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

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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

![Chemical structures](images)

- 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$_2$O+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 $\log k$

Retention characteristics (II)

- Polycratic study: measurement of $\log k$ at different modifier concentrations in the mobile phase

- 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: \( \varphi_0 \)

- \( \varphi_0 \) is the value of \( \varphi \) for which \( \log k \) equals 0

- Calculated through:

  \[
  \log k = \log k_0 - n \times \varphi
  \]

  \[
  \log k = 0 \Rightarrow 0 = \log k_0 - n \times \varphi_0
  \]

  \[
  \Rightarrow \varphi_0 = -\frac{\log k_0}{S}
  \]

- 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\textsuperscript{1} and Carr\textsuperscript{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 $\pi$ electrons.
  - $S =$ solute dipolarity/polarizability. Models dipole-dipole interactions.

- Small letters: coefficients calculated through multiple linear regression analysis, characteristic of the chromatographic system used
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 \(\mu\)
  - electron excess charge on the most negatively charge atom \(\delta_{\text{min}}\)
  - solvent accessible molecular surface area \(A\)

- \(\mu\) accounts for dipole-dipole and dipole-induced dipole interactions

- \(\delta_{\text{min}}\) accounts for polar interactions and hydrogen bonding

- \(A\) accounts for dispersive interactions

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The study

**Chromatographic conditions**
- Berger Minigram SFC system
- stationary phase: 2-ethyl-pyridine, 4.6x50 mm, 60 Å, 5μm
- mobile phase main component: CO₂
- 3 different modifiers
  - a) pure methanol (MeOH)
  - b) 0.1% v/v ethyl-dimethyl-amine (EDMA) in MeOH
  - c) 0.6mM ammonium acetate (NH₄OAc) 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

![Graph showing log k values]

- \( \log k = -0.13 \)
- \( \log k = 0.78 \)
- \( \log k = 1.24 \)
Practical range of retention (II)

- Getting the retention factor within the range:

  - *slow eluting compounds*: no difficulties since increasing $\varphi$ affords earlier elution

  - *fast eluting compounds*: decreasing $\varphi$ 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 \( \phi \)
  - some compounds did not exhibit satisfactory chromatographic behaviour
  - such behaviour usually resulted in large errors
Linearity of $\log k = f(\varphi)$ (I)

- From 32 compounds, 3 early eluters and 2 non-linear analytes were removed from study
- $\log k = \log k_0 + S \times \varphi$ 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 $\varphi$ vs. $\log k$: $R^2 > 0.98$
Linearity of $\log k = f(\varphi)$ (II)

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

- $\log k_0, S$ and $\varphi_0$ calculated for the studied compounds with all 3 mobile phases
Correlating retention data with compounds properties

- Retention characteristics log $k_0$ and $S$ and $\varphi_0$ 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 $\mu$
  - surface area $A$
  - volume $V$
  - electronic charges on single atoms

- Multiple regression analysis performed to correlate properties with retention characteristics
Modifier: pure MeOH

MeOH:

\[
\log k_0 = 0.108 \mu - 0.002 A - 1.257 \delta_{\text{min}} - 1.305
\]

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

\[
\varphi_0 = 2.805 \mu + 0.056 A - 28.69 \delta_{\text{min}} - 29.35
\]
Modifier: MeOH+EDMA, MeOH+NH₄OAc

- **MeOH + 0.1% v/v EDMA:**
  - R = 0.92
  - R = 0.85
  - R = 0.82

- **MeOH + 0.6mM NH₄OAc:**
  - R = 0.94
  - R = 0.80
  - R = 0.87
Experimental vs. Predicted $\phi_0$

- Prediction gives reasonable estimate of $\phi_0$
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 \( \log k = f(\varphi) \)

- Measured retention characteristics correlated with calculated molecular descriptors \( \mu, A \) and \( \delta_{\text{min}} \). Calculated \( \varphi_0 \) give good estimate of experimental values

  \[
  \log k_0 = a_1 \mu + b_1 A + c_1 \delta_{\text{min}} + d_1 \quad ; \quad S = a_2 \mu + b_2 A + c_2 \delta_{\text{min}} + d_2
  \]

  \[
  \varphi_0 = a_3 \mu + b_3 A + c_3 \delta_{\text{min}} + d_3
  \]

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

Thank you!

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