DIONEX

Application Note 529

Capillary PS-DVB Monolithic Column of 500-µm i.d. for Peptide and Protein Separations in Top-Down Proteomics Studies

INTRODUCTION

Polymeric monolithic stationary phases offer an alternative to the classical microparticulate sorbents, bringing important advantages to sample analysis. In contrast to the traditional stationary phases, which consist of packed particles the monolithic separation medium is made of a continuous, rigid polymeric rod with a porous structure. The lack of intraparticular void volume improves mass transfer and separation efficiency, allowing for fast, high-quality separations.

Polystyrene-divinylbenzene (PS-DVB) capillary monolithic columns with internal diameters (i.d.) of 100–200 µm have successfully been applied in combination with ESI and MALDI MS for the separation and identification of peptides and proteins.

The wide dynamic concentration range in which proteins are present in complex biological matrices, necessitates the use of large sample amounts. Top-down and bottom-up proteomics LC separations therefore require columns with enough sample capacity. Here the application of PS-DVB capillary monolithic columns with an i.d. of 500 µm is described for separation of peptides and proteins. The chromatographic performance is demonstrated with separations of standard peptide and protein samples as well as proteins from an *Eschericia coli* cell lysate.

EXPERIMENTAL

LC system:	UltiMate™ 3000
Column:	PS-DVB monolithic column 500-µm i.d. × 5 cm (P/N 164087)
Solvent A:	Water, 0.05% TFA
Solvent B:	MeCN, 0.04% TFA
Gradient:	5–35% MeCN in 7.5 min for peptides 15–50% MeCN in 20 min for proteins
Flow Rate:	25 µL/min
Temperature:	60 °C
Samples:	(1) Cytochrome c digest, 8 pmol injected (2) Standard proteins, 0.05 µg injected (3) Ion-exchange fraction of *E.coli* cell lysate
Detection:	UV, 214 nm, 45-nL flow cell

RESULTS

Peptides

To determine the performance of the 500-µm i.d. PS-DVB monolithic column for peptide separations, a digest of cytochrome c digest was injected. The separation was performed with a steep gradient of 5–35% MeCN in 7.5 min and is shown in Figure 1. Peak width at half height was between 0.08 and 0.10 min for the tryptic peptides. Under these conditions the peak capacity for peptides of this monolithic column is approximately 50. The peak capacity is calculated with the formula $P = 1 + T_g/w$, where P is the peak capacity, T_g is the gradient time and w is the average peak width at 13.4% of the peak height. Shallower gradients can be applied to increase the peak capacity up to around 150 for a gradient time of 90 min.

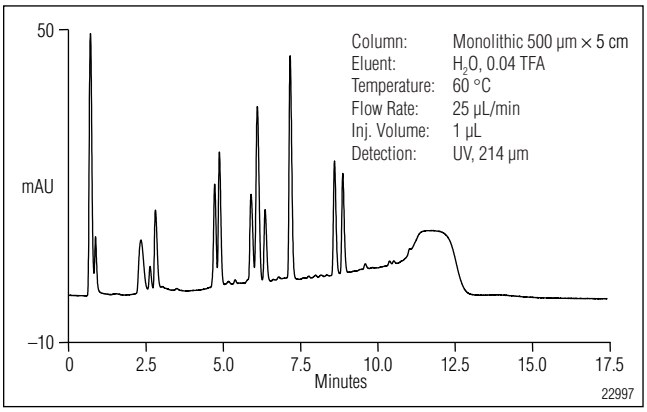

Figure 1. Separation of a cytochrome c digest separated on a monolithic capillary column of 500-µm i.d. × 5 cm, gradient 5–35% MeCN in 7.5 min, sample amount 8 pmol, flow rate 25 µL/min.

Proteins

The PS-DVB monolithic columns can also be used for protein separations using the same mobile phases. To elute proteins, the MeCN concentration must be increased compared to the elution of peptides. In Figure 2, a chromatogram is shown for the separation of 8 standard proteins. All proteins were baseline separated in 20 min with peak widths between 0.1 and 0.2 min at half height. The peak capacity for proteins is approximately 60 for a 20 min gradient.

The injected amount was 25 ng for the standard proteins. The maximum loading capacity for proteins allowing a 10% increase in peak width at half-height was around 10 pmol.

