University
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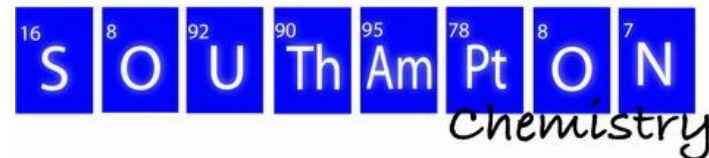
SOUThAmPtON
Chemistry
THE ELEMENTS OF SUCCESS

Properties-retention study on Supercritical Fluid Chromatography coupled to Mass Spectrometry (SFC-MS). Analysis of a sulfonamide library.

HPLC conference 2007
Ghent

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Outline

➤ **Introduction**

➤ **The library**

➤ **Retention characteristics**

➤ **Quantitative Structure-Retention Relationships (QSRR)**

➤ **The study**

➤ **Results**

➤ **Conclusion**

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 analyte**

➢ **Is there a 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 to be screened by SFC**

Design and synthesis of the compounds

➤ **Library based on 4 sulfonylchlorides and 8 amines**
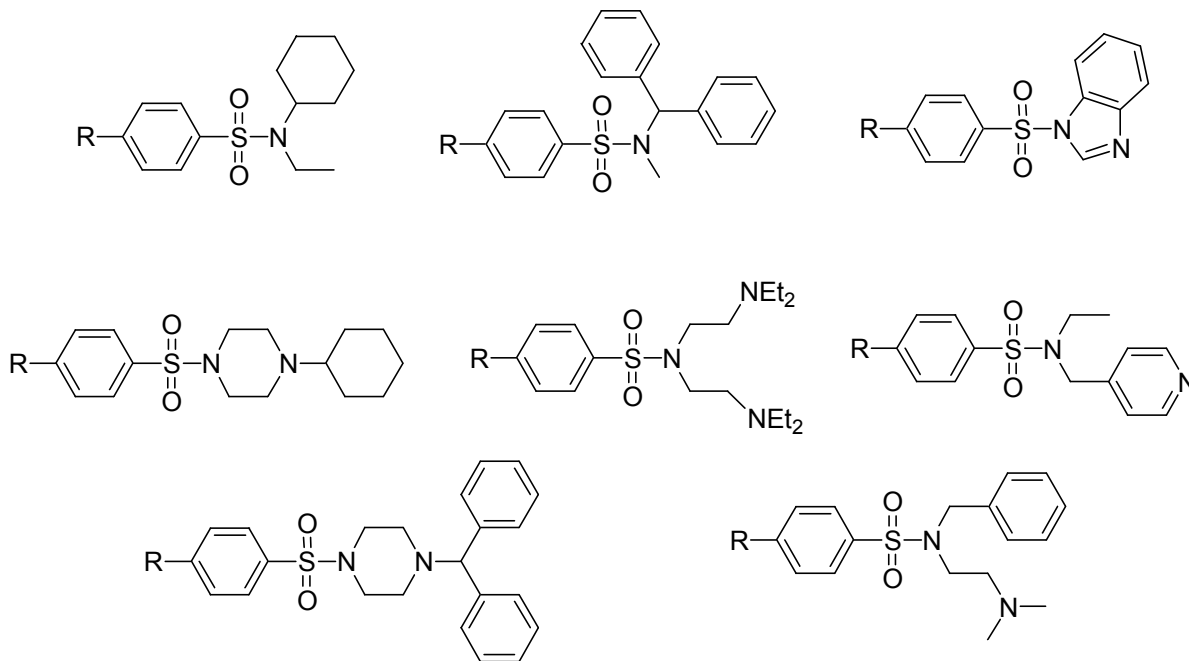
R = H, F$_3$C, phenyl, H$_2$N

➤ **Design of the library:**
  - Choice of sulfonamides (widely used, quick synthesis)
  - Choice of subsituents to have neutrals and basics, comply with
    Lipinski's rule of five and include privileged structures

Retention characteristics (I)

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

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

➢ **Successful models of retention developed by Snyder[1] and Soczewinski[2], in reverse phase systems like ODS/H_2O+MeOH**

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

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

$$\log k = \log k_0 + S \times \log \varphi$$

➢ **Intercept log k_0: standardised parameter more reliable than arbitrary isocratic log k**

1. Snyder, L. R., *Principles of Adsorption chromatography*. First ed.; M. Dekker: New York, **1968**
2. Soczewinski, E. *Journal Chromatogr. A* **2002,** 965, (1-2), 109-116

