

## Stability Testing of Biopharmaceuticals

***Since 2005, the rate of approvals of biopharmaceuticals has slowed down. Dave Scott, General Manager, Tepnel Research Products & Services, investigates some of the considerations which must be taken into account when stability testing biological derived drugs.***

Stability testing of a drug substance or a finished product is a vital part of the data package submitted in support of new drug applications or marketing authorisations. The principle ethos of stability testing is to provide evidence to a regulatory body that the quality of the product or raw material doesn't vary with time, under the influence of a range of different factors including temperature, humidity and light.

With the evolution of drug development to include whole new classes of molecules, the application of the International Conference on Harmonisation (ICH) guidelines remains the same but the level of technical complexity in designing stability programmes that are fit for purpose has increased dramatically. Therapeutic agents that include proteins and peptides or monoclonal antibodies pose a different set of practical challenges in providing data which meets the needs of both the regulatory authorities and the developers of these drug products.

This article will consider the regulatory requirements for both drug substances and finished products whilst comparing the scientific considerations that come into play when stability testing biological derived drugs compared with conventional small molecule products and substances.

### Stability Testing and Harmonisation

The stability program and subsequent testing of both the Active Pharmaceutical Ingredient (API) and the finished product plays a pivotal part in the drug development pipeline. It is crucial that any programme undertaken is sustainable and is also compliant with current regulations of the Medicines and Health Regulatory Authority (MHRA), Food and Drug Administration (FDA) or other competent regulatory organisations.

Stability testing provides the scientific evaluation of both API and finished product in determining the shelf life consideration for the label and governs also the suitability of any formulations for clinical trial to be suitably active for the duration of any intended dosing regime. The intent is to establish that under certain pre-prescribed conditions the item under investigation will both retain its original properties and therefore efficacy whilst not undergoing any significant changes in either its composition or structure which could cause deleterious harm to the eventual recipient.

Stability testing also plays a significant role in the development of a product down the drug development pipeline providing information which is useful in facilitating decisions and understanding the product/formulation in greater detail. This can include information on purity, strength and quality of both the drug substance and finished product; expiration dating and compliance with CGMP requirements; information on product characteristics when exposed to stress conditions; degradation pathways; facilitation of analytical methods development; facilitation of the design of the formulation and the manufacturing process; information on the compatibility of the container closure and/or delivery systems; finalisation of the release and stability specifications, and any in-use conditions which may be vital to the successful efficacy of the product; and a comparability assessment after any manufacturing changes have been made.

“The history of medicinal product registration, in much of the industrialised world, has followed a similar pattern which could be described as: *Initiation, Acceleration, Rationalisation and Harmonisation.*”<sup>(1)</sup>

This statement is taken directly from the ICH website and summarises the process of steps that have taken place since the early 90's to arrive at a situation today whereby, the

mechanism for the assessment of the stability of a Drug Substance or API, Finished Product or Investigational Medicinal Product (IMP) is uniform and in principle universally accepted by regulatory authorities across the globe. The ICH in itself is a joint initiative that has involved both regulators and industry, crucially as equal partners, in the scientific and technical discussions that have centred on the testing procedures that are required to ensure and to assess the safety, quality and efficacy of medicines. It is composed of six member bodies with three observers who represent the regulatory bodies and the research-based industry in the European Union, Japan and the USA.

A review of the current ICH Guidelines establishes that there are six documents which are specific in their application to the stability testing of pharmaceutical products and components both small molecule and biopharmaceutical. These six guidance documents are summarised in Table 1.

Table 1: Stability specific ICH guidance

<b>Guidance</b>	<b>Title</b>	<b>Status</b>
Q1A(R2):	Stability Testing of New Drug Substances and Products (Second Revision)	Status : Step 5 February 2003
Q1B:	Photostability Testing of New Drug Substances and Products	Status : Step 5 November 1996
Q1C:	Stability Testing for New Dosage Forms	Status : Step 5 November 1996
Q1D:	Bracketing and Matrixing Designs for Stability Testing of Drug Substances and Drug Products	Status : Step 5 February 2002
Q1E:	Evaluation of Stability Data	Status : Step 5 February 2003
Q1F <sup>(*)</sup> :	Stability Data Package for Registration Applications in Climatic Zones III and IV	Status: Withdrawal June 2006
Q5C:	Quality of Biotechnological Products : Stability Testing of Biotechnological/Biological Products	Status : Step 5 November 1995
Q7:	Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients	Status : Step 5 November 2000

(\*) - ICH Q1 F defined storage conditions for stability testing in countries located in Climatic Zones III (hot and dry) and IV (hot and humid), In the course of the discussions which led to the development of the guideline, a survey amongst member states to find consensus on 30°C/65% RH as the long-term storage conditions for hot and humid regions was reached on the basis of no significant objections being raised during this survey. However, based on new calculations and discussions, some countries in Climatic Zone IV expressed their wish to include a larger safety margin for medicinal products to be marketed in their region. As a consequence, several countries and regions have revised their own stability testing guidelines, defining up to 30°C/75 % RH as the long-term storage conditions, therefore due to this divergence in global stability testing requirements, the ICH decided to withdraw ICH Q1F.

It is recognised that the quality guidance documents are by definition interlinked; however, the table above notes those document's which give specific guidance on the conduct of stability studies.

The regulatory requirements of Q1A(R2) versus Q5C are in principle the same i.e. to assess the stability characteristics of either the drug substance or the finished product, to determine storage conditions and expiration dates and the application of the remaining Q1 guidance does not vary for either class of molecule.

