Key Factors Influencing Nano-Electrospray Ionization Efficiency of Tryptic Peptides from Fused Silica Emitters During Reversed Phase Liquid Chromatography Separations

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ABSTRACT

Purpose: Determine key factors that influence ionization efficiencies of tryptic peptides.

Methods: The ionization efficiency of tryptic peptides was studied using reversed-phase LC separation and mass spectrometry detection on a Thermo Scientific™ Fusion™ Lumos™ Tribrid™ Mass Spectrometer. Sheath gas flow rate and emitter-to-inlet distance were varied.

Results: Electrospray dynamics were found to principally impact proteome coverage. Used in conjunction with tapered fused silica emitters, use of sheath gas (nitrogen, < 1 L/min) provided more reproducible proteome coverage across different emitter/column pairs. Principal component analysis (PCA) provided useful insight into desolvation mechanisms during gradient elution.

INTRODUCTION

It is well established when coupling liquid chromatography with mass spectrometry via electrospray ionization, that the highest ionization efficiency is achieved at flow rates less than 500 nL/min due to the formation of smaller and more highly charged droplets. During gradient elution, a decreasing eluent surface tension changes the size distribution of droplets with constant conditions (voltage, distance from mass spectrometer inlet, temperature and gas flux). In this work, we investigate the key variables principally affecting ionization efficiency and the transport of peptide ions formed from tapered fused silica emitters at nano flow rates during reversed-phase gradient elution.

MATERIALS AND METHODS

Sample Preparation

For data dependent analysis, 200 ng/µL HeLa cell protein digest (Thermo Fisher Scientific) was prepared in water containing 0.1 % formic acid and 2% acetonitrile.

Test Methods

Reversed-phase LC separation using a Thermo Scientific™ RSLCnano with mass spectrometry detection on a Fusion Lumos Tribrid Mass Spectrometer was used to test a prototype ion source. The ion source was fabricated to allow for the addition of a coaxial flow of nitrogen sheath around the fused silica emitter. The protrusion of the emitter from the nozzle was ~1.5 mm. Note that sheath gas values are set according to pressure (psi) that is proportional to a flow rate. A range of sheath gas flow rates between ~0.1 – 1.0 L/min were used in conjunction with a 250 µm nozzle resulting in linear gas velocities in the subsonic range. The emitter-to-inlet distance was varied between 2 – 4 mm.

Samples were analyzed using 50 cm x 75 um columns packed with 2 µm Thermo Scientific™ PepMap™ particles at 300 nL/min. In all cases, a 1 µL injection volume was used. The gradient was ramped from 3% to 40%B (where [A]: 0.1% formic acid in water, [B]: 0.1% formic acid in 80:20 acetonitrile:water) over 30 or 120 minutes. Method parameters are listed in Table 1.

Table 1. DDA method parameters for both analytical gradient lengths. The Orbitrap and Ion Trap (Turbo scan speed) were used for MS1 and MS2 events, respectively. Note that 30 min gradients were employed, unless otherwise noted.

	30 min	120 min
Cycle Time, s	1	3
MS1 Resolution	60k	240k
Dynamic Exclusion, s	5	20
Quadrupole Isolation	1.2	1.2
MS2 AGC Target	3e4	3e4
Ion Injection Time, ms	10	15
Product m/z Range	200-1400	200-1400

Data Analysis

Raw files were searched in Thermo Scientific™ Proteome Discoverer™ 2.3 software using Sequest HT and Percolator to filter results to a 1% FDR at the peptide level. The software was also used to perform PCA and label free quantitation.

RESULTS

Principal Component Analysis

- Replicate measurements spatially cluster together suggesting that analytical conditions are repeatable and of high precision allowing differentiation of different emitter positions and sheath gas flow rates (Fig 1a)
- PC1 accounts for a majority of the variance; ~70% of the variance accounted for with 2 PCs
- When any sheath gas is present, data generally cluster by emitter-to-inlet distance, not sheath gas flow rate (Fig 1b and c)

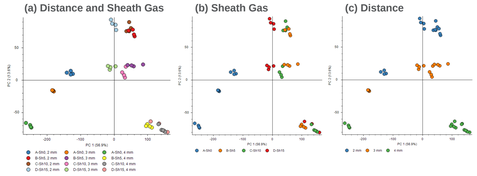

Figure 1. PCA scores plots shown as a function of experimental conditions listed above.

- The region of the loadings plot with highest density was divided into three clusters (Fig 2a). Representative peptide abundances from each cluster are shown with respect to experimental conditions (Fig 2b).
- For clusters 1 and 2, sensitivity decreases with increased emitter-to-inlet distance without sheath gas. For these clusters, eluting over a majority of the gradient, a sensitivity increase is observed when sheath gas is present. As evidenced by cluster 3, a trend toward greater sensitivity with larger emitter-to-inlet distance and higher sheath gas flow rates is observed later in the gradient.

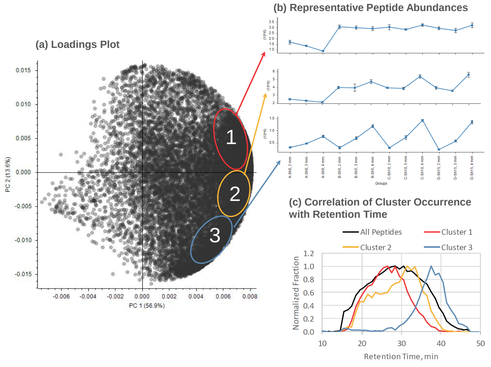

Figure 2. (a) PCA loadings plots with representative peptides shown from each cluster (b). The normalized fraction for clusters 1-3 are shown with respect to retention time in (c).

