Rapid determination of polycyclic aromatic hydrocarbons in tea infusion samples by high-performance liquid chromatography and fluorimetric detection based on solid-phase extraction

The Analyst

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Received 27th May 1998, Accepted 3rd August 1998

This paper describes a method for the determination of PAHs in black, green and decaffeinated tea infusion samples. The method is based on the solid phase extraction of the PAHs using Sep-Pak vac $_t$C-18 cartridges. The PAHs are then eluted from the cartridges with dichloromethane. Quantification and detection are carried out by HPLC with a fluorimetric detector using a program of excitation and emission wavelength pairs. Recoveries at concentration levels in the range 190–1790 ng l^{-1} were higher than 65% for all PAHs except dibenz[*a,h*]anthracene, for which it was around 54%. The mean content of PAHs was in the range 28.7–112 ng l^{-1} in the tea infusions, with relative standard deviations between 2 and 18% (n = 4).

Introduction

Polynuclear aromatic hydrocarbons (PAHs) are well known environmental pollutants present at low concentrations. Owing to their mutagenic and carcinogenic potential, environmental samples such as air, water, marine sediments and soils have been analyzed.[1–5] Traces of PAHs have been detected in many foods, including vegetable oils, fruits, sea food, grilled and roasted meat, smoked fish, tea and coffee; in particular, benzo[*a*]pyrene has been found in these samples at concentration levels between 0.1 and 100 μg kg^{-1}.[6–10] Food pollution is due to deposition of PAHs from the air or water or results from preservation, drying and cooking procedures.[1,6] Most of the methods applied to the determination of PAHs in food samples are based on previous saponification with a KOH–methanolic solution and liquid–liquid extraction (LLE) with hexane, cyclohexane, or isooctane, then the extract is cleaned-up on a silica and/or alumina column.[7,8] Supercritical fluid extraction (SFE) and solid-phase extraction (SPE) with alumina, silica and C_{18} cartridges have also been used.[9,10] The fractions eluted from the columns are further analyzed by high-performance liquid chromatography (HPLC) with photometric or fluorimetric detection or by gas chromatography (GC) with flame ionization or mass spectrometric detectors.[8–11] Only a few research studies regarding the PAH content in tea samples have been published; they are based on LLE with hexane followed by clean-up on silica or alumina columns.[12–14] This method is not ideal and has significant disadvantages. The major disadvantage of LLE is the use of large volumes of expensive, high-purity organic solvents, and it is extremely time consuming. Other drawbacks include emulsion formation and a lack of sensitivity for more volatile analytes. In this paper we report a simple method for PAH determination in tea infusions, based on SPE using Sep-Pak vac $_t$C-18 cartridges. Elution of PAHs was carried out with much smaller volumes of organic solvent than with LLE.

Experimental

Apparatus and materials

The HPLC system consisted of a Milton Roy CM 4000 high-pressure gradient pump (Rivera Beach, FL, USA), a Rheodyne Model 7125 injection valve with a 20 μl loop (Cotati, CA, USA), a Perkin-Elmer LS 30 luminescence spectrometer (Norwalk, CT, USA) and a Milton Roy CI 4100 integrator. A Nova-Pak C_{18} 4 μm particle size column (150 × 3.9 mm id) (Waters, Milford, MA, USA), thermostated in a P-Selecta Precisterm bath (Barcelona, Spain) at 22 °C was used. Sep-Pak vac $_t$C-18 (500 mg), cartridges (Waters) were used for the extraction and preconcentration of PAHs from tea infusion samples. A vacuum flask (1 l) (Pobel, Madrid, Spain), separating funnel (250 ml) (Pobel), a Barna-vacio vacuum pump and a Visiprep vacuum manifold system (Supelco, Bellefonte, PA, USA) were used. A P-Selecta ultrasonic bath was also used for the preparation of PAH solutions. Solvents for preparing the mobile phase were filtered through Lida nylon membrane filters (Kenosha, WI, USA) with 0.45 μm pore size and the sample extracts were also filtered with PTFE (13 mm, 0.5 μm) (MFS, Dublin, CA, USA). Whatman filter-papers (Maidstone, UK) were used to filter tea infusions.

