UNIVERSITY of LIEGE

THE DEVELOPMENT OF LC GENERIC ANALYTICAL METHODS TO FIGHT COUNTERFEIT NSAIDS USING DESIGN SPACE OPTIMISATION STRATEGY

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Agenda

1. Introduction
2. Objectives
3. Methodology
4. Results
5. Conclusion

1. Introduction

Pictures

A picture of results published from a large scale study was challenging.

- 1/3 of antimalarial medicines sampled in three African nations were found to be substandard or counterfeit.

- 80 % of counterfeit reported in some African's regions.

Sources : USP-USAID-WHO report: http://www.usp.org/worldwide , Feb. 8, 2010.

1. Introduction

Consequences

Public health

- Therapeutic failure,
- Emergence of resistance,
- Death,
- Lack of health security.

1. Introduction

Consequences

Socio-economic

- Impoverishment of the population,
- Loss in market share,
- Loss of credibility,
- The government / other worldwide organisations: loss of jobs

2. Objectives

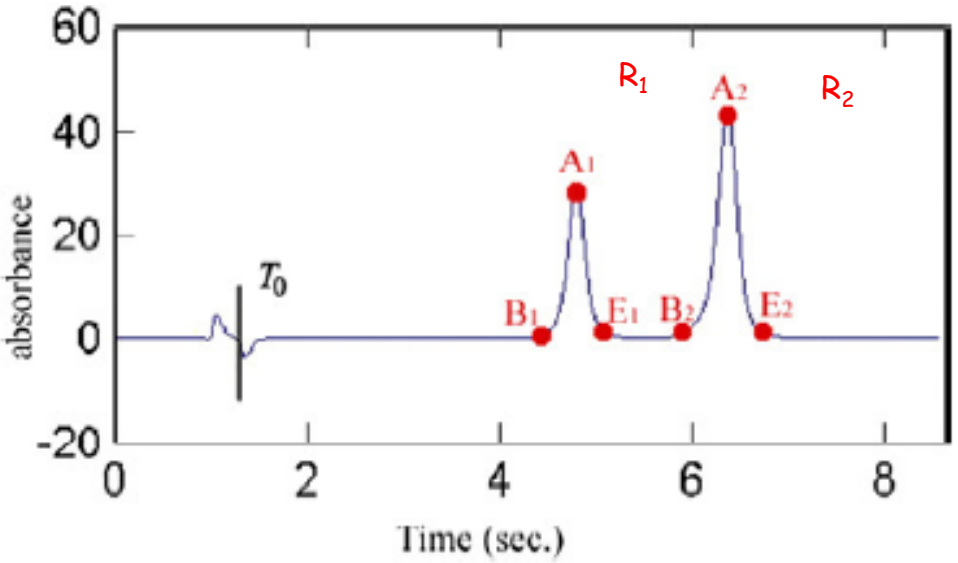
Development of generic analytical methods

❑ Detecting and tracing (screening) simultaneously several substances belonging to a pharmacological group / with substances of interest

- In this context, interest of separative techniques such as HPLC-UV
- Combination of experimental design with *Design Space* optimization strategy

❑ Presentation of the results of NSAIDS (Non steroidal anti-inflammatory drugs) method

3. Methodology



- Analytical responses :

Peak retention time RT at
Begining 'B', Apex 'A', End 'E'

- Modelled responses : Log (w)

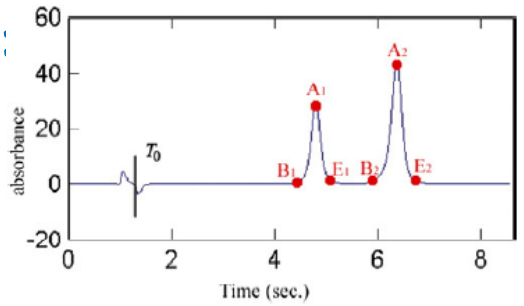
$w_B = k_R - k_B$, $w_E = k_E - k_R$ and $w_A = k_R$

P. Lebrun et al., Chemom. Intell. Lab Sys., 91, pp. 4-16 (2008).

3. Methodology

❖ Criteria to evaluate the quality of a chromatogram :

Separation (S) between peaks : $RT_{B\ peak2} - RT_{E\ peak1}$



❖ Design space (DS) : area in which the probability for a criteria meet the acceptance limits be superior to the selected level quality (π)

$$DS = \left\{ \mathbf{x}_0 \in \chi \middle| E_\theta[P(S > \lambda)|\hat{\theta}] \geq \pi \right\}$$

