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Simultaneous analysis of antibiotics in biological samples by ultra high performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

A rapid and reliable multiclass method was developed for the simultaneous analysis of 21 antibiotics (beta-lactams, aminoglycosides, penicillins, cephalosporins, carbapenems or quinolones) in urine, serum, cerebrospinal fluid (CSF) and bronchial aspirations by ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS). Prior to chromatographic determination, the analytes were extracted from human biological fluids by simple sample treatments, which imply dilution, liquefaction, or protein precipitation. Several chromatographic conditions were optimized in order to obtain a fast separation (<6 min for each chromatographic run). MS/MS conditions were evaluated in order to increase selectivity and sensitivity and all compounds were detected in electrospray (ESI) positive ion mode, except clavulanic acid and sulbactam, which were monitored in negative ion mode. The developed method was validated in terms of linearity, selectivity, limits of detection (LODs) and quantification (LOQs), trueness, repeatability and interday precision. The LOQs ranged from 0.01 to 1.00 mg/L for urine, serum and CSF. In case of bronchial aspirations, the LOQs were between 0.02 and 0.67 mg/kg. In all matrices the recovery results were in the range 70–120% and interday precision was lower than 25%. Finally, the optimized method was applied to the analysis of biological samples from 10 patients in the intensive care unit (ICU) of a hospital located in Almeria (Spain). Several antibiotics (e.g., amoxicillin, tobramycin, levofloxacin, or linezolid) were found in the studied samples, observing that the highest concentrations were obtained in urine samples.

1. Introduction

Antibiotics are used to prevent and treat diseases and microbial infections in animals and humans [1,2] and global consumption has been estimated between 100,000 and 200,000 tons/year [3].

Antimicrobial treatments have several benefits, but when large quantities of a specific type of antibiotic are supplied, allergic reactions and another adverse effects, as drug resistant microorganisms, can appear [4–8]. In the case of drug-resistance bacteria, hospital stay should be longer and patients should be treated with more costly, and sometimes, more toxic antibiotics [9–11]. For these reasons, the rational use of antibiotics, the correct dosing and treatment duration are key factors to prevent possible resistances [12]. In this sense, it is important to determine the concentration of antibiotics present in different tissues, in order to know the effectiveness of treatment.

Analytical chemistry is essential to improve clinical outcomes, because it can provide a suitable methodology for a rapid determination of antibiotics in biological matrices. This is important for critically ill patients, who need an early and reliable diagnosis. Moreover, since substances such as drugs, metabolites or endogenous compounds can be bio-accumulated in human body at levels that may be toxic, the determination of these compounds in biological fluids is very important in clinical research [13].

Antibiotics are usually combined in order to cover a broad spectrum of activities, minimizing toxicity and increasing the efficiency of the treatment [14]. Therefore, it is necessary to develop multi-class methods for the simultaneous determination of several antibiotics in the same analysis. Furthermore, analytical techniques should be rapid, accurate, automated, selective and sensitive.

Traditionally, microbiological methods have been used [15,16]. However, despite they are simple and inexpensive, these methods are time-consuming, they present poor selectivity and they do not differentiate among several types of drugs [17,18]. In order to overcome these problems, liquid chromatography (LC)

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coupled to conventional detection as fluorescence [19] and chemiluminescence [20,21] has been used, although complex and laborious derivatization steps are required [22]. LC coupled to UV detection has also been applied [23], but problems related to selectivity can be found [24]. Furthermore, LC has been coupled to amperometric [25] and electrochemical detectors [26], but both detection techniques require tedious sample preparation and chromatographic steps. Nowadays, tandem mass spectrometry (MS/MS) has been widely used, because its higher sensitivity and selectivity [22,27,28], which implies simple sample treatments, increasing sample throughput. Finally, in order to reduce the total analysis time, ultra-high performance liquid chromatography (UHPLC) has been developed in the last years [29,30] allowing the analysis of a large number of samples in a short period of time [31].

One of the main problems in multiclass methods is the complexity of the extraction steps and the need for including clean-up and preconcentration stages before instrumental analysis. For this purpose, different extraction techniques have been used, such as solid phase extraction (SPE) [32], liquid–liquid extraction (LLE) [33], pressurized liquid extraction (PLE) [34] and matrix solid phase dispersion (MSPD) [35], which include laborious steps (evaporation, elution or cleanup) [36], and therefore, they are time consuming and costly techniques.

Bearing in mind that most of the current methods are mainly focused on the determination of a few compounds, the aim of this work was the development of new a method for the simultaneous determination of antibiotics belonging to different families (aminoglycosides, macrolides, β-lactames, quinolones, penicillins, cephalosporins, carbapenems, glycopeptides and polypeptides), which were widely used in the intensive care units of hospitals. In addition to plasma and urine, two more biological fluids such as bronchial aspirations and cerebrospinal fluid (CSF) were analyzed bearing in mind that some of the selected compounds can achieve these fluids [37] and the concentration could be more meaningful than in plasma or urine [38]. The final method was based on rapid and simple protein precipitation or dilution stages of samples and determination by UHPLC–MS/MS.

