



Australian Government
Department of Health
Therapeutic Goods Administration

Evaluation of biosimilars

Version 1.0, July 2013

TGA Health Safety
Regulation

Historical document

Guideline under review

20 April 2015

The understanding of biosimilar medicines is evolving and as a result the current guideline 'Evaluation of Biosimilars' may need to be updated.

Therefore, in addition to TGA undertaking a review of the policy about the naming convention for biosimilars (see below), the TGA is currently reviewing the rest of the TGA guideline on the Evaluation of biosimilars.

If you want further information or are intending to submit an application to register a biosimilar medicine, you should seek advice early about the evaluation of biosimilars by emailing your query to streamlinedsubmission@tga.gov.au.

Naming conventions for biosimilars

21 January 2015

Following recent international developments in the area of biosimilar naming the TGA will not be continuing with the previously proposed naming convention for biosimilars while a review of the policy is undertaken.

In July 2013 the TGA published guidance on biosimilar naming based upon the combination of a WHO *Programme on International Nonproprietary Names (INN)* issued biosimilar identifier with the Australian biological name (ABN). In July 2014 the WHO - INN published a draft policy '*Biological Qualifier - An INN Proposal*'. This proposal has superseded the previous INN position on which the TGA policy was based. This means the TGA biosimilar naming convention described below cannot be implemented and the TGA is undertaking a review of the policy.

In the interim biosimilars will use the Australian biological name without a specific biosimilar identifier suffix, for example a biosimilar to the reference product Neupogen filgrastim would be named 'TRADENAME' filgrastim.

Check the TGA website for up-to-date guidance

The most up-to-date information about prescription medicine registration in Australia is on the [TGA website](http://www.tga.gov.au) <<http://www.tga.gov.au>>. Now that guidance is presented in a series of web pages, updates are likely to be more common than in the past. If you subscribe to the TGA guidelines email alert service, you will be emailed every time the TGA web guidance is updated.

TGA web pages are dated, and can be printed.

A PDF format is being provided during the transition between the former version of the ARGPM (Australian Regulatory Guidelines for Prescription Medicines) and the new web format. Please note that information in the PDF should not be relied upon to be up-to-date.

About the Therapeutic Goods Administration (TGA)

- The Therapeutic Goods Administration (TGA) is part of the Australian Government Department of Health, and is responsible for regulating medicines and medical devices.
- The TGA administers the *Therapeutic Goods Act 1989* (the Act), applying a risk management approach designed to ensure therapeutic goods supplied in Australia meet acceptable standards of quality, safety and efficacy (performance), when necessary.
- The work of the TGA is based on applying scientific and clinical expertise to decision-making, to ensure that the benefits to consumers outweigh any risks associated with the use of medicines and medical devices.
- The TGA relies on the public, healthcare professionals and industry to report problems with medicines or medical devices. TGA investigates reports received by it to determine any necessary regulatory action.
- To report a problem with a medicine or medical device, please see the information on the TGA website <<http://www.tga.gov.au>>.

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Version history

Version	Description of change	Author	Effective date
V1.0	Original publication	Office of Medicines Authorisation	1/07/2013

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Historical document

Introduction

'Evaluation of biosimilars' is an initial guideline on the regulation of biosimilar products. As the TGA's understanding of these products is still evolving, this document will be updated from time to time. If you would like to provide feedback to the TGA on this document please contact info@tga.gov.au.

The purpose of the guidance is to:

- assist sponsors to identify the data necessary to support applications for the registration of biosimilars
- clarify the scientific and regulatory principles used by the TGA to evaluate those applications.

This guidance refers solely to the evaluation of biosimilars.

Most biosimilars are likely to contain biotechnology-derived proteins as the active substance(s), but this guidance also applies to other biosimilars such as those consisting of:

- vaccines and monoclonal antibodies
- polysaccharides, such as low molecular weight heparins.

What is a biosimilar?

A biosimilar or similar biological medicinal product (SBMP) is a version of an already registered biological medicine that:

- has a demonstrable similarity in physicochemical, biological and immunological characteristics, efficacy and safety, based on comprehensive comparability studies
- has been evaluated by the TGA according to this guideline and other relevant EU guidelines adopted by the TGA.

Although referred to as biosimilars in Australia, the term 'similar biological medicinal products' (SBMPs) is derived from the EU guidelines adopted by the TGA. The terms may be used interchangeably. In other jurisdictions, they also are variously referred to as:

- similar biotherapeutic products (WHO)
- follow-on biologics
- subsequent entry biologics.

