

Improvement of the liquid chromatographic analysis of protein tryptic digests by the use of long capillary monolithic columns.

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Introduction:

The standard method of protein identification consists of tryptic digestion of the protein(s), followed by LC-MS⁽ⁿ⁾ analysis of the resulting digest. For this goal, highly effective separation methods are needed. Peak capacity is the primary parameter for evaluation of efficiency in gradient chromatography. Peak capacity for a given stationary phase system can be increased by the use of a longer column. A Bovine Serum albumin (BSA) tryptic digest was separated with two capillary monolithic silica columns of 150 and 750 mm length with various gradient times, in order to show the effect of column length on separation and protein identification (through Mascot database searching).

Experimental:

Sample:

- Bovine Serum Albumin
- Reduce disulfide bonds with DTT
- Alkylate thiols with iodoacetamide
- Digest with porcine trypsin
- Dilute to 3 μM

System:

- Agilent HP1100 nanoLC
- ATAS-GL MU701 UV (215 nm)
- Agilent LC/MSD trap XCT MS
- GL Sciences silica monolith
 - 150 x 0.1 mm
 - 750 x 0.2 mm

Separation:

- 5-50% ACN gradient
- 3-75 minute gradient length
- 150 x 0.1 mm
 - 0.25 μl injection (0.75 pmol)
 - 0.5 μl/min (1.06 mm/s)
- 750 x 0.2 mm
 - 1.00 μl injection (3 pmol)
 - 2.0 μl/min (1.06 mm/s)

Results:

LC-UV

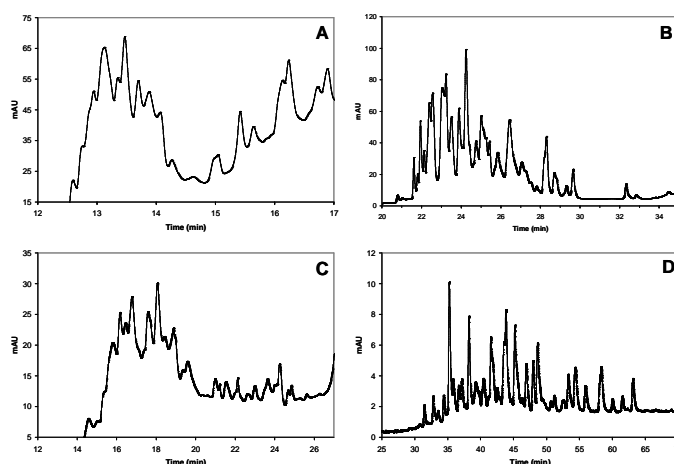


Figure 1: LC-UV chromatograms of a separation of a tryptic BSA digest on silica monolithic columns. A; 150 x 0.1mm column, 5-50% ACN in 3 min. B; 750 x 0.2 mm column, 5-50% ACN in 15 min. C; 150 mm column, 5-50% in 15 min. D; 750 mm column, 5-50% in 75 min.

When comparing the separation for both columns, it is clear that using a longer column leads to a better separation. This is also expressed in the peak capacity ratios (table 1), which, as expected, are close to the square root of the column length ratio (~2.24).

Table 1: Chromatographic parameters

150 x 0.1 mm				750 x 0.2 mm				PC ratio
t _G	W _{av} ^a	Δt ^b	PC ^{**}	t _G	W _{av} ^a	Δt ^b	PC ^{**}	
3 min	0.24	3.05	12.6	15 min	0.39	11.6	29.7	2.36
15 min	0.44	11.0	25.0	75 min	0.77	31.7	41.0	1.64

^a Average width of 7 to 12 selected peaks

^b Elution window between the first and last eluting peptides

LC-MS

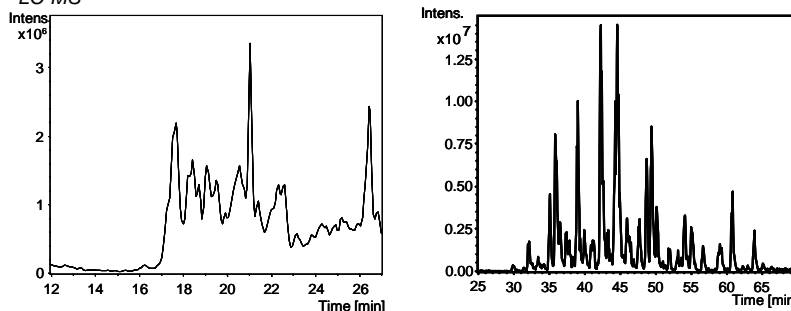


Figure 2: Base peak chromatograms of tryptic BSA separation. Left panel; 15 minute gradient on 150 x 0.1 mm column. Right panel: 75 minute gradient on 750 x 0.2 mm column.

Mascot scores per peptide were higher for the separations on the long column (52-60 vs. 42-51). Together with a larger number of identified peptides, this adds up to a higher Mascot score. Mascot scores decrease at longer gradient times, probably due to the increase in peak width, leading to lower peak concentration.

Conclusion:

Longer columns improve peptide separation and protein identification, a side-effect of this approach is longer run times. Therefore these columns are most suited for the analysis of complex peptide mixtures, where the resolution of short columns is insufficient.

