

Polycyclic Aromatic Hydrocarbons (PAHs): Occurrence of Organic Pollutants in Edible Oils Commonly Consumed in Bangladesh

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Keywords: Polycyclic aromatic hydrocarbons, Edible oil, GC-MS, Carcinogenic

Posted Date: September 23rd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11069221/v1>

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Additional Declarations: No competing interests reported.

Abstract

This disquisition deals with investigation the presence of selected priority carcinogenic polycyclic aromatic hydrocarbons (PAH₇) namely naphthalene (Nap), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), pyrene (Pyr), benzo[a] anthracene (BaA) and chrysene (Chr) in eleven brands of two categories edible oil samples like as rice bran oil (Five samples) and mustard oil (Six samples) congregated from Bangladeshi markets with Gas Chromatography-Mass Spectrometry (GC-MS) technique. The samples were hydrolyzed in methanolic hydroxide and then PAH₇were extracted into 1:4 mixture of dichloromethane and Hexane according to standard method. Gas chromatography and Mass spectroscopy technique showing high accuracy and precision with low detection limit, has been applied for analysis of aforesaid targeted polycyclic aromatic hydrocarbons (PAH₇). It was found from analysis that all of the above targeted PAH₇ were not present at a time in all edible oils. Results showed naphthalene was present almost in each investigated sample. Highest concentrations of naphthalene in rice bran oil and mustard oil were 370µg/kg and 52.6 µg/kg, on the contrary lowest concentrations of naphthalene in rice bran oil and mustard oil were 17.7 µg/kg and 9.83 µg/kg respectively. Except naphthalene no one PAH was found in mustard oil whereas rice bran oil contained further two carcinogenic PAHs which were chrysene and benzo [a] anthracene. The highest concentrations of chrysene and benzo[a]anthracene were 1346.8µg/kg and 359 µg/kg in rice bran oil respectively. Fluorene, phenanthrene, anthracene, pyrene were found below the detection limit for both types of test samples. In this study the spike recovery of aforesaid PAH₇compounds wereranged from 63.20 to 90.7% which confirmed the perfect consistency and accuracy of applied working method.

1. INTRODUCTION

Edible oil is an essential ingredient for our cooking foods and the use of it's in our daily food items is an important part to make our food delicious and enchanting. Good oil in our diet helps to improve absorption and assimilation of food and nutrients. It is good for improving memory and lubricants inside and outside of the whole body including connective tissue and organs thus making the body flexible and youthful. Oil intake assists with many ailments especially constipation, dry skin, stiffness, arthritis and other diseases of the nervous system. Organic pollutants named as polycyclic Aromatic Hydrocarbons (PAHs) are considered as toxicants (Viegaset al., 2012) and these toxicants resulting from the incomplete combustion or pyrolysis of organic matter during industrial processing and various human activities. **(Tareq ARM et al., 2022)**. PAHs are subsequently found in soil, water, and air which are widely spread throughout the environment (Shen et al., 2008). These compounds are also widely present in the environment due to their hydrophobic properties which allow their adsorption on atmospheric particles. Humans are exposed to PAHs primarily through inhalation, ingestion, and skin absorption (Linares et al., 2010). These originate from diverse sources such as tobacco smoke, engine exhausts, petroleum distillates, and coal-derived products and with combustion sources predominating (Silvaet al., 2011). Certain PAHs are highly carcinogenic and have been implicated in various types of human cancers, including breast, lung, and colon cancers. For the first time Jung and Morand in 1962 reported the

