RESIDUES AND TRACE ELEMENTS

Determination of Polycyclic Aromatic Hydrocarbons in Honey by Matrix Solid-Phase Dispersion and Gas Chromatography/Mass Spectrometry

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A multiresidue method was developed for the determination of 16 polycyclic aromatic hydrocarbons (PAHs) in unifloral and multifloral honeys. The analytical procedure is based on the matrix solid-phase dispersion of honey on a mixture of Florisil and anhydrous sodium sulfate in small glass columns and extraction with hexane–ethyl acetate (90 + 10, v/v) with assisted sonication. The PAH residues are determined by gas chromatography with mass spectrometric detection using selected-ion monitoring. Average recoveries for all the PAHs studied were in the range of almost 80 to 101%, with relative standard deviations of 6 to 15%. The limits of detection ranged from 0.04 to 2.9 μg/kg. The simultaneous extraction and cleanup of samples makes this method simple and rapid, with low consumption of organic solvents.

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds formed by ≥2 fused benzene rings, some of which are known or suspected carcinogens or mutagens that are widespread pollutants in the environment. These compounds are introduced in the environment from both natural sources (incomplete combustion of organic matter) and anthropogenic sources (oil spills, waste incineration, traffic, burning of fossil fuels, factory discharge, etc.). PAHs have been studied in numerous environmental matrixes such as water, soil, vegetables, and aquatic organisms.

Contamination of honey with PAHs may come from several sources, such as forest fires, stubble burning, location of beehives near industrial sites, or inadequate practices by beekeepers. In the atmosphere, PAHs are present in the vapor phase or as particles that can travel long distances and that can be deposited onto grains, fruits, and vegetables.

Although large amounts of PAHs are found in nature, the U.S. Environmental Protection Agency (EPA) has suggested a list of 16 as priority pollutants on the basis of their frequency and carcinogenicity. The list includes naphthalene (Naph), acenaphthylene (Acyl), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phen), anthracene (Anth), fluoranthene (F), pyrene (Py), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DBahA), benzo[g,h,i]perylene (BghiP), and indeno[1,2,3-c,d]pyrene (IcdPy). Figure 1 shows the chemical structures of these compounds.

The extraction procedures most often used for the determination of PAHs in environmental and food samples have been liquid–liquid extraction (1) for water samples or solid-phase extraction (SPE; 2, 3) for water and beverages. Solid-phase microextraction (SPME; 4, 5), supercritical fluid extraction (SFE; 6–8), and microwave-assisted extraction (MAE; 9) have also been used recently for the determination of these compounds in food, especially food of animal origin. Before extraction by these procedures, Soxhlet extraction was used for complex samples (10, 11). Biological tissues, such as meat or marine animals, usually need a previous saponification step because of their lipid content. Generally, the extraction solvents used are acetonitrile (8), methylene chloride (9), cyclohexane (12), hexane (13), or mixtures of these compounds (3).

Matrix solid-phase dispersion (MSPD) is a simultaneous extraction and cleanup technique that requires less time and solvent than do conventional methods. This technique is based on dispersion of the sample on an adsorbent, usually Florisil or C_{18}. Because compounds such as waxes and pigments are retained on the surface of the adsorbent, a further cleanup step is not necessary, and the extract can be analyzed directly.

PAHs are mainly determined by liquid chromatography with fluorescence detection (2, 3, 8, 11, 14) or by gas chromatography (GC) with mass spectrometric detection (6, 10, 12, 15). GC coupled with mass spectrometry (GC/MS) has the advantages of high selectivity and sensitivity that allow the determination of numerous PAHs in a single analysis. Synchronous spectrofluorimetric determination, based on the simultaneous variation of emission and excitation wavelengths, has been used mainly to determine PAHs in water samples (13, 16).

