Capture scheduled retention time window shift in large scale of peptide quantitation using a modified Orbitrap hybrid mass spectrometer

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Abstract

Purpose: A high-throughput targeted method (~800 peptides in a 30-minute gradient) was developed to dynamically adjust scheduled data acquisition windows at narrower time intervals to eliminate missing values that compromise data quality. This is the first time adaptive RT has been used on hybrid Orbitrap mass spectrometer (MS) in large-scale targeted assays.

Methods: A large-panel targeted PRM method was developed with a 0.6-minute RT window to accommodate approximately 800 peptides. Using the same column and gradient, a reference file was generated and subsequently embedded into the targeted PRM method for adaptive RT real-time alignment.

Results: Using adaptive RT, the scheduled retention time (RT) windows were adjusted in real-time to capture peptides with shifted RTs. This eliminated the need for rescheduling RT windows, saving time in large-scale targeted quantitation assays. Consequently, 800 peptides were measured in a 30-minute gradient with excellent linearity, accuracy, and precision.

Introduction

Plasma proteomics using mass spectrometry is a promising method for discovering disease biomarkers due to the ease of sample collection. However, large-scale peptide quantitation using Orbitrap PRM requires a robust mass spectrometry setup with compromising protein identification, dynamic range, and analysis precision. This necessitates a scheduled retention time approach, which could cause missed peaks due to shifted retention time. Rescheduling the retention time window can be tedious.

The adaptive RT on a the Thermo Scientific™ Orbitrap™ Excedion Pro hybrid mass spectrometer (MS) can solve this problem. This method dynamically adjusts scheduled data acquisition windows at narrower time intervals to eliminate missing values that compromise data quality. This is the first time adaptive RT has been used on an Orbitrap hybrid MS in large-scale targeted assays.

Materials and methods

Sample preparation

PQ500™ peptides were obtained from Biognosys AG. Peptide standard was resuspended following the manufacturer's instruction. Thermo Scientific™ Pierce™ neat plasma digest was used as matrix for adaptive RT and peptide sensitivity evaluation.

Test method

A Thermo Scientific™ Vanquish™ Neo UHPLC system was coupled with a Orbitrap Excedion Pro hybrid mass spectrometer. Mobile phase A was 0.1% FA in H2O, and mobile phase B was 0.1% FA in 80% ACN. Autosampler temperature was set at 7°C. Source parameters, including spray voltage and ion transfer tube temperature were optimized. To verify the accuracy, precision, linearity and sensitivity, peptides were diluted into 400ng/µl digested human plasma with a 2 times serial dilution. The concentration of the dilution curve had a wide dynamic range for different heavy peptides, which was from 1x manufacturer's concentration = 100% to 0.1% with a 100% plasma blank as the final level.

Data analysis

Skyline software was used for peptide peaks retention time visualization and data analysis.

Figure 1. LC-MS setup for the large-scale targeted assay with adaptive RT

Build a retention time alignment method using adaptive RT function for ~800 PQ500 peptides

Workflow to build a retention time alignment PRM method

First, PQ500 peptides in pure solvent were run using unscheduled methods created from a transition list in Skyline. Based on the retention times from these unscheduled data files, a 1.2-minute RT window PRM method was developed. This 1.2-minute RT method was then applied to run PQ500 peptides in a plasma digest. A reference file was generated by selecting "Acquire Reference" in the Adaptive RT Full Scan experiment (Figure 2; Table 1a and b). Using the retention times from the 1.2-minute RT window data files, a 0.6-minute narrow RT window PRM method was created through PRM Conductor in Skyline. This reference file was embedded into the narrow window PRM assay for real-time alignment.

The final adaptive RT method included three experiments (Table 1a): an Adaptive RT Full Scan experiment, a full MS experiment, and a scheduled PRM method. Detailed parameters are listed in Table 1b-d.

Figure 2. The workflow to build a retention time alignment method

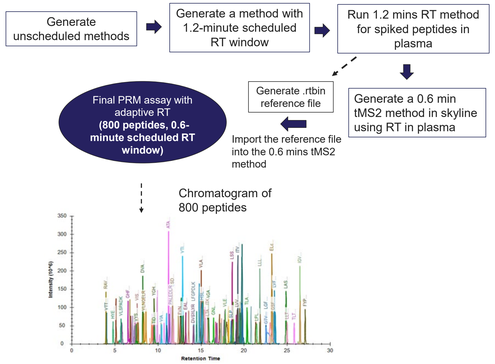
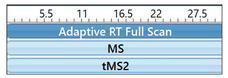
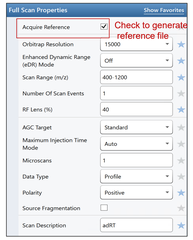


Table 1. The LC-MS/MS parameters in the adaptive RT PRM method: (a) Adaptive RT method includes 3 experiments; (b) MS parameters in adaptive RT full scan experiment; (c) LC gradient; (d)) MS parameters in targeted MS2 experiment

(a)



(b)



(c)

30-minutes gradient (EASY-Spray™ HPLC ES906 column)			
Gradient	Time	%B	Flow (µl/min)
	0	3	0.8
	22.5	30	0.8
	7.5	45	0.8
	Column Wash		
	0.2	99	0.8
	2.8	99	0.8
	Stop Run		
	Column Equilibration		

(d)

tMS2 experiment parameters	
Isolation Window (m/z)	4
Activation Type	HCD
HCD Collision Energy (%)	30
AGC Target	Standard
Maximum Injection Time Mode	Auto
Microscans	1
Loop Control	Time
Time(s)	3
Dynamic Time Scheduling	Adaptive RT
Reference File	Generated from Adaptive RT Full Scan experiment when "Acquire Reference" was checked
Orbitrap Resolution	15000
RF Lens (%)	50
Scan Range Mode	Auto

