Ionisation Prediction Summit Webinar Series

Flavonoids and Some Examples of Challenged pK_a determination

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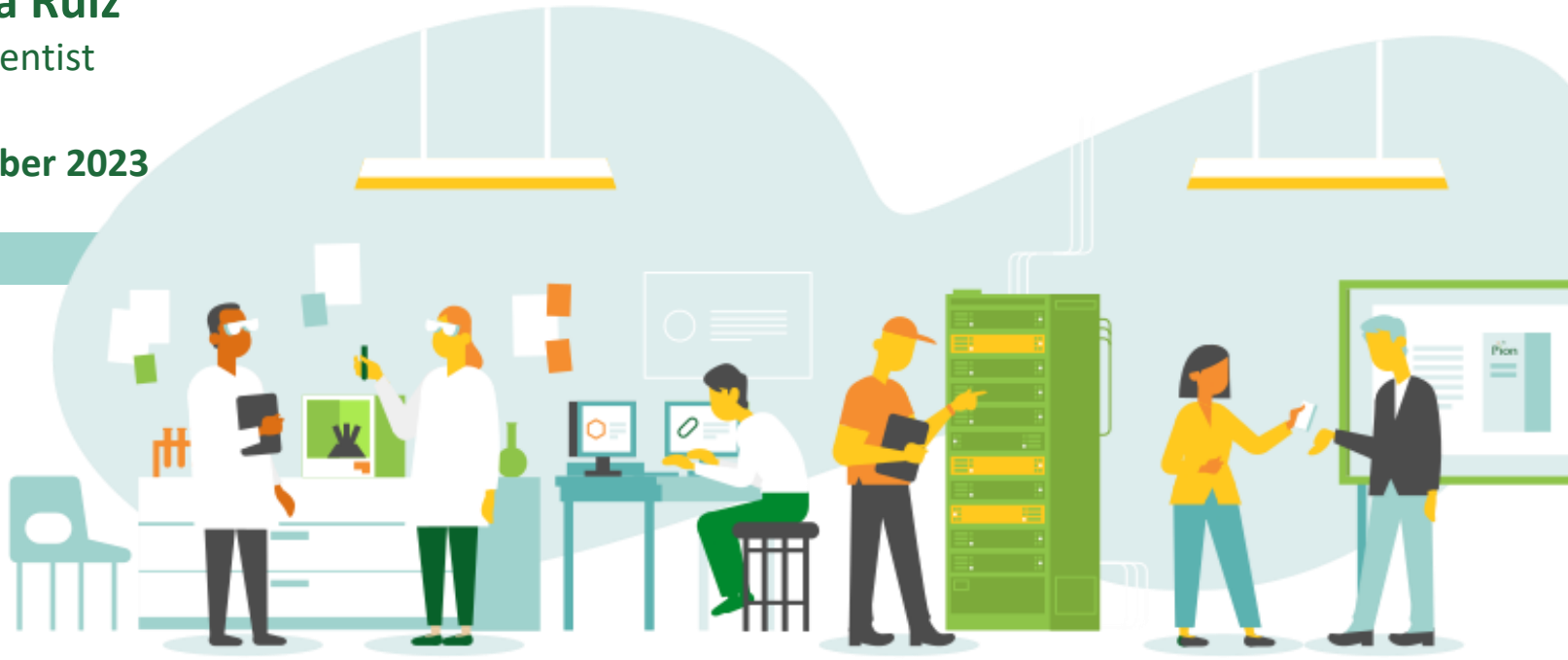
14th November 2023



Pion

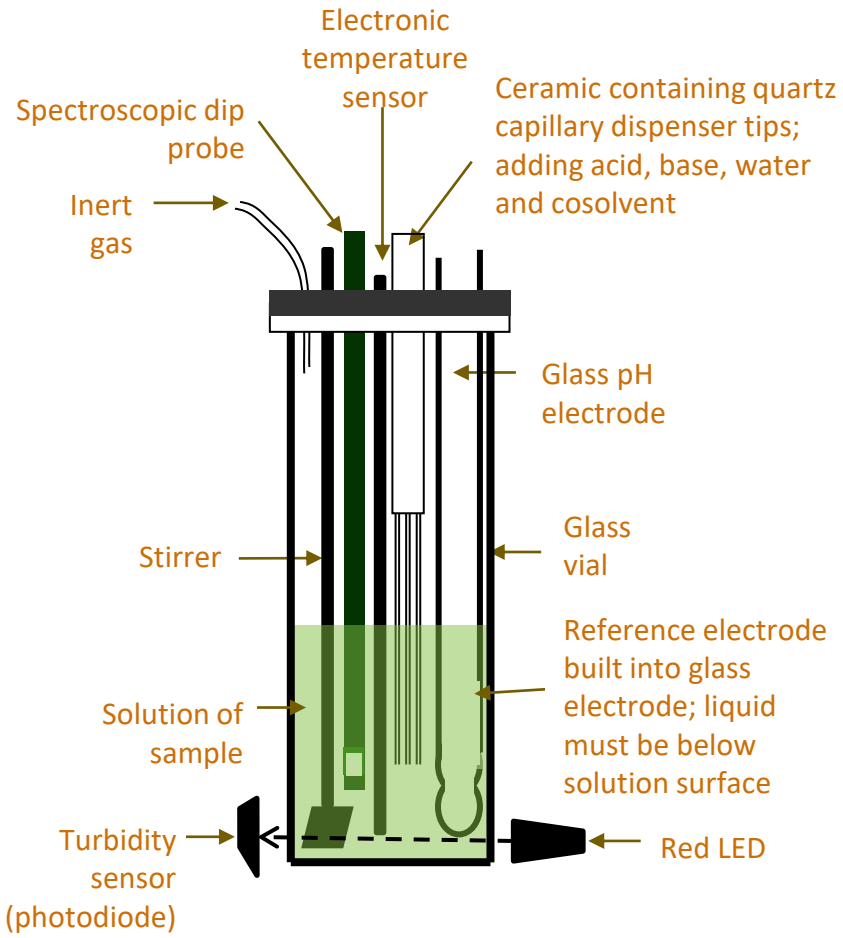
Pion SiriusT3

Pion SiriusT3

SiriusT3 Probes

Typical Experimental Conditions:

- 1.5 mL ISA-water/cosolvent 0.15M (ionic strength adjusted KCl)
- pH between 2 and 12 (UV-metric between 1.5 and 12.5)
- Starting from the pH where sample is ionised
- Temperature controlled at 25/37°C (from 20°C to 50°C)
- Under argon atmosphere
- Standardised solutions 0.5M KOH and 0.5M HCl as titrants
- Spectrometric (UV-active drugs) and Potentiometric (non UV-active drugs) Determination
- Triple Titrations



T3 Measurement cell

Pion

pK_a – Techniques: Spectrometric

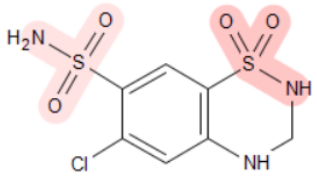
Fast UV (Screening)

- Fast method for pK_a(s) between pH 2 and 12
- 6 min/titration (20 min triple assay)
- Saved Reference Spectrum

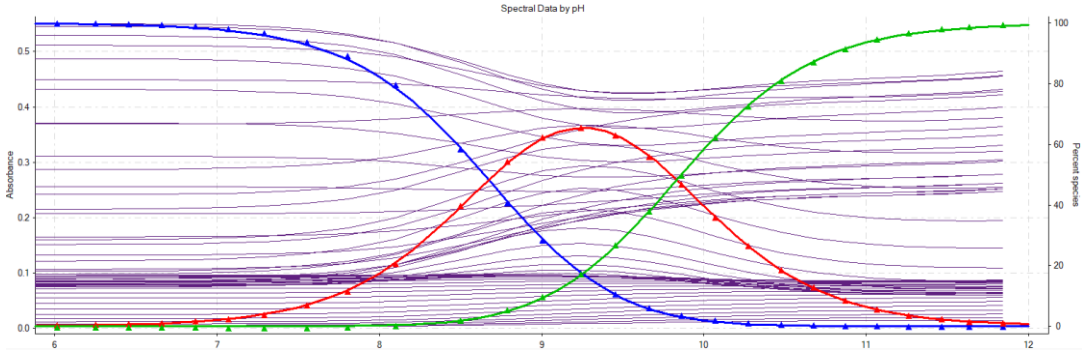
UV-metric

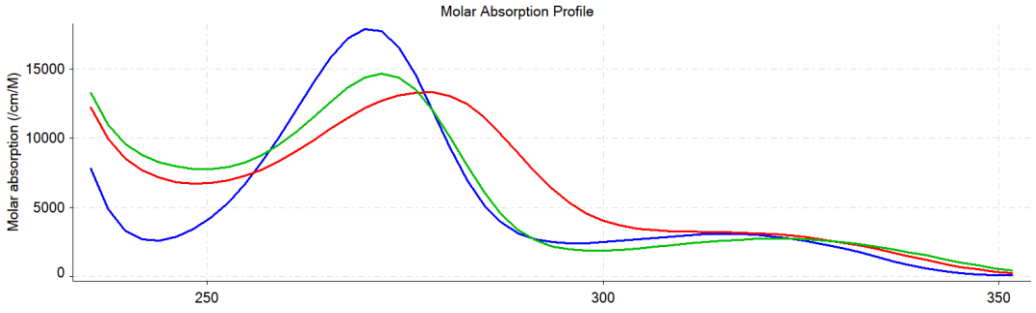
- Measures pK_a(s) between pH 1.5 and 12.5
- 20 min/titration (1 h triple titration)
- Fresh Reference Spectrum

- 0.02 mg of sample (5uL of 10mM DMSO stock solution)
- **Requires a change in UV with ionisation state**
- Aqueous or co-solvent media ; methanol, MDM, dioxane, etc.



