FlashNote

Sub-ppm HCP Screening in 15 minutes using dia-PASEF

By taking advantage of the unique capabilities of the timsTOF Pro 2, you can now confidently detect sub-ppm levels of host cell proteins in 15 minutes. Use of dia-PASEF benefits from the correlation between ion mobility and m/z to maximize ion utilization, enabling the measurement of low abundant Host Cell Proteins in therapeutic protein preparations. The workflow utilises a standard UHPLC at analytical flow rates to maximize ease of use and robustness.

Challenge

There is a growing demand for high-throughput screening of Host Cell Protein impurities in biopharmaceuticals. Process development teams need detailed information that ELISA alone cannot provide, and the testing of a wide range of purification parameters requires a high throughput which must not come at a cost in sensitivity. Due to their intrinsic low-abundance, HCP signals can be weak and therefore an additional metric to confirm identification would be highly beneficial.

Solution

The proven robustness of the timsTOF Pro 2 is well suited to continuous analysis of large sample sets [1], demonstrated here coupled to analytical flow UHPLC. Data independent acquisition in the form of dia-PASEF captures up to 100% of peptide precursor ions, maximising the sensitivity boost created by the Vacuum Insulated Probe Heated-ESI (VIP-HESI) ion source [2]. Ion mobility is integral to dia-PASEF, increasing specificity and sensitivity [3], and is also used for matching data with spectral libraries. Precise collisional cross sections (CCS) are measured for all ions, increasing confidence of HCP identifications.

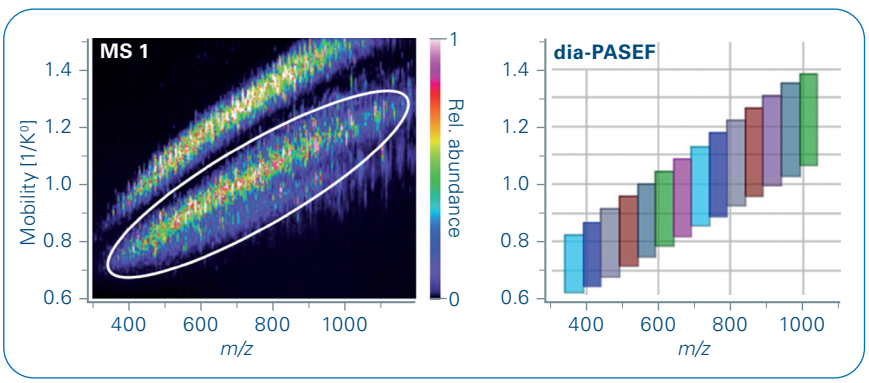

Figure 1: dia-PASEF method optimized for comprehensive sampling of HCP 2+ and 3+ precursors in a 1 sec cycle

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dia-PASEF: Exploiting the data independent approach for HCPs

Experimental setup

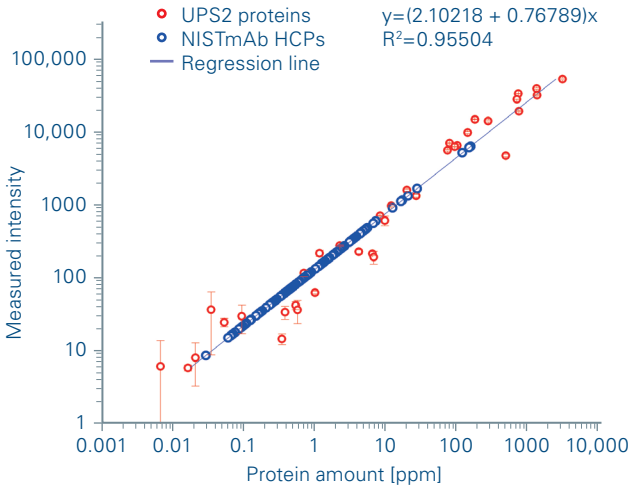
Spectral library generation: Equimolar Universal Proteomics Standard 1 (UPS1, Sigma) was spiked at 1 in 50 into NISTmAb (Merck) and digested under native conditions as previously reported [4]. Peptides were separated on a CSH C18 1.7 μm 2.1 x 100 mm column (Waters) with a 150 min gradient at a flow rate of 200 µL/min. The Elute UHPLC was interfaced with the timsTOF Pro 2 via the VIP-HESI ion source (all Bruker) and spectra were acquired using PASEF and searched against mouse and UPS databases in Spectronaut (Biognosys).

dia-PASEF: Dynamic Universal Proteomics Standard 2 (UPS2, Sigma) was spiked into NISTmAb (1 in 100) and digested and analysed in triplicate as above, this time using data independent acquisition (dia-PASEF) and a 15 min gradient. Spectra were searched against the mouse and UPS spectral library in Spectronaut and quantified using the Top3 method.

