Contrasting Selectivity Between HPLC and SFC using Phenyl-type Stationary Phases: A Study on Linear Polynuclear Aromatic Hydrocarbons.

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Introduction

The polynuclear aromatic hydrocarbons (PAHs) are often by-products of petroleum processing or combustion. Many PAHs are carcinogenic, teratogenic and mutagenic [1]. It is therefore of large interest to study these compounds. Gas chromatograph is often used for low molecular weight volatile PAHs; however, for thermally labile and low volatile solutes reversed phase high performance liquid chromatography (RPHPLC) utilizing C18 phases are most commonly used [2]. Also supercritical fluid chromatography (SFC) has shown promising characteristics for analysing PAHs [3].

In a series of prior studies we evaluated the retention behaviour of linear PAHs on phenyl-type stationary phases in reversed phase high performance liquid chromatography (RPHPLC). Two of the columns that were tested in these prior works were a Synergi-Polar RP (Synergi) stationary phase, and a Cosmosil 5PBB (Cosmosil) stationary phase. The Synergi phase comprises a phenyl ring that is tethered to a silica substrate via an ether linked propyl chain. The Cosmosil phase comprises a pentabromo-phenyl ring that is tethered to the silica substrate also via a propyl chain, but with no ether group in the alkyl chain. These two phases were selected for testing in SFC in order to explore whether the SFC environment offered similar separation behaviour compared to RPHPLC.

In RPHPLC separation of non-ionic solutes, such as PAHs, the separation variables at hand are either the (1) stationary phase (2) type of mobile phase or (3) the composition of the mobile phase. Utilising SFC as opposed to RPHPLC provides a substantial change in the nature of the mobile phase, and this brings with it significant opportunities to vary not only the composition of the mobile phase, but also the state of the mobile phase, i.e., variation of density of phases from sub- to supercritical fluids. At any given composition of mobile phase in SFC, that is, the proportion of CO_2 to organic modifier, the density of the mobile phase can be varied by control of the pressure and temperature in the system. From a practical perspective this also requires a better temperature, pressure and modifier control of the system to ensure reproducible data [4,5]. High pressures effectively produce a higher density liquid-like phase, while lower pressures give a less dense liquid-like state. In contrast, the density of the mobile phase for RPHPLC is almost independent of pressure. The effect of mobile phase composition on retention optimisation is therefore more complex in SFC than in RPHPLC, and as a consequence, the changes in selectivity may potentially be more substantial.

Generally, SFC separations are more akin to normal phase separation modes, since the mobile phase is largely non-polar sub/super-critical CO_2, with small quantities of polar modifiers, such as methanol. The stationary phases are usually either polar, or as is often the case, chiral, thus, enabling alternatives to the less environmentally friendly normal-phase HPLC separation mode. Nevertheless, for strongly retained species, such as non-polar PAHs, retention in SFC environments that incorporate reversed phase HPLC columns is a viable option to RPHPLC separation protocols. In this work we present an unusual, and unexpected outcome in the retention behaviour of two different types of phenyl bonded reversed phase stationary phases for the separation of linear PAHs.

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Experimental

Chemicals

Supercritical CO_2 was obtained using food grade CO_2 purchased from Coregas, Yennora, Vic., Australia. HPLC grade methanol was used as an organic mobile phase modifier and HPLC grade tetrahydrofluran (THF) was used for the dissolution of the polynuclear aromatic hydrocarbons (PAH). Both methanol and THF were purchased from Honeywell Burdick & Jackson (Taren Point, NSW, Australia). Polynuclear aromatic hydrocarbon standards were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia).

