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Asep Kurnia

Indonesian Agricultural Environment Research Institute (IAERI), Pati 59182, Indonesia,
asep_balingtan@yahoo.com

Lee Wah Lim

Department of Applied Chemistry, Faculty of Engineering, University of Gifu, Gifu 501-1191, Japan

Toyohide Takeuchi

Department of Applied Chemistry, Faculty of Engineering, University of Gifu, Gifu 501-1191, Japan

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Cover Page Footnote

Special gratitude goes to IAARD that has given the opportunity to continue studies and completed this research, and to all those who have provided assistance for the implementation of this research.

Determination of *Polycyclic Aromatic Hydrocarbons (PAHs)* Using Environmentally Friendly Liquid Chromatography

Asep Kurnia^{1*}, Lee Wah Lim², and Toyohide Takeuchi²

1. Indonesian Agricultural Environment Research Institute (IAERI), Pati 59182, Indonesia
2. Department of Applied Chemistry, Faculty of Engineering, University of Gifu, Gifu 501-1191, Japan

*E-mail: asep_balingtan@yahoo.com

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Abstract

An analytical method to determine polycyclic aromatic hydrocarbons (PAHs) is required to obtain high-quality analytical results. The purpose of this study is to achieve good separation of a few PAHs by using environmentally friendly liquid chromatography. Accordingly, a liquid chromatograph incorporating a fluorescence detector, UV detector, and a capillary column is employed herein to simultaneously minimize the use of chemicals and obtain analytical results better than those obtained using a conventional column. Observation parameters include single analysis of each PAH, method validation, the new stationary phase, the effect of mobile phase concentration, and Quencher effect. The PAHs tested include naphthalene, phenanthrene, anthracene, fluoranthene, and pyrene. The test results show that when using an acetonitrile concentration of 100%, the retention times of different PAHs do not differ significantly. Furthermore, upon decreasing the acetonitrile concentration, the retention time increases, and better separation was achieved. The value ranges of precision, LOD, LOQ, and linearity are 3.43–12.42%, 4.7–15.1 mg/L, 15.6–50.5 mg/L, and 0.87–0.99, respectively. The new Sil-S-ImC30 stationary phase showed good results in terms of separation of PAHs. Likewise, the use of 60, 70, 80, and 90% acetonitrile as the mobile phase in combination with 0.03 M acrylamide as the quencher affected retention time but not separation.

Abstrak

Penentuan Polisiklik Aromatik Hidrokarbon (PAHs) Menggunakan Kromatografi Cair yang Ramah Lingkungan. Sebuah metode analisis polisiklik aromatik hidrokarbon (PAH) sangat diperlukan untuk memperoleh hasil analisis yang berkualitas tinggi. Tujuan dari studi ini adalah untuk memperoleh pemisahan beberapa PAH menggunakan kromatografi ramah lingkungan. Pada studi ini digunakan kromatografi cair yang dilengkapi dengan detektor fluoresen, detektor UV dan kolom kapiler untuk meminimalisasi penggunaan bahan kimia dan memperoleh hasil analisis yang lebih baik dibandingkan dengan kolom konvensional. Parameter pengamatan terdiri dari analisis tunggal masing-masing PAH, validasi metode, fase diam yang baru, pengaruh fase gerak, pengaruh panjang gelombang dan *quencher*. Beberapa PAH yang diuji antara lain naphthalen, phenanthrene, anthracene, fluoranthene dan pyrene. Hasil uji menunjukkan menggunakan asetonitril 100 % waktu retensi dari tiap PAH tidak berbeda nyata. Lebih jauh, dengan menurunkan konsentrasi asetonitril maka waktu retensi dan pemisahan lebih bagus. Nilai presisi, LOD, LOQ dan linearitas berkisar antara 3.43–12.42%, 4.7–15.1 mg/L, 15.6–50.5 mg/L, and 0.87–0.99. Fase diam baru Sil-S-ImC30 menunjukkan hasil pemisahan PAH yang bagus. Penggunaan 60, 70, 80, 90% asetonitril yang dikombinasikan dengan 0,03 M acrylamide sebagai *quencher* berpengaruh terhadap waktu retensi tetapi tidak berpengaruh terhadap pemisahan.

Keywords: PAHs, chromatography, analysis, capillary column, separation

Introduction

There are approximately 10,000 polycyclic aromatic hydrocarbon (PAHs) compounds, and the most well-known of those are acenaphthene (ACE), acenaphthylene (ACY), anthracene (ANTH),

benzo[a]anthracene (BaA), benzo[a]pyrene (BaP), benzo[k]fluoranthene (BkF), fluoranthene (FLT), naphthalene (NAPH), and phenanthrene (PHEN). PAHs are colorless, white or pale yellow-green solid organic compounds that contain at least two fused six-sided benzene rings that include only carbon and hydrogen.

