FlashNote

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.

Challenge

HCP analysis places huge demands on the LC-MS platform to truly meet the demands of the biopharma industry. The ideal platform should deliver short gradients to allow the required sample throughput, the sensitivity to detect HCPs in the low and sub-ppm range, and the robustness to ensure reproducibility whilst avoiding instrument downtime.

Solution

It is well documented that the timsTOF Pro 2 leads the field for robustness [1], and PASEF acquires MS/MS spectra at 120 Hz MS/MS with ion mobility focussing to ensure optimal speed and sensitivity. For HCP analysis the timsTOF Pro 2 is coupled with the Elute UHPLC to provide a robust and stable platform using analytical flow rates, which benefits from increased sensitivity due to optimal ionization in the Vacuum Insulated Probe Heated-ESI (VIP-HESI) ion source [2]. This combination allows the use of shorter gradients whilst retaining deep HCP coverage, leading to cost and time savings.

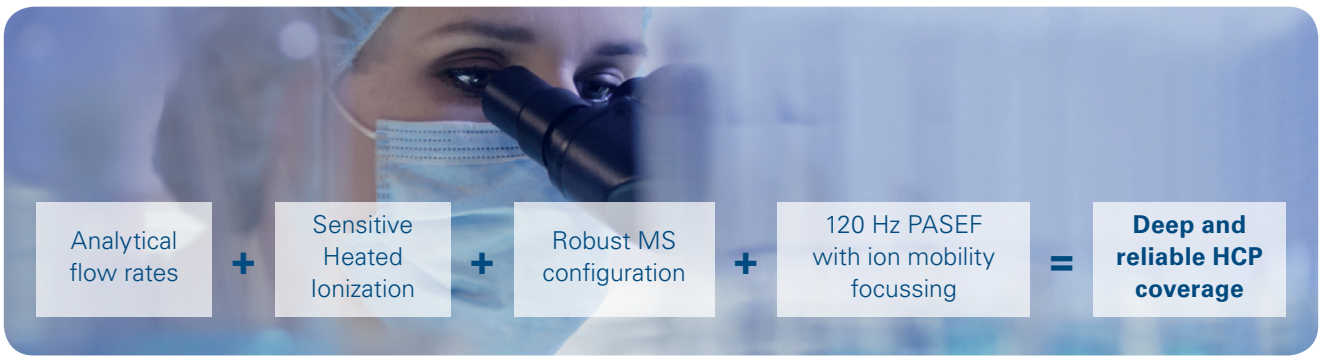

Figure 1: Formula for success in HCP analysis

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High Confidence for HCP Identifications

Experimental setup

NISTmAb (Merck) was digested under native conditions and analyzed by LC-MS as previously reported [2], using the same sample amount (50 µL) and gradient (120 min, for comparison a 40 min gradient was also used) as described. Peptides were separated on a CSH C18 1.7 µm 2.1 x 100 mm column (Waters) 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 at 120 Hz with a 1.1 sec cycle. Spectra were searched against the mouse sequence database using PEAKS Studio 10.6 and applying 1% FDR at the protein level; proteins with 2 or more unique peptides are reported.

Results

188 HCPs were identified in the NISTmAb sample with 2 or more peptides using a 120 min gradient. To illustrate the suitability of the platform for higher throughput use, 125 HCPs were identified using a 40 min gradient. These results are compared to recent literature [3-5] in Figure 2. The high correlation with published data and the additional identification of 92 previously unreported NISTmAb HCPs is shown in Figure 3. The run-to-run reproducibility over an 8-week period is approximately 85% (Figure 4).

Summary

- The data reported here demonstrate discovery of new HCPs in biopharmaceuticals and suitability of the workflow for higher throughput applications.
- The HCP identifications from NISTmAb greatly exceed those reported in the literature whilst showing excellent correlation for high confidence results.
- The timsTOF Pro 2, coupled to the Elute UHPLC with VIP-HESI source, provides a robust and reliable platform for sensitive HCP analysis, well-suited to 24/7 operation.

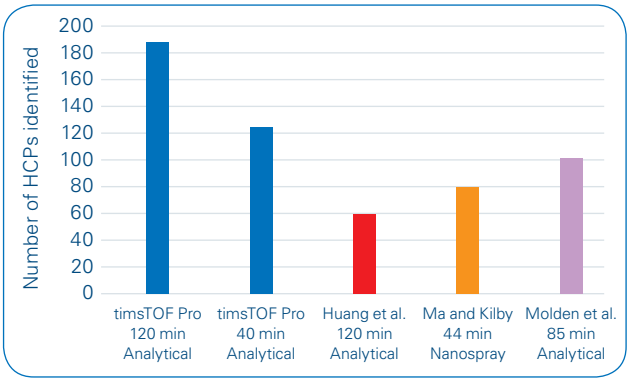

Figure 2: Number of HCPs identified with 2 or more peptides with Elute UHPLC-VIP-HESI-timsTOF Pro 2 compared to recent literature

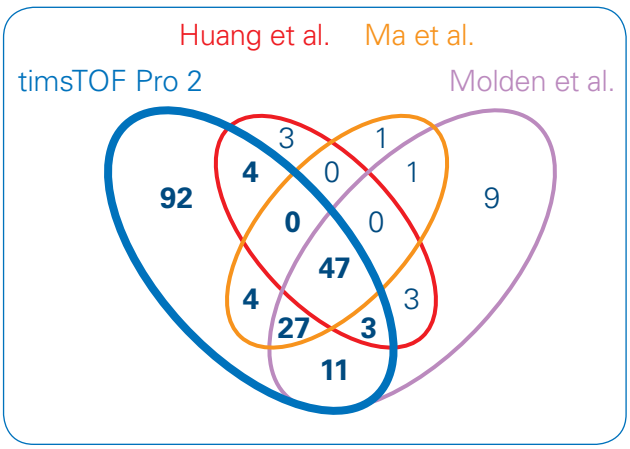

Figure 3: Overlap of HCPs identified by UHPLC-VIP-HESI-timsTOF Pro 2 and recent literature

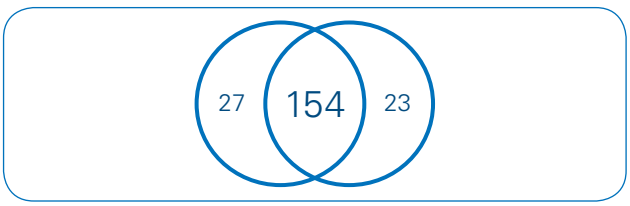

Figure 4: Overlap of HCP identifications between 2 runs acquired 8 weeks apart from 2 different digest batches

References

[1] Meier et al. (2018), Mol. Cell. Proteom. **17**: 2534-2545
[2] VIP-HESI dual source brochure (Bruker)
[3] Huang et al. (2017), Anal. Chem, **89**, 5436-5444
[4] Ma and Kilby, J Proteome Res. **202**;19(8):3396-404
[5] Molden et al. (2021), Mabs, Jan-Dec;**13**(1):1955432
[6] Venn (2007-2015). Diagram created using Venny 2.1: Oliveros JC

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