Retention characteristics (II)

➢ **Polycratic study: measurement of log *k* at different modifier concentrations in the mobile phase**

φ vs. log k

$$\log k = S \times \varphi + \log k_0$$
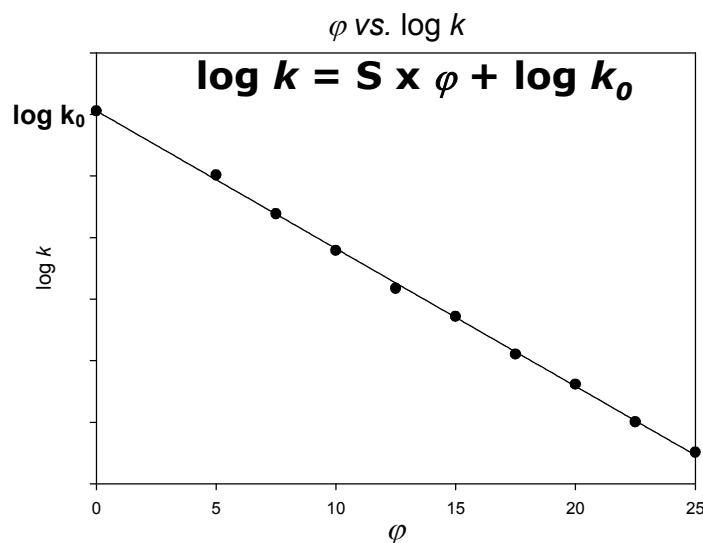
➢ **Regression analysis: calculation of intercept and slope of the curve**

➢ **Aim: calculation of intercept log k_0 and slope *S* for a series of compounds**

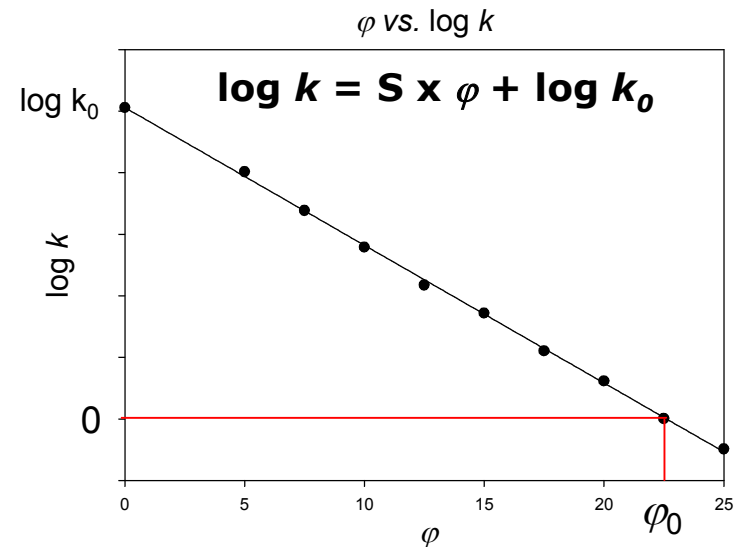# Variation of retention with mobile phase composition (III)

➢ **Another parameter of interest:** φ_0

➢ φ_0 **is the value of** φ **for which log** k **equals 0**

➢ **Calculated through:**

$$\log k = \log k_0 - n \times \varphi$$

$$\log k = 0 \Rightarrow 0 = \log k_0 - n \times \varphi_0$$

$$\Rightarrow \varphi_0 = -\frac{\log k_0}{S}$$

φ vs. log k

log k **= S x** φ **+ log** k_0

➢ **Practically, log** k **equals 0 means that the analyte spends as much time in mobile phase as in stationary phase** *i.e.* $t_R = 2\ t_0$

➢ **Easier to interpret than log** k_0 **and** S

QSRRs

➢ **QSSR: Quantitative Structure-Retention Relationships**

➢ **Statistical relationships between chromatographic characteristics of a series of analytes and measured or calculated quantities accounting for their structural differences**

➢ **Based on linear free-energy relationships (LFER)**

➢ **Used for, *e.g.***

- retention prediction
- meaningful descriptors identification
- retention mechanism elucidation
- classification of stationary phases

The solvation parameter model

➢ **Introduced and developed by Abraham[1] and Carr[2]**

➢ **Based on linear solvation energy relationship**

➢ **Retention process is divided into contributions for intermolecular interactions**

$$\log k = eE + sS + aA + bB + vV + c$$

➢ **Capital letters: molecular descriptors of the characterising the analytes**

E = excess molar refraction.
Models polarizability contributions
from n and π electrons.