Many PAHs are insoluble in water, but a few of them vaporize easily. Although about 10,000 PAHs exist in the environment, the US Environmental Protection Agency (US EPA) recognizes only 16 of them as priority pollutants owing to their carcinogenic properties. These 16 PAHs are NAPH, ACY, ACE, fluorene (FLU), ANTH, FLT, pyrene (PYR), BaA, chrysene (CHR), benzo[b]fluoranthene (BbF), BkF, BaP, dibenzo[a,h]anthracene, benzo[g,h,i]perylene (BghiP), and indeno[1,2,3-cd]pyrene (INPY). PAHs are derived from the incomplete combustion of oil, coal, petrol, wood, garbage, tobacco, meats, and other carbon-containing organic materials. Few PAHs are known for their utilities, for example, NAPH is known for its use as mothballs, and ANTH is used for making dyes, explosives, plastics, lubricants, and moth repellents. The products that contain coal tars contain PAHs at low concentrations as well. The primary sources of PAH emissions are smoke, automobile emissions, and industrial exhausts of petroleum refineries, fossils fuel power plants, and coking plants, paper mills. In addition, naturally occurring fires, such as bush or forest fires, and active volcanoes release PAHs [1].

Methods of determining PAH compounds usually employ chromatographic techniques, such as gas chromatography and liquid chromatography. Research on the analysis of PAHs has been conducted, including the development of extraction methods for different types of samples and assembly of various types of stationary phases [2-5]. Research on analytical methods to detect PAHs is growing, including research to increase sensitivity and find the separation factor of chromatography to detect these compounds. Researchers continue to develop PAH separation methods that are easier and cheaper in practice [6-7]. Moreover, the environmental friendliness of analysis methods has been a topic of increasing focus of late. Since the 1970s, miniaturization of the separation columns used in LC has been undertaken [7-8]. Compared to the conventional column size, that is, 4.0–6.0 mm I.D., capillary columns have a smaller diameter, that is, approximately 0.1–0.8 mm I.D. The use of microcolumns in LC offers advantages such as increased mass sensitivity owing to a decrease in column cross-sectional area; low consumption of solvent, reagent, and packing material; and use of exotic mobile phase and mobile phase additives. Most importantly, micro-column LCs can be directly coupled to mass spectrometry (MS) apparatus, which is the best detector for chromatography. However, decreasing the size of the separation column also decreases its concentration sensitivity owing to the limit of sample injection, which is usually 0.2 L for most injectors. Capillary liquid chromatography is more efficient in terms of chemical usage, and cost is a concern when selecting the method used. In this study, we have attempted to find an alternative method for effective separation of PAHs by using environmentally friendly liquid chromatography.

Materials and Methods

Reagents and materials. Commercial grade PAHs (naphthalene, phenanthrene, anthracene, fluoranthene, pyrene) were obtained from Nacalai Tesque (Kyoto, Japan). Acetonitrile of HPLC grade was obtained from Wako Pure Chemical Industries (Osaka, Japan). Ultra-pure water was obtained from Simplicity UV system (Milipore, Bedford, MA, USA) with an electrical resistivity of $18.2 \text{ M}\Omega \text{ cm}^{-1}$, and it was used in all experiments. Stock solution of PAHs, as well as their diluted mixtures, were prepared in acetonitrile. Acrylamide was purchased from Nacalai (Kyoto, Japan). All solutions were filtered through PTFE $0.45 \mu\text{m}$ membrane filters (GL chromatodisc). Develosil C30-UG was obtained from Nomura Chemical, Seto, Japan. The reagents used for preparing the Sil-S-ImC30 stationary phase were obtained from Nacalai Tesque, except 3-Aminopropyltriethoxylane (APTES), which was purchased from Tokyo Chemical Industry.

Instruments. The capillary LC system used in this work consists of an L.TEX 8301 micro-feeder equipped with a MS-GAN 050 gas-tight syringe (0.5 mL Ito, Fuji, Japan) as a pump, Rheodyne-7520 microinjection valve (Cotati, California, USA) with an injection volume of $0.2 \mu\text{L}$ as an injector, $0.32 \text{ mm i.d} \times 0.45 \text{ mm o.d} \times 100 \text{ mm}$ micro-column, Jasco UV-2075 as UV Detector, and FP920 fluorescence detector (Jasco, Tokyo, Japan). Data were acquired using a CDS ver. 5 data processor (LAssoft, Chiba, Japan).

Single and Mix analysis of PAHs. At the beginning, single analysis of PAHs was conducted using 100% acetonitrile as the mobile phase, C30 UG 5 develosil as the stationary phase, UV detector with a wavelength of 254 nm, flow rate of $4 \mu\text{L}/\text{min}$, and injection volume of $2 \mu\text{L}$. Column size was $0.32 \text{ mm I.D.} \times 100 \text{ mm}$. The concentration of each PAH was set to 10 ppm. The aim of the experiment was to determine the suitability of acetonitrile as a mobile phase for determining of PAHs. In terms of mixing analysis, the condition was the same as that in the single analysis, but different concentrations of water were used in the mobile phase. The acetonitrile:water ratio was varied as 60:40, 70:30, 80:20, and 90:10.

