Chromatography

HEADSPACE–MASS SPECTROMETRY WITH ALTERNATIVE CHROMATOGRAPHIC SEPARATION USING A COLUMN SWITCHING SYSTEM FOR THE SCREENING AND DETERMINATION OF BTEX AND STYRENE IN COMESTIBLE OIL

Carla Toledo,[1,2] Pedro Enríquez,[2] Mariano Garrido,[3] Beatriz Fernández-Band,[3] and Pablo Richter[1]

[1]*Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile*

[2]*Laboratorio Química Ambiental y Alimentaria, Servicio Agrícola y Ganadero, Santiago, Chile*

[3]*Laboratorio de FIA, Departamento de Química, Universidad Nacional del Sur, Bahia Blanca, Argentina*

A headspace–mass spectrometry with alternative chromatographic separation using a column switching system was developed for the screening and confirmation of BTEX and styrene in comestible oils. According to the position of the switching valve, the chromatographic column can be bypassed and the volatile sample constituents are transferred directly from the headspace sampler to the mass spectrometer providing a global, non resolved, signal in less than 1 min after injection. In this way, a set of samples can be rapidly processed in order to determine if they are (or not) contaminated with BTEX and styrene. Subsequently, only the samples with positive response in the previous screening can be processed by gas chromatography–mass spectrometry in the same analytical system by switching the position of the valve, thus confirming the presence of the analytes in the sample.

The method presents good analytical features and it is applicable to the analysis of real samples. Detection limits were lower than 0.1 ng mL^{-1}, and recoveries were between 97 and 105% with relative standard deviations lower than 4%.

Analysis of real comestible oils showed the presence of toluene, benzene, and styrene in some samples packed in plastic bottles.

Keywords: BTEX; Column switching system; Headspace; Mass spectrometry; Styrene

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Address correspondence to Pablo Richter, Departamento de Química Inorgánica y Analitica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Casilla Box 233, Santiago, Chile. E-mail: prichter@ciq.uchile.cl

INTRODUCTION

Volatile organic compounds, such as benzene, toluene, ethylbenzene, xylenes (BTEX), and styrene, are a family of important environmental pollutants which can also be present in foods. Because their high toxicity and common occurrence, the development of new methods for their analytical determination in different matrixes has been important for decades. The level of exposure to BTEX by food is not as high as by air. The compounds, BTEX, however, have been detected in some bottled water, liquor, and food. Benzene can be present in some food contact materials, appearing as a breakdown product of organic chemicals used in the production of plastics or as a contaminant in the starting materials used in polymer synthesis (Food Standards Agency 1994a). Similarly, styrene is used as an intermediate in the manufacture of plastics, elastomers, and resins which, in turn, are used in packaging foods, food products, and various other processes in the food industry. Such packaging is known to contain a residual styrene monomer which can migrate into food (Food Standards Agency 1994b). On the other hand, decarboxylation of the biophenol present in the olives, cinamic acid, can also produce the appearance of styrene residues in the olive oil (Peña et al. 2004a).

The determination of volatile compounds in foods commonly implies the employment of headspace technology (HS), purge and trap (PT), and solid phase microextraction (SPME) coupled to chromatographic systems such as GC or HPLC, for their determination (Zini et al. 2002; Fleming-Jones and Smith 2003; De Lacy Costello et al. 2001; Cuevas-Glory et al. 2007).

The development of screening methods, avoiding a chromatographic separation for the resolution and determination of different analytes is of great interest owing to their speed. For instance, the direct coupling of mass spectrometry with headspace (HS-MS) sampling (Peréz Pavón et al. 2003; Shiers and Squibb 1998; Berdagué et al. 1998; Marcos Lorenzo, Pérez Pavón, Fernández Laespada, García Pinto, and Moreno Cordero 2002; Pérès, Begnaud, and Berdagué 2002; Marcos Lorenzo, Pérez Pavón, Fernández Laespada, García Pinto, Moreno Cordero, Henriques et al. 2002), has been developed for the analysis of foods. This coupling provided "fingerprints" of the products under analysis, and the information, suitably processed by applying chemometric data treatment, can be used to determine different analytes in a sample.

