Trap-Refocusing-Elute nano liquid chromatography: how to take advantage of both direct injection and trap-elute mode?

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LC-MS is the method of choice for differential bottom-up proteomics where sample complexity requires high resolution and sensitivity. The two primary LC methodologies include direct injection (DI), where samples are introduced directly onto the analytical column, and trap-and-elute (TE), where samples are trapped on a first column before elution onto the analytical column. DI typically achieves superior chromatographic resolution compared to TE but is limited to small volumes of injection (±1 µL) and does not allow online sample desalting.

We introduce a novel LC method termed "Trap-Refocusing-Elute" (TRE), which integrates the strengths of trap-elute technique with enhanced chromatographic resolution capabilities. TRE involves a post-trap column dilution step to increase water content before the analytical column using an additional LC pump. This new approach has been evaluated, in comparison to DI and TE with a standard solution of HeLa cells tryptic digest by monitoring peptide peak widths as an indicator of LC resolution. The dilution factor (trap column flow / analytical column flow) has been optimized to reach the DI resolution minimizing the impact of the dilution.

TRE represents a promising practical advancement for achieving high LC resolution, online desalting, and efficient sample handling for proteomics workflows. This methodological innovation holds potential for enhancing the sensitivity and robustness required in high throughput complex proteomic analyses.