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Rapid Qualitative Analysis and Absolute Quantification of Plasma Proteins Using SONAR with Biognosys PQ500 for Proteomic Clinical Research Studies

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Abstract

This study highlights the qualitative and quantitative advantages of SONAR for high throughput proteomics.

Benefits

- High throughput proteomics providing qualitative and highly precise quantitative data
- Absolute protein quantitation achieved using SONAR and Biognonsys PQ500 kit with average CV's of 6%
- Quantification of the plasma proteome over a wide dynamic range

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- Applicable solution which could be routinely implemented into a proteomic clinical research workflow

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Introduction

Quantitative proteomics often incorporates the use of stable labeled isotopes (SILs) in order to provide absolute quantification. Recent advancements have seen the introduction of peptide panels allowing the quantification of more than 500 proteins in plasma sample sets. However, this is technically challenging when attempting to acquire the data using more traditional MS acquisition modes such as multiple reaction monitoring (MRM), since the duty cycle of the instrument is compromised and therefore results in under sampling of the data. An alternative approach is to apply SONAR, a data independent analysis (DIA) methodology, allowing for high throughput while also ensuring high specificity and maintaining quantitative performance. SONAR has previously been described,[1,2] highlighting the utilization of a fast scanning quadrupole, enabling the technique to be compatible with fast chromatography, high throughput workflows. Clinical research using proteomics is one high throughput example which requires the analytical benefits provided by SONAR. Here, we present the applicability of SONAR for high throughput, absolute quantification of plasma proteins from human samples consisting of controls, chronic obstructive pulmonary disease (COPD), and asthma.

Experimental

Sample preparation

Undepleted human plasma (Innovative Research, MI) originating from controls (n=6) and confirmed COPD (n=6) and asthma (n=6) source samples were reduced, alkylated, and tryptically digested overnight. Prior to LC-MS analysis, samples corresponding to their individual groups were pooled and spiked with PQ500 SIL peptides (Biognosys AG, Schlieren, Switzerland).

LC conditions

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LC system:	ACQUITY UPLC M-Class
Column(s):	Peptide CSH C_{18} 130Å, 1.7 µm, 300 µm $\times$ 100 mm (p/n 186009255)
Column temp.:	55 °C
Flow rate:	7 µL/min
Mobile phase A:	Water (0.1% formic acid)
Mobile phase B:	Acetonitrile (0.1% formic acid)
Gradient:	3% to 40% B in 15 or 45 min
Injection volume:	5 µL (5 µg)

MS conditions

MS system	Xevo G2-XS QTof
Ionization mode	ESI (+) at 2.2 kV
Cone voltage	30 V
Acquisition mode	SONAR
Acquisition range	50 to 2000 *m/z* both functions (low and elevated energy)
Acquisition rate	0.5 s both functions (low and elevated energy)

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Quadrupole scan range:	330 to 1100 *m/z*
Isolation window	20 Da
Collision energy	6 eV (low energy function) and from 14 eV to 36 eV (elevated energy function)
Resolution	30,000 FWHM

Informatics

LC-MS data were processed with Spectronaut Pulsar X (Biognosys AG, Schlieren, Switzerland). Data were searched against a human plasma specific spectral library, generated from SONAR processed data with Progenesis QI for Proteomics. Additional data visualization and statistical analysis was performed using Metaboanalyst.[1]

Results and Discussion

The principle of SONAR, a scanning quadrupole based DIA method, is illustrated in the left hand side image of Figure 1. In short, alternate datasets are acquired in low (MS1) and elevated (MS2) collision energy mode.[2,3] During each low and elevated energy segment, the quadrupole isolation window is scanned linearly between two user-selected positions and 200 TOF spectra are acquired. The quadrupole scan duration is application/chromatographic peak width dependent and typically varies from 0.1 s to 1 s. In the elevated energy mode, the collision energy can be ramped between two values, which are selected to optimize fragmentation efficiency at each quadrupole position. The selectivity of the acquisition method is illustrated by the middle image where, dependent on the position of the quadrupole, i.e. transmission *m/z* window, precursor (even those close in mass) and product ions can be exclusively isolated.

