Validated Stability-indicating HPLC-UV Method for the Simultaneous Determination of Flurbiprofen and Triclosan in Dental Nanogel Formulation

by

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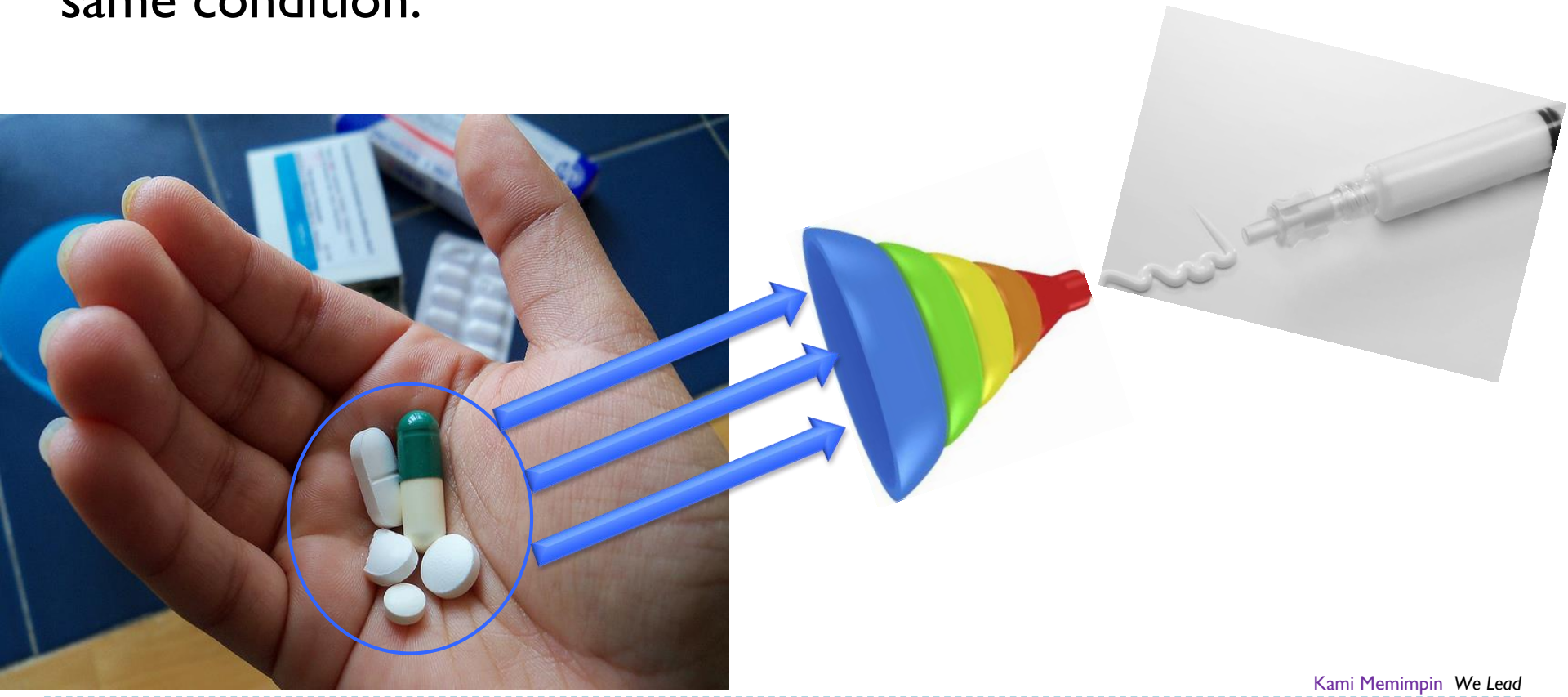
Introduction

Combination therapy (or polytherapy) is a broad term for the use of multiple medications or therapies, in order to fight the same condition.



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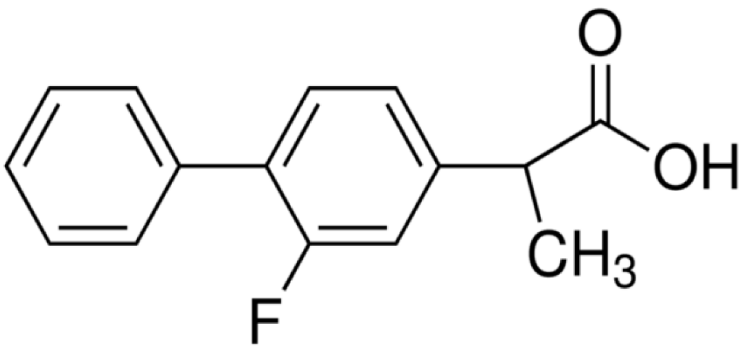
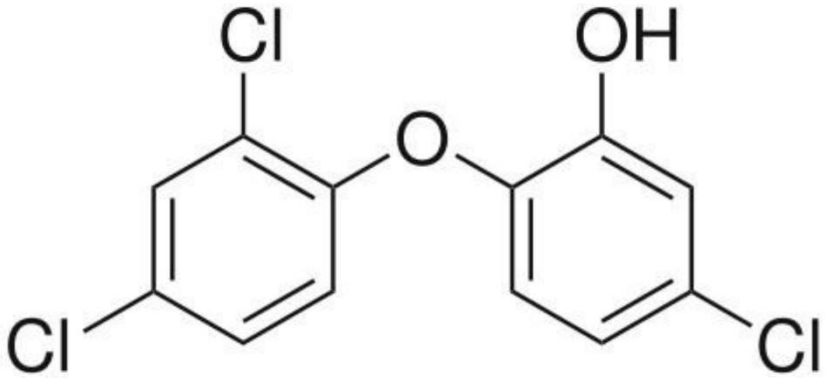


Flurbiprofen: *(RS)*-2-(2-fluorobiphenyl-4-yl) propanoic acid

- Anti-inflammatory; Analgesic; Anti-pyritic
- Indicated for dental pains, arthritis & soft tissue injuries
- Also efficacious when used as a topical rinse and as systemic medication in periodontitis management.
- Aid in healing and significantly lowering alveolar bone loss in periodontitis.



Triclosan: 5-Chloro-2-(2,4-dichlorophenoxy) phenol

- Broad spectrum antimicrobial agent with **antibacterial**, antifungal and antiviral effects which is being used since last four decades
- Has a recognized efficacy against several plaque-forming bacteria
- Effective anti-plaque and anti-gingivitis agent
- Included in several oral health care products – dentifrices & rinses
- Used for the treatment of periodontitis
- Relatively nontoxic to humans and other mammals at standard dose

