HPLC method development for ibuprofen quantification

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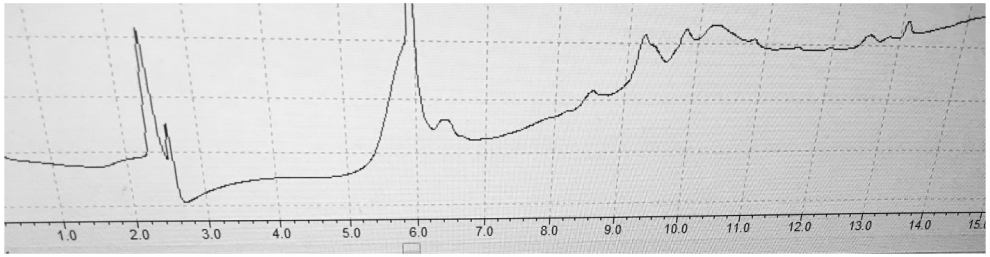
Project

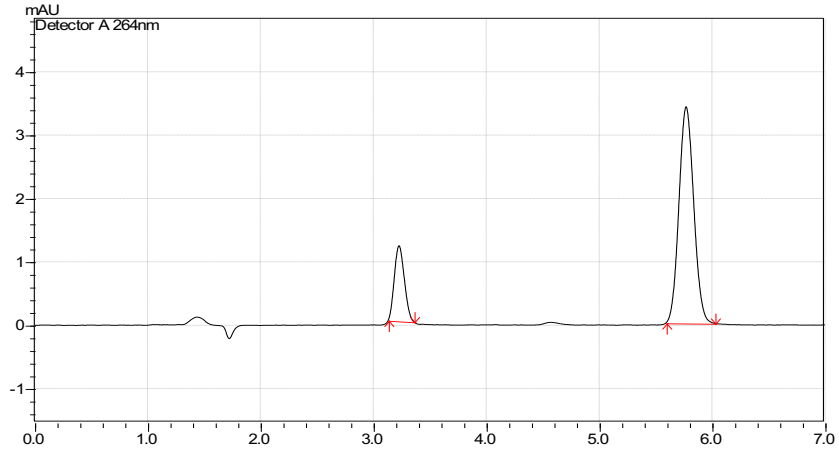
Develop the HPLC method to quantify ibuprofen and its release from the formulation

Outline of my presentation

- Method development and optimization
- Method validation
- Sample preparation and application of the HPLC method

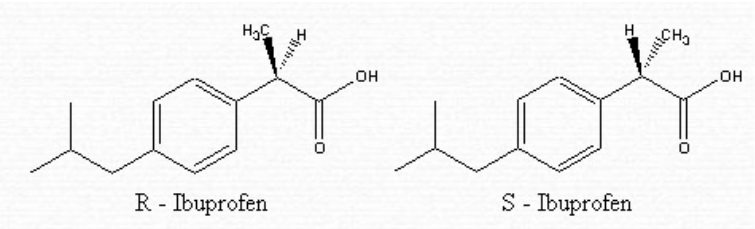


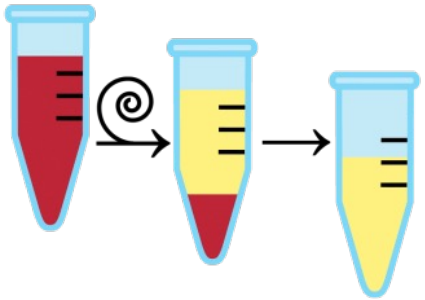
I am sorry that I only have this blurry picture for this chromatogram as the computer was infected with a virus and everything was erased from the computer.



