

Elaborating European Pharmacopoeia monographs for biotherapeutic proteins using substances from a single source

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ABSTRACT

For more than twenty years, the European Pharmacopoeia (Ph. Eur.) monographs for biotherapeutic proteins have been elaborated using the multisource approach (Procedure 1), which has led to robust quality standards for many of the first-generation biotherapeutics. In 2008, the Ph. Eur. opened up the way towards an alternative mechanism for the elaboration of monographs (Procedure 4-BIO pilot phase), which is applied to substances still under patent protection, based on a close collaboration with the Innovator company, to ensure a harmonised global standard and strengthen the quality of the upcoming products. This article describes the lessons learned during the P4-BIO pilot phase and addresses the current thinking on monograph elaboration in the field of biotherapeutics. Case studies are described to illustrate the standardisation challenges associated with the complexity of biotherapeutics and of analytical procedures, as well as the approaches that help ensure expectations are met when setting monograph specifications and allow for compatibility with the development of biosimilars. Emphasis is put on monograph flexibility, notably by including tests that measure process-dependent microheterogeneity (e.g. glycosylation) in the Production section of the monograph. The European Pharmacopoeia successfully concluded the pilot phase of the P4-BIO during its 156th session on 22-23 November 2016.

KEYWORDS

Biotherapeutics, complexity, public standards, flexibility, production, monograph specifications, reference standards.

1. INTRODUCTION

Since the approval of human insulin, derived from recombinant DNA technology in the early 1980s, numerous biotherapeutics have received regulatory approval in Europe.

Public pharmacopoeial standards (documentary and reference standards) for biotherapeutic proteins are necessary because they provide the means for an independent judgement as to the overall quality of a substance. Many first-generation biotherapeutics have come to the end of their patent protection and may face competition from biosimilar products. In light of the emergence of these biosimilar products it is even more important that public standards are in place at the time of their patent expiry. The European Directorate for the Quality of Medicines & HealthCare (EDQM) has been keen to assure that public standards which meet the needs of regulators, control laboratories and industry will be available to ensure a harmonised global standard and strengthen the quality of the upcoming biotherapeutics.

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2. HISTORY AND BACKGROUND

The governing body of the European Pharmacopoeia (Ph. Eur.), the Ph. Eur. Commission, launched a pilot phase in 2008 to ensure that there are suitable public standards in place for biotherapeutics as they come off-patent. The Ph. Eur. Commission opted to use a procedure (Procedure 4; P4), that had been successfully used for chemical entities, to elaborate monographs for which a single interested manufacturer has been identified and which is usually applied to substances still under patent protection where there is potential for future production of generics. P4 is based on close collaboration with the manufacturer responsible for innovating the substance/product. This is in contrast to the more commonly used Procedure 1 (P1) where multiple manufacturers of a substance have been identified to contribute to the elaboration of a monograph. It should be noted that whichever procedure is used, Ph. Eur. monographs take account of approved products on the European market and the principle basis for elaborating the monograph is the approved licensing specification(s) backed up by batch data.

Given regulator and industry interest in insulins and coagulation factors, this P4 pilot phase for biotherapeutics (P4-BIO) began with the elaboration of monographs for an insulin analogue (insulin glargine) and two recombinant human coagulation factors (factors VIIa and IX). In 2011, the P4-BIO pilot phase was enlarged with the aim of covering more diverse and complex classes of biotherapeutics licensed in Europe, such as hormones (e.g. parathyroid hormone), fusion proteins, pegylated products and hyperglycosylated proteins. This saw the addition of teriparatide, etanercept, pegfilgrastim and darbepoetin alfa to the Ph. Eur. monograph elaboration process.

