



PhysChem Measurements -more than just a number

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Physical and Analytical Sciences UCB

PhysChem Forum June 2007

Overview



PhysChem Report

Overview of the Assays run at UCB

More to learn from AKAS and HTLogD

Summary

Questions?

PhysChem Report



No.	AKAS (uM)				HTLogD	ACDLabs Predicted pKa values			ACDLabs calculated values				Comments	Progress
	pH3	pH5	pH7.4	pH9	pH7.4	pKa 1	% ionised @ pH7.4	pKa 2	PSA+S	PSA-S	HBD	HBA		
1	>350	139	137	119	3.61	5.42	1.0	2.36	141	113	3	9	Basic ionisation event between pH3 and pH5 due to protonation of thiazole	FAIL
2	7	2	1	2	4.50	4.47	0.1		96	42	0	4	Basic ionisation event between pH3 and pH5 due to protonation of pyridine	FAIL
3	>350	>350	>350	>350	3.84	3.81	100.0	3.53	127	99	2	10	Spectral change due to basic ionisation event between pH5 and pH7.4 due to protonation of diaminopyrimidine	PASS
4	>350	>350	162	41	3.94	4.24	0.1	3.53	115	87	1	9	Spectral change due to basic ionisation event between pH5 and pH7.4 due to protonation of diaminopyrimidine	PASS
5	>350	>350	>350	29	3.95	8.86	96.6	3.62	86	58	1	6	Basic ionisation event between pH7.4 and pH9 due to amine	PASS
6	>350	>350	>350	40	4.23	7.39	49.4	6.33	86	58	1	6	Basic ionisation event between pH7.4 and pH9 due to amine	PASS
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8	ND	ND	ND	ND	ND	10.32	99.9	7.37	106	78	1	6	Compound 82% pure, therefore all measured data is unreliable and will not be returned. Any data present in spotfire will be removed.	FAIL

	ND	Poor	Modest	Good	
AKAS	ND	<30mM	30-150mM	150-350mM	>350mM
PSA		>113	83-113	<83	

AKAS Automated Kinetic Aqueous Solubility



All NCE's

10mM DMSO Stock Solution



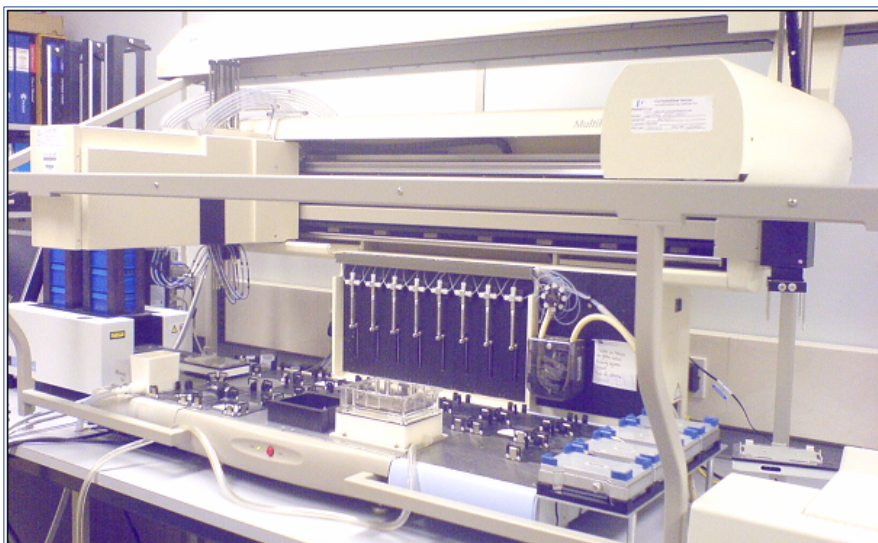
Aqueous Buffer

Kinetic



Fastest form to dissolve not necessarily the most stable

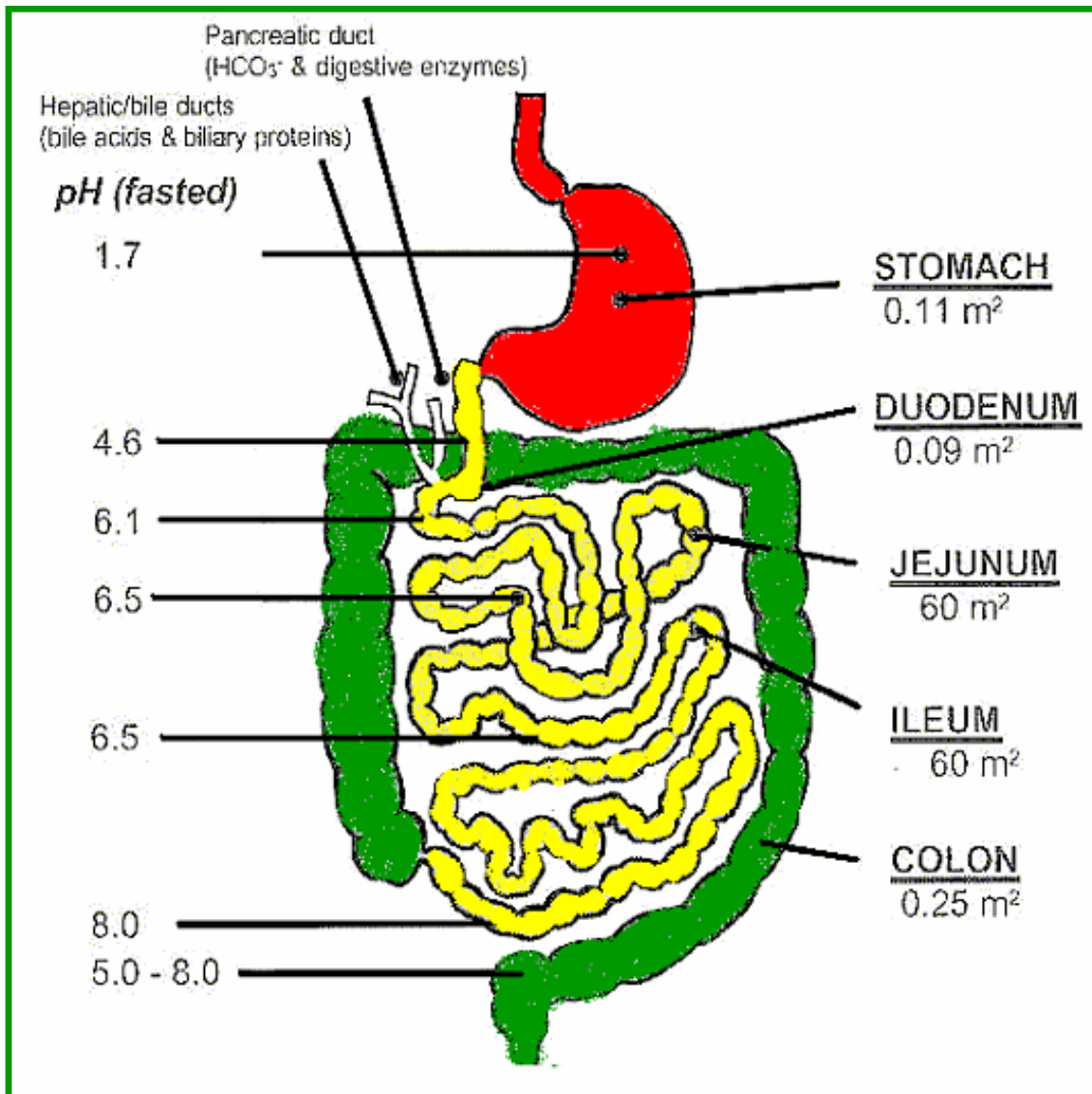
HT MultiPROBE II



44 x compounds in 4hrs
Run twice a week

Final DMSO content 5%

AKAS at 4 pH's



Run at 4 pH's to mimic the varying pH range of the GI tract:

pH3, pH5, pH7.4, pH9.

Stomach pH 1 - 3

Duodenum pH 5 - 7

Blood pH7.4

Colon pH 7 - 8

Solubility Assays



1st Tier Solubility

AKAS

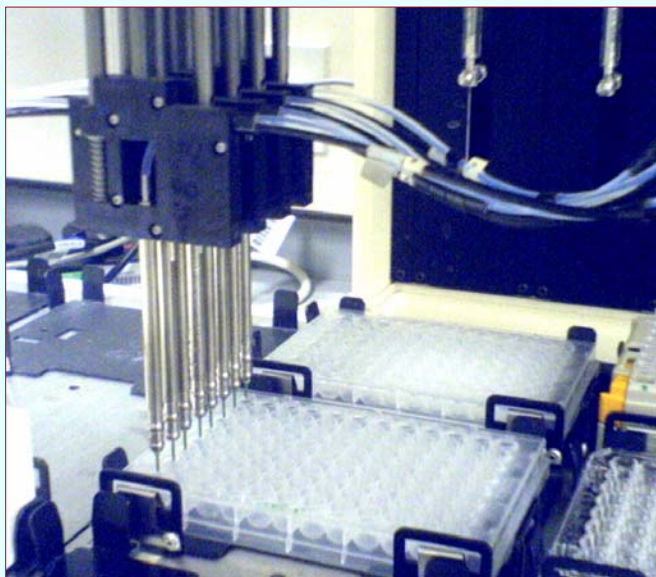
Passes at
>150uM

2nd Tier Solubility

QuickSol

Kinetic - from DMSO Stock

Pseudothermodynamic - from solid

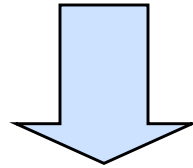


Solubility Assays - QuickSol



pH7.4 (pH5 on request)

Buffer



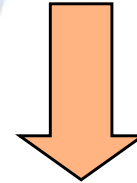
shaken for 90 mins

Solid Sample

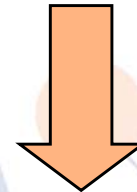
Analysis by HPLC



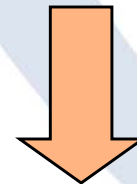
Pseudo-thermodynamic



Starts from solid but not in excess.



Dissolution may not be complete in 90 mins.

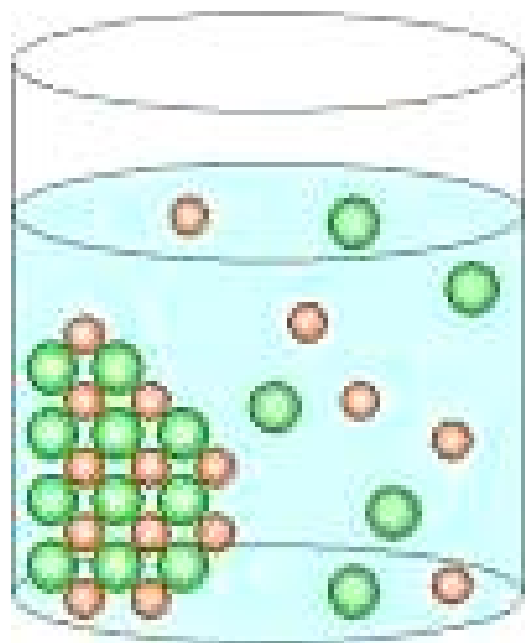


Only a single time point not necessarily at the equilibrium.

Why is QSol Important?

Crystal forms can have different solubility

Depending on the dissolution energy of the crystal lattice



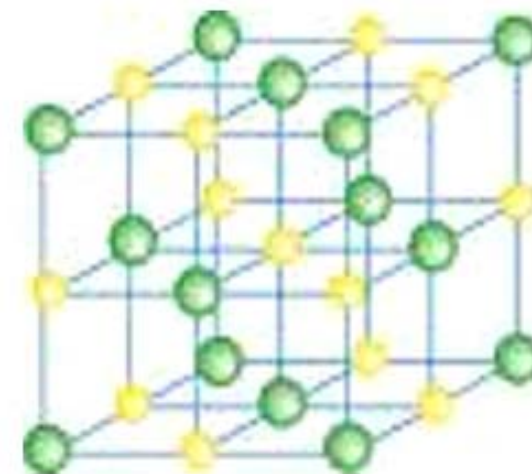
Batches can have different crystal forms



Batch to Batch Variation



Do not Average Batches



No DMSO present so no co-solvent effects - AKAS has 5% DMSO

Use the Right Assay for your Question



A Biologist



Assays start
from DMSO Stock

= AKAS

DMPK

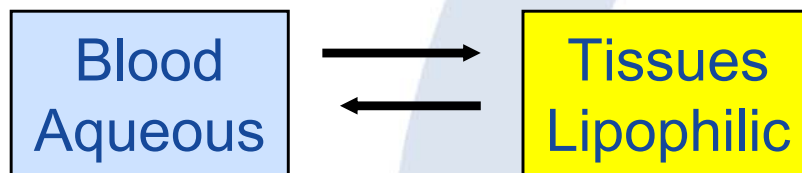


Using Solid
to dose animals

= QSol

Lipophilicity

Distribution Around The Body



HTLogD_{7.4} assay

Sample added to octanol and water and shaken for 90 minutes

Partition

Octanol

Water

Layers are separated and analysed by HPLC

$$D(o/w) = \frac{[\text{ionised}]_{\text{oct}} + [\text{neutral}]_{\text{oct}}}{[\text{ionised}]_{\text{aq}} + [\text{neutral}]_{\text{aq}}}$$