Hydrochlorothiazide
pK_as; 8.75, 9.88





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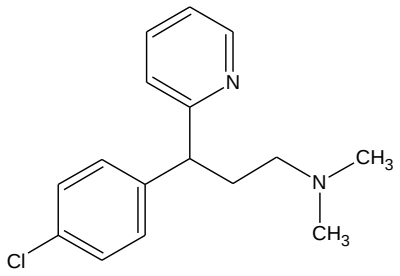
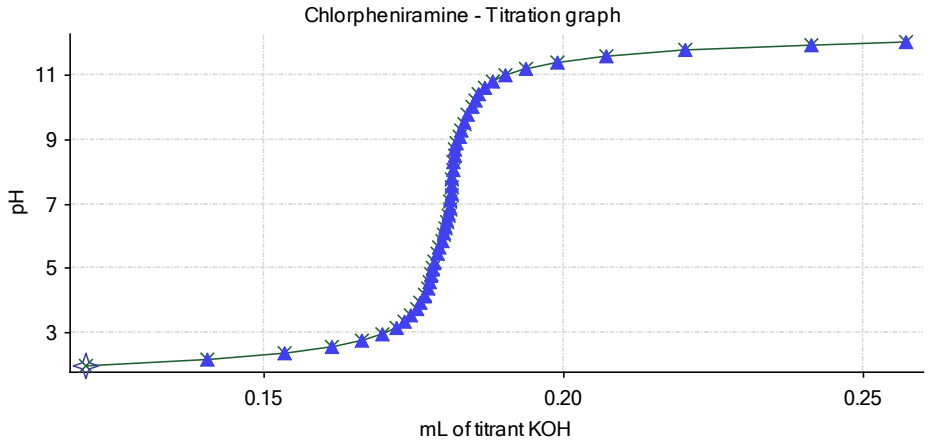
pK_a – Techniques: Potentiometric

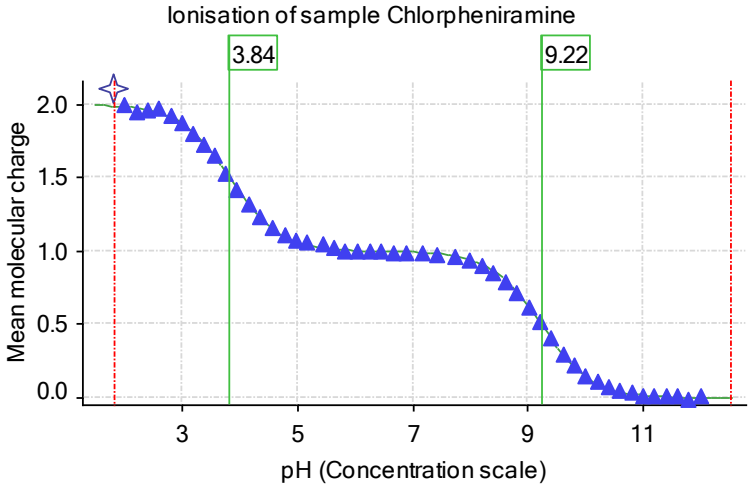
pH-metric

- Measures pK_a(s) between pH 2.0 and 12.0
- Typically, 0.5 - 1 mg of sample
- ~20 min/titration (~ 1h)
- **Does not require UV absorbance**
- Aqueous or co-solvent media for poorly soluble samples; range of solvents available: methanol, MDM, dioxane, etc.



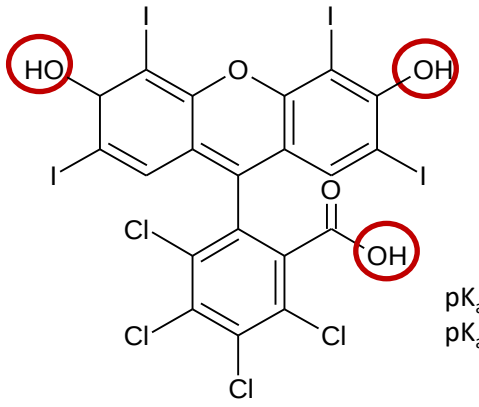
Chlorpheniramine





Rose Bengal

$pK_{a\ ACD}$ = 10.4
$pK_{a\ Galas}$ = 13.8



$pK_{a\ ACD}$ = 5.7
$pK_{a\ Galas}$ = 6.8

$pK_{a\ ACD}$ = 1.3
$pK_{a\ Galas}$ = 2.1

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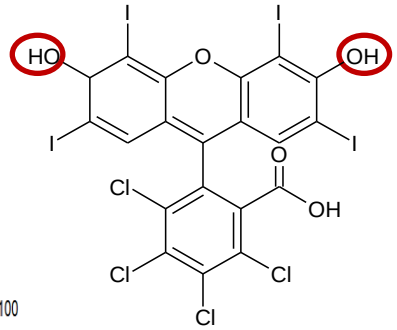
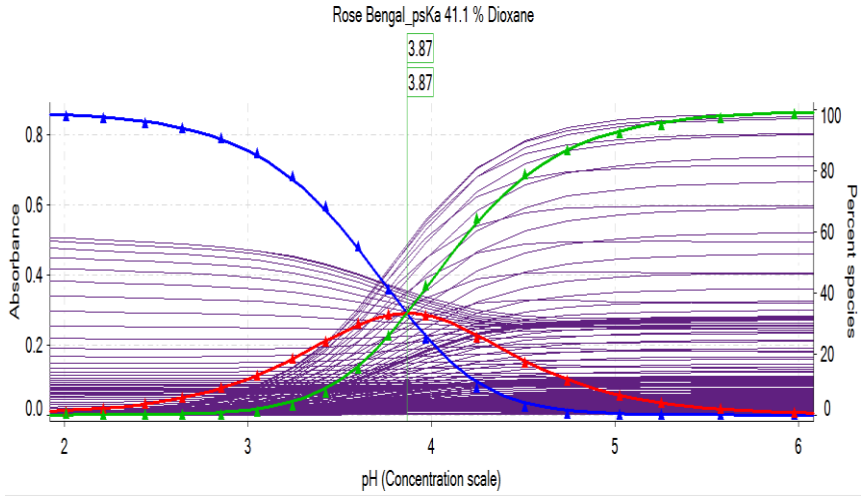
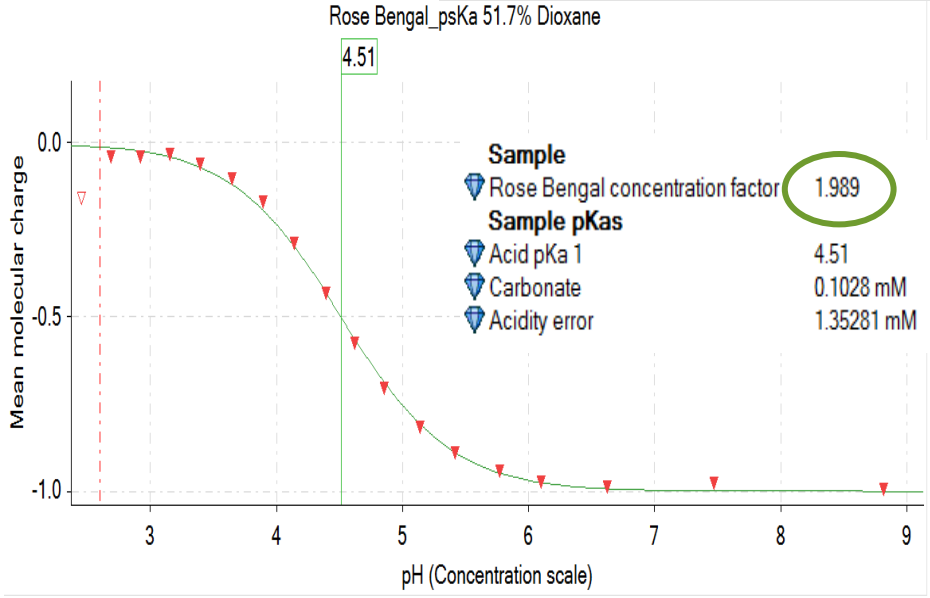
Rose Bengal – pK_a determination



UV-metric technique - pK_a

1.5 mL total volume
41.1% dioxane-water
Ionic strength 0.15M KCl
25°C
p_sK_a = 3.87





pH-metric technique - pK_a

1.5 mL total volume
51.7% dioxane-water
Ionic strength 0.15M KCl
25°C
p_sK_a = 4.51

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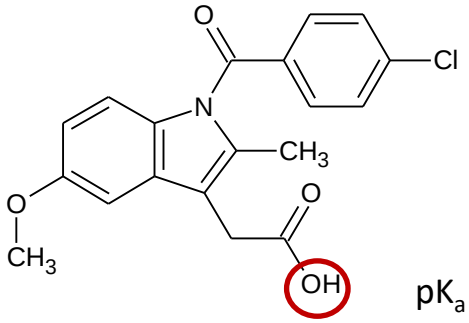
Indomethacin



$pK_{a\ ACD}$ = 4.0
$pK_{a\ Galas}$ = 4.5

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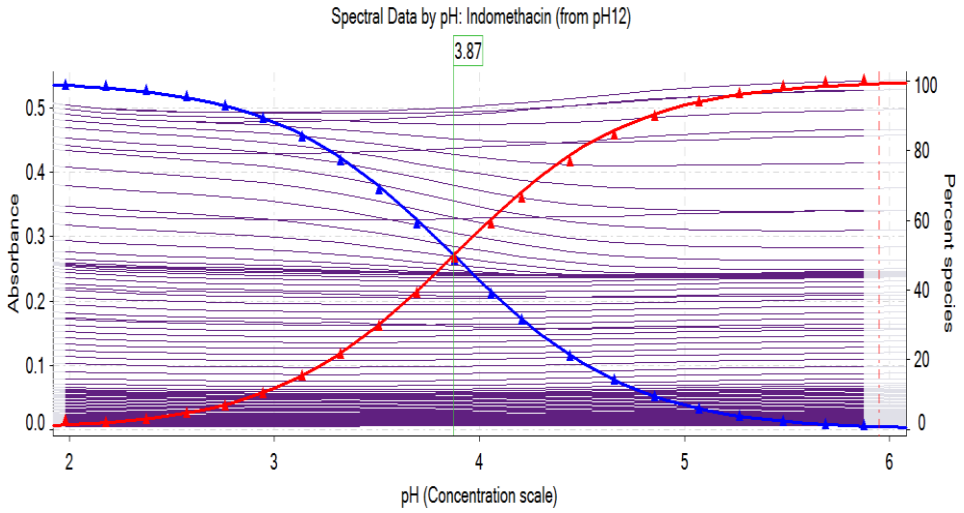
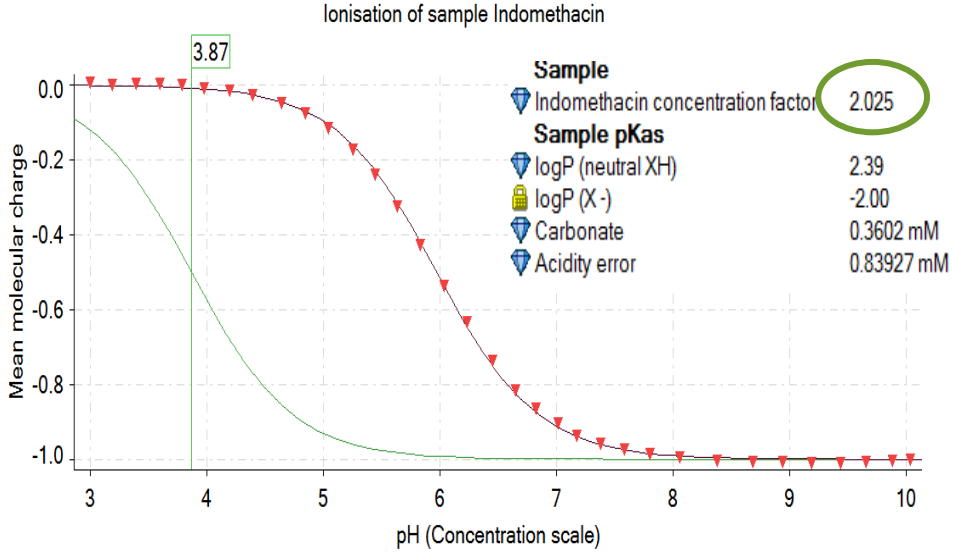
Indomethacin – pK_a and logP determination



