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A polydimethylsiloxane rod extraction-based method for the determination of pharmaceuticals and triclosan by liquid chromatography in water samples

--Manuscript Draft--

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Abstract:	A new analytical method for the determination of naproxen, ketoprofen, diclofenac, carbamazepine, and triclosan in water samples by liquid chromatography is developed and validated. The method is based on the extraction of the analytes by a polydimethylsiloxane (PDMS) rod. The different parameters affecting extraction, such as the addition of salt, pH, initial volume, extraction and elution times and elution solvent, as well as the application of sonication, are studied. The results showed that the detection limits are all in the 0.1-0.3 μgL−1 range except for carbamazepine (6 μgL−1) with relative standard deviations in the range of 0.4–9.7%. The method developed, which was validated by analysing spiked surface water samples at 10, 25 and 75 μgL−1 gave recoveries of between 84.8 and 111.2%. In the case of carbamazepine, a recovery of 99.1% was obtained at 75 μgL−1. The main advantage of the developed method is that allows high performance liquid chromatography- diode array (HPLC-DAD), which is widely available in non-specialised laboratories, to be applied for pharmaceuticals and triclosan determination in surface waters after performing a preconcentration/clean-up step with PDMS rods as it has been shown by analysing real water samples.

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The original manuscript has been adapted including the associate editor comments in order to clarify some questions and to increase the quality of the manuscript. All changes in the manuscript have been highlighted in **red**. We express our gratitude to the reviewer for providing helpful comments and suggestions, which have clearly contributed to the improvement of our manuscript**.** We are confident that, after the recommended revision, the manuscript is now both clearer and more interesting, and we hope that the current revised version will meet the requirements to be accepted for publication in **Bulletin of Environmetal Contamination and Toxicology (BECT)**.

1) Revise abstract and indicate recovery range (including carbamazepine) for all compounds at 10 ug/L. The detection limits are misleading and must be revised. As it is written you are implying the method works for all compounds near the LOD. Change "real" to "spiked river". Discuss low recovery for carbamazepine including likely reasons for low recovery. Finally add a sentence explaining where this method may be used for environmental samples. Compare SPME detection limits to instrument detection limits and EF for each compound using HPLC -DAD.

   The abstract has been revised and the recovery range has been given as requested, except for the case of CBZ. The reason for the low recovery of CBZ is that this compound is not well adsorb by the PDMS due to it is less apolar than the other compounds as is discussed on page 4 (lines 154-155). When all the method is applied the lowest CBZ concentration that can normally be detected is 6 ug/L and, hence, CBZ peak can be observed in the chromatogram at 10 ug/L (Fig. 6 A). However, taking into account that the quantification limit for CBZ is 18 ug/L, quantification was not performed. This is also the reason why the recovery of CBZ from the spiked river water samples was only calculated at the highest spiked level. Hence, you are right that the method does not work for all the compounds at the same level of sensitivity and this is now clearly explained in the manuscript. We have also revised the calculations of the LODs and LOQs for the other compounds included in the method by using the calibration data obtained at different days and by eliminating outliers resulting in an improvement of the values.

   We have changed “real” to “spiked” as requested.

   The reason why this method can be used for environmental samples are commented on pg. 11 (lines 314-324). Moreover, it is important to note that there are no regulations about the concentrations of these compounds in environmental waters so there are currently no requirements regarding the method detection limits.

   SPME-HPLC-DAD detection limits as well as extraction efficiencies for these compounds are now included in Table 3 (pg. 8). As can be seen, the LODs reported for pharmaceuticals are higher than those obtained with our method, except for carbamazepine.

2) In the discussion, add a paragraph (line 322) describing the limitations of this

method improvement over direction injection IDL, and include practical uses at concentrations >20 ug/L. What are likely interferences in environmental samples based on the river water sample? Explain the difficulty in resolving peaks using HPLC-DAD, and possible solutions to these issues. For example, can confidence in identification be improved by using matrix standards at several concentration levels?

We have introduced a sentence (pg. 11, lines 324-326) explaining the advantages of our method over direct injection that are clear in the case of all of the target compounds except CBZ, recognising that direct injection can be faster than our method depending on the type of clean-up method used to treat the environmental water samples.

Concentrations >20 ug/L are not commonly found but, in any case, we can say that the advantage of the method presented here in these cases is, as before, that a separate clean-up step is avoided, although this comes at the cost of being a longer method, taking some 24 hours.

River water samples are representative samples of surface waters and can contain a wide variety of compounds that can interfere with the analysis such as suspended solids (humic and fulvic acids), other pollutants, etc. Peak resolution in HPLC-DAD can be improved by optimization of the chromatographic conditions through the composition of the mobile phase and the gradient as well as the mobile phase flux. In the proposed method these conditions were optimised using standard solutions containing the target compounds.

In the proposed methodology, the use of PDMS rods allows the performance of a clean-up and preconcentration step at the same time. The apolar characteristics of the PDMS rod avoid the adsorption of interfering polar compounds on the rod and the desorption with methanol prevents the desorption of the most apolar compounds, that are not soluble or poor soluble in methanol. Taking into account these aspects and as can be seen in the chromatogram of the spiked river water, the clean-up of the sample is very effective and no interferences are observed (Fig. 6A). It was surprising for us that after validating the method with spiked river water samples obtaining very good chromatograms (Fig. 6A), when the water sample from another river was analysed (Fig.6B), more peaks were observed in the chromatogram. This result is explained by the fact that other compounds with similar physical and chemical characteristics to the target analytes may be present in the sample. Hence, the use of matrix standards at several concentration levels can help to improve the method in the case of low resolved analyte peaks. Moreover, this problem can be also solved by optimizing the flux of the mobile phase and the gradient in order to improve the resolution of the peaks.

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The original manuscript has been adapted including the reviewer and the associate editor comments in order to clarify some questions and to increase the quality of the manuscript. All changes in the manuscript have been highlighted in **red**. We express our gratitude to the reviewers for providing helpful comments and suggestions, which have clearly contributed to the improvement of our manuscript**.** We are confident that, after the recommended revision, the manuscript is now both clearer and more interesting, and we hope that the current revised version will meet the requirements to be accepted for publication in **Bulletin of Environmetal Contamination and Toxicology (BECT)**. N the

We have carefully revised all the experimental data and we found out that there was a mistake in the extraction conditions as all the data reported was obtained at pH 2. Hence, this figure has been changed thorough all the manuscript. We apologize for this error.

Reviewer #1: This is a revised manuscript regarding the extraction and HPLC analysis of various commonly used NSAIDs which make their ways to drinking water via river waters and lakes. Measuring NSAIDs in drinking water is an environmentally significant problem as it relates to animal and human health. Authors have answered several comments posted earlier, but my enthusiasm for this revised manuscript is still not improved due to not having used an internal standard as a marker of extraction efficiency and characterization of tiny peaks by LC-MS/MS or other mass spectrometric methods.

The adsorption efficiency was evaluated by determining the concentrations of the analytes in the solution after equilibrium was reached by LC-DAD analysis using a calibration curve in ultrapure water. When the desorption conditions were studied, a calibration curve in methanol was used. So, since the full sample treatment step consisted of adsoption by the PDMS rod followed by methanol desorption, analyte solutions in ultrapure water were used and the final concentrations of these analytes in the desorption solution were determined using a calibration curve in methanol media.

In order to evaluate the matrix effect and the accuracy of the method, spiked river water samples were analysed and the results were interpolated in a calibration curve obtained by applying the full procedure to the standard solutions. The calibration curve data is included in Table 1. This means that PDMS rods were immersed in the river water samples and the aqueous standard solutions, in which the optimum amount of salt was added and the pH was adjusted. The solutions were then agitated until equilibrium and, finally, they were desorbed in methanol. It is important to take into account that this sample treatment allows both the preconcentration of the analytes and the clean-up of the samples. Only peaks of the analytes were obtained in the chromatogram with these solutions.

On the other hand, in the case of the analysis of river water samples, more peaks were found but they were not analysed since our objective was to determine the target analytes in the samples not to characterize the whole sample composition. The identification of the analytes in river water samples was made by the retention time that was monitored at three wavelengths and the quantification by interpolating the peak area in the calibration curve in accordance with previous calibrations. We agree with

the reviewer that the use of an MS detector would allow quantification and identification of the analytes. However, the purchase and maintenance costs of the HPLC-MS/MS instrumentation makes it unviable for routine laboratories, hence the development of preconcentration methods allowing HPLC-DAD for use in the determination of pharmaceuticals and antibacterial compounds is of great concern. Moreover, even when the most sensitive MS detection is used, the application of a clean-up/preconcentration step is required prior to chromatographic analysis, taking into account matrix effects on the ionization source. As is commented in the manuscript, the developed preconcentration method can be also used with HPLC-MS/MS.

