Short Communication

Comparison of five different HPLC columns with different particle sizes, lengths and make for the optimization of seven polycyclic aromatic hydrocarbons (PAH) analysis

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Abstract
The aim of this study is to evaluate the performance of five different analytical columns for the analysis of seven carcinogenic polycyclic aromatic hydrocarbons namely Benz[*a*]anthracene, Chrysene, Benzo[j]fluoranthene, Benzo[*e*]Pyrene, Benzo[*k*]fluoranthene, Benzo[*a*]Pyrene, Dibenzo[*a*,*h*]anthracene and select the best column in terms of separation, peak shape and analysis time and to use the selected column under optimized analytical condition for the detection and estimation of polycyclic aromatic hydrocarbons (PAHs) in light cycle oil; one of aromatic rich petroleum stream. The performance of five different analytical columns with different lengths, particle sizes and make were compared for analysis of above PAHs using HPLC with PDA detector and monitoring the HPLC–UV signals at 254 nm wavelength. Chromatographic parameters including retention time (t_R), resolution (R), limit of detection, limit of quantification, number of theoretical plates (N), height equivalent to theoretical plate (HETP) and reduced plate height (h) were evaluated and compared on mentioned columns for analysis of PAHs under study. The mobile phase acetonitrile (95%):water (5%) was used at flow rate of 1.5 mL/min and the injection volume in all the case was 20.0 µL. The best results w.r.t time and acceptable resolution were obtained when these PAHs were analyzed using the shorter Agilent Eclipse PAH column having 1.8 µm, 100 mm × 4.6 mm id.

Keywords Chromatography · HPLC columns · UV–visible detector · PAH

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) or polynuclear aromatic hydrocarbons are organic compounds that are mostly present as colorless, white, or pale yellow solids [1]. Polycyclic aromatic hydrocarbons consist of two or more single fused aromatic rings with a pair of carbon atoms shared between rings in their molecules. PAHs with six fused aromatic rings are often known as "small" PAHs, and those with more than six aromatic rings are called "large" PAH. The general characteristics of PAHs are high melting points, boiling points, occurrence as solids, low vapor pressure and low aqueous solubility that further decrease with increasing molecular weight. PAHs have good solubility in organic solvents because they are highly lipophilic.

Generally, PAHs can be formed during incomplete combustion of organic matter from both natural combustion sources (forest and brush fires, volcanic eruption) or man-made combustion sources (automobile emissions, cigarette smoke, and aircraft). The food processing include (such as drying and smoking) and cooking of food at high temperatures (grilling, roasting, frying) are major sources of PAH production. Contamination of vegetable oils (including olive residue oils) with PAH usually occurs during technological processes like direct fire drying. Polycyclic aromatic hydrocarbons (PAHs) can be formed in

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processes of production/distillation of high-boiling petroleum products as a result of thermal cracking.

They are omnipresent in environment and formed of group of several chemical compounds, they are persistent in environment with different structures and varied toxicity [2–6]. They have toxic effects and cause inhibition in the function of cellular membranes as well as with enzyme systems in organism and they also affect environment (air, water and soil). PAHs can cause carcinogenic, mutagenic, teratogenic effects [7, 8] and are potent immunesuppressants. The International Agency for Research on Cancer classifies some PAHs as carcinogenic to humans (Group 1, 2A or 2B). It is because of harmful effect of some of PAHs, that different regulatory bodies have put restrictions on their content in various industries. In this context, it becomes imperative to have methods that may help in precise detection and quantitative estimation of PAHs in different matrices.

The institute of petroleum standard IP346 uses a standard procedure [9] for the isolation and determination of PAH in polycyclic aromatic hydrocarbons (PCA) containing fraction in unused additive free lubricating base oils which is widely used all over the world. The procedure is based on a gravimetric determination of the fraction soluble in dimethyl sulfoxide at room temperature. However this method gives information on total PAH content and does not tell anything about specific PAHs. ISO/TS 16190 [10] illustrates a method quantitative evaluation of polycyclic aromatic hydrocarbons (PAH) in footwear materials by using *n*-hexane at 60 °C in an ultrasonic bath for 1 h to take out the test material. An aliquot is then analysed using either GC with MS or HPLC without prior purification of the sample extract. EN method 16143 establishes a scheme for the determination of benzo[*a*]pyrene and other selected PAHs in petroleum products [11]. The product is dissolved in *n*-pentane and submitted to a double cleaning step using silica-based column chromatography. The final extract is then analysed by unit resolution GC–EIMS.

Historical development on various analytical methods in liquid chromatography (LC) and gas chromatography (GC) for determination of 16 U.S. EPA priority Polycyclic Aromatic Hydrocarbons (PAHs) has been reported by Wise et al. [12]. The current researches for the determination of PAHs in the environment include gas chromatography with flame ionization detection (GC/FID) [13], gas chromatography with mass spectrometry [14] and high-performance liquid chromatography (HPLC) [15] with ultraviolet or fluorescence detection. While GC/FID is the more sensitive technique, it is subject to background interferences from other carbonaceous sources. HPLC is the preferred method of analysis [16], because it provides the necessary sensitivity in combination with higher specificity. These experiments are, however, very difficult and time and solvent-consuming, and, because they involve long and complex procedures, are unsuitable for routine analysis. Thus rapid and accurate analyses of specific PAHs are required.

The most widely used technique for the separation of the PAHs is HPLC. Although separation can be achieved with both normal and reversed phase columns, reverse phase systems are commonly used. Among the different detection technologies, fluorescence detection [17] is most often used in the analysis of PAHs since PAH exhibit natural fluorescence.

In recent years significant improvements have been introduced in the analysis of PAHs especially in column chromatography. The use of packing materials with particle sizes < 2 µm and the use of chromatographs that can support very high pressure have led to dramatic reduction of analysis times. Although promising results have obtained with these adaptations the technology is not often available in many laboratories due to the high cost implications.

