferrara, Italy

Original Paper

Signal processing to evaluate parameters affecting SPE for multi-residue analysis of personal care products

This paper discusses the development of a comprehensive method for the simultaneous analysis of personal care products (PCPs) based on SPE and GC-MS. The method was developed on 29 target compounds to represent PCPs belonging to different chemical classes: surfactants in detergents (alkyl benzenes), fragrances in cosmetics (nitro and polycyclic musks), antioxidants and preservatives (phenols), plasticizers (phthalates) displaying a wide range of volatility, polarity, water solubility. In addition to the conventional C_{18} stationary phase, a surface modified styrene divinylbenzene polymeric phase (Strata™ X SPE cartridge) has been investigated as suitable for the simultaneous extraction of several PCPs with polar and non-polar characteristics. For both sorbents different solvent compositions and eluting conditions were tested and compared in order to achieve high extraction efficiency for as many sample components as possible. Comparison of the behavior of the two cartridges reveals that, overall, Strata-X provides better efficiency with extraction recovery higher than 70% for most of the PCPs investigated. The best results were obtained under the following operative conditions: an evaporation temperature of 40°C, elution on Strata-X cartridge using a volume of 15 mL of ethyl acetate (EA) as solvent and operating with slow flow rate (~10 KPa). In addition to the conventional method based on peak integration, a chemometric approach based on the computation of the experimental autocovariance function ($EACVF_{tot}$) was applied to the complex GC-MS signal: the percentage recovery and information on peak abundance distribution can be evaluated for each procedure step. The PC-based signal processing proved very helpful in assisting the development of the analytical procedure, since it saves labor and time and increases result reliability in handling GC complex signals.

Keywords: Emerging contaminants / Gas chromatography-mass spectrometry / Multi-residue method / Personal care products (PCPs) / Solid phase extraction

Received: December 22, 2008; revised: February 4, 2009; accepted: February 6, 2009

DOI 10.1002/jssc.200800749

1 Introduction

Water pollution from emerging pollutants such as personal care products (PCPs) is one of the major focuses of current environmental research because they have such a great effect on the environment and human health [1, 2]. Most PPCPs are man-made organic chemicals that are constantly being introduced into the environment by anthropogenic inputs and not removed by sewage treatment works (STW) [3 – 6].

To investigate the influence of PCPs on the environment, efficient and reliable analytical methods are critically needed to address the occurrence, concentration, and fate of these chemicals. GC is an excellent method for the analysis of volatile, semi-volatile, and non-polar compounds, and recent developments in GC have substantially reduced analysis times and improved sensitivity and selectivity. Despite this, the rapid, accurate, and simultaneous determination of a large number of PCPs in complex environmental matrices continues to be a major and fascinating challenge for researchers not only because of the diversity in PCP chemical properties, but also because of the generally low concentrations (usually

Correspondence: Professor Maria Chiara Pietrogrande, Department of Chemistry, University of Ferrara, Via L. Borsari, Ferrara, Italy
E-mail: chiara.pietrogrande@unife.it
Fax: +39-0532-240709

Abbreviations: DM, dichloromethane; **EA,** ethyl acetate; **$EACVF_{tot}$,** experimental autocovariance function; **MeOH,** methanol; **PCP,** personal care product; **SC,** single components; **SIM,** single ion monitoring

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

on the order of ng/L – pg/L levels) and the complexity of the matrices [7 – 11].

Suitable preparation techniques need to be applied to the samples in order to isolate and pre-concentrate the analytes prior to their determination. Sampling and sample pre-treatment are the bottleneck in GC analysis since the tedious sample pre-treatment typically accounts for over 60% of the total analysis time, and the quality of this step largely determines the success of the complex matrix analysis. Therefore, the development of faster, more cost-effective, more environment-friendly procedures is a compulsive requirement.

SPE is the method of choice for comprehensive chemical profiling of aqueous samples: it is particularly well suited to extraction and concentration of many compounds displaying a wide range of polarity and physicochemical properties. A wide range of SPE sorbents have been used to extract target PCPs: C_8 and C_{18} bonded phases on silica, ion-exchange phase, and polymeric phase [12 – 14].

The objective of this study is to develop a multi-residue method for simultaneous extraction of several PCPs with polar and nonpolar characteristics, without a derivatization step, to be applied to water matrices as an alternative to methods devoted to a small number of PCPs belonging to the same chemical class [15 – 18]. The target compounds were chosen to represent a wide variety of compound classes such as surfactants in detergents (alkyl benzenes), fragrances in cosmetics (nitro and polycyclic musks), antioxidants and preservatives (phenols), plasticizers (phthalates).

Different parameters affecting the performance of the SPE procedure were investigated to define improved experimental conditions yielding higher extraction efficiency for all analytes: the chemical properties of the SPE sorbent, the composition and volume of the elution solvent, some operative procedure conditions such as evaporation temperature and elution flow rate.

To evaluate each step of the SPE procedure, in addition to the conventional method based on peak integration, a signal processing procedure is used to extract chemical information from the GC-MS separations in order to save labor and time, and increase result reliability in handling GC complex signals [19 – 21].

2 Signal processing procedure: the autocovariance function method

The chemometric approach is based on the computation and study of the experimental autocovariance function ($EACVF_{tot}$): it can be directly computed from the whole experimental chromatogram acquired in digitized form (Fig. 1a), using the following expression [19]:

$$EACVF_{tot}(\Delta t) = \frac{1}{N_p}\sum_{j=1}^{N_p-k}\left(Y_j - \hat{Y}\right)\left(Y_{j+k} - \hat{Y}\right)$$

$$k = 0, 1, 2, \ldots M-1 \qquad (1)$$

where Y_j is the digitized chromatogram signal formed by N_p number of points, $\hat{Y}$ its mean value and M the truncation point in the $EACVF_{tot}$ computation. The time Δt is the inter-distance between subsequent points in the chromatogram and assumes discrete k values ranging from 0 to (M – 1). $EACVF_{tot}$ is reported *versus* the inter-distance Δt to obtain an $EACVF_{tot}$ plot, *i.e.*, Fig. 1b (solid line) shows the $EACVF_{tot}$ plot computed on the chromatogram of Fig. 1a.

Theoretical models have been developed to relate $EACVF_{tot}$ (Δt) to the chromatogram parameters so that the main information on the sample – number of components, m_{tot}, abundance distribution, separation performance, σ, and retention pattern – can be extracted from the experimental signal [21].

The most general case is a multicomponent chromatogram containing m_{tot} single components (SCs) displaying a Poissonian retention pattern, *i.e.*, a completely disordered separation where SC positions are uniform randomly distributed over the retention axis (as the chromatogram reported in Fig. 1a). In this case the $EACVF_{tot}$ value computed at the origin (Δt = 0) is given by [19]:

$$EACVF_{tot}(0) = \frac{A_T^2\left(\sigma_M^2/a_M^2 + 1\right)}{4\pi m_{tot}\sigma X} \qquad (2)$$

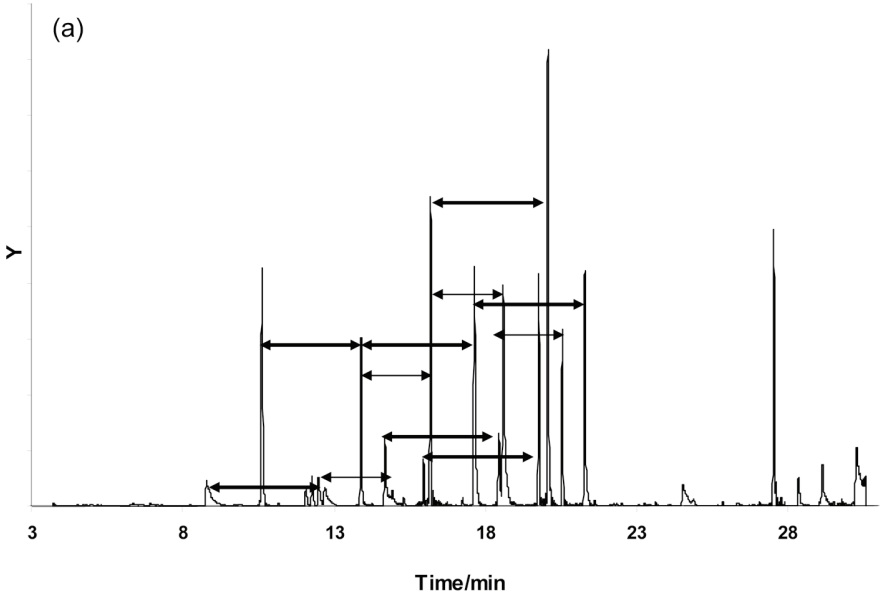
where A_T is the total area of the chromatogram, X the total chromatogram time range, σ the mean peak standard deviation (SD). The parameter σ_M^2/a_M^2 is the peak maximum dispersion ratio derived from the mean, a_M, and the variance, σ_M^2, of peak maxima computed from the observed peak maxima in the chromatogram: it describes the relative abundance distribution of the m_{tot} components present in the mixture. Equation 2 shows that the $EACVF_{tot}$ is characterized by a Gaussian peak at the origin Δt = 0 and that the shape of this peak represents the average shape computed over all the SC peaks present in the mixture (solid line in the enlarged inset in Fig. 1b). From the half height width, $d_{1/2}$, of this $EACVF_{tot}$ peak, it is possible to simply estimate the mean separation performance, expressed by the mean peak SD, σ (see solid line in inset in Fig. 1b):

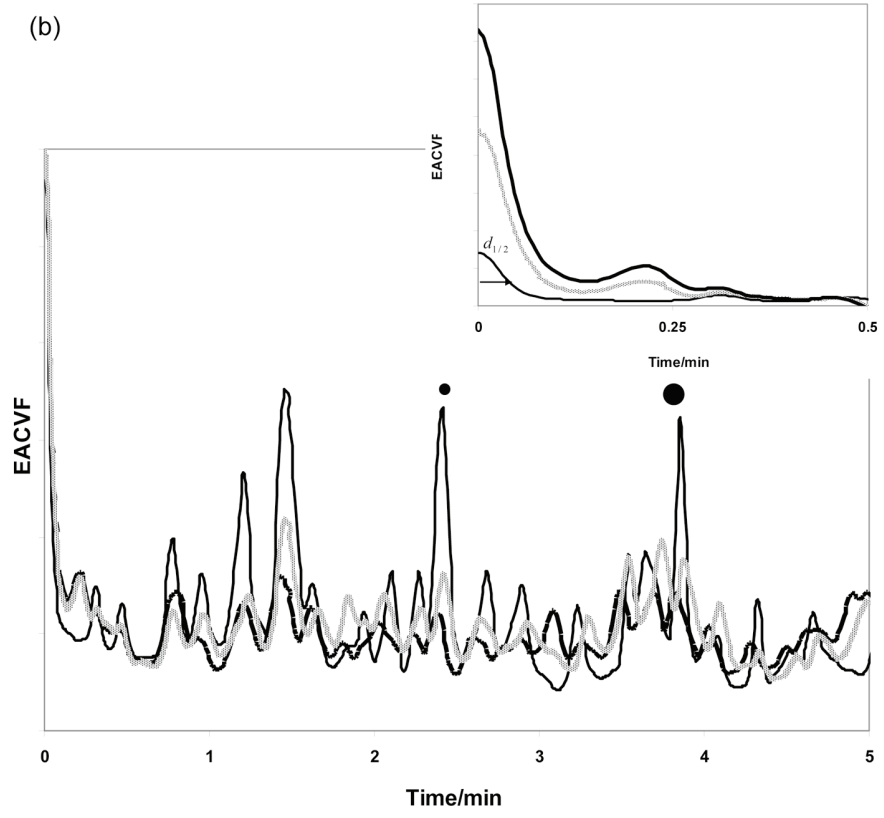
$$\sigma = \frac{d_{1/2}}{1.665} \qquad (3)$$

Information on the separation pattern is contained in the second part of the $EACVF_{tot}$ plot: if some Δt inter-distances are repeated in the chromatogram (arrows in the chromatogram in Fig. 1a), the $EACVF_{tot}$ plot computed on it shows well-defined peaks at the corresponding Δt values (solid line in Fig.1b, peaks signed by points) [21]. The

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

$EACVF_{tot}$ peak height is related to the abundance of the repetitiveness in the chromatogram, *i.e.*, the combination of the number of repeated peaks and their heights. Therefore, the $EACVF_{tot}$ plot can be considered a simplified fingerprint of the complex signal still retaining information on the quantitative chemical composition of the mixture. This property is due to two concomitant abilities of $EACVF_{tot}$: it cancels the effect of the randomness of SC peak positions while it amplifies the recursivity of the repeated inter-distances [21].





© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

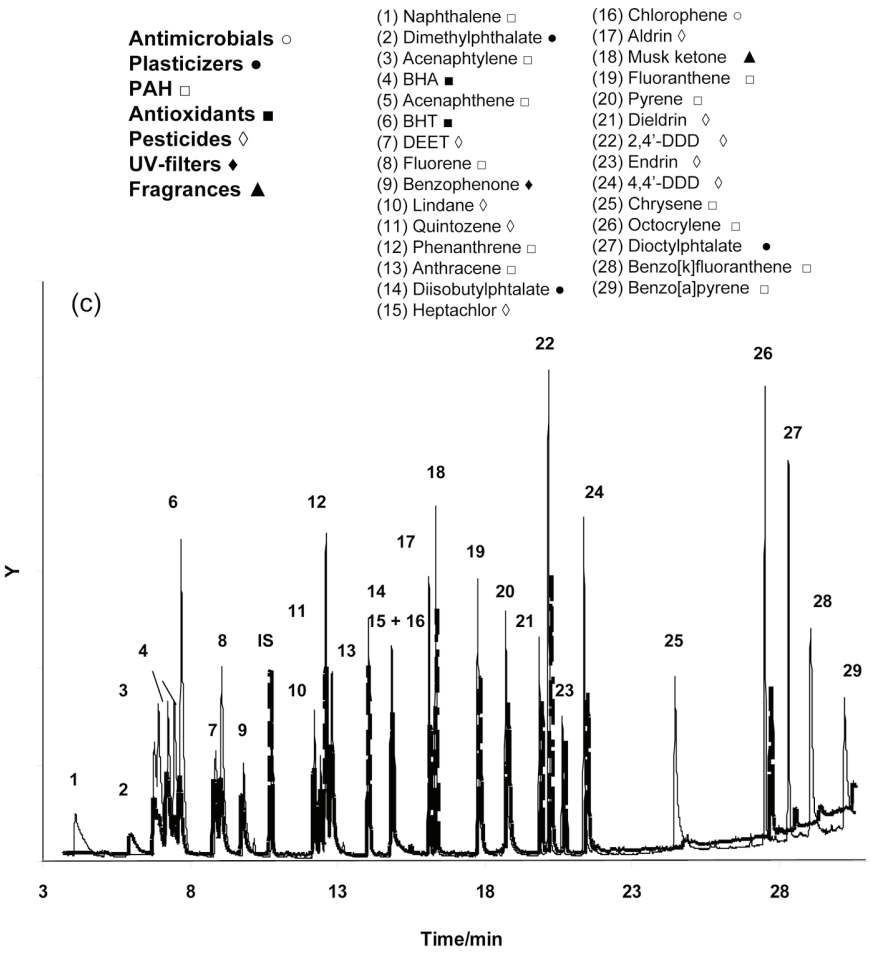


Figure 1. SPE method on Strata-X eluting with EA as solvent: (a) GC-MS TIC chromatogram of a water sample (nearly 200 mL of water mixture containing 7.5 ng of each PCPs) eluted from Strata-X using 10 mL of EA; the solid and bold arrows indicate the inter-distances between the most abundant peaks; (b) $EACVF_{tot}$ plots computed on the GC-MS signals. Solid line: $EACVF_{tot}$ computed on the sample eluted with 10 mL of EA (Fig. 1a): the black points indicate the most abundant inter-distances (arrows in Fig.1a); bold line: $EACVF_{tot}$ computed on the reference mixture (Fig 1c); shadow bold line: $EACVF_{tot}$ computed on the sample eluted with 15 mL of EA; enlarged detail: first part of the $EACVF_{tot}$ plots; (c) GC-MS TIC chromatogram of MeOH reference mixture of 29 target PCPs (7.5 ppm for each PCPs); dotted bold line: GC-MS TIC chromatogram of a water sample (nearly 200 mL of water mixture containing 7.5 ng of each PCPs) eluted from Strata-X using 15 mL of EA.

2.1 Application to evaluate SPE strategy

In the present application, $EACVF_{tot}$ is computed on the GC-MS signals to evaluate different steps of the SPE procedure in order to assist the development of the analytical method. The $EACVF_{tot}$ computed on each chromatogram makes it possible:

i. Estimation of the chromatographic properties, *i.e.*, X, σ, σ_M^2/a_M^2 and m_{tot} (Eq. 2). Using the total area of the chromatogram, A_T – computed from the digitized chromatogram, and the m_{tot} value, estimated from the $EACVF_{tot}$ – it is possible to calculate the mean area, A_m, defined as:

$$A_m = {}^{A_t}/_{m_{tot}} \quad (4)$$

Both the parameters A_T and A_m can be used to quantitatively calculate the content of the target PCPs in the sample: the total area A_T expresses the total amount of compounds present in the mixture, while A_m represents the mean amount of each compound present in the mixture [20].

ii. Evaluation of the peak abundance distribution, since the second part of the $EACVF_{tot}$ plot can be regarded as a fingerprint of the complex mixture retaining information on the chemical composition of the sample. By investigating the $EACVF_{tot}$ plot computed on sample signal in comparison to that of the reference standard solution it is possible to evaluate the qualitatitive and quantitative variation in sample

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 1. List of the 29 studied target compounds belonging to different PCP classes with their physico-chemical properties: molecular weight (MW), boiling point (b.p., °C); octanol–water partition coefficient ($\log K_{ow}$)

Compounds	Compound class	MW	b.p. (°C)	$\log K_{ow}$	Ion mass (*m*/*z*)
Naphthalene	PAH	294	218	3.3	128
Dimethylphthalate	Plasticizer	194	284	1.6	163
Acenaphthylene	PAH	152	270	3.6	152
Butylated hydroxyanisole (BHA)	Anti-oxidant	180	264	5.1	137
Acenaphthene	PAH	154	279	3.9	153
Butylated hydroxytoluene (BHT)	Antioxidant	220	265	4.2	205
Diethyltoluamide (DEET)	Pesticide	191	290	–	119
Fluorene	PAH	166	295	4.2	165
Benzophenone	UV-blocker	182	305	3.2	105
Lindane	Pesticide	110	323	3.5	181
Quintozene	Pesticide	202	328	–	214
Phenanthrene	PAH	361	336	4.5	178
Anthracene	PAH	178	340	4.5	178
Diisobutyl phthalate	Plasticizer	278	320	4.1	149
Chlorophene	Antiseptic	219	327	–	218
Heptachlor	Pesticide	373	140	6.7	100
Aldrin	Pesticide	365	145	6.4	263
Musk ketone	Fragrance	291	395	4.3	279
Fluoranthene	PAH	202	375	5.2	202
Pyrene	PAH	178	404	5.0	202
Dieldrin	Pesticide	381	385	6.2	79
2,4′-DDD	Pesticide	320	–	5.9	235
Endrin	Pesticide	382	–	4.9	243
4,4′-DDD	Pesticide	320	350	6.0	235
Chrysene	PAH	228	448	5.9	228
Octocrylene (Parsol 340)	UV-blocker	128	218	6.9	232
Dioctylphtalate	Plasticizer	390	222	7.9	149
Benzo[k]fluoranthene	PAH	252	480	6.8	252
Benzo[a]pyrene	PAH	252	311	6.3	252

The reported *m*/*z* values are the characteristic fragments selected for SIM detection of each PCP.

composition related to specific experimental conditions of the SPE procedure.

3 Materials and methods

3.1 Chemicals and standards

Twenty-nine PCP target compounds were selected to represent different chemical classes: the choice was based on the widest occurrence in wastewaters and representatively large range of physico-chemical properties affecting their environmental impact, *i.e.*, water solubility, octanol–water partitioning coefficient, and volatility. Table 1 reports a list of the studied compounds with some physico-chemical properties, *i.e.*, molecular weight, boiling point, octanol–water partition coefficient.

The standard PCPs were dissolved in pure methanol (MeOH) at concentration levels of 100 and 1000 ppm, then they were properly diluted in water to obtain a sample (nearly 200 mL) containing a standard quantity of 7.5 μg for each compound.

A MeOH sample containing a concentration level of 7.5 ppm was also prepared and used as standard reference mixture.

All standards were obtained from Lab Service Analytical S.R.l (Bologna, Italy), Carlo Erba Reagenti (Milano, Italy), VWR International S.R.l (Pennsylvania, USA) and Sigma–Aldrich (Steinheim, Germany). All standards and reagents used were of the highest purity commercially available. All solvents were trace analysis grade from 99.7%.

3.2 SPE

The investigated SPE cartridges were: C_{18} cartridge, 3 mL, 500 mg (Restek U.S., Bellefonte, PA, USA) and Strata-X SPE cartridge, 3 mL, 500 mg (Phenomenex, Torrance, CA, USA).

The SPE cartridges were placed in a 12-port Visiprep SPE Vacuum Manifold Disposable Liner (Supelco, Bellefonte, PA, USA) and conditioned by passing 5 mL of eluent, 5 mL of MeOH, and finally 5 mL of Milli-Q water. Then the sample (nearly 200 mL of the standard solution containing 7.5 μg for each target PCP) was siphoned through the cartridge adjusting the vacuum at a pressure of between −10 and −50 KPa. The solid phase in the cartridge was not allowed to become dry at any time. After loading the sample into the SPE cartridge, it was

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

dried under vacuum for 5 min. Afterwards, the PCPs were eluted under vacuum conditions with a variable solvent volume, using from 10 to 30 portions with a volume of 0.5 mL each. The eluate was then evaporated at different temperatures and the residue obtained was dissolved by adding an adequate volume of internal standard (5 μL of a 1 μg/μL solution of heptadecane in toluene). Finally, the appropriate volume of MeOH was added to bring it up to a final volume of 0.2 mL.

Analyses of a blank sample were performed together with every set of samples.

3.3 GC-MS

The GC-MS system consisted of a Focus GC, PolarisQ GC-MS^n Benchtop IT Mass Spectrometer (Thermo Fisher Scientific (Bellefonte, PA, USA). Helium was used as carrier gas (flow 1 mL/min). A fused-silica column RTX-5MS (DB5 30 m 0.25 mm ID, d_p 0.25 μm) was purchased from Thermo Fisher Scientific (Bellefonte, PA, USA). Injection was performed with a split/splitless injector at a temperature of 250°C. Splitless time was 1.5 min. Injection volume was 1 μL. The GC oven was programmed as follows: 1 min at 100°C, first ramp 15°C/min to 160°C, 2 min at 160°C, second ramp 5°C/min to 290°C, 3 min at 290°C. The total analysis time for one GC run was approximately 32 min. The GC-MS interface temperature and ion source was kept at 280 and 250°C, respectively. MS acquisition was performed in the positive electron impact mode at 70 eV under full-scan mode (40 – 400 m/z range) and single ion monitoring (SIM) mode (m/z values in Table 1). For each PCP a characteristic fragment was selected to be used in SIM detection to identify and quantify the compound.

3.4 Recovery estimation

The analyte quantization was performed using internal standard calibration: heptadecane at a constant quantity of 5 μg was added to each sample. The concentration of each PCP compound was estimated by relating its chromatographic peak area, A_i, to that of the IS, A_{IS}, to obtain the relative chromatographic peak area, A_i/A_{IS}. The recovery of each procedure step was computed by relating the chromatographic area of the extracted solution to the area of the original MeOH standard solution.

Each recovery experiment was repeated three times: it displayed a good degree of reproducibility, with percent RSD of less than 10% for all compounds, the reported values are the mean values computed on experimental data.

4 Results and discussion

The target PCPs were analyzed in the GC-MS system, from the full-scan spectra (40 – 400 m/z range) characteristic ion fragments were selected for each PCP to be used in SIM detection to identify and quantify the compounds (m/z values in Table 1).

The temperature programming conditions were properly optimized to achieve the separation of all the target compounds. Figure 1c reports the chromatogram of the MeOH standard mixture containing 7.5 ppm of each PCP (1 μL injected). The sole exception is the co-eluted pair chlorophene – heptachlor (peak 15 + 16 in Fig. 1c) which can be quantified by the SIM detection at m/z = 218 and 100, respectively. The 7.5 ppm concentration level was chosen for each compound to represent the trace level at which PCPs are usually present in wastewater.

Selecting an appropriate SPE procedure is a difficult task when simultaneous determination of several classes of compounds is required, the solution to the problem needs to be a compromise between the conditions providing the best recoveries for each class of compounds.

In addition to the universal RP C_{18} sorbent, a surface modified styrene divinylbenzene polymeric phase (Strata-X SPE cartridge) has been investigated since it is known to give better recovery of both polar and non-polar compounds and to have greater capacity than alkyl-bonded silicas. This ability is due to a mixed sorption mechanism based on both RP and polar functionalities [22, 23].

The main factors affecting extraction performance were investigated, evaporation procedure, elution flow rate, composition, and volume of the solvents used for washing and eluting the cartridge. The best extraction procedure was tailored to the final analysis of the 29 target PCPs representing different chemical classes.

4.1 The effect of evaporation procedure

In the early stages of method development, evaporation proved to be a critical step, particularly for the more volatile compounds. Previous studies showed that some volatile compounds could be lost in the evaporation step and thus result in very low (< 30%) recovery [8].

The effect of evaporation temperature on some volatile target PCPs was investigated in order to find the best operating conditions to reduce evaporation losses. A MeOH standard solution (1 mL of MeOH) containing nine PCPs (7.5 ppm) was evaporated to dryness under N_2 flow operating at room temperature (nearly 1 – 2 days) or under gentle heating at 40 and 75°C to save time (5 and 2 h, respectively). The performance of each evaporation step was estimated in terms of percentage recovery of the analytes (Fig. 2).

All the PCPs studied are nearly totally lost by heating at 75°C while the highest recoveries can be achieved by evaporating at room temperature, although this procedure requires a great deal of time. Therefore, gentle heating at 40°C appears to be the best condition as it offers a

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

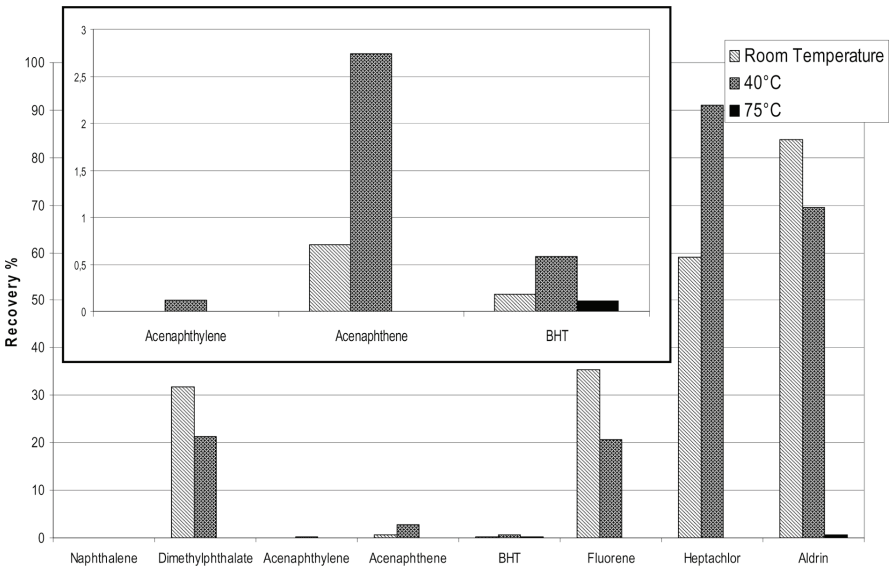


Figure 2. Recovery % values obtained for some target PCPs by evaporating at different temperatures: room temperature, heating at 40°C and 75°C. Enlarged detail: recovery % values for the most lost compounds, *i.e.*, acenaphthylene, acenaphthene, and BHT.

good compromise between recovery (obtaining values about 10% lower than those obtained at room temperature) and speed (some hours compared to days). The evaporation temperature of 40°C was chosen and applied in the following.

4.2 The effect of the elution rate

The performance of the SPE procedure can be significantly improved by using an SPE vacuum manifold where the elution flow rate can be carefully controlled by means of a positive pressure to enhance the reliability of the results obtained [8, 12]. The effect of elution flow rate on the analyte recovery was tested, two different flow rate conditions were investigated by controlling vacuum: a pressure of about −10 KPa for slow flow rate and −50 KPa for the high speed procedure. Such conditions were applied to water solutions containing 29 PCPs: nearly 200 mL of a water mixture containing 7.5 ng of each PCPs was charged on a Strata-X SPE cartridge and eluted with 15 mL of a solvent mixture (EA/DM 50:50).

Different combinations were applied to define the operating conditions yielding the highest recovery values, a vacuum condition of −50 KPa for both the sample loading and elution steps (fast in Table 2), vacuum values of −10 KPa for both the procedure steps (slow) and a mixed condition of vacuum value of −50 KPa for sample loading, and −10 KPa for the elution step (fast-slow in Table 2).

The obtained results (Table 2, 1st–3rd columns) show that slowing down the elution flow rate significantly increases the yield with this procedure. In fact, most analytes are poorly recovered when fast loading and elution steps were applied (mean value 25%, 1st column in Table 2) and greater quantitative recovery is achieved by applying slow sample loading conditions (mean value 33%, 2nd column) or slow loading and elution steps (mean value 56%, 3rd column). Therefore, the lower flow rate obtained in −10 KPa vacuum was selected as the best operative condition and applied in the following part of the study.

