

Introduction:

Stationary phases providing selectivities, which are different from commonly-used C₁₈ or C₈ silica gels are appreciated in high performance liquid chromatography. Particularly aromatic selectivity from phenyl groups with π-π molecular interactions are a welcome add-on to pure van der Waals interactions of alkyl-substituted phases^{1,2}. This study examines the retention of 86 organic compounds on 10 alkyl- and arylmodified silica gel columns and describes the different selectivity patterns.

Methods

LC system: Thermo Scientific Vanquish UHPLC System with PDA
Column dimension: 125 mm x 4 mm
Mobile Phase: ACN and MeOH in combination with water or 0.2 % TFA/water.
(The composition was adjusted to achieve sufficient retention but for each compound only one composition is used on each column)
Flow: 1 mL/min
Temperature: 30 °C
PDA-Detection: 200–350 nm
Injection volume: 1 µL
Void volume marker: Uracil

All stationary phases are commercially available except for NUCLEODUR® Biphenyl. Biphenyl-silyl substituted NUCLEODUR® has been synthesized analogous to NUCLEODUR® π². The solutes are various organic compounds. The list is available from the author on request. For evaluation and graphic illustration the following values were calculated:

$$k'_{rel}(i, col) = \frac{k'(i, col)}{k'(ref col)}$$

Relative capacity factor of compound i on column col in comparison to reference column NUCLEODUR® C₁₈ Gravity.

$$\overline{k'_{rel}(col)}$$

Averaged relative capacity factor over all solutes as a measure of the strength of the interaction of each stationary phase in comparison with NUCLEODUR® C₁₈ Gravity.

$$RSD = \frac{\sqrt{\frac{1}{n} \sum [k'_{rel}(i, col) - \overline{k'_{rel}(col)}]^2}}{\overline{k'_{rel}(col)}}$$

Relative standard deviation as a measure of the dispersion of the relative capacity factors.

