CZE method development for more hydrophobic pharmaceuticals

and

an investigation of the orthogonality between RP-HPLC and CZE

CE-users meeting KVCV-KNCV

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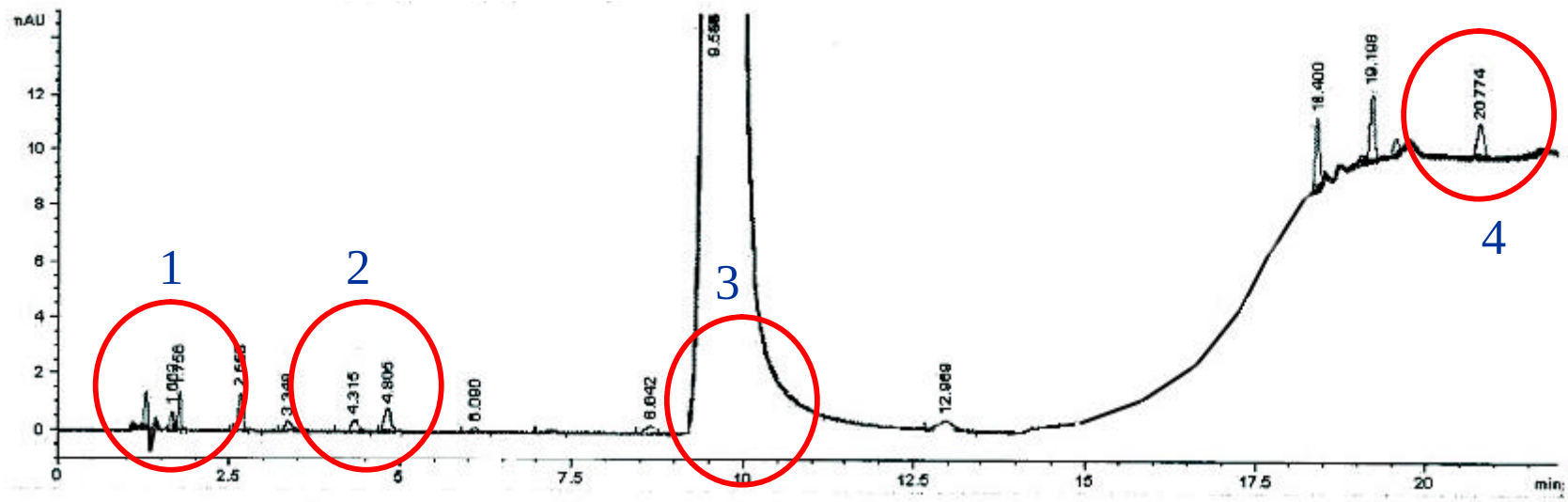
Setting

- Pharmaceutical products need to be fully characterized
- ICH-guidelines [1] require either reporting, identification or qualification of all impurities

[1] International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, -Q*3A*-*R2*- (2006) & -Q*3B*-*R2*- (2006)

Setting

- RP-HPLC is capable of this demanding task
- Stability indicating power of the validated method
- Critical regions in chromatogram regarding co-elution
- Demand for **orthogonal** techniques:
  - By definition a different separation obtained
  - Not a 2D-instrument, but parallel instruments
  - Indicates strengths and weaknesses of methods
  - Increases confidence in validated method
  - Techniques like NPLC, RPLC, GC and CZE



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  - By definition a different separation obtained
  - Not a 2D-instrument, but parallel instruments
  - Indicates strengths and weaknesses of methods
  - Increases confidence in validated method
  - Techniques like NP-HPLC, GC and CZE

[2] M. Jimidar, M. De Smet, et.al. -*Capillary electrophoresis as an orthogonal technique in HPLC method validation*- Journal of Capillary Electrophoresis and Microchip technologies 008 3/4 (2003) 45-52

Objectives

- Part I:
  - Experimental conditions to analyse more hydrophobic, $3 < log(P) < 7$, SLV-compounds using CZE
    => Generic BGEs to serve as smart starting points for CZE method development

