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DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN VEGETABLE OILS BY CAFFEINE COMPLEXATION AND GLASS CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

Glass capillary gas chromatography was used for the determination of polycylic aromatic hydrocarbons (PAHs). Reproducible PAH profiles were obtained from vegetable fats and oils by complexing them with caffeine in formic acid solution. The caffeine-complexable materials were then separated by column liquid and thin-layer chromatography, respectively. Glass capillary gas chromatographic results for PAHs specified by the U.S. Environmental Protection Agency were obtained after split injection within about 25 min. Quantitative and qualitative results are given for several commercial vegetable oils. Recoveries of PAHs were in the range 68–95%. The detection limit was 16 ng of coronene in 100 g of oil. Grapeseed oil was shown to contain the lowest "polycyclic aromatic hydrocarbons burden".

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) have been implicated in oncogenesis. The level of their environmental occurrence is one of the variables used in determining the extent of voluntary or involuntary human exposure to the combined action of carcinogens, procarcinogens and syncarcinogens. Consequently, numerous analytical methods have been developed to monitor the total carcinogenic burden by accurate measurements of the PAH level in various environmental sources. A large part of the work has focused on food and food products. It has been established that the average D.D.R. citizen ingests up to 85 mg of benzo[a]pyrene during his lifetime[1]. Recently, this carcinogenic compound has also been demonstrated to produce atherosclerotic lesions to chickens when fed on diets at 0.1–10 mg kg^{-1} levels for up to 20 weeks[2]. Fifteen other PAHs are included in the list of "priority pollutants" issued by the U.S. Environmental Protection Agency (EPA)[3].

The traditional techniques for the isolation of PAHs from food usually involve an alkaline digestion followed by liquid–liquid partition with organic solvents and liquid–solid chromatography. These combined procedures have been described with specific methods applicable to different materials[4-7]. Although reproducible results are obtained for relatively small samples analysed for the benzo[a]pyrene level

alone[8-12], the approach remains unsuitable for profile PAH analyses owing to the lack of any reliable definition of the oncogenetic extracts. The liquid–liquid extraction step is often the major cause of poor profile reproducibility, especially with emulsion-forming samples.

Recently, Sagredos and Sinha-Roy[6,7] described two methods involving complexation of PAHs in caffeine–formic acid solution[13]. The principle of these methods represents a particular advantage because it defines the PAH profile as that fraction of the oil which is complexable by caffeine. This approach, similar to that used by Van Heddeghem et al.[14], improves the reproducibility of PAH profiles and their evaluation by internal standard methods. We have adapted this rapid procedure for the determination of PAHs in fats and oils by glass capillary gas chromatography (GC).

Several workers[15-19] have presented glass capillary GC profiles PAHs obtained by various extraction and enrichment procedures. Until now, glass capillary GC analyses involving cold on-column injection techniques have been preferred to those linked with the inlet splitter injection system, which was reported to cause sample discrimination[20,21]. In a recent paper[22] we described the glass capillary GC conditions under which the split injection of PAH standards provided satisfactory quantitative results. This paper illustrates the application of the same experimental conditions to the screening of refined vegetable fats and oils.

EXPERIMENTAL

Benzo[b]chrysene, benzo[a]pyrene, benzo[e]pyrene and benzo[j]fluoranthene were supplied by the Community Bureau of Reference (Brussels, Belgium) and coronene, dibenz[a,c]anthracene, perylene, pyrene and triphenylene by Fluka (Buchs, Switzerland). Sixteen PAHs specified by the EPA protocol were supplied by Supelco (Crans, Switzerland). Otherwise analytical materials were as stated elsewhere[6,7].

