REVIEW ARTICLE

Determination of Polycyclic Aromatic Hydrocarbons in Human Blood Samples Using Solid Phase Extraction and Gas Chromatography Mass Spectrometry- A Review

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants with toxic effects and adverse health impacts on general population. Several methods of extraction had been applied to extract PAHs from human blood samples such as solid phase extraction (SPE). The SPE represents one of the most common techniques for extraction and clean-up procedures as it needs low quantity of solvents with less manual efforts. Similarly, various analytical instruments like gas chromatography coupled to mass spectrometry (GC-MS) was used to measure the PAHs levels. Gas chromatography is a simple, fast, and very efficient method for solvents and small organic molecules. This review provides an overview of the measured concentrations of PAHs in human blood samples through the application of SPE and GC-MS during the last ten years. While these studies used various solvents, their application of SPE method and GC-MS revealed rewarding results about the determination of PAHs levels in the human samples.

Keywords: Polycyclic aromatic hydrocarbons (PAHs), Serum, Whole blood, Solid phase extraction (SPE), Gas chromatography-mass spectrometry (GC-MS)

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) represent a group of common pollutants around the globe produced concurrently with incomplete combustion of organic compounds and delivered to the environment through vehicles emissions, industrial sources, cigarette smoke, and waste incineration (1). The United States Environmental Protection Agency (USEPA) specified 16 PAHs as priority compounds due to their toxicity on human health. These 16 PAHs include naphthalene (Nap), acenaphthene (Ace), acenaphthylene (A), fluorene (F), anthracene (Ant), phenanthrene (Phe), pyrene (Pyr), fluoranthene (Flu), benz(a)anthracene (BaA), benzo(b+k)fluoranthene (B(b+k)Flu), chrysene (Chr), benzo(a)pyrene (Bap), dibenzo(a,h)anthracene (DA), benzo(g,h,i)perylene (BP), and indeno(1,2,3-cd) pyrene (IcdP) (2). Based on the number of benzene rings in their structures, PAHs can be classified into low molecular weight (LMW-PAHs) (2-3 rings), medium molecular weight (MMW-PAHs) (4 rings), and high molecular weight (HMW-PAHs) (5-6 rings) (3). While LMW-PAHs are only toxic, the HMW-PAHs are both toxic and carcinogenic (2). (2).

Human are exposed to PAHs in both occupational and non-occupational settings through various routes of exposure such as inhalation, ingestion, and dermal contact. Moreover, some exposures could affect the total absorbed dose by having more than one route simultaneously (such as inhalation and dermal exposures from contaminated air) (4). Interestingly, the emissions of complex mixtures of PAHs into the atmosphere depict a possible health risk to the human being. Furthermore, the short-term impacts of PAHs on an individual count massively on the exposure duration, concentrations, toxicity, and route of exposure to PAHs (4). These acute health effects include impaired lung function in asthmatics and thrombotic effects in people affected by coronary heart disease (5). However, the chronic health effects of PAHs include its carcinogenic possibility, specifically lung malignancy in addition to skin tumour and bladder cancer (6).

Recognition of these health risks is very crucial and may be achievable when enough data about the amount and duration of PAHs exposure are available. Even though ambient air concentrations of PAHs may be applied to assess these exposures, body biomarkers such as whole blood, serum or urine samples give more precise data about the amount of exposure as well as retention of these compounds (7)Furthermore, most PAHs can be detected in blood samples while their metabolites are found in urine samples (8). There are various extraction methods of PAHs from biological samples such as solid phase extraction (SPE) and liquid-liquid extraction as well as their corresponding analytical method e.g. gas chromatography coupled to mass spectrometry (GC-MS) and high- performance liquid chromatography (HPLC) (8, 9).

SPE offers many benefits and advantages over other traditional sample preparation techniques (such as liquid-liquid extraction) including high recoveries and excellent concentrations of the analytes, highly purified extracts, ease of automation, compatibility with instrumental analysis, and reduction in organic solvent consumption (10). Selection of solvent for SPE is based on the type of SPE column such as reverse-phase or normal-phase type. Furthermore, activation solvent should be able to wet the SPE column sorbent well and compatible with the solvent for equilibration. Also, solvents for equilibration should be compatible with the activation solvent, wet the sorbent well, and of low elution power. Solvents for collection should be compatible with previously used elution solvent, and can remove the targeted compounds with small volume and easy to evaporate.

