AN AUTOMATED LC/MS/MS PROTOCOL TO ENHANCE THROUGHPUT OF PHYSICO-CHEMICAL PROPERTY PROFILING IN DRUG DISCOVERY

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INTRODUCTION

The synthesis of large, focused chemical libraries allows pharmaceutical companies to rapidly screen large numbers of compounds against disease targets. Active compounds, or hits, resulting from these screens are traditionally ranked based on their activity, binding and/or specificity. Turning these hits into leads requires further analysis and optimization of the compounds based upon their physicochemical and ADME characteristics. The critical factor to consider in physicochemical profiling is throughput. The bottlenecks to throughput include MS method optimization for a large variety of compounds and data management for the large volume of data generated.

Currently, experiments including solubility, chemical and biological stability, water/octanol partioning, PAMPA, Caco-2, and protein binding are used to generate physicochemical profiles of compounds in drug discovery. The measurement of physicochemical properties from these studies is easily enabled using chromatographic separation and quantitation using LC/MS/MS/UV. While the sample analyses may be efficient, the processing of the data and the interpretation of the results often requires tedious and time-consuming manual manipulation and calculation.

This paper describes an approach to solving these problems by the use of a novel software package that allows for the design of experiments, data acquisition, and the processing as well as report generation in a fully automated manner.

To demonstrate the usage of this software package we have developed an automated LC/MS/MS protocol for data generation. The data acquired from multiple assays were processed by a single processing method, all in an automated fashion. As a result, the physicochemical profiling process was significantly simplified and throughput increased.

LC/MS/MS CONDITIONS

LC Conditions

Instrument: Waters® Alliance® HT HPLC System
Column: Waters Sunfire™ C18 Column
2.1 x 30 mm IS column, 3.5 um, 35°C
Sample Temp : 5° C
Injection Volume: 5 uL
Mobile Phase: A. 10 mM NH4OAc in AcN/Water 10/90, pH 5.0
B. 10 mM NH4OAc in AcN/Water 90/10, pH 5.0

Gradient: Time	A%	B%	Curve	Flow
0.00	95%	5%	6	0.60 ml/min
0.80	20%	80%	6	0.60 ml/min
1.00	0%	100%	1	0.60 ml/min
3.00	95%	5%	1	0.60 ml/min

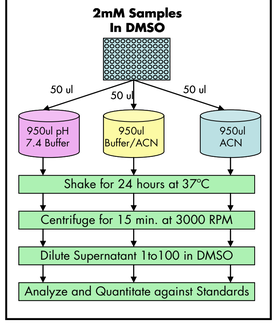
MS Conditions

Instrument: Waters Micromass® Quattro micro™ API Tandem Quadrupole MS
Software: MassLynx™ 4.0 SP4 with ProfileLynx™
Tune Page Parameters:

ESI Capillary Voltage:	0.5 kV	Polarity:	Positive
Source Temp.:	130°C	Inter-scan Delay:	20 ms
Desolvation Temp.:	400°C	Inter-Channel Delay:	20 ms
Desolvation Gas Flow:	800 L/Hr	Dwell:	100ms
Cone Gas Flow:	50 L/Hr		

PROPERTY PROFILING ASSAYS

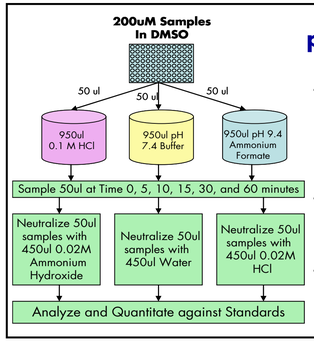
Solubility



Experimental

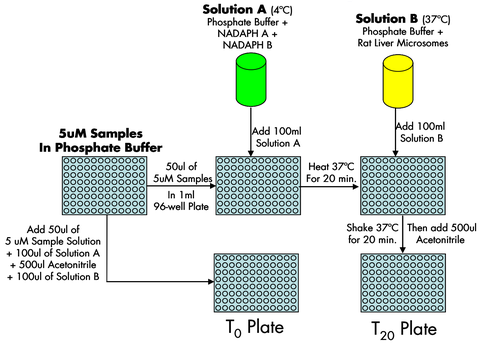
- A set of 18 commercially available compounds were randomly chosen to demonstrate the **ProfileLynx™ Application Manager**.
- QuanOptimize™ allows for the automated optimization of the MS MRM conditions for each compound.
- Each compound and a reference standard were analyzed by solubility, pH stability, LogP/LogD, and microsomal stability assays based on methods previously published.[1,2,3]
- For quantitative experiments, a six standard calibration from 0.06—2.0 uM was constructed.
- To mimic the current practice in discovery labs, 96-well plate formats were used in this study.

pH Stability



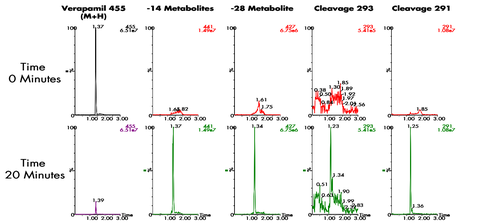
- pH Stability assays were carried out at three different pH's:
  - Stomach—pH 1.0
  - Blood—pH 7.4
  - Colon—pH 9.4
- Solutions were sampled and quenched at Times 0, 5, 10, 15, 30, and 60 minutes.
- Fractions were quantified against standard calibration curves.

