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Development of headspace solid-phase microextraction–gas chromatography method for the determination of solvent residues in edible oils and pharmaceuticals

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Abstract

The application of headspace solid-phase microextraction for isolation and enrichment of solvent residues from oils and pharmaceuticals is discussed. The optimal parameters for isolation and preconcentration of common process solvents (hexane, benzene, toluene and selected chloroderivatives of hydrocarbons) were established. Four fiber types (100 μm polydimethylsiloxane (PDMS), 75 μm Carboxen–PDMS, 65 μm PDMS–divinylbenzene and 85 μm polyacrylate) were evaluated to choose the most efficient coating, able to absorb the greatest amount of analytes. GC–flame ionization detection (FID) and GC–electron-capture detection systems were used for quantitative and qualitative analysis, adequately to the appropriate group of the analytes. For all compounds the limit of detection (LOD), linearity, dynamic range, repeatability and intermediate precision were estimated.

Keywords: Solid-phase microextraction; Headspace; Gas chromatography; Solvents residues; Vegetable oils; Pharmaceuticals

1. Introduction

Increasing demands of consumers and higher competition on the market emphasize the importance of food and drug analysis. The accurate assessment of quality and freshness is especially important to ease anxiety and to benefit consumers.

The quality and stability of pharmaceutical substances and products can be affected by the presence of volatile impurities. Volatile impurities (in drug and food products) are often residual solvents used in the synthesis, crystallization that escape during drying or in the extraction (in the case of oils) [1]. These solvents have a negative influence, not only on the quality of oils and pharmaceuticals, but also on human health [2].

Such a situation obligates analysts to develop better, less labour-consuming, faster and more accurate analytical procedures. However, this is not simple, since foodstuffs and pharmaceuticals contain a broad range of components. The ideal method should combine one-step isolation, preconcentration and quantitative determination of analytes, which are usually present on trace levels, independently on the complexity of the matrix. Most of the modern measuring techniques are not sensitive enough to allow direct analysis of the samples without the necessity of isolation and preconcentration of analytes. It should also be realized that each additional step in the analytical procedure increases the probability of analyte loss or sample pollution. Therefore, it is desirable to minimize the number of steps in sample preparation without reducing the quality of the analysis [3–6].

It seems that headspace solid-phase microextraction (HS-SPME) is fulfilling most of the requirements mentioned above. HS-SPME is a fast, universal, sensitive, solventless and economical method for isolation and preconcentration of volatile analytes from complex matrices for gas chromatographic (GC) analysis [7–9].

Since the early 1990s, when the presence of the benzene hydrocarbons in declared virgin olive oils was found, the determination of solvent residues becomes one of the most important tasks in analytical chemistry. There