

Parallel LC with Capillary PS-DVB Monolithic Columns for High-Throughput Proteomics

INTRODUCTION

Peptide sequencing by nano LC-ESI-MS/MS is a widespread technique used for protein identification in proteomics. Typically a protein digest sample is separated in one hour, employing 75 μm i.d. packed nano columns. For large scale proteomics analysis, e.g., multidimensional analysis, this approach is rather time consuming and, therefore, typically applied for samples that do not reveal enough information with MALDI-MS. With the chromatographic separation being the time limiting factor, the focus of this study was on decreasing the analysis time in LC-ESI-MS/MS. Monolithic columns allow for very fast gradient analysis of peptides and proteins due to the absence of intraparticle void volume and favorable mass transfer kinetics. The replacement of a packed capillary column with a monolithic column will decrease the analysis time by a factor of 3–4. When applied in a parallel column setup, monolithic columns can be used to maximize the MS sample throughput. The column switching applied in the parallel LC setup allows almost continuous MS detection of peptides, due to the overlap of peptide elution from one column with the wash and equilibration of the second column. With typical run times of 7.5 min, eight samples per hour have been routinely analyzed with the system. The system has been used as a screening tool and as a tool for enhanced identification of proteins.



Figure 1. UltiMate 3000 in parallel LC configuration.

EXPERIMENTAL

- Nano LC system: UltiMate™ 3000 (LC Packings, a Dionex Company)
- Columns: PS-DVB Monolithic 200 μm i.d. x 5 cm column (P/N 161409)
PS-DVB Monolithic 200 μm i.d. x 5 mm trap columns (P/N 163972)
Both thermostatted at 60 °C
- Mobile Phase: A: H₂O, 0.05% TFA
B: H₂O/ACN, 50:50, 0.04% TFA
- Loading Solvent: H₂O, 0.05% HFBA
- Gradient: 10 – 100% in 7.5 min, wash and equilibration
- Inj. Volume: 1 μL
- Flow Rate: 2.5 $\mu\text{L}/\text{min}$

Detection: UV, 214 nm, 3 nL flow cell
 MS, Bruker Equire 3000+, positive ion mode
 Mass range: 200–2000 m/z ; cycle time 0.12 min
 Mass tolerance of 1.0 Da for the parent ion and 0.5 Da for the fragment ions
 Protein IDs defined by at least two peptides
 Peptide ion score ≥ 14 .

Samples: Cytochrome C digest, 1 pmol/ μL (P/N 161089)
 Six protein digest: Alcoholdehydrogenase, Apo-transferrin, Bovine serum albumin, Betagalactosidase, Cytochrome C, Lysozyme, 1 pmol/ μL of each protein (P/N 161088)
 Platelet proteins, digested, 0.5–1.0 μg

A schematic overview of the fluidics of the parallel LC setup on the UltiMate 3000 is represented in Figure 2. The entire fluidics is in the thermostatted column compartments and a low dead volume nanovalve is used to minimize postcolumn bandbroadening when switching either column in-line with the detector.

The aim of a parallel LC setup is to obtain a higher sample throughput without LC gradient modification, therefore the gradient in this study was fixed to 7.5 min and only the other parameters were tuned to maximize throughput.

Optimizing the gradient overlap in combination with the column switching is the most important approach for higher sample throughput. Two examples of the gradient overlap are represented in Figures 3 and 4. They will be referred to as gradient A and B, respectively.