2. Materials and methods

2.1. Chemicals and reagents

Amoxicillin, levofloxacin, amikacin, ceftriaxone, vancomycin, ampicillin, tobramycin, tazobactam, piperacillin, ceftazidime and linezolid were obtained from Sigma–Aldrich (Madrid, Spain). Imipenem, sulbactam, cefepime, moxifloxacin and teicoplanin were purchased from European Pharmacopeia (Strasbourg, France). Moxifloxacin and clavulanic acid were provided from Fluka (Steinheim, Germany). Meropenem was supplied by LGC Stantandars (Barcelona, Spain) and tigecycline from Wyeth (Madrid, Spain). Daptomycin was supplied from Novartis (Barcelona, Spain) and clarithromycin from Dr. Esteve (Barcelona, Spain). Stock standard solutions of individual compounds (with concentrations between 200 and 300 mg/L) were prepared by exact weighing of the powder and dissolved in 50 mL of methanol (HPLC grade, Sigma), which were stored at −20 °C in the dark. A multicompound working standard solution at a concentration of 10 mg/L of each compound was prepared by appropriate dilutions of the stock solutions with methanol and stored in screw-capped glass tubes at −20 °C in the dark. This solution was stable for three weeks, after which it was replaced by a new fresh solution.

HPLC-grade acetonitrile was supplied by Sigma. Formic acid and ammonium formate was purchased from Panreac (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q Gradient water system (Millipore, Bedford, USA). A solution of DL-dithiothreitol (DTT) obtained from Sigma–Aldrich was prepared in water (1%, w/v).

2.2. Equipment

Chromatographic analyses were performed using an Acquity UPLC system (Waters, Milford, MA, USA), and separations were achieved using an Acquity UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 μm particle size) from Waters. Mass spectrometry analysis was carried out using a Waters Acquity TQD tandem quadrupole mass spectrometer (Waters, Manchester, UK). The instrument was operated using electrospray ionization (ESI). Data acquisition was performed using MassLynx 4.0 software with QuanLynx program (Waters). Chromatographic separation was carried out with a mobile phase consisting of methanol (eluent A) and an aqueous solution of formic acid, 0.01%, v/v (eluent B), at a flow rate of 0.3 mL/min.

Each sample was injected twice: one in ESI positive ion mode and one in ESI negative ion mode (for the detection of clavulanic acid and sulbactam). To elute the compounds detected in positive ion mode, the following gradient profile was used: the elution started at 20% of eluent A, which was linearly increased up to 100% A in 3.5 min, keeping constant for 1 min before being returned to the initial conditions in 0.5 min. Finally, the total run time, including a re-equilibration step was 6 min. For the elution of the compounds detected in negative ion mode, the gradient profile was slightly different: the elution started at 10% eluent A and it was increased linearly to 100% in 3 min. This composition was kept 1 min before being returned to the initial conditions in 0.3 min, keeping constant for 1.2 min. The total time of analysis was 5.8 min. In all cases, injection volume was 5 μL, and column temperature was set at 30 °C. Autosampler temperature was set at 8 °C. For MS/MS detection, the ionization source parameters in positive and negative ion mode were the same. The capillary voltage and the extractor voltage were 3.0 kV and 2 V, respectively. The source temperature was 120 °C and desolvation temperature 350 °C. The cone gas (nitrogen) and desolvation gas (also nitrogen) were set at flow rates of 80 L/h and 600 L/h, respectively, and the collision-induced dissociation was performed using argon as the collision gas at the pressure of 4×10^{-3} mbar in the collision cell. The specific MS/MS parameters for each antibiotic are shown in Table 1.

Centrifugations were performed in a high-volume centrifuge from Centronic (Barcelona, Spain). An analytical AB204-S balance (Mettler Toledo, Greinfesse, Switzerland) was also used. Cartridges C18 Sep-Pak (500 mg) and Oasis HLB (500 mg) cartridges were obtained from Waters (Milford, MA, USA) and they were used during optimization of the extraction procedure of serum samples.

2.3. Sample collection

Urine, serum, CSF and bronchial aspirations samples were collected from patients admitted in the intensive care unit (ICU) in a hospital from Almeria (Spain). These patients were randomly selected from those subjected to several treatments with some of the antibiotics included in the developed method. Samples were collected in sterile containers and they were immediately frozen at −20 °C with the aim of avoiding a possible antibiotic degradation during transport. Samples were analyzed within 24 h after they were acquired, and before analysis, they were thawed to room temperature.

2.4. Extraction procedure for urine, serum, CSF and bronchial aspiration samples

The optimized procedures for the extraction of antibiotics from the different matrices were: