Multiresidue analytical method for pharmaceuticals and personal care products in sewage and sewage sludge by online direct immersion SPME on-fiber derivatization – GCMS

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Abstract

The work here presented aimed at developing an analytical method for the simultaneous determination of 22 pharmaceuticals and personal care products, including 3 transformation products, in sewage and sludge. A meticulous method optimization, involving an experimental design, was carried out. The developed method was fully automated and consisted of the online extraction of 17 mL of water sample by Direct Immersion Solid Phase MicroExtraction followed by On-fiber Derivatization coupled to Gas Chromatography - Mass Spectrometry (DI-SPME – On-fiber Derivatization – GC - MS). This methodology was validated for 12 of the initial compounds as a reliable (relative recoveries above 90% for sewage and 70% for sludge; repeatability as %RSD below 10% in all cases), sensitive (LODs below 20 ng L^{-1} in sewage and 10 ng g^{-1} in sludge), versatile (sewage and sewage-sludge samples up to 15,000 ng L^{-1} and 900 ng g^{-1}, respectively) and green analytical alternative for many medium-tech routine laboratories around the world to keep up with both current and forecast environmental regulations requirements. The remaining 10 analytes initially considered showed insufficient suitability to be included in the final method. The methodology was successfully applied to real samples generated in a pilot scale sewage treatment reactor.

Keywords: DI-SPME • GC-MS • On-Fiber Derivatization • PPCPs • Sewage sludge • Wastewater

1 Introduction

The development of analytical methodologies for the determination of pharmaceuticals and personal care products (PPCPs) in environmental matrices has boomed in the past years. In this context, Zwiener and Frimmel [1] reported that the analysis of PPCPs has been traditionally dominated by Liquid Chromatography detected by tandem Mass Spectrometric (LC-MS/MS) techniques. Fischer et al. [2] recently observed major trends in the use of Ultra High Performance Liquid Chromatography (UHPLC) [3] and High Resolution Mass Spectrometry (HRMS) [4-6] like Time Of Flight (TOF) and Orbitrap [7] analyzers. However, these techniques require costly instrumentation not affordable by many laboratories worldwide. In contrast, Gas Chromatography coupled to single quadrupole Mass Spectrometry (GC-MS) is an analytical configuration far more common in routine analysis laboratories around the world, including developing countries. Despite PPCPs are mainly polar compounds and not readily analyzable by GC, Lopez-Serna et al. [8] recently showed how GC-MS is a valid instrumental technique for the analysis of emerging contaminants in environmental matrices like sewage, when a derivatization step is included in the method. In terms of sample preparation, Solid-Phase Extraction (SPE) represents nowadays the most popular technique for the extraction of pollutants from environmental aqueous samples, and recent developments in this field have mainly focused on SPE automation [9]. In addition, a great effort has been lately made to develop new analytical methodologies able to perform direct analyses using miniaturized equipment, thereby achieving high enrichment factors, minimizing solvent consumption and reducing waste [7, 10] in accordance to the requirements of green analytical chemistry. Solid-Phase MicroExtraction (SPME) was firstly developed in the 1990s by Pawliszyn and coworkers [11]. Since then many configurations have been successfully implemented, which can be classified into static and dynamic techniques [12]. Static procedures are typically carried out in stirred samples, including fiber SPME, and constitute the most common format for this technique. Fiber SPME

utilizes a sorbent coating on the outer surface of a fused silica fiber to extract the analyte(s) from the sample matrix in a process that occurs through direct immersion (DI-SPME) or from the sample headspace in a closed container (HS-SPME) [10]. Thus, analytes that exhibit a high vapor pressure can be extracted either by immersing the fiber into the aqueous sample or by sampling its headspace. In contrast, analytes that exhibit a low vapor pressure could only be extracted by immersion. Fiber SPME has become a very popular technique, especially for volatile compounds, due to its simplicity, relatively short extraction time, solvent-free nature, full automation potential and easy coupling with chromatography [12]. These advantages eventually reduce the contamination of the original sample and the loss of analytes. In addition, SPME can also be used for onsite sample extraction and is able to obtain good results even for trace analytes in complex matrices [12]. However, its application to the environmental analysis of polar compounds has been poorly explored, especially when this sample pretreatment is coupled to GC. This application implies the addition of a derivatization step, which is essential for the analysis of non-volatile and/or thermolabile compounds by GC. Today, two approaches are commonly used to carry out derivatization when SPME is the pretreatment technique. The first one, namely *in-situ* derivatization, is based on the addition of the derivatizing agent directly to the sample and the collection of the derived volatile analytes by SPME in the headspace of a closed vial. In the second approach, namely *on-fiber* derivatization, analyte extraction occurs via direct fiber immersion in the sample combined with a headspace derivatization by exposing the analytes-loaded fiber to the vapors of the derivatizing agent. This second approach is environmentally and economically preferred, because the derivatizing agent can be reused for a large number of analyses (with the subsequent decrease of reagent consumption).