Chemicals

The PAHs phenanthrene, fluoranthene, pyrene, benz[*a*]anthracene, benzo[*e*]pyrene, benzo[*a*]pyrene, dibenzo[*a,h*]anthracene and benzo[*ghi*]perylene (Sigma, St. Louis, MO, USA) were used. Stock standard methanolic solutions of PAHs at concentration levels of 36.0–305 mg l^{-1} were prepared. These solutions were stored in glass bottles at 4 °C. Working standard PAH mixtures were prepared by dilution of the above stock standard solutions with methanol according to their sensitivities

to fluorescence detection. HPLC-purity acetonitrile, methanol and dichloromethane (Scharlau, Barcelona, Spain) were used. The other solvents and chemical reagents were also of HPLC purity. Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA).

Tea samples

Black tea (five samples), green tea and decaffeinated tea samples, all purchased in a supermarket, were analyzed.

Sample preparation

About 35 g of tea (20 tea bags) were placed in 500 ml of boiling water for 10 min, then the tea infusion was filtered through a filter-paper by means of a vacuum system and 99 ml of the tea infusion were mixed with 1 ml of methanol for 5 min in an ultrasonic bath.

Preconcentration

The Sep-Pak cartridges were previously conditioned, first with 3 ml of dichloromethane, then with 6 ml of methanol, twice, and then with 4 ml of Milli-Q water. A 100 ml volume of the tea–methanol (99 + 1) sample, unspiked or spiked with PAHs in the range 5.5–268 ng, was passed at a flow rate of 10 ml min^{-1} through a conditioned cartridge, where the PAHs were retained. Subsequently, the cartridge was dried in the vacuum system for 3 min. PAHs were eluted from the cartridge, first with 2 ml of dichloromethane and then with 1 ml of the same solvent at a flow rate of 0.5 ml min^{-1}. The eluate was collected in a graduated glass tube and the solvent was evaporated with a vacuum manifold system; to facilitate solvent evaporation, the tube was inserted in a glass vessel containing water at 22 °C. The residue was dissolved in 1 ml of methanol using the ultrasonic bath. This solution was filtered through a PTFE filter and analyzed by HPLC. The volume injected into the HPLC column was 20 µl and the calibration procedure was applied for the quantification of PAHs; the retention times were used for identification purposes.

Calibration

External calibrations were obtained with PAH solutions at five concentration levels in the range 4–268 ng ml^{-1}. The gradient was prepared by mixing water (solvent A) and acetonitrile (solvent B). The gradient profile for the separation of the PAHs was 55% A–45% B (0 min), which was held for 1 min, and subsequently changed linearly to 60% B in 17 min and to 85% B in 12 min, which was held for 8 min. The flow rate was 1 ml min^{-1} and the analytical column was thermostated at 22 °C. Fluorescence detection was performed with a program of seven excitation and emission wavelength pairs (Table 1). The injection volume was 20 µl. The areas of the peaks were used for quantification of PAHs. The mobile phase was degassed with helium.

Table 1 Program of excitation and emission wavelength pairs

Compound detected[a]	Time/min	λ_{ex}/nm	λ_{em}/nm
Phenanthrene	0	250	365
Fluoranthene	18.5	285	465
Pyrene	22.9	270	390
B[*a*]a	24.0	287	388
B[*e*]p	28.5	290	390
B[*a*]p	30.8	295	405
Db[*a,h*]a, B[*ghi*]p	32.5	290	418

[a] B[*a*]a, benz[*a*]anthracene; B[*e*]p, benzo[*e*]pyrene; B[*a*]p, benzo[*a*]pyrene; Db[*a,h*]a, dibenzo[*a,h*]anthracene; B[*ghi*]p, benzo[*ghi*]perylene.