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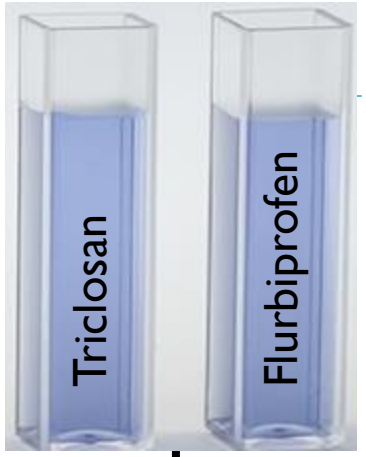
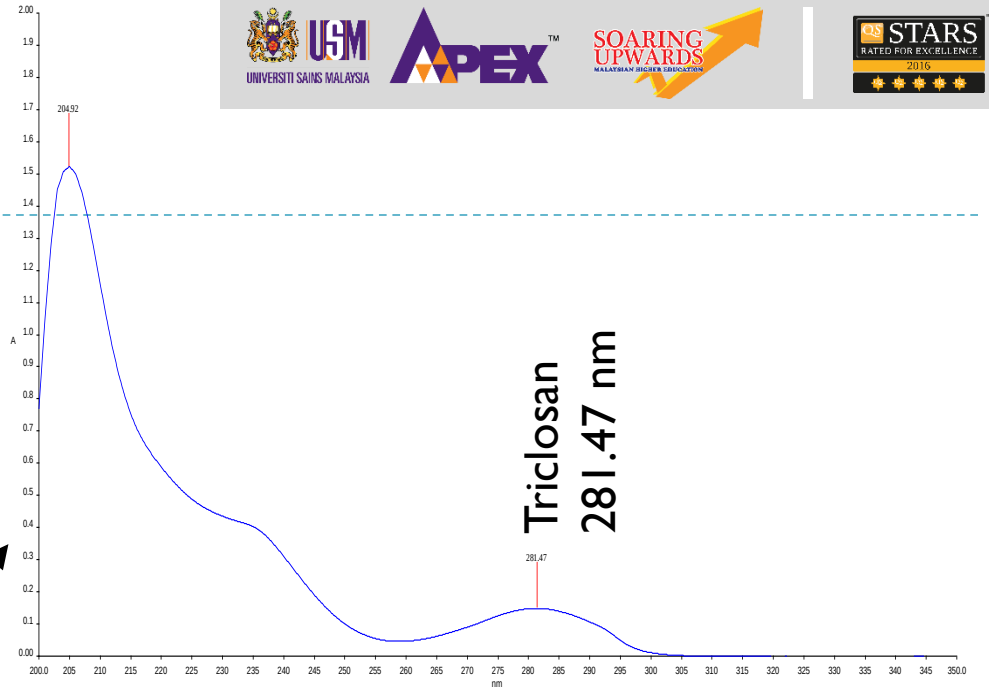
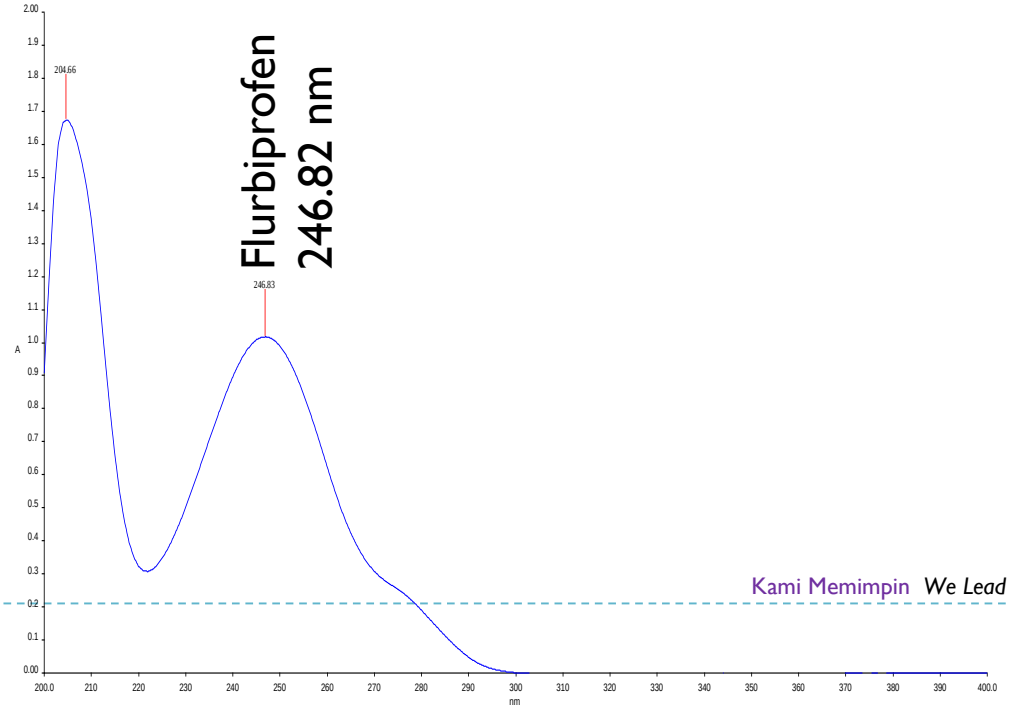