This review is aimed to briefly assess the concentrations of PAHs in blood samples and their influences on the human health, and to discuss the SPE method used in their extraction as well the GC-MS used in their analysis from the year 2009 to 2019. In addition, this study compares the total sera concentrations of PAHs, the highest and lowest PAH compounds, and the HMW-PAHs and LWM-PAHs concentrations among studies done over the world in the last ten years. These serum samples may highly be associated with PAHs exposure in addition to their retention, therefore giving a clear picture about PAHs exposure.

METHODOLOGY

This article reviewed the concentrations of PAHs in blood samples and their influences on the human health from the year 2009 to 2019 (3, 10-14). In addition, the SPE methods used in their extraction as well as the GC-MS used in their analysis were discussed. These data were compiled through literature databases such as Google Scholar and Scopus. Various concentrations of blood serum PAHs were reviewed in tandem with their extraction and analysis methods. Besides that, the health effects of these PAHs were briefly discussed in this review.

RESULTS AND DISCUSSION

Extraction and analysis of PAHs in human blood samples

Six studies were reviewed according to the using of SPE and GC-MS methods in the extraction and analysis of PAHs compounds in blood serum and whole blood samples (Table I). Although all selected studies were using SPE method for extraction and clean-up processes, each study applied different solvents to extract the PAHs compounds. In this section, details concerned with solvents and the type of SPE cartridge were reviewed. Also, the limit of detection (LOD) which is defined as the smallest concentration of analyte in the test sample that can be reliably distinguished from zero, and recoveries of PAHs in these selected studies were discussed.. Recovery rate represents the accuracy of the extraction and is determined by the percentage of analyte that was measured after being processed through the SPE device.

Tsang et al. (2011) (11) used dichloromethane (DCM), methanol, milli-q water, and n-hexane during the extraction and clean-up procedures. The clean-up procedure included SPE (High Capacity C18 end-capped, Alltech Associates Inc.) and micro-florisil column. The limit of detection (LOD) was not mentioned in the study while the recovery rate of PAHs was ranged between (71-126%).

Moreover, Wang et al. (2015) (12) and Yu et al. (2011) (14) were using similar solvents such as acetonitrile, sodium sulfate, n-hexane, and a chromatography column filled with the silica gel during the SPE and clean-up methods of PAHs. However, different matrices were selected in their studies including blood serum and whole blood, respectively (12, 14). Also, the detection limits in both studies were applied different units of measure. While the LOD was 0.05-0.10 ng/mL in the first study, the LOD in the second study was based on fresh weight (fw) and was 0.012-0.025 ng/g fw. Despite that, the recovery rates in both studies were approximately close with values of 67.4-106% and 73.3-121%, respectively (12, 14).

Methanol, M phosphate buffer, acetonitrile, n-butyl chloride, and ethyl acetate were utilized as solvents during the SPE extraction and clean-up processes by Ramesh et al. (2015) (3). However, the type of SPE columns, which used in this study, was not specified. Besides that, the PAHs LOD as well as their recoveries were not reported, despite mentioning the LOD 20-5000 ng/mL in relation to benzoylecgonine (the primary metabolite of cocaine).

In another study by Guo et al. (2012) (10), who included some solvents such as n-hexane, water ethanol, sodium hydroxide, milli-q ultrapure water, and DCM for the SPE extraction and clean-up procedures. Unlike the study

Table I: Type of matrix, number of PAHs analysed, reported concentration of PAHs, method of extraction and its reagents, sample clean-up method, type of analytical instrument and sensitivity of each reviewed studies

Matrix	Sample size	PAHs	Reported concentration of PAHs	Sample extraction & Reagents	Sample clean-up	Analytical instrument	LOD & Recovery	Reference
Blood serum	1-2 mL	16 PAHs	- 1461 ng/g fat (maternal serum) - 1158 ng/g fat (cord blood serum)	SPE - DCM - Methanol - Milli-Q water - n-hexane	SPE column - High Capacity C18 - Micro-florisil column	GC-MS	- NA - 71-126%	(10)
Blood serum	2 mL	27 PAHs	- 5839 ng/g fat (cases) - 2668 ng/g fat (controls)	SPE - Acetonitrile - Sodium sulfate - n-hexane	- 200-300 silica gel mesh	GC-MS	- 0.05-0.10 ng/mL - 67.4-106%	(11)
Blood serum	2 mL	16 PAHs	- 0.3-19 ng/mL	SPE - Methanol - M phosphate buffer - n-butyl chloride - Ethyl acetate	SPE column - Sigma SPE columns	GC-MS	- 20-5000 ng/mL - NA	(9)
Blood serum	2 mL	16 PAHs	- 206 ng/g fat	SPE - Formic acid - Ethanol - n-hexane - DCM	- Column filled with pre-activated silica gel and sodium sulfate	GC-MS	- 0.013-0.912 ng/mL - 70.2-103.4%	(12)
Umbilical cord blood	5 mL	15 PAHs	- 2560 ng/g fat	SPE - Acetonitrile - Sodium sulfate - n-hexane	- 200-300 silica gel mesh	GC-MS	- 0.012-0.025 ng/g fresh weight - 71%	(13)
Umbilical cord blood	NA	7 PAHs	- 108.05 ng/mL (cases) - 79.36 ng/mL (controls)	SPE - n-hexane - Water ethanol - Sodium hydroxide - Mill-Q ultrapure - DCM	SPE column - Supelco RP-18 cartridges	GC-MS	- 0.03-0.09 ng/mL - 78.2-105.6 %	(8)

