# Analysis of Polynuclear Aromatic Hydrocarbons (PAHs) in Edible Oil to 0.5 ppb

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**Polynuclear Aromatic Hydrocarbons (PAHs) are found in many edible oils, which are typically introduced during the refining and manufacturing process, but can also be the result of environmental contamination through transportation and storage. Some of these compounds are known to be carcinogens so testing for them is critically important in order to protect public health. The European Union (EU) has recently introduced new directives for all foodstuffs including edible oils requiring maximum levels of 0.9 ppb for three PAHs, including benzo(a)anthracene, chrysene and benzo(b)fluoranthene in addition to the original target of benzo(a)pyrene, which was not considered a sufficient toxicity marker on its own. This study will therefore investigate the development of a method, including sample cleanup to meet a reporting limit of 0.5 ppb in palm oil using gas chromatography (GC) coupled with a single quadrupole mass spectrometer (MS).**

## EU Regulations for the Measurement of PAHs in Foodstuffs

In 1970 the U.S. EPA proposed the monitoring of a set of 16 PAHs which are frequently found in environmental monitoring samples. The European Union (EU) in conjunction with the Scientific Committee on Food (SCF), now a part of the European Food Safety Authority (EFSA), further refined the list based on evaluations carried out by various international food research groups, which prioritised compounds based on the health risk rather than on occurrence in food. As a result, it suggested a shorter list, including eight high molecular weight compounds, but recommended the use of benzo[a] pyrene as a significant marker, based on an assessment of PAH toxicity profiles in food and a carcinogenicity study in mice. They also stressed the importance of continuing to collect data on other PAHs in order to evaluate the contamination of food materials and any possible changes in the PAH profile. These directives came out in 2006 and were in place until 2011 when the European Union (EU) introduced new directives for all foodstuffs including edible oils requiring maximum levels of 0.9 ppb for three additional PAHs plus the original target of benzo(a)pyrene. Finally in 2015, the EU updated these regulations and included a suite of additional natural food products [1]. A summary of the timeline of the implementation of these regulations is shown below:

- 2006: Regulation (EC) No 1881/2006 which targets benzo(a)pyrene as a marker [2].
- 2011: The regulation was updated with Regulation (EU) No 835/2011, which added benzo(a)anthracene, chrysene and benzo(b)fluoranthene to the original target of benzo(a)pyrene, which was not considered a sufficient toxicity marker on its own [3].
- 2015: The EU published Regulation (EU) No 2015/1933, which set maximum levels for PAHs in cocoa fibre, banana chips, food supplements, dried herbs and dried spices [4].

## Analytical Methodology

### Instrumentation

The PerkinElmer Clarus 680 Gas Chromatograph (GC) [5] coupled with the SQ8 single quadrupole Mass Spectrometer [6] was used for this study, utilising TurboMass™ chromatography data system (CDS) for instrument control, data processing and reporting [7]. The GC was also configured with a split/splitless programmed temperature vaporiser (PTV) injection port, which will be described later [8].

### Sample Preparation

Removing and extracting the PAHs from the oil is a critically important component of this analysis to maximise the recoveries of PAHs and to minimise the presence of interfering compounds in the extract. A variety of extraction options were considered including gel permeation chromatography (GPC), solid phase extraction (SPE) and molecular imprinted polymer (MIP) cartridges. All three gave similar extraction performance, but the MIP approach was chosen because of its ease of use compared to the other extraction methods. The fundamental principles of MIP technology are described below.

### Molecular Imprinted Polymers

This extraction technique uses a process by which selected functional monomers are allowed to self-assemble around a template molecule and subsequently polymerised in the presence of a crosslinked molecule [9]. These cartridges are designed to retain the compounds of interest and to remove the sample matrix, which in this case is the palm oil. The cartridge is first rinsed with a solvent. Then the sample is loaded or placed into the cartridge. For this method, a 50:50 solution of palm oil:cyclohexane was used. Then the cartridge is rinsed with 20 mL volume of cyclohexane or enough volume to elute the oil from the material. Cyclohexane has the effect of leaving the PAHs retained in the MIP while removing the palm oil from the cartridge. After successfully removing as much oil as possible, 2 mL of ethyl acetate is used to remove the PAHs from the cartridge. The extraction protocol is described below.

