Simultaneous Peptide Quantification and Identification using High Resolution MS

Using the Profile workflow on the AB SCIEX TripleTOF™ 5600 System

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Protein quantification based on mass spectral data falls into two categories: MS/MS based approaches, such as isobaric amine labeling strategies or Multiple Reaction Monitoring (MRM), and MS based approaches, such as non-isobaric labeling or label free approaches. With the TripleTOF™ 5600 system, all of these protein quantification workflows can be performed on a single instrument for the first time. For MS based approaches, the high resolution, high mass accuracy TOF MS spectra on the TripleTOF™ 5600 system enables profiling of peptides in medium complexity biological samples across several orders of dynamic range. Concurrent to this TOF MS profiling, high sensitivity, high resolution MS/MS spectra are acquired at fast acquisition rates for in-depth sample characterization. This combination makes the TripleTOF™ 5600 system a unique solution for both peptide identification and quantification in a single experiment using the Profile workflow.

To assess qualitative and quantitative reproducibility of this Profile workflow (Figure 1), a standard proteomic sample consisting of 5 test proteins and the corresponding heavy isotope labeled lysine and arginine tryptic peptides was analyzed[1].

Key Features of the Profile Workflow for Proteomic Samples

The key benefits of this qualitative and quantitative profiling are:

- Full scan high resolution TOF MS profiling with cycle time optimized to obtain 6-8 scans across LC eluting peaks
- High resolution and high mass accuracy TOF MS spectra allows for post-acquisition generation of extracted ion chromatograms (XICs) at resolutions of >20,000 resolution (0.05 Da extraction window) for increased specificity
- Fast Information Dependant Acquisition (IDA) of high resolution, high mass accuracy MS/MS peptide spectra enables highest confidence identification of many peptides and proteins

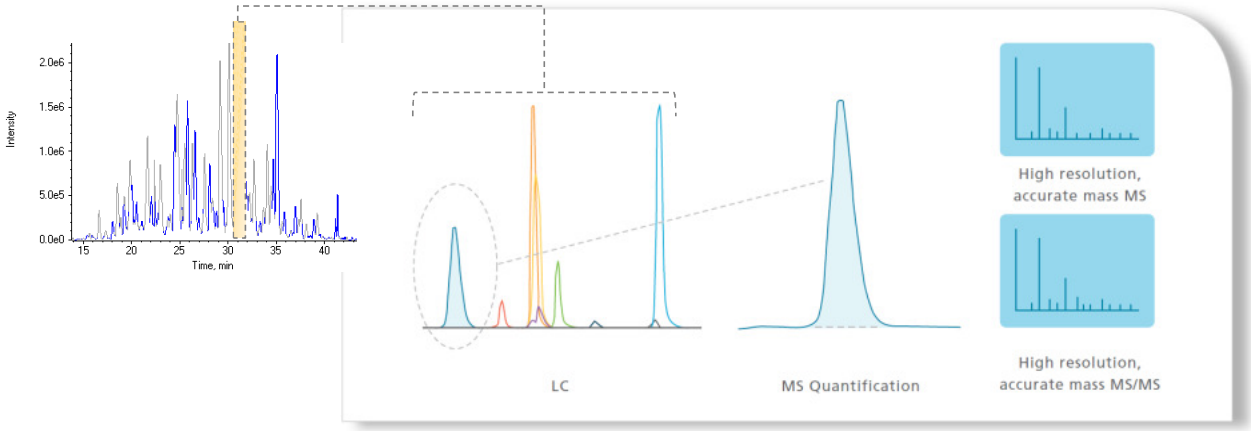

Figure 1. Profile Workflow for Simultaneous Protein Identification and High Resolution MS Peptide Quantification. From an LCMS separation, many features are resolved in the MS data, especially when using a high resolution TOF MS scan. In this workflow, the quantitative information for many of the peptides identified from the high resolution and high mass accuracy MS/MS data can be extracted from the accompanying MS data.

Methods

Sample Preparation: The ABRF sPRG2009 proteomic standard mixture contains 5 target proteins and a set of heavy labeled peptide analogues (Table 1). This sample was diluted to a working concentration of low fmol/µL as indicated in Table 1.

