

PRODUCT INFORMATION INTACT MASS AND PEPTIDE MAPPING ANALYSIS

Introduction

ICH Topic Q6B (1) provides a uniform set of internationally accepted principles for characterization of biotechnological products to support new marketing applications. The document suggests that analyses should be performed to provide the following information for biological or biopharmaceutical products, including:

Molecular weight or size

The guidelines state that “Molecular weight (or size) is determined using size exclusion chromatography, SDS-polyacrylamide gel electrophoresis (under reducing and non-reducing conditions), mass-spectrometry, and other appropriate techniques”.

Peptide mapping

The guidelines state “Selective fragmentation of the product into discrete peptides is performed using suitable enzymes or chemicals and the resulting peptide fragments are analyzed by HPLC or other appropriate analytical procedure. The peptide fragments should be identified to the extent possible using techniques such as amino acid compositional analysis, N-terminal sequencing or mass-spectrometry. Peptide mapping of the drug substance or drug product using an appropriately validated procedure is a method that is frequently used to confirm desired product structure for lot release purposes” [1].

The techniques of Electrospray Mass Spectrometry (ES-MS) using an atmospheric pressure ionization source [2] and Matrix Assisted Laser Desorption Ionization (MALDI) Mass Spectrometry [3] have created the possibility of accurately measuring the intact molecular weight of biopolymers up to at least 130 kDa and 500 kDa respectively.

These techniques are very powerful and powerful strategies were developed for MAPPING proteins and glycoproteins by ES-MS and MALDI-MS. The coupling of on-line microbore HPLC with Electrospray mass spectrometric detection of separated digest products has increased the power and scope of this technique (4 and references therein).

The concept is to map out peptides generated from specific enzymatic or chemical digests by their molecular weights. Since the probability of two peptides from any one digest of a protein having the same molecular weight is fairly small, particularly when the specificity of the digest used is taken into account, the MAP thus produced is an accurate diagnostic for the presence (and thus correctness) or absence of the component parts of the predicted protein sequence. Since the mass of a substance is a definitive physical quantity, a point mutation (with the exception of Leu/Ile or Lys/Gln) will show up as a mass shift and the amount of shift, or difference in mass, reveals the identity of the replacement amino acid. Similar logic applies to the identification and location of blocking groups eg. increasing the peptide mass by 42 for acetyl – or 28 for formyl – N termini. Various operations may be carried out on the peptide mixtures to aid interpretation, including Edman degradation, acetylation, esterification, further digestion or high sensitivity MS/MS (5). A definite advantage of the MAPPING procedure is that there is an equal probability of observing the C-terminal as well as the N-terminal regions of the protein.

References

1. ICH Topic Q 6 B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products
2. Fenn, J.B., Mann, M., Meng, C.K., Wong, S.F. and Whitehouse, C.M. (1989) *Science*. 246, 64-70.
3. Hillenkamp, F., Karas, M., Beavis, R.C., Chait, B.T., *Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry of Biopolymers*, (1991) *Anal. Chem.* 63, 1193-1203.
4. Dell, A. and Morris, H.R. *Science* (2001), 291, 2351-2356
5. Morris, H.R., Paxton, T., Dell, A., Langhorn, J., Berg, M., Bordoli, R.S., Hoyes, J., and Bateman R.H. *Rapid Commun. Mass Spectrom.* (1996) 10, 889-896.