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Rapid determination of BTEXS in olives and olive oil by headspace-gas chromatography/mass spectrometry (HS-GC–MS)

Bienvenida Gilbert-López, José Robles-Molina, Juan F. García-Reyes, Antonio Molina-Díaz*

Analytical Chemistry Research Group, Department of Physical and Analytical Chemistry, University of Jaén, Campus Las Lagunillas, Edif. B-3, 23071 Jaén, Spain

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ABSTRACT

In this work, a straightforward, reliable and effective automated method has been developed for the direct determination of monoaromatic volatile BTEXS group (namely benzene, toluene, ethylbenzene, *o*-, *m*- and *p*-xylenes, and styrene) in olives and olive oil, based on headspace technique. Separation, identification and quantitation were carried out by headspace-gas chromatography–mass spectrometry (HS-GC–MS) in selected ion monitoring (SIM) mode. Sample pretreatment or clean-up were not necessary (besides olives milling) because the olives and olive oil samples are put directly into an HS vial, automatically processed by HS and then injected in the GC–MS for chromatographic analysis. The chemical and instrumental variables were optimized using spiked olives and olive oil samples at 50 $\mu g\,kg^{-1}$ of each targeted species. The method was validated to ensure the quality of the results. The precision was satisfactory with relative standard deviations (RSD (%)) in the range 1.6–5.2% and 10.3–14.2% for olive oil and olives, respectively. Limits of detection were in the range 0.1–7.4 and 0.4–4.4 $\mu g\,kg^{-1}$ for olive oil and olives, respectively. Finally, the proposed method was applied to the analysis of real olives and olive oil samples, finding positives of the studied compounds, with overall BTEXS concentration levels in the range 23–332 $\mu g\,kg^{-1}$ and 4.2–87 $\mu g\,kg^{-1}$ for olive oil and olives, respectively.

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1. Introduction

Virgin olive oil is an outstanding commodity due to its content in bioactive phenolic compounds with antioxidant properties, and also because of its fatty acid composition and the benefits associated in the regulation of cholesterol levels and prevention of cardiovascular diseases [1,2]. For this reason, olive oil consumption has increased worldwide over 1.100 t throughout the last 19 years [3]. In the current scenario and with the aim of assessing the food safety and quality of virgin olive oil and derivate products, strict monitoring programs are undertaken targeting different potential chemical contaminants that may become a threat to health if the concentration detected is upper the established limits. Amongst them, pesticides [4–7], heavy metals [8,9], and polycyclic aromatic hydrocarbons [10,11] are probably amongst those which have been more extensively studied in the last decade.

Mono-ring aromatic hydrocarbons (MAHs) are widely distributed in the environment and can also be present in foods either naturally or as contaminants. Amongst them, BTEXS [benzene, toluene, ethylbenzene, the three xylene isomers (*ortho*, *meta* and *para*) and styrene (also called vinylbenzene)] are a subclass of volatile organic compounds with boiling points between 80 and 150 °C. BTEXS are considered hazardous carcinogenic and neurotoxic compounds and are classified as priority pollutants by Environment Canada [12] and the U.S. Environment Protection Agency (EPA) [13]. Note particularly the case of benzene, which has a very low tolerance standard in drinking water (5 $\mu g\,L^{-1}$). The relatively high solubility of BTEXS in water, together with the chronic toxicity associated with the single aromatic ring presents in their structure, address this group with a high pollution potential that may pose a risk to health.

The presence of BTEXS in olives and olive oil can be attributed to several factors such as biological processes in the fruit, the production technology used, contamination by fuel vapors, etc. [14]. For instance, styrene is frequently used in food industry to produce plastics by polymerization; those plastics are used as containers for many different food products, which could be contaminated by migration of styrene monomers. On the other hand, decarboxylation of the cinnamic acid naturally present in the olive pulp may also produce the appearance of styrene residues in olive oils.

Since 1996, when the European Union Commission expressed concern about the dietary exposure to volatile aromatic compounds, BTEXS, with particular reference to olive oil [15], a limited number of studies have been performed to establish the typical concentration profiles of these compounds in olive oil and olives.

* Corresponding author. Tel.: +34 953 212 147; fax: +34 953 212 940.
E-mail address: amolina@ujaen.es (A. Molina-Díaz).

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Only a few methods have been proposed for the determination of these compounds in olive oil due to the complex nature of this matrix. For virgin olive oils, some methodologies such as headspace solid-phase microextraction (HS-SPME) [16], purge-and-trap with GC–MS [17], headspace-mass spectrometry [18] and thermal desorption [19,20] have become of great interest for the analysis of volatile compounds including BTEXS. The use of HS-GC–MS for the measurement of benzene hydrocarbons in virgin olive oil has been proposed, and seems to be the more valuable methodology for the targeted application because almost no sample treatment is required [17,21,22]. Recently, a method based on a preliminary liquid–liquid extraction (LLE) [23] has been proposed in order to enhance the sensitivity and LODs, with regards to the conventional direct HS-GC–MS method.

