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Title: Online hyphenation of reversed-phase liquid chromatography (RPLC) and supercritical fluid chromatography (SFC) for comprehensive on-line two-dimensional analysis of neutral compounds in complex samples

Couplage en ligne de la chromatographie liquide en phase inverse et de la chromatographie en fluide supercritique pour l'analyse compréhensive bidimensionnelle de composés neutres dans des échantillons complexes

Comprehensive two-dimensional chromatography provides such a large peak capacity that it is foreseen as the most efficient separative tool for the analysis of complex samples, especially when coupled to mass spectrometry detection. One of the challenge in the development of such a technique is however the choice of orthogonal conditions to benefit from most of the 2D separation space. While RPLCxRPLC is powerful for ionizable molecules, it may be less attractive for neutral compounds due to a strong correlation between separation mechanisms. The most orthogonal combination for neutral compounds in liquid chromatography is the coupling of reversed phase and normal phase mechanisms (RPLC x NPLC). However, immiscibility of solvents used as mobile phases makes such a development in LCxLC very challenging. Supercritical fluid chromatography (SFC) is a separation technique using a non-polar CO₂-based mobile phase in a normal phase mode. Hence the combination of SFC and RPLC is expected to be as attractive as the combination of NPLC and RPLC for the 2D analysis of neutral compounds. While online SFC x RPLC requires depressurization strategies including packed loops and make-up flow to avoid sample loss, online RPLC x SFC is more straightforward. Furthermore SFC separations are much faster which is an additional advantage compared to SFC x RPLC. However online RPLCxSFC involves the transfer of hydroorganic sample solvent onto the SFC column. Our team has recently demonstrated that it was possible to directly transfer the fractions from the first RPLC to the second SFC dimension by simply using empty loops^{1,2}.

The objective of this work is to analyze neutral molecules in complex samples by on-line RPLCxSFC hyphenated to UV and mass spectrometry. Related to our previous studies, fundamental understanding of the transfer behavior of solutes, improvement of predictive tools, suitable hyphenation to mass spectrometry will be the main goals of this thesis. Relevant applications will be carried out in collaboration with our industrial partners. The candidate will join the "Chromatography and Hyphenated techniques" team, long-time expert in the optimization of chromatographic methods, especially focusing on multidimensional separations³⁻⁵.

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