Why we measure $\text{LogD}_{7.4}$ not LogP



LogP

P = Partition coefficient

Octanol Neutral species

Water Neutral species

LogP can only be run at a pH where all of the compound is neutral



Would have to be run at a different pH for each compound depending on its pKa

LogD_{7.4}

D = Distribution coefficient

Octanol Neutral and Ionised species

Water Neutral and Ionised species

LogD_{7.4} can be run at the same pH for all compounds



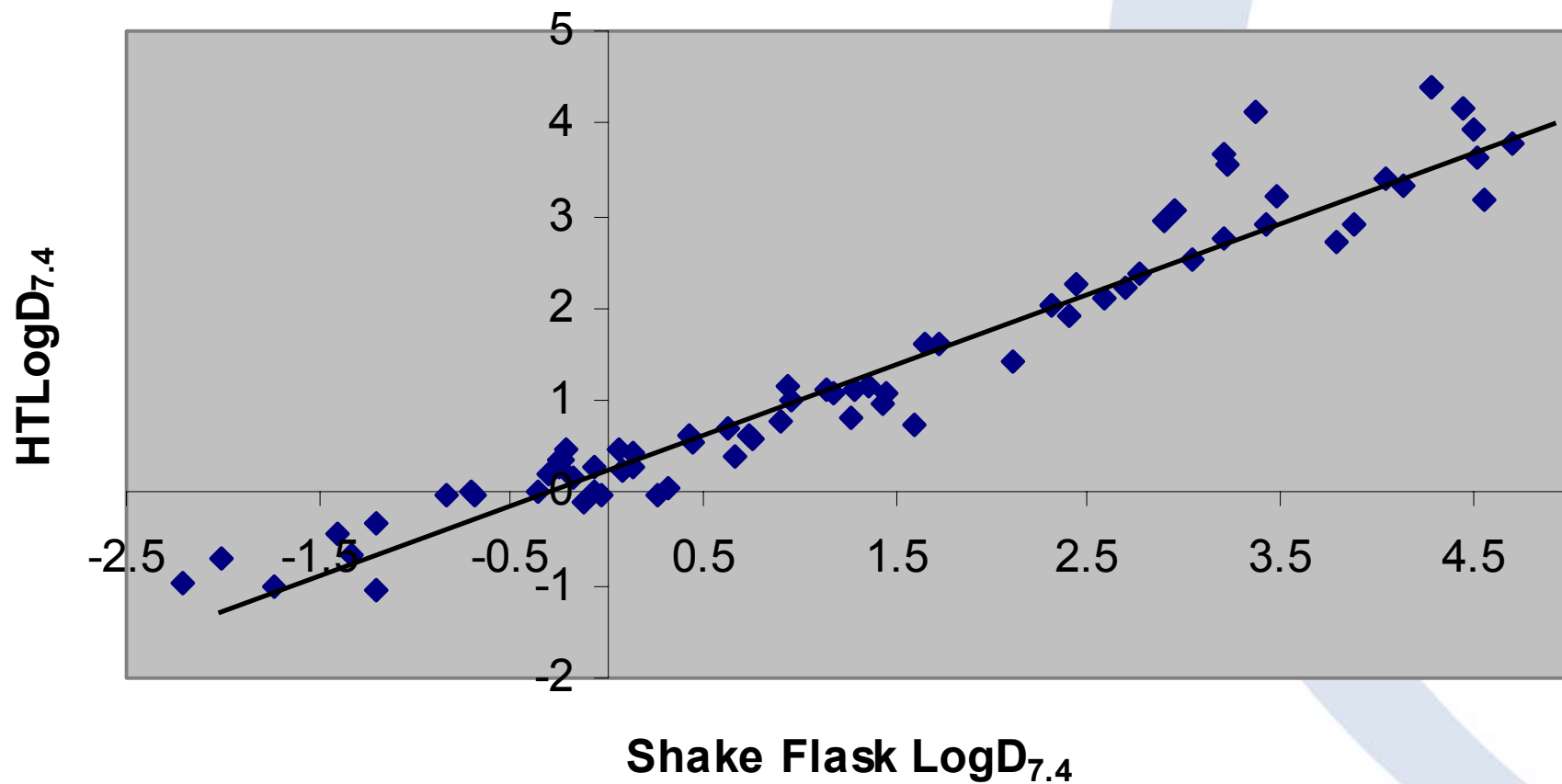
It's run at a pH of interest eg pH7.4 for blood

Ratio

Shake flask $\text{LogD}_{7.4}$ vs HTLog $\text{D}_{7.4}$



$R^2 = 0.94$



There's more to learn from AKAS than just solubility



If a compound is ionisable

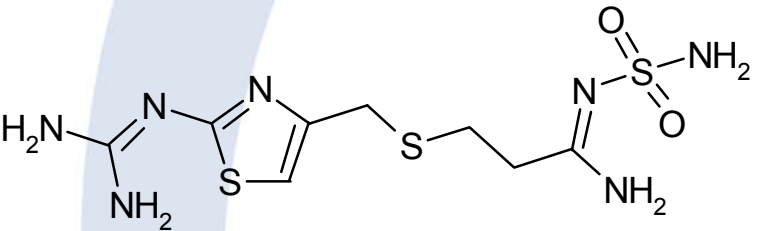
pKa Approximation

Stability

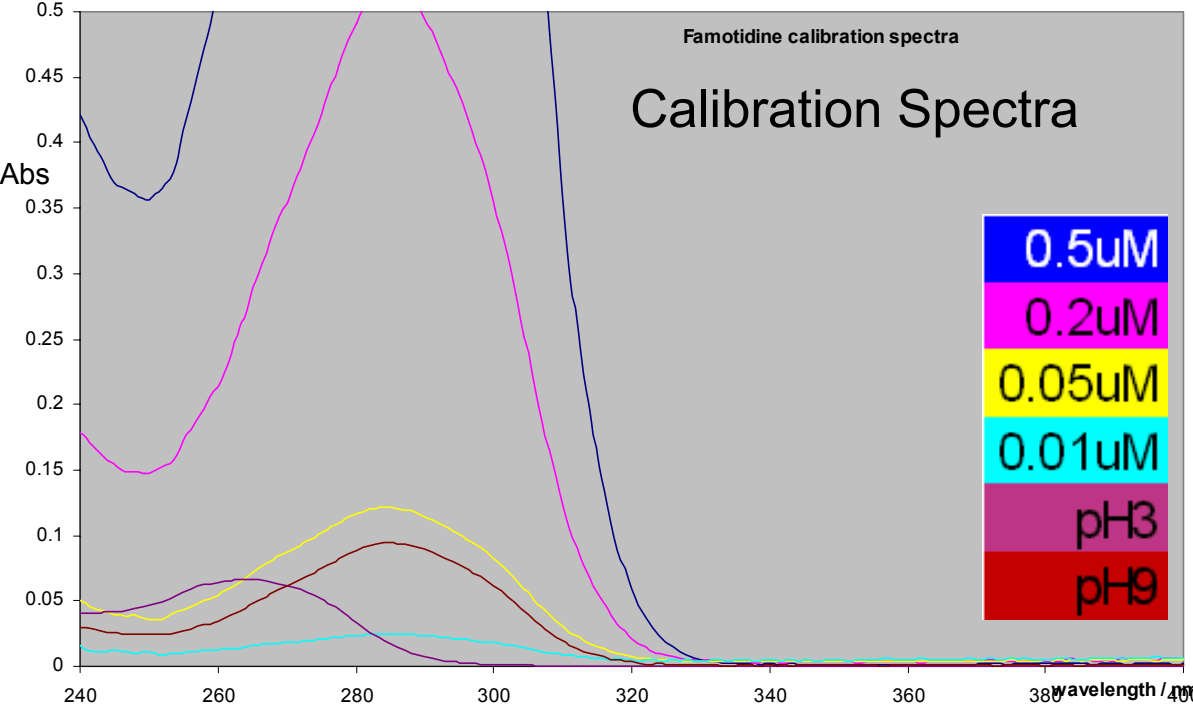




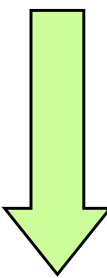
Ionisable compounds



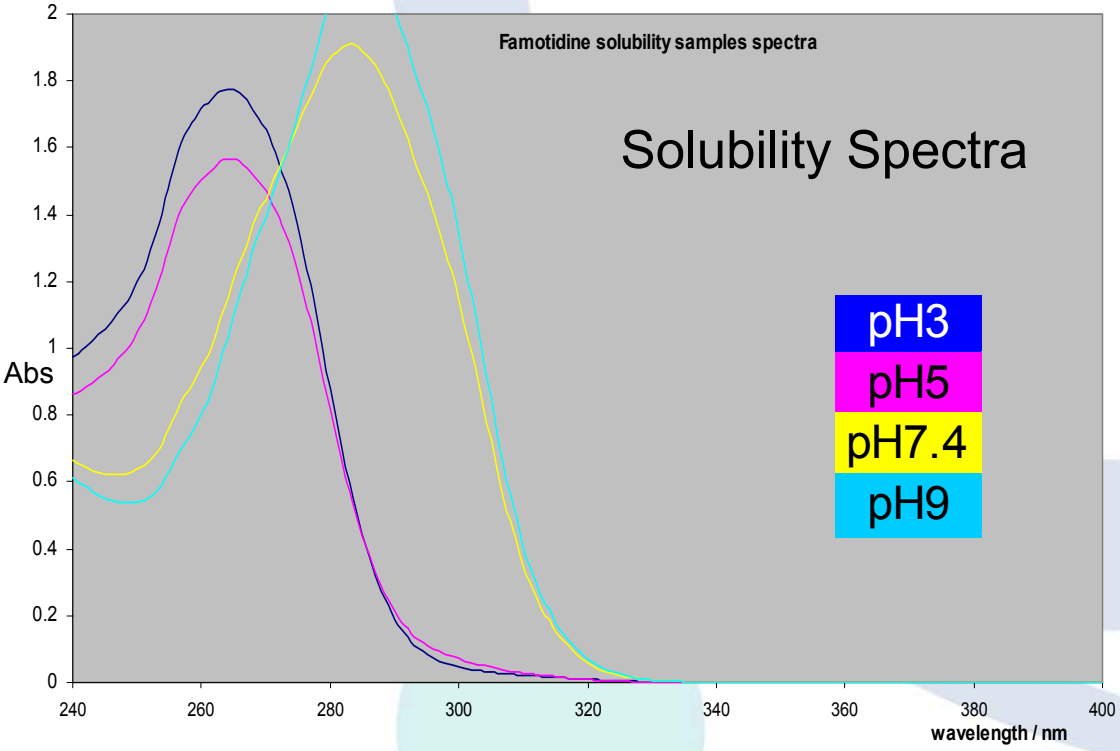
Famotidine



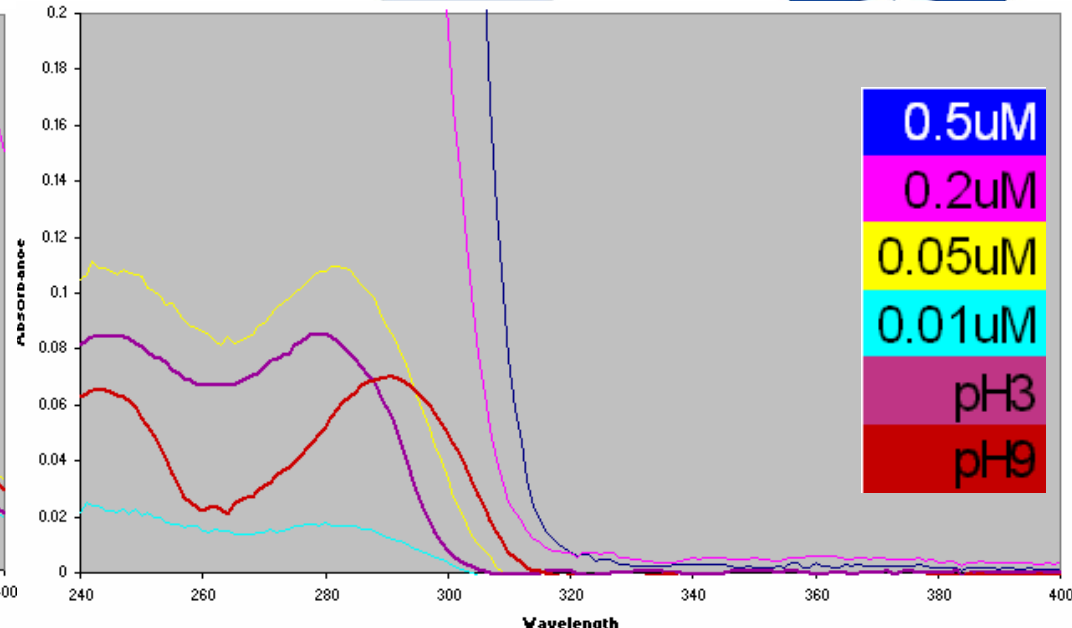
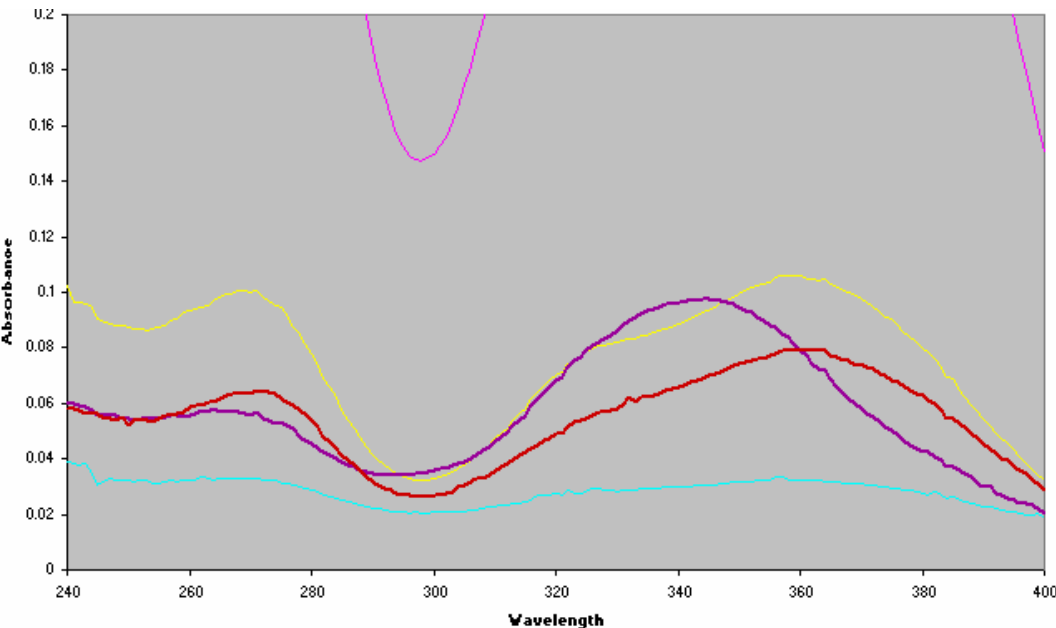
Four point calibration



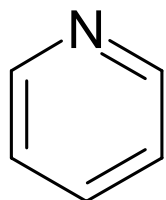
Spectral change in the solubility



Acidic or Basic Ionisation?