Chromatography: The sample was analyzed using the Eksigent nanoLC-Ultra™ 2D System combined with the cHiPLC™-Nanoflex system. A gradient of 5-35% acetonitrile (0.1% formic acid) in 30 min gradient was used on a nano cHiPLC column (75 µm x 15 cm ChromXP C18-CL 3 µm 300 Å).

Mass Spectrometry: The acquisition method on the TripleTOF™ 5600 System consisted of a high resolution TOF MS survey scan followed by 20 MS/MS per second. The acquisition method on the QTRAP® 5500 system consisted of 100 MRM transitions to both the heavy and light peptides, created using the Scheduled MRM™ algorithm. The MRM experiments were acquired at Q1 and Q3 settings of Unit resolution. The resulting cycle time on both instruments was <1.4 sec to ensure a minimum of 6 data points across the chromatographic peaks at half height for optimal quantification.

Table 1. sPRG2009 Quantitative Standard Protein Description. The five protein mixture was developed to assess quantitative reproducibility across multiple instruments and labs.

Protein	Accession #	Amount of Protein Analyzed (fmol)	Light/Heavy Ratio
Ubiquitin - UBIQ	P62988.1	10	10
Histidyl-tRNA synthetase - SYHC	P12081.2	5	3
Albumin - ALBU	P02768.2	1	1
Ribosyldihydronicotinamide dehydrogenase - NQO2	P16083.4	1	1
Peroxiredoxin 1 - PRDX1	P06830.1	0.1	0.1

Data Processing: Both datasets were processed using MultiQuant™ software. The QTRAP® 5500 system MRM data was processed directly by integrating the area under every LC peak. For the TripleTOF™ 5600 system data, precursor masses were specified and XICs were generated from the TOF MS data automatically using a peak width of 0.05 Da.

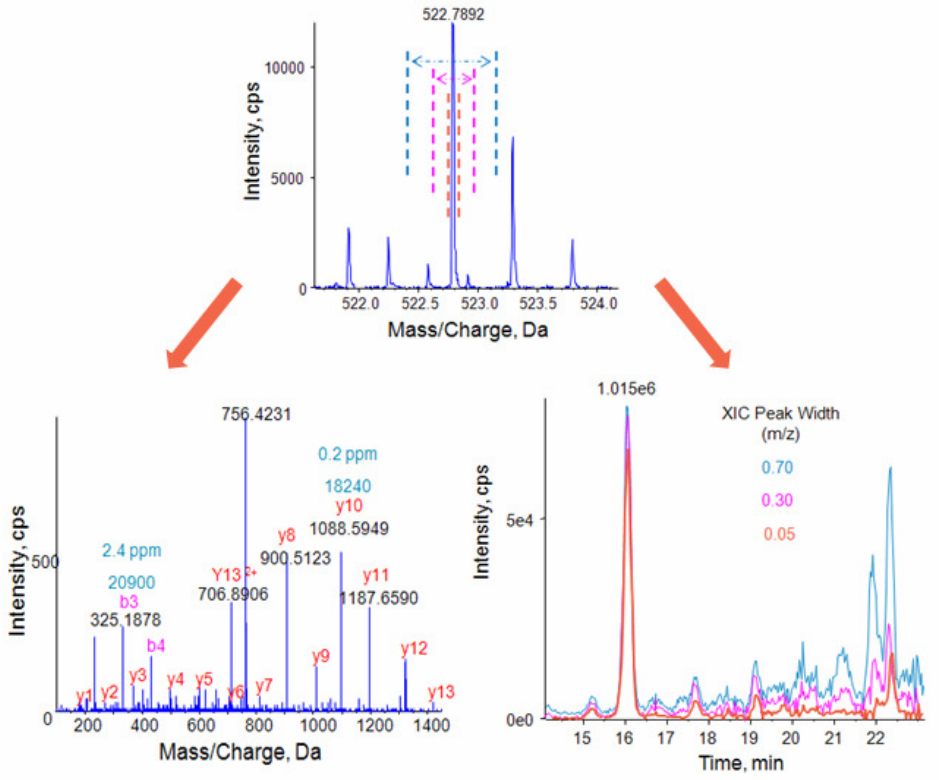

Figure 2. High Resolution Extracted Ion Chromatograms (XIC). Because of the high resolution TOF MS data and the high mass accuracy that is easily attained across extended LCMS operation, it is possible to generate MS XICs at high resolution for highest specificity. In the same run, high quality MS/MS spectra for identification are generated.

