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Determination of selected pharmaceutical compounds in biosolids by supported liquid extraction and gas chromatography–tandem mass spectrometry☆

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ABSTRACT

In this work, an analytical method was developed for the determination of pharmaceutical drugs in biosolids. Samples were extracted with an acidic mixture of water and acetone (1:2, v/v) and supported liquid extraction was used for the clean-up of extracts, eluting with ethyl acetate:methanol (90:10, v/v). The compounds were determined by gas chromatography–tandem mass spectrometry using matrix-match calibration after silylation to form their *t*-butyldimethylsilyl derivatives. This method presents various advantages, such as a fairly simple operation for the analysis of complex matrices, the use of inexpensive glassware and low solvent volumes. Satisfactory mean recoveries were obtained with the developed method ranging from 70 to 120% with relative standard deviations (RSDs) ≤ 13%, and limits of detection between 0.5 and 3.6 ng g^{-1}. The method was then successfully applied to biosolids samples collected in Madrid and Catalonia (Spain). Eleven of the sixteen target compounds were detected in the studied samples, at levels up to 1.1 μg g^{-1} (salicylic acid). Ibuprofen, caffeine, paracetamol and fenofibrate were detected in all of the samples analyzed.

1. Introduction

Pharmaceutical substances are a source of increasing environmental concern and together with endocrine disrupting chemicals have been classified as the most frequently detected organic contaminants in the environment [1]. The determination of pharmaceuticals in the environment has focused on the aquatic compartment because it has been reported that wastewater treatment plants (WWTPs), especially those with conventional technology, cannot accomplish the removal of these compounds before the effluents are discharged to surface waters [2]. Microbial degradation and the high tendency of some pharmaceuticals to remain adsorbed onto activated sludge are mechanisms that may explain the removal of pharmaceuticals during wastewater treatment [3]. Biosolids are the nutrient-rich organic materials resulting from the treatment of sewage sludge. When treated and processed, sewage sludge becomes biosolids, which can be safely recycled and applied as fertilizer to sustainably improve and maintain productive soils and stimulate plant growth. In the European Union, around 4 million metric tons (dry weight) of biosolids are annually applied to agricultural land [4]. Therefore, it is important to develop analytical methods for the detection of pharmaceuticals at trace levels to study their occurrence, behavior and fate in this complex matrix. The analysis of pharmaceutical drugs in sludge or biosolids has been focused on a limited set of compounds, mainly antibiotics [5–7] or non-steroidal anti-inflammatory drugs (NSAIDs) (e.g. naproxen, diclofenac, ketoprofen, and ibuprofen) [3,8]; however, recent studies have included many more compounds belonging to several therapeutic classes that exhibit very different physicochemical properties [9–11].

However, the determination of these compounds in solid environmental samples is still scarcely documented, due primarily to a lack of appropriate analytical methods. Several methodologies have been developed for determination of pharmaceuticals in sewage sludge using pressurized liquid extraction [3,7,10–12], ultrasound assisted extraction [1,6,13], microwave assisted extraction [8] and QuEChERS [9].

Most published works regarding the analysis of pharmaceutical compounds in the environment were initially performed using mass spectrometry coupled to gas chromatography (GC–MS)

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[8,14–17] or liquid chromatography (LC–MS) [18]. Nowadays, the majority of the analytical methods for the separation and detection of pharmaceuticals uses liquid chromatography–tandem mass spectrometry (LC–MS/MS) [10,19–23]. Nevertheless, GC–MS/MS is an interesting alternative to LC due to its high resolution, lower operation costs and reduced solvent waste. Moreover, there is a clear impact on electrospray ionization of these compounds when working with complex matrices such as biosolids producing either ion enhancement or suppression that hinders the quantification of target compounds [1]. GC–MS/MS was used in the determination of pharmaceuticals in sewage water with very low detection limits [24]; however, its performance has not been evaluated for biosolid samples. Most pharmaceuticals possess functional groups with active hydrogen atoms (amines, amides, hydroxyl or phenolic groups) and a chemical derivatization of these groups, to reduce their polarity while increasing their thermal stability and volatility, is needed before their analysis by GC. Silylation with different reagents that lead to the formation of trimethylsilyl (TMS) or *t*-butyldimethylsilyl (*t*BDMS) derivatives is an effective approach for their determination.

Supported liquid extraction (SLE) is a relatively new technology that has been developed to replace classical liquid–liquid extraction (LLE). SLE involves the immobilization of aqueous samples over a solid inert phase, generally high purity diatomaceous earth that has a high capacity to retain water. The subsequent extraction is performed with any solvent that is immiscible with water. This technique presents several advantages over LLE, such as the reduced sample and solvent volumes required and no formation of emulsions [25]. Since emulsions are not an issue, solvent mixtures can be used regardless of density [26]. One advantage of SLE over solid-phase extraction (SPE) is that no preconditioning of the column is required; hence, the sample is directly loaded into the solid support, which shortens the whole extraction procedure. Unlike SPE, the whole sample is absorbed onto the solid support and there is no flow-through. This technique has been mainly applied with biological fluids such as plasma [25,27], although it has also been used in the determination of chemical warfare agents in water [28], polyphenols in wine [29] and pesticides in honey [30].

