Clinical Biochemistry 49 (2016) 587–595

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Multiple response optimization of a liquid chromatographic method for determination of fluoroquinolone and nitroimidazole antimicrobials in serum and urine

Sai Sandeep Mannemala, Valliappan Kannappan *

Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar, Tamil Nadu 608002, India

ARTICLE INFO

Article history:
Received 30 September 2015
Received in revised form 16 December 2015
Accepted 19 December 2015
Available online 14 January 2016

Keywords:
High performance liquid chromatography
Fluoroquinolones
Design of experiments
Nitroimidazoles
Antimicrobial bioanalysis

ABSTRACT

Objectives: Development and validation of a sensitive, selective and robust SPE assisted HPLC method for the quantification of fluoroquinolones and nitroimidazoles in human serum and urine using design of experiments methodology.

Design and methods: Design of experiments was employed for method optimization (Box–Behnken design) and robustness testing (Plackett–Burman design). Sample preparation involved a simple solid phase extraction, which offered a satisfactory recovery (≥94%). Analytes were separated on a phenyl hexyl column with mobile phase comprising water, acetonitrile and triethyl amine in ratio of 74:26:0.15 *v*/*v*, with a flow rate of 1.1 mL/min.

Results: Calibration curves were linear over selected range (≥0.995) for all the analytes. The method was sensitive with detection limits of 0.06–0.16 μg/mL in serum and urine samples. Inter and intra-day precision data (in terms of %RSD) was found to be less than 7%. Stability studies were carried out to assess freeze thaw, short term and long term stability and all analytes were found to be stable. The method was successfully applied for determination of antimicrobial drugs in spiked serum and urine.

Conclusion: The obtained results corroborated the potential of the proposed method for determination of all the four antimicrobial drugs in therapeutic drug monitoring, bioequivalence and drug–drug interaction studies.

1. Introduction

Discovery and development of antimicrobials revolutionized the 20th century medicine, along with vaccination it led to near eradication of infective diseases like tetanus, diphtheria and tuberculosis [1]. Although, antimicrobials are safe to use, the success in the treatment of infectious diseases has led to a new public health challenge — the emergence of microorganisms that are resistant to standard antimicrobial therapy. For higher efficacy and better patient compliance, fixed dose combinations of fluoroquinolones (FQs) with nitroimidazoles (NIMs) are widely prescribed [2]. In India, the most popular combinations of FQs and NIMs are ofloxacin (OFL) or norfloxacin (NOR) with ornidazole (ORN) or tinidazole (TIN) [2]. The combination therapy also demands selective and sensitive chromatographic techniques for estimation of these drugs in biological fluids (serum and urine) from pharmacokinetic and therapeutic drug monitoring perspectives. Therefore, it is essential to devise an analytical strategy for bioanalysis of these drugs. Hence, it is important that the developed chromatographic methods are well characterized, fully validated in terms of sensitivity, selectivity, accuracy, precision and documented to a satisfactory standard, in order to yield reliable results.

OFL, (Fig. 1a) designated as [(±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyridol (1,2,3,-de)-1,4-benzoxazine-6-carboxylic acid] and NOR, (Fig. 1b) designated as [1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid] belongs to second generation fluoroquinolone antibiotics. They act by inhibiting the bacterial DNA gyrase or the topoisomerase IV enzyme, thus preventing DNA replication and transcription, resulting in rapid bacterial death. ORN, (Fig. 1c) designated as [1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol] and TIN, (Fig. 1d) designated as [1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole] belong to the class of nitroimidazole antibiotics, used in treatment of bacterial and protozoan infections.

In recent years, there have been many reports on individual determination of selected FQs [3–9] and NIMs [10–13] in pharmaceuticals

Abbreviations: HPLC, high performance liquid chromatography; SPE, solid phase extraction; BBD, Box–Behnken design; PBD, Plackett–Burman design; RSD, relative standard deviation; OFL, ofloxacin; NOR, norfloxacin; ORN, ornidazole; TIN, tinidazole; QC, quality control.

* Corresponding author.
E-mail addresses: reddysince1989@gmail.com (S.S. Mannemala), vallikonnect@gmail.com (V. Kannappan).

