Comparison of Sensitivity and Spectra between the AP-MALDI LC/MSD Trap SL System and Traditional Vacuum MALDI-TOF

Application Note

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Introduction

Matrix-assisted laser desorption ionization (MALDI) is a popular technique for protein and peptide analysis.[1, 2] Historically, MALDI analyses have been accomplished with the sample under vacuum in combination with time-of-flight (TOF) mass analyzers, MALDI-TOF. More recently, MALDI has been performed at atmospheric pressure in combination with ion trap (IT) mass analyzers: AP-MALDI-IT.[3–6]

The AP-MALDI-IT and the vacuum MALDI-TOF approaches each have known advantages. The advantages of vacuum MALDI-TOF are:

- Greater mass range, permitting analysis of intact proteins
- Potentially better mass accuracy using time lag focusing and assuming recent mass calibration
- Greater resolving power for reflectron TOF systems

The advantages of AP-MALDI-IT are:

- Decouples ionization factors such as laser intensity and sample plate position from mass accuracy and resolution
- Permits generation of true MS/MS data for full peptide sequence information, even at subfemtomole sample levels, whereas post-source decay (PSD) in MALDI-reflectron TOF systems does not generate full sequence information, even at picomole levels
- Provides high sensitivity, high quality MS/MS data with a less expensive ion trap mass spectrometer rather than a more expensive Q-TOF or TOF-TOF instrument
- Because the ionization process takes place at atmospheric pressure, switching between AP-MALDI and LC/MS/MS on the same instrument takes only a few minutes

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Agilent's second-generation AP-MALDI ion source is considerably more sensitive than those of other mass spectrometer vendors. In the current work, experiments were conducted to compare sensitivity and spectral features in MS-only mode for tryptic digests analyzed using the Agilent 1100 Series LC/MSD Trap SL ion trap mass spectrometer equipped with the Agilent AP-MALDI ion source and two modern MALDI-TOF systems. This work demonstrates that while there were many spectral similarities between AP-MALDI-IT and MALDI-TOF, the Agilent AP-MALDI Trap, overall, showed better sensitivity. An additional benefit of the AP-MALDI Trap was MS/MS capability at a significantly lower price than MALDI Q-TOF or MALDI TOF-TOF mass spectrometers. The use of MS/MS greatly increases the specificity of database searches when compared with searches that use only the peptide mass fingerprint (PMF) technique commonly employed with MALDI-TOF mass spectrometers.

Experimental

Sample preparation

Tryptic digests were obtained from Michrom BioResources, Inc. (Auburn, CA) as lyophilized materials. All proteins were reduced with dithiothreitol (DTT), alkylated with iodoacetic acid, and digested with TPCK-treated trypsin. Digested proteins were taken to dryness and stored frozen prior to use. Each digest was dissolved in 15% isopropyl alcohol in water, mixed with α-cyano-4-hydroxycinnamic acid matrix, and spotted onto the MALDI plate.

Instrument conditions

Samples were analyzed in MS-only mode using the Agilent AP-MALDI Trap system and also using two popular commercial MALDI-TOF systems. An additional sample was analyzed at the subfemtomole level in both MS and MS/MS modes using the AP-MALDI Trap. Instrument conditions for the Agilent system are given in Table 1. The MALDI-TOF systems were operated under the participating laboratory's standard conditions for peptide analyses.

Table 1. Agilent AP-MALDI Trap operating conditions

Parameter	Setting
Instrument	Agilent 1100 Series LC/MSD Trap SL
Polarity	Positive
Dry gas flow rate	5 L/min
Dry gas temperature	325°C
Mass range mode	Standard, 50–2200 *m/z*
Scan resolution	Peak width 0.5–0.65 u, at a scan speed of 13,000 u/sec
Scan range	400–2200 Da
Number of MS scans for averaging	10

Results and Discussion

Sensitivity

Figures 1–4 compare sensitivity among the various instruments. For this comparison, tryptic digests of cytochrome c, lysozyme, myoglobin, and bovine serum albumin (BSA) were prepared in exactly the same manner and analyzed at the 5 femtomole (fmol) level on all instruments. Overall, the Agilent system showed the best signal-to-noise.