Separations

All chromatographic separations were performed on a Agilent 1260 Infinity Analytical SFC System, utilizing a Fusion A5 (G4301A) SFC system, 1260 degasser (G1322A), HPLC-SFC binary pump (G4302A), SFC autosampler (G4303A), column compartment (G1316C), DAD UV-detector (G1313C, set at 304 nm), and Agilent Chem Station software on an Intel Core 2 Duo 3.16 GHz processor (Mulgrave, Victoria, Australia). The chromatography columns used in this study were a Synergi polar-RP (4 µm P_d, 150 × 4.6 mm, 80 Å) and a Cosmosil 5PPB (5 µm P_d, 150 × 4.6 mm, 120 Å) purchased from Phenomenex (Lane Cove West, NSW, Australia).

The PAH stock standards were dissolved in THF and made up in concentrations of 10 mg/mL; injectable samples were then prepared by dilution with THF to 1 mg/mL. Each column was tested using five different mobile phase compositions of CO_2 and methanol at a flow rate of 3 mL/min, with a column temperature set at 35 ºC and backpressure regulated at 110 bar. Each sample was injected onto the column using a 5 µL injection loop with an overfill factor of 3 and duplicates were performed for each injection.

Retention factors were determined using void volumes calculated by the inflection point of the solvent front resulting from the minor disturbance generated by the injection plug.

Results and Discussion

In order to quantify the retention behaviour of small solutes the retention factor, k, may be expressed as a function of the mobile phase composition Φ. One model that could be used to describe this relationship is the Linear Solvent Strength (LSS) theory [6,7]. LSS theory provides the following relationship:

$$\log k = \log k_0 - S\Phi \qquad (1)$$

where k_o is the retention factor of the solute in the weak solvent (i.e., water in reversed phase and CO_2 for SFC), and S is the rate of change in log k with Φ. Plots of log k versus Φ are important as they provide a visual depiction of how selectivity changes as the solvent composition changes and the S parameter provides a means to quantify the expected degree

of separation – or global selectivity, and then allow the determination of the optimum solvent composition required to bring about the desired level of separation. The relationship between log k and Φ is generally linear when the range of retention factors considered is limited to between 1 to 10, beyond which a quadratic relationship is often observed []. Previously it has be reported an higher sensitivity of the retention times in SFC compared to RPHPLC with respect to the methanol fraction in the eluent [4,8]. In the present study we evaluated the relationship between retention factor and solvent composition to assess the changes in selectivity of a homologue series of linear PAHs on both the Synergi phase and the Cosmosil phase.

The first observation regarding the retention behaviour of the PAHs on these non-polar phenyl-type stationary phases in SFC environments with CO_2/methanol mobile phases was in essence similar to reversed phase HPLC. That is the addition of methanol to the CO_2 (in SFC) or to the water (in RPHPLC) resulted in a decrease in the retention of the non-polar solutes. Furthermore, the retentivity of the PAHs on the Cosmosil stationary phase was far greater than on the Synergi phase, which was consistent with our findings in RPHPLC []. For example, the retention factor of pentacene on the Cosmosil phase using a mobile phase comprising 70% CO_2 and 30% methanol was in the order of 86, whereas, on the Synergi phase, the retention factor of pentacene was just 21 when the mobile phase was 99 (v/v)% CO_2 and 1% methanol. But retentivity just reveals one aspect of the retention behaviour; it is the relationship between retention and solvent composition on these two phases that is far more interesting and somewhat surprising.

To evaluate the retention behaviour of the PAHs, retention was tested using a range of solvent compositions. On the Synergi stationary phase, for example, the composition of methanol in the mobile phase was varied between 1 to 5%, the upper limit being restricted to 5% because retention of the smaller PAH species was insufficient above 5% methanol. Since the retentivity was greater on the Cosmosil phase, the methanol range varied between 30% to an upper limit of 40%.

