FlashNote

High Throughput HCP Screening is here: > 50 HCPs identified in 21 minutes

Monitoring of host cell proteins (HCPs) for biotherapeutics is required during manufacture and prior to product release. Bottom-up mass spectrometry approaches are established for analysing HCPs, but the low abundance of many of these proteins presents a significant challenge for confident detection of HCPs, especially when using short gradients. Here, the high speed of PASEF, (parallel accumulation and serial fragmentation) as implemented on the timsTOF Pro, coupled with the Evosep One LC system were used to detect HCPs using a 21 minute gradient.

Challenge

HCPs are present in biotherapeutic drug preparations, often at low ppm concentration levels, and must be monitored due to stability, efficacy and immunogenicity concerns. Sensitive detection of HCPs usually requires high sample amounts to be loaded on the HPLC column which are then separated using a long elution gradient. The resulting sample-to-sample times are not suitable for routine high-throughput monitoring of HCP removal during development and manufacture of biotherapeutics.

Solution

PASEF is a novel data-dependent method for MS/MS fragmentation at spectra acquisition rates up to 120 Hz which features automatic re-sampling of low abundant precursors, generating high quality MS/MS spectra with high sensitivity. The speed of this instrument is a perfect fit with the Evosep One, which efficiently delivers short gradients using single use and disposable Evotip trap-columns to prevent carryover between samples and ensures reliable data from each analysis. This system provides high sample turnarounds of 60 samples per day with a 21 minute gradient for HCP analysis.

EVOSEP

Fast, High-Throughput, High Confidence HCP Analysis!

timsTOF Pro and Evosep One combine to provide a fast, sensitive and straight-forward workflow for high-throughput HCP analysis.

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Method

Digestion

Twenty five µg NISTmAb (NIST Reference Material 8671, Humanized IgG1k Monoclonal Antibody) was digested with Trypsin (Promega) according to the protocol published by Huang et al. (Anal. Chem. 2017, 89, 5436–5444). In this method reduction is not performed prior to digestion, the mAb remains largely intact and is removed prior to LC-MS/MS by precipitation.

LC-MS/MS

The sample was loaded onto an Evotip. This serves as a disposable pre-column for the Evosep One, which features Gradient Offset Focussing™. Using this method the separation gradient is 21 minutes with a total runtime of 24 minutes, allowing 60 samples to be measured per day.

Data were acquired on the timsTOF Pro applying a 0.5 sec cycle consisting of 1 TIMS MS scan and 4 PASEF MS/MS scans. PASEF scans were searched against the mouse SwissProt database at 1% FDR using PEAKS Studio 8.5 (Bioinformatics Solutions Inc.).

Results

61 HCPs were identified using a 21 minute gradient, 28 with two or more peptides. Example MS/MS spectra are shown which matched to adenylate kinase 2, mitochondrial, a NISTmAb HCP reported at a concentration of 2 ppm (Huang et al., 2017).

Description	Coverage	#Peptides	#Unique	Avg. Mass
Fructose-bisphosphate aldolase A OS=Mus m...	74%	35	30	39356
Glucose-6-phosphate isomerase OS=Mus mus...	46%	26	26	62767
Fructose-bisphosphate aldolase C OS=Mus m...	53%	18	13	39395
Semaphorin-4B OS=Mus musculus GN=Sema...	10%	7	7	91392
Ig gamma-3 chain C region OS=Mus musculu...	7%	7	6	43929
Protein ABHD11 OS=Mus musculus GN=Abhd1...	35%	6	6	33561
Protein disulfide-isomerase A6 OS=Mus musc...	13%	4	4	48100
Low affinity immunoglobulin gamma Fc region...	11%	4	4	36695
Polypeptide N-acetylgalactosaminyltransferase...	7%	3	3	71537
Syntaxin-12 OS=Mus musculus GN=Stx12 PE=...	18%	3	3	31195
NSFL1 cofactor p47 OS=Mus musculus GN=Ns...	16%	3	3	40710
Fumarate hydratase, mitochondrial OS=Mus ...	9%	3	3	54357
Methionine--tRNA ligase, cytoplasmic OS=Mus...	5%	3	3	101431
Nucleoside diphosphate kinase B OS=Mus mu...	26%	3	3	17363
Adenylate kinase 2, mitochondrial OS=Mus m...	21%	3	3	26469

Figure 1: HCPs identified in 21 minutes with 3 or more peptides

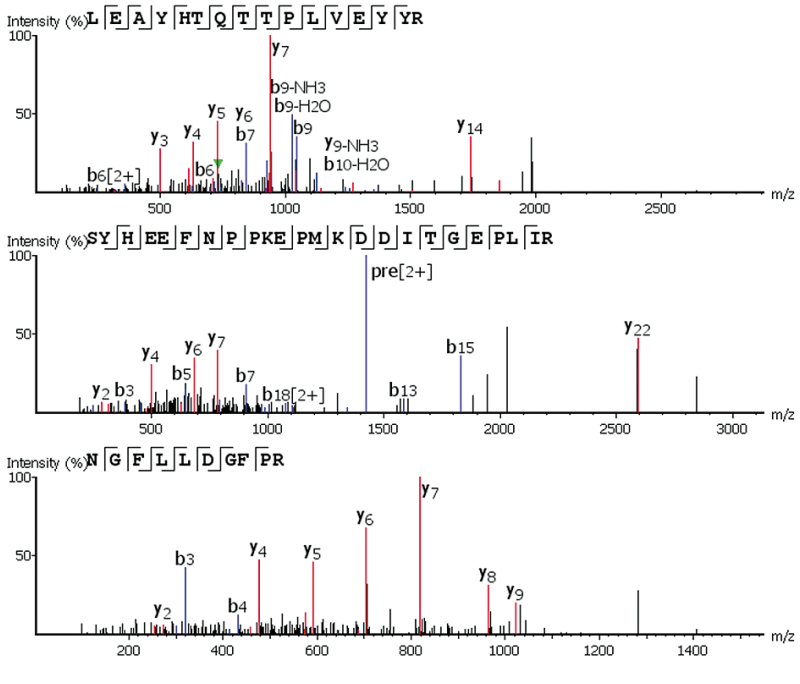

Figure 2: MS/MS Spectra for adenylate kinase 2, mitochondrial

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