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

Caroline Green, Seona McKee and Ken Saunders, Automation Team, Department of Drug Metabolism
Pfizer Global Research and Development, Sandwich Laboratories, Sandwich, Kent CT13 9NJ, UK.

- **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.

**Results & Discussion**

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