To overcome this problem, recently, many manufacturers are producing packing materials with, C18; Kinetex, green PAH, eclipse PAH etc. to offer faster and efficient separation without causing much increase in pressure of system.

The aim of this study was to assess the performance of five different columns for the analysis of seven polycyclic aromatic hydrocarbon such as Benz[*a*]anthracene, Chrysene, Benzo[*j*]fluoranthene, Benzo[*e*]Pyrene, Benzo[*k*] fluoranthene, Benzo[*a*]Pyrene, Dibenzo[*a*,*h*]anthracene (Fig. 1). These priority PAHs were chosen for this study because these PAHs are present in various petroleum stream [18] and are presumed carcinogen (Group 1B) according to CLP classification [19]. The limit of detection (LOD), the limit of quantification (LOQ), resolution between the peaks and other chromatographic parameters [number of theoretical plates (N), height equivalent to a theoretical (HETP) and reduced plate height (h) were evaluated. The different columns were assessed for the analysis of targetted PAHs in petroleum matrix of LCO obtained from refinery's processes. Light cycle oil (LCO) is a middle distillate petroleum stream generally produced from the petrochemical industries and Refineries [20, 21]. The high content of the aromatic compound and sulfur nitrogen make it unattractable choice as a fuel. But high level of aromatic compound in LCO also make it high value aromatic product feed stock specially, Benzene, Toulene, Xylene.

2 Materials and methods

2.1 Reagents and standards

Acetonitrile, dicholromethane and *n*-Hexane (free from PAHs) were obtained from M/s Rankem, India. Highly pure

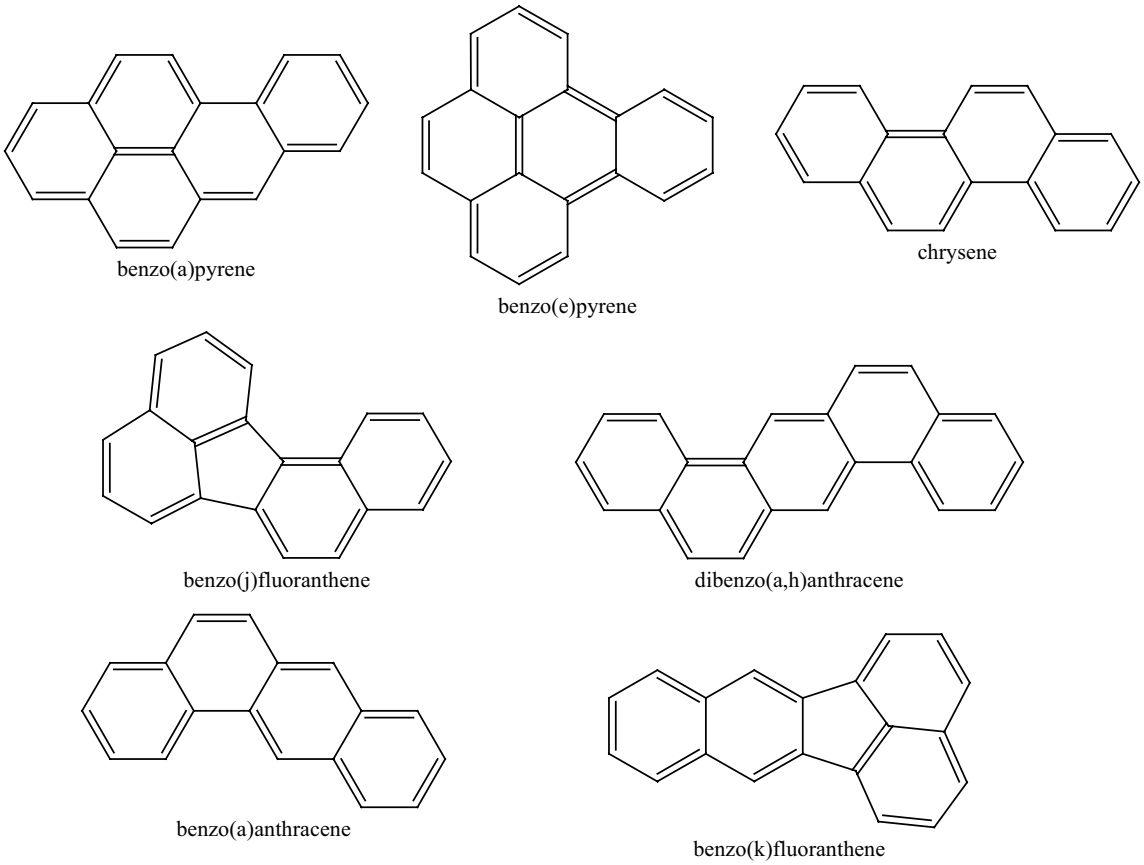


Fig. 1 Structure of some targeted PAHs used in this comparative study

(> 99%) Benz[*a*]anthracene, Chrysene, Benzo[j]fluoranthene, Benzo[*e*]Pyrene, Benzo[*k*]fluoranthene, Benzo[*a*]Pyrene, Dibenzo[*a*,*h*]anthracene were procured from M/s Aldrich Chemical Company, Inc., Milwaukee, WI, USA and used directly without any purification. All solvents were of HPLC grade. Pure water was obtained from a Milli-R/Q water system (Millipore, Bedford, MA, USA).

2.2 Standard preparation

Stock solution of above PAH standards were prepared in the range of 90–110 ppm in hexane at 30 °C by weighing appropriate weight. Appropriate volume of stock solutions of these polycyclic aromatic hydrocarbons were placed in 10 mL volumetric flask and were let to dry under gentle stream of N_2 at 35 °C. After drying these STD were diluted (1.0–0.0625 ppm) using Acetonitrile:water mixture in the ratio of 80:20.

