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Development and validation of a MEPS-UHPLC-PDA method for determination of ulifloxacin in human plasma and urine of patients with peripheral arterial disease

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ABSTRACT

A novel sensitive analytical method based on the use of a semi-automatic microextraction by packed sorbents (MEPS) techniques combined with ultra high-performance liquid chromatography (UHPLC) with PDA detection has been developed and validate for the analysis of ulifloxacin, the active metabolite of prulifloxacin using danofloxacin as internal standard in human plasma and urine. Different experimental parameters were optimized and validated according to international guidelines. Complete separation of the analytes was achieved with a Waters BEH C_{18} (50 × 2.1 mm I.D., 1.7 μm particle size) analytical column, a mixture of 10 mM ammonium acetate (pH 3.0) (A) with and acetonitrile (B) both containing 1% triethylamine were used as mobile phase, at a flow rate of 0.6 mL/min in gradient elution, and detection wavelength of 272 nm. This method is linear in concentration range of 0.02–10.0 μg/mL for plasma and urine, respectively. The limit of quantitation was 20 ng/mL for the two fluids. The recoveries of the method were 95% for ulifloxacin in human plasma and urine and 95.5% for the internal standard. Intra- and inter- assay precision and accuracy for ulifloxacin were lower than 10% at all tested concentrations. The proposed method was successfully applied to measure plasma and urine concentrations of ulifloxacin in patients suffering from Peripheral Arterial Disease and for pharmacokinetics study.

1. Introduction

Prulifloxacin is an oral fluoroquinolone antibacterial agent, its chemical name according to IUPAC nomenclature is (RS)-6-Fluoro-1-methyl-7-[4-(5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl-1-piperazinyl]-4-oxo-4H-[1,3] thiazeto [3,2-a]quinoline-3-carboxylic acid (Fig. 1B). After oral administration prulifloxacin is absorbed in the upper small intestine and then metabolized to ulifloxacin [1–3]. Ulifloxacin or 6-Fluoro-1-methyl-4-oxo-7-(1-piperazinyl)-4H-[1,3] thiazeto [3,2-a]quinoline-3-carboxylic acid (Fig. 1A), has a broad-spectrum activity *in vitro* against various Gram-negative and Gram-positive bacteria and acts directly on bacterial DNA-gyrase, inhibiting cell reproduction that leads to cell death [4,5]. Its spectrum of activity and pharmacological properties make ulifloxacin useful in the treatment of diabetic foot infection [5]. Peripheral arterial disease is a common cardiovascular complication in patients with diabetes [5]. The presence of significant peripheral arterial disease in an infected limb impairs delivery of the required dose of prulifloxacin to the infected tissues [5]. Several methods that have been developed for the determination of fluoroquinolones in biological fluids include high-performance liquid chromatography (HPLC) [3,6–9]. Separation is typically performed by HPLC coupled with ultraviolet (UV) [3,6–8], fluorescence (FL) [9–11], capillary sone electrophoresis [12–14], or tandem mass spectrometry (MS/MS)detection [15,16]. Difficulties may be met in the analysis of fluoroquinolones both in extraction and in the chromatographic steps. These molecules are weak heterocyclic amino acids with two reactive sites: an amino group which can be protonated and a carboxylic group which can lose a proton. Due to this amphoteric character of fluoroquinolones, they exist in cationic, zwitterionic and anionic forms.

The most widely used columns are packed with C_{18} sorbent and the chromatographic elution systems are typically binary with acidified aqueous polar solvent, such, phosphoric acid, or formic

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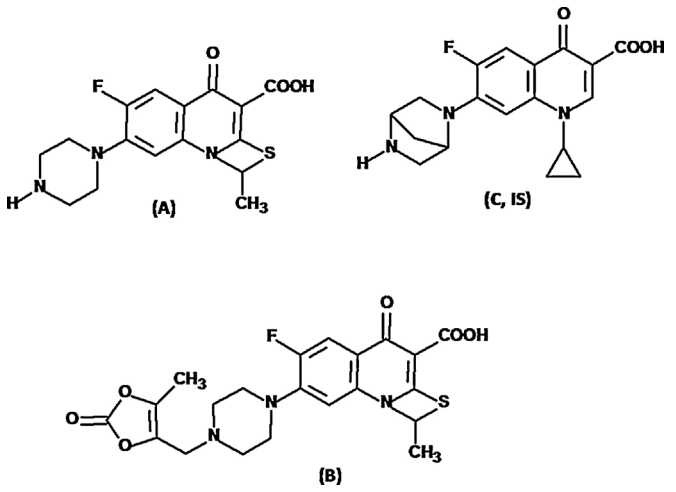

Fig. 1. Chemical structures of ulifloxacin (A), prulifloxacin (B), and (C) danofloxacin (internal standard).

acid and a less polar organic solvent, such as methanol or acetonitrile. In these separation methods, the sample preparation is performed by liquid–liquid extraction (LLE) [6,7,9] molecular polymers imprinted (MIP) [17], matrix solid-phase dispersion (MSPD) [18], solid-phase extraction (SPE) [8,19–21], and chemiluminesciences [12,14]. However, some of these sample preparation procedures are often time-consuming, laborious and expensive. In addition, some of them have deleterious effects on the environment due to the large quantities of organic solvents that need to be used and discarded. Therefore, recent developments in the field of sample preparation have been directed toward miniaturisation and automation. One of the latest developments is microextraction by packed sorbent (MEPS). Microextraction by packed sorbent has emerged as an attractive alternative for sample preparation owing to its simplicity, low solvent consumption and ease of connection on-line to either GC or LC chromatographic systems. This technique combines sample extraction, pre-concentration and clean-up in a single device composed of the following two parts, the MEPS syringe and the MEPS cartridge, also known as the BIN (Barrel Insert and Needle). The BIN contains the packed MEPS bed, a solid support that retains the target analytes when the sample passes through it built into the syringe needle. In general, MEPS is an adaptation of SPE that incorporates all the desirable characteristics into a miniaturized device with a typical void volume of less than 10 μL. MEPS has been employed for the analysis of drugs in biological fluids [22–28].

