

## MICRO-ANALYSIS of POLYNUCLEAR AROMATIC HYDROCARBONS in PETROLEUM

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It is well known that petroleum and related oils are largely used in our communities and that these oils contain many kinds of polynuclear aromatic hydrocarbons (PAH) in trace amounts. Some PAH are carcinogenic to experimental animals and are suspected to be carcinogenic to man. In fact, occupational cancer has been observed in various types of worker groups having an occupational contact with petroleum and related products such as fuel oil, lubricating oil, paraffin oil, waxes and so on (1). These indicate the need of a reliable method for determining PAH in petroleum and related oils. We have devised dual band thin-layer chromatography (TLC). This TLC has been proved to be useful as a tool for routine microanalysis of PAH in various samples such as heavy oil (2), kerosene (2), gasoline (3), air-borne particulates (4,5), cigarette smoke (6), asbestos (7), coal tar (8,9), pitch (9) and so on.

This paper describes two analytical methods for analysing PAH in petroleum and related oils. The first method, multiple PAH analysis, is useful for analysing many kinds of PAH. The second method, major component analysis, is useful for determining 5 - 10 PAH which are prevalent in petroleum and related oils.

### 1. Multiple PAH Analysis

Multiple PAH analysis consists of the following 3 procedures; selective extraction of PAH by a series of liquid - liquid partition, separation of the extract into each component by two dimensional dual band TLC, and identification and quantitative determination of the separated compounds by spectrofluorometry.

A known amount of petroleum are dissolved in cyclohexane. PAH in this solution is selectively extracted by a series of liquid - liquid partition of the cyclohexane solution - dimethyl sulfoxide (DMSO), [ DMSO + (1 + 4) Hydrochloric acid, 1:1,v/v] - cyclohexane, 70% sulfuric acid - cyclohexane, and 5% sodium hydroxide aqueous solution - cyclohexane.

At the first partition, aromatic fraction in the sample is extracted to DMSO phase, remaining aliphatic fraction in cyclohexane phase. The aromatic fraction contain PAH, aromatic quinones, acidic and basic aromatic compounds. At the 2nd partition, PAH, aromatic quinones and some of acidic compounds are extracted to cyclohexane phase, and separated from basic aromatic compounds and water soluble acidic compounds. Aromatic quinones and trace amounts of basic aromatic compounds in the cyclohexane phase are removed by the 3rd liquid - liquid partition. Acidic aromatic compounds are removed by the 4th partition. The final cyclohexane solution thus obtained contains PAH selectively. Table 1 shows the partition coefficients for 30 aromatic compounds in these liquid - liquid partitions, and also shows the number of times required for extracting 99% or more of these compounds.

The cyclohexane solution which contains very rich PAH is washed with water. After removing the residual water in the solution by adding small amounts of sodium sulfate anhydride, the solution is dried up at a low temperature ( 40°C) under a reduced pressure. The residue is dissolved in a known amount of benzene (0.5 to 1.0 ml).

PAH in this benzene extract are separated into each component by two dimensional dual band TLC. The TLC plate consists of an aluminum oxide layer ( 4 x 20, cm) and a 26% acetylated cellulose layer (16 x 20,cm). The acetylated cellulose was prepared by acetylation of microcrystalline cellulose for TLC (Avicel SF) using the method of Wieland et al (10). After the application of a few microliters of the benzene extract onto the aluminum oxide layer, the first development is

carried out with n-hexane - ether (19:1,v/v) to the 15 cm mark on the aluminum oxide layer. This is done at about 20% relative humidity, easily achievable by placing a container of saturated aqueous solution of potassium acetate in a developing chamber. Developing time is about 35 min.

PAH on the aluminum oxide layer are then separated into each component on the acetylated cellulose layer by the second development with methanol - ether - water (4:4:1,v/v). It requires about 60 min. for the developer to reach 10 cm from the layer boundary.