2.3 Instrumentation

HPLC (Dionex UltiMate 3000/DAD-3000/WPS-3000, M/s Dionex Corporation, Sunnyvale, California, USA) with the suitable diode array detector and autosampler was used for polycyclic aromatic hydrocarbon (PAH) analysis at 254 nm wavelength. Isocratic mobile phase acetonitrile (CH_3CN) and water (95%):(5%) was used at flow rate was 1.5 mL/min and the injection volume in all the cases was 20.0 µL. Separation was achieved within 5–10 min including column cleaning. The equipment was controlled and the resulting data processed using Chromeleon 7.0 chromatography data system software.

Five different columns were used for this study:

(1) Phenomenex Kinetex C_{18}, 5 µm, 250 mm × 4.6 mm (M/s Phenomenex, Inc., Torrance, CA, USA)
(2) Dionex C_{18}, 5 µm, 250 mm × 4.6 mm (M/s Dionex Corporation, Sunnyvale, California, USA)
(3) Hypersil Green PAH 5 µm, 250 mm × 4.6 mm (M/s Thermofisher Scientific, Waltham, Massachusetts, USA)
(4) Agilent Eclipse PAH column 5 µm, 250 mm × 4.6 mm (M/s Agilent Technologies, Inc., Santa Clara, California, USA)

(5) Agilent Eclipse PAH column 1.8 µm, 100 mm × 4.6 mm (M/s Agilent Technologies, Inc., Santa Clara, California, USA)

2.4 Chromatographic parameters calculations [22]

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using Eqs. (1) and (2) respectively:

$$LOD = 3.3 \times \sigma/s \quad (1)$$

$$LOQ = 10 \times \sigma/s \quad (2)$$

where s is slope of calibration curve and σ is the standard deviation of response.

The chromatographic resolution (Rs) between the peaks was calculated using Eq. (3):

$$Rs = t_{R2} - t_{R1}/0.5\left(t_{w1} + t_{w2}\right) \quad (3)$$

where t_R is the retention time and t_w are the tangents' width of the peak at the base line.

The number of theoretical plates (N) was calculated using Eq. (4):

$$N = 16\left(t_R/W_b\right)^2 \quad (4)$$

where t_R is the retention time and W_b is the width of the peak at the base line.

The Van-Deemter equation describes the height equivalent to a theoretical plate (HETP) and was calculated in order to compare between different columns using Eq. (5):

$$HETP = L/N \quad (5)$$

where L is the length of the column and N is the number of theoretical plates. And finally, the reduced plate height (h), that is a dimensionless parameter that allows the direct comparison of the efficiency of two or more columns packed with different particle size packing materials, was calculated using Eq. (6):

$$h = HETP/dp \quad (6)$$

where dp is the mean particle size (µm).

2.5 LCO sample preparation

Flow diagram for the LCO sample preparation is given in Fig. 2. An open column is filled with the 50 g activated silica by wet slurry method. Activated silica is mixed with the hexane and poured into the column taking care to avoid the air gap in the silica bed filling. A 2 g of the LCO sample by weight is loaded to the column over the silica bed. Enough care was taken to form a very thin bed of sample. Now the sample loaded column is ready for the chromatography.10 mL of mobile phase (hexane) is poured onto the ready silica column containing LCO sample and the eluent is collected in weighed beaker by opening the knob of the glass column. This is collected as fraction 1st. About 25 such fractions of 10 mL each were collected separately in different beaker. The fractions were checked using HPLC for presence of different hydrocarbon classes (Saturates, monoaromatics, diaromatics or Polycyclic aromatic hydrocarbons). The fraction found from 1 to 12 (about 120 mL) mainly contains saturates. After this started elution of monoaromatics then diaromatics and in the last polycyclic aromatic hydrocarbons. The polycyclic aromatic hydrocarbons fraction is taken for the preparation of 1 ppm sample solution by taking appropriate weight and drying the hexane and make up with the mixture of acetonitrile and water (80:20) and added few drop of DCM.

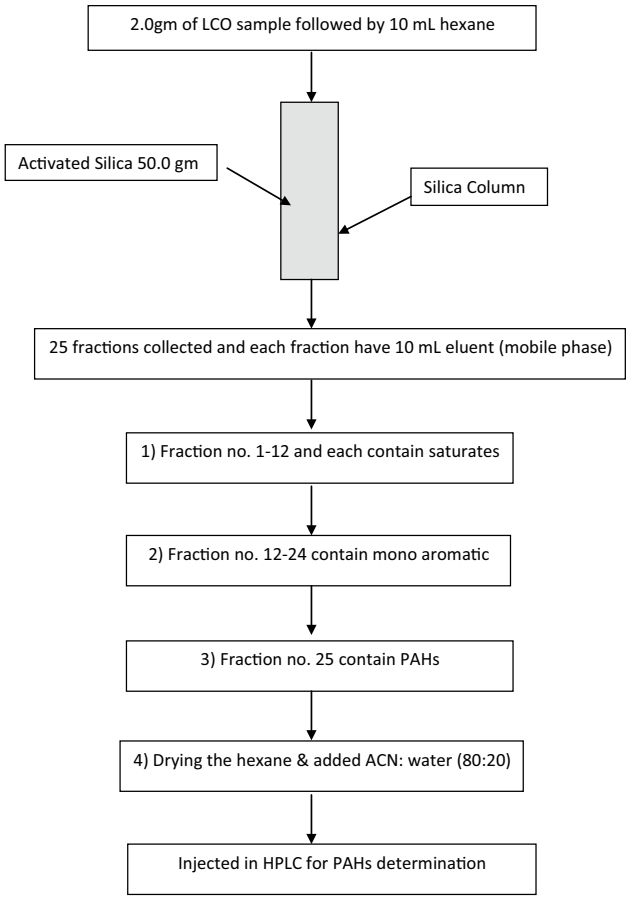


Fig. 2 Flow diagram for the LCO sample preparation

3 Results and discussion

3.1 Retention times

The different retention times obtained, for different concentrations of PAHs with different columns, were

studied. A chromatogram obtained by the injection of PAHs on different columns can be seen in Fig. 3. Retention times for all seven PAHs and five columns studied are shown in Tables 1 and 2.