Method development

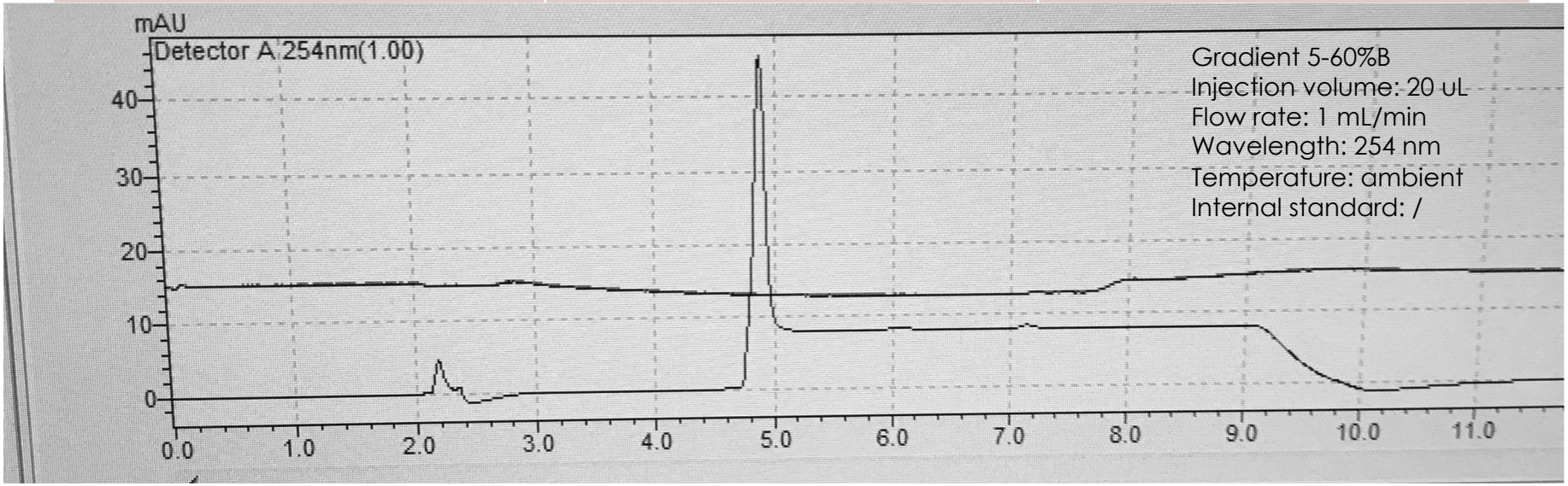
Stationary phase	Mobile phase	Chromatographic time (mins)	pH	Flow rate(mL/min)	Detection
CLC Shim-pack C8 column (2504.6 mm, 5-lm particle size)	methanol and 0.05M dihydrogen phosphate buffer (75:25, v=v)	7	6.5	1	fluorescence-detection at 295nm
Chiracel OJ-RH column (150 × 2.1 mm, 5 µm)	mixture of 0.1% (v/v) acetic acid in methanol/water (90:10, v/v)	6	/	0.15	MSMS
(R, R)-Whelk-02 (4.6 mm diameterx 250 mm leng th, I 0 ~lm partic le size	EtOH-water (30 + 70 v/v) containing I 00 mtvl AmAc	12	/	1.3	diode array detector 220nm
LuxCellulose 3 (250 × 4.6 mm, 5 m particle size)	0.1% (v/v) acetic acid in mixture of methanol and water in ratio90:10	14		0.6	MS
Agilent ZORBAX Eclipse Plus C18 100 mm × 4.6 mm,3.5 mm column	methanol	4		/	DAD 221nm
Luna® 5 µm C18(2) 100 Å, LC Column 250 x 4.6 mm, Ea	Acetonitrile / Water (60:40) with 20mM Chloroacetic acid	6	3	2	254 nm
C18 column (Hypersil BDS, 150 x 4.6 mm, 5 µm)	Acetate buffer (triethylamine & ortho phosphoric acid) andACN in the ratio of 40:60 % (v/v).	4		1.5	220 nm





