

Applications of AP-MALDI-HR Ion Source with Thermo Scientific™ Orbitrap Fusion™ MS and Thermo Scientific™ TSQ Quantiva™ MS for Peptide Analysis and Imaging

Berk Oktem, MassTech

Inc.

Introduction

MassTech's AP-MALDI-HR source is now available for *Thermo Scientific™ Orbitrap Fusion™*, *Thermo Scientific™ TSQ Quantiva™* and *TSQ Endura™* MS systems. This note describes uses of the AP-MALDI HR ion source with a high repetition rate laser and includes preliminary data.

AP-MALDI HR Parameters

Laser Repetition Rate	: 1 kHz
Laser Energy	
(with 100 μm fiber)	: ~3-5 μJ
Spot Size (elliptic)	: ~85x170 μm

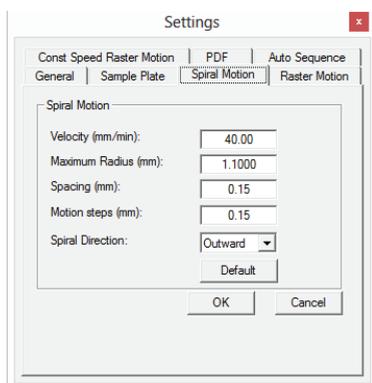


Figure 1: AP-MALDI Spiral Motion Parameters

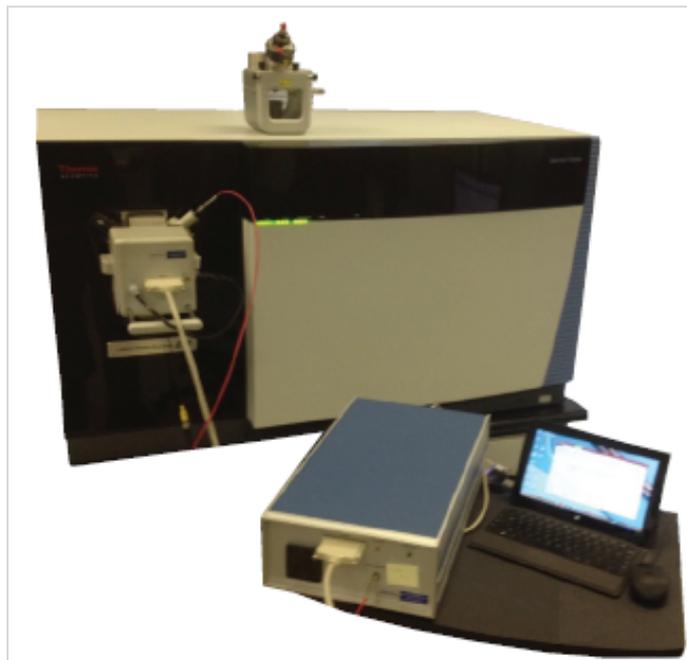
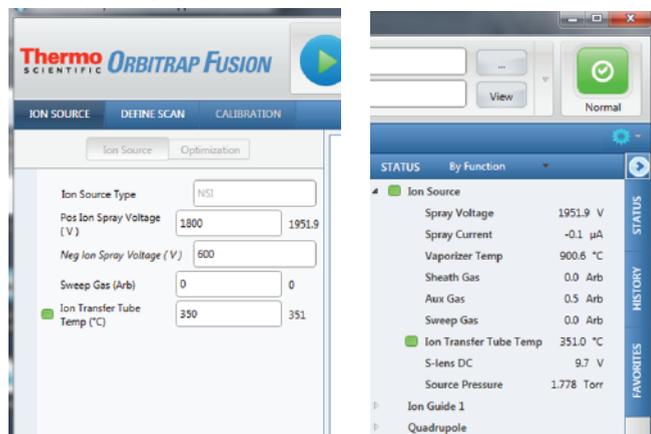


Figure 2: Experimental Setup

MS Parameters



Ion Injection Time	: 100 ms
Number of microscans	: 3
Plate (Ion Spray) Voltage	: 1900 V
Ion Transfer Tube	: 350 °C

Figure 3: MS Parameters

Keywords:

AP/MALDI
Thermo Scientific™
Orbitrap Fusion™ MS
TSQ Quantiva™ MS
TSQ Endura™ MS

Peptide Analysis

Several model peptides were used: Angiotensin II -human (MW: 1046.18), Bradykinin Fragment 1-7 (MW: 756.85), P₁₄R (MW: 1533.85), Substance P (MW: 1347.63) and Bovine Serum Albumin digest. The laser was continuously fired while Target software moved the sample in spiral motion. After the scanning was completed laser burn pattern was recognizable. (see Figure 4). User did not intervene to reposition the laser spot. Due to quick depletion of sample at a fixed spot, the velocity of the spiral motion is set to be very high at 40 mm/min (see Figure 1). A 1 minute average peptide spectrum is shown on Figure 5 using Angiotensin II (10 fmol/μL), using Orbitrap mode (3 μscans; 1.93 sec/scan).