Evaluation between retention times found from different C_{18} column chemistries showed that slight reduction of retention times from 5.6 to 3.09 min is obtained from Dionex C_{18} column to Kinetex C_{18} column. Benzo[*a*]anthracene and chrysene coelute on Dionex C_{18} column (at 4.2 min) as well as on Kinetex C_{18} column (at 2.58 min). Similarly benzo[*a*]pyrene, and dibenzobenzo[*a*,*h*]anthracene are found to coelute on Dionex C_{18} column (at 5.6 min) and Kinetex C_{18} column (at 3.09 min). Hence above two columns are not suitable for the effective separation of seven PAHs at given chromatographic conditions (Table 1). Comparison between retention times obtained from different PAH columns showed that reduction of particle size from 5 to 1.8 µm led to impressive reduction of the retention times from 7.14 to 2.47 min.

The average reduction in retention time in % terms while shifting from eclipse 5 µm column to eclipse 1.8 µm for different PAHs is as mentioned- Benzo[*a*]anthracene 63.94%, Chrysene 64.08%, Benzo[j]fluoranthene 64.91%, B[*e*]P 65.09%, B[*k*]F 65.06%, B[*a*]P 65.27%, Dibenzo[*a*,*h*] anthracene 65.40%. This allowed the reduction of total analysis time by 5 min (approximately 65%) excluding column cleaning time. The best results w.r.t time and acceptable resolution were obtained when these PAHs were analyzed using the shorter Agilent Eclipse PAH column having 1.8 µm particle size, 100 mm length and 4.6 mm id. This reduction in retention times allowed an even higher decrease in the total analysis time per sample from 10 to 5 min (including column cleaning).

3.2 Linearity and limit of detection (LOD) and limit of quantification (LOQ)

Calibration curves were built with data obtained from all three PAH columns (Agilent Eclipse PAH column 1.8 µm; Agilent Eclipse PAH column 5 µm and Hypersil Green PAH 5 µm) and data points fitted to a straight line using Microsoft Excel (Fig. 4). For three PAH columns and all PAHs the R^2 values are found to be in the range of ≥ 0.991, showing remarkable linear response of the UV detector in the range of the concentrations tested

LOD and LOQ were calculated [23, 24] and the results are shown in Table 3. Substantial enhancement in both LOD and LOQ were observed with decrease of particle size of column. The PAH can be estimated at trace level up to 0.0001 ppm using the shorter Agilent Eclipse PAH column having 1.8 µm particle size, 100 mm length and 4.6 mm id.

3.3 Columns performance

The chromatographic parameters calculated after the injection of various PAH standard concentration with different columns are shown in Table 4.

3.3.1 Columns resolution

Regarding the peak resolution, the best results w.r.t acceptable resolution and analysis time for all PAHs were obtained with the column containing particle size 1.8 µm and 100 mm length (Eclipse PAH). The resolution values are ≥ 1.5 for all PAH except between the Benzo[j]fluoranthene and Benzo[*e*]pyrene for which it is found to be 1.11. Generally the Resolution values ≥ 1.5 are considered appropriate for good analysis, demonstrating that the peaks will be effectively separated. The peak resolution values obtained between different PAHs are tabulated in Table 5. It can be seen that the peaks were effectively resolved on all three columns namely 1.8 µm eclipse PAH, 5 µm eclipse PAH and 5 µm Hypersil green PAH. From the table we note that the best performances were obtained from the column 1.8 µm particle size.

3.3.2 Number of theoretical plates and height equivalent to a theoretical plate

The numbers of theoretical plates increase with the reduction of particle size and with the increase in the length of column. The outcome is less apparent when we compared three columns having variation in both particle size and column length. For Eclipse PAH column having particle size 1.8 µm and length 100 mm, while the comparative lower particle size favors increase in theoretical plate, at the same time its lower length (40% as compared to other two columns under study) works to decrease the theoretical plate. The number of theoretical plate and HETP is gathered for all three columns and tabulated in Table 4. It can be seen, despite smaller length (which tends to decrease the HETP value), the column with 1.8 µm particle size is found to have lower value of HETP or we can say, it is found to perform better as compared to other two column.

3.3.3 Comparison of the reduced plate heights

The reduced plate height; a dimensionless parameter had been introduced by Giddings [25] that allows the direct comparison of the efficiency of two or more columns packed with different particle size packing materials. According to the theory, a well packed column should have a reduced plate height (h) in the range of 2–3. It also depends on other factors, such as the velocity of the mobile phase through the porous matrix. The

