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Simultaneous determination of volatile and semi-volatile aromatic hydrocarbons in virgin olive oil by headspace solid-phase microextraction coupled to gas chromatography/mass spectrometry

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Abstract

A reliable, simple and relatively fast method for the simultaneous determination of volatile and semi-volatile aromatic hydrocarbons in virgin olive oil was developed, based on headspace solid-phase microextraction (HS-SPME). The investigation regarded eco-contaminants such as alkylated monoaromatic hydrocarbons from C1- to C4-benzenes and light polyaromatic hydrocarbons up to four aromatic rings. Sampling and chromatographic conditions were optimized by using standard solutions in deodorized olive oil and the analytical performances of the method were determined. The proposed method was then applied to real samples of virgin olive oil were the target hydrocarbons could be identified and quantified. Several of them had not been previously quantified in virgin olive oil. Moreover, by the analysis of olive oil samples an additional number of C4-benzenes could be tentatively identified.

Keywords: Volatile aromatic hydrocarbons; Light polycyclic aromatic hydrocarbons; Contaminants; Virgin olive oil; Solid-phase microextraction; SPME; Gas chromatography; Mass spectrometry

1. Introduction

A wide number of mono- and polyaromatic hydrocarbons are well known as ubiquitous contaminants and several of them are considered as priority environmental pollutants by the Environmental Protection Agency (EPA) [1]. Monoaromatic hydrocarbons (MAHs), comprising benzene and alkyl benzene homologues, as well as polyaromatic hydrocarbons (PAHs) are mainly emitted into the atmosphere by fuel oils spill, combustion and evaporation, by vehicular and industrial emissions and geochemical processes [2–4]. MAHs, in particular benzene, toluene, ethylbenzene, xylenes (collectively known as BTEX) also widely occur in industrial solvents, paints and chemical products [4,5], and PAHs are formed in incomplete combustion and pyrolysis of several forms of organic matters [6].

In terms of safety of aromatic hydrocarbons, certain carcinogenic, immunological, reproductive, fetotoxic, and genotoxic effects have been associated with some MAH compounds [7,8]; 1,3,5-trimethylbenzene is considered as a severe systemic toxic [9]; tetramethylbenzenes possess genotoxic and mutagenic effect [10] and finally PAHs are known to be human carcinogens [11].

Human exposition to these compounds mainly occurs by inhalation, but they also pollute the food chain and are ingested by the consumer. The high solubility of aromatic hydrocarbons in organic rather than in aqueous matrix leads to their accumulation in edible oils and fats, that may be contaminated by environmental pollution and processes prior to

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refining. For the absence of refining processes which lead to a decrease of total volatile compounds, virgin olive oil could maintain higher levels of volatile contaminants. For this reason, the assessment of volatile contaminants in olive oil deserves a special attention. Nine monoaromatic hydrocarbons were identified for the first time in virgin olive oil in 1984 [12] and some studies were carried out to assess BTEX levels in virgin olive oil, related with air contamination and extraction process [13–15]. As well, due to the absence of refining process, in virgin olive oil have been observed higher levels of light PAHs than in other vegetable oils [16]. Distinct techniques have been employed for the determination of aromatic hydrocarbons in olive oils. PAHs are most frequently extracted by means of liquid–liquid partition or solid phase extractions [6,16–18]. Concerning MAHs, few studies on their presence in virgin olive oils have been carried out employing the dynamic headspace technique [12–15]. Only Page and Lacroix [19] describe the application of the solid-phase microextraction (SPME) to assess BTEX and halogenated volatile contaminants in vegetable oils.

In the present work, headspace solid-phase microextraction (HS-SPME) was proposed for the simultaneous determination of volatile and semivolatile aromatic hydrocarbons in virgin olive oil, where they can be especially abundant for the absence of deodorizing and refining processes. The investigation regarded alkylated monoaromatic hydrocarbons from C1- to C4-benzenes and light polyaromatic hydrocarbons up to four aromatic rings. Sampling and chromatographic conditions were optimized by means of using standard solutions in deodorized olive oil and the developed method was then applied to real samples of virgin olive oil.

2. Experimental

2.1. Reagents and standards

Ethylbenzene, *o*-, *m*- and *p*-xylene, 1,3,5-, 1,2,3- and 1,2,4-trimethylbenzene, 2-and 3-ethyltoluene, butylbenzene, 1,2,3,4-tetramethylbenzene and *p*-cymene were purchased by TCI Ltd. (Tokyo, Japan). Standard solutions of the aromatic hydrocarbon mix (acenaphthene, acenaphthylene, anthracene, fluoranthene, pyrene, fluorene, naphthalene, α-methylnaphthalene, 2-methylnaphthalene), ethylbenzene-d10, indene, acenaphthene-d10 and phenanthrene-d10 were purchased by Supelco Ltd. (Bellefonte, PA, USA). Toluene was from Sigma–Aldrich (St. Louis, Missouri, USA).

2.2. Standard solutions

A standard solution in deodorized olive oil was prepared, at a concentration of 10 mg/L, containing toluene, C2-, C3-, and C4-benzenes and the standard polyaromatic hydrocarbon mix. Oil standard mixtures at various concentrations in the range 1–70 μg/kg were then obtained by spiking deodorized olive oil with this stock solution.

