

# IMPLEMENTATION OF DESIGN SPACE CONCEPT FOR THE DEVELOPMENT OF ROBUST ANALYTICAL METHODS

B. Debrus<sup>1</sup>, P. Lebrun<sup>1</sup>, B. Boulanger<sup>2</sup>, E. Rozet<sup>1</sup>, G. Caliaro<sup>3</sup>, A. Ceccato<sup>4</sup>, Ph. Hubert<sup>1</sup>

<sup>1</sup> Laboratory of Analytical Chemistry, CIRM, University of Liege, Belgium, <sup>2</sup> Arlenda Laboratory Solutions, Liege, Belgium  
<sup>3</sup> Orailac Quality Solutions, Brussels, Belgium, <sup>4</sup> Odyssea Pharma, Grâce-Hollogne, Belgium

## 1. Introduction

Nowadays, the concept of Quality by Design and Design Space (DS) have become widespread in the field of pharmaceutical and analytical science. In the framework of method development, the DS computation can be carried out using design of experiment approach (DoE). The combination of these methodologies (DoE-DS) are applied to optimize the operating conditions governing a separation and to estimate the robustness of the developed method, simultaneously.

### 1.1. What is the Design Space?

ICH Q8(R2) guideline provides a harmonized guidance to improve the robustness and reliability of pharmaceutical development. In this guideline, 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". In the method development framework, the DS defines a space wherein the separation is complete taking into account the uncertainty in models, measurements and process. This guideline follows with "working within the design space is not considered as a change". Therefore, the DS can be considered as a zone of theoretical robustness as the method parameters modifications do not result in significant variations in terms of separation quality.

### 1.2. How to compute the Design Space?

First, DoE are used to model the retention factors against some chromatographic factor (e.g. pH, gradient time, temperature, etc.). The beginning, the apex and the end of each peak (i.e. respectively,  $t_{R1}$ ,  $t_R$  and  $t_{R2}$ ) are individually modelled by multiple linear regressions.

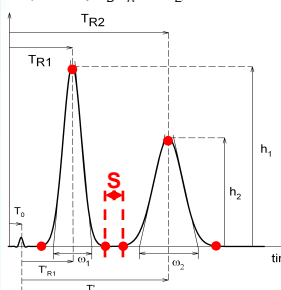


Fig. 1: Calculation of S for the critical pair

Second, a criterion is selected to quantify the quality of a separation. The chromatographic resolution ( $R_S$ ) is generally used to do it. But, the division taking place in its calculation leads to inaccurate estimation of the error that affects this latter. Thus, a simpler criterion is introduced, the separation criterion, S, as shown on Fig. 1. S is computed from the modelled retention times and prediction can be made over the experimental domain (i.e. for each experimental conditions within the workspace).

Third, the error affecting the modelled responses is propagated to S using Monte Carlo method. This step is very important as it significantly improves the prediction confidence. The DS can then easily be computed as the space where the separation is complete ( $S > 0$  min) and where the error is relatively low ( $P(S > 0)$  is high) as shown by Eq. 1.

$$DS = \{x_0 \in \chi | E_{\theta} [P(S > \lambda) | \theta] \geq \pi\} \quad \text{Eq. 1.}$$

Where  $x_0$  is a point in the experimental domain,  $\lambda$  is the threshold on this criterion,  $\pi$  is the selected probability of acceptance. Symbols P and E respectively correspond to probability and mathematical expectation.

Thus, the robustness is evaluated by the quality criteria ( $\pi$ ) and the DS shape. A high  $\pi$  and a large DS size means that the method is robust.

## 2. Results

**2.1. Pharmaceutical formulation.** The first example is the optimization of the separation of a common cold pharmaceutical formulation containing phenylephrine, chlorpheniramine maleate, paracetamol, E110 (sunset yellow) and PVP-K30. A full factorial design was used to model the retention times against the pH and the gradient time.

Factors	Levels				
pH	2.6	4.4	6.3	8.1	10.0
Gradient time ( $T_G$ , min)	10	20	30		

Table 1: Full factorial design

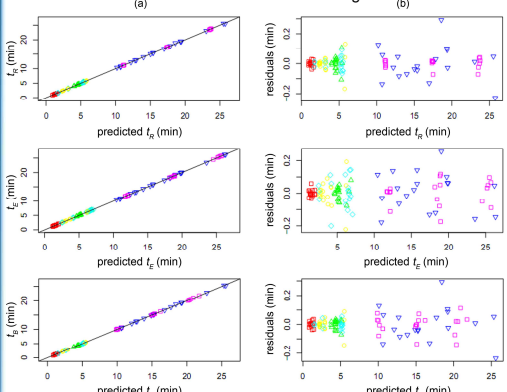


Fig. 2: (a) Predicted versus experimental values for  $t_{R1}$ ,  $t_E$  and  $t_{R2}$ . (b) Corresponding residuals plots.

This design leads to 17 experiments. ( $n = 15+2$  independent repetitions at the centre)

As depicted on Fig 2, the adequacy between the experimental retention times and the predicted one is very good ( $R^2 > 0.99$ ). Furthermore, the corresponding residuals were normally distributed between -0.2 and 0.2 min.

