Automation of ADME Applications

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Abstract

In recent years there has been a trend to incorporate ADME/TOX screening assays earlier in the drug discovery pipeline as part of the lead optimization process. The synergistic use of automation, 96-well filter based assays and flexible analytical detection techniques have greatly enabled this process, allowing scientists to screen large numbers of compounds with relative ease, increased throughput, decreased time commitments and precious sample quantities.

Such screening assays, including solubility, permeability and compound plasma levels (total drug analysis) are essential pieces obtained in the development of a compound's physicochemical profile. Complete automation of these three assays; Aqueous Compound Solubility, PAMPA and Total Drug Analysis are detailed here using a Hamilton MICROLAB® STAR automation system. 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 method[1] described in this poster allows many compounds to be screened quickly to determine their relative solubility. The use of the Hamilton MICROLAB STAR system with a 96-well MultiScreen® Solubility filter plate (Millipore Corp.) allows 96 samples to be processed in 1 hour and 48 minutes.

Another important characteristic of a drug that needs to be identified during the drug discovery process is the PK (pharmacokinetics) of a drug. The PK for a drug is calculated by knowing the concentration of the drug in the plasma or serum at various time points after administrating the drug. The concentration can be determined by doing Total Drug Analysis. Organic solvents, typically acetonitrile, are used to precipitate proteins and salts from plasma or serum samples and as a result the drug is dissolved in the organic solvent. Once extracted, the sample is then analyzed by using LC-MS/MS to determine its concentration. This assay[2] can be done using a 96-well MultiScreen Deep Well Solvinert Plate (Millipore Corp.). The total time for 96 samples (not including LC-MS/MS analysis) is 10 minutes when using a Hamilton MICROLAB STAR system.

The passive transcellular permeability of a compound can be determined with the use of a 96-well MultiScreen Permeability filter plate (Millipore, Corp). The HDM-PAMPA assay[3] places a lipophillic barrier (hexadecane/hexane artificial membrane) on the polycarbonate membrane support. The rate at which a compound diffuses from the donor to the acceptor compartment across the membrane can then be determined. The rate is predictive of compound absorption and can be used as an early screen to rank the permeability rates for compounds. The combination of this 96-well plate and the Hamilton MICROLAB STAR system provides a fast and easy way to process up to 96 samples in 1 hour and 17 minutes (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 90 minutes at 300 rpm.
2. Filter at 10"Hg 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 192 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 shake for 5 minutes.
5. Read both plates on plate reader at 280nm, 300nm, 320nm, 340nm, 360nm and 800nm.

Automation vs Manual

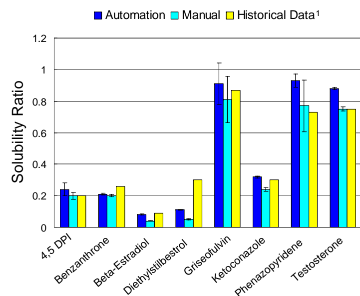


Figure 1. The Solubility Ratio was determined for 8 drug compounds (n=12) using a Millipore MultiScreen Solubility filter plate (MSSLBPC10) and the Hamilton MICROLAB STAR system. The manual control was done on the same day. Absorbance analysis was performed using a SpectraMax® Plus reader, from Molecular Devices (Sunnyvale, CA). The results obtained by automation are equivalent to previously published data[1] as well as the same day manual control.

Hamilton MICROLAB STAR

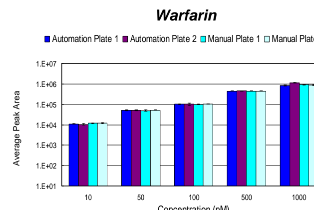
Figure 2. The Hamilton MICROLAB STAR system equipped with 96 head, 8 channel head, iSWAP, shaker and Automated Vacuum System was used for the ADME applications. The Solubility assay uses the 96 head, while the HDM-PAMPA assay uses both the 96 and 8 channel heads. The Total Drug Analysis assay can be done with either the 96 head or the 8 channel head.