PAH separated on the acetylated cellulose layer are detected as small spots by their fluorescence under UV ray (253 & 365 nm). Detection limit is very low and nanogram order of PAH are usually detectable. Most of PAH are stable on the acetylated cellulose layer. Fig. 1 shows two dimensional dual band thin-layer chromatograms of the benzene extracts from a heavy oil C and an ethylene bottom oil. Heavy oil C, which is widely used as a fuel oil, contained 42 PAH and the ethylene bottom oil contained 69 PAH. PAH in each spot can be analysed by spectrofluorometry. At the present time, 10 PAH in the heavy oil C and 21 PAH in the ethylene bottom oil have been identified as shown in Fig.1. Many PAH in the spots on the chromatograms are left for future identification mainly due to the difficulty in getting the reference substances.

This multiple PAH analysis has revealed that various kinds of PAH are contained in petroleum and related oils such as kerosenes (22 - 39 PAH), heavy oils (32 - 42 PAH), paraffin oil (66 PAH) and gasoline (76 PAH).

## 2. Major Component Analysis

It is often necessary to analyse 5 - 10 PAH which are abundant in petroleum and related oils. These PAH can be analysed by the major component analysis. This method differs from the multiple PAH analysis only in separation procedure. That is, a one dimensional dual band TLC is used instead of the two dimensional dual band TLC mentioned above. The one dimensional dual band TLC has the following advantages as compared with the usual one dimensional TLC: 1) It has a higher separation efficiency. 2) Separation efficiency is not affected by the spot size at the origin. 3) Sample solution up to a few milliliters can be tested. 4) Quantitative sample application is easily achievable. 5) Detectability of PAH is very high. Therefore, this method is especially useful for the analysis of a sample which contains very low amounts of PAH.

The dual band TLC plate used consists of a kieselguhr layer (4 x 20,cm) and a 26% acetylated cellulose layer (16 x 20,cm). The former layer is used for the application of a sample and the latter layer for separating the sample. Fig. 2 shows a one dimensional dual band thin-layer chromatogram of PAH in a gasoline. In this case, gasoline sample was applied to the kieselguhr layer without any purification by liquid-liquid partition, because gasoline sample evaporated rapidly leaving PAH and related compounds on the layer. Development was carried out with methanol - ether - water (4:4:1,v/v) until the developer reach 10 cm from the layer boundary. It required about 60 min. PAH and related compounds in a gasoline are usually separated into 21 - 24 rectangular spots on the acetylated cellulose layer.

After extraction with 4 ml of DMSO, PAH in each spot is identified by spectrofluorometry in due consideration of its R<sub>f</sub> value. Spectrofluorometry is useful for the identification of PAH in a spot containing several kinds of PAH and related compounds. For example, PAH in the spot C in the chromatogram in Fig. 2 were easily identified as pyrene and fluoanthene. In this method, 10 PAH are usually identified from a gasoline sample. They are benzo(a)pyrene, chrysene, benzo(b)fluoranthene, benz(a)anthracene, anthanthrene, benzo(k)fluoranthene, perylene, pyrene, fluoanthene, and benzo(ghi)perylene. The first 4 PAH are carcinogen and the last 3 PAH are cocarcinogen.

The identified PAH except benzo(b)fluoranthene can be determined quantitatively by a narrow base line method (6). Benzo(b)fluoranthene can be analysed after complete separation with benzo(a)pyrene by repeat development with methanol

- ether - water (4:4:1,v/v). The narrow base line method has been proved to be very effective in eliminating interference from other PAH and related compounds coexisting in a test solution. For example, pyrene in a solution which also contained fluoranthene, perylene and benzo(e)pyrene was analysed by this method. Observed value of pyrene differed by only 3% from the theoretical values, even when the concentrations of the latter 3 PAH (100 ng/ml) were 5 times higher than pyrene (20 ng/ml). It was also found that observed value of benzo(a)pyrene in a mixed PAH solution differed only 0.8% from the theoretical value, even when the solution contained benzo(b)fluoranthene, chrysene, anthanthrene and dibenzo(a,h)pyrene in 10 times higher concentration than benzo(a)pyrene.

