

Automation of ADME Screening Assays

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Abstract

In recent years there has been a trend to move ADME assays earlier in the drug discovery pipeline as part of the lead optimization process. The use of automation and 96-well plates have aided in this process by allowing scientists to screen larger numbers of compounds with relative ease, increasing throughput, simplifying sample handling and decreasing the amount of compound required.

Solubility and permeability are two such screening assays which are essential for classification of compounds in the BCS (Biopharmaceutics Classification System). Automation of aqueous compound solubility and PAMPA (Parallel Artificial Membrane Permeability Assay) are detailed here using 96-well filter plates from Millipore and a Sciclone ALH 3000 Advance Liquid Handler system from Caliper Life Sciences. Results show correlation with manual testing methods as well as a decrease in variability through the use of automation.

Introduction

Early screening methods are critical in the drug discovery process. It is important to determine a compound's solubility and permeability earlier because they are often classified based on these properties (Biopharmaceutics Classification System). If a compound has low solubility, it can produce unreliable results during in-vitro testing. Unreliable data can result in time and money being spent on qualifying a compound which will fail further characterization assays. The aqueous solubility method¹ described in this poster allows many compounds to be screened quickly to determine their relative solubility. The use of the Sciclone ALH 3000 workstation with a 96-well MultiScreen[®] Solubility filter plate (Millipore Corp.) allows 96 samples to be processed in 1 hour and 45 minutes.

Another early screening method (PAMPA) utilizes artificial membranes to model the passive transport properties of drugs across the cell membrane. The first PAMPA assay (PAMPA-Lipid²), places a lipid barrier on a PVDF membrane of the MultiScreen-IP filter plate (Millipore, Corp.). The two lipids used in this poster were chosen because they are easy to aspirate and dispense with the Sciclone in position the PVDF membrane. The lipids are slightly different in composition which results in slightly different transport rates.

The second assay, PAMPA-HDM³ assay, places a lipophilic barrier (hexadecane/hexane) on a polycarbonate membrane support of the MultiScreen Permeability filter plate (Millipore, Corp.). In both assays the rate at which a compound diffuses from the donor to the acceptor compartment across the membrane is determined. This rate is predictive of compound absorption and can be used as an early screen to rank the permeability rates for compounds. The combination of both types of 96-well filter plates and the Sciclone ALH 3000 workstation provide a fast and easy way to process up to 96 samples. PAMPA-Lipid takes 15 minutes (this does not include the 16 hour incubation period), while PAMPA-HDM takes about in 1 hour and 15 minutes (this does not include the 5-7 hours incubation).

Drug Solubility Using MultiScreen Solubility Filter Plate

1. Dispense 190 μ L PBS buffer into each well of the MultiScreen Solubility plate. Add 10 μ L of drug compound (10 mM DMSO stock). Cover and shake for 90 minutes at 300 rpm.
2. Filter at 10⁴Hg for 1 minute.
3. Transfer 160 μ L of the filtrate to a UV 96-well plate, add 40 μ L acetonitrile. Cover and shake for 5 minutes.
4. Dispense 192 μ L 80% PBS/20% Acetonitrile to a UV 96-well plate. Add 8 μ L of drug compound (10 mM DMSO stock) to create the standards plate. Cover and shake for 5 minutes.
5. Read both plates on plate reader at 280nm, 300nm, 320nm, 340nm, 360nm and 800nm.

Automation vs Manual

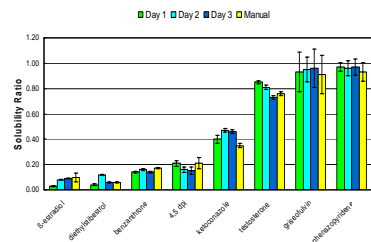


Figure 1. The Solubility Ratio was determined for 8 drug compounds (n=12) using a Millipore MultiScreen Solubility filter plate (MSLSPC10) and the Caliper LifeSciences Sciclone ALH 3000 workstation. The manual control shown is from the Day 3 automation run. Absorbance analysis was performed using a Molecular Devices (Sunnyvale, CA) SpectraMax[®] Plus reader. The results obtained by automation are equivalent to a manual control.

Sciclone ALH 3000

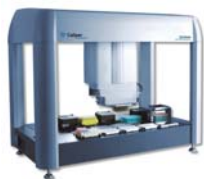


Figure 2. The Sciclone ALH 3000 system equipped with a 96 pipetting head, a gripper, a microplate shaker and a positive pressure filtration system was used for the ADME applications. The solubility assay utilizes the positive pressure filtration system. All three assays utilize the ability to aspirate and dispense out of covered plates while holding the cover with the gripper.

PAMPA-Lipid Using MultiScreen-IP PAMPA Plate

1. Dispense 5 μ L of a lipid in each well of the PAMPA plate.
2. Dispense 300 μ L 5% DMSO buffer to an acceptor plate (MSSACCEPTOR, Millipore, Corp.).
3. Create daughter plate by dispensing 285 μ L PBS buffer into a v-bottom polypropylene plate. Add 15 μ L drug compound. Mix.
4. Transfer 150 μ L from the daughter plate to the MultiScreen PAMPA plate.
5. Place the MultiScreen PAMPA plate on-top of the acceptor plate, cover and incubate for 16 hours at room temperature.
6. Create the equilibrium plate: transfer 80 μ L from the initial donor plate (daughter plate) to a UV 96-well plate. Add 170 μ L 5% DMSO buffer. Read the plate on a plate reader at 280nm, 300nm, 320nm, 340nm, and 360nm.
7. After 16 hour incubation, transfer 250 μ L from the acceptor plate to a second UV 96-well plate and analyze with a plate reader.

