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Development of a fast high performance liquid chromatographic screening system for eight antidiabetic drugs by an improved methodology of *in-silico* robustness simulation

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ABSTRACT

Robustness of RP-HPLC methods is a crucial method quality attribute which has gained an increasing interest throughout the efforts to apply quality by design concepts in analytical methodology. Improvement to design space modeling approaches to represent method robustness was the goal of many previous works. Modeling of design spaces regarding to method robustness fulfils quality by design essence of ensuring method validity throughout the design space. The current work aimed to describe an improvement to robustness modeling of design spaces in context of RP-HPLC method development for screening of eight antidiabetic drugs. The described improvement consisted of *in-silico* simulation of practical robustness testing procedures thus had the advantage of modeling design spaces with higher confidence in estimated of method robustness. The proposed *in-silico* robustness test was performed as a full factorial design of simulated method conditions deliberate shifts for each predicted point in knowledge space with modeling error propagation. Design space was then calculated as zones exceeding a threshold probability to pass the simulated robustness testing. Potential design spaces were mapped for three different stationary phases as a function of gradient elution parameters, pH and ternary solvent ratio. A robust and fast separation for the eight compounds within less than 6 min was selected and confirmed through experimental robustness testing. The effectiveness of this approach regarding definition of design spaces with ensured robustness and desired objectives was demonstrated.

1. Introduction

Since the introduction of quality by design (QbD) concepts into the pharmaceutical manufacturing and development, there have been many discussions about the opportunities for analytical method development to follow a QbD based approach similar to that described by ICH Q8(R2). In QbD frameworks, a systematic approach is followed that begins with predefinition of objectives and implements sound science and risk assessment [1]. The main outcome of this approach is to obtain a robust method over wider ranges of conditions with fewer routine application problems [2,3].

Design space (DS) is a key element of QbD frameworks. In QbD based HPLC method development, DS is the combination of chromatographic factors ranges which achieved the desired levels of method critical quality attributes (CQAs) [1,4]. Analytical methods CQAs are the response variables which provide the mathematical representation of the method quality such as resolution, minimum or maximum retention time limits. In the light of QbD concepts, DS can be regarded as a zone of theoretical robustness as changes to method parameters within the DS will not impact method CQAs. Robustness could be defined as the ability of the method to retain its quality criteria significantly unaffected upon small deliberate shifts of method conditions [5].

Fulfillment of robustness is crucial for DS modeling to gain the DS benefit of ensuring validity of any point within the DS [1]. Subsequently, different approaches for robustness incorporation in the DSs modeling were proposed. Some of these approaches expressed robustness as the probability of the method to pass predefined acceptance criteria after propagation of the modeling error to the predicted CQAs [4,6]. Some reports proposed the propagation of both of modeling error and the method conditions implementation error to the predicted CQAs [7,8]. In the later work, modeling error was emphasized as the major source of uncertainty upon estimation of simulated robustness [8].

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However, the aforementioned approaches differed from the commonly applied procedures for robustness testing in the light of ICH Q2 (R1) guideline in which a full factorial design of small deliberate shifts in method conditions around the tested point was performed. Furthermore, these deliberate shifts differed in magnitude and distribution from those obtained by simulated method implementation error described in previous literature. Therefore, the DSs obtained from these approaches may not reflect method robustness from the practical aspects [9].

Recently, a simulation of full-factorial experimental design for robustness testing of a selected point was evaluated [10–12]. Robustness of the selected point was calculated as the probability to pass a minimum resolution threshold. However, this approach was applied to a selected point within the potential DS rather than the whole DS mapping. Furthermore, modeling error uncertainty was not considered despite its significant impact of on the accuracy of modeled quality criteria [13].

Consequently, an extension of the robustness simulation application for potential DSs mapping with modeling error propagation will have a significant added value throughout the continuous endeavors for improving method robustness in the modeled DSs.

Numerous screening systems for multiple drugs were reported. Development of a single HPLC screening system of many drugs has the advantage over screening each compound with its specific HPLC system regarding saving time and resources. HPLC methods were preferred over more expensive techniques such as near IR or Raman spectroscopy [14]. HPLC screening systems have been applied in biological and environmental analysis as well as counterfeit medicines monitoring which is a global problem that affects either the developing countries in Africa, Asia and Latin America [15–17] especially for life saving medicines or in Europe and North America especially for lifestyle medicines [18].

Robustness of HPLC screening methods is crucial especially upon their transfer between different laboratories. To date, traditional strategies of HPLC method development do not ensure method robustness during method development and selection [19]. Only few reported HPLC screening systems in literature applied the concepts of QbD based method development such as for antimalarial drugs [20], nonsteroidal anti-inflammatory drugs [21] and antibiotics [6].

