

Adjusting selectivity with column chemistry in LC/MS

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Introduction

Optimisation of a separation in reverse phase HPLC can be achieved altering either:

- Mobile phase (composition or pH)
- Temperature
- Stationary phase

In this poster the selectivity changes that can be obtained by altering the stationary phase will be discussed. Three different stationary phases have been chosen to investigate the effect that endcapping and a different functionality have on the selectivity of the stationary phase. Three modes of interaction are investigated using six specific probes. The modes of interaction under investigation are:

- 1) Hydrophobicity**
Hydrophobic retention (HR) – the capacity factor of a hydrophobic hydrocarbon, pentybenzene, give a broad measure of hydrophobicity.
- 2) Steric selectivity (SS)**
Steric selectivity is the ability of the stationary phase to distinguish between molecules with similar structures, but different shapes. The selectivity factor between *o*-terphenyl and triphenylene is indicative of steric selectivity as the former has the ability to twist and bend, while the latter has a fairly rigid structure and will be retained quite differently.
- 3) Hydrogen bonding capacity (HBC)**
Selectivity factor between caffeine and phenol, which provides a measure of the number of available silanol groups and the degree of endcapping.

Other analytes used to compare selectivity include uron herbicides and catechins. These were run under typical LC/MS mobile phase conditions, with a generic gradient.

Materials & Methods

Instrumentation:

Thermo Scientific Surveyor HPLC system fitted with MayLab Column Switcher

Columns:
Experimental High Surface Area C18, 5 μ m, 100 x 4.6 mm; Experimental High Surface Area aQ, 5 μ m, 100 x 4.6 mm; Experimental High Surface Area Phenyl, 5 μ m, 100 x 4.6 mm.
Mobile phase: H₂O / Methanol (35:65); **Flow rate:** 1.5 mL/min; **Temperature:** 40°C;
Detection: 254 nm; **Injection volume** 10 μ L.

TABLE 1. Characterization test parameters.

Parameter	Probe
Hydrophobic retention	K: pentybenzene
Hydrophobic Selectivity	α (pentybenzene, butylbenzene)
Steric selectivity	α (triphenylene, <i>o</i> -terphenyl)
H-bonding capacity	α (caffeine, phenol)

Results

FIGURE 1. Comparison of hydrophobicity, steric selectivity and hydrogen bonding capacity on C18, polar endcapped (aQ) and Phenyl phases.

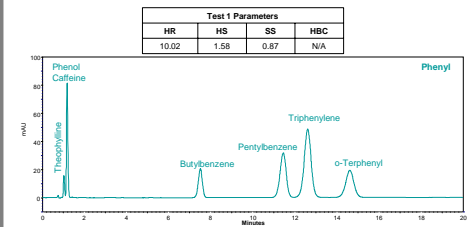
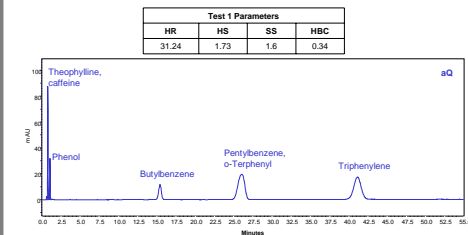
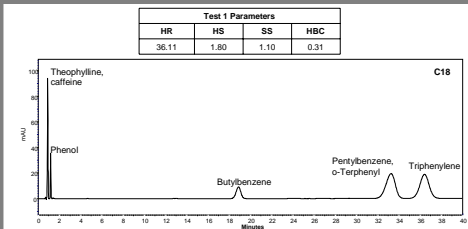


Figure 1 compares the results of the six probe chromatographic test on the C18, polar endcapped C18 (aQ) and phenyl phases. The C18 shows the greatest hydrophobic retention (HR) and hydrophobic selectivity (HS), although the aQ phase values are very close, which is expected based on the percentage of carbon for the phases. The aQ phase shows the highest value for steric selectivity, but with the Phenyl phase there is actually a reversal of the elution order of triphenylene and *o*-terphenyl. This is likely to be caused by *o*-terphenyl's ability to twist and bend and therefore interact with the phenyl structure at more contact points.

FIGURE 2. Selectivity comparison of a mixture of catechins on C18, polar endcapped C18 (aQ) and Phenyl phases.

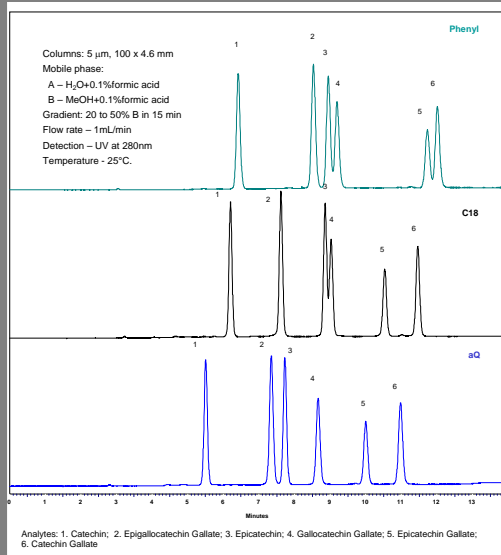
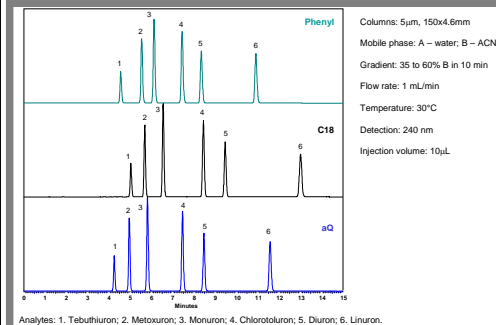


FIGURE 3. Selectivity comparison of a mixture of herbicides on C18, polar endcapped C18 (aQ) and Phenyl phases.



In Figures 2 and 3 the changes in selectivity when changing from a C18 to a polar endcapped C18 or to a phenyl phase are illustrated for a mixture of catechins and a mixture of uron herbicides. In both comparisons a generic gradient typical of LC/MS analysis was used. The separations were optimised on the aQ phase and then run on the C18 and phenyl.

Conclusions

- The six probe chromatographic test used to compare C18, polar endcapped C18 and phenyl phases confirmed that the C18 has the highest hydrophobic selectivity.
- The phenyl phase shows a reversal of the elution order of triphenylene and *o*-terphenyl. This is likely to be caused by *o*-terphenyl's ability to twist and bend, and therefore interact with the phenyl structure at more contact points.
- The C18 material shows excellent column to column reproducibility for the analysis of zidovudine according to the USP method.
- The complementary selectivity of C18, polar endcapped C18 and phenyl phases was illustrated for mixtures of catechins and uron herbicides.

For additional information, please visit our Chromatography Resource Centre which can be found at:

www.thermo.com/chem/chrresourc.htm

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