Assay Reproducibility – Avanti Lipid

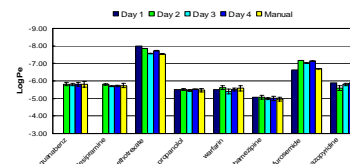


Figure 3. The Log P_e was determined for 8 compounds (n=12) using Millipore MultiScreen-IP PAMPA filter plate (MAIPN) and the Sciclone ALH 3000 workstation. A manual control was also run on Day 2 at the same time. The lipid used was a DOPC lipid (Synthetic Phospholipid Blend 1, Avanti Polar Lipids) and the donor concentration for all the drugs was 500 μ M. Absorbance analysis was performed using a SpectraMax Plus reader. Log P_e -6.00 or below are the result of the compounds being at the limit of detection of UV/Vis and therefore should be analyzed using a more sensitive method, i.e. LC/MS. The results obtained through automation processing are equivalent to data obtained manually.

Automation vs Manual – pION Lipid

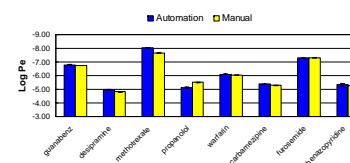


Figure 4. The Log P_e was determined for 8 compounds (n=12) using Millipore MultiScreen-IP PAMPA filter plate (MAIPN) and the Sciclone ALH 3000 workstation. A manual control was also run at the same time. The lipid used is manufactured by pION (#110618) and the donor concentration for all the drugs was 500 μ M. Absorbance analysis was performed using a SpectraMax Plus reader. Log P_e -6.00 or below are the result of the compounds being at the limit of detection of UV/Vis and therefore should be analyzed using a more sensitive method, i.e. LC/MS. The results obtained through automation processing are equivalent to data obtained manually.

PAMPA-HDM Using MultiScreen Permeability Plate

1. Dispense 15 μ L of 5% hexadecane in hexane in each well of the permeability plate. Allow to dry for 60 minutes.
2. Dispense 300 μ L 5% DMSO buffer to an acceptor plate (MSSACCEPTOR, Millipore, Corp.).
3. Create daughter plate by dispensing 285 μ L PBS buffer into a v-bottom polypropylene plate. Add 15 μ L drug compound. Mix.
4. Transfer 150 μ L from the daughter plate to the MultiScreen Permeability plate.
5. Place the MultiScreen Permeability plate on-top of the acceptor plate, cover and incubate for 5 hours at room temperature.
6. Create the equilibrium plate: transfer 80 μ L from the initial donor plate (daughter plate) to a UV 96-well plate. Add 170 μ L 5% DMSO buffer. Read the plate on a plate reader at 280nm, 300nm, 320nm, 340nm, and 360nm.
7. After 5 hour incubation, transfer 250 μ L from the acceptor plate to a second UV 96-well plate and analyze with a plate reader.

Automation vs Manual

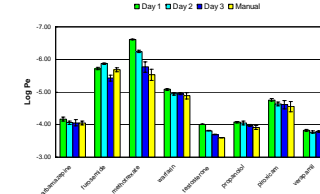


Figure 5. The Log P_e was determined for 8 compounds (n=12) using Millipore MultiScreen Permeability filter plate (MAPBM) and the Sciclone ALH 3000 workstation. A manual control was run on Day 3. The donor concentration for all the drugs was 500 μ M except for testosterone which was 100 μ M. Absorbance analysis was performed using a SpectraMax Plus reader. Log P_e -6.00 or below are the result of the compounds being at the limit of detection of UV/Vis and therefore should be analyzed using a more sensitive method, i.e. LC/MS. The results obtained through automation processing are equivalent to data obtained manually.

Summary

- ADME applications (Solubility, PAMPA-Lipid and PAMPA-HDM) can be easily automated on the Sciclone ALH 3000 workstation.

- Data obtained from automation are equivalent from day to day and to manual data demonstrating robust protocols.

- Automation data shows equal to or in most cases lower standard deviation than manual processed samples. This demonstrates a reduction in variability as a result of automation.

- Early characterization of compounds through the use of ADME automation reduces the time and money needed to in secondary screening. Furthermore, selected compounds with improved drug-like properties potentially lead to faster drug approvals.

- ADME automation allows scientists to increase throughput and productivity.

References

1. Onofrey, T.; Kazan, G. *Performance and Correlation of a 96-well High Throughput Screening Method to Determine Aqueous Drug Solubility*, Millipore Corporation Application Note, 2003; Lit. No. AN1731EN00.
2. Schmidt, D.; Lynch, J. *Evaluation of the Reproducibility of Parallel Artificial Membrane Permeation Assays (PAMPA)*, Millipore Corporation Application Note, 2003; Lit. No. AN1728EN00.
3. Schmidt, D.; Lynch, J. *The Evaluation of the Reproducibility of Passive, Transcellular Drug Permeability Assays*, Millipore Corporation Application Note, 2002; Lit. No. AN1725EN00.