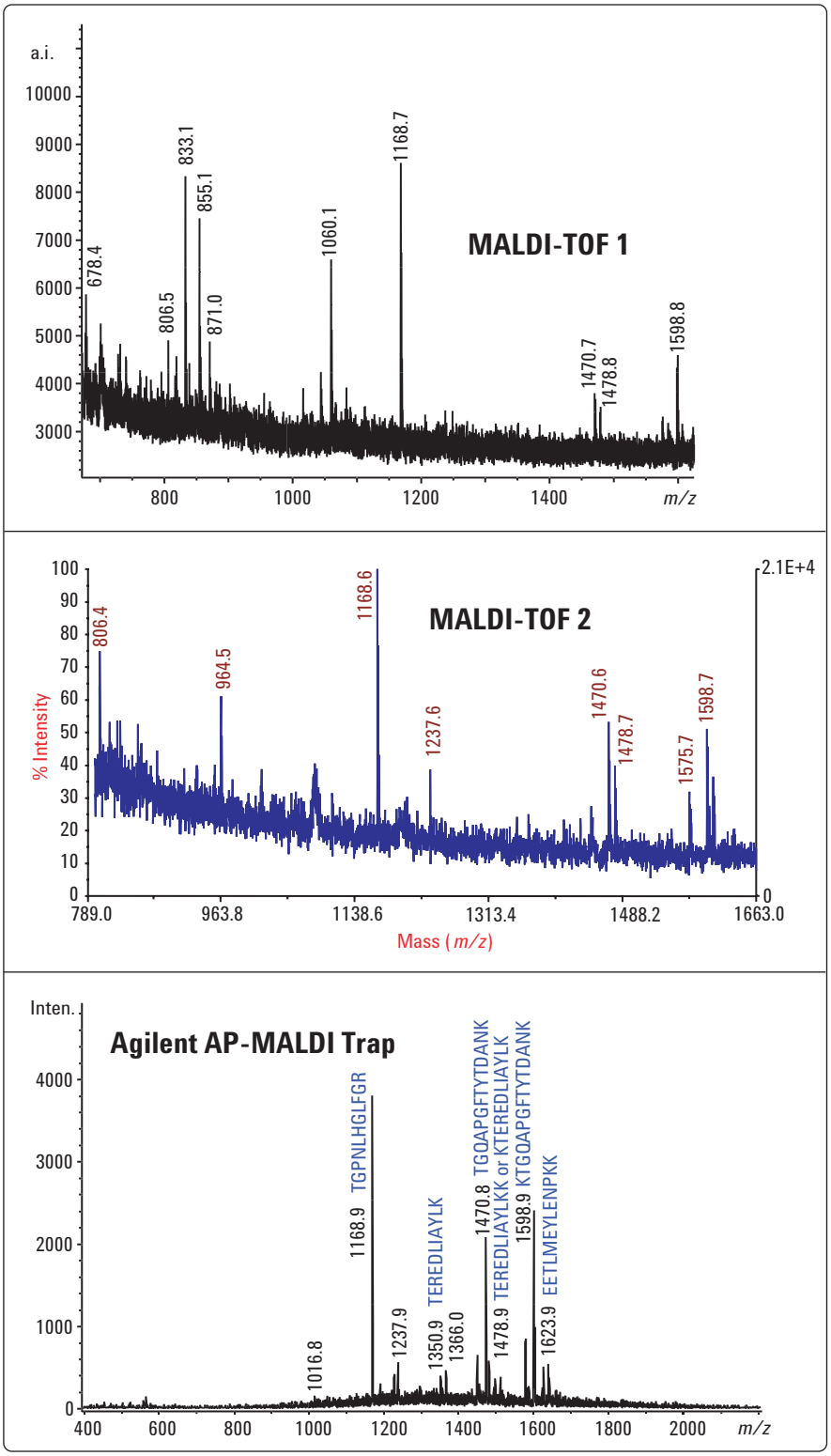