Isolation of PAH

A 400-ml volume of cyclohexane, 100 g of vegetable oil (or melted fat) and 50 μl of internal standard solution (5 mg of benzo[b]chrysene dissolved in 100 ml of toluene) were placed in a 1000-ml separating funnel. The mixture was extracted twice for 120 sec with 100 ml of caffeine–formic acid solution (90 % formic acid containing 15 % of caffeine). After standing for 15 min, the caffeine–formic acid phase was drawn off into a 3000-ml separating funnel containing 1500 ml of 2 % sodium chloride solution. After agitation for 1 min, the sodium chloride phase was extracted twice for 60 sec with 250 ml of cyclohexane. The combined cyclohexane extracts were allowed to stand for 20 min, then the excess of water was drawn off. The extract was further dried over 30 g of anhydrous sodium sulphate (Merck, Darmstadt, G.F.R.), which was added through the top of the 3000-ml separating funnel, then passed slowly through fluted filter-paper (diameter 18 cm) containing ca. 5 g of anhydrous sodium sulphate, into a 1000-ml round-bottomed flask. The volume of the sample was reduced to ca. 10 ml at 40°C using a vacuum rotary evaporator.

Liquid chromatographic clean-up procedure

A 5-g amount of silica gel for adsorption chromatography (Woelm, Eschwege, G.F.R.) with a 15 % water content were packed into a 20 × 1 cm I.D. glass column.

The prepared extract was chromatographed on the column by elution with 110 ml of cyclohexane. After evaporation to *ca*. 3–4 ml, 1 ml of toluene was added to the eluate. A 10-ml conical test-tube was used for further concentration of the sample to *ca*. 200–300 μl under a stream of nitrogen at 30°C. Additional details of the method have been described elsewhere[4,6,7].

Thin-layer chromatographic clean-up procedure

High-performance thin-layer chromatography (HPTLC) plates (Merck) were used for the TLC clean-up of the concentrated column eluate. The eluate was loaded on to the HPTLC plate as a 70-mm band, using a Linomat III apparatus fitted with a 500-μl syringe (Camag, Mutenz, Switzerland). Then the remaining starting area of the plate was loaded with PAH standard solution, after which the plate was eluted twice with isooctane in a darkened TLC tank. Before the second elution, the plate was dried with a stream of nitrogen for about 1 min. The chromatograms were examined under UV light (254 and 366 mm) for approximately 5 sec. The PAH zone was detached using a plate scraper (Camag) and then rapidly homogenized in an 8-ml screw-capped test-tube (Sovirel, Paris, France) with a glass rod. To extract the PAHs, 4 ml of toluene were added to the test-tube, the contents of which were then heated to 45°C. After agitation for 1 min with a Vortex Genie Mixer (Scientific Industry, Bohemia, NY, U.S.A.) the sample was centrifuged at 2300 $\pm$ 100 g and then the supernatant evaporated in a conical test-tube to *ca*. 200 μl under a stream of nitrogen. The volume of the sample was further reduced to *ca*. 10 μl in a 500 μl Reacti-Vial, prior to glass capillary GC split injection.

Glass capillary GC analysis

Analyses were carried out using an HP 5830 A gas chromatograph fitted with a flame ionization detector (FID) and a home-made inlet splitter injection system, including septum purge. The GC conditions were as follows: 30 m × 0.3 mm I.D. glass-capillary column coated with OV-17–SE-30 (1:1) stationary phase, which was prepared using the ammonia etching pre-treatment[23]; injection temperature, 260°C; detector temperature, 280°C; oven temperature, 150°C, then programmed at 6°C min^{-1} and held at 280°C; pre-set splitting ratio, 1:10; carrier gas hydrogen at 0.55 bar.

The injector port thread was first lined with *ca*. 6 cm PTFE tape and then closed with a modified septum holder[22]. The latter was designed to guide the syringe through a 3 × 0.5 mm diameter hole into the injection chamber. A 5-μl syringe (Hamilton, Reno, NE, U.S.A.) fitted with a cemented needle (0.46 mm O.D.) was used for injection. The split injection of PAHs was carried out as follows. The syringe was pre-washed several times with toluene, so that clean solvent remained in the injection needle. The syringe was loaded successively with 1 μl of air, 1 μl of sample and 1 μl air (air–sample–air injection). The sample was injected immediately after smooth septum penetration. The whole operation was timed with a stop-watch. The syringe was removed from the vaporizing chamber 30 sec after the injection. Chromatograms were recorded and computed using an HP 18850 A GC terminal.

