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Capability measurement of size-exclusion chromatography with a light-scattering detection method in a stability study of bevacizumab using the process capability indices

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ABSTRACT

In this study, we investigated if the size-exclusion chromatography coupled with light-scattering and refractive index detection (SEC/LS/RI) method is fitted for its intended purpose and checked if the analytical method is able to provide enough conforming results. For this, the process capability indices C_p, C_{pk}, and C_{pm} were computed. The traditional X-chart and moving range (MR) chart were used by the same analyst to monitor the equipment in the laboratory over a 1-year period. For this, a bovine serum albumin (BSA) sample (0.3 mg mL^{-1}) with a nominal M_w of 66.4 kDa was analyzed each working day. The results confirmed that the analytical method is in-control and stable. To determine whether the given process meets the present capability requirement and runs under the desired quality conditions, the Pearn and Shu (2003) method based on the lower confidence bound C on C_{pm} was used. The estimator C_{pm} was 1.81, and the lower confidence bound C was 1.40. We therefore conclude that the true value of the method capability C_{pm} is no less than 1.40 with a 95% level of confidence. This result indicates that the method is satisfactory and no stringent precision control is required. The usefulness of this method was applied in the characterization of bevacizumab commercial pharmaceutical preparations stored under different conditions that lead to aggregation. In this case, the computed C_{pm} index was 0.98 (0.70, 1.26), which indicates that the method does not comply with the specification limits and needs to be revised. The quality improvement effort should: (1) reduce the uncertainty in the absolute M_w determination; (2) either move the process mean closer to the target value or reduce the process variation, i.e. improve the method accuracy (μ–T) and precision (σ^2). On this point, the Bayesian posterior distribution of the mean and standard deviation pointed out the need to control the precision but specially accuracy in order to reduce the overall uncertainty of analytical method and thus, the method is capable.

1. Introduction

Monoclonal antibodies (mAb) are glycoproteins used as therapeutics for the treatment of cancer, inflammatory diseases, complications upon organ transplantation, and infectious and cardiovascular diseases [1]. Bevacizumab (Avastin®, Genetech, San Francisco, USA) is a recombinant humanized monoclonal IgG1 antibody that prevents or reduces the formation of blood vessels (angiogenesis), thereby preventing or reducing metastatic disease progression [2]. Although bevacizumab was approved in 2004 for metastatic cancer of the colon or rectum, it has not gained approval by the FDA for intravitreal use and therefore can be utilized only in an off-label setting. In these cases, it has been shown to be effective as an adjunctive treatment for neovascularization of the iris and neovascular glaucoma with or without vitreous haemorrhage [3]. An improvement in vision has even been reported in approximately 25–40% of age-related macular degeneration (MAD) patients at 1-year follow-up and appears to be maintained at 2 years [4].

Proper protein-formulation development is crucial for optimal therapeutic performance of biopharmaceuticals and its design should be geared to avoid both physical and chemical degradation, aggregation being the most common degradation process. Selection of excipients, physical state and storage conditions are critical factors, to avoid loss of therapeutic value and induction of immune responses [5]. A key to controlling protein aggregation is understanding the mechanism of protein aggregation. Kinetic studies, data curve-fitting, an analysis are, in turn, keys to rigorous mechanistic studies. The kinetics and products of protein aggregation have been measured using different analytical techniques [6]. The validation of quantitative analytical methods is therefore critical to

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ensure the safety and efficacy of biopharmaceutical drugs. Many regulatory organizations have addressed this issue in the chemical and pharmaceutical industry (e.g. International Conference on the Harmonization-ICH, the Food and Drug Administration-FDA or Eurachem) [7,8]. However, in this type of study it is necessary to know the identity of starting proteins, products and intermediates of protein aggregation, the first rule of mechanistic science, since the steps in any proposed mechanism must be added to those observed compounds.

Various analyses are used to characterize the biomolecules. Molecular weight (M_w), conformation, size and shape, and state and extent of aggregation are a few of the physicochemical properties studied. Recent technological advances have significantly increased the speed of characterizing proteins. It is now feasible to measure light-scattering, refractive index and the intrinsic viscosity signal, along with UV-absorption characteristics of protein component peaks separated on a size-exclusion chromatography (SEC) column in real time by coupling four detectors online with HPLC equipment. A combination of two or more of these four-detector units has been used in several laboratories for determination of protein M_w, hydrodynamic radius, protein aggregation, and protein glycosylation [9–13].

Size-exclusion chromatography (SEC) is used in the purification process, which is the main part of the downstream portion of recombinant protein production; its elution profiles provide the first indication of protein purity, homogeneity and oligomeric state. However, most proteins are subjected to other quality assessment methods to determine additional biophysical properties [14]. In this case, SEC coupled with online laser light-scattering (LS) and refractive index (RI) detection provides an excellent approach to determine the absolute M_w of proteins and products of aggregation [9–11]. However, proper calibration of the SEC/LS/RI system is crucial to obtain high precision and accuracy since the experimentally determined M_w depends on the precision of the instrument calibration constant and the M_w of the calibration standard [9–13]. The theory behind the use of LS and RI signals to calculate M_w distribution is described in detail in several of the earlier reports and reviews [15–17].

