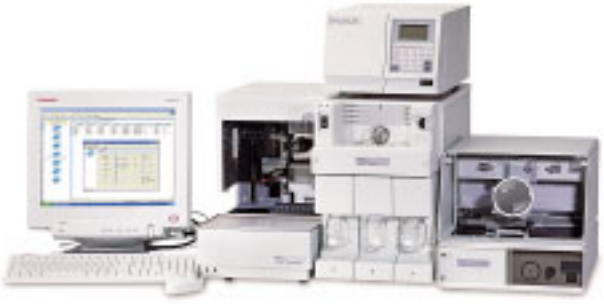
Performance PerSPECtives

Waters LC-MALDIprep™ Sample Collection Module: Improved Peptide Mass Fingerprinting from Continuous Sample Tracks

Introduction: Peptide mass fingerprinting (PMF) is a useful method for rapid identification of proteins using MALDI mass spectrometry. PMF identifies proteins based on two factors: 1) the protein's unique sequence of amino acids, and 2) the fact that the digestion enzyme cleaves the protein at one or two specific amino acids. The digestion of the protein generates a set of peptides that is unique. Mass spectrometry measures the masses of these peptides. The resulting masses are then searched against a protein database to find a matching sequence. Traditionally, PMF is performed by MALDI-TOF/MS analysis of non-separated digests deposited onto sample wells. While effective for mixtures of a few proteins of equi-molar concentration, this spotting approach has limited dynamic range. In addition, poor sensitivity is observed for low abundance proteins when mixed with high abundance species. The addition of LC-MALDI/MS technology to the analysis of protein digests can improve the dynamic range for the analysis, enable the performance of PMF on more complex protein mixtures, and yield greater sensitivity for samples containing both low and high abundance proteins. This report describes the separation of a four-protein digest mixture on Waters CapLC® System. The separated material was continuously collected onto a matrix pre-coated MALDI target (along a 45 mm long by 2 mm wide sample track) using Waters LC-MALDIprep™ Sample Collection Module. The MALDI/MS instrument is then used to scan along this track to generate a mass chromatogram of the separation for use in PMF.

Figure 1: The LC-MALDI/MS System consists of Waters CapLC® System, the LC-MALDIprep™ Sample Collection Module, and a MALDI/MS instrument (not shown).



Experimental: The analyzed sample consisted of four protein standards (Sigma) digested using porcine trypsin (Promega). The amount of protein loaded onto Waters CapLC® System was 1270 fmol for apomyoglobin, and 10 fmol each for actin, ADH and cytochrome *c*. The flow rate was 10 uL/min with a 2% change in acetonitrile concentration per minute (from 3 to 53% Acn) using a water / acetonitrile (0.1 % TFA) binary solvent system. The separation was performed at 30°C on a Waters Symmetry® C_{18}, 3.5 µm, 0.32 x 50 mm column. Eluent was collected onto the alpha-Cyano-4-hydroxycinnamic acid pre-coated MALDI target at a rate of 2.81 mm/min. The initial desolvation temperature was 65°C. The desolvation temperature was gradually lowered during the gradient separation to a final temperature of 50°C. A MALDI-TOF/MS (Micromass U.K. Ltd.) instrument was used to scan along the length of the track to generate a mass chromatogram. This mass chromatogram was processed to retrieve a list of peptide mass values for PMF analysis.

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Figure 2: LC-MALDI/MS mass chromatogram of the four-protein digest. Some of the apomyoglobin tryptic peptide peaks are labeled.

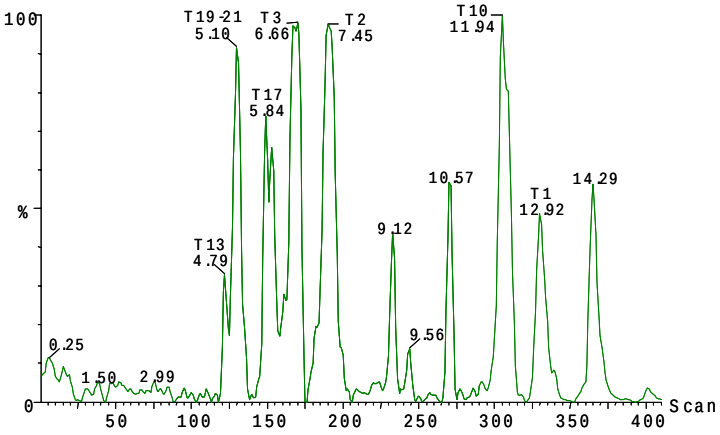

Figure 3: Comparing mass spectra of three of the tryptic peptides from the 10 fmol actin protein digest. The LC-MALDI/MS analysis is in the top view. The traditional spotted sample MALDI-TOF/MS results are in the bottom view. Signal-to-noise for LC-MALDI/MS is significantly higher than from the traditional spot method.

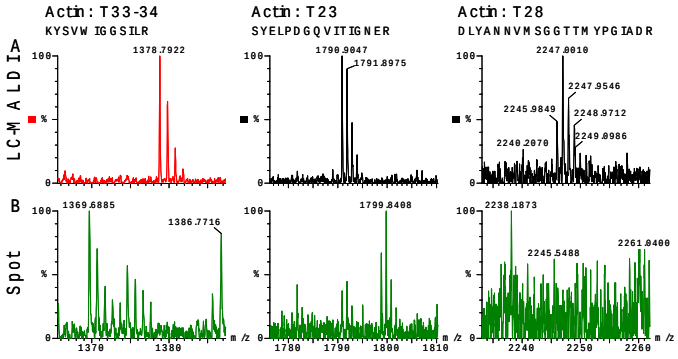

Table 1: PMF Results from LC-MALDIprep versus Sample Spotting Preparation Method for Actin Tryptic Peptides (10 fmol) in a Four-Protein Digest Mixture

LC-MALDIprep Method	Load (fmol)	Matched Peptides	% Seq. Cov
Actin	10	9	31
Sample Spot Method	Load (fmol)	Matched Peptides	% Seq. Cov
Actin	10	1	4

Results: The mass spectral signal-to-noise ratio from the LC-MALDI/MS samples was at least 4 times greater than observed using a traditional sample spotting method for the 10 fmol peptides (Figure 3). This is due to 1) improved dynamic range using the CapLC separation and 2) improved sensitivity due to improved concentration (3X increase) of separated samples during LC-MALDIprep compared to use of traditional MALDI sample wells. The PMF results (Table 1) indicate that many more peptides can be detected from the LC-MALDI/MS system versus use of traditional sample spotting techniques. This effect is even more pronounced as the protein mixture increases in complexity and dynamic range.

Summary:

1. Waters LC-MALDIprep Sample Collection Module can be used as an interface between LC separations and MALDI/MS for peptide mass fingerprinting.
2. LC-MALDI/MS technology yields significantly improved signal-to-noise compared to use of traditional sample spotting techniques.
3. PMF is significantly improved using LC-MALDI/MS technology, especially for low abundance protein digests mixed with high abundance protein digests.
4. This approach could be applied to the analysis 1-D or 2-D gel digests where low abundance proteins are difficult to detect using traditional sample spotting methods.