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A workflow for column interchangeability in liquid chromatography using modeling software and quality-by-design principles

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ABSTRACT

The goal of the present study was to develop a generic workflow to evaluate the chromatographic resolution in a large design space and easily find some replacement column for the method. To attain this objective from a limited number of initial experiments, modern LC modeling software (Drylab) was employed to study the behaviour of the compounds and visually compare the parts of design spaces obtained with different columns, where a given criterion of critical resolution is fullfilled. A zone of robust space can then easily be found by overlapping design spaces. By using 50 × 2.1 mm columns packed with sub–2 μm fully porous particles (UHPLC), the resolution in the entire design space can be modeled on the basis of only 2–3 h experimental work *per* column.

To demonstrate the applicability of the developed procedure, amlodipine and its related pharmacopeia impurities were selected as a case study. It was demonstrated that two columns from different providers (Waters Acquity HSS C18, Thermo Hypersil Gold C18) can be interchanged, providing a sufficient resolution at the same working point and a high degree of robustness around this condition.

1. Introduction

Nowadays thousands of liquid chromatographic columns are available. If only octadecyl (C18) phases are taken into account, then we have the possibility to choose from more than 500 products. On one hand, this can make the method development easier since the chromatographer can select the most suitable stationary phase for a given separation. On the other hand, it can be a heavy task to find an appropriate replacement (alternative) column, which provides a very similar separation as the original column. Today, it is indeed required to suggest an alternative column in pharmaceutical analytical laboratories, and to prove its equivalency during the method validation process. In fact, the pharmaceutical regulatory guidelines mention that method robustness has to be checked on columns from different batches and also on other manufacturer's column providing similar separation quality [1].

The column interchangeability in the U.S. Pharmacopeia Convention is quite straightforward. The liquid chromatography columns are classified in 'L' groups according to their chemical modification [2]. All the columns with C18 bonding belong to the L1 group, which is defined as: "octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 1.5–10 μm in diameter, or a monolithic rod". This definition is quite broad, since it contains all the phases with irregular silica particles, high metal ion content and low surface coverage as well as the widely used hybrid silica endcapped phases with high surface coverage, showing significant differences in retention properties. These phases are clearly not interchangeable and it can even occur that C18 and C8 ligands that are attached to the same silica particle show more similar retention properties than C18 ligands bonded to different silica particles [3].

To compare different reversed phase (RP) materials, various tests have been proposed in the literature [4–7]. Available databases are also based on these tests. The limitation of such tests is that they provide information only on a limited number of compounds, measured under "one constant set" particular conditions. Those tests cannot predict the applicability of columns for impurity profiling or assays, which are the most common applications in pharmaceutical analysis. One of the most popular databases is based on the "hydrophobic-subtraction model" proposed by Snyder and coworkers in the early 2000 s [8]. This model takes the hydrophobicity (*H*), hydrogen bond basicity (*B*), ionic interactions at two pH (*C(2.8)* and *C(7.0)*), hydrogen bond acidity (*A*) and steric selectivity (*S*) into account. These parameters are used for the calculation of similarity factor (*Fs*) between columns. $Fs < 3$ corresponds to an excellent selectivity similarity between the compared columns; between $3 < Fs < 5$, the selectivity similarity is moderate;

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between $5 < Fs < 10$, there is a questionable but still fair comparability of selectivity, and for $Fs > 10$, the selectivity is considered as different [9]. This approximation gives a scientific based comparison against the USP classification, but it does not consider peak shape and peak width, which are crucial for pharmaceutical impurity profiling.

Modern silica gels (Type B silica) have low metal ion content, and acidic pK_a value of surface silanol groups typically ranges between 3 and 5. The amount of residual silanol groups is around 8 μmol/m^2, half of which being covered after alkyl modification. The number of residual silanol groups can be further reduced by endcapping, but unreacted silanol groups are always present. Depending on the pH conditions, these acidic silanols can be ionized and are then able to offer strong electrostatic interaction with basic compounds which are also ionized. To demonstrate the differences between C18 phases, it is therefore important to select chromatographic conditions where the residual silanol groups are ionized and the sample should contain some basic, acidic and neutral-like compounds as well. This is for example the case when using a mobile phase pH comprised between 3 and 6 [10–12].

In previous studies, the simulated robustness testing, included within commercial modeling softwares, was systematically studied and compared to experimental measurements and DoE based predictions [13,14]. The reliability of this "early stage" simulated robustness approach was critically evaluated for real-life separations applying short narrow bore columns (50 × 2.1 mm) and fast separations. Moreover, as a continuation of robustness study, the column interchangeability was further investigated, using four different C18 columns packed with sub–2 μm particles. By properly varying the method variables, the separation was feasible on all columns within the same timescale (less than 4 min). This work demonstrates the accuracy of simulated robustness testing and shows that nearly the same quality of separation can be achieved on different stationary phases.