As far as we know, no analytical method for the determination of PAHs in honey has been published in the scientific literature. The aim of this work was to develop a method for the determination of PAHs in different kinds of honey, based on

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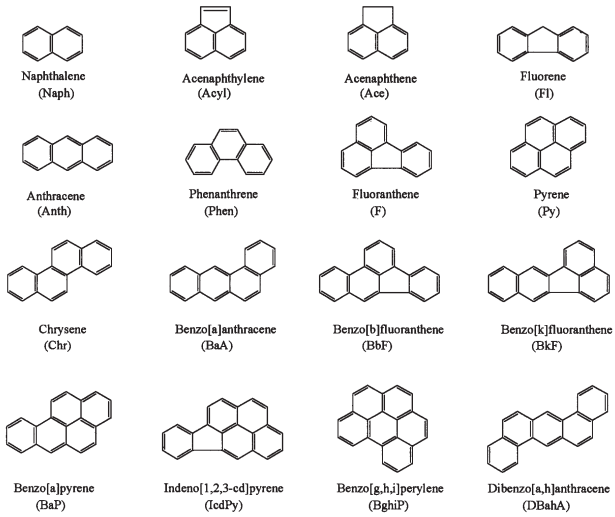

Figure 1. Chemical structures of the PAHs studied.

the MSPD of samples on Florisil. PAH residues were determined by GC/MS with selected-ion monitoring (SIM).

METHOD

Reagents

(**a**) *Solvents*.—Residue analysis grade methanol, hexane, and ethyl acetate (Scharlau, Barcelona, Spain); acetonitrile, cyclohexane, and methylene chloride (Panreac, Barcelona, Spain).

(**b**) *Anhydrous sodium sulfate*.—Reagent grade (Sigma-Aldrich, Steindhem, Germany).

(**c**) *Florisil (60–100 mesh)*.—Research grade (Fluka Chemie, Buchs, Switzerland). The adsorbent was heated for 24 h at 140°C before use.

(**d**) *Standard stock solutions*.—A standard solution of the 16 EPA-priority PAHs (each at 2000 μg/mL) in methylene chloride–benzene (50 + 50) was supplied by Sigma-Aldrich. The mixture was diluted with ethyl acetate to a concentration of 20 μg/mL for each PAH, and the final standard stock solution was stored at 4°C.

(**e**) *Standard working solutions*.—Prepare a set of standard mixtures for fortification of honey samples. Transfer 5 mL stock solution to a 25 mL volumetric flask, and dilute to volume with methanol to give a concentration of 4 μg/mL. Transfer 10 mL working solution to a 50 mL volumetric flask, and dilute to volume with methanol to give a concentration of 0.8 μg/mL. Transfer 5 and 0.5 mL of this last working solution to 20 mL volumetric flasks, and dilute to volume with methanol to give concentrations of 0.2 and 0.02 μg/mL, respectively.

(**f**) *Samples*.—Several Spanish honeys were purchased: 4 unifloral (eucalyptus, lavender, rosemary, and thyme) and 1 multifloral.

Apparatus

(**a**) *Extraction columns*.—Glass, 20 mL, with Whatman No. 1 filter paper circles of 2 cm id (Whatman, Maidstone, UK).

(**b**) *Ultrasonic water bath*.—Raypa (Barcelona, Spain).

Table 1. Main ions and their relative abundance in the mass spectra of the PAHs studied

Compound	PAH		t_R, min	*m/z* (% relative abundance)
	Name	Abbreviation		
1	Naphthalene	Naph	7.08	127(13), 128[a](100), 129(11)
2	Acenaphthylene	Acyl	11.93	150(13), 151(16), 152[a](100), 153(12)
3	Acenaphthene	Ace	12.52	152(48), 153[a](100), 154(94)
4	Fluorene	Fl	14.12	163(14), 165(87), 166[a](100)
5	Phenanthrene	Phen	17.14	176(18), 178[a](100), 179(16)
6	Anthracene	Anth	17.28	176(18), 178[a](100), 179(15)
7	Fluoranthene	F	21.02	101(11), 200(21), 202[a](100)
8	Pyrene	Py	21.78	101(12), 200(21), 202[a](100)
9	Benzo[a]anthracene	BaA	26.52	226(25), 228[a](100), 229(20)
10	Chrysene	Chr	26.68	226(28), 228[a](100), 229(21)
11	Benzo[b]fluoranthene	BbF	31.18	126(13), 250(22), 252[a](100), 253(22)
12	Benzo[k]fluoranthene	BkF	31.29	126(15), 250(21), 252[a](100), 253(22)
13	Benzo[a]pyrene	BaP	32.78	126(11), 250(23), 252[a](100)
14	Indeno[1,2,3-c,d]pyrene	IcdPy	40.35	138(19), 276[a](100), 277(26)
15	Dibenzo[a,h]anthracene	DBahA	40.69	138(14), 276(31), 278[a](100), 279(26)
16	Benzo[g,h,i]perylene	BghiP	42.43	138(18), 274(21), 276[a](100), 277(25)

[a] Quantitation ion.