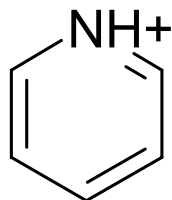


Neutral at pH9

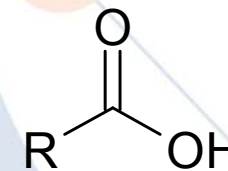


Basic Shift

Ionised at pH3

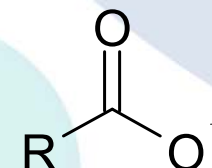


Neutral at pH3



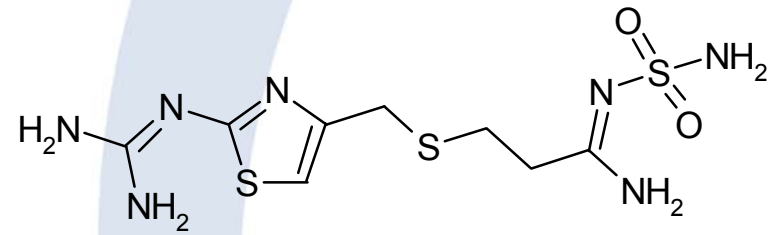
Acidic Shift

Ionised at pH9

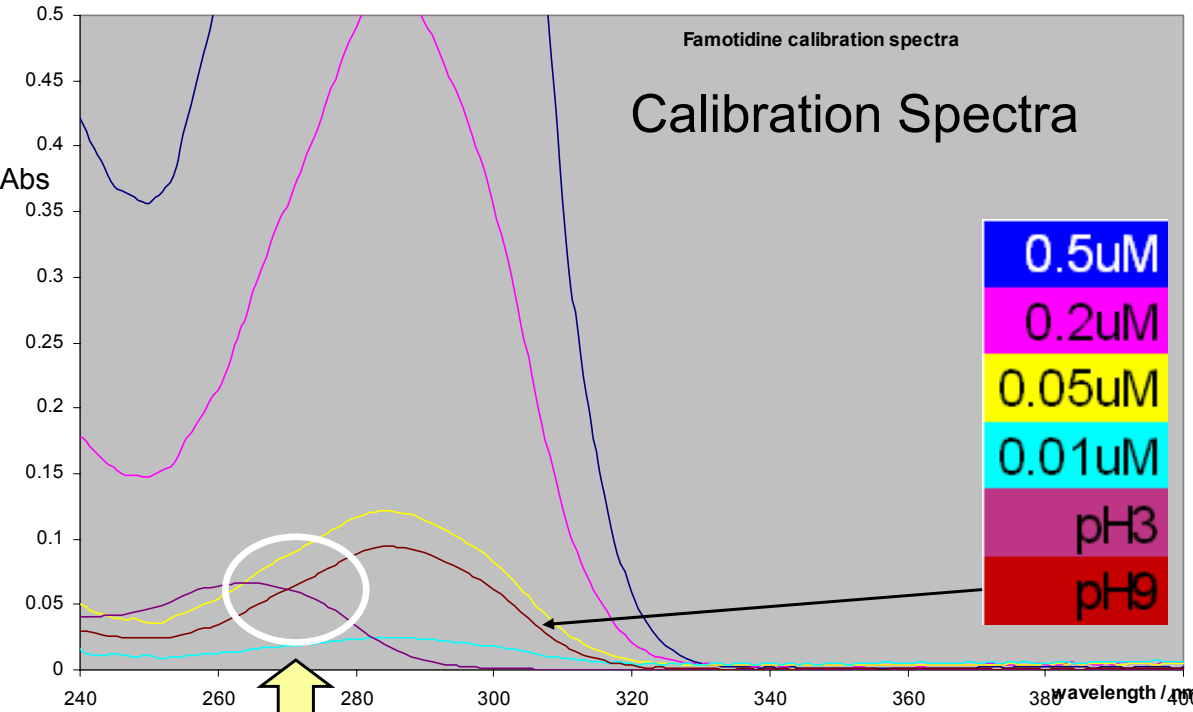




Ionisable compounds



Famotidine



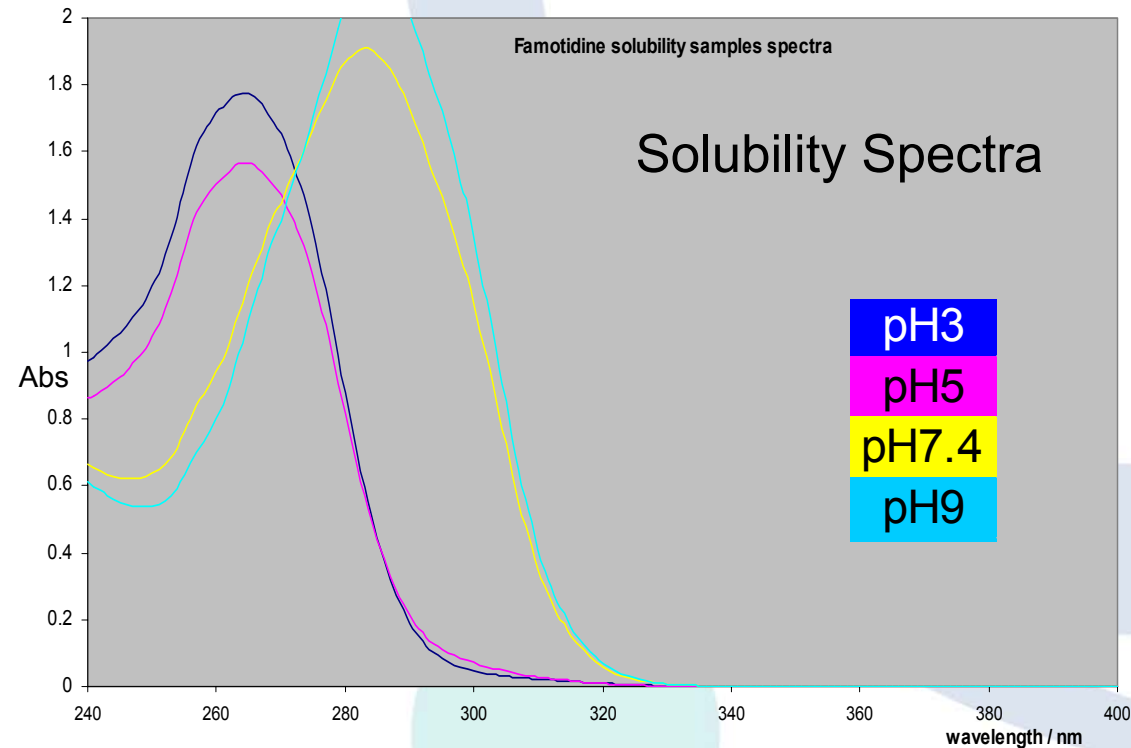
Isosbestic point

Neutral and charged curves cross: absorbance is the same

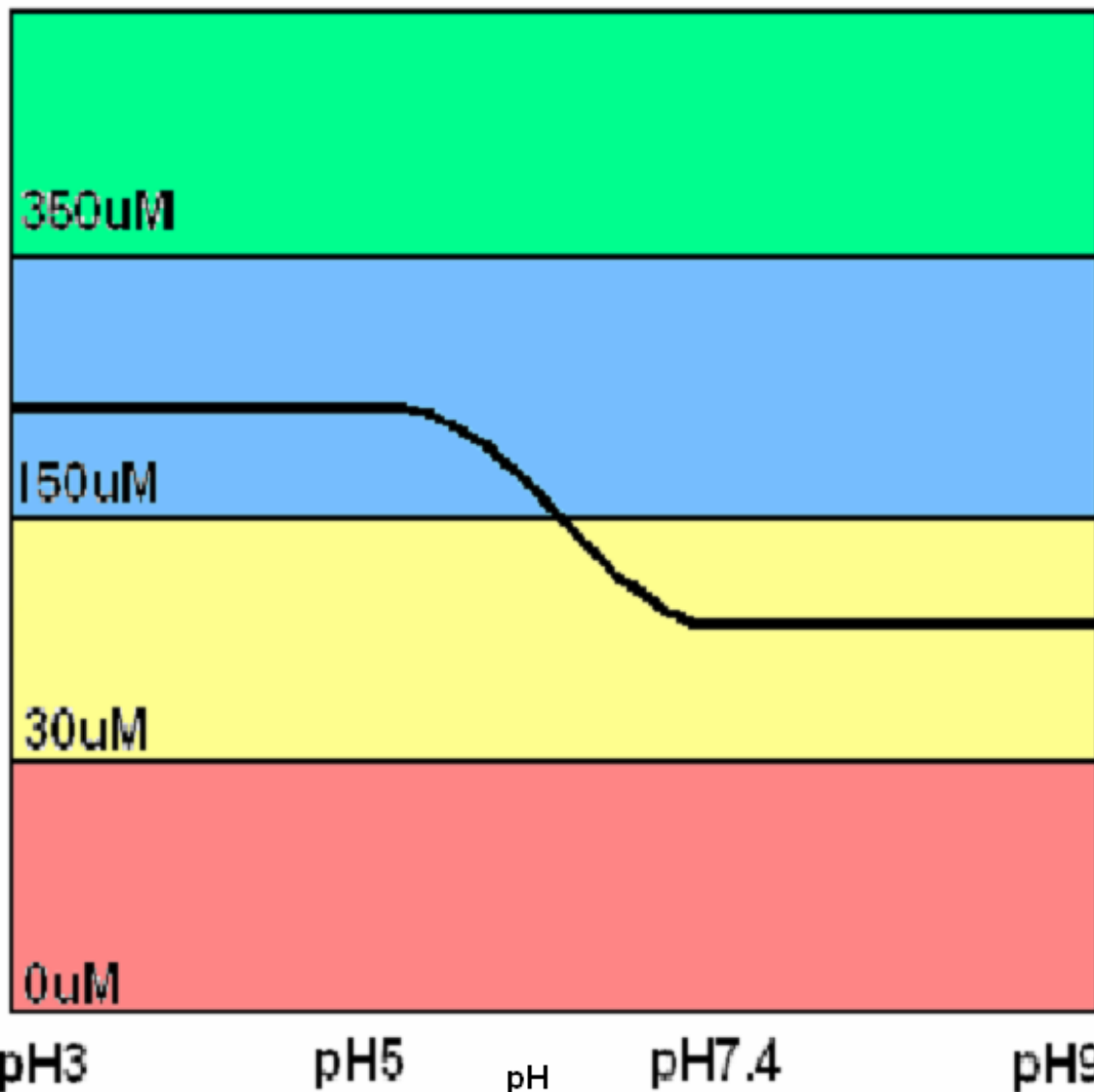
Basic ionisation event between pH5 and pH7.4