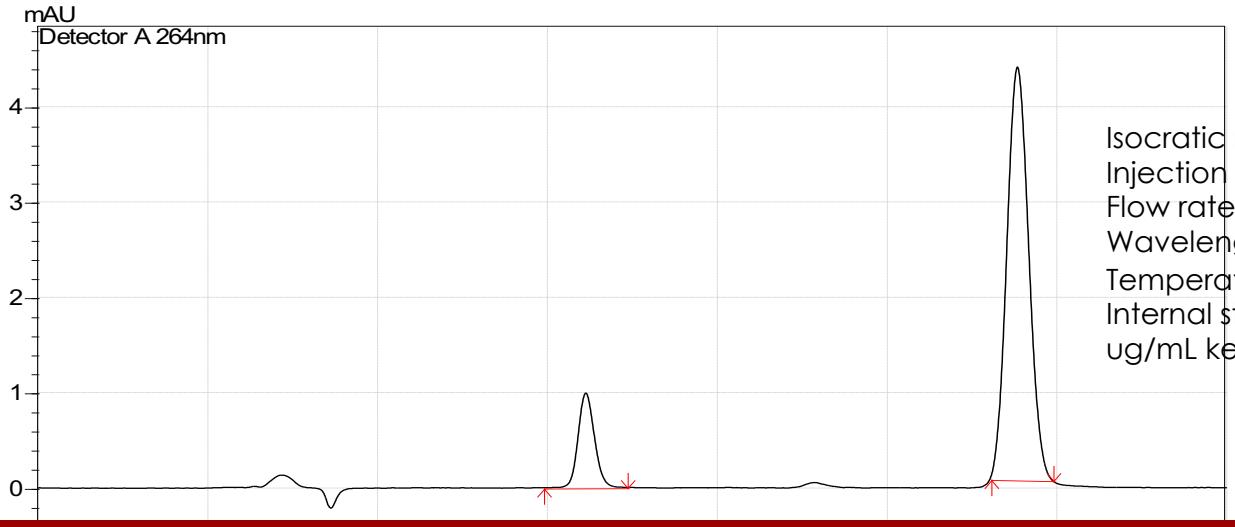
Method optimization

Parameter	Original USP method	Changes
Column length (cm)	25	15
Flow rate (mL/min)	2	1
Acid used	chloroacetic acid	formic acid



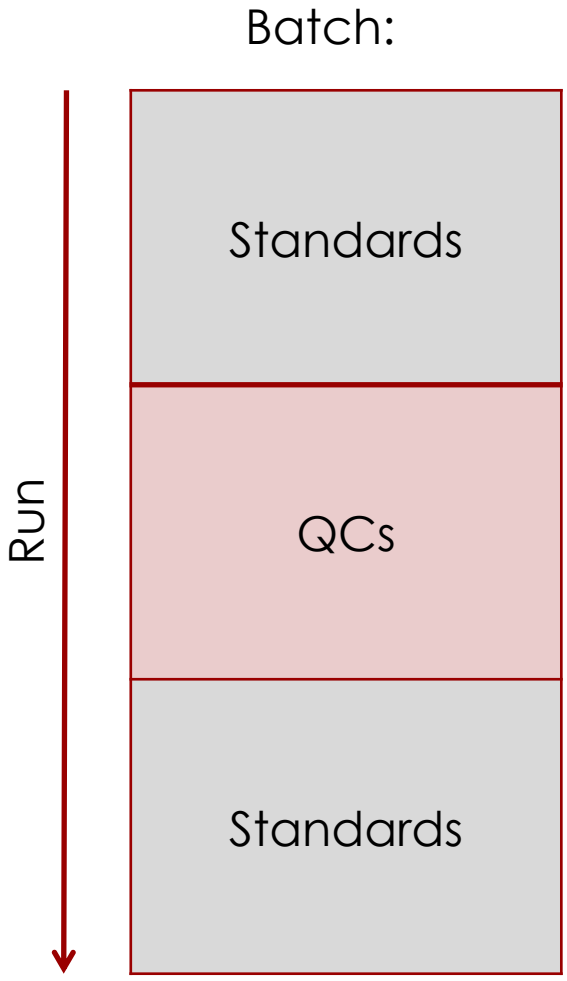
Chromatogram

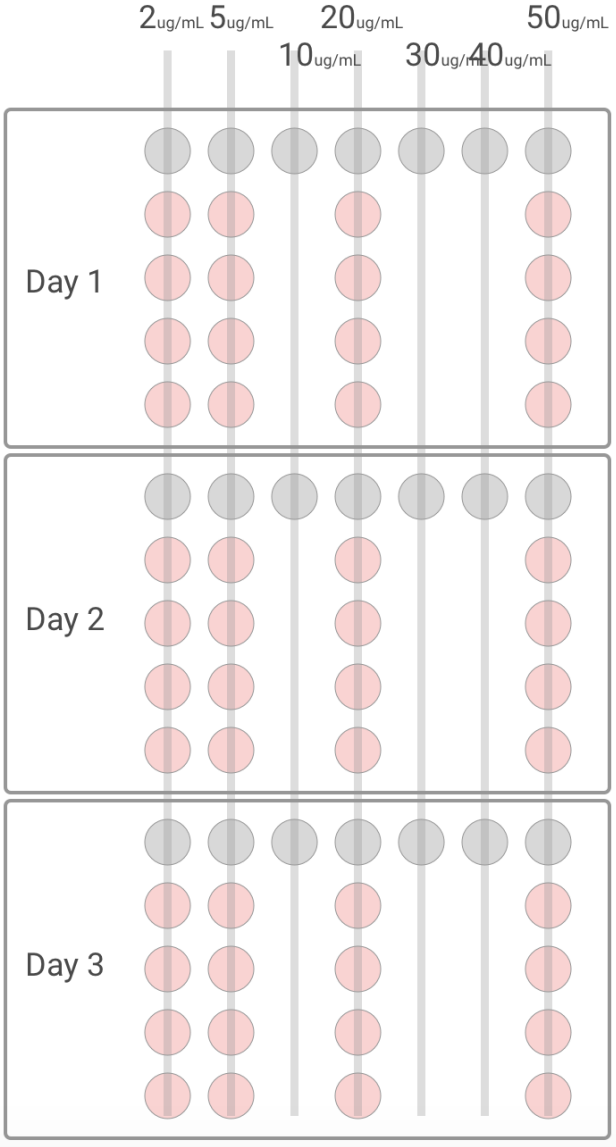
- Run time: less than 7 minutes
- Sharp peaks



