

Increased Sensitivity for Protein Digest Analysis with a High Capacity AP-MALDI Trap

Patrick D. Perkins, Bryan D. Miller, Christine A. Miller, Alex Mordehai

Agilent Technologies, Santa Clara, CA 95051 USA

Proteolytic digests of complex protein samples (e.g., whole cell lysates or tissue extracts) generate a wide range of individual peptide abundances. Detection of peptides representing the low abundance proteins is of increasing interest in proteomics studies. AP-MALDI with ion trap mass spectrometry is an emerging technique for high sensitivity detection and identification of peptides in digest mixtures. Analytical throughput and sensitivity performance are determined by the number of ions measured per unit time. In this work, we examine the performance of a newly optimized 3D quadrupole ion trap mass analyzer for this application.

Standard protein digests were analyzed using the AP-MALDI source on a newly designed, high capacity ion trap. Samples were spotted on target plates using CHCA matrix that was pre-mixed with the sample. MS and MS/MS sensitivity were determined using protein digests and peptide standards. In addition, dynamic range was investigated.

Full scan and MS/MS data could readily be acquired on the AP-MALDI high capacity trap below 1 fmol on the target if careful attention was paid to sample preparation, target cleanliness, and instrument parameters. The ultimate sensitivity was approximately 60 amol BSA protein digest in full scan mode (Figure 1), or approximately 125 amol protein digest in MS/MS mode (Figure 2). These amounts are roughly four-fold less than reported previously [1,2], and the improvement is attributed primarily to the higher effective ion capacity of the trap. The dynamic range for an analysis of two protein digests (ratio of the amounts of the two) was determined to be about 20, though it is understood the protein digest response depends on many factors—completeness of digestion, identity of the starting protein, enzyme, etc.

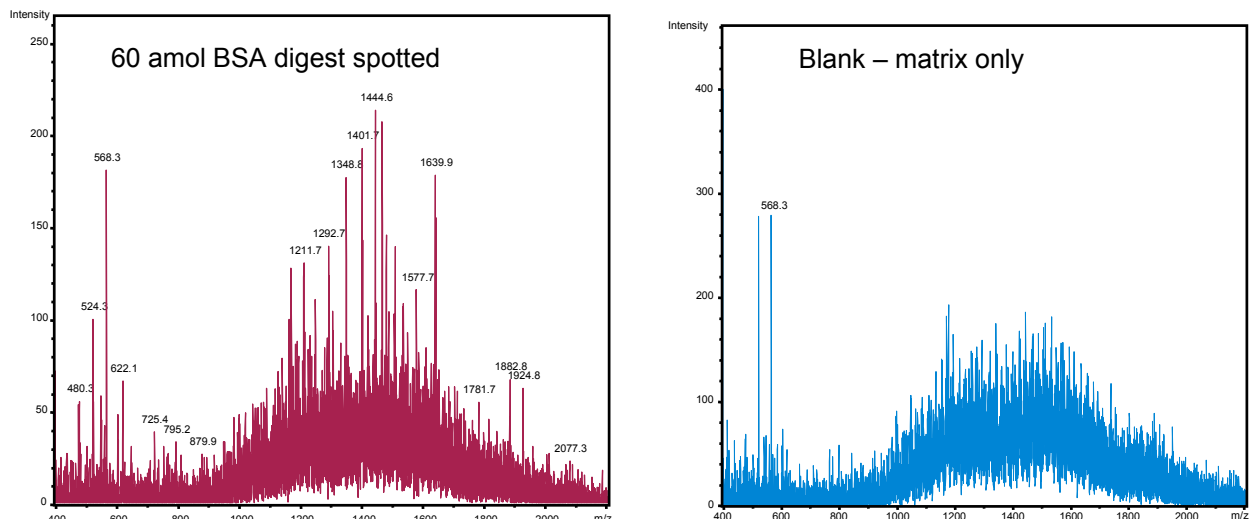


Figure 1. 60 amol BSA digest, full scan MS mode, and analysis of blank matrix

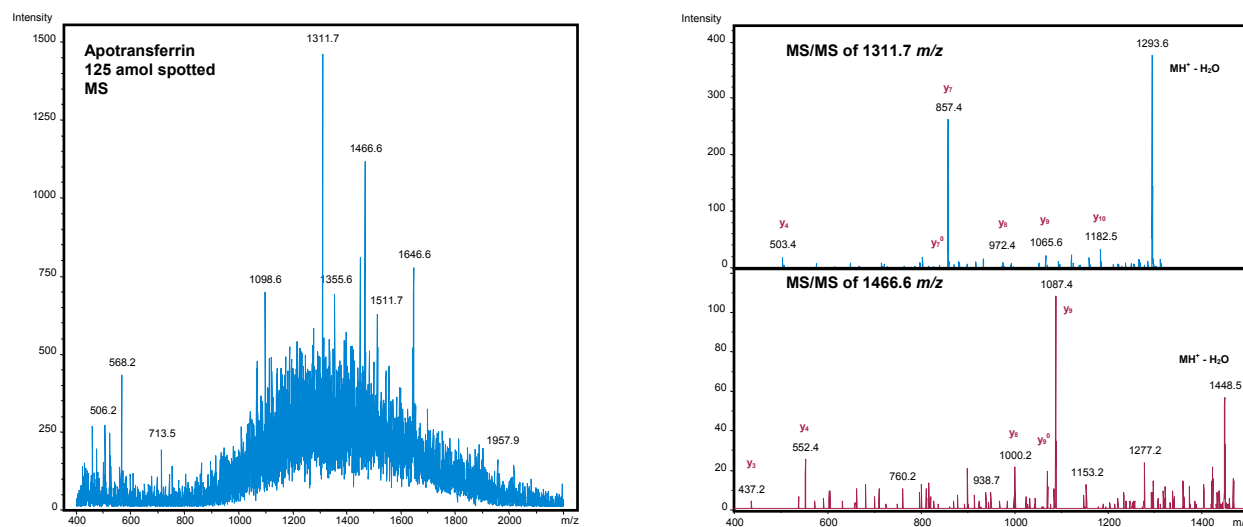


Figure 2. 125 amol apotransferrin digest, MS/MS mode, and representative MS/MS spectra

1. Doroshenko, V. M.; Laiko, V. V.; Taranenko, N. I.; Berkout, V. D.; Lee, H. S.; *Int. J. Mass Spectrom.* **2002**, *221*, 39–58.
2. Miller, C. A.; Yi, D.; Perkins, P. D.; *Rapid Commun. Mass Spectrom.* **2003**; *17*, 860–868.