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Multiresidue trace analysis of sulfonamide antibiotics and their metabolites in soils and sewage sludge by pressurized liquid extraction followed by liquid chromatography–electrospray-quadrupole linear ion trap mass spectrometry

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ABSTRACT

The present study describes the development, validation and a practical application of a fully automated analytical method based on pressurized liquid extraction (PLE) followed by solid-phase extraction-liquid chromatography–tandem mass spectrometry (SPE-LC–MS/MS) for the simultaneous determination of 22 sulfonamides, including five acetylated metabolites, in sewage sludge and soil samples. Both matrix matched calibration curves and standard calibration curves were built in order to evaluate the potential matrix effects during analysis, and different internal standards were used to compensate these effects during quantification. The recovery efficiencies were found to be 60–130% for the majority of the sulfonamides in both matrices and at two spike levels. The intra-day and inter-day precisions, expressed by the relative standard deviation (RSD), were below 23%. The method limits of detection (MLODs) achieved were in the range 0.03–2.23 ng g^{-1} for sewage sludge and 0.01–4.19 ng g^{-1} for soil samples. The methodology was applied to evaluate the occurrence of the target sulfonamides in several sewage sludge and soil samples taken in different wastewater treatment plants (WWTPs) and agricultural areas. Results confirmed the wide presence of sulfonamides in both matrices, being sulfathiazole and sulfamethazine the sulfonamides most frequently detected in sewage sludge and soil samples, respectively. Maximum concentrations corresponded to sulfamethazine in both cases (139.2 ng g^{-1} and 8.53 ng g^{-1} for sewage sludge and soils respectively). Levels were generally lower in soils. Three of the five acetylated metabolites were detected in sewage sludge and two of them in soils, at concentrations not higher than 9.81 ng g^{-1}.

1. Introduction

Several studies have demonstrated the widespread presence of sulfonamides (SAs) in the environment and identified their main entrance pathways [1–5]. The occurrence of their main metabolites, the acetylated conjugates, at similar concentrations has become evident recently, especially in environmental waters [6,7]. It has been demonstrated that these metabolites, once excreted, can deconjugate and revert back to the original parent drug both in water and manure [2,8,9]. Therefore, the inclusion of these metabolites in environmental occurrence surveys for SAs is key to obtain more complete and reliable information on the actual levels, avoiding potential underestimations. Up to date, data on the presence of these metabolites in solid matrices such as soils or sludge from wastewater treatment plants (WWTPs) are scarce, and to our knowledge only one publication by Stoob et al. included acetylated metabolites in its scope [10]. However, the presence of the acetylated conjugates in groundwaters has been demonstrated in different works [1,6,11,12], indicating that these metabolites probably reach the aquifers percolating through the upper soil, in which residual concentrations may still be present. Liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) has been the analytical technique of choice in most of the monitoring studies carried out for SAs, due mainly to its versatility and high selectivity for the analysis of complex samples. However, despite the number of new analytical methodologies developed in the last decade for the detection of SAs in solid matrices, such as soils, sediments or sewage sludge, they have been detected only scarcely in these matrices, and generally at low concentrations [10,13–15]. This is mainly due to their high polarity and generally low tendency to adsorb onto solid particles [16,17], which make them very mobile contaminants once released into the environment. The method limits of detection (MLODs) of the method can be also compromised by the high organic content of the samples. Furthermore, despite the many advantages of LC–MS/MS analysis, matrix effects when using the electrospray ionization source (ESI) have become the main drawback during the analysis of environmental samples, leading to the potential suppression/enhancement of the signal. Solid

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matrices extracts usually have a high content of organic components such as humic acids, phenols, and lipids which increase the viscosity of the sample and the superficial tension of the droplets generated in the ESI source, hindering the evaporation efficiency of the target analytes. These interfering compounds can also contribute to the coprecipitation of the analytes, limiting their transfer to the gas phase, or even competing with them to reach the droplet surface for the maximum evaporation efficiency [18,19]. Different approaches can be followed to minimize matrix effects before analysis, based on more efficient clean up of the samples and improved chromatography with a better separation of the matrix compounds (i.e. ultra performance liquid chromatography, UPLC) to obtain a total separation of the analytes of interest from the interfering compounds. Compensating measures are also frequently used, such as the use of internal standards or matrix matched calibration curves for quantification.

The aim of the present study was to develop a new analytical method, based on pressurized liquid extraction (PLE) followed by LC–MS/MS analysis for the determination of 17 SAs and 5 of their acetylated metabolites in sewage sludge and soil samples. The potential matrix effects were evaluated in order to obtain the most accurated quantification approach and improve MLODs. After its validation, the new method was applied to the analysis of several agricultural soils and sewage sludge samples from different wastewater treatment plants (WWTPs) located in Catalonia and along the Ebro River basin.

