Enhanced separation performance with reduced carry-over for proteomics analyses using novel nanoLC and the timsTOF HT

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Introduction

The rapid and extraordinary development of modern mass spectrometry instrumentation, especially of the ion-mobility applications, led to significantly higher peptide and protein identifications and better quantitation. However, even with the latest separation technologies, innovative stationary phases and instrumentation analytes originating from a previously injected sample are detectable in the subsequent run. This is a significant problem causing delays and issues with quantitative and qualitative analysis.

Here, we show an innovative approach for reducing carryover in proteomics analysis. We use a novel nanoLC system with an increased sample loading speed and advanced wash of the injection path hyphenated with the timsTOF HT. Thus, the sample throughput increases, and redundant cleaning steps are reduced.

Methods

All separations were performed using an innovative nanoLC separation system with a dedicated and tunable wash procedure of the injection path. Sample analysis was performed using direct sample injection on the separation and trap columns. Tryptic HeLa peptides and mice retina tissue lysates were injected either into a 5 mm trap column connected to 25 cm C18 PepSep Ultra separation column (25cmX75µm, 1,5µm particles) connected to a 10µm emitter, or directly into the analytical column (both settings being accessible by a software switch with no further hardware modifications). The nanoLC coupled to a TimsTOF HT (Bruker Daltonics) mass spectrometer (Fig.1). The HPLC gradient was generated by mixing solvent A (water with 0,1% formic acid) and solvent B (acetonitrile with 0,1% formic acid), and using a 22-minute active gradient. All MS acquisitions were performed using the dia-PASEF mode.

Data were analyzed using DIA-NN 2.0.1 in library-free mode, with the SwissProt Homo sapiens database for HeLa samples (release 02-2025, 20,417 entries) and the Mus musculus database for tissue lysates (release 01-2025, 17,229 entries). The analysis was performed without using the match-between-runs functionality

Results

High-speed injection procedures with the novel nanoLC system have shortened the time for the direct sample injection on the nano separation column and, simultaneously, helped protect the sensitive packed column bed by reducing the pressure shock during the injection procedure.

We have used an innovative procedure for cleaning the injection path during the separation without interfering with the analysis and reducing the carryover. These procedures are tunable and can be adapted depending on the sample composition and complexity and the length of the separation gradient, thus enabling a large extent of flexibility. The first tests performed with a Hela cell line digest (Fig.3) showed similar ID performance for the 1column standard and fast loading method, while the 2-columns setup allowed a more than 2-fold reduction of the carryover for a 6% protein ID number tradeoff.

A similar analysis performed in the frame of a clinical research project using a mice tissue lysates (Fig.4) allowed to reach similar conclusions. The Protein group ID was limited to 3% for the 2-column setup while the carryover observed for the Fast loading method was increased compared to the two others.