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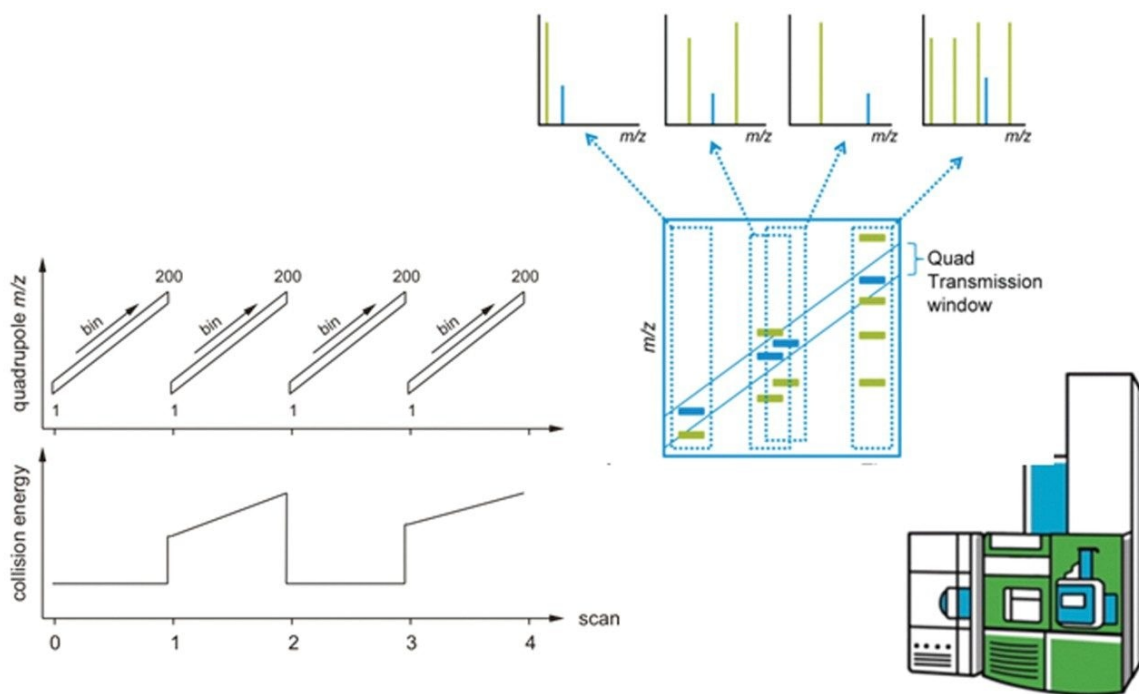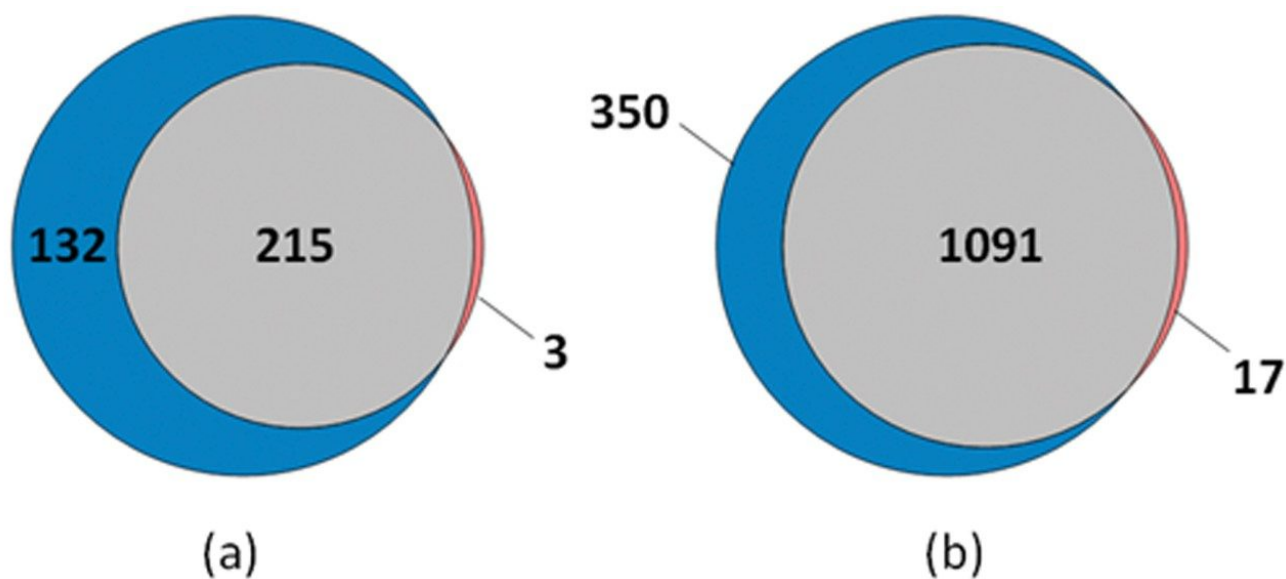
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Figure 1. A schematic of the SONAR acquisition method shows the behavior of the collision energy, which alternates between a constant low value and a linear ramp to generate informative precursor and fragmentation data, respectively, for as many peptide precursor ions as possible. The quadrupole scans with the same parameters in both the low and high energy CID scans.