presence of Polycyclic Aromatic Hydrocarbons (PAHs) in vegetable oil and fats and then further investigation on PAHs was followed by Sagredos and Sinharoyin 1979). Whatever PAHs have been detected in various food samples, including tea, roasted coffee, fruits, vegetables, oils, milk, smoked cheese, smoked meat, and smoked fish, at ng/g concentrations (Ishizaki et al., 2010). PAHs are known as highly stable contaminants present in many foods (**Speer et al., 1990**). Chemically PAHs are diversified of more than 100 lipophilic organic contaminants composed of two or more fused aromatic rings (Guillen, 1994). These contaminants are formed organic matter as a consequence of a series of natural processes by incomplete combustion (**Moret and Conte, 2002**). Due to their carcinogenic activity, PAHs have been included in the European Union (EU) and the United States Environmental Protection Agency (USEPA) priority pollutant lists. Larger or Heavy PAHs compounds are less volatile and adsorbed on other combustion particles like soot and thus widely distributed, extremely volatile compounds comprise Smaller or Light PAHs. (Singh et al, 2016). These PAHs cause metabolic activation in mammalian cells via di-epoxides which results errors in deoxyribonucleic acid (DNA) replication and mutation. (**USEPA, 2002**; Armstrong et al., 2004; **US Yoon et al., 2007**; Janoszka et al., 2004). Many studies have demonstrated that diet is the major non-occupational source of PAHs for non-smokers (Martorell et al., 2010; Ciecierska and Obiedziński, 2013) and contributes more than 70% of their total exposure. (Gilbert J 1994). In recent years, many researchers also have detected PAHs in different food categories such as oils and fats, vegetables, fruits, seafood, tea, coffee, sugar, toasted bread, infant foods, and smoked food products. (**Camargo R and Toledo 2003**) and it has been proved that PAH ingestion from edible oils and fats account for one third of all ingestions from food (Ibañez et al., 2005). In vegetable oils, these are found at high levels because of their lipophilic nature. A number of authors revealed the presence of PAHs in different vegetable oils as a consequence of oil processing and/or to a lesser extent as a result of environmental contamination (Moret et al., 2005). Many reports indicate that foods are likely to become contaminated by environmental PAHs and as a result of food-processing methods such as smoking, drying, roasting, baking, and frying (Stolyhwo and Sikorski 2005; Chung et al., 2011). European Union priority PAHs need to be analyzed in different food groups. The European Food Safety Authority (EFSA) has established an on-line analytical database in collaboration with European Union Member States in order to collect data for sixteen priority PAHs (Wenzl et al., 2006). Cooking effects with edible oil during frying, grilling, barbecuing, toasting, and roasting on the formation of 27 different PAHs in foods with charcoal plus wood chips creates the greatest formation of PAHs in foods (Rose et al., 2015). Nonetheless vegetable oils provide a plethora of benefits such as reducing the risk of cardiovascular diseases, better metabolism and digestion, lowers chances of breast cancer, and provides omega-3 fatty acids to the body. Reactive metabolites of some PAHs have become one of the major health concerns because of their potential to bind to cellular proteins and DNA with toxic effects, despite the presence of some not metabolized PAHs (Armstrong et al., 2004). The resulting biochemical disruption and cell damage can lead to mutations, development malformations, tumors, and cancer (Bach et al., 2003). Evidence indicates that mixtures of PAHs may be more carcinogenic to humans than individual PAHs. The evidence comes primarily from occupational studies of workers exposed to mixtures of PAHs.

Despite various views on the health benefits and risks of consuming vegetable oils, they are important in our diet. However, understanding the composition of edible oil and its effects on health and human body is must desired. With a view to studying the present scenario of PAHs in edible oils of Bangladesh, we performed our tests with proper analytical study by with chromatography and mass spectroscopy (GC-MS) technique. In Bangladesh, there is no up to date systematic studies on the levels of PAH in edible oil which are highly consumed in our country. This research will therefore serve as the reference line for further possible research on contaminated and carcinogenic polycyclic aromatic hydrocarbons in numerous comestible diets.

2. EXPERIMENTAL

2.1. Reagents and Chemicals

Cyclohexene, dichloromethane, methanol, hexene and other solvents from Merck, Germany were of HPLC grade. The Standard polycyclic aromatic hydrocarbons like as naphthalene, fluorine, anthracene, phenanthrene, pyrene, chrysene, benzo (a) anthracene, benzo (a) pyrene were purchased from Sigma, USA and their purity ranged from 96-99.9%. Anhydrous sodium sulfate (Merck, Germany) was further dried by heating at 180°C before use. Due to enhancing the activation of silica gel (Merck, Germany) it was activated at 400°C for 12 hours prior to use. Water was purified by a deionized system of Telemecanique made in U.S.A.

2.2. Sample collection

In this present study we used two different sorts of total eleven numbers edible oil (rice bran oil and mustard oil) samples from different local arcades of Dhaka metropolitan city in june2018 and marked those as S1 to S11 correspondingly. The samples were preserved cautiously into a freezer compartment at 4°C prior to starting analysis work. At the launching of analysis, the conserved samples were brought out at customary room temperature. About 5g of each oil sample were separately weighted and transferred into different pre-cleaned round bottom flask.

2.3 Preparation of standard solution

The known amounts of the PAH₇ standards were dissolved in definite amount of solvent (dichloromethane) to prepare 100 ppm mix PAH₇ standard solution. The solution was shake and sonicated with intermittent shaking to dissolve completely into solvent. This was stock standard solution and this prepared solution was stored in the freezer of a refrigerator. At the time of preparing working standards, the stock standard solution was kept in outside of the refrigerator to reach room temperature. Required amount of stock standard solution was diluted into suitable measured pre-cleaned and dried volumetric flask for preparing of 1ppm, 1.50ppm, and 2.50 ppm and 5ppmsolutions respectively and made it up to volume with dichloromethane (DCM) solvent.

2.4. Samples preparation

2.4.1 Alkaline Hydrolysis (methylation):

Owing to removing oily components from oil samples, alkaline Hydrolysis (methylation) process was carried on. Each of the 5g samples were hydrolyzed in 100 ml 2M methanolic KOH solution. The resulting mixtures were placed on a horizontal shaker and vigorously shaken for 30 minutes, after which they were refluxed in the water bath at 45°C for two hours. After completion of saponification, the resulting mixtures were centrifuged for 15 minutes at 3000rpm. Afterwards the centrifuged samples were filtered through Whatman cellulose filter paper and filtered liquid samples were collected into a clean conical flask.

