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Solubility Screening by UPLC-MS/MS

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Abstract

This application note describes the 23 compounds which were analyzed with a UPLC-MS/MS protocol including MS multiple reaction monitoring (MRM) parameter optimization, MS acquisition method creation,

data acquisition, data processing, and report generation.

Introduction

Determining solubility has become an essential step in the early stages of the drug discovery process. Solubility is important because the ratio of the anticipated dose of a given drug to its solubility, together with the dissolution rate, determine the fraction of the dose available for absorption. Because gastric and intestinal fluids are a complex mixture of natural surfactants, salts, and buffers, it is also important to determine the effect of pH, salts, and surfactants on a drug's solubility.

Potentiometric titration and nephelometry are two popular methods for the fast determination of drug solubility. While these methods may be high throughput, the solubility range is limited by the sensitivity of the detection methods and the concentration of the stock DMSO solutions.

Results obtained via these methods are also less reliable for designing in vivo animal toxicity and drug metabolism and pharmacokinetics (DMPK) studies, in which solubilization of the drug substance in proper media is often required to prepare liquid formulations at various concentrations.

UltraPerformance LC (UPLC) technology can be used to measure compound solubility in either an aqueous buffer solution or mixtures of co-solvents with relatively high-throughput capability, while maintaining adequate accuracy of results.

Given the selectivity and sensitivity of MS/MS detection, UPLC-MS/MS analysis with the Waters ACQUITY TQD System (Figure 1) in combination with specialized software, ProfileLynx and QuanOptimize Application Managers, is the ideal choice for quantitation of a solubility screen.

Figure 1. ACQUITY TQD System.

Experimental

A set of 23 commercially available compounds were randomly chosen to demonstrate the ProfileLynx Application Manager.

Samples

Individual stock solutions of the 23-compound library were prepared in a 96-well plate at a concentration of 5 mM in dimethylsulfoxide (DMSO). The three different pH buffer solutions were prepared in the following manner:

- pH 1.0 - A solution of hydrochloric acid (HCl) was prepared at approximately 0.1 M and adjusted to pH 1.0 with either additional HCl or water.
- pH 7.4 - A solution of phosphate buffered saline (PBS, pH~7) was pH adjusted to 7.4 with ammonium hydroxide.
- pH 9.4 - A solution of PBS was pH adjusted to 9.4 with ammonium hydroxide.

For each different pH assay, 50 µL of each 5 mM stock solution was transferred to a 2-mL, 96-well plate. 950 µL of the appropriate pH buffer was added to each well in the plate resulting in compound concentrations of 250 µM.

The plate was shaken gently on a shaker for 1.5 hours at room temperature. The well contents were transferred to a Sirocco plate and were vacuum-filtered for ~30 to 60 seconds into a collection plate.

The sample/pH buffer solutions were diluted 1:100 in 50:50 acetonitrile/water, resulting in sample concentrations of a maximum of 2.5 µM (assuming that the compound was completely soluble at 250 µM in pH buffer). Samples were injected (5 µL injections) at this concentration.

Standards

A three-point calibration of standards was used for quantitation of the sample solutions. Standards were prepared at concentrations of 0.25 µM, 1.25 µM, and 2.5 µM. 5 mM stock solutions of the compounds in DMSO were diluted 1:100 in 50:50 acetonitrile/water to a concentration of 50 µM.

The 50 µM stock solutions were diluted 1:20 in 50:50 acetonitrile/water resulting in the 2.5 µM standard solutions. The 2.5 µM standard solutions were diluted 1:10 to give 0.25 µM standard solutions, which were then diluted 1:2 to give 1.25 µM standard solutions.

These samples and standards were analyzed by UPLC-MS/MS. The QuanOptimize Application Manager was used for the automated optimization of the MS multiple reaction monitoring (MRM) conditions for each compound.

LC Conditions

LC system:	Waters ACQUITY TQD System
Column:	ACQUITY UPLC BEH C_{18} Column 2.1 x 50 mm, 1.7 µm
Column temp:	40 °C
Flow rate:	600 µL/min
Mobile phase A:	0.1% Formic acid in water

Mobile phase B:	0.1% Formic acid in acetonitrile
Gradient:	5 to 95% B/1.3 min

MS Conditions

MS system:	Waters TQ Detector
Ionization mode:	ESI Positive
Capillary voltage:	3200 V
Source temp:	150 °C
Desolvation temp:	450 °C
Desolvation gas:	900 L/hr
Cone gas flow:	50 L/hr
Inter-scan delay:	20 ms
Inter-channel delay:	5 ms
Dwell:	200 ms
Acquisition range:	100 to 1000 *m/z*

Calculations

Because the solubility assay of the compounds is carried out at 250 µM and the samples are diluted 1:100 before UPLC analysis, the concentrations determined by UPLC-MS/MS must be multiplied by a factor of 100 to get the final solubility. If this final measured concentration is 250 µM, then the compound has a solubility greater than or equal to 250 µM at the pH of the buffer.

