HOW TO SELECT ORTHOGONAL CONDITIONS IN RPLC TO ACHIEVE COMPREHENSIVE SEPARATIONS ACCORDING TO THE NATURE OF COMPOUNDS

Ramia AL BAKAIN (1)*, Isabelle RIVALS (2), Patrick SASSIAT (1), Didier THIEBAUT (1), Marie-Claire HENNION (1), Guillaume EUVRARD (2), Jérôme VIAL (1)

(1) Laboratoire des Sciences Analytiques, Bioanalytiques et Miniaturisation UMR-CNRS-UPMC- PECSA 7195, ESPCI ParisTech, (2) Équipe de Statistique Appliquée. ESPCI - 10 rue Vauquelin, 75231 Paris CEDEX 05. ramia.bakain@espci.fr

1- INTRODUCTION

The demand for characterization of complex samples, i.e. containing several hundreds of compounds, requires advanced analytical tools such as comprehensive liquid chromatography. Its high separation power is obtained via the coupling of two separation modes exhibiting different mechanisms, i.e. orthogonal. In this study, a wide set of probe solutes was considered. Principle component analysis (PCA) was used to map the distribution of solutes. Chromatographic behavior was then evaluated by Analysis Of Variance (ANOVA) to establish which factor has a significant effect on orthogonality, as a function of the nature of compounds. Finally, an optimized subset of test compounds was proposed to evaluate orthogonality.

2- EXPERIMENTAL CONDITIONS

- 63 Solutes → Basic, Acidic, Neutral
  - logP values from -1.67 to 7.72,
  - M.wt from 76 to 1486 g/mol,
  - p*K*a values from 0.6 to 14.0

- 8 Stationary phases:
1) HS F5, 2) HS PEG, 3) C8(2),
4) Capcell Pak C18, 5) Phenyl-hexyl,
6) Zorbax SB-CN, 7) Kromasil C18,
8) XBridge RP18.

Two organic modifiers: methanol (MeOH) and acetonitrile (MeCN)

