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Reliability assessment of a gas chromatographic method for polycyclic aromatic hydrocarbons in olive oil

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ABSTRACT

A quality control test was developed for a gas chromatographic method to determine polycyclic aromatic hydrocarbons in olive oil. Fifteen oil specimens were fortified with eight three- to six-ring polycyclic aromatic hydrocarbons at levels of between 3.0 (approximate detection limit) and 360 μg/kg. Three sets of five equally fortified specimens were obtained and assayed at random by three operators. For each fortification level, the means of recovery yield were in the range 56–107%, and were independent of the polycyclic aromatic hydrocarbon congener specificity and the operator's capability. Excluding subsets of data associated with both the fortification level at the detection limit and a deviant polycyclic aromatic hydrocarbon term (benzo[*ghi*]perylene), an overall mean accuracy of 96% and a precision of 7% were achieved.

INTRODUCTION

The determination of polycyclic aromatic hydrocarbons (PAHs) in evironmental samples is currently of major interest, owing to their ubiquitousness and to the carcinogenic and mutagenic activity shown by many of them [1,2]. PAHs result from the combustion, pyrolysis and pyrosynthesis of organic matter. They have been inplicated as possible contaminants of foodstuffs mainly through polluted air, sorption from water and soil, or food preparation methods. This topic has been extensively reviewed by Fazio and Howard [3]. In particular, PAHs appear to be generally present in vegetable oils at trace levels. For such foods, biochemical synthesis has been suggested as a possible additional source of contamination; however, according to the reviewers, this route seems to be considerably controversial.

Identification and assessment of PAHs in commercial olive oil have previously been carried out by UV and fluorescence spectrophotometry [4–8] and, more recently, by gas chromatography–mass spectrometry [9] and high-performance liquid chromatography [10,11]. PAHs have been extracted from oil by various liquid–liquid partition schemes [4,6,9,10], in some cases preceded by a saponification step [8,11] or even by a caffeine–formic acid complexation [10]. Clean-up of the extract has been per-

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formed by column chromatography on silica gel [9–11], alumina [6,7], XAD-2 resin [10], and Florisil, alone [8] or followed by two-step thin-layer chromatography (TLC) on cellulose and cellulose acetate [4].

In a previous paper [12], results were reported of an investigation carried out to assess the PAH content of "extra-virgin" olive oil derived from plants contaminated with industrial pitch condensate. (The "extra-virgin" oil is an unrefined quality obtained traditionally from the olive-pressing process or alternatively, in recent times, by centrifugation in presence of warm water.) In that paper, a procedure was developed which enabled determination of complex mixtures of PAHs in a relatively short time and with limited amounts of reagent. The procedure is reported here below. The aim of this paper is to present the results of a quality control test carried out to evaluate the reliability of the procedure, especially in terms of accuracy and precision, at contamination levels of practical interest (from a few μg/kg to a few hundreds of μg/kg).

EXPERIMENTAL[a]

Reagents

Reference PAHs were selected to cover a wide range of gas chromatographic retention times and had three to six rings (see Table I). PAHs were obtained from Analabs (Norwalk, CT, USA) and Fluka (Buchs, Switzerland). *p*-Terphenyl (p-T) and β,β′-binaphthyl (bNA) were purchased from Fluka and K&K Labs (Plainview, NY, USA), respectively, and used as internal standards; reference solutions were made in cyclohexane at 219 and 314 μg/ml, respectively.

All solvents were obtained from Carlo Erba (Milan, Italy). *n*-Pentane and dimethyl sulfoxide (DMSO) were UV-spectroscopy grade. The other solvents were of analytical grade; however, acetone, cyclohexane and dichloromethane (DCM) were distilled in a glass apparatus prior to use.

Silica gel 70–230 mesh was purchased from Merck (Darmstadt, Germany). TLC was performed on 20 × 20 cm, 1-mm-thick layer, ready-for-use silica gel plates provided by Merck; plates were washed with acetone before use.

