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Comparison of Analytical Method validation guidelines used for release, stability in Biosimilar Manufacturing process.

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Abstract

Analytical method validation is the process of verifying a method for its purpose of fit, whether it suffices its intended application or not. Analytical method validation is a mandatory requirement to be fulfilled for measuring critical quality attributes (CQA) during the manufacturing process to get a drug approval for human, and veterinary use. As the drug approval process differs from one country to another in a similar way method validation guidelines also differ from one country to another, these requirements will be added as your country of approval differs, In the present review an attempt was made to bring all method validation guidelines in a comparative manner by comparing country-specific requirements these countries/organizations hold the major pharma market and the Stringent regularity countries. The paper majorly focuses on the analytical method used in the Biosimilar manufacturing process and its validation approach by comparing the method validation guidelines from the International Council for Harmonisation of Technical Requirements of Pharmaceuticals for Human Use (ICH), USA, European Medicines Agency (EMA), Japan and India with the CQAs monitored during the biosimilar manufacturing process.

Key Words: Food and Drug Administration, European Medicines Agency, ICH, critical quality attribute, Method Validation, QTPP.

Introduction:

According to the US Food and Drug Administration (FDA) and European Medicines Agency (EMA), a biosimilar is a biological medicine highly similar to an already approved biological drug (commonly referred to as a Reference product) in that respective region (1,2). Biosimilars and generics show similar effects to the innovator product/Reference product which undergoes an extensive analytical characterization followed by minimal clinical studies in comparison with the innovator product/ Reference product (1). Significant differences exist between the generic drug/biosimilar drug and reference product considering the various stages starting from the Synthesis stage till it encounters the market (3,4). Generic drugs are chemical entities with low molecular weight and are produced majorly through chemical synthesis, where the process and its critical quality attribute (CQA) are more controlled, and the final product will be an exact match with the innovator product/Reference product. whereas in the case of biosimilars,, the molecules are produced

in live cells, due to the complex nature of cells and other factors the control on the process and CQA's are considerably less when compared with generic molecules and by the definition of FDA and EMA, it is clearly understood that the Biosimilars are highly similar molecules but not the exact match to the reference product (1-5). Due to the complex nature of Biosimilars, the Analytical methods used for Quality attribute monitoring should be highly robust and should serve the intended purpose at each stage of the Manufacturing operations, release, stability, and in assessing the Analytical similarity/Biosimilarity.

Critical quality attribute involved in biosimilars:

As per the International Council for Harmonisation of Technical Requirements of Pharmaceuticals for Human Use (ICH) Q8 CQA is defined as physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. CQAs are generally associated with the drug substance, excipients, intermediates (in-process materials), and drug product (6). As per ICHQ8 the CQA's are defined based on the QTPP data generated from the reference product along with prior product and process development knowledge and excipients (As depicted in the Figure 1). CQA's represents all product characteristics like structure, quality, safety, and efficacy[6].

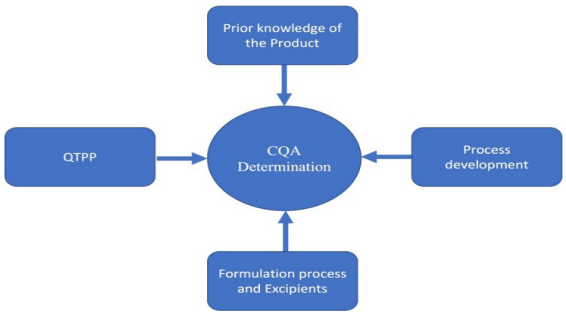

Figure 1:Determination of the CQA as per ICHQ8

CQA's play an important role in determining the Structure, Quality, safety, and Efficacy of the Drug product used for patients. CQA's are measured by various methods at each step of the biosimilar process.

Typical biosimilar manufacturing and approval process

Any typical Biosimilar Manufacturing process involves 5 major steps, Amino acid sequencing of Reference product, cloning development and optimization, cell culture process development, Purification process development (Capture step, polishing step, filtration process) and Formulation and fill finish (7,8).

