



LogD Measurement by Agilent HPLC with iChemExplorer

Michael Lopez
Reaction Analytics Inc.



LogD by HPLC

- Traditional Method
 - Shake API with two phases in flask
 - allow to separate
 - sample and analyze for API
- LogD measures API partition between water and octanol mimicking the biological partition between aqueous cytoplasm and greasy phospholipids.

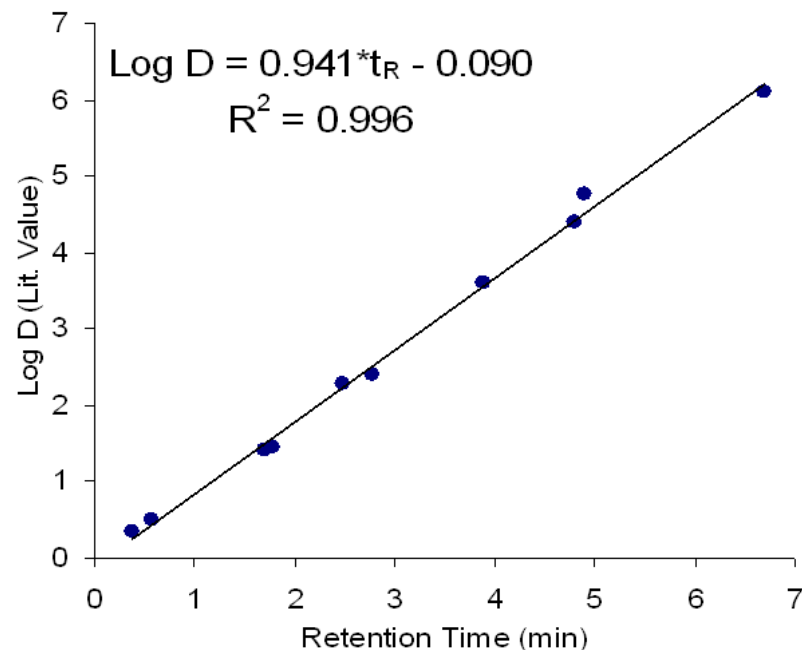




Open Access with Auto Calibration

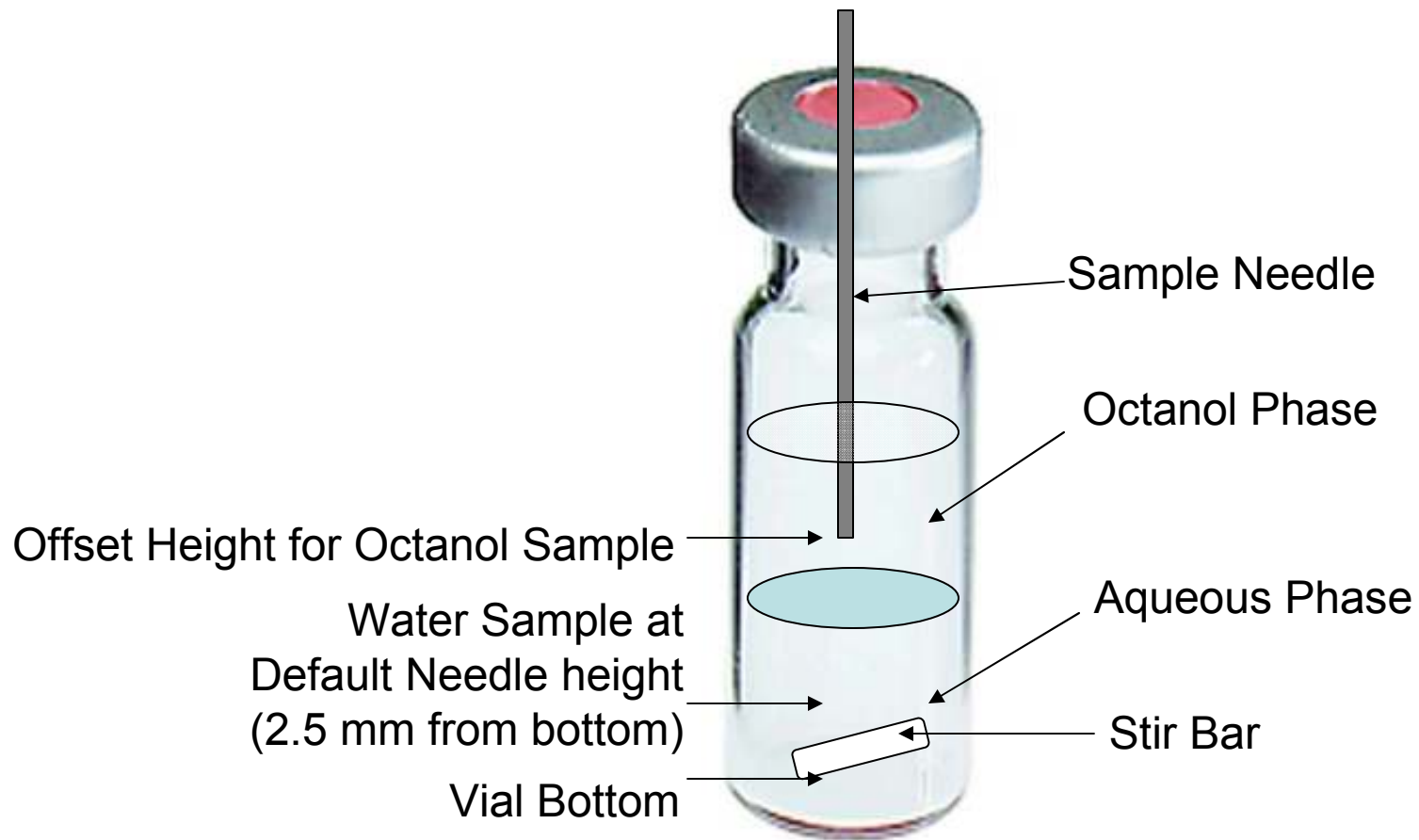
In order to evaluate system performance, a quality control (QC) check is performed daily using a set of 10 commercial compounds with known $\log D$ values ranging from 0.50 – 6.10