2.4.2 Extraction of PAHs

With a view to extracting PAHs from previously treated filtered sample, it was transferred into pre cleaned separating funnel and three cycles liquid-liquid extraction was performed by using 1:4(v/v) of di-chloro-methane (DCM) and hexane mixtures with sample solution. Firstly, about 25mL of DCM and hexane mixture was added with sample solution and it was shaken for 30 minutes. Between two layers bottom layer was transferred into a clean beaker and upper layer was transferred into another clean beaker. Afterwards about 15mL of DCM and hexane mixture was added with bottom layer sample solution which was re-shifted into separating funnel and shaken for 15 minutes for the further extraction of PAHs. Here from bottom layer was transferred into a clean beaker and top layer was collected in prior top layer sample solution containing beaker. At last, by adding of 10mL of DCM and hexane mixture, identical procedure was maintained for the extraction of PAHs. All eleven samples were extracted just following analogous methods.

2.4.3 Removal of residual water from extracted sample:

Residual water was removed from the extract by treating with anhydrous sodium sulphate. Sodium sulphate (3 to 4 grams) was placed into the total extracted sample containing beaker and kept it for about 2 hours. Then the sample was filtered through whatman-40 filter paper and filtered sample was collected into another clean and dried conical flask. The treated extract was stored in a refrigerator for further examination.

2.4.4 Clean up and pre-concentration procedure of the extract

Due to reduce color from extracted sample solution, this was passed through a clean-up column which was prepared by placing cotton (one inch), silica gel (seven inch), sodium sulfate (two inch) and cotton layers (one inch) successively from bottom to top within a burette. About five mL of di-chloro-methane (DCM) was passed through column to wash and saturate the cotton, silica gel, sodium sulfate and cotton layers. After reducing color, the extracts were reduced to a volume of 1–2 mL concentrated solution by using nitrogen gas passing through sample solution. Now the pre-concentrated solution was passed through C₁₈ containing SPE cartridge filter which was then cleaned by 5 ml DCM. Again, the

solution was pre-concentrated to 1–2 mL solution by using nitrogen gas which was ready for the analysis with GC-MS and it was preserved in a refrigerator at 4°C for further use.

2.5 GC-MS Analysis

The identification and quantification of polycyclic aromatic hydrocarbons (PAH₇) was accomplished by using a Varian CP-3800 GC system equipped with Varian Saturn-2200 MS system and a VF-5ms fused silica capillary column (30m x 0.25 i.d., film thickness 25µm). An electron ionization system with the ionization energy of 70 eV was used for the detection of MS. Selected Ion monitoring (SIM/SIS) mode was preferred for the analysis of samples. In case of PAH₇ the GC oven temperature was programmed from initially 50°C (1 min hold) to 320°C at 10°C/min and hold for 5 minutes. Helium was selected as the carrier gas at a constant flow rate of 1 ml min⁻¹ and at constant pressure at 27 psi. Injector temperature was 280°C and injected volume was 1 µL (split at 30). The transfer line temperature, injector temperature, and manifold temperature of MS at 200°C, 250°C, and 40°C respectively. GC-MS methods usually operating in Selected Ion storage (SIS/SIM) where mass to charge ratio (m/z) were 128, 166, 178, 202, 228 respectively preferred for the analysis of PAH₇. In this method total run time was 32 minutes for identifying of PAH₇. The system suitability of the method was evaluated by the intra and inter day precision and accuracy of replicates. The accuracy was evaluated through recovery studies by adding known amounts of the standard solution to the extracts. Identification of the standard compounds were determined by based on computerized matching mass spectra from GC-MS NIST Library. Identification of the preferred compounds in the pre-concentrated samples were determined matching of its retention times with standard solution. Quantification of the PAHs every standard and sample solution was injected by ensuring identical above conditions.

3. Results and Discussion

In this present study more detrimental aforesaid PAH₇ were investigated in five brands of rice bran oil and six brands of mustard oil and they are marked as S₁ to S₅ for rice bran oil and S₆ to S₁₁ for mustard oil samples. The identification and quantification of PAH₇ like naphthalene (Nap), fluorine (Flu), phenanthrene (Phe), anthracene (Ant), pyrene (Pyr), benzo (a) anthracene (BaA) and chrysene (Chr) were done by external calibration curve method. The peaks of the mentioned compounds were confirmed by matching of their retention times with reference standard peaks. After preparing of standard solutions, it was run through GC-MS instrument and calibration curves of above PAH₇ originated by plotting the area of four different spots vs the concentration. Retention time and detection limit for naphthalene, fluorene, phenanthrene, anthracene, pyrene, benzo (a) anthracene and chrysene were 9.53 min and 1.92 µg/kg, 14.92 min and 1.13 µg/kg, 17.27 min and 0.15 µg/kg, 17.33 min and 0.78 µg/kg, 20.62 min and 1.03 µg/kg, 23.62 min and 0.12 µg/kg, 23.64 min and 0.21 µg/kg respectively. The GC–MS chromatogram of the selected polycyclic aromatic hydrocarbons (PAHs), including naphthalene, fluorene, phenanthrene, anthracene, pyrene, benzo(a)anthracene, and chrysene along with their respective retention times, is presented in Fig. 1

Standard calibration curve was constructed individually using different known concentrations of each aforesaid compound and **Figure-2** showing the calibration curve of naphthalene.

