HIGH THROUGHPUT SCREENING DEGRADATION STUDY: DETERMINATION OF STORAGE OPTIONS AND RETENTION LIMITS

L.E. Williams, B.A. Kozikowski, T. Burt, B. Kuzmak, K. L. Morand

Procter & Gamble Pharmaceuticals

Abstract

Currently, all High Throughput Screening compound plates are stored wet in anhydrous DMSO at room temperature. A yearlong study was designed to investigate the effects of these storage conditions on our compound collection. Flow Injection Analysis (FIA) using positive and negative electrospray ionization (ESI) mass spectrometry was utilized to monitor the chemical stability of a series of 116 plates containing a total of 9,280 selected compounds. Each compound was assessed at three time points throughout the duration of the study. In this poster we will be presenting a general overview and results for the long term storage options and retention limits for compound screening collections.

Current Storage Conditions

- **All compounds are stored in 99.8% anhydrous DMSO solutions at ambient temperature. They are kept within the Kardex plate storage system.**

Compounds Tested

- **A diverse subset was selected from among those available within the P&G screening repository. (see diagram)**
- **9,280 individual compounds were assessed at three time intervals throughout the study.**

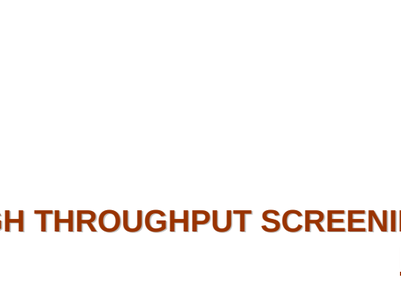
T=0 Initial submissions for all plates

T=1 Incremental monthly submissions for all plates

T=2 Final submission ~ 1 year from initial date

- **There were 27,840 total data points.**

Diversity Analysis



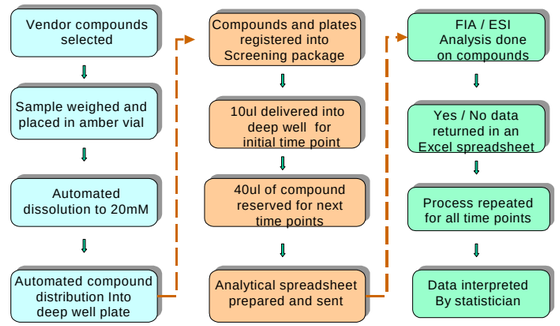
Three views of the relationship of the diversity of the stability data set to the diversity of the P&GP Repository

Stability data set

P&GP Repository

(Dave Stanton, Diversity Analysis)

Study Layout



FIA/ESI

Samples were diluted 50:50 in methanol/acetonitrile using a Robbins 96-Hydra. They were mixed and covered with foil to await analysis.

Samples were analyzed on a micromass Platform II mass spectrometer, with an HP 1100 series HPLC system and a Gilson 215 multi-probe autosampler. The mobile phase consisted of methanol with 0.2% formic acid and 0.2 mM ammonium acetate pumped at 5ml per minute.

Flow injection analysis (FIA) was performed with positive/negative electrospray ionization mass spectrometry for a mass range from 100 Da to 800 Da and a scan rate of 1000-Da per second. Data was processed using Micromass Openlynx© software and evaluated by the analyst. Yes-No data was returned in an excel spreadsheet.

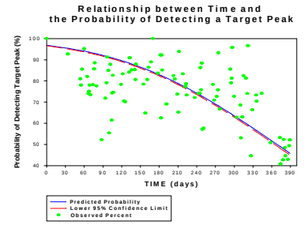
PLATE SUBMISSIONS

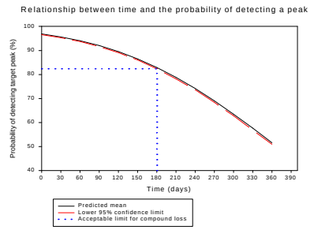
Order	Barcode	Analytical Plate #	Date #1 (t=0)	Day Interval for reanalysis	Date #2	Date #3 (t=final)
1	45415	278	10/8/99	30	1/30/00	12/31/00
22	45462	299	11/3/99	330	9/28/00	11/3/00

The above spreadsheet shows an example of the intervals used for plate submissions

Statistical Analysis

A generalized linear mixed model was used to model the effect of time on the presence/absence of a target peak. The maximum length of storage will be determined from where the lower one-sided 95% confidence interval intersects a predetermined probability of detecting a target peak. Both the slope and the intercept were highly statistically significant.





Conclusions

- **Storage retention limits for compound plates kept at ambient temperature will be approximately 6 months.**
- **This storage retention limit was based on the study results and a pre-determined acceptable limit for compound loss.**

Next Steps

- **Correlate structural classes with the stability data**
- **Use LC/MS to generate quantitative information on sample loss**