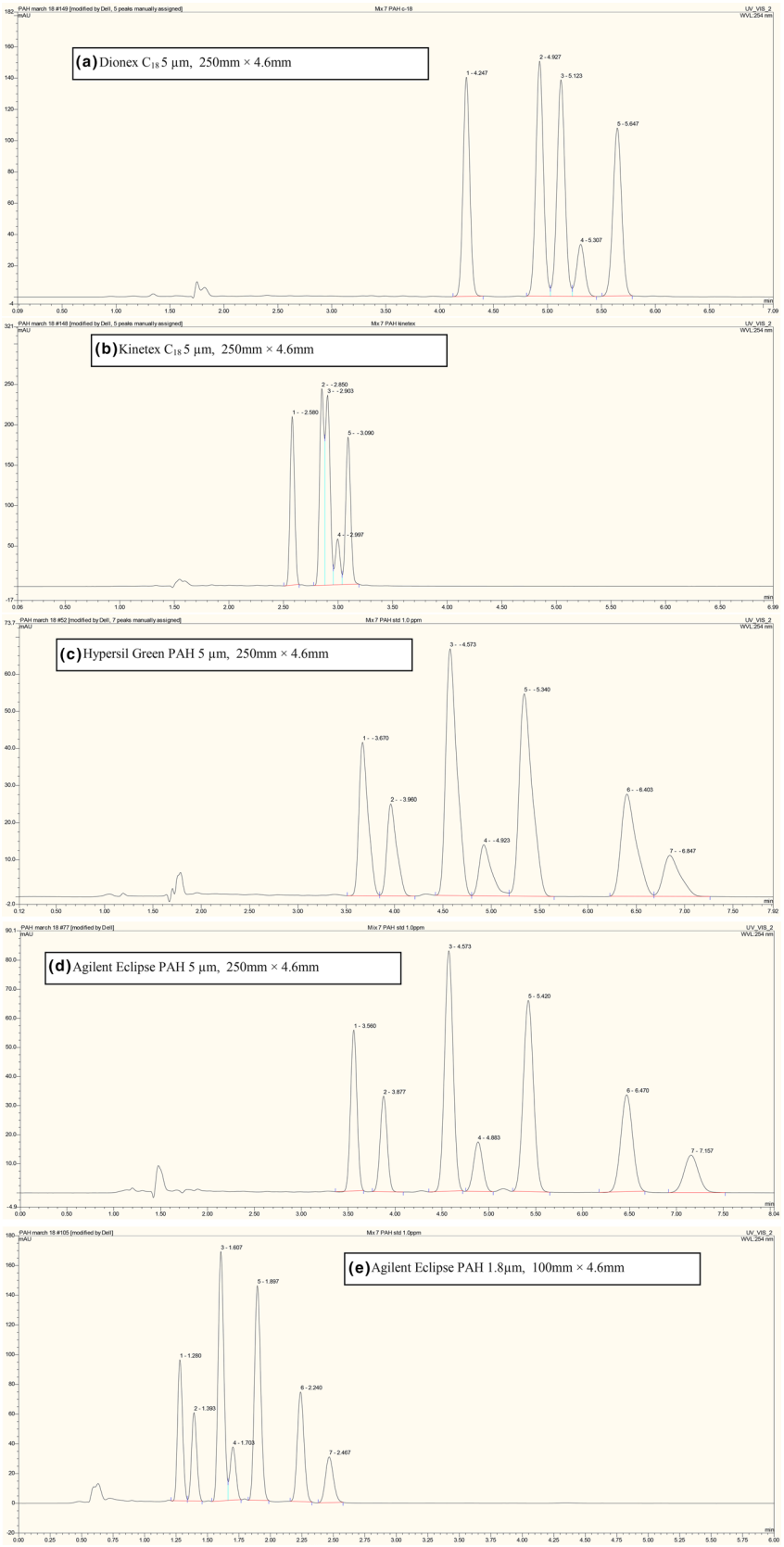


Fig. 3 HPLC-UV(254 nm) chromatograms (**a**–**e**) of a standard solution containing benzo[*a*]anthracene, chrysene, benzo[*j*]fluoranthene, benzo[*e*]pyrene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, and dibenzobenzo[*a*,*h*]anthracene on different columns

Table 1 Column comparisons of benzo[*a*]anthracene, chrysene, benzo[j] fluoranthene, benzo[*e*] pyrene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, and dibenzobenzo[*a*,*h*]anthracene the RT obtained with column with different particle size, length and make

Column name and dimension	Retention time		HPLC observations
	Dionex[b] C_{18} 5 µm	Kinetex[a] C_{18} 5 µm	
PAH compounds			
1 Benzo[*a*]anthracene	4.247	2.580	Peak Co elute
2 Chrysene	4.247	2.580	
3 Benzo[j]fluoranthene	4.927	2.850	
4 Benzo[*e*]pyrene	5.123	2.903	
5 Benzo[*k*]fluoranthene	5.307	2.997	
6 Benzo[*a*]pyrene	5.647	3.090	Peak Co elute
7 Dibenzobenzo[*a*,*h*]anthracene	5.647	3.090	

Average values from 5 different injections with different concentrations

[a]Phenomenex Kinetex C_{18}, 5 µm, 250 mm × 4.6 mm

[b]Dionex C_{18}, 5 µm, 250 mm × 4.6 mm

Table 2 Column comparisons using retention time of benzo[*a*]anthracene, chrysene, benzo[j]fluoranthene, benzo[*e*] pyrene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, and dibenzobenzo[*a*,*h*]anthracene with respect to particle size, length and make

Column name and dimension	Agilent 1.8[a] µm		Agilent 5[b] µm		Hypersil 5[c] µm	
	t_R	± SD	t_R	± SD	t_R	± SD
PAH compounds						
1 Benzo[*a*]anthracene	1.277	0.001	3.557	0.003	3.670	0.003
2 Chrysene	1.393	0.002	3.877	0.004	3.963	0.003
3 Benzo[j]fluoranthene	1.607	0.003	4.563	0.004	4.577	0.005
4 Benzo[*e*]pyrene	1.703	0.002	4.876	0.006	4.923	0.004
5 Benzo[*k*]fluoranthene	1.897	0.003	5.417	0.005	5.343	0.006
6 Benzo[*a*]pyrene	2.237	0.003	6.452	0.007	6.407	0.008
7 Dibenzobenzo[*a*,*h*]anthracene	2.467	0.003	7.143	0.007	6.853	0.012

The data is collected using average values from 5 different injections with different concentrations

