THE EFFECT OF FREEZE THAWING AND STORAGE TIME ON DEGRADATION OF COMPOUNDS AT 4ºC UNDER HUMIDITY CONTROL USING EXPERIMENTAL DESIGN

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ABSTRACT

Compound stability may be affected by storage in DMSO under a variety of conditions. There are a number of factors that can affect stability including storage format, concentration, temperature, water uptake and freeze thawing. A number of stability studies have been carried out to define best practice for storage of liquid samples at GlaxoSmithKline.

This study was carried out to determine the effect of storage time and freeze-thaw cycles on the stability of samples held in the GSK Automated Liquid Store using factorial experimental design (i.e. experiments designed to study the effects of more than one factor simultaneously). The conditions tested in the experiment were the stability of samples held in 384 deepwell blocks stored at 4?C and at 5% relative humidity. Samples were analysed for degradation using Liquid Chromatography-Mass Spectrometry and the % water uptake by DMSO was monitored by Karl Fischer titration.

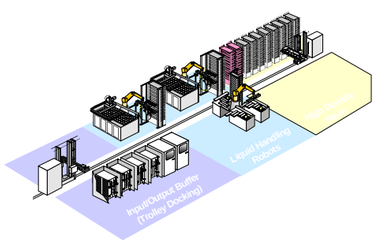
The effects of freeze thawing and storage time on the degradation of compounds held in neat DMSO solution at 4?C and low humidity will be discussed.

INTRODUCTION

Samples for High Throughput Screening (HTS) are currently stored in DMSO in the Automated Liquid Store (ALS). Working samples are stored at a concentration of 2mM in lidded but unsealed blocks at 4?C under reduced relative humidity (5%RH) to minimise water uptake.

This experiment was designed to determine the effect on stability of samples in 384 deepwell blocks stored in the ALS area at 4?C and at 5% relative humidity. The storage time and the number of times a block of samples is frozen and thawed was investigated. The % water uptake by DMSO was also monitored during this process.

ALS and LHR integrated facility



EXPERIMENTAL DESIGN

The process was investigated by looking at combining stability and freeze thawing in a factorial design experiment.

This factorial design experiment combined the freeze thawing and block storage as a single experiment. Factorial designs are used primarily for screening significant factors, but can also be used sequentially to model and refine a process.

A response surface regression analysis was performed to assess the linear and quadratic effects of freeze thawing and time. The response used in the analysis was the square root of the proportion of the compound not degraded.

This experiment used 17 combinations of freeze thaw and storage times.
The limits of the factors of the experiment are from 2 weeks to 6 months (storage time) and from 5 to 25 freeze-thaw cycles. Currently the experiment has used 13 out of the 17 combinations and one extra (0 freeze thaw) was included.

17 Experiments

9	Std	Run	Block	Factor A:Time months	Factor B:Freeze	Response Amount
	9	1	Block 1	3.00	15.00	
	12	2	Block 1	3.00	15.00	
	17	3	Block 1	3.00	23.00	
	6	4	Block 1	5.00	15.00	
	8	5	Block 1	3.00	25.00	
	11	6	Block 1	3.00	15.00	
	2	7	Block 1	4.00	10.00	
	14	8	Block 1	0.50	10.00	
	16	9	Block 1	3.00	7.00	
	10	10	Block 1	3.00	15.00	
	3	11	Block 1	2.00	20.00	
	5	12	Block 1	1.00	15.00	
	1	13	Block 1	2.00	10.00	
	13	14	Block 1	3.00	15.00	
	15	15	Block 1	6.00	15.00	
	4	16	Block 1	4.00	20.00	
	7	17	Block 1	3.00	5.00	

Following LC-MS analysis of the samples, the areas under the UV peak were recorded as the responses and the software determined the effects of stability and freeze thawing on the degradation of samples.

SAMPLE SELECTION + DISTRIBUTION

- A set of 160 compounds was randomly selected to cover a range of diverse structural types.
- The 160 samples were distributed in a 384 well plate (80 samples per plate) each sample was surrounded by DMSO.

EXPERIMENTAL

A number of identical deep well blocks were prepared by Liquid Stores and deposited in the ALS area. The deepwell blocks contained 125µL of sample at 2mM concentration. DMSO was added to all of the empty wells to check for % water uptake. Control samples were also added to the blocks and were replicated on each plate.

2 blocks were analysed immediately so a time zero result could be obtained in duplicate.

At each time point or freeze-thaw cycle a block was taken out of the ALS area, allowed to defrost and then analysed by LC-MS. One block remained in the store and did not undergo any freeze thaw cycles. Each freeze thaw cycle took 90 minutes.

