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Validation of HPLC method for determination of priority polycyclic aromatic hydrocarbons (PAHS) in waste water and sediments

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ABSTRACT

A simple method of extraction and determination of sixteen priority polycyclic aromatic hydrocarbons (PAHs) from waste water and sediment using high performance liquid chromatography (HPLC) has been validated with limits of detection (LOD) and limits of quantification (LOQ), method recovery and reproducibility and other factors. HPLC parameters, such as mobile phase composition and flow standardized for determination of PAHs using ultra violet-diode array detector (UV-DAD). PAH extraction was carried out by liquid-liquid and Ultrasonication using dichloromethane and acetone/hexane solvents for water and sediment, respectively. Silica gel column chromatography was carried out for extract clean-up. Linearity of calibration curves was good for all sixteen PAH (R^2, 0.991-0.996) in the concentration range 2.5-300 ppb. Analysis of standard spiked water and sediment samples resulted in good recoveries between 78-100 % and 82-106 %, respectively. The estimated LOD and LOQ ranged between 0.01-0.51 ppb and 0.03-1.71 ppb, respectively. The method described has been used for determination of the sixteen PAHs contents in water and sediment samples collected from municipal drains.

Key words: Priority PAHs, HPLC, Method validation, Waste water, Sediment

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INTRODUCTION

Polycyclic aromatic hydrocarbons or polyaromatic hydrocarbons are group of organic arene compounds composed of two or more aromatic benzene rings with molecular masses ranging from 128 Dalton to 278 Dalton. There are hundreds of polycyclic aromatic hydrocarbons (PAHs) in the environment, among them; sixteen PAHs have been classified as priority pollutants by the United States Environmental Protection Agency [1-2]. The sixteen priority PAHs includes naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo(a)pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene.

They are matter of concern because of their toxicity and tendency to accumulate in sediments, soils and through bioaccumulation, biomagnifications in the food chain [3]. Bioconcentration and bioaccumulation of PAHs in organisms occurs through various routes including ingestion, inhalation or dermal contact pathways. World Health Organization's International Agency for Research on Cancer (IARC) has classified the carcinogenicity of individual PAH compounds. PAHs are toxic and their carcinogenicity is initiated by their metabolic conversion to peroxides that bind covalently to cellular macromolecules, including DNA, causing an increase of elevated levels of DNA adducts and developing errors in DNA replication which cause carcinogenesis in both humans and other organisms [4-7].

PAHs are released to the environment predominantly from petroleum products (petrogenic sources) and anthropogenic activities of incomplete combustion processes involving coal, petroleum products and biomass (pyrogenic sources) [4]. Due to their low vapor pressure, non-polar, lipophilic and highly hydrophobic in nature, they are globally distribution in atmospheric, terrestrial and aquatic systems. Low molecular weight PAH compounds have the higher water solubility which decreases with increasing molecular mass. PAHs generally tend to be more easily adsorbed onto organic matter. In the environment, PAHs are readily associated with organic substances such as biopolymers, humic substances and black carbon. In the aquatic environment, however, they occur either as free molecules or associated with dissolved organic matter and particulate phases and finally in sediments.

Human exposure to PAHs occurs mainly through the consumption of contaminated food, and releases in the occupational environments. The amount of PAHs in water and sediments/soils and the close proximity to biota including humans lead to human exposure [8-9]. Therefore, assessments of levels of PAHs in different compartments of the environmental need much attention. Several analytical methods have been frequently used for determination of PAHs or their derivatives in the environment, biological or food matrices. Most frequently, chromatographic methods are GC or HPLC with mass spectrometry detection [10-17]. HPLC methods are more suitable for analysis of PAHs, as in comparison to GC, the thermally labile or low volatile compounds can be analyzed easily. Reliable analytical methods are required for compliance with national and international regulations [18]. The aim of this work was to validate a simple method for extraction of priority 16 PAH compounds in waste water and sediments using LLE and Sonication extraction techniques, respectively and quantification by high performance liquid chromatography with diode array detector (HPLC-DAD).

