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*Review*

# Analytical techniques for the detection of benzo[a]pyrene and other polycyclic aromatic hydrocarbons in food

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**Abstract** Polycyclic aromatic hydrocarbons are persistent environmental contaminants, several of which, including benzo[a]pyrene (BaP), are potent carcinogens. Their presence in food poses significant health risks, necessitating accurate and sensitive monitoring. This review summarizes analytical approaches used for the extraction, separation, and detection of BaP and other Polycyclic aromatic hydrocarbons in food and beverages. Conventional chromatographic methods, particularly liquid chromatography with fluorescence detection (LC-FLD) and gas chromatography-mass spectrometry (GC-MS), remain the reference techniques for quantitative analysis, achieving detection limits of 0.01-10 μg/kg across various matrices. Immunological methods such as enzyme-linked immunosorbent assay and lateral flow immunoassay offer rapid, cost-effective screening, with sensitivities of 0.03-0.1 μg/kg. Recent spectroscopic innovations, including Raman, surface-enhanced Raman, and fluorescence spectroscopy, enable non-destructive, solvent-free detection of BaP at sub-ppb levels. These techniques support the growing shift toward high-throughput, portable analytical platforms for food safety surveillance. Overall, while immunological and spectroscopic tools provide excellent preliminary screening capabilities, chromatographic methods, especially LC-FLD and GC-MS, remain the most reliable and widely validated options for routine food analysis due to their superior accuracy, selectivity, and regulatory acceptance.

**Keywords** polycyclic aromatic hydrocarbons, benzo[a]pyrene, analytical methods



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## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants released from both natural and anthropogenic processes, primarily through the incomplete combustion of organic materials such as fossil fuels, wood, and biomass (Abdel-Shafy and Mansour, 2016; Stogiannidis and Laane, 2015). They are also generated during high-temperature food processing methods like smoking, drying, roasting, and grilling. Structurally, PAHs consist of fused aromatic rings and are broadly divided into low-molecular-weight (two to three rings) and high-molecular-weight (four or more rings) groups, the latter being more stable and toxic (Nzila, 2018; Stogiannidis and Laane, 2015).

Among these compounds, benzo[a]pyrene (BaP) is of particular concern due to its environmental persistence and carcinogenic potential (Han and Kang, 2020; Nisbet and Lagoy, 1992). Its rigid, hydrophobic structure limits biodegradation and promotes bioaccumulation (Abdel-Shafy and Mansour, 2016; Rentz et al., 2008). Major sources include cigarette smoke, diesel emissions, grilled and smoked foods, and industrial by-products (Boström et al., 2002; Fromberg et al., 2007). Although minor natural emissions occur through volcanic activity and wildfires, anthropogenic

inputs dominate.

BaP exposure in humans has been linked to cancer and developmental, reproductive, and immunotoxic effects (Barnes et al., 2018; Verma et al., 2012). Consequently, BaP is listed among the United States Environmental Protection Agency (USEPA) top ten priority pollutants. Regulatory bodies, including the European Food Safety Agency (EFSA) and the European Union (EU), have set a maximum residue limit of 2 μg/kg in food and 10 ng/L in drinking water (Bortolato et al., 2008; Wang and Guo, 2010). It is classified by the International Agency for Research on Cancer (IARC) as a Group 1 human carcinogen (Zachara et al., 2017), with estimated dietary intake ranging between 6-8 ng/kg body weight per day (Danyi et al., 2009). Elevated levels in foods such as chocolate and smoked cheese (Fromberg et al., 2007) have prompted mitigation efforts, including the Code of Practice for the Reduction of PAH Contamination in Food (Raters and Matissek, 2014).

Given the trace levels at which BaP and other PAHs occur, their accurate quantification requires sensitive and selective analytical methods. Traditional chromatographic approaches such as liquid chromatography with fluorescence detetection (LC-FLD) and gas chromatography-mass spectrometry (GC-MS) remain the reference standards but are often time-consuming and solvent-intensive. Therefore, recent advances emphasize the need for faster, greener, and high-throughput alternatives, such as immunoassays, biosensors, and spectroscopic techniques, that offer comparable sensitivity for reliable food safety monitoring. This review, therefore, provides an overview of analytical methodologies for determining PAHs, with a particular focus on BaP in food and beverage matrices. Emphasis is placed on extraction and cleanup procedures, as well as on the instrumental techniques used for detection and quantification.

## 2. Extraction and cleanup

### *2.1. Fatty matrices*

#### 2.1.1. Liquid matrices

Fatty matrices pose a significant challenge in the analysis of PAHs due to their high lipid content, making the extraction of PAHs from these complex matrices an arduous task (Moret and Conte, 2000). It is essential to eliminate these lipids to achieve lower detection limits and to maintain the sensitivity of the analytical instruments. The need for high sensitivity of instruments is substantiated by the low amounts of PAHs set as maximum permitted levels in many countries (Simon et al., 2008; Wenzl et al., 2006).

One of the most common fatty food products studied for BaP contamination is edible oil. Common routes of exposure to PAHs through edible oils include solvent evaporation during heating (Bogusz et al., 2004) and the drying of raw materials before oil extraction (Moret and Conte, 2002). Reported methods of extraction and cleanup usually involve sample dilution using *n*-hexane (Moret and Conte, 2000), followed by a liquid-liquid extraction (LLE) step and solid-phase extraction (SPE) cleanup (Barranco et al., 2003; Guillén et al., 2004; Mottier et al., 2000). In contrast, other authors reported a single SPE step after dilution (Luo et al., 2007; Weißhaar, 2002). Typical solvents used for LLE include dimethyl sulfoxide (DMSO), cyclohexane, petroleum ether, and other solvent mixtures.

In some literature, a saponification step is introduced before LLE to lower the lipid content, particularly triacylglycerols. This involves the use of ethanol- or methanol-based solutions of sodium or potassium hydroxide (Moreda et al., 2001; Simon et al., 2008). Although this approach is practical for lipid removal, it has been associated with partial BaP losses due to its distribution in the alcoholic phase (Mottier et al., 2000) and degradation of chemically unstable compounds (Moret and Conte, 2000). Caffeine-assisted complexation of PAHs, followed by disruption with aqueous sodium chloride, has also been explored (Moreda et al., 2001; Moret and Conte, 2000). Although conceptually promising, the method has not advanced to routine application, suggesting limitations in practicality or performance.