Compound	Lit. $\log D$	t_R (min)
Cimetidine	0.35	0.37
Fluconazole	0.50	0.58
Chlorpheniramine	1.41	1.70
Prednisone	1.46	1.78
Tetracaine	2.29	2.48
Imipramine	2.40	2.78
Triflupromazine	3.61	3.89
Loratadine	4.40	4.79
Bifonazole	4.77	4.90
Amiodarone	6.10	6.69





Shake Flask in a Vial





Developed for Agilent HPLC

- Designed to Fit Together
- Specifications and Operation stay the same
- Add Heating and Stirring
- Add Reporting in Microsoft Excel





LogD as Measure of Partition

Direct Measurement by HPLC as...

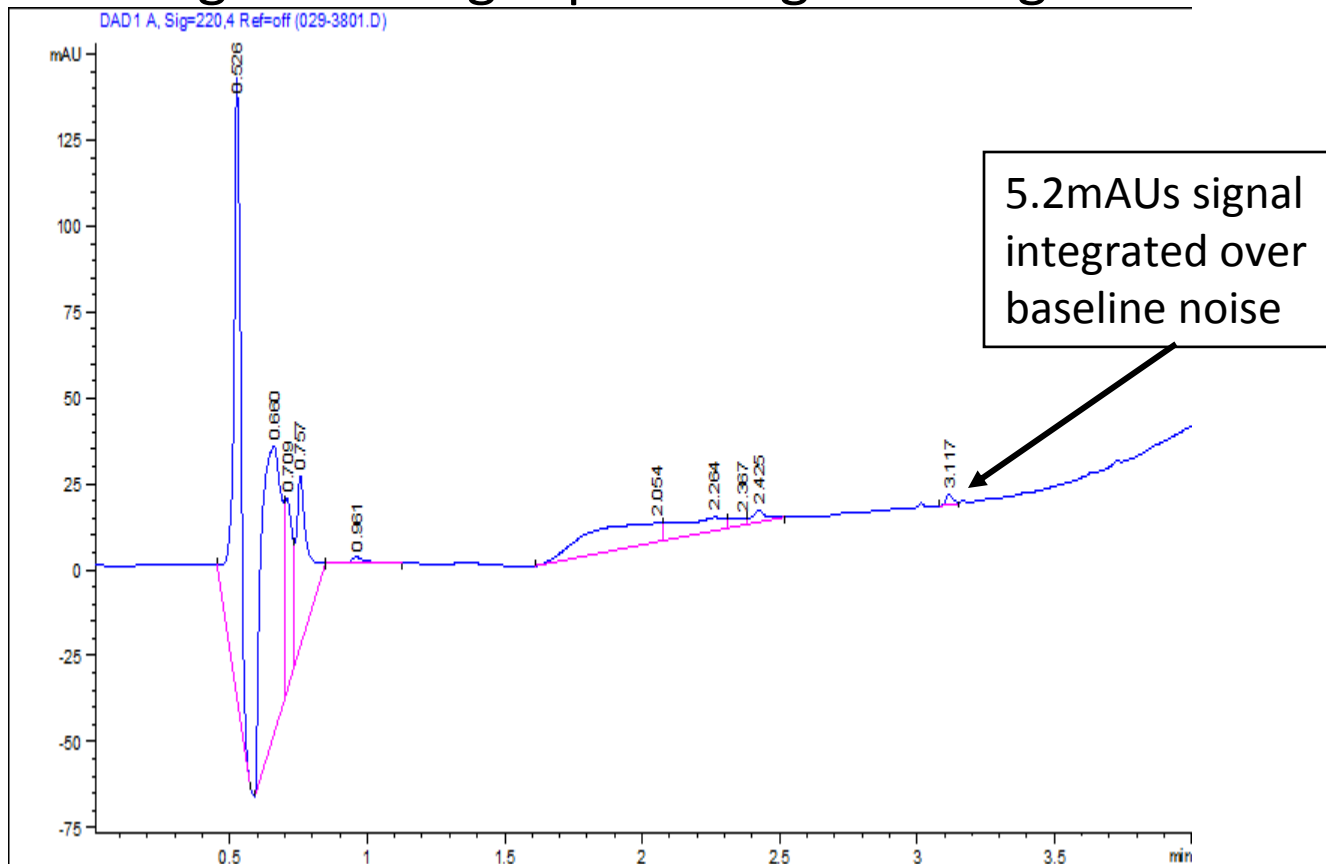
$$\text{LogD} = \text{Log}(\text{Peak Area}_{(\text{Octanol})} / \text{Peak Area}_{(\text{Water})})$$

- LogD = 0 1:1
- LogD = 1 10:1
- LogD = 2 100:1
- LogD = 3 1000:1
- LogD = 4 10000:1 carry over < 100 ppm
- LogD = 5 100000:1 carry over < 10 ppm



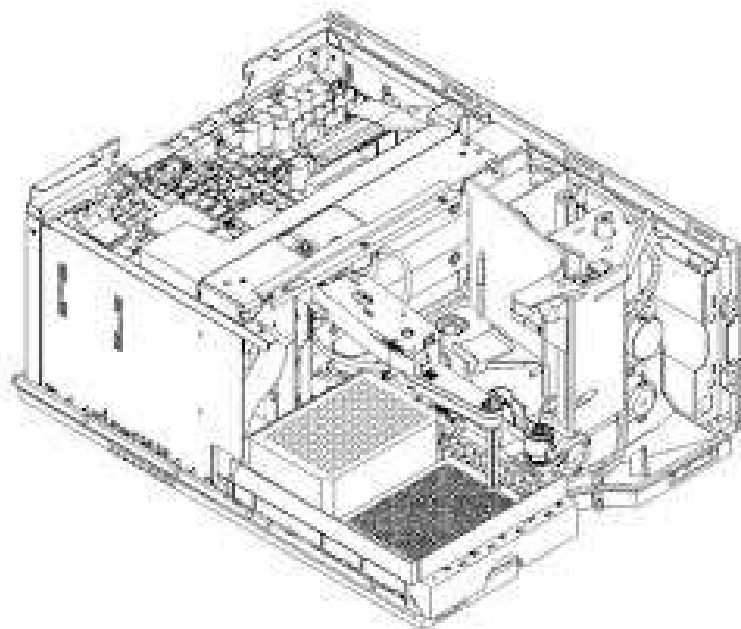
HDR Dual Light Path Solution

- Short 10 mm light path for high concentration
- Long 60 mm light path length for high sensitivity





