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Trace determination of β-lactam antibiotics in environmental aqueous samples using off-line and on-line preconcentration in capillary electrophoresis☆

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Abstract

A sensitive and reliable method using capillary zone electrophoresis with UV-diode array detection (CZE-DAD) has been developed and validated for trace determination of β-lactam antibiotics in waste, well and river water matrices. Due to the lack of sensitivity of the UV–vis detection, a solvent extraction/solid-phase extraction (SPE) method applied for off-line preconcentration and cleanup of water samples, in combination with an on-line preconcentration methodology named large volume sample stacking (LVSS) have been applied. The analytes included nafcillin, dicloxacillin, cloxacillin, oxacillin, ampicillin, penicillin G and amoxicillin. Average recoveries for water samples fortified with the studied β-lactams at different concentration levels (1.0, 2.0 and 4.0 μg/L) were ranging between 94 and 99%, with relative standard deviations (RSDs) lower than 10%. The precision, calculated as intra-day and inter-day standard deviations fell within acceptable ranges (3.3–7.2%). The limits of detection were estimated to range between 0.08 and 0.80 $\mu g\,L^{-1}$ for the studied compounds. All the samples analyzed were negative for all the analytes at these levels of concentration and the method showed its usefulness for the detection of these widely applied β-lactam antibiotics in different kinds of waters.

Keywords: β-Lactam antibiotics; Capillary zone electrophoresis; Sample stacking; Wastewater; River water; Well water

1. Introduction

In addition to pesticides, industrial chemicals and their metabolites, pharmaceutical substances have experienced a fast growing interest and recent studies have shown that a multitude of drugs are present in aquatic systems [1]. The importance of the determination of antibiotic residues in environment samples arises from the fact that they are suspected of being responsible for the appearance of bacterial strains that are resistant to antibiotics [2]. Also, it is important to consider that the large amount of antibiotics which are continuously introduced to the environment make them potential pollutants that are incorporated from a variety of sources, including discharges from domestic wastewater treatment plants and pharmaceutical companies, runoff from animal feeding operations, infiltration from aquaculture activities or from compost made of animal manure containing antibiotics [3,4].

β-Lactam antibiotics have been the most widely used as antimicrobial drugs for more than 80 years and still constitute the most important group of antibiotics. These antibiotics are used to treat bacterial infection of various organs [5]. A high percentage of antibiotics consumed by humans and animals in hospitals or by prescription are excreted uncharged via urine and feces into domestic sewage, and are discharged to wastewater treatment plants effluent into the aquatic environment. Thus, the origin of antibiotic contamination in surface and ground waters is considered to be point and non-point source discharges of municipal and agricultural wastewater [6]. Even the poor stability of the β-lactam ring that can be opened by β-lactamases, a widespread enzyme in bacteria, or by chemical hydrolysis, it is not possible to exclude the presence of these compounds in aquifers, where manure or sludge is applied to soils, or where hospital wastewaters is discharged, via a relatively small sewage treatment plant, into the nearest stream [7,8].

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Several analytical methods have already been described for simultaneous determination of antibiotic residues in the aqueous environment. Most of the described methods refer to the use of HPLC both with UV [9,10] or mass spectrometry (MS) [11,12] detection and GC–MS [13–15], which require large volumes of solvents and/or derivatizating treatments. Usage of GC–MS also involves high costs, thus limiting accessibility. Specifically for β-lactams, solid-phase extraction (SPE) and LC with DAD have been recently applied to detect these residues or a combination with other pharmaceutical compounds in water samples [9,16] and with MS detection for its control in tap and surface water [17] and in surface water and urban wastewater [8].

In recent years, the use of capillary electrophoresis (CE) for the determination of antibiotics has gained considerable importance because it is an effective and economic approach for the separation of a large variety of substances, as well as the increasing availability of automated CE instruments that have promoted the exploration of an increasing number of CE methods for routine analysis [18–23]. Mainly, micellar electrokinetic capillary chromatography (MEKC) has been applied to the simultaneous separation of several penicillins [24–31] in different matrixes and specifically to the screening of penicillin compounds in farm water samples [32]. Capillary isotachophoresis has been applied for the analysis of penicillins and cephalosphorins in pharmaceutical preparation [33]. Also, CZE has been used for the analysis of penicillin G [34] and amoxicillin [35] together with other [36] and for the simultaneous screening of six antibiotics, including four penicillins (penicillin G, amoxicillin, ampicillin and cloxacillin) in milk [37].