A biosimilar is not a generic biological medicine

By their nature biotechnological products are not composed of a single, pure substance, but are invariably complex, microheterogeneous mixtures of isoforms of the desired substance.

While biosimilars have some conceptual parallels with generic versions of medicines containing chemically-derived small molecules as the active substances, this complexity and microheterogeneity mean that the principles relevant to the evaluation and use of generic medicines cannot be simply extrapolated to biosimilars.

Legislative provisions for the evaluation of biosimilars

As with New Chemical Entities (NCEs) or New Biological Entities (NBEs), the legislative basis for the evaluation and registration of biosimilars is Section 25 of the *Therapeutic Goods Act (1989)*, and with reference to *Therapeutic Goods Regulations (1990)* Subregulations 16C and 16D.

Related Therapeutic Goods Orders:

- [TGO No. 50 - General Standard for Pyrogen and Endotoxin Content of Therapeutic Goods:](#)
- [TGO No. 69 - General requirements for labels for medicines](#)
- [TGO No. 77 - Microbiological Standards for Medicines.](#)

The evaluation of biosimilars

General process and data requirements

Applications to register biosimilars are:

- managed through the Prescription Medicines Registration Process
- to be submitted in Common Technical Document (CTD) format.

Related information and guidance

- [Pre-Submission Planning Form \(PPF\)](#)
- [Information for sponsors completing the PPF](#)
- [Mandatory requirements for an effective application](#)
- [General submission dossier requirements](#)
- [CTD module 1](#)
- [CTD modules 2,3, 4 and 5](#)
- [Risk management plan guideline](#)

Related European Guidelines

The TGA has adopted a number of EU guidelines outlining data requirements specific to biosimilars as well as an ICH guideline on the assessment of comparability:

- [CHMP/437/04: Guideline on similar biological medicinal products](#)
- [EMA/CHMP/BWP/49348/2005: Guideline on similar biological medicinal products containing Biotechnology-Derived Proteins as Active Substance: Quality Issues](#)
- [CPMP/ICH/5721/03 ICH Topic Q5E: Comparability of Biotechnological/Biological Products Note for Guidance on Biotechnological/Biological Products Subject to Changes in their Manufacturing Process](#)
- [EMA/CHMP/BWP/42832/2005: Guideline on similar biological medicinal products containing Biotechnology-Derived Proteins as Active Substances: Non-Clinical and Clinical Issues](#)
- [CPMP/BWP/101695/2006: Guideline on Comparability of Biotechnology-Derived Medicinal Products after a change in the Manufacturing Process - Non-Clinical and Clinical Issues](#)
- [EMA/CHMP/BWP/14327/2006: Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins](#)
- [Product-specific guidelines detailing the clinical and safety data requirements.](#)

What to include in the application to register a biosimilar

- Provide the results of the studies outlined by the relevant guidelines above.

Provide :

- chemistry, manufacturing and quality control data (Module 3)
- preclinical data (Module 4)
- clinical data (Module 5)
- a Risk Management Plan.

Include the details of these in a [PPF](#), and ensure that the SBMP box on the PPF is ticked.

Pre-submission meeting

If desired, it is possible to arrange a pre-submission meeting to discuss proposed biosimilar application with the relevant clinical evaluation unit of OMA prior to lodging the application.

The evaluation process for biosimilars

Once the PPF is accepted by the TGA, milestones are set by the TGA for the submission, evaluation, feedback and decision.

Evaluation of the CTD Modules

[Modules 1-5](#) are evaluated simultaneously.

If reduced Module 4 and 5 datasets are submitted evaluation will commence on the assumption that the Module 3 data will demonstrate sufficient comparability of the proposed biosimilar to the reference product.

If the Module 3 evaluation fails to demonstrate that the proposed biosimilar is sufficiently comparable to the reference product, the application may be:

- withdrawn, and
- resubmitted as a New Biological Entity (NBE) with full clinical and pre-clinical datasets.

If it is not withdrawn it is probable that the application will be rejected.



Note

Application and [evaluation fees](#) for the biosimilar will not be refunded if the application is withdrawn after completion of the first round evaluation.

