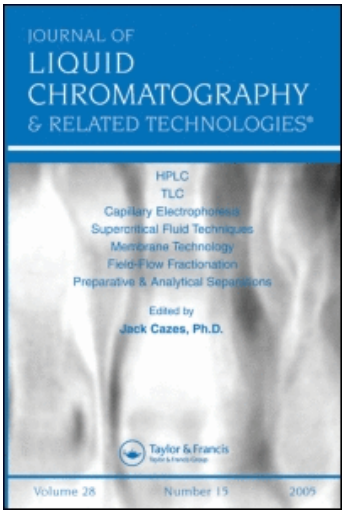
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A NEW METHOD FOR THE DETERMINATION OF SELECTED PAHs IN COFFEE BREW SAMPLES BY HPLC WITH FLUORIMETRIC DETECTION AND SOLID-PHASE EXTRACTION

M. N. Kayali-Sayadi[a]; S. Rubio-Barroso[a]; M. P. Cuesta-Jimenez[a]; L. M. Polo-Díez[a]
[a] Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, Madrid, Spain

Online publication date: 25 February 1999

To cite this Article Kayali-Sayadi, M. N. , Rubio-Barroso, S. , Cuesta-Jimenez, M. P. and Polo-Díez, L. M.(1999) 'A NEW METHOD FOR THE DETERMINATION OF SELECTED PAHs IN COFFEE BREW SAMPLES BY HPLC WITH FLUORIMETRIC DETECTION AND SOLID-PHASE EXTRACTION', Journal of Liquid Chromatography & Related Technologies, 22: 4, 615 — 627
To link to this Article: DOI: 10.1081/JLC-100101686
URL: http://dx.doi.org/10.1081/JLC-100101686

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

J. LIQ. CHROM. & REL. TECHNOL., 22(4), 615–627 (1999)

A NEW METHOD FOR THE DETERMINATION OF SELECTED PAHS IN COFFEE BREW SAMPLES BY HPLC WITH FLUORIMETRIC DETECTION AND SOLID-PHASE EXTRACTION

M. N. Kayali-Sayadi, S. Rubio-Barroso,*
M. P. Cuesta-Jimenez, L. M. Polo-Díez

Department of Analytical Chemistry
Faculty of Chemistry
Complutense University of Madrid
28040 Madrid, Spain

Downloaded At: 10:35 24 January 2011

ABSTRACT

A rapid analytical method is proposed for the determination of PAHs (fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[e]pyrene, benzo[a]pyrene, dibenzo[a,h]anthracene and benzo[g,h,i]perylene) in coffee brew samples. The method is based on solid phase extraction with Sep-Pak Vac tC-18 cartridges and elution with ethyl ether. The extracts were analyzed by RP-HPLC with a Green PAH column and fluorescence detection under an acetonitrile-water mobile phase gradient. Some PAHs were detected in the samples studied and their mean contents were 1.65-2.87 ng/L coffee brew. The relative standard deviations were in the range 2-13% for four replicates.

Copyright © 1999 by Marcel Dekker, Inc. www.dekker.com

INTRODUCTION

Polynuclear aromatic hydrocarbons (PAHs) are toxic compounds. Due to their carcinogenic power, they have been included in the European Community (EC) 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 much less attention.[1-9] 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.[1-4]

The analytical procedures most often used for PAHs determination in foods are based, according with the sample type, on previous treatment with potassium or sodium hydroxide in ethanol-water,[10-13] followed by liquid-liquid extraction (LLE) with organic solvents such as cyclohexane, hexane,[11-13] or solid phase extraction (SPE) on alumina, silica, or C_{18} cartridges.[10,14] Supercritical-fluid extortion (SFE) has also been used.[15] When necessary, a clean-up step is carried out by column chromatography.[11,13,14,16] Eluted fractions from the column are analyzed by gas chromatography (GC) with flame ionization or mass spectrometry detectors,[10,11,14] or reverse phase high performance liquid chromatography (RP-HPLC) with spectrophotometric (UV) or fluorometric (FL) detection.[12,13,15,16] 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 liquid-liquid extraction (LLE) with cyclohexane followed by a clean-up on silica gel column.[17-20]

In this paper we present a simple method for the PAHs determination based on solid phase extraction (SPE) of the coffee brew sample, and RP-HPLC with fluorometric detection. The method was applied to several types of coffee samples available commercially, prepared by different manufacture processes.

