

## Biotechnological/Biological Products: About Its Stability Testing

Vivek P. Chavda<sup>1\*</sup>, Dharmesh Shaliya<sup>2</sup>, Bhargav Patel<sup>3</sup>, Ashish Gabani<sup>4</sup>

<sup>1</sup>Department of Pharmaceutics, B.K. Mody Government Pharmacy College, Rajkot, Gujarat, India

<sup>2</sup>Quality Assurance, Intas Pharmaceuticals Ltd., Ahmedabad, Gujarat, India

<sup>3</sup>Quality Assurance, Zydus cadila pharmaceuticals Ltd., Ahmedabad, Gujarat, India

<sup>4</sup> Intas Biopharmaceuticals Ltd., Ahmedabad, Gujarat, India

\*[vivek7chavda@gmail.com](mailto:vivek7chavda@gmail.com)



### ABSTRACT

The drug delivery systems have made some of the technological advances especially in the case of biopharmaceuticals. When one talks about biotechnological product stability of drug product becomes main culprit due to dynamic nature of drug molecule. This review is grafted using ICH and other regulatory guidelines to provide an overview to stability testing of such molecules.

**KEYWORDS:** Biologicals; Stability testing; rDNA technology

### INTRODUCTION

When we talk about biological; the basic difference comes in our mind with respect to it is dynamic nature of API in comparison to static nature of API in conventional pharmaceuticals. Some basic feature with respect to stability testing is common for both type of drug product. However, biotechnological/biological products do have distinguishing characteristics to which consideration should be given in any well-defined testing program designed to confirm their stability during the intended storage period. For such products, in which the active components are typically proteins and/or polypeptides, maintenance of molecular conformation and, hence of biological activity, is dependent on noncovalent as well as covalent forces. The products are particularly sensitive to environmental factors such as temperature changes, oxidation, light, ionic content, and shear. In order to ensure maintenance of biological activity and to avoid degradation, stringent conditions for their storage are usually necessary.

The evaluation of stability may necessitate complex analytical methodologies. Assays for biological activity, where applicable, should be part of the pivotal stability studies. Appropriate physicochemical, biochemical and immunochemical methods for the analysis of the molecular entity and the quantitative detection of degradation products should also be part of the stability program

whenever purity and molecular characteristics of the product permit use of these methodologies.

With the above concerns in mind, the applicant should develop the proper supporting stability data for a biotechnological/biological product and consider many external conditions which can affect the product's potency, purity and quality. Primary data to support a requested storage period for either drug substance or drug product should be based on long-term, real-time, real-condition stability studies. Thus, the development of a proper long-term stability program becomes critical to the successful development of a commercial product. The purpose of this document is to give guidance to applicants regarding the type of stability studies that should be provided in support of marketing applications. It is understood that during the review and evaluation process, continuing updates of initial stability data may occur.

### SCOPE

The guidance expressed in this article applies to very much portrayed proteins and polypeptides, their subordinates and results of which they are segments, and which are secluded from tissues, body liquids, cell societies, or delivered utilizing rDNA innovation. Along these lines, the report covers the era and accommodation of steadiness information for items, for example, cytokines (interferons, interleukins, settlement fortifying variables, tumor corruption

elements), erythropoietins, plasminogen activators, blood and immunizations comprising of all around portrayed proteins or polypeptides. Moreover, the direction laid out in the accompanying areas might apply to different sorts of items, for example, ordinary immunizations, after discussion with the suitable administrative powers. The archive does not cover anti-microbials, allergenic concentrates, heparins, vitamins, entire blood, or cell blood segments.

## SELECTION OF BATCHES <sup>[1, 2]</sup>

### Drug Substance (Bulk Material)

Where bulk material is to be stored after manufacture but prior to formulation and final manufacturing, stability data should be provided on at least 3 batches for which manufacture and storage are representative of the manufacturing scale of production. A minimum of 6 months stability data at the time of submission should be submitted in cases where storage periods greater than 6 months are requested. For drug substances with storage periods of less than 6 months, the minimum amount of stability data in the initial submission should be determined on a case-by-case basis. Data from pilot-plant scale batches of drug substance produced at a reduced scale of fermentation and purification may be provided at the time the dossier is submitted to the regulatory agencies with a commitment to place the first 3 manufacturing scale batches into the long-term stability program after approval.

The quality of the batches of drug substance placed into the stability program should be representative of the quality of the material used in preclinical and clinical studies and of the quality of the material to be made at manufacturing scale. In addition, the drug substance (bulk material) made at pilot-plant scale should be produced by a process and stored under conditions representative of that used for the manufacturing scale. The drug substance entered into the stability program should be stored in containers which properly represent the actual holding containers used during manufacture. Containers of reduced size may be acceptable for drug substance stability testing provided that they are constructed of the same material and use the same type of container/closure system that is intended to be used during manufacture.