The novelty of the present work is the practical use of the recently introduced Column Comparison module in DryLab modeling software. In this module, various 3D resolution maps can be compared by overlapping two or more cubes, which can help studying the measured points – in a design space – of the different phases and find a common zone where the sample components are all separated with sufficient selectivity and resolution. For this illustration, amlodipine and related impurities have been selected as model samples. The pharmacopeia suggests a 60 min long conventional separation for amlodipine impurity profiling which has already been shortened drastically in our previous study [15].

2. Experimental

2.1. Chemicals, columns

The mobile phase used in this work was a mixture of acetonitrile and water buffered with 10 mM ammonium-acetate buffer. Acetonitrile (gradient grade), acetic acid, ammonium hydroxide and standard reference buffers (pH 2.00, 4.01 and 7.00) were purchased from Merck (Darmstadt, Germany). For the measurements, water was prepared freshly using ELGA Purelab UHQ water (ELGA, Lane End, UK).

Sample was prepared from amlodipine API (0.5 mg/mL) and spiked with all the impurities at 0.5% level. Amlodipine and its impurities [15] were purchased from European Directorate for the Quality of Medicines & HealthCare (EDQM, Strasbourg, France). Sample solvent was acetonitrile:water 30:70 (v/v).

The columns used in this study were selected on the basis of the following criteria: all of them should be based on porous silica gel (to neglect differences in morphology), with similar particle size (to have comparable specific surface area and efficiency). We focused on differences and effects of accessible free silanols.

The Acquity HSS C18 and HSS C18 SB columns (50 × 2.1 mm, 1.7 μm) were purchased from Waters (Milford, USA), Hypersil GOLD C18 column (50 × 2.1 mm, 1.9 μm) was purchased from Thermo Scientific (Waltham, USA), Titan C18 column (50 × 2.1 mm, 1.9 μm) was purchased from Sigma-Aldrich (St. Louis, USA). Acquity HSS C18 and Hypersil GOLD C18 colums have relatively high surface coverage with endcapping, Titan C18 column has medium surface coverage with endcapping and Acquity HSS C18 SB possesses low surface coverage without endcapping (see Table 1).

2.2. Equipment and software

UHPLC experiments were performed on a Waters Acquity UPLC I-Class system (Milford, USA) equipped with binary solvent delivery pump, autosampler, photodiode array detector and Empower 3 software. This UHPLC system had flow-through-needle (FTN) sample injector and 500 nL flow cell. The dwell volume of the system was measured as 0.1 mL.

The MP 225 pH-meter was purchased from Mettler-Toledo (Mettler-Toledo, Greifensee, Switzerland).

UHPLC method development and modeling was performed by using DryLab® 4, v.4.3.1 optimization software (Molnár-Institute, Berlin, Germany).

3. Results and discussion

3.1. Preliminary experiments

As previously mentioned, the goal of this study was to introduce a strategy where – beside method optimization – a substitution (alternative) column can be offered as part of the robustness testing. About robustness the ICH Q2 (R1) guideline contains the following "*The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. . . . In the case of liquid chromatography, examples of typical variations are . . . rent columns (different lots and/or suppliers)*" [1].

Based on former experiments, amlodipine and its impurities were found to be relatively lipophilic, so the starting mobile phase composition was set as 30% acetonitrile. However, the ImpA compound was highly lipophilic, so high acetonitrile content (90%) is required at the end of the gradient to elute this substance. In addition, it is also important to mention that there is structural similarity between amlodipine, ImpD, ImpE and ImpF and all of them contain a primary amino group (p$K_{a>}$ 10). Therefore, all these substances will be ionized under common RP conditions. The ImpH impurity has acidic character, due to the carboxylic acid group attached to an aromatic structure (p$K_a \sim 4$), so depending on the RP conditions, it can be either fully ionized or neutral [3,14,15].

During the preliminary experiments, four C18 columns belonging to the USP L1 group were chosen. The reference column was the Acquity HSS C18 and our goal was to find the appropriate replacement column. During the initial experiments at pH = 4.5, it occurred that Acquity HSS SB C18 column showed high silanol activity under these conditions, since the peaks of the basic substances were broad and tailed, with a significant increase in retention (Fig. 1*b*), below). For all these reasons, this column was excluded.

In the case of Titan C18 column, which has medium surface coverage and endcapping, the peak shapes of basic compounds were more asymmetrical than the peaks of acidic or neutral compounds, but they could be evaluated during method optimization (Fig. 1*d*), below).