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Reliability of simulated robustness testing in fast liquid chromatography, using state-of-the-art column technology, instrumentation and modelling software

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ABSTRACT

The goal of this study was to evaluate the accuracy of simulated robustness testing using commercial modelling software (DryLab) and state-of-the-art stationary phases. For this purpose, a mixture of amlodipine and its seven related impurities was analyzed on short narrow bore columns (50 × 2.1 mm, packed with sub-2 μm particles) providing short analysis times. The performance of commercial modelling software for robustness testing was systematically compared to experimental measurements and DoE based predictions. We have demonstrated that the reliability of predictions was good, since the predicted retention times and resolutions were in good agreement with the experimental ones at the edges of the design space. In average, the retention time relative errors were <1.0%, while the predicted critical resolution errors were comprised between 6.9 and 17.2%. Because the simulated robustness testing requires significantly less experimental work than the DoE based predictions, we think that robustness could now be investigated in the early stage of method development.

Moreover, the column interchangeability, which is also an important part of robustness testing, was investigated considering five different C8 and C18 columns packed with sub-2 μm particles. Again, thanks to modelling software, we proved that the separation was feasible on all columns within the same analysis time (less than 4 min), by proper adjustments of variables.

1. Introduction

A fundamental criteria of quality in a High Performance Liquid Chromatographic (HPLC) separations, is robustness [1]. Guidelines define the robustness of an analytical procedure as "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. . ." providing ". . .an indication of its reliability during normal usage" [2]. Historically, robustness testing was usually carried out as the final step of a method development process, during the validation stage which often led to unexpected observations [1,3]. However, since a method considered as non-robust should be adapted/redeveloped and revalidated, this could lead to a substantial increase of development time and costs. Therefore, robustness is verified earlier in the lifetime of a method, i.e. at the method development stage or at the beginning of the validation procedure [4–6].

Generally two approaches are used to evaluate robustness according to the ICH definition in pharmaceutical analytical practice. Either a one-factor-at-a-time (OFAT) procedure or an experimental design (DoE) procedure could be applied. The OFAT procedure varies the levels of a given factor, while keeping the other factors at nominal levels, to evaluate the effect of the former factor on the method response(s) [4]. The results obtained after varying one factor, are then compared to that of the experiment with all factors at nominal levels. This univariate approach is sometimes performed when a factor is varied in a relatively wide range to understand the peak movements. In the past, this approach was frequently used for method development and screening purposes. But for other reasons, this OFAT approach is not recommended for robustness testing. The most important one is that when the factors are examined in given intervals, the effects are estimated for a smaller domain around the nominal levels with the OFAT compared to the experimental design approach. When applying a DoE, the effect of a given factor is calculated at several level combinations of the other factors, while with the OFAT approach this is only at one level. Thus, in DoE, a reported factor effect is an average value for the whole domain, and it represents more globally what happening around the nominal situation. Moreover, the univariate approach requires more experiments and time, especially when the number of examined factors becomes larger, and secondly, the

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importance of factor interactions cannot be taken into account [4]. In pharmaceutical industrial practice, the DoE approach is clearly preferred. Plackett-Burman, full factorial, nested factorial, fractionated factorial and asymmetrical factorial experimental designs are often carried out [7–10]. These types of robustness testing typically allow the investigation of 3–15 factors (variables) based on 8–16 experiments. Beside continuous quantitative factors e.g. gradient program, mobile phase composition, pH, temperature or flow rate, the effect of qualitative factors such as column or instrument (laboratory) could also be studied and included in robustness testing.

The development of a method cannot only be based on quality but has also to be based on assurance of quality, taking into account the variability of the quality [11]. Evaluating the robustness of a method is equivalent to find its design space (DS), 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" [12–14]. Therefore, it is obviously preferred to perform the robustness testing during the method development. For that purpose, state-of-the-art chromatographic modelling softwares offer a very efficient and straightforward way of robustness testing incorporated in method development, based on modelling the retention properties. Computer modelling programs can be employed to improve the analysis throughput as well as maximize information about method specificity during method development. One of the most successful and widespread modelling programs (DryLab) optimizes the DS mainly by measuring and visualizing the effects of the mobile phase composition: gradient time and shape, pH, ionic strength, ternary eluent, additive concentrations and temperature [15–18]. For this purpose, the program suggests a relatively well-defined number of experiments on a particular stationary phase; furthermore it can predict the separation inside the DS based on changes in mobile phase composition, mode of elution (either isocratic or gradient), temperature, pH or column parameters such as column length, internal diameter, particle size and flow-rate [19].