The internal standards solution of ethylbenzene-d10, indene, acenaphthene-d10 and phenanthrene-d10 was also prepared by dilution in deodorized olive oil.

2.3. SPME conditions

The SPME fibre used was a divinylbenzene/carboxen/polydimethylsiloxane 50/30 μm, 2 cm long (DVB/Car/PDMS), from Supelco Ltd.

Various sampling temperatures were tested in order to improve the extraction efficiency. Two grams of a 10 μg/kg oil standard mixture were placed into a 10 mL vial fitted with a silicone septum, then in silicon oil bath whose temperature was fixed at 40, 60, 80 and 100 °C, successively. Temperature was tested first at an arbitrary time, fixed at 30 min in order to make faster the analysis. The oil was maintained under magnetic stirring (700 rpm). After 2 min of sample conditioning, the fibre was exposed to the sample headspace during 30 min and immediately desorbed in the gas chromatograph injector. The sampling temperature of 100 °C was chosen to perform the analysis.

To determine the optimal time of exposition of the fibre to the sample headspace, the fibre was held to the headspace of the standard mixture at 100 °C for time periods of 15, 30, 45 and 60 min, and after comparison of the relative chromatographic responses, the sampling time of 60 min was chosen to perform the analysis.

2.4. GC–MS analysis

GC analyses were performed on a Agilent Technologies 6890N Network gas chromatograph coupled to a Agilent Technologies 5973 Network quadrupole mass selective spectrometer and provided with a split-splitless injection port. Helium was the gas carrier, at a linear velocity of 38 cm/s. Separation of compounds was performed on a Supelcowax-10 (Supelco Ltd., Bellefonte, PA, USA) and on a HP-5MS (Hewlett-Packard, Avondale, PA, USA) capillary columns (both 30 m × 0.25 mm I.D., 0.25 μm film thickness). Column temperature was held at 40 °C for 3 min and increased to 75 °C at 4 °C/min, then at 8 °C/min to 250 ° holding 10 min. The injector temperature was 265 °C and the time of desorption of the fibre into the injection port was fixed at 5 min.

The temperature of the ion source and the transfer line was 175 and 280 °C, respectively. Electron impact mass spectra were recorded at 70 eV ionization energy, 2 scan/s.

GC-MS analysis in the complete scanning mode (SCAN) in the 40–300 amu mass range was performed to allow the identification of compounds in samples and oil standard mixtures. Quantitative assessment of aromatic hydrocarbons was carried out in the selected ion monitoring mode (SIM) in order to improve the detection limits, and the ions analyzed were: *m/z* 91, 98 and 106 (group 1); 105, 116, 119, 120 and 134 (group 2); 128, 142 (group 3); 152, 153 and 164 (group 4); 166, 178, 188 and 202 (group 5). Base peak ions were used for quantification of compounds.

2.5. Analytical performances

Response factors and linearity were calculated by analysing oil standard mixtures at concentrations of 1, 2.5, 5, 10, 20, 40 and 70 μg/kg. Concentration of the internal standards was maintained at 10 μg/kg. To evaluate the competition effect due to the interference of sample volatiles, standard mixtures in the range 1–70 μg/kg were prepared in quadruplicate using either deodorized olive oil and virgin olive oil. The means of linear regressions slopes were then compared by the Student *t*-test.

Intra- and inter-day repeatability of the method were tested by repeating six and five times, respectively, the analysis of a 10 μg/kg oil standard mixture.

Limits of detection (LOD) and quantification (LOQ) were calculated on virgin olive oil as $LOD = 3\delta/m$ and $LOQ = 10\delta/m$, according with Long and Winefordner [20] and IUPAC [21] definitions, where δ is the standard deviation of the blank and m is the slope of the calibration curve.

Accuracy of the analytical assay was determined as the percentage of the theoretical standard hydrocarbons recovered from a virgin olive oil fortified at two concentration levels (10 and 20 μg/kg).

2.6. Distribution constants

The distribution constant between sample matrix and fibre coating (K_{fs}) was obtained as the product of the sample/headspace (K_{sh}) and the headspace/fibre (K_{hf}) distribution constants [22]. K_{sh} and K_{hf} at the equilibrium were calculated by the following expression: $K_{sh} = (A_h/V_h)/(A_s/V_s)$ and $K_{hf} = (A_f/V_f)/(A_h/V_h)$ where A_h, A_f, A_s are the chromatographic areas given by analytes determined in the headspace, in the oil sample and adsorbed on fibre, respectively; V_h, V_f, V_s are the volumes of headspace, sample and fibre, respectively. A_h was obtained by performing a static headspace (SHS) analysis of 10 μg/kg oil standard mixture by a 1 mL gas syringe (V_f), after conditioning at 100 °C; A_f was from a 10 μg/kg oil standard mixture extracted by SPME until equilibration, considering V_f to be 1 μL [23]; finally, A_s was the area obtained by direct injection of 0.02 μg of standards in hexane, considering negligible the depletion of analyte due to equilibration with headspace and fibre previously checked. V_s was of 2.22 mL and corresponded to the volume of 2 g of oil.

2.7. Olive oil samples

The SPME method was applied to 10 samples of extra virgin olive oil from local retails and producers. SPME sampling of the oils was carried out as described for standard solutions. Compounds were identified by comparison of their mass spectra and retention times with those of standard compounds or else by comparison of the mass spectrum with those of the mass spectra library Wiley 6. Moreover, Kovat's retention indices were compared with retention indices of the compounds available in the literature.

