__ Was the API prepared at approximately the same batch size (e.g. within +25% linear scale of the validated batch size)?

Requirements, acceptance criteria, and conclusions for the homogeneity study may be included in the process validation documents, or may be presented in separate documentation that is referenced in the validation documents.

Materials to be tested

__ Homogeneity shall be demonstrated for finished APIs unless otherwise justified and documented.

__ The need to show homogeneity of isolated critical intermediates should be considered on a case-by-case basis depending on how the intermediate is used in subsequent processing. In general, studying the homogeneity of an intermediate is of less importance than that of a final

API, especially if the intermediate will be dissolved in the next step of the processing. If homogeneity of the intermediate is critical to the quality of the final API prepared from it, demonstration of intermediate homogeneity should be considered.

__ Homogeneity testing is typically not needed when the API is a liquid, because of the inherent homogeneous nature of such materials.

Selection of Test Methods for Examining Homogeneity

Three measurements are typically considered for a given homogeneity study: one to demonstrate chemical homogeneity, one to demonstrate physical homogeneity (if appropriate), and one to demonstrate the effectiveness of the drying process (if appropriate). Appropriately chosen analytical tests in these categories usually eliminate the need to perform other analytical tests to show homogeneity.

__ Chemical Homogeneity: Process impurity testing is generally the preferred analytical methodology for examining chemical homogeneity of small molecules, but other analytical techniques may be used. For large molecules, the consistency of the profile of heterogeneity of product-related molecular variants is demonstrated by appropriate techniques.

__ Physical Homogeneity: Evaluation for physical homogeneity may include tests such as those for particle size distribution, crystal form uniformity, and/or bulk volume. Where physical quality attributes have not been established for the API, physical homogeneity need not be demonstrated. If this is the case, it should be explained in the validation protocol.

__ Effectiveness of Drying: This may be important because residual solvents (including water) are considered process impurities. This assessment is especially important for higher risk cases such as vacuum tray driers used for static drying operations. Attention should be focused on either the last solvent used in the process, and/or the solvent that is most difficult to remove from the API.

complete mapping studies (of temperature, pressure, or air flow) on freeze driers, shelf driers, and ovens.

Acceptance Criteria for Evaluating Homogeneity Test Results

__ Chemical homogeneity results are often evaluated using a statistical criterion. An F-Test is appropriate for this evaluation and is used to compare the variability of the results of homogeneity samples from the batch to the variability of the test method (4). The purpose of the evaluation is to see if the variability of results seen with the samples from the batch is significantly greater than the variability that arises because of the test method. An assessment of method precision that examines the full analytical system is not needed for this

F-test comparison, because the sample set from a batch is typically run within a short period of time, and often by just one analyst. A set of six replicate determinations (including separate sample preparations) or recent system suitability results are generally regarded as a sufficient assessment of the method variability for this purpose.

Some considerations for use of the F-test:

__ When performing the F-Test, a generally accepted criterion is based on 95% confidence that the variability between results for the set of homogeneity samples is not different than the variability exhibited by the test method. Critical F values for a one-tailed test are used since one is only testing the data to determine if the variability of samples results is greater than method variability.

__ When the magnitude of the numbers being examined approaches that of the uncertainty of the measurement, the F-test is less reliable and may not be appropriate. For instance, it is recommended that HPLC impurity amounts below about 0.2% not be evaluated using the F-test. In these cases, rather than requiring a statistical analysis on a different measure (such as API purity), examine the range of impurity results for the individual samples from a given batch. When all results for the batch are qualitatively similar, the results support homogeneity of the material.

__ There is no indication of inhomogeneity in the case of method variability being greater than variability of sample results, thus obviating the need for a two-tailed test.

__ **Appendix I** provides an example of the application of the F-test.

__ Effectiveness of drying: A statistical criterion such as the F-test recommended for evaluating chemical homogeneity may be used for evaluating effectiveness of drying (especially for residual solvents), such as when experience with the process indicates consistent control of
drying. Some processes, most notably those involving hydrates or solvates, may exhibit more variability in moisture levels, and thus may not be not well suited for

Method variability: replicate determinations on sample 8 of Batch 101	Results for Impurity A
Determination 1	0.40
Determination 2	0.36
Determination 3	0.39
Determination 4	0.42
Determination 5	0.44
Determination 6	0.39
Mean	0.4000
Standard deviation of Method for Impurity A	0.0276
Method variance for Impurity A ($= S^2_{method}$)	0.0007600

Use of the F-test begins with the assumption of a null hypothesis, H_0: "The variability of the sample set is not different than the variability of the method, with 95% confidence." The 95% confidence level is a standard degree of certainty that is widely accepted for evaluations such as this. The null hypothesis is true when the calculated F value, a ratio of variances, is less than a value of Critical F obtained from a statistical table (or see reference 4 for an on-line resource for finding Critical F values). using values for a one-tailed test with P = 0.05 (i.e., probability of 5% that null hypothesis is not true, which is the same as 95% confidence that the null hypothesis is true).

The F function used for obtaining Critical F values should be based on a one-tailed test, which is appropriate for this application because we are concerned only about values where sample set variance is greater than method variance, and not the inverse situation. Thus, in circumstances where method variance is greater than the variance from the sample set being examined, no calculation of F is needed because the sample data shows little variability, confirming homogeneity. The Critical F value obtained from the table is also dependent on the number of "degrees of freedom" from the numerator and denominator used to calculate F from the data being analyzed. The degrees of freedom of each variance determination = number of determinations minus 1. Thus, if ten data points were used to determine the variance of the numerator and six data points were used to determine the variance of the denominator, the degrees of freedom from the numerator and denominator are nine and five, respectively, and

therefore critical F is 4.772, as obtained from a statistical table of critical F values for a one-tailed test with P = 0.05.

