THE EFFECT OF SEALING VERSUS LIDDING PLATES ON DEGRADATION AND WATER UPTAKE OF COMPOUNDS HELD IN DMSO AT 4ºC

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ABSTRACT

Compound stability may be affected by storage in DMSO in a variety of formats, concentrations and temperatures. Compounds may also be affected by water uptake, due to exposure to the atmosphere or by freeze thawing. A number of stability experiments have been carried out to define best practice for storage of liquid samples at GlaxoSmithKline.
This study investigates the effect of water uptake on sample stability at 4?C and examines two experimental conditions, lidded vs. sealed 384 deepwell blocks. Samples were analysed for degradation using Liquid Chromatography-Mass Spectrometry and the % water uptake by DMSO was monitored by Karl Fischer titration.
The % water uptake and its effect on degradation of compounds will be discussed.

INTRODUCTION

Previous studies at GlaxoSmithKline have demonstrated that significant water uptake occurs when samples are stored unsealed in DMSO, especially in the absence of humidity control.

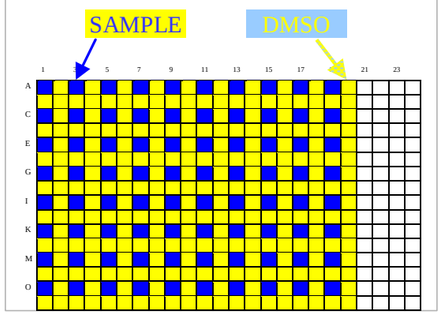
The intention of this study is to demonstrate:

- The difference in water uptake between samples in DMSO that are stored sealed and samples in DMSO that are stored with a loose fitting lid.
- The effect of water uptake on sample stability/solubility

SAMPLE SELECTION + DISTRIBUTION

- A set of 160 compounds was randomly selected to cover a range of diverse structural types.
- A set of 80 unstable compounds was selected from previous stability studies to form a biased set, in which each compound has demonstrated some instability/precipitation in DMSO upon storage.

The 240 samples were distributed in 384 well plates (80 samples per plate) being surrounded by DMSO as shown below .



STORAGE CONDITIONS

Samples are stored for up to 3 months at 4ºC, no humidity control.
Plates were covered using one of two methods:

- Beckman foil seal technology
- Greiner universal loose fitting lid

EXPERIMENTAL PROCEDURE

20 µL of sample was analysed by Karl Fischer for water content (selected wells).
50 µL of 10 mM sample was taken and diluted to 500 µL.
The samples were transferred to a 96 well microtitre plate and analysed by LC-MS. Time zero was done in duplicate.
Stability measurements were performed via comparison of DAD peak area at 1, 2 and 3 months with time zero .
The identity of every compound was checked by MS.

LC/MS SYSTEM

The LC/MS system comprised of a Gilson 233XL autosampler and an Agilent 1100 HPLC system fitted with a Supelcosil ABZ+ column (3.3 cm x 4.6 mm I.d.).
A Micromass Platform LC Mass Spectrometer fitted with an electrospray source was used as the MS detector.

Solvent A: 0.1 % aqueous formic acid + 10 mM ammonium acetate.
Solvent B: 90% acetonitrile + 0.07% formic acid + 10 mM ammonium acetate.
Flow rate: 1 ml/min.

Time (min)	Solvent A	Solvent B
0	95	5
1.0	95	5
4.6	0	100
8.0	0	100
9.0	95	5
10.0	95	5

DATA PROCESSING

Controls and test mixes were used to validate the process.

The % change in peak area is due to the the following factors:

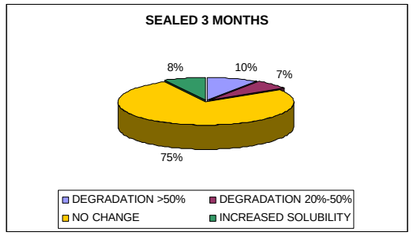
- degradation of compound
- change of concentration due to water uptake
- precipitation
- increase in solubility

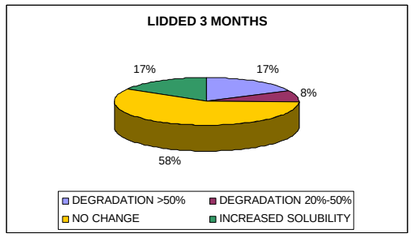
In this study, % change is due to degradation if a decrease in area is accompanied by an appearance of degradation peaks.