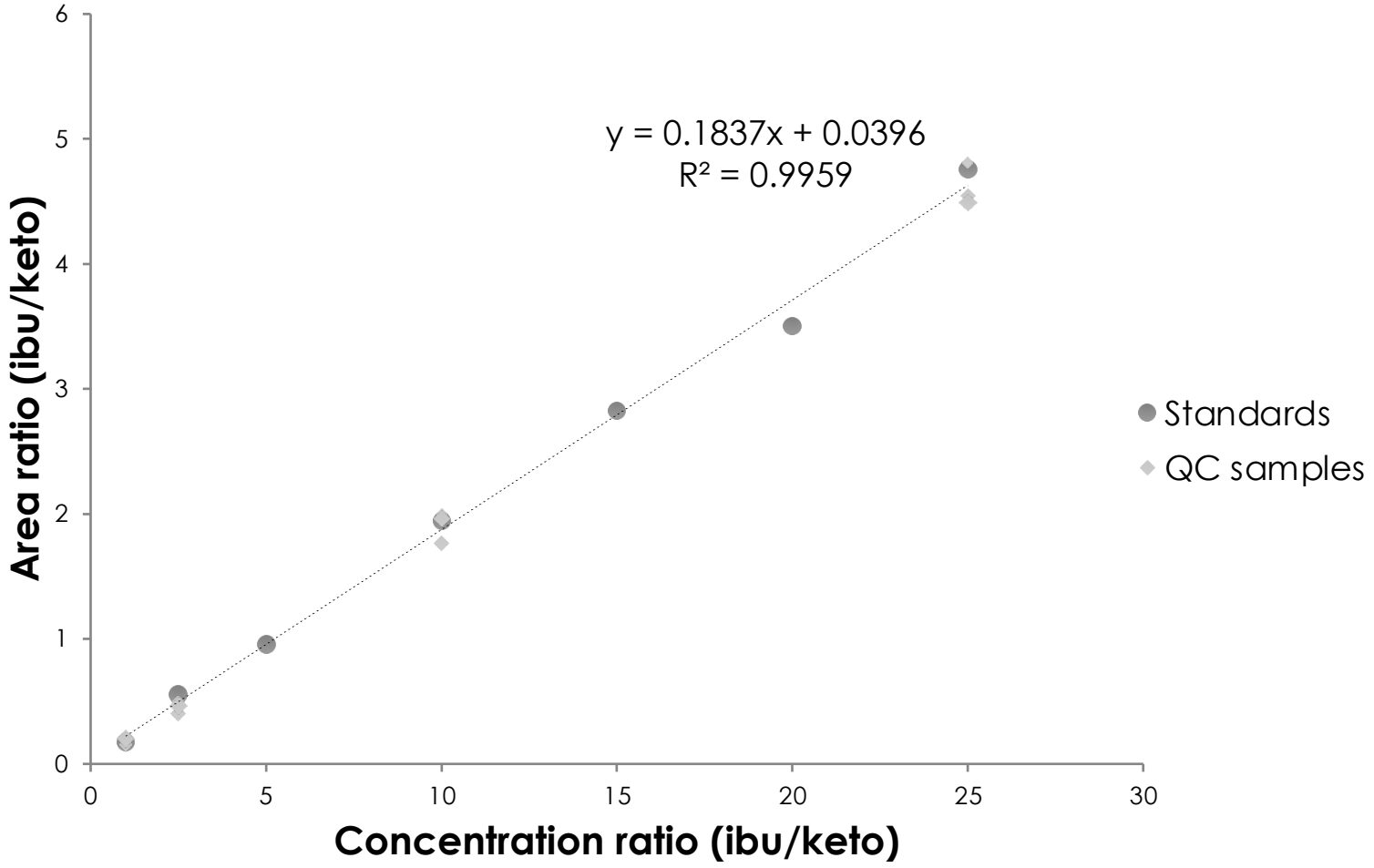
Isocratic run 60%B
Injection volume: 10 uL
Flow rate: 1 mL/min
Wavelength: 264 nm
Temperature: 40°C
Internal standard: 2 ug/mL ketoprofen

