Characterisation of Protein and Peptide Drug Products

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INTRODUCTION

In the drive to bring new drugs to market, researchers face many obstacles along the road from discovery to regulatory approval. One such hurdle is to find and develop reliable analytical tools which can determine the physicochemical structure of the product, so that it may be manufactured consistently within a GMP environment. This characterisation presents a particularly difficult challenge for conventional analytical techniques when dealing with biotechnology products. In contrast to small molecule drugs, protein and peptide pharmaceuticals are complex entities, often comprising multiple disulphide-bridged and/or glycosylated molecules (i.e. carbohydrate residues attached to the protein). In particular, they require methods capable of determining not only the primary protein structure of the amino-acid backbone, but also of detecting and assigning post-translational modifications and microheterogeneities. So, new analytical techniques which can help smooth the development pathway are always welcome. One such breakthrough has been the use of mass spectrometry for final product characterisation.

MASS SPECTROMETRY METHODS

Mass spectrometry (MS) has become an essential tool for the analysis of “well-characterised” biomolecules. Complementary to “classical” Edman degradation protein sequencing techniques, MS has the advantage of being able to probe areas inaccessible to conventional sequencing. Its use can be extended to not only synthetic peptide products and recombinant proteins, but also large glycoproteins such as monoclonal antibodies. Indeed, the same MS instruments which deduce protein structure can also provide valuable information on the glycan moiety when combined with particular carbohydrate chemistries.

Over the past 20 years or more, various MS techniques have been developed and applied at all stages of the drug pipeline. These versatile and sensitive methods can firstly detect, then identify and even quantify changes in the protein product structure. Unfortunately, there is no one ideal or universal mass spectrometer for this application; all have their advantages and disadvantages (usually cost!), and it is normally necessary to use more than one type during a complex biopharmaceutical study. The most commonly utilised ionisation techniques include Electrospray (ES-MS) and its variations: ionspray, nanospray and on-line LC-MS, Matrix Assisted Laser Desorption Ionisation (MALDI) and, for derivatised carbohydrates, Fast Atom Bombardment (FAB) and GC-MS. In fact, it was the advent of FAB-MS in the early 1980’s that generated the interest in the use of MS for proteins and peptides, but nowadays this technique has been largely superseded.

Electrospray ionisation allows the mass range of ionisable molecules to encompass intact proteins up to approx. 150kDA. This technique is widely applied using small or benchtop quadrupole instruments, often a triple-quadrupole, capable of MS/MS experiments to produce sequence or fragment data as well as molecular weight information. One advantage of ES-MS is that it can be directly coupled to a liquid on-line sample introduction method such as HPLC, allowing separation and simultaneous detection of the components of complex mixtures. Indeed on-line LC-MS has become a routine workhorse for bioanalytical and pharmacokinetic studies in the pharmaceutical and CRO industries.

MALDI ionisation, usually with a Time-Of-Flight (TOF) analyser and Delayed Extraction (DE) technology is also capable of intact mass measurement of large molecules - up to approx. 500kDA.

A new generation of instruments has emerged recently, the design consisting of a quadrupole analyser followed by a collision cell with an orthogonal injection of ions into a TOF analyser. These instruments (for example, Q-TOF and Q-STAR (pictured)) provide the most powerful tandem MS/MS sequencing available today. These advances in mass spectrometry technologies have opened the door to the use of MS in drug development and QC. Moreover, these new instruments have major applications at discovery level too. “Proteomic” approaches to the characterisation of potentially novel proteins in complex mixtures e.g. cells and tissues, involve various strategies, including gel or multidimensional chromatographic separation. Whichever approach is used, for the subsequent detection and identification of peptides at sub-picomole levels, the sensitivity of the new MS/MS instruments mentioned above is essential.

At a more advanced stage in the development path of a biopharmaceutical, for example, QC release, it is imperative to confirm that the product has the correct anticipated structure, i.e. that the gene sequence has been correctly translated and that there have been no errors, insertions, deletions or mutations. The first step is usually a simple molecular weight measurement. Depending on the size, this is usually performed by MALDI-TOF or ES-MS. This measurement would “flag” any discrepancy between the theoretical mass and the actual mass, and depending on the mass range and resolution of the technique, may provide a clue to the type of modification(s).

But in order to take a closer look at any potential modifications, MS-MAPPING procedures must be carried out.[1] Analogous to LC peptide mapping, the molecule is initially digested into smaller parts using enzymic or chemical means and then the mixture of peptides produced is analysed using ES-MS or MALDI. If the mixture is too complex, it can be separated using on-line LC-MS, where the HPLC is directly linked to the mass spectrometer, bringing the additional dimension of molecular weight to the peptides separated in the UV profile. Differences between the measured masses and the theoretical masses of the anticipated peptides can be spotted and the corresponding peptides isolated and collected for further study. As the MS technique relies on measuring mass changes/differences, non-protein modifications such as sulphation, phosphorylation or addition of lipid or carbohydrate, can therefore be detected. Using the same types of instruments, the carbohydrate portions of glycoproteins can be characterised too.

M-Scan first introduced “MS Mapping” into the biopharmaceutical characterisation field.[2] Now, MALDI-TOF “Mapping” performed on specific enzymic digested peptide mixtures of a target protein/glycoprotein, or on-line ES-MS, provide reliable methods of confirming the overall amino-acid structure and can pinpoint areas for further investigation. Sequence analysis can then be carried out using ES-MS/MS for further confirmation of assignments if required.

REGULATORY REQUIREMENTS

Full characterisation of a biopharmaceutical product is critical for regulatory purposes. M-Scan have developed, applied and validated MS procedures to address the problems of bioproduct characterisation at both pre-clinical and GMP phases. Working closely to the recommendations of the ICH guidelines, particularly the requirements of ICH Topic Q6B (Specifications: test procedures and acceptance criteria for biotechnological/biological products).[3] M-Scan provide contract analytical services (GLP/GMP level) which fulfil the requirements listed in Table 1.

Table 1.
ICH Topic Q6 B
(Specifications: test procedures and acceptance criteria for biotechnological/biological products)

6.1 Appendix for Physiochemical Characterisation

6.1.1 Structural characterisation and confirmation

a) Amino acid sequence
b) Amino acid composition
c) Terminal amino acid sequence
d) Peptide map
e) Sulfhydryl groups and disulphide bridges
f) Carbohydrate structure

6.1.2 Physicochemical properties

a) Molecular weight or size
b) Isoform pattern
c) Extinction coefficient
d) Electrophoretic patterns
e) Liquid chromatographic patterns
f) Spectroscopic profiles

For example, intact molecular weight determination is particularly useful for Quality Control I.D. testing of synthetic peptides and oligonucleotides. If appropriate specifications have been set, then this can be a reliable release test assay. ES MS/MS sequencing of peptides can then be carried out to confirm their anticipated sequence if required. Sequence confirmation of oligonucleotide products is generally performed using MALDI-TOF. Here, the masses of fragments generated following digestion with two different phosphodiesterases: snake venom (3’ to 5’direction) and bovine spleen (5’ to 3’) are used to build up the sequence in a step-wise manner.

In conclusion, various Mass Spectrometry techniques have become established throughout the entire drug discovery pipeline to aid biotechnology researchers to bring their product to market successfully. Methods developed over the years by M-Scan have the capability to provide molecular weight and sequence data, detect errors of translation etc, identify post-translational modifications, identify sites of glycosylation (including carbohydrate structure and sequence), identify heterogeneity at the N- and C- termini and assign disulphide bridges amongst other things! These procedures have been developed, applied and validated to address the problems of characterisation for regulatory body purposes. Moreover, these techniques will be further refined to keep pace with the ever changing demands of the biopharmaceutical industry.

References
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3.) ICH Harmonised Tripartite Guideline, Topic Q6B. Specifications: Test Procedures and Acceptance Criteria for Biotechnological/ Biological products. Step 4, Consensus Guideline, March 1999 (CPMP/ICH/365/96).

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