HT-UHPLC AS SECOND DIMENSION IN COMPREHENSIVE ON-LINE RPLC X RPLC: SELECTION OF SUITABLE ANALYTICAL CONDITIONS FOR THE SEPARATION OF IONISABLE COMPOUNDS

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Nowadays, comprehensive on-line LCxLC has emerged as a powerful technique for the separation of complex samples such as pharmaceutical, biological or environmental ones. Yet, generating much higher peak capacities than those obtained with a single chromatographic system while keeping a reasonable analysis time remains a challenging task. The aim of this work is to demonstrate that high temperature liquid chromatography (HTLC) combined with very high pressure (HT-UHPLC) is very attractive as second dimension in comprehensive on-line RPLC x RPLC, particularly in case of ionisable compounds.

Strategies for both separations are considered. Best operating conditions (column geometry, injection volumes, flow-rates) in each dimension are selected from a calculation tool, based on theoretical equations. The calculations take into account the numerous instrumental constraints (maximum pressure, maximum column length, maximum flow-rate, dwell volumes, time required for the injection…). On the other hand, the choice of reversed-phase systems in both dimensions (RPLC x RPLC) is attractive as it offers the possibility of a large panel of analytical conditions and hence of selectivities for ionisable compounds. In addition, the problem of eluent compatibility is reduced. The degree of orthogonality between different chromatographic systems (stationary phase-mobile phase-temperature) is assessed by means of a two-dimensional display of gradient data. Based on both theoretical results and illustrative examples, the potential of HT-UHPLC will be discussed and compared to more conventional systems both in terms of peak capacity and gain in time.