Preparation of Sil-S-ImC30. Octadecylsilica is commonly used as a stationary phase for separating polar and non-polar compounds, but these compounds have weaknesses such as incompatibility with highly mobile aqueous phase and low selectivity toward polar solution. Chemical modification of silica-based packing material by using ionic liquids (ILs) is one approach to solve the above problem. IL-modified silica has been used for the separation of various solutes such as inorganic and organic anions, cations, bases, phenols, PAHs. One of the ILs that

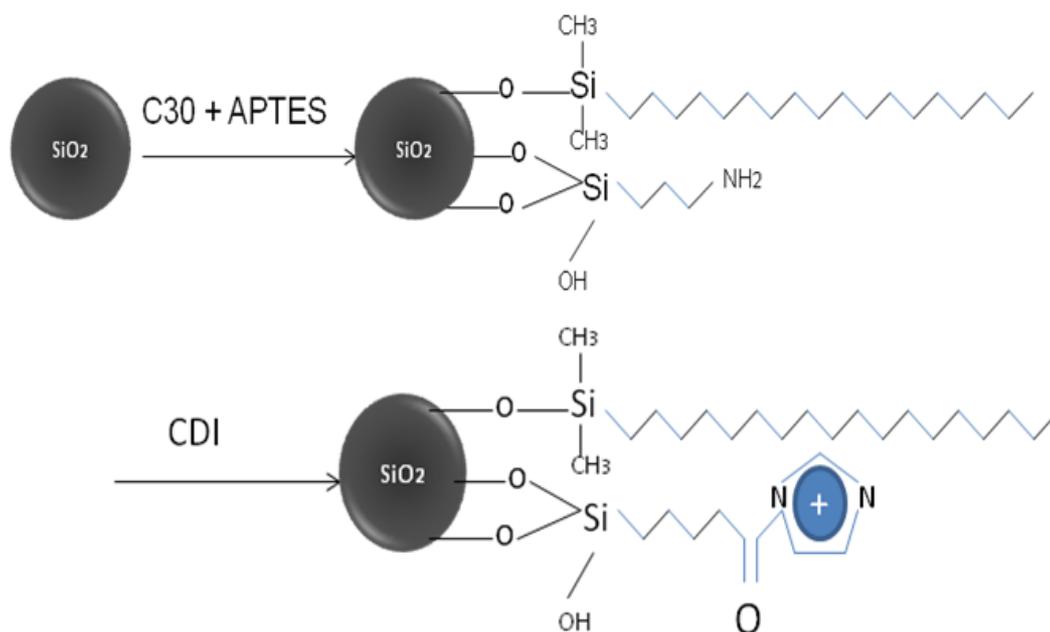


Figure 1. Expected Reaction of Sil-S-ImC30

is usually used as a novel column packing material is imidazolium. Imidazolium-modified silica can interact with PAHs through π - π interaction. π -interactions are a type of non-covalent interactions that involve π systems. As in an electrostatic interaction, where a region of negative charge interacts with a positive charge, the electron-rich π system can interact with a metal (cationic or neutral), an anion, another molecule, and even another π system, as in aromatic-aromatic interactions (π stacking), which involves interactions of aromatic molecules with each other [9-12].

Imidazolium-spaced octadecyl is prepared by co-immobilization of two silane coupling agents (Dimethyl octadecyl chlorosilane and 3-Aminopropyltriethoxysilane) to silica, followed by quaternization of carbonyldiimidazole to form a polar-spaced phase. The multiple interactions arising from the combination of polar groups and lipophilic moieties, such as π - π , hydrogen bonding and hydrophobic interactions, endow SCIL SPs with unique chromatographic performances, such as fine hydrophobic and aromatic selectivity, and recommendable isomer selectivity [13-16].

The reaction procedure (Figure 1) was as follows. 0.2 g Silica, 0.2 g C30, 0.2 mL 3-Aminopropyltriethoxysilane, and 3 ml toluene were mixed homogeneously by using a magnetic stirrer, and the mixture was treated in a stainless tube oven at 110 °C for 20 h. The resultant was washed with dry toluene and dried at 75 °C for 4 h. Thereafter, 0.2 g 1,1 Carbonyl diimidazole was mixed with 3.5 mL toluene, treated in the oven at 80 °C for 24 h, and washed with methanol.

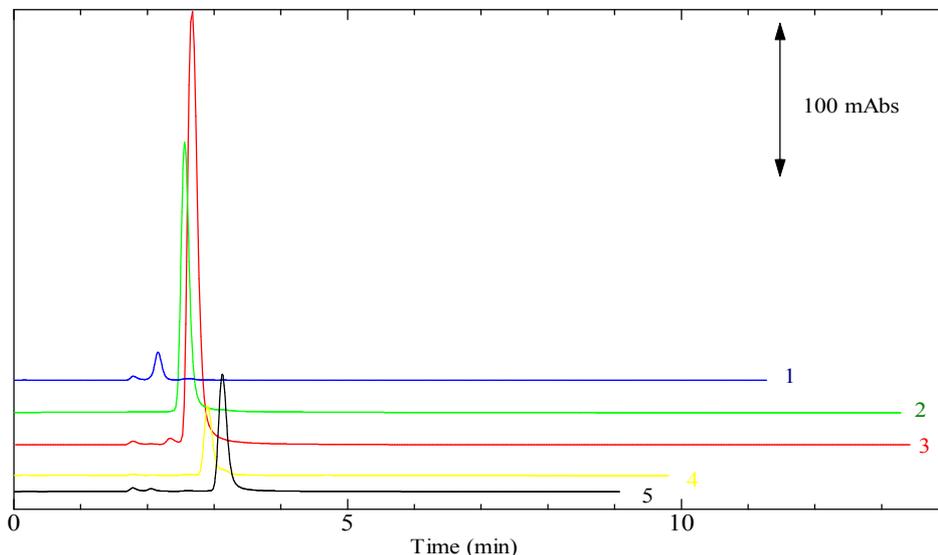
Acrylamide As Quencher. Acrylamide was used as the quencher in this experiment. The acrylamide concentration was set to 0.03 M. Acrylamide solution was added to the mobile phase to the extent of 25% volume of the total mobile phase. The concentration of the mobile phase was varied as 60%, 70%, 80%, and 90%, respectively. The mobile phase comprised acetonitrile and water. The function of acrylamide as a quencher in combination with variation of the mobile phase concentration was to increase sensitivity and degree of separation of each PAH compound when using a fluorescent detector. The excitation and emission wavelengths of the fluorescent detector were 260 nm and 400 nm, respectively. The stationary phase, flow rate, and injection volume used were C30, 4 μ L/min, and 2 μ L, respectively.

