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〈論 文〉

Determination of Polycyclic Aromatic Hydrocarbons in Chimney Soot Extract by High-Resolution Gas Chromatography

고분리능 기체크로마토그라피에 의한 굴뚝재추출물안의 다환방향족 탄화수소의 정량에 관한 연구

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국 문 초 록

대기오염물질 속에 함유되어 있는 다환방향족 탄화수소(PAHs)는 분자식 구조가 비슷한 수십개의 이성질체가 여러 종류의 유기화합물과 혼합되어 존재한다.

본 연구에서는 환경오염 시료중의 PAHs를 분석하는데 있어서 분석방법과 결과를 비교하기 위하여 지침이 될 수 있는 환경표준 기준물을 개발할 목적으로 굴뚝 안쪽벽에서 긁어 채취한 검댕을 시료로 선택하여 액체/액체 용매 추출방법에 의해 PAHs 분류부분을 얻었다. Phenanthrene 이외의 30여종의 PAHs화합물을 가스크로마토그라피의 머무른시간과 가스크로마토그라피/질량분석기에 의하여 분리, 판명하였고 5종의 주 PAHs 화합물을 정확하게 정량 분석하였다. 정량분석 결과의 신뢰도, 정확도, 정밀도는 미국 NBS의 표준기준물 1647을 분석하여 검정치와 비교함으로써 평가하였다.

INTRODUCTION

Numerous polynuclear aromatic hydrocarbons (PAHs) have been reported as potential health hazard materials. They are generally found in various sources such as atmosphere[1], coal soot [2], coal tar[3], mineral oil used to lubricate the machine[4] and even charcoal-broiled food [5-6] with an enormous complexity of their structure. Because particular PAHs among hundreds of PAHs have been closely related to carcinogenic and/or mutagenic properties, there exists a need for efficiently separating, identifying and quantifying an accurate amount of these compounds.

When one wants to analyze PAHs in complex combustion-related materials, one should compare his analytical method and results with those from previously analyzed standard material having similar matrices. The object of this research was to develope an analytical measurement method and standard reference materials (SRMs) for PAH measurement which would be readly available to environmental analysts in Korea. As a preliminary step for development of PAH SRMs, chimney soot was chosen since it was expected that lots of PAHs exist in chimney soot. According to Henry's report[7] a mean annual death rate for chimney sweepers was

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755 per 1,000,000, compared to 4.2 per 1,000,000 for the population as a whole.

High performance liquid chromatography (HPLC) is the most popular analytical method for studying PAHs. For the determination of PAH in air particulate, diesel exhaust particulate and coal tar, HPLC with ultraviolet (UV) and fluorescence detection system [8-15], capillary gas chromatography (GC) with flame ionization and gas chromatography/mass spectrometry (GC/MS) system [14-16] have been reported. For the purpose of developing SRM and an alalytical method which can be easily available in Korea, HPLC with fluorescence detection system is, however, less favorable than GC is more widely used here. Some PAH isomers are not separable on GC, but capillary GC combined with flame ionization detector provides an excellent resolution and reproducibility.

In this study, firstly a pure solution of PAH SRM issued by the National Bureau of Standard (NBS), U.S.A.[17] was rigorously analyzed with LC and GC and then our accuracy of PAH measurement was evaluated by comparing our results with the NBS certified value. Secondly, a chimney soot chosen as a future SRM was separated by a easily available liquid-liquid partitioning method, analyzed by LC, GC, and GC/MS. Accurate quantification was performed by GC.

MATERIAL AND METHODS

The standard PAHS were purchased from Supelco. Co. (Supelco park, PA, U.S.A), Aldrich Chemical Co. (Milwaukee, WI, U.S.A) and Fluka Co. (Buch, Switzerland). The solvents used for extraction were distilled in glass from Burdick & Jackson Lab. (Muskegon, MI, U.S.A.) and the HPLC solvents were HPLC grade from J.T. Baker Chemical Co. (Philipsburg, NJ, U.S.A.). Chimney soot chosen as a preliminary material for developing an environmental SRM was collected by scrubbing the inside of chimney wall of power plant, dried in an ambient air for a week, thoroughly mixed in a ball mixer and bottled. Randomly selected four bottles were used for analytical measurement.

