columns

Monolithic Capillary Columns

Monolithic capillary LC columns are specially designed for the fast separation of proteins and peptides by capillary LC. Monolithic capillary columns are based on a polystyrene divinylbenzene copolymer bed. The monolithic bed structure of the column offers a high-quality alternative to traditional microparticulate sorbents, providing important advantages to the chromatographic separation. High-sensitive proteomics applications are easily performed using these columns.

- *Polymeric monolithic stationary phases*
- *High-speed protein and peptide separations (<10 min)*
- *Highest separation efficiency of >200,000 plates/m routinely possible*
- *Highest column-to-column reproducibility*
- *Highest sensitivity in LC/MS*
- *Superior lifetime*

Alternative to Packed Columns

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 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 featuring superior pH stability (1–13). The lack of intraparticular void volume improves mass transfer and separation efficiency. These columns enable fast, high-resolution separations of biomolecules, such as proteins and peptides, using a short 5-cm-long capillary column with 200-µm i.d. For optimal ruggedness and ease of use, a PEEK housing protects the column. The inlet and outlet fitting form an integral part of the housing, allowing for zero dead volume connections.

DIONEX

Analysis of Peptides

For the evaluation of the Monolithic capillary columns, peptide and protein mixtures were analyzed. Figure 2 shows separation of a test mixture consisting of 9 peptides using a gradient from 0–25% acetonitrile in water, 0.05% trifluoracetic acid (TFA). In only 7 min, peptides were separated with baseline-to-baseline resolution. Peak widths at half height (PWHH), ranging from 1.6 to 3.5 s, illustrate the high resolution achievable using Monolithic capillary columns (see Table 1).

Analysis of Proteins

In proteomics, the analysis of protein digests is commonly performed for protein identification. The separation of intact proteins can sometimes yield additional information about the proteome or protein complexes. Therefore, the same Monolithic column has been evaluated for the separation of proteins. Figure 3 shows the separation of a protein mixture using a gradient of 20–50% acetonitrile in water, 0.05% TFA, in 15 min. Similar separation efficiencies are achieved for the peptides, illustrating the excellent performance of Monolithic capillary columns for both peptides and proteins.

Comparison of Peptide Separations with Formic Acid and TFA

In LC-MS applications, formic acid (FA) is preferred as a mobile phase additive. The use of weaker acids reduces the discrimination effect for high-sensitive LC/MS applications such as nanoelectrospray. Figure 4 shows the comparison using both additives for the separation of the peptide test mixture. Independent of the mobile phase additive—TFA or formic acid—an excellent separation has been obtained. Using formic acid as ion pair, PWHH increases only marginally and the peak height decreases by only 20–30%.

