

QUALITY OF BIOTECHNOLOGICAL PRODUCTS: STABILITY TESTING OF BIOTECHNOLOGICAL/ BIOLOGICAL PRODUCTS *)

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Additional Notes	This note for guidance concerns the application of Part 2, sections C and F of the Annex to Directive 75/318/EEC as amended, with a view to the granting of a marketing authorisation for a new medicinal product of biological or biotechnological origin. It is an annex to the <i>Stability Testing of New Active Substances and Medicinal Products</i> guideline which should be consulted for basic principles.

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1 PREAMBLE

The guidance stated in the ICH Harmonised Tripartite Guideline *Stability Testing of New Drug Substances and Products*, hereafter called Tripartite Guideline on Stability, (published in this volume under the title *Stability Testing of New Active Substances and Medicinal Products*) applies in general to biotechnological/biological products. 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 physico-chemical, 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 an active substance or medicinal product should always 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.

2. SCOPE OF THE ANNEX

The guidance stated in this annex applies to well-characterised proteins and polypeptides, their derivatives and products of which they are components, and which are isolated from tissues, body fluids, cell cultures, or produced using rDNA technology. Thus, the document covers the generation and submission of stability data for products such as cytokines (interferons, interleukins, colony-stimulating factors, tumour necrosis factors), erythropoietins, plasminogen activators, blood plasma factors, growth hormones and growth

factors, insulins, monoclonal antibodies, and vaccines consisting of well-characterised proteins or polypeptides. In addition, the guidance outlined in the following sections may apply to other types of products, such as conventional vaccines, after consultation with the appropriate regulatory authorities. The document does not cover antibiotics, allergenic extracts, heparins, vitamins or whole blood.

3. TERMINOLOGY

For the basic terms used in this annex the reader is referred to the “Glossary” in the Tripartite Guideline on Stability. However, since manufacturers of biotechnological/biological products sometimes use traditional terminology, traditional terms are specified in parentheses to assist the reader. A supplemental glossary is also included that explains certain terms used in the production of biotechnological/biological products.

4. SELECTION OF BATCHES

4.1 Active 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 three batches for which manufacture and storage are representative of the manufacturing scale of production. A minimum of six months stability data at the time of submission should be submitted in cases where storage periods greater than six months are requested. For active substances with storage periods of less than six 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 active 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 three manufacturing scale batches into the long-term stability program after approval.

The quality of the batches of active substance placed into the stability program should be representative of the quality of the material used in pre-clinical and clinical studies and of the quality of the material to be made at manufacturing scale. In addition, the active 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 active 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 active 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.

4.2 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.

4.3 Medicinal product (Final Container Product)

Stability information should be provided on at least three 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 six months data at the time of submission should be submitted in cases where storage periods greater than six months are requested. For medicinal products with storage periods of less than six 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 medicinal product may be provided at the time the dossier is submitted to the regulatory agencies with a commitment to place the first three 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 pre-clinical and clinical studies, the applicant should notify the appropriate regulatory authorities to determine a suitable course of action.

4.4 Sample selection criteria

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 medicinal 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 three 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.

5. STABILITY-INDICATING PROFILE

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.

5.1 Protocol

The dossier accompanying the application for marketing authorisation should include a detailed protocol for the assessment of the stability of both active substance and medicinal 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.

5.2 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 standards exists, 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 substance(s) to a second moiety or binding to an adjuvant. Dissociation of the active substance(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 physico-chemical 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.

5.3 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 active substance and medicinal product used in the pre-clinical and clinical studies.

The use of relevant physico-chemical, biochemical and immunochemical analytical methodologies should permit a comprehensive characterisation of the active substance and/or medicinal product (e.g. molecular size, charge, hydrophobicity) and the accurate detection of degradation changes that may result from deamidation, oxidation, sulphoxidation, 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 pre-clinical and clinical studies.

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

5.4 Other Product Characteristics

The following product characteristics, though not specifically relating to biotechnological/biological products, should be monitored and reported for the medicinal 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 medicinal product. If there is any indication during preliminary stability studies that reaction or degradation of such materials adversely affect the quality of

the medicinal 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).

6. STORAGE CONDITIONS

6.1 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.

6.2 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.

6.3 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 active substance and medicinal 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 active substance or medicinal 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 active substance or medicinal 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.

6.4 Light

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

6.5 Container/Closure

Changes in the quality of the product may occur due to the interactions between the formulated biotechnological/biological product and container/closure. Where the lack of interactions cannot be excluded in liquid products (other than sealed ampoules), stability studies should include samples maintained in the inverted or horizontal position (i.e., in contact with the closure), as well as in the upright position, to determine the effects of the closure on product quality. Data should be supplied for all different container/closure combinations that will be marketed.

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.

6.6 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.

7. 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 five 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.

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

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

While the testing intervals listed above may be appropriate in the pre-approval or pre-license 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. nine-month testing) for post-approval/post-licensure, long-term studies.

8. SPECIFICATIONS

Although biotechnological/biological products may be subject to significant losses of activity, physico-chemical changes, or degradation during storage, international and national regulations have provided little guidance with respect to distinct release and end of shelf life specifications. Recommendations for maximum acceptable losses of activity, limits for physico-chemical changes, or degradation during the proposed shelf life have not been developed for individual types or groups of biotechnological/biological products but are considered on a case by case basis. Each product should retain its specifications within established limits for safety, purity, and potency throughout its proposed shelf life. These specifications and limits should be derived from all available information using the appropriate statistical methods. The use of different specifications for release and expiration should be supported by sufficient data to demonstrate that clinical performance is not affected as discussed in the Tripartite Guideline on Stability.

9. LABELLING

For most biotechnological/biological substances and products, precisely defined storage temperatures are recommended. Specific recommendations should be stated, particularly for active substances and medicinal products that cannot tolerate freezing. These conditions, and where appropriate, recommendations for protection against light and/or humidity, should appear on containers, packages, and/or package inserts. Such labelling should be in accordance with relevant national regional requirements.

GLOSSARY

Conjugated Product

A conjugated product is made up of an active substance (for example, peptide, carbohydrate) bound covalently or noncovalently to a carrier (for example, protein, peptide, inorganic mineral) with the objective of improving the efficacy or stability of the product.

Degradation Product

A molecule resulting from a change in the active substance (bulk material) brought about over time. For the purpose of stability testing of the products described in this guideline, such changes could occur as a result of processing or storage (e.g. by deamidation, oxidation, aggregation, proteolysis). For biotechnological/biological products some degradation products may be active.

Impurity

Any component of the active substance (bulk material) or medicinal product (final container product) which is not the chemical entity defined as the active substance, an excipient, or other additives to the medicinal product.

Intermediate

For biotechnological/biological products, a material produced during a manufacturing process which is not the active substance or the medicinal product but whose manufacture is critical to the successful production of the active substance or the medicinal product. Generally, an intermediate will be quantifiable and specifications will be established to determine the successful completion of the manufacturing step prior to continuation of the manufacturing process. This includes material which may undergo further molecular modification or be held for an extended period of time prior to further processing.

Manufacturing-Scale Production

Manufacture at the scale typically encountered in a facility intended for product production for marketing.

Pilot-Plant Scale

The production of the active substance or medicinal product by a procedure fully representative of and simulating that to be applied at manufacturing scale. The methods of cell expansion, harvest, and product purification should be identical except for the scale of production.