UNIVERSITE de LIEGE

New Methodology for the Development of Chromatographic Methods

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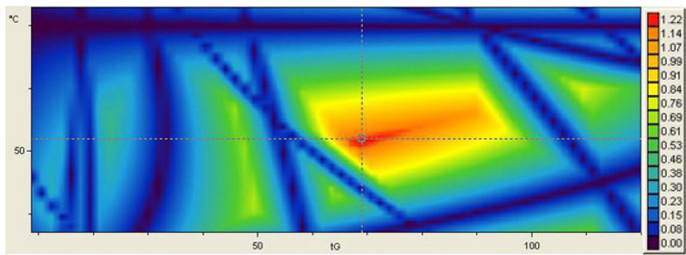
Outline

1. Introduction

- Rapid development of methods using commercially available products.
  - DryLab®
  - Chromsword®
  - ACD/LC simulator®
  - Osiris®

Chromatographic Theory:
→ Prediction of R_S



What probability of success?

1. Introduction

Regulatory requirement

International Conference on Harmonization (ICH)

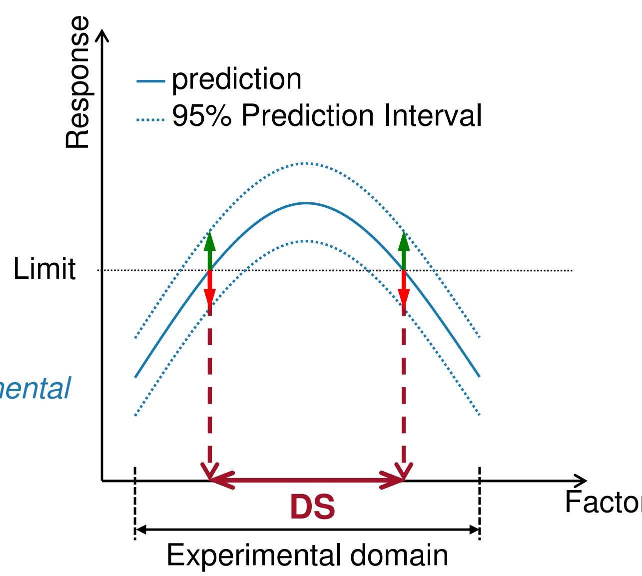
•ICH Q8(R2): Pharmaceutical development

➔ Quality by Design (QbD)

➔ Design Space (DS)

"*the multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide assurance of quality.*"

→ *DS is a sub-region of the experimental domain in which the objectives are reached with a defined probability.*



1. Introduction

Regulatory requirement

International Conference on Harmonization (ICH)

- ICH Q8(R2): Pharmaceutical development
  - ➔ Quality by Design (QbD)
  - ➔ Design Space (DS)

"*working within the DS is not considered as a change.*"

⇕

ICH Q2(R1): Analytical method validation

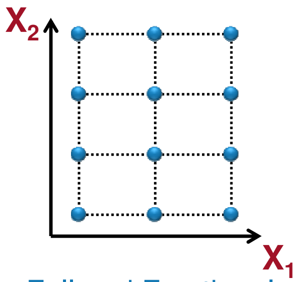
"The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage."

Outline

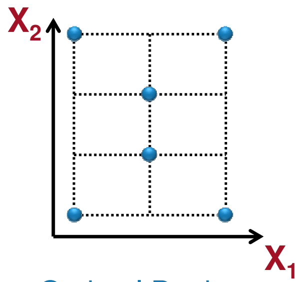
1. Introduction
2. The methodology
   - 2.1. Designs of Experiments and modeling
   - 2.2. Error propagation
   - 2.3. Automatic reading of chromatograms
3. Application of the methodology
   - 3.1. Mixture of 9 compounds
   - 3.2. Pharmaceutical formulation
   - 3.3. Improvement of EurPh Monography
   - 3.4. Application to Legal Toxicology Sciences
   - 3.5. Chiral separation
4. Conclusions

2.1. DoEs and Modeling

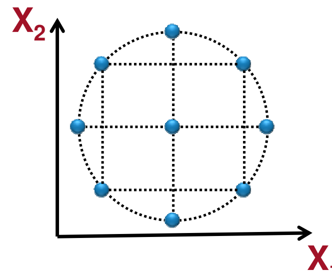
Model chromatographic behaviour through **Design of Experiments (DoEs)**.



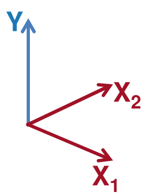
Full and Fractional Factorial Designs



Optimal Designs (D-optimal)



Central Composite Designs

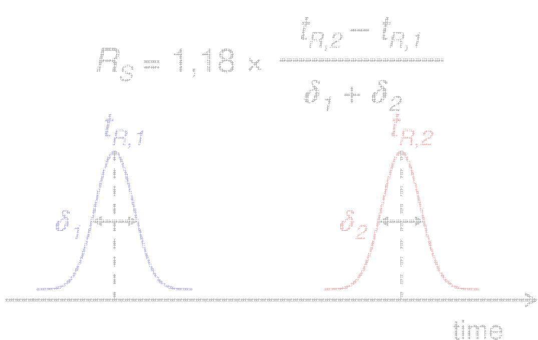


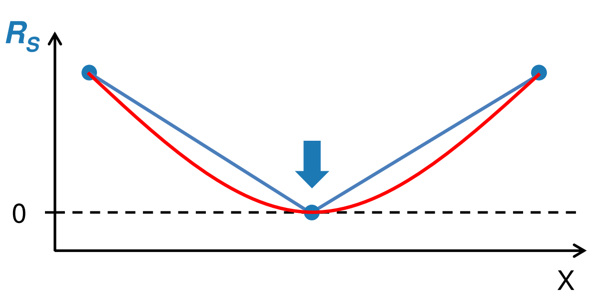
$$\underbrace{Y}_{\text{response}} = \beta_0 + \beta_1 \times \underbrace{X_1}_{\text{factors}} + \beta_2 \times X_2 + \ldots + \underbrace{\varepsilon}_{\text{error}}$$

2.1. DoEs and Modeling

Selection of a response

Selection of factors

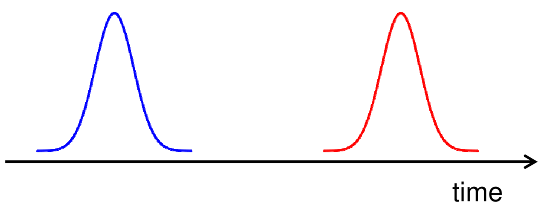


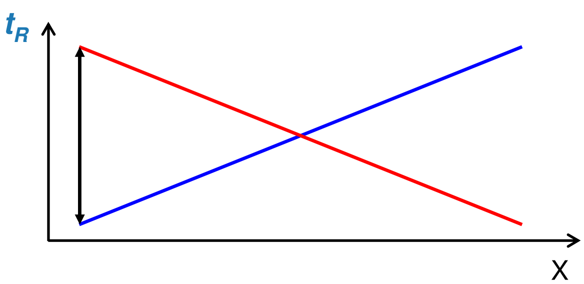


2.1. DoEs and Modeling

Selection of a response

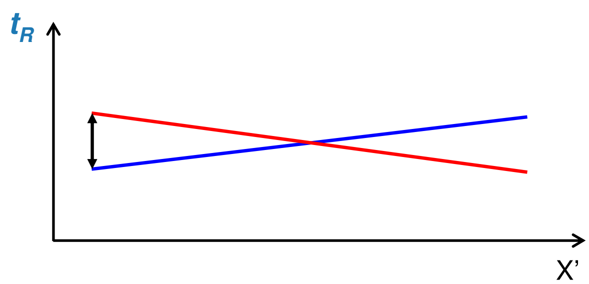
$\boxed{t_R = \boldsymbol{f}(X)}$



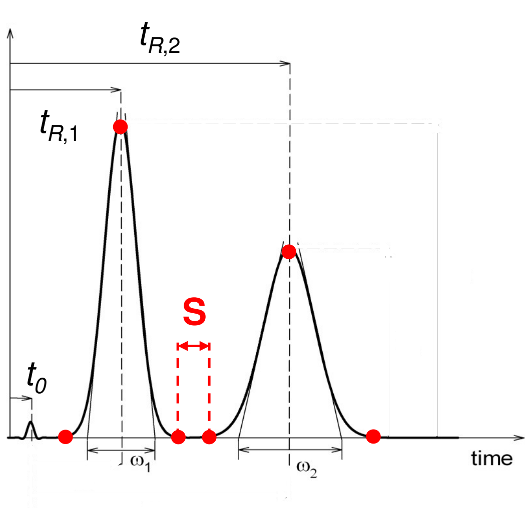


Selection of factors

Factors having most effect:
-Stationnary phase
-Organic modifier
-pH
-Gradient time
-Temperature
-…



2.1. DoEs and Modeling



Modeled Responses

- $\log(k)$ $k = (t_R - t_0)/t_0$
- $\log(\omega_{/2\ left})$
- $\log(\omega_{/2\ right})$

