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Application Data Sheet

No.48

LC-MS
Liquid Chromatograph Mass Spectromrter

Accelerating quantitative proteomics analysis with Shimadzu UFMS and Skyline

The Key challenge for quantitative proteomics researchers is to accurately quantitate proteins and peptides in complex biological systems with high sensitivity. Multiple reaction monitoring (MRM) is the main current approach for highly confident protein and peptide quantification.

In a method development of quantitative proteomics, thousands of MRM are needed to be monitored to exhaustively pursue an optimum condition. Skyline[1] can simplify the workflow for both method set up and data processing. On the other hand, in typical triple quadrupole mass spectrometers, it is mandatory for users to divide the experiment into multiple methods to monitor over a thousand MRMs with adequate sensitivity. In other words, multiple injections are required which wastes time and precious samples. Shimadzu UFMS features an ultra fast MRM analysis (UF-MRM®) capability which enables the reduction of number of injections. Figure 1 illustrates an example of method development with trypic digest of BSA by using a single injection. A total of 1710 MRM transitions were monitored in 8 minutes. By combining intuitive features of Skyline and UF-MRM® of Shimadzu UFMS LCMS-8050, the throughput in quantitative proteomics is greatly improved.

1 Skyline is software developed by MacCoss Lab of Biological Mass Spectrometry at University of Washington, WA, US.

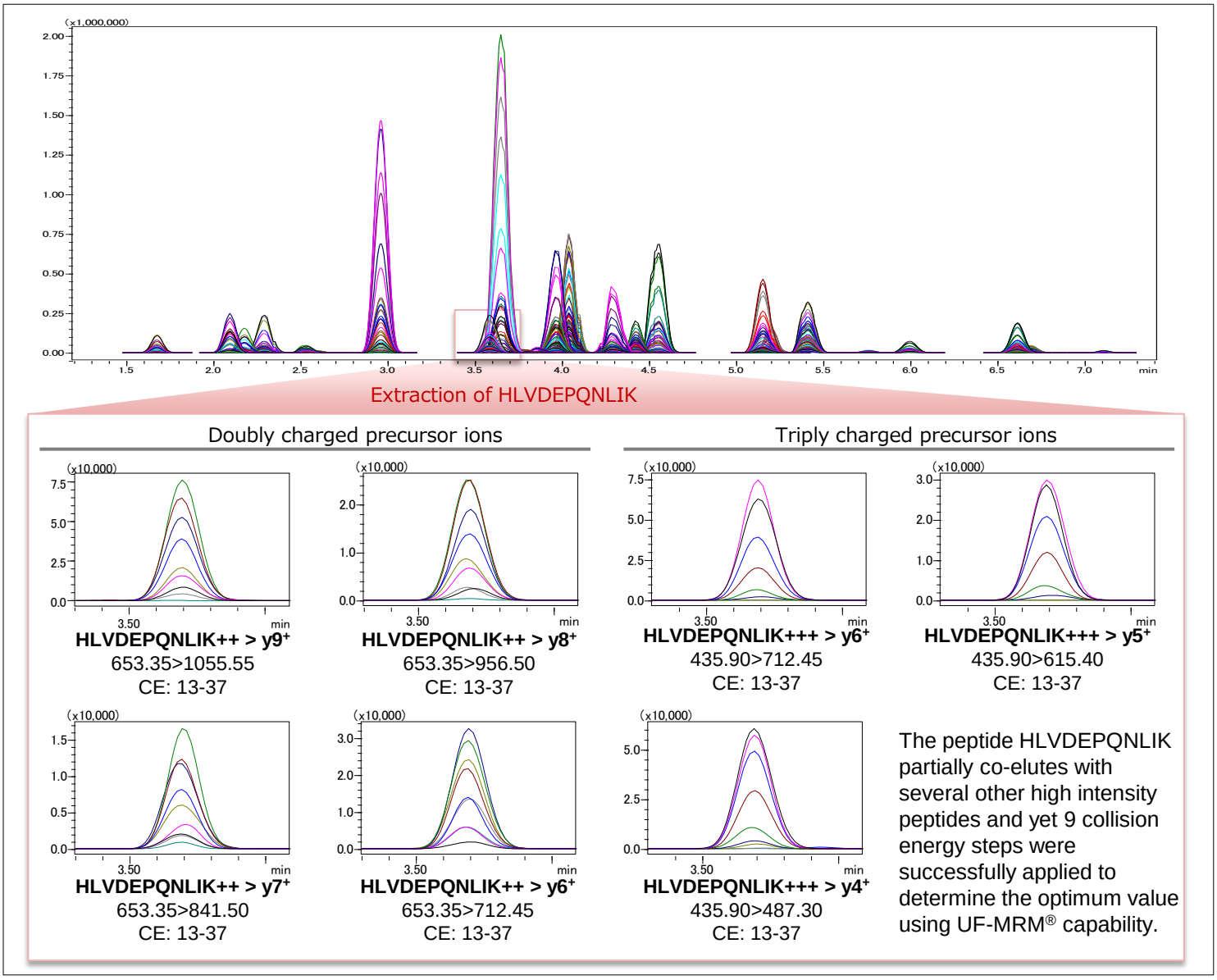
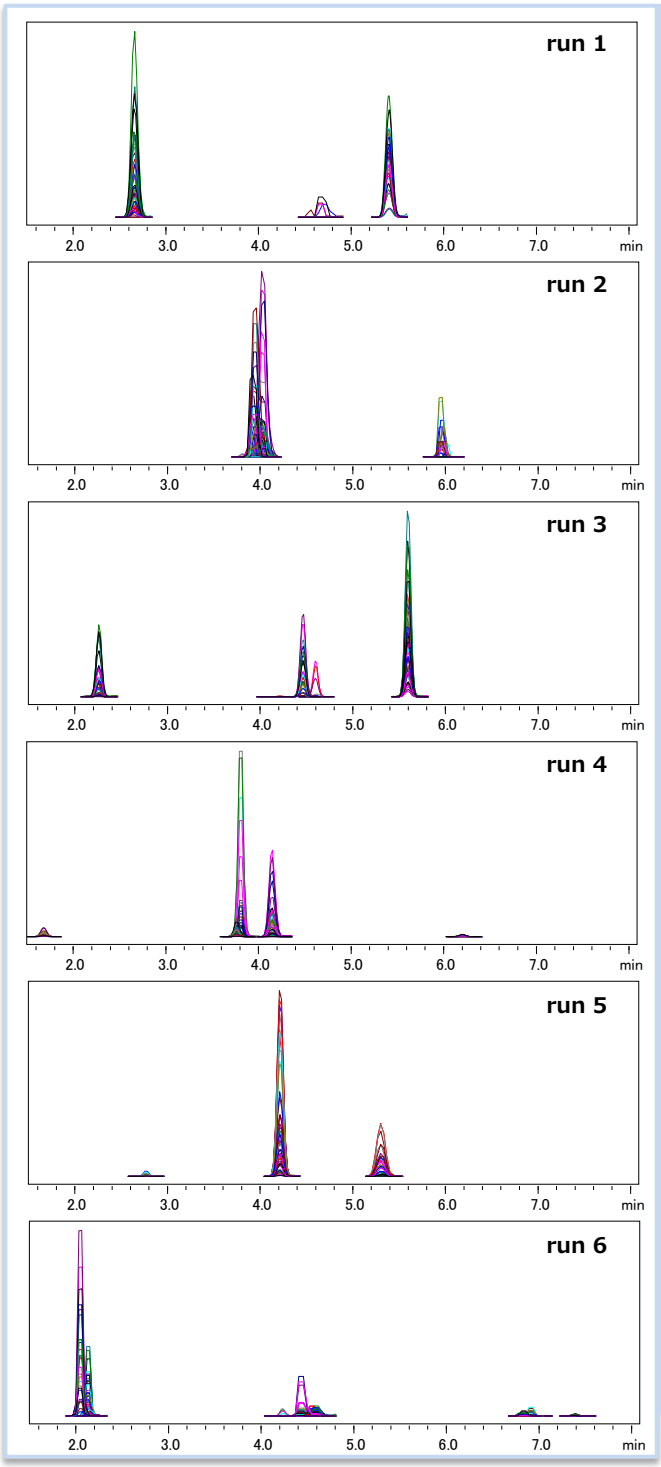
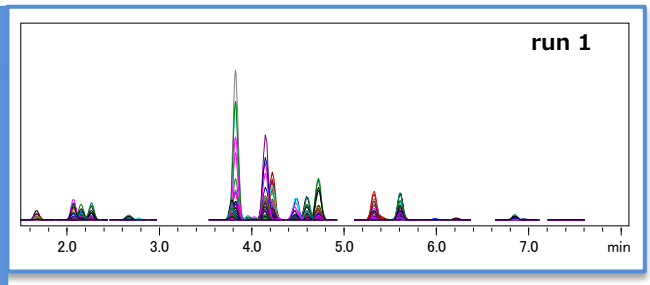