4.3 The effect of SPE elution solvent

4.3.1 C_{18} silica sorbent

To set up a SPE procedure suitable for all the studied PCPs, a C_{18} cartridge was first investigated as a universal sorbent whose retention mechanism is primarily governed by hydrophobic interactions [8, 12]. On the basis of the published data, different solvents displaying increasing elution strengths were selected for eluting the sample, pure MeOH, pure ethyl acetate (EA), and a combination of dichloromethane (DM/MeOH) with different compositions: 50:50, 30:70, and 70:30 v/v, respectively. Before loading the sample, the sorbent was washed and conditioned with the same solvent subsequently used for elution. The solvent volumes used for these steps were chosen in compliance with the literature and customer suggestions, a volume of 3 mL was used for washing, conditioning, and eluting the cartridge. The solvent performance was evaluated as recovery percentage value computed for each PCP from the GC-MS signal of the eluted sample (Table 3). Most of the studied PCPs were not eluted by the most polar solvent MeOH (Table 3, 1st column). Low recovery values were obtained for most PCPs eluting with EA (Table 3, 2nd column), while significantly better results were achieved by increasing the sol-

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 2. Recovery values (recovery % related to reference MeOH solution) obtained for each target PCP by eluting samples from Strata-X SPE cartridges.

	EA/DM fast	EA/DM fast-slow	EA/DM slow	EA/DM	EA	DM	TM	MeOH	ACN	EA/Ex	EA/ACN
Dimethyl phthalate	0	2.8	17	13	1.9	0.2	0.4	0.4	100	28	7.9
BHA	0.4	8.3	0.5	5.2	1.6	1.4	22	1.4	109	17	3.1
DEET	6.6	30	70	68	43	20	33	16	110	99	30
Fluorene	0	0.9	0.1	0.2	0	0	12	0	20	12	5.8
Benzophenone	0	6.2	0.1	3.0	1.9	0.7	26	1.2	105	50	29
Lindane	0.5	24	3.9	14	17	8.1	35	2.4	85	68	30
Quintozene	0	29	13	12	41	0	0	2.0	59	0	0
Phenanthrene	0	17	1.4	15	14	8.8	34	1.7	34	63	31
Anthracene	0.4	19	25	19	17	10	32	0.2	27	71	35
Diisobutyl phthalate	12	50	60	58	72	57	0	84	102	[a]	82
Heptachlor	0.1	16	1.7	14	16	14	34	2.5	65	71	42
Chlorophene	87	[a]	110	79	110	0	0	1.8	[a]	0	33
Aldrin	1.8	23	5.8	14	28	21	33	6.6	40	58	33
Musk ketone	50	110	110	73	[a]	63	71	64	110	[a]	72
Fluoranthene	12	57	92	69	86	60	42	0.9	42	85	48
Pyrene	21	61	108	70	101	68	44	1.4	39	84	52
Dieldrin	20	48	83	64	80	71	55	59	70	110	60
2,4′-DDD	71	61	110	65	110	64	43	55	62	78	52
Endrin	85	110	[a]	108	[a]	110	110	102	101	[a]	110
4,4′-DDD	91	59	110	55	102	66	50	51	48	92	51
Chrysene	30	9.8	89	25	29	78	43	3.0	4.3	93	50
Octocrylene	46	25	110	20	94	31	28	33	42	53	25
Dioctyl phthalate	4.8	6.4	10	12	14	8.8	11	3.2	7.5	11	4.1
Benzo(k)fluoranthene	39	10	110	27	35	50	36	2.5	2.6	68	32
Benzo(a)pyrene	45	21	110	41	69	49	35	2.2	7.1	58	29
Mean values	25	33	56	38	47	34	33	20	65	58	38

Columns 1st – 3rd: effect of different flow rate conditions. Eluent: 15 mL of the EA/DM (50:50) mixture. Fast: vacuum condition of – 50 KPa for both the sample loading and elution steps; fast – slow: vacuum value of –50 KPa for sample loading and –10 KPa for the elution step; slow: vacuum value of –10 KPa for both the sample loading and elution steps. Columns 4th – 11th: effect of different solvents under vacuum value of –10 KPa (slow). Eluent: 10 mL of EA/DM 50:50, EA, DM, TM, ACN, EA/*n*-hexane (Ex) 50:50, EA/ACN 50:50.

[a] Recovery values higher than 110% due to co-eluted interferences.

vent strength and eluting with DM/MeOH mixtures (Table 3, 3rd – 5th columns), the highest recovery values – close to 40% – were achieved for all the compounds with the mixture composition DM/MeOH 70:30 (Table 3, 5th column).

4.3.2 Strata-X sorbent

As an alternative to the conventional C_{18} sorbent, the Strata-X SPE cartridge was investigated as it offers unique selectivity for a wide spectrum of analytes. It has been found suitable to provide simultaneous extraction of polar (Log P < 3) and nonpolar (Log P > 3) analytes, since it displays multimode retention mechanisms involving hydrophilic, hydrophobic as well as H-bonding, and π-π retention interactions [8, 22 – 25].

Different elution solvents were tested to search for the most suitable conditions for analyzing different PCPs with different polarities: starting from the solvents displaying the highest (EA, DM, and trichloromethane (TM)) and the lowest (MeOH) elution strength, solvents with intermediate elution strength (ACN) and mixtures thereof (EA/DM, EA/Ex; EA/ACN 50:50) were investigated. In this preliminary step, a volume of 10 mL was selected for elution, in compliance with the literature and customer suggestions. The recovery values obtained for each PCP with different solvents are reported in Table 2 (4th – 11th columns).

Acceptable results (on average recoveries higher than 30% for most PCPs) can be obtained by all the investigated solvents, with the exception of MeOH (Table 2, 8th column). EA, ACN, and a solvent mixtures containing EA proved to be the most effective solvents since they yield recovery values close to 60% for most of the target PCPs (Table 2, 4th, 5th, 9th – 11th columns).

A comparison of the recovery values obtained with Strata-X and C_{18} showed that the former was clearly superior due its ability to adsorb polar and apolar moieties present in the PCP molecules [8, 22, 24].

Although acceptable results were obtained with the developed procedure, they are quite far from the ideal recoveries (higher than 80%). Therefore, to improve the SPE method the solvent volume used for elution was

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 3. Recovery values (recovery % related to reference MeOH solution) obtained for each target PCP by eluting water samples from C_{18} SPE cartridges.

	MeOH	EA	DM/MeOH 50:50 v/v	DM/MeOH 30:70 v/v	DM/MeOH 70:30 v/v
Dimethyl phthalate	0.3	4.4	0	4.4	6.5
BHA	0	0.2	3.2	0.7	0.4
DEET	1.9	40	19	49	47
Fluorene	0	1.5	0	1.5	2.5
Benzophenone	0.1	17	1.7	15	21
Lindane	0	48	20	49	56
Quintozene	0	14	4.9	14	25
Phenanthrene	0.2	14	6.2	19	30
Anthracene	0	17	4.7	14	24
Diisobutyl phthalate	0.2	57	38	69	78
Heptachlor	0	12	6.9	18	33
Chlorophene	0	0	0	0	0
Aldrin	0	16	8.8	14	21
Musk ketone	1.1	45	40	5	59
Fluoranthene	1.1	26	40	42	56
Pyrene	2.1	27	44	44	60
Dieldrin	1.2	0	49	47	64
2,4′-DDD	5.1	25	40	46	48
Endrin	0.9	19	58	46	69
4,4′-DDD	12	24	41	49	48
Chrysene	18	23	40	41	34
Octocrylene	26	19	36	44	37
Dioctyl phthalate	25	19	36	41	32
Benzo(k)fluoranthene	20	21	32	29	20
Benzo(a)pyrene	16	11	33	28	21
Mean values	5.2	20	24	31	36

Eluent: 10 mL of different solvents: MeOH, EA, DM/MeOH mixtures with different compositions: 50:50, 30:70, 70:30.

changed as another important parameter strongly affecting the elution recovery.

4.4 The effect of elution volume

The preliminary results (Tables 2–3) were obtained by eluting samples with the conventional solvent volumes of 3 mL for C_{18} cartridge and 10 mL for Strata-X, as reported in the literature and in compliance with customer suggestions [8, 11]. For the Strata-X cartridge, the effect of the eluent volume was evaluated for the solvents: EA, ACN and the EA/DM (50:50) mixture. For each solvent, three different elution volumes were investigated: 5, 10 and 15 mL. The results obtained show specific behavior for the three eluents (Table 4). With EA and the EA/DM mixture the elution volume must be increased to 15 mL to reach satisfactory recovery values (most higher than 60%, Table 4, 3rd, 6th columns), since changing the volume from 5 to 10 mL only yields a slight improvement. On the other hand, with ACN an elution volume of 10 mL is enough to reach recovery values higher than 60%, levels, which remained constant even after the volume was further increased to 15 mL (Table 4, 8th–9th columns).

From the reported results it is evident that a volume of 15 mL is the best choice since it ensures a satisfactory recovery (on average 60%) independently of solvent composition.

4.5 The chemometric approach

The usefulness of the chemometric approach based on the $EACVF_{tot}$ in assisting the method optimization was tested by applying the method to the GC-MS signals obtained from the samples eluted on Strata-X cartridges under different operative conditions [19–21].

4.5.1 Estimation of recovery %

The main chromatographic properties – σ_M^2/a_M^2, σ and m_{tot} – were estimated for the GC-MS signals of the samples (Table 5, 1st–11th row) and of the reference standard solution (Table 5, 12th row). The total area of the chromatogram, A_T, was directly computed from the digitized chromatogram and from it the mean area, A_m, was estimated using m_{tot} (Eq.4): both the parameters were used to quantitatively calculate the content of the target PCPs in the sample. To make different chromatograms comparable, the A_T values of all the chromatograms studied are nor-

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 4. Recovery values (recovery % related to MeOH standard solution) obtained for each target PCP by eluting water samples from Strata-X SPE cartridges.

	5 mL EA	10 mL EA	15 mL EA	5 mL EA/DM	10 mL EA/DM	15 mL EA/DM	5 mL ACN	10 mL ACN	15 mL ACN
Dimethyl phthalate	19	1.9	81	0.9	13	17	3.6	100	74
BHA	0.8	1.6	90	0.3	5.2	0.5	0.3	109	91
DEET	61	43	110	49	68	70	38	110	110
Fluorene	0	0	61	0	0.2	0.1	0	20	23
Benzophenone	0.1	1.9	95	0	3.0	0.1	0	105	91
Lindane	1.0	17	91	2.8	14	3.9	0	85	96
Quintozene	1.9	41	110	8.3	12	13	0	59	62
Phenanthrene	0.4	14	84	1.0	15	1.4	0	34	40
Anthracene	1.6	17	71	4.3	19	25	0	27	31
Diisobutyl phthalate	16	72	98	51	58	60	14	102	88
Heptachlor	0.9	16	107	2.7	14	1.7	0.4	65	60
Chlorophene	106	110	[a]	110	79	110	21	[a]	[a]
Aldrin	1.1	28	64	1.9	14	5.8	1.4	40	37
Musk ketone	36	[a]	106	70	73	110	45	110	97
Fluoranthene	22	86	91	63	69	92	2.0	42	43
Pyrene	28	101	90	70	70	108	1.7	39	43
Dieldrin	31	81	100	75	64	83	26	70	67
2,4′-DDD	49	110	87	79	65	110	41	62	55
Endrin	62	[a]	110	106	108	[a]	43	101	93
4,4′-DDD	44	102	81	81	56	110	35	48	58
Chrysene	85	29	16	20	25	89	0	4.3	11
Octocrylene	56	94	58	40	20	110	49	42	44
Dioctyl phthalate	43	14	13	9.5	12	10	9.7	7.5	7.9
Benzo[k]fluoranthene	89	35	17	23	27	110	0	2.6	9.5
Benzo[a]pyrene	73	70	38	44	41	110	0	7.1	13
Mean values	33	47	78	37	38	56	13	58	56

Different volumes (5, 10, 15 mL) of different solvents: EA, ethyl acetate; EA/DM mixture 50:50, ACN.

[a] Recovery values higher than 110% due to co-eluted interferences.

Table 5. Results obtained from the EACVF$_{tot}$ procedure applied to the GC-MS chromatograms of water samples submitted to the SPE procedure on Strata-X SPE under different operative conditions.

Sample	σ_M^2/a_M^2	σ(s)	m_{tot}	Recovery % on A_{tot}	Recovery % on A_m
15 mL EA/DM 50:50 fast	0.22	1.2	30 ± 5	27%	26%
15 mL EA/DM 50:50 fast-slow	0.23	1.3	29 ± 5	40%	40%
15 mL EA/DM 50:50 slow	0.21	1.2	31 ± 6	62%	58%
5 mL EA/DM 50:50 slow	0.22	1.2	29 ± 5	37%	37%
10 mL EA/DM 50:50 slow	0.20	1.2	30 ± 5	39%	38%
5 mL EA slow	0.22	1.2	28 ± 5	32%	33%
10 mL EA slow	0.77	1.3	31 ± 5	87%	81%
15 mL EA slow	0.23	1.3	30 ± 5	82%	79%
5 mL ACN slow	0.22	1.2	28 ± 5	14%	14%
10 ml ACN slow	0.21	1.2	30 ± 5	67%	65%
15 mL ACN slow	0.23	1.2	30 ± 5	66%	64%
Reference standard solution	0.23	1.3	29 ± 5	–	–

malized by referring them to the peak area of the internal standard (5 μg of heptadecane).

The percentage variation of A_T and A_m among samples eluted on SPE and the reference standard solution indicates the percentage recovery during the procedure step. Table 5, reports the % recovery values based on A_T and A_m values (4th and 5th columns, respectively). In this manner, each step of the SPE procedure can be evaluated by controlling the variation of m_{tot} and the mean loss in target PCP concentration. The % recovery results are compared with the mean data computed on each separated PCP for the different operative conditions (Tables 2–4, last row). Some data calculated from A_T appeared erroneously over-estimated (Table 5, 1st, 3rd, 5th, 7th, 8th, 10th, 11th row): they correspond to the eluted samples containing some interfering, cartridge-released com-

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

pounds, since in these cases the estimated number of components (m_{tot}, Table 5, 3rd column) is higher than the real number $m_{tot} = 29$. These interferring compounds yield a significant increase in the total area, A_T, with the consequent over-estimation in the recovery values. On the other hand, the A_m values are a more correct estimation of the total PCP content since they are not so severely affected by interference: higher A_T values are compensated for by higher m_{tot}. Therefore, the A_m values can be used for a correct evaluation of % recovery values.

This result proves the reliability of the $EACVF_{tot}$ method as a rapid, simple, and precise method for estimating the recovery values directly from the whole chromatogram.

4.5.2 Evaluation of the elution pattern

Moreover, the $EACVF_{tot}$ method can be used to extract information on peak abundance distribution and relate it to the specific chemical composition of the analyzed sample. In fact, the $EACVF_{tot}$ plot may be considered a fingerprint of the complex chromatogram since it represents a simplified behavior still retaining information on the retention pattern [21]. This ability was tested in evaluating the performance of the SPE procedure steps.

An example is the sample eluted from Strata-X with 10 mL of EA (GC-MS signal in Fig. 1a) in comparison with the reference MeOH mixture (chromatogram in Fig. 1c, solid line). The first signal shows some high peaks (peaks 14, 15, 18, 19, 20, 22, 24) indicating that such PCPs (diisobutyl phthalate, chlorophene, musk ketone, fluoranthene, pyrene, 2,4′-DDD, and 4,4′-DDD) are selectively eluted from the cartridge with higher recovery than that found for the other PCPs, as confirmed by the higher recovery % values computed for these compounds (Table 4, 2nd column).

This selective peak abundance distribution can be singled out by a simple visual inspection of the $EACVF_{tot}$ plot computed on the GC-MS signal (solid line in Fig. 1b). It clearly shows a specific pattern characterized by high peaks located at $\Delta t = 2.4$ min and $\Delta t = 3.8$ min (small and large points in Fig. 1b) which correspond to the inter-distance values between the most abundant peaks (solid and bold arrows in Fig. 1a). Such a selective pattern can be simply identified by comparison with the $EACVF_{tot}$ plot computed on the reference mixture (bold line in Fig. 1b). This selectivity in eluting the target PCPs disappears if elution is performed with 15 mL of EA (chromatogram in Fig. 1c, shadow bold line), since this higher volume yields a recovery close to 80% for most PCPs (Table 4, 3rd column). Accordingly, the $EACVF_{tot}$ plot computed on the corresponding GC-MS signal displays a pattern very close to that of the MeOH standard solution with no specific selectivity effects (shadow bold line compared to bold line in Fig. 1b).

These results strongly show that the $EACVF_{tot}$ plot is quite suitable to simply identify different peak abundance distributions and can be proposed as a data handling tool to assist optimization of an analytical procedure.

4.5.3 Comparison of SPE procedures

To summarize all the information acquired in the present study, $EACVF_{tot}$ was computed on CG signals obtained under different operative conditions: they refer to elution on Strata-X using 15 mL of EA, ACN and ACN/DM mixture. The obtained $EACVF_{tot}$ plots were compared (Fig. 3, where the reference MeOH solution is also reported): a simple visual inspection of the plots makes it possible to extract two main pieces of information relevant for evaluating and optimizing the analytical method:

(i) The $EACVF_{tot}$ plots of all the studied mixtures nearly overlap: this means that none of the investigated conditions displays a selective retention or elution versus any particular PCPs;

(ii) The $EACVF_{tot}$ of each signal can be used to estimate the properties of each separation, in particular A_T and A_m to compute recovery % values (enlarged detail in Fig. 3).

The obtained results are fully confirmed by the data obtained from the computation on each PCP signal (Tables 2–4). This further confirms that the $EACVF_{tot}$ approach can be suggested as a PC-based signal processing method to assist the optimization of the analytical strategy by reducing the labor and time requirements and the subjectivity introduced by human intervention.

4.6 Analytical parameters

The results obtained makes it possible to conclude that the best operative conditions consist of an evaporation temperature of 40°C, elution on Strata-X cartridge using a volume of 15 mL of EA as solvent and operating with slow flow rate (–10 kPa).

Experiments were conducted under these selected conditions in order to investigate the analytical performance and the applicability of the method.

The LOD for each compound was calculated as three times the SD of the background noise of the extracted ion chromatogram baseline from a solution of standards. The LOD instrumental limits, expressed as picograms injected, ranged from 5 to 10 pg. If we extrapolate these values and consider the analytical procedure (200 mL water extracted and final extract re-concentrated to 200 μL) the method LOD ranged from 5 to 10 ng/L, respectively. These values at ppt level are very similar to the values obtained in other works concerning each class of compounds [6–18].

The procedure was applied for the determination of the selected PCPs in real water samples: three samples

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

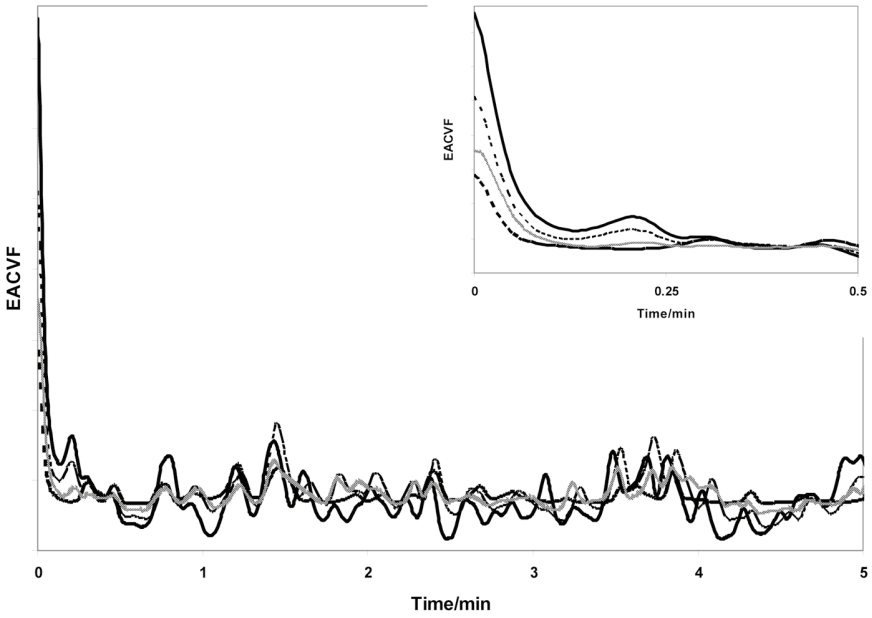


Figure 3. $EACVF_{tot}$ plots for comparing different solvents used to elute water sample (nearly 200 mL of water mixture containing 7.5 ng of each PCPs) from Strata-X. Enlarged detail: first part of the $EACVF_{tot}$ plots. Bold line: $EACVF_{tot}$ computed on the GC-MS signal of the reference mixture (MeOH mixture containing 7.5 ppm of each PCPs, Fig. 1c, solid line); dotted line: $EACVF_{tot}$ computed on the GC-MS signal of the sample eluted with 15 mL of ACN; shadow bold line: $EACVF_{tot}$ computed on the GC-MS signal of the sample eluted with 15 mL of EA (Fig. 1c, dotted bold line); dotted bold line: $EACVF_{tot}$ computed on the GC-MS signal of the sample eluted with 15 mL of EA/DM mixture.

Table 6. Validation data for the method under the best operative conditions.

Analyte	Compound class	LOD (pg)	Recovery %		Precision RSD %, $n = 3$	
			50 ng/L	100 ng/L	50 ng/L	100 ng/L
BHA	Antioxidant	5	91	91	8.5	4.8
Benzophenone	UV-blocker	10	96	94	9.8	5.0
Lindane	Pesticide	7	91	91	9.3	5.1
Phenanthrene	PAH	5	78	78	8.2	4.8
Aldrin	Pesticide	5	66	70	9.4	5.3
Fluoranthene	PAH	5	92	92	9.0	5.7
Pyrene	PAH	5	90	91	8.9	5.2
Dieldrin	Pesticide	5	98	99	8.2	4.8
4,4′-DDD	Pesticide	5	82	84	9.1	5.0
Octocrylene	UV-blocker	5	90	93	8.9	5.1

LOD (pg), precision (RSD%, $n = 3$), and accuracy (recovery %) of triplicate analysis of a real water sample fortified at a concentration level of 50 and 100 ng · L^{-1} with ten target PCPs.

were collected from effluent of an urban treatment plant (Ferrara municipality wastewater treatment plant (WWTP)) from industrial and domestic influents and analyzed in duplicate. The results obtained revealed that only some of the target PCPs (musk ketone, anthracene, 2,4′-DDD) can be detected, but at a concentration lower than method LOQ.

To check the precision and accuracy of the proposed method, the less contaminated water sample was spiked with ten target PCPs at two different concentration levels of 50 to 200 ng/L for each analyte and analyzed in triplicates. Table 6 shows the LOD values, mean recoveries and RSDs. Recoveries varied from 66 to 99%, with RSD% values lower than 10% in all cases.

These data demonstrate that the PCPs studied can be simultaneously separated and determined from water samples by the proposed method with good accuracy and precision.

5 Concluding remarks

The results of this study confirm that the effect of the eluent properties, *i.e.*, chemical composition and elution volume, on the SPE performance is very complex and cannot be predicted in detail when a wide range of compounds are to be analyzed. Therefore, in this case an experimental investigation is needed to optimize the operative parameters. This search is particularly challenging for multi-residue methods, such as the present case of a large variety of PPCPs, since they require the joint extraction and determination of many compounds displaying a broad range of polarities.

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Under the selected conditions the procedure achieves a recovery higher than 70% for most PCPs investigated, this result makes the developed method suitable for comprehensive chemical profiling of PCPs in various aqueous matrices. It is clear that higher recovery values can be achieved only for selected PCPs using specific target-compound methods.

The developed method may be the basis of wastewater monitoring for temporal and spatial changes of both target and nontarget compounds. It will be also applied to monitoring the fate of PCPs in the WWTP effluents to evaluate the efficiency of innovative treatments to remove PCPs, *i.e.*, in constructed wetlands using natural processes involving wetland vegetation, filling media, and their associated microbial colonies.

The use of a PC-based signal processing proved very helpful in aiding optimization of the analytical strategy. It saves labor and time and reduces the subjectivity introduced by human intervention. Moreover, the method seems to offer a promising tool for high-throughput processing of the large datasets generated by chemical monitoring in environmental water analysis.

This work was supported by the University of Ferrara and by the L.A.R.A., Land Network Laboratory for Waters of Emilia Romagna Region.

The authors declared no conflict of interest.

6 References

[1] Daughton, C. G., Jones-Lepp, T. L., Pharmaceuticals and personal care products (PPCPs) in the environment. Scientific and Regulatory Issues, ACS Symp. Ser. 791, Oxford University Press, Washington, USA 2001.

[2] Daughton, C. G., *Environ. Impact Assess. Rev.* 2004, *24*, 711 – 732.

[3] Ellis, J. B., *Environ. Pollut.* 2006, *144*, 184 – 189.

[4] Lishman, L., Smyth, S. A., Sarafin, K., Kleywegt, S., Toito, J., Peart, T., Lee, B., Servos, M., Beland, M., Seto, P., *Sci. Total Environ.* 2006, *367*, 544 – 558.

[5] Matamoros, V., Bayona, J. M., *Environ. Sci. Technol.* 2006, *40*, 5811 – 5816.

[6] Soliman, M. A., Pedersen, J. A., Park, H., Castaneda-Jimenez, A., Stenstrom, M. K., Suffet, I. H., *Water Environ. Res.* 2007, *79*, 156 – 167.

[7] Richardson, S. D., *Anal. Chem.* 2006, *78*, 4021 – 4045.

[8] Peck, A. M., *Anal. Bioanal. Chem.* 2006, *386*, 907 – 939.

[9] Barcelo, D., Petrovic, M., *Anal. Bioanal. Chem.* 2007, *387*, 1141 – 1142.

[10] Kot-Wasik, A., Debska, J., Namiesnik, J., *TrAC Trends Anal. Chem.* 2007, *26*, 557 – 576.

[11] Pietrogrande, M. C., Basaglia, G., *TrAC Trends Anal. Chem.* 2007, *26*, 1086 – 1103.

[12] Pichon, V., *J. Chromatogr., A* 2000, *885*, 195 – 215.

[13] Hyotylainen, T., Riekkola, M. L., *TrAC Trends Anal. Chem.* 2007, *26*, 557 – 576.

[14] Hyotylainen, T., Riekkola, M. L., *Anal. Chim. Acta* 2008, *614*, 27 – 37.

[15] Trenholm, R. A., Vanderford, B. J., Holady, J. C., Rexing, D. J., Snyder, S. A., *Chemosphere* 2006, *65*, 1990 – 1998.

[16] Goncalves, C., Alpendurada, M. F., *J. Chromatogr., A* 2002, *968*, 177 – 190.

[17] Hao, C., Zhao, X., Yang, P., *TrAC Trends Anal. Chem.* 2007, *26*, 569 – 580.

[18] Hao, C., Zhao, X., Tabe, S., Yang, P., *Environ. Sci. Technol.* 2008, *42*, 4068 – 4075.

[19] Pietrogrande, M. C., Zampolli, M. G., Dondi, F., Szopa, C., Sternberg, R., Buch, A., *J. Chromatogr., A* 2005, *1071*, 255 – 261.

[20] Pietrogrande, M. C., Ghedini, D., Velada, G., Dondi, F., *Analyst* 1998, *123*, 1199 – 1204.

[21] Pietrogrande, M. C., Zampolli, M. G., Dondi, F., *Anal. Chem.* 2006, *78*, 2579 – 2592.

[22] Huq, S., Dixon, A., Kelly, K., Kallury, K. M. R., *J. Chromatogr., A* 2005, *1073*, 355 – 361.

[23] Biesaga, M., Jankowska, A., Pyrzynska, K., *Microchim. Acta* 2005, *150*, 317 – 322.

[24] D'Archivio, A., Fanelli, M., Mazzeo, P., Ruggieri, F., *Talanta* 2007, *71*, 25 – 30.

[25] Michalkiewicz, A., Biesaga, M., Pyrzynska, K., *J. Chromatogr., A* 2008, *1187*, 18 – 24.

