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Food Chemistry 90 (2005) 643–647
Food Chemistry
www.elsevier.com/locate/foodchem

Minimal clean-up and rapid determination of polycyclic aromatic hydrocarbons in instant coffee

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Received 16 February 2004; received in revised form 22 March 2004; accepted 22 March 2004

Abstract

The essential aim of this work is the development of a simple, fast, quantitative and economic method for polycyclic aromatic hydrocarbons (PAHs) potentially generated by roasting coffee beans, which is the most important process in the coffee industry for the development of the characteristic flavour of the bean mix. The PAHs were chosen because they differed in the number of aromatic rings, had different polarity, have low residual limits, are commonly widespread in the environment and are generated by roasting. The key issue is whether or not the most polar PAHs, those with lower molecular weight or less rings, appear in the water extracts of ground roasted coffee beans, taking into account that those PAHs with lower molecular weight are those with higher volatility. The proposed analytical method is also broadly applicable to other roasted foods or their aqueous extracts. The method was evaluated by constructing calibration curves, measurement of recovery and precision, and the limits of detection. The method involves extraction with hexane, clean-up with a silica cartridge, concentration to dryness and injection of the acetonitrile solution of the residue for HPLC analysis with fluorescence detection. The method allowed to confirm or not the presence of the selected PAHs in instant coffees.

Keywords: Polycyclic aromatic hydrocarbons; HPLC-fluorescence; Instant coffee

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are toxic compounds. Due to the in carcinogenic activity, they have been included in the European Union (EU) and the Environmental Protection Agency (EPA) priority pollutant lists. These compounds are known to be present in the atmosphere, water, sediments, tobacco smoke, and food. The presence of PAHs in environmental samples such as water, sediments, and particulate air has been extensively studied but food samples have received less attention (Stall & Eisenbrand, 1988; Vo-Dinh, 1989; Shanahan, 1992; Baird, 1995; Kayali-Sayadi, Rubio-Barroso, & Polo-Díez, 1995; Kayali-Sayadi, Rubio-Barroso, & Polo-Díez, 1994; Kayali-Sayadi, Rubio-Barroso, Beceiro-Roldán, & Polo-Díez, 1996; Koester & Clement, 1993; Eisert & Levsen, 1996). The presence of PAHs in foods is due both to deposition of PAHs containing particles from the air on the surface of plants, and the pollution resulting from manufacture processes such as drying, roasting, or smoking (Stall & Eisenbrand, 1988; Vo-Dinh, 1989; Shanahan, 1992; Baird, 1995). Foods are controlled in the EU through maxima residual limits (MRLs) of pollutants which stipulate the amounts permitted to appear in foods; there is a proposal not yet accepted to limit benzo[*a*]pyrene at 1 μg/kg in foods. For enforcement purposes analytical methods are required that can be used to determine whether or not such limits have been exceeded.

The principal problems associated with the determination of PAHs in foods are the low detection levels required and the diversity of potential interferences present in the sample matrix. For removing interferences, the use of chromatographic techniques to determine selected PAHs has become widespread in recent years, mainly because their separating capacities allow

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0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.03.059

easy avoidance of matrix effects, above all when selective detection techniques are used. Thin layer chromatography was gradually replaced by high performance liquid chromatography (HPLC), which offered advantages such as the ability to handle larger samples, higher resolution and reliable quantitative detection (Lodovici, Dolaras, Casalini, Ciappellano, & Testolin, 1995; de Vos, van Dokkum, Schouten, & de Jong-Berkhout, 1990; Jarvenpaa, Huopalahti, & Tapanainen, 1996; Gomaa, Gray, Rabie, López-Bote, & Booren, 1993). Analysis of the selected PAHs was also performed by gas chromatography (Rivera, Curto, Pais, Galcerán, & Puignou, 1996; Hernández, Machado, Corbella, Rodríguez, & García-Montelongo, 1995; Nyman, Perfetti, Joe, & Diachenko, 1993). Compared to gas chromatography, liquid chromatography presents numerous advantages when applied to the study of organic contaminants in foods: a complex clean-up is unnecessary, reducing the likely sources of error and increasing absolute recoveries; and there is no need to use "appropriate" internal standards to correct for poor recovery and/or instrumental errors due to injection discrimination. Regarding coffee samples, scarce data are available and they are mainly related to benzo[*a*]pyrene determination in coffee brew; the procedure used for sample preparation is based on organic solvent extraction followed by a clean-up procedure (De Kruijf, Schouten, & van der Stegen, 1987; Lintas, De Matthaeis, & Merli, 1979; Hischenhuber & Skijve, 1987; Belitz, 1991) or on solid phase extraction (Kayali-Sayadi, Rubio-Barroso, Cuesta-Jiménez, & Polo-Díez, 1999), before chromatography.