Two pH values of the mobile phase: 2.5 and 7.0

8x2x2 = 32 systems → *496 couples of chromatographic systems*

3- ORTHOGONALITY EVALUATION

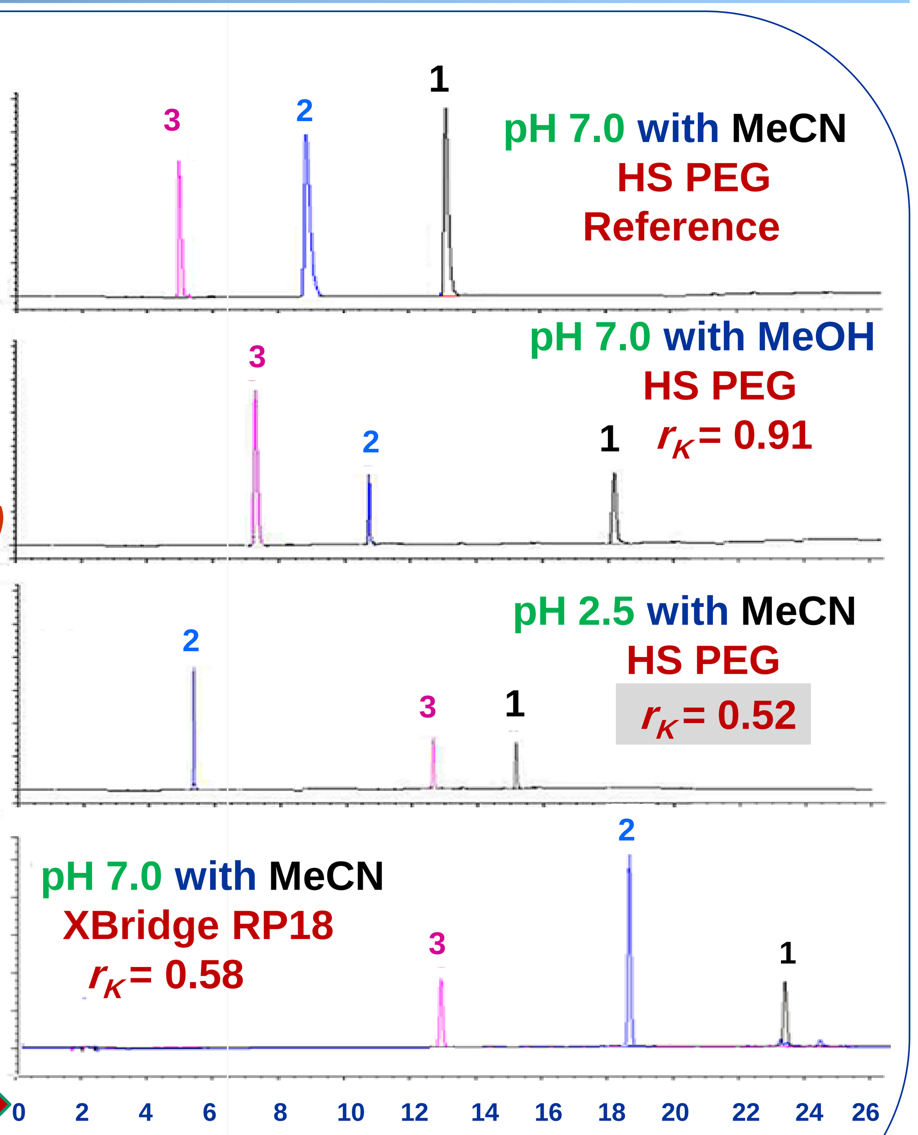
3 criteria were used for evaluation

1) Pearson's correlation coefficient, (r_p)
2) Spearman's correlation coefficient, (r_S)
3) Kendall's correlation coefficient, (r_K)

Kendall's correlation coefficient (r_K) is the most sensitive criterion [1].

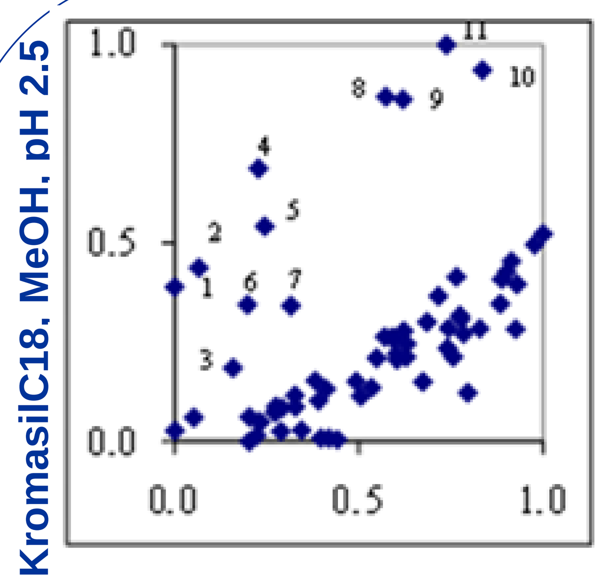
pH > Stationary phase > Mobile phase [1].