AKAS results

pH3	pH5	pH7.4	pH9
244	162	83	75



pKa approximation from AKAS



pH3	pH5	pH7.4	pH9
244	162	83	75



pKa Estimation

100%

% Ionised

0%

Basic ionisation event
between pH5 and pH7.4

Soluble at Lower pHs

Ionisable Compounds

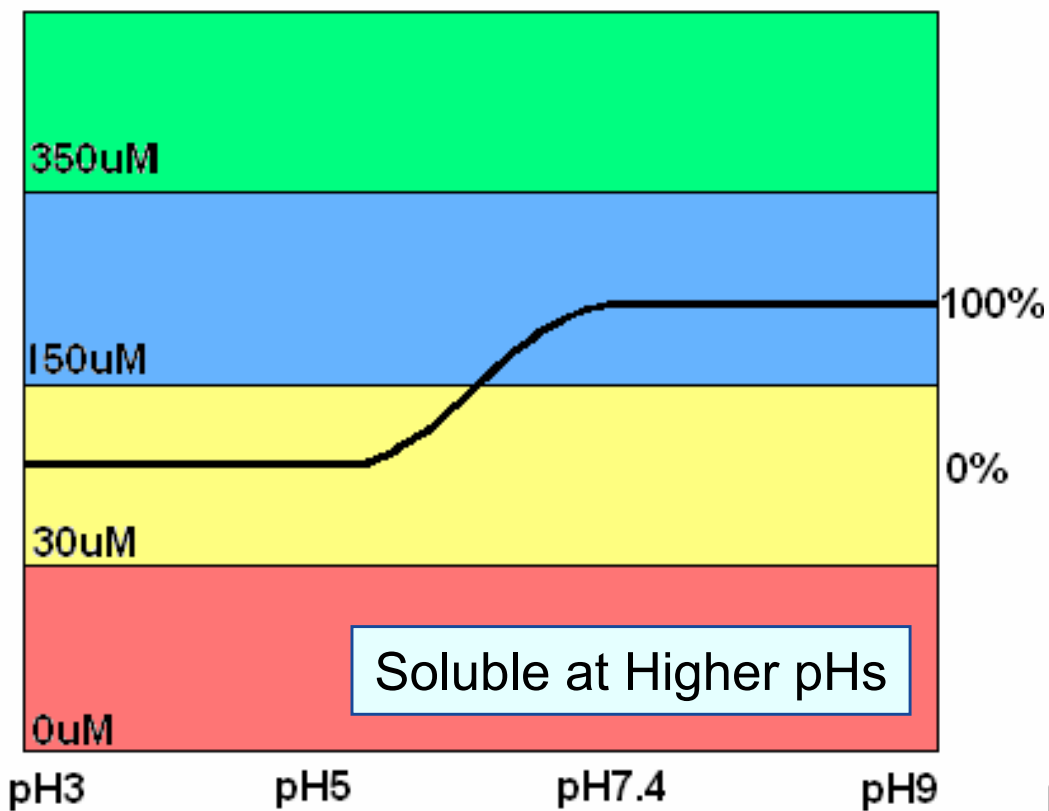


pH3	pH5	pH7.4	pH9
82	89	163	184

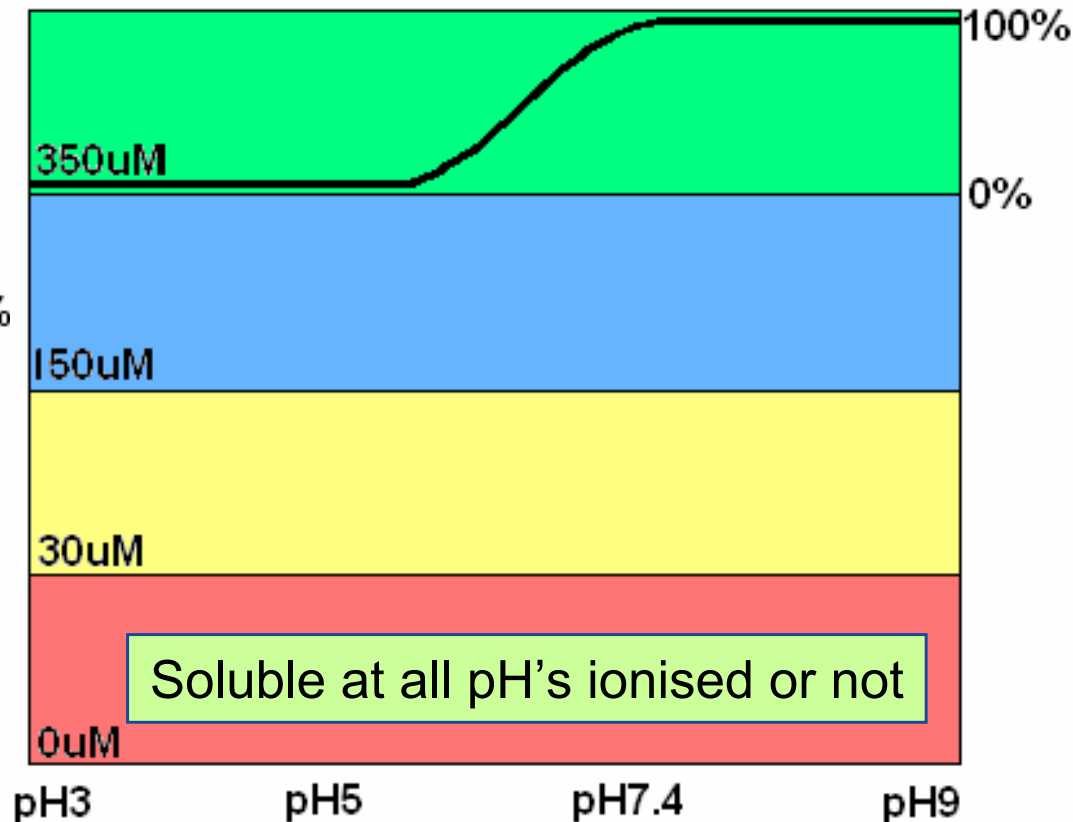
↑
pKa Estimation

pH3	pH5	pH7.4	pH9
>350	>350	>350	>350

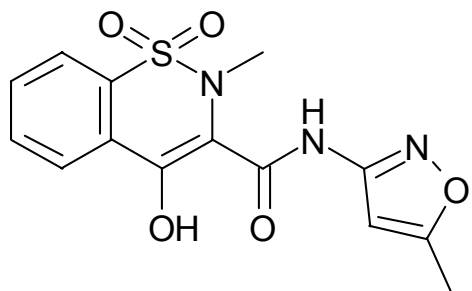
Ibuprofen



Acidic ionisation event between pH5 and pH7.4

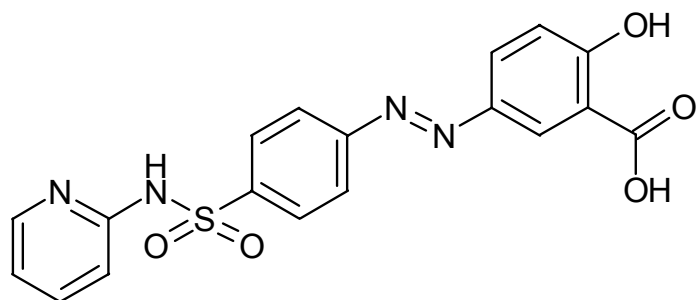


Examples of AKAS approximating pKa



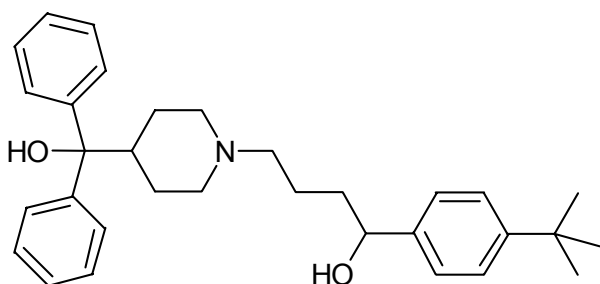
Isoxicam			
AKAS			
pH3 (uM)	pH5 (uM)	pH7.4 (uM)	pH9 (uM)
2	>350	>350	>350

↑
pKa



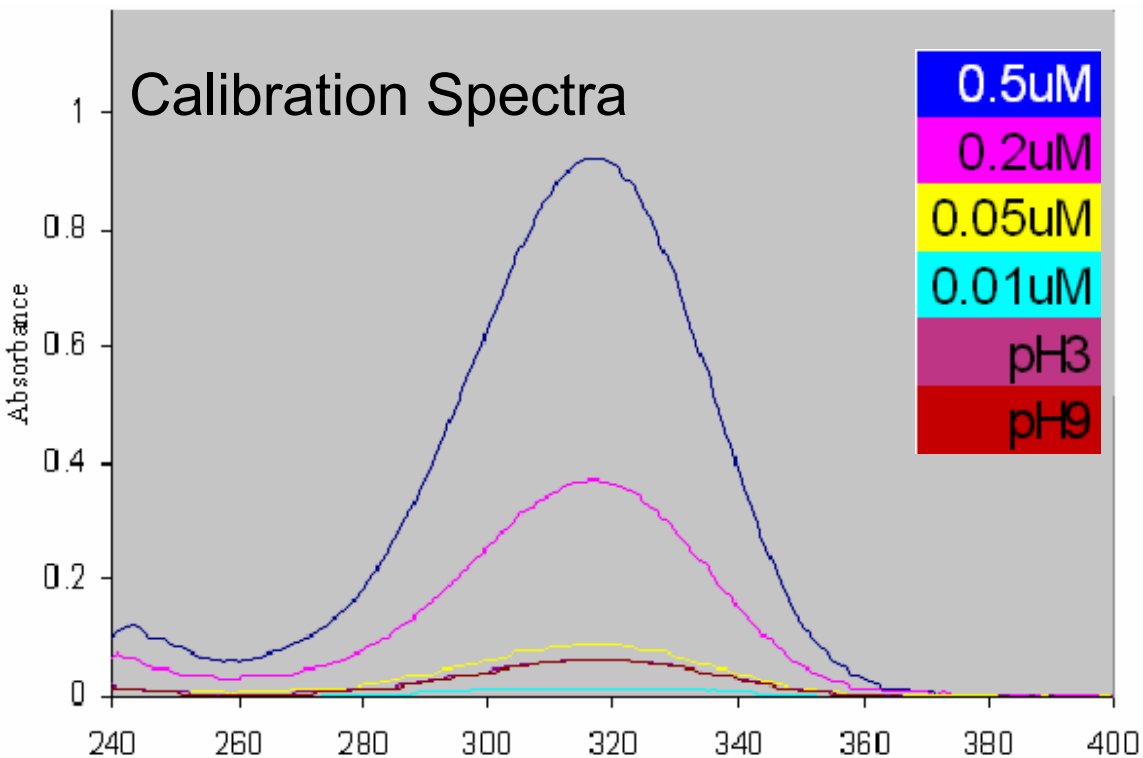
Sulfasalazine			
AKAS			
pH3 (uM)	pH5 (uM)	pH7.4 (uM)	pH9 (uM)
144	>350	>350	>350

↑
pKa

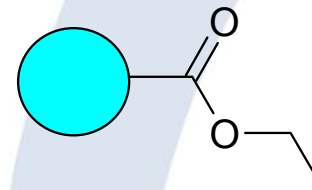


Terfenadine			
AKAS			
pH3 (uM)	pH5 (uM)	pH7.4 (uM)	pH9 (uM)
>350	>350	240	0

↑
pKa



pH Stability



Compound X

Unstable ester at pH9?