Protein Identification with High Mass Accuracy

The TripleTOF™ 5600 system data were submitted for protein database searching using the ProteinPilot™ Software. In total, 32 proteins and 501 unique peptides were identified at a 1% global false discovery rate and high sequence coverage was observed for the 5 protein targets as shown in Table 2. Mass accuracy calculations were carried out using the ProteinPilot Descriptive Statistics Template using the outputs from the ProteinPilot™ Software searches. High mass accuracy was observed in the MS data for the precursor ions of the detected peptides (Figure 3), the RMS of the ppm error was 1.30.

Table 2. Qualitative Analysis of the Standard Protein Sample. Significant sequence coverage was obtained for the protein targets in addition to the heavy and light peptides of interest.

Protein	Sequence Coverage at > 50% Confidence
Ubiquitin - UBIQ	87%
Histidyl-tRNA synthetase - SYHC	74%
Albumin - ALBU	68%
Ribosyldihydronicotinamide dehydrogenase - NQO2	77%
Peroxiredoxin 1 - PRDX1	53%

Mass Error Summary Statistics Table

	Std. Deviation	RMS	Average Error
Delta m/z error	0.00093	0.00094	-0.00016
Delta ppm error	1.30	1.32	-0.26
Delta Sqrt m/z error	1.84E-05	1.86E-05	-3.02E-06

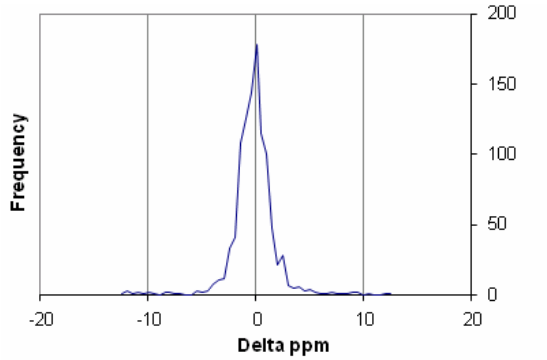

Figure 3. Mass Accuracy Observed for Detected Peptides. Stable, high mass accuracy is crucial when performing high resolution XIC analysis for quantitation as the total MS peak must remain within the XIC window. Very good mass accuracy was obtained here which contributes to the good analytical accuracy obtained.