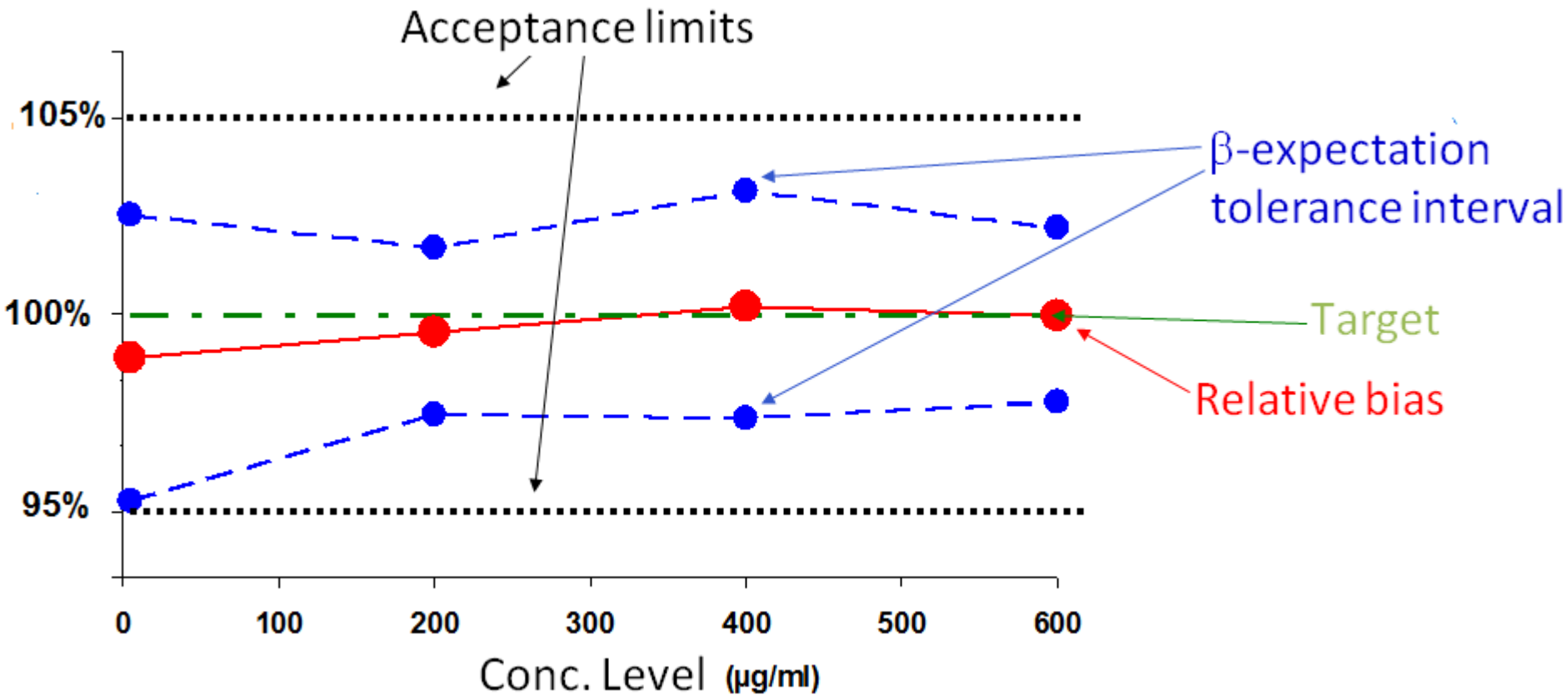
$\lambda = 0$ (acceptance limit)

P. Lebrun et al., Chemom. Intell. Lab Sys., 91, pp. 4-16 (2008).

3. Methodology

❖Method validation : total error approach

Accuracy profile



Hubert et al., J. Pharm. Biomed. Anal., 45, pp.82-96 (2007)

3. Methodology

18 NSAIDs + 9 molecules of interest

18 NSAIDs	
Ibuprofen	Sulindac
Diclofenac	Phenylbutazone
Mefenamic acid	Flurbiprofen
Ketoprofen	Suprofen
Nimesulide	Naproxen
Dextropropoxyphene	Tiaprofenic acid
Niflumic acid	Fenoprofen,
Tenoxicam	Indomethacin
Piroxicam	Acetylsalicylic acid

9 molecules of interest
Chlorzoxazone
Caffeine
Paracetamol
Salicylic acid
Nipagine
Nipasol
Butylated hydroxyanisole
Butylated hydroxytoluene
Sodium benzoate

