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Gas chromatography-mass spectrometry using microvial insert thermal desorption for the determination of BTEX in edible oils

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Abstract

Microvial insert thermal desorption is evaluated as a sample introduction system for gas chromatography-mass spectrometry (GC-MS) determination of benzene, toluene, ethylbenzene and xylene isomers (BTEX) in vegetable oil samples. Under the optimized conditions, the BTEX contained in a 50 µL oil sample placed in the microvial insert were thermally vaporized at 140 ºC for 5 min while helium at a flow of 120 mL min^{-1} and pressure of 4.3 psi propelled the analytes to the programmable temperature vaporizator (PTV), where they were focused at 15 ºC. The compounds were finally injected into the GC column by rapidly heating the PTV at 270 ºC. The proposed method provided good linearity between 10 and 200 µg L^{-1}. High sensitivity, with detection limits in the 0.7-1.2 µg L^{-1} range, depending on the compound, was achieved with a low sample volume and minimum sample handling. Fourteen edible vegetable oil samples were analyzed and the analytes were found in a wide concentration range.

1.　Introduction

Benzene, toluene, ethylbenzene and xylene isomers, commonly known as BTEX, are volatile organic compounds commonly found in a wide range of petroleum derivatives. BTEX are emitted to the environment during a large variety of processes, including the combustion of wood and fuels, or as consequence of their use as additives in industrial adhesives, degreasing agents, dyes, resins, detergents or paints.[1] The sum of the concentrations of these chemicals is used as an indicator of contamination, particularly in the case of soil or groundwater. Apart from harming the environment, they tend to accumulate in foodstuffs because of their lipophilic nature, especially in fatty products and edible oils.[2] A study of the origin of BTEX in edible vegetable oils concluded that both contamination and biosynthetic reactions taking place during vegetable maduration are responsible.[3]

BTEX are health hazards due to their toxic characteristics. Benzene has been identified as a carcinogenic agent, involved in the development of leukaemia, for which reason it has been included in the International Agency for Research on Cancer (IARC) Group 1. Ethylbenzene has also been classified as a possible carcinogenic agent and is included in IARC Group 2, while toluene and xylene, neither of which has shown any carcinogenic effect to date, are included in IARC Group 3. In addition, toluene, ethylbenzene and xylenes have harmful effects on the central nervous system. When chronically ingested, all BTEX may cause liver and kidney damage.[4] Such toxicological data, joined to the fact that BTEX have been detected in edible oil samples, led the European Union Commission Scientific Committee on Food to express concern about

dietary exposure to these compounds through olive oil.[5] Nevertheless, no regulations have been introduced related to the presence of BTEX in these food products.

Analysis of the headspace (HS) of samples by gas chromatography-mass spectrometry (GC-MS)[3,6–10] is the analytical strategy most widely used for the determination of BTEX in edible oils, although direct HS-MS coupling[9,11] has also been used for the global determination of BTEX. In order to minimize matrix interference and to improve the separation of the analytes from oil sample, some authors include a step prior to HS, for example liquid-liquid extraction (LLE)[6] or distillation.[10]

HS sampling, despite being a clean and simple technique, usually requires an equilibrium time of between 15 and 30 min. This implies that sensitivity is limited by the relative vapour pressure of the studied compound, meaning that the technique is only applied to volatile analytes at relative high concentrations. Solid-phase microextraction (SPME) in the headspace mode has also been proposed for the determination of all or some BTEX compounds in oils.[12–14] From those published SPME procedures, only Vichi et al.[14] provide the method performance.

Amirav and Dagan developed the named direct sample introduction (DSI) injection GC technique,[15] which, among large volume injection (LVI) techniques, can be catalogued as rapid, sensitive, simple and inexpensive. When applied to liquid samples, the technique is also known as microvial insert thermal desorption or microvial insert large volume injection.[16] The liquid sample, with a volume of up to 150 µL, is placed in a glass microvial and introduced into the programmed temperature vaporizator (PTV) or a thermodesorption unit (TDU)

attached to the PTV inlet. Non-volatile interfering matrix components remained in the vial, which can be removed and discarded after each assay, while volatile compounds are vaporized and transferred to the GC column for separation.[17] DSI has previously been used for the determination of pesticides in eggs and vegetables,[18,19] odour-related compounds in wine and fruits[16,20] and phthalate esters in cleaning products.[21] The literature mentions the application of DSI for the determination of volatile compounds in oils,[22] but only toluene is included in the characterization and no quantitative data are provided.

