Checklist for Protocol Analysis and Analytical Method Validation (Biologics Products)

These checklists are intended to provide guidance on the submission of documents/ information for analytical method validation/ verification for biologics drug products. The following checklists are not exhaustive and Centre for Quality Control (CQC), National Pharmaceutical Regulatory Agency (NPRA) reserves the right to request additional data whichever it deems necessary. All submitted documents must be arranged and labelled accordingly. Otherwise CQC, NPRA reserves the right to reject the documents.

Table A shows the information required for protocol of analysis. Table B, C and D illustrate validation parameters and documents required for validation of identification/ characterisation test, assay/content test and related substances test respectively. Justification or explanation must be provided if any information listed in tables below is not available. The tests listed in Table A are examples and Product Registration Holder (PRH) shall provide relevant documents for all the tests specified in the Drug Product specifications.

The following verification parameters are required (if applicable) for **COMPENDIAL METHOD** and **SECOND SOURCE***:

a) Specificity
b) Precision (intermediate precision)
c) LOD/ LOQ (applicable to impurity test only)
d) System Suitability tests

* Verification must be conducted using the samples and resources from second source.

These checklists shall come into force on **10th October 2016.**

Table A: Checklist for Protocol Analysis

TEST	INFORMATION REQUIRED		AVAILABILITY
Physical Tests	**Statement according to pharmacopoeias or photocopies from pharmacopoeias shall not be accepted. Details of test methods shall include the following items:**		
• Appearance • Colour, Clarity and Opalescence • Visible particles • Subvisible particles • pH • Osmolarity • Moisture content • Extractable volume • Dissolution time • Homogeneity test • Others	1	List of equipment and apparatus (if applicable)	
	2	List of chemical, reagents and media (if applicable)	
	3	Preparation of solutions such as sample, reference standard (if applicable), medium, buffer, etc	
	4	Volume and temperature of sample solution (if applicable)	
	5	Setting up of analytical instrumentation (if applicable)	
	6	Testing condition/ parameter (if applicable)	
	7	Testing procedure	
	8	System suitability tests (if applicable)	
Identification / Characterisation Tests	**Details of test methods shall include the following items:**		
• Peptide Mapping • Identification of preservative and active substance • Precipitate reaction • Microscopic examination • Colony morphology • Virus identification • Others	1	List of equipment and apparatus	
	2	List of chemical, reagents, media and cell line (if applicable)	
	3	Preparation of solutions such as sample, standard, system suitability solution, mobile phase, medium, buffer, etc (the amount of chemical/sample/ standard and volume of diluents used in the preparation must be stated)	
	4	Setting up of analytical instrumentation	
	5	Testing condition/ parameter such as HPLC parameter, etc	
	6	Testing procedure	
	7	System suitability tests and acceptance criteria of system suitability test.	
	8	Complete formula for calculation (if applicable) and interpretation of results	
	9	Image of SDS PAGE/ IEF/ electropherogram/ HPLC chromatogram/ etc for blank, sample, standard and system suitability solution	

Assay/ Content Tests	**Details of test methods shall include the following items:**		
• Protein concentration • Content of preservative • Bioassay/ Potency (animal- based, cell culture- based and biochemical- based) • Others	1	List of equipment and apparatus	
	2	List of chemical, reagents and media	
	3	Preparation of solutions such as sample, standard, system suitability solution, mobile phase, medium, buffer, etc (the amount of chemical/sample/ standard and volume of diluents used in the preparation must be stated)	
	4	Setting up of analytical instrumentation	
	5	Testing condition/ parameter such as HPLC parameter, animal criteria, etc	
	6	Testing procedure	
	7	System suitability tests and acceptance criteria of system suitability test.	
	8	Data analysis such as complete formula for calculation (the formula must provide in the unit stated in COA) and interpretation of results	
	9	HPLC chromatogram/ UV spectrum (if applicable) for blank, sample, standard and system suitability solution	
	10	Complete testing report for finished product (only for bioassay and potency test – any batch) *Testing report should comprise information such as passage cell, viability cell, relative light units, 4- parameter logistic curve, F- test, L- term and others	
Purity/ Impurities Tests	**Details of test methods shall include the following items:**		
• Known impurities • Unknown Impurities • High Molecular Weight Protein • Monomer • Dimer • Aggregates • Residual solvent	1	List of equipment and apparatus	
	2	List of chemical, reagents and media	
	3	Preparation of solutions such as sample, standard, system suitability solution, mobile phase, medium, buffer, etc (the amount of chemical/sample/ standard and volume of diluents used in the preparation must be stated)	
	4	Setting up of analytical instrumentation	
	5	Testing condition/ parameter such as HPLC parameter, etc	
	6	Testing procedure	
	7	System suitability tests and acceptance criteria of system suitability test.	
	8	Data analysis such as complete formula for calculation (the formula must provide in the unit stated in COA) and interpretation of results	
	9	Image of SDS PAGE/ IEF/ electropherogram/ HPLC chromatogram/ etc for blank, sample, standard and system suitability solution.	