3. Results and discussion

3.1. SPME conditions

Not many studies describe the use of SPME in the quantification of volatiles in lipid samples, and in particular few data are available on the application of SPME for the quantitative determination of volatile contaminants in lipid samples. The principal difficulty of the HS-SPME analysis of lipid samples is the matrix effect, that causes the decrease of SPME efficiency. Indeed, lipid sample participates in the distribution equilibrium of volatiles as well as fibre coatings, having a high affinity with organic compounds. In order to optimize the efficiency and sensitivity of the method of analysis, is necessary to identify the most suitable SPME sampling conditions. Basically the type of fibre coating, temperature and time of extraction are the parameters to be taken into account, since in the case of lipids the amount of sample does not affect the mass of analyte absorbed by the SPME coating [19].

Among the commercially available fibres, the Carboxen-based coatings show the better efficiency for a wide number of volatile organic compounds [19,24]. In this study, the three-phases coating PDMS/Car/DVB was choosen on the basis of its affinity for compounds of both low and medium molecular weight. In fact, while Carboxen micropores are ideal for extracting small molecules, discriminating compounds with higher molecular weight, DVB mesopores are suitable for trapping analytes up to 15 carbon atoms [25].

Several extraction temperatures were tested to observe the behaviour of the distinct classes of aromatic hydrocarbons, in order to identify the conditions allowing the better uptake either of volatile and semi-volatile compounds. High extraction temperatures enhance the mass transfer of analytes from the sample to the headspace and increases their concentration in the gas phase. However, as the adsorption of analytes by the fibre coating is an exothermic process, the partition coefficient decreases by increasing temperature, negatively affecting the adsorption of analytes [26]. Fig. 1 reports the mass of the standard aromatic hydrocarbons adsorbed by the fibre, expressed as percentages. As expected, the uptake of less volatile compounds increased at high temperatures because of the improvement of the mass-transfer process from the sample to the headspace. In particular, the most of PAHs uptakes showed an exponential curve of increase. Nevertheless, C1- and C2-benzenes uptake decreased by increasing the extraction temperature over 40 °C, while C4- and in particular C3-benzenes responses decreased at temperatures above 60°. That can be attributed to a decrease of the headspace-coating distribution constant with temperature. Although it caused a loss in some MAHs uptake, the extraction temperature was fixed at 100 °C, allowing a remarkable improvement of sensitivity for other less volatile aromatic hydrocarbons. This

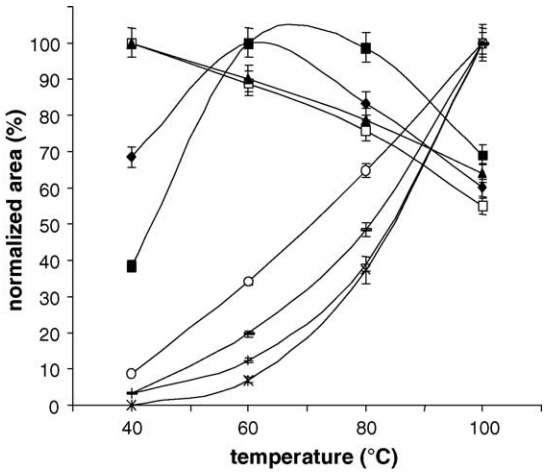

Fig. 1. Uptakes of the standard aromatic hydrocarbons at different SPME extraction temperatures and 30 min of extraction time. Amounts are expressed as normalized chromatographic areas. (□) C1-benzene; (▲) C2-benzenes; (♦) C3-benzenes; (■) C4-benzene; (◯) naphthalenes; (▬) acenaphthene + acenaphthylene; (+) phenanthrene + anthracene + fluorene; (×) fluoranthene + pyrene.

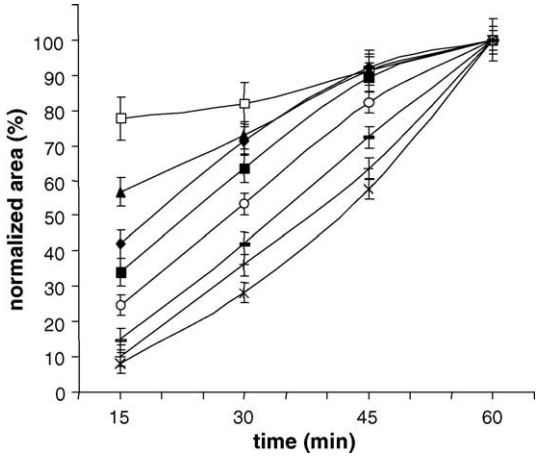

Fig. 2. Uptakes of the standard aromatic hydrocarbons at different SPME extraction periods and extraction temperature of 100 °C. Amounts are expressed as normalized chromatographic areas. (□) C1-benzene; (▲) C2-benzenes; (♦) C3-benzenes; (■) C4-benzene; (◯) naphthalenes (▬) acenaphthene + acenaphthylene; (+) phenanthrene + anthracene + fluorene; (×) fluoranthene + pyrene.

temperature favours the extraction of PAHs, which in olive oil are less abundant than MAHs, according with previous works carried out by other authors [13,14,16,18].