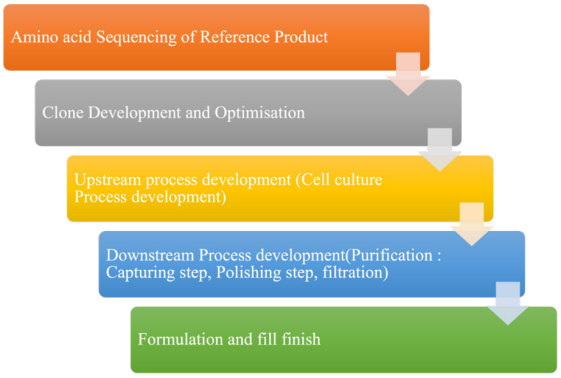

Figure 2: Typical Biosimilar Manufacturing Process.

Amino acid sequencing of Reference product: The Biosimilar program development starts with the sequencing of reference products using various basic analytical techniques.

Clone Development and Optimization: Clone development starts with the synthesis of the gene with a similar amino acid sequence obtained from the Reference product, followed by cloning this gene into a microbial or mammalian cell line by using a suitable vector (7). Based on the titer, clonality of the cell line, and minimal quality attributes (charge variants, glycosylation pattern, aggregates), the top clone will be selected followed by the generation of MCB with a set CQA specifications. Upstream process development: MCB generated from the

top clone selected will be used for the generation of WCB followed by process development during which various conditions required for cell growth and production of the desired product are optimized. Optimized process will be scaled up in step-by-step increments up to commercial scale ensuring the quality of the desired product is maintained (8).

Downstream process development: The downstream process majorly concentrates on the isolation and purification of the desired product utilizing various types of chromatography by keeping the CQA within the set specifications range. Various types of filters are also evaluated during downstream process development to ensure the product is free of viral and other contaminants. Optimized process will be scaled up in step-by-step increments up to commercial scale ensuring the quality of the desired product is maintained (8). Formulation and fill finish: The Purified Product is then concentrated/diluted based on the target set concentration/dosage along with the addition of excipients at the set range kept for the formulation process followed by sterile filling. The final drug product will be tested and compared with the reference product (7,8) ensuring similarity between both. During the biosimilar manufacturing process as depicted in Figure 2 various tests are involved at each stage of the process to ensure the final product CQA is comparable with Reference product (7,8). The technique/type of the method used for monitoring the CQA is referenced in Table 1. Methods used in biosimilar manufacturing can be categorized into various types based on its usage and its purpose.

The method outlined in the table above should be robust and their intended purpose should be fulfilled. The major objective of the analytical method validation is to demonstrate that the method is fit for its intended use and to identify the errors that might occur deliberately during the analytical procedure execution. Data generated during the analytical method validation [9,10,11,12] defines the robustness and the variability of the method. As per the ICH, Japa-

Table 1: List of Techniques/Methods used in monitoring the CQA and other Quality attributes (9,10).

Steps in Biosimilar Manufacturing	Methods/ Technique	Purpose	Classification of the methods based on purpose
Amino Acid sequencing	LC-MS	Amino acid identification	Identity
Clone Development / Master Cell bank creation	Protein A/ UV-spectroscopy	Product Titer Quantification	Assay
	SDS_PAGE	Product Identification/Size Variants	Identification/Purity
	SEC_H(U) PLC/CE-SDS	Size variants Quantification	Purity
	IEX_H(U) PLC/ icIEF	Charge variants Quantification	Purity
	Glycan	Glycan Content estimation	Purity
	Sialic acid	Sialic Acid Estimation	Assay
	DNA Barcode assay	Identity test for the cell line	Identity
	Sterility test	Microbiological Contaminants	Identification
	PCR	Viral contaminants and specific virus tests	Identification
Upstream Development	Protein A/ UV-spectroscopy/ Solo-VPE	Product Titter Quantification	Assay
	SEC_H(U) PLC/CE-SDS	Size variants Quantification	Purity