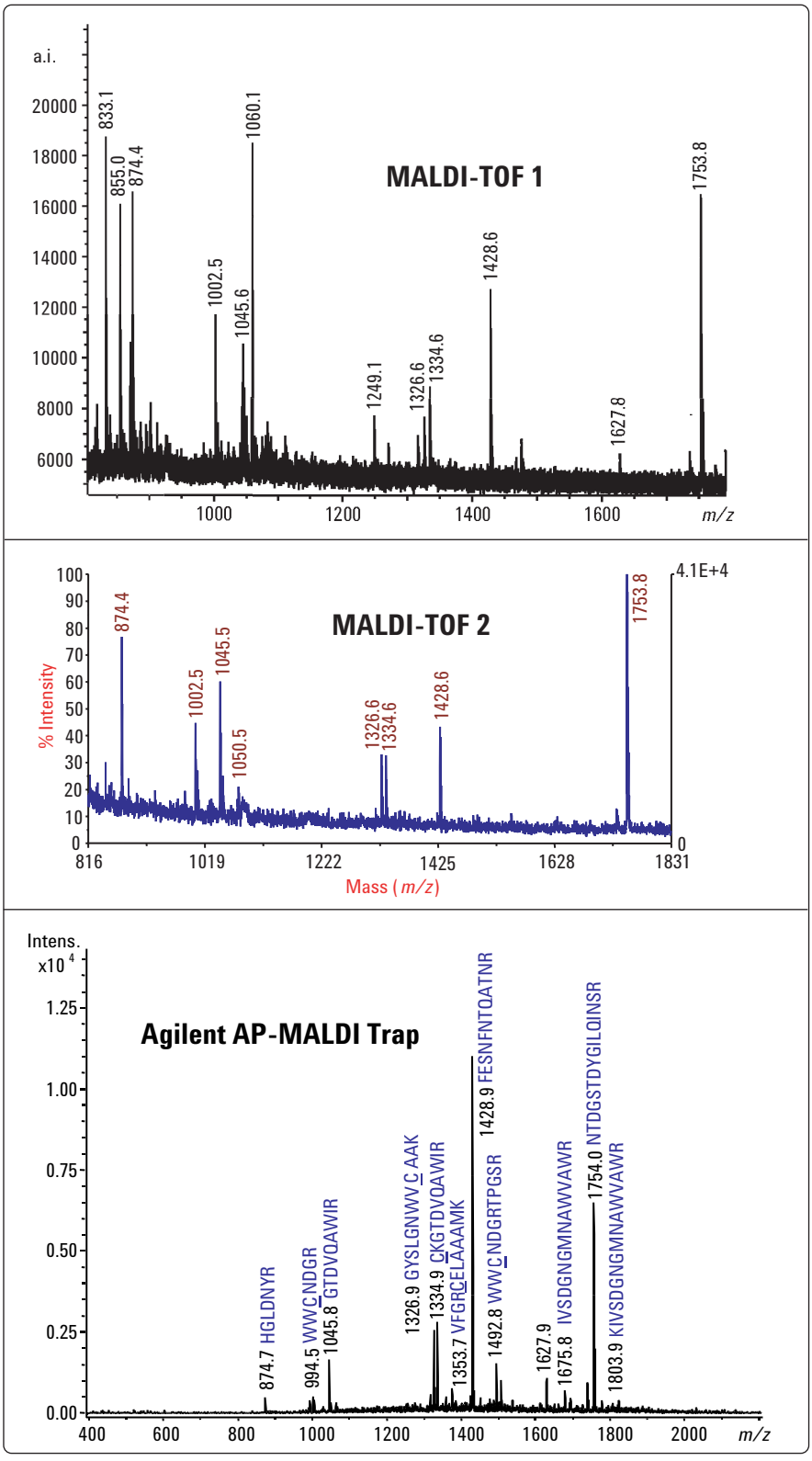