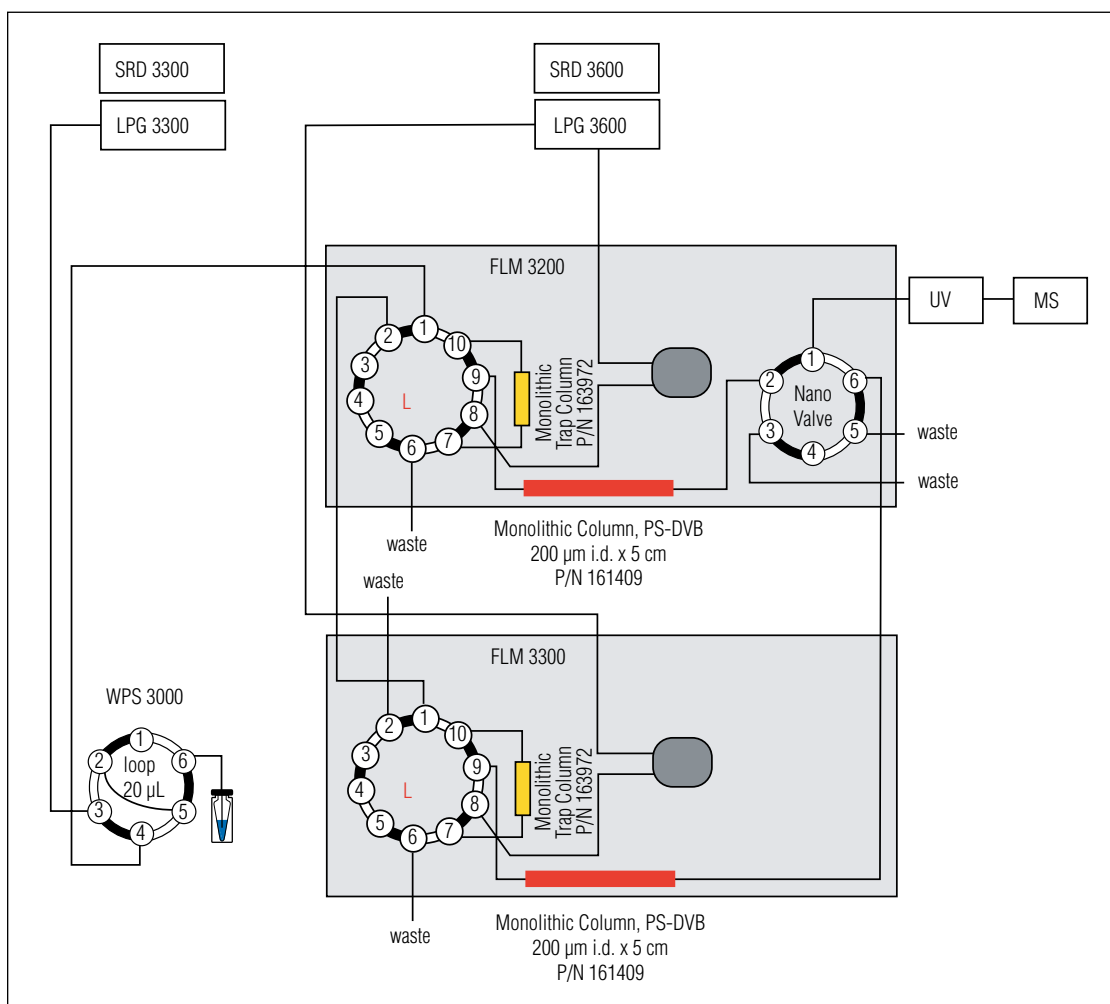


Figure 2. Setup for parallel LC with capillary monolithic columns.

In gradient A (Figure 3) the run time is 7.5 min and the time it takes to perform an injection is used as the column wash time. Typically an injection takes up to 1–2 min and for PS-DVB monolithic columns this is sufficient. After the injection one of the columns is equilibrated and the other is eluted. A total of 6 samples per hour can be analyzed this way.

For some samples no wash is required, because the gradient from 10–100% B is sufficient to elute all compounds in 7.5 min. In those cases, elimination of the injection time means there is no column wash and another increase in sample throughput is obtained. For the WPS-3000 autosampler, a Chromeleon® command exists that prepares the next injection during the analysis (i.e., at run start, the loop is already filled and only the inject valve is required to switch). When this PrepareNextSample feature is used with gradient A, it is possible to analyze a total of eight samples in one hour.

The absence of intraparticle void volumes allows for very fast equilibration of PS-DVB monolithic columns. A shorter equilibration time produces a higher throughput. When the equilibration time is shortened, the gradient has to be interrupted because the gradient time and equilibration time are no longer equal. The elimination of the injection time allows for this gradient interruption without having an isocratic portion in the chromatogram. The compromise for this extra throughput (12 samples in one hour) is that a 5 min detection window has to be used with a 7.5 min gradient and, therefore very hydrophilic/phobic peptides will be undetected (Figure 4).

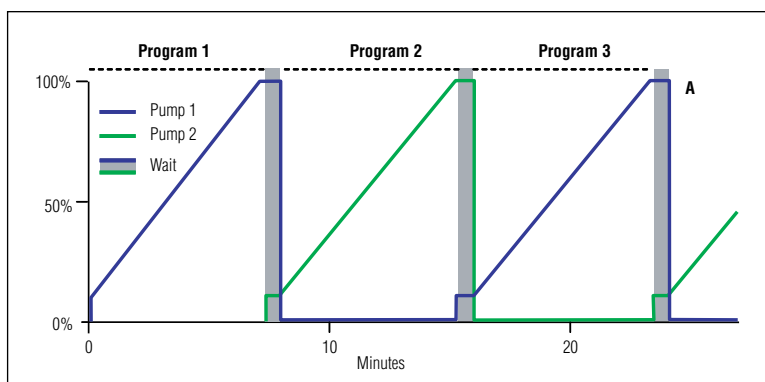


Figure 3. Run time of 7.5 min; the inject is used as the column wash time.