This study aimed at developing and optimizing a fully automated method consisting of Online DI-SPME - *On-Fiber* Derivatization - GC-MS for the analysis of 19 PPCPs and 3 of their Transformation Products (TPs) in sewage (SW) and sludge (SS) using statistical experimental design. To the authors' knowledge, there are only two other publications [13, 14] proposing the use of this technique for the analysis of PPCPs in sewage and none for sludge. However, none of them included the level of

automation here presented. Finally, the analytical limitations encountered during the application of this innovative methodology were also discussed.

2 Material and methods

2.1 Chemicals

The standards for all PPCPs and their TPs, provided in **Table S1** as supplementary data, were of high purity grade (>95%). They were purchased from Sigma-Aldrich (Tres Cantos, Madrid, Spain) as neutral non-solvated molecules, except for amoxicillin (acquired as trihydrate), atorvastatin (acquired as calcium salt) and diclofenac (acquired as sodium salt). The isotopically labelled compounds Diclofenac-d4, Ibuprofen-d3, Salicylic acid-d4, Naproxen-d3, Propylparaben-d7 and Triclosan-d3 were obtained from TRC Canada (Toronto, ON, Canada).

Individual stock solutions at 1 g L^{-1} for both PPCPs standards and isotopically-labelled-internal-standards were prepared on a weight basis in methanol (MeOH), except for the fluroquinolones (ciprofloxacin, levofloxacin and norfloxacin), which were dissolved in a water-methanol (H_2O/MeOH) mixture (1:1) containing 0.2% v/v hydrochloric acid (HCl) due to their low solubility in pure MeOH [15]. From them, a stock solution with all the analytes was then prepared in MeOH at 20 mg L^{-1}. Serial aqueous dilutions were subsequently prepared from it. A separate mixture of isotopically labelled internal standards and further dilutions were also prepared. After preparation, all stock solutions were stored at -20 °C in darkness.

High purity solvents, i.e., SupraSolv® GC-MS grade MeOH by Merck Millipore (Madrid, Spain), LC-MS Chromasolv® grade Ethyl Acetate (EA) by Fluka (Madrid, Spain), Sodium chloride (NaCl) and 37% HCl were supplied by Panreac (Barcelona, Spain). Acetone, 99% pure, was supplied by Cofarcas (Burgos, Spain). N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide, with a purity >99%, (MTBSTFA), was obtained from Regis Technologies Inc. (Morton Grove, IL, USA). SPME fibers were purchased from Supelco (Tres Cantos, Madrid, Spain). Milli-Q® grade water was in-house produced. Helium 99.999% (He) was purchased from Abelló Linde S.A. (Alcalá de Henares, Madrid, Spain).

2.2 Sewage analytical methodology

The development of the analytical method, further explained in **Sections SD.1.1 and SD.1.2** within the **Supplementary data (SD)**, was carried out in Milli-Q® water and validated for sewage as detailed in **Section 3.2.1**. In addition, the optimized method based on Online DI-SPME – On-Fiber Derivatization – GC – MS was applied to the analysis of raw and treated wastewater from a pilot scale activated sludge reactor, and the results are presented in **Section 3.2.2**.

2.2.1 Online DI-SPME – On-Fiber Derivatization

Water samples (100 mL) were supplemented with NaCl at 30 % (wt./vol.). After stirring for 20 min to assure complete dissolution, the resulting water sample pH was adjusted to 3 by adding as few drops of diluted solutions of HCl (1%, 0.1% and/or 0.01%) as needed. A volume of 17 mL of the resulting solution was placed in a 20-mL SPME vial along with 200 µL of an aqueous mixture of the isotopically labelled internal standards at 0.5 mg L^{-1}.

The resulting vial was placed in the sample rack of a CTC PAL RSI autosampler. A SPME tool held a 2-cm long 50/30-µm thick Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) StableFlex/SS fiber that was protected inside a 23 Ga needle. The fully automated DI-SPME method included a fiber pre-conditioning for 15 min at 270 °C in the spare GC inlet, followed by 120 min sample extraction at a penetration depth of 60 mm, which entailed that the fiber was fully immersed in the sample (DI-SPME). On-fiber derivatization of the analytes absorbed onto the fiber was then carried out by introducing the fiber in another 20-mL SPME vial containing 1 mL of the derivatizing agent MTBSTFA for 48 min at a penetration depth of 60 mm. Thus, the fiber was exposed to the vapors of the MTBSTFA in the headspace of the vial. Both the DI-SPME and On-Fiber Derivatization were carried out at a constant temperature of 50 °C under orbital agitation at 500 rpm with a stirring regime of 6s on / 30 s off. The fiber, loaded with the derivatized analytes, was then taken to the GC inlet connected to the GC column for desorption at 250 °C for 3 min. Finally, the fiber was post-conditioned for 15 min at 270 °C in the spare GC inlet prior to the next analysis.