Microsomal Stability

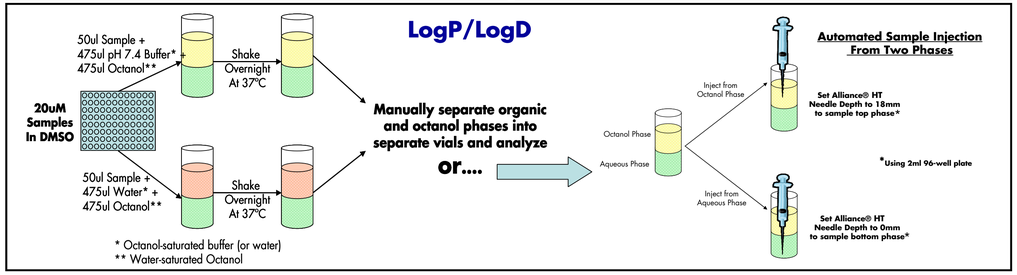


To ensure the integrity of the rat liver microsomes, verapamil was analyzed using MS full scan detection. The results below show only trace levels of metabolites at T_0. At T_{20}, 92.5% of the verapamil has been metabolized.

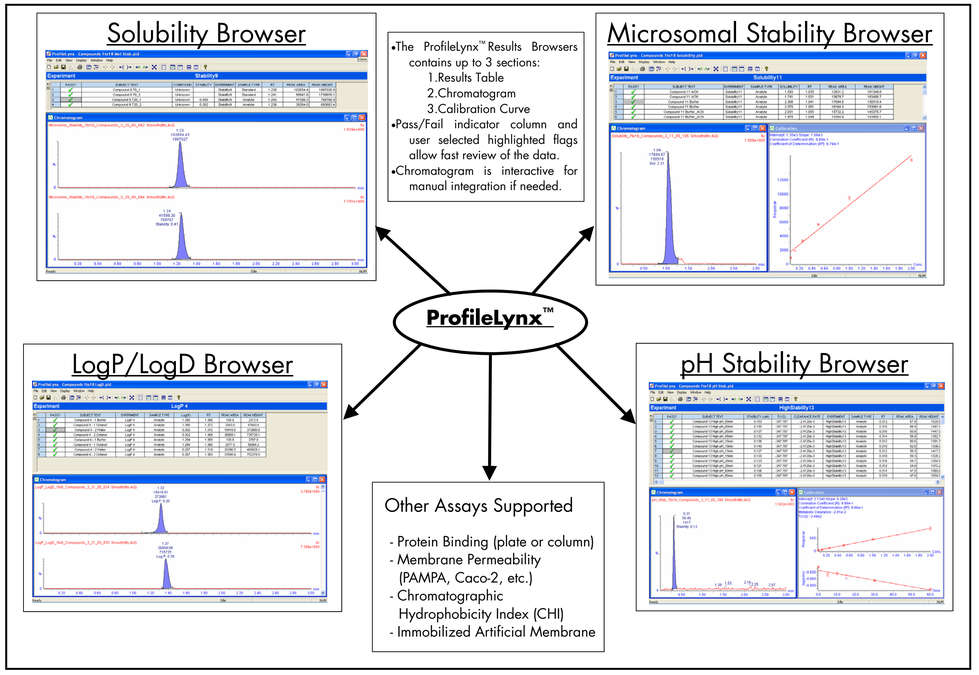
Verapamil and Metabolites



LogP/LogD



DATA PROCESSING AND REPORT GENERATION



DISCUSSION

- The 18 compounds were analyzed with the LC/MS/MS protocol including MS MRM parameter optimization, MS acquisition method creation, data acquisition, data processing, and report generation.
- The data generated from the variety of assays were all processed with the same software automatically.
- A single report was created for the 18 compounds containing the results from all Property Profiling assays, increasing throughput.
- Results are displayed in an interactive, graphical summary format based on sample or experiment.
- Additional improvements to throughput were achieved for the LogP/LogD assay by utilizing the needle height adjustment of the Alliance® HT system to inject directly from the two phases of the octanol-water mixture without the need to manually separate the two phases.

CONCLUSIONS

Using ProfileLynx™/QuanOptimize™ allows for:

- —Automated MS Method Development and Data Acquisition
- —Single Approach for Data Processing and Report Generation from Multiple Assays
- —Complete Automated Analysis/Processing/Reporting
- —Increased Laboratory Throughput

REFERENCES

1. Kerns E, Journal of Pharmaceutical Sciences (2001), **90**, No. 11, 1838-1858
2. US Pharmacopia (2000), **24**, p. 2236
3. Di L, Kerns E, Hong Y, Kleintop T, McConnell O, Journal of Biomolecular Screening 8(X), 2003

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