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

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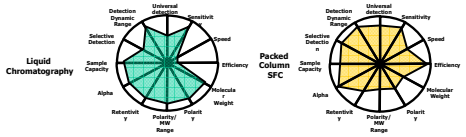
1. Introduction

• 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 sulphonamides.

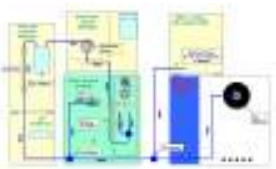
2. Instrumentation and Method

• **Instrumentation:**

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

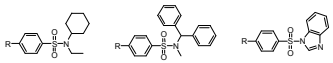
✓ In addition to the UV detector, a *Mass Spectrometer Platform LCZ* is fitted to the system *via* a T-piece immediately after the UV detector outlet.

✓ 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.

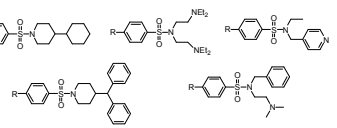


• **Test Compounds:**

Neutral Set



Basic Set



R = H, F_3C, phenyl, H_2N

• **Method:**

✓ *"Polycratic" study:* capacity ratios k measured for each compound at a number of different eluent compositions φ

✓ Over $1 < k < 10$, *log k vs. φ relationship is linear:*[1] $\log k = \log k_0 + S \times \phi$.

✓ *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 and $\log k_0$ with the calculated molecular descriptors.

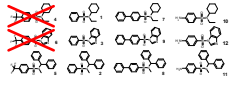
3. Results

• **Preliminary Study: Neutral Set**

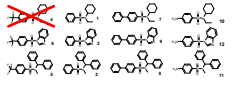
✓ *Columns:* neutral analytes were studied on four different packed SFC columns (**2-ethyl-pyridyl** [2-EP], **cyano-propyl**, **diol** and **bare silica**, 4.6 x 250mm, 60Å pores, 6 μ m particle size).

✓ *Mobile phase:* pure methanol (MeOH) in CO_2

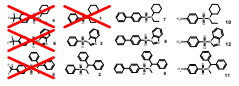
✓ *Studied Compounds:* $1 < k < 10$ could not be satisfied for all compounds on all four columns, some compounds were removed from study:



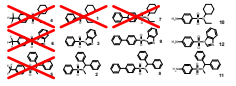
Compounds studied on 2-EP column



Compounds studied on cyano column



Compounds studied on diol column



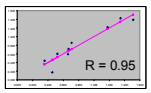
Compounds studied on silica column

✓ *Linearity of the log* $k = \log k_0 + S \times \varphi$ *:*[2] good linearity observed ($R^2 > 0.92$), $\log k_0$ and S calculated for each compound on each column.

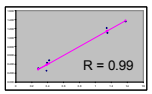
✓ *Correlation of* $\log k_0$ *and S with molecular descriptors:*[2] retention characteristics of the analytes were found to be correlated with calculated molecular descriptors: total dipole moment μ, surface area A and atomic charge on the most negatively charged atom δ_{min}.

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

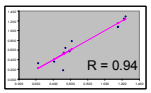
✓ *Plotting experimental vs. predicted values of log* k_0*:*



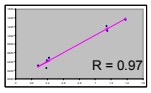
2-EP column



silica column



cyano column



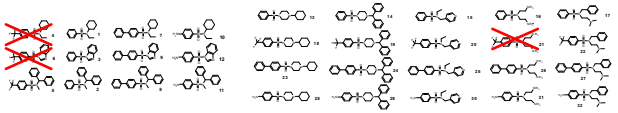
diol column

• **Further investigation: Neutral and Basic Sets**

✓ *Column:* Neutral and basic analytes were so far studied on **2-EP**, 4.6 x 50mm, 60Å pores, 5 μ m particle size.

✓ *Mobile phase:* pure MeOH and MeOH + 0.1% v/v diethylmethylamine (DMEA) in CO_2.

✓ *Studied Compounds:* $1 < k < 10$ could not be satisfied for all compounds on the 2-EP column, some compounds were removed from study:



✓ *Linearity of the log* $k = \log k_0 + S \times \varphi$ *:*[2] good linearity observed with MeOH and MeOH + DMEA ($R^2 > 0.90$).

✓ *Correlation of* $\log k_0$ *and S with molecular descriptors:*[2]

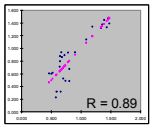
• *MeOH as modifier:* relationships found between retention characteristics of the neutral analytes and their calculated molecular descriptors could not be extended to the basic set.

• *MeOH + DMEA as modifier:* retention characteristics of the analytes were found to be correlated with their μ, A and δ_{min}.

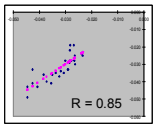
$$\log k_0 = a\,\mu + b\,A - c\,\delta_{min} + d \quad ; \quad S = e\,\mu + f\,A - g\,\delta_{min} + h$$

✓ *Plotting experimental vs. predicted values of log* k_0 *and S:*

Predicted log k_0 *vs.* Experimental log k_0



Predicted S *vs.* Experimental S



4. Conclusion

• *Polycratic retention studies* carried out for 12 neutral sulphonamides on 2-EP, cyano, diol and bare silica stationary phases and for 20 basic sulphonamides on 2-EP stationary phase.

• Neutral set-only study: *when* $0 < \log k < 1$, on all four columns, linear relationships found with pure MeOH as a modifier:

$$\log k = \log k_0 + S \times \varphi \qquad R^2 > 0.90$$

• Neutral and basic sets study: *when* $0 < \log k < 1$, on 2-EP column, linear relationships found with pure MeOH and MeOH + DMEA as a modifier.

• When MeOH + DMEA is used as a modifier, *retention characteristics log* k_0 *and S* of neutral and basic compounds are correlated with molecular descriptors μ, A and δ_{min}: $\log k0 = a\,\mu + b\,A - c\,\delta_{min} + d \quad ; \quad S = e\,\mu + f\,A - g\,\delta_{min} + h$

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 *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
PRINCETON
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.