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Determination of sulfonamides in serum by on-line solid-phase extraction coupled to liquid chromatography with photoinduced fluorescence detection

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ABSTRACT

An analytical method based on on-line solid-phase extraction coupled to liquid chromatography with photoinduced fluorescence detection has been developed to determine sulfonamides in serum. A home-made setup was used to percolate 3 mL of sample through a solid-phase extraction column. Analytes were retained onto the sorbent by an anion exchange mechanism which ensures an optimum compatibility with the subsequent chromatographic separation using a C-18 column and an on-line photoreactor in order to derivatize sulfonamides, which do not present native fluorescence. The method allowed the determination of 7 sulfonamides in serum samples previously deproteinized in less than 18 min and with limits of detection ranging between 1.8 and 3.6 mg/L. Relative recoveries between 91.5% and 102.1% were obtained with satisfactory precision since relative standard deviations were always below 10.5%.

1. Introduction

Sulfonamides are antibiotics that belong to the group of antibacterials. They inhibit a broad-spectrum of both gram positive and gram-negative bacteria, presenting chemotherapeutic activity against the infections caused by them as well as some protozoa. They are commonly prescribed in human and veterinary medicine against infection of digestive and respiratory tracts, affections of the skin, as well as for therapy of coccidiosis in animals [1,2].

In humans, for medical purposes, sulfonamides are normally administered orally through pharmaceutical preparations in a concentration range from 250 to 500 mg/L. Sulfonamides are well distributed in all body tissues. High concentrations can be found in bile, cerebrospinal fluid, prostatic fluid and sputum. They are metabolized in the liver but are primarily excreted unchanged in the urine. Taking into account their absorption rate, metabolism and elimination paths, typical concentrations in urine range between 0.001 and 1 μg/mL [3]. Regarding human blood, it is a common practice to maintain the blood levels of sulfonamides at approximately 0.1–100 μg/mL [3]; however a significant variation in blood levels might be found when analyzing blood from different patients treated with the same doses. In general, levels ranging between 50 and 150 μg/mL are considered therapeutically effective for most infections and levels between 120 and 150 μg/mL are considered optimal for serious infections.

Several reviews have been published including the different techniques and methodologies for sample treatment in the determination of sulfonamides in a great variety of matrices such as food, environmental samples, drugs or biological fluids [3–6]. With regard to sample preparation, mainly for cleaning up and preconcentration, traditionally liquid–liquid extraction (LLE) and mainly solid phase extraction (SPE) have been two of the most commonly employed procedures [3], in spite of other modern microextraction techniques such as salting-out liquid–liquid extraction (SALLE) [31], liquid phase microextraction (LPME) [7], hollow fiber LPME [8], QuEChERS [9,10] or dispersive liquid–liquid microextraction (DLLME) [10,11].

In general, SPE continues to be the most applied sample preparation method in routine analysis [3,12] showing some advantages including the easy automation and on-line coupling with liquid chromatography (LC) which involves reduction of the exposure to hazardous solvents and higher sensitivity [13,14]. However, compatibility between the elution step and the chromatographic separation must be taken into account. Solvents with high eluotropic strength are usually used to elute analytes from SPE cartridge but if analytes are injected in this solvent they would be poorly retained in the LC column, which would not be convenient for on-line SPE. In order to overcome this disadvantage an alternative is to use SPE sorbent based on ionic interactions. Analytes could be eluted just by changing the pH and without the

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need of high percentages of organic solvents. If the pH required for eluting the analytes is similar to the pH of the mobile phase an optimum compatibility between SPE and LC will be ensured. Some of the anion exchange sorbents used were PRP-X100 poly(styrene–divinylbenzene) based trimethylammonium to determine glyphosate and aminomethyl phosphonic acid in water [15], Isolute SAX to determine insulin derivatives in biological matrices [16], methylcellulose-immobilized-strong anion-exchanger to determine aspirin and metabolites in plasma [17], Dionex IonPac AG16 guard column to determine iodide and thiocyanate in powdered milk and infant formula [18], weak anion exchange monolithic column to determine cefazolin sodium and cefotaxime sodium in human urine [19], Biomax AX to determine intracellular triphosphate metabolites in peripheral blood mononuclear cells [20,21], hypercrosslinked polymer resin (HXLPP) modified with 1,2-ethylenediamine to determine a group of pharmaceuticals in water [22], Strata X-CW for therapeutic and abuse drugs in urine [23], Hysphere MM to determine folate catabolites in human biofluids [24] and Biobasic AX for domoic acid in shellfish [25]. Recently this strategy has been applied for the determination of quinolones in tap water and human urine using HyperSep Javelin Direct-Connect Retain-AX or HyperSep Uniguard Retain-AX without any sample pretreatment, only dilution of urine in water [26].

Reversed phase liquid chromatography (RP-LC) has been traditionally the most employed technique and in the last few years ultra high-performance liquid chromatography (UHPLC) has increasingly been used for the rapid separation of these compounds, mainly with mass spectrometry (MS) detection [9,27–30]. Other detection systems coupled with LC comprise UV and DAD [11,31–33], fluorescence previous derivatization with fluorescamine [10,34,35], chemiluminescence [36] or electrochemical detection [37].