Data was collected for undepleted plasma which was separated over either 45 or 15 min gradients, resulting in 347 and 218 protein identifications respectively (Figure 2). Regardless of the gradient conditions employed, statistical analysis showed clear separation between the three cohorts, with all three technical replicates clustering together. Likewise, a comparison of protein abundances using hierarchical clustering on the basis of Euclidean distances, showed distinct clusters of proteins which produced similar levels of protein expression under both gradient conditions (Figure 3).

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Figure 2. Venn diagrams representing the number of protein groups (a) and peptides (b) for gradients corresponding to 45 min (blue) and 15 min (red). Common protein identifications between both gradients are shown in grey.
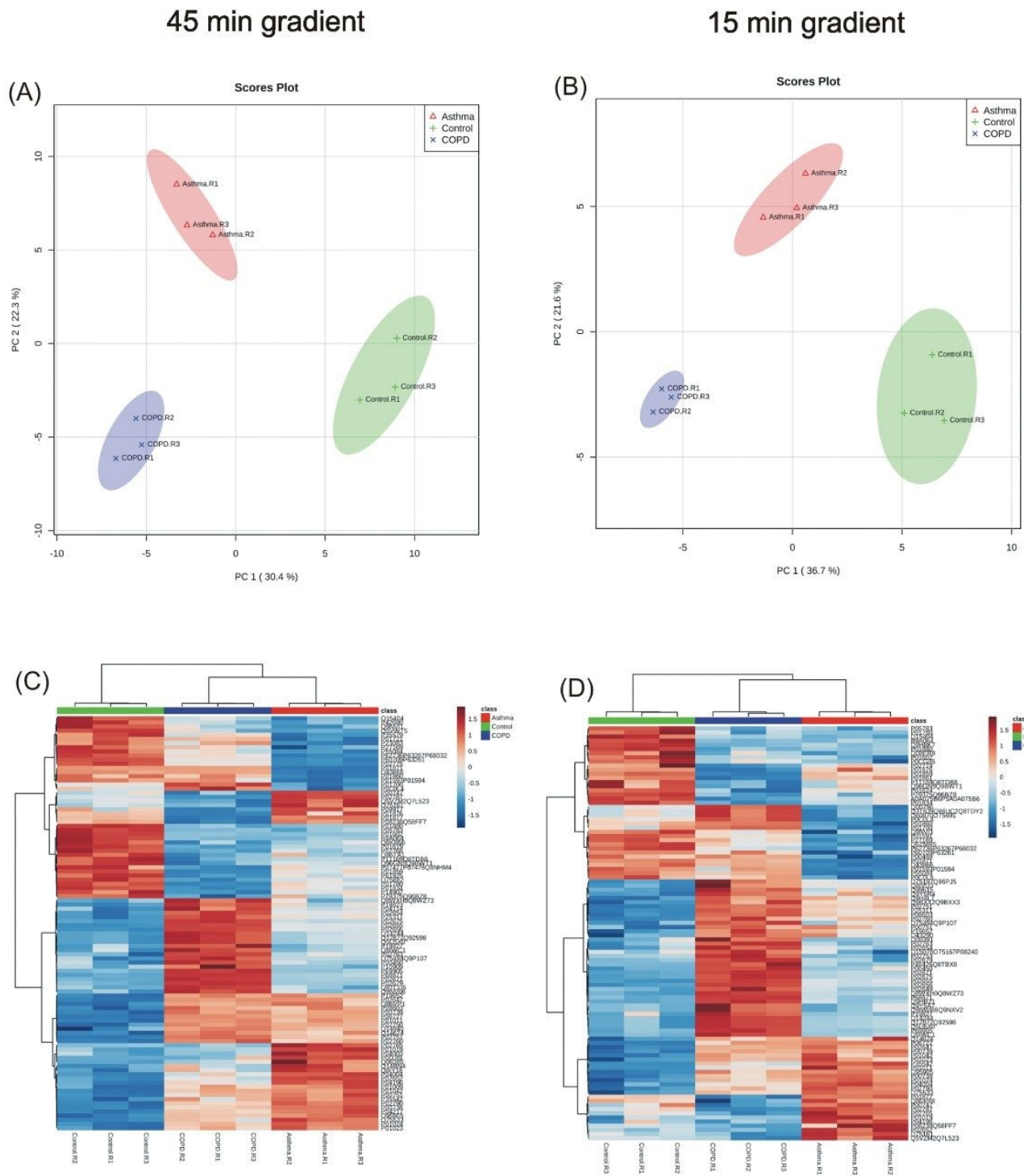
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45 min gradient