Results

Fig. 1. Retention behavior using acetonitrile as organic modifier

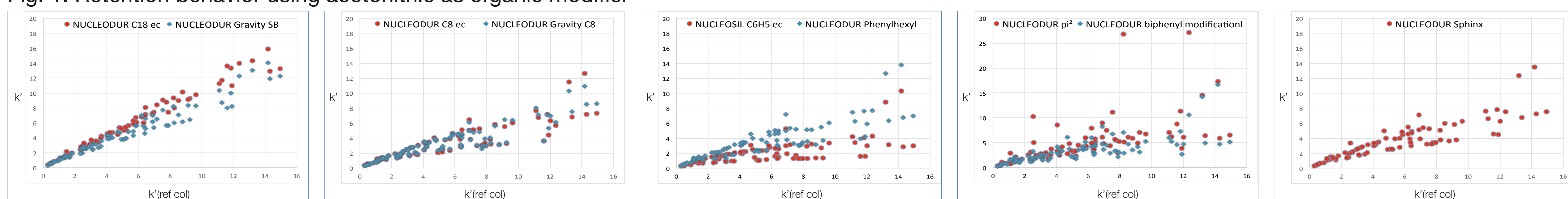


Fig. 2. Retention behavior using methanol as organic modifier

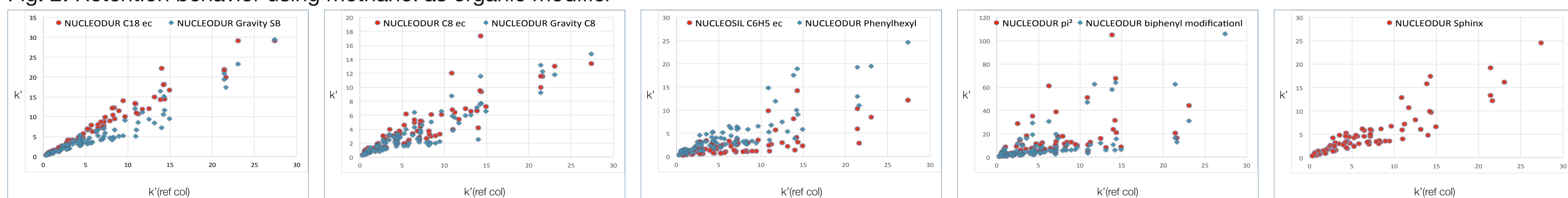
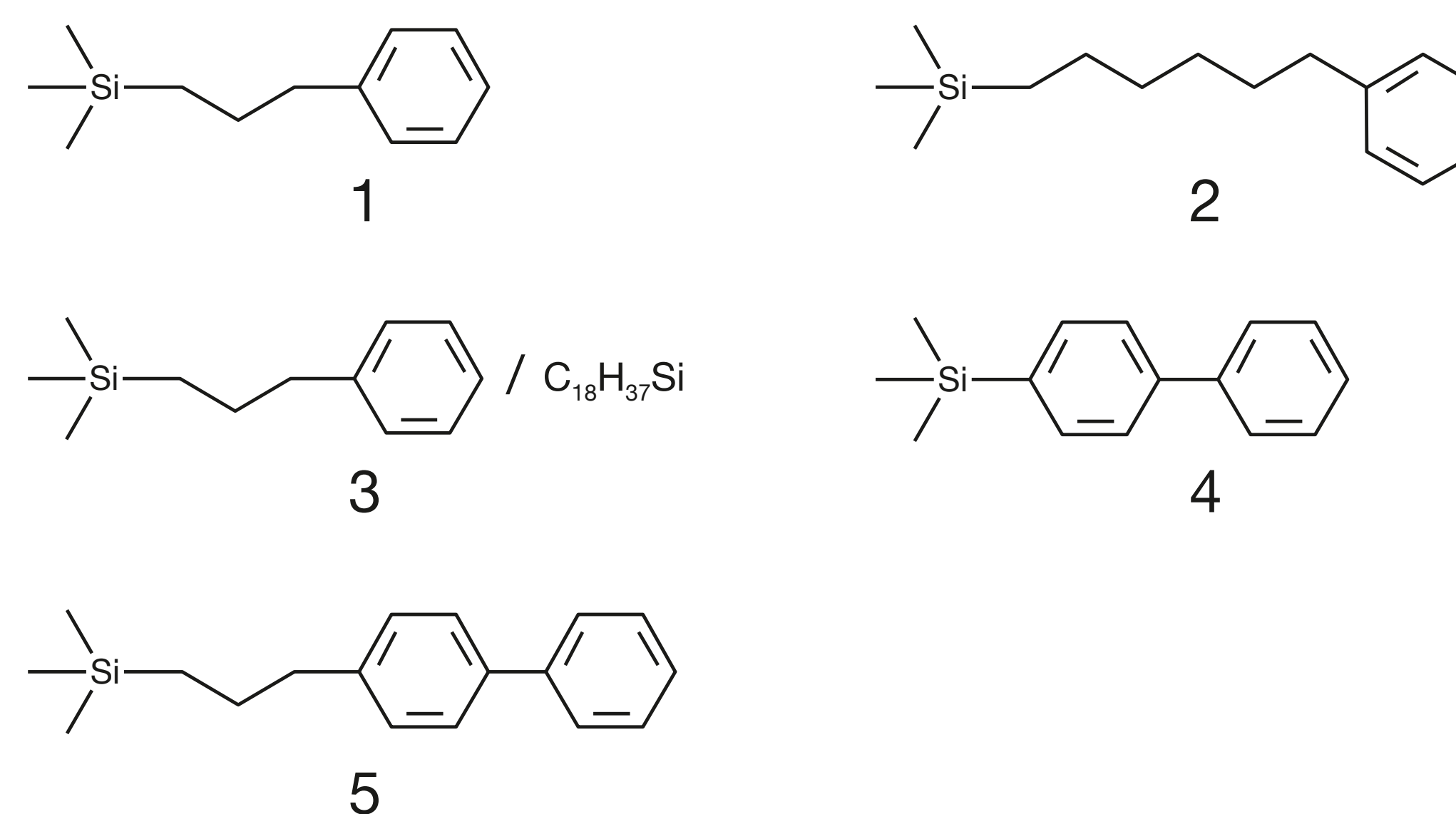