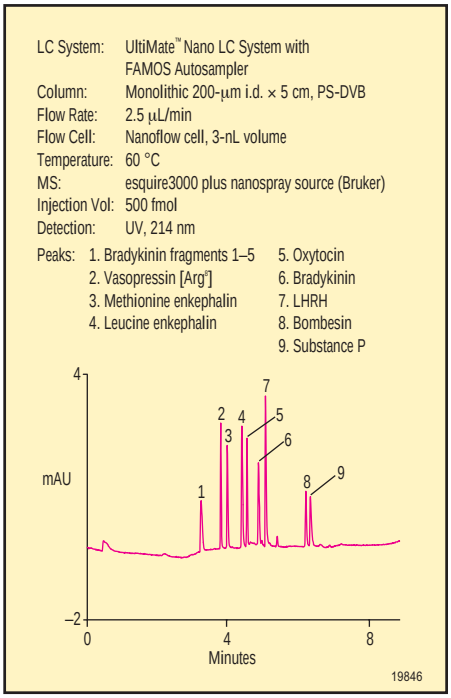

***Figure 2**. Separation of peptide test mixture*

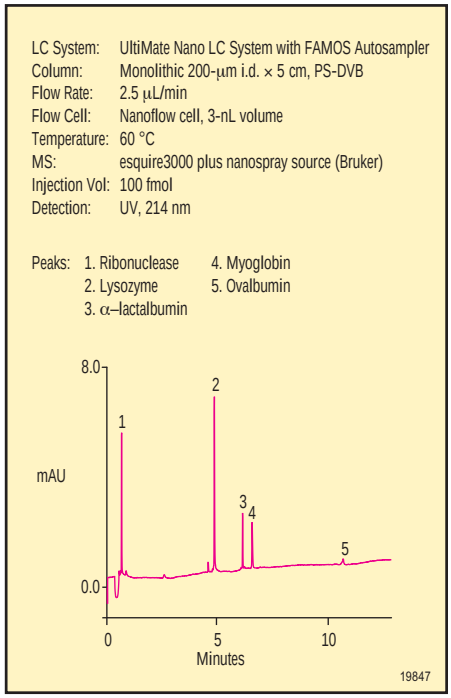

***Figure 3**. Separation of proteins.*

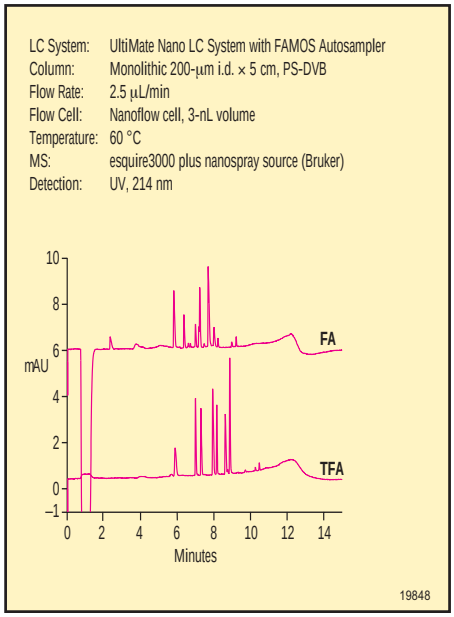

***Figure 4**. Separation of peptide test mixture.*

Table 1. Peak Width at Half Height (PWHH) for Peptides Separated on a Monolithic Columns

Peptide Number	Retention Time Minutes	PWHH Seconds
1. Bradykinin fragment 1–5	3.3	3.5
2. Vasopressin [Arg^8]	3.8	1.6
3. Methionine enkephalin	4.0	1.9
4. Leucine enkephalin	4.4	2.3
5. Oxytocin	4.6	1.6
6. Bradykinin	4.9	2.5
7. LHRH	5.1	1.9
8. Bombesin	5.1	1.9
9. Substance P	6.4	2.6

FAMOS™ and UltiMate™ Nano LC System

Sample Loadability of the Monolithic Columns

Figure 5 shows the sample loadability of the Monolithic capillary columns for both peptides and proteins. The elution behavior of proteins is shown for overloading conditions. The relative loading ability of Monolithic capillary columns has been tested for a 6-protein mixture using a gradient from 0% to 100% B in 20 min, (A) water/acetonitrile 98:2, 0.05% TFA, and (B) water/acetonitrile 20:80, 0.04% TFA. The following six proteins used in peak elution order were: (1) ribonuclease A, (2) cytochrome c, (3) lysozyme, (4) α-lactalbumin, (5) myoglobin, (6) ovalbumin. One protein was always in a constant low concentration, whereas the concentration of the other five proteins increased in the ratio 1:1, 1:10, 1:50 and 1:100. The protein chosen in low concentration is cytochrome c, always in a concentration of 0.1 pmol/µL. The analysis of the chromatographic results revealed a very good peak separation for concentration ratios of 1:1 and 1:10, whereas cytochrome c is only detected by a mass spectrometer in concentration ratios of 1:50 and 1:100, and identified in all four tested ratios. The deconvoluted spectra show the characteristic ion pattern for cytochrome c (right).