More recently, mass spectrometry coupled to gas chromatography (GC-MS) has also been described for screening purposes (Peña et al. 2004a,b; Richter et al. 2006) by using two different approaches, with the advantage that the whole system can be used alternatively for confirmative purposes after rapid screening of the samples. In one of these approaches, the GC-column temperature is keeping at a level high enough to avoid chromatographic resolution of the target analytes (Peña et al. 2004a,b). In these conditions, a global (not resolved) signal is obtained in approximately 1 min. The second approach was developed for our research team (Richter et al. 2006) for the determination of perchloroethylene in air, and since it is based in the use of a switching valve and a transfer line, the response is faster than in the previous approach. The global signal in this case is obtained in less than 1 min after sample injection, because the chromatographic column is bypassed. The global signal is obtained by whichever of these methods can be differentiated on the basis of

chemometric data treatment, making possible the presence/absence or estimation of the analyte concentrations. In addition, both screening/confirmatory systems provide the additional advantage that samples containing the analyte near an imposed threshold, or samples in which the presence of the analyte is doubtful, can be subjected in the same system to conventional gas chromatographic mass spectrometry detection for confirmatory purposes.

The aim of this study was to assess a headspace-mass spectrometry/gas chromatography-mass spectrometry (HS-MS/HS-GC-MS) method based on a switching column approach for the screening and determination of BTEX and styrene in comestible oil samples.

EXPERIMENTAL

Reagents

Benzene, toluene, ethylbenzene, xylenes, and styrene 99.9% (Supelco, Bellefonte, PA, USA) were used for calibration purposes in the determination of BTEX and styrene in olive oil samples. Working standards in the interval 1–50 μg/l were prepared by dilution of this standard with methanol (Supelco, Bellefonte, PA, USA). Helium 5.0 UHP (AGA, Chile) was used as a carrier gas and also for the headspace sampler cleaning process.

Nitrogen 4.5 (AGA, Chile) was used for purging gas in the headspace sampler.

Instruments and Apparatus

The HP 7694 headspace autosampler was used to introduce samples automatically directly into the MS or GC-MS system.

The quantitation was performed using a gas chromatograph Hewlett-Packard model 5890 series II coupled to a mass selective detector Fisons Instruments model MD 800.

A 6-port selecting valve (Valco, Houston, TX, USA) was assembled in the upper part of the chromatograph (Figure 1) in order to select subjecting the sample directly to MS (position 1) or to GC-MS (position 2). The transfer line connected to the 6 position valve was a 5 m fused silica capillary (250 μm i.d).

General Procedure

The 20 ml headspace glass vials were filled with 10 ml of sample oil, tightly sealed with PTFE/silicone septa and placed in the autosampler. The samples were heated in the oven at 150°C under stirring, to ensure the equilibration of the analytes between the head space gas phase and oil sample; afterwards, by pressurizing (15 psi) and venting (4.0 psi) the sample vial, the loop (100 μl) connected to the injection valve was filled with the headspace of the sample and injected to the MS or GC-MS, depending of the switching valve position (Figure 1).

For screening HS-MS, the switching valve was kept in position 1 (Figure 1). The injector was kept at 280°C, while the switching valve and chromatographic oven were kept at 150°C. Flow rate of the carrier gas (He) was 1 ml min^{-1}. The MS transfer line was held at 280°C. Determination of analytes was carried out by using

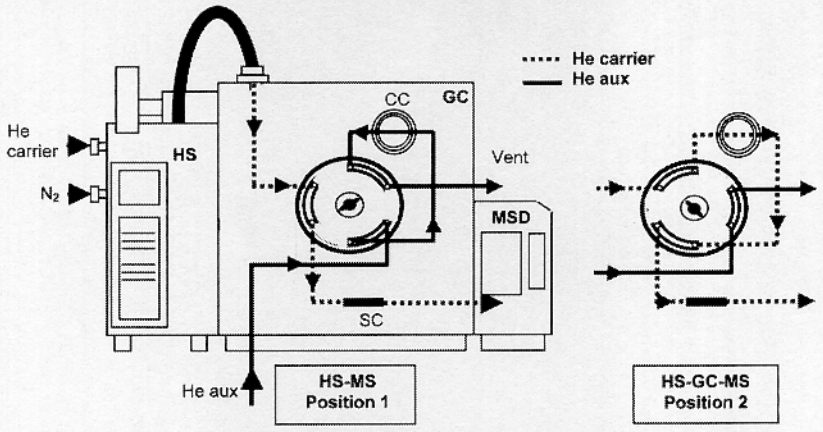

Figure 1. HS-MS switching column system with alternative chromatographic separation. HS, head space sampler; GC, gas chromatograph; CC, chromatographic column; He_{aux}, auxiliary helium gas; SC, silica capillary; MSD, mass selective detector.

the Selective Ion Recording (SIR) mode (m/z: 51, 52, 65, 77, 78, 79, 91, 92, 103, 104, 105, 106), thus it was possible to perform the screening of BTEX and styrene quickly in the samples.