To date, five Ph. Eur. drug substance monographs have been adopted by the Ph. Eur. Commission:

Monograph	Ph. Eur. edition	Implementation date
Insulin glargine (2571)	8	January 2014
Human coagulation factor VIIa (rDNA), concentrated solution (2534)	8	January 2014
Human coagulation factor IX (rDNA), concentrated solution (2522)	8.2	July 2014
Teriparatide (2829)	9	January 2017
Etanercept (2895)	9.3	January 2018

and one monograph for *Pegfilgrastim (2889)* was published in Pharmeuropa 28.2 (April 2016) for public comment. In addition, a quality framework for finished product monographs for biotherapeutics has been established by developing a new monograph for *Human coagulation factor IX (rDNA) powder for solution for injection (2994)*, which has been adopted by the Ph. Eur. Commission in November 2016.

3. COMPLEXITY OF BIOTHERAPEUTICS AND ITS INFLUENCE ON MONOGRAPH ELABORATION

Biotherapeutics are generally not composed of a single chemical entity (unlike the classical chemically-derived drugs). Instead, they may be composed of complex mixtures of closely related variants (post-translationally modified forms, e.g. with naturally occurring heterogeneity in glycosylation). Thus, biological manufacturing is more complex, and sophisticated tests and controls are required to demonstrate the identity, quality, potency and purity of the drug substance. All these necessitate a thorough understanding of the manufacturing process and product attributes, which are assessed through a combination of physico-chemical and biological testing. In addition, as a principle, biotherapeutics are defined by the way they are manufactured with changes in manufacturing processes leading to distinct quality attributes including changes in the purity/impurity profile. Common examples of this include differences in glycan structure, charge heterogeneity and chemical modification. The complexity and naturally occurring heterogeneity of the molecular structure, dependence on production in living cells and complicated manufacturing process, as well as the diversity of analytical methods make the public standard setting a demanding exercise, with a unique set of challenges.

4. LESSONS LEARNED AND CRITICAL POINTS TO BE CONSIDERED

4.1. Monograph flexibility

In establishing monographs for biotherapeutics the Ph. Eur. has recognised that because of their structural complexity, biotherapeutics require more flexible monographs compared to monographs on chemically-defined substances. A key challenge has been how to translate this flexibility into a public standard that provides comprehensive and sufficiently prescriptive quality requirements and allows for the development of follow-on versions.

4.2. Production section

One of the ways that flexibility has been achieved in the P4-BIO pilot phase is by defining the quality attributes linked to the process in the Production section of the monograph; a section which draws attention to particular aspects of the manufacturing process but is not necessarily comprehensive. Specifically when drafting the monographs for biotherapeutic products it was recognised that because protein glycosylation, which plays a critical role in protein structure/conformation and its effector function, is a source of heterogeneity its analysis cannot be included in the Tests section of the monograph as a direct transfer of the lot-release specifications of the innovator product. This is because glycosylation is manufacturing process-dependent and its analysis requires multiple purification procedures and the use of an in-house reference preparation which is only available to the manufacturer. Additionally, acceptance criteria in the form of numerical limits for specific glycan species may not be suitable for all registered products and therefore should not form part of the monograph specifications. By adopting this approach for biotherapeutic product monographs they are able to reflect a suitable set of expectations that could be universally applied, which makes the monographs compatible with the development of biosimilars.

4.3. Monograph vs release specifications

The basis for monograph elaboration is the data relating to the substance provided by the Innovator company, which consists of a set of tests and acceptance criteria carefully chosen based on the specific control strategy in place. However, the Innovator may have applied release specifications that may not be appropriate for a public standard, for example tests removed or replaced by other tests or tests being performed as in-process controls or on intermediates may lead to the set of specifications provided not covering all quality attributes. Where there are important quality attributes of the substance that need to be monitored during the course of product development and manufacturing (such as O-glycan occupancy as a measure of glycosylation consistency for etanercept or the need for quality assessment of the PEG reagent for pegfilgrastim) these have been included in the Production section of the monograph.

Additionally, setting monograph specifications for process-related impurities derived from chemical modification of the protein moiety in conjugated proteins presents challenges. Although specifications in the data provided by the Innovator reflect the understanding of the impurity risk and process capacity to produce a product with a safe impurity profile, these may not be sufficient or appropriate for a public standard.