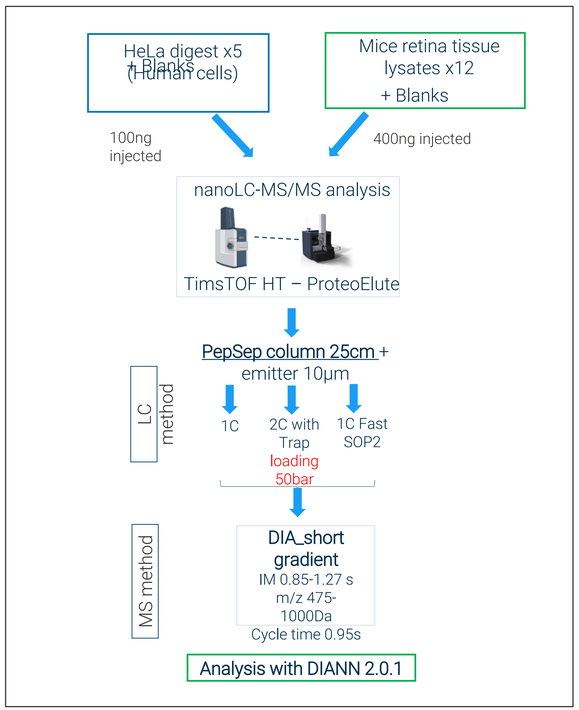

Fig. 1 : Experimental setup display

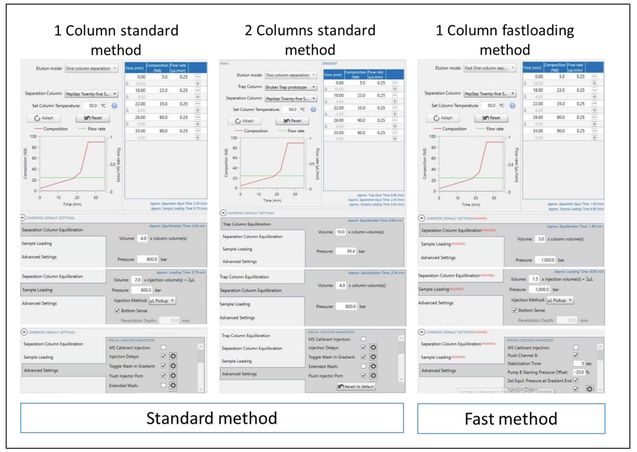

Fig. 2 Chromatograhic methods setup. All three methods use the same 22 min active gradient method. They differ by there equilibration and loading protocols, namely the wash volume and equilibration / loading pressure. The total run time is 55 minutes for the 1C method, 47 minutes for the 2C method and the 1C fast loading method.

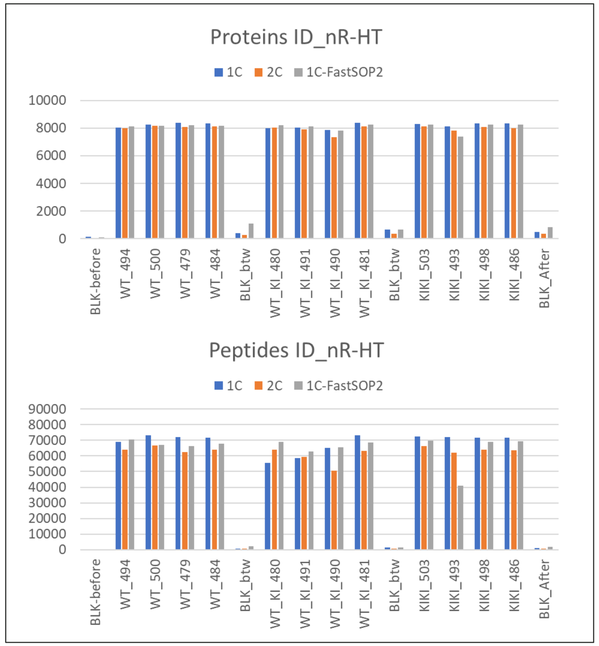

Fig. 4: Outcome for the method test on a mice tissue lysates. Injections are presented in their chronological order, from left to right.

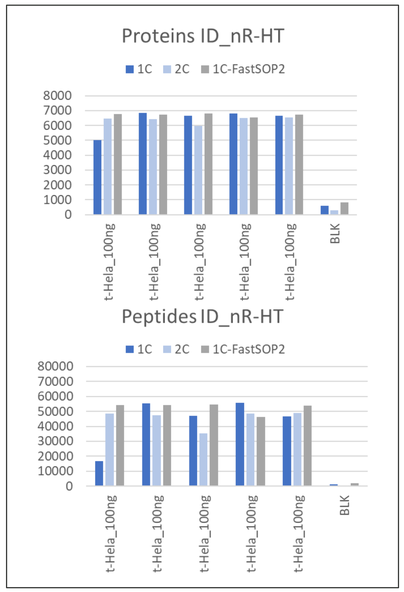

Fig. 3 : First results obtained from a Hela cell line digest injection (using a new column, which probably explains the lower ID results for the 1st injection. Injections are presented in their chronological order, from left to right.

Conclusions

- The new, fast-loading method allowed to increase the global throughput while preserving the Identification performances.
- The lowest carryover was observed with the 2-column setup, for a closeby throughput, at a 3-5% cost in terms of protein group ID's.

Technology