Maleate  
Phenylephrine  
Saccharine  
Paracetamol  
Chlorpheniramine  
PVP-K30

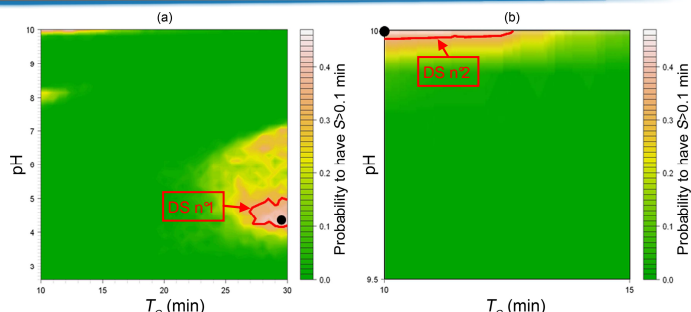


Fig. 3: (a) Probability surface for the separation criterion (acceptance limit  $\lambda=0.1$  min) optimum n1 is depicted by the black circle (pH 4.3 -  $T_G=28.0$  min). (b) Magnification of the upper left zone, optimum n2 is depicted by the black circle (pH 10 -  $T_G=10$  min).

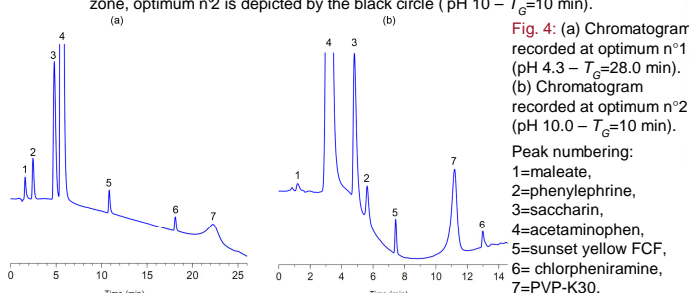


Fig. 4: (a) Chromatogram recorded at optimum n1 (pH 4.3 -  $T_G=28.0$  min). (b) Chromatogram recorded at optimum n2 (pH 10.0 -  $T_G=10$  min).

Peak numbering:  
1=maleate,  
2=phenylephrine,  
3=saccharin,  
4=acetaminophen,  
5=sunset yellow FCF,  
6= chlorpheniramine,  
7=PVP-K30.

**2.2. Test sample.** The second example is the optimization of the separation of a system suitability test mixture. The same full factorial design (see Table 1) was used.

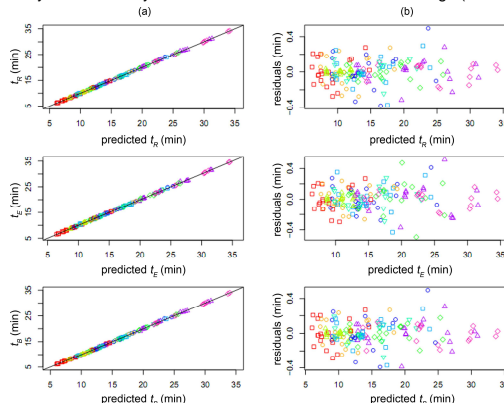


Fig. 5: (a) Predicted versus experimental values for  $t_{R1}$ ,  $t_E$  and  $t_{R2}$ . (b) Corresponding residuals plots.

As depicted on Fig 5, the adequacy between the experimental retention times and the predicted one is also very good ( $R^2 > 0.995$ ). Furthermore, the corresponding residuals were normally distributed between -0.4 and 0.4 min.

Peaks labels and numbering  
1) Atenolol  
2) Pindolol  
3) Oz290  
4) Propranolol  
5) Indoprofen  
6) Naproxen  
7) Warfarin  
8) RA-Imp2  
9) Retinoic Acid

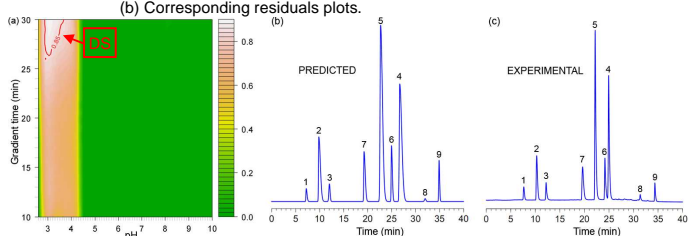


Fig. 6: (a) depicts the probability surface to obtain a separation (S) of at least 0 min The quality level is set to 85%. (b) predicted chromatogram at pH 3.0 and with  $T_G = 30$  min. (c) chromatogram recorded at this operating condition.

This DoE-DS methodology was also successfully applied on a cannabinoids mixture [B. De Backer *et al.*, J. Chrom. B 877 (2009) 4115], on the separation of sulindac and its related impurities [F. Krier *et al.*, Drug Analysis 2010 poster], on the separation of 18 antimalarial drugs [R.D. Marini *et al.*, Drug Analysis 2010 poster] on extracts of *spirosporum penduliformum* [M. Rafamantanana *et al.*, Drug Analysis 2010 poster] and on extracts of *strychnos usambarensis* [I. Nistor *et al.*, Drug Analysis 2010 oral communication]

## 3. Conclusions

Even if the number of experiments needed ( $n$ ) might seem high regarding to conventional optimization approaches, the DoE-DS methodology is able to simultaneously model the chromatographic behaviour of each compound and provides optimal conditions in which the prediction error was analyzed in order to evaluate the method robustness.

Check our publications on <http://orbi.ulg.ac.be>