[APPLICATION NOTE]

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AN AUTOMATED LC/MS/MS PROTOCOL TO ENHANCE THROUGHPUT OF PHYSICOCHEMICAL 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, that result 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 partitioning, PAMPA, Caco-2, and protein binding are used to generate physicochemical profiles of compounds in drug discovery. The measurement of physico-chemical properties from these studies is easily enabled using chromatographic separation and quantitation using LC/MS/MS/UV. While the sample analyses may be efficient, processing the data and interpreting the results often requires tedious and time-consuming manual manipulation and calculation.

This application note describes an approach to solving these problems by using MassLynx™ Software's ProfileLynx™ Application Manager, a fully automated software package that allows for the design of experiments, data acquisition, and data processing as well as report generation.

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

ACQUITY TQD with the TQ Detector.

EXPERIMENTAL

LC conditions

Instrument:	Waters® ACQUITY UPLC® System
Column:	ACQUITY UPLC BEH C_{18} Column 2.1 x 50 mm, 1.7 µm
Column temp.:	40 °C
Sample temp.:	20 °C
Injection volume:	5 µL
Mobile phase A:	0.1% Formic acid in water
Mobile phase A:	0.1% Formic acid in acetonitrile

Gradient:

Time	A%	B%	Curve	Flow
0.00	95%	5%	6	0.60 mL/min
1.00	5%	95%	6	0.60 mL/min
1.30	0%	100%	1	0.60 mL/min
2.50	95%	5%	11	0.60 mL/min

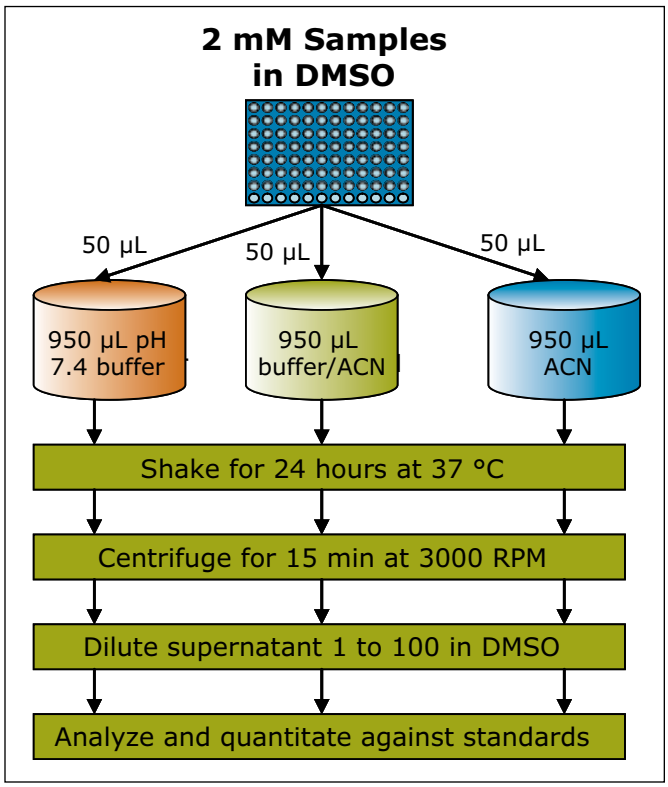
MS conditions

MS system:	Waters TQ Detector
Software:	MassLynx 4.1 with ProfileLynx
ESI Capillary voltage:	3.20 kV
Polarity:	Positive
Source temp.:	150 °C
Inter-scan delay:	20 ms
Desolvation temp.:	450 °C
Inter-channel delay:	5 ms
Desolvation gas flow:	900 L/Hr
Dwell:	200 ms
Cone gas flow:	50 L/Hr

