Waters

A Rapid Automated Approach to the Generation and Visualization of *in vitro* Metabolism, Solubility, and Log D Using LC/MS/MS and UPLC/MS/MS

Paul M Lefebvre, Warren B Potts III, Robert Plumb, Kate Yu Waters Corporation, 34 Maple Street, Milford MA 01757

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 Liquid Chromatography (UPLC[™]), which makes use of very small column particles (<2 μ m) 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 analysed 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 analysed and processed in under two hours.

Optimising 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

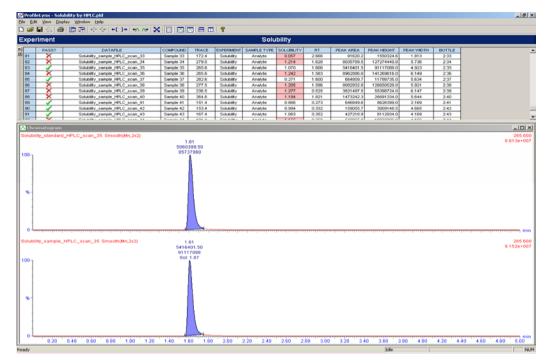
Factors to consider when increasing the sample throughput

- Having sufficient number of data points for integration
- Fast scan, short inter-scan delay and narrow scan window give fastest scan rate

unction:1 MS	Scan	X
Mass (m/z)		Method
<u>S</u> tart	130	Ionization Mode
En <u>d</u>	500	D <u>a</u> ta Centroid 💌
Time (Mins)		Scan Duration (secs)
Start	0	Sca <u>n</u> Time 0.07
<u>E</u> nd	0.6	Inter-Scan Delay
Cone Voltage		APcl Probe
🔽 Use Tune P	age	🔲 Use Tune Page Settings
Cone Voltage (*	y 20	Pro <u>b</u> e Temp
🔲 Use Cone 🗹		
	amp	OK Cancel

Solubility Results

HPLC/MS



ProfileLynx Results Browser window of the HPLC solubility results.

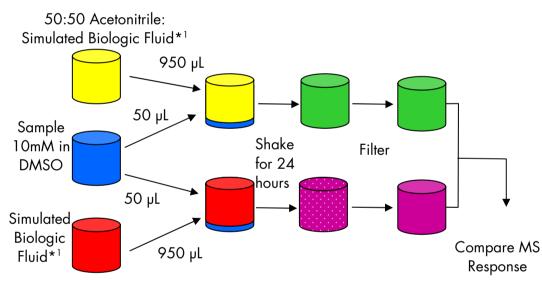
- The ProfileLynx Results Browser contains up to 3 sections
 - 1. Results table
 - 2. Chromatogram
 - 3. Calibration Curve (when doing retention time based experiments)
- Pass / Fail indicator column and user selected highlighted flags make for fast review of the data
- Chromatogram is interactive for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

UPLC/MS

🧭 Prof	leLynx - S	slubility by UPLC.pld											X
Ele Ed	R Yew (jisplay Window Help											
D 🚅		🚇 🛅 🖼 🗠 🗇 I+(-)+ I+A A# 🗙			8								
Expe	riment					Solu	bility						
비	PASS?	DATAFLE	COMPOUND	TRACE	EXPERIMENT	SAMPLE TYPE	SOLUBILITY	RT	PEAK AREA	PEAK HEIGHT	PEAK WIDTH	BOTTLE	
85		Solubility_sample_UPLC_scan_037	Sample 37	282.6	Solubility	Analyte	0.796	0.471	1360182.5	30546714.0	4.480	85	_
06		Solubility_sample_UPLC_scan_038	Sample 30	277.5	Solubility	Analyte	0.003	0.469	3624632.3	02472192.0	4.305	06	1
87		Solubility_sample_UPLC_scan_039	Sample 39	236.5	Solubility	Analyte	1.012	0.317	1678406.6	59642176.0	2.935	87	
88		Solubility_sample_UPLC_scan_040	Sample 40	364.6	Solubility	Analyte	0.957	0.345	318484.6	12411271.0	2.681	88	
09		Solubility_sample_UPLC_scan_041	Sample 41	151.4	Solubility	Analyte	0.900	0.189	352747.0	10900414.0	3.310	09	1

Methods

Solubility Assay



* Simulated Biologic Fluid prepared per USP Simulated Intestinal Fluid TS¹.

