Innovative green Supercritical Fluid Chromatography development for the separation of neurotransmitters using Design Space strategy

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1. Introduction

In the context of analytical green chemistry, the interest of supercritical fluid chromatography (SFC) was investigated. In order to prove the potential of SFC, polar compounds were selected. Indeed, polar compounds are not the classical compounds separated by SFC and consist of a chromatographic challenge. Neurotransmitters such as catecholamines were mainly selected as model compounds for this study.

Polar compounds have been widely studied by many chromatographic techniques such as reverse-phase liquid chromatography with ion pairing reagents or more recently hydrophilic interaction liquid chromatography (HILIC). This last one showed an improvement of retention but used a very large amount of organic solvent, usually acetonitrile at a percentage higher than 80 %. SFC could be an interesting and greener alternative to the methods previously developed.

Opposite to empirical strategies to develop analytical methods, pharmaceutical guidelines ICH Q8 (R2) recommend a systematic approach called quality by design (QbD). These guidelines define the design space (DS) as "the multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide assurance of quality". The DS may be expressed as:

$$DS = \{ x_0 \in \chi : P(CQAs \in \Lambda \mid x_0, data) \geq \pi \}$$

In other words, the DS is a region of an experimental domain – χ – where the posterior probability that the critical quality attributes (CQAs) are within acceptance criteria Λ, is higher than a specified quality level π, conditionally on the available data.

This innovative design space strategy was used to develop a robust SFC method by modeling the logarithm of retention factor to predict the separation between peaks.

2. Screening

A screening design was first planned to select best qualitative parameters before the method optimization. Stationary phase chemistry, modifier and additive type were the factors of a full factorial design in order to study the effect of the factors and their interactions.

Factors	Levels			
Stationary phase	Bare silica	Diol	Ethylpyridine	Silica-C
Modifier	Methanol	Acetonitrile	Isopropanol	
Additive	TFA 10 mM	Diisopropylamine 0.1% (v/v)	Ethylene glycol 0.1 % (v/v)	

Table 1. Factors and levels of screening design

The main objective of this screening was to select stationary and mobile phase by maximizing the number of peaks, minimizing the number of co-elutions and maximizing peak capacity. A total desirability approach was developed to model the results.

Maximizing the global desirability gave the following levels for the three factors : 2-ethylpyridine stationary phase, methanol as modifier and TFA as additive *(Fig. 1 C).* This combination of stationary/mobile phase eluted all the compounds with a single co-elution within a 12 minutes run time. The results of the screening were used to establish starting chromatographic conditions for the optimization design.

A screening design involving the nature of stationary and mobile phases as factors is inescapable for unexplored chromatographic methods, especially for difficult compounds, such as polar ones in SFC.

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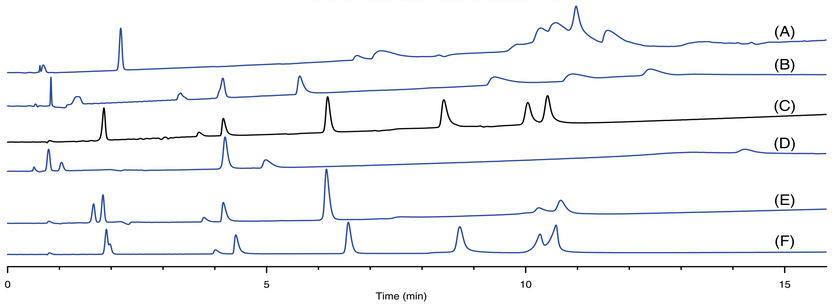

Figure 1. Screening chromatograms (A) bare silica, MeOH, TFA; (B) diol, isopropanol, TFA ; (C) ethylpyridine, MeOH, TFA; (D) diol, MeOH, DIP; (E) ethylpyridine, isopropanol, TFA ; (F) ethylpyridine, MeOH, ethyleneglycol

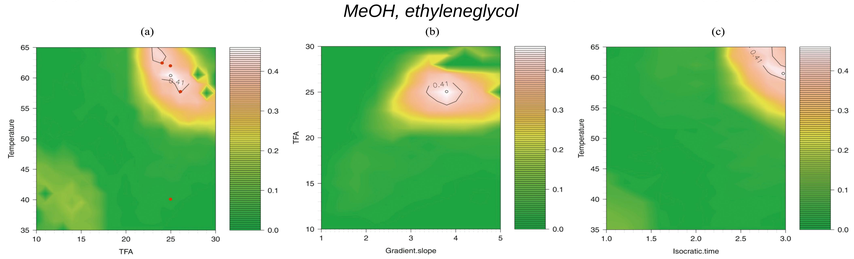

Figure 2. Probability surfaces (i.e. P(S > 0 ; t_{tot} < 15). (a) temperature (° C) vs TFA (mM), (b) TFA (mM) vs gradient slope (% modifier min^{-1}), (c) temperature (° C) vs isocratic time (min). The DS (π = 41%) is encircled by a dark line.

