Novel ion source with integrated separation column and replaceable emitters facilitates high-throughput multiplexed analysis

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Abstract

Purpose: This study is to evaluate the OptiSpray ion source, comprised of an integrated separation column and replaceable emitters could enhance the quantitative robustness of the Stellar MS platform.

Method: A high-speed hybrid nominal mass platform (Thermo Scientific™ Stellar™ mass spectrometer) combined with an Thermo Scientific™ OptiSpray™ ion source to carry out Parallel Reaction Monitoring (PRM-MS) analysis of a modified 57-protein multiplexed Health Surveillance Panel (HSP)

Results: The OptiSpray ion source provides improved ionization efficiency and ensures consistent and stable spray performance, leading to reliable and reproducible results. The user-friendly design simplifies setup and operation, while its compatibility with various mass spectrometry systems enhances versatility and integration into existing workflows.

Introduction

Targeted MS-based proteomics approaches, such as multiple reaction monitoring (MRM) and parallel reaction monitoring (PRM), are used to validate candidate biomarkers. The Stellar MS platform is a recent innovation that combines a dual-pressure linear ion trap with a triple quadrupole. To potentially further enhance biomarker workflows, a novel ion source with integrated cartridges containing a column, liquid junction, and replaceable emitter assembly has been developed. We evaluated this new configuration with a multiplexed health surveillance panel, HSP comprised of 57 proteins, using ultra high-throughput PRM-MS and a novel ion source with integrated separation column and replaceable emitters.

Materials and methods

PRM-MS method development and optimization were conducted for a modified 2nd-generation 57-protein multiplexed Health Surveillance Panel (HSP) (bioRxiv. 2025 PMID: 40161722) on Stellar MS with Thermo Scientific™ Vanquish™ Neo UHPLC system combined with a OptiSpray ion source consisting of a prototype Thermo Scientific™ OptiSpray cartridge with a Thermo Scientific™ PepMap™ Neo 150 µm x 15 cm column and a tapered emitter. The “capillary flow quick optimization” automated routine was used to determine the optimal position for the emitter. All data was acquired with the emitter in this position. The 2nd generation HSP assay includes **83 prototypic peptides**, their corresponding SIL peptides, and a 14 Peptide Retention Time Calibration (PRTC) mixture (Thermo Fisher Scientific), totaling 180 peptides. We assessed three different throughputs: 100, 144 and 180 samples per day (SPD). Methods were developed and compared for both the Thermo Scientific™ EASY-Spray™ ion source and OptiSpray ion source using Skyline™ software and PRM Conductor.

Results

OptiSpray technology makes nanoflow easy

The OptiSpray technology, which comprises of the OptiSpray ion source and column cartridges addresses the challenges of the nanoflow spray stability and complex column installation procedures that can lead to irreproducible results. The three main features are plug-and-play cartridge installation, automated emitter positioning, and exchangeable integrated emitters. Additionally, the OptiSpray ion source minimizes contamination of the MS inlet by controlling the position of the cartridge during a sequence. There are two positions that the cartridge alternates between: 1. the active position, which is the automated routine-determined position, and 2. the inactive position, which is approximately 1.5 cm to the right of the atmospheric inlet, preventing most ions from entering the mass spectrometer (Figure 1C). Moving the emitter away from the inlet while maintaining the electrospray process going prevents emitter degradation compared to other methods such as turning off the electrospray voltage.

In summary, the OptiSpray ion source proves to have better reproducibility, increased points per peak, and more reliable quantification than EASY-Spray ion source highlighting OptiSpray ion source’s advantages in streamlining multiplex proteomics workflows.

Source installation and emitter optimization
A minimal intervention strategy

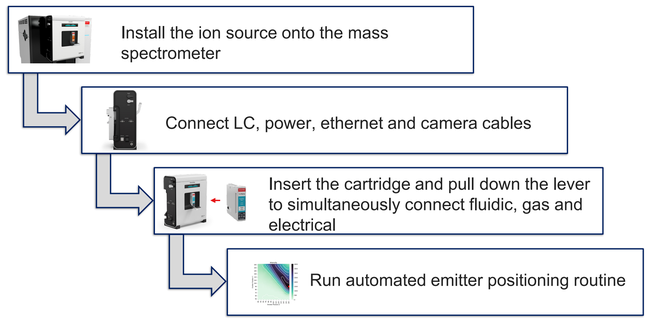

Fig. 1. Simple and hands-off installation and use. The OptiSpray ion source and column cartridges aim to provide a simple, hands-off approach to low flow separations. The source is installed on the Stellar mass spectrometer, the LC is connected, then the column cartridge is installed with one flip of a lever. The emitter is positioned by an automated routine that is based on ion signal--using this routine eliminates user-to-user variation.

