Rev. Int. Contam. Ambie. 34 (2) 355-366, 2018
DOI: 10.20937/RICA.2018.34.02.15

Technical Note / Nota Técnica

ANALYTICAL PERFORMANCE OF MICROWAVE-ASSISTED SOLVENT EXTRACTION (MASE) FOR THE ROUTINE DETERMINATION OF PAHs IN POLLUTED SOILS BY GAS CHROMATOGRAPHIC-MASS SPECTROMETRY (GC-MS)

José Enrique SÁNCHEZ-URÍA[1]* and Estela DEL CASTILLO-BUSTO[2]

[1] Departamento de Química Física y Analítica, Universidad de Oviedo, Julián Clavería 8, E33006 Oviedo, España
[2] Département Biomédical et Chimie Inorganique, Laboratoire National de Métrologie et d´Essais, 1, Rue Gaston Boissière, 76724 Paris Cedex 15, France
*Author for correspondence; jesu@uniovi.es

(Received January 2016; accepted August 2017)

Key words: pollutants, analysis, method, solid, samples

ABSTRACT

The extraction of polycyclic aromatic hydrocarbons (PAHs) from polluted soil samples using microwave-assisted solvent extraction (MASE) with subsequent determination by gas chromatography-mass spectrometry (GC-MS) was investigated. The optimum conditions for the extraction were established and, after cleansing the extracts, pollutants were quantified by GC-MS. The detection limits for the 16 USEPA members of the family, analysis time, precision and linear range of the analysis were also established. The proposed extraction methodology was compared with the most conventional Soxhlet extraction technique, and validated by applying MASE to a reference soil sample. Good recoveries for the majority of PAHs (when the calibrations were carried out by using internal standards), a significant shortening of the time of analysis and the volume-reduction of solvent used were observed. Therefore, the MASE sample preparation methodology is a very good alternative to the extraction of PAHs from soil samples before final determination by GC-MS.

Palabras clave: contaminantes, análisis, método, muestras sólidas

RESUMEN

Se investiga la extracción de hidrocarburos aromáticos policíclicos (HAP) de muestras de suelo utilizando extracción con solventes asistida por microondas (MASE) y la posterior determinación de contaminantes mediante cromatografía de gases con detección de espectrometría de masas (GC-MS). Se establecieron las condiciones óptimas para la extracción y tras la limpieza de los extractos, los contaminantes fueron cuantificados por GC-MS. Se establecieron los límites de detección para los 16 miembros de la familia según la clasificación de la USEPA, el tiempo de análisis, la precisión y el rango dinámico lineal. Se comparó la metodología de extracción propuesta con el método convencional Soxhlet y se evaluó el método que se propone, aplicándolo a una muestra de suelo de referencia. Se obtuvieron buenas recuperaciones para la mayoría de los HAP (cuando las calibraciones se realizaban mediante patrón interno) y se observó una importante reducción del tiempo de análisis así como del volumen de solvente utilizado. Se demostró que la metodología de preparación de muestras de suelo mediante MASE es una buena alternativa para la extracción de HAP antes de la determinación por GC-MS.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous and persistent contaminants of anthropogenic origin affecting a given ecosystem. Waters, soils and sediments are the receivers in the last instance of such pollutants coming from a contaminated atmosphere (where they accumulate as a result of the incomplete combustion of coal, oil and wood) (Wilde and Jones 1995). The heaviest compounds are quickly condensed or adsorbed onto particles and then they fall to the ground or into surface waters (including wet or dry deposition), industrial effluents (Moore and Ramamoorthy 1994) or municipal wastewater (Manoli and Samara 1999). As result of the hydrophobic characteristic of PAHs, their water solubility is very low when they appear in water adsorbed on suspended particles (Mahafley et al. 1988). The mobility in the soil-plant system also seems to be very slow.

PAHs are regarded as persistent organic pollutants in the environment with mutagenic and carcinogenic properties, and they have been included on the USEPA and European Union lists of pollutants (Bouzige et al. 1999). The USEPA has identified 16 PAHs as priority pollutants, some of which are considered as probable human carcinogens. On the other hand, the European list contains eight target PAHs, including benzo(a)anthracene, chrysene, dibenzo(a,h)anthracene, benzo(a) pyrene, benzo(k)fluoranthene, benzo(g,h,i)perylene, and indeno(1,2,3-cd)pyrene (Menzie et al. 1992).

The extraction of PAHs from contaminated soils for their analysis can be achieved using a number of well-established methods wherein Soxhlet extraction (USEPA 1986, Lara-Gonzalo 2015) is the most conventional method. This sample preparation technique is rather time consuming (12-24 h), with the additional drawbacks of high solvent consumption as well as energy demand. Therefore, alternative extraction techniques have been developed including sonication (Cejpeck et al. 1995), ultrasonic micellar extraction (Pino et al. 2001), supercritical fluid extraction (Librando et al. 2004), accelerated solvent extraction (Richer et al. 1996), pressurized hot water extraction (Andersson et al. 2002), and microwave assisted solvent extraction (MASE) (Letellier and Budzinski 1999a). A comparative study of several extraction methods for PAHs in contaminated soils was carried out by Song et al. (2002), concluding that no significant differences in the extraction efficiency of the methods had been observed for less polluted soils.

From the first publication about the use of microwave energy (Ganzler et al. 1986) to enhance the extraction of organic compounds from solid samples, different oven designs have been used for such purpose. In fact, domestic microwave ovens were initially used in laboratories, while today a number of manufacturers supply microwave ovens specially designed for MASE analytical applications with high security features.

In the present study, the determination of PAHs by gas chromatography-mass spectrometry (GC-MS) in polluted soils is attempted by using the MASE system Ethos Sel® Microwave Labstation (Milestone, CT, USA). This extraction system has several advantages over the more conventional system: it allows the use of pure non-polar solvents (as hexane) with low microwave power by using a magnetic stirring of “weflon” (fluoropolymer that absorbs microwave energy), continuous control of the temperature and power supply into the vessel, detection of organic vapor in the microwave cavity, self-close action when the vessel is open by overpressure and a vacuum system allowing for fast filtration of extracts and solvent evaporation after extraction. The equipment allows for rapid extraction of such analytes from the soil samples, and rapid filtration of extracts and the evaporation of solvents in a single microwave unit, prior to the final cleaning and sample solution reconstitution for the final injection into the GC-MS.

