







2.5 Precision - the degree of agreement between replicate analyses of an homogenous sample, usually measured as the relative percent difference (RPD) between duplicates or the relative standard deviation (RSD) of a set of replicates. Normally the precision required for an assay of the active ingredients is on the order of 2%, which may require an internal standard method. Precision for trace impurities, where the instrument may be working closer to the detection limit, is often higher (15-20%). The ICH Guideline defines three precision measurements: (a) repeatability or short term precision as defined above, (b) intermediate precision which is essentially the same as intra-lab ruggedness, and (c) reproducibility which is essentially the same as inter-lab ruggedness. Replicate sample preparations should be analyzed as described above for accuracy.

Note that the homogeneity of a sample will depend on sample size, particularly for solids. Adequate sample size should be used to insure the sample is representative of the test article, as demonstrated by the reproducibility between aliquots.

- 2.6 Internal Standard (IS) - a compound of similar chemistry and structure to the analyte which is used to correct the response for variations in injection amount and other instrument and chromatographic variables. In LC methods, the IS compound should elute at a similar retention time, preferably after the drug to avoid potential interference from faster eluting, more polar metabolites.
- 2.7 Specificity (Selectivity) - the degree of bias (or lack thereof) caused by expected sample components and common interferences, determined by measuring the analyte with and without anticipated interferences. For example, it has been recommended that when developing an analysis for a drug in blood or plasma, that at least six independent sources of blank matrix be tested for interferences. A known reference material may be spiked with known impurities and product excipients (i.e. placebo). Degradation and metabolite products of the matrix and drug may be obtained from stress tests. See also 2.14 Stability Indicating Methods. Peak purity should be addressed, commonly through the use of a second detector.
- 2.8 Detection Limit (LOD or DL) - the lowest concentration which can be detected with confidence (usually at the 99% confidence level), estimated as three times the standard deviation (SD) of the background signal of low level sample spikes (3-5X the estimated DL). An instrument detection limit (IDL) may be determined from low level standards or method blanks as opposed to a sample LOD determined from low level sample spikes. The 3 X SD gives a concentration which is statistically known as the 99% confidence level at which the experimental value is known to be above the background concentration (zero). When using standards or spikes, the concentration must be reduced to a level which challenges the analysis, i.e. a level at which the precision of the determination increases over the errors normally experienced at higher concentrations. For example, if precision at 100 ppm is generally demonstrated by an RSD of 10%, the concentration should be reduced until the precision is significantly greater than 10% (perhaps 15-50% RSD).

- 2.9 Limit of Quantitation (LOQ) - the concentration level above which the concentration can be determined with acceptable precision (typically RSD < 10 - 25%) and accuracy (typically 80-120% recovery), usually estimated as ten times the SD of the background or low level sample spikes. A standard near the LOQ should be included in the calibration curve for method used for the quantitation of impurities and degradation products.
- 2.10 Range and Linearity - the variance in the response with concentration, measured as the RSD of response factors or the correlation coefficient ( $R^2$ ) from a linear regression fit. Note that a normal, unweighted regression fit normally weights the high concentration and understates errors at low concentration. For this reason a response factor graph is preferable. For an assay method, six determinations should be made in the range of 25-125% of anticipated or specified levels. For quantitative determination of impurities and degradation products, the lower level should be extended to the LOQ or background level. Calibration curves are not forced through zero.
- 2.11 Robustness - the capacity of a method to remain unaffected by small, deliberate variations in the method parameters that one might experience during normal usage, determined by varying reagent and eluent composition (typically 2% variation) and pH, columns (at least two from different lots of the same part number), column temperature, flow rates, extraction times, etc. Robustness may be proposed as part of a separate study after other validation parameters are demonstrated.
- 2.12 Ruggedness (intermediate precision) - the variance in the analysis of homogenous samples between analysts, instruments, columns, and on different days. May include inter-laboratory performance.
- 2.13 Stability - Replicates of a homogenous sample should be stored for "worst case" holding times (1-2 weeks) before analysis under typical storage conditions and compared to results from analysis performed immediately after the samples were prepared. Stability of the sample and standard solutions, dilutions, or digests should be addressed.
- 2.14 Stability Indicating Method (SIM) - a validated analytical procedure that accurately and precisely measures active ingredients (drug substance or drug product) free from potential interferences like degradation products, process impurities, excipients, or other potential impurities. The FDA recommends that all assay procedures for stability studies be stability indicating. This involves demonstrating that interferences are not produced during degradation of the sample. This requires degraded samples to be tested during the development or validation phase of the study. The goal of the SIM is to obtain baseline resolution of all the resulting products (the API and all the degradation products) with no coelutions. Forced degradation of samples by acid and base hydrolysis, oxidation, heat, and light is beyond the scope of this SOP, as it is typically performed by our clients and based on their knowledge about degradation pathways and products that could form during storage. Degradation of at least 10-30% of the assay peak is recommended.