Final confirmation was carried out by GC-MS using a HP-5MS fused silica capillary column (30 m × 0.25 mm i.d., and 0.25 μm film thickness) coated with 5% phenyl–95% methylpolysiloxane. One hundred microliters of HS was injected into the column (position 2 switching valve). The injector temperature was 280°C. Column temperature was maintained at 36°C for 2 min, raised up to 60°C at 5°C min^{-1} held to 60°C for 3 min, and raised up to 85°C at 5°C min^{-1}. A constant flow of helium as the carrier gas, 1.0 ml min^{-1} was used. The MS transfer line was held at 280°C and the quantitation was based on calibration with standard analytes using the mass spectrometric parameters (SIM mode). The ions mentioned for each analyte in Table 1 were used for quantitation (target ion) and confirmation (qualifier ions). The relative abundance ion ratio should match the comparison standard within ±20%.

Study of Variables for Head-Space Sampler

An experimental design (factorial 2^4 plus two central points $16 + 2 = 18$ experiments) was carried out to study the HS variables. For this study, a blank olive oil

Table 1. Selected ions (m/z) used in the SIM mode

Analyte	Target ion (m/z)	Qualifier ions (m/z)
Benzene	78	77, 51
Toluene	91	92
Ethylbenzene	91	106
Xylene	91	106, 105
Styrene	104	103, 78

sample (checked by GC-MS) was spiked with a known standard concentration (50 ng ml^{-1}) containing BTEX and styrene. Studied variables were: headspace vial temperature, vial pressure, equilibration time, and stirring mode.

Recovery Study

Ten olive oil samples were spiked with a known amount (50 ng mL^{-1}) of BTEX and styrene standard. The analytical procedure was followed, the samples were subjected HG-GC-MS and the recoveries were calculated by reference to the calibration graphs.

Determination of BTEX and Styrene in Commercial Oils

Seven commercial samples were analyzed (5 virgin olive oils, 2 vegetable oils). Two of the analyzed samples were packed in plastic containers.

RESULTS AND DISCUSSION

The instrumental manifold used for screening or confirmation of BTEX and styrene is shown in Figure 1. The switching valve in position 1 allows carrying the sample directly to the MS detector through a silica capillary, preventing the sample from passing through the chromatographic column. Contrarily, by switching the selecting valve of the manifold to position 2 (Figure 1) the sample can be inserted into the chromatographic column in order to separate the analytes previous to the MS detection. Under these conditions a typical chromatogram is obtained, which can be used to confirm the presence of the analytes. In the present chromatographic conditions resolution of o-xylene and styrene was not possible. In order to resolve the signals, it is necessary to use a fused silica capillary of 45 m as established previously (Peña et al. 2004), which unfortunately was not available in this work. In any case, it is known that mass spectrometry allows distinguishing each of these compounds according to the mass ratio between target and qualifier ions monitored.

Comparison between both alternatives reveals that the time saved in analysis is evident; the screening signal appears 1 min after injection, whereas the chromatogram is recording in about 15 min.

On the other hand, the lack of resolution in the screening signal could be avoided by means of the specific ion monitoring. For example, in a previous research made by our team (Richter et al. 2006), it was possible to determine directly by MS (without chromatographic separation) perchloroethylene in air in presence of other volatile organic compounds, because this compound possesses a selective ion m/z 166. In the present case (BTEX and styrene), none of the analytes possesses a totally selective mass fragment; in consequence, the screening alternative only allows establishing the presence or absence of the analytes in the sample.

It was observed, as expected, that the sensitivity of the transient signal obtained for both methods depends on the variables associated to the HS sampler. In this context, these variables were studied for each analyte through the screening method taking into account that this alternative provides faster results.

Figure 2 shows the Pareto chart obtained from the experimental design for all the analytes. It is observed that pressure and temperature are the significant

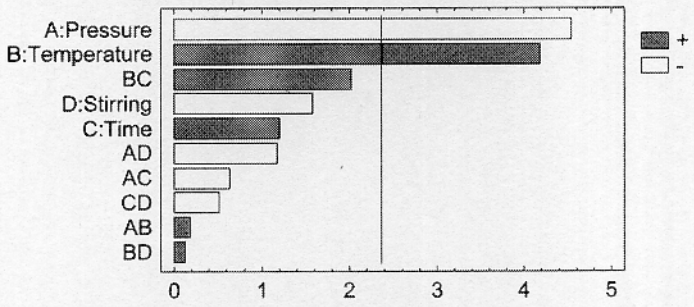

Figure 2. Pareto chart for variables involved in HS-MS for a mixture of BTEX and styrene.

variables. Figure 3 shows the response surface given by the statistical design. Here it is observed that the maximum response for the pressure and temperature is reached at 5 psi and 150°C, respectively. On the other hand, the design gives a maximum response for an equilibration time of 10 minutes and a low stirring mode.