MATERIALS AND METHODS

Chemical and reagents

A primary mixture standard solution with a concentration of 2000 µg/mL of each component of the 16 PAHs in a benzene/dichloromethane mix from AccuStandard was used. Deuterated internal standards (IS) containing naftalene-d_8, acenaphtene-d_{10}, phenantrene-d_{10}, chrysene-d_{12}, and perylene-d_{12} each at 4000 µg/mL in dichloromethane were obtained from Hewlett-Packard.

The secondary mixture standard solution (20 µg/mL for each of the 16 PAHs) was prepared by the dilution of 1 mL of the PAH primary mixture to 100 mL with hexane. This solution was stored in a refrigerator in brown glass bottles and in the dark. A second solution (40 µg/mL) of IS was also prepared by dilution of 1 mL of a commercial solution of IS to 100 mL with hexane. Finally, calibration solutions were prepared by weight from different amounts of the secondary standard solution of the 16 PAHs, 40 µL of 40 µg/mL IS standard solution, being the final volume 1.5 mL. The PAH concentration of the calibration solutions ranged between 0.10 µg/mL and 1.95 µg/mL. These solutions were prepared at the moment of calibration.

High purity chromatographic quality hexane, acetone and dichloromethane were used. The validation of the methodology was carried out by the analysis of a certified soil (CRM-104-100 [LGC Promochem]) with PAH levels 0.77 and 24.8 mg/kg.

Instrumentation

A Hewlett-Packard (Palo Alto, CA, USA) model 6890 gas-chromatograph coupled to a Hewlett-Packard 5973 mass spectrometric detector working in single ion monitoring mode (SIM) were used for PAHs analytical separation and detection with an automatic injection system 7683 from Hewlett-Packard.

The GC separation column was a 30 m × 0.33 mm i.d. × 25 µm thin thickness DB-XLB (equivalent to 5 % phenyl, 95 % methylpolysiloxane) fused silica capillary column (Hewlett- Packard). An electronic pressure control was utilized to maintain a constant gas carrier helium flow of 1.0 mL/min throughout the oven program. The injector and detector port temperatures were 300 and 230 ^oC, respectively. The oven program temperature was 60 ^oC (hold 1.5 min), rate 8 ^oC/min to 270 ^oC (hold 10 min), and rate 5 ^oC/min to 280 ^oC (hold 13 min). Split mode was used.

The mass spectrometric detector was operated in electron ionization mode (EI) with ionization energy of 70 eV; the source and quadrupole temperatures were 230 and 150 ^oC, respectively; the dwell time was 100 ms for all the ions.

A Milestone Ethos Sel was used for microwave-assisted organic extraction. This device is furnished with accessories for filtration (FilterEx-12), evaporation (EvaporEx-12) and software (Easywave), and the microwave oven incorporates a vacuum system (Milestone Vac 2000).

Several special materials for microwaves, e.g. Teflon pumps, microfilters, vials for filtration, weflon stirrers, etc., were provided for the Milestone Ethos Sel. A system for Soxhlet extraction (Selecta, Spain), a vacuum manifold (Whatman, Middlesex, England) and a rotatory evaporator (Heidolph, Nüremberg, Germany) were also used.

Separation of different PAHs by gas chromatography and mass spectrometry detection

By using a standard sample of PAHs containing the 16 USEPA pollutants, chromatographic separation was carried out with the internal standard (IS) technique in order to correct any possible instrumental drift. The deuterated homologous of PAHs (naftalene-d_8, acenaphtene-d_{10}, phenantrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}) were used as IS. These compounds are not present in real samples, and they have physical and chemical properties similar to those analytes providing interference-free GC-MS signals.

The mass-spectrum of perfluorotributylamine was used for a wide mass-calibration range. Detection was carried out using the selection ion monitorization (SIM) mode, by measuring only the m/z ratio of the most abundant molecular fragments.

Table I shows retention times of the main and secondary ions of PAH and internal standards monitored, selected for each ion.

TABLE I. RETENTION TIMES AND MAIN AND SECONDARY IONS OF PAHs AND INTERNAL STANDARDS MONITORED SELECTED FOR EACH ION

Retention times (min)	Compound	Main ion	Secondary ion
10.59	Naphtalene d-8	136	----
10.65	Naphtalene	128	129
15.52	Acenaphthylene	152	153
15.98	*Acenaphthene d-10*	164	----
16.77	Acenaphthene	154	153
17.76	Fluorene	166	165
20.68	*Phenanthrene d-10*	188	----
20.75	Phenanthrene	178	179
20.95	Anthracene	178	179
24.55	Fluoranthene	202	101
25.24	Pyrene	202	101
29.19	Benzo (a) anthracene	228	226
29.25	*Chrisene d-12*	240	----
29.35	Chrisene	228	226
35.03	Benzo (b) fluoranthene	252	253
35.87	Benzo (k) fluoranthene	252	253
37.34	Benzo (a) pyrene	252	253
37.81	*Perylene d-12*	264	----
46.97	Indene (1,2,3-cd) pyrene	276	138
47.14	Dibenzo (a,h) anthrathene	278	139
49.80	Benzo (g,h,i) perylene	276	138

Extraction, filtration and preconcentration of PAHs from the soil samples procedure

The extraction of 16 PAHs from soil samples was carried out in the Milestone Ethos Sel microwave oven. One of the 12 vessels, with the same volume of solvent as the samples, was aimed at measuring the applied temperature. The vial of filtration belonging to each vessel was of use to test a microfilter, a performance disc (for microfilter scaling), a coated magnet on quartz and the base of the vial made from weflon. Two grams (2.0000 g) of sample soil were weighed and deposited in the filtration vial and then 25 mL of a hexane/acetone 1:1 mixture was added to the filtration vial allowing 10 mL of the mixture to remain in the extraction vessel. Then the extraction vessels were opened, the microwave power was set to 500 W, the stirring to 100 % and the system was started following the optimized program. A temperature of 120 ^{o}C was selected in two steps, a first step from room temperature to 120 ^{o}C for 10 min, and then temperature was maintained for another 10 min.