Constructed calibration curves of different standard solutions of PAH₇ showed in excellent linearity response and high correlation coefficients (R^2) values of the standard PAH₇(**Table-1**). The correlation coefficients (R^2) values of Naphthalene; Fluorene; Phenanthrene; Anthracene; pyrene; Benzo(a)anthracene; Chrysene were 0.997528; 0.997958; 0.994490; 0.899974; 0.992527; 0.994027; and 0.967713, respectively indicating that the method is applicable with a wide range. The relative standard deviations found in the present study for the compounds were 9.21 to 19.44 indicating that the method is fit for purpose. The recoveries studies have been performed in order to check the accuracy of the analysis method. The recovery determination has been carried out by spiking technique. In the present study, the recovery has been found from 68.9 to 90.4%

Table-1: Linearity Response, Correlation Coefficients (R^2), RSD%, Detection Limit and Recovery Rates of PAH₇ samples.

PAH ₇	RT (min)	R ²	RSD%	DL (µg/kg)	Recovery (%)
Naphthalene	9.53	0.997528	9.21	1.92	90.4
Fluorene	14.92	0.997958	12.29	1.13	70.2
Phenanthrene	17.22	0.994490	18.47	0.15	71.1
Anthracene	17.22	0.899974	14.15	0.78	68.9
Pyrene	20.62	0.992527	19.44	1.03	88.5
Benzo(a) anthracene	23.64	0.994027	12.63	0.12	69.8
Chrysene	23.604	0.967713	7.471	0.21	76.1

To determine the concentrations of extracted PAH₇ from mustard oil and rice bran oil samples by organic solvent mixture (DCM and hexane), previously prepared and pre-concentrated samples preserved in fridge at 4⁰C were injected to GC-MS after normalize the temperature of the samples to room temperature. Identification of the chromatographic for PAH peak in sample solutions among of PAH₇ was done by comparison with chromatogram of standard solution. Characteristic consecutive chromatograms of rice bran oil and mustard oil samples which were achieved in our analysis in consort with standard sample are shown in **Figure-3** and **Figure-4** at a glance as staked representation.

The resultant **Figure-5** of the investigated collected edible oil samples showing that almost each sample contained Naphthalene. The content of Naphthalene in rice bran oil samples which were depicted as S1 to S5 were found as 21.2 µg/L; 17.7 µg/L; 52.6 µg/L; 148.7 µg/L; 370 µg/L respectively. All Rice bran oil samples didn't contain approximate amounts of naphthalene. It was varied significantly from one

sample to another sample. Rice bran oil sample S5 contained the highest amount of naphthalene whereas S2 contained the lowest amount of naphthalene. Among six mustard oil samples which were depicted as S6 to S11 only four samples contained naphthalene at significant amounts. In S7 and S8 mustard oil Samples we didn't found naphthalene but the others samples showed the naphthalene contents as 9.83 µg/L; 52.6 µg/L; 9.3 µg/L; 9.83 µg/L for S6; S9; S10 and S11 respectively. There is a plausible cause of naphthalene is much higher in rice bran oil than mustard oil due to its manufacturing process which going through under high temperature (232°C) operational conditions. Also, production process of rice bran oil is more sophisticated than mustard oil, so there is a great chance of contamination by naphthalene which may come from during production and from atmosphere. The highest concentration of naphthalene was in rice bran oil 370 µg/L and the lowest was 9.30 µg/L in mustard oil sample.

The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) concluded that naphthalene is possibly carcinogenic to humans. An assessment on naphthalene was prepared by Agency for Toxic Substances and Disease Registry (**ATSDR**) on January 21 in 2015 where it has shown the permissible limit of naphthalene in water and air. National Institute of Occupational Safety and Health (**NIOSH**) in this report showed that 10 parts per million (ppm) for the level of naphthalene in workplace air over an 8-hour workday is suitable for human health. Environmental protection agency (**EPA**) recommends in this report that children not drink water with over 0.5 ppm naphthalene for more than 10 days. Even though here the tolerance limit of naphthalene is shown in air and water, nevertheless it provides an assumption to us on the basis of this report that the content of naphthalene in edible oil relatively higher. So, the highest amount of naphthalene in edible oils should be a concern issue for food safety control authority in Bangladesh to reduce the potent human health risk.

In the case of Benzo(a)anthracene, it was not found in all edible oil samples. Benzo (a) anthracene was found in a sample of rice bran oil which was marked as S1 among five number samples. In this sample the quantity of benzo(a)anthracene was 359 µg/kg. In a investigation of vegetable oil sample the content of benzo (a) anthracene was found 619.2 µg/kg by (**Princewill et al., 2015**) whereas our result showed almost half fold of its. Benzo[a]anthracene is considered to be carcinogenic to human health (**Wegrzyn et al., 2006; IARC, 1987**) so these high contents of this compound indicate an alarming phenomenon before intake edible oil.

Hossain et al., (2012) did not find Chrysene in edible oil samples. But in this study, we have found carcinogenic Cyrene in one rice Bran oil sample (S6) and the content of Chrysene was 1346.8 µg/L. This higher amount of Chrysene was unquestionably an alarming message to consume edible oil which is contaminated by it for human being.