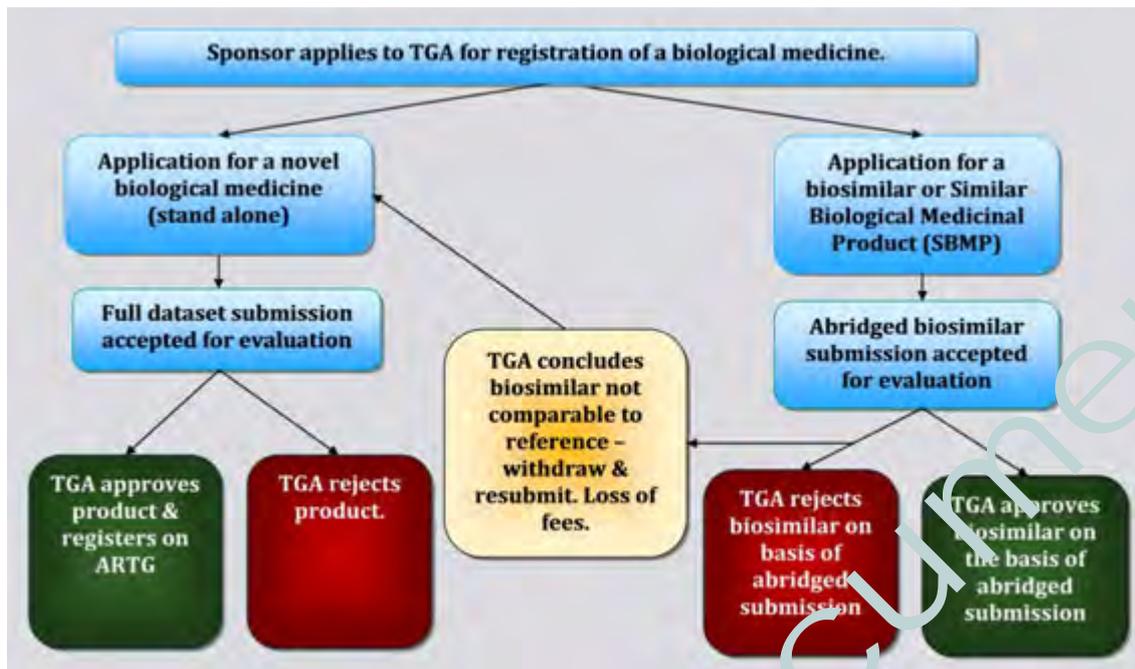
Advisory committee advice about biosimilars

During the evaluation of a biosimilar application, the TGA may refer the application to the following advisory committees for advice:

- the Advisory Committee on Prescription Medicines (ACPM)
- the Pharmaceutical Subcommittee (PSC)

- the Advisory Committee on the Safety of Medicines (ACSOM).

Flowchart outlining possible outcomes of a biological medicine submission



Possible outcomes of a submission to register a biological medicine

This is a text only description of the flowchart image above.

When the sponsor applies for registration, if they elect to not use the biosimilar process, it is processed as a novel biological medicine or stand-alone. A full data set is submitted and evaluated and on the basis of that evaluation, the biological medicine is either approved or rejected.

If the sponsor elects to use the biosimilar process, an abridged submission with a comparability study is made and evaluated. The product may be approved or rejected as a biosimilar on the basis of the evaluation of the abridged data set.

If the comparability study is evaluated to show the biosimilar is not sufficiently comparable to the reference product, the sponsor may elect to withdraw the submission, lose the submission and evaluation fees and resubmit with a full data set as a novel biological medicine.

Reference products for biosimilars

Ensure the same reference product is used for both drug substance and drug product and in all three (Quality, Safety and Efficacy - Modules 3-5) parts of the submission.

Ensure the reference product is clearly identified by:

- brand name
- pharmaceutical form
- formulation

- strength
- origin or place of purchase
- batch numbers and dates of expiry.

Ensure the biosimilar has the same formulation, strength and dosage form as the reference product, or include a scientific justification for any differences.

Use state-of-the-art analytical methods to directly compare the reference product to the biosimilar.

Notes about the reference product

Ensure the reference product:

- is not an international standard
- is registered in Australia
- is not itself an biosimilar, but is a biological medicine registered by means of a full data submission in all Modules
- has been marketed for a suitable duration and have a volume of marketed use so that there is likely to be a substantial body of acceptable data regarding the safety and efficacy.