MATERIALS AND METHODS

Chemicals, Solvents and Standards

HPLC grade solvents (hexane, acetone and dichloromethane), sodium sulphate (AR grade), water (HPLC grade) and acetonitrile (HPLC gradient grade) procured from Fisher scientific and Merck, India used in processing and analysis. Solvents used in mobile phase were degassed by Sonication before use. Silica gel (100–200 mesh) procured from Supelco (Sigma-Aldrich, USA) was activated at 130 $^{\circ}$C for 16 h and used as absorbent during chromatographic column cleanup. Anhydrous sodium sulphate (Merck, India) was cleaned with solvents in Soxhlet and stored in the sealed desiccator. Individual standard solutions of 16 PAHs compounds [naphthalene (Npt), acenaphthene (ANe), acenaphthylene (ANy), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo(a)pyrene (BaP), dibenzo[a,h]anthracene (DBA), indeno[1,2,3-cd]pyrene (Ind), and benzo[g,h,i]perylene (BghiP)] and EPA's priority 610 mixture solutions were purchased from Supelco (Sigma-Aldrich, USA). Working standard solutions were prepared by diluting the stock solutions as required. Ultra pure water was prepared using a Mili-Q plus water purifications system.

Instrumentation

All the glassware was cleaned with detergent followed by deionised water and finally solvents rinse and dried in hot air oven. LLE and Sonication techniques using separating funnel and Ultrasonic water bath were followed for PAHs extraction from water and sediments, respectively. Vacuum rotary evaporator (Eyela, Tokyo, Japan), Turbovap (Caliper, USA) and Minivap (Supelco, USA) were used for extract concentrations. HPLC system (Series 1100, Agilent Technology Inc., Santa Clara, CA, USA) was used in combination with a quaternary solvent delivery system with vacuum degasser unit, auto sampler, column oven, and UV-DAD (ultra violet- diode array detector) (λ=254 nm) for the chromatographic analysis.

Table 1: Gradient flow of mobile phase with composition and time

Time (min)	Water (%)	Acetonitrile (%)	Flow (mL/min)
0.01	0	100	1.0
15	35	65	1.0
20	15	85	1.0
25	5	95	1.0
30	0	100	1.0
45	0	100	1.0
50	0	100	1.0

Analytical Conditions

The chromatographic separation was performed on a LC-PAH Supelcosil^{TM} , an analytical column (25cm x 4.6 mm, 5 µm film) and Eclipse XDB-C8 (4.6 x 12.5 mm, 5 µm film) as guard column by 20 µL sample injection and a gradient flow with acetonitrile and water @1.0 ml/min. The temperature in a column oven was set to 30◦C. Mobile phase with multi-step gradient elution conditions were used with total run time of 50 min per sample extract injection (Table 1).

Sample Extraction

One (1L) water sample was taken in separatory funnel and extracted by dichloromethane thrice (50, 30, 20 ml) by vigorous shaking for 2 min each. The organic phase was passed through anhydrous sodium sulphate to remove traces of water contents and the extracts were concentrated to near 5 ml by vacuum rotary evaporator (Eyela, Tokyo, Japan). The concentrated extract volume was reduced under gentle stream of purified nitrogen gas using Turbo Vap (Caliper, USA) and Minivap (Supelco, USA) and solvent exchanged to acetonitrile (1.0 ml).

Ultrasonication technique was used for PAHs extraction from sediment samples. During this process, approximately 20 g of sediment sample was thoroughly mixed with anhydrous sodium sulphate to get free flowing powder and extracted with 50 ml mixture of acetone-hexane (1:1 v/v) for 30 min in ultrasonic bath. After Sonication, the extracts were allowed to settle and solvent layer was filtered through a Whatman 41 filter paper. The process was repeated for two more times. The pooled solvent extract was concentrated to near 1.0 ml under reduced pressure in a 40 ^{0}C water bath using a rotary evaporator (Eyela, Tokyo, Japan).

