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ScienceDirect
Journal of Chromatography A, 1130 (2006) 72–82
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of antimicrobials in sludge from infiltration basins at two artificial recharge plants by pressurized liquid extraction–liquid chromatography–tandem mass spectrometry

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Available online 5 July 2006

Abstract

This work describes the optimization of a multi-residue analytical approach for the simultaneous determination of 11 antimicrobials (9 sulphonamides and 2 penicillins) in sludge from infiltration basins. The method is based on pressurized liquid extraction (PLE) followed by solid-phase extraction (SPE) for pre-concentration and purification, and analysis by liquid chromatography–tandem mass spectrometry using electrospray in the positive ionization mode (LC–(ESI+)–MS/MS). Limits of detections (LODs) between 1 pg/g and 0.2 ng/g and limits of quantifications (LOQs) between 5 pg/g and 0.6 ng/g were achieved. Good recovery values (57.6–104%) were obtained for sulfamethazine, sulfapyridine, sulfadiazine and sulfamethoxypyridazine, while medium recovery values (14–47%) were afforded for sulfadimethoxine, sulfathiazole and sulfamethoxazole. However, only a poor recovery (<1%) could be possible for both penicillins and two sulphonamides, namely nafcillin, dicloxacillin, sulfisoxazole and sulfamethizole. These low recoveries were attributed to the presence of ionic suppression effects (even after thorough extraction and purification) rather than to an inefficient extraction. The method developed was applied to the analysis of sludge samples from the infiltration basins of two artificial recharge plants located in Sweden and Denmark. All target compounds were found to be present in at least one sample. Sulfadimethoxine, nafcillin and dicloxacillin were detected in all the samples analysed.

Keywords: Sulphonamides; Penicillins; Antimicrobials; Environmental analysis; Pressurized liquid extraction; Liquid chromatography–tandem mass spectrometry; Solid-phase extraction; Sludge

1. Introduction

Generally, pharmaceuticals are incompletely absorbed by the organism after intake and are further subjected to metabolic reactions such as hydroxylation, acetylating, cleavage or biodegradation. Therefore, after administration the drug is discarded to the environment through excretion as the parent compound and/or in a conjugated form that can be readily converted back to the dosed drug [1]. Antimicrobials are widely prescribed with both therapeutically and prophylactic purposes against microbial infections and also as growth promoting substances at sub-therapeutic levels in animal farms and aquaculture. Recently, concern has been expressed that continuous sub-lethal levels of antibiotics in the aquatic environment have led to the emergence of antibiotic resistant, harmful bacteria strains [2].

In order to assess the occurrence and fate of pharmaceutical residues in the environment sensitive analytical methods enabling the simultaneous analysis of multiple compounds at rather low concentration levels (typically in the low ng/L or ng/g range) are required. Pharmaceuticals in the environment, including antimicrobials, have already been studied at national scale for example in Germany [3], Sweden [4], USA [5], Canada [6] and Italy [7]. Environmental solid matrices, i.e. soils, sludge and sediments have been scarcely investigated in comparison with water [8–10]. This might be due to the generally greater complexity of the solid matrices which require comprehensive extraction and clean-up procedures to remove the interferences present in the sample.

So far, most of the analytical methods reported in the literature for pharmaceutical residue analysis are based on gas chromatography–mass spectrometry (GC–MS) [3,11], which often requires derivatization. However, in the last years liquid chromatography–mass spectrometry (LC–MS) and LC–tandem MS (LC–MS/MS) [12,13] has seen a continuously increasing

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doi:10.1016/j.chroma.2006.05.076

application in drug residue analysis. LC–MS methods enable the determination of polar, non-volatile and thermo labile compounds without tedious derivatizations and are not limited by such factors as the molecular weight of the analytes. Nevertheless, the extremely selective fluorescence detection is still preferred to tandem MS detection for selected compounds as would be the case of fluoroquinolone antibiotics [14].

To our knowledge, the use of LC–MS/MS based methods for the multi-residue analysis of antimicrobials in environmental solid samples has only been reported in a few works [15,16]. Löffler and Ternes [15] analysed 11 acidic pharmaceuticals, 9 antibiotics (including 4 sulphonamides), and the parasiticide ivermectin using three independent methods based on solvent extraction with ultrasonic assistance, solid phase extraction (SPE) clean-up and analysis by LC–MS/MS with different ionization sources (electrospray (ESI) and atmospheric pressure chemical ionization (APCI)), in sediments of laboratory batch experiments. Göbel et al. [16] determined five sulphonamides, among other antimicrobials, in sludge from wastewater treatment plants with a method consisting of PLE enrichment and SPE purification prior to LC–(ESI+)–MS/MS analysis. PLE was optimized and recovery rates for target analytes were compared with those obtained by ultrasonic (USE) extraction under similar extraction conditions. Results evidenced the high extraction power of PLE for all compounds investigated, especially notorious for sulphonamides as compared to ultrasonic extraction.