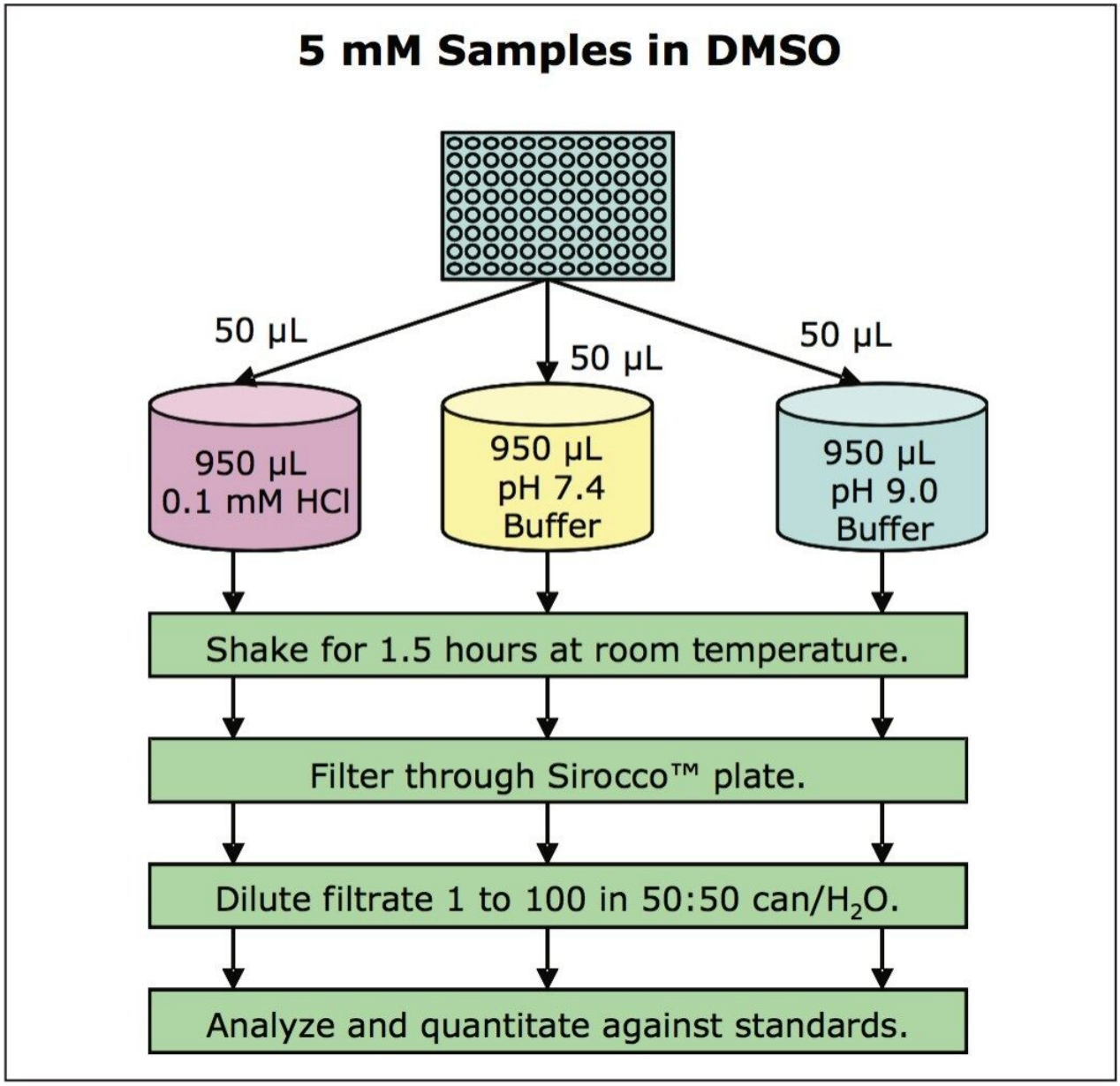

Figure 2. Flowchart of solubility assay procedure.