Extract Cleanup

Clean up of the extract is required to remove co-extracted compounds that could interfere during the instrumental analysis. Sample extracts were cleaned using silica gel column chromatography. For this purpose, a glass chromatographic column (25 cm × 10 mm i.d.) packed with plug of glass wool, 10 g activated silica gel (100-200 mesh), 1 cm layer of sodium sulphate and plug of glass wool was used to separate the required analytes fraction from other interfering compounds. The concentrated extracts and two 2-ml hexane rinses of the sample flask were transferred on to top of the column. The column was first eluted with 30 ml of hexane containing aliphatic hydrocarbons and that was discarded. Subsequently, final elution was carried out with 35 ml of dichloromethane at the flow rate of ~2 ml min^{-1} and retained for PAHs quantification. PAHs containing fraction was concentrated to near 1 ml with rotary evaporator. An additional 20 ml hexane was added to the concentrated extracts and evaporated to remove traces of dichloromethane. Final extract was concentrated to 1 ml under gentle stream of pure nitrogen, using Turbo Vap (Caliper, USA) and Minivap (Supelco, USA) and immediately solvent exchanged to acetonitrile for PAH analysis by HPLC.

Basic Analytical Quality Control

The method was performed with quality assurance (replicate sample, instrument calibration verification and repeatability check of the instrument). Two replicate blanks were processed as real samples to check any cross contaminations or loss of the analytes. A set of working stock standard solution was prepared by diluting aliquots of the stock solutions. Calibration PAHs standard solutions were prepared by suitable dilution of the stock solutions with a mixture of acetonitrile/water (50:50, v/v) to give appropriate concentrations of each PAH compound (Table 2). Calibration standard solutions were stored at 4 ºC in the dark and were stable for approximately three months. Calibration of the instrument was carried out by injecting active amount of the five level PAHs concentrations as a function of peak area using linear fit. The peak identification of the analytes was conducted by the accurate retention time of each standard. Calibration was verified by analyzing the middle level calibration standard and the relative percent difference between the five-point calibrations (Table 2). The instrument was calibrated with every batch of sample analysis. The calibration curves followed the Beer's law in the investigation range of PAHs injected in the column. Other statistical data for instrument calibration, i.e. regression equation, standard deviation (SD) and the value of the multiple correlation coefficients (R^2) of the instruments are listed in Table 2. Measurements were repeated three times for each sample and the results were averaged and expressed relative to the average result for the method blank (concentration, <DL "BDL").

The method detection limits or limit of detection (LOD) and limit of quantification (LOQ) were calculated for a valid quantifiable peak at signal to noise ratio >3:1 and 10:1, respectively. Limit of detection (LOD) and limit of quantification (LOQ) were obtained by processing the eight aliquots of a spiked sample to produce a detectable response (s/n >3) and multiplying the standard deviation by 3 ($t_{students}$ value for eight replicates at 99% confidence

level) and 10, respectively. Statistically calculated detection limits for all sixteen PAHs were presented in Table 2. The method performance and matrix effects were checked by analyzing samples spiked with surrogate standard (1-fluoronaphthalene). The results of the recovery study are reported in Table 3.

Calculation of Results
The results were to be calculated as follows:

PAHs concentration (µg/l or µg/kg)=(AxB/C)

Where:
A* = Concentration of PAH obtained from instrument (ng)
B = Final extract volume (ml)
C = Initial sample volume taken (L or Kg)
*Based upon the average of 3 separate determinations of each solution. Blank value must be deducted.

Application of Method
To assess application of this method, determination of priority sixteen polycyclic aromatic hydrocarbons was carried out in municipal drain water and sediment samples. Water and sediment samples were collected from different sites of municipal drains in Delhi. The observed concentrations of individual PAH compounds were presented in Table 5.

