Journal of Pharmaceutical and Biomedical Analysis 85 (2013) 83–92

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Application of an innovative design space optimization strategy to the development of LC methods for the simultaneous screening of antibiotics to combat poor quality medicines

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ARTICLE INFO

Article history:
Received 9 May 2013
Received in revised form 27 June 2013
Accepted 30 June 2013
Available online 12 July 2013

Keywords:
Antibiotics and associated substances
Poor quality medicines
Design of experiments
Design space
Method transfer
Accuracy profile

ABSTRACT

The poor quality of medicines is a crucial problem of public health. Therefore, it is important to have analytical tools to attend decisions of the legal authorities while combating this offense. In this context, the main objective of this study was to develop generic methods able to trace, screen and determine several antibiotics and common associated molecules by mean of liquid chromatographic techniques. For that purpose, an innovative Design Space optimization strategy was applied, targeting 16 antibiotics and 3 beta-lactamase inhibitors. The robustness of the developed method allowed using its use in an environment where operational factors such as temperature are not easy to control and eased its transfer to Ultra High Performance Liquid Chromatography. To demonstrate its ability to quantify the targeted molecules, the developed and transferred method was fully validated for two active ingredients commonly used in association, sulbactam and ceftriaxone, using the accuracy profile as decision tool. Based on this successful step, the method was then used for the quantitative determination of these two active ingredients in three pharmaceutical brands marketed in the Democratic Republic of Congo. Two out of the three pharmaceutical products did not comply with the specifications.

1. Introduction

The manufacture and the sale of poor quality medicines increase worldwide, causing serious consequences to the public health and to the socio-economy. Although, precise and detailed data on such medicines is not easy to obtain, one can find information of their trade that is ranging from 1% in the developed countries to over 10% in the developing countries, depending on the geographical area and on the period of survey [1,2]. Poor quality medicines can be classified into three main categories: counterfeit (falsifying with an intention to avoid the right of intellectual property), substandard (poor quality control during manufacture due mainly to neglecting without any intention) and degraded (chemical and biological instabilities especially in tropical climates) [3].

Counterfeiters are very active in developing countries where medicines are largely used such as antibiotics and antiparasitics [4]. According to the literature, a wide range of antibacterial agents have been found to be substandard or counterfeit [4]. Although no part of the world is exempted, Southeast Asia and Africa seem to be particularly plagued by poor quality antibacterial agents [4–12].

Some causes of the large diffusion of pharmaceutical counterfeiting in developing countries are lack of controls at importation and insufficient quality control of medicinal products at different levels of the distribution chain including import, wholesalers, official and informal vendors [1]. To ensure the quality of medicines and contribute in fighting against poor quality medicines, the development of screening analytical methods that can simultaneously trace several of the most commonly used molecules is an essential analytical strategy [13]. In this context, the separative technique stays as one of the best options to analyze simultaneously several molecules. Over the last decade, several liquid chromatographic (LC) methods were developed and published for the concurrent screening of potentially counterfeit medicines [1,13–17]. However, none of these includes an exhaustive list of the antibiotics molecules, limiting their use when screening complex or unknown

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http://dx.doi.org/10.1016/j.jpba.2013.06.036

mixture of this pharmacological group. For instance, M.C. Gaudiano et al. (2008), optimized a LC method for the separation of six antibiotics regardless the major pharmaceutical products such as ceftriaxone, ciprofloxacin, etc., or other main molecules often associated to betalactams such as sulbactam, tazobactam, etc. [1]. In the present study, we focused on LC techniques targeting a subset of 19 molecules that are marketed as single or combined antibiotics. Thus, our objective was to optimize the separation conditions for 19 of these molecules among which 16 are antibiotics: amoxicillin (AMO), ampicillin (AMP), cefadroxil (CFA), cefotaxime (CFO), ceftriaxone (CFT), chloramphenicol (CHL), ciprofloxacin (CIP), clindamycin (CLI), doxycycline (DOX), levofloxacin (LEV), metronidazole (MET), norfloxacin (NOR), phenoxymethylpenicillium (PENI-V), sulfamethoxazole (SLF), tetracycline (TET) and trimethoprim (TRI). The remaining molecules, clavulanic acid (CLA), sulbactam (SUL) and tazobactam (TAZ) are beta-lactamase inhibitors, often associated with β-lactams antibiotics.

Nowadays, LC method development can be achieved using different methodologies. In this study, a distinct and innovative methodology combining design of experiments (DoE) and design space (DS) as suggested in ICH Q8(R2) [18,19] was exploited to simultaneously optimize the separation and evaluate the method robustness over the examined experimental domain (i.e. the knowledge space).

As a second objective, the HPLC method developed was transferred to Ultra High Performance Liquid Chromatography (UHPLC) by means of a geometric transfer in order to verify that the DoE-DS strategy can ease the development of robust fast analytical methods.

The third objective was to validate the transferred UHPLC method using the accuracy profile as decision tool for the determination of the tested compounds [20,21]. For that purpose, an antibiotic association containing ceftriaxone and sulbactam powders for injection (intramuscular and intravenous) and marketed in some African countries was used.

Finally, the validated method was used to analyze several drugs often targeted by counterfeit marketed in the Democratic Republic of Congo (DRC).

