Comparative Analysis of Skyline and SmartPeak Performance in Quantifying a 60-Biomarker Panel in Plasma from Inflammatory Bowel Disease Patients

Dragana Noe[1], Chi D. L. Nguyen[1], Jonathan T. Bui[1], Jennifer E. Van Eyk[2], Susan M. Mockus[1]

[1]Precision Biomarker Laboratories, Cedars-Sinai Medical Center, Beverly Hills, CA; [2]Advanced Clinical Biosystems Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA

Precision Biomarker Laboratories

Cedars Sinai

Introduction

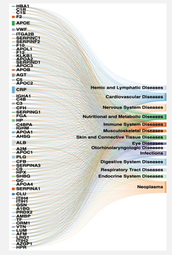
Accurate data analysis is pivotal in clinical proteomics for the precise quantification of plasma biomarkers, which is crucial for both disease detection and monitoring. Most targeted proteomics research relies on open-source software, such as Skyline and SmartPeak. This study compares the performance of Skyline and SmartPeak using a 60-biomarker Health Surveillance Panel (HSP) in plasma from inflammatory bowel disease (IBD) patients, utilizing an automated, highly multiplexed liquid chromatography-targeted mass spectrometry workflow. Precision Biomarker Laboratories' HSP panel is illustrated in Fig A. Our assessments include Limit of Detection (LOD), Limit of Quantification (LOQ), linear range, and endogenous peptide quantification. Our aim is to rigorously compare the performance of Skyline and SmartPeak in analyzing the HSP, highlighting the crucial role of robust data analysis in effectively interpreting plasma biomarkers.

Methods

The calibration plate design comprised stable isotope-labeled (SIL) peptides at varied concentrations, diluted in digested human plasma matrix, with 10 dilution points (from 0.1 to 8000 fmol) on column (B). The sample plate design included cases (IBD patients), controls, and quality control (QC) samples, measured in triplicates across 42 cases and 42 controls. QC samples included human plasma, chicken plasma, and defined ratios, serving as a reference set for accurate quantification. The HSP multiple reaction monitoring (MRM) assay involved 111 monitoring peptides, each with 2 measuring transitions. Thermo Scientific Ultimate 3000 LC system (30-minute gradient at 500 µL/min) coupled with a Sciex triple quadrupole MS system were employed to acquire all sample measurements. Data analysis used both Skyline and SmartPeak software.

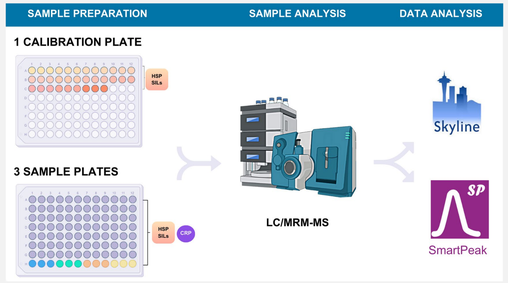
Figures

A. PBL Health Surveillance Panel (HSP)

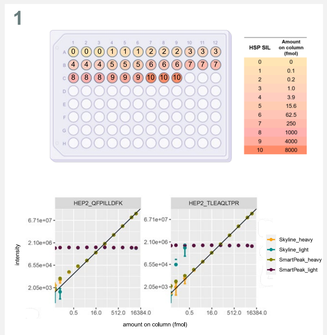


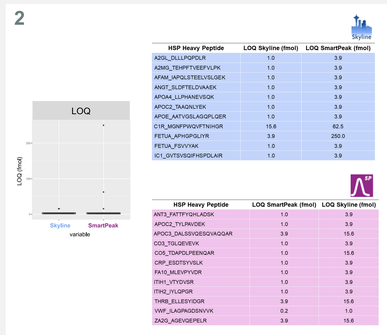
- Off-the-shelf 60 biomarker targeted MRM assay
- 31 biomarkers on FDA approved list
- Holistic view of health and wellness
- Insights for more than 14 disease states
- Measures inflammation, immunity, multiple organ function, or health status after surgery

B. Workflow overview

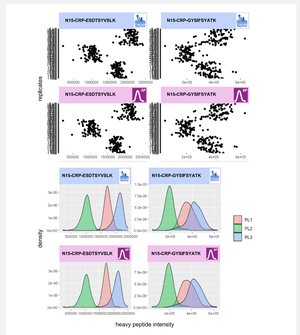


C. Calibration plate | LOD/LOQ & Linear Range

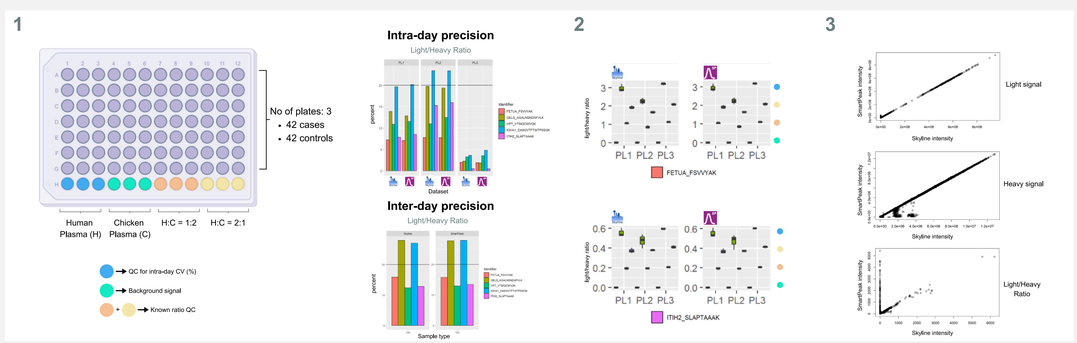




D. Digestion QC



E. IBD samples plate | Reproducibility & Precision



Results

Calibration Plate

- Both Skyline and SmartPeak generated calibration curves within the desired linear range for the 60-biomarker HSP, meeting LOQ criteria (C-1).
- Skyline excelled in detecting 11 heavy peptides at lower concentrations, while SmartPeak exhibited superior sensitivity in identifying 12 peptides (C-2).

QC Analysis

- N-15 labeled CRP peptides, serving as internal controls, displayed high consistency in CVs, intensity, and density between Skyline and SmartPeak (D).
- Human plasma QC samples showed consistent CV values between Skyline and SmartPeak, with the majority below the 20% CV threshold (E-1).
- The reference set of assay samples demonstrated a robust correlation between Skyline and SmartPeak (E-2).

Samples Plates

- Correlation plots indicated good agreement between Skyline and SmartPeak for both heavy and light signals, although SmartPeak missed detecting a notable portion of heavy signals and Skyline faced challenges with 25 out of 111 peptides in light signals, which were appropriately not reported after manual inspection (E-3).

Conclusions

While both platforms demonstrated strengths and limitations in peptide detection and quantification, Skyline showed superior performance in detecting heavy peptides at lower concentrations and exhibited robust noise handling. SmartPeak exhibited higher sensitivity in identifying certain peptides but faced challenges in detecting a notable portion of heavy signals. Overall, our findings underscore the importance of understanding platform-specific signal detection and noise handling mechanisms for effective plasma biomarker interpretation in clinical proteomics.

Conflict of Interest: None to declare.

Contact Information: dragana.noe@cshs.org