LC PACKINGS
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Application Note 525

LC-MALDI MS Using Monolithic Capillary Columns

INTRODUCTION

Monolithic capillary columns, based on a polystyrene-divinylbenzene polymer (PS-DVB), show excellent separation performance for both proteins and peptides. Using short 5-cm length columns results in very fast protein and peptide separations with a peak width at half height (PWHH) of only a few seconds. Another advantage of the monolithic structure is the very robust column bed, resulting in zero voiding and a superior column lifetime.

EXPERIMENTAL

A complex protein digest sample was separated on 200-µm i.d. by a 5-cm Monolithic capillary column using an UltiMate™ Plus Capillary LC System. This capillary LC system was equipped with a capillary flow sensor to assure precise flow delivery and was on-line coupled to a Probot™ Microfraction Collector for subsequent fraction collection. Fractions were collected every 10 s on MALDI targets. Probot's robotic system moves only the collection table and not the collection needle (Figure 1), thus unique precision of ±2 µm is achieved routinely. During fractionation, an alpha-cyano-4-hydroxy cinnamic acid (CHCA) solution was added 1:1 to the effluent as matrix solution. The fractionated peptides were further analyzed by MALDI-TOF MS.

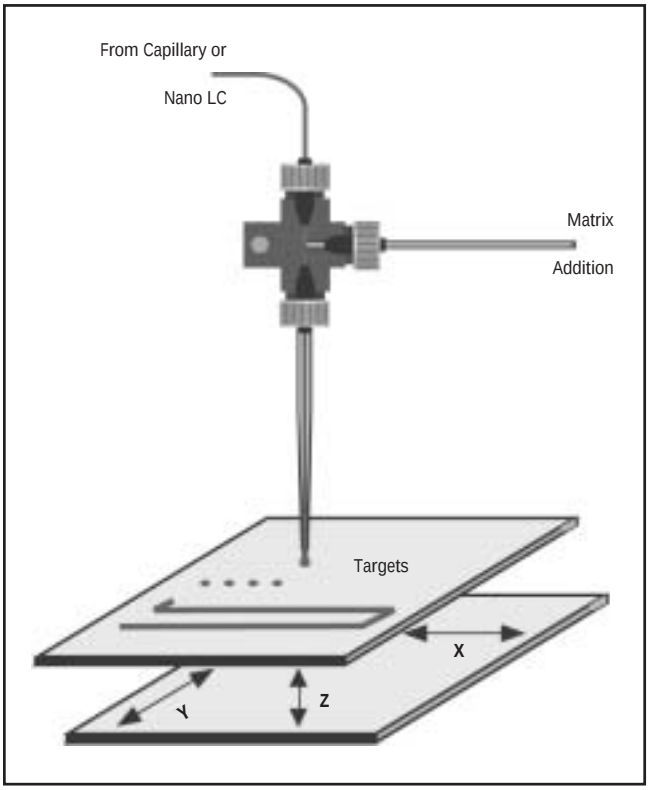

***Figure 1**. X, Y, Z table movement with static needle for high-precision sample spotting on MALDI targets.*

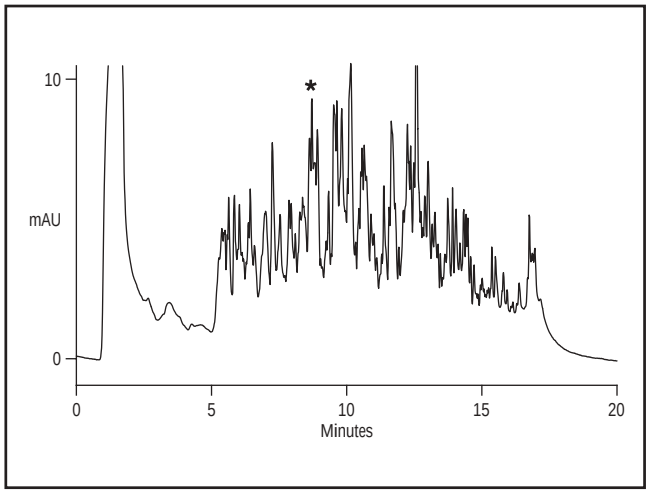

***Figure 2**. UV trace of a digested protein mixture separated on a 200-µm i.d. × 5 cm Monolithic capillary column and subsequent on-line fraction collection on MALDI targets.*

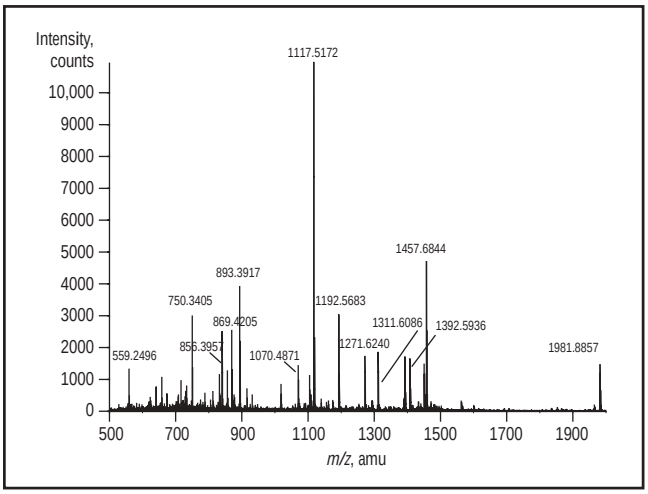

***Figure 3**. MS data from spot #41 (marked with an asterisk in Figure 2).*

Figure 2 shows the separation of the digested proteins. A gradient from 0 to 70% acetonitrile is performed in 10 min, resulting in a rapid separation of the peptides. Individual peaks were collected on a high-density MALDI target for subsequent MS analysis. Figure 3 shows the MS spectrum of an arbitrarily chosen peak marked with an asterisk (spot #41).

Coupling Monolithic capillary columns to MALDI-MS results in very fast and sensitive LC-MS analysis, making these columns ideally suited for high-throughput LC-MS proteomics.

CONDITIONS

LC system:	UltiMate Plus (P/N 163628) FAMOS™ (P/N 163654) Probot (P/N 160560)
Loop:	1 µL (full-loop injections)
Column:	Monolithic capillary column (P/N 161409) 200-µm i.d. × 5 cm
Flow rate:	2 µL/min
UV-Vis wavelength:	214 nm
Mobile phase:	(A) H_20 – 0% acetonitrile + 0.05% TFA; (B) H_20 – 50% acetonitrile + 0.04% TFA
Gradient:	0–70%B in 10 min
Matrix solution:	10 mg/mL alpha-cyano-4-hydroxy cinnamic acid (CHCA)
Sample	Tryptic digest of 17 proteins

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* Designed, developed, and manufactured under an NSAI registered ISO 9001 Quality System.

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