



PS-DVB Monolithic Columns Applied in a Microcolumn Switching Setup for the Analysis of Peptides and Proteins

Remco Swart and Irina Dragan
LC Packings – A Dionex Company

Polystyrene-divinylbenzene (PS-DVB) trap columns have been evaluated for microcolumn switching applications. In contrast to 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 absence of intraparticle void volume increases separation efficiency, allowing for faster separations. Column lifetime is higher compared to packed columns.

This article demonstrates that the use of Monolithic Trap Columns for pre-concentration and desalting of peptides and proteins does not negatively influence chromatographic performance or sample recovery. Sample capacity of the Monolithic Trap Columns (200- μm i.d. \times 5 mm) is 100 pmol for both peptides and proteins.

Direct Injection Versus Microcolumn Switching

The influence of the Monolithic Trap Column on chromatographic performance was evaluated by the separation of tryptic peptides of cytochrome c. Figure 1 shows a comparison between direct injection and microcolumn switching with a Monolithic Trap Column. Table I lists peak widths at half height (PWHH) for the tryptic peptides with and without preconcentration.

Protein Separation After Preconcentration Using Monolithic Columns

Figure 2 shows the separation of 16 standard proteins on a Monolithic Capillary Column with sample loading on a Monolithic Trap Column. PWHH were typically between 4 and 8 s.

Conclusion

The experiments demonstrate that Monolithic Trap Columns can be used for preconcentration and desalting of samples consisting of peptides and proteins, without negatively influencing the chromatographic performance or recovery of the compounds. The sample capacity of the Monolithic Trap Columns (200- μm i.d. \times 5 mm) is in the range of 100 pmol for both peptides and proteins (data not shown).

Table I: PWHH for tryptic peptides of cytochrome c separated on monolithic columns

Peak No.	Peptide Sequence	Mass	PWHH (s)	
			Direct Injection	Column Switching
1	KTGQAPGFSYTDANK	1584.71	2.2	2.4
2	TGQAPGFSYTDANK	1456.53	2.2	2.5
3	MIFAGIK	779.01	3.5	3.7
4	TGPNLHGLFGR	1168.32	4.5	4.8
5	EDLIAYLK	964.13	3.4	3.4
6	IFVQKCAQCHTVEK	1633.94	4.1	4.5
7	GITWGEETLMEYLENPKK	2138.42	2.8	3.1
8	GITWGEETLMEYLENPK	2010.25	2.4	2.6

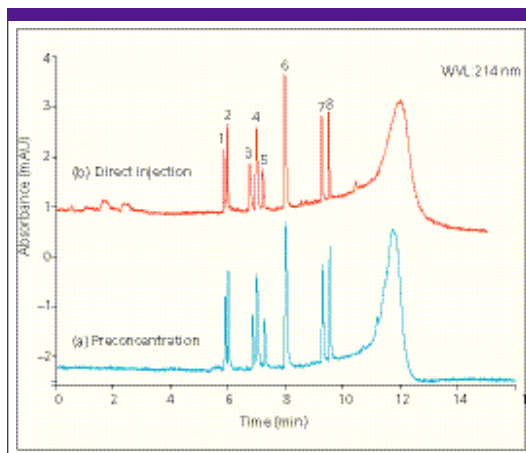


Figure 1: Direct injection (b) versus microcolumn switching with Monolithic Trap Column of a cytochrome c digest (a) with 1 pmol injected.

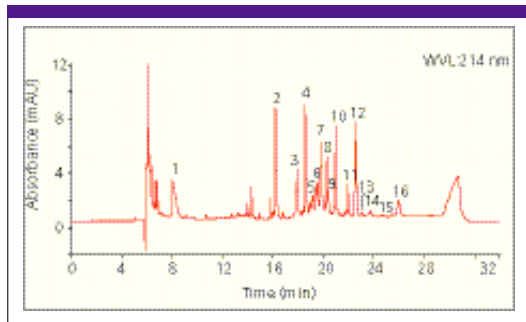


Figure 2: Separation of 16 intact proteins (5 ng each injected) on a Monolithic Capillary Column after preconcentration on a Monolithic Trap Column. Peaks: 1 = ribonuclease, 2 = cytochrome c, 3 = lysozyme, 4 = transferrin, 5 = α -lactalbumin, 6 = BSA, 7 = conalbumin, 8 = α -chymotrypsinogen, 9 = α -chymotrypsin, 10 = myoglobin, 11 = carbonic anhydrase, 12 = β -lactoglobulin, 13 = β -casein, 14 = alcohol dehydrogenase, 15 = β -galactosidase, 16 = ovalbumin.

Dionex Corporation

1228 Titan Way, P.O. Box 3603, Sunnyvale, CA 94088-3603
tel. (408) 737-0700, fax (408) 730-9403
www.dionex.com

For More Information Circle 9