Checklist for Protocol Analysis and Analytical Method Validation

These checklists are intended to provide guidance on the submission of documents/ information for protocol of analysis and analytical method validation/ verification. The following checklists are not exhaustive and Centre for Quality Control (CQC), National Pharmaceutical Regulatory Division (NPRA) reserves the right to request additional data whichever it deems necessary. All submitted documents must be uploaded accordingly in QUEST 3+. Otherwise CQC, NPRA reserves the right to reject the documents.

Table A shows the information required for protocol of analysis. Table B, C, D and E illustrate validation parameters and documents required for validation of identification/ characterisation test, assay/potency/content test, related substances test and dissolution test respectively. Table E displays commonly acceptance criteria for each validation parameter. Justification or explanation must be provided if any information listed in tables below is not available.

The following verification parameters are required 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 **1st July 2018.**

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	
	2	List of chemical, reagents and media	
	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 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	Complete formula for calculation (if applicable) and interpretation of results	
	9	Image of SDS PAGE/ IEF/ electropherogram/ TLC/ UV spectrum/ IR spectrum/HPLC chromatogram etc for blank, sample, standard and system suitability solution	

Assay/ Potency/ Content/ Dissolution test	**Details of test methods shall include the following items:**		
• Protein concentration • Content of active ingredient and preservative • Bioassay/ Potency (animal- based, cell culture- based and biochemical- based) • Dissolution test • 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). Stability and storage condition of sample and standard solutions	
	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, 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	
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) Stability and storage condition of sample and standard solutions	
	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, 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/ TLC/ 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 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/ IR spectrum etc 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	
System Suitability Testing (if applicable)	1	Parameter of system suitability	
	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 C: Checklist for Assay/Potency/Content Test

TEST	ASSAY/CONTENT TEST		
PARAMETER	**No.**	**DOCUMENTS REQUIRED**	**AVAILABILITY**
Specificity	1	Testing Method	
	2	Acceptance criteria	
	3	Chromatogram/spectrum for following solutions:- a) Standard b) Sample c) Blank/Placebo	
Linearity	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum five (5) levels of standard solutions	
	4	Data such as: a) linear regression equation b) r^2/ r c) linearity graph	
Range	1	80% - 120% (Assay and Potency Test), 70% - 130% (Content Test)	
Accuracy	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum three (3) levels of concentration in triplicates covering the specified range	
	4	Data and result such as theoretical and observed value, % recovery/ difference between mean and accepted true value and confidence interval	
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 and result such as result in unit as per stated in COA eg: mg/ml or IU/ml or percentage or others, standard deviation, relative standard deviation (coefficient of variant) and confidence interval	
Precision (intermediate precision/	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum three (3) levels of concentration in triplicates covering the specified range , OR	

ruggedness)		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 and result such as result in unit as per stated in COA eg: mg/ml or IU/ml or percentage or others, standard deviation, relative standard deviation (coefficient of variant) and confidence interval	
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 Related Substances

TEST	RELATED SUBSTANCES		
PARAMETER	**No.**	**DOCUMENTS REQUIRED**	**AVAILABILITY**
Specificity	1	Testing Method	
	2	Acceptance criteria	
	3	Force degradation studies should be conducted (if applicable) 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	
Linearity	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum five (5) levels of standard solutions	
	4	Data such as: a) linear regression equation b) r^2/ r c) linearity graph	
Range	1	From the reporting level of an impurity to 120% of the specification	
Accuracy	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum three (3) levels of concentration in triplicates covering the specified range (LOQ – 120% of specification)	
	4	Data and result such as theoretical and observed value, % recovery/ difference between mean and accepted true value and confidence interval.	
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 and result such as result in unit as per stated in COA eg: mg/ml or IU/ml or percentage or others, standard deviation, relative standard deviation (coefficient of variant) and confidence interval	
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 and result such as result in unit as per stated in COA eg: mg/ml or IU/ml or percentage or others, standard deviation, relative standard deviation (coefficient of variant) and confidence interval	
Quantitation Limit (quantitative related substances test)	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, chromatograms/ images for following solutions:- a) placebo + spike standard at quantitation limit or b) sample solution at quantitation limit	
	4	If based on calibration curve method a) Minimum five (5) levels of standard solutions b) Peak area values and HPLC chromatograms or related data for all concentrations c) Data for linear regression equation, r^2, linearity graph and standard deviation	
	5	Value of signal and noise (if applicable)	
	6	Calculation/formulation (if applicable)	
	7	Value of quantitation limit	
	8	Validate estimated quantitation limit by independent analysis of suitable number of samples known to be near or prepared at the quantitation limit. Related data for independent analysis must be provided.	
Detection Limit (qualitative related substances test)	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, chromatograms/ images for following solutions:- a) placebo + spike standard at detection limit or b) sample solution at detection limit	
	4	If based on calibration curve method a) Minimum five (5) levels of standard solutions	

