Miniaturisation of an Aqueous Solubility assay using Acoustic Dispensing and 384-Well Based Workflows

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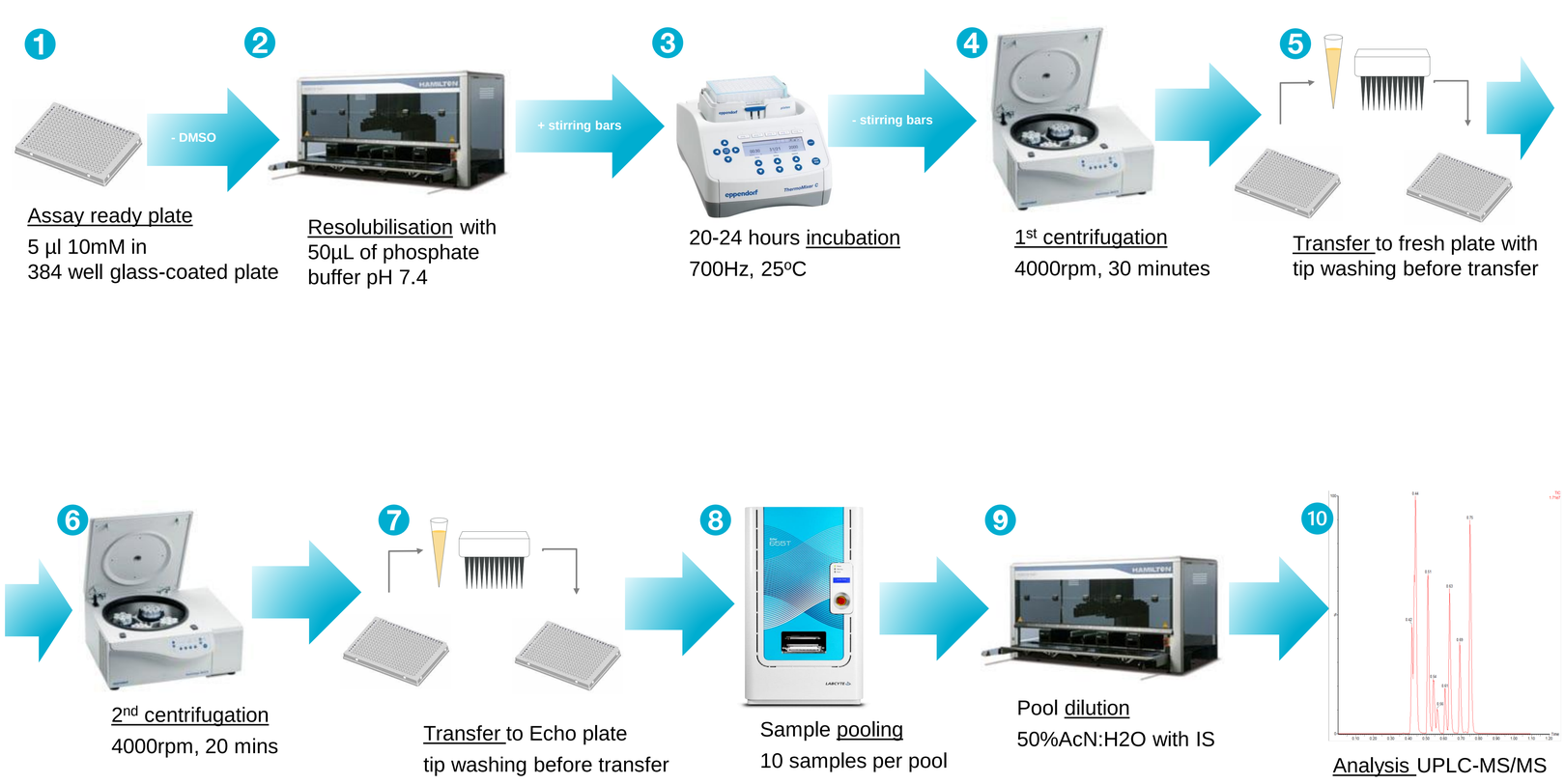
Introduction

Drug discovery serves to optimize and qualify candidate drugs for testing in man. This process requires concurrent optimization of physicochemical properties and metabolic stabilities alongside potency on the intended target to achieve efficacy. A critical property that affects observed activities, as well as the ability to formulate a compound for *in vivo* studies, is aqueous solubility.

Given the significant challenges associated with accurate prediction of solubility, there is a strong need for robust and efficient experimental methods. To maximise impact, such methods must be available at a scale where they can support structure-activity relationships during chemistry optimisation. Assay miniaturisation and automation towards this objective is however known to be challenging [1]. Here we disclose what we believe is the first 384-well dried-DMSO solubility assay that demonstrate concordance with results from thermodynamic solubility measurements.

