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Ultrasound-assisted emulsification–microextraction of phenolic preservatives in water

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ABSTRACT

Simultaneous ultrasound-assisted emulsification–microextraction (USAEME) and derivatization combined with gas chromatography–tandem mass spectrometry (GC–MS/MS) is proposed for the first time for the analysis of parabens, triclosan and related phenols in water samples. In situ acetylation was successfully applied for the derivatization of target compounds with high efficiency using non-expensive reagents. The proposed method exhibits many advantages such as simplicity, efficiency, low cost, and minimum solvent consumption. In addition, the whole analytical process, including sample preparation and determination, is performed in only 20 min.

A multifactorial experimental design was employed to study and optimize the main variables potentially affecting the microextraction and derivatization processes (extraction solvent, phase ratio, sodium chloride concentration, extraction time, and acetic anhydride volume).

The performance of the method was studied in terms of accuracy, linearity, precision, and enrichment factor. Quantitative recoveries (≥85%) were obtained for all target compounds, and method precision was also satisfactory (RSD ≤ 13%) even for complex samples. Enrichment factors ranging from 100 to 200 were obtained, allowing achieving limits of detection at the low picogram per millilitre for most of the target compounds.

Several real samples, including wastewaters, river waters and swimming pool water, were analyzed. Since matrix effects were not observed, quantification can easily be performed using external calibration with acetylated standards, allowing a high sample throughput.

1. Introduction

The increasing concern about residues from personal care products (PCPs) demands the evaluation of their fate and occurrence in the environment. This group of emerging pollutants encompasses a wide range of chemicals, including several phenolic compounds such as triclosan (TCS), and the esters of *p*-hydroxybenzoic acid, commonly known as parabens. They are extensively employed as biocides and preservative agents in products intended for personal care and hygiene, such as deodorants, shower gels, shampoos, creams, and tooth pastes [1–4]. Parabens are also used as preservatives in pharmaceuticals, as well as in food and beverage processing.

As in the case of other personal care chemicals, they are continuously released into the environment through domestic and industrial wastewater and, although they are removed in a considerable extension during conventional sewage treatment plant (STP) processes [5–7], their presence has been detected in river water samples [8–10].

The acute toxicity of these compounds is supposed to be low. However, parabens can act as weak endocrine disrupter chemicals (EDCs) [11,12], whereas triclosan can be converted, under certain conditions, into more toxic and persistent compounds, such as chlorophenols, dioxins or methyl triclosan [13–15].

Thus, the development of analytical methodologies that allow investigating these pollutants in the aqueous environment is a topic of growing interest.

Few methods have been reported for the determination of parabens in water samples. Most of them rely on the use of solid-phase extraction (SPE) followed by liquid chromatography (LC) [16] or gas chromatography (GC) with mass spectrometry detection (MS) [5,17]. Recently, a method based on solid-phase

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microextraction (SPME) and gas chromatography coupled to tandem mass spectrometry (GC–MS/MS) has also been reported [18].

Regarding triclosan and related phenols, SPE is again the most common extraction technique, previously to their determination by gas or liquid chromatography [14,19]. Nevertheless, new approaches based on microextraction techniques have recently been reported. Thus, triclosan has been extracted from water samples using SPME [20], stir bar sorptive extraction (SBSE) [21], hollow-fibre liquid-phase microextraction (HF-LPME) [22] and dispersive liquid–liquid microextraction (DLLME) [10]. All these techniques allow eliminating the disadvantages of conventional extraction methods, such as solvent and time consumption, while achieving low limits of quantification.

Due to their polar nature, these compounds are often derivatized for GC analysis to reduce their adsorption in the chromatographic system, improving sensitivity, peak separations and peak symmetry [19,23]. Although analytical derivatizations are effective, they usually involve additional steps, which increase even more the time required for sample preparation.

A on-fibre silylation procedure using N-methyl-N-(*tert*-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) has been applied for derivatization of parabens and triclosan after SPME extraction from water samples [18,20]. The same silylating reagent has been recently employed for the determination of triclosan in water using a simultaneous derivatization and extraction by DLLME [10]. Pentafluoropropionic acid anhydride (PFPA) has also been used to form pentafluoropropionyl derivatives of endocrine disrupter phenols and acids, including triclosan and parabens [5].

In situ acetylation with acetic anhydride is one of the most common derivatization procedures for phenolic compounds [24,25]. The reaction can be performed in aqueous samples, in a few minutes, with high efficiency and using low cost reagents, much more comparing with silylating agents. This reaction has been successfully used in the determination of triclosan in water samples using a HF-LPME method [22].

In a previous work of this research group, a new microextraction technique for aqueous samples, known as ultrasound-assisted emulsification–microextraction (USAEME), was developed [26]. This approach is based on the emulsification of a microvolume of organic extractant in an aqueous sample by ultrasound radiation, and further separation of both liquid phases by centrifugation. The application of ultrasonic radiation accelerate the mass-transfer process between two immiscible phases, which together with the large surface of contact between both phases leads to an increment in the extraction efficiency in a minimum amount of time [27–29]. Thus, ultrasound-assisted emulsification–microextraction (USAEME) can be employed as a simple and efficient extraction and preconcentration procedure for organic compounds in aqueous samples. This technique was for the first time applied to the determination of synthetic musk fragrances, phthalate esters and lindane [26], and very recently, Fontana et al. [30] reported the successful determination of polybrominated diphenyl ethers in environmental waters using USAEME.

The aim of the present work is to develop a simple and rapid method of USAEME with in situ derivatization and gas chromatography–tandem mass spectrometry (GC–MS/MS) for the analysis of parabens, triclosan and related phenols in water samples.

In order to obtain an analytical approach applicable to complex water samples, method development and performance are completely carried out using STP wastewaters.

In situ acetylation using acetic anhydride is proposed, to the best of our knowledge, for the first time as a new alternative for the derivatization of parabens. In addition, the current work describes the first application of simultaneous derivatization and extraction by USAEME.

A multifactorial experimental design is employed to study and optimize main experimental parameters potentially affecting the simultaneous microextraction and derivatization process. Accuracy, precision, linearity, enrichment factor and detection limits (LoDs) are evaluated in order to assess the performance of the proposed method. Several environmental water samples, including wastewaters, are analyzed to demonstrate the applicability of the proposed method.

