Achieving robust quantitative analysis of proteomes using Vacuum insulated probe heated electrospray ionization (VIP-HESI) coupled with microflow chromatography and timsTOF-Mass-Spectrometer

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Introduction

Nanoflow liquid chromatography mass spectrometry (nLC-MS/MS) is popular in proteome research due to its sensitivity and sample efficiency but has restrictions in throughput and robustness. The Vacuum Insulated Probe Heated ElectroSpray Ionization source (VIP-HESI) coupled with the microflow liquid chromatography (µLC) and Bruker timsTOF mass spectrometer, enhances chromatography signals compared to nanospray using CaptiveSpray (CS) and ElectroSpray Ionization (ESI) sources. Using the VIP-HESI source ,we characterize the efficiency in proteomic analysis of synthetic peptide mixtures, proteomes of HeLa and K562 cells, and with undepleted mouse plasma using Slice-PASEF and dia-PASEF methods to advance proteomic analysis capabilities and address nLC-MS/MS limitations.

Methods

- VIP-HESI, ESI, and CS sources were compared using SCIEX PepCalMix solution.
- Linear response was assessed across different sample amounts (12.5fmol to 800fmol) using VIP-HESI and ESI sources.
- Comparison of injection amounts (0.4µg to 40µg) with CS and VIP-HESI was performed using mouse plasma sample.
- High-throughput analysis of 284 plasma samples was conducted using VIP-HESI.
- VIP-HESI performance in slice-PASEF mode for low sample amounts was evaluated.
- Spectronaut 16.0 and DIA-NN 1.8.2 tools were used for identification and quantification analysis.

Results

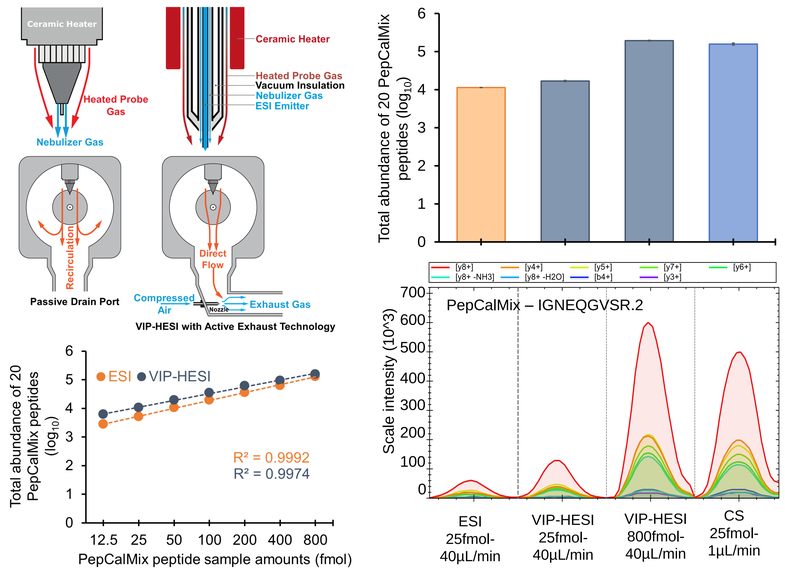

Figure 1. Schematic diagram and comparative performance of VIP-HESI with ESI and CS sources setups using PepCalMix measurements.

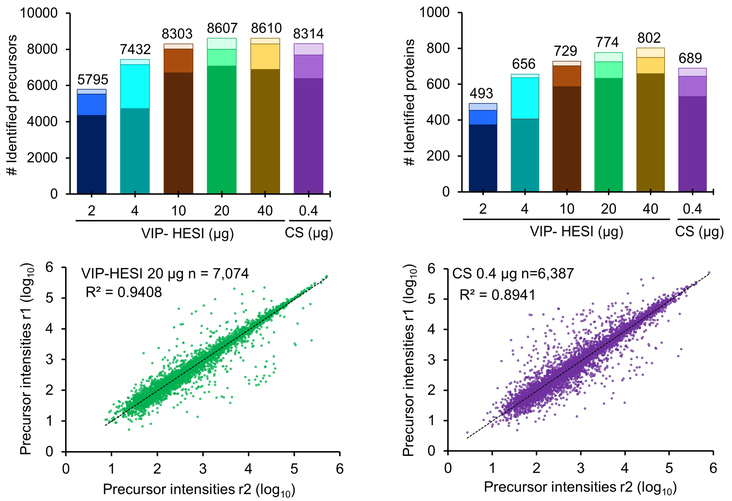

Figure 2. Comparative performance assessment of VIP-HESI and CS ion sources using different injection amounts of mouse plasma samples.