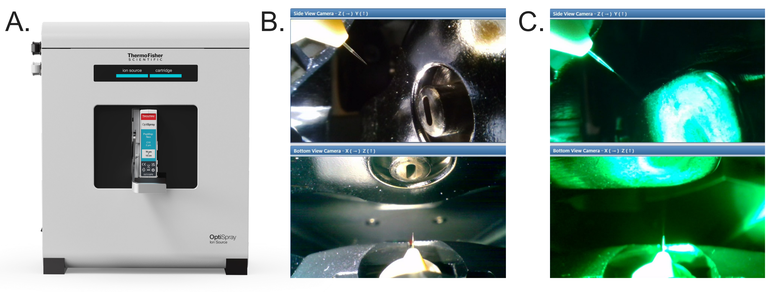

Fig. 2. OptiSpray Ion Source emitter position. The Capillary Quick Optimization placed the emitter several mm from the ion transfer tube with the emitter tip pointing towards its center. The optimized emitter position was saved to the cartridge memory. When the cartridge is removed from the ion source and reinserted, it returns to the stored optimized position. When a new cartridge is inserted, a popup appears that indicates that quick optimization has not yet been performed on this cartridge. A. OptiSpray ion source with cartridge installed. B. Optimized emitter position for PepMap Neo 150 µm x 15 cm column and a tapered emitter with white lighting, C. Inactive position with green lighting that illuminates the spray.

Comparison of signal intensities of EASY-Spray and OptiSpray sources

The OptiSpray ion source illustrates equivalent quantification performance with lower CVs

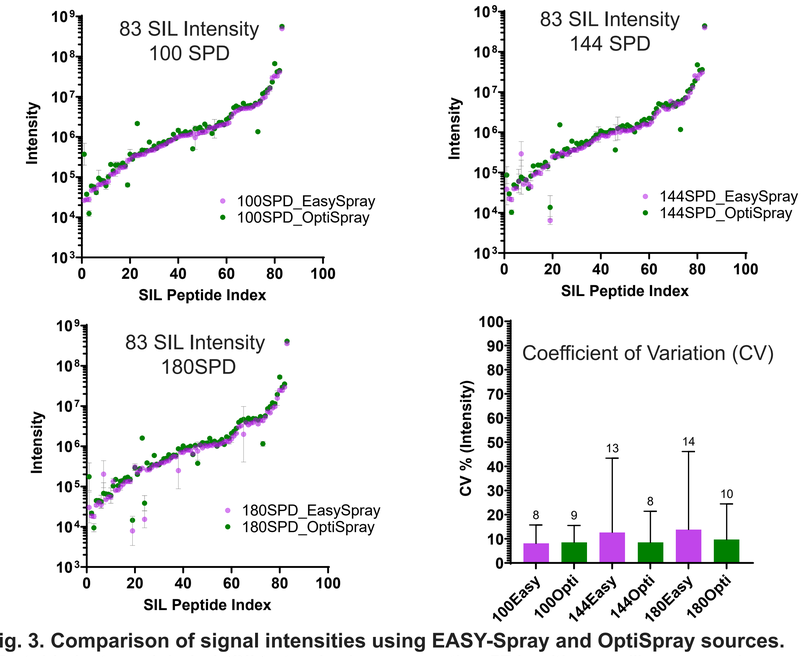

Fig. 3. Comparison of signal intensities using EASY-Spray and OptiSpray sources. 83 stable isotope–labeled (SIL) standards representing 57 health surveillance panel proteins were used to assess signal intensities and reproducibility. Ten injections were performed with each source at throughput levels of 100, 144, or 180 SPD. PepMap Neo 150 µm × 15 cm columns (ES906) were used with both EASY-Spray and OptiSpray sources, with the ES906 column integrated into the OptiSpray cartridge.