In contrast, there are several methods available in the literature dealing with the LC–MS/MS determination of such compounds in other matrices, e.g. groundwater [17–19], surface water [19,20], wastewaters [19,21,22] and tap water [19], and a variety of methods to quantify sulphonamides in foodstuffs (animal edible tissues, eggs, milk, honey, etc.) [23–26].

Regarding extraction, PLE has been recently applied in the extraction of tetracyclines, macrolides, ionophores, sulphonamides and tiamulin from agricultural soil samples [27,28] and fluoroquinolones from sludge and sludge-treated soil [29]. PLE allows rapid extraction under high pressure and elevated temperature with excellent reproducibility [30]. However, on the whole, most extraction methods are based on mechanical shaking or ultra-sonication, using a quite high volume of several organic solvents. On the other hand, a further clean-up step is always needed to minimize interferences and pre-concentrate target analytes, which is generally, carried out by SPE with a range of different SPE sorbent materials.

Today, the need for reference analytical methods able to determine a high number of antimicrobials with low solvent consumption and providing an unequivocal identification of the target compounds is clear. In this context, the objective of the present work was to develop a selective, sensitive and reliable LC–(ESI+)–MS/MS multi-residue method for the quantitative, simultaneous determination of sulphonamides (SAs) and penicillins (PENs), namely, sulfadiazine, sulfamethoxazole, sulfathiazole, sulfisoxazole, sulfadimethoxine, sulfamethoxypyridazine, sulfamethizole, sulfapyridine, sulfamethazine, nafcillin and dicloxacillin in sludge from infiltration basins at trace levels. The antimicrobials investigated were selected from those that are currently in the European market for treatment and prevention of human and animal microbial infections. Especial care has been paid to the extraction and purification procedures for such complex matrix. The method was validated by evaluating a set of parameters, including linearity, precision, accuracy, and limits of detections (LODs) and quantifications (LOQs). The proposed method was applied to the determination of the target sulphonamides and penicillins in sludge samples from infiltration basins at different stages of the treatment process in two artificial water recharge plants.

2. Experimental

2.1. Chemicals

Sulphonamides and penicillins were purchased from Sigma–Aldrich (Seelze, Germany): sulfadimethoxine (99.9%), sulfamethizole (99.9%), sulfisoxazole (99.8%), sulfathiazole (99.9%), sulfadiazine (99.8%), sulfapyridine (98%), sulfamethoxypyridazine (99.8%), nafcillin sodium salt monohydrate (99.9%) and dicloxacillin sodium salt monohydrate (97.1%). The chemical structures of these compounds, the CAS No. and their main physical–chemical properties are shown in Table 1.

Individual antimicrobial stock standard solutions were prepared by dissolving 50 mg of the individual drug in 100 mL methanol. A stock standard solution of the mixture of all compounds at a concentration of 10 μg/mL was made up by mixing 2 mL of the individual antimicrobial stock standard solutions with methanol in a 100 mL flask. Working standard solutions of the mixture in the range 1000–1 ng/mL were freshly made by appropriate dilution of the stock standard mixture in methanol. Solutions were transferred to amber bottles and stored in the dark at 4 °C to minimize analyte degradation.

Water, methanol (MeOH), acetone, *n*-hexane, acetonitrile (ACN) and dichloromethane (DCM) were of HPLC grade; formic acid and ammonium formate were of MS grade; Na_2EDTA, HCl, acetic acid, ammonium acetate, citric acid monohydrate and Na_2HPO_4 of analytical grade. All they were purchased from Merck (Darmstadt, Germany). High quality nitrogen 5.0 was supplied by Abelló Linde (Barcelona, Spain).

Glass-fiber filters (19.8 mm diameter) were from Dionex (Sunnyvale, CA, USA), and the Chem. Tube-Hydromatrix (part No. 198003), used for drying the lyophilized sediment samples and to reduce the void volume of the 33-mL pressure resistant steel PLE extraction cells, was from Varian (Palo Alto, USA),

For SPE, Oasis HBL cartridges (60 mg, Waters, Milford, MA, USA) were used.

2.2. Instrumentation

Extraction of antibiotics from sludge was performed by PLE in an ASE 200 (accelerated solvent extractor) from Dionex.

SPE clean-up was carried out with an ASPEC XL autosampling and dilutor processor (Gilson Inc., Middleton, USA) controlled by the Gilson 721 ASPEC Controller software.

LC–tandem MS analyses were performed in a system consisting of a Waters Alliance 2690 LC pump equipped with