RESULTS AND DISCUSSION

Sample Preparation and Cleanup
The objective of our work was to develop a simple, one-step cleanup procedure suitable for water and sediments. Water samples were prepared merely by manual extraction with dichloromethane. Sediment samples were homogenized with anhydrous sodium sulphate and the extraction was carried out using Sonication technique. Generally, clean water matrix does not required cleanup step. However, sediment sample extracts were clean by simple silica gel column chromatography to separate aromatic hydrocarbons from aliphatic hydrocarbons. Injection of unclean sample extracts resulted in generation of substantial backpressure in the analytical column, reduced retention reproducibility and interferences in peak identification [19]. The problem disappeared when silica gel cleanup was used and it was also found the method could be used on sediment samples. Both the methods are cost effective, easy to perform and used by several workers [11,20-22] for PAHs quantifications for environmental and human health risk assessment.

Instrumental Quantification
HPLC and GC methods have been considered to be equally valid approaches to analyse PAHs by USEPA and other environmental agencies. In this study, a simple HPLC method was validated for the separation and detection of PAH in the water and sediment samples. Separation of polycyclic aromatic hydrocarbons is usually performed on a reversed-phase column with acetonitrile–water mobile phases using ultraviolet-diode array detection [15-18,20,23]. Under these conditions separation is depends on retention of PAHs in column, which is proportional to sample molecular weight, i.e. hydrophobicity [24]. Gradient elution conditions of HPLC mobile phase are given in Table 1.

Method Validation Parameters
Validation of analytical method plays a major role in achieving consistent, reliable and accurate data during analytical measurements. Various parameters have been defined [18, 25-28] for the validation of analytical methods, which are described briefly in the following paragraphs.

Selectivity/Specificity:
Specificity is the ability of an analytical method to differentiate and quantify the analytes in the presence of other compound in the sample. Specificity in liquid chromatography is obtained by choosing optimal columns and setting chromatographic conditions, such as mobile phase composition, column temperature and detector wavelength. Besides chromatographic separation, the sample preparation step can also be optimized for best selectivity [18, 25, 28]. Optimized specific HPLC conditions were followed for selectivity by analyses of blank samples (pre-extracted water and sediment samples) in triplicates. Each blank sample tested for interference, and selectivity was lower than limit of detection (LOD). The specificity of the method was determined by analyzing the sample solution containing all the sixteen PAH compounds. For this purpose 20 µL of one of the sample solutions was injected into the HPLC

system and the specificity of the method was measured in terms of the resolution between the two peaks (retention times) without overlapping of the peaks (Table 2).

Table 2: HPLC performance parameters for quantification of priority sixteen PAHs

PAH Compounds	Ret. time (min)	Cal. range (ppb)	Cal. ver. (±%)	Regression Equation	Linearity (R^2)	LOD (ppb)	LOQ (ppb)
Naphthalene	7.75	25-150	0.03	y = 26.54x + 56.33	0.995	0.12	0.41
Acenaphthylene	9.01	50-300	0.08	y = 33.68x + 89.23	0.993	0.51	1.71
Acenaphthene	11.42	25-150	0.05	y = 10.65x + 34.15	0.992	0.31	1.03
Fluorene	12.04	5-30	0.01	y = 25.00x + 57.20	0.994	0.12	0.41
Phenanthrene	14.29	2.5-15	0.01	y = 32.98x + 86.65	0.993	0.03	0.11
Anthracene	16.97	2.5-15	0.01	y = 61.90x + 248.8	0.991	0.02	0.06
Fluoranthene	19.66	5-30	0.01	y = 14.00x + 29.60	0.995	0.01	0.03
Pyrene	21.39	2.5-15	0.01	y = 6.255x + 13.98	0.995	0.04	0.14
Benzo(a)Anthracene	25.15	2.5-15	0.01	y = 15.46x + 27.81	0.996	0.03	0.10
Chrysene	26.03	2.5-15	0.01	y = 22.67x + 46.30	0.996	0.02	0.06
Benzo(b)Fluoranthene	28.59	5-30	0.02	y = 35.10x + 64.21	0.996	0.02	0.05
Benzo(k)Fluoranthene	29.95	2.5-15	0.01	y = 10.63x + 20.99	0.996	0.04	0.14
Benzo(a)Pyrene	31.35	2.5-15	0.02	y = 11.80x + 22.28	0.995	0.02	0.06
Benzo(g,h,i)Perylene	33.23	5-30	0.13	y = 7.683x + 13.28	0.996	0.03	0.08
Dibenzo(a,h)Anthracene	34.78	5-30	0.01	y = 11.13x + 16.73	0.996	0.07	0.25
Indeno(1,2,3-Cd)Pyrene	35.84	2.5-15	0.01	y = 14.56x + 29.60	0.995	0.04	0.14