Well Plate Autosampler



Holds TWO
Well Plates



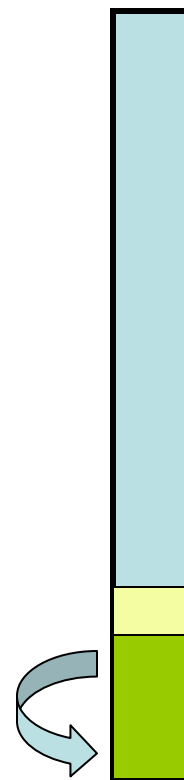
Identify by
Sample
Needle in
Robot Arm

Agilent Autosampler
For Well Plates
1367 and 4226



How to Manage Contamination

- Plug of pure phase in needle tip
- Draw Sample
- Spit
- Wash outside
- Inject
- Wash inside of needle
- Draw Aqueous samples first then Octanol





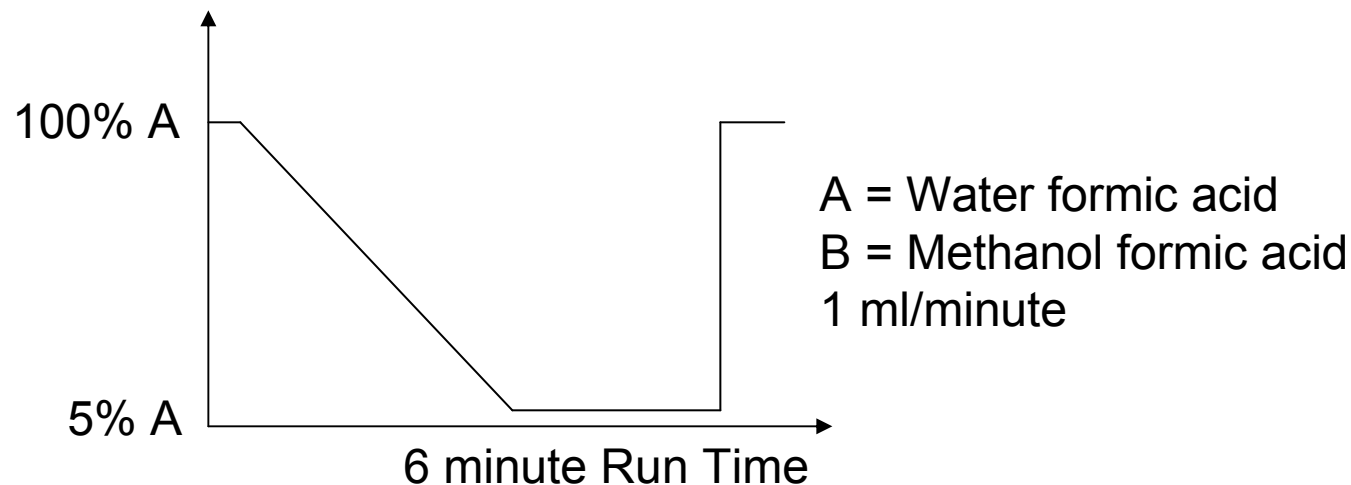
System Description

- Agilent HPLC with Bio-Inert autosampler and High Dynamic Range Detectors
- ICEbox and ICEtray for 2 ml vials
- iChemExplorer software v.9.4.9+
- Agilent Chemstation Revision C
- Windows 7 and Microsoft Excel 2003



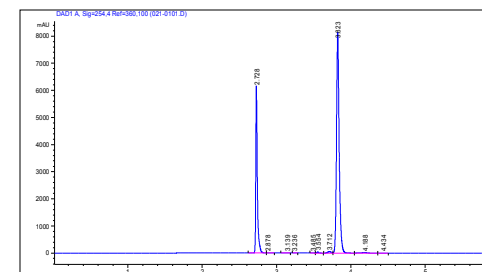
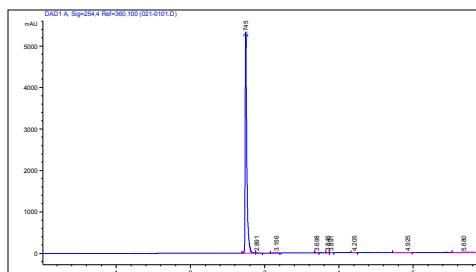


Analytical Method Validation



Combined Standard 4 ul sample

- Caffeine at 3 mg/ml
- Naphthalene at 3 mg/ml



Log D	literature	Measured
Caffeine	0.1	0.2
Naphthalene	3.4	3.1

Over 16 samples with 0.2 error



Solution Preparation

- Weigh out API 3 mg/ml-buffer solution
- Add API to Buffer Solution – water with buffer for pH 7.4
- Add stir bar; Add 500 ul buffer solution
- Add 500 ul Octanol; seal crimp top
- Stir and wait for partitioning
- Measure height of partition from vial bottom for offset to sample Octanol phase