LC/MS Analysis

HPLC/MS

- Waters[®] Alliance[®] 2795 System, Photodiode Array Detector, 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

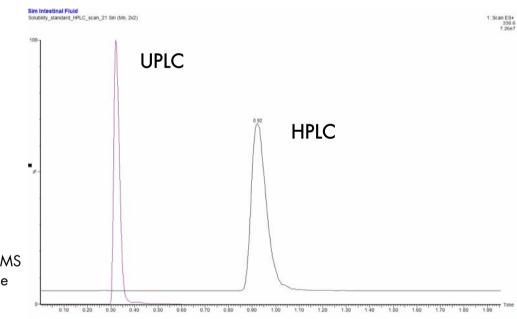


Waters Alliance-Quattro micro LC/MS.

UPLC/MS

- Waters ACQUITY UPLC[™] System, Quattro micro Mass Spectrometer
- ACQUITY UPLC 1.7μm C₁₈ 2.1 x 50 mm

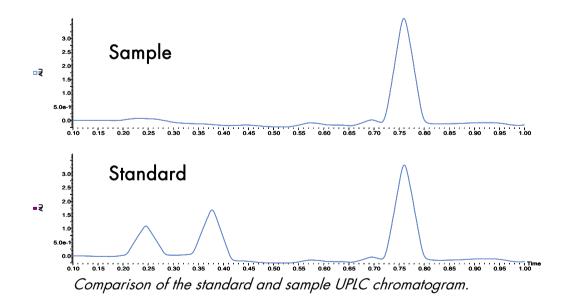
- MS Scan Method window.
- The setting shown allows for 8.3 scans / second at 3083 amu/ second
- Chromatographic Performance



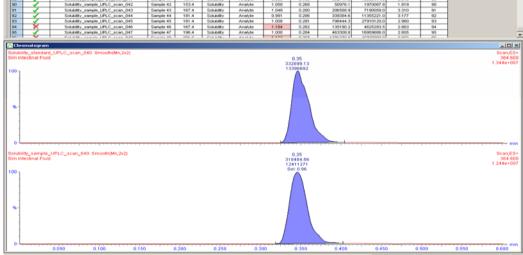
Overlay of UPLC and HPLC Chromatograms

Why UPLC/MS

- Normal solubility assay methods look at the total absorbance
- Impurities can add to the total absorbance



- MS identifies the target peak
- Measured solubility by LC/MS = 100%
- Solubility by total absorbance = 60%
- The increased throughput of UPLC now allows more analysis time to be invested for more accurate and precise data compared to current methods.



ProfileLynx Results Browser window of the UPLC solubility results.

• UPLC results are within <u>+</u> 5% of those from HPLC

Multiple Measurements from One Injection

CHI and Solubility

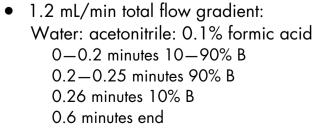
• The retention time of the solubility samples can be used to calculate one of the CHI values, with the additional injections of the CHI calibrants^{2,3}