For reference products that are registered in Australia but manufactured overseas

A reference product manufactured and sourced overseas may be used, provided:

- the product is registered in Australia
- a bridging comparability study between the Australian-sourced product and all batches of the reference product is provided.



Note

The bridging comparability study may be abbreviated if evidence is provided that the product marketed in Australia is sourced from the same manufacturing facility as that used for the reference product.

Related information and guidance

- [EMA/CHMP/BWP/49348/2005 Guideline on similar biological medicinal products Containing Biotechnology-Derived Proteins as Active Substances: Non-Clinical and Clinical Issues](#)
- [WHO/BS/09.2110 Guidelines on Evaluation of Similar Biotherapeutic Products \(SBPs\).](#)

In-house primary reference standard for biosimilars

Provide evidence to demonstrate the biosimilar manufacturer has established an in-house primary reference standard comparable to the reference preparation in the comparability study.

Following any subsequent significant manufacturing process changes, the sponsor must provide evidence that the post variation product is comparable to both the in-house primary reference standard and the pre-variation product.

All comparability studies done to show similarity between the biosimilar and the reference product should be in accordance with the following three guidelines:

- [EMA/CHMP/BWP/49348/2005 Guideline on Similar Biological Medicinal Products containing Biotechnology-Derived Proteins as Active Substance: Quality Issues](#)
- [CPMP/ICH/5721/03 ICH Topic Q 5 E: Comparability of Biotechnological/Biological Products Note for Guidance on Biotechnological/Biological Products Subject to Changes in their Manufacturing Process](#)
- [CHMP/BMWP/101695/2006: Guideline on Comparability of Biotechnology-Derived Medicinal Products after a change in the Manufacturing Process - Non-Clinical and Clinical Issues.](#)

The comparability study should directly compare the reference standard and the biosimilar, preferably drug substance manufactured by a single process used to make the biosimilar product for both clinical trials and commercial distribution.

It is undesirable to change the manufacturing process during later pharmaceutical development. However, if the biosimilar manufacturing process changes significantly between clinical trial and commercial stages:

- include the reference product in the second comparability study (according to ICH Q5E) to show the clinical trial and commercial products are similar
- clearly identify such second comparability studies in the application data submitted.

The use of more than two comparability studies is not acceptable as it is not possible to make robust comparison between the reference product and batches of biosimilar material made using different or evolving processes.

Where direct comparison is not possible (e.g. if the concentration of the active substance in the reference product is too low or there are interfering excipients such as human serum albumin) then extraction or concentration techniques may be used, but these must be:

- declared in the dossier
- described in full
- validated for use.

Analytical techniques used in comparability studies

The analytical techniques should be:

- carefully selected and optimised to maximise the potential for detecting relevant differences in quality attributes
- sufficiently broad in scope to capture the full range of quality attributes. Batch release testing or testing specified in a relevant monograph is insufficient, hence additional characterisation tests should also be utilised
- intensive enough to fully investigate each physicochemical property or biological activity. Apply more than one analytical procedure to evaluate the same quality attribute (e.g. molecular weight, impurities, secondary/tertiary structures). Each method should employ different physicochemical or biological principles (i.e. orthogonal approaches) to collect data for the same parameter to maximise the likelihood of detecting any differences between the biosimilar and the reference substance.

A list of suggested techniques which may be used in comparability studies is provided in [Appendix 1](#).

Extrapolation of indications

As stated in the adopted EU guideline: [EMA/CHMP/BMWP/42232/2005: Guideline on similar biological medicinal products Containing Biotechnology Derived Proteins as Active Substances: Non-Clinical and Clinical Issues](#)

"In case the originally authorised medicinal product has more than one indication, the efficacy and safety of the medicinal product claimed to be similar has to be justified or, if necessary, demonstrated separately for each of the claimed indications. In certain cases, it may be possible to extrapolate therapeutic similarity shown in one indication to other indications of the reference medicinal product. Justification will depend on e.g. clinical experience, available literature data, whether or not the same mechanisms of action or the same receptor(s) are involved in all indications. Possible safety issues in different subpopulations should also be addressed."

Post registration regulation of biosimilars

As for all newly-registered biological medicines, biosimilars are placed on batch release as a condition of registration. This usually entails:

- the submission of release certification and shipping records for all batches
- the submission of samples for all batches having unique drug substances which may be tested by the TGA for compliance
- the continuance of these conditions until satisfactory batch consistency has been demonstrated (usually at least five batches of unique drug substance).

