

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

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## Introduction

A method often used for protein identification consists of tryptic digestion of a protein-mixture, followed by LC-MS 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. The peak capacity can be increased by using longer columns. A bovine serum albumin (BSA) tryptic digest was separated using 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 (by Mascot database searching).

## Experimental

### System:

- Agilent HP1100 nanoLC
- Atas-GL MU 701 UV detector (215 nm)
- Agilent LC/MSD trap XCT MS
- GL Sciences C18-silica monolith
  - 35 x 0.2 mm trapping column
  - 750 x 0.1 mm separation column
- Merck chromatolith monolith
  - 150 x 0.1 mm separation column

### Experimental set-up:

- 5-50% acetonitrile gradient
- 9-225 min gradient length
- 400 nl/min flow rate
- 1.0 µl injection (1.5 pmol)

## Results

### 150 mm column:

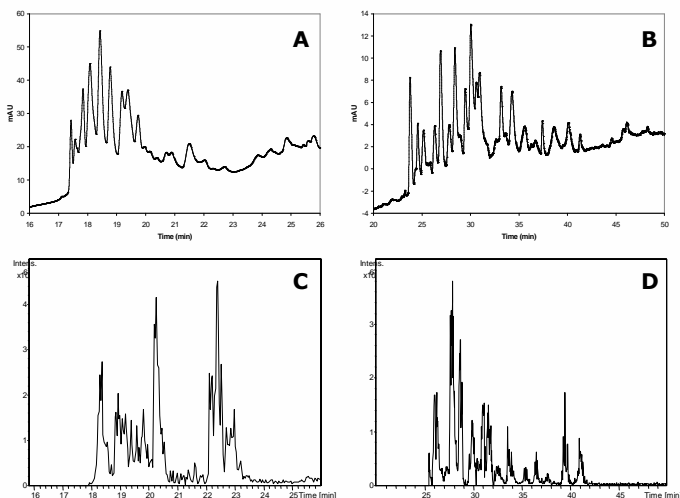


Figure 1: Chromatograms of a BSA tryptic digest. Column: a 150 x 0.1 mm C18-silica monolithic. A: LC-UV, 9 min gradient, B: LC-UV, 45 min gradient, C: LC-MS, 9 min gradient, D: LC-MS, 45 gradient.

Gradient	$w_{av}$	$\Delta t$	Peak Capacity	Mascot Score	Coverage
9 min	0.242	4.59	19.0	151	11%
45 min	0.582	17.51	30.1	109	11%

### 750 mm column:

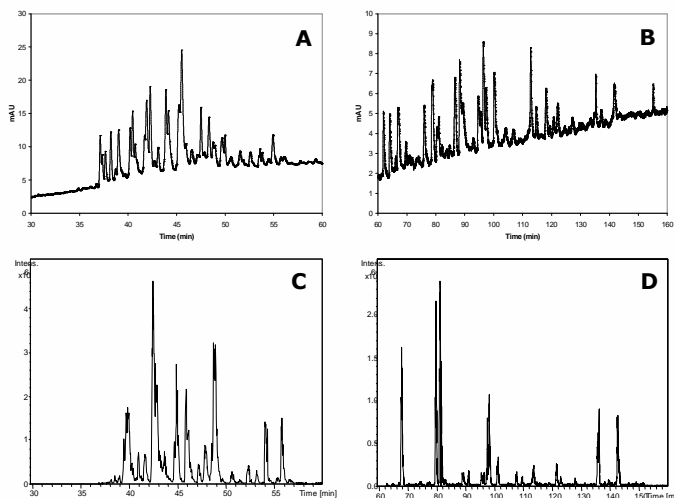


Figure 2: Chromatograms of a BSA tryptic digest. Column: a 750 x 0.1 mm C18-silica monolithic. A: LC-UV, 45 min gradient, B: LC-UV, 225 min gradient, C: LC-MS, 45 min gradient, D: LC-MS, 225 gradient.

Gradient	$w_{av}$	$\Delta t$	Peak Capacity	Mascot Score	Coverage
45 min	0.498	18.95	38.0	204	16%
225 min	1.006	82.75	82.3	216	17%

## Conclusions

Longer columns improve peptide separation, illustrated by an increase in peak capacity by a factor roughly proportional to the square root of the column length ratio (PC ratio is 2.01 for the 9/45 min gradient pair and 2.73 for the 45/225 min pair). The use of long columns also leads to an increase in protein sequence coverage with database searching. The use of longer columns also causes longer run-times, therefore these columns are most suited for analysis of complex peptide mixtures, where shorter columns provide insufficient resolution.