Automated Screening of Aqueous Compound Solubility in Drug Discovery

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Abstract

It is desirable and increasingly more common in drug discovery to characterize compound solubility prior to biological testing. A filter-based method has been developed for both semi-quantitative and quantitative solubility determinations. Compounds solubilized in water-miscible solvents are added to buffer in a filter plate and incubated on a shaker deck. The filter plate is then transferred to a vacuum manifold and samples are filtered. A fixed volume of each filtrate is transferred to a UV-transparent analysis plate. Depending on the type of analysis - semi-quantitative or quantitative - a single calibrator or standard solutions are transferred to the plate and absorbance is measured. Results from the filter-based method correlate well with values obtained using standard methodology (shake flask). The filter-based assay can be fully automated on a number of laboratory robots. Depending on calibration and replicate number, the method is capable of producing results on hundreds of samples per day.

Introduction

Determining compound solubility in water has become an essential early measurement in the drug discovery process. Poor water-solubility can cause problems in many different *in vitro* testing techniques leading to unreliable results and/or reproducibility problems. Consequently, candidate compounds can fail early in their development due to unfavorable physicochemical profiles. An even larger problem results when insoluble precipitates cause false positives in bioassays, potentially wasting valuable resources. Such issues can add significant cost and time to drug development activities.

The standard protocol to determine the solubility of a compound is to use the shake-flask solubility method.[1] This method is inherently low-throughput, labor intensive, and necessitates the addition of drug in powder form – a requirement which can be incompatible with how compounds are generally maintained (e.g., in DMSO[2,3]). The shake-flask method involves adding an excess quantity of solid material to a volume of buffer at a fixed pH. This saturated solution is agitated (shake-flask) until equilibrium is reached, generally after 24 to 48 hours (empirically determined). Following separation by filtration or centrifugation, the compound in solution is analyzed and quantified by UV/Vis spectroscopy or HPLC.

Presented herein are two methods carried out in a 96 well format for the fast and accurate determination of aqueous compound solubility. Using a single point calibration for classification, or a calibration curve for quantitation, the solubility of hundreds of compounds can be determined in less than four hours. The method is automation compatible, high throughput and can be used in conjunction with existing laboratory equipment. The solubility assay has excellent correlation with the shake-flask method.

Features

- MultiScreen® Solubility plate designed and optimized for the determination of aqueous solubility
- 96-well format allows for analysis of multiple drugs in a single plate
- Compatible with standard laboratory robotics and analytical equipment
- Low non specific binding/high drug recovery (see **Chart 5**)
- Low sample amounts required (e.g., 10 µL at 10 mM = 100nMoles)
- Direct quantitation of compound in solution
- Functional over a wide pH range and compatible w/multiple excipients
- Good particle retention removes insoluble compound
- Compatible with aqueous organic solutions (e.g., ≤ 5% DMSO)
- Reproducible and repeatable results

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Method Correlation

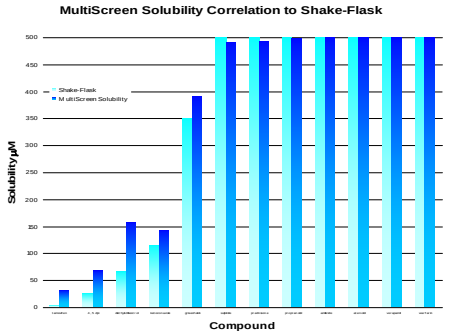


Chart 1: The solubility of more than 30 commercially available compounds was determined using the MultiScreen solubility quantitation method and the shake-flask method. Results for 12 commercially available compounds are presented demonstrating the correlation between the quantitative method and the traditional shake flask method over a range of compound solubilities.

Correlation of the Screening Method

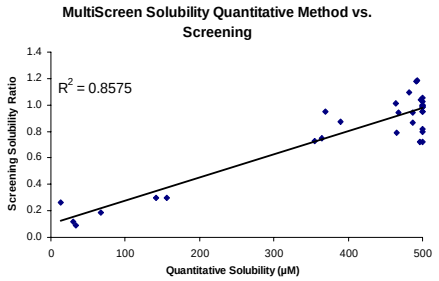
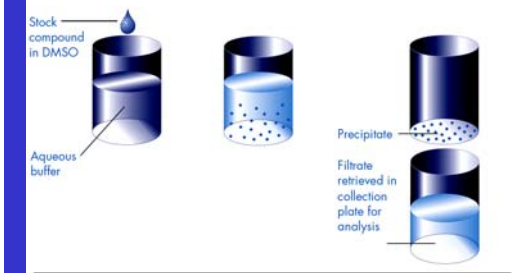


Chart 2: The correlation of the MultiScreen solubility quantitative method to the screening method for 30+ commercially available compounds is illustrated.

