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Citation for published version (APA):

Claessens, H. A., Rhemrev, M. M., Wevers, J. P., Janssen, A. A. J., & Brasser, L. J. (1991). Comparison of extraction methods for the determination of polycyclic aromatic hydrocarbons in soot samples. *Chromatographia*, 31(11-12), 569-574. <https://doi.org/10.1007/BF02279477>

DOI:

[10.1007/BF02279477](https://doi.org/10.1007/BF02279477)

Document status and date:

Published: 01/01/1991

Document Version:

Publisher's PDF, also known as Version of Record (includes final page, issue and volume numbers)

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
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Comparison of Extraction Methods for the Determination of Polycyclic Aromatic Hydrocarbons in Soot Samples

H. A. Claessens^{1*} / M. M. Rhemrev² / J. P. Wevers¹ / A. A. J. Janssen¹ / L. J. Brassier¹

¹Eindhoven University of Technology, Dept. of Chem. Engineering, Lab. Instrum. Analysis, P.O. Box 513, 5600 MB Eindhoven, The Netherlands

²TNO Environmental and Energy Research, Department of Analytical Chemistry, P.O. Box 217, 2600 AE Delft, The Netherlands

Key Words

Column liquid chromatography
Gas chromatography
Polycyclic aromatic hydrocarbons
Soot and air samples
Liquid CO₂ extraction

Summary

The results of the analysis of polycyclic aromatic hydrocarbons (PAH) in soot and similar types of sample are often dependent on the extraction method applied, owing to carbonaceous substances present in these samples.

The recoveries of three extraction methods, an ultrasonic ether, a Soxhlet toluene and a Soxhlet extraction with liquid CO₂ were compared for a typical soot sample.

Additionally the CO₂ extraction was optimized with respect to the necessary number of transfers during the extraction and the proper method to remove the CO₂ after extraction.

Finally, the liquid chromatographic pretreatment of the extract, prior to gas chromatographic analyses of the PAH, was improved.

Introduction

Industrial processes, traffic and many other human activities contribute to the pollution of the environment. To study the impact of these activities reliable analytical procedures for the determination of harmful components should be available.

From an analytical point of view the main problem very often lies in the proper isolation of the components of interest from a complex sample prior to the final chromatographic analysis. In many cases the sample pretreatment procedure is of crucial importance to achieve reliable qualitative and quantitative results. This is also the case for the determination of polycyclic aromatic hydrocarbons (PAH) in smoke and similar samples. PAH, of which a

number have mutagenic and carcinogenic properties, are associated in smoke with aerosol particles up to 90 % of which are in the respirable range [1-6].

Before PAH and other components in smoke aerosols can be analysed, sample pretreatment must be applied to isolate and preconcentrate these components. Such a pretreatment method should meet the following conditions: selectivity for the components of interest, high efficiency in short time, no degradation of the sample and no losses during the preconcentration step.

Many reliable isolation procedures, like Soxhlet, ultrasonic extraction and vacuum sublimation techniques for PAH from several types of sample have been described [4, 7-11]. Problems arise when the sample matrix is complex and strongly absorbs the components of interest like, for example, PAH in carbonaceous samples.

In earlier studies it was shown that large differences in recovery of PAH could be observed between different extraction procedures applied to a specific sample matrix [4, 9, 12]. In a number of cases the same is true when one typical extraction procedure is applied to a sample. It was also shown that the recovery of spiked PAH material on carbonaceous samples is poor [12].

The above studies tend to the conclusion that qualitative and quantitative results of PAH analysis of complex samples strongly depend on the isolation procedure applied, which of course is a very undesirable situation. In this study we concentrate on the comparison of a number of extraction methods applied to one specific soot sample.

Ultrasonic extraction with diethylether; Soxhlet extraction with toluene and modified Soxhlet extraction with liquid CO₂ were compared with respect to recoveries and reproducibilities of the PAH under study. These methods were also studied applying two of the selected procedures, in different sequence, to the aerosol sample. The relatively recent procedure with liquid CO₂ was investigated in more detail concerning the necessary number of transfers for complete extraction and the proper method to remove the CO₂ after completion of the extraction process. Opening the valve to allow evaporation of the liquid CO₂, which is in general suggested [13, 14] showed strongly decreased recoveries of the PAH. An improved method will be discussed.

