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Optimizing Throughput of Physicochemical Property Analyses

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Overview

The physicochemical properties of new chemical entities (NCE's) are used to evaluate their viability as a drug candidate. These properties include hydrophobicity, solubility, membrane permeability, chemical stability, metabolic stability and protein binding. With the "fail sooner faster" approach for lead optimization, larger sample sets are being screened. This puts additional strain on the drug discovery laboratories and generates large quantities of data to be processed and reviewed.

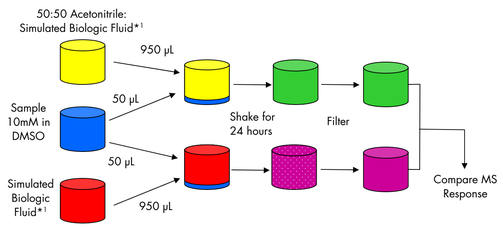
Enhancements to HPLC and LC/MS technologies have provided useful tools to improve the throughput and accuracy of these assays. These include the use of short columns with rapid gradients, fast mass spectrometers for cassette analysis and the use of parallel LC (MS) systems. In addition, throughput can be increased with the new technology of Ultra Performance LC™ (UPLC™), which makes use of very small column particles (<2 µm) and high operating pressure (>10,000 psi); which results in a up to 10 fold increase in throughput with a 3 fold increase in sensitivity.

Data management is also a critical issue of throughput. These new fast technologies result in a large volume of data that must be processed and reviewed to rank order the screened NCE's. In order to process and review the large amount of data generated we have created a dedicated software package (ProfileLynx™). Now compounds can be analyzed and the data from different assay brought together in one efficient data visualization package. In addition the results can be exported to third party (in house) data management systems in a facile manner for across site use and review.

In this poster we illustrate how these tools have been applied to the solubility analysis using a lead optimization sample set. This includes UPLC-MS analysis with less than 1 minute sample run times such that a microtiter plate can be analyzed and processed in under two hours.

Methods

Solubility Assay



* *Simulated Biologic Fluid prepared per USP Simulated Intestinal Fluid TS[1]*

LC/MS Analysis

HPLC/MS

- Waters® Alliance® 2795, 2996, Quattro Micro™ Mass Spectrometer
- Sunfire™ C18 3.5µm 2.1 x 50 mm
- 0.8 mL/min total flow gradient:
  Water: Acetonitrile: 0.1% Formic Acid
  0—3 minutes 10—90% B
  3—3.5 minutes 90% B
  3.5—3.55 minutes 90—10% B
  5 minutes end

Waters Alliance—Quattro micro LC/MS

UPLC/MS

- Waters ACQUITY UPLC™ System, Quattro micro Mass Spectrometer
- ACQUITY UPLC 1.7µm C_{18} 2.1 x 50 mm
- 1.2 mL/min total flow gradient:
  Water: acetonitrile: 0.1% formic acid
  0—0.2 minutes 10—90% B
  0.2—0.25 minutes 90% B
  0.26 minutes 10% B
  0.6 minutes end

Waters ACQUITY UPLC System

Test samples are a mixture of drug like compounds

Optimizing the Analysis

Increasing sample cycle time

Methodology	Analysis Time	Sample to Sample Cycle Time	Total Run Time*
HPLC	5 minutes	6.15 minutes	9.85 hours
UPLC	36 seconds	50 seconds	1.33 hours

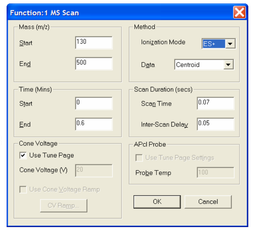
* *Total Run Time is for 48 samples: 1 injection of the each standard and analyte (96 total injections)*

- ***7.5 fold increase in throughput with UPLC***

Factors to consider when increasing the sample throughput

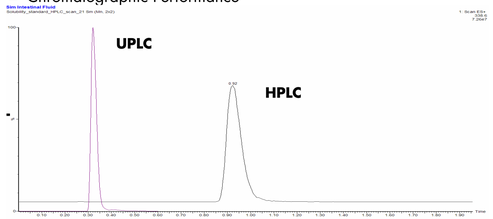
– Having sufficient number of data points for integration

- Fast scan, short inter-scan delay and narrow scan window give fastest scan rate
- The setting shown allows for 8.3 scans / second at 3083 amu/second