UV-metric technique - pK_a

1.5 mL total volume
Aqueous media
pH 12 → 2
Ionic strength 0.15M KCl
25°C
pK_a = 3.87



pH-metric technique - logP

1.5 mL total volume
Water/octanol
Octanol sat with water at
Ionic strength 0.15M KCl
pH 12 → 2
25°C
pK_a = 3.87

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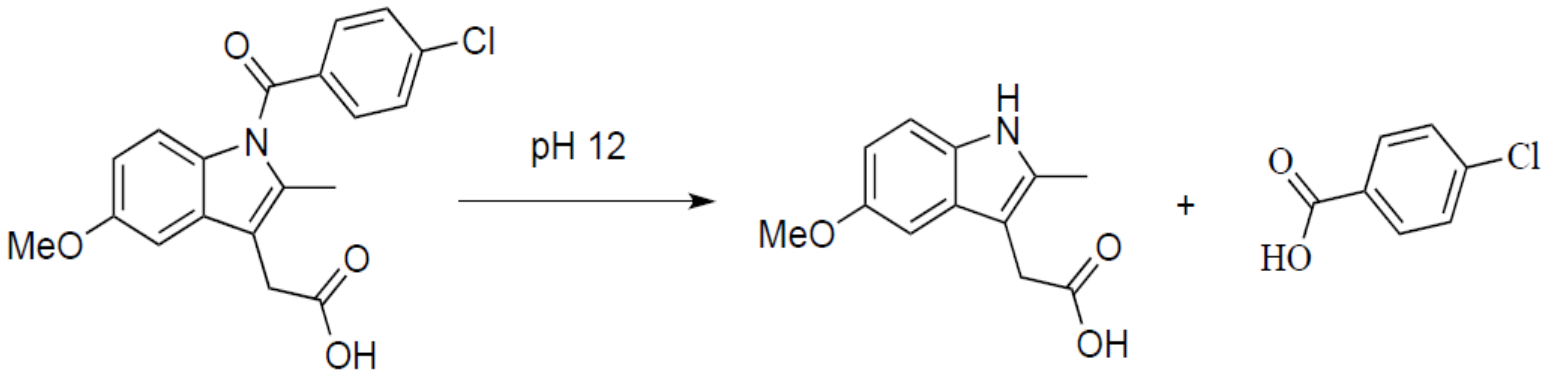
Indomethacin– Decomposition



Indomethacin

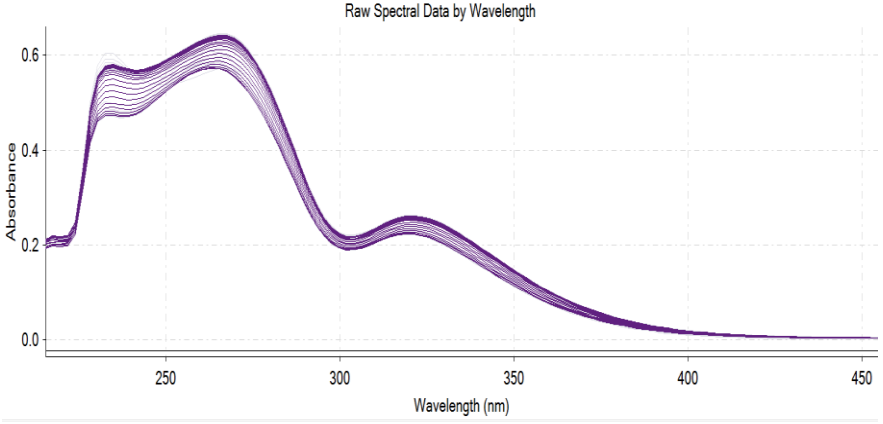
pK_a = 4.01

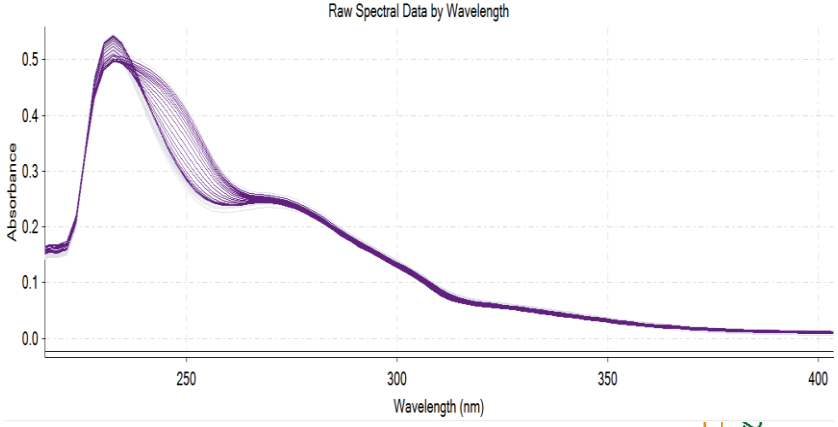
5-methoxy-2-methyl-3-indoleacetic acid

pK_a = 4.42

p-chlorobenzoic acid

pK_a = 3.75





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Indomethacin– Decomposition

The intrinsic aqueous solubility of indomethacin

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[2]*Unilever Centre for Molecular Informatics, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK*
[3]*School of Pharmacy & Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK*

Abstract

A value of 8.8 µg/mL was measured for the intrinsic solubility of indomethacin. Evidence of a form with a solubility of about 77 µg/mL was also obtained. Solubility measurements were conducted using the CheqSol and Curve Fitting methods using a maximum pH of 9. It is also demonstrated that a published intrinsic solubility of 410 µg/mL was in error due to decomposition of indomethacin at pH 12. The decomposition of indomethacin at pH 12 was investigated. Decomposition products comprising p-chlorobenzoic acid and 5-Methoxy-2-methyl-3-indoleacetic acid were isolated and characterised.

Keywords: Indomethacin, solubility, CheqSol, *p*-chlorobenzoic acid, decomposition

ADMET & DMPK ***2(1)*** *(2014) 18-32; doi:* ***10.5599/admet.2.1.33***

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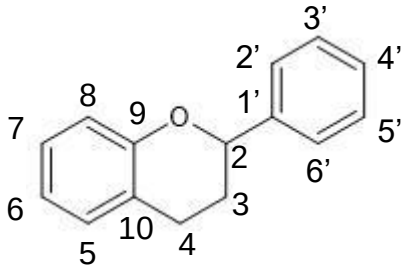
Flavonoids



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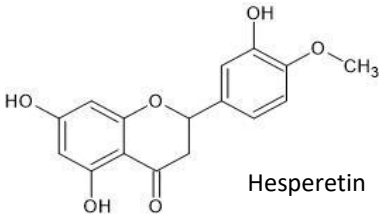
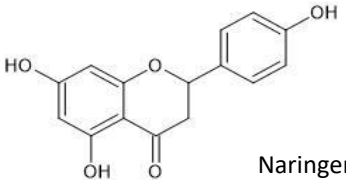
Flavonoids - Structures

Flavanones



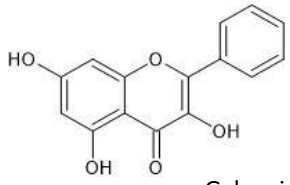
Hesperetin



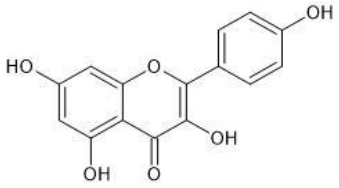
Naringenin

Polyphenolic compounds, which are starting to emerge as a potential new class of drugs due to their extent of pharmacological activity. They play multiple roles in biological processes, especially related to their antioxidant efficiency.

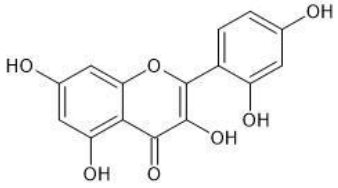
Flavonols



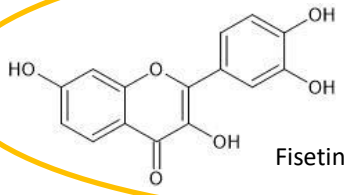
Galangin



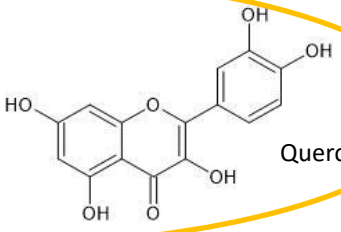
Kaempferol



Morin

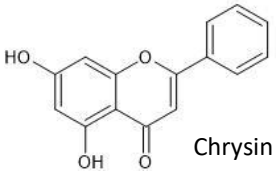


Fisetin

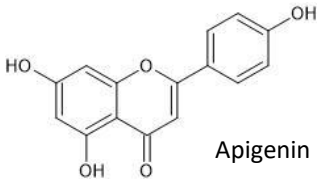


Quercetin

Flavones



Chrysin



Apigenin

E. Fuget, C. Rafols, M. Mañe, R. Ruiz, E. Bosch. Talanta 253 (2023) 124096

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Flavonoids – Literature values and Predictions