The experience gained from elaborating the monographs has shown that a way to address this is to include (on a case-by-case basis) considerations/additional tests on quality attributes, which are not part of release specifications but need to be controlled during the manufacturing process or downstream purification.

4.4. Selection of tests for the monograph

The selection of tests to be included in the monograph specifications is product-specific and takes into account the set of quality attributes reflected in release specifications as well as the manufacturing process performance. In addition, sufficiently detailed data for structural elucidation and confirmation is used to help understand the protein structure and physico-chemical properties and define a relevant set of quality requirements for the monograph.

Although information on characterisation has been found useful for the method verification, it is not the intent of the monograph to provide characterisation tools and any additional (characterisation) information provided by the manufacturer is not included in the monograph. Nevertheless, on a case-by-case basis, physico-chemical characterisation tests may be part of monograph specifications.

4.4.1. *Characterisation tests adapted in the monograph*

One example is peptide mapping which is part of the extensive product characterisation to establish identity, but is not required of routine release testing. The use of a peptide map in a monograph does not require a complete characterisation of the individual peptide peaks nor 100 % protein sequence coverage. Given the complex nature of biotherapeutics and the complexity of peptide mapping analysis, the monograph prescribes a set of acceptance criteria based on a comparative procedure with a peptide map obtained with a suitable reference standard.

4.4.2. *Diversity of analytical methods*

For biotherapeutics, a significant number of physico-chemical and biological parameters have to be tested, and each of them requires advanced methods and equipment, whereas assay and impurities content for chemicals are generally assessed through one common HPLC method, and a determination of potency via a bioassay is not performed.

Analytical specifications for biotherapeutics comprise identification, assay and purity testing methods. For identification, methods used include peptide mapping, capillary or gel electrophoresis, charge heterogeneity, glycan analysis and in some cases potency determination.

The analytical methods used to determine the purity of the drug substance are quantitative or semi-quantitative methods, which may be similar to those used for identification. They typically include: quantitative determination of protein variants/related species (ion-exchange chromatography, RP-HPLC, hydrophobic interaction chromatography), of high molecular species (dimers and aggregates by size exclusion chromatography), of post-translational modifications, including gammacarboxylation for coagulation factors (LC) etc. and semi-quantitative determination of protein variants based on charge and size (electrophoretic methods). Additional tests applicable to biotherapeutics consist of microbiological tests (bacterial endotoxins test, microbiological contamination) and of procedures designed to minimise or eliminate agents of infection.

Finally an assay for protein content determination or potency determination is used. In some cases it is possible to employ the same procedure (e.g. HPLC) for both assay of the drug substance and quantitation of impurities.

4.4.3. *Method verification*

Although the analytical methods proposed for a monograph are validated and robust as per ICH Q2 (R1) *Validation of Analytical Procedures: Text and Methodology* (with validation data provided in the manufacturer's data package submitted for monograph elaboration), their robustness, transferability and suitability for a pharmacopoeial standard are confirmed experimentally. In addition, the information provided by the manufacturer on method robustness verification allows definition of a set of key parameters and anticipation of potential technical issues.

The diversity and complexity of analytical procedures to be included in a recombinant protein monograph requires extensive method verification and this generally involves two laboratories (including the EDQM Laboratory) as a minimum requirement. This work may lead to significant amendments to the manufacturer's methods and additional validation studies. Overall, the successful drafting of a monograph strongly relies on the extensive verification of the methods performed by individual Official Medicines Control Laboratories who participate in the P4-BIO pilot phase.