When the extraction program was finished and temperature was lower than 40 ^{o}C (after approximately 20 min), the filtration vessels were opened and the filtration was carried out using the FilterEx-12 system by connecting the vacuum pump. After filtration, the vials were coupled to the EvapEx-12 evaporation system and this set was introduced into the oven. Then, the microwave power was tuned to 135 W, while a vacuum was applied simultaneously. In 14.5 min the volume was reduced to 2 mL. This extract was cleansed by solid phase extraction (SPE) using two coupled silica gel cartridges, previously conditioned with hexane. The PAHs were retained there and they were eluted by 5 mL (two times) of a hexane/acetone 7:3 mixture. This extract was pre-concentrated again to 2 mL by evaporation (applying the vacuum commercial system to 135 W of power during 12 min). After the adequate addition of internal standards and apposite dilutions (1 preconcentrate:1 hexane) dilution for naphthalene, acenaphthylene, acenaphthene, fluorene, and dibenzo(a,h)anthracene determination; (1 preconcentrate:10 hexane) dilution for phenanthrene, anthracene, pyrene, venzo(a)anthracene, indene(1,2,3-cd)pyrene, and venzo(g,h,i)perylene determination; and (1 preconcentrate:50 hexane) dilution for fluorantene, chrysene, venzo(b,k)fluorantene, and venzo(a)pyrene determination, the resulting treated and diluted sample solutions were injected in the gas chromatograph.

RESULTS AND DISCUSSION

Optimization of the microwave-assisted solvent extraction

The optimum experimental parameters for the extraction of PAHs by MASE were investigated by using a sample soil contaminated with these pollutants.

Extraction temperature

Temperatures of 80, 100, 115, 120, 120* and 130* ^{o}C were selected according to the literature (López-Ávila et al. 1994, Barnabas et al. 1995, Chee et al. 1996). The asterisks mean that in such cases the applied temperature program was carried out as follows: a first step from room temperature to final temperature (10 min) and then the final temperature was maintained for 10 min. The rest of the parameters (extraction time, volume of the solvent and solvent) were maintained constantly.

Table II shows the concentrations for the 16 studied PAHs according to the applied temperatures with the exception of dibenzo(a,h)anthracene. As can be seen, for PAHs containing 2-4 aromatic rings, better extraction conditions were obtained by applying the extraction program in two steps at 120* ^{o}C, being 100 ^{o}C the best option for PAHs with 5-7 aromatic rings. In subsequent studies, we selected a temperature of 120* ^{o}C in two steps, as a compromise solution. This temperature allows for the extraction of higher amounts of most PAHs, especially the most volatile ones (with lower molecular mass), which can be found in soils at lower concentrations.

Extraction time

A extraction temperature of 120 ^{o}C (*) in two steps was selected (first, from room temperature to 120 ^{o}C in 10 min for all cases, and a second step at 120 ^{o}C for 5, 10, 15 and 20 min), and the same parameters referred in the previous paragraph were maintained constantly. The obtained results are shown in **table III**. As can be seen, similar considerations as those formulated in the study of temperature can be applied: for compounds having 2-4 aromatic rings in their molecule, the optimum extraction time was 20 min at 120 ^{o}C, but for PAHs with 5-7 aromatic rings, the time was 15 min, although differences of concentrations for both times are not significant as has been mentioned in the literatures. Budzinski et al. (1999) and Letelier and Budzinski (1999b) have in mind the same criterion for the study of the temperature. A compromise solution of 20 min as extraction time in the second step was selected.

TABLE II. CONCENTRATIONS OF PAHs (mg/kg) IN A CONTAMINATED SAMPLE OF SOIL USING MASE EXTRACTION ACCORDING WITH VARYING TEMPERATURE

PAH	80 ºC (1*) (n=3)			100 ºC (1*) (n=3)			115 ºC (1*) (n=3)			120 ºC (1*) (n=3)			120 ºC (2*) (n=3)			130 ºC (2*) (n=3)		
	Mean	S	RSD%	Mean	S	RSD%	Mean	S	RSD%	Mean	S	RSD%	Mean	S	RSD%	Mean	S	RSD%
Naphthalene	8.4	1	14	11	0.2	2	11	2	18	7	0.4	6	12	0.1	0.7	11	0.6	6
Acenaphthylene	0.9	0.5	57	0.8	0.3	35	2	1	10	3	0.2	5	2	0.7	33	0.9	0.1	2
Acenaphthene	7	1	15	7	0.1	1	7	2	26	4	0.1	1	9	0.4	5	7	0.2	3
Fluorene	23	2	10	22	1	5	23	3	13	15	0,2	1	29	4	13	19	2	10
Phenanthrene	20	8	40	17	1	7	16	2	13	9	0.3	3	17	2	12	12	1	7
Anthracene	116	25	22	112	24	21	218	126	56	65	0.1	1	242	53	16	454	17	4
Fluoranthene	29	9	32	22	3	14	23	4	17	12	1	8	25	2	9	15	2	10
Pyrene	22	7	32	17	2	12	15	3	17	9	1	7	20	2	10	12	1	9
Benzo(a)anthracene	59	20	35	51	6	12	34	2	6	25	7	27	50	1	2	32	3	9
Chrysene	78	31	40	104	2	2	123	56	40	56	6	10	198	11	6	126	9	7
Benzo(b)fluoranthene	79	34	43	72	1	1	57	25	44	64	8	12	41	3	8	23	1	3
Benzo(k)fuoranthene	157	68	43	233	38	16	188	59	31	215	54	25	167	20	12	130	13	11
Benzo(a)pyrene	70	32	46	73	2	2	60	21	35	63	8	13	46	4	8	27	1	5
Indeno(1,2,3-cd)pyrene	28	13	47	31	2	6	27	12	44	28	9	32	20	1	6	12	1	6
Dibenzo(a,h)anthracene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d
Benzo(g,h,i)perylene	272	142	52	374	182	49	318	194	81	400	152	38	201	3	2	179	13	7
Total PAH	970	388	40	1154	130	12	1123	149	13	976	242	35	1179	58	8	1059	31	3