Method validation





Results (1)

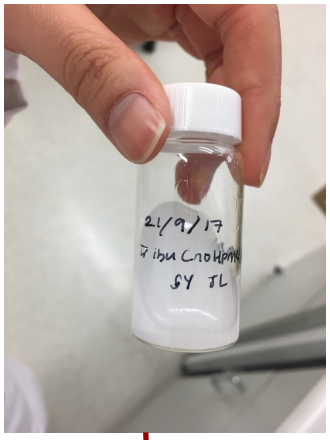

y = 0.1837x + 0.0396
R² = 0.9959
Area ratio (ibu/keto)
Concentration ratio (ibu/keto)
0
1
2
3
4
5
6
0
5
10
15
20
25
30
Standards
QC samples

Results (2)

Concentration (ug/mL)	Accuracy (%)					Precision (%)				
	Within-run			Between-run	Acceptance criteria +/-	Within-run			Between-run	Acceptance criteria +/-
	1	2	3			1	2	3		
2	114	78	101	100	20	9	2	7	20	20
5	98	99	97	98	10	8	4	7	6	10
20	96	103	105	93		1	6	4	6	
50	96	100	97	91		8	6	4	5	

The linearity, accuracy and precision of the method were successfully validated within the concentration range of 5-50 ug/mL.

Sample preparation



Gelling test

1 mL

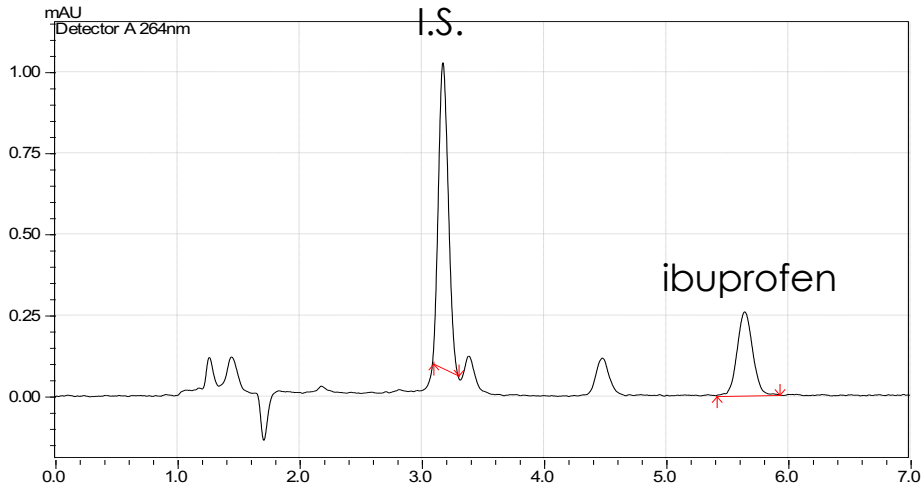
0.9 mL

Centrifugation
1 min

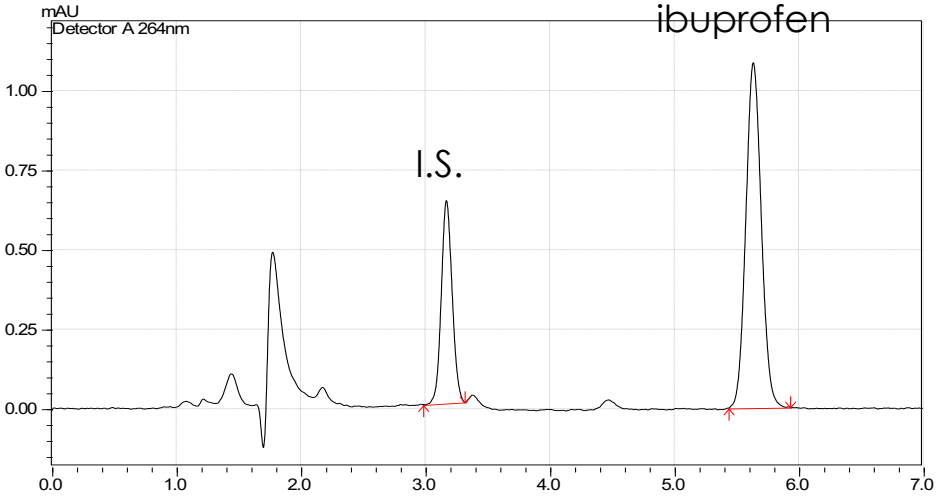
Centrifugation
1 min

-1:13.3 dilution
-addition of I.S.

