

High Resolution Precursor Ion Selection on the 4800 MALDI TOF/TOF™ Analyzer



Introduction

High resolution selection of precursor ions is a prerequisite for complex mixture analysis by MS/MS techniques. Without such resolution, peptides separated from the precursor of interest by a few daltons may also be selected for MS/MS, resulting in a complex fragmentation pattern containing product ions from more than one peptide. This will significantly complicate database searching, *de novo* sequence information as well as accurate quantitation using iTRAQ™ reagents obtained on that peptide.

A major advantage of the 4800 MALDI TOF/TOF™ Analyzer is high selectivity of the QuanTIS™ timed ion selector (TIS), an improved precursor ion selector which can select a narrower range of ions *without* a loss of sensitivity across the broad mass range that is typically employed in proteomics and biomarker studies.

Key Benefits

- High resolution precursor ion selection over a wide mass range without significant reduction the isolated precursor intensity
- Unrivaled precursor selection among all tandem time-of-flight instruments
- Equivalent precursor ion resolution to commercially available ion traps and quadrupole instrumentation
- Improved confidence in MS/MS database search results and *de novo* sequence elucidation

Discussion

The performance of the 4800 MALDI TOF/TOF™ Analyzer TIS was demonstrated by the isolation of three peptides of distinctly different molecular weight from a mixture that also contained two additional peptides (in each molecular weight range) that were of similar molecular weight to the peptide targeted for isolation.

A mixture of 9 peptides (3 of mass 900.5 to 904.5, 3 of mass 1293.7 to 1299.6 and 3 of mass 2199.9 to 2208.7) was prepared and deposited onto a MALDI plate using alpha-cyano-4-hydroxycinnamic acid as matrix. These were mixed in equal proportions at a concentration of ~100 fmol/uL.

Figure 1 is the result of an experiment designed to isolate the peptide of m/z 902.5 (RKRSRAE) with minimal contribution from the other two peptides of similar molecular weight (m/z 900.5, RPKPQQF and m/z 904.5, PPGFSPFR). The top panel of Figure 1 shows data collected with the TIS wide open to demonstrate the presence of the three peptides in the mixture. Collection of MS/MS data under these conditions is of limited value to a discovery proteomics experiment as the resulting spectrum would be a composite of the fragment ions of all three components, and this would significantly increase the difficulty of sequence elucidation of each peptide. The bottom panel of Figure 1 depicts analysis of the same sample with a higher TIS resolution (resolution = 400). In this example, the peptide of m/z 902.5 has been clearly isolated from the higher molecular weight (m/z 904.5) component. The lower molecular weight peptide (m/z 900.5) has been significantly suppressed (>95%). Under these experimental conditions high precursor ion specificity was achieved with in this mass range with minimal suppression of the signal detected for the component of interest.

This precursor selection resolution is comparable to that achievable by a trap or quadrupole isolating ions formed by ESI. In ESI, the peptides at nominal mass 900Da would likely be observed in the 2⁺ charge state making the isotope spacing 0.5 *m/z*. Running the trap or quad in the tight “unit” resolution mode would still allow leak through of the neighboring peaks with a reduction in precursor intensity greater than that observed on the 4800.

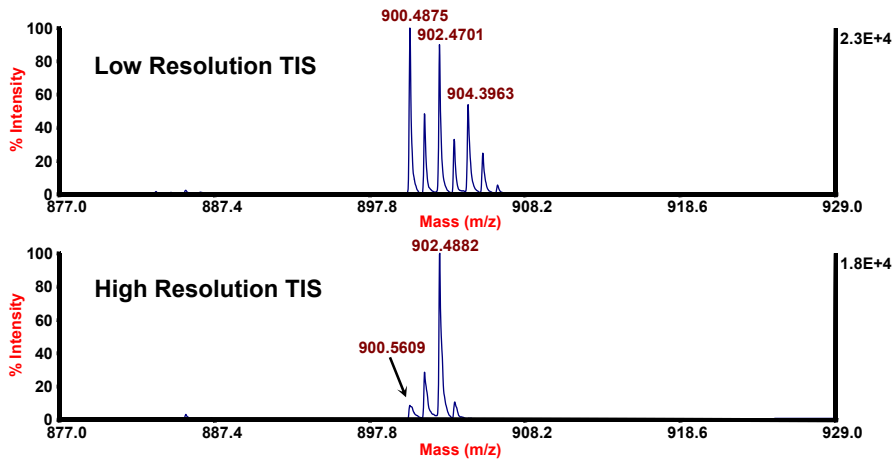


Figure 1 Specific isolation a peptide of *m/z* 902.5 (RKRSRAE) when present in a mixture containing two neighboring peptides at *m/z* 900.5 (RPKPQQF) and *m/z* 904.5 (PPGFSPFR). Top panel demonstrates an open TIS, with all peptide ions isolated simultaneously. Bottom panel demonstrates high resolution ($r \approx 400$) isolation of the peptide of *m/z* 902.5

