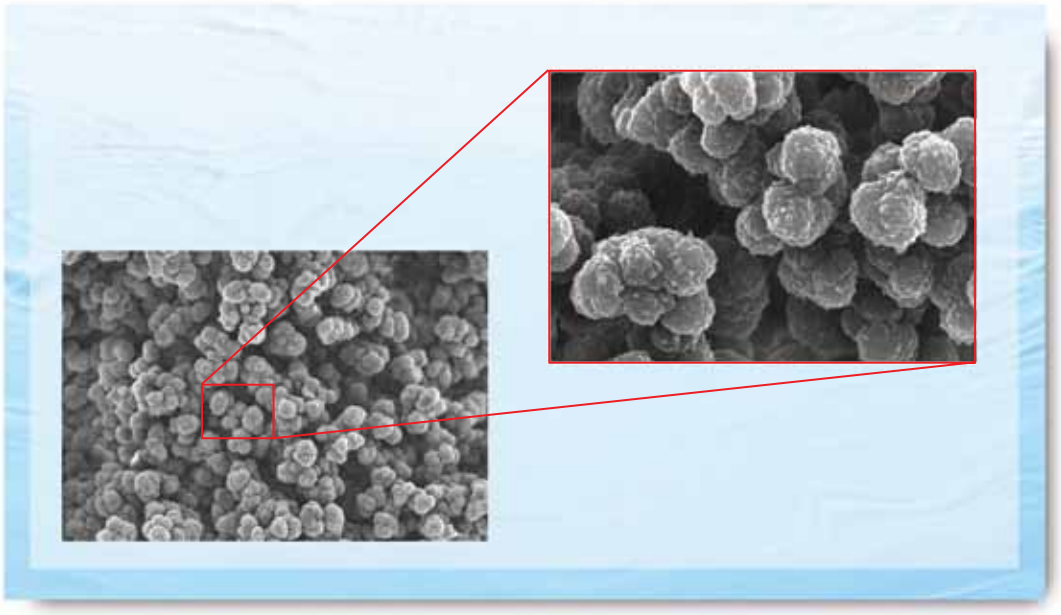
columns

PepSwift and ProSwift Capillary Monolithic Reversed-Phase Columns



PepSwift™ and ProSwift® monolithic columns are specially designed for high-resolution LC/MS analysis in protein identification, biomarker discovery, and systems biology. Based on a polystyrene divinylbenzene copolymer bed, the monolithic structure offers a high-quality alternative to traditional microparticulate sorbents, providing important advantages to the chromatographic separation. High-sensitivity proteomics and biotech applications are easily performed using these columns.

Now sold under the Thermo Scientific brand

Thermo SCIENTIFIC

High-Resolution Biomolecule Separations

- High-resolution for protein identification, biomarker discovery, and systems biology
- High-speed peptide and protein separations (<15 min)
- Highest sensitivity for LC/MS
- Highest column-to-column reprodu cibility
- Wide range of column i.d.s and lengths available
- Superior lifetime
- nanoViper™ fittings for easy column installation

Unique Bed Structure for Optimal LC Performance

Monolithic capillary columns, based on a polystyrene divinylbenzene copolymer, offer an alternative to the classical microparticulate sorbents, bringing important advantages to sample analysis. In contrast to traditional stationary phases, which consist of packed particles, the monolithic separation medium comprises a continuous, rigid, polymeric rod with a porous structure featuring superior pH stability (1–10). The lack of intraparticular void volume improves mass transfer and separation efficiency. These columns provide fast, high resolution separations of biomolecules, such as proteins and peptides.

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Separation of Peptides

PepSwift capillary monolithic columns are uniquely suited for the analysis of complex peptide samples. Available in diameters between 100 µm and 500 µm, these columns deliver fast, high-resolution separations. The excellent separation performance for peptides is demonstrated in Figure 1 by the analysis of a peptide mixture. Eight peptides are separated with baseline-to-baseline resolution in only 7 min, using a gradient from 0–25% acetonitrile in water, with 0.05% trifluoracetic acid (TFA). Peak widths at half height ranging from 1.6 to 3.5 s illustrate the high resolution achievable using PepSwift monolithic capillary columns. To achieve this efficiency, the use of a dedicated nano LC system with minimal dead volume is required.

