High Throughput Screening for Host Cell Proteins with sub ppm sensitivity using dia-PASEF with a 15 minute gradient

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Introduction

Host Cell Proteins (HCPs) usually remain in biopharmaceutical drug preparations such as monoclonal antibodies and need to be removed as much as possible using a combination of purification steps. Mass spectrometry has emerged as a method for identifying and monitoring HCPs throughout the manufacturing pipeline. Here we apply parallel accumulation – serial fragmentation combined with data-independent acquisition (dia-PASEF(***1***)) in a high throughput approach, demonstrating HCP detection and quantitation with sub ppm sensitivity.

Methods

NISTmAb 8671 (Merck) was spiked with either Universal Proteomics Standard 1 (UPS) 1 or 2 (Sigma) and digested with trypsin using native digest method (2). UPS1 (equimolar) was spiked at 1 in 50 for generating the spectral library, UPS2 (dynamic) was spiked at 1 in 100 for high throughput library screening.

Peptides were separated using an Elute UHPLC interfaced with a timsTOF Pro via a VIP-HESI ion source (***2***) and analysed by PASEF (spectral library creation, 150 min gradient) or dia-PASEF (library screening, 15 min gradient) (***3***).

Raw data were processed using Spectronaut (Biognosys). NISTmAb UPS1 data were searched against a mouse and UPS database to generate a spectral library against which the dia-PASEF spectra were searched

A regression line was calculated from the UPS 2 data and used for calculating the amounts of NISTmAb HCPs present in the sample.

Results

36/48 UPS2 proteins were identified and quantified in all 3 dia-PASEF runs, spanning all six concentration levels (***Fig 2, red***). At the spike level of 1 in 100, UPS2 proteins were detected in the range from 0.006 ppm to 3118 ppm relative to the NISTmAb (ng/mg), with an estimated LLOQ of 0.3ppm (***Fig 2***). A regression line was calculated from this data and used for calculating the amounts of NISTmAb HCPs present in the sample (***Fig 2, blue***). In total, 140 NISTmAb HCPs were identified and quantified in all replicates between 0.03 ppm and 158 ppm (ng/mg). For some examples of the HCPs identified and their amount relative to NISTmAb see ***Fig 3***. The quantitation and identifications correlate and overlap very well with previously published data (***4,5***) whilst extending coverage of NISTmAb HCPs (***Fig 4***). Mobility data is recorded for all peptides and can be used as an additional identification metric, reducing validation efforts (***Fig 5***).

References

1. Meier et al., Mol. Cell. Proteom. 2018, 17: 2534-2545
2. VIP-HESI dual source brochure (Bruker)
3. Meier et al., Nat Methods. 2020 Dec ;17(12) :1229-1236
4. Huang et al., Anal. Chem 2017, 89, 5436-5444
5. Molden et al., Mabs 2021, Jan-Dec;13(1):1955432
6. Venn Diagram created using Venny 2.1: Oliveros J.C. (2007-2015)

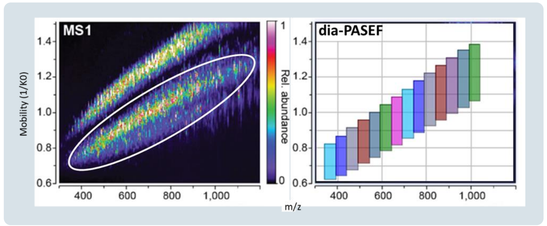

Figure 1: dia-PASEF method optimized for comprehensive sampling of HCP 2+ and 3+ precursors in a 1 sec cycle

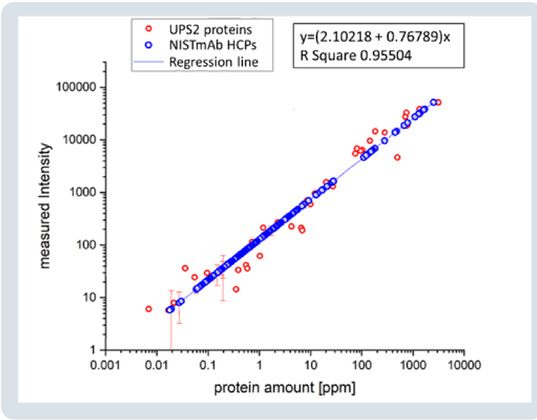

Fig. 2 Quantitation of NISTmAb HCPs. Average intensity (n=3) of UPS2 proteins (red dots) spiked into NISTmAb (1 in 100) and calculation of average NISTmAb HCP amounts based on the UPS2 regression line (ppm) within the same sample. Error bars are shown for the UPS2 proteins - many error bars are within the circles and not visible

NISTmAb HCP	Amount (ppm)	Peptides (n)
Protein disulfide-isomerase A6	158.7	7
Glucose-6-phosphate isomerase	28.1	25
Beta-2-microglobulin	12.7	4
Prostaglandin reductase 1	5.5	7
Syntaxin-12	3.8	6
Fumarate hydratase mitochondrial	2	9
UMP-CMP kinase	0.8	4
Selenoprotein M	0.5	2
RNA polymerase II-associated protein 3	0.1	2

Figure 3: Average amount of selected NISTmAb HCPs quantified with dia-PASEF using a 15 min gradient (n=3)

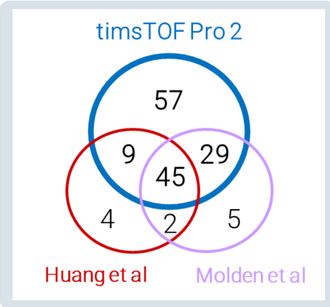

Figure 4: The number of NISTmAb HCPs quantified by the timsTOF Pro 2 compared to recent literature (***4,5***)

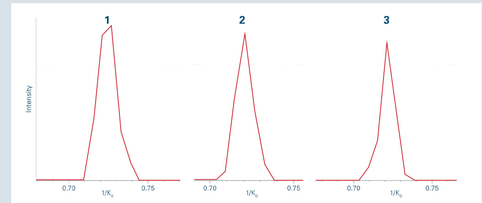

Figure 5: Mobility profiles for ILHDFYIER 3+ peptide from RNA polymerase II-associated protein 3 quantified at 0.1 ppm in NISTmAb sample across 3 dia-PASEF runs

Conclusions

- 140 NISTmAb HCPs were quantified in the range from 0.03 to 158 ppm with a 15 min gradient
- High correlation with published data, whilst providing more HCP identifications
- Robust analytical flow platform with very high sensitivity from PASEF acquisition modes and VIP-HESI source
- HCP identifications even at 0.1 ppm level validated by peptide mobilograms
- Potential for CCS spectral libraries to enable low concentration HCP identification without MS/MS

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