5.1.1.6 Limit of Detection
5.1.1.7 Limit of Quantitation
5.1.1.8 Robustness
5.1.1.9 Reproducibility (for biological test methods only)

5.1.2 Validation parameters applicable to different types of methods are indicated by an "X" in the tables below:

Table 1: For Non-Compendial Analytical and Biological Test Methods

Parameter	Identification	Impurity Quantitation	Impurity Limit Test	Assay	Dissolution	Biological Test Method	Physical Properties
Specificity	X	X	X	X	X	X	(a)
Linearity		X		X	X	X	(a)
Range		X		X	X	X	(a)
Accuracy		X		X	X	X	(a)
Precision Repeatability Intermediate Reproducibility		 X X (b) X (b)		 X X (b) X (b)	 X X (b) X (b)	 X X X	 X X (b) X (b)
Limit of Detection		X	X			(a)	(a)
Limit of Quantitation		X				X	(a)
Robustness	(a)	X	(a)	X	X	(a)	(a)

(a) To be considered if critical to the test method
(b) Reproducibility is not required for analytical test method validation, but its results may be used in place of intermediate precision.

Table 2: For Compendial Analytical and Biological Test Methods

Parameter	Identification	Impurity Quantitation	Impurity Limit Test	Assay	Dissolution	Biological Test Method	Physical Properties
Specificity	X (a)	X (a)	X (a)	X (a)	X (a)	X (a)	
Linearity		X (a)		X (a)	X (a)		(c)
Accuracy		X (a)		X (a)	X (a)	X	(c)
Precision Repeatability Intermediate Reproducibility		 X (a) X (a&b)		 X (a) X (a) X (a&b)	 X (a) X (a&b)	 X (a) X (a) X (a)	 X (a) X (b)
Limit of Detection		X (a)	X (a)				(c)
Limit of Quantitation		X (a)					(c)

known) or at approximately 1.0% of the analytical concentration of the active. If necessary, individual solutions of each degradant are prepared for the aid of identification. The chromatograms are inspected for interference. Photo-diode array detector or mass spectrometry can also be employed to assess peak purity. The known potential degradation products should be separated from each other and/or from other components in the same matrix.

5.2.1.2.7 Forced Degradation for API or Finished Products: If impurities or degradation products are not available, forced degradation can be carried out as per the following guidelines:

5.2.1.2.7.1 Light: Prepare 50 mL of the sample solution as per the analytical method. Store the sample in a light cabinet 2000Lux for one month (equivalent to 1.4 million Lux hours) or leave the sample under sunlight for 8 hours. Compare the chromatogram to that of a control chromatogram.

5.2.1.2.7.2 Heat: Prepare 50 mL of the sample solution as per the analytical method. Store in a 105ºC oven for 7 days or boil it on a waterbath for 7 hours). Compare the chromatogram to that of a control chromatogram.

5.2.1.2.7.3 Acid: Add 2 mL of 1 M HCl to 50 mL of the sample solution prepared as per the analytical method (Proposed: 2 mL of conc. HCl in 50 mL sample and boil for 1 hours on waterbath). Store for 7 days. Compare the chromatogram to that of a control chromatogram.

5.2.1.2.7.4 Base: Add 2 mL of 1 M NaOH to 50 mL of the sample solution prepared as per the analytical method (Proposed: 2 mL of 5 M NaOH in 50 mL sample and boil for 1 hours on waterbath). Store for 7 days. Compare the chromatogram to that of a control chromatogram.

5.2.1.2.7.5 Oxidation: Add 5 mL of 1.0%w/v hydrogen peroxide to 50 mL of the sample solution. Mix and store for 7 days. (Proposed: 1 mL of 30%w/v hydrogen peroxide in 50 mL sample and boil for 1 hours on waterbath) Compare the chromatogram to that of a control chromatogram.

5.2.1.2.7.6 Stress conditions can be changed since the aim of forced degradation is to ensure that the product / API is definitely degraded so that the method can be used to demonstrate that separation of active peak from any possible break-down peaks is achieved. Much stronger conditions, combined with heat, have better chances to achieve degradation and can also reduce waiting time and speed up the validation process.

5.2.1.3 For Dissolution Tests: Specificity needs to discriminate between the compound(s) of interest and any other compounds that are likely to be in the sample solution such as excipients, dissolution media reagents.

