Use of Column Selectivity Tables to Predict Separations

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Overview

Purpose: To investigate the usefulness of the data acquired from stationary phase characterisation tests in predicting the nature of interaction of selected chromatographic probes on Thermo Scientific Accucore columns.

Methods: The hydrophobic and steric selective properties of the Accucore™ C18, PFP and Phenyl-Hexyl were investigated. Experimental test probes were analyzed on three different columns.

Results: Hydrophobicity and steric selectivity of the Accucore C18, PFP and Phenyl-Hexyl were demonstrated. The data collected provides a useful tool in selecting a column for a reversed-phase separation.

Introduction

The retention and selectivity of column stationary phases can be categorized into different modes of interaction; hydrophobic, steric, hydrogen bonding, ion exchange and chelation.

To fully characterise the nature of a stationary phase, a series of diagnostic tests can be performed (based on those developed by Tanaka [1]). These tests characterize analyte/stationary phase interactions using a combination of chromatographic probes.

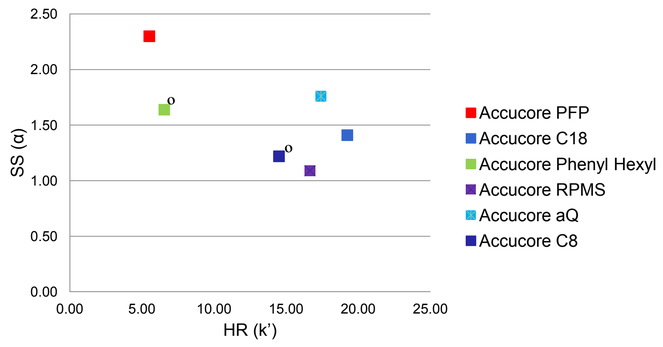
The tests for characterizing two of the primary modes of interaction are described as follows:-

1) Hydrophobicity
Hydrophobic retention (HR) – the capacity factor of a hydrophobic hydrocarbon, pentylbenzene, give a broad measure of hydrophobicity.
Hydrophobic selectivity (HS) - The selectivity factor between pentylbenzene and butylbenzene provides a measure of the surface coverage of the phase; these two alkylbenzenes differ by one methylene group and their selectivity is dependent on ligand density.

2) Steric selectivity (SS)
Steric selectivity is the ability of the stationary phase to distinguish between molecules with similar structures and hydrophobicity 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

The analyte/stationary phase interactions of the Accucore series have been previously characterized using these testing procedures [2].

FIGURE 1. Scatter plot of Hydrophobic Retention (HR) against Steric Selectivity (SS), for Accucore columns. HR is calculated using the capacity factor of pentylbenzene. SS is calculated using the selectivity factor between o-terphenyl and triphenylene. (ºElution order for SS was reversed for Accucore Phenyl Hexyl and C8 columns. These were calculated as 1/α).



Based on this data the effect of two of the modes of interaction; hydrophobic and steric interactions, were investigated further on the Accucore C18, PFP and Phenyl-Hexyl columns, using different chromatographic probes, to the ones described above. This would demonstrate that the prediction of selected separations based on the data acquired is possible.

Methods

Instrumentation:
Thermo Scientific Accela 1250 UHPLC system

Columns:
Accucore C18, 100 mm x 2.1 mm, 2.6 µm
Accucore PFP, 100 mm x 2.1 mm, 2.6 µm
Accucore Phenyl-Hexyl, 100 mm x 2.1 mm, 2.6 µm

HPLC conditions are summarized in Table 1. The chromatographic probes used for each test are summarized in Table 2.

TABLE 1. Description of experimental conditions

	Hydrophobic Retention	Steric Selectivity
Mobile phase	H_2O / Methanol (30:70)	H_2O / Methanol (30:70)
Flow rate (mL/min)	0.5	0.4
Temperature (°C)	40	40
Detection (nm)	254	254

TABLE 2. Description of chromatographic probes for the Hydrophobic and Steric Selectivity. Physiochemical properties for each compound were obtained from www.Chemspider.com. o-, m- and p-Terphenyl are positional isomers. (*predicted LogP, using ACD/Labs' ACD/Physchem suite)

Variable	Chromatographic probes	Molecular Formula	Peak Number	Structure Type	LogP
Hydrophobic Retention	Ethylbenzene	C_8H_{10}	2	Alkylbenzene	3.15
	Propylbenzene	C_9H_{12}	3	Alkylbenzene	3.72
	Butylbenzene	$C_{10}H_{14}$	4	Alkylbenzene	4.38
	Pentylbenzene	$C_{11}H_{16}$	5	Alkylbenzene	4.76*
	Heptylbenzene	$C_{13}H_{20}$	6	Alkylbenzene	5.78*
	Octylbenzene	$C_{14}H_{22}$	7	Alkylbenzene	6.29*
Steric Selectivity	Acenaphthene	$C_{12}H_{10}$	2	PAH	3.73*
	Phenanthrene	$C_{14}H_{10}$	3	PAH	4.46
	Anthracene	$C_{14}H_{10}$	4	PAH	4.45
	Triphenylene	$C_{18}H_{12}$	5	PAH	5.73*
	o-Terphenyl	$C_{18}H_{14}$	6	Rotational, Aromatic	5.17*
	m-Terphenyl	$C_{18}H_{14}$	7	Rotational, Aromatic	5.63*
	p-Terphenyl	$C_{18}H_{14}$	8	Rotational, Aromatic	5.63