Perkin Elmer, Lambda 25; Singapore





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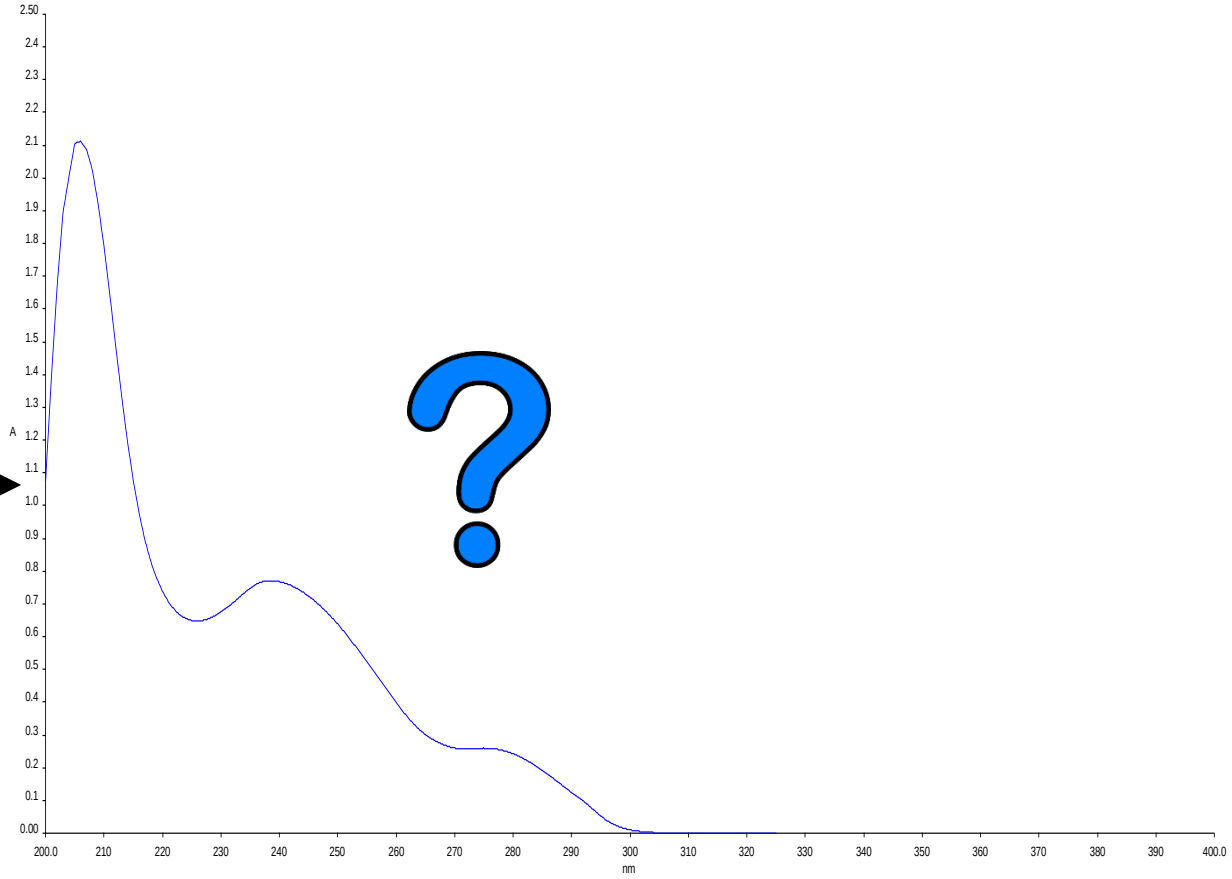
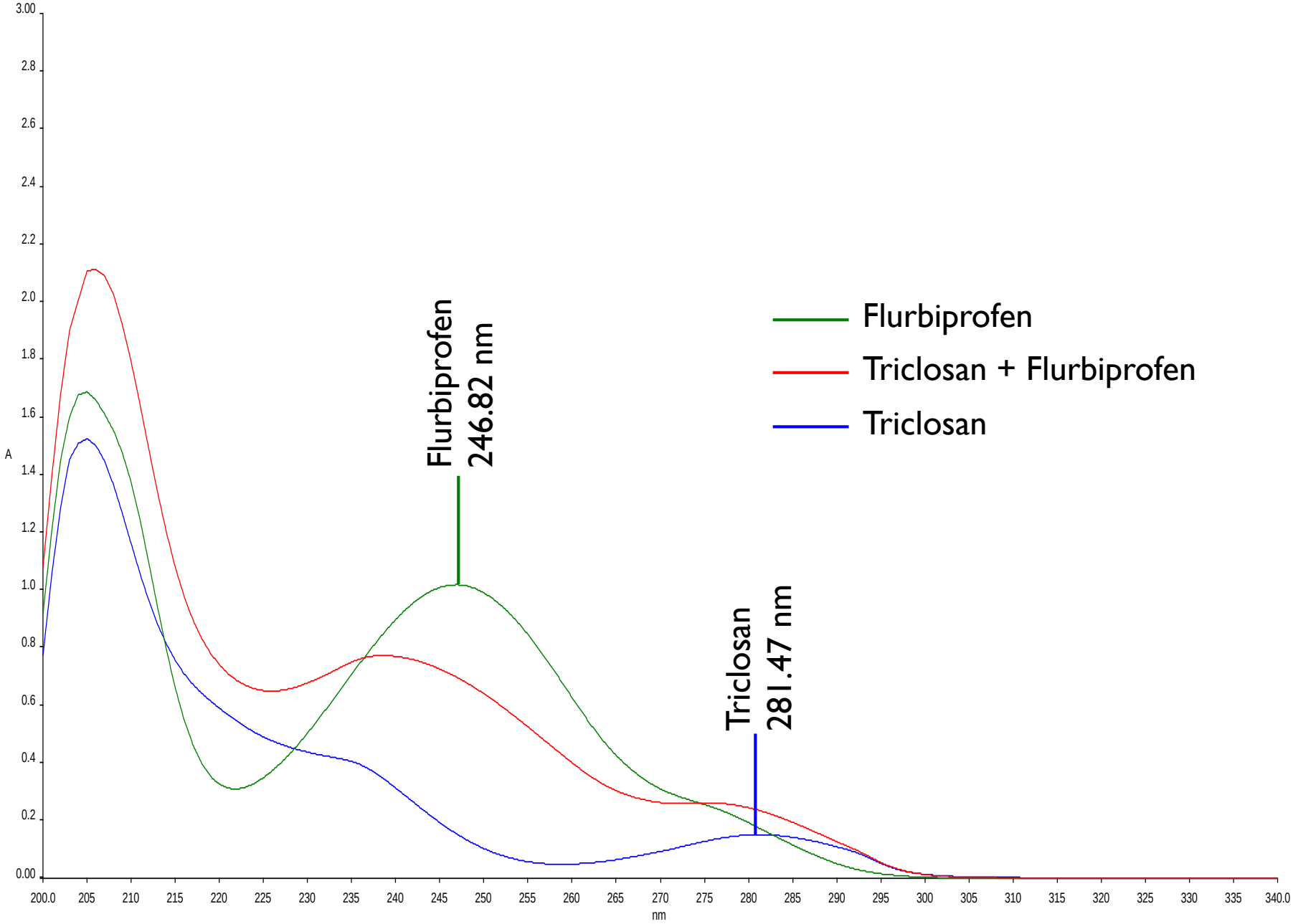
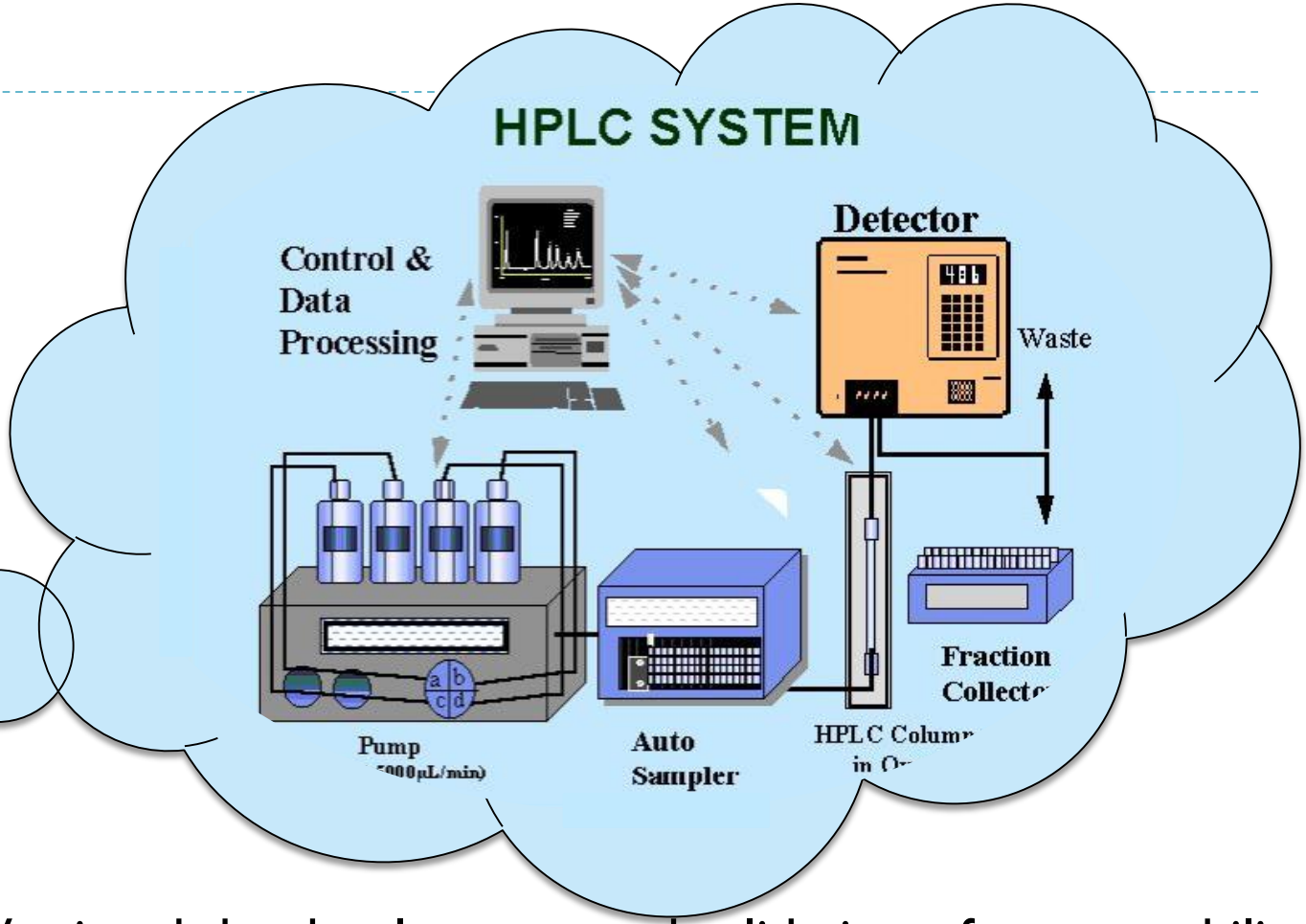