Bioassay. *In vitro* cell-based potency assays are largely used for biotherapeutics, and rely on the quantification of a measurable response to a biological preparation in a clonal cell line in order to determine potency relative to a standard preparation. These *in vitro* bioassays are considered to be amongst the most complex and problematic of all analytical methods, including having challenging method transfer. Due to the high complexity of bioassays, a high level of variability is reached in analyses (e.g. RSD up to 30-40 % compared to 1-2 % for chromatographic assays). This variability requires careful control of the critical parameters and assessment of sources of variations (e.g. dilutions, cells, etc.); therefore, further optimisation to achieve consistent performance may be needed before the inclusion of the bioassay in the monograph. Additionally, as a Ph. Eur. monograph cannot prescribe the use of reagents protected by trade mark or patent, non-proprietary bioassays need to be included in the monograph. This requires manufacturers to supply data on bioassays supported by assay inter-changeability and validation and in those cases where such data is not available, the development and validation of (new) alternative methods will be needed.

4.5. Reference standards

Ph. Eur. monographs for biotherapeutics invoke the use of reference standards, which are an integral part of the quality standard. Ph. Eur. chemical reference standards (CRSs) may need to be established to support physico-chemical analytical methods described in a monograph; they may serve qualitative (peak identification, impurity and system suitability) and quantitative (assay/protein content determination) purposes.

As regards determination of biological activity, a repertoire of WHO International Standards has been developed for the new generation biotherapeutics and may serve as a basis (case-by-case) for setting/calibration of Ph. Eur. method-specific biological reference preparations (BRPs). In some cases, WHO may be establishing the International Standard simultaneously with the monograph elaboration, which leads to joint efforts to assure compatibility of strategies between EDQM and WHO.

An initial reflection on a CRS/BRP strategy is made early and adapted at a later stage (i.e. method verification) based on the requirements/needs of the methods detailed in the monograph, availability of the substance, storage/dispatch conditions for the future CRS/BRP (e.g. freeze-dried or liquid preparation), and sustainability of CRS/BRP supply throughout the life of the monograph.

5. CONCLUSION AND STEPS FORWARD

This P4-BIO pilot phase for the elaboration of biotherapeutic product monographs was essential because it has established the first monographs which allow for the control of the quality of new single source biotherapeutics whilst recognising their inherent complexity. The monographs for these biotherapeutics also firmly establish the link between product quality and production process. These monographs have allowed for flexibility in the approach to setting specifications by typically including complex tests that measure process-dependent microheterogeneity (e.g. glycosylation) in the Production section of the monograph.

Additionally, the thinking on what are the important features of a recombinant protein monograph has evolved in order to reflect the product complexity. For most of the biotherapeutics in question, the understanding of the important product characteristics affecting quality, efficacy and safety and of the methods needed to monitor them strongly depended on a sufficient level of information including information on product characterisation. The critical evaluation of the Innovator methods and careful selection of the tests included in the specifications has been crucial – the specifications provided by the Innovator have been treated as a base for elaboration of the monograph but not directly copied. Sometimes the submitted methods were outdated and efforts were taken to align them with already existing monographs, perform extensive method verification, achieve more harmonised methods and take a broader view to consider newer methods, while keeping in mind that public standards should be applicable by everyone (highly robust methods are preferred to highly sophisticated ones).

The work performed during the P4-BIO pilot phase successfully proved that it is possible and extremely useful to elaborate monographs on complex biotherapeutics; in the specific case of etanercept, the monograph elaboration showed that complex molecules and complicated assays can be standardised.

The elaboration of monographs for biotherapeutics has achieved important milestones thanks to this pilot phase with five monographs published in the Ph. Eur. While the monographs have been elaborated taking into consideration data from a single manufacturer, the standards have been drafted in a way that allows for future products to be approved on the European market. It is to be noted that the Ph. Eur. monographs will be revised, if needed, in consideration of new data submissions as soon as additional products appear on the market.

In the light of recent developments and positive outcome of this significant work, the P4-BIO pilot phase was concluded at the 156th session of the Ph. Eur. Commission in November 2016. With this, the P4 procedure has become a well-established mechanism within the Ph. Eur. framework devoted to setting public standards for biotherapeutic products.

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