

HIGH PERFORMANCE QUALITATIVE AND QUANTITATIVE PROTEOME ANALYSIS FOR BIOMARKER DISCOVERY WITH **SPECTRONAUT® 19** AND **timSTOF HT**

In this application note you will learn about:

- the advantages of using Bruker timSTOF HT in combination with Spectronaut® 19 for efficient protein identification and accurate quantitative analysis.
- the results of a controlled quantitative experiment comparing the performance of Spectronaut® 19 with DIA-NN 1.9 for the identification of proteins and peptide precursors.
- the superior performance of Spectronaut® 19 for the recovery of true candidates and the detection of small-fold changes and how this demonstrates its practical value for biomarker discovery.

INTRODUCTION

Liquid chromatography-mass spectrometry (LC-MS) is a powerful tool for analyzing and profiling the proteome of complex biological samples. The efficient and accurate analysis of data from these experiments is crucial for basic research and drug discovery processes, including biomarker discovery, elucidating drug modes of action, and deconvoluting drug targets.

To showcase the benefits of this unique combination for processing dia-PASEF data and its suitability for proteome profiling, we conducted a controlled quantitative experiment (CQE). CQEs are routinely used as an objective and convenient way to assess metrics in quantitative workflows. In this study, we performed analyses using Spectronaut® 19 and another widely accessible DIA data analysis tool, DIA-NN 1.9.

However, the high dynamic range and inter-individual variability of the proteome presents huge challenges that must be overcome to achieve consistent and reproducible results.

The Bruker timsTOF HT mass spectrometer offers deep proteome profiling at high throughput. In dia-PASEF® mode, the timsTOF HT collects all fragment ions across a predefined mass-to-charge range with high-resolution precursor separation, providing accurate and reproducible data with minimal missing values.

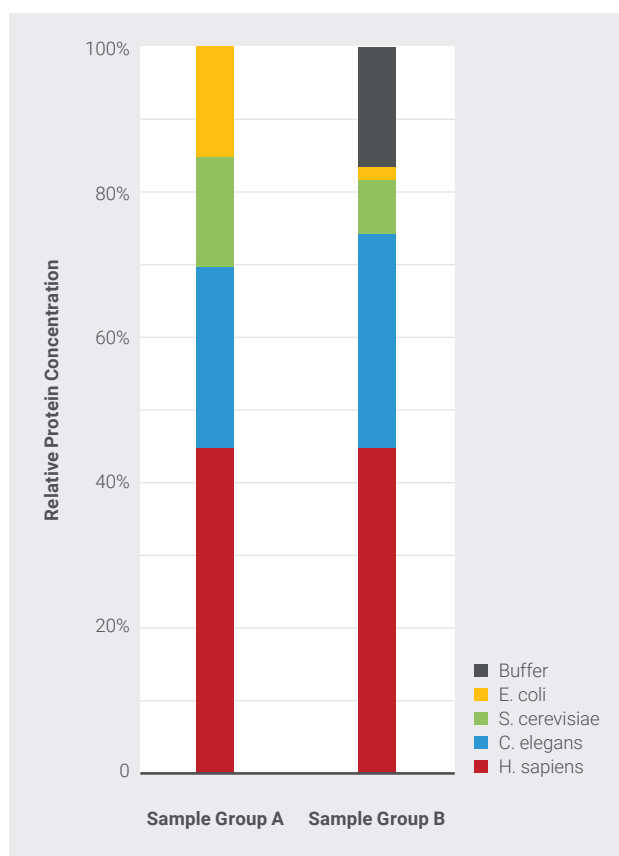
Biognosys' Spectronaut® software analyzes dia-PASEF data with cutting-edge algorithms, characterizing thousands of proteins. Combining our software with the power of the timsTOF HT provides a seamless workflow for analyzing timsTOF data at the speed and scale necessary in the fast-paced drug discovery field without compromising on analytical depth.

METHODS

A four-species CQE was acquired using timsTOF HT and varying gradient lengths (Figure 1). The CQE was conducted using mixtures with 30%, 100%, and 900% changes in the proteomes. Mixed proteome samples were prepared under two different protein abundance conditions, as seen in Figure 1 below (Sample Group A:B - H .sapiens 1:1, S. cerevisiae 2:1, E. coli 10:1, C. elegans 1:1.3).

Each condition was analyzed in triplicate using dia-PASEF on a timsTOF HT mass spectrometer with 7-, 21-, and 40-minute elution gradients. The data analysis was performed using a library-free approach in both Spectronaut® 19 and DIA-NN 1.9 with identical protein databases and false discovery rate (FDR) settings. Their performance in terms of analytical depth, precision, and accuracy was evaluated and validated with statistical testing.