Plots of log k versus Φ, expressed as volume fraction of methanol in supercritical CO_2, are shown in Figure 1 for both the Synergi and Cosmosil stationary phases. In all cases these plots were linear over the solvent composition ranges tested. No retention of benzene beyond the void time was possible the Synergi stationary phase, even in 99% CO_2. Napthalene was only slightly more retentive with the retention factor varying from 0.53 to 0.60 over the 5% range in methanol composition. However, for pentacene, the retention factor varied from around 11 in 95% CO_2 to 21 in 99% CO_2. These substantial differences in retention and selectivity as a function of the number of rings are apparent in the plots of log k versus Φ in Figure 1. Firstly, the magnitude of log k increased as the number of rings increased, and secondly the slope of the relationship increased as the ring number increased, in most ways consistent with RPHPLC retention behaviour. This verified that selectivity is dependent on the composition of the mobile phase. In the studies undertaken in RPHPLC, the S values (slope) changed from 2.71 for benzene to 4.91 for pentacene, however, in SFC, the S values showed a much greater range, practically zero for benzene (however, more or less un-retained), to 7.1 for pentacene, signifying far greater selectivity in SFC than in HPLC, albeit,

with limited retention for the smaller solute species and subsequently limited modifier compositions available in order to optimise the separation.

In contrast, however, the retention behaviour on the more retentive Cosmosil phase was substantially different. Retention of all linear PAHs, including benzene, was found, although, the retention factor of benzene was just 0.35 in 70% CO_2 compared to 86 for pentacene. Clearly, the Cosmosil phase offered great scope with respect to retention, however, gaining selectivity as a function of the mobile phase composition was more limited. The plots of log k versus Φ in Figure 1, for example, show that the slopes of these plots were almost independent of the number of rings, that is, selectivity effectively was independent of the amount of methanol modifier. S values ranged from 0.7 for benzene to 1.5 for pentacene; S being about 20% that observed on the Synergi phase. In contrast, when RPHPLC was employed, the S values ranged from 2.61 to 4.25. This outcome was unexpected, and showed that the separation was almost independent of the mobile phase composition, with a decrease in retention for each species, with little change in resolution. The relative degree of separation as a function of the solvent composition on the Cosmosil phase is illustrated in the normalised retention plots shown Figure 2a; the separation achieved in 70% CO_2 is almost exactly the same as the separation obtained in 60% CO_2, the latter being completed in 35 minutes, compared to 45 minutes. There was almost no change in resolution between each of the linear PAHs, in contrast to the predictions set out by the classic resolution equation, applicable in HPLC:

$$R_s = \frac{\sqrt{N}}{4}\frac{k}{1+k}\frac{\alpha-1}{\alpha} \qquad (2)$$

where N is the efficiency α is the selectivity and R_s is the resolution.

There were, however, significant changes in the selectivity for the contamination products (unidentified) in the PAH samples, as shown for example, the change in retention for the compounds labelled as ‘a’. A comparison of normalised chromatograms obtained on the Synergi phase, however, showed substantial changes in selectivity when the solvent composition changed even by as little as 5% methanol, as shown in Figure 2b. On the Synergi phase, the contamination products showed even greater selectivity, in fact, their migration changes were difficult to conclusively identify. The changes in selectivity between each of the linear PAHs as a function of the solvent composition on both the Synergi and Cosmosil phases are given in Table 1.

Conclusion

These preliminary investigations provide a snapshot of the selectivity differences between RPHPLC and RPSFC. In this work a study was undertaken using linear PAHs on two types of phenyl bonded stationary phases that were shown in RPHPLC to provide strong retention and diverse selectivity for these test compounds. However, in RPSFC environments, the retention of the smaller PAHs on the Synergi polar phase was limited, yet the selectivity across the group containing 1 to 5 ring PAHs was substantial, much more so than in RPHPLC. In contrast, the retention of the linear PAHs on the Cosmosil phase was substantial, but the selectivity was almost independent of the mobile phase composition.

Based on a comparison in the retention behaviour of the linear PAHs and 'impurity' peaks, it may be that this type of behaviour is very solute class dependent. Future work will explore this in more detail.