Results and Discussion

Effect of acetonitrile concentration on retention time. The results of single analysis using 100% acetonitrile as the mobile phase showed that distinct peaks with different heights and areas of all PAHs appeared. The peak with the greatest height and area was that of anthracene, followed by phenanthrene, pyrene, fluoranthene, and naphthalene. When using 100% acetonitrile as the mobile phase, the retention times of each the PAHs did not differ significantly, with the least being that of naphthalene, followed by phenanthrene, anthracene, fluoranthene, and pyrene. This could be ascribed to the presence of a thin aqueous layer between the stationary phase and the analyte, meaning the analyte could latch on the stationary phase only briefly, and separation would not be good. According to the data

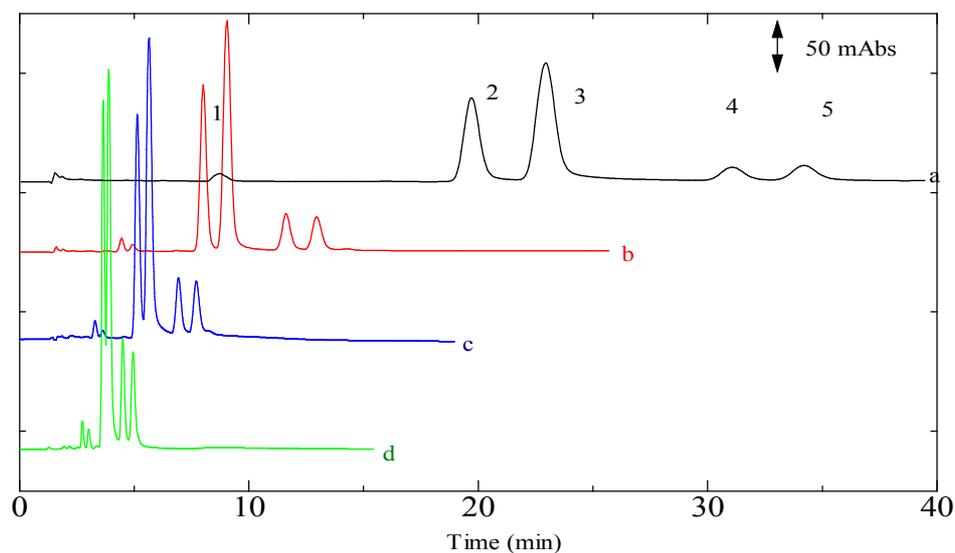
in Figure 2, a greater amount of water must be added to the mobile phase to create a substantial aqueous layer. It is important to determine the percentage of water that must be mixed with acetonitrile.

The result of mixed analysis using various acetonitrile:water concentration ratios showed that increasing the water content of the mobile phase increases the retention time (Figure 3). This is because the proportion



Column : C-30 UG5 Develosil (0.32 mm I.D x 100 mm)
 Eluent : ACN 100%
 Detector : UV 254 nm
 Flow rate : 4 μ L/min
 Injection Vol : 0,2 μ L
 Sample : 1. Naphthalene, 2. Phenanthrene, 3. Anthracene, 4. Fluoranthene, 5. Pyrene (each 10 ppm)

Figure 2. Single Analysis of Each PAH by using 100% Acetonitrile as Mobile Phase



Column : C-30 UG5 Develosil (0.32 mm I.D x 100 mm)
 Eluent : (ACN:Water) a. (60:40), b (70:30), c (80:20), d. (90:10)
 Detector : UV 254 nm
 Flow rate : 4 μ L/min
 Injection Vol : 0,2 μ L
 Sample : 1. Naphthalene, 2. Phenanthrene, 3. Anthracene, 4. Fluoranthene, 5. Pyrene (each 10 ppm)

Figure 3. Mixed Analysis of PAHs using Various Concentrations of Acetonitrile

of water in the mobile phase affects the retention time, as shown in [17-19]. In reversed-phase chromatography, adding water to the organic solvent as the mobile phase could increase the solution polarity, causing the analyte to elute at a slower rate depending on its polarity, but the separation performance would be better.