	IEX_H(U) PLC /icIEF	Charge variants Quantification	Purity
	Glycan	Glycan Content estimation	Purity
	Sialic acid	Sialic Acid Estimation	Assay
	Process related impurity generated/ added to improve the process efficiency	Estimation and clearance	Assay
	Bioburden	Estimation and Identification	Assay and identification
	BET	Estimation	Assay
Down-stream	Protein A/ UV-spectroscopy/ Solo-VPE	Product Titter Quantification	Assay
	SEC_H(U) PLC/CE-SDS	Size variants Quantification/fragments	Purity
	IEX_H(U) PLC /icIEF	Charge variants Quantification	Purity
	Process related impurity generated during Upstream/ down-stream	Estimation and clearance	Assay
Formulation and fill finish	Protein A/ UV-spectroscopy/ Solo-VPE	Product Titer Quantification	Assay
	SEC_H(U) PLC/CE-SDS	Size variants Quantification	Purity
	IEX_H(U) PLC /icIEF	Charge variants Quantification	Purity
	pH	Estimation	Measurement
	Osmolality	Estimation	Measurement
	Colour/ Clarity/Appearance	Identity	Identity
	Potency/ Biopotency	Identity and estimation	Identity and estimation
	BET	Estimation	Assay
	Bioburden	Estimation/ Identification	Assay and Identification
	Sterility	Identification	Identification
	Fill volume	Estimation	Assay
	Visible/ Sub visible Particles	Estimation	Assay
	Excipients	Estimation	Assay
	Impurity	Estimation	Clearance
	Elemental impurities	Estimation	Clearance

nese Pharmacopoeia (JP), United States Pharmacopoeia (USP), FDA and other major guidelines, various analytical methods are classified into four major types based on their intended purpose: (i) Identification tests (ii) Quantitative tests for impurities' content (iii) Limit tests for the control of impurities (iv) Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Parameters that has to be evaluated are defined based on the classification of the method into types mentioned above. The method validation parameters are depicted in the Figure 3.

Comparison of analytical method validation guidelines used for release, stability in biosimilar manufacturing process

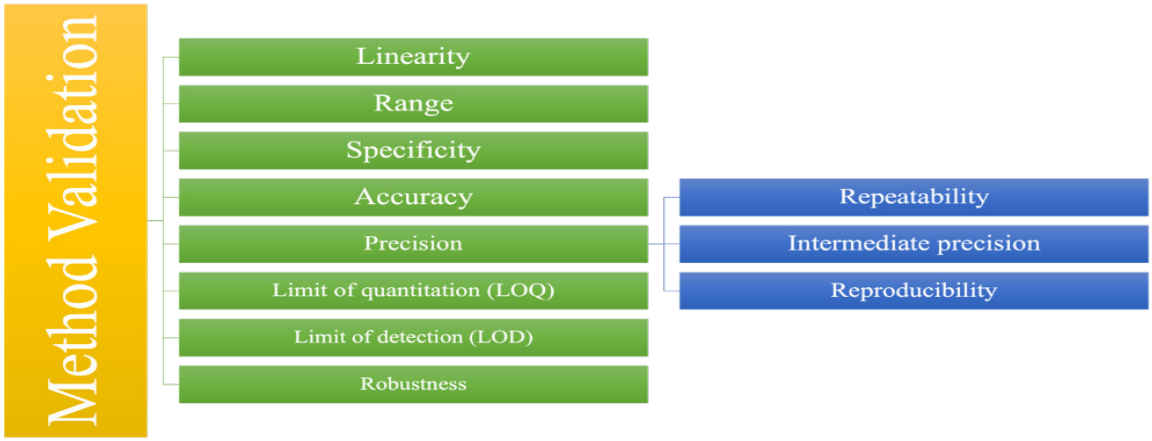

Figure 3:Analytical method validation parameters

As mentioned before, the extent of method validation depend on the purpose of the method and its regulatory requirements. The detailed explanation about each method was extensively mentioned in the further sections. Table 2 depicts the method validation requirements based on the methods categorified as per the Table 1.