Calibration Range and Linearity:
In this method, the five point calibration curves were prepared with different concentration levels for different PAH compounds (Table 2). The calibration range of this method is the interval from the upper to the lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity. The minimum specified range is 80 to 120 percent of the test concentration. Calibration curve of individual PAH compounds function plausibly as linear, passes through the origin, and is unaffected by the matrix of the test material. Linearity of an analytical method is its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample. Linearity is determined by a series of five to six injections of five or more standards whose concentrations span 80–120 percent of the expected concentration range [25]. The observed linearity (R^2) ranged between 0.991-0.996 for all the sixteen PAH compounds, which is within acceptable range [29]. Linearity and matrix effects were tested by a plot of linear regression equation and standard addition method for individual PAHs (Table 2). A linear regression equation applied to the results should have an intercept not significantly different from zero. A significant nonzero intercept was obtained, which demonstrated that this linearity has no effect on the accuracy of the method.

Limit of determination and limit of quantification (LOD and LOQ):
To assess the sensitivity of the instrument the detection limits were calculated [25, 26, 28]. The Limit of detection (LOD) and limit of quantification (LOQ) were obtained by processing the eight aliquots of a spiked sample with smallest quantity of the standard materials to produce a valid quantifiable peak at signal to noise ratio >3:1 (s/n >3) for a 20 µL of injection. The LOD was calculated as per the USEPA method. LOD was calculated as:

Limit of detection (LOD)=Std Dev X $t_{student}$

Where $t_{student}$ is n-1 (degree of freedom and n is number of observations) at 99% confidence level. The limit of quantification (LOQ) was calculated for a valid quantifiable peak at signal to noise ratio >10:1. The LOQ was determined as:

LOQ=Std Dev X 10

The calculated LOD and LOQ ranged between 0.01-0.51 ppb and 0.03-1.71 ppb, respectively (Table-2).

Precision:
Precision is the closeness of agreement between independent test results obtained under validated conditions. It is usually specified as standard deviation or relative standard deviation. For single-laboratory validation, two sets of repeatability conditions were tested (a) precision observed during a single run, and (b) precision observed during

run-to-run conditions. The observed standard deviation during single run of known standard ranged between 0.01-0.13 percent (Table 2). Repeatability test was carried out for water and sediment replicate sample analysis (Table 3). The standard deviation (SD) for water and sediment repeat analysis ranged between 0.03-0.07 and 0.01-0.17, respectively and their relative standard deviation (RSD) (1.39-2.02 and 0.11-0.50, respectively) was less than acceptable limit of central value [26].

Table 3: Instrument repeatability for sixteen PAHs spiked water and sediment samples