Mathematical Models

(multiples linear equation)
e.g. gradient time (t_G) and pH

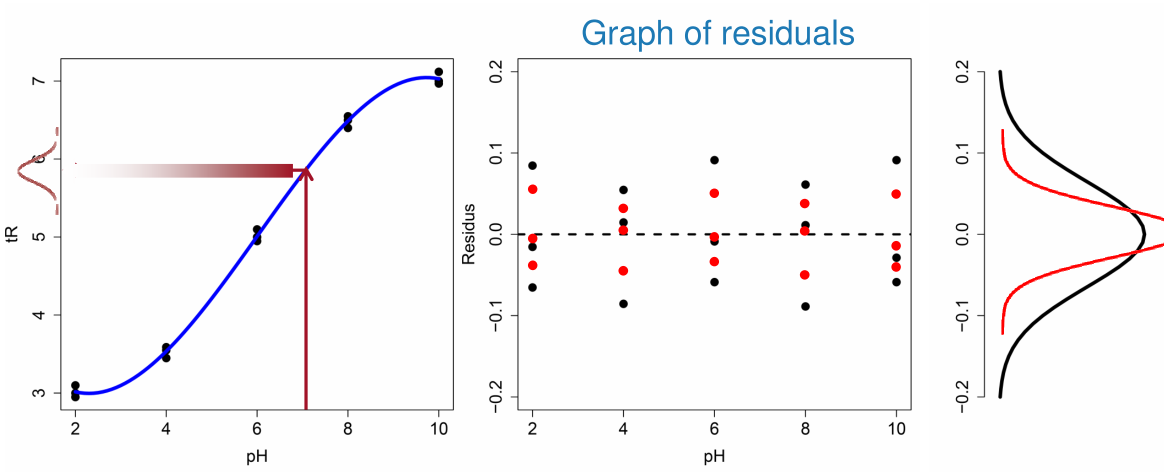
$$\log(k) = \beta_0 + \beta_1 \cdot t_G + \beta_3 \cdot t_G^2 + \beta_4 \cdot pH + \beta_5 \cdot pH^2 + \beta_6 \cdot pH^3 + \beta_7 \cdot pH^4 + \beta_8 \cdot t_G \cdot pH + \varepsilon$$

Optimised criteria

- R_S
- $S = t_{R,begin} - t_{R,end}$
- Analysis time
- …

P. Lebrun et al., Chemom. Intell. Lab. Syst. 91 (2008) 4.

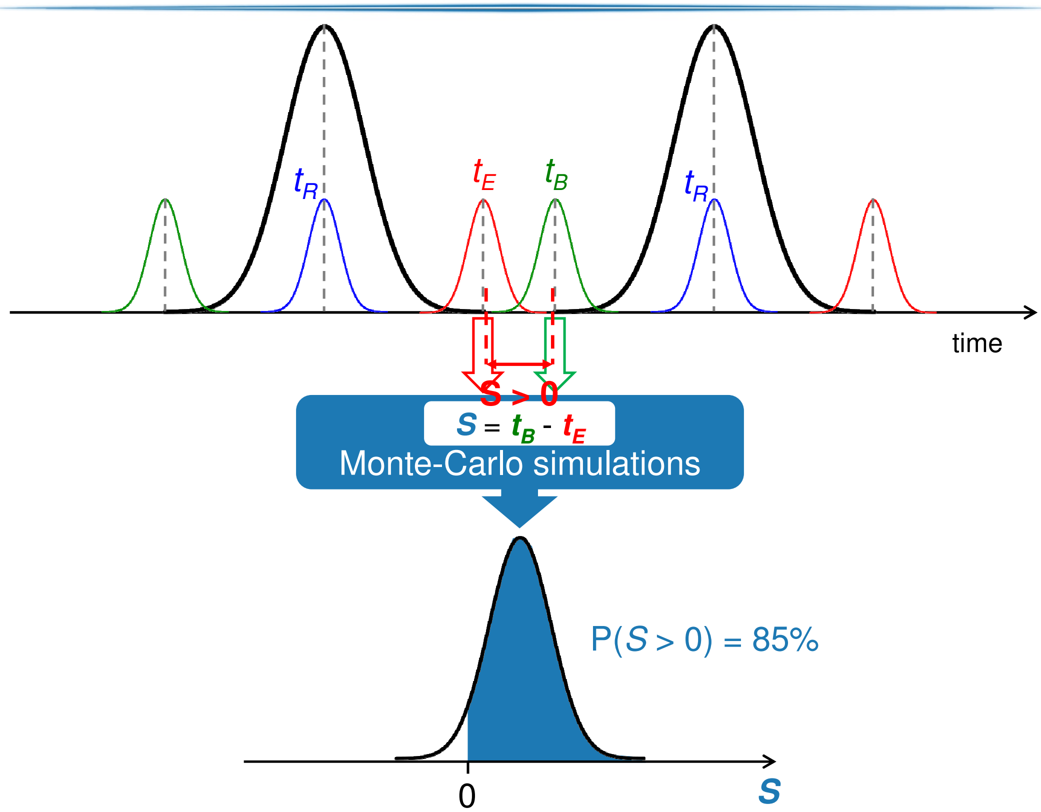
2.2. Error propagation and DS



$$t_R = 4{,}39 - 1{,}32 \times pH + 0{,}36 \times pH^2 - 0{,}02 \times pH^3 + \varepsilon$$

P. Lebrun et al., Chemom. Intell. Lab. Syst. 91 (2008) 4.

2.2. Error propagation and DS



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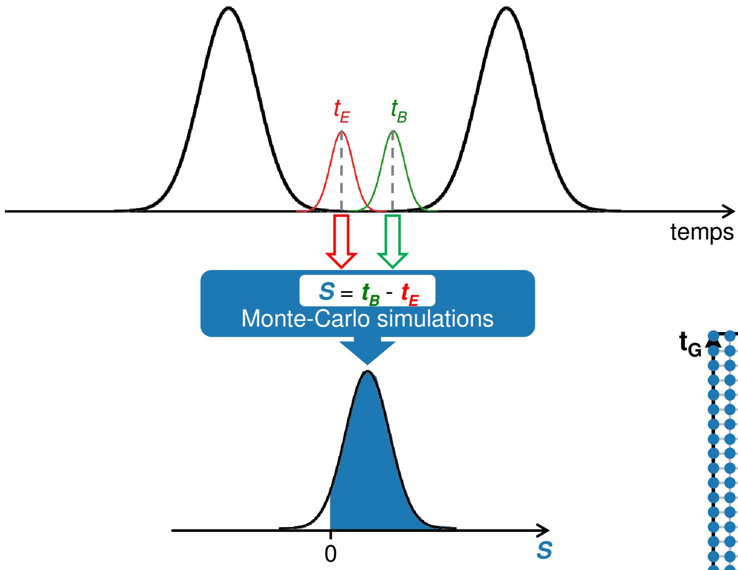
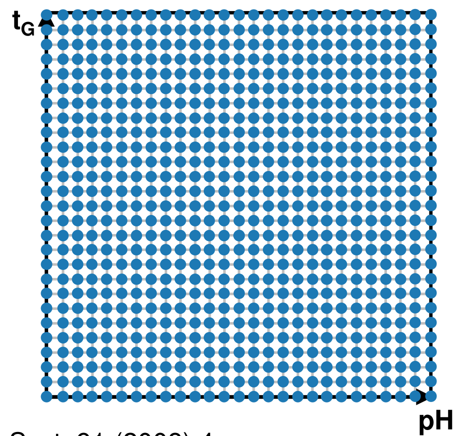




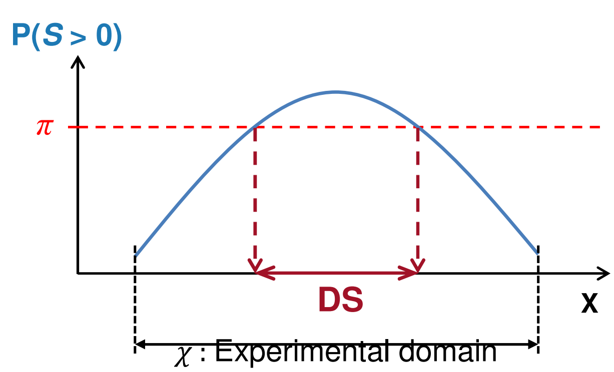
P. Lebrun et al., Chemom. Intell. Lab. Syst. 91 (2008) 4.

2.2. Error propagation and DS

The design space → region of probability of success

$$\mathrm{DS} = \{\underline{x_0 \in \chi} \,|\, E_{\hat{\theta}}[\underline{\mathrm{P}(S > \lambda)}|\hat{\theta}] \geq \underline{\pi}\}$$

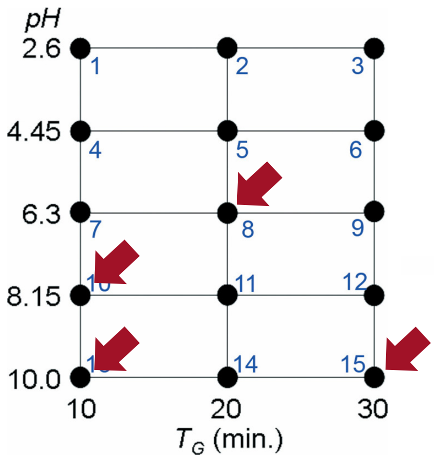
$$\hat{\theta} = (\beta_1, \ldots, \beta_{10}, \varepsilon)$$

