A Comparison of the Analysis of Covariance (ANCOVA) and Range-Based Approaches for Assessing Batch-to-Batch Variability of the Stability of Pharmaceutical Products

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Stability data were generated by the Monte Carlo method, and batch-to-batch variability was evaluated by analysis of differences in slope and intercept according to the analysis of covariance (ANCOVA) approach recommended in the FDA Guidance. Using the same generated data, batch-to-batch variability was also evaluated by assessing the equivalence of shelf lives estimated for individual batches based on the range (Range-based approach) in order to compare the ability of the two approaches to detect stability differences among batches. The results of the study indicated that the Range-based approach can detect a 30% difference in the slope of degradation curves among batches with a similar β error as the ANCOVA approach, provided that degradation data are obtained with assay errors below 0.5. The range-based approach appears to be useful as an alternative method to ANCOVA, if it is modified such that the variance of estimates is taken into account.

Key words stability; shelf-life; equivalence; range; analysis of covariance (ANCOVA)

The shelf life of pharmaceutical products in which a quantitative characteristic changes with time is usually estimated as the time at which the 95% one-sided confidence limit for the mean curve intersects the acceptance criterion.[1)] As the amount of data used for shelf life estimation increases, the confidence interval becomes narrower, thus allowing for longer shelf life estimates. The ICH Tripartite guideline of stability testing for new drug substances and products[2)] allows for the combination of stability data from different batches into one overall estimate, provided that batch-to-batch variability is small. Batch-to-batch variability can be assessed based on differences in the slope and intercept of the mean curve for each batch by analysis of covariance (ANCOVA). However, the power of ANCOVA depends largely on the assay error, such that it decreases markedly with increasing error.[3)] Thus, stability differences among batches is more likely to be overlooked in data with a larger assay error.

The authors previously proposed an approach for assessing the equivalence of shelf life estimates obtained for individual batches based on the range (Range-based approach)[4)] as an alternative method to ANCOVA. The effect of assay error on the ability of the Range-based approach to detect stability differences was found to be much smaller than that of ANCOVA. However, in the previous study, the Range-based approach was compared with the ANCOVA approach in which the differences in slope and intercept are simultaneously assessed by testing the uniformity of regression. This ANCOVA approach is different from the ANCOVA approach recommended in the FDA Guidance,[5)] in which the differences in slope and intercept are assessed separately.

In the present study, using data generated by the Monte Carlo method, batch-to-batch variability was assessed by the ANCOVA approach recommended in the FDA Guidance. PASG Excel routine for shelf-life estimation[6)] developed based on the FDA SAS Drug Formulation Stability Program,[7)] was used in the study. Using the same generated data, batch-to-batch variability was also assessed by the Range-based approach in order to compare the ability of the two approaches to detect stability differences among batches.

Experimental

Data Generation Five hundred sets of stability data from three batches were generated using the Monte Carlo method under the assumption that degradation of a drug product can be described by zero-order kinetics. The intercept of the degradation curve was assumed to be 100%, and the slope was assumed to be 0.1%/month for two batches and 0.12%/month or 0.13%/month (a 20 or 30% larger slope) for the remaining batch. Experimental assay data with errors were obtained at 0, 3, 6, 9, 12 and 18 months by adding random numbers selected from a normal distribution (a mean of zero and a standard deviation of 0.02—2.0%) to the theoretical value at each time point. Two samples from each batch were assayed at each time point. Microsoft Excel 2000 was used to generate the data. As shown in Fig. 1, the distribution of assay errors added to the theoretical drug content was consistent with a normal distribution, as represented by the solid line. Typical degradation data generated are shown in Fig. 2.

Assessment of Batch-to-Batch Variability by ANCOVA Approach The batch-to-batch variability of the 500 sets of generated stability data was evaluated using PASG Excel routine. Upper and lower specification limits were assumed to be 105 and 95%, respectively. The probability of overlooking the stability differences among batches (β error) was calculated from the probability of the conclusion that the regression lines from different batches have a common slope and a common zero-time intercept.