Check HPLC System Ready

- All Modules Ready
- Leak Check
- Mobile Phase
- Wash Source
- Waste
- Chemstation Shows Ready



iChemExplorer Log D Tool

pKa/logD Sampling Tool

File

Sampling Properties

Number of Samples: 3

Measurements per sample: 1

Sampling method: TEST2

pH Settings for pKa

Minimum pH: 1

Maximum pH: 14

Equilibration Temp/DegC: 25

Equilibration Time/h: 2

Create Sampling Sequence and Temp Ramp

Start Experiment

Select logD or pKa

logD pKa

- Select LogD
- Vials to sample up to 57
- Measurements per sample – each from aqueous and octanol
- Select sample Method from Chemstation Method folder
- Equilibration Temperature set at entered value
- Equilibration time before first sample in Sequence Table
- Select Create to create Sequence Table in Chemstation and Heat Table in iChemExplorer



FILE sampling properties

Sampling Properties

Octanol Spacer Octanol Source Vial 78 Octanol Spacer Volume (ul) 5	Aqueous Spacer Water Source Vial 79 Water Spacer Volume (ul) 8
Octanol Layer Sampling Sample Volume (ul) 1 Needle Offset (mm) 6.5	Aqueous Layer Sampling Sample Volume (ul) 50 Needle Offset (mm) 1.5
External Needle Wash Wash Time (secs) 5	Internal Needle Flush <input checked="" type="radio"/> On <input type="radio"/> Off Flush Time (secs) 10

Internal Needle Flush
Draw Spacer
Draw Sample
External Needle Wash
Inject Sample

Apply

- Octanol and Aqueous Source Vials fixed at locations 78 and 79
- Spacer Volume is volume drawn before sample
- Sampling Volume is selected for Octanol and Aqueous Phases; Same volumes for all samples
- Needle Offset is from Default position 2.5 mm from bottom of vial
- External needle wash time
- Internal Needle Flush to waste



Sample Injector Steps

Setup Method

Binary Pump HiP Sampler HiP Sampler Injector Program Column Comp. DAD Instrument Curves

Use Injector Program

Function	Parameter
▶ Valve	Switch valve to "Main In" from sample for 10 s using default offset
Valve	Switch valve to "Bypass"
Draw	Draw 20 μL from location "78" with 100 $\mu\text{L}/\text{min}$ using offset 0 mm
Draw	Draw 10 μL from sample with 100 $\mu\text{L}/\text{min}$ using offset 6.5 mm
Wash	Wash needle in flushport for 10 s
Inject	Inject

Offset
to draw from
Octanol phase
as set In File
sampling parameters

Created by iChemExplorer in Agilent Chemstation
with selections from LogD Tool and File parameters



Vial Locations for Log D



- ⊙ W = 79 = Water
- ⊙ O = 78 = Octanol
- ⊙ = 26 = Antipyrine
- ⊙ = 25 = Desipramine
- ⊙ = 24 = Bifonazole
- ⊙ = 23 = Acebutalol
- ⊙ = 22 = Chlorpromazine
- ⊙ = 21 = Caffeine/Naphthalene