Therefore, UV spectrophotometry is not suitable

Objective



We aimed the development and validation of a new stability-indicating HPLC method for the simultaneous determination of flurbiprofen and triclosan in nanogel formulation

HPLC Instruments and Analytical Conditions

- UV-VIS detector
- Solvent delivery unit (LC-20AD)
- Degasser
- Column oven
- Auto sampler
- Communication bus module
- LabSolutions software installed in a desktop computer system

HPLC Instruments and Analytical Conditions

- The chromatographic fractionation was accomplished with Agilent ZORBAX SB-C18 column (5 µm, 4.6 X 250 mm)
- Mobile phase: Mixture of acetonitrile and 8X10-4 mol/L citric acid, pH 3.13 (90:10, v/v)
- Elution mode: Isocratic
- Flow rate: 0.3 mL min–1
- The UV detection: 242 nm

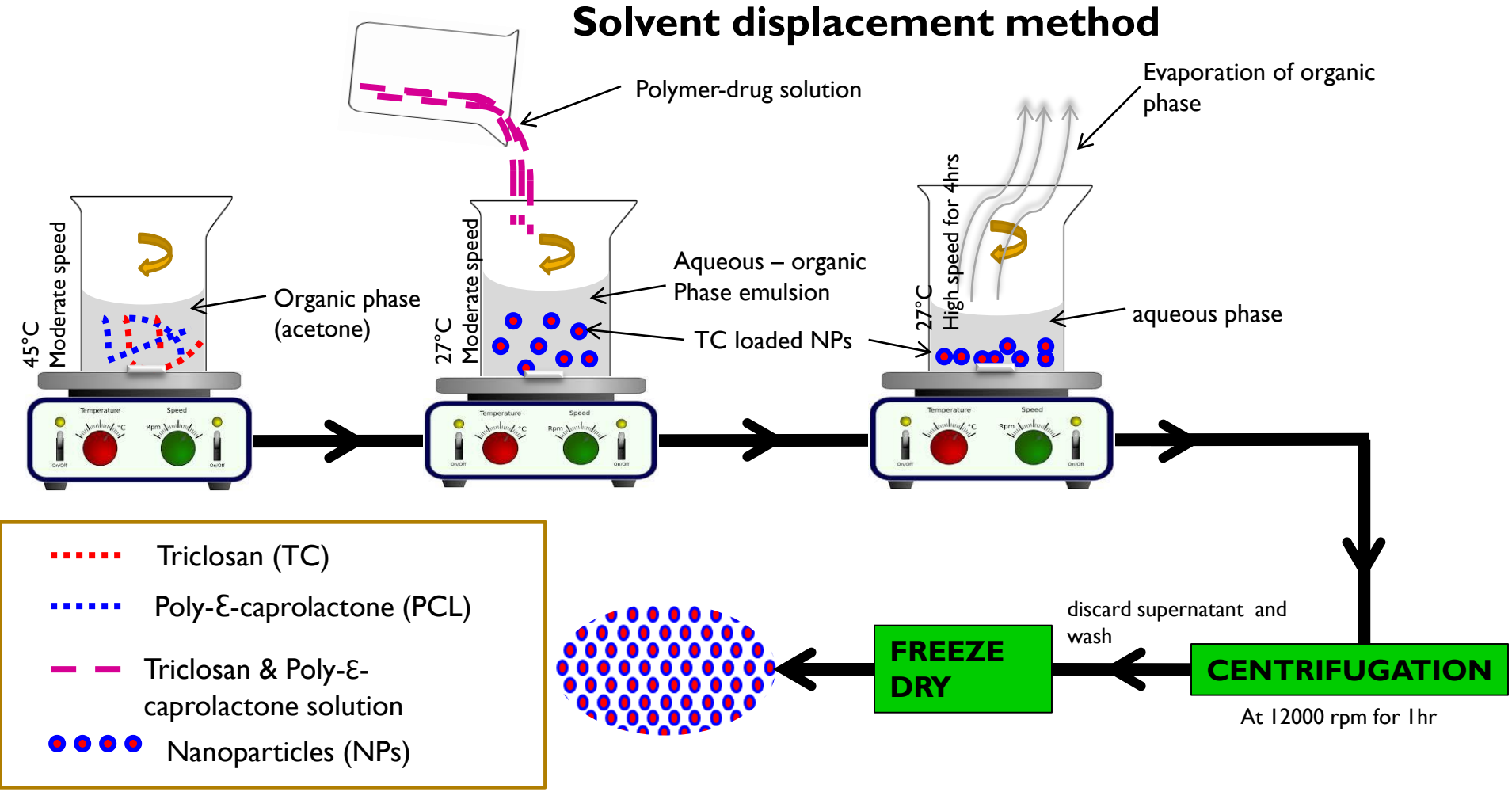
Samples preparation

Standard solutions for calibration curves was prepared in methanol comprising the following concentrations:

Flurbiprofen (μg mL^{-1})	Triclosan (μg mL^{-1})
15	10
30	20
60	40
90	60
120	80
180	120
240	160
300	200