Specifications

Plate	Polystyrene
Membrane	0.4 µm PCTE
Well Capacity	0.3 mL
Maximum Vacuum	12" Hg
Recommended Vacuum	10" Hg

Method Overview



1. Add compound dissolved in organic solvent to aqueous buffer.
2. Shake for 90 minutes to allow insoluble compound to precipitate.
3. Apply vacuum to filter solution into collection plate. Precipitates remain on membrane. Analyze filtrate in collection plate to quantitate amount of compound still in solution.

Factors Influencing Solubility

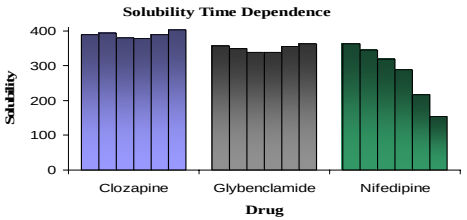


Chart 3: The effect of time on compound solubility. Solubility was determined for each of the compounds at time = 0, 0.5, 1.5, 3, 6, and 16 hours. For the majority of compounds (e.g., clozapine and glybenclamide), an incubation time of 1.5 hours was sufficient to reach equilibrium. A 1.5 hour incubation may not permit super-saturated compounds to reach equilibrium as some compounds may crystallize slowly in the solvent system. This effect is illustrated with nifedipine, whose solubility decreased from 350 µM initially to 150 µM after 16 hours.

DMSO Effects

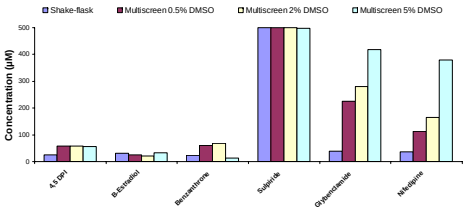


Chart 4: DPI, ß-estradiol, benzanthrone, all relatively low solubility compounds, and sulpiride, a relatively high solubility compound, are essentially unaffected by the concentration of DMSO, while the apparent solubility of glybenclamide and nifedipine are elevated by an order of magnitude in the presence of 5% DMSO.

Data Analysis

The quantitative solubility of a drug is calculated from the absorbance of a sample divided by the slope of the line generated from the calibration curve:

$$\text{Aqueous Solubility} = \left(\frac{A\max \, Filtrate}{slope}\right) \times 1.25$$

The screening ratio is calculated using six wavelengths for both the sample and standard as detailed below. The resultant screening ratio is classified as follows:

$$\text{Screening Ratio} = \left(\frac{\sum \text{AU @ 280, 300, 320, 340, 360 minus AU @ 800nm Sample}}{\sum \text{AU @ 280, 300, 320, 340, 360 minus AU @ 800nm Standard}}\right)$$

Screening ratio < 0.5	then solubility < 100 µM
Screening ratio > 0.5 and <1	then solubility > 100 µM and < 500 µM
Screening ratio ≈ 1.0	then solubility ≥ 500 µM

Drug Recovery

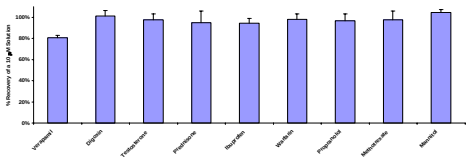


Chart 5: Nine drugs were analyzed for drug recovery in a Multiscreen Solubility plate. Compound recovery was at least 80 %, with the majority of compounds being recovered at 90 to 100 %.

Reproducibility

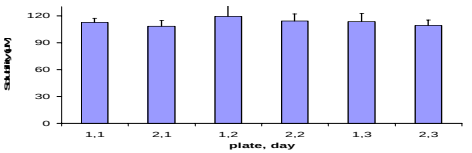


Chart 6: Ketoconazole was analyzed in a total of 6 plates (45 wells per plate) on 3 different days. The average and standard deviation from a given plate on a specific day is plotted in µM units.

Summary

The MultiScreen Solubility Filter plate and screening method provide an automation compatible, high throughput means to estimate the aqueous solubility of hundreds of compound per day. Using a single point calibration, the screening ratio is easily and quickly derived, and compound solubility is readily approximated. Multiple samples, each requiring approximately 200 nanomoles (~100 µg) per result, can be run in parallel. The method allows for the analysis of approximately 45 compounds (duplicate determinations) per plate with the capability of completing four or more plates in a standard 8-hour day. The assay is inherently compatible with the manner in which most compound libraries are produced (e.g., as stock solutions in DMSO, etc.), fully automatable and is easily integrated into existing chemical profiling and early ADME workflows.

1. ASTM International, E1148-02.
2. Bevan, C. D.; lloyd, R. S. *Anal. Chem.* **2000**, *72*, 1781-7.
3. Ruell, J., Avdeef, A. *Mod. Drug Disc.* **2003,** 47-9.