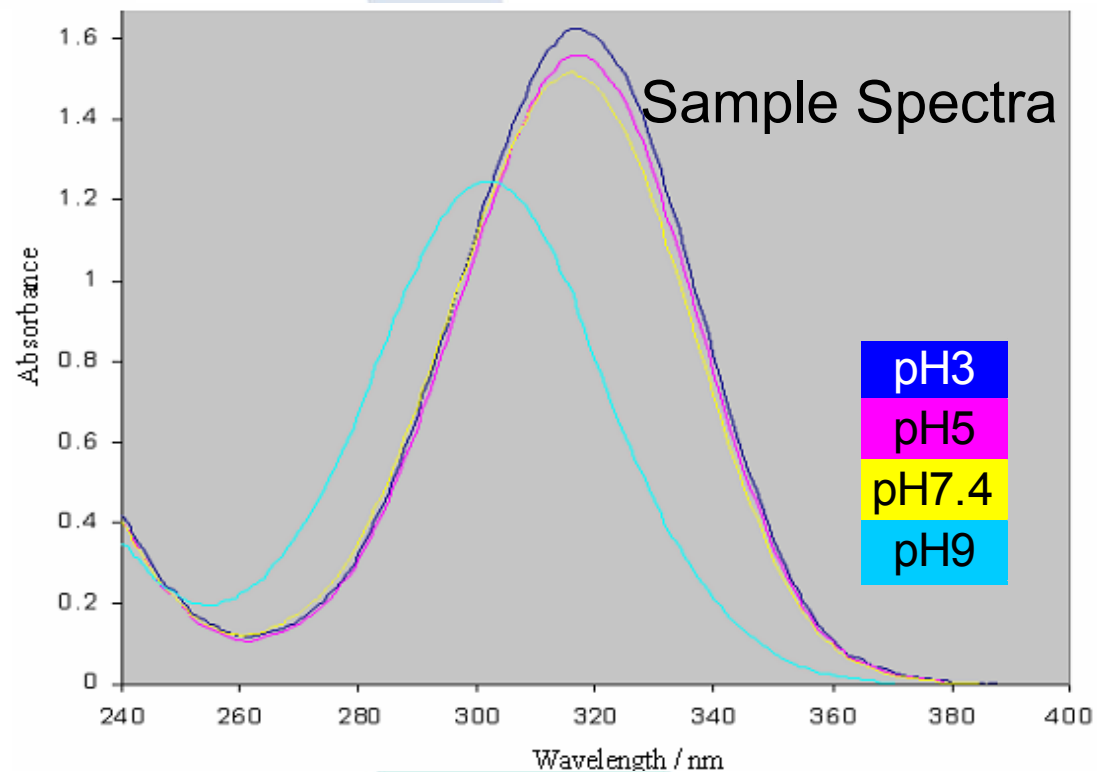
pH3	pH5	pH7.4	pH9
>350	>350	>350	ND

No spectral change in the calibration

Possible stability issues

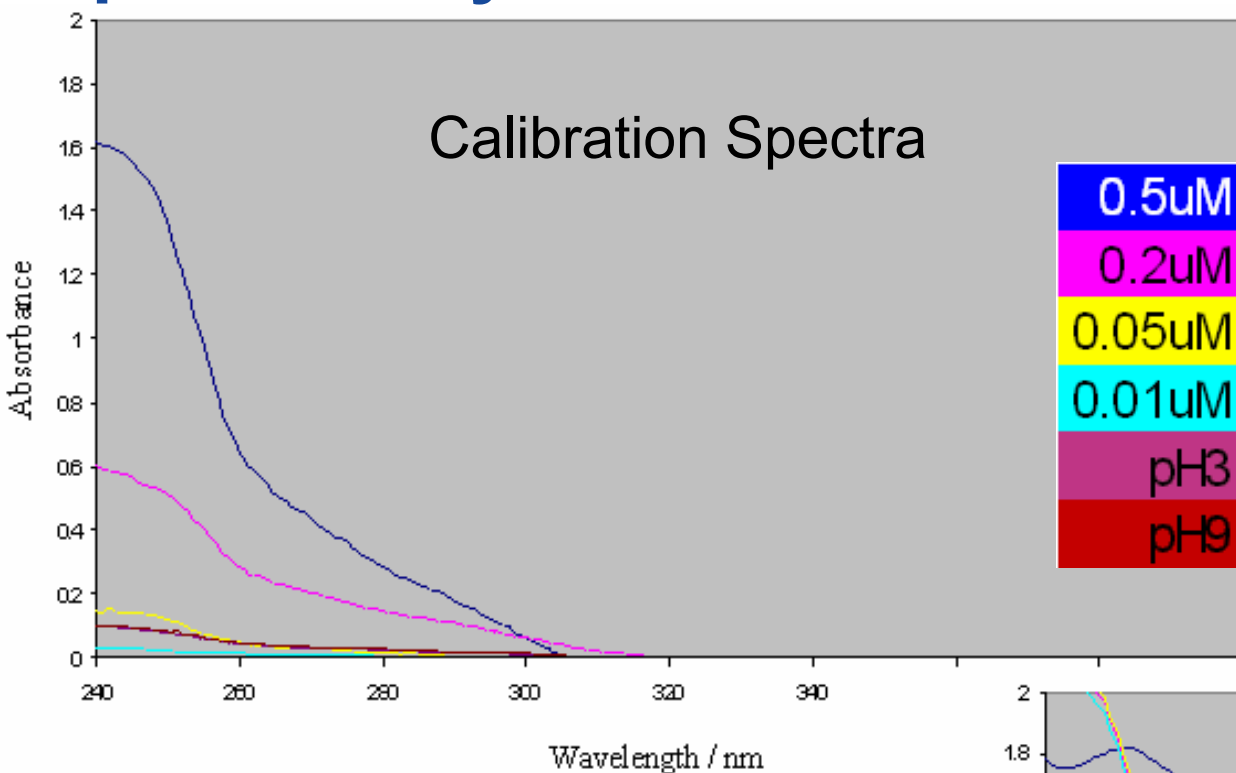
90min shake

Spectral change in the solubility



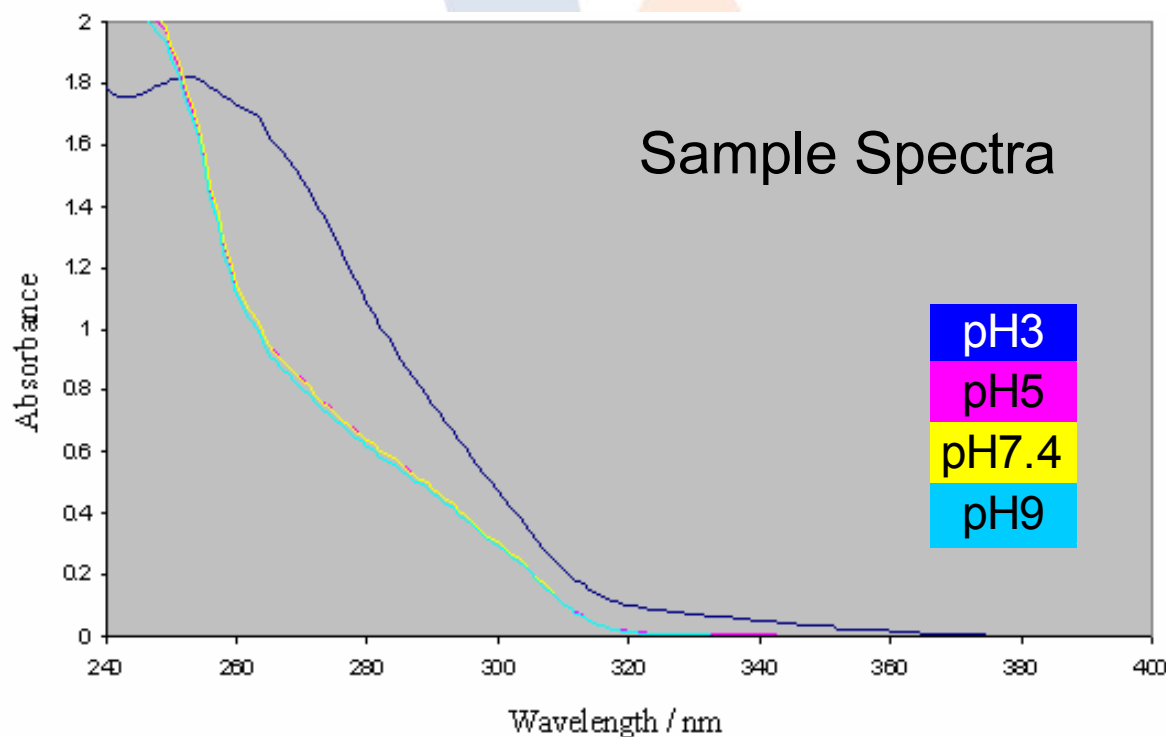


pH Stability issues — from AKAS UV spectra



pH3	pH5	pH7.4	pH9
ND	>350	>350	>350

Unstable at pH3?



There's more to learn from AKAS than just solubility



So in 6hrs we have discovered:

If a compound is ionisable

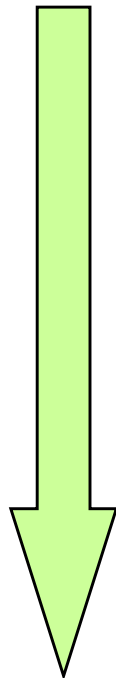
pKa Approximation

Stability

Why we run a purity check before HTLogD_{7.4}



QC Purity Check by HPLC



HTLogD_{7.4} Assay

Indicates which HPLC
pH method to use

Accept over 90% purity

Using QC as a time zero we can
identify if the compound has
degraded over time

QC and HTLogD_{7.4}



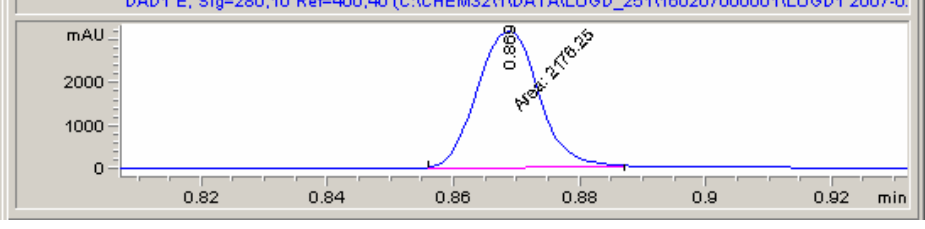
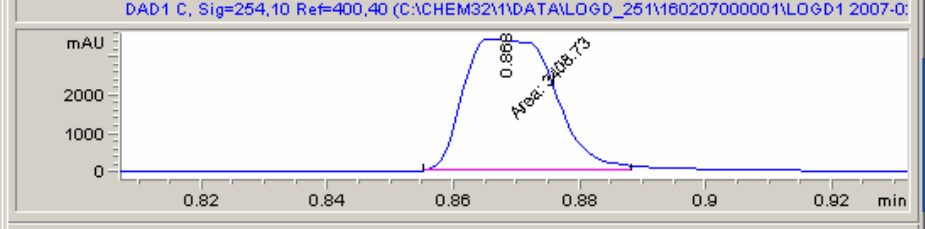
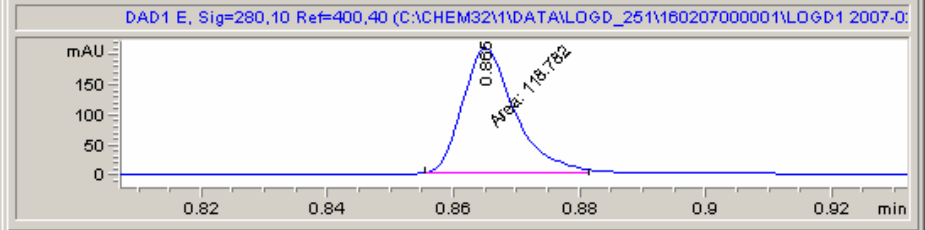
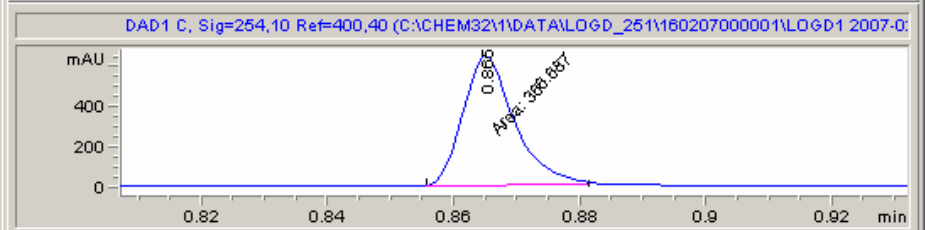
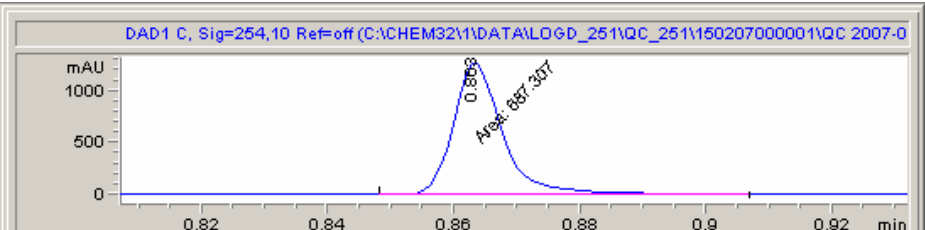
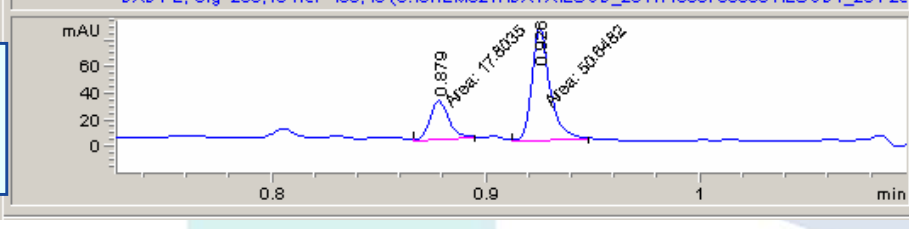
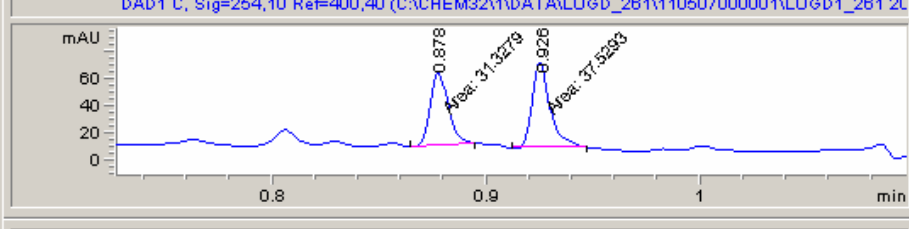
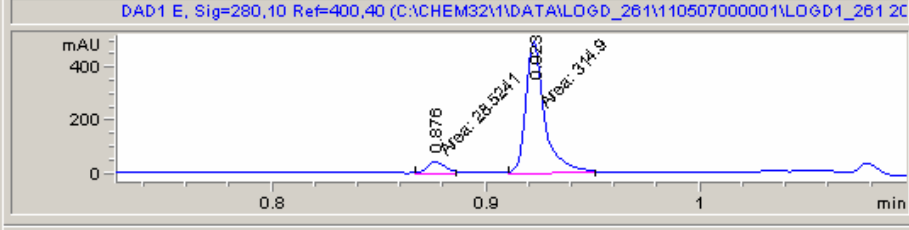
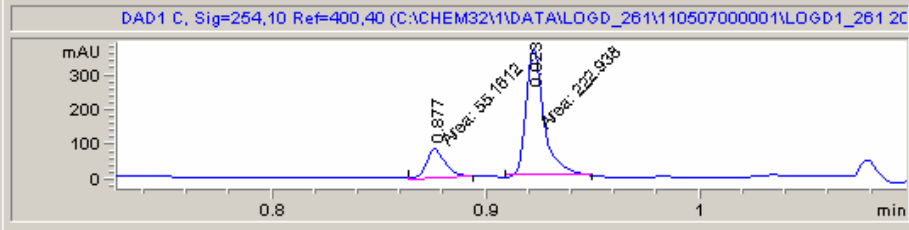
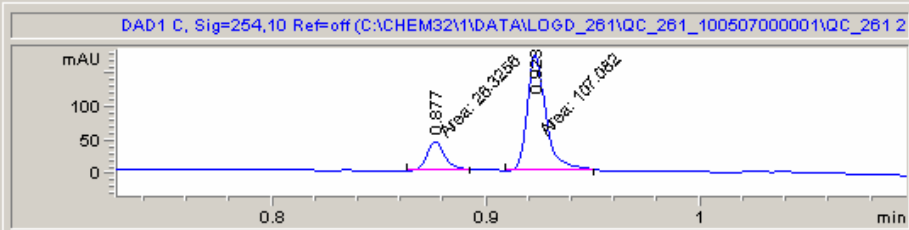
QC
254nm