Other Safety Test	**Details of test methods shall include the following items:**		
• Pyrogen Test • Bacterial Endotoxins Test • Sterility Test	1	Refer to DRUG REGISTRATION GUIDANCE DOCUMENT (DRGD) under GUIDELINE FOR THE SUBMISSION OF PROTOCOL OF ANALYSIS (POA)	
Others	**Details of test methods shall include the following items:**		
• Test for absence of virulent mycobacteria • Test for excessive dermal reactivity • Specific toxicity test • Abnormal toxicity test (innocuity) • Others	1	List of equipment and apparatus	
	2	List of chemical, reagents and media	
	3	Preparation of solutions such as sample, standard, medium, buffer, etc	
	4	Testing condition /animal criteria	
	5	Testing procedure	
	6	Calculation of the result (if applicable) or calculation method used	
	7	animal test: - specific requirement for the animal used such as weight, age, sex (if applicable) etc - dose used and injection technique	

Table B: Checklist for Analytical Method Validation for Identification/ Characterisation Test

TEST	IDENTIFICATION/ CHARACTERISATION TEST (QUANTITATIVE TEST METHOD)		
PARAMETER	**No.**	**DOCUMENTS REQUIRED**	**AVAILABILITY**
Specificity	1	Testing Method	
	2	Acceptance criteria	
	3	Chromatograms/Images/ Electropherogram for following solutions (if applicable):- a) Standard b) Sample c) Blank/Placebo d) Markers (if applicable) e) Any supporting data to prove the method is specific	
	4	If involve calculation, an example of calculation and all the data which are needed to obtain the result must be provided	
System Suitability Testing (if applicable)	1	Parameters of system suitability	
	2	Acceptance criteria	
	3	Provide evidence such as HPLC chromatograms/UV spectrum (if applicable), result and any other data which are able to prove the system suitability tests are fulfilled	

Table C: Checklist for Analytical Method Validation for Assay/Content Test

TEST	ASSAY/CONTENT TEST		
PARAMETER	**No.**	**DOCUMENTS REQUIRED**	**AVAILABILITY**
Specificity	1	Testing Method	
	2	Acceptance criteria	
	3	Chromatogram/spectrum/ dose response profile for following solutions:- a) Standard b) Sample c) Blank/Placebo	
	4	Optical density/ absorbance reading or any data which is able to prove the acceptance criteria of specificity is fulfilled (if applicable)	
Linearity	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum five (5) levels of standard solutions unless otherwise justified	
	4	Data such as: a) Theoretical value and observed value or any data such as peak area/absorbance/ optical density which are needed to plot linearity graph b) Linear regression equation c) R^2 or R d) Linearity graph	
	5	An example of calculation to calculate theoretical value and observed value (if applicable/ depend on the test method)	
	6	Related data generated from validated software (if applicable) or data from one representative batch to demonstrate calculation	
Range	1	80% - 120% or sufficient to cover the specification range	
Accuracy	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum three (3) levels of concentration in triplicates covering the specified range	
	4	Data & result such as: a) Theoretical and observed value and any data such as peak area/absorbance/ optical density which are needed to perform the calculation	