In this work, we propose a microvial insert thermal desorption GC-MS method for the determination of BTEX in different vegetable oil samples. The use of microvial insert large volume injection allows introduction of the edible oil into the chromatographic system and the selective vaporization of the BTEX, while avoiding any contamination problem related to the oil matrix components.

2.   Experimental

2.1   Chemicals and reagents

A benzene, toluene, ethylbenzene, o-xylene, m-xylene and p-xylene solution, each at 200 mg L^{-1} in methanol, was obtained from Sigma (St. Louis, MO, USA). Working solutions (2 mg L^{-1}) were prepared by diluting the commercial products with acetone, and kept at −18 ºC in darkness. Pure grade acetone was also provided by Sigma. Helium (99.9999% purity) was supplied by Air Liquide (Madrid, Spain)

2.2 Instrumentation

The sample introduction system was composed of a Thermal Desorption Unit (TDU-2) equipped with an autosampler (MPS-2) and a Programmed Temperature Vaporization (PTV) Cooled Injector System (CIS-4) provided by Gerstel (Mullheim an der Ruhr, Germany). The experimental conditions used for the sample introduction system are summarized in Table 1.

GC analyses were performed on an Agilent 6890N (Agilent, Waldbronn, Germany) gas chromatograph coupled to an Agilent 5973 quadrupole mass selective spectrometer equipped with an inert ion source. Under the selected conditions (Table 1) the compounds eluted at retention times between 3 and 10 min, corresponding to benzene and p-xylene, respectively (Table 2). The compounds were quantified in the selected ion monitoring (SIM) mode in order to improve the detection limits using different ions (Table 2). Identification was confirmed by the retention time of the target ion and the qualifier-to-target ion ratios for each compound.

2.3 Samples and analytical procedure

A total of 14 edible oil samples, including corn, olive, sunflower, sesame and peanut, were obtained from local supermarkets. All of the edible oils were sold in plastic (PET) or glass bottles. Once opened, samples were kept in darkness at 4 °C until analysis.

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A 50 µL-aliquot of oil was placed in a 150 µL glass microvial insert using a microsyringe, and then introduced into a glass desorption tube. The whole assembly was submitted to the thermal desorption procedure by placing the desorption tube in the TDU and using the experimental conditions given in Table 1.

For validation purposes, three oil samples (corn, olive and sunflower) were spiked at two different concentrations, 20 and 140 µg L^{-1}, and allowed to stand for 1 h before being submitted in triplicate to the analytical procedure.

3.   Results and discussion

3.1   Optimization of GC-MS conditions

Different temperature programmes were tested to separate the analytes, and the best resolution was achieved when an initial temperature of 40 ºC was maintained for 5 min, which allowed the elution of benzene and toluene; next a ramp of 2.5 ºC min^{-1} was applied to increase the column temperature to 50 ºC, ethylbenzene eluting at 8.4 min and both m- and p-xylene 0.5 min later. This temperature was maintained 1 min to elute o-xylene. All the experiments carried out to separate m- and p-xylene isomers were unsuccessful, as other authors have found.[6-8,13] Consequently, m- and p-xylene were therefore quantified as the sum of both isomers, taking into account that there are no differences between their ions. The GC-MS analysis of oil samples in the scanning mode (SCAN) in the 40-300 m/z range allowed identification of the analytes and

confirmation of the need to increase the column temperature beyond the elution of o-xylene to elute matrix components of the oil (Fig. 1A). Quantification was carried out in the SIM mode in order to increase sensitivity (Table 2).

3.2   Selection of microvial insert thermal desorption conditions

Microvial insert thermal desorption is a complex step that may be influenced by a large number of experimental variables. The effect of seven of these variables (desorption time and temperature, gas flow-rate and pressure, PTV liner filling and focusing and heating temperature) in the BTEX response and in its ratio *versus* the matrix response was evaluated at different levels. For this purpose, 50 µL of olive oil spiked with each analyte at 100 µg L^{-1} was placed in the microvial and introduced into the gas chromatograph using the TDU system.

Five different thermal vaporization temperatures (from 80 to 160 °C) and vaporization times (from 1 to 5 min) were tested. In general, higher temperatures and longer vaporization times may facilitate the release of analytes from the sample, but may reduce the overall efficiency due to retention losses in the PTV injector, as well as increase interference from the oil matrix. Maximum sensitivity was attained for all compounds, except for toluene, when 140 °C was applied as desorption temperature, while all analytes increased their responses with at a desorption time of 5 min (Fig. 2).

