ELECTROSPRAY IONIZATION SOURCE COMPARISON AND OPTIMIZATION ON A CAPILLARY-FLOW SUREQUANT ASSAY

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INTRODUCTION

- Capillary-flow liquid chromatography/mass spectrometry (LC/MS) is widely used for proteomics assays in clinical research because of its sensitivity and robustness
- To optimize a capillary-flow (5 µl/min) proteomics assay for human plasma, we compared multiple Orbitrap-based source configurations and utilized stable isotope-labeled (SIL) peptides at a fixed concentration for quantitative evaluation
- Four configurations of electrospray ionization (ESI) were used to inject PQ500 reference peptides and were evaluated for spray stability, reproducibility, and assay sensitivity
- The configurations include 3 ESI sources, in which the Thermo Nanospray Flex Ion source was customized with both the CoAnn and New Objective emitters

OBJECTIVE

- To investigate the spray stability, reproducibility, and sensitivity of ESI emitters and sources in detecting and quantifying SIL peptides on the Orbitrap Exploris 480

METHODS

Sample Preparation

- 1 µL of commercially available human plasma sample (BioIVT) was aliquoted into each well of a 96-well plate and digested using a PreOmics iST HT 192X kit
- All peptides were quantified using a Quantitative Fluorometric Peptide Assay kit (Pierce)
- Peptides were reconstituted in a Loading Buffer (98% Optima-grade water, 2% Acetonitrile, 0.1% Formic Acid) and 1.5 µg of digested plasma was injected on to the column
- PQ500 SIL peptides were spiked into each well according to the vendor's guidance (Biognosys)

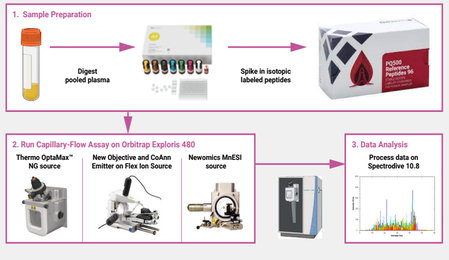
LC/MS

- A Thermo Orbitrap Exploris 480 equipped with Dionex Ultimate 3000 RSLCnano was configured with a Thermo PepMap column (0.3 mm x 150 mm; 2 µm) and operated at a 5 µL/min flowrate
- Data were collected with a SureQuant method on 3 ESI sources: Thermo OptaMax™ NG (TNG), Thermo Nanospray Flex Ion, and Newomics MnESI. The Thermo Nanospray Flex Ion source was equipped with either a New Objective or CoAnn emitter, and the Newomics MnESI source had an M3 emitter
- Data were processed with Spectrodive 10.8 (Biognosys), in which only PQ500 SIL peptides were analyzed

Experimental Design

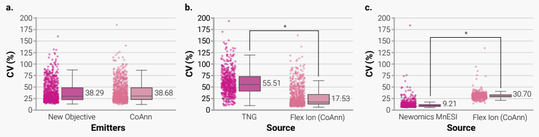
- Filtered Human K2EDTA Plasma was digested in all 3 experiments and injected by each configuration with 1 injection/well:
  1. New Objective vs CoAnn: 84 wells of plasma were digested and injected on each emitter
  2. TNG vs Flex Ion (CoAnn): 11 wells of plasma were digested and injected with each source
  3. Flex Ion (CoAnn) vs Newomics MnESI: 40 wells of plasma were digested and injected with each source

FIGURE 1. Experimental design for capillary-flow SureQuant assay on Orbitrap Exploris 480.



RESULTS

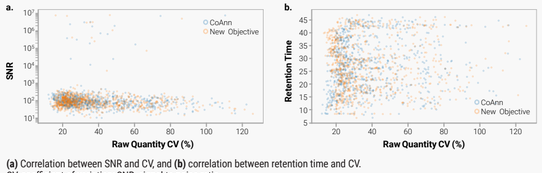
FIGURE 2. Newomics MnESI source provided superior reproducibility over standard source configurations.



CV of mean AUC for: **(a)** New Objective and CoAnn emitters on ambient Flex Ion source; **(b)** captive TNG source and ambient Flex Ion source with CoAnn emitter; and **(c)** ambient Flex Ion source with CoAnn emitter and captive Newomics MnESI source.
*p-value<0.05; 2-tailed t-test for independent means used to calculate p-value.
CV, coefficient of variation; AUC, area under the curve; TNG, Thermo OptaMax™.

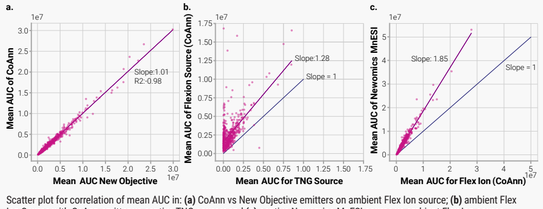
- On the Flex Ion source, the New Objective and CoAnn emitters had comparable reproducibility (Figure 2a), although CoAnn had greater reproducibility when the source was covered with aluminum foil (Figure 2b)
- Between the ambient CoAnn emitter and captive Newomics MnESI source, the latter yielded the lowest CV (<10%) (Figure 2c)

FIGURE 3. No correlation was observed between peptide SNR/retention time and CV in the ambient Flex Ion source.



(a) Correlation between SNR and CV, and **(b)** correlation between retention time and CV.
CV, coefficient of variation; SNR, signal to noise ratio.

