A "Universal" Data-dependent Mass Spectrometry Method That Eliminates Time-Consuming Method Optimization for Achieving Maximal Identifications from Each Sample

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Overview

Purpose: To develop a unique mode of LC-MS/MS analysis that is optimal for all sample types to eliminate time-consuming and sample intensive method development.

Methods: We analyzed a Yeast lysate, HeLa lysate, and immunoprecipitation samples using standard and custom data-dependent LC-MS/MS methods on a Thermo Scientific™ Orbitrap Fusion™ coupled to a Thermo Scientific™ Easy-nLC 1000 ultra-high pressure LC.

Results: Method optimization for varying sample types (ie. Variable concentration or complexity) involves modifying maximum injection times and ion targets. We have developed a novel method that varies maximum injection time and ion targets on-the-fly based on the available parallelizable time on the Orbitrap Fusion. This method successfully identified maximal number of peptides in each sample without optimization of instrument parameters and wasting often precious sample.

Introduction

Choosing the parameters for an LC-MS/MS method occurs by a variety of modes which include significant method optimization, “copy-cat”, or “pick-and-stick”.

- Significant method optimization – a significant amount of time and sample are used for method parameter optimization with multiple replicate runs. Distinct parameters are varied and the effect on identifications are determined for each run until the optimal method is established. This is a common mode used for MS focused labs with expertise in both LC and MS analyses where sample and instrument time are readily available. Researchers from the University of Wisconsin-Madison identified 4,000 proteins from a yeast lysate with a 70 minute LC-MS/MS method.[1]
- “Copy-cat” - this mode of method development is often used by non-MS experts who are usually experts in other fields. Here, a reference paper is used to copy the method using a similar sample type or instrument. Usually these samples pose real-life challenges such as low abundance and unknown concentration. When sample type and concentration/complexity or instrument type are not equivalent between the users sample and the reference paper, these methods may not be optimal.
- “Pick-and-stick” method – this mode of method development is often used by both non-MS experts as well as MS-experts with limited time/too many samples or not enough sample. In this mode, a single method is used for most samples to increase throughput. This is common for MS facilities.

Non-optimized methods may lead to decreased identifications. When building methods based on the pick-and-stick or the copy-cat mode of method development, the methods often do not contain the optimal parameters for the sample. Knowingly or unknowingly, peptide identifications are sacrificed for time/sample savings.

Herein, we will explore the development of a “universal” data-dependent LC-MS/MS method for all sample types and concentrations that does not require method optimization and wasted sample/instrument time.

Methods

Sample Preparation

For these experiments, we analyzed a tryptic yeast digest (provided by Steve Gygi, Harvard), a commercially available tryptic HeLa digest (Pierce, Rockford, IL), and immunoprecipitation samples (provided by Nevan Krogan, UCSF).

Liquid Chromatography

We separated the mixture with an Easy-nLC 1000 ultra-high pressure LC on a Thermo Scientific™ PepMap™ C18 column (75 µm x 25 cm). Solvent A consisted of 0.1% formic acid in H_2O, and B consisted of 0.1% FA in 100% ACN. Gradients were modified based on sample type – Yeast lysate and immunoprecipitations (70 min) or HeLa lysate (140 min or as indicated). For HeLa gradient: peptides were eluted with a linear gradient of 3% to 22% B in 110 minutes, followed by 22% to 35% in 10 minutes, and 35% to 95% in another 10. We finished by holding a 95% B for 10 minutes.

Mass Spectrometry

The eluting peptides were interrogated with an Orbitrap Fusion mass spectrometer running data-dependent LC-MS/MS methods on Orbitrap Fusion Instrument Control Software version 1.1.

- All methods included FTMS1 spectra collected using the following parameters: scan range 400-1500 *m/z*, resolving power 120k, AGC target 4E5, and maximum injection time of 50 ms.
- All methods consisted of 3 second Top Speed mode where precursors were selected for a maximum 3 second cycle. We only interrogated precursors with an assigned monoisotopic m/z and a charge state of 2-6. We filtered precursors using a 60 second dynamic exclusion window and an intensity threshold of 5000.
- All methods consisted of ITMS2 analyses using the following parameters: rapid scan rate, CID NCE 30, 1.6 *m/z* isolation window.
- Each standard method used an AGC target 1E4 and maximum injection time of 35-500 ms (as indicated).
- The universal method used an AGC target of 1E2 and maximum injection time of 250 ms with use all available parallelizable time enabled.