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159 **2.2.2 GC – MS**

160 Chromatographic runs started concomitantly with fiber desorption in a pulsed
161 splitless mode at 250 °C in the split/splitless back inlet. A SPME injection sleeve, 0.75
162 mm i.d., was used as a liner. The tests were performed in an Agilent 7890B GC System
163 coupled to a 5977A MSD. A capillary HP-5MS GC column (30 m length, 0.25 mm i.d.,
164 0.25 µm film thickness) was used for the chromatographic separation with He as
165 carrier gas at a constant flow rate of 1.2 mL min^{-1}. Injector temperature was set at 250
166 °C, while the GC oven temperature increased from 70 °C (held for 3 min during fiber
167 desorption) to 120 °C at 20 °C min^{-1}, then to 250 °C at 10 °C min^{-1} and finally to 300 °C
168 (held for 5 min) at 5 °C min^{-1}. The total analysis time for each GC run was 33.5 min. The
169 multimode front GC inlet was set at 270 °C in split mode to facilitate the elimination of
170 residual compounds during fiber pre- and post-conditioning.

171 Mass detection was obtained in electron impact ionization mode (70 eV) with
172 selected ion monitoring (SIM) and a filament delay of 12 min. The GC–MS interface,
173 ion source and quadrupole temperatures were set at 280, 230 and 150 °C,
174 respectively. Quadrupole resolution was set at low. Target compounds were recorded
175 in five acquisition windows along the run time. **Table 1** shows the primary (in italics)
176 and the two secondary ions monitored per compound. Acquisition stopped at min 26.
177 Instrument control and data acquisition were performed by Agilent Technology Mass
178 Hunter B.07.03.2129 software.

179

180 **2.3 Sewage sludge analytical methodology**

181 Aerobic sludge was used to develop and validate the methodology further
182 discussed in **Sections SD.1.3 and 3.2.1**, respectively. The sewage sludge analytical
183 method was designed as follows: 1) One hundred milliliters of fresh sludge sample
184 were freeze-dried. 2) A known amount of dried sludge (~800 mg) was weighed into a
185 20-mL glass vial, along with 200 µL of a mixture of the isotopically labelled internal
186 standards at 20 mg L^{-1} in acetone. 3) The mixture was thoroughly vortex-stirred and
187 remained overnight to allow solvent evaporation and internal standard fixation. 4) A
188 volume of 12 mL of MilliQ® water at pH 9 was then added to the vial, which was then
189 vigorously vortex-stirred to obtain a homogenous suspension. 5) Then, the vial

underwent Ultrasound Assisted Extraction (UAE) for 30 min at room temperature in a JP Selecta Univeba ultrasonic bath of 50 W and 60 Hz (Barcelona, Spain). 6) Subsequently, the suspension was centrifuged for 5 min at 2,655 x g in a Fisher Bioblock Scientific Centrifuge 2-16P (Madrid, Spain). 7) The resulting supernatant was then collected with a glass pipette and transferred to a 20-mL glass vial. 8) Steps 4-7 were repeated once more and the supernatants were pooled together. 9) The resulting solution was analyzed by Online DI-SPME – On-fiber derivatization – GC-MS using the optimized method described in **Section 2.2**, except for the addition of internal standards as they were already added in step 2.

2.4 Experimental design

As a first approach, a screening design was carried out. Hence, the key parameters influencing the performance of the Online DI-SPME – On-Fiber Derivatization methodology were identified for the development of the instrumental leg of both sewage and sludge methods. As a result, a total of 18 parameters were sorted out in four categories, depending on the target of their influence, i.e., DI-SPME extraction, On-Fiber Derivatization, Fiber Desorption and Carry-Over avoidance (**Table S2**). Afterwards, technical limitations to this innovative methodology were pointed out, which narrowed down to 6 the number of parameters admitting further optimization. Nonetheless, 4 of them, i.e., fiber coating, sample Ionic strength, sample pH and derivatization temperature could easily be optimized by a one-factor-at-a-time approach as they are discrete variables or otherwise consolidated references exist in the scientific literature which drastically delimits the range of variation. Eventually, only two parameters remained as significant, extraction and derivatization times, and in need of further optimization. Thus, a response surface methodology (RSM), consisting of a full factorial 2^2 with a central point repeated five times and extended with 4 star points, was applied to them. Thus, a set of 13 experiments was randomly performed. Afterwards, the software Statgraphics Centurion XVII was used to process the acquired experimental data and mathematically fit it to a second order polynomial model through the least squares method.