		b) Peak area values and HPLC chromatograms or related data for all concentrations c) Data for linear regression equation, r^2, linearity graph and standard deviation d) Validate estimated detection limit by independent analysis of suitable number of samples known to be near or prepared at the detection limit. Related data for independent analysis must be provided.	
	5	Value of signal and noise (if applicable)	
	6	Calculation/formulation (if applicable)	
	7	Value of detection 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	

* For qualitative related substances/ limit test, only parameters specificity, detection limit and system suitability tests are required.

Table E: Checklist for Dissolution

TEST	DISSOLUTION		
PARAMETER	**No.**	**DOCUMENTS REQUIRED**	**AVAILABILITY**
Specificity	1	Testing Method	
	2	Acceptance criteria	
	3	Chromatogram/spectrum for following solutions:-	
		a) Standard	
		b) Sample	
		c) Blank/Placebo	
Linearity	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum five (5) levels of standard solutions	
	4	Data such as: a) linear regression equation b) r2/ r c) linearity graph	
Range	1	Dissolution testing: ± 20% over the specified range Example 1: if the specification is NLT 75% *(Q)* of the labelled amount is dissolved in 45 minutes, the validated range would be 60 – 100% of the label claim Example 2: if the specification for a controlled released product cover a region from 20% after 1 hour, up to 90%, after 24 hours, the validated range would be 0 – 110% of the label claim	
Accuracy	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum three (3) levels of concentration in triplicates covering the specified range	
	4	Data and result such as theoretical and observed value, % recovery/ difference between mean and accepted true value and confidence interval.	
Precision (Repeatability)	1	Testing Method (using sample/product as the test solution)	
	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 WC	
	4	Data and result such as result in unit as per stated in COA eg: mg/ml or IU/ml or percentage or others,	

		standard deviation, relative standard deviation (coefficient of variant) and confidence interval	
Precision (intermediate precision/ ruggedness)	1	Testing Method (using sample/product as the test solution)	
	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 WC	
	4	Cover at least 2 parameters among variation of analyst, date and equipment	
	5	Data and result such as result in unit as per stated in COA eg: mg/ml or IU/ml or percentage or others, standard deviation, relative standard deviation (coefficient of variant) and confidence interval	
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	

Table D: Commonly Acceptance Criteria for Analytical Method Validation (Pharmaceutical Products)

NO	PARAMETER	ACCEPTANCE CRITERIA
1	Specificity	Absence of interfering peaks in the placebo, impurity demonstrate specificity
2	Linearity	$r^2 \geq 0.995$
		y-intercept at 100% working concentration ≤ 2%
3	Accuracy	Measured recovery within 95% - 105% or mean difference ± 2%
4	Precision (Repeatability)	RSD ≤ 2.0%
5	Precision (intermediate precision/ruggedness)	RSD ≤ 2.0%
6	Detection Limit	LOD peak must be visible
		If based on standard deviation of the response and the slope method, DL = 3.3 Ó/S
		If based on signal to noise, S/N= 3:1 or 2:1
7	Quantitation Limit	if based on visual observation method, accuracy and precision data at LOQ must be ± 20%
		If based on standard deviation of the response and the slope method, DL = 10 Ó/S
		If based on signal to noise, S/N= 10:1
8	System Suitability Testing (chromatographic method)	RSD ≤ 2%
		Theoretical plate/column efficiency, N ≥ 2000
		Tailing factor < 2
		Resolution > 2
9	System Suitability Testing (other method)	RSD ≤ 5%