Figure 4: Laser Burn Pattern After the Spiral Motion

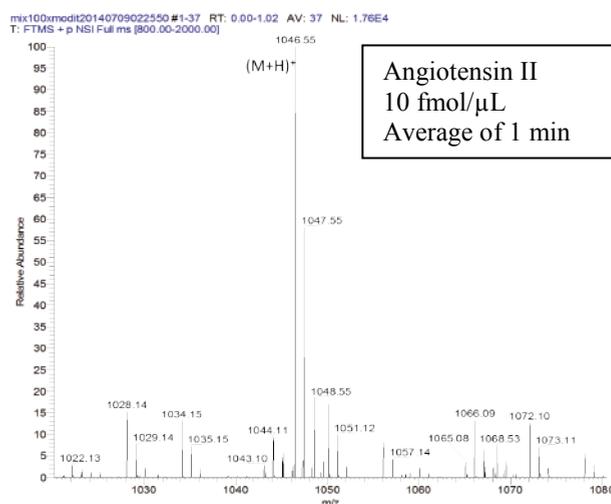


Figure 5: Angiotensin II Mass Spectrum-High Resolution/Orbitrap mode

Other peptides were tested as well. The two component peptide mixture: Angiotensin II -human, and P₁₄R is shown in Figure 6. ; (100 fmol/μL) . Similarly, Substance P is shown in Figure 7 ; (100 fmol/μL) ; as well as BSA digest (130 ng/mL)in Figure 8.

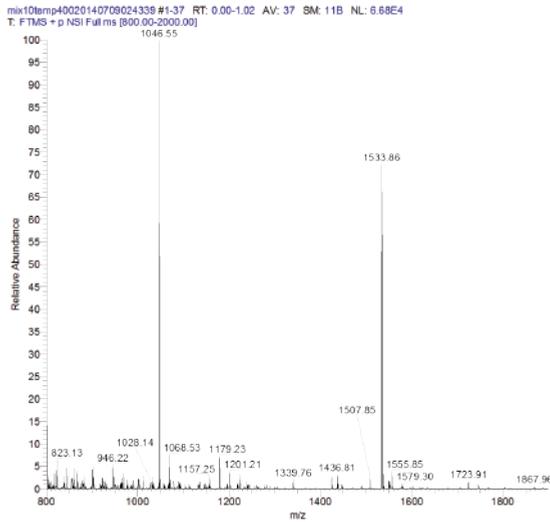


Figure 6:
Angiotensin II and
P₁₄R mixture Mass
Spectrum-High
Resolution/Orbitrap
mode