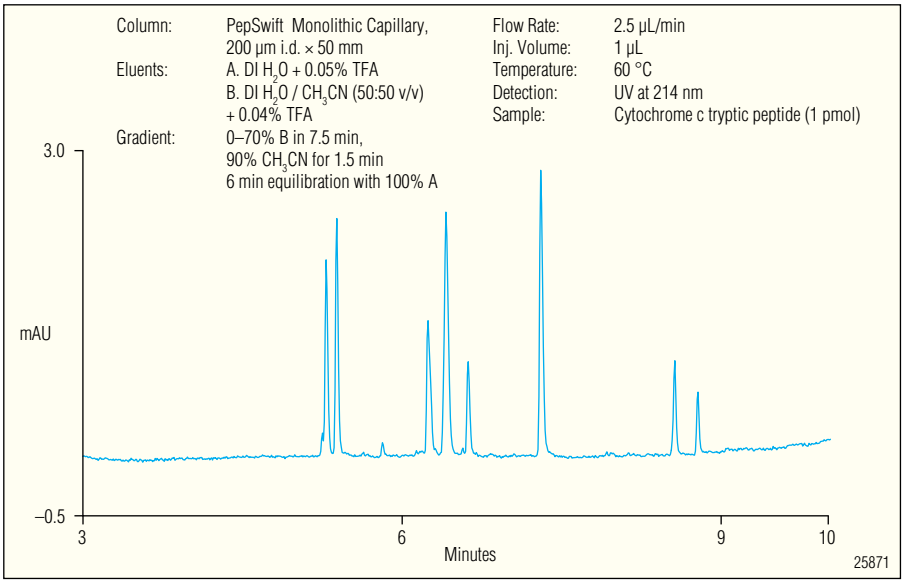

Figure 1. Separation of cytochrome c tryptic peptides using a PepSwift RP column.

High-Speed, High-Resolution LC-MS/MS Peptide Mapping

PepSwift capillary monolithic columns are ideally used with tandem mass spectrometers for peptide sequencing, both in on-line (ESI, nanospray) mode and off-line (MALDI) mode. In LC-MS applications, formic acid (FA) is often preferred as a mobile phase additive. The use of weaker acids reduces the discrimination effect for high-sensitivity LC/MS applications such as nano-electrospray. Independent of the mobile phase additive—TFA or FA—excellent separation efficiencies are routinely obtained. Figure 2 shows the MS base peak chromatogram of a fast and highly efficient LC-MS separation of six digested proteins.

MALDI MS/MS can also be applied in combination with PepSwift monolithic columns for peptide sequencing and protein identification. To preserve the chromatographic resolution of the separation on the PepSwift column, the use of a fast MALDI spotter such as the Probot™ is required.

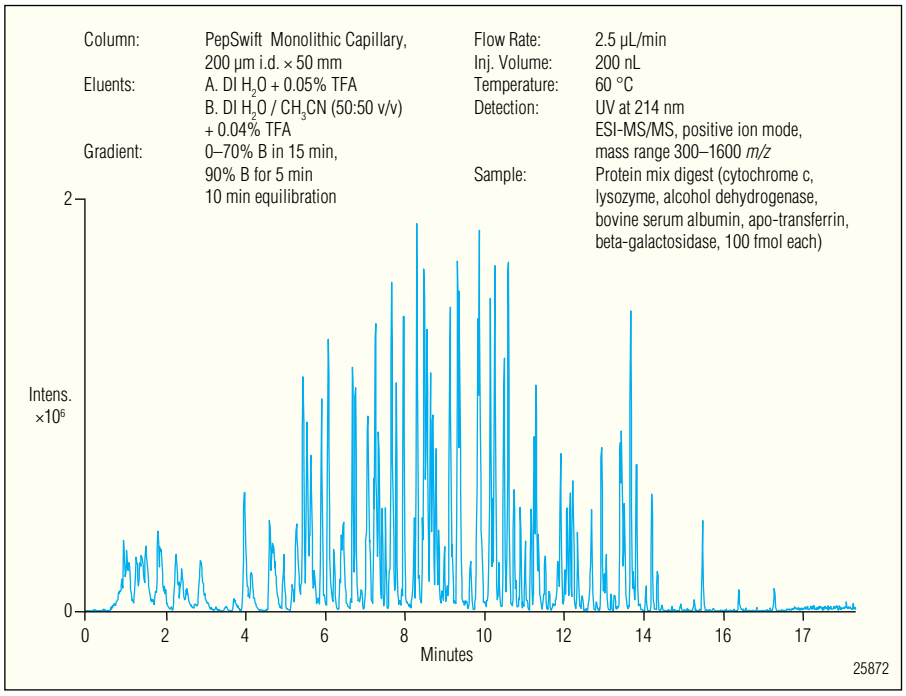

Figure 2. MS chromatograms of a capillary LC separation of over 100 peptide fragments, obtained in less than 15 min, using a PepSwift RP column.

