Statistical Evaluation of Accelerated Stability Data Obtained at a Single Temperature. I. Effect of Experimental Errors in Evaluation of Stability Data Obtained

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Accelerated stability data obtained at a single temperature is statistically evaluated, and the utility of such data for assessment of stability is discussed focussing on the chemical stability of solution-state dosage forms. The probability that the drug content of a product is observed to be within the lower specification limit in the accelerated test is interpreted graphically. This probability depends on experimental errors in the assay and temperature control, as well as the true degradation rate and activation energy. Therefore, the observation that the drug content meets the specification in the accelerated testing can provide only limited information on the shelf-life of the drug, without the knowledge of the activation energy and the accuracy and precision of the assay and temperature control.

Keywords drug stability; accelerated stability test; experimental error; stability data analysis; shelf-life; statistical evaluation

Drug degradation in a solution-state generally follows simple kinetics, and the degradation rate constant conforms to the Arrhenius relationship. For dosage forms such as oral, parenteral and ophthalmic solutions, therefore, the time required for 10% degradation at 25 °C, $t_{90(25)}$, can be extrapolated from the rates observed at several levels of elevated temperature. The $t_{90(25)}$ obtained by such extrapolation can support the shelf-life determined from the stability data at 25 °C, though the shelf-lives are determined by not only the change of drug content but also by the changes in the other characteristics of the product. Estimation of $t_{90(25)}$ requires accelerated test data obtained at several temperatures; data obtained at a single elevated temperature are not enough. Accelerated test data in the stability documents for new drug applications, however, usually include single temperature (40 °C) data. Therefore, we statistically evaluated the feasibility of using accelerated testing data obtained at a single temperature, 40 °C, to support the shelf-life. As a result, experimental errors in drug assay or temperature control were found to have a large effect on results extrapolated from the accelerated test data.

This paper describes the effect of experimental errors on the ability to correctly assess the stability from the fact that the drug content of a product is observed to be within the lower specification limit in an accelerated test carried out at 40 °C, and discusses how the accelerated test data should be interpreted.

Theoretical

Assay errors generally follow a normal distribution. The probability that a certain value of drug content, x, is observed in the assay can be related to the true drug content, μ, by Eq. 1:

$$P(x)=\frac{1}{\sqrt{2\pi}\sigma}\exp\left[-\frac{(x-\mu)^2}{2\sigma^2}\right] \quad (1)$$

where σ is the standard deviation of the assay. Assuming zero-order kinetics, the time required for 10% degradation at 25 °C, $t_{90(25)}$, can be related to the percent remaining at time t at 40 °C by Eq. 2:

$$t_{90(25)}=C/(100-\mu) \quad (2)$$

where μ is the true value of the percent remaining at time t, and C is represented by Eq. 3:

$$C=10\left\{\exp\left[-\frac{E_a}{\boldsymbol{R}}\left(\frac{1}{313}-\frac{1}{298}\right)\right]\right\}\cdot t \quad (3)$$

In Eq. 3, E_a represents the activation energy and $\boldsymbol{R}$ is the gas constant. Inserting Eq. 2 in Eq. 1 yields:

$$P(x)=\frac{1}{\sqrt{2\pi}\sigma}\exp\left[-\frac{(100-C/t_{90(25)}-x)^2}{2\sigma^2}\right] \quad (4)$$

Integrating Eq. 4 in the range where x is larger than 90%, provides the probability that the drug content is observed to be larger than 90% as a function of $t_{90(25)}$. Programs for the calculation were written in BASIC and compiled on a PC-9801 VX (NEC, Tokyo).

Results

Assuming that the drug product is assayed every two months (2nd, 4th, and 6th month) during storage at 40 °C, the probability that the drug content is observed to be larger than 90% at each time depends on the $t_{90(25)}$, E_a and the standard deviation of the assay, as represented by Eq. 4. Figure 1 shows the probability plotted against $t_{90(25)}$ assuming 2% of the standard deviation and 20 kcal/mol of E_a. The solid line represents the probability that the drug

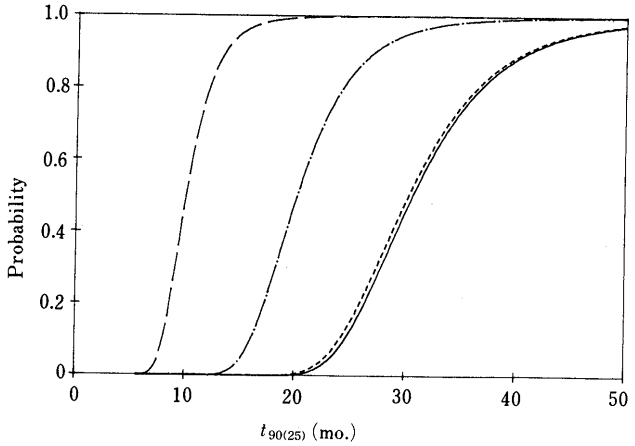

Fig. 1. Probability That Drug Content Is Observed to Be Larger than 90% as a Function of $t_{90(25)}$

Testing period: ——, 2; —·—, 4; ------, 6 months. E_a: 20 kcal/mol. Assay standard deviation: 2%. The solid line represents the probability for the drug to meet the specification in all the tests after 2, 4 and 6 months.