Assay workflow



Scheme 1. Schematic representation of solubility assay workflow

Assay validation

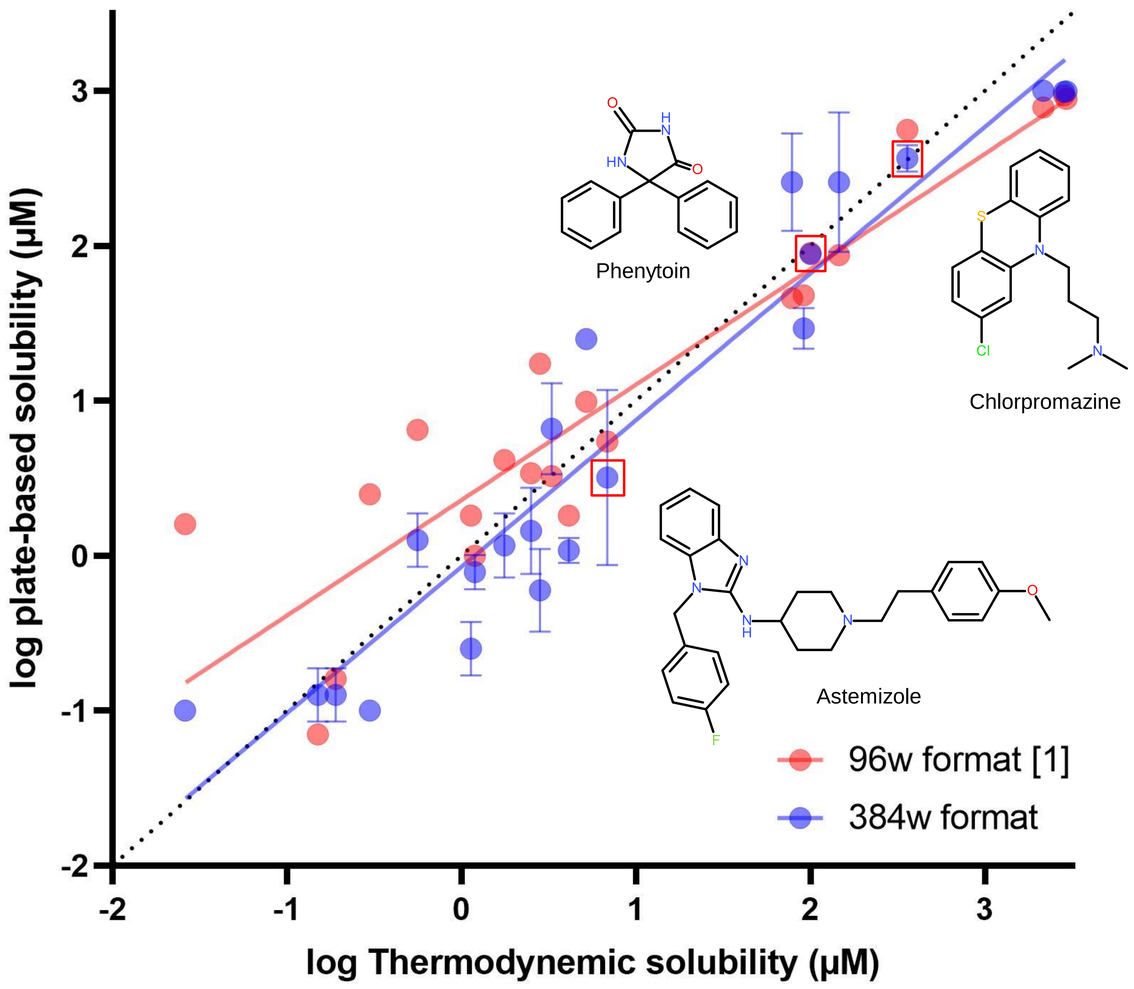


Figure 1. Correlation between thermodynamic assay results (y-axis) vs dried-DMSO method in 96-well and 384-well based formats (x-axis).

Validation test set

Clofazimine	Terfenadine	Loratadine	Phenytoin
Bifonazole	AZ1569	Astemizole	Testosterone
Cinnarizine	Tamoxifen	Estriol	Flutamide
Amiodarone	Albendazole	Nimodipine	Chlorpromazine
Fenofibrate	Mebendazole	Haloperidol	Piretanide
	Diclofenac	Enalapril	

Assay performance was validated for our acoustic dispensing enabled dried-DMSO assay in 384w plate format (Scheme 1) through comparison with a classical thermodynamic solubility test [2]. We also made comparisons with historical values from a 96w dried-DMSO assay [3]. Excellent agreement was observed between data in the new 384w assay with thermodynamic solubilities for a validation set of 22 compounds, the majority of which are approved drugs.

Linear regression gave a slope of 0.95 (0.87-1.0) and a r^2 value of 0.91, representing an improvement from the previous 96w assay (**Figure 1**). A key observation in this work was the necessity to retain a second centrifugation step after incubation and resting to avoid overestimation of poorly soluble compounds. As illustrated in Figure 1 assays are quality controlled by randomly placed reference compounds in each run.

Conclusions

The new 384w dried-DMSO aqueous solubility assay comes with an almost 50% reduction in sample need, as it starts from 6 µl of 10 mM DMSO stock solution instead of 11 µl. It also comes with an increased capacity allowing for 762 samples at each test occasion. We achieve this assay efficiency by doing sample pooling prior to analysis with LC-MS.

Moving forward this 384w assay will replace the 96w version within our DMPK Wave1 panel [3], which is an integral part of the design-make-test-analysis cycle at AstraZeneca.

- ☑ **only 6 µl 10mM stock required (~50% less)**
- ☑ **ARP preparation by Echo dispensing**
- ☑ **assay capacity 762 samples/run**
- ☑ **robust supernatant aliquoting**
- ☑ **sustainability improvements**
- ☑ **cost reductions**

References

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2. Llinàs et al. (2008). Solubility Challenge: Can You Predict Solubilities of 32 Molecules Using a Database of 100 Reliable Measurements? J. Chem. Inf. Model, 48, 1289-1303.
3. Wernevik et al. (2020). A Fully Integrated Assay Panel for Early Drug Metabolism and Pharmacokinetics Profiling. Assay and drug development technologies, *18*(4), 157–179.

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