

# Accelerated Stability Studies by High Performance Liquid Chromatography

Greg Serna, Tamara Tarbox, Isil Dilek

2012 Sigma Aldrich® Summer Intern Program

## Abstract

The purpose of this study was to perform accelerated stability studies to predict the shelf lives of 21 Cerilliant products and compare the results to available real time stability information. From the rates of degradation, an Arrhenius plot was constructed for each product to yield the rate of degradation at the control temperature and the energy of activation for thermolytic degradation. Thermolytic degradation was assumed to be the only decomposition pathway.

## Experimental Design

Twenty two ampules of each product were placed in different storage conditions for a total of 28 days. All products are ampuled solution standards, flame-sealed under argon.

| 22 Total Ampules                               | 5 Time points (in days) |
|--|-------------------------|
| 2 @ Freezer (control)                          | T=0                     |
| 5 @ Refrigerator, Room Temperature, 40°C, 60°C | T=3, 7, 14, 21, 28      |

At each timepoint, one ampule was transferred from each storage condition to the freezer. After 28 days timepoint, all samples were analyzed for purity by HPLC.



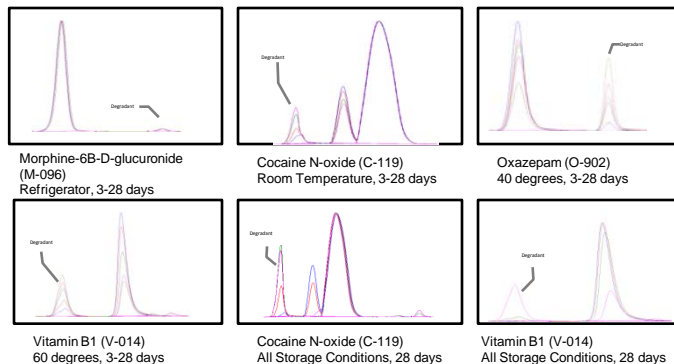
**Left:** Samples of D-069 pulled from the 60°C storage condition. From left to right: sample after 3, 7, 14, 21, 28 days. The darkened color and precipitate are due to degradation.

**Right:** HPLC was used for the analysis of samples. Stability indicating HPLC methods were developed for the analyses.

## Products tested for the Study

|                           |       |                         |       |                        |       |
|---------------------------|-------|-------------------------|-------|------------------------|-------|
| Oxazepam                  | O-902 | (-)-Δ <sup>9</sup> -THC | T-005 | 7-Aminoclonazepam      | A-916 |
| Oxazepam glucuronide      | O-023 | Propofol                | P-076 | Noroxycodone HCl       | N-011 |
| Norbuprenorphine          | N-059 | Cocaine-N-Oxide         | C-119 | Tacrolimus             | T-049 |
| Morphine-6β-D-glucuronide | M-096 | Benzoylcegonine         | B-004 | Butylone HCl           | B-045 |
| Vitamin B1 (Thiamine HCl) | V-014 | Propoxyphene            | P-011 | Rufinamide             | R-015 |
| Mycophenolic Acid M-106   |       | Desmethyloanzapine      | D-069 | Green Tea Catechin mix | G-016 |
| Thyroid                   | T-075 | Dopamine                | D-081 | 25-Hydroxyvitamin D3   | H-083 |

## Degradation Effects on Selected Products



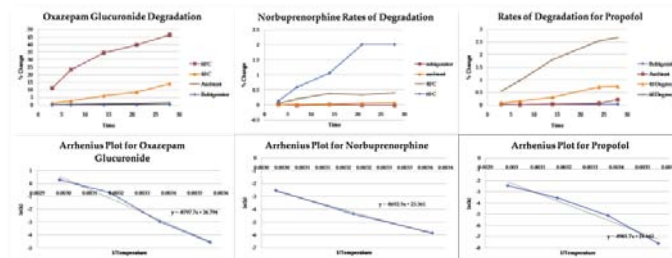
These select chromatogram portions show the amount of degradation over time and with increasing temperature.