N.A. stands for data not available from the study

reported by Ramesh et al. (2015) (3), the type of SPE column that used for cleaning up the sample was stated in this study and it was RP-18 cartridge (Supelco) (10). Moreover, this study showed better LOD of PAHs 0.03-0.09 ng/mL in addition to recovery rates with values of 78.2-105.6% (10).

Furthermore, formic acid, ethanol, n-hexane, and DCM were used as solvents by Yin et al. (2017) (13). A column chromatography packed with pre-activated silica gel and sodium sulfate was applied for the clean-up process. The LOD in this study was 0.013-0.912 ng/mL, which was the best among all selected studies (3, 10-12, 14). Likewise, the recovery range of PAHs in this study was also the best compared to other studies that ranged from 83.1% to 122% (13).

Concentration of PAHs in human blood samples and their influences on health

A total of six studies from the past ten years were reviewed in terms of concentration of PAHs in human blood samples (Table I). While four of these studies used blood serum (2 mL) for the detection of PAHs, only two studies used whole blood for measuring concentration of PAHs (Table I).

Approximately (30-50 mL) of blood was withdrawn from thirty participants, and serum was obtained subsequently via blood centrifuging at 3500 rpm (for 5 min) in a study done in Hong Kong (11). Also, lipid contents in blood serum were determined gravimetrically and the PAHs analysis was carried out using GC-MS. The total concentrations of sixteen PAHs found in maternal and cord sera of the subjects were 1461 and 1158 ng g−1 fat, respectively. This difference between maternal and cord sera PAHs could be belong to the difference in lipid compositions of both sera (maternal serum 0.53% vs cord serum 0.45%). The manifestation of cord serum PAHs represents antenatal exposure of foetus to those harmful compounds. While Nap had the highest levels in both types of serum (maternal serum: 331 ng g-1 fat, cord serum: 348 ng g-1 fat), B(b+k)Flu had the lowest levels in both types of serum (maternal serum: 16 ng g-1 fat, cord serum: 20 ng g-1 fat). LMW-PAHs were found in 100% of all human serum samples. However, HMW-PAHs compounds were not found in the serum samples except B(b+k)Flu (11).

In another study carried out by Wang et al. (2015) (12), the relationship between maternal serum concentrations of PAHs and neural tube defect (NTD) risk in offspring was investigated using a case-control study design. The study was conducted in Northern China and 117 women who delivered NTD-affected infants (cases) and 121 women who had healthy pregnancies (controls) were included. Blood samples were collected from all participants during delivery or termination of NTD-affected pregnancies, and maternal serum was gathered within one hour of

collection. Triglycerides and cholesterol levels were measured using the oxidative method, and the GC-MS was used for the analysis of PAHs. The sum of serum LMW-PAHs, HMW-PAHs, and all PAHs in cases were double the values determined in the controls (4712 vs 2068 ng g-1 fat, 1164 vs 557 ng g-1 fat, 5839 vs 2668 ng g–1 fat), respectively. Concurrently, the sum of HMW-PAHs was reported to have greater odd ratio (OR) values in comparison to the sum of LMW-PAHs. This could be explained by the fact that five out of the six detected HMW-PAHs are carcinogenic, and their greater potency could lead to extra oxidative stress damage than that shown by the LMW-PAHs, thereby results in a greater risk for NTDs in the exposed pregnancies. PHE had the highest serum levels in both cases and controls (1820 vs 796 ng g-1 fat), which possesses structure of three rings in addition to represent a very common element in traffic emissions. Also, PHE has shown greater serum levels in traffic policemen in, China (15). However, BkF had the lowest serum levels in both cases and controls (32.2 vs 11.3 ng g-1 fat).