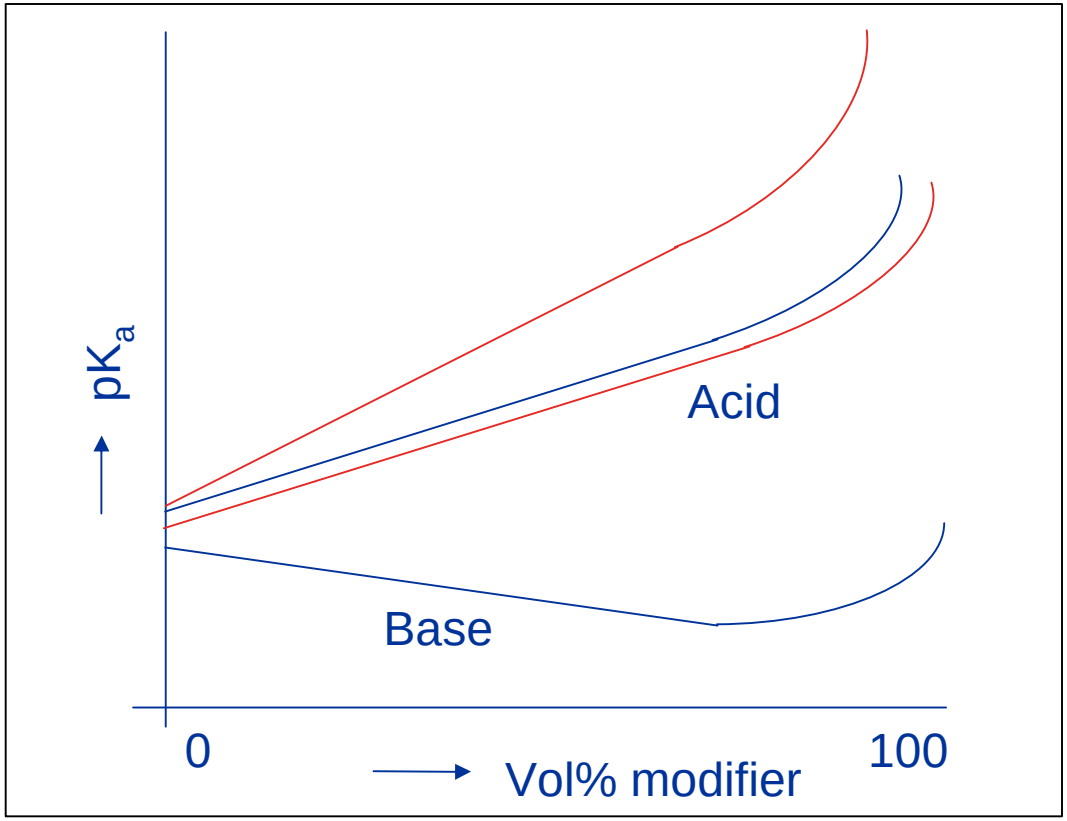
- Part II
  - Tool to determine the level of orthogonality between analytical methods and especially CZE vs RP-HPLC
    => value of CZE

Results (part I)

- Fraction of organic solvent in BGE:
  - At least uncommon in CZE
  - Initiates several side-effects:
    - Evaporation of solvents during analysis
    - Requires compatibility of buffer salts and additives
    - Different ${}^{s}_{w}pH$ of BGE due to medium effect
    - Shift in pK_a of compounds due to medium effect
    - Different analyte ionisation level a and effective migration μ_{eff}

Results (part I)

- Impact of the medium effect [3]:



If %modifier ?

Weaker acid

(buffer salt),

Stronger base

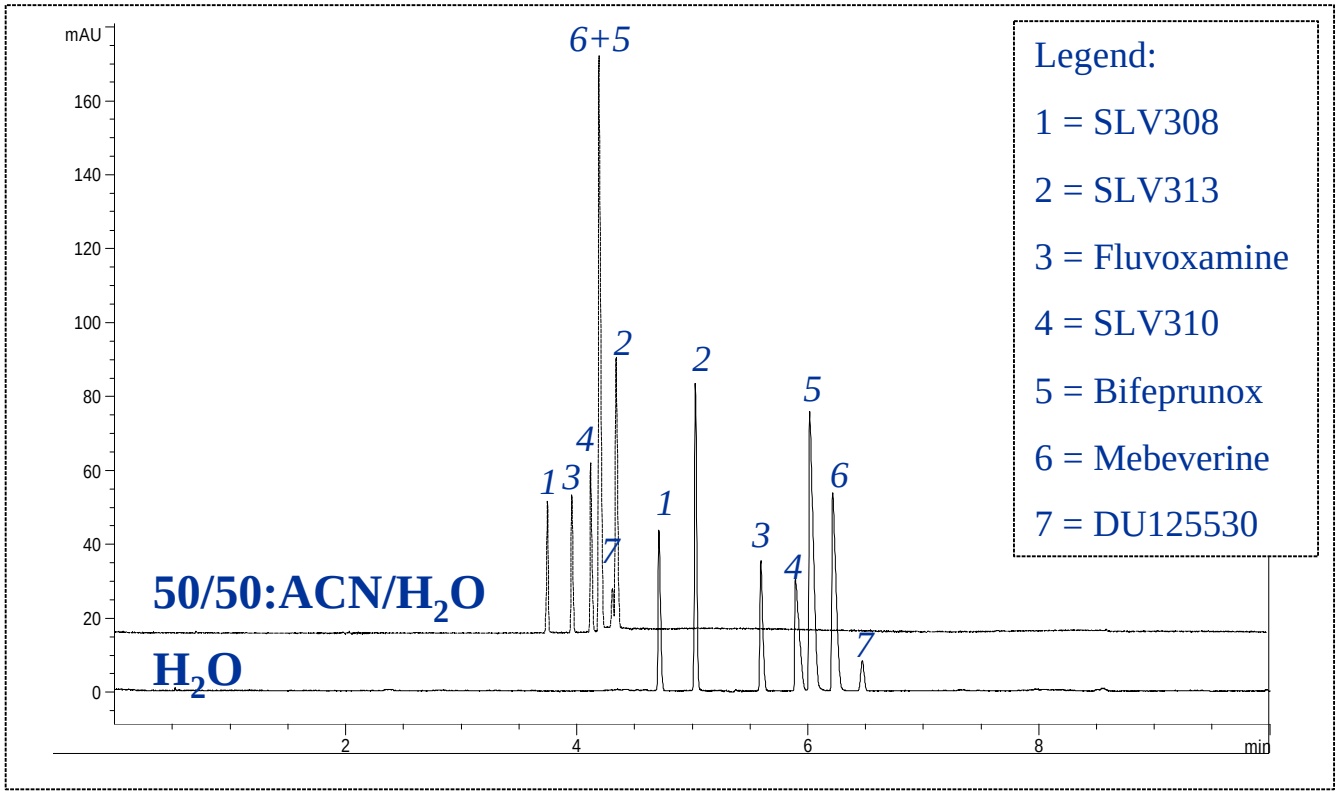
(SLV-compound)

