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New Quadrupole-Ion Trap-Orbitrap Mass Spectrometer Combined with Real Time Search Enhances Proteome Coverage and Quantification Accuracy in Multiplexing Workflows

Xiangyun Yang, Xiujie Sun, Yue Zhou, Thermo Fisher Scientific, Building 6, 27 Xinjinqiao Road, Shanghai, China, 210006

ABSTRACT

Purpose: Evaluate the performance of Orbitrap Eclipse Tribrid mass spectrometer including modified hardware, real time search in accuracy, precision, and sensitivity for TMT based quantitation.

Methods: Thermo Scientific™ Pierce™ TMT11plex yeast digest standard, TMT-10plex cell line and plasma sample were analyzed with MS2, SPS-MS3 and Real Time Search based SPS-MS3 (RTS-SPS-MS3).

Results: RTS-SPS-MS3 method on Orbitrap Eclipse instrument showed deep proteome coverage and enhanced quantitative accuracy performance in multiplexing workflows.

INTRODUCTION

Multiplexing quantification with isobaric reagents (e.g., TMT) is a widely-used and powerful strategy for proteomics studies. A challenge of this technique is the limited quantification accuracy caused by interference from co-isolated peptides and co-fragmentation in MS2 spectra. To address this challenge, a new technique called Real-time Search-MS3 (RTS-MS3) has been developed for the Thermo Scientific™ Orbitrap Eclipse™ quadrupole-ion trap-Orbitrap instrument, building on the SPS-MS3 method used with previous generations of this platform[1]. Here, we evaluate the sensitivity and quantification accuracy of RTS-MS3 on the Orbitrap Eclipse mass spectrometer for TMT-based quantitation using TMT11plex yeast digest standard, TMT10plex cell samples and depleted plasma samples containing a 6-proteins digest.

MATERIALS AND METHODS

Sample Preparation

TMT11-labeled triple knockout (TKO) yeast standards (P/N A40938), Thermo Scientific™ Pierce™ Top14 Abundant Protein Depletion Mini Spin Columns (P/N A36369) and Pierce™ Intact Protein Standard Mix (P/N A33527) were obtained from Thermo Fisher Scientific.

High abundant proteins of human plasma were depleted using Top 14 Abundant Protein Depletion Spin Columns. Trypsin digested human 293T cell, depleted plasma and Pierce intact protein standard mix were prepared in lab follow common digestion workflow[2]. 0.5 to 48 pmol trypsin digested 6 protein standard were added to 20 µg trypsin digested human 293T cell and plasma, and then 15µg peptides were labeled with TMT 10plex regent. As needed, TMT labeled 293T cell peptides and plasma were separated via reversed phase chromatography (Figure 1).

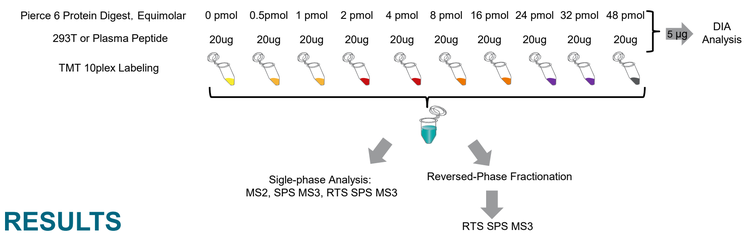
Test Method

Thermo Scientific™ Pierce™ TMT11plex yeast digest standard is used for optimizing method and assessing the sensitivity of RTS-MS3 quantitation. In order to evaluate the accuracy, precision, and sensitivity, 1µg of TMT labeled peptides containing a 6-proteins digest mixtures were analyzed with Orbitrap Eclipse using MS2, SPS-MS3 and RTS-SPS-MS3. For comprehensive proteome characterization, each unlabeled samples was analyzed with DIA method. Meanwhile, each fraction of TMT labeled samples was analyzed using RTS-MS3 method on an Orbitrap Eclipse, with Xcorr value set to 1 and ΔCn set as 0.1.

