High Sensitivity Peptide Analysis Using the 6550 Q-TOF with iFunnel Technology

Application Note

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Introduction

Sensitivity is a key requirement for peptide analysis in areas such as protein identification, profiling, and quantitation. The Agilent 6550 Quadrupole Time-of-Flight (Q-TOF) Mass Spectrometer incorporates Agilent iFunnel technology to achieve greater levels of sensitivity for the detection of peptides. Agilent iFunnel technology combines highly efficient electrospray ion generation and focusing of Agilent Jet Stream technology with a hexabore capillary sampling array and dual-stage ion funnel for increased ion sampling and transmission. This innovative design includes offset alignment of the dual-stage ion funnel to allow improved removal of the increased gas load and removal of neutral background, enabling a 10-fold increase in sensitivity for selected molecules as compared to an earlier generation Q-TOF lacking iFunnel technology.

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Improved Sensitivity with iFunnel Technology

Increased sensitivity due to the iFunnel technology was assessed using a synthetic peptide, LVNEVTEFAK, derived from trypsinized human serum albumin (HSA). A comparison was performed using an Agilent 1290 Infinity LC (2.1 x 50 mm column operated at 400 µL/min) interfaced to either an Agilent 6540 Q-TOF (no ion funnel) or an Agilent 6550 Q-TOF (with ion funnel). Both instruments were configured with an Agilent Jet Stream source which uses thermal gradient ion focusing electrospray ionization to provide a 3-5x improvement in sensitivity for peptides over a conventional electrospray source. Analyses were performed on both Q-TOF instruments in full scan MS (2 Hz) mode as well as targeted MS/MS (5 Hz) mode. Results of full scan MS mode for the synthetic peptide on both the 6550 iFunnel Q-TOF system (blue trace) and the 6540 Q-TOF (red trace) are shown in Figure 1. A clear increase in signal height is seen from 300,000 to over 3 million counts.

In targeted MS/MS mode, MS/MS is performed only on a list of user-specified precursors, and the MS/MS cycle can be repeated over the entire analysis time or for just a selected retention time window. Targeted MS/MS mode thus collects full scan MS/MS data and quantitation can be done on product ion(s) selected post-acquisition. The results of targeted MS/MS mode for the target peptide on both the Agilent 6550 iFunnel Q-TOF system (blue trace) and the Agilent 6540 Q-TOF (red trace) are shown in Figure 2. Again, the Agilent 6550 iFunnel Q-TOF data (blue trace) shows a significant increase in signal over the Agilent 6540 Q-TOF (red trace).

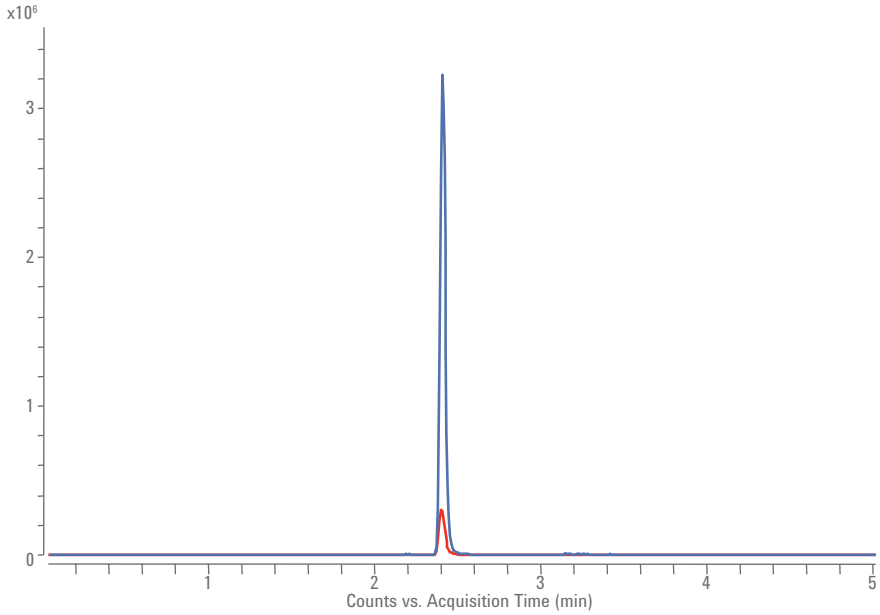


Figure 1. Extracted ion chromatogram (EIC) of *m/z* 575.3111, the doubly charged species of the peptide LVNEVTEFAK, using a ±5 ppm window. Data shows a 10x increase in sensitivity with the iFunnel-based Q-TOF.

