DETERMINATION OF BENZO(a)PYRENE IN VEGETABLE OILS

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ABSTRACT

Determination of benzo(a)pyrene in vegetable oils was carried out using aluminium oxide for clean-up followed by reverse phase high performance liquid chromatographic (HPLC). A quantity of oil was dissolved in petroleum ether and added to an aluminium oxide column which retained the lipids. Benzo(a)pyrene in the oil was eluted from the column with petroleum ether and the collected eluate was concentrated by evaporation of the solvent. Identification and quantification was performed by HPLC using fluorescence detection at the optimized wavelength. The retention time of a benzo(a)pyrene standard was used for identification and the concentration was calculated by external calibration.

The method was tested on an oil matrix, then validated by determining the recoveries of benzo(a)pyrene from vegetable oil samples spiked with polycyclic aromatic hydrocarbons at 0.01 μg kg^{-1} to 0.5 μg kg^{-1}. Recoveries and coefficients of variation for the oils spiked with benzo(a)pyrene standard were 90% to 102% and 1.23%-2.03% respectively. This method was then used for monitoring the level of benzo(a)pyrene in vegetable oils.

Keywords: liquid chromatography, benzo(a)pyrene, vegetable oils.

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), produced during incomplete combustion, or pyrolysis of organic materials, are toxic contaminants of the environment. Contamination of foodstuffs, including edible oil from plant sources are usually associated with the presence of these compounds in the environment through the uptake by the plants from contaminated soil. Other potential occurrence during the processing of food by direct drying contaminated plant materials and contamination through extraction solvents.

PAHs present in oils and fats are likely due to direct drying of oilseeds with fuel gases, where combustion products come into contact with raw material. For example, grape seeds have to be dried prior to oil extraction. PAHs found in coconut oil and olive pomace oil are of health concern for oils and fats producers because of the potential carcinogenicity of some of these PAHs.

In a report on United Kingdom diet surveys, Dennis *et al.* (1991) indicated that the groups of cereals, and oils and fats, each contributed about one-third of the daily total PAHs intake. This was estimated to be 3700 Ωg per person and about 80% of the total daily dietary intake was benzo(a)pyrene (250 Ωg per person). Margarine was found to be a major source in the oils and fats group, contributing 70% of the benzo(a)pyrene in the total intake of the contaminant. PAHs were also high in retail vegetables oils with an average of benzo(a)pyrene content of 1.29 Ωg g^{-1} and also high concentrations of benzo(a)pyrene (maximum 2.2 Ωg g^{-1}) were found in cereal-derived products and puddings, biscuits and cakes, containing edible oils as their ingredient (Moffart and Whittle, 1999).

The presence of PAHs in vegetable oils was reported by a number of workers and the detected levels of benzo(a)pyrene in the various oils analysed (corn, soyabean, sunflower, rapeseed, palm) were in the range of 1.1 μg kg^{-1} to 68.0 μg kg^{-1}. Furthermore, food processing such as smoking, drying, barbecuing or roasting was found to be the main source of

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significant amounts of PAHs and the groups of food commonly listed as contributors to PAHs intake were grilled meat, cereals, vegetable fats and oils (Dennis *et al*., 1991; De Vos *et al*., 1990; Kazerouni *et al*., 2001). Jung and Morand (1962; 1963; 1964) isolated pyrene and benzo(a)pyrene from different oils by repeated column chromatography on alumina and silica gel (sometimes preceded by saponification of oils) followed by paper chromatography. The PAH's compounds were identified by ultraviolet and fluorescence procedures.

Howard *et al*. (1966) developed a method for the isolation and determination of PAHs in refined vegetable oils by diluting them with an aliphatic solvent, extracting with dimethyl sulfoxide, isolation using column chromatography then thin layer chromatographic separation followed by quantification using ultraviolet and spectrofluorometric detection. The average recoveries of benzo(a)pyrene, dibenz(a,h)-anthracene, benz(a)anthracene and benzo(g,h,i)perylene from 200 g oil samples containing 2.0 μg kg^{-1} of PAHs was reported to range from 71% to 100%.