Olives are the raw material used for the production of olive oil, and probably the main contributor of the presence of BTEXS in olive oil, occurring either naturally or from anthropogenic sources. However, to the best of our knowledge, there is no analytical methodology described in the literature for the direct detection of BTEXS in olives. Anticipating the detection of BTEXS in the raw material could be used as an effective tool to trace and detect contaminated lots of olives, and also to elucidate the origin of possible anthropogenic contamination events of olives with BTEXS, due to for instance proximity to a gas-station or a highway. In this article, a rapid method for the determination of BTEXS in olives is described for the first time. A detailed study has been accomplished to optimize analytical methodologies for BTEXS detection in both olives and virgin olive oil samples. The proposed methodologies are based on the use of HS-GC–MS. For olives samples analysis a minimal sample preparation is required, only comprising olives milling and the subsequent addition of water and potassium chloride to the homogenized paste. Programmable temperature vaporizer (PTV) injection port parameters, and the addition of modifiers such as organic solvents and inorganic salts in order to promote salting-out effect have been studied in detail. The method was tested with different olives and olive oil samples.

2. Experimental

2.1. Reagents and standards

All the standards were of analytical grade or better. Standards of benzene, toluene, ethylbenzene, styrene and the three isomers of xylenes (*ortho*, *meta* and *para*) were purchased from Riedel-de-Haën (Seelze, Germany), OEKANAL® quality. Stock standard solutions of each analyte were prepared in methanol at a concentration of 1.0 mg mL^{-1} and stored in amber glass capped vials at −20 °C. A standard solution containing the mixture of BTEXS at individual concentration of 100 μg mL^{-1} was prepared in methanol by appropriate dilution of the stocks. Working standard solutions were prepared by spiking the standard solution to olives and olive oil samples previously analyzed to check the content of BTEXS. HPLC-grade methanol was obtained from Merck (Darmstadt, Germany). 20 mL glass flat-bottomed vials (22.7 mm OD × 75 mm) as well as magnetic PTFE-silicone seals (3.0 mm i.d.) were purchased from Supelco (Madrid, Spain). PTFE-encapsulated magnetic stirring bars (6 mm × 12 mm) were purchased from Varian Inc. (Walnut Creek, CA, USA). Potassium chloride (reagent grade) was obtained from Panreac (Barcelona, Spain). A Milli-Q-Plus ultra-pure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain the HPLC-grade water used during the analyses. Olive samples were obtained from a Spanish oil manufacturer company, and commercial virgin olive oil samples were purchased at local markets.

2.2. Gas chromatography/mass spectrometry

2.2.1. Gas chromatography

The separation of the species targeted was carried out using a CP-3800 gas chromatograph (Varian Inc., Walnut Creek, CA, USA) equipped with electronic flow control (EFC) and a 1079 universal capillary injector that allows programmed temperature injection (a PTV injection port). The gas chromatograph was also equiped with an autosampler (CombiPAL autosampler, CTC Analytics) with capacity for 32 headspace vials composed of an oven for sample heating/headspace generation and a robotic arm where the headspace syringe was placed. Helium was employed as carrier gas. The operating conditions of the headspace were: incubation time: 30 min; incubation temperature: 90 °C; magnetic stirring speed: 700 rpm. Then 1 mL of the vial headspace was injected into the GC inlet heated at 200 °C, operated in splitless mode and equipped with a split open deactivated insert liner of 5 mm OD × 54 mm × 3.4 mm ID (Varian Inc., Walnut Creek, CA, USA). The column oven temperature was set at 30 °C and remained constant during 10 min. After this time, the temperature raised up to 60 °C at 5 °C/min, and after 1 min it raised up again to 200 °C at 20 °C/min. Then was kept at 200 °C for 2 min. A constant column flow of 1.5 mL/min of helium was used. A Varian FactorFour VF-5ms capillary column of 30.0 m × 0.25 mm i.d. and 0.25 μm of film size (Varian Inc., Walnut Creek, CA, USA) was used for chromatographic separation.

2.2.2. Mass spectrometry

The gas chromatograph was connected to a triple quadrupole mass spectrometer Varian 300-MS TQ MS (Varian Inc., Walnut Creek, CA, USA) by an inert transfer line heated at 280 °C. The source and manifold (QqQ) temperatures were kept at 250 and 40 °C, respectively. Electron impact ionization (EI) was operated at 70 eV. A filament current of 50 μA and a multiplier voltage of 1300 V were used in MS mode. Specific SIM ions were recorded for each compound analyzed (167 ms acquisition time for each ion). A filament multiplier delay of 2.3 min was fixed in order to prevent detector overload/saturation. The mass spectrometer was calibrated as needed with perfluorotributylamine (PFTBA). Varian WorkStation software (version 6.9) was used for automated analysis and data acquisition and Varian MS Data Review was used for data processing.

2.3. General procedure

2.3.1. HS-GC–MS determination of BTEX in olive oil and olives

2.3.1.1. Olive oil sampling. 12.5 g of olive oil (*ca.* 15 mL) were weighed into a 20-mL HS glass vial with a PTFE-encapsulated magnetic stirring bar and was immediately sealed with a PTFE-silicone septum.

2.3.1.2. Olives pretreatment (mill). Approximately 500 g of olives (including the kernel) were first crushed by means of a mill manufactured by Talleres Lopera (Priego de Córdoba, Córdoba, Spain) and designed specially for crushing up olives (*molino triturador-reductor (M-R)*, 45 cm (length) × 51.5 cm (high) × 35 mm (width), 40 kg (weight)). The mill consisted in a hopper that led the olives to a worm gear connected to a rotor (1.1 kW). This rotor rips the olives and olive kernel, then obligating them to pass through a sieve of small orifices (5.0 mm i.d.). As a result, a homogenized paste is obtained and collected in an appropriate food-container. The milling step was performed at room temperature, and no significant heating of the paste was observed during the procedure. This is important to avoid analyte losses because of the relatively high vapor pressure of the targeted analytes.