The scientific principles in stability testing have not changed significantly in recent years but there is now a far greater understanding of the complexities and complications that biopharmaceuticals intrinsically carry. There are a number of subtle differences which come into play and which mean that a blanket approach to stability testing of both types of molecules cannot be applied.

### **NCEs versus NBEs**

There are a multitude of definitions as to what constitutes a biopharmaceutical however, a recent survey of those involved in biopharmaceutical development and production agree that biopharmaceuticals are pharmaceuticals which are inherently biological in nature due to their method of manufacture. Specifically, these would involve the use of a biotechnology process which involves the use of live organisms. In such cases the API is referred to as a New BioPharmaceutical Entity or NBE.

The predominance of pharmaceutical products currently licensed to the marketplace are New Chemical Entities (NCEs) or more generally referred to as small molecule API's. While stability testing for small molecule drugs is well established, its counterpart for NBE derived products is still evolving and the complexity of both the NBE and the process by which it is manufactured dictates that a very different view must be taken when considering stability testing.

If we consider NCEs versus NBEs at 4 basic levels we can start to understand the complexities which are inherent in the NBE and consequently play a major component in the design of the testing required in a biopharmaceutical stability testing regime.

### ***Manufacturing***

The first area of interest and impact is the process of manufacturing. Small molecule API's and products are the end result of a chemical synthetic process which is on the whole well characterised, understood and controlled and therefore the end product demonstrates a high degree of homogeneity from batch to batch production runs. In contrast to this, NBE's are usually the end product of either a single or series of steps, within a biological system. Biological systems are often poorly understood or characterised and as a consequence there is a large amount of heterogeneity that can exist within both the process and the end product. It is therefore very difficult to design the component parts of a stability study when the end point of the manufacturing process can vary from batch to batch. Consideration must therefore be given to establishing that inter batch variation as a consequence of the inherent variability of the process are not mistaken as losses in the stability of either the API or the finished product.

### ***Structure***

We must consider the entity itself and any impurities that arise as a part of the manufacturing process. In relative terms small molecule API's and products are simple, the API is structurally well defined and the presence of any impurities are easily qualified and quantified in either manufacturing standards or specifications. NBEs in contrast are complex molecules, many orders of magnitude of size larger than NCEs whose activity depends on their complicated structure which can be based on secondary, tertiary and even sometimes quaternary folding structures. The end result of the manufacturing process should lead to a homogeneous batch of product but as these are biological systems slight variances in the environmental conditions can have a major impact on the system which can result in both intra and inter batch heterogeneity. It is therefore extremely difficult to both define what impurities can exist and which should be quantified.

### **Methods of Analysis**

There are a plethora of well developed NCE platforms, analytical methods and techniques that will allow for the characterisation of the drug substance both in bulk and in formulation which are sensitive, provide good analytical standards to aid accurate quantification and which discriminate the analyte of interest from impurities of constituents of the final product. NBEs often have structures that cannot be fully defined with our present set of analytical techniques or approaches for potency testing. There are therefore a set of limitations on the available methods for quantification of activity, potency and impurity profiles which are present before any assessment of stability can commence. It is not always inevitable but it is often required that a number of different methods be applied to provide the same end point data. This is especially true when we consider the measurement of potency. Where as small molecule potency and purity is predominately measured using High Performance Liquid Chromatography, the potency of an NBE can be quantified using HPLC, electrophoretic techniques, and biological assays. Within these methods the level of accuracy and precision vary; therefore consideration must be given to finding not necessarily the most efficient method of analysis but rather those methods which are most effective in demonstrating what is required to meet the regulators expectations.

### **Biological Contamination**

In general, extraneous or intrinsic biological contamination of NCE drug substances or finished products is minimal. The chemical synthetic process is often very restrictive towards opportunities for microbial growth and contamination. This is not to say that there are circumstances whereby biological contamination can occur and certainly products which are classified as parenteral will always be checked for both sterility and endotoxin content at the end of the process. NBEs are derived from biotechnological processes all of which will contain various sources for contamination from adventitious agents. These can include elements of the cell substrate such as endogenous viruses, exogenous microbial contamination, cell culture reagents both animal and non-animal derived and environmental agents. With such a large number of possible biological contaminants, the concern from a stability indicating point of view would not just be the presence of these from a contamination perspective but the consideration that the moiety of these contaminants could lead to results which were wrong due to the relative specificity of some of the analytical methods employed.

### **Conclusion**

Stability testing of small molecule API's and finished products is at a mature level of development. The requirements of the regulatory authorities, the scientific principles and techniques for accumulating, analysing and presenting the data is well understood and documented in the ICH guidelines. With every advancement in the production and manufacture of biopharmaceuticals additional knowledge and understanding is gained; however, the goalposts are also changing and the requirements of the regulators and the mechanisms of meeting these needs are under constant review. As we have noted the basic principle behind stability testing has not changed; however, the inherent variability and difficulties in manufacturing biopharmaceuticals is making effective stability testing an increasing difficult art.

2005 saw a record year for approvals of biopharmaceuticals within the FDA with 21 products receiving official FDA approval. However, in the subsequent years there has been a slow down in approvals. There are a number of reasons behind this and the inference is not that this is linked to stability testing failure but it is indicative that the regulatory requirements for a biopharmaceutical are evolving and that success of a biopharmaceutical can be enhanced through constant and continued dialogue coupled with a full understanding of the process, the product and the variability that lies within.

(1) - <http://www.ich.org/>

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