In our investigation we found three PAH₃ namely as naphthalene, benzo (a) anthracene and chrysene among PAH₇. The rest PAH₄ namely as fluorene, phenanthrene, Anthracene and pyrene were found below detection limit in our analyzed sample (S1 to S11). In research of PAHs performed by **Hossain et al., (2012)**, there we have seen that they have found fluorene, phenanthrene, Anthracene and pyrene in edible

oil samples which were collected from Bangladeshi markets. In their research they showed that only mustard oil samples were contaminated by fluorene whereas both mustard oil and coconut oil samples were contaminated by phenanthrene and pyrene. By anthracene all sorts of tested samples like soybean, mustard and coconut oil were contaminated. In a comparison of our result with **Hossain et al., (2012)**, it is seen that naphthalene was common contaminant in mustard oil but the result showed a huge difference of contamination level by this contaminant. The maximum level of naphthalene was 0.0614 µg/L and 52.6µg/L in **Hossain et al., (2012)**, and our research work respectively for mustard oil sample. **Table-2** Showing overall analyzed results for each contaminant at a glance.

Table-2: PAH ₇ contents in edible oil samples.

PAH ₇	Analyzed samples										
	Rice bran oil (µg/L)						Mustard oil (µg/L)				
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
Nap	21.2	17.7	52.6	148.7	370	9.83	<DL	<DL	52.6	9.3	9.83
Flu	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Phe	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Ant	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Pyr	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
BaA	359	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Chr	1346.8	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
<DL - below detection limit											

Conclusion

In this research, it was investigated to determine the degree of contamination level of highly consumed edible oil by carcinogenic organic PAHs pollutants viz., naphthalene, fluorene, phenanthrene, anthracene, pyrene, benzo[a] anthracene and chrysene which easily contaminate edible oil during various process. Carcinogenic naphthalene was found in both sorts of edible oil (rice bran oil and mustard oil) whereas benzo(a)pyrene and chrysene were found in one type of edible oil (rice bran oil). Total nine among eleven samples of rice bran and mustard oil were severely contaminated by naphthalene and only one rice bran oil was contaminated by naphthalene, benzo[a] anthracene and chrysene. As the finding results showed that most of the samples were highly contaminated by naphthalene, hence this research propose that awareness must be created among the denizens of Bangladesh to consume good quality edible oil. We are also optimistic that this research article based on different brands of Bangladeshi rice bran and

mustard oil samples will help to monitor the agencies and departments who are concerned with the quality of food and health for ensuring low public ingestion with these PAHs by taking proper initiative.

Declarations

Ethical Approval

The Author(s) confirm that the research complies with ethical guidelines and regulations, including but not limited to obtaining necessary approvals from ethics committees. The manuscript does not contain any form of plagiarism, falsification, or fabrication of data.

Consent to Participate:

The Author(s) affirm that the manuscript is original, has not been published previously, and is not under consideration for publication elsewhere. The Author(s) confirm that they have made significant contributions to the research and writing of the manuscript and have the right to submit the work for publication.

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Competing Interests:

The Author(s) declares any potential conflicts of interest that may influence the objectivity of the research. Any financial, institutional, or personal relationships related to the manuscript must be disclosed.

Funding:

N/A

Author Contribution

Abu Rayhan Mohammad Tareq: Supervision, Visualization, Writing-Review and Editing. AKM Atique Ullah: Formal analysis, Visualization. Amrita Saha Hashi: Visualization, Formal analysis and Data curation. Sanjida Afrin: Visualization, Formal analysis and Data curation. Md. Shamim Hossen: Visualization,

Formal analysis and Writing – original draft. Ronia Sultana: Formal analysis and Data curation. Quamrun Nahar: Supervision. Rehana Begum: Supervision and Visualization.

Acknowledgements

This work was supported by the Nuclear and Advanced Chemistry Division, Atomic Energy Centre Dhaka, Bangladesh Atomic Energy Commission, to procure the necessary chemicals for this investigation. I would like to express my sincere gratitude to the members of the research team who contributed to the successful completion of this study. I am also indebted to Md. Zaidul Islam, the support staff who facilitated the smooth operation of our research activities. Finally, we extend our heartfelt appreciation to our families and peers for their encouragement and unwavering support throughout this work.

Data Availability Statement:

The Author(s) agree to provide access to data supporting the manuscript's findings upon reasonable request. The Journal encourages open data sharing in compliance with scientific best practices.

