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Determination of Product Shelf Life and Activation Energy for Five Drugs of Abuse

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Estimating the shelf life of reagents and controls is a critical step in evaluating new formulations. We describe several stability assessment techniques, giving particular attention to experimental design. Activation energy was experimentally determined for five major drugs of abuse and metabolites: morphine, 11-nor-Δ-9-tetrahydrocannabinol-9-carboxylic acid, amphetamine, phencyclidine, and benzoylecgonine. The use of activation energy in establishing shelf life is illustrated for SENTRY™ (Hycor Biomedical Inc.) Drugs of Abuse Screen Control, a liquid urine-based product.

Additional Keyphrases: *Arrhenius plots · accelerated stability testing · control materials · bracket table · Q Rule · activation energy*

The shelf life of a product may be defined as the time that essential performance characteristics are maintained under specific handling conditions. Although it may be estimated by accelerated stability testing protocols, real-time product stability testing is necessary to validate stability claims for clinical chemistry reagents and reference material. Manufacturers' quality-assurance criteria typically require that a product must recover at least 90% of the initial value throughout its life. Applying this performance criterion to the results of stability testing thereby determines shelf life. However, alternative criteria are sometimes advisable.

Theory

Real-Time Stability Testing

In real-time stability testing, the duration of the test period should be long enough to allow significant product degradation under recommended storage conditions. At the least, the testing protocol must permit one to distinguish percent degradation from interassay variation. For example, data may be collected at an appropriate frequency such that a trend analysis may discern instability from day-to-day imprecision. The reliability of data interpretation can be increased by including in each assay a single lot of reference material with established stability characteristics. Sample recovery between assays can be thereby normalized to this reference, minimizing the impact of systematic drift and interassay imprecision. Frequently, however, an appropriate reference material is not available for use as a control.

When one measures the stability of a reference material, imprecision may be introduced by changes in both reagents and instrumentation. Ideally, reagents should be sufficiently stable that a single lot provides unchanging performance throughout the stability study and instrument performance should remain constant. However, one must monitor system performance and control for drift and discontinuity resulting from changes in both reagents and instrumentation.

Accelerated Stability Testing

Accelerated stability testing is often used in the development of clinical reagents to provide an early indication of product shelf life and thereby shorten the development schedule. To this end, a product is stressed at several high (warmer than ambient) temperatures and the amount of heat input required to cause product failure is determined. This information is then projected to predict product shelf life or used to compare the relative stability of alternative formulations.

A reasonable statistical treatment in accelerated stability projections requires that at least four stress temperatures be used. In addition, more nearly accurate stability projections are obtained when denaturing stress temperatures are avoided. This finding is particularly true for reagents containing labile, proteinaceous components.

Accelerated stability testing protocols allow one to stress samples, refrigerate them after the stressing, and then assay them simultaneously. Because the duration of the analysis is short, the likelihood of instability in the measurement system is reduced. Accelerated stability protocols additionally permit one to compare the unstressed product with stressed material within the same assay, the stressed sample recovery being expressed as a percent of unstressed recovery. This utilization of product as an internal control is especially valuable when no suitably stable reference material is available (*1*).

The Arrhenius Equation and Activation Energy

Most accelerated testing models are based on the Arrhenius equation (*2*):

$$\log\left(\frac{k_2}{k_1}\right) = \frac{-E_a}{2.303R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

where k_2 and k_1 are rate constants at temperature T_2 and T_1, respectively; E_a is the activation energy; and R is the gas constant. Temperature is in kelvins.

This equation describes the relationship between storage temperature and degradation rate. Use of the Arrhenius equation permits a projection of stability from the degradation rates observed at high temperatures (*3*). Activation energy, the independent variable in the

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equation, is equal to the energy barrier that must be exceeded for the degradation reaction to occur. When the activation energy is known (or assumed), the degradation rate at low temperatures may be projected from those observed at "stress" temperatures.