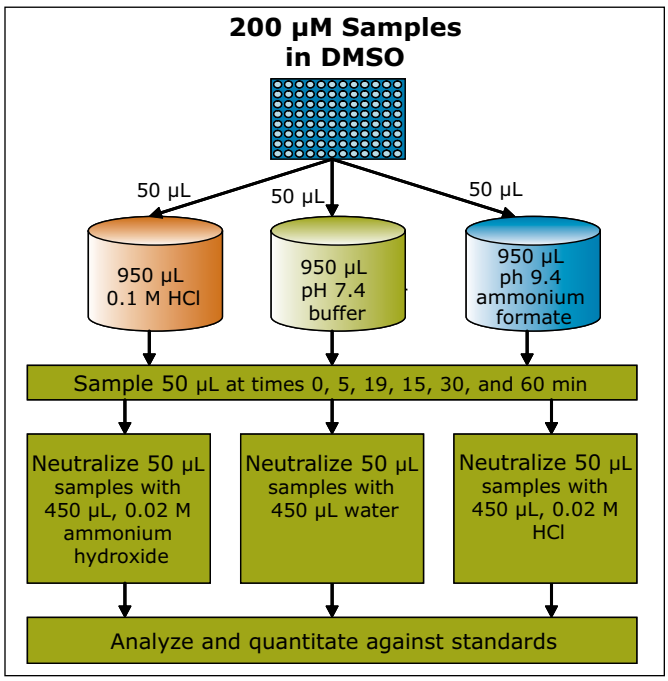
Property profiling assays

- A set of 30 commercially available compounds were randomly chosen to demonstrate the ProfileLynx Application Manager.
- QuanOptimize™ Application Manager allows for the automated optimization of the MS multiple reaction monitoring (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, single point or multipoint calibration curves were used.
- To mimic the current practice in discovery labs, 96-well plate formats were used in this study.
- pH stability assays were carried out at three different pHs: stomach (pH 1.0), blood (pH 7.4), and colon (pH 9.4).
- Solutions were shaken overnight and vacuum filtered through a Sirocco™ plate.
- Fractions were quantified against single point 1 µM calibration standards.

