Tr. J. of Chemistry
20 (1996) , 269 – 275.
© TÜBİTAK

A Comparison of Normal(-CN) and Reversed (C-18) Phase Chromatographic Behaviour of Polycyclic Aromatic Hydrocarbons

Ş. Fatma AYGÜN
Ondokuz Mayıs University,
Faculty of Arts and Science, Samsun-TURKEY
Mustafa ÖZCİMDER
Ondokuz Mayıs University,
Faculty of Education, Samsun-TURKEY

Received 12.12.995

Because of the well-known carcinogenic and mutagenic character of polycyclic aromatic hydrocarbons (PAHs), still new methods have been tried for their qualitative and quantitative determinations in various smaples. Liquid chromatography is one of the most widely applied techniques. In this study we used liquid chromatography in normal and reversed phase modes for the determinations of PAHs and tried to find a relation between the retention times and various parameters, such as molecular shape, water solubility and number of carbon atoms.
Key Words: High-Pressure Liquid Chromatography, Polycyclic Aromatic Hydrocarbons, Normal Phase Chromatography, Reversed Phase Chromatography.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed throughout the environment. They appear in water[3], air[2], soil [3], food[4] and so on. They come mainly from two sources;

1. Natural: Forest fires, volcanic activity, petroleum.
2. Man-made: Burning fuels, metallurgical furnaces, exaust gases, smoking, etc.

Although many PAHs exist, only sixteen of them draw significant attention and appear in the list of environmental protection and control institutes. These are presented in Table 1.

Analytical techniques applied for PAH analysis use mostly chromatographic[4] and spectroscopic[3,5–6], in particular fluorometric[3], methods. The detection limits of various analytical techniques used in quantitative determination of PAHs[6] are shown in Table 2. From this table, the fluorimeter has the lowest detection limits[7]. For years, since the resolution power is much higher than that of spectroscopic techniques, gas chromatography has been the preferred technique. However, during recent decades, high performance (pressure) liquid chromatography has been replacing gas chromatography for the determination of PAHs because of their relatively high boiling points[5–10]. Liquid chromatography is used either in normal phase mode

or in the reversed phase mode. In normal phase, a polar mobile phase is chosen as n-hexane, n-pentane. The stationary phase can be either silica, alumina or a modified silica, which are generally called "bonded-phases", such as Si-CN, Si-NH_2. On the other hand, in reversed phase mode the mobile phase is either a pure polar solvent or a mixture of various polar solvents while the stationary phase is a nonpolar solid or a liquid. In reversed phase mode, water is one of the components in mobile phase. This is a real advantage of reversed phase mode over normal phase[10–12,13]. However, the chromatographic retention and resolution mechanisms of many components in reversed phase mode are still being studied. For instance, Ohtsuka et al.[14] investigate the retention behaviour of metal chelates in ion-pair reversed-phase liquid chromatography as a function of mobile phase composition. Other[15–18,7,19] researchers are still involved in the mechanisms of various materials of HPLC columns. In the mean time, different types of stationary phases are being developed for the separation of various compounds[20]. These show that reversed phase separation of various compounds is under investigation. In this study, we tried to find a relation between the retention times and the physical parameters of PAHs in reversed phase mode and compared the resolution power with that of normal phase under selected experimental conditions.

Table 1. Priority Pollutant Aromatic Hydrocarbons

1. Naphthalene	9. Benz(a)anthracene
2. Acenaphthalene	10. Chrysene
3. Acenaphthene	11. Benzo(b)fluoranthene
4. Fluorene	12. Benzo(k)fluoranthene
5. Phenanthrene	13. Benzo(a)pyrene
6. Anthracene	14. Dibenz(a,h)anthracene
7. Fluoranthene	15. Benzo(g,h,i)perylene
8. Pyrene	16. Indeno(1,2,3-c,d)pyrene

Table 2. Detection Limits of Some QPAHs of Different Techniques(6)