Table 1
Studied chromatographic parameters for the optimization of BTEXS separation. FS-3 conditions were selected for the final procedure.

Method	Column flow (mL/min)	Injector temperature (°C)	Split	Oven temperature (°C)	HS injection volume (mL)	Acquisition delay time (min)
FS-1	1.5	PTV-1[a]	S-1[b]	R-1[d]	1	1.6
FS-2	1.5	180	1:15	R-2[e]	1	2.0
FS-3	1.5	200	S-2[c]	R-3[f]	1	2.3
FS-4	1.5	200	S-2[c]	R-4[g]	1	2.3

[a] PTV-1: starts at 70 °C (1 min constant), increases at 150 °C/min until reach 200 °C. Then remain constant during 10 min.
[b] S-1: starts at 1:80 during 0.5 min, followed by 1:20 for 4.5 min; then 1:100.
[c] S-2: at the beginning is off (splitless), followed by 1:0.01 for 15 min; then 1:100.
[d] R-1: starts at 35 °C (constant 3 min), rising up at 5.0 °C/min until 60 °C and remaining constant during 1 min. Finally it increases to 200 °C at 20 °C/min, and was kept during 2 min.
[e] R-2: starts at 30 °C (constant 3 min), rising up at 5.0 °C/min until 60 °C and remaining constant during 1 min. Finally it increases to 200 °C at 20 °C/min, and was kept during 2 min.
[f] R-3: starts at 35 °C (constant 10 min), rising up at 5.0 °C/min until 60 °C and remaining constant during 1 min. Finally it increases to 200 °C at 20 °C/min, and was kept during 2 min.
[g] R-4: starts at 27 °C (constant 10 min), rising up at 5.0 °C/min until 60 °C and remaining constant during 1 min. Finally it increases to 200 °C at 20 °C/min, and was kept during 2 min.

2.3.1.3. Olives sampling. 5 g of homogenized paste from the olives were weighed into a 20-mL HS glass vial with a PTFE-encapsulated magnetic stirring bar. Then 10 mL of milli-Q water plus, and 1.5 g of KCl were added and the vial was immediately sealed with a PTFE-silicone septum.

The HS vial containing the sample (either olive oil or olive paste) was placed in the CombiPal autosampler furnished with a heating module for automated unattended heating with stirring (700 rpm) for 30 min at 90 °C in order to ensure the equilibration between gas-phase and sample. An aliquot of the gas phase above the sample (1 mL) was automatically injected into the GC–MS system. During GC–MS analysis specific SIM ions were recorded for each target compound.

2.3.2. Calibration curves and spiking procedure

2.3.2.1. Matrix-matched calibration curves for olive oil. An spiked olive oil sample were prepared by adding known amounts of working methanolic solution containing the BTEXS to the olive oil matrix. Several standards of 12.5 g (*ca.* 15 mL) with concentrations ranging 5–500 $\mu g\,kg^{-1}$ (5, 10, 20, 30, 50, 80, 100 and 500 $\mu g\,kg^{-1}$) were prepared.

2.3.2.2. Matrix-matched calibration curves for olives. Representative portions of crushed olives homogenized sample were weighted and fortified homogenously with appropriate volume of working standard solution to reach 5, 10, 20, 30, 50, 80, 100 and 500 $\mu g\,kg^{-1}$ of the studied BTEXS in the portions of crushed olives. The mixtures were then gently blended for 30 min, to assess the homogeneity of the spiked sample. Then the samples were incubated overnight at +4° C. Finally, three replicates (5 g each) of each concentration level spiked sample (calibration standard) were measured by HS-GC–MS with the proposed procedure.

2.3.2.3. Spiking procedure for precision study. A representative portion of the homogenized olive oil matrix or the crushed olives homogenized sample was weighted and fortified homogeneously with an appropriate volume of working standard solution to reach 10 $\mu g\,kg^{-1}$ of the studied compounds in the spiked sample. The mixture was then gently blended for 30 min, to assess the homogenization of the spiked sample. Then the sample was incubated overnight at +4° C. Finally, eight replicates of the spiked sample were analyzed by HS-GC–MS under the optimized conditions. The same procedure was followed in order to perform the precision study at 50 $\mu g\,kg^{-1}$ concentration level.

3. Results and discussion

3.1. GC–MS method optimization

The optimization and selection of the chromatographic parameters (i.e. carrier gas flow, injector temperature, split conditions, initial oven temperature, temperature gradient program, and headspace injection volume) were carried out by analyzing virgin olive oil spiked with 50 $\mu g\,kg^{-1}$. Different conditions for oven and injector temperatures, as well as split ratios, were studied to obtain a proper separation of chromatographic peaks corresponding to BTEXS. A summary of the studied conditions is shown in Table 1. Best peak separation and sensitivity were obtained using method FS-3, although two coelutions are unavoidable: (1) *m*-xylene and *p*-xylene; (2) *o*-xylene and styrene. The *m*- and *p*-xylene overlapped cannot be solved by using mass spectra features as it was the case of the pair *o*-xylene-styrene. Both compounds coelute at 12.6 min, but the monitoring of compound specific ions (*m*/*z* 91, 105 and 106 for *o*-xylene, and *m*/*z* 78, 103 and 104 for styrene) allows the correct identification and quantitation of the peaks, as long as SIM chromatograms yield individual and well resolved peaks (see Fig. 1). This overlapping of xylene isomers, described elsewhere [24] has been solved by using a longer column [25] or a chiral column [26]. We decided to preserve a standard method based on a common universal column that can be implemented easily in any laboratory using a standard capillary column, and quantify the samples using the sum of both isomers. Once the optimum values for chromatographic parameters were established, the more abundant ions of the targeted compounds were chosen in order to create the acquisition method. The selected BTEXS SIM ions along with their relative abundance and retention times in the optimized GC conditions are shown in Table 2. The use of SIM mode enhances the method sensitivity and selectivity compared to full-scan mode.