2. Experimental

2.1. Materials

Cefotaxime (94.3%), ceftriaxone (93.9%), clavulanic acid (43.3%, a mixture of potassium clavulanate and microcrystalline cellulose as excipient (1:1)), levofloxacin (99.0%), norfloxacin (99.1%), sulbactam (91.5%) and tazobactam (99.2%) were purchased from Molekula Limited (Dorset, UK). Amoxicilline (99.1%), ciprofloxacin (>98%), clindamycine (95.8%), doxycycline (97.6%), metronidazole (99.9%), penicillin-V (100.2%), sulfamethoxazole (99.9%), tetracycline (96.6%) and trimethoprime (99.2%) were purchased from Fagron N.V. (Waregem, Belgium). Ammonium acetate (98.0%), ammonium hydroxide (32%), hydrochloric acid (37%), methanol (HPLC gradient grade) and sodium chloride (>99.5%) were purchased from Merck (Darmstadt, Germany). Ammonium formate (98.1%) and ammonium hydrogen carbonate (97.5%) were purchased from BDH Prolabo (Almere, The Netherlands). Ampicillin (98.0%) was purchased from Applichem Biochemica (Darmstadt, Germany). Cefadroxil (97.0%) was purchased from DR. Ehrenstorfer Gmbh (Augsbourg, Germany). Chloramphenicol (99.2%) was purchased from N.V LEPETIT BELGILA S.A (Brussels, Belgium). Ultrapure water was obtained from a Milli-Q Plus 185 water purification system (Millipore, Billerica, MA, USA). For the preparation of validation standards, a matrix formulation of powder for injection containing 1000 mg of ceftriaxone, 500 mg of sulbactam and 170 mg of sodium chloride was provided by an Indian manufacturing laboratory legally authorized in the DRC.

2.2. Standard sample preparation

2.2.1. Mixture preparation groups

Different mixtures of antibiotics were prepared as following:

Group 1 (see section 3.1): In a first step, 10 mg of AMO, CFT, MET, SLF, TRI, PENI-V and LEV, 40 mg of CIP, CHL and DOX, 30 mg of SUL and AMP, were dissolved in a 10.0 mL volumetric flask with methanol. This solution was annotated S1. In a second step, 70 mg of CLI were dissolved in a 10.0 mL volumetric flask with 1 mL of solution S1 and with methanol that was used to complete to volume. This last antibiotic was used at higher concentration level due to its weak absorptivity in the UV range. The final solution obtained was diluted twice (2.5 mL/5.0 mL) in a mixture of water and methanol (92%/8%, v/v) prior to injection at the HPLC system, and was diluted tenth (1.0 mL/10.0 mL) in the same solvent, prior to injection at the UHPLC system. Before analysis, an aliquot of each solution was filtered with 0.20 μm PTFE syringe filtration disks into a vial for injection in the HPLC and UHPLC systems.

Group 2 (see section 3.1): In a first step, 10 mg of TAZ, CFT, MET, CFO, NOR, TET, PENI-V and LEV, 40 mg of CHL and DOX, 30 mg of SUL and AMP, were dissolved in a 10.0 mL volumetric flask with methanol. This solution was annotated S2. In the second step, 70 mg of CLI and 40 mg of CLA were dissolved in a 10.0 mL volumetric flask with 1 mL of solution S2 and completed to volume with methanol. Prior to their use to the HPLC and UHPLC systems, the finals solutions were prepared as for group 1, dilution twice and dilution tenth, respectively, followed by filtration with 0.20 μm PTFE syringe filtration disks.

Group 3 (see section 3.1) was prepared as group 1, replacing amoxicillin by cefadroxil.

All these solutions were prepared sheltered from light to avoid degradation of light-sensitive antibiotics. The ultrasonic bath was necessary to ensure a complete dissolution.

2.2.2. Solution used for calibration and validation

A stock solution containing CFT and SUL was prepared by dissolving 100 mg and 50 mg, respectively, in 100 mL water. Another stock solution was prepared by dissolving 100 mg of sodium chloride in 100 mL of water.

For the calibration standards (CS), dilutions were performed in water in order to obtain solutions at three concentration levels of 160 μg/mL, 320 μg/mL and 480 μg/mL for CFT and the corresponding concentration levels of 80 μg/mL, 160 μg/mL and 240 μg/mL for SUL.

For validation standards (VS), independent stock solutions of CFT and SUL were prepared in the same way as described for the CS. For the matrix, the same sodium chloride solution was added into each working solution to obtain an amount of sodium chloride of 17% relative to the amount of CFT. Subsequent dilutions in water were carried out in order to obtain solutions at five different concentration levels namely 160 μg/mL, 240 μg/mL, 320 μg/mL, 400 μg/mL and 480 μg/mL of CFT, and 80 μg/mL, 120 μg/mL, 160 μg/mL, 200 μg/mL and 240 μg/mL of SUL. The VS were independently prepared in the matrix, in such a way to simulate as much as possible the corresponding antibiotic formulation and its routine analysis.

2.3. Instrumentation and chromatographic conditions

The optimization was performed on a HPLC system comprised of a Waters 2695 separation module coupled to a Waters selector valve 7678 and a Waters 996 Photodiode array (PDA) detector