Results

Hydrophobic Retention

FIGURE 2. Hydrophobic retention on the Accucore C18 (a), Accucore PFP (b) and Accucore Phenyl-Hexyl (c). The chromatograms are shown to scale to demonstrate the differences in Hydrophobic Retention. Theophylline is used as a t_0 marker. (1) Theophylline (2) Ethylbenzene (3) Propylbenzene (4) Butylbenzene (5) Pentylbenzene (6) Heptylbenzene (7) Octylbenzene

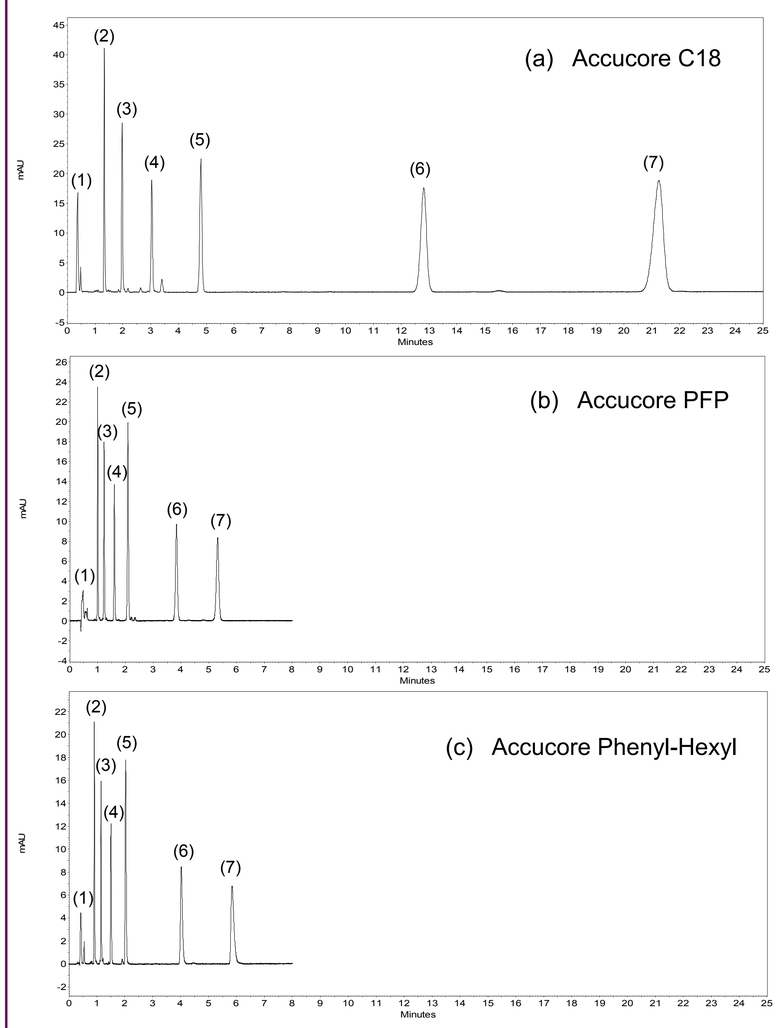
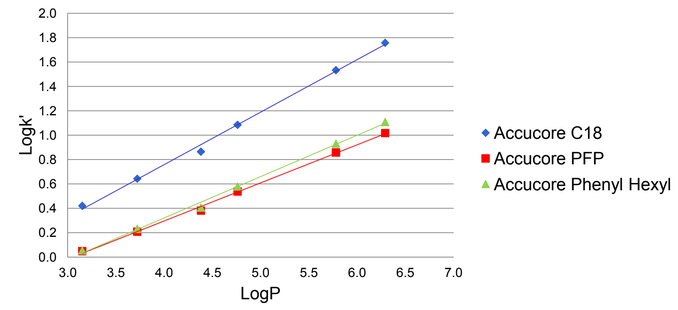


FIGURE 3. Linear plots of LogP against Logk', which illustrate increasing retention with increasing LogP, i.e. Hydrophobicity, of the alkylbenzene probes analysed on the Accucore C18, PFP and Phenyl-Hexyl.



