Screening of a Sulfonamides Library by Supercritical Fluid Chromatography coupled to Mass Spectrometry (SFC-MS). Preliminary properties-retention study.

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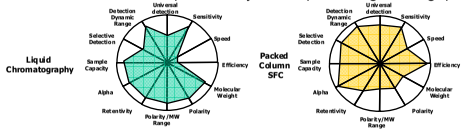
1. Introduction

• 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 set of 12 neutral sulfonamides.

2. Instrumentation and Method

• Instrumentation:

• Experiments carried out on *SFC Berger MiniGram System* from Mettler Toledo.

• The system consists of:

✓ a *flow control module*, CO_2 and modifier pumped separately and mixed in a mixing chamber

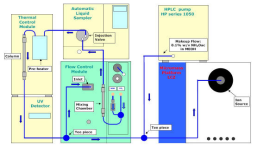
✓ a *liquid autosampler* fitted with a 10 μL loop

✓ a *thermal control module*: fluid brought to required temperature in a pre-heater, column thermostated in an oven

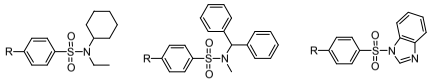
✓ a *UV detector*.

• 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 a number of different eluent compositions φ on four different packed SFC columns (2-ethyl-pyridyl [2-EP], cyano-propyl, diol and bare silica, 4.6 x 250mm, 60Å pores, 6 μm particle size).

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

• *Regression analysis* performed to obtain values of the slope S and intercept $\log k_0$.

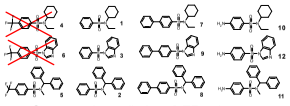
• *Various molecular descriptors* (log P, total dipole moment μ, atomic formal charges and electron density surfaces) calculated using Spartan'02 software.

• *Multiple regression analysis* performed to correlate S and $\log k_0$ with the calculated molecular descriptors.

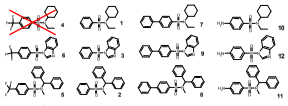
3. Results

• Studied Compounds:

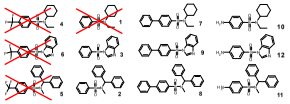
$1 < k < 10$ could not be satisfied for all compounds on all four columns, some compounds were removed from study:



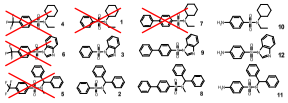
Compounds studied on 2-EP column



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]

Compounds	2-EP			Silica			Cyano			Diol		
	R^2	$\log k_0$	S	R^2	$\log k_0$	S	R^2	$\log k_0$	S	R^2	$\log k_0$	S
1	**0.985**	0.228	-0.012	**nd**	nd	nd	**0.993**	0.282	-0.015	**nd**	nd	nd
2	**0.999**	0.601	-0.020	**0.923**	0.138	-0.008	**0.995**	0.547	-0.020	**0.990**	0.285	-0.014
3	**0.995**	0.444	-0.018	**0.995**	0.428	-0.017	**0.994**	0.403	-0.016	**0.994**	0.439	-0.017
4	**nd**	nd	nd	**nd**	nd	nd	**nd**	nd	nd	**nd**	nd	nd
5	**0.980**	0.171	-0.010	**nd**	nd	nd	**0.918**	0.182	-0.015	**nd**	nd	nd
6	**nd**	nd	nd	**nd**	nd	nd	**0.933**	0.325	-0.068	**nd**	nd	nd
7	**0.996**	0.593	-0.022	**nd**	nd	nd	**0.997**	0.574	-0.022	**0.974**	0.279	-0.017
8	**0.999**	0.859	-0.024	**0.970**	0.308	-0.015	**0.999**	0.784	-0.025	**0.983**	0.509	-0.022
9	**0.999**	0.714	-0.022	**0.997**	0.484	-0.020	**0.999**	0.644	-0.022	**0.991**	0.582	-0.021
10	**0.995**	1.218	-0.035	**0.995**	1.105	-0.037	**0.998**	1.077	-0.035	**0.987**	1.088	-0.033
11	**0.996**	1.427	-0.033	**0.994**	1.214	-0.037	**0.996**	1.289	-0.036	**0.990**	1.142	-0.030
12	**0.995**	1.390	-0.032	**0.996**	1.362	-0.037	**0.996**	1.245	-0.036	**0.983**	1.285	-0.030

nd: not determined

$R^2 > 0.97$ (except for compound **2** on silica, $R^2 = 0.923$; and compounds **5** and **6** on cyano, $R^2 = 0.918$ and $R^2 = 0.933$ respectively).

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

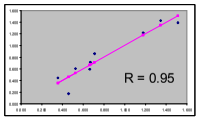
✓ No correlation between lipophilicity constants and retention characteristics

✓ Correlation between retention characteristics and calculated molecular descriptors: total dipole moment μ, surface area A and atomic charge on the most negatively charged atom δ_{min}.

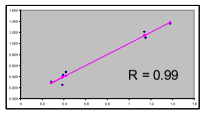
$$\log k_0 = a\,\mu + b\,A - c\,\delta_{min} + d$$
$$S = e\,\mu + f\,A - g\,\delta_{min} + h$$

Coefficients		2-EP		Silica		Cyano		Diol	
$\log k_0$	a	0.232		0.119		0.063		0.124	
	b	0.001	R = 0.95	-0.001	R = 0.99	0.001	R = 0.94	$2x10^{-4}$	R = 0.97
	c	-1.325		-1.714		-1.579		-1.409	
	d	-2.200		-1.178		-1.25		-1.173	
S	e	-0.003		$-8x10^{-4}$		-0.011		$-8x10^{-4}$	
	f	$-2x10^{-5}$	R = 0.90	$-8x10^{-5}$	R = 0.84	$-2.2x10^{-5}$	R = 0.84	$-8x10^{-5}$	R = 0.95
	g	0.270		0.040		0.071		0.040	
	h	-0.023		-0.012		-0.040		-0.012	

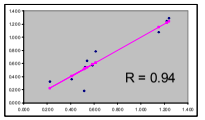
• Plotting experimental *vs.* predicted values of $\log k_0$:



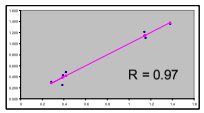
2-EP column



silica column



cyano column



diol column

4. Conclusion

• *Polycratic retention studies* of 12 neutral sulfonamides carried out on 2-EP, cyano, diol and bare silica stationary phases.

• *When 0 < log k < 1:* on all four columns, linear relationships found:

$$\log k = \log k_0 + S \times \varphi \qquad R^2 > 0.97$$

• *Retention characteristics log k_0 and S* correlated with molecular descriptors total dipole moment μ, surface area A and atomic charge on the most negatively charged atom δ_{min}.

• *Encouraging results for log k_0* which are correlated with molecular descriptors with R > 0.94 on all four stationary phases.

• *Less promising results for* S, especially on cyano and silica columns (R = 0.84).

• *Results to be considered with care*, since obtained on small set of structurally similar compounds.

5. Future work

• The analysis of a set of 20 basic sulfonamides is currently under way.

• The study has to be extended to compounds of different structures.

• Recalculation of molecular descriptors by more powerful algorithms is under way.

• Regression analysis using other descriptors has to be undertaken in an attempt to highlight better correlation.

Acknowledgements

evotec, gsk GlaxoSmithKline, Mettler Toledo, Pfizer, Princeton Chromatography Inc.

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.