After the initial batch release phase is completed, the sponsor of the biosimilar is required to submit an annual report of all batches and to provide samples to the TGA for testing in periodic product surveys.

Variation of the conditions of registration can occur under section 9D using the normal Category 3 and self-assessable request processes. If there is a significant change in manufacturing process, a comparability study between the in-house reference standard as well as the pre- and post-change product will be required.

Pharmacovigilance of biosimilars

As clinical trial data are usually insufficient to identify rare adverse effects, the general pharmacovigilance requirements applied to biosimilars are the same as those for any biological medicine. The sponsor must develop a comprehensive Risk Management Plan outlining the pharmacovigilance procedures to be implemented as detailed in the Australian and adopted EU guidelines:

- [Risk Management Plans for prescription medicines](#)
- [Australian requirements and recommendations for pharmacovigilance responsibilities of sponsors of medicines](#)
- [EMA/CHMP/BMWP/42832/2005 Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues](#)

This Risk Management Plan is to be submitted with the biosimilar application.

In brief, the mandatory post-registration requirements for pharmacovigilance are that sponsors must:

- notify the TGA of the person responsible for fulfilling the sponsor obligations
- submit Periodic Safety Update Reports (PSURs) and Adverse Events (AR) reports to the TGA
- notify the TGA when they become aware of any significant safety issues
- ensure that any request from the TGA for the provision of additional information is answered fully and within the requested time frame.

In addition, any provisions included in the Risk Management Plan that have been imposed as conditions of registration should be fulfilled.

Because all biotechnology-derived products are inherently variable, the established safety record of the reference product does not necessarily apply to the biosimilar. For this reason, it is possible that after assessment of the Risk Management Plan, active pharmacovigilance measures may be applied on a case by case basis.

In submitting adverse event reports, sponsors, patients and health professionals should ensure they clearly identify the product suspected to have caused the adverse event. They should enter:

- the trade name
- the entire non-proprietary name (including any biosimilar identifier) N.B. both the trade name and the non-proprietary name should be given wherever possible
- the AuscR number
- the batch number and expiry date
- the dosage form and presentation.

Naming conventions for biosimilars

Australian Biological Names (ABN)

Under the *Therapeutic Goods Regulations (1990)* Schedules 12 & 13, all medicines are required to use Australian Approved Names (AAN) in both:

- Product Information (PI)
- Consumer Medicine Information (CMI).

In the case of a biosimilar, this will be drawn from the Biologicals List or Australian Biological Names (ABN).

Therapeutic Goods Order No. 69 *General requirements for labels for medicines (2001)*, Section 3(10) mandates the use of the AAN/ABN for all active ingredients and excipients on labels.

A biosimilar is not identical to its reference product and must be assumed to be different to any other biosimilar as no direct comparability study has been conducted. As small differences between biosimilars can give rise to differences in clinical behaviour, in particular in immunogenic effects, certain additional nomenclature provisions are necessary to ensure that it is possible to distinguish between biosimilars and clearly identify the reference product.

The TGA therefore requires that the ABN for a biosimilar be composed of:

- the reference product ABN, thus identifying the reference product with which the biosimilar has demonstrable comparability
- a biosimilar identifier, consisting of: the prefix *sim(a)-* and a three letter code issued by the WHO International Non-proprietary Name (INN) Committee, according to its draft policy.

The object of this naming policy is to allow prescribers to identify the reference product and to distinguish clearly between biosimilars.

These distinctions are also important for pharmacovigilance purposes.

Nomenclature example

The fictitious company *gPharm* registers a biosimilar for the monoclonal antibody *infliximab*.

The product uses the ABN **infliximab simfam**. This is used in full in all labelling and in each reference to the product in the PI and CMI.

Although the biosimilar ABN will be partly based on the approved ABN used by the reference product, sponsors will need to apply:

- to the WHO INN Committee for a biosimilar three letter code
- to the ABN Committee for the use of the INN three letter code in the biosimilar identifier
- for this identifier to then be added to the list of [Australian Approved Names](#).

**Note**

An application for the use of a specific biosimilar identifier may be rejected if:

- it does not comply with WHO INN naming policy
- it could otherwise be confused with an existing non-proprietary name or trademark.