EXPERIMENTAL

Chemicals

Solvents and PAHs

Analytical standard PAHs from Sigma (St. Louis, MO) and HPLC grade solvents: acetonitrile and methanol from Carlo Erba; (Milan, Italy) were used. Other solvents and chemical reagents were also of HPLC purity. Purified water was obtained using a Milli-Q apparatus from Millipore (Milford, MA).

Downloaded At: 10:35 24 January 2011

Downloaded At: 10:35 24 January 2011

Standard solutions

Stock solutions containing 25.5, 295, 152, 83.1, 208, 59.5, 84.1, and 35.4 µg/mL of fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[e]pyrene, benzo[a]pyrene, dibenzo[a,h]anthracene and benzo[g,h,i]perylene, respectively, were prepared by dissolving the solid standards in methanol. These solutions were stored in glass bottles under refrigeration at 4°C. Diluted individual standards were mixed at different concentrations according to their sensitivities to fluorometric detection.

Apparatus and materials

The HPLC system consisted of the following components: a Milton-Roy CM4000 high-pressure-gradient pump (Rivera Beach, FL); a Rheodyne 7125 valve with a 20 µL loop injector (Cotati, CA); a Perkin Elmer LS 30 luminescence spectrometer (Norwalk, CT) and a Milton Roy CI 4100 integrator. The Hypersil Green PAH (100x4,6mm), 5 µm particulate size column by Shandon (England) was thermostatized in a P-Selecta Precisterm bath (Barcelona, Spain) which was used to maintain the column temperature at 22±1°C. Sep-Pak Vac $_t$C-18 (500 mg, Waters; Milford, MA) cartridges were used to extract the PAHs from coffee brew samples. A vacuum flask (1000 mL, Pobel; Madrid, Spain) and separatory funnels (250 mL, Pobel), a Barna Vacio vacuum pump and a Visiprep solid phase extraction vacuum manifold from Supelco (Bellefonte, PA) were also used. All PAHs solutions were prepared using a P-Selecta ultrasonic bath. Solvents used to prepare the mobile phase and sample eluates were filtered through Lida nylon membrane filters with 0.45 µm pore size (Gelman Sciences, Ann Arbor, MI) and MFS-13 PTFE membrane filters with 0.5 µm pore size (MFS, Dublin, CA). The coffee brew samples were obtained with a Moulinex electric coffee maker.

Coffee samples

Milled coffee samples were those available at the supermarket, prepared by different manufacturing processes; torrefied coffee (coffee roasted in the presence of sugar) three samples, roasted coffee, decaffeinated and green.

Procedures

Sample preparation

The aqueous coffee brew samples were obtained using an electric coffee maker equipped with a paper filter. In all cases, 50 g of commercial milled coffee

were treated by passing 300 mL of water during 5 minutes. The samples were stored in amber bottles at 4°C and were analyzed within 24 hours of collection. Afterwards, 93 mL of the coffee brew and 7 mL of methanol were mixed in a glass flask by sonication, and then the PAHs were extracted using Sep-Pak Vac $_t$C-18 cartridges at a flow rate of 10 mL/min. The cartridges were dried for 5 minutes using the vacuum system.

The adsorbed PAHs were eluted first with 3 mL and then with 1 mL of ethyl ether at a flow rate of 0.8 mL/min. The cartridges were firstly cleaned with 3 mL of ethyl ether, then conditioned with 3 mL of methanol, twice, and finally with 3 mL of Milli-Q water.