Analytical procedure

A scheme of the analytical procedure is presented in Fig. 1. Each oil specimen (10.0 g) was spiked with 5.0 μl of the p-T solution, dissolved in 20 ml *n*-pentane, and partitioned three times with 10 ml DMSO, according to a procedure adapted from Natusch and Tomkins [13]. The combined DMSO extracts were diluted with 60 ml water (caution: considerable heat can be generated when mixing DMSO and water) and back-partitioned three times with 50 ml cyclohexane. The combined cyclohexane extracts were washed with 100 ml distilled water and filtered through an 8-cm-diameter funnel containing (from bottom to top): a glass wool plug, a 1-cm silica gel layer and a 2-cm anhydrous sodium sulphate layer. After filtration, the funnel was washed with an additional 10 ml of cyclohexane and two 5-ml portions of DCM. The filtrate

[a] *Precautions.* Many PAHs have carcinogenic activity, for which there are different degrees of experimental evidence [1,2]. Every possible precaution must be taken to avoid human exposure when handling PAHs as a class. Spent samples and unused standards should be disposed of safely.

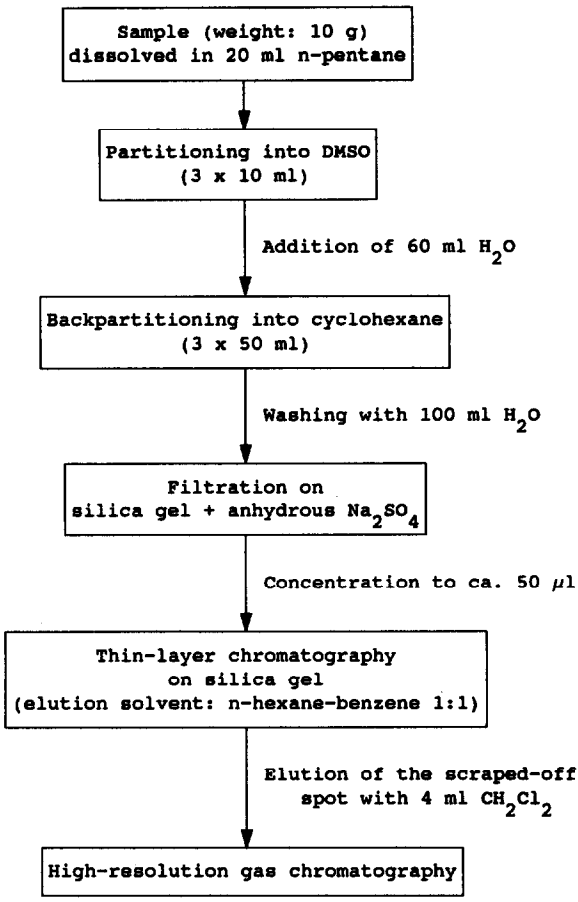

Fig. 1. Scheme of the analytical procedure to assess PAHs in olive oil.

and washings were combined, concentrated to about 50 µl, and subjected to TLC. The TLC plate was developed with 1:1 (v/v) *n*-hexane–benzene to a height of 12 cm. The PAH spot (R_F: *ca.* 0.8) was detected with 366-nm UV light. The silica gel spot was then scraped off, quantitatively transferred to a 2.5-cm I.D. glass tube equipped with a sintered glass disc, and eluted in three successive stages with a total of 4 ml DCM. The eluate was gently evaporated to dryness under nitrogen, then 5.0 µl of the bNA solution were added, and the solution was made up to 100 µl volume with cyclohexane.

Instrumental determination

A Carlo Erba Model 2960 gas chromatograph, equipped with a cold on-column injector (temperature at initial oven temperature), a 30 m × 0.32 mm I.D. fused-silica SPB-1 capillary column and a flame ionization detector (temperature 300°C), was employed for quantitative assessment. A Hitachi Model D-2000 integrator was used

for data acquisition and processing. The oven temperature was held at 85°C for 1 min, raised to 180°C at 25°C/min, and then to 300°C at a rate of 7°C/min, where it was held isothermally. Helium was used as a carrier gas at a flow-rate of 5 ml/min. PAH identification and quantification were carried out by comparing sample chromatograms against calibration solutions. Integrator peak height measurements were used for quantifications; they were corrected against the mean value of the internal standard bNA, obtained by averaging all its determinations carried out during this study. The estimated detection limit in specimens was about 3 μg/kg.

Quality control program

PAHs were weighed (approximately 2.5 mg each; precision ±0.01 mg) and dissolved in 25 ml cyclohexane to obtain eight individual standard solutions. Working solutions were prepared at concentrations of about 1 and 10 ng/μl. Fifteen 10.0-g specimens of blank olive oil were spiked with known quantities of the working solutions, so that three sets of five equally fortified specimens each were obtained (Table I). Added volumes ranged from 29.3 to 334.6 μl. Blank oil was an "extra-virgin" olive oil previously analyzed and found to contain few PAHs at relatively low background levels [mean values (n = 3): AN, 6 μg/kg; FA, 19; PY, 18; BaF, 3; TRI, 5. For PAH abbreviations, see Table I].