Thus *a* decreases

[3] S. Porras, E. Kenndler -*Capillary zone electrophoresis in non-aqueous solutions: pH of the background electrolyte*-, Journal of Chromatography A 1037 (2004) 455-465

Results (part I)

- Example of generic BGE with Δ%ACN:



Electropherograms collected using: HP 3D CE system, Agilent standard capillary (L_{tot} 48.5 cm, L_{eff} 40.0 cm, I.D. 50 µm and T = 30 °C), PDA (?=200 nm and $?_{ref}$=450 nm), injection performed with 50 mbar for 5 s, 25 kV applied starting with voltage ramp 0-25 kV in 0.2 min. All analyte concentrations were about 1 mg/mL. Electrolyte system consisted of 0.1 M TRIS adjusted to $^{w}_{w}$pH 2.5 by adding *o*-phosphoric acid (85% m/m)

Results (part I)

- In BGE fraction of 50% (v/v) ACN:
  - Increased solubility strength
  - Rather low impact of medium effect (resembling compounds)
  - Adequate electrophoretic migration, separation pattern
  - Not all SLV-compounds rapidly dissolved

Results (part I)

- Full replacement of water in BGE:
  - Mode is called non-aqueous CZE (NACE)
  - Applications described in literature, small part of all CZE
  - Restricted solvent choice
  - ${}^{s}_{w}pH$ not determined, make use of constant fraction of acid