Sequence Table LogD Tool

One hour wait step to stir sample
Blank injection to prepare column

Analyze aqueous first

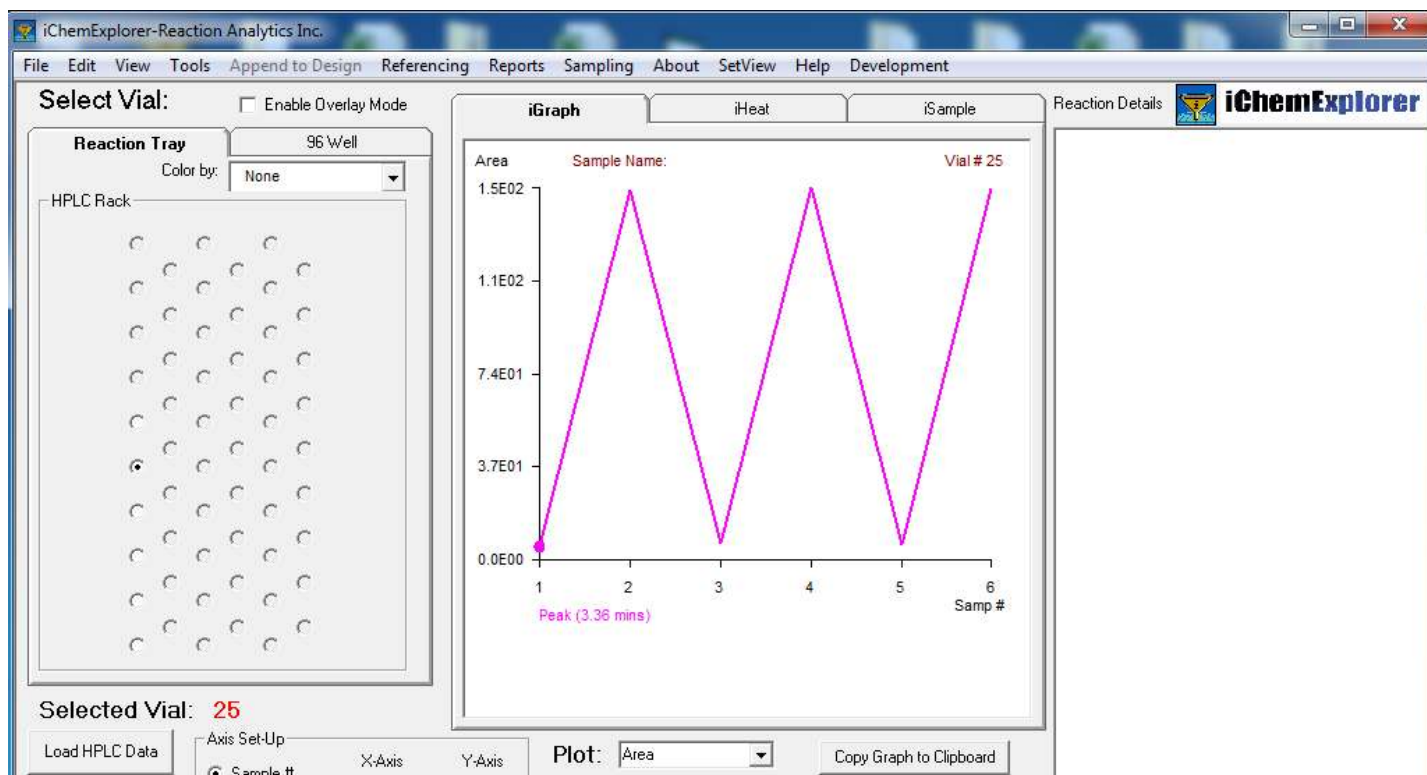
Analyze octanol second

Sequence Table: HPLC1

Line	Sample Con...	Sample ...	Sample Name	Method Name	Injection Source	Injection Vol...	Inj/Loc	Sample Type	Cal
▶ 1	Use Curr...	1		WAITSOL	As Method	1	2	Sample	
2	Use Curr...	21		CONDITION	As Method	10	1	Sample	
3	Use Curr...	21		AQUEOUSS...	As Method	10	1	Sample	
4	Use Curr...	22		AQUEOUSS...	As Method	10	1	Sample	
5	Use Curr...	23		AQUEOUSS...	As Method	10	1	Sample	
6	Use Curr...	21		OCTANOLS...	As Method	10	1	Sample	
7	Use Curr...	22		OCTANOLS...	As Method	10	1	Sample	
8	Use Curr...	23		OCTANOLS...	As Method	10	1	Sample	
9	Use Curr...	21		AQUEOUSS...	As Method	10	1	Sample	
10	Use Curr...	22		AQUEOUSS...	As Method	10	1	Sample	
11	Use Curr...	23		AQUEOUSS...	As Method	10	1	Sample	
12	Use Curr...	21		OCTANOLS...	As Method	10	1	Sample	
13	Use Curr...	22		OCTANOLS...	As Method	10	1	Sample	
14	Use Curr...	23		OCTANOLS...	As Method	10	1	Sample	
15									



Success in a Vial



Classic Partition pattern – low peak area in aqueous samples and high peak area in octanol samples. We have success



