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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.

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, López-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

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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 material 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 material 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 6 s 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.

2.2.2. GC – MS

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

Mass detection was obtained in electron impact ionization mode (70 eV) with selected ion monitoring (SIM) and a filament delay of 12 min. The GC–MS interface, ion source and quadrupole temperatures were set at 280, 230 and 150 °C, respectively. Quadrupole resolution was set at low. Target compounds were recorded in five acquisition windows along the run time. Table 1 shows the primary (in italics) and the two secondary ions monitored per compound. Acquisition stopped