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Paper 3

M.C. Pietrogrande, G. Basaglia, “Enantiomeric resolution of biomarkers in space analysis: Chemical derivatization and signal processing for gas chromatography-mass spectrometry analysis of chiral amino acids” – Journal of Chromatography A, Vol. 1217 (2010) 1126-1133

Journal of Chromatography A, 1217 (2010) 1126–1133

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Enantiomeric resolution of biomarkers in space analysis: Chemical derivatization and signal processing for gas chromatography–mass spectrometry analysis of chiral amino acids

M.C. Pietrogrande*, G. Basaglia

Department of Chemistry, University of Ferrara, Via L. Borsari, 46, I-44100 Ferrara, Italy

ARTICLE INFO

Article history:
Available online 26 September 2009

Keywords:
Amino acid enantiomeric resolution
Derivatization for GC–MS analysis
Alkyl chloroformate
Perfluorinated alcohols and anhydrides
Signal processing
In situ space analysis

ABSTRACT

The work compares two GC–MS methods for enantioselective separation of amino acids as suitable candidate for stereochemical analysis of chiral amino acids on board spacecrafts in space exploration missions of solar system body environments. Different derivatization reagents are used: a mixture of alkyl chloroformate–alcohol–pyridine to obtain the alkyl alkoxy carbonyl esters and a mixture of perfluorinated alcohols and anhydrides to form perfluoroacyl perfluoroalkyl esters. 20 proteinogenic amino acids were derivatized with the two procedures and submitted to GC–MS analysis on a Chirasil-L-Val stationary phase. The results were then compared in terms of the enantiomeric separation achieved and intensity of MS response. The combination of methyl chloroformate (MCF) and heptafluoro-1-butanol (HFB) allows separation of 14 enantiomeric pairs, five of which display a resolution ($R_s \geq 1.2$) supposed to be sufficient to quantify the enantiomeric excess. Three mixtures of trifluoroacetic (TFAA) and heptafluorobutyric (HFBA) anhydrides were combined with the corresponding perfluorinated alcohols – TFE (2,2,2-trifluoro-1-ethanol) and HFB (2,2,3,3,4,4,4-heptafluoro-1-butanol) – to give three different reagents (TFAA–TFE, TFAA–HFB, HFBA–HFB): the derivatives obtained show separation of the same number of proteinogenic amino acids (14 of 20) at a temperature lower than column bleeding limit (200 °C) and 8 of them give a separation with $R_s \geq 1.2$. Linearity study and limit of detection (X_{LOD}) computation show that both methods are suitable for quantitative determination of several amino acid diastereomers at trace level ($X_{LOD} \approx 0.5$ nmol as derivatized quantity). Both the procedures were coupled with automatic data handling to increase their suitability for space analysis: the simplified data treatment is especially helpful to handle the low quality data recovered from space experiments and labor and time are saved, as imposed by the space experiments requiring a rapid delivery of the results. To achieve this aim, a chemometric approach based on the computation of the Autocovariance Function (ACVF) was applied to extract information on the enantiomeric pairs present in the sample and the enantioseparation achieved on the chiral column.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

The detection of organic molecules, such as amino acids and sugars, in extraterrestrial environments is one of the most challenging goals for future space missions, since they can be biomarkers indicating life, both extant and extinct [1,2]. Furthermore, it is known that only one on the two enantiomeric structures of these molecules are used to build the biotic macromolecules (L for amino acids and D for sugars) whereas racemic mixtures (L and D in equal parts) are present in abiotic systems. Therefore, the search for homochirality and characterization of the enantiomeric excess in amino acids are of primary relevance as organic signatures of present or extinct life in space [2–5].

Particular efforts are being devoted to Mars because intense exploration, started in the 1990s, revealed that all the ingredients required for life to emerge (liquid water, organic molecules, and energy) should have gathered early in Martian history [6–11].

The analytical instruments suitable for space missions must meet the severe requirements imposed by flight conditions: automation, remote control operations, short analysis times and low energy consumption. Moreover, they must also provide the lowest possible detection limit since biomarker concentrations in extraterrestrial environments are not well known. Gas chromatography (GC) has proved to be the best analytical technique for in situ search for organic molecules in extraterrestrial environments [7–14], among the many conventional bench-top scale instrumentations available for chiral separation of amino acids—including

* Corresponding author. Tel.: +39 0532 455 152; fax: +30 0532 240 709.
E-mail address: mpc@unife.it (M.C. Pietrogrande).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.09.055

HPLC, GC and CE, also applicable to lab-on-a-chip system [15]. Recently, a chiral stationary phase formed by a mixed binary chiral selector has been developed for the simultaneous GC enantioseparation of racemic compounds of exobiologic interest to be used in future space experiments [16].

The GC analysis of low-volatile compounds, such as amino acids, requires a preliminary derivatization step to convert them into more stable, volatile compounds suitable for GC separation [16–18]. This is the basis for space instrument sub-systems developed for the in situ analysis of extraterrestrial atmosphere and soil: i.e., COSAC (COmetary Sampling And Composition experiment) of the Rosetta space mission [4,19] and SAM (Sample Analyses at Mars) on the 2009 Mars Science Laboratory (MSL) rover [8,10,20]. However, further search for suitable procedure for space application has to be developed, since the procedures applied do not allow enantiomer separation (MTBSTFA, used for the COSAC experiment) or make it possible with poor detection sensitivity (DMF-DMA used the SAM experiment). For this reason, other derivatization techniques were investigated as possible candidates for future space experiments to yield derivatives preserving the enantiomeric configuration of amino acid pairs and avoiding racemization phenomena in order to achieve identification and quantitation of an enantiomeric excess [16–18].

To meet these requirements for analyzing amino acids enantiomers in space, two derivatization procedures for enantiomeric separation of amino acids have been recently developed as simple, automatic GC methods that may be suitable candidates for in situ space analysis. One method is based on a derivatization reaction that employs an alkyl chloroformate–alcohol–pyridine mixture to obtain the N(O,S)-alkyl alkoxy carbonyl esters of amino acids [21]. The other is a one-step procedure that obtains the N(O,S)-perfluoroacyl perfluoroalkyl derivatives by using a mixture of perfluorinated anhydride and perfluoro alcohols to simultaneously perform esterification and acylation [22]. The separation was performed on a commercially available GC column coated with Chirasil-Val: its advantage is the availability of the stationary phase in the D- and L-forms, thereby making it possible to reverse the elution order of the enantiomers [23]. Moreover, this type of column is used in the COSAC experiment which currently flies to a comet because it was demonstrated to resist to the space constraints [4,19].

In this paper the derivatization reactions are investigated and compared on the basis of the following properties:

(1) enantiomeric resolution of the derivatives on the Chirasil-L-Val chiral stationary phase under energy saving conditions (short analysis time, low analysis temperature);
(2) analytical performance in terms of MS detectability, i.e., detection (X_{LOD}) and quantification limits (X_{LOQ}).

The possibility of automating data handling is also investigated as an helpful tool to increase the method suitability for high-throughput analysis of the data from space mission experiments: it facilitates the treatment of the low quality data recovered from in situ space analysis and saves labor and time in the data treatment, as imposed by the space experiments requiring a rapid delivery of the results. In addition to the conventional GC–MS data analysis, a chemometric approach was applied to handle complex signals and extract all the analytical information hidden therein, in particular those concerning the enantiomeric pair composition of the sample and the enantioseparation on the chiral column.

2. Signal processing procedure based on Autocovariance Function

In space research, the interpretation of complex chromatographic signals and the extensive amounts of data generated by hyphenated techniques is particularly helpful in decoding chromatograms recovered from space missions, as well as in designing analytical equipment for future space missions [24–32]. In particular, the chromatograms resulting from analytical procedures involving derivatization steps may be crowded with peaks since, besides the intrinsic complexity of the sample containing other interfering organics, artifacts can even result from sample chemical derivatization [31].

Among the many signal processing procedures developed for this problem, a chemometric approach based on the AutoCovariance Function (ACVF) of the chromatographic signal has been developed by the Authors and widely applied to experimental chromatograms [24–32]. The method has proved to be a powerful tool for interpreting chromatograms of complex mixtures, extracting accurate information on the mixture composition and the presence of classes of compounds with correlated structures.

The chemometric approach studies the Experimental Autocovariance Function (EACVF) that can be directly computed from the experimental chromatogram acquired, in digitized form, using the following expression [27]:

$$\mathrm{EACVF}(\Delta t) = \frac{1}{M}\sum_{j=1}^{N-k}(Y_j - \hat{Y})(Y_{j+k} - \hat{Y}) \quad k = 0, 1, 2, \ldots \mathrm{M} - 1 \qquad (1)$$

where Y_j is the digitized chromatogram signal, $\hat{Y}$ its mean value, M the truncation point in the EACVF computation. The correlation time Δt is the interdistance between the subsequent digitized positions, and assumes discrete values with k ranging from 0 to $(M-1)$:

$$\Delta t = k\tau \qquad (2)$$

where τ is the time interval between the subsequent digitized positions.

EACVF values can be plotted as a function of the time interdistance Δt to obtain the EACVF plot: as an example, the EACVF plot computed on the GC–MS signal of Fig. 1a is reported in Fig. 1b. The EACVF study makes it possible to characterize chromatographic signal complexity in terms of a set of statistical parameters describing both the sample complexity and the chromatographic separation. In particular, the following information can be obtained [27–32]:

1. *The mean peak standard deviation*, σ: The first region in the EACVF plot ($\Delta t \leq 4\sigma$) resembles half of a Gaussian peak showing a shape averaged over the shape of all the peaks present in the chromatogram: in the simplified approach, a constant width was assumed as this can be experimentally obtained under optimized programmed temperature conditions [27]. The mean peak standard deviation can be estimated from the width of the EACVF peak close the origin $\Delta t = 0$ using the simple equation (see Fig. 1b):

$$\sigma = d_{h/2}/1.665 \qquad (3)$$

where $d_{h/2}$ is the half-height width of the EACVF peak.

2. *The number of single components* (SC) present in the mixture, m_{tot}, can be estimated from the value of EACVF at the origin ($\Delta t = 0$) using the following equation (Fig. 1b):

$$m_{tot} = \frac{A_T^2(\sigma_h^2/a_h^2 + 1)}{\mathrm{EACVF}(0)d_{h/2}2.129\ X} \qquad (4)$$

where A_T^2 and X are the total area and the total time range of the chromatogram, respectively. The value σ_h^2/a_h^2 is the peak maximum dispersion ratio derived from the mean, a_h, and the

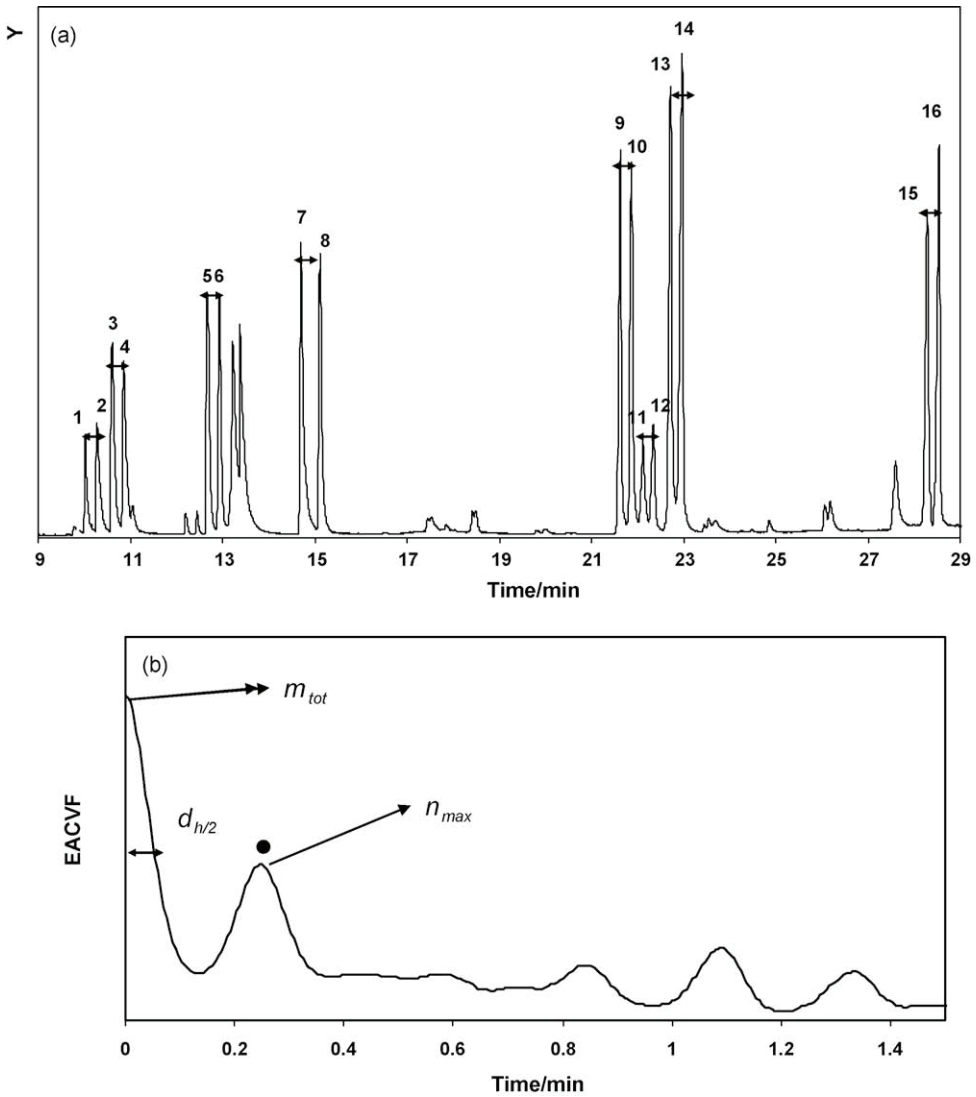


Fig. 1. Separation of TFAA–TFE derivatives. (a) GC–MS signal obtained under the optimized conditions: linear temperature increasing from 40 to 200 °C at 4.4 °C/min rate, followed by isothermal conditions. Arrows: constant interdistance $\Delta t = 0.25$ min between the enantiomeric pairs. D,L-Val [1,2], D,L-Ala [3,4], D,L-Ile [5,6], D,L-Leu [7,8], D,L-Met [9,10], D,L-Glu [11,12], D,L-Phe [13,14], D,L-Tyr [15,16]. (b) EACVF plot computed on the GC–MS signal.

variance, σ_h^2, of peak height computed from the separated peaks observed in the chromatogram [27].

3. *Information on the separation pattern*: The second part of the EACVF plot, for $\Delta t \geq 4\sigma$, shows a specific pattern dependent on the distribution of SC peak positions over the separation axis [28]. In particular, the EACVF method has proved efficient in identifying the presence of retention repetitivities inside the chromatogram, i.e., peaks located at constant interdistance values b repeated in the chromatogram (arrows in the chromatogram of Fig. 1a). Such order can be related to structural regularity in the molecular properties of the mixture components—i.e., a common molecular scaffold or constant structural modifications to yield constant interdistances $\Delta t = b$ in different regions of the chromatogram. In this case, the EACVF plot displays well-defined deterministic peaks located at interdistance b (first deterministic peak, signed by the point in Fig. 1b) and multiple values $\Delta t = bk$, if the interdistance is repeated k times in the chromatogram, as in the case of homologous series. The appearance of these peaks is diagnostic to identify the presence of ordered structures in the chromatogram, and their height, i.e., EACVF(bk) value computed at $\Delta t = bk$, is related to the abundance of the repetitiveness in the chromatogram, i.e., the combination of the number of repeated peaks and their heights. From the height of the first peak, the EACVF(b) value, the number of compounds $n_{\max}$ located at constant interdistance $\Delta t = b$ can be estimated according to the equation (see Fig. 1b) [28–30]:

$$\mathrm{EACVF}(b) = \frac{\sqrt{\pi}\sigma a_h^2 n_{\max}}{X}\left[\frac{\sigma_h^2}{a_h^2} + 1\right] \quad (5)$$

3. Experimental

3.1. Amino acids and reagents

Twenty proteinogenic amino acids were studied: Ala, Val, Pro, Ile, Leu, Asp, Thr, Asn, Met, Cys, Glu, Gln, Phe, His, Lys, Tyr, Ser, Arg, and Trp in their D- and L-enantiomeric forms plus glycine. They were purchased from Aldrich (Milan, Italy) and from Fluka (Milan, Italy). Stock standard solutions of the individual pairs of D- and L-amino acids were prepared in a concentration range from 1×10^{-2}

to 4×10^{-2} M using deionized water or 0.1 M HCl in some cases (Asp, Glu, Tyr, and Trp).

A standard solution of methyl laurate 5×10^{-3} M in acetonitrile was prepared as internal standard (IS). Methyl laurate (methyl dodecanoate, 97%) was purchased from Fluka (Milan, Italy).

The derivatization reagents were: 2,2,3,3,4,4,4-heptafluoro-1-butanol (HFB, 95%), methyl chloroformate (MCF, 97%), ethyl chloroformate (ECF), pyridine (Py, 99%), trifluoroacetic anhydride (TFAA), heptafluorobutyric anhydride (HFBA), 2,2,2-trifluoroethanol (TFE). They were purchased from Sigma–Aldrich (Milan, Italy) and Fluka (Milan, Italy). Also the solvents, chloroform (99.8%), methanol, ethanol, acetonitrile, ethyl acetate and acetonitrile, were purchased from Fluka (Milan, Italy). All these compounds were analytical grade reagents.

3.2. GC analysis

GC–MS analysis was performed on a QMD 1000 GC–MS system (Fisons, Milan, Italy). The Electron Impact ionization mode operated at 70 eV. The MS system operated in scan mode (mass range 38–550 u at 1 scan/s, solvent delay: 5 min). The detector and injector temperatures were 250 °C, the carrier gas Helium at a flow rate of 1 ml min^{-1}. Split injection was used with a split ratio of 1:20.

The chromatographic column was a Chirasil-L-Val fused-silica (L-valine-tert-butylamide modified polydimethylsiloxane) 25 m × 0.25 mm I.D. capillary column (Varian, The Netherlands) with a 0.12 μm film coating. Its maximum operating temperature was 200 °C. The enantiomeric resolution of the chloroformate and anhydride derivatives was investigated and compared under the same column temperature program: temperature was linearly increased from 60 to 180 °C at a rate of 4 °C/min and held at 180 °C for 15 min. These conditions present a compromise between short retention times, low column temperature (imposed by energy saving constraint) and good resolution for almost all amino acids [21,22]. To obtain reliable and reproducible quantitative data, the internal standard procedure was used, by selecting methyl laurate as IS: detector response was expressed as peak area value (A_{aa}) relative to IS peak area (A_{IS}), i.e., A_{aa}/A_{IS}.

3.3. Derivatization procedure

3.3.1. Chloroformate derivatization procedure

Standard amino acid stock solutions (25 μl plus 25 μl of IS) were transferred into a silanized screw capped 2 ml vial and the reagents were added in the following order: 60 μl of heptafluoro-1-butanol (HFB), 15 μl of pyridine and 15μl of methyl chloroformate (MCF). For each enantiomeric pair the analyzed quantity ranged from 1×10^{-6} to 5×10^{-7} moles. The previously described procedure was followed [21]. The mixture was immediately shaken for 1 min in an ultrasound bath kept at a constant temperature of 25 °C. The MCF derivatives were extracted from the reactive mixture by adding 200 μl of chloroform and a small volume (20 μl) of saturated NaCl solution. The solution was then shaken for 10 s and, after waiting 2 min to reach phase separation, 1 μl of the bottom chloroform phase was injected into the GC system.

3.3.2. Anhydride derivatization procedure

Derivatization was performed according to the procedure previously described in Ref [22]. Standard amino acid stock solution was transferred into a 2-ml ampoule and evaporated to dryness. For each enantiomeric pair the analyzed quantity was 2.4×10^{-7} moles. The two derivatizing agents (50 μl of perfluoroalcohol and 100 μl of perfluoroanhydride) were added to the dry residue and the ampoule was sealed and kept at 100 °C for 1 h without stirring. After being cooled to room temperature, the reagents were removed using a nitrogen stream. Then, the residues were dissolved in 100 μl of ethyl acetate, and 20 μl of IS solution was added; 1 μl of this solution was injected into the GC–MS.

4. Results and discussion

The two derivatization procedures were applied to twenty proteinogenic amino acids and their performance was investigated and compared by GC–MS analysis of the obtained derivatives in terms of enantiomeric resolution and quantitative sensitivity for the target amino acid pairs.

For both the procedures the chromatographic separation was optimized by selecting proper temperature program conditions to obtain the best enantiomeric resolution for most of the enantiomeric pairs.

4.1. Enantiomeric resolution

20 proteinogenic amino acids were derivatized with chloroformate and perfluoroacylated anhydrides and submitted to GC–MS analysis on a Chirasil-L-Val stationary phase. The same column temperature program (linear increase from 60 to 180 °C at 4 °C/min) was used to investigate and compare the enantiomeric resolution obtained for both the derivative classes. Among different combinations of chloroformates (methyl chloroformate, ethyl chloroformate and isobutyl chloroformate) and alcohols having an identical or different alkyl chains, the combination methyl chloroformate (MCF) and heptafluoro-1-butanol (HFB) was chosen since it allows operation at a lower column temperature and this ensures better chiral separation and reduced energy consumption [21,33].

Among the 20 proteinogenic amino acids analyzed, 14 enantiomeric pairs could be separated in these operating conditions with a constant elution order since the D form always eluted first. Most of these derivatives (6 out of 14) were strongly retained – with retention time longer than 20 min – displaying, in general, lowest resolution for the most retained compounds. Six enantiomeric pairs displayed good resolution values R_s higher than 1.4: Ala, Val, Ile, Leu, Met, and Glu (1st column in Table 1). Eight pairs showed lower resolution ($0.5 \geq R_s \geq 1.5$): they were the heaviest amino acids (Thr, Phe, Lys, Tyr, and Trp) or compounds yielding the bi-esterified (Asp) and bis-acylated (Gln, Ser) derivative as a more stable product. No enantiomeric separation was obtained for Pro, mono-acylated Ser, His and Asn. Arginine was not detected at the studied concentration level because its derivatization yield was very low, given the low reactivity of the guanidine group in the molecule under these derivatization conditions. Glutamine is not reported since it is converted into glutamine acid during the derivatization reaction [34].

Table 1
Enantiomeric resolution of amino acid pairs after chloroformate (1st column) and perfluoro anhydride derivatization (2nd–4th columns).

Amino acids	HFB/MCF	TFAA-TFE	TFAA-HFB	HFBA-HFB
	R_s			
DL-Ala	2.11	1.79	1.84	1.71
DL-Val	2.39	1.49	2.26	1.20
DL-Ile	6.24	1.56	1.93	1.44
DL-Leu	3.88	3.22	3.97	4.13
DL-Met	2.43	2.25	2.93	2.31
DL-Glu	1.38[a]	3.43	2.31	2.07
DL-Phe	0.93	2.23	2.27	2.05
DL-Tyr	0.85[a]	1.61	1.60	1.46

Comparison between R_s values on Chirasil-L-Val under the same program temperature conditions: linear increase from 60 to 180 °C at a rate of 4 °C/min. Absolute quantity submitted to derivatization: 5×10^{-7} moles for chloroformate; 2.4×10^{-7} moles for perfluoroanhydride reaction.

[a] bis-Esterified derivative.

Table 2
X_{LOD} and X_{LOQ} (derivatized nmol) values calculated from the calibration curves of the L-forms of a series of HFB/MCF and HFBA–HFB derivatives of 9 amino acids.

Amino Acids	X_{LOD} (derivatized nmol) HFB/MCF	X_{LOQ} (derivatized nmol)	X_{LOD} (derivatized nmol) HFBA–HFB	X_{LOQ} (derivatized nmol)
L-Ala	1.64	5.47	4.70	15.6
L-Val	0.72	2.4	1.13	3.77
L-Ile	1.28	4.27	0.58	1.93
L-Pro	6.68	22.3	2.71	9.03
L-Leu	6.08	20.3	0.50	1.66
L-Met	3.92	13.0	0.43	1.43
L-Glu	6.28	20.9	0.86	2.86
L-Phe	2.56	8.53	0.31	1.03
L-Tyr	5.8	19.3	0.36	1.20

Trifluoroacetic (TFAA) and heptafluorobutyric (HFBA) anhydrides have been found the most useful reagents for esterification–acylation reaction for quantitative GC determination of amino acids: they are both strong, highly reactive acylating agents that form stable derivatives [35,36]. The procedure was applied using three different combinations of TFAA and HFBA anhydrides with the corresponding perfluorinated alcohols TFE (2,2,2-trifluoro-1-ethanol) and HFB (2,2,3,3,4,4,4-heptafluoro-1-butanol) to give three varieties of amino acid derivative combinations (TFAA–TFE, TFAA–HFB, HFBA–HFB, Table 1) [22]. In addition to mono-derivatives, the bis- and tris-derivatives were also obtained when the functional groups were esterified (Asp, Glu) or acylated (Gln, Trp).

The retention behavior of the derivatives and their enantiomeric separation was investigated on chiral Chirasil-L-Val capillary column (linear temperature program from 60 to 180 °C at 4 °C/min). 14 of the 20 proteinogenic amino acids could be separated at a temperature lower than the column bleeding limit (200 °C), even the heaviest amino acid derivatives (Tyr, Gln, Lys, and Trp). Comparison among the obtained results shows that three reagent combinations display similar retention time patterns (11–40 min range) and chiral separations (Table 1, 2nd–4th columns). For all three combinations, 8 enantiomeric pairs could be well separated yielding a good resolution ($R_s \geq 1.4$): Ala, Val, Ile, Leu, Met, Glu, Phe, and Tyr (Table 1, 2nd–4th columns). Other amino acids gave poorly separated peaks (Pro, Thr, Asp, and Lys) or low signals due to the formation of by-products or degradation products (Gln, Trp). In general, the TFAA–TFE derivatives displayed the best selectivity on the Chirasil-L-Val column, while the TFAA–HFB compounds presented the greatest separation problems, in particular coelution of Ala–Val, Pro–Thr, and Glu–Phe enantiomer pairs. This motivates the selection of the TFAA–TFE derivatization for qualitative analysis [22]. However, it must be underlined that the separation of all the studied compounds is far to be achieved under these conditions: a promising alternative may be the use of GC columns coated with different selectors such as one cyclodextrin (CD) or their binary mixture in combination of chiral selectors with complementary enantioselectivity [16,22].

4.2. Quantitative analysis

The performance of the two methods for quantitative analysis was investigated and compared in terms of sensitivity and linearity.

The relative sensitivity was investigated by comparing the response factor measured as the relative peak area A_{aa}/A_{IS}. For the chloroformate derivatives using the HFB/MCF combination, a comparable sensitivity has been found for most of the studied amino acids: the exceptions were lysine and histidine (containing an additional aminic group), serine and glutamic acid (containing the additional hydroxyl group) which gave the lowest reaction yields [21].

Since MS response increases with the size of the protecting groups, when the esterification–acylation procedure was used, the HFBA–HFB derivatives yielded the highest response, making this combination the reagent of choice for quantifying amino acid enantiomers. Comparable sensitivity was found for most of the studied amino acids: L-Met and L-Phe displayed the highest sensitivity which was nearly triple that of L-Pro and L-Trp (bis-acylated) which was the least sensitive [22].

For quantitative analysis, linearity and sensitivity were evaluated by computing the calibration lines: the amino acids studied were L-Ala, L-Val, L-Ile, L-Pro, L-Leu, L-Met, L-Glu, L-Phe and L-Tyr. Different quantities of the L-form (30–500 nmol for HFB/MCF and 3–300 nmol for HFBA–HFB reagents) were submitted to derivatization and MS analysis. From the calibration lines, displaying good linearity over the wide concentration range exploited, the detection limit X_{LOD} and quantification limit X_{LOQ} were determined: X_{LOD} was computed as the analyte concentration yielding a signal value of $X_{LOD} = \bar{y}_b + 6\sigma_b$, where $\bar{y}_b$ is the blank average signal of 10 blank responses and σ_b its standard deviation. The quantification limit X_{LOQ} was determined as the analyte concentration corresponding to a signal value $X_{LOQ} = \bar{y}_b + 20\sigma_b$ to ensure that the quantitative determination gave satisfactory measurement precision (RSD% ≤5%) [37].

The obtained results show the comparable sensitivity of the methods, yielding low detection and quantification limits, X_{LOD} ≤6 nmol and X_{LOD} ≤20 nmol (Table 2). In particular, the chlorofor-

Table 3
Enantiomeric resolution R_s values for amino acid pairs under GC optimized temperature program. Absolute quantity submitted to chloroformate and perfluoroanhydride derivatization: 5×10^{-7} moles. Operating conditions for chloroformate derivatives (1st column): isotherm at 90 °C for 10 min, linear increase to 160 °C at 4 °C/min, isotherm at 160 °C for 10 min, linear increase to 180 °C at 4 °C/min, isotherm at 180 °C for 15 min. Operating conditions for TFAA–TFE perfluoro anhydride derivatives (2nd column): linear increase from 40 to 200 °C at 4.4 °C/min rate, followed by isothermal conditions; operating conditions for HFBA–HFB perfluoro anhydride derivatives (3rd column): isotherm at 60 °C for 27 min, linear increase to 120 °C at 3 °C/min, isotherm at 120 °C for 10 min, increase to 155 °C at 10 °C/min, isotherm at 155 °C for 5 min, increase to 200 °C at 3 °C/min, followed isothermal conditions.

Amino acids	HFB/MCF	TFAA–TFE	HFBA–HFB
		R_s	
DL-Ala	2.11	2.19	2.42[a]
DL-Val	2.39	2.21	2.52[a]
DL-Ile	6.24	2.03	2.30
DL-Pro	NR	LR	LR
DL-Thr	LR	LR	LR
DL-Leu	3.88	2.11	2.91
DL-Asp	0.81	LR	LR
DL-Met	2.43	2.08	2.28
DL-Glu	1.38[a]	1.88	2.14
DL-Phe	0.93[a]	1.95	2.24
DL-Tyr	0.85	2.06	1.96
DL-Gln (bis-acylated)	LR	1.23	0.89
DL-Lys	0.73	0.69	0.87
DL-Trp (bis-acylated)	LR	0.80	0.65

LR: resolution value $R_s \leq 0.6$.
[a] Enantiomeric resolution is possible only under SIM detection.