Compound	LC-MS*	UV*	Fluorescence*
Naphthalene	10	2.40	400.00
Acenaphthylene	10	3.72	800.00
Acenaphthene	10	7.22	-
Fluorene	10	0.40	-
Phenanthrene	10	0.21	-
Anthracene	10	0.06	1.10
Fluoranthene	10	0.65	0.36
Benz(a)anthracene	10	50.00	17.80
Chrysene	10	3.31	400.00
Benzo(b)fluoranthene	10	0.19	0.07
Benzo(k)fluoranthene	10	0.41	0.04
Benzo(a)pyrene	10	0.49	0.07
Dibenz(a,h)anthracene	10	2.00	4.00
Benzo(g,h,i)perylene	10	1.40	0.55
Indeno(1,2,3-c,d)pyrene	10	1.24	0.36

*mg/L

Material and Methods

Polycyclic aromatic hydrocarbons used in this study were all purchased from the Community Bureau of Reference Materials in Belgium. All solvent and mobile phases, acetonitrile, n-hexane, dichloromethane (all Merck) were of spectroscopic grade. Deionized and bidistilled water were used as mobile phase. Varian 5560 Liquid chromatograph (HPLC) equipped with a high pressure pump capable of pumping liquids up to 10 mL/min. was used. The detector was Varian-200 UV spectrometer with a detector volume of $4.5\text{x}10^{-3}$ mL coupled to HPLC. Absorptivities were measured at 254 nm. The chromatographic conditions were as follows:

Reversed Phase Chromatography

Column	:	Stainless steel filled with LiChrosorb RP C 18 (Altech) 5 μm, 25 cm length and 4.6 mm internal diameter.
Mobile phase	:	85% acetonitrile 15% water, 1.5 mL/min flow rate.
Detector	:	254 nm, 0.05 abs sensitivity.
Recorder	:	Packard dual pen 10 mV/f.s.d., 1.0 cm/min chart speed.

Normal phase Chromatography

Column	:	Stainless Steel filled with Si-CN (bonded-phase) (Altech) 25
	:	cm length and 4.6 mm internal diameter.
Mobile phase	:	n-hexane, 1.5 mL/min flow rate.
Detector	:	254 nm, 0.05 abs sensitivity.
Recorder	:	Packard dual pen, 10 mV/f.s.d., 30 cm/hr chart speed.

Standard Solutions

10 mg/L solutions of PAHs were prepared in n-hexane and 5 μL aliquots of samples were injected to both types of chromatographic system using SGE 10-μL injector (5 times). Capacity factors were calculated with the following equation:

$$k' = (t - t_s)/t_s$$

t = retention time of a PAH, minutes

t_s = retention time of a reference material, minutes

Reference materials, being the non-retarded compound in the particular column, are phenol (very polar), for the reversed phase column and n- pentane (non-polar) for the normal phase column.

Results and Discussion

Reversed-phase

The capacity factors, k', of thirteen PAHs are shown in Table 3 along with some parameters. The k' volues were used to plot Figure 2 and 3 showing the relationship between the capacity factors and the number of carbon atoms and shape factor[13], Figure 1, shows the ratio of length to breadth of molecules. As shown in Figure 2, retention on reversed phase column increases with the number of carbon atoms. In other words, there is a linear relationship between k' and n ($r^2 = 0.98222$).

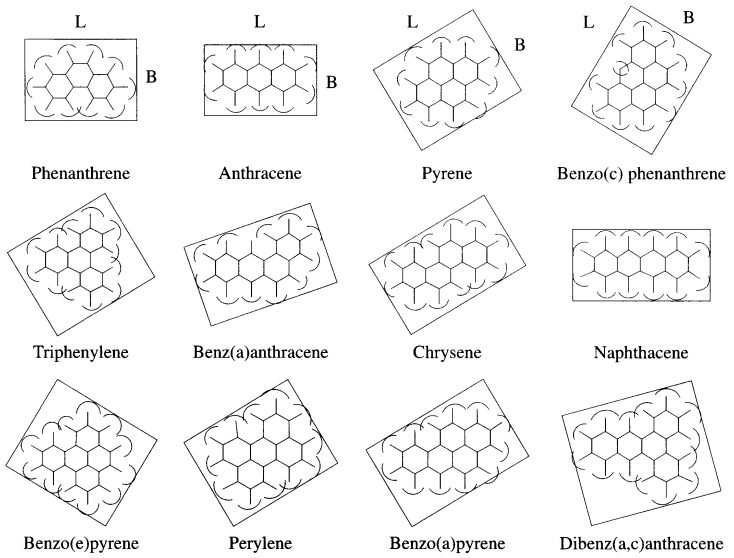

Figure 1. Structures and maximized length-to-breadth ratio rectangles for several parent PAHs(13)

