Chromatographic separation of a pharmaceutical formulation using Design of Experiment and Design Space.

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1 Introduction

The optimization of chromatographic separation of pharmaceutical formulation is often complicated. Indeed, it can be tricky to separate components due to the similarities between their chromatographic behaviors or to elute them all well separated when some components have widely distinct properties (e.g. polarities, pKa, Log P …). A lot of improvements in the fields of method development have been done those last years [1, 2, 3] and it is thus possible to foresee different strategies that could be applied to find optimal solutions. The main difficulty is that these strategies need to give accurate and robust predictions before validation and/or transfer steps. Design of experiment (DOE) is one of these strategies. The selection of the responses is frequently a critical step. The use of resolution (R_S) of the critical pair (i.e. the two most proximate peaks) as the response to model can be hazardous because of its non-linear and discontinuous behavior. Furthermore, inaccurate predictions are usually obtained as the error committed on the prediction is omitted. Therefore, in this paper we have set up an experimental design to model the retention times of the components of a common-cold drug, Pharmagrip, from Cinfa laboratory. Additionally, an estimation of the influence of the error in the models is also carried out through Monte-Carlo simulation. The criteria to optimize are the separation between critical pair and the total analysis time. They were combined using special Desirability functions. Finally, a design space is calculated and if existing, tested. An optimal separation is thus discovered and the robustness is checked by stress testing.

2 Theory

The responses of the models are the retention times (or capacity factors) of each compound at the beginning, the apex and the end of each peak. Each part of each compound is then modeled by one equation, leading to the knowledge of the behavior of the compounds across the entire experimental domain. Based on this knowledge, it is easy to compute predicted value of some chromatographic criteria (separation, total analysis time, etc.), giving response surfaces which are combined into one multi-criteria global desirability value using special desirability functions. This approach can also be used to compute a Design Space (DS) [4]. We define the DS as the set of points where the criteria are better accomplished than a selected threshold value (e.g. separation bigger than 0.2 and analysis time smaller than 20 min.) with a given probability (i.e. 95%). This is achieved by using step functions as desirability functions. The DS is defined by equation (1).

$$x_0 \in \mathbf{DS} = [x_0 \in \chi \mid P(cr_z > \lambda_z) > \gamma\%] \,\forall\, z \text{ in } 1,...,Z \tag{1}$$

Where x_0 is a point in the experimental domain χ, cr_z is the zth criterion, λ_z is the threshold on this criterion, γ is the selected probability of acceptance and Z is the number of criteria considered in the optimization process.

In summary, a grid can be defined over the experimental domain. Monte-Carlo simulations are used in each points of the grid to estimate the influence of the error in the models on the responses (retention times), and to propagate this error through the different criteria used in the optimization process (separation, total analysis time, etc.).

3 Material and methods

3.1 Chemical and reagents

Pharmagrip common-cold formulation (Lot A032) was obtained from Cinfa (Huarte, Spain). Methanol and acetonitrile were purchased from Sigma-Aldrich (Steinheim, Germany). Ultra pure water was obtained from an Academic A10 Milli-Q system (Millipore, Eschborn, Germany). Acetic acid (>98%) was purchased from Fluka (Steinheim, Germany), ammonium formate (99%) and ammonium hydrogencarbonate (99.7%) were purchased from Sigma-Aldrich.

3.2 Apparatus

All analysis were performed on a Waters 2695 separation module coupled to a Waters selector valve 7678 and a Waters 996 Photodiode array detector (Waters, Eschborn, Germany). The Empower 2.0 software was used to manage chromatographic data. The analytical column used was an XBridge C18 (Waters) (100x2.1 mm i.d. ; particle size 3.5 μm).

3.3 Chromatographic conditions

The elution gradients were performed in 10, 20 or 30 minutes from 5% to 95% of organic modifier (methanol or acetonitrile) with the adequate buffer at a constant flow rate of 0.25 ml/minute at 30 °C. The buffer solutions were prepared with ammonium formate or ammonium hydrogencarbonate and were adjusted at the desired level of pH with diluted formic acid or ammoniac solution. All the buffers solutions were prepared at the concentration of 10mM. The chromatograms were recorded between 210 and 400 nm and peak integration was performed at 215 nm.

3.4 Experimental design

The parameters of the *d*-optimal experimental design are the pH of the aqueous part of the mobile phase, the time of gradient applied during elution and the organic modifier present in the mobile phase. The parameters selection is summarized in Table 1.

Parameters	Number of levels	Values				
pH	5	2.6	4.45	6.3	8.15	10.0
Gradient time (min.)	3	10	20	30		
Organic modifier	2	Methanol	Acetonitrile			

Table 1 – Parameters of the experimental design.

The selected *d*-optimal design counted 18 experiments and the central points (pH 6.3, gradient of 20 minutes with methanol and acetonitrile) were independently repeated once for a total of 20 experiments.

