University of Southampton

SOUThAmPtON Chemistry
THE ELEMENTS OF SUCCESS

Screening of a Sulfonamides Library by Supercritical Fluid Chromatography Coupled to Mass Spectrometry (SFC-MS). Preliminary properties-retention study.

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Cambridge
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OUTLINE

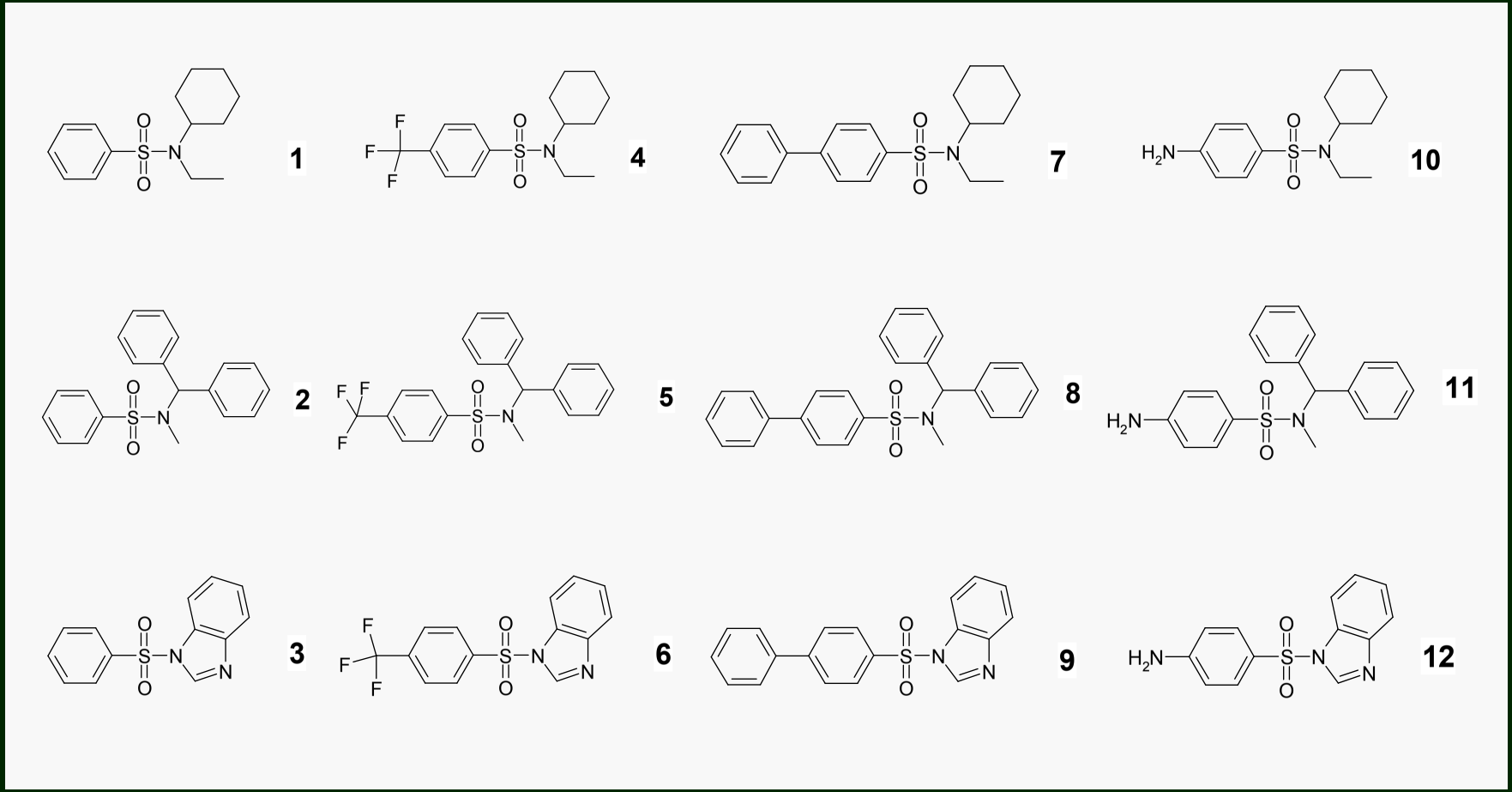
- **Introduction**
- **SOTLIB Library**
- **Isocratic study**
- **Retention models in chromatography**
- **Polycratic study**
  - Looking for linear relationships
  - Correlating data with physico-chemical properties
- **Conclusion and future work**

INTRODUCTION

- SFC-MS appears more and more as a technique complementary to HPLC for high throughput analysis
- Importance of knowing which technique is more suitable for a specific type of analytes
- Possibility of outlining a set of properties-based rules allowing prediction of the retention of a given compound by SFC ?
- To investigate those questions: design of a small library of sulfonamides compounds to be screened by SFC

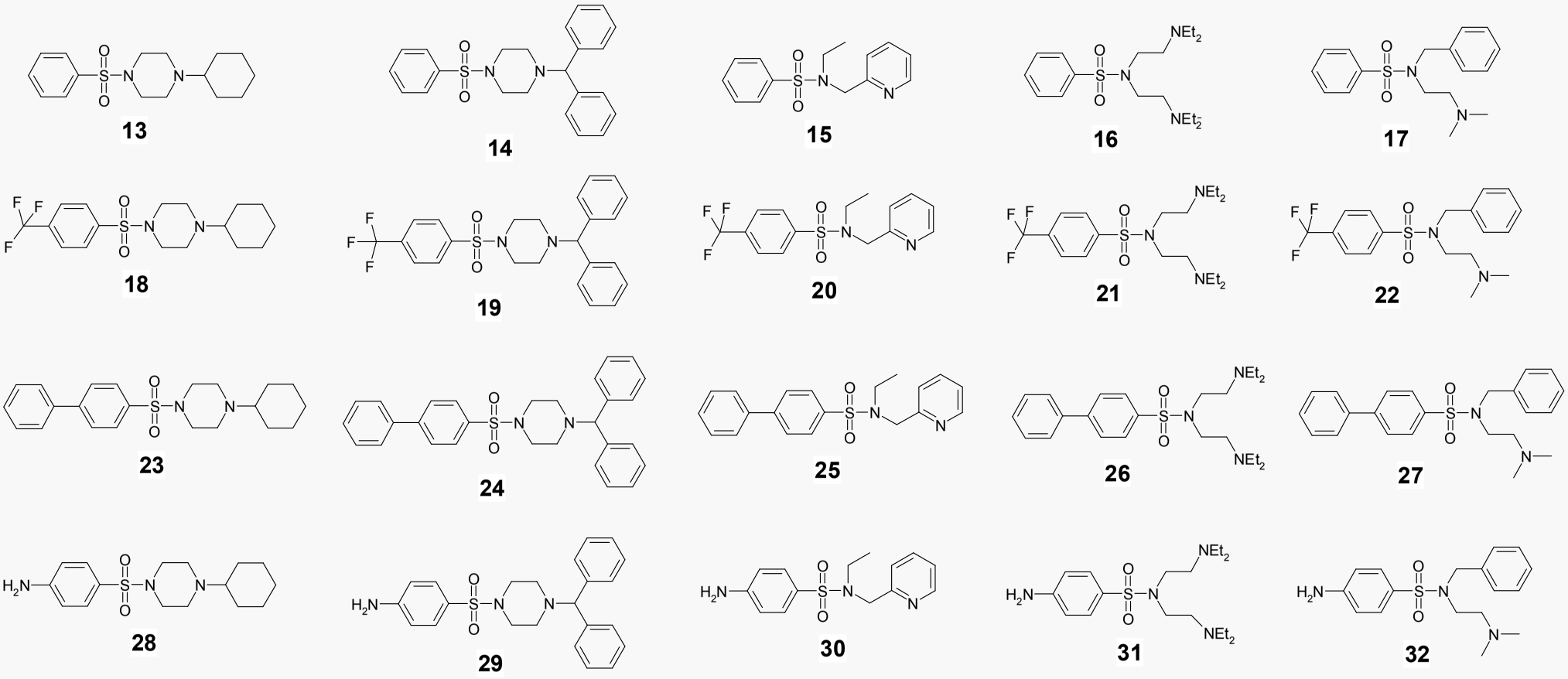
SOTLIB LIBRARY: NEUTRAL SET

- Coupling of 4 sulfonyl chlorides with 3 amines
- All compounds synthesized and analysed by SFC