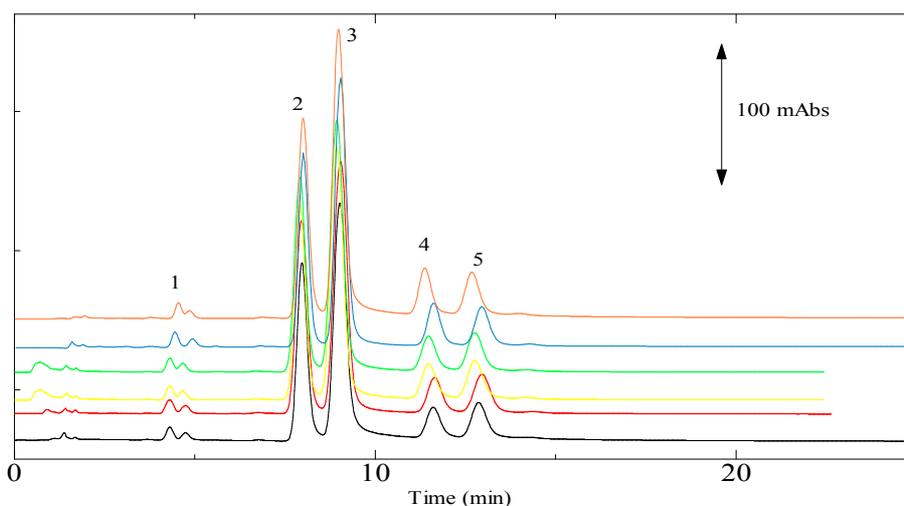
The values of retention and separation are usually describe in terms of capacity/retention factor (k) and relative retention/separation factor (α), respectively. When using an acetonitrile concentration of 90%, the capacity factors of naphthalene, phenanthrene, anthracene, fluoranthene, and pyrene were 0.72, 1.28, 1.43, 1.81, and 2.09, respectively, while the separation factors between each PAH were 2.55, 1.18, 1.38, 1.11, respectively. When using an acetonitrile concentration of 80%, the capacity factors of naphthalene, phenanthrene, anthracene, fluoranthene, and pyrene were 1.06, 2.21, 2.53, 3.33, and 3.81, respectively, and the separation factors between each PAH were 2.25, 1.16, 1.34, 1.14, respectively. When using an acetonitrile concentration of 70%, the capacity factors of naphthalene, phenanthrene, anthracene, fluoranthene, and pyrene were 1.78, 4.00, 4.66, 6.25, and 7.09, respectively, and the while separation factors between each PAH were 2.08, 1.14, 1.32, 1.14, respectively. When using an

acetonitrile concentration of 60%, the capacity factors of naphthalene, phenanthrene, anthracene, fluoranthene, and pyrene were 4.44, 11.31, 13.31, 18.41, and 20.38, respectively, and the separation factors between each PAH were 1.78, 1.11, 1.27, 1.16, respectively. An increase in the water concentration in the mobile phase caused an increase in the capacity factor of each PAH and the separation factor between each pair of PAHs. The data are listed in Table 1.

Analytical Performance The use of LC with a capillary column minimizes the amount of chemical compounds used for analysis. In this study, some conditions were employed to determine the analytical performance of this LC on the analysis of PAHs. The stationary phase used in this study was C-30 UG5 Develosil, which comprised slurry packed in a 0.32 mm \times 100 mm fused silica column, 70% acetonitrile as the mobile phase, UV detector with an excitation wavelength of 254 nm, 4 μ L/min flow rate, 0.2 μ L injection volume. Six replications with a PAH concentration of 10 ppm were conducted to determine the values of precision (%RSD), LOD, and LOQ (Figure 4), while linearity (R^2) was determined using three levels of concentration of 1.5, 5, and 10 ppm (Figure 5).

Table 1. Retention and Separation Factors for Various Acetonitrile Concentrations

Mobile Phase (ACN: Water)	Naphthalene		Phenanthrene		Anthracene		Fluoranthene		Pyrene
	K	α	K	α	K	α	K	α	K
60%	4.44	2.55	11.31	1.18	13.31	1.38	18.41	1.11	20.38
70%	1.78	2.25	4.00	1.16	4.66	1.34	6.25	1.14	7.09
80%	1.06	2.08	2.21	1.14	2.53	1.32	3.33	1.14	3.81
90%	0.72	1.78	1.28	1.11	1.43	1.27	1.81	1.16	2.09