2. Materials and methods

2.1. Reagents and materials

Methyl 4-hydroxybenzoate (methylparaben, MP), ethyl 4-hydroxybenzoate (ethylparaben, EP), propyl 4-hydroxybenzoate (propylparaben, PP), butyl 4-hydroxybenzoate (butylparaben, BP), 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP) and 5-chloro-2-(2,4-dichlorophenoxy)phenol (Triclosan, TCS) were purchased from Aldrich (Milwaukee, WI, USA). Table 1 shows the chemical abstract service (CAS) registry numbers, molecular weights, octanol–water partition coefficients ($\log K_{ow}$) and chemical structures of the target compounds.

Deuterated methylparaben (methyl 4-hydroxybenzoate-2,3,5,6-d4) was obtained from C/D/N Isotopes (Quebec, Canada) whereas carbon-13 labeled triclosan (TCS-13C) was provided by Cambridge Isotope Laboratories (Andover, MA, USA) as 100 μg mL^{-1} solution in nonane. PCB-166 (2,3,4,4′,5,6-hexachlorobiphenyl) was purchased as 10 μg mL^{-1} solutions in isooctane from Dr. Ehrenstorfer (Augsburg, Germany).

Methanol, ethyl acetate, *n*-hexane, chloroform and acetic anhydride (Ac_2O) were provided by Merck (Darmstadt, Germany). Chlorobenzene and 1,1,1-trichloroethane were obtained from Sigma (St. Louis, MO, USA), whereas carbon tetrachloride was purchased from Fluka (Buchs, Switzerland). Individual stock solutions of each compound were prepared in methanol. Further dilutions and mixtures were prepared in *n*-hexane and methanol. The latter were employed for spiking water samples. Working solutions were made by appropriate dilution and then stored in amber glass vials at −20 °C.

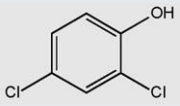
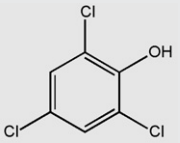
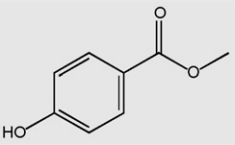
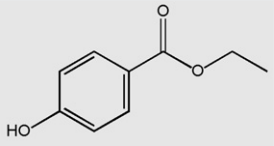
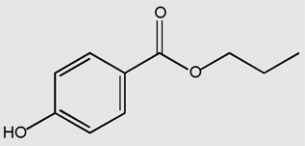
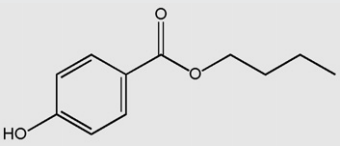
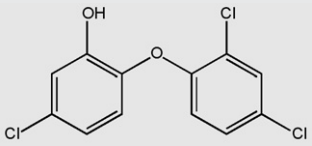
Sodium hydroxide, sodium hydrogen phosphate heptahydrate and sodium thiosulphate were purchased from Alfa Aesar (Karlsruhe, Germany). Potassium hydrogen carbonate was obtained from Aldrich (Milwaukee, WI, USA) and sodium chloride was provided by VWR Prolabo (Fontenay-sous-Bois, France). All solvents and reagents were of analytical grade. Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Billerica, MA, USA).

Different real water samples, including river water, urban wastewater and swimming pool water, were collected in amber glass containers. The excess of free chlorine in the swimming pool water sample was removed by addition of sodium thiosulphate (0.1 mg mL^{-1}). Water samples were filtered through 0.22 μm Millipore GV membrane filters (Billerica, MA, USA) and stored in glass bottles at 4 °C until analysis.

2.2. Ultrasound-assisted emulsification–microextraction (USAEME) with in situ derivatization

For the simultaneous USAEME and derivatization, aliquots of 10 mL water samples were placed in 15 mL conical-bottom glass centrifuge tubes, where 0.1 g sodium hydrogen phosphate were previously weighted. Prior to extraction, 1 ng of deuterated methylparaben and carbon-13 labeled triclosan (in methanol) were added to each sample as surrogate standards. Under final optimized conditions, 100 μL of 1,1,1-trichloroethane containing 2 ng of PCB-166

Table 1
Physicochemical properties and structure of the studied compounds.

Compound	CAS number	MW	pK_a	Log K_{ow}	Structure
2,4-DCP	120-83-2	163.0	7.90[a]	2.98[b]	
2,4,6-TCP	88-06-2	197.4	6.10[a]	3.56[b]	
MP	99-76-3	152.2	8.47[c]	1.91[d]	
EP	120-47-8	166.2	8.50[c]	2.34[d]	
PP	94-13-3	180.2	8.47[c]	2.94[d]	
BP	94-26-8	194.2	8.47[c]	3.50[d]	
TCS	3380-34-5	289.5	4.5[e]	4.8[f]	

[a] [31].
[b] [32].
[c] [33].
[d] [34].
[e] [35].
[f] [7].

(internal standard) and 200 μL acetic anhydride were added as extractant solvent and derivatization reagent, respectively.

The tube was immediately immersed into an ultrasonic water bath Selecta Ultrasounds (Barcelona, Spain) in such a way that the level of both liquids (bath and sample) was the same. Extractions were performed at 40 kHz of ultrasound frequency and 100 W of power for 5 min at 25 ± 3 °C at the beginning of every experiment.

As a result, oil-in-water (O/W) emulsions of 1,1,1-trichloroethane (dispersed phase) in water (continuous phase) were formed. Emulsions were disrupted by centrifugation at 5000 rpm for 3 min and the organic phase sedimented at the bottom of the conical tube from where it was removed by using a 100 μL Hamilton syringe (Reno, NV, USA) and transferred to a 100 μL glass insert placed in a 1.8 mL gas chromatography vial. The extracts were stored at −20 °C until analysis by GC–MS/MS.

2.3. Gas chromatography–mass spectrometry

The GC–MS/MS analysis was performed using a Varian 450-GC gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA, USA) coupled to an ion trap mass spectrometer Varian 240-MS (Varian Chromatography Systems) with a waveboard for multiple MS (MS^n) analysis. The system was operated by Saturn GC–MS Workstation v6.9 software.

Separation was carried out on a J&W HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) from Agilent Technologies (Palo Alto, CA, USA). Helium (purity 99.999%) was employed as carrier gas at a constant column flow of 1.0 mL min^{-1}. The GC oven temperature was programmed from 60 °C (held 2 min) to 200 °C at 30 °C min^{-1} (held 2 min) and then until 280 °C at 40 °C min^{-1} (held 2 min) (total analysis time = 13 min).

Table 2
Selected MS/MS experimental parameters and retention times of the target compounds.