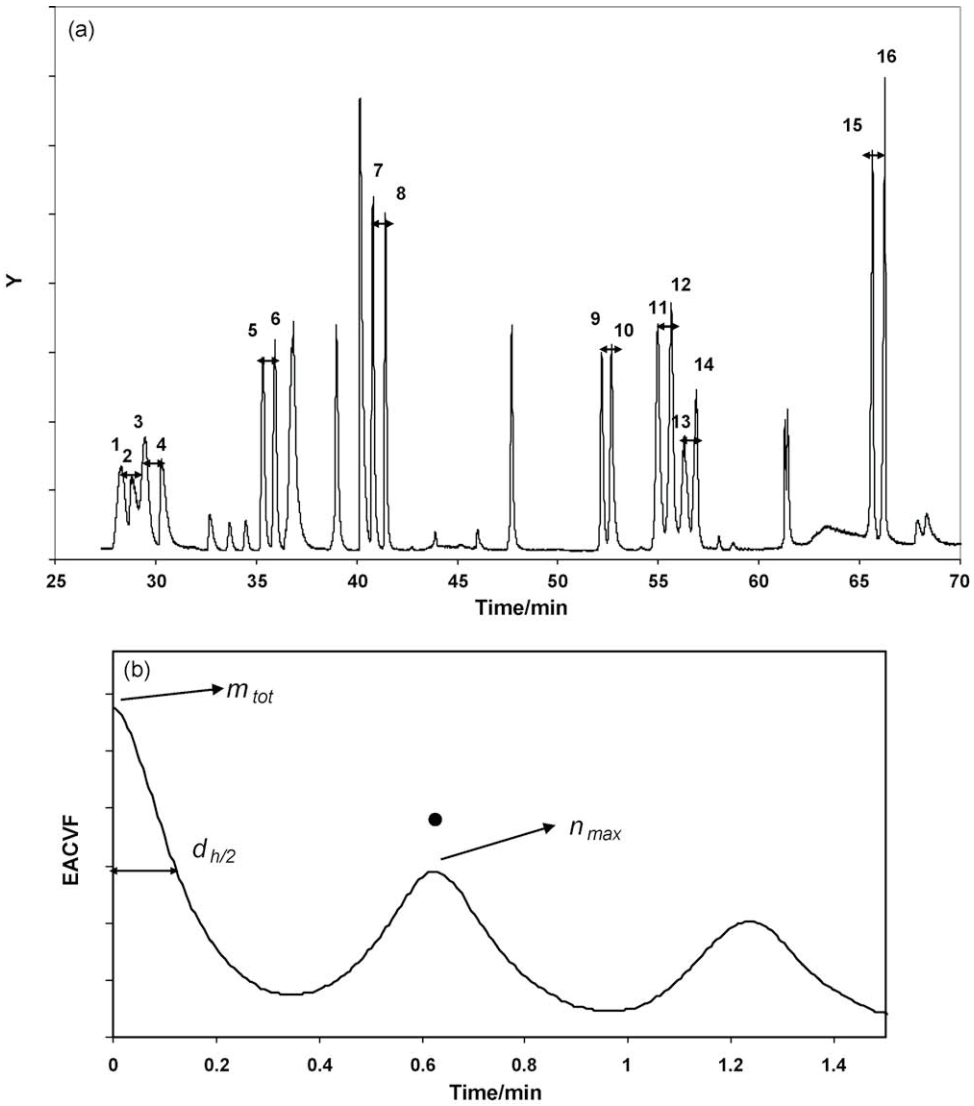


Fig. 2. Separation of HFBA–HFB derivatives. (a) 25–70 min region of the GC–MS signal obtained under the optimized conditions: an isotherm at 60 °C for 27 min followed by a three-stage temperature program: from 60 to 120 °C at 3 °C/min, 10 min at 120 °C, from 120 to 155 °C at 10 °C/min, 5 min at 155 °C, from 155 to 200 °C at 3 °C/min, followed isothermal conditions. Arrows: constant interdistance $\Delta t = 0.63$ min between the enantiomeric pairs. D,L-Val [1,2], D,L-Ala [3,4], D,L-Ile [5,6], D,L-Leu [7,8], D,L-Met [9,10], D,L-Phe [11,12], D,L-Glu [13,14], D,L-Tyr [15,16]. (b) EACVF plot computed on the GC–MS signal.

mate derivatives showed higher sensitivity for the lighter amino acids, L-Ala and L-Val, achieving $X_{LOD} \approx 1$ nmol, while the HFBA–HFB combination showed higher sensitivity for the least volatile amino acids, L-Met, L-Phe and L-Tyr, giving $X_{LOD} \leq 0.4$ nmol derivatized quantity. The obtained X_{LOD} and X_{LOQ} values are compatible to in situ analysis of extraterrestrial environments, where amino acids are expected to be present at the sub-nmol trace level, as suggested by the concentration level found in meteorites on Hearth [1–3].

4.3. Enantiomeric separation of amino acid mixtures

The performance of the described methods in separating amino acid enantiomers was checked on a mixture containing 15 amino acids: Gly and enantiomeric pairs Ala, Val, Pro, Ile, Leu, Asp, Thr, Met, Phe, Gln, Glu, Lys, Tyr and Trp (the absolute quantity submitted to derivatization was 5×10^{-7} moles for each amino acid enantiomer). After chloroformate and anhydride derivatization, the derivatives were submitted to GC–MS analysis on Chirasil-L-Val column: a number of trials were performed to select the best temperature program conditions yielding the best separation of the highest number of enantiomeric pairs.

For the chloroformate derivatives, the best separation was achieved in nearly 1 h by adding an initial isotherm at 90 °C for 10 min and inserting an isotherm step (160 °C for 10 min) in the linear temperature program (from 90 to 180 °C at a rate of 4 °C/min). Under these conditions, 13 of the 14 enantiomeric pairs were separated, in addition to Gly (R_s values reported in Table 3, 1st column), by combining the resolution power of the Chirasil-L-Val column and the high selectivity of the SIM (selected ion monitoring) detection mode. The overlapped peak formed by co-eluting derivatives of Phe and Glu can be solved by operating in SIM detection at $m/z = 91$ for Phe and $m/z = 84$ for Glu. Two esterification–acylation procedures were investigated: the TFAA–TFE derivatives yielding the best separation and the HFBA–HFB compounds giving the highest signal response for quantitative determination. After a number of trials, the proper chromatographic conditions were identified to yield acceptable enantiomeric separations for 14 of amino acid pairs (R_s values reported in Table 3, 2nd and 3rd columns).

The best separation of the TFAA–TFE derivatives was achieved under a linear temperature increasing from 40 to 200 °C at a rate of 4.4 °C/min, followed by isothermal conditions. The GC–MS signal obtained under these conditions (chromatogram in Fig. 1a) shows

that 8 enantiomeric pairs can be properly separated with $R_s \approx 2$ and 3 amino acids, DL-Gln, DL-Lys, and DL-Trp, satisfactory separated with $R_s \approx 0.8$ (R_s data in Table 3, 2nd column).

Separation of the HFBA–HFB derivatives of the same mixture is more difficult: a long analytical run of 85 min is required for the complete elution of all compounds. An isotherm at 60 °C for 27 min is introduced to separate DL-Ala from DL-Val enantiomers followed by a three-stage temperature program: from 60 to 120 °C at 3 °C/min, 10 min at 120 °C, from 120 to 155 °C at 10 °C/min, 5 min at 155 °C, from 155 to 200 °C at 3 °C/min, followed by isothermal conditions. The GC–MS chromatogram of the region 25–70 min containing most of the separated amino acid pairs is reported in Fig. 2a. Also, for these derivatives, 8 enantiomeric pairs can be satisfactory separated with $R_s \geq 2$, while low resolution ($R_s \approx 0.9$) was obtained for DL-Lys, DL-Gln and DL-Trp di-acylated derivatives (R_s data in Table 3, 3rd column). To solve the peak overlapping between DL-Ala and DL-Val pairs, the SIM detection mode was applied by selecting specific $m/z = 240$ value for Ala, and $m/z = 268$ for Val.

4.4. GC–MS signal processing using EACVF

The EACVF approach was applied to handle the complex GC–MS signals of the optimized separation of the derivatized 29-amino acid mixture (Gly and 14 enantiomeric pairs). Under these conditions a constant interdistance $\Delta t = b$ between the separated enantiomeric pairs can be experimentally achieved. The EACVF peak at $\Delta t \leq 4\sigma$ contains information on the separation parameters—the standard deviation σ and the number of components m_{tot} (Eqs. (3) and (4))—while the EACVF peak at $\Delta t = b$ gives information to characterize the enantioseparation achieved—number of separated chiral compounds (Eq. (5)) and mean R_s values.

Unlike the chloroformate derivatives, the perfluoroalkyl esters display a common retention behavior for the different amino acids in the test mixture, hence, under optimized conditions, most of the separated enantiomers exhibited the same chiral separation. This may be an experimental evidence that the chiral interactions between L-Val selector and chiral analyte moiety involved in the recognition process are similar for the studied amino acids.

Among the TFAA–TFE derivatives, 8 amino acids show a nearly constant interdistance $\Delta t = 0.25$ min between the separated enantiomeric pairs: DL-Val, DL-Ala, DL-Ile, DL-Leu, DL-Met, DL-Glu, DL-Phe and DL-Tyr (signed by arrows in Fig. 1a).

The $EACVF_{tot}$ was computed on the chromatographic signal in the 9–29 min region containing 25 amino acids, including the 8 enantiomeric pairs resolved ($EACVF_{tot}$ plot Fig. 1b). From the half-height width of the first EACVF peak, the mean peak standard deviation is estimated as $\sigma = 0.03$ min. From the EACVF (0) value the number of components present in the sample, m_{tot}, can be estimated as $m_{tot} = 28 \pm 5$, according to Eq. (3). The presence of a well-defined peak at $\Delta t = 0.25$ min can be used to identify the presence of enantiomeric pairs and, from its value, the number of the separated enantiomers $n_{max} = 15$ can be correctly estimated.

The HFBA–HFB derivatives of the same enantiomeric pairs (DL-Val, DL-Ala, DL-Ile, DL-Leu, DL-Met, DL-Phe, DL-Glu, and DL-Tyr) are separated by a constant interdistance $\Delta t = 0.63$ min (signed by arrows in the GC–MS signal reported in Fig. 2a). As a consequence, the EACVF plot computed on the signal (reported in Fig. 2b) clearly shows a well-defined peak at $\Delta t = 0.63$ min that is diagnostic for the presence of separated enantiomeric pairs. From the half-height width of the first EACVF peak, the mean peak standard deviation is estimated as $\sigma = 0.07$ min; such a high σ value, describing low separation efficiency, may be expected under these conditions of slow separation (all the retention times are higher than 28 min). From the EACVF(0) value the number of components present in the sample, m_{tot}, can be estimated as $m_{tot} = 29 \pm 5$, according to Eq. (3). From the height of the EACVF peak at $\Delta t = 0.63$ min, the number of the separated stereoisomers can be correctly estimated as $n_{max} = 15$.

Since the computed Δt and σ parameters measure the mean properties of the chromatogram, the ratio $\Delta t/4\sigma$ represents the mean resolution R_s for enantiomeric pairs: Δt is the mean interdistance between the separated enantiomeric pairs and 4σ the mean peak width. For the chromatograms of the TFAA–TFE and HFBA–HFB derivatives (Figs. 1a and 2a) R_s values, respectively, of $R_s = 2.08$ and $R_s = 2.38$ are obtained. These results perfectly agree with the values computed as average on R_s for each separated enantiomeric pair (data in Table 3, 2nd–3rd columns): $R_s = 2.06$ and $R_s = 2.35$ for the TFAA–TFE and HFBA–HFB derivatives, respectively. These results are proof of the reliability and robustness of the EACVF method in evaluating complex GC separations and its applicability to identify and characterize specific retention patterns such as the enantiomeric separation.

5. Conclusions

The results show that the described procedures enable the enantiomeric separation and quantification of 14 enantiomeric pairs of amino acids by combining the chiral selectivity of the commercially available Chirasil-L-Val capillary column and the high selectivity of the SIM detection mode.

Both the methods are simple and fast procedures, based on one-step derivatization reactions, and both display a wide linearity range at trace level (nmol detection limits) for quantitative determinations: these properties make the methods suitable candidates for designing instrumental devices for the in situ analysis of chiral organic compounds of exobiological interest onboard space exploration probes. The space suitability can be enhanced by applying a signal processing method to interpret the data recovered from space GC–MS experiments. Information on the chemical composition of samples collected in space missions, in particular characterization of the enantiomeric excess in amino acids, can be extracted with a simple and automatic procedure reducing the labor and time required as well as the subjectivity introduced by human intervention.

A subsequent challenge is full automation of the entire procedure, making it compatible with remote control conditions. Then, it could be integrated into space instrument sub-systems used to perform extraction, evaporation and derivatization in a single reactor coupled to GC–MS for in situ analysis in extraterrestrial environments [7–9]. Further developments of the enantioseparation system will also focus on instrument miniaturization or implementation into two-dimensional GC × GC apparatus for the pre-separation of achiral components prior to enantiomer resolution [38].

Acknowledgements

The authors thank F. Dondi (Department of Chemistry, University of Ferrara) for the fruitful discussion and suggestions.

This work was financially supported by the University of Ferrara.

References

[1] C. Rodier, C. Laurent, C. Szopa, R. Sternberg, F. Raulin, Chirality 14 (2002) 527.
[2] N. Fujii, Orig. Life Evol. Biosph. 32 (2002) 103.
[3] M.P. Bernstein, J.P. Dworkin, S.A. Sandford, G.W. Cooper, L.J. Allamandola, Nature 416 (2002) 401.
[4] W.H.P. Thiemann, U. Meierhenrich, Orig. Life Evol. Biosph. 31 (2001) 199.
[5] L. Cagliati, O. Holczknecht, N. Fujii, C. Zucchi, G. Palyi, Orig. Life Evol. Biosph. 36 (2006) 459.
[6] H.P. Klein, Rev. Geophys. Space Phys. 17 (1979) 1655.
[7] A. Buch, D.P. Glavin, R. Sternberg, C. Szopa, C. Rodier, R. Navarro-Gonzalez, F. Raulin, M. Cabane, P.R. Mahaffy, Planet. Space Sci. Special Astrol. Issue 54 (2006) 1592.

[8] D. Meunier, R. Sternberg, F. Mettetal, A. Buch, D. Coscia, C. Szopa, C. Rodier, P. Coll, M. Cabane, F. Raulin, Adv. Space Res. 39 (2007) 337.
[9] F. Stalport, P. Coll, C. Szopa, F. Raulin, Adv. Space Res. 42 (2008) 2014.
[10] A. Buch, R. Sternberg, C. Szopa, C. Freissinet, C. Garnier, El J. Bekri, C. Rodier, R. Navarro-Gonzalez, F. Raulin, M. Cabane, M. Stambouli, D.P. Glavin, P.R. Mahaffy, Adv. Space Res. 43 (2009) 143.
[11] A. Banin, R.L. Mancinelli, Adv. Space Res. 15 (1995) 163.
[12] C. Szopa, G. Freguglia, R. Sternberg, M.J. Nguyen, P. Coll, F. Raulin, M.C. Pietrogrande, H. Niemann, J. Chromatogr. A 1131 (2006) 215.
[13] C. Szopa, R. Sternberg, D. Coscia, F. Raulin, C. Vidal-Madjar, H. Rosenbauer, J. Chromatogr. A 953 (2002) 165.
[14] C. Szopa, R. Sternberg, F. Raulin, H. Rosenbauer, Planet. Space Sci. 51 (2003) 863.
[15] G. Danger, D. Ross, Electrophoresis 29 (2008) 4036.
[16] D. Kreidler, H. Czesla, V. Schurig, J. Chromatogr. B 875 (2008) 208.
[17] V. Schurig, M. Juza, M. Preschel, J.M. Nicholson, E. Bayer, Enantiomer 4 (1999) 297.
[18] V. Schurig, Trends Anal. Chem. 21 (2002) 647.
[19] F. Goesmann, H. Rosenbauer, R. Roll, C. Szopa, F. Raulin, R. Sternberg, G. Israel, U. Meierhenrich, W. Thiemann, G. Munoz-Caro, Space Sci. Rev. 128 (2006) 257.
[20] M. Cabane, P. Coll, C. Szopa, G. Israel, F. Raulin, R. Sternberg, P. Mahaffy, A. Person, C. Rodier, R. Navarro-Gonzale, H. Niemann, D. Harpold, W. Brinckerhoff, Adv. Space Res. 33 (2004) 2240.
[21] M.G. Zampolli, G. Basaglia, F. Dondi, R. Sternberg, C. Szopa, M.C. Pietrogrande, J. Chromatogr. A 1150 (2007) 162.
[22] M.G. Zampolli, D. Meunier, R. Sternberg, F. Raulin, C. Szopa, M.C. Pietrogrande, F. Dondi, Chirality 18 (2006) 279.
[23] G. Lai, G. Nicholson, E. Beyer, Chromatographia 26 (1988) 229.
[24] M.C. Pietrogrande, I. Tellini, A. Felinger, F. Dondi, C. Szopa, R. Sternberg, C. Vidal-Madjar, J. Sep. Sci. 26 (2003) 569.
[25] M.C. Pietrogrande, I. Tellini, L. Pasti, F. Dondi, C. Szopa, R. Sternberg, C. Vidal-Madjar, J. Chromatogr. A 1002 (2003) 179.
[26] M.C. Pietrogrande, M.G. Zampolli, F. Dondi, C. Szopa, R. Sternberg, A. Buch, F. Raulin, J. Chromatogr. A 1071 (2005) 255.
[27] N. Marchetti, A. Felinger, L. Pasti, M.C. Pietrogrande, F. Dondi, Anal. Chem. 76 (2004) 3055.
[28] M.C. Pietrogrande, M.G. Zampolli, F. Dondi, Anal. Chem. 78 (2006) 2579.
[29] M.C. Pietrogrande, M. Mercuriali, L. Pasti, Anal. Chim. Acta 594 (2007) 128.
[30] M.C. Pietrogrande, M. Mercuriali, L. Pasti, F. Dondi, Analyst 134 (2009) 671.
[31] M.C. Pietrogrande, I. Tellini, C. Szopa, A. Fellinger, P. Coll, R. Navarro-González, R. Sternberg, C. Vidal-Madjar, F. Raulin, F. Dondi, Planet. Space Sci. 51 (2003) 581.
[32] M.C. Pietrogrande, P. Coll, R. Sternberg, C. Szopa, R. Navarro-Gonzalez, C. Vidal-Majar, F. Dondi, J. Chromatogr. A 939 (2001) 69.
[33] I. Abe, N. Fujimoto, T. Nishiyama, K. Terada, T. Nakahara, J. Chromatogr. A 722 (1996) 221.
[34] S. Casal, M.D. Oliveira, M.A. Ferreira, J. Chromatogr. A 866 (2000) 221.
[35] S.L. MacKenzie, D. Tenashuk, J. Chromatogr. 322 (1985) 228.
[36] I. Spanik, J. Krupcik, I. Skacani, P. Sandra, D.W. Armstrong, J. Chromatogr. A 1071 (2005) 59.
[37] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics: Part A, Elsevier Science, Amsterdam, NL, 1988, pp. 171.
[38] M. McGuigan, J.H. Waite, J. Hunter, H. Imanaka, R.D. Sacks, J. Chromatogr. A 1132 (2006) 280.

Paper 4

R.Rodil, J.B. Quintana, G. Basaglia, M.C. Pietrogrande, R. Cela, “Determination of synthetic phenolic antioxidants and their metabolites in water samples by downscaled solid-phase extraction, silylation and gas chromatography-mass spectrometry” – Journal of Chromatography A,Vol.1217 (2010) 6428-6435

Journal of Chromatography A, 1217 (2010) 6428–6435

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Determination of synthetic phenolic antioxidants and their metabolites in water samples by downscaled solid-phase extraction, silylation and gas chromatography–mass spectrometry

Rosario Rodil[a,*], José Benito Quintana[a,*], Giulia Basaglia[b], Maria Chiara Pietrogrande[b], Rafael Cela[a]

[a] Department of Analytical Chemistry, Nutrition and Food Sciences, IIAA - Institute for Food Analysis and Research, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain
[b] Department of Chemistry, University of Ferrara, Via L Borsari 46, I-44100 Ferrara, Italy

ARTICLE INFO

Article history:
Received 18 June 2010
Received in revised form 29 July 2010
Accepted 6 August 2010
Available online 13 August 2010

Keywords:
Phenolic antioxidants
Butylated hydroxytoluene (BHT)
Butylated hydroxyanisole (BHA)
Solid-phase extraction (SPE)
Derivatisation
Gas chromatography–mass spectrometry (GC–MS)
Water analysis

ABSTRACT

The development and performance evaluation of an analytical method dedicated to the comprehensive determination of the most relevant antioxidants and their metabolites in aqueous environmental samples is presented. This was achieved by a miniaturised solid-phase extraction (SPE) with 10 mg Oasis HLB cartridges, which allow to achieve a concentration factor of 200, reducing organic solvent wastes (1 mL of ethyl acetate suffices for complete elution) and SPE costs and eliminating the need for solvent evaporation that otherwise compromises the recoveries of butylated hydroxytoluene (BHT) and 2,6-di-*tert*-butylcyclohexa-2,5-diene-1,4-dione (BHT-Q). Analytes were then determined by gas chromatography–mass spectrometry (GC–MS) after derivatisation with N-methyl-N-(*tert*-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) in a single run. BHT-d_7 and *n*-propyl-paraben-d_4 (PrP-d_4) were used as surrogate internal standards. These surrogates allowed obtaining relative recoveries in the 80–110% range for all analytes even with complex wastewater samples and LODs at the 2–44 ng L^{-1} level taking into account blank issues often associated to antioxidants analysis. The method was applied to sewage and river waters, showing that the seven analytes could be detected in raw wastewater. BHT and BHT-Q were the most concentrated species in that type of sample (in the 275–871 ng L^{-1} range). On the other hand two metabolites of BHT, 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (BHT-CHO) and 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (BHT-COOH) appeared to be the most ubiquitous species, being found in all samples in the 10–150 ng L^{-1} concentration range.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Antioxidants are substances which prolong the shelflife of foodstuffs by protecting them against deterioration caused by oxidation, such as fat rancidity and colour changes. Since natural antioxidants are usually of poor stability, manufacturers prefer to use synthetic antioxidants. Many synthetic compounds are active as antioxidants, but only a few are used because of very strict safety regulations. The most frequently used are the synthetic phenolic antioxidants (SPA). FDA [1] and EU [2] have established the permitted food phenolic antioxidants and amounts of their allowable usage. SPAs currently permitted for use in food are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), *tert*-butylhydroquinone (TBHQ), propyl gallate, octyl gallate and dodecyl gallate, usually at concentrations up to 100–200 μg g^{-1} of SPAs in oils or fats, either singly or in combination. The use of SPAs is not restricted to foodstuffs. Thus, they are permitted in many types of packaging materials, in adhesives that come in contact with food and also in cosmetics, personal care products and pharmaceuticals. Among the SPAs, BHA and BHT are the most used antioxidants.

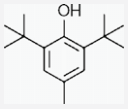
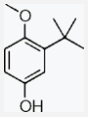
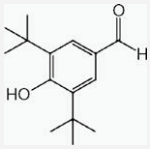
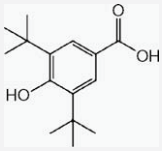
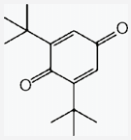
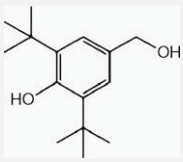
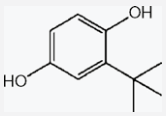
The results of scientific studies about the consumption of these additives are controversial since several studies have shown a potential link between BHA, BHT and cancer [3,4], while other studies have shown no link [5,6], and even a protective effect [7]. Nevertheless, their degradation products should be evaluated since they may pose an environmental or human health risk [8].

Studies on the metabolism of BHT have revealed that there are two main metabolic processes [9]; that is, oxidation of the alkyl substituent and oxidation of the aromatic ring system. 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (BHT-COOH) is a major metabolite formed by oxidation of the alkyl substituent and may be generated via the corresponding alcohol (BHT-OH) and aldehyde (BHT-CHO). Moreover, oxidation of the π-system of BHT leads, amongst others,

* Corresponding authors.
E-mail addresses: rosario.rodil@usc.es (R. Rodil), jb.quintana@usc.es (J.B. Quintana).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.08.020

Table 1
Analyte abbreviations, structures and other relevant data.

Abbreviation	IUPAC name	CAS	Formula	Estructura	Monisotopic MW	Log K_{ow}[a]	pK_a[a]	P_v (Torr)[a]
BHT	2,6-di-*tert*-Butyl-4-methylphenol	128-37-0	$C_{15}H_{24}O$		220.18	5.319 ± 0.235	12.75 ± 0.4	0.00624
BHA	2-*tert*-Butyl-4-methoxyphenol	25013-16-5	$C_{11}H_{16}O_2$		180.12	2.998 ± 0.235	11.82 ± 0.18	4.46E−3
BHT-CHO	3,5-di-*tert*-Butyl-4-hydroxybenzaldehyde	1620-98-0	$C_{15}H_{22}O_2$		234.16	4.769 ± 0.279	8.33 ± 0.40	1.28E−3
BHT-COOH	3,5-di-*tert*-butyl-4-Hydroxybenzoic acid	1421-49-4	$C_{15}H_{22}O_3$		250.17	4.796 ± 0.253	4.77 ± 0.10	3.28E−5
BHT-Q	2,6-di-*tert*-Butylcyclohexa-2,5-diene-1,4-dione	719-22-2	$C_{14}H_{20}O_2$		220.15	3.902 ± 0.381	–	2.81E−3
BHT-OH	2,6-di-*tert*-Butyl-4-(hydroxymethyl)phenol	88-26-6	$C_{15}H_{24}O_2$		236.18	3.675 ± 0.251	12.00 ± 0.40	3.37E−4
TBHQ	2-*tert*-Butylbenzene-1,4-diol	1948-33-0	$C_{10}H_{14}O_2$		166.10	2.333 ± 0.225	10.78 ± 0.18	1.12E−3

[a] Software calculated value, from SciFinder Scholar Database 2006: http://www.cas.org/products/sfacad/.

to 2,6-di-*tert*-butylcyclohexa-2,5-diene-1,4-dione (BHT-Q). On the other hand, the degradation of BHA produces TBHQ.

Most of the methods described in the literature for the quantitative analysis of antioxidants or antioxidant mixtures have been developed for the analysis of foodstuffs and food packaging [10]. In these cases, liquid chromatography with UV detection was the most common determination technique following the extraction by liquid–liquid extraction (LLE) or solid-phase extraction (SPE) of the sample. However, those methods are not applicable for trace analysis in environmental matrices because they do not offer the necessary selectivity and sensitivity. Hence, the establishment of sensitive and selective analytical methods to monitor the widespread in the environment of antioxidants and their degenerative products is a real need.

In aqueous environmental samples (i.e. wastewater samples and river water) most of the data of occurrence of these analytes have been obtained in multi-residue studies which evaluated the presence of one or two antioxidants together with a wide range of other organic compounds such as pharmaceuticals, phthalates, phenols, etc. In those methods, LLE [11], solid-phase microextraction (SPME) [12] and mainly SPE [13,14] have been used as pre-concentration techniques followed by GC–MS determination. However, studies dedicated to the development of analytical methods and subsequently the occurrence of SPAs and their metabolites in the aqueous environment are very scarce. The only exceptions are the works of Fries and Püttman, who studied BHT together with its metabolite BHT-CHO in river, ground and wastewater samples of Germany, where these pollutants were typically detected in the 10–2000 ng L^{-1} range, depending on the sample nature [15,16].

It is then necessary to develop analytical methods that allow the determination of antioxidants and a broader range of metabolites in the aqueous environment. Therefore, the goal of this work was the development and performance evaluation of a method that allows the determination of the three main synthetic phenolic antioxidant (i.e. BHT, BHA and TBHQ) together with their four most relevant metabolites (BHT-CHO, BHT-COOH, BHT-OH and BHT-Q; TBHQ is also a metabolite of BHA) in water by GC–MS combined to SPE for the enrichment of samples. Moreover, critical aspects associated

Table 2
GC–MS experimental data.

Abbreviation	Retention time (min)	M^+	Quantification ion (*m/z*)	Qualifier ions (*m/z*)
BHT-Q	8.43	220	177	220, 205
BHT-d7	8.86	227	212	227
BHT	8.92	220	205	220, 177
BHA	12.08	294	237	294, 181
PrP-d4	13.16	298	241	298
BHT-OH	14.12	350	293	276, 219
TBHQ	14.81	394	394	281, 337
BHT-CHO	15.12	348	291	333, 348
BHT-COOH	15.62	364	307	263, 233

with the determination of these analytes at trace level have also been considered and discussed.

2. Materials and methods

2.1. Chemicals and stock solutions

Analytes' names, abbreviations and other relevant data are shown in Table 1. BHT, BHA, BHT-COOH, BHT-Q, BHT-OH and TBHQ were obtained from Sigma–Aldrich (Steinheim, Germany) and BHT-CHO from TCI Europe (Zwijndrecht, Belgium). Deuterated BHT (2,6-di-(*tert*-butyl-d_1)-4-methyl-d_3-phenol-3,5-d_2; BHT-d_7) and *n*-propyl paraben (*n*-propyl 4-hydroxybenzoate-2,3,5,6-d_4; PrP-d_4), used as surrogate internal standards (ISs) were obtained from CDN Isotopes (Quebec, Canada).

Methanol and ethyl acetate (all of chromatographic analysis grade) were purchased from Merck (Darmstadt, Germany). Hydrochloric acid was purchased from Panreac (Castellar del Vallès, Spain). Pure water was obtained from a Milli-Q system (Millipore, Billerica, MA, USA).

The derivatisation reagents N-methyl-N-(*tert*-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA), bis(trimethylsilyl)trifluoroacetamide (BSTFA) and N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) were supplied by Aldrich.

Individual stock solutions were prepared in acetone at the 2 mg mL^{-1} level. Mix standard solutions were prepared at the 20 μg mL^{-1} in acetone and subsequently diluted as necessary. Calibration standards were prepared in ethyl acetate.