### Extraction Protocol

The cartridge was rinsed with 5 mL of cyclohexane prior to loading the sample. Approximately 1g of sample was weighed accurately and diluted with 1 mL of cyclohexane. This was then loaded into the cartridge followed by rinsing with cyclohexane, which has the effect of leaving the PAHs trapped in the MIP and removing the oil. After rinsing with cyclohexane, the PAHs are eluted from the cartridge with 2 mL of ethyl acetate. To optimise detection limits, the PAHs can be concentrated in the extract by evaporating a known volume of solvent. The final volume will depend upon the detection limit requirements; however, in this case, a 1 mL extract remained.

Concentration is performed in a hood with a slow nitrogen purge into the vial. The amount of flow depends upon how many samples are being concentrated at the same time. Bubbling of the solvent should be avoided, so the correct flow is indicated by a small dimple in the solvent caused by the nitrogen flow.

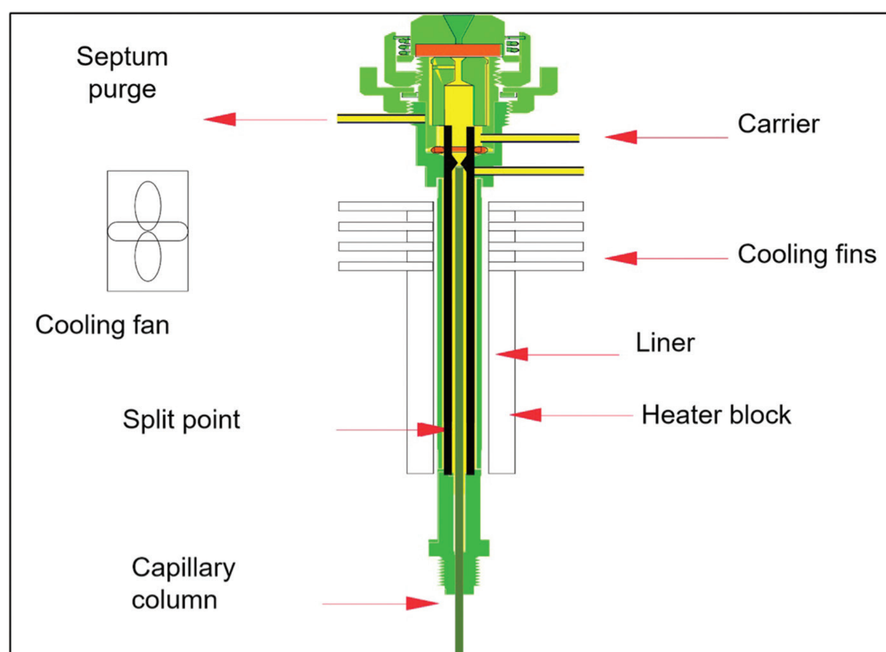


*Figure 1: Schematic of the temperature programmable split/splitless injector in the on-column configuration.*

### Large Volume Injection

Large Volume Injection (LVI) was employed in this investigation to enhance the detection limits [10]. A temperature programmable split/splitless injection port (PSS), which is also known as a programmed temperature vaporisation (PTV) was used to concentrate the analytes in the injection port. After the solvent is purged from the inlet, the split vent is closed and the injector port rapidly heats to volatilise the analytes making a splitless injection into the column. The benefits of the PSS injector are that it is 'universal' which means it is capable of hot volatilisation (split/splitless), temperature programmed volatilisation (split/splitless), on column and solvent purge using both small and large volumes. The PSS also has a low thermal mass, so it heats up and cools down very rapidly. The cooling fan and cooling fins, help to dissipate the heat so it is ready to inject when the GC is back to the initial temperature. The PSS injector, which is shown in Figure 1 in the on-column (not split/splitless) configuration, can heat up to a maximum of 500°C at a rate of 200°C per minute.