1. **Illustration of the 4-species CQE Design:** C. elegans, S. cerevisiae, and E. coli proteins had differential abundances, while H. sapiens was held constant.

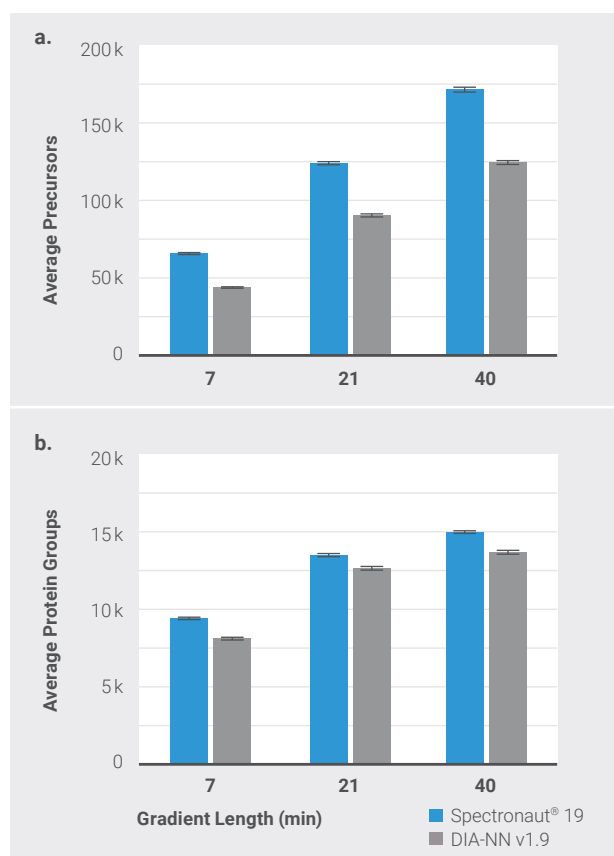


RESULTS

Impressive Depth and Proteome Coverage

To assess the depth of analysis, the number of proteins and peptide precursors identified by Spectronaut® 19 and DIA-NN 1.9 were measured across all three gradient lengths (7, 21, and 40 minutes). Spectronaut® 19 identified, on average, 32% more peptide precursors than DIA-NN 1.9 across all three gradient lengths (Figure 2a). Furthermore, Spectronaut® 19 identified, on average, 7% more protein groups and achieved an impressive depth of 15,438 protein groups in the 40-minute gradient length (Figure 2b).

2. **Peptide Precursor and Protein Identifications across all three Gradient Lengths by Spectronaut® 19 and DIA-NN v1.9**



Highly Effective Recovery of Differentially Abundant Protein Groups

Biomarker candidates exhibit differential abundance as they are indicators of specific biological states or conditions. To showcase the effectiveness of Spectronaut® 19 in detecting even subtle quantitative changes in the proteome, the software was applied together with DIA-NN 1.9 in a CQE analysis. The ability of the two software tools to detect differentially abundant proteins in mixtures of *H. sapiens*, *S. cerevisiae*, *E. coli*, and *C. elegans* was then evaluated.

First, regulation analysis was performed to determine true candidates (i.e. protein groups with abundance changes across sample groups) using an unpaired t-test of protein quantities from MS2 data. All regulated candidate proteins were then sorted by p-value and categorized as true or false positives based on the ground truth where *H. sapiens* is constant across sample groups (Figure 1).

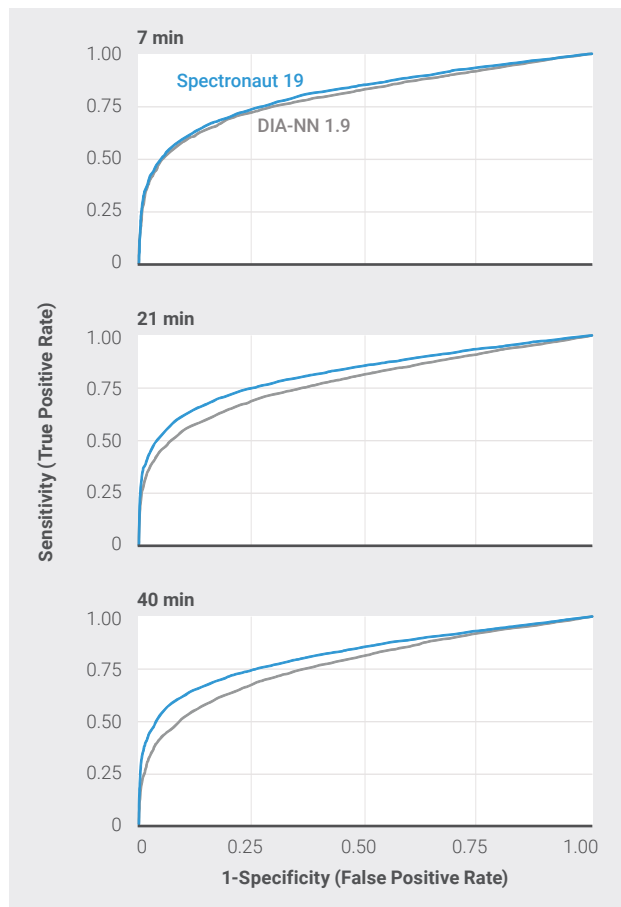
The true candidate discovery rate (Sensitivity) was plotted as a function of the false candidate recovery rate (1-Specificity) for each gradient (Figure 3). Results show Spectronaut® 19 outperformed DIA-NN 1.9 in recovering true candidates with a higher area under the curve across all three gradient lengths (Figure 3).