The chromatogram coming out from HPLC-DAD may not reveal the target molecule(s) due to complexity of metrics and other contaminants present in the samples unless identified by mass spectrometric detector. Moreover, authors responded on page 3, as "The chromatographic method was previously validated (data not shown)" is not acceptable. This should be supported either by the proper citation or method details.

As has been explained above, the retention time was used to identify whether or not the analyte is present in the river water sample. The sentence “the chromatographic method…..” referred to instrumental chromatographic method which details are explained in lines 107-114 and the detection limits in lines 130-132. I would like to remark that these detection limits were considerable improved using the developed method (see Table 1).

Associate Editor: I believe that the novelty of the extraction method provides some merit for publication. However, several sections must revised. Make sure all abbreviations, including "SR", are defined in the text.

We have change SR (silicone rod) for polydimethylsiloxane (PDMS) rod and the rest of abbreviations are now defined thoroughly the manuscript as requested.

Line 60: Provide equation for calculation of extraction efficiency;

We have now included in the text a sentence (lines 150-152) explaining how extraction efficiency was calculated but given that its simplicity we didn’t add any equation.

Extraction efficiency = (m_s mass of the analyte in the PDMS rod) / (m_0 initial mass of the analyte in the solution) x100

- **m_s** mass of the analyte in the PDMS rod = V(volum of solution)x C_0 (initial concentration) – Vx C_{eq} (remaining analyte concentration at the equilibrium)
- **m_0** initial mass of the analyte in the solution = V(volum of solution)xC_0 (initial concentration).

Line 120 - Provide on-column instrument detection limits of HPLC method and final method of calibration. Were calibration standards extracted from water?

We have provided the instrumental detection limits in lines 131-133 and those of the final method in Table 1. The calibrations standards were prepared in ultrapure water as is made clear in lines 254-255.

Line 129 - what additional sample types were evaluated?

Only river water was evaluated.

Line 141 - What is the minimum tolerance for extraction efficiency?

Since extraction efficiency is calculated as the ratio between the mass of the analyte in the PDMS rod (m_s) and the initial mass of the analyte in the solution (m_0) as it has been explained above. These values are calculated through the determination of the analyte concentration in the aqueous phase at the equilibrium. The minimum tolerance is related to the detection limit of the instrumental calibration method.

Should carbamazepine be dropped from the method?

In fact, carbamazepine was not preconcentrated by PDMS rods given its polar characteristics.

Figure 6 - suggest including extracted standard and spiked sample at 10 ug/L instead of 100 ug/L. Add representative chromatogram for spiked treated wastewater at same concentration.

We have now opted to include the chromatogram of a spiked river water sample at 10 ug/L rather than the standard sample at 100 ug/L. Unfortunately, the journal's space requirements don't allow us to include both. The peak corresponding to Carbamazepine can also be observed.

The reason LC-MS-MS is used for these is that concentrations are typically at sub-ppb levels, even in wastewater. Provide some justification in the abstract, introduction and conclusions of the need for a SR- HPLC-DAD method. Where and how can this method be used as described?

The preconcentration/clean-up method that we propose result in detection limits being lowered to sub-ppb levels for the most hydrophobic compounds, permitting the use of an HPLC-DAD instrument after preconcentration, which is of course much more practical for monitoring laboratories. This is now made clear in the abstract (lines 20-24), introduction (lines 74-79) and conclusions (319-323). Moreover, this PDMS-rod-based method can also be applied when a LC-MS-MS instrument will be used as a clean-up method.

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A polydimethylsiloxane rod extraction-based method for the determination of pharmaceuticals and triclosan by liquid chromatography in water samples

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Abstract

A new analytical method for the determination of naproxen, ketoprofen, diclofenac, carbamazepine, and triclosan in water samples by liquid chromatography is developed and validated. The method is based on the extraction of the analytes by a polydimethylsiloxane (PDMS) rod. The different parameters affecting extraction, such as the addition of salt, pH, initial volume, extraction and elution times and elution solvent, as well as the application of sonication, are studied. The results showed that the detection limits are all in the 0.1-0.3 μgL^{-1} range except for carbamazepine (6 μgL^{-1}) with relative standard deviations in the range of 0.4–9.7%. The method developed, which was validated by analysing spiked surface water samples at 10, 25 and 75 μgL^{-1} gave recoveries of between 84.8 and 111.2%. In the case of carbamazepine, a recovery of 99.1% was obtained at 75 μgL^{-1}. The main advantage of the developed method is that allows high performance liquid chromatography- diode array (HPLC-DAD), which is widely available in non-specialised laboratories, to be applied for pharmaceuticals and triclosan determination in surface waters after performing a preconcentration/clean-up step with PDMS rods as it has been shown by analysing real water samples.

Keywords: polydimethylsiloxane rod, pharmaceuticals, triclosan, high performance liquid chromatography, UV-vis, microextraction techniques.

Introduction

Pharmaceuticals and antibacterial compounds are classified as emerging contaminants as they are regarded as possible threats to the aquatic environment and human health (Bu et al. 2013; Liu and Wong 2013). Although these compounds are normally not regulated, diclofenac has recently been included in the European Union watch list (Directive 2013/39/EU) and triclosan has been categorized as a high priority pollutant by the Environmental Protection Agency (EPA) in the aggregate risk assessment (Chen et al. 2013).

Due to their poor biodegradability, these contaminants are normally not eliminated in sewage treatment plants, and therefore are able to make their way into drinking water via river waters and lakes (Delgado et al. 2013; Gros et al. 2012). Anti-inflammatory drugs, such as diclofenac (DCF), naproxen (NAP) and ketoprofen (KET), have been detected in surface waters in concentrations of 10 μgL^{-1}, 121μgL^{-1}, and 102μgL^{-1}, respectively (Yang et al. 2017). Triclosan (TCS) was the most commonly found antibacterial in surface water with concentrations of up to 24 μgL^{-1} (Blair et al. 2013; Kasprzyk-Hordern et al. 2008).

Liquid chromatography with ultraviolet (UV-vis) and mass spectrometry (MS) detection are among the most important analytical methodologies to measure trace levels of pharmaceuticals and triclosan, although they require sample enrichment steps prior to chromatographic analysis (Richardson and Ternes 2014). The extraction of the analytes with solid-phase extraction (SPE) while having some advantages requires relatively large volumes of toxic solvents and is laborious and costly (Togunde et al. 2012). The use of solid-phase microextraction (SPME), in which the analytes are extracted by a polydimethylsiloxane (PDMS) fibre, has the disadvantages of the fragility of the fibre and the need for a special device when combined with high performance liquid chromatography (HPLC) (Płotka-Wasylka et al. 2015). Stir-bar sorptive extraction (SBSE) consists of a magnetic bar covered by a thin layer of a sorptive phase, generally PDMS, and provides improved extraction efficiencies in comparison with SPME (He et al. 2014). Other stir extraction techniques are stir-rod-sorptive extraction, stir-cake-sorptive extraction (SCSE), and rotating disk sorptive extraction (RDSE) that share the same sorptive principle as SPME (Cárdenas and Lucena 2017). In recent years, new microextraction techniques such as bar adsorptive micro-extraction (BAμE) (Neng et al. 2010; Almeida et al. 2017) and dynamic fabric phase sorptive extraction (DFPSE) have been applied in the determination of pharmaceuticals and triclosan (Lakade et al. 2016).

All these microextraction techniques have great potential, however, some of them requires of the synthesis of polymeric sorbent phases (Ahmad et al. 2017; Almeida et al. 2017). Hence, there is a need for less costly and simpler methods by combining efficient extraction/preconcentration techniques, using commercial sorbents, with chromatographic techniques such as HPLC-DAD, that is available in routine monitoring laboratories and that can determine the target analytes at low μg/L concentration levels. The extraction efficiency of technical silicone sorbents such as PDMS rods, which were introduced by Popp et al. 2004, meets analytical requirements in terms of purity, inertness and thermal stability and were applied to extract pharmaceuticals (Paschke et al. 2007). Other advantages of PDMS rods are their greater flexibility and robustness, together with the fact that they can be discarded after a single use, eliminating problems of carryover (van Pinxteren et al. 2010). Moreover, PDMS rods can be used as sorptive materials in passive sampling (Seethapathy and Górecki 2012).

The objective of this study is to develop a new analytical method for the determination of NAP, KET, CBZ, DCF, and TCS based on their extraction and preconcentration by PDMS

rods followed by liquid desorption and high performance liquid chromatography (HPLC-DAD) analysis. The method is validated by analysing spiked surface water samples and applied to the determination of target compounds in river waters. The analytical parameters of the developed method are compared with those obtained with other micro-extraction based techniques.