PAH Compounds	**Water (n=7)** (ppb)			**Sediment (n=8)** (ppb)		
	Mean	SD	RSD	Mean	SD	RSD
Naphthalene	1.95	0.03	1.55	24.24	0.04	0.17
Acenaphthylene	3.94	0.07	1.73	55.13	0.17	0.31
Acenaphthene	2.03	0.03	1.59	49.59	0.10	0.21
Fluorene	3.86	0.07	1.70	25.36	0.04	0.16
Phenanthrene	1.95	0.03	1.69	4.92	0.01	0.22
Anthracene	2.04	0.03	1.65	2.48	0.01	0.22
Fluoranthene	3.92	0.07	1.74	2.57	0.00	0.11
Pyrene	1.95	0.04	2.02	4.92	0.01	0.29
Benzo(a)Anthracene	1.96	0.04	1.83	2.48	0.01	0.41
Chrysene	1.96	0.03	1.61	2.46	0.01	0.24
Benzo(b)Fluoranthene	3.93	0.07	1.71	2.49	0.00	0.20
Benzo(k)Fluoranthene	2.00	0.03	1.72	4.90	0.01	0.29
Benzo(a)Pyrene	2.00	0.04	1.99	2.48	0.01	0.25
Benzo(g,h,i)Perylene	3.90	0.06	1.44	2.34	0.01	0.35
Dibenzo(a,h)Anthracene	3.88	0.05	1.39	4.99	0.02	0.50
Indeno(1,2,3-Cd)Pyrene	1.94	0.03	1.49	4.86	0.01	0.28

Table 4: Recovery study data for priority 16PAHs in water and sediment sample (n=8)

PAH compounds	**Spiked level** (ppb)	**Water (%)**			**Sediment (%)**		
		Range	Mean	SD	Range	Mean	SD
Naphthalene	50	76-80	78	3.20	78-89	82	11
Acenaphthylene	100	82-101	89	3.86	91-101	95	5.22
Acenaphthene	50	79-102	85	5.83	92-101	96	4.36
Fluorene	10	93-104	98	3.84	93-100	96	3.88
Phenanthrene	5	82-101	95	6.36	92-101	95	4.60
Anthracene	5	83-102	96	6.60	92-101	94	6.03
Fluoranthene	10	91-101	99	3.18	90-101	96	4.55
Pyrene	5	90-101	97	3.18	93-101	96	4.58
Benzo(a)Anthracene	5	94-101	97	2.10	93-101	95	4.15
Chrysene	5	85-101	95	5.36	90-113	100	14.68
Benzo(b)Fluoranthene	10	95-101	98	2.23	94-101	96	4.64
Benzo(k)Fluoranthene	5	85-101	97	5.38	92-100	96	4.50
Benzo(a)Pyrene	5	96-102	100	4.45	92-101	96	5.35
Benzo(g,h,i)Perylene	10	90-103	96	4.76	91-101	96	5.50
Dibenzo(a,h)Anthracene	10	90-126	97	12.27	93-105	104	11.03
Indeno(1,2,3-Cd)Pyrene	5	83-101	94	7.24	91-100	96	4.56
1-Fluoronaphthalene*	50	96-101	99	2.03	101-109	106	3.34

**surrogate standard*

Accuracy or Recovery in terms of Trueness or Bias:

Accuracy of an analytical method is the closeness of agreement between the conventional true value or an accepted reference value and the value found. Trueness or bias was determined by comparing the response of the method to a reference material with the known value assigned to the material. In this study 1-fluoronaphthalene and known concentrations of individual PAH compounds were spiked and analysed as real sample. The average recovery of 1-fluoronaphthalene was 99% and 106%, respectively in water and sediment matrix and assumed to be satisfactory. The recoveries of individual sixteen PAHs varied from 78 (±2.23%) to 100 (±12.27%) and from 82 (±3.88%) to100 (±14.68%), respectively for water and sediment matrices. The average percent recovery was calculated using the following equation:

Recovery (%) = ($Concentration_{observed}$/$Concentration_{spiked}$) x 100

Where, $Concentration_{observed}$ is the concentration observed in the samples and $Concentration_{spiked}$ is the initial concentration spiked to the sample. Obtained method spiking/recovery data for individual PAHs was presented in detail in Table 4.