Recoveries were determined from 100 mL coffee brew:methanol (93:7) samples spiked with the PAH mixture, in the range 3.67-867 ng/L. The eluate was collected in a small graduated glass tube and the solvent was evaporated to near dryness in the vacuum manifold system; for this purpose, the tube was placed in a water glass vessel at 22°C. The residue was redissolved in 0.5 mL of methanol in the ultrasonic bath for 5 minutes. This solution was filtered through a PTFE membrane filter and then analyzed by RP-HPLC. The PAHs were identified by means of their retention times and quantified by applying the calibration procedure.

Downloaded At: 10:35 24 January 2011

Calibration

External calibration graphs at five concentration levels were prepared from standard solutions containing the PAHs in the range 0.734-173 ng/mL. The acetonitrile-water mobile phase gradient specified in Table 1 was employed with a flow rate of 1 mL/min at 22°C using the program of six excitation and emission wavelength pairs indicated in Table 2. The mobile phase was degassed with helium. The peak areas were used for their quantitation.

RESULTS AND DISCUSSION

Preliminary Studies

In order to identify the PAHs present in coffee brew samples, the procedure specified in literature[7] was applied and retention factors were used for PAHs identification. These studies show the presence of eight PAHs: fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[e]pyrene, benzo[a]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene. On the other hand these PAHs were confirmed by gas chromatography-mass spectrometry.

Table 1

Linear Gradient of the Mobile Phase

Time, Min	Acetonitrile, %	Water, %
0.0	50	50
3.0	50	50
25.0	85	15
28.0	100	---

Table 2

Program of Excitation and Emission Wavelength Pairs

Detected Compound*	Time, s	λ_{ex}, nm	λ_{em}, nm
1. Fluoranthene	0	285	465
2. Pyrene	1020	270	390
3. B[a]a,	1350	270	384
4. Chrysene			
5. B[e]p	1650	290	390
6. B[a]p	1800	295	405
7. Db[a,h]a,	2070	290	418
8. B[g,h,i]p			

* B[a]a, Benzo[a]anthracene; B[e]p, Benzo[e]pyrene; B[a]p, Benzo[a]pyrene; Db[a,h]a, Dibenzo[a,h]anthracene; B[g,h,i]p, Benzo[g,h,i]perylene.

Downloaded At: 10:35 24 January 2011

Optimization of the Chromatographic Characteristics

Mobile phases acetonitrile or methanol-water were tested in the ranges 50-100% and 70-100% respectively, at a flow rate of 1 mL/min; the best results were obtained by using a mobile phase based on acetonitrile-water; the gradient specified in Table 1 was used. In order to attain maximum sensitivity, the program of excitation and emission wavelength pairs specified in Table 2 was used.[5] Figure 1 shows a chromatogram of the standard PAHs mixture.

Analytical Characteristics for Standards

Standard PAH solutions were analyzed in the range 0.734-173 ng/mL. The correlation coefficients were higher than 0.996 in all cases. Table 3 summarizes the

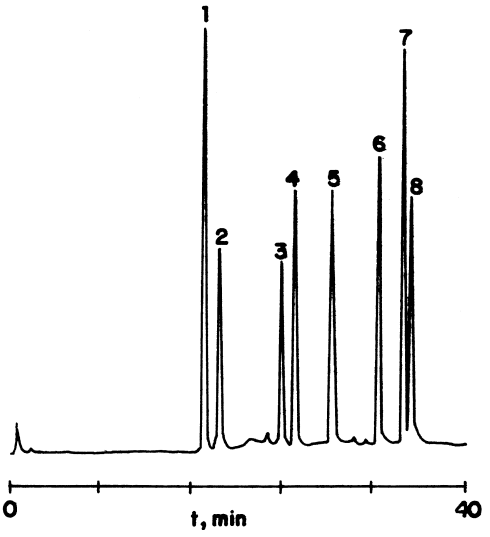


Figure 1. Chromatogram of a standard mixture of eight PAHs. Conditions: Hypersil Green PAH (100 x 4.6 mm) column; Temperature, 22°C; Mobile phase, gradient of acetonitrile/water, see Table 1; Flow-rate, 1 mL/min; Fluorimetric detection, see Table 2; Injection volume, 20 µL. Peaks: 1, fluoranthene; 2, pyrene; 3, B[a]a; 4, Chrysene; 5, B[e]p; 6, B[a]p; 7, Db[a,h]a; 8, B[g,h,i]p.

