Mass Spectrometric Analysis of Plasma Proteins Using an Experimental Design: Challenges & Perspectives

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Introduction

Proteomics methods based on mass spectrometry hold special promise for the discovery of novel biomarkers, but to date their contribution to the diagnostic field has been disappointing. This is due in part to the lack of a coherent pipeline connecting marker discovery with well-established methods for validation (1). In the present discusion, uses of experimental design for optimization of liquid chromatography tandem mass spectrometry (LC-MS/MS) integrated methods will be discussed (2). An attempt was made to find solutions to the questions? Such as: What are the optimization criteria, how do we implement appropriate optimization strategies/procedures, and how do we interpret the data obtained? It has been our endeavor to present and explore different parameters associated with an LC-MS/MS hyphenated experimental set-up, utilizing mainly electrospray ionization (ESI) along with suitable application.

Methods

Standard MATLAB program was applied for screening of chromatographic and mass spectrometric factors; factorial design was applied for optimization of essential factors for the robustness study. A linear model was postulated and a 2^3 full factorial design was employed to estimate the model coefficients for intermediate precision

Results

Earlier we have applied this experimental design process for quantification of oxcarbazipine (3), same design we have implemented for the quantification of selected cytokines in diabetic and healthy patients. The use of experimental design during method validation for biomarker discovery constitutes a basic feature of multivariate optimization particularly for validation parameters such as robustness and intermediate precision, which if appropriately used can solve several problems and constitutes a powerful tool in the hands of proteomic scientists. More specifically, experimental design helps the researcher to verify if changes in factor values produce a statistically significant variation of the observed response.

Innovative aspects

- The application of different mathematical tools is a prerequisite for the realization of the robust results!
- Possible limitations when it comes to choosing the setting of a specific parameter and a stepwise optimization strategy using an experimental design will be discussed that hopefully will aid the reader to optimize the performance of such an experimental design approach for mass spectrometric method development and validation.
- We can get three dimensional graphs for understanding step by step change of each parameter with respect to the results (Ex fig1)

References

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(3) Srinubabu G et al, Development and Validation of LC-MS/MS Method for the Quantification of Oxcarbazepine in Human Plasma Using an Experimental Design- *Chem. Pharm. Bull*. **56**(1) 28—33 (2008).

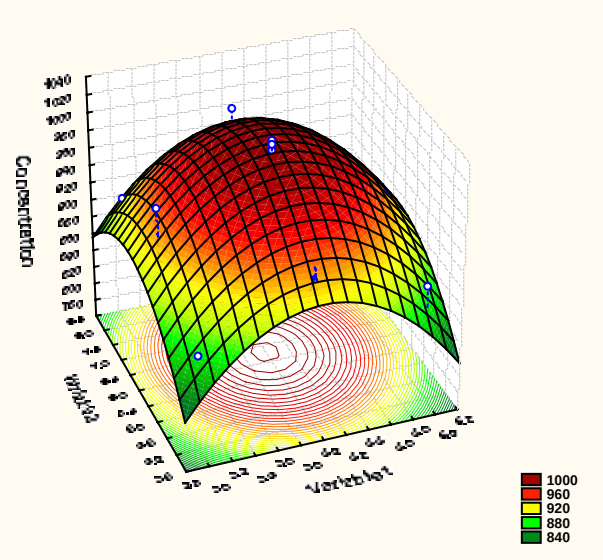

Figure 1. Three-dimensional plot of the response surface for Y (found concentration). Variation of the response Y as a function of variable 1 and variable 2