The effect of cold on-column injection on the linearity of PAH profiles was also examined using a Carlo Erba Model 4160 gas chromatograph, fitted with two injectors (cold on-column and split/splitless). The GC conditions were as follows: 30 m ×
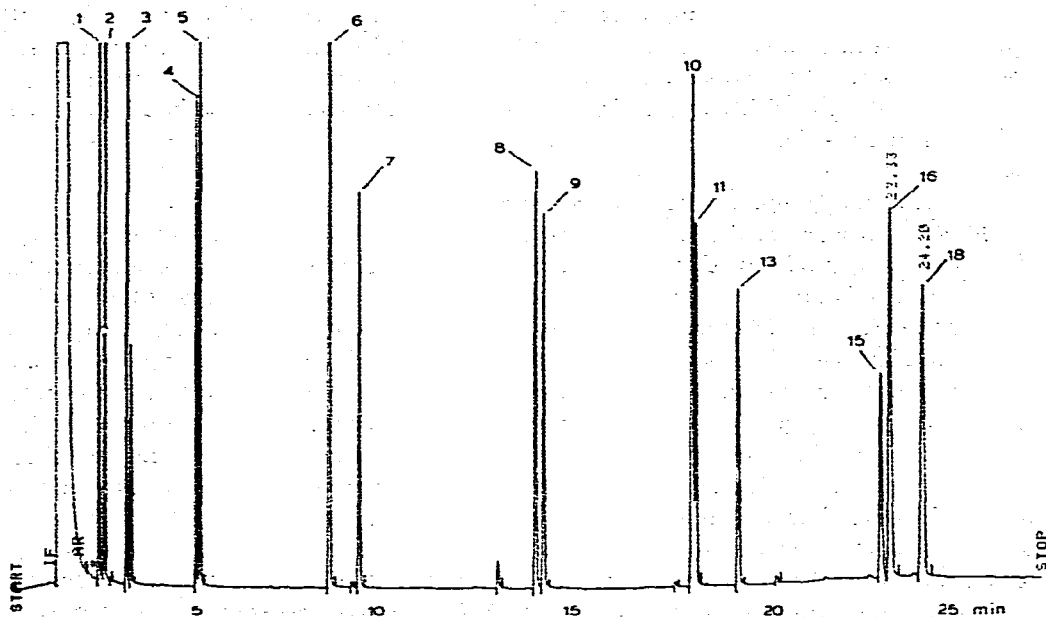
Fig. 1. Glass capillary GC separation of PAH standards from the list of EPA priority pollutants. All identified peaks are listed in Table I. GC conditions: 30 m × 0.3 mm I.D. glass capillary column coated with OV-17–SE-30 (1:1); oven temperature, 150°C, then programmed at 6°C min⁻¹ and held at 280°C; FID temperature, 280°C; injector temperature, 260°C; carrier gas, hydrogen at 0.55 bar; pre-set splitting ratio, 1:10.

0.3 mm I.D. glass-capillary column coated with OV-17–SE-30 (1:1) stationary phase; cold on-column injection; 1 min isothermal at 80°C, then programmed at 35°C min⁻¹ to 150°C, again 1 min isothermal, then programmed at 6°C min⁻¹ and held at 290°C; detector FID; detector temperature, 350°C; carrier gas, hydrogen at 0.65 bar.