STATISTICS

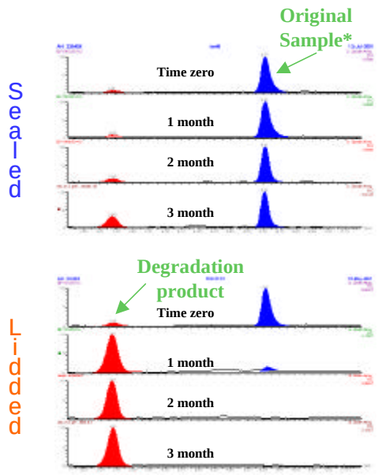
A statistical model was fitted in ANOVA (analysis of variance) using compound method sealed vs. lidded and time as explanatory variables. The response used was proportion of compound not degraded.

STABILITY STUDY RESULTS



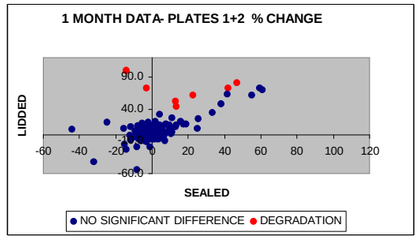


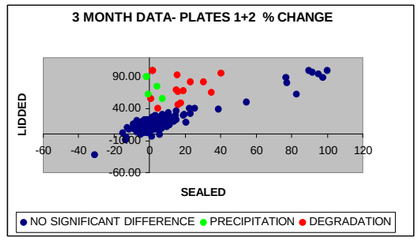
SAMPLE DEGRADATION



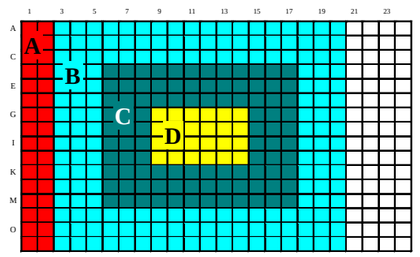
*sample 43, plate 1 (well I5)

GRAPHS: SEALED vs. LIDDED PLATES 1 & 2



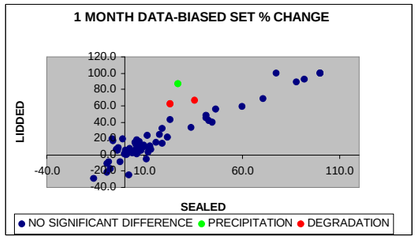


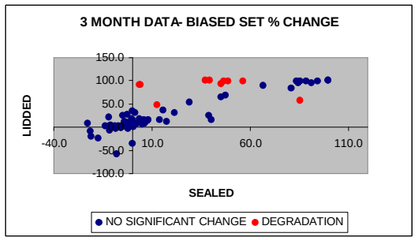
% Water Uptake by DMSO during the Stability Study Period



	% Water					
	Sealed			Lidded		
Zone	1 month	2 month	3 month	1 month	2 month	3 month
A	11	14	14	18	22	26
B	0	0	0	8	15	20
C	0	0	0	2	10	13
D	0	0	0	0	0	1

GRAPHS: SEALED vs. LIDDED BIASED SET (PLATE 3)





CONCLUSIONS

It was observed that more water uptake occurs in lidded plates than sealed and the water uptake was greater at the edges and corners of the plate than the centre.

After 3 months, 10.8% (17 out of 157) of the lidded randomly selected compounds showed more degradation and precipitation (sd>20%) than the sealed ones.
After 3 months, 11.6% (8 out of 69) of the lidded biased compounds showed more degradation (sd>20%) than the sealed ones.

Statistical analysis showed that the method (sealed vs. lidded) had a significant effect on the amount of degradation. p=0.0068 for the unbiased compounds and p= 0.014 for the biased with lidded showing greater degradation.
Also the effect of time was highly significant (p<0.0001) with greater degradation with increasing time. (If p<0.05, there is a significant difference between methods sealed and lidded).

RECOMMENDATION

It is recommended that samples are stored sealed to avoid problems such as degradation, precipitation, increased solubility and change in concentration.

GlaxoSmithKline