Checklist for Analytical Method Validation (Chemical)

These checklists provide guidance for the validation/verification of the analytical procedures included as part of registration applications submitted to Centre for Quality Control (CQC), National Pharmaceutical Control Bureau (NPCB). Please take note the following checklists are not exhaustive and CQC, NPCB reserves the right to request additional data whichever it deems necessary. Furthermore, these checklists do not intend to provide direction on how to accomplish validation/verification of analytical procedures. Please take note also all documents submitted to CQC, NPCB must be arranged and labelled accordingly. Otherwise CQC, NPCB reserves the right to reject the documents submitted.

Table A shows the parameters required for the validation of assay and related substances (quantitative test method) analytical procedure. Table B illustrates the parameters required for the validation of related substances using limit test or qualitative analytical procedure. Table C addresses the parameters required for the validation of dissolution test. Table D displays commonly acceptance criteria for each validation parameter.

The following verification parameters are required for **COMPENDIAL METHOD** (assay/related substances/dissolution):

a) Specificity
b) Precision (intermediate precision)
c) System Suitability tests

These checklists shall come into force on **1st March 2015.**

TEST	ASSAY/RELATED SUBSTANCES (QUANTITATIVE TEST METHOD)		
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	
		d) Stress/spike solution	
		e) System suitability tests	
	4	Impurities available	
		a) Peak purity for PDA detector or spike solution for non-PDA detector	
	5	Impurities not available	
		a) Stress data (minimum data for humidity, heat and light)	
		b) Peak purity (LC method only)	
		c) Relative Retention Time (RRT) (chromatographic method only)	
Linearity	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum five (5) levels of standard solutions	
	4	Data for peak area/absorbance, linear regression equation, Y-intercept, slope, r^2 and linearity graph	
Range	1	For the assay of drug substances: 80% - 120% of working concentration (WC)	
	2	For the determination of an impurity: 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	
	4	Result : reported as percent recovery by the assay of known added amount of analyte in the sample, OR as the difference between the mean and the accepted true value together with the confidence intervals	
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	Result : SD, RSD 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	Result : SD, RSD and confidence Interval	
Quantitation Limit (related substances only)	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, chromatogram for following solutions:-	
		a) placebo + spike standard at quantitation limit	
		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 for all concentrations	
		c) Data for linear regression equation, Y-intercept, slope, r^2 and linearity graph.	
	5	Calculation/formulation where applicable	
	6	Value of quantitation limit	
	7	Accuracy and precision data known to be near or prepared at the quantitation limit must be provided	
System Suitability Testing (chromatographic method)	1	RSD, tailing factor, theoretical plate	
	2	Resolution (if two or more components)	
System Suitability Testing (other method)	1.	RSD	
Robustness (not mandatory)	1	Testing Method	
	2	Acceptance criteria	
	3	Result : refer acceptance criteria for accuracy and precision (repeatability)	

Table A: Checklist for Assay/Related Substances (quantitative test method)

TEST	RELATED SUBSTANCES (LIMIT TEST / QUALITATIVE TEST METHOD)		
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	
		d) Stress/spike solution	
		e) System suitability tests	
	4	Impurities available	
		a) Peak purity for PDA detector or spike solution for non-PDA detector	
	5	Impurities not available	
		a) Stress data (minimum data for humidity, heat and light)	
		b) Peak purity (LC method only)	
		c) Relative Retention Time (RRT) (chromatographic method only)	
Detection 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, chromatogram for following solutions:-	
		c) placebo + spike standard at detection limit	
		d) sample solution at detection limit	
	4	If based on standard deviation of the response and the slope method	
		a) Minimum five (5) levels of standard solutions	
		b) Peak area values for all concentrations	
		c) Data for linear regression equation, Y-intercept, slope, r^2 and linearity graph.	
		d) Accuracy and precision data known to be near or prepared at the detection limit must be provided	
	5	Calculation/formulation where applicable	
	6	Value of detection limit	
System Suitability Testing	1	RSD, tailing factor, theoretical plate	
	2	Resolution (if two or more components)	

(chromatographic method)			
System Suitability Testing (other method)	1.	RSD	
Robustness (not mandatory)	1	Testing Method	
	2	Acceptance criteria	
	3	Result : refer acceptance criteria for accuracy and precision (repeatability)	

Table B: Checklist for Related Substances (limit test / qualitative test method)

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	
		d) System suitability tests	
Linearity	1	Testing Method	
	2	Acceptance criteria	
	3	Minimum five (5) levels of standard solutions	
	4	Data for linear regression equation, Y-intercept, slope, r^2 and 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	Result : reported as percent recovery by the assay of known added amount of analyte in the sample, OR as the difference between the mean and the accepted true value together with the confidence intervals	
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	Result : SD, RSD and confidence Interval	
Precision (intermediate	1	Testing Method (using sample/product as the test solution)	
	2	Acceptance criteria	

precision/ ruggedness)	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	Result : SD, RSD and confidence Interval	
System Suitability Testing (chromatographic method)	1	RSD, tailing factor, theoretical plate	
	2	Resolution (if two or more components)	
System Suitability Testing (other method)	1	RSD	
Robustness (Not Mandatory)	1	Testing Method	
	2	Acceptance criteria	
	3	Result : refer acceptance criteria for accuracy and precision (repeatability)	

Table C: Checklist for Dissolution

NO	PARAMETER	ACCEPTANCE CRITERIA
1	Specificity	Absence of interfering peaks in the placebo, impurity demonstrate specificity
		Pass peak purity test (particularly for related substances test)
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% & CI
4	Precision (Repeatability)	RSD ≤ 2.0% & CI
5	Precision (intermediate precision/ruggedness)	RSD ≤ 2.0% & CI or mean difference ± 2% & CI
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%
10	Robustness (not mandatory)	Refer acceptance criteria for accuracy and precision (repeatability)

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