The second example (Figure 2) demonstrates specific isolation of Angiotensin I (DRVYIHPFHL, *m/z* 1296.7) from the nine component mixture and specifically from two peptides of similar molecular weight (*m/z* 1293.7 Ac-ASQKRPSQRHG and *m/z* 1299.6 SYSMEHFRWG). The concentration of each peptide in the mixture was ~100 fmol/ μ L. The top panel of Figure 2 shows data collected with the TIS wide open to demonstrate the presence of these three peptides. Again, collection of data under these conditions would yield

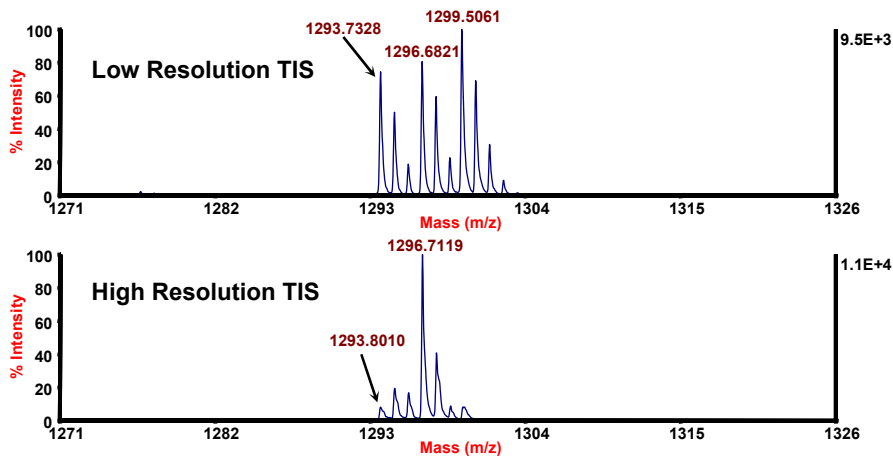


Figure 2 Specific isolation of Angiotensin I (DRVYIHPFHL, *m/z* 1296.7) when present in a mixture containing eight other peptides two of which were detected at *m/z* 1293.7 (Ac-ASQKRPSQRHG) and *m/z* 1299.6 (SYSMEHFRWG). Top panel demonstrates an open TIS, with all peptide ions isolated simultaneously. Bottom panel demonstrates high resolution ($r \approx 400$) isolation of Angiotensin I.

an MS/MS spectrum comprised of the fragment ions of all three components, and this would significantly decrease the probability of sequencing all three peptides. The bottom panel of Figure 2 depicts analysis of the same sample with a TIS resolution set to 400. In this example, while suppression of both the high and low mass components was > 95% the signal for Angiotensin I (m/z 1296.7) was unaffected and clearly isolated from both components. Again, a trap or quadrupole ion selector would have difficulty achieving this level of selectivity with no suppression on ions formed by ESI ionization.