S = Standard deviation. RSD% = Relative standard deviation. n.d. = not detected. (1*) One step temperature program. (2*) Two step temperature program

TABLE III. CONCENTRATIONS OF PAHs (mg/kg) IN A CONTAMINATED SAMPLE OF SOIL USING THE MASE EXTRACTION WITH VARYING TIMES

PAH	15 min (n=3)			20 min (n=3)			25 min (n=3)			30 min (n=3)		
	Mean	S	RSD%	Mean	S	RSD%	Meab	S	RSD%	Mean	S	RSD%
Naphthalene	12.4	1.5	11.8	12.2	0.1	0.7	12.2	0.3	2.1	12.1	0.5	4.5
Acenaphthylene	0.5	0.3	72	2.1	0.7	12.2	0.3	2.1	12.1	21	0.5	4.5
Acenaphthene	7.6	0.5	6.2	8.6	0.4	4.9	9.2	0.7	7.2	8.7	0.3	4.0
Fluorene	23.5	1.1	4.8	29	4	13	26	1.3	4.9	28	7	25
Phenanthrene	16.3	0.5	3.3	17	2	12	16	2	13	15	3	21
Anthracene	219	124	57	342	53	16	314	17	5	270	10	4
Fluoranthene	24	2	8	25	2	9	25	3	13	22	3	15
Pyrene	18.6	1.7	9	20	2	10	10	2	12	17	3	16
Benzo(a)anthracene	53	10	19	50	1.2	2.3	49	7	12	27	11	42
Chrysene	108	39	36	190	11	6	172	31	18	116	26	22
Benzo(b)fluoranthene	56	23	42	41	3	8	39	1.5	4	38	3	9
Benzo(k)fuoranthene	160	26	18	167	20	12	177	8	3	157	25	16
Benzo(a)pyrene	57	14	25	45	4	8	45	4	8	43	3	7
Indeno(1,2,3-cd)pyrene	23	4	19	20	1.2	6.2	20	0.9	4.5	20	3	14
Dibenzo(a,h)anthracene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Benzo(g,h,i)perylene	353	40	11	201	3	2	207	18	9	215	29	13
Total PAH	1143	144	13	1179	58	5	1124	61	5	991	62	6

S = Standard Deviation. RSD% = Relative standard deviation. n.d. = not detected

Volume of solvent

The volume of solvent used for MASE is another parameter that has an effect on the extraction of PAHs from soil samples. In order to analyze its influence on the extraction of analytes by using an hexane/acetone 1:1 mixture as extraction solvent, several volumes (20, 25, 30, and 35 mL) of such mixture were used under two conditions: (1) the volume of solvent must be enough to cover the soil sample and (2) 10 mL of this volume must be added to the extraction vessel to secure the direct contact of the sensor with the solution, and the rest 10, 15, 20 and 25 mL should be added to the filtration vial. The capacity of the vials (26 mL) limits the use of a higher volume. Obviously, for the reduction to a final volume of 2 mL by using microwave energy (135 W) and vacuum together, different time lapses must be applied: 12 min for 20 mL, 14.5 min for 25 mL, 17 min for 30 mL and 20 min for 35 mL. During this study, the rest of the parameters remained constant: 2.0000 g of contaminated soil, 120 °C as extraction temperature in two steps: 20 min as extraction time, 500 W for the microwave power supply, stirring 100 % and 20 min as a final cooling step. **Table IV** shows the concentrations of PAHs found on the sample soil when different volumes of solvent mixture were used for the extraction. Again, slight differences in concentration values for different volumes of solvent were observed, thus it can be said that the volume of solvent is not an important factor for the extraction of PAHs from soil samples, as has been previously mentioned (Barnabas et al. 1995, Chee et al. 1996). For subsequent experiments, a volume of 25 mL of a hexane/acetone 1:1 mixture, added into the filtration vial, was selected as the optimum alternative for the majority of PAHs.

Choice of solvent

Acetone has been recommended as a more suitable solvent than hexane for the extraction of PAHs from soil samples (Barnabas et al. 1995). The acetone molecule has a permanent dipole that can induce dipole-dipole interactions with numerous π-type electrons from PAHs. On the other hand, hexane is a non-polar solvent, which means that it is unable to generate such interactions. We have analyzed whether there is a difference between using a mixture of hexane/acetone and acetone alone for the extraction. Once the previous parameters were optimized, the results obtained by using the hexane/acetone mixture were compared with those obtained by using acetone with the same purposes. In this case, the constant parameters were 20 min as extraction time, 500 W for the microwave power supply, stirring 100 % and 20 min as a final cooling step. The results obtained are shown in **table V,** where we can verify that the

TABLE IV. CONCENTRATIONS OF PAHs (mg/kg) IN A CONTAMINATED SAMPLE OF SOIL USING THE MASE EXTRACTION ACCORDING TO VOLUME OF SOLVENT SELECTED