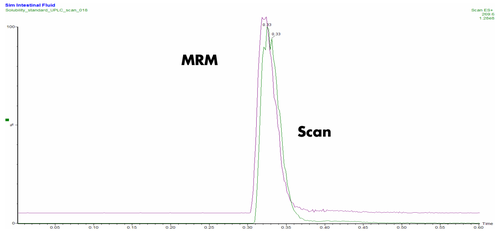
MS Scan Method window

– Chromatographic Performance



- UPLC gives narrower peak than HPLC
- The 5% DMSO has a greater negative impact on the rapid UPLC analysis
- Typical physicochemical samples are greater than 85% pure

– Full Scan versus MRM data acquisition



- Similar results when mass spectrometer is fast enough in scan mode
- MRM is more selective, however, sample matrix is not complex
- MRM requires optimization of the cone voltage and collision energy
  – Can be done automatically using Quan-Optimize™

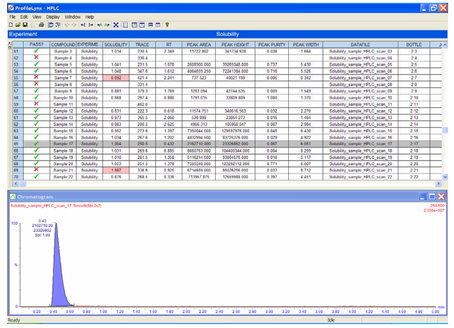
Data Processing and Reviewing

ProfileLynx Application Manager

- Automates the data processing
- Capable of processing both quantitative (solubility, stability, protein binding) and retention time based (CHI, IAM) experiments
- Results are displayed in a graphical summary format based on sample or experiment
- Vendor supported solution—No internal IT support required

Solubility Results

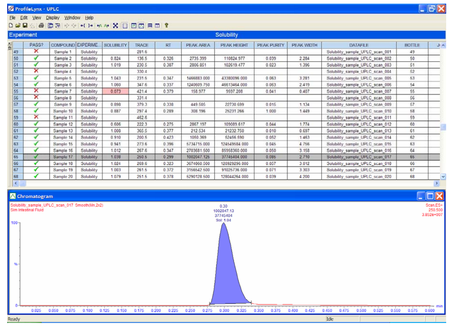
HPLC/MS



ProfileLynx Results Browser window of the HPLC solubility results

- The ProfileLynx Results Browser contains up to 3 sections
  1. Results table
  2. Chromatogram
  3. Calibration Curve (when doing retention time based experiments
- Pass / Fail indicator column and user selected highlighted flags make for fast review of the data
- Chromatogram is interactive for manual integration

UPLC/MS



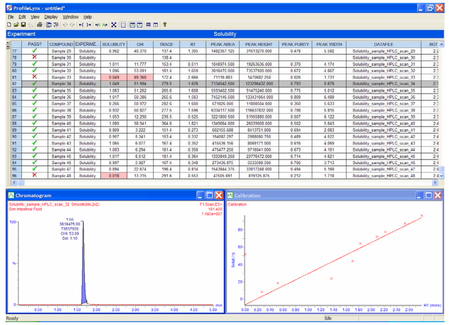
ProfileLynx Results Browser window of the UPLC solubility results

- ***UPLC results are with ± 5% of those from***

Multiple Measurements from One Injection

CHI and Solubility

- The retention time of the solubility samples can be used to calculate one of the CHI values, with the additional injections of the CHI calibrants[2,3]



ProfileLynx Results Browser window of the HPLC solubility and CHI results

- Both results are displayed in the results table and are individually flagged
- CHI for the undetected solubility samples can still be calculated using the standards retention time (not shown)

Conclusions

- The analysis of solubility assay samples can be performed with HPLC/MS, however UPLC/MS gives a 7.5 fold increase in throughput while giving equivalent results
- ProfileLynx automates the data processing and presents the data in a simple, interactive, user friendly format
- Multiple physicochemical measurements can be made from a single injection with the appropriate experiment design

References

1. US Pharmacopeia **24**, 2000, p. 2236
2. Du, CM, Valko K, Bevan C, Reynolds D, Abraham MH, *Anal. Chem.* (1998) **70**, 4228—4234.
3. Valzo K, Bevan C, Reynolds D, *Anal. Chem.* (1997), **69**, 2022—2029.