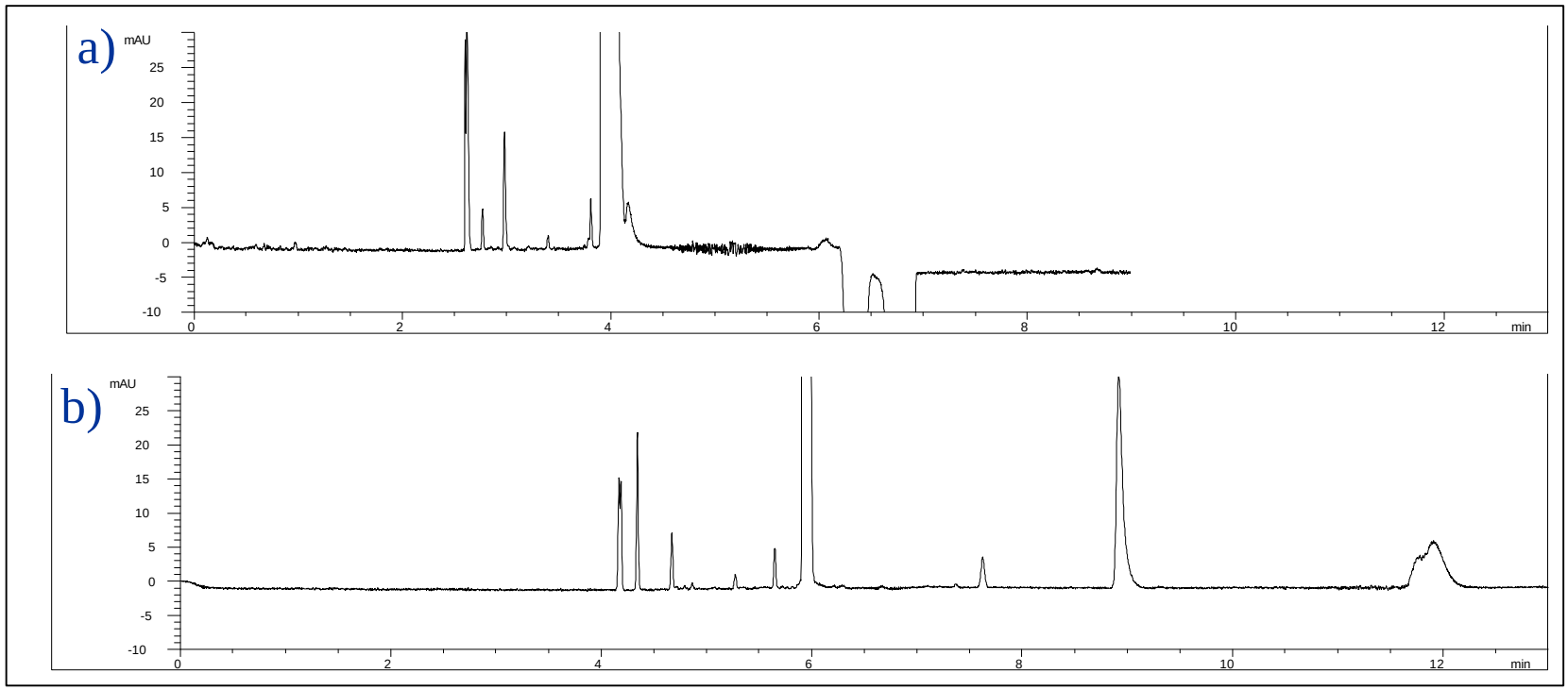
Results (part I)

- NACE resulted in:
  - Even higher solubility, all basic compounds dissolved
  - Solvent evaporation could be easily prevented
  - Poor m_{eff} if pK_a low $\Rightarrow$ low a due to medium effect
  - Various initiatives to increase the a were unsuccessful
    - ↳ *Preference: the BGE should contain 50%H_2O*
  - Poor peak shape $\Rightarrow$ poor mobility match
    - ↳ *Requirement: the BGE must contain a proper co-ion*

Results (part I)

- NACE versus aqueous-organic BGEs

BGE: HCOOH in 50/50 ACN/MeOH (a) and TRIS in 50/50 ACN/H_2O (b)



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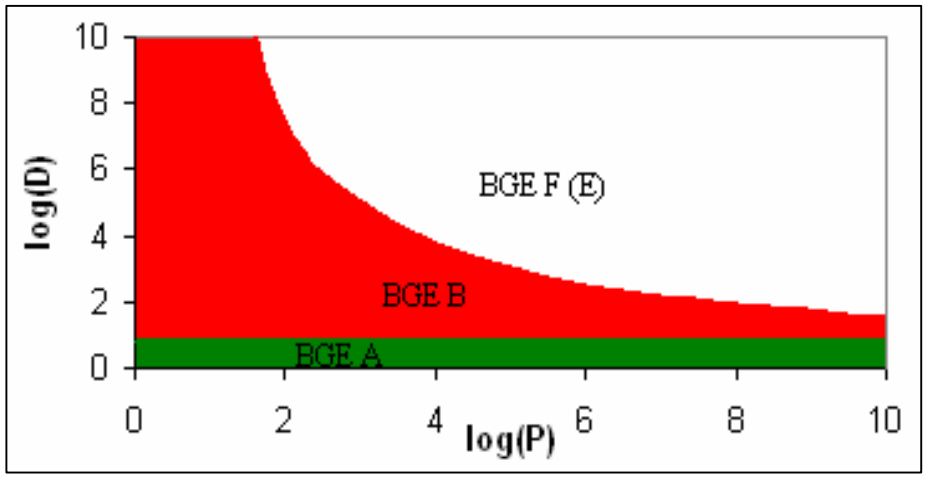
Conclusion (part I)

- Addition of organic solvent lifts solubility strength of BGE
- Main side-effect is a decrease in *a* if modifier?
- For the vast majority of compounds H_2O fraction in BGE preferred
- CZE-analysis of pharmaceuticals requires a proper co-ion
- CZE can handle moderately hydrophobic compounds and has high separation potency towards impurities
- Generic BGE conditions determined for basic compounds

Conclusion (part I)