5.2.4.6.1 For assay methods (including methods for content uniformity), biological methods and quantitative impurity methods for APIs and drug products, accuracy shall be assessed using minimum of nine (9) determinations over a minimum of three (3) concentration levels covering the specified range of the test method [e.g., three (3) concentrations with three (3) replicate sample preparations each]. Results from each of the nine (9) determinations shall be 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.

5.2.4.6.2 For dissolution methods, accuracy shall be assessed using eighteen (18) determinations over a minimum of three (3) concentration levels covering the specified range of the test method [i.e., three (3) different concentrations, each consisting of six (6) replicate samples from six dissolution vessels]. Results from each of the eighteen (18) determinations must be reported. Individual recovery results must be within 97% to 103% of the input.

5.2.4.6.3 Accuracy shall be reported for each concentration level as percent recovery by assay of a known added amount of analyte in the sample or as the difference between the mean and accepted true value together with the confidence intervals.

5.2.5 **Precision**

5.2.5.1 Validation of tests for assay (including dissolution and content uniformity), quantitative determination of impurities and physical properties should include precision. Precision is expressed as the variance, standard deviation, or relative standard deviation (RSD).

5.2.5.2 Precision will be considered at three levels: (1) Repeatability, (2) Intermediate precision, and (3) Reproducibility.

5.2.5.3 Either intermediate precision or reproducibility is evaluated. It is not necessary to evaluate both.

5.2.5.4 **Repeatability**

5.2.5.4.1 For assay methods (including content uniformity), biological methods and quantitative impurity methods:

5.2.5.4.1.1 If the method requires composite sample to be ground, repeatability shall be assessed using a minimum of nine determinations over a minimum of three concentration levels over the specified range of the analytical method. [e.g., three different sample weights used providing three different concentration levels (e.g. 80%, 100% and 120%), each consisting of three replicate sample preparations].

5.2.5.4.1.2 If the method requires whole tablets to be used, six replicate samples are to be prepared and analysed as per method.

S = the slope of the calibration curve.

5.2.7.5 The slope S is estimated from the calibration curve of the analyte generated using a minimum of five points. The value of s shall be estimated using one of the following approaches:

5.2.7.5.1 Based on the Standard Deviation of the Blank
Measurement of the magnitude of the analytical background response is performed by analysing a minimum of five blank samples and calculating the standard deviation of the responses.

5.2.7.5.2 Based on the Calibration Curve
A specific calibration curve/s shall be studied using samples containing the analyte in the range of QL. The residual standard deviation (root mean square) of the regression line or the standard deviation of y-intercepts of multiple regression lines shall be used as the standard deviation.

5.2.7.6 The QL will be validated by the analysis of a minimum of three samples in the product matrix, known to be at the concentration of the QL, and demonstrating accuracy and precision.

5.2.8 **Robustness**

5.2.8.1 Robustness shall be considered during either the method development phase or validation on the type of procedure under study.

5.2.8.2 Robustness shall demonstrate the reliability of an analysis with respect to small deliberate variations in analytical parameters. If measurements are susceptible to variations in analytical conditions, the analytical conditions must be controlled or a precautionary statement included in the procedure.

5.2.8.3 Data collected during robustness studies shall be used to set or verify system suitability requirements.

5.2.8.4 Examples of typical variations include:
5.2.8.4.1 Stability of analytical solutions;
5.2.8.4.2 Sample extraction time;
5.2.8.4.3 Influence of variations of pH in HPLC mobile phase;
5.2.8.4.4 Influence of variations in HPLC mobile phase composition;
5.2.8.4.5 Different HPLC columns;
5.2.8.4.6 Different HPLC flow rates;
5.2.8.4.7 Different column temperatures;
5.2.8.4.8 Different filters;
5.2.8.4.9 Influence of variation of reagent quantity in dissolution media;
5.2.8.4.10 Influence of variation of instrument operation parameters for biological test.

5.2.8.5 Stability of reference standard and sample solutions involves the storing of solutions in the laboratory environment and in a refrigerated environment is compared to freshly prepared standard solutions. Suitable time points include 0, 6, 12, 24, 48, 72 and 120 hours. For reference standard solutions degradation of 1% or

