Properties-Retention Study on Supercritical Fluid Chromatography Coupled to Mass Spectrometry (SFC-MS). Analysis of a Sulfonamide Library.

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1. Introduction

• Quality and safety requirements expected for new drug compounds confront analytical chemists to the necessity of developing new analytical methods capable of quick, highly-efficient separations for the characterization of all compounds and impurities.

• Until recently HPLC-MS has been preferentially used for this purpose. However SFC-MS appears more and more as a complementary technique for high throughput analysis.

• Because HPLC and SFC are complementary, more and more analytical departments are being equipped with both types of instrumentation. To avoid time-consuming double analysis, it is of interest to be able to determine which technique will be the most efficient, for a given sample, prior to analysis.

2. Objectives

• Kaliszan and co-workers have studied prediction of the retention of diverse drug-like compounds in HPLC using three molecular descriptors: total dipole moment, μ; electron excess charge of the most negatively charged atom, δ_{min}; and water-accessible molecular surface area, A_{was}.[1] In SFC, studies have been undertaken for restricted sets of structurally similar analytes.[2]

• The applicability of SFC to drug-like compounds and the possibility of applying Kaliszan's model to predict the retention behaviour of a given compound are studied herein by screening a library of 32 sulfonamides.

3. Instrumentation and Method

• **Instrumentation:**
  ✓ Experiments undertaken on _SFC Berger MiniGram System_ from Mettler Toledo.
  ✓ In addition to the UV detector, a _Mass Spectrometer Platform LCZ_ is fitted to the system _via_ a T-piece immediately after the UV detector outlet.
  ✓ In order to guarantee good ionisation of the analytes, a _makeup flow_ is pumped into the system by a HPLC pump through another T-piece immediately before the MS inlet.

• **Method:**
  ✓ _"Polycratic" study:_ capacity ratios _k_ measured for each compound at 10 different eluent compositions φ.
  ✓ Over 1 < k < 10, _log k vs. φ relationship proved linear:_[3] log k = log k_0 + S x φ.
  ✓ _Regression analysis_[4] performed to obtain values of the slope S and intercept log k_0.
  ✓ _Various molecular descriptors_ (total dipole moment μ, atomic formal charges and electron density surfaces) calculated using Spartan'02 software.
  ✓ _Multiple regression analysis_ performed to correlate S, log k_0 and φ_0 = -logk_0/S with the calculated molecular descriptors.[5]
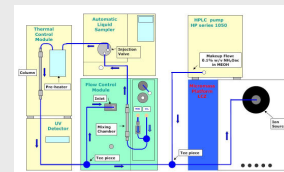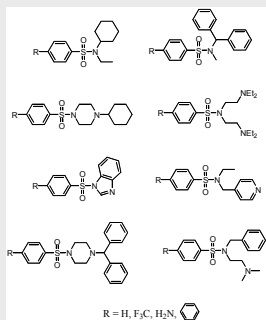
4. Results

• **Acquisition of chromatographic data and restriction of the test set**
  ✓ _Stationary phase:_ test analytes were studied on packed **2-ethyl-pyridyl** column (4.6 x50mm, 60Å pores, 6 mm particle size).
  ✓ _Mobile phase:_ CO_2 was modified with methanol (MeOH) containing either 0.6mM of NH_4OAC or 0.1% v/v of ethyl-dimethyl-amine (DMEA).
  ✓ _Adjusting retention within 0 < k < 1:_
    • retention of _late eluting compounds_ could be adjusted by increasing the modifier proportion in the mobile phase.
    • three _early eluting analytes_ were removed from the study due to lack of retention even at very low modifier concentration:
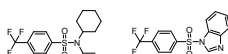
  ✓ _Linearity of log k = log_ k_0 _+ S x φ:_ good linearity was observed (R^2 > 0.98) for all the analytes but two. Those two outliers were studied at concentrations of MeOH less than 10% v/v which is believed to explain the non-linearity.