Y Sample 20 Solubility 1014 </th <th>perim</th> <th>ent</th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th>Solubility</th> <th></th> <th></th> <th></th> <th></th> <th></th>	perim	ent							Solubility					
Y Sample 30 Solubility 101 11777 113.4 0.511 1048974 500 19283636.000 0.570 4.174 Solubility, sample 31, 194.0 19 ✓ Sample 31 Solubility 1.011 11.777 115.4 0.511 1048974 500 19283636.000 0.570 4.174 Solubility, sample 31, 194.0 60 ✓ Sample 33 Solubility 0.043 83.950 1124 2.666 71116.803 1417620 0.025 17.731 Solubility, sample 31, Solubilit	T	PASS?	COMPOUND	EXPERIME	SOLUBILITY	CHI	TRACE	RT	PEAK AREA	PEAK HEIGHT	PEAK PURITY	PEAK WIDTH	DATAFILE	BO
10 X Sample 30 Subulity 11 13 1 13 1 13 1 13	7	1	Sample 29	Solubility	0.962	40.370	137.4	1.305	1482367.125	31613270.000	0.478	5.582	Solubility sample HPLC scan 29	2
0 ✓ Sample 32 Solubity 1.99 \$3.011 11.4 1.99 33.1147.6.00 77.21 4.687 Solubity Somple 34 1 X Sample 33 Solubity 0.443 85.360 117.4 2.666 71116.863 1470682.250 0.025 1.731 Solubity Solu	3	×	Sample 30				138.4						Solubility sample HPLC scan 30	
1 Sample 33 Solubility 0.443 83.800 172.4 2.666 71116.85 147062.250 0.028 1.731 Solubility, sample, HPLC, 1 2 V Sample 33 Solubility 1.89 172.4 2.666 71116.85 147062.250 0.028 1.731 Solubility, sample, HPLC, 1 2 V Sample 35 Solubility 1.69 65.0250 0.1733 5.678 Solubility, sample, HPLC, 1 4 Sample 35 Solubility, sample, HPLC, 1 1.600 65.025 0.028 1.731 Solubility, sample, HPLC, 1 6 Sample 35 Solubility, sample, HPLC, 1 1.000 67.020 0.000 6.489 Solubility, sample, HPLC, 1 6 Sample 35 Solubility, sample, HPLC, 1 1.966 67.1716.000 0.900 6.489 Solubility, sample, HPLC, 1 7 Sample 35 Solubility, 1.683 1.220 2.286 0.625 3.22100.00 0.996 5.643 Solubility, sample, HPLC, 1 7 Sample 35 Solubility, 1.675 <t< td=""><td>3</td><td>1</td><td>Sample 31</td><td>Solubility</td><td>1.011</td><td>11,777</td><td>153.4</td><td>0.511</td><td>1048974.500</td><td>19263636.000</td><td>0.370</td><td>4.174</td><td>Solubility sample HPLC scan 31</td><td></td></t<>	3	1	Sample 31	Solubility	1.011	11,777	153.4	0.511	1048974.500	19263636.000	0.370	4.174	Solubility sample HPLC scan 31	
2 V Sample 31 Salubility 1 649 6 1 944 27 95 1 628 7 13952 500 1 2328632 000 0 733 5 6.878 Solubility, sample, 14PLC, 0 V Sample 35 Solubility 1 603 5 5242 2 666 1 608 5 53412 560 1 73351246 500 0 775 5 012 Solubility, sample, 14PLC, 6 V Sample 37 Solubility 0 765 5 012 Solubility, sample, 14PLC, 6 449 Solubility, sample, 14PLC, 6 449 Solubility, sample, 14PLC, 6 449 Solubility, sample, 14PLC, 6 455 5 506 5 506 5 506 5 506 5 500)	1	Sample 32	Solubility	1.096	53.091	181.4	1.659	3818475.000	73537920.000	0.672	4.867	Solubility_sample_HPLC_scan_32	