Data Analysis

All TMT data analysis was performed using Thermo Scientific™ Proteome Discoverer™ 2.4 software with a 10ppm MS1 and 0.6 Da MS2 mass tolerance, TMT6plex (229.163 Da) set as a static modification, co-isolation threshold set to 75. False-discovery rate of PSM, peptide and protein was set to 1%. DIA data was analyzed with DIA-NN software[3].

Figure 1. TMT-labeling strategy for the preparation of 293T cell and depleted plasma peptides containing a 6-proteins digest mixtures. 0.5 to 48 pmol trypsin digested 6 protein standard were added to 20 µg trypsin digested human 293T cell and plasma peptide. Peptide mixtures were analyzed using MS2, SPS-MS3, RTS-SPS-MS3 and DIA method.



RESULTS

SPS-MS3 based methods provide higher accuracy than HR-MS2 methods for TMT quantitation. However, conducting an MS3 scan takes time, and not every MS3 scan is useful. Besides, quantitative accuracy depends on which fragments are selected for MS3 fragmentation. The real-time Search capability enables instruments to make an intelligent decision on when to perform an MS3 scan and speeds up this workflow. Using the optimized method, we could quantify more than 1500 proteins using RTS-MS3 or MS2 based method, while just about 900 proteins could be quantified using SPS-MS3 method from TMT11plex yeast digest standard (Figure 2). What's more, Real-time search can determine which fragment should be chosen for MS3 fragmentation. The TMT-11plex yeast result showed that the interference could be reduced by 20% compared to MS2-quantification (Figure 3).

Figure 2. Real-time search can elevate the proteome depth of SPS MS3 quantitation. 500ng of TMT11plex Yeast Digest Standard was analyzed on a 60min gradient using MS2, SPS MS3, or RTS SPS MS3 methods. We evaluated the effect of Real Time Search for TMT identification rates on PSM(2A.), peptide (2B.) and protein(2C.) levels as well as the SPS mass match distribution at PSM level (2D, 2E).

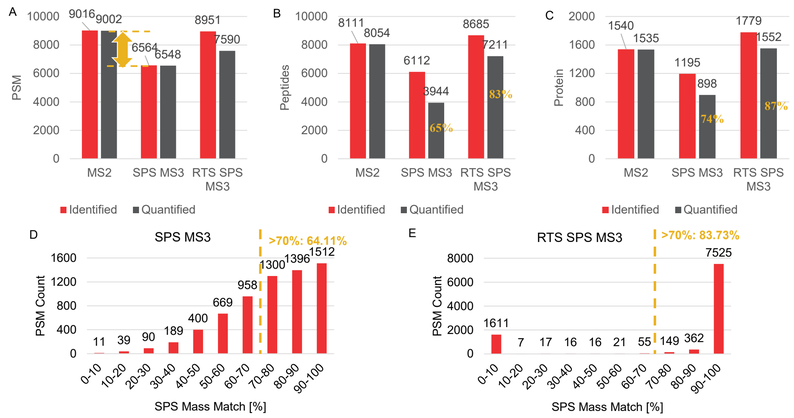


Figure 3. Intelligent acquisition strategies of real-time search can improve the TMT quantification accuracy. Pierce TMT11plex Yeast Digest Standard including three triplicated knock-out strains (Met6Δ, His4Δ, or Ura2Δ) (3A.). These three knock out proteins can be used to evaluate the interference free index[4] (IFI) at the protein level (3B.) or peptide level (3C.).