When the variable study was made for each compound separately, the results were equivalent to those obtained for the mixture.

Analytical Features

Under the selected conditions of the headspace variables: pressure, 5 psi; temperature, 150°C; equilibration time, 10 min and a low stirring mode, the analytical features of the HG-GC-MS method were determined. Two linear ranges were established for all compounds ranging from 0.1 to 5 ng mL^{-1} and from 5 to 80 ng mL^{-1} with correlation coefficients (R^2) over 0.997. Regarding to the limits of detection, they were calculated on the basis of the standard deviation of residuals (Sy/x) by constructing calibration graphs in presence of the oil matrix from individual standard solutions containing benzene, toluene, ethylbenzene, xylene, and styrene at concentrations between 1 and 5 ng ml^{-1}. The detection limits, expressed as three times the Sy/x divided by the slope of calibration graphs, were always lower than 0.1 ng mL^{-1}. Reproducibility, expressed as relative standard deviation was lower than 4% and recoveries were in the interval 97–105% (Table 2).

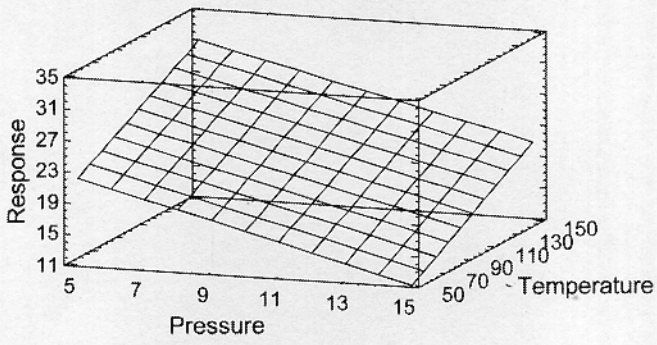

Figure 3. Response surface for variable temperature and pressure in HS-MS for a mixture of BTEX and styrene.

Table 2. Analytical features of the HS-GC-MS method

Analyte	Limit of detection, $ng\,mL^{-1}$	Reproducibility, RDS, %	Recovery %
Benzene	0.07	2	97
Toluene	0.06	2	99
Ethylbenzene	0.05	4	104
Xylenes	0.05	2	105
Styrene	0.08	4	102

Table 3. Application of the screening and confirmative methods to real samples

Sample	HS-MS screening response	Concentration determined by HS-GC-MS, $ng\,mL^{-1}$		
		Benzene	Toluene	Styrene
O1	Negative	–	–	–
O2	Negative	–	–	–
O3	Negative	–	–	–
O4	Negative	–	–	–
O5	Positive	0.17	0.34	0.12
V1	Positive	ND	0.23	0.12
V2	Positive	ND	0.22	0.11

O, Olive oil; V, Vegetable oil; ND, not detected.

Analysis of Real Samples

Seven comestible oil samples (5 olive oils, 2 vegetable oils) were analyzed by the proposed screening-confirmation method. Three of these samples gave rise to a global signal by the screening method. These three samples were also analyzed by HS-GC-MS in order to confirm the screening response provided by the HS-MS alternative. As can be seen in Table 3, positive screening responses were confirmed by HS-GC-MS. It is important to establish that all positive oil samples were packed in plastic bottles, which suggest that residues of these analytes could have migrated from the plastic to the oil matrix.

CONCLUSIONS

A screening/confirmative method by HS-MS/HS-GC-MS was developed for determination of BTEX and styrene in comestible oil samples. The proposed system may be rapidly alternated in order to be used directly as an MS detector for screening purposes or alternatively as a common GC-MS, for confirmation. Consequently it is possible to rapidly screen a large amount of samples which can be confirmed by GC-MS only if required. This integrated screening/confirmation system, through the use of a switching valve, prevents the sample from passing through the chromatographic column. Thus, the lifetime of the column could be extended and screening time is considerably shortened than in other similar approaches.

Some real samples analyzed by this approach contain traces of the analytes, probably because they are packed in plastic bottles from which the analytes can migrate to the oil matrix.

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