The relationship between drug concentration [D] after time (t) for a first-order equation is

$$\frac{-d[D]}{dt} = K_1 [D]$$

where K_1 is the reaction rate constant at a given temperature (*2*). Integrating from $t = 0$ to $t = t$, one obtains

$$\ln[D] = \ln[D]_0 - K_1 t$$

$$\text{or } \log[D] = \log[D]_0 - (K_1 t/2.303)$$

where $[D]_0$ is the drug concentration at time 0. By substituting $0.90\ [D]_0 = [D]$ in the equation above, the time required to reach 90% of the original drug concentration can be shown to be

$$t_{90} = 0.105/K_1$$

A common practice of manufacturers in pharmaceutical and diagnostic reagent industries is to utilize various shortcuts, e.g., bracket tables (*4*) and the Q Rule (*2*), to estimate product shelf life. These techniques (see below) share the advantage that decisions may be made by analyzing only a few stressed samples. However, they are based on assumptions about the activation energy of product components and are valid only insofar as these assumptions are accurate. Whatever method is chosen, the validity of product stability projections depends on analytical precision, the use of appropriate controls within the experimental design, the assumptions embodied in the mathematical model, and the assumed or measured activation energy of product components.

Reaction rate is influenced by pH, tonicity, the presence of stabilizers, and so forth. Key product component(s) may degrade (or otherwise become unavailable) through multiple mechanisms (*2, 3*). In complex chemical systems, therefore, minor variation in formulation can profoundly affect lot-to-lot stability and, indeed, the activation energy of product degradation. Consequently, shelf life projected from accelerated studies must be validated by appropriate real-time stability testing.

Rapid Techniques for Projecting Shelf Life

Examples of shortcuts for projecting product shelf life for storage at 5 °C include the Q Rule and bracket table methods.

Q Rule. The Q Rule states that a product degradation rate decreases by a constant factor (Q_{10}) when the storage temperature is lowered by 10 °C. The value of Q_{10} is typically set at either 2, 3, or 4 because these correspond to reasonable activation energies. For larger shifts in temperature, the rate constant changes exponentially with temperature, and is proportional to $(Q_{10})^n$, where n equals the temperature change (°C) divided by 10. For example, the estimated decrease in degradation rate caused by lowering the storage temperature by 50 °C is $(Q_{10})^5$ because $n = 50/10 = 5$ (see Table 1). This model falsely assumes that the value of Q does not vary with temperature. A more detailed treatment has been published (*2*).

A Q_{10} value of 2 provides a conservative estimate, and results calculated with this value are considered *probable*. A Q_{10} value of 4 is less conservative and yields results considered to be *possible*.

To illustrate the application of the Q Rule in predicting shelf life, assume that 90% of phencyclidine (PCP) is recovered after 26 days at 55 °C. The stability of PCP under refrigerated (5 °C) conditions may be estimated [26 days/$(Q_{10})^5$] as follows:

(*a*) PCP is *probably* stable for 832 days (2.3 years) if $Q_{10} = 2$.

(*b*) PCP *may be* stable for 6318 days (17 years) if $Q_{10} = 3$.

(*c*) PCP is *possibly* stable for 26 624 days (73 years) if $Q_{10} = 4$.

Bracket tables. The bracket table technique assumes that, for a given analyte, the activation energy is between two limits, e.g., between 10 and 20 kcal. As a result, a table may be constructed showing "days of stress" at various stress temperatures (Table 2). The use of a 10 to 20 kcal bracket table is reasonable, because broad experience indicates that most analytes and reagents of interest in pharmaceutical and clinical laboratories have activation energies in this range (*4, 5*).