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Figures

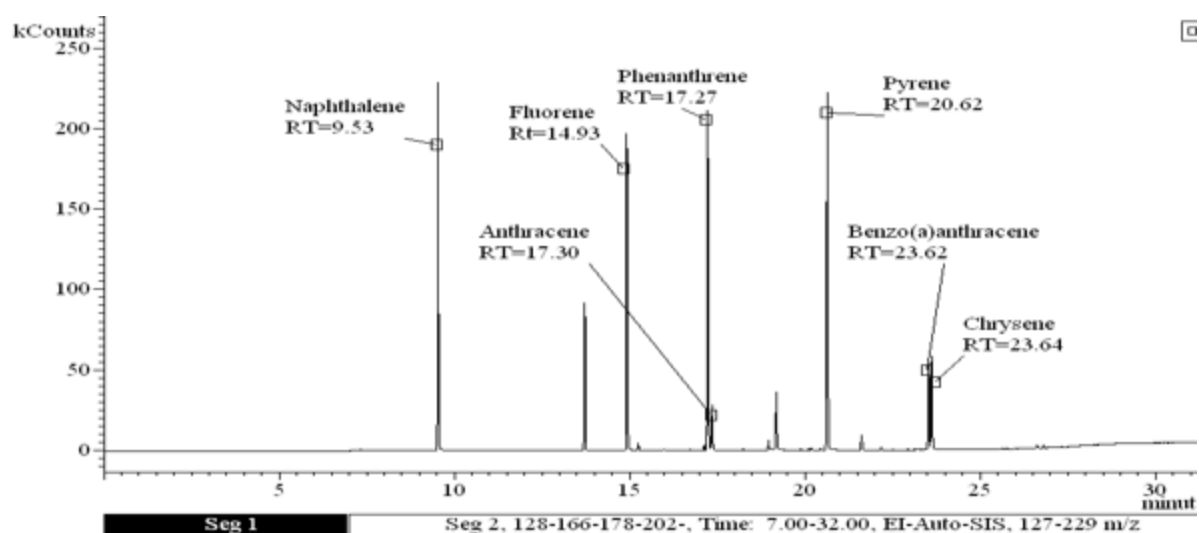


Figure 1

GC-MS chromatogram of PAH₇ mix standard solution

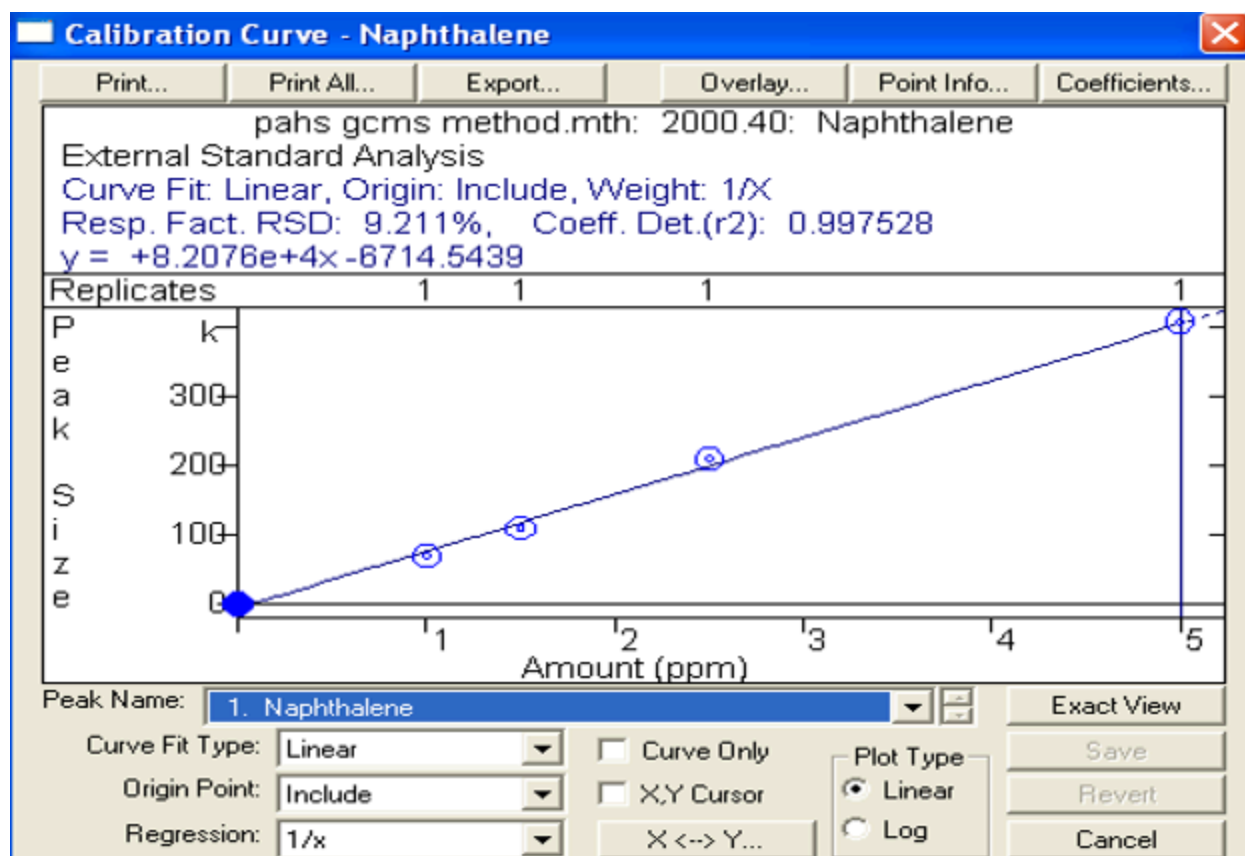