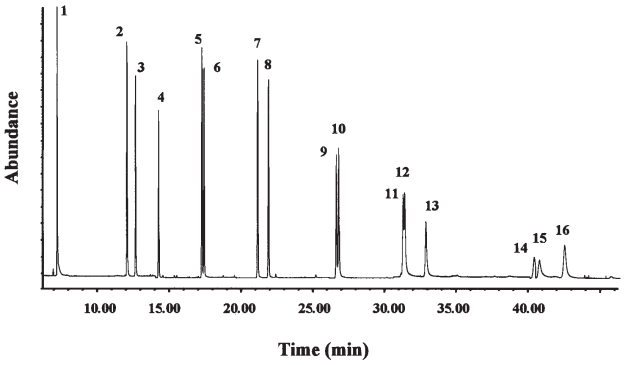

Figure 2. Chromatogram obtained by GC/MS in the SIM mode for a standard mixture of PAHs at 0.01 μg/mL. *See* Table 1 for peak identification.

(**c**) *Vacuum manifold*.—Supelco Visiprep (Madrid, Spain).

(**d**) *GC/MS system*.—Hewlett-Packard Model 6890 gas chromatograph (Waldbronn, Germany) equipped with a Hewlett-Packard Model HP 7683 automatic injector and a Hewlett-Packard 5973 series mass-selective detector. The mass spectrometer was operated in the electron-impact ionization mode (ionizing energy of 70 eV) scanning from *m/z* 50 to 450 at 3.62 scan/s. The ion source and quadrupole temperatures were 230 and 150°C, respectively. A fused-silica capillary column (ZB-5MS), 30 m × 0.25 mm id, with 5% phenyl polysiloxane, 0.25 mm id, as the nonpolar stationary phase was supplied by Phenomenex (Torrance, CA). Operating conditions were as follows: injector port temperature, 290°C; injection volume, 2 μL, in pulsed splitless mode (pulsed pressure, 45 psi for 1.5 min); helium as the carrier gas at a flow rate of 1 mL/min; GC/MS interface temperature, 250°C; oven temperature program: 80°C for 0.5 min, from 80 to 230°C at 8°C/min, from 230 to 280°C at 5°C/min, and held at 280°C for 17 min; solvent delay, 6 min. The total analysis time was 46.25 min, and the equilibration time was 2 min.

SIM was used with 10 acquisition windows for MS analysis as follows: (*1*) from 0 to 11.0 min, *m/z* 128, 129; (*2*) from 11.0 to 13.9 min, *m/z* 152, 153; (*3*) from 13.9 to 16.9 min, *m/z* 165, 166; (*4*) from 16.9 to 19.5 min, *m/z* 178, 179; (*5*) from 19.5 to 26.0 min, *m/z* 101, 202; (*6*) from 26.0 to 30.5 min, *m/z* 226, 228, 229; (*7*) from 30.5 to 40.0 min, *m/z* 250, 252; (*8*) from 40.0 to 40.5 min, *m/z* 138, 276; (*9*) from 40.5 to 42.0 min, *m/z* 138, 276, 278; and (*10*) from 42.0 to 46.2 min, *m/z* 276, 277. The dwell time for the ions monitored was 100 ms.