The 27 materials were divided into 5 groups based on the pharmaceutical form of the NSAIDs

3. Methodology

Group 1 Compounds often presented in combination in tablet or capsule	Paracetamol – acetylsalicylic acid – ibuprofen – diclofenac – chlorzoxazone – dextropropoxyphen – nimesulid – ketoprofen – mefenamic acid – salicylic acid – caffeine
Group 2 Compounds presented in combination in syrup and suspension	Paracetamol – ibuprofen – nimesulid – mefenamic acid – nipagine – nipasol – sodium benzoate – BHA – BHT
Group 3 NSAIDs found alone in tablet or capsule	Indomethacine – tenoxicam – piroxicam – flurbiprofen – tiaprofenic acid – naproxen – suprofen – sulindac – phenylbutazone – fenoprofen – niflumic acid

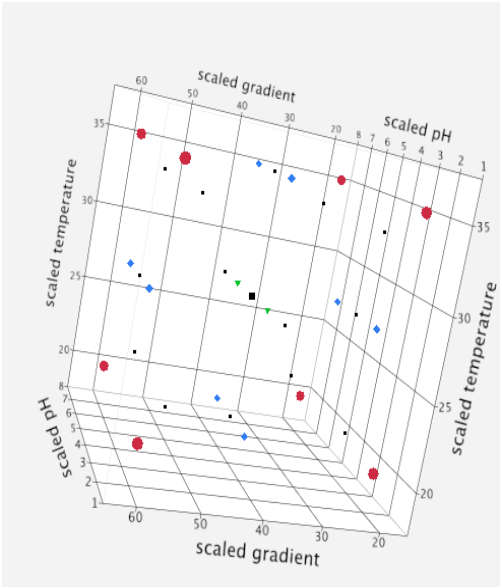
3. Methodology

Group 4 Pharmaceutical combinations presented in tablet or capsule	1	Paracetamol - acetylsalicylic acid - caffeine
	2	Paracetamol – ibuprofen
	3	Paracetamol – diclofenac
	4	Paracetamol – diclofenac – chlorzoxazone
	5	**Paracetamol – ibuprofen - caffeine**
	6	Paracetamol – mefenamic acid
	7	Paracetamol - dextropropoxyphen
	8	Paracetamol – dextropropoxyphen - caffeine
Group 5 Compounds presented in syrup and suspension	1	Paracetamol – nipagine – nipasol – sodium benzoate – BHA - BHT
	2	Paracetamol – ibuprofen – nipagine – nipasol – sodium benzoate – BHA - BHT
	3	Ibuprofen – nipagine – nipasol – sodium benzoate – BHA - BHT
	4	Nimésulide – nipagine – nipasol – sodium benzoate – BHA – BHT
	5	Mefenamic acid – nipagine – nipasol – sodium benzoate – BHA - BHT

3. Methodology

Factors	Levels						
pH	1.85	2.42	3.14	4.42	5.71	6.42	7
Gradient time (TG, min)	-	20	24.5	40	55.5	60	-
Temperature (Temp, °C)	-	20	21.7	27.5	33.3	35	-

DOE : **central composite design** with 3 factors



32 experimental conditions.