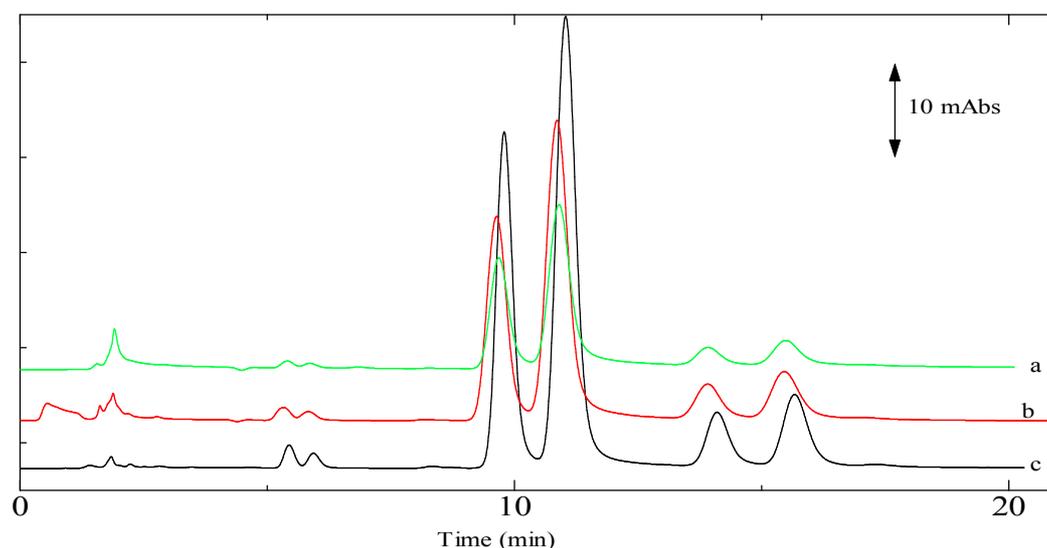
Column : C-30 UG5 Develosil (0.32 mm I.D x 100 mm)
 Eluent : ACN 70 %
 Detector : UV 254 nm
 Flow rate : 4 μ L/min
 Injection Vol : 0,2 μ L
 Sample : 1. Naphthalene, 2. Phenanthrene, 3. Anthracene, 4. Fluoranthene, 5. Pyrene (each 10 ppm)

Figure 4. Chromatogram of Mixed Analysis of PAHs with Six Replications

Table 2 shows that %RSD by retention time of each of the PAHs was in range of 0.54–1.87, while the ideal standard value of %RSD was $\leq 2\%$. Therefore, the % RSD by retention time obtained in this work is within the ideal standard value. Furthermore, the %RSD by area value of naphthalene, phenanthrene, anthracene, fluoranthene, and pyrene are 3.43, 6.34, 6.46, 12.42, and 5.12, respectively. The R^2 values of naphthalene, phenanthrene, anthracene, fluoranthene, and pyrene are 0.8791, 0.9992, 0.9983, 0.9022, and 0.999, respectively. The LOD values of naphthalene, phenanthrene, anthracene, fluoranthene, and pyrene are 4.7, 5.0, 5.4, 15.1, and 4.9, respectively. The LOQ values of naphthalene, phenanthrene, anthracene, fluoranthene, and pyrene are 15.6, 16.6, 17.9, 50.5, and 16.2, respectively. The expected %RSD Horwitz for 10 ppm concentration is 11, but the maximum acceptable %RSD is 22. According to the above data, all %RSD values all PAHs were under

the maximum acceptable value. The standard value of R^2 is 0.95, and the R^2 values of naphthalene and fluoranthene are under the standard value, but those of phenanthrene, anthracene, pyrene are above the standard value.

Separation of PAHs Using Sil-S-ImC30. In two-dimensional chromatography, two types of chromatography system are used. Sil-S-ImC30 has two function both hydrophobic and hydrophilic, so that by employing this column, two retention mechanism would occur. First, the hydrophobic retention mechanism works in the presence of a long carbon chain of C30 from Sil-S-Im30. Then, second, the hydrophilic retention mechanism works in the presence of NH_2 chain on the compound. While imidazole serves two functions of hydrophobic and hydrophilic stationary phase.



Column : C-30 UG5 Develosil (0.32 mm I.D x 100 mm)
 Eluent : ACN 70 %
 Detector : UV 254 nm
 Flow rate : 4 $\mu\text{L}/\text{min}$
 Injection Vol : 0,2 μL
 Sample : 1. Naphthalene, 2. Phenanthrene, 3. Anthracene, 4. Fluoranthene, 5. Pyrene
 Sample Conc : a. 1.25 ppm, b. 5 ppm, c. 10 ppm

Figure 5. Chromatogram of Mixed Analysis of PAHs with Three Levels of Concentration

Table 2. Values of Precision (%RSD), Linearity (R^2), Limit of Detection (LOD), and Limit of Quantification (LOQ)

PAHs	RSD by Retention Time	RSD by Area	R^2	LOD	LOQ
	%				ppb
Naphthalene	1.87	3.43	0.8791	17.1	57.0
Phenanthrene	0.63	6.34	0.9992	0.9	3.1
Anthracene	0.75	6.46	0.9983	0.8	2.6
Fluoranthene	0.54	12.42	0.9022	20.2	67.5
Pyrene	0.62	5.12	0.9990	4.9	16.4