Sample Extraction and Cleanup

This method is based on a previously published method for the determination of pesticide residues in honey (17, 18). Samples of different commercial honeys were heated at 45°C in a water bath to reduce their viscosity before handling. A 1.5 g portion of honey was placed in a glass tube with screw stopper and blended with 1.5 mL methanol or with 1.5 mL PAH mixture to produce a final concentration in the range of 0.02–0.8 μg/g. The mixture was homogenized by using a Vortex mixer for complete dissolution; 2 mL of the resulting honey solution was transferred to a glass column filled with

Table 2. Effect of various extraction solvents on the recovery (%)[a] of PAHs from lavender honey fortified at 0.5 μg/g

Compound	Hexane	Cyclohexane	Methylene chloride	Hexane–ethyl acetate (90 + 10, v/v)
Naph	70.5 ± 19.0	92.0 ± 3.9	53.8 ± 5.3	85.3 ± 9.2
Acyl	70.8 ± 17.1	96.0 ± 3.7	65.8 ± 7.2	92.0 ± 7.5
Ace	70.0 ± 16.8	93.0 ± 3.9	63.3 ± 7.2	91.0 ± 6.4
Fl	74.8 ± 18.8	100.0 ± 4.4	70.5 ± 8.7	97.8 ± 7.3
Phen	72.5 ± 19.1	99.0 ± 5.9	74.0 ± 10.1	99.3 ± 8.5
Anth	65.3 ± 18.2	94.5 ± 8.2	65.5 ± 7.7	93.0 ± 8.8
F	64.0 ± 18.5	88.0 ± 3.6	60.8 ± 11.1	88.0 ± 6.7
Py	63.5 ± 18.6	87.0 ± 3.6	60.3 ± 10.8	87.3 ± 6.8
BaA	52.5 ± 19.9	81.7 ± 7.1	58.3 ± 15.7	84.8 ± 8.7
Chr	55.0 ± 20.0	83.7 ± 5.8	55.0 ± 14.9	86.5 ± 7.2
BbF	39.4 ± 18.7	71.7 ± 10.1	49.7 ± 15.4	77.7 ± 7.1
BkF	42.5 ± 19.7	71.7 ± 8.5	47.0 ± 15.2	78.2 ± 6.9
BaP	37.2 ± 20.1	75.7 ± 15.8	55.6 ± 18.8	89.5 ± 10.5
IcdPy	30.4 ± 16.9	63.8 ± 7.3	51.9 ± 22.9	96.1 ± 9.6
DBahA	20.4 ± 12.1	44.6 ± 15.9	40.0 ± 19.4	73.8 ± 8.8
BghiP	33.9 ± 17.2	69.0 ± 12.3	45.4 ± 18.9	81.1 ± 9.1

[a] Each value is recovery ± relative standard deviation, % (*n* = 4).

Table 3. Recovery (%) of Naph, Acyl, Ace, Fl, Phen, Anth, F, Py, and BaA from honey samples[a]