As an example, Fig. 1 shows the total ion chromatogram and SIM chromatograms for (a) a virgin olive oil fortified with 20 $\mu g\,kg^{-1}$ of BTEXS, and for (b) a crushed olives sample fortified with at the same concentration level (20 $\mu g\,kg^{-1}$) of BTEXS.

3.2. Optimization of HS variables and chemical modifiers addition

The optimization of the HS variables was carried out with a headspace sampling module so that the entire HS-GC–MS method is fully automated (heating, stirring, injection, etc.). The main parameters of headspace technique that need to be carefully studied are sample volume or weight, heating temperature and heating

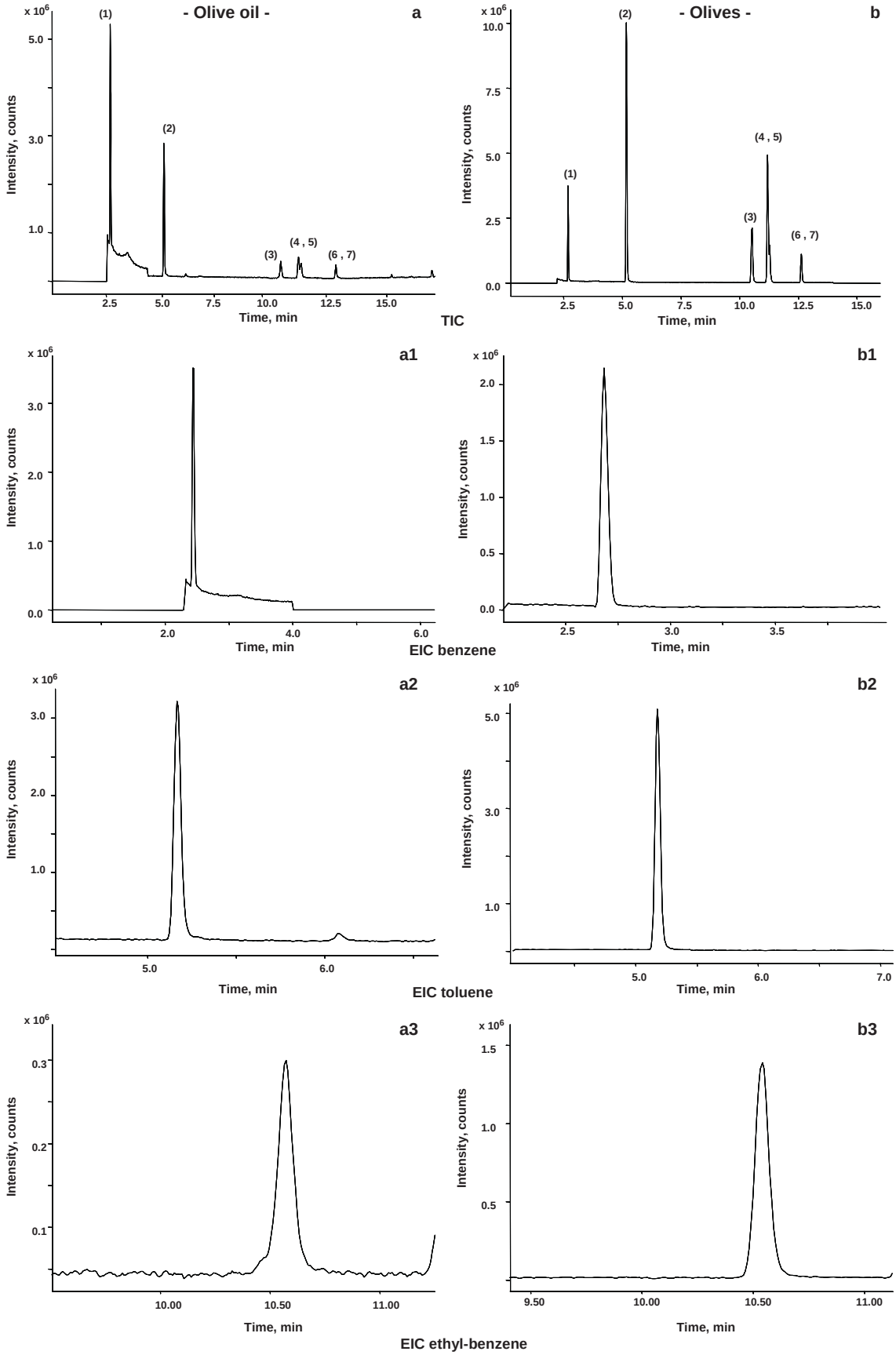

Fig. 1. Total ion chromatogram and SIM chromatograms for (a) a virgin olive oil fortified with 20 μg kg^{-1} of BTEXS, and for (b) an olive paste sample fortified with the same concentration level (20 μg kg^{-1}) of BTEXS.