In this paper we propose a simple method for the PAHs determination in instant coffees based on extraction with hexane and subsequent clean-up on a silica cartridge before separation by reversed-phase HPLC with fluorescence detecion. The method was applied to several types of water-soluble instant coffee from the market.

2. Method

2.1. Chemicals, solutions, disposables and instant coffee samples

The seven PAHs studied (benzo[*b*]fluoranthene (B[*b*]F, 98%), benzo[*k*]fluoranthene (B[*k*]F, 98%), benzo[*a*]pyrene (B[*a*]P, 97%), benzo[*ghi*]perylene (B[*ghi*]P, 98%), indeno[1,2,3-*cd*]pyrene (I[1,2,3-*cd*]P, 98%), benzo[*a*]anthracene (B[*a*]A, 98%) and dibenzo[*ah*]anthracene (DB[*ah*]A, 97%)) were purchased from Aldrich (Germany) and Supelco (USA). The first five are indicators of drinking water quality, whereas it is important to monitor the rest in environmental (wild animals, soil, particulate matter in air, ash, etc.) and food (oil, fried foods, etc.) samples. Acetonitrile, water and hexane of HPLC grade were supplied by Merck.

Independent 100 mg/l stock solutions of PAHs were prepared by dissolving about 0.01 g of the different PAHs in a small amount of hexane and diluting to 100 ml with the same solvent. From this solution, a PAHs mix solution at levels ranging from 1 to 20 mg/l for the different PAHs was prepared in hexane. These solutions were stored in amber flasks at 4 °C and were then stable for at least 6 months. From the PAHs mix solution, a new solution at levels ranging from 25 to 350 μg/l for the different PAHs was prepared into acetonitrile by evaporating the hexane; this solution was diluted to construct calibration lines for the PAHs. All these solutions were preserved for at least 6 months in the same storage conditions.

Waters Sep-Pak silica plus (690 mg) cartridges were used as solid-phase extraction (SPE) minicolumns for purification of the hexane extract. A Visiprep Solid Phase Extraction Vacuum Manifold was used to simultaneously process up to 24 SPE cartridges. Other small apparatus such as an analytical scale, an up-and-down shaker, a rotary evaporator and a vortex shaker were used. Amongst other disposables used are nylon filters (0.45 μm), micropipettes (200–1000 μl) and injection vials (2 ml) provided with screw caps and PTFE-lined butyl rubber septa and inserts (0.35 ml).

Instant coffee samples were obtained in a chain store selecting different trade names. A total of 12 samples were bought; six with caffeine and six without; three of each six were obtained by natural roasting and the others by torrefy roasting (with added sugar).

3. Liquid chromatograph and operating conditions

All HPLC measurements were taken using a Thermo Separation Products (TSP) P2000 binary pump, equipped with a TSP AS1000 autosampler, a TSP SCM1000 vacuum membrane degasser and a Jasco FP-1520 fluorescence detector. The chromatographic data were collected and processed using the Chrom-Card software. The optimized instrumental parameters for the chromatographic analysis of PAHs were as follows (Fig. 1):

Injection loop: 50 μl.

Column: A 25 cm × 4.6 mm i.d. stainless steel analytical column packed with 5 μm Supelcosil LC-PAH (Supelco).

Elution conditions: 32 min linear gradient elution from 80:20 acetonitrile/water to 97:03 acetonitrile/water, followed by 3 min linear gradient to 100% acetonitrile, keeping 100% acetonitrile for 2 min. Flow rate was 1 ml/min throughout. Elution temperature was maintained at 33 °C.

Fluorescence detection: 16 min λex at 274 nm and λem at 414 nm, followed by 6 min λex at 300 nm and λem at