2.2. Samples

Surface water and WWTP influent and effluent samples were used along this study. The WWTP is located near Santiago de Compostela (Galicia, NW Spain) and receives urban and hospital wastewater from ca. 100,000 inhabitants. Surface water was collected from the river Sar in Galicia (NW Spain), 4 km downstream the WWTP effluent discharge. All samples were taken in amber glass bottles previously rinsed with Milli-Q water and methanol and stored in the dark at 4 °C for a maximum of 48 h prior to their analysis. Samples were filtered using cellulose acetate membranes (47 mm diameter, 0.45 μm pore size).

2.3. Sample extraction

SPE of samples was carried out with a Visiprep SPE manifold (Supelco, Bellefonte, PA, USA). Oasis HLB (10 and 60 mg) obtained from Waters (Mildford, MA, USA) were used.

In the optimised method, samples (200 mL) were adjusted to pH 2.5 and spiked with the deuterated ISs (200 ng L^{-1}). Oasis HLB (10 mg) cartridges were sequentially conditioned with 1 mL ethyl acetate, 1 mL MeOH and 1 mL pure water (pH 2.5). Cartridges were then loaded with the samples, washed with 3 mL Milli-Q water (pH 2.5) and dried with nitrogen for 30 min. Finally, elution of analytes was performed with ethyl acetate (1 mL).

tert-Butyldimethylsilyl derivatives were obtained by addition of 50 μL of MTBSTFA to an extract aliquot (50 μL). The derivatisation was performed at 80 °C during 90 min

2.4. GC–MS determination

GC–MS determination was performed on an HP 7890A system (Agilent Technologies, Palo Alto, CA, USA) equipped with a mass spectrometer detector MSD 5975C and a 7693 autosampler. The injector was set to 270 °C and injection volume was 2 μL. An HP-5MS capillary column (30 m × 250 μm i.d., 0.25 μm film thickness) was used with the following oven temperature program: 1 min at 90 °C, first ramp at 10 °C min^{-1} to 270 °C and second ramp at 25 °C min^{-1} to 290 °C (held for 10 min). Helium was used as carrier gas with a constant flow of 1 mL min^{-1}. Transfer line, MS quad

Table 3
Experimental domain and relative importance (with their sign) of the main effects associated to each factor and second order interactions in the Box–Behnken design.

Factors	Temperature (°C)	Time (min)	MTBSTFA/extract volume ratio	Interactions					
Low Level	40	15	0.1						
Central Level	60	52.5	0.55						
High Level	80	90	1						
Relative effects	A	B	C	AA	BB	CC	AB	AC	BC
BHA	++	++	++	+	+	+	+	++	++
BHT-OH	–	+	++	+	+	+	+	+	++
TBHQ	++	++	++	+	+	+	+	++	++
BHT-CHO	–	–	++	+	+	–	+	–	+
BHT-COOH	–	–	–	++	+	+	–	+	+
Selected conditions	80	90	1						

++ or – – indicate a statistically significant effect (95% confidence level), positive or negative respectively.
+ or – indicate that the effect was not statistically significant.

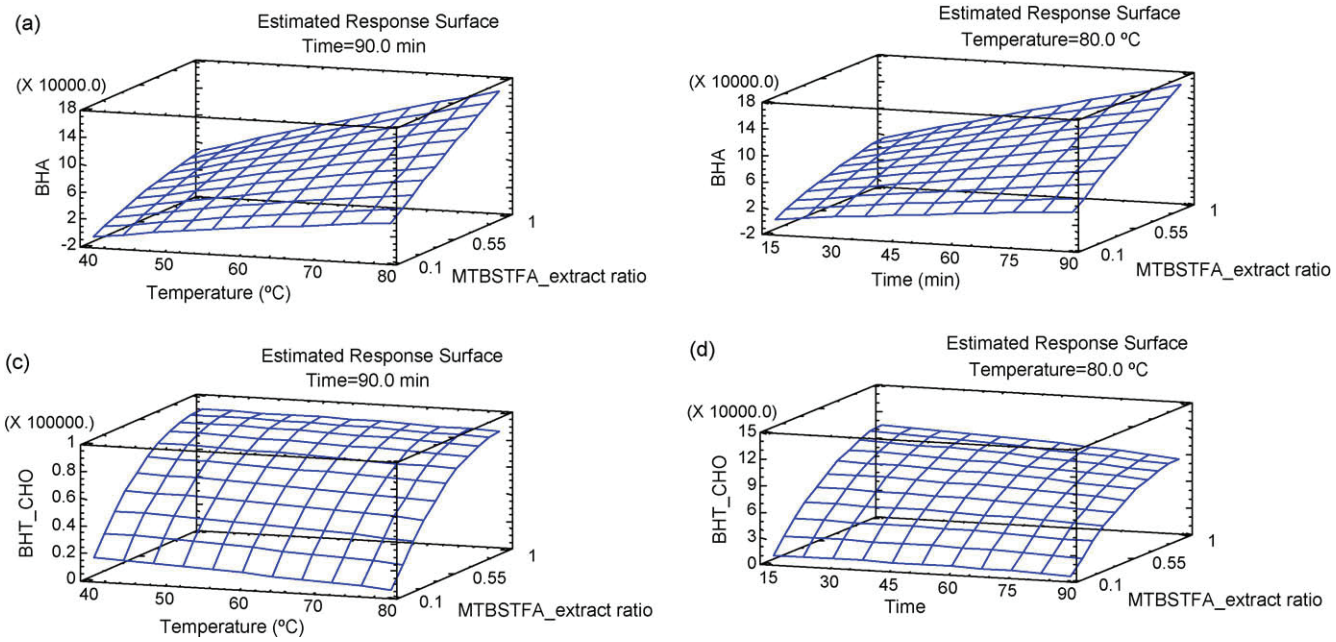


Fig. 1. Response surface plots for BHA (a and b) and BHT-CHO (c and d).

and MS source were maintained at 290, 150 and 230 °C, respectively. Detection was carried out by electron ionisation (70 eV) in single ion monitoring (SIM) mode, by considering two (in the case of ISs) or three (analytes) characteristic ions for each compound. The characteristic ions together with the substance-specific GC retention times for each studied compound are shown in Table 2.

2.5. Software

Experimental design creation and analysis was performed with the software package Statgraphics 5.1 (Statpoint Technologies, Warrenton, VA, USA).

3. Results and discussion

3.1. Derivatisation–GC–MS

In general, the studied compounds contain groups (phenols, alcohols and carboxylic acids) which can be derivatised to improve the chromatographic properties and separation on the GC-column [17]. The most common derivatisation procedure of compounds containing –OH and $-CO_2H$ groups is silylation [18,19]. Among the many possibilities of silylating agents, derivatisation experiments were performed by considering BSTFA, MSTFA and MTBSTFA. Derivatisation was accomplished by addition of 100 μL of the derivatisation reagent to a solution (900 μL) of the analytes in ethyl

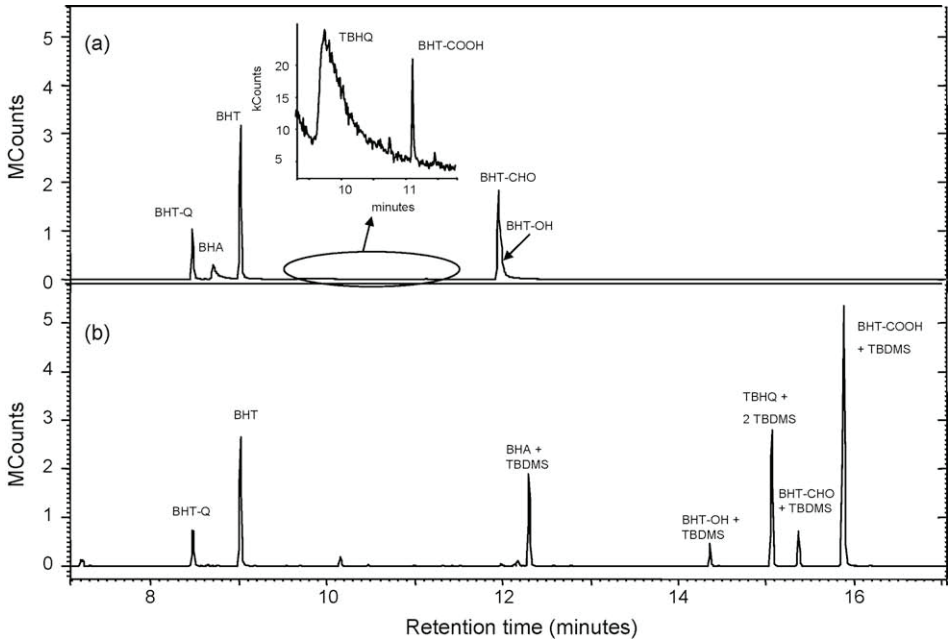


Fig. 2. Chromatograms of 1 μg mL^{-1} standard: (a) non-derivatised and (b) derivatised with MTBSTFA.

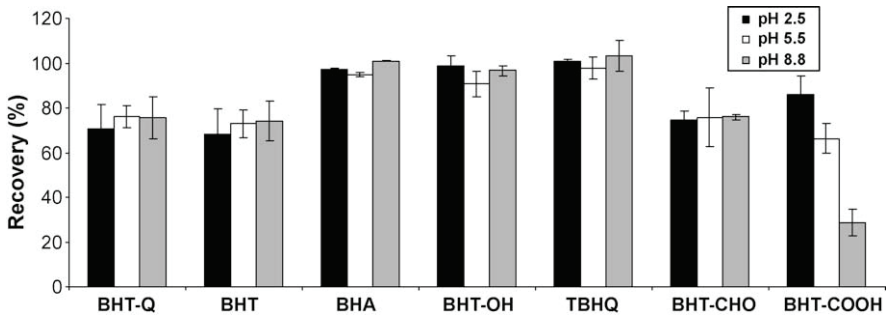


Fig. 3. Effect of sample pH on the SPE recoveries with 10 mg Oasis HLB cartridges. Sample volume: 100 mL; $n = 3$.

Table 4
Repeatability, linearity and detection and quantification limits of the GC–MS method.

	Internal standard	Repeatability (RSD, %) [a]	Linearity (R^2) [b]	LOD ($\mu g\,L^{-1}$) [c]	LOQ ($\mu g\,L^{-1}$) [c]
BHT-Q	BHT-d_7	2	0.999	0.6	2.1
BHT	BHT-d_7	4	0.997	0.2	0.6
BHA	PrP-d_4	3	0.994	0.8	2.7
BHT-OH	PrP-d_4	3	0.995	0.3	1.2
TBHQ	PrP-d_4	2	0.996	0.03	0.1
BHT-CHO	PrP-d_4	6	0.994	0.9	2.9
BHT-COOH	PrP-d_4	6	0.993	0.1	0.5

[a] 200 $\mu g\,L^{-1}$ standard ($n = 6$).
[b] 2–2000 $\mu g\,L^{-1}$; 10 levels in duplicate.
[c] Calculated for a signal-to-noise ratio of 3 (LODs) or 10 (LOQs).

acetate (1 $mg\,L^{-1}$). The mixtures were heated at 60 °C for 1 h prior to their injection in the chromatographic system. The proposed silylation reaction with MTBSTFA produced the successful derivatisation of all the studied compounds, except BHT, whose hydroxyl group is sterically hindered by two *tert*-butyls, and the quinone BHT-Q. Nevertheless both BHT and BHT-Q are easily determined by GC without the need of derivatisation. This derivatisation reaction leads to the formation of mono-*tert*-butyldimethylsilyl (TBDMS) derivates of BHA, BHT-OH, BHT-CHO and BHT-COOH while it produces the di-TBDMS derivate of TBHQ. On the other hand, the use of BSTFA and MSTFA leads to mixture of mono- and di-trimethylsilyl (TMS) derivates of BHT-CHO. Therefore, MTBSTFA was selected as the derivatisation agent because its ability to derivatise the studied compounds and the proved thermal and hydrolytic stability of the TBDMS derivates [20–22]. In addition, the resulting TBDMS derivatives produce very characteristics mass spectra by electron ionisation-mass spectrometry, with a molecular ion quite weak or even absent but dominated by the loss of the *tert*-butyl moiety $[M\text{-}57]^+$ (Table 2) [21–23].

The yield of derivatisation is affected by several variables such as derivatisation temperature and time and MTBSTFA/solvent volumes ratio. In order to optimise the derivatisation process, a Box–Behnken experimental design (with 3 central points; i.e. 15 experiments), was carried out. The use of Box–Behnken experimental design minimizes the number of experiments for 3 factors [24]. The factor levels were selected according to the literature [21–23], and bearing also in mind that: a higher MTBSTFA/solvent ratio would result in excessive dilution; temperature was limited to 80 °C to avoid overpressure in the vial and 90 min was considered the maximum reasonable derivatisation time. The experimental domain and the results of the analysis of the experimental design are shown in Table 3. Maximisation of peak area of the derivatised analytes was the target of the optimisation process. A pooled extract obtained from several treated wastewater samples spiked at 1 $mg\,L^{-1}$ for each compounds was used for the optimisation.

As shown in Table 3, the MTBSTFA/extract volume ratio had a statistically significant positive effect for most analytes (i.e. BHA, BHT-OH, TBHQ and BHT-CHO) except BHT-COOH. Temperature and time were only statistically significant for BHA and TBHQ and its effect was positive on the derivatisation yield. Finally, the MTBSTFA/extract volume ratio showed a positive statistically significant interaction with temperature and time for both BHA and TBHQ. Fig. 1 exemplarily shows the surface plots for BHA and BHT-CHO. Thus, the highest levels for all factors were selected as optimium for further experiments: 80 °C during 90 min with a ratio MTBSTFA/extract of 1. Fig. 2 presents the chromatograms of a standard

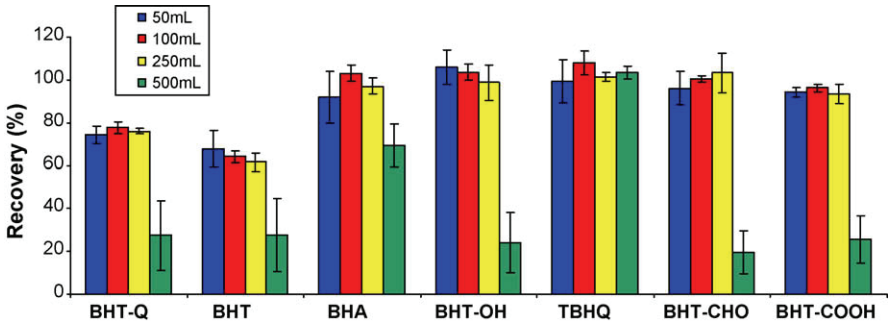


Fig. 4. Breakthrough volume study with Oasis HLB 10 mg cartridges. Sample pH adjusted to 2.5; $n = 3$.

Table 5
Relatives recoveries ($n = 4$) of analytes considering the surrogate ISs and detection limits of the whole analytical method.

	% Relative recovery (%RSD)				LODs[a] (ng L^{-1})	
	Milli-Q water (50 ng L^{-1})	Milli-Q water (500 ng L^{-1})	Treated wastewater (500 ng L^{-1})	Raw wastewater (1000 ng L^{-1})	Milli-Q, river and treated WW	Raw WW
BHT-Q	100 (7)	111 (7)	81 (8)	98 (18)	17[b]	39[b]
BHT	95 (10)	111 (6)	87 (4)	104 (8)	19[b]	44[b]
BHA	95 (4)	84 (5)	88 (3)	93 (12)	3	4
BHT-OH	100 (5)	80 (8)	83 (12)	98 (2)	8	8
TBHQ	81 (1)	84 (4)	80 (14)	83 (12)	2	3
BHT-CHO	86 (9)	110 (6)	98 (9)	110 (3)	10[b]	14[b]
BHT-COOH	98 (7)	95 (5)	108 (4)	108 (8)	2[b]	2[b]

[a] Calculated for a signal-to-noise ratio of 3 (LODs) from the second most intense ion.
[b] Calculated as three times the standard deviation of the blanks ($n = 6$).

either subjected or not to the final derivatisation procedure. Under these conditions, a complete derivatisation was observed, thus, no-derivatised analytes were not found in the chromatograms

Table 4 summarises the figures of merit of the derivatisation–GC–MS procedure. The developed GC–MS chromatographic method exhibits excellent linearity ($R^2 > 0.993$) in the 2–2000 μg L^{-1} range (duplicate injection, ten levels), and precision from the injection of a 200 μg L^{-1} standard (RSD < 6%), with detection limits (for S/N = 3) between 0.03 and 0.9 μg L^{-1}. BHT-d_7 and PrP-d_4 were selected as internal standards in order to correct possible variation in the whole process. BHT-d_7 was selected for the non-derivatised analytes (i.e. BHT-Q and BHT) while PrP-d_4 was selected for the derivatised ones.

3.2. Sample preparation

For the solid-phase extraction of the analytes, Oasis HLB cartridges were selected because of their ability to retain both non-polar and polar compounds providing good recoveries [25–27]. Thus, both parent compounds and their more polar metabolites can be recovered simultaneously with a single protocol and allow the possibility of decreasing SPE sorbent amount in order to decrease the organic solvent consumption and analytical costs. Hence, for a primary screening, different sorbent amounts (10 and 60 mg) were tested on their suitability for the SPE of the antioxidants into a single extraction method. Different parameters were evaluated for both phase amounts: elution volume of ethyl acetate, sample-pH and breakthrough volume.

First, the volume of ethyl acetate needed for a complete elution of the analytes was studied. Thus, 100 mL Milli-Q water samples spiked at the 20 μg L^{-1} level were percolated through the cartridges and eluted with 4 × 0.5 mL ethyl acetate (10 mg cartridges) or 4 × 1 mL ethyl acetate (60 mg cartridges), and the fractions were derivatised and analysed by GC–MS. The results of this study showed that a volume of 1 mL is enough for the complete elution of 10 mg cartridges. In the case of 60 mg cartridges, the elution is completed with 2 mL, except for TBHQ which needed 3 mL of ethyl acetate for the complete elution (data not shown).

Moreover, considering the different acidic character of the analytes, different sample-pH values were evaluated: 2.5, 5.5 and 8.8. Thus, 100 mL Milli-Q water samples adjusted to the different pH values indicated and spiked at the 20 μg L^{-1} level were percolated through the cartridge and eluted with 1 mL ethyl acetate (10 mg cartridges) or 3 mL ethyl acetate (60 mg cartridges). As expected (Fig. 3), the effect of the sample-pH is only significant for the most acidic compound (BHT-COOH); thus, the response decrease when sample-pH increases. Then, sample-pH should be adjusted to pH 2.5 prior to the extraction.

Finally, different sample volumes (50, 100, 250 and 500 mL) of river water spiked with the analytes (1 μg) were extracted with both cartridges in order to determine the breakthrough volume. Using 60 mg cartridges, breakthrough was never observed (data not shown), while it occurred for sample volumes higher than 250 mL for 10 mg cartridges (Fig. 4). Thus, in the case of 10 mg cartridges the extraction volume was limited to 200 mL.

In order to increase the enrichment factor, evaporation of ethyl acetate extracts (3 and 1 mL) containing the analytes to dryness and reconstitution in 100 μL ethyl acetate was performed. Possible losses of analytes during the extract evaporation step were studied, as this has been found as a critical step [28]. Absolute recoveries below 40% were obtained for all the analytes. In order to overcome this limitation, new experiments were performed evaporating the extract directly to 100 μL (avoiding dryness of the extract). Under these conditions absolute recoveries were slightly higher but still low (≈50%) and also showed a poor repeatability (RSD ≈ 40%). Since losses could not be overcome, no evaporation was performed in further experiments. Thus, in order to obtain the higher enrichment factor 10 mg cartridges were selected since 1 mL of ethyl acetate is enough for a complete elution and this critical step is no longer necessary. A further advantage of 10 mg cartridges is their lower price.

Under these conditions, absolute recoveries ($n = 4$) were between 70 and 115% in the evaluated matrices (Milli-Q water: 50 and 500 ng L^{-1}, treated wastewater: 500 ng L^{-1} and raw wastewater: 1000 ng L^{-1}) for all analytes but for BHT and BHT-Q (35–61%). Thus, possible losses of BHT and BHT-Q during different steps of

Table 6
Concentration (ng L^{-1}) ± standard deviation of antioxidants found in river and wastewater samples ($n = 3$ replicates of the same sample).

	February 2010			March 2010		
	River	Treated	Raw	River	Treated	Raw
BHT-Q	nd	nd	771 ± 100	nd	228 ± 22	871 ± 155
BHT	32 ± 5	nd	275 ± 21	112 ± 12	251 ± 30	801 ± 34
BHA	nd	nd	100 ± 4	nd	nd	135 ± 19
BHT-OH	nd	nd	55 ± 7	nd	nd	64 ± 6
TBHQ	nd	nd	9 ± 1	nd	nd	9 ± 2
BHT-CHO	26 ± 2	57 ± 3	144 ± 15	13 ± 1	24 ± 4	31 ± 6
BHT-COOH	13 ± 1	90 ± 9	67 ± 7	24 ± 2	61 ± 12	65 ± 8

nd: not detected (<LOD).

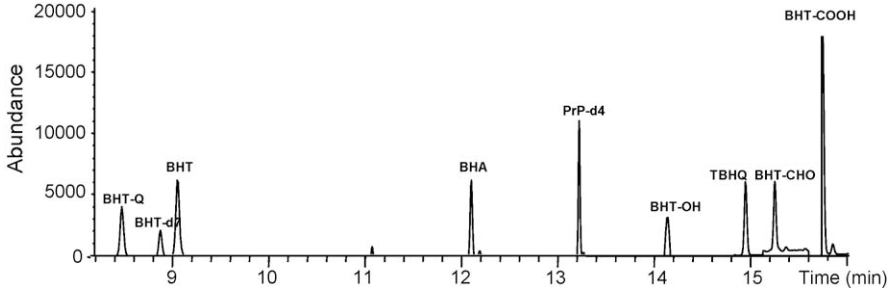


Fig. 5. Chromatogram of a real raw wastewater sample taken in March 2010. See Table 6 for concentration details.

the procedure were evaluated, viz. filtration, adsorption on SPE glassware and tubing, cartridges drying and degradation of the analytes. Filtration was evaluated by analysis of Milli-Q water spiked with the analytes (2 $\mu g\,L^{-1}$) before and after the filtration and then submitted to SPE. No losses were observed during filtration (data not given). Obviously, this does not mean that some antioxidants/metabolites adsorbed to particulate matter will remain on the filter, so that only the dissolved fraction is measured. Possible adsorption of analytes on glassware and SPE tubing was studied by addition of different percentages of MeOH (0, 2.5, 5 and 10%) to a Milli-Q sample (2 $\mu g\,L^{-1}$) prior its extraction by SPE. No improvement on SPE were observed at the different levels of methanol, only a slight decrease in the response at 20% methanol due to breakthrough of analytes at this high alcoholic level (data not shown). In order to estimate possible losses during cartridges drying, Oasis HLB cartridges were directly spiked with the analytes (4 μg) and dried for different periods of time (0, 30 and 60 min) and then eluted and analysed. No losses were observed (data not shown). Finally, degradation of BHT and BHT-Q was evaluated by analysis of Milli-Q water spiked with a sole analyte, either BHT or BHT-Q at high concentration levels (20 $\mu g\,L^{-1}$) and submitted to the sample preparation protocol. Although, dimerisation of BHT has been reported in the literature [15], formation of these dimers or any other degradation products was not observed.

Thus, the explanation for low recoveries of BHT and BHT-Q remains unclear. Fortunately, the labelled standard (BHT-d_7) is commercially available, permitting recovery correction and thus achieving good accuracy for these compounds (see Section 3.3).

3.3. Performance of the analytical method

Table 5 summarises the figures of merit of the whole analytical procedure, including SPE, derivatisation and final GC–MS determination. After the enrichment of 200 mL Milli-Q water samples spiked at two levels (50 and 500 ng L^{-1}) the obtained relative recoveries were satisfactory for all compounds (80–111%). Recoveries were also evaluated from raw and treated wastewater samples spiked at 500 and 1000 ng L^{-1} level, respectively. Each sample was processed in quadruplicate. Non-spiked aliquots of each sample were also analysed and obtained peak areas subtracted from those corresponding to the spiked ones. The use of the surrogated IS (BHT-d_7) corrected satisfactorily the losses and the relative recoveries obtained for BHT and BHT-Q were between 81 and 104% with RSD values in the 4–18% range independently of the sample matrix. For the remaining analytes, PrP-d_4 was used as surrogate IS, as no other isotopically labelled analogous compounds were commercially available and PrP-d_4 is also derivatised with MTBSTFA. For those five analytes, relative recoveries (81–110%) and RSD (2–12%) values were also satisfactory (Table 5).

LODs were calculated by two different approaches: based on blank assays of Milli-Q water samples ($n = 6$) as blank signal plus 3 times the standard deviation of the blank; and defined for a signal-to-noise ratio of 3 and calculated on the basis of extracts of Milli-Q water samples spiked at 50 ng L^{-1} level from the second most intense ion. LODs were established based on the highest value of these two approaches for each compound. The obtained LODs ranged from 2 to 19 ng L^{-1}. In the literature, BHT blank levels up to 2 $\mu g\,L^{-1}$ have been reported due to plastic cartridges usage that authors could only reduce to the 25 ng L^{-1} by replacing plastic 200 mg Oasis HLB cartridges by glass ones [13]. In this work, with 10 mg Oasis HLB plastic cartridges, no such high blank levels were found, and blanks remained bellow the 5 ng L^{-1} level. Thus, in spite of the measures taken with glassware, etc. blank contamination problems cannot be completely eliminated, but however the levels of BHT, BHT-Q, BHT-CHO and BHT-COOH could be maintained at a constant level in this work, which permitted to obtain acceptable LOD levels.

3.4. Application to samples

The SPE–GC–MS method was applied to the determination of antioxidants in 2 sets of (raw and treated) wastewater and 2 river water samples collected in February and March 2010. Blanks (from Milli-Q water) were processed with each SPE sample lot and subtracted for concentration calculations. Also, positives were confirmed from the ratio of the quantification and qualifiers ions, according to the European Union *Commission Decision 2002/657/EC*. A chromatogram of a raw wastewater sample is presented in Fig. 5.

As summarised in Table 6, BHT-Q and BHT were the two analytes detected in higher concentrations in raw wastewater samples (between 275 and 871 ng L^{-1}). Also, BHA was found at high concentration levels in raw wastewater (100 and 135 ng L^{-1}). However, these compounds seem to be partially removed during the wastewater treatment and lower concentrations were found in treated wastewater samples. These results are in agreement with the ones found in the literature for BHT and BHA, since these compounds were consistently detected in concentration at several hundred parts-per-trillion (ng L^{-1}) level in raw wastewater while the concentration in treated wastewater effluents were at from several tenths to hundred parts-per-trillion (ng L^{1}), suggesting that conventional wastewater treatment plants are capable of removing these chemicals with efficiencies varying between 65 and 99% [13].

It is noteworthy, that BHT-CHO and BHT-COOH were found in all the analysed samples, including in river water, and the concentration of BHT-COOH is constant and even increases during the treatment of wastewater. BHT-CHO was also previously detected in river water samples at higher concentrations levels (up to 233 ng L^{-1}) [15,16]. Obviously, these are grab samples that do not represent average concentrations and a deeper study is needed.

4. Conclusions

An SPE–derivatisation–GC–MS method has been developed for the determination of two synthetic phenolic antioxidants and their

five main metabolites in water. This is the first published method dedicated solely to the determination of this chemical class in water samples.

Extraction on 10 mg Oasis HLB cartridges provides a satisfactory enrichment factor for environmental samples avoiding the need of solvent evaporation and reducing SPE costs and organic solvent wastes. After extraction, polar metabolites are derivatised with MTBSTFA to produce stable, less polar analytes that are determined by GC–MS at low levels. The usage of two surrogate internal standards results in a method providing good accuracy, with relative recoveries between 80 and 110%, and limits of detection (2–44 ng L^{-1}).

The application of the method to wastewater and river samples showed BHT and BHT-Q as the compounds in higher concentrations in wastewater (up to 800 ng L^{-1}) and the metabolites BHT-CHO and BHT-COOH as the most resistant to water treatment, being at the 10–100 ng L^{-1} in sewage and river samples.

Acknowledgements

This research was funded by the Spanish Ministry of Science and Innovation (Ministerio de Ciencia e Innovación) and FEDER funds: project no. CTQ2009-08377. RR and JBQ extend their gratitude to the Spanish Ministry of Science and Innovation (Ramón y Cajal research program). Finally, we are indebt to Aquagest for kindly providing access to wastewater samples.