(A)

15 min gradient

(B)

(C)

(D)

Figure 3. Principal component analysis (PCA) showing clear separation between controls, COPD and asthma

groups for 45 min (a) and 15 min (b) gradients. Hierarchal clustering represented as heatmaps, highlight

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differences in protein abundances between the three cohorts for both gradient conditions (c, d).

The ability to sufficiently gain a high number of proteins, while achieving high quantitative precision is critical for large cohort, high throughput applications. Implementation of a fast scanning quadrupole, allows this to be achieved. The importance of peak sampling frequency and its effect on quantitative precision is described in more detail elsewhere.[2] Figure 4 shows the complexity which is introduced with co-eluting peptides for a 15 min gradient, providing significant opportunities for interference effects. Increased specificity and high rates of data acquisition provided by SONAR, result in non-interfered detection and extraction of MS2 fragment ions allowing proteins to be quantified over a wide dynamic range (>4 orders). The quantitative variance in terms of average %CV within each cohort (over technical replicates) is demonstrated to be 6% for data acquired using a 45 min gradient. Reducing the gradient to 15 min shows the average %CV to be maintained at 6% across the three groups. Data representing the shorter gradient also provides less variance overall, with all measurements falling within 20% CV (Figure 5). Absolute quantitation is provided for proteins corresponding with the PQ500 SIL peptides but also identifications external to the kit (Figure 6).