Samples preparation

1st stage

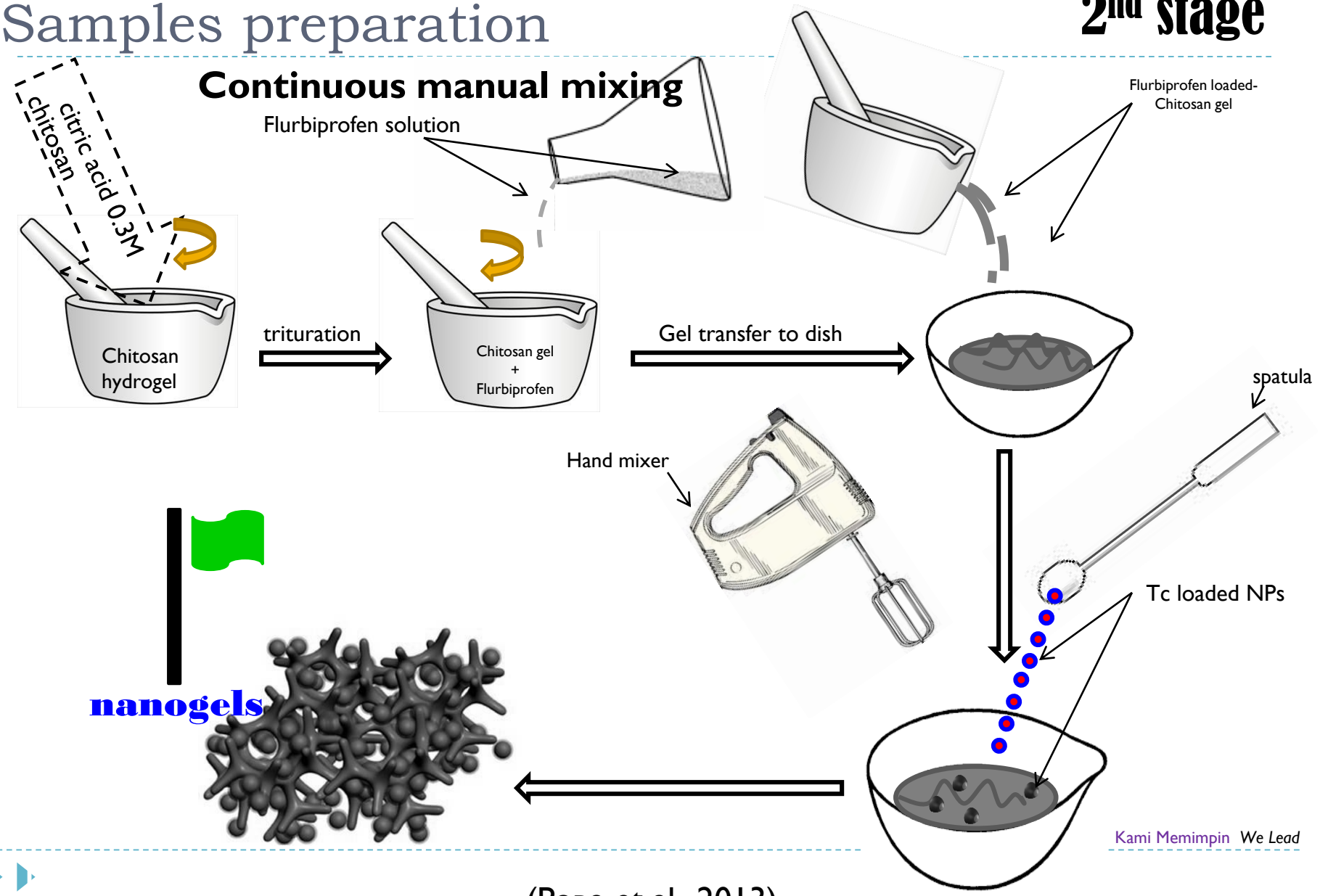


(Vega et al., 2006; Espuelas et al., 1997)

Samples preparation

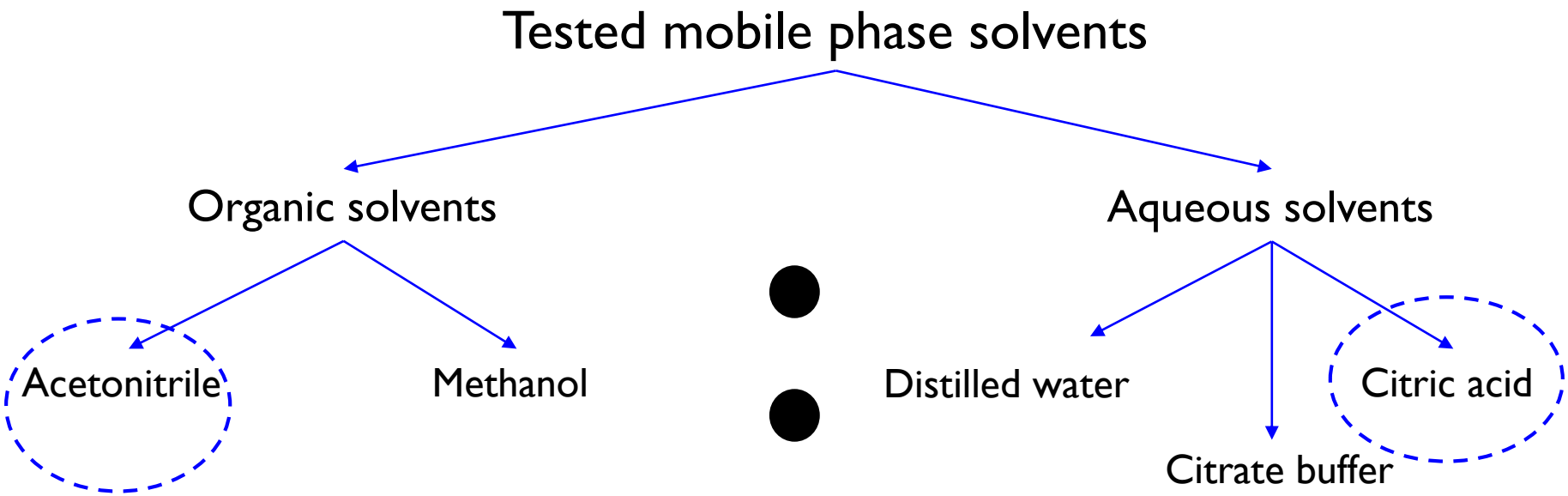
2nd stage

Continuous manual mixing



(Popa et al., 2013)