PAH added, μg/g	Naph	Acyl	Ace	Fl	Phen	Anth	F	Py	BaA
					Lavender				
0.8	99.5 ± 14.8	94.9 ± 5.4	92.5 ± 4.3	98.9 ± 5.0	99.8 ± 5.5	98.0 ± 5.8	90.5 ± 6.0	89.9 ± 5.5	91.8 ± 8.3
0.2	79.3 ± 2.3	90.6 ± 3.5	89.3 ± 3.5	97.9 ± 3.4	102.4 ± 2.9	90.9 ± 3.8	91.9 ± 3.7	88.8 ± 3.8	87.9 ± 6.9
0.02	93.8 ± 12.4	87.5 ± 6.5	88.9 ± 5.7	92.3 ± 14.6	102.8 ± 10.9	96.0 ± 6.7	95.6 ± 7.7	91.6 ± 9.6	97.6 ± 14.7
					Eucalyptus				
0.8	90.8 ± 15.2	91.7 ± 7.9	90.2 ± 8.4	96.3 ± 9.4	97.2 ± 8.2	96.2 ± 9.4	88.0 ± 6.3	87.5 ± 8.1	89.3 ± 7.8
0.2	79.4 ± 4.7	89.4 ± 5.9	88.6 ± 5.2	96.6 ± 6.2	101.6 ± 10.1	87.9 ± 7.6	89.9 ± 9.5	85.1 ± 8.1	92.6 ± 5.4
0.02	92.8 ± 10.1	83.0 ± 5.5	86.3 ± 5.1	98.5 ± 6.4	107.5 ± 4.5	85.6 ± 8.3	90.6 ± 9.7	88.8 ± 4.3	95.8 ± 8.6
					Rosemary				
0.8	96.8 ± 9.9	103.0 ± 3.6	102.3 ± 4.7	108.9 ± 5.8	109.1 ± 5.6	105.1 ± 5.9	98.0 ± 4.6	98.0 ± 5.3	96.4 ± 7.2
0.2	86.8 ± 2.7	100.5 ± 3.2	98.8 ± 2.7	108.0 ± 3.0	107.0 ± 4.0	97.4 ± 3.4	102.1 ± 5.6	96.1 ± 4.0	92.0 ± 6.2
0.02	93.0 ± 15.8	93.3 ± 15.5	95.4 ± 18.6	95.9 ± 17.6	113.3 ± 18.4	99.7 ± 15.9	106.5 ± 18.9	99.0 ± 19.1	102.6 ± 18.8
					Thyme				
0.8	92.0 ± 6.8	95.9 ± 5.3	93.9 ± 4.6	100.1 ± 4.8	100.4 ± 4.9	96.6 ± 5.0	89.3 ± 5.2	88.9 ± 5.4	83.1 ± 5.8
0.2	84.4 ± 5.6	94.9 ± 3.4	93.9 ± 3.2	101.9 ± 3.6	100.5 ± 4.1	92.6 ± 3.9	94.3 ± 3.7	89.5 ± 3.6	90.4 ± 5.9
0.02	84.4 ± 16.2	85.3 ± 12.0	81.0 ± 13.0	92.3 ± 15.3	100.3 ± 17.7	83.3 ± 15.6	93.2 ± 11.4	85.0 ± 5.3	82.1 ± 11.2
					Multifloral				
0.8	93.1 ± 18.0	90.5 ± 7.2	89.1 ± 5.5	94.8 ± 5.1	95.1 ± 5.5	92.5 ± 6.3	85.8 ± 7.9	85.3 ± 6.7	89.2 ± 7.6
0.2	89.4 ± 3.0	105.8 ± 2.1	103.6 ± 1.5	94.8 ± 12.1	114.9 ± 4.4	104.4 ± 3.6	112.1 ± 6.0	106.4 ± 6.2	103.8 ± 10.9
0.02	100.0 ± 14.3	94.5 ± 9.3	93.3 ± 13.8	97.8 ± 15.9	115.9 ± 11.6	102.2 ± 12.6	117.5 ± 10.9	112.5 ± 15.8	115.2 ± 12.7
Average	89.4 ± 11.3	92.1 ± 7.4	94.2 ± 6.9	96.4 ± 8.6	101.1 ± 7.8	99.5 ± 7.5	95.4 ± 9.6	93.3 ± 8.6	92.1 ± 9.7

[a] Each value is recovery ± relative standard deviation, % (n = 4).

Table 4. Recovery (%) of Chr, BbF, BkF, BaP, IcdPy, DBahA, and BghiP (DbahA, BghiP, and IcdPy) from honey samples[a]