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208 Figure 1. Plots of log k versus Φ (solvent fraction of methanol in mobile phase) for the
209 linear PAHs on the Synergi and Cosmosil Stationary phases.

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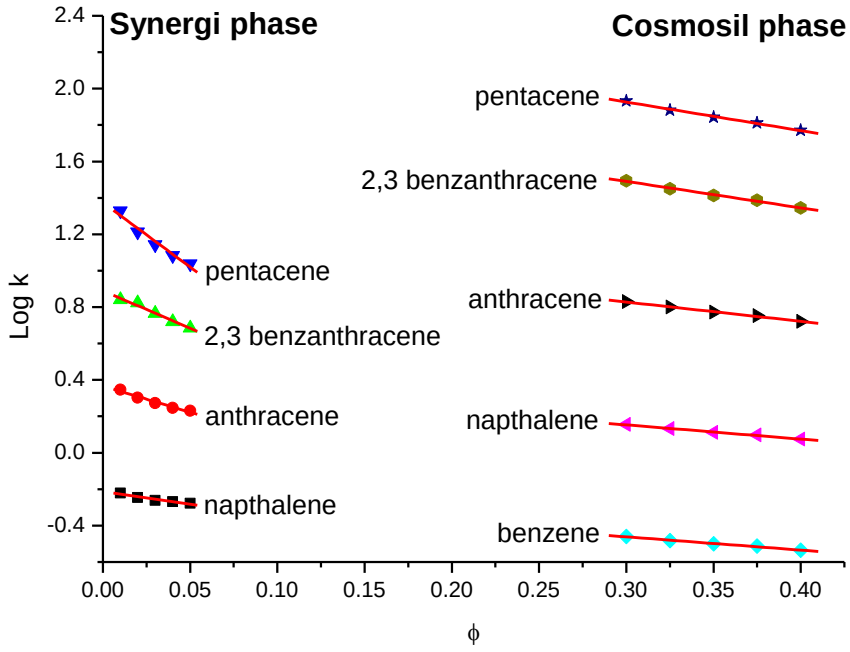

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Figure 2a Normalised chromatograms illustrating the separation of the linear PAHs on the Cosmosil phase using a mobile phase with either 40% or 30% methanol modifier. The separations were normalised in time with respect to pentacene and in intensity, with respect to naphthalene. Peaks: 1 (benzene), 2 (naphthalene), 3 (anthracene), 4 (2,3-benzeanthracene), 5 (pentacene).

