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Comparison of *core–shell* and totally porous ultra high performance liquid chromatographic stationary phases based on their selectivity towards alfuzosin compounds☆

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ABSTRACT

This paper focuses on the application of a column classification system based on the Katholieke Universiteit Leuven for the characterization of physicochemical properties of *core–shell* and ultra-high performance liquid chromatographic stationary phases, followed by the verification of the reliability of the obtained column classification in pharmaceutical practice. In the study, 7 stationary phases produced in *core–shell* technology and 18 ultra-high performance liquid chromatographic columns were chromatographically tested, and ranking lists were built on the F_{KUL}-values calculated against two selected reference columns. In the column performance test, an analysis of alfuzosin in the presence of related substances was carried out using the brands of the stationary phases with the highest ranking positions. Next, a system suitability test as described by the European Pharmacopoeia monograph was performed. Moreover, a study was also performed to achieve a purposeful shortening of the analysis time of the compounds of interest using the selected stationary phases. Finally, it was checked whether methods using *core–shell* and ultra-high performance liquid chromatographic columns can be an interesting alternative to the high-performance liquid chromatographic method for the analysis of alfuzosin in pharmaceutical practice.

1. Introduction

Over the past decades, high-performance liquid chromatography (HPLC) has become the dominant analytical technique, being used at every stage of production in the pharmaceutical industry, from the research of raw materials and intermediates to finished products [1]. It is also a valuable analytical tool used to determine active substances in various biological matrices, which is important in pharmacokinetic studies and real clinical applications [2]. Its rapid growth results not only from its capabilities of qualitative analysis and accurate and precise quantitative analysis of multi-component mixtures of chemical compounds, but it is also related to the evolution of the packing materials used to effect the separation, including also the fillings packed in *core–shell* technology (*CS*). An important factor is also the underlying principle of HPLC, which dictates that as the column packing particle size decreases, the efficiency, and thus the resolution increases [3,4]. As a result, many different HPLC stationary phases with even smaller particle diameters have been progressively introduced to the market. Further advances in chromatography have led to the creation of ultra-high performance liquid chromatography (UHPLC), where a substantial reduction of the dwell volume from approximately 1 to 2 mL to about 120 μL was achieved. Moreover, a stationary phase with a particle diameter of less than 2 microns as well as an inner diameter of the column within 2.0–2.1 mm are used [5]. It should be emphasized that UHPLC technology is perfectly suited for coupling with mass spectrometry. Of great importance is also the fact that due to the shorter duration of the analysis and lower reagent consumption, the UHPLC technique is more environmentally friendly and economically efficient [6,7]. In consequence, the UHPLC method can be an interesting alternative to HPLC methods for the pharmaceutical industry and clinical laboratories. On the other hand, the columns used in UHPLC technology can generate several times higher pressure in the chromatographic system than that achieved in HPLC systems, which requires an adaptation of HPLC methods to UHPLC conditions. It should be noted that an extremely important step during a classic transfer of HPLC

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methods to UHPLC conditions or during the development of new UHPLC methods is the selection of a suitable column to the specific pharmaceutical or biomedical applications. In the case of HPLC methods for this purpose, various column classification systems can be used like, *e.g.* the hydrophobic-subtraction model [8,9], quantitative structure-relation relationships (QSRRs) [10–15], the sum of ranking differences (SRD) [16,17], the Tanaka method [18,19] and its extended modifications [20–22], as well as others [23,24] whose credibility in pharmaceutical and clinical practice has been confirmed in many scientific reports. Among them, an interesting alternative is a system developed by researchers at the Katholieke Universiteit Leuven in Belgium (so called KUL) [25–28], which refers to columns with a C_{18} chain and is based on three well-defined procedures that allow for the appointment of the following parameters: the retention factor of amylbenzene (k'_{amb}) describing hydrophobicity, the relative retention factor of benzylamine/phenol at pH 2.7 ($rk'_{ba/ph\,pH\,2.7}$), which reflects silanol activity, the relative retention factor of triphenylene/o-terphenyl ($rk'_{tri/o-ter}$) indicating the possibility for steric selectivity, and the retention factor of 2,2′-dipyridyl ($k'_{2,2'-dip}$) evaluating silanol activity and metal impurities. As a result, in the KUL system all columns are described by the values of those parameters, and this in turn allows the determination of the parameter F_{KUL} which characterizes the similarity of these determined parameters with respect to the reference column. Therefore, firstly, four reference parameters corresponding to a chosen reference column or the selection of a defined reference column should be defined, and then on the basis of the following formula the value of the parameter F_{KUL} is determined:

$$F_{KUL} = (k'_{amb,ref} - k'_{amb,i})^2 + (rk'_{ba/ph\,pH\,2.7,ref} - rk'_{ba/ph\,pH\,2.7,i})^2 + (rk'_{tri/o-ter,ref} - rk'_{tri/o-ter,i})^2 + (k'_{2,2'-dip,ref} - k'_{2,2'-dip,i})^2 \quad (1)$$

where *ref*, reference column; *i*, tested column.