PAH	20 mL Hexane/Acetone (1:1) (n=3)			25 mL Hexane/Acetone (1:1) (n=3)			30 mL Hexane/Acetone (1:1) (n=3)			35 mL Hexane/Acetone (1:1) (n=3)		
	Mean	S	RSD%	Mean	S	RSD%	Meab	S	RSD%	Mean	S	RSD%
Naphthalene	12.4	0.7	5.6	12.2	0.1	0.7	14	3	10	12.4	0.3	2.5
Acenaphthylene	0.4	0.1	32	2.1	0.7	32	0.5	0.1	16	1.1	0.7	60
Acenaphthene	8.5	0.3	2.1	8.6	0.4	4.9	8.2	0.3	4.1	9.4	6.4	4.4
Fluorene	23	0.5	2	29	4	13	25	0.9	3.7	24	1	4
Phenanthrene	14.5	1.0	6.8	17	2	12	17	5	29	17	3	9
Anthracene	328	18	6	342	53	16	274	21	8	200	81	41
Fluoranthene	21	2	8	25	2	9	23	4	16	23	3	12
Pyrene	16.5	1.3	7.7	20	2	10	18	3	14	18	2	13
Benzo(a)anthracene	45.2	1	2	50	1.2	2.3	39	6	18	49	12	20
Chrysene	142	7	5	206	12	6	137	14	10	126	40	12
Benzo(b)fluoranthene	29	3	10	41	3	8	36	6	17	35	5	14
Benzo(k)fuoranthene	177	21	12	176	20	12	214	43	23	187	7	4
Benzo(a)pyrene	38	4	10	46	4	8	46	8	17	44	4	9
Indeno(1,2,3-cd)pyrene	16	0.3	1.7	20	1.2	6.2	19	2	13	17	2	12
Dibenzo(a,h)anthracene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Benzo(g,h,i)perylene	283	24	8	200	3	2	264	44	12	306	32	10
Total PAH	1154	7	1	1193	58	5	1135	106	9	1068	128	12

S = Standard deviation. RSD% = Relative standard deviation. n.d. = not detected

TABLE V. CONCENTRATIONS OF PAHs (mg/kg) IN A CONTAMINATED SAMPLE OF SOIL USING THE MASE EXTRACTION WITH VARYING SOLVENTS

PAH	Acetone (n=3)			Hexane/Acetone (1:1) (n=3)		
	Mean	S	RSD%	Mean	S	RSD%
Naphthalene	8.8	0.7	7.9	12.2	0.1	0.8
Acenaphthylene	2.4	0.1	2.5	2.1	0.7	33
Acenaphthene	6.8	0.1	0.7	8.6	0.4	4.9
Fluorene	20	1.7	8.5	29	4	13
Phenanthrene	15	2.0	3.0	17	2	12
Anthracene	376	58	15	342	53	15
Fluoranthene	21	2	8	25	2	9
Pyrene	16.1	1.3	8.1	20	2	10
Benzo(a)anthracene	64	23	36	50	1.2	2.4
Chrysene	114	23	20	206	12	6
Benzo(b)fluoranthene	26	3	12	41	3	7
Benzo(k)fuoranthene	91	10	11	176	20	11
Benzo(a)pyrene	31	1.4	4.5	46	4	9
Indeno(1,2,3-cd)pyrene	16	2	13	20	1.2	6
Dibenzo(a,h)anthracene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Benzo(g,h,i)perylene	211	32	15	200	3	2
Total PAH	1018	131	13	1193	58	5

S = Standard deviation. RSD% = Relative standard deviation. n.d. = not detected mixture hexane/acetone is more suitable for PAHs extraction from soil samples, which agrees with prior research (López-Ávila et al. 1994, Chee et al. 1996).

Analytical performance characteristics

To evaluate the linearity of detector responses after MASE-GC-MS, standard solutions containing all 16 PAHs in a range of concentrations between 35 and 2000 ng/mL were prepared. The results are summarized in **table VI**. For all PAHs, the responses were linear over the range tested, with regression coefficients better than 0.9940 (n = 3). The linear range was established by plotting the quotient area of the analytic/area of IS vs. the quotient concentration of analyte/concentration of IS. The limits of detection (LOD) and limits of quantification (LOQ) were calculated from equations defining calibration lines by measuring (by triplicate) three solutions containing 16 PAHs in very low concentrations (35, 155 and 254 ng/mL) with the Long and Winefordner (1985) criterion. The values found were 10-32 ng/mL for LOD and 32-108 ng/mL for LOQ, which shows that quantification should be possible for PAHs at levels found in soils and sediments (Popp et al. 1997, Saim et al. 1997, Schantz et al. 1997). Repeatability, which was evaluated for two levels of concentrations (155 and 531 ng/mL), with and without IS, was satisfactory with standard relative deviations (RDS) between 1% and 6% if IS was employed.

The analytical advantages of the MASE-GC-MS procedure were previously evaluated in samples of polluted soil collected near a coke oven by comparing the observed results with those obtained for the PAHs of such samples previously extracted by using the classical Soxhlet method recommended by the USEPA(1995). In this latter method, the soil amount was 5.0000 g, the volume of the solvent used was 350 mL of a 1:1 mixture of hexane/acetone, and the extraction time was 18 hours at the rate of 3-4 cycles per hour. The cleaning and pre-concentration of extracts were carried out by rotoevaporation-SFE-rotoevaporation. Then the cleaned extracts were analyzed by GC-MS. The results obtained for both PAHs extraction methods under scrutiny (MASE and Soxhlet) in mg of PAH/kg of soil are shown in **table VII**. As can be seen, both extraction techniques provided similar values for the majority of PAHs with the exception of chrysene, benzo(b)fluoranthene and benzo(k)fluoranthene.

Validation of the MASE method and real sample application

In order to validate the proposed method, the new set-up was used to extract PAHs from the CRM 104-100 soil. The reference material came from Elisabeth River in the Chesapeake Bay, VA, USA, containing 16 PAHs (the values for benzo(k,b)fluoranthene and dibenzo(a,h)anthracene are only recommended). These two compounds of the reference material were extracted by both MASE and Soxhlet techniques.