The fortified oil specimens were shipped to another laboratory, together with three blank unspiked specimens (10.0 g) and a PAH standard mixture (each PAH approximately 50 ng/μl). All specimens were kept refrigerated from the time of their preparation until analysis. Fortified and blank oil specimens were analyzed by three operators of the receiving laboratory. The operators were not informed of specimen identities. In addition, three reagent blanks were also analyzed. Three calibration solutions were prepared from the PAH standard mixture at concentration levels of 0.5, 5 and 20 ng/μl to obtain analytical levels of 5, 50 and 200 μg/kg: for each compound, calibration graphs were found to be linear over the whole range of concentrations.

TABLE I

FORTIFICATION LEVELS OF OIL SPECIMENS (μg/kg)

Each set includes five equally fortified specimens.

Compound[a]	Abbreviation	Mol. wt.	Number of rings	Set A	Set B	Set C
Anthracene	AN	178	3	15	50	80
Fluoranthene	FA	202	4	60	120	160
Pyrene	PY	202	4	50	180	120
11H-benzo[*a*]fluorene	BaF	216	4	3	35	18
Triphenylene	TRI	228	4	30	70	220
Benzo[*e*]pyrene	BeP	252	5	20	20	300
Perylene	PE	252	5	8	140	40
Benzo[*ghi*]perylene	BPE	276	6	5	9	360
Total 8 PAHs				191	624	1298

[a] Ranked according to increasing molecular weight and, for isomers, increasing gas chromatographic retention time.

RESULTS AND DISCUSSION

Table II shows the results of the study with reference to each PAH term. Results were corrected for background (see *Quality control program* section). The recovery yields, per individual PAH and fortification level (FL), are means of five independent determinations which vary between 56 and 107%. The higher figure is associated with an FL at the detection limit (3.0 μg/kg for BaF), the lower figure with the highest FL adopted in the study (360 μg/kg for BPE). However, BPE also exhibits lower mean recovery yields (76%) at the other two FLs (for which signal-to-noise ratio ≥ 5), and at 360 μg/kg FL its recovery yield may drop to as low as 39%. Based on these findings, it may be presumed that BPE exhibits a certain degree of deviance with respect to the other congeners. If, in the light of the above, the data associated with BPE and with the only level at the detection limit (five-datum subsets 1, 2, 4 and 24) are neglected, then the remaining 20 subsets exhibit mean recovery yields between 78 and 106% (see also Table III, "all operators"). Coefficients of variation (C.V.) vary between 8 and 20%, except for one case (PE at a 40 μg/kg FL: C.V. = 30%). Lastly, if individual recovery yield figures are averaged by a specific congener ($n = 15$ for each mean), *i.e.* regardless of FLs, the new means are very similar from congener to congener (except for BPE) and range between 89 and 95%.

Table III shows the recovery yields rearranged to show their dependence on FL (see also Fig. 2) and on the operator's capability (operator's repeatability). The three operators seem to perform differently with respect to accuracies and precisions (Table III, lower section). When the entire set of data ($n = 120$) is considered, deviance from expectancy is 5% for operator III, 10% for operator I and 20% for operator II; when the set of data omitting subsets 1, 2, 4 and 24 ($n = 100$) is considered, the deviance is 4, 8 and 18%, respectively. Inter-operator precision shows even greater variations: based on C.V. values, operator III is about two-fold more precise than operators I and II. In general, this is true with both data sets, *i.e.* for $n = 120$ and $n = 100$. Each operator provides Gaussian-distributed findings, although operators I and II exhibit distributions (unshown) which are broader than that of operator I.

The recovery data set ($n = 120$) has a Gaussian distribution (Fig. 2). The overall mean of recovery yield data is 88%; when subsets 1, 2, 4 and 24 are excluded the mean improves to 90%.

CONCLUSIONS

The analysis of the results obtained makes it possible to conclude that the tested procedure is a reliable tool for assessing PAHs in olive oil. This is borne out by the individual recovery yields ($n = 120$) which are all within the range 39–133%: this approximately 1:3 variation appears to be small if one considers the number of variables dealt with, *i.e.* FL, PAH congener specificity and operator capability. It would also be acceptable in risk assessment analysis. Moreover, if one considers the set of recovery yield means estimated at each FL, (five-datum subsets 1–24), then the overall variation is only from 56 to 107%, *i.e.* it improves to somewhat less than 1:2.