Octanol
254nm

Octanol
280nm

Aqueous
254nm

Aqueous
280nm



PhysChem Report



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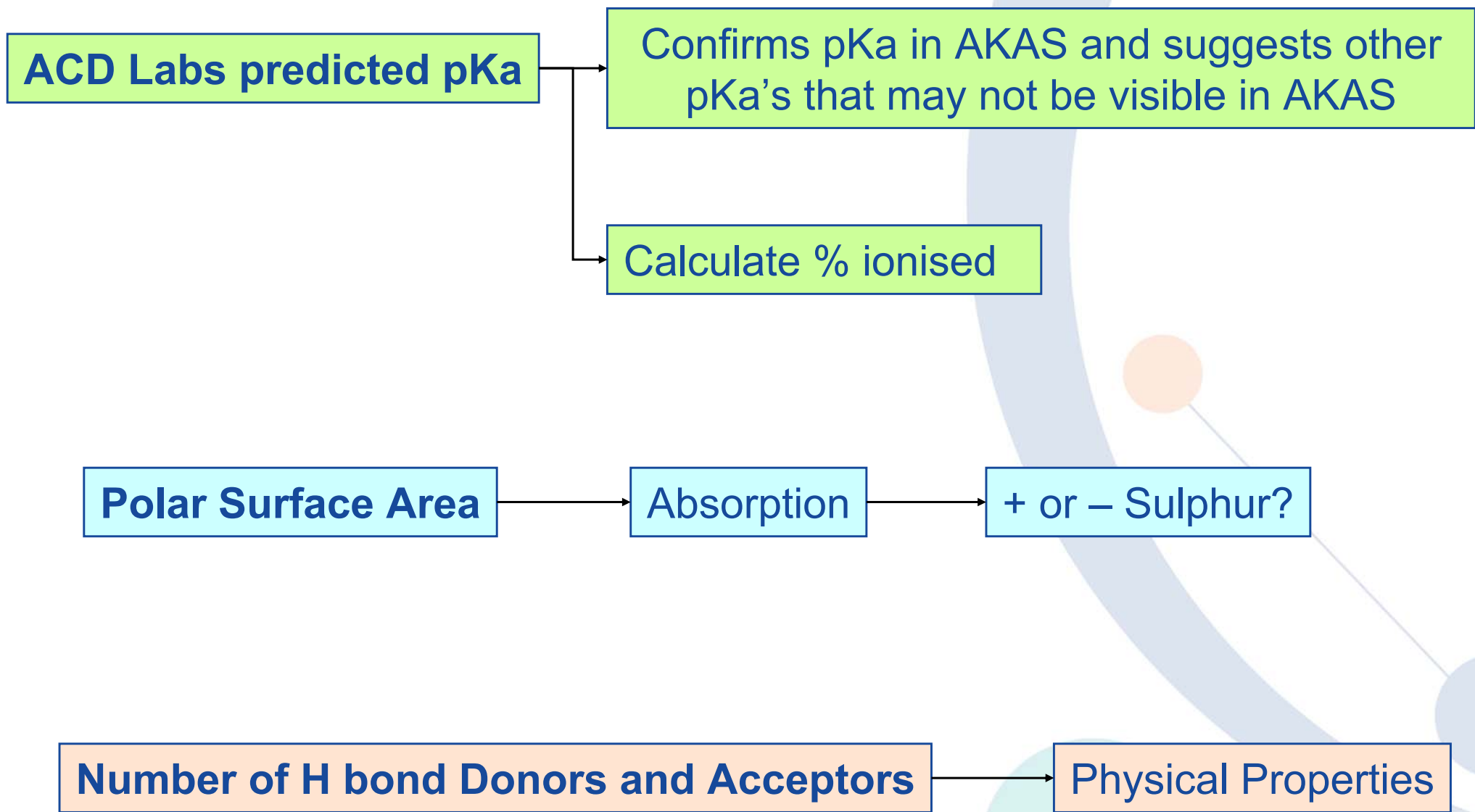
PhysChem Report



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AKAS	ND	<30mM	30-150mM	150-350mM	>350mM
PSA		>113	83-113	<83	

Calculated values



Benefit of Our Report Over Databases



Averaged and concatenated data

Comments not looked at – stability issues highlighted

Difficult to see PhysChem data as a package

ACD labs pKa

Which AKAS results do I use?



AKAS_Solubility µM_pH=3.0_Average	AKAS_Solubility µM_pH=5.0_Average	AKAS_Solubility µM_pH=7.4_Average	AKAS_Solubility µM_pH=9.0_Average	AKAS_Solubility µM_pH=3.0_Concat	AKAS_Solubility µM_pH=5.0_Concat	AKAS_Solubility µM_pH=7.4_Concat	AKAS_Solubility µM_pH=9.0_Concat
AKAS_Solubility µg/ml_pH=3.0_Average	AKAS_Solubility µg/ml_pH=5.0_Average	AKAS_Solubility µg/ml_pH=7.4_Average	AKAS_Solubility µg/ml_pH=9.0_Average	AKAS_Solubility µg/ml_pH=3.0_Concat	AKAS_Solubility µg/ml_pH=5.0_Concat	AKAS_Solubility µg/ml_pH=7.4_Concat	AKAS_Solubility µg/ml_pH=9.0_Concat

Average?

SUBSTANCE_ID	AKAS_Solubility µM_pH=3.0_Average	AKAS_Solubility µM_pH=5.0_Average	AKAS_Solubility µM_pH=7.4_Average	AKAS_Solubility µM_pH=9.0_Average
1	99	92	82	88
2	85	90.67	81.67	75
3	134	95	81	52

or Concatenated?

SUBSTANCE_ID	AKAS_Solubility µM_pH=3.0_Concat	AKAS_Solubility µM_pH=5.0_Concat	AKAS_Solubility µM_pH=7.4_Concat	AKAS_Solubility µM_pH=9.0_Concat
1	99	92	82	88
2	40, 96, 119	83, 85, 104	77, 77, 91	65, 74, 86
3	134	95	81	52

Use concatenated as batches may differ

PhysChem Report



No.	AKAS (uM)				HTLogD	ACDLabs Predicted pKa values			ACDLabs calculated values				Comments	Progress
	pH3	pH5	pH7.4	pH9	pH7.4	pKa 1	% ionised @ pH7.4	pKa 2	PSA+S	PSA-S	HBD	HBA		
1	>350	139	137	119	3.61	5.42	1.0	2.36	141	113	3	9	Basic ionisation event between pH3 and pH5 due to protonation of thiazole	FAIL
2	7	2	1	2	4.50	4.47	0.1		96	42	0	4	Basic ionisation event between pH3 and pH5 due to protonation of pyridine	FAIL
3	>350	>350	>350	>350	3.84	3.81	100.0	3.53	127	99	2	10	Spectral change due to basic ionisation event between pH5 and pH7.4 due to protonation of diaminopyrimidine	PASS
4	>350	>350	162	41	3.94	4.24	0.1	3.53	115	87	1	9	Spectral change due to basic ionisation event between pH5 and pH7.4 due to protonation of diaminopyrimidine	PASS
5	>350	>350	>350	29	3.95	8.86	96.6	3.62	86	58	1	6	Basic ionisation event between pH7.4 and pH9 due to amine	PASS
6	>350	>350	>350	40	4.23	7.39	49.4	6.33	86	58	1	6	Basic ionisation event between pH7.4 and pH9 due to amine	PASS
7	>350	>350	144	>350	3.62	4.17	0.1		104	76	2	7	Increase in solubility at pH9 does not fit with structure - treat data with caution	PASS
8	ND	ND	ND	ND	ND	10.32	99.9	7.37	106	78	1	6	Compound 82% pure, therefore all measured data is unreliable and will not be returned. Any data present in spotfire will be removed.	FAIL

	ND	Poor	Modest	Good	
AKAS	ND	<30mM	30-150mM	150-350mM	>350mM
PSA		>113	83-113	<83	

Summary



The PhysChem Report

Measured Values, AKAS, QSol, HTLogD.

Can approximate pKa from AKAS

Possible to highlight stability issues from AKAS and HTLogD

Calculated Values, pKa, PSA, H-bond Donors/Acceptors

Limitations with Spotfire

Acknowledgements

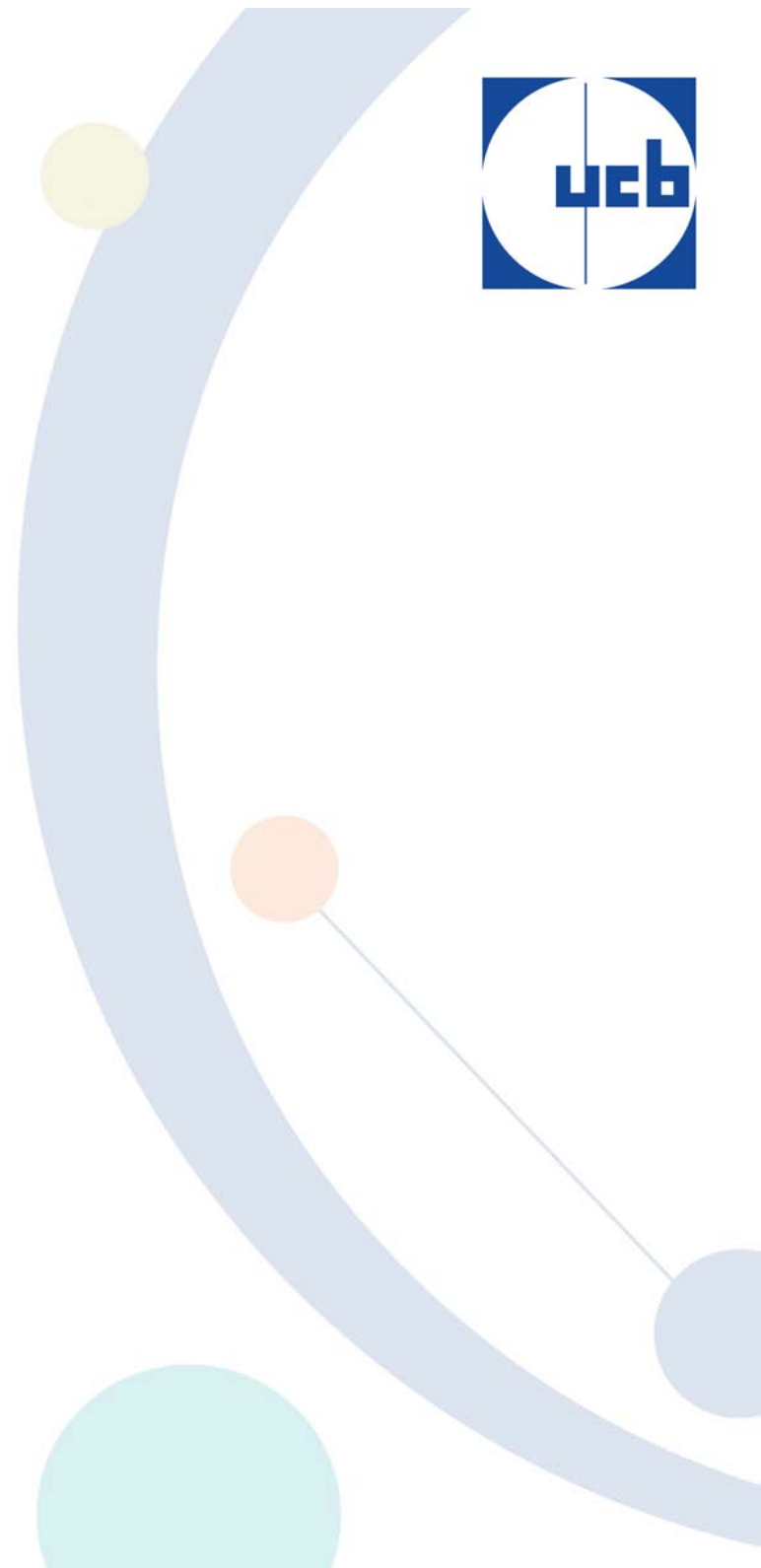


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 - Barbara Mason
 - Christine Prosser
 - Justin Staniforth
 - Femi Kumuyi
- PhysChem, Braine, Belgium
 - Luc Quere
 - Liliane Ellens
 - Benoit Matthew
 - Geraldine Longfils
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 - Benedicte Fau
 - Dave Sherwood
- Informatics, Slough
 - Mike Sanderson
 - Sarah Archibald
 - Dave Lee
 - Phil Ashworth
 - Steve Guise
 - Matt Page

Questions



Questions?