3 Results and discussion

3.1 Analytical method development and optimization for sewage and sludge

A selection of 22 PPCPs, in particular, 5 pharmaceuticals and 2 of their TPs as well as 14 personal care products and 1 of their TPs, were initially chosen as target analytes.

The protocol followed to develop and optimize the analytical method, including an experimental design, is described in the supplementary data **SD** file. In brief, after the GC-MS leg was developed, the sample pretreatment part of the methodology was optimized. Hence all the parameters with a role during the Online DI-SPME – On-Fiber Derivatization were identified and some technical limits were set. Afterwards, some preliminary experiments were carried out in a one-factor-at-a-time approach to optimize the Type of Fiber Coating, Sample Ionic Strength, Sample pH and Derivatization temperature. Finally, as the extraction and derivatization time could interfere with each other, a response surface method was designed based on a full factorial 2^2 with a central point repeated five times and extended with 4 star points. TS/N was selected as the response variable during the optimization, in order to get a compromise among the performance of all the compounds. As a result, the optimum value for the response variable obtained corresponded to an extraction time of 120 min and a derivatization time of 48 min. That is graphically shown in **Figure 1**.

After the optimization, ten of the initial target PPCPs, including the analgesics acetaminophen and acetylsalicylic acid, the lipid regulator atorvastatin, and the antibiotics amoxicillin, ciprofloxacin, levofloxacin, norfloxacin, sulfamethoxazole, erythromycin and clarithromycin turned out to be unsuitable for their analysis by Online DI-SPME – On-Fiber Derivatization – GC-MS, as they exhibited a very weak or even no response whatsoever. Therefore, they were ruled out and not included in the method.

The final methods, which allowed for the analysis of 12 PPCPs including 3 TPs, are summarized in **Sections 2.2 and 2.3.** Representative SIM chromatograms, obtained from MilliQ® water and sewage sludge samples spiked with the target PPCPs at 4 μg L^{-1} and 400 ng g^{-1}, respectively, using the optimized method conditions, are illustrated in **Figure 2**.

3.2 Method validation and application

3.2.1 Method validation

Several regulatory bodies (like the United States Food and Drug Administration (FDA) [17] or Eurachem [18]), standardization agencies (like the International Association of Official Analytical Chemists (AOAC International) [19]), and working groups and committees (like the Food and Agricultural Organization/World Health (FAO/WHO) [20]) have published guidelines and requirements for method validation. In addition, the European Union adopted a decision [21] implementing a directive concerning the performance of analytical methods and the interpretation of results. It refers to animal products. However, it has been widely used as an illustrative reference in the design of customized validation protocols for environmental analysis like in [22-25], as well as in the present study because of the lack of specific guidelines.

Hence, five validation parameters, i.e., accuracy, ME, precision, sensitivity and dynamic range were determined for all 12 target analytes included in the method (clofibrate, 1,4-benzoquinone, methylparaben, ethylparaben, clofibric acid, ibuprofen, propylparaben, salicylic acid, p-hydroxybenzoic acid, naproxen, triclosan, diclofenac) in sewage and sludge. In addition, a carryover test was also performed to ensure the absence of contamination between samples during the instrumental leg of the analysis. Two meaningful levels of concentration per matrix –100 and 1000 ng L^{-1}, and 100 and 400 ng g^{-1}– typical for the target compounds in real sewage and sludge samples, respectively, were tested for the four first parameters, as recommended by [23, 24]. Each test was run in triplicate with the optimized method. The results, average of both concentration levels, which are discussed below, are shown in **Table S4**.

1) Accuracy: Absolute recoveries (%) were determined by comparing the peak areas obtained from spiked samples analyzed using the optimized methods with the areas obtained from direct injections (2 µL) of equivalent amounts of standards in EA solutions. As both sewage and sludge can contain some of the target compounds, non-spiked samples were also analyzed and the peak areas were afterwards subtracted from the spiked samples in order to calculate the absolute recovery. **Table S4A** shows very variable absolute recoveries for sewage. Hence, SPME supported good

recoveries of compounds like clofibrate, 1,4-benzoquinone, propylparaben and diclofenac from sewage, with absolute recoveries above 80%. In contrast, recoveries were below 30% for compounds like methylparaben, ethylparaben, clofibric acid, salicylic acid or *p*-hydroxybenzoic acid. The absolute recoveries were lower in sewage sludge for all the target compounds (**Table S4B**). This poor accuracy was overcome by using an appropriate quantification approach based on a matrix-matched calibration curve prepared in the same matrix and run by the same optimized analytical method. In addition, the use of 6 internal standards (isotopic analogues to 6 of the target analytes) was included in the method. The assignment for the other 6 target compounds was carried out by choosing the one that better corrected their losses in the extraction recovery. The assignments are shown in **Table S4A and S4B**. The combination of these two quantification approaches, i.e matrix-matched and internal standard, resulted in a high method reliability. Hence, relative recoveries (**Table S4A and S4B**), which were determined as the ratio between the absolute recoveries for each compound and the recoveries of their corresponding internal standard, remained above 90% and 70% for sewage and sludge, respectively, which are similar or better than the ones reported in other methodologies for sewage [23, 26], sewage sludge [27, 28] and other solid environmental matrices recently published [3, 7], where recoveries where under 70% for many compounds, especially the most polar/acidic ones.