The accurate amount of soot wrapped in a prepurified glass wool was soxhlet extracted with 125 mL of dichloromethane for 8h, filtered with a preextracted filter paper and concentrated to 50 mL by a rotary evaporation. The general scheme for the separation of PAHs in soot extract is shown in Fig. 1. The neutral PAHs were obtained by eliminating an acidic and a basic fraction through acid-base wash steps and then further separated according to their polarities using 10% aqueous dimethyl formamide and cyclohexane. The final 60 mL of cyclohexane fraction was concentrated to 3 mL by a kuderna-danish concentrator, and then further concentrated to 0.5 mL by nitrogen. For the quantification of selected PAHs present in soot an internal standard solution of benzo(b)fluoranthene was added to the soot samples prior to extraction. The internal standard solution was prepared in acetonitrile and added to soot sample.

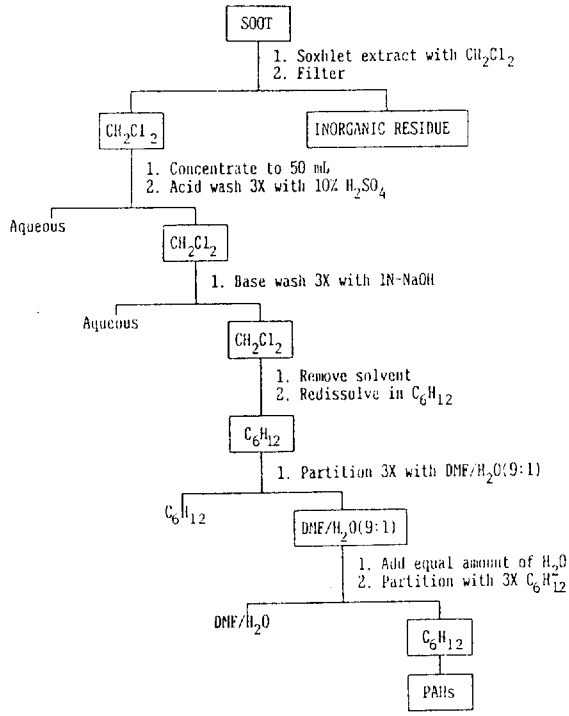

Fig. 1. Scheme for the separation and the fractionation of PAHs in chimney soot.

HPLC analysis

HPLC separation was achieved using a Waters liquid chromatographic system with a variable wavelength UV detector (Model 450), a fluorescence detector Model (420) and a digital integrator (Data Module M730).

Reverse phase PAH columns (5μm, 250mm x 4mm I.D.; Waters Associates, Milford, MA and Supelcosil Co., Supelco park, PA, U.S.A.) were used. The samples were injected by a Waters injector (Model U6K) with a 10μL sample loop. A gradient solvent program was employed for the separation of a standard mixture, NBS SRM 1647. The program consisted of 30% - 60% aqueous acetonitrile, programmed to 100% acetonitrile in 30 min using a flow rate of 1 mL/min. Triphenylene was added to the calibration solutions as an internal standard. The UV and fluorescence detector responses of each compound were determined individually.

GC analysis

The gas chromatographic analysis was carried out on a Hewlett-Packard 5880A GC-Flame Ionization detector (FID) using a 25m x 0.25mm i.d. fused silica capillary column coated with a 0.25 μm film of SE-54 (Hewlett-Packard, Palo Alto, CA, U.S.A.) The following GC conditions were used for analysis: injector and flame ionization detector temperature, 250°C and 310°C, respectively; oven temperature program, 100°C for 2 min, then linear temperature program at 8°C/min to 290°C then hold for 4 min. The GC had a spilt flow injector port and helium was used as the carrier gas with a head pressure of 16 psi. Calibration solutions were prepared for the quantification of 5 identified PHAs in soot. Response factors of those PAHs were determined by using benzo(b) fluoranthene as an internal standard.

Quantitative GC analysis of NBS SRM 1647 in validating measurement technique of our lab was performed by using the same conditions as mentioned above except that another calibration solutions which contains 11 PAHs were employed to determine response factors.

GC/MS analysis.