Successively, after fixing the optimal temperature, the behaviour of aromatic hydrocarbons during time extraction was tested.

Fig. 2 shows the uptakes of distinct classes of aromatic hydrocarbons at several sampling times (15, 30, 45 and 60 min). As the equilibrium was not reached within the entire range of time tested, the highest uptakes (100%) corresponded with the longest time of sampling, that was fixed at 60 min. Longer periods of extraction were considered

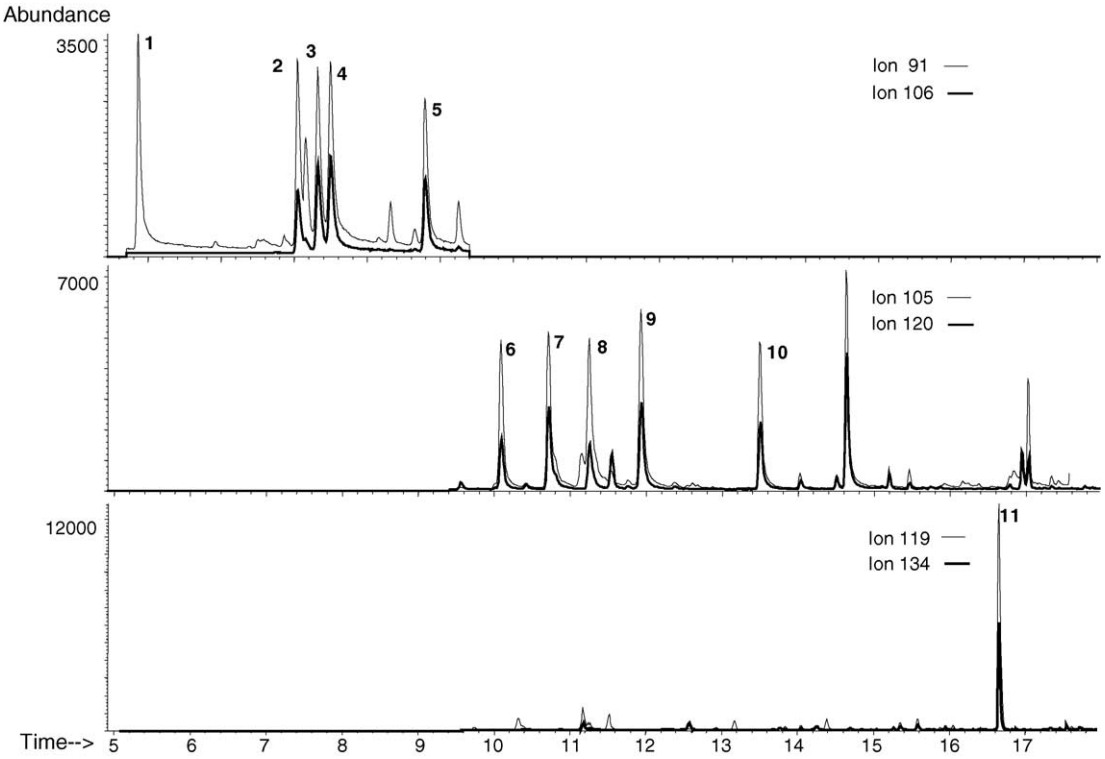

Fig. 3. Extracted ion chromatogram of monoaromatic standard hydrocarbons, obtained by analysing a 10 μg/kg oil mixture. Separation was performed on a Supelcowax capillary column and peaks are identified according to Table 1.

to be unsuitable for making the analysis excessively time-expensive.

3.2. Method performances

The sensitivity and selectivity of the method were enhanced by using the single ion monitoring (SIM) mode for the determination of the target compounds. Figs. 3 and 4 display the chromatograms of mono- and polyaromatic standard hydrocarbons, respectively, obtained by analysing a 10 μg/kg oil mixture.

The linearity of response of the aromatic hydrocarbons as a function of their concentration was evaluated by means of *r* values of linear regressions. Table 1 shows relative response factors (slope) and *r* values. All the compounds tested resulted in a satisfactory linearity within the entire range of concentration investigated (1–70 μg/kg). In the case of vegetable oils of the same origin, the composition in triglycerydes is comparable and it probably only slightly affect the analytes partition. On the contrary, and in particular in the case of unrefined oils, the composition of the volatile fraction can largely differ in each sample, causing different competition effects

