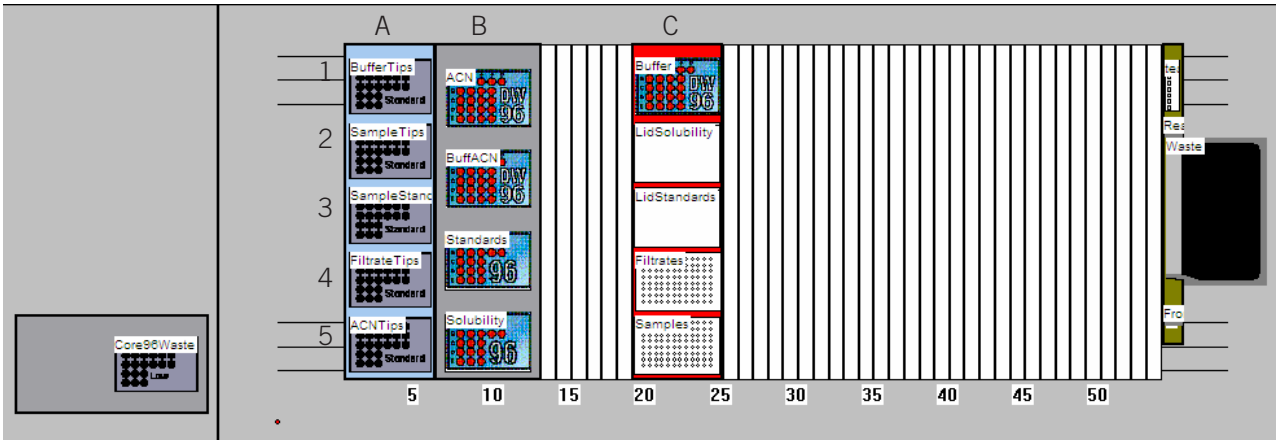
AUTOMATING AQUEOUS COMPOUND SOLUBILITY USING THE 96-WELL MULTISCREEN® SOLUBILITY FILTER PLATE ON A HAMILTON MICROLAB® STAR WORKSTATION

Introduction

Determining aqueous compound solubility has become an essential tool in the early stages of the drug discovery process. Solubility of a compound is important in the Biopharmaceutics Classification System (BCS). BCS classifies compounds based on their solubility and permeability. Low solubility can lead to unreliable results during in-vitro testing. Also, insoluble precipitates have been shown to cause false positives in bioassays, wasting valuable time and resources. Such issues will typically add significant cost to drug research projects. In addition to these factors, the standard shake-flask method used to evaluate drug solubility is inherently low throughput and labor intensive.

Here we describe an automated method for determining drug solubility using Millipore's MultiScreen Solubility filter plate on the Hamilton MICROLAB STAR workstation. The procedure requires minimal manual intervention by utilizing the iSWAP for all plate movements on the deck, but vacuuming occurs offline. The vacuuming occurs off-line because the Hamilton's Automated Vacuum System does not have a support grid needed for the MultiScreen Solubility plate. Eight compounds were tested in twelve replicates and the results were compared to those achieved through the manual method to demonstrate the viability of the automated protocol. It takes about 1 hour and 48 minutes to process one plate of 96 samples (this includes 90 minute shaking on the deck with a VARIOMAG® shaker). This translates to about six or more plates being able to be processed in a standard 8-hour day.

Configuration of the MICROLAB STAR Work Surface



Important:

- Program created using Hamilton Vector Software V 3.1.0.2160 on a MICROLAB STAR Workstation.
- Program written for a system with 96 CO-RE pipetting head and an iSWAP gripping mechanism.

HAMILTON

Prior to starting program (Millipore Solubility2.med), make sure the deck configuration is as follows:

A: Disposable Tip Carrier (tip_car_480_a00.tml)
1. 300 µL pipette tips (addition of buffer)
2. 300 µL pipette tips (addition of samples to Solubility plate)
3. 300 µL pipette tips (addition of samples to standard plate)
4. 300 µL pipette tips (transfer filtrate to UV plate)
5. 300 µL pipette tips (addition of acetonitrile to UV plate)

B: Front Shaker Carrier (front shaker_position4.tml)
1. Acetonitrile (Hamilton reagent trough)
2. 80% Universal Buffer, pH 7.4/20% Acetonitrile (Hamilton reagent trough)
3. UV 96-well plate for standards
4. MultiScreen Solubility Filter Plate on VARIOMAG shaker. After off-line filtration a then the UV 96-well plate for sample analysis is placed here.