2 Sample 35 Solubility 1063 51 262 2266 1 608 593102 500 9175 6 012 Solubility_sample_sHPLC_ 4 Sample 35 Solubility 1 017 56 306 2656 1 508 7452146 500 13331064 000 0 0775 6 012 Solubility_sample_sHPLC_ 5 Sample 37 Solubility 0 326 50 927 2775 1 596 6 573117 500 11603504 000 0 350 5 6.813 Solubility_sample_sHPLC_ 6 Sample 38 Solubility 0 322 526 627 2775 1 596 6 573117 500 1 50500 0 300 0 563 Solubility_sample_sHPLC_ 7 Sample 38 Solubility 0 322 526 625 3221000 0 500 0 500 0 5162 Solubility_sample_HPLC_ 8 Sample 40 Solubility 1.03 1 546 1 121 1 149564 000 0 500 0 502 56-643 Solubility_sample_HPLC_ 9 Sample 41 Solubility 1 571 0 322 15608 14137	1	×	Sample 33	Solubility	0.049	89.360	172.4	2.666	71116.883	1470682.250	0.026	1.731	Solubility_sample_HPLC_scan_33	
1 ✓ Sample 35 Solubility 1017 50.396 256.6 1.583 7.45746.600 10331064.000 0.009 6.489 Solubility_sample_FPLC_1 5 ✓ Sample 35 Solubility 0.266 6.972 282.6 1.600 671826.000 1100504.000 0.909 6.489 Solubility_sample_FPLC_1 6 ✓ Sample 38 Solubility 1.553 122.277.5 1.506 653117.500 11657781200 0.907 6.132 Solubility_sample_FPLC_1 7 ✓ Sample 39 Solubility 1.055 122.290 228.6 0.525 3221800.500 51685 Solubility_sample_FPLC_1 8 ✓ Sample 41 Solubility 0.693 5.815 Solubility_sample_FPLC_1 9 ✓ Sample 42 Solubility 0.693 5.841 Solubility_sample_FPLC_1 9 ✓ Sample 41 Solubility 0.322 1514.4 0.332 145462 0.489 4.522 Solubility_sample_FPLC_1 9 ✓ Sample 41 Solubility 0.326 475477250 9716410	2	1	Sample 34	Solubility	1.049	51.994	279.5	1.628	7134542 500	123296432.000	0.793	5.678	Solubility_sample_HPLC_scan_34	3
Sample 37 Sample 38 Sample 37 Sample 38 Sample 38 Sample 38 Sample 38 Sample 38 Sample 39 Sample 39 Sample 39 Sample 39 Sample 39 Sample 39 Sample 38 Sample 38 Sample 38 Sample 38 Sample 38 Sample 39 Sample 31 Sample 31 <t< td=""><td>3</td><td>1</td><td>Sample 35</td><td>Solubility</td><td>1.063</td><td>51.262</td><td>265.6</td><td>1.608</td><td>5593402.500</td><td>91475240.000</td><td>0.775</td><td>5.012</td><td>Solubility sample HPLC scan 35</td><td></td></t<>	3	1	Sample 35	Solubility	1.063	51.262	265.6	1.608	5593402.500	91475240.000	0.775	5.012	Solubility sample HPLC scan 35	
6 ✓ Sample 38 Solubility 0.932 50.827 277.5 1.596 6:33117.500 1157.7122.000 0.799 5.815 Solubility, sample 34 7 ✓ Sample 38 Solubility 1.063 12.290 236.6 0.525 321600.500 0.007 6.122 Solubility, sample 34 Plot.01 8 ✓ Sample 41 Solubility 1.060 5.64.1 1.021 1.13964.600 20039500.000 0.522 5.64.3 Solubility, sample 34 Solubility, sampl	1	1	Sample 35	Solubility	1.017	50.385	265.6	1.583	7452146.500	133431064.000	0.809	6.489	Solubility_sample_HPLC_scan_36	1
