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

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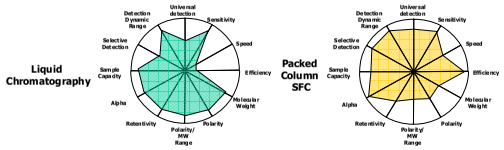
1. Introduction

• 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.



• The possibility of outlining a set of properties-based rules allowing prediction of the retention of a given compound by SFC is being studied on a small library of sulfonamides.

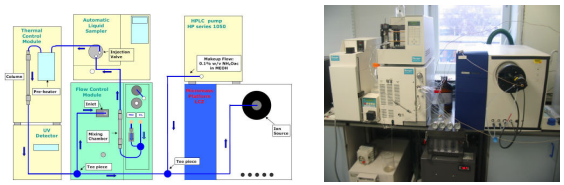
2. Instrumentation and Method

• **Instrumentation:**

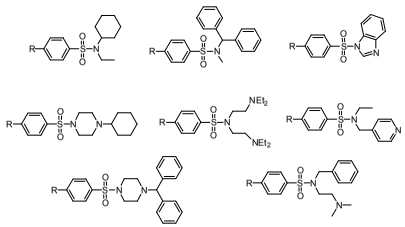
✓ Experiments undertaken on *SFC Berger MiniGram System* from Mettler Toledo.

✓ 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.



• **Test Compounds:**



R = H, F_3C, phenyl, H_2N

• **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:*[1] $\log k = \log k_0 + S \times \varphi$.

✓ *Regression analysis* 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 $\varphi_0 = -\log k_0/S$ with the calculated molecular descriptors.

3. Results

• **Acquisition of chromatographic data and restriction of the test set**

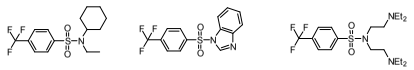
✓ *Stationary phase:* test analytes were studied on packed **2-ethyl-pyridyl** column (4.6 x50mm, 60Å pores, 6 μm particle size).

✓ *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:*

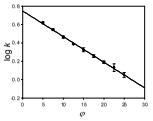
• retention of *late eluting compounds* could be adjusted by increasing the modifier proportion in the mobile phase.

• three *early eluting analytes* were removed from the study due to lack of retention even at very low modifier concentration:

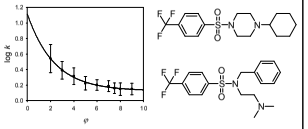


✓ *Linearity of log* $k = \log k_0 + S \times \varphi$: good linearity was observed ($R^2 > 0.98$) for all the analytes but two. Those two outliers were studied at concentrations of MeOH less than 10% v/v which is believed to explain the non-linearity.

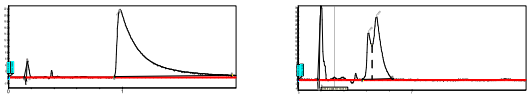
Example of good linearity:



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.

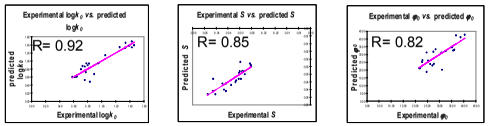
• **Correlation of log k_0, *S* and φ_0 with molecular descriptors**: retention characteristics of the analytes were found to be correlated with calculated molecular descriptors: total dipole moment μ, surface area *A* and atomic charge on the most negatively charged atom δ_{min}.

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

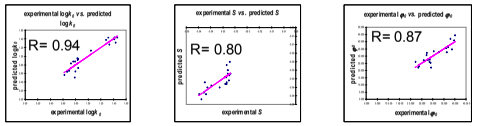
$$\varphi_0 = i\,\mu + j\,A - l\,\delta_{min} + m$$

• **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.

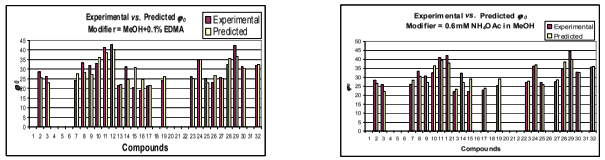
✓ MeOH + 0.1% v/v EDMA:



✓ MeOH + 0.6mM NH_4OAc:



• **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).



4. Conclusion

• *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 $\log k = \log k_0 + S \times \varphi$ $R^2 > 0.98$

•*When NH_4OAc used as additive*: 21 compounds exhibited satisfactory retention and peak as well as linearity of the relationship $\log k = \log k_0 + S \times \varphi$ $R^2 > 0.98$

• With both additives, *retention characteristics log* k_0, S and φ_0 are correlated with molecular descriptors μ, *A* and δ_{min}:

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

$$\varphi_0 = i\,\mu + j\,A - l\,\delta_{min} + m$$

5. Future work

• *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

evotec, METTLER TOLEDO, PRINCETON CHROMATOGRAPHY INC, BMSS, GlaxoSmithKline, Pfizer

References

1. Schoenmakers, P. J.; Billiet, H. A. H.; De Galan, L., Influence of organic modifiers on the retention behaviour in reverse-phase liquid chromatography and its consequences for gradient elution. *Journal of Chromatography* **1979,** 185, 179-195.
2. Regression analysis performed using the regression analysis tool of Microsoft® Office Excel.