Rapid Host Cell Protein (HCP) Detection with Advanced Trapped Ion Mobility Spectrometry (TIMS) Technology

The biopharmaceutical field is rapidly expanding, with biotherapeutic analysis and bioprocess development seeing particularly significant growth in recent years. The market has a projected growth of 8.59% CAGR from 2019–2024[1], which will continue to help the industry address the unmet medical needs for the world's most challenging diseases.

The percentage of large molecules in the pharmaceutical pipeline rose from 30% in 2010 to 42% in 2017[2] and there are nine Food and Drug Administration (FDA) approved biosimilars on the market, five of which are from 2017. There is, therefore, an ever-increasing requirement for analytical methods with rigorous process-related impurity testing, to monitor these contaminants during manufacture and prior to product release.

During biotherapeutic manufacturing, process-related impurities derived from the host organism can contaminate drug products. For example, during the expression of a recombinant protein drug, host cell systems can express numerous endogenous proteins, known as host cell proteins (HCPs). These contaminants may significantly affect drug efficacy and sometimes cause immunogenicity, making their removal highly important to ensure drug safety and purity. Biotherapeutics, such as monoclonal antibodies (mAbs), are purified using chromatographic techniques designed to remove residual HCP, DNA and viruses, as well as product-related impurities, such as fragments and aggregates. However, low-level HCPs often remain after purification, so the detection and quantification of these residual impurities is critical for biopharmaceutical companies to comply with regulatory guidelines.

Impact of Residual HCPs

There are several ways in which biotherapeutics contaminated with residual HCPs could compromise patient safety. The drug's efficacy and toxicity can be affected, and the therapeutic window in which the drug acts can be altered. Immunogenicity is the primary concern, where the HCP can invoke an unwanted immune response and cause damage to the patient, even at trace levels. For example, the clinical trial of IXinity (formerly IB1001) for the treatment of haemophiliacs was suspended in 2012, due to the development of antibodies to the drug in 26% of patients[3].

The stability of biotherapeutics may also be compromised by the presence of residual HCPs. They can contain enzymes, such as oxidases and lipases, which gradually break down the therapeutic protein or excipient, affecting the stability of the product over time. HCPs are also capable of mimicking the action of therapeutic proteins in assays, possibly resulting in the misformulation of the product outside the therapeutic window [4].

Meeting Regulations

The amount of residual HCP in a drug product is generally considered a critical quality attribute (CQA), due to its ability to impact product safety and efficacy. The International Conference on Harmonisation (ICH) Q6B requires the detection and quantification of process-related impurities, such as cell substrates (including HCPs and host cell DNA), as well as cell culture or downstream processing[5]. It also stipulates that, where possible, the biological activity of impurities should be evaluated.

HCPs are measured in parts per million (ppm), often expressed as nanograms per milligram of the intended recombinant protein. The general target for analysis is to ensure identification of HCPs at least down to the 100 ppm level. However, many contaminating enzymes in the HCP mixture can still be active at levels below 100 ppm, so highly sensitive detection technologies are, therefore, desirable for biopharmaceutical manufacturing organisations.

Impurity Testing Technologies

Comprehensive HCP analysis allows biopharmaceutical manufacturers to ensure quality control between batches, and facilitates comparisons between biologic drugs and biosimilars. The gold standard technique for HCP analysis is the enzyme-linked immunosorbent assay (ELISA), which is a commonly-used immunoassay for measuring antibodies, antigens, proteins and glycoproteins in biological samples. Despite its high throughput, sensitivity and selectivity capabilities, the ELISA can overlook weak and non-immunoreactive HCPs, which still carry the potential to impact drug safety and efficacy. It is also process-specific, meaning a new assay is required after each process change, which may take several months.

Many biopharmaceutical companies are now looking towards orthogonal methods, such as mass spectrometry (MS), as an alternative or complementary technique for HCP analysis. MS can rapidly monitor and identify multiple protein analytes in the same sample, and detect very low amounts of HCP in a non-targeted manner. This is crucial, considering that even very low-level HCPs can cause immunogenicity and impact drug quality. Whereas an ELISA can only measure the total HCP, MS can provide detailed information on the level of each individual HCP. Advanced MS techniques not only monitor, but characterise several impurities using a single method, bringing the high discriminatory power needed to separate impurities, while providing high sensitivity to detect and quantify low-level HCPs.