C: 5 Position Plate Archived Carrier (plt_car_l5ac.tml)
1. Universal Buffer, pH 7.4 (Hamilton reagent trough)
2. Lid for solubility plate
3. Lid for standards plate
4. Filtrates (v-bottom poly-propylene plate) – after off-line filtration
5. Drug Compounds (v-bottom poly-propylene plate)

*Refer to application notes AN1730EN00 or AN1731EN00 for description of solutionpreparation.

Procedure:

1. Distribute 190 µL aliquots of the Universal buffer (C1) to the MultiScreen Solubility plate (B4) with the Buffer Tips (A1).
2. Distribute 192 µL aliquots of the 80% Universal buffer (B2) to the UV standards plate (B3) with the Buffer Tips (A1).
3. Add 10 µL of drug compound from the sample plate (C5) to the MultiScreen Solubility plate (B4) using Sample Tips (A2).
4. Add 8 µL of drug compound from the sample plate (C5) to the standards UV plate (B3) using Sample Standards Tips (A3).
5. The iSWAP moves the cover (C3) onto the standards plate and prompts the user to manually remove the standards UV plate (B3). The standards plate will need to shake for 5 minutes at 300 rpm off-line. It is shaken off-line, so it can be read while the solubility plate is shaking for 90 minutes.
6. The iSWAP moves the cover (C2) to the MultiScreen Solubility plate. Shake on the VARIOMAG shaker for 90 minutes at 300 rpm.
7. Filter the MultiScreen Solubility plate off-line for 1 minute at 10"Hg.
8. Return the filtrates (v-bottom poly-propylene plate) to the deck at position C4. Place a UV analysis plate on the VARIOMAG shaker (B4) and the cover at C2.
9. Transfer 160 µL of the filtrate from the filtrate plate (C4) to the UV analysis plate (B4) using the Filtrate Tips (A4).
10. Distribute 40 µL of Acetonitrile (B1) to all wells of the UV analysis plate (B4) using the ACN Tips (A5).
11. The iSWAP moves the cover (C2) to the UV analysis plate (B4). Shake at 300 rpm for 5 minutes.

Drug Solubility

	Hamilton Automation Method		Manual Method	
Drug	$\left(\frac{\sum AU\ Filtrate}{\sum AU\ Standard}\right)$		$\left(\frac{\sum AU\ Filtrate}{\sum AU\ Standard}\right)$	
Sample	Mean	Standard deviation	Mean	Standard deviation
4,5 DPI	0.24	0.04	0.20	0.02
benzanthrone	0.21	0.01	0.20	0.01
β-estradiol	0.08	0	0.04	0
diethylstilbestrol	0.11	0	0.05	0
griseofulvin	0.91	0.13	0.81	0.15
ketoconazole	0.32	0.01	0.24	0.01
phenazopyridene	0.93	0.04	0.77	0.16
testosterone	0.88	0.01	0.75	0.01

Table 1. Absorbance was measured in endpoint mode at 280, 300, 320, 340, 360, and 800 nm on a UV vis microplate spectrophotometer (SpectraMax® Plus, Molecular Devices). Aqueous solubility of each drug was calculated from the maximum absorbance units (AU) at each wavelength.