Methods and Materials

Triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol), naproxen ((2S)-2-(6 methoxynaphthalen-2-yl)propanoic acid), ketoprofen (2-(3-Benzoylphenyl)propanoic acid), carbamazepine (benzobenzazepine-11-carboxamide), and sodium diclofenac (sodium;2-[2-(2,6-dichloroanilino)phenyl]acetate) were purchased from Sigma–Aldrich (Germany). Working solutions of pharmaceuticals and triclosan ranging from 10 to 150 μgL^{-1} were prepared with ultra-pure water by dilution of a 500 mgL^{-1} stock methanol solution. Chromatographic grade acetonitrile (Fisher, USA), sodium chloride (Carlo Erba, Italy) and analytical grade anhydrous sodium acetate, acetic and hydrochloric acids (Sigma-Aldrich, Germany) were used. Ultrapure water with conductivity of 18.2 MΩ/cm was obtained from a water purification system (Millipore, USA).

An Agilent 1200 series high performance liquid chromatography system equipped with two pumps and a DAD detector was used. The analytes were separated in a C18 Luna column (50 × 2 mm, 2.5 µm) (Phenomenex, USA) using a gradient of mobile phase: (A) 0.1 % acetic acid and 4.7 mM of sodium acetate in ultra-pure water, and (B) acetonitrile (0 min, 90% A; 5 min, 75%A; 10 min, 65% A; 15 min, 20% A) at a flow rate of 0.3 $mLmin^{-1}$.The detection wavelength was set at 242 nm for CBZ, KET and TCS; 250 nm for NAP; and 280nm for DCF. Water samples were filtered with a 0.2 µm nylon membrane (Supelco, USA) before injection.

Commercial 10 mm elastomer PDMS rods (approx. 0.037 g) were cut from a flexible 2 mm diameter PDMS cord (Goodfellow, England). These were then cleaned and stored in methanol and, immediately prior to use, were dried with a lint-free tissue. The PDMS rod was immersed in 50 mL of a 100 μgL^{-1} solution of all the compounds in ultrapure water and 15% w/v of NaCl. The pH was adjusted as required (2, 3, and 6) and the extraction was performed for different periods of time (3, 5, 8, 10 and 24 h). The experiments were performed three times using a ten-point magnetic shaker (MultiMix D, Ovan, Spain) at 200 rpm. After extraction, the PDMS rod was removed with clean tweezers and then dried with a lint-free tissue. The rod was then placed into a tapered glass insert containing 200 µL of methanol allowing the desorption process to take place for times ranging from 15 to 45 minutes with and without sonication in an ultrasonic bath (J.P. Selecta, Spain). The PDMS rod was removed and 10 µL of the extract were then injected into the liquid chromatograph. During the experiments performed to find out the best adsorption and desorption conditions, aliquots of the desorbed solution were measured by interpolation in a calibration curve obtained using standard solutions in methanol. The detection limits of the instrumental method were: 2.42 μgL^{-1} for KET, 3.65 μgL^{-1} for NAP, 3.99 μgL^{-1} for DCF, 4.48 μgL^{-1} for CBZ, and 5.45 μgL^{-1} for TCS.

Water samples were collected in 1 L amber glass bottles from the Onyar, Ter and Fluvià rivers (Girona, Spain). Samples were transported to the laboratory under refrigeration and then stored at 4°C before characterization by determining their conductivity, chemical oxygen demand and ionic composition. The samples were filtered using a 0.45 µm nylon membrane

(Supelco, USA). After filtration, one of the samples was spiked with KET, NAP, DCF, CBZ, and TCS at different concentration levels (10, 25, 75 μgL^{-1}) and recovery experiments were carried out in triplicate. The other samples were analysed with the developed method.

Results and discussion

A systematic study of several parameters was undertaken to find the best extraction and desorption conditions for the preconcentration of pharmaceuticals and triclosan.

Extraction time was evaluated with 50 mL of a 100 μgL^{-1} solution containing 15% NaCl and all the studied compounds at pH 3. Five different extraction periods (3, 5, 8, 10, and 24 h) were studied by analysing the remaining concentrations in the aqueous solution. Equilibrium was reached at 10 h for all compounds. The extraction efficiency, calculated as the ratio between the amount of analyte extracted by the PDMS rod (m_s) and its initial mass in the aqueous phase (m_0), followed the order CBZ (6%), KET (17%), NAP (19%), DCF (56%), and TCS (75%), which corresponds to their hydrophobicity order (log K_{ow}): CBZ(2.45) < KET (3.1) < NAP (3.12) <DCF(3.91)< TCS (4.7), showing that the PDMS has the greatest affinity to those compounds that have log K_{ow}> 3 (Prieto et al. 2010) and a very low affinity to CBZ, which is the compound with the lowest hydrophobicity (log K_{ow} =2.45).

The effect of pH on the extraction efficiency was studied at different pH values (2, 3, and 6) by immersing a 10 mm PDMS rod in 50 mL of 100 μgL^{-1} solution of the target analytes for 10 h. After equilibrium, the rod was exposed to 200 µL of methanol for 30 min. The best results in terms of the concentrations of the analytes in the desorption solution were obtained at pH 2 for TCS and KET, and at pH 3 for NAP whereas for CBZ and DCF, no significant differences were obtained between pH 2 and 3 (Fig.1). Finally, pH 2 was selected as a compromise, particularly taking into account the need to improve the preconcentration of KET. At this pH, all the analytes were present in their non-ionized forms: pH < pK_a (DCF pKa 4.3, NAP pK_a 4.15, KET pK_a 4.45, TCS pk_a 8.14, and CBZ pK_a 13.9).

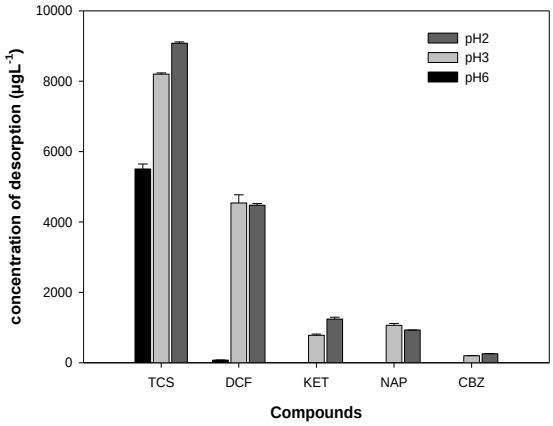

Fig. 1 Effect of pH on the extraction (n=3). Initial: 50 mL of 100 μgL^{-1} of pharmaceuticals and triclosan and 15% NaCl. Desorption volume: 200 µL and desorption time: 30 min.

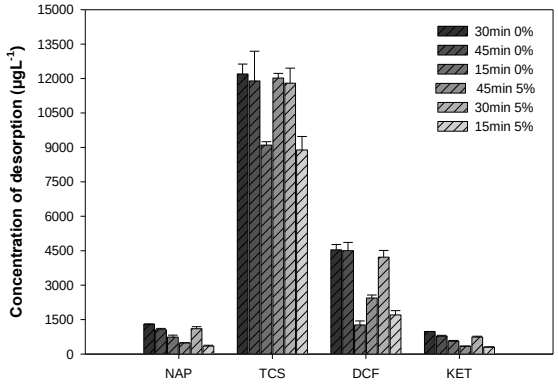

Fig. 2 Effect of the addition of methanol on the extraction (n=3) and of the desorption time. 50 mL of 100 μgL^{-1} of the target analytes at pH 2 and 15% NaCl with 5% of MeOH and without modifier. Desorption volume: 200µL.

The addition of matrix modifiers such as methanol and NaCl to aqueous solutions is common in SBSE and SPME techniques. Here we study the addition of 5% methanol to the sample, which is added to reduce the adsorption of organic analytes on the glassware (Valls-Cantenys

et al. 2014) together with desorption time (15 min, 30 min and 45 min). The concentrations in the desorption solutions increased without adding methanol (Fig. 2). With 5% methanol, concentrations in the desorption solutions increased at 30 min and 45 min, although less so than when no methanol was used, which consequently was the chosen option.

We also studied the salting-out effect (Valls-Cantenys et al. 2014) at concentrations of NaCl of 0, 5, 10 and 15% (w/v). The progressive addition of salt resulted in a significant increase in the extraction efficiency, which is seen in the increase in the concentrations of TCS, NAP, KET, DCF and CBZ in the desorption solution when the percentage of NaCl was increased to 15%. (Fig. 3). Therefore, the addition of 15% NaCl to the aqueous solution was found to be optimum for extracting the analytes.