2) Matrix effect: Absolute recoveries were indicators of the overall analytical procedure efficiency. Experiments were performed to determine the part of the inefficiency due to the matrix effect. To quantify the matrix effect associated to sewage, MilliQ® water samples were prepared and spiked identically to the sewage samples used in the validation step, and run using the same optimized method. For sewage sludge, empty glass vials were spiked at the same concentrations as the sewage sludge samples used in the validation step, and subjected to the same optimized methodology. The differences between the areas obtained in the samples with and without matrix were attributed to matrix effect, which are shown in **Table S4A and S4B** as percentage of signal suppression. Negative values should be interpreted as a signal enhancement. In light of the results observed in sewage, depending on the compound, matrix suppressed between 17 to 61% of the expected

signal, except for salicylic acid which showed enhancement. These results are, mostly, in accordance with previously reported similar methodologies for sewage [23, 26]. As expected, matrix effect in sewage sludge was, in general, more acute than in sewage. This was also observed by [27] where signal suppression reached up to near 100% for many compounds in sludge. In any case, these deficiencies were encompassed within the method accuracy discussed above, and therefore corrected by the matrix-matched and internal standard quantification approaches.

3) Precision: The overall method repeatability, calculated as the relative standard deviation (%RSD) of equivalent samples in triplicate (n=3) run by the optimized methods described above, was satisfactory for both sewage and sludge, with %RSD values lower than 10 % for most of the compounds when the analyses compared were made in different days (interday precision). In addition, the %RSD values for intraday precision were even lower (**Table S4A and S4B**). This showed an improved method precision in comparison to previous methodologies for sewage [23, 26], sludge [27, 28] or other environmental matrices like compost or fish tissue [3, 7], where %RSD was commonly surpassing 10% in intraday replicates or even 30% when the analysis were repeated in different days.

4) Sensitivity: Limits of detection (LODs) and quantification were experimentally determined for each target compound in each matrix as the concentration providing a signal-to-noise ratio of 3 and 10, respectively (**Table S4C and S4D**). LODs were below 20 ng L^{-1} for most of the target compounds in sewage and 30 ng g-1 in sewage sludge, which were considered sufficient for the trace analysis of the target compounds in the matrices analyzed. In addition, these sensitivity levels coincided with, or even improved, the upper LODs validated in similar multiresidue methods based on GC-MS [28] and even LC-MS/MS [23, 26]. Differences in the detection technique had a stronger impact in environmental solid matrices, where LOQs in units of parts-per-trillion have been reported [3, 7, 27].

5) Instrumental carryover: Tests were carried out to ensure lack of a significant carryover effect during the instrumental analysis since the same SPME fiber

and derivatizing agent were used for a large number of samples in the methodology proposed. Generally, fiber life time was extended for around 60 injections, after which the fiber-protecting needle ended up breaking apart (as explained below) before any signs of performance decay was observed. Both SPME fiber and derivatization agent were then replaced. Thus, blank MilliQ® water samples were run with the optimized instrumental method after spiked sewage and sludge samples at the validation levels. The peak areas obtained in the blanks, sewage and sludge samples were then compared. Most of blank samples contained less than 5% of the preceding signal of sewage and sludge samples (**Table S4C and S4D**). Therefore, carryover phenomena were deemed negligible, and desorption and fiber conditioning were satisfactorily validated.

6) *Dynamic range:* Quantification based on peak areas was concurrently performed by both matrix-matched and internal standard approaches in both matrices. Eleven and eight-point calibration curves were built by spiking blank sewage and sludge aliquots, respectively, between 58 and 46512 ng L^{-1}, and 37.5 and 1500 ng g^{-1} for all target compounds. The linear equations shown in **Table S4C and S4D** provided coefficients of determination (R^2) above 0.99 within the concentration ranges indicated, i.e., up to 5 and 3 orders of magnitude for sewage and sewage sludge, respectively. Similar or poorer linearity ranges have been reported with up to 3 [26] and 6 orders of magnitude [23] in sewage, and up to 2 in sludge [28], compost [3] and fish [7].

7) Other observations: The applied mechanical agitation stressed on the fiber-protecting needle, which prematurely broke in several occasions for this reason. Therefore, an agitation regime of 6s ON and 30s OFF was set (versus the original 5s ON, 2s OFF) in order to increase the fiber lifespan. This decrease in the fiber lifespan showed that mechanical agitation is not an appropriate agitation mode during DI-SPME. In this context, magnetic stirring would extend the lifetime of the expensive fibers.