Results

36/48 UPS2 proteins were identified and quantified in all 3 dia-PASEF runs, spanning all six concentration levels (Figure 2a, red). 4/8 UPS2 proteins were identified in the lowest two concentration levels. 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 0.3 ppm (Figure 2a). A regression line was calculated from this data and used for calculating the amounts of NISTmAb HCPs present in the sample (Figure 2, blue). In total, 140 NISTmAb HCPs were identified and quantified in all replicates between 0.03 ppm and 158 ppm (ng/mg). Some examples of the HCPs identified and their amount relative to NISTmAb are shown in Figure 2b. The quantitation and identifications correlate and overlap very well with previously published data (Figure 3) whilst extending the coverage of NISTmAb HCPs by identification of additional low abundant HCPs, predominantly in the sub-ppm range (Figure 4). Mobility data is recorded for all peptides and can be used as an additional identification metric, reducing validation efforts (Figure 5).

A



B

NISTmAb HCP	Amount (ppm)	Peptides (n)
Protein disulfide-isomerase A6	158.7	7.0
Glucose-6-phosphate isomerase	28.1	25.0
Beta-2-microglobulin	12.7	4.0
Prostaglandin reductase 1	5.5	7.0
Syntaxin-12	3.8	6.0
Fumarate hydratase mitochondrial	2.0	9.0
UMP-CMP kinase	0.8	4.0
Selenoprotein M	0.5	2.0
RNA polymerase II-associated protein 3	0.1	2.0

Figure 2: Quantitation of NISTmAb HCPs. (A) 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. (B) Average amount of selected NISTmAb HCPs quantified with dia-PASEF using a 15 min gradient (n=3)

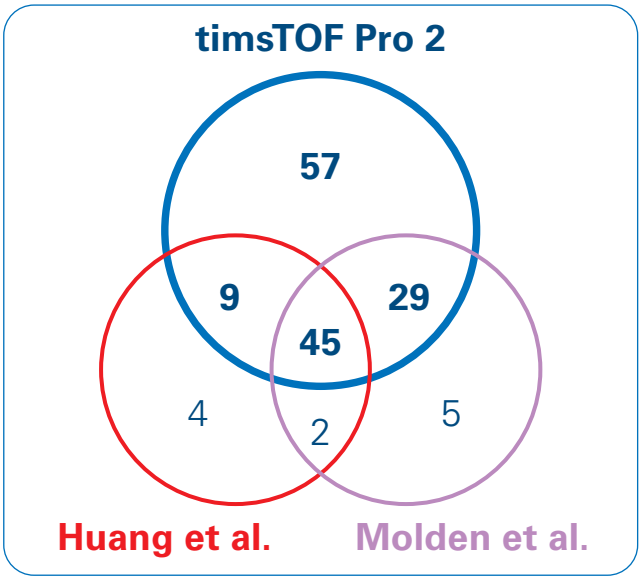


Figure 3: The number of NISTmAb HCPs quantified by the timsTOF Pro 2 in comparison to recent literature [4,5]

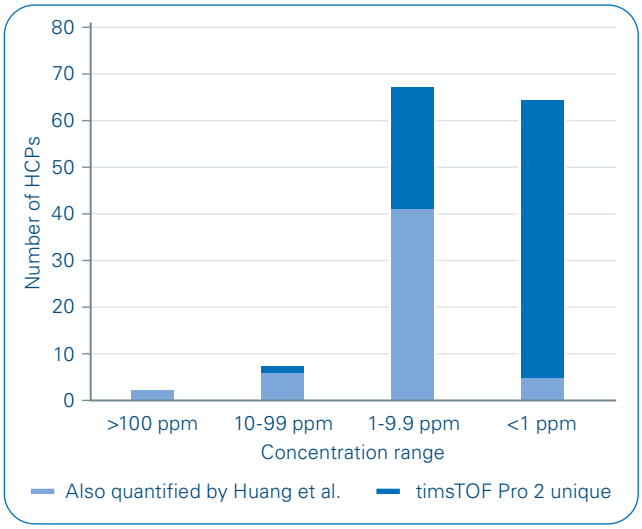


Figure 4: The distribution of NISTmAb HCPs quantified by timsTOF Pro 2 using diaPASEF with a 15 min gradient, sorted by concentration amount (ppm) and whether or not also identified by Huang et al. [4]. 6 HCPs reported by Huang et al (all under 2 ppm) were not identified in this experiment.