Figure 1. Sensitivity comparison for 5 fmol cytochrome c digest

Figure 2. Sensitivity comparison for 5 fmol lysozyme digest. C = carboxymethylated cysteine



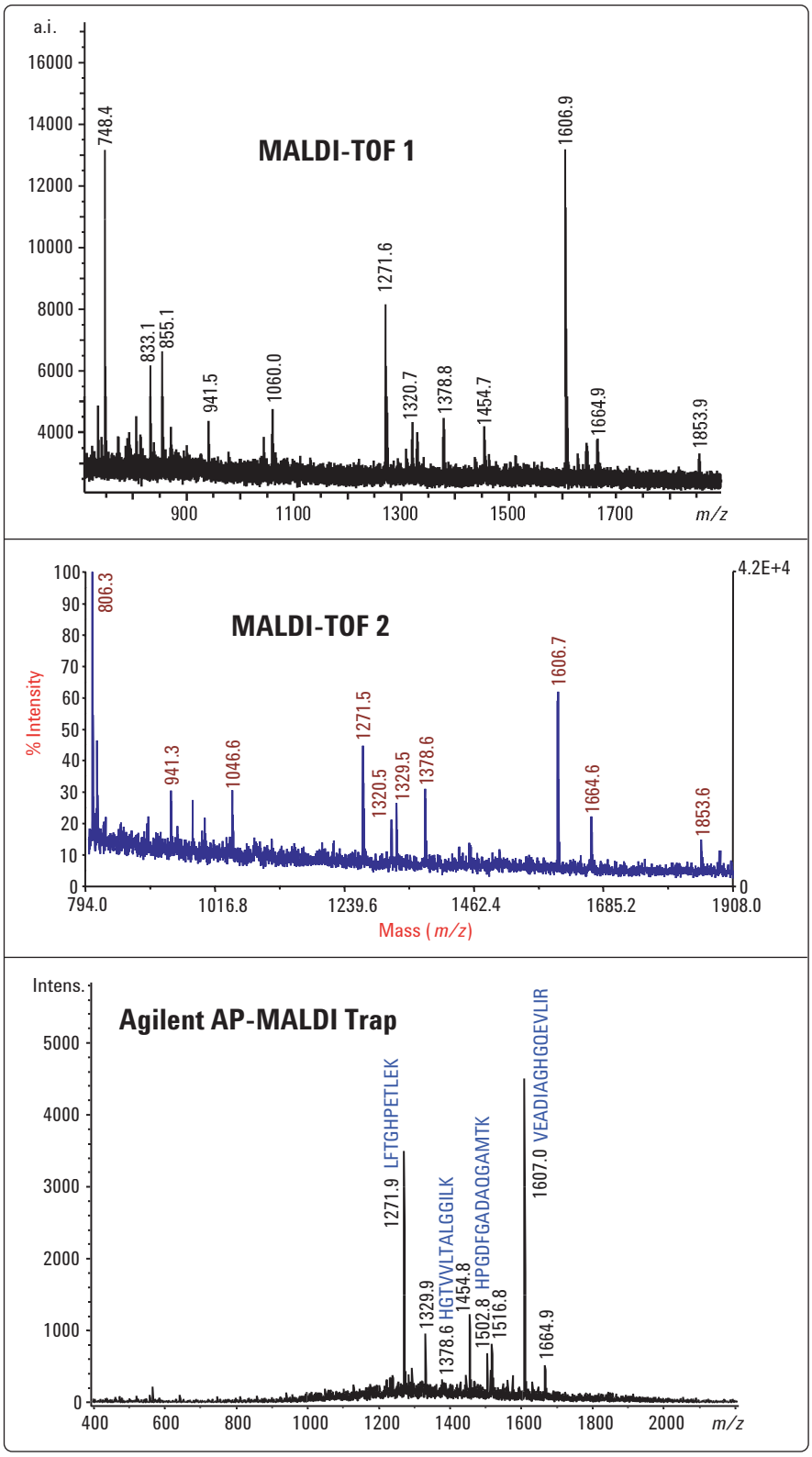


Figure 3. Sensitivity comparison for 5 fmol myoglobin digest

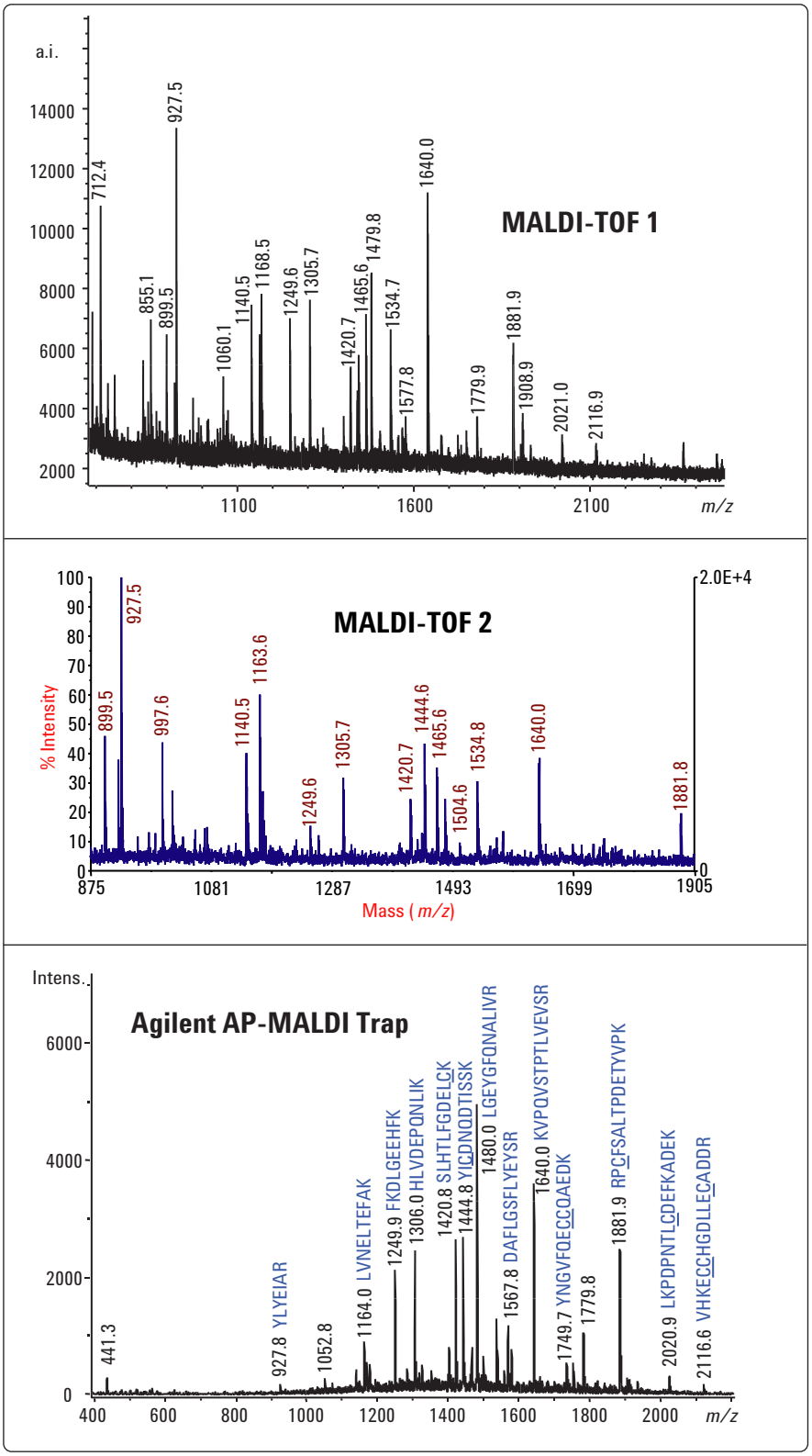


Figure 4. Sensitivity comparison for 5 fmol BSA digest. C = carboxymethylated cysteine

Spectral comparison

Tables 2–5 compare data at the 5 fmol level from the Agilent AP-MALDI Trap and the two MALDI-TOF systems. The mass values in the tables are displayed to 0.1 u, which is appropriate for the mass accuracy of these three instruments. All *m/z* listed are for the respective $[M + H]^+$ ions.

Table 2. Peptide sequences identified and *m/z* assigned for cytochrome c tryptic digest

Sequence*	Theoretical (*m/z*)	AP-MALDI (*m/z*)	MALDI-TOF 1 (*m/z*)	MALDI-TOF 2 (*m/z*)
YIPGTK	678.4		678.4	
KYIPGTK	806.5		806.5	806.4
*			833.1	
*			855.1	
*			871.0	
EDLIAYLK				964.5
*			1060.1	
TGPNLHGLFGR	1168.6	1168.9	1168.7	1168.6
*		1237.9		1237.6
TEREDLIAYLK	1350.7	1350.9		
*		1366.0		
TGQAPGFTYTDANK	1470.7	1470.8	1470.7	1470.6
TEREDLIAYLKK or KTEREDLIAYLK**	1478.8	1478.9	1478.8	1478.7
*				1575.7
KTGQAPGFTYTDANK	1598.8	1598.9	1598.8	1598.7
EETLMEYLENPKK	1623.8	1623.9		

*Entries without sequence identification indicate *m/z* that did not match those of peptides predicted by theoretical digestion.