Downloaded At: 10:35 24 January 2011

Table 3

Analytical Characteristics

PAH	Range of Linearity ng/mL*	C ng/mL	RSD,** %	LD,*** ng/mL	k	RSD,** %
Fluoranthene	1.07-26.6	16	3.0	0.021	21.4	0.51
Pyrene	2.40-60.0	36	10	0.17	23.1	1.0
B[a]a	1.80-45.0	27	6.0	015	30.4	0.86
Chrysene	1.53-38.4	23	6.0	0.10	32.2	0.81
B[e]p	6.94-173	104	8.0	0.41	36.3	0.69
B[a]p	0.734-18.4	11	5.0	0.035	41.3	0.36
Db[a,h]a	4.14-103	62	4.0	0.19	44.3	0.40
B[g,h,i]p	2.34-58.4	35	10	0.17	45.0	0.39

* Studied range. ** Relative Standard Deviation (n=4). *** Detection Limit, DL = 3S/N.

analytical characteristics obtained. Relative standard deviations (RSD) at concentration levels of 11-104 ng/mL, at the middle point of the calibration graphs, were between 3-10% from four replicates. Detection limits (DL, 3 times the signal-to-noise ratio) were lower than 0.4 ng/mL; the lowest values were for fluoranthene 0.021 ng/mL and benzo[a]pyrene 0.035 ng/mL. The table also shows the retention factors of the PAHs; void time was determined with KNO_3 12 mM.

Recovery Study from Coffee Brew Samples Using SPE

Nature and volume of the eluent

100 mL of the coffee brew sample spiked with PAHs in the range 55.1-520 ng/L, were passed directly through the $_tC$-18 cartridge, previously conditioned. Various solvents such as ethyl ether, methylene chloride, and ethyl ether:methylene chloride (1:1) were tested for PAH elution. Using 2 mL of these eluents the mean recoveries were below 50% and these results would not improve greatly by increasing to 4 mL. This could be attributed to the collapse of the C_{18} phase, described for reversed HPLC column,[21] which would decrease the retention capacity of the cartridge. The effect of the presence of methanol in the coffee brew sample was studied.

We tested several methanol percentages, in the range 0-10%, in 100 mL of coffee brew sample spiked with the PAHs. Recoveries increased significantly even for low methanol contents, the optimum values being obtained using 7% methanol. Higher percentages yielded poor recoveries; obviously retention decreases because methanol competes with the active sites of the cartridge. The elution of PAHs from the cartridge was carried out with 2 mL ethyl ether, with a mean recovery of 64%.

On the other hand, as the recoveries are a function of the number of stages and eluent volume of the desorption stage, volumes of 2, 3, and 4 mL of ethyl ether were tested. It was observed that best results were achieved with 3 mL, the mean recovery being 70%.

Maximum recovery and reproducibility were obtained when elution was carried out in two stages, firstly with 3 mL and then with 1 mL, collecting the two fractions in a graduated glass tube; the mean recovery was 78%. A washing effect seems to appear related with the flow rate and the non-equilibrium of the elution stage.

In order to eliminate potential interferences retained in the cartridge, the effect of clean-up was tested passing through the cartridge 1-4 mL of 5% methanol-water solution. In all cases the recoveries were lower due to the methanol-water elution power.