PAH added, μg/g	Chr	BbF	BkF	BaP	IcdPy	DBahA	BghiP
			Lavender				
0.8	94.1 ± 7.7	86.4 ± 11.1	89.1 ± 9.9	97.0 ± 11.6	78.6 ± 13.6	72.1 ± 12.3	80.5 ± 10.1
0.2	88.6 ± 5.2	83.3 ± 9.4	82.8 ± 8.3	91.6 ± 10.7	78.0 ± 19.5	72.3 ± 19.0	80.5 ± 14.4
0.02	98.0 ± 8.5	96.0 ± 7.9	85.9 ± 13.4	99.0 ± 13.2	87.8 ± 13.1	73.5 ± 19.8	73.8 ± 11.4
			Eucalyptus				
0.8	83.9 ± 15.4	77.1 ± 15.8	81.0 ± 16.0	86.9 ± 18.2	71.6 ± 3.4	66.2 ± 3.0	73.0 ± 4.9
0.2	83.1 ± 11.5	77.6 ± 15.3	77.0 ± 12.2	81.1 ± 16.3	74.2 ± 18.5	69.1 ± 18.3	71.1 ± 15.9
0.02	98.3 ± 6.8	86.9 ± 12.8	80.4 ± 11.4	98.2 ± 12.2	99.0 ± 13.1	65.5 ± 16.2	68.5 ± 8.9
			Rosemary				
0.8	97.4 ± 7.5	89.5 ± 10.9	91.9 ± 9.4	98.1 ± 9.1	82.6 ± 16.4	72.7 ± 17.2	84.1 ± 13.3
0.2	94.4 ± 5.1	89.1 ± 7.1	88.9 ± 5.3	86.1 ± 5.8	76.9 ± 7.1	71.7 ± 6.1	83.7 ± 8.5
0.02	104.9 ± 18.5	101.6 ± 18.9	94.4 ± 15.0	104.6 ± 15.9	98.4 ± 19.0	86.1 ± 16.9	81.6 ± 10.5
			Thyme				
0.8	83.8 ± 6.3	79.0 ± 5.6	80.0 ± 5.2	90.6 ± 12.6	86.3 ± 15.3	74.8 ± 19.5	74.8 ± 14.0
0.2	92.9 ± 5.9	89.6 ± 7.5	89.0 ± 5.7	97.8 ± 8.2	88.6 ± 10.4	86.1 ± 10.5	86.3 ± 10.0
0.02	93.3 ± 13.1	88.3 ± 15.1	81.0 ± 11.4	97.7 ± 17.2	85.2 ± 14.7	85.0 ± 9.3	72.3 ± 16.2
			Multifloral				
0.8	88.2 ± 9.7	84.4 ± 10.0	82.3 ± 5.7	97.7 ± 6.8	88.9 ± 6.6	69.0 ± 4.6	87.6 ± 7.4
0.2	101.5 ± 7.5	108.3 ± 14.8	99.6 ± 9.6	105.7 ± 9.3	105.0 ± 8.3	89.4 ± 2.9	93.0 ± 5.9
0.02	98.0 ± 18.3	105.0 ± 9.3	109.0 ± 16.2	111.3 ± 10.0	105.3 ± 13.0	82.5 ± 4.9	91.0 ± 17.2
Average	91.7 ± 10.0	89.4 ± 13.8	87.4 ± 11.6	89.8 ± 13.1	88.4 ± 15.3	78.9 ± 13.9	78.2 ± 12.1

[a] Each value is recovery ± relative standard deviation, % (n = 4).

3.5 g Florisil–anhydrous sodium sulfate (2.5 + 1, w/w). The honey sample, dispersed throughout the column by the methanol used in the preparation of the sample was extracted with 5 mL hexane–ethyl acetate (90 + 10, v/v) for 15 min in an ultrasonic bath at room temperature. The water level of the bath was adjusted to the solvent level inside the column. The column was supported upright in a tube rack and closed with a 1-way stopcock. After sonication, the column was placed in a vacuum manifold, where the eluate was collected in a 10 mL graduated glass tube. This step was repeated with another 5 mL extraction solvent. For the highest spiking level, the combined eluates were diluted to 10 mL with the same solvent. For the other 2 spiking levels, the eluates were concentrated with a gentle stream of air to an appropriate volume (2 mL for the lowest level and 5 mL for the intermediate level) before GC analysis.

Quantitation

Samples were analyzed by GC/MS. The concentration of each compound was determined by comparing the ratios of the peak areas obtained for the samples with those found for standard mixtures of known concentration.

Results and Discussion

PAHs are known to be light-sensitive. Therefore, to minimize the possible photodecomposition of the PAHs studied, working solutions (stored in foil-wrapped volumetric flasks) and fortified honey samples were prepared on the same day. Because these compounds tend to remain adsorbed on the walls of their containers, glass columns instead of propylene columns were used in the sample extraction procedure to reduce possible losses.