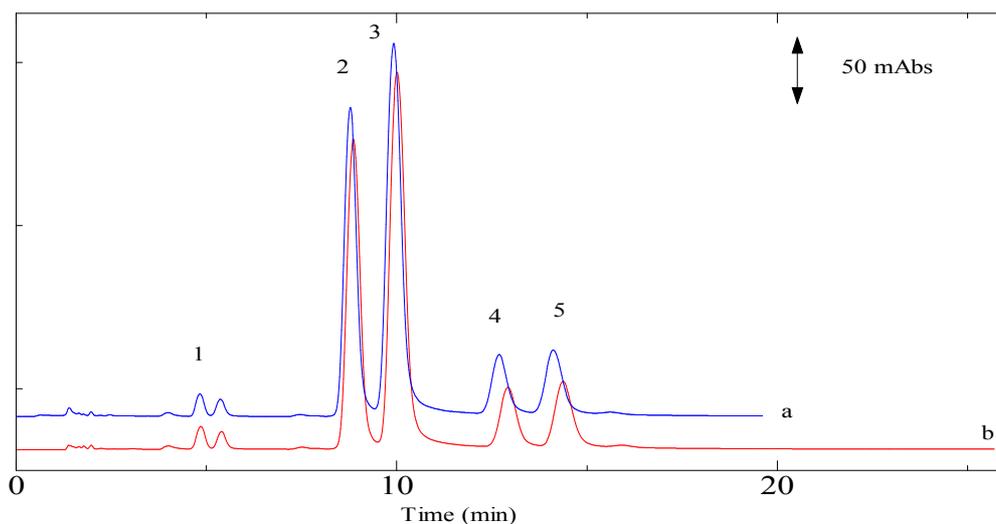
A test mixture of PAHs composed of naphthalene, phenanthrene, anthracene, fluoranthene, pyrene was used in the chromatographic evaluation of silica-spaced imidazole C-30 stationary phase. By regulating acetonitrile contents, excellent separation of the PAH mixture was achieved with acetonitrile–water (70:30, v/v) as the mobile phase. Figure 5 shows symmetric peaks and better resolutions of PAHs. These PAHs have conjugative rings or large electronic clouds, so interactions such as hydrophobic, electrostatic, and π - π interactions, with the stationary phase are strong, resulting in strong retention. Compared to the commercial ODS, it was found that Sil-S-ImC30 shows the same selectivity for the six PAHs.

Given that a few imidazole groups were bound to the carbon chain in the stationary phase molecule, this stationary phase may have a hydrophobic characteristic. PAHs are the best specimens for showing the hydrophobic characteristics of the stationary phase. The retention mechanism of the Sil-S-ImC30 stationary phase was based on the existence of a few hydrophobic and π -to- π interactions in the separation of PAHs.

Table 3 shows the column efficiencies of naphthalene, phenanthrene, anthracene, fluoranthene, and pyrene with the Sil-S-ImC30 column and the C30 column (Figure 6), and the corresponding values are 2042, 1677, 2172, and the corresponding values are 2042, 1677, 2172,

Table 3. Value of Column Efficiency (N), Separation Factor (α), and Retention Factor (k) of Sil-S-ImC30 and C30

PAHs		Value	
		Sil-S-ImC30	C30
Naphthalene	N	2042	2085
	K	16.00	5.39
	α	1.81	1.81
Phenanthrene	N	1677	1716
	K	29.00	9.78
	α	1.14	1.14
Anthracene	N	2172	2216
	K	33.00	11.11
	α	1.28	1.29
Fluoranthene	N	3574	3688
	K	42.33	14.33
	α	1.11	1.11
Pyrene	N	4406	4531
	K	47.00	15.89



Column : a. Sil-ImC30 (0.32 mm I.D x 100 mm), b. C30 (0.32x100mm)
 Eluent : ACN 70 %
 Detector : UV 254 nm
 Flow rate : 4 μ L/min
 Injection Vol : 0.2 μ L
 Sample : 1. Naphthalene, 2. Phenanthrene, 3. Anthracene, 4. Fluoranthene, 5. Pyrene (each 10 ppm)

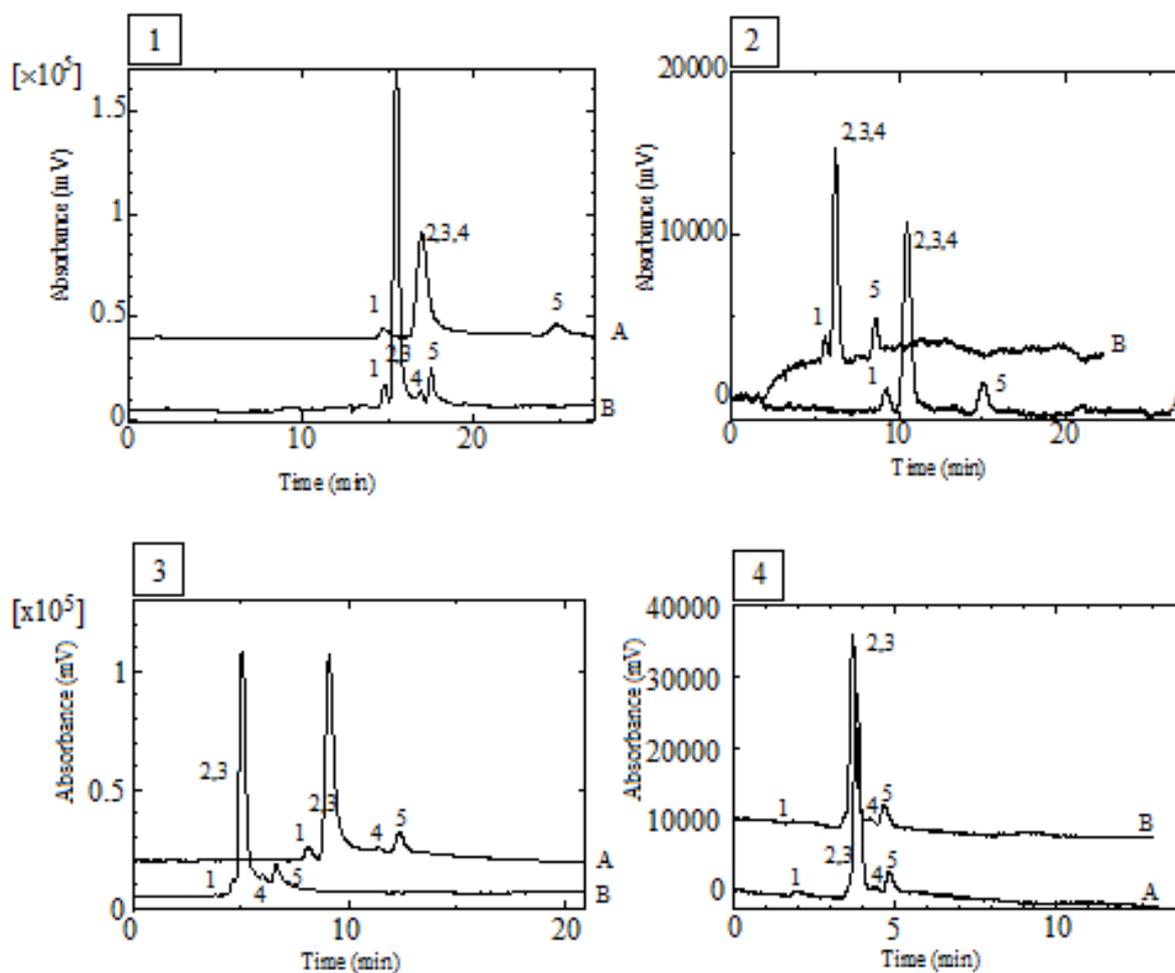
Figure 6. Performance of Sil-s-ImC30 Column