RESULTS AND DISCUSSION

Fig. 1 illustrates the glass-capillary GC resolution of fifteen PAH standards that conform to the EPA priority pollutants programme[3]. Ammonia etching pre-treatment of uncoated, persilylated glass capillaries, combined with the effect of increased polarity of OV-17–SE-30 mixed stationary phase (similar to OV-7, but containing a gum phase) contributed substantially to the rapidity and efficiency of the glass capillary GC analysis[23]. This, together with data from our previous investigation on the linearity of PAH split injection[22], allowed us to select the principal GC parameters: a higher initial oven temperature (150°C) and a faster programming rate (6°C min⁻¹), which reduced the time required for the analysis of the above PAH standards to ca. 25 min. The only rejected target compound was naphthalene (mol. wt. 128). The separation of this compound from the solvent peak could be achieved only with a lower initial oven temperature.

All of the compounds listed in Table I were identified according to retention times recorded for pure PAH standards. High-chart-speed chromatograms were also

TABLE I

LEVELS OF POLYCYCLIC AROMATIC HYDROCARBONS IN SOME COMMERCIAL VEGETABLE OILS

Average values after statistical evaluation of four glass capillary GC measurements.

Peak No.	PAH	PAH content ($\mu g\ kg^{-1}$)***						
		Grapeseed	*Rapeseed*	*Sunflower*	*Cocoa butter*	*Soybean*	*Peanut A*	*Peanut B*
1	Acenaphthylene	3.48	1.09	4.36	1.22	0.86	—	0.90
2	Acenaphthene	2.47	0.59	2.49	0.47	0.81	—	0.93
3	Fluorene	2.10	0.68	1.96	0.43	0.79	—	0.93
4	Phenanthrene	1.57	1.10	2.31	2.71	2.17	—	6.19
5	Anthracene	0.35	t	t	1.35	2.10	—	t
6	Fluoranthene	2.80	1.98	6.70	16.35	8.95	17.14	19.88
7	Pyrene	3.08	0.57	4.97	8.47	2.57	7.17	5.79
8 8*	Chrysene Triphenylene	0.49	1.04	1.70	3.65	17.35	63.31	10.26
9	Benzo[a]anthracene	0.86	1.69	3.11	6.07	21.93 ·	78.52	15.54
10	Benzo[b]fluoranthene	0.56	2.30	2.21	3.19	24.83	85.29	11.63
11 11*	Benzo[k]fluoranthene Benzo[j]fluoranthene	0.53	1.96	2.01	2.54	27.61	98.77	13.19
12*	Benzo[e]pyrene	1.89	3.15	4.11?	4.91	25.25	87.63	10.01
13	Benzo[a]pyrene	0.60	2.14	1.51	2.58	28.45	105.74	10.69
14*	Perylene	0.46	1.77	0.60	2.76	10.00	36.17	2.92
15	Indeno[1,2,3-cd]pyrene	0.27	4.05	1.32	0.32	22.82	80.63	9.01
16 16* 17**	Dibenzo[a,h]anthracene Dibenzo[a,c]anthracene Benzo[b]chrysene	0.24	1.31	t	t	4.74	12.92	2.24
18	Benzo[g,h,i]perylene	0.49	5.90	1.68	0.76	16.86	65.75	8.43
19*	Coronene	0.16	0.39	0.31	t	2.14	7.43	0.92
Σ PAH ($\mu g\ kg^{-1}$)		22.93	34.61	41.35	57.78	220.23	750.25	129.10
S.D. ($\mu g\ kg^{-1}$)		2.45	3.18	3.14	3.89	15.08	29.68	10.59

* Unspecified by EPA.
** Internal standard (22.74 $\mu g\ kg^{-1}$) added in each oil.
*** ? = merged peak; t = trace.