S = solute dipolarity/polarizability.
Models dipole-dipole interactions.

A and *B* = hydrogen bond acidity and basicity.
Model overall hydrogen-bond interactions.

V = McGowan characteristic volume.
Models dispersive interactions.

➢ **Small letters: coefficients calculated through multiple linear regression analysis, characteristic of the chromatographic system used**

1.   P.C. Sadek, P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft, M.H. Abraham, *Anal. Chem.* **1985**, 57, 2971.
2.   P.W. Carr, *Microchem. J.* 1993, 48, 4.

Reduction of number of descriptors

➢ **Kaliszan _et al._[1] showed that retention in HPLC could be modelled using only three easily calculated molecular descriptors**

- total dipole moment μ
- electron excess charge on the most negatively charge atom δ_{min}
- solvent accessible molecular surface area A

➢ μ **accounts for dipole-dipole and dipole-induced dipole interactions**

➢ δ_{min} **accounts for polar interactions and hydrogen bonding**

➢ *A* **accounts for dispersive interactions**

1. Kaliszan, R., van Straten, M. A., Markuszewski, M., Cramers, C. A., Claessens, H. A. _J. Chromatogr._ A **1999**, 855, 455-486

The study

➢ **Chromatographic conditions**

- Berger Minigram SFC system
- stationary phase: 2-ethyl-pyridine, 4.6x50 mm, 60 Å, 5μm
- mobile phase main component: CO_2
- 3 different modifiers

  a) pure methanol (MeOH)

  b) 0.1% v/v ethyl-dimethyl-amine (EDMA) in MeOH

  c) 0.6mM ammonium acetate (NH_4OAc) in MeOH

- *flow*: 4 mL min^{-1}
- *temperature*: 35°C
- *outlet pressure*: 100 bar
- detection: UV, mass spectrometry (ESI)

➢ **Polycratic study**

- each compound analysed at 10 modifier concentrations
- analyses performed three times to ensure validity of data

Practical range of retention (I)

➢ **Requirement: keep log *k* within a range of "practical interest" defined as**

$$0 < \log k < 1$$

➢ **log *k* < 0: the analyte is almost not retained and elutes with solvent**

➢ **log *k* > 1: the analyte hardly moves through the column, peaks get broader and might tail**

log *k* = - 0.13

log k = 0.78

log k = 1.24

A.U

260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20

1   2   3   4   5

t_R (min)
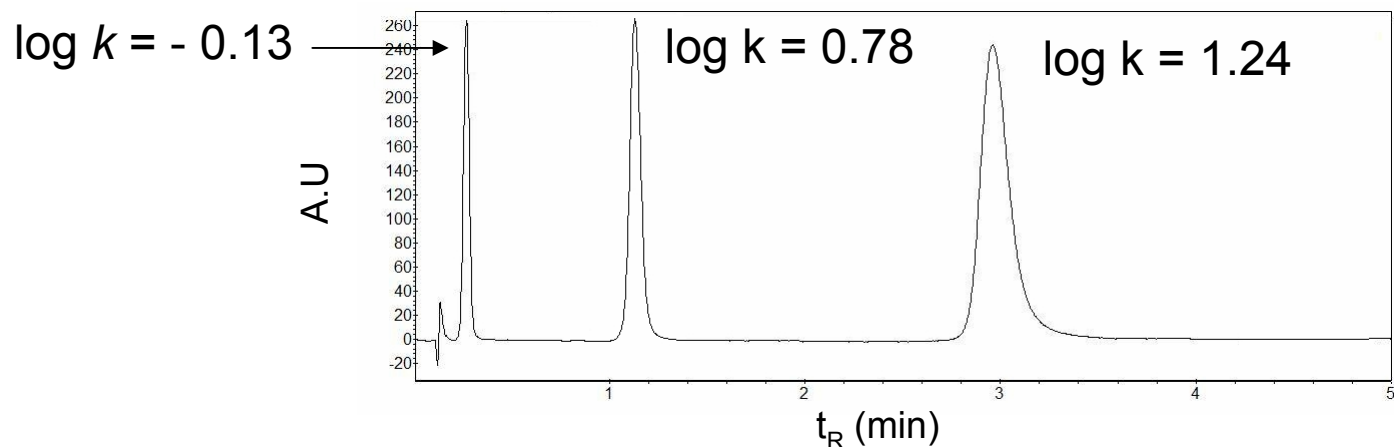
Practical range of retention (II)