4. Results

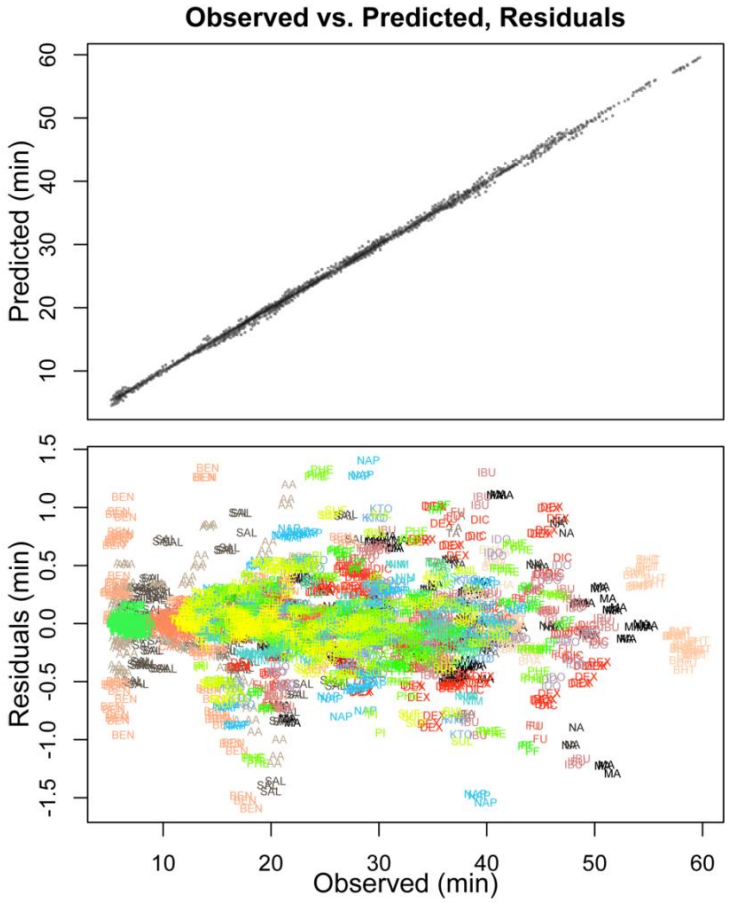
- Modelling : $Y = XB + E$

$$Log\,(w_{(A)}) = \beta_0 + \beta_1 * pH + \beta_2 * pH^2 + \beta_3 * pH^3 + \beta_4 * pH^4 + \beta_5 * T_G + \beta_6 * T_G^2 + \beta_7 * T° + \beta_8 * T°^2 + \beta_9 * pH * T_G + \beta_{10} * pH * T° + \beta_{11} * T_G * T° + \beta_{12} * pH * T_G * T° + \epsilon$$

- Validation of the models :