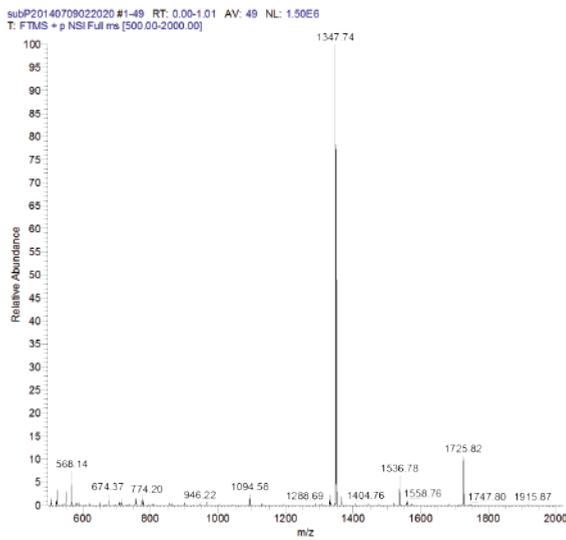


Figure 7: Substance
P spectrum. High
Resolution/Orbitrap
mode

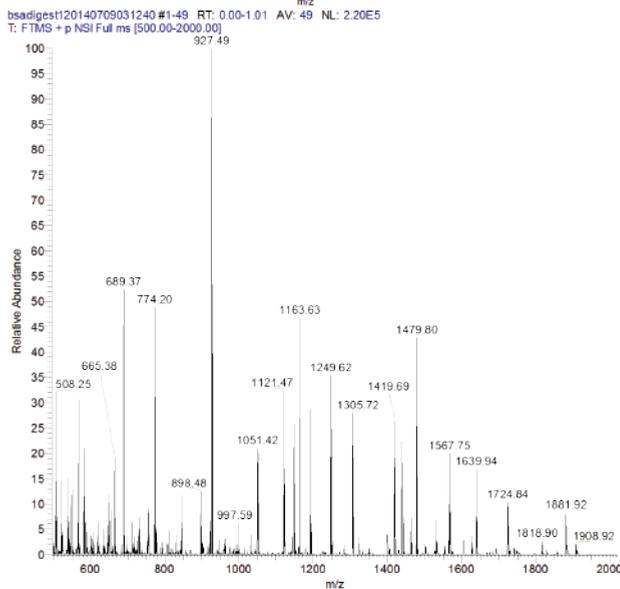


Figure 8: BSA
digest. High
Resolution/Orbitrap
mode

Automated Analysis

Automation was tested with *Target's* auto sequence mode, where *XCalibur* software was enabled with contact closure feature and a sequence is set up. This enables multiple samples to be acquired without user intervention: multiple spots are selected, *XCalibur* Software is set to acquire equal number of files, and then data was acquired in sequence.

Imaging Mode

A new feature available beginning *Target Version 7.0* is the Zoom Mode. It is available by two soft keys added at *Target* soft key panel as highlighted on Figure 9.



Figure 9: Target Zoom Mode buttons

The sample areas can be scanned under the “zoom mode” of *Target*. The two scanning modes available are: Full sync of each pixel with the MS (Pixel Map) and the continuous motion with each row synced with the MS (CSR-Constant Speed Raster).

The *Target* software can also run in an internal timing mode, where MS acquisition will start when signal is received from *Target*.

Imaging test was done with a single peptide spot to show data compatibility between *Orbitrap Fusion* and *Target* software. Peptide was spotted on an ABI-opti-TOF plate (1.5 mm size). Data was then processed by *Thermo ImageQuest* software. The test shown on Figure 10 demonstrates full data compatibility.

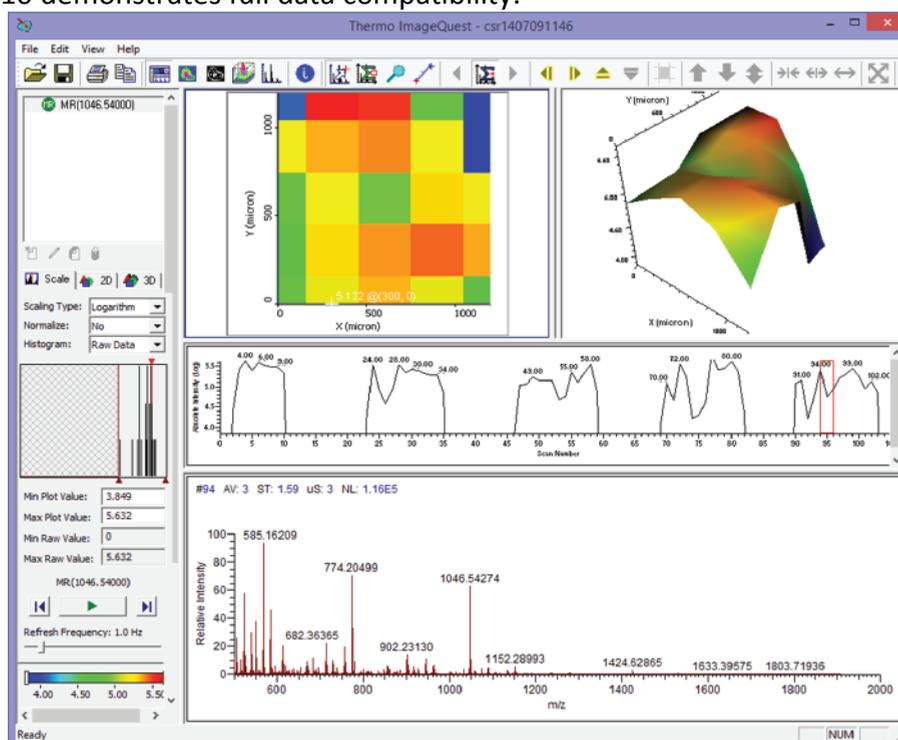


Figure 10: ImageQuest view of AP-MALDI generated data

AP-MALDI on Thermo Scientific™ TSQ Quantiva™ MS

AP-MALDI was tested on TSQ Quantiva MS. The three component peptide mixture: Angiotensin II -human, Bradykinin Fragment 1-7 and P₁₄R is shown at Figure 11.