Method development

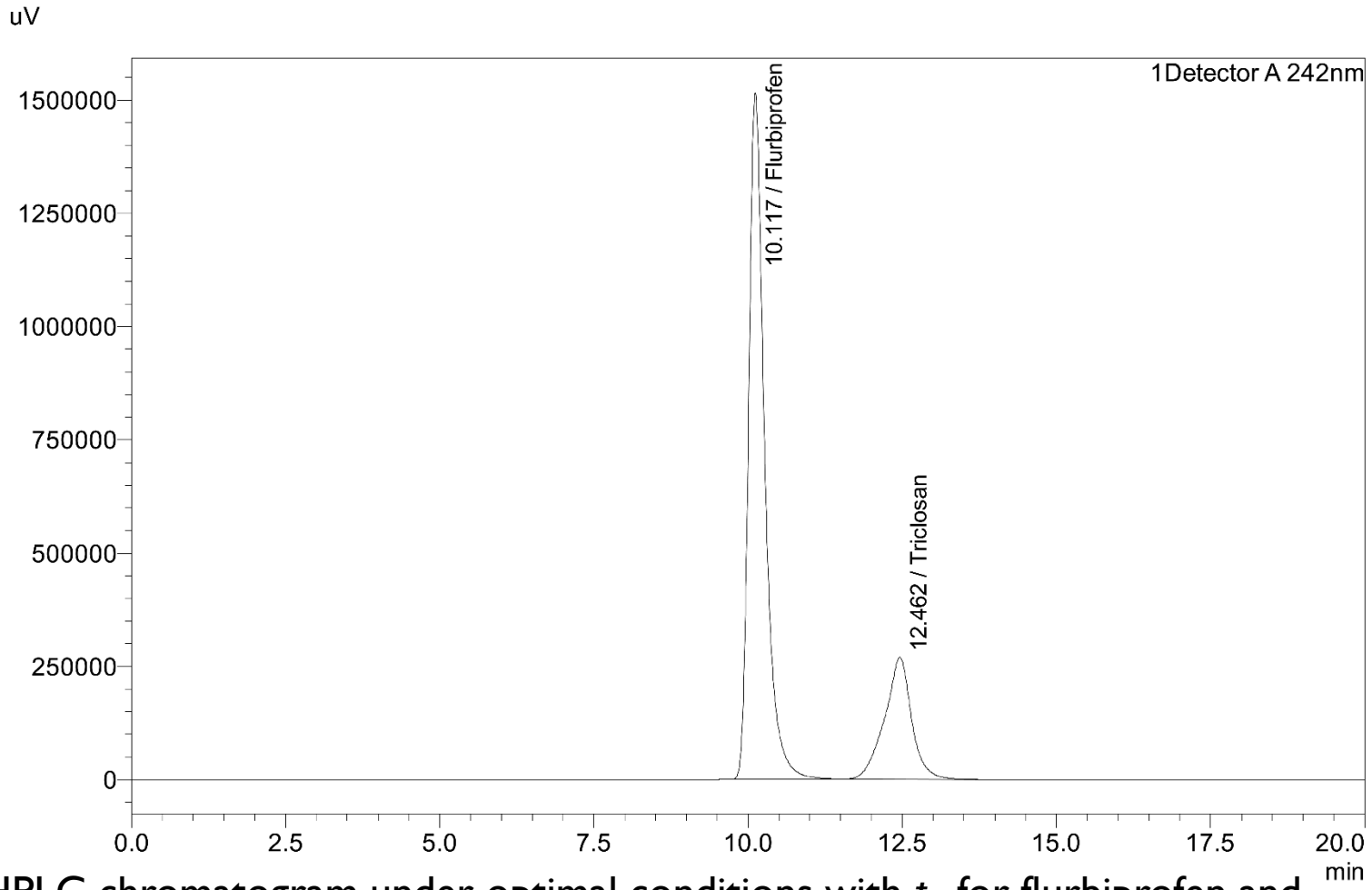


Acetonitrile and 8X10^{-4} mol/L citric acid, pH 3.13 (90:10, *v*/*v*)

Determining parameters:

Separation of analyte peaks from the excipients, impurities, or other agents; reproducibility of retention times (t_R); peak's sharpness, resolution, and symmetry

Method development



The HPLC chromatogram under optimal conditions with t_R for flurbiprofen and triclosan at 10.1 min and 12.5 min, respectively

Method validation

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

VALIDATION OF ANALYTICAL PROCEDURES:
TEXT AND METHODOLOGY
Q2(R1)

Validation characteristics were assessed

- Specificity
- Accuracy
- Linearity
- Precision
- Range
- System suitability
- Limit of detection (LOD)
- Limit of quantification (LOQ)

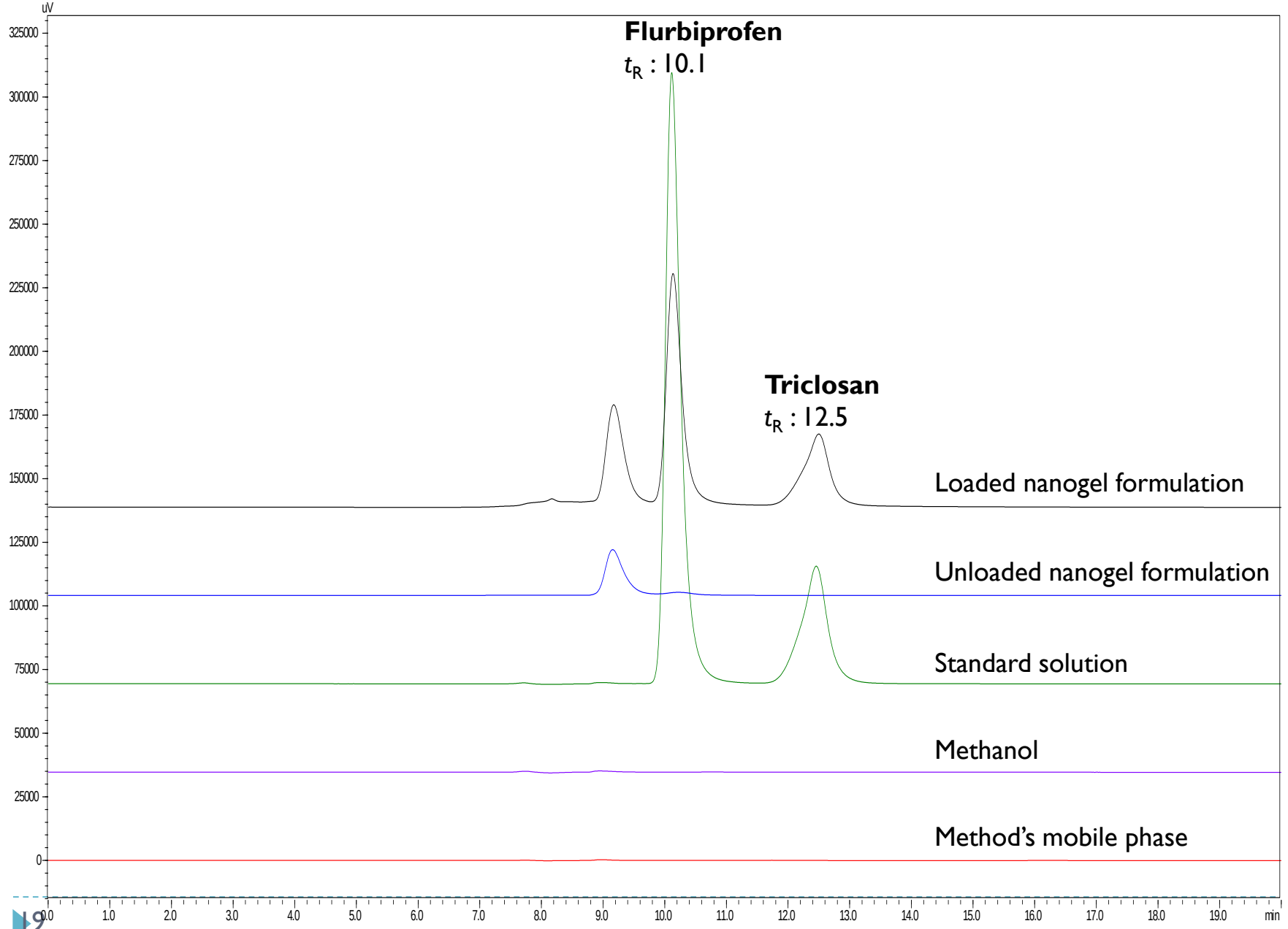
Method validation results

Parameter	Flurbiprofen	Triclosan	Acceptable limits
System suitability (*n* = 10)			
t_R (±SD)	10.11 (0.01)	12.65 (0.03)	–
Tailing factor (±SD)	1.45 (0.07)	0.98 (0.04)	≤ 2
Number of theoretical plates (±SD)	8160 (592)	4575 (238)	> 2000
Resolution (±SD)	2.50 (0.13)	3.97 (0.30)	> 2
Injection repeatability[1] (% RSD)	0.14	0.09	≤ 1%
Linear regression			
Regression equation	y = (149431) x + (-118634)	y = (67450.0) x + (59777.6)	–
Slope	149431	67450.0	–
y-intercept	-118634	59777.6	–
R^2	0.9991	1	> 0.999
Ranges (µg mL^{-1})	15 - 300	10 - 200	–
Accuracy			
Average recovery (%)	97.93[2a], 103.18[2b]	96.07[2a], 99.28[2b]	100 ± 2%
Average R.E. (%)	-2.07[3a], 3.18[3b]	-3.93[3a], -0.72[3b]	± 2%
Average RSD (%)	0.19[4a], 0.76[4b]	0.06[4a], 0.30[4b]	–
Precision			
Intra-day precision[5] (% RSD)	0.61	0.20	≤ 2%
Inter-day precision[6] (% RSD)	0.38	0.33	≤ 2%
LOD (µg mL^{-1})	0.01	0.02	–
LOQ (µg mL^{-1})	0.03	0.06	–