Compound	Retention time (min)	Parent ion (*m*/*z*)	Excitation storage level (*m*/*z*)	Waveform type	Excitation amplitude (V)	Quantification ions (*m*/*z*)	Identification ions (*m*/*z*)
2,4-DCP	6.42	164	70	Resonant	0.72	126	128, 164
2,4,6-TCP	6.88	198	85	Resonant	0.78	162	134, 198
MP	7.06	152	70	Non-Resonant	52	121	151, 152
MP-d4 (SS)	7.06	156	70	Non-Resonant	52	125	154, 156
EP	7.46	138	60	Non-Resonant	52	121	122, 138
PP	8.12	138	60	Non-Resonant	52	121	122, 138
BP	8.95	138	60	Non-Resonant	52	121	122, 138
TCS	10.98	290	130	Resonant	0.78	218	255, 290
TCS-13C (SS)	11.00	300	130	Resonant	0.52	230	265, 300
PCB-166 (IS)	11.98	360	160	Resonant	2.20	290	325, 360

Pulsed splitless mode was used for injection with a pressure pulse of 30 psi during the splitless time (2 min). Split flow was set at 20 mL min^{-1} and the injector temperature was kept at 280 °C. Injection volume was 1 μL.

The ion trap mass spectrometer was operated in the electron impact (EI) ionization positive mode (+70 eV) using an external ionization configuration. Manifold, ion trap, ion source and transfer line temperatures were maintained at 40, 150, 180 and 280 °C, respectively. Helium was also used as damping gas at a flow of 0.8 mL min^{-1}.

In the full scan mode the mass range was varied from 35 to 500 *m*/*z* at 0.6 s $scan^{-1}$. For MS/MS analysis, general parameters were as follows: filament/multiplier delay, 5 min, filament emission current, 80 μA, electron multiplier potential, 1500 V, multiplier offset, +100 V, and AGC target value, 8000 counts. Specific MS/MS conditions and retention times for each target compound are listed in Table 2. The analytes were positively identified by comparison of their mass spectra and retention times to those of standards.

3. Results and discussion

3.1. GC–MS/MS optimization

Optimization of the chromatographic conditions was accomplished using a standard mixture solution of all target compounds in *n*-hexane. Direct analysis of the compounds produced peaks with appreciable tailing due to the interaction of hydroxyl groups with the chromatographic system. Therefore, a derivatization step by acetylation was introduced prior to GC analysis to improve the chromatographic properties of compounds. The procedure to obtain a standard solution of the corresponding acetylated compounds was based on a previous work dealing with the acetylation of other phenolic species [36]. Derivatives were prepared by adding 200 μL acetic anhydride and 5 μL pyridine to 1 mL of a 10 μg mL^{-1} standard solution in *n*-hexane. The mixture was maintained at 80 °C for 30 min, and then allowed to cool down to room temperature. Further dilutions were prepared in ethyl acetate. Reaction yield was considered quantitative under these conditions since the peaks of the underivatized compounds were not detected in the chromatograms. The acetylated standards were stable for at least 3 months.

3.2. Several criteria were employed to confirm the formation of the acetylated derivatives

First, retention times were shifted to higher values than those of the corresponding underivatized compounds. Secondly, chromatographic peaks showed much more symmetrical peak shapes, indicating the absence of the hydroxyl group on the molecules.

Regarding their mass spectra, acetylated derivatives resembled the corresponding underivatized compounds, since molecular ions were not present and only small differences in the intensity of the most abundant ions were observed. The absence of molecular ions in mass spectra has also been reported for acetylated derivatives of other phenols as a result of the loss of the acetyl group upon ionization [37].

In order to improve the selectivity and sensitivity of the determinations, the MS/MS detection mode was chosen. Working conditions were optimized using the automated method development (AMD) tool implemented in the software of the Saturn GC–MS Workstation. The effect of the collision induced dissociation (CID) amplitude was studied in the resonant and non-resonant modes for every compound. The non-resonant mode provided better results for parabens, whereas the resonant waveform type was required for obtaining a suitable dissociation of the chlorinated compounds. Multiple reaction monitoring (MRM) was used for the simultaneous MS/MS analysis of the isotopically labelled surrogates and the corresponding compounds. Optimized MS/MS conditions for each target compound are detailed in Table 2. Fig. 1 displays the GC–MS/MS extracted ion chromatograms for a standard mixture solution at a concentration of 10 ng mL^{-1} for triclosan and 30 ng mL^{-1} for the rest of compounds.

3.3. Preliminary experiments

As previously commented, a derivatization step is necessary due the polar nature of target compounds. Acetylation with acetic anhydride in the presence of hydrogen carbonate or carbonate is one of the most simple and cheap derivatization procedures for phenolic compounds in aqueous media, including chlorinated and brominated phenols [25]. Nevertheless, to the best of our knowledge it has not yet been used for the derivatization of parabens.

Therefore, preliminary experiments were conducted to study if this acetylation procedure is suitable for parabens and if it can be simultaneously performed with the extraction. Ultrapure water spiked with the target analytes at a concentration of 5 ng mL^{-1} was employed for these experiments. Derivatization was performed according to the reported conditions for other phenolic species [38], whereas the extraction procedure was based on previous experience with USAEME [26]. In brief, experiments were carried out using aliquots of 10 mL water sample, 3% (w/w) sodium hydrogen carbonate, 100 μL chloroform as extractant solvent and finally, 100 μL acetic anhydride as derivatization reagent. The mixture was US irradiated for 10 min and the resulting emulsion was disrupted by centrifugation.

During the collection of the organic phase sedimented at the bottom of the centrifuge tube, an important amount of carbon dioxide bubbles was observed. They are produced as a consequence of decomposition of carbonic acid generated by carbonates in the presence of the acetic acid formed from the anhydride hydrolysis. This fact makes difficult to separate the extractant solvent from the aqueous sample.