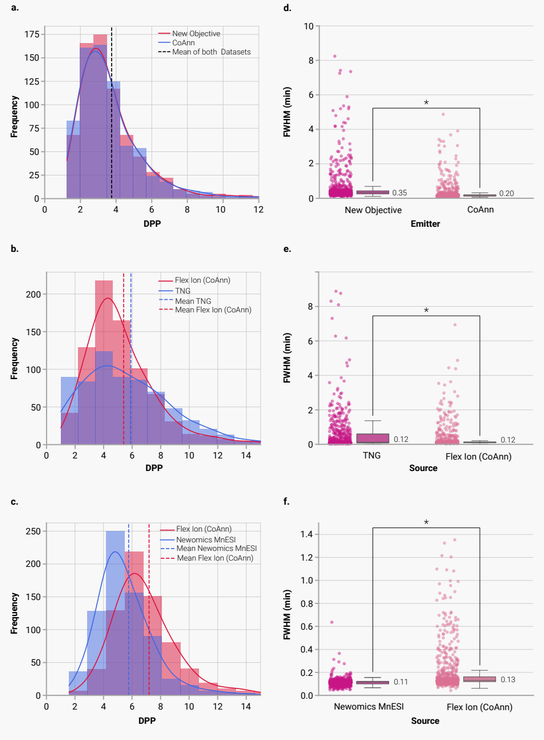
FIGURE 4. Newomics MnESI source had higher sensitivity over standard pulled emitters.



Scatter plot for correlation of mean AUC in: **(a)** CoAnn vs New Objective emitters on ambient Flex Ion source; **(b)** ambient Flex Ion Source with CoAnn emitter vs captive TNG source; and **(c)** captive Newomics MnESI source vs ambient Flex Ion source with CoAnn emitter.
AUC, area under the curve; TNG, Thermo OptaMax™ NG.

- New Objective and CoAnn emitters on a Flex Ion source had comparable sensitivity (Figure 4a), the CoAnn emitter on a Flex Ion source had slightly higher sensitivity than the TNG source (Figure 4b), and the Newomics MnESI source had higher sensitivity than the CoAnn emitter on the Flex Ion source (Figure 4c)

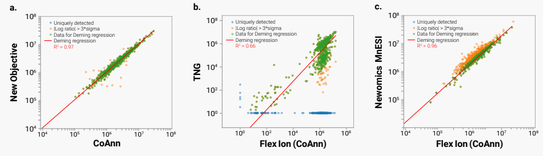
FIGURE 5. Newomics MnESI source provided superior DPP and FWHM compared to other source/emitter configurations.



Kernel density estimate distributions of average DPP and box plots of mean FWHM for: **(a,d)** New Objective vs CoAnn emitters on ambient Flex Ion source; **(b,e)** captive TNG source vs ambient Flex Ion Source with CoAnn emitter; and **(c,f)** ambient Flex Ion source with CoAnn emitter vs captive Newomics MnESI source. *p-value<0.05; 2-tailed t-test for independent means used to calculate p-value.
DPP, data points per peak; TNG, Thermo OptaMax™ NG; FWHM, full width at half maximum.

- The CoAnn emitter had comparable DPP to the New Objective emitter but had higher DPP compared to the captive TNG source (Figure 5a, b, d, and e)
- Based on the t-test results of boxplot comparisons, the difference in FWHM between all configurations was statistically significant and meaningful (Figure 5f)

FIGURE 6. Newomics MnESI source provided improved sensitivity compared to other source/emitter configurations when comparing raw quantity of heavy SIL peptides.



Raw quantity (peak area) of heavy SIL peptides between: **(a)** ambient Flex Ion source with New Objective emitter vs CoAnn emitter; **(b)** captive TNG source vs ambient Flex Ion source with CoAnn emitter; and **(c)** captive Newomics MnESI source vs ambient Flex Ion Source with CoAnn emitter.
TNG, Thermo OptaMax™ NG.

- Different emitters on the Flex Ion source had a strong correlation (Figure 6a)
- The Flex Ion source and TNG Source had low correlation, with more peptides and proteins detected on the Flex Ion source (Figure 6b)
- The Flex Ion and Newomics MnESI sources also had a strong correlation, with higher sensitivity on the Newomics MnESI source (Figure 6c)

TABLE 1. Summary comparison of all source and emitter combinations tested on Orbitrap Exploris 480.

	Flex Ion (New Objective)	Flex Ion (CoAnn)	TNG	Newomics MnESI
Source Type	Ambient	Ambient	Captive	Captive
Average CV (%) Median	38.3%	29.0%	55.5%	9.2%
Expected PQ500 SIL Quantified (%)	99.9%	96.6%[a]	63.1%	100%

[a]Averaged across all 3 experiments.
CV, coefficient of variation; SIL, stable isotope label; TNG, Thermo OptaMax™ NG.

CONCLUSIONS

- Under our experimental conditions, we observed that these ESI sources can have CVs ranging from 9-55% and 2-2.5-fold differences in the mean AUC of the SIL standard peptides
- CoAnn and New Objective emitters had comparable results on the Flex Ion source, but an ambient source is susceptible to high CV from variables like temperature or air flow fluctuations
- The Flex Ion and Newomics MnESI sources demonstrated similar PQ500 SIL peptide detection rates, while the TNG source demonstrated poor sensitivity at 5 µL/min flow rates
- The Newomics MnESI source demonstrated superior sensitivity and reproducibility on the Orbitrap Exploris 480 over both the Flex Ion and TNG sources
- Future experiments will evaluate the impact of different flowrates (5-10 µL/min) on sensitivity, reproducibility, and robustness with a Newomics MnESI source equipped Orbitrap Exploris 480

DISCLOSURES

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