Strijve and Hischenhuber (1987) described a simplified method for the determination of 12 significant PAHs in various commodities such as tea, coffee, herbs, spices, spice extracts, smoked meat and fish, vegetable oils and roasted cereals, by subjecting the samples to saponification, solvent partition and column clean up before PAHs separation and determination using HPLC with a fluorescence detector. An optional method, using thin layer chromatography (TLC) with flourodensitometric measurement, was also reported (Strijve and Hischenhuber, 1987) and the recoveries of PAHs spiked in various substrates at levels of 2.5 – 75 μg kg^{-1} were 82% –103%.

Speer *et al*. (1990) extracted PAHs from vegetable oil, smoked fish products, mussels and oysters by using liquid-liquid partition technique with dimethylformamide- water- cyclohexane for the isolation of PAHs. After extraction, the sample was cleaned up using silica gel chromatography and gel permeation chromatography. Finally, the PAHs were analysed by capillary gas chromatography-mass spectrometry.

Perrin *et al*. (1991) described a rapid technique where PAHs were isolated from oils by donor, acceptor complex chromatography. The neutral lipids and minor compounds of the oil were eluted with a mixture of hexane/methyltributyl ether in the ratio of 75:25. The PAHs, as an electron donor were collected and eluted with pure dichloromethane and then analysed by reversed phase chromatography. The recoveries of different PAHs were nearly 100% and the detection limit was 0.1 μg kg^{-1}.

In this study, benzo(a)pyrene in an oil matrix was determined using HPLC with octadecyl C18 column and solvent mixture of water and acetonitrile as a mobile phase for the separation. Monffart and Whittle (1991) stated that the detection with flurorescence detector would allow a degree of selectivity through optimization of excitation and emission wavelengths. The detectable amount of individual PAHs determined by HPLC-fluoresence detection was as low as 0.8 pg with the limit of quantification in the range 0.02 Ωg g^{-1} to 3Ωg g^{-1}.

The method in this paper described the determination of benzo(a)pyrene in an oil matrix and the procedure was validated by determining the recoveries of benzo(a)pyrene from vegetable oils spiked with benzo(a)pyrene standard solutions.

EXPERIMENTAL

Reagents and Standards

Petroleum ether (40°C-60°C) (PE), deactivated aluminium oxide, activity super 1, anhydrous sodium sulphate and tetrahydrofuran with 99% purity were purchased from Merck Company. Analytical grade acetonitrile with 99.9% purity was supplied by Sigma Aldrich and benzo(a)pyrene standard of 99% purity was purchased from Dr Erhenstofer Brand in Germany. Deionized water/ HPLC water used obtained by using Milipore purification system. The HPLC mobile phase was a mixture of acetonitrile/water (88%/12%).

Aluminium oxide activity IV was prepared by adding 10 g distilled water to 90 g aluminium oxide activity super 1 and was shaked about 15 min and equilibrated in a closed vessel at room temperature for 24 hr before using.

Apparatus

Glass chromatography column (300 x 15 mm with Teflon tap). The HPLC system used in this study consisted of a Hewlett-Packard model 1100 series (Palo Alto, USA) equipped with a G1311 Quatpump, G1322A-Degasser, G1313A-Autosampler and 1046A-Fluorescence detector (Hewlett Packard) with emission and excitation wavelength at 436 nm and 292 nm respectively. A computer was linked to the system for data analysis. The mobile phase system of acetonitrile: water was delivered to the (250 mm length x 4.6 mm id) reverse phase column, at a flow-rate of 1.0 ml min^{-1}, under isocratic condition.

Samples

The samples used in this study were crude palm oil (CPO); crude palm kernel oil (CPKO); refined, bleached and deodorized palm oil (RBD PO); refined, bleached and deodorized palm kernel oil (RBD PKO); sunflower oil; coconut oil; groundnut

oil; canola oil; soyabean oil; palm fatty acid distillate (PFAD); bleached palm oil (BPO) and mixed vegetable oil.