[a]Agilent Eclipse PAH column 1.8 µm, 100 mm × 4.6 mm

[b]Agilent Eclipse PAH column 5 µm, 250 mm × 4.6 mm

[c]Hypersil Green PAH 5 µm, 250 mm × 4.6 mm

results obtained from the analysis of the standard solutions show that further development of the method would be possible by varying the flow rate. With this approach "h" would have been optimized. However, it should be noted that we propose the analysis of seven PAHs in single run, with the first compound having a shortest retention time is observed to have h = 12.09, h = 4.04 and h = 7.27 for the columns with 1.8 µm eclipse PAH, 5 µm eclipse PAH and 5 µm Hypersil green PAH particles respectively. The flow rate was fixed at 1.5 mL/min bearing in mind that at this flow rate we get acceptable level of resolution (peak resolution ≥ 1.5) for most of PAH. Further increase in flow rate will compromise the peak resolution and decrease in flow rate will enhance the analysis time and both of these potential effects are undesirable. Despite the velocity of mobile phase could have been lower and the h values obtained were not optimum, the 1.8 µm particles column presented higher performance and shorter analysis time for the separation of these seven PAH compounds.

3.4 Analysis of poly aromatic fraction of LCO sample

Due to its excellent performance in the analysis of seven PAHs standards the Agilent Eclipse PAH having these dimension 1.8 µm particle size, 100 mm length and 4.6 mm id, was selected for analyzing LCO fraction for PAH identification. Four carcinogenic PAHs were identified in LCO sample based on retention time and these were benzo[j] fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, and dibenzobenzo[*a*,*h*]anthracene (Fig. 5). Since in HPLC–UV many PAHs can co-elute at same RT, we have used the PDA spectra fpr unambiguous assignmement. In PDA detector, each of PAHs has characteristic and unique spectral profile. UV–Vis profile is used for quick scanning of the sample for presence of PAHs under study. Presence of signal at the retention time of PAHS, indicate the possibility of presence of the corresponding PAHs. The peak at this RT is scanned for PDA profile to unambiguously to see the presence/absence of PAHs under study. Corresponding UV–Vis spectrum

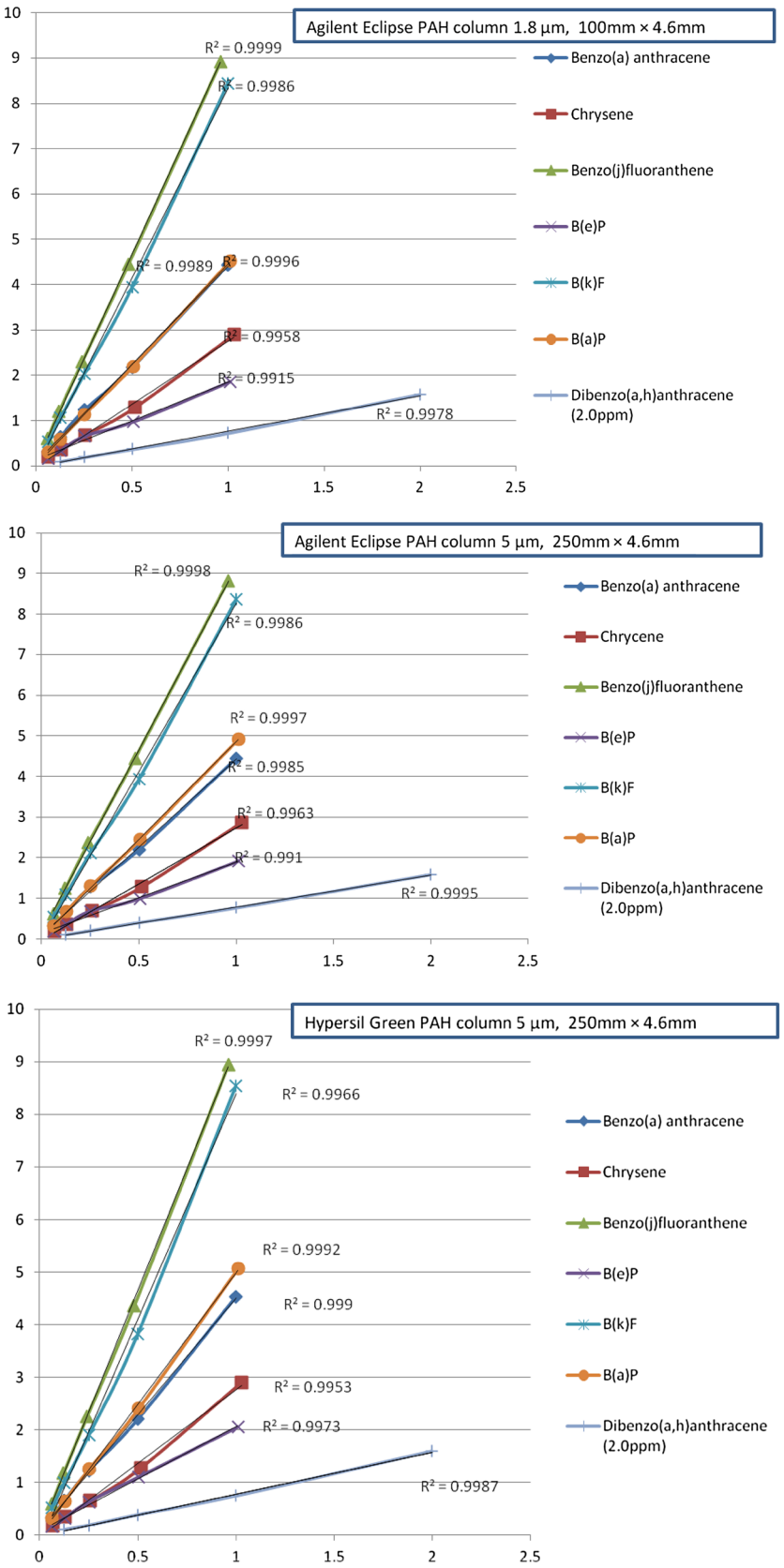


Fig. 4 Comparison of correlation coefficients for different PAHs obtained with three PAH columns assayed