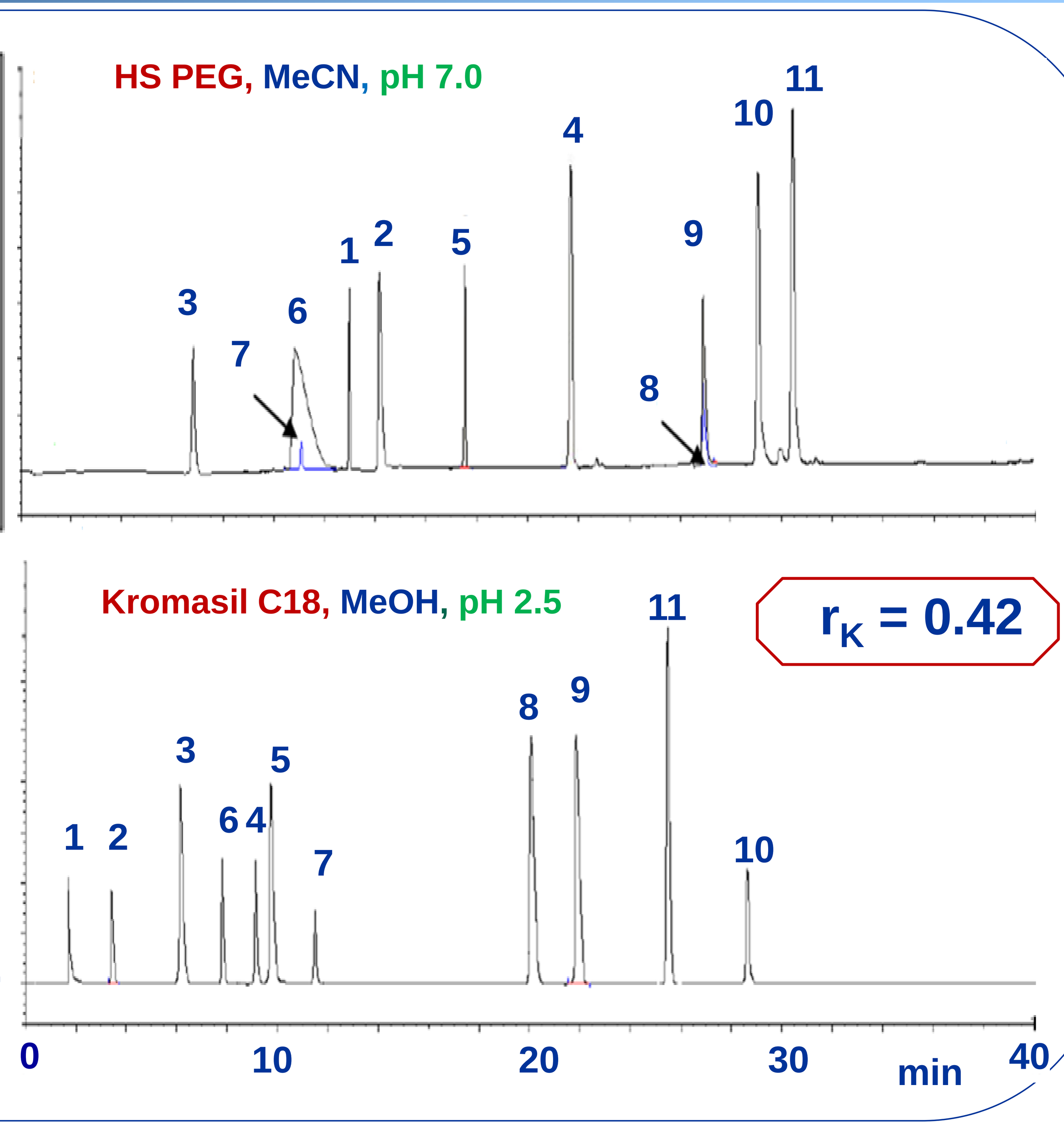
Chromatograms show the effect of changing pH, organic modifier and stationary phase.



1) Phenanthrene, 2) Prometryn, 3) Ibuprofen

4- MOST ORTHOGONAL COUPLES OF SYSTEMS





The solutes are numbered as follows:
(1) nicotine, (2) benzylamine, (3) vancomycin, (4) strychnine, (5) atropine, (6) (+)- tubocurarine, (7) 1, 2- phenylendiamine, (8) imipramine, (9) loperamide, (10) amiodarone, (11) clofazimine.