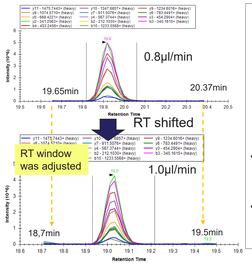
Results

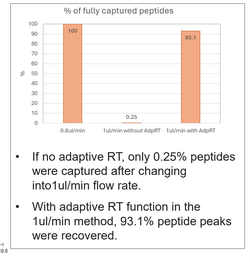
1. **Adaptive RT evaluation with different conditions**

1.1 Test adaptive RT using various column flow rates

To evaluate if adaptive RT is functional, all method parameters were kept the same except that the flow rate was changed from 0.8 µL/min to 1.0 µL/min. Data showed that the retention time shifted by up to 1 minute for different peptides. However, the scheduled retention time windows were also adjusted in real-time analysis to capture the shifted retention times of the peptide peaks.

Figure 3. Scheduled RT window was adjusted in different flow rate by adaptive RT during real time analysis

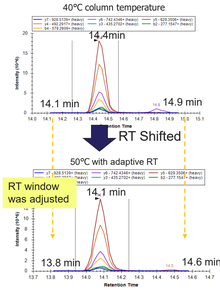


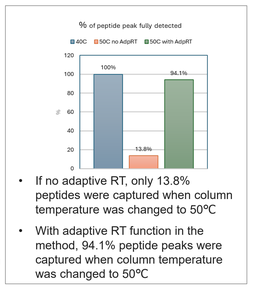


- If no adaptive RT, only 0.25% peptides were captured after changing into1ul/min flow rate.
- With adaptive RT function in the 1ul/min method, 93.1% peptide peaks were recovered.

1.2 Adaptive RT evaluation using different column temperatures

Figure 4. Scheduled RT window was adjusted by adaptive RT function when retention time shifted because of column temperature



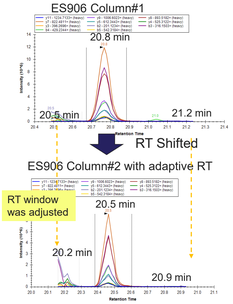


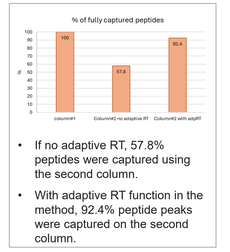
- If no adaptive RT, only 13.8% peptides were captured when column temperature was changed to 50ºC
- With adaptive RT function in the method, 94.1% peptide peaks were captured when column temperature was changed to 50ºC

1.3 Adaptive RT evaluation using different column with the same gradient

When a different ES906 column was used, 92.4% peptides were covered with adaptive RT function, increased 34.6% compared with no adaptive RT alignment in the method.

Figure 5. Scheduled RT window was adjusted by adaptive RT on column#2





- If no adaptive RT, 57.8% peptides were captured using the second column.
- With adaptive RT function in the method, 92.4% peptide peaks were captured on the second column.

2. 800 peptides were quantified using tMS2 method with 0.6-minute scheduled retention time window

PQ500 peptides were diluted into 400 ng of human plasma to measure calibration curves. The linearity was determined based on peptide peak area and concentrations. More than 94.6% of peptides had R^2 values no less than 0.9. More than 98% of peptides had R^2 values no less than 0.8, showing good linearity of the mass spectrometer for large-panel peptide analysis. More than 94.5% of peptides had CV values below 25% in 25 replicate analyses. Limits of quantification and detection (LOQ and LOD) were determined using 3 and 10 times the signal-to-noise ratio, respectively. Many peptides had an LOD of less than 50 attomoles. The LOQ of an example peptide, TLAFPLTIR, was determined as 13.7 amol on column. The accuracy of the peptide is within (100±20)%, while the precision is within 10%.

Figure 6. CV values of 800 peptides using the PRM method with 0.6-minute scheduled retention time window (n=25)

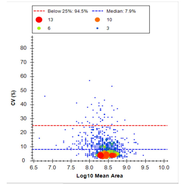


Figure 7. The range of R^2 values from the linearity of 800 peptides

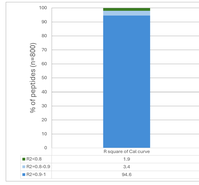
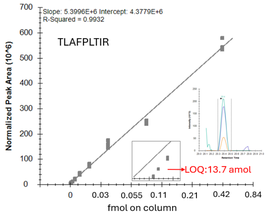


Figure 8. Linearity, accuracy and precision of an example peptide TLAFPLTIR using the 30 minutes PRM method with 0.6-minute scheduled RT window



	fmol (on column)	Accuracy (%)	CV (%, n=3)
level 1	3.5	99.5	0.9
level 2	1.75	91.8	2.9
level 3	0.88	111.5	5.7
level 4	0.44	112.9	7.1
level 5	0.22	119.2	7.8
level 6	0.11	117.2	8.4
level 7	0.055	115.6	7.8
level 8	0.027	111.5	5.0
level 9	0.014	102.0	4.7

Conclusions

- **Peptide analysis with adaptive RT**: Retention time alignment during real time analysis.
- **High throughput**: 800 peptides in 30 mins using 0.6-minute scheduled RT window
- **Time efficiency**: No need to reschedule RT windows after column or condition changes
- **Enhanced data quality**: Adaptive RT adjustment eliminates missing values
- **Innovative technology**: First use of Adaptive RT on Orbitrap hybrid MS for large-scale targeted assays
- **Superior linearity**: PQ500 dilution curves in human plasma show linearity across wide dynamic ranges
- **Exceptional sensitivity**: Achieve low amol level detection and quantitation limits

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