Table 2: Method validation parameters based on the method requirement (12,13).

Parameter	Identity Methods	Assay methods	Purity/Impurity	Clearance
Linearity	N	Y	Y	Y
Range	N	Y	Y	Y
Specificity	Y	Y	Y	Y
Accuracy	N	Y	Y	Y
Precision				
Repeatability	N	Y	Y	Y
Intermediate Precision	Y	Y	Y	Y
Reproducibility	N	Y	Y	Y
LOQ	N	Y	Y	Y
LOD	N	May be required	May be required	Y
Robustness	Y	Y	Y	Y

N – Validation of the specific parameter is not required, Y – Validation of the parameter is required.

Linearity

Definition

The linearity of a method is expressed as the ability of a method to elicit a response in the form of linear or a mathematical expression form proportional to the amount of the analyte present in the sample. The linear or mathematical response majorly depends on the instrument and the detector used. For HPLC, UV, and the majority of the methods the relationship between the amount of analyte present in the sample and the response should be linear. For CAD detectors based on methods, the response can be linear, or quasi-linear [9,10,11,12,13,14].

Procedure

The linearity of the method will be evaluated graphically by plotting the response from the detector connected to the analytical instrument and to the theoretical concentration or the content of the analyte present in the sample being tested. The responses should be directly proportional or proportional by means of the amount of analyte present in the sample. The relation should be explained either by a linear equation or by an appropriate mathematical/statistical equation as applicable. Linearity has to be tested around the range as applicable[12,13,14]. For the majority of the methods the Linearity was expressed in terms of Linear regression as mentioned in the

, R^2

Here the Y–axis represents the analyte response generated from the Instrument detector.

X – axis represents the theoretical concentration of the analyte present in the sample (the sample will be diluted into multiple determinations to cover the range of the method with the representative diluent/buffer)

m – slope of the curve

R^2 – coefficient of regression

Number of determinants/concentrations used for linearity majorly depends on the range required for the method, a minimum of 5 determinants should be used.

Acceptance criteria/test requirements

The correlation coefficient, Y-intercept, slope of the regression line, and the sum of least squares should be submitted.

Table 3: Linearity Test acceptance criteria (15,16).

Parameter	**ICH**[9]	**USA**[15]	**EU**[16]	**Japan**[10]	**India**[13]
Minimum number of concentration	05	05	05	05	05
Regression coefficient (R^2)	-	-	-	-	0.999*
Graphical plot – Response Versus Concentration	Required	Required	Required	Required	Required

∗ *For methods with usage of CAD as a detector, criteria can be relaxed with proper justification and supporting data.*

Range

Definition

The range of a method will be derived from the linearity studies covering the lower and upper limit of analyte concentration to be measured with that analytical method. All the determinants present in the range should have acceptable degree of linearity, accuracy and precision.

Procedure and acceptance criteria

The range of a methodwill be derived from the linearity data showing acceptable linearity, accuracy and precision. Table 4 represents the minimum ranges that has to be considered for range based on its application of use:

Table 4:Minimum acceptable ranges based on its intended use (15,16).

Nature of the analyte	Purpose of the method	Minimum Range to be tested				
		ICH (9)	USA (15)	EU (16)	Japan (10)	India (13)
Drug substance/ Drug product	Assay	80 – 120 %	80 – 120 %	80 – 120 %	80 – 120 %	80 – 120 %
Impurity	Assay	Reporting level to 120 % of Specification	Reporting level to 120 % of Specification	Reporting level to 120 % of Specification	Upper , lower and middle limit	Reporting level to 120 % of Specification
Impurity	Assay/Clearance	LOQ/LOD till upper limit/120 % of specification	LOQ/LOD till upper limit/120 % of specification	LOQ/LOD till upper limit/120 % of specification	Upper , lower and middle limit	Reporting level to 120 % of Specification

In order to cover the impurity for clearance/assay it is advisable to perform the range test from the LOQ/LOD level till the 150 % of the upper limit of specification (17).

