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Determination of some polycyclic aromatic hydrocarbons in wood smoke by HPLC-FLD following steam-extraction and liquid-liquid extraction

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Abstract
Most polycyclic aromatic hydrocarbons (PAHs) are carcinogenic to animals and humans, and most of them are produced by the incomplete combustion of organic substances. This study looks at developing a new procedure for the extraction of polycyclic aromatic hydrocarbons (PAHs) from wood smoke. The procedure employed liquid-liquid extraction following steam-extraction. This technique will have advantages of simplicity, affordability, availability and minimal waste generation, hence, environment friendly. 4 g of *Klanedoxia gabonensis* wood was completely burnt and the PAHs scrubbed off the smoke by steam-extraction and pre-concentrated by liquid-liquid extraction. LC-FLD was used for the analysis. The qualitative analysis was accomplished by comparing the retention times of PAHs in a standard mixture of 10 PAHs, with those in the smoke sample. The quantitative analysis of the smoke sample gave 2.49 µg of naphthalene/g of wood, 4.04 µg of fluorene/g of wood, 3.00 µg of phenanthrene/g of wood, and 1.70 µg of anthracene/g of wood. Determination of the coefficients of variation for seven replicate measurement indicated high precision of this technique. The technique may not have extracted the higher molecular mass PAHs due to their high hydrophobicity. Further research is required to actualize complete extraction of PAHs using this method.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are included in the European Union and US Environmental Protection Agency priority pollutant lists because PAHs represent the largest group of compounds that are mutagenic, carcinogenic, and teratogenic[1-3]. Exposure to PAHs occurs mainly by inhalation of air and by ingestion of food and drinking water[4-5]. Although food can be contaminated with PAHs from the environment (air, dust and soil), PAHs in food are mainly formed during industrial processing and food preparation, for example smoking, roasting, baking, drying, frying, or grilling[6]. For this reason, their detection and monitoring has become an important problem and this has led to the development of new analytical methods with improved selectivity and sensitivity[7-10].

Analytical techniques for sample preparation such as extraction with nonionic surfactant polyoxyethylene-10-lauryl ether[10], solid-phase extraction (SPE)[9] and analytical techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) with mass spectrometric (MS) detection have been reviewed[11-15].

A review of the literature indicates a focus on extraction techniques for PAHs in solids (biological matrices, soil and sediment samples) and liquid samples. However, the extraction of PAHs directly from smoke has been by using disposable smoke pads which are not readily available in most laboratories. The aim of this work was to design a steam-extraction of PAHs from smoke produced from the incomplete combustion of wood. It is simple, less expensive, easily constructed with cheap and readily available materials and not disposable.

2. Experimental

2.1. Reagents

All reagents were analytical or HPLC grade. Acetonitrile, PAHs, toluene, and sodium sulphate were bought from Sigma-Aldrich (St Louis, MO, USA). The water used was from a milliQ system (Milford, Mass, USA). Mobile phase was filtered through a Whatman membrane filter (47 mm diameter and 2µm pore size. A G-1321A Scanning Fluorescence Detector (Agilent Technologies, Palo Alto, USA); Agilent Chemstation software for controlling LC and data analysis; Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA, USA) and Column (Agilent Pursuit PAH, 100 x 4.6 mm, 3µm).

2.1.1. Preparation of Standard Solutions of PAHs

Standard stock solutions (1 mg/mL) were prepared by dissolving 10 mg of the desired PAH in 10 mL acetonitrile and stored at 4^{o}C in the dark. All working solutions were prepared fresh daily by serial dilutions with acetonitrile. The stock solution comprised of naphthalene, fluorine, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a] pyrene, chrysene, benzo[b] fluoranthene and benzo[k] fluoranthene.

2.2. Extraction/Pre-Concentration

PAHs were steam-extracted from wood-smoke using the laboratory set-up as shown in Figure 1. The steam was then cooled to liquid (PAH-containing liquid).

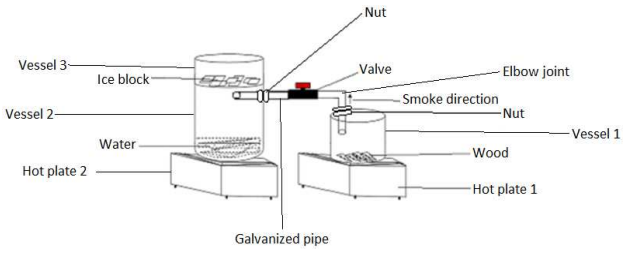

Figure 1. *Improvised laboratory set-up for steam-extraction of PAHs*

All the components of the laboratory set-up were made of either galvanized iron or borosilicate glassware. The nuts help to fix the position of the galvanized pipes in the two vessels (1 and 2). 4 g of the *Klanedoxia gabonensis* wood was burnt in vessel 1 and the smoke directed in the pipe to vessel 2 with the valve at open position. The valve was closed when the wood was completely burnt at a pre-determined time of 5 minutes. At this point, vessel 2 was saturated with smoke and hot plate 2 was turned on at reasonable temperature just enough to produce steam in order to effect steam-extraction. However, the PAH-containing steam was condensed to water with the aid of iced cubes in vessel 3. Thirty milliliteres (30ml) of water was initially put in the vessel 2 but 20 mL was obtained at the end of the extraction.