7 Sample 39 Sububity 1.053 12.290 22.65 0.525 322100.500 51.92 Sububity Sububity 1.050 50.941 364.6 1.921 1.349564.000 25039509.000 0.602 5.643 Golubity sububity Sububity 1.600 50.941 364.6 1.921 1.349564.000 25039509.000 0.602 5.643 Golubity sububity Sububity 0.605 5.843 Golubity Sububity 0.605 5.843 Golubity sububity 0.664 2.003 Solubity Solubity sububity 0.666 5.643 Golubity sububity 0.666 5.643 Golubity sububity 0.664 2.003 Solubity Solubity sububity 0.666 5.643 Golubity sububity 0.666 4.602 2.063 Solubity Solubity sububity 5.643 Golubity Solubity Solubity sububity 5.643 Golubity Solubity Solubity Solubity Solubity Solubity Solubity Solubity 5.643 Solubity Solubity Solubity Solubity <td< td=""><td>5</td><td>1</td><td>Sample 37</td><td>Solubility</td><td>0.266</td><td>50.972</td><td>282.6</td><td>1.600</td><td>671826.000</td><td>11808504.000</td><td>0.360</td><td>5.633</td><td>Solubility sample HPLC scan 37</td><td></td></td<>	5	1	Sample 37	Solubility	0.266	50.972	282.6	1.600	671826.000	11808504.000	0.360	5.633	Solubility sample HPLC scan 37	
2 Sample 40 Solubility 1.060 58.911 364.6 1.821 1.349664.000 2603960.000 0.502 5.643 Golubility, sample, IPLC, 0 2 Sample 41 Solubility 0.005 3.222 151.4 0.277 602155.608 60.1157.1000 0.684 2.003 Solubility, sample, IPLC, 0 3 Sample 41 Solubility 0.015 5.341 155.44 0.332 14640.227 2.013 Solubility, sample, IPLC, 0 4 Sample 43 Sububility 1.056 6.077 197.4 0.332 14640.227 2.01680.00 0.616 4.069 Solubility, sample, IPLC, 0 2 Sample 44 Solubility 0.364 153.44 0.358 475.547.250 0.714 4.621 Solubility, sample, IPLC, 0 2 Sample 44 Solubility 0.364 153.445.156 0.05017.000 0.673 4.101 Solubility, sample, IPLC, 0 2 Sample 45 Solubility 0.374 0.340 27330.076 0.0714 4.621 <td< td=""><td>3</td><td>1</td><td>Sample 38</td><td>Solubility</td><td>0.932</td><td>50.827</td><td>277.5</td><td>1.596</td><td>6334117.500</td><td>116637832.000</td><td>0.798</td><td>5.815</td><td>Solubility sample HPLC scan 38</td><td></td></td<>	3	1	Sample 38	Solubility	0.932	50.827	277.5	1.596	6334117.500	116637832.000	0.798	5.815	Solubility sample HPLC scan 38	
1 ✓ Sample 41 Solubility 0.099 3.222 151.4 0.273 602155.608 8417571.000 0.694 2.003 Solubility, sample 420 2 ✓ Sample 42 Solubility 0.967 6.341 153.4 0.332 1544082.297 2988080.760 0.489 4.522 Solubility, sample 440.C 2 ✓ Sample 43 Solubility 1.065 6.077 167.4 0.362 415534.150 0.06171.000 0.516 4.069 Solubility, sample 440.C 2 ✓ Sample 43 Solubility 1.005 6.274 1161.4 0.352 415534.150 000171.000 0.516 4.069 Solubility, sample 440.C 2 ✓ Sample 43 Solubility 1.005 6.274 1161.4 0.352 415534.150 000171.000 0.516 4.069 Solubility, sample 440.C 3 ✓ Sample 44 Solubility 0.977 6.527 1074.4 0.358 17547.000 0.714 4.621 Solubility, sample 440.C 6.016 5.060 7.00 3.713 Solubility, sample 440.C 6.016 5.060 3.0172.800 0.044 5.0160 5.0160.117, sample 440.C 5.0160.117, sample 440 5.0160.117, sample 440.0	7				1.053	12 290	236.5	0.625	3221800.500	51955880.000	0.007	6.122	Solubility sample HPLC scan 39	1.1
