Library Maintenance for the Smaller Pharma

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MEDICINE ON THE BODY'S OWN TERMS

Introduction

Best practices in compound management of screening collections have been discussed widely in the pharmaceutical industry as there has been much concern regarding degradation of organic compounds following long-term storage as solutions (Ref 1).

The complexities of managing a collection, the large number of samples being handled and the problems encountered when storing compounds in DMSO solution have led many companies to install large dedicated individual use tube storage facilities. In particular, DMSO hydration and the consequent compound precipitation, have been demonstrated (Ref 2-4). As a result the larger pharmaceutical companies have chosen to store and supply samples under inert atmospheres thus increasing the costs of the storage facility.

Smaller pharmaceutical companies do not have at their disposal these dedicated automated compound handling facilities. When we initiated a bulk purchase of a new library we had the opportunity to explore new, affordable processes for storing and maintaining approximately 120,000 library compounds.

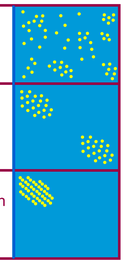
Our main considerations were compound integrity, limited storage space and readily available equipment. We decided to avoid long-term storage in DMSO as we were unable to provide the optimal inert atmosphere solution storage conditions. Our previous experience handling in-house medicinal chemistry libraries in DMSO stored at -20°C was that wells hydrate on freeze-thaw despite the use of cap-mats. As a result many compounds precipitated.

The most practical solution to fulfil our requirements was dry-film, which has since been validated for long-term storage (Ref 5). This created a set of plates that could be directly transferred into the screening assay and was achieved using our existing laboratory equipment.

Library Compound Selection

Compound selection for the new library was based on our requirements for tractable hits taking into account Lipinski rules (Ref 6), diversity, clustering our interest in GPCRs *etc.* and therefore compounds were sourced from a number of different suppliers.

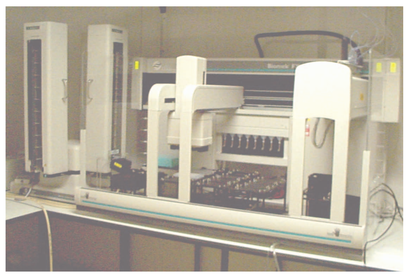
Library selected for diversity of chemical space.

Diverse	completely random	hit-seeking	
Targeted	to a target class, e.g. GPCR's, proteases, agonists/antagonists	hit-seeking	
Focused	to a particular lead/target combination	lead-optimisation	

Description of Equipment Used

In our laboratory we routinely use a Genevac DD-4 centrifugal evaporator for removing solvent. This was readily converted to accommodate 16 microtitre plates having SBS standard footprint. This instrument allows 16 x 384-well plates to be evaporated to dryness in 25 minutes at 40°C.

Liquid handling for our HTS comprises 2 Beckman Coulter BioMek FX instruments one each of 96 and 384 capability.



Compound Handling and Library Preparation

Compounds were solubilised, reformatted from 96 to 384 well plates then dried down for storage. The overall process is described below.

Library Format On Receipt

The library was purchased in 96-well plate format (Nunc 500µl plates) as dry film at 80 compounds/plate and 1µmole/well. Fresh DMSO, spectrophotometric grade (Sigma), was purchased to ensure dry solvent used to start.

Preparation of Library Copies

Day One - Solubilisation:

Add 100µl DMSO to each well containing 1 µmole (80 comp's A01 to H10) and then left overnight at room temperature to dissolve.

Day Two - Plate Reformatting:

Transfer of four 96 well plates into quadrants of one 384 well plate.

Materials: 4 x 96 well plates [Nunc 267245] (80 comp's A01 to H10)

2 x 384 well plates [Matrix 4314 polypropylene]

Dimethyl sulphoxide [Sigma 154938]

Methods:

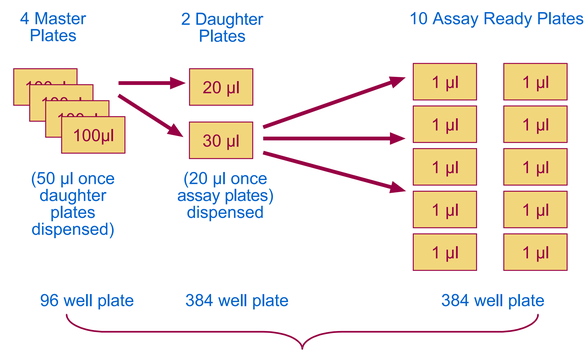
Biomek FX 96 using 250µl tips

80µl of the 100µl well contents mixed 5 times and then 20µl added to one 384well plate (A01 to P20) and 30µl added to the other (A01 to P20).