### 3.0 PROCEDURE






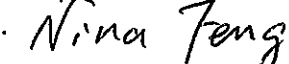



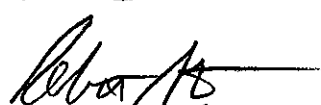
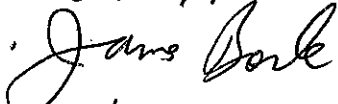
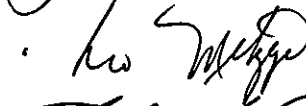


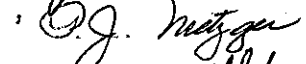
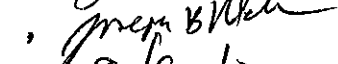



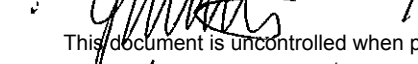
- 3.1 Analysts will be assigned by the Study Director, Technical Director, or Group Leader for conducting the validation work. They must have documentation of training on the associated instrumentation and/or technique. If ruggedness is an included parameter, a second analyst will be assigned.
- 3.2 Prior to method validation, when method development is first contracted, clear goals or guidelines should be identified for performance characteristics.
- 3.3 Create a draft protocol that addresses the elements of method validation pertinent to the analysis (sec. 1.3). If any elements are declined by the client, a scientifically valid justification must be included in the protocol. A signed copy of the protocol must be filed in the Job Envelope before the analysis of samples begins. Validation protocols should include the following items:
  - 3.3.1 Description of the analyte of interest (e.g. chemical name, structure, etc.).
  - 3.3.2 List of standards, placebos, and samples (including identification numbers) to be used.
  - 3.3.3 References relevant to the protocol (e.g. method development reports, methods or previous validations).
  - 3.3.4 Acceptance criteria for validation.
  - 3.3.5 The protocol must be approved by the client; the technical reviewer (the Study Director, Technical Director, or Group Leader), and Quality Assurance.
  - 3.3.6 Attachments: the draft SOP to be validated, the specification, or other relevant product information.
- 3.4 Assemble any required equipment and reagents. Ascertain that equipment has been qualified and reagents are within expiry. Perform any suitability tests required by the method.
- 3.5 Conduct the tests as outlined in the signed, approved protocol.
- 3.6 Any deviations must be documented in the data package and corrected or justified. Any observed deficiencies in the method will be noted in the report. Risk assessment and toxicological evaluation of method deficiencies are the responsibility of the client.
- 3.7 The analyst, as assigned by the Group Leader, will prepare the draft method validation report.

3.8 Submit the data package and draft report for review by a Senior Chemist (or above), Group Leader, or Technical Director.

3.9 Submit the data package and draft report for QA review.

3.10 Following QA review, the draft report is submitted to the client and the final report prepared. The final reports will include a copy of the raw data.

The following people have read this SOP and are currently using these procedures in the laboratory:

Signature	Date
	06.28.09
	06/29/09
	06/29/09
	06/29/09
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	7/15/09
	07/21/09

· Jones 07-21-09  
 · Louis Albanese 07-21-09  
 · [Signature] 07-21-09

SOP Review Log

<u>Reviewed by</u>	<u>Date</u>	<u>Approved by</u>	<u>Date</u>
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