In industrial activities, it is necessary to obtain information about the performance of the process when it is operating under statistical control. For this, the capability indices are calculated, to evaluate whether the process under study is able to provide sufficient conforming units. When an analytical procedure is performed on a sample, this is itself a process just as the manufacturing operation is a procedure. By analogy, capability indices could be used to evaluate whether the analytical method is only able to provide enough conforming results to check if a method is fitted for its intended purpose [18]. Various examples of the usefulness of capability indices in the framework of analytical methods validation can be found in the literature [19–21].

As stated in the ICH-Q2 (R1): "The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated" [7]. It is commonly accepted that the reason for validation of an analytical procedure is to demonstrate that it is suitable for its "intended purpose". For any analytical method, performance characteristics which constitute desired "suitability" must be defined.

The aim of our research was to check the suitability of data from SEC/LS/RI method in the characterization of bevacizumab commercial pharmaceutical preparations stored under different conditions that lead to aggregation (i.e. identity of starting proteins, products and intermediates of protein aggregation). For this, several process capability indices were used to determine whether the process is capable. These indices use both the process variability (precision and accuracy of analytical method) and the process specifications, which constitute the two validation characteristics which need to be evaluated. For this, the validity of the analytical method was controlled and verified during routine use of the method using the X-mean and moving range (MR) control charts. To determine whether a given process meets the present capability requirements and runs under the desired quality condition, the Pearn and Shu method based on the lower confidence bound was used [22]. The Bayesian posterior distribution of the mean and standard deviation was used to define the overall uncertainty of analytical method [23]. Finally, the Monte-Carlo simulation method was used to check results.

2. Theory

Process capability indices (PCIs) were introduced to give an indication of the capability of a process or analytical method. They are designed to quantify the relation between the desired specifications and the actual performance of the process or analytical method. A capability index is generally a function of the process parameters, such as the mean μ, standard deviation σ, target value T, lower specification limit (LSL) and upper specification limit (USL) of X. Four common capability indices found in the literature are:

$$C_p = \frac{\mathrm{USL} - \mathrm{LSL}}{6\sigma} \tag{1}$$

$$C_{pk} = \min\left\{\frac{\mathrm{USL} - \mu}{3\sigma}, \frac{\mu - \mathrm{LSL}}{3\sigma}\right\} \tag{2}$$

$$C_{pm} = \frac{\mathrm{USL} - \mathrm{LSL}}{6\sqrt{\sigma^2 + (\mu - T)^2}} \tag{3}$$

$$C_{pmk} = \min\left\{\frac{\mathrm{USL} - \mu}{3\sqrt{\sigma^2 + (\mu - T)^2}}, \frac{\mu - \mathrm{LSL}}{3\sqrt{\sigma^2 + (\mu - T)^2}}\right\} \tag{4}$$

C_p is the most used index. It aims at measuring if the dispersion of the studied variable is more or less greater than the interval defined by the process specification limits. It measures the percentage of the specification interval used by the variable measured. Indeed, the C_{pk} index measures simultaneously the position and dispersion of the variable, i.e. it considers the process yield. However, these indices do not give any information about the position of the variable with respect to a target value. For this, Chan, Cheng, and Spiring [24] considered this fact in developing C_{pm}, which considers process loss and variation from the target. Later Pearn et al. [25] added C_{pmk}, which incorporates the yield-based index C_{pk} and the loss-based index C_{pm}, taking into account the process yield (whether it fulfils the method specifications) as well as the process loss (variation from the target). C_{pmk} can reveal more information about the location of the process mean and also is more sensitive than other capability indices to any deviations from the relative process mean [26]. Details about PCIs and their statistical properties can be found in these two monographs by Kotz and Lovelace [27] and Kotz and Johnson [28]. For a review of the work on PCIs during the period 1990–2002, see the article by Kotz and Johnson [29] and Spiring et al. [30].

In fact, C_{pm} was developed because C_{pk} is observed to be an inadequate measure of process centering, although C_{pk} was developed to deal with the case of a process with mean μ that is not centered between the specification limits, whereas C_p is inadequate in process centering. As a matter of fact, when μ is within the interval of the specification limits, LSL and USL, C_{pk} depends inversely on the process standard deviation σ (i.e. systematic error, $E[X-\mu]^2 = \sigma^2$) and becomes large as σ gets closer to zero. This means a large value of C_{pk} does not actually give any information about the location of the mean in the specification limits interval (i.e. process centering). In that case, the C_{pm} index, which is a better indicator of process centering, would be much more useful [31].Consequently, the C_{pm}