Table 2 shows contents of 9 PAH in regular and premium gasolines as well as coefficient of variation for each PAH obtained in this analysis. The coefficient of variation is low for all PAH analysed. Furthermore, this method is so sensitive that it can analyse PAH quantitatively at a concentration of 1 ng/ml or less.

Another major component analysis is achievable by high speed liquid chromatography instead of one dimensional dual band TLC. A spectrofluorometer is used as a detector of a high speed liquid chromatograph. Fig. 3 demonstrates an example in which a liquid-liquid partition extract from a gasoline is separated and detected at the spectrofluorometric condition for analysing benzo(a)pyrene, benzo(k)fluoranthene, benzo(ghi)perylene and anthracene. The other PAH in the extract can also be analysed in a suitable spectrofluorometric condition. It has been proved that this major component analysis is useful for analysing several kinds of PAH in petroleum and related oils as well as combustion products of these oils.

These analytical methods described in this paper have a wide range of uses, producing reliable data on PAH in petroleum and related oils. These methods will be useful for analysing PAH in various kinds of samples in the environment.

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Table 1. Partition Coefficients of Aromatic Compounds

Substance		A Phase		DMSO		Cyclohexane		Cyclohexane		Cyclohexane	
		B Phase		Cyclohexane		DMSO + (1+4)		70% H <sub>2</sub> SO <sub>4</sub>		5% NaOH	
		K	N	K	N	K	N	K	N	K	N
Hydrocarbon	Anthracene	3.9	3	>100	1	>100	1	>100	1	>100	1
	Phenanthrene	3.9	3	40	2	>100	1	14	2		
	Fluorene	7.9	3	>100	1	>100	1	>100	1		
	Pyrene	4.6	3	>100	1	>100	1	>100	1		
	Chrysene	9.8	2	>100	1	>100	1	>100	1		
	Benzo(a)pyrene	13	2	>100	1	>100	1	>100	1		
	Fluoranthene	5.3	3	>100	1	>100	1	>100	1		
	Benzo(b)fluoranthene	10	2	>100	1	>100	1	>100	1		
	Benzo(ghi)perylene	14	2	66	2	>100	1	>100	1		
	Coronene	14	2	40	2	>100	1	>100	1		
Quinone	Anthraquinone	12	2	49	2	0.77	9	>100	1		
	Benzanthrone	20	2	30	2	0.017	270	>100	1		
	Benz(a)anthraquinone	13	2	>100	1	19	2	>100	1		
	p-Benzoquinone	—	—	0.26	7	0.010	460	0.0035	1300		
	1,2-Naphthoquinone	>100	1	0.13	38	0.052	91	—	—		
	1,4-Naphthoquinone	22	2	2.9	4	0.078	62	0.0015	3100		
	Nicotinic Acid	—	—	0.051	92	—	—	0.027	180		
Acid	o-Hydroxybenzoic Acid	—	—	0.115	43	0.086	56	0.001	4600		
	Terephthalic Acid	—	—	0.032	150	—	—	0.031	150		
	Phenazine	4.0	3	4.6	3	—	—	14.5	2		
Base	Carbazole	>100	1	0.83	8	0.14	36	>100	1		
	Lepidine	6.2	3	0.047	100	—	—	—	—		
	Benzo(h)quinoline	7.0	3	0.019	240	—	—	—	—		
	Benzo(f)quinoline	12	2	<0.001	>4600	—	—	—	—		
	Acridine	8.8	3	<0.001	>4600	0.016	290	—	—		
	1-Naphthylamine	55	2	0.007	660	0.001	4600	—	—		
	2-Aminoanthracene	>100	1	0.077	63	—	—	—	—		
	2-Naphthylamine	>100	1	<0.001	>4600	—	—	—	—		
	2-Aminofluorene	>100	1	<0.001	>4600	—	—	—	—		
	2-Aminochrysene	>100	1	0.001	4600	0.055	87	—	—		

