LC PACKINGS
A DIONEX Company

Application Note 522

Monolithic Capillary Columns in LC-MS Proteomics

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 that 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, which allows for very fast separations of biopolymers.

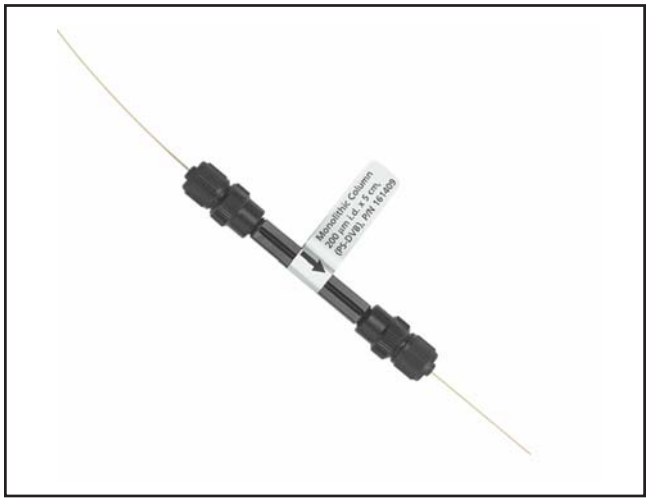

Figure 1. *Monolithic capillary column in protective housing, 200-μm i.d. × 5 cm.*

INSTRUMENTATION

All experiments were performed on the UltiMate™ Plus Nano and Capillary LC System equipped with a special 3-nL UV flow cell, and the FAMOS™ Micro Autosampler. The Monolithic capillary column, 200-μm i.d. × 5 cm, made of PS-DVB (polystyrene-divinylbenzene polymer), was thermostatted at 60 °C, using the UltiMate column oven. UV detection was performed at 214 nm, and the flow rate was 3 μL/min (gradient mode). For LC-MS, the system was coupled on-line to a fast-scanning, ion-trap MS (esquireHCT, Bruker-Daltonics) equipped with a nanospray source.

HIGH-RESOLUTION PEPTIDE SEPARATIONS

Figure 2 shows the separation of a test mixture consisting of 9 peptides (see Table 1). A gradient from 0–25% acetonitrile in acidified water (0.05% TFA) is performed in 7 min, resulting in a fast baseline separation of all peptides. Peak widths at half height (PWHH) of only 1.6–3.5 s illustrate the fast separations that are achievable using a Monolithic capillary column.

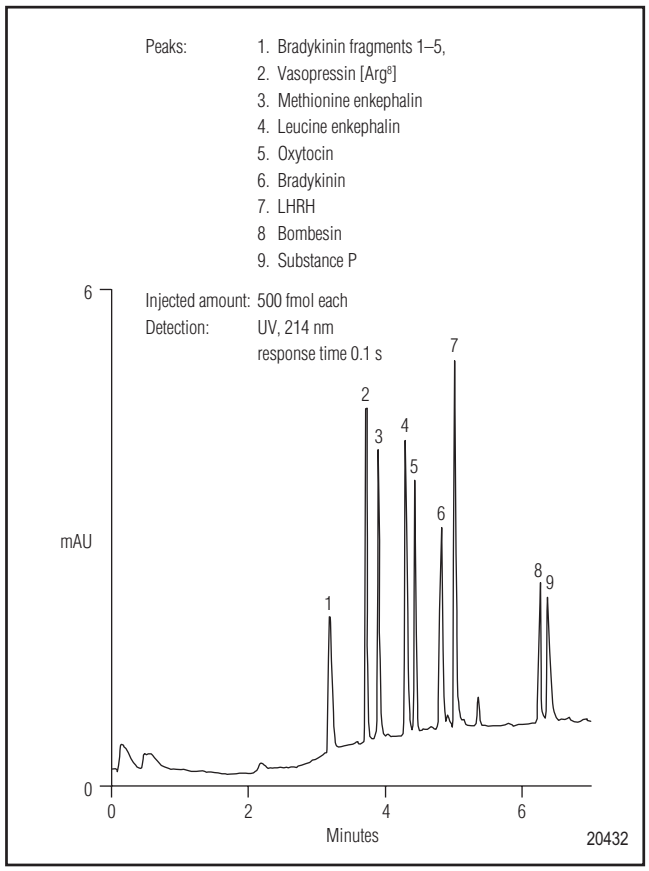

Figure 2. *Separation of peptide test mixture.*

TABLE 1. PEAK WIDTH AT HALF HEIGHT (PWHH) FOR PEPTIDES SEPARATED ON A MONOLITHIC CAPILLARY COLUMN

Peptide	Retention time min	PWHH s
1. Bradykinin fragment 1–5	3.3	3.5
2. Vasopressin [Arg^8]	3.8	1.6
3. Methionin enkephalin	4.0	1.9
4. Leucin 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	6.3	2.0
9. Substance P	6.4	2.6

FAST LC/MS ANALYSIS

Figure 3 shows the fast separation of the tryptic peptides from 13 proteins. Peak capacities of up to 150 peaks in less than 15 min are routinely observed. PWHH is in the range of only 3 s, illustrating the tremendous separation performance of Monolithic capillary columns.