Results and discussion

Preliminary studies

In order to identify the PAHs present in the tea samples studied, after preparation of analytical solutions according to procedures specified in literature,[3] a green PAH specific column was used. Retention factors were used for identification purposes and the PAHs specified in Table 1 were detected. The smaller PAHs such as naphthalene, fluorene and acenaphthene were not detected, possibly owing to losses of these more volatile analytes during tea sample preparation when adding boiling water. Moreover, the PAHs were confirmed by GC-MS after changing the solvent to hexane. Because pairs such as fluorene–acenaphthene were not detected in these samples, a cheaper, non-specific C_{18} column could be used. Consequently, the column specified in the Experimental section was employed in the rest in the work.

Optimization of the chromatographic characteristics

Eight PAHs, phenanthrene, fluoranthene, pyrene, benz[*a*]anthracene, benzo[*e*]pyrene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene and benzo[*ghi*]perylene, were studied. The chromatographic parameters were selected after a previous systematic study; methanol–water and acetonitrile–water mobile phase gradients with compositions in the range 45–90% and flow rates in the range 0.8–2 ml min^{-1} were tested, maintaining the chromatographic column at 22 °C. The acetonitrile–water mobile phase gradient specified in the Experimental section, at flow rate of 1 ml min^{-1}, was selected because the chromatogram obtained was lower and the resolution and sensitivity were better. In order to attain maximum sensitivity, the excitation and emission wavelength pair program showed in Table 1 was applied. Fig. 1(a) shows a chromatogram obtained under these conditions.

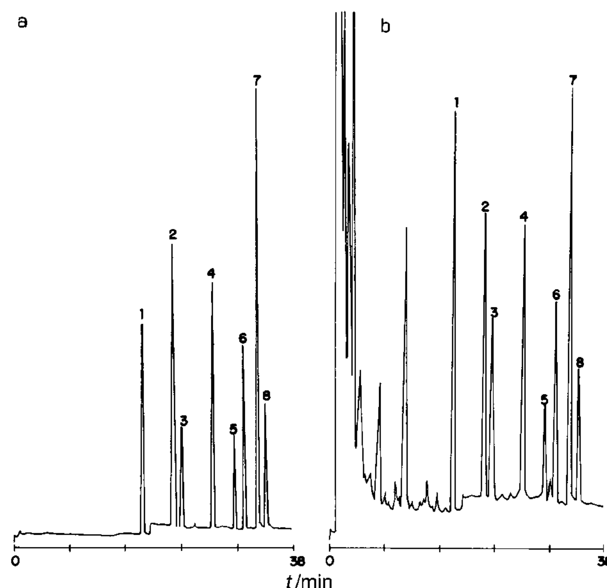

Fig 1 Chromatograms of (a) standard mixture of eight PAHs and (b) tea infusion sample spiked with PAHs. Conditions: Nova-Pak C_{18} (column 150 × 3.9 mm id); temperature, 22 °C; mobile phase, gradient of acetonitrile–water (see Experimental section); flow rate, 1 ml min^{-1}; fluorimetric detection (see Table 1); injection volume, 20 µl at concentration levels of 12–113 ng ml^{-1}. Peaks: 1, phenanthrene; 2, fluoranthene; 3, pyrene; 4, B[*a*]a; 5, B[*e*]p; 6, B[*a*]p; 7, Db[*a,h*]a; 8, B[*ghi*]p.

Analytical characteristics for standards

The PAH concentrations tested were in the range 4–268 ng ml^{-1}. Linearity was observed in all the cases with regression coefficients higher than 0.999. The relative standard deviations (RSDs) at concentration levels of 12–113 ng ml^{-1} in the middle of the linear ranges studied were 5.3–11% (n = 4) and the detection limits, defined as a signal-to-noise ratio of 3 (DL = 3S/N) were between 0.016 and 0.14 ng ml^{-1}; these limiting values are for fluoranthene and benzo[*e*]pyrene, respectively. The retention times, with RSD values in the range 0.1–1.8% (n = 4), (are given in Table 2).

Recovery of PAHs from tea infusion samples

Because the PAH concentrations in tea infusion samples are at the ng l^{-1} level,[13] a previous preconcentration step was necessary. SPE was used to retain the PAHs from tea samples, using Sep-Pak vac $_t$C-18 cartridges. Several experimental variables were optimized.