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

- *slow eluting compounds*: no difficulties since increasing φ affords earlier elution

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

➤ **Consequence: 3 compounds to be removed from study**
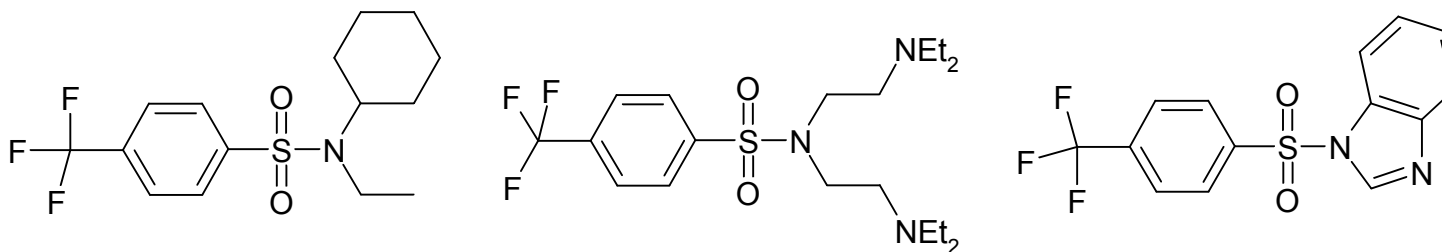
Restriction of test set

➢ **Experimental data:**

• below 10% of modifier in the mobile phase, log k does not vary linearly with φ
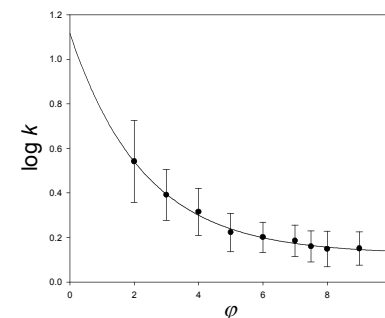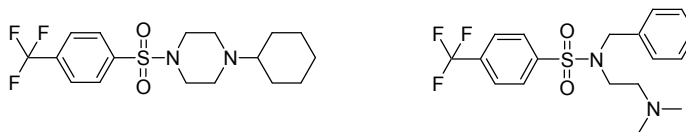
• some   compounds   did   not   exhibit   satisfactory chromatographic behaviour
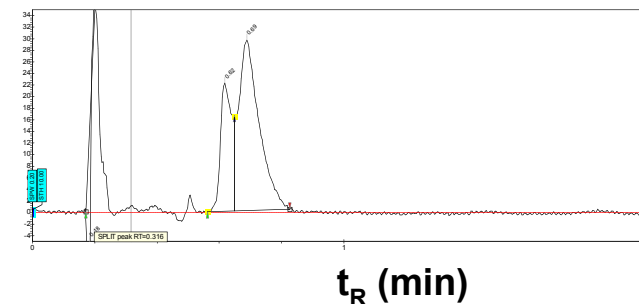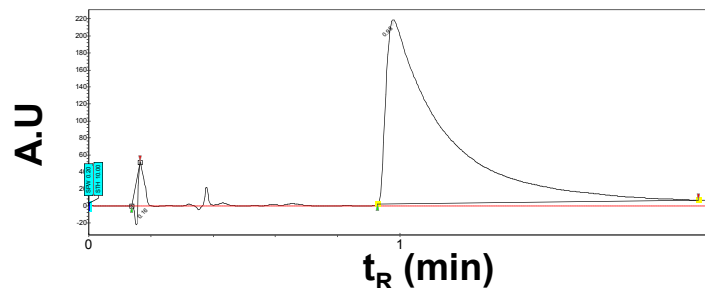
• such behaviour usually resulted in large errors
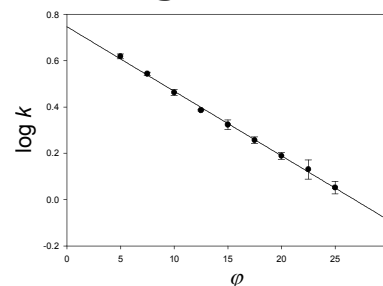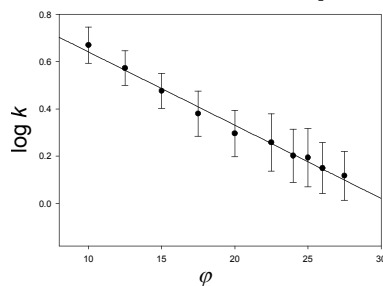
Linearity of log k = f(φ) (I)