Data Analysis

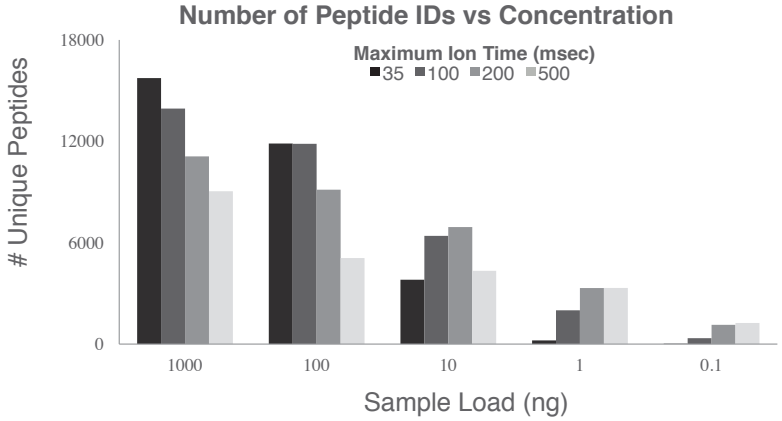
MS/MS spectra were searched using Thermo Scientific™ Proteome Discoverer™ software. We searched the UniProt Human database with tryptic specificity, allowing for up to 2 missed cleavages. We used an MS1 tolerance of 20 ppm and an MS2 tolerance of 1 Da. Oxidation (M) was treated as a variable modification, and carbamidomethyl (C) was treated as fixed. Search results were trimmed to a 1% FDR using Percolator.

Results

How does maximum injection time affect peptide identifications at varying sample load?

During a 70 minute LC-MS/MS analysis of the yeast lysate, the effect of maximum injection time on peptide identifications with variable amounts of peptides on column was evaluated.

FIGURE 1. Demonstration of affect of maximum injection time on number of identifications using a complex tryptic yeast digest.



Achieving maximal identifications requires obtaining a balance between injection time (relates to how many ions are in a spectra and, ultimately, to spectral quality) and scan rate (how many spectra are acquired and may be identified).

- If concentration/complexity are high and injection times are long, spectral quality will be high but scan rate will be too low to obtain maximal identifications.
- If concentration/complexity are low and injection times are short, lots of spectra will be acquired but the quality may be too low to obtain maximal identifications.

Can we create a "Universal" method that achieves optimal peptide identifications without any knowledge of sample concentration/complexity?

Orbitrap Fusion MS, owing to its unique architecture with multiple analyzers, offers many areas of parallelization of activities including isolation of precursors, fragmentation of precursors, and detection of high resolution (Orbitrap) and high sensitivity (ion trap) spectra.

FIGURE 2. Increasing MS/MS spectral quality without decreasing scan rate by using all parallelizable time *between precursor injection and MS/MS detection*. Top Panel: Using standard method with short injection times, parallelization is near complete. Middle Panel: When injection times are shorter than detection times, there is idle time that could be used for longer injection times without affecting scan rate. Bottom Panel: The Universal Method extends all precursor injection times to match the scan out time of the previous ion, increasing spectral quality without decreasing number of scans.