Table 3 Comparison of the LOD and LOQ obtained with the columns assayed

	LOD			LOQ		
	1.8[a] µm	5[b] µm	5[c] µm	1.8[a] µm	5[b] µm	5[c] µm
PAH compounds						
Benzo[*a*]anthracene	0.0002	0.0005	0.0006	0.0006	0.0015	0.0018
Chrysene	0.0002	0.0018	0.0013	0.0007	0.0055	0.0039
Benzo[j]fluoranthene	0.0001	0.0002	0.0007	0.0004	0.0007	0.0020
Benzo[*e*]pyrene	0.0006	0.0013	0.0026	0.0018	0.0038	0.0079
Benzo[*k*]fluoranthene	0.0002	0.0004	0.0005	0.0005	0.0013	0.0016
Benzo[*a*]pyrene	0.0002	0.0009	0.0010	0.0006	0.0028	0.0031
Dibenzobenzo[*a*,*h*]anthracene	0.0006	0.0023	0.0036	0.0018	0.0070	0.0107

[a]Agilent Eclipse PAH column 1.8 µm, 100 mm × 4.6 mm

[b]Agilent Eclipse PAH column 5 µm, 250 mm × 4.6 mm

[c]Hypersil Green PAH 5 µm, 250 mm × 4.6 mm

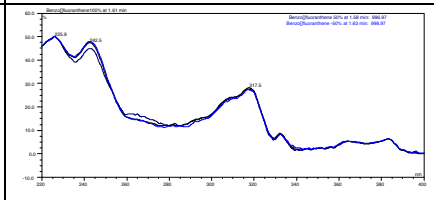
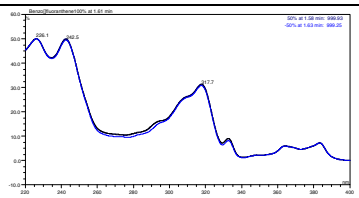
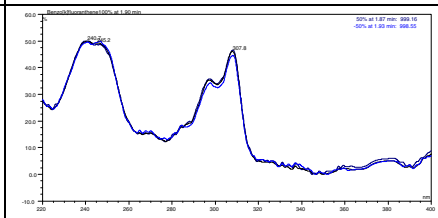
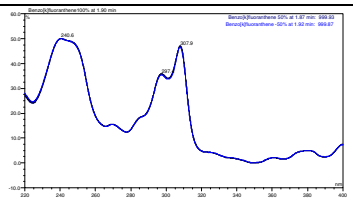
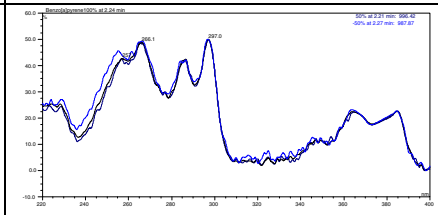
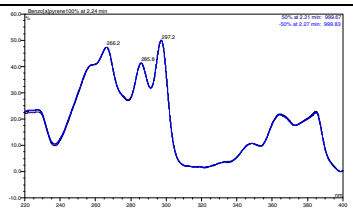
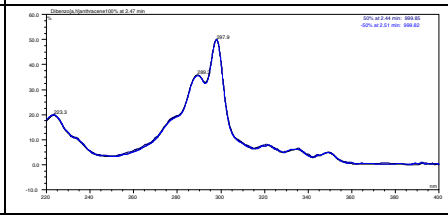
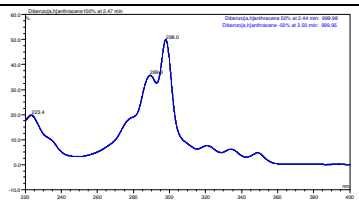
Table 4 Average and standard deviation of peak resolution (R), number of theoretical plate (N), height equivalent to theoretical plate (HETP) and reduced plate height (h) obtained for columns with different particle size and length columns

	Additive	Peak resolution	± SD	N	± SD	HETP	± SD	h	± SD
Agilent Eclipse PAH 1.8 µm, 100 mm × 4.6 mm	Benzo[*a*]anthracene		± 0.024	4596	± 26.8	21.76	± 0.13	12.09	± 0.07
	Chrysene	1.47	± 0.019	5055	± 72.7	19.79	± 0.29	10.99	± 0.16
	Benzo[j]fluoranthene	2.61	± 0.02	5714	± 28.6	17.5	± 0.09	9.72	± 0.05
	Benzo[*e*]pyrene	1.11	± 0.018	6010	± 37.9	16.64	± 0.1	9.24	± 0.06
	Benzo[*k*]fluoranthene	2.16	± 0.018	6623	± 29	15.1	± 0.07	8.39	± 0.04
	Benzo[*a*]pyrene	3.58	± 0.024	8209	± 39.4	12.18	± 0.06	6.77	± 0.03
	Dibenzobenzo[*a*,*h*]anthracene	2.16	± 0.024	7396	± 178.5	13.53	± 0.32	7.52	± 0.18
Agilent Eclipse PAH 5 µm, 250 mm × 4.6 mm	Benzo[*a*]anthracene		± 0.038	12,350	± 47	20.24	± 0.08	4.05	± 0.02
	Chrysene	2.37	± 0.095	11,888	± 851	21.14	± 1.62	4.23	± 0.32
	Benzo[j]fluoranthene	4.43	± 0.025	11,431	± 76	21.87	± 0.15	4.37	± 0.03
	Benzo[*e*]pyrene	1.74	± 0.01	11,289	± 238	22.15	± 0.47	4.43	± 0.09
	Benzo[*k*]fluoranthene	2.79	± 0.016	11,425	± 71	21.88	± 0.13	4.38	± 0.03
	Benzo[*a*]pyrene	4.77	± 0.017	11,909	± 52	20.99	± 0.09	4.20	± 0.02
	Dibenzobenzo[*a*,*h*]anthracene	2.68	± 0.038	10,680	± 207	23.42	± 0.45	4.68	± 0.09
Hypersil Green PAH 5 µm, 250 mm × 4.6 mm	Benzo[*a*]anthracene		± 0.028	6883	± 246	36.37	± 1.29	7.27	± 0.26
	Chrysene	1.61	± 0.035	7150	± 237	35.00	± 1.15	7.00	± 0.23
	Benzo[j]fluoranthene	3.07	± 0.061	7348	± 172	34.04	± 0.80	6.81	± 0.16
	Benzo[*e*]pyrene	1.56	± 0.036	7409	± 753	34.06	± 3.30	6.81	± 0.66
	Benzo[*k*]fluoranthene	1.79	± 0.046	7672	± 167	32.60	± 0.71	6.52	± 0.14
	Benzo[*a*]pyrene	3.97	± 0.015	7665	± 234	32.64	± 0.99	6.53	± 0.20
	Dibenzobenzo[*a*,*h*]anthracene	1.47	± 0.028	7502	± 241	33.36	± 1.08	6.67	± 0.22