Downloaded At: 10:35 24 January 2011

Table 4

Infuluence of PAH Concentration in the Study of PAH Recoveries from Coffee Brew Samples*

PAHs	C_1, ng/L	R_1, %	C_2, ng/L	R_2, %	C_3, ng/L	R_3, %	C_4, ng/L	R_4, %
Fluoranthene	5.33	60	26.7	60	80.0	70	133	81
Pyrene	12.0	110	60.0	111	180	102	300	47
B[a]a	9.00	81	45.0	95	135	81	225	76
Chrysene	7.67	45	38.3	46	115	87	192	42
B[e]p	34.7	40	173	38	520	81	867	88
B[a]p	3.67	64	18.4	67	55.1	71	91.8	25
Db[a,h]a	20.7	65	103	65	310	68	517	20
B[g,h,i]p	11.7	40	58.3	42	175	67	292	11
Mean recoveries		62		66		78		49

Coffee brew volume: 100 mL; R, %; percentage recovery.
* Mean of four determinations. Relative standard deviations are in the range 3-12%.

Downloaded At: 10:35 24 January 2011

Amount of sample and breakthrough volume

As the PAHs concentrations in coffee is low, the maximum amount of sample was evaluated. Due to the potential matrix effect, recoveries were determined from spiked coffee brew samples at PAHs levels close to those expected for benzo[a]pyrene.[17-20] Firstly, increasing weights of solid coffee samples were extracted and the volume of the analytical solution was increased accordingly in the range 50-200 mL to keep the final concentration constant; PAH amounts spiked were in the range 55.1-520 ng/L. The maximum recovery was obtained from 100 mL solutions of coffee brew sample. Using higher sample amounts or volumes the recovery decreased.

Secondly, 100 mL of coffee brew samples were spiked with 40, 200, 600, and 1000 µL of PAHs solution with concentration levels between 9.18-86.7 ng/mL. The PAH concentration and the recoveries found are shown in Table 4. As it can be seen, the best results were obtained when the PAHs concentrations in the coffee brew sample were in the range 55.1-520 ng/L; all recoveries were higher than 80% except for benzo[a]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene, the lower being for benzo[g,h,i]perylene due to its insolubility in water.

The relative standard deviations were in the range 3-12% from four replicate measurements. The extract volume was adjusted to 0.5 mL. Figure 2 shows a chromatogram obtained from one of the samples.

Downloaded At: 10:35 24 January 2011

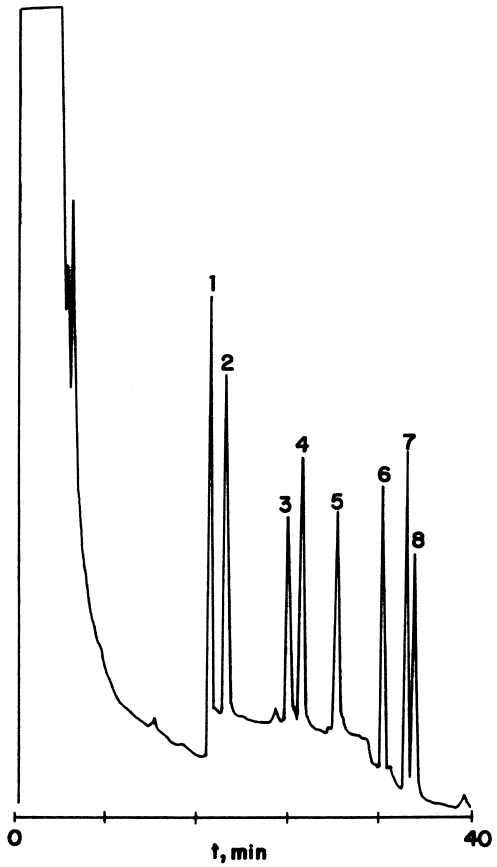


Figure 2. Chromatogram of coffee brew sample number 2 spiked with PAHs in the range 55.1-520 ng/mL. Conditions: Hypersil Green PAH (100 x 4.6 mm) column; Temperature, 22°C; Mobile phase, gradient of acetonitrile/water, see Table 1; Flow-rate, 1 mL/min; Fluorometric detection, see Table 2; Injection volume, 20 µL. Peaks: 1, fluoranthene; 2, pyrene; 3, B[a]a; 4, Chrysene; 5, B[e]p; 6, B[a]p; 7, Db[a,h]a; 8, B[g,h,i]p.