Therefore, a temperature programme increasing from room temperature to 140 °C at 200 °C min^{-1}, with a hold time of 5 min, was applied in the TDU for vaporization purposes. The behaviour of each analyte with the seven TDU variables studied is shown in ESI section – Fig. S1.

A carrier gas is necessary to propel the analytes to the PTV injector while they are being thermally vaporized. TDU vaporization and subsequent PTV focusing of the BTEX may be influenced by the flow-rate and pressure of this gas. High flow rates generally lead to the effective propulsion of the vaporized compounds, but increase their speed through the PTV, reducing their residence time, and thus the retention efficiency in this device. Low pressures during the vaporization step facilitate the transfer of the BTEX from the oil sample to the gas phase. Five different flow-rates (ranging from 40 to 120 mL min^{-1}) and pressures (from 4.3 to 10 psi) were tested and, as expected, best responses were obtained for all BTEX when 120 mL min^{-1} and 4.3 psi were applied (Fig. 2).

Vaporized compounds are focused in the PTV before entering the chromatographic column. This device is cooled by a Peltier unit, which allows cooling to temperatures slightly below room temperature, so five different focusing temperatures (from 15 to 23 ºC) were tested. As shown in Fig. 2, lower temperatures increased their retention efficiency and minimized analyte losses, so the lowest assayed temperature (15 ºC) was selected.

The PTV liner is usually filled with different inert supports to facilitate the retention of the analytes. Several liner filling materials, including fibreglass (FG), polydimethylsiloxane (PDMS) foam, graphitized carbon black adsorbent (CB) or 2,6-diphenylphenylene oxide (DPO), and an empty baffled (BF) liner were tested for this purpose. Best responses were obtained for benzene, toluene and ethylbenzene with CB filling, while the response of xylene was maximum with DPO. Taking into account the average response (Fig. 2), as well as the oil

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matrix response, which was higher with DPO, CB was selected as PTV liner filling material.

Once the vaporization step is over, the PTV is heated in order to elute the BTEX retained in the liner into the chromatographic column. Five different PTV heating temperatures, ranging from 270 ºC to the maximum recommended for the CB filling, 350 ºC, were evaluated. This factor did not significantly affect the responses of the studied compounds (Fig. 2). Nevertheless, the temperature increase led substantially enhanced the matrix response; taking into account the BTEX/matrix response ratio, 270 ºC was adopted. Therefore, a temperature programme increasing from 15 to 270 ºC at 540 ºC min^{-1}, with a hold time of 5 min, was applied in the PTV after the vaporization step.

3.3   Method performance

Calibration graphs using the standard additions method were obtained for three edible oil samples of different vegetable origin (sunflower, olive and corn oils). The graphs were linear in the 10-200 µg L^{-1} range ($r > 0.99$). The statistical comparison of the resulting slopes by means of an ANOVA test confirmed the suitability of using the matrix matched calibration method ($0.09 < p < 0.87$), and a single edible oil calibration graph for the quantification of different oil samples (see ESI section – Table S1).

The repeatability of the method was calculated using the average relative standard deviation (RSD) of 10 replicate analyses of an olive oil sample spiked with the analytes at a concentration of 40 µg L^{-1}. RSD values ranged from 6.5 to 9.9%, depending on the compound (Table 2).

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The sensitivity was evaluated from the detection limits (LODs) (Table 2), which were calculated considering a signal-to-noise ratio of 3. The LOD values ranged between 0.7 and 1.2 µg L^{-1}, depending on the compound.

A comparison of the proposed method with other methods previously published for the determination of BTEX in edible oils is summarized in Table 3. The method here optimized provided good sensitivity, with very low sample consumption and a rapid sample treatment, allowing to propose it for the routine monitoring of BTEX in oil samples

Since no reference material was commercially available, the method was validated by recovery assays, fortifying three different oil samples (sunflower, olive and corn) at two concentration levels (20 and 140 µg L^{-1}). Recoveries in the 88-112% range were obtained for the lowest level (n=45), while recoveries of 96-101% were obtained for the highest one (n=45).

3.4   Analysis of samples

The optimized procedure was applied for the determination of BTEX in commercial vegetable edible oil samples. Fourteen real samples, including a wide variety of oil types (olive, sunflower, peanut, sesame and corn), from different brands and with different packages, were analyzed in triplicate. As shown in Table 4, BTEX were found in all of the samples. Benzene was the most commonly found compound, with an average concentration of 108 µg L^{-1}, while the highest total BTEX concentration was found in an olive oil sample. None of the samples exceeded the maximum recommended total BTEX concentration of 2000 µg L^{-1}.[6] The concentrations of the studied compounds

found in the edible oils analyzed were in concordance with previously reported values for other edible oil samples in the bibliography.[6-8]

Fig. 1 shows a typical chromatogram obtained by microvial insert TD-GC-MS for a spiked oil sample at 40 µg L^{-1} using SCAN and SIM modes. This figure illustrates the absence of interfering peaks at the retention time of the analytes, which were identified by comparing their retention times and mass spectra in samples and standard solutions.