References

[1] Food and Drug Administration, Code of Federal Regulations, Title 21: Food and Drugs, Office of the Federal Register, Washington, DC, 2001 (Chapter I).
[2] Council Directive 95/2/EC of 20 February 1995 on Food Additives Other than Colours and Sweeteners, OJ L 61, 18.3.1995, Brussels, 1995, p. 1.
[3] H.C. Grice, Food Chem. Toxicol. 26 (1988) 717.
[4] H.P. Witschi, Food Chem. Toxicol. 24 (1986) 1127.
[5] A.A.M. Botterweck, H. Verhagen, R.A. Goldbohm, J. Kleinjans, P.A. van den Brandt, Food Chem. Toxicol. 38 (2000) 599.
[6] J. Whysner, G.M. Williams, Pharmacol. Ther. 71 (1996) 137.
[7] F. Iverson, Cancer Lett. 93 (1995) 49.
[8] S. Oikawa, K. Nishino, S. Inoue, T. Mizutani, S. Kawanishi, Biochem. Pharmacol. 56 (1998) 361.
[9] M. Matsuo, K. Mihara, M. Okuno, H. Ohkawa, J. Miyamoto, Food Chem. Toxicol. 22 (1984) 345.
[10] C. Andre, I. Castanheira, J.M. Cruz, P. Paseiro, A. Sanches-Silva, Trends Food Sci. Technol. 21 (2010) 229.
[11] M.A. Soliman, J.A. Pedersen, H. Park, A. Castaneda-Jimenez, M.K. Stenstrom, I.H. Suffet, Water Environ. Res. 79 (2007) 156.
[12] F. Hernández, T. Portoles, E. Pitarch, F.J. López, Anal. Chem. 79 (2007) 9494.
[13] R.A. Trenholm, B.J. Vanderford, J.E. Drewes, S.A. Snyder, J. Chromatogr. A 1190 (2008) 253.
[14] M. Remberger, L. Kaj, A. Palm, J. Sternbeck, E. Kvernes, E. Brorström-Lundén, Screening tertiary butylphenols, methylphenols, and long-chain alkylphenols in the Swedish environment, IVL Swedish Environmental Research Institute, Stockholm, 2003.
[15] E. Fries, W. Püttmann, Water Res. 36 (2002) 2319.
[16] E. Fries, W. Püttmann, Sci. Total Environ. 319 (2004) 269.
[17] L. Guo, M.Y. Xie, A.P. Yan, Y.Q. Wan, Y.M. Wu, Anal. Bioanal. Chem. 386 (2006) 1881.
[18] J.M. Halket, V.G. Zaikin, Eur. J. Mass Spectrom. 9 (2003) 1.
[19] M. Rompa, E. Kremer, B. Zygmunt, Anal. Bioanal. Chem. 377 (2003) 590.
[20] I. González-Mariño, J.B. Quintana, I. Rodríguez, R. Cela, J. Chromatogr. A 1217 (2010) 1748.
[21] T. Heberer, H.J. Stan, Anal. Chim. Acta 341 (1997) 21.
[22] I. Rodríguez Pereiro, R. González Irimia, E. Rubí Cano, R. Cela Torrijos, Anal. Chim. Acta 524 (2004) 249.
[23] I. Rodríguez, J.B. Quintana, J. Carpinteiro, A.M. Carro, R.A. Lorenzo, R. Cela, J. Chromatogr. A 985 (2003) 265.
[24] S.L.C. Ferreira, R.E. Bruns, H.S. Ferreira, G.D. Matos, J.M. David, G.C. Brandão, E.G.P. da Silva, L.A. Portugal, P.S. dos Reis, A.S. Souza, W.N.L. dos Santos, Anal. Chim. Acta 597 (2007) 179.
[25] N. Fontanals, R.M. Marce, F. Borrull, Trac-Trends Anal. Chem. 24 (2005) 394.
[26] R. Rodil, J.B. Quintana, P. López-Mahía, S. Muniategui-Lorenzo, D. Prada-Rodríguez, J. Chromatogr. A 1216 (2009) 2958.
[27] S. Weigel, R. Kallenborn, H. Huhnerfuss, J. Chromatogr. A 1023 (2004) 183.
[28] M.C. Pietrogrande, G. Basaglia, F. Dondi, J. Sep. Sci. 32 (2009) 1249.

Paper 5

G. Basaglia, L. Pasti, M.C. Pietrogrande, “Multi-residual GC-MS determination of Personal Care Products in waters using Solid Phase Micro Extraction” – Analytical and Bioanalytical Chemistry, 399 (2011) 2257-2265

Anal Bioanal Chem (2011) 399:2257–2265
DOI 10.1007/s00216-010-4609-4

ORIGINAL PAPER

Multi-residual GC-MS determination of personal care products in waters using solid-phase microextraction

G. Basaglia · L. Pasti · M. C. Pietrogrande

Received: 27 September 2010 / Revised: 25 November 2010 / Accepted: 13 December 2010 / Published online: 9 January 2011
© Springer-Verlag 2011

Abstract A multi-residual method is described for the simultaneous determination of 23 personal care products (PCPs), which display a wide range of physicochemical properties, present at trace levels in water samples. A one-step procedure was developed based on solid-phase microextraction (SPME) coupled with GC-MS analysis. A chemometric approach consisting of an experimental design (design of experiments) was applied to systematically investigate how four operating parameters—extraction temperature and time and desorption temperature and time—affect extraction recovery of PCPs in water. The optimum SPME procedure operating conditions, those yielding the highest extraction recovery for all the compounds, were determined; they correspond to an extraction time of 90 min and temperature of 80 °C and a desorption time of 11 min and temperature of 260 °C. Under these optimized conditions, the SPME procedure shows good analytical performance characterized by high reproducibility (RSD% intra-day accuracy varying in the 0.01–1.3% range) as well as good linearity and low detection limits (LODs lower than 2 ppb for most of the investigated PCPs).

Keywords Personal care products · Solid-phase microextraction · Gas chromatography–mass spectrometry · Multi-residue method · Response surface model

G. Basaglia · L. Pasti · M. C. Pietrogrande (✉)
Department of Chemistry, University of Ferrara,
Via L. Borsari, 46,
44100 Ferrara, Italy
e-mail: Chiara.pietrogrande@unife.it

Introduction

Personal care products (PCPs) constitute a broad class of chemical compounds widely used in everyday human and veterinary activities, e.g., food additives, sunscreens, lotions, cosmetics, insect repellents, dental care products, soaps, shampoos, and deodorants [1, 2]. Together with various pharmaceuticals, they constitute the pharmaceuticals and PCPs (PPCPs), a broad class of important "unrecognized" or "emerging" pollutants found in wastewater and surface waters [1, 3, 4]. Given the constant anthropogenic input of PPCPs into the environment and their possible ecotoxicological impact, there has been growing public interest in monitoring this class of contaminants, and following the principle of precaution, the EU Water Framework Directive has identified some PCPs as future emerging priority candidates for monitoring and regulation [5, 6].

Therefore, there is critical need for efficient and reliable analytical methods to address the occurrence, concentration, and fate of the PCPs in natural waters, i.e., where they have been identified in very low concentrations, ranging from nanograms per liter to hundreds of micrograms per liter [7]. Gas chromatography–mass spectrometry (GC-MS) has been the approach of choice because of its superior separation and identification capabilities. In addition, the development of faster, more cost-effective, more environment-friendly sample preparation procedures is a mandatory requirement, since tedious sample pre-treatment causes an analysis bottleneck that typically accounts for over 60% of the total analysis time.

In this paper, a solid-phase microextraction (SPME) procedure is investigated. Since it is a solvent-free isolation technique, integrating sampling, extraction, concentration and sample GC injection into a single step (the procedure involves only sorption and desorption, indeed) [8–10], it is

a suitable candidate as extraction and pre-concentration procedure for PCPs analysis from water samples. In comparison with the more popular solid-phase extraction (SPE) [11, 12], SPME requires less sample volume and shows a high concentrating capacity and selectivity. SPME methods have been successfully employed for the determination of a wide range of water pollutants with similar polarities, structures or activities, such as musk fragrances, phthalates, polychlorinated biphenyls, brominated flame retardants, and phenolic compounds [13–25].

This paper describes a fast, accurate multi-residue method for the simultaneous determination of a large number of PCPs and their bioactive metabolites, which display a wide range of physicochemical properties and are present at trace level in water samples [2, 7, 26, 27]. Thus, the development of a SPME multi-residue method requires optimization of the operative parameters (extraction temperature and time, desorption temperature and time) for the highest sensitivity of all the analytes. In this regard, multivariate methods of optimization, including factorial design and response surface methods have been used to evaluate the main and interactive effects of the variables in relation to analytical response, as well as to optimize the variables simultaneously with a reduced number of experiments.

Materials and methods

Chemicals and standards

Twenty-three PCP compounds were investigated. All standards were of the highest purity commercially vailable; they were obtained from Lab Service Analytical S.r.l (Bologna, Italy), Carlo Erba Reagenti (Milano, Italy), VWR International (Pennsylvania, USA) and Sigma–Aldrich (Steinheim, Germany). All solvents were trace analysis grade from 99.7%.

Eight of them were selected as target molecules for the SPME procedure optimization to represent the different chemical classes (Table 1, numbered from 1 to 8): the choice was based on a representatively large range of physicochemical properties affecting their environmental impact, i.e., volatility, water solubility, and octanol–water partitioning coefficient (Table 1). Pentachloronitrobenzene (quintozene, Table 1, last row) was used as internal standard in the GC-MS analysis.

The standard PCPs were individually dissolved in pure methanol (MeOH) at 1,000 ppm, and then diluted with MeOH in mixed solutions at concentration levels of 100 ppb and 1, 15, 50, and 100 ppm. The SPME optimization was carried out using a final concentration of 30 ppb (100 μl of 15 ppm solution) in 50 ml of MilliQ water (Waters MA USA) in 60 mL vial.

For the eight target PCPs (compounds 1–8 in Table 1) a nine-point calibration curve was constructed by diluting the concentrate MeOH solution with MilliQ water to achieve concentration levels of 0, 0.1, 1, 5, 15, 30, 50, 100, and 150 ppb. In addition, six-point calibration curves were computed for all the PCPs (compounds 1–23 in Table 1) by analyzing standard solutions in tap water (concentration of 0, 0.9, 3.1, 9.3, 15.5, 27.9, and 58.9 ppb). The drinking water was previously filtered in 0.45 μm mixed cellulose ester filter (Whatman GmbH).

All the calibration curves were carried out by appropriately diluting the concentrate solutions in 50 mL of water. In all the diluted solutions, a constant MeOH volume was added (0.2%) in order to avoid changes in extraction yield due to the methanol content. All the materials used in the analysis have to first be proved free of interferences, and this is done by running reference matrix method blanks. Proper cleaning of glassware is extremely important, because glassware may not only contaminate the samples but it can even remove the analytes of interest by adsorption on the glass surface.

Solid-phase microextraction

The used SPME fibers were coated with polydimethylsiloxane at 100 μm thickness (supplied by Supelco, Bellefonte, USA) and housed in a manual holder. This sorbent was selected because of its highest versatility: indeed, it is generally used for extraction of a wide range of analytes with different polarities and volatilities [2, 22, 23, 25–28].

Following the conditioning guidelines, the fibres were conditioned under helium at a flow rate of nearly 1.0 mL/min with the split valve open (to reduce the amount of impurities entering the column) in the hot injection port of a gas chromatograph at 250 °C for 1 h prior to use. Additionally, the SPME fibers were conditioned for 15 min at 250 °C every day before use and they were systematically cleaned at 250 °C for 20–30 min after every extraction. The blanks were tested by thermal desorption (5 min in the injection port) followed by GC analysis to confirm that all compounds were desorbed and prevent the fiber's memory effect.

Preliminary experiments were performed to evaluate the fixed extraction condition. A direct immersion mode was used for extraction from a fixed sample volume of 50 ml of water in a 60-ml vial. Samples were immersed in a thermostatic water bath at a given temperature (having a precision of ±1 °C) for 10 min to equilibrate before SPME insertion.

The samples were maintained under controlled agitation with a magnetic stirrer (500 rpm) in order to transport analytes from the bulk of the solution to the vicinity of the fiber and facilitate rapid extraction. After exposure to the sample, the fiber was inserted into the GC injector for analysis.

Table 1 List of studied compounds

	Compound	Use	PM	b.p. (°C)	Formula	Log Kow	t_r (min)	Ion mass *m/z*
1	Butylated hydroxyanisole	Anti-oxidant	180.25	264	$C_{11}H_{16}O_2$	5.1	9.4	137+165
2	Fluorene	PAH	166.22	295	$C_{13}H_{10}$	4.2	11.5	165
3	Benzophenone	UV-blocker	182.218	305	$C_{13}H_{10}O$	3.2	12.5	105+182
4	Phenanthrene	PAH	361.48	336	$C_{14}H_{10}$	4.5	15.1	178+152
5	Aldrin	Pesticide	364.9	145	$C_{12}H_8Cl_6$	6.4	18.1	263+261
6	Musk ketone	Fragrance	290.8	395	$C_{14}H_{18}N_2O_5$	4.3	18.3	279+43
7	2.4'-DDD	Pesticide	320.05	No data	$C_{14}H_{10}Cl_4$	5.9	22.2	235+237
8	Chrysene	PAH	228.29	448	$C_{18}H_{12}$	5.9	26.6	228+226
9	Naphthalene	PAH	294.31	218	$C_{10}H_8$	3.4	8.6	128+102
10	Butylated hydroxytoluene	Antioxidant	220	265	$C_{15}H_{24}O$	4.2	10	205+177
11	Lindane	Pesticide	110.1	323	$C_6H_6Cl_6$	3.6	14.3	181+183
12	Galaxolide	Fragrance	258.4	304	$C_{18}H_{26}O$	5.9	15.8	243+213
13	Musk xylene	Fragrance	297.27	no data	$C_{12}H_{15}N_3O_6$	4.9	16	282+128
14	Di-iso-butylphthalate	Plasticizer	278	320	$C_{16}H_{22}O_4$	4.1	16.1	149
15	Heptachlor	Pesticide	373.34	140	$C_{10}H_5Cl_7$	6.7	16.8	100+272
16	Fluoranthene	PAH	202.26	375	$C_{16}H_{10}$	5.2	20.4	202+88
17	Pyrene	PAH	178.23	404	$C_{16}H_{10}$	5	21.2	202+100
18	Dieldrin	Pesticide	380.93	385	$C_{12}H_8Cl_6O$	6.2	22	79+77
19	4,4'-DDD	Pesticide	320.05	350	$C_{14}H_{10}Cl_4$	6.0	23.5	235+65
20	Octocrylene	UV-blocker	128.17	218	$C_{24}H_{27}NO_2$	6.9	28.1	232+204
21	Dioctylphtalate	Plasticizer	390	222	$C_{24}H_{38}O_4$	7.9	28.6	149
22	Benzo[k]fluoranthene	PAH	252.32	480	$C_{20}H_{12}$	6.8	29.7	252+250
23	Benzo[a]pyrene	PAH	252.32	311	$C_{20}H_{12}$	6.4	30.7	252+250
24	Quintozene	Pesticide	202.26	328	$C_6Cl_5NO_2$	4.6	14.5	237+214

The retention times refer to GC temperature program explained in paragraph GC-MS

Gas chromatography–mass spectrometry

The GC-MS system consisted of a Focus GC, PolarisQ GCMSn Benchtop IT Mass Spectrometer (Thermo Fisher Scientific, Bellefonte, PA, USA). Helium (99.999%) was used as carrier gas at a constant head pressure of 50 kPa. A fused-silica column RTX-5MS (DB5 30 m×0.25 mm I.D., 0.25 μm film thickness) was purchased from Thermo Fisher Scientific (Bellefonte, PA, USA).

In order to define the optimum GC condition for the best analyte separation, the PCP solution (1 μl at 15 ppm) was injection into a split/splitless injector in splitless mode; splitless time was 1.5 min, the injector temperature was maintained at 260 °C.

For the SPME optimization procedure, an injector temperature varying in the 190–270 °C range was investigated; an optimized temperature of 260 °C was used for the other analyses.

A preliminary investigation on the appropriate SPME needle height in the gas chromatography injection port (adjusting the black needle guide) showed that a 4 cm depth yields the highest desorption.

The GC oven was programmed as follows: 2 min at 50 °C, first ramp, 25 °C/min to 160 °C and 2 min at 160 °C; second ramp, 5 °C/min to 230 °C; and third ramp, 10 °C/min to 300 °C and 5 min at 300 °C. The total analysis time for one GC run was approximately 35 min (retention times of each target PCP in Table 1, 6th column).

The GC-MS interface and ion source temperature were kept at 280 and 270 °C, respectively. MS acquisition was performed in the positive electron impact mode at 70 eV under full-scan mode (40–400 *m/z* range) and single ion monitoring mode, specific fragments were selected to identify and quantify each target PCP (*m/z* values of the most abundant characteristic ion fragments in Table 1, 7th column).

Experimental design

The experimental design approach using the inscribed central composite design (CCD) model [29–31] was employed to investigate four factors, namely extraction temperature and time, desorption temperature and time, in order to optimize the SPME extraction yield. Response surface methodology was applied to mathematically fit the

experimental domain studied in the design of experiments through a response function. The CCD permits the response surface to be modeled by fitting by second-order polynomial models through the least squares method. [29, 30]. For all statistical work, the MATLAB™ 7.0 software program was used for statistical evaluation of data obtained in all optimization procedures.

Results and discussion

Optimization of SPME operative parameters

From the literature [22–26] and from the results of screening, four factors were identified as able to influence extraction recovery, namely: extraction temperature and time and desorption temperature and time. An inscribed CCD approach was employed, where the design is divided into three groups of design points: a two-level factorial design with axial (coded with ±1), "star" (coded ±α), and center points (coded 0) (Table 2). The star points allow curvature estimation in the model; the center points permit estimation of the "pure error" in the system. In the present work, each variable was set at five separate coded levels: −1, −α, 0, +α, and +1 (Table 2) to yield a total of 27 experiments, with three repetitions of the central point (Table 3).

The factors were investigated inside the operative limits imposed by experimental requirements: 30–90 min and 40–80 °C for extraction time and temperature, respectively, and 2–12 min and 190–270 °C for desorption temperature and time (Table 2). The upper desorption temperature limit is given by the thermal stability of the fiber.

The optimization study was performed on eight target PCPs (Table 1, compounds 1–8). All the experiments were performed in duplicate, and their order was randomized to avoid possible carryover effects of the analytical apparatus. The area of a single PCP or their sum (as relative value referred to the internal standard area) was selected as response function (Y) (Table 3, 6th column, mean values of the two repeated measurements).

Table 2 Experimental design domain and optimized values (last line)

	Extraction		Desorption	
	Time t_{ex} (min)	Temperature T_{ex} (°C)	Time t_{des} (min)	Temperature T_{des} (°C)
−1	30	40	2	190
−α	45	50	4.5	210
0	60	60	7	230
+α	75	70	9.5	250
+1	90	80	12	270
Optimized	90	80	11	260

The general empirical equation of the second polynomial containing quadratic terms was examined to describe the response values, Y, explaining the nonlinear nature of response. A second-order pure quadratic equation gave the best fitting degree, according to the general equation:

$$Y = b_0 + b\,x_1 t_{ex} + b\,x_2 T_{ex} + b\,x_3 t_{des} + b\,x_4 T_{des} + b\,x_1{}^2 t_{ex}{}^2 + b x_2{}^2 T_{ex}{}^2 + b\,x_3{}^2 t_{des}{}^2 + b x_4{}^2 T_{des}{}^2 \quad (1)$$

Where Y is the extraction yield, b_0 the intercept; bx_1, bx_2, bx_3, and bx_4 the constants or equation parameters; t_{ex}, the extraction time; T_{ex}, the extraction temperature; t_{des}, the desorption time; T_{des}, the desorption temperature. The model was applied to fit the total response Y_{tot} computed by adding the response area for the eight investigated PCPs as well as the response of each single PCP. In order to ensure a good model, the significance of the regression model and individual model factor were tested by applying the analysis of variance. The statistical significance of the estimated parameters was evaluated by applying Student's t test: the variables were considered irrelevant when the significance levels (p) were greater than 0.05. Final predictive equations were computed after ruling statistically insignificant terms out of the models (Table 4). The obtained results show that only the first order extraction temperature and time are statistically significant (p value< 0.05) for the total extraction recovery as well for the response of most PCPs (Table 4). The exceptions are fluorene (FLU), benzophenone (BP), phenanthrene (PHEN), and musk ketone (MK) response, for which also other terms are statistically significant, i.e., the second-order term of extraction temperature (FLU and PHEN, Table 4, 2nd and 3rd rows), as well as extraction time (FLU, 2nd row) or desorption temperature (BP and PHEN, 3rd and 4th rows), or desorption time (MK, 6th row). No statistically significant parameters were found to describe the BHA response: this result may be the consequence of the low concentration level (30 ppb) used in the optimization study, i.e., comparable to the Limit of Quantification for this compound (Table 5, 1st row).

In addition, the same full quadratic model was applied to fit the values describing data dispersion, Y_D, computed as the mean values of the two repeated measurements, obtaining the following equation:

$$Y_D = 7.39 - 1.38\,t_{ex} + 0.67\,T_{ex} + 1.54\,t_{des} + 0.38\,T_{des} - 1.74\,t_{ex}{}^2 - 4.27 T_{ex}{}^2 - 5.27 t_{des}{}^2 - 2.08 T_{des}{}^2 \quad (2)$$

The Student's t test investigation of the equations calculated shows that no terms are statistically significant for data dispersion (at 95% of confidence level).

Table 3 Central composite design experiments (with three repetitions of the central point) and results for all analytes referred to internal standards

	Extraction		Desorption		Total response
Experiment no.	Time (min)	Temperature (°C)	Time (min)	Temperature (°C)	∑ peaks areas/internal standard area
1	45	50	4.5	210	8.75
2	45	50	4.5	250	8.77
3	45	50	9.5	210	8.22
4	45	50	9.5	250	8.64
5	45	70	4.5	210	10.23
6	45	70	4.5	250	11.29
7	45	70	9.5	210	11.21
8	45	70	9.5	250	11.22
9	75	50	4.5	210	8.98
10	75	50	4.5	250	9.80
11	75	50	9.5	210	9.82
12	75	50	9.5	250	9.55
13	75	70	4.5	210	12.12
14	75	70	4.5	250	16.08
15	75	70	9.5	210	15.00
16	75	70	9.5	250	16.17
17	60	60	7	230	10.72
18	60	60	7	230	9.90
19	30	60	7	230	9.97
20	90	60	7	230	10.83
21	60	40	7	230	8.12
22	60	80	7	230	15.23
23	60	60	2	230	9.78
24	60	60	12	230	9.90
25	60	60	7	190	9.70
26	60	60	7	270	10.13
27	60	60	7	230	10.55

To better visualize the effect the significant parameters have on the recovery yield, the fitted models are separately plotted as a function of a single variable (i.e., extraction temperature or time) in the exploited range, keeping the other factors constant (Fig. 1a, b). The experimental parameters show specific effects on the recovery yield for each single PCP: both positive and negative effects are described by the plots, the line slopes proportional to the

Table 4 Second-order pure quadratic equations describing the PCP response for all the PCPs (first line) and each individual compound (2nd–8th lines)

compound	Equation	Variable of significance
Total response	$Y = 10.29 + 1.74t_{ex} + 3.75T_{ex} + 0.442{t_{ex}}^2 + 1.72{T_{ex}}^2$	t_{ex}, T_{ex}
FLU	$Y = 0.59 - 0.17t_{ex} - 0.29T_{ex} + 0.12{t_{ex}}^2 + 0.12{T_{ex}}^2$	t_{ex}, T_{ex}, ${t_{ex}}^2$, ${T_{ex}}^2$
BP	$Y = 0.037 - 0.0083\,t_{ex} - 0.017T_{ex} + 0.01\,T_{des} + 0.011\,{t_{ex}}^2 - 0.0094\,{T_{ex}}^2 + 0.0005\,{T_{des}}^2$	t_{ex}, T_{ex}, T_{des}
PHEN	$Y = 0.91 - 0.13\,t_{ex} - 0.39\,T_{ex} + 0.048\,T_{des} + 0.093\,{t_{ex}}^2 + 0.16\,{T_{ex}}^2 + 0.048\,{T_{des}}^2$	t_{ex}, T_{ex}, T_{des}, ${T_{ex}}^2$
ALD	$Y = 3.5 + 0.96\,t_{ex} + 1.7\,T_{ex} + 0.10\,{t_{ex}}^2 + 0.61\,{T_{ex}}^2 -$	t_{ex}, T_{ex}
MK	$Y = 0.40 - 0.042\,t_{ex} - 0.13\,T_{ex} + 0.0017\,t_{des} + 0.031\,{t_{ex}}^2 + 0.026\,{T_{ex}}^2 - 0.009\,{t_{des}}^2$	t_{ex}, T_{ex}, t_{des}
2,4′-DDD	$Y = 2.5 + 0.75\,t_{ex} + 1.3\,T_{ex} - 0.036\,{t_{ex}}^2 + 0.65\,{T_{ex}}^2$	t_{ex}, T_{ex}
CRY	$Y = 1.25 + 0.37\,t_{ex} + 1.49\,T_{ex} + 0.14\,{t_{ex}}^2 + 0.15\,{T_{ex}}^2$	t_{ex}, T_{ex}

Equations were calculated considering only the statistically significant variables (listed in the 3rd column)

FLU fluorene, *BP* benzophenone, *PHEN* phenanthrene, *ALD* aldrin, *MK* musk ketone, *CRY* chrysene

Table 5 Evaluation of the analytical performance of the SPME procedure under the optimized conditions: upper linearity range, detection and quantification limits, and reproducibility

Analyte	Up-linear range (ppb)	r^2	LOD (ppb)	LOQ (ppb)	RDS%[c]
MilliQ water[a]					
BHA	150	0.990	5.3	21	
FLU	100	0.998	0.035	0.75	
BP	150	0.991	7.3	13	
PHEN	100	0.998	0.55	0.79	
ALD	15	0.990	0.051	1.5	
MK	50	0.996	0.71	1.5	
2.4′-DDD	15	0.989	0.024	0.98	
CRY	50	0.998	0.71	1.5	
Tap water[b]					
BHA	58.9	0.936	3.8	6.8	1.32
FLU	27.9	0.932	0.071	1.6	0.021
BP	58.9	0.910	8.5	14.3	1.24
PHEN	27.9	0.941	0.83	2.1	0.056
ALD	15	0.978	0.08	2.5	0.34
MK	58.9	0.989	0.9	2.1	0.044
2.4′-DDD	27.9	0.993	0.072	1.9	0.057
CRY	58.9	0.921	2.2	5.3	0.014
NAP	58.9	0.936	3.2	7.1	0.18
BHT	58.9	0.935	0.014	3.5	0.26
LIN	58.9	0.889	5.1	10.2	1.35
HHCB	15.5	0.988	0.0010	0.87	0.058
MX	27.9	0.998	0.28	0.72	0.007
DIBP	58.9	0.987	0.8	2.2	0.069
HEPT	58.7	0.991	0.53	1.7	0.033
FLNT	58.9	0.983	2.6	6.2	0.17
PYR	58.9	0.943	2.8	6.3	0.040
HEOD	58.9	0.993	0.005	1.5	0.029
4,4′-DDD	58.9	0.988	0.27	0.81	0.011
OC	27.9	0.998	0.05	0.13	0.014
DOP	27.9	0.953	0.95	1.9	0.024
BKF	58.9	0.937	1.8	3.2	0.030
BAP	58.9	0.954	1.6	3.3	0.012

FLU fluorene, *BHA* butylated hydroxyanisole, *BP* benzophenone, *PHEN* phenanthrene, *ALD* aldrin, *MK* musk ketone, *CRY* chrysene, *NAP* naphthalene, *BHT* butylated hydroxytoluene, *LIN* lindane, *HHCB* galaxolide, *MX* musk xylene, *DIBP* di-isobutylphthalate, *HEPT* heptachlor, *FLNT* fluoranthene, *PYR* pyrene, *HEOD* dieldrin, *OC* octocrylene, *DOP* dioctylphtalate, *BKF* benzo[k]fluoranthene, *BAP* benzo[a]pyrene

[a] Extraction from spiked MilliQ water samples (eight points in the 0.1 to 150 ppb range; 1st–8th rows)

[b] Extraction from spiked tap water samples (six points in the 0.1 to 58.9 ppb range; 9th–32nd rows)

[c] Intra-day reproducibility of PCP extraction from 50 ml of tap water, at a concentration of 58.9 ppb

degree of the effect. The extraction temperature shows a positive trend for FLU, ALD, CRY, 2,4'-DDD, MK, and PHEN, while only BP shows a negative dependence (Fig. 1a). The dependence of the recovery yield on the extraction time is positive for most of the target PCPs, displaying a positive dependence with the time; the exception being 2,4'-DDD which shows a slightly negative trend (Fig. 1b).

With the aim of developing a multi-residue method, the optimum operating conditions have to be selected according to the total cumulative response by combining individual target PCP patterns (green triangles in Fig. 1a, b): a linear positive dependence prevails suggesting that the recovery yield increases as extraction temperature and time increase. In addition, the lowest data dispersion is obtained at the upper level of the investigated values. It must be noted that the exploited domain is limited by instrumental operating constraints so that operative conditions cannot be experimentally extended to higher values. Therefore, the highest SPME yield can be obtained by operating at the upper limits of an extraction temperature of 80 °C for a duration of 90 min. In all the fitted models, the desorption parameters (temperature and time) display no statistically significant effects on the response of each target PCP; desorption temperature shows a slightly positive effect only for BP and PHEN and desorption time only for MK (Table 4, 3rd, 4th, and 6th rows). Therefore, parameter values close to the border values were selected for desorption temperature and time, i.e., 260 °C and 11 min, respectively, since they ensure higher performance and smaller data dispersions for the totality of investigated PCPs.