Considering that these compounds are most likely to be found at very low concentrations in the different aquatic environments, like sewage treatment plants, influents and effluents, surface, ground and drinking water, it is necessary to use sufficiently sensitive analytical techniques. This is normally a problem for CE using UV–vis detection. Recently MEKC has been combined with a very sensitive detection technique, laser-induced fluorescence (LIF) detection, for the separation and determination of ampicillin, amoxicillin, cephradine and cephalexin in environmental waters, involving a previous derivatization step top from the fluorescent products and obtaining limits of detection (LODs) from 30 to 45 ng L^{-1} [38]. Several preconcentration procedures have been shown to be useful for determining analytes at low concentrations [39], based on the manipulation of the electrophoretic velocity of the analyte (sample stacking) or related to the ability of the analyte to partition into a pseudostationary phase (sweeping and analyte concentrators). The simple-one is sample-stacking (SS) which is an inherent and exclusive feature of CE, taking place when the sample compounds encounter isotachophoretic concentration at the interface between sample zone and buffer (isotachophoretic sample stacking, ITPSS) or when the conductivity of the sample is smaller than that of the buffer (field-amplified sample stacking, FASS) [40]. Additionally, large-volume sample stacking (LVSS), also named stacking with matrix removal (SWMR), has demonstrated to improve detection limits of charged analytes by more than 1000-fold and it can be easily automated and controlled by software [41]. Using this methodology, slightly modified, the analysis of eight penicillins by MEKC has been carried out [42]. Field amplified sample injection (FASI) has also been applied for the CZE analysis of amoxicillin [26] and LVSS was applied for the detection of oxacillin, cloxacillin and dicloxacillin [43].

The aim of this work was to develop a very sensitive method to the analysis of β-lactams at trace level in environmental water samples of different origins. The chemical structures of the studied compounds are shown in Fig. 1. For this purpose an on-line preconcentration procedure based on LVSS has been optimized and combined with the utilization of a SPE cartridge for the off-line preconcentration of the analytes in aquatic samples. The CE separation method has been optimized using DAD.

2. Experimental

2.1. Chemicals and solvents

The β-lactam antibiotics nafcillin, CAS [147-52-4], dicloxacillin, CAS [3116-76-5], cloxacillin, CAS [61-72-3], oxacillin, CAS [66-79-5], ampicillin, CAS [69-53-4], penicillin G, CAS [61-33-6], amoxicillin, CAS [26787-78-0], were obtained from Sigma (St. Louis, MO, USA). The organic solvents employed (acetonitrile, methanol, and ethanol) were purchased from Merck (Darmstadt, Germany), and all reagents were of analytical reagent grade.

The running buffer was prepared from tris(hydroxymethyl)aminomethane supplied by Merck and the pH was adjusted to 8 with 1 M sodium hydroxide obtained from Panreac-Química (Madrid, Spain). Methylparaben, used as internal standard (I.S.), was also purchased from Sigma.

Ultrapure water (Milli–Q plus system, Millipore Bedford, MA, USA) was used throughout the work.

Extraction cartridges containing an Oasis hydrophilic–liphophilic balance (HLB; 60 mg, 3 mL; Waters, Milford, MA, USA) and alumina N (500 mg, 3 mL; E. Merck) in laboratory-prepared cartridges were used.

2.2. Preparation of standards

Stock standard solutions (1000 μg mL^{-1}) of each compound were prepared by dissolving the appropriate amount of each substance in deionized water, stored at 4 °C in the dark, and then diluted to the desired concentration prior to use. Under such conditions, they were stable for at least 2 months.

A stock solution of 0.5 mg L^{-1} of the methylparaben (I.S.) was prepared by dissolving 0.5 mg of the product in 1000 mL of water. The solution was stable for at least 1 month.

2.3. Instrumentation

The pH of the running buffer was adjusted with a pH meter (Crison model pH 2000, Barcelona, Spain) and was employed with a resolution of ±0.01 pH unit. Solid-phase extraction was carried out on a vacuum manifold system from Supelco (Bellefonte, PA, USA) coupled with a vacuum pump (Büchi model B-121, Flawil, Switzerland).