Sample preparation. First, the effect of the presence of methanol in the sample was tested. The PAHs were eluted from the cartridges with 2 ml of dichloromethane. The mean recoveries from 100 ml aqueous infusion tea samples, for the eight PAHs at levels of 190–1790 ng l^{-1}, are about 60%. The effect of the presence of methanol was tested at the same PAH concentration levels; the mean recoveries increased by about 20% in the presence of 1% of methanol but decreased at methanol concentrations of 3 and 5%. The positive effect of small amounts of methanol on the hydrophobicity seems to be overcome by the negative influence of large percentages of methanol as a result of competitive effects on the stationary phase.

Nature and volume of the eluent. The nature and volume of the eluent were also studied; on the basis of a previous study,[3] diethyl ether, dichloromethane and diethyl ether–dichloromethane (1 + 1) were tested for elution of the PAHs using 2 ml of solvent. The highest recoveries were obtained using dichloromethane, the mean recovery being about 70%, so this solvent was adopted as the eluent. Several eluent (dichloromethane) volumes, in the range 2–4 ml, were tested. The highest mean recovery was obtained when elution was carried out in two steps, first with 2 ml and then with 1 ml of dichloromethane.

Effect of sample volume and PAH concentration in tea infusion samples. Tea infusion sample volumes of 50, 100, 150

Table 2 Analytical characteristics

PAH	Range of linearity/ ng ml^{-1a}	RSD^b (%) (n = 4)	DL^c/ ng ml^{-1}	t_R/min	RSD (%) (n = 4)
Phenanthrene	21–161	5.3	0.090	17.5	0.1
Fluoranthene	6–41	7.1	0.016	22.0	1.8
Pyrene	14–96	5.5	0.050	23.3	0.2
B[*a*]a	10–68	8.1	0.030	27.4	0.7
B[*e*]p	40–268	11	0.140	30.4	0.4
B[*a*]p	4–30	5.3	0.024	31.7	0.3
Db[*a,h*]a	24–159	5.5	0.046	33.5	0.2
B[*ghi*]p	13–87	10	0.045	34.9	0.2

^{a} Studied range. ^{b} Relative standard deviation at a concentration level in the middle of the range studied. ^{c} Detection Limit, DL = 3S/N.

Table 3 Influence of PAH concentration on the study of their recoveries from tea infusion samples. Tea infusion volume: 100 ml

PAH	$C_1{}^a$	$R_1{}^b$ (%)	$C_2{}^a$	$R_2{}^b$ (%)	$C_3{}^a$	$R_3{}^b$ (%)	$C_4{}^a$	$R_4{}^b$ (%)
Phenanthrene	320	50	640	55	1070	65	1610	40
Fluoranthene	80	69	160	73	260	100	390	76
Pyrene	190	100	380	103	630	79	940	47
B[*a*]a	130	81	260	92	440	78	660	61
B[*e*]p	535	80	1070	73	1790	81	2680	36
B[*a*]p	55	56	110	50	190	75	280	24
Db[*a,h*]a	315	44	630	48	1050	54	1580	31
B[*ghi*]p	170	50	340	50	570	67	860	30
Mean recovery (%)		66		68		75		43

^{a} C_1, C_2, C_3 and C_4: PAH concentration in ng l^{-1}. ^{b} R, mean percentage recovery of four determinations. RSDs are in the range 3–17%.

