

# Pulsed Elution and Active Modulation in 2D reversed phase LC: Increasing Flexibility

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The interest in online comprehensive two-dimensional liquid chromatography (LC×LC) have increased as it offers ways to improve the performance of separations in terms of peak capacity and selectivity compared to one-dimensional liquid chromatography. However, the price for greater resolving power in LC×LC is a more complex system, where parameterization of the first dimension constrains the second dimension and vice versa. In this study a way to release these constrains is presented.

The developed method addresses several of the challenges encountered in LC×LC. This includes in particular: loss of peak capacity due to undersampling of the first dimension, limited peak capacity in the second dimension due to short second dimension analysis time and limitations in selection of column dimensions and flow rates.

The method is based on a strategy where the sample is eluted of the first dimension column by pulses of increasing eluotropic strength and width. In between the pulses the first dimension is kept in a no-elution state by applying a flow of mobile phase with weak eluotropic strength. The eluate from the first dimension is diluted with a make-up flow of water before it is trapped on trap columns in the modulator and re-injected onto the second column.

It is demonstrated that the LC system is capable of delivering the required pulses of strong solvent and by tuning the length and eluotropic strength of these pulses, analytes with retention factors in water ( $k_w$ ) above 150 can be manipulated to elute in 3-4 pulses. In between the pulses the first dimension can be kept in a no-elution state for up to 10 minutes without changes in which pulse and the number of pulses analytes with  $k_w$  above 350 elute in. For analytes with  $k_w$  equal to 150 minor changes in the pulse position of elution was observed, when the no-elution time was increased from 1 to 10 min. The initial experiments were done using 27 nitrogen containing aromatic compounds and UV-detection, and the method applied to alkaline fractions of vacuum gas oils with positive electrospray ionization and time-of-flight detection. Peak capacities of 4018 and 4610 (corresponding to a peak production rate of 7.4 peaks/min and 4.5 peaks/min) was obtained for a 540 min and 1040 min analysis, respectively. The pulsed elution approach combined with a refocussing step between the two dimensions offers great potential with respect to increasing the flexibility of LC×LC.