Total Drug Analysis Using MultiScreen Deep Well Filter Plate

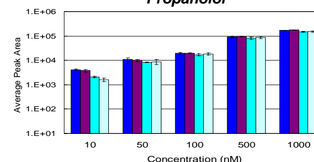
1. Dispense 1000 µL acetonitrile into each well of a MultiScreen Deep Well Solvinert filter plate.
2. Aspirate 125 µL serum sample (with drug), aspirate 125 µL acetonitrile (from the Deep Well plate), and dispense both back into the MultiScreen Deep Well Solvinert Filter plate.
3. Repeat step 2 again.
4. Shake for 2 minutes at 900 rpm.
5. Filter into a deep well collection plate at 20" Hg for 1 minute.
6. Analyze filtrate on LC-MS/MS.

Automation vs Manual

Warfarin



Propanolol



Testosterone

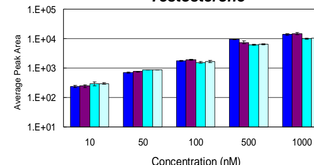


Figure 3. The peak area was determined for 3 compounds (Warfarin, Propanolol and Testosterone) using Millipore MultiScreen Deep Well Solvinert filter plates (MDRPNP410) and the Hamilton MICROLAB STAR system. Two plates were run on the same day on the robot and two controls were done manually. Analysis was performed using LC-MS/MS. The results obtained from the both plates using automation are equivalent to data obtained manually.

HDM-PAMPA Using MultiScreen Permeability Plate

1. Dispense 15 µL of 5% hexadecane in hexane in each well of the permeability plate. Allow to dry for 60 minutes.
2. Dispense 300 µL 5% DMSO buffer to a acceptor plate (MSSACCEPTOR, Millipore, Corp.).
3. Create daughter plate by dispensing 285 µL PBS buffer into a v-bottom polypropylene plate. Add 15 µL drug compound. Mix.
4. Transfer 150 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 initial donor plate: transfer 100 µL from daughter plate to a UV 96-well plate. Read the plate on a plate reader at 280nm, 300nm, 320nm, 340nm, and 360nm.
7. Create the equilibrium plate: transfer 80 µL from the initial donor plate to a second UV 96-well plate. Add 170 µL 5% DMSO buffer. Read with a plate reader at the same wavelengths described above.
8. After 5 hour incubation, transfer 250 µL from the acceptor plate to a third UV 96-well plate and analyze with a plate reader.
9. Transfer 100 µL from the MultiScreen Permeability plate to a fourth UV-96 well plate and analyze with a plate reader.

Automation vs Manual

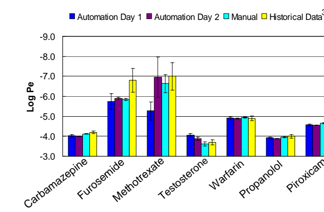


Figure 4. The Log Pe was determined for 7 compounds (n=12) using Millipore MultiScreen Permeability filter plate (MAPBM) and the Hamilton MICROLAB STAR system. A Manual control was also run at the same time. The donor concentration for all the drugs was 500 µM except for testosterone which was 100 µM. Absorbance analysis was preformed using a SpectraMax Plus reader. The results obtained through automation processing are equivalent to data obtained manually and data previously generated[3].

Summary

•ADME applications (Solubility, HDM-PAMPA and Total Drug Analysis) can be easily automated.

•Data obtained from automation are equivalent to manual data obtained on the same day and to previously generated data demonstrating robust protocols.

•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. *High Throughput Sample Preparation for the Quantitation of Drug Compounds in Serum Samples*, Millipore Corporation Application Note, 2004; Lit. No. AN2004EN00.
3. Schmidt, D.; Lynch, J. *The Evaluation of the Reproducibility of Passive, Transcellular Drug Permeability Assays*, Millipore Corporation Application Note, 2002; Lit. No. AN1725EN00.

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