Table 1. Comparison of interaction strength and dispersion

Stationary phase	Description of the surface modification	Results acetonitrile		Results methanol	
		$\overline{k'_{rel}(col)}$	RSD	$\overline{k'_{rel}(col)}$	RSD
NUCLEODUR® C ₁₈ Gravity	Dense C ₁₈ layer. reference column	1.00	–	1.00	–
NUCLEODUR® 100-5 C ₁₈ ec	C ₁₈ layer	1.08	0.09	1.17	0.13
NUCLEODUR® C ₁₈ Gravity SB	C ₁₈ layer with bulky side chains	0.91	0.14	0.81	0.24
NUCLEODUR® C ₈ Gravity	Dense C ₈ layer	0.67	0.15	0.55	0.38
NUCLEODUR® 100-5 C ₈ ec	C ₈ layer	0.67	0.20	0.68	0.43
NUCLEOSIL® 100-5 C ₆ H ₅ ec	C ₆ H ₅ structure 1	0.44	0.54	0.45	0.90
NUCLEODUR® Phenylhexyl	C ₆ H ₅ structure 2	0.70	0.31	0.75	0.50
NUCLEODUR® Sphinx RP	C ₆ H ₅ /C ₁₈ structure 3	0.72	0.29	0.75	0.40
NUCLEODUR® Biphenyl	Biphenyl structure 4	0.72	0.43	1.73	1.00
NUCLEODUR® π ²	Biphenyl structure 5	0.96	0.62	3.10	1.61



The strength of the interactions $\overline{k'_{rel}(col)}$ of C₁₈ modified phases is relatively similar. Since the base material (silica gel) is identical, the differences can be mainly attributed to the surface modification. C₈ stationary phases reduce the interactions on average by one third. In both cases the RSD values are low due to similar retention mechanisms of the alkyl groups.

The strength of interactions using ligands with phenyl groups is between 44 and 75% depending on the alkyl portion in the chain.

Switching to methanol as a mobile phase has only a little effect on the strength of interaction of alkyl and phenyl derivatized phases, while dispersion is increased and differences become more evident.

Stationary phases containing biphenyl ligands especially biphenylpropyl have moderately increased retention times using acetonitrile organic modifiers. However we observed that these phases interact much stronger in MeOH/water mobile phase systems and the dispersion increases up to 1.61. Therefore the Y axis for these phases has to be resized. Nevertheless, in case of methanol there are 3 values outside the chart area.

Strong interactions with biphenylpropyl ligands were found for steroids, sulfa drugs, polynuclear aromatics and nitroaromatic compounds.

Conclusion

Stationary phases with biphenyl ligands are an interesting alternative to alkyl-substituted HPLC phases due to their considerable orthogonal selectivity.

With NUCLEODUR® π² a comparable interaction strength in acetonitrile to C₁₈ phases can be achieved in acetonitrile/water mobile phases which can be increased by a factor of 2 by replacing ACN by methanol.

The dispersion is a measure of the orthogonal selectivity of NUCLEODUR® π² in comparison to the investigated sorbents and most pronounced in acetonitrile (0.62) and methanol (1.61).

References

- M. R. Euerby, P. Peterson, W. Campbell, W. Roe J. Chromatogr. A 1154 (2007) 138–151
- K. Croes, A. Steffens, D. H. Marchand, L. R. Snyder J. Chromatogr. A 1098 (2005) 123–130