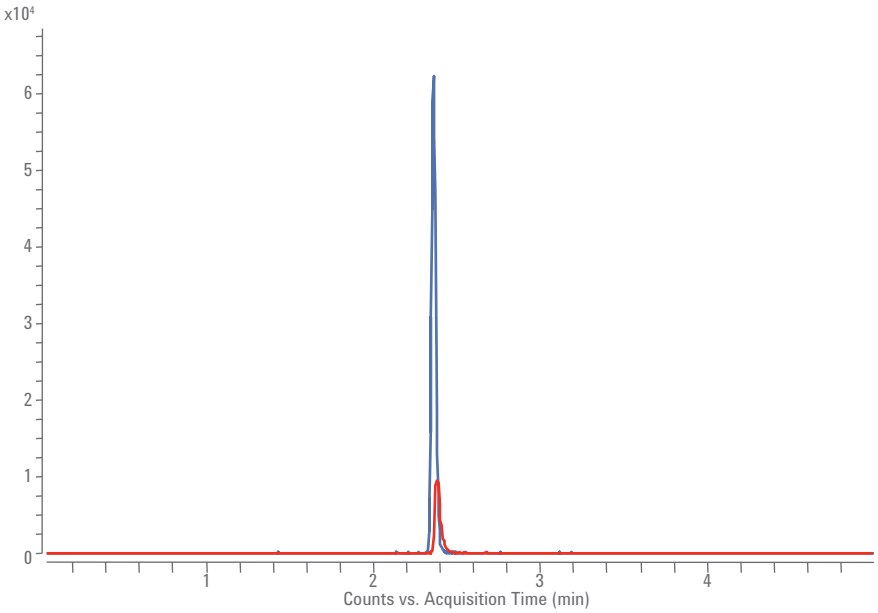
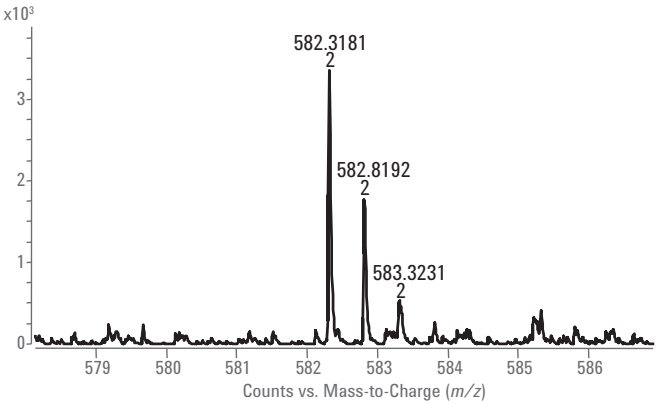


Figure 2. EIC of the product ion *m/z* 937.4625, from the precursor at *m/z* 575.3111, were created using a ±5 ppm window. Data shows a 7x increase in sensitivity with the Agilent 6550 iFunnel Q-TOF.

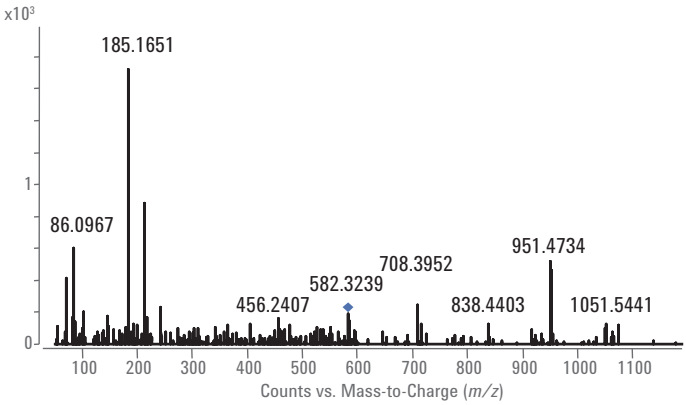
Improved Sensitivity for Protein Identification