### Intermediates

During manufacture of biotechnological/biological products, the quality and control of certain intermediates may be critical to the production of the final product. In general, the manufacturer should identify intermediates and generate in-house data and process limits that assure their stability within the bounds of the developed process. While the use of pilot-plant scale data is permissible, the manufacturer should establish the suitability of such data using the manufacturing scale process.

### Drug Product (Final Container Product)

Stability information should be provided on at least 3 batches of final container product representative of that which will be used at manufacturing scale. Where possible, batches of final container product included in stability testing should be derived from different batches of bulk material. A minimum of 6 months data at the time of submission should be submitted in cases where storage periods greater than 6 months are requested. For drug products with storage periods of less than 6 months, the minimum amount of stability data in the initial submission should be determined on a case-by-case basis. Product expiration dating will be based upon the actual data submitted in support of the application. Since dating is based upon the real-time/real-temperature data submitted for review, continuing updates of initial stability data should occur during the review and evaluation process. The quality of the final container product placed on stability studies should be representative of the quality of the material used in the preclinical and clinical studies. Data from pilot-plant scale batches of drug product may be provided at the time the dossier is submitted to the regulatory agencies with a commitment to place the first 3 manufacturing scale batches into the long term stability program after approval. Where pilot-plant scale batches were submitted to establish the dating for a product and, in the event that product produced at manufacturing scale does not meet those long-term stability specifications throughout the dating period or is not representative of the material used in preclinical and clinical studies, the applicant should notify the appropriate regulatory authorities to determine a suitable course of action.

### Sample Selection

Where one product is distributed in batches differing in fill volume (e.g., 1 millilitre (ml), 2 ml, or 10 ml), unitage (e.g., 10 units, 20 units, or 50 units), or mass (e.g., 1 milligram (mg), 2 mg, or 5 mg) samples to be entered into the stability program may be selected on the basis of a matrix system and/or by bracketing. Matrixing, i.e., the statistical design of a stability study in which different fractions of samples are tested at different sampling points, should only be applied when appropriate documentation is provided that confirms that the stability of the samples tested represents the stability of all samples. The differences in the samples for the same drug product should be identified as, for example, covering different batches, different strengths, different sizes of the same closure and possibly, in some cases, different container/closure systems. Matrixing should not be applied to samples with differences that may affect stability, such as different strengths and different containers/closures, where it cannot be confirmed that the products respond similarly under storage conditions.

Where the same strength and exact container/closure system is used for 3 or more fill contents, the manufacturer may elect to place only the smallest and largest container size into the stability program, i.e., bracketing. The design of a protocol that incorporates bracketing assumes that the stability of the intermediate condition samples are represented by those at the extremes. In certain cases, data may be needed to demonstrate that all samples are properly represented by data collected for the extremes.

### STABILITY-INDICATING PROFILE <sup>[1, 2]</sup>

On the whole, there is no single stability-indicating assay or parameter that profiles the stability characteristics of a biotechnological/biological product. Consequently, the manufacturer should propose a stability-indicating profile that provides assurance that changes in the identity, purity and potency of the product will be detected.

At the time of submission, applicants should have validated the methods that comprise the stability-indicating profile and the data should be available for review. The determination of which tests should be included will be product-specific. The items emphasised in the following subsections are not

intended to be all-inclusive, but represent product characteristics that should typically be documented to adequately demonstrate product stability.

### Protocol

The dossier accompanying the application for marketing authorisation should include a detailed protocol for the assessment of the stability of both drug substance and drug product in support of the proposed storage conditions and expiration dating periods. The protocol should include all necessary information which demonstrates the stability of the biotechnological/biological product throughout the proposed expiration dating period including, for example, well-defined specifications and test intervals. The statistical methods that should be used are described in the tripartite guideline on stability.

### Potency

When the intended use of a product is linked to a definable and measurable biological activity, testing for potency should be part of the stability studies. For the purpose of stability testing of the products described in this guideline, potency is the specific ability or capacity of a product to achieve its intended effect. It is based on the measurement of some attribute of the product and is determined by a suitable quantitative method. In general, potencies of biotechnological/biological products tested by different laboratories can be compared in a meaningful way only if expressed in relation to that of an appropriate reference material. For that purpose, a reference material calibrated directly or indirectly against the corresponding national or international reference material should be included in the assay.

Potency studies should be performed at appropriate intervals as defined in the stability protocol and the results should be reported in units of biological activity calibrated, whenever possible, against nationally or internationally recognised standard. Where no national or international reference standards exist, the assay results may be reported in in-house derived units using a characterised reference material.