➢ **From 32 compounds, 3 early eluters and 2 non-linear analytes were removed from study**

➢ **log k = log k_0 + S x φ was considered linear when R^2 was 0.98 or higher**

➢ **MeOH as modifier:**
- 11 compounds removed due to tailing or splitting
- 16 remaining compounds, plot φ vs. log k: R^2 > 0.98
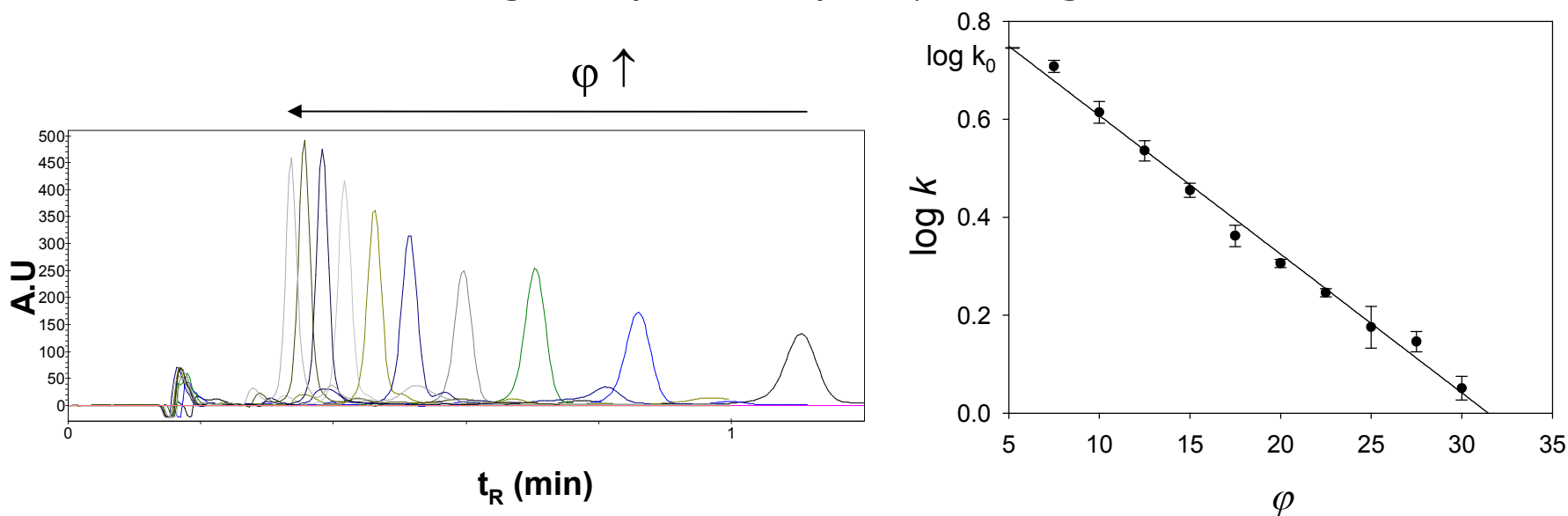
Linearity of log k = f(φ) (II)

➢ **MeOH + 0.1% v/v EDMA:**
- 4 compounds removed due to tailing and splitting
- 23 remaining compounds, plot φ vs. log k: $R^2 > 0.98$

➢ **MeOH + 0.6 mM NH$_4$OAc:**
- 6 compounds removed due to tailing or splitting
- 21 remaining compounds, plot φ vs. log k: $R^2 > 0.98$

➢ **log k_0, S and φ_0 calculated for the studied compounds with all 3 mobile phases**

Correlating retention data with compounds properties

➢ **Retention characteristics log k_0 and S and φ_0 could not 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 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**

Modifier: pure MeOH

➢ MeOH:

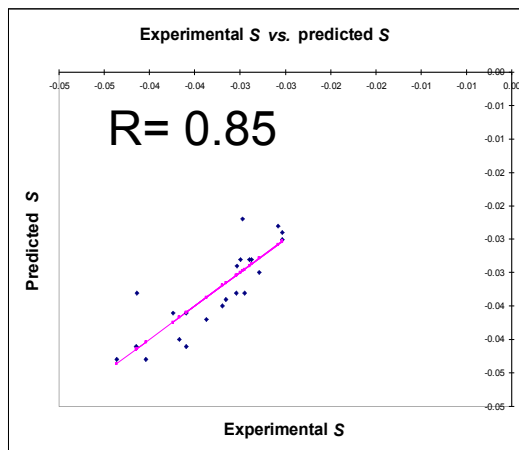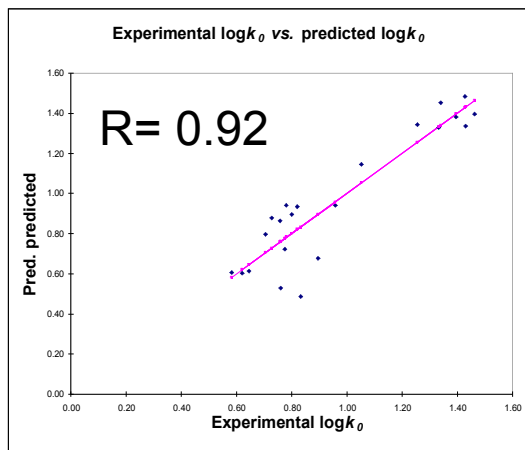$$\log k_0 = 0.108\ \mu - 0.002\ A - 1.257\ \delta_{min} - 1.305$$

$$S = 0.0004\ \mu - 9.5 \times 10^{-7}\ A + 0.011\ \delta_{min} - 0.020$$

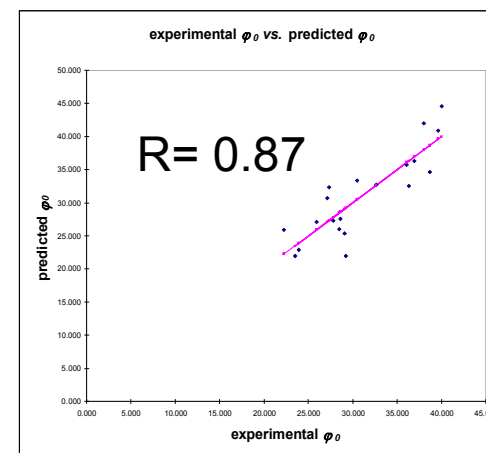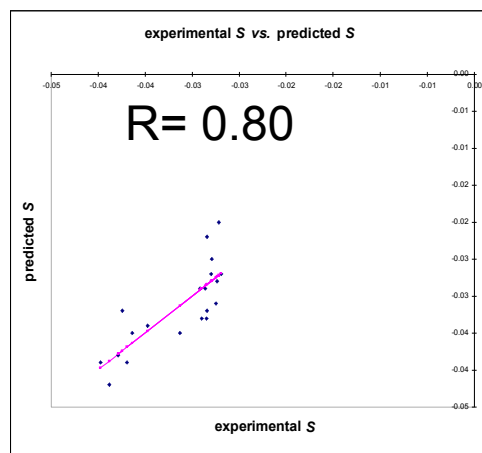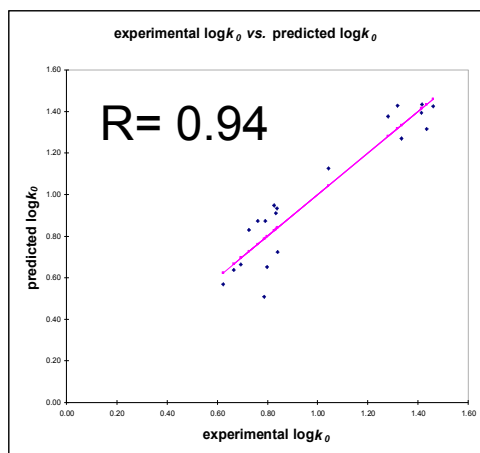$$\varphi_0 = 2.805\ \mu + 0.056\ A - 28.69\ \delta_{min} - 29.35$$