**MS/MS may resolve ambiguity

Table 3. Peptide sequences identified and *m/z* assigned for lysozyme tryptic digest

Sequence*	Theoretical (*m/z*)	AP-MALDI (*m/z*)	MALDI-TOF 1 (*m/z*)	MALDI-TOF 2 (*m/z*)
*			833.1	
*			855.0	
*			871.0	
HGLDNYR	874.4	874.7	874.4	874.4
WWCNDGR	994.4	994.5		
*		1002.8	1002.5	1002.5
*			1044.1	
GTDVQAWIR	1045.5	1045.8	1045.6	1045.5
CELAAAMKR	1050.5	1050.7	1050.5	1050.5
*			1060.1	
*			1249.1	
GYSLGNWVCAAK	1326.6	1326.9	1326.6	1326.6
CKGTDVQAWIR	1334.7	1334.9	1334.6	1334.6
VFGRCELAAAMK	1353.7	1353.7		
FESNFNTQATNR	1428.7	1428.9	1428.6	1428.6
WWCNDGRTPGSR	1492.6	1492.8		
*		1627.9	1627.8	
IVSDGNGMNAWVAWR	1675.8	1675.8		
NTDGSTDYGILQINSR	1753.8	1754.0	1753.8	1753.8
KIVSDGNGMNAWVAWR	1803.9	1803.9		

*Entries without sequence identification indicate *m/z* that did not match those of peptides predicted by theoretical digestion.

C = carboxymethylated cysteine

The AP-MALDI and MALDI-TOF spectra showed many of the same masses, although the ion ratios varied between the instruments. The MALDI-TOF spectra tended to exhibit more low- and mid-mass ions and/or higher abundances at these masses. Low-mass ions were not as abundant in the AP-MALDI spectra because in AP-MALDI, ions are cooled by multiple collisions at atmospheric pressure, resulting in lower internal energies and less fragmentation. Also, the ion optics of the Agilent AP-MALDI Trap system were set to reduce the transmission of low-mass matrix ions that often create significant interferences.

Table 4. Peptide sequences identified and *m/z* assigned for myoglobin tryptic digest

Sequence*	Theoretical (*m/z*)	AP-MALDI (*m/z*)	MALDI-TOF 1 (*m/z*)	MALDI-TOF 2 (*m/z*)
ALELFR	748.4		748.4	
*			806.3	806.3
*			833.1	
*			855.1	
YKELGFQG	941.5		941.5	941.3
*				1046.6
*			1060.0	
LFTGHPETLEK	1271.7	1271.9	1271.6	1271.5
*			1320.7	1320.5
*		1329.9	1329.6	1329.5
HGTVVLTALGGILK	1378.8	1378.6	1378.8	1378.6
*		1454.8	1454.7	
HPGDFGADAQGAMTK	1502.7	1502.8		
*		1516.8		
VEADIAGHGQEVLIR	1606.9	1607.0	1606.9	1606.7
*		1664.9	1664.9	1664.6
GHHEAELKPLAQSHATK	1854.0		1853.9	1853.6

*Entries without sequence identification indicate *m/z* that did not match those of peptides predicted by theoretical digestion.