http://dx.doi.org/10.1016/j.clinbiochem.2015.12.013
0009-9120/

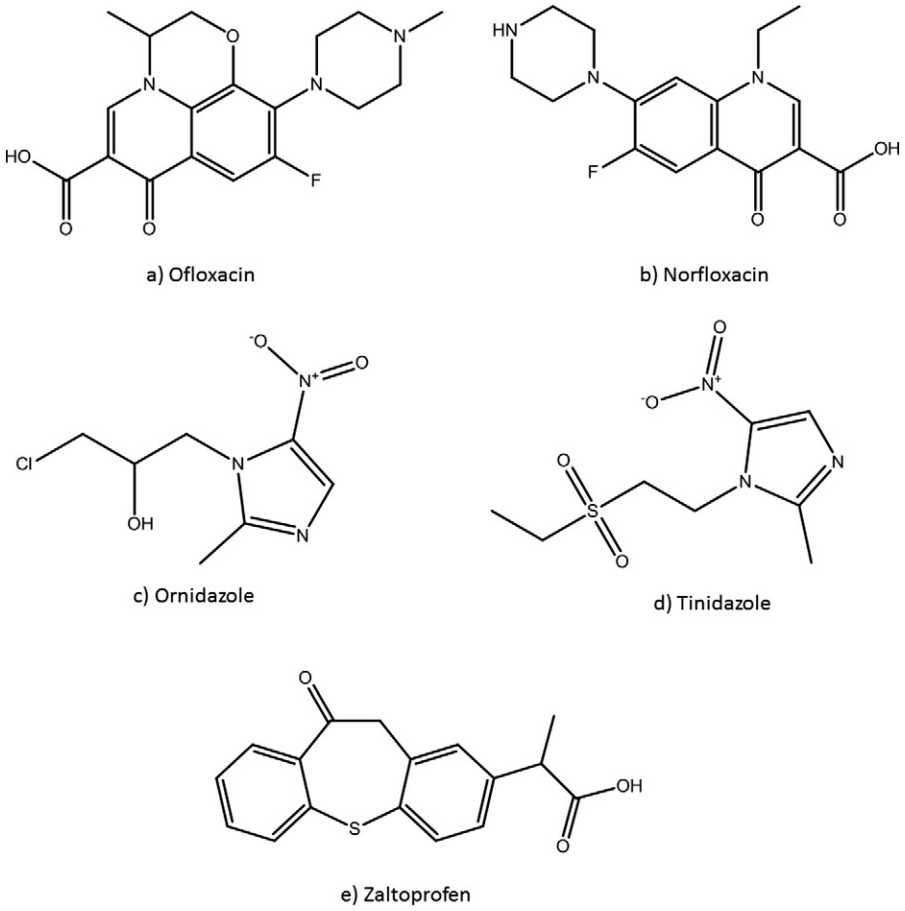

Fig. 1. Chemical structures corresponding to four antimicrobials under study and internal standard.

and biological matrices. Additionally, many liquid chromatographic methods are reported for the simultaneous determination of OFL and ORN [14–16]; OFL and TIN [17–19]; NOR and TIN [20–23]. Furthermore, most of these methods suffer from limitations such as poor retention (FQs), complicated procedure, expensive instrumentation and low detection capability for estimation in biological matrices.

However, an intense literature search revealed some bioanalytical methods available for determination of OFL, NOR and TIN. Kobayashi et al. [24] developed a method for simultaneous determination of NOR and enoxacin in human serum and urine. Although, the method employs simpler mobile phase system, the method quantifies only NOR in serum and urine and showed high retention time (10 min). Samanidou et al. [7] reported a method for determination of four fluoroquinolones in blood serum by HPLC. The method suffers from limitation of poor resolution between NOR and OFL ($Rs < 1.5$). Further, the reported method quantifies only two analytes understudy (OFL and NOR). Rizk et al. [20] reported a micellar liquid chromatographic method for simultaneous determination of NOR and TIN in human plasma. The method suffers from weakness like using complex mobile phase system and low sensitivity (LOD ≥ 5 μg mL^{-1}), which may limit the routine usage of this method for clinical studies. In their study, Helmy S.A. [21] proposed a HPLC method for simultaneous determination of NOR and TIN in human plasma. Although, the method employs simpler mobile phase, the method suffers limitation of poor retention behavior of NOR. Abou-Taleb et al. [23] reported a micro-emulsion liquid chromatographic method for determination of NOR and TIN in plasma and formulation. Although, the method employs design of experiments approach, it employs usage of complex mobile phase which makes the method impractical for routine use.

Owing to the poor retention of the quinolones or higher detection limits of analytes in the reported methods, the simultaneous estimation of selected antimicrobial drugs in biological matrices is not possible. Together, these reported methods emphasize the demand of a liquid chromatographic method which is selective, sensitive, short and precise as well as economic for simultaneous quantification of all aforementioned antimicrobials in serum and urine.

Nevertheless, to the best of our knowledge, there seems to be no HPLC method available that can simultaneously quantify all the aforementioned antimicrobials to date in serum and urine. On the other hand, except for the method proposed by Abou-Taleb et al., none of other reported methods employed systematic optimization approach for separation of antimicrobial drugs. Systematic optimization involves utilization of chemometric tools like design of experiments (DoE) to assess the effects of various factors on quality of chromatographic separation. In DoE, Box–Behnken designs (BBD) belong to class of rotatable or nearly rotatable second-order designs based on three-level incomplete factorial designs. In this work, a three level (BBD) was used. BBD's are proved to be more efficient than central composite design and full factorial design, when the researcher knows safe operating zone for the process [25]. Usually, it is almost uncommon that one patient would be prescribed more than two of the antimicrobial drugs at once, but the developed method offers advantage of providing suitability for analyzing four combinations of antimicrobials for therapeutic drug monitoring, bioequivalence studies and drug–drug interaction studies.

The current study describes optimization and validation of a rapid, sensitive, and cost-effective SPE-HPLC-PDA method for simultaneous estimation of OFL, NOR, ORN and TIN in human serum and urine.