Application Note

Aqueous Solubility Testing with the MultiScreen®$_{HTS}$ – PCF Filter Plate

INTRODUCTION

Water solubility testing is an important part of many scientific studies, including the early drug discovery process. However, the standard shake-flask method for solubility testing is labor intensive and suited solely for low-throughput work. To overcome these challenges, Millipore has developed a new filtration-based assay using the MultiScreen$_{HTS}$ – PCF plate.

This new solubility assay uses a high-throughput 96-well plate with a specially-developed membrane to improve particle retention and achieve reproducible results in the micromolar range. The plate is compatible with aqueous organic solutions (<10% DMSO) and both manual and automated systems.

RESULTS & DISCUSSION

The MultiScreen solubility assay is well suited for a number of compound screening applications. As summarized in Table 1, it achieves results similar to the standard shake-flask method, but more easily and efficiently. In this case, aqueous solubility was determined with UV spectroscopy from 260-500 nm (data shown are at pH 7.4). In most instances, the apparent solubility as determined with the MultiScreen$_{HTS}$ – PCF plate is somewhat higher than that measured using the shake-flask method. This is probably due to the presence of 5% DMSO in the MultiScreen method.

Table 1. Comparison of the shake-flask & MultiScreen solubility tests at pH 7.4

Solubility Drug	Shake-Flask	MultiScreen
Tamoxifen	2	24
4,5 dpi	20	57
β-estradiol	25	27
Glybenclamide	31	>400
Nifedipine	35	370
Diethylstilbestrol	53	125
Clozapine	77	>400
Ketoconazole	91	216
Prednisone	>400	394
Testosterone	251	292
Phenazopyridene	163	284
2-naphthoic acid	>400	>400
Amiloride	>400	>400
Amitryptiline	>400	389
Atenolol	>400	>400
Caffeine	>400	>400
Chloramphenicol	>400	>400
Chlorpromazine	>400	374
Diclofenac	>400	>400
Furosemide	>400	>400

The MultiScreen plate is also compatible with automated systems. Unlike the limited, time-consuming shake-flask method, the Multiscreen plate can provide aqueous solubility data in triplicate for up to 120 drug compounds in an eight-hour day. In addition, pKa data on compounds with ionizing groups can also be obtained when solubility is determined versus pH.

There are, however, some important method limitations. Compounds, especially standards, must remain soluble over the duration of the assay. Certain compounds that are not soluble in 20% aqueous acetonitrile may produce visible precipitates and cloudiness which can interfere with the UV spectroscopic analysis. If a precipitate is found, other analytical methods, such as HPLC or LC/MS/MS, may be used. Some compounds may contain color-producing chromophores, so the spectral range should be increased beyond 500 nm. It is also possible to overestimate a compound's solubility in a purely aqueous solution, as samples are made up in a 5% (v/v) DMSO solution. Lowering the amount of DMSO (e.g., 0.5%) may improve the correlation between the aqueous solubility method and the shake-flask method. Sample purity will also affect results—if the compound is less than 95% pure, the UV spectroscopy method may not be suitable. A complex mixture would require some sort of chromatographic separation prior to analysis. Finally, it is essential that the compounds being investigated have sufficient UV spectroscopic molar absorptivities (extinction coefficients) to provide the requisite analytical sensitivity.

With this in mind, the MultiScreen solubility assay is still a suitable high-throughput tool for numerous applications. Scientists wishing to determine structure-solubility relationships and establish appropriate dosing concentration ranges for subsequent *in vitro* testing programs will find this assay to be particularly useful.

MATERIALS & METHODS

Equipment:

- MultiScreen$_{HTS}$ – PCF 96-well filter Plate (Catalogue No. MS SLB PC 10) with lid.
- Compounds in concentrated organic solution form (e.g., ACN, DMSO stocks)
- Stock buffer solution
- Stericup® filter unit
- 96-well disposable UV-Star™ analysis plate or equivalent for UV micro-plate reader
- 96-well polypropylene, V-bottom collection plate
- 96-well polypropylene, 2.4 mL deep-well plate
- Single-channel pipette, 0.2-10 µL, or automated liquid handling
- Multichannel pipette, 50-1200 µL, or automated liquid handling
- Titer-plate shaker
- Vacuum pump and manifold system
- UV spectroscopic micro-plate reader or HPLC-UV or LC/MS/MS

GENERAL CONSIDERATIONS

The recommended sample volume per well is 200 µL.
The presence of any amount of DMSO in the sample may increase the apparent aqueous concentration of some organic compounds. (Lower levels of DMSO can be used with more concentrated stocks and/or for a lower dynamic range to check for the effect of DMSO on a compound's aqueous solubility. 500 µM is the maximum measurable solubility for a 5% final DMSO concentration [when initial is 10 mM stock]).

Mixing and incubation of solution takes place in the MultiScreen plate, which is then operated in a vacuum mode to retrieve the filtrate for further analysis (solubility) by UV spectroscopy. LC/MS/MS and HPLC can also be used to determine compound solubility, especially for compounds with low UV absorbance (greater than 270 nm), and/or compounds with lower purity (<90%). For quantification of aqueous solubility, it is recommended that a standard calibration curve be completed and analyzed for each unknown compound prior to aqueous solubility determinations (see Millipore Protocol Note PC2445EN00 for more detail).

For UV spectroscopic analysis, use the purest form of compound possible. Impurities may interfere with the ability to determine solubility using UV spectroscopy. An alternate detection method such as HPLC, can be used to increase method reliability.

Compounds with very low solubility and/or low molar extinction coefficients in the 260 to 500 nm range may not be quantifiable by UV spectroscopy.

Some compounds may not be fully soluble in 20% acetonitrile, so an alternate analytical method may be necessary.

OPERATING PROCEDURE

1. Make pH 7.4 buffer (see Millipore Protocol Note PC2445EN00) and filter with a Stericup to remove any particulates. Store at 4°C for up to one month prior to use.
2. Using a multichannel pipette, dispense 190 µL per well of pH 7.4 buffer from step 1 into a MultiScreen$_{HTS}$ – PCF plate.
3. Using a multichannel pipette, dispense 10 µL per well of stock compound (normally at 10 mM in DMSO) from a 96-well polypropylene V-bottom plate directly into the buffer in the MultiScreen solubility plate.
4. Cover with lid, and mix with gentle shaking (100-300 rpm) at room temperature for 1.5 hours.
5. After mixing, place the MultiScreen plate on a vacuum manifold with a grid and filter (10-12″ Hg) into a clean polypropylene, 96-well V-bottom collection plate. (Filtration by vacuum requires that there is liquid in all 96 wells of the MultiScreen solubility plate.)
6. After filtration, use an appropriate method (UV spectroscopy, HPLC, LC/MS/MS) to quantify dissolved compound versus standard curves (see Millipore Protocol Note PC2445EN00 for more detail).

ORDERING INFORMATION

Description	Qty/pk	Catalogue No.
MultiScreen$_{HTS}$-PCF Filter Plate	10/pk	MSSL BP C10
	50/pk	MSSL BP C50
MultiScreen$_{HTS}$-Vacuum Manifold	1/pk	MSVM HT S00

Related Products

96-well Polypropelene Collection Plate	100/pk	MSCP NP P00
96-well UV Analysis Collection Plate	40/pk	MSCP NU V40
96-well Deep Well Collection Plate	50/pk	MSCP N2 M50

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