SOTLIB LIBRARY: BASIC SET

- Coupling of 4 sulfonyl chlorides with 5 amines
- All compounds synthesized and analysed by SFC



ISOCRATIC STUDY: NEUTRAL SET (I)

- **Screening of the library using isocratic elution**

- **Neutral set studied on:**

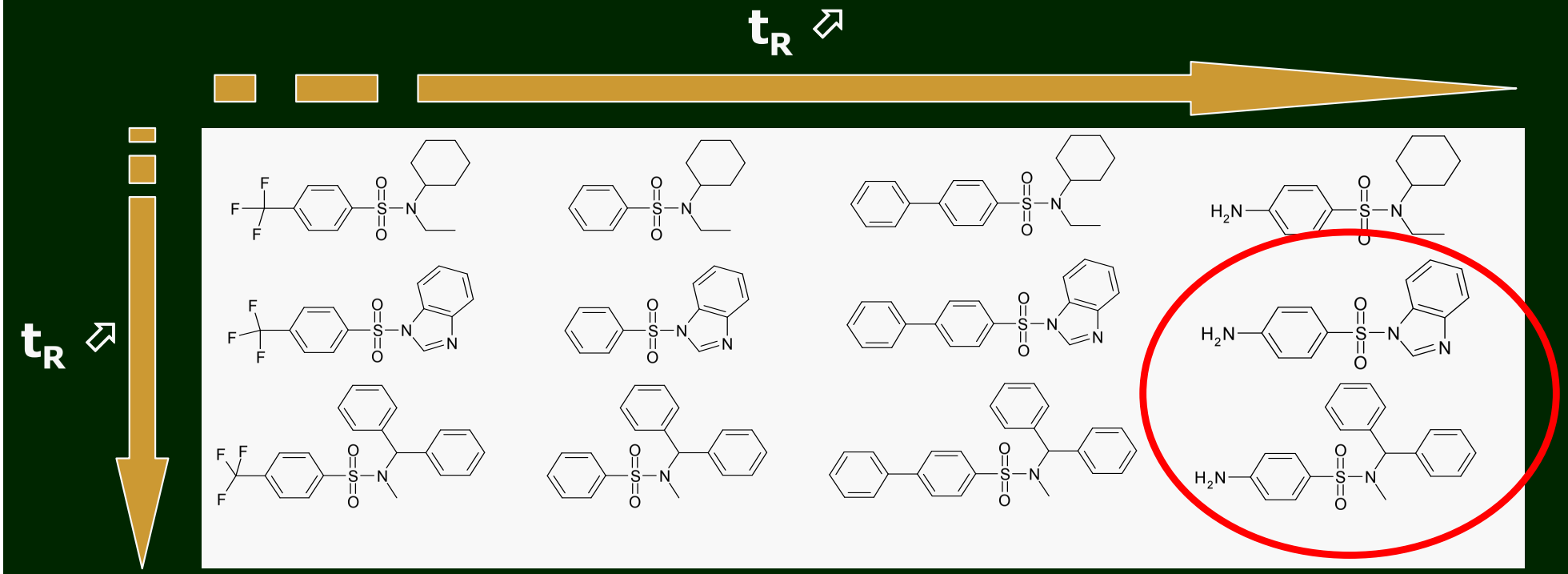
  - *2-ethyl-pyridyl* column 4.6x250mm
  - *cyano* column 4.6x250mm
  - *diol* column 4.6x250mm
  - *bare silica* column 4.6x250mm

- **Chromatographic Conditions:**

  - *mobile phase*: 20% v/v MeOH in CO_2
  - *flow*: 4mL.min^{-1}
  - *temperature*: 35°C
  - *outlet pressure*: 100 bars
  - *injection volume*: 4µL (~0.5mg.mL^{-1})

ISOCRATIC STUDY: NEUTRAL SET (II)

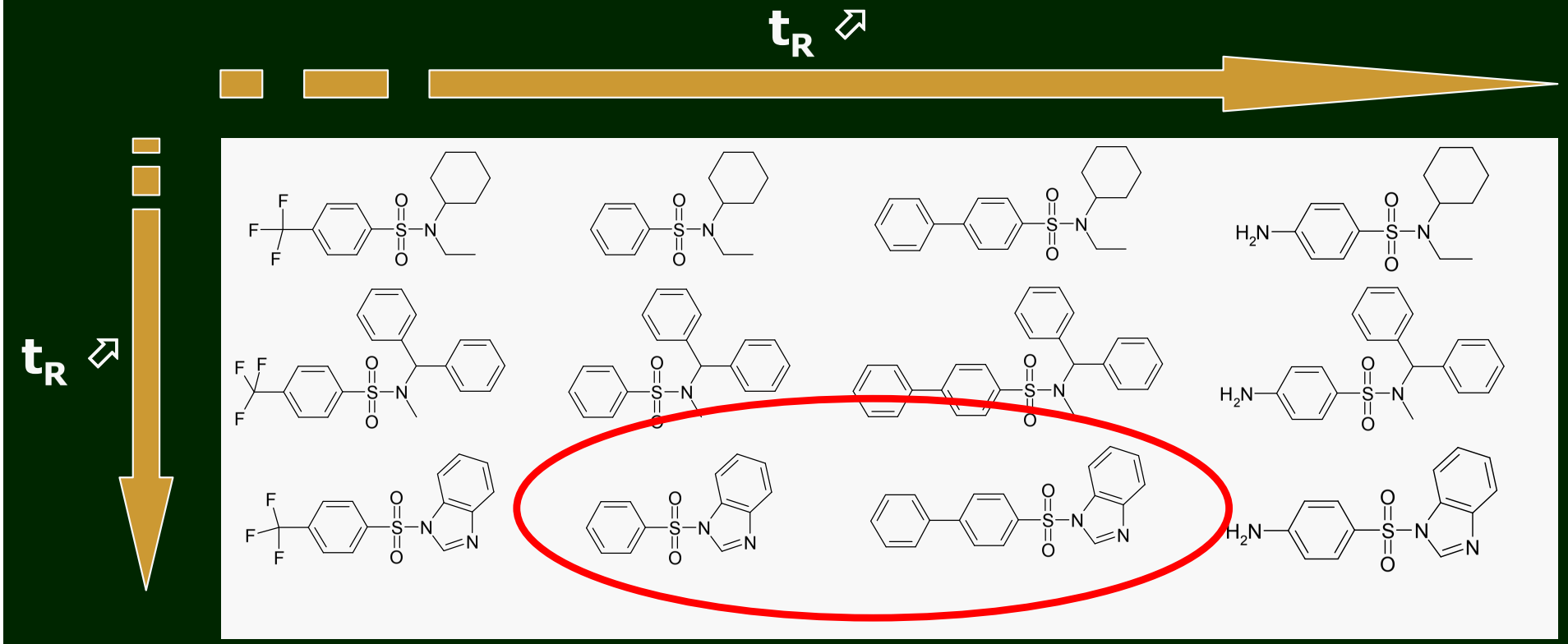
- Retention time related to structure of compounds
- 2-ethyl-pyridyl (2-EP) and cyano columns:



- On 2-EP phase: co-elution of 2 compounds, despite distinct physico-chemical properties.

