A fully automated kinetic solubility screen in 384-well plate format using nephelometry

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- **Nephelometry as a rapid, reliable and low cost method for solubility screening in 384-well plate format**
- **Kinetic solubility determination of 24 compounds in 75 min**
- **Special fit method for solubility curve**

Introduction

Within the drug discovery industry there is a growing trend towards measuring ADME and physical chemical properties for larger numbers of compounds at an earlier stage, and at a higher throughput, in an attempt to highlight potential ADME issues and to reduce attrition. Solubility is one of the most important properties of a compound and recognising solubility issues at an early stage is invaluable. Not only are low solubility compounds more difficult to develop, obtaining reproducible data for ADME screens such as Caco-2 and lipophilicity is also more time-consuming and costly. Therefore, a rapid, low cost method for determining solubility prior to running the more costly ADME screens is a useful tool.

Laser nephelometry has been shown to be a reliable technique for the measurement of kinetic solubility in 96-well plate format. Laser nephelometry is the measurement of forward scattered light when a laser beam is directed through a solution. The more particulate there is in the solution, the greater the amount of forward scattered light (measured as counts). This work shows how this technique has been advanced into a fully automated and rapid kinetic solubility screen in 384-well plate format.

Materials & Methods

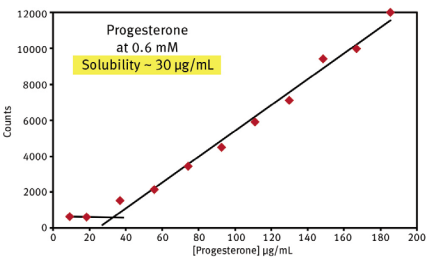
- A liquid handling robot has been integrated with a BMG LABTECH NEPHELOstar® *Plus* to produce a fully automated kinetic solubility screening system for discovery compounds
- The robot is fitted with 1 mL syringes, fixed tips and a ROMA arm.
- The liquid handling, pipetting volumes, plate type, plate reading, time scheduling and processing software have been optimised resulting in an analysis and data processing time of 75 minutes for 24 compounds (8 compounds in quadruplicate per 384-well plate).
- This system has been validated with 38 commercial and in-house compounds.

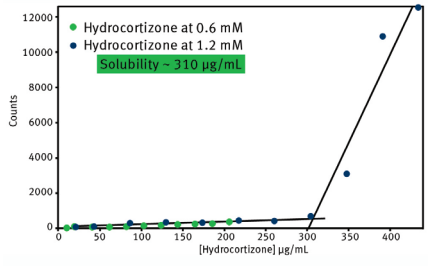
Experimental

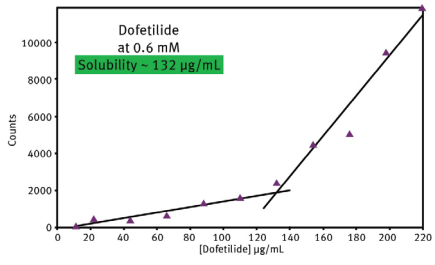
- Stock solutions were prepared at 0.6 mM or 1.2 mM in 5% DMSO: 95% PBS buffer manually.
- For a single batch of 24 compounds (3 plates), the stock solutions were diluted to decreasing molarity across the plate with 5% DMSO: 95% PBS buffer by the robot.
- The optimum net volume for all samples was found to be 100 µL. This is the maximum volume to fit into a 384-well plate, whilst minimising pipetting errors at low volumes (< 5 µL).
- Each plate was read vertically, with a gain of 100 and a laser intensity of 90% to produce raw data of counts per well.
- All raw data were processed using BMG LABTECH MARS data analysis software.

Results & Discussion

The following graphs from below (figure 1) show: mean counts (n=4) plotted against concentration for progesterone, hydrocortizone, dofetilide and paracetamol.







Rev. 10/2004
Keywords: **Dofetilide, Hydrocortizone, Paracetamol, Progesterone, Solubility**

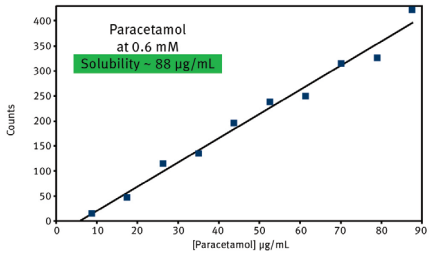


Fig. 1: Kinetic solubilities for 4 compounds evaluated from the fitted mean counts (n=4).

Compound	This work[a] µg/mL	Literature[b] µg/mL
Hydrocortizone	289	>181
Tyrosine	63	91
Propanolol	>156	41 (10)[c]
Paracetamol	>88	Na
Progesterone	30	20
Dofetilide	132	Na
Acetazolamide	>118	>111
Nitrofurazone	>119	>99
Theobromine	80	90
Ibuprofen	>123	52 (1)[c]

[a]Measured solubility in 384-well plate format at pH 7.4.
[b]Measured solubility in 96-well plate format at pH 7.4.
[c]Measured solubility in 96-well plate format at pH given in parentheses.

For the first 3 compounds there is a dramatic increase in counts which corresponds to the compound precipitating out of solution. Two linear lines are fitted to the data and the point at which they cross is taken as the kinetic solubility. The plot for paracetamol shows no point of precipitation which indicates complete solubility over the concentration range covered.

Hydrocortizone has been chosen as a control compound to assess the batch to batch variability of the system. Figure 2 shows the results for hydrocortizone run over 6 different days (n=14). The mean result is (289 ± 14) µg/mL. In the future, the result for hydrocortizone run in each batch will have to fall within this range for the batch to be accepted. The batch to batch variability of the system is 5%.

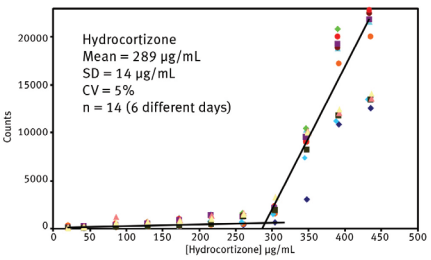


Fig. 2: Batch to batch variability for hydrocortizone as a control compound. Results over 6 different days (n=14) are shown.

38 compounds have been run on the system described here as part of the validation. It was possible to obtain measured solubilities for 35 out of the 38 compounds. A non-result was due to the plots of mean counts against concentration being too scattered to fit any reasonable line through. 10 of the compounds measured in this work have been measured previously using nephelometric techniques in 96-well plate format. Table 1 shows that the results using the fully automated 384-well plate system and the manual 96-well plate method compare favourably.

Conclusion

- This novel, fully automated kinetic solubility screen in 384-well plate format can be used to analyse 24 compounds in 75 minutes with a batch to batch variability of 5%.
- The screen can be used to measure and rank the kinetic solubility of approximately 90% of discovery compounds submitted to the screen.
- Results will be reported as measured values and flagged as green for soluble (>100 µg/mL), amber for partially soluble (15 - 100 µg/mL) and red for poorly soluble (<15 µg/mL) compounds.
- The system can be easily adapted to work with alternative buffers and pHs.

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