Determination of PAHs in coffee brew samples

Seven coffee brew samples, specified in the experimental section, were analyzed by applying the proposed method. A chromatogram obtained from a torrefied coffee brew sample is shown in Figure 3, and Table 5 shows the PAHs content in these coffee brew samples. The PAHs were confirmed by spiking the samples with the PAHs detected. As it can be seen the PAHs content depends on the sample elaboration; for example, decaffeinated and green coffees contain mean

Downloaded At: 10:35 24 January 2011

Figure 3. Chromatogram of coffee brew sample number 1. Conditions: Hypersil Green PAH (100 x 4.6 mm) column; Temperature, 22°C; Mobile phase, gradient of acetonitrile/water, see Table 1; Flow-rate, 1 mL/min; Fluorometric detection, see Table 2; Injection volume, 20 µL. Peaks: 1, fluoranthene; 2, pyrene; 3, B[a]a; 4, Chrysene; 6, B[a]p; 7, Db[a,h]a; 8, B[g,h,i]p.

concentrations, of 1.99 and 1.65 ng/L coffee brew, respectively, and the contents in torrefied coffee correspond to the higher concentration, 2.87 ng/L coffee brew, found in one of the samples. In general, the roast process increases the PAHs content in the samples.[1] On the other hand, all the samples contain the most toxic PAHs, benzo[a]pyrene and dibenzo[a,h]anthracene. The relative standard deviations were in the range 2-13%, n=4. These results are in good agreement with available data for benzo[a]pyrene in coffee brew, which are at the level of ng/L; there are no data in the literature regarding other PAHs.[17-20]

Table 5

PAHs Amount in Coffee Brew Samples, ng/L

		Torrefied Coffee		Roasted Coffee	Decaf. Coffee	Green Coffee
PAH	**1**	**2**	**3**	**4**	**5**	**6**
Fluoranthene	2.27	1.13	1.37	0.74	1.78	0.975
Pyrene	10.9	9.02	6.80	7.77	7.55	7.35
B[a]a	1.21	2.17	2.00	1.25	---	---
Chrysene	1.72	1.27	1.38	---	---	0.883
B[e]p	---	---	---	1.25	---	1.13
B[a]p	3.20	1.60	2.17	2.38	3.40	---
Db[a,h]a	3.38	3.02	---	3.02	3.15	2.92
B[g,h,i]p	0.31	0.29	---	0.41	---	---
Mean	2.87	2.31	1.72	2.10	1.99	1.65

Relative standard deviations, %= 2-13% (n=4). 1-6: Sample number.

In any case, the PAH contents in the samples are below the maximum permitted limits of 0.2 µg/L in water samples.[22] On the other hand, the proposed method was applied to determine the PAHs in tap-water as it had been used to prepare the coffee brew samples; but PAHs were not detected.

CONCLUSIONS

The developed method for the determination of trace amounts of PAHs at levels of ng/L in coffee brew is more selective, rapid, and economic than those referred in the literature, based on liquid-liquid extraction; this is due to the use of solid-phase extraction. All samples analyzed contained benzo[a]pyrene and dibenzo[a,h]anthracene at levels of ng/L coffee brew. One of the torrefied coffees was the most polluted; green coffee being the one which resulted in lower levels of PAHs.

ACKNOWLEDGMENTS

We acknowledge financial support of the Spanish DGICYT for this research under the project PB 96-0642 and Servicio de Espectrometría de Masas del Centro de Espectroscopía de la UCM, Madrid (Spain).

Downloaded At: 10:35 24 January 2011

Downloaded At: 10:35 24 January 2011

REFERENCES

1. W. Stall, G. Einsenbrand, "Determination of Polynuclear Aromatic Hydrocarbons and Nitrosamines," in **HPLC in Food Analysis**, R. Macrae, ed., Academic Press, New York. NY, 1988. pp 377-408.