ISOCRATIC STUDY: NEUTRAL SET (III)

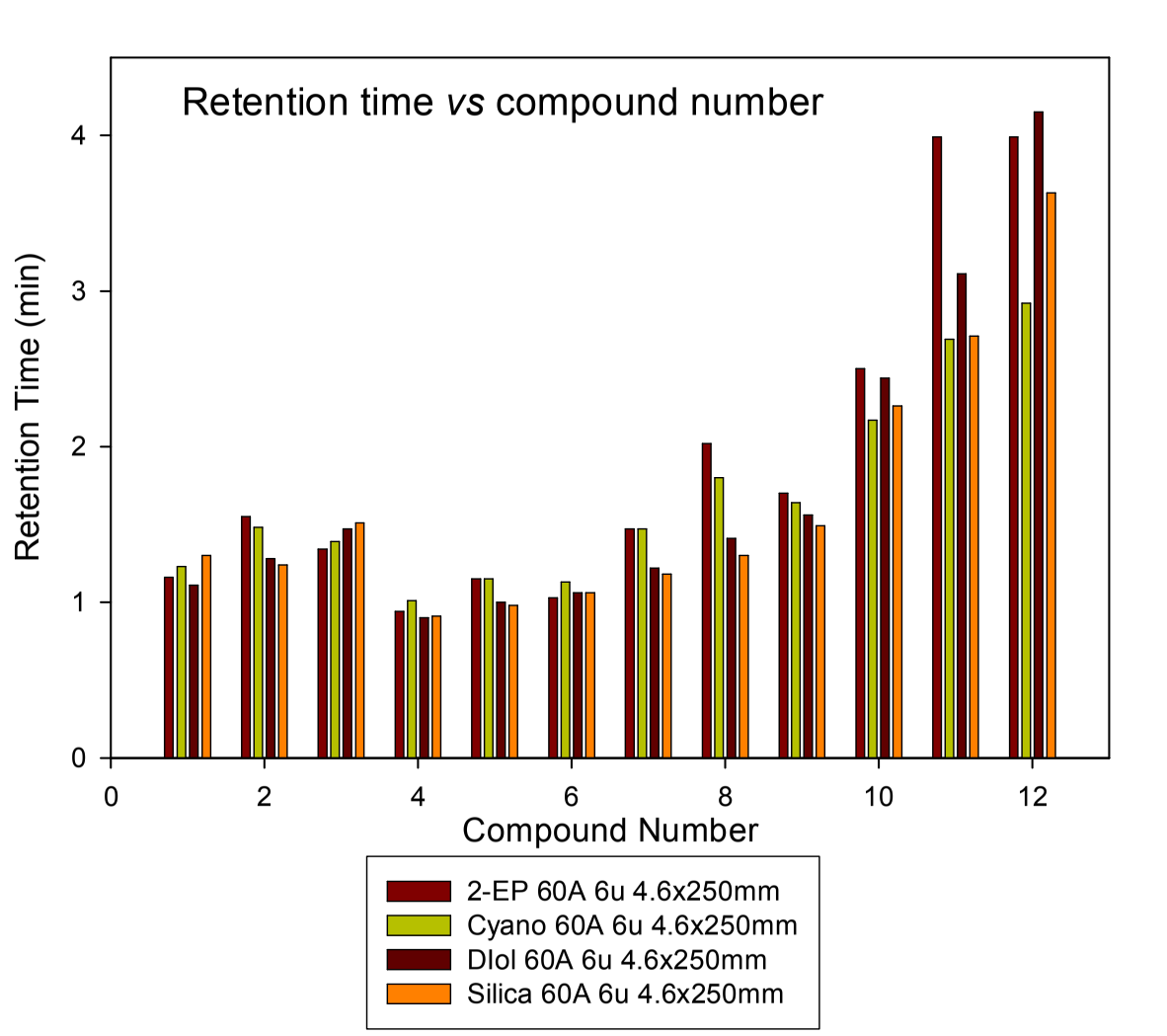
- Different trend observed on diol and silica phases: benzimidazole derivatives eluted the latest



- On bare silica column: compounds 3 and 9 co-eluting

COLUMN COMPARISON

- **Method: isocratic 20% MeOH, 4mL/min, 35°C, 100 bars**



- **Compounds not more retained by a particular column**
- **Same interactions between compounds and all four columns ?**
- **Retention mechanism mainly due to free silanol groups ?**

ISOCRATIC STUDY: BASIC SET (I)

- **Screening of the library using isocratic elution**

- **Basic set studied on:**

  - 2-EP column 4.6x250mm
  - *cyano* column 4.6x250mm
  - *diol* column 4.6x250mm

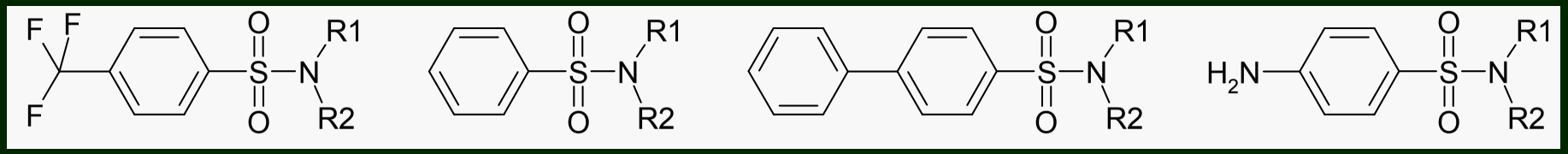
- **Chromatographic Conditions:**

  - *modifier*: MeOH + 0.1% v/v DEA
  - *mobile phase*: 20% v/v modifier in CO_2
  - *flow*: 4mL.min^{-1}
  - *temperature*: 35°C
  - *outlet pressure*: 100 bars
  - *injection volume*: 4µL (~0.5mg.mL^{-1})

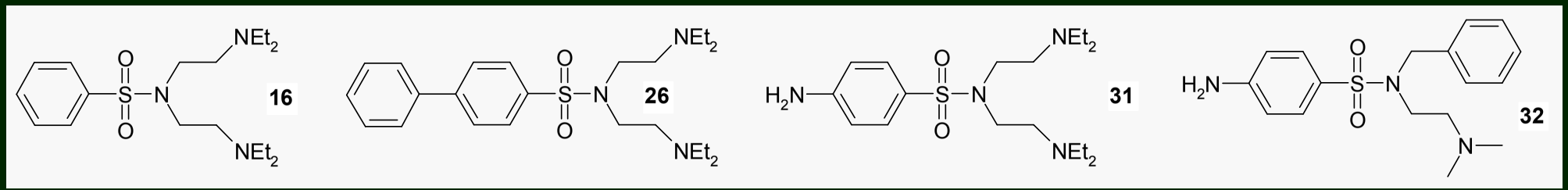
ISOCRATIC STUDY: BASIC SET (II)

