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Ultra-high performance liquid chromatographic determination of levofloxacin in human plasma and prostate tissue with use of experimental design optimization procedures

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ABSTRACT

Fluoroquinolones are considered as gold standard for the prevention of bacterial infections after transrectal ultrasound guided prostate biopsy. However, recent studies reported that fluoroquinolone- resistant bacterial strains are responsible for gradually increasing number of infections after transrectal prostate biopsy. 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 minimal inhibitory concentration). Such approach, however, has no relation to the treated tissue characteristics and might be highly misleading. Thus, the objective of this study was to develop, with the use of Design of Experiments approach, a reliable, specific and sensitive ultra-high performance liquid chromatography- diode array detection method for the quantitative analysis of levofloxacin in plasma and prostate tissue samples obtained from patients undergoing prostate biopsy. Moreover, correlation study between concentrations observed in plasma samples *vs* prostatic tissue samples was performed, resulting in better understanding, evaluation and optimization of the fluoroquinolone-based antimicrobial prophylaxis during transrectal ultrasound guided prostate biopsy.

Box-Behnken design was employed to optimize chromatographic conditions of the isocratic elution program in order to obtain desirable retention time, peak symmetry and resolution of levofloxacine and ciprofloxacine (internal standard) peaks. Fractional Factorial design 2^{4-1} with four center points was used for screening of significant factors affecting levofloxacin extraction from the prostatic tissue. Due to the limited number of tissue samples the prostatic sample preparation procedure was further optimized using Central Composite design. Design of Experiments approach was also utilized for evaluation of parameter robustness. The method was found linear over the range of 0.030–10 μg/mL for human plasma and 0.300–30 μg/g for human prostate tissue samples. The intra-day and inter-day variability for levofloxacine from both plasma and prostate samples were less than 10%, with accuracies between 93 and 108% of the nominal values. The limit of detection and the limit of quantification for human plasma were 0.01 μg/mL and 0.03 μg/mL, respectively. For the prostate tissue, the limit of detection and the limit of quantification were 0.1 μg/g and 0.3 μg/g, respectively. The average recoveries of levofloxacin were in the range from 99 to 106%. Also, the method fulfills requirements of robustness what was determined and proved by Design of Experiments. The developed method was successfully applied to examine prostate tissue and plasma samples from 140 hospitalized patients enrolled into the clinical study, 12 h after oral administration of LVF at a dose of 500 mg. The mean (± SD) LVF concentration in prostate was 6.22 ± 3.52 μg/g and in plasma 2.54 ± 1.14 μg/mL. Due to simplicity of the method and relative small amount of sample needed for the assay, the method can be applied in clinical practice for monitoring of LVF concentrations in plasma and prostate gland.

1. Introduction

Each antimicrobial agent, in order to exhibit its pharmacological activity, must gain its minimal inhibitory concentration (MIC).

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When the administered dose of an antimicrobial drug is not sufficient to provide MIC in a certain organ, tissue or body fluid, implemented treatment may not only be ineffective, but also might lead to health-worsening conditions. The risk of ineffectiveness is also hidden in quitting the treatment immediately after subsiding of the symptoms as neglecting physician's instructions result in incomplete pathogen eradication. Microbes which were not eradicated due to insufficient treatment time or antimicrobial agent dose may survive and adapt or proliferate under favorable conditions. Pronounced development of resistance among bacteria is aggravated by overconsumption of antimicrobial preparations associated with irresponsible attempts to cure the infections caused by viruses or other insensitive pathogens. Misusage of antibiotics, along with their careless usage, greatly contributes to the development of resistant strains of bacteria [1].

This phenomenon was already predicted in 1945 by Sir Alexander Fleming, who said the following words at the Nobel Prize ceremony: *"Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant"* [2].

Bacterial resistance to antimicrobial agents has remained a serious public health problem for many years. New resistant strains are emerging every day and consequences for the wide-spread of antibiotic resistance are grave. For this reason, the World Health Organization (WHO) has issued recommendations with possible activities against antimicrobial resistance. One of them relies on the development of new antibacterial agents, whereas another emphasizes an optimization of the use of currently prescribed antimicrobial medicines [3,4]. To deal with spreading of inappropriate use of antibacterial agents, evaluation of the currently recommended regimen is required.

