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HPLC-Based Determination Of Benzo[A]Pyrene In Different Brands Of Coffee Samples

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Abstract
Coffee is a widely consumed beverage that may contain trace amounts of polycyclic aromatic hydrocarbons (PAHs) like Benzo[a]pyrene (BaP) due to environmental contamination or roasting processes. A method for extracting and quantifying Benzo[A]pyrene (B[a]P)was evaluated using varieties of coffee samples. The samples were extracted with acetone, followed by saponification and cyclohexane extraction. The extracts were purified by chromatography. BaP is a known carcinogen, so it is important to monitor levels in coffee as it's a popular beverage consumed by people on regular basis. This study developed and validated an HPLC method for identifying and quantifying BaP in different coffee brands. The quantification was done by HPLC with a reverse-phase HPLC system of C18 Kromasil 100 column and UV-visible detection under isocratic conditions. The developed HPLC method is simple, sensitive, and suitable for routine monitoring of BaP in coffee samples to ensure consumer safety.

Keywords: Benzo[a]pyrene, B[a]P, Coffee, HPLC

Introduction:

Benzo(a)pyrene (BAP) is a needle-like, pale yellow chemical found in cigarette smoke, grilled foods, and industrial operations. Benzo[a]pyrene (BaP) is a polycyclic aromatic hydrocarbon (PAH) known as an environmental pollutant as it is seen in coal tar, automobile exhaust fumes of diesel engines, in smoke from combustion of organic material, cigarette smoke and in residential wood burning [1].It is a potent carcinogen affecting the lungs [2],skin, and gastrointestinal tract [3].

Research has found Benzo(a) pyrene (BaP) levels in various media, including air, household dust, and food samples. It has been found in urine from pregnant women and children, and in placenta, cord blood, maternal blood, and breast milk.

Exposure to Benzo[a]pyrene during pregnancy in animal experiments raises the likelihood of many cancer types and hampers the development of the respiratory and immunological system.[4].

Benzo[a]pyrene exposure in infants and children as seen in Table 1, have been found to lead to several health concerns [5] as continuous exposure can have persistent effects on the function of the immune system[6],[7].

Benzopyrenes are harmful because they form carcinogenic and mutagenic metabolites (such as (+)-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide from benzo[a]pyrene) which intercalate into DNA, interfering with transcription. The mechanism of action of benzo[a]pyrene-related DNA modification has been investigated extensively and relates to the activity of cytochrome P450 subclass 1A1

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(CYP1A1). These adducts can potentially impair DNA replication and increase the susceptibility to certain types of malignancies.[8].

There have been studies to show the presence of BaP in broiled food, and higher amounts of the hydrocarbon were found in foods that were grilled on the barbecue [9]. Cooked meat products have been shown to contain up to 4 ng/g of BaP [10],[11]. It is often used to indicate the presence of PAHs in food and beverages.

Coffee is a widely consumed beverage globally, and there have been reports showing evidence of trace amounts of BaP in coffee samples due to the roasting process [12], which can lead to the formation of PAHs. As consumer awareness and regulatory scrutiny regarding food safety increases, monitoring and controlling harmful contaminants like BaP in coffee becomes imperative.

High-performance liquid chromatography (HPLC) is a powerful analytical technique widely used for separating, identifying, and quantifying complex mixtures. Its high resolution, sensitivity, and versatility make it an ideal choice for determining chemical components in food and beverage samples. The application of HPLC in the determination of BaP in coffee allows for precise quantification [12] and helps ensure that the levels of this carcinogen are within the permissible limits set by food safety authorities.

This research focuses on the HPLC-based determination of Benzo[a]pyrene in different brands of coffee. The study aims to quantify the Benzo(a)pyrene levels in various coffee samples, assess the variability in amounts of BaP among different brands, and evaluate any potential health risks associated with BaP consumption through coffee. By providing a comprehensive analysis of BaP in coffee, this research contributes to the ongoing efforts to ensure food safety and protect consumer health.

Table1: Benzo(a) pyrene exposure risk areas for children:

Exposure area	Risk level	Factors responsible for detection of Benzo(a)pyrene in risk area
Outdoor air	High	Outdoor air contains numerous releases from metal processing plants, automobile emissions, wood stoves, coal tar and cigarette smoke.
Indoor air	High	Indoor air where people smoke cigarettes causing passive smoking effect on children living in the house, cooking and grilling, and smoke from burning wood or coal in heating stoves or fireplaces of room heating areas.
Sediment in soil	High	Sedimentation of BaP when exposed to UVrays of sunlight.
Diet	Medium	Grilling of foods and charcoal broiled foods.
Drinking water	Lower	Found in drinking water when sources are contaminated with BaP as it binds to particulate matter in water. However it can be removed by filtration before domestic use.