- Selection of proper generic BGE for CZE method development



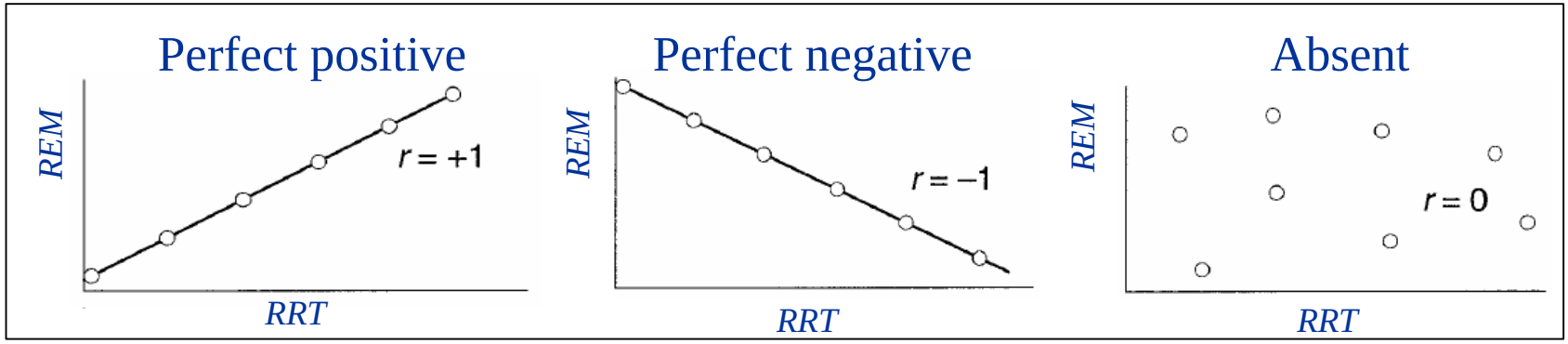
Co-ion	TRIS		TEtOHA	
Solvents	H_2O	ACN/ H_2O	H_2O/ACN/MeOH	MeOH/ACN
Composition	100	50/50	50/25/25	50/50
phosphate buffer, pH 2.5	A	B		
formate buffer, 4% v/v			F	E

Results (part II)

- Tools to determine level of orthogonality:
  - Described in literature, 3 principles selected:
    - Data variance: PCA
    - Spatial distances: WPGMA clustering
    - Correlation: Colormaps, HAAL clustering, Orthogonality factor *F*
  - Processing data:
    - Chromatographic (RRT) and electrophoretic behavior (REM)
    - Included 34 SLV-compounds
    - 16 RP-HPLC systems (4 buffer salts, always C_{18} as stationary phase)
    - 4 CZE systems (BGE A, B, E and F)

Results (part II)

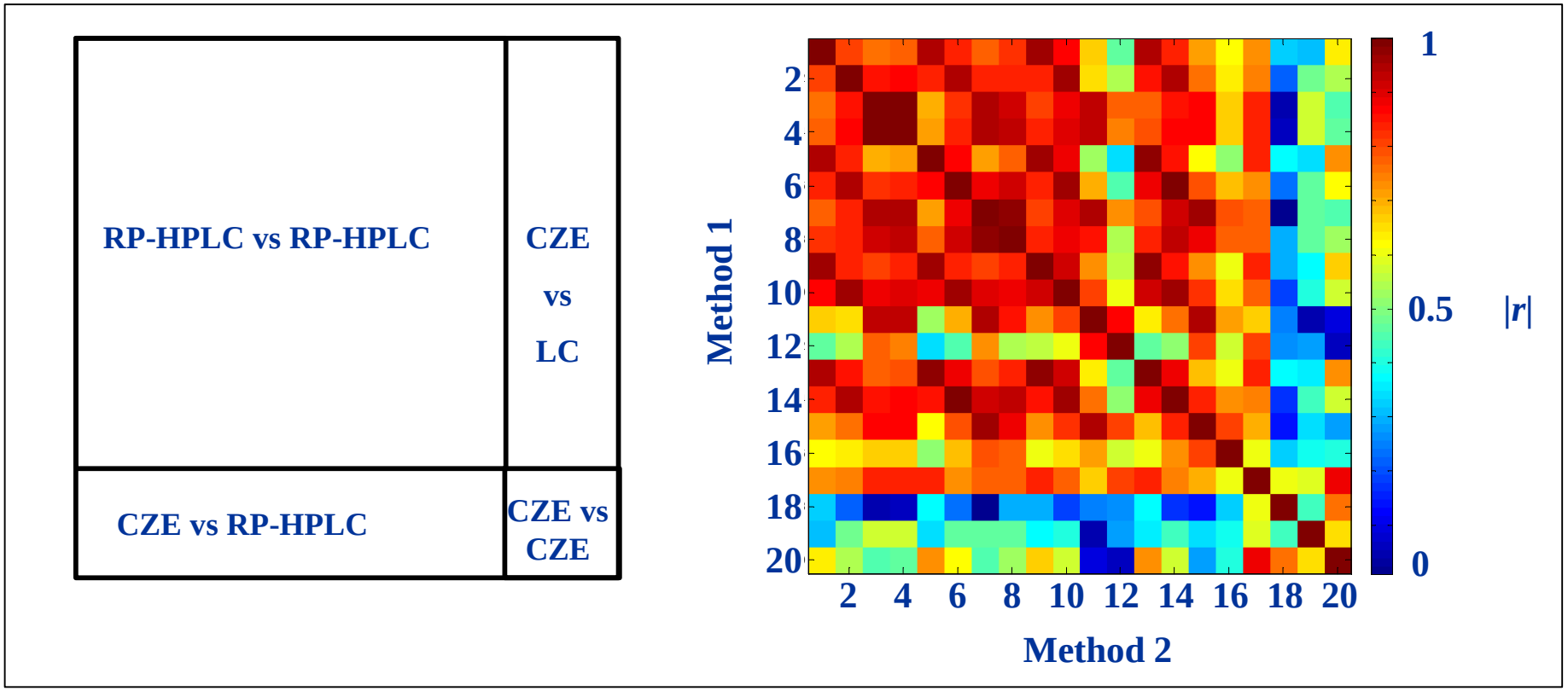
- Principle of the correlation coefficient r [4]:
  - $$r = \frac{\sum_i \left((x_i - \bar{x})(y_i - \bar{y})\right)}{\sqrt{\left(\sum_i (x_i - \bar{x})^2\right)\left(\sum_i (y_i - \bar{y})^2\right)}} \qquad \{1\}$$
  - Value: $-1 \leq r \leq 1$
  - Examples of r:



[4] J. Miller, J. Miller –*Statistics and Chemometrics for Analytical Chemistry*-, ISBN 0130228885, Pearson Education Limited, Dorset Press, 4th edition (2000)

Results (part II)

- Colormap to indicate |*r*| between methods [5]



[5] E. Van Gyseghem, et.al. -*Orthogonality and similarity within silica-based reversed-phased chromatographic systems*- Journal of Chromatography A 1074 (2005) 117-131

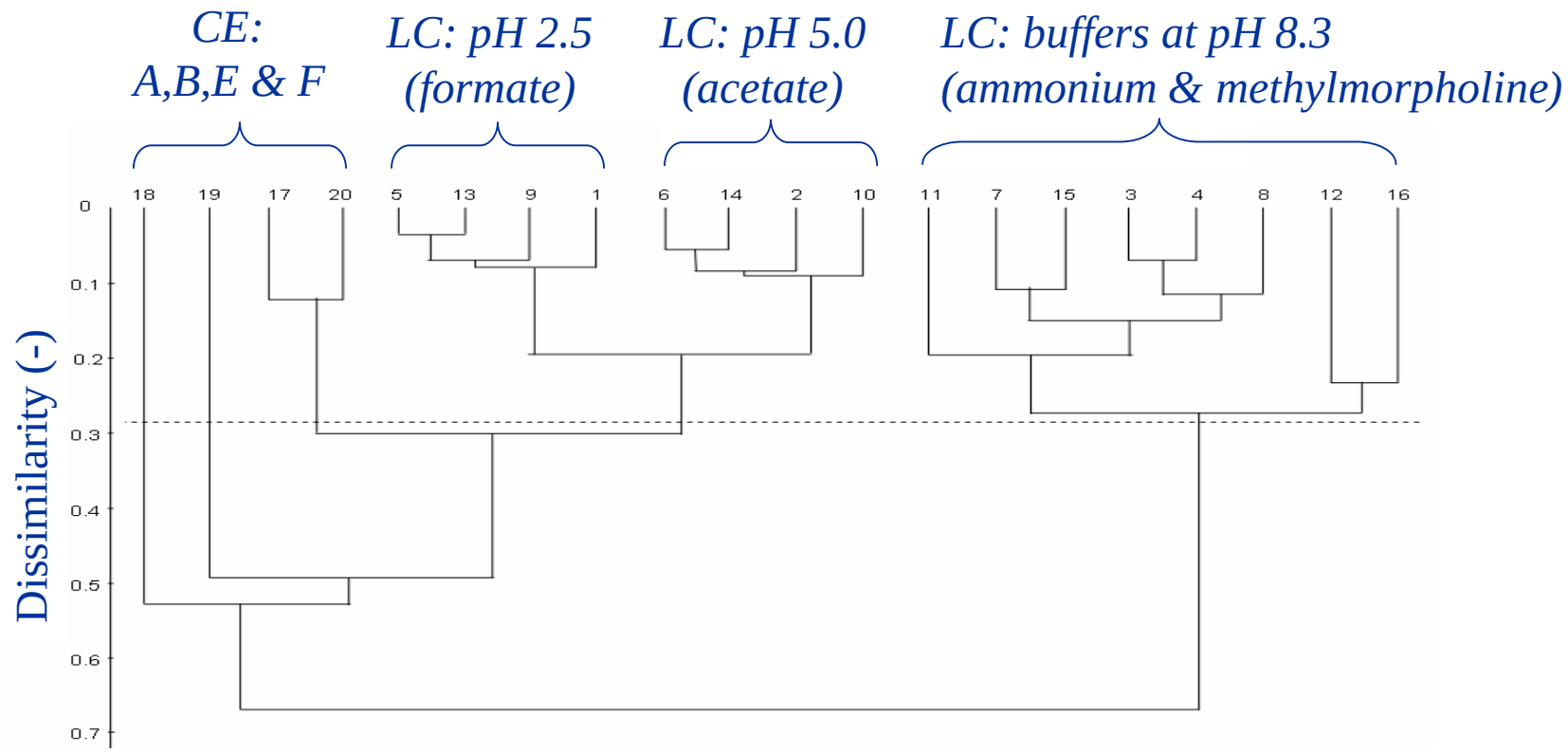
Results (part II)