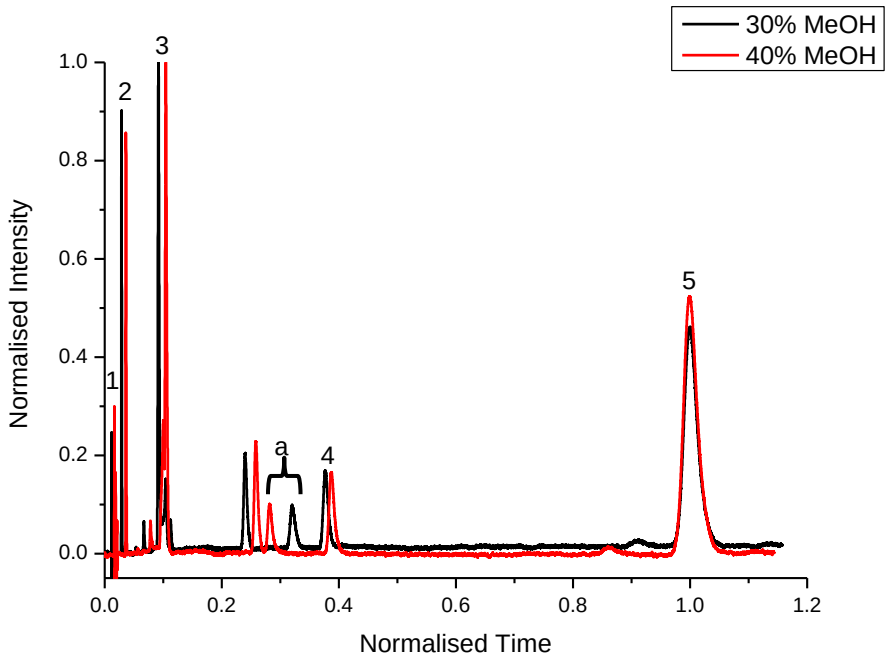
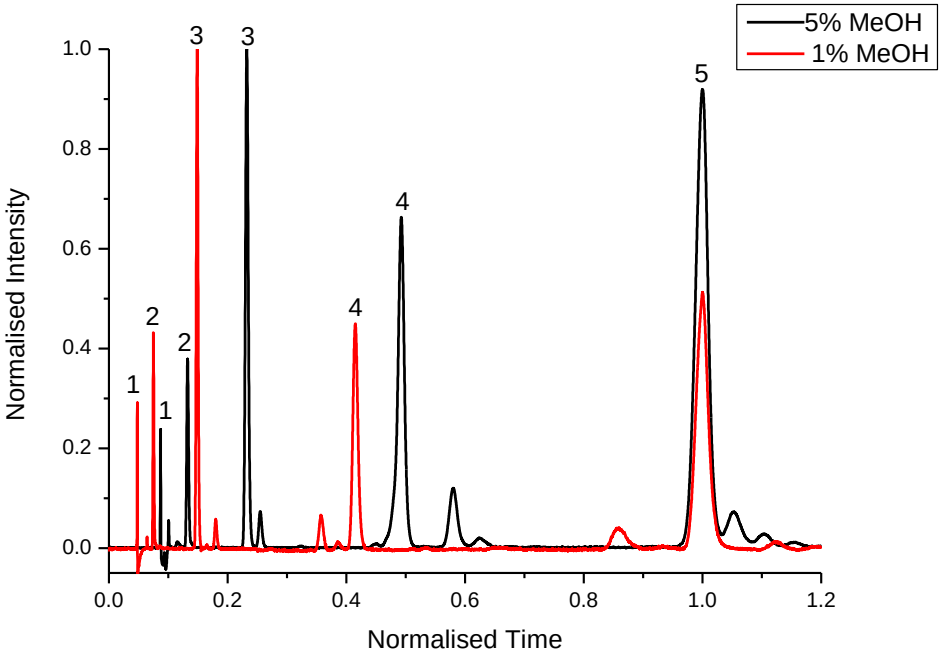


Figure 2b Normalised chromatograms illustrating the separation of the linear PAHs on the Synergi phase using a mobile phase with either 1% or 5% methanol modifier. The separations were normalised in time with respect to pentacene and in intensity, with respect to anthracene. Peaks: 1 (benzene), 2 (naphthalene), 3 (anthracene), 4 (2,3-benzeanthracene), 5 (pentacene).



229 Table 1. Selectivity as a function of the solvent composition, Φ, for the Synergi and
230 Cosmosil stationary phases.

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Φ (%MeOH)	Synergi Stationary Phase Selectivity (ring x/y)			ϕ (%MeOH)	Cosmosil Stationary Phase Selectivity (ring x/y)			
	3/2	**4/3**	**5/4**		**2/1**	**3/2**	**4/3**	**5/4**
1.0	3.70	3.11	3.08	**30.0**	4.11	4.73	4.63	2.74
2.0	3.53	3.31	2.46	**32.5**	4.12	4.65	4.47	2.70
3.0	3.42	3.11	2.38	**35.0**	4.09	4.57	4.37	2.69
4.0	3.28	2.96	2.32	**37.5**	4.07	4.52	4.31	2.66
5.0	3.23	2.84	2.26	**40.0**	4.07	4.43	4.20	2.67
Range (%)	*14.5*	*9.5*	*36.4*	***Range (%)***	*1.0*	*6.7*	*10.1*	*2.5*

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