i ✓ Sample 41 Sububity 0.099 3.222 151.4 0.273 602155.608 84117571.000 0.694 2.003 Sububity_sample.1PLC_1 i ✓ Sample 41 Sububity 0.967 5.341 153.4 0.332 1544082.297 2988080.760 0.489 4.522 Sububity_sample.1PLC_1 i ✓ Sample 43 Sububity 1.065 6.077 167.4 0.362 415534.156 900171.000 0.516 4.069 Sububity_sample.1PLC_1 i ✓ Sample 44 Sububity 1.005 6.027 107.4 0.352 415534.156 900171.000 0.516 4.069 Sububity_sample.1PLC_1 i ✓ Sample 44 Sububity 1.005 6.027 107.4 0.352 415534.56 900171.000 0.516 4.069 Sububity_sample.1PLC_1 i ✓ Sample 44 Sububity 0.877 107.4 0.340 273500.700 0.714 4.621 Sububity_sample.1PLC_1 i ✓ Sample 44 Sububity 0.877 197.4 0.340 273500.700 0.494 5.160 Sububity_sample.1PLC_1 i ✓ Sample 47 Sububity 0.818 1		1	Sample 40	Solubility	1.060	58.941	364.6	1.821	1349664.000	26039608.000	0.502	5.643	Solubility sample HPLC scan 40	
Image 42 Solubility 0.957 5.341 153.4 0.332 156082.297 298000.750 0.499 4.522 Solubility, sample, 14PLC, Image 43 Solubility 1.003 6.294 157.4 0.332 156052.297 298000.750 0.499 4.522 Solubility, sample, 14PLC, Image 43 Solubility 1.003 6.294 151.4 0.358 475477.250 9716041.000 0.673 4.101 Solubility, sample, 14PLC, Image 43 Solubility 1.003 6.524 151.4 0.368 475477.250 9716041.000 0.673 4.101 Solubility, sample, 14PLC, Image 43 Solubility 1.007 6.512 151.4 0.364 173349.250 2776472.000 0.714 4.621 Solubility, sample, 14PLC, Image 43 Solubility 0.084 12.674 0.544 154.4 0.364 173304724.000 0.494 5.166 Solubility, sample, 14PLC, Image 43 Solubility 0.018 13.315 291.6 0.543 47505.691 819125.875 0.212 1.718 Solubility, sample, 14PLC, Image 44 Solubility 0.018 13.315 291.6 0.553 47505.691 819125.875 0.212 <		1	Sample 41	Solubility	0.809	3.222	151.4	0.273	602155.688	8413751.000	0.694	2.083	Solubility sample HPLC scan 41	
Image 44 Sokubity 1033 6.294 1914 0.356 475477 250 971641100 0.673 4.101 Sokubity Sokubity 1015 Sokubity Sokubity 1015 Sokubity Sokubity Sokubity Sokubity 1017 6.512 1814 0.364 1733349.250 2716472.000 0.711 4.621 Sokubity Sokubity Sokubity Sokubity 0.897 5.927 107.4 6.346 273545.072 0033060.056 0.765 3.713 Sokubity Sokubity Sokubity Sokubity 0.897 5.927 107.4 0.346 27456.072 0033060.056 0.765 3.713 Sokubity		1	Sample 42	Solubility	0.957	5.341	153.4	0.332	154082 297	2988080.750	0.489	4.522	Solubility sample HPLC scan 42	