### Controlled Volatilisation

The injection technique required for performing LVI is referred to as a solvent purge injection since the solvent is being purged from the injector. During this purge time, the injector port is kept at very low temperatures mainly for two reasons. First, there would be enormous vapour expansion of the solvent if the injector port was hot. Secondly, the semi-volatile components can't volatilise until the split vent is closed after the solvent purge to optimise detection limits.

There are many benefits of controlled volatilisation, compared to the limitations of flash volatilisation in a hot injector port, which are outlined below:

- Flash hot injections may result in a solvent vapour expansion that exceeds the volume of the liner. This is sometimes referred to as a backflash when the sample pressure is so great it contaminates the pneumatics and the plumbing lines.
- Even if the sample doesn't get into the pneumatics, the sample is not contained in the liner and as a result will not find its way to the column, which could potentially cause poor precision and low recoveries particularly with higher boiling compounds.
- Hot injection can lead to the breakdown of thermally labile compounds, which is often attributed to high inlet temperatures required for fast sample vaporisation.
- Syringe fractionation can result from hot injection, where the lighter components vaporise first and leave the syringe needle quickly, while heavier compounds take longer to evaporate and leave the syringe at a slower rate resulting in sample discrimination.

It's also important to emphasise that solvent purge is a type of splitless injection technique, which is used to provide lower detection limits for semi-volatile compounds. As a result, solvent purge enhances recoveries and repeatability over classical splitless injections because of this controlled volatilisation process. In addition, controlled volatilisation can be used for classical split and splitless injection without solvent purge by ramping the injector rapidly after injection. Because vapour expansion is greatly reduced, larger volumes may be used.

### Experimental

The solvent purge time was 0.5 minutes at an inlet temperature of 70°C and a split flow of 150 mL/min. A 5 uL injection was used.

After the split vent was closed, the injector was heated at 200°C/min to a final temperature of 330°C.

The chromatography was performed on a PerkinElmer Elite 5 stationary phase column with dimensions of 30 m x 0.25 mm x 0.25 µm. The initial GC oven temperature was set to 70°C with a hold time of 2 minutes. It was then ramped at 5°C/min to a final temperature of 300°C and held for 2 minutes.

As mentioned previously, the most recent version of the EU regulation requires four PAHs benzo(a)anthracene, chrysene, benzo(b)fluoranthene and benzo(a)pyrene to be reported. Initially, the MS acquisition was performed in simultaneous full mass scan