	$pK_{a,1}$	$pK_{a,2}$	$pK_{a,3}$	$pK_{a,4}$	$pK_{a,5}$	Exp. or Calc. Approach
Flavanones						
Hesperetin	6.67	8.76	11.54	-	-	Spectrophotometry
	7.12	*9.19*	*10.28*	-	-	**SPARC*
	7.5	*10.0*	*11.5*	-	-	**Percepta*
Naringerin	6.7	9.1	13.05	-	-	Spectrophotometry
	7.11	*9.76*	*10.52*	-	-	**SPARC*
	7.6	*10.2*	*11.4*	-	-	**Percepta*
Flavones						
Apigenin	7.86	-	-	-	-	Spectrophotometry
	6.90	*8.26*	*10.63*	-	-	**SPARC*
	8.2	*9.2*	*13.1*	-	-	**Percepta*
Crysin	7.9	11.40	-	-	-	Spectrophotometry
	8.37	12.37	-	-	-	Potentiometry
	8.0	11.9	-	-	-	Spectrophotometry
	6.90	*10.51*	-	-	-	**SPARC*
	8.2	*12.9*	-	-	-	**Percepta*
Flavonols						
Galangin	6.8	9.4	-	-	-	Spectrophotometry
	7.6	9.5	-	-	-	spectrophotometric Titrations
	6.9	*9.7*	*10.9*	-	-	**SPARC*
	8.1	*10.3*	*13.6*	-	-	**Percepta*
Kaempferol	8.2	9.5	-	-	-	Spectrophotometry
	7.05	9.04	11.04	-	-	Capillary Zone Electrophoresis
	7.89	-	-	-	-	Spectrophotometry
	7.49	9.12	10.90	11.69	-	Spectrophotometry
	6.98	*8.84*	*10.19*	*11.63*		**SPARC*
	7.8	*8.6*	*11.4*	*13.8*	-	**Percepta*
Morin	3.46	8.1	-	-	-	Spectrophotometry
	5.06	8.64	10.62	-	-	Capillary Zone Electrophoresis
	5.18	8.11	10.03	11.45	12.94	Spectrophotometry
	4.99	8.29	10.33	-	-	Potentiometry
	4.99	8.23	10.34	-	-	Potentiometry
	6.93	*8.81*	*9.95*	*11.02*	*12.43*	**SPARC*
	7.4	*8.3*	*9.1*	*12.5*	*14.2*	**Percepta*
Fisetin	7.36	9.71	-	-	-	Capillary Zone Electrophoresis
	7.57	9.34	-	-		Potentiometry
	7.70	9.54	-	-		Potentiometry
	-	8.87	10.31	13.2	-	Spectrophotometry
	7.31	*8.27*	*11.11*	*13.23*	-	**SPARC*
	7.8	*8.7*	*11.6*	*13.9*	-	**Percepta*
Quercetin	7.3	8.4	-	-	-	Spectrophotometry
	6.62	9.7	-	-	-	Potentiometry
	7.19	9.36	11.56	-	-	Capillary Zone Electrophoresis
	6.74	9.02	11.55	-	-	Spectrophotometry
	7.71	9.44	11.46	-	-	Potentiometry
	7.59	9.33	11.56	-	-	Potentiometry
	7.76	-	-	-	-	Spectrophotometry
	6.41	7.81	10.19	11.53	12.91	Spectrophotometry
	6.95	8.21	10.11	11.71	13.33	**SPARC*
	7.7	*8.5*	*10.9*	*13.5*	*14.3*	**Percepta*

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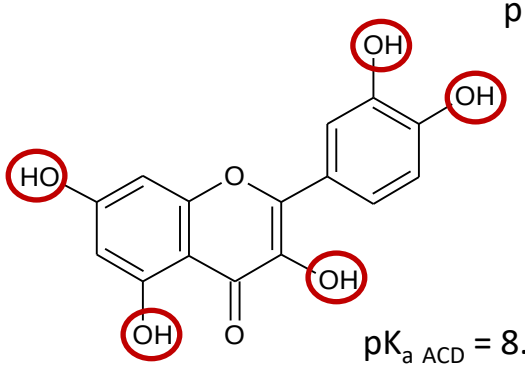
Quercetin



$pK_{a\ ACD} = 9.9$
$pK_{a\ Galas} = 14.3$

$pK_{a\ ACD} = 13.0$
$pK_{a\ Galas} = 7.7$

$pK_{a\ ACD} = 7.5$
$pK_{a\ Galas} = 8.5$

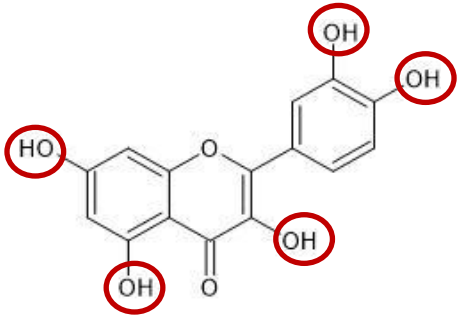
$pK_{a\ ACD} = 8.7$
$pK_{a\ Galas} = 10.9$

$pK_{a\ ACD} = 6.3$
$pK_{a\ Galas} = 13.5$

Pion

Spectrometric Technique – UV-metric:

Experimental Data

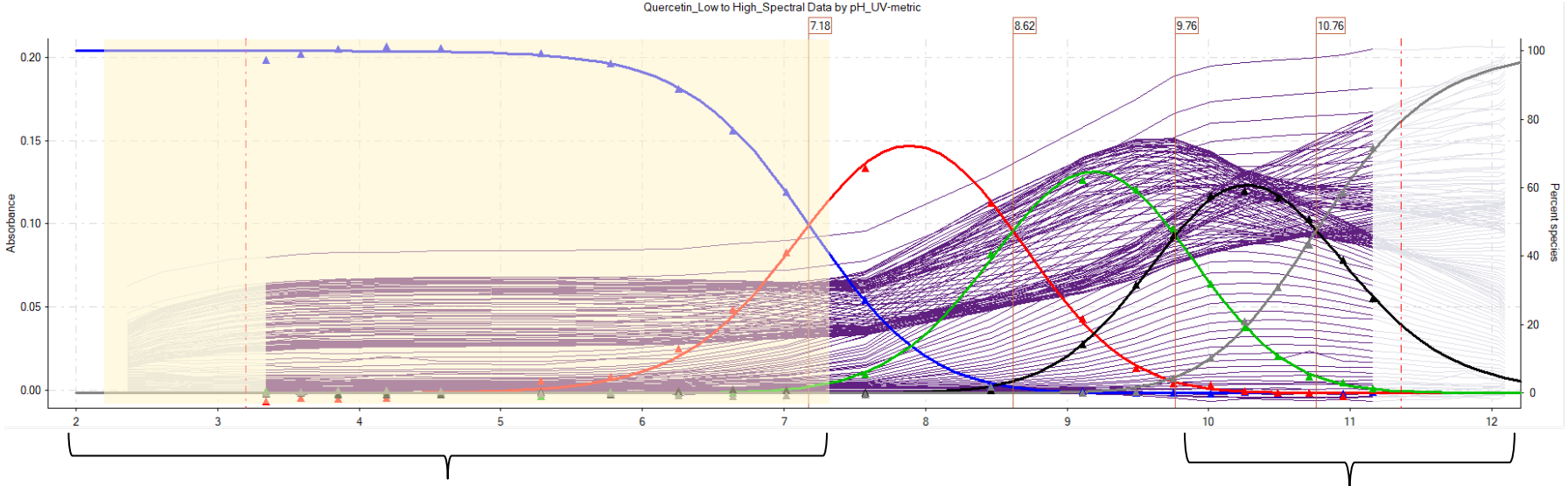


Quercetin

UV- metric Experimental conditions (20min/titration)

- Reference: 5µL DMSO and 25µL Phosphate buffer (15mM)
- Sample: 5µL (10mM DMSO stock) and 25µL Phosphate buffer (15mM)
- Aqueous media (I = 0.15 M KCl) at 25°C under Argon conditions
- Direction of the Titration from pH 2 to pH 12
- Titrants: HCl 0.5M and KOH 0.5M

