Ultra-fast Solubility Sample Analysis using SPE-TOF

Panos Hatsis¹, Michelle V. Romm², Vaughn P. Miller², William A. LaMarr², Jakal Amin¹, and Shawn Harriman¹
¹Novartis Institutes for Biomedical Research, Cambridge, MA; ²BIOCIUS Life Sciences, Wakefield, MA

Objective
Evaluate the capability of an ultrafast SPE-TOF system using generic conditions to analyze drug discovery solubility assays

Abstract
Biomedical groups across the pharmaceutical industry are interested in improving the sample throughput/capacity of in vitro ADME assays to keep pace with increasing sample volumes and to afford timely feedback about chemical series properties. While many assays have seen improvements recently, the solubility assay has received relatively little attention, and facilitates formulation development. We investigated the utility of solid phase extraction (SPE) coupled with mass spectrometry for improving sample throughput of the solubility assay.

The current method for solubility sample analysis employs HPLC with ultraviolet absorbance detection (UV). We investigated a SPE-TOF system consisting of an ultra-fast SPE system (RapidFire®) linked to an Agilent 6530 Q-TOF mass spectrometer. The two analytical methods were compared using a set of compounds from Novartis’ drug discovery programs that covered a wide range of chemical space and properties. Solubility was measured in triplicate in pH 6.8 buffer and in FASSIF buffer. A four point calibration curve was prepared to quantify the solubility of each sample.

The SPE-TOF method had a cycle time of eight seconds compared to two minutes for the HPLC-UV method. This represents a 15-fold improvement in cycle time, which can be used to increase the capacity of this assay. The SPE-TOF method was able to maintain data quality as evidenced by linear standard curves ($r^2 = 0.99$) and back-calculated standards within 30% Moreover a correlation plot of the solubility determined using SPE-TOF vs. HPLC/UHPLC was linear with a slope of approximately one and an $r^2 = 0.98$.

Workflow

1. Mini-Prep Filter Vial
2. Add Buffer to 10x Dilution
3. Filter Vial Evaporation
4. Analysis
5. Transfer to Plate
6. Sample Analysis
7. Data Delivery
8. SPE/MS

RapidFire Conditions

Samples were analyzed at a rate of 6-8 seconds per sample using a RapidFire® RFS30 system interfaced to an Agilent 6550 Q-TOF (RF-TOF).

- Buffer A: Water with 0.09% formic acid, 0.01% TFA; 1.5 mL/min flow rate
- Buffer B: 100% acetonitrile with 0.09% formic acid, 0.01% TFA, 1.25 mL/min flow rate
- SPE Column: Inert-silica 
- All samples were analyzed using the same generic MS source conditions and data acquisition from 150-1500 m/z. Each compound and an internal standard were monitored simultaneously in all experiments.

HPLC-UV Conditions

Samples were analyzed on an Agilent 1100 HPLC system. The column used is a Waters Symmetry C18 (3.5 x 150 mm, 3.25 µm particle size). Flow rate is 1 mL/min and the A/B gradient is 30% A, 70% B. The gradient is as follows (2 min cycle time including autosampler overhead): 0-2 min: 100% B; 2.1 min: <1% B; 2.2 min: 100% B; 2.3 min: 10% B; 2.4 min: 100% B; 2.5 min: 100% B; 2.6 min: 100% B.

Data Analysis

Comparison of a typical daily analysis is not illustrative for the analysis of 16 compounds with a 1.25 UV cycle time of 2-min per sample versus a 1.1 TOF cycle time of 12 seconds per sample. Each compound had 16 replicates consisting of a 10-standard curve and 12 samples.

Conclusions

- A generic solid phase extraction method was established and successfully used with a large set of drug discovery compounds with diverse chemical properties.
- Solubility correlation plots generated for the comparison of HPLC/UHPLC vs. SPE-MS yielded a slope of approximately 1 and correlation coefficients of approximately 0.8 for 149 proprietary compounds.

- The results from HPLC/UHPLC and SPE-MS were within accepted error ranges for a drug discovery workflow.
- The SPE-TOF system improved sample analysis cycle times 15-fold.
- 7 seconds/sample versus 2 minutes/sample for a standard HPLC-UV method. SPE-TOF analyzed a single 96 well plate of samples in 16 minutes compared to 3.2 hours using HPLC/UHV.

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[Insert figure showing solubility data analysis]

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