Table 5. Peptide sequences identified and *m/z* assigned for BSA tryptic digest

Sequence*	Theoretical (*m/z*)	AP-MALDI (*m/z*)	MALDI-TOF 1 (*m/z*)	MALDI-TOF 2 (*m/z*)
AWSVAR	689.4		689.4	
SEIAHR	712.4		712.4	
NYQEAK	752.4		752.4	
*			833.1	
LSQKFPK	847.5		847.5	847.5
*			855.1	
LCVLHEK	899.5		899.5	899.5
IETMREK	922.5		922.5	922.5
YLYEIAR	927.5	927.8	927.5	927.5
*				997.6
*			1060.1	
CCTESLVNR	1140.5		1140.5	1140.5
LVNELTEFAK	1163.6	1164.0	1163.6	1163.6
CCTKPESER	1168.5	1168.9	1168.5	1168.5
FKDLGEEHFK	1249.6	1249.9	1249.6	1249.6
HLVDEPQNLIK	1305.7	1306.0	1305.7	1305.7
SLHTLFGDELCK	1420.7	1420.8	1420.7	1420.7
RHPEYAVSVLLR	1439.8	1439.5	1439.8	1439.8
YICDNQDTISSK	1444.6	1444.8	1444.6	1444.6
TCVADESHAGCEK	1465.6	1465.7	1465.6	1465.6
LGEYGFQNALIVR	1479.8	1480.0	1479.8	1479.8
QTALVELLKHKPK	1504.9	1504.7		1504.6
LKECCDKPLLEK	1534.7	1534.8	1534.7	1534.8
DDPHACYSTVFDK	1555.6	1555.7		
DAFLGSFLYEYSR	1567.7	1567.8		
LKPDPNTLCDEFK	1577.8	1577.7	1577.8	
KVPQVSTPTLVEVSR	1639.9	1640.0	1640.0	1640.0
YNGVFQECCQAEDK	1749.7	1749.7		
*		1779.8	1779.9	
RPCFSALTPDETYVPK	1881.9	1881.9	1881.9	1881.8
NECFLSHKDDSPDLPK	1902.9	1902.8		
LFTFHADICTLPDTEK	1908.9	1909.0	1908.9	
LKPDPNTLCDEFKADEK	2021.0	2020.9	2021.0	
VHKECCHGDLLECADDR	2116.8	2116.6	2116.9	

*Entries without sequence identification indicate *m/z* that did not match those of peptides predicted by theoretical digestion.

C = carboxymethylated cysteine

Subfemtomole MS and MS/MS data from the AP-MALDI Trap

In contrast with many MALDI-TOF instruments, the AP-MALDI ion trap is capable of generating both MS and MS/MS results at the subfemtomole level. Figure 5 shows both MS and MS/MS spectra from the analysis of 250 attomole (amol) BSA digest. The red diamonds in the upper spectrum indicate the ions that were selected automatically for MS/MS analysis, with two of the resulting MS/MS spectra below.

Note that few matrix ions are apparent in the MS spectrum, which has not been corrected for background. A combination of hardware design improvements and ion optics adjustment allows discrimination against the transmission of lower mass matrix ions into the trap. The results are cleaner spectra and better sensitivity.