Preparation of Aluminium Oxide Column

The extraction glass column was filled to half its height with petroleum ether and 22 g of aluminium oxide of activity 1V was rapidly weighed and transferred to the column. The column was gently tapped to settle the aluminium oxide in the column. Then, about 3 cm of anhydrous sodium sulphate was added to the top of the column. The column-tap was opened to allow the petroleum ether to fall to the level of the top of the sodium sulphate. Care must be taken not to allow the top of the column bed to dry out, or the effectiveness of the stationary phase will be impaired. Furthermore, channeling of the column will affect the separation.

Sample Extraction

The 2.0 g each of the various oil or fat samples was dissolved in petroleum ether (PE) in a 10 ml volumetric flask. The volumetric flasks were made up to the 10.0 ml mark with PE solvent. Then, 2.0 ml of the oil solution was transferred onto the aluminium oxide column and a 20.0 ml measuring cylinder flask was placed under the tap. The tap was opened to allow the petroleum ether to elute into the measuring cylinder at a rate of approximately 1 ml min^{-1}. The petroleum ether was allowed to fall to the level of the top of the sodium sulphate layer. After discarding the first 20 ml of eluent, 60 ml of fresh PE solvent was introduced onto the column. The eluate was collected at a rate of 1 ml min^{-1} in a 100 ml round bottom flask. Boiling chips were added to the round bottom flask and the solvent was evaporated using a water bath. A rotary evaporator can be used instead of a water bath but care must be taken to prevent cross contamination by cleaning the system thoroughly between consecutive sample evaporation. The concentrated solution was then transferred into a pre-weighed vial and evaporated to almost dryness using a stream of nitrogen at temperature of about 35°C. The round bottom flask was rinsed twice with 1-2 ml mobile phase and the rinsings were quantitatively transfered into the vial followed by evaporation to dryness. Lastly, the vial was weighed again (to the nearest 0.1 mg) to determine the weight of the residue (*R mg*). The vial was immediately capped with a Teflon layer septum and an aluminium cap and stored in a refrigerator (below 4°C).

Calibration Curve

Stock solutions. A stock solution of benzo(a)pyrene (BaP stock solution) was prepared by dissolving 12.5 mg benzo(a)pyrene standard with toulene in a 25 ml volumetric flask. The concentration of the stock solution was 0.5 mg ml^{-1} and it was stored in a refrigerator until use. The shelf life of BaP stock solution in toluene , if stored in refrigerator (dark, below 4°C), is at least six months.

Working standard solutions. Five working standard solutions were prepared in the range of 0.01-0.5 μg ml^{-1} by appropriate dilutions of the standard stock solution with acetonitrile in 100 ml volumetric flasks.

Recovery Study

Spiked oil. The 2.0 g oil samples were weighed into 10 ml volumetric flasks. The appropriate volumes of working standard solutions were added to obtain concentrations of 0.01, 0.05, 0.1, 0.2 and 0.5 mg ml^{-1} respectively. The volumetric flasks were filled to the 10 ml mark with PE solvent. The extraction procedure for the spiked samples were same as the procedue for real sample.

Blank Test

The 100 ml petroleum ether were reduced to about 1-5 ml and transferred to a vial. Evaporation of the petroleum ether was then continued till dryness. The vial was immediately capped using an aluminium cap with a teflon layered septum and stored in a refrigerator (below 4°C) until analysis.

HPLC Analysis

Calibration. A five point calibration curve was established by injecting working standard solutions containing 0.5 μg ml^{-1}, 0.2 μg ml^{-1}, 0.1 μg ml^{-1}, 0.05 μg ml^{-1} and 0.01 μg ml^{-1} B(a)P to the HPLC equipment. The same injection volume (V_{inj}μL) of 100 ml was also used for the samples.