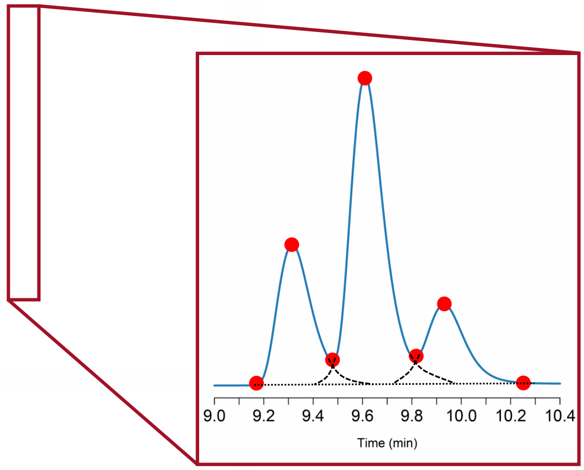


P. Lebrun et al., Chemom. Intell. Lab. Syst. 91 (2008) 4.

2.3. Automatic reading

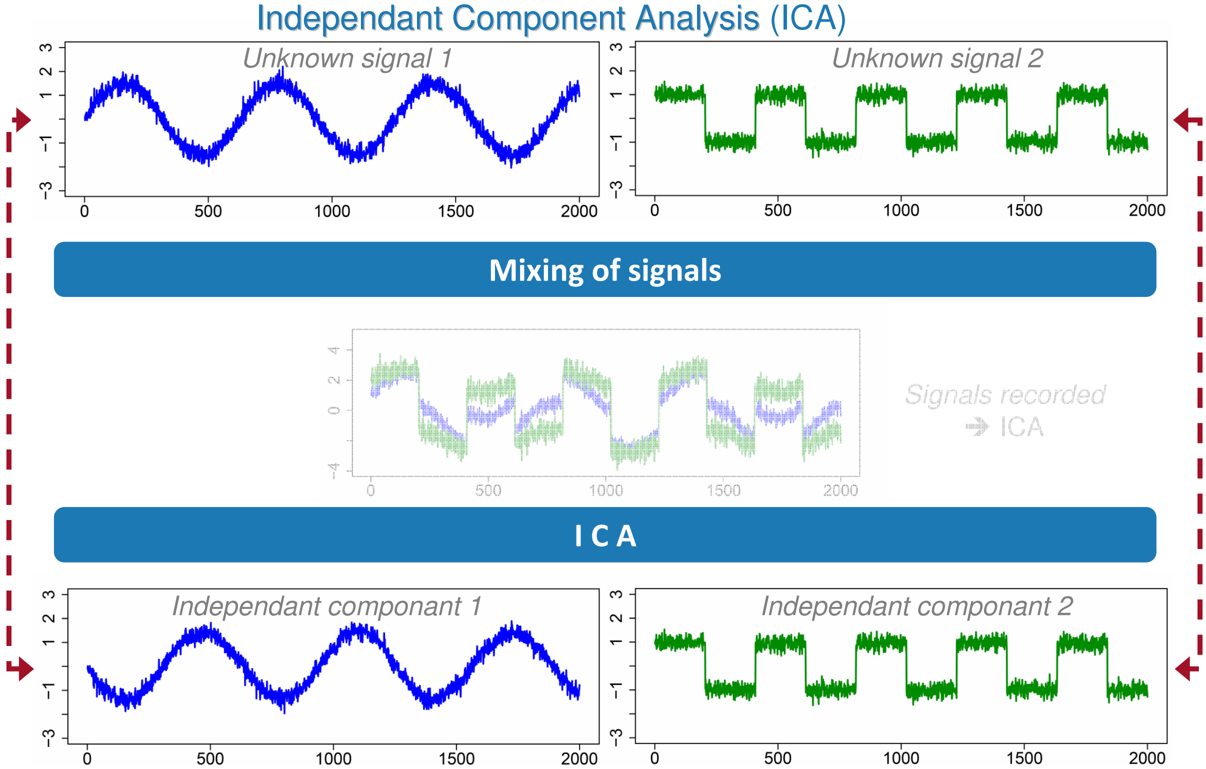
Problem stating





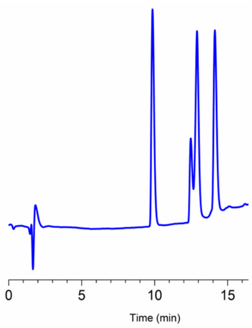
2.3. ICA in liquid chromatography

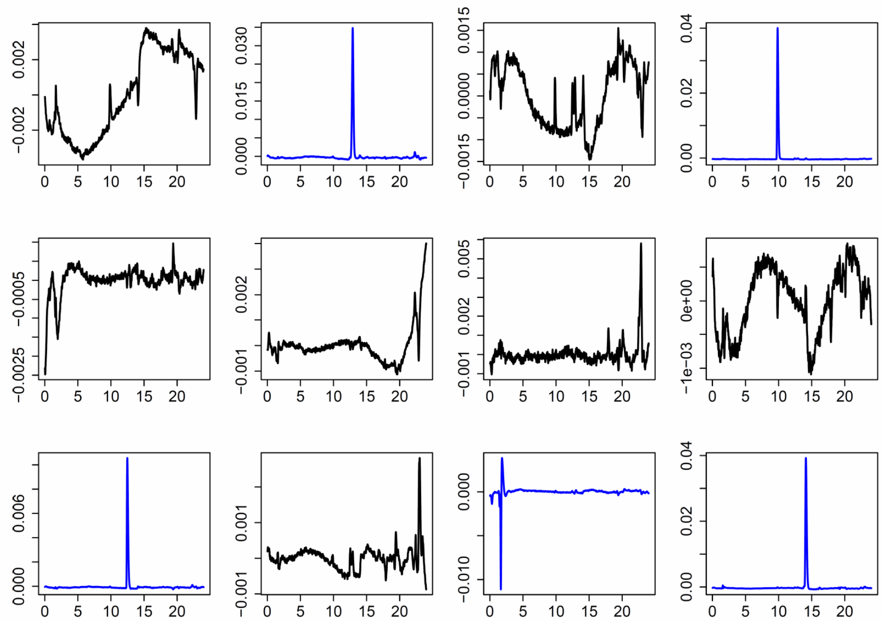
Independant Component Analysis (ICA)



2.3. ICA in liquid chromatography

ICA (nbr of sources = 12)



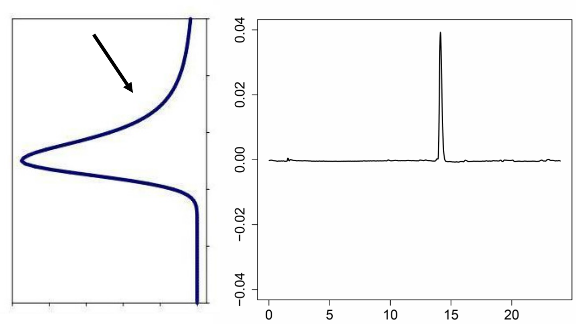


200 nm – 400 nm → 240 nm

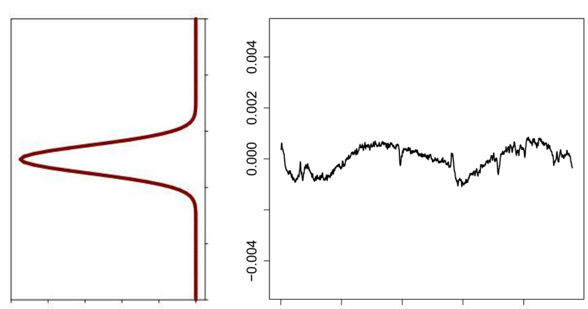
B. Debrus, et al., Talanta 79 (2009) 77.

2.3. ICA in liquid chromatography

Asymetrical distribution
Non gaussian



Gaussian distribution



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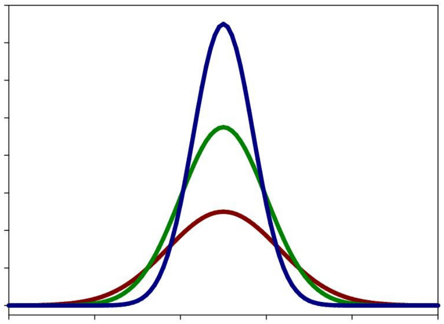
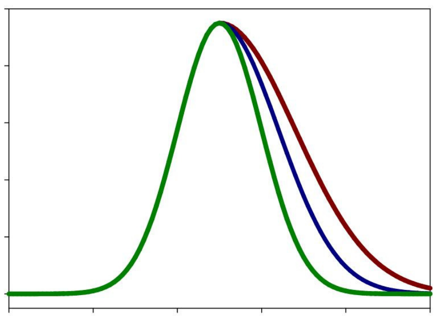
2.3. ICA in liquid chromatography

Kurtosis



Kurtosis > 3 Over-normal (leptokurtic)
Kurtosis = 3 Normal Distribution (mesokurtic)
Kurtosis < 3 Sub-normal (platikurtic)