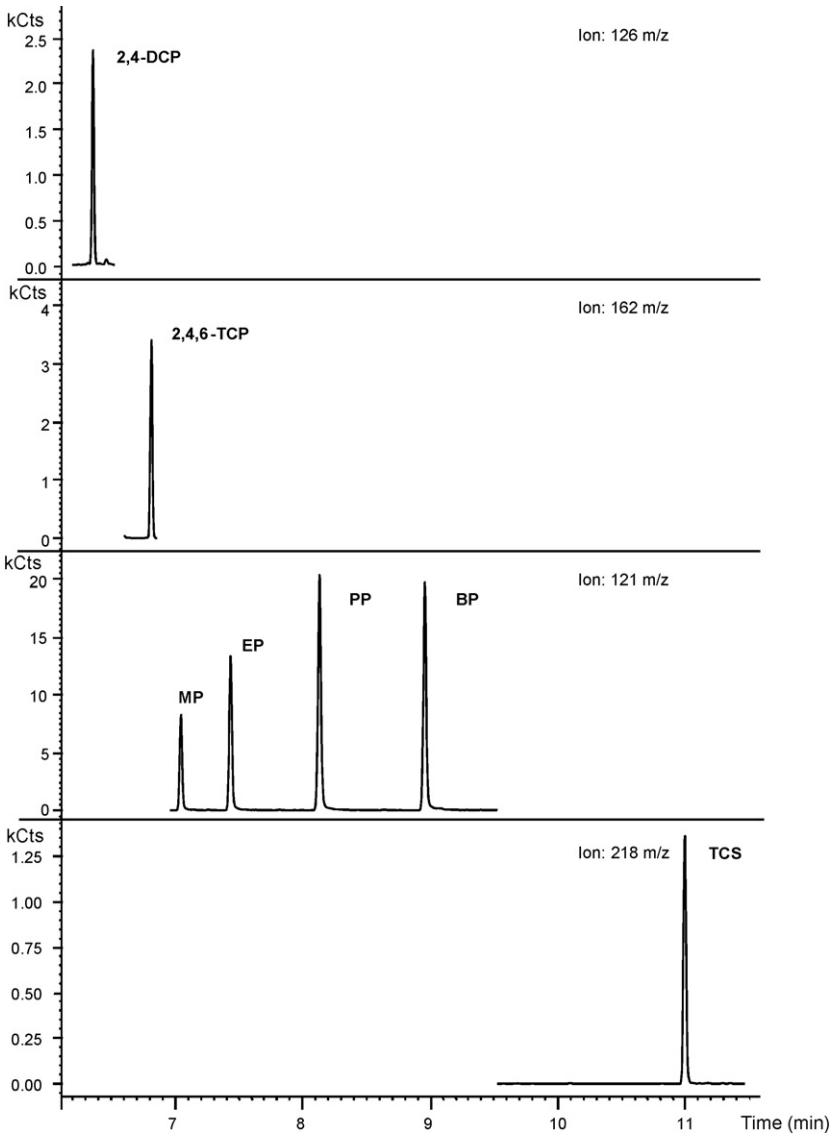


Fig. 1. GC–MS/MS extracted ion chromatograms of a standard mixture of the target compounds at a concentration of 10 ng mL^{-1} for triclosan and 30 ng mL^{-1} for the rest of compounds.

Similar problems have also been reported in other liquid microextraction techniques such as LPME [22,39], due to the instability of the organic drop caused by carbon dioxide bubbles.

The use of non-carbonate salts for adjusting the pH of water samples was proposed as the main solution to bubbling. Sodium hydroxide has been employed with this purpose in HF-LPME for acetylation of bisphenol A [40] and triclosan [22] in aqueous samples. However, its strong base character may cause abrupt pH changes complicating pH adjustment.

Sodium hydrogen phosphate heptahydrate is proposed for the first time as a new alternative to carbonate salts for the acetylation of phenols with acetic anhydride. Similar pH values can be obtained, but avoiding the drawbacks derived from bubbling. Amounts of this salt of 0.1 and 0.4 g were solved in 10 mL water, giving pH values of 8.7 and 9.1, respectively. Extraction was hence performed using aliquots of 10 mL water sample, 0.4 g sodium hydrogen phosphate, 100 μL chloroform and 100 μL acetic anhydride. No bubbling was observed, so organic phase could be correctly collected after centrifugation. Analysis of the resulting extracts showed the presence of acetylated compounds in a high extent and a minute quantity of underivatized phenols, which indicated that USAEME and derivatization can be simultaneously carried out under these conditions.

To study the effect of amount of sodium hydrogen phosphate on the derivatization process, various experiments were performed by adding different quantities of this salt (0.1–0.4 g) to 10 mL wastewater effluent sample spiked at 1 ng mL^{-1}. Other experimental conditions were kept constant. The responses obtained were very similar, so 0.1 g sodium hydrogen phosphate were used in all subsequent experiments.

Selection of a suitable extractant for USAEME is limited by several characteristics that are necessary for emulsification in the presence of ultrasonic radiation. Some of these characteristics are a higher density than water and low water solubility. Besides, selected solvent must be compatible with the separation and detection technique and therefore, a good gas chromatographic behaviour is another desirable characteristic.

In a first optimization step, several halogenated solvents were tested in order to evaluate their emulsification and extraction capacities. Dichloromethane (CH_2Cl_2), carbon tetrachloride (CCl_4), chloroform ($CHCl_3$) and 1,1,1-trichloroethane ($C_2H_3Cl_3$) were initially considered as possible extracting solvents. Their main

Table 3
Physicochemical properties of the solvents considered as possible extractants.

Solvents	Density 20 °C ($g\,mL^{-1}$)	Vapour pressure 20 °C (kPa)	Water solubility 20 °C ($g\,mL^{-1}$)	Log K_{ow}	Dipole moment 20 °C (D)
CH_2Cl_2	1.33	47.4	0.013	1.25	1.14
$CHCl_3$	1.48	21.2	0.008	1.97	1.15
CCl_4	1.59	12.2	0.0008	2.64	0
$C_2H_3Cl_3$	1.34	13.3	0.0005	2.49	1.78

Table 4
Factors and levels selected for the factorial design optimization.

Factors	Key	Levels	
		Lower level (−)	Upper level (+)
Extraction solvent	A	CCl_4	$C_2H_3Cl_3$
Phase ratio ($\beta_{s/e}$)	B	100	200
NaCl concentration (%)	C	0	20
Extraction time (min)	D	5	10
Ac_2O volume (μL)	E	50	200

physical properties are shown in Table 3. Aliquots of 10 mL wastewater effluent sample spiked at 1 $ng\,mL^{-1}$ were US extracted using 100 μL of every solvent. In all experiments, PCB-166 was previously added to the extracting solvents as internal standard at a concentration of 20 $ng\,mL^{-1}$. The ratio of peak area of each analyte to that of internal standard was used as analytical signal, avoiding possible problems resulting from lack of repeatability in volumes of sedimented phases. Emulsification was observed in all cases with the exception of CH_2Cl_2. The higher water solubility and volatility of dichloromethane is supposed to be the cause of no emulsion formation, so this solvent was ruled out for further optimization. Emulsions were then separated by centrifugation and extracts were analyzed by GC–MS/MS. No significant differences were obtained in the responses of target compounds, so chloroform was discarded since its higher water solubility usually leads to a lower repeatability in the volume of sedimented extracts. Therefore, carbon tetrachloride and 1,1,1-trichloroethane were considered for the next steps of method development.