The procedure can provide a high degree of accuracy (within 4% of expectancy) and very good precision (C.V. ± 7%). These figures, taken from the "best" operator's selected ($n = 100$) results, are independent of FL and the congener specificity.

TABLE II

RECOVERY YIELDS OF PAHs ADDED TO BLANK OLIVE OIL FOR QUALITY CONTROL TEST: EFFECT OF CHEMICAL COMPOUND

PAH[a]	Fortification level (μg/kg)	Recovery yield (%)[b]														$\bar{x}$	S.D.	C.V.	All samples		
		Set A					Set B					Set C									
		I	I	II	II	III	II	III	II	III	III	III	II	II	I	I				$\bar{x}$	S.D.	C.V. (%)
AN	15	100	93	73	87	93											89	10	11			
	50						78	94	72	94	106						89	14	15			
	80											91	65	93	90	101	88	14	15			
																				89	12	13
FA	60	105	85	100	87	97											95	9	9			
	120						78	101	87	91	98						91	9	10			
	160											92	61	89	89	99	86	15	17			
																				90	11	12
PY	50	106	88	94	94	104											97	8	8			
	120											96	67	88	90	98	88	13	14			
	180						73	88	76	81	94						82	9	11			
																				89	11	12

BaF	3.0	133	100	100	67	133											107	28	26			
	18											89	56	94	72	89	80	16	20			
	35						69	97	89	91	94						88	11	13			
																				92	22	24
TRI	30	113	90	97	70	103											95	16	17			
	70						76	100	89	84	99						89	10	11			
	220											99	60	90	76	84	82	15	18			
																				89	14	16
BeP	20	120	105	110	90	105											106	11	10			
	20						85	110	105	100	110						102	10	10			
	300											89	65	90	65	80	78	12	16			
																				95	17	17
PE	8.0	100	113	100	75	100											98	14	14			
	40											108	53	108	65	83	83	25	30			
	140						76	100	89	94	92						90	9	10			
																				90	17	19
BPE	5.0	80	80	80	60	80											76	9	12			
	9.0						67	78	78	78	78						76	5	7			
	360											67	49	70	39	56	56	13	22			
																				69	13	19

[a] For abbreviations, see Table I.

[b] As obtained by three operators identified by I, II and III.