Analysing HCPs with MS

Although MS is gaining popularity as a powerful technique, there are still some limitations to overcome. A mass spectrometer with up to six orders of magnitude dynamic range is required to directly detect 1 to 100 ppm of HCPs in the sample, which is out of the range of many current instruments[6]. One way of overcoming the issue of dynamic range for HCP characterisation is to use ion mobility. Some modern instruments use trapped

ion mobility spectrometry (TIMS) quadrupole time-of-flight (QTOF) MS to achieve high confidence in identification and deep coverage, without compromising speed.

TIMS is a separation technique in gas phase, which resolves sample complexity with an added dimension of separation, in addition to high performance liquid chromatography (HPLC) and MS. This method increases peak capacity and confidence in compound characterisation and, combined with parallel accumulation serial fragmentation (PASEF) technology, enables high sequencing speeds while generating high-quality spectra. The high resolving power of TIMS MS makes it useful for the analysis of complex samples, such as those of biological origin.

PASEF scans implemented on TIMS QTOF MS/MS can combine fast acquisition speeds (>100 Hz MS/MS) with high resolution detectors, for high resolution quantifiable data from a complex sample, in a single run. When coupled with an advanced HPLC system, TIMS QTOF MS/MS with PASEF can detect HCPs using a gradient as short as 21 minutes. PASEF enables almost 100% duty cycle, for maximum use of all ions and therefore superior sensitivity.

There are two approaches to HCP analysis by MS:

1. HCP discovery

This method is useful in early process development, where critical problems arise in production or stability testing. HCP discovery uses longer runs for increased depth of analysis, typically two hours or more. Quantitation is not always necessary, as the primary concern is identifying all the proteins in the mixture. Maximum depth of coverage can be achieved, to identify low-level proteases/lipases that can affect stability, as well as other problematic proteins. Hundreds of peptides can be identified in a single run (Figure 1).

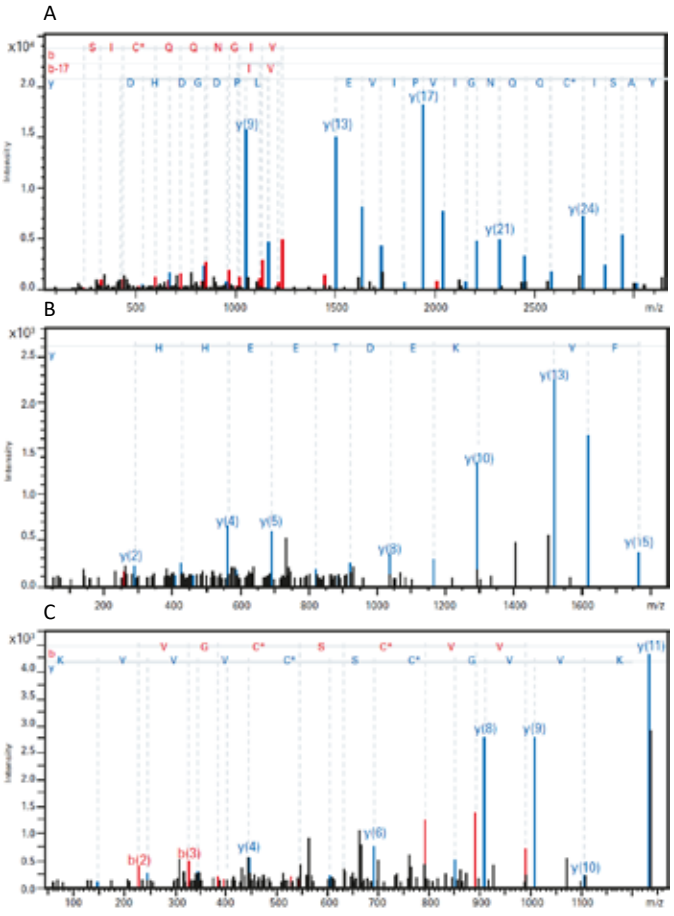

Figure 1: High-quality TIMS QTOF MS/MS with PASEF spectra (timsTOF Pro, Bruker Daltonics), featuring high sequencing speeds of > 100 Hz and sensitive detection of low abundant proteins (A) Fructose-biphosphate adolase A, (B) Heterogeneous nuclear ribonucleoprotein A1 and (C) 40S ribosomal protein S12.