$R^2_{Adjusted}$ and residual graphes

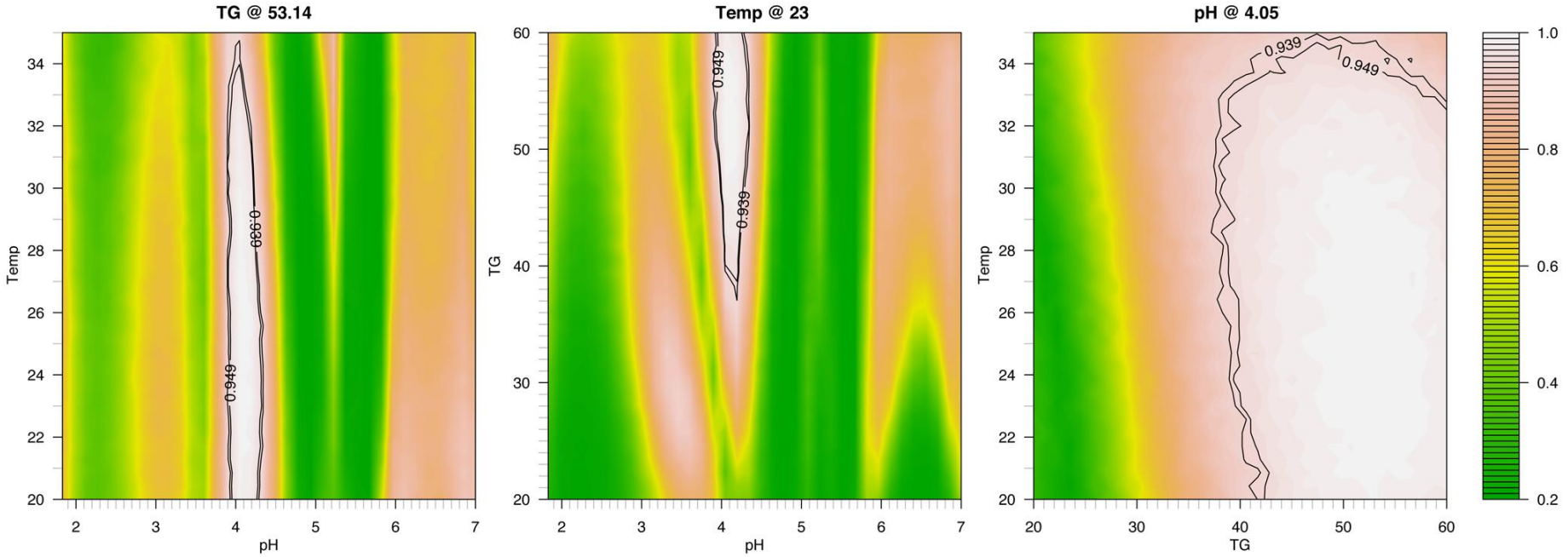
$R^2_{Adjusted} \sim 1$



4. Results

Prediction from DS

Group 2

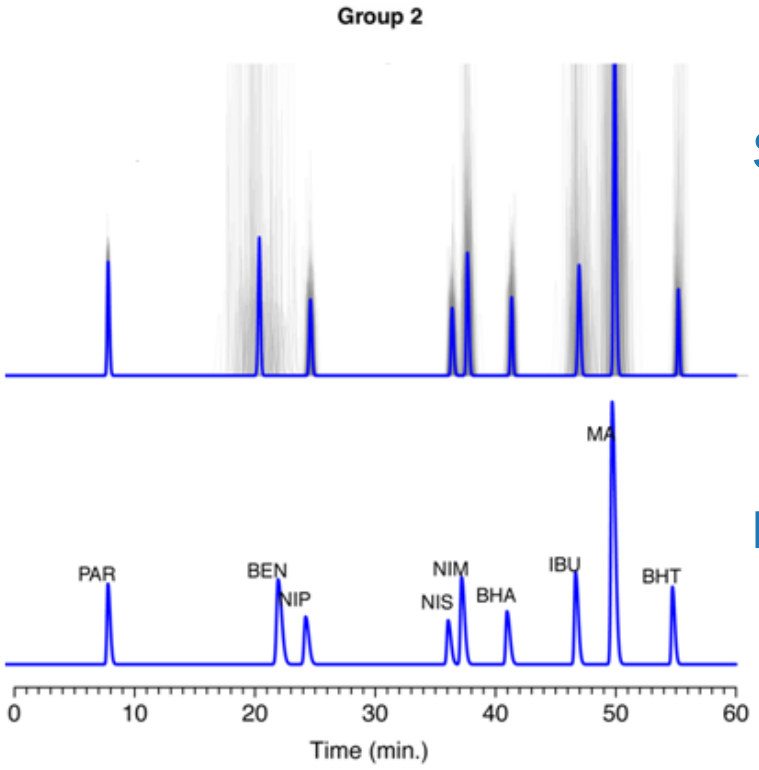


Optimal conditions	Optimal P(S>0)	pH	Gradient time (minutes)	Temperature (° C)
Group 1	67. 0%	3.05 (2.80-3.10)	49. 30 (40.00-60.00)	34. 5 (20.0-35.0)
Group 2	94. 9%	4.05 (3.90-4.30)	53. 14 (40.00-60.00)	23. 0 (20.0-35.0)
Group 3	23. 0%	7.00 (6.90-7.00)	60. 00 (57.00-60.00)	21. 7 (20.0-27.0)
Group 4	99. 9%	3.00 (1.83-3.50)	20. 00 (20.00-30.00)	27. 0 (20.0-35.0)
Group 5	99. 9%	6.14 (6.05-6.20)	35. 00 (34.00-36.00)	29. 4 (23.0-30.0)

4. Results

Chromatogram experimental

Validation of the optimal condition for group 2. (Grey) simulations showing the uncertainty of prediction.



