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Robust HPLC–MS/MS method for levofloxacin and ciprofloxacin determination in human prostate tissue

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ABSTRACT

Fluoroquinolones are the drugs of choice in the prevention of bacterial infections after transrectal ultrasound guided prostate biopsy. In order to improve assessment of antibacterial efficacy in the target tissue a simple, selective, rapid and robust HPLC-ESI–MS/MS method for the determination of levofloxacin and ciprofloxacin concentrations in human prostate bioptates was developed and validated. Preparation procedure for prostate samples (10 mg) was carried out using homogenization and filtration steps. Analyses were performed within 3.5 min using RP C_{18} column in the isocratic elution mode with mobile phase composed of a mixture of 0.1% formic acid aqueous solution and 0.1% formic acid methanol solution (*v/v*; 79:21). The method was linear between 0.3 μg/g and 15 μg/g for levofloxacin and ciprofloxacin with coefficient of correlation (r) ≥0.999. The limit of detection and the limit of quantification for levofloxacin were 0.06 μg/g and 0.2 μg/g and for ciprofloxacin were 0.04 μg/g and 0.13 μg/g, respectively. Average concentrations (±SD) of levofloxacin and ciprofloxacin obtained from patients tissue were 5.4 ± 2.2 μg/g and 3.9 ± 1.5 μg/g, respectively. Additionally, during validation procedure a novel, experimental design approach was applied for the robustness study. For evaluation of analytical method robustness, Plackett-Burman design was employed and for sample preparation method robustness Fractional Factorial design was used.

The developed and validated method was successfully applied to examine prostate tissue samples obtained from patients enrolled into a clinical study. Up to now, there has been no other HPLC-ESI-MS/MS method reported for the simultaneous determination of levofloxacin and ciprofloxacin in human prostatic tissue.

1. Introduction

Transrectal ultrasound guided prostate biopsy (TRUS-Bx) is a standard procedure for diagnosis of prostate cancer. However, biopsy procedure is burdened with risk of relatively frequent and serious infectious complications, which enforces antimicrobial prophylaxis [1]. Prostate and blood contamination by the intestinal flora may lead to febrile urinary tract infections, prostatitis or even to life-threatening urosepsis. According to the European Association of Urology (EAU) Guidelines, fluoroquinolones are the drugs of choice and the most frequently used antimicrobial agents in the prevention of bacterial infections after TRUS-Bx [2]. However, in recent few years, the rapid increase in the number of such complications has been reported in several studies [3–5].

In accordance to recent studies, fluoroquinolone-resistant bacterial strains lead to increased number of infections after TRUS-Bx [6,7]. In daily clinical practice, antibacterial efficacy is evaluated only *in vitro*, by measuring the reaction of bacteria with an antimicrobial agent in culture media (i.e. calculation of Minimum Inhibitory Concentration, MIC). This approach might be very misleading, because actual effectiveness of implemented antibacterial prophylaxis depends not only on susceptibility of bacteria to a certain drug, but also on its bioavailability in the treated tissue. The actual effectiveness of peri-biopsy prophylaxis is determined not only by the antimicrobial susceptibility of *E. coli* strains to fluoroquinolones but also by the degree of antibiotic penetration into the prostatic tissue. The latter is an equally important factor as prostate gland is characterized by exceptionally low pH and with

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probably no secretory or active transport mechanisms through the plasma-prostate barrier [8]. Levofloxacin (LVF) and ciprofloxacin (CPR), widely used fluoroquinolones, are regarded as drugs that can easily reach the prostatic fluid [9–13]. However, data about penetration of LVF and CPR to the prostate gland are rather limited and on the contrary to TRUS-Bx, a popular diagnostic procedure, might be considered insufficient, especially in the era of personalized medicine. Therefore there is a need for an objective tool for fluoroquinolone penetration assessment to the prostatic tissue in an individual patient.

Concentration of LVF and CPR in human prostatic tissue has been the subject of only few studies [9,14–18]. Regarding analytical techniques applied in mentioned articles, quantitative determination of LVF or CPR was performed with traditional techniques, like HPLC with UV detection [9,17,18] or microbiological assays [14,15], such as agar well diffusion method. Most of these methods lacked validation protocols [9,14,15,17] and required large amounts of prostate tissue (up to 500 mg per sample) [15,17]. Until now, no study concerning simultaneous determination of the above-mentioned chemotherapeutics in prostate tissue with the use of the novel LC-ESI-MS/MS technique has been published.

Hence, in the current study, a simple, selective, rapid and robust high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry method (HPLC-ESI-MS/MS) for monitoring of LVF and CPR concentrations in the prostate gland of patients undergoing TRUS-Bx procedure was proposed.

Another aspect of the project was implementation of quality-by-design approach into the analytical robustness study assessment. During robustness test various factors, which may potentially affect the results of an analytical method are examined. To evaluate significance of each factor settings, different approaches can be applied, so called univariate and multivariate approaches. A classically employed univariate procedure is the one-variable at-a-time (OVAT) approach. It has some disadvantages as the numerous experiments have to be conducted as the number of factors increases, interactions between factors are not considered and only a small part of the experimental domain is examined so the global optimum might not be found [19,20].