Because the bracket table provided in Table 2 does not specify stability requirements at a stress temperature of

Table 1. Q_{10} Factors

Q_{10}	E_a, kcal/mol	$(Q_{10})^5 = Q_{50}$
2.0	12.2	32
3.0	19.4	243
4.0	24.5	1024

Table 2. Bracket Table[a]

	Days of stress to predict stability at 5 °C for							
	6 months		1 year		2 years		3 years	
Stress temp., °C	20 kcal	10 kcal	20 kcal	10 kcal	20 kcal	10 kcal	20 kcal	10 kcal
14.5	55.3	100	111	201	221	402	332	603
25	16.1	54	32	108	64	217	97	326
35.5	5.1	30.6	10	61	20	122	31	183
47.5	1.5	16.6	3	32	6	66	9	100
60	0.5	9.2	0.9	18	1.9	37	2.8	55

[a] To use this table, select the column appropriate to the stability claim to be evaluated, e.g., 1 year. Acceptable performance after the number of days of stress in the 20-kcal column predicts that it is *possible* the selected claim will be observed (e.g., stressed for 32 days at 25 °C). Acceptable performance indicated in the 10-kcal column predicts that it is *probable* the selected claim will be observed.

55 °C, a conservative use of the table requires that projections be taken from the 47.5 °C data. The PCP stability assumed in the Q Rule illustration (26 days) exceeds the six-month stability requirement (16.6 days) for the 10 kcal model. Thus, the interpretation of the bracket table is that PCP is *probably* stable at 5 °C for at least six months. Furthermore, because the assumed 26 days also exceeds the nine days stability required for the three-year 20 kcal model, it is *possible* that PCP is stable for at least three years.

Prudent use of either of these rapid techniques would dictate that data at three or four higher temperatures be incorporated into the projection of refrigerated shelf life. To evaluate the usefulness of the Q Rule and the bracket table in this example, one must determine the activation energy for PCP and then project the refrigerated shelf life by using the Arrhenius equation.

Materials and Methods

The activation energies of the five drugs of abuse in the SENTRY™ (Hycor Biomedical Inc., Garden Grove, CA 92641) liquid urine control were determined in three steps. First, we stressed the samples at high temperatures and determined the recovery of each drug. Rate constants were then derived by plotting analyte recovery vs days of heat stress at each temperature. Finally, we calculated the activation energy of each drug, using the Arrhenius equation.

Individual vials were stressed in constant-temperature incubators under the conditions summarized in Table 3. Semi-quantitative drug concentrations of each stressed sample were measured with the TD_x™ system (Abbott Labs., North Chicago, IL 60064), with fluorescence polarization immunoassay reagents. The manufacturer's recommendations were followed for instrument operation and maintenance. TD_x controls were assayed and the acceptability of each run was determined by comparing the recovery of the control with the manufacturer's stated acceptance range.

Because neither the 5 °C nor the −15 °C control values changed significantly with time, we used the 5 °C control results as "100% recovery" in each run. That is, drug recovery in stressed samples was expressed as a percent of that recovered in the 5 °C control. For each stress temperature, relative recovery values were plotted against days of heat input. The slope of the best-fit line was taken as the degradation rate constant, k.

The activation energy, E_a, for the chemical degradation of each drug was calculated from the Arrhenius equation by inserting the corresponding degradation rate constants.

Results

The percent drug recovery relative to that of the 5 °C control was calculated for samples at each stress condition; these data were analyzed by regression analysis to determine the slope of the best-fit line (Figure 1). These slopes, equal to the rate constants, were used to calculate the activation energies by use of the Arrhenius equation (Table 4).

Figure 2 illustrates for each drug the observed relationship between degradation rate and stress temperature. Note that 278 K is equivalent to 5 °C; the inverse