The chromatographic analysis of the honey samples is based on SIM. The mass spectra of PAHs have a characteristic fragmentation pattern and, as a result of their weak fragmentation, the molecular ion is the main and most abundant ion. Therefore, the other ions in the mass spectra of PAHs have low relative abundance, around 20%. Table 1 shows the main ions of each compound as well as their relative abundance. The identification of PAHs by GC/MS in the SIM mode is based on the main ion of the characteristic mass spectrum of each

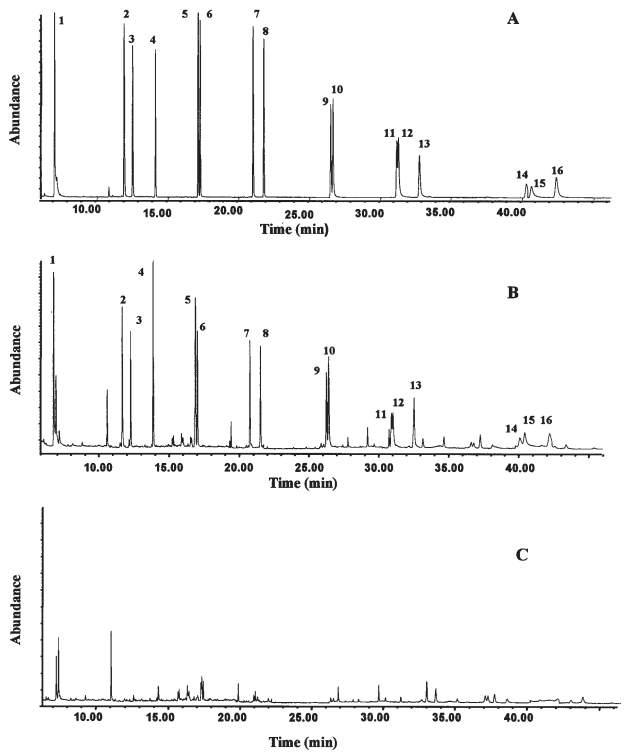

Figure 3. Chromatograms obtained by GC/MS in the SIM mode for (A) thyme honey spiked at 0.2 μg/g; (B) lavender honey spiked at 0.02 μg/g; and (C) a blank lavender honey sample. *See* Table 1 for peak identification.

compound together with the relative abundance of the other ions selected for each PAH, and on the chromatographic retention time of the PAH. The weak fragmentation of PAHs enhances the sensitivity of the method when the molecular ion is used for quantitation. Figure 2 shows a chromatogram obtained in the SIM mode for a standard mixture of PAHs at 10 ppb. Initially, the scan mode was applied to a standard mixture to determine the retention time and the main ions of each PAH.

The proposed method was used to determine 16 PAHs in honey. PAHs in environmental or food samples were extracted with various organic solvents, generally of low polarity (9, 12, 13). Several extraction solvents were evaluated: acetonitrile, hexane, cyclohexane, and methylene chloride, together with hexane–ethyl acetate (90 + 10, v/v), which was used with good results in previous work on residue analysis of honey for pesticides (17, 18). Sonication-assisted extraction of residues was used in the proposed method because of the improvement in recoveries obtained with this technique in previous work. Table 2 shows the recovery results obtained with various solvents for lavender honey samples spiked at 0.5 μg/g. Recoveries with cyclohexane as the extraction solvent were acceptable for most of the PAHs. On the other hand, extraction with acetonitrile formed emulsions; the polarity of this solvent probably allowed the extraction of the methanol used in the standard solutions to spike the honey samples, together with some water from the honey; thus, acetonitrile was discarded as the extraction solvent. The best results were obtained with hexane–ethyl acetate (90 + 10, v/v). The improvement in recovery was more noticeable for the PAHs with higher molecular weights, which gave values of around 30% with the other solvents; recoveries obtained with hexane–ethyl acetate (90 + 10, v/v) were > 70%.

Tables 3 and 4 show the recovery results obtained with different types of honey. Honey samples, previously analyzed to verify the lack of PAHs, were fortified at 0.8, 0.2, and 0.02 μg/g before extraction. The average recoveries obtained ranged from around 80 to 101%, with relative standard deviations between 6 and 15%. The recoveries of PAHs with higher molecular weights were on the lower end of the range obtained, probably because of the tendency of these compounds to remain adsorbed on Florisil (19).