3574, 4406 and 2085, 1716, 2216, 3688, 4531. With increasing conjugative systems of molecules in naphthalene, phenanthrene, anthracene, fluoranthene, and pyrene, the retention factors obtained when using Sil-S-ImC30 increased from 16 to 47 and those obtained when using C30 increased from 5.39 to 15.89 with 70% (v/v) acetonitrile–water as the mobile phase. The separation factors between Sil-S-ImC30 and C30 are almost equal, ranging from 1.11 to 1.81 with a tolerance of about 1-2. Sil-S-ImC30 provided a better retention factor value than C30, but its separation factor and column efficiency are not significantly different from those of C30. C30 is known to be a good stationary phase for non-polar compounds, but Sil-S-ImC30 facilitates multiple interactions by combining polar groups and lipophilic moieties, such as π - π , hydrogen bonding and hydrophobic interactions, which endows SCIL SPs with unique

chromatographic performance, such as fine hydrophobic and aromatic selectivity, and commendable isomer selectivity [13-16]

Effect of Quencher. Quenching is a process that decreases the intensity of fluorescence, and as such, may be the result of a variety of processes. Acrylamide is usually used as quencher in the determination of proteins, and good results have been obtained in a few studies when using acrylamide as a quencher. Acrylamide quenching is very sensitive to the degree of tryptophan accessibility to the solvent containing acrylamide [20].

In this case, it is expected that any contact quenching during the addition of acrylamide would increase sensitivity, meaning detection and separation would be better. In contact quenching, the donor and acceptor molecules



Remarks:

Sample: 1. Naphthalene, 2. Phenanthrene, 3. Anthracene, 4. Fluoranthene, 5. Pyrene (each 10 ppm)

Mobile phase: Chromatogram 1. A. Acetonitrile 60%, B. Acetonitrile 60% + Acrylamide 0.03 M

Chromatogram 2. A. Acetonitrile 70%, B. Acetonitrile 70% + Acrylamide 0.03 M

Chromatogram 3. A. Acetonitrile 80%, B. Acetonitrile 80% + Acrylamide 0.03 M

Chromatogram 4. A. Acetonitrile 90%, B. Acetonitrile 90% + Acrylamide 0.03 M

Figure 7. Effect of Acrylamide with Variation of Mobile Phase Concentration on Separation

interact by means of proton-coupled electron transfer through the formation of hydrogen bonds. In aqueous solutions, electrostatic, steric, and hydrophobic forces control the formation of hydrogen bonds. When the hydrogen-bonded acrylamide-PAHs complex absorbs energy from light, the excited state immediately returns to the ground state without emission of a photon, and the molecules do not emit fluorescent light. A characteristic of contact quenching is a change in the absorption spectra of the two molecules when they form a complex [21]. Using a fluorescent detector and acrylamide as quencher had no impact on the separation and sensitivity in the determination of PAHs. The concentration of the mobile phase impacted the retention time in the determination of PAHs. Theoretically, the quencher must have an impact on detection sensitivity, but any such effect was not observed, probably because acrylamide as a quencher was suspended shortly (Figure 7).

Conclusion

A LC system equipped with a capillary column offers good PAH determination performance. The separation factor increased with increasing water content in the mobile phase. Sil-S-ImC30 provided a good separation factor, while the use of acrylamide as a quencher had no impact on separation.

Acknowledgments

Special gratitude goes to IAARD that has given the opportunity to continue studies and completed this research, and to all those who have provided assistance for the implementation of this research.

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