Specificity

Definition

Specificity is the ability of the analytical method to measure the analyte unequivocally in the presence of other inevitable sample components. For example the sample used for testing should contain impurities, Degradation products, or process raw materials or excipients. Method used should specifically identify the analyte (DS/DP/Impurity/Degradation product) of measurement without the interference of other sample matrix components.

Procedure

The specificity of the method can be established in multiple ways depending on the usage of the method. (i) DS/DP, purified Impurity, Degradants of the products, and other analytes were spiked into the matrix /Placebo and their response was evaluated over the amount of materials spiked into the respective matrix (15). (ii) In a Situation where impurities/ degradants products/Standards are not available for spiking , then a well characterised alternative/orthogonal method should be employed. It is preferable to use the method which is compendial or validated to check the specificity (16). (iii) For chromatographic methods respective impurities/ degradants product peaks should be collected and analysed by using Mass spectroscopy technique. (iv) For Assay and impurity tests, the test sample and the respective buffer should be analysed side by side to check the interference of the product. There should not be any interface at/with the peak of analyte (17).

Acceptance criteria

No interference should be observed in the measurement of the analyte. The requirements for the specificities across the selected are mentioned in the

Table 5:Country specific requirements for Specificity (15,16).

ICH (9)	**USA** (15)	**EU** (16)	**Japan** (10)	**India** (13)
All the components in the sample from the result should be properly labeled with proper resolution	All the components in the sample from the result should be properly labeled with proper resolution	All the components in the sample from the result should be properly labeled with proper resolution	All the components in the sample from the result should be properly labeled with proper resolution	All the components in the sample from the result should be properly labeled with proper resolution
Impurities and other samples should be spiked into the placebo/ Sample matrix	Impurities and other samples should be spiked into the placebo/Sample matrix	Impurities and other samples should be spiked into the placebo/ Sample matrix	Impurities and other samples should be spiked into the placebo/Sample matrix	Impurities and other samples should be spiked into the placebo/Sample matrix
When Impurities are not available other well characterized method should be used to demonstrate specificity	When Impurities are not available other well characterized method should be used to demonstrate specificity	When Impurities are not available other well characterized method should be used to demonstrate specificity	When Impurities are not available other well characterized method should be used to demonstrate specificity	When Impurities are not available other well characterized method should be used to demonstrate specificity
-	For a stability indicating method Accelerated stress samples should be used to show the specificity for degradants	–	In case the reference standard of the impurities/degradants are not available then Accelerated stress samples should be used to show the specificity	-

Accuracy

Definition

The accuracy of the analytical method defines how close is the method result value to the actual value (amount of analyte present in the sample) (15). Generally accuracy of the method is expressed in terms of recovery of the analyte present in the solution to the amount of analyte spiked into the solution.

Procedure acceptance criteria

The accuracy of the method was performed by spiking the known amount of analyte into placebo/Background buffer/sample matrix, and the results were expressed in terms of percentage recovery as mentioned in the below equation (16).

$$\% \text{ Recovery} = \frac{(\text{Amount of analyte present in the sample})}{(\text{Amount of analyte spiked or added ino the palcebo})} \times 100$$

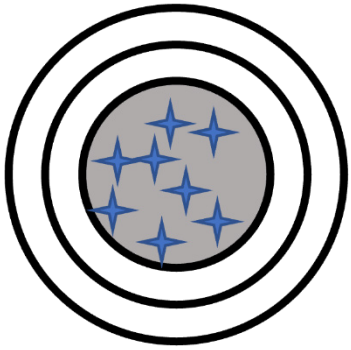

Figure 4: Accuracy of the analytical method

In the Figure 4 stars represent the results obtained from the analytical method whereas the circle with the coloured portion is the true value with accepted method variabili-

ty. The accuracy of a method should cover the linearity and range of the method with at least 9 determinants covering the entire range of a method with at least 3 concentrations (upper limit, middle and lower limit of the range).

