MILLIPORE

Application Note

Date: August 2004

Literature no: AN1240EN00

Title: MultiScreen® Solubility Filter plate: Integration with Detection Techniques

ABSTRACT

The use of the MultiScreen Solubility Filter Plate and supporting methodology in conjunction with the Molecular Devices SpectraMax® Plus microtiter plate reader with SoftMax® Pro software enables the classification of aqueous compound solubility for hundreds of samples per day. When aqueous compound solubility cannot be determined due to low sample concentration and/or a poor extinction coefficient, the sample generated from the filter plate can be readily analyzed by potentially more sensitive detection techniques such as HPLC-UV. Following the prescribed methodology, aqueous compound solubility can be determined for a significant majority (>95%) of compounds.

BACKGROUND

With the high demand on the pharmaceutical industry to introduce new drugs to market quickly and in a cost-effective manner, it has become increasingly important to determine compound absorption, distribution, metabolism and excretion (ADME) properties earlier in the drug discovery process. Perhaps the most critical and important first characteristic to measure is compound solubility.

Requiring minimal sample consumption, the MultiScreen Solubility Filter plate with supporting methodology provides an efficient, high throughput and automation compatible method to determine aqueous compound solubility. Aqueous compound solubility can be readily classified for multiple compounds using a Molecular Devices SpectraMax Plus microtiter plate reader with supporting software.

INTRODUCTION

The screening methodology with the Multiscreen Solubility filter plate is designed to classify aqueous compound solubility as **high** (500 µM or greater); **moderate** (between 100 and 500 µM); or **marginal** (less than 100 µM). Ranking is based the compound's screening ratio which is determined by dividing the (sum of) absorbance of the compound filtrate by the (sum of) the absorbance of the compound standard solution (normally at 500 µM) (**Figure 1, Table 1**).
Such classifications are based on a highest concentration of 500 µM. In such instances when a higher or lower dynamic range is required, *e.g.* ranging from 2.0 mM to 10 µM, or 100 µM to 0.50 µM respectively, the screening ratio can be adjusted accordingly. In such circumstances, the upper limit is defined as the highest possible concentration while the lower limit is defined by the sensitivity of the analytical method.

$$\textbf{Screening Ratio} = \left(\frac{\sum(\text{AU}\,@\,280, 300, 320, 340, 360) - 800\,\text{nm of the Filtrate}}{\sum(\text{AU}\,@\,280, 300, 320, 340, 360) - 800\,\text{nm of the Standard}} \right)$$

Figure 1: The equation used to determine the screening ratio which uses the filtered and sample absorbance values at 280, 300, 320, 340, 360 and 800 nm.

Screening Ratio	Aqueous Solubility Classification	Solubility
≅ 1	[conc] ≥ 500 µM	High

<1 but > 0.5	500µM > [conc] > 100	Moderate
< 0.5	[conc] ≤ 100 µM	Marginal

Table 1: Aqueous compound solubility classification based on the screening ratio

The Softmax Pro software (v. 4.7) is equipped with a protocol that provides the user with the solubility classification as described (See Millipore Application Note Number: AN1731AN00).
The software requires the absorbance readings for each standard and sample to meet specific acceptance criteria to generate screening classification results. The acceptance criteria can be user defined; the default values are listed in **Table 2**. The default acceptance criteria include "Ratio Acceptance," in which the ratio of the sample to standard cannot exceed; 1.2; "800 Acceptance," in which the absorbance at 800 nm cannot exceed 0.1 (Absorbance Units) AU, generally employed as a measure of sample clarity; and "Sum Acceptance" in which the sum of the absorbances at 280, 300, 320, 340, and 360 nm must exceed 0.03 AU.

Acceptance Criteria	User defined limit (default settings)	Error message
Ratio acceptance	1.2	'High ratio'
800nm acceptance	0.1 AU	'800nm too high'
Sum acceptance	0.03 AU	'Low Sum'

Table 2: Acceptance criteria and default values for the Solubility Screening Assay on the SpectraMax Plus plate reader.

The final acceptance criterion, "Sum Acceptance" is included to discriminate signal from sample noise. An error message of "Low Sum" is generated if the absorbance is indistinguishable from instrument noise. In such instances, this can generally be attributed to compounds with poor UV-Vis chromophore or low sample concentration. Alternative means of detection such as HPLC-UV or LC-MS/MS are recommended to classify or quantify compound solubility.

MATERIALS, EQUIPMENT AND PROTOCOL

For a complete list of materials, equipment and protocols required to determine aqueous compound solubility with the MiltiScreen Solublity Filter Plate, please refer to the following documents which can be found at **http://www.millipore.com/catalogue.nsf/docs/C8875**.