The analytical procedure consists of a sample pretreatment step by HPLC described earlier [15] followed by final analysis of the HPLC fractions by capillary GC. Losses of PAH of up to 30 % sometimes observed during pretreatment were studied. PAH standard mixtures and ambient air samples were employed to investigate the pretreatment step in more detail.

From this it turned out that the loss of PAH and also the laborious conditioning of the silica stationary phase, necessary before sample treatment, could be eliminated by the use of a diol-modified silica.

Experimental

Samples and Chemicals

In order to compare the extraction procedures properly, all experiments were carried out with a specific soot sample from a domestic stove with wood as a fuel. The sample was passed through a 175 μm sieve and homogenized by intensive mixing. It was stored in a refrigerator at 253 °K.

For the qualitative and quantitative measurements of 16 Priority Pollutant PAH were used as standards (Serco Inc., Roseville, MI, USA). Carbon dioxide for extraction was from Hoekloos (Amsterdam, NL). All other chemicals were of analytical grade (Merck, AG, Darmstadt, FRG).

To determine the recovery of PAH after HPLC pretreatment (with silica and diol modified silica stationary phases) a PAH-mix (TNO Environmental and Energy Research, Dept. of Analytical Chemistry, Delft, NL) and an ambient air sample were used. The organic solvents for extraction of the ambient air samples (methanol), HPLC pretreatment (hexane, dichloromethane, acetonitrile and 2,2-dimethoxypropane) and analysis by reversed-phase HPLC (water and methanol), were of HPLC-grade (Rathburn, Walkerburn, UK).

Extraction Procedures

Extraction procedures for soot samples. The Soxhlet extraction for PAH by liquid CO_2 from the aerosol samples were performed in equipment described earlier [14]. The thermocouple sensor, situated above the extraction tube, detected the number of transfers during the extraction process. The cold finger condenser was cooled with a stream of nitrogen at 253 °K.

After extraction the extractant was removed by either direct evaporation, opening the needle valve in the container or by sublimation. In the latter case the CO_2 was frozen by putting the container in liquid N_2 . Then the container was opened and the solid CO_2 permitted to sublimate from the glass Soxhlet vessel. To study the efficiency of both methods known amounts of toluene and n-decylbenzene as test components in n-hexane were put in the Soxhlet vessel. After some transfers, to mimic the extraction, the CO_2 was removed by one of the methods. The 4-step ultrasonic extraction with diethylether as an extractant was as described earlier [15].

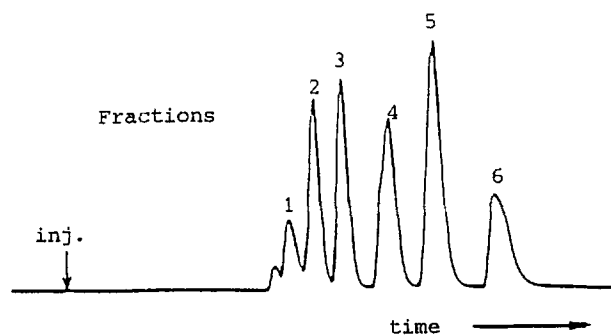
The Soxhlet extraction with toluene took 8 h. Subsequently, the toluene was removed by a rotating vacuum evaporator. For the extractions 0.25–1.5 g sample was used.

Extraction of ambient air sample. The particles from ambient air were collected with a Sartorius HV100 'Staubsammelgerät' (high-volume samplers) on Sartorius SM13400 glass fibre filters ($\varnothing = 25.7 \text{ cm}$); flow rate $100 \text{ m}^3 \cdot \text{h}^{-1}$, calibrated with a flowmeter. The filter was extracted with 170 ml methanol for 8 h under nitrogen and solvent removed with a rotary evaporator at 30 °C under reduced pressure.

HPLC Pretreatment

HPLC fractionation of soot samples. HPLC fractionation was as described earlier [15]. To overcome the problems of the necessary frequent activation of the silica columns, a diol-modified silica column ($250 \times 4 \text{ mm i.d.}$; $d_p = 5 \mu\text{m}$; Merck AG, Darmstadt, FRG) with n-hexane as eluent was also investigated.