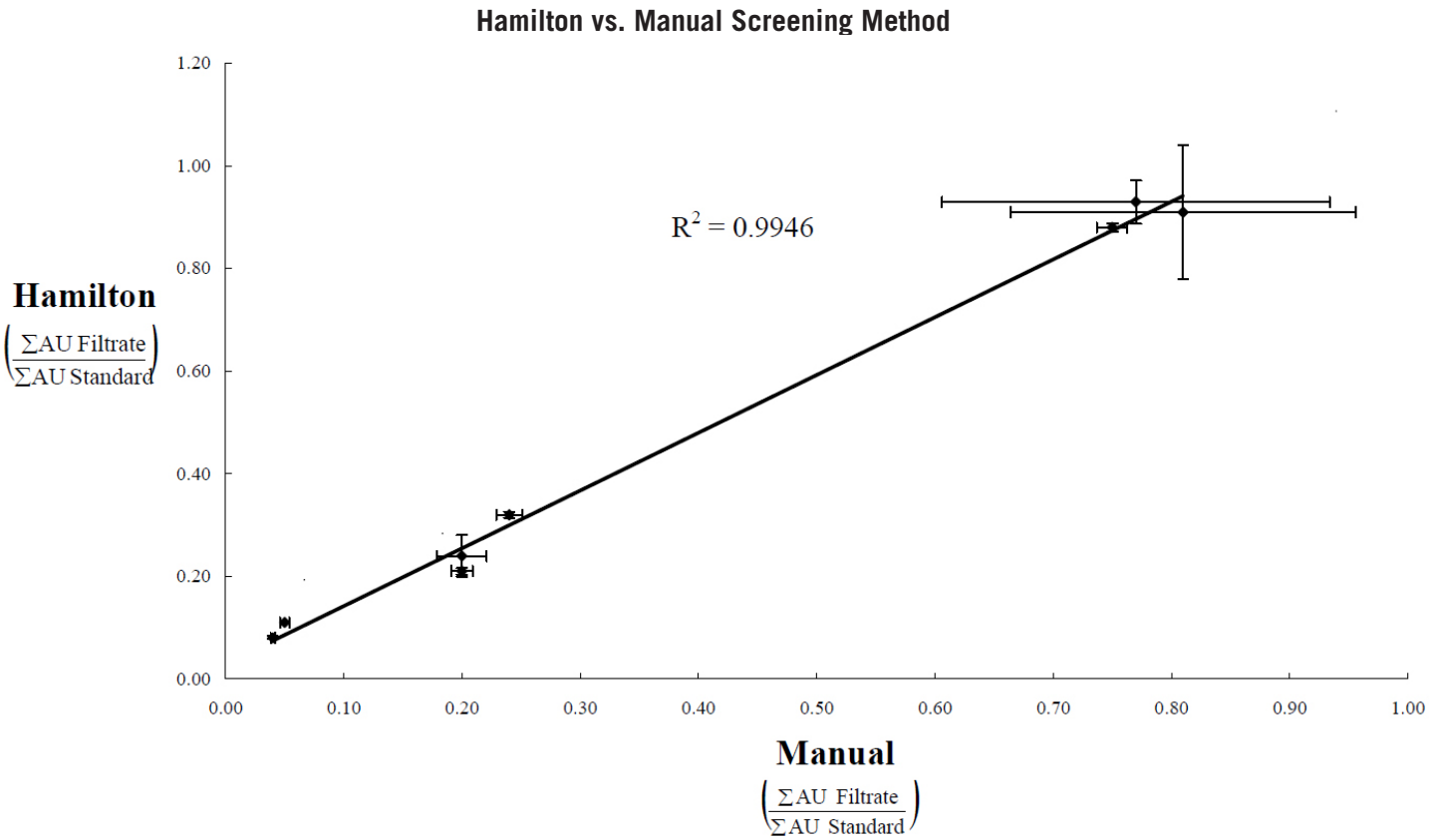


Figure 1. Data represents the correlation of manual and automated screening methods. Each data point represents the solubility ratio of the drug from one plate per method. Hamilton ratio, n=12. Manual ratio, n=12.

Conclusion:

The use of the Hamliton MICROLAB STAR with the MultiScreen Solubility plate produces screening ratio values similar to manually run plates. The variation within sample replicates during automation was equal to or less than the manual variation. The MultiScreen Solubility plate provides a high throughput means to estimate the aqueous solubility of hundreds of compounds per day. The use of the single point calibration allows for the screening ratio to be derived quickly thus allowing for compound solubility approximations. 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 per plate (in duplicate) with the capability of completing six or more plates in a standard 8-hour day.

Millipore Ordering Information:

	Part Number	Package Size
MultiScreen Solubility Plate	MS SLB PC 10	10/pk

Hamilton Accessories:

Item	Part Number
1 – Disposable Tip Carrier (TIP_CAR_480_A00)	182085/00
1 – 4 Position Front Shaker (Front Shaker_Position4)	187149, 187144, 187001
1 – 5 Position Archived Plate Carrier (PLT_CAR_L5AC_A00)	182090/00
3 - Hamilton Reagent Trough	4750-01
300 µL disposable pipetting tips	235902
96-CO-RE Pipetting Head	173084
iSWAP Gripper Mechanism	182600

Note: Hamilton accessories are U.S. part numbers and are subject to change. Please check with Hamilton prior to any purchase.

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