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

EXPERIMENT DATES

ALS 4^0C Study		
DATE		**RUN**
Tues 21th March 2001		start
Tues 4th April 2001		8
Tues 20th April 2001		12
Tues 21st May 2001		11,13
Tues 21st June 2001		1,2,3,5,6,9,10,14,17
Tues 20th July 2001		7,16
Tues 21st August 2001		4
Tues 21st September 2001		15
Plate 2		
Tues 5th June 2001		start
Fri 15th June 2001		8
Thurs 5th July 2001		12
Mon 6th August 2001		11,13
Wed 5th September 2001		1,2,3,5,6,9,10,14,17
Fri 5th October 2001		7,16
Mon 5th November 2001		4
Wed 5th December 2001		15

WATER UPTAKE DURING SAMPLE STORAGE

	TIME ZERO % WATER UPTAKE	2 WEEK % WATER UPTAKE	1 MONTH % WATER UPTAKE	2 MONTH % WATER UPTAKE	3 MONTH % WATER UPTAKE
A2	2.04	5.61	5.80	9.09	10.15
A20	1.86	7.28	6.07	11.57	10.74
B2	1.37	4.57	3.39	7.76	9.37
B10	0.69	1.87	3.48	5.68	6.31
D8	0.60	1.16	1.24	0.85	1.31
D16	0.58	3.00	3.29	3.85	1.65
F3	0.78	1.51	1.53	1.49	3.93
F6	0.70	1.03	1.01	0.41	0.91
F19	0.56	2.44	1.82	5.44	7.34
H1	2.47	3.66	5.17	6.58	8.27
H12	0.60	0.89	0.60	0.65	0.73
J7	0.75	1.74	0.86	0.75	0.71
L4	0.60	1.73	1.14	0.79	1.67
L10	0.59	0.91	0.85	0.90	5.59
L18	0.60	1.86	1.22	4.54	7.17
P1	3.24	6.74	7.05	10.57	11.57
P2	1.29	5.34	6.32	7.61	10.69
P12	0.72	2.75	6.49	8.60	10.67
P19	1.05	5.29	6.68	9.46	12.38
P20	1.59	6.73	7.43	10.50	12.95

Corner of plate
Edge of plate
Inner part of plate

The results demonstrate a clear correlation between the position of samples on the plate (edges, corners and interior) with water uptake. Water uptake also increases with time.

STATISTICS

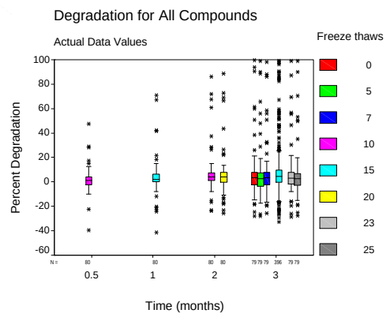
A response surface regression analysis was performed, including the factors time and freeze thawing as linear and quadratic components in a model. Variation between compounds were also adjusted for in the model. The model was defined as:

$$E(Y_{ijk}) = \beta_0 + \beta_1 freeze_j + \beta_2 time_k + \beta_3 freeze_j^2 + \beta_4 freeze_j * time_k + \beta_5 time_k^2$$

for each compound i, freeze thaw j and time k. The response Y was the square root of the proportion of compound not degraded.

DATA SUMMARY - BOX PLOT

Box plots are a summary of the data based on the median, quartiles and extreme values. The box contains the middle 50% of the data values. The whiskers are lines that extend from the box. The length of the whisker is defined as one and a half times the size of the box. Outliers, marked with a star, are the most extreme values in the data. A line across the box indicates the me

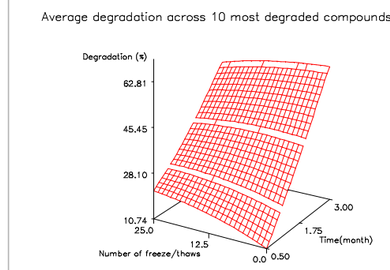


RESULTS

The overall results from the 80 compounds showed that storage time has a significant linear effect (i.e. degradation increased as storage time increased). There was no linear effect of freeze thaw on degradation (see box plot and regression analysis, the graph shown below).

The 10 most degraded compounds were investigated separately as the freeze thaw and storage time would be expected to have more of an effect on these compounds. The extent of degradation for the 10 most degraded compounds at 3 months was between 13.3% and 90.5%.

The effect of storage time was highly significant (p<0.0001). From the plot below this can be seen to be due to the linear increase in degradation with time. There was no significant effect of freeze thawing.



CONCLUSION

The results show that compound degradation increases with storage time. There is no evidence that degradation increases with the number of freeze thaws.
Based on these results and previous experiments, we are confident that samples can be stored in the Automated Liquid Store for at least 3 months under these conditions.

FURTHER WORK

Plate 1, months 4, 5 and 6 are due to be completed by the end of September 2001. Plate 2, the other 80 compounds are due to be completed by the end of December 2001.

ACKNOWLEDGEMENTS

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