K: Partition Coefficient (Concentration in A/Concentration in B)

N: Number of times required for extracting 99% or more of a compound from B phase to A phase

Table 2. Contents of polynuclear aromatic hydrocarbons in gasoline

Sample No.	Pyrene Mean C.V.	Fluoranthene Mean C.V.	Chrysene Mean C.V.	Benz(a)-anthracene Mean C.V.	Benzo(a)-pyrene Mean C.V.	Benzo(k)fluoranthene Mean C.V.	Perylene Mean C.V.	Anthracene Mean C.V.	Benzo(ghi)perylene Mean C.V.
1	2.9*	1.4*	0.24	0.20	0.05*	0.04	0.02	0.02	0.25
2	5.8*	2.2*	0.75	0.40	0.18*	0.09	0.06	0.04	0.74
3	14.6*	2.6*	0.58	0.20	0.16*	0.02	0.01	0.04	1.09
4	4.8	1.3	0.38	0.28	0.19**	0.05	0.02	0.05**	0.52
5	11.4	2.8	0.93	0.64	0.32	0.09	0.06	0.05	1.07
6	2.5	1.0	0.34	0.22	0.12	0.06	0.03	0.03	0.28
7	16.4	0.6	0.56	0.25	0.22	0.03	0.02	0.05	1.36
8	1.3	2.3	0.11	0.10	0.05	0.03	0.01	0.02	0.13
1	4.6*	1.8*	0.54	0.34	0.06*	0.07	0.03	0.03	0.38
2	10.6*	4.1*	1.07	0.46	0.25*	0.11	0.08	0.08	0.80
3	17.1*	2.9*	0.79	0.26	0.17*	0.02	0.01	0.04	1.65
4	3.4	3.5	0.45	0.39	0.15*	0.04	0.01	0.03**	0.39
5	16.9	1.7	1.31	1.14	0.50	0.15	0.10	0.11	1.22
6	3.4	0.5	0.50	0.29	0.18	0.09	0.03	0.05	0.43
7	20.0	0.8	0.86	0.35	0.35	0.04	0.02	0.09	2.00
8	14.3	3.7	1.28	1.00	0.56	0.23	0.11	0.22	1.64

Mean of 4 determinations, \* Mean of 3 determinations, \*\* Mean of 7 determinations,

C.V.: Coefficient of variation (%)