3 mm Emitter-to-Inlet Distance

- Over a majority of the gradient, a sensitivity improvement is observed when sheath gas is present at all flow rates. However, no gain and in some cases, a sensitivity loss is observed when sheath gas is present at the gradient extremes.

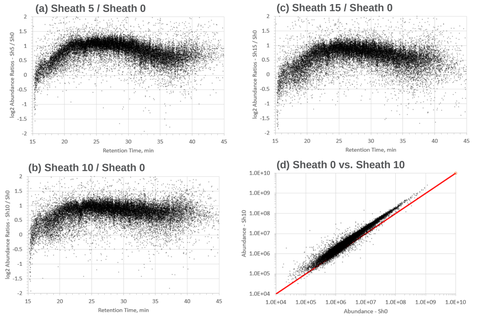

Figure 3. (a-c) log2 abundance ratios as a function of retention time for various sheath gas flow rates. (d) Abundance comparison of with and without sheath gas at a distance of 3 mm.

Peak Area Reproducibility

- As expected, %CV values generally improve with greater sensitivity (Fig 4b and c). Fig 4a shows that %CV distributions are similar in all cases, though all distributions with sheath gas are slightly broader. For all conditions, >93% of all peptides identified have %CV values <20%.

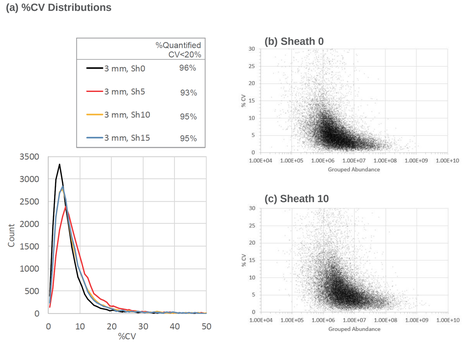

Figure 4. (a) Peak area reproducibility across n = 4 replicates for each condition. For sheath gas values of 0 and 10 psi, the %CV distribution is plotted with respect to abundance (b and c).

2 and 4 mm Emitter-to-Inlet Distance

- Decreasing the emitter-to-inlet distance (to 2 mm) without sheath gas slightly improves sensitivity across the entire gradient (Fig 5a). At this distance, the addition of sheath gas has minimal effect early in the gradient but causes sensitivity loss for hydrophobic peptides (Fig 5b).
- Increasing the emitter-to-inlet distance (to 4 mm) without sheath gas decreases sensitivity early in the gradient, and interestingly, improves sensitivity for peptides eluting near the end of the gradient. (Fig 5c). At this distance, the addition of sheath gas induces a minor sensitivity improvement early in the gradient and again, additional gains for hydrophobic peptides were observed (Fig 5d).

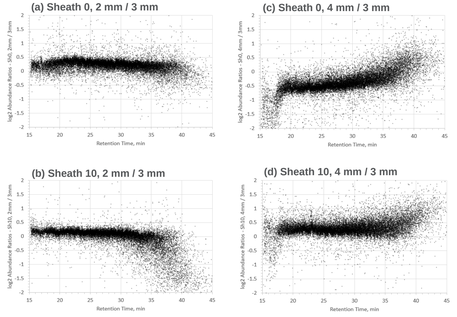

Figure 5. log 2 abundance ratios without (a and c) and with (b and d) sheath gas. In each case, 3 mm distance was used as the baseline.

- Trends observed in Fig 5 at the gradient extremes correspond to the low end of the dynamic range (Fig 6). Peptides with greater abundances (>1e8) are not significantly affected by the experimental conditions.

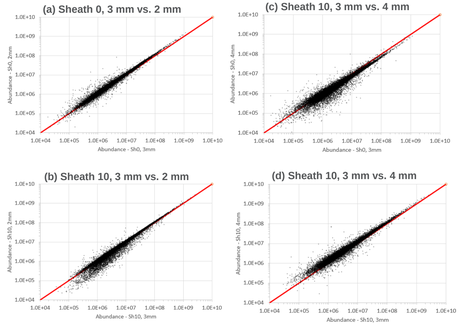

Figure 6. Abundance comparisons without (a and c) and with (b and d) sheath gas. In each case, 3 mm distance was used as the baseline.

Proteome Coverage

- HeLa proteome coverage across four different emitter/column pairs was evaluated with and without sheath gas, and also as a function of distance. On emitter/columns #1 and #2, sensitivity was superior across a majority of the dynamic range when no sheath gas was used; the converse was true for #3 and #4. The greatest variation in coverage was observed without sheath gas. The reproducibility across different emitter column pairs was considerably improved with the addition of sheath gas.

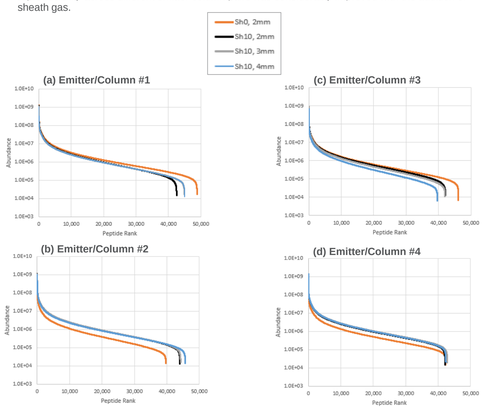

Figure 7. Peptide abundance as a function of rank order. Each line consists of the cumulative result of three technical replicates. The gradient length was 120 min and the HeLa load was 200 ng.

CONCLUSIONS

- PCA proved useful in providing insight into desolvation mechanisms during gradient elution
- Used in conjunction with tapered fused silica emitters, sheath gas (nitrogen, < 1 L/min) provided more reproducible proteome coverage across different emitter/column pairs
- This work suggests that the electrospray dynamics were found to significantly impact proteome coverage

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TRADEMARKS/LICENSING

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