compared. Coronene eluted *ca.* 33 min after injection. This six-ring PAH could be detected and quantitated at levels as low as 16 ng per 100 g of vegetable oil (Table I). A systematic repetition of a whole series of peaks was observed in different PAH profiles. Such a tendency offers the advantage of a fundamental analytical description of the glass capillary GC pattern typical for fats and oils in relation to the method of isolation via caffeine complexes. Erroneous results are often linked to the unexpected components that arise during clean-up procedures and from analytical equipment. In our work, the hot injection chamber generated a limited series of homologues that were missing from the chromatograms obtained by cold on-column injection. Gieger and Schaffner[15] described similar effects as a possible result of processes in the traditional injection system. The effect appears to be more pronounced in relation to the
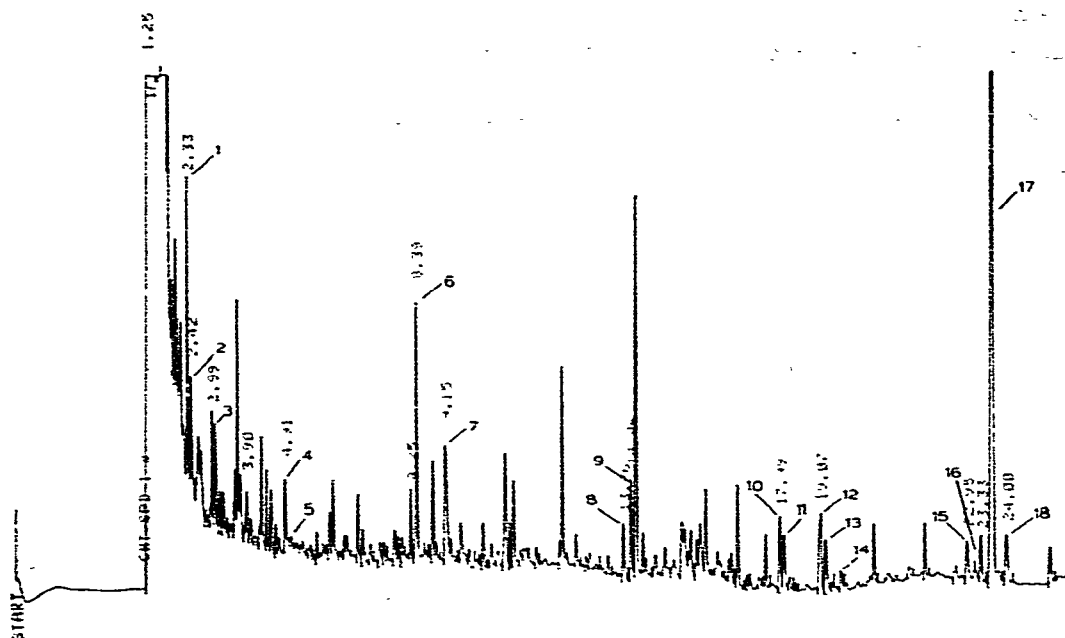
Fig. 2. Glass capillary GC profile of PAHs isolated from sunflower oil. All identified peaks are listed in Table I. GC conditions as in Fig. 1.

hot-needle injection of PAH standards[22]. Fig. 2 shows a non-target compound, benzo[e]pyrene, merging with one such artefact.

The original method of PAH isolation via caffeine complexes[6] was modified after unsuccessful attempts to extract PAHs from coffee oils. A small amount of the natural caffeine, even as low as 20–600 ppm, when present in these oils enhances the complexation of PAH and their consecutive solubilization in the 90% formic acid pre-washing phase. As other compounds in fats and oils, so far still unspecified, may induce PAH complexation, some procedures described in the previous method[6] were avoided. The modification resulted in the successful isolation of PAH profiles from the coffee oils (to be published later).

Liquid-adsorption chromatography on silica gel columns has been widely accepted as a rapid and reliable clean-up procedure in PAH analysis[4–6]. A less favourable attitude has been adopted towards in situ PAH evaluation by TLC under UV light[24,25]. The rapid TLC clean-up procedure described in this paper was shown to produce no deleterious effects on the stability of PAH standards eluted (see Table II, procedure E). However, rapid, probably photochemical, changes were observed with caffeine–complexable materials, which migrated below rather than along with the PAH band. Their fluorescence took on a red to deep red coloration under UV light within a few seconds. A comparative study, using different TLC supports, e.g., acetylated cellulose, will be useful in establishing whether these differently migrating compounds may be derivatives of the PAH formed during the TLC procedure. Nevertheless, HPTLC plates proved to be useful in the rapid clean-up of caffeine-complexable materials from refined vegetable fats and oils (compare Figs. 2–4 and Fig. 5).