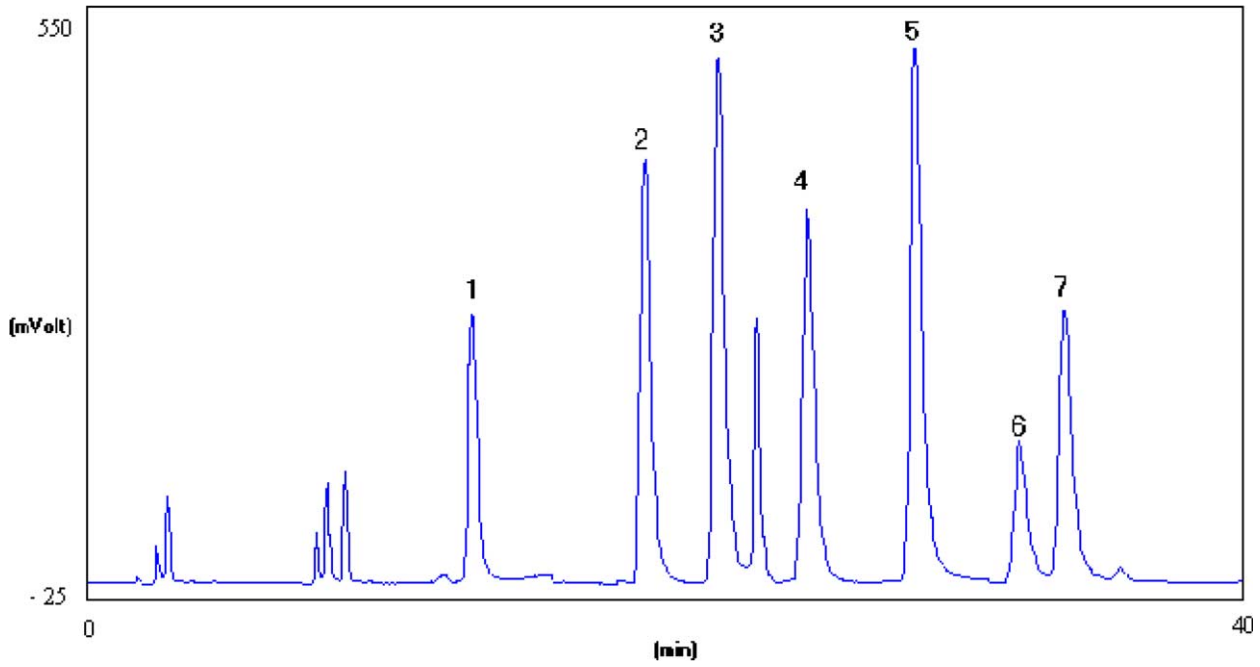

Fig. 1. Chromatogram obtained after applying the method to a spiked sample of instant coffee. Peaks identification: 1, B[*a*]A; 2, B[*b*]F; 3, B[*k*]F; 4, B[*a*]P; 5, D[*ah*]A; 6, B[*ghi*]P; 7, I[*1,2,3-cd*]P.

446 nm, followed by 9 min λex at 296 nm and λem at 406 nm, and followed by 6 min λex at 300 nm and λem at 470 nm. Gain was 1000; slit was kept at 40 nm.

3.1. Instant coffee sample pre-analytical treatment with minimal clean-up

Homogenized instant coffee (0.5 g) was extracted with hexane (15 ml) in an up-and-down shaker for 10 min. The solvent was separated by filtering through nylon membranes (0.45 μm) and then was cleaned-up with a silica cartridge, passing additional hexane (7 ml) through the cartridge to complete PAHs elution. The collected hexane solution was taken to dryness by rotary evaporation, and the residue obtained was redissolved in acetonitrile (0.5 ml); 50 μl were automatically sampled into the chromatographic system by means of 0.35 ml inserts in 2-ml vials.

4. Results and discussion

4.1. Performance characteristics of the method proposed

Several extraction strategies were tested and dismissed. Amongst them, first of all, the efficiency of passing an aqueous solution of instant coffee through a C18 minicolumn to selectively retain PAHs from the coffee matrix was tested. Problems arose with soluble solids blocking the cartridge, which contributed to the rejection of this procedure. Subsequently, organic solvent extraction of instant coffee samples assisted by microwaves was checked, but low stability of several PAHs under the tested conditions ruled out the use of microwaves to facilitate PAHs extraction. The last extraction strategy that was abandoned was based on stir bar sorptive extraction (SBSE). The reasons were the low recoveries obtained compromising detection and the variability obtained with different coffee samples due to matrix interferences.

PAHs extraction from instant coffee was then optimized having taken into account parameters such as extraction solvent and number of consecutive extractions, together with extract clean-up. Different non-polar solvents were assayed. The decreasing order of performance was: hexane> dichloromethane >ethyl acetate. The first extraction with hexane proved to be quantitative since recoveries were generally higher than 87% for the different PAHs. After hexane evaporation and redissolution in water, SBSE was tried once more to recover PAHs, but still low recoveries and variability amongst samples was found, even although alkaline hydrolysis of the extracted fat and acetonitrile addition to increase fat solubility into water were assayed. Another approach based on the use of different polar minicolumns for the hexane extract clean-up was tested: neutral alumina, florisil and silica were evaluated. PAHs were successfully eluted retaining most of the co-extracted interferences in the cartridge, especially in that made of silica. An instant coffee sample was then fortified with all PAHs, and the spiked sample was further analysed after being left overnight protected from light