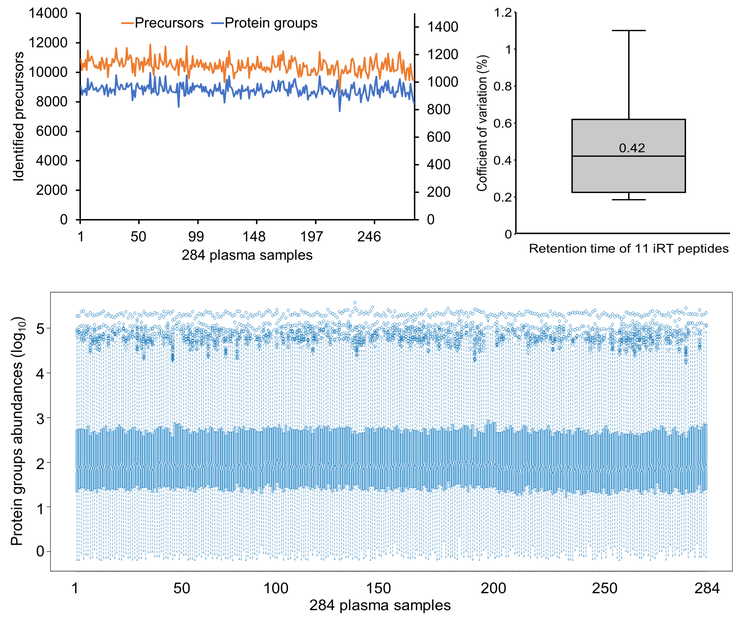

Figure 3. Large-scale quantitative analysis of undepleted mouse plasma samples using VIP-HESI coupled with microflow LC–MS/MS system.

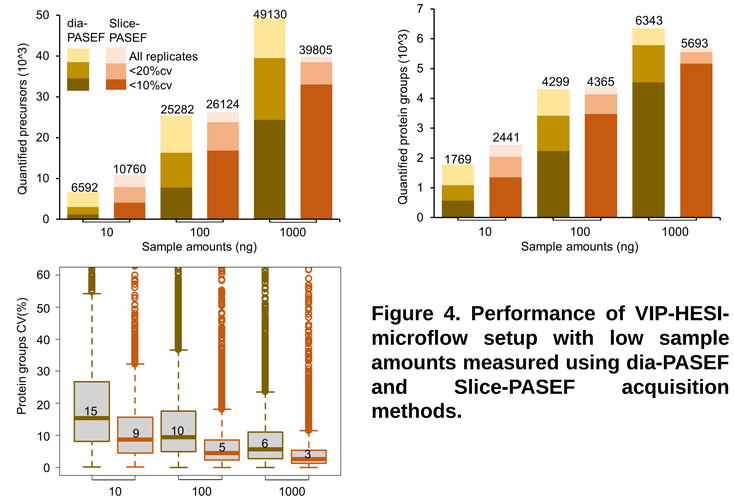

Figure 4. Performance of VIP-HESI-microflow setup with low sample amounts measured using dia-PASEF and Slice-PASEF acquisition methods.

Conclusion

- High linearity (R^2 >0.99) observed for 20 PepCalMix peptides.
- VIP-HESI exhibits 48% and 23% higher abundance than ESI and CS respectively.
- VIP-HESI (20µg) outperforms CS (0.4µg) with higher plasma proteins, show positive correlations (R^2 = 0.94 and 0.89, respectively).
- Consistent identification of peptides (8,627-11,885) and proteins (788-1,067) across 284 samples.
- Spiked-in iRT peptides show high chromatographic reproducibility (CV 0.4%).
- VIP-HESI with Slice-PASEF significantly increases identifications, offering improved quantitative precision at low sample amounts.

Conflict of Interest Disclosure

The authors declare no competing financial interest.

Citation

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