Guidance Number: 027

Appendix I. Example Use of F-test as a statistical evaluation criterion for homogeneity

The following information provides an example of homogeneity data for three batches. A statistical evaluation and discussion of these data is provided.

Sample number	Batch 101 – results for Impurity A	Batch 102 – results for Impurity A	Batch 103 – results for Impurity A
S1	0.39	0.52	0.24
S2	0.40	0.47	0.30
S3	0.37	0.50	0.38
S4	0.42	0.53	0.22
S5	0.39	0.55	0.35
S6	0.41	0.46	0.37
S7	0.40	0.49	0.25
S8	0.40	0.49	0.19
S9	0.37	0.51	0.30
S10	0.39	0.46	0.37
S11	0.39	No sample	0.21
S12	0.36	No sample	No sample
Mean	0.391	0.498	0.289
Standard deviation (s)	0.0173	0.0301	0.0708
Variance (s^2_{lot})	0.0002992	0.0009067	0.005009

The statistical evaluation involves use of the F test to compare the variability (or "variance") observed within a set of samples from a given batch to the variance observed for the analytical test method. Method variance is calculated from replicate determinations (involving multiple sample preparations and assays) on a single sample. These data may be information generated from replicate determinations on a reference sample, such as what may have been done during validation of the assay method, or that from verifying performance of the method as part of routine lab operations, or data obtained specifically for the validation study by performing additional determinations. In any case, one should be certain that method variability data was generated for the same version of the test method used to analyze the homogeneity samples.

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.

The table below summarizes the statistical calculations of F from the data compiled in the above tables. As illustrated with batch 101, F_{calc} (the ratio of variances) need only be determined when the variance for the sample set is greater than that for the method.

Batch 101	
$F_{calc} = S^2_{lot} / S^2_{method}$	S^2_{method} is larger than S^2_{lot} so method variance is greater than that for sample set.
Conclusion	Null hypothesis H_0 (batch is homogeneous) is supported.
Batch 102	
$F_{calc} = S^2_{lot} / S^2_{method}$	1.19
Critical $F_{(9,5)}$	4.772
Conclusion	F_{calc} is less than Critical F, so H_0 is supported .
Batch 103	
$F_{calc} = S^2_{lot} / S^2_{method}$	0.005009 / 0.0007600 = 6.591
Critical $F_{(10, 5)}$	4.735
Conclusion	Here, F_{calc} is greater than critical F, so H_0 is not supported by the statistical analysis. However, examining the data, it is apparent the variability of the results for the sample set is not very large (standard deviation = 0.0708). If from a practical standpoint all the results are similar, and if all results are within specification, a conclusion that the lot is homogeneous is appropriate.