This greatly simplifies the ranking system of columns, because there is a relationship where the smaller value of the parameter F_{KUL} confirms that the stationary phase is more similar to the reference column. In the literature there are many reports which confirm that the KUL method is a useful analytical tool which facilitates the selection of the optimal conventional RPLC stationary phase for a specific chromatographic separation in pharmaceutical and clinical practice [29–35]. On the other hand, no KUL results are published describing the physicochemical properties of the columns produced in *core–shell* technology. Moreover, so far the KUL procedure could not be directly used for the testing of UHPLC stationary phases because of the excessive pressure increase in the UHPLC system.

The first aim of the present work was to characterize the *CS* columns with the KUL procedure, and next to evaluate the reliability of the obtained theoretical classification results in pharmaceutical practice. The second task of the study was an adaptation of the column classification system based on the KUL method to testing of UHPLC columns, and then the verification of the suitability of the adapted protocol of the KUL procedure in the real chromatographic separation of the selected active substance. Thus, an evaluation of column classification based on the KUL approach was performed using the analysis of alfuzosin and its impurities A and D (Fig. 1), where conventional HPLC methodology in accordance with the European Pharmacopoeia (Ph. Eur.) monograph [36] was optimized in terms of using *CS* and UHPLC columns. In this study, 7 *CS* columns and 18 UHPLC stationary phases were characterized by the KUL method, and next the F_{KUL}-values were calculated for the tested stationary phases relative to the Inertsil ODS2 (Inert) and XBridge Shield RP18 (XbridShield) columns

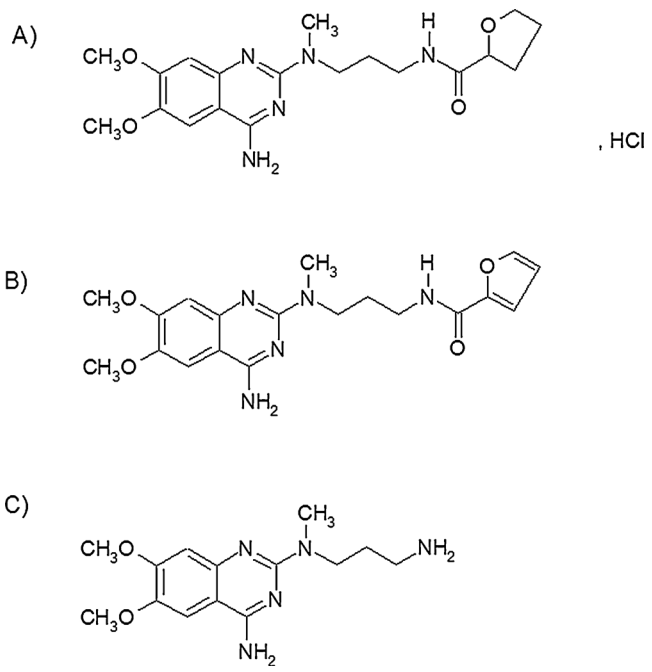

Fig. 1. Chemical structure of: (A) alfuzosin hydrochloride; (B) impurity A; and (C) impurity D.

as references. The Inert column having high hydrophobic character and hydrophobic selectivity was recommended by the Ph. Eur. for the HPLC analysis of alfuzosin in accordance with the knowledge database [37]. The inclusion of the XbridShield an embedded stationary phase having high polar selectivity as a reference was the result of our research reported in Ref. [34]. According to the published data, it was shown that this column offered much more effective separation of the parent substance and related compounds (SST = ∞) than the recommended Inert (SST = 23). Then, the verification test was performed on the basis of comparing the retention times (t_R), peak resolution (R_s), and the number of theoretical plates (N) for the test analytes, which clearly describe the run of each chromatographic analysis obtained for the examined stationary phases. Particular attention was paid to the results of the Ph. Eur. system suitability test (SST), which in the case of alfuzosin requires that the peak-to-valley (p/v) ratio designated for impurities A and the parent substance was at least 5.0. Moreover, to assess whether the optimized method for the analysis of alfuzosin and its impurities using *CS* and UHPLC columns could be an interesting alternative with respect to Ph. Eur., the HPLC method was also conducted.

2. Experimental

2.1. Reagents

The analytes used in the KUL method, such as amylbenzene, benzylamine, 2,2′-dipyridyl, *o*-terphenyl, triphenylene and uracil were supplied by Sigma–Aldrich (St. Louis, MO, USA), while phenol was supplied by POCH (Gliwice, Poland). *Alfuzosin for system suitability CRS* containing alfuzosin hydrochloride – (2RS)-N-[3-[(4-amino-6,7-dimethoxyquinazolin-2-yl) methylamino]propyl]tetrahydrofuran-2-carboxamide hydrochloride, impurity A – N-[3-[(4-amino-6,7-dimethoxyquinazolin-2-yl)methylamino]propyl]furan-2-carboxamide, and impurity D – N-[4-amino-6,7-dimethoxyquinazolin-2-yl)methylpropane-1,3-diamine was purchased from EDQM (Strasbourg, France). A standard solution from *alfuzosin for system suitability CRS* containing a parent substance at a concentration of 0.4 mg/mL and the