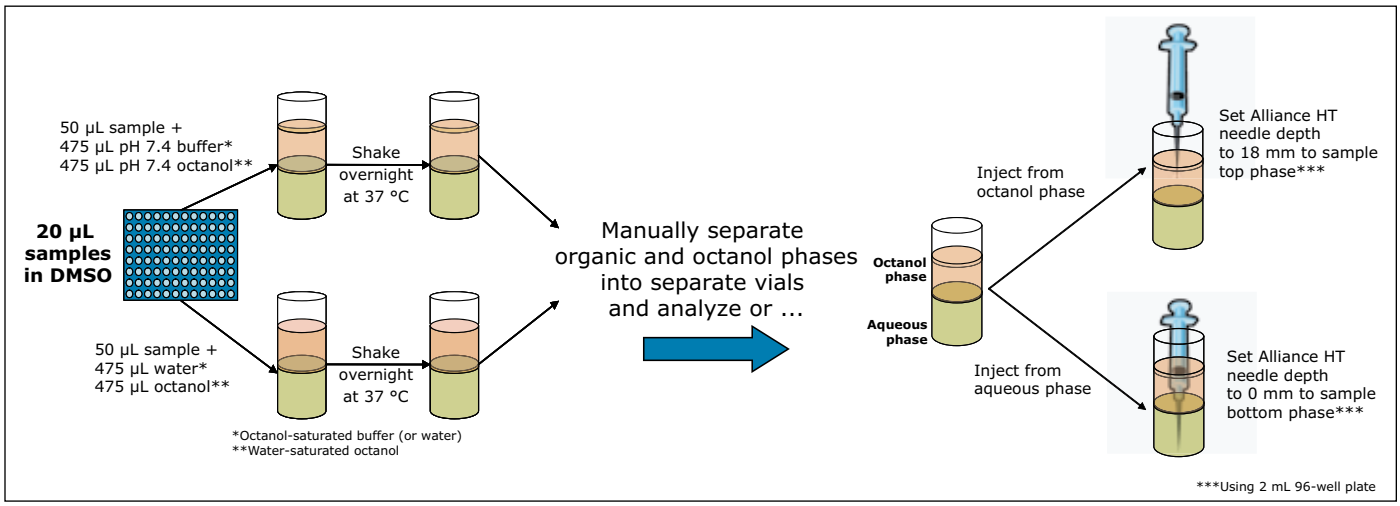
Solubility



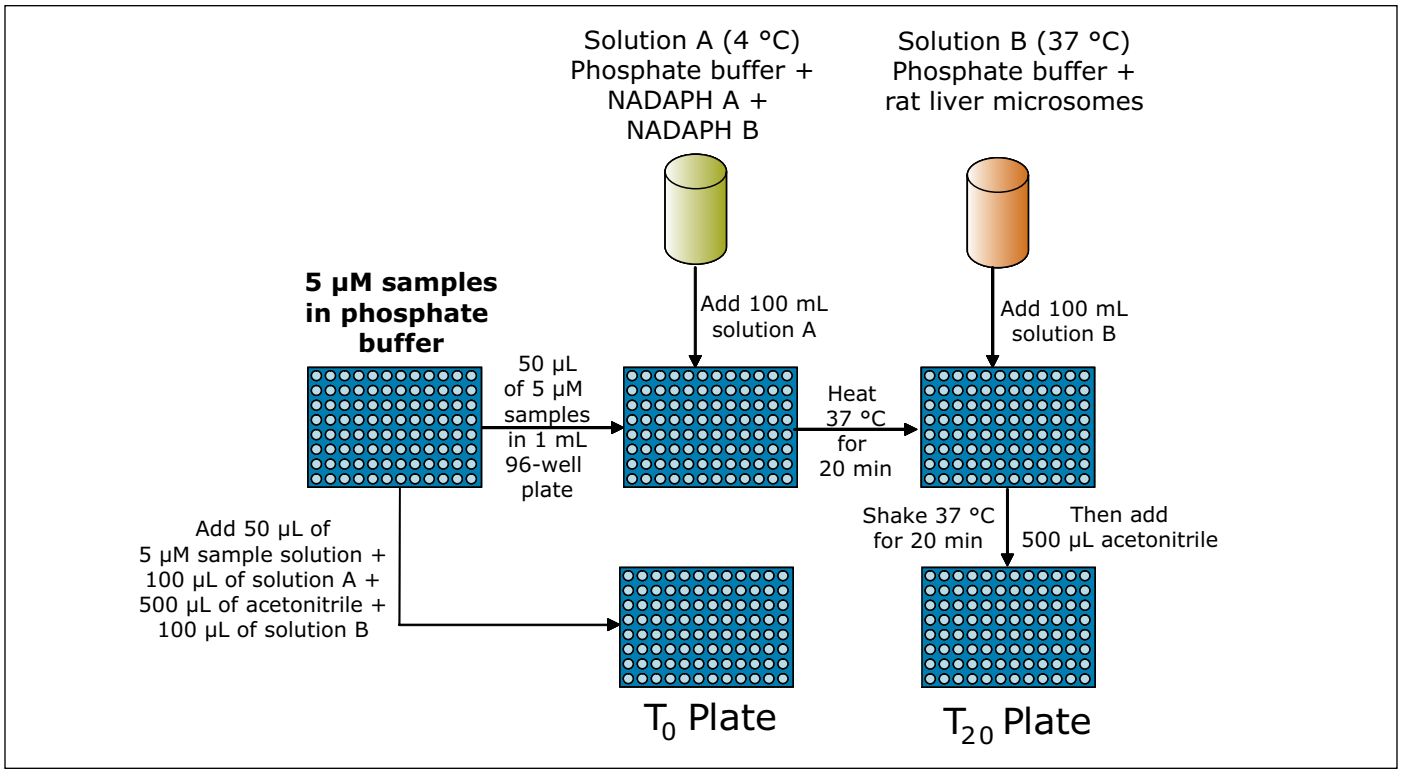
pH stability



LogP/LogD



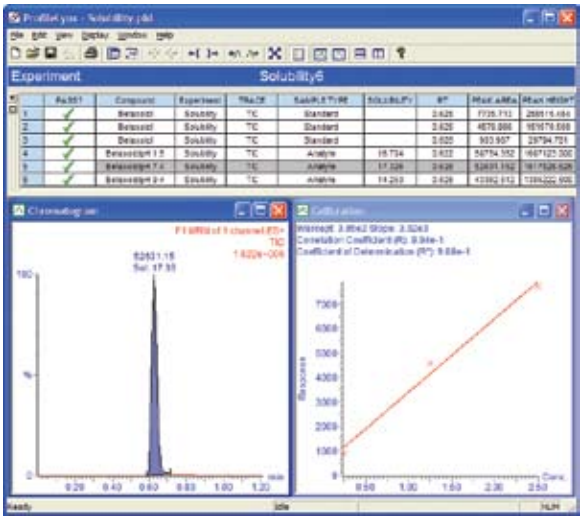
Microsomal stability



Data processing and report generation

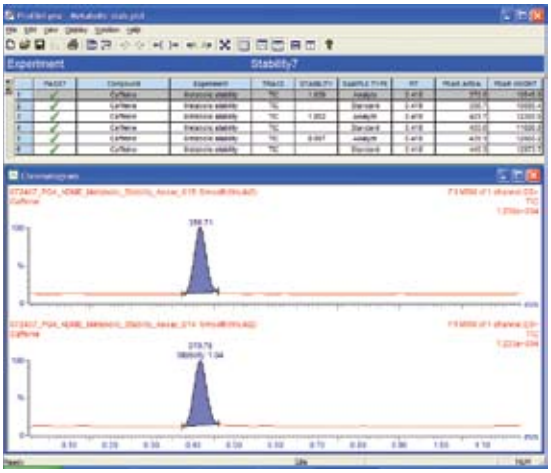
- The ProfileLynx results browser contains up to three sections: a results table, the chromatogram, and the calibration curve.
- A pass/fail indicator column and user-selected highlight flags allow fast review of the data.
- The chromatogram is interactive for manual integration if needed.

Solubility browser

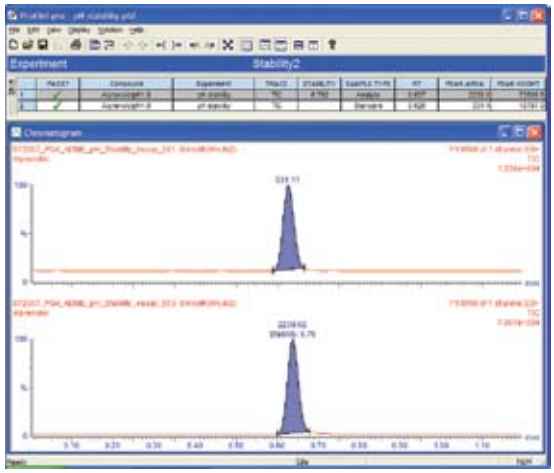


LogP/LogD browser

Metabolic stability browser



pH stability browser



DISCUSSION

- The 30 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 30 compounds that contained 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.

Other assays supported:

- Protein binding (plate or column)
- Membrane permeability (PAMPA, Caco-2, etc.)
- Chromatographic hydrophobicity index (CHI)
- Immobilized artificial membrane

CONCLUSION

Using the ProfileLynx and QuanOptimize Application Managers allows for:

- Automated MS method development and data acquisition.
- A single approach for data processing and report generation from multiple assays.
- Complete and automated analysis, processing, and reporting.
- Increased laboratory throughput.

References

1. Kerns E. Journal of Pharmaceutical Sciences. 2001; 90 (11): 1838-1858.
2. US Pharmacopia. 2000; 24: 2236.
3. Di L, Kerns E, Hong Y, Kleintop T, McConnell O. Journal of Biomolecular Screening. 2003; 8(X).

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