Innovative green supercritical fluid chromatography development for the separation of neurotransmitters using design space strategy

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Recently, the number of publications about SFC knew an important increase. This phenomenon could be explained by the necessity of rapid, effective and green analytical methods. In order to prove the potential of SFC, the aim of our work was to develop a SFC method for the separation of very polar compounds such as catecholamines and pharmaceutical compounds associated using an innovative chemometric approach.

Following the context of pharmaceuticals guidelines ICH Q8 R2[1], an innovative methodology based on design space (DS) was used to develop a SFC method. Briefly, DS could be defined as a subspace of the experimental domain into the chromatographic conditions will ensure the quality of the separation. This methodology was previously developed and tested on liquid chromatography[2,3].

First a screening design was used to select the stationary phase and the nature of the mobile phase based on a maximization of the number of peaks eluted, a minimization of the number of co-eluted peaks and a maximization of efficiency. Ethylpyridine stationary phase and CO_2/methanol/TFA mobile phase were selected by modeling screening design results.

Then a central composite design with orthogonal blocks defined a set of experiments used to model the retention times of each peak at the beginning, the apex and the end. The critical quality attributes (CQA), the separation (S) between peaks of the most critical pair and the analysis time were the responses considered to assess the quality of the separation. The DS was computed as the multidimensional subspace where the probability for the separation and analysis time criteria to be within acceptance limits ($S > 0$; $t_{tot} < 15$ min) was higher than a defined quality level. The DS was computed propagating the prediction error from the modeled responses to the quality criterion using Monte Carlo simulations. The optimal condition was predicted at a gradient slope of 3.8%/min to linearly modify the modifier proportion between 5 and 40 %, a isocratic time of 3 minutes, a concentration of TFA of 25 mM and a temperature of 60.5°C. This optimal condition was experimentally tested to confirm the prediction. Furthermore, chromatographic conditions included into the DS and on the limits of the DS were experimentally tested to confirm the prediction ad to assess the robustness of the developed SFC method.

This work was the first separation of underivatized catecholamines in SFC, showing a very interesting, green and low cost alternative to HILIC method generally used for polar compounds. Moreover, the DS strategy was successfully applied to prove the potential of SFC in the field of pharmaceutical analysis.

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