Status of the biosimilar identifier

Addition of a biosimilar identifier to the AAN list by the ABN Committee does not in any way imply endorsement or acceptance by the TGA of the substance as a biosimilar, but only that the sponsor's application for the use of a biosimilar identifier has been approved.

The assessment of biosimilarity or comparability is made by evaluators on the basis of a complete dataset, not by the ABN Committee.

If the TGA determines that the substance is not biosimilar:

- do not use the ABN of the reference product
- do not use the biosimilar identifier
- apply for a new and unique ABN.

Tradenames

Biosimilars are required to have a clearly distinguishable tradename from all other products, especially the reference product and other biosimilars.

Basing the tradename on "the active ingredient name with the company identified" as indicated in [Best practice guideline on prescription medicine labelling](#) is not appropriate for biosimilars. This is because it may:

- give the impression that the biosimilar is a generic medicine
- lead to confusion at both prescribing and dispensing. (As noted by Dr Annemarie Hellebek in the *EMA Medication errors workshop report* (28 Feb-1Mar 2013) [EMA/144458/2013](#), "generic product names using common stems may... cause name confusion".)
- contribute to difficulties in traceability following adverse event reporting
- be considered an unacceptable presentation under section 25(1)e of the Act, as it has the potential to mislead.

For these reasons the use of the active ingredient ABN in the tradename of a biosimilar is not acceptable.

Related information and guidance

- [Therapeutic Goods Order No. 69 General requirements for labels for medicines \(2001\)](#)
- [Best practice guideline on prescription medicine labelling](#)

Labels, product information (PI) and consumer medicine information (CMI) for biosimilars

As for all NCE and NBE applications, biosimilar applications are required to comply with:

- [TGO No. 69 - General requirements for labels for medicines](#) for labelling
- the Product Information guideline and Schedule 13 of the Regulations for the PI.

The CMI must:

- be consistent with the PI
- contain the information required by Schedule 12 of the Regulations.

Relevant clinical trial information generated on the reference product and reported in the reference product PI may be incorporated into the PI for the biosimilar. However, these data should be clearly identified as having been produced using the reference product not the biosimilar.

As biosimilars are not generic versions of their reference products, to inform the prescriber the text of the PI should include words to the effect of:

"The comparability of **[biosimilar product name]** with **[Reference product name (AustR nnnnnn)]** has been demonstrated, with regard to particular physicochemical characteristics and efficacy and safety outcomes **[see PHARMACOLOGY and CLINICAL TRIALS]**. The level of comparability that has been shown supports the use of **[biosimilar product name]** for the listed indication[s]. The level of comparability that has been shown is not sufficient to designate this product as a generic version of **[Reference product name]**. Replacement of **[Reference product name]** with **[biosimilar product name]**, or vice versa, should take place only under the supervision of the prescribing medical practitioner."

as the first paragraph under **Precautions**.

In addition the approval letter is likely to include text similar to the following:

"The application for registration of **[biosimilar product name]** included data that established to the TGA's satisfaction that the product is a biosimilar to **[Reference product name (AustR nnnnnn)]**.

A biosimilar is a version of an already registered biological medicinal product with demonstrated similarity in physicochemical, biological and immunological characteristics, efficacy and safety, based on a comprehensive comparability analysis.

It is the TGA's view that it is not currently possible to determine a degree of similarity, between a biosimilar and an already registered biological medicine sufficient to support a designation by the TGA of 'bioequivalence'."

Appendix 1 - Suggested techniques for inclusion in comparability studies

This list is neither prescriptive nor exhaustive, but is an indication of the characteristics to be addressed and some suggestions on what techniques may be used.

- [Physicochemical Properties](#)
- [Biological activity](#)
- [Content, purity and impurity profile](#)
- [Glycosylation \(if applicable\)](#)
- [Immunochemical \(if applicable e.g. monoclonal antibodies\)](#)

Physicochemical Properties

- **Primary structure**
 - Edman degradation
 - peptide mapping with Liquid Chromatography with MS detection (LC-MS)
 - C-terminal sequencing
 - amino acid analysis.
- **Secondary structure**
 - Peptide mapping with reduced/non-reduced hydrolysis and Edman degradation or MS analysis to show disulphide bonding and other structural forms
 - near ultraviolet(UV) Circular Dichroism CD.
- **Tertiary and Quaternary structure**
 - far Ultraviolet (UV) Circular Dichroism (CD)
 - NMR
 - FTIR
 - X-Ray crystallography.
- **Molecular mass**
 - Mass spectrometry - Matrix Assisted Laser Desorption Ionisation (MALDI) and Electrospray Ionisation MS (ESI-MS)
 - ultracentrifugation
 - Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)
 - Size Exclusion High Performance Liquid Chromatography (SE-HPLC) (same as GF-HPLC)
 - laser light scattering.
- **Isoforms**