Long Monolithic Columns For Maximum Peak Capacity Separations

PS-DVB monolithic columns target fast and high resolution biomolecule separations. The short (50 mm) columns are best suited for fast separations; long columns (250 mm) are the best choice for ultrahigh resolution separations. An example of a ultrahigh resolution peptide separation is shown in Figure 3. The separation of an *E.coli* tryptic digest sample was obtained in a 1 m PepSwift column by connecting four 25 cm long columns in series. A peak capacity of > 1000 was obtained using a 600 min gradient.

PepSwift Monolithic Trap Column for Desalting and Preconcentration

PepSwift monolithic trap columns can be used for preconcentration and desalting of samples consisting of peptides and proteins. The sample capacity of the PepSwift precolumns (200 µm i.d. × 5 mm) is in the range of 100 pmol for both peptides and proteins. The highest trapping efficiency is obtained when adding heptafluorobutyric acid as an ion-pairing agent.

PepSwift monolithic columns and trap columns are tailored to work with the UltiMate® 3000 RSLCnano and UltiMate 3000 Nano LC systems, providing highest efficiencies in standard as well as 2D-LC analysis.

Fast and High-Resolution Separations of Intact Proteins

The polymer-based PepSwift and ProSwift RP-10R monolithic columns provide excellent separation performance for both intact proteins and peptides. The use of short (5 cm) columns results in fast protein and peptide separations with sharp peaks, small peak volumes, and optimal peak shape. Thus, combined with the observed high peak capacity, these monolithic columns are perfectly suited for routine and high-throughput applications in proteomics and biopharmaceutical analysis.

Typical examples of high-efficiency and ultrafast protein separations are demonstrated in Figure 4A and 4B, respectively.

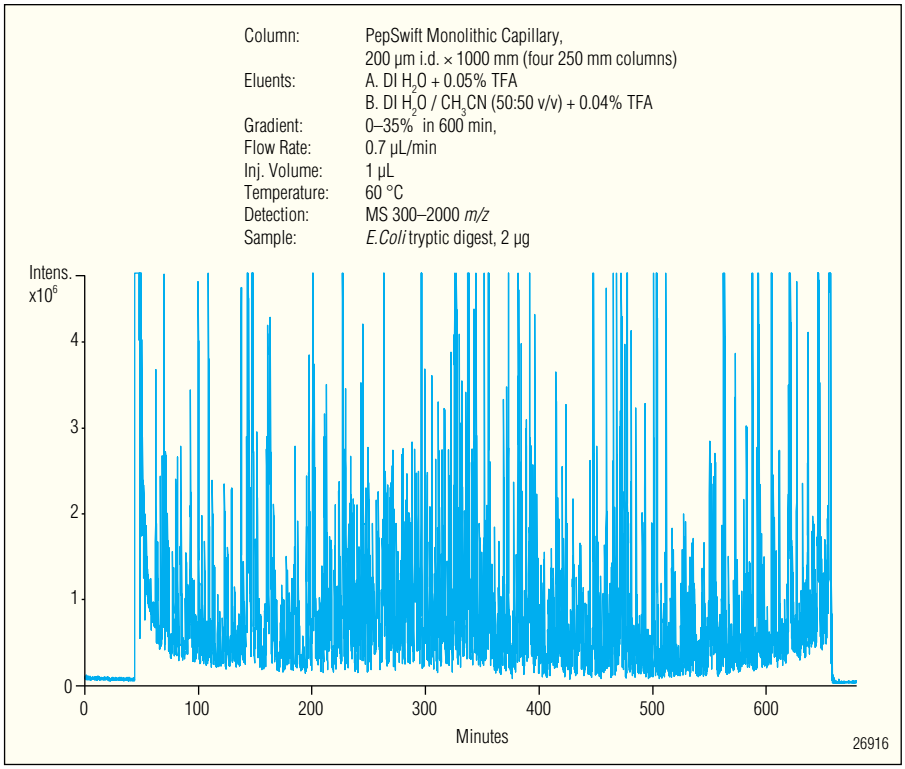

Figure 3. Ultrahigh resolution separation of an E. coli *digest on a 200 µm × 1000 mm long column (four 250 mm columns in series).*