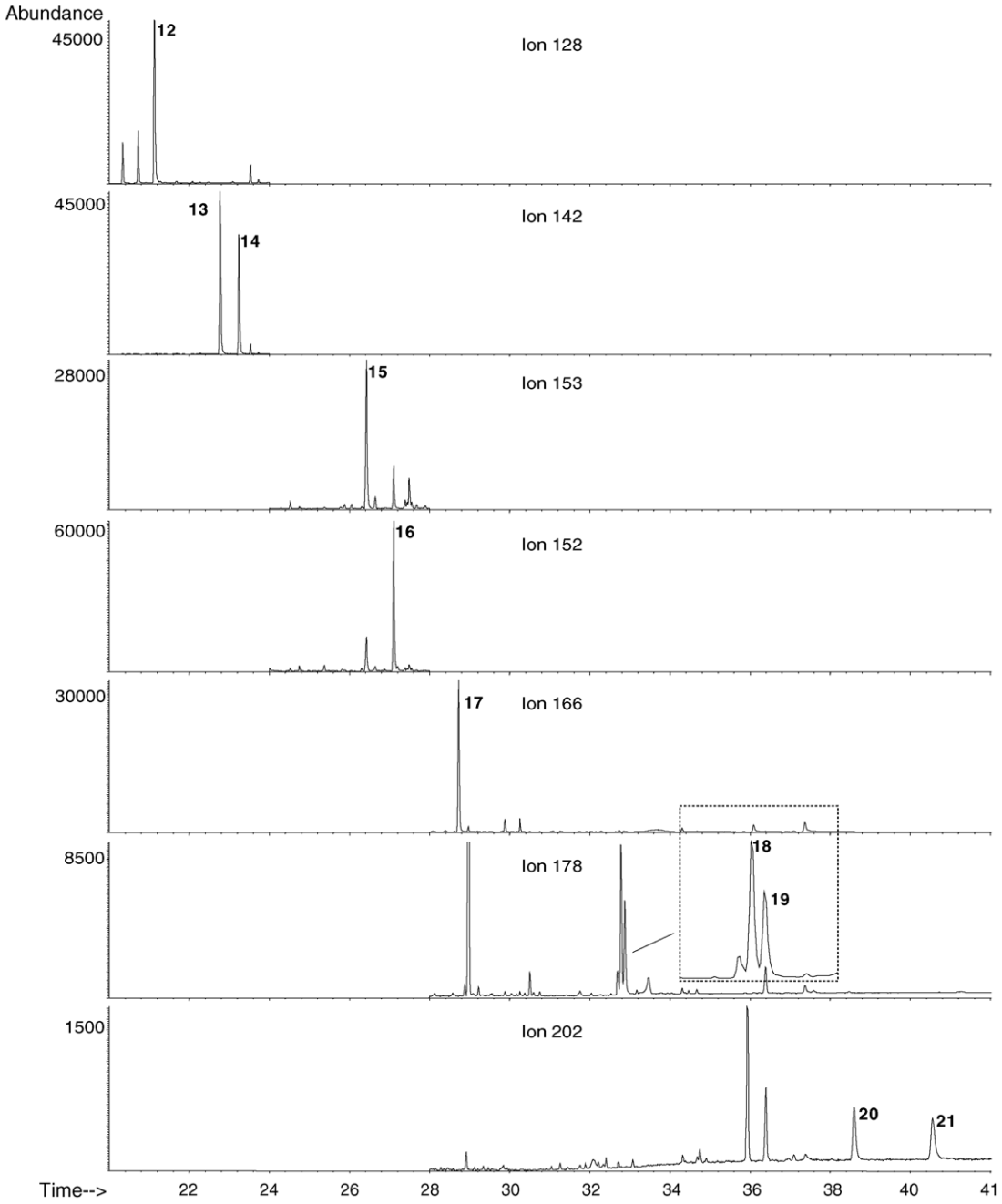

Fig. 4. Extracted ion chromatogram of polyaromatic standard hydrocarbons, obtained by analysing a 10 μg/kg oil mixture. Separation was performed on a Supelcowax capillary column and peaks are identified according to Table 1.

Table 1
Linear retention indices, target ions, linearity and relative response factors (expressed as *r* and slope, respectively) of the standard aromatic hydrocarbons

		KI[a]	KI[b]	Target ions	*r*	slope[c]
IS[d]	Ethylbenzene-d10	1105	852	98	–	–
IS	Indene	1467	1041	116	–	–
IS	Acenaphthene-d10	2119	1478	164	–	–
IS	Phenanthrene-d10	2713	1776	188	–	–
1	Toluene[e]	1023	669	91	0.9881	0.76
2	Ethylbenzene[e]	1109	855	91[f], 106	0.997	0.92
3	*m*-Xylene[e]	1118	864	91[f], 106	0.9994	0.87
4	*p*-Xylene[e]	1123	864	91[f],106	0.9976	0.85
5	*o*-Xylene[e]	1166	889	91[f], 106	0.9972	0.84
6	3-Ethyltoluene[e]	1209	957	105[f], 120	0.9986	1.28
7	1,3,5-Trimethylbenzene[e]	1229	964	105[f], 120	0.9963	1.30
8	2-Ethyltoluene[e]	1246	975	105[f], 120	0.9974	1.30
9	1,2,4-Trimethylbenzene[e]	1267	989	105[f], 120	0.9988	1.49
10	1,2,3-Trimethylbenzene[e]	1322	1019	105[f], 120	0.9991	1.24
11	1,2,3,4-Tetramethylbenzene[g]	1476	1151	119[f], 134	0.9977	0.74
12	Naphthalene[g]	1730	1171	128	0.9984	3.35
13	2-Methylnaphthalene[g]	1845	1289	142	0.9972	3.19
14	α-Methylnaphthalene[g]	1880	1306	142	0.9946	2.32
15	Acenaphthene[h]	2132	1447	153	0.9986	1.28
16	Acenaphthylene[h]	2188	1483	152	0.9988	2.62
17	Fluorene[i]	2331	1582	166	0.9902	2.90
18	Phenanthrene[i]	2723	1780	178	0.9928	1.12
19	Anthracene[i]	2733	1789	178	0.9944	0.63
20	Fluoranthene[i]	3096	2062	202	0.9925	0.17
21	Pyrene[i]	3160	2114	202	0.9897	0.12