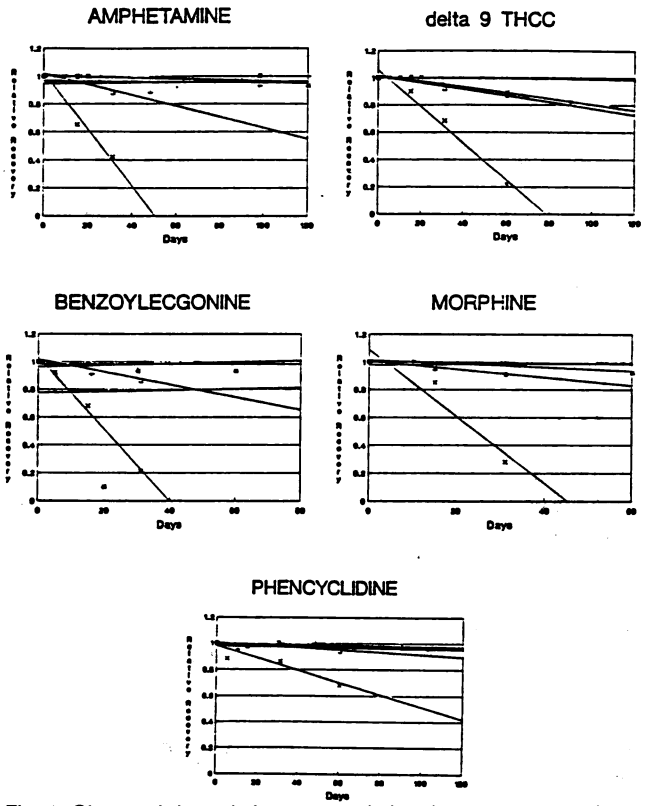

Fig. 1. Observed degradation rates: relative drug recovery vs time
The rate of drug loss at a given temperature is equal to the degradation rate constant, k. The dependence of this constant on temperature is illustrated for each drug by the slope of each line: ●, 15 °C; +, 25 °C; *, 35.5 °C; □, 45 °C; X, 55 °C; delta 9 THCC, 11-nor-Δ-9-tetrahydrocannibinol-9-carboxylic acid

Table 3. Accelerated Stress Conditions Used to Calculate Degradation Rate Constants for the Drugs of Abuse Study

Temp., °C	Days of stress								
−15	5	10	15	30	48	60	90	120
5	5	10	15	30	48	60	90	120
15				30		60	90	120
25			15	30		60	90	120
35		10		20			90	120
45	5		15	30				
55	5		15	30				

Table 4. Calculated Degradation Rate Constants and Activation Energies

Drug	Rate constants, $k \times 10^3$					E_a, kcal/mol
	15 °C	25 °C	35 °C	45 °C	55 °C	
Morphine	0.20	0.30	1.3	2.7	22	30
Delta-9-THCC[a]	0.01	0.11	0.20	2.2	34	35
Amphetamine	0.25	0.63	0.42	3.4	19	24
Phencyclidine	0.20	0.40	0.50	1.0	5.3	18
Benzoylecgonine	0.02	0.02	2.2	3.7	25	33

[a] See legend to Fig. 1.

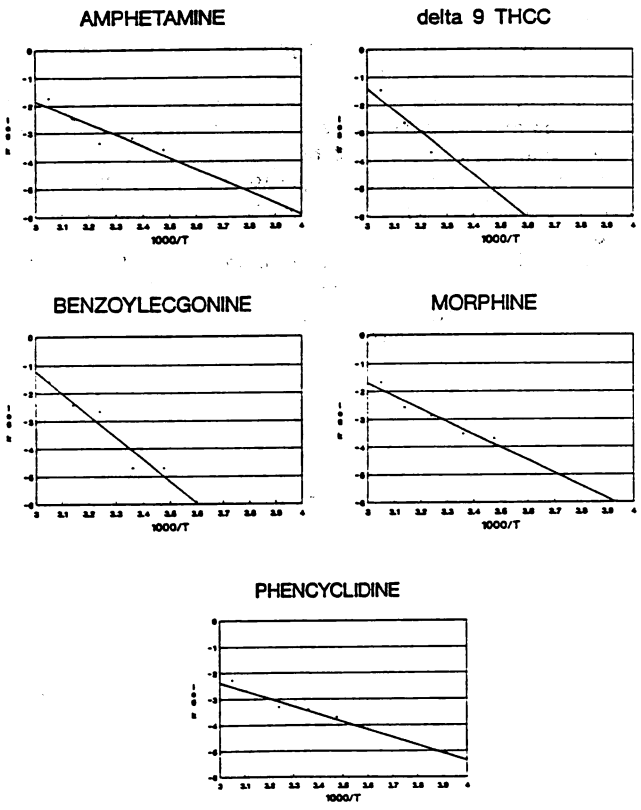

Fig. 2. Arrhenius plots

The log *k* values derived in this study are plotted vs inverse temperature (K). Projected values of *k* at 5 °C (3.5 on the *abscissa*) are useful in determining drug stability at refrigerated temperatures

