Integrated tools for scripted automation of tuning, review and analysis pipelines

Philip M. Remeš[1], Nicholas Shulman[2], Cristina C. Jacob[1], Courtney Patterson[1], Qingling Li[1], David Kotol[3], Alan Atkins[1], Wes Rogers[1]
[1]Thermo Fisher Scientific, San Jose CA; [2]University of Washington Dept. of Genome Science, Seattle WA; [3]ProteomEdge, Stockholm Sweden

Abstract

Purpose: Automate targeted method development and analysis steps including Finding LC peaks and optimizing MS2 and MS3 parameters.

Methods: Acquisition Composer platform implements iterative loops of pre-acquire, acquire, post-acquire steps integrated into LC/MS workflows, using Skyline for data extraction and Python scripts for data analysis.

Results: Automation provides tools to unlock the potential of the full feature set of Stellar MS™ at scale, including MS2, MS3, and HCD/CID activation types.

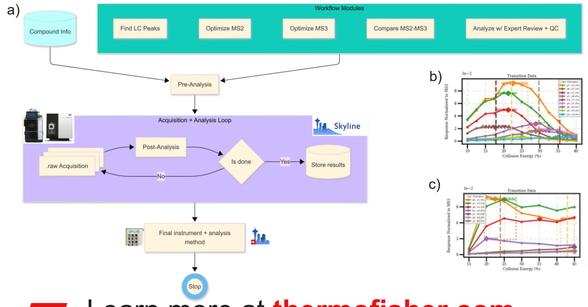
Introduction

Targeted LC/MS analyses provide high-quality data through analyte-specific method customization, but both method development and data analysis can become burdensome as the number of compounds in an assay increases. The Thermo Scientific™ Stellar™ mass spectrometer enables targeted analysis at unprecedented scale, achieving high selectivity through MS3 analysis and post-acquisition MS2 transition selection.[1] Here, we present preliminary results from workflows under development for a platform called Acquisition Composer. We performed MS2 and MS3 parameter optimization and generated dilution curves for the ProteomEdge™ Discovery Edge (DE175) kit and a panel of drugs of abuse.

Methods

Acquisition Composer is a platform that converts compound inputs into MS instrument and analysis methods through iterative cycles of analysis and LC injections. Several workflows are currently under development, including LC peak detection, optimization of MS2 and MS3 parameters, and routine acquisition workflows with Expert Review peak picking and QC-triggered actions. Data extraction from raw files is performed using the Skyline CLI, leveraging the new spectrum filtering feature to separate data acquired under different parameter settings.

Figure 1. a) Overview of Acquisition Composer package. Typical breakdown curves, shown for DE175 peptide LETPDFQLFK for b) collision cell HCD, and c) resonance CID.



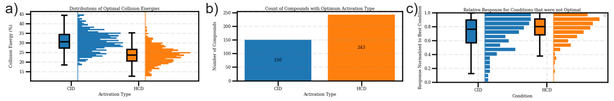
ProteomEdge Discovery Edge 175

393-Peptide Plasma Analysis Kit

The DE175 a 96-well plate kit containing dried down Quantitative Recombinant Protein Standards (qRePS™) produced by ProteomEdge for routine plasma analysis[2]. The qRePS yield a set of heavy-labeled peptides that normalize for digestion and instrument variability. The ProteomEdge assay, originally developed using Evosep 40 SDP and EV1112 75µm x150 mm column, was transferred to Vanquish Neo™ and comparable ES75150PN column using the Find LC Peaks module (see last column).

Optimal MS2 conditions were then determined by varying activation type and energy across four LC injections. The optimum normalized collision energies were 30% for resonance CID using 2 ms activation time and q=0.25, and ~25% for collision cell HCD (Figure 2a). HCD yielded higher signal for 62% of the peptides (Figure 2b). The relative improvement for one activation type compared to the other was typically not large, about 20% (Figure 2c).

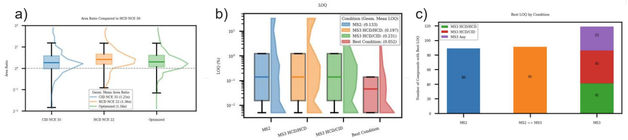
Figure 2. a) Optimal collision energies for DE175 peptides, b) counts of best activation type, c) signal for non-optimal condition relative to optimal condition.



Stellar MS instrument methods currently use a default activation energy of 30% for HCD. This study shows that a lower HCD energy of ~22% gives an average of 35% higher signal, with little or no improvement to be had through further optimization (Figure 3a).