Superior Protein Differentiation

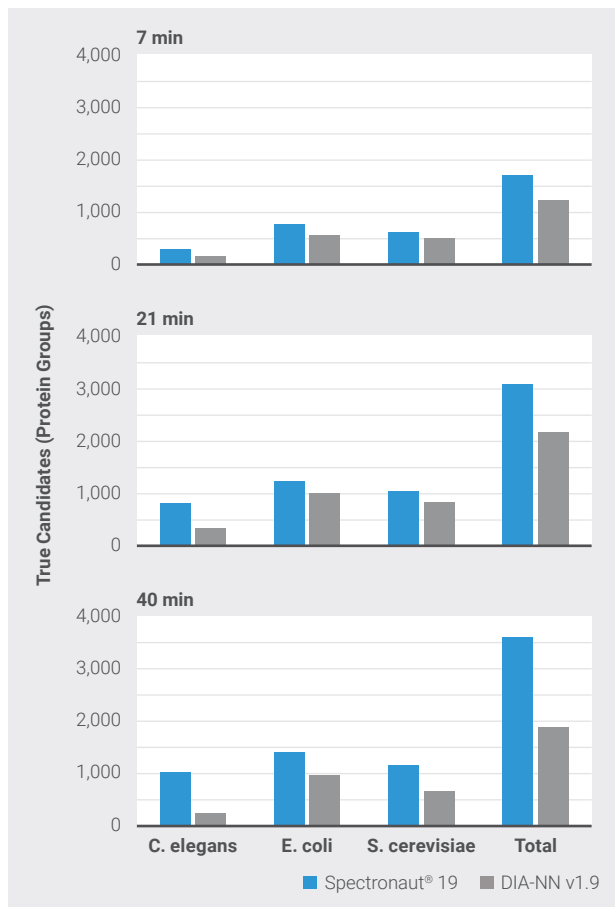
Comparing the total number of true candidate protein groups (Figure 4) at a fixed false recovery rate of 5% further demonstrates the superior sensitivity of Spectronaut® 19.

The variation in candidate recovery between software was most prominent for *C. elegans*, which had the least change in the mixed-proteome controlled ratio experimental design (Figure 1).

3. True Candidate Recovery by Spectronaut® 19 and DIA-NN v1.9 per Dataset



4. True Candidate Recovery by Spectronaut® 19 and DIA-NN v1.9 per Species



CONCLUSION

Despite the small change in abundance of the *C. elegans* proteome, Spectronaut® displayed excellent sensitivity and found 311% more true candidates than DIA-NN 1.9 in the 40-minute gradient length (Figure 4).

As the gradient length increased, the performance gap between Spectronaut® 19 and DIA-NN 1.9 widened, with Spectronaut® 19 surpassing DIA-NN 1.9 by 37,8% for 7-minute gradient, 42% for 21-minute and 91% for 40-minute gradients.

In conclusion, results from the controlled quantitative experiment show that Spectronaut® 19 consistently outperforms DIA-NN 1.9 in proteome coverage, identification depth and the detection of differentially abundant proteins across varying gradient lengths.

The combination of timsTOF® HT and Spectronaut® 19 is unmatched in terms of true candidate recovery at a high analytical speed, showcasing its power to advance high-throughput proteome analysis for biomarker discovery.

HOW TO GET SPECTRONAUT®

Here at Biognosys, we are committed to making our next-generation proteomics solutions widely available to researchers. Therefore, we offer flexible licensing options to best fit your personal and organizational needs.

If you are interested in a Spectronaut demo, a free trial license, or you would like to proceed to purchase, contact us via biognosys.com/contact.

At Biognosys, we believe that deep proteome insights hold the key to breakthrough discoveries that transform science for better lives. We make the proteome actionable to empower research, drug development, and clinical decision-making with our versatile portfolio of mass spectrometry-based proteomics research services, software, and kits. These solutions provide a multi-dimensional view of protein expression, function, and structure in all biological species and sample types.

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