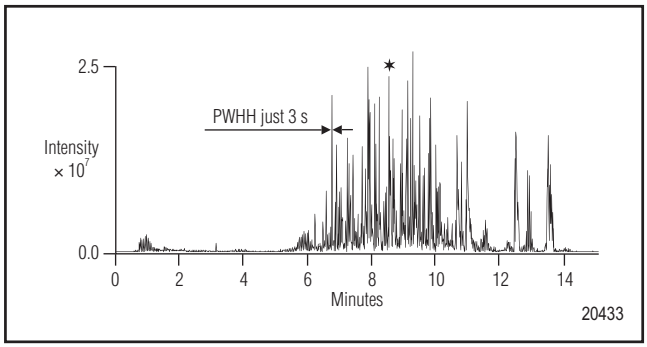

Figure 3. *Capillary LC-MS separation of 13 digested proteins. Up to 150 peptides separated in less than 15 min (courtesy Dr. Detlev Suckau, Bruker-Daltonik, Bremen, Germany).*

From an arbitrarily chosen peak eluting at 8.6 min and marked with an asterisk (*), a full MS scan was performed (Figure 4). The MS scan revealed this peak consisted of 4 precursor ions. With a scan speed of 26,000 amu/s and unit resolution by the esquireHCT, it was still possible to perform MS-MS experiments of each precursor ion and identify the corresponding peptide. Overall, more than 184 peptides from this 15 min chromatographic run could be identified by MS-MS (Figure 5).

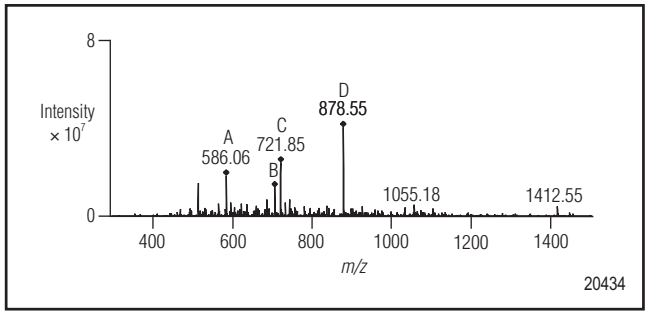

Figure 4. *MS data of the peak eluting at 8.6 min (courtesy Dr. Detlev Suckau, Bruker-Daltonik, Bremen, Germany).*

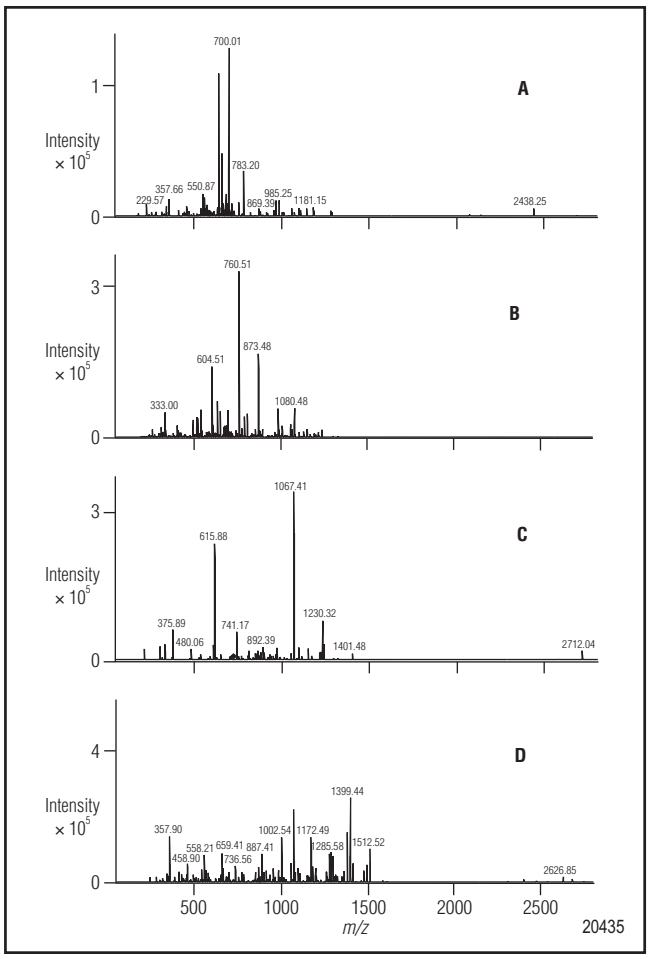

Figure 5. *MS-MS scan of the 4 detected precursor ions (courtesy Dr. Detlev Suckau, Bruker-Daltonik, Bremen, Germany).*

CONCLUSIONS

Monolithic capillary columns (polymer-based) show excellent separation performance. The same column can be used for both protein and peptide separations. Using short columns of 5 cm length very fast peptide separations with PWHH of a few seconds and of high sensitivity are achieved. Another advantage of the monolithic structure is the very robust column bed, resulting in zero voiding and superior column lifetime. Coupling these columns to ESI-MS results in very fast and sensitive LC-MS analysis, making these columns ideally suited for high-throughput LC-MS proteomics.

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