Materials and Methods:

Chemical and reagents:

Silica gel 60-particle 0.063-0.200 mm(70-230 mesh ASTM), Potassium hydroxide (pellets), Anhydrous Sodium Sulphate, Cyclohexane, Acetone, and Methanol. These reagents were analytical grade. For liquid chromatography, HPLC-grade acetonitrile and water were used. The benzo [a] pyrene standard used was of sigma grade. Four different brands of coffee samples with code numbers were used for analysis.

Preparation of standard

2gms of Benzo (a) pyrene standard was extracted in 25ml acetone. The acetone evaporated at 40 degrees Celsius. The residue was saponified with 0.14 gm of potassium hydroxide in 5ml methanol-

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water. After complete saponification, 12ml distilled water is added slowly through the condenser. The mixture was then transferred to a separatory funnel and shaken with 4 ml of cyclohexane for 2 minutes. After separating the layers, the aqueous layer was twice extracted with fresh portions of 3ml of cyclohexane for 2min.

The combined cyclohexane extracts were dried over 5 gm of anhydrous sodium sulphate pre-washed with 2 ml of cyclohexane, then concentrated in a rotatory evaporator at 40 degrees Celsius to approximately 5 ml.

The concentrated extract was applied at the top of a 3gms silica gel column. The column was prewashed with 2 ml of cyclohexane and eluted with 10 ml of cyclohexane. The first 2 ml of cyclohexane eluate was discarded; the rest of the eluate was collected and concentrated in a rotatory evaporator at 40 degrees Celsius. Up to 4 ml of eluate was concentrated and dried. The residue was dissolved in 0.3 ml of HPLC acetonitrile and analyzed by HPLC.

Extraction and clean up of sample

A 2gm portion of ground homogenized coffee samples was extracted in 25ml acetone. The acetone was evaporated at 40 degrees Celsius. The residue was saponified with 0.14 gm of potassium hydroxide in 5 ml methanol-water. After complete saponification, 12ml distilled water is added slowly through the condenser.

The mixture was then transferred to a separatory funnel and shaken with 4 ml of cyclohexane for 2 minutes. After separating the layers, the aqueous layer was twice extracted with fresh portions of 3ml of cyclohexane for 2min. The combined cyclohexane extracts were dried over 5 gm of anhydrous sodium sulphate pre-washed with 2 ml of cyclohexane, then concentrated in a rotatory evaporator at 40 degrees Celsius to approximately 5 ml.

The concentrated extract was applied at the top of a 3 gm silica gel column. The column was prewashed with 2ml of cyclohexane and eluted with 10ml of cyclohexane. The first 2ml of cyclohexane eluate was discarded; the rest of the eluate was collected and concentrated in a rotatory evaporator at 40 degrees Celsius. Upto 4ml eluate was concentrated and dried. The residue was dissolved in 0.3 ml of HPLC acetonitrile and analyzed by HPLC.

Separation was achieved on a 250 × 4.6mm Kromasil 100 c18 operated at room temperature. The mobile phase acetonitrile-water (80:20,v/v)was used at a 1.0 ml /min flow rate. Aliquots of 20 µL coffee samples was injected into the HPLC column of LC 6600 Chemito make. The UV detector was operated with a wavelength at 295nm and a measurement at 495 nm. The chromatographic process was done in 15min. Quantification was performed by comparing sample peak areas with those obtained using a standard solution.

Chromatographic parameter :

Mobile phase ACN:Water – 80:20 V/V
Pressure - 30 psi
Column - 250 × 4.6mm kromasil 100 c18
Flow rate - 1.0ml/min
Injection volume - 20 µL
Detector - The UV detector
Temperature - Room temperature