3.4. Optimization of USAEME process: screening factorial design

The influence of the main variables potentially affecting the efficiency of ultrasound-assisted emulsification–microextraction was evaluated by using a multifactorial screening design. The study consisted of a half fraction 2^{5-1} design plus 2 centerpoints, involving a total of 18 randomized experiments. The selected design has resolution V, which means that it is capable of evaluating all main effects and all two-factor interactions. Numerical analysis of data resulting from the experimental design was made with the statistical software package Statgraphics XV Centurion (Manugistics, Rockville, MD, USA).

In contrast to most of optimization studies in extraction techniques, which are usually carried out using ultrapure water, experiments were performed using STP wastewaters aiming to obtain a method applicable even to complex samples. Thus, 10 mL aliquots of an effluent wastewater sample spiked with the analytes at a concentration of 5 $ng\,mL^{-1}$ were employed.

The selection of an appropriate extractant is an important parameter for all LLE-based processes. Two organic solvents previously selected, carbon tetrachloride and 1,1,1-trichloroethane, were tested in the experimental design in an attempt to achieve the highest extraction efficiency for the target compounds.

The influence of the volumes of both liquid phases was also considered in this study through the phase ratio ($\beta_{s/e}$), defined as the ratio of the volume of aqueous sample to the volume of extract. The volume of sample was kept constant at 10 mL whereas the organic solvent volume ranged from 50 to 100 μL. Thus, the phase ratio was studied at values of 200 and 100, respectively. Obtained responses for every compound were divided by the used volume in order to obtain a relative measure of the effect of this factor.

The salting-out effect has been frequently used in LLE, SPME and LPME. Generally, addition of salt can decrease the solubility of analytes in the aqueous phase and promote the transfer of the analytes towards the organic phase. Therefore, the concentration of sodium chloride in the aqueous solution was evaluated at two levels, 0% (no addition) and 20% (w/v).

Extraction time is usually an important factor in most extraction procedures. The effect of this factor was examined from 5 to 10 min. The volume of derivatization reagent was also studied in the experimental design at two levels, 50 and 200 μL.

In brief, five variables were screened in this design, namely extracting solvent, phase ratio, sodium chloride concentration, extraction time and acetic anhydride volume. Studied levels of each factor and the corresponding identification keys are listed in Table 4. The selected design allows to interpret the results using statistical tests and graphic tools in order to determine which factors have a statistically significant effect, as well as which are the significant interactions between factors.

Pareto charts for main factors and two-factor interactions are shown in Fig. 2. The length of each bar is proportional to the absolute value of its associated standardized effect. The standardized effect is obtained by dividing the estimated effect of each factor or interaction by its standard error. Vertical dotted line in the graphs represents the statistically significant bound at the 95% confidence level. As can be seen, concentration of sodium chloride was the most relevant factor, showing statistical significance for all studied compounds with the only exception of EP. Volume of acetic anhydride presented a significant effect for all compounds except for 2,4-DCP and MP, whereas extractant was significant for MP, EP and PP. Phase ratio had not statistical significance for any of the target compounds, which means that extraction efficiency is not affected by the volumes in the studied range. Therefore, the highest phase ratio can be used to improve the method sensitivity as necessary. However, 100 μL extractant were employed for the rest of experiments since the use of this volume provides limits of detection low enough (see Section 3.4) and favours the autosampler injection during the GC analysis. Extraction time was not significant for any of the compounds, so the low level of this factor was selected in order to increase the throughput of the method.

Regarding the two-factor interactions, that between phase ratio and sodium chloride concentration (BC) was significant only for EP, whereas the interaction between extracting solvent and volume of derivatization reagent (AE) presented statistical significance only for TCS.

Fig. 3 shows the main effect plots for several representative compounds. This kind of plots shows the main effects with a line drawn between the low and the high level of the corresponding factors. The length of the lines is proportional to the effect magnitude of each factor in the extraction process, and the sign of the slope indicates the level of the factor that produces the highest response.

Sodium chloride concentration was a significant factor for most of compounds and its influence is clearly appreciated in these plots. Response decreased in the presence of sodium chloride for all compounds except for MP and EP. Extraction efficiency of MP was improved by the salting-out effect, whereas the response for EP was not significantly affected. Salt addition should be favourable for the most polar species (Table 1) since it decreases the water solubility of analytes enabling a higher mass transfer towards the organic phase. An increase in viscosity with the ionic strength might play a negative role on extraction since ultrasound mechanical energy is partially damped and converted in heat, leading to a reduction

