USEFULNESS OF DESIGN SPACE FOR THE OPTIMIZATION OF CHROMATOGRAPHIC SEPARATIONS

Benjamin Debrus[1], Pierre Lebrun[1], Attilio Ceccato[2],
Gabriel Caliaro[3], Javier Rupérez[4], Coral Barbas[4], Bruno Boulanger[5], Philippe Hubert[1]
[1]*University of Liège, Laboratory of Analytical Chemistry, CIRM, Liège, Belgium,* [2]*Mithra Pharmaceuticals, Liège, Belgium,* [3]*Orailac Quality Solutions, Belgium,* [4]*University San Pablo-CEU, Analytical Chemistry, Madrid, Spain,* [5]*UCB Pharma SA, R&D Clinical Pharmacometrics, Brussels, Belgium*

The development of chromatographic methods is often complicated. The similarities or distinctness between the chromatographic behaviours of a set of compounds can make this a baffling problem, taking into account that the objective is to separate all the compounds in a short time. Practical and automated methodologies are evolving continuously since some decades and different strategies are now well established (1,2). However, the accuracy and the robustness of optimal separations remain the essential needs before validation and/or transfer steps. Among the possible strategies, Design of Experiment (DOE) is really appropriate for the purpose of liquid chromatography separations. Nevertheless, the conventional model of resolution (R_S) can be risky because of its non-linear and discontinuous behaviour. Furthermore, inaccurate predictions are usually obtained as the error committed on the prediction is omitted. Therefore, an experimental design was established to model the retention times (or capacity factor) at the beginning, the apex and the end of each peak. Each part of each compound is then modelled by one equation, leading to the knowledge of the behaviour of the compounds across the entire experimental domain. Moreover, an estimation of the influence of the error in the models is also carried out through Monte-Carlo simulation. The optimized criteria are the separation between critical pair (i.e. the two most proximate peaks) and the total analysis time. Desirability step functions are used to access a global desirability over all possible operating conditions. The use of step functions as desirability functions makes possible to compute a Design Space (DS) (3) which can be defined 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%). The DS can be considered as a theoretical zone of robustness which can be verified by stress testing analysis. This methodology was tested on actual samples such as pharmaceutical formulations and plant extracts. Results will be presented.

References

(1) L.R. Snyder, J.J. Kirkland, J.L. Glajch: Practical HPLC Method Development, Wiley & Sons, 2nd Edition.
(2) W. Dewé, R. Marini, P. Chiap, P. Hubert, J. Crommen, B. Boulanger: Development of response models for optimizing HPLC methods. *Chemom Intell Lab Sys* 2004, 74:263–268.
(3) P. Lebrun, B. Govaerts, B. Debrus, A. Ceccato, G. Caliaro, Ph. Hubert, B. Boulanger: Development of a new predictive modelling technique to find with confidence equivalence zone and design space of chromatographic analytical methods. *Chemom Intell Lab Sys* 2008, 91(1):4-16.