Steric Selectivity

FIGURE 4. Chromatograms of steric selectivity probes on the Accucore C18 (a), Accucore PFP (b) and Accucore Phenyl-Hexyl (c). The chromatograms are shown to scale to demonstrate the differences in retention and selectivity. Theophylline is used as a t_0 marker. (1) Theophylline (2) Acenaphthene (3) Phenanthrene (4) Anthracene (5) Triphenylene (6) o-Terphenyl (7) m-Terphenyl (8) p-Terphenyl

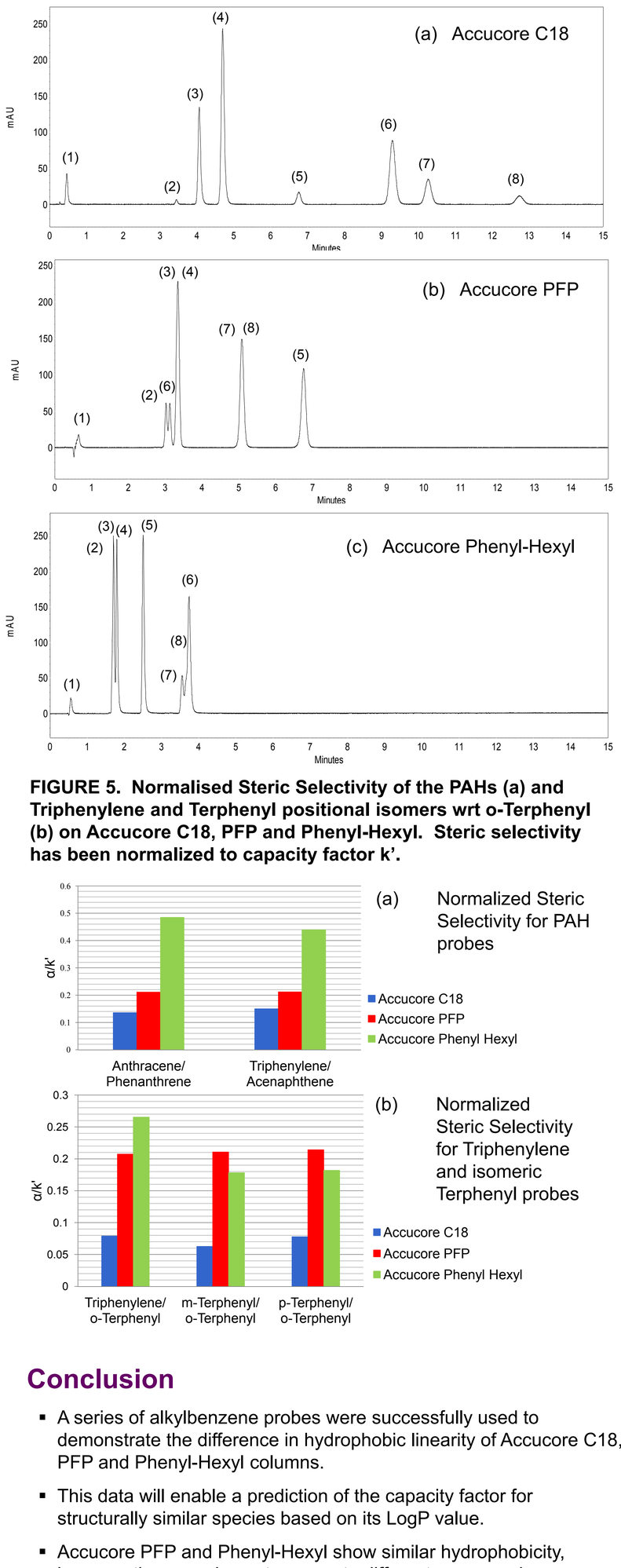


FIGURE 5. Normalised Steric Selectivity of the PAHs (a) and Triphenylene and Terphenyl positional isomers wrt o-Terphenyl (b) on Accucore C18, PFP and Phenyl-Hexyl. Steric selectivity has been normalized to capacity factor k'.

Conclusion

- A series of alkylbenzene probes were successfully used to demonstrate the difference in hydrophobic linearity of Accucore C18, PFP and Phenyl-Hexyl columns.
- This data will enable a prediction of the capacity factor for structurally similar species based on its LogP value.
- Accucore PFP and Phenyl-Hexyl show similar hydrophobicity, however they are shown to separate different compounds.
- Steric selectivity data was normalised to retention factor (k') to see if the differences in selectivity observed were solely due to steric interactions with the stationary phase.
- Normalised steric selectivity data indicates that the Accucore PFP shows optimum selectivity for rotational aromatic positional isomers and the Accucore Phenyl-Hexyl shows optimum selectivity for PAHs.
- The data collected provides a useful tool in selecting a column for a reversed-phase separation based on a combination of LogP and steric interactions of a compound.

Future Directions

The effect of mobile phase composition, including organic content and the difference between methanol and acetonitrile in regard to steric interactions of compounds with different column types will be investigated in future studies.

References

[1} K. Kimata, K. Iawguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, N. Tanaka, Journal of Chromatographic Science, 27 (1989) 721-728

[2] Thermo Scientific Accucore HPLC columns technical guide, 2011, BRCCSACCUCORE 0611

For additional information, please visit our Chromatography Resource Centre which can be found at: www.thermoscientific.com/chromatography

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