4.   Conclusions

The microvial insert thermal desorption procedure for GC-MS analysis allows a rapid, robust and reliable method for the quantification of BTEX in vegetable oils. The high sensitivity attained, joined to the low sample volume consumption, minimum sample handling, as well as the low time required, allow us to propose the optimized procedure for the routine monitoring of BTEX in oil samples.

Acknowledgements

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Legends for the Figures

Fig. 1   Microvial insert TD-GC-MS chromatogram obtained for an olive oil sample spiked with the studied compounds at 40 µg L^{-1} in SCAN (A) and SIM (B) modes. (1) Benzene, (2) toluene, (3) ethylbenzene, (4) m,p-xylene and (5) o-xylene.

Fig. 2   Effect of TDU temperature, TDU heating time, gas flow rate, gas pressure, PTV cooling temperature, PTV liner filling and PTV heating temperature on mean analyte responses. Error bars indicate standard deviation for n=15.
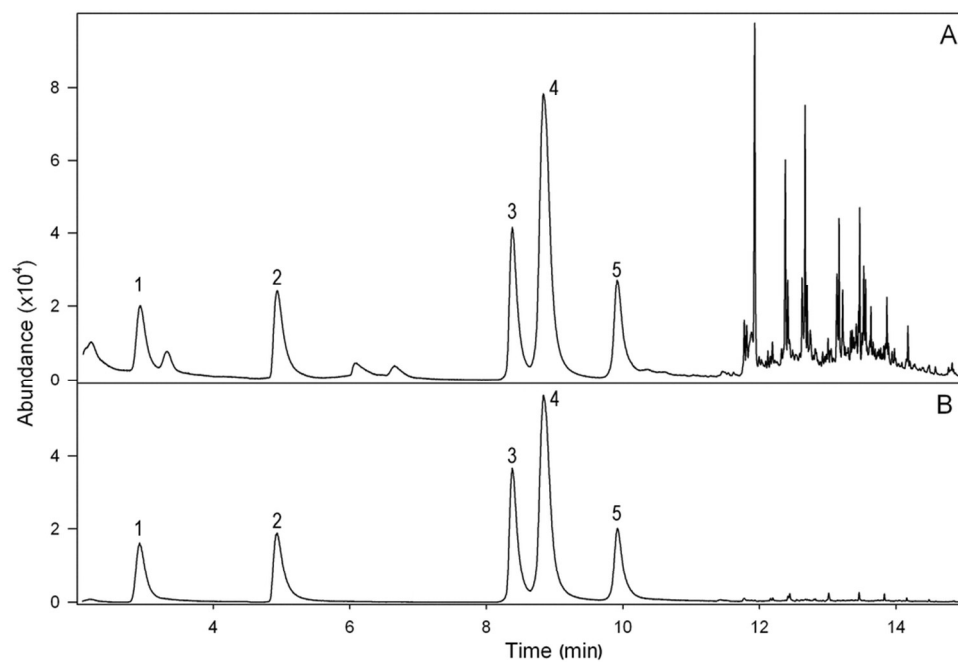
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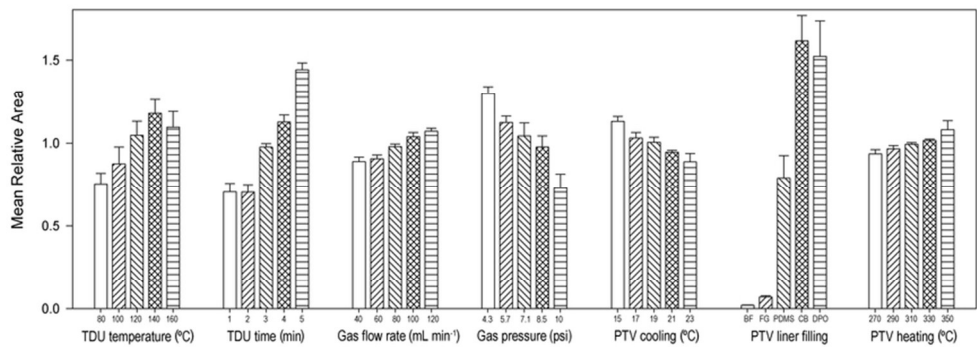
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Table 1 Experimental conditions of the TD-GC-MS procedure