Shapiro-Wilk Test

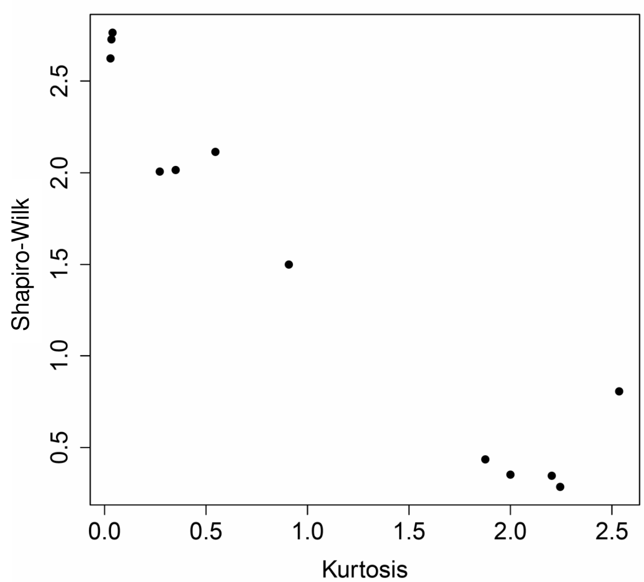


$W_{S\text{-}W} = 1 > p_{S\text{-}W} > p_{S\text{-}W}$

$\downarrow$

Normal Distribution

2.3. ICA in liquid chromatography



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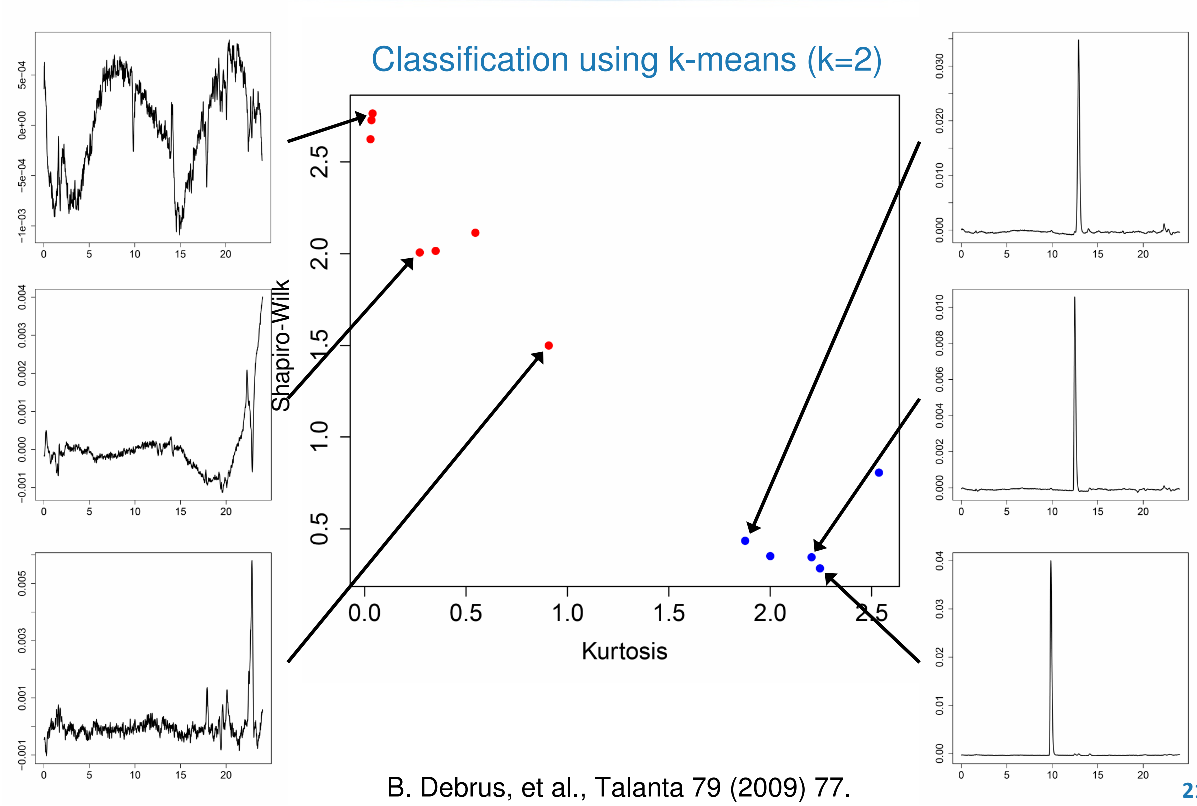

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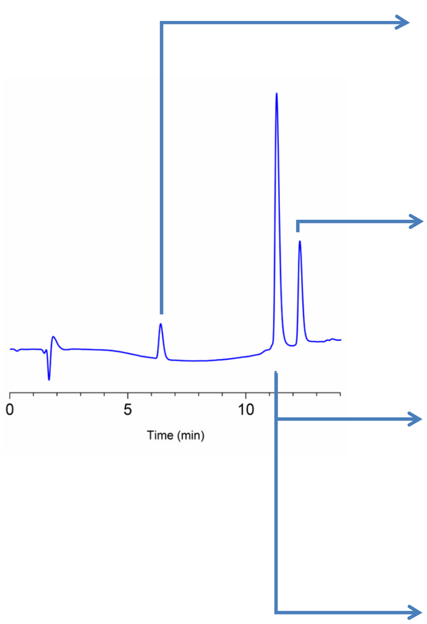
2.3. ICA in liquid chromatography



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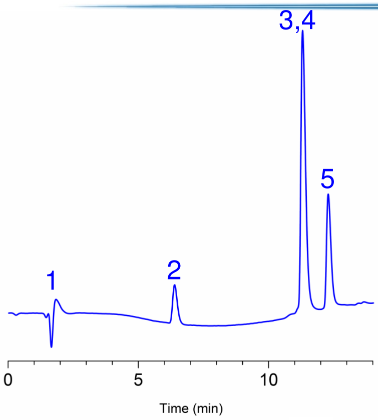
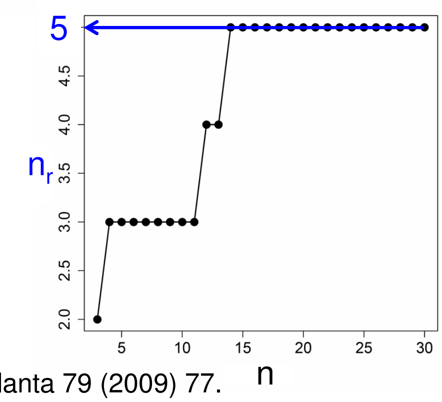
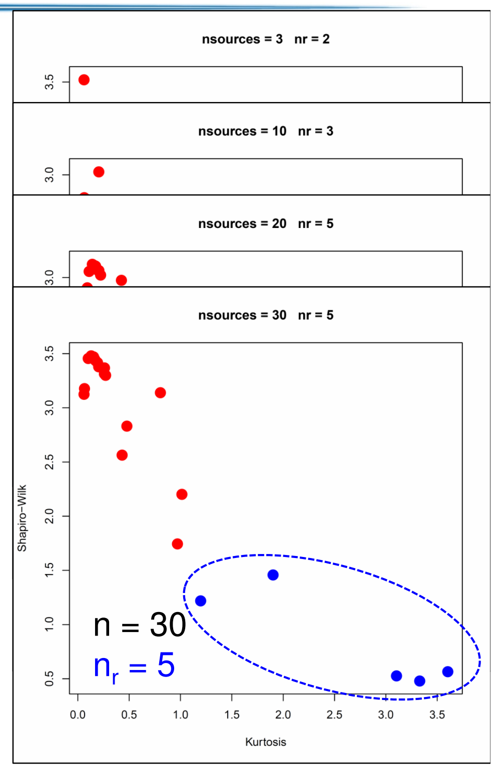
2.3. ICA in liquid chromatography



n = ?





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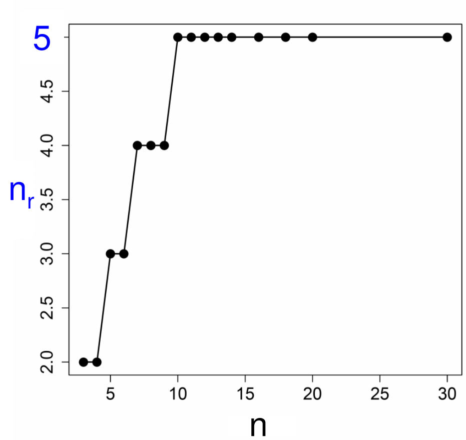
2.3. ICA in liquid chromatography