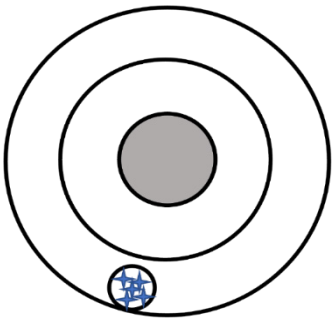

Figure 5: Results are Precise but not accurate

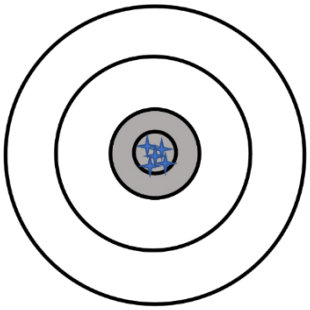

Figure 6: Results are Accurate and Precise

of an analytical method but it can't assure the accuracy of the method. Figure 5 and Figure 6 illustrate the precision and the relationship between accuracy and precision.

It was expressed in three ways as mentioned below: (i) *Repeatability/Intra-assay precision,* (ii) *Intermediate precision,* (iii) *Reproducibility*

Repeatability/Intra-assay precision

Definition

The repeatability of a method was assessed by defining the variability of the method within a short period of time under the same conditions with the homogenous sample (15,16). The variability of the method was estimated with the same operator, same instrument and sample etc.

Precision

Precision is measured as the closeness of the analytical results obtained from a sample with allowed variability. the precision of the method estimates how close the measurement

Procedure

The repeatability of a method was estimated by analyzing the sample multiple times with the same analyst, in fact, the repeatability merely depends on the analytical instrument, and the detector responsible for measurement (17). Repeatability depicts the precision of the instrument as well.

ICH (9)	**USA** (15)	**EU** (16)	**Japan** (10)	**India** (13)
A minimum of nine determinants covering the entire range of the method with 3 replicates covering 3 Concentrations of the range	A minimum of nine determinants covering the entire range of the method with 3 replicates covering 3 Concentrations of the range	A minimum of nine determinants covering the entire range of the method with 3 replicates covering 3 Concentrations of the range	Variance and standard deviation should be established with 90 % confidence intervals	A minimum of nine determinants covering the entire range of the method with 3 replicates covering 3 Concentrations of the range
A minimum of 6 determinants with 100 % test sample concentrations	A minimum of 6 determinants with 100 % test sample concentrations	A minimum of 6 determinants with 100 % test sample concentrations		A minimum of 6 determinants with 100 % test sample concentrations

Acceptance Criteria

The repeatability of the method will be established by keeping the % RSD criteria for the output value between the determinants analyzed for the study (18). The % RSD criteria depend on the type of method and its requirements for its uses in the process.

Intermediate precision

Definition

Intermediate precision of the method estimates the variability of the method within laboratories, different days, analysts, equipment, etc.,

Procedure

Repeatability sequence can be repeated with different analysts, days, equipment, lots of chemicals, columns, etc., used in the method (19). The variations in the method execution can be done one factor at a time or by using a design of experiments.

Acceptance criteria

Intermediate precision of the method was established by keeping the % RSD criteria for the output value between the determinants analyzed for the study (20,21). The % RSD criteria depend on the type of method and its requirements for its uses in the process.

Reproducibility:

Definition

Reproducibility is the precision expressed between the two different laboratories.

Procedure

The reproducibility experiment will be done by executing two sets of experiments as described in repeatability in two different laboratories.

Acceptance criteria

The repeatability of the method was established by keeping the % RSD criteria for the output value between the determinants analyzed for the study (21,22). The % RSD criteria depend on the type of method and its requirements for its uses in the process.

Limit of quantification(LOQ)

Definition

Estimation of the Lowest concentration or amount of the analyte present in the sample with accuracy and precision, in other words, lowest concentration of an analyte measured by the analytical method (22,23).

Procedure

The LOQ of a method is estimated by using the known concentration of the sample at the lowest amount with acceptable accuracy and precision (24,25). For the Chromatographic methods, the LOQ of the method was established by using the signal to Nosie ratio with acceptable accuracy and Precision.