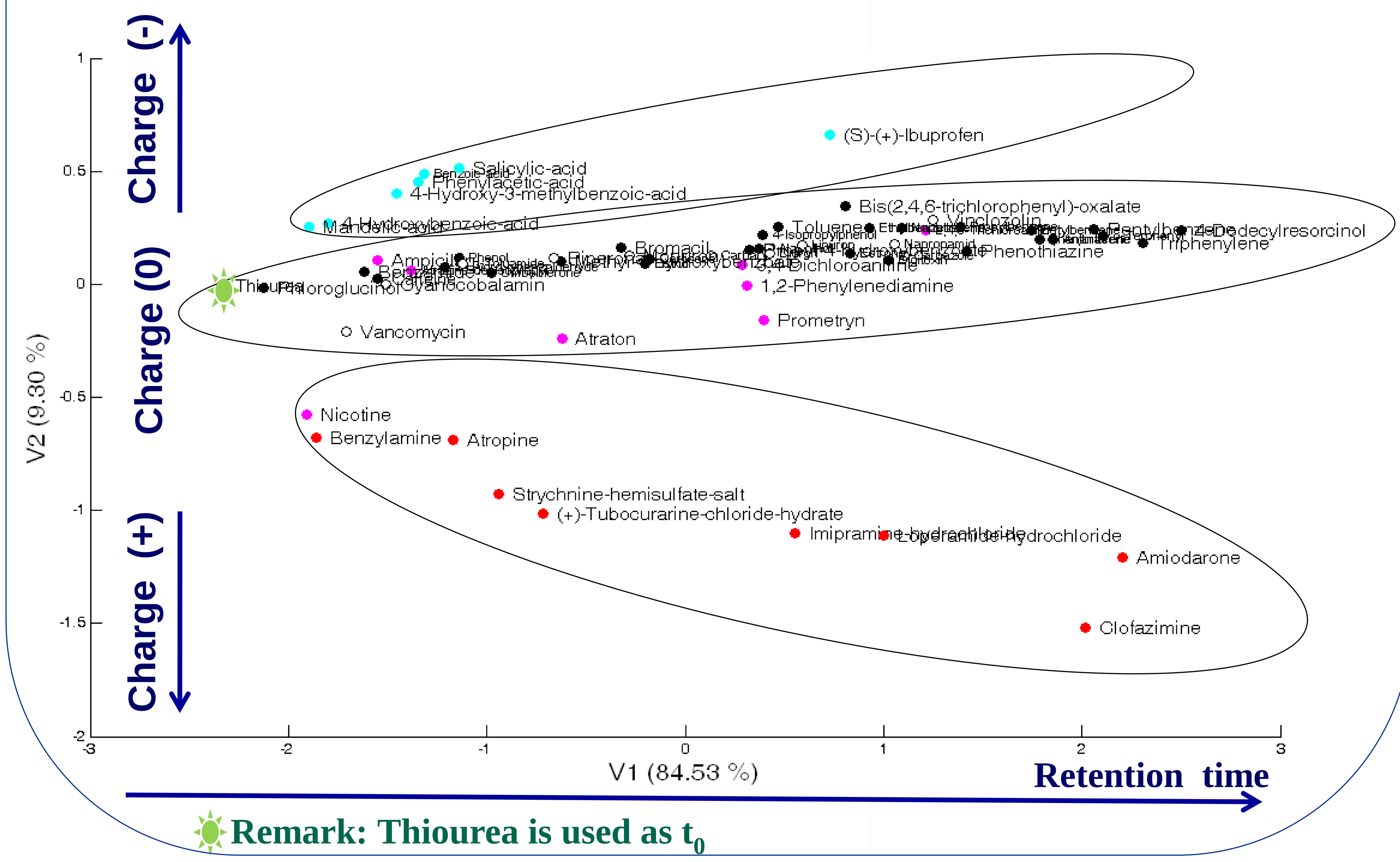
5- SOLUTE MAPPING BY PCA

The 63 probe solutes are a priori classified according to their charges into five groups:

- solutes with positive charge,
- solutes with negative charge,
- solutes with positive charge according to pH,
- neutral solutes,
- ○ (white) for non classified compounds.