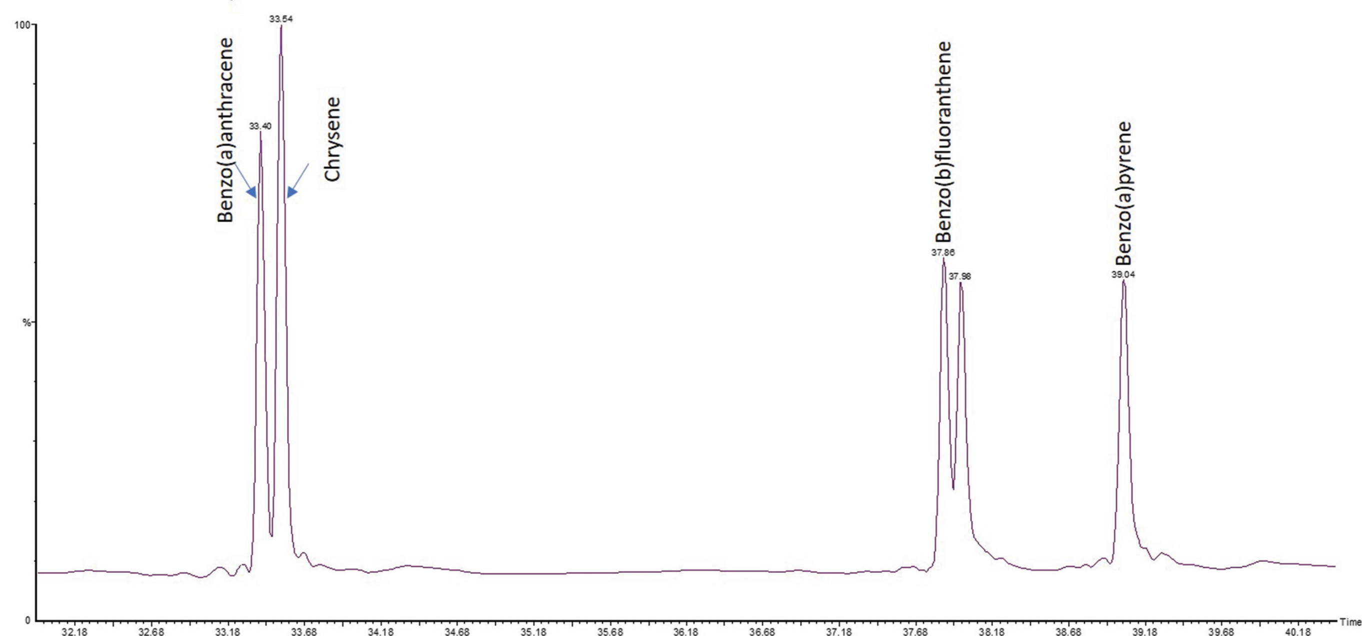


*Figure 2: Mass chromatograms of chrysene, benzo(a)anthracene, benzo(b)fluoranthene and benzo(a)pyrene, showing additional peaks from the Restek 16-PAH mixture.*

and single ion monitoring (SIM) modes. Full scan was collected to ensure the sample was successfully cleaned of the oil, whereas for calibration and quantitation, SIM acquisition was performed using masses 228 amu for benzo(a)anthracene and chrysene and 252 amu for benzo(b)fluoranthene and benzo(a) pyrene. Repeatability (precision) was performed at a concentration of 2 ppb, while a matrix spike to determine recoveries was performed at 5 ppb.

### Calibration

A five-point internal standard linear regression calibration was performed using a standard mix purchased from Restek Corporation - Catalog # 31011 [11], which contained 16 PAHs. However, only the four compounds of interest were investigated. The standards were diluted in a 50:50 blend of ethyl acetate and cyclohexane, using deuterated chrysene-d12 as the internal standard. Note: The 'zero' point was not included in the calibration and the line was not forced through zero.

## Discussion and Results

The Elite-5 phase stationary phase column described earlier is commonly used for the analysis of semivolatile compounds including PAHs. The mass chromatograms of the four investigated PAHs are shown in Figure 2. The additional peaks are the isomers that were in the 16 PAH standard mix but were not required for this investigation.

Figure 3 exemplifies the injection made in full scan mode to ensure the sample matrix, after cleanup, was not interfering with the analysis. There were many other clean-up procedures investigated to assess the most robust method with regard to reduction of sample interferences and optimisation of instrument uptime, but they are outside the scope of this study.

The analytical performance is demonstrated in Table 1 including the results of the calibration, the signal to noise determined on the quantitative SIM ion for each compound and the results from the