Despite SPME has been shown to be a proficient pretreatment technique, its destructive nature entails that each sample can only be analyzed once. However, the

small sample size needed compensates this problem, as equivalent aliquots can be analyzed. Finally, the fact that the method has been successfully validated for a large number of compounds with very different physical-chemical properties highlights its high versatility and would allow to increase the method multicomponent feature in the future [14, 29].

3.2.2 Method application

The method was applied to the analysis of real samples from a completely mixed aerobic activated sludge pilot reactor treating domestic wastewater. The experimental set-up, which consisted of a 5-L activated sludge reactor connected to a 16-L circular settler, was operated indoors at the Department of Chemical Engineering and Environmental Technology of University of Valladolid (Spain) at 23±1 ºC. The reactor was daily fed with synthetic sewage ([30]). The system was preconditioned during 28 days before PPCPs were incorporated in the synthetic sewage (ISW). Six PPCPs, i.e., Ibuprofen, Propylparaben, Salicylic acid, Naproxen, Triclosan and Diclofenac, were selected based on their biodegradability, adsorption and solubility properties. The purpose of this 16-weeks study was to assess the system capacity to remove these PPCPs at different Hydraulic Retention Times (HRT_1 = 4.9 and HRT_2 =7.2 h) and initial PPCP concentrations. The two levels of PPCPs concentrations were selected based on real concentrations recorded in wastewater treatment plants in Spain [31-33]: $ISW_{Ibuprofen1}$: 8.1 µg L^{-1}, $ISW_{Ibuprofen2}$: 12.1 µg L^{-1}; $ISW_{Propylparaben1}$: 0.25 µg L^{-1}, $ISW_{Propylparaben2}$: 0.37 µg L^{-1}; $ISW_{Salicylic\ acid1}$: 21.6 µg L^{-1}, $ISW_{Salicylic\ acid2}$: 32.4 µg L^{-1}; $ISW_{Naproxen1}$: 0.5 µg L^{-1}, $ISW_{Naproxen2}$: 5 µg L^{-1}; $ISW_{Triclosan1}$: 0.28 µg L^{-1}, $ISW_{Triclosan2}$: 0.4 µg L^{-1}; $ISW_{Diclofenac1}$: 0.24 µg L^{-1}, $ISW_{Diclofenac2}$: 0.36 µg L^{-1}. Thus, combinations of these two levels were performed in 4-week legs: weeks 1-4 (HRT1 and ISW1), weeks 5-8 (HRT2 and ISW1), weeks 9-12 (HRT1 and ISW2) and weeks 13-16 (HRT2 and ISW2). A total of 12 samples of ISW and 20 samples of treated sewage (ESW) along each four-week leg were drawn and analyzed using the validated methodology above presented. Average concentrations along with PPCPs removal efficiencies are shown in **Table S5**. HRT2 supported a more efficient PPCPs removal, while no significant influence of the initial ISW concentration on PPCPs removal was observed. Ibuprofen, followed by salicylic acid and propylparaben, were the compounds more effectively removed regardless of

414 the operational conditions. On the other hand, diclofenac and naproxen were always
415 the most recalcitrant compounds.
416
417 **4 Conclusions**
418 The demand of multicomponent methods for the analysis of emerging
419 contaminants in environmental matrices is a reality today. However, conventional
420 techniques based on Solid Phase Extraction (SPE) coupled to Liquid Chromatography
421 Mass Spectrometry (LC-MS) are very often only available in high-tech laboratories. A
422 cost-competitive methodology was successfully developed and validated here. It
423 consists of an innovative method for the analysis of 19 PPCPs and 3 TPs in sewage and
424 sludge using a fully automatized online DI-SPME – On-fiber Derivatization – GC-MS.
425 Ten of the compounds were dismissed along the optimization of the methods based
426 on their unsuitability to be quantitatively determined by the analytical technique or
427 compromised method conditions. The validated method was proven to be reliable,
428 thanks to the combination of two quantification approaches, i.e., matrix-matched and
429 internal standard, as well as sensitive (LODs below 20 ng L^{-1} for most of the target
430 compounds in sewage and 30 ng g^{-1} in sewage sludge), versatile and green for the
431 analysis of 12 PPCPs, including the 3 TPs. The method was successfully applied to real
432 samples from a pilot scale aerobic reactor treating domestic wastewater.

433 This methodology will certainly increase the number of laboratories around the
434 world able to carry out PPCPs analysis, and therefore will help to fill the existing gap
435 between the current environmental needs and analytical technological capacities.