- Retention time related to structure of compounds
- BUT: only with regards to sulfonyl part
- Same trend as observed for neutrals:

t_R ⇗

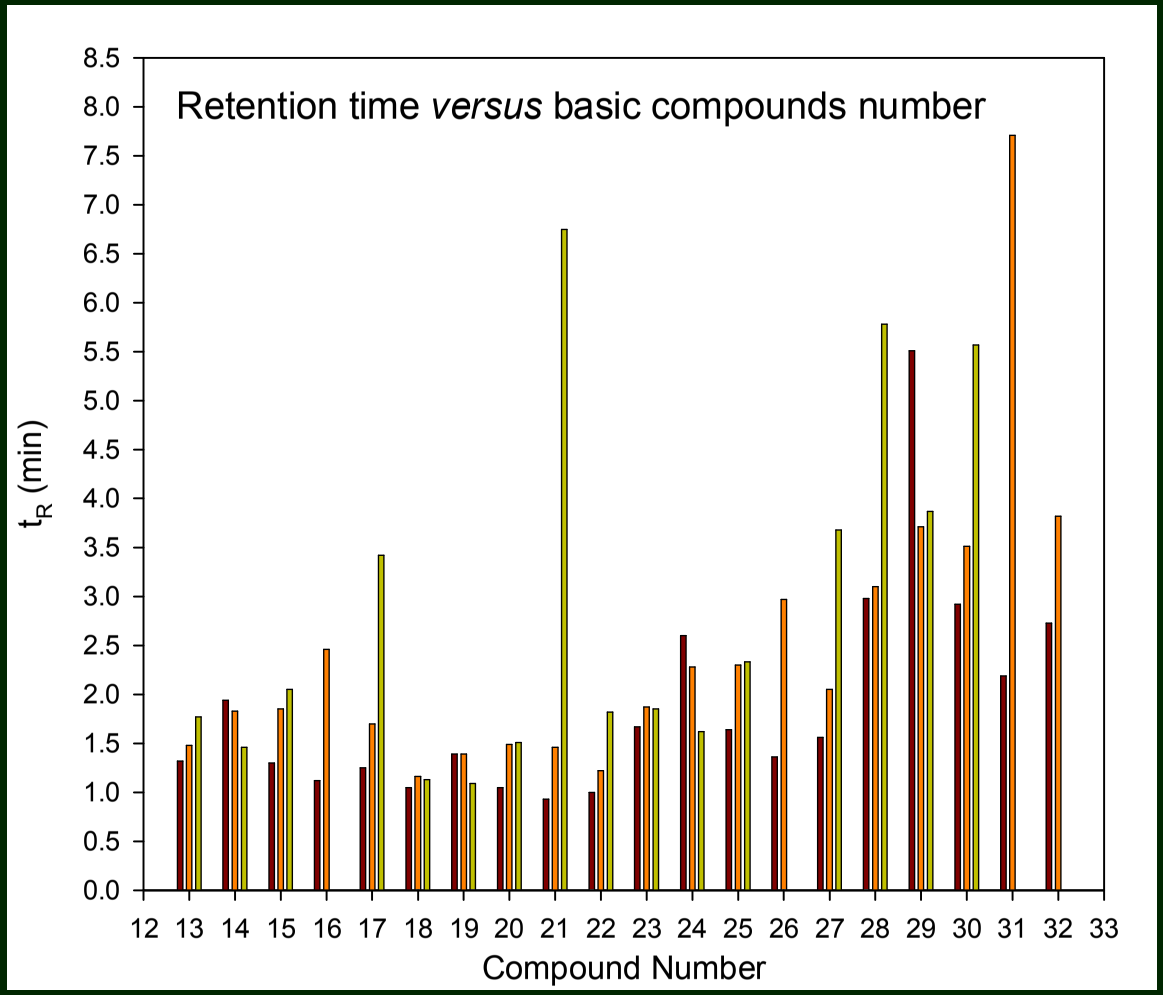


- Diol columns: 4 compounds not eluted:



COLUMN COMPARISON

- **Isocratic elution, 20% MeOH+0.1%DEA, 4mL/min, 35°C, 100 bars**



- **More differences than observed with neutral set**
- **However retention close on all columns for most of the compounds**
- **Retention mechanism mainly due to free silanol groups ?**

LIMITS OF THE ISOCRATIC APPROACH

- No simple correlation highlighted between physico-chemical properties and t_R
- Is the isocratic approach right?
- Indeed: methanol composition (20%) chosen arbitrarily
- So, why 20% and not 30% ?
- Retention time: the most obvious parameter, but is it the most appropriate ?

RETENTION MODEL IN CHROMATOGRAPHY (I)

- More than the retention time, the capacity ratio *k* is a convenient way to normalize the retention of an analyte:

$$k = \frac{t_R - t_0}{t_0}$$

- Most successful models of retention developed by Snyder[1] and Soczewinski[2], in reverse phase systems like ODS/H_2O+MeOH:

$$\log k = \log k_w - n\varphi$$

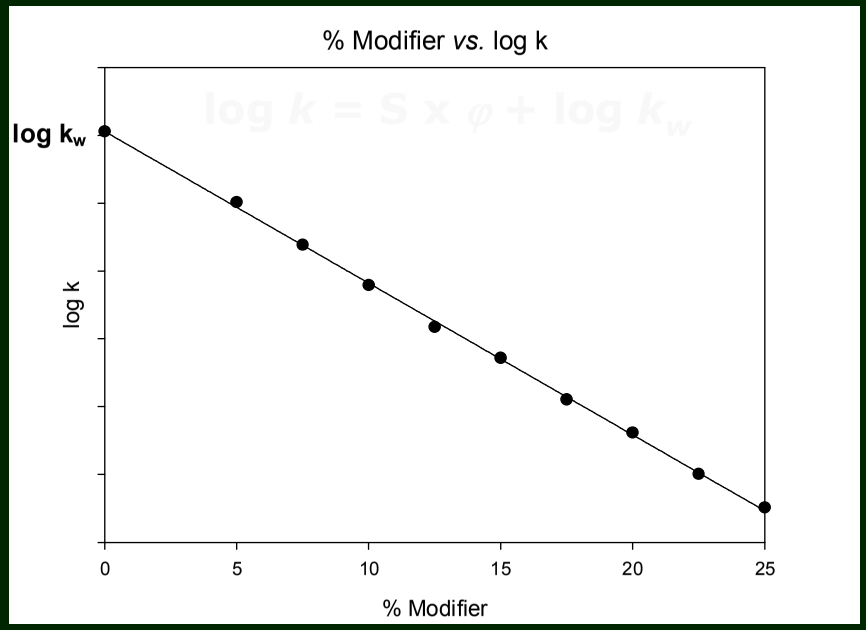
- In normal phase system, strong deviation observed, but linear correlation can be found in log-log coordinate system:

$$\log k = \text{const} - m\log\varphi$$

1. Snyder, L. R., *Principles of Adsorption chromatography*. First ed.; M. Dekker: New York, **1968**
2. Soczewinski, E., Mechanistic molecular model of liquid-solid chromatography - Retention-eluent composition relationships. *Journal of Chromatography A* **2002,** 965, (1-2), 109-116

RETENTION MODEL IN CHROMATOGRAPHY (II)

- Regression analysis: calculation of intercept log k_w and slope
- log k_w: standardised parameter more reliable than arbitrary isocratic log k
- Start of "polycratic" study, aim: calculation of intercept "log k_0" and slope S



« POLYCRATIC » STUDY

- **Requirement: keep log *k* within a range of "practical interest" as defined by Schoenmaker *et al.*[1]:**

$$0 < \log k < 1$$

- **Getting the retention factor within the range:**

  - *slow eluting compounds*: no difficulties since increasing φ allows to elute them soon enough

  - *fast eluting compounds*: decreasing φ doesn't systematically allow sufficient increase in retention to comply with requirement