Thermal Desorption Unit	
Mode	Splitless
Temperature programme	40 – 140 ºC at 200 ºC min^{-1}, held 5 min
Desorption flow	120 mL min^{-1}
Desorption flow pressure	4.3 psi
Cooled Injector System	
Mode	Solvent venting
Liner	Graphitized carbon black, 2 mm i.d.
Temperature programme	15 – 270 ºC (5 min) at 540 ºC min^{-1}
GC-MS	
Capillary column	HP-5MS, 5% diphenyl-95% dimethylpolysiloxane (30 m x 0.25 mm, 0.25 µm)
Carrier gas	Helium (1 mL min^{-1})
Oven programme	40 ºC, held 5 min
	40 – 50 ºC at 2.5 ºC min^{-1}, held 1 min
	50 – 240 ºC at 50 ºC min^{-1}, held 1 min
Transfer line temperature	300 ºC
Quadrupole temperature	150 ºC
Ion source temperature	230 ºC
Ionization	Electron-impact mode (70 eV)

Table 2 Method characteristics

Compound	RT (min)	Monitored ions (*m/z*)	RSD[a] (%)	LOD[b] (µg L^{-1})
Benzene	3.0	<u>78</u>, 77 (16), 52 (15)	9.9	0.9
Toluene	5.0	<u>91</u>, 92 (78), 65 (12)	6.5	0.7
Ethylbenzene	8.4	<u>91</u>, 106 (37), 51 (9)	7.7	0.9
m-Xylene / p-Xylene	8.9	<u>91</u>, 106 (69), 77 (12)	7.0	1.0
o-Xylene	10.0	<u>91</u>, 106 (63), 77 (10)	6.5	1.2

Underlined numbers correspond to *m/z* of the target ion, and values in brackets represent the qualifier-to-target ion ratios as percentage

[a] n=10

[b] Calculated using S/N=3

Table 3 Comparison of the proposed method with other previously developed methods based on GC-MS for the determination of BTEX in edible oils

Sample treatment	Sample consumption	Treatment time (min)	Approximate total time analysis (min)	Linearity (μg L^{-1})	LOD (μg L^{-1})	Reference
MWCNTs-LLE-HS	9 mL	~ 15	35	1-200	0.25-0.43	6
HS	15 mL	30	60	12.5-625	0.6-5.5	7
HS	10 mL	25	40	10-1000	2.8-7.4	8
SPME	2 g	30	75	1-70[a]	0.4-0.7[a]	14
Microvial insert TD	50 μL	5	20	10-200	0.7-1.2	This work

MWCNTs, Multiwalled carbon nanotubes

[a] ng g^{-1}

Table 4 Analysis of oil samples (µg L^{-1})

Sample	Benzene	Toluene	Ethylbenzene	m,p-Xylene	o-Xylene	Total
Corn	177.6±5.1	56.3±7.5	11.6±0.8	14.3±0.9	32.1±5.2	291.9
Olive	38.3±2.3	ND	2.3±0.2	ND	NQ	44.6
Olive	13.6±2.1	ND	2.7±0.4	ND	22.8±2.7	39.1
Olive	267±16	46.4±3.7	17.0±1.4	5.3±0.3	32.3±0.7	368
Olive	ND	ND	65.6±0.6	ND	ND	65.6
Olive	96.3±7.8	28.8±3.6	5.1±0.3	7.2±0.5	15.7±0.9	153.1
Olive	24.6±3.1	55.5±8.2	ND	7.4±0.4	32.3±4.2	119.8
Peanut	17.4±2.3	13.9±1.2	ND	NQ	7.4±0.6	39.9
Sesame	ND	16.5±1.2	ND	NQ	24.0±0.5	42.0
Sunflower	233±15	71.0±4.6	3.3±0.2	3.8±0.2	ND	311.1
Sunflower	85.5±5.8	10.3±1.1	6.5±0.4	ND	14.0±0.8	116.3
Sunflower	155±11	15.1±2.1	8.1±0.4	5.7±0.4	43.6±8.4	227.5
Sunflower	86.5±7.4	23.5±1.5	NQ	8.4±1.2	47.8±3.9	169.2
Sunflower	ND	5.5±0.5	2.6±1.0	ND	54.9±4.5	63.0

Values are mean ± standard deviation (n=3), ND means not detected, NQ means not quantified

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A rapid analytical procedure for BTEX determination involving minimum sample handling

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