TABLE II

RECOVERY OF POLYCYCLIC AROMATIC HYDROCARBONS AFTER THE DIFFERENT PROCEDURES

(A) PAH standard mixture used in steps B, C, D and E; (B) mixture A after addition of known amount of benzo[b]chrysene (solution 1); (C) mixture A recovered after evaporation of 500 ml of cyclohexane and addition of solution 1; (D) mixture A recovered after caffeine complexation, extraction, concentration, LC clean-up and addition of solution 1; (E) mixture A recovered after TLC clean-up and addition of solution 1; (F) total recoveries after steps D and E. The amount of benzo[b]chrysene used in steps B, C, D and E (solution 1) was 16.85 μg kg^{-1}. All values are averages of five determinations.

Procedure	Results	Phenan-threne	Pyrene	Chrysene	Benzo-[a]pyrene	Benzo-[b]chrysene
A	Concentration present (%)	20.97	20.89	18.21	20.13	19.85
	S.D. (%)	0.55	0.50	1.04	0.57	0.96
	Recovery (%)	–	–	–	–	–
B	Concentration present (%)	16.41	16.94	15.53	17.29	33.83
	S.D. (%)	0.70	0.64	0.42	0.47	0.79
	Recovery (%)	–	–	–	–	–
C	Concentration present (%)	11.24	15.66	15.00	16.70	40.21
	S.D. (%)	0.35	0.72	0.66	0.73	0.69
	Recovery (%)	68	92	97	97	84
D	Concentration present (%)	10.82	14.27	14.75	16.42	43.73
	S.D. (%)	1.30	1.80	0.63	0.70	2.70
	Recovery (%)	66	84	95	95	77
E	Concentration present (%)	16.92	16.97	14.58	17.28	33.24
	S.D. (%)	1.09	0.95	0.27	0.42	1.33
	Recovery (%)	103	100	94	100	102
F	Recovery (%)	68	84	89	95	79

Other workers[26-31] have also demonstrated successful TLC separations of PAHs. With crude and heat-abused oils, the approach is lacking in efficiency. We were unsuccessful in obtaining satisfactory PAH recoveries from alumina sheets[6], using toluene as the extraction solvent. At present studies are under way using acetylated cellulose in the TLC clean-up of caffeine-complexable materials from crude or heat-abused vegetable oils.

The data in Table II show that major divergences in the recoveries of PAH were caused after application of procedure C. Procedure D also involves evaporation of cyclohexane: ca. 500 ml from extraction and ca. 110 ml from LC clean-up. Comparison between the recoveries in procedures C and D suggests that caffeine complexation and extraction, cyclohexane extraction and LC clean-up, have less pronounced effects on recoveries of PAHs than concentration of the cyclohexane.

The relative amounts of "target compounds" in the caffeine-complexable materials are apparent on rapid inspection of HPTLC plates under UV light. This was particularly noticeable when the TLC plates of soybean and peanut oil A were compared, and which reflected the higher PAH burden of the latter (Table I).