Table 1
Performance of the proposed method for the determination of PAHs in instant coffees

PAHs	Absolute[a] recovery			Instrument linearity[b] range (μg/l)	r^2	LOD[a] (μg/kg)	LOQ[a] (μg/kg)
	(μg/kg)	(%)	± RSD				
B[*a*]A	0.5	87	6	0.1–5	0.9990	0.03	0.1
B[*b*]F	0.5	93	8	0.1–5	0.9992	0.03	0.1
B[*k*]F	0.3	95	5	0.04–2	0.9997	0.01	0.04
B[*a*]P	0.3	101	5	0.04–2	0.9994	0.01	0.04
D[*ah*]A	0.5	89	6	0.1–5	0.9997	0.03	0.1
B[*ghi*]P	0.7	103	6	0.2–10	0.9998	0.05	0.2
I[*1,2,3-cd*]P	0.7	87	5	0.2–10	0.9997	0.05	0.2

[a] ($n = 7$).
[b] ($n = 10$) determinations.

under refrigeration conditions; the results are summarized in Table 1; most recovery percentages were higher than 87%, while RSD% were lower than 8%. There are no matrix effects affecting results as is proved by the excellent results obtained, equivalent to those obtained by repeating the procedure with different instant coffee samples. Limits of detection (LOD) and Limits of quantitation (LOQ) were evaluated on the basis of the noise obtained with the analysis of blank instant coffee samples. LOD and LOQ were defined as the concentration of the analyte that produced a signal-to-noise ratio of three and ten, respectively (American Chemical Society, 1980), and were then tested experimentally. The quantification by HPLC used the chromatographic peak area, and the calibration lines were constructed by plotting peak areas against standard concentrations; standard solutions were injected directly into the chromatographic column for calibration purposes since the method proved to be quantitative and reproducible. It was found that use of an internal standard was not necessary as the method performance characteristics were already satisfactory. Fluorescence detection was selected to maximize the analyte response, while reducing interferences from the matrix to a minimum. The ratio of sample mass to extractant volume can be increased to improve detection when the extraction solvent does not extract fat from the coffee.

4.2. Content of polycyclic aromatic hydrocarbons in instant coffees

While considerable research has been conducted on the generation of PAHs during roasting, there are a lack of studies on the water extraction of those PAHs in instant coffees. In this work, was a systematic study performed on the contents of the selected PAHs in 12 market samples of instant coffees with different trade names: six with caffeine and six without; three of each six were obtained by natural roasting and the others by torrefy roasting (with added sugar). The results showed that the PAHs were not extracted into water in the preparation of instant coffees; in only two torrefied samples (high roast), both without caffeine, B[*b*]F, B[*k*]F and B[*a*]P were found at levels between the detection and quantification limits of the method, 0.03–0.1 μg/kg for B[*b*]F and 0.01–0.04 μg/kg for the others, far below any potential legal restriction.

Results obtained are in the same order of those for B[*a*]P found by other authors in roasted coffee grounds (0.1–0.4 μg/kg) and in the brews prepared by the filter-drip method (2–7 ng/l) ([Hischenhuber & Skijve, 1987). We have to take into account that instant coffee can be considered a product between roasted coffee grounds and coffee brews, or even better, a dried coffee brew. In The Netherlands ([De Kruijf et al., 1987), the B[*a*]P levels in the roasted coffee samples analysed range from approximately 0.1 to 0.5 μg/kg; in coffee brew the levels found indicate B[*a*]P extraction yields of 1% for different preparation methods. Other authors determined selected PAHs in coffee brew samples and their findings for all the PAHs determined are in the range of 0.3–9 ng/l (Kayali-Sayadi et al., 1999).

5. Conclusions

The versatility of HPLC as analytical tool makes it an ideal technique for analytical quality control and research and development laboratories in the food and beverage industry. The determination of PAHs in instant coffees at the sub-ppb level was performed in a short time and can be run automatically. The analysis procedures proved not to affect their stability and are quantitative as indicated by the method recoveries (in general better than 87%) and precisions (between 5% and 8%). Detection and quantification limits for the PAHs in instant coffees were found to be satisfactory and much lower than the restrictions given in proposals of EU Directives for B[*a*]P (1 μg/kg). The methods are recommended for the determination of the extraction of those PAHs from ground roasted coffee into water in the preparation of instant coffee. The analytical methodology developed could eventually be used for studies of other similar PAHs in other foods with low fat content.

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