However, in chromatographic method development and validation, predominantly two or more factors have to be tested which can be performed with the aid of multivariate optimization strategies. In contrary to univariate procedure, in multivariate approach several factors are tested simultaneously [19]. Multivariate approaches contain simultaneous procedures, in which a predefined number of experiments is conducted, according to a defined experimental set-up, namely design of experiments (DoE).

In the current study, DoE procedure, in combination with statistical evaluation of the data, was implemented to test robustness of the LC–MS/MS method and of the sample preparation procedure. A Plackett-Burman design, which was used for LC–MS/MS method robustness study, is an orthogonal two-level experimental design, allowing to screen a relatively large number of factors in a relatively small number of experimental runs. What is more, this design is a good choice for robustness testing, as interaction effects are assumed to be negligible and only main effects are estimated. A Fractional Factorial design, applied in the sample preparation method robustness study, is very effective for reducing the number of experiments, thus, it is particularly favorable when there are limitations in the number of experimental runs, that can be performed, e.g. due to high cost or small amount of samples [20,21].

The aim of this study was to develop a robust, rapid, selective and easily applicable HPLC–MS/MS method for the simultaneous determination of LVF and CPR in prostate tissue with the use of experimental design approach during validation study. Finding the relationship between administered dose and concentration of LVF and CPR in prostate tissue may lead to evaluation and hence optimization of fluoroquinolone-based antimicrobial prophylaxis before TRUS-Bx. Determination of the actual concentrations of LVF and CPR in prostatic tissue during TRUS-Bx might also help to verify the trueness of applied dose and to decide whether another antimicrobial drug should be implemented.

2. Materials and methods

2.1. Materials

The reference standards of levofloxacin hemihydrate and ciprofloxacin hydrochloride were acquired from Polpharma SA (Pharmaceutical Works Polpharma SA, Starogard Gdański, Poland). Enrofloxacin (applied as an internal standard; IS) and methanol (LC–MS grade) were purchased from Sigma Chemical Co. (Sigma-Aldrich, Saint Louis, MO, USA). The structures of LVF, CPR and IS are shown in Fig. 1. Pure, deionized water for mobile phase was prepared using a Mili-Q system (Millipore, Milford, MA, USA). 97% formic acid (FA), a mobile phase component, was obtained from Alfa Aesar (A. Johnson Matthy Company, Karlsruhe, Germany).

2.2. Instrumentation

Chromatographic analysis was performed with the use of Agilent 1260 Infinity LC System (Agilent Technologies, Palo Alto, CA, USA), which consisted of degasser, binary pump, thermostated autosampler and thermostated column compartment, coupled with mass spectrometer, 6430 Series Triple Quadrupole (QqQ) from Agilent Technologies. The software used for controlling the equipment, acquiring and processing the data was MassHunter Workstation (Agilent Technologies, Santa Clara, CA, USA).

Prostate tissue samples were weighted using Radwag XA 60/220/X (Radwag, Radom, Poland) analytical balance. Samples were mixed on IKA MS3 Vortex (IKA®, Staufen, Germany) and centrifuged with Beckman Coulter centrifuge (Microfuge 16, Beckman-Coulter, Missisauga, Ontario, Canada). Heating process was performed in Thermoblock TB-951S (JW Electronic, Warsaw, Poland). Solutions were degassed by sonication with Sonic 6 (Polsonic, Warsaw, Poland).

2.3. LC–MS/MS analysis

An electrospray ionization source (ESI), working in positive ionization mode was chosen. Multiple reaction monitoring mode (MRM) was applied for quantitative analysis. The nebulizer pressure and capillary voltage were set at 30 psi and 1500 V, respectively. As a drying gas, nitrogen was used (12 l/min, 330 °C). The fragmentor and collision energy voltages were selected for both analytes and internal standard individually. The collision energy voltages for quantitative and qualitative ion mass transitions were set at 17 and 25 V for LVF, respectively. For CPR these values were set at 21 and 37 V, respectively, whereas for IS the collision energy voltage was 21 V. The optimized fragmentor voltages for LVF, CPR and IS were as follows: 130, 105 and 130 V, respectively. The quantitative ion mass transitions were: m/z 362.2 → 318.2 (LVF), m/z 332.1 → 314.1 (CPR) and m/z 360.2 → 342.2 (IS), whereas the qualitative ion mass transitions were: m/z 362.2 → 261.1 (LVF) and m/z 332.1 → 231.0 (CPR).

Separation of analytes was performed with the use of a Poroshell EC-C_{18} column from Agilent Technologies (2.1 × 50 mm, particle size 2.7 μm). Isocratic elution mode was applied. Mobile phase contained 79% of eluent A (water with 0.1% FA) and 21% of eluent B (methanol with 0.1% FA). Prepared solutions were degassed ultrasonically for 20 min at ambient temperature. One chromatographic run lasted 3.5 min. In order to avoid potential sample carryover in isocratic elution mode, needle was washed with 50% aqueous