Table VIII shows the certified values for different PAHs with their confidence limits; concentrations (mg/kg) of the pollutants obtained by the MASE and Soxhlet extraction methodologies; and recovery values for the different PAHs with final determination by GC-MS. In both cases, MASE and Soxhlet, we found that our values fall into the confidence limits established for the reference material. However, the recovery values obtained for PAHs turned out to be higher than those obtained with MASE (anomalous values were observed for fluoranthene (–147 %) and benzo(g,h)perylene (–124 %). Nevertheless, once clean extracts were obtained for the analysis by GC-MS, it was necessary to handle the extracts at three dilution levels: (1 preconcentrate:1 hexane) dilution for naphthalene, acenaphthylene, acenaphthene, fluorene, and dibenzo(a,h)anthracene determination; (1 preconcentrate:10 hexane) dilution for phenanthrene, anthracene, pyrene, venzo(a) anthracene, indene(1,2,3-cd)pyrene, and venzo(g,h,i) perylene determination; and (1 preconcentrate:50 hexane) dilution for fluorantene, chrysene, venzo(b,k) fluorantene, and venzo(a)pyrene determination. The necessary dilutions are the main drawback of the GC-MS determination, but they are necessary following both PAHs extraction methods.

Figure 1 shows the chromatogram of PAHs contained in the CRM104-100 soil extract using MASE as the extraction technique and 1:10 dilution.

CONCLUSION

This research demonstrated that microwave-assisted solvent extraction (MASE) was the best alternative for conventional sample preparation to extract PAHs from polluted soil samples for final GC-MS determinations. The main advantages of the proposed extraction technique for PAHs are: i) the smaller size of the sample (2 g for MASE vs. 5 g for Soxhlet); ii) substantial smaller volume of solvent extraction (25 mL for MASE vs. 350 mL for Soxhlet); iii) and substantial saving of time (40 min vs. 24 h). Moreover, this technique allows for simultaneous operation of twelve samples. On the other hand, the validation results and limits of quantification achieved for all the PAHs by GC-MS, after purification and pre-concentration of

TABLE VI. CALIBRATION EQUATIONS, CORRELATION COEFFICIENTS, LIMITS OF DETECTION (L.D.), LIMITS OF QUANTIFICATION (L.Q.), WITH OR WITHOUT INTERNAL STANDARD (IS) FOR 16 PAH UNDER STUDY

PAH	Calibration Equation	R^2	L.D. ng/mL	L.Q. ng/mL	[1] RSD% (n=3) With IS	[1] RSD% (n=3) Without IS	[2] RSD% (n=3) With IS	[2] RSD% (n=3) Without IS
Naphthalene	$y=0.0281+0.7038x$	0.9983	10	32	1.5	6.5	0.6	3.3
Acenaphthylene	$y=0{,}0954+1.0932x$	0.9945	25	82	0.5	5.9	0.8	4.6
Acenaphthene	$y=0.0431+0.6895x$	0.9965	15	53	0.7	6.7	0.5	4.3
Fluorene	$y=0.0462+0.7576x$	0.9978	20	68	6.8	0.9	0.3	4.0
Phenanthrene	$y=0.0455+0.7220x$	0.9958	17	58	0.5	1.2	0.3	3.9
Anthracene	$y=0.0602+1.4182x$	0.9952	15	51	0.3	1.0	1.2	6.4
Fluoranthene	$y=0.0442+0.7758x$	0.9977	22	72	0.6	1.4	0.6	4.2
Pyrene	$y=0.0370+0.8292x$	0.9983	16	54	9.3	1.0	3.9	3.7
Benzo(a)anthracene	$y=0.0181+0.5805x$	0.9990	25	84	1.0	2.9	3.3	7.4
Chrysene	$y=0.0759+0.2998x$	0.9946	11	38	0.5	6.1	2.3	6.1
Benzo(k,b)fluoranthene	$y=0.0210+1.5101x$	0.9995	28	86	0.8	4.3	2.6	9.3
Benzo(a)Pyrene	$y=-0.0090+0.6441x$	0.9977	25	62	1.3	5.0	1.8	13.7
Indeno(1,2,3-cd)pyrene	$y=-0.0103+0.5432x$	0.9952	32	106	4.1	7.7	6.1	18.2
Dibenzo(a,h)anthracene	$y=-0.0072+0.3546x$	0.9952	25	85	3.2	6.9	3.7	12.8
Benzo(g,h,i)perylene	$y=-0.0111+0.5447x$	0.9947	32	108	4.2	7.6	6.0	18.1

"y" = peak area. "x" = concentration of PAH in ng/mL

[1] Standard Solution of 155 ng/mL

[2] Standard Solution of 531 ng/mL

TABLE VII. MEASURED CONCENTRATIONS OF PAHs (mg/kg) IN A CONTAMINATED SAMPLE OF SOIL USING THE SOXHLET AND OPTIMIZED MASE EXTRACTIONS

PAH	Soxhlet extraction n=3			MASE n=3		
	Mean	S	RSD%	Mean	S	RSD%
Naphthalene	10.50 ± 0.09	0.03	0.27	12.15 ± 0.10	0.1	0.72
Acenaphthylene	2.60 ± 0.61	0.2	8.1	2.1 ± 0.7	0.7	32.8
Acenaphthene	10.20 ± 0.61	0.2	2.0	8.6 ± 0.5	0.4	4.9
Fluorene	21.80 ± 2.74	0.9	4.0	29 ± 4	4	13
Phenanthrene	14.20 ± 2.73	0.9	6.1	17 ± 2	2	12
Anthracene	328 ± 6	2	1	342 ±53	53	16
Fluoranthene	27 ± 6	2	9	25 ± 2	2	9
Pyrene	21 ± 6	2	9	20 ± 2	2	10
Benzo(a)anthracene	56 ± 30	10	17	50.3 ± 1.2	1.2	2.3
Chrysene	50 ± 12	4	7	198 ±11	11	6
Benzo(b)fluoranthene	78 ± 9	3	4	41 ± 3	3	8
Benzo(k)fuoranthene	166 ± 6	2	1	167 ±20	20	12
Benzo(a)pyrene	101 ± 9	3	1	45 ± 4	4	8
Indeno(1,2,3-cd)pyrene	38.4 ± 0.6	0.2	0.4	20.0 ± 1.2	1.2	6.2
Dibenzo(a,h)anthracene	n.d.	n.d	n.d.	n.d.	n.d.	n.d.
Benzo(g,h,i)perylene	515 ± 130	43	8	201 ± 3	3	2
Total PAH	1440	63	4	1179	58	5

n.d. = not detected

TABLE VIII. CERTIFIED VALUES, FOUND CONCENTRATIONS (mg/Kg) AND RECOVERIES FOR PAHs IN REFERENCE SOIL SAMPLE CRN 104-100 BY GC-MS AFTER SOXHLET AND MASE EXTRACTION