Interestingly, a study was done in US to report the concentrations of PAHs in blood sera samples of 650 autopsied human who died due to different causes (3). SPE and clean-up were used to extract PAHs from two millilitre of blood serum. PAHs analysis was done using GC-MS, however the lipid contents were not assessed. The PAHs concentrations reported in this study were (0.3-19 ng/mL), and were within the range measured in other studies [(0.37–260 ng/mL) (9); (0.15–5.45 ng/mL) (16); (3.0–231 pg/mL) (17)] for normal people. Despite that, some concentrations measured in this study showed great values, which resulted from mixed exposures as well as the environmentally poor quality of study area. Moreover, several autopsied samples belonged to individuals with drug abuse, who mostly could have been subjected to tobacco as well as narcotics smoke. However, this study did not mention the highest and lowest PAH compounds.

The concentrations of 16 PAHs compounds and their possible impact on levels of reproductive hormone in the umbilical cord (UC) serum of 98 mother-neonate pairs was assessed by Yin et al. (2017) (13). Briefly, two millilitre of blood serum was required for the extraction and clean-up processes that followed the SPE methods in order to evaluate the exposure to PAHs in the sera samples of mothers and their neonates. Total fat in the serum was measured and was applied to normalize the concentration of every compound. Besides that, the reproductive hormones such as testosterone, estradiol, follicle-stimulating hormone (FSH), luteotropic hormone (LH) and Anti-Mullerian hormones (AMH), were measured using enzyme-linked immunosorbent assay (ELISA) kits. The mean value of total serum concentrations of the sixteen PAHs was 206 ng g-1 fat, and it showed lesser value than those reported among the previous studies (3, 11, 12). The reason behind that may be the rural area, where the samples were taken, has lesser industrialization in comparison to the developed cities as the body burden of PAHs may be related to that. While pyrene represented the highest PAH compound with a mean value of 98 ng g-1 fat, acenaphthylene showed the lowest PAH compound with a mean value of 0.297 ng g-1 fat. Additionally, LMW-PAH pollutants were the majority (68%) in UC serum in comparison to only (32%) represented by HMW-PAHs pollutants.

Albeit the preceding four studies were done on blood serum, the next two studies were carried out using whole blood. Firstly, Yu et al. (2011) (14) investigated the PAHs concentrations in 40 non-smoking mothers as 30-100 mL UC blood were collected from each participant at the time of delivery in Beijing, China. The mean value of total concentration of fifteen PAHs in the UC blood sample was 2560 ng/g fat. While the LMW-PAHs were the predominant PAHs, HMW-PAHs showed relatively low levels. Furthermore, PHE was the highest PAH compound with a value of 763 ng/g fat. Nevertheless, IcdP showed the lowest value 4.73 ng/g fat among all PAHs compounds.

Secondly, Guo et al. (2012) (10) had conducted a study in order to assess the correlation between serious environmental pollution of PAHs emitted from unregulated electronic-waste recycling and probable neonatal health risk in Guiyu, China. One hundred three UC blood samples were gathered from exposed area, and eighty samples from the control area to evaluate the body burden of seven carcinogenic PAHs. The PAHs were extracted and cleaned up using solid phase methods, then determined by GC-MS. The median value of all 7 PAHs concentration was greater in Guiyu 108.05 ng/mL in comparison to samples from control area 79.36 ng/mL. Living in Guiyu and prolonged process of food cooking at the time of pregnancy were strongly correlated with total levels of PAHs. BaA, Chr, and BaP were contributing to short neonatal height and reduced gestational age. In addition, these congeners showed significantly higher levels in infants with unfavourable birth outcomes in comparison to infants with healthy outcomes. Therefore, mothers who exposed to PAHs could suffer potential accumulation of toxic PAHs, and thereby could cause adverse effects on the health of their neonatal.

CONCLUSION

Currently, studies tend to use human blood samples to assess the PAHs exposure as non-metabolized PAHs represent excellent biomarker to reflect body burden. Although different solvents were used in these studies, SPE method showed highly promising sensitivity in extraction of PAHs compounds. Moreover, GC–MS is still needed to determine the level of PAHs in the biological samples, and its use to analyse PAHs has been prevalent with robust results in many studies. Further

studies are demanded to identify the best solvents and SPE columns used for the extraction and clean-up procedures of PAHs. Also, these futuristic studies will provide extra information on the dose and retention of PAHs in biological samples as well as the toxic impacts of PAHs on the human health.

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