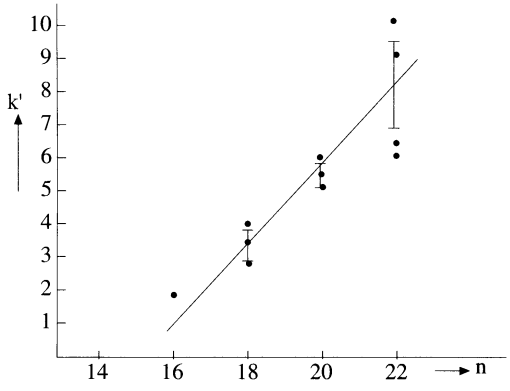

Figure 2. Graph of k' vs. n of reversed phase mode

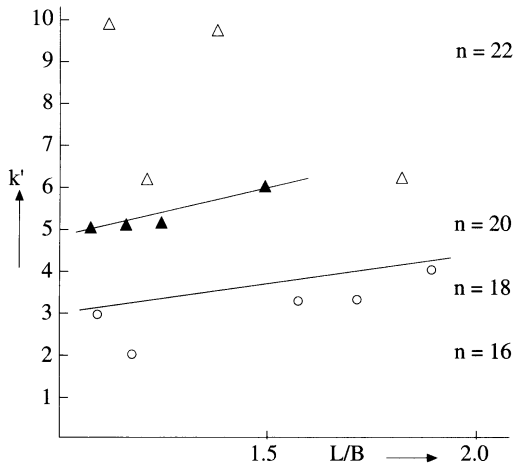

Figure 3. Graph of k' vs. L/B of reversed phase mode

This is to be expected after examining the Table 3 again. In Table 3, we see that as n increases, water solubility decreases, giving rise to increased capacity factors. This proves that water solubility is a determining factor. Therefore, as water solubility increases, retention time decreases when water is a major component of the mobile phase. It may also be deducted from the same figure that PAHs can be resolved according to their number of carbon atoms. The resolution of the PAHs with the same number of carbon atoms is still a problem. In order to show that the shape factor is an important parameter, Figure 3 was drawn. For n=18, retention increases as L/B increases, with r^2 as 0.75 ($r^2 = 0.9099$ excluding chrysene) For n=20 even a better linearity is observed with an r^2 of 0. 94729. For 20 carbon-containing PAHs, as molecules attain a higher L/B value retention increases. That is generally the case because longer and smaller (narrower) molecules can easily penetrate into the holes between the C 18 molecules (Figure 4), where they can stay longer as their length/breadth ratio gets higher. For higher molecules, the retention mechanism seems rather complex. The k' values show that good resolution exists between the molecules. Capacity factors are 6.33, 6.44, 9.88 and 10.00 for dibenz(a,h)anthracene, dibenz(a,c)anthracene, indeno(1,2,3-c,d)pyrene and benzo(g,h,i)perylene, respectively, while the shape factors are 1.79, 1.24, 1.40 and 1.12. This means that there is no linear relationship between shape factor and retention time. Water solubility of high molecular

compounds is very low, and at the same time they seem very close to each other. However, one may still claim that molecular shape and water solubility probably play a dominant role together in the elution of PAHs.

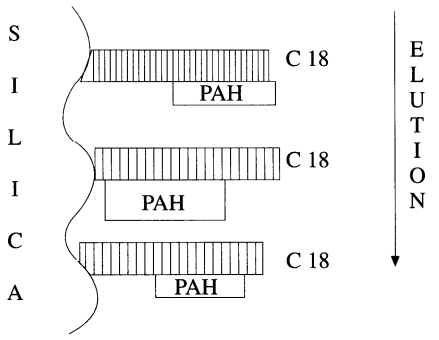

Figure 4. Penetration of a molecule into the hole between C 18 molecules in reversed phase chromatography

Table 3. Capacity Factors of Some PAHs in Reversed Phase-Column, Shape Parameters, and Aqueous Solubility