pH 2 → pH 12



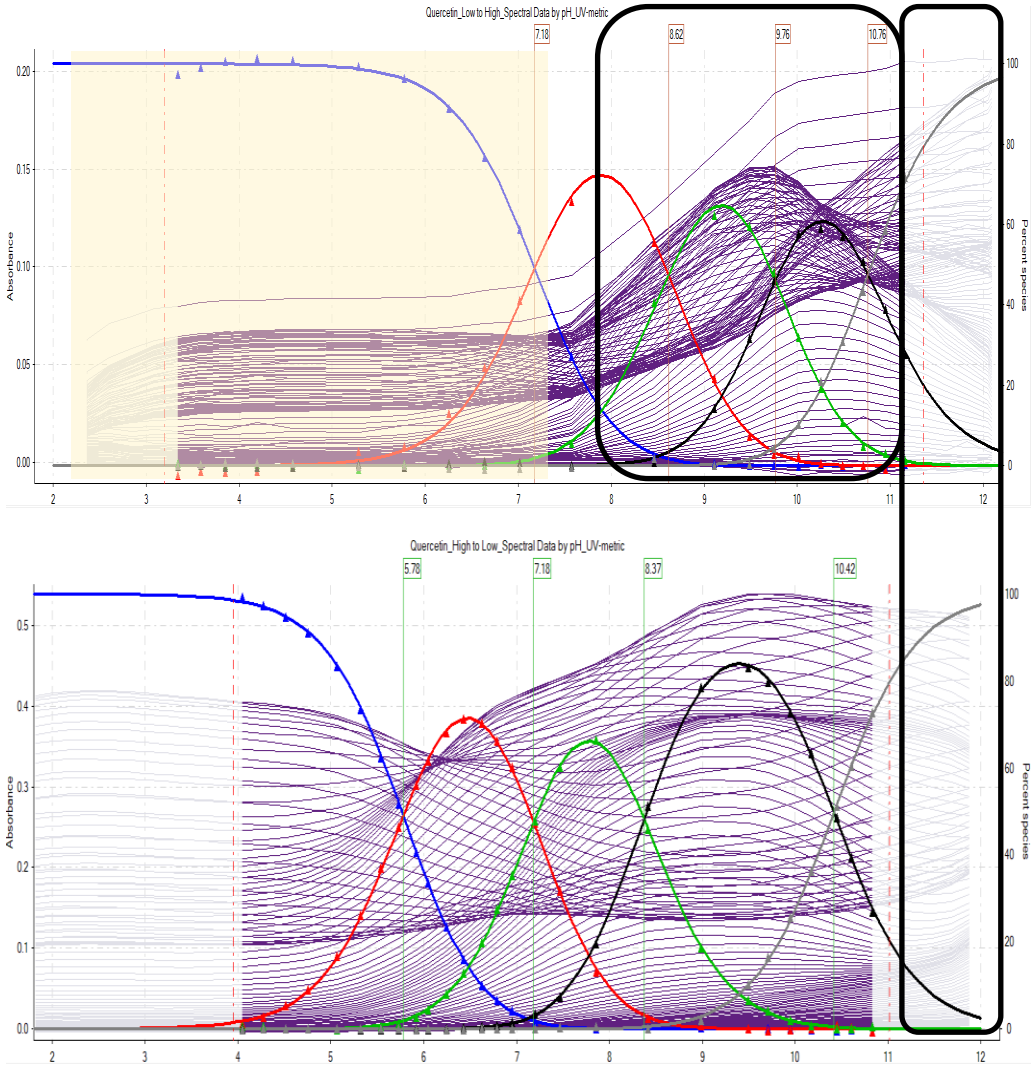
Precipitation detected

Decomposition observed at high pH

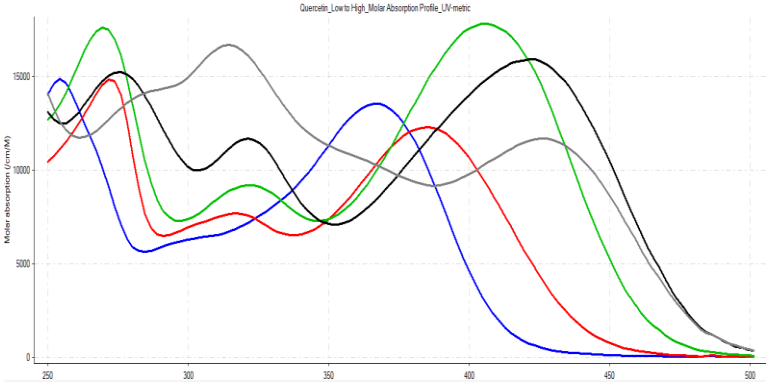
Pion

Spectrometric Technique – UV-metric:

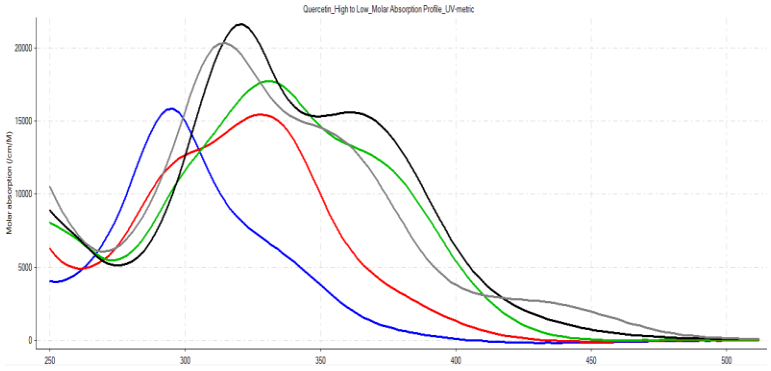
Decomposition at high pH



pH 2 → pH 12



pH 12 → pH 2
Decomposition



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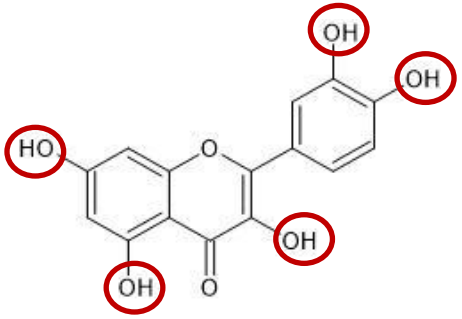
Spectrometric Technique – Fast UV:

Experimental Data

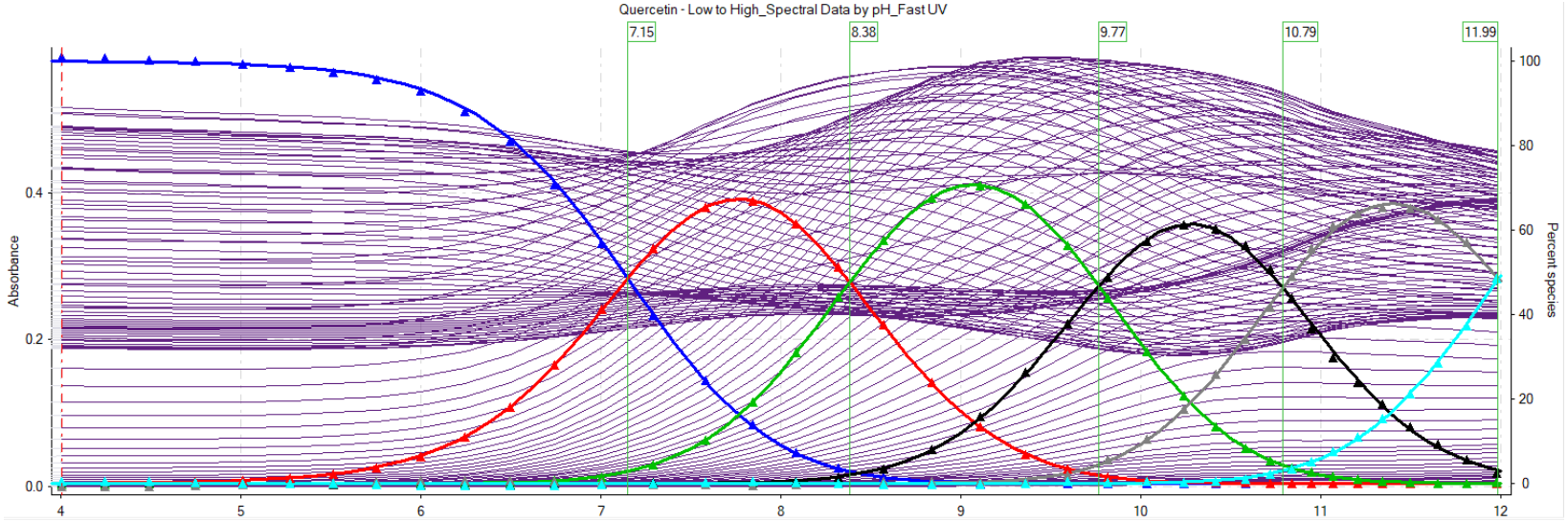


Quercetin

Fast UV Experimental conditions (6min/titration)

- Reference: 5µL DMSO and 25µL Neutral Linear Buffer*
- Sample: 5µL (10mM DMSO stock) and 25µL Neutral Linear Buffer* Aqueous media (I = 0.15 M KCl) at 25°C under Argon conditions
- Direction of the Titration from pH 2 to pH 12
- Titrants: HCl 0.5M and KOH 0.5M

pH 2 → pH 12



**Pion's Proprietary Information*

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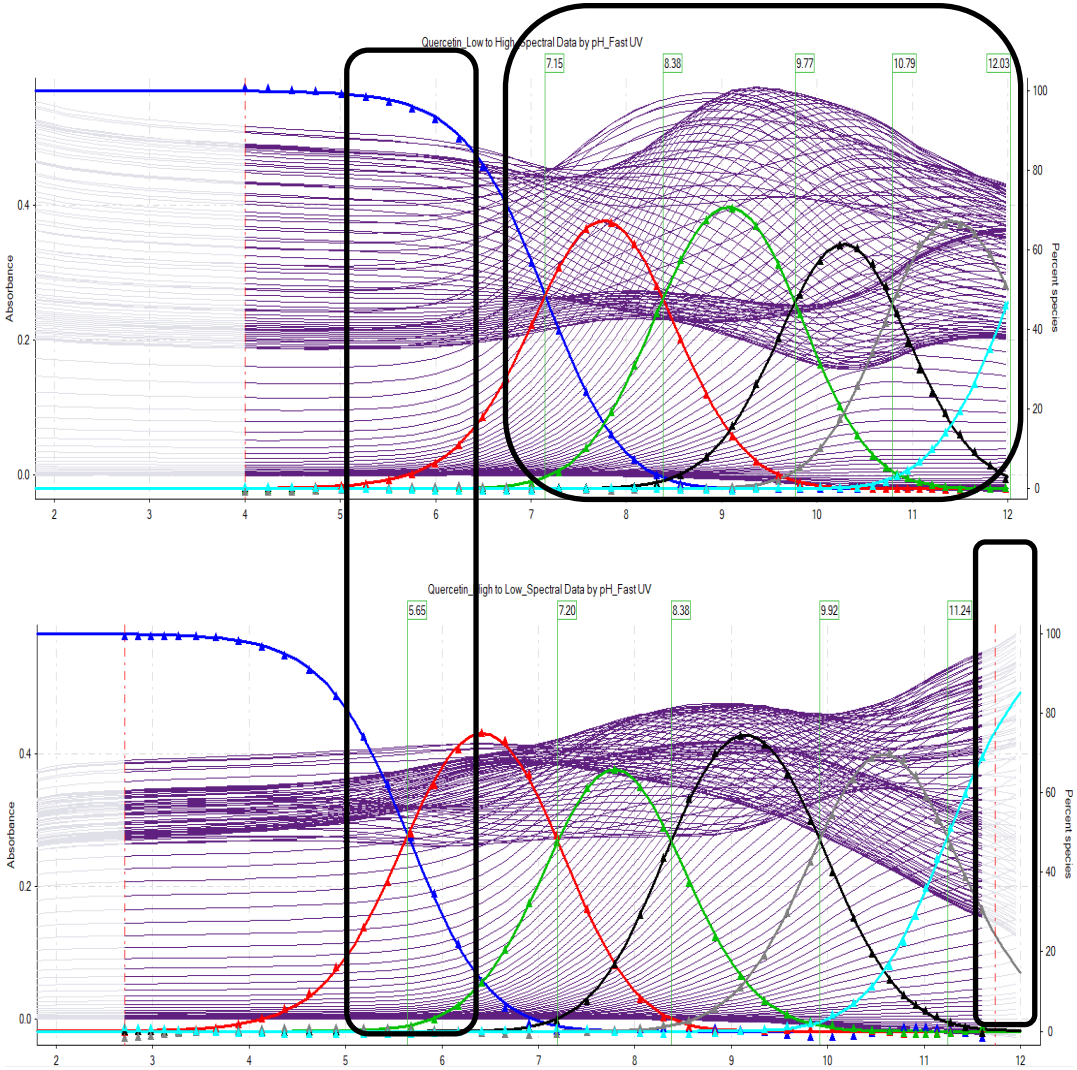
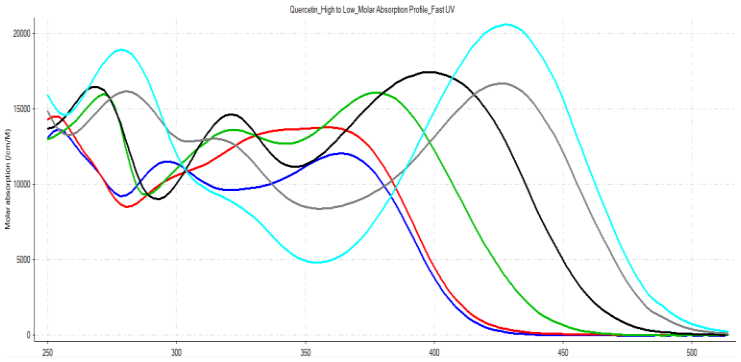
Spectrometric Technique – Fast UV:

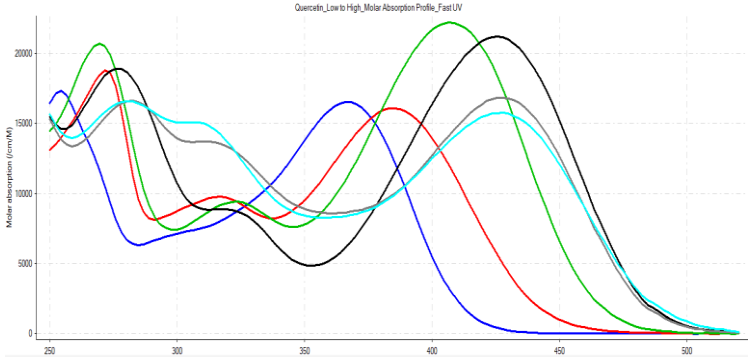
Decomposition at high pH



pH 2 → pH 12



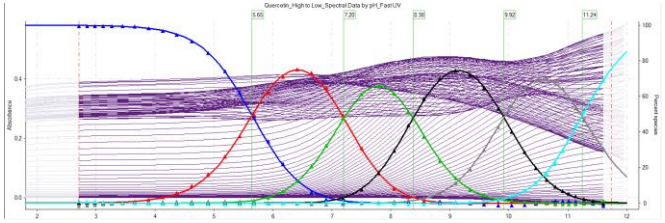
pH 12 → pH 2
Decomposition

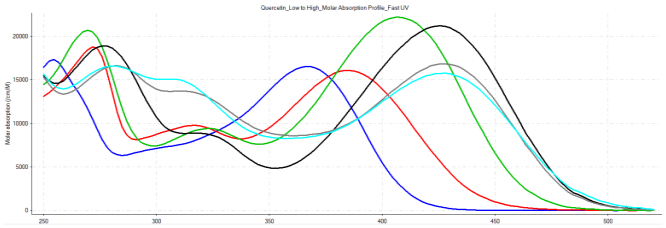


Pion

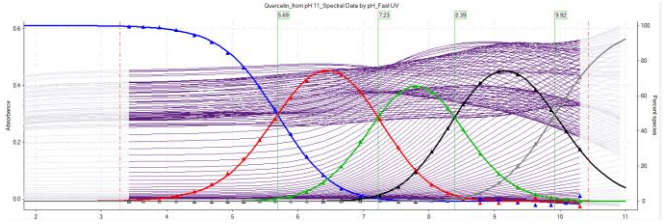
Study of the decomposition conditions – Quercetin

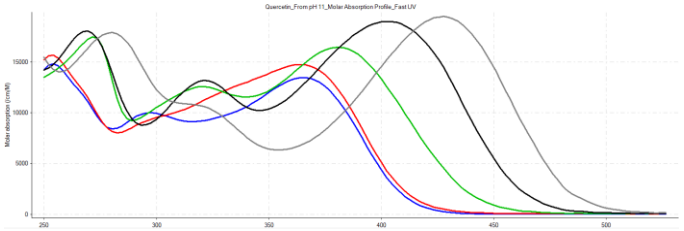
pH 12 → pH 2



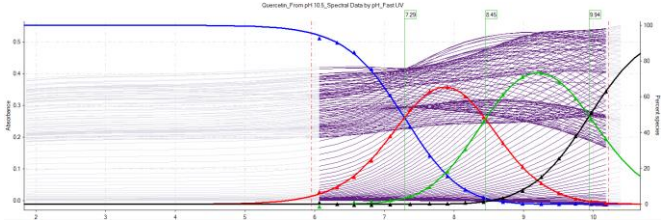


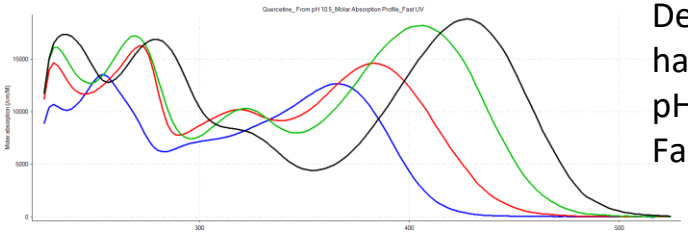
pH 11 → pH 2





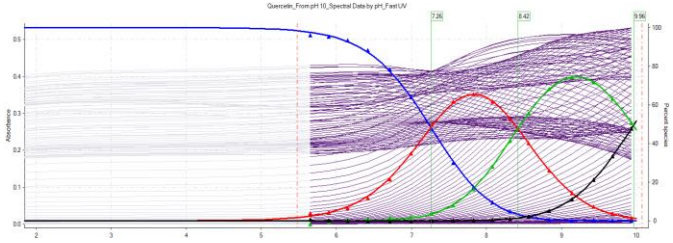
pH 10.5 → pH 2

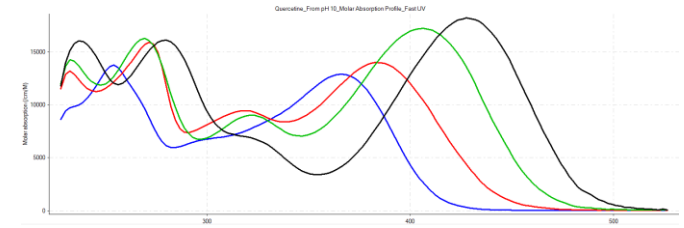




Decomposition happens above pH 10.5 using Fast UV

pH 10 → pH 2

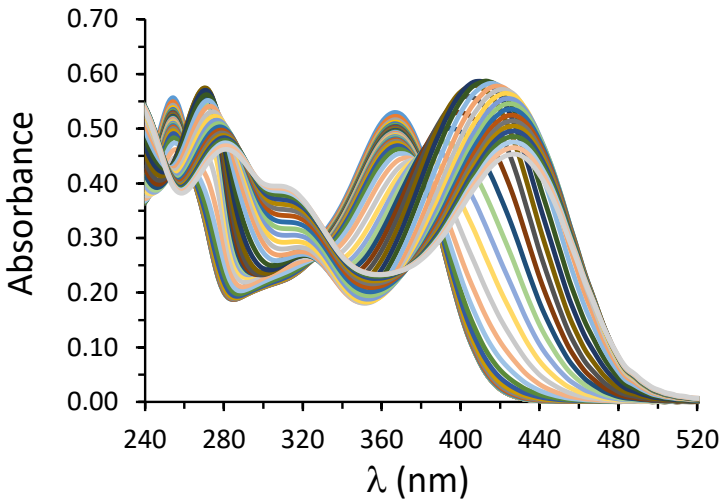




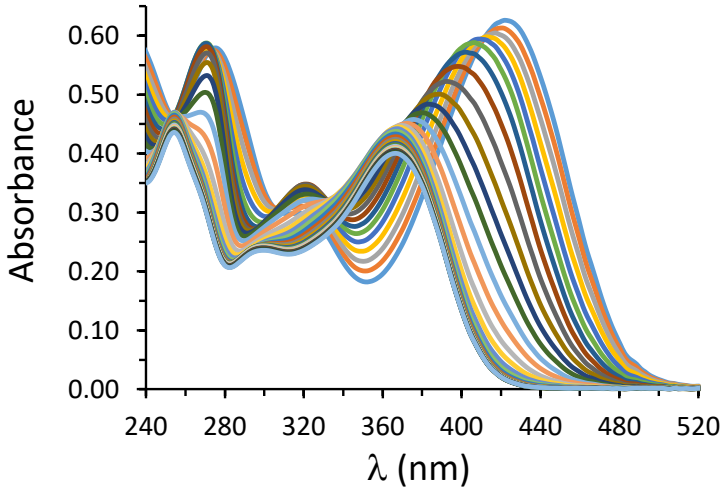
Pion

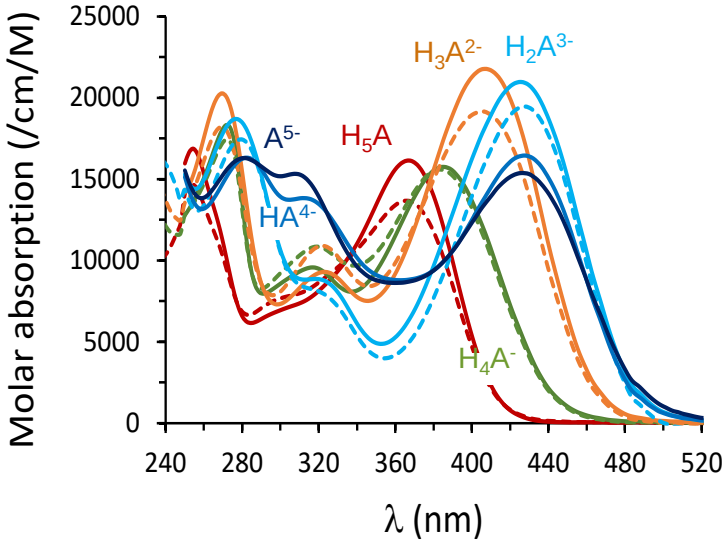
Study of the decomposition – Quercetin

pH 2 → pH 12



pH 12 → pH 2





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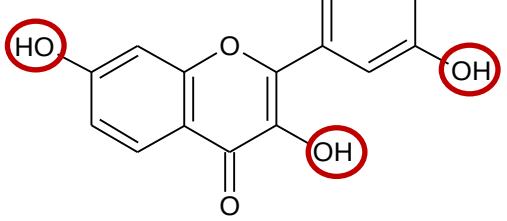
Fisetin



