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

These checklists shall come into force on **1st July 2018.**

**Table A: 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):-<br>a) Standard<br>b) Sample<br>c) Blank/Placebo<br>d) Markers (if applicable)<br>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 B: 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:-<br>a) Standard<br>b) Sample<br>c) Blank/Placebo | |
| Linearity | 1 | Testing Method | |
| | 2 | Acceptance criteria | |
| | 3 | Minimum five (5) levels of standard solutions | |
| | 4 | Data such as:<br>a) linear regression equation<br>b) $r^2$/ r<br>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<br>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 C: 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:-<br>a) Standard<br>b) Sample<br>c) Blank/Placebo<br>d) Stress solution<br>e) System suitability tests | |
| Linearity | 1 | Testing Method | |
| | 2 | Acceptance criteria | |
| | 3 | Minimum five (5) levels of standard solutions | |
| | 4 | Data such as:<br>a) linear regression equation<br>b) $r^2$/ r<br>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<br>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<br>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:-<br>a) placebo + spike standard at quantitation limit or<br>b) sample solution at quantitation limit | |
| | 4 | If based on calibration curve method<br>a) Minimum five (5) levels of standard solutions<br>b) Peak area values and HPLC chromatograms or related data for all concentrations<br>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:-<br>a) placebo + spike standard at detection limit or<br>b) sample solution at detection limit | |
| | 4 | If based on calibration curve method<br>a) Minimum five (5) levels of standard solutions | |

| | | | |
|---|---|---|---|
| | | b) Peak area values and HPLC chromatograms or related data for all concentrations<br>c) Data for linear regression equation, $r^2$, linearity graph and standard deviation<br>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 D: 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:<br>a) linear regression equation<br>b) r2/ r<br>c) linearity graph | |
| Range | 1 | Dissolution testing: ± 20% over the specified range<br>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<br>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 E: 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%<br>or<br>mean difference ± 2% |
| 4 | Precision (Repeatability) | RSD ≤ 2.0% |
| 5 | Precision<br>(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<br>(chromatographic method) | RSD ≤ 2% |
| | | Theoretical plate/column efficiency, N ≥ 2000 |
| | | Tailing factor < 2 |
| | | Resolution > 2 |
| 9 | System Suitability Testing<br>(other method) | RSD ≤ 5% |