[a] Kovats indices on Supelcowax column.
[b] Kovats indices on HP-5MS column.
[c] Relative area in function of concentration.
[d] Internal standards.
[e] IS: ethylbenzene-d10.
[f] Ions used for quantification.
[g] IS: indene.
[h] IS: acenaphthene-d10.
[i] IS: phenanthrene-d10.

on the adsorption of analytes on the fibre coating. The influence of the volatile composition of the sample on the uptake of aromatic hydrocarbons was evaluated by comparing the slopes of linear regressions obtained by spiking two different olive oil matrices with standard compounds: deodorized olive oil (Table 1) and a virgin olive oil (data not shown). The response factors calculated for the aromatic hydrocarbons in two olive oils with different volatile composition did not result significantly different by the Student *t*-test ($p > 0.05$).

Repeatability values (Table 2) calculated at the concentration of 10 μg/kg resulted in a relative standard deviation lower than 10% for the most of compounds. A slightly lower precision was observed for fluoranthene and pyrene, the compounds with the lowest responses (Fig. 4).

Limits of detection (LOD) and quantification (LOQ) are also reported in Table 2. MAHs are readily detected and quantified at less than 1 and 2.3 μg/kg, respectively, excepting tetramethylbenzene, which showed a LOQ of 3.4 μg/kg. Among light PAHs LODs were below 1 μg/kg, excepting α-methylnaphthalene and fluorene whit a slightly higher LOD. LOQ ranged from 0.2 μg/kg for acenaphtylene to 5.2 μg/kg for fluorene.

Concerning the accuracy of the method, the lowest percentages of theoretical hydrocarbon amounts recovered (Table 2) were found for fluoranthene and pyrene (74 and 77) in the oil spiked with 10 μg/kg of standard, while the other compounds showed higher recoveries at both of the fortification levels.

The efficiency of the SPME extraction was evaluated by calculating the distribution constants of the target compounds at the temperature applied in the analysis (Table 3). The uptake of analytes is related to the equilibration process between sample, headspace and fibre coating. The overall distribution constant between sample matrix and fibre coating (K_{fs}) is defined as the product of the sample/headspace (K_{sh}) and the headspace/fibre (K_{hf}) distribution constants [22] and both depend on temperature. As expected, K_{sh} were inversely correlated to molecular weight of aromatic hydrocarbons ($r = -0.545$, $p < 0.05$), and thus proportional to their volatility. On the contrary, at the extraction temperature of 100 °C aromatic hydrocarbons with high molecular weight showed higher K_{hf} than did more volatile compounds. The highest K_{fs} were obtained for two to three rings aromatic hydrocarbons and for toluene, followed by four-rings aro-

Table 2
Repeatability values, limits of detection and quantification and accuracy for aromatic hydrocarbons

	Compound	Intra-day RSD (%)[a] ($n = 6$)	Inter-day RSD (%)[a] ($n = 5$)	LOD[b] (μg/kg)	LOQ[c] (μg/kg)	Accuracy		
						Initial[d] (μg/kg)	% Level 1[e]	% Level 2[f]
1	Toluene	9.8	14.8	0.4	1.4	48.9	89	109
2	Ethylbenzene	6.1	6.7	0.6	1.9	17.2	96	102
3	*m*-Xylene	4.2	4.2	0.7	2.2	78.0	108	97
4	*p*-Xylene	4.5	4.7	0.4	1.5	46.5	111	111
5	*o*-Xylene	7.8	7.2	0.6	1.9	12.8	116	93
6	3-Ethyltoluene	5.3	7.6	0.6	2.1	16.0	106	94
7	1,3,5-Trimethylbenzene	5.0	10.0	0.6	2.0	6.1	103	91
8	2-Ethyltoluene	8.2	7.2	0.6	2.1	8.9	103	98
9	1,2,4-Trimethylbenzene	5.3	6.7	0.5	1.8	23.7	114	97
10	1,2,3-Trimethylbenzene	5.0	6.6	0.7	2.3	6.8	99	98
11	1,2,3,4-Tetramethylbenzene	4.3	5.9	1.0	3.4	3.5	85	104
12	Naphthalene	3.9	3.5	0.7	2.2	13.9	108	103
13	2-Methylnaphthalene	5.6	4.1	1.0	3.2	7.0	111	103
14	α-Methylnaphthalene	5.0	5.1	1.1	3.8	3.8	113	103
15	Acenaphthene	4.5	1.1	0.1	0.4	2.8	87	92
16	Acenaphthylene	6.9	1.8	0.05	0.2	2.6	94	97
17	Fluorene	5.0	6.2	1.6	5.2	29.1	128	80
18	Phenanthrene	2.9	3.7	0.5	1.8	15.0	105	98
19	Anthracene	7.9	6.3	0.7	2.3	5.6	105	101
20	Fluoranthene	14.9	9.2	0.2	0.5	6.8	77	113
21	Pyrene	15.8	14.4	0.2	0.8	8.0	74	119

[a] Relative standard deviation calculated by analysing a 10 μg/kg standard oil mixture.
[b] Limit of detection calculated as 3× standard deviation of noise signal/slope.
[c] Limit of quantification, calculated as 10× standard deviation of noise signal/slope.
[d] Initial concentration of the virgin olive oil spiked for the determination of accuracy.
[e] Accuracy (%) calculated by spiking a virgin olive oil with 10 μg/kg of standard aromatic hydrocarbons.
[f] Accuracy (%) calculated by spiking a virgin olive oil with 20 μg/kg of standard aromatic hydrocarbons.