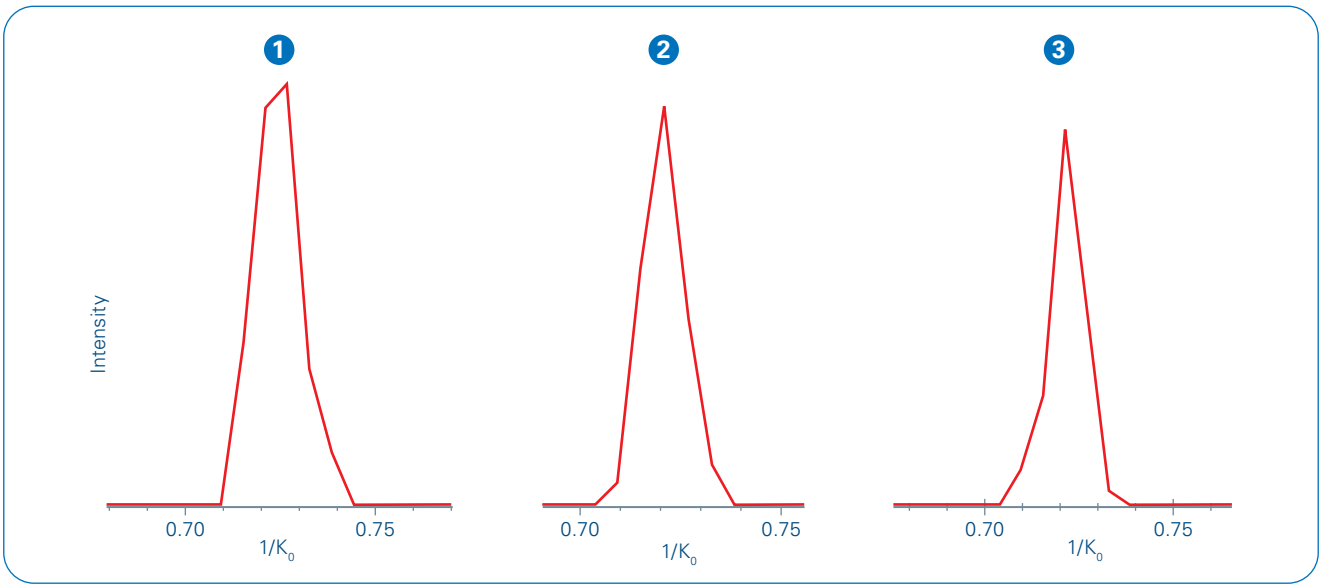


Figure 5: Mobility profiles for ILHDFYIER 3+ peptide from RNA polymerase II-associated protein 3 quantified at 0.1 ppm in NISTmAb sample across three dia-PASEF runs

Summary

- The unique features of the timsTOF Pro 2 enable a 15 min dia-PASEF method to be used for the rapid, robust and sensitive detection and quantitation of 140 NISTmAb Host Cell Proteins.
- High sensitivity allows enhanced HCP quantitation in sub-ppm range using analytical flow LC.
- The quality of HCP peptide mobilograms even at 0.1 ppm allows their use during peptide validation and may enable peptide identification and quantification in CCS-ready spectral libraries without an available MS/MS spectrum at the highly purified drug substance level.

References

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

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FlashNote

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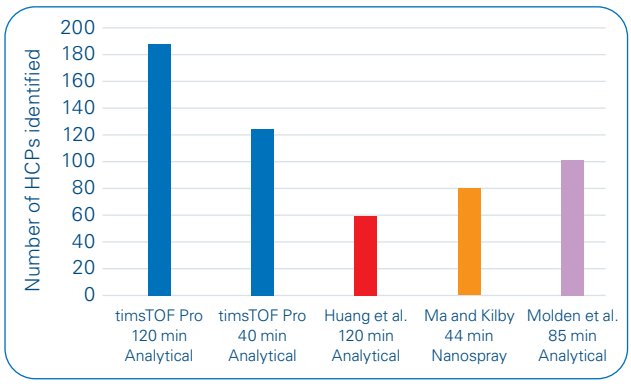
No Compromises: Fast, Sensitive and Robust HCP Analysis with timsTOF Pro 2

The timsTOF Pro 2 enables the discovery of previously unidentified low abundant Host Cell Proteins in therapeutic protein preparations. Samples are analyzed by RP-LC with the Elute UHPLC and VIP-HESI ion source at analytical flow rates, a configuration which can also be adapted for highly sensitive routine QC analysis by using shorter gradients.



Number of HCPs identified with 2 or more peptides with Elute UHPLC-VIP-HESI-timsTOF Pro 2 compared to recent literature

Data-dependent acquisition using PASEF greatly extends the depth of NISTmAb HCP coverage whilst showing excellent correlation with current literature.

For more details, refer to FN-21:
No Compromises: Fast, Sensitive and Robust HCP Analysis with timsTOF Pro 2

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