Results



Sample 1



Sample 4

Next steps

- Robustness of the method
- Method suitability test

Acknowledgement

- Thank you Syaza, Natalie, Given and Gracia
- This project would not have been possible without your support and guidance.

Calculations

	Concentration (mg/mL)	Stock solution (mL)	ACN (mL)	Total volume (mL)
Ibuprofen stock solution	10			
Diluted solution A	1	0.1	0.9	1
Diluted solution B	0.1	0.1	0.9	1
Ketoprofen stock solution	10			
Diluted solution C	1	0.1	0.9	1
Diluted solution D	0.1	0.1	0.9	1

Using diluted solution B and D:					
	Concentration (ug/mL)	Solution B (ibu) (uL)	ACN (uL)	Solution D (keto) (uL)	Total volume (mL)
Standard solution 1	2	20	978	2	1
Standard solution 2	5	50	948	2	1
Standard solution 3	10	100	898	2	1
Standard solution 4	20	200	798	2	1
Standard solution 5	30	300	698	2	1
Standard solution 6	40	400	598	2	1
Standard solution 7	50	500	498	2	1
Standard solution 8	2.5	25	973	2	1
Standard solution 9	3	30	968	2	1

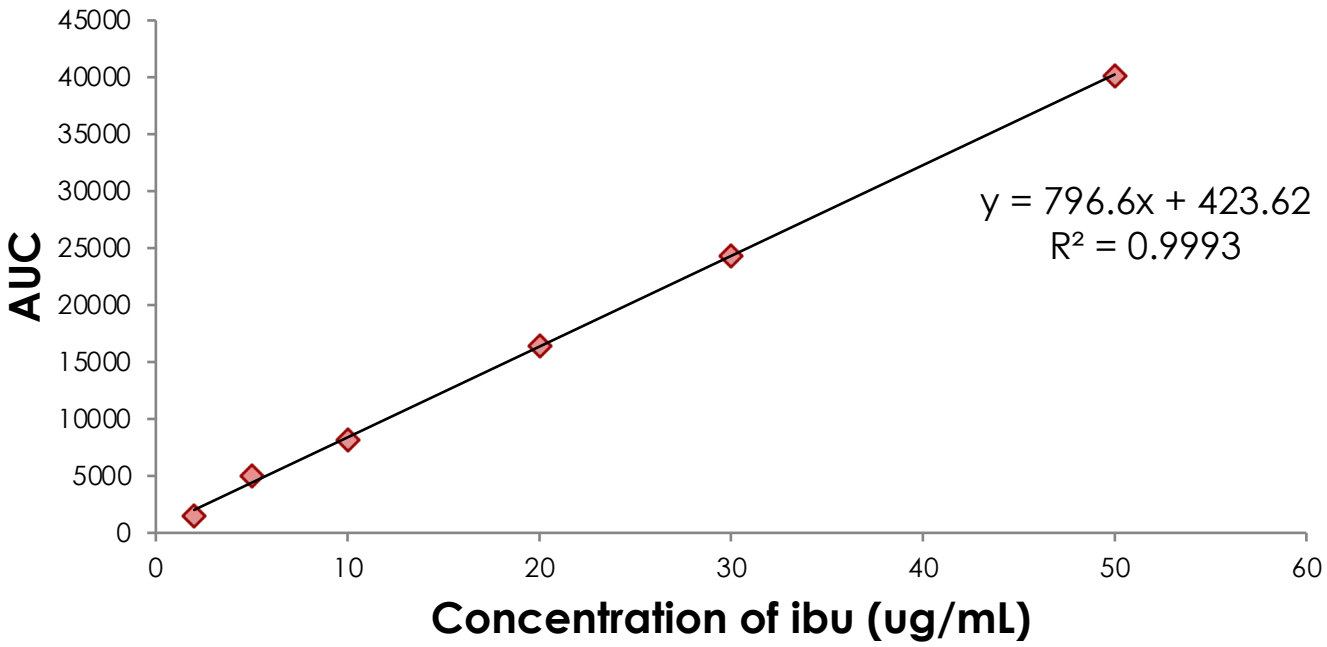
DOE

		Effect of	Effect of	Effect of	Effect of	Effect of	Effect of	Effect of