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441
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Figure 1: Response surface after applying an experimental design 2^2 + star + 5 central points

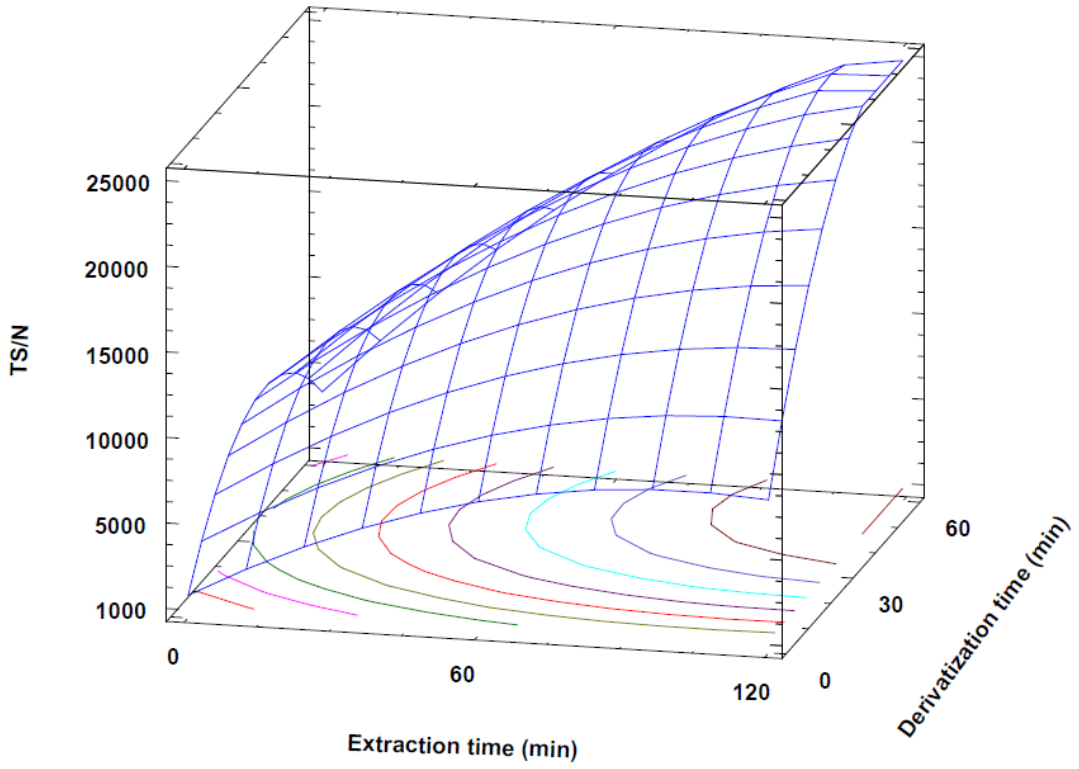
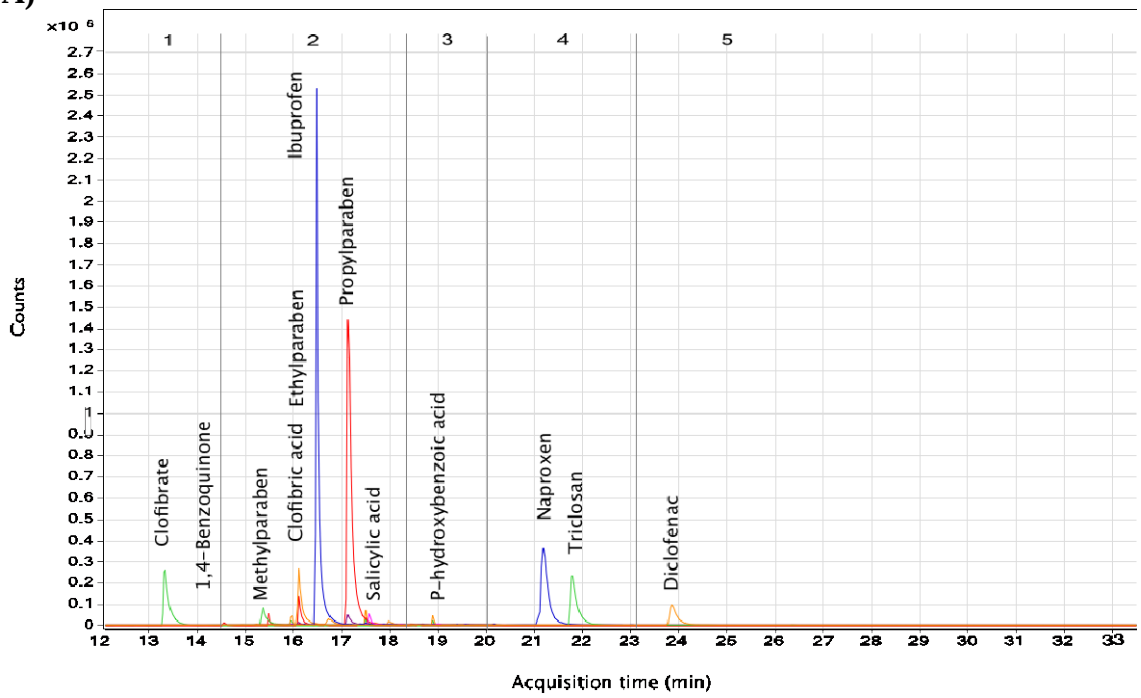