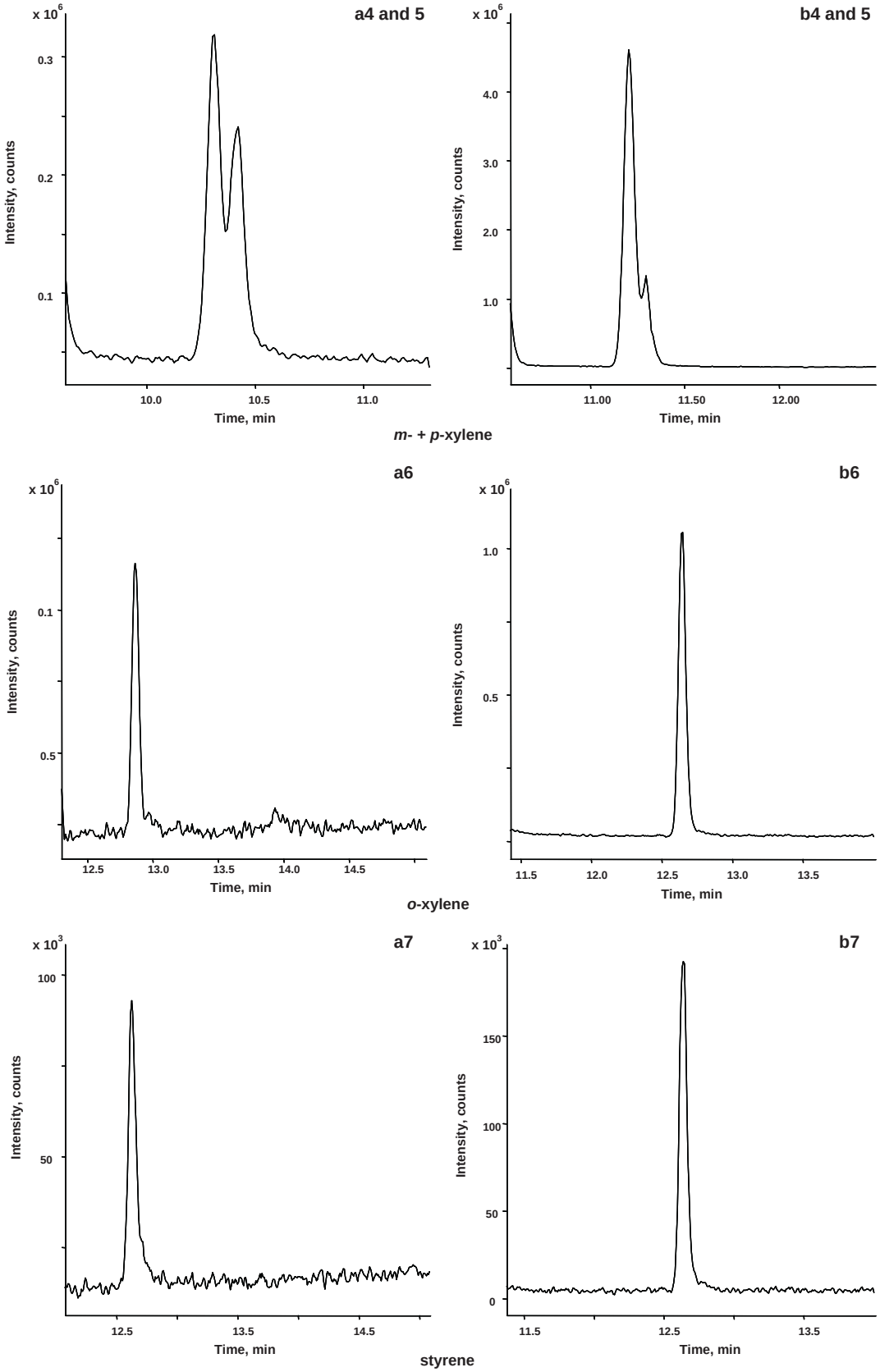

Fig. 1. (*Continued*).

Table 2
BTEXS retention times, SIM ions and relative abundance.

Compound	MW	RT (min)	SIM ions (rel. abundance, %)		
			Quantitation	Confirmation	Qualifiers
Benzene	78	2.7	78 (100)	77 (23)	51 (30)
Toluene	92	5.2	91 (100)	92 (66)	65 (13)
Ethyl-benzene	106	10.5	91 (100)	106 (36)	56 (29)
m-Xylene	106	11.2	91 (100)	106 (67)	105 (27)
p-Xylene	106	11.3	91 (100)	106 (68.5)	105 (46)
o-Xylene	106	12.6	91 (100)	106 (50)	105 (21.5)
Styrene	104	12.6	104 (100)	103 (64)	78 (15)

time of the samples (incubation time), and magnetic stirring rate. In addition, some modifiers can be added to the samples in order to achieve better sensitivity.