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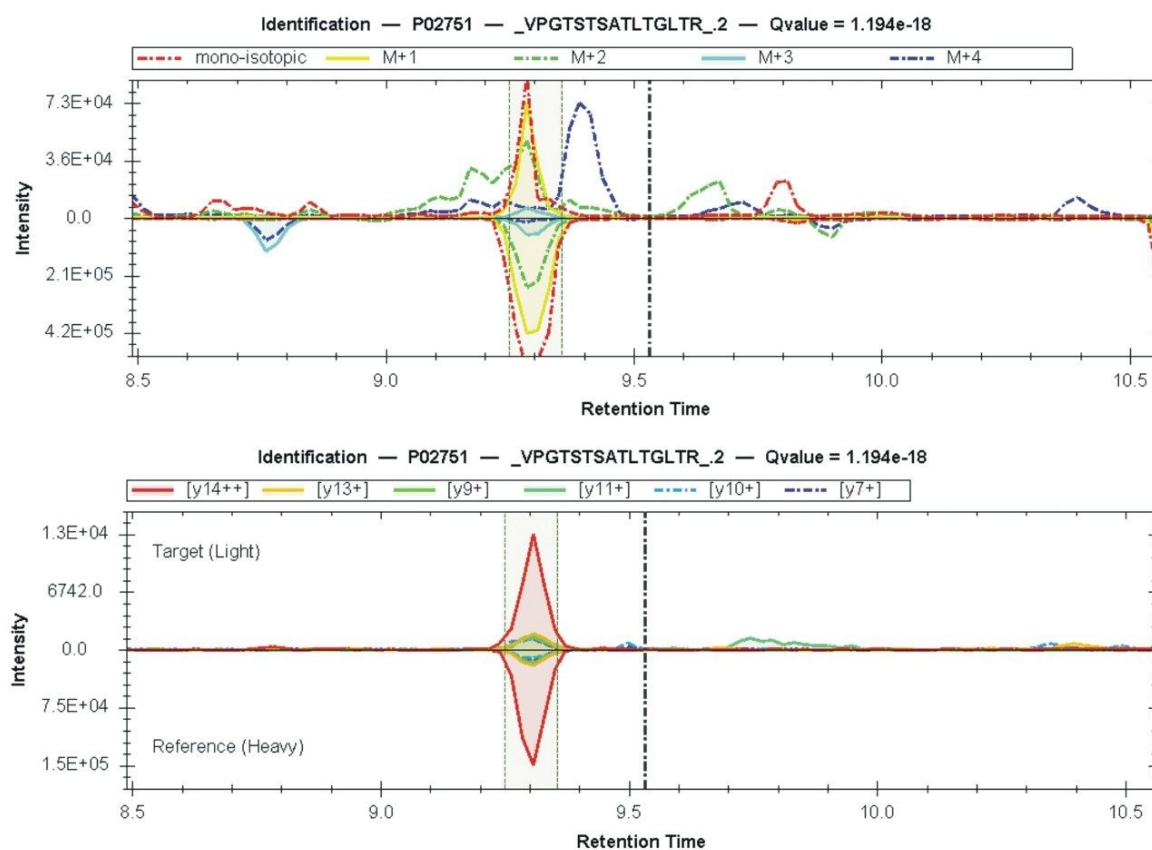
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Figure 4. Selectivity benefits increase quantitative precision using SONAR. MS1 data representing a fibronectin related peptide, shows potential interference from co-eluting peptides (upper). Increased SONAR specificity provides a clean, non-interfered MS2 spectrum (lower).

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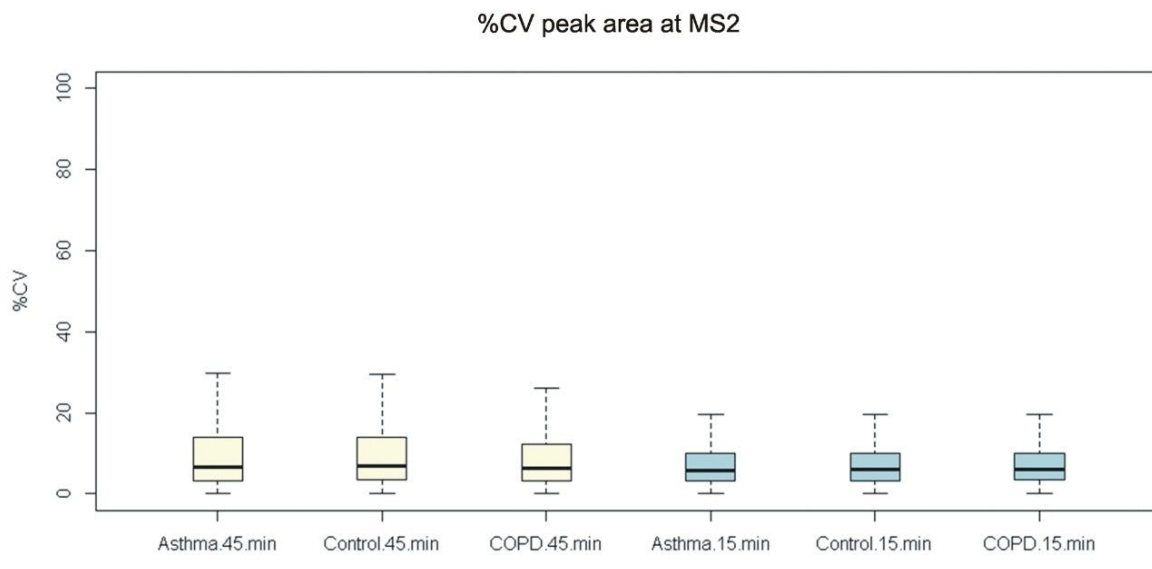
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%CV peak area at MS2