Experimental $\log k_0$ vs. predicted $\log k_0$ — R=0.90

Experimental S vs. predicted S — R=0.51

Experimental φ_0 vs. predicted φ_0 — R=0.83
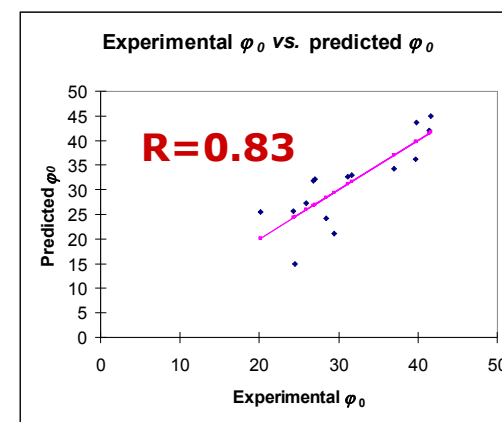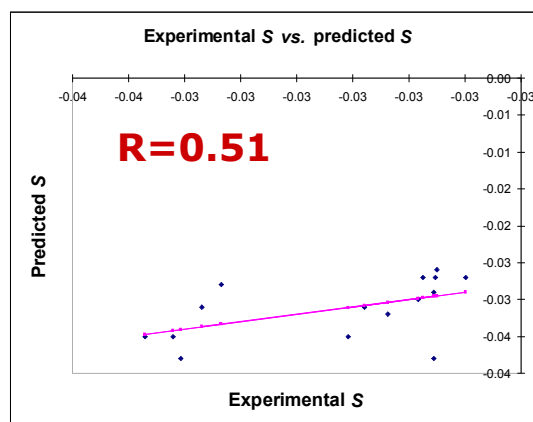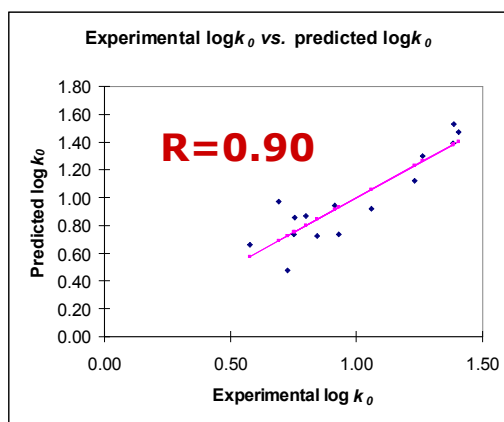
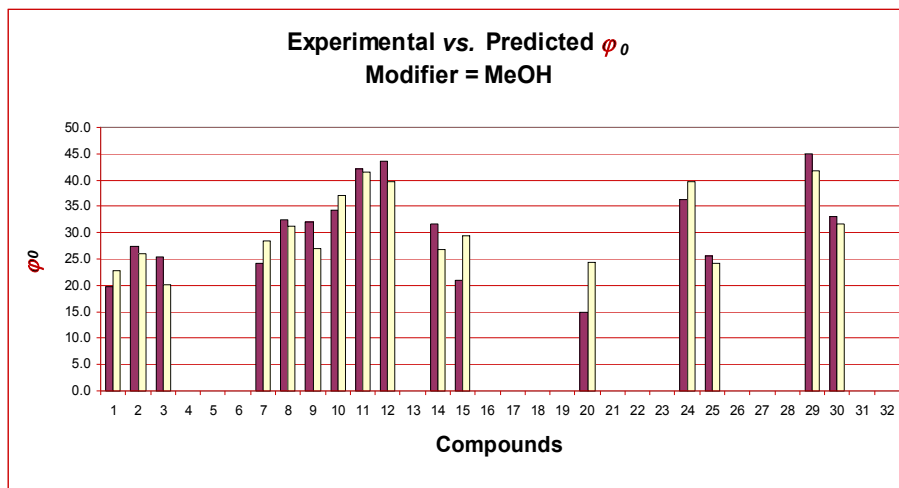# Modifier: MeOH+EDMA, MeOH+NH$_4$OAc
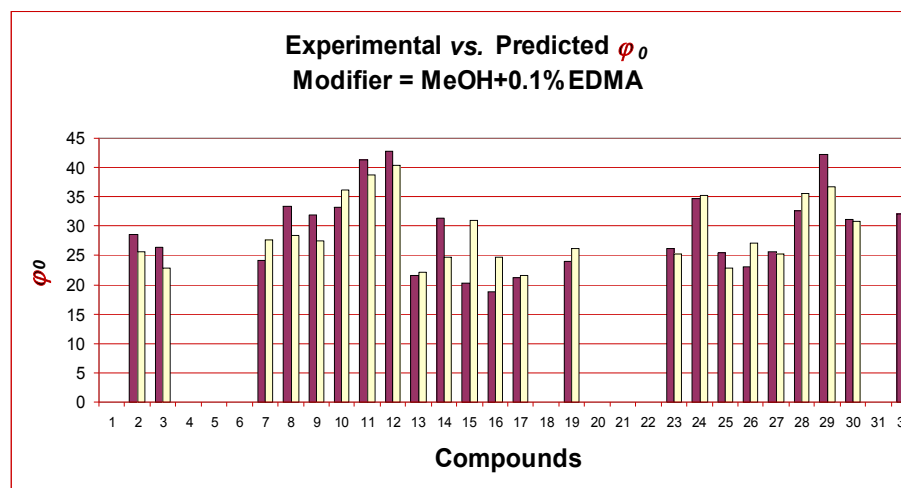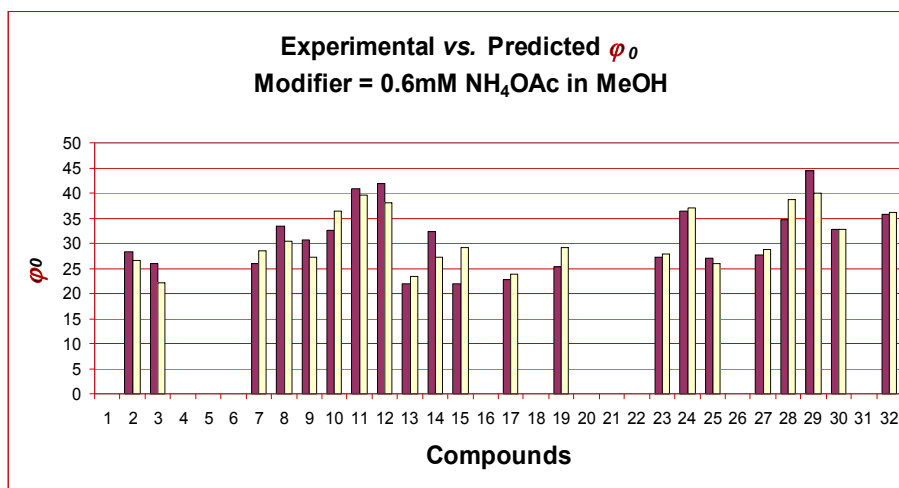
➢ **MeOH + 0.1% v/v EDMA:**
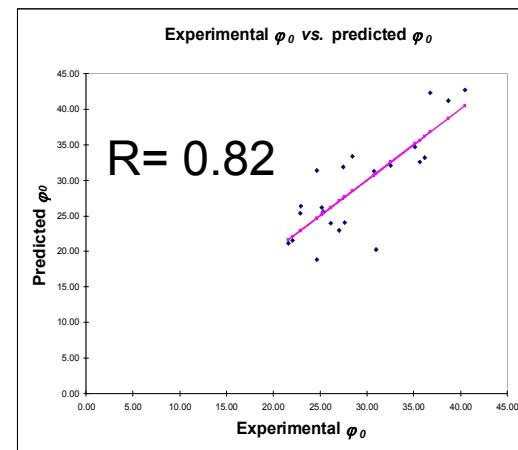
➢ **MeOH + 0.6mM NH$_4$OAc:**