3.2.1. Olive oil

Optimization of HS variables was carried out by using an olive oil sample spiked with 50 μg kg^{-1} of the studied compounds. 10 g of sample were used in the preliminary experiments. Heating time was evaluated in the interval 20–40 min. Signal increased as raised equilibration time of the sample inside the oven, but time values higher than 30 min led to a slight decrease in the analytical signal (Fig. 2). Thus, 30 min was chosen as optimum heating time for samples. Heating temperatures were evaluated in the interval 75–90 °C, and signal increased with oven temperature. Therefore, 90 °C was stated as optimum value. Magnetic agitation rate was studied in the range 600–750 rpm. Signal slightly increased as raised the rate of agitation, but values up to 700 rpm kept signal constant, so 700 rpm was selected as optimum. Sample amount was studied in the interval 5–12.5 g, being observed an increase of the signal with sample volume. 12.5 g was considered the optimum. Finally, the addition of selected modifiers (organic solvents was evaluated in terms of signal enhancement). Methanol, n-hexane and ethyl acetate were added (800 μL) to olive oil samples and the results were compared with those obtained from an "unmodified" sample. Analyte response of benzene was enhanced slightly (<10%) when using adding n-hexane to the sample as modifier. However, better sensitivities were obtained for the rest of analytes without modifier. Therefore, no modifier was used for further olive oil analysis.

3.2.2. Olives

For the analysis of olives samples, HS parameters optimized for olive oil were used, except the amount of sample and additives (heating time: 30 min; heating temperature: 90 °C, magnetic agitation rate: 700 rpm). To carry out the optimization of additives, homogenized olives (spiked with 50 μg kg^{-1} of BTEXS) were used. A representative portion of 5 g was used to carry out the experiments. However, solid samples were difficult to stir by a magnet, so the addition of ultrapure water was investigated in order to facilitate homogeneous sample stirring and heating. As a result, better precision and sensitivity was obtained when the olive paste was mixed with water. The volume of water added to the HS vial was evaluated in the range 2.5–10 mL (using 5 g of spiked olives), resulting in an increase of signals with the volume of water added. Therefore, 10 mL of ultrapure water were selected as optimum. The effect the addition of acids (nitric acid) and salt were also studied. The use of HNO_3 0.1 M did not cause any improvement. In contrast, the addition of potassium chloride caused the increase of analytes signal (Fig. 3), as long as better reproducibility among standards replicates. This is due to the salting-out effect that foster the transfer of relatively apolar molecules to gas-phase, thus enhancing the analytical performance of the method. As shown in Fig. 3, this effect is slightly more significant for benzene. The evaluation of potassium chloride addition was carried out in the range 0.5–2.0 g. Signal increased with the amount of KCl, but amounts higher than 1.5 g did not increase the signal significantly. Finally, 1.5 g of KCl was selected as optimum value.

3.3. Analytical performance

All matrix-matched standards were analyzed by triplicate, and the calibration plots were constructed using the averaged area of each concentration level standards. Calibration curves were obtained by plotting the peak area against the analyte concentration for each compound, except in the case of *m*-xylene and *p*-xylene, in which both were identified and quantified together since their corresponding peaks were partially overlapped. For virgin olive oil, calibration curves were constructed in the interval 10–500 μg kg^{-1} obtaining correlation coefficients in the range from 0.9961 to 0.9994. For olives, calibration curves were constructed in the interval 10–500 μg kg^{-1} obtaining coefficients better than

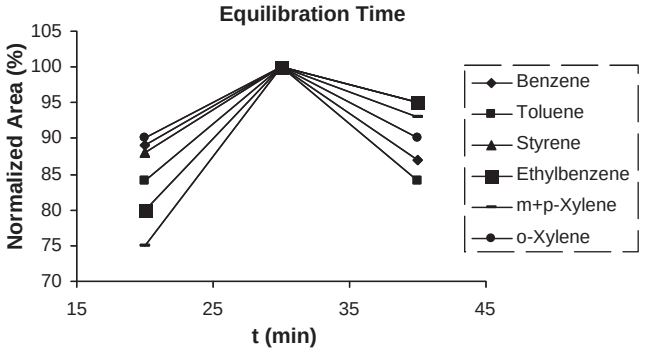

Fig. 2. Optimization of equilibrating time by using a virgin olive oil spiked at 50 μg kg^{-1} of BTEXS.

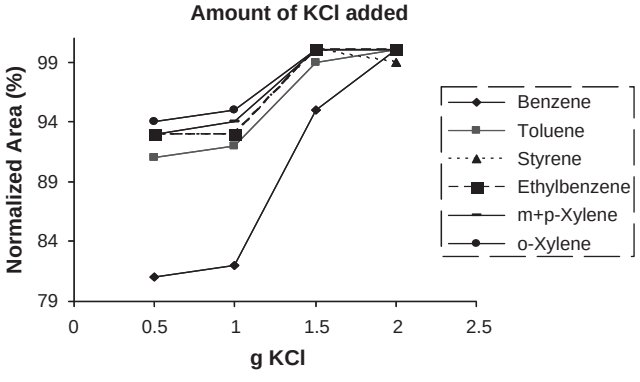

Fig. 3. Optimization of the addition of salt to a homogenized crushed olives sample spiked at 50 μg kg^{-1}.

Table 3
Linearity, limits of detection and limits of quantitation achieved in olives and olive oil by the proposed method.