During the robustness testing in pharmaceutical industry, among the several method variables, the column itself is always of great interest. A method validation report has to suggest an alternative column that is able to perform nearly the same quality of separation as the one using the "primary column". Finding the alternative column (column interchangeability) is often difficult. Generally, the method is developed using one given column and then an alternative column is considered at the validation procedure under the optimized conditions. In many cases, the alternative column has not the same working point (optimal conditions in a robust zone) as the primary column. Therefore, this "trial and error" approach at the end of method development often fails. However, it is worth mentioning that the alternative column is also probably able of separating the analytes, but under different analytical conditions. Column databases could be helpful for selecting an appropriate column but generic stationary phase tests (e.g. Tanaka test, hydrophobic subtraction model [20,21]) are not always able to predict certain column similarity for special separations.

In this study, the simulated robustness testing, included within commercial modeling software, was systematically studied and compared to experimental measurements and DoE based predictions. 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 a previous study, the column interchangeability was further studied applying five different C8 and C18 sub-2 μm packings. By varying properly the 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.

2. Experimental

2.1. Chemicals, columns

The mobile phase used in this work was a mixture of acetonitrile and 10 mM citrate buffer. Acetonitrile (gradient grade), citric acid, sodium hydroxide, 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). The buffer was filtered before use on regenerated cellulose filter membrane, 0.2 μm pore size (Sartorius, Göttingen, Germany).

The test samples contained 10 μg/ml Amlodipine and its European Pharmacopoeia (Ph. Eur.) impurities (A, B, D, E, F, G, H). Real life samples were prepared from amlodipine API (1 mg/ml) and spiked with all the impurities at 0.1% level. Amlodipine and its impurities were purchased from European Directorate for the Quality of Medicines & HealthCare (EDQM, Strasbourg, France). Sample solvent was acetonitrile:water 30:70 (v/v).

The Acquity columns (50 × 2.1 mm, 1.7 μm BEH C18, BEH C8, HSS C18) were purchased from Waters (Milford, USA), Hypersil columns (50 × 2.1 mm, 1.9 μm GOLD C18, GOLD C8) were purchased from Thermo Scientific (Waltham, USA).

2.2. Equipment and softwares

UHPLC experiments were performed on a Waters Acquity UPLC system (Milford, USA) equipped with binary solvent delivery pump, autosampler, photodiode array detector and Empower software. This UHPLC system had 5 μl injection loop and 500 nl flow cell. The dwell volume of the system was measured as 125 μl.

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

Modelling was carried out using DryLab v.4.0 and the quantitative robustness evaluation of generated models was performed in the latest DryLab Robustness Module v.1.0. (Molnár-Institute, Berlin, Germany). StatSoft Statistica v.11 was used for the evaluation of robustness testing (StatSoft Inc., Tulsa, OK, USA).

2.3. Apparatus and methodology

2.3.1. Considerations and initial runs for setting up the model

The selected example describes a fast and efficient method development for the determination of impurities and degradation products of a long-acting calcium channel blocker dihydropyridine (DHP) class active pharmaceutical ingredient (amlodipine), utilizing the separation power of sub-2 μm packed columns. Due to the basic character of the solutes, the mobile phase pH should play an important role in tuning the selectivity; therefore this factor was considered as a variable of robustness testing and method development. When dealing with low molecular weight analytes, the most common strategy in method development consists in selecting suitable stationary phase chemistry, organic modifier nature and mobile phase pH [11]. In a second instance, the gradient program and mobile phase temperature are optimized as complementary parameters, for fine tuning the optimum after selection of the correct combination of stationary phase, organic modifier and mobile phase pH.

Snyder, Dolan and co-workers recommended initial basic runs for multifactorial experimental designs already in the 1990s [22]. A general methodology is to simultaneously model the effect of temperature and gradient steepness on selectivity with a given RP column [23,24]. Thanks to the recent developments in chromatographic modelling softwares, it is now possible to model the effect of three variables simultaneously on a given separation and calculate the effect of additional factors like flowrate, column length,