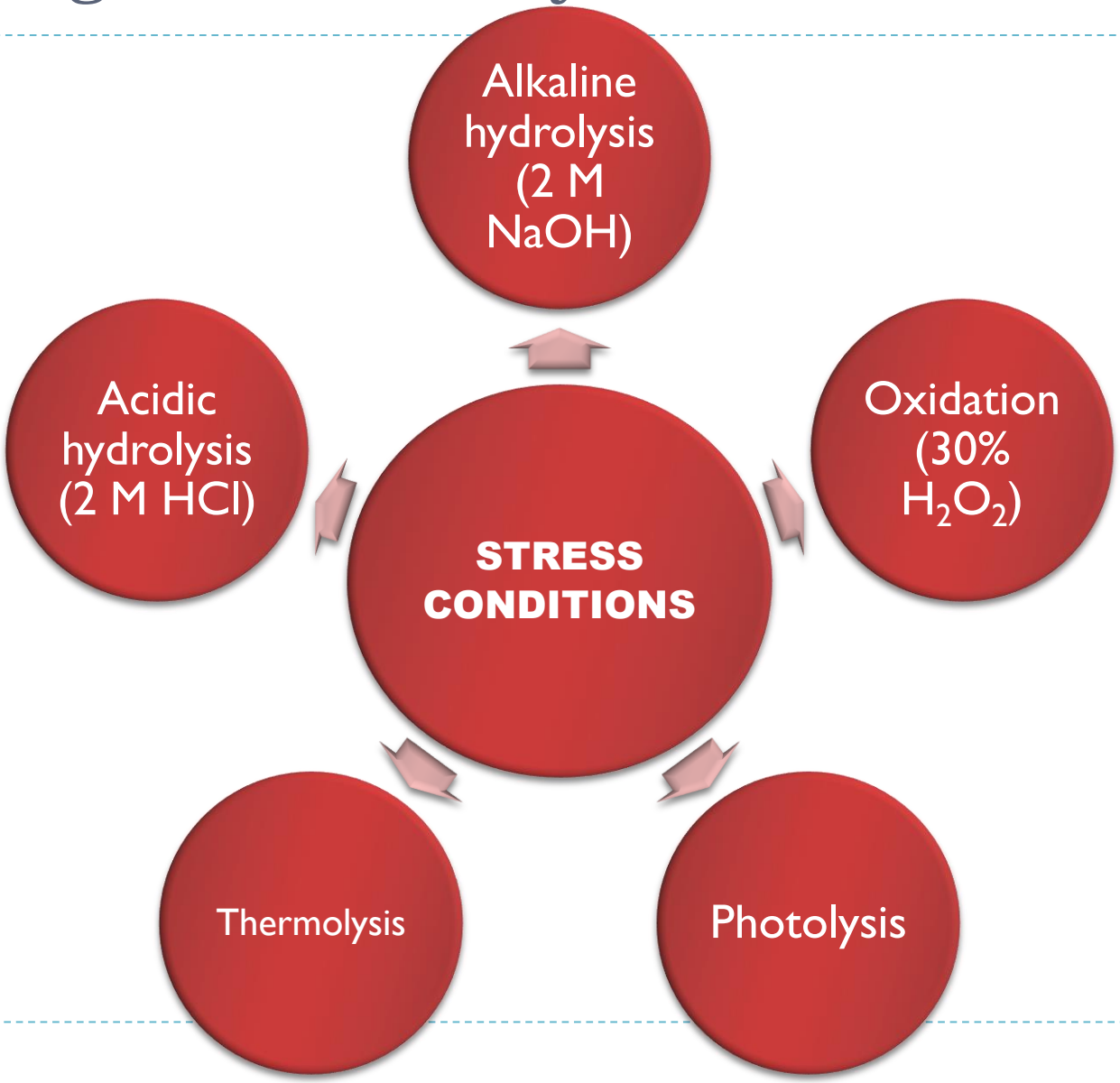
Interpretation of method validation results

- $t_{R,}$ retention time (in minutes); SD, standard deviation; RSD, relative standard deviation; R.E., relative error; LOD, limit of detection; LOQ, limit of quantification
- [1] RSD values for injection repeatability of the method (n = 10)
- [2a] Percentage recovery values of analyte from nanogel formulation (n = 6)
- [2b] Percentage recovery values of analyte from spiked samples at three concentration levels (n = 6)
- [3a] Percentage relative error values of analyte from nanogel formulation (n = 6)
- [3b] Percentage relative error values of analyte from spiked samples at three concentration levels (n = 6)
- [4a] RSD values for recoveries of analyte from nanogel formulation (n = 6)
- [4b] RSD values for recoveries of analyte from spiked samples at three concentration levels (n = 6)
- [5] RSD values for precision of six samples of the analytes analysis within the same day (n = 6)
- [6] RSD values for precision of analytes samples analysis for six consecutive days (n = 6)