Assessment of Batch-to-Batch Variability by Range-Based Approach Using the 500 sets of generated stability data, shelf life was estimated for individual batches by PASG Excel routine. The range of shelf life estimates obtained for individual batches (the difference between the largest and

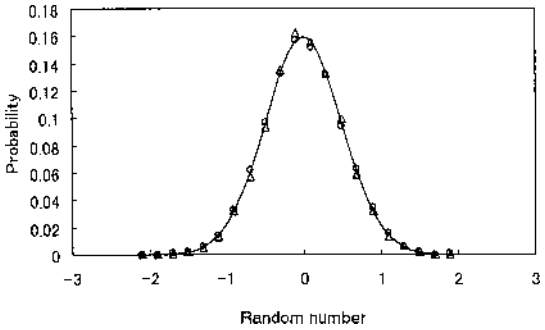

Fig. 1. Distribution of Random Numbers Used for Generating the Data of 30% Stability Difference among Batches and 0.5% Assay Error (○), and the Data of 20% Stability Difference and 0.5% Assay Error (△)

Solid line represents theoretical normal distribution.

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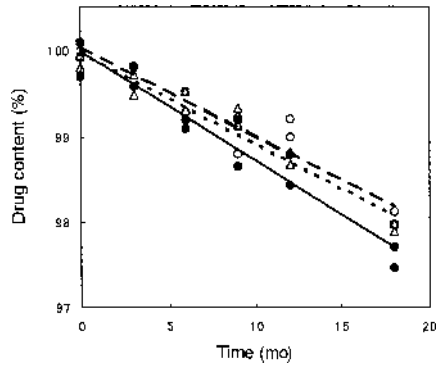

Fig. 2. Typical Degradation Data Generated for Three Batches

Batch A (○), batch B (△), and batch C (●). Lines are regression curves for batch A (···), batch B (– – –), and batch C (——). The slope of degradation curve was 0.1%/month for batches A and B, and 0.13%/month for batch C. Assay error: 0.2%.

smallest of three estimates) was calculated. If the range was smaller than 15% of the largest shelf life estimate, the shelf life estimates for individual batches were considered to be equivalent. The probability of overlooking the stability difference among batches (β error) was calculated from the probability of falsely determining that shelf lives estimated for individual batches were equivalent. The critical point of 15% was chosen based on the previous discussion.[4)]

Results and Discussion

It is often important in practical new drug applications to determine if a product has a shelf life longer than 36 months from stability data observed up to 18 months. In the present simulation, assay data from two samples were generated at 0, 3, 6, 9, 12 and 18 months. The slope of the mean degradation curve for the two stable batches was assumed to be 0.1%/month such that a shelf life of approximately 36 months is obtained at a lower specification limit of 95% and an assay error with a standard deviation of 0.5%. The slope for the remaining batch was assumed to be 20 or 30% larger so as to give a shelf life approximately 6 months shorter than that of the other two batches. The time at which the mean curve intersects the lower acceptance criterion (theoretical shelf life) was calculated from the generated data for individual batches. The time at which the lower 95% confidence limit for the mean curve intersects the lower specification limit (estimated shelf-life) was also calculated from the generated data. Figure 3 shows the distributions of theoretical shelf lives (B) and shelf life estimates (A) for individual batches. A small scale (size 500) of simulation is generally considered to provide a large variance of estimates. However, it is suggested that the 500 sets of generated stability data in the present study is sufficient to compare the ANCOVA and Range-based approaches, since a similar distribution was observed for the two stable batches having the same slope.