Figure 3 shows representative chromatograms obtained for honey samples fortified at the intermediate and lowest levels and for a blank honey sample. The peaks in the chromatogram for the blank honey sample (Figure 3C) do not match any of the PAH peaks; therefore, compounds such as waxes and pigments do not interfere in the determination of the compounds studied.

Table 5 summarizes the calibration data, instrumental detection limits (IDLs), and limits of detection (LODs) for the PAHs studied. The responses of all the PAHs were linear for the concentration range studied, from 0.01 to 0.08 μg/mL, with good correlation coefficients that ranged from 0.996 to 1.000. The IDLs were determined by considering a value equal to or higher than 3 times the background noise obtained for a standard mixture solution of PAHs at 10 ppb. The LODs were determined by considering a value equal to or higher than 3 times the background noise obtained for a blank honey sample; they ranged from 0.04 to 2.90 μg/kg. The limit of quantitation (LOQ), supported by the recovery data presented, was 20 μg/kg for each compound. Nevertheless, a lower LOQ could be obtained for the PAHs studied on the basis of their LOD values.

The proposed method shows good results in comparison with those reported by other researchers for the determination of PAHs in various foods, mainly meat products. The average recoveries obtained with the present method are in the higher part of the range, and the LODs are in the lower part of the range of values previously published for those matrixes (10, 11, 15).

The developed MSPD method was used to analyze various commercial Spanish honeys; no residues of the PAHs studied were found at levels above the LODs in these samples.

Conclusions

An MSPD multiresidue method was developed for the determination of 16 PAHs in honey by GC/MS with SIM. The proposed method allows the extraction and cleanup of samples in a single step, which makes it simple and rapid. This procedure is a good alternative to conventional liquid–liquid

Table 5. Calibration data, instrumental detection limit (IDL) values, and limit of detection (LOD) values for the PAHs studied

		Calibration data[a]			
Compound	PAH	Correl. coeff. (r)	Equation	IDL, pg	LOD, μg/kg
1	Naph	1.000	$2.26 \cdot 10^8 \times - 4.12 \cdot 10^5$	0.3	0.2
2	Acyl	0.999	$2.02 \cdot 10^8 \times - 5.86 \cdot 10^5$	0.1	0.1
3	Ace	0.999	$1.76 \cdot 10^8 \times - 4.61 \cdot 10^5$	0.1	0.1
4	Fl	0.999	$1.35 \cdot 10^8 \times - 4.30 \cdot 10^5$	0.1	0.04
5	Phen	0.999	$1.82 \cdot 10^8 \times - 5.84 \cdot 10^5$	0.4	0.3
6	Anth	0.997	$1.71 \cdot 10^8 \times - 7.76 \cdot 10^5$	0.8	0.5
7	F	0.998	$1.79 \cdot 10^8 \times - 7.30 \cdot 10^5$	0.1	0.05
8	Py	0.998	$1.74 \cdot 10^8 \times - 7.28 \cdot 10^5$	0.2	0.2
9	BaA	0.996	$1.01 \cdot 10^8 \times - 4.06 \cdot 10^5$	1.2	0.8
10	Chr	0.997	$1.35 \cdot 10^8 \times - 7.08 \cdot 10^5$	0.9	0.6
11	BbF	0.999	$7.43 \cdot 10^7 \times - 3.55 \cdot 10^5$	0.8	0.5
12	BkF	0.997	$1.23 \cdot 10^8 \times - 7.68 \cdot 10^5$	1.9	1.3
13	BaP	0.999	$6.80 \cdot 10^7 \times - 2.76 \cdot 10^5$	1.7	1.1
14	IcdPy	1.000	$2.91 \cdot 10^7 \times - 1.66 \cdot 10^5$	2.8	1.9
15	DBahA	0.999	$4.53 \cdot 10^7 \times - 2.11 \cdot 10^5$	4.3	2.9
16	BghiP	0.999	$5.45 \cdot 10^7 \times - 1.08 \cdot 10^5$	1.6	1.1

[a] Concentration range: 0.01–0.08 μg/mL.

or Soxhlet extractions, and its low consumption of organic solvents decreases the risk of using toxic chemicals. Moreover, the described MSPD method allows the detection of PAHs at the low limits needed in monitoring programs.

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