The Effect of Freeze/Thaw Cycles on the Stability of Compounds in DMSO

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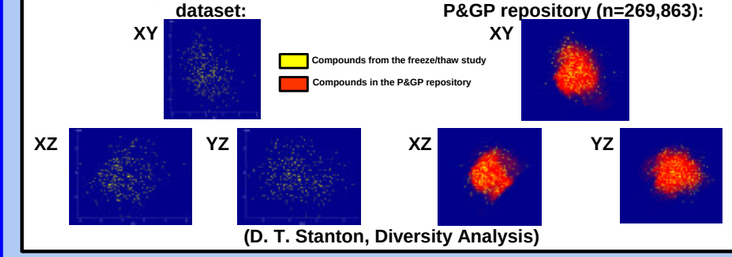
Abstract

A study was designed to evaluate the effects of multiple freeze/thaw cycles on the stability of compounds in DMSO. Microtiter plates containing DMSO solutions of 320 compounds were stored at 4ºC under argon in pressure canisters to simulate a low humidity environment. The plates were subjected to 25 freeze/thaw cycles and were exposed to ambient conditions after each thaw to simulate the time that compound plates are exposed to the atmosphere during liquid handling. HPLC-ELSD-MS was used to quantitate the amount of compound remaining after every fifth freeze/thaw cycle. Control plates were either stored at room temperature under argon or at 4ºC under argon with no freeze/thaws, and were evaluated at the midpoint and the endpoint of the study. Experimental procedures and results obtained from this study will be presented in this poster.

Compounds Tested

- 320 diverse compounds from the P&GP Repository were dissolved to 20 mM in 100% DMSO

Diversity of the freeze/thaw dataset:



Comparison of this dataset to the P&GP repository (n=269,863):

(D. T. Stanton, Diversity Analysis)

Storage Method

- Modified pressure canisters (D. L. Garbutt) were used to generate a low humidity environment. A positive pressure of argon was maintained in each canister.

Study Design: Three Storage Conditions

Storage Condition #1: Freeze/thaw plates under argon at 4C

Daily routine over the 5 week study duration:
- Plates thawed under argon
- Plates exposed to the atmosphere for ~2 hours
- Plates purged with argon & stored at 4C
- Aliquot sent for MS analysis after every 5th freeze/thaw

Storage Condition #2: Frozen controls (2 plates) under argon at 4C

- Plate #1 thawed under argon at week 2 and submitted for MS analysis
- Plate #2 thawed under argon at week 5 and submitted for MS analysis

Storage Condition #3: Room temperature controls under argon

Daily routine over the 5 week study duration:
- Plates exposed to the atmosphere for ~2 hours
- Plates purged with argon & stored at room temperature
- Aliquot sent for MS analysis after week 2 and week 5

Analytical Procedures

Detector: Micromass Platform-II Mass Spectrometer with APCI ionization

HPLC/Autosampler: HP1050 Quaternary System

Mobile phase: A: 95% ACN/5% H_2O/0.002% TFA B: 3% ACN/97% H_2O/0.002% TFA
C: 3% ACN/97% H_2O/0.002% TFA D: 95% ACN/5% H_2O/0.002% TFA
Flow rate: 3 ml/min
Gradient: 50/50 B/C to 50/50 A/D over 3 min, hold 1.5 min
Equilibrate at 50/50 B/C for 1.5 min (total run time=6 min)

Column: Waters Symmetry Shield Rx C8, 4.6 x 50 mm, 3.5 ? m particle size

10 ? l of sample was diluted with 200 ml of DMSO and was transferred to an HP vial fitted with a microinsert. 5 ? l was then injected and analyzed via High Pressure Liquid Chromatography-Evaporative Light Scattering Detection-Atmospheric Pressure Chemical Ionization-Mass Spectrometry (HPLC-ELSD-APCI-MS). Samples, standards (from 0.2 to 30 mg/ml), and QCs were monitored via three detectors: ELSD (detector of choice for purity and quantitation determination), UV @ 254 nm (secondary detector-not utilized except as a check of the system), and MS with APCI ionization in both the positive and negative mode (detector and ionization method of choice for molecular weight confirmation). Study samples and QCs were quantitated against an averaged-value ELSD calibration curve constructed utilizing two surrogate compounds with MW of 331 and 706 amu.

Once all study samples were quantitated for the five timepoints, measured concentrations were compared to associated concentrations determined at the initial T=0 timepoint to determine a '% Compound Remaining' for each successive freeze/thaw cycle or control.

Statistical Analysis and Results

A mixed model was used to model the effect of the three different storage conditions on the '% remaining'. Multiple treatment comparisons were made using t-test and Tukey's test. The three methods of storage were statistically different from each other (see Figure 1).

A repeated measures regression model was used to describe the relationship between the number of thaws and the '% remaining'. Both the slope and intercept were highly statistically significant. The maximum number of freeze/thaw cycles can be determined from where the lower one-sided confidence interval intersects a predetermined '% remaining' (see Figure 2).

Figure 1. Relationship between time and the % remaining

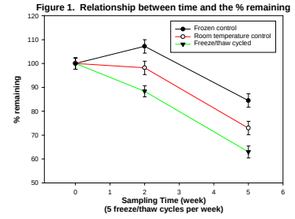
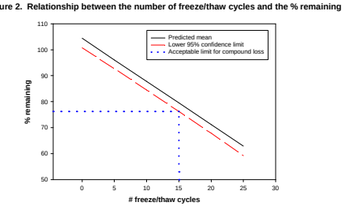


Figure 2. Relationship between the number of freeze/thaw cycles and the % remaining



Sample loss was not due to compound degradation based on the HPLC-ELSD-APCI-MS data but was attributed to compound precipitation upon freeze/thaw cycling.

Conclusions

- The '% remaining' decreased for all three storage methods
- 4ºC > room temperature > freeze/thaw cycled
- Based on the data from this study, samples will undergo a maximum of 15 freeze/thaw cycles before being discarded

Next Steps

- Evaluate the relationship between chemical structure and freeze/thaw stability