Compound	LODs ($\mu g\,kg^{-1}$)		LOQs ($\mu g\,kg^{-1}$)		LDR tested ($\mu g\,kg^{-1}$)		*r* coefficient	
	Olives	Olive oil	Olives	Olive oil	Olives	Olive oil	Olives	Olive oil
Benzene	6.1	0.5	20.0	2.2	10–500	10–500	0.9975	0.9994
Toluene	7.4	0.4	24.4	1.25	10–500	10–500	0.9993	0.9979
Ethyl-benzene	0.1	3.7	0.4	14.4	10–500	10–500	0.9997	0.9972
m-Xylene + *p*-xylene	0.3	4.4	1.0	15.0	10–500	10–500	0.9998	0.9974
o-Xylene	1.0	2.6	3.3	10.0	10–500	10–500	0.9997	0.9993
Styrene	0.6	3.5	2.1	13.3	10–500	10–500	0.9998	0.9961

0.997. Limits of detection (LODs) and limits of quantitation (LOQs) were estimated using the signal-to-noise criteria (S/N = 3 and S/N = 10 for LODs and LOQs, respectively) with spiked olives and olive oil samples at concentration levels in the range 5–10 $\mu g\,kg^{-1}$. Linear dynamic range (LDR), LODs and LOQs obtained for olives and olive oil matrixes are shown in Table 3.

In order to explore the ruggedness of the proposed confirmatory HS-GC–MS method, a precision test was carried on both olives and olive oil samples. The study was carried out at two concentration levels, 10 and 50 $\mu g\,kg^{-1}$ ($n = 8$), following the spiking procedure detailed in Section 2.3. The precision was satisfactory with relative standard deviations (RSD (%)) in the range 1.6–5.2% and 10.3–14.2% for olive oil and olives, respectively. The relative standard deviation (%) values obtained from the analysis of olives were slightly higher than those obtained from the analysis of olive oil. This is due to the difficult homogenization of crushed olives with the kernels, which may involve a higher uncertainty than that of a more homogeneous sample such as olive oil (Table 4).

Table 4
Reproducibility studies carried out in olives and olive oil.

Compound	10 $\mu g\,kg^{-1}$ (RSD%)		50 $\mu g\,kg^{-1}$ (RSD%)	
	Olives	Olive oil	Olives	Olive oil
Benzene	13.30	5.23	12.71	4.55
Toluene	10.09	3.05	12.48	2.39
Ethyl-benzene	13.54	4.84	10.32	4.21
m-Xylene + *p*-xylene	14.20	3.71	10.41	4.42
o-Xylene	12.40	3.95	11.68	4.41
Styrene	10.91	2.36	11.81	1.61

3.4. Application to real samples

The proposed method was applied to the analysis of 13 virgin olive oil samples purchased in local markets. Samples were analyzed by triplicate, being reported the mean concentration value. The results are shown in Table 5. The overall concentration of BTEXS found in the studied olive oil samples was in the range from 11.2 to 332.2 $\mu g\,kg^{-1}$.

In addition, the method was applied to 10 olives samples collected in the province of Jaén (Spain), during the harvesting time in 2009 (from November-2009 to January-2010). Concentration levels of BTEXS found in olives were lower than those found in olive oil (see Table 5). To our knowledge, this is the first report on the presence of BTEX in olives. As shown in Table 5, the overall BTEXS concentration in olives is significantly lower than that found in the olive oil samples tested. The overall concentration of BTEXS found in the studied olive samples was in the range from 2.4 to 79.8 $\mu g\,kg^{-1}$. Fig. 4 shows some examples of positive findings of BTEXS at low concentration levels (<15 $\mu g\,kg^{-1}$) in olives and in virgin olive oil samples. These chromatograms clearly show the ability of the proposed method to control trace levels of these contaminants in the studied samples.

Table 5
Results of analyzed olives and virgin olive oil samples ($\mu g\,kg^{-1}$).

Compound/sample	Benzene	Toluene	Ethyl-benzene	*m*-Xylene + *p*-Xylene	*o*-Xylene	Styrene	$\sum$BTEX
Olive oil							
A-01	2.9	21.5	ND	<LOQ	<LOQ	22.3	46.7
A-02	<LOQ	15.9	ND	<LOQ	<LOQ	16.2	32.1
A-03	<LOQ	11.2	ND	<LOQ	<LOQ	ND	11.2
A-04	3.6	19.9	15.0	25.8	11.8	16.4	92.5
A-05	6.1	14.7	<LOQ	<LOQ	<LOQ	37.9	58.7
A-06	<LOQ	12.8	ND	<LOQ	ND	18.4	31.3
A-07	<LOQ	6.7	ND	LOD	ND	16.5	23.2
A-08	18.4	20.9	<LOQ	<LOQ	<LOQ	15.0	54.3
A-09	<LOQ	21.7	15.8	22.9	13.3	20.4	94.0
A-10	6.4	123.0	36.7	56.3	47.4	62.3	332.2
A-11	<LOQ	21.9	<LOQ	20.7	13.3	105.9	161.7
A-12	<LOQ	19.6	31.2	53.1	28.8	ND	132.7
A-13	3.6	51.6	15.7	30.1	21.1	39.8	161.9
Olives							
O-01	ND	ND	0.9	2.1	<LOQ	5.4	8.4
O-02	ND	ND	ND	1.4	LOD	4.2	5.6
O-03	ND	<LOQ	1.5	3.3	<LOQ	75.0	79.8
O-04	ND	ND	1.0	2.9	<LOQ	24.3	28.2
O-05	ND	ND	0.7	1.4	ND	2.1	4.2
O-06	ND	ND	ND	1.0	ND	17.2	18.2
O-07	ND	<LOQ	1.1	2.6	<LOQ	5.4	9.1
O-08	ND	<LOQ	0.5	1.9	<LOQ	<LOQ	2.4
O-09	ND	<LOQ	0.6	2.1	<LOQ	4.8	7.5
O-10	ND	<LOQ	1.2	3.9	<LOQ	4.0	9.1