While the full MS3 optimization results are still in progress, dilution curve experiments were performed for three conditions: optimized MS2, MS3 using HCD/HCD, and MS3 using HCD/CID, with collision energies of 22% and 35% applied in both MS3 methods. These MS3 conditions were selected based on smaller-scale PRTC studies. After data acquisition, the analysis transitions were further optimized to achieve the lowest limits of quantitation (LOQs). Selection of the best activation condition for each peptide yielded about 2.5x lower LOQs (Figure 3b). For 70% of the compounds, MS3 produced LOQs that were the same or better than the MS2 LOQs, while 40% of the time MS3 was better than MS2. Future studies will repeat these analyses using peptide-specific MS3 settings, and wider Q1 isolation widths for MS3.

Figure 3. a) Signal relative to previous default HCD 30%, b) limits of quantitation, c) counts of condition with best limit of quantitation.



Drugs of Abuse Assay

32 Common Drugs in a 5-minute Method

This assay is being developed using the Vanquish Horizon™ LC system. In contrast to peptides, the optimal collision-cell HCD energies for MS2 varied substantially across the small-molecule drugs analyzed (Figure 4a), whereas resonance CID at 35% produced the best results for most compounds. Similar trends were observed at the MS3 level (Figure 4b). For both MS2, the relative performance differences between activation types were roughly comparable (Figure 5a). The MS3 result also gave responses that were usually within a factor of 2x in response (Figure 5b).

Figure 4. a) Optimal collision energies for drugs of abuse for a) MS2, and b) MS3

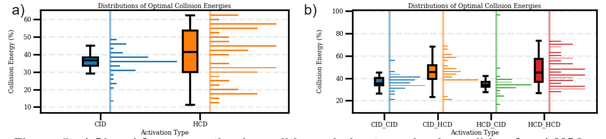
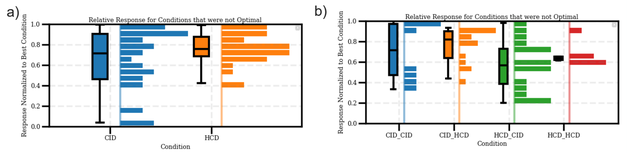
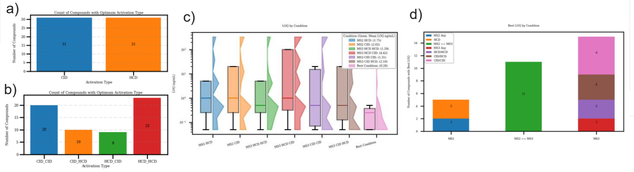


Figure 5. a) Signal for non-optimal condition relative to optimal condition for a) MS2, and b) MS3



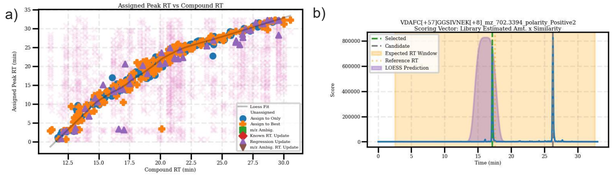
Results showed an even split between optimal activation types for MS2 (Figure 6a), while HCD/HCD or CID/CID often gave the highest MS3 signal (Figure 6b). In some cases, MS3 provided substantially improved selectivity and better analytical performance metrics, as demonstrated for zolpidem (Figures 6c,d). Selection of the best activation condition for each molecule yielded about 6.3x lower LOQs (Figure 6c). MS3 typically gave the same or lower LOQ compared to MS2, where when a clear MS3 modality was favored, often the first activation type was CID (Figure 6d). For 84% of the molecules, MS3 gave LOQs that were the same or better than MS2 LOQs, and 48% of the time MS3 was better than MS2.

Figure 6. Counts of optimal activation type by signal response for a) MS2, and b) MS3, c) distributions of limits of quantitation, d) counts of condition with best limit of quantitation.



Acquisition Composer supports automated assignment of LC features to compounds. At minimum, the workflow requires precursor m/z information, while the inclusion of expected retention times and/or spectral libraries increases assignment confidence. Transferring the assay from the Evosep system to the Vanquish platform was straightforward because ProteomEdge had previously established the expected elution time trends (Figure 7). The observed outliers were attributed to missed-cleavage peptides that were likely absent in the Thermo studies.

Figure 7. a) Comparison of assigned RT on Vanquish Neo compared to Evosep method. b) Disambiguation performed using local regression analysis.



Conclusions

The Acquisition Composer workflows automate key targeted method tasks.

- Peptide activation parameters need little optimization. However, selection of the best MS3 or MS2 condition yielded 2.5x better LOQ than MS2 HCD.
- Optimization of activation parameters for small molecule drugs is essential. Selection of the best MS3 or MS3 condition yielded 6.3x better LOQs than MS2 HCD.
- Future work will build out an integrated user interface that focuses at first on the workflows in Figure 1.

References

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2. Kotol et. al., Targeted proteomics analysis of plasma proteins using recombinant protein standards for addition only workflows. Biotechniques. 2021, 71, 3, 476-483

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