The effect of the initial volume was tested by using volumes of 25, 50 and 100 mL, of a 20 μgL^{-1} solution containing all the analytes and 15% NaCl at pH 2. Analyte desorption was performed with 100 µL of methanol in an ultrasonic bath for 30 min. Results are presented as enrichment factors (EF), defined as the ratio of analyte concentration (C_{desor}) in the desorbed methanol solution and the initial concentration in the aqueous phase (C_0) (Fig. 4). EF of 134 for TCS, 110 for DCF, 32 for NAP, 28 for KET and 2 for CBZ were obtained with a sample volume of 50 mL (Fig. 4). EF for TCS increased significantly as the sample volume was raised to 100 mL, whereas the increase in DCF was relatively slight. EFs for KET and NAP remained almost unchanged with 25 and 50 mL and decreased with 100 mL, while for CBZ the EF was only calculated for 50 mL, which was the volume selected for the following experiments.

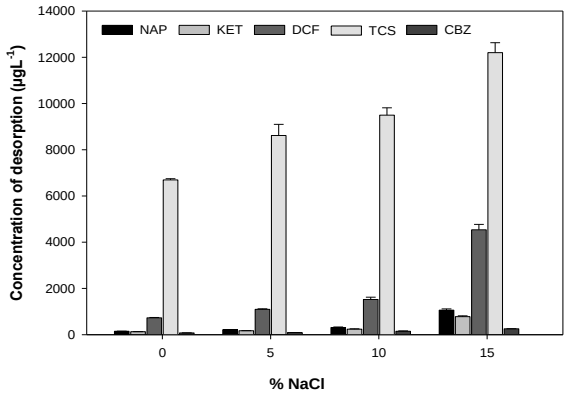

Fig.3 Effect of the addition of NaCl on the extraction (n=3). 50 mL of 100 μgL^{-1} of pharmaceuticals and triclosan solution at pH=2. Desorption volume: 200 µL; desorption time: 30 min.

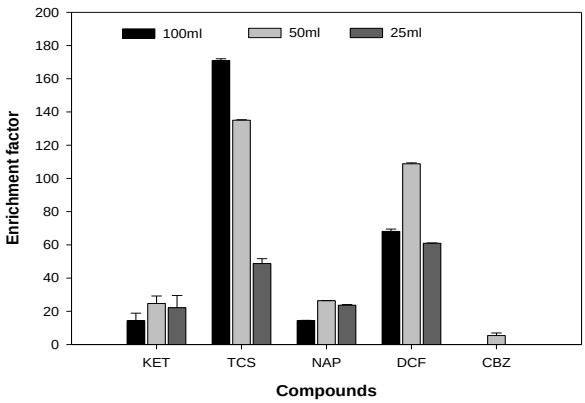

Fig.4 Enrichment factors obtained with different sample volumes (n=3). Initial concentration: 20 μgL^{-1} of pharmaceuticals and triclosan at pH 2 and 15% NaCl. Desorption volume: 100 µL; desorption time: 30 min.

Methanol and acetonitrile were tested as desorption solvents to strip the target compounds from the polymeric phase. Triplicate extractions were performed a 100 μgL^{-1} solution in the previously described conditions. Then, three consecutive desorptions of 30 min each were performed and two solvent volumes of 100 and 200 µL were tested. Acetonitrile is slightly better than methanol in desorbing TCS and DCF, which are the most lipophilic compounds, whereas no differences between methanol and acetonitrile were found for KET and NAP.

Methanol (200 µL) was finally selected as the desorption solvent, facilitating the chromatographic analysis.

After selecting the desorption solvent, the back-extraction time was also evaluated at different periods (15, 30 and 45 min). In order to accelerate the stripping of the adsorbed compounds, ultrasonic treatment was also tested, except in the case of 45 min where it was preferred to avoid the risk of breaking the vial. No significant difference was found between 30 min and 45 min with or without sonication for TCS, NAP and KET, whereas 15 min of sonication was only efficient in the case of DCF (Fig. 5). Given its greater simplicity, a desorption time of 30 min without sonication was selected.

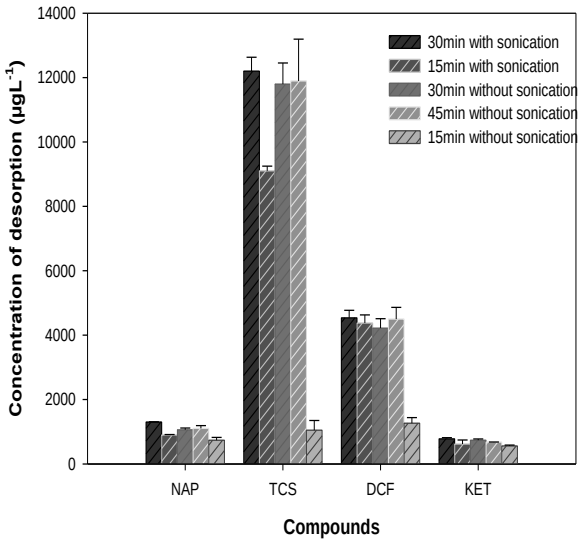

Fig. 5 Effect of desorption time and sonication on the desorption of the extracted pharmaceuticals and triclosan (n=3). 50 mL of 100 µgL^{-1} solution of pharmaceuticals and triclosan at pH=2 and 15% NaCl. Desorption volume: 200 µL.

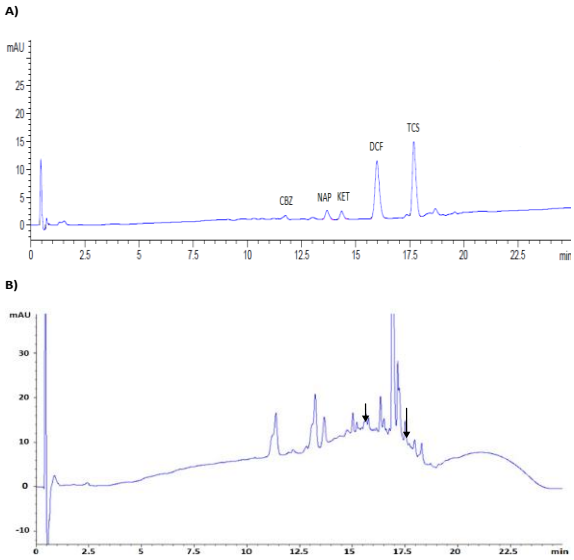

Fig. 6 A) Chromatogram of a river water sample spiked at 10 µgL^{-1} of pharmaceuticals and triclosan, and B) Chromatogram of a river water sample in which only DCF (15.5 min) and TCS (17.5 min) were detected, obtained with the PDMS-rod-HPLC-DAD method.

Linearity was evaluated by extracting ultrapure water samples spiked in triplicate with all the target compounds at five different concentration levels: 10, 25, 50, 75 and 100 µgL^{-1}. The concentrations were selected taking into account the different EFs obtained for each compound, since in the case of carbamazepine the EF was the lowest the calibration curve was built with standards ranging from 25 to 150 µgL^{-1}.The method was linear for all compounds and determination coefficients (r^2) were higher than 0.990 (Table 1). The LODs and LOQs were calculated using the Excel regression analysis tool and considering a signal-to-noise ratio of 3 and 10, respectively. LODs ranged from 0.1 to 0.3 µgL^{-1}, except for carbamazepine, which was 6.04 µgL^{-1}. LOQs ranged from 0.4 to 1.0 µgL^{-1}, except for carbamazepine, which was 18.33 µgL^{-1}.The precision of the method, expressed as RSD%, was evaluated by replicate analysis (n=6) of ultrapure water samples spiked at two concentration levels (25 and 100 µgL^{-1}). Intraday precision was in the range of 0.4–9.7% at both levels and interday precision was between 3.8 and 10.5%, except for carbamazepine, which was 18.8%.

Table 1 Calibration curves, LODs, LOQs and precision of the method.