- Cluster analysis tool [6]:
  - Processes dissimilarity values D ? $D = 1 - |r|$
  - In each clustering step methods with smallest D are linked
  - Using hierarchical agglomerative average linking (HAAL):
    - Linked methods are represented by their averaged D-values

[6] P. Forlay-Frick et.al. -*Selection of orthogonal chromatographic systems based on parametric and non-parametric statistical tests*-, Analytica Chimica Acta 539 (2005) 1-10

Results (part II)

- Dendrogram after HAAL-clustering:



Conclusion (part II)

- Tools, from literature, were applied on RRT/REM-pattern
- Dataset not enormous, strong indications after using different tools
- Tools based on correlation were found most favorable, since these are simple and can point out orthogonal methods
- Low level of orthogonality within one technique, especially within generic RP-HPLC-methods
- RP-HPLC and CZE had high level of orthogonality
- CZE was proven to be orthogonal towards RP-HPLC

Summary

- More hydrophobic compounds can be analyzed by CZE
- Even generic CZE methods show extreme selectivity
- Aqueous-organic BGEs can be applied easily
- Medium effect occurred only for highly organic BGEs
- Selection of proper co-ion can increase resolution

- Comparison of the elution and migration order of APIs with impurities
- A large dataset is of major importance
- Relative poor orthogonality observed within one technique
- Much more orthogonality between RP-HPLC and CZE methods
- CZE was proven to be orthogonal towards RP-HPLC