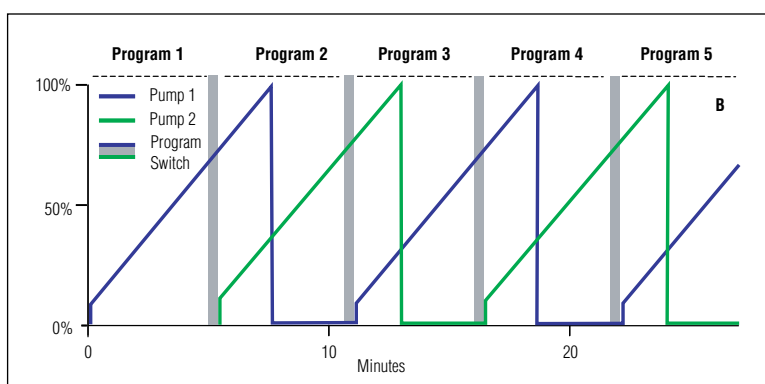


Figure 4. Run times of 5 min with 7.5 min gradient. By efficient column switching only the first and the last part of the gradient are not detected.

RESULTS AND DISCUSSION

The separation of a Cytochrome C digest was routinely performed and the capillary parallel LC system stability was maintained over a long period of time. In Figure 5 the results of six successive runs using gradient A (Figure 3) are shown. The efficiency of the monolithic columns is not represented in the TIC traces due to the low scanning speed of the MS detection in MS/MS mode; the sampling rate is too low for sufficient data points. However, when searched against Mass Spectrometry protein sequence DataBase (MSDB), Cytochrome C is identified with 49% sequence coverage.

By optimization of the column switching, it was possible to analyze up to 12 samples per hour. The protein digest mixture was analyzed with gradient B (Figure 4). Figure 5 shows that 12 runs per hour can be achieved and although the gradient time (7.5 min) exceeds the detection window (5 min), almost all peptides of the protein digest mixture can be detected.

To stress the system and evaluate its applicability with real samples, a digest of platelet proteins was analyzed using the parallel LC method, however instead of utilizing only the speed of the setup, the unique ability of having two column systems was used to analyze each sample on both column systems. Although the MS traces show great similarity for each column system, differences were found in the identified proteins on each column system (Figure 6). However, when the data of both column systems was merged, a total number of 67 platelet proteins was identified. The difference in detected peptides with each column system is not due to the LC separation, but instead to the low scanning speed of the MS in MS/MS mode and the subsequent data-dependent data acquisition.

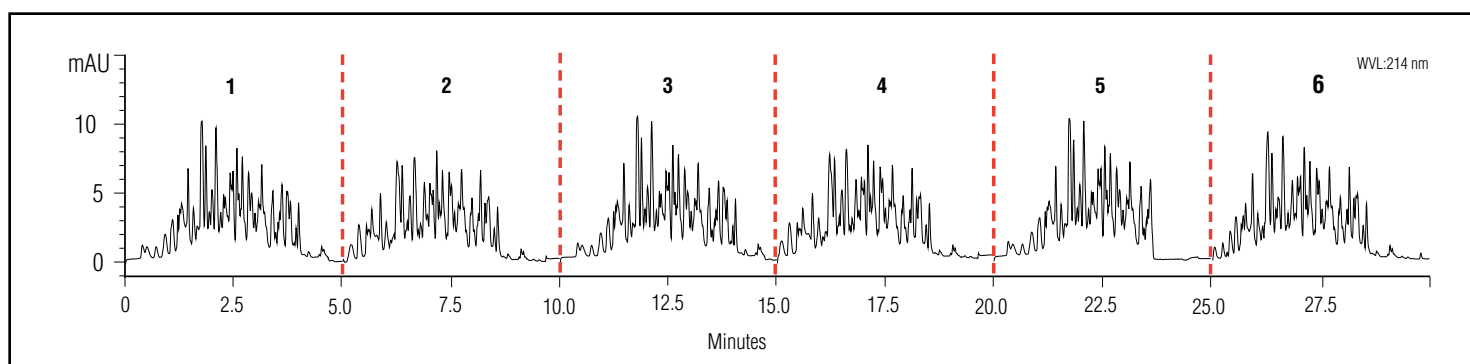


Figure 5. UV trace of six successive protein mix digest separations analyzed with gradient B and detected in a 5-min window allowing a maximum of 12 samples in one hour.