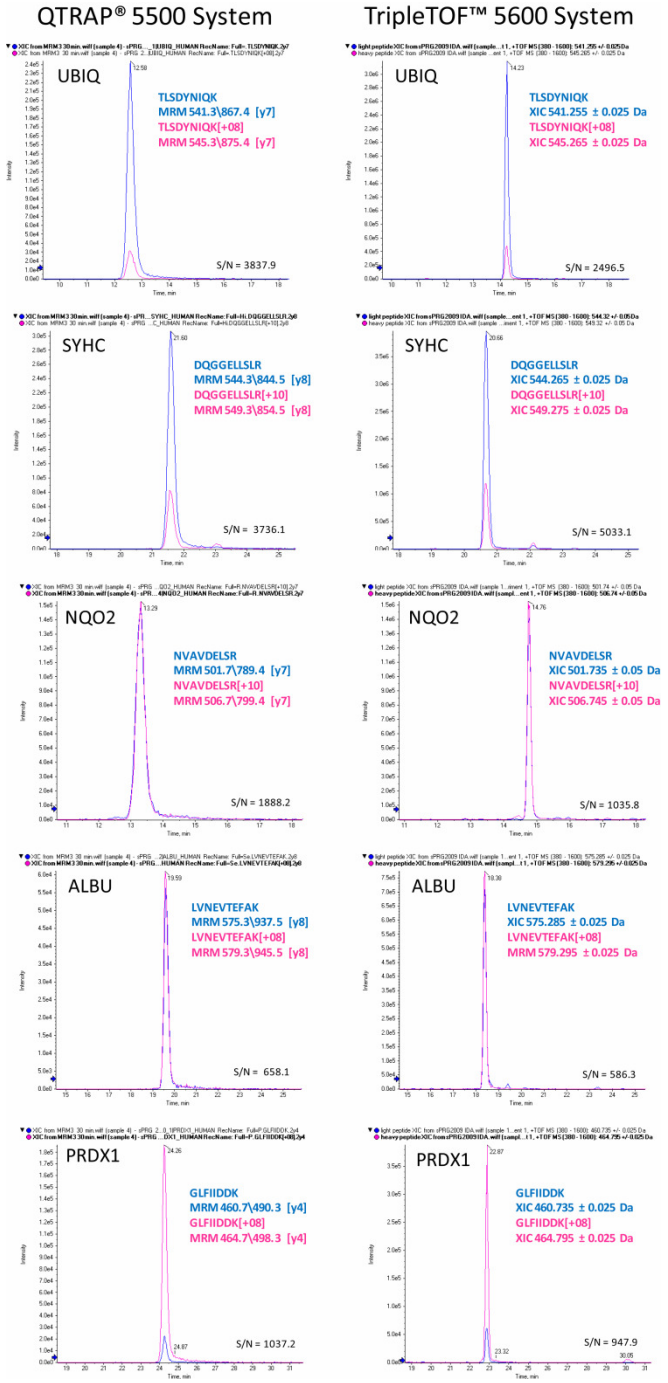

Figure 4. Comparing Quantitation Quality. Shown is a comparison between the quantitation obtained from extracted ion chromatograms (XICs) from TOF MS on the TripleTOF™ System and the best MRM transition acquired on the QTRAP® 5500 system for the light [blue trace] and heavy [pink trace] labeled peptide set for each target protein.

Table 3. Comparing Quantitative Accuracy. The reproducibility and accuracy in measuring the quantitative differences on both the TripleTOF™ 5600 system and the QTRAP® 5500 system were compared.

		TripleTOF™ 5600 System			QTRAP® 5500 System		
Protein	Peptide	Average L/H XIC Ratio	Average L/H Protein Ratio	% Error from Expected	Average L/H MRM Ratio	Average L/H Protein Ratio	%Error from Expected
Ubiquitin	TITLEVEPSDTIENVK	8.254			8.350		
	TLSDYNIQK	7.558	9.16 ± 0.50	9.19	8.884	9.36 ± 0.59	6.84
	ESTLHLVLR	11.665			10.844		
SHYC	AALEELVK	3.559			3.678		
	DQGGELLSLR	3.629	3.43 ± 0.52	12.50	3.907	3.86 ± 0.26	13.50
	GLAPEVADR	3.074			3.900		
	IFSIVEQR	3.461			4.390		
Albumin	LVNEVTEFAK	1.042			0.929		
	SLHTLFGDK	0.989	0.98 ± 0.09	2.00	1.220	1.03 ± 0.12	2.48
	AEFAEVSK	0.909			1.025		
NQO2	NVAVDELSR	0.980	0.98	2.00	1.006	1.01	0.57
PRDX1	DISLSDYK	0.105			0.107		
	ADEGISFR	0.117	0.11 ± 0.07	9.01	0.108	0.11 ± 0.01	9.01
	GLFIIDDK	0.106			0.108		
	LVQAFQFTDK	0.102			0.120		

AB SCIEX TripleTOF™ System has High Quantitative Accuracy

The direct comparison of the MRM quantification from the QTRAP® 5500 system with the high resolution TOF MS quantification obtained on the TripleTOF™ 5600 system is shown in Figure 4. The best MRM transition for both heavy and light peptides are aligned with the extracted TOF MS XICs for each protein. Shown in Table 3 are the quantitative metrics for these two datasets. The measured light / heavy protein ratios for each protein agreed very well with the expected ratio with the relative % experimental error from expected below 12.5% for all measurements with an average of 7%. This result paralleled the quantitative accuracy achieved on the QTRAP® 5500 system by MRM analysis.

Conclusions

On medium complexity samples, the TripleTOF™ 5600 System delivers triple quadrupole type quantification using high resolution MS using the *Profile Workflow*. The analysis of a proteomic quantitative standard containing 5 protein targets and their stable isotopic labeled standard peptides achieved high quantitative accuracy, relative to the expected light/heavy peptide ratios. In the same acquisition, ~500 unique peptides and 32 distinct proteins were identified through the acquisition of high quality MS/MS spectra in a short analysis time.

References

1. ABRF sPRG2009 Study; Development of Quantitative Proteomics Standards. D Arnott, JG Farmar, AR Ivanov, JA Kowalak, WS Lane, K Mechtler, BS Phinney, RR Ogorzalek Loo, MR Raida, and ST Weintraub, Association of Biomolecular Resource Facilities.

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