Figure 2

Calibration curve of naphthalene

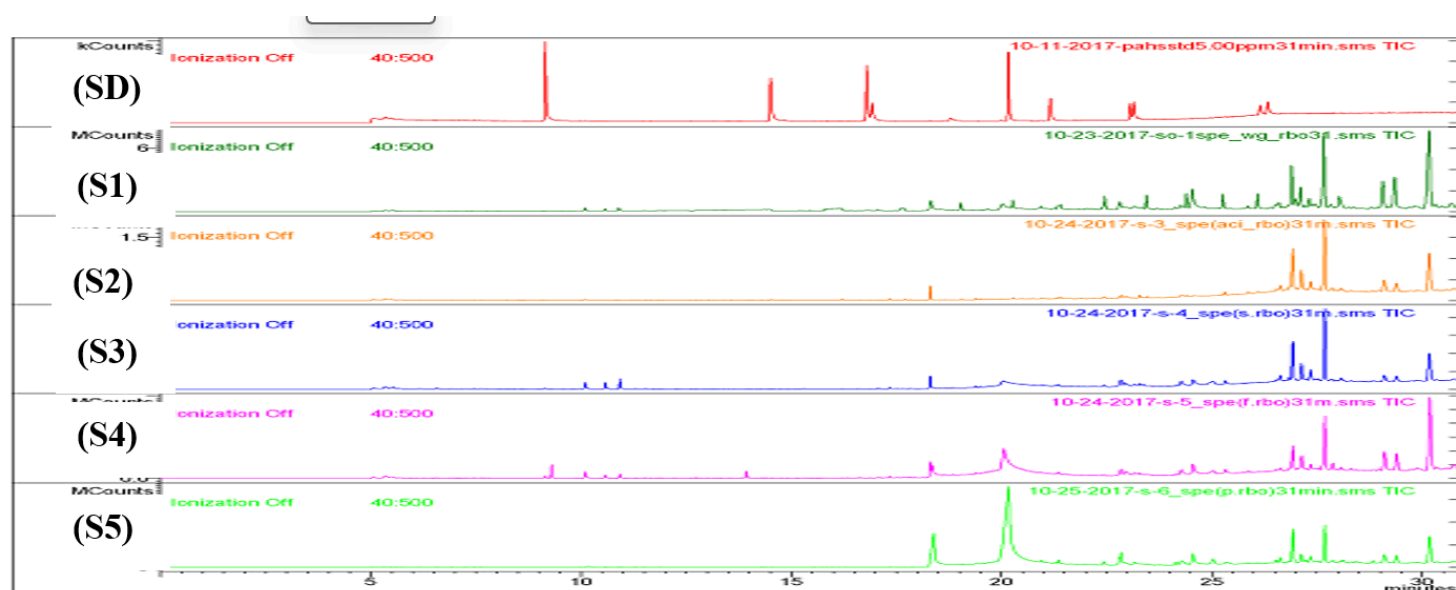


Figure 3

Chromatograms of PAHs for standard (SD) and Rice bran oil samples (S₁ to S₅)