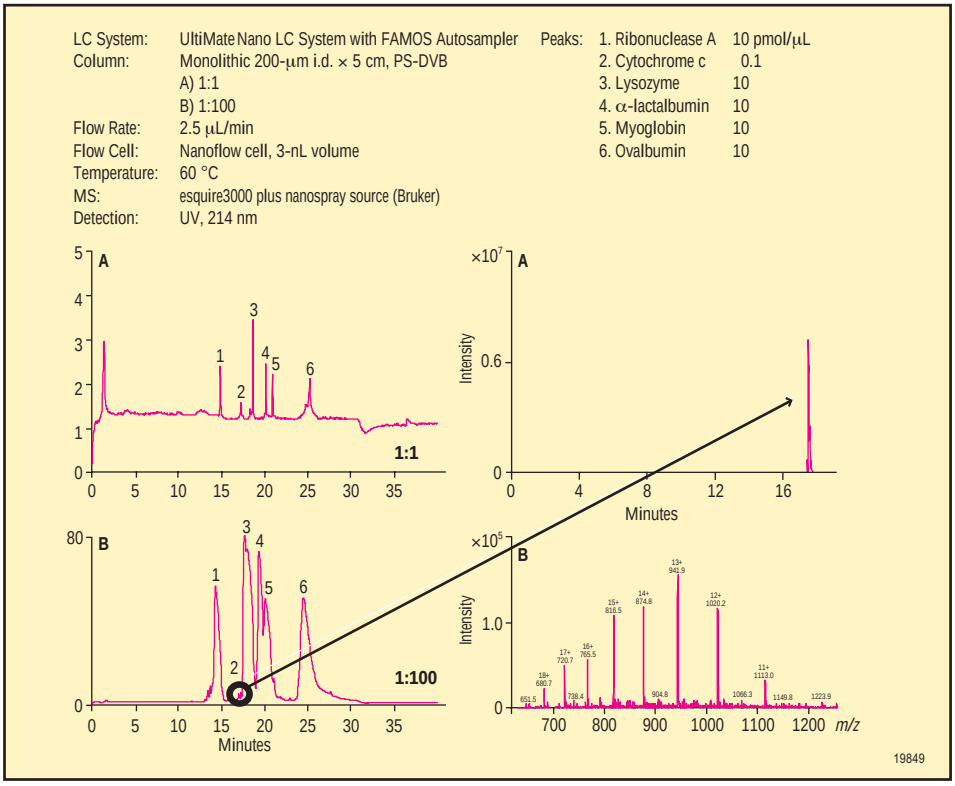

***Figure 5**. Sample loadability. Left: Chromatograms showing loading ability of Monolithic capillary column for cytochrome c vs other proteins: (A) ratio 1:1 and (B) 1:100. Inj. amount cytochrome c 0.1 pmol/µL, other proteins 10 pmol/µL. Proteins: (1) ribonuclease A, (2) cytochrome c, (3) lysozyme, (4) α-lactalbumin, (5) myoglobin, (6) ovalbumin (in peak elution order). Right: cytochrome c; protein mix 1:100, Extracted ion chromatogram (EIC) (above) and deconvolution of cytochrome c mass spectrum (below). EIC (A) and MS spectrum (B) of cytochrome c, both from chromatogram left, below.*

Excellent Separation Performance for Proteins and Peptides

Monolithic capillary columns (polymer-based) show excellent separation performance for both proteins and peptides, and the same column can be used for peptides and intact proteins. Using short 5-cm-long columns results in very fast protein and peptide separations with PWHH of a few seconds only and an optimal peak shape with extremely narrow peaks and small peak volumes. Thus, combined with the observed high peak capacity, Monolithic capillary columns are perfectly suited for routine and high-throughput applications in proteomics. The fast separations result in a considerable reduction of analysis time.

Coupled to a mass spectrometer, these columns allow for sensitive analysis independent of the mobile phase additive (TFA or FA). The rigid structure results in excellent column lifetime due to the absence of any voiding and an improved chemical stability at extreme pH. Monolithic capillary columns have remarkably high plate counts of 200,000–250,000 plates/meter. To achieve separation efficiencies of up to 250,000 plates/meter, the use of a dedicated nano HPLC system with zero dead volumes is required.

Monolithic capillary columns are manufactured according to strict specifications to ensure column-to-column reproducibility. Each column is shipped with a certificate of analysis and an individual test chromatogram.

ORDERING INFORMATION

To order 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.

Description	Part Number
Monolithic capillary column, 200-µm i.d. × 5 cm (PS-DVB)	161409
UltiMate upgrade kit for Monolithic capillary columns	161407
UltiMate upgrade kit for Monolithic capillary columns with nanoflow cell	161438
UltiMate calibrator cartridge for Monolithic columns, 200-µm i.d.	161406

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