Table 3
Distribution constants calculated at 100 °C by analysing 10 μg/kg standard oil mixture

	Compound	K_{sh}[a]	K_{hf}[b]	K_{fs}[c]
1	Toluene	1.4×10^{-2}	1.9×10^{3}	26.1
2	Ethylbenzene	3.7×10^{-3}	3.6×10^{3}	13.3
3	*m*-Xylene	2.8×10^{-3}	2.8×10^{3}	7.8
4	*p*-Xylene	2.8×10^{-3}	2.7×10^{3}	7.4
5	*o*-Xylene	1.8×10^{-3}	4.3×10^{3}	7.6
6	3-Ethyltoluene	9.1×10^{-4}	8.1×10^{3}	7.4
7	1,3,5-Trimethylbenzene	1.1×10^{-3}	6.2×10^{3}	6.6
8	2-Ethyltoluene	9.8×10^{-4}	5.7×10^{3}	5.6
9	1,2,4-Trimethylbenzene	9.5×10^{-4}	7.4×10^{3}	7.0
10	1,2,3-Trimethylbenzene	9.0×10^{-4}	8.0×10^{3}	7.2
11	1,2,3,4-Tetramethylbenzene	7.1×10^{-4}	1.0×10^{4}	7.3
12	Naphthalene	3.9×10^{-4}	6.6×10^{4}	25.6
13	2-Methylnaphthalene	2.2×10^{-4}	1.2×10^{5}	25.7
14	α-Methylnaphthalene	1.9×10^{-4}	9.5×10^{4}	18.1
15	Acenaphthene	1.2×10^{-4}	1.5×10^{5}	18.3
16	Acenaphthylene	1.1×10^{-4}	2.7×10^{5}	30.0
17	Fluorene	8.8×10^{-5}	4.4×10^{5}	39.1
18	Phenanthrene	1.1×10^{-4}	4.4×10^{5}	46.4
19	Anthracene	7.6×10^{-5}	6.4×10^{5}	49.2
20	Fluoranthene	8.6×10^{-5}	1.3×10^{5}	11.3
21	Pyrene	8.2×10^{-5}	1.1×10^{5}	8.9

[a] Sample/headspace distribution constant.
[b] Fibre/headspace distribution constant.
[c] Sample/fibre distribution constant.

matic hydrocarbons, C2-, C3-, and C4-benzenes. In the case of toluene the high K_{fs} was due to its volatility, while for polyaromatic hydrocarbons the low volatility was compensated by high K_{hf}. The K_{fs} values highlighted that at these conditions the better efficiency of SPME extraction was obtained for semivolatile compounds as light PAHs. A higher efficiency for semivolatile aromatic hydrocarbons is suitable because their concentration in virgin olive oil is generally lower than concentration of monoaromatic hydrocarbons [14–17].

3.3. Analysis of virgin olive oils headspace

GC–MS analysis of the samples in the complete scanning mode (SCAN) allowed the identification of all the investigated hydrocarbons. Moreover, a number of compounds showing the spectrum of a C4-benzene was also detected. Fig. 5 displays the profile of a virgin olive oil where can be detected *p*-cymene, butylbenzene, 1,2,3,4-tetramethylbenzene and the tentatively identified C4-benzenes (Table 4). In the same figure their common mass spectrum is shown. Among C4-benzenes, only tetramethylbenzene had been previously reported in literature to be present in virgin olive oil [12], while no quantitative data of C3- and C4-benzenes were available.

In the samples analysed in this study, *p*-xylene and toluene showed the highest concentrations, between 21–91 μg/kg and 18–372 μg/kg, respectively. The rest MAHs concentrations