A liquid-liquid extraction was carried out on this aqueous sample with a total of 60 mL toluene, running three cycles of extractions (20 mL of toluene for each cycle) using a separatory funnel. The total organic phase (toluene phase) was dried of its possible water content with sodium sulphate. The dried extract was evaporated on a water bath (40ºC) under a stream of nitrogen and diluted with 5 mL acetonitrile for chromatographic analysis.

2.3. HPLC Analysis

HPLC analysis was performed with a flow rate of 0.8 mL min^{-1} at 25°C. The injection volume was 20 µL. The column was stabilized at 25°C for 1 h before chromatography. The mobile phase was a gradient prepared from water (component A) and acetonitrile (component B). Details of the gradient are given in Table 1. Excitation (Ex) and Emission (Em) wavelengths were programmed as reported in Table 2.

Table 1. *Mobile phase gradient for HPLC separation*

Time (min)	% water	% acetonitrile
0	60	40
7	0	100
15	0	100
20	60	40

Table 2. *Wavelength Changes of the Fluoresence Detector*

Time (min)	PAHs	Ex/Em wavelength (nm)
8.5	Naphthalene fluorene	270/385
12.5	phenanthrene anthracene fluoranthrene	256/446
20.0	pyrene benzo[a]anthracene chrysene benzo[b]fluoranthene benzo[k]fluoranthene	274/507

2.4. Repeatability Test

The extraction and pre-concentration steps were repeated 7 times on 7 consecutive days and the extracts analyzed by LC-FLD. After the steam extraction, the different components of the steam-extraction set-up were immediately washed with slightly soapy water and washed copiously with hot water before any subsequent steam-extraction. The washing step was necessary to eliminate the carry-over of PAHs on the

equipment in the preceding extraction.

3. Results and Discussion

The HPLC analysis was carried out with the conditions spelt out in the experimental sections and Figure 2 shows the chromatograms obtained. Traces A, B, and C respectively show the chromatograms of the blank, sample, and PAH standard mixture. The figure shows the presence of naphthalene, fluorene, phenanthrene, anthracene in the sample and the absence of PAHs in the blank.

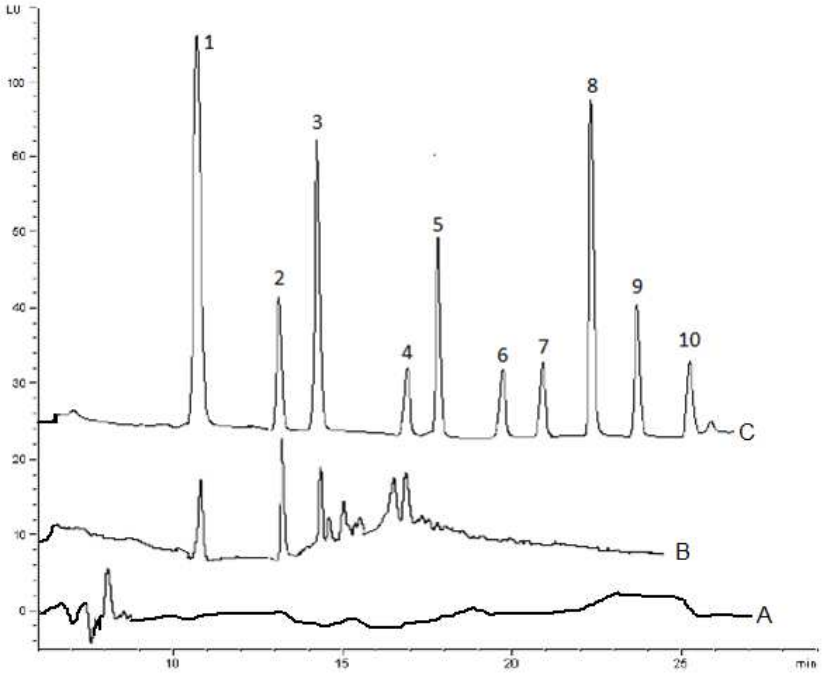

Figure 2. *LC-FLD chromatograms: Trace C: Standard mixture of 10 PAHs (30 ng/mL each). Peak identification: 1 = naphthalene, 2 = fluorene, 3 = phenanthrenef , 4 = anthracene, 5 = fluoranthrene, 6 = pyrene, 7 = benzo[a]anthracene, 8 = chrysene, 9 = benzo[b]fluoranthene, 10 = benzo[k]fluoranthene. Trace B: sample. Trace A: blank*