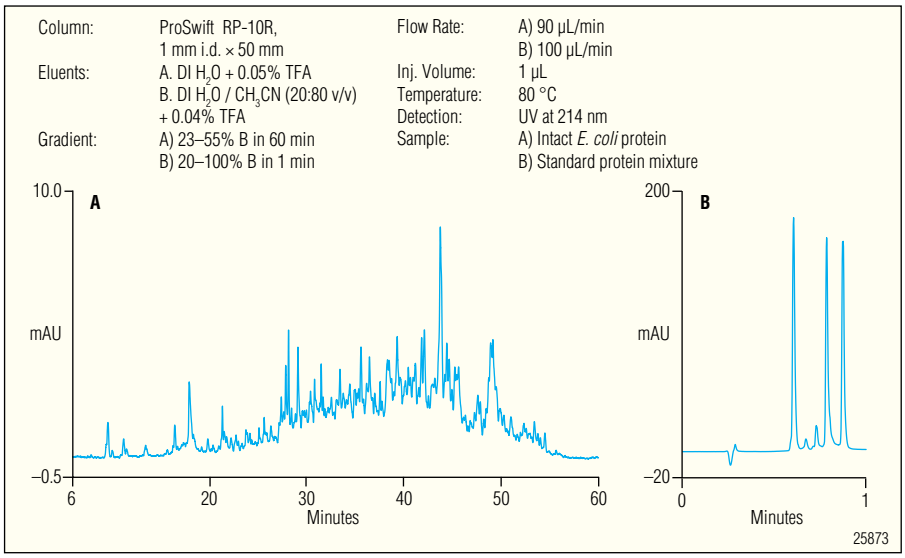

Figure 4. Examples of A) high-peak-capacity separation of intact E. coli *proteins, and B) ultrafast separation of a standard mixture of proteins, both obtained using the 1 × 50 mm ProSwift RP-10R monolithic column.*

Multi-Dimensional LC Separations for Proteomics

PepSwift and Proswift monolithic columns are ideally used in 2D-LC separations of peptides and proteins to improve the resolution of complex samples. Taking advantage of the short run times that can be achieved on monolithic columns, an entire 2D-LC/MS analysis can be performed within hours. The UltiMate 3000 Proteomics MDLC system is designed for desalting and gradient separations of peptides/proteins and for off-line and on-line separations. Proteins or peptides are desalted and preconcentrated on a monolithc trap column using a typical preconcentration setup followed by a gradient separation and UV or (tandem) MS detection.

Monolithic columns applied in 2D-LC typically yield much higher peak-capacity separations than one-dimensional LC. In off-line 2D-LC, the autosampler is used for injection and automated fraction collection. Figure 5 shows high-resolution second-dimension chromatograms using the 1 × 50 mm ProSwift RP-10R monolithic column, of different ion-exchange fractions obtained after the first-dimension *E.coli* separation (total peak capacity of 4900 obtained within 6 h).