In edible oils, following dilution with *n*-hexane, *n*-heptane, or isohexane/butyldimethylether, the extraction of BaP and other PAHs was achieved using LLE (Barranco et al., 2003; Pandey et al., 2004), SPE using silica (Moret and Conte, 2002), humic acid-bonded silica (Luo et al., 2007), and polystyrene/divinyl benzene (PS-DVB) (Weißhaar, 2002). Cleanup was achieved by column chromatography (Pandey et al., 2004), donor-acceptor column chromatography (Barranco et al., 2004), and a variety of SPE cartridges, including C18/C8 (Barranco et al., 2003) and aminopropyl/C18 (Mottier et al., 2000).

Extraction of BaP from olive oil and vegetable oils has also been widely reported in the literature. Solid-phase

microextraction (SPME) is a standard method used for extracting BaP from vegetable oils. This is usually preceded by dilution using *n*-hexane (Purcaro et al., 2007a; Purcaro et al., 2007b). Vichi et al. (2005) reported the application of headspace-solid phase microextraction (HS-SPME) to extract BaP from olive oil. Solid-liquid extraction (SLE) (Ballesteros et al., 2006) and LLE (Ballesteros et al., 2006; Guillén et al., 2004) have also been used to extract PAHs from olive oil. Cleanup can be achieved by using SPE (silica) or Soxhlet (Guillen et al., 2004), thin-layer chromatography (TLC) (Diletti et al., 2005), or gel-permeation chromatography (GPC) (Ballesteros et al., 2006).

A study compared the effectiveness of SPE and matrix solid-phase dispersion (MSPD) for extracting PAHs from olive oil (Bogusz et al., 2004). In MSPD, a small sample (about 0.5 g) is combined with a solid sorbent like C18 and then processed using an SPE cartridge. While MSPD has benefits, such as using less solvent and being easier to use than LLE, it showed lower recovery rates and less consistency than SPE, with relatively high standard deviation (RSD) values.

Milk has received less attention than edible oils in studies of PAHs, despite containing fat, albeit in lower amounts than oils. Like oil matrices, LLE is the primary method used, although it usually requires fewer extraction steps (Grova et al., 2002; Kishikawa et al., 2003; Lutz et al., 2006). Lutz et al. (2006) used a combination of LLE and SPE for cleanup, with specific procedures for PAHs and hydroxy-PAHs. Interestingly, no SPE-based methods were found for the initial extraction of PAHs from milk. Saponification has also been investigated, with ethanol concentration affecting both analyte recovery and matrix interference. Microextraction techniques, such as HS-SPME (Aguinaga et al., 2008) and SPME (Aguinaga et al., 2007), have been used with PDMS-DVB fibers, and SPME involved prior dilution of the sample. While HS-SPME did not work well for higher molecular weight PAHs, it was effective for compounds with up to four aromatic rings and offered better precision than LLE. In summary, both HS-SPME and SPME showed improved recovery compared to traditional LLE methods. Table 1 summarizes the extraction and analytical methods for PAHs in liquid samples.

While traditional LLE and SPE combinations remain reliable for oil matrices, they are laborious and solvent-intensive. Saponification effectively reduces lipid interference but can cause analyte loss. In contrast, SPME and HS-SPME provide comparable or higher recoveries with improved precision and minimal solvent use. SPE continues to dominate routine analysis, but microextraction techniques offer a greener, more efficient alternative for complex lipid systems.

### 2.1.2. Solid matrices

In terms of food products, meat and fish are among the most studied sources of BaP and other PAHs. In meat, especially smoked products, PAHs contamination is mainly connected to both traditional and industrial smoking methods. In fish, it is widely agreed that vertebrate fish quickly metabolize PAHs, which limits their accumulation in muscle tissue. However, PAHs can accumulate in fatty tissues, and fish are still vulnerable to environmental exposure.

Chen et al. (1996) developed a method that utilizes ultrasound-assisted extraction (USE) on lyophilized samples. This process includes a cleanup step using SPE with Florisil. They compared this method to a more complicated one that involved saponification with Soxhlet extraction, several LLE steps, and final SPE. While USE provided similar recoveries and took less time and fewer solvents, the Soxhlet method was favored because saponification is crucial for accurate PAH quantification. Similarly, Chiu et al. (1997) highlighted the importance of saponification in their extraction process.

Wang et al. (1999) introduced pressurized liquid extraction (PLE) for PAH analysis in meat. They used a dichloromethane/acetonitrile solvent along with C18 or C8 sorbents and sodium sulfate in the extraction cell. While PLE allowed for some automation, it still required extensive cleanup, which involved sulfuric acid partitioning and Florisil column chromatography. Later studies (Djinovic et al., 2008a; Jira, 2004) improved the method by adding GPC with either column chromatography or SPE. They usually employed *n*-hexane and polymeric styrene-divinylbenzene (DVB) columns to remove lipids. Although GPC effectively reduced lipid content, further cleanup was needed. Jira (2004) proposed GPC as an alternative to saponification, using silica gel chromatography for remaining polar compounds. Adsorption losses of certain PAHs on sea sand and drying agents resulted in their exclusion. Overall, GPC combined with column chromatography provided better recoveries and lower RSDs than GPC with SPE. However, the latter allowed for the detection of a broader range of PAHs (Djinovic et al., 2008a; Djinovic et al., 2008b).