$pK_{a\ ACD}$ = 6.8
$pK_{a\ Galas}$ = 8.7

$pK_{a\ ACD}$ = 13.0
$pK_{a\ Galas}$ = 7.8

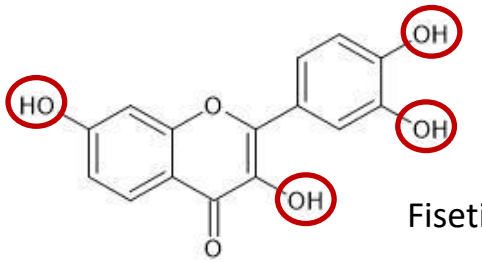
$pK_{a\ ACD}$ = 9.9
$pK_{a\ Galas}$ = 13.9

$pK_{a\ ACD}$ = 9.1
$pK_{a\ Galas}$ = 11.6

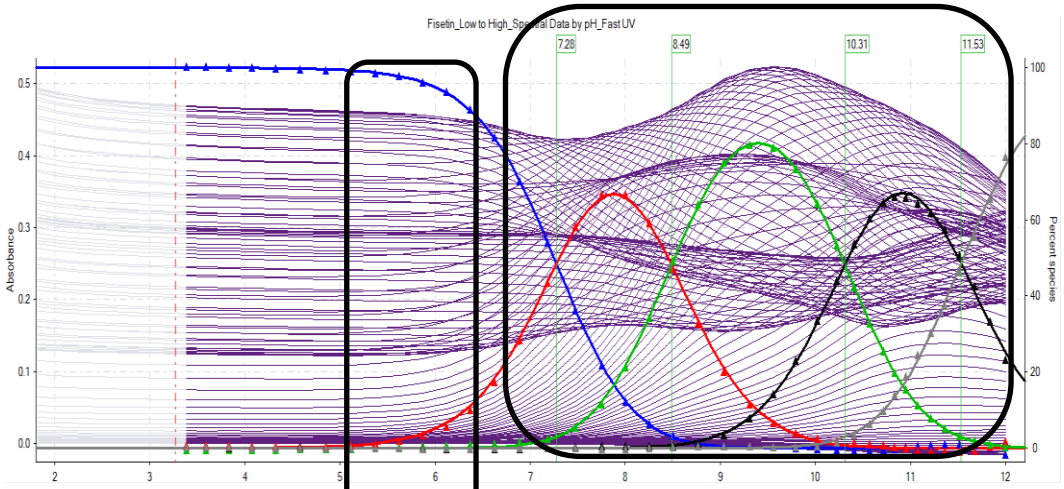
Pion

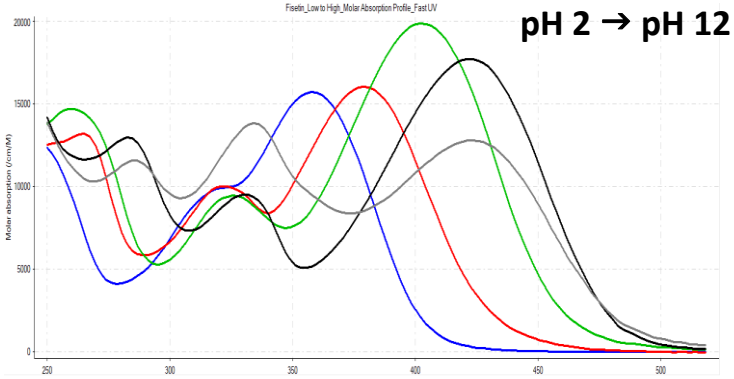
Spectrometric Technique – Fast UV:

Decomposition at high pH

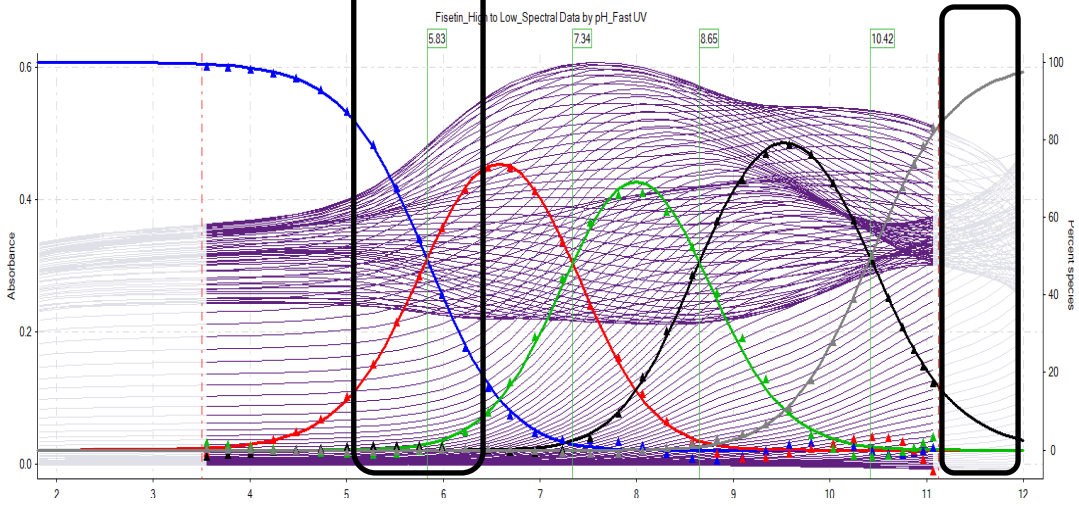


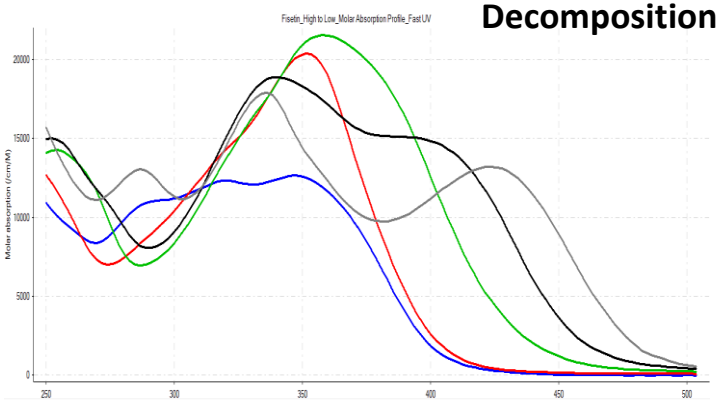
Fisetin





pH 2 → pH 12



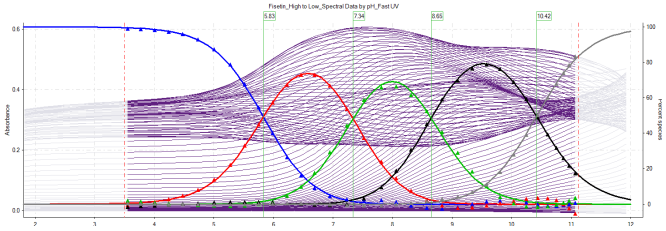


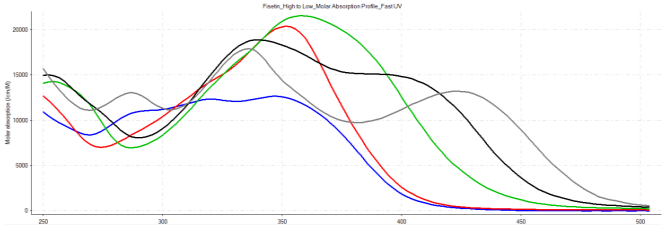
pH 12 → pH 2
Decomposition

Pion

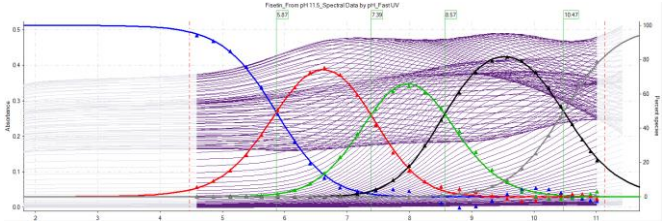
Study of the decomposition conditions – Fisetin