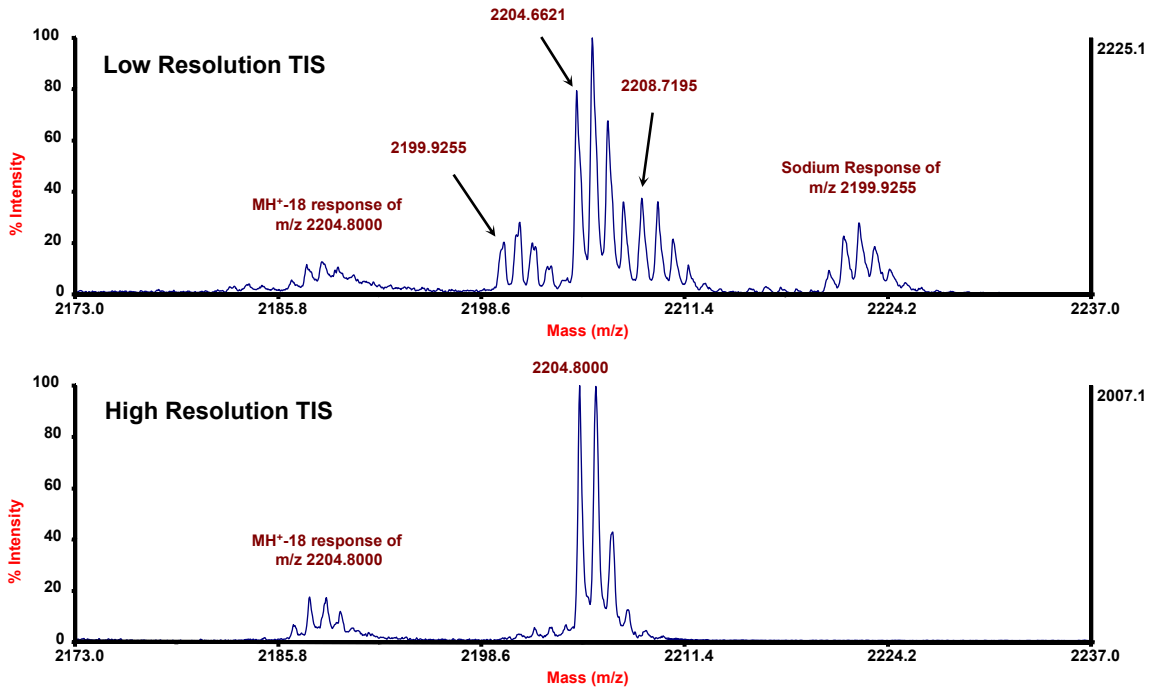


Figure 3 Specific isolation of peptide of m/z 2204.0 (DEGPYRMEHFRWGSPPKD) when present in a mixture containing eight other peptides two of which were detected at m/z 2199.1 (GWTLSAGYLLGPQQFFGLM-NH₂) and m/z 2208.1 (CTHGIRPVVSTQLLLNGSLAE). Top panel demonstrates an open TIS, with all peptide ions isolated simultaneously. Bottom panel demonstrates high resolution ($r \approx 400$) isolation of peptide of m/z 2204.0.

The third example (Figure 3) demonstrates specific isolation of a higher mass peptide at m/z 2204.0 (DEGPYRMEHFRWGSPPKD) from the nine component mixture and specifically from two peptides of similar molecular weight (m/z 2199.1 GWTLSAGYLLGPQQFFGLM-NH₂ and m/z 2208.1 CTHGIRPVVSTQLLLNGSLAE). The concentration of each peptide in the mixture was ~ 1 pmol/uL for the peptides of m/z 2204.0 and m/z 2208.1 and ~ 5 pmol/uL for the peptide of m/z 2199.1 (this peptide was added to the mixture at a higher concentration due to facile adduction of sodium). As for the previous two examples, the top panel of Figure 3 shows data collected with the TIS wide open and demonstration of the presence of all three peptides with a nominal molecular weight of 2200. The bottom panel of Figure 3 depicts analysis of the same sample using a higher TIS resolution. In this example the peptide of m/z 2204.0 was clearly isolated with high specificity from both of the other components of similar molecular weight with no observed loss in signal intensity. As ions of this nominal mass would likely be observed in the 3⁺ charge state in ESI, this selection is very competitive with that of quadrupole and ion trap devices.

Summary

The high resolution precursor ion selection advantage of the 4800 MALDI TOF/TOF™ Analyzer was demonstrated by specific isolation of three peptides of different molecular weight (peptide ions were detected nominally at m/z 900, m/z 1300 and m/z 2200). Two contaminating peptides of similar molecular weight to each of the peptides targeted for isolation were also components of the mixture. In all m/z ranges the targeted peptide was isolated with high specificity with minimal suppression of the target precursor ion. In contrast suppression of both high and low mass contaminants was $> 95\%$ in all case. This allows very specific isolation of peptides within the mass range that is typically used to analyze complex mixtures and demonstrate a benefit to the use of the 4800 MALDI TOF/TOF™ Analyzer. This performance is unrivalled by other commercially available TOF/TOF™ instruments and equivalent to ion trap and quadrupole based instrumentation.

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