# THE DETERMINATION OF BENZO(A)PYRENE IN OIL MATRIX BY HPLC AND FLUOREESENCE DETECTION

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# Introduction.

Polycyclic aromatic hydrocarbons (PAHs) are highly stable contaminants which occur in air, soil and food. Some of these components have toxic properties. Benzo(a)pyrene, the best known of the carcinogenic PAHs, has been found at varies concentration in several foods and beverages including water, cereal, oils and smoked meat<sup>2,3</sup>. The possible source of PAHs contamination in vegetable oils are atmospheric contamination of plant material, direct drying of the plant material with combustion smoke before extraction, contamination through the extraction solvent and uptake by the oil plants from contaminated soils<sup>7,8</sup>. The presence of PAHs in vegetables oils has been reported by a number of workers<sup>1,5,6,4,8</sup> and the detected level of benzo(a)pyrene in the analyzed oils ( corn, soybean, sunflower, rape seed, palm) were in the range 1.10  $\mu$ g/kg to 68.6  $\mu$ g/kg. The investigation of benzo(a)pyrene content in palm oil products was initiated by validating the proposed method by IUPAC for used with palm oil and palm oil industry to monitor products for contamination by this carcinogenic pollutant

# Materials and methods.

All chemicals used were pro-analysis. Full blank analysis were carried out to confirm that the chemicals are suitable for trace analyses.

# Materials

- Petroleum ether  $(40^{\circ}\text{C} 60^{\circ}\text{C})$
- Deactivated aluminium oxide, activity Super 1
- Aluminium oxide, activity IV, prepared by adding 10g distilled water to every 90g aluminium oxide of activity super I. Shaken for about 15 min and equilibrated in a closed vessel at room temperature for 24 hours before using.
- Anhydrous sodium sulphate, purity 99%
- Tetrahyrofuran, purity  $\geq$  99%
- Acetonitrile gradient grade, minimum purity 99.8%

- HPLC eluant: acetonitle/water (88/12% v/v)
- Benzo(a)pyrene standard, 99% purity
- Toluene
- Benzo(a)pyrene stock solution 0.5mg/ml. Calibration standard solution prepared in the range of 0.01µg/µl to 0.05 µg/µl by diluting aliquots of the standard stock solution with acetonitrile
- Glass chromatography columns (300mm x15mm, with teflon taps)
- HPLC- fitted with a reversed phase column (Jones Chromatography C18, 4 μm 250mm x 4.6 mm) and fluorescence detector (emission wavelength 410nm, excitation wavelength 292 nm)

# Methods.

The following flow chart shows the method for extracting and analysing benzo(a)pyrene in a sample of palm oil.

2g benzo(a)pyrene spiked sample of palm oil

dissolve in 10 ml volumetric flask with Petroleum ether (PE)

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Transfer 2 ml of sample solution onto aluminium oxide column

Introduce 60ml of PE into the column

Collect the eluent at rate approximately 1 ml/min

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Evaporate under a nitrogen stream of about 25ml/min at 35°C

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Transfer into vial and evaporate until dryness then make up to 1 ml with tetrahydrofuran

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Inject 100 µl into HPLC column

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Determine the peak area by fluorescence detection

## **Results And Discussions.**

This method was previously evaluated in a collaborative study using crude fish oil and rapeseed oil. Based on the results obtained, the method was adopted by IUPAC. In our investigation we tested the method on palm oil and validated the procedure by determining the recoveries of benzo(a)pyrene from palm oil and palm kernel oil sample spiked with the polycyclic aromatic hydrocarbon at levels ranging from  $0.01\mu$ g/ml to  $0.5\mu$ g/ml. The recoveries were found to be in the range of 92% to 101%. This method is useful for monitoring the levels of polycyclic aromatic hydrocarbons such as benzo(a)pyrene in the vegetable oil industry.

# Acknowledgement.

The authors would like to thank the Malaysian Palm Oil Board for permission to present out this paper.

Thanks are also due to research assistants for their technical help.

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