Although Soxhlet extraction has its disadvantages, such as


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**Table 1. Analytical methods for benzo[a]pyrene in some fatty and non-fatty liquid samples**

| Matrix | Extraction method | Cleanup | Separation/detection | References |
|---|---|---|---|---|
| Edible oil (F) | Dilution (n-hexane); LLE | SPE (C18/C8) | LC-FLD | Barranco et al. (2003) |
| | Dilution (n-hexane) | DACC column; Column chromatography | LC-FLD | Barranco et al. (2004) |
| | Dilution (n-heptane); LLE (DMSO; cyclohexane; water) | Column chromatography | LC-FLD | Pandey et al. (2004) |
| Olive oil (F) | SPE (C18 Nucleoprep + Florisil) MSPD (C18 + Florisil) | | GC-MS, LP-GC-MS, LC-FLD | Bogusz et al. (2004) |
| | Dilution (n-hexane); LLE | SPE (silica); Soxhlet; LLE | GC-MS | Guillén et al. (2004) |
| | HS-SPME | | GC-MS | Vichi et al. (2005) |
| | HS | | GC-MS(/MS) | Arrebola et al. (2006) |
| Oil, food mixture (F) | PLE | SPE (PS-DVB) | GC-MS/MS | Veyrand et al. (2007) |
| Olive, olive-pomace oil (F) | SLE or LLE | GPC | GC-MS/MS | Ballesteros et al. (2006) |
| Vegetable oil (F) | Dilution (n-hexane); SPME | | GC-MS | Purcaro et al. (2007a) |
| | Dilution (n-hexane); SPME | | GC × GC-MS | Purcaro et al. (2007b) |
| Fish oil, fish (F) | Homogenization; Saponification; LLE | SPE (Florisil) | GC-MS(/MS) | Ehrenhauser et al. (2010) |
| Milk (F) | HS-SPME (PDMS-DVB) | | GC-MS | Aguinaga et al. (2008) |
| | Dilution (water), SPME (PDMS-DVB) | | GC-MS | Aguinaga et al. (2007) |
| | Addition: Sodium oxalate; LLE | Column chromatography | GC-MS | Grova et al. (2002) |
| | Saponification; LLE | | LC-FLD | Kishikawa et al. (2003) |
| Coffee (NF) | LLE | SPE | LC-FLD | García-Falcón et al. (2005) |
| | MIP-SPE | | LC-FLD | Lai et al. (2004) |
| Coffee brew (NF) | SPE | | LC-FLD | Houessou et al. (2005) |
| Tea (NF) | SPE | | LC-FLD | Kayali-Sayadi et al. (1998) |
| Beverages (NF) | Addition 10% MeOH; MASE | | GC-MS | Rodil et al. (2007) |
| Sugarcane juice (NF) | SBSE-TD; MASE | | GC-MS | Zuin et al. (2006) |
| Cachaca (NF) | LLE | Column chromatography | LC-FLD | Tfouni et al. (2007) |

F, fatty matrices; NF, non-fatty matrices; LLE, liquid-liquid extraction; DMSO, dimethylsulfoxide; SPE, solid-phase extraction; MSPD, matrix solid-phase dispersion; HS, headspace; HS-SPME, headspace solid-phase microextraction; PLE, pressurized liquid extraction; SLE, supported liquid extraction; PDMS-DVB, polydimethylsiloxane/divinylbenzene; MIP-SPE, molecularly imprinted polymer solid-phase extraction; MeOH, methanol; MASE, microwave-assisted solvent extraction; SBSE-TD, stir bar sorptive extraction-thermal desorption; DACC, donor-acceptor complex chromatography; PS-DVB, polystyrene-divinylbenzene; GPC, gel permeation chromatography; LC-LFD, liquid chromatography with fluorescence detector; GC-MS, gas chromatography-mass spectrometry; LP-GC-MS, low pressure-gas chromatography-mass spectrometry; GC-MS/MS, gas chromatography-tandem mass spectrometry; GC × GC-MS, comprehensive two-dimensional gas chromatography coupled with mass spectrometry.

high solvent use, lengthy procedures, and low selectivity, it remains a popular method in PAH analysis due to its effectiveness (Anyakora et al., 2005; Araki et al., 2001; Jánská et al., 2004). Typical solvents include dichloromethane and *n*-hexane. Sample preparation steps, such as lyophilization (Jie and Kai-Xiong, 2007) and homogenization with sodium sulfate (Anyakora et al., 2005; Araki et al., 2001), typically occur before extraction. Since there are often high levels of co-extracted material, post-extraction cleanup is important. GPC is frequently used (Jánská et al., 2004; Jie and Kai-Xiong, 2007) for this purpose; however, other methods, such as saponification, LLE, and column chromatography, have also been mentioned (Al-Omair and Helaleh, 2004; Araki et al., 2001). However, GPC typically requires chlorinated solvents,

which raise environmental and safety issues. While Soxhlet-based methods provide acceptable recoveries, they often show high relative standard deviations of 2 to 20%, likely due to the complexity and length of the procedures. Saponification followed by LLE steps is less time-consuming than Soxhlet extraction followed by GPC or LLE; however, its recovery results are lower (Perugini et al., 2007).

PLE has become an effective alternative to traditional methods such as Soxhlet and ultrasound-assisted extraction (USE) because it shortens extraction time (Jánská et al., 2004; Martinez et al., 2004; Wang et al., 1999). However, its non-selective nature requires extra cleanup. Wang et al. (1999) found that strong acid treatment during cleanup damaged several PAHs, while a milder acid (9 M $H_2SO_4$) prevented such losses. Martinez et al. (2004) achieved good recoveries using saponification with a different solvent mix, and they discovered that PLE and USE performed better than Soxhlet in terms of recovery and precision. Similarly, Jánská et al. (2004) noted no significant differences in PAH recovery among the three methods, although PLE showed better repeatability. They recommended using water-miscible solvents in PLE to enhance extraction from moist, fatty matrices like fish.

Microwave-assisted extraction (MAE) combined with saponification has been used to shorten extraction time; however, subsequent SPE cleanup was necessary, limiting analysis to seven PAHs (Pena et al., 2006). Direct SPE or GPC cleanup of MAE extracts has also been employed, yielding satisfactory results on certified reference materials, albeit with limited recovery and precision data (Akpambang et al., 2009; Navarro et al., 2006; Purcaro et al., 2009).

HS-SPME has been applied for PAHs with up to four rings in fish and seafood, using polyacrylate or PDMS-DVB fibers (Aguinaga et al., 2008; Guillén and Errecalde, 2002). Solid samples can be analyzed directly in HS vials or after being mixed with a solvent, but comparative performance data are lacking.

MSPD was employed for the extraction of six PAHs in fish, utilizing sulfuric acid-impregnated silica for lipid removal (Pensado et al., 2005). Although some analytes were retained on the sorbent, recoveries were sufficient and RSDs low, addressing MSPD's usual reproducibility issues. Sulfuric acid is still an effective agent for lipid removal in these matrices.