		factor 1	factor 2	factor 3	interaction	interaction	interaction	interaction
level	average	Tween 80	sodium citrate	sodium alginate	IA12	IA13	IA23	IA123
level -1		0	0	150				
level 0		50	150	200				
level +1		100	300	250				
unit	in 10 mL	mg	mg	mg				
exp1	1	-1	-1	-1	1	1	1	-1
exp2	1	1	-1	-1	-1	-1	1	1
exp3	1	-1	1	-1	-1	1	-1	1
exp4	1	1	1	-1	1	-1	-1	-1
exp5	1	-1	-1	1	1	-1	-1	1
exp6	1	1	-1	1	-1	1	-1	-1
exp7	1	-1	1	1	-1	-1	1	-1
exp8	1	1	1	1	1	1	1	1
CP1		0	0	0	0	0	0	0
CP2		0	0	0	0	0	0	0
CP3		0	0	0	0	0	0	0

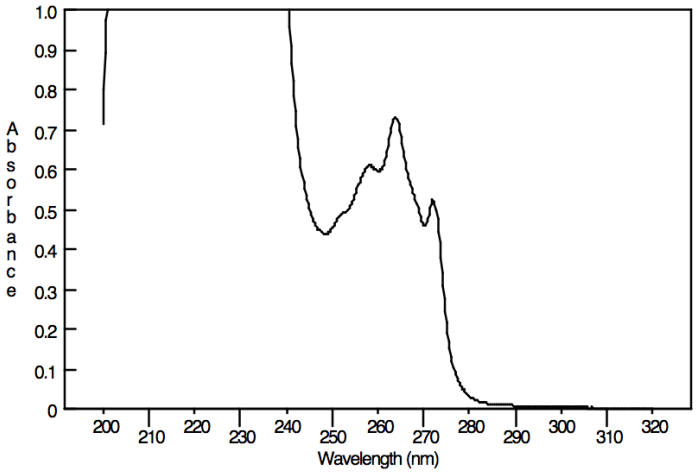
Calculation (sample prep)

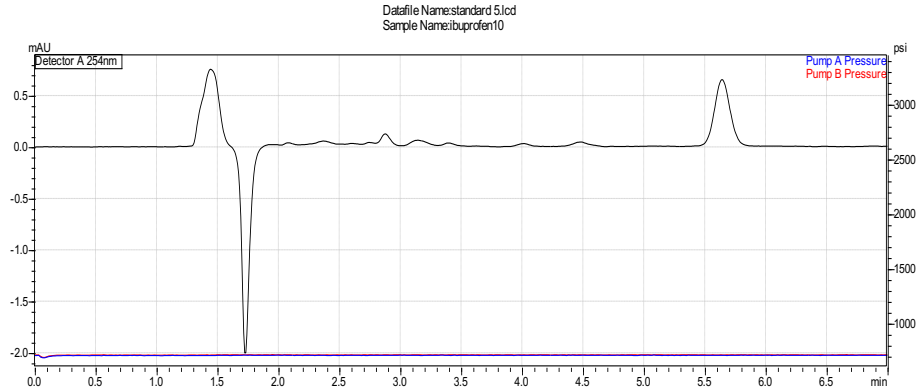
- Concentration of ibu in the formulation is 20 mg/10 mL (2 mg/mL)
- Test of release of the formulation: 600 ug/mL to 3 mL of HCl (0.4 mg/mL)
- Dilution method: assumed all ibu is released,
- Make it up to '30 ug/mL'
- 74 uL sample solution was used (1:13.3 dilution)
- we don't want it to go over the concentration of 50 ug/mL which is the limit of the validated calibration graph.

Use of internal standard?

