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Application News

Liquid Chromatography Mass Spectrometry

No. LCMS-053

Rapid and Reproducible Analysis of Peptides using the Triple Quad LCMS-8050

■ Summary

Recent innovations with Skyline have resulted in a version of Skyline that can upload and analyze *.lcd files directly. We applied this analysis to demonstrate the integration of Shimadzu products into the Skyline environment. Using this workflow, we identified CVs of less than 2 % for several experimental approaches.

■ Background

Two peptides were analyzed at the concentrations of 100 ng/mL, corresponding to 0.1313 and 0.0606 pmol/ µL, respectively. Peptide transitions (generated from Skyline and empirically validated), and generic gradient conditions were used for initial optimization work.

■ Method

For initial method optimization, 2 µL of sample was injected using flow injection analysis (FIA). Under these conditions, precursor and product ions were validated and optimized using Q1, Q3 and product ion scans. MRM transitions were built and the interface and CID conditions optimized using 1 µL FIA injections. Interface conditions included probe voltage, drying, nebulizing and heating gases, interface temperature, heating block temperature and desolvation line (DL) temperature. The important CID variables were CID gas pressure, dwell time, pause time and collision energy. Each parameter was tested and some variables were tested for interaction effects.

Following MS optimization, a Perfinity Halo C18 column was installed and the samples were run using the listed conditions (**Table 1 and Figure 1**). Five consecutive injections followed by blank injection were carried out. Data were uploaded to Skyline daily v2.5.1.5963 and processed using standard approaches. Samples were grouped by experiment (dwell time, pause time) and areas, CV and retention time statistics were generated.

Table 1: LCMS-8050 QQQ Parameter Optimization Values.

MS Parameter	Value
Nebulizing Gas	3 L/min
Heating Gas	20 L/min
Drying Gas	3 L/min
Interface Temperature	400 °C
Heating Block Temperature	400 °C
DL Temperature	150 °C
CID Gas Pressure	300 kPa
Probe Voltage	**1 kV**
CE (Peptide 1)	-17 V
CE (Peptide 2)	-34 V
Column Temperature	40 °C
Dwell Time	**1 - 3 ms**

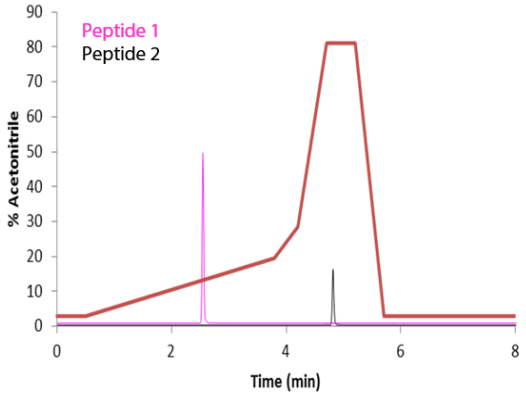

Figure 1: LC gradient used for analysis of the two peptides. Injection volumes were 10 µL for sample analysis following optimization. The gradient utilized for analysis of the peptides was water supplemented with 0.1 % formic acid (FA) and acetonitrile supplemented with 0.1 % FA at a flow rate of 0.5 mL/ min. The column used was a Halo C18, 2.1x100mm 2.7µm, P/N P281-2602 (Perfinity Biosciences, West Lafayette IN).