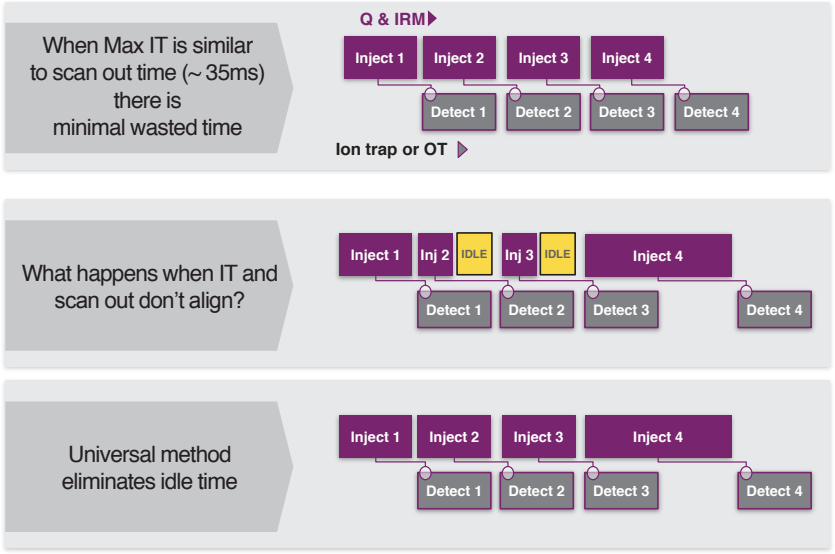


FIGURE 3. Increasing MS/MS spectral quality without decreasing scan rate by using all parallelizable time *during full scan detection* for MS/MS precursor injection and detection. Top Panel: When using a standard method with short injection times, the quadrupole and ion trap may sit idle while the Orbitrap finishes acquiring and processing before a new list of precursors is determined. Bottom Panel: When using the Universal Method, injection times are automatically adjusted to use the full scan time as the total maximum injection time.

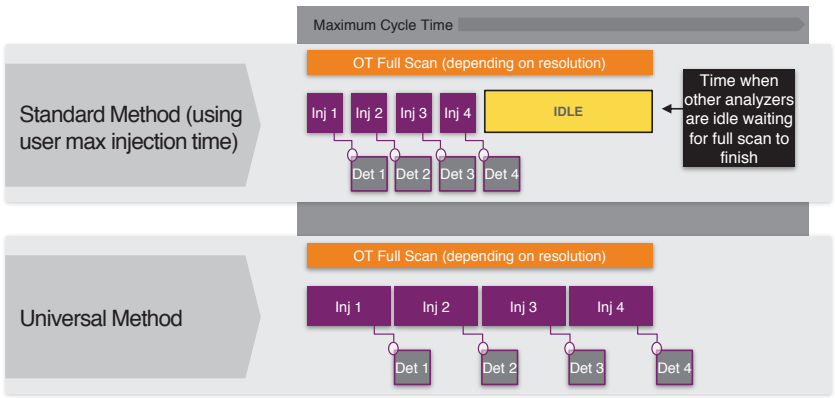
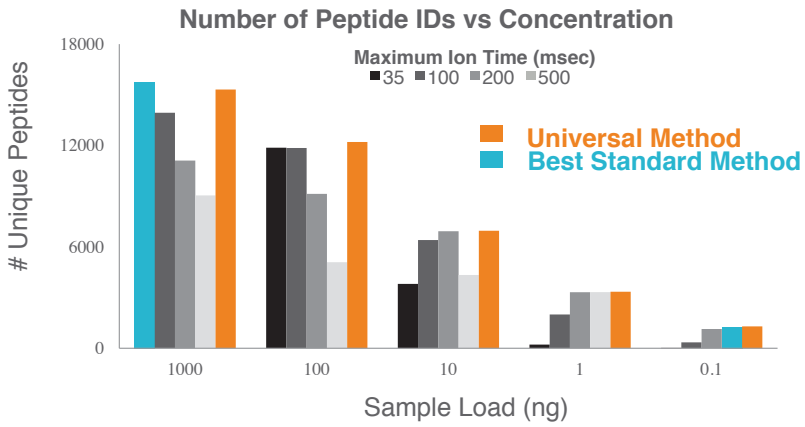


FIGURE 4. Demonstration of the success of a single Universal Method on number of identifications using a complex tryptic yeast digest with multiple variable sample loads.



Achieve maximal identifications using the Universal method by making on-the-fly decisions about the length of injection time based on the number and intensity of precursors selected and the available time available as described in Figure 2 and 3. Ensuring that all time is used between MS/MS scans (ie. Figure 2 bottom panel) enables a decreased dependence on parameters. In the case of the Universal method developed here for MS analysis in the Orbitrap and MS/MS analysis in the ion trap, we were able to decrease the AGC target to 1e2 and increase the maximum injection time to 250 ms. In this method, long injection times are only used when the time is available for "free" (ie. it is parallelizable and does not affect scan rate) or if the intensity is so low that lengthy injection times are required for quality spectra. The Universal method achieves a balance between injection time (relates to how many ions are in a spectra and, ultimately, to spectral quality) and scan rate (how many spectra are acquired and may be identified). When concentration/complexity is low, even at a given time in the chromatographic elution, injection times will automatically be increased. If concentration/complexity is high, injection times will be decreased to maximize scan rate.