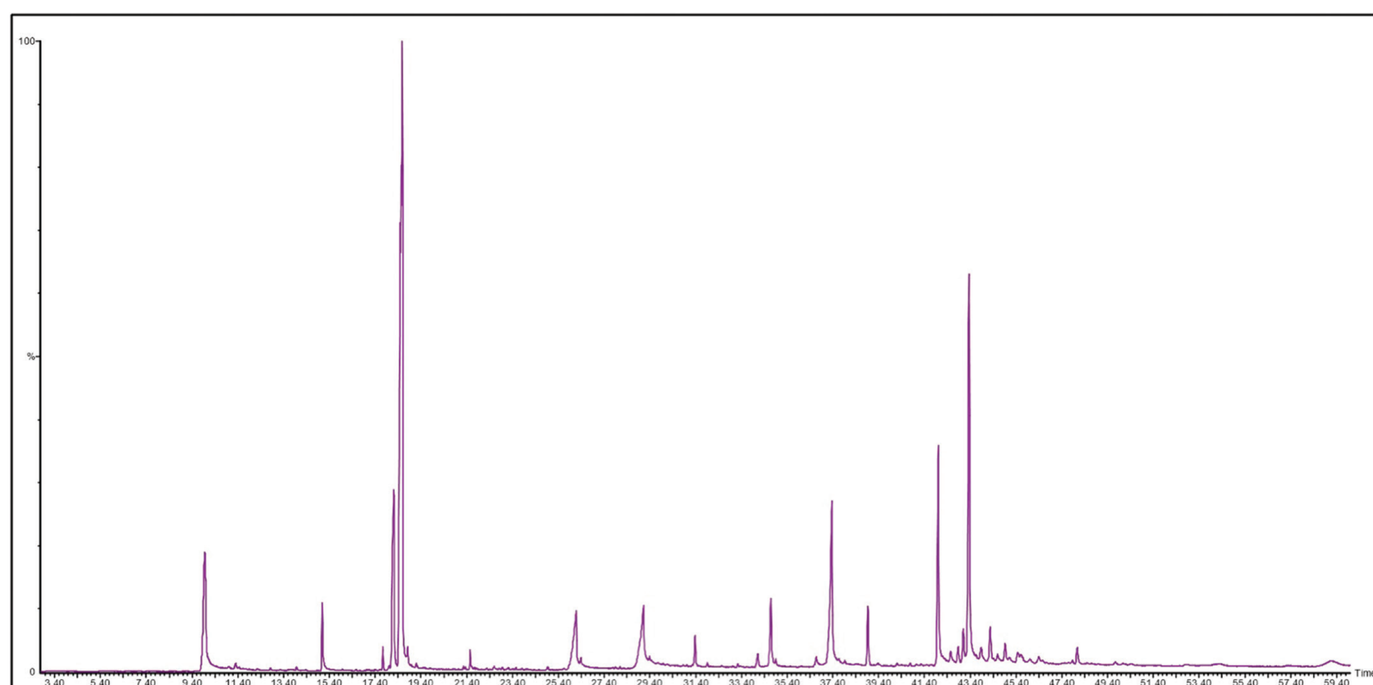

*Figure 3: Full scan total Ion chromatogram (TIC) representation of the sample matrix.*

| Compound | Linearity (range 0.5 to 10 ug/Kg) $r^2$ | Precision (n=5) | Reporting Limit S/N at 0.5 µg/Kg | Quant Ion | Matrix Spike PPB | % Recovery |
|---|---|---|---|---|---|---|
| Benzo(a)anthracene | 0.9947 | 4.87 | 2034:1 | 228 | 3.91 | 78.2 |
| Chrysene | 0.9976 | 3.47 | 445:1 | 228 | 6.10 | 122.0 |
| Benzo(b)fluoranthene | 0.9996 | 8.20 | 453:1 | 252 | 5.04 | 101.0 |
| Benzo(a)pyrene | 0.9967 | 4.80 | 603:1 | 252 | 4.19 | 83.8 |

*Table 1: Results from analytical performance and validation experiments. Note: Precision data represents %RSD of the 2.0 ppb standard.*

repeatability experiment, where five injections of the 2.0 ppb standard were carried out from the same vial.

On reviewing the signal to noise, the authors feel very confident that the reporting limit is in a robust quantitation range and could be further reduced by another factor of 10. In addition, the results of the 5 ppb matrix spikes of palm oil were all in an acceptable recovery range.

## Conclusion

The results obtained in this investigation have clearly achieved the EU regulatory limits. As a result, this method is currently the technique of choice for many food-based organisations that require reporting a suite of four PAHs to exceed the EU requirement of 0.9 ppb. In addition, the data suggests that this methodology can easily achieve detection capability down to 0.05 ppb for a larger suite of PAH compounds. This work will be the basis of a future study to look at additional PAHs and at lower reporting limits for foodstuffs.

## Acknowledgement

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