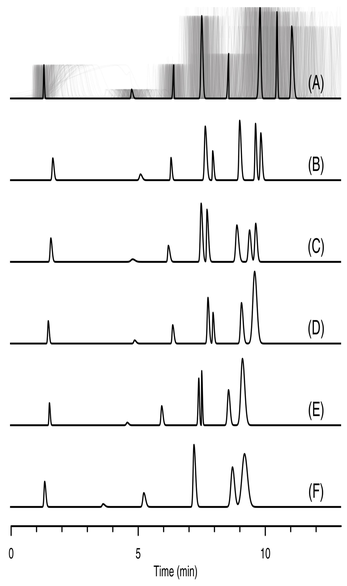

Figure 3. (A) predicted chromatogram and predictive uncertainty (shaded grey area) at the optimal condition : S_G 3.8% min^{-1}, t_{iso} 3 min, TFA 25 mM, T 60.5° C; (B) respective experimental chromatogram; (C) experimental chromatogram recorded at S_G 3.8 % min^{-1}, t_{iso} 3 min, TFA 25 mM, T 61.5° C; (D) experimental chromatogram recorded at the limit of the DS S_G 3.8% min^{-1}, t_{iso} 3 min, TFA 24 mM, 62° C (E) experimental chromatogram record at the limit of the DS S_G 3. % min^{-1}, t_{iso} 3 min, TFA 26 mM, 57.5° C (F) experimental chromatogram recorded outside DS limits S_G 3.8% min^{-1}, t_{iso} 3 min, TFA 25 mM, 40° C.
Elution order: 1. CAF; 2. PSE; 3. PAR; 4. CET; 5. HIS; 6. DOP; 7. NOR; 8. SER

3. Method optimization

Secondly, the DS chromatographic method optimization strategy was used for the first time in SFC. A four factors central composite design with orthogonal blocks was selected. The central point was independently repeated six times (i.e. carried out twice per block).

Factors	Levels				
T_{iso} (min)	1	1.5	2	2.5	3
S_G (% modifier min^{-1})	1	2	3	4	5
TFA (mM)	10	15	20	25	30
T (°C)	35	42.5	50	57.5	65
block	*1*		*2*		*3*

Table 2. Factors and respective levels of central composite design

The model was established using noradrenalin as model compound (eluted at the end of the gradient). The block effect was investigated because two column of two different batches were used to carry out the experiments. The model developed involving two block because none significant differences were observed between block 2 and 3. An original model was developed involving the linear and quadratic contributions of the factors and their interactions, including interactions between factors and block effect (R^2_{adj} = 0.972 ; p-value < 0.001 for reference compound)

$$\log(k_{tR}) = \beta_0 + \beta_1 . b + \beta_2 . S_G + \beta_3 . S_G^2 + \beta_4 . t_{iso} + \beta_5 . t_{iso}^2 + \beta_6 . TFA + \beta_7 . TFA^3 + \beta_8 . T + \beta_9 . b . S_G + \beta_{10} . b . TFA + \beta_{11} . b . T + \beta_{12} . S_G . t_{iso} + \beta_{13} . S_G . TFA + \beta_{14} . S_G . T + \beta_{15} . t_{iso} . TFA + \beta_{16} . t_{iso} . T + \beta_{17} . TFA . T + \beta_{18} . S_G . t_{iso} . TFA + \beta_{19} . S_G . TFA . T + \beta_{20} . t_{iso} . TFA . T + \varepsilon$$

where $\beta_0 \ldots \beta_{20}$ are the estimated parameters and ε is the estimated error of the model.

Using Monte-Carlo simulations, the predictive probabilities for S to be greater than 0 min and run time to be lower than 15 min were calculated (*Fig . 2*).

One optimal separation condition was identified at S_G 3.8 % min^{-1}, t_{iso} 3 min, TFA 25 mM, and T set at 60.5° C, with a quality level π of 0.41. The predictive probability for S> 0 and t_{tot}<15 minutes is 0.46 for the optimal condition. The chromatogram recorded at the optimal condition is presented in *Fig .3.*

As recommended by the USP proposal, the robustness of the chromatographic conditions proposed by the DS strategy was assessed, three experimental conditions included in and at the limits of the DS were tested. Within the DS limits, the quality criterion was maintained (S > 0) but, the co-elution of the most critical peaks pair (NOR-SER) was observed at the limits of the DS.

4. Conclusion

This work was the first demonstration of analysis of underivatized catecholamines by SFC. This SFC method using CO_2/methanol is clearly a greener alternative to HILIC. Thus, SFC could contribute to sustainable development in the field of pharmaceutical analysis.

The value of DS methodology for SFC separation was successfully demonstrated in this work. Indeed, polar compounds could be considered as a very challenging case for SFC method but our methodology allowed us to develop a robust method to separate eight compounds within 12 minutes by means of 66 experiments, screening and optimization designs included.

This study was the first demonstration of the interest of this methodology to develop green methods and could be useful for any SFC method development.

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