PAH	Concentration (mg/Kg)			Recovery (%)	
	Certified Value	Soxhlet (n=3)	MASE (n=3)	Soxlet (n=3)	MASE (n=3)
Naphthalene	0.77 ± 0.18	0.78 ± 0.04	0.70 ± 0.06	101 ± 3	91 ± 3
Acenaphthylene	1.21 ± 0.39	1.18 ± 0.01	1.08 ± 0.05	97 ± 1	89 ± 1
Acenaphthene	0.77 ± 0.10	0.71 ± 0.02	0.53 ± 0.09	92 ± 2	69 ± 4
Fluorene	0.65 ± 0.09	0.62 ± 0.01	0.48 ± 0.12	96 ± 1	73 ± 2
Phenanthrene	5.79 ± 0.86	6.32 ± 0.10	5.33 ± 0.03	109 ± 2	92 ± 1
Anthracene	1.44 ± 0.29	1.52 ± 0.01	0.70 ± 0.09	105 ± 3	48 ± 3
Fluoranthene	24.8 ± 4.34	36.6 ± 3.0	27.5 ± 0.05	147 ± 8	111 ± 4
Pyrene	15.0 ± 3.41	16.5 ± 0.01	15.5 ± 0.08	110 ± 2	103 ± 6
Benzo(a)anthracene	7.98 ± 1.30	9.50 ± 0.10	9.27 ± 0.11	118 ± 1	116 ± 4
Chrysene	8.60 ± 1.11	9.06 ± 0.01	8.26 ± 0.14	105 ± 2	96 ± 3
Benzo(b+k)fluoranthene	14.79 *	11.37 ± 0.30	7.69 ± 0.20	77 ± 3	52 ± 4
Benzo(a)pyrene	5.09 ± 0.77	5.45 ± 0.90	3.56 ± 0.16	107 ± 1	70 ± 3
Indeno(1,2,3-cd)pyrene	4.46 ± 1.01	5.02 ± 0.90	4.09 ± 0.10	113 ± 1	92 ± 2
Dibenzo(a,h)anthracene	1.55 *	1.7 ± 0.2	1.17 ± 0.14	111 ± 2	75 ± 1
Benzo(g,h,i)perylene	3.58 ± 0.98	4.45 ± 0.47	3.52 ± 0.39	124 ± 4	98 ± 3
Total PAH	94.73	110.68	89.36	117	94

*Recommended value

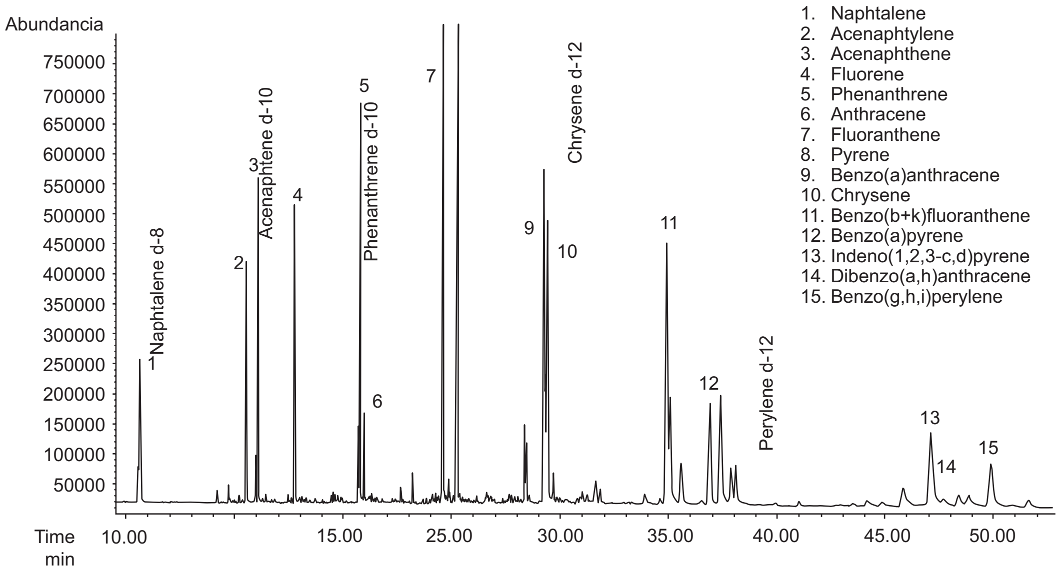

Fig. 1. Chromatogram from the CRM 104-100 soil extract after Microwave Assisted System Extraction (Dilution 1:10)

extracts, clearly reveals that such determination in soil by sample preparation and pre-concentration is reliable. Finally, good recoveries (over 70 %) were attained for the vast majority of PAHs scrutinized (except for anthracene and benzo(b+k)fluoranthene), and the repeatability is also satisfactory when calibrations were done by using internal standards.

ACKNOWLEDGMENTS

The authors thank FICYT (Asturias, Spain) for the financial support under the framework of the Project Ref. PC-CIS01-20. We also thank E. Fernández (from Cogersa, Asturias, Spain) for the assistance provided during the realization of this work.