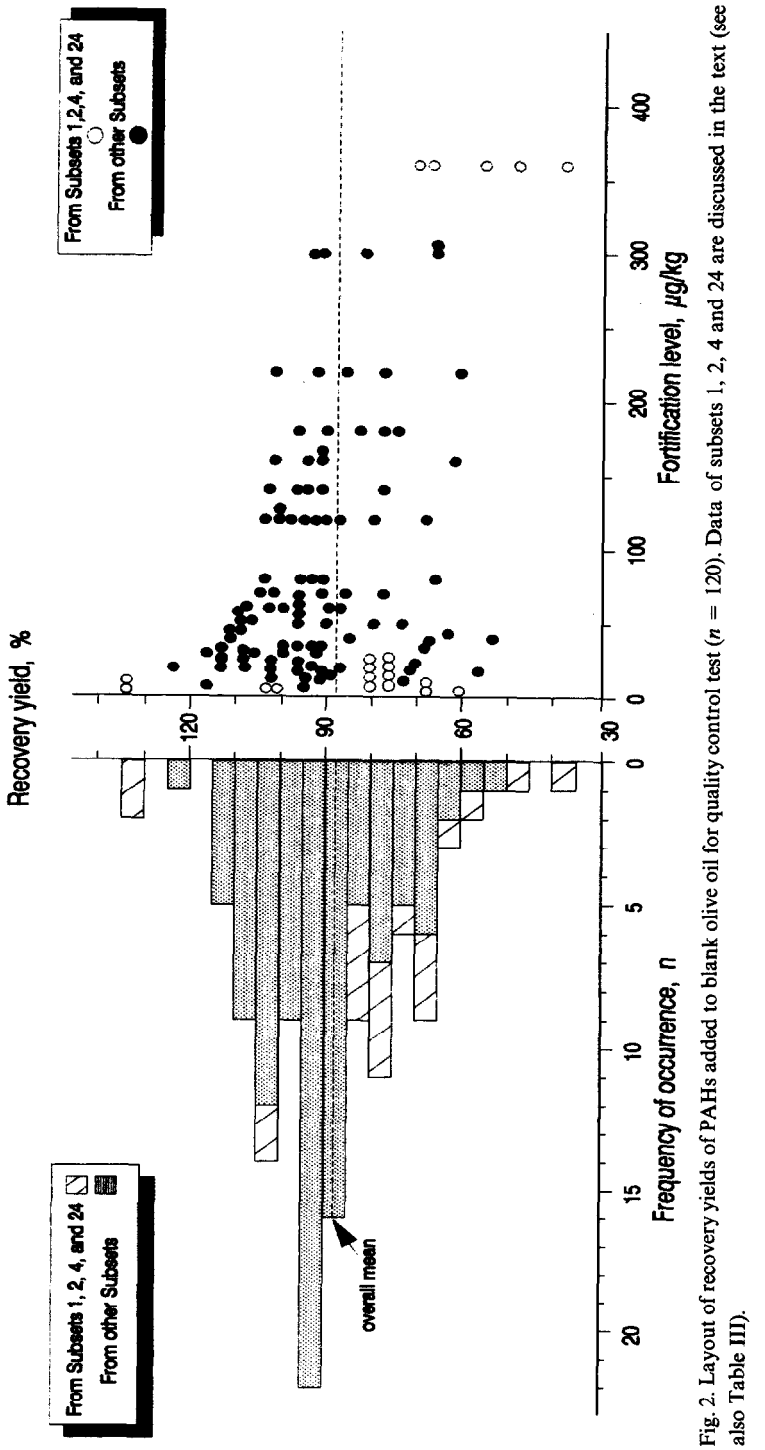

Fig. 2. Layout of recovery yields of PAHs added to blank olive oil for quality control test (n = 120). Data of subsets 1, 2, 4 and 24 are discussed in the text (see also Table III).

TABLE III

RECOVERY YIELDS OF PAHs ADDED TO BLANK OLIVE OIL FOR QUALITY CONTROL TEST: EFFECT OF FORTIFICATION LEVEL AND OPERATOR

Subset[a]	Fortification level (μg/kg)	PAH[b]	Recovery yield (%)[c]									
			Operator I		Operator II		Operator III			All operators		
										$\bar{x}$	S.D.	C.V. (%)
1	3.0	BaF	133	100	100	67	133			107	28	26
2	5.0	BPE	80	80	80	60	80			76	9	12
3	8.0	PE	100	113	100	75	100			98	14	14
4	9.0	BPE			67	78	78	78	78	76	5	7
5	15	AN	100	93	73	87	93			89	10	11
6	18	BaF	72	89	56	94	89			80	16	20
7	20	BeP	120	105	110	90	105			106	11	10
8	20	BeP			85	105	110	100	110	102	10	10
9	30	TRI	113	90	97	70	103			95	16	17
10	35	BaF			69	89	97	91	94	88	11	13
11	40	PE	65	83	53	108	108			83	25	30
12	50	AN			78	72	94	94	106	89	14	15
13	50	PY	106	88	94	94	104			97	8	8
14	60	FA	105	85	100	87	97			95	9	9
15	70	TRI			76	89	100	84	99	89	10	11
16	80	AN	90	101	65	93	91			88	14	15
17	120	FA			78	87	101	91	98	91	9	10
18	120	PY	90	98	67	88	96			88	13	14
19	140	PE			76	89	100	94	92	90	9	10
20	160	FA	89	99	61	89	92			86	15	17
21	180	PY			73	76	88	81	94	82	9	11
22	220	TRI	76	84	60	90	99			82	15	18
23	300	BeP	65	80	65	90	89			78	12	16
24	360	BPE	39	56	49	70	67			56	13	22
$\bar{x}$			90		80		95			88		
S.D.			19		15		11			16		
C.V.(%)			21		18		12			18		
n			32		48		40			120		
$\bar{x}$[d]			92		82		96			90		
S.D.			14		14		7			14		
C.V.(%)			15		17		7			15		
n			26		40		34			100		

[a] Subsets 1 and 2: fortification levels at, or near to, detection limit; subsets 2, 4 and 24 associated with a deviant PAH term (see text).

[b] For abbreviations, see Table I.

[c] Same values as reported in Table II, but ranked according to increasing fortification level and grouped according to operators.

[d] Estimates not including subsets 1, 2, 4 and 24.

Finally, although a mean recovery greater than 50% may be accepted for the assessment of the environmental contaminants (risk analysis), the study shows that as far as possible particular care should be taken when analyzing the heavier congeners such as BPE.

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