Compounds	**Retention time** (min)	**Equations of calibration curve**	Linearity (R^2)	**RSD interday (%)** (n=6)		**RSD intraday (%)** (n=2)		**LOD** (μgL^{-1})	**LOQ** (μgL^{-1})
				25 μgL^{-1}	**100** μgL^{-1}	**25** μgL^{-1}	**100** μgL^{-1}		
KET	13.8	$y = 9.9x + 1.5$	0.999	3.8	4.7	0.4	6.0	0.2	0.5
TCS	17.5	$y = 29.1x - 1.2$	1	4.5	4.7	1.6	2.3	0.1	0.4
NAP	13.3	$y = 3.4x + 1$	0.999	10.2	10.5	0.5	0.4	0.3	1.0
DCF	15.5	$y = 13.1\ x + 0.4$	0.999	5.9	5.6	5.8	2.2	0.2	0.7
CBZ	11.12	$y = 0.5x - 1.7$	0.997	8	18.8	7.7	9.7	6.0	18.3

To evaluate the applicability of the present methodology to real samples, assays were performed by analysing spiked river water samples at concentrations of 10, 25, and 75 μgL^{-1} of all the target compounds. However, CBZ's recoveries could only be calculated at the 75 μgL^{-1} as the spiking level of 25 μgL^{-1} was too close to the LOQ. The recoveries obtained were in the range of 84.8–108.0% at the lowest concentration level, 87.3–111.2% for the medium concentration level, and 86.5–104% for the highest concentration level (Table 2). Before performing the recovery experiments, the river water samples were analysed by HPLC-MS/MS in order to ensure that the target compounds were not present. As can be seen in the chromatogram of the river water sample spiked at 10 μgL^{-1} (Fig. 6 A), the peaks of all the target analytes are separated between them and of the baseline. CBZ was not quantified as the peak area was below the corresponding to the LOQ.

Table 2 Recoveries (%) of the target analytes by the developed methodology at three spiking levels

Compounds	Concentration (μgL^{-1})		
	10	**25**	**75**
CBZ	-	-	99.1±1.6
KET	97.7±5.6	100.7±0.4	96.1±3.8
TCS	84.8±3.4	87.3±7.1	109.4±2.4
NAP	91.2±2.6	92.0±7.1	86.5±1.1
DCF	108.0±7.5	111.2±7.9	104.0±8.1

The developed method was compared with other microextraction techniques followed by HPLC-DAD analysis (Table 3). The proposed methodology had better recovery levels for KET, NAP, DCF and TCS than SBSE coated with PDMS (Silva and Nogueira 2008; Silva et al. 2008), polyurethane (PU) (Silva et al. 2008), and synthesized ionic liquids (IL) (Fan et al. 2014). BaμE coated with an N-vinylpyrrolidone polymer (NVP) (Ahmad et al. 2017) and SPME with a PDMS/divynilbenzene (DVB) fibre (Vera-Candioti et al. 2008) gave better recoveries with the method developed here, except for CBZ. Similar recoveries were obtained

using a BaμE coated with a synthetic polymer (P5) (Almeida et al. 2017). On comparing the amount of the sorbent phases used and their chemical properties, it was found that smaller amounts such as those reported in Ahmad et al. 2017; Almeida et al. 2017; Silva and Nogueira 2008; Silva et al. 2008), led to lower recoveries being obtained, except in the case of BaμE (P5) and BaμE (NVP). Both polymeric-based (P5 and NVP) sorbents improved the sensitivity and selectivity of HPLC-DAD determination given that a mixed hydrophobic and π-π interaction is involved in the sorption process. The LODs achieved by the developed method are almost as good both in terms of order and number as those of other microextraction techniques used in combination with HPLC-DAD (Silva et al. 2008, Vera-Candioti et al. 2008) and they can be improved by reducing the desorption volume. Another strategy to improve sensitivity is to combine the use of a commercial PDMS rod with LC-MS/MS.

Table 3 Comparison of the LODs and average recovery of different static microextraction techniques for the determination of pharmaceuticals and triclosan

Analytes	Static micro-extraction technique	Recovery (%)	LOD ($\mu g L^{-1}$)	Amount (g) or μL	Ref.
NAP	BAμE (P5)	100.1	0.025	0.001	(Almeida et al. 2017)
	SBSE (IL)	52.7	0.31	30 μL	(Fan et al. 2014)
	SBSE (PDMS) SBSE (PU)	9.8 78.3	1 0.4	0.1201 0.1	(Silva et al. 2008) (Silva et al. 2008)
	SPME (PDMS/DVB)	117.9	0.5	65 μL	(Vera-Candioti et al. 2008)
	PDMS rod	**86.5**	**0.3**	**0.037**	**Present study**
KET	BAμE (P5)	101	0.05	0.001	(Almeida et al. 2017)
	SBSE (IL)	51.6	0.27	30 μL	(Fan et al. 2014)
	SPME (PDMS/DVB)	106.2	2.0	65 μL	(Vera-Candioti et al. 2008)
	PDMS rod	**96.1**	**0.2**	**0.037**	**Present study**
DCF	BAμE (P5)	99.1	0.1	0.001	(Almeida et al. 2017)
	BAμE (NVP)	87.4	0.02	0.0025	(Ahmad et al. 2017)
	SBSE (PDMS) SBSE (PU)	34.6 77.7	1.6 0.7	0.1201 0.1	(Silva et al. 2008) (Silva et al. 2008)
	SPME (PDMS/DVB)	107.1	1.5	65 μL	(Vera-Candioti et al. 2008)
	PDMS rod	**104.0**	**0.2**	**0.037**	**Present study**
CBZ	BAμE (NVP)	102.4	0.02	0.0025	(Ahmad et al. 2017)
	SPME (PDMS/DVB)	79.4	3.0	65 μL	(Vera-Candioti et al. 2008)
	PDMS rod	**99.1**	**6.0**	**0.037**	**Present study**
TCS	SBSE (PDMS)	78.5	0.1	0.1201	(Silva and Nogueira, 2008)
	BAμE (NVP)	74.5	0.03	0.0025	(Ahmad et al. 2017)
	PDMS rod	**109.4**	**0.1**	**0.037**	**Present study**

The developed method was applied to the analysis of water samples from three different rivers in north-east Spain. TCS and DCF seemed to be detected although quantification was

not carried out due to the poor resolution between the adjacent peaks (Fig.6 B). DCF at μgL^{-1} concentration levels have been detected in surface waters from different regions at mean concentration levels of 2.20 μgL^{-1} and a maximum concentration of 18.74 μgL^{-1} was found in the Llobregat river (Ginebreda et al. 2010) while in river water of South Africa was 9.69 μgL^{-1} (Madikizela and Chimuka 2017).

The sensitive, effective and low-cost method, based on the combination of PDMS rod extraction with HPLC-DAD that has been developed here allows the determination of four pharmaceuticals (NAP, KET, CBZ, DCF) and TCS in surface water samples resulting in detection limits in the 0.1-0.3 μgL^{-1}range, except 6.04 μgL^{-1} for carbamazepine. These LODs are more than ten times lower that those obtained with the instrumental method, except for CBZ, and can be improved by using a lower volume of the desorption solvent which makes this method environmentally friendly. The main advantages of PDMS rods are that they are commercial and more economical than other sorbents, and are single use, so avoiding carryover and contamination issues and allowing HPLC-DAD, which is widely available in non-specialised laboratories, to be applied for pharmaceuticals and TCS determination in surface waters. It should, of course, be taken into account that there is a greater time requirement than for direct injection, although the later requires a clean-up step and it fails to achieve such a good level of sensitivity.

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A polydimethylsiloxane rod extraction-based method for the determination of pharmaceuticals and triclosan by liquid chromatography in water samples

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Abstract

A new analytical method for the determination of naproxen, ketoprofen, diclofenac, carbamazepine, and triclosan in water samples by liquid chromatography is developed and validated. The method is based on the extraction of the analytes by a polydimethylsiloxane (PDMS) rod. The different parameters affecting extraction, such as the addition of salt, pH, initial volume, extraction and elution times and elution solvent, as well as the application of sonication, are studied. The results showed that the detection limits are all in the 0.47 to 1.02 μgL^{-1} range except for carbamazepine (3.4 μgL^{-1}) with relative standard deviations in the range of 0.4–9.7%. The method developed, which was validated by analysing spiked surface water samples at trace levels, gave recoveries of between 84.8 and 111.2%. The main advantage of the developed method is that allows high performance liquid chromatography-diode array (HPLC-DAD), which is widely available in non-specialised laboratories, to be applied for pharmaceuticals and triclosan determination in surface waters after performing a preconcentration/clean-up step with PDMS rods as it has been shown by analysing real water samples.

Keywords: polydimethylsiloxane rod, pharmaceuticals, triclosan, high performance liquid chromatography, UV-vis, microextraction techniques.

Introduction

Pharmaceuticals and antibacterial compounds are classified as emerging contaminants as they are regarded as possible threats to the aquatic environment and human health (Bu et al. 2013; Liu and Wong 2013). Although these compounds are normally not regulated, diclofenac has recently been included in the European Union watch list (Directive 2013/39/EU) and triclosan has been categorized as a high priority pollutant by the Environmental Protection Agency (EPA) in the aggregate risk assessment (Chen et al. 2013).