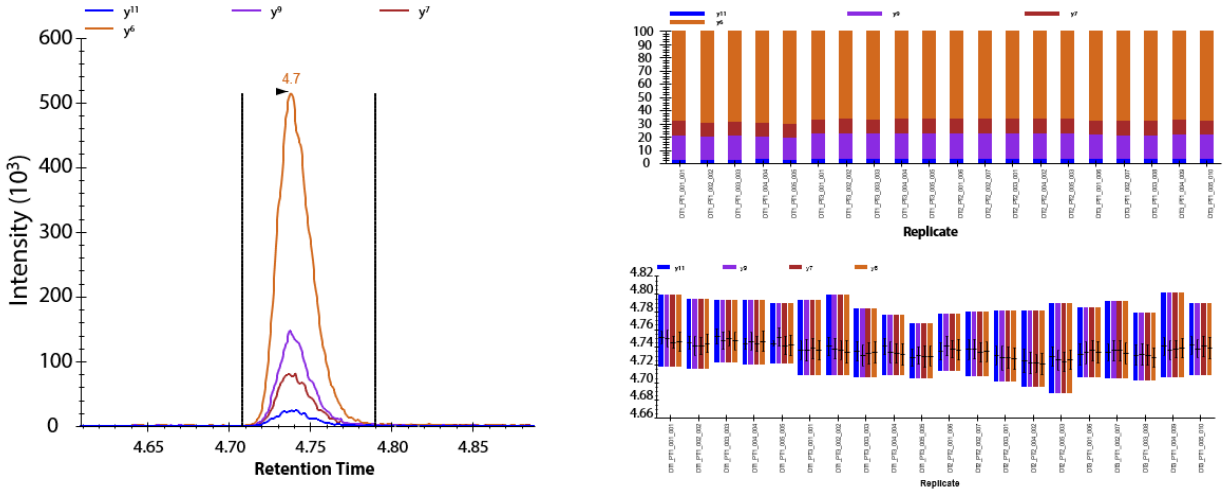

Figure 2: Reproducibility of the acquired LC-MS data. Various acquisition parameters were tested (dwell time and pause time combinations). The selected product ions were monitored for relative abundance and retention time (right side bar charts). The samples showed retention time stability and consistent fragmentation.

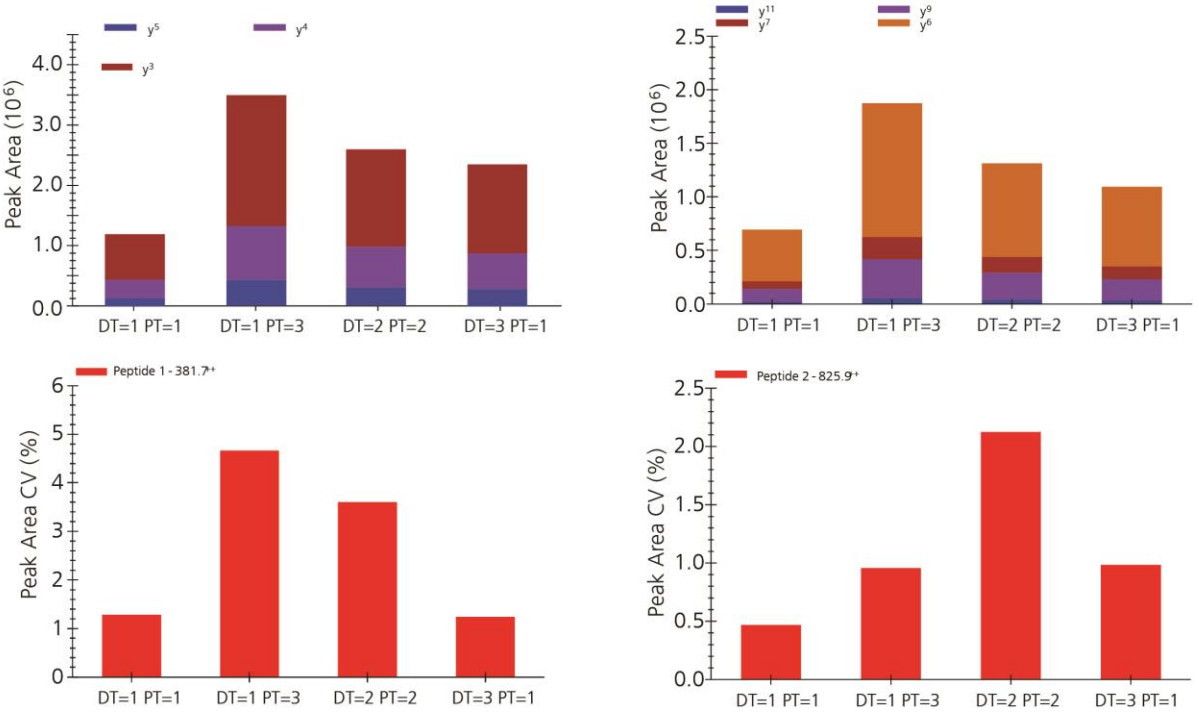

Figure 3: Quantitative analysis of peptide acquisitions. The peak area and CVs for Peptide 1 and Peptide 2 were compared using four conditions. Dwell time (DT) of 1-3 ms and pause time (PT) of 1-3 ms were used. Signal, measured as peak area, was highest with a DT of 1 ms and PT of 3 ms, but optimal CVs of less than 1 % were observed with the shorter pause time.