Results and Discussion

Solubility was determined using MassLynx Software's ProfileLynx Application Manager. Each compound was identified within the sample list and the sample type (standard or analyte) was specified. The solubility concentration of the analytes was determined using a calibration curve generated by a concentration series

of each standard.

Any solubility values outside of a specified minimum and maximum range were automatically flagged within the ProfileLynx Results Browser (Figure 3). For this experiment, the minimum was set at 0.0 mg/mL and the maximum at 2.5 mg/mL.

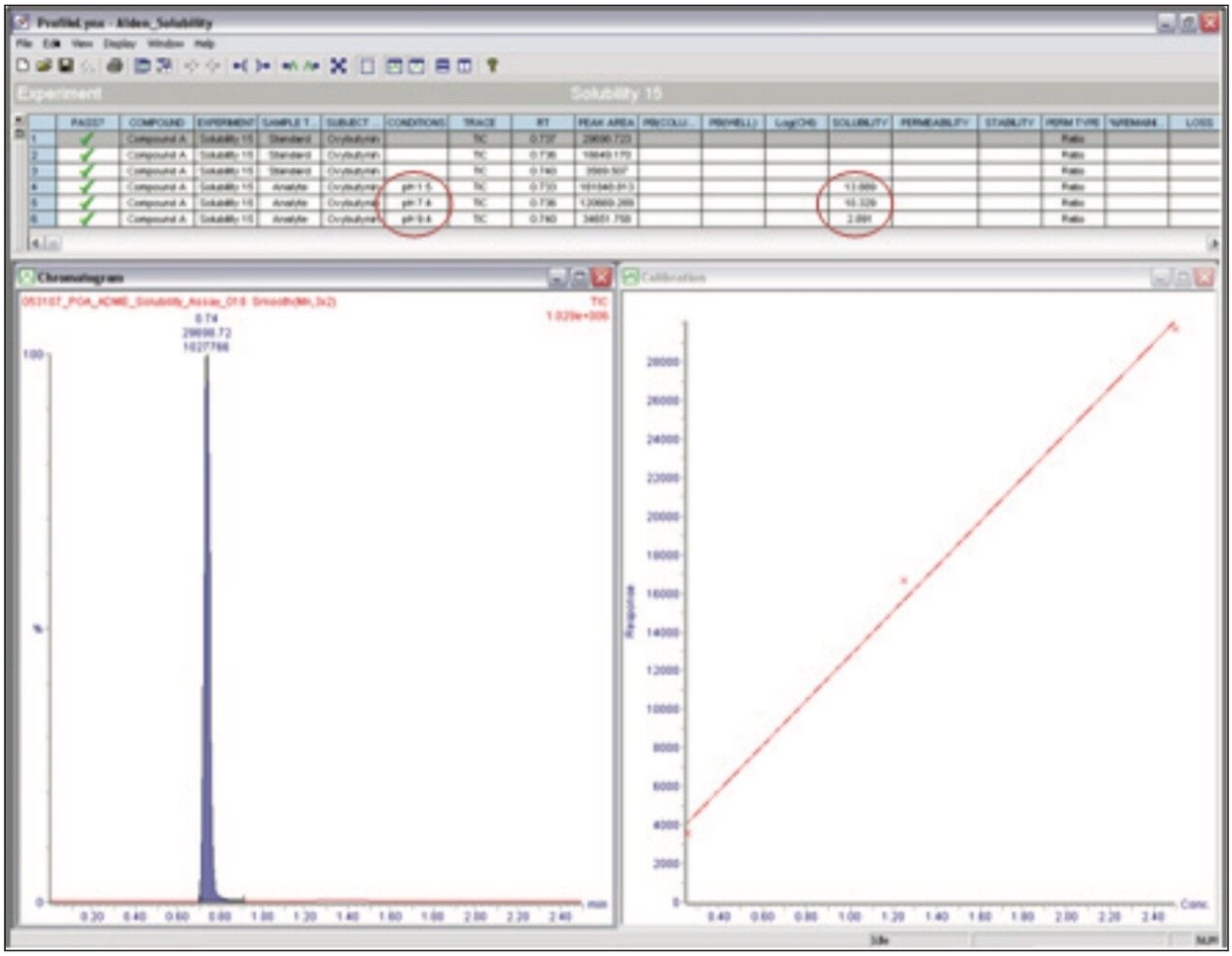

Figure 3. Results for Oxybutynin displayed in the ProfileLynx browser.