While the quantitative results may not always be comparable to previously published data, this may simply reflect the influence of industrial processing, storage and environment. The PAH burden has been shown to decrease after deodorization and bleaching[32-34]. On the other hand, the low PAH burden in the grapeseed oil is
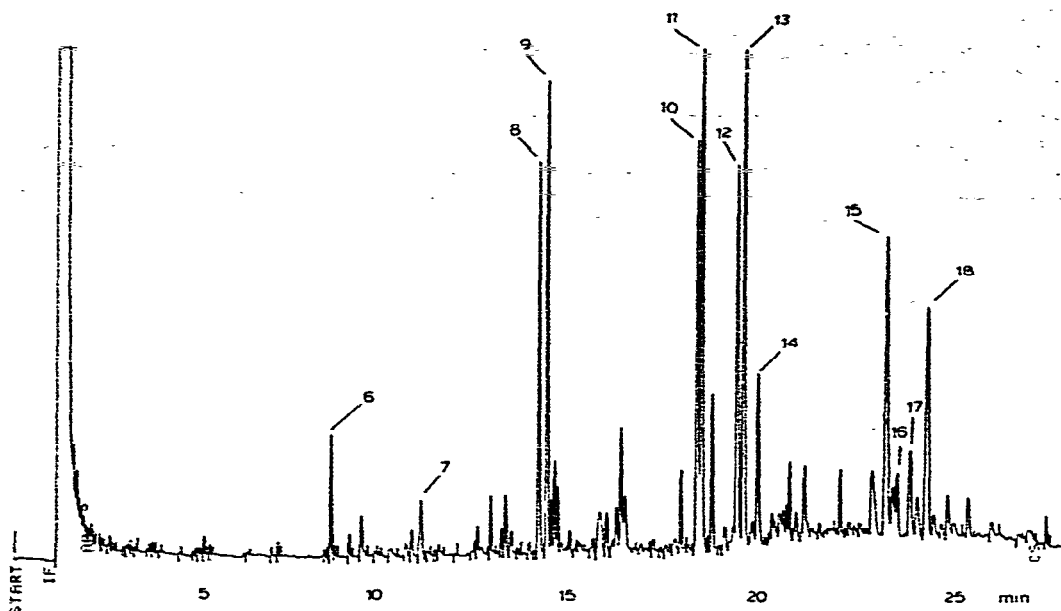
Fig. 3. Glass capillary GC profile of PAHs isolated from peanut oil A. All identified peaks are listed in Table I. GC conditions as in Fig. 1.
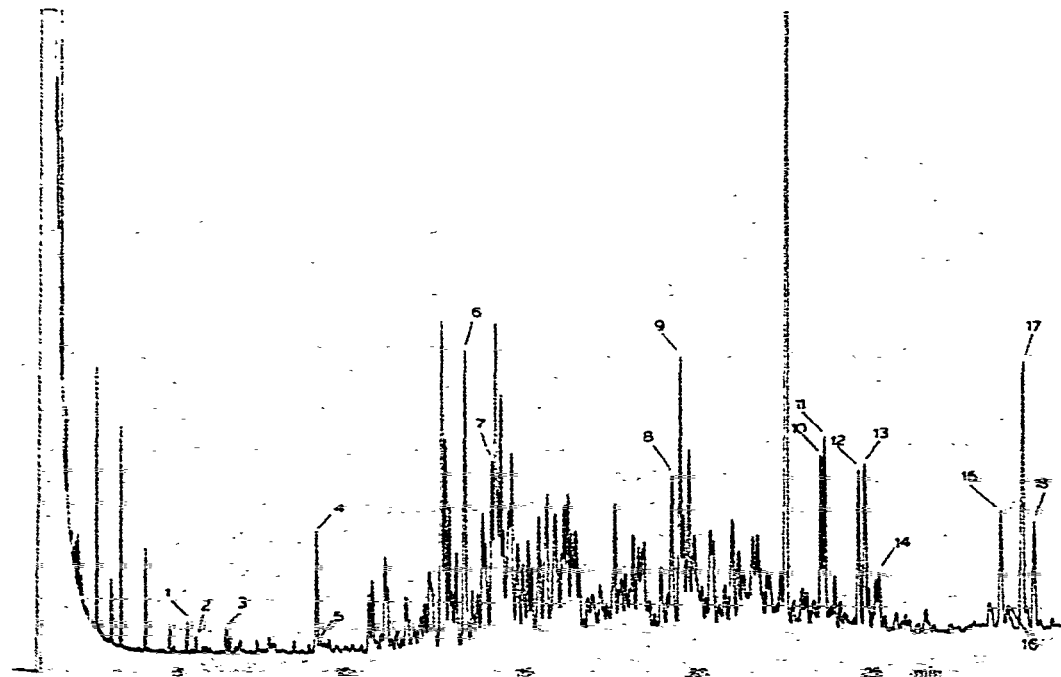