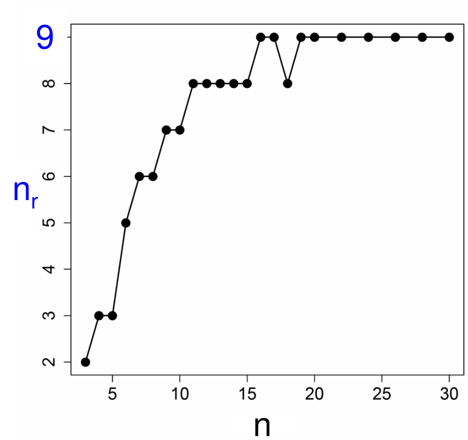
Mixture of 4 compounds

24 experimental conditions
➔ 26 chromatograms



Mixture of 9 compounds

15 experimental conditions
➔ 17 chromatograms



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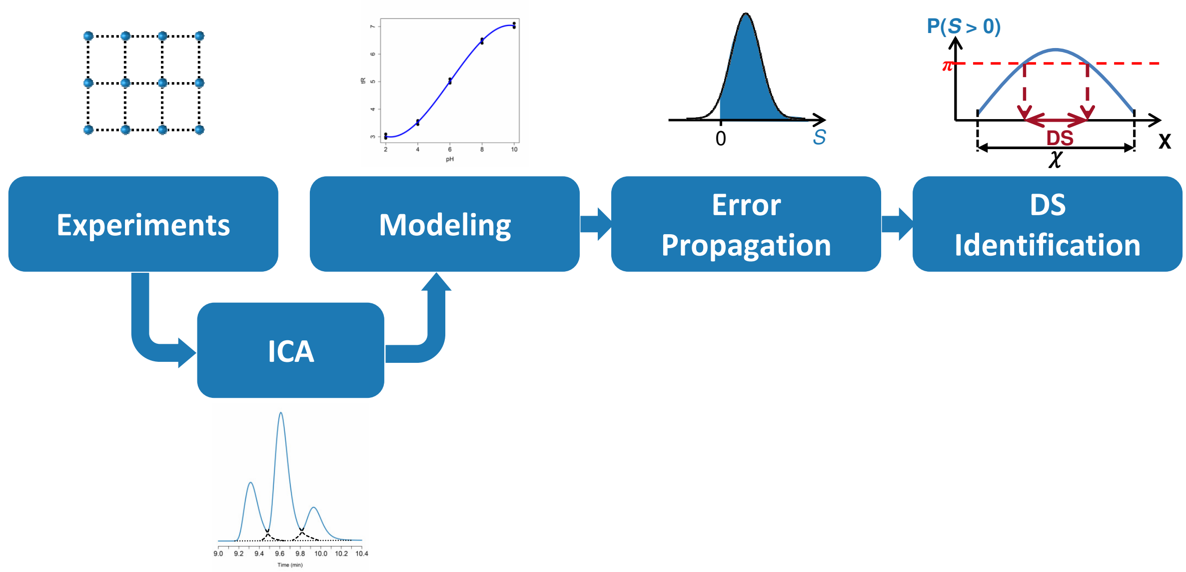
DoE-ICA-DS methodology



Outline

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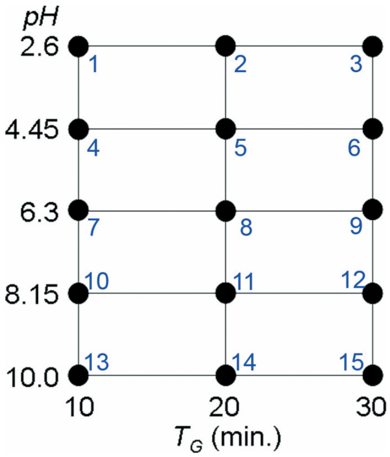
3. Application of the methodology
- 3.1. Mixture of 9 compounds
- 3.2. Improvement of EurPh Monography
- 3.3. Antimalarial compounds
- 3.4. Application to Legal Toxicology Sciences
- 3.5. Chiral separation

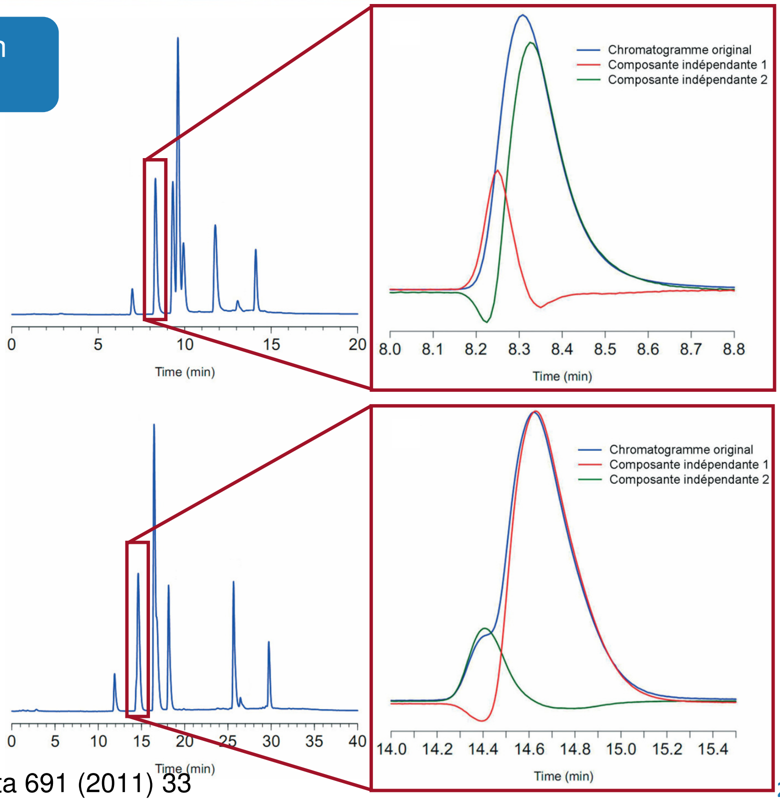
4. Conclusions

3.1. Application - 1

1. Mixture of 9 unknown compounds



XBridge C18
100 × 2.1 mm
3.5 µm



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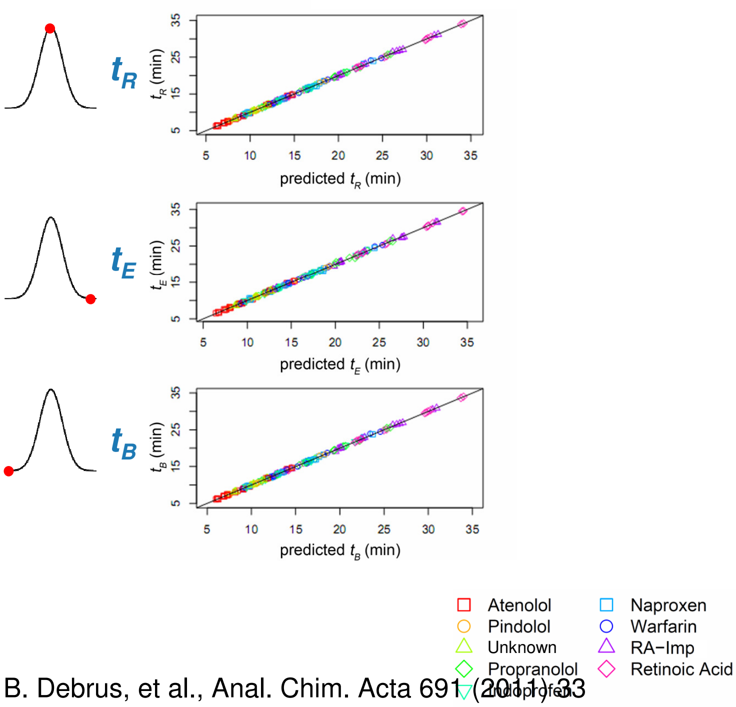
3.1. Application - 1

Normality test
of Shapiro-Wilk



p-value = 0.111

p-value = 0.129

p-value = 0.080

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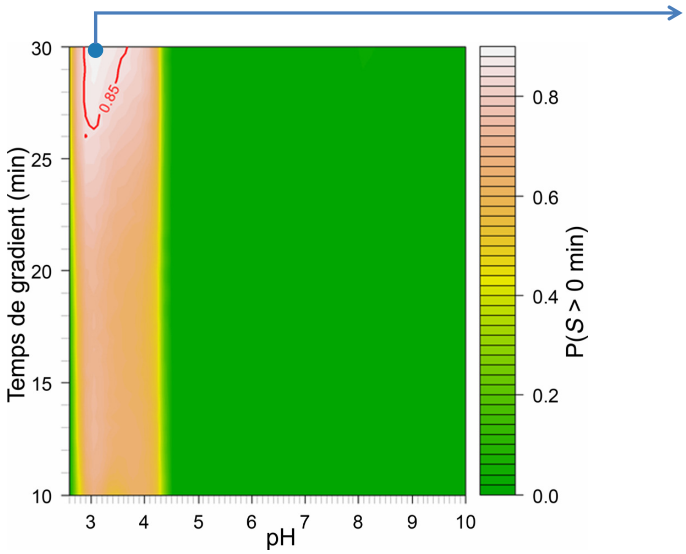
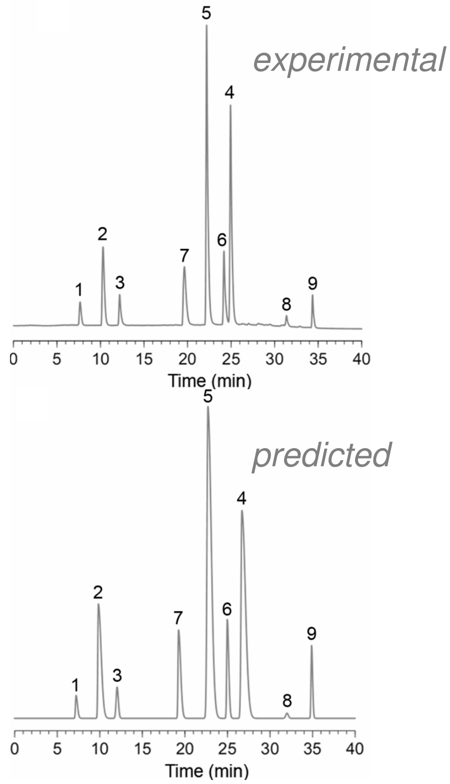
3.1. Application - 1

DS (π = 85%)