The aim of this work was to develop a rapid and sensitive multiresidue method based on SLE followed by GC–MS/MS for determination of 16 frequently used pharmaceuticals in biosolid samples. GC–MS/MS was selected to analyze a high number of compounds of different classes in a single run with a high selectivity. The selected pharmaceuticals present wide range of physico-chemical properties and belong to different therapeutic classes: NSAIDs, antiepileptics, antidepressants, lipid regulators, nervous stimulants and β-blockers. The simultaneous determination of pharmaceutical drugs with different physico-chemical characteristics requires a compromise in the selection of experimental conditions for all the compounds studied. A significant advantage of the described procedure is the reduction in the consumption of organic solvents and time versus previously published methodologies. To the best of our knowledge; this is the first time that SLE has been applied in the multiresidue determination of pharmaceutical drugs in biosolids.

2. Materials and methods

2.1. Sewage sludge collection

Pelletized biosolids from Madrid (Spain) were used in the development and optimization of the analytical method. Samples were put in amber glass jars (250–500 g) and stored at −20 °C until processing. The developed method was applied to the analysis of biosolids from WWTPs located in Madrid and Catalonia (Spain).

2.2. Reagents and standards

Methanol, ethyl acetate, acetone and acetonitrile (ACN), residue analysis grade, were purchased from Scharlab (Barcelona, Spain). A Milli-*Q* water purification system from Millipore (Bedford, MA, USA) was used to provide ultrapure water in this study. Formic acid was purchased from Sigma-Aldrich (St Louis, MO, USA). ISOLUTE SLE+ 1 mL supported liquid extraction columns were purchased from Biotage (Uppsala, Sweden).

N-*t*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA, purity > 97%), a mixture of MTBSTFA and *t*-butyldimethylchlorosilane (*t*BDMCS) (99:1, v/v), *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, purity > 99%) and the mixture of BSTFA and trimethylchlorosilane (TMCS) (99:1, v/v), used as silylation reagents, were purchased from Aldrich (Steinheim, Germany). Pyridine was purchased from Panreac (Barcelona, Spain).

The standards of clofibric acid, ibuprofen, caffeine, salicylic acid, paracetamol, allopurinol, gemfibrozil, fenoprofen, amitriptyline, metoprolol, naproxen, mefenamic acid, ketoprofen, carbamazepine, diclofenac and fenofibrate were of analytical grade (purity >99%) and purchased from Sigma-Aldrich (St Louis, MO, USA).

Individual stock standard solutions were prepared in methanol at a concentration of 5 μg mL^{-1} and stored in amber vials at −18 °C in the dark. A working mixture solution containing 1000 ng mL^{-1} of all compounds was prepared in ACN weekly by dilution of the stock solution.

2.3. Sample preparation

An amount of 0.5 g pelletized biosolids, previously ground, was weighed into a 15 mL screw-cap glass tube and for the recovery assays, aliquots of the working mixture solution were added to reach final concentrations of 100, 50 and 25 ng g^{-1} allowing 24 h before extraction at 4 °C to reach equilibrium. After the addition of 2 mL acetone-1% aqueous formic acid (2:1, v/v), the mixture was stirred intensively by magnetic agitation for 60 min. Then, the extract was centrifuged at 4000 rpm for 4 min. A 500 μL aliquot of the supernatant was transferred to a glass tube and diluted (1:1, v/v) with 1% aqueous formic acid. The diluted sample was loaded on the SLE column and left 5 min for the sample being completely absorbed. Then, it was placed in a multiport vacuum manifold (SupelcoVisiprep, Madrid, Spain) and the analytes were eluted with 2 × 5 mL of ethyl acetate:methanol (90:10, v/v), applying vacuum for 5 min to complete elution. The extract was evaporated to dryness using a Genevac EZ-2 evaporator (NET Interlab, S.A.L., Spain) and the analytes reconstituted in 100 μL acetonitrile and pipetted into a 2 mL vial with a micro insert. The *t*BDMS derivatives were prepared by the addition of 50 μL of MTBSTFA:*t*BDMCS (99:1, v/v), then the vial was capped and placed in an oven at 70 °C for 1 h. After the derivatization step, the vial was left to cool down before performing the chromatographic analysis.

2.4. Gas chromatography–tandem mass spectrometry analysis

GC–MS/MS analysis was performed with an Agilent 7890A gas chromatograph equipped with a multimode inlet (MMI) and coupled to a triple quadrupole mass spectrometer, Model 7000 (Waldbronn, Germany). The MMI was operated in solvent-vent mode with gas liner with deactivated glass wool. During the 2 μL injection, at a rate of 40 μL min^{-1}, the split vent was open for 0.18 min with an inlet pressure of 5 psi and a flow rate of 100 mL min^{-1}. Once the entire sample has been injected, the inlet was switched to splitless mode for analyte transfer. After 2.68 min, the purge valve was activated at a 60 mL min^{-1} flow rate. The MMI program started at 60 °C kept for 0.18 min after injection,