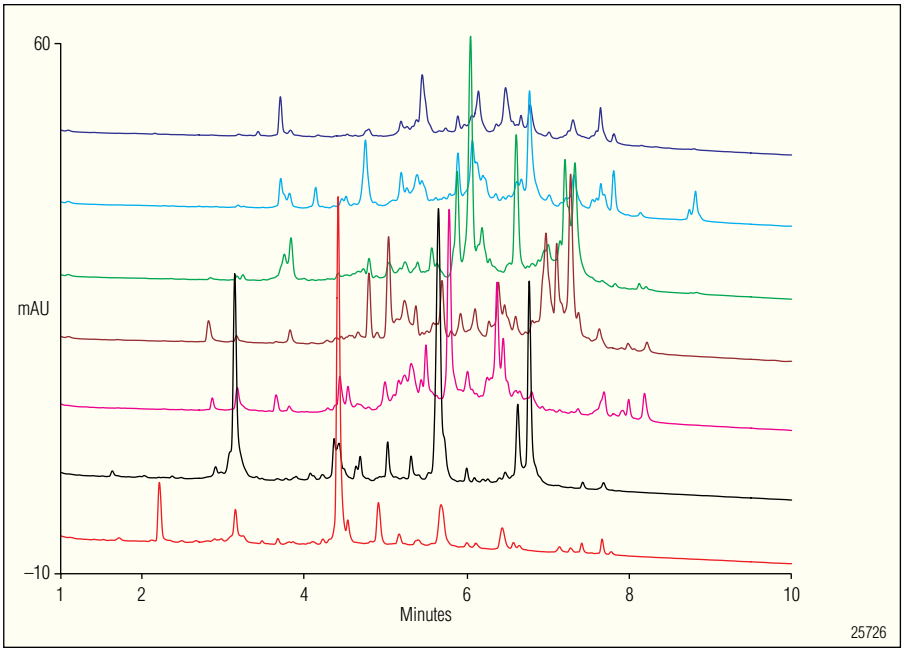

Figure 5. Second-dimension chromatograms of intact E. coli *proteins from first-dimension ion-exchange fractions, separated using the 1 × 50 mm ProSwift RP-10R monolithic column.*

SPECIFICATIONS

	PepSwift RP			ProSwift RP-10R
Typical Applications	Peptides, proteins, and oligonucleotides			Proteins and oligonuleotides
Column Dimensions	100 µm × 50 mm	200 µm × 50 mm	500 µm × 50 mm	1 mm × 50 mm
Base Material	Polystyrene/divinyl benzene	Polystyrene/divinyl benzene	Polystyrene/divinyl benzene	Polystyrene/divinyl benzene
Chemistry	Phenyl	Phenyl	Phenyl	Phenyl
Recommended Flow Rate	700–1000 nL/min	2–3 µL/min	15–25 µL/min	40–60 µL/min
Temperature	80 ˚C	80 ˚C	80 ˚C	80 ˚C
Maximum Pressure	4350 psi (30 MPa)	4350 psi (30 MPa)	4350 psi (30 MPa)	4350 psi (30 MPa)
Recommended Sample Quantity	5 ng*	20 ng*	0.12 µg*	2 µg**
Sample Capacity***	0.2 ng	0.6 ng	3.8 ng	0.5 µg
pH Stability	1–10	1–10	1–10	1–10
Solvent Compatibility	Most common RP solvents	Most common RP solvents	Most common RP solvents	Most common RP solvents (≥ 5% organic solvent)

*Tryptic digest of six proteins
***E. coli* proteins
*** Dynamic loading capacity is defined as the 10% increase in peak width measured at half height induced by injected mass of a single peptide (tryptic peptide of cytochrome c for PepSwift RP) and intact protein (cytochrome c for ProSwift RP-10R), respectively

ORDERING INFORMATION

In the U.S., call (800) 346-6390 or contact the Dionex Regional Office nearest you. Outside the U.S., order through your local Dionex office or distributor. Refer to the following part numbers:

PepSwift and ProSwift Monolithic Columns	Part Number
PepSwift Monolithic Nano Column, 100 µm i.d. × 5 cm	162348
PepSwift Monolithic Nano Column, 100 µm i.d. × 5 cm, nanoViper	164584
PepSwift Monolithic Nano Column, 100 µm i.d. × 25 cm, nanoViper	164543
PepSwift Monolithic Capillary Column, 200 µm i.d. × 5 cm	161409
PepSwift Monolithic Capillary Column, 200 µm i.d. × 5 cm, nanoViper	164557
PepSwift Monolithic Capillary Column, 200 µm i.d. × 25 cm, nanoViper	164542
PepSwift Monolithic Capillary Column, 500 µm i.d. × 5 cm	164087
PepSwift Monolithic Capillary Column, 500 µm i.d. × 5 cm, nanoViper	164585
ProSwift RP-10R Monolithic Capillary Column, 1 mm i.d. × 5 cm	164397
ProSwift RP-10R Monolithic Capillary Column, 1 mm i.d. × 5 cm, nanoViper	164586
PepSwift Trap Columns	
PepSwift Monolithic Trap Column, 200 µm i.d. × 5 mm (set of 2)	163972
PepSwift Monolithic Trap Column, 200 µm i.d. × 5 mm, nanoViper (set of 2)	164558

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