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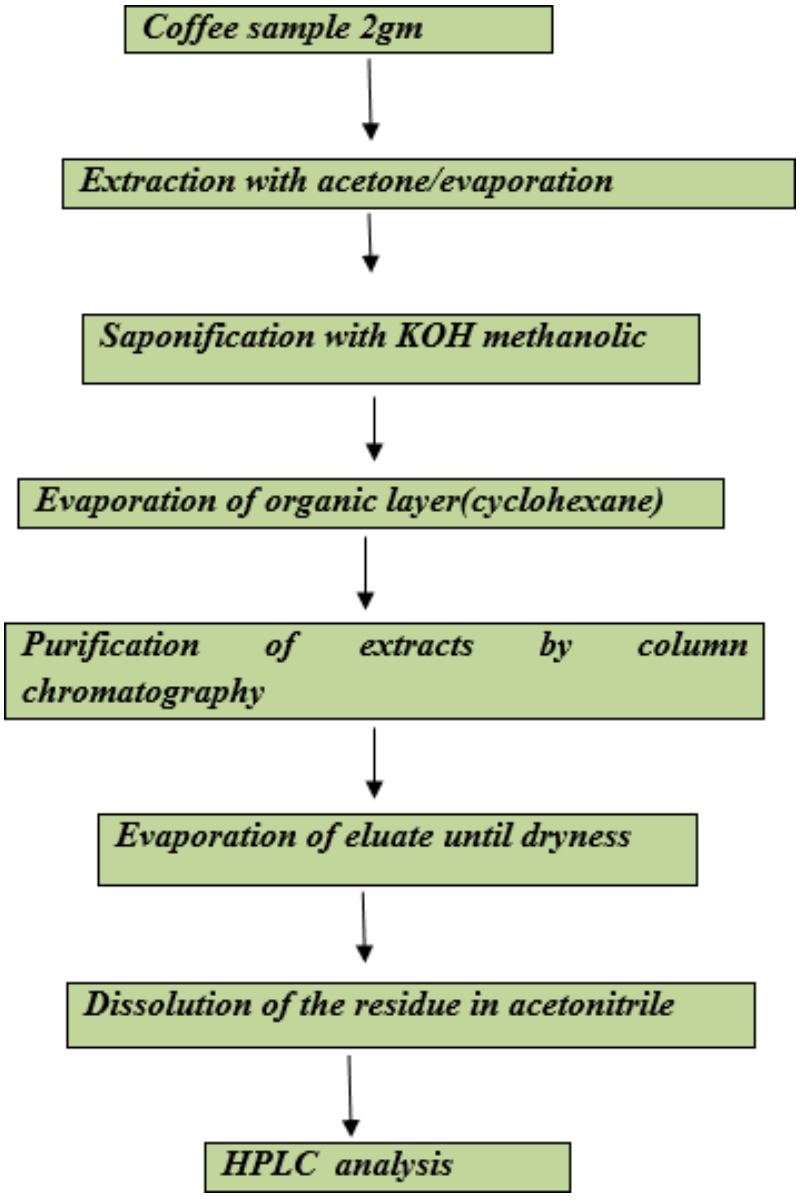
Flow sheet for sample prepararion and detection of BaP



RESULTS AND DISCUSSIONS:

Four different brands of coffee samples with code numbers were analyzed by HPLC technique for checking the presence of Benzo(a)pyrene. As seen in Table 2: No peak has been observed in the brand, whereas all other samples BC, NC, CCDC had shown significant presence of BaP.

Table2: Interpretation data of HPLC

SAMPLE	RETENTION TIME[MIN]	AREA [mV]	HEIGHT	AREA[%]	HEIGHT[%]	W05[MIN]
Benzo(a)pyrene standard	**3.493**	**4282.601**	**53.982**	**100**	**100**	**1.26**
BC	**3.780**	**1390.871**	**19.795**	**100**	**100**	**1.06**
NC	**3.887**	**1317.046**	**14.403**	**100**	**100**	**1.35**
CCDC	**4.113**	**419.990**	**4.312**	**100**	**100**	**1.43**
FC	-	-	-	-	-	-

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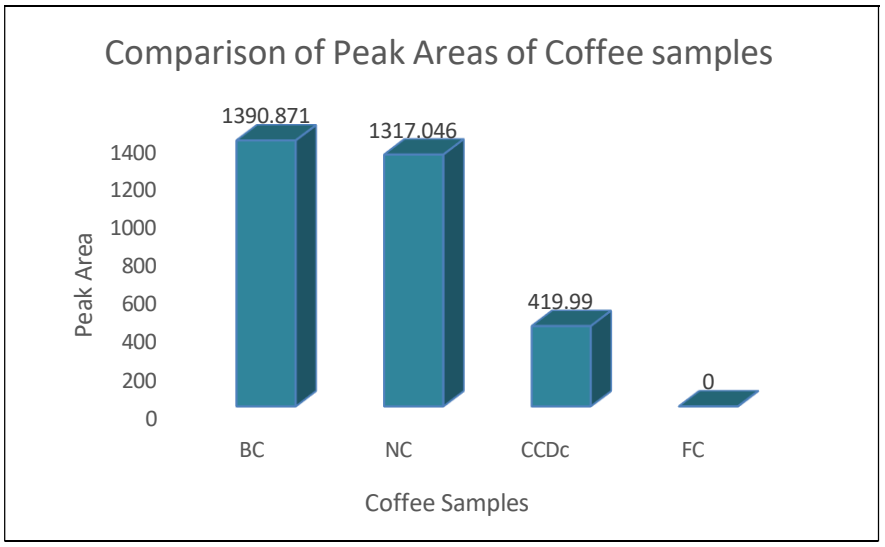