However, its application to the analysis of fluoroquinolones in biological samples has not yet been published. In the literature there is only one article about application of MEPS for extraction of fluoroquinolones from the environmental water samples [29].

The objective of this study was to develop and validate a MEPS-UHPLC-PDA method for the quantification of ulifloxacin, the active metabolite of prulifloxacin in human plasma and urine using danofloxacin as an internal standard. MEPS has shown to be a rapid procedure for the determination of the selected fluoroquinolone drug in human plasma and urine, allowing reducing the handling time and the costs of analysis. The MEPS parameters affecting the extraction efficiency, such as the type of BIN, extraction time, solvents used for the conditioning, loading, and washing, sample flow-rate, elution solvent, type and volume were optimized to selected the best option in terms of sensitivity and precision of drugs retention. Furthermore, UHPLC-PDA has demonstrated to be a powerful tool for the quantitation of the studied analyte, obtaining a good selectivity and sensitivity using a sample volume of as low as 100 μL. This work provides a novel application for the first time of MEPS as procedure for the pre-concentration and extraction of ulifloxacin and danofloxacin in human plasma and urine and applied for clinical pharmacokinetic studies. A simple, sensitive and fast UHPLC-PDA method was also developed and validated for the analysis of this drug in human plasma and urine. Once optimized, the MEPS-UHPLC-DAD procedure was applied to analysis of selected drug in human plasma and urine obtained from patients. To our knowledge this is the first MEPS-UHPLC-PDA method for the quantification of ulifloxacin, in human plasma and urine.

2. Experimental

2.1. Chemicals and reagents

Ulifloxacin (CAS 112984-60-8; >98% purity index) was purchased from Suzhou Bichal Biological Technology Co. Ltd (Jiangsu, China). Prulifloxacin (CAS 3447-62-1; ≥98% purity index), ofloxacin (CAS 82419-36-1; ≥99% purity index), Norfloxacin (CAS 70458-96-7; ≥98% purity index), Fleroxacin (CAS 79660-72-3; ≥98% purity index), Pefloxacin (CAS 149676-40-4; ≥98% purity index), and danofloxacin (Fig. 1) (CAS 112398-08-0 >99% purity index) were supplied from Sigma-Aldrich (Sigma-Aldrich Milan, Italy). Acetonitrile (ACN) (HPLC-grade), methanol (HPLC-grade) and triethylamine (TEA) were purchased from Carlo Erba Reagents (Milan, Italy). Ammonium acetate (>99% purity index) was purchased from FlukaChemika – BioChemik (Buchs, Switzerland). Pooled drug-free human plasma was obtained from Sigma-Aldrich (Milan, Italy) while urine was purchased from FAR Diagnostic (Verona, Italy). HPLC-grade water was obtained by passage through an Elix 3 and Milli-Q Academic water purification system (Millipore, Bedford, MA, USA). All the chemicals and reagents used were of the highest purity that was commercially available.

2.2. Apparatus and chromatographic conditions

The analysis of Ulifloxacin was carried out on an Acquity H-Class UHPLC system (Waters, Milford, MA, USA) equipped with a quaternary solvent manager, a sample manager, a column heater, a photodiode array detector and a degassing system. Data handling was managed by Empower v. 3.0 software (Waters). The mobile phase used was a mixture of 10 mM ammonium acetate (pH 3.0) (A) with acetonitrile(B) both containing 1% triethylamine in gradient elution. At a flow rate of 0.6 mL/min, giving a maximum back pressure of 10,200 psi, which is within the capabilities of the UHPLC system, an isocratic plateau (88:12 A:B v/v) was programmed for the first 1.0 min. Then,within 1 min eluent B linearly increased from 12% to 33% (B), then maintained in isocratic condition (67:33 A:B v/v) for 1 min, then the initial conditions were regained within 1 min and the column was re-equilibrated for 2 min. The extracts (5 μL) were injected into the UHPLC system equipped with a Waters BEH C_{18} (50 × 2.1 mm I.D.,1.7 μm particle size) column protected by Van Guard pre-column with a $BEHC_{18}$ (5 × 2.1 mm) provided by Waters (Waters, Milford, MA, USA). The column was thermostated at 25 °C and the samples were kept at 20 °C in the sample manager. All samples, standards and eluents were filtered through a 0.22 μm PTFE filter (Millipore, Milford, USA) before injection in order to remove solid impurities. Acetonitrile was filtered through a 0.45 μm WTP membrane, while phosphate buffer solutions were filtered through a WCN 0.5 μm membrane (Whatman, Maidstone, UK). For quantification, the PDA wavelength was set at 272 nm. Ulifloxacin identification was based on the comparison with retention times and spectra given by standard solutions of individual compounds.

The MEPS syringe (250 μL) and the MEPS C_{18}BIN 4 mg, were supplied from SGE (Ringwood, Australia). This sorbent has irregu-