Values are obtained from 6 different injections at concentration (low, medium and high)

of identified four PAHs in LCO samples with STD mix are given in Table 5. The result proved that the column is apt qualitative analysis in laboratories for the detection of above PAHs in LCO matrices.

Table 5 Comparison UV–Vis spectrum of indentified PAHs in LCO sample with PAHs STD mix

Name of PAHs	Spectrum of indentified PAHs in LCO sample	Spectrum of PAHs in STD mix
Benzo[j]fluoranthene		
Benzo[*k*]fluoranthene		
Benzo[*a*]pyrene		
Dibenzo[*a*,*h*]anthracene		

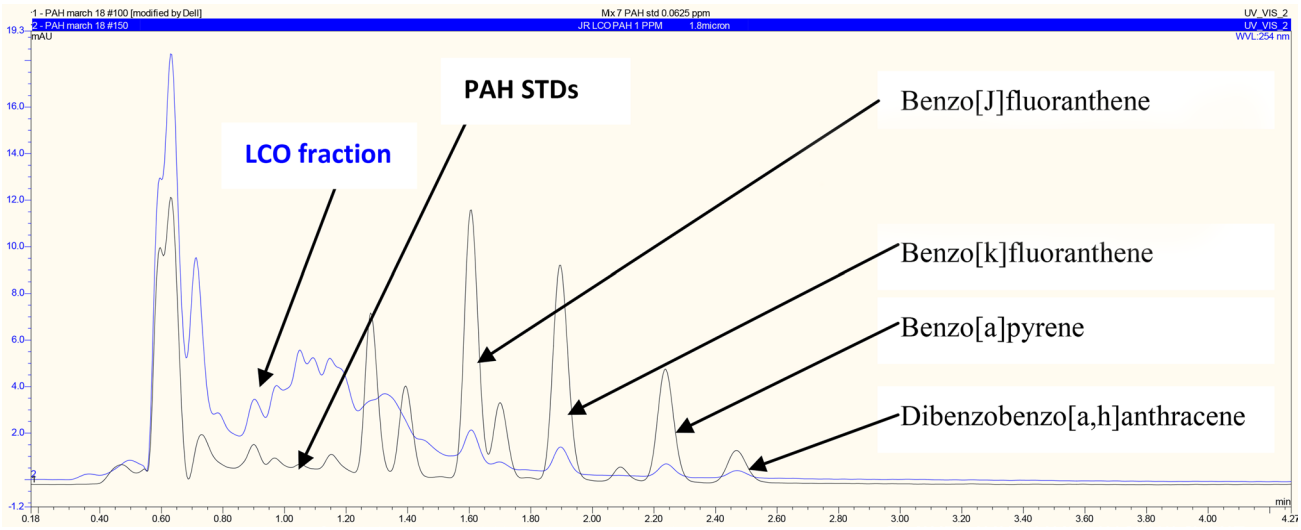


Fig. 5 Overlay of poly aromatic fraction of LCO sample with PAH STD on Agilent Eclipse PAH having these dimension 1.8 µm particle size, 100 mm length and 4.6 mm id

4 Conclusion

The use of the new PAH column with particle size 1.8 µm currently produced by various manufacturers, is found to be suitable for the analysis of seven PAHs. Eclipse PAH column having particle size 1.8 µm and length 100 mm led to reduction of the analysis time by 65% and 64% with respect to Agilent Eclipse PAH column 5 µm, 250 mm × 4.6 mm and Hypersil Green PAH 5 µm, 250 mm × 4.6 mm respectively (Table 2). The resolution (R) values for PAH column 1.8 µm are ≥ 1.5 for all PAH except between the Benzo[j]fluoranthene and Benzo[*e*]pyrene for which it is found to be 1.11. Theoretical plates (N) are calculated for each PAHs and found to vary in range of 4000–9000 on this column. Even though HETP value is low for this column, it is found to perform better as compared to other two columns (Table 4**)**.

The developed method on above column is also suitable for detection and quantitative estimation of four PAHs in LCO sample namely benzo[j]fluoranthene, benzo[*k*] fluoranthene, benzo[*a*]pyrene, and dibenzobenzo[*a*,*h*] anthracene based on retention time and unique PDA profile. The developed method not only led to reduction in consumption of organic solvents but also increased the throughput of sample analysis owing to reduction in analysis time.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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