Sample analysis. The 20 μl of THF were injected into the vial to dissolve the dried residue prepared from the recovery studies. Care must be taken to ensure that the residue was thoroughly and uniformly dissolved. The sample was then injected into the HPLC. The calibration curve mentioned above can be used for oil samples containing 0.1 to 3.5 μg kg^{-1} benzo(a)pyrene. If the benzo(a)pyrene content were higher than 3.5 μg kg^{-1}, a larger quantity of THF would have added to the vial or a smaller volume of the residue solution be injected. However, not more than 1.5 mg residue should be introduced into the column. If a larger amount of residue were present, the quantity of THF must be adjusted accordingly.

Quantification. The amount of benzo(a)pyrene was calculated from the calibration data obtained for the five concentrations of working standard. The

benzo(a)pyrene content of the sample was obtained using the following formula.

$$C_{BaP} = \frac{N}{V} \times [V_{vial} + R] \times \frac{V_{samp}}{M_{samp} \times V_{AIO}}$$

where

C_{BaP} = concentration of benzo(a)pyrene in the sample (μg kg^{-1});
N = amount (Ωg) of benzo(a)pyrene obtained from the calibration curve;
V_{vial} = amount of THF (μl) added to the vial;
V_{inj} = injection volume (μl) of THF;
V_{sample} = volume (ml) in which the oil was dissolved (usually in 10 ml volumetric flask);
V_{AIO} = volume (ml) of oil sample introduced into the aluminium oxide column; and
M_{sample} = mass of the sample dissolved (g).
R = residue (mg); '1.25 x R' is the volume (ml) of the residue.

RESULTS AND DISCUSSION

This method required the use of harmful reagents and the appropriate laboratory safety precautions were duly followed. Benzo(a)pyrene was a carcinogenic compound. Preparation of the standard stock solutions were performed in a fume cupboard to minimize exposure. Safety gloves were worn at all times during the analysis and contaminated materials such as tissues and gloves were disposed collected in a sealed plastic bag. A number of factors may affect the efficiency of benzo(a)pyrene extraction from the aluminium oxide column. The purity of the aluminium oxide and the solvent, petroleum ether, were two of the most important considerations. The equilibrated activated aluminium oxide was sealed and stored at ambient temperature. The benzo(a)pyrene present was identified by its retention time compared to that of a standard. HPLC with fluorescence detector. It was the most common method used for benzo(a)pyrene analysis since the analyte fluoresces and the optimized excitation and emission wavelengths are 384 nm and 406 nm respectively. For the quantification, a linear calibration curve was plotted using data from the five concentrations of working standard solutions. The limit of detection using HPL-fluorescence detector was 0.0005 μg ml^{-1}. A linear calibration curve with Y = 214.42 was obtained from the analyses of benzo(a)pyrene working standard solutions and $R^2 = 0.9998$ (*Figure 1*), with Y = response area of benzo(a)pyrene standard while X = concentration of the injected benzo(a)pyrene (Ωg) standard. The concentration range of the calibration curve was 0.1-3.5 μg kg^{-1}. For quantification, the concentration of benzo(a)pyrene (Ωg) was presented as N. The coefficients of variation were less than 10%. These indicated that the variation due to operator and instrument were within acceptable limits. The extraction efficiency of the method was evaluated by recovery studies on CPO, CPKO and RBD PO samples obtained from the industry. Preliminary analyses for benzo(a)pyrene were carried out on these oil samples to ensure that they were free from benzo(a)pyrene before using them for the recovery procedure. The results of the samples spiked with known amounts of benzo(a)pyrene at 0.01-0.5 μg ml^{-1} for CPKO, CPO and RBD PO are summarized in *Table 1.*

The average recovery ranges were as follows: 90%-99% for CPKO, 99%-102% for CPO and 92%-98% for RBD PO with relative standard deviations ranging from 1.2% to 23%. The recovery experiments showed that the method was feasible for determining benzo(a)pyrene in oil samples. One hundred and forty-eight samples were collected from the industry and analysed for benzo(a)pyrene content using this method. Benzo(a)pyrene content in 145 samples or 99% of the analysed sample were less than 0.1 μg kg^{-1}. Benzo(a)pyrene was detected in three coconut oil samples with concentration of 0.1 μg kg^{-1} to 6.0 μg kg^{-1}. *Table 2* shows the result for the analysed samples.