GC/MS analysis was performed on a Hewlett Packard 5890 gas chromatograph interfaced to a HP 5988 quadrupole mass spectrometer with a HP 1000 data system containing 40,000 compounds' library system. The ion source temperature was 200°C and GC outlet was directly connected to an ion source through an interface. The interface temperature was maintained at 250°C. The mass spectrometer was operated in the electron impact mode and was scanned repetitively from 40 to 350 amu at a rate of 1.38 scans per second. The GC conditions were the same as in GC analysis with the exception of column was a 25m x 0.32mm i.d. fused silica capillary column coated with a 0.17μm of film of ultra 2 (Hewlett-Packard, Palo Alto, CA, U.S.A.).

RESULTS AND DISCUSSION

It was very difficult to perform complete analysis of environmental samples for PAHs by GC or LC alone because of sample complexity. For complex environmental samples to be accurately analyzed they should be simplified into less complex subsamples by separating compounds into different classes based on their physical and chemical properties prior to GC/MS analysis. In order to obtain a neutral fraction from a chimney soot, the partitioning method described in Bjorseth (1977)[18] was used with a slight modification. May and Wise (1984) also employed this technique to collect the neutral fraction of air particulate and they further fractionated the neutral fraction on the normal phase LC and analyzed each fraction by reversed phase LC with UV or fluorescence detector. However, since it was expected that chimney soot samples may have much less complexity than air particulate extracts due to the fact that many portion of material from combustion are already exhausted to the atmosphere, the neutral PAH fraction was directly analyzed on GC and GC/MS.

Table I. Identified PAH compounds in chimney soot extract.

Peak No.	Compound	Mol wt.	Retention time, min	Method of identification*
1	naphthalene	128	4.125	a.b.c
2	fluorene	166	10.091	a.b.c
3	methylfluorene	180	11.695	a.b.c
4	dibenzothiophene	184	11.967	a.b
5	phenanthrene	178	12.398	a.b.c
6	methylphenanthrene	198	13.665	a.b.c
7	phenanthrenedione	208	14.690	a.b.c
8	anthracenedione	208	14.797	a.b.c
9	2-phenylnaphthalene	204	14.911	a.b
10		198,154	15.265	a.b
11	phenylnaphthalene	204	15.652	a.b
12	fluoranthene	202	16.061	a.b.c
13	phenanthro [4,5-bcd] thiophene	208	16.382	a.b
14		222	16.432	a.b
15	pyrene	202	16.602	a.b.c
16	benzo (a) fluorene	216	17.486	a.b.c
17	methlpyrene isomer	216	17.714	a.b
18	methlpyrene isomer	216	17.782	a.b
19	dimethylpyrene	230	19.210	a.b
20	benzo (b) naphtol [1,2,-d] thiophene	234	19.552	a.b
21	4H-cyclopental [cd] pyrene	226	19.758	a.b
22		234,230	19.590	a.b
23	benz(a) anthracene	228	20.303	a.b.c
24	chrysene/triphenylene	228	20.441	a.b.c
25	benzo (b or k) fluoranthene	252	23.621	a.b
26	benzo (a) pyrene	252	24.187	a.b.c
27		254	25.798	a.b
28	benzo (ghi) peryene	276	26.926	a.b.c
29	indeno (1,2,3-cd) pyrene	276	27.157	a.b.c
30		276	27.809	a.b

* (a) Identified by molecular weight from mass spectrum
(b) Identified by NBS library search
(c) identified by GC retention time and mass spectrum of authentic reference standard

Table I lists the compounds identified or tentatively identified in the neutral fraction of chimney soot. Approximately 30 compounds were identified, primarily based on GC retention behavior of pure standards and GC/MS data. Capillary gas chromatography sufficiently separated the neutral fraction, but the neutral fraction itself was not free of interferences from homologues of aliphatic hydro-

carbons. However, as mass spectra of aliphatic and aromatic hydrocarbon were clearly distinguished from each other, many PAHs from naphthalene to benzo (ghi) perylene could be detected and identified. Several structure isomers eluted on the similar retention time were not tried to identify rigorously because gas chromatography and mass spectrometry do not give assured informations about structure isomers. The GC analysis of soot extract is illustrated in Fig. 2.