AN1731EN00: MultiScreen Solubility Filter Plate: Performance and correlation of a 96-well high throughput screening method to determine aqueous drug solubility

AN1730EN00: MultiScreen Solubility Filter Plate: Quantitative method to determine drug aqueous solubility

PC2445EN00: MultiScreen Solubility Filter Plate: Determination of aqueous compound solubility using a 96-well filter plate

TN1177EN00: The MultiScreen® Solubility Screening Method Protocol With Data Acquisition and Analysis using Molecular Devices SpectraMax®Plus with SoftMax® Pro Software

HPLC analyses were completed with a Waters HPLC-UV system equipped with two Waters 510 pumps and Waters WAT46980 C-18 column with a guard cartridge using Waters Millennium 3.2 software.
Solvent A: 0.05% Phosphoric acid in water, pH 3.0; Solvent B: 100 % Acetonitrile;
Injection volume: 100 µL;

Flow rate: 1.0 mL/min.
A gradient elution of 100 % A to 100% B over 10 minutes then 100 % B for 6 minutes.
Samples were monitored at λ = 214 and 254 nm.

RESULTS AND DISCUSSION

More than 50 compounds have been screened for aqueous solubility following the referenced protocol and classified as having marginal, moderate or high solubility. A partial list is detailed in **Table 3**.

Entry	Compound	Screening Ratio	Solubility Classification	Shake flask (M)[a]
1	tamoxifen	0.12	marginal	3
2	glybenclamide	0.13	marginal	39
3	4,5 DPI	0.18	marginal	25
4	benzanthrone	0.26	marginal	23
5	diethylstilbestrol	0.3	marginal	66
6	nifedipine	0.46	marginal	35
8	phenazopyridene	0.73	moderate	204
9	testosterone	0.75	Moderate	315
10	methotrexate	0.91	high	500
11	thioridazine	0.95	high	500
12	diclofenac	0.98	high	500
13	verapamil	0.99	high	500
14	warfarin	0.99	high	500
15	amiloride	1	high	500
16	atenolol	1.04	high	500
17	propranolol	1.04	high	500
18	2-naphthoic acid	1.05	high	500
19	naproxen	1.1	high	500
20	ß-estradiol	low sum		21[b]
21	clotrimazole	low sum		1
22	digoxin	low sum		83[b]
23	ibuprofen	low sum		500[b]
24	mannitol	low sum		500[b]
25	taxol	low sum		2
26	terfenadine	low sum		8

Table 3: Aqueous solubility screening classification results for numerous compounds determined with the MultiScreen Solubility Filter plate and supporting methodology. The screening ratio and solubility classification were determined using the SpectraMax Plus plate reader and supporting software. A comparison to aqueous shake flask solubility has been provided.
[a] Unless otherwise indicated, shake flask solubility was determined according to ASTM standards.[1] [b]Shake flask values according to literature.[2]

As can be seen in **Table 3**, aqueous solubility classification using the SpectraMax Plus plate reader could be determined for the majority of compounds. However, seven of the compounds (ß-estradiol, clotimazole, digoxin, ibuprofen, mannitol, Taxol, and terfenadine) had inadequate absorbance values to permit analysis. The low absorbance values triggered the error message "Low Sum" based on a minimal absorbance requirement. Consequently, compound solubility was determined for these compounds using HPLC-UV. The results are displayed in **Table 4**.

Entry	Compound	Screening ratio	Solubility classification	Shake Flask (M)
20	β-estradiol	0.09	marginal	21
21	clotrimazole	0.06	marginal	1
22	digoxin	0.05	marginal	83
23	ibuprofen	0.98	high	> 500

24	mannitol	N/A	N/A	> 500
25	taxol	0.12	marginal	2
26	terfenadine	0.44	marginal	8

Table 4: Aqueous compound solubility for compounds determined by HPLC-UV. N/A = not available

Using HPLC-UV, it was possible to classify the solubility of six of the seven compounds. All results were in agreement with shake flask values. Mannitol, which does not contain a UV chromophore was undetectable with both a UV-Vis plate reader and HPLC-UV. Any compounds that do not have a UV-Vis chromophore will require a detection mechanism such as LC/MS.

CONCLUSION

The MultiScreen Solubility Filter plate provides a highly flexible, fast, high throughput, and automation-compatible means of determining compound solubility. Analysis and data reporting is easily performed using the Molecular Devices SpectraMax Plus microtiter plate reader with SoftMax Pro software. In those instances when the UV/Vis plate reader does not provide adequate sensitivity, the software template identifies the problem and an alternative analytical technique can be considered. As demonstrated in this applications note, HPLC-UV analysis can be used successfully to obtain results on the majority of samples that can't be screened using the higher throughput plate reader.

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[1] ASTM: E 1148-02, Standard test methods for measurement of aqueous solubility, Book of Standards Volume 11.05.

[2] Handbook of Physical Properties of Organic Compounds ed. Howard, P. H. and Meylan, W. M.; copyright 1997 by CRC Press, Inc., Boca Raton, FLA.

Millipore Lit. No. AN1240EN00