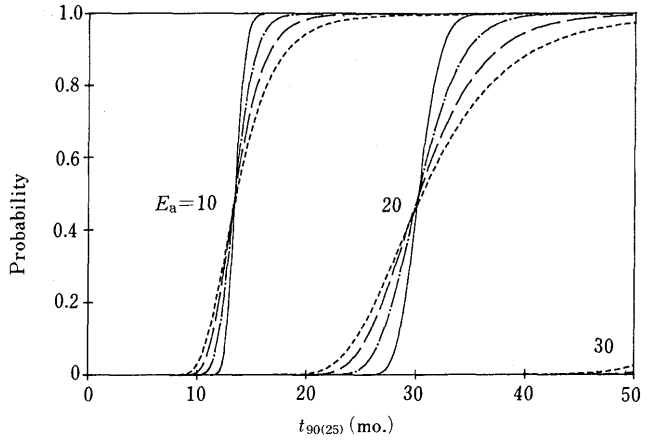

Fig. 2. Effect of Assay Errors on Probability That the Drug Content Is Observed to Be Larger than 90% at 2nd, 4th and 6th Month for Various Values of E_a

Assay standard deviation: ——, 0.5; —·—·, 1; – – –, 1.5; ------, 2%. Numbers represent the E_a (kcal/mol).

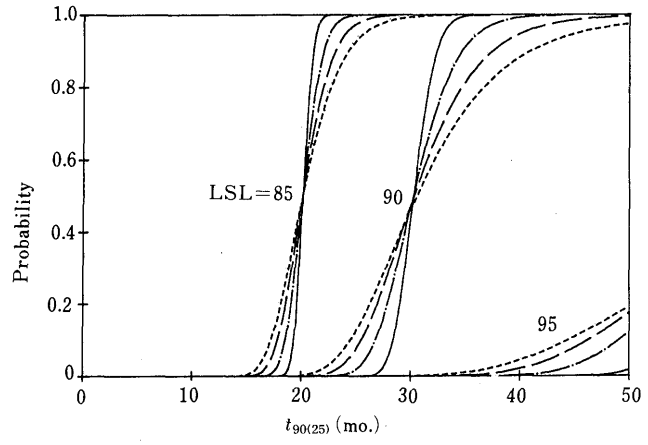

Fig. 3. Effect of Assay Errors on Probability That the Drug Content Is Observed to Be Larger than the Low Specification Limit at 2nd, 4th and 6th Month

Assay standard deviation: ——, 0.5; —·—·, 1; – – –, 1.5; ------, 2%. E_a: 20 kcal/mol. Numbers represent the lower specification limit, LSL (%).

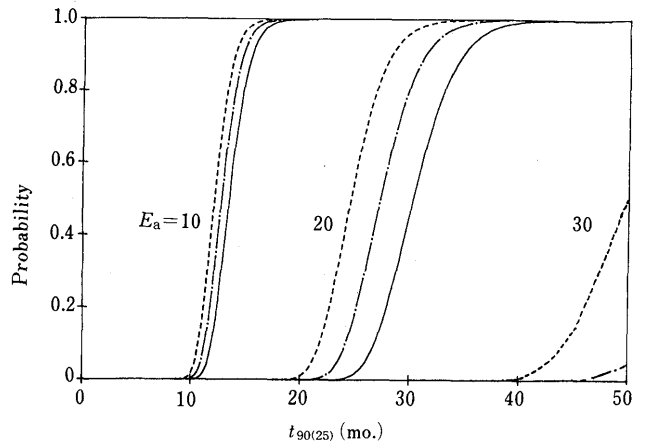

Fig. 4. Effect of Temperature Control Errors on Probability That Drug Content Is Observed to Be Larger than 90% at 2nd, 4th and 6th Month

Temperature: ——, 40; – – –, 39; ------, 38 °C. Assay standard deviation: 1%. Numbers represent the E_a.