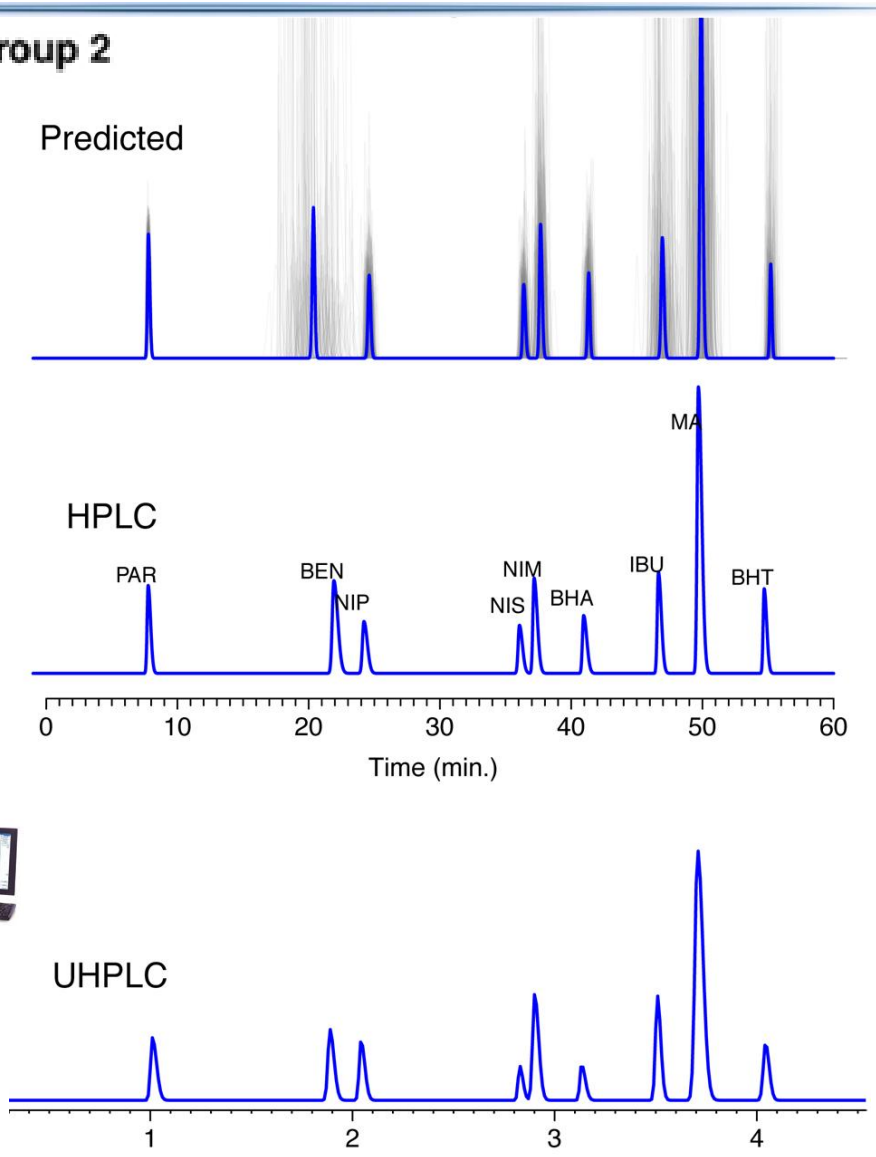
LC conditions: Analytical column: XBrigde (250 x 4.6 mm; i.d.) packed with octadecyl silica particles (5 µm dp). Mobile phase: mixture of methanol and ***20mM ammonium formate buffer at pH 4.05****. Gradient increased linearly methanol proportion from* ***15 % to 95 % in 53,14 minutes****. Flow rate: 1.0 mL.min-1, column temperature: 23°C. UV-detection: 220 nm.*

4. Results

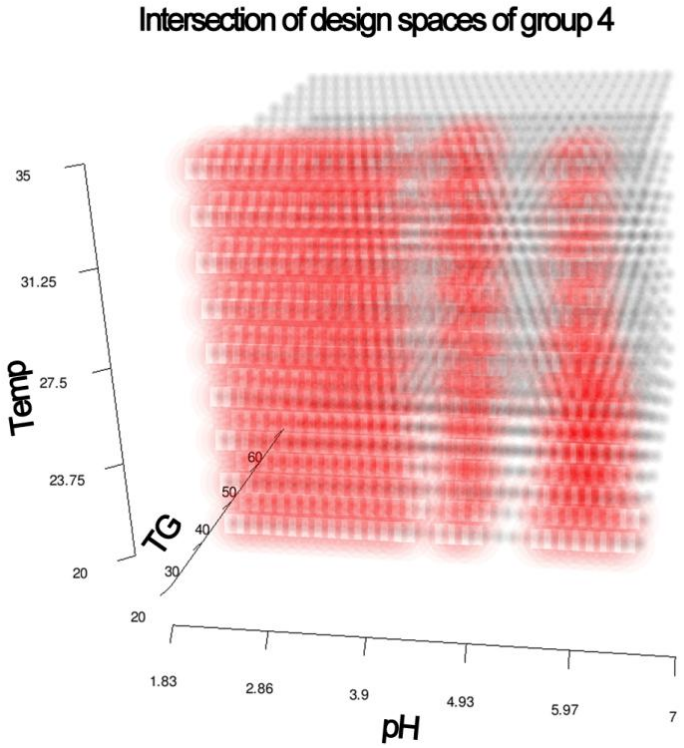
HPLC transfer to UHPLC

Compound	HPLC		UHPLC
	Pred. RRT	Obs. RRT	Obs. RRT
PAR	0.141	0.146	0.150
BEN	0.369	0.406	0.396
NIP	0.446	0.447	0.438
NIS	0.659	0.662	0.657
NIM	0.682	0.682	0.680
BHA	0.749	0.750	0.743
IBU	0.850	0.854	0.849
MA	0.904	0.910	0.908
BHT	1.000	1.000	1.000

UHPLC conditions: Analytical column: Acquity BEH *(50 mm×2.1 mm i.d., particle size 1.7 µm). Flow rate: 0.613 µL.min-1*