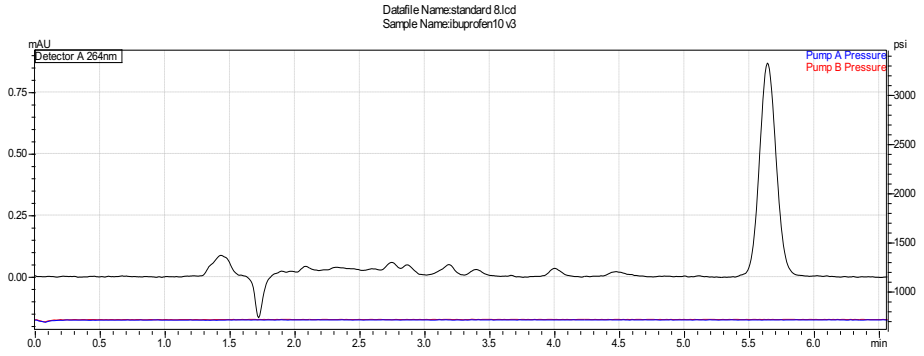


Wavelength





10 ug/mL 254 nm



10 ug/mL 264 nm

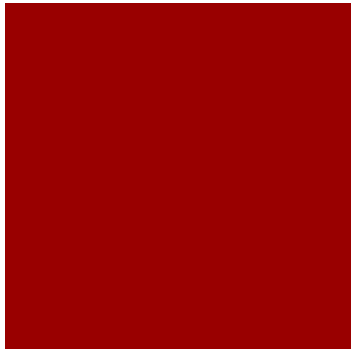
Composition of formulation

- The solution is prepared by mixing sodium alginate, T80, calcium carbonate and sodium citrate.
- HCl

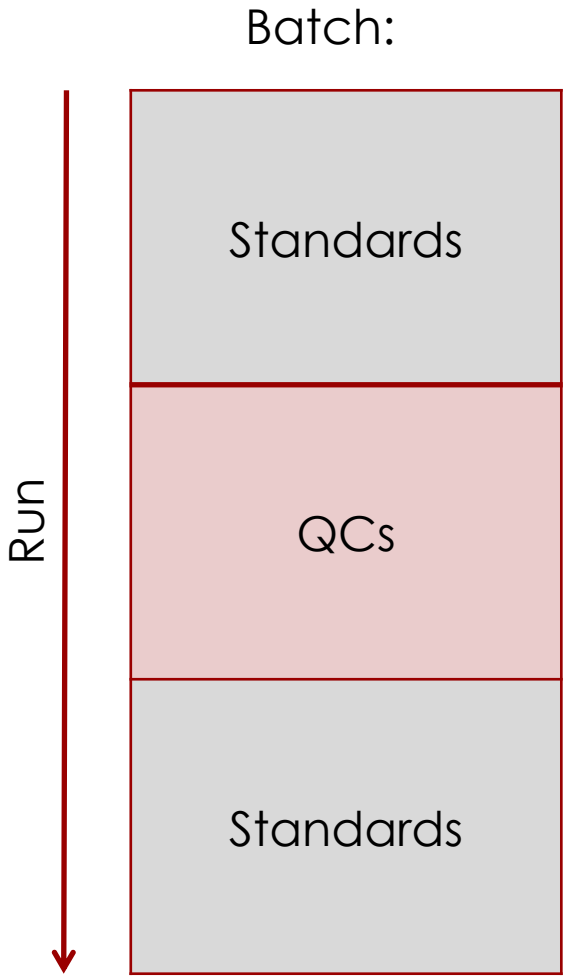
May I remove outliers?

- If an outlying value can be traced back to a failure in the system (e.g. injection error, bad chromatography, pipetting error, etc.) then it is permissible to remove it or better yet to repeat the measurement in question. If such a retrace does not come up with any failure then the outlying value should be considered as a real but rare incident and kept in the data.

How it treats JA



Bracketing QCs



Increase in temperature

Baseline drift

Possible cause	Solution
Column temperature fluctuation. Even small changes cause cyclic baseline rise and fall. Most often affects refractive index, UV and conductivity detectors at high sensitivity	Control column and mobile phase temperature, use heat exchanger before detector

Interday results

Concentration	Day 1	Day 2	Day 3	average	sd	%accuracy	%precision
2	2.186	1.516	2.074	1.99183333	0.4030391	100%	20%
2	2.765	1.573	1.933				
2	2.53	1.561	2.195				
2	2.142	1.563	1.864				
5	4.672	4.658	4.544	4.89625	0.30462052	98%	6%
5	4.75	5.149	5.091				
5	4.721	4.921	5.15				
5	5.531	5.051	4.517				
20	18.977	22.29	20.811	18.6695	5.98521219	93%	32%
20	0	20.676	22.184				
20	19.183	19.757	20.112				
20	19.292	19.483	21.269				
50	44.025	48.527	47.01	45.599	11.6255877	91%	25%
50	48.954	54.339	50.601				
50	51.129	48.862	46.886				
50	9.568	47.826	49.461				