Method validation: Specificity



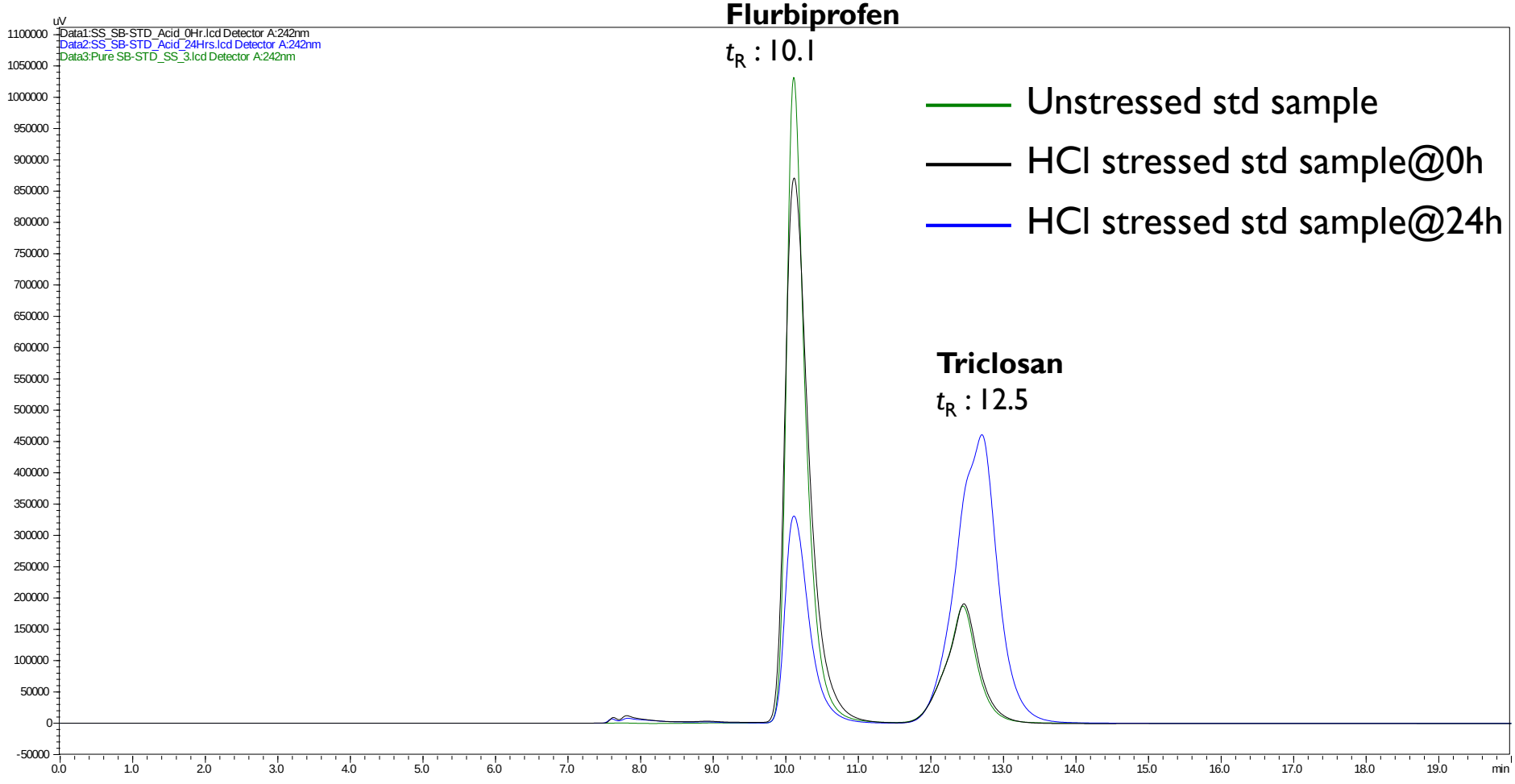
Stress degradation study



Results for stress degradation studies

		Standard		Nanogel formulation	
		Recovery (%) (±SD, n = 3)		Recovery (%) (±SD, n = 3)	
Analyte	Degradation condition	At 0 h	At 24 h or 2 h for heat	At 0 h	At 24 h or 2 h for heat
FLB	No treatment	99.56 ± 0.03	99.27 ± 0.09	99.23 ± 0.02	99.32 ± 0.09
	Acidic (2 M HCl)	104.06 ± 0.02	39.93 ± 0.19	95.19 ± 0.04	54.40 ± 0.01
	Alkaline (2 M NaOH)	102.82 ± 0.01	102.81 ± 0.06	98.18 ±0.01	105.22 ± 0.03
	Oxidative (30% H_2O_2)	104.19 ± 0.04	99.52 ± 0.21	97.85 ± 0.02	96.47 ± 0.02
	Photolytic (at a wavelength of 365 nm)	98.21 ± 0.02	98.43 ± 0.01	95.08 ± 0.04	95.08 ± 0.01
	Heat (at 80°C)	99.15 ± 0.08	110.83 ± 0.03	99.22 ± 0.07	98.16 ± 0.01
TCS	No treatment	99.90 ± 0.18	99.87 ± 0.21	98.82 ± 0.15	98.95 ± 0.12
	Acidic (2 M HCl)	105.82 ± 0.07	327.26 ± 0.2	95.56 ± 1.07	283.44 ± 0.13
	Alkaline (2 M NaOH)	103.73 ± 0.16	103.70 ± 0.13	97.70 ± 0.15	98.43 ± 0.21
	Oxidative (30% H_2O_2)	103.23 ± 0.09	101.16 ± 0.06	95.01 ± 0.05	95.67 ± 0.09
	Photolytic (at a wavelength of 365 nm)	106.85 ± 0.09	105.32 ± 0.05	98.95 ± 0.54	93.45 ± 0.08
	Heat (at 80°C)	99.92 ± 0.18	119.20 ± 0.08	98.86 ± 0.21	98.28 ± 0.12

Results for stress degradation studies



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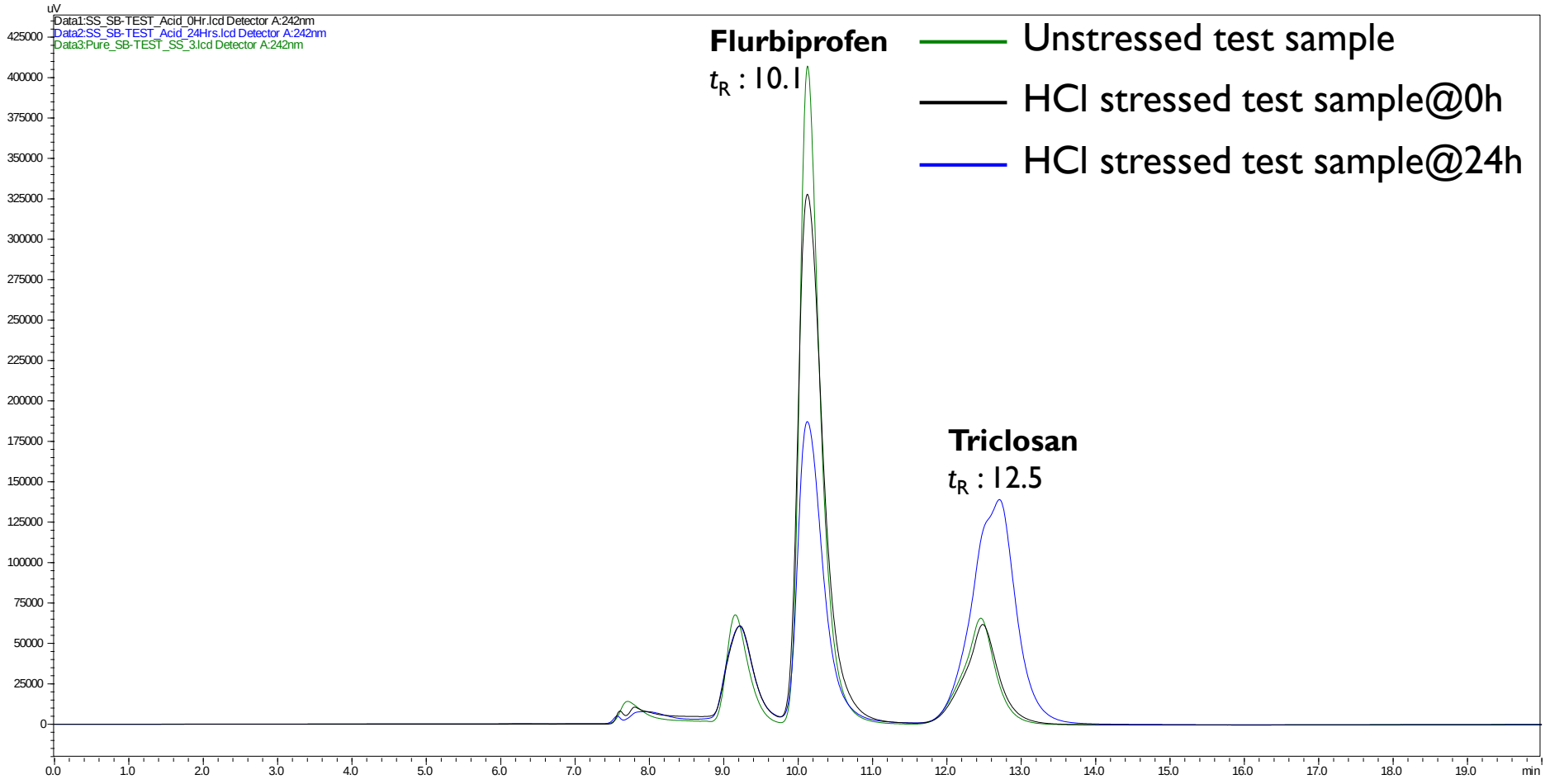

Conclusion

- A new, simple, specific, accurate and stability-indicating HPLC method for the quantification of flurbiprofen and triclosan was successfully developed and validated.
- The analytes were extracted from the nanogel formulation and quantified without any interference from the excipient constituents or impurities.
- The stress degradation studies revealed that acidic hydrolysis exert antagonistic and synergistic effects on flurbiprofen and triclosan, respectively.
- The developed method is suitable for simultaneous determination of flurbiprofen and triclosan in dental formulations and can be utilised for routine quality control evaluations.

Thank You!

Questions are most welcome