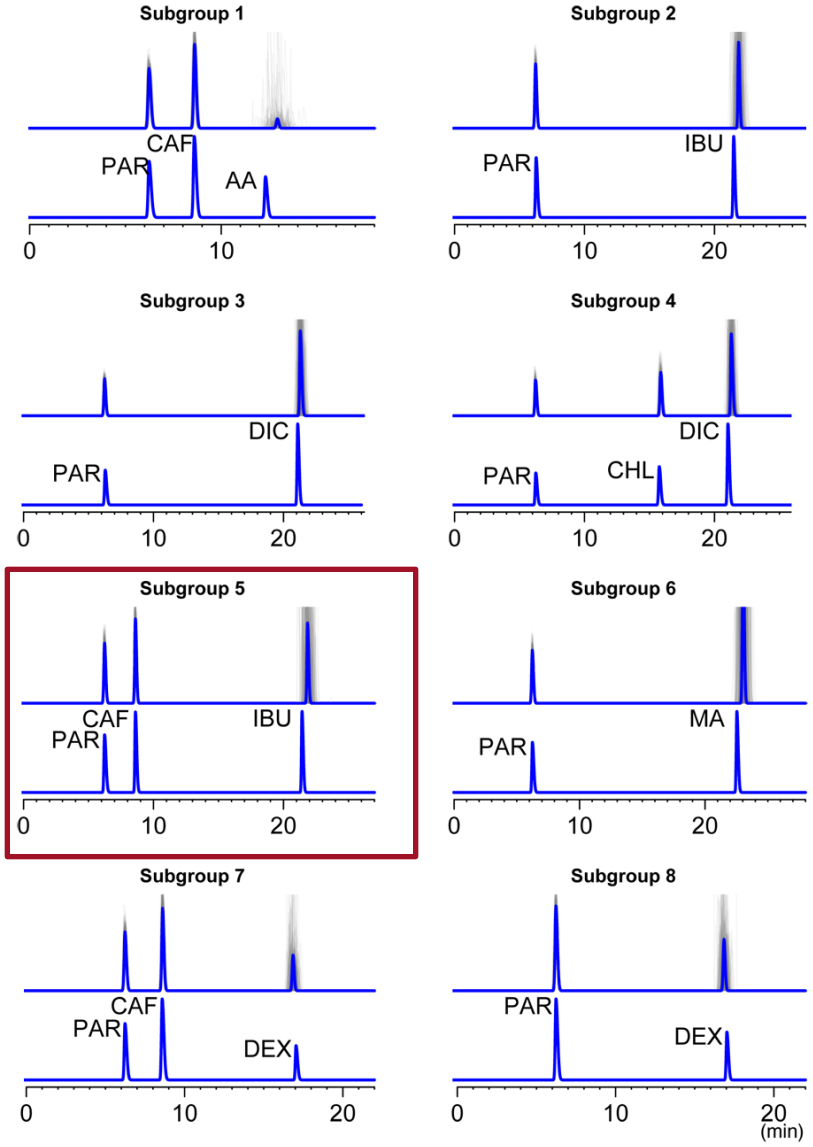


4. Results



Optimal condition

P(S>0) 99,9 %, π 0.95
pH 3 (1.83 – 3.5)
Gradient time 20 min (20 – 30)
Température 27 °C (20.0 – 35.0)



4. Results

Method validation

Accuracy profiles for quantitative methods validation

(Red) bias (%).
(Black) acceptance limit (±5%).
(Blue) 95% b-expectation tolerance interval.
(Green) individual measures.