of this absolute temperature is "3.6" on the Arrhenius plot. The Arrhenius plots obtained are approximately linear for each drug. We used regression analysis to project the 5 °C rate constant for each drug. The period over which at least 90% of the initial drug is recovered was calculated from the equation: stability (days) = 0.105/*k*. Finally, the calculated stabilities were supported by the observed 18-month recovery of these drugs in the SENTRY Control maintained at 5 °C (Table 5).

Discussion

The projected stability of a refrigerated product is determined exclusively by the calculated 5 °C degradation rate constant. This projected stability depends on the activation energy and cannot reliably be projected by monitoring drug recovery at a single stress temperature.

In the present study, we used simple linear-regression analysis to fit experimental data. Alternatively, Arrhenius analysis can be fine-tuned by using estimates of analytical error to establish a confidence interval for each rate constant (*2, 3*). This analysis permits a *weighted* least-squares statistical treatment and produces a more statistically valid projection of product stability at lower temperatures. One can calculate the fit of observed rate constants to the Arrhenius relationship and thereby evaluate the validity of the accelerated stability study (*6*).

As shown in Table 4, amphetamine has the highest observed degradation rates at 15 and 25 °C. At 35, 45, and 55 °C, benzoylecgonine has the highest rates. However, the 5 °C degradation rate constants calculated from the Arrhenius equation demonstrate that PCP has the highest rate constant, and is therefore the least stable under refrigerated conditions. The calculated activation energy for morphine is in fair agreement with a published value (*5*) of 23 kcal/mol. As previously mentioned, the reaction rate, and hence the activation energy, can be influenced by the chemical matrix in which drug stability is tested.

The quality-assurance risk inherent in using any method for stability estimation, including the Q Rule or bracket tables, is that one might assume the activation energy to be greater than it actually is. The stability in this case will likely be overestimated. On the other hand, if one assumes an activation energy lower than actual, the estimated stability may be so "conservative" that it is of little practical value.

Recall the assumed PCP stability in our example examination of the Q Rule and bracket table. This 26-day stability interval is in fact the experimentally observed stability of PCP in the current study. The activation energy of PCP, as well as its projected stability is based on data obtained at five stressing temperatures; the projected 5 °C stability of PCP is 10 years (Table 5).

The Q Rule provides a conservative estimate (2.3 years) if $Q_{10} = 2$. The estimated stability (17 years) is significantly higher, if $Q_{10} = 3$, despite the fact this Q_{10} value corresponds to an activation energy (19.4 kcal/mol, Table 1) near the calculated value (18 kcal/mol, Table 4). This estimation error likely results from the erroneous assumption that Q does not vary with temperature.

The bracket table method provides a good but conservative estimate of stability for PCP, the stability estimate from the 20 kcal column being at least three years.

In summary, accelerated stability testing and the various models available for interpreting these data can provide valuable information for evaluating reagents and control products. By optimizing the analytical precision and other aspects of test protocol design, one can

Table 5. Calculated and Observed Stability

Drug	Projected Stability, years	Observed recovery, %[a]
Morphine	57	92
Delta-9-THCC[c]	364	101
Amphetamine	18	100[b]
Phencyclidine	10	97
Benzoylecgonine	114	103

[a] After 18 months at 5 °C.

[b] 100% recovery was observed after six months at 5 °C; subsequent data showed an increased recovery owing to a specificity change in TD_x reagents for amphetamine.

[c] See legend to Fig. 1.

expect both real-time and accelerated stability studies to provide more valid information. For analytes with high activation energies, such as the drugs of abuse studied here, both bracket tables and the Q Rule provide useful information when they are applied conservatively. Use of published or experimentally derived activation energy values can significantly lower the risks inherent in projecting product shelf life.

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