In some biotechnological/biological products, potency is dependent upon the conjugation of the active ingredient(s) to a second moiety or binding to an adjuvant. Dissociation of the active ingredient(s)

from the carrier used in conjugates or adjuvants should be examined in real-time/real-temperature studies (including conditions encountered during shipment). The assessment of the stability of such products may be difficult since, in some cases, *in vitro* tests for biological activity and physicochemical characterisation are impractical or provide inaccurate results. Appropriate strategies (e.g., testing the product prior to conjugation/binding, assessing the release of the active compound from the second moiety, *in vivo* assays) or the use of an appropriate surrogate test should be considered to overcome the inadequacies of *in vitro* testing.

### Purity and Molecular Characterisation

For the purpose of stability testing of the products described in this guideline, purity is a relative term. Due to the effect of glycosylation, deamidation, or other heterogeneities, the absolute purity of a biotechnological/biological product is extremely difficult to determine. Thus, the purity of a biotechnological/biological product should be typically assessed by more than one method and the purity value derived is method-dependent. For the purpose of stability testing, tests for purity should focus on methods for determination of degradation products.

The degree of purity, as well as individual and total amounts of degradation products of the biotechnological/biological product entered into the stability studies, should be reported and documented whenever possible. Limits of acceptable degradation should be derived from the analytical profiles of batches of the drug substance and drug product used in the preclinical and clinical studies.

The use of relevant physicochemical, biochemical and immunochemical analytical methodologies should permit a comprehensive characterisation of the drug substance and/or drug product (e.g., molecular size, charge, hydrophobicity) and the accurate detection of degradation changes that may result from deamidation, oxidation, sulfoxidation, aggregation or fragmentation during storage. As examples, methods that may contribute to this include electrophoresis (SDS-PAGE, immunoelectrophoresis, Western blot, isoelectrofocusing), high-resolution chromatography (e.g., reversed-phase chromatography, gel filtration,

ion exchange, affinity chromatography), and peptide mapping.

Wherever significant qualitative or quantitative changes indicative of degradation product formation are detected during long-term, accelerated and/or stress stability studies, consideration should be given to potential hazards and to the need for characterisation and quantification of degradation products within the long-term stability program. Acceptable limits should be proposed and justified, taking into account the levels observed in material used in preclinical and clinical studies.

For substances that cannot be properly characterised or products for which an exact analysis of the purity cannot be determined through routine analytical methods, the applicant should propose and justify alternative testing procedures.

### Other Product Characteristics

The following product characteristics, though not specifically relating to biotechnological/biological products, should be monitored and reported for the drug product in its final container: Visual appearance of the product (colour and opacity for solutions/suspensions; colour, texture and dissolution time for powders), visible particulates in solutions or after the reconstitution of powders or lyophilised cakes, pH, and moisture level of powders and lyophilised products.

Sterility testing or alternatives (e.g., container/closure integrity testing) should be performed at a minimum initially and at the end of the proposed shelf-life.

Additives (e.g., stabilisers, preservatives) or excipients may degrade during the dating period of the drug product. If there is any indication during preliminary stability studies that reaction or degradation of such materials adversely affect the quality of the drug product, these items may need to be monitored during the stability program.

The container/closure has the potential to adversely affect the product and should be carefully evaluated (see below).

## STORAGE CONDITIONS

### Temperature

Since most finished biotechnological/biological products need precisely defined storage temperatures, the storage conditions for the real-

time/real-temperature stability studies may be confined to the proposed storage temperature.

#### **Humidity**

Biotechnological/biological products are generally distributed in containers protecting them against humidity. Therefore, where it can be demonstrated that the proposed containers (and conditions of storage) afford sufficient protection against high and low humidity, stability tests at different relative humidities can usually be omitted. Where humidity-protecting containers are not used, appropriate stability data should be provided.

#### **Accelerated and Stress Conditions**

As previously noted, the expiration dating should be based on real-time/real-temperature data. However, it is strongly suggested that studies be conducted on the drug substance and drug product under accelerated and stress conditions. Studies under accelerated conditions may provide useful support data for establishing the expiration date, provide product stability information for future product development (e.g., preliminary assessment of proposed manufacturing changes such as change in formulation, scale-up), assist in validation of analytical methods for the stability program, or generate information which may help elucidate the degradation profile of the drug substance or drug product. Studies under stress conditions may be useful in determining whether accidental exposures to conditions other than those proposed (e.g., during transportation) are deleterious to the product and also for evaluating which specific test parameters may be the best indicators of product stability. Studies of the exposure of the drug substance or drug product to extreme conditions may help to reveal patterns of degradation; if so, such changes should be monitored under proposed storage conditions. While the tripartite guideline on stability describes the conditions of the accelerated and stress study, the applicant should note that those conditions may not be appropriate for biotechnological/biological products. Conditions should be carefully selected on a case-by-case basis.