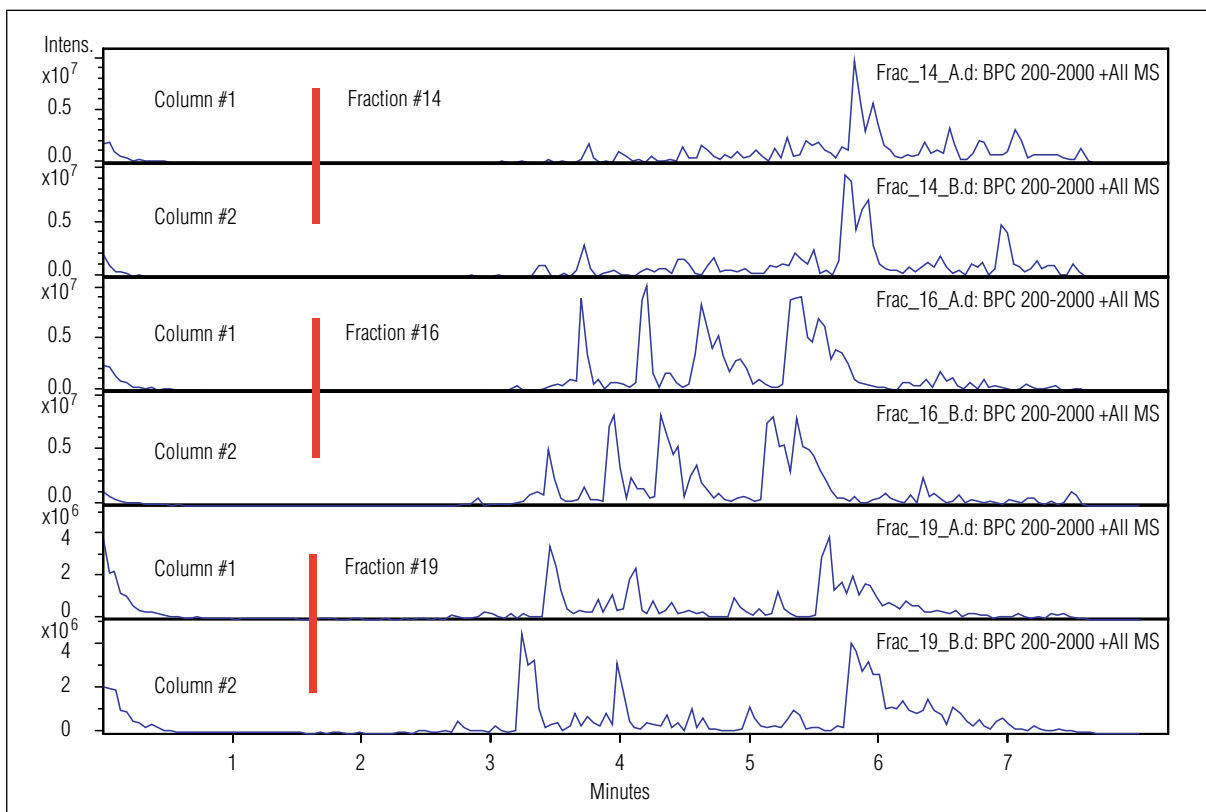


Figure 6. Base peak chromatograms of three platelet digest fractions on both column systems.

CONCLUSIONS

A high throughput LC system based on parallel LC with monolithic columns was used to analyze peptide standards and a digest of platelet proteins. The high separation speed, typical for PS-DVB monolithic columns, was fully exploited in this parallel LC setup. As many as 12 runs in one hour were performed with the parallel LC system. In another application each sample was injected twice to improve the protein sequence coverage. The data-dependent data acquisition of the MS will obtain partially

overlapping data, but when using active exclusion lists in MS software, this overlap could be decreased to twice the amount of data in the same time. A stable system performance of the parallel capillary LC system was maintained over a long period of time. This setup is now routinely used for the MS analysis of multidimensional separations in our lab. In this research the capillary parallel LC was interfaced with ESI-MS. With minor modifications this setup could be interfaced with the Probot™ Microfraction Collector to allow high-throughput MALDI target preparation.

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