2. T. Vo-Dinh, "Significance of Chemical Analysis of Polycyclic Aromatic Compounds and Related Biological Systems," in **Chemical Analysis of Polycyclic Aromatic Compounds**, T. Vo-Dinh, ed., John Wiley and Sons, New York, NY, 1989. pp 1-30.

3. I. Shanahan, "Air Pollution Analysis," in **Chemical Analysis in Complex Matrices**, M.R. Smyth, ed., Ellis Hoorwood. London, England, 1992. pp 192-240.

4. C. Baird, "Toxic Organic Chemicals," in **Environmental Chemistry**, W. H. Freeman and Company. New York. NY, 1995. pp 215-286.

5. M. N. Kayali, S. Rubio-Barroso, L. M. Polo-Díez, J. Chromatogr. Sci., **33(4)**, 181-185 (1995).

6. M. N. Kayali, S. Rubio-Barroso, L. M. Polo-Díez, J. Liq. Chromatogr., **17(17)**, 3623-3640 (1994).

7. M. N. Kayali-Sayadi, S. Rubio-Barroso, C. Beceiro-Roldan, L. M. Polo-Díez, J. Liq. Chromatogr. Relat. Technol., **19(19)**, 3135-3146 (1996).

8. C. J. Koester, R. E. Clement, Crit. Rev. Anal. Chem., **24(4)**, 263-316 (1993).

9. R. Eisert, K. Levsen, J. Chromatogr., **733(1-2)**, 143-157 (1996).

10. L. Rivera; M. J. C. Curto; P. Pais, M. T. Galceran, L. Puignou, J. Chromatogr. **731(1-2)**, 85-94 (1996).

11. J. E. Hernández; I. T. Machado, R. Corbella, M. A. Rodríguez, F. García-Montelongo, Bull. Environ. Contam. Toxicol., **55(3)**, 461-468 (1995).

12. M. Lodovici, P. Dolaras, C. Casalini, S. Ciappellano, G. Testolin, Food Addit. Contam., **12(5)**, 703-713 (1995).

13. R. H. de Vos, W. van Dokkum, A. Schouten, P. de Jong-Berkhout, Fd. Chem. Toxic., **28(4)**, 263-268 (1990).

14. P. J. Nyman, G. A. Perfetti, F. L. Joe, Jun and G. W. Diachenko, Food Addit. Contam., **10(5)**, 489-501 (1993).

15. E. Jarvenpaa, R. Huopalahti, P. Tapanainen, J. Liq. Chromatogr. Relat. Technol., **19(9)**, 1473-1482 (1996).

16. E. A. Gomaa, J. I. Gray, S. Rabie, C. López-Bote, A. M. Booren, Food Addit. Contam., **10(5)**, 503-521 (1993).

17. N. De Kruijf, T. Schouten, G. H. D. van der Stegen, J. Agric. Food Chem., **35(4)**, 545-549 (1987).

18. C. Lintas, M. C. De Matthaeis, F. Merli, Fd. Cosmet. Toxicol., **17**, 325-328 (1979).

19. C. Hischenhuber, T. Skijve, Detsch. Lebensm-Rundsch., **83(1)**, 1-4 (1987).

20. Herrn. Prof. Dr. Hans-Dieter Belitz, Detsch. Lebensm-Rundsch, **87(3)**, 69-75 (1991).

21. J. W. Dolan, LC-GC INT, **8(3)**, 381-382 (1995).

22. Boletín Oficial del Estado, nº 17, January 20, 1982, pp. 1278-1281.

Received January 4, 1998
Accepted July 3, 1998
Manuscript 4718

Downloaded At: 10:35 24 January 2011

Downloaded At: 10:35 24 January 2011

Request Permission or Order Reprints Instantly!

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/Reprints Here" link below and follow the instructions. Visit the U.S. Copyright Office for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on Fair Use in the Classroom.

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our Website User Agreement for more details.

Order now!

Reprints of this article can also be ordered at

http://www.dekker.com/servlet/product/DOI/101081JLC100101686