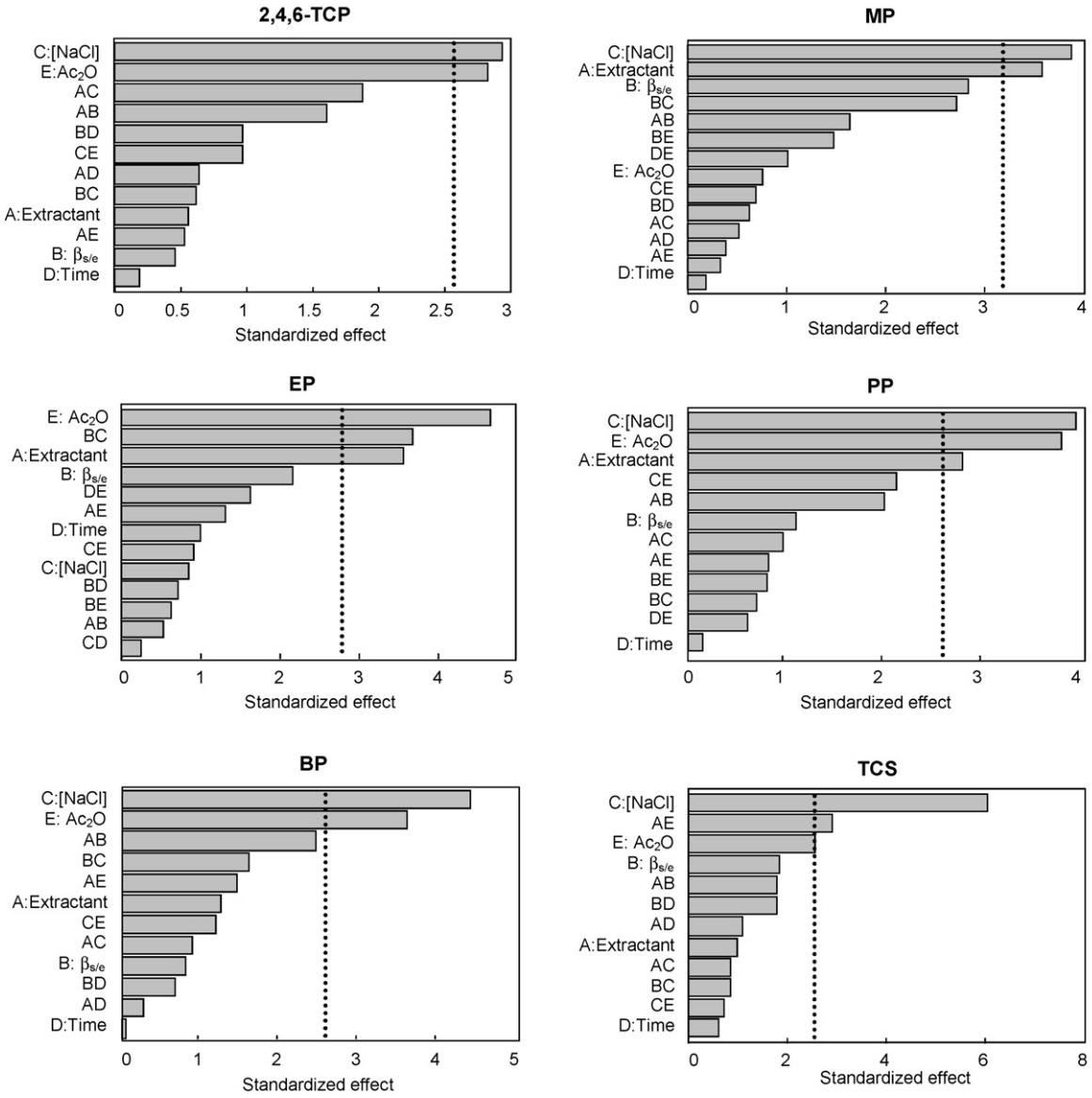


Fig. 2. Pareto charts for selected target compounds.

in the efficiency of the organic phase fragmentation and thus, to a reduction of the solvents interface area [28]. Additionally, viscosity decreases the diffusion flux of the analyte towards the solvents interface, due to a reduction in the acoustic fluxes in its vicinity, and the increase of the diffusion layer thickness. However, the obtained results demonstrated that extraction efficiency was independent of the extraction time (see Fig. 3) and so, the negative effect of salt addition on the extraction of the less polar species cannot be explained by kinetic factors. A negative influence of salt addition on the extraction yield of other species has been reported in USAEME [26] and other microextraction techniques such as SPME and LPME [41,42].

Regarding the extractant, higher extraction efficiencies were observed when 1,1,1-trichloroethane was used, although this factor was only significant for the most polar species of the group, MP, EP and PP. A possible explanation for this effect might be related to the higher dipole moment of this solvent (1.78 D) in comparison with carbon tetrachloride (0 D), allowing a better interaction between solvent molecules and the most polar compounds.

Volume of derivatization reagent showed a positive influence for all compounds except for 2,4-DCP and MP, which were not significantly affected by this factor. Thus, a volume of 200 μL acetic anhydride is preferred for the simultaneous derivatization and extraction process.

In view of the results of the optimization study, the experimental conditions selected for the for the simultaneous microextraction of the target compounds from water samples were as follows: 1,1,1-trichloroethane as extractant, a phase ratio of 100, no addition of sodium chloride, an extraction time of 5 min and 200 μL of acetic anhydride.

3.5. Method performance

The performance of the proposed method was evaluated in terms of accuracy, precision, linearity, enrichment factor and limits of detection. In order to assess its feasibility, experiments were carried out using real water samples.

Wastewaters are expected to present very complex matrices, so they were selected to study possible matrix effects. Accuracy of the method was evaluated using an effluent wastewater sample spiked at concentrations of 50, 500 and 5000 pg mL^{-1}, respectively. Recoveries were calculated by dividing the difference between the

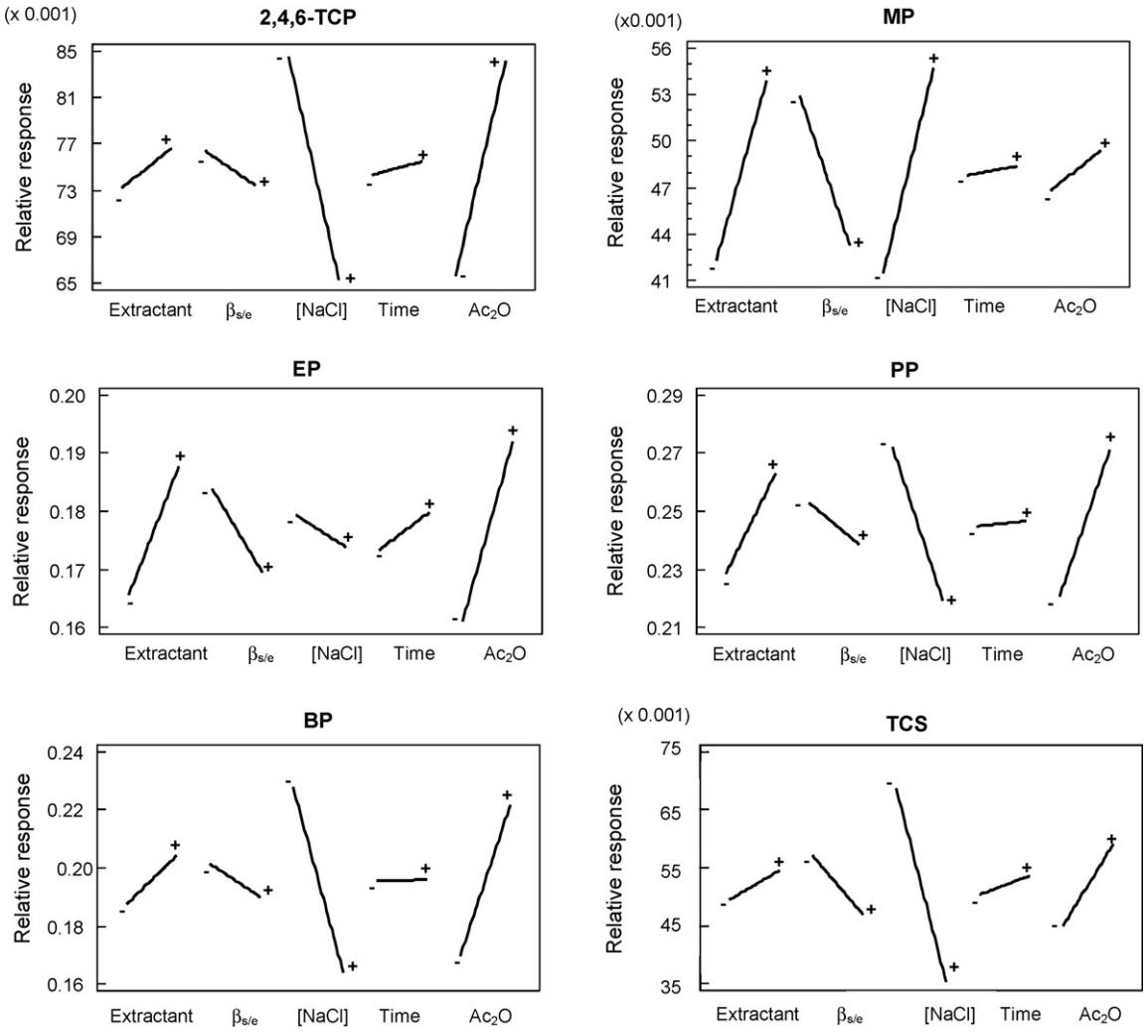


Fig. 3. Main effects plots for representative target compounds.