Image 44 Sokubity 1.003 6.294 181.4 0.358 475477.250 9716041.000 0.673 4.101 Solubity sample 44 Sokubity 1.003 6.294 181.4 0.358 475477.250 9716041.000 0.673 4.101 Solubity sample 45 Sokubity 1.017 6.552 181.4 0.364 1733249.250 2771647.200 0.714 4.621 Sokubity_sample_PLPC_ Image 46 Sokubity 0.897 5.927 107.4 0.344 275456.075 6.050.00 0.714 4.621 Sokubity_sample_PLPC_ Image 47 Sokubity 0.897 5.927 107.4 0.344 275456.075 6.050.00 0.713 Sokubity_sample_PLPC_ Image 47 Sokubity 0.897 2.2674 196.4 0.814 27565.00 3.91728.000 0.434 5.168 Sokubity_sample_PLC_ Image 476 Sokubity 0.018 13.315 201.6 0.581 47565.001 819125.875 0.212 1.7118 Solubitiiy, sample_PLC_ <td></td> <td>1</td> <td>Sample 43</td> <td>Solubility</td> <td>1.065</td> <td>6.077</td> <td>167.4</td> <td>0.352</td> <td>415536.156</td> <td>8069171.000</td> <td>0.616</td> <td>4.069</td> <td>Solubility sample HPLC scan 43</td> <td></td>		1	Sample 43	Solubility	1.065	6.077	167.4	0.352	415536.156	8069171.000	0.616	4.069	Solubility sample HPLC scan 43	
✓ Jample 40 Sububity 0.957 0.927 107.4 0.349 273.500 050 0.705 3.713 3tubity_sample_JPLC_0 ✓ Sample 47 Solubity 0.894 22.674 1964 0.814 151384375 3301728.000 0.494 6.169 Solubity_sample_JPLC_0 ★ Sample 48 Solubity 0.018 13.315 291.6 0.534 47505.691 511258.075 0.212 1.718 Solubity_sample_JPLC_0 Chromatogram F13cm35+ F13cm35+ F13cm35+ 6 Solubity_sample_JPLC_0 Solubity_sa		1			1.003	6.294	181.4	0.358	475477 250		0.673		Solubility sample HPLC scan 44	-
✓ Sample 40 Subdity 0.597 0.597 0.016 273.500 0.00 0.710 30bbilly_sample_IPLC_0 ✓ Sample 47 Sobbility 0.897 0.597 0.0270 0.0300 0.09 0.710 30bbility_sample_IPLC_0 ✓ Sample 48 Sobbility 0.884 22.674 1964 0.814 1543484.375 3301728.000 0.494 6.168 Sobbility_sample_IPLC_0 ✓ Sample 48 Sobbility 0.018 13.315 291.6 0.534 47505.691 61125.875 0.212 1.718 Sobbility_sample_IPLC_0 Chromatogram F13.028.514 F30.6764 0.212 1.718 Sobbility_sample_IPLC_0 Sample 490		1	Sample 45	Solubility	1.017	6.512	181.4	0.364	1333849.250	23776472.000	0.714	4.621	Solubility sample HPLC scan 45	
X Sample 48 Solubitity 0.018 13.315 291.6 0.553 47505 591 819125.875 0.212 1.718 Solubitity_sample_HPLC_ Chromatogram bests/stravior F13ccn85+ 106 341847500 F13ccn85+ 7.465en07 Calibration		1			0.997		167.4				0.706		Solubility sample HPLC scan 46	-
Chromatogram Calibration Life_stamps_VPLC_scan_32 Smooth(Mn.2x2) F13can.55* 181400 7.485+470 Calibration 166 33184750 16 1		1	Sample 47	Solubility	0.894	22.674	196.4	0.814	1543844.375	33817248.000	0.494	5.168	Solubility sample HPLC scan 47	
Chromatogram		×	Sample 48	Solubility	0.018	13.315	291.6	0.553	47505.691	819125.875	0.212	1.718	Solubility sample HPLC scan 48	
3818475.00	bility_	sample_H		mooth(Mn,2x2	0			F1	IScan,ES+ Calibra 181,400				_	
CH: 53.09 Sol 1.10			3818475 735379 CHI: 53	5.00 20 09				,		-			X X X	×
									Solution	.×		×	×	

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





Waters ACQUITY UPLC System.

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
- 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. Valko K, Bevan C, Reynolds D, Anal. Chem. (1997), 69, 2022-2029.