Ultra-fast Solubility Sample Analysis using SPE-TOF

Panos Hatsis[1], Michelle V. Romm[2], Vaughn P. Miller[2], William A. LaMarr[2], Jakal Amin[1], and Shawn Harriman[1]
[1]Novartis Institutes for Biomedical Research, Cambridge, MA; [2]BIOCIUS Life Sciences, Wakefield, MA,

Objective

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

Abstract

Bioanalytical 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 offer timely feedback about chemical series properties. While many assays have seen improvements recently, the solubility assay has received relatively little attention, even though solubility helps drive oral exposure 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 a 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 ($r^2 \approx 0.99$) and back-calculated standards within 30%. Moreover, a correlation plot of the solubility determined using SPE-TOF vs. HPLC/UV was linear with a slope of approximately one and an $r^2 > 0.8$.

Assay Analysis Conditions

RapidFire Conditions

Samples were analyzed at a rate of 6-8 seconds per sample using a RapidFire RF360 system interfaced to an Agilent 6530 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 A (reversed-phase C_4 chemistry)

All samples were analyzed using the same generic MS source conditions and data acquisition from 150-1000 m/z. Each compound and an internal standard were monitored simultaneously in all experiments.

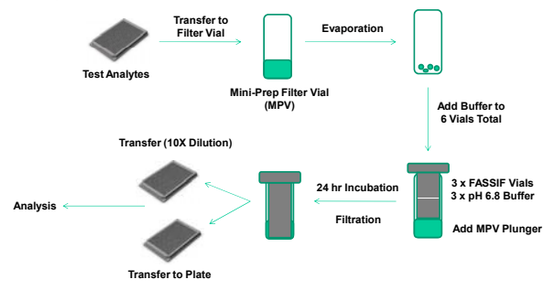
HPLC-UV Conditions

Samples were analyzed on an Agilent 1100 HPLC system. The column used is a Waters Symmetry C18 (50 x 2.1 id), 3 um particle size. Flow rate is 1 mL/min and the A mobile phase is water with 0.1% TFA and the B mobile phase is acetonitrile with 0.1% TFA. The gradient is as follows (2 min cycle time including autosampler overhead):

0 min	**5% B**	**1.35 min**	**100% B**
0.2 min	**5% B**	**1.37 min**	**5% B**
0.85min	**100 %B**	**1.60 min**	**5% B**

RapidFire 360

Workflow



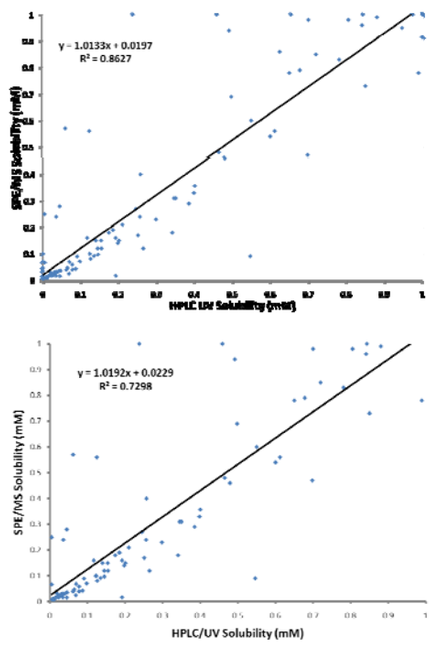
Compounds are first solubilized at 10 mM in DMSO. The DMSO stock solution was then transferred into the chamber of 6 mini-prep filter vials. The DMSO solvent was dried with a GeneVac solvent evaporator. Triplicate samples were tested for each compound in each of the two buffer solutions FaSSIF (Fasted State Simulated Intestinal Fluid) and pH 6.8 buffer. Buffer solution is added to each vial and a plunger which incorporates a filtration membrane (Whatman, PVDF filter with 0.45 µm pore size) on the bottom was added. The system was shaken for 24 hours at room temperature. Afterwards, filtrates are transferred to a 96-well shallow plate and a 1:10 dilution plate was created followed by a thorough mixing process. Both plates were submitted for analysis. Plates submitted for SPE/MS analysis were diluted an additional 200-fold.

Time Savings

Comparison of a typical daily workflow in our laboratory for the analysis of 64 compounds with a LC-UV cycle time of 2 mins per sample versus a RF-TOF cycle time of 7.2 seconds per sample. Each compound had 16 injections consisting of a 4 pt standard curve and 12 samples.

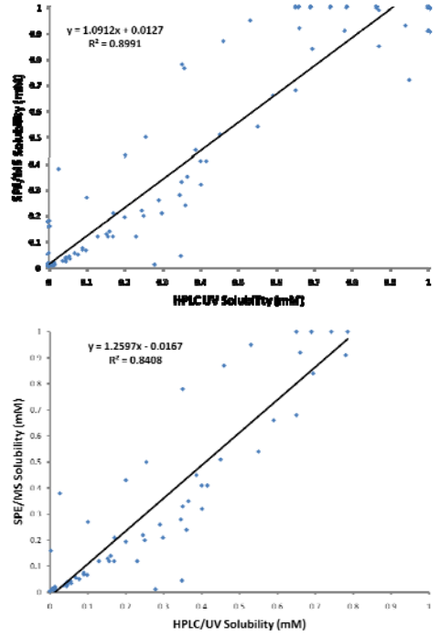
Results

pH 6.8 Buffer



156 chemically diverse discovery compounds were analyzed. Seven compounds were removed due to inconclusive results (due to impurities, degradation etc.) by the LC-UV and the SPE-MS assay. These correlation plots represent the data in pH 6.8 buffer. The top graph represents all 149 compounds investigated. The bottom graph removes the extreme values, 9pts >1mM and 49pts <0.005mM.

FASSIF Buffer



156 chemically diverse discovery compounds were analyzed. Seven compounds were removed due to inconclusive results (due to impurities, degradation etc.) by the LC-UV and the SPE-MS assay. These correlation plots represent the data in FASSIF buffer. The top graph represents all 149 compounds investigated. The bottom graph removes the extreme values, 9pts >1mM and 45pts <0.005mM.

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/UV to SPE-MS yielded a slope of approximately 1 and correlation coefficients of approximately 0.8 for 149 proprietary compounds.**
- **The results from HPLC/UV and SPE-MS were within accepted error ranges for a discovery workflow.**
- **The SPE-TOF system improved sample analysis cycle time 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/UV**

We would like to acknowledge Gina Geraci and Liping Zhou for their technical expertise preparing the samples.

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