Figure 1: Comparison Graph of samples and Peak area:

Table3: Comparison of values of chromatogram peak area of different coffee samples

Sample	Peak Area
BC	**1390.871**
NC	**1317.046**
CCDc	**419.990**

Quantification of Benzo[a] pyrene present in coffee samples:

The peak areas as seen in Figure 1, and Table 3, of the coffee samples and that of the standard with the amount of sample and standard taken were compared and the amount of Benzo[a]pyrene was determined.

The results obtained after calculation were as follows as seen in Table 4.:

Table 4: Quantity of Benzo (A) pyrene (BAP) in coffee samples:

1)	**BC**	**0.649 gm/kg**
2)	**NC**	**0.615 gm/kg**
3)	**CCDC**	**0.195 gm/kg**
4)	**FC**	**0**

The absence of a peak in filter coffee indicates that no Benzo[a]pyrene is present in the coffee sample. This is because the coffee sample were not roasted. The remaining samples were roasted, and as a result, they contained a certain quantity of Benzo[a]pyrene.

Based on theobservation as per Table 4, it has been observed that the amount of Benzo(a)pyrene in FC is nil and among the other samples tested for the presence of BaP least amount was detected in CCDC beans, while the amount observed in BC coffee is the significantly high. Coffee beans from CCDC are processed in a manner distinct from that of the other types of coffee beans. For the purpose of preparing coffee, some contemporary methods are utilized, which result in the roasting process being done less frequently. Therefore, the quantity that is found in them is relatively low. However, BC and NC are produced in factories that use a different roasting process, which results in a slightly higher amount of Benzo(a)pyrene during the manufacturing process.

Since this is the case, one can draw the conclusion that FC is safer to consume. In contrast, it has been

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observed that if Benzo (a) pyrene (BaP) is present in any food item at a concentration ranging from 0.03 to 0.75 gm/kg, then it does not pose a significant risk of cancer to human beings. Consequently, all three samples can be consumed without any concerns because they fall within the range.
However from the current study it is realized that consuming coffee on alternating days or just once per day is not detrimental to health due to presence of traces of Benzo (a) pyrene.

Conclusion:

Benzo(a) pyrene (BaP) is a needle-like, pale yellow chemical found in cigarette smoke, grilled foods, and industrial operations. It is metabolized in humans and animals to create hazardous metabolites, including DNA adducts, which may affect DNA replication and raise the risk of various cancers. Benzo (A) pyrene (BAP) has a mutagenic effect for tumour growth and requires metabolic activation to become carcinogenic. Exposure during pregnancy increases the risk of several types of cancer in the new born infant, impairs immune system development and function. The European Commission limits benzo[a]pyrene in some foods, and it is chemically inert and hydrophobic.

Research has found Benzo (a) pyrene (BaP) levels in various media, including air, household dust, and food samples. It has been found in urine from pregnant women and children, and in placenta, cord blood, maternal blood, and breast milk. BaP can create Benzo (a) pyrene (BaP) -DNA adducts, disrupting DNA replication and affecting adult reproductive organs. The exposure media for children's exposure include ambient air, indoor air, sediment, soil, diet, drinking water, and groundwater.

A study using HPLC analysis was conducted using different coffee samples with appropriate codes. There was no peak indicating presence of BaP in only one of the coffee samples. The remaining samples were found to have varying amounts of Benzo[a]pyrene due to roasting of coffee seeds during manufacturing process in factories.
The study concluded that Benzo(a) pyrene (BaP) in coffee at concentrations ranging from 0.03 to 0.75 gm/kg does not pose a significant risk of cancer to humans. Consuming coffee on alternating days or once per day is not detrimental to health due to Benzo(a) pyrene.

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