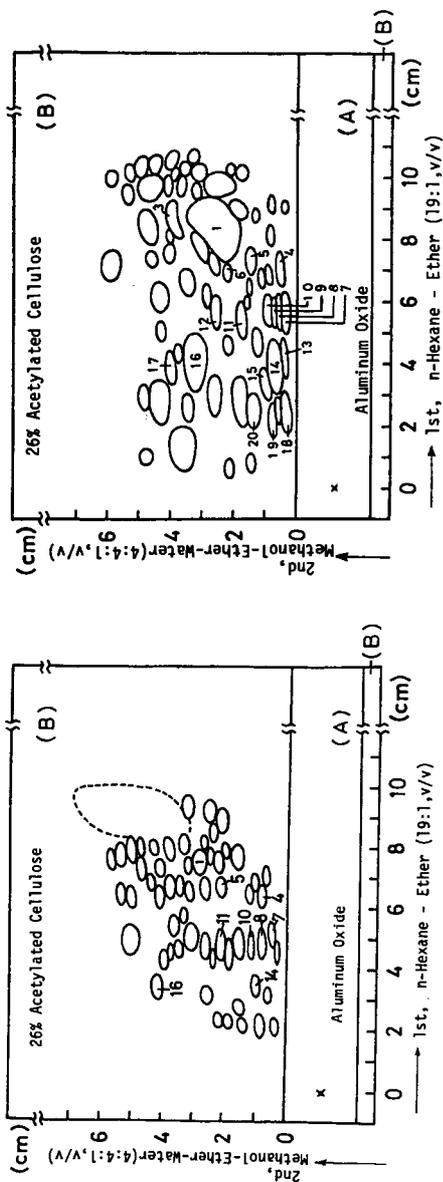


Fig. 1. Two dimensional dual band thin-layer chromatograms of the benzene extracts from heavy oil C (left) and ethylene bottom oil (right)

Identified polynuclear aromatic hydrocarbons and their spot numbers: pyrene and fluoranthene (spot 1), methyl derivatives of pyrene and fluoranthene (3), chrysene (4), benz(a)anthracene (5), benzo(b)fluorene (6), benzo(a)pyrene (7), benzo(b)fluoranthene (8), benzo(j)fluoranthene (9), benzo(k)fluoranthene (10), perylene (11), benzo(e)pyrene (12), anthanthrene (13), indeno(1,2,3-cd)pyrene (14), benzo(b)chrysene (15), benzo(ghi)perylene (16), coronene (17), dibenzo(a,h)pyrene (18), dibenzo(a,i)pyrene (19), and tribenzo(a,e,i)pyrene (20).

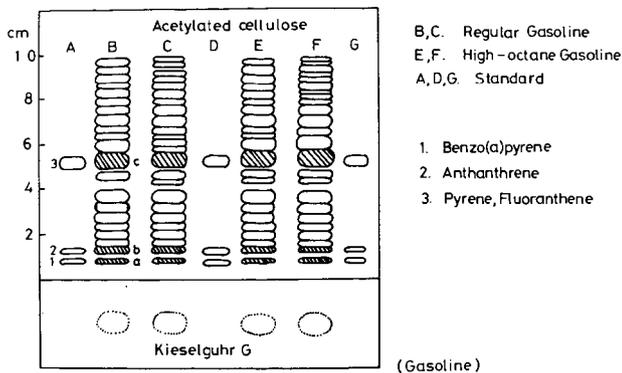


Fig. 2. One dimensional dual band thin-layer chromatogram of gasoline

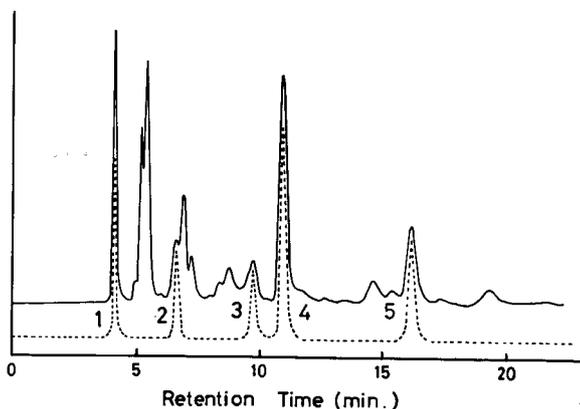


Fig. 3. High speed liquid chromatograms of the liquid-liquid partition extract from gasoline (—) and standard solution having 16 PAH

Column: Zorbax ODS (4.6mm x 25cm), Mobile phase: Methanol-Water (85:15,v/v), Detector: Spectrofluorometer (excitation 370nm, emission 410nm), 65°C, 1100psi.  
1. anthracene, 2. 9-phenylanthracene, 3. benzo(k)fluoranthene, 4. benzo(a)pyrene, 5. benzo(ghi)perylene  
Standard solution is a mixture of 1 - 5 PAH, naphthalene, fluorene, 3,4-benzofluorene, fluoranthene, chrysene, 7-ethylchrysene, benz(a)anthracene, 7-methylbenz(a)anthracene, perylene, benzo(b)fluoranthene and benzo(e)pyrene.