No	PAH	k'	L/B(13)	n	m(13,14) (mg/kg)	MW
1.	Fluoranthene	1.88	1.22	16	0.2060	202
2.	9-10 Dibenzanthracene	3.00	1.12	18	0.043	228
3.	Benz(a)anthracene	3.44	1.58	18	0.0094	228
4.	Chrysene	3.44	1.72	18	0.0018	228
5.	2-3 Dibenzanthracene	4.33	1.89	18	0.00057	228
6.	Benzo(b)fluoranthene	5.22	1.12	20	0.0015	252
7.	Benzo(k)fluoranthene	5.22	1.20	20	0.008	252
8.	Perylene	5.44	1.27	20	0.0004	252
9.	Benzo(a)pyrene	6.25	1.50	20	0.0012	252
10.	Dibenz(a,h)anthracene	6.33	1.79	22	0.0005	278
11.	Dibenz(a,c)anthracene	6.44	1.24	22	-	278
12.	Indeno(1,2,3-c,d)pyrene	9.88	1.40	22	0.00019	276
13.	Benzo(g,h,i)perylene	10.00	1.12	22	0.0007	276

k'=Capacity factor
L=Molecular length
B=Breadth of a molecule
n=Number of carbon atoms
m=Water solubility (25°C)
MW=Molecular weight

Normal-phase

The capacity factors of thirteen PAHs obtained on a Si-CN bonded phase column together with shape factors are given in Tabel 4. As expected, the retention times increase with the number of carbon atoms. In Figure 5, capacity factors vs. number of carbon atoms were plotted, giving a straight line (r^2=0.9841). Figure 6 gives the relation between L/B vs. k'. In Figure 6 it is difficult to talk about straight lines. Only for 18 carbon atoms does regression analysis show a straight line with a regression coefficient of 0.6906. By excluding benz(a)anthracene (BaA) a much better straight line with a regression coefficient of 0.9934 was obtained. Negative slopes of k' vs. L/B being -0.204 (-0.244 excluding BaA) mean that as the L/B ratio gets higher retention times decrease. From these figures, one can say that resolution based on number of carbon atoms is achivable but PAHs with the same number of carbon atoms can not be separated in normal phase mode.

Table 4. Capacity Factors of Some PAHs in Si-CN Normal Phase Column

No	PAH	k'	L/B(13)	n	m(13,14) (mg/kg)	MW
1.	Fluoranthene	0.57	1.22	16	0.2060	202
2.	2-3 Dibenzanthracene	0.67	1.89	18	0.00057	228
3.	Benz(a)anthracene	0.76	1.58	18	0.0094	228
4.	Chrysene	0.81	1.72	18	0.0018	228
5.	9-10 Dibenzanthracene	0.86	1.12	18	0.043	228
6.	Benzo(b)fluoranthene	0.91	(1.12)	20	0.0015	252
7.	Benzo(k)fluoranthene	1.05	(1.20)	20	0.0008	252
8.	Perylene	1.09	1.27	20	0.0004	252
13.	Benz(g,h,i)perylene	1.19	1.12	22	0.0007	276
12.	Indeno(1,2,3-c,d)pyrene	1.29	1.40	22	0.00019	276
10.	Dibenz(a,h)anthracene	1.48	1.79	22	0.0005	278
11.	Dibenz(a,c)anthracene	1.52	1.24	22	-	278

* Abbreviations are as given in Table 3.