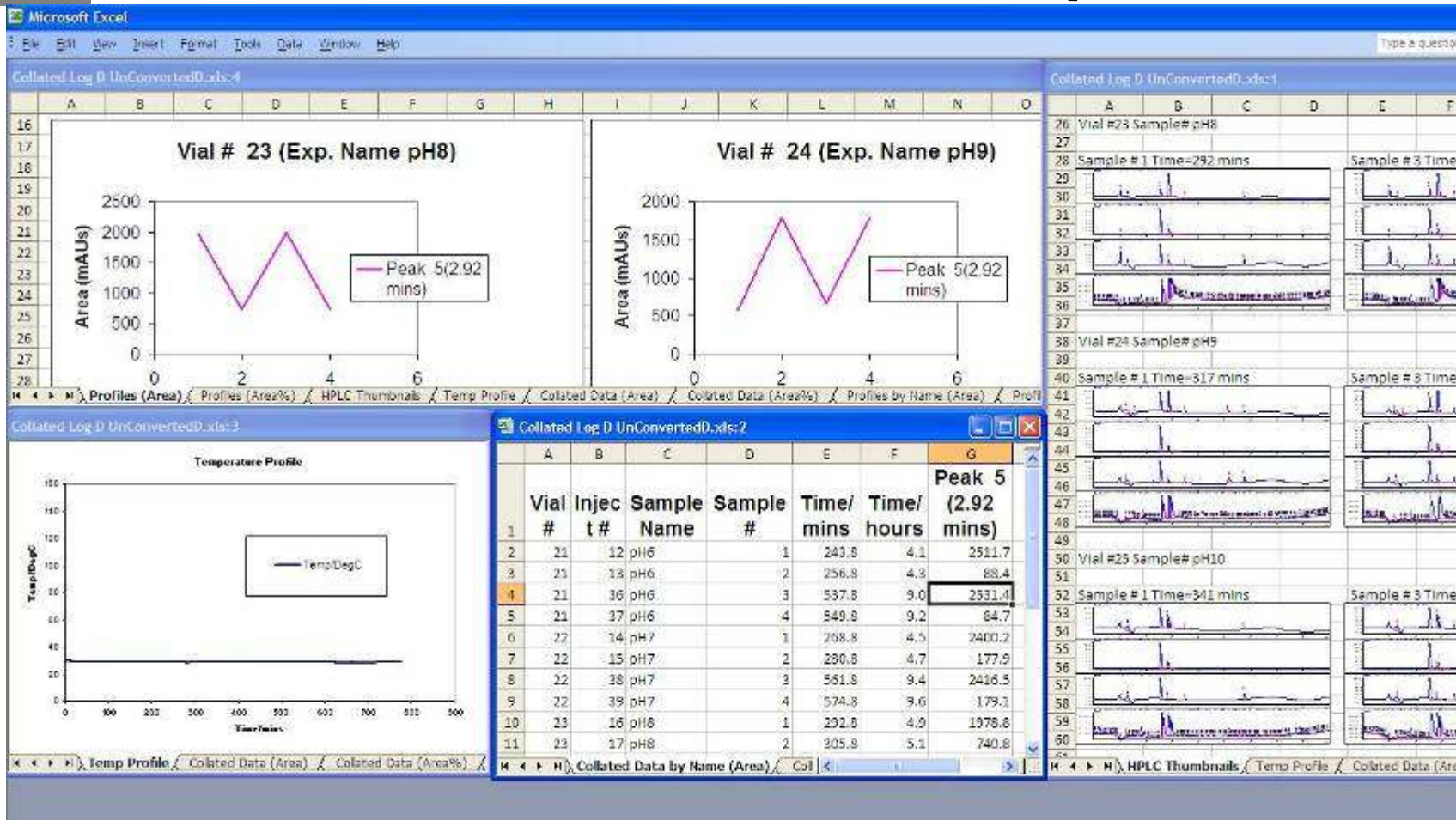
Log D Report



Peak Name	logD (measured)	logD (Lit.)
Caffeine	-0.2	-0.1
Chlorpromazine	3.5	3.5
Acebutalol	0.1	-0.3
Bifonazole	4.5	4.8
Desipramine	1.4	1.3
Antipyrine	0.2	0.4



Excel Collated Report





Conclusion for LogD by LC

- Fit for Purpose
 - Good For Measurement from -1 to +4.5
 - Repeatable to 0.3
- Fit for Application
 - Direct measurement adaptable to MS
 - Small sample requirement – 3 mg or less
 - Adaptable to HPLC on the bench



Acknowledgments

- Dr. Takahiro Takeuchi *See HPLC 2015
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