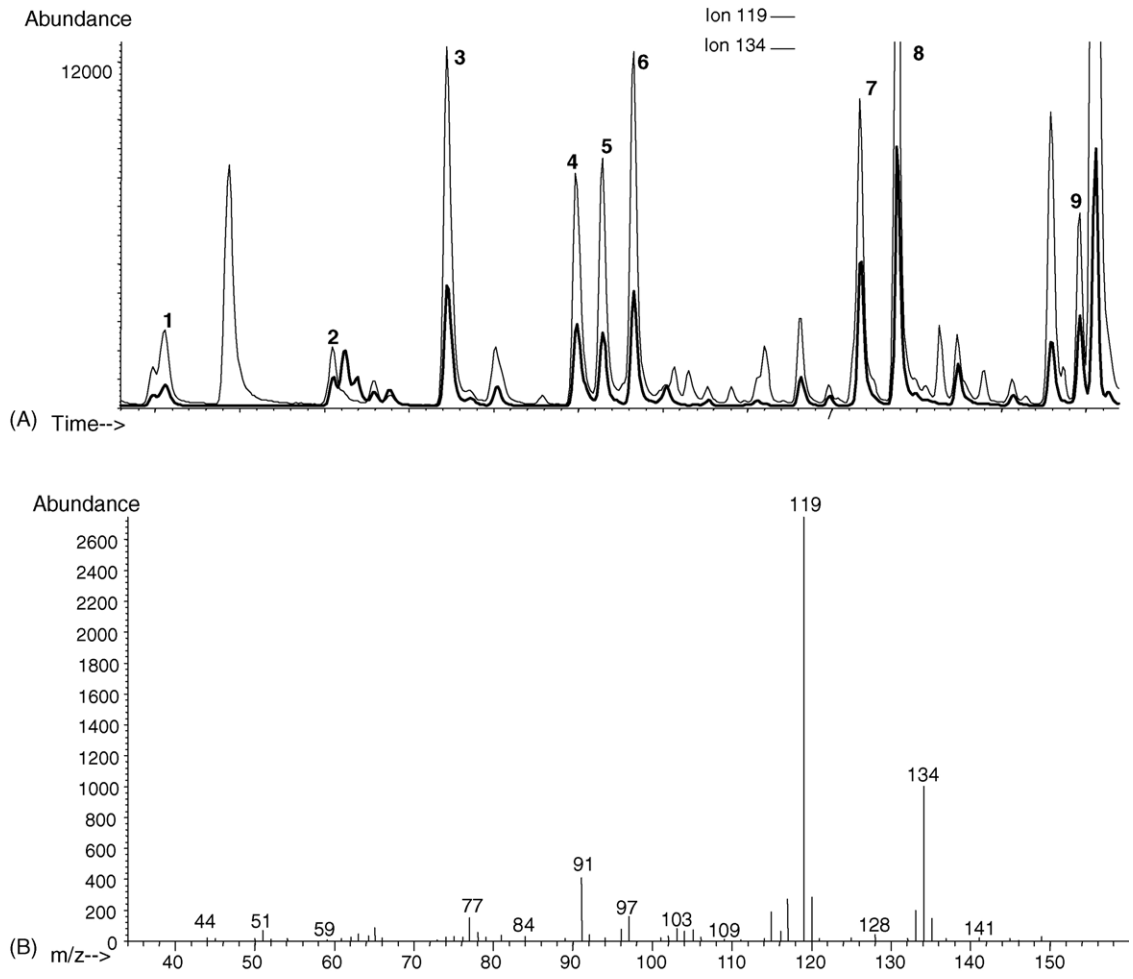

Fig. 5. (A) Extracted ion chromatogram of the tentatively identified C4-benzenes detected in virgin olive oil samples. Separation was performed on a Supelcowax capillary column and peaks are identified according with Table 4. (B) Mass spectrum of the tentatively identified C4-benzenes.

were in the following order: C2- > C3- > C4-benzenes. C2- and C3-benzenes, excepting toluene and *p*-xylene, were in the range of 3–198 μg/kg. C4-benzenes (quantified with the response factor calculated for 1,2,3,4-tetramethylbenzene) ranged within detectable but no quantifiable concentrations (n.q.) to 8 μg/kg. Among polyaromatic hydrocarbons the most abundant were naphthalene (5–16 μg/kg) and phenanthrene (8–26 μg/kg), while lower concentrations were observed for methylnaphthalenes (n.q. to 7 μg/kg), acenaphthene (0.4–1 μg/kg), acenaphthylene (0.4–5.7 μg/kg), fluo-

Table 4
Identification parameters of C4-benzenes detected in virgin olive oil

	Compound	Target ions	KI[a]	Ref.[b] KI[a]	KI[c]	Ref. KI[c]
1	*p*-Cymene [d]	119, 134	1255	–	1023	1008 [29]
2	Butylbenzene[d]	91, 134	1297	1309 [27],1302[28]	–	1039 [29]
3	1,3-Dimethyl-5-ethylbenzene[e]	119, 134	1309	1320 [27]	1059	1041 [29]
4	1,4-Dimethyl-2-ethylbenzene[e]	119, 134	1341	1343 [27], 1335[28]	1077	1059 [29]
5	1,3-Dimethyl-4-ethylbenzene[e]	119, 134	1348	1350 [27], 1343[28]	1078	1061 [29]
6	1,2-Dimethyl-4-ethylbenzene[e]	119, 134	1355	1357 [27], 1351[28]	1083	1066 [29]
7	1,2,4,5-Tetramethylbenzene[e]	119, 134	1411	1406 [27], 1401[28]	1131	1098 [29]
8	1,2,3,5-Tetramethylbenzene[e]	119, 134	1422	1416 [27], 1411[28]	1137	1100 [29]
9	1,2,3,4-Tetramethylbenzene [d]	119, 134	1476	1461 [27], 1456[28]	1151	1130 [29]

[a] Kovats retention indices on Supelcowax capillary column.
[b] Kovats retention indices reported in literature.
[c] Kovats retention indices on HP-5 capillary column.
[d] Identified by comparison with standard compounds.
[e] Tentatively identified.

rene (n.q.), anthracene (n.q. to 5 μg/kg), fluoranthene (n.q. to 11 μg/kg) and pyrene (n.q. to 11 μg/kg).

In conclusion, this HS-SPME method may be a suitable tool for the quantitative and qualitative analysis of aromatic hydrocarbons in virgin olive oil. It allows the simultaneous assessment of monoaromatic and light polyaromatic hydrocarbons with a high reliability, avoiding the use of solvents in the concentration steps and minimizing sample manipulation and contamination. This relatively rapid, simple and solvent-free method allows the determination of aromatic hydrocarbons in a large number of virgin olive oil samples.

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