Table 5
Recovery, repeatability and limits of detection of the proposed method.

Compound	Recovery (%, $n=3$)			RSD (%, $n=3$)			LoD (pg mL^{-1})
	50 pg mL^{-1}	500 pg mL^{-1}	5000 pg mL^{-1}	50 pg mL^{-1}	500 pg mL^{-1}	5000 pg mL^{-1}	
2,4-DCP	n.c.	87	89	n.c.	11	9	27.5
2,4,6-TCP	100	91	85	11	9	7	10.8
MP	n.c.	86	89	n.c.	7	10	16.4
EP	85	90	87	9	10	9	12.5
PP	92	95	94	13	11	11	7.70
BP	87	88	89	8	8	8	3.90
TCS	86	94	92	12	8	10	5.84

n.c.: not calculated (addition level < LoQ).

measured concentrations for spiked and non-spiked samples by the added concentrations. Table 5 shows that recoveries ranged from 85% to 100% for all three addition levels. These values can be considered quantitative and therefore, an exhaustive extraction of analytes is assumed. Such knowledge is very important for practical reasons, since quantification can be performed by external calibration using standards in 1,1,1-trichloroethane.

The precision of the method was evaluated by calculating the relative standard deviation (RSD) at the same three concentration levels and results are also shown in Table 5. Values varied from 8% to 13% at the lowest level, whereas they ranged from 7% to 11% at the highest concentration.

Table 6
Study of method linearity.

Compound	R^2	LoF test	
		F-ratio	p-Value
2,4-DCP	0.9994	2.43	0.0563
2,4,6-TCP	0.9991	1.69	0.1696
MP	0.9996	0.36	0.9137
EP	0.9998	0.39	0.9002
PP	0.9998	0.33	0.9294
BP	0.9996	0.98	0.4718
TCS	0.9997	0.24	0.9589

Table 7
Analysis of the target compounds in different waters.

Compound	Concentration (pg mL^{-1})					
	Influent wastewater	Effluent wastewater	River water 1	River water 2	River water 3	Swimming pool water
2,4-DCP	177.7 ± 8.2	n.q.	n.q.	n.q.	n.d	n.q.
2,4,6-TCP	186 ± 24	84.0 ± 3.5	n.q	n.q.	n.q.	n.q.
MP	n.q.	n.q.	n.d.	n.d.	n.d.	n.q.
EP	n.q.	n.d.	n.d.	n.d.	n.d.	n.q.
PP	2784 ± 352	n.q.	n.d.	n.d.	n.d.	n.q.
BP	318 ± 28	n.q.	n.q.	n.d.	n.d.	13.7 ± 0.7
TCS	343 ± 38	n.d.	n.q.	n.d.	n.d.	n.d.

n.d.: not detected (<LoD); n.q.: not quantified (<LoQ).

Linearity was tested using acetylated standards prepared in 1,1,1-trichloroethane at seven different concentrations. Linear ranges were from 1 to 1400 ng mL^{-1} for most of compounds. Determination coefficients (R^2) for the calibration curves are shown in Table 6. All compounds showed good correlation with R^2 values higher than 0.9991. To validate the regression data, an analysis of variance (ANOVA) was performed. The lack-of-fit (LoF) test is designed to determine whether the selected model is adequate to