Protein identification workflows typically use data-dependent acquisition followed by protein database searching to identify the peptides and thus the proteins in a biological sample. Improved sensitivity increases the number of high-quality MS/MS spectra and thus increases the number of peptides and proteins identified. In addition, the increased sensitivity results in a decrease in the amount of sample needed for protein identification, minimizing consumption where the amount of sample is limited. In order to assess the sensitivity of the 6550 iFunnel Q-TOF in data-dependent mode, a trypsinized bovine serum albumin (BSA) standard (Michrom, Agilent p/n: G1900-85000) was analyzed on the Agilent 6550 Q-TOF coupled to an HPLC-Chip/MS interface. The nanoflow LC/MS results demonstrated that BSA was correctly identified at the 10 amol on-column level with 4-8 unique peptides (n = 3). Figure 3 shows the MS and MS/MS spectra at 10 amol as well as the Spectrum Mill Protein Database search results. Table 1 summarizes the results for all the levels tested.

A.



B.



C.

Run #	Run Name	Group (#)	Subgroup (#)	Spectra (#)	Distinct Peptides (#)	Distinct Summed MS/MS Search Score	% AA Coverage	Total Protein Spectral Intensity	Species	Database Accession #	Protein Name
1	C2-cm0815-10amol-HSA-r003	1	1	8	5	68.05	11.8	2.11e+005	BOVIN	P02769	Serum albumin

#	Filename	z	Score	Discriminant	SPI (%)	Spectrum Intensity	Sequence	RT (min)	Peak Width (sec)	Averagine Chi Squared	m/z Measured (Da)	MH+ Matched (Da)
1	C2-cm0815-10amol-HSA-r003.1177.1177.4	4	15.11	15.11	80.7	4.95e+004	(K) VHKECCHGDLLECADDR (A)	4.02	4.36	0.98	529.9664	2116.837
2	C2-cm0815-10amol-HSA-r003.1232.1232.3	3	12.04	12.04	71.1	8.81e+003	(K) ECCHGDLLECADDR (A)	4.23	0.00	0.77	584.8788	1752.615
3	C2-cm0815-10amol-HSA-r003.1235.1235.3	3	7.95	7.95	91.4	8.81e+003	(K) ECCHGDLLECADDR (A)	4.23	0.00	0.77	584.8788	1752.615
4	C2-cm0815-10amol-HSA-r003.1347.1349.2	2	14.99	14.99	82.8	2.55e+004	(K) LVNELTEFAK (T)	4.80	3.85	0.99	582.3184	1163.631
5	C2-cm0815-10amol-HSA-r003.1351.1351.3	3	7.40	7.40	80.9	4.47e+003	(R) RPCFSALTPDETYVPK (A)	4.80	0.00	0.97	627.9706	1881.905
6	C2-cm0815-10amol-HSA-r003.1444.1444.4	4	10.62	10.62	77.9	3.95e+003	(K) TVmENFVAFVDKCCAADDKEACFAVEGPK (L)	5.32	0.00	0.71	832.6024	3311.425
7	C2-cm0815-10amol-HSA-r003.1504.1504.4	4	18.51	18.51	82.8	5.47e+004	(K) TVMENFVAFVDKCCAADDKEACFAVEGPK (L)	5.68	3.33	0.91	828.6148	3311.425
8	C2-cm0815-10amol-HSA-r003.1506.1506.4	4	15.36	15.36	90.8	5.47e+004	(K) TVMENFVAFVDKCCAADDKEACFAVEGPK (L)	5.68	3.33	0.91	828.6148	3311.425

Figure 3. Results for the analysis of 10 amol of BSA digest using nanoflow LC/MS on the Agilent 6550 iFunnel Q-TOF. The MS spectrum (A) and MS/MS spectrum (B) allow successful identification of BSA based on multiple unique peptides (C).