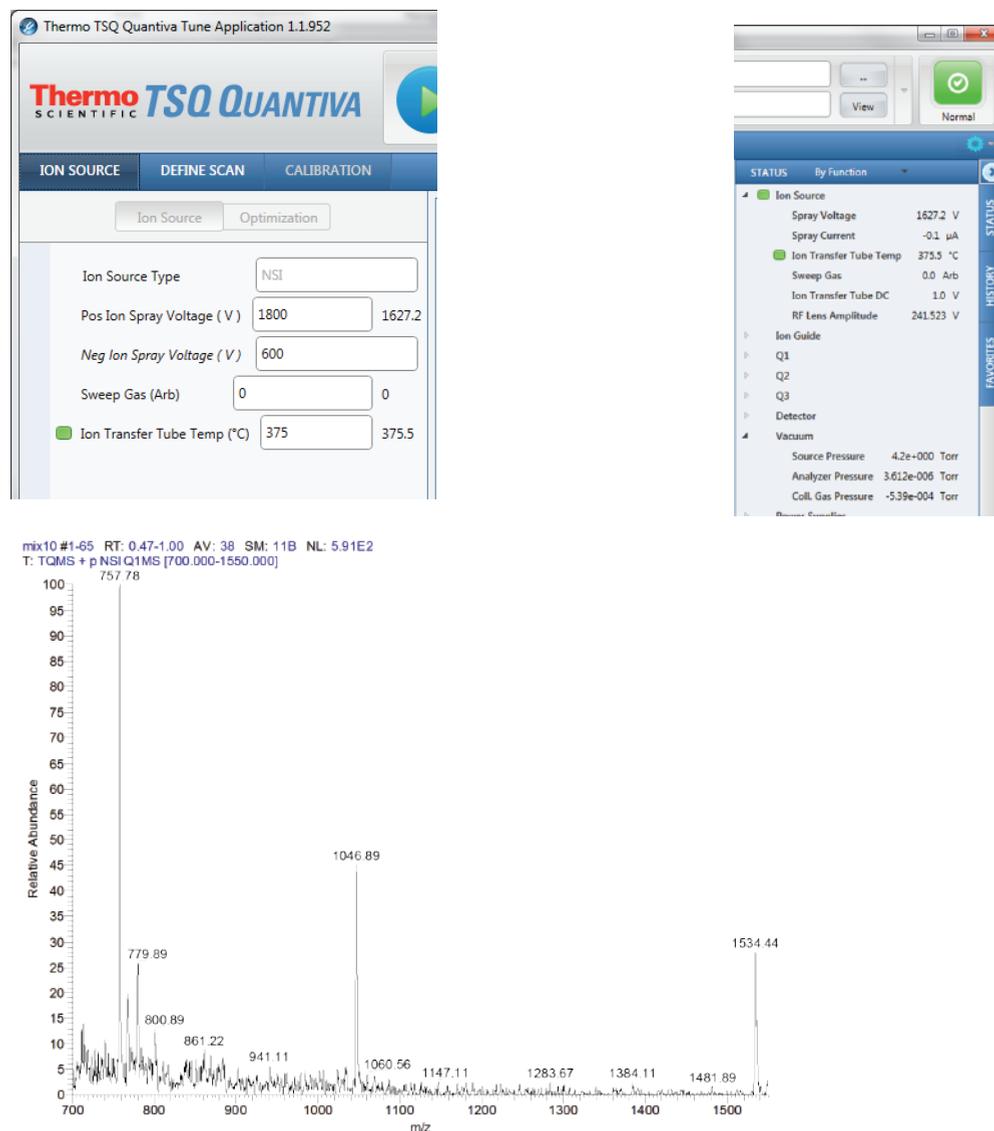


Figure 11: Tri-peptide mixture with TSQ Quantiva MS:
100 fmol /µL; 0.5 min average

Acknowledgements

The author thanks Josef Ruzicka and Joshua J. Nicklay of Thermo Scientific for help in data acquisition and helpful discussions .