Due to their poor biodegradability, these contaminants are normally not eliminated in sewage treatment plants, and therefore are able to make their way into drinking water via river waters and lakes (Delgado et al. 2013; Gros et al. 2012). Anti-inflammatory drugs, such as diclofenac (DCF), naproxen (NAP) and ketoprofen (KET), have been detected in surface waters in concentrations of 10 μgL^{-1}, 121μgL^{-1}, and 102μgL^{-1}, respectively (Yang et al. 2017). Triclosan (TCS) was the most commonly found antibacterial in surface water with concentrations of up to 24 μgL^{-1} (Blair et al. 2013; Kasprzyk-Hordern et al. 2008).

Liquid chromatography with ultraviolet (UV-vis) and mass spectrometry (MS) detection are among the most important analytical methodologies to measure trace levels of pharmaceuticals and triclosan, although they require sample enrichment steps prior to chromatographic analysis (Richardson and Ternes 2014). The extraction of the analytes with solid-phase extraction (SPE) while having some advantages requires relatively large volumes of toxic solvents and is laborious and costly (Togunde et al. 2012). The use of solid-phase microextraction (SPME), in which the analytes are extracted by a polydimethylsiloxane (PDMS) fibre, has the disadvantages of the fragility of the fibre and the need for a special device when combined with high performance liquid chromatography (HPLC) (Płotka-Wasylka et al. 2015). Stir-bar sorptive extraction (SBSE) consists of a magnetic bar covered by a thin layer of a sorptive phase, generally PDMS, and provides improved extraction efficiencies in comparison with SPME (He et al. 2014). Other stir extraction techniques are stir-rod-sorptive extraction, stir-cake-sorptive extraction (SCSE), and rotating disk sorptive extraction (RDSE) that share the same sorptive principle as SPME (Cárdenas and Lucena 2017). In recent years, new microextraction techniques such as bar adsorptive micro-extraction (BAμE) (Neng et al. 2010; Almeida et al. 2017) and dynamic fabric phase sorptive extraction (DFPSE) have been applied in the determination of pharmaceuticals and triclosan (Lakade et al. 2016).

All these microextraction techniques have great potential, however, some of them requires of the synthesis of polymeric sorbent phases (Ahmad et al. 2017; Almeida et al. 2017). Hence, there is a need for less costly and simpler methods by combining efficient extraction/preconcentration techniques, using commercial sorbents, with chromatographic techniques such as HPLC-DAD, that is available in routine monitoring laboratories and that can determine the target analytes at low μg/L concentration levels. The extraction efficiency of technical silicone sorbents such as PDMS rods, which were introduced by Popp et al. 2004, meets analytical requirements in terms of purity, inertness and thermal stability and were applied to extract pharmaceuticals (Paschke et al. 2007). Other advantages of PDMS rods are their greater flexibility and robustness, together with the fact that they can be discarded after a single use, eliminating problems of carryover (van Pinxteren et al. 2010). Moreover, PDMS rods can be used as sorptive materials in passive sampling (Seethapathy and Górecki 2012).

The objective of this study is to develop a new analytical method for the determination of NAP, KET, CBZ, DCF, and TCS based on their extraction and preconcentration by PDMS rods followed by liquid desorption and high performance liquid chromatography (HPLC-DAD) analysis. The method is validated by analysing spiked surface water samples and applied to the determination of target compounds in river waters. The analytical parameters of the developed method are compared with those obtained with other micro-extraction based techniques.

Methods and Materials

Triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol), naproxen ((2S)-2-(6 methoxynaphthalen-2-yl)propanoic acid), ketoprofen (2-(3-Benzoylphenyl)propanoic acid), carbamazepine (benzobenzazepine-11-carboxamide), and sodium diclofenac (sodium;2-[2-(2,6-dichloroanilino)phenyl]acetate) were purchased from Sigma–Aldrich (Germany). Working solutions of pharmaceuticals and triclosan ranging from 10 to 150 μgL^{-1} were prepared with ultra-pure water by dilution of a 500 mgL^{-1} stock methanol solution. Chromatographic grade acetonitrile (Fisher, USA), sodium chloride (Carlo Erba, Italy) and analytical grade anhydrous sodium acetate, acetic and hydrochloric acids (Sigma-Aldrich, Germany) were used. Ultrapure water with conductivity of 18.2 MΩ/cm was obtained from a water purification system (Millipore, USA).

An Agilent 1200 series high performance liquid chromatography system equipped with two pumps and a DAD detector was used. The analytes were separated in a C18 Luna column (50 × 2 mm, 2.5 µm) (Phenomenex, USA) using a gradient of mobile phase: (A) 0.1 % acetic acid and 4.7 mM of sodium acetate in ultra-pure water, and (B) acetonitrile (0 min, 90% A; 5 min, 75%A; 10 min, 65% A; 15 min, 20% A) at a flow rate of 0.3 $mLmin^{-1}$.The detection wavelength was set at 242 nm for CBZ, KET and TCS; 250 nm for NAP; and 280nm for DCF. Water samples were filtered with a 0.2 µm nylon membrane (Supelco, USA) before injection.

Commercial 10 mm elastomer PDMS rods (approx. 0.037 g) were cut from a flexible 2 mm diameter PDMS cord (Goodfellow, England). These were then cleaned and stored in methanol and, immediately prior to use, were dried with a lint-free tissue. The PDMS rod was immersed in 50 mL of a 100 μgL^{-1} solution of all the compounds in ultrapure water and 15% w/v of NaCl. The pH was adjusted as required (2, 3, and 6) and the extraction was performed for different periods of time (3, 5, 8, 10 and 24 h). The experiments were performed three times using a ten-point magnetic shaker (MultiMix D, Ovan, Spain) at 200 rpm. After extraction, the PDMS rod was removed with clean tweezers and then dried with a lint-free tissue. The rod was then placed into a tapered glass insert containing 200 µL of methanol allowing the desorption process to take place for times ranging from 15 to 45 minutes with and without sonication in an ultrasonic bath (J.P. Selecta, Spain). The PDMS rod was removed and 10 µL of the extract were then injected into the liquid chromatograph. During the experiments performed to find out the best adsorption and desorption conditions, aliquots of the desorbed solution were measured by interpolation in a calibration curve obtained using standard solutions in methanol. The detection limits of the instrumental method were: 2.42 μgL^{-1} for KET, 3.65 μgL^{-1} for NAP, 3.99 μgL^{-1} for DCF, 4.48 μgL^{-1} for CBZ, and 5.45 μgL^{-1} for TCS.

Water samples were collected in 1 L amber glass bottles from the Onyar, Ter and Fluvià rivers (Girona, Spain). Samples were transported to the laboratory under refrigeration and

then stored at 4°C before characterization by determining their conductivity, chemical oxygen demand and ionic composition. The samples were filtered using a 0.45 µm nylon membrane (Supelco, USA). After filtration, one of the samples was spiked with KET, NAP, DCF, CBZ, and TCS at different concentration levels (10, 25, 75 μgL^{-1}) and recovery experiments were carried out in triplicate. The other samples were analysed with the developed method.

Results and discussion

A systematic study of several parameters was undertaken to find the best extraction and desorption conditions for the preconcentration of pharmaceuticals and triclosan.

Extraction time was evaluated with 50 mL of a 100 μgL^{-1} solution containing 15% NaCl and all the studied compounds at pH 3. Five different extraction periods (3, 5, 8, 10, and 24 h) were studied by analysing the remaining concentrations in the aqueous solution. Equilibrium was reached at 10 h for all compounds. The extraction efficiency, calculated as the ratio between the amount of analyte extracted by the PDMS rod (m_s) and its initial mass in the aqueous phase (m_0), followed the order CBZ (6%), KET (17%), NAP (19%), DCF (56%), and TCS (75%), which corresponds to their hydrophobicity order (log K_{ow}): CBZ(2.45) < KET (3.1) < NAP (3.12) <DCF(3.91)< TCS (4.7), showing that the PDMS has the greatest affinity to those compounds that have log K_{ow}> 3 (Prieto et al. 2010).

The effect of pH on the extraction efficiency was studied at different pH values (2, 3, and 6) by immersing a 10 mm PDMS rod in 50 mL of 100 μgL^{-1} solution of the target analytes for 10 h. After equilibrium, the rod was exposed to 200 µL of methanol for 30 min. The best results in terms of the concentrations of the analytes in the desorption solution were obtained at pH 2 for TCS and KET, and at pH 3 for NAP whereas for CBZ and DCF, no significant differences were obtained between pH 2 and 3 (Fig.1). Finally, pH 2 was selected as a compromise, particularly taking into account the need to improve the preconcentration of KET. At this pH, all the analytes were present in their non-ionized forms: pH < pK_a (DCF pKa 4.3, NAP pK_a 4.15, KET pK_a 4.45, TCS pk_a 8.14, and CBZ pK_a 13.9).