## Treatment of Data

The change in % area value vs. time was plotted for each temperature. The change in % area was defined as the absolute difference between the freezer sample's % area and the % area of other samples. Microsoft Excel was used to plot this change over time for each storage condition and determine a linear trend line. The slope (k) of this trend line gives the rate of degradation at a given temperature. A plot of  $\ln(k)$  vs.  $1/T$  was also created for each product using the slopes from each storage condition and was used to determine the shelf-lives reported. The slope of this line was also used to determine the activation energy for thermolytic degradation using the Arrhenius relationship  $k=Ae^{-(E_a/RT)}$  where  $k$ = degree of rate constant,  $A$ =pre-exponential factor,  $E_a$ =Activation energy,  $R$ =universal gas constant and  $T$ =temperature in Kelvin.

## Degradation and Arrhenius Plots

Selected examples of degradation analysis are shown below.



## Results

| Name of Product           | Rates of Degradation (%/day) |                  |        |         | Predicted Shelf Life (years) | Assigned Date from Real Time Study (years) | Energy of Activation (kcal/mol) |
|---------------------------|------------------------------|------------------|--------|---------|------------------------------|--|---------------------------------|
|                           | Refrigerator                 | Room Temperature | 40°C   | 60°C    |                              |  |                                 |
| Oxazepam                  | 0.0133                       | 0.1723           | 1.8971 | 1.2927  | 2.8                          | 4  | 16.7632                         |
| Oxazepam glucuronide      | 0.0104                       | 0.0513           | 0.5    | 1.328   | 5.9                          | 5  | 17.4714                         |
| Norbuprenorphine          | -0.0005                      | 0.0029           | 0.0127 | 0.0803  | 197.3                        | 5  | 17.2632                         |
| Morphine-6β-D-glucuronide | 0.0022                       | 0.0027           | 0.0117 | -0.0046 | 96.6                         | 3  | 8.6436                          |
| Mycophenolic Acid         | 0.0092                       | 0.3215           | 0.4663 | 0.2516  | 0.9 On Retest                |  | 10.7088                         |
| (-)-Δ <sup>9</sup> -THC   | 0.0039                       | 0.0049           | 0.017  | 0.0931  | 16.9                         | 5  | 14.059                          |
| Cocaine N-oxide           | 0.0212                       | 0.2974           | 0.1442 | 0.0516  | 28.7 On Retest               |  | 11.1625                         |
| Propofol                  | 0.0005                       | 0.006            | 0.0287 | 0.0865  | 193.3 On Retest              |  | 17.6779                         |
| Benzoylcegonine           | 0.0019                       | 0.0148           | 0.0931 | 0.7222  | 63.1                         | 5  | 20.4051                         |

The data for products not included in this section had not been analyzed at the time of this poster's submission.

## Discussion

This study was conducted under the assumption that all of the compounds followed the Arrhenius relationship. The compounds were assumed to have the same degradation mechanism at all temperatures and that the rate of the degradation stayed constant for each temperature condition. The storage conditions were chosen so that % change outside the range of analytical variation would be observed in the samples. Most of the shelf lives estimated from the Arrhenius plots agree with real time data. Real time data is only collected on products for up to five years and the experimental data predicts shelf lives that go beyond 5 years. In example s of oxazepam and morphine-6β-D-glucuronide results did not match the real time studies. The degradation rates for these compounds were non-linear at one or more temperatures and the Arrhenius plots could not accurately predict a shelf-life.

## Conclusion

An accelerated stability study was performed on 21 selected Cerilliant products. The products were stressed in a variety of temperature conditions and the predicted shelf-lives were compared to the available real time stability data. This method of predictive stress degradation can be used on new Cerilliant products to estimate a shelf-life before real time data is available. Cerilliant introduces ~100 new products a year and accelerated stability studies could provide customers with critical long term storage information.