Ramalhosa et al. (2009) used the QuEChERS method, initially developed for pesticide analysis, to detect PAHs in fish. This method works well for volatile PAHs because it skips evaporation steps, which reduces losses. It also yields consistent results for heavier PAHs, as confirmed by a certified reference material. QuEChERS is a simpler, more efficient option than traditional techniques such as Soxhlet or LLE, while still delivering good performance.

Smoked cheese, a fatty matrix, has not been studied as much. Reported methods include Soxhlet with GPC (Suchanová et al., 2008) approaches based on LLE (Pagliuca et al., 2003), and saponification (Anastasio et al., 2004), along with SPE cleanup using silica sorbents. Recovery rates range from 52 to 96%. However, volatile compounds often have low recovery rates. This issue is less important because these compounds pose a lower cancer risk (Suchanová et al., 2008). Table 2 summarizes the extraction and analytical methods for PAHs in solid samples.

Soxhlet extraction offers high recovery and robustness but suffers from long run times and high solvent demand. PLE and USE shorten extraction time and enhance reproducibility, but they require additional cleanup steps, such as GPC or SPE. MAE and MSPD reduce solvent usage but often yield lower precision. Overall, PLE and USE balance efficiency and accuracy best, while integrated extraction-cleanup systems remain a future improvement focus.

## 2.2. Non-fatty matrices

### 2.2.1. Liquid matrices

Non-fatty liquid foods, such as coffee, tea, alcoholic drinks, and fruit juices, have been tested for BaP and other PAHs. Extracting these substances is usually easier than extracting them from fatty foods because they contain less fat and cause less interference. Common methods include LLE followed by SPE cleanup with silica sorbents (García-Falcón et al., 2005) and LLE with silica gel column chromatography (Tfouni et al., 2007). SPE using reversed-phase or polymeric sorbents must consider PAH solubility and possible adsorption onto glassware. This issue can be mitigated by adding a small amount of organic solvent, such as methanol, acetonitrile, or propan-2-ol.

SPE has been widely applied for PAH analysis in coffee, tea, and spirits using cartridges such as PS-DVB and C18 (Galinaro et al., 2007; Houessou et al., 2006; Kayali-Sayadi et al., 1998). PS-DVB is generally preferred due to π-π


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**Table 2. Analytical methods for benzo[a]pyrene in some fatty and non-fatty solid samples**

| Matrix | Extraction method | Cleanup | Separation/detection | References |
|---|---|---|---|---|
| Meat (F) | Freeze-drying; Soxhlet; LLE | SPE (Florisil) | GC-MS | Chen et al. (1996) |
| Smoked meat (F) | PLE | GPC | GC-EI-MS | Djinovic et al. (2008) |
| | Saponification | SPE (Florisil) | LC-UV, LC-FLD, GC-EI-MS | Chiu et al. (1997) |
| | SPME-DED | | GC-MS | Martin and Ruiz (2007) |
| | PLE | GPC | GC-MS | Jira (2004) |
| | MAE | SPE (Silica) | LC-FLD | Purcaro et al. (2009) |
| Fish (F) | Homogenization, Soxhlet | Water addition; LLE; Column chromatography | GC-MS | Araki et al. (2001) |
| | HS-SPME | | GC-MS | Guillén et al. (2002) |
| | MAE; Centrifugation | SPE (Silica) | LC-FLD | Pena et al. (2006) |
| | Lyophilization; MSPD | Simultaneous SPE | LC-FLD | Pensado et al. (2005) |
| | Homogenization; Soxhlet | Column chromatography | GC-MS | Anyakora et al. (2005) |
| | QuEChERS | | LC-FLD | Ramalhosa et al. (2009) |
| Fish, seafood (F) | Saponification; Water addition; LLE | | LC-FLD | Perugini et al. (2007) |
| | MAE | SPE (Florisil); GPC | GC-MS | Navarro et al. (2006) |
| Fish (F), palm dates (NF) | Soxhlet | Column chromatography | GC-MS | Al-Omair et al. (2004) |
| Shellfish (NF) | Freeze-drying; Soxhlet | GPC; Column chromatography | GC-MS | Jie and Kai-Xiong (2007) |
| Mussel(F) | Lyophilization; PLE | Saponification | GC-MS | Martinez et al. (2004) |
| Cheese (F) | Saponification; LLE | SPE | LC-FLD | Anastasio et al. (2004) |
| | Soxhlet | GPC | LC-FLD | Pagliuca et al. (2003) |
| Fruits, vegetables (NF) | Saponification; LLE | Column chromatography | LC-FLD; GC-MS | Camargo and Toledo (2003) |
| Foodstuff (NF) | Soxhlet | Column chromatography | LC-FLD | Bordajandi et al. (2004) |
| Cane sugar (NF) | SLE; LLE | Column chromatography | LC-FLD | Tfouni and Toledo (2007) |
| Tea leaves (NF) | Ultrasonication | Column chromatography | LC-UV | Lin and Zhu (2004) |
| | Soxhlet | SPE | GC-EI-MS | Fiedler et al. (2002) |
| Vegetables (NF) | Soxhlet | SPE | GC-MS | Zohair et al. (2006) |

F, fatty matrices; NF, non-fatty matrices; LLE, liquid-liquid extraction; PLE, pressurized liquid extraction; SPME-DED, solid-phase microextraction direct extraction device; MAE, microwave-assisted extraction; HS-SPME, headspace solid-phase microextraction; MSPD, matrix solid-phase dispersion; SPE, solid-phase extraction; GPC, gel permeation chromatography; GC-MS, gas chromatography-mass spectrometry; GC-EI-MS, gas chromatogrphy-electron ionization-mass spectrometry; LC-UV, liquid chromatography with ultraviolet detector; LC-LFD, liquid chromatography with fluorescence detector.

interactions that enhance PAH retention and improve reproducibility compared to C18. Small amounts of methanol or acetonitrile are added to reduce PAH adsorption onto glass or sorbent surfaces, though optimal percentages vary by matrix. Molecularly imprinted polymer SPE (MIP-SPE) has also been used for BaP extraction in coffee, showing superior recovery compared to C18 (Lai et al., 2004).

