FlashNote

Same Day HCP Workflow Has Become Reality!

Host cell proteins (HCPs) are process-related impurities encountered in biotherapeutic drug development. Presence of HCPs in the final drug product, even at very low levels, can affect drug efficacy and stability, and may cause immunogenic responses in patients treated with the drug product. Here, we employ a mass spectrometry approach to enhance identifications of HCPs by combining an optimized sample preparation strategy using the novel iST-BCT (inStageTip-biopharmaceutical) Kit with PASEF (parallel accumulation and serial fragmentation) acquisition mode on the timsTOF Pro instrument.

Challenge

HCPs are usually present at low ppm concentration in biotherapeutic drug preparations, and even these small amounts can adversely affect drug stability and/or efficacy. There is therefore a need for rapid, robust methods for sample processing and analysis which attain the high level of sensitivity required for HCP detection.

Solution

The iST-BCT Kit (PreOmics) is a robust, reproducible and time-saving sample preparation kit that has been optimized for the enzymatic digestion of biopharmaceuticals. The timsTOF Pro delivers highly sensitive MS/MS analysis using PASEF via 100% ion usage and a robust instrument configuration which requires minimal maintenance. The instrument is interfaced to the nanoElute uHPLC with the CaptiveSpray ion source to provide stable nanospray sensitivity in a simple to use design.

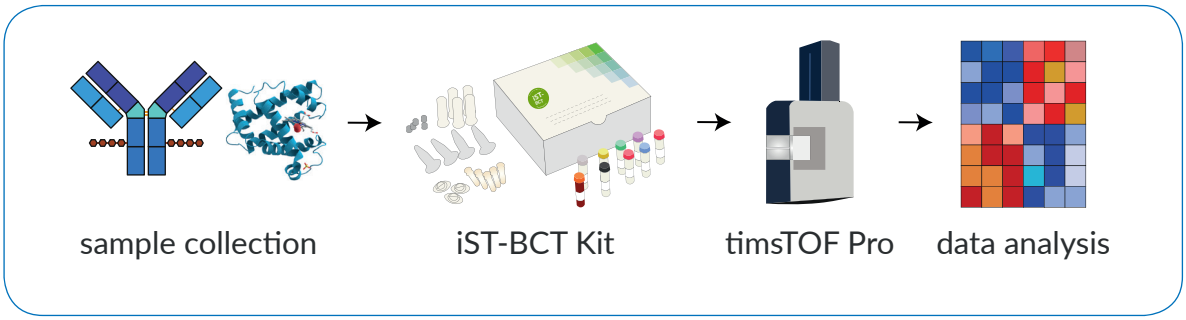

Figure 1: Simplified HCP workflow using the iST-BCT Kit (PreOmics) and the timsTOF Pro (Bruker Daltonics)

Streamline Your HCP Workflow Today!

Authors: Katrin Hartinger[1], Stuart Pengelley[2], Philipp Strohmidel[2], Fabian Hosp[2], Nils Kulak[1]
[1] PreOmics GmbH, Planegg/Martinsried, Germany, [2] Bruker Daltonik GmbH, Bremen, Germany

Optimized Same Day HCP Processing

The iST-BCT Kit was developed to enable the robust processing of body fluids, HCPs and therapeutic proteins such as monoclonal antibodies, and has been optimized to minimize artificial modifications (e.g. deamidation, oxidation) and increase the alkylation rate. The kit provides an all-in-one solution for processing samples in less than two hours and with less than 30 minutes hands-on-time.

Material and Methods

50 µg NIST antibody standard (NIST Monoclonal Antibody Reference Material 8671) was processed with either iST-BCT kit (in accordance with the manufacturer`s instructions) or digested with trypsin (Promega) using a standard overnight protocol (reduction using DTT in TFE solution, alkylation with iodoacetamide, overnight digestion with trypsin). Data was acquired on the timsTOF Pro using the PASEF MS/MS scan mode, coupled to the nanoElute LC system via the Captive-Spray ion source (all Bruker Daltonics). 200 ng sample was separated using an Aurora 25 cm x 75 µm ID, 1.6 µm C18 column (ionopticks), 2-37% acetonitrile gradient in 100 mins with a 400 nL/min flow rate. PASEF scans were searched against the mouse SwissProt database at 1% FDR using Mascot in BioPharma Compass software (Bruker Daltonics).

Results

Nearly 70 HCPs were identified with 2 or more peptides with the timsTOF Pro after being prepared using the newly developed iST-BCT kit, a 1.5-fold improvement over the standard overnight protocol. This was accompanied by improved sequence coverage, thereby increasing the confidence in HCP identification.

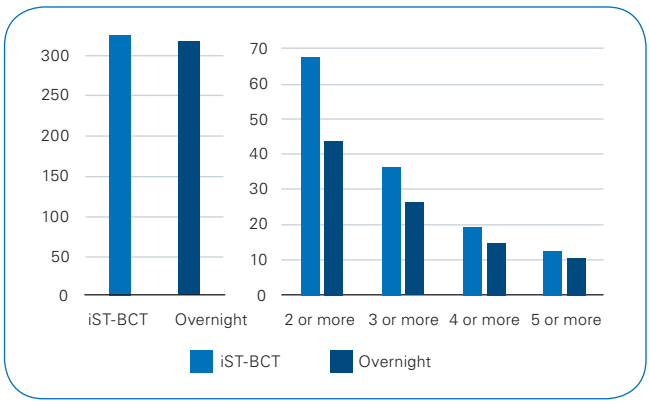

Figure 2: The average number of HCPs identified by ≥ 1 peptide (left panel) and ≥ 2 to ≥ 5 peptides (right panel) on the timsTOF Pro after preparing the samples with the iST-BCT kit (n=4) and the standard overnight protocol (n=2)

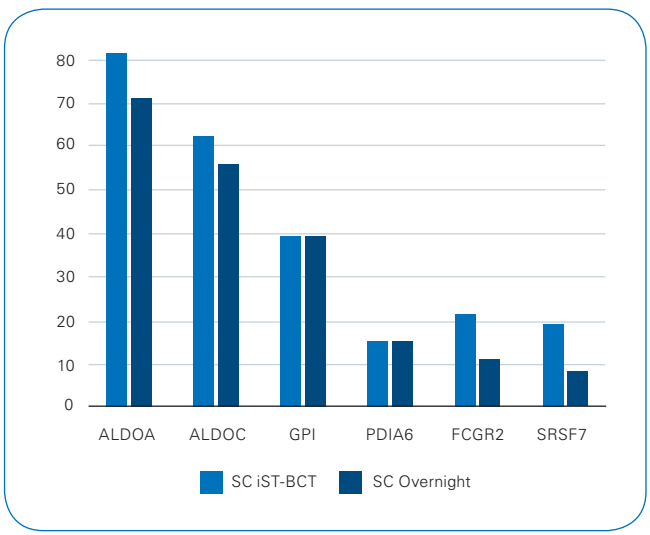

Figure. 3: Sequence coverage (%) for 6 HCPs identified on the timsTOF Pro after preparing the samples with the iST-BCT kit and the standard overnight protocol

Bruker Daltonik GmbH

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com – www.bruker.com

You are looking for further Information?
Check out the Link or scan the QR Code.

www.bruker.com/timsTOFPro