Experimental *vs.* Predicted φ_0

> **Prediction gives reasonable estimate of φ_0**

Conclusion

- **Polycratic study undertaken on a test set of 32 sulfonamides to determine their retention characteristics**

- **Some compounds had to be removed**
    - **early eluters: increase retention by changing T and/or P**
    - **Tailing/splitting compounds: increase [additive]**

- **For other compounds: good linearity of log k = f(φ)**

- **Measured retention characteristics correlated with calculated molecular descriptors μ, A and δ_{min}, calculated φ_0 give good estimate of experimental values**

$$\log k_0 = a_1\,\mu + b_1\,A + c_1\,\delta_{min} + d_1 \qquad ; \qquad S = a_2\,\mu + b_2\,A + c_2\,\delta_{min} + d_2$$

$$\varphi_0 = a_3\,\mu + b_3\,A + c_3\,\delta_{min} + d_3$$

- **Study to be extended to excluded compounds and to different structures (acidic compounds, standard compounds)**

Acknowledgements
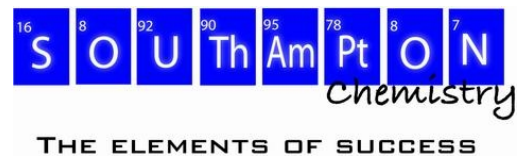
Acknowledgements

Thank you !

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