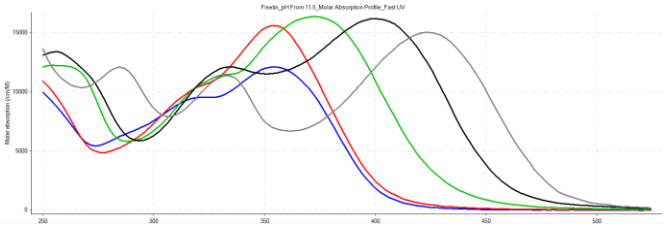
pH 12 → pH 2



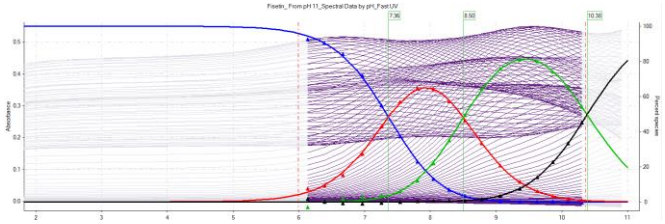


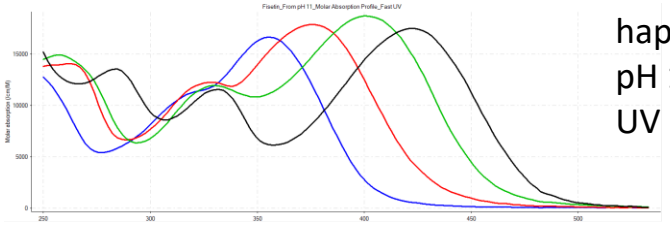
pH 11.5 → pH 2





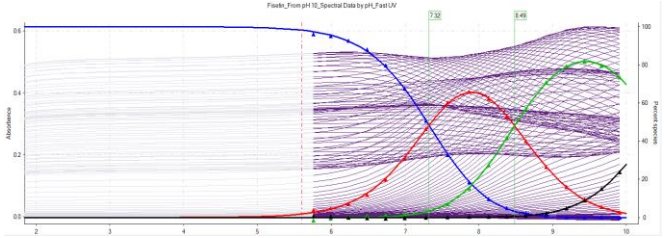
pH 11 → pH 2

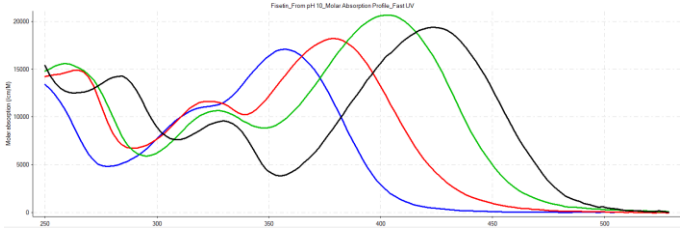




Decomposition happens above pH 11 using Fast UV

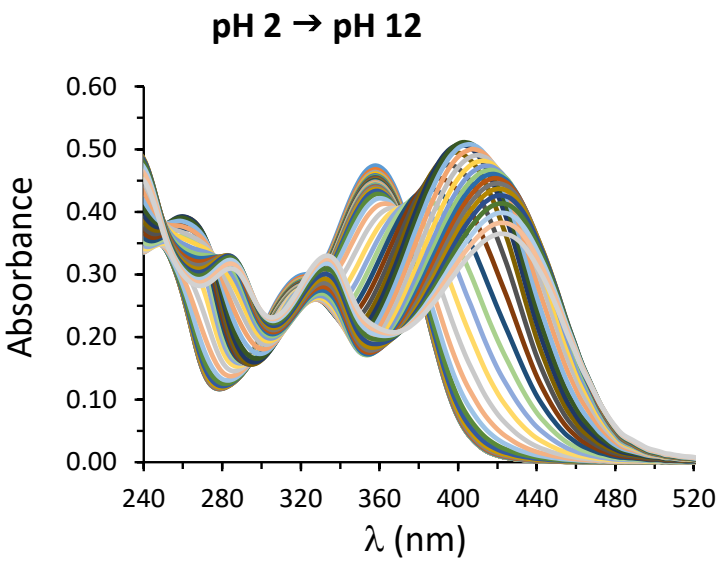
pH 10 → pH 2

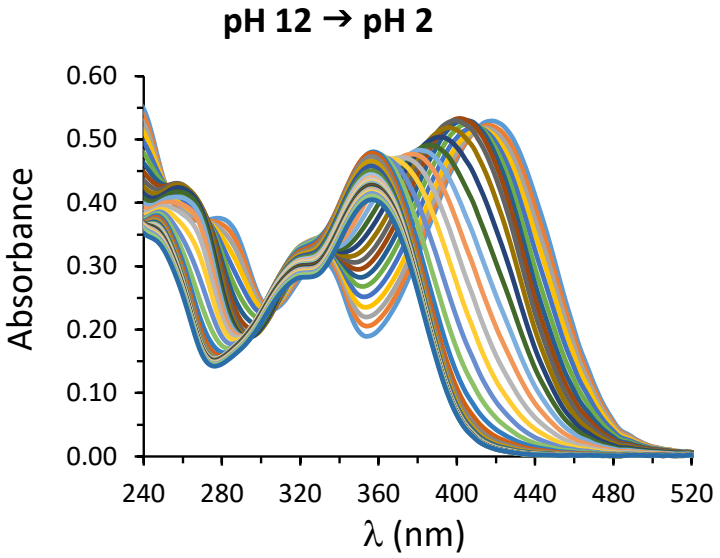


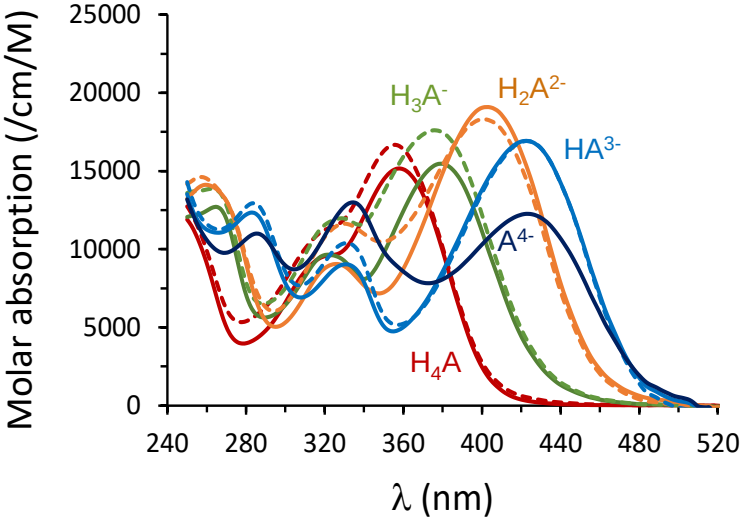


Pion

Study of the decomposition – Fisetin







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Results

	$pK_{a,1}$	$pK_{a,2}$	$pK_{a,3}$	$pK_{a,4}$	$pK_{a,5}$	Exp. (literature) or Calc. Approach
Fisetin	7.36	9.71	-	-	-	Capillary Zone Electrophoresis]
	7.57	9.34	-	-		Potentiometry
	7.70	9.54	-	-		Potentiometry
	-	8.87	10.31	13.2	-	Spectrophotometry
	7.31	*8.27*	*11.11*	*13.23*	-	**SPARC*
	7.8	*8.7*	*11.6*	*13.9*	-	**Percepta*
Quercetin	7.3	8.4	-	-	-	Spectrophotometry
	6.62	9.7	-	-	-	Potentiometry
	7.19	9.36	11.56	-	-	Capillary Zone Electrophoresis
	6.74	9.02	11.55	-	-	Spectrophotometry
	7.71	9.44	11.46	-	-	Potentiometry
	7.59	9.33	11.56	-	-	Potentiometry
	7.76	-	-	-	-	Spectrophotometry
	6.41	7.81	10.19	11.53	12.91	Spectrophotometry
	6.95	8.21	10.11	11.71	13.33	**SPAR*
	7.7	*8.5*	*10.9*	*13.5*	*14.3*	**Percepta*

		pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}	pK_{a5}
Fisetin	pH 2 → pH 12	7.27 (0.01)	8.49 (0.01)	10.31 (0.01)	11.54 (0.02)	
	pH 11 → 2	7.34 (0.01)	8.53 (0.04)	10.41 (0.02)		
	Average	**7.33 (0.03)**	**8.52 (0.04)**	**10.37 (0.06)**	**11.54 (0.02)**	
Quercetin	pH 2 → pH 12	7.15 (0.01)	8.38 (0.01)	9.77 (0.01)	10.79 (0.03)	11.95 (0.10)
	pH 10.5 → 2	7.27 (0.02)	8.45 (0.03)	9.94 (0.08)		
	Average	**7.24 (0.05)**	**8.44 (0.04)**	**9.89 (0.10)**	**10.79 (0.03)**	**11.95 (0.10)**

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Concluding Remarks

- Several techniques and different assay settings must be used to be able to confirm results, as different techniques are complementary giving enough information for a better understanding of the behaviour of the drug.

- Performing two titrations in opposite pH directions allows stability evaluation and the correct determination of the pK_as.

- Fast UV was proved to be a technique which avoids – in this study- decomposition and precipitation in comparison with the UV-metric technique.

- The use of pK_a-predictions helps to design the experimental conditions- saving sample - and offering additional information in order to evaluate the results.

- For the first time two completely new ionisation constants have been determined in this study.

Pion

Acknowledgments

Pion Analytical Services Team

Andy Kennedy
Cezary Nowak
Daniel Bowdery

Sam Lee

University of Barcelona

Dr Clara Rafols
Dr Elisabeth Bosch
Dr Elisabet Fuguet
Meritxell Mañe

Thank you!

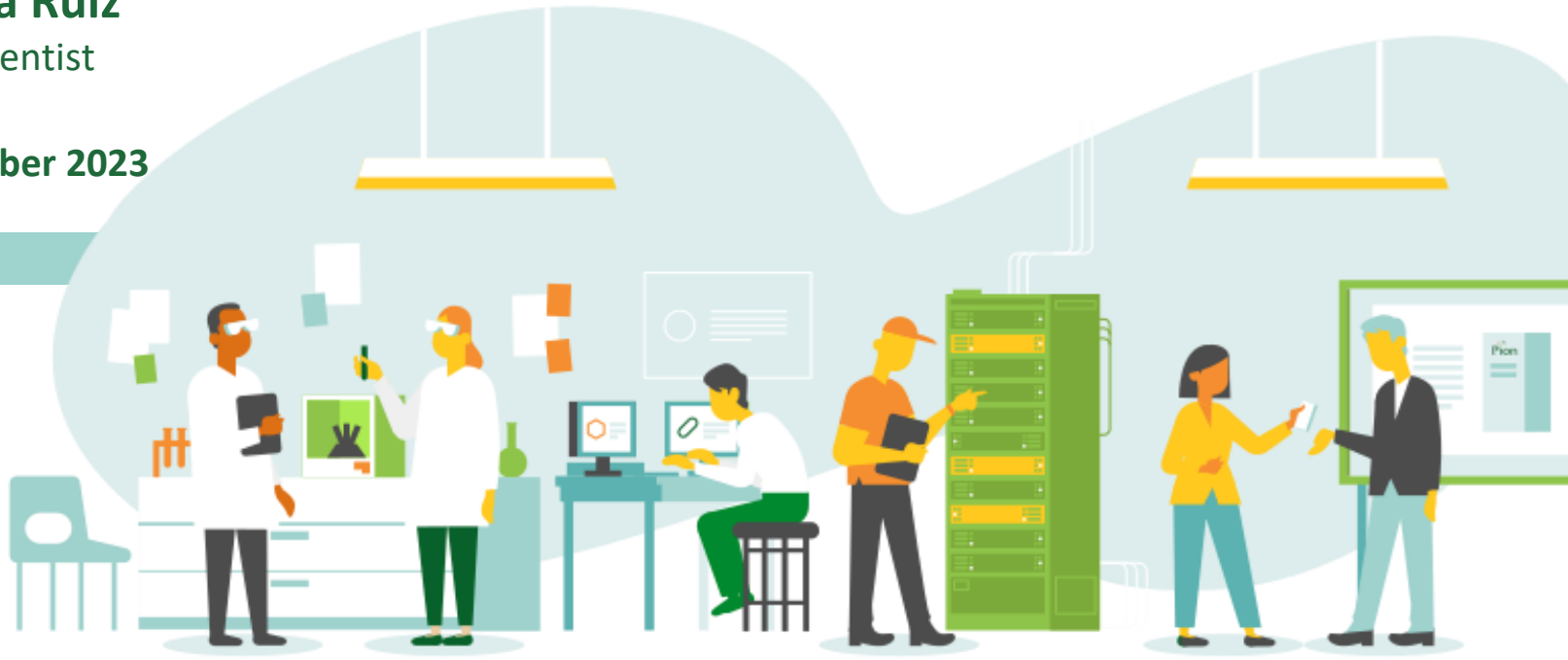
Pion

Ionisation Prediction Summit Webinar Series

Flavonoids and Some Examples of Challenged pK_a determination

Dr Rebeca Ruiz
Principal Scientist

14th November 2023



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