The PAH sample gave six distinct fractions on both columns silica and diol. The fractions were collected manually and consisted of 1–3 ml of eluent, (Figure 1) which were subjected directly to capillary GC analysis.



No.	Component	Fractions					
		1	2	3	4	5	6
1	Naphthalene	x					
2	Acenaphthylene	x					
3	Acenaphthene	x					
4	Fluorene						
5	Phenanthrene		x				
6	Anthracene		x				
7	Fluoranthene			x			
8	Pyrene			x			
9	Benz(a)anthrene					x	
10	Chrysene				x		
11	Benzo(b)fluoranthene					x	
12	Benzo(k)fluoranthene					x	
13	Benzo(a)pyrene					x	
14	Dibenzo(a,h)anthracene						x
15	Benzo(g,h,i)perylene						x
16	Indenol(1,2,3-c,d)pyrene						x

Figure 1

Chromatogram from HPLC-fractionation of 16 parent PAH, including occurrence in different fractions. Column: diol modified silica, $250 \times 4 \text{ mm}$; eluent: n-hexane; flow: 0.7 ml min^{-1} .

HPLC fractionation of ambient air sample. HPLC fractionation of the ambient air sample was employed with a Model 655A LC-pump (Hitachi; Merck AG, Darmstadt, FRG) controlled by a Model L-5000 gradient controller (Hitachi, Merck). A Model 770 variable UV-absorption detector was used at 254 nm (Schoeffel, Applied Biosystems, Maarssen, NL).

The fractions (7 ml) were collected with the Model FRAC-300 collector (Pharmacia, LKB Biotechnology, Uppsala, Sweden). Injections of 250 μ l sample were made with a Model AH60 pneumatic injector (Valco, Houston, TX, USA). Fractions 5, 6 and 7 were collected for the PAH-analysis by RP-HPLC.

In this study a RSIL-silica column (250 \times 10 mm i.d., d_p = 10 μ m, Alltech Ass. Inc., IL, USA) as well as a diol-modified silica column, Hibar Lichrosorb Diol (250 \times 10 mm i.d., d_p = 7 μ m, Merck, Darmstadt, Germany) were investigated.

The activation and separation conditions for the silica column are in Table I.

PAH-Analysis

PAH analysis of soot samples. HPLC-fractions were analyzed by capillary gas chromatography. The instrument consisted of a Carlo Erba Gas Chromatograph equipped with FID, controller 490, on-column injection device and temperature programmer model 430 (Carlo Erba Strumentazione, Milan, Italy).

Injections of 3 μ l samples were made in a retention gap (3 \times 0.35 mm) connected to the separation column with CP-SIL 8CB stationary phase (Chrompack, Middelburg, NL).

After injection a temperature program was employed for optimum separation. The program included: t_1 = 70 $^{\circ}$ C constant 2 min; t_2 = 230 $^{\circ}$ C, 8 $^{\circ}$ C min^{-1} ; t_3 = 278 $^{\circ}$ C, 4 $^{\circ}$ C min^{-1} ; t_4 = 278 $^{\circ}$ C constant, 3 min.

The data were collected and processed by a Spectra Physics 4000 system using an ADC interface, model 2 SP-4020 (Spectra Physics, Santa Clara, CA, USA).

PAH-analysis of ambient air sample. The LC-fractions 5, 6 and 7 collected were analyzed by RP-HPLC. The LC system comprised two Millipore-Waters (Etten-Leur, NL) Model 6000A solvent delivery modules, controlled by a Millipore-Waters automated Gradient controller. Injection was with a Millipore-Waters model WISP710B autoinjector. Column effluent was monitored with a Schoeffel (Applied Biosystems, Maarssen, NL) model FS 970 LC fluorimeter. Data were recorded with the Beckman Peak-Pro software system. For separation an LC-PAH (250 \times 4.6 mm i.d., d_p = 5 μ m) (Supelco Inc., Bellefonte, PA, USA) column was used at ambient temperature under the following conditions: injection volume = 20 μ l; flow = 1 ml min^{-1} ; excitation wavelength = 250 nm and emission wavelength > 389 nm; eluent A = 70 v/v % methanol in water; eluent B = methanol. The elution program included: from 85 % A/15 % B linear to 45 % A/55 % B in 80 minutes, from 45 % A/55 % B linear to 100 % B in the next 40 minutes, and during the next 30 minutes 100 % B constant.