	10 amol		100 amol		200 amol		300 amol		400 amol		500 amol		5 fmol		10 fmol	
#	Unique peptides	Coverage	Unique peptides	Coverage	Unique peptides	Coverage	Unique peptides	Coverage	Unique peptides	Coverage	Unique peptides	Coverage	Unique peptides	Coverage	Unique peptides	Coverage
1	8	11.3	17	24.7	14	21	18	28	24	34.5	21	31.1	37	54.6	45	63.9
2	4	8.8	10	14.8	17	25.3	20	29.1	22	32.9	23	33.6	41	57.9	43	61.1
3	5	11.8	6	8.8	8	11.8	20	28.6	23	32.9	25	36.2	41	58.4	44	58.1
4			7	11.2	13	19.9	20	28.8	20	28.5	20	29.9	41	60.4	45	60.2
5			6	8.8	15	23	14	21.5	22	32.6	23	32.7	40	56.8	43	60.2
6			10	17.2	17	25.3	21	30.8	22	31.7	23	32.9	37	53.8	41	57

Table 1. Summary of the protein identification results obtained for the nanoflow LC/MS analysis of BSA.

Improved Sensitivity for Peptide Quantitation

While triple quadrupole mass spectrometry is typically the instrument of choice for routine, high-throughput quantitation[1], the Agilent 6550 iFunnel Q-TOF system permits high sensitivity qualitative and quantitative analysis to be performed on a single system. Sensitivity and linearity were evaluated using the synthetic peptide, LVNEVTEFAK, derived from trypsinized human serum albumin (HSA). In order to mimic quantitation in a complex matrix, this peptide standard was spiked into a trypsinized *Saccharomyces cerevisae* lysate. The analysis was done by nanoflow LC/MS using the 6550 Q-TOF coupled to an HPLC-Chip/MS interface. Since accurate mass Selected Ion Monitoring (SIM) offers a lower level of specificity for a complex matrix, targeted MS/MS mode was employed. For quantitative analysis, a product ion was selected and its EIC was extracted with a narrow extraction window (10 ppm). The results (Figure 4) demonstrate linearity over 4 orders of dynamic range and a limit of detection of 10 amol on-column for the target peptide in the presence of 150 ng of digested yeast lysate.

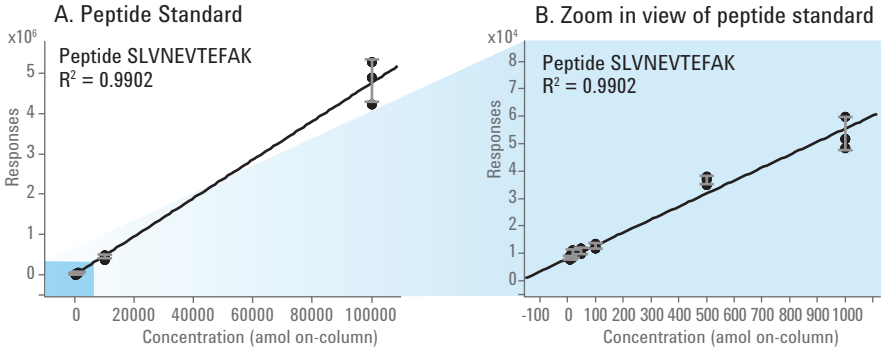


Figure 4. Results for the targeted MS/MS analysis of a peptide standard spiked into a yeast lysate digest. The product ion EIC for 575.3111 → 937.4625 was used to generate the calibration curve from 10 amol to 100 fmol on-column (A). Panel B shows a zoom in view of the peptide standard from a concentration of 0-1000 amol.

Conclusions

The increase sensitivity achieved with the 6550 iFunnel Q-TOF provides a significant enhancement for peptide analysis. The improved performance has been demonstrated in full scan MS, data-dependent MS/MS, and targeted MS/MS modes, which will impact LC/MS analysis of peptides for a variety of qualitative and quantitative applications. Where sample or peptide amounts are limited, nanoflow LC/MS on the 6550 Q-TOF offers the ultimate sensitivity. The outstanding sensitivity of the iFunnel Q-TOF now clears the path for the use of high-throughput UHPLC methodology instead of nanoflow LC/MS for many protein and peptide applications.

Reference

1. The 6490 Triple Quad LC/MS Enables the Highest Sensitivity for Peptide Quantitation in Plasma. *Agilent Publication*, **2010**, publication number 5990-6513EN.

www.agilent.com/chem/QQQ

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