For a 1.5 µg NISTmAb tryptic digest, nanoLC provides a 210-minute gradient for deep coverage of HCPs. Sample preparation can either be a standard tryptic digest of the entire mixture (220 HCPs identified), or a native tryptic digest (using protocol from[6]), where the mAb is left in its native structure, which provides a better dynamic range (280 HCPs identified).

2. Fast HCP screening

The method provides rapid analysis of downstream processes, with fast runs and high throughput. Quantitation is often necessary. Faster analysis is advantageous for more routine applications, but coverage of 50+ HCPs is still attainable at 1% false discovery rate (FDR) using a 21-minute gradient (Figure 2).

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=Nfs...	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 2: Host cell proteins (HCPs) identified in 21 minutes with three or more peptides. Data were acquired on the timsTOF Pro (Bruker Daltonics) applying a 0.5 sec cycle consisting of one TIMS MS scan and four PASEF MS/MS scans.

Both methods provide unbiased analysis of proteins, accurate mass measurement and, because of the high resolution of both MS and MS/MS, isotopic fidelity. They also provide collisional cross-section (CCS) values for a new dimension of confidence. These are physical parameters of peptides, often more reproducible than LC retention times. Novel MS instrumentation, such as the timsTOF Pro with PASEF (Bruker Daltonics), is now enabling users to combine HCP discovery and screening in one workflow, and accurately and reproducibly measure CCS values. Highly reproducible CCS values eliminate the need to include retention times (RT) in confidence measurements, enabling easy changing of LC methods, columns, flows, and other instrument parameters.

In instances that require deeper coverage of HCPs, samples can be analysed using a standard proteomics setup using nano ultra-high-performance liquid chromatography (UHPLC).

The Future of HCP Analysis

The low abundance of HCPs continues to present a challenge to the biopharmaceutical industry, and work on the removal of impurities has traditionally required specialised setups. PASEF scans implemented on advanced TIMS TOF MS instrumentation

can be applied to HCP analysis using both routine analytical and nanoflow configurations, to achieve sensitive detection with enhanced speed and data quality.

The depth of HCP identification provided by PASEF technology allows fingerprinting of biomanufacturing processes and ability to easily identify the effects of changes in these procedures. Sensitive, rapid, in-depth techniques for HCP analysis are driving the field of biotherapeutics, which is now transitioning into a new phase, thanks to decreased run times conferred by PASEF implemented on TIMS TOF MS. Such technologies are enabling biopharmaceutical manufacturers to accurately identify and quantify HCPs as CQAs and, therefore, comply with increasingly stringent regulatory guidelines.

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Guillaume Tremintin

Guillaume Tremintin is Market Manager BioPharma at Bruker Daltonics where he manages the development and commercialisation of Bruker mass spectrometry solutions for the biopharmaceutical industry.

Email: guillaume.tremintin@Bruker.com

Stuart Pengelley

Stuart Pengelley is a Senior Scientist at Bruker Daltonics where he develops solutions and methodologies for biopharma applications.

Email: stuart.pengelley@bruker.com

Michael Greig

Michael Greig, Director, US Pharma/Biopharma at Bruker Daltonics. Mike Greig joined Bruker in 2018 as the Director of the US Pharma/Biopharma Business Unit for Bruker Daltonics. Michael previously worked at Pfizer in Drug Discovery, directing labs performing everything from high-throughput analysis of small molecule libraries using supercritical fluid-MS, protein NMR, native mass spectrometry, HDX-MS for structural biology, protein turnover, fragment-based drug design, to proteomics. He also spent several years at Ionis Pharmaceuticals managing an oligonucleotide-based mass spectrometry research lab, and worked at Revlon Science Institute as a polymer and analytical chemist. He has taught over 25 mass spectrometry classes worldwide. He was a keynote speaker at the International Mass Spectrometry Conference, is a member of the Lab Automation Scientific Committee (now SLAS), is a member of National High Magnetic Field Laboratory FTICR MS Advisory Panel, and has over 50 scientific publications.

Email: michael.greig@bruker.com