- isoelectric focussing
- capillary electrophoresis
- Ion Exchange High Performance Liquid Chromatography (IE-HPLC).
- **Crystal structure**
 - microscopy (where crystal structure is necessary for action e.g. protamine zinc insulin)
- **Sugar composition and linkage**
 - quantitative monosaccharide analysis (for polysaccharide biological medicines like heparin).

Biological activity

- *In vivo* activity - measuring therapeutic effect in animals
- *In vitro* activity - measuring therapeutic effect in cells, for instance:
 - cell proliferation or inhibition of proliferation
 - cell senescence
 - measurable changes in cell size or content (e.g. mRNA).
- Enzyme assays
- Receptor-binding assays
- Promotion or inhibition of coagulation by chromogenic or turbidometric techniques.

Content, purity and impurity profile

- **Protein content**
 - Protein assay (e.g. Beldam, Lowry, Bradford)
 - Absorbance at 280 or 230 nm
 - High Performance Liquid Chromatography (HPLC)
 - Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE).
- **Purity**
 - High Performance Liquid Chromatography (HPLC)
 - Reverse Phase High Performance Liquid Chromatography
 - Size Exclusion High Performance Liquid Chromatography (same as GF-HPLC)
 - Ion Exchange High Performance Liquid Chromatography HPLC (IE) or Hydrophobic Interaction HPLC (HI)
 - Capillary Electrophoresis (CE)
 - Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE).
- **Impurities - process-derived:**

- **DNA** - Threshold®, Real Time Polymerase Chain Reaction (RT-PCR)
- **Host protein** - Enzyme-linked Immunosorbent Assay (ELISA)
- **Cell culture components and/or antibiotics** - ELISA, antibiotic assays
- **Leachates** - High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC)
- **Protein A** - Enzyme-linked Immunosorbent Assay (ELISA).
- **Impurities - product-derived:**
 - **Aggregates, subunits, fragments, truncation** - Size Exclusion High Performance Liquid Chromatography (SE-HPLC) (same as GF-HPLC), Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)
 - **Oxidised** - Reverse Phase High Performance Liquid Chromatography (RP-HPLC), peptide mapping
 - **Deamidated** - Reverse Phase HPLC (RP-HPLC), test kit
 - **N-terminal cyclisation** - peptide mapping, Liquid Chromatography with MS detection (LC-MS), Edman degradation
 - **Phosphorylation, sulphation** - Isoelectric Focussing (IEF), Ion Exchange HPLC (IE-HPLC), peptide mapping.

Glycosylation (if applicable)

- **Glycosylation sites and site occupancy**
 - peptide mapping, LC-MS
- **Glycosylation content** (total and site specific)
 - peptide mapping/quantitative hydrolysis
 - Liquid Chromatography with MS detection (LC-MS).
- **Monosaccharide content**
 - quantitative hydrolysis with colorimetric reaction
- **Sialic acid content and type**
 - quantitative hydrolysis with colorimetric reaction/HPLC
- **Glycan profile** (total and site-specific)
 - High pH Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD)
 - Ion Exchange Liquid Chromatography (IE-HPLC) or Normal Phase High Performance Liquid Chromatography (NP-HPLC) for fluorophore-derivitised glycans,
 - Fluorophore-Assisted Carbohydrate Electrophoresis (FACE) (with identification of the individual peaks or bands).
- **Oligosaccharide linkages**

- enzyme digestion with profiling
- Mass Spectrometry carried out to n levels of disintegration (MSn).

Immunochemical (if applicable e.g. monoclonal antibodies)

- **Binding specificity**
 - ELISA
 - Surface Plasmon Resonance (SPR)
 - histochemical staining, immunoblotting
 - Western blotting.
- **Binding avidity**
 - SPR
 - binding assays
 - competitive ELISA.

Where delivery to specific intracellular sites of action are required for therapeutic effect, e.g. lysosomal enzyme replacement, the rate and proportion of the active ingredient in the site of action should be demonstrated and compared to the reference product.

Historical document

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