References



Li Di and E H Kerns. *Profiling drug-like properties in discovery research*. Current opinion in Chemical Biology 2003, 7:402-408

Li Di and E H Kerns. *Physicochemical profiling: overview of the screens*. DDT. Vol.1, No.4 2004

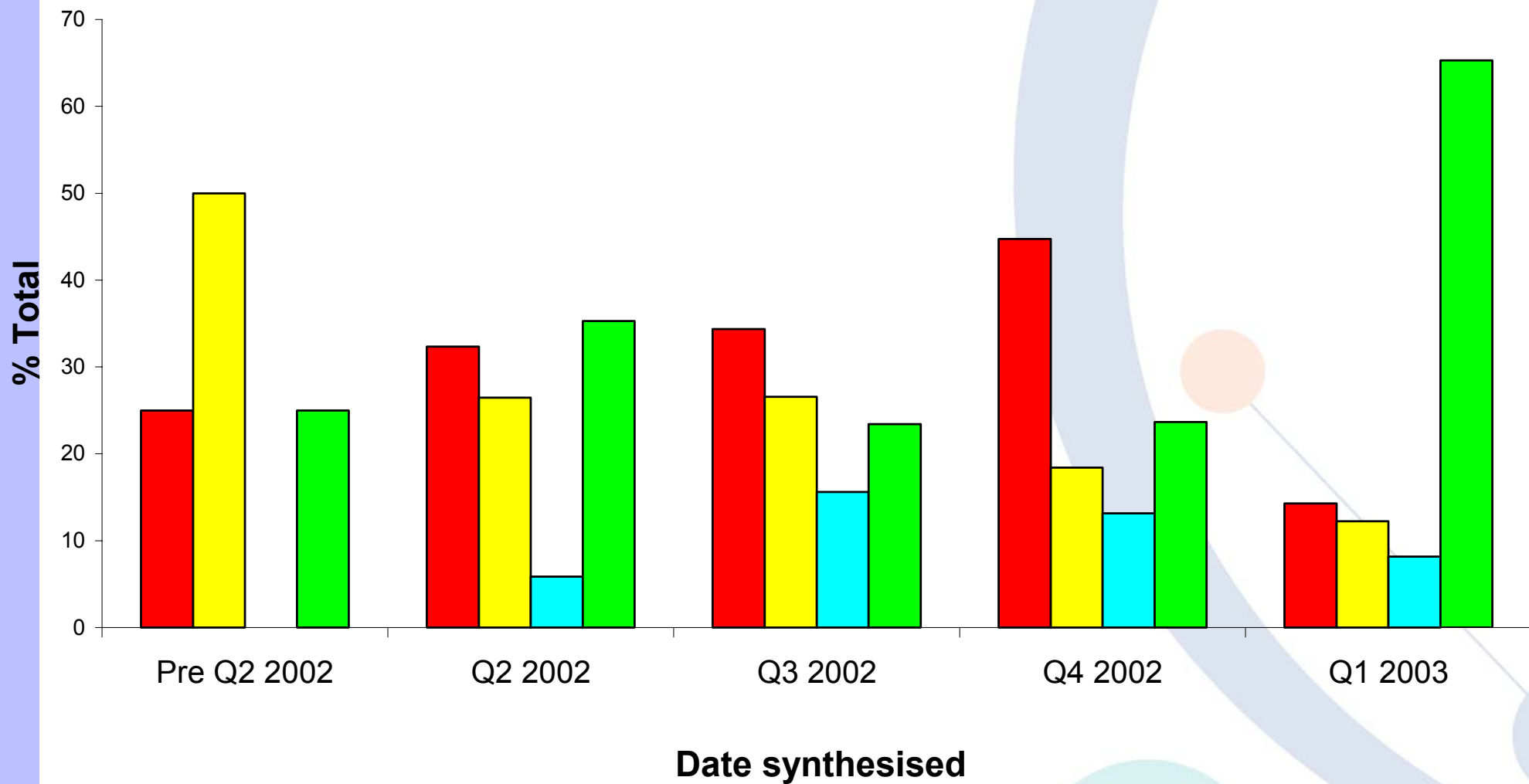
Li Di and E H Kerns. *Pharmaceutical profiling in drug discovery*. Therapeutic focus. DDT Vol.8 No.7 April 2003

T Hartman, J Schmitt. *Lipophilicity-beyond octanol/water: a short comparison of modern technologies*. DDT Vol.1 No.4 2004

Alex Avdeef. *Absorption and Drug Development Solubility, Permeability and Charge State*. pION, Inc. John Wiley & Sons 2003

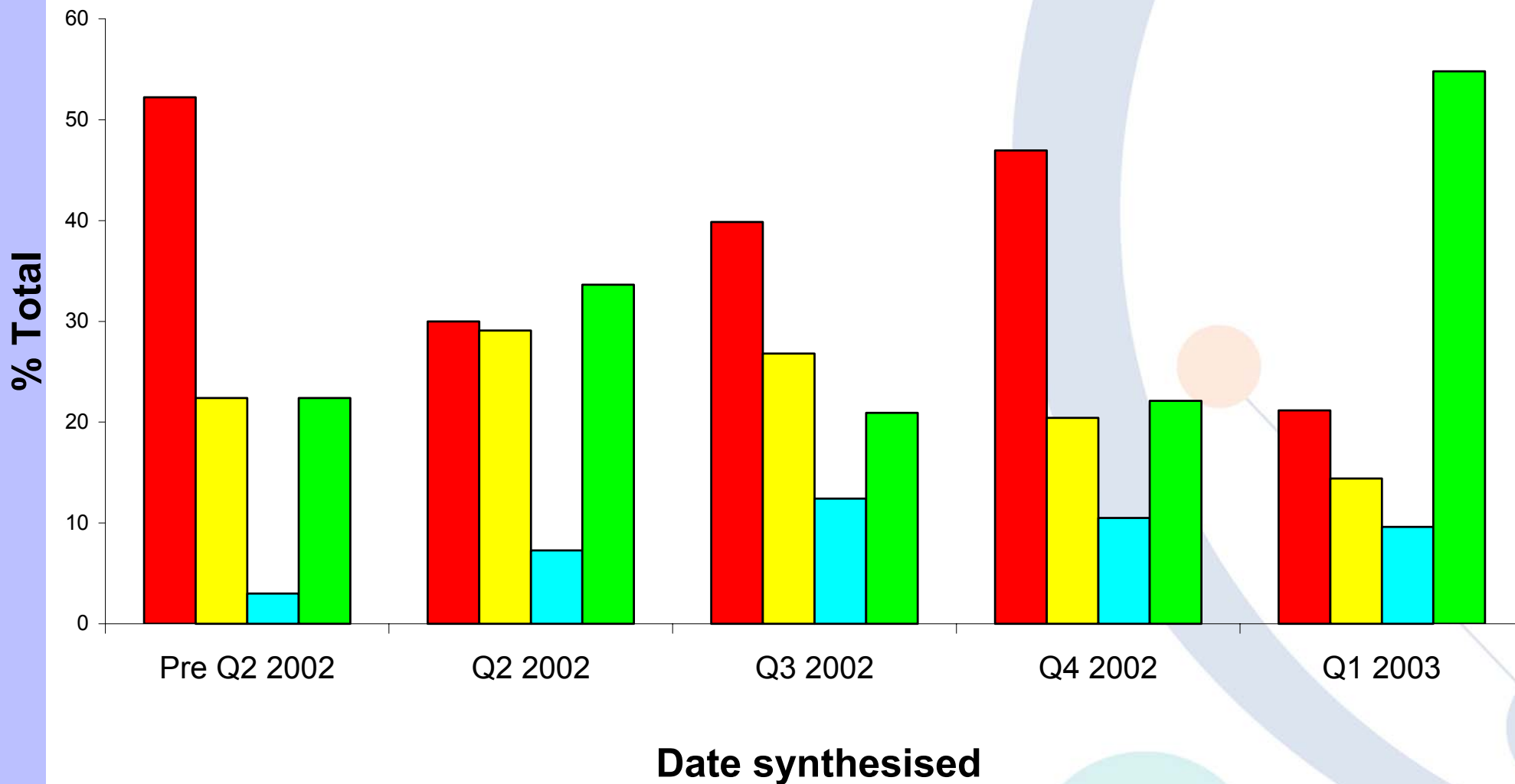


AKAS solubility of naphthalene/quinoline series (vs time)

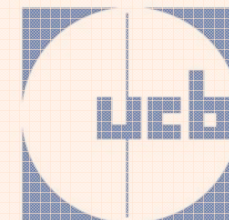




AKAS solubility of P38 compounds (vs time)



pKa Estimation With AKAS



Tamoxifen

Basic ionisation event between
pH5 and pH7.4

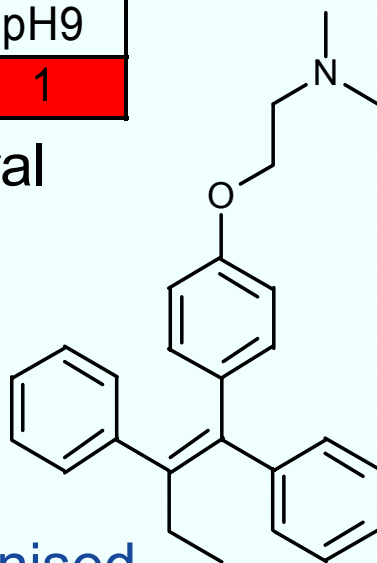
pH3	pH5	pH7.4	pH9
>350	>350	16	1

Ionised

pKa

Neutral

Base



Soluble at low pH as ionised.

Fluorescein

Acidic ionisation event between
pH3 and pH5

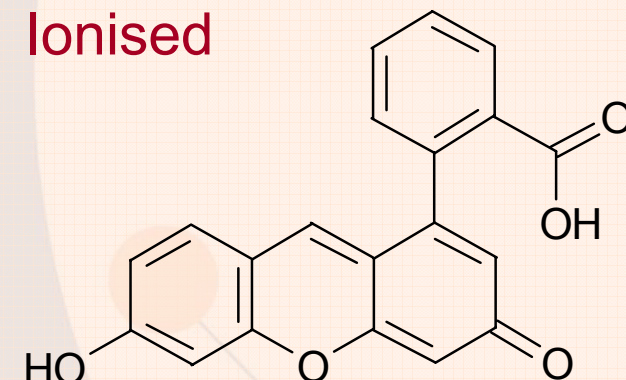
pH3	pH5	pH7.4	pH9
113	>350	>350	>350

Neutral

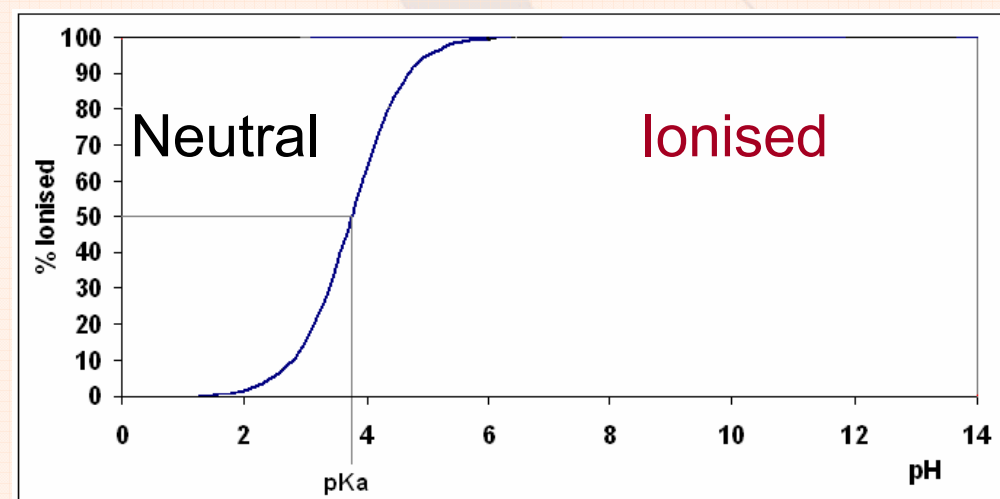
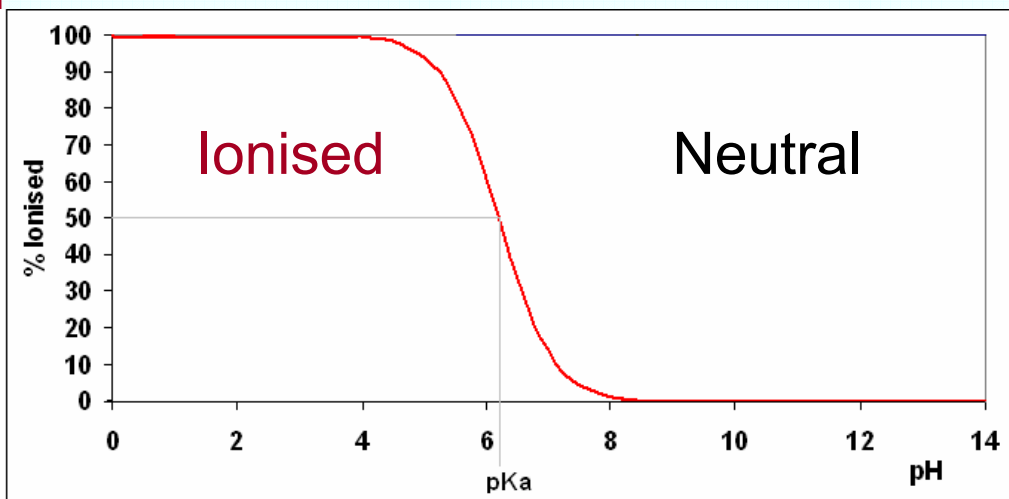
pKa