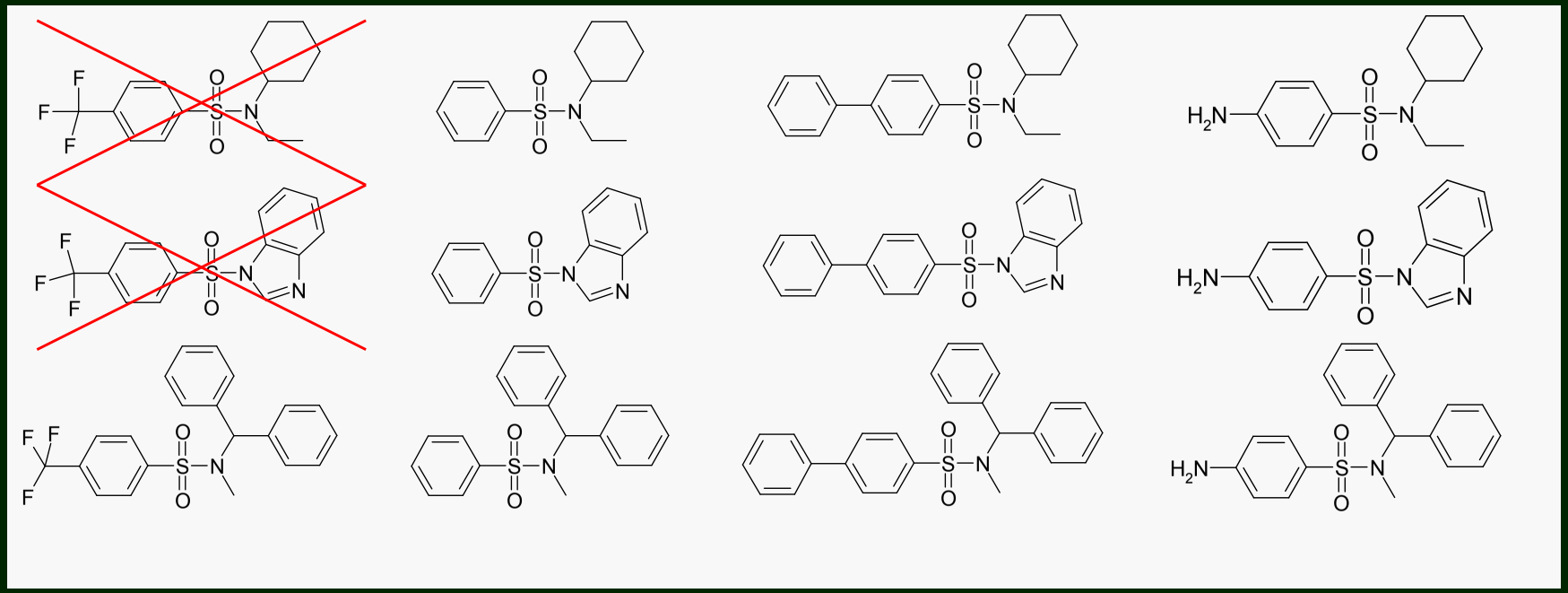
- **Consequence: some compounds to be removed from study**

- **Analyses carried out three times, plotting of mean values**

1. Schoenmaker *et al. Journal of Chromatography* **1979**, *185*, 179-195

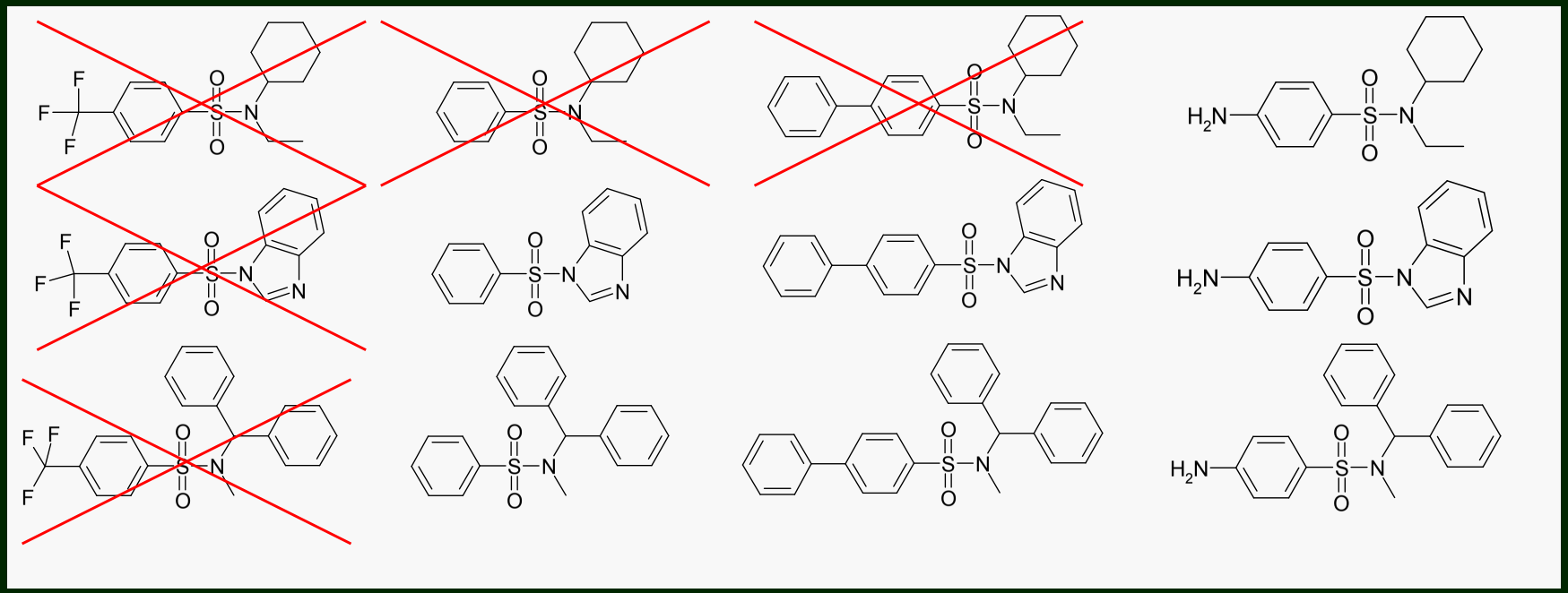
2-EP COLUMN

- 10 compounds within *k* range
- plot φ *vs.* log *k*: $R^2 > 0.98$
- log k_0 and *S* calculated for the 10 compounds