Automated emitter positioning and ion source parameters

Vanquish Neo system

OptiSpray source & Stellar mass spectrometer

Ion source parameter	Set value
Pos ion voltage (V)	1800
Sheath gas (arb)	10
Sweep gas (arb)	0
Ion transfer tube temp (°C)	250
Cartridge temp (°C)	45

Fig. 4. In one automated routine available in the Tune software, the OptiSpray ion source rapidly optimizes the emitter position based on signal intensity. The capillary flow quick optimization was executed with a solvent composition of 90% B at 1.5 µL/min. Ion source parameters used for LC-MS data acquisition (Table above) were also applied during the routine.

Evaluation of 3 PRM methods with repeated injections

In three distinct high-throughput PRM analyses, OptiSpray ion source delivered excellent signal intensities, consistent peak coverage, and stable retention times.

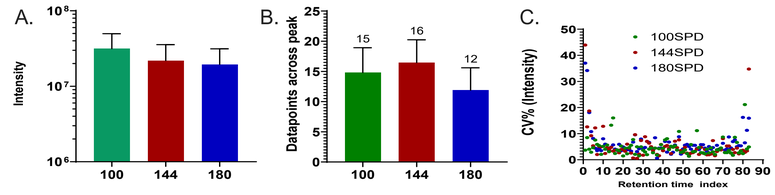

Fig. 5. Evaluation of 3 PRM methods with repeated injections. The performance of the 3 SPD methods was initially evaluated using 40 fmol of the 83 SIL peptides spiked into 50 ng of digested control plasma (n=5, technical replicates). A. The median peak intensities. B. The median cross-peak points. C. Coefficient of variation (CV) of the signal intensities for each peptide. The peptides order was sorted by retention time (from low to high).

Evaluation of the sensitivity and linearity of 3 three SPD

OptiSpray Achieving outstanding sensitivity and quantification performance, including low limits of detection (LOD), limits of quantification (LOQ), and robust linearity.

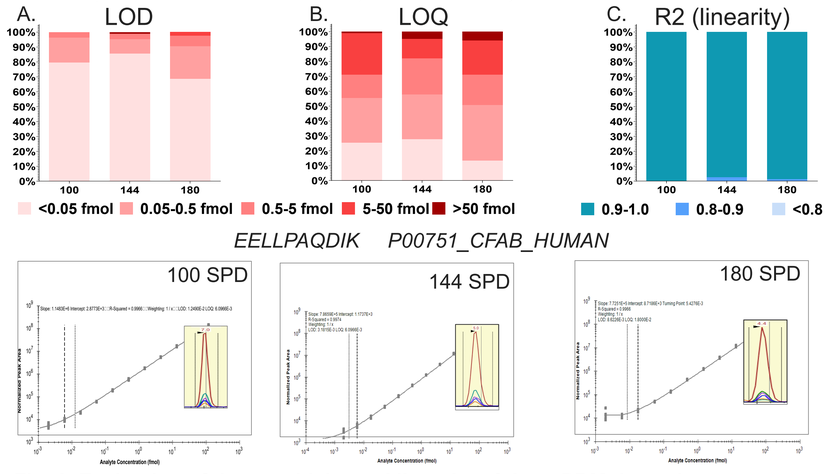

Fig. 6. Evaluation of the sensitivity and linearity of three PRM throughput methods. 0.002-120 fmol 83 SIL/50 ng pooled plasma peptides was evaluated for each SPD method (11-point dilution curve; n=5, technical replicates). A. The LLOD was calculated using the Skyline software’s bilinear regression fit (blank+3x standard deviation). The results are binned into 5 ranges. B. The LLOQ was also determined as coefficient of variation (CV) <20% and a bias <20%. The results are binned into the same 5 ranges. C. Median linear response (R2) for all 83 SIL peptides over the dilution curve. D. Examples of linear regression curves for EELLPAQDIK peptide (P00751-CFAB, Complement factor B) are presented for 100, 144, and 180 SPD. The peaks displayed are from Skyline software showing integrated peaks of the same peptides.

Conclusion

The Stellar MS with retention time adaptor algorithm and PRM conductor provides high sensitivity and ensures precise quantification of target peptides, making it an excellent platform for targeted PRM assays required for biomarker validation and quantitative proteomics. Additionally, the Stellar MS for PRM with the OptiSpray ion source ensures durability and long-term use, minimizing maintenance and downtime. The combination of the Stellar MS with the adaptive RT algorithm, PRM conductor with the automated OptiSpray ion source, provides a seamless workflow and a robust platform to quantify highly multiplexed and scheduled analytes in high-throughput studies for PRM assays.

General Laboratory Equipment – Not For Diagnostic Procedures.