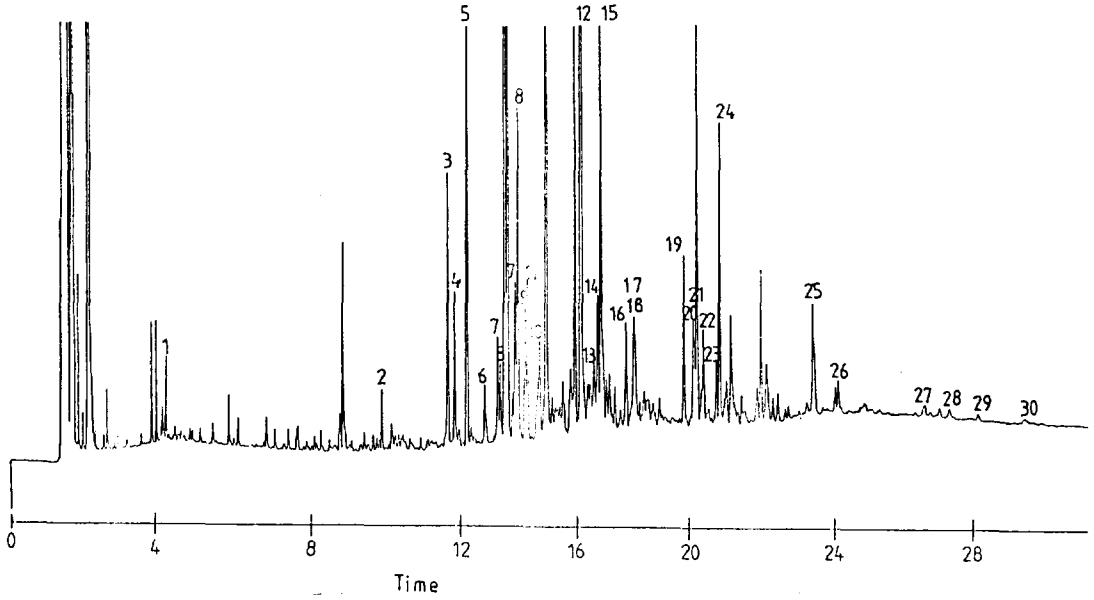

Fig. 2. Gas chromatogram of PAHs fraction of chimney soot extract. peaks as numbered in Table I. GC conditions are in the text.

Quantification of selected PAH

For quantification of selected PAH, a validation test based upon the accuracy and precision of PAH measurement was initiated in our laboratory. The NBS SRM 1647 which is a simple calibration solution of the 16 priority pollutant PAH was chosen and its certified values were compared with our results. Ten of sixteen priority pollutant PAHs were measured by an internal standard calibration method. A known amount of triphenylene as an internal standard was added to the NBS SRM and to the four different concentration of calibration solutions for HPLC-UV analysis. At least three injections were made for each sample and the averaged peak area or height was used in order to derive ratios of PAH against to the internal standard. The results from HPLC-UV analysis are presented in The measurement results in our laboratory were very close to the NBS values despite the fact that different columns and different instruments were used. Concentration of nine compounds among ten compounds were within a 95% confidence limits of NBS results. Benzo(a)pyrene was the only compound whose measured concentration was slightly higher than that of NBS. This may be due to several reasons, including unstable instrument response against benzo(a)pyrene, weighing error or an interference peak eluted on the time which is very closed to the retention time of benzo(a)pyrene.

Assuming that an amount of fluoranthene in NBS SRM 1647 was an absolute value, it was used as an internal standard for GC analysis, since labeled PAH or other PAH compound such as alkylated PAH which were used as internal standards in NBS could not be purchased at the time of an experi-