Antidiabetic drugs included a wide range of compounds with diverse potencies and cost. For example, glyburide is a cheap drug while repaglinide and pioglitazone were relatively expensive. Anticounterfeit screening HPLC systems therefore were required to detect counterfeiting expensive APIs with cheaper ones [14].

A previous example for separation of six antidiabetic drugs was provided by Yao et al. [14]. Another determination of five antidiabetics in formulation and plasma was described [22]. In addition, a screening system for identification of nine antidiabetic drugs with a run time of 46 min was reported [23]. Another isocratic system was described to separate four antidiabetics within 8 min [24].

The aim of this work was to describe an improved approach that ensures more confidence of produced DS regarding robustness estimation and avoids drawbacks of the previous approaches. This improved approach modeled robustness through simulation of the actual practice of robustness testing in accordance to robustness definition in ICH Q2 (R1) guideline. The basic concept of this work was that it is more logic to map robustness by simulation of its actually applied testing procedure within the validation study. This approach yielded DSs which better described robustness and thus fulfilled the basic QbD concept of retaining the method validity and robustness at any conditions combination within the described DS.

Accordingly, this approach consisted of simulation of robustness testing by simulated full factorial design of small deliberate shifts around each point in the modeled subspace. Modeling error was propagated to the simulated results before calculation of CQAs. Robustness of each point in potential DS was estimated as the probability of the method CQAs to pass the predefined acceptance criteria upon robustness simulation. These probability values were mapped as a function of the chromatographic conditions. A previous study compared the DS produced by simulated robustness with the previous approaches [9]. This study concluded that the previously reported modeling approaches produced significant subspaces on boundaries of their DSs which did not guarantee method robustness in practical sense as compared with the proposed simulated robustness procedure [9].

The approach described in this work was applied in context of QbD based development of a fast screening HPLC method of ensured robustness for separation of eight antidiabetic compounds (Fig. 1); pioglitazone (PZ), rosiglitazone (RZ), glyburide (GB), glimepiride (GM), gliquidone (GQ), gliclazide (GL), glipizide (GP), and repaglinide (RG). Three different stationary phases were experimented to explore more selectivity options for this separation. The value of simulated robustness modeling approach regarding obtaining DSs and methods with required quality criteria and ensured robustness was demonstrated and evaluated.

2. Experimental

2.1. Materials

Acetonitrile and methanol were obtained from CDH (Newdelhi, India). Working reference materials of RZ was kindly provided from Biopharm pharmaceuticals (Cairo, Egypt), PZ (hydrochloride salt), GM and GL from Medical union pharmaceuticals Co. (Ismailia, Egypt), GQ from Minapharm pharmaceuticals (Cairo. Egypt), GB from Sigma pharmaceuticals (Quesna, Egypt), GP from Chemical industries development; CID (Giza, Egypt) and RG from Amoun Pharmaceutical Co. (Cairo, Egypt).

A sample from the pharmaceutical product, Glimepiride plus®, in tablet containing 30 mg PZ and 4 mg GM (Al-Andalous Medical Company (Cairo, Egypt) was purchased from local market and employed as a model for method validation.

2.2. HPLC system and chromatographic conditions

A Shimadzu Class-VP HPLC system (Shimadzu Corporation, Tokyo, Japan) was used through the study. The system included two binary pumps with a high pressure gradient mixer (mixing volume = 0.5 ml), a Rheodyne manual injector with 5 μl loop and a variable wavelength spectrophotometric detector. Column temperature was controlled by a thermostated column compartment, Replete RPL-D2000 (Dalian Replete Science and Technology, Dalian, China).

Three chromatographic columns were used in this study; Zorbax SB-C8 150 mm × 4.6 mm 5 μm (Agilent technologies, Santa Clara, CA, USA), Luna C18(2) 150 mm × 4.6 mm 5 μm (Phenomenex,Torrance, CA, USA) and Nucleodur C18ec 75 mm × 4.6 mm 3 μm (Macherey-NagelGmbH & Co. KG,Düren, Germany).

All initial experiments were conducted in gradient elution mode with flow rate of 2 ml/min except for Nucleodur column as flow rate was adjusted to be 1.5 ml/min. Column temperature was fixed at 40 °C. Detection wavelength was performed using dual wavelength detection mode at 230 and 270 nm. A degassed filtered solution of a buffer mixture of 20 mM sodium dihydrogen phosphate–20 mM sodium acetate was adjusted at different pH values and was used as the mobile phase aqueous solution. Methanol, acetonitrile or blends of both solvents at different ratios were used as the mobile phase organic portions.