		b) Theoretical and observed value and % recovery	
	5	An example of calculation to calculate theoretical value and observed value (if applicable/ depend on the test method)	
	6	Related data generated from validated software (if applicable) or data from one representative batch to demonstrate calculations	
Precision (Repeatability)	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum three (3) levels of concentration in triplicates covering the specified range , OR minimum six (6) replicates at 100% of working concentration	
	4	Data & result such as: a) Peak area/absorbance/ optical density/ any data which are needed to calculate result in unit as per stated in COA eg: mg/ml or IU/ml or percentage or others b) Result in unit as per stated in COA eg: mg/ml or IU/ml or percentage or others	
	5	An example of calculation to calculate theoretical value and observed value (if applicable/ depend on the test method)	
	6	Related data generated from validated software (if applicable) or data from one representative batch to demonstrate calculations	
Precision (intermediate precision/ ruggedness)	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum three (3) levels of concentration in triplicates covering the specified range , OR minimum six (6) replicates at 100% of the working concentration	
	4	Cover at least 2 parameters among variation of analyst, date and equipment	
	5	Data & result such as: a) Peak area/absorbance/ optical density/ any data which are needed to calculate result in unit as per stated in COA eg: mg/ml or IU/ml or percentage or others b) Result in unit as per stated in COA eg: mg/ml or IU/ml or percentage or others	
	6	An example of calculation to calculate theoretical value and observed value (if applicable/ depend on the test method)	
	7	Related data generated from validated software (if applicable) or data from one representative batch to demonstrate calculations	

System Suitability Testing	1	As per protocol of analysis	
	2	Acceptance criteria	
	3	Provide evidence such as HPLC chromatograms/UV spectrum, result and any other data which are able to prove the system suitability tests are fulfilled	

Table D: Checklist for Analytical Method Validation for Related Substances Test

TEST	RELATED SUBSTANCES		
PARAMETER	**No.**	**DOCUMENTS REQUIRED**	**AVAILABILITY**
Specificity	1	Testing Method	
	2	Acceptance criteria	
	3	Force degradation studies must be conducted and related chromatograms/ images must be provided.	
	4	Chromatogram/Image for following solutions:- a) Standard b) Sample c) Blank/Placebo d) Stress solution e) System suitability tests (if applicable)	
Linearity	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum five (5) levels of standard solutions unless otherwise justified (For methods where impurities are not available, standard levels spiked with degraded material are allowed.)	
	4	Data such as: a) Peak area (or theoretical value and observed value or any data which are needed to plot linearity graph) b) Linear regression equation c) R^2 or R d) Linearity graph	
	5	An example of calculation to calculate theoretical value and observed value (if applicable)	
Range	1	From the reporting level of an impurity to 120% of the specification unless otherwise justified	
Accuracy	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum three (3) levels of concentration in triplicates covering the specified range (LOQ – 120% of specification) unless otherwise justified	
	4	Data & result such as: a) Theoretical and observed value or any data such as peak area which are needed to perform the calculation	

		b) Theoretical and observed value and % recovery	
	5	An example of calculation to calculate theoretical value and observed value.	
Precision (Repeatability)	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum three (3) levels of concentration in triplicates covering the specified range , OR minimum six (6) replicates at 100% of the working concentration	
	4	Data & result such as: a) Peak area or any data which are needed to calculate result in unit as per stated in COA eg: mg/ml or IU/ml or percentage or others b) Result in unit as per stated in COA eg: mg/ml or IU/ml or percentage or others	
	5	An example of calculation to calculate result in unit as per stated in COA	
Precision (intermediate precision/ ruggedness)	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum three (3) levels of concentration in triplicates covering the specified range , OR minimum six (6) replicates at 100% of the working concentration	
	4	Cover at least 2 parameters among variation of analyst, date and equipment	
	5	Data & result such as: a) Peak area or any data which are needed to calculate result in unit as per stated in COA eg: mg/ml or IU/ml or percentage or others b) Result in unit as per stated in COA eg: mg/ml or IU/ml or percentage or others	
	6	An example of calculation to calculate result in unit as per stated in COA	
Quantitation Limit	1	Testing Method : visual observation / signal-to-noise / standard deviation of the response and the slope	
	2	Acceptance criteria	
	3	If based on visual observation / signal-to-noise, one representative chromatogram/ image for following solutions:- a) Placebo + spike standard at quantitation limit b) Diluted sample solution which showed main peak or peak of impurity c) Sample solution at quantitation limit	

	4	If based on calibration curve method a) Minimum five (5) levels of standard solutions b) Peak area values or related data for all concentrations c) One representative HPLC chromatogram at quantitation limit d) Data for linear regression equation, r^2, linearity graph and standard deviation	
	5	Value of signal and noise (if applicable)	
	6	Calculation/formulation where applicable	
	7	Value of quantitation limit	
	8	Validation data from the analysis of a suitable number of samples known to be near or prepared at the quantitation limit	
System Suitability Testing	1	As per protocol of analysis	
	2	Acceptance criteria	
	3	Provide evidence such as HPLC chromatograms, result and any other data which are able to prove the system suitability tests are fulfilled	