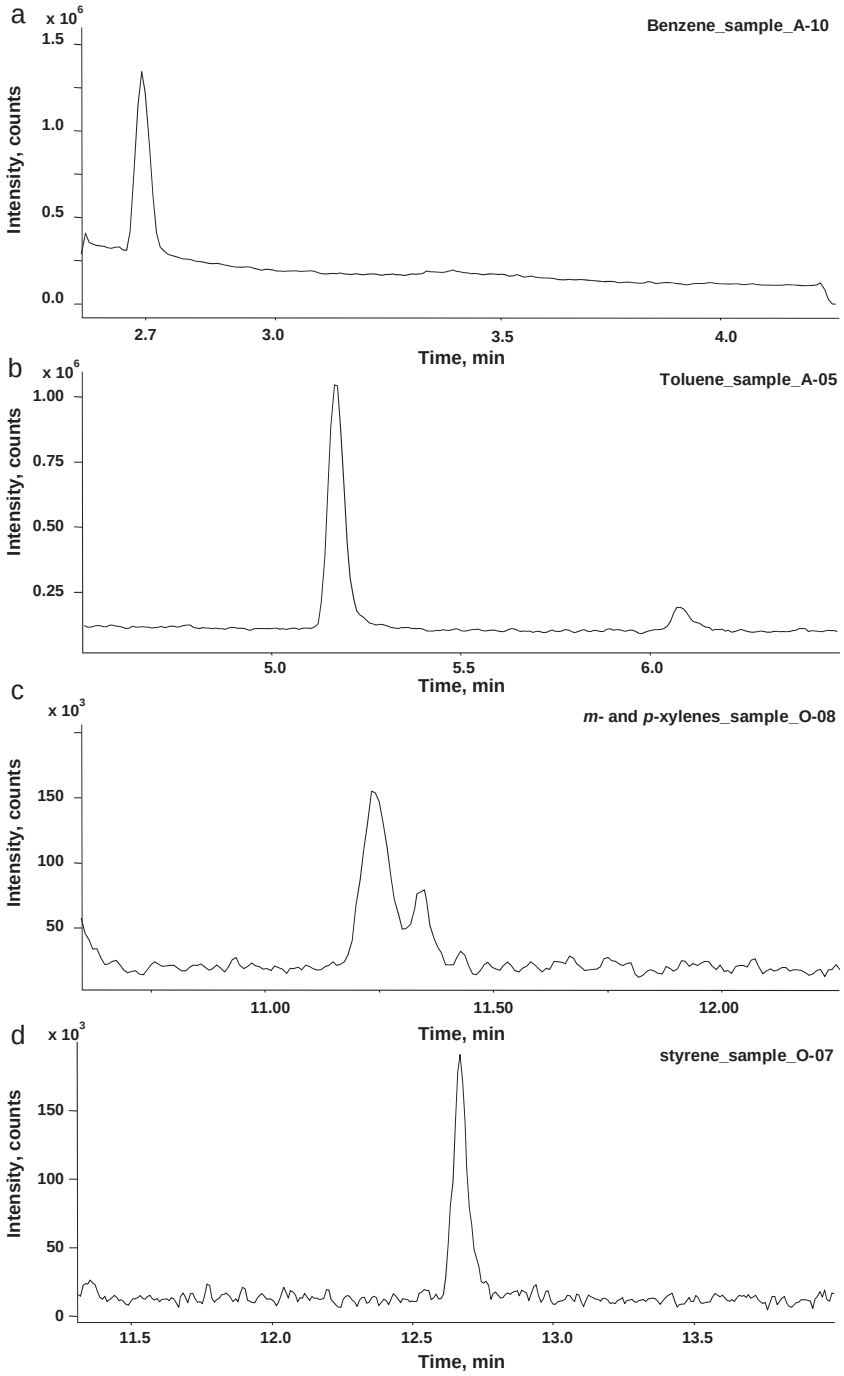

Fig. 4. Example of positive findings of BTEXS at low concentration levels (<15 μg kg^{-1}) in real samples: (a) benzene in virgin olive oil sample A-10; (b) toluene in virgin olive oil sample A-05; (c) *m*- and *p*-xylene in crushed olives sample O-08; (d) styrene in crushed olives sample O-07.

4. Conclusions

In this work, we have reported the first analytical methodology for the direct determination of BTEXS compounds in olive samples. The proposed method which is based on the headspace technique is fully automated, sensitive, straightforward and reliable. Sample pretreatment or clean-up stages were not necessary because samples are almost put directly into an HS vial, automatically processed by HS and then injected in the GC–MS for chromatographic analysis. For olive samples analysis, a minimal sample preparation is required, only comprising olives milling and the subsequent addition of water and potassium chloride to the homogenized paste. The proposed methodology (developed also for olive oil) was successfully applied to the analysis of BTEXS in 23 olive and olive oil samples. To our knowledge, this is the first study reporting data on BTEX concentration in olives. We observed that the overall BTEXS concentration present in olive oil samples was significantly higher than that found in olives. The proposed methodology could be used as an effective tool to trace and detect contaminated lots of olives, and also to elucidate the origin of possible anthropogenic contamination events of olives with BTEXS, due to, for instance, proximity to a gas-station or a highway.

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