Ionised

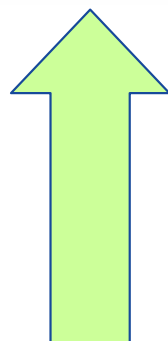
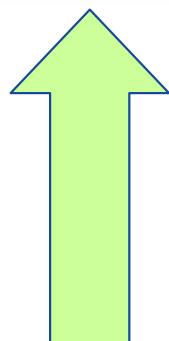
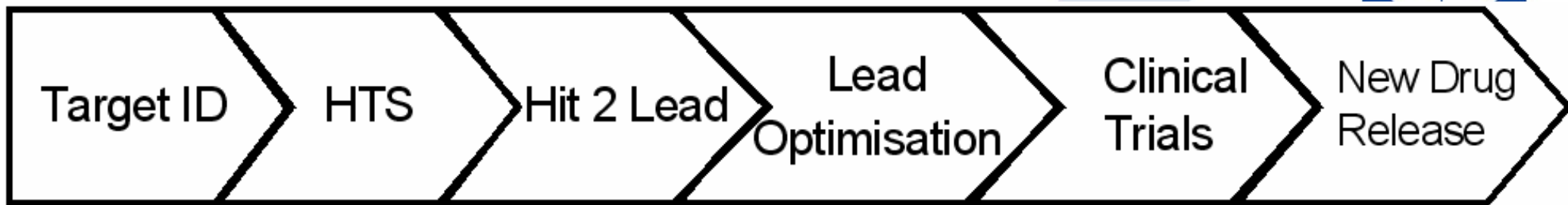
Acid



Soluble at high pH as ionised



Where Discovery PhysChem Fits in UCB

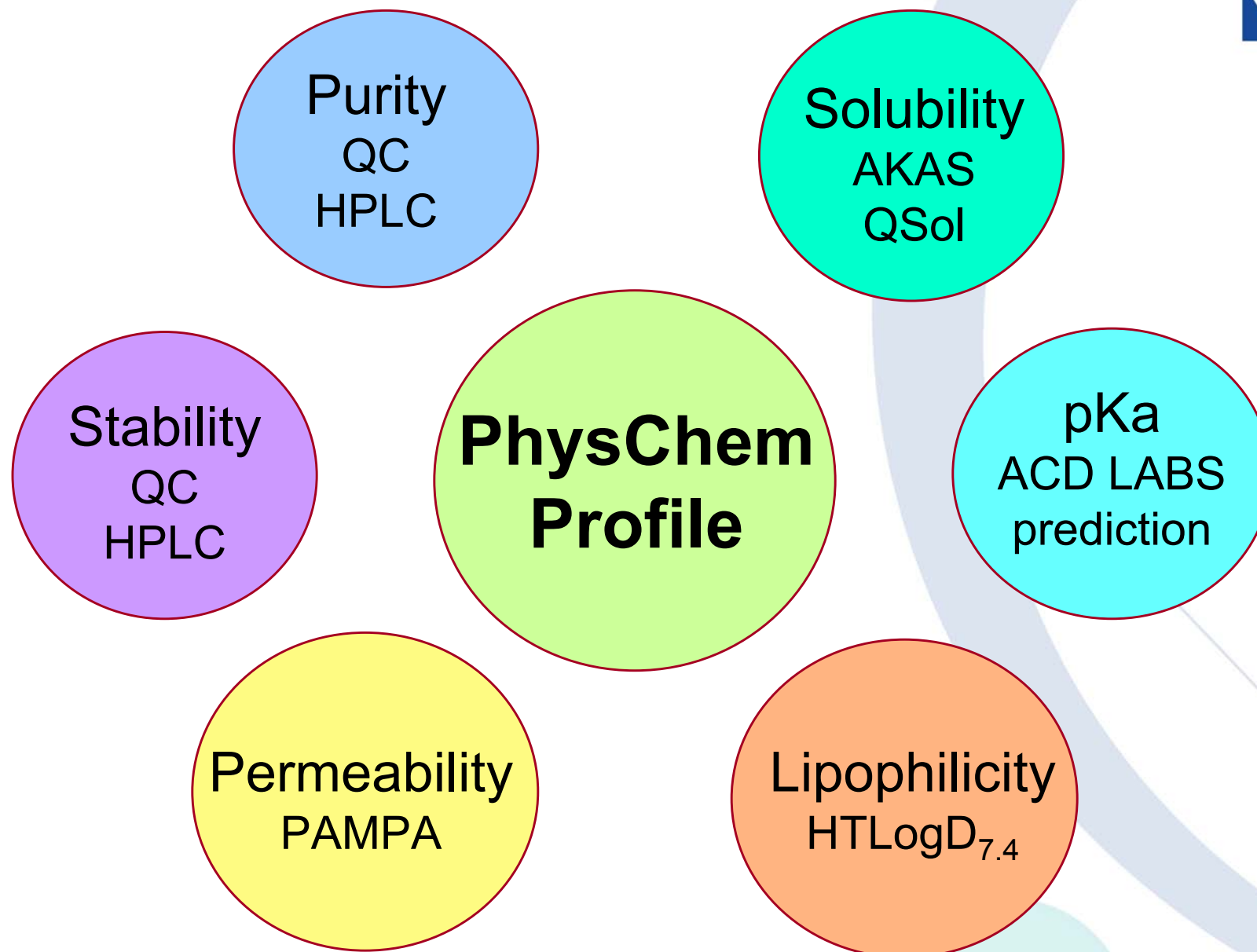


Discovery PhysChem
Early stage Solubility and
Permeability data

Later stage research
supported by colleagues
in Cambridge and Braine.

To avoid late stage PhysChem problems screen early in discovery

PhysChem Profile



Purity
QC
HPLC

Solubility
AKAS
QSol

Stability
QC
HPLC

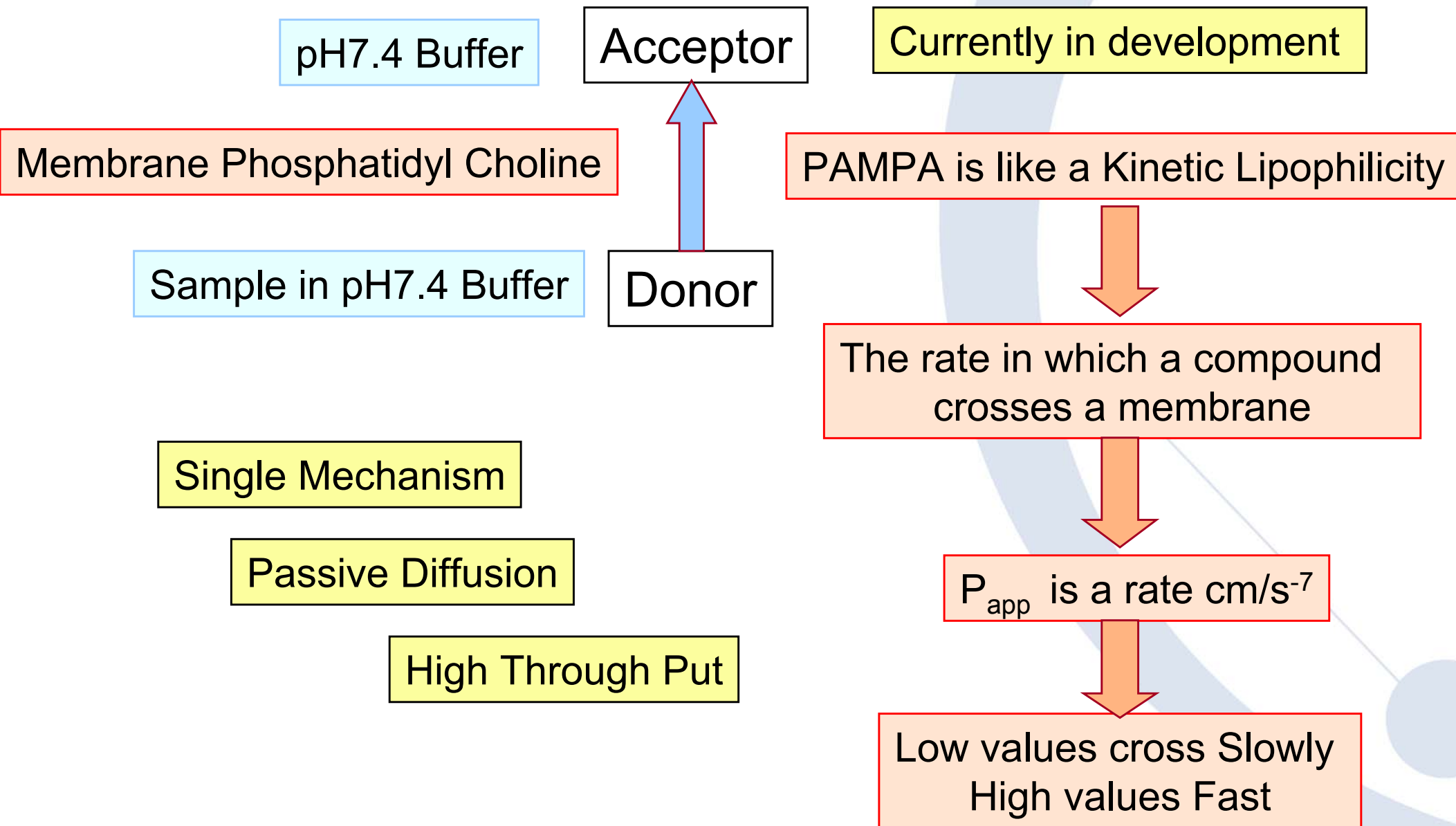
**PhysChem
Profile**

pKa
ACD LABS
prediction

Permeability
PAMPA

Lipophilicity
HTLogD_{7.4}

PAMPA in development



Cascade



10 mM DMSO Stock

QC
(all NCEs)

Discovery PhysChem (Slh)

PhysChem (Braine + Cam)

DMPK (Slh)

Solubility

AKAS
(all NCEs)

QSol
(request)

SnapSol
(request)

BioSol
(all NCEs)

TSol
(request)

Lipophilicity

HT Log D_{7.4}
(all NCEs)

pKa
(request)

Log D
(request)

Log P
(request)

Permeability

GI PAMPA
(all NCEs)

BIO-PAMPA
(all NCEs)

BBB PAMPA
(all NCEs)

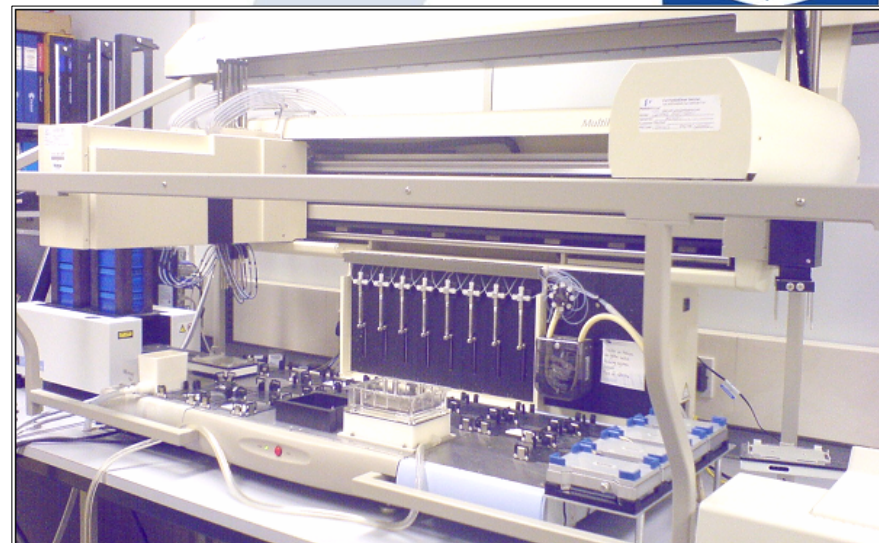
Caco-2
(request)

Solubility Assays – SnapSol – BioSol



SnapSol
(request)

- **SnapSol** – kinetic – 1pH
- Can run 190 compounds in 2hrs
- Data processing is very fast
- Compound detected by UV plate reader



HT MultiPROBE II

BioSol
(all NCEs)

- **BioSol** – Kinetic – at the conditions used in the biological primary assays.



Compound ID	AKAS (uM)				HTLogD	ACDLabs Predicted pKa Values				ACDLabs Calculated Values				Comments	QSol Progress
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6	>350	>350	>350	>350	2.51	5.35	0.9			90	62	2	5		PASS
7	>350	>350	>350	>350	4.20	6.55	12.4	5.72		121	92	2	8	Spectral change due to basic ionisation event between pH5 and pH7.4 due to protonation of pyridiazine	PASS
8	>350	>350	>350	40	4.23	7.39	49.4	6.33		86	58	1	6	Basic ionisation event between pH7.4 and pH9 due to amine	PASS
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