In numerous cases it is challenging to assess whether particular doses of administered antimicrobial drugs are sufficient to achieve desired concentrations in target tissues. An example of such situation is evaluation of the antimicrobial prophylaxis during transrectal ultrasound guided prostate biopsy (TRUS-Bx). This procedure has become a gold standard for the diagnosis of prostate cancer. Due to the fact, that the transrectal approach for prostate biopsy may promote contamination by the intestinal flora, consequently leading to frequent infectious complications, preventive actions are necessary [5,6]. According to the European Association of Urology (EAU) Guidelines, fluoroquinolones are routinely used to prevent bacterial infections in patients undergoing TRUS-Bx [7]. Levofloxacin (LVF) and ciprofloxacin (CPR) belong to the third- and the second-generation of quinolones, respectively. They exhibit activity against gram-positive and gram-negative bacteria and can be used in monotherapy or in combination with other drugs. They are both considered as drugs of excellent bioavailability after oral administration and distribute widely to peripheral tissues including the prostate. Therefore, they are commonly used in clinical practice [8–11] . Unfortunately, according to recent studies, fluoroquinolone-resistant bacterial strains contribute to gradually increased number of infections after TRUS-Bx [12,13]. In every day clinical practice, antibacterial efficacy is assessed only *in vitro*, by measuring in culture media the bacterial susceptibility to an antimicrobial agent, estimated by MIC. This approach has no relation to the treated tissue characteristics and may be insufficient. For this reason, in our study, the effectiveness of fluoroquinolone-based prophylaxis implemented during TRUS-Bx procedure was evaluated by measuring LVF concentrations in the plasma and prostate tissue of patients.

Chromatographic method development and validation involve application of different optimization strategies. In case when only one factor is optimized, a simple univariate procedure can be utilized. In the traditional univariate approach, one-variable-at-a-time (OVAT) procedure, the effect of the studied factors on the response is measured by changing the level of one factor while keeping the other factors at nominal levels [14]. Small experimental domain around the nominal levels, which is explored during the study, and large number of experimental analyses with no investigation of interactions between factors, are the main disadvantages of the OVAT procedure. Therefore, the OVAT approach, despite its common use during chromatographic development and validation study, will not be discussed in this article. On the other hand, in Design of Experiments (DoE), the effect of each factor is measured simultaneously in combination with other factors via a multivariate approach. Therefore, all interactions between factors are taken into account and the reported factor effects are an average value for the whole domain, representing the experimental design space more completely [15].

In our study, several statistical DoE methods, such as Box-Behnken design (BBD), Fractional Factorial design (FFD), Central Composite design (CCD) and Plackett-Burman design (PBD) were applied for chromatographic method development and robustness testing.

BBD can be used for the optimization of the separation step of chromatographic methods in pharmaceutical analysis. However, its application in analytical chemistry is still rare [16]. FFD can be applied for screening of important factors in sample preparation procedure optimization, however, this was reported in just few research studies [17–19]. FFD is very efficient for reducing the number of experimental analyses and hence, particularly favorable, when there exist limitations in the number of experiments that can be performed, for example due to high cost or small amount of samples [14] . CCD delivers high quality predictions in studying linear, quadratic and interaction effect factors which influence a system, while interactions are unobserved in the normal orthogonal design and single factor tests. Moreover, CCD belongs to so called subset designs, which are considered as designs robust to missing observations. CCD might be applied in situations, in which some observations are missing due to some accidents or cost constraints, as this design performs well in case of loss of one or two observations, and parameters of the assumed model can still be estimated without much loss of efficiency [20].

One of the numerous applications of DoE is an assessment of method robustness. It evaluates an influence of small changes in parameter values around a specified set of conditions. A robust method does not require a rigorous control over method parameters in order to operate as designed and planned [14,15]. PBD allows to screen a relatively large number of factors in rather small number of experimental analyses. PBD is a good choice for robustness testing, as interaction effects are assumed to be negligible and only main effects are estimated [14].

In recent years several specific and sensitive chromatographic methods have been developed to determine LVF in human plasma [21–42]. Such described methods include traditional techniques like high performance liquid chromatography with UV detection [21–23,27–30,32,34,36,41] and high performance liquid chromatography with fluorescence (FL) detection [24,26,31,33,37,40,42] as well as new techniques as high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) [25,38,39], ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS) [35]. Not all of these assays are appropriate for rapid pharmaceutical analysis of LVF in clinical and research practice. MS technique is not widely available for many laboratories because of the high cost of the equipment, reagents and analyses.

To date, only one analytical method has been reported for the determination of LVF in human plasma using reversed phase ultra-high performance liquid chromatography (UHPLC) with ultraviolet (UV) detection [43]. However, to our best knowledge, there has been no reported UHPLC-UV method for LVF analysis in human