The precision of estimates of degradation rate from accelerated degradation tests is much improved by

- increasing the range of temperatures at which the samples are stored, even if the total statistical weight is kept constant;
- increasing the total statistical weight by having replicate samples tested;
- increasing the duration of the study.

A separate analysis of the measured response from the reference samples (e.g., if a radial diffusion assay, the ring diameter; if an ELISA assay, the absorbance; or if an HPLC assay, the peak height) will indicate the between-assay variation. The analysis of duplicates of the reference samples will indicate the within-assay variation.

Common Problems Seen with Accelerated Degradation Studies

Reduction in Degradation Rate with Time

This may be seen with freeze-dried preparations and is likely to be caused by an irreversible consumption of residual oxygen or moisture present as a contaminant of the atmosphere within the container or within the freeze-dried material itself.

Discontinuity in the Relationship Between Degradation Rate and Temperature

The analytical models assume that the rate of molecular diffusion (i.e., viscosity) in the stored samples does not significantly alter over the range of temperatures studied.

A significant change in viscosity can occur in the accelerated degradation testing of freeze-dried preparations containing a large proportion of substances that do not crystallize on freezing but form a glass, for example, some proteins and carbohydrates, used as bulking materials or cryoprotectants. If, during subsequent storage, the temperature of the freeze-dried material exceeds the T_g' of the glassy matrix, for example, by storage at some elevated degradation storage temperature, there is progressive collapse of the previously stable glass by the water released from the glass. Subject to the magnitude of the water released, the glass progressively collapses into a deformable rubber, a viscous syrup fluid, and finally to a mobile fluid.

Over the temperature range where this progressive transformation occurs, there will be a marked change in the rate of diffusion and the Arrhenius/Heyring equations are not valid above this point of discontinuity. This may be revealed by a discontinuity in the relationship between ln(relative potency) or degradation rate, and the reciprocal of absolute temperature.

For information, at temperatures near the glassy transformation point, the kinetics is said to be better described by William Landel Ferry equation

$$\log(k) = \frac{C1(T - T_g)}{C2 + (T - T_g)},$$

where

- k is the rate constant,
- T the absolute temperature (Kelvin),
- T_g the glass transition absolute temperature (Kelvin), and
- $C1$ and $C2$ are constants.