Table I Activation and separation conditions on silica columns.

Program for the activation of silica				
time (min)	% hexane	% dichloromethane	% acetonitrile	% DMP ^{*)}
0,0	0	0	0	100
15,0	0	0	0	100
15,1	0	100	0	0
22,1	0	100	0	0
22,2	0	0	100	0
29,2	0	0	100	0
29,3	0	100	0	0
34,3	0	100	0	0
34,4	100	0	0	0
49,4	100	0	0	0
Separation conditions				
Time (min)	% hexane	% dichloromethane	% acetonitrile	% DMP ^{**)}
0,0	100	0	0	
20,0	20	80	0	
20,1	0	100	0	
25,1	0	100	0	
25,2	0	50	50	
35,2	0	50	50	
35,3	0	0	100	
50,3	0	0	100	
55,3	0	100	0	
60,3	100	0	0	

*) DMP = hexane/acetic acid/2,2-dimethoxypropane: 90:10:2.5 v/v/v.

**) flow = 3.5 ml min^{-1}

Results and Discussion

For both columns used for the HPLC--prefractionations mass balances of PAH were made by injecting and subsequently analysing known amounts of these components.

Figure 2 shows the results of the HPLC pretreatment of a soot sample with silica and diol modified silica as stationary phase. Table II shows similar results for an ambient air sample.

From these results it can be concluded that, using a silica column, the recoveries of PAH vary between 0–100%. Especially important PAH such as perylene, benz(a)pyrene, 3-methylcholanthrene and anthanthrene show low recoveries. Although the reason for these losses is not clear, it might be assumed that they are due to degradation and/or absorption of the PAH on the highly activated silica. The assumption of absorption is supported by the observation that decreased yields of PAH are less pronounced when real samples are used rather than test solutions (see Table II). The interfering components from the sample are absorbed on the most active sites so inhibiting irreversible absorption of PAH.

Moreover, it turned out that the silica column has to be reactivated and rinsed frequently, while for the diol-column a simple rinsing procedure with dichloromethane proved to be sufficient. In this study the HPLC-fractionation was continued on the diol. column.

Efficient removal of CO₂, after extraction, by direct evaporation and by sublimation was investigated.

For toluene and n-decylbenzene as test substances, efficiencies of 96 and 100% were observed removing the CO₂ by sublimation, while the corresponding values were 65 and 86%, respectively for the direct evaporation method. The

losses observed in the latter method may be caused by the strong turbulence of the evaporating CO₂, displacing the substances. Removal of the CO₂ was therefore continued by the sublimation method.

The extraction yields of PAH depend in part on the number of extraction steps or transfers in the different extraction methods. For both ultrasonic and Soxhlet extraction, the procedures applied here proved to isolate completely from the soot sample PAH accessible under the experimental conditions.

The number of transfers to complete the extraction by CO₂ was studied by extracting 1 g of the soot sample 4 times, 25 transfers each in succession. The results are shown in Table III. From these data, it follows that at least 50 transfers are necessary to obtain sufficient yields of extraction. This relatively high number may be due to the complex structure of the sample matrix and the limited solubility of the PAH in liquid CO₂. However, supplementary studies

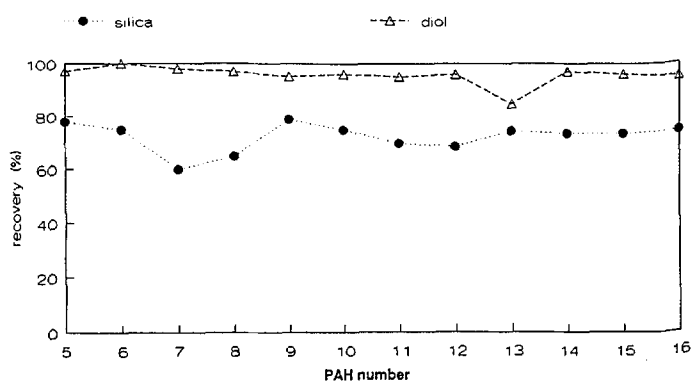


Figure 2
Recoveries of PAH in HPLC-fractionation for silica and diol columns.