Acceptance criteria

For chromatographic methods, the LOQ will be identified at a concentration where the signal-to-noise ratio is equal or more than 10 with acceptable accuracy and precision. The LOQ is estimated by injecting the same sample six times with an acceptable % RSD between the six preparations of the sample.

Limit of detection (LOD)

Definition

Detection of Lowest concentration or amount of the analyte present in the sample, in other words, the LOD depicts the presence of the analyte in the sample which is not accurate in terms of quantity.

Procedure

The LOD method is estimated by evaluating the response which should be higher than the response for the blank sample (25). For the Chromatographic methods, the LOQ of the method was established by using the signal to Noise ratio.

Acceptance criteria

For chromatographic methods, the LOD will be determined at a concentration where the signal-to-noise ratio is equal to or more than 3.

Robustness

Definition

The robustness of a method is the ability of the method to remain unaffected with small deliberate changes with are unavoidable during the method execution (26,27). Expected changes are purposely introduced during the robustness study to understand the effect of these variations on the analytical method outcome.

Procedure

Expected changes/variations that might occur in the routine analysis will be introduced during the method validation. For example pH of the buffer and column temperature will be varied and will be studied during the robustness activity. The stability of the prepared sample(diluted/undiluted) solution will also be evaluated.

Acceptance criteria

Based on the outcome of the results, the method parameters with a defined range will be finalized for routine analysis. The Method validation parameters vary depending on the above types and based on the application of the method in the Bio-pharmaceutical /pharmaceutical manufacturing (28). In the current review process, the method validation approaches will be majorly categorized based on their application, type of method and manufacturing process requirements. Method validation guidelines from different regulatory bodies resulted in certain method validation characteristics and the same are discussed further.

For identification methods

The identification methods are used to confirm the identity of the analyte present in the sample. These tests are performed based on the spectrum of the method or in comparison with the reference standard available (29). Specificity, intermediate precision, and Robustness study are evaluated for the methods used for the identification of analyte such as Amino Acid sequencing by LC-MS, SDS PAGE, PCR, DNA bar code assays method. For Colour/Clarity/Appearance testing methods the whole sample consisting of a drug substance/drug product with its impurities along with its background buffer/matrix will be evaluated as there is no estimation of a specific analyte. The majority of the time this method of analysis is of pharmacopeial methods, and the validation/verification will be done by analyzing the three batch samples or a single batch in triplicates which is intended for commercial process and the similarity of the results will be considered (29). For the microbial methods like Sterility and bioburden matrix/background interference, evaluation estimates the condition buffer for the microbial growth along with positive and negative control.

For assay methods:

For better ease of understanding the application of assay methods, the assay methods are divided in to various types as mentioned in Figure 7.

Figure 7: Assay methods and its types

Assay measurement

Methods like pH, and osmolality estimation majorly come under assay measurement, where the solution will be analyzed rather than a specific analyte. As pH, and osmolality plays a major role in the stability of the molecule and their interaction within the body when injected, their control is very critical in the process[29]. The method validation will be done by measuring pH and osmolality of three batch samples intended for commercial process or a single batch in triplicates and the similarity of the results will be

considered[30]. These methods has to be applied for release as well as for stability as the sample/ solution should be within the limits till the end of the shelf life of the molecule.

Assay concentration estimation

The method used for estimation of product titer, size variants, charge variants and other quality attributes comes under this category. For the methods used to estimate the active substance in in-process samples, drug substance and drug product, validation will be performed considering the parameters as mentioned in Table 2. However, the method used to estimate active substance in DS and DP method need not be validated for LOQ and LOD limits as the concentration of the analyte in DS and DP samples will be as per the dosing requirement of the concerned medicine (29,30). Also, the range of these methods will be validated as per the process limits with a variation of 10 – 20 % from the lower and upper side of the process limits. These methods should be applied for release as well as for stability as the molecule concertation should be within the limits till the end of the shelf life of the molecule.