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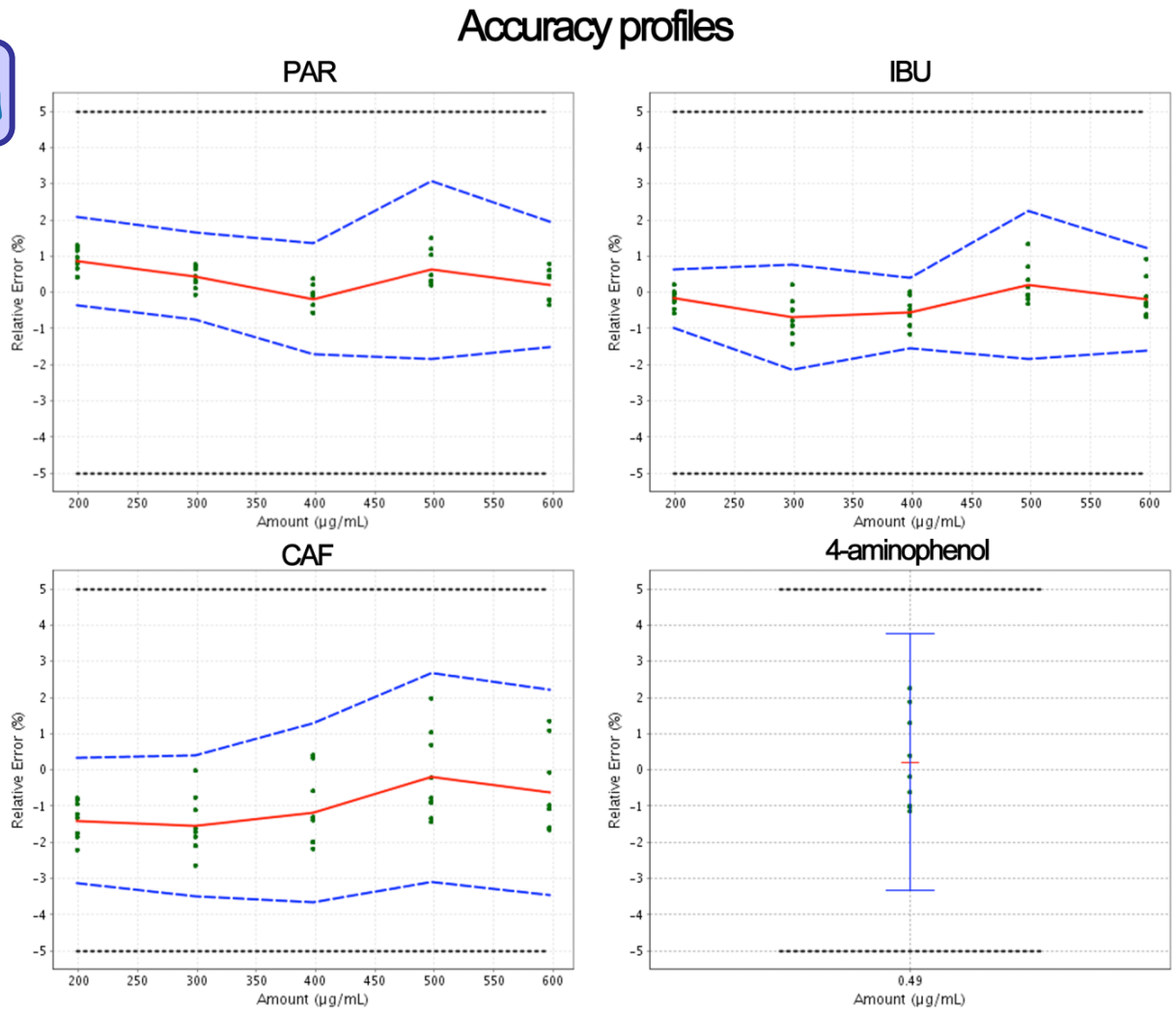
For the 4-aminophenol, a one-level calibration is used

0.1% of 4-aminophenol (0.5 µg/mL) in 100% paracetamol (500 µg/mL)
LLOQ : 0.1 µg/mL



Legend : PAR. Paracetamol-IBU. Ibuprofen - CAF. Caffeine

4. Results

Quantification

Coded specialities (brands)	Declared amount Content (mean% ± S.D.; n = 3)			Specifications (%) (European Medicines Agency)
	Paracetamol	Caffeine	Ibuprofen	
A	325 mg 98.4 ± 0.41	30 mg 90.7 ± 1.49	200 mg 103.7 ± 0.74	95.0 – 105.0
B	325 mg 100.0 ± 0.35	40 mg 94.7 ± 0.63	200 mg 103.0 ± 0.58	
C	200 mg 90.4 ± 0.22	40 mg 85.2 ± 0.79	400 mg 91.1 ± 0.73	
D	325 mg 78.2 ± 0.39	40 mg 74.5 ± 0.44	400 mg 77.9 ± 0.15	
E	325 mg 78.9 ± 0.28	40 mg 75.9 ± 0.31	400 mg 80.6 ± 0.35	

5. Conclusion

- Counterfeit of medicines is a crucial problem of public health. It is so important to develop analytical tools to support decision making of the legal authorities.

- The main objective of this work was to develop generic methods able to trace, screen and determine multiple NSAIDs and common associated molecules, in order to detect potential counterfeit drugs.

- DS strategy enables robust method development, confirmed by successful transfer method. these validated methods can easily used in quality control laboratory.

5. Conclusion

- Transfer/ Development of other methods are currently carried out with view on advantages allowing use them efficiently and continuously.

Thanks for your attention

- See our publications at: http://orbi.ulg.ac.be/; and our posters

Contact: jmbinze@student.ulg.ac.be

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