- Solutes are distributed according to their charges into 3 groups:
  - A) 7 Compounds with negative charge (blue),
  - B) 47 compounds contain: neutral compounds (black), (white) for non classified compounds, and others.
  - C) 9 compounds with positive charge (red).

- x-axis reflects the retention time and y-axis the charge.



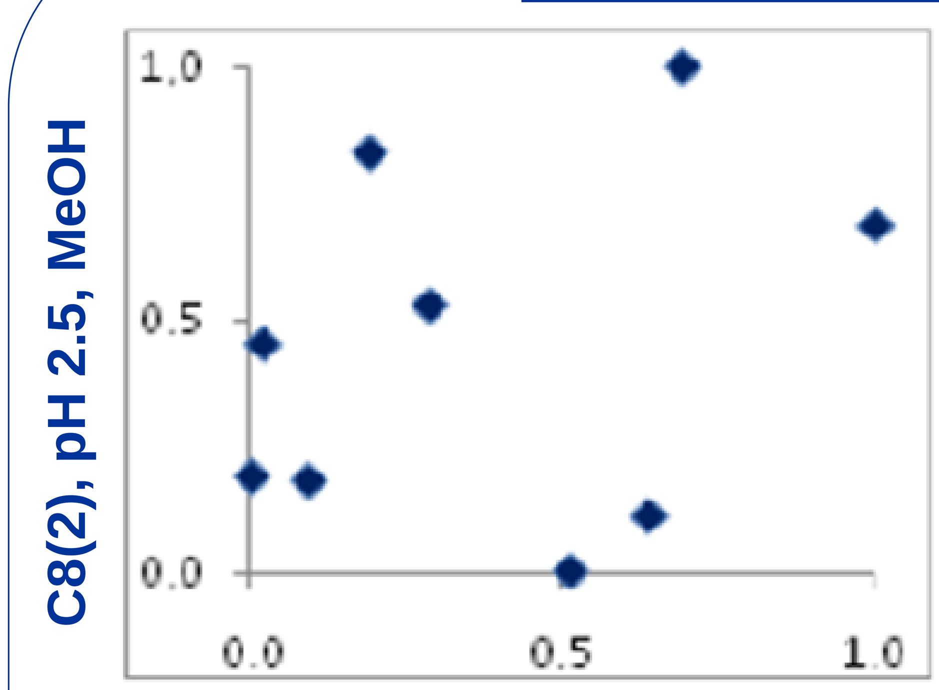
Remark: Thiourea is used as t_0

6- ORTHOGONALITY & PROBE SOLUTE SET

Influence of the factors (ANOVA)

63 compounds: pH > Stationary phase > Mobile phase
9 basic compounds: pH > Stationary phase ≈ Mobile phase
7 acidic compounds: pH > Stationary phase > Mobile phase
47 neutral compounds: Stationary phase > Mobile phase > pH

Optimal subset of 9 compounds



9 compounds (acidic, basic and neutral): ibuprofen, salicylic acid, mandelic acid, benzamide, bromasil, dodecylresocinol, clofazimine, tubocurarine and benzylamine.

Same results as with 63 probes

pH > Stationary phase > Mobile phase
Kendall's correlation coefficient (r_K) is the most sensitive.

7- CONCLUSION

This study revealed that orthogonality strongly depends on the composition of the probe solute set in acidic, basic or neutral compounds. An optimized subset of 9 solutes can produce the same results as a 63 probe solute set.

8- REFERENCES

[1] R. Al Bakain, I. Rivals, P. Sassiat, D. Thiébaut, M.-C. Hennion, G. Euvrard, J. Vial, Journal of Chromatography A, 1218 (2011) 2963