2. Materials and methods

2.1. Chemicals and reagents

HPLC-grade solvents (water, methanol (MeOH), acetone and acetonitrile (ACN)) and formic acid (HCOOH, 98–100% purity) were supplied by Merck (Darmstadt, Germany). High purity standards (>99%) of the 18 SAs studied, namely sulfisomidin (SSD), sulfamerazine (SZI), sulfacetamide (SCT), sulfadoxine (SDX), sulfabenzamide (SBZ), succinylsulfathiazole (SuSTZ), sulfaquinoxaline (SQX), sulfadiazine (SDZ), sulfadimethoxine (SDM), sulfamethazine (SMZ), sulfamethizol (SMT), sulfamethoxazole (SMX), sulfamethoxypyridazine (SMP), sulfapyridine (SPY), sulfathiazole (STZ), sulfisoxazole (SSX), sulfanitran (SNT) and N^4-acetylsulfamethazine (AcSMZ), were purchased from Sigma–Aldrich (St. Louis, MO, USA). The standards of the remaining acetylated metabolites N^4-acetylsulfamethoxazole (AcSMX), N^4-acetylsulfapyridine (AcSPY), N^4-acetylsulfadiazine (AcSDZ) and N^4-acetylsulfamerazine (AcSZI), together with the isotopically labeled compounds d_4-sulfamethoxazole (d_4-SMX), d_4-sulfathiazole (d_4-STZ), d_4-sulfadiazine (d_4-SDZ) and d_4-sulfamethazine (d_4-SMZ), used as surrogate and internal standards, were purchased from Toronto Chemical Research (North York, Ontario, Canada). Stock standard solutions for each of the analytes were prepared in MeOH at 1 mg mL^{-1} and stored at −4 °C until use. Standard solutions of the mixtures of all compounds at appropriate concentrations were prepared by the corresponding dilution of the stock solutions in MeOH.

Oasis HLB solid phase extraction (SPE) cartridges (200 mg, 6 mL) were purchased from Waters (Milford, MA, USA).

2.2. Sampling site

Digested sludge samples were collected in June 2009 from different full-scale WWTPs located in Catalonia (Spain) and along the Ebro River basin. The water treatment applied in all the WWTPs consisted of sedimentation (primary settler) followed by biological treatment with P and/or N removal. Sludge from the primary and secondary settlers was thickened by gravity, digested under anaerobic conditions and finally dewatered by centrifugation. Soil samples were taken in rural areas under a significant farming pressure in Catalonia (6 samples) and also along the Ebro River basin (9 samples). Both sludge and soil samples were transported in cool conditions, freeze-dried upon arrival to the laboratory (−50 °C, 0.044 bar vacuum) and kept at −30 °C until analysis.

2.3. Extraction and clean up

Soil and sludge samples were extracted by PLE using an ASE 300 accelerated solvent extractor (Dionex, Sunnyvale, CA). Samples were grinded and homogenized in order to decrease the size particle and facilitate the PLE process. Prior to extraction, d^4-SMX was added as surrogate standard to the weighted samples at a concentration of 100 ng g^{-1}. The samples were mixed in the extraction cells with Hydromatrix dispersing agent, in order to prevent particle clumping of the sample particles and reduce interstitial volume in the cells [20]. Homogenized mixtures of the different soil and sludge samples, respectively, were used to optimize the extraction procedure in terms of sample weight, solvents and temperature. The optimized conditions for the PLE procedure were the following:

Sludge: 2 g, ACN–water (25:75, v/v), 50 °C;
Soil: 5 g, MeOH–water (90:10, v/v), 100 °C;

For both matrices, a preheating period of 5 min was chosen, and 3 static cycles of 5 min each were carried out; a total flush volume of 100% and 60 s of nitrogen purge were applied. Pressure was set to 1500 psi as it has been demonstrated that its variations are not decisive in the extraction efficiency [20]. The PLE extracts obtained were further purified by solid phase extraction (SPE), using a Baker vacuum system (J.T. Baker, The Netherlands). In all cases, the extracts (20 mL) were diluted with HPLC grade water (200 mL) to reduce the content of organic solvent to less than 5%, in order not to interfere in the SPE procedure, and filtrated through 0.45 μm nylon filters (Whatman, Maidstone, UK). Oasis HLB cartridges were conditioned with 3 mL of MeOH in 50 mM HCOOH, followed by 3 mL of acetone in 50 mM HCOOH and 2 mL of HPLC grade water at neutral pH with 5% of MeOH [21]. After the sample loading, the cartridges were rinsed with 3 mL of HPLC grade water at neutral pH with 5% of MeOH, to remove potential retained interferences. Cartridges were dried under vacuum (around 30 min) and then eluted with 5 mL of MeOH in 50 mM HCOOH and 5 mL of acetone in 50 mM HCOOH. SPE extracts were dried under a gentle N_2 stream and reconstituted with 1 mL of HPLC grade water–ACN (75:25, v/v). Before LC–MS/MS analysis, the corresponding internal standards were added to the reconstituted extracts at a concentration of 50 ng mL^{-1}.

2.4. Instrumental analysis

LC analysis was performed with a Symbiosis™ Pico System (Spark Holland, Emmen, The Netherlands), equipped with a HPLC system consisting of an Alias™ autosampler, a loop injector and two binary pumps with a four-channel solvent selector for each one. Chromatographic separation was performed using an Atlantis C_{18} LC-column (Waters, 150 mm × 2.1 mm, 3 μm of particle size) preceded by a guard column with the same packing material. The flow rate was set to 0.2 mL min^{-1}, being eluent A HPLC grade water acidified with 10 mM HCOOH, and eluent B ACN with 10 mM HCOOH. The elution gradient started with 25% of eluent B, increasing to 80% in 10 min and to 100% in 11 min. During the next 2 min the column was kept at 100% B, readjusted to the initial conditions in 3 min and equilibrated for 7 min. MS/MS analyses were carried out in a 4000 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with