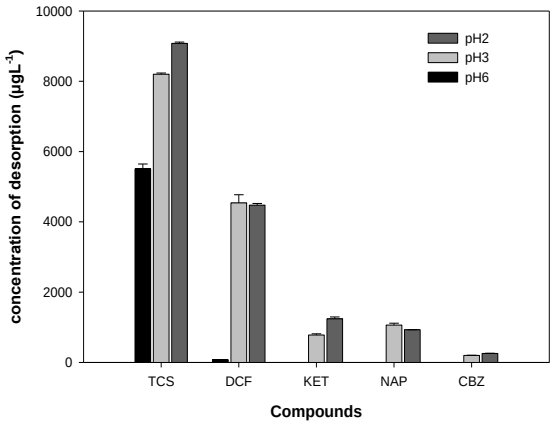

Fig. 1 Effect of pH on the extraction (n=3). Initial: 50 mL of 100 μgL^{-1} of pharmaceuticals and triclosan and 15% NaCl. Desorption volume: 200 µL and desorption time: 30 min.

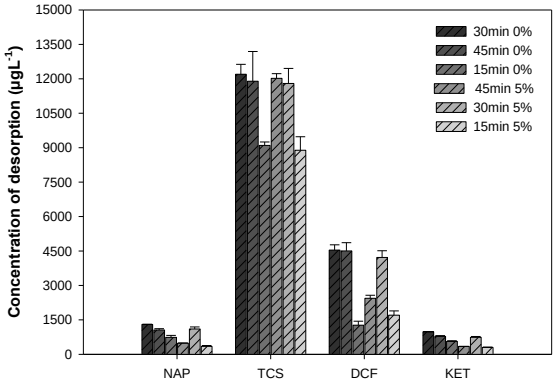

Fig. 2 Effect of the addition of methanol on the extraction (n=3) and of the desorption time. 50 mL of 100 μgL^{-1} of the target analytes at pH 2 and 15% NaCl with 5% of MeOH and without modifier. Desorption volume: 200µL.

The addition of matrix modifiers such as methanol and NaCl to aqueous solutions is common in SBSE and SPME techniques. Here we study the addition of 5% methanol to the sample,

which is added to reduce the adsorption of organic analytes on the glassware (Valls-Cantenys et al. 2014) together with desorption time (15 min, 30 min and 45 min). The concentrations in the desorption solutions increased without adding methanol (Fig. 2). With 5% methanol, concentrations in the desorption solutions increased at 30 min and 45 min, although less so than when no methanol was used, which consequently was the chosen option.

We also studied the salting-out effect (Valls-Cantenys et al. 2014) at concentrations of NaCl of 0, 5, 10 and 15% (w/v). The progressive addition of salt resulted in a significant increase in the extraction efficiency, which is seen in the increase in the concentrations of TCS, NAP, KET, DCF and CBZ in the desorption solution when the percentage of NaCl was increased to 15%. (Fig. 3). Therefore, the addition of 15% NaCl to the aqueous solution was found to be optimum for extracting the analytes.

The effect of the initial volume was tested by using volumes of 25, 50 and 100 mL, of a 20 μgL^{-1} solution containing all the analytes and 15% NaCl at pH 2. Analyte desorption was performed with 100 µL of methanol in an ultrasonic bath for 30 min. Results are presented as enrichment factors (EF), defined as the ratio of analyte concentration (C_{desor}) in the desorbed methanol solution and the initial concentration in the aqueous phase (C_0) (Fig. 4).

EF for TCS increased significantly as the sample volume was raised to 100 mL, whereas the increase in DCF was relatively slight. EFs for KET and NAP remained almost unchanged with 25 and 50 mL and decreased with 100 mL, while for CBZ the EF was only calculated for 50 mL (Fig. 4). A 50 mL sample volume was selected for the following experiments.

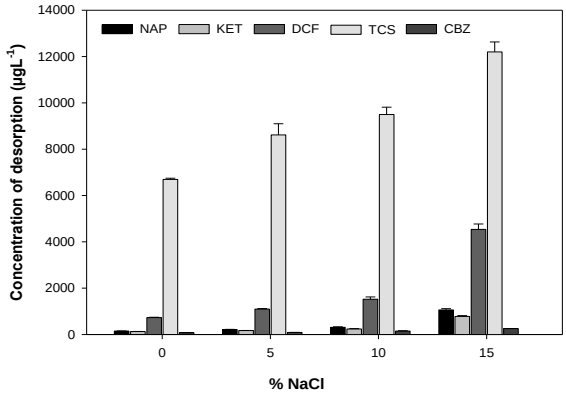

Fig.3 Effect of the addition of NaCl on the extraction (n=3). 50 mL of 100 μgL^{-1} of pharmaceuticals and triclosan solution at pH=2. Desorption volume: 200 µL; desorption time: 30 min.

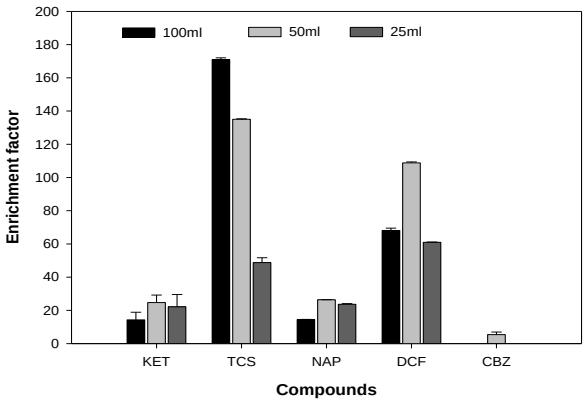

Fig.4 Enrichment factors obtained with different sample volumes (n=3). Initial concentration: 20 μgL^{-1} of pharmaceuticals and triclosan at pH 2 and 15% NaCl. Desorption volume: 100 µL; desorption time: 30 min.

Methanol and acetonitrile were tested as desorption solvents to strip the target compounds from the polymeric phase. Triplicate extractions were performed a 100 μgL^{-1} solution in the previously described conditions. Then, three consecutive desorptions of 30 min each were performed and two solvent volumes of 100 and 200 µL were tested. Acetonitrile is slightly better than methanol in desorbing TCS and DCF, which are the most lipophilic compounds, whereas no differences between methanol and acetonitrile were found for KET and NAP

Methanol was finally selected as the desorption solvent, facilitating the chromatographic analysis.

After selecting the desorption solvent, the back-extraction time was also evaluated at different periods (15, 30 and 45 min). In order to accelerate the stripping of the adsorbed compounds, ultrasonic treatment was also tested, except in the case of 45 min where it was preferred to avoid the risk of breaking the vial. No significant difference was found between 30 min and 45 min with or without sonication for TCS, NAP and KET, whereas 15 min of sonication was only efficient in the case of DCF (Fig. 5). Given its greater simplicity, a desorption time of 30 min without sonication was selected.

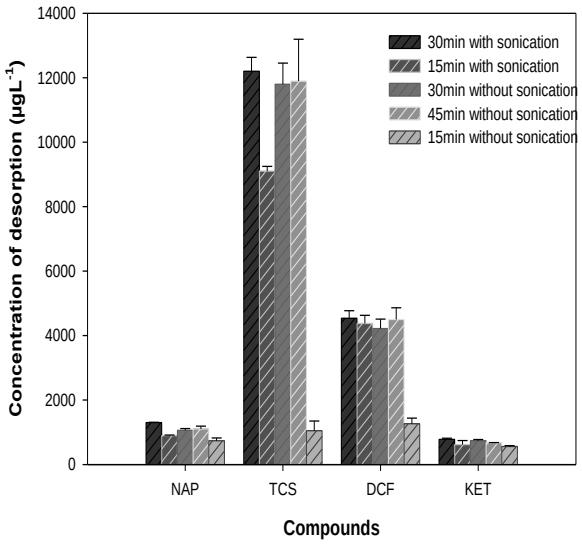

Fig. 5 Effect of desorption time and sonication on the desorption of the extracted pharmaceuticals and triclosan (n=3). 50 mL of 100 µgL^{-1} solution of pharmaceuticals and triclosan at pH=2 and 15% NaCl. Desorption volume: 200 µL.