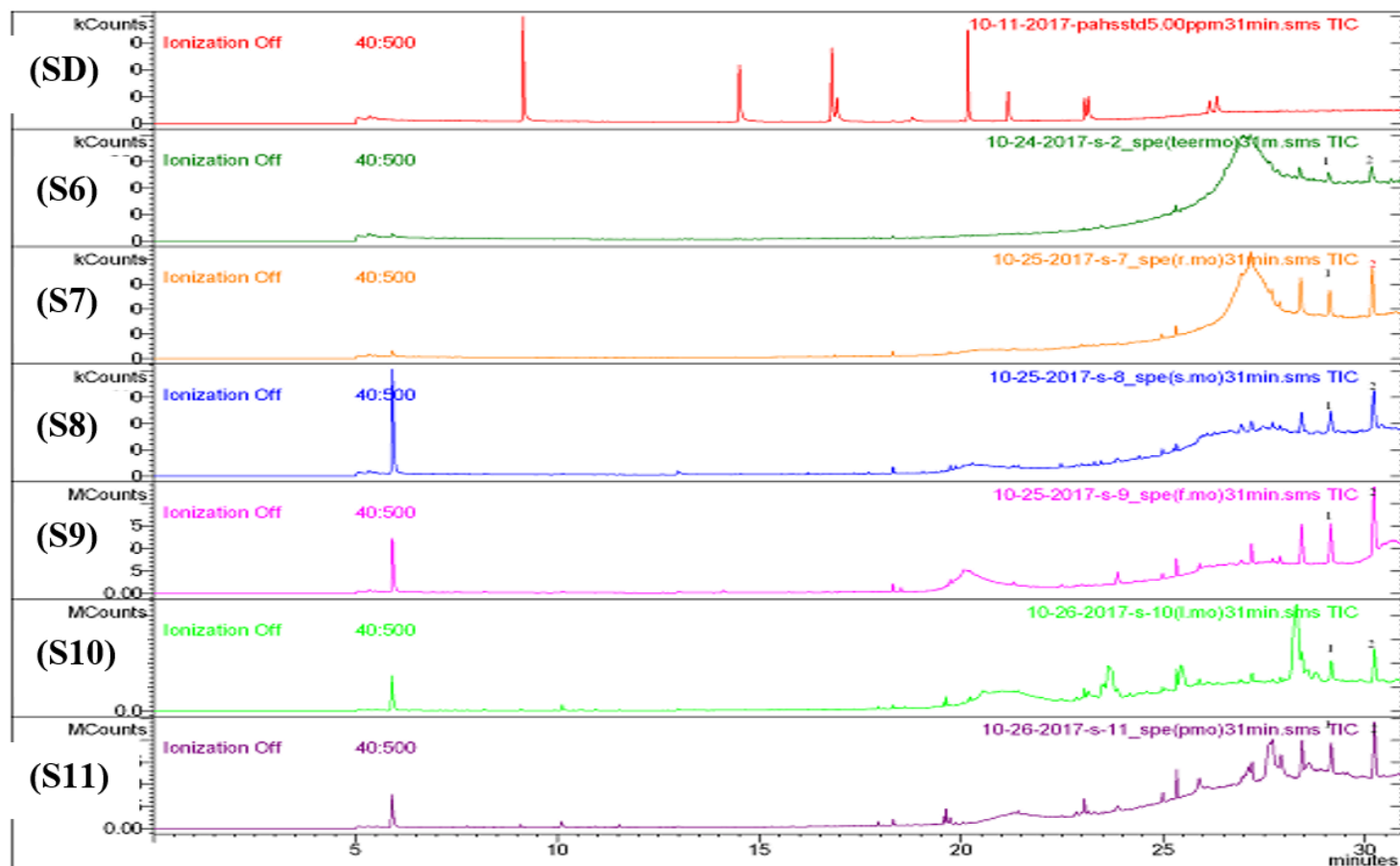


Figure 4

Chromatograms of PAHs for standard (SD) and Mustard oil samples (S₆ to S₁₁)

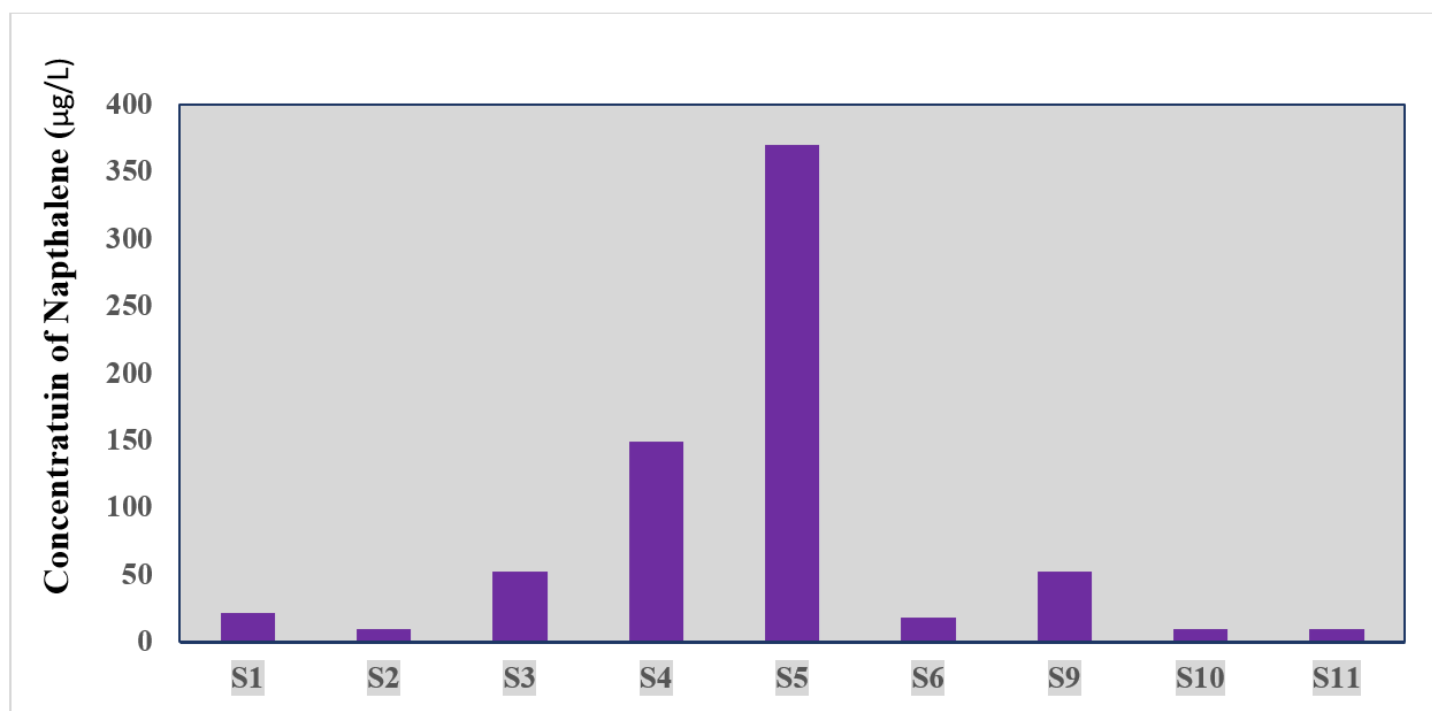


Figure 5

Concentration of naphthalene in oil samples

Here [S1] = Rice Bran oil; [S2] =Rice oil; [S3] = Rice Bran oil; [S4] = Rice Bran oil; [S5] =Rice Bran oil; [S6] =Mustardoil; [S9] = Mustard oil; [S10] =Mustard oil; [S11] = Mustard oil.