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Evaluation of the quantitative performances of supercritical fluid chromatography: From method development to validation

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ABSTRACT

Recently, the number of papers about SFC increased drastically but scientists did not truly focus their work on quantitative performances of this technique. In order to prove the potential of UHPSFC, the present work discussed about the different steps of the analytical life cycle of a method: from development to validation and application. Moreover, the UHPSFC quantitative performances were evaluated in comparison with UHPLC, which is the main technique used for quality control in the pharmaceutical industry and then could be considered as a reference. The methods were developed using Design Space strategy, leading to the optimization of robust method. In this context, when the Design Space optimization shows guarantee of quality, no more robustness study is required prior to the validation. Then, the methods were geometrically transferred in order to reduce the analysis time. The UHPSFC and UHPLC methods were validated based on the total error approach using accuracy profile. Even if UHPLC showed better precision and sensitivity, UHPSFC method is able to give accurate results in a dosing range larger than the 80–120% range required by the European Medicines Agency. Consequently, UHPSFC results are valid and could be used for the control of active substance in a finished pharmaceutical product. Finally, UHPSFC validated method was used to analyse real samples and gave similar results than the reference method (UHPLC).

1. Introduction

Supercritical fluid chromatography (SFC) is an old technique hidden in the shadow of gas chromatography (GC) and liquid chromatography (LC) for almost fifty years [1,2]. Recently, the interest of manufacturers and scientists for SFC increased leading to the development and the improvement of the SFC instrumentation. Moreover, the involvement of SFC in the worldwide effort for green chemistry helped to its success. Ultra high performance supercritical fluid chromatography (UHPSFC) is now presented as a really powerful technique complementary to GC and LC.

Nowadays, the advantages and interests of SFC, such as high throughput or improved chromatographic performances, are worldwide approved. Although the number of publications increased significantly in recent years, most scientists did not truly consider (UHP)SFC as a quantitative method but more for fundamental studies and chiral applications. Moreover, few publications described validation process of SFC method. The validation of chiral separation of a drug compound was previously described [3] assessing several validation criteria such as selectivity, linearity and precision. Unfortunately, surprising results (i.e. intraday repeatability with relative standard deviation (RSD) value of 9%) were mentioned without explanation about acceptance criteria. Xiang et al. described the validation of nine chiral compounds considering repeatability and linearity [4]. Whang et al. described partial validation of SFC method, considering the selectivity as critical in order to demonstrate the orthogonality between two methods [5]. Only one publication presents the validation results SFC method for the quality control of medicines [6]. In the evaluation of the precision criterion, the intra-day RSD values were superior than the inter-day RSD for some concentration levels. Thus, these peculiar results cannot be considered as adequate indicators of the quantitative performances of the method. Moreover, the accuracy was tested using standards addition method; unfortunately, the dosing range investigated was not the same than the other criteria tested (precision and linearity). To our best of knowledge, a full validation considering total error approach of SFC method was not yet published.

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Validation is one of the main steps in analytical method life cycle. The aim of validation process is to demonstrate the analytical performances of the developed analytical method in accordance to its intended use. Validation of analytical methods is well established and described in the literature. Nevertheless, despite many regulatory documents (GMP, ISO, FDA, etc.) the conclusion about the method acceptance criteria remains confused. For that purpose, Hubert et al. proposed a common strategy for the validation of quantitative an analytical method [7–10], introducing the concept of total error approach as a decision tool. In the pharmaceutical industry, the validation of analytical procedure is required before its use in the quality control (QC) laboratory. The objective of the present work is to investigate the life cycle of a UHPSFC method from development to validation, including its application to real samples.

The concept of Quality by Design (QbD) is now well established in pharmaceutical development. The QbD is defined by ICH Q8 R2 [11] as "*a systematic approach to development that begins with predefined objectives and emphasises product and process understanding based on sound science and quality risk management*". Furthermore, the QbD concept was recently introduced in the field of analytical method development and validation [12,13]. Indeed, an analytical method can be seen as a process that must have an output of acceptable quality. Borman et al. [14] demonstrated that the QbD concept for manufacturing processes could also be applied to analytical methods. Design of Experiments (DoE) considering risk management by means of error propagation is considered as a keystone to optimize process in the QbD environment [15].