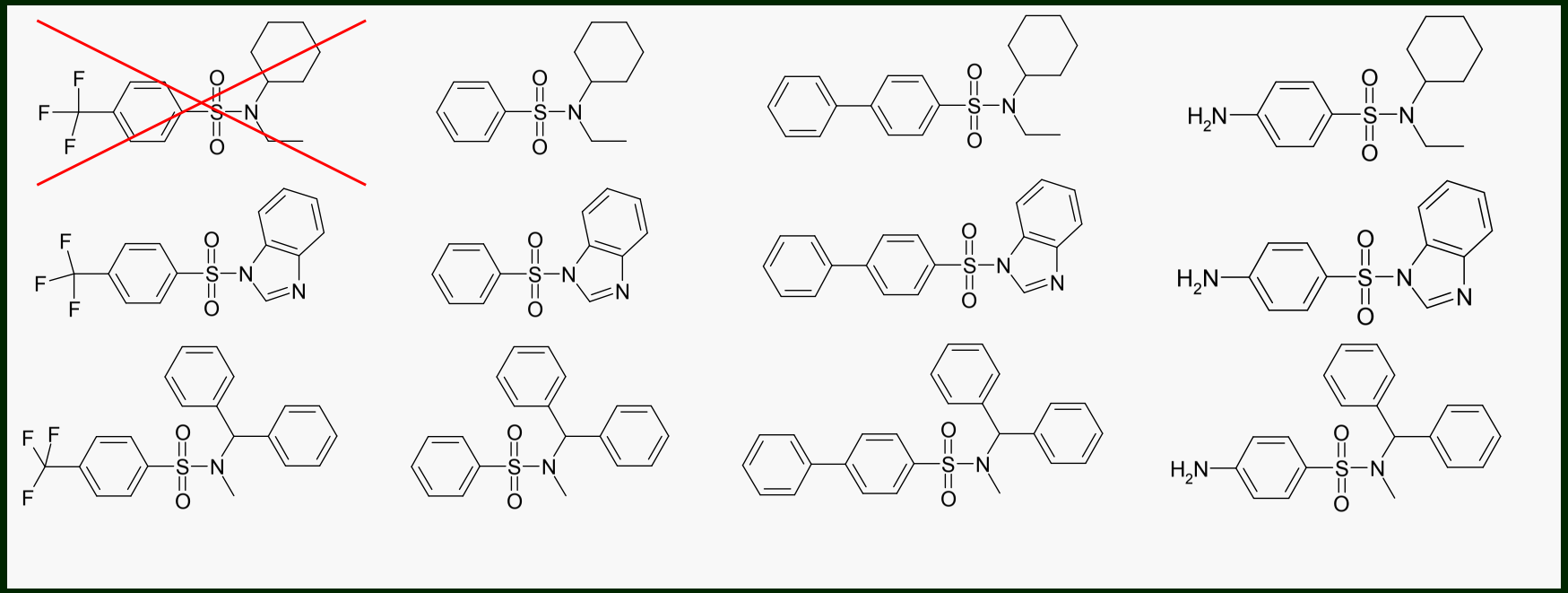
SILICA COLUMN

- 7 compounds within *k* range
- plot φ *vs.* log *k*: $R^2 > 0.99$
- log k_0 and *S* calculated for the 7 compounds



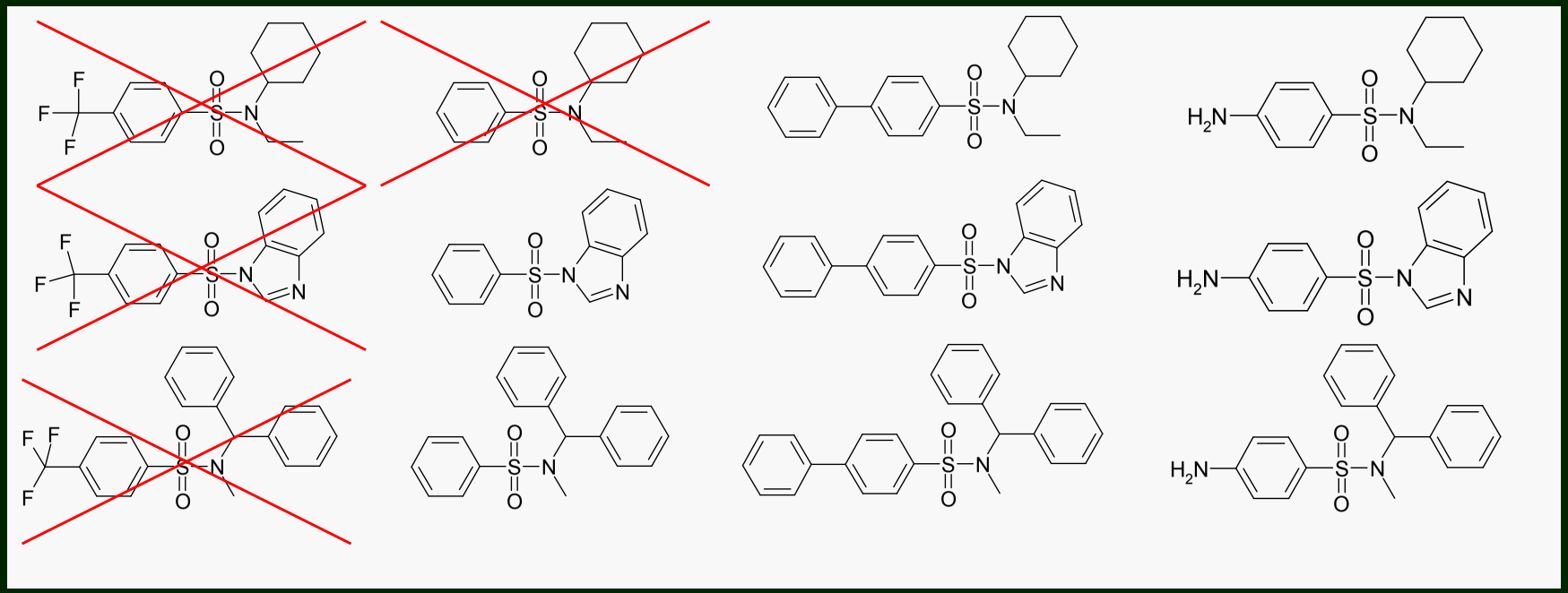
CYANO COLUMN

- 11 compounds within *k* range
- plot φ *vs.* log *k*: $R^2 > 0.99$ (except compound 6 at $R^2=0.92$)
- log k_0 and *S* calculated for the 11 compounds



DIOL COLUMN

- 8 compounds within *k* range
- plot φ *vs.* log *k*: $R^2 > 0.97$
- log k_0 and *S* calculated for the 8 compounds



CORRELATING DATA WITH COMPOUNDS PROPERTIES (I)

- **On none of the columns, log k_0 and S could be correlated with lipophilicity constants of the analytes (log P [ACD and Spartan], log D at pH 7.4) nor with pKa values.**

- **However, other properties of the neutral set compounds calculated with Spartan, *e.g.*:**

  - total dipole moment μ
  - surface area A
  - volume V
  - electronic charges on single atoms

- **Multiple regression analysis performed to correlate properties with retention characteristics log k_0 and S**

CORRELATING DATA WITH COMPOUNDS PROPERTIES (II)

➢ **On 2-EP column:**

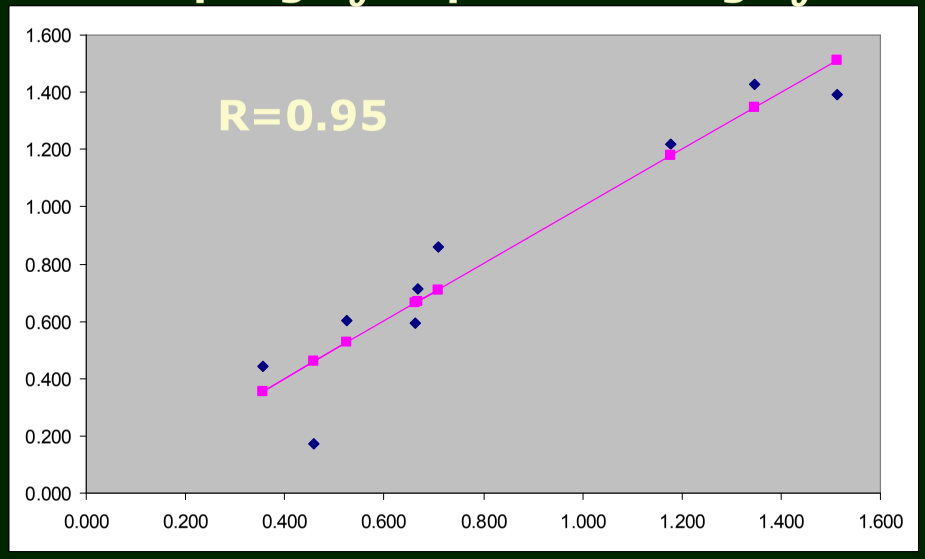
- when regressing log k_0 *vs.* μ, *A* and δ_{min}:

log k_0 = 0.232 μ + 0.001 *A* – *1.325* δ_{min} – 2.200 R=0.95

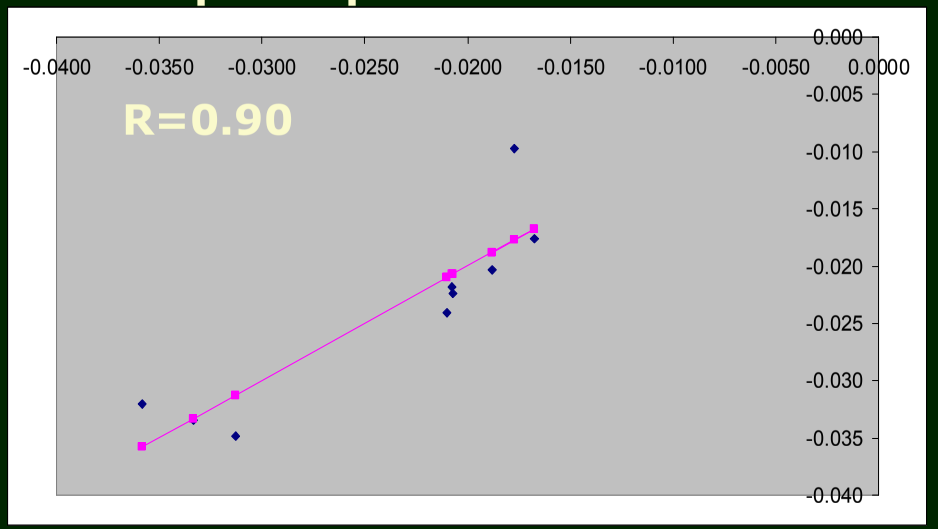
- when regressing *S vs.* μ, *A* and δ_{min}:

S = -0.003 μ - 1.6x10^{-5} *A* + *0.27* δ_{min} – 0.023 R=0.90

exp log k_0 *vs* predicted log k_0



exp S *vs* predicted S



CORRELATING DATA WITH COMPOUNDS PROPERTIES (III)

- **On cyano column:**

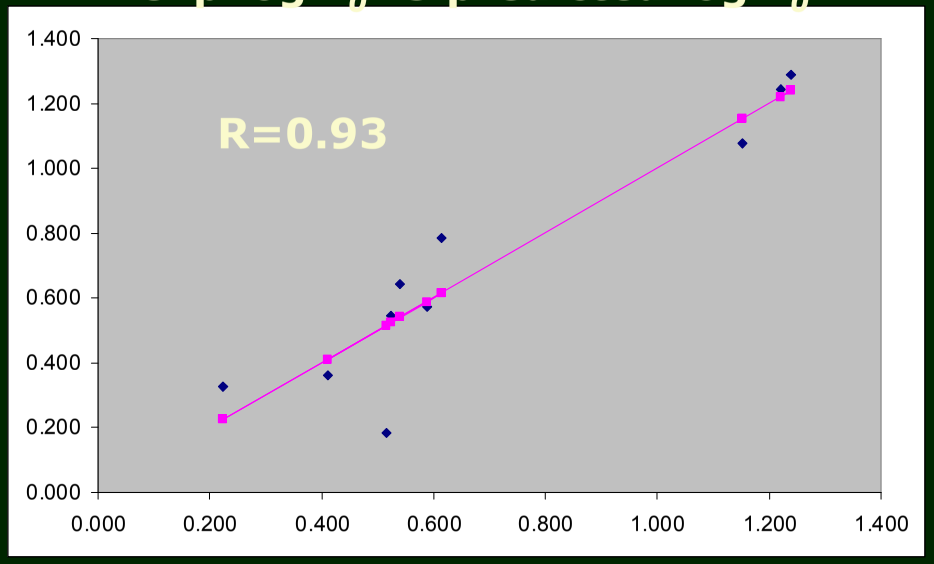
  - when regressing log k_0 *vs.* μ, *A* and δ_{min}:

log k_0 = 0.063 μ + 0.001 *A* – *1.579* δ_{min} – 1.25 R=0.94

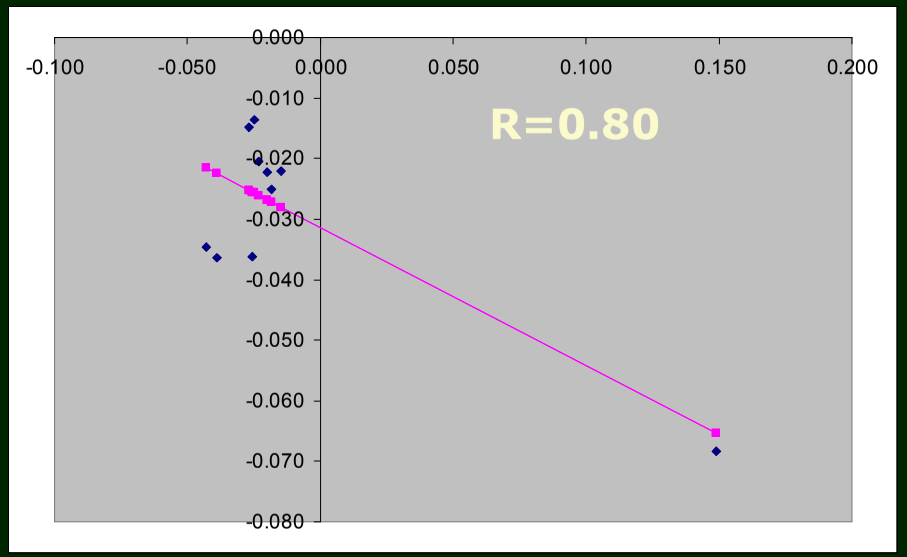
  - when regressing *S vs.* μ, *A* and δ_{min}:

S = -0.011 μ - 2.2x10^{-5} *A* + *0.071* δ_{min} – 0.040 R=0.84

exp log k_0 *vs* predicted log k_0



exp S *vs* predicted S



CORRELATING DATA WITH COMPOUNDS PROPERTIES (IV)