The HPLC chromatograms for benzo(a)pyrene standard solution at the lowest (0.01 μg ml^{-1}) and highest (0.5 μg ml^{-1}) concentration are shown in *Figures 2* and *3*, while the chromatograms for the oil samples are shown in *Figure 4* for none detected sample and *Figure 5* for detected sample which, benzo(a)pyrene peak was eluted together with other impurities.

TABLE 1. BENZO(a)PYRENE RECOVERIES FROM CPO, CPKO AND RBD PO SAMPLES

Concentration (μg ml^{-1})	CPO			CPKO			RBDPO		
	%	SD	CV	%	SD	CV	%	SD	CV
0.5	99	0.128	0.69	99	0.232	0.56	97	0.069	0.14
0.2	99	0.091	0.98	98	0.279	0.37	97	0.136	0.81
0.1	102	0.145	1.23	90	0.017	0.24	90	0.129	1.46
0.05	99	0.023	1.54	98	0.037	1.53	98	0.031	0.07
0.01	100	0.012	1.36	99	0.013	1.74	92	0.024	2.08

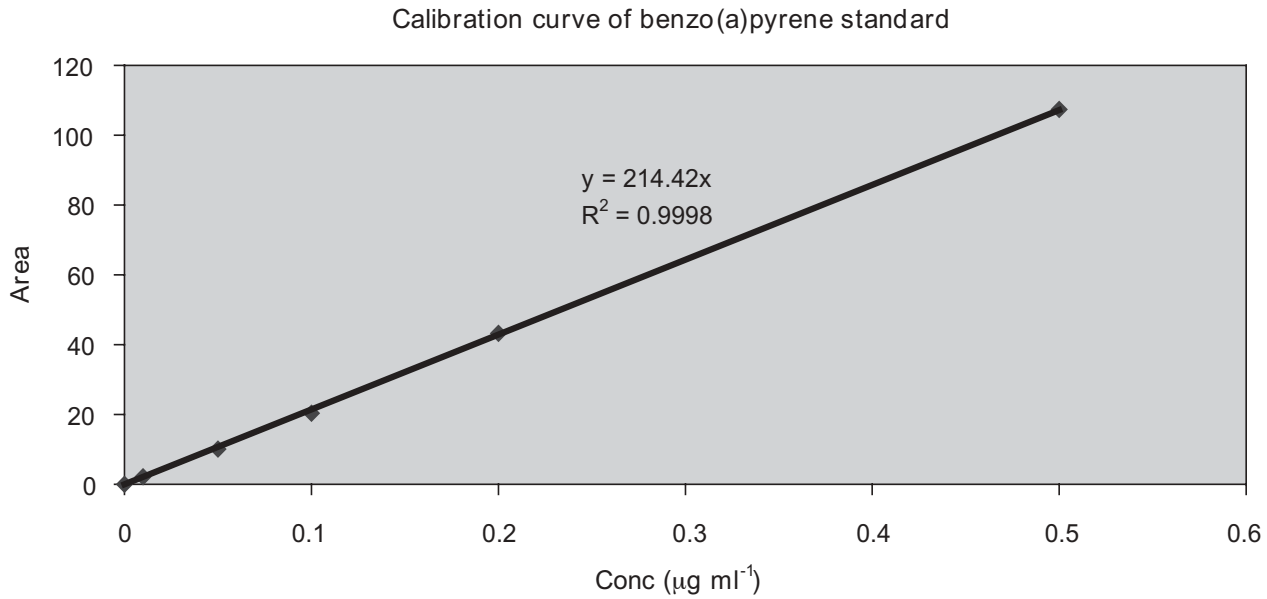

Figure 1. Calibration curve for benzo(a)pyrene standard solution.

