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 throughout, 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 Sciclove ALH 3900 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. The aqueous solubility method described in this poster allows many compounds to be screened quickly to determine their relative solubility. The use of the Sciclove ALH 3900 workstation with a 96-well MultiScreen Solubility filter plate (Millipore Corp.) allows 96 samples to be processed in 1 hour and 15 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-Lipid), 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 Sciclove onto the PVDF membrane. The lipids are slightly different in composition which results in slightly different transport rates.

The second assay, PAMPA-HDM 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 Sciclove 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 1 hour and 15 minutes (this does not include the 5.7 hours incubation).

Drug Solubility Using MultiScreen Solubility Filter Plate

1. Dispense 190 µL PBS buffer into each well of the MultiScreen Solubility plate. Add 10 µL of drug compound (10 mM DMSO stock). Cover and shake for 5 minutes.
2. Filter at 100 µm for 1 minute.
3. Transfer 160 µL of the filtrate to a UV 96-well plate, add 40 µL acetonitrile. Cover and shake for 5 minutes.
4. Dispense 100 µL 80% PBS/20% Acetonitrile to a UV 96-well plate. Add 8 µL of drug compound (10 mM DMSO stock) to create the standards plate. Cover and incubate for 16 hours at room temperature.
5. Read both plates on a plate reader at 280nm, 300nm, 340nm, and 360nm.

Automation vs Manual

PAMPA-Lipid Using MultiScreen-IP PAMPA Plate

1. Disperse 5 µL of a lipid in each well of the PAMPA plate.
2. Dispense 350 µL 5% DMSO buffer to an acceptor plate (MISSECEPTOR, Millipore, Corp.).
4. Transfer 15 µL 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 µL from the initial donor plate (daughter plate) to a UV 96-well plate. Add 170 µL 5% DMSO buffer. Read the plate on a plate reader at 280nm, 300nm, 320nm, 340nm, and 360nm.
7. After 16 hour incubation, transfer 250 µL from the acceptor plate to a second UV 96-well plate and analyze with a plate reader.

PAMPA-HDM Using MultiScreen Permeability Plate

1. Dispense 15 µL of 5% hexadecane in hexane in each well of the permeability plate. Allow 5-7 hours for solubilization.
2. Dispense 300 µL 5% DMSO buffer to an acceptor plate (MISSECEPTOR, Millipore, Corp.).
4. Transfer 15 µL 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 µL from the initial donor plate (daughter plate) to a UV 96-well plate. Add 170 µL 5% DMSO buffer. Read the plate on a plate reader at 280nm, 300nm, 320nm, 340nm, and 360nm.
7. After 5 hour incubation, transfer 250 µL from the acceptor plate to a second UV 96-well plate and analyze with a plate reader.

Summary

• ADME applications (Solubility, PAMPA-Lipid and PAMPA-HDM) can be easily automated on the Sciclove 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