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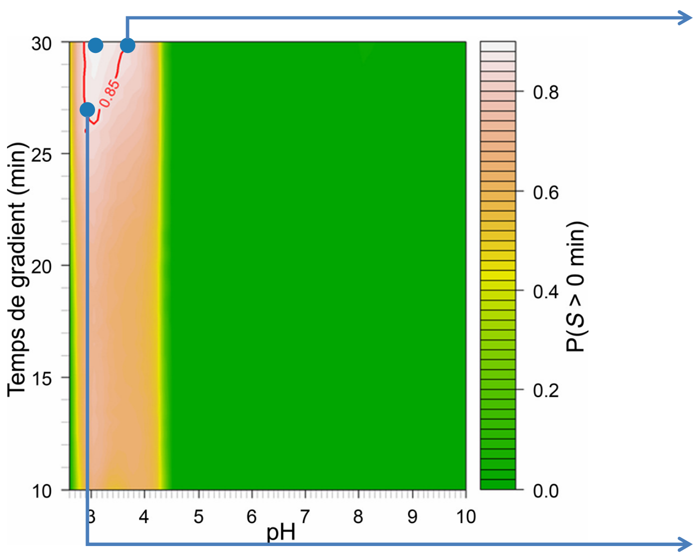
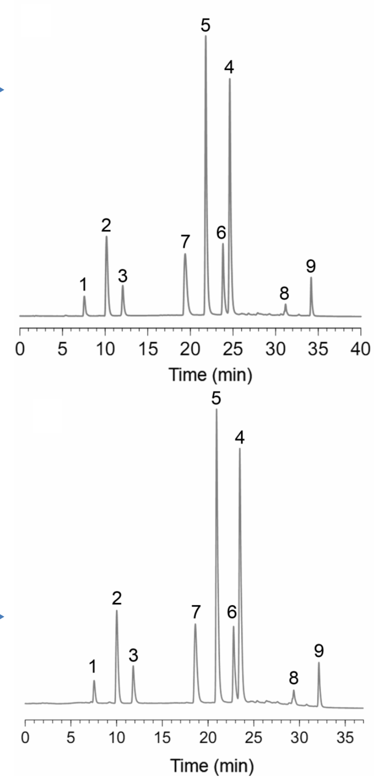
3.1. Application - 1

DS (π = 85%)





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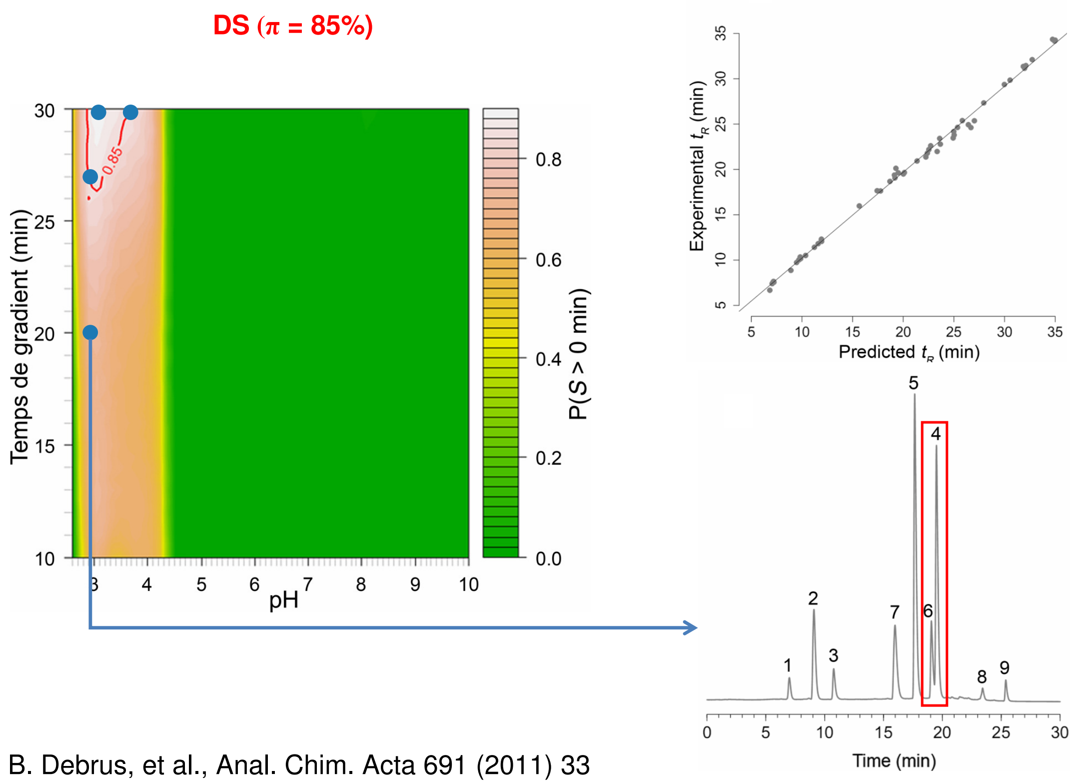
3.1. Application - 1



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3.2. Application - 2

2. Antimalarial compounds

19 antimalarial drugs	
chloroquine	proguanil
amodiaquine	mefloquine
sulfalene	Lumefantrine
sulfadoxine	Atovaquone
cinchonine	Piperaquine
quinine	Artesunate
pyriméthamine	Arteether
primaquine	Artemether
halofantrine	Artemisinine
dihydroartemisinine	

Full Factorial Design

pH	t_G (min)	T (°C)
2.5	20	25
4	40	30
6	60	35
8		

Precolumn
XBridge C18
20 × 4.6 mm
5 µm

Column
XBridge C18
250 × 4.6 mm
5 µm

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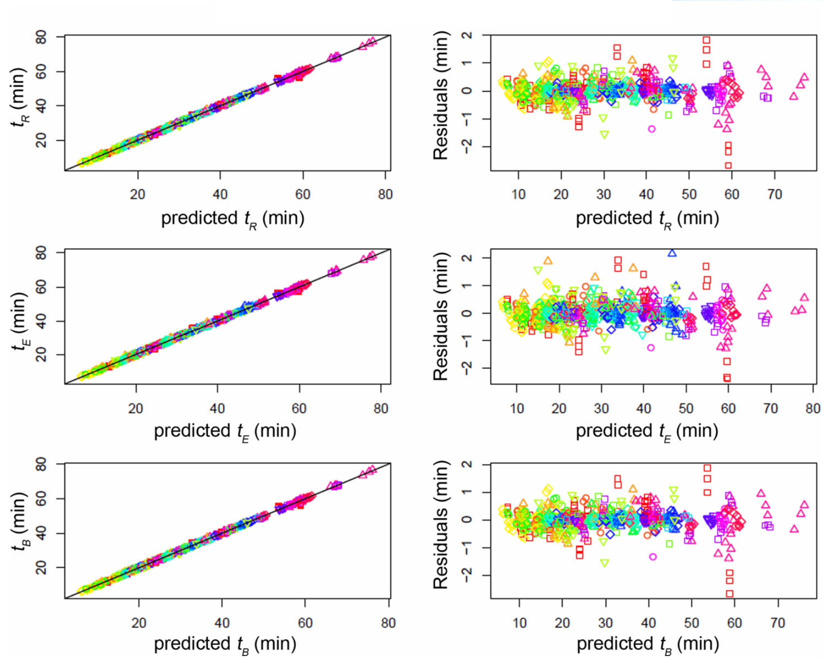
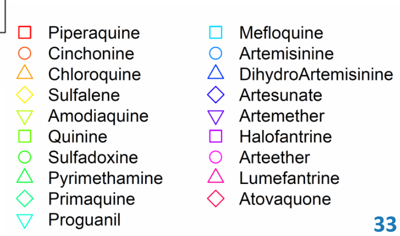
3.2. Application - 2



Normality tests of Shapiro-Wilk

p-values > 0.05



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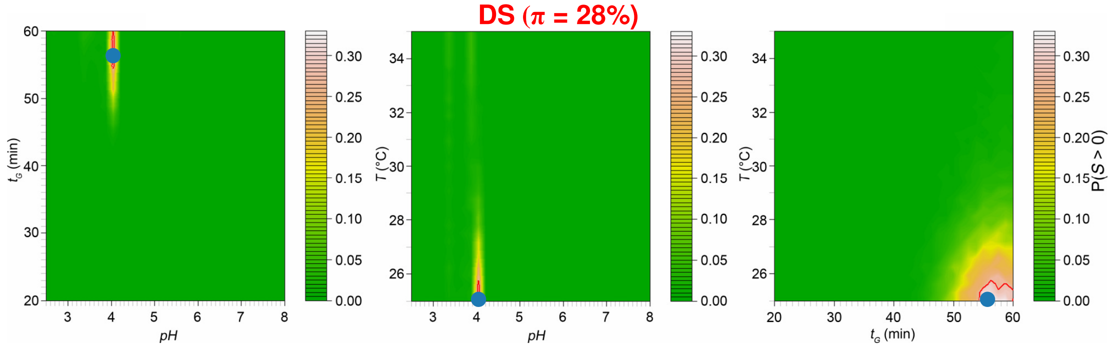
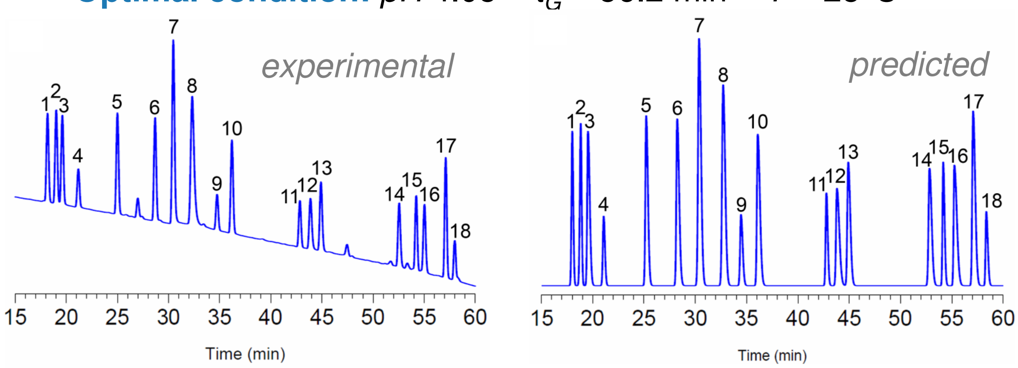
3.2. Application - 2