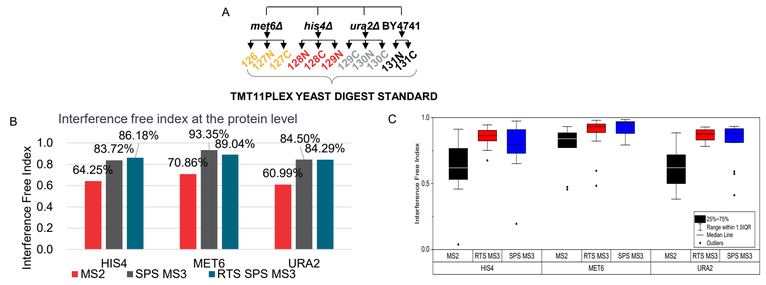
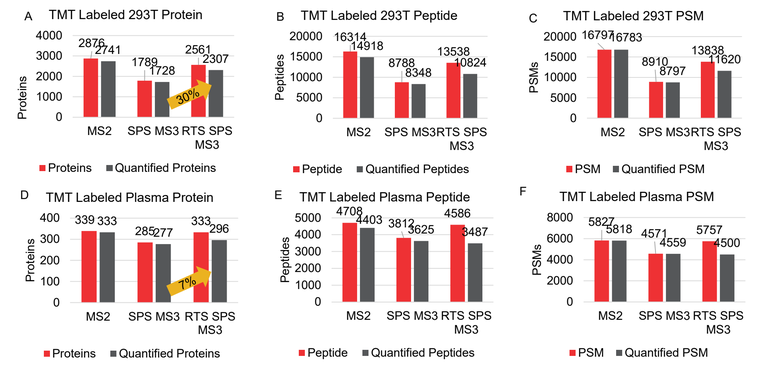


Figure 4. Evaluate the performance of real time search with single-phased TMT labeled peptides using HRMS2, SPS-MS3 and RTS-MS3. 1ug of 293T or plasma peptides was analyzed on a 120min and 90min gradient separately.



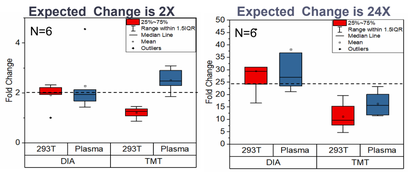
For deep human proteome characterization, we also tested RTS-MS3 using fractionated TMT-10plex cell line and plasma sample for a total analysis time of 24h or 18h, respectively. We observed more 9728 protein groups and more than 80000 peptide groups from 12µg TMT labeled 293T samples, and 1411 protein groups and 10000 peptide groups from 12µg top-14 abundant protein depleted plasma samples (Table 1) . Pierce 6-proteins digest mixtures were spiked in various ratios into the 293T cell or plasma peptides (Figure 1). 1ug unlabeled peptides were measured with DIA method. The total analysis time of DIA is equal to TMT analysis time.

Compared with MS2-based quantification, the measured ratio of the six proteins in RTS-MS3 experiment was closer to the expected value. With the same analysis duration, the proteome coverage of TMT-quantification was greater than DIA method (Table 1). Meanwhile, compared to TMT quantification, we observed that the quantitative accuracy of DIA is higher (Figure 5).

Table 1. Proteins identified from trypsin digest of 293T cell or top-14 abundant protein depleted human plasma with TMT or DIA method.

	Method	Proteins	Quantified Proteins
293T_TMT10plex_24h	RTS SPS MS3	9728	9347
Plasma_TMT10plex_18h	RTS SPS MS3	1411	1248
293T_TMT10plex_25h	DIA	7245	682
Plasma_TMT10plex_18h	DIA	809	6985

Figure 5. Evaluation of TMT quantification accuracy and comparison to DIA method.



CONCLUSIONS

- We use a set of complex models including Pierce™ TMT11plex yeast digest standard and, TMT-10plex cell line and plasma sample to evaluate.
- 6 proteins digest with known relative abundances were spike into cell and plasma proteome. This allowed us to study the accuracy and precision of TMT based quantification.
- RTS-MS3 method on Orbitrap Eclipse instrument is the new gold standard for highest DDA quantitative accuracy and proteome depth.
- DIA quantification can be used to further verify the results from TMT quantification. With these techniques, researchers will be able to find more reliable quantifications, which will finally be translated into meaningful biological findings.

REFERENCES

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