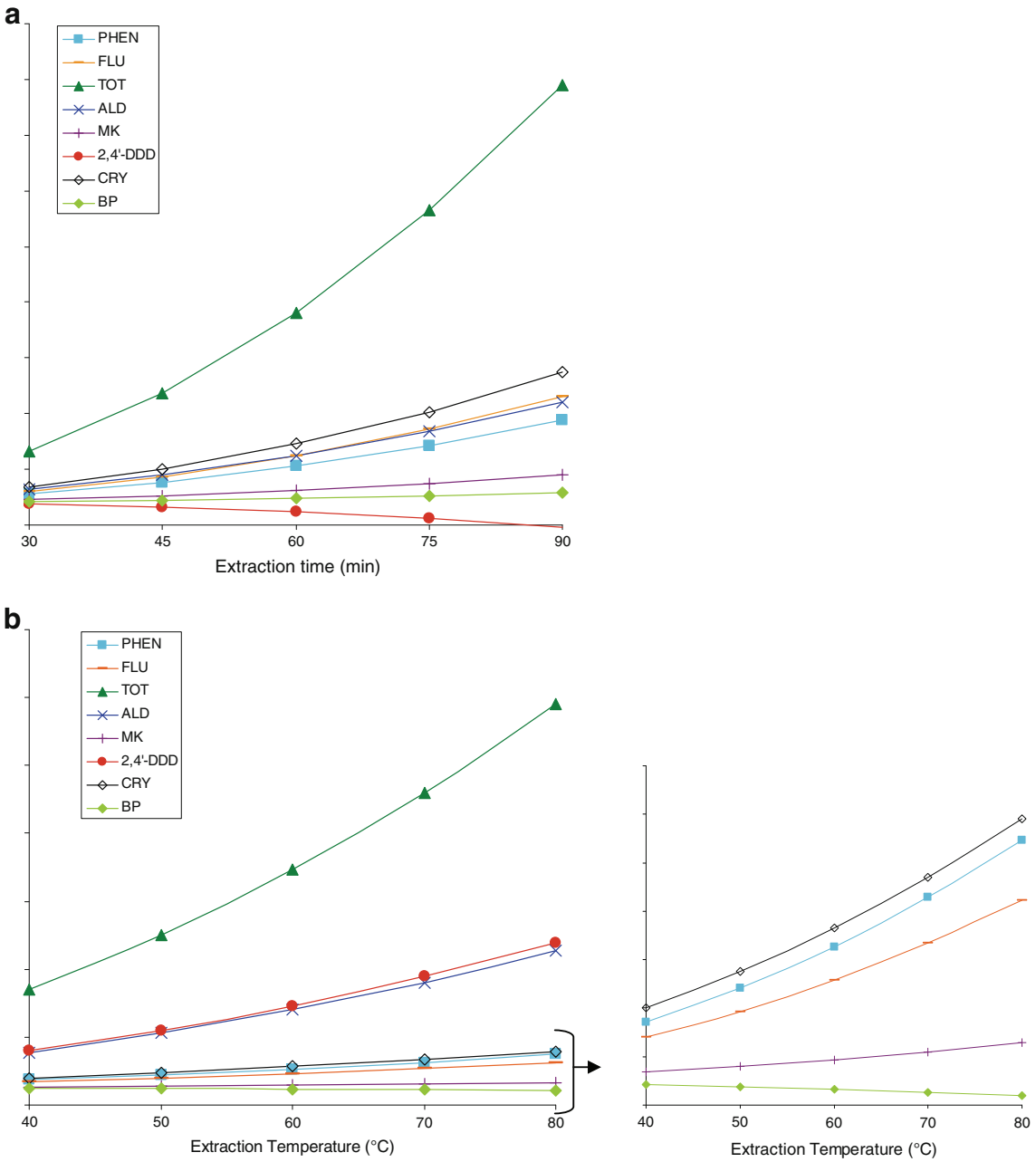


Fig. 1 Dependence of extraction yield as a function of the extraction time (**a**) and extraction temperature (**b**), behavior of single compounds (where this factor is significant for recovery) and plots for the total response (*green triangles*) and each single PCP: *sky blue squares*, PHEN; *orange dash*, FLU; *blue error marks*, ALD; *violet plus sign*, MK; *red circles*, 2,4'-DDD; *black empty diamonds*, CRY; and *light green filled diamonds*, BP

Consequently, for all the compounds, the optimum SPME operating conditions have been defined as: extraction time, 90 min; extraction temperature, 80 °C; desorption time, 11 min; desorption temperature, 260 °C (Table 2, last row).

Evaluation of method analytical performance

The analytical performance of the SPME procedure under the optimized conditions was investigated by evaluating measurement reproducibility and method accuracy for quantitative determinations. It has been widely reported that the main drawback of the SPME procedure is its low reproducibility, since the recovery yield strongly depends on many uncontrollable operative conditions [22–26]. Therefore, specific experimental measurements were devoted to testing the precision of the optimized procedure.

The inter-day reproducibility of Quintozene peak area was evaluated during the analysis period: percent relative

standard deviation (RSD%) was 8.4% in 8 days. The inter-day precision was also evaluated for all the PCPs by measuring the GC-MS signals (concentration level of 3.1 ppb) on different days: relative standard deviations lower than 8% were obtained for all target PCPs.

The intra-day precision was evaluated for each target PCP at a concentration level of 58.9 ppb: a good measure precision was obtained for all the investigated PCPs with RSD% values varying in the 0.01–1.3% range (Table 5, 6th column).

Method linearity for quantitative analysis was tested by evaluating the calibration curves: standard solutions of the eight target PCPs (compounds 1–8 in Table 1) were analyzed at different concentration levels in MilliQ water varying in the 0.1 to 150 ppb range (0.1, 1, 5, 15, 30, 50, 100, and 150 ppb). Each concentration was analyzed twice. The linearity range was evaluated and instrument detection limits (LOD) were computed as 3:1 signal-to-noise value while the quantification limits (LOQ) were computed as 10:1 signal-to-noise values (Table 5, 1st–8th rows). Good linearity was observed for most of the PCPs, correlation coefficients ranging from 0.989 to 0.998. The limits of detection were between 0.024 and 7.3 ppb, similar to those reported in the literature on SPME methods for a single, or max two, PCP class(es) [22–26, 31–34].

The evaluation of the procedure performance was also extended to the whole list of 23 PCPs belonging to different chemical classes (Table 1, compounds 1–23). In order to test the applicability to real matrices, the standard solutions were prepared in tap water (filtered in 0.45 μm filter before the extraction) and the extraction was performed on 50 ml of the sample (containing 0.2% of methanol). The method linearity was evaluated on six-point calibration curves (0, 0.9, 3.1, 9.3, 15.5, 27.9, and 58.9 ppb). The obtained results (Table 5, 9th–31st rows) show good analytical performance described by good linearity (correlation coefficients between 0.910 and 0.998) and high sensitivity, i.e., LOD values varying from 0.005 to 8.5 ppb.

Conclusions

The results of this study confirm that optimization using central composite design and response surface methodologies is an extremely efficient tool for fast, complete optimization of the parameters affecting the extraction efficiency throughout the analytical procedure. A simple SPME method has been developed for the simultaneous GC-MS determination of various PCPs at trace levels in water. As a solvent-free method for sample preparation, SPME offers the benefits of high sensitivity and high sample throughput, thus making it a good alternative to conventional LLE and SPE for complex environmental matrices. These properties are particularly relevant for multi-residue methods, such as the present case of a large variety of PPCPs, since they require the joint extraction and determination of many compounds displaying a broad range of polarities. Under the optimized conditions the procedure provides low detection limits (≤4 ppb) and satisfactory reproducibility (RSD%≤1%) for most of the PCPs investigated: this result makes the developed method suitable for comprehensive chemical profiling of PCPs in various aqueous matrices. The developed method may be used as the basis for wastewater monitoring of temporal and spatial changes in target PCPs and can easily be extended to nontarget compounds with similar physicochemical characteristics.

References

1. Daugthon CG, Jones-Lep T, Washington D (2001) Pharmaceuticals and personal care products in the environment: scientific and regulatory issues. In: ACS symposium series 791, American Chemical Society. Oxford University Press, Washington
2. Peck AM (2006) Anal Bioanal Chem 386:907–939
3. Daughton CG (2004) Environ Impact Assess 24:711–732
4. Barcelo D, Petrovic M (2007) Anal Bioanal Chem 387:1141–1142
5. U.S. Environmental Protection Agency (2009) Pharmaceuticals and personal care products as pollutants. Available at: http://www.epa.gov/esd/chemistry/ppcp/. Accessed 15 December 2009
6. European Commission Environment (2010) Available at: http://ec.europa.eu/environment/water/
7. Richardson SD (2006) Anal Chem 78:4021–4045
8. Pawliszyn J (1997) Solid phase micro extraction: theory and practice. Wiley, New York
9. Arthur CL, Pawliszyn J (1990) Anal Chem 62:2145–2148
10. Lord H, Pawliszyn J (2000) J Chromatogr A 885:153–193
11. Pietrogrande MC, Basaglia G, Dondi F (2009) J Sep Sci 32:1249–1261
12. Rodil R, Quintana JB, Basaglia G, Pietrogrande MC, Cela R (2010) J Chromatogr A 1217:6428–6435
13. Magdic S, Pawliszyn JB (1996) J Chromatogr A 723:111–122
14. Buchholz KD, Pawliszyn J (1993) Environ Sci Technol 27:2844–2848
15. Llompart M, Blanco B, Cela R (2000) J Microcol Sep 12:25–32
16. Landin P, Llompart M, Lourido M, Cela R (2001) J Microcol Sep 13:275–284
17. Llompart M, Lourido M, Landin P, Garcia-Jares C, Cela R (2002) J Chromatogr A 963:137–148
18. Polo M, Llompart M, García-Jares C, Cela R (2005) J Chromatogr A 1072:63–72
19. Polo M, Gómez-Noya G, Quintana JB, Llompart M, García-Jares C, Cela R (2004) Anal Chem 76:1054–1062
20. Polo M, Gómez-Noya G, Llompart M, Garcia-Jares C, Bollaín MH, Cela R (2006) J Chromatogr A 1124:11–21
21. Polo M, García-Jares C, Llompart M, Cela R (2007) Anal Bioanal Chem 338:1789–1798
22. Regueiro J, Llompart M, Garcia-Jares C, Cela R. J (2009) Chromatogr A 1216:2816–2824
23. Regueiro J, Becerril E, Garcia-Jaresa C, Llompart M (2009) J Chromatogr A 1216:4693–4702
24. Bianchi F, Mattarozzi M, Careri M, Mangia A, Musci M, Grasselli F, Bussolanti S, Basini G (2010) Anal Bioanal Chem 396:2639–2645
25. Fernandez-Alvarez M, Lamas JP, Sanchez-Prado L, Llompart M, Garcia-Jares C, Lores M (2010) J Chromatogr A 1217:6634–6639

26. Pietrogrande MC, Basaglia G (2007) Trends Anal Chem 26:1086–1103
27. Scheyer A, Morville S, Mirabel P, Millet M (2006) Anal Bioanal Chem 384:475–487
28. Mitra S (2003) Sample Preparation Techniques in Analytical Chemistry. Wiley, New York
29. Leardi R (2009) Anal Chim Acta 652:161–172
30. Massart DL, Vandeginste BGM, Deming SN, Michotte Y, Kaufman L (1988) Chemometrics: a textbook. Elsevier, Amsterdam
31. Dejaegher B, Vander Heyden Y (2009) Acta Chromatogr 21:161–201
32. Poiger T, Buser H-R, Balmer ME, Bergqvist P-A, Müller MD (2004) Chemosphere 55:951–963
33. Llompart M, García-Jares C, Salgado C, Polo M, Cela R (2003) J Chrom A 999:185–193
34. Ferrari F, Sanusi A, Millet M, Montury M (2004) Anal Bioanal Chem 379:476–483

Paper 6

G. Basaglia, M.C. Pietrogrande, “Optimization of multi-residual GC-MS determination of Pharmaceuticals and Personal Care Products in water using Solid Phase Micro Extraction with On-Fiber derivatization”, *submitted for publication*

Optimization of multi-residual GC-MS determination of Pharmaceuticals and Personal Care Products in waters using Solid Phase Micro Extraction with On-Fiber derivatization

G. Basaglia, M. Chiara Pietrogrande

Department of Chemistry, University of Ferrara, Via L. Borsari, 46, I-44100 Ferrara, Italy.
E. mail: Chiara.pietrogrande@unife.it

Abstract

A headspace solid phase microextraction coupled to gas chromatography with mass spectrometry detection (HS-SPME-GC-MS) method was optimized for the simultaneous determination in water samples of 21 target Pharmaceuticals and Personal Care Products (PPCPs), including fragrances, UV-filters, antiseptics, estrogens, antinflammatory drugs, and pesticides. Microextraction was carried out over water sample in direct immersion using a polyacrylate fiber (PA, 85 μm) fibre, coupled with silylation in the headspace of a 2 ml vial. A chemometric approach consisting of an experimental design (design of experiments, DOE) was applied to systematically investigate and optimize the operative parameters that affect the extraction recovery, namely, extraction temperature and time, derivatization time, desorption temperature and time.

The optimum operating conditions, those yielding the highest recovery for all the compounds, were: an extraction time of 125 min and temperature of 40°C, derivatization time of 30.5 min and a desorption time of 2 min and temperature of 300°C.

In these optimized conditions a good reproducibility was assessed as RDS% values less than 10% and less than 20%, for underivatized and derivatized compounds, respectively. The method detection limit (LOD) was computed from the calibration curves: the best sensitivity was obtained for less polar compounds with LOD values lower than 5.0 ppb, while the most of more polar derivatized PPCPs show LOD values lower than 40 ppb. The method accuracy was evaluated on tap water spiked samples: recoveries varied from 75 to 110 % and from 85 to 103% for derivatized and non derivatized compounds, respectively.

These values prove that the optimized procedure achieves analytical performance similar to that of the existing methods concerning a single or max two, PPCP class, and this result makes it suitable for the determination of PPCPs in waters at trace level.
Keywords: Pharmaceuticals and personal care products, Solid-phase microextraction, On-fiber SPME derivatization, Gas chromatography-mass spectrometry, Multi-residue method, Experimental design.

Introduction

During the last decade, Pharmaceutical and Personal Care Products (PPCPs) have been classified the most frequently detected organic contaminants in the aquatic environment. Consequently great research interest has been devoted to assess their toxic effects on environment and living organisms.[1-7]. Given the constant anthropogenic input of PPCPs, due to their widespread use in various human activities, significant concentrations (from ng/L to hundreds of μg/L [4,5,8,9]) have been found in different environmental water. Therefore, there has been growing public interest in monitoring this class of contaminants, and following the principle of precaution, the EU Water Framework Directive has identified some PPCPs as future emerging priority candidates for monitoring and regulation [10,11].

Therefore, there is critical need for efficient and reliable analytical methods to address the occurrence, concentration, and fate of the PPCPs in natural waters. Based on current analytical protocols, methods of PPCP monitoring consist of extraction for isolation and preconcentration of the target analytes, purification procedures, chromatographic separation and mass spectrometric characterization and detection.

Among the several extraction techniques used up to date to extract and preconcentrate PPCPs from water samples, solid phase microextraction (SPME) is favoured as a simple, easily automated technique that does not require organic solvents [12-25]. One advantage of this technique is integration of sampling, extraction, concentration and sample GC injection into a single step to strongly reduce labour and time of sample pre-treatment, that typically accounts for over 60% of the total analysis time [12,24,25], Nowadays, SPME

has been widely used in a broad field of analyses, including the environmental, food and drug abuse areas [13-23].

Usually, analysis is mainly focusing on only one group of compounds with similar physicochemical properties (e.g. molecular weight, Log Kow, water solubility and pKa) and structures, while multi-residue methodologies have to be preferred in environmental monitoring in order to achieve the simultaneous analysis of a wide range of analytes.

In a previous work a SPME-GC procedure coupled with GC-MS method has been developed for a multi-residue determination of a large number of PCPs, including pesticides, fragrances, PAHs, UV-filters [26]. Here the method is extended to pharmaceuticals such as estrogens, anti-inflammatory drugs[2,8,13,27]. The target analytes were selected among those most frequently found in the environment, the most world's people consumed and the most potential toxicologically damaging.

If Gas chromatography-mass spectrometry (GC-MS) is chosen for PPCP analysis, because of its superior separation and identification capabilities, sample derivatization is required for polar acidic compounds , such as antinflammatories, estrogens, antiseptics, BisphenolA, in order to increase analyte volatility and thus improve GC separation and detection sensitivity. Different derivatization approaches could be used in combination with SPME procedure: 1. derivatization in the sample matrix, 2. derivatization in the GC injector port, and 3. derivatization in the SPME fiber coating.

The headspace derivatization on-fiber is usually preferred because it minimizes the matrix interferences and prolongs the fibre coating lifetime.

To find optimal operative conditions it is not a simple task, due to the large number of variables involved in the analytical procedure, namely, derivatization conditions, temperature and time of extraction and temperature of desorption. For this reason, multivariate methods of optimization, including central composite design and response surface methods, have been used to investigate and optimize the variables to obtain the highest sensitivity of all the analytes, with a reduced number of experiments.

2. Experimental

2.1 Reagents and standards

Twenty one PPCP compounds were investigated. All standards were of the highest purity commercially available; they were obtained from Lab Service Analytical S.r.l. (Bologna, Italy), Carlo Erba Reagenti (Milano, Italy), VWR International (Pennsylvania, USA) and Sigma–Aldrich (Steinheim, Germany). All solvents were trace analysis grade from 99.7%.
Eight of them were selected as target molecules for the SPME procedure optimization to represent the different chemical classes (Table 1, numbered from 1 to 8): the choice was based on a representatively large range of physico-chemical properties affecting their environmental impact, i.e., volatility, water solubility, octanol–water partitioning coefficient (Table 1).
Pentachloronitrobenzene (quintozene, Table 1, last row) was used as internal standard in the GC-MS analysis.
Reagent used for the derivatization (BSTFA, 1% trimethylchlorosilane) was obtained from Aldrich Chemical Co. (Milan, Italy). Preliminary derivatization experiments were done in order to check the products of reaction. For each eleven derivatized compounds (indicated in Table 1 with prefixes TMS- and di-TMS) only one product was formed, no subproducts were detected.
The standard PPCPs were individually dissolved in pure methanol (MeOH) at 1000 ppm, and then diluted with MeOH in mixed solutions at concentration levels of 150 ppm, 250 ppm and 280 ppm. The SPME optimization was carried out using a final concentration of 125 ppb for BHT, ALD and PYR, 75 ppb for HHCB and 1.05 ppm for the other compounds, in 40 ml of tap water in 40 mL amber vial. The drinking water was previously filtered in 0.45 μm mixed cellulose ester filter (Whatman GmbH).
The derivatizing agents, as well as the individual and composite standard solutions, were stored at 4°C.
For the 21 target PPCPs (Table 1) an eight point calibration curve was constructed by diluting the concentrate MeOH solution with tap water to achieve concentration levels of 0,

3.75, 7.5, 15, 18.75, 37.5, 67.5, 135 ppb for less polar compounds and 0, 15, 30, 60, 75, 150, 270, 540 ppb for derivatized analytes.

All the calibration curves were carried out by appropriately diluting the concentrate solutions in 40 mL of water. In all the diluted solutions, a constant MeOH volume was added (1%) in order to avoid changes in extraction yield due to the methanol content. All the materials used in the analysis have to first be proved free of interferences and this is done by running reference matrix method blanks. Proper cleaning of glassware is extremely important, because glassware may not only contaminate the samples but it can even remove the analytes of interest by adsorption on the glass surface.

2.2 SPME

The used SPME fibers were coated with polyacrylate (PA) at 85 µm film thickness (supplied by Supelco, Bellefonte, USA) and housed in a manual holder. This sorbent was selected because of it shows better efficiency in the extraction of polar compounds [14,28-32]. Some preliminary proofs were performed testing polydimethylsiloxane (PDMS) at 100 µm thickness, because of the highest versatility: indeed, it is generally used for extraction of a wide range of analytes with different polarities and volatilities [2,13,26,27,33-36], however this fiber shows no possibility of derivatization: when it was in contact with derivatization agent vapours the fiber support broken. Shao et al. [19] observed that the PDMS fiber swell in the BSTFA vapour phase treatment during the derivatization step, which would inevitably damage the fiber coating and support; this could be the cause of the support breaking.

Following the conditioning guidelines, the fibres were conditioned under helium at a flow-rate of nearly 1.0 mL/min with the split valve open (to reduce the amount of impurities entering the column) in the hot injection port of a gas chromatograph at 280°C for 1 h prior to use. Additionally, the SPME fibers were conditioned for 15 minutes at 280°C every day before use and they were systematically cleaned at 280°C for 20-30 minutes after every extraction. The blanks were tested by thermal desorption (5 min in the injection port)

followed by GC analysis to confirm that all compounds were desorbed and prevent the fiber's memory effect.

Preliminary experiments were performed to evaluate the fixed extraction condition. A direct immersion (DI-SPME) mode was used for extraction from a fixed sample volume of 40 ml of water in a 40 ml amber vial. Samples were immersed in a thermostatic water bath at a given temperature (having a precision of ±1°C) for 10 minutes to equilibrate before SPME insertion. The samples were maintained under controlled agitation with a magnetic stirrer (500 rpm) in order to transport analytes from the bulk of the solution to the vicinity of the fiber and facilitate rapid extraction.

After finishing the extraction step the SPME fiber was removed and dried in a nitrogen stream in order to eliminate the presence of water. The fiber was then placed in the headspace of 2 ml vial containing 40 µl of BSTFA (1% TMCS), stored in a thermo block (2050-1CESUP, Barnstead/Lab-Line, Melrose Park, IL, USA) at 35°C. The derivatizing agent was left for 10 minute in the thermo block to equilibrate before SPME derivatization. The derivatization temperature was fixed at a relative low temperature because of the fiber coating preservation and to prevent the possible lost of extracted compounds from the fiber. It's known that the BSTFA would destroy the fiber coating at high silylation temperature, Luan et al. [16] report that a PA fiber can only be used six times at 60°C.

After extraction and derivatization, the fiber was inserted into the GC injector for analysis.

2.3 GC-MS Apparatus

The GC-MS system consisted of a Focus GC, PolarisQ GCMSn Benchtop IT Mass Spectrometer (Thermo Fisher Scientific, Bellefonte, PA, USA). Helium (99.999%) was used as carrier gas at a constant head pressure of 50 kPa. A fused-silica column RTX-5MS (DB5 30 mx0.25 mm I.D., 0.25 µm film thickness) was purchased from Thermo Fisher Scientific (Bellefonte, PA, USA).

In order to define the optimum GC condition for the best analyte separation, the PPCPs solution (after derivatization) was injection into a split/splitless injector in splitless mode; splitless time was 1.5 min, the injector temperature was maintained at 260°C.

For the SPME optimization procedure, an injector temperature varying in the 220°C-300°C range was investigated; an optimized temperature of 300°C was used for the other analyses. A preliminary investigation on the appropriate SPME needle height in the GC injection port (adjusting the black needle guide) showed that a 4 cm depth yields the highest desorption.
The GC oven was programmed as follows: 2 min at 50°C, first ramp 20°C/min to 130°C, second ramp 10°C/min to 300°C, 5 min at 300°C. The total analysis time for one GC run was approximately 35 min (retention times of each target PPCP in Table 1).
The GC-MS interface and ion source temperature were kept at 280 and 250°C respectively, MS acquisition was performed in the positive electron impact mode at 70 eV under full-scan mode (40–650 m/z range) and single ion monitoring (SIM) mode: specific fragments were selected to identify and quantify each target PPCP (m/z values of the most abundant characteristic ion fragments in Table 1, last column).

2.4. Data treatment and experimental design

An experimental design approach using the Face Centered Central Composite Design (CCD) has been applied for the optimization of the operative parameters that influence the SPME yield, as a proper methodology that allows optimization of analytical methods by carrying out a relatively small number of experiments. The Face Centered Central Composite Design model was chosen due to its widespread use and versatility [37-39]. Response surface methodology, RSM, was applied to mathematically fit the experimental domain studied in the DOE through a response function. The CCD permits the response surface to be modeled by fitting by second order polynomial models through the least squares method. [37,38].
For all statistical work, the MATLAB™ 7.0 software program was used for statistical evaluation of data obtained in all optimization procedures.

3. Results and discussion

3.1. Optimization of SPME operative parameters

From the literature data [13,14,26,28,29,31,33-35] and the preliminary studies five factors have been established as the most significant variables affecting the extraction efficiency, namely: extraction temperature and time, derivatization time, and desorption temperature and time. Preliminary studies have shown that derivatization temperature in the range between 30°C and 50°C has little, or none effect, on the reaction yield: values higher than 70°C favour the loss of the derivatized analytes and damages the fiber coating.

The optimization process involves three major steps, namely, performing the statistical design experiments, estimating the coefficients of the mathematical model fitting the experimental values, and predicting the response in order to check the adequacy of the model.

A Face Centered CCD model was employed: itconsists of a factorial design with center and star points, at the center of each face of the factorial space, so $\alpha=\pm1$. This design provides relatively high quality predictions over the entire design space and do no require using points outside the original factor range. The experimental domain was defined taking into account operative limits imposed by experimental requirements, namely: 5-125 min and 30°C-70°C for extraction time and temperature respectively, 0.5-30.5 min for derivatization time, and 2-12 min and 220°C-300°C for desorption temperature and time. The upper desorption temperature limit is given by the thermal stability of the fiber; longer extraction and desorption times as well as derivatisation times greater would determine long analysis times. Moreover no longer derivatization time is requiring, as results from literature [14,26,28,29,31].

The CCD model requires three levels of each factor, coded : -1, 0, +1 (Table 2 shows the different factor levels) to yield a total of 36 experiments, including face centered points and 10 replicates of the central point, with the purpose of obtaining a good estimation of the experimental variability. Factor experimental values for each design point are detailed in Table 3.

The optimization study was performed on 8 target PPCPs (Table 1, compounds 1-8). The order of all the experiments was randomised to avoid possible carryover effects of the analytical apparatus. As response function (Y) the chromatographic area of each single PPCP was considered or the sum of the logarithms (for more homogeneous response scale) of all the PPCP areas for a comprehensive model (Table 3, last column, sum of area logarithms).

The general empirical equation of the second polynomial containing quadratic and interaction terms was examined to describe the response values, Y, explaining the non-linear nature of response. A second-order full quadratic equation gave the best fitting degree, according to the general equation:

$$Y_x = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i \neq j} \beta_{ij} x_i x_j + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \varepsilon_x$$

where Y_x is the extraction yield, n is the number of factors, Y_x denotes the response observed for combination X = (x_1, x_2, . . ., x_n), and ε_X the random error variables. The parameter β_i represents the linear effect of the i^{th} factor. The parameter β_{ii} represents the quadratic effect of the i^{th} factor, and β_{ij} represents the cross-product effect, or interaction effect, between the i^{th} and j^{th} factors.

The model was applied to fit the response of each single PPCP, as well as the total response Y_{tot} computed by adding the logarithm of response areas for the 8 investigated PPCPs. For each regression equation, the main and interaction effects were calculated. In order to ensure a good model, the significance of the regression model and individual model factor were tested by applying the analysis of variance (ANOVA). The statistical significance of the estimated parameters was evaluated by applying Student's *t*-test: the variables were considered irrelevant when the significance levels (*p*) were greater than 0.05. Final predictive equations were computed after ruling statistically insignificant terms out of the models (Table 4).

The results obtained from the response of each single PPCP (table 4, 1st-8th lines) show that the extraction time gives significant effects for all the less polar compounds: BHT, HHCB, ALD, and PYR. The sign of this effect is positive showing that the signal is improved by increasing the extraction time level.

While there's not a common significant factor for the polar analytes, i.e. BP3, TCS, BPA, and E2 Extraction temperature negatively influences the yield of BHT, TCS and BPA. Desorption conditions are significant only concerning temperature for BP3 and BPA. The term describing derivatization time is significant for TCS and BPA yield, displaying a positive influence; in addition it's statistically significant for BHT, in the interaction term with extraction time, displaying a negative effect. No statistically significant parameters were found to describe the E2 response (Table 4, 9th line), probably because of the low reproducibility of its determination that is about 25% (inter-day RDS%).

For describing the total extraction recovery Y_t the most statistically significant factor is the second order extraction temperature (99.99% of confidence level), in addition to other factors significant at 95% of confidence level, namely extraction temperature and time, and their interaction with desorption temperature and reaction time (Table 4, 1th line).

With the aim of developing a multi-residue method, the optimum operating conditions have to be selected according to the total cumulative response, in combination with individual target PPCP patterns.

A positive dependence prevails for extraction time and desorption temperature, suggesting that the recovery yield increases with parameter values: therefore, the optimized conditions are selected at the upper level of the exploited domain, i.e., 125 min of extraction time and 300°C for desorption temperature. An opposite pattern is shown by extraction temperature and desorption time parameters, to yield the highest response for the lowest ore close the lowest parameter values, i.e., extraction temperature of 40°C and desorption time of 2 min. The derivatization time parameter displays a controversial pattern: it negatively affects the cumulative response of all the studied PPCPs, so that the highest yield is obtained at the lower level of 0.5 min (Figg.1a and 1b). The same pattern is also displayed by the BHT recovery but an opposite behaviour is shown for TCS and BPA, to yield the highest response derivatizing for the longest time of 30.5 min. In order to clarify the behaviour of the experimental design model for total response and BHT, Fig. 1 shows the response surfaces for the pairs of variables involving in the statistically significant interaction term: derivatization and extraction time. In each case, the other variables not considered are set at their optimum values obtained from the CCD model.

As a consequence, the selection between lower, 0.5 min, or higher, 30.5 min, values is questionable, also by considering the aim of the present paper, namely the development a general multiresidue SPME procedure also extendible to more polar PPCPs requiring chemical derivatization, as TCS and BPA.

The experimental design model was validated by performing some replicated measurements using the experimental setting providing the optimized conditions (Table 2, last line), using both the derivatization time conditions: the experimental responses were measured for the cumulative response (refer to the sums of the PPCP area logarithms) and each PPCP signal. For each response, the predicted values were calculated using the equations reported in Table 4, and the confidence interval considering the standard deviation and the model leverage. A good agreement between predicted and experimental values was observed, demonstrating the validation of both the models (last lines in Table 3 report predicted and experimental values of the total response for both detivatization time conditions).