Table II Recoveries (%) of PAH in HPLC-pretreatment step for silica and diol columns.

Component	PAH-Mix (silica)	Air sample (silica)	PAH Mix (diol)
Fenanthrene	95	50*	100
Anthracene	87	82	103
Fluoranthene	105	104	112
Pyrene	102	109	109
3,6-dimethylfenanthrene	100	58*	104
Trifenylen	105	103*	109
Benz(b)fluorene	104	110	109
Benz(a)anthracene	100	107	110
Chrysene	101	93	110
Benz(e)pyrene	102	94	111
Benz(j)fluoranthene	107	110	112
Perylene	0–92	86	109
Benz(b)fluoranthene	104	95	111
Benz(k)fluoranthene	103	96	111
Benz(a)pyrene	65	99	111
Dibenz(a,j)anthracene	97	–**	113
Dibenz(a,l)pyrene	66	–**	109
Benz(g,h,i)perylene	96	95*	113
Dibenz(a,h)anthracene	97	–**	118
Indenol(1,2,3,-c,d)pyrene	107	84	118
3-Methylcholanthrene	8–86	88	108
Anthanthrene	0–19	69	111

*) Since peak in chromatogram is not efficiently separated from other (unknown) compounds, maximum amount of analyzed compound is given.

***) These compounds not found in analyzed sample.

showed that extraction could be completed in 25 transfers by decreasing the sample to about 0.3 g. An example of the recoveries of PAH using liquid CO₂ is given in Figure 3. A number of tests were carried out on the soot sample by the three extraction methods; the results are summarized in Figure 4.

The data show that CO₂ extraction gives increased yields for the PAH to chrysene, while for the PAH from benzo(b)fluoranthene the other two methods are better.

Table III Yields (%) of PAH from 1 g of soot sample after liquid CO₂ extraction. Numbers 1-4 represent 25 transfers each.

Component	Yields (%)			
	1	2	3	4
5	77	18	5	1
6	78	20	2	0
7	79	16	5	1
8	79	16	5	1
9	78	14	8	0
10	77	15	7	0
11	74	18	9	1
12	74	17	9	0
13	69	21	10	0
14	75	25	0	0
15	100	0	0	0
16	77	23	0	0

Complete performance of the three extraction procedures takes for the ultrasonic, the CO₂ and the Soxhlet extraction, about 2h, 6h and 16h, respectively, including the evaporation/sublimation time in both the toluene and CO₂-Soxhlet procedures, which took at least 3h.

From the above it can be concluded that CO₂ extraction is rather time consuming and is not significantly more efficient compared to the other methods, within the framework of these experiments.

On the other hand CO₂ extraction, being carried out in an oxygen-free and dark environment, may contribute to improved efficiencies in the case of volatile and unstable substances.

Moreover, in both the ultrasonic and Soxhlet procedures, the removal of the extractant may easily give rise to losses due to evaporation. Evaporation of CO₂ by sublimation is superior in this respect.

Since the three extraction methods gave different yields of PAH, combinations of two of these procedures were applied successively to the soot sample in order to study to what extent recoveries might be increased.

The soot sample was therefore subjected to ultrasonic extraction and after that the sample residue to a CO₂ extraction and vice versa. An example is given in Figure 5.

From these data it is seen that the recoveries from extractions carried out in the sequence CO₂/ether and CO₂/toluene were significantly higher than for the reversed sequences ether/CO₂ and toluene/CO₂.