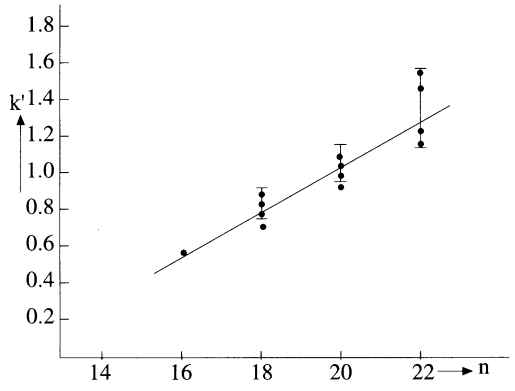

Figure 5. Graph of k' vs. n of normal phase mode

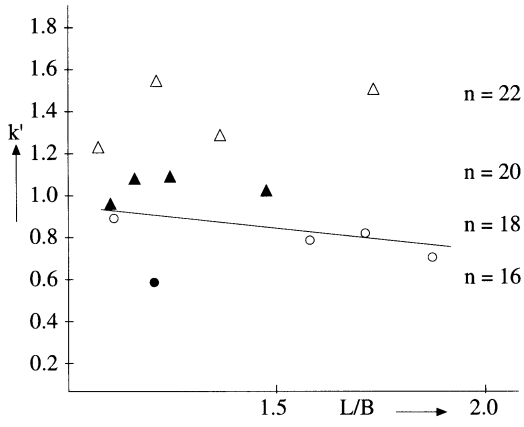

Figure 6. Graph of k' vs L/B of normal phase mode

Comparison of Normal and Reversed Phase Modes

1. Resolution due to number of carbon atoms is better in reversed phase, which is indicated by the higher slope of reverse phase 1.03, than that of normal phase, -0.204.

2. For 18 and 20 carbon atoms, there exists a linear relationship between the molecular shape and capacity factor for reversed phase, which is not the case for normal phase mode.

3. For the reversed phase elution of the same number of carbon atoms (for 18 and 20), as L/B gets higher, k' gets higher, which is the reverse in normal phase mode. In other words there is no direct relationship between shape parameter and capacity factor for normal phase.

4. Chromatographic separations of PAHs are, in general, much better in reversed phase than in normal phase elution.

References

1. H. Shiralshl, N. H, Pilkington, A. Otsuki, and K. Fowa, **Environ. Sci. Technol., 19,** 585-590 (1985).
2. G. W. Kelly, K. D. Bartle, A. A. Clifford, and D. Scammells, **J. Chromatogr. Sci., 31,** 73-76 (1985).
3. C. G. Pinto, J. L. P. Pavon, and B. M. Cordero, **Anal, Chem., 66,** 874-881 (1994).
4. S. L. Simonich, and R. A. Hites, **Environ. Sci. Technol., 28,** 939-943 (1994).
5. M. E. Snook, W. J. Chamberlain, R. G. Severson, and O. T. Chortyk, **Anal. Chem., 47,** 1155-1157 (1975).
6. J. W. Giles, R. Somack, and V. S. McKay, "Detection and Identification of Polynuclear Aromatic Hydrocarbons by HPLC" American Chem Annual Meeting, Washington, D.C., 1979.
7. M. C. Hennion, C. Picard, C. Combellas, M. Cude, and R. Posst, **J. Chromatogr., 210,** 211-228 (1981).
8. J. C. Fetzer, **J. Chromatogr. Sci., 31,** 70-72 (1993).
9. D. L. Vassilaros, R. G. Kong, D. W. Later, and M. L. Lee, **J. Chromatogr., 252,** 1-2 (1982).
10. A. L. Colmjö, and J. C. MacDonald, **Chromatographia, 13,** 350-352 (1980).
11. M. Popl. V. Dolansky, and J. Mostecky, **J. Chromatogr., 117,** 117-127 (1976).
12. S. A. Wise, and W. E. May, **Anal. Chem., 55,** 1479-1485 (1983).
13. S. A. Wise, W. J. Bonnett, F. R. Guenther, and W. E. May, **J. Chromatogr. Sci., 19,** 457-465 (1981).
14. C. Ohtsuka, K. Matsuzawa, H. Wada, and G. Nakagawa, **Anal. Chim. Acta., 294,** 69-74, (1994).
15. S. J. Sheikh, H. Cho, and J. C. Touchstone, **J. Liq. Chromatogr., 14,** 1235-1245 (1991).
16. C. L. Copper, and M. J. Sepaniak, **Anal. Chem., 66,** 147-154 (1994).
17. A. A. Ben-Bassat, and E. Grushka, **J. Liq. Chromatogr., 14,** 1051-1111 (1991).
18. W. E. Acree, Jr., S. A. Tucker, and D. C. Wilkins, **J. Phys. Chem., 97,** 11199-11203 (1993).
19. R. E. Boehm, and D. E. Martire, **J. Phys. Chem., 98,** 1317-1327 (1994).
20. L. C. Sander, K. E. Sharpless, N. E. Craft, and S. A. Wise, **Anal. Chem., 66,** 1667-1674 (1994).