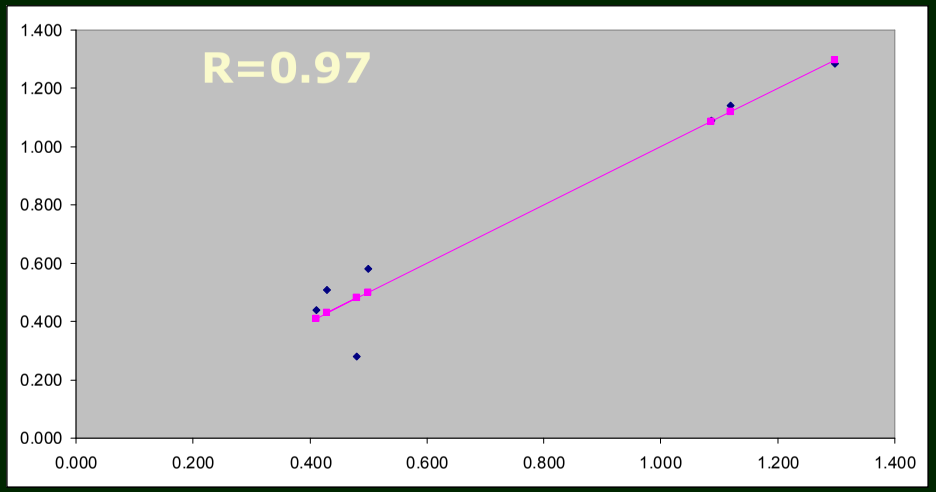
- **On diol column:**
  - when regressing log k_0 *vs.* μ, *A* and δ_{min}:

log k_0 = 0.124 μ + 0.0002 *A* – *1.409* δ_{min} – 1.173 R=0.97

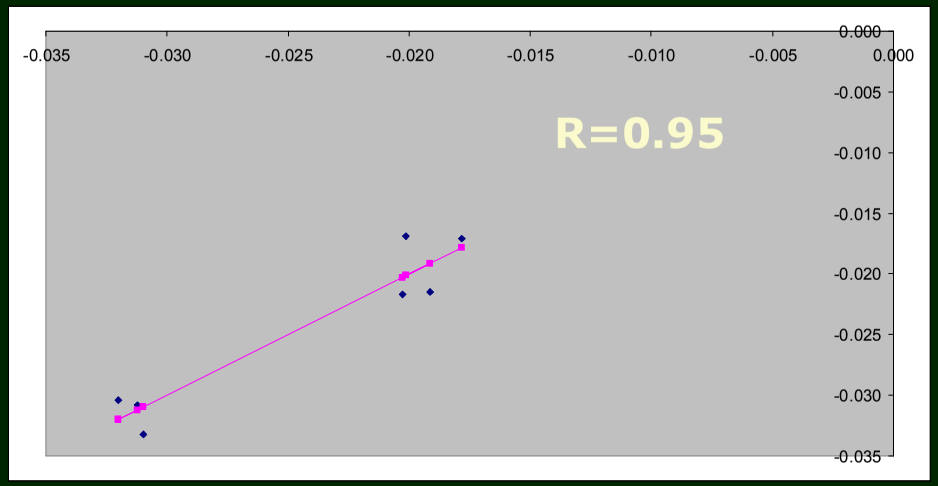
  - when regressing *S vs.* μ, *A* and δ_{min}:

S = -0.0004 μ - 1.1x10^{-5} *A* + *0.030* δ_{min} – 0.008 R=0.95

exp log k_0 *vs* predicted log k_0



exp S *vs* predicted S



CORRELATING DATA WITH COMPOUNDS PROPERTIES (V)

➢ **On silica column:**

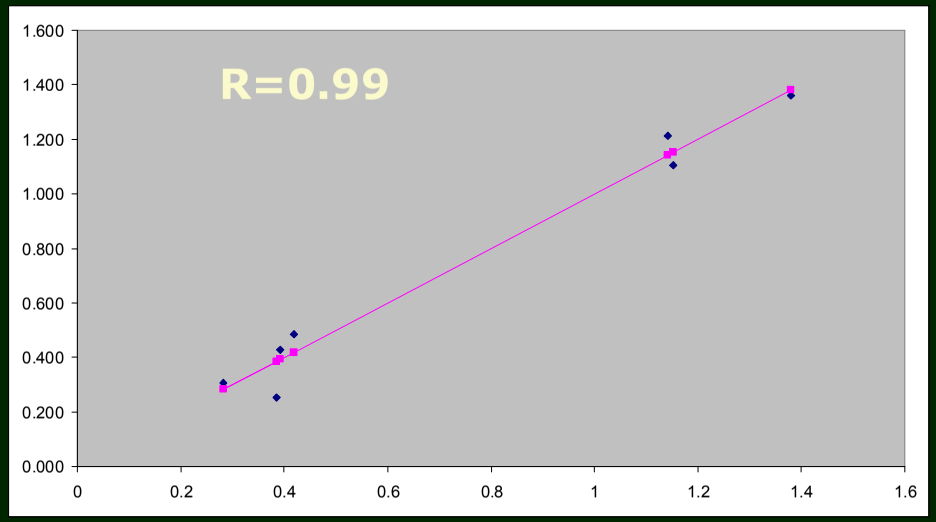
- when regressing log k_0 *vs.* μ, *A* and δ_{min}:

log k_0 = 0.119 μ - 0.001 *A* – 1.714 δ_{min} – 1.178 R=0.99

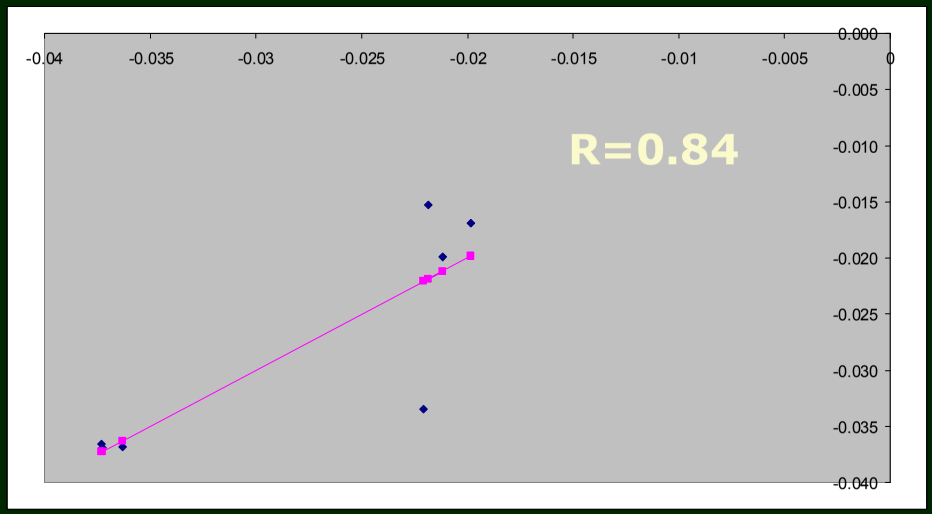
- when regressing *S vs.* μ, *A* and δ_{min}:

S = -0.0008 μ - 7.7x10^{-5} *A* + 0.040δ_{min} – 0.012 R=0.84

exp log k_0 *vs* predicted log k_0



exp S *vs* predicted S



CONCLUSION (I)

- **Isocratic approach: highlighting of trends in retention**
- **Polycratic study:**

  - supposed *better characterization* of the retention

  - *no correlation* of log k_0 or S with *lipophilicity constants or pKa*

  - *study of 12 neutral sulfonamides* on 2-EP, cyano, diol and bare silica stationary phases

  - linearity found for $\log k = \log k_0 + S \times \varphi$ $R^2>0.97$

CONCLUSION (II)

- **Correlating retention characteristics and molecular descriptors**

  - *Retention characteristics log* k_0 *and S correlated with* total dipole moment μ, surface area *A* and atomic charge on the most negatively charged atom δ_{min}

  - *Encouraging results for log k0* which are correlated with molecular descriptors with R > 0.94 on all four stationary phases

  - *Less promising results for S*, especially on cyano and silica columns (R = 0.84).

  - *Results to be considered with care*, since obtained on small set of structurally similar compounds.

FUTURE WORK

- Analysis of a set of 20 basic sulfonamides currently under way
- Study to be extended to compounds of different structures
- Recalculation of molecular descriptors by more powerful algorithms under way
- Regression analysis using other descriptors has to be undertaken in an attempt to highlight better correlation (experimental log *P* to be measured)

ACKNOWLEDGEMENTS