Figure 5. Co-efficient of variance (CV) for MS2 based quantitation. All three cohorts demonstrate a %CV of 6% for analyses over 45 min gradients. Reducing the analysis time to 15 min, results in the %CV being maintained at 6% over the three cohorts. Acquiring data over a shorter gradient also appears to reduce the spread of CV, maintaining CV's <20% for all measurements.
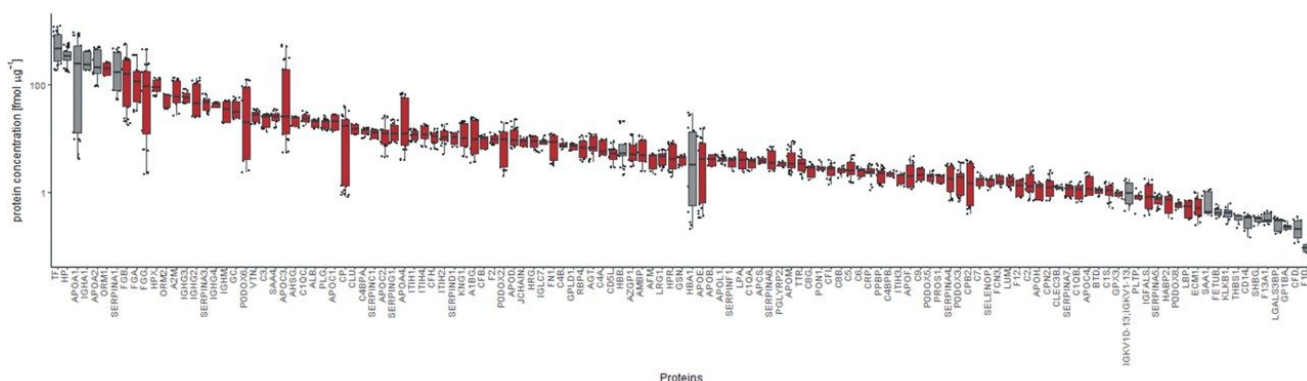

Figure 6. Absolute quantitation regression of identified plasma proteins. Protein concentrations corresponding to the PQ500 SILs (red box-whisker) are provided in addition to proteins identified external to the PQ500 kit (grey box-whisker).

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Conclusion

Overall, results from this study highlight the qualitative and quantitative advantages of SONAR for high throughput proteomics. The implementation of a scanning quadrupole with SONAR acquisitions allow data to be acquired at a faster rate, therefore providing high numbers of protein identifications with higher quantitative precision. A combined workflow with the PQ500 kit demonstrates absolute quantification using the DIA approach, which overcomes the technical challenges provided by traditional, targeted MS schema.

References

- Chong J, et al. MetaboAnlyst 4.0: Towards More Transparent and Integrative Metabolomics Analysis. *Nucl. Acids Res.* (doi: 10.1093/nar/gky310).

- Gethings LA, et al. Lipid Profiling of Complex Biological Mixtures by Liquid Chromatography/ Mass Spectrometry Using a Novel Scanning Quadrupole Data-Independent Acquisition Atrategy. *Rapid Commun Mass Spectrom*. 2017 Oct 15;31(19):1599–1606. doi: 10.1002/rcm.7941.

- Moseley AM, et al. Scanning Quadrupole Data-Independent Acquisition, Part A: Qualitative and Quantitative Characterization. *J of Proteome Res*. 2018 17 (2), 770–779. doi: 10.1021/acs. jproteome.7b00464

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