Biomek FX384 using 30µl tips

30µl plate taken to FX384 and then 1µl dispensed into 10 x 384 well plates. (25µl air gap, 3µl aspirated from source plate, 2µl dispensed into source plate, then 1µl dispensed in destination plate with 25µl air gap blown out at bottom of well, with a move across the bottom of well at the same time, to remove droplets).

Preparation of Library Plate Copies



All copies dried down using Genevac DD4

Drying Down

Method:

Genevac

All plates dried down in S DD-4, Rotor Temp set at 44°C and Drum Temp set at 40°C.

50µl 96 well plates dried for 2 hours, 20µl 384 well plate dried for 1.5 hours and 1µl plates for 25 minutes.

Plates then stored in 10 sets of screening plates in bags in boxes at room temperature until needed.

References

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3. Kozikowski BA, Burt TM, Tirey DA, Williams LE, Kuzmak BR, Stanton DT, Morand KL, Nelson SL. *J Biomol Screen*. 2003, Apr;8(2):210-5
4. Kozikowski BA, Burt TM, Tirey DA, Williams LE, Kuzmak BR, Stanton DT, Morand KL, Nelson SL. *J Biomol Screen*. 2003, Apr;8(2):205-9
5. Topp A, Zbinden P, Wehner HU, Reigenass U. *JALA*. 2005 4 (Vol. 10, Issue 2): 88-97
6. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. *Adv Drug Deliv Rev*. 2001 Mar 1;46(1-3):3-26

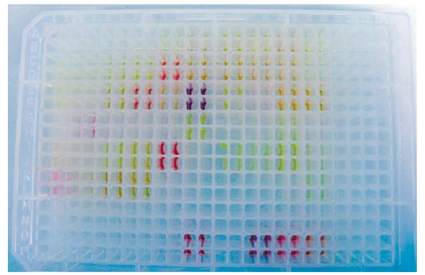
Solubilisation of HTS Compound Plates

Due to the centrifugation step in the dry film preparation the compounds in the 384 well plate are in effect spun to the outer corners of the well (see Picture 1). The addition of small volumes of solubilising DMSO solvent is insufficient to cover the bottom of the well resulting in little contact with the compound dry-film. To achieve effective solubilisation the centrifugation step is repeated and both compound and solvent are in the same position within the well.

Method:

2µl of DMSO added to each well and then plate returned to the Genevac for a 2 minute spin to position the DMSO over the dried down compound. Plates left overnight at room temperature to re-solubilise the compounds.

Picture 1: Compound/solvent position after spin



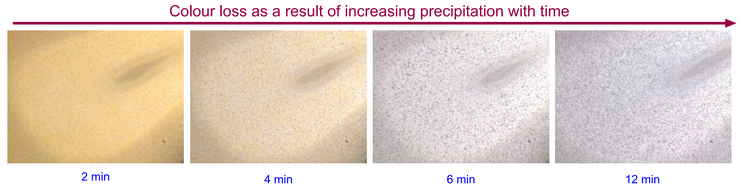
Aqueous Dilution

Aqueous dilution of the solubilised compound plates is achieved immediately prior to addition to the final assay plate, to ensure that the compound has the best chance of being in solution. The effect of just 6 minutes contact with aqueous medium can be observed in Picture 2.

Method:

Water added immediately prior to the plates use in the assay, either on FX96 and then FX384 or FX384 alone depending on the amount of water to be added. The aqueous solutions are mixed 5 times. 1µl - 5µl is then added to assay plate using the Biomek FX384, dependent on final assay concentration required.

Picture 2: Time-course of compound precipitation in aqueous solution



Further Work - Understanding Compound Integrity

We have now started to analyse the compound integrity of the dry film library. This may be compared to the integrity of compounds from our "historic" library that have been stored in DSMO at -20°C. This analysis was carried out LC/MS. The two methods we currently use to determine Peak Detection & Purity Estimation for our medicinal chemistry libraries are:

ELSD (evaporative light scattering detector)

- will detect all compounds equally regardless of how well they ionise or how they absorb in the UV
- volatile compounds are not detected well
- excellent reliability

UV @ 254nm

- compound must have a chromophore, the larger the chromophore the bigger the absorbance
- at lower wavelength get more interferences from DMSO solvent peaks

CONCLUSIONS

We have discussed a dry film solution that has addressed both the compound integrity and physical storage issues using standard liquid and compound handling techniques, most suited to a smaller pharmaceutical environment. The result is that we have assay ready boxed sets of screening plates for HTS, reducing the time for compound preparation for at least the next 10 screens.