#### **Light**

Applicants should consult the appropriate regulatory authorities on a case-by-case basis to determine guidance for testing.

#### **Container/Closure**

Changes in the nature of the item might happen because of the associations between the planned biotechnological/organic item and holder / conclusion. Where the absence of communications can't be prohibited in fluid items (other than fixed ampoules), strength studies ought to incorporate specimens kept up in the upset or level position (i.e., in contact with the conclusion), and in the upright position, to decide the impacts of the conclusion on item quality. Information ought to be supplied for all distinctive holder/conclusion mixes that will be promoted.

In addition to the standard data necessary for a conventional single-use vial, the applicant should demonstrate that the closure used with a multiple-dose vial is capable of withstanding the conditions of repeated insertions and withdrawals so that the product retains its full potency, purity, and quality for the maximum period specified in the instructions-for-use on containers, packages, and/or package inserts. Such labelling should be in accordance with relevant national/regional requirements<sup>[1, 2]</sup>.

#### **Stability after Reconstitution of Freeze-Dried Product**

The stability of freeze-dried products after their reconstitution should be demonstrated for the conditions and the maximum storage period specified on containers, packages, and/or package inserts. Such labelling should be in accordance with relevant national/regional requirements.

#### **TESTING FREQUENCY**

The shelf-lives of biotechnological/biological products may vary from days to several years. Thus, it is difficult to draft uniform guidelines regarding the stability study duration and testing frequency that would be applicable to all types of biotechnological/biological products. With only a few exceptions, however, the shelf-lives for existing products and potential future products will be within the range of 0.5 to 5 years. Therefore, the guidance is based upon expected shelf-lives in that range. This takes into account the fact that degradation of biotechnological/biological products may not be governed by the same factors during different intervals of a long storage period.<sup>[1, 2]</sup>

When shelf-lives of 1 year or less are proposed, the real-time stability studies should be conducted monthly for the first 3 months and at 3 month intervals thereafter.

For products with proposed shelf-lives of greater than 1 year, the studies should be conducted every 3 months during the first year of storage, every 6 months during the second year, and annually thereafter.

While the testing intervals listed above may be appropriate in the pre-approval or pre-licence stage, reduced testing may be appropriate after approval or licensure where data are available that demonstrate adequate stability. Where data exist that indicate the stability of a product is not compromised, the applicant is encouraged to submit a protocol which supports elimination of specific test intervals (e.g., 9 month testing) for post-approval/post-licensure, long-term studies.<sup>[1, 2]</sup>

### SPECIFICATIONS

Although biotechnological/biological products may be subject to significant losses of activity, physicochemical changes, or degradation during storage, international and national regulations have provided little guidance with respect to distinct release and end of shelf-life specifications.<sup>[1]</sup>

Proposals for greatest adequate misfortunes of action, points of confinement for physicochemical changes, or debasement amid the proposed timeframe of realistic usability have not been created for individual sorts or gatherings of biotechnological/natural items however are considered on a case-by-case premise. Every item ought to hold its details inside set up breaking points for wellbeing, immaculateness, and power all through its proposed time span of usability. These determinations and cutoff points ought to be gotten from all accessible data utilizing the fitting measurable routines. The utilization of distinctive

determinations for discharge and close ought to be upheld by adequate information to exhibit that clinical execution is not influenced a Proposals for greatest adequate misfortunes of action, points of confinement for physicochemical changes, or debasement amid the proposed timeframe of realistic usability have not been created for individual sorts or gatherings of biotechnological/natural items however are considered on a case-by-case premise. Every item ought to hold its details inside set up breaking points for wellbeing, immaculateness, and power all through its proposed time span of usability. These determinations and cutoff points ought to be gotten from all accessible data utilizing the fitting measurable routines. The utilization of distinctive determinations for discharge and close s talked about in the tripartite rule on dependability.<sup>[2]</sup>

### LABELLING

For most biotechnological substances and drug items, absolutely characterized capacity temperatures are suggested. Particular proposals ought to be expressed, especially for medication substances and drug items that can't endure solidifying. These conditions, and where proper, suggestions for security against light and/or stickiness, ought to show up on compartments, bundles, and/or bundle embeds. Such naming ought to be as per important national/local prerequisites.<sup>[1]</sup>

### CONCLUSION

Since these products are quiet delicate as these are proteneous in nature requires special stability testing attributes. The dynamic nature of molecule has making it prone to be altered by diverse environmental factors. Due to above mentioned credential its stability testing is quiet important regarding its storage and self life.

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