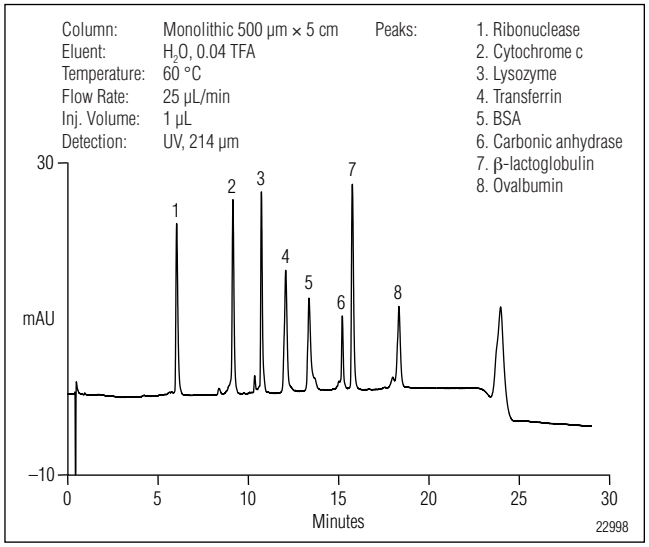

Figure 2. Separation of eight standard proteins (Table 1) on a monolithic capillary column (500-µm i.d. × 5 cm). Gradient 15–50% MeCN in 20 min. Injected amount 0.025 µg of each protein, flow rate 25 µL/min.

Proteins From an E. coli *Cell Lysate*

To demonstrate the suitability of the 500-µm i.d. PS-DVB monolithic column for more complex samples, an ion-exchange fraction of an *E. coli* total cell lysate was separated on the column. Approximately 2 µg in 50 µL ion-exchange buffer was loaded onto the column. The chromatogram is shown in Figure 3.

Several proteins were fractionated in a 384 well plate for further identification. The procedure involved mobile phase evaporation, in-well digestion and LC-MS/MS analysis. The protein identification results are shown in Table 2.

Table 1. Peak Parameters for Eight Standard Proteins Separated on PS-DVB Monolithic Columns

Peak #	Protein	Retention Time (min)	Peak Width at 50% Height (min)
1	Ribonuclease	6.0	0.11
2	Cytochrome c	9.1	0.11
3	Lysozyme	10.2	0.09
4	Transferrin	12.1	0.16
5	BSA	13.4	0.16
6	Carbonic anhydrase	15.2	0.09
7	β-lactoglobulin	15.8	0.12
8	Ovalbumin	18.3	0.19

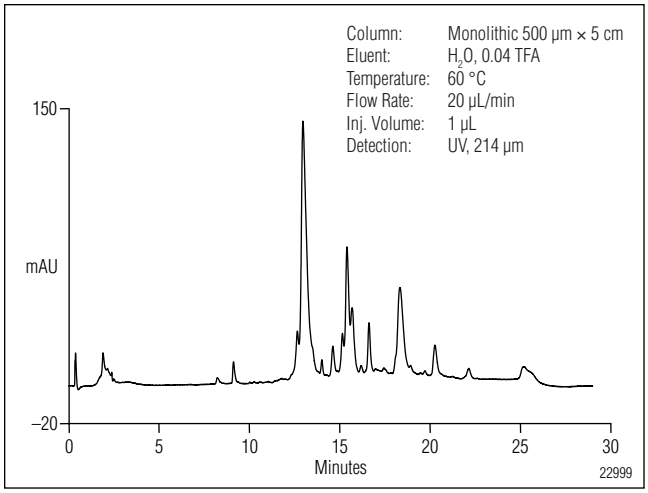

Figure 3. Separation of E. coli *proteins (ion-exchange fraction) on a monolithic capillary column (500-µm i.d. × 5 cm). Gradient 10–50% MeCN in 20 min, flow rate 20 µL/min, injected amount approximately 2 µg.*

Table 2. Protein Identification Results from the Separation Shown in Figure 3 Separated on Capillary PS-DVB Monolithic Columns

Identified Protein	Sequence Coverage (%)
Chain A, Yoda From *Escherichia Coli* crystallized with zinc ions	51
High-affinity branched-chain amino acid transport protein	43
Putative adhesin	41
High-affinity leucine-specific transport system; periplasmic binding protein	38
Phosphoglycerate mutase 1	34
Transaldolase B	51
Fructose-biphosphate aldolase, class II	18
Phosphoserine aminotransferase	49
Alkyl hydroperoxide reductase C22 protein	45
5-methyltetrahydropteroyltri-glutamate-homocysteine methyltransferase	8
Aspartate-semialdehyde dehydrogenase	24
Phosphoserine aminotransferase	8

CONCLUSIONS

PS-DVB monolithic columns of 500-µm i.d. allow for fast and efficient separations of peptides and proteins with peak width at half-height between 0.1 and 0.2 min. Peak capacities for proteins are approximately 60 using a 20 min gradient and 50 for peptides using a steep gradient in 7.5 min and can be increased by using shallower gradients.

The relatively high sample capacity of the capillary PS-DVB monolithic columns allows for micropreparative fractionation of proteins and their identification. In an alternative approach, this column can be directly coupled to ESI-TOF-MS instruments, with or without postcolumn splitting.