In this context, the Design Space (DS) was introduced as a key component of analytical method development [13,16]. The DS is defined as "*the multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality*" [1]. Thus, the DS is a subspace of the experimental domain in which the assurance of quality has been proved. As previously described [16], the Design Space could be defined as a region of an experimental domain – χ – where the posterior probability that the critical quality attributes (CQAs) are within acceptance criteria Λ, is higher than a specified quality level π, conditionally on the available data.

$$\mathrm{DS} = \{x_0 \in \chi : P(\mathrm{CQAs} \in \Lambda | x_0, \mathrm{data}\} \geq \pi \quad (1)$$

CQAs provide some indications about the overall achievement of the analytical method. In chromatography, CQAs may be the resolution (Rs) or the separation (S) of a critical pair of peaks, while the acceptance criteria Λ may be Rs > 1.5 and/or S > 0. In this context, a result given as a predictive probability that the CQAs will be within the acceptance interval establishes the assurance of quality. For chromatographic method development, the DS could be defined as the space of chromatographic conditions that will ensure the quality of the separation. Therefore, the method robustness is guaranteed inside the DS limits.

Method robustness should be evaluated prior or post validation step. Only a few examples of SFC robustness studies were published [17]. The interest of robust optimization strategy, especially for SFC methods development, was previously described [18,19]. USP requires robustness evaluation, before the initiation of method validation during the development/optimization step [20]. This important step is no more required if robust method optimization was performed and acceptable guarantee level π was found. Thus, DS strategy fulfils the USP recommendations and at the same time allows speeding up the analytical life cycle.

The main objective of this work is to investigate the interest of UHPSFC as a quantitative method used for the quality control of manufactured medicines. For that purpose, robust method optimization and validation QbD compliant were performed. Because of their weight in the current pharmaceutical therapy, antibiotics drugs were selected using the following model compounds: phenoxymethylpenicillin (penicillin V), doxycycline, levofloxacin, metronidazole, amoxicilline, trimethoprim and clindamycin. These drugs are frequently used in Democratic Republic of Congo (DRC) [21]. Counterfeiters are very active in developing countries, such as DRC, where medicines are largely used such as antibiotics and antiparasitics. In this context, the development of screening analytical methods that can simultaneously trace several molecules is an essential strategy in order to fight against poor quality medicines. Caffeine was included in the studied model mixture as system suitability compound. The studied compounds, their structures, pK_a and log P are shown in Table 1.

Nowadays, the most popular method used for the quality control in pharmaceutical industry is HPLC because of the wide range of compounds that could be analysed and the good quantitative performances of the technique. Previously, a HPLC method was developed for the screening of a wide range of antibiotic drugs [21] using DS strategy. This method was transferred to UHPLC in order to get a faster technique. UHPLC could be used as a reference quantitative technique to evaluate and compare the potential of UHPSFC in the field of quantitative analysis, considering the analytical performances (method validation) and the analysis of real samples.

2. Material and methods

2.1. Chemicals and reagents

Levofloxacine (99.0%) was purchased from Molekula Limited (Dorset, UK). Amoxicilline (99.1%), caffeine (100.1%) clindamycin (95.8%), doxycycline (97.6%), metronidazole (99.9%), penicillin-V (100.2%) and trimethoprim (99.2%) were provided by Fagron N.V. (Waregem, Belgium).

Methanol (HPLC gradient grade) was purchased from J.T. Baker (Deventer, Netherlands). 2-Propanol (HPLC gradient grade), n-heptane (HPLC grade), ammonium acetate (98.0%, analytical grade), hydrochloric acid (37%, analytical grade) and formic acid (98%, analytical grade) were obtained from Merck Millipore (Darmstadt, Germany). Carbon dioxide (99.995%) was purchased from Westfalen (Brussels, Belgium). Ultrapure water was obtained from a Milli-Q Plus 185 water purification system (Millipore, Billerica, MA, USA).

2.2. Standard samples preparation

2.2.1. Mixture preparation

According to their UV absorbance and solubility, the antibiotics were divided in three groups. All stock solutions were prepared in pure methanol. Stock solution of group 1 (S_1) was obtained by dissolving 40 mg of penicillin-V, 50 mg of doxycycline, 25 mg of levofloxacine and 20 mg of metronidazole in a volumetric flask of 5.0 mL. Solution stock of group 2 (S_2) was obtained by dissolving 10 mg of amoxicilline in a volumetric flask of 5.0 mL. Solution stock of group 3 (S_3) was obtained by dissolving 20 mg of caffeine and 25 mg of trimethoprim in a volumetric flask of 20.0 mL. All stock solutions were sonicated in an ultrasonic bath for 12 min to ensure a complete dissolution. Stock solutions were stored at −27 °C. Intermediate and working solution were prepared daily by dilution of stock solutions.

Intermediate solution (SI) was prepared by dissolving, and diluting in 2-propanol, 100 mg of clindamycin, 1.0 mL of S_1, 2.0 mL of S_2 and 1.0 mL of S_3 in a volumetric flask of 10.0 mL. Working solution was obtained by diluting SI twice in 2-propanol/n-heptane (50/50, v/v) mixture. Thus, the final sample diluent is a mixture methanol/2-propanol/n-heptane (20/55/25, v/v/v). This solvent