FIGURE 5. Demonstrating the success of a single Universal Method with short gradients (60 min) for highly complex HeLa digest.

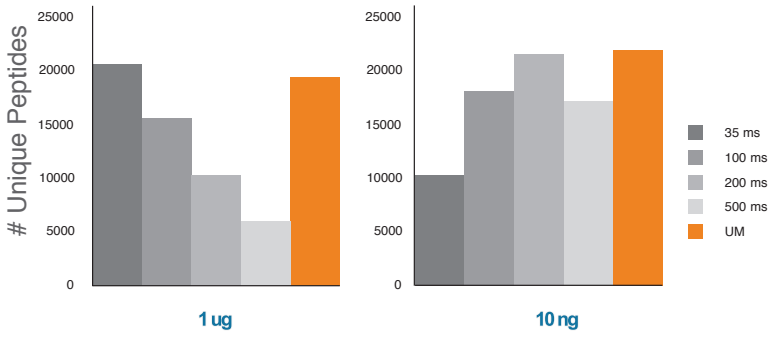
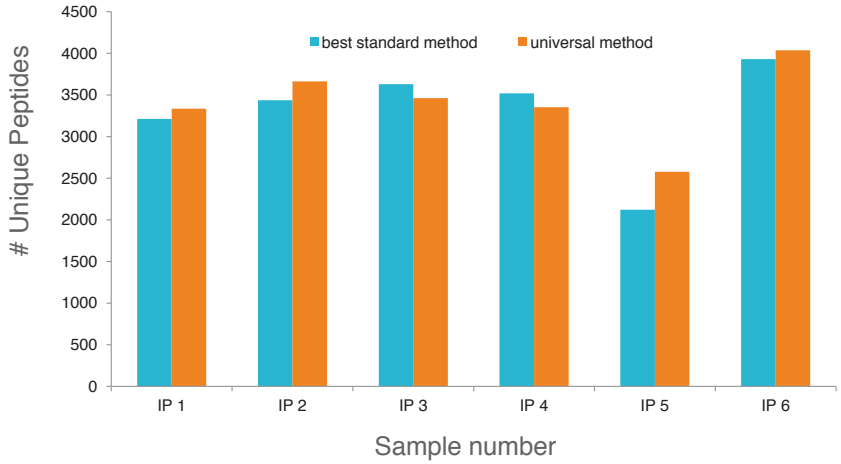


FIGURE 6. Demonstration of the success of a single Universal Method on number of identifications using lower complexity immunoprecipitations of unknown concentrations.



Many samples analyzed by mass spectrometry are injected on column without knowledge beforehand of the concentration or complexity. Prime examples of this are enrichments (such as phosphopeptide enrichments), fractionations (such as SCX), or immunoprecipitations. Method building for such samples can be difficult as often the sample is limited so method scouting is not an option. The universal method provides ease of sample analysis by LC-MS/MS on the Orbitrap Fusion MS.

Conclusion

Developing optimal methods for samples, particularly those of unknown complexity and/or concentration can be incredibly time and sample consuming. Often, time and sample limit the ability to fully optimize the method and maximize the number of identifications.

- The Orbitrap Fusion MS, using the Universal Method, is the first mass spectrometer to know more about your samples than you do.
- The Universal method determines optimal injection times and scan rate on-the-fly depending on the number of precursors available, their intensity, and the available parallelizable time within the system.
- The Universal method has been developed with and evaluated on complex mixtures (Yeast and HeLa lysates) as well as immunoprecipitations of unknown concentration and complexity.

References

1. Hebert AS, Richards AL, Bailey DJ, Ulbrich A, Coughlin EE, Westphall MS, Coon JJ. The one hour yeast proteome. *Mol. Cell. Proteomics*, 3, 339-47 (2014)

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