Mixture of 18 molecules

DS (π = 28%)

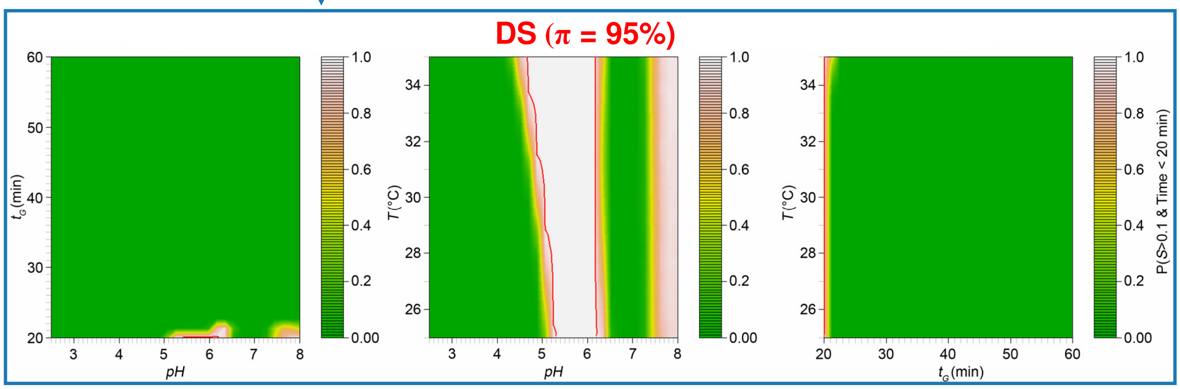


Optimal condition: pH 4.05 – t_G = 56.2 min – T = 25 °C



3.2. Application - 2

Mixture 1	Mixture 2	Mixture 3	Mixture 4	Mixture 5
Amodiaquine	Sulfalene	Artemether	Sulfalene	Piperaquine
Artesunate	Pyrimethamine	Lumefantrine	Pyrimethamine	Dihydroartemisinine
	Artesunate		Dihydroartemisinine	



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3.2. Application - 2

Mixture 1	Mixture 2	Mixture 3	Mixture 4	Mixture 5
Amodiaquine	Sulfalène	Arteméther	Sulfalène	Pipéraquine
Artésunate	Pyriméthamine	Luméfantrine	Pyriméthamine	Dihydroartemisinine
	Artésunate		Dihydroartemisinine	

Mixture 1	Mixture 2	Mixture 3	Mixture 4	Mixture 5
pH = 5,6 t_G = 20 min	pH = 5,4 t_G = 20 min	pH = 4,5 t_G = 20 min	pH = 4,7 t_G = 20 min	pH = 2,5 t_G = 20 min

T° = 35°C

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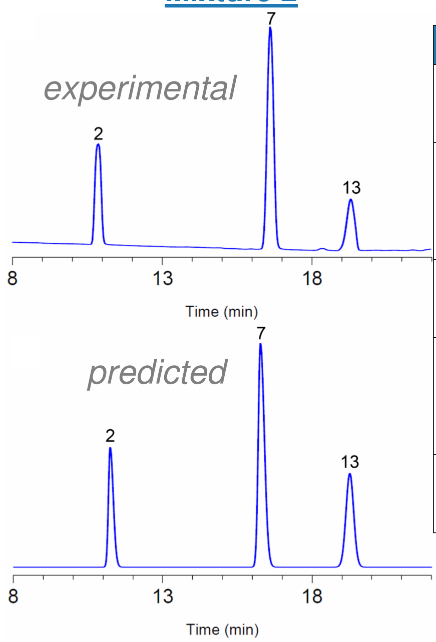
3.2. Application - 2

Mixture 2



Mix.	Compound	t_R pred.	t_R observed	error
1	Amodiaquine	13,51	13,85	-0,34
	Artésunate	19,10	19,26	-0,16
2	Sulfalène	11,23	10,83	0,40
	Pyriméthamine	16,27	16,60	-0,33
	Artésunate	19,26	19,37	-0,11
3	Artéméther	23,09	23,17	-0,08
	Luméfantrine	25,99	26,90	-0,91
4	Sulfalène	11,29	10,97	0,32
	Pyriméthamine	15,45	15,42	0,03
	Dihydroartémisinine	20,00	20,11	-0,11
5	Piépraquine	7,45	8,14	-0,69
	Dihydroartémisinine	20,08	20,12	-0,04
			Mean |error|	0,29

B. Debrus, et al., J. Chromatogr. A, 1218 (2011) 5205

3.3. Application - 3

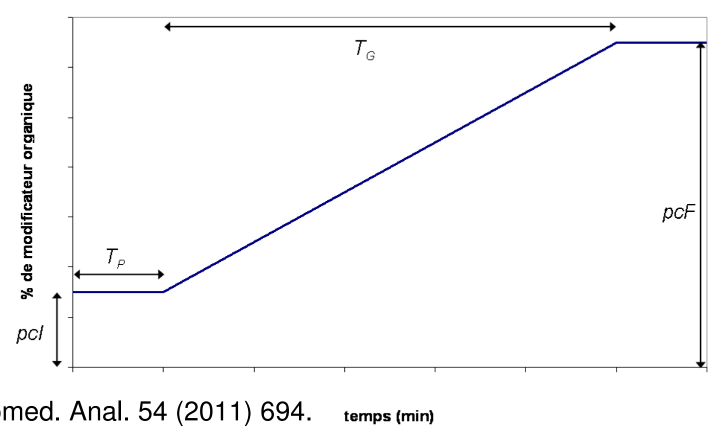
3. Eur Ph. Monography

4 compounds:
- sulindac (sulfoxide – Z isomer)
- sulfone
- sulfide
- E-sulindac

Rocket Platinum C18
53 mm × 7 mm, 1.5 µm

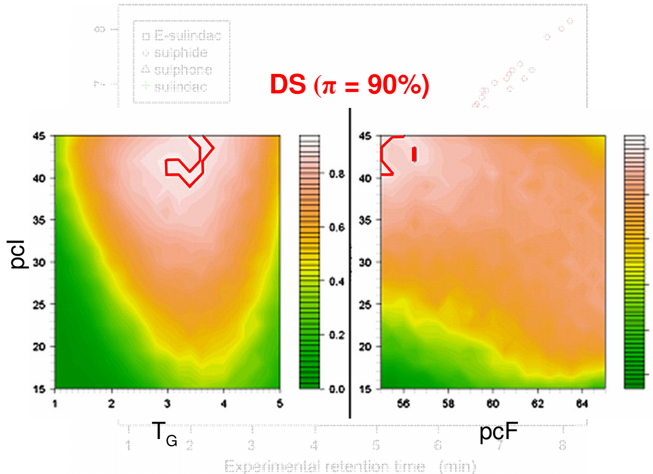
Full factorial design

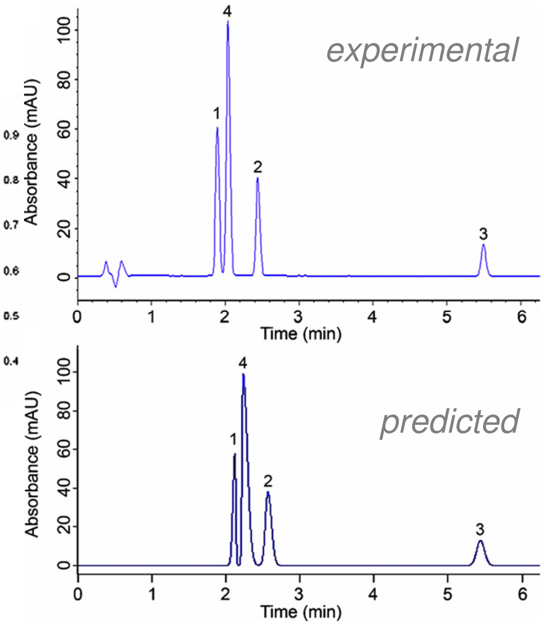
Factors	Levels		
T_P (min)	0	1	
pcI (%)	15	30	45
T_G (min)	1	3	5
pcF (%)	55	60	65



F. Krier et al., J. Pharm. Biomed. Anal. 54 (2011) 694.

3.3. Application - 3





Optimal conditions

Tp = 1 min
pcl = 40.5%
T_G = 3.6 min
pcF = 55%

1: sulindac
2: E-sulindac
3: sulfone
4: sulfide

F. Krier et al., J. Pharm. Biomed. Anal. 54 (2011) 694.

3.4. Application - 4

4. Legal Toxicology

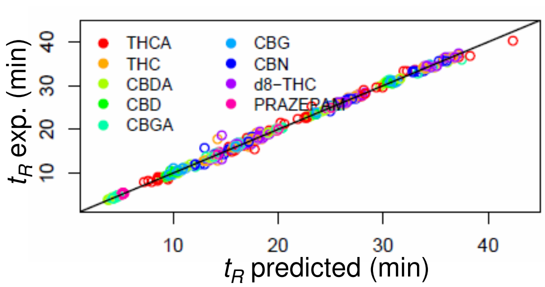
9 compounds:

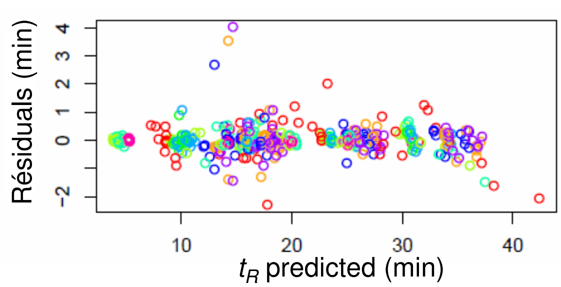
Prazepam (IS)
CBDA, CBGA
CBD, CBG
THCA, d9-THC,d8-THC
CBN

Full factorial design

Factors	Levels				
pH	2.6	4.45	6.3	8.15	10
T_G (min, $\%_{ini} \rightarrow 95\%$)	10	20	30		
$\%_{ini}$ (MeOH)	5	40	75		

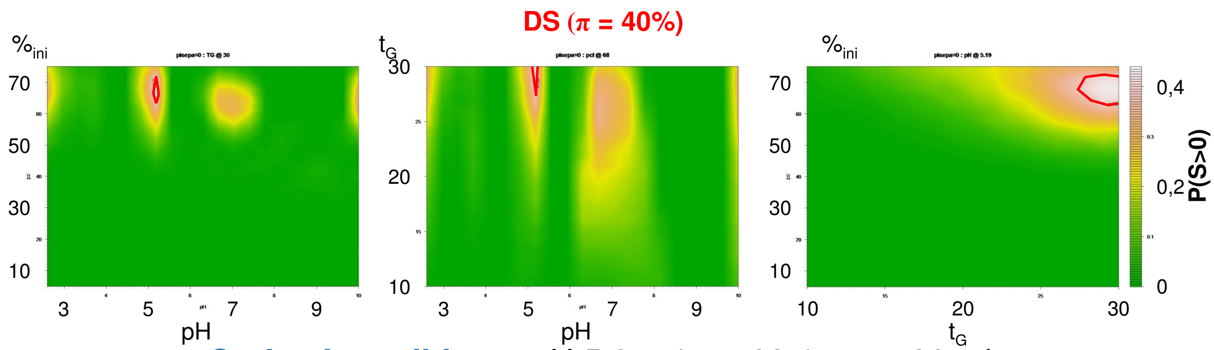
XBridge C18 150 mm × 4.6mm, 5µm



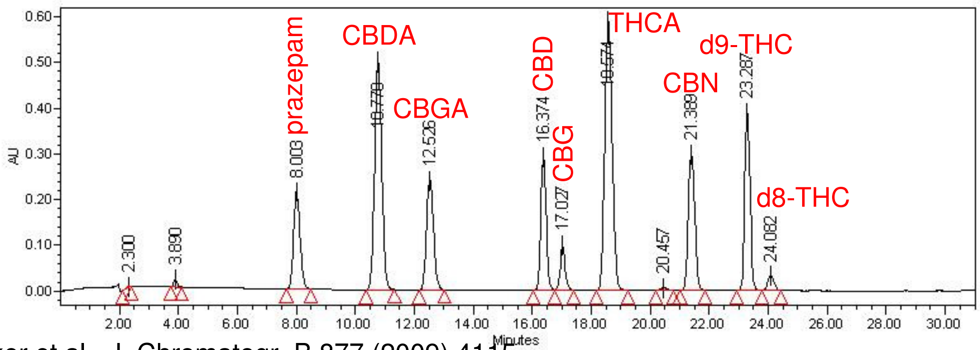


De Backer et al., J. Chromatogr. B 877 (2009) 4115.

3.4. Application - 4



Optimal conditions: pH 5.2 - $\%_{ini}$ = 68% - t_G = 30 min



De Backer et al., J. Chromatogr. B 877 (2009) 4115.

3.5. Application - 5

5. Chiral Separation

1 basic compound
2 chiral centers

Central Composite design

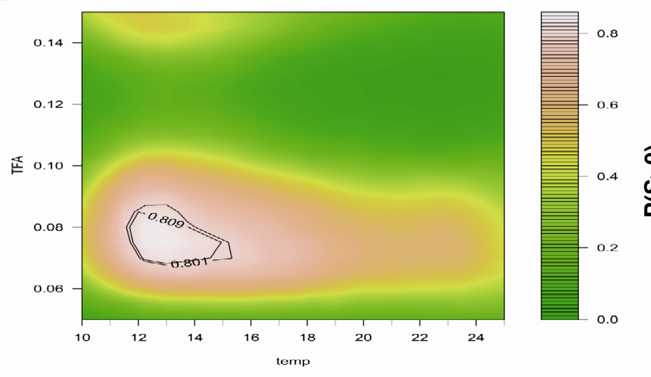
Factors	Levels	
%TFA	0.05	0.15
%n-hexan	1	10
t°C	10	25

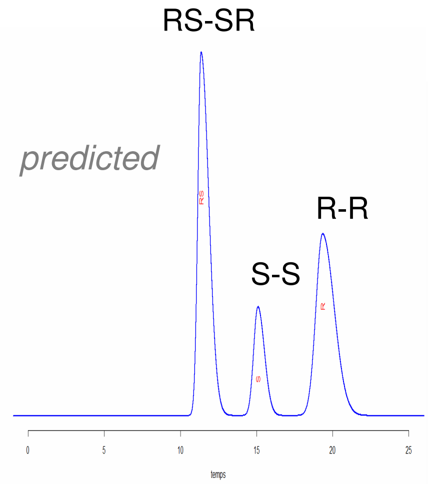
Chiral selector:
cellulose tris(4-chloro-3-methylphenylcarbamate)
DEA 0.1%

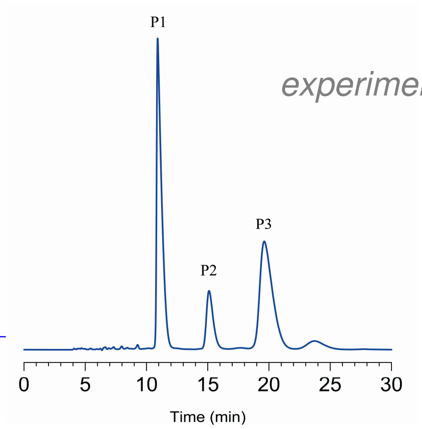
3.5. Application - 5

Work still in progress

Optimal conditions:
8.65% n-hexane,
13℃,
0.08% TFA



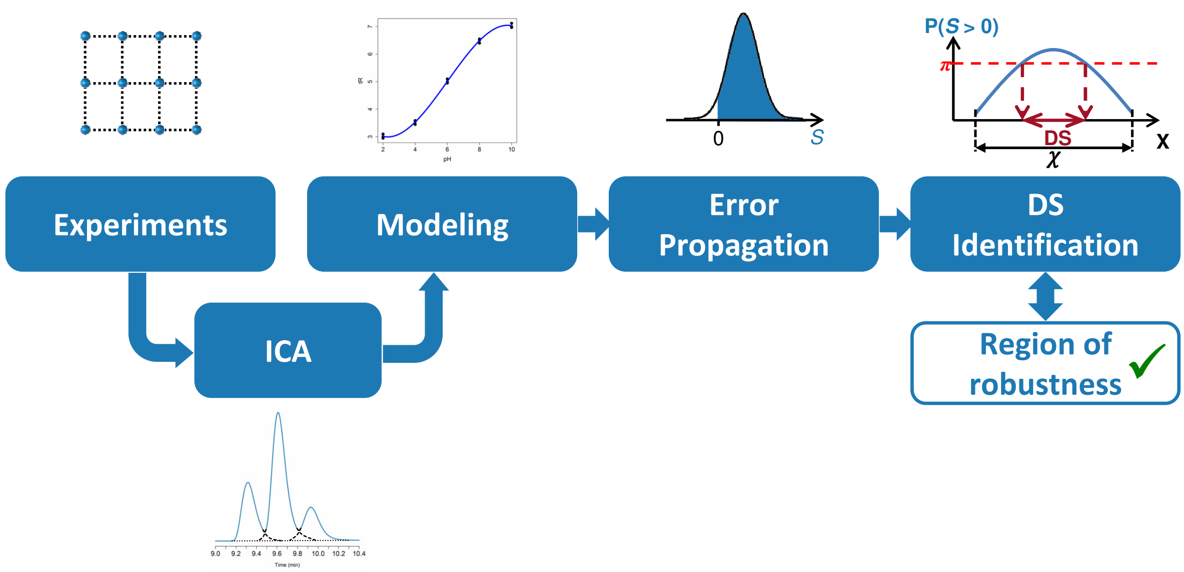




Conclusions

- The proposed methodology allows modeling the chromatographic behaviour of the studied compounds.
- It allows to propagate prediction uncertainty and thus allows to define the *design space* and is compliant with the concept of *Quality by Design (QbD).*
- ICA allows the separation of coeluted peaks and the automatic reading of chromatograms (detection and indexing peaks).
- The global methodology DoE-ICA-DS has demonstrated its capacity to allow the development of chromatographic methods that are optimal and robust.
- Not limited to chromatography, nor to analytical chemistry … any process

DoE-ICA-DS methodology



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