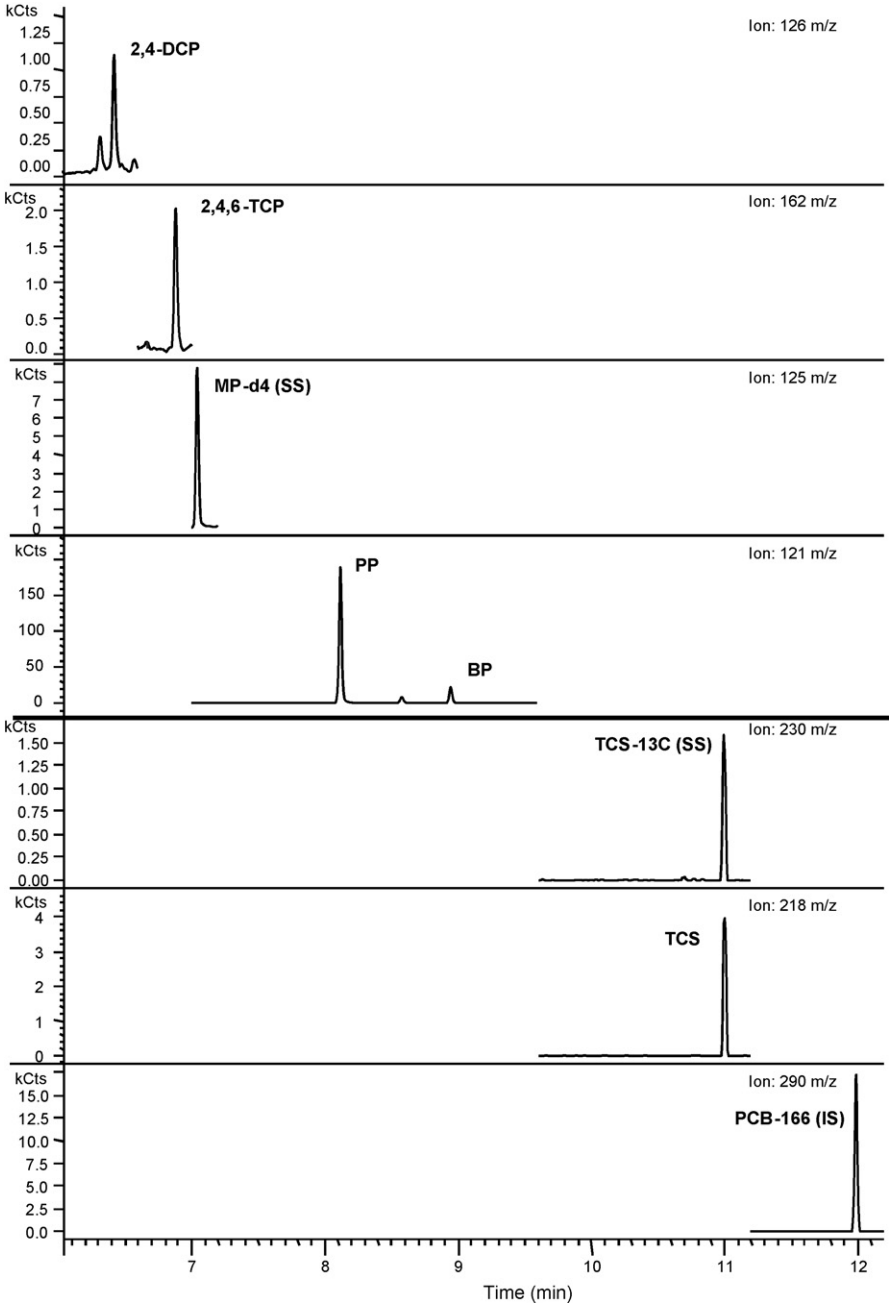


Fig. 4. GC–MS/MS extracted ion chromatograms for a STP influent wastewater sample.

describe the experimental data. The test compares the variability of the proposed model residuals to the variability between observations at replicate values of the independent variable. Results of the LoF test for the calibration range considered, at a confidence level of 95% are also shown in Table 6. Since *p*-values for LoF test are greater than 0.05 for all compounds, the linear regression models appear to be adequate for the experimental data.

Limits of detection (LoDs), defined for a signal-to-noise ratio of 3 (S/N = 3), were estimated employing real wastewater samples. Since the presence of 2,4-DCP and MP was observed in the procedural blanks, the LoDs for these compounds were estimated as those corresponding to the average amount of analyte giving a response that is the blank signal plus three times the standard deviation (LoD = blank signal + 3SD). The source of 2,4-DCP and MP in blanks might be due to their presence at low levels in the ultrapure water or to contamination through the analytical process. As shown in Table 5, values ranged from 3.90 to 12.5 pg mL^{-1} for all compounds except for MP and 2,4-DCP, which presented LoDs of 16.4 and 27.5 pg mL^{-1}, respectively. Although these LoDs can be considered as very low, they are slightly higher than those reported for the determination of parabens and triclosan in water using N-methyl-N-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) as derivatization reagent and SPME as the extraction technique [18,20]. Nevertheless, the use of in situ acetylation and USAEME constitutes an advantageous alternative since it is cheaper and less time consuming, and allows a higher throughput of analysis.

The enrichment factor, defined as the ratio of the concentration of analyte in the extract to that of the primary sample, is supposed to be approximately equal to the phase ratio ($\beta_{s/e}$) in an exhaustive extraction [43]. Therefore, an enrichment factor about 100 is obtained using an extractant volume of 100 μL and 10 mL aqueous sample. It should be underlined that if required, an improvement of sensitivity could be achieved increasing the enrichment factor by reducing the extractant volume since no loss of extraction efficiency was observed in the optimization study (Section 3.3) at least up to an extractant volume of 50 μL.

3.6. Application to real samples

The proposed method was applied to the analysis of several non-spiked water samples, including wastewaters from an urban treatment plant, several river water samples and one indoor swimming pool water sample (Table 7). Surrogate standards were added to each sample prior to the extraction to ensure the absence of matrix effects. Recoveries of the surrogates were within the range of 93–108% for all the analyzed samples.

The target chlorophenols were detected in most of samples, although they were above the quantification limit only in wastewaters, at concentrations ranging from 84.0 to 186 pg mL^{-1}. Similar values were previously reported in river [21] and wastewater samples [21,25].

Methyl, ethyl, *n*-propyl, and *n*-butyl paraben were present in the influent wastewater, being the *n*-propyl the most abundant paraben (2784 pg mL^{-1}). These compounds were also found in the effluent sample at concentrations below their quantification limits, indicating a >90% removal of the most lipophilic parabens during the sewage treatment processes. These results are in agreement with those reported in wastewater samples from Canada [5]. Parabens were also detected in the swimming pool water, although only butyl paraben was at a quantifiable concentration. These parabens were virtually absent in the river water samples as their levels were either close to or below their detection limits.

Triclosan was only determined in the influent wastewater at a level of 343 pg mL^{-1}, although it was also found below its quantification limit in one river sample. Since this compound was not detected in the effluent wastewater, it suggests a high removal efficiency during the sewage treatment. Obtained results are similar to those recently reported by other authors for river and wastewater samples [10,21].

In Fig. 4, the GC–MS/MS extracted ion chromatograms for the STP influent water sample are shown (see concentrations in Table 7).

4. Conclusions

A novel and simple method based on ultrasound-assisted emulsification–microextraction (USAEME) coupled to GC–MS/MS has been developed for the analysis of parabens, triclosan and related chlorophenols in water samples. The proposed method, that has been developed and performed well with complex water samples, exhibits many advantages such as efficiency, low cost and minimum solvent consumption and residues, which is in agreement with the criteria of green chemistry. In addition, a high sample throughput is attained since the whole analytical process, including sample preparation and determination, is performed in about 20 min.

In situ derivatization with acetic anhydride demonstrated to be successful under optimized conditions, and to the best of our knowledge this is the first time that this derivatization procedure is applied for the determination of parabens in water. The use of sodium hydrogen phosphate instead of a carbonate salt is advantageous to easily obtain a suitable basic media while avoiding drawbacks derived from bubbling. In this way, the proposed approach can be useful not only for USAEME but also for other liquid microextraction techniques such as LPME or SDME, where the stability of the organic phase plays an important role.

The influence of the most important variables involved in the extraction and derivatization processes was evaluated and the performance of the method was studied in terms of accuracy, linearity, precision, and enrichment factor. Quantitative recoveries (≥85%) were obtained for all target compounds and method precision was also satisfactory (RSD ≤ 13%) even for complex samples. Limits of detection at the low picogram per millilitre for most of target compounds were achieved with enrichment factors of 100–200.

The proposed method was applied to the analysis of several real samples including wastewaters, river waters and swimming pool water. Since no matrix effects were observed, quantification could be easily performed using external calibration with acetylated standards, allowing increased sample throughput and procedural simplicity if compared with non-exhaustive extraction techniques such as SPME. It should be underlined that the simultaneous USAEME and derivatization method might also be applied to the determination of other phenolic species in water samples.

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