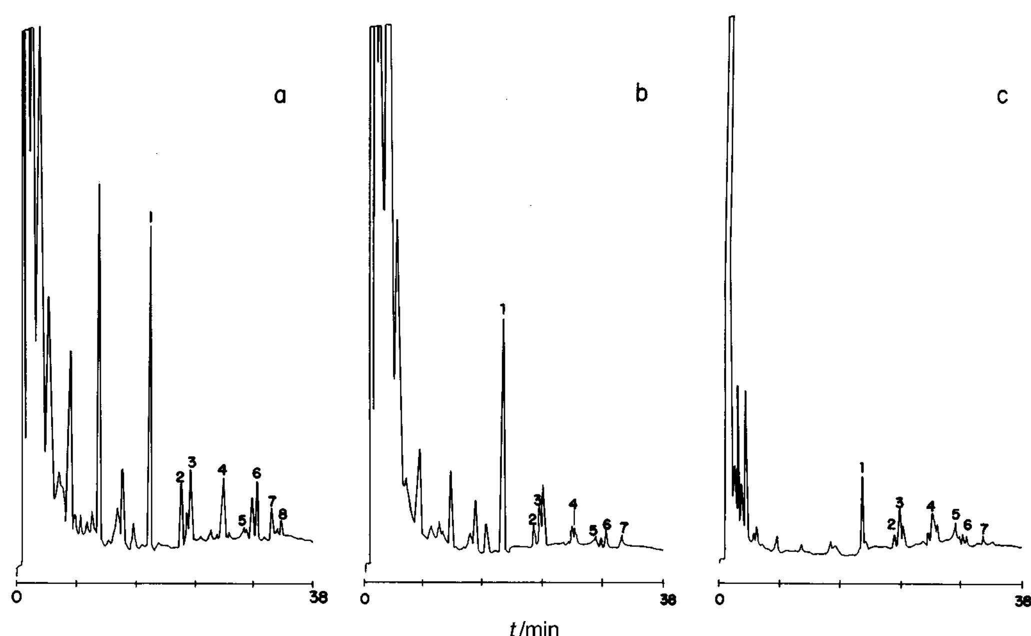

Fig 2 Chromatograms of tea infusion samples: (a) black number 4; (b) green; and (c) decaffeinated tea. Conditions: Nova-Pak C_{18} (150 × 3.9 mm id) column; temperature, 22 °C; mobile phase, gradient of acetonitrile–water (see Experimental section); flow rate, 1 ml min^{-1}; fluorimetric detection (see Table 1); injection volume, 20 µl. Peaks: 1, phenanthrene; 2, fluoranthene; 3, pyrene; 4, B[*a*]a; 5, B[*e*]p; 6, B[*a*]p; 7, Db[*a,h*]a; 8, B[*ghi*]p.

Table 4 Determination of PAHs in tea infusion samples (ng l^{-1})[a]

PAH	Black					Green	Decaffeinated
	1	2	3	4	5		
Phenanthrene	212	448	273	658	420	504	119
Fluoranthene	7	21	7	30.1	13.3	11.9	4.2
Pyrene	70	147	168	61	108	23.8	18.4
B[*a*]a	2.8	7	14	43.4	9.8	5.6	25.4
B[*e*]p	9.7	19.3	7	30.5	8.2	11.7	56
B[*a*]p	2.1	14	2.1	23.8	7	14.7	4.2
Db[*a,h*]a	4.9	7	5.6	23.7	9.1	8.4	2.3
B[*ghi*]p	5.6	7	4.2	27.3	—	—	—

[a] $n = 4$; RSD in the range 2–18%.

and 200 ml containing amounts of PAHs between 19 and 179 ng were tested. The mean recovery decreased from 70% for a volume of 100 ml to 50% for a volume of 200 ml. It was not possible to pass volumes higher than 200 ml through the cartridge owing to the nature of the sample, which contains solid particles that block the cartridge. For volumes in the range 150–200 ml a decrease in the sample flow rate through the cartridge was observed, which led to lower recoveries.

Several PAH concentrations were also tested. Recoveries from 100 ml tea infusion samples at four PAH concentration levels in the range 55–2680 ng l^{-1}, specified in Table 3, were studied. For the highest concentration tested, the recoveries were lower owing to cartridge overloading and/or the low solubility of PAHs in aqueous samples. The RSDs were in the range 3–17% for four replicates. In general, the recoveries decreased when the PAH molecular mass increased; consequently, benzo[*a*]pyrene, dibenz[*a,h*]anthracene and benzo[*ghi*]perylene showed very low recoveries. Fig. 1(b) shows a chromatogram obtained under these conditions.