REFERENCES

Andersson T., Hartonen K., Hyötiläinin T. and Riekkola M.L. (2002). Pressurised hot water extraction and thermal desorption of polycyclic aromatic hydrocarbons from sediment with use of a novel extraction vessel. Anal. Chim. Acta 466, 93-97.
DOI: 10.1016/S0003-2670(02)00543-3

Barnabas I.J., Dean J.R., Fowlin I.A. and Owen S.P. (1995). Extraction of polycyclic aromatic hydrocarbons from highly contaminated soils using microwave energy Analyst 120, 1897-1901.
DOI: 10.1039/AN9952001897

Budzinski H., Letellier M., Garrigues P. and Le Menach K. (1999). Optimisation of the microwave-assisted extraction in open cell of polycyclic aromatic hydrocarbons from soils and sediments: Study of moisture effect. J. Chromatogr. A 837, 187-192.
DOI: 10.1016/S0021-9673(99)00067-9

Bouzige M., Pichon V. and Hennion M.C. (1999). Class-selective immunosorbent for trace-level determination of polycyclic aromatic hydrocarbons in complex sample matrices, used in off-line procedure or on-line coupled with liquid chromatography/fluorescence and diode array detections in series. Environ. Sci. Technol. 33, 1916-1921.
DOI: 10.1021/es9810311

Cejpec K., Hajslovà J., Jehlickovà Z. and Merhaut J. (1995). Simplified extraction and cleanup procedure for the determination of PAHs in fatty and protein-rich matrices. Int. J. Environ. Anal. Chem. 61, 65-69.
DOI: 10.1080/03067319508026237

Chee K.K., Wong M.K. and Lee H.K. (1996). Optimization of microwave-assisted solvent extraction of polycyclic aromatic hydrocarbons in marine sediments using a microwave extraction system with high-performance liquid chromatography-fluorescence detection and gas chromatography-mass spectrometry. J. Chromatogr. A 723, 259-265.
DOI: 10.1016/0021-9673(95)00882-9

Ganzler K., Salgo A. and Valko K. J. (1986). Microwave extraction : A novel sample preparation method for chromatography. J. Chromatogr. A 371, 299-305.
DOI: 10.1016/S0021-9673(01)94714-4

Lara-Gonzalo A. (2015). Análisis forense ambiental de la contaminación multicomponente de un terreno industrial abandonado. Ph.D. thesis. Universidad de Oviedo, 178-179.

Letellier M. and Budzinski H. (1999a). Microwave assisted extraction of organic compounds. Analusis 27, 259-263. DOI: 10.1051/analusis:1999116

Letellier M. and Budzinski H. (1999b). Influence of sediment grain size on the efficiency of focused microwave extraction of polycyclic aromatic hydrocarbons. Analyst 124, 5-11. DOI: 10.1039/A807482H

Librando V., Hutzinger O., Tringali G. and Aresta M. (2004). Supercritical fluid extraction of polycyclic aromatic hydrocarbons from marine sediments and soil samples. Chemosphere 54, 1189-1191. DOI: 10.1016/j.chemosphere.2003.07.008

Long G.L. and Winefordner J.D. (1983). Limit of detection. A closer look at the IUPAC definition. Anal. Chem. 55, 712-724. DOI: 10.1021/ac00258a001

López-Ávila V., Young R. and Beckert W.F. (1994). Microwave-assisted extraction of organic compounds from standard reference soils and sediments. Anal. Chem. 66, 1097-1102. DOI: 10.1021/ac00079a027

Mahafley W.R., Gibson D.T. and Cernighia C.E. (1988). Bacterial oxidation of chemical carcinogens: formation of polycyclic aromatic acids from benz[a]anthracene. Appl. Environ. Microb. 54, 2415-2418.

Manoli E. and Samara C. (1999). Polycyclic aromatic hydrocarbons in natural waters: sources, occurrence and analysis. TRAC-Trend. Anal. Chem. 18, 417-423. DOI: 10.1016/S0165-9936(99)00111-9

Menzie C.A., Potcki B.B. and Santodonato J. J. (1992). Exposure to carcinogenic PAHs in the environment. Environ. Sci. Technol. 26, 1278-1284. DOI: 10.1021/es00031a002

Moore S.W. and Ramamoorthy A. (1994). Organic chemicals in natural waters – Applied monitoring and impact assessment. Springer Verlag, N.Y., 292 pp. DOI: 10.1007/978-1-4613-9538-6

Pino V., Ayala J. H., Afonso A.M. and González V. (2001). Ultrasonic micellar extraction of polycyclic aromatic hydrocarbons from marine sediments. Talanta 54, 15-22. DOI: 10.1016/S0039-9140(00)00622-6

Popp P., Keil P., Moder M., Paschke A. and Thuss U.J. (1997). Application of accelerated solvent extraction followed by gas chromatography, high-performance liquid chromatography and gas chromatography–mass spectrometry for the determination of polycyclic aromatic hydrocarbons, chlorinated pesticides and polychlorinated dibenzo-p-dioxins and dibenzofurans in solid wastes. J. Chromatogr. A 774, 203-207. DOI: 10.1016/S0021-9673(97)00337-3

Richer B.E., Jones B.A., Ezzell J.L. and Poter N.L. (1996). Accelerated solvent extraction: A technique for sample preparation. Anal. Chem. 68, 1033-1038. DOI: 10.1021/ac9508199

Saim N. Dean J.R., Abdullah M.P. and Zakaria Z. (1997). Extraction of polycyclic aromatic hydrocarbons from contaminated soil using Soxhlet extraction, pressurised and atmospheric microwave-assisted extraction, supercritical fluid extraction and accelerated solvent extraction. J. Chromatogr. A 791, 361-366. DOI: 10.1016/S0021-9673(97)00768-1

Schantz M.M., Nichols J.J. and Wise S.A. (1997). Evaluation of pressurized fluid extraction for the extraction of environmental matrix reference materials. Anal. Chem. 69, 4210-4213. DOI: 10.1021/ac970299c

Song Y.F., Jing X., Fleischmann S. and Wilke B.M. (2002). Chemosphere 48, 993-998.

USEPA (1986). Method 8100. Determination of polycyclic aromatic hydrocarbons in soil waste by gas chromatography. US Environmental Protection Agency, Washington DC.

USEPA (1995). Test Methods for evaluating solid waste. Method 3540 C. Soxhlet extraction. Washington DC.

Wild S.R and Jones K.C. (1995). Polynuclear aromatic hydrocarbons in the United Kingdom environment: A preliminary source inventory and budget. Environ. Pollut. 88, 91-95. DOI: 10.1016/0269-7491(95)91052-M