The quantitative analysis showed that the 1g of wood respectively gave (2.49 ± 0.02) µg of naphthalene, (4.04 ± 0.03) µg of fluorene, (3.00 ± 0.02) µg of phenanthrene, and (1.70 ± 0.12) µg of anthracene. Table 3 shows the retention times, equations of the calibration curves, r^2 values and concentrations of PAHs in the test samples. However, Fluoranthrene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, and benzo[k]fluoranthene do not have equation of straight line, r-squares and concentration values because they were not detected. This could be as a result of using steam extraction. According to Inengite et al[16], 2- and 3- ringed PAHs are hydrophilic while 4-6 ringed PAHs are hydrophobic. This method may have extracted only the hydrophilic PAHs (low molecular weight, 2- to 3- ringed PAHs), leaving out the hydrophobic PAHs (high molecular weight 4- to 6- ringed PAHs).

To be sure that the low molecular weight PAHs were actually from the combustion of wood, diagnostic PAH ratios were applied to Anthracene and Phenanthrene. Budzinski et al[17] stated that PAH (anthracene/anthracene + phenanthrene) ratio <0.10 usually is taken as an indication of petroleum while a ratio >0.10 indicates a dominance of combustion. Comparing this to the Anthracene/Anthracene+Phenanthene which was 0.36, gives a clue that the PAHs should be from the combustion of wood.

Table 3. *Results of qualitative and quantitative analyses*

PAH	Ret. Time (min)	Equation	R^2	Concentration (µg/g of wood)
Naphthalene	10.87	$y = 0.050x + 0.0097$	0.998	2.49 ± 0.02
Fluorene	13.10	$y = 0.020x + 0.0150$	0.990	4.04 ± 0.03
Phenanthrene	14.40	$y = 0.036x + 0.0015$	0.999	3.00 ± 0.02
Anthracene	15.98	$y = 0.061x + 0.5290$	0.997	1.70 ± 0.12
Fluoranthene	17.87	ND	ND	ND
Pyrene	19.65	ND	ND	ND
Benzo[a]anthracene	20.97	ND	ND	ND
Chrysene	22.46	ND	ND	ND
Benzo[b]fluoranthene	23.57	ND	ND	ND
Benzo[k]fluoranthene	25.36	ND	ND	ND

ND: Not Detected in Sample

3.1. Descriptive Statistics of Test Results

The extraction and HPLC analysis was repeated 7 times on 7 consecutive days and the results are as presented in Tables 4. The descriptive statistics, showing the coefficients of variation is as stated in Table 5.

Table 4. *Daily concentration values of PAHs in µg/g of wood*

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Naphthalene	2.5	2.49	2.5	2.51	2.48	2.47	2.51
Fluorene	4.06	4.04	4.01	4.07	4.06	4	4.02
Phenanthrene	3 .00	3.02	2.98	2.99	3.02	2.99	3.01
Anthracene	1.76	1.77	1.76	1.55	1.76	1.54	1.77

Table 5. *Descriptive Statistics of the Cncentrations of PAHs µg/g of wood*

	Naphthalene	Fluorene	Phenanthrene	Anthracene
Mean	2.49	4.04	3.00	1.70
Standard Error	0.01	0.01	0.01	0.04
Median	2.50	4.04	3.00	1.76
Mode	2.50	4.06	3.02	1.76
Standard Deviation	0.02	0.03	0.02	0.11
Sample Variance	0.00	0.00	0.00	0.01
Range	0.04	0.07	0.04	0.23
Minimum	2.47	4.00	2.98	1.54
Maximum	2.51	4.07	3.02	1.77
Count	7	7	7	7
Confidence Level (95.0%)	0.01	0.03	0.02	0.10
Coefficient of Variation	0.80	0.74	0.67	6.47

The coefficients of variation indicate that the precision of the method is quite high. The precision of the method for the determination of the four PAHs detected followed the order Phenanthrene> Fluorene > Naphthalene > Anthracene.

4. Conclusion

The sampling method employed liquid-liquid extraction following steam-extraction. This novel technique has advantages of simplicity, affordability and availability. There is also minimal waste generation and is therefore environment friendly. The coefficients of variation are low, indicating high precision of the method. The method may not have extracted the higher molecular mass PAHs due to their high hydrophobicity rather the lower molecular mass PAHs were extracted, still due to their hydrophilic nature. Further research is required to actualize complete extraction of PAHs using this method.

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