4   Results and discussion

4.1 Models

The retention times of each compound are modeled and the computation of all the selected criteria (separation, analysis time) is carried out. The accuracy of the model is checked by the correlation existing between the observed retention times and the predicted ones and mostly by the analysis of residuals. This can be seen on Figure 1. The model are then created, for each response, using stepwise regression to minimize AIC (Akaike's information criterion) and maximize adjusted R^2.
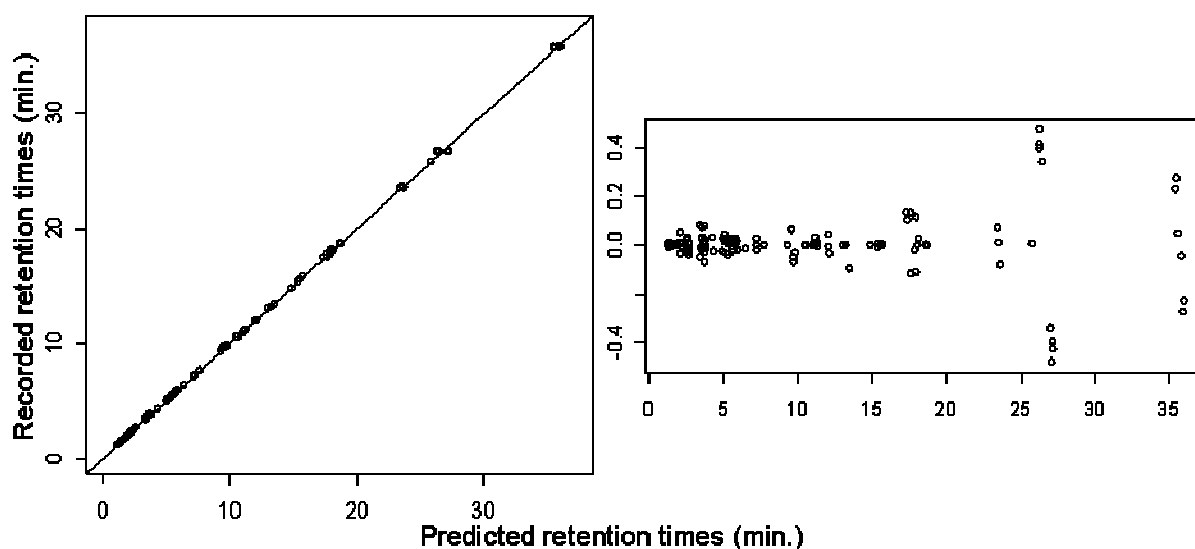

Figure 1 – Recorded retention times versus predicted retention times (left) and residuals (right)

The adjusted R^2 are greater than 0.98 for the logarithm of the retention times for each compound and the RMSE of the retention times is equal to 0.11.

4.2 Design Space

A DS can be found for each criterion with its selected thresholds and the criteria can be combined to find an optimal solution using desirability functions. If a design space exists, it means that a total separation of all the compounds in the formulation is possible. The DS shown in Figure 2 is sufficiently large to carry out a stress testing analysis. Three operating conditions are selected within the design space and the experiments are conducted. The criteria variation was small enough to validate the design space. In fact, the DS illustrated on Figure 2 is computed with a threshold on separation of 0.5 minute. Within this DS the probability is mostly equal to one and it is not possible to differentiate the optimal solution from the surrounding ones. The threshold is then decreased until the DS disappear and the last point belonging to the DS is considered as optimal. The optimal operating condition is a gradient from 5% to 95% of methanol in 12.4 minutes with a buffer solution at pH 7.3.
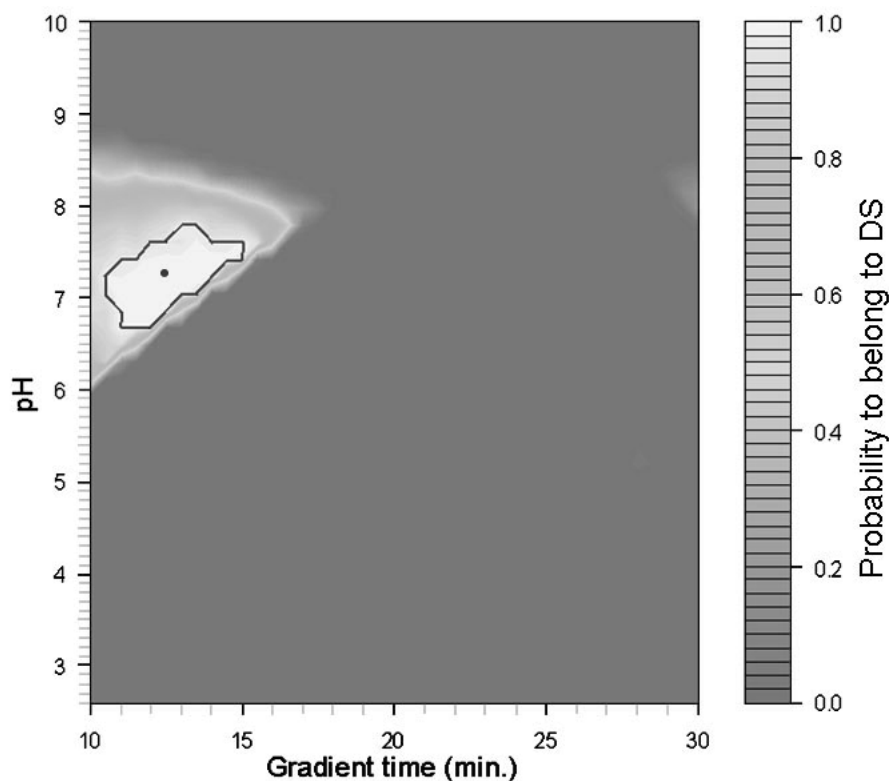
Figure 2 – Probability surface for separation criterion only (threshold at 0.5 min) with the design space (γ=95%) hedged-in the red line and the optimal operating condition shown by the red point (for methanol).

5   Conclusion

Design of experiments and multi criteria strategy using step desirability functions and error propagation is a powerful tool for the development of new chromatographic methods. It is also very flexible because the choice and the threshold of each criterion are given to the analyst. It enables to define a Design Space which is the set of analytical conditions giving a satisfactory solution. The robustness was checked with a stress testing analysis around the optimum. The model of retention times using one equation by compound is accurate and broadens the number of possible solutions, e.g. complete separation of all compounds or separation of one target compound for quantification in a shorter analysis time.

6   References

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