Fig. 4. Glass capillary GC profile of PAHs isolated from peanut oil B. On-column injection. All identified peaks are listed in Table I. GC conditions: column as in Fig. 1; cold on-column injection, 1 min isothermal at 80°C, then programmed at 20°C min^{-1} up to 150°C, 1 min isothermal, followed by 6°C min^{-1} and held at 280°C; FID temperature, 350°C; carrier gas, hydrogen at 0.65 bar.
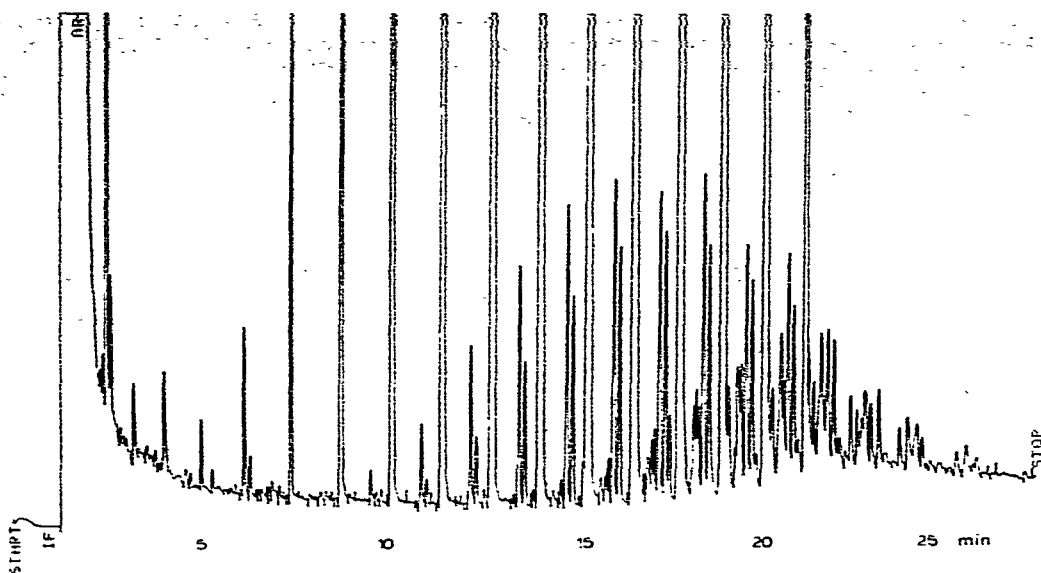
Fig. 5. A typical broad-spectrum glass capillary GC profile of caffeine-complexable materials before TLC clean-up, extracted from vegetable oils. The major homologous series was confirmed by GC–MS to be saturated hydrocarbons. GC conditions as in Fig. 1.

possibly explained by the botanical constitution of the parent fruit.

Fig. 5 represents a typical GC profile of the caffeine-complexable materials before their TLC clean-up. GC–mass spectrometry (MS) analytical data confirmed the major homologous series to be saturated hydrocarbons. The definition of these broad-spectrum profiles requires further and systematic investigation in order to establish qualitative and quantitative changes in caffeine-complexable materials obtained from different oils before and after exposure to thermo-oxidative processes[11]. Sufficient amounts of caffeine-complexable materials for these purposes can be obtained from only a few grams (*ca.* 5 g) of oil. A recent publication summarizes the possible interrelationships between fat and cancer[35]. The potential of the caffeine-complexable materials (see Fig. 5) to affect the oncogenetic response induced by benzo[a]pyrene and other carcinogens remains to be studied.

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