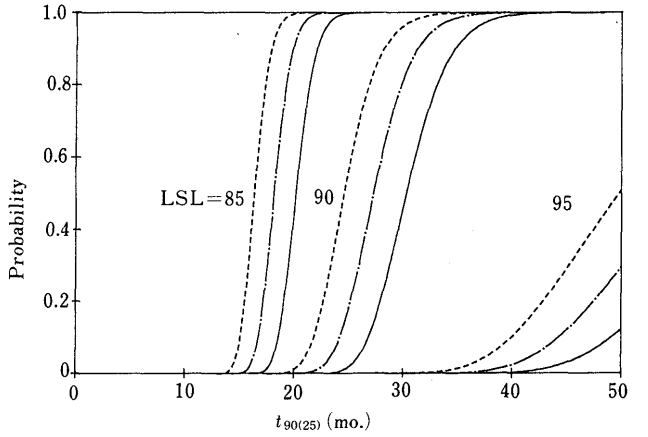

Fig. 5. Effect of Temperature Control Errors on Probability That Drug Content Is Observed to Be Larger than the Lower Specification Limit at 2nd, 4th and 6th Month

Temperature: ——, 40; —·—·, 39; ------, 38 °C. E_a: 20 kcal/mol. Assay standard deviation: 1%. Numbers represent the lower specification limit, LSL (%).

content is observed to be larger than 90% every time, *i.e.* the product of the values of probability at 2nd, 4th and 6th month (hereinafter simply denoted as "probability"). The probability increases with true $t_{90(25)}$, *e.g.* the drug content of a product having 30 months of $t_{90(25)}$ is observed to be larger than 90% with about 0.5 of probability.

The profile of this probability is highly dependent on the magnitude of the E_a and the assay errors. Figure 2 shows the effect of assay errors on the probability that the drug content is observed to be larger than 90% for various values of E_a. The probability decreases with increasing E_a; in the case of a drug product having 30 months of $t_{90(25)}$, the drug content is observed to be larger than 90% with a probability close to 1 when the degradation of E_a is 10 kcal/mol. On the other hand, the drug content is rarely observed to be larger than 90% when E_a is 30 kcal/mol.

Figure 3 shows the effect of assay errors on the probability that the drug content is observed to be larger than 85, 90 and 95%, respectively, for 20 kcal/mol of E_a. As expected, the probability increases with a decreasing lower limit.

Figures 2 and 3 indicate that the probability is largely affected by the assay error; an increase in the assay standard deviation results in increasing the probability for a drug product having shorter $t_{90(25)}$ and descreasing the probability for a product having longer $t_{90(25)}$. In other words, the drug content is often observed to be smaller than 90% (lower limit) even for a product having considerably long $t_{90(25)}$, while it is observed to be larger than 90% even for a product having considerably short $t_{90(25)}$, when the assay error is large.

Temperature control errors also affect the probability profiles. Figure 4 shows the probability that the drug content is observed to be larger than 90% when temperature is maintained lower than 40 °C (*i.e.* 38 and 39 °C). Figure 5 shows the probability that the drug content is observed to be larger than 85, 90 and 95%, respectively, under the same temperature conditions. The 1 °C difference has a large effect on the probability.

Discussion

Accelerated test data obtained at a single temperature (40 °C), *per se*, cannot be used for extrapolation to 25 °C. What does the observation that the drug content of a

product remains within the lower specification limit in a single-temperature accelerated test mean?

The probability for the drug content of a product to meet the specification depends on the experimental errors in the assay and temperature control (Figs. 2—5), as well as the true $t_{90(25)}$ and E_a of the degradation, and the lower specification limit. Without knowing the values of E_a, and the accuracy and precision of the assay and temperature control, the observation that the drug content remains within the lower specification limit cannot play any supportive role in $t_{90(25)}$ assessment.

It is known that the E_a values of chemical reactions generally range from 10 to 30 kcal/mol. If we intend to assure that the $t_{90(25)}$ of a product is not less than a specified value by the results obtained in the single-temperature accelerated test, we should treat the data assuming that the E_a is 10 kcal/mol. If we intend to use the accelerated test data in estimation of the maximum value of $t_{90(25)}$ which the drug product could give, the data should be treated assuming that the E_a is 30 kcal/mol. In both cases, information on the accuracy and precision of the assay and temperature control should be required (Figs. 2 and 4).

Increasing the lower specification limit results in a decrease of the probability that drug content is observed to be larger than the limit (Figs. 3 and 5). If the limit is high, we can assure that $t_{90(25)}$ of the product is not less than a specified value with a larger probability.

In conclusion, the effect of experimental errors on the probability that drug content is observed to be larger than the lower limit in the single-temperature accelerated test can be interpreted graphically. This probability is highly dependent on the experimental errors in the assay and temperature control, as well as the true degradation rate and activation energy. The observation that the drug content meets the specification in the single-temperature accelerated test can provide only limited information on the drug stability, without the knowledge of the assay error and temperature deviation.