TABLE 2. BENZO(a)PYRENE CONTENT IN THE ANALYSED SAMPLES

Sample type	Total sample	Sample		Range of benzo(a)pyrene content (μg kg^{-1})
CPKO	44	ND	44	< 0.1
		D	-	-
RBD PKO	33	ND	33	< 0.1
		D	-	-
RBDPO	20	ND	20	< 0.1
		D	-	-
CPO	21	ND	21	< 0.1
		D	-	-
PFAD	8	ND	8	< 0.1
		D	-	-
BPO	5	ND	5	< 0.1
		D	-	-
Soyabean oil (crude + RBD)	3	ND	3	< 0.1
		D	-	-
Canola oil (crude + RBD)	3	ND	3	< 0.1
		D	-	-
Sunflower oil (crude + RBD)	3	ND	3	< 0.1
		D	-	-
Groundnut oil (crude + RBD)	3	ND	3	< 0.1
		D	-	-
Coconut oil				
- Crude	4	ND	1	< 0.1
- RBD		D	3	0.1- 4.0
Mixed vegetable acid	1	ND	1	< 0.1
		D	-	-
Total	148			

Notes: ND: not detected. D: Detected.
Limit of determination: 0.1 μg kg^{-1}.

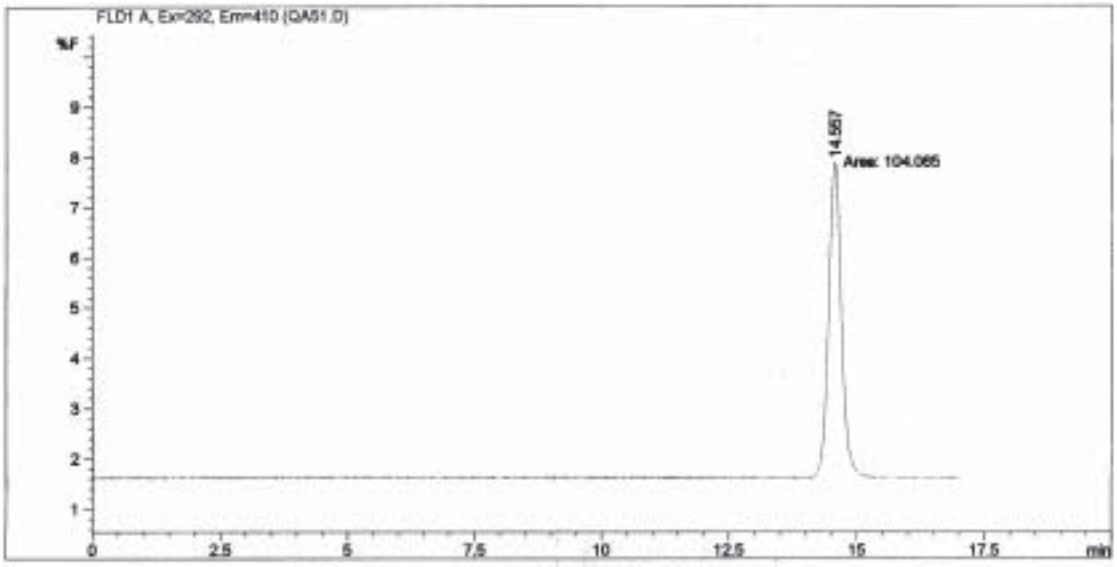

Figure 2. Chromatogram of benzo(a)pyrene standard solution (0.5 μg ml^{-1}.)