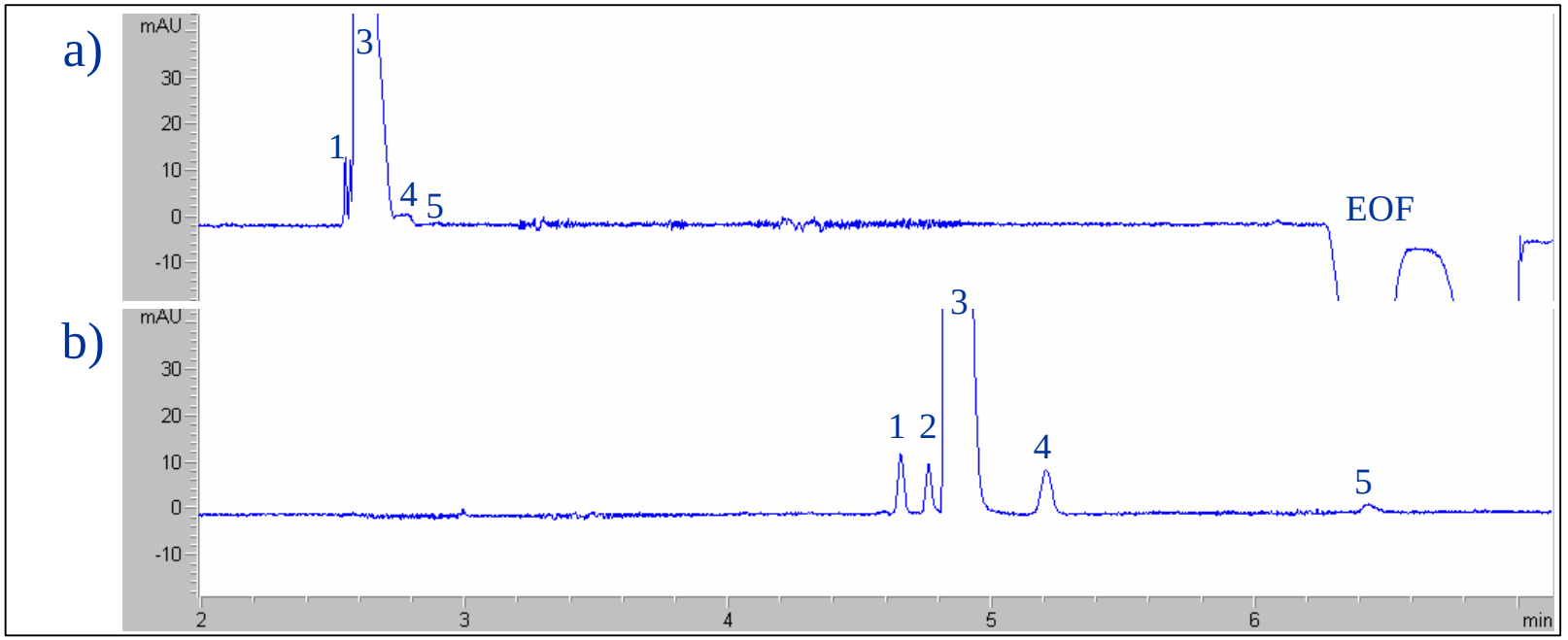
Questions or Discussion

Thanks goes to following Solvay Pharmaceutals researchers:
Marco Ruijken, Norbert Lammers, Piet Hoogkamer and Pim Muijselaar

Results (part I)

- Improved peak shape due to proper co-ion

  BGE: 4% v/v HCOOH in MeOH/ACN:50/50 (v/v), with addition of buffersalt NH_4OOCH (a) or TEtOHA (b)



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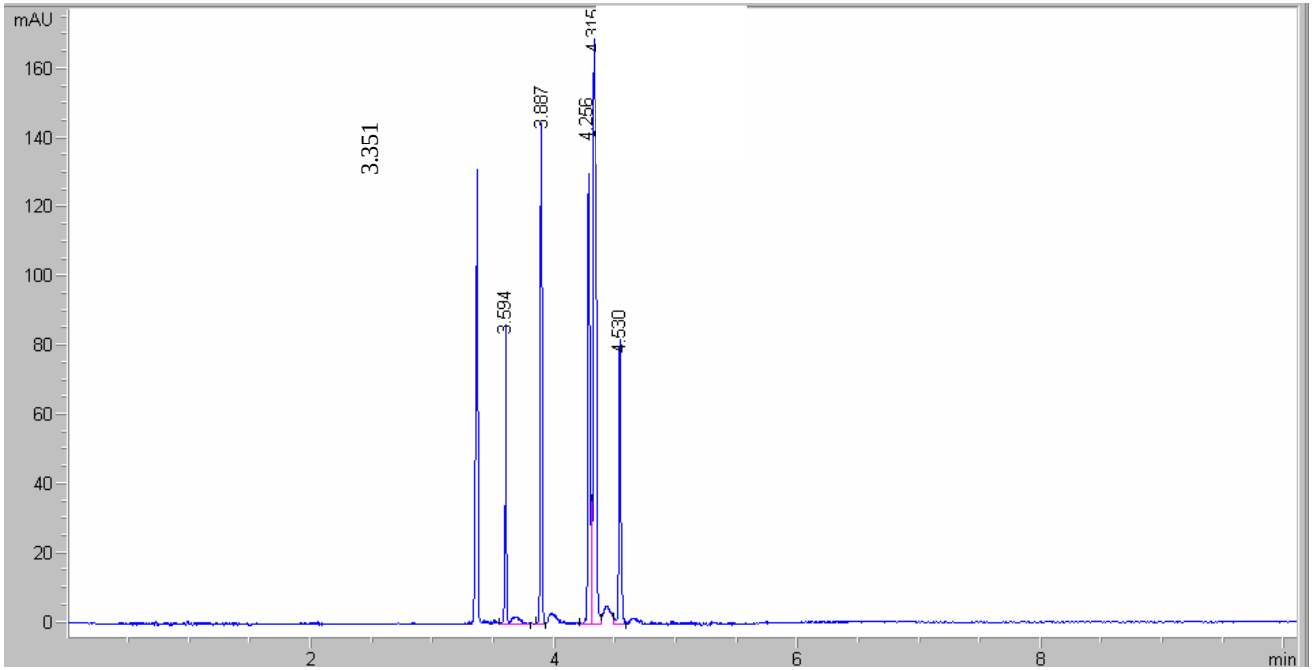
Results (part I)

- Sample: Mebeverine and 5 impurities (all ± 1 mg/mL),
  BGE: 100 mM TRIS and *o*-phosphoric acid at $^{w}_{w}pH$ = 2.5
  in H_2O/ACN : 50 / 50



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