  Example of good linearity: Example of non-linear compound:
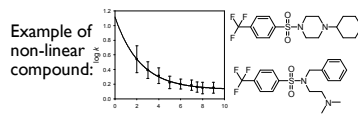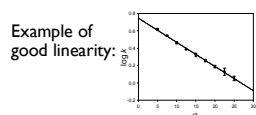
  ✓ A few other studied analytes were not taken into account in the final calculations due to tailing or splitting peaks, examples are given below:

  ✓ All in all, 23 compounds fulfilled all requirements and were included in the final calculation with EDMA as additive and 21 with NH_4OAc as additive.

• **Correlation of log k_0, S and φ_0 with molecular descriptors**: retention characteristics of the analytes were found to be correlated with calculated molecular descriptors: total dipole moment μ, surface area A and atomic charge on the most negatively charged atom δ_{min}.

$$\log k_0 = a\, \mu + b\, A - c\, \delta_{min} + d \qquad S = e\, \mu + f\, A - g\, \delta_{min} + h$$
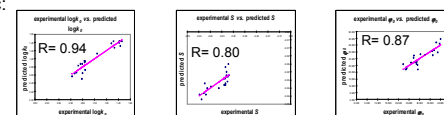
$$\varphi_0 = i\, \mu + j\, A - l\, \delta_{min} + m$$

• **Plots of experimental _vs._ predicted values:** these equations allow for the calculations of predicted values of the retention characteristics, that can be plotted _vs._ the experimental values with good correlation coefficient.
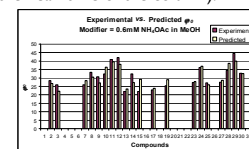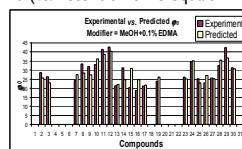
  ✓ MeOH + 0.1% v/v EDMA:

  R= 0.92   R= 0.85   R= 0.82

  ✓ MeOH + 0.6mM NH_4OAc:

  R= 0.94   R= 0.80   R= 0.87
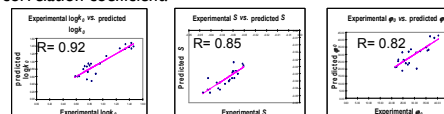
• **Predicted values give good estimates of experimental data**, especially for φ_0, which practically represents the proportion of modifier needed in the mobile phase to achieve k = 0 (_i.e._ retention time equals 2 times the dead time of the column).

5. Conclusion and future work

• _Polycratic retention studies_ carried out for 32 sulfonamides on 2-ethyl-pyridyl stationary phase.

• _When EDMA used as additive_: 23 compounds exhibited satisfactory retention and peak as well as linearity of the relationship log k = log k_0 + S x φ R^2 > 0.98.

• _When NH_4OAc used as additive_: 21 compounds exhibited satisfactory retention and peak as well as linearity of the relationship log k = log k_0 + S x φ R^2 > 0.98.

• With both additives, _retention characteristics log_ k_0, S and φ_0 are correlated with molecular descriptors μ, A and δ_{min}.

• _Results to be considered with care_, since obtained on small set of structurally similar compounds.

• The study has to be extended to _higher concentration of additive_ in the modifier, _other stationary phases_ and _compounds of different structures_.

• Regression analysis using _other descriptors_ has to be undertaken in an attempt to highlight better correlation.

Acknowledgements

References

1. T. Baczek, R. Kaliszan, _Journal of Chromatography A_ **2003**, 987, 29-37.
2. G.A. Alvarez, W. Baumann _Zeitschrift Fur Naturforschung Section a-a Journal of Physical Sciences_ **2005**, 60 (2005) 61-69.
3. Schoenmakers, P. J.; Billiet, H. A. H.; De Galan, L. _Journal of Chromatography_ **1979,** 185, 179-195.
4. Regression analysis performed using SigmaPlot 9.0.
5. Multiple regression analysis performed using Microsoft® Office Excel.