3.2. Extension to a wider list of PPCPs

With the aim of developing a general SPME procedure suitable for the simultaneous analysis of several PPCPs displaying a wide range of chemical properties - polarities, structures or activities - the optimized procedure was applied to a wider list of 21 PPCPs, including such as musk fragrances, phenolic compounds, estrogens and anti inflammatory drugs analysis (Table 1, lines 9-21). The tap water standard solutions (at a concentration level of 15 ppb for each less polar compound and 60 ppb for more polar ones) were submitted to the SPME procedure. Based on preliminary research and literature data, the tap water pH was adjusted to a value of 3, in order to guarantee high extraction yield for acidic compounds, such as the non-steroidal acidic anti-inflammatory drugs, namely NSAIDs: IP, KP and NP, [14,15].

In order to select the optimal derivatization time between the two values 0.5 and 30.5 min, the SPME optimized conditions (Table 2, last line) were applied to analyse the spiked tap water samples containing 21 PPCPs, varying the derivatization time. To estimate the

change in extraction yield, a % relative ratio was computed by comparing the response obtained by derivatizing for 30.5 min in comparison with a derivatization time of 0.5 min. Fig. 2 shows the % variation of extraction yield (mean values of three repeated measurements) for the analyzed compounds displaying a significant variation (higher than 1%). By increasing extraction time form 0.5 to 30.5 min, strongly increases the extraction yield for several compounds, in particular for IP, NP and KP showing an increase close to 100%. The only exception is Galaxolide, displaying a decrease of -8%. As consequence of this result, the longer derivatization time of 30.5 min was selected as the best operative condition for analysis of all the 21 PPCPs (Table 2, last line).

3.3. Evaluation of method analytical performance

The analytical performance of the SPME procedure under the optimized conditions was investigated by evaluating measurement reproducibility and method accuracy for quantitative determinations.

This investigation was performed using tap water standards, rather than MilliQ water solutions, in order to better describe matrix effects: it's known that the matrix complexity, due to the presence of concurrent dissolved compounds, decreases the yield of the SPME extraction for the most polar compounds, probably because of an higher decreasing of partition coefficients between the fiber and the sample [28].

The reliability for quantitative determination was investigated by computing the calibration curves using multicomponent standard solutions the 21 PPCPs at different concentration levels in tap water, varying in the 3.75 to 135 ppb range for less polar (numbered 1-3,6,11,13,14,17,19 and 21 in Table 1) and in the 15 to 540 ppb range for more polar PPCPs submitted to derivatization (numbered 4,5,7-10,12,15,16,18 and 20 in Table 1). Three independent samples were analyzed for each calibration point.

The method detection limit (LOD) was computed as the concentration that corresponds to three times the noise signal (LOD, Table 5, 3rd column) and the quantification limit (LOQ) was evaluated as 10:1 signal-to-noise value (Table 5, 4th column).

The best analytical performance was obtained for less polar compounds showing a correlation coefficients ranging from 0.929 to 0.990, with LOD values between 0.1 and 5.0 ppb. The more polar derivatized PPCPs show correlation coefficients ranging from 0.775 to 0.977, and LOD values lower than 40 ppb, with the exception of IP (LOD=71 ppb). These values prove that the optimized procedure achieves analytical performance similar to that reported by various Authors describing SPME methods properly developed for a single, or max two, PPCP class(es) [13,26,28,32-35,39-43].

The reproducibility was assessed through the relative standard deviation (RSD%) of the triplicate measurements performed on standard mixtures containing each target PPCP at a concentration level of at 15 ppb for underivatized analytes and of 60 ppb for derivatized ones: RDS% values were less than 10% and less than 20%, for underivatized and derivatized compounds, respectively. These results show satisfactory reproducibility of the analytical procedure, according with that reported in bibliography [9,28,31,44].

In order to evaluate accuracy of the method, tap water samples were spiked with different PPCP concentrations (30 and 40 ppb level for less polar compounds and 120 and 135 ppb for derivatized ones). The extraction recoveries of the analytes were determined by comparing the peak areas of the spiked samples after SPME procedure with those of the corresponding standard mixture solution. Good recoveries were obtained, since they varied from 75 to 110 % and from 85 to 103% for derivatized and non derivatized compounds, respectively.

4. Conclusions

The results of this study confirm that optimization using central composite design and response surface methodologies is an extremely efficient tool for fast, complete optimization of the parameters affecting an analytical procedure.

A precise, sensitive, and solvent free method for the determination of a wide range of compounds at trace levels in water samples has been developed. Compared to previously developed SPE and SPME methodologies [2,28-33,45-47], the present method offers the benefits of high sensitivity and high sample throughput to efficiently extract a wide range

of target PPCPs present at trace levels in various aqueous matrices with a significant reduction in the sample treatment time. These properties are particularly relevant for multi-residue methods, such as the present case of a large variety of PPCPs, since they require the joint extraction and determination of many compounds displaying a broad range of polarities, also requiring chemical derivatization of polar compounds. The on-fiber silylation of the oxydrilic compounds can be performed in only 30.5 minutes with a very small consumption of silylation reagent and at low temperature. The derivatization reaction is performed using the derivatizing agent vapour, rather than the pure liquid or a solution; this should favour desirable kinetics and regioselectivity. Also, steps involving the removal of the derivatizing agent are eliminated, reducing a likely source of sample loss, error in the method and time.

This optimized SPME procedure, combining extraction, derivatization, and analysis, is very straightforward. It's noted that the SPME fibers hold as well throughout the process, as over 80 complete analysis have been performed, with no apparent fiber degradation.

Under the optimized conditions the procedure provides low detection limits (≤ 1 ppb for the non polar analytes and ≤ 35 ppb for the derivatized ones) and satisfactory reproducibility (RSD% ≤ 10% or 20%) for most of the PPCPs investigated: this result makes the developed method potentially suitable for comprehensive chemical profiling of PPCPs as the basis for wastewater monitoring and can be easily extended to non-target compounds with similar physico-chemical characteristics. Finally, the method should be amenable to automation.

References

[1] C.G. Daugthon, T. Jones-Lep, D. Washington (2001) Pharmaceuticals and personal care products in the environment: scientific and regulatory issues. ACS symposium series 791. American Chemical Society, Washington, Oxford University Press, Washington, USA, 2001

[2] A.M. Peck, Anal. Bioanal. Chem. 386 (2006) 907.

[3] C.G. Daughton, T.A. Ternes, Environ. Health Perspect. 107 (1999) 907.

[4] G.R. Boyd, J.M. Palmeri, S. Zhang, D.A. Grimm, Science of the Total Environment 333 (2004) 137.

[5] J.M. Brausch, G.M. Rand, Chenosphere 82 (2011) 1518.

[6] C.G. Daughton, Environ Impact Assess 24 (2004) 711.

[7] D. Barcelo, M. Petrovic, Anal. Bioanal. Chem. 387 (2007) 1141.

[8] S.D. Richardson, Anal. Chem. 78 (2006) 4045.

[9] R.A. Trenholm, B.J. Vanderford, J.C. Holady, D.J. Rexing, S.A. Snyder, Chemosphere 65 (2006) 1990.

[10] U.S. Environmental Protection Agency. Pharmaceuticals and Personal Care Producs as Pollutants: http://www.epa.gov/esd/chemistry/ppcp/. Accessed 15 December 2009

[11] European Commission Environment. http://ec.europa.eu/environment/water/

[12] H. Lord, J. Pawliszyn, J. Chromatogr. A 885 (2000) 153.

[13] M.C. Pietrogrande, G. Basaglia, Trends Anal Chem 26 (2007) 1086.

[14] I. RodrÍguez, J. Carpiteiro, J.B. Quintana, A.M. Carro, R.A. Lorenzo, R. Cela, J. Chromatogr. A. 1024 (2004) 1.

[15] K.D. Buhholz, J. Pawliszyn, Anal. Chem. 66 (1994) 160.

[16] T. Luan, G. LI, Z. Zhang, Anal. Chimica Acta 424 (2000) 19.

[17] A.F. Lagalante, M.A. Felter, J. Agric Food Chem. 52 (2004) 3744.

[18] M. Fernandez-Alvarez, J.P. Lamas, L. Sanchez-Prado, M. Llompart, C. Garcia-Jares, M. Lores, J. Chromatogr. A. 1217 (2010) 6634.

[19] Y. Shao, P. Marriott, H. Hügel, Chromatographia Supplement 57 (2003) 349.

[20] A.M. Carro, P. González, N. Fajar, R.A. Lorenzo, R. Cela, Anal. Bioanal. Chem. 394 (2009) 893.

[21] I. Rodríguez, F. Calvo, J.B. Quintana, E. Rubí, R. Rodil, R. Cela, J. of Chromatography A 1108 (2006) 158.

[22] J.B. Quintana, I. RodrÍguez, Anal. Bioanal. Chem. 384 (2006) 1447.

[23] A.M. Peck, Anal. Bioanal. Chem. 386 (2006) 907.

[24] Pawliszyn J (1997) Solid phase micro extraction: theory and practice. Wiley, New York

[25] C.L. Arthur, J. Pawliszyn, Anal Chem 62 (1990) 2145.

[26] G. Basaglia, L. Pasti, M.C. Pietrogrande, Anal. Bioanal. Chem. 399 (2011) 2257.

[27] A. Scheyer, S. Morville, P. Mirabel, M. Millet, Anal. Bioanal. Chem. 384 (2006) 475.

[28] J. Carpiteiro, J.B. Quintana, I. Rodríguez, A.M. Carro, R.A. Lorenzo, R. Cela, J. Chromatogr. A 1056 (2004) 179.

[29] P. Viñas, N. Campillo, N. Martínez-Castillo, M. Hernández-Córdoba, Anal. Bioanal. Chem. 397 (2010) 115.

[30] P.D. Okeyo, N.H. Snow, J. Microcolumn Separation 10 (1998) 551.

[31] P. Canosa, I. Rodriguez, E. RubÍ, R. Cela, J. Chromatogr. A. 1072 (2005) 107.

[32] L. Xiangli, L. Li, Z. Shichun, L. Chongyu, L. Tiangang, Chinese J. of Anal Chem 34 (2006) 325.

[33] J. Regueiro, M. Llompart, C. Garcia-Jares, R. Cela, J. Chromatogr. A 1216 (2009) 2816.

[34] R. Regueiro, E. Becerril, C. Garcia-Jaresa, M. Llompart, J. Chromatogr. A 1216 (2009) 4693.

[35] M. Fernandez-Alvarez, J.P. Lamas, L. Sanchez-Prado, M. Llompart, C. Garcia-Jares, M. Lores, J. Chromatogr. A 1217 (2010) 6634.

[36] S. Mitra (2003) Sample Preparation Techniques in Analytical Chemistry. Wiley, New York

[37] R. Leardi, Analytica Chimica Acta 652 (2009)161.

[38] D.L. Massart, B.G.M. Vandeginste, S.N. Deming, Y. Michotte, L. Kaufman, (1988) Chemometrics: a textbook. Elsevier, Amsterdam

[39] B. Dejaegher, Y. Vander Heyden, Acta Chromatographica 21 (2009) 161.
[40] T. Poiger, H.-R. Buser, M.E. Balmer, P.-A. Bergqvist, M.D. Müller, Chemosphere 55 (2004) 951.
[41] M. Llompart, C. García-Jares, C. Salgado, M. Polo, R. Cela, J. Chromatogr. A 999 (2003) 185.
[42] F. Ferrari, A. Sanusi, M. Millet, M. Montury, Anal. Bioanal. Chem. 379 (2004) 476.
[43] N. Negreira, P. Canosa, I. RodrÍguez, M. Ramil, E. RubÍ, R. Cela, J. Chromatogr. A 1178 (2008) 206.
[44] R. Rodil, M. Moeder, J. of Chromatography A, 1179 (2008) 81.
[45] M.C. Pietrogrande, G. Basaglia, F. Dondi, J. Sep. Sci. 32 (2009) 1249.
[46] R. Rodil, J.B. Quintana, G. Basaglia, M.C. Pietrogrande, R. Cela, J. Chromatogr. A 1217 (2010) 6428.
[47] M. Polo, C. García-Jares, M. Llompart, R. Cela, Anal. Bioanal. Chem. 338 (2007) 1789.

Table 1 List of studied compounds. PM refers to not derivatived analytes; TMS means trimethylsilyl derivatives, di-TMS when the derivatization products are bi-substituted. The retention times refers to GC temperature program explained in paragraph GC-MS.

	compound	use	PM	b.p. °C	formula	Log Kow	tr (min)	Ion mass m/z
1	Butylated hydroxytoluene (BHT)	Antioxidant	220	265	$C_{15}H_{24}O$	4.2	10.9	205+177
2	Galaxolide (HHCB)	Fragrance	258.4	304	$C_{18}H_{26}O$	5.9	14.6	243+213
3	Aldrin (ALD)	Pesticide	364.9	145	$C_{12}H_8Cl_6$	6.4	16	263+261
4	(TMS-)Benzophenone-3 (BP3)	UV-blocker	228.2	224-227	$C_{14}H_{12}O_3$	3.8	16.9	285+242
5	(TMS-)Triclosan (TCS)	Antiseptic	289.5	120	$C_{12}H_7Cl_3O_2$	4.8	17.3	200+345
6	Pyrene (PYR)	PAH	178.2	404	$C_{16}H_{10}$	5	17.5	202+100
7	(diTMS-)b-estradiol (E2)	Estrogen	272.4	no data	$C_{18}H_{24}O_2$	3.9	21.8	285+416
8	(diTMS)-Bisphenol A (BPA)	Plastics component	228.3	220 (4mmHg)	$C_{15}H_{16}O_2$	1.2	23.3	357+372
9	(TMS-)Chloroxylenol (PCMX)	Antiseptic	156.6	246	C_8H_9ClO	3.3	9.8	228+213
10	(TMS-)Ibuprofen (IP)	Antinflammatory	206.3	157 (4mmHg)	$C_{13}H_{18}O_2$	2.2	12	160+263
11	Benzophenone (BP)	UV-blocker	182.2	305	$C_{13}H_{10}O$	3.2	12.4	105+182
12	(TMS-)Chlorophene (OBPC)	Antiseptict	218.7	327	$C_{13}H_{11}ClO$	1.2	15.2	275+290
13	Heptachlor (HEPT)	Pesticide	373.3	140	$C_{10}H_5Cl_7$	6.7	15.3	100+272
14	Musk ketone (MK)	Fragrance	290.8	395	$C_{14}H_{18}N_2O_5$	4.3	15.8	279+43
15	(TMS-)Naproxene (NP)	Antinflammatory	230.3	198-199	$C_{14}H_{14}O_3$	3.3	16.8	185+243
16	(TMS-)Ketoprofen (KP)	Antinflammatory	254.3	no data	$C_{16}H_{14}O_3$	3.1	17.9	282+295
17	2,4'-DDD	Pesticide	320.1	no data	$C_{14}H_{10}Cl_4$	5.9	18	235+237
18	(diTMS-)Diethylstibestrol (DES)	Estrogen	268.4	no data	$C_{18}H_{20}O_2$	5.1	19.1	412+217
19	Chrysene (CRY)	PAH	228.3	448	$C_{18}H_{12}$	5.9	20.4	228+226
20	(diTMS-)Estrone (E1)	Estrogen	270.4	no data	$C_{18}H_{22}O_2$	3.4	21.5	342+257
21	Benzo[a]pyrene (BAP)	PAH	252.3	311	$C_{20}H_{12}$	6.4	23.4	252+250
	Quintozene	Pesticide	202.3	328	$C_6Cl_5NO_2$	4.6	13.7	237+214

Table 2 Experimental design domain and optimized values (last line)

	Extraction		Derivatization	Desorption	
	time t_{ex} (min)	temperature T_{ex} (°C)	time t_{der} (min)	time t_{des} (min)	temperature T_{des} (°C)
- 1	5	30	0.5	2	220
0	65	50	15.5	7	260
+1	125	70	30.5	12	300
Optimized	125	40	30.5	2	300

Table 3 Central composite design experiments (with 10 repetitions of the central point), and results for all analytes refer (sums of area logarithms). Last four line refer experimental design model validation, the predicted values were calculated derivatizing in 0.5 and 30.5 min.

	Extraction		**Derivatization**	**Desorption**		**Total Response**
Experiment no.	time (min)	temperature (°C)	time (min)	time (min)	temperature (°C)	∑ logarithm of peaks areas
1	5	30	0.5	2	300	45.45368
2	5	30	0.5	12	220	43.42690
3	5	30	30.5	2	220	40.24876
4	5	30	30.5	12	300	46.67908
5	5	70	0.5	2	220	39.99816
6	5	70	0.5	12	300	45.47592
7	5	70	30.5	2	300	45.63154
8	5	70	30.5	12	220	45.04213
9	125	30	0.5	2	220	47.51600
10	125	30	0.5	12	300	47.00053
11	125	30	30.5	2	300	48.72249
12	125	30	30.5	12	220	45.64620
13	125	70	0.5	2	300	46.91947
14	125	70	0.5	12	220	47.46235
15	125	70	30.5	2	220	43.56150
16	125	70	30.5	12	300	38.45968
17	5	50	15.5	7	260	46.05175
18	125	50	15.5	7	260	48.83293
19	65	30	15.5	7	260	44.08215
20	65	70	15.5	7	260	41.77626
21	65	50	0.5	7	260	45.15121
22	65	50	30.5	7	260	49.34941
23	65	50	15.5	2	260	48.86296
24	65	50	15.5	12	260	48.84349
25	65	50	15.5	7	220	47.42164
26	65	50	15.5	7	300	48.15585
27	65	50	15.5	7	260	46.47769
28	65	50	15.5	7	260	44.29809
29	65	50	15.5	7	260	47.77212
30	65	50	15.5	7	260	46.12907
31	65	50	15.5	7	260	47.59089
32	65	50	15.5	7	260	48.72512
33	65	50	15.5	7	260	47.52185
34	65	50	15.5	7	260	47.90185
35	65	50	15.5	7	260	48.94665
36	65	50	15.5	7	260	47.65171
Experimental	125	40	0.5	2	300	48.7903 ± 4.3535
Predicted from model	125	40	0.5	2	300	50.6893 ± 2.1397
Experimental	125	40	30.5	2	300	45.4281 ± 4.3666
Predicted from model	125	40	30.5	2	300	48.7199 ± 2.1397

Table 4 Second order quadratic equations describing the response for all the PPCPs (first line) and each individual compound (2^{nd}-8^{th} lines). Equations were calculated considering only the statistically significant variables (listed in the 3^{rd} column).

compound	Equation	Variable of significance
Total response	$Y=47.538+0.8951 * t_{ex} -0.8027 * T_{ex} – 0.8013 t_{ex} * T_{ex}-0.9847 t_{ex} * t_{de}r -1.0901 t_{ex} * t_{des} -1.1006 t_{ex} * T_{des} -1.2102 t_{des} * T_{des} -2.9212 T_{ex}^2$	T_{ex}^2, $t_{ex}*t_{des}$, $t_{ex}*T_{des}$, $t_{des}*T_{des}$, t_{ex} , T_{ex}, $t_{ex}*T_{ex}$, $t_{ex}*t_{der}$
BHT	$Y = 13215000 + 3946900*t_{ex} – 2159900t_{ex}*t_{der} -4151700 * T_{ex}^2$	t_{ex}, T_{ex}^2, $t_{ex}*t_{der}$
HHCB	$Y = 11119000 + 4403500 * t_{ex}$	t_{ex}
ALD	$Y = 8205700 + 3827000 * t_{ex}$	t_{ex}
PYR	$Y = 10040000 + 3572500 * t_{ex}$	t_{ex}
TMS-BP3	$Y = 25773 + 14689 * T_{des}$	T_{des}
TMS-TCS	$Y = 1535800 – 483980 * T_{ex} + 507580 * t_{de}r$	T_{ex} , t_{der}
diTMS-BPA	$Y = 36986 – 10090 * T_{ex} + 15495 * t_{der} +11975 * T_{des}$	t_{der},T_{ex}, T_{des}
diTMS-E2	-	-

Table 5 Evaluation of the analytical performance of the SPME procedure under the optimized conditions: upper linearity range, detection and quantification limits.

Analyte	Up-linear range (ppb)	r^2	LOD (ppb)	LOQ (ppb)
TMS-PCMX	540	0.922	37	223
BHT	135	0.979	0.8	11.2
TMS-IP	540	0.943	71	293
BP	135	0.995	5.0	33.4
HHCB	37.5	0.970	0.1	1.8
TMS-OBPC	270	0.971	7	64
HEPT	37.5	0.979	1.0	1.7
MK	135	0.990	1.4	2.3
ALD	67.5	0.959	0.1	18.7
TMS-NP	540	0.923	21	47
TMS-BP3	540	0.947	34	36
TMS-TCS	540	0.977	0.5	36.9
PYR	67.5	0.989	0.1	4.4
TMS-KP	150	0.953	34	97
2,4'-DDD	67.5	0.962	0.3	10.2
diTMS-DES	540	0.961	16	104
CRY	67.5	0.942	0.1	23.4
diTMS-E1	540	0.964	21	152
diTMS-E2	540	0.914	41	195
diTMS-BPA	150	0.775	39	52
BAP	67.5	0.929	0.3	19.2

Fig.1 Behaviour of Total response (a) and BHT (b) yield depending on derivatization time. The other variables not considered are set at their optimum values (Table 2, last line)

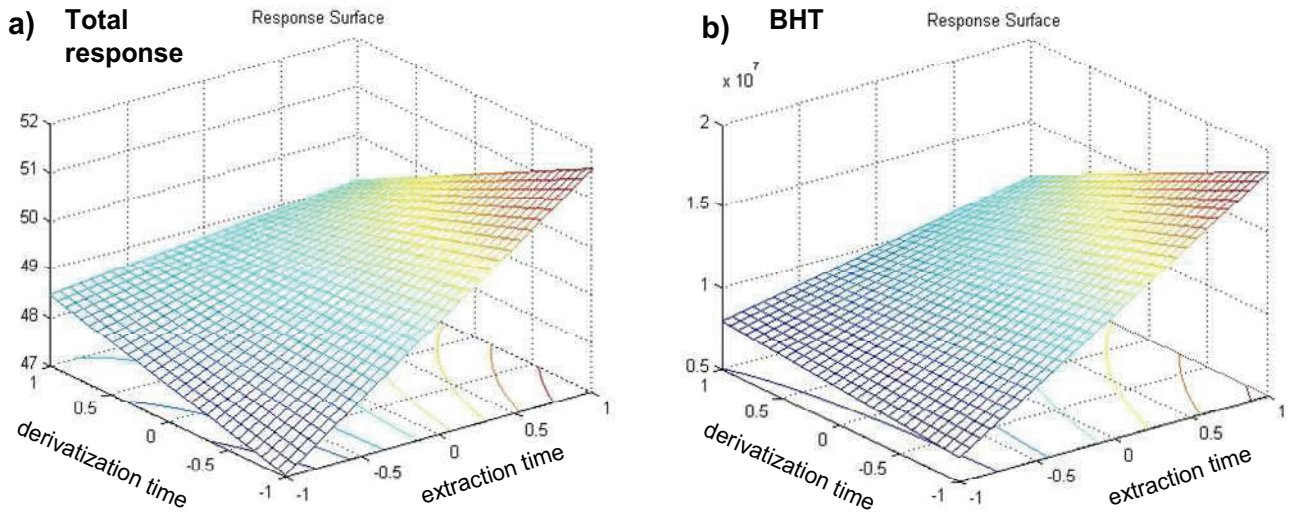
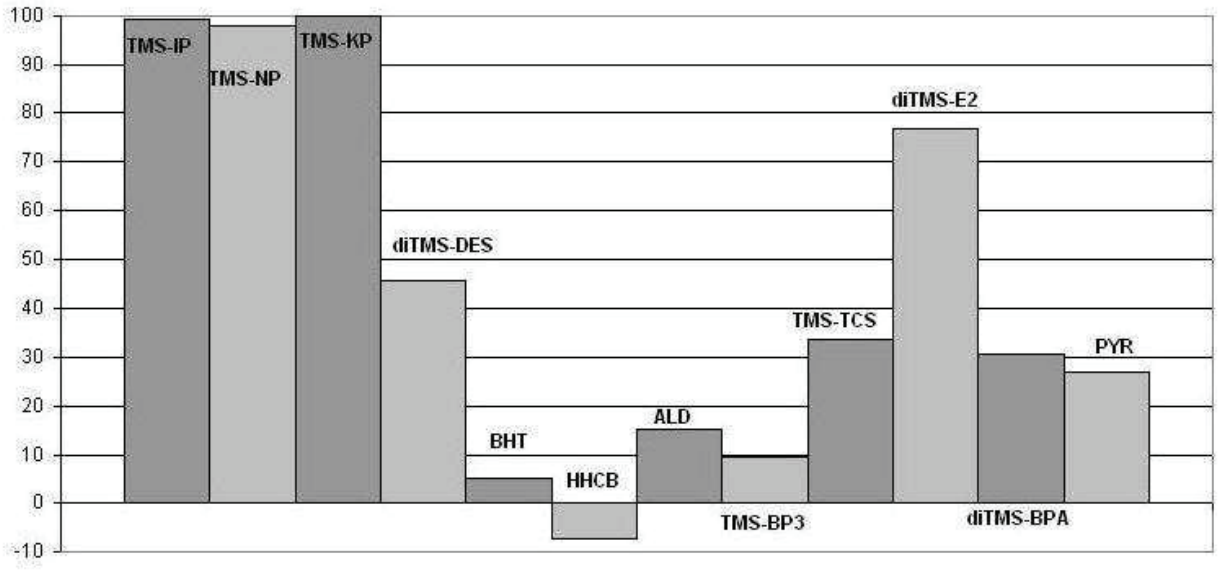


Fig 2. Percentual difference in PPCPs extraction yield increasing the derivatization time from 0.5 min to 30.5 min. The analytes not showed displayed no relevant difference (less than 1%).



UNIFE

FORMAZIONE POSTLAUREA

Your E-Mail Address
giulia.basaglia@gmail.com
Subject
Dichiarazione di conformità Tesi di dottorato
Io sottoscritto Dott. (Cognome e Nome)
Basaglia Giulia
nato a
Ferrara
Provincia
Ferrara
il giorno
10 Gennaio 1981
avendo frequentato il corso di Dottorato di Ricerca in:
Chimica Analitica ed Ambientale
Ciclo di Dottorato
XXIII
Titolo della tesi in Italiano
Sviluppo di metodiche analitiche avanzate per la determinazione di contaminanti emergenti in matrici ambientali acquose
Titolo della tesi in Inglese
Development of advanced analytical methods for the determination of emerging pollutants in environmental waters
Titolo della tesi in altra Lingua Straniera
Tutore - Prof:
Maria Chiara Pietrogrande
Settore Scientifico Disciplinare (SSD)
CHIM/01
Parole chiave (max 10)
pharmaceuticals and personal care products; emerging pollutants; gas chromatography-mass spectrometry; extraction techniques; water samples; experimental design
Consapevole - Dichiara
CONSAPEVOLE --- 1) del fatto che in caso di dichiarazioni mendaci, oltre alle sanzioni previste dal codice penale e dalle Leggi speciali per l'ipotesi di falsità in atti ed uso di atti falsi, decade fin dall'inizio e senza necessità di alcuna formalità dai benefici conseguenti al provvedimento emanato sulla base di tali dichiarazioni; -- 2) dell'obbligo per l'Università di provvedere al deposito di legge delle tesi di dottorato al fine di assicurarne la conservazione e la consultabilità da parte di terzi; -- 3) della procedura adottata dall'Università di Ferrara ove si richiede che la tesi sia consegnata dal dottorando in 4 copie di cui una in formato cartaceo e tre in formato .pdf, non modificabile su idonei supporti (CD-ROM, DVD) secondo le istruzioni pubblicate sul sito : http://www.unife.it/dottorati /dottorati.htm alla voce ESAME FINALE – disposizioni e modulistica; -- 4) del fatto che l'Università sulla base dei dati forniti, archivierà e renderà consultabile in rete il testo completo della tesi di dottorato di cui alla presente dichiarazione attraverso l'Archivio istituzionale ad accesso aperto "EPRINTS.unife.it" oltre che attraverso i Cataloghi delle Biblioteche Nazionali Centrali di Roma e Firenze. --- DICHIARO SOTTO LA MIA RESPONSABILITA' --- 1) che la copia della tesi depositata presso l'Università di Ferrara in formato cartaceo, è del tutto identica a quelle presentate in formato elettronico (CD-ROM, DVD), a quelle da inviare ai Commissari di esame finale e alla copia che produrrò in seduta d'esame finale. Di conseguenza va esclusa qualsiasi responsabilità dell'Ateneo stesso per quanto riguarda eventuali errori, imprecisioni o omissioni nei contenuti della tesi; -- 2) di

prendere atto che la tesi in formato cartaceo è l'unica alla quale farà riferimento l'Università per rilasciare, a mia richiesta, la dichiarazione di conformità di eventuali copie; -- 3) che il contenuto e l'organizzazione della tesi è opera originale da me realizzata e non compromette in alcun modo i diritti di terzi, ivi compresi quelli relativi alla sicurezza dei dati personali; che pertanto l'Università è in ogni caso esente da responsabilità di qualsivoglia natura civile, amministrativa o penale e sarà da me tenuta indenne da qualsiasi richiesta o rivendicazione da parte di terzi; -- 4) che la tesi di dottorato non è il risultato di attività rientranti nella normativa sulla proprietà industriale, non è stata prodotta nell'ambito di progetti finanziati da soggetti pubblici o privati con vincoli alla divulgazione dei risultati, non è oggetto di eventuali registrazioni di tipo brevettale o di tutela. --- PER ACCETTAZIONE DI QUANTO SOPRA RIPORTATO

Firma Dottorando

Ferrara, lì 24/01/2011 Firma del Dottorando Giulia Bosoglia

Firma Tutore

Visto: Il Tutore Si approva Firma del Tutore