Table II. Concentration of PAH in NBS SRM 1647 measured by HPLC-UV

Peak No.	Compound	Concentration (μg/mL) Our Value[a]	RSD[b]	NBS value[c]	RSD
1	naphthalene	22.6±0.25	1.2%	22.4±0.5	2.2%
2	acenaphthylene	19.8±0.36	1.9	19.2±0.5	2.6
3	acenaphthene	–		21.2±0.4	1.9
4	fluorene	–		4.96±0.18	3.6
5	phenanthrene	4.98±0.17	3.4	5.12±0.18	3.5
6	anthracene	3.18±0.19	6.0	3.33±0.10	3.0
7	fluoranthene	9.35±1.26	13.4	10.3±0.5	4.9
8	pyrene	9.45±1.02	10.8	9.85±0.58	5.9
9	benz(a) anthracene	4.90±0.19	3.7	5.12±0.14	2.7
10	chrysene	5.16±0.17	3.2	4.69±0.15	3.2
11	benzo(b) fluoranthene	–		5.13±0.21	4.1
12	benzo(k) fluoranthene	–		5.06±0.15	3.0
13	benzo(a) pyrene	5.66±0.15	2.6	5.32±0.13	2.4
14	dibenz(a,h) anthracene	4.50±0.39	8.6	4.09±0.30	7.3
15	indeno (1,2,3-cd) pyrene	–		3.73±0.12	3.2
16	benzo (ghi) perylene	–		4.11±0.15	3.6
	average RSD		4.2%		3.6%

a. Results produced from our laboratory

b. RSD was calculated from triplicate injections. Uncertainty is ±1 standard deviation of the mean.

c. NBS LC result which is specified in the certification of NBS SRM 1647

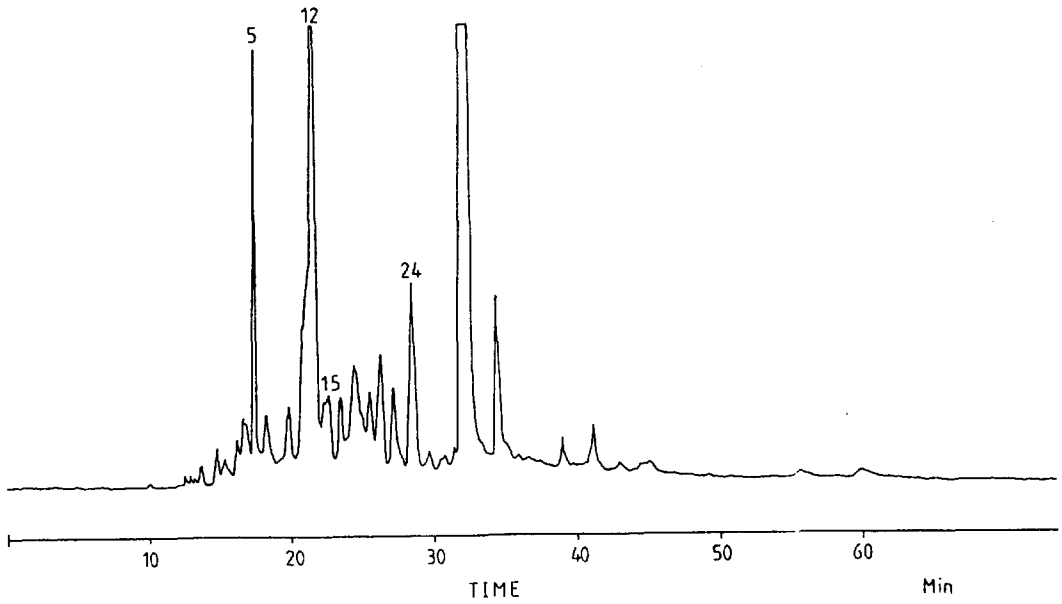

Fig. 3. Liquid chromatogram of PAHs fraction of chimney soot extract. (Fluorescence detection at excitation 254 nm, emission 375 nm). peaks as numbered in Table I. LC conditions are in the text.

ment. Calibration solutions for only GC analysis were reprepared and six injections were made for each sample. Calculation procedure was the same as described in LC analysis.

The results of the GC analysis are presented in Table III. Comparing our GC results with the NBS results[17], concentration of all compounds with the exception of benzo(a)pyrene were within a confidence limit of certified NBS values. NBS. Our higher concentration of benzo(a)pyrene compared with the NBS value probably came from a weighing error during the preparation of standard solution because GC-FID response of benzo(a)pyrene was stable and there was no interference peak. Reproducibility of our GC results (average RSD; 2.9%) was also as good as NBS (average RSD ~ 3%). Therefore, we concluded that our measurement technique was validated, so that we may produce our SRMs relating to PAHs in our laboratory.