In relation to the application of on-line SPE coupling with LC for the determination of sulfonamides, some works have been reported using Oasis HLB for waters [38–40], multiwalled carbon nanotubes in eggs and pork [41] or alumina for soils [42]. To the best of our knowledge, there is no report of the use of an anion exchange sorbent for their determination by on-line SPE coupled with LC.

Although sulfonamides present poor native fluorescence, some methods have been proposed for their determination based on their native fluorescence or sensitized fluorescence [43]. Nevertheless, the use of different derivatization procedures to sensitize their fluorescent detection is a common practice. Chemical agents, mainly fluorescamine, have been typically employed for fluorescence labeling [10,34,35]. Enhanced fluorescence signals have been discovered and exploited for analytical methodology development, based on the formation of inclusion complexes of sulfonamides in cyclodextrins [44]. A special case of derivatization includes the use of light, which gives rise to photochemically-induced fluorescence (PIF) methods. The photochemical decomposition of sulfonamides has been reported [45–47] as well as the fluorescence properties of some important sulfonamides and their respective photoproducts in aqueous medium [48], concluding that UV irradiation induces enhanced fluorescence signals for heterocyclic sulfonamides meanwhile it induces decrease of fluorescence for non-heterocyclic ones. Sulfacetamide, sulfaguanidine and sulfametazine have been directly determined in milk and pharmaceutical preparations [49]. Other methodologies based on PIF for the monitoring of sulfonamides involved the use of first and second derivative techniques [50–53], the coupling with flow injection analysis (FIA) [43,54], the application of multivariate calibration strategies [55] or the proposal of a fluorimetric multioptosensor [56].

The aim of this work is to check the performance of the on-line coupling of SPE with LC using an anion exchange sorbent for the monitoring of sulfonamides in a biological matrix, such as serum, with minimum sample conditioning. Also, in order to increase sensitivity, PIF is proposed as detection system, using an on-line photochemical derivatization unit after the chromatographic separation. The proposed system could offer a high degree of automation and a satisfactory sensitivity combining on-line both SPE and photodegradation of analytes to obtain fluorescent products.

2. Materials and methods

2.1. Chemicals, reagents and samples

All the reagents were analytical reagent grade, solvents were HPLC grade and sulfonamides were analytical standard grade (Vetranal, Sigma-Aldrich, St. Louis, MO, USA). Methanol (MeOH) and acetonitrile (MeCN) were supplied by Panreac (Madrid, Spain). Acetic acid and formic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA).

A stock standard solution of 1000 μg/mL of each sulfonamide (sulfadiazine: SDZ; sulfapiridine: SPD; sulfamerazine: SMR; sulfamethazine: SMZ; sulfachloropiridazine: SCP; sulfamethoxazole: SMX; sulfadoxine: SDX) was prepared by dissolving 10 mg of the product in 10 mL of MeOH. The solutions were stable for at least 2 months, stored in the dark at 4 °C. The working solutions were prepared by dissolving the appropriate volume in deionized water.

Filters of 25 mm with 0.22 μm polyethersulfone membrane (Agela Technologies, DE, USA) were used for sample filtration.

Ultrapure water (18.2 MΩ cm^{-1}, Milli-Q Plus system, Millipore Bedford, MA, USA) was used throughout all the work.

A serum pool from anonymous patients was obtained from a local hospital.

2.2. Instrumentation

The chromatographic separation was carried out on a Jasco LC system consisting of a quaternary pump (PU-2089), an autosampler with 100 μL loop (AS-2055) coupled to a UV Derivatization module (LCTech, Dorfen, Germany) and subsequently to a fluorescence detector (Model FP 2020, Jasco). LC-Net II/ADC was used as the hardware interface between the LC system and the computer. Fluorescence chromatograms were acquired using ChromNav version 1.18.03. For on-line SPE experiments, the autosampler was removed and a setup consisting of a multisyringe pump with programmable speed (MultiBurette 4S, Crison Instruments, Alella, Barcelona, Spain), a low pressure selection valve (Rheodyne 5011, Supelco, Bellefonte, PA, USA) and a high pressure 6-port injection valve (Rheodyne 7725i, Supelco, Bellefonte, PA, USA) were used instead. The SPE procedure was executed and controlled by software written in-house using Visual Basic 2010 (Microsoft, Redmond, WA, USA). The software was designed to control the position of commutation valves and the speed and direction of piston movement on the multisyringe pump. Fig. 1 shows the on-line SPE–HPLC setup with the corresponding times for each step.

2.3. Chromatographic conditions

LC separation was performed in a Luna C_{18} column (150 × 0.5 mm, 5 μm) from Phenomenex (Torrance, CA, USA). Mobile phase consisted of 5% acetic acid in water (solvent A) and 5% acetic acid in MeCN (solvent B) at a flow rate of 1.2 mL/min. The eluent gradient profile was as follows: 0 min: 5% B; 15 min: 25% B; 23 min: 60% B. Finally it was back to 5% B in 2 min and maintained for 5 min for column equilibration. The temperature of the column was 40 °C and the injection volume was 3 mL. Fluorescence detection was performed at their maximum excitation/emission wavelength 240/350 nm. Gain detector was set to 100.