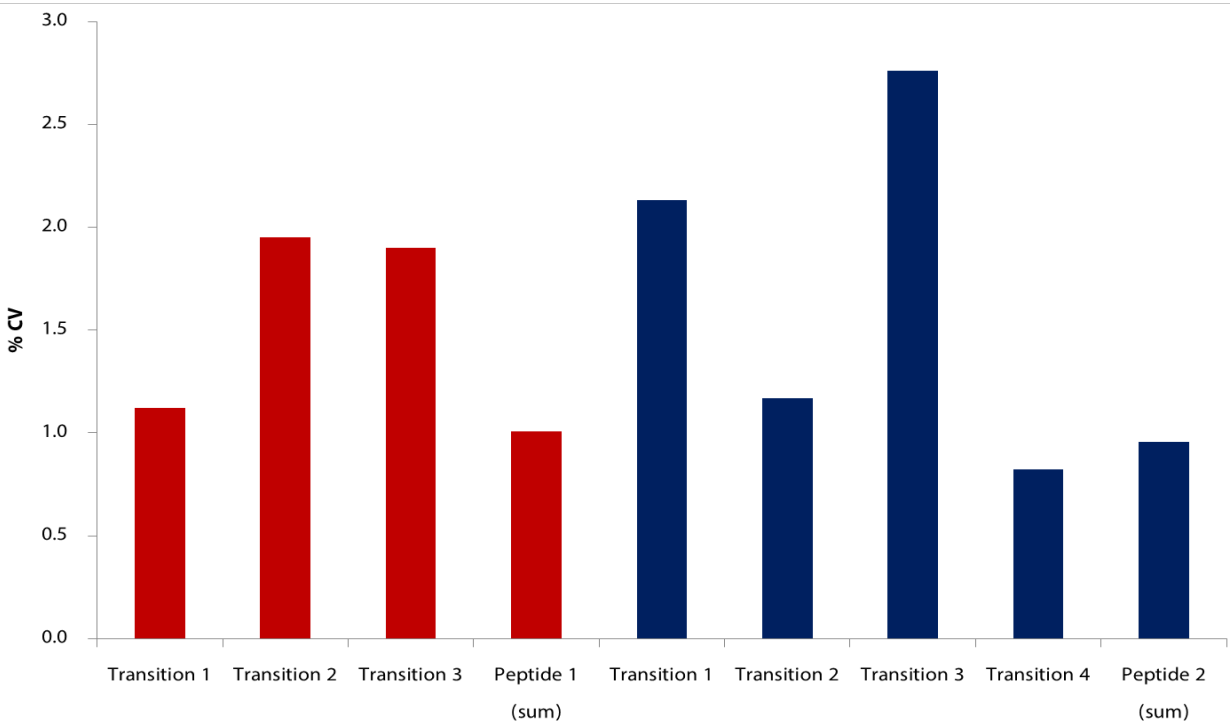

Figure 4: Reproducibility of the acquired LC-MS data. Using Skyline, the CVs for each transition and summed peptide were calculated (n = 5 injections). The CVs were all under 3 % for each transition, and the summed transitions (CV of total area of all transitions) were below 1 %.

■ Results and Discussion

Using Skyline, the effects of dwell time and pause time were analyzed. Using a longer dwell time of 3 ms in conjunction with a short pause time (1 ms) allowed both increased signal detection and improved CVs for the two peptides in comparison to other combinations of these variables.

Overall, in both cases the optimum CV values were at or below 1 % for five replicate injections. Both peptides showed transition ion abundances that were in accordance with the predicted ions. However, both sequences were not present in the reference database (NIST human reference database) to be compared to library spectra for expected ion abundance.

■ Conclusion

The LCMS-8050 demonstrated optimal performance for both target peptides with combined dwell and pause times below 5 ms. By directly importing Shimadzu files into Skyline for integration and statistical analysis, the reported CVs for summed transitions for both peptides were at or below 1 % for five replicate injections. The LCMS-8050, in conjunction with its' associated software tools, offers a robust solution for peptide analysis and quantification using ultra-fast mass spectrometry.

ULTRA FAST MASS SPECTROMETRY

LCMS-8030 LCMS-8040 LCMS-8050 LCMS-2020 LCMS-IT-TOF

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First Edition: October, 2012