In the procedure of quantification of selected PAH in soot extracts such as phenanthrene, fluoranthene, pyrene, benz(a)anthracene, chrysene/triphenylene and benzo(a)pyrene, a known amount of benzo(b)fluoranthene was spiked to the soot sample prior to extraction since benzo(b)fluoranthene was rarely present in soot according to preliminary analysis result. In the direct analysis of neutral fraction of soot extract by LC fluorescence, it was not possible to accurately quantify all PAHs identified due to the other compounds which are eluted on

Table III. Concentration of PAH in NBS SRM 1647 measured by GC-FID

Peak No.	Compound	Concentration (μg/mL)		
		Our value[a]	RSD[b]	NBS value[c]
1	naphthalene	22.32±0.14	6.4%	22.5±0.2
2	acenaphthylene	19.44±0.56	1.8	19.1±0.2
3	acenaphthene	20.34±0.53	2.6	21.0±0.4
4	fluorene	5.13±0.3	0.7	4.92±0.10
5	phenanthrene	4.12±0.12	2.3	5.06±0.10
6	anthracene	–	1.4	3.29±0.10
7	fluoranthene	–		10.1±0.2
8	pyrene	10.01±0.12	1.2	9.84±0.10
9	benz(a) anthracene	4.85±0.2	0.6	4.97±0.06
10	chrysene	5.29±0.18	3.6	4.68±0.06
11	benzo(b) fluoranthene	–		5.09±0.06
12	benzo(k) fluoranthene	–		4.99±0.10
13	benzo(a) pyrene	6.24±0.14	2.7	5.31±0.19
14	benzo(ghi) perylene	–		4.02±0.06
15	dibenz (a,h) anthracene	3.62±0.31	8.7	3.63±0.07
16	indeno (1,2,3-cd) pyrene	–		3.63±0.07
	average RSD		2.9%	

a. Result produced from our laboratory

b. RSD was calculated from six injections. Uncertainty is expressed as ±1 standard deviation of an average result from six injections.

c. NBS GC result which is specified in the certification of NBS SRM 1647

the same or the very close retention time and an unstable baseline. Since capillary GC produced a much better chromatogram in terms of resolution and baseline, only GC analysis was employed to quantify five PAHs. The results of PAH concentration obtained from quadruplicate analysis are shown in Table IV. Only five PAH compounds could be accurately quantified at this moment. This soot material as a standard reference material to assist in validating the accuracy of PAH measurement will be available after an enough stability test period.

Table IV. Concentration of selected PAHs in soot extract by GC

Compound	Concentration ($\mu g/g$)[a]				
	Sample 1[b]	Sample 2	Sample 3	Sample 4	Average
phenanthrene	11.7±1.1[c]	12.8±0.6	12.1±0.4	12.2±0.8	12.2±0.4
fluoranthene	19.8±2.0	20.3±1.1	19.9±1.0	20.0±1.4	20.0±0.2
pyrene	9.94±0.9	10.2±0.7	10.2±0.6	10.1±0.6	10.1±0.1
chrysene/ triphenylene[d]	5.49±0.3	5.35±0.2	5.29±0.2	5.18±0.07	5.33±0.1
benzo(a) pyrene	1.37±0.1	1.23±0.04	1.55±0.04	1.45±0.12	1.40±0.1

a. Each concentration was obtanied from the result of 6 injection.
b. Sample number means quadruplicate extractions
c. Uncertainty is ±1 standard deviation.
d. The values are the concentration of chrysene and triphenylene combined, because they are not separated on GC.

CONCLUSION

NBS SRM 1647 which is a simple solution of PAHs was throughly analyzed by liquid and capillary gas chromatography and compared data obtained from our lab with NBS certified values. Accuracy and precision of PAH measurement were validated. Preliminary SRM, a chimney soot, was analyzed by LC, GC, and GC/MS. Five PAH compounds such as phenanthrene, fluoranthene, pyrene, chrysene/triphenylene and benzo(a)pyrene was precisely quantified for the purpose of developing SRMs. This soot material will be able to available after an accomplishment of enough stability test.

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