Figure 2: Chromatograms obtained from A) 4000 ng L^{-1} MilliQ water and B) 400 ng g^{-1} sludge samples after the optimized methods were applied

A)



B)

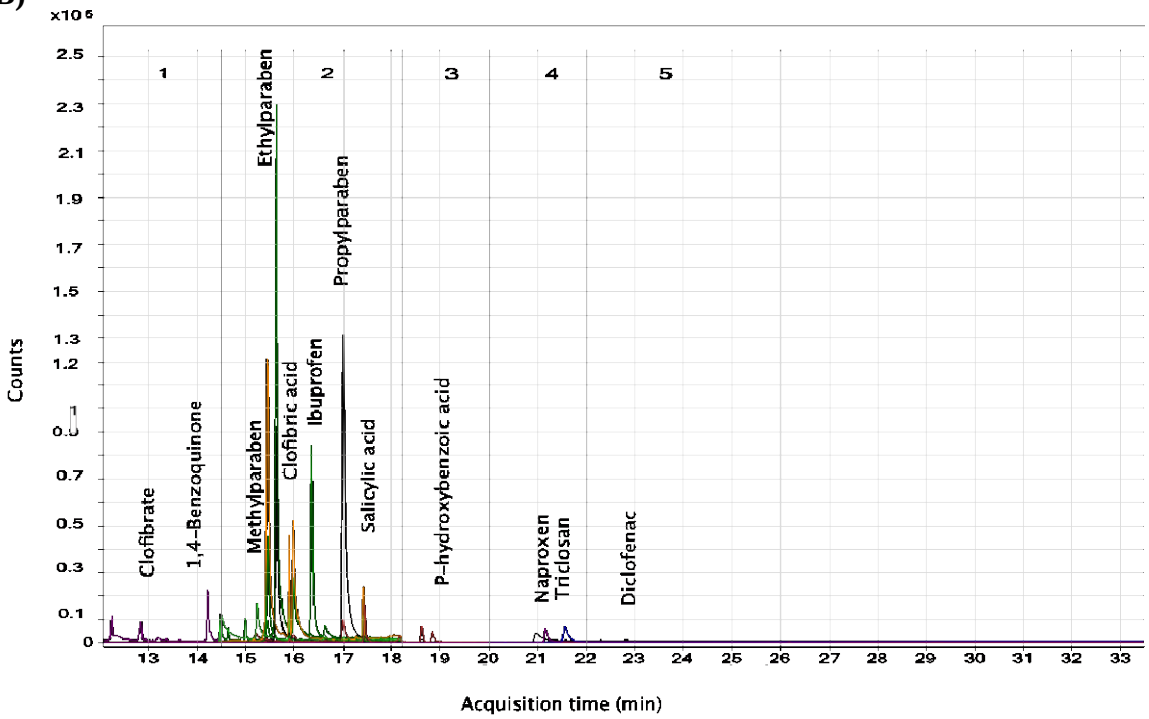


Table 1: MS parameters for the final target compounds and internal standards

[1]IS	Analyte	Chemical Name	Acquisition window #	[2]t_R (min)	[3]SIM ions, m/z		
	1	Clofibrate	1	13.17	*128*	130	169
	2	1,4-Benzoquinone		13.70	*281*	338	282
	3	Methylparaben	2	15.23	*209*	210	135
	4	Acetylsalicylic acid		15.28	*195*	237	135
	5	Ethylparaben		16.02	*223*	224	151
	6	Clofibric acid		16.02	*143*	271	185
	7	Ibuprofen		16.38	*263*	264	117
1		Ibuprofen-d3		16.34	*266*	267	164
	8	Propylparaben		17.04	*237*	238	151
2		Propylparaben-d7		16.96	*244*	245	152
	9	Salicylic acid		17.50	*309*	310	195
3		Salicylic acid-d4		17.45	*313*	314	312
	10	Acetaminophen	3	18.24	*208*	265	166
	11	P-hydroxybenzoic acid		18.91	*309*	265	310
	12	Naproxen	4	21.07	*287*	185	288
4		Naproxen-d3		20.95	*290*	188	207
	13	Triclosan		21.66	*347*	345	200
5		Triclosan-d3		21.54	*350*	348	200
	14	Diclofenac	5	23.73	*352*	214	354
6		Diclofenac-d4		23.73	*356*	218	358

[1]IS: Internal Standard; [2]tR: Retention Time; [3]SIM: Selected Ion Monitoring

1