Identity method and assay

The Method used to estimate the potency of the molecule can be used as identity and assays. In the Bioassay analysis, the DS/ DP binding with its respective molecular target will be used and the potency will be estimated, as binding is highly specific the method can be used as identity also (30). Potency can be measured, and it is linked to the molecule's efficacy towards the molecule. These methods are majorly done by ELISA or cell-based methods (30). These methods should be applied for release as well as for stability as the molecule concertation should be within the limits till the end of the shelf life of the molecule.

Limit tests for impurities

Limit tests for impurities will be used to estimate the amount the impurities present in the sample, in the biologics manufacturing process there are impurities that majorly come from various stages in the manufacturing process Figure 8 represents the impurities generated in the manufacturing process.

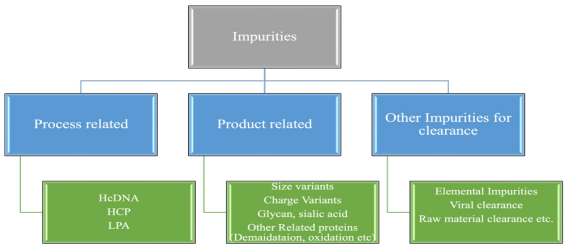

Figure 8: Impurities generated in the Biologics manufacturing process

Process-related impurities

The Process-related impurities like HcDNA , LPA and HCP have a defined calculation for the amount as per agency requirement, the method should be validated to the limits based on daily intake and the process consistency (29,30,31). The method validation must include LOQ and LOD limits. The method validation range should include the process limits as well as the respective health agencies requirement (31). These methods have to be applied for the DS/DP Release and is not required for stability as these impurities will not change based on the time.

Product-related impurities

Product-related impurities like size variants, charge variants, deamidation, oxidation etc, generate over time and increase as the product nears the end of its shelf life. The method validation range for this limit should include the lower limit and the upper limit of the particular impurity, the limit will be decided based on the safety and efficacy data generated from the clinical trial, In the case of biosimilars these limits are set based on the QTPP data generated form the innovator drug procedure from the indented marketing region (30,31). In case where

the impurity limit is very high than the LOQ of the method then the LOD of the method is not required to be generated. These methods have to be applied for the DS/DP Release and is not required for stability as these impurities will not change based on the time (30). The Impurities like Glycan and Sialic acid will not increase over the period of time due to this the method will be used for release and need not be tested on stability.

Other impurities for clearance

Impurities like elemental impurities clearance should be performed at the DP stage based on the elemental impurities limits linked to their safety, for method validation of this method the LOQ and LOD have to be established as the limits will be highly minimal except few elemental impurities (31). These will be demonstrated based on the consistency of the process and need not be used for routine release however the measurement of impurities will be evaluated case by case and respective metal ions will be estimated as per the requirement (31). For the method used to test for Viral impurities and other advantageous elements method should be validated with LOD and LOQ, as these impurities absence should be demonstrated in the method, in these cased the amount of some impurities will be spiked as a positive control to show the absence of the impurities, as these will not increase over the period of time due this the method will be used for release and need not to be tested on stability.

Methods used for the Assay of Excipients:

The excipients method will be used to check its content in the final DS and DP, as per the label claim or not, for the validation of these methods the range will be established along with the limits as per the label claim, the establishment of LOQ and LOD is not required as per the excipients until there is special consideration of the method (30,31). or if the lower limit of the method coincides/is near the LOQ limit.

Conclusion:

The method validation is one of the important requirements for the health agencies to check its performance as the safety, and efficacy of the drug entirely depend on CQA and the stability of the drug which are governed by the analytical method used to estimate the Quality attributes of the method being used. Validation of the method majorly relies on the requirements of the process and the regulators it is always recommended to use the validation parameters to cater to all the agencies' requirements as depicted in the present paper. The Validation should always be planned in such a way as to cover the requirements of all the regulatory agencies, and this requirement should be seen not only to fulfill the requirements of the countries but also in a scientific way these are important for checking the suitability of the method also.

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