APPLICATION OF EXPERIMENTAL DESIGN FOR THE HPLC-UV-MS SEPARATION OF APORPHINE ALKALOIDS FROM LEAVES OF *SPIROSPERMUM PENDULIFLORUM* THOUARS

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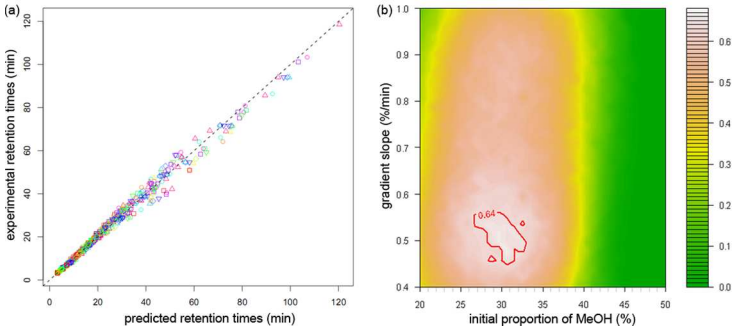
Spirospermum penduliflorum Thouars (Menispermaceae) is an endemic species of Madagascar and traditionally used for the treatment of hypertension. Recently, two aporphine alkaloids known to possess antihypertensive properties (dicentrine and neolitsine) were isolated and identified from this plant. Therefore, we decided to develop an analytical method to separate these molecules to control the quality and effectiveness of this plant.

The optimization of chromatographic methods for plant extracts is often intricate and can be time consuming. In fact, it is a thorny problem to separate components due to the similarities between their chromatographic behaviours or to elute them all well separated when some components have widely distinct properties (e.g. polarities, pKa, Log P …). In order to optimize the separation and simultaneously evaluate the method robustness, experimental design and design space methodologies were applied [1].

Three common chromatographic parameters (mobile phase pH, initial proportion of methanol and gradient slope) were selected to construct a full factorial design of 36 experiments. The times at the beginning, the apex (i.e. retention time) and the end of each peak were recorded. The logarithm of the retention factors and the semi-widths were then modelled by multi-linear regressions and these were used to optimize the separation. The relationship between predicted and experimental retention times is depicted in Figure a. The corresponding residuals were normally distributed. Subsequently, the predictive error propagation was analyzed to evaluate the ability of the method to remain unaffected by small parameters variations. Figure b shows the probability surface (at pH 3.5) to reach a selected threshold of the separation criterion (S>0 min, i.e. baseline resolved peaks). The design space (DS) is defined as the zone where the probability to attain S>0, is higher than the quality level. The DS is surrounded in red for a quality level of 64% in Figure b.



The application of design of experiments and design space led to separate 13 molecules including dicentrine and neolitsine. An isomer of a dicentrine was also evidenced. Other molecules were identified by the comparison of literature data and our data bank.

References:

[1] P.Lebrun, B. Govaerts, B. Debrus, A. Ceccato, G. Caliaro, P. Hubert, B. Boulanger. Chemom. Intell. Lab. Sys.: 91 (2008) 4-16.