Figure 1. Collision Energy (CE) optimization using Skyline and UF-MRM® (a total of 1710 MRM transitions were monitored in 8 minutes).

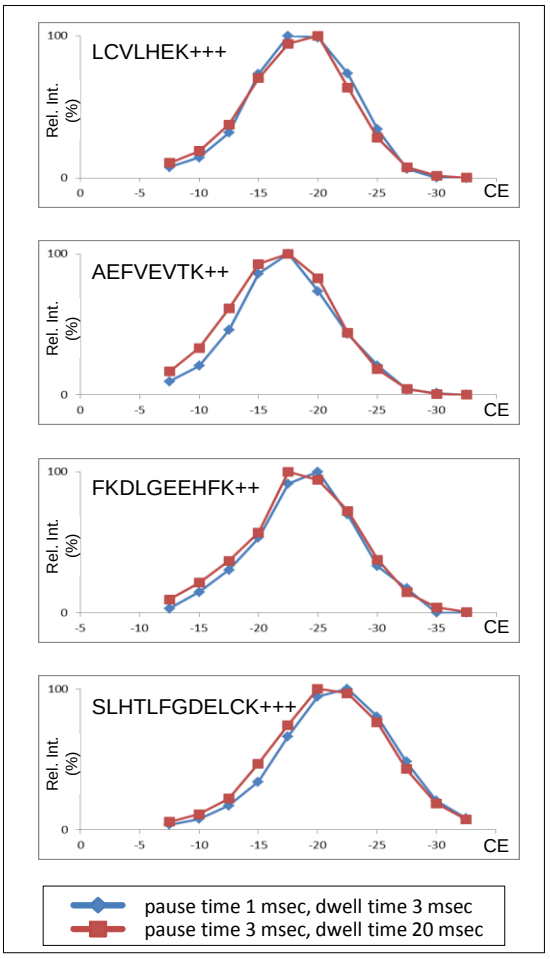
To detect and quantify a high number of peptides with high sensitivity requires CE (collision energy) optimization for each peptide. In conventional technologies this process would typically involve repeated sample injections to achieve an appropriate data quality (in this example, using a pause time of 3 msec and a dwell time of 20 msec required 6 injections to achieve a sufficient number of data points across a peak). However, using UF-MRM® method technologies, a single injection can be used without compromising the data quality. Figure 2 highlights the same data quality acquired with UF-MRM® CE optimization for 1710 MRM transitions using a single injection compared to multiple sample injections acquired with slower, conventional pause and dwell times.



a) Conventional analysis (multiple injections with segmented methods) required 6 injections to acquire sufficient data points to describe a peak.



b) UF-MRM® chromatogram using a dwell time of 3msec an a pause time of 1msec following a single sample injection. Signal intensity and sensitivity are not compromised by high speed data acquisitions.



c) Comparing the signal response using different CE settings between conventional methods and UF-MRM®. Despite a single injection approach used in UF-MRM® the data quality is not compromised.

Figure 2. Comparison between multiple injections and single injection by UF-MRM®

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