Determination of PAHs in tea infusion samples

The proposed method was used to determine eight PAHs in tea infusion samples. Black (five samples), green and decaffeinated tea were analyzed and the chromatograms obtained are shown in Fig. 2. Retention times were used to identify the PAHs and the external calibration procedure was applied for quantification. Table 4 gives the results obtained from seven samples. Benzo[*a*]pyrene and dibenz[*a,h*]anthracene were found in all samples, their contents being very low, at the ng l^{-1} level. The high contents of phenanthrene and pyrene with respect to the other PAHs could be due to their high solubility in water (1290, 135 and 0.3 μg l^{-1} at 25 °C for phenanthrene, pyrene and benzo[*ghi*]perylene, respectively[15]). The least contaminated sample was the decaffeinated tea, with a mean content of 28.7 ng l^{-1}. The RSDs for the whole method were in the range 2–18% ($n = 4$). The results obtained are in accordance with published values, which are of the order of ng l^{-1}.[12,13]

Conclusions

A method for the determination of PAHs in tea infusions is proposed. This method is based on SPE using Sep-Pak vac $_t$C-18 cartridges; the determination is carried out by HPLC with fluorimetric detection. The method is more selective, rapid and economical than those described in the literature for tea samples, which are based on LLE. The method is very sensitive; PAH concentration levels in the low ng l^{-1} range can be detected and quantified. Seven samples of black, green and decaffeinated tea were analyzed; the least contaminated sample was the decaffeinated tea.

Acknowledgements

The financial support of the Spanish CICYT, project PB96-0642, and the Servicio de Espectrometría de Masas del Centro de Espectroscopía de la UCM (Madrid, Spain) is acknowledged.

References

1 S. E. Manahan, *Environmental Chemistry*, Lewis, Boca Raton, FL, 1994, pp. 311, 318, 686 and 776
2 M. N. Kayali, S. Rubio-Barroso and L. M. Polo-Diez, *J. Chromatogr. Sci.*, 1995, **33**(4), 181.
3 M. N. Kayali-Sayadi, S. Rubio-Barroso, C. Beceiro-Roldan and L. M. Polo-Diez, *J. Liq. Chromatogr. Relat. Tech.*, 1996, **19**(19), 3135.
4 Y. Yang, A. Gharaibeh, S. B. Hawthorne and D. J. Miller, *Anal Chem.*, 1995, **67**(3), 641.
5 S. B. Hawthorne and D. J. Miller, *Anal. Chem.*, 1994, **66**(22), 4005.
6 W. Stall and G. Einsenbrand, in *HPLC in Food Analysis*, ed. R. Macrae, Academic Press, New York, 1988, pp. 377–408
7 J. E. Hernandez, I. T. Machado, R. Corbella, M. A. Rodriguez and F. Garcia-Montelongo, *Bull. Environ. Contam. Toxicol.*, 1995, **55**(3), 461.
8 M. Lodovici, P. Dolaras, C. Casalini, S. Ciappellano and G. Testolin, *Food Addit. Contam.*, 1995, **12**(5), 703.
9 E. Jarvenpaa, R. Huopalahti and P.Tapanainen, *J. Liq. Chromatogr. Relat. Technol.*, 1996, **19**(9), 1473.
10 L. Rivera, M. J. C. Curto, P. Pais, M. T. Galceran and L. Puignou, *J. Chromatogr.*, 1996, **731**(1–2), 85.
11 L. Webster, L. Angus, G. Topping, E. J. Dalgarno and C. F. Moffat, *Analyst*, 1997, **122**, 1491.
12 Y. Tonogal, S. Ogawa, M. Toyoda,Y. Ito and M. J. Iwaida, *Food Protect.*, 1982, **45**(2), 139.
13 T. Stijve and C. Hischenhuber, *Dtsch. Lebensm-Rundsch.*, 1987, **83**(9), 276.
14 C. Lintas, M. C. De Matthaeis and F. Merli, *Food Cosmet. Toxicol.*, 1979, **17**, 325.
15 D. Mackay and W. Y. Shiu, *J. Chem. Eng. Data*, 1977, **22**, 399.

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