The interactive browser allowed for the editing of peak integration. Peak assignments were easily changed and peak integrations were quickly optimized. Results were then exported in a format amenable to the corporate database.

The data in Figure 3 show a typical solubility result. The solubility of Oxybutynin is nearly 10-fold lower at high pH than at low pH. Table 1 summarizes the solubility results for all 23 compounds tested.

COMPOUND	BUFFER	Injection µM SOLUBILITY	Final µM SOLUBILITY	COMPOUND	BUFFER	Injection µM SOLUBILITY	Final µM SOLUBILITY
Alprenolol	pH 1.5	17.436	>250 µM	Nortriptyline	pH 1.5	15.576	>250 µM
Alprenolol	pH 7.4	16.776	>250 µM	Nortriptyline	pH 7.4	13.873	>250 µM
Alprenolol	pH 9.4	14.826	>250 µM	Nortriptyline	pH 9.4	11.548	>250 µM
Amitriptyline	pH 1.5	15.581	>250 µM	Oxprenolol	pH 1.5	14.377	>250 µM
Amitriptyline	pH 7.4	13.282	>250 µM	Oxprenolol	pH 7.4	13.618	>250 µM
Amitriptyline	pH 9.4	6.923	>250 µM	Oxprenolol	pH 9.4	13.151	>250 µM
Betaxolol	pH 1.5	16.771	>250 µM	Oxybutynin	pH 1.5	13.889	>250 µM
Betaxolol	pH 7.4	16.611	>250 µM	Oxybutynin	pH 7.4	10.329	>250 µM
Betaxolol	pH 9.4	14.406	>250 µM	Oxybutynin	pH 9.4	2.891	>250 µM
Caffeine	pH 1.5	12.989	>250 µM	Pindolol	pH 1.5	11.423	>250 µM
Caffeine	pH 7.4	14.25	>250 µM	Pindolol	pH 7.4	11.093	>250 µM
Caffeine	pH 9.4	12.955	>250 µM	Pindolol	pH 9.4	10.726	>250 µM
Colchicine	pH 1.5	16.051	>250 µM	Propranolol	pH 1.5	13.158	>250 µM
Colchicine	pH 7.4	16.838	>250 µM	Propranolol	pH 7.4	12.264	>250 µM
Colchicine	pH 9.4	15.842	>250 µM	Propranolol	pH 9.4	11.249	>250 µM
Diltiazem	pH 1.5	15.738	>250 µM	Sulfadimethoxine	pH 1.5	14.94	>250 µM
Diltiazem	pH 7.4	14.309	>250 µM	Sulfadimethoxine	pH 7.4	15.426	>250 µM
Diltiazem	pH 9.4	12.692	>250 µM	Sulfadimethoxine	pH 9.4	15.272	>250 µM
Diphenylamine	pH 1.5	22.981	>250 µM	Timolol	pH 1.5	15.618	>250 µM
Diphenylamine	pH 7.4	16.101	>250 µM	Timolol	pH 7.4	14.44	>250 µM
Diphenylamine	pH 9.4	17.7	>250 µM	Timolol	pH 9.4	13.529	>250 µM
Doxepin	pH 1.5	15.492	>250 µM	Tolazamide	pH 1.5	8.985	>250 µM
Doxepin	pH 7.4	13.986	>250 µM	Tolazamide	pH 7.4	18.217	>250 µM
Doxepin	pH 9.4	10.82	>250 µM	Tolazamide	pH 9.4	13.894	>250 µM
Lidocaine	pH 1.5	14.352	>250 µM	Tolbutamide	pH 1.5	24.375	>250 µM
Lidocaine	pH 7.4	13.519	>250 µM	Tolbutamide	pH 7.4	28.877	>250 µM
Lidocaine	pH 9.4	13.078	>250 µM	Tolbutamide	pH 9.4	27.486	>250 µM
Loperamide	pH 1.5	13.344	>250 µM	Verapamil	pH 1.5	13.956	>250 µM
Loperamide	pH 7.4	10.836	>250 µM	Verapamil	pH 7.4	13.072	>250 µM
Loperamide	pH 9.4	---	---	Verapamil	pH 9.4	9.151	>250 µM
Metoprolol	pH 1.5	14.818	>250 µM	Zolpidem	pH 1.5	14.657	>250 µM
Metoprolol	pH 7.4	13.77	>250 µM	Zolpidem	pH 7.4	13.656	>250 µM

- Single approach for data processing and report generation from multiple assays
- Complete automated analysis, processing, and reporting
- Increased laboratory throughput

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