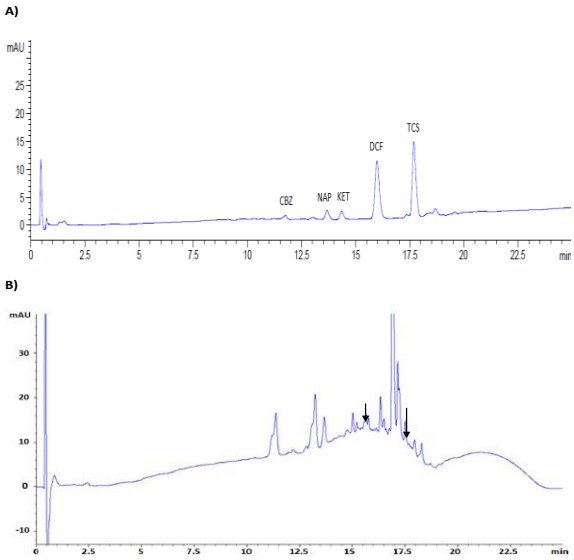

Fig. 6 A) Chromatogram of a river water sample spiked at 10 µgL^{-1} of pharmaceuticals and triclosan, and B) Chromatogram of a river water sample in which only DCF (15.5 min) and TCS (17.5 min) were detected, obtained with the PDMS-rod-HPLC-DAD method.

Linearity was evaluated by extracting ultrapure water samples spiked in triplicate with all the target compounds at five different concentration levels: 10, 25, 50, 75 and 100 µgL^{-1}. The concentrations were selected taking into account the different EFs obtained for each compound, since in the case of carbamazepine the EF is practically 1 where as in the case of triclosan, the most hydrophobic compound tested, the EF is 174.The method was linear for all compounds and determination coefficients (r^2) were higher than 0.990 (Table 1). The LODs and LOQs were calculated using the Excel regression analysis tool and considering a signal-to-noise ratio of 3 and 10, respectively. LODs ranged from 0.47 to 1.02 µgL^{-1}, except for carbamazepine, which was 3.40 µgL^{-1}. LOQs ranged from 1.44 to 3.17 µgL^{-1}, except for carbamazepine, which was 10.33 µgL^{-1}.The precision of the method, expressed as RSD%, was evaluated by replicate analysis (n=6) of ultrapure water samples spiked at two concentration levels (25 and 100 µgL^{-1}). Intraday precision was in the range of 0.4–9.7% at both levels and interday precision was between 3.8 and 10.5%, except for carbamazepine, which was 18.8%.

Table 1 Calibration curves, LODs, LOQs and precision of the method.

Compounds	**Retention time** (min)	**Equations of calibration curve**	Linearity (R^2)	**RSD interday (%)** (n=6)		**RSD intraday (%)** (n=2)		**LOD** (μgL^{-1})	**LOQ** (μgL^{-1})
				25 μgL^{-1}	**100** μgL^{-1}	**25** μgL^{-1}	**100** μgL^{-1}		
KET	13.8	$y = 14.15x - 34.01$	0.999	3.8	4.7	0.4	6.0	1.02	3.17
TCS	17.5	$y = 96.5\,x - 76.68$	1	4.5	4.7	1.6	2.3	0.47	1.44
NAP	13.3	$y = 7.538\,x + 13.41$	0.999	10.2	10.5	0.5	0.4	0.56	1.70
DCF	15.5	$y = 38.62\,x + 88.79$	0.999	5.9	5.6	5.8	2.2	0.75	2.24
CBZ	11.12	$y = 2.094x - 54.82$	1	8	18.8	7.7	9.7	3.40	10.33

To evaluate the applicability of the present methodology to real samples, assays were performed by analysing spiked river water samples at concentrations of 10, 25, and 75 μgL^{-1} of all the target compounds. The chromatogram of the river water sample spiked at 10 μgL^{-1} is shown in Fig. 6 A. The recoveries obtained were in the range of 84.8–108.01% at the lowest concentration level, 87.31–111.18% for the medium concentration level, and 86.53–103.98% for the highest concentration level (Table 2). Before performing the recovery experiments, the river water samples were analysed by HPLC-MS/MS in order to ensure that the target compounds were not present.

Table 2 Recoveries (%) of the target analytes by the developed methodology at three spiking levels

Compounds	Concentration (μgL^{-1})		
	10	**25**	**75**
CBZ	-	-	99.07±1.59
KET	97.66±5.65	100.67±0.43	96.1±3.84
TCS	84.8±3.97	87.31±7.06	109.45±2.36
NAP	91.25±2.65	91.96±7.06	86.53±1.11
DCF	108.01±7.54	111.18±7.93	103.98±8.1

The developed method was compared with other microextraction techniques followed by HPLC-DAD analysis (Table 3). The proposed methodology had better recovery levels for KET, NAP, DCF and TCS than SBSE coated with PDMS (Silva and Nogueira 2008; Silva et al. 2008), polyurethane (PU) (Silva et al. 2008), and synthesized ionic liquids (IL) (Fan et al. 2014). BaμE coated with an N-vinylpyrrolidone polymer (NVP) (Ahmad et al. 2017) gave better recoveries with the method developed here, except for CBZ with BaμE (NVP) (Ahmad

et al. 2017). Similar recoveries were obtained using a BaμE coated with a synthetic polymer (P5) (Almeida et al. 2017). On comparing the amount of the sorbent phases used and their chemical properties, it was found that smaller amounts such as those reported in Ahmad et al. 2017; Almeida et al. 2017; Silva and Nogueira 2008; Silva et al. 2008), led to lower recoveries being obtained, except in the case of BaμE (P5) and BaμE (NVP). Both polymeric-based (P5 and NVP) sorbents improved the sensitivity and selectivity of HPLC-DAD determination given that a mixed hydrophobic and π-π interaction is involved in the sorption process. The LODs achieved by the developed method are almost as good both in terms of order and number as those of other microextraction techniques used in combination with HPLC-DAD (Silva et al. 2008) and they can be improved by reducing the desorption volume. Another strategy to improve sensitivity is to combine the use of a commercial PDMS rod with LC-MS/MS.

Table 3 Comparison of the LODs and average recovery of different static microextraction techniques for the determination of pharmaceuticals and triclosan

Analytes	Static micro-extraction technique	Recovery (%)	LOD (μgL^{-1})	Amount (g) or μL	Ref.
NAP	BAμE (P5)	100.1	0.025	0.001	(Almeida et al. 2017)
	SBSE (IL)	52.7	0.31	30 μL	(Fan et al. 2014)
	SBSE (PDMS)	9.8	1	0.1201	(Silva et al. 2008)
	SBSE (PU)	78.3	0.4	0.1	(Silva et al. 2008)
	PDMS rod	**86.53**	**0.56**	**0.037**	**Present study**
KET	BAμE (P5)	101	0.05	0.001	(Almeida et al. 2017)
	SBSE (IL)	51.6	0.27	30 μL	(Fan et al. 2014)
	PDMS rod	**96.1**	**1.02**	**0.037**	**Present study**
DCF	BAμE (P5)	99.1	0.1	0.001	(Almeida et al. 2017)
	BAμE (NVP)	87.4	0.02	0.0025	(Ahmad et al. 2017)
	SBSE (PDMS)	34.6	1.6	0.1201	(Silva et al. 2008)
	SBSE (PU)	77.7	0.7	0.1	(Silva et al. 2008)
	PDMS rod	**103.98**	**0.75**	**0.037**	**Present study**
CBZ	BAμE (NVP)	102.4	0.02	0.0025	(Ahmad et al. 2017)
	PDMS rod	**99.07**	**3.40**	**0.037**	**Present study**
TCS	SBSE (PDMS)	78.5	0.1	0.1201	(Silva and Nogueira, 2008)
	BAμE (NVP)	74.5	0.03	0.0025	(Ahmad et al. 2017)
	PDMS rod	**109.45**	**0.47**	**0.037**	**Present study**

The developed method was applied to the analysis of water samples from three different rivers in north-east Spain. TCS and DCF seemed to be detected although quantification was not carried out due to the poor resolution between the adjacent peaks (Fig.6 B). DCF at μgL^{-1} concentration levels have been detected in surface waters from different regions at mean concentration levels of 2.20 μgL^{-1} and a maximum concentration of 18.74 μgL^{-1} was found in the Llobregat river (Ginebreda et al. 2010) while in river water of South Africa was 9.69 μgL^{-1} (Madikizela and Chimuka 2017).

The sensitive, effective and low-cost method, based on the combination of PDMS rod extraction with HPLC-DAD that has been developed here allows the determination of four pharmaceuticals (NAP, KET, CBZ, DCF) and TCS in surface water samples resulting in detection limits in the 0.47 to 1.02 μgL^{-1}range, except 3.40 μgL^{-1} for carbamazepine. These LODs can be improved by using a lower volume of the desorption solvent which makes this method environmentally friendly. The main advantages of PDMS rods are that they are commercial and more economical than other sorbents, and are single use, so avoiding carryover and contamination issues and allowing HPLC-DAD, which is widely available in non-specialised laboratories, to be applied for pharmaceuticals and TCS determination in surface waters.

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