Measurement of PAHs in Water and Sediment

This method was used for extraction and determination of sixteen priority PAHs in municipal drain water and sediments from Delhi region. Observed concentrations of individual and total 16PAHs were presented in Table 5. The total 16PAHs concentration in water and sediments ranged between 5.87-35.32 µg L^{-1} and 921-18795 µg kg^{-1}, respectively. The frequently detected PAHs in water samples were fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]-fluoranthene and benzo[k]fluoranthene. However, in sediment samples, naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo(a)pyrene, indeno[1,2,3-cd]pyrene, and benzo[g,h,i]perylene detected in most of the sediment samples.

PAHs concentrations observed in municipal drains water and sediments from Delhi were compared with the recent measurements around the world including India. The total concentrations of 16 PAHs in water samples collected from municipal drains were lower than those reported for various surface water from India [30-31] and other countries [17,23,32-34]. In some other studies, low concentrations of PAHs were reported for water samples from India [35-37] and other countries [14-15, 38-42]. Concentrations in sediments were found to be lower than reported by other workers from India [30,43-47] and world [22,33,40,48-49]. However, lower concentrations have been reported in other studies from India [30,50-51] and World [14,42].

Table 5: Concentrations of priority sixteen PAH compounds in municipal drain water and sediments

PAH compounds	Water (ppb)						Sediments (ppb)					
	Site-1	Site-2	Site-3	Site-4	Site-5	Site-6	Site-1	Site-2	Site-3	Site-4	Site-5	Site-6
Naphthalene	1.13	BDL*	BDL	5.34	0.35	BDL	148	390	19	BDL	41	1090
Acenaphthylene	1.85	3.83	BDL	BDL	3.58	6.64	BDL	3690	BDL	BDL	BDL	1860
Acenaphthene	BDL	9.05	BDL	7.34	BDL	BDL	BDL	1310	BDL	BDL	92	1260
Fluorene	0.60	1.25	1.20	5.05	10.16	2.18	146	280	BDL	50	105	310
Phenanthrene	0.80	0.48	1.08	1.05	1.26	0.69	319	1250	108	360	346	990
Anthracene	0.18	0.17	0.63	0.90	0.71	0.51	71	340	19	60	91	280
Fluoranthene	0.75	BDL	1.08	2.56	BDL	0.47	419	1060	256	400	777	1000
Pyrene	BDL	BDL	0.59	0.38	1.01	BDL	277	1290	72	500	960	920
Benzo(a)Anthracene	BDL	BDL	0.75	0.91	0.50	0.35	167	680	152	110	855	290
Chrysene	0.21	5.41	BDL	0.13	BDL	0.13	114	420	102	80	772	160
Benzo(b)Fluoranthene	0.31	0.25	BDL	0.59	BDL	0.23	139	411	100	90	1618	110
Benzo(k)Fluoranthene	3.41	3.97	0.55	3.38	BDL	1.66	291	1240	BDL	30	1301	110
Benzo(a)Pyrene	BDL	BDL	BDL	1.39	0.30	0.45	114	940	47	80	1294	60
Benzo(g,h,i)Perylene	BDL	1.47	BDL	6.30	BDL	2.31	1245	2790	BDL	70	7455	250
Dibenzo(a,h)Anthracene	BDL	BDL	BDL	BDL	BDL	BDL	230	2840	BDL	BDL	1840	220
Indeno(1,2,3-Cd)Pyrene	BDL	BDL	BDL	BDL	BDL	BDL	220	390	45	50	1248	110
Total 16PAHs	**9.24**	**25.88**	**5.87**	**35.32**	**17.85**	**15.62**	**3900**	**19321**	**920**	**1880**	**18795**	**9020**

**BDL-Below detection limit*

CONCLUSION

A validated HPLC method has been developed for the extraction and determination of priority sixteen polycyclic aromatic hydrocarbons in waste water and sediments. Method described in this paper has shown the analytical precision, accuracy, sensitivity and selectivity for the determination of sixteen polycyclic aromatic hydrocarbons in waste water and sediments. The developed method was successfully applied to the quantitative analysis of sixteen polycyclic aromatic hydrocarbons in the waste water and sediments.

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