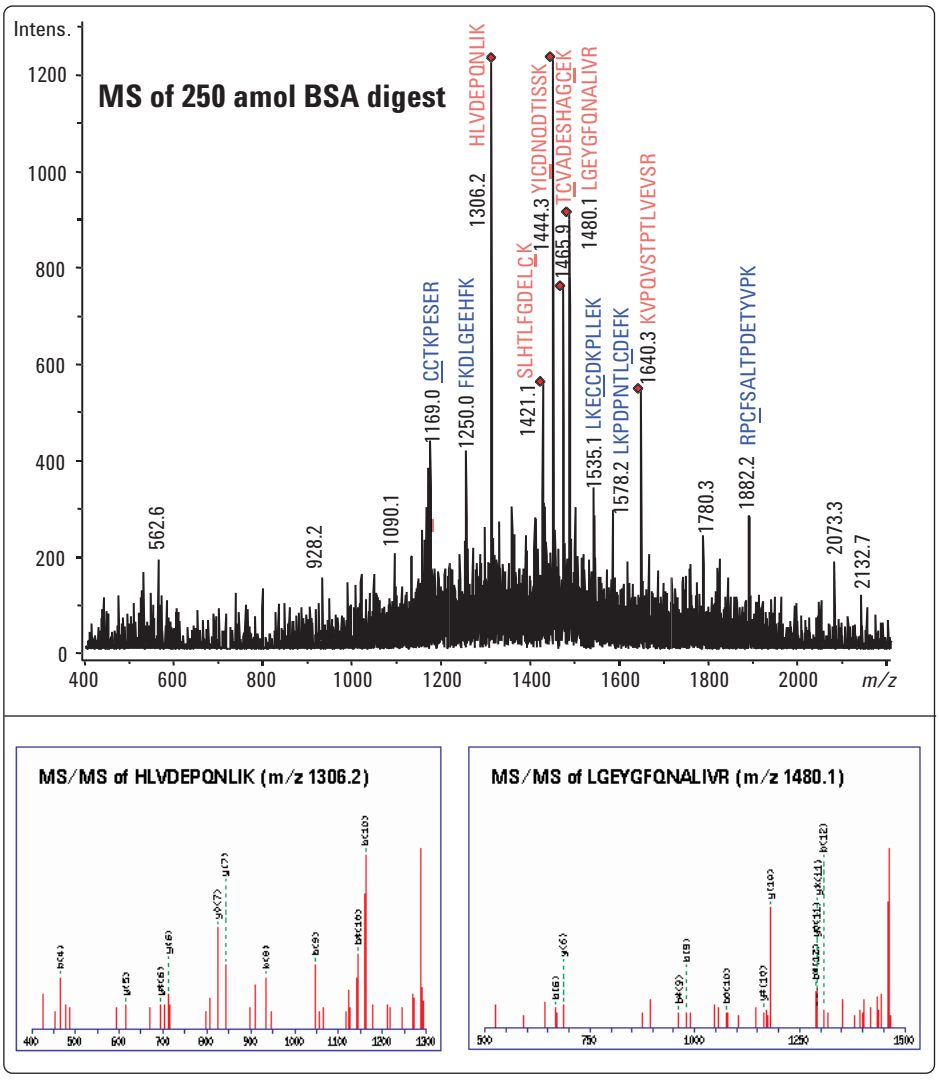


Figure 5. AP-MALDI Trap MS spectrum (top) and MS/MS spectra (bottom) from 250 amol BSA digest. The MS/MS spectra show b and y ions labeled by Mascot database search software.

Figure 6 shows results of a Mascot protein database search from an AP-MALDI Trap MS/MS analysis of 500 amol BSA digest. The database search results were unambiguous even at this low sample level.

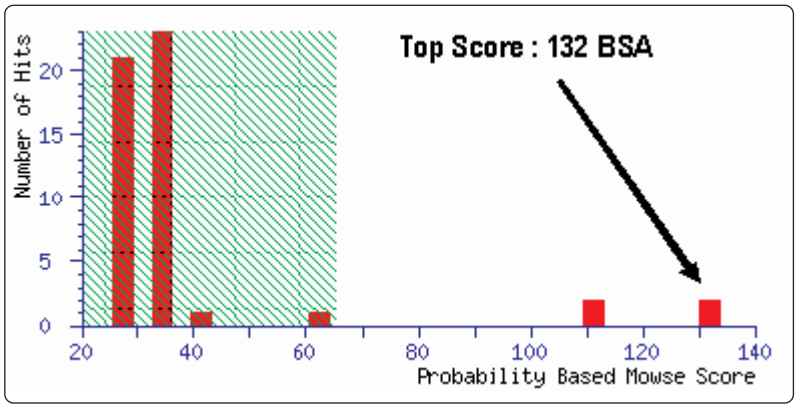


Figure 6. Mascot protein database search results from AP-MALDI Trap MS/MS analysis of 500 amol BSA digest

Conclusions

This study compared sensitivity and spectra among two vacuum MALDI-TOF instruments and the Agilent AP-MALDI Trap. The Agilent AP-MALDI Trap exhibited the best overall signal-to-noise. Furthermore, second-generation design improvements in the Agilent AP-MALDI Trap enabled peptide analyses in the 250–500 attomole level, significantly below the levels attained using many vacuum MALDI-TOF instruments.

The AP-MALDI Trap maintained the added advantage of producing high quality, high sensitivity MS/MS data.

There were many similarities between the AP-MALDI and vacuum MALDI spectra, making it easy to compare data between these two techniques. The two types of spectra showed many of the same ions, although in different ratios. AP-MALDI, being a softer ionization process, produced fewer ions of lower mass than did vacuum MALDI.

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