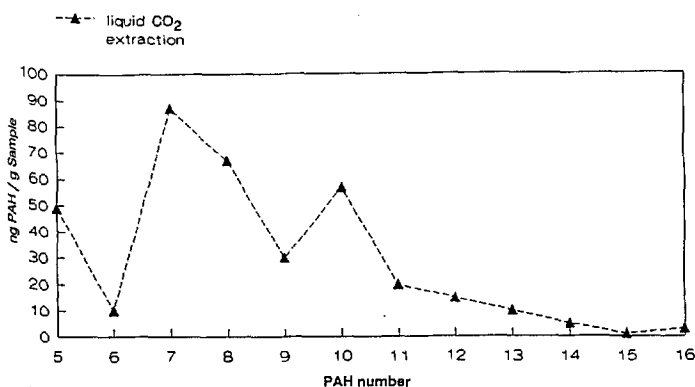


Figure 3 Yields in ng g⁻¹ sample of PAH No 5-16 from soot sample, using liquid CO₂ extraction.

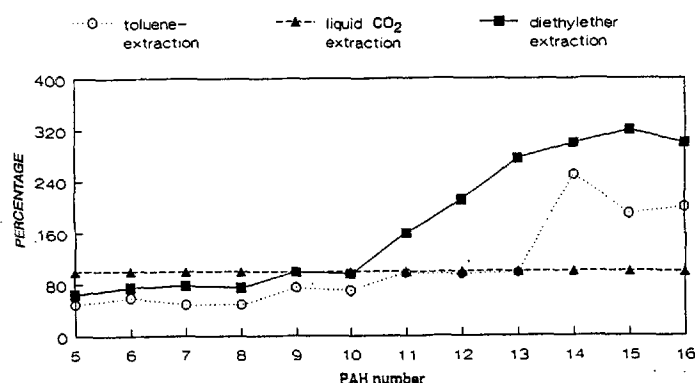


Figure 4 Comparison of yields of PAH of soot sample by three extraction procedures; results normalized to data of CO₂ extraction (100 %).

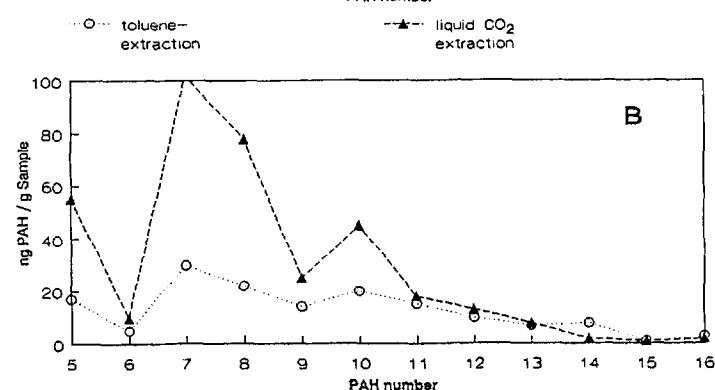
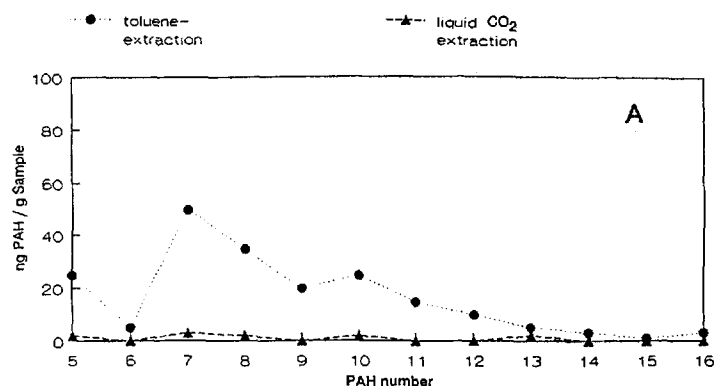


Figure 5 Recoveries of extractions in sequences toluene/CO₂ (A) and CO₂/Toluene (B) of soot sample.

The total amounts of the extracted PAH were for the combinations CO₂/ether; ether/CO₂; toluene/CO₂ and CO₂/toluene: 403, 315, 211 and 488 µg PAH/g, respectively.

From these results it can be concluded that the extraction yields are not only dependent on a specific extraction method and extractant, but also on the conditioning of the sample by the first extraction.

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Received: Jan. 15, 1991
Accepted: Apr. 4, 1991
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