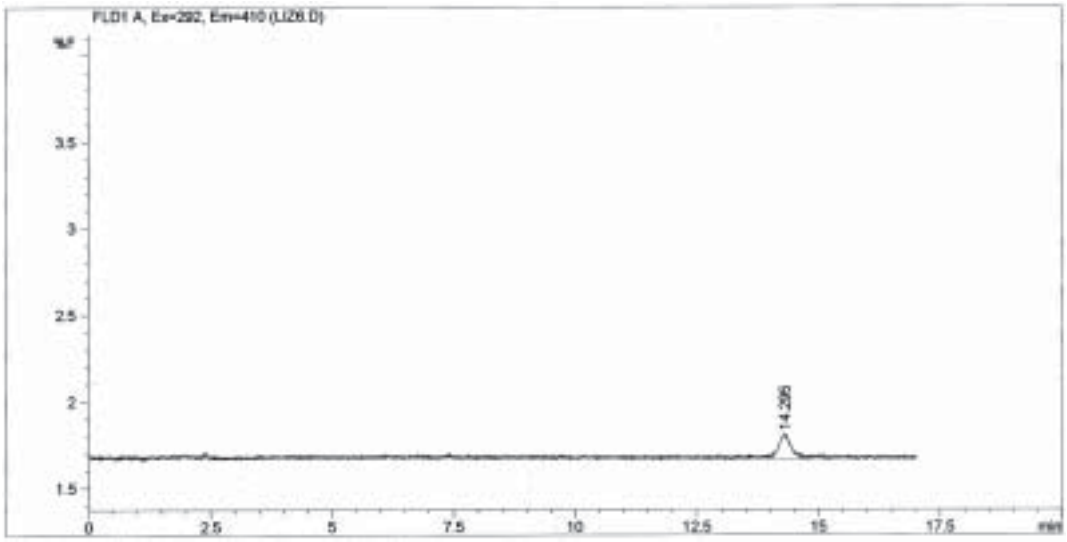

Figure 3. Chromatogram of a benzo(a)pyrene standard solution (0.01 μg ml^{-1}).

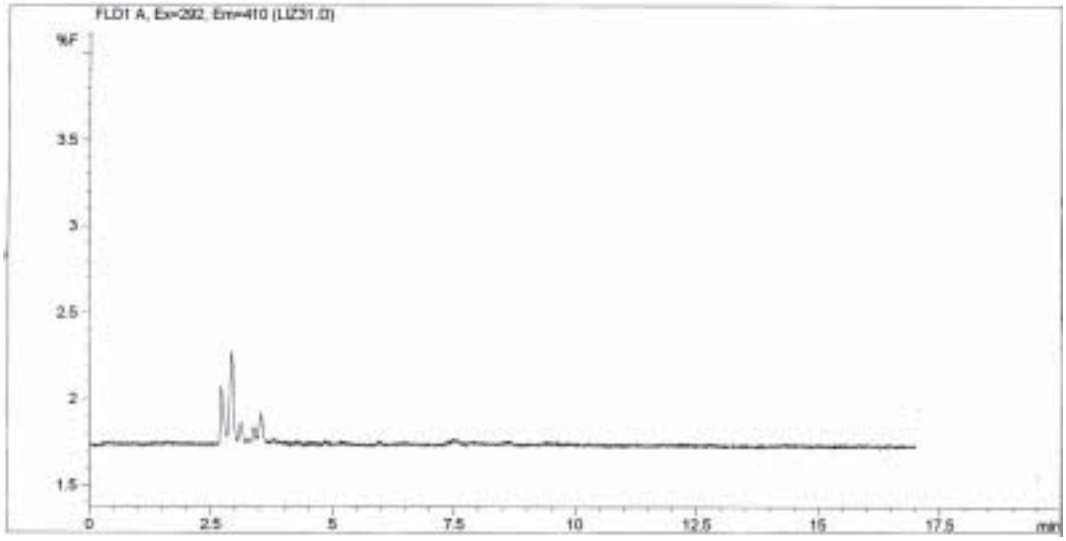

Figure 4. Chromatogram of a blank coconut oil sample.

Figure 5. Chromatogram of a coconut oil sample with benzo(a)pyrene detected.

CONCLUSION

In conclusion, the procedure described in this paper is sufficiently sensitive for the determination of benzo(a)pyrene in oil matrix. This method can be used to monitor benzo(a)pyrene in all vegetable oils. The detected levels of benzo(a)pyrene were lower than the acceptance limit of 2.0 μg kg^{-1}. The benzo(a)pyrene content in various vegetable oils analysed using ISO method showed no B(a)P detected in the samples (< LOD).

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