Figure 4 shows the probability of overlooking stability differences among batches (β error) observed in the ANCOVA approach and the Range-based approach. The β error of the ANCOVA approach increased with assay error. On the other hand, the Range-based approach exhibited β errors with a maximum at a certain assay error value. For a 30% difference in the slope of degradation curve among batches, the ANCOVA approach showed smaller values of β error than the Range-based approach at assay errors up to 0.3%. However, the ANCOVA approach exhibited a greater β error at an assay error of 0.5%, and the difference in β error between

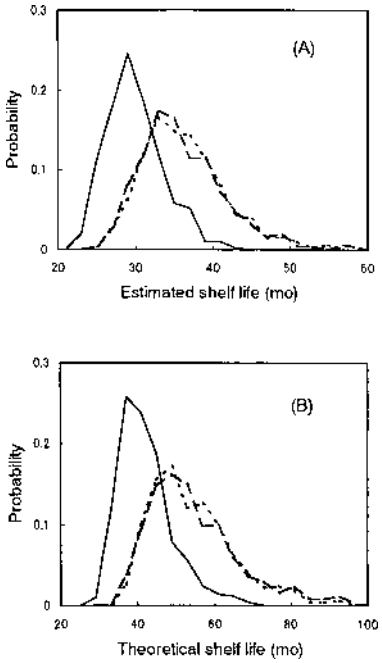

Fig. 3. Distributions of Estimated Shelf Lives (A) and Theoretical Shelf Lives (B) from Three Batches

··· batch A, – – – batch B,—— batch C. The slope of degradation curve was 0.1%/month for batches A and B, and 0.13%/month for batch C. Assay error: 0.5%. Theoretical shelf life is the time at which mean degradation curve intersects the acceptance criterion (95%).

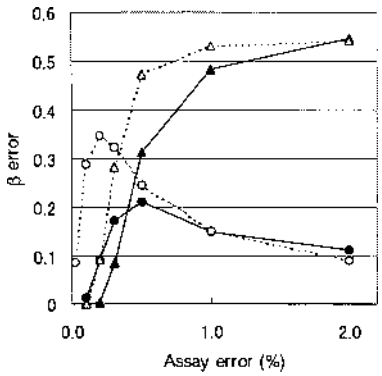

Fig. 4. Effect of Assay Error on the β Error of the ANCOVA Approach (△▲) and the Equivalence Approach (○●)

Stability difference among batches : 20% (△○) and 30% (▲●).

these two approaches increased with assay error. If degradation data are obtained at assay errors below 0.5%, both ANCOVA and Range-based approaches can be considered to have a similar ability to detect stability differences among batches. At larger assay errors, the Range-based approach exhibits a much smaller probability of overlooking the stability differences.

When stability differences among batches decreased to 20%, the β errors of both ANCOVA and Range-based approaches increased. At an assay error of 0.3%, no large difference in β error was observed between the two approaches. At smaller assay errors, the β error of the Range-based approach was larger than that of the ANCOVA approach, and *vice versa*.

The ANCOVA approach analyzes differences in slope

among batches based on the sum of all deviations from a mean slope. On the other hand, the Range-based approach assesses differences in shelf life estimates based on a single value of range calculated from the longest and shortest shelf life estimates. Thus, the variance of predicted shelf lives is not taken into account. Therefore, the sensitivity of the approach may be affected by the number of batches used in the test. Further studies may be needed to establish another approach for assessing the difference in shelf life estimates that takes into account the variance of estimates to avoid this problem. However, the results obtained in the present study suggest that the Range-based approach may be an alternative to the ANCOVA approach, since it was found that the Range-based approach can detect a 30% difference in the slope of the degradation curve among batches with a similar value of β error as the ANCOVA approach, provided that degradation data are obtained with assay errors below 0.5.

The critical point of 15% used in the Range-based approach was chosen not to provide β error higher than 20%, as previously reported.[4] In order to justify this value of critical point, not only β error but also α-error should be taken into account. Although the present study compared the ANCOVA and Range-based approaches on β error, further studies may be needed to compare these approaches based on α- error.

References and Notes

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