HS-SPME has been used for PAH analysis in tea infusions, with PDMS-DVB fibers providing the best results among polar, non-polar, and medium-polarity options (Viñas et al., 2007). Limitations include fiber overloading due to thin coatings (Zuin et al., 2005). Stir bar sorptive extraction

(SBSE) addresses this by offering greater coating capacity, higher adsorption, and reduced co-extracted matrix material, making it more environmentally friendly. SBSE can be coupled with thermal desorption (SBSE-TD) or solvent-assisted desorption, with minimal solvent use. SBSE-TD successfully quantified BaP in sugarcane juice but showed higher RSDs than standard SBSE (Zuin et al., 2006). Membrane-assisted solvent extraction (MASE) has also been applied, allowing monitoring of more PAHs (16 compounds) with improved recoveries and acceptable results for volatile PAHs due to the absence of evaporation steps (Rodil et al., 2007).

In non-fatty liquid samples, SPE using polymeric or MIP sorbents provides excellent selectivity and reproducibility compared to conventional C18. LLE is simpler but less efficient for trace analysis. Miniaturized techniques such as HS-SPME and SBSE minimize solvent use and enable rapid, on-site analysis with comparable sensitivity. Hence, SPE remains the benchmark, but microextraction offers an eco-friendly alternative for high-throughput applications.

#### 2.2.2. Solid matrices

BaP and other PAHs have been identified in a variety of non-fat solid food matrices, including tea leaves (Fiedler et al., 2002; Lin and Zhu, 2004), vegetables (Nieva-Cano et al., 2001; Zohair et al., 2006), fruits (Camargo et al., 2003), bread (Nieva-Cano et al., 2001), sugar (Tfouni and Toledo, 2007), mixed foods (Veyrand et al., 2007), coffee (Houessou et al., 2006), and dates (Al-Omair and Helaleh, 2004). Extraction strategies for these matrices are similar to those for fatty foods but require modifications due to lower lipid content and different matrix compositions.

Classical Soxhlet extraction remains reliable and widely applied for solid samples. Bordajandi et al. (2004) demonstrated consistent recoveries across diverse food types using a Soxhlet-based protocol, though the method is labor-intensive and solvent-demanding. Alternative approaches such as USE and PLE have gained attention for their shorter processing times and reduced solvent requirements. Nieva-Cano et al. (2001) achieved satisfactory recoveries using sonication followed by direct high-performance liquid chromatography (HPLC)-FLD without extensive cleanup, while Camargo et al. (2003) reported comparable efficiency with PLE in fruits and vegetables.

To improve selectivity, a saponification step followed by SPE or GPC is often used to remove pigments, sugars, and other interfering compounds. Houessou et al. (2006) optimized LLE combined with SPE for ground coffee, whereas Tfouni and Toledo (2007) achieved 80-95% recoveries from sugar using n-hexane extraction and silica purification. Studies on vegetables and fruits (Camargo et al., 2003; Zohair et al., 2006) highlight that surface deposition and thermal processing largely influence PAH accumulation rather than intrinsic matrix composition.

Overall, Soxhlet extraction offers robustness and reproducibility but is being progressively replaced by greener, faster techniques such as PLE and USE. These alternatives maintain comparable accuracy while minimizing solvent use and analysis time. Nonetheless, efficient cleanup remains essential to ensure sensitivity and reproducibility. Future work should emphasize miniaturized, solvent-free extractions (e.g., QuEChERS, SPME) and automated detection systems to enhance throughput and support sustainable PAH monitoring in non-fatty solid foods.

## 3. Detection methods

### *3.1. Chromatographic method*

In recent years, chromatography and mass spectrometry have become more important for analyzing complex chemical mixtures (Jeong et al., 2024). They provide detailed compositional data and highly sensitive measurements. Techniques such as HPLC, GC, and liquid chromatography-mass spectrometry (LC-MS) are commonly used. They work with various detectors, including electron impact ionization (EI), flame ionization (FID), and mass selective (MSD). These tools enable scientists to detect compounds at extremely low concentrations, often at parts per million or even less (Chen et al., 2012). These methods are beneficial for monitoring aromatic compounds such as PAHs. PAHs are persistent in the environment and pose potential health risks. Typically, PAHs are analyzed by LC-FLD or UV-visible detection (LC-UV). GC-MS is also used. These techniques provide the sensitivity and accuracy needed for reliable trace-level detection.

#### 3.1.1. Liquid chromatography with different detectors

LC remains one of the principal techniques for separating


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trace-level and non-volatile polar PAHs. However, its resolution is constrained by column efficiency and environmental factors such as temperature and humidity (Kumar et al., 2017). Compared with GC and HPLC, conventional LC provides less analytical detail for individual PAHs or their alkylated derivatives. The integration of advanced detection systems, however, has significantly enhanced its analytical potential.

FLD has become the most widely applied detector for PAH analysis due to its high sensitivity, selectivity, and relatively low cost (Barranco et al., 2003; Galinaro et al., 2007; Moret and Conte, 2002; Poster et al., 2006). It enables variable excitation and emission wavelengths, ideal for fluorescent PAHs such as anthracene and perylene. However, it lacks an adequate response to non-fluorescent compounds such as chrysene and cyclopenta[cd]pyrene, which are better suited to UV detection (Simon et al., 2008). Hybrid LC systems combining FLD with UV-diode array detectors (DAD) have improved compound confirmation and spectral profiling (Miege et al., 1998). LC-FLD has thus been incorporated into official monitoring protocols for food and beverages because it provides an optimal balance between performance, cost, and operational simplicity (Wenzl et al., 2006).

Despite these advantages, FLD faces limitations in selectivity-particularly for distinguishing alkylated PAHs and isotopically labeled compounds that share similar fluorescence properties (Simkó, 2002; Simon et al., 2008). These issues often necessitate confirmatory analysis using GC-MS for compound-specific validation (Camargo et al., 2003; Houessou et al., 2006; Mottier et al., 2000).

Mass spectrometric detection (LC-MS/MS) has emerged as a complementary and more definitive approach, offering improved structural characterization and lower detection limits (Zhang et al., 2015). However, its application to food matrices remains limited due to instrument cost, ionization challenges, and matrix effects (Lien et al., 2007; Robb et al., 2000; Van De Wiele et al., 2004). Alternative ionization methods such as ESI, APCI, and APPI have been evaluated for hydroxy- and non-polar PAHs, showing promise for extending LC applicability (Ehrenhauser et al., 2010; Rey-Salgueiro et al., 2009).

In summary, LC-FLD remains the method of choice for routine PAH screening due to its affordability and robustness. At the same time, LC-MS/MS serves as a confirmatory tool where higher specificity and quantification accuracy are required.

### 3.1.2. Gas chromatography with mass spectrometry

GC is widely used for the determination of volatile and semi-volatile nonpolar PAHs. It provides superior separation efficiency and molecular resolution through rapid analyte interaction with the stationary phase. However, its use is limited to thermally stable compounds, as excessive volatility or degradation during injection can compromise accuracy.

Among GC-based techniques, GC-MS has become the preferred analytical platform for PAH determination in food matrices, surpassing LC-FLD in selectivity and structural elucidation (Berset et al., 1999). Its combined separation and mass spectral capabilities enable precise identification of PAHs that lack fluorescence or exhibit weak optical responses, such as cyclopenta[cd]pyrene, naphthalene, acenaphthylene, acenaphthene, and fluorene (Cai et al., 2009). GC-MS has been increasingly adopted in standardized monitoring protocols, including USEPA Method 8100, and in recent analytical studies across diverse matrices (Dinaintang Harikedua et al., 2024; Gómez-Ruiz and Wenzl, 2009; Martin and Ruiz, 2007; Nácher-Mestre et al., 2009).

Several modifications of GC-MS have further improved sensitivity and selectivity. Multi-dimensional GC coupled with quadrupole MS (GC×GC-qMS) and SPME allows separation of PAH isomers (Lamani et al., 2015), while ultrasound-assisted emulsification microextraction (USAEME) and GC-FID provide reliable quantification in aqueous matrices (Saleh et al., 2009). Similar advancements, such as two-dimensional gas chromatography-sulfur chemiluminescence detector (GC-SCD) for sulfur-containing PAHs (Dijkmans et al., 2014) and microwave-assisted extraction coupled with HS-SPME (Ratola et al., 2012; Ré et al., 2015), have expanded GC's analytical versatility.

GC-MS methods demonstrate high reproducibility and low detection limits (ng-pg range), as shown in smoked meats (Jira, 2004), aquatic organisms (Aguinaga et al., 2007), and beverages (Rodil et al., 2007). Studies comparing extraction and cleanup techniques, such as ASE, GPC, SPE, and MAS, confirm that GPC is often most effective for complex matrices due to its lipid-removal efficiency and reduced matrix interference (Navarro et al., 2006; Zuin et al., 2006).

In summary, GC-MS offers unmatched specificity and sensitivity for PAHs, especially non-fluorescent compounds,

but requires elaborate sample preparation and cleanup compared to LC-FLD. Consequently, LC-FLD remains preferred for rapid routine monitoring, while GC-MS serves as the definitive confirmatory tool for regulatory and trace-level analysis.

## 3.2. Immunological methods

Regulatory requirements for monitoring a broad spectrum of chemical contaminants in food have driven the need for analytical screening tools that are simple, cost-effective, rapid, sensitive, and capable of detecting multiple analytes simultaneously in a high-throughput, automated format (Wenzl et al., 2006). While chromatographic methods offer high sensitivity and accuracy, they are often time-consuming, labor-intensive, costly, and require extensive sample preparation (Cai et al., 2009; Danyi et al., 2009; Naccari et al., 2008). Consequently, there has been a growing interest in developing alternative screening tools that address these limitations. Among these, immunosensors have emerged as valuable platforms for the rapid and selective detection of BaP in various sample matrices (Boujday et al., 2009; Shimomura et al., 2001).

### 3.2.1. ELISA-based assays

Enzyme-linked immunosorbent assays (ELISAs) are the most established immunoassays for PAH determination, with proven applicability across environmental, food, and biological samples. Early competitive ELISAs using monoclonal antibodies (MAbs) demonstrated strong specificity and cross-reactivity. Meng et al. (2015) produced a MAb using pyrene-protein conjugates, achieving an impressive detection limit of 65.08 pg/mL and excellent recoveries (99-100%) in water samples. Similarly, Xi et al. (2016) developed an indirect competitive ELISA (ic-ELISA) based on clone 2E12, showing an $IC_{50}$ of 0.779 μg/L, detection sensitivity of 0.054 μg/L, and high accuracy in spiked beef samples (recoveries 81-94%). The assay's precision (<6% CV) and minimal cross-reactivity support its potential for food monitoring. Wu et al. (2022) extended the ic-ELISA approach to aquatic products, achieving detection limits of 0.43-0.98 μg/L and strong correlation with HPLC-FLD results. Jeeno et al. (2024) demonstrated a cost-effective ic-ELISA using IgY antibodies from egg yolk, suitable for BaP screening in grilled meat, though with higher $IC_{50}$ values. Collectively, ELISA-based assays combine simplicity, reproducibility, and strong quantitative performance, but their dependence on laboratory instrumentation and potential matrix effects limit on-site applicability.

### 3.2.2. Lateral flow and rapid immunochromatographic assays

To improve portability and speed, lateral flow immunoassays (LFIA) and gold nanoparticle-based immunochromatographic assays (GICA) have been developed for point-of-care detection. Beloglazova et al. (2011) introduced a handheld immunochemical test with detection limits of 4-40 ng/L, validated against HPLC-FLD for food supplements such as garlic and radish. Yuan et al. (2025) advanced this concept by integrating Prussian blue nanoparticles (PBNPs) with monoclonal antibodies into an LFIA platform for the analysis of edible oils. The method achieved detection limits of 0.035-0.106 μg/kg within 14 minutes and demonstrated up to a 285-fold improvement in sensitivity over conventional assays. Li et al. (2024) further applied a gold nanoparticle-based dual T-line GICA to BaP detection in oilfield chemicals, achieving a broad dynamic range (0.42-300 mg/kg) and excellent recoveries (88-106%) even under harsh conditions.

These portable assays combine ease of use, rapid response, and minimal pre-treatment requirements. However, their semi-quantitative nature and relatively narrower precision windows compared to ELISA still necessitate confirmatory chromatographic analysis for regulatory reporting.

### 3.2.3. Advanced and hybrid immunoassays

Beyond traditional ELISAs and LFIA systems, hybrid immunoassays integrate nanotechnology, fluorescence enhancement, or molecular design to improve sensitivity and selectivity. Li et al. (2016) reported a label-free fluorescence immunoassay exploiting fluorescence resonance energy transfer (FRET) between BaP and its antibody, enhancing fluorescence by 3.1-fold and yielding a detection limit of 0.06 ng/mL with good recoveries in cereal matrices. Karsunke et al. (2011) developed an automated flow-through biochip chemiluminescence immunoassay (CLEIA), achieving rapid screening (<5 min) with $IC_{50}$ values of 0.31-0.92 μg/L. Georgiadis et al. (2012) presented a sandwich chemiluminescence immunoassay (SCIA) for PAH-DNA adducts, offering a detection limit of 3 adducts per $10^9$ nucleotides and validated


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applicability for large-scale population studies. Ma and Zhuang (2018) demonstrated a real-time biotin-streptavidin immuno-PCR (BA-IPCR) for BaP detection in food, reaching an ultra-low limit of 2.85 pg/L with recoveries above 90%. These hybrid formats combine the specificity of immunoassays with the sensitivity of molecular amplification or luminescence detection, enabling trace-level quantification and high-throughput screening.

#### 3.2.4. Supporting and complementary approaches

Complementary advances such as molecularly imprinted solid-phase extraction (MISPE) coupled with ELISA (Pschenitza et al., 2014) have further improved sample cleanup and selectivity. The MISPE-ELISA combination achieved recoveries of 63-114% in vegetable oils and reduced lipid interference, though minor overestimation occurred due to cross-reactivity among PAHs. Similarly, flow cytometry- based immunoassays (Meimaridou et al., 2010) and handheld immunosensors (Beloglazova et al., 2011) have expanded automation and multiplexing capabilities, allowing simultaneous analysis of multiple PAHs in complex matrices.

Immunological techniques have evolved from laboratory-bound ELISAs to portable, multiplexed, and hybrid systems capable of detecting BaP at sub-ng/L levels. ELISA-based assays remain the benchmark for quantitative accuracy and are well-validated for regulatory use, though they require benchtop instrumentation. Lateral flow assays (LFIA/GICA) provide unmatched portability and speed, offering qualitative or semi-quantitative detection suitable for rapid field screening and preliminary risk assessment. Hybrid assays (CLEIA, BA-IPCR, FRET-based, and SCIA) represent the next generation, achieving ultra-low detection limits (in the pg/L range) and multiplexing capabilities, but often require specialized reagents and instrumentation, which limit widespread use.

Overall, hybrid and nanomaterial-enhanced immunoassays show the most significant promise for commercialization, thanks to their balance of speed, sensitivity, and potential for miniaturization. Nonetheless, ELISA remains the most practical choice for routine laboratory monitoring, while LFIA and biochip formats are emerging as viable tools for on-site screening and rapid decision-making in food safety control.

### *3.3. Spectroscopic method*

Spectroscopic techniques play a vital role in both the qualitative and quantitative analysis of substances, particularly in scientific research. These methods rely on the interaction of electromagnetic radiation with matter, enabling the detection and measurement of compounds by analyzing the energy distribution within molecules at a given moment. PAHs, which consist of conjugated π-electron systems, are ideal for such analyses, especially when distinguishing compounds with similar molecular weights but different chemical structures. Spectroscopy, including UV-Vis, IR, Raman, X-ray fluorescence, atomic absorption, and atomic emission techniques, operates on the principle that molecules absorb or reflect specific wavelengths of light. The intensity of this interaction correlates with the concentration and identity of the analyte, allowing for the detection of even trace amounts, often at sub-ppm levels. These tools are widely used to assess sample purity, determine component percentages in mixtures, and investigate chemical interactions or color changes, providing high accuracy in both qualitative and quantitative investigations.

#### 3.3.1. Raman and surface-enhanced raman spectroscopy (SERS)

Raman and SERS techniques are increasingly being explored for PAH detection due to their high molecular specificity and minimal sample preparation requirements. Liu et al. (2023) demonstrated that integrating Raman spectroscopy with machine learning algorithms enables accurate qualitative and quantitative analysis of BaP in peanut oil, achieving a 97.5% classification accuracy and a correlation coefficient of 0.9932 using random forest modeling. The study highlights how chemometric tools enhance the interpretability of complex Raman data, providing a pathway for automated PAH screening.

Similarly, Chen et al. (2014) reported the Raman and SERS spectra of seven PAHs, supported by density functional theory (DFT) calculations to assign vibrational modes. These findings supply valuable spectral fingerprints for method standardization. Costa et al. (2006) improved SERS substrate stability and reproducibility by introducing self-assembled monolayers (SAMs) on gold films, reducing analyte degradation and enabling detection in the ppm range. Gu et al. (2013) employed a metal “sandwich” substrate with silver cavities and 1,10-decanedithiol linkers, achieving nanomolar detection

limits for anthracene and pyrene. Guerrini et al. (2009) enhanced PAH sensitivity using viologen-functionalized silver nanoparticles (AgNPs), achieving zeptomole detection levels, while Fu et al. (2015) quantified trace BaP levels (1-5 μg/L) in oils using inositol hexaphosphate-stabilized gold nanoparticles (AuNPs).

Across studies, SERS-based detection provides ultra-high sensitivity, rapid analysis, and minimal sample pretreatment. Functionalized metallic nanostructures significantly enhance Raman signals and improve selectivity toward hydrophobic PAHs. Nonetheless, variability in substrate fabrication and the absence of standardized calibration protocols hinder reproducibility and regulatory acceptance. Integrating chemometric and machine learning models enhances analytical reliability, but further validation across food matrices is required for routine application.

### 3.3.2. Fluorescence spectroscopy

Fluorescence-based methods offer high sensitivity for detecting BaP due to its strong native fluorescence and ability to form stable excimers in organic matrices. Orfanakis et al. (2023) introduced an extraction-free fluorescence approach for BaP detection in extra virgin olive oil (EVOO), achieving ppb-level sensitivity consistent with EU regulatory limits. Their partial least squares (PLS) regression model confirmed the method's robustness and suitability for rapid quality control. Similarly, García-Falcón et al. (2000) combined microwave-assisted extraction and saponification with second-derivative synchronous spectrofluorimetry, attaining low detection (0.05 mg/kg) and quantification limits (0.12 mg/kg) and recoveries near 90%.

Li et al. (2011) enhanced PAH resolution using nonlinear variable-angle synchronous fluorescence scanning, allowing simultaneous quantification of BaP, benzo[k]fluoranthene, and anthracene in tea with detection limits of 0.18-0.89 μg/kg and recoveries up to 116%. Bortolato et al. (2008) developed a solvent-free fluorescence technique using nylon membranes coupled with chemometric modeling (PARAFAC and U-PLS/RBL), achieving the quantification of BaP and dibenzo[a,h]anthracene at levels below 10 ng/L, even in the presence of complex PAH mixtures. Andrade Eiroa et al. (2000) further demonstrated that second-derivative synchronous luminescence (SDCESL) surpasses constant-wavelength approaches in terms of precision and detection limit (0.007 ng/mL), fully complying with EU drinking water directives.

Fluorescence spectroscopy remains one of the most sensitive and accessible tools for BaP detection, capable of meeting or exceeding regulatory thresholds in food and environmental samples. Its significant advantages lie in rapidity, low cost, and minimal solvent use. However, spectral overlap and matrix fluorescence can complicate quantification in complex samples. Chemometric algorithms have mitigated these limitations, suggesting that fluorescence spectroscopy is a viable method for high-throughput screening when confirmatory chromatographic methods are impractical.

### 3.3.3. Infrared spectroscopy

Infrared (IR) spectroscopy provides complementary structural and compositional information, especially useful for characterizing PAH molecular features and predicting their environmental behavior. Tommasini et al. (2016) analyzed 51 PAHs and correlated mid-IR absorption bands (600-900 $cm^{-1}$) with edge topology and hydrogen connectivity using DFT simulations. The identification of vibrational patterns (SOLO, DUO, TRIO, and QUATRO) provides a framework for structure-spectra relationships and the modeling of graphene-like edge structures. Izawa et al. (2014) examined near-IR reflectance spectra of 47 PAHs and identified diagnostic overtone regions (880-1,860 nm) sensitive to ring connectivity and heteroatom substitution, offering potential for non-destructive classification. Zhang et al. (2016) applied mid-IR spectroscopy coupled with a hybrid chemometric algorithm (DPSO-WPT-PLS) to determine BaP in cigarette smoke, achieving superior predictive accuracy compared with conventional PLS methods and demonstrating the feasibility of real-time monitoring in tobacco quality control.

Infrared-based approaches enable molecular fingerprinting and non-destructive quantification of PAHs in complex matrices. The integration of chemometrics and machine learning enhances predictive capability, though sensitivity remains lower than fluorescence or SERS techniques. IR spectroscopy is best suited for structural elucidation, qualitative screening, and complementing other detection platforms rather than serving as a standalone quantitative tool.

In general, spectroscopic methods, particularly fluorescence, Raman/SERS, and IR, offer rapid, solvent-free, and environmentally sustainable alternatives to chromatographic analyses. Their key advantages include minimal sample preparation, reduced cost, and suitability for real-time, in situ monitoring. However, challenges remain regarding quantitative


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standardization, inter-laboratory reproducibility, and matrix-specific interference, which currently limit their integration into official regulatory frameworks. Fluorescence spectroscopy is the most mature for regulatory use, with demonstrated compliance with EU BaP limits and validated correlation to chromatographic standards (García-Falcón et al., 2000; Orfanakis et al., 2023). Raman and SERS methods show extraordinary sensitivity and potential for miniaturization but require standardized substrate fabrication and calibration models before widespread adoption. Infrared spectroscopy, while non-destructive and information-rich, remains primarily a complementary tool for qualitative and structural analysis.

Future progress will depend on the development of harmonized validation protocols, portable sensor integration, and chemometric-assisted calibration models to ensure reproducibility and regulatory acceptance. Overall, spectroscopic methods represent a rapidly advancing frontier in PAH analysis, bridging the gap between laboratory-based confirmation and field-deployable screening systems.

## 4. Conclusions

The detection and quantification of PAHs, particularly BaP, in food remain critical for public health protection and regulatory compliance. Extraction and cleanup efficiency largely depend on matrix composition, especially lipid content. Traditional methods such as Soxhlet extraction, LLE, and SPE remain reliable but are time- and solvent-intensive. Modern alternatives like PLE, UAE, MAE, and QuEChERS offer faster, greener, and more sustainable options, though performance differences across matrices highlight the need for systematic cross-validation.

Chromatographic techniques, including LC-FLD and GC-MS or GC-MS/MS, continue to serve as the benchmark for PAH quantification. LC-FLD is cost-effective for routine use, whereas GC-MS provides superior selectivity for confirmatory analysis. However, both methods are resource-intensive and unsuitable for rapid screening. As a result, immunological and spectroscopic methods have gained attention as complementary tools for large-scale or field-based monitoring.

Immunoassays such as ELISA, LFIA, and CLEIA combine high specificity, rapid turnaround, and affordability, enabling high-throughput screening. Spectroscopic techniques, including fluorescence, Raman, and IR spectroscopy, offer solvent-free, non-destructive detection with increasing sensitivity through nanomaterial enhancement and chemometric modeling. Despite these advantages, their broader regulatory adoption is limited by variability, matrix dependence, and the lack of standardized validation.

Key gaps persist in harmonized method validation across food matrices, inter-laboratory reproducibility, and unified reporting of analytical performance parameters. Addressing these limitations requires standardized reference materials, cross-platform calibration protocols, and performance criteria aligned with international standards (ISO, AOAC, Codex).

Future PAH monitoring will benefit from integrating rapid screening and confirmatory platforms. Hybrid systems combining microextraction, portable immunoassays, and spectroscopic readouts, supported by chemometrics and machine learning, could achieve accurate, automated, and real-time contamination assessment. Moreover, aligning analytical workflows with green chemistry principles will reduce solvent use, waste, and energy demand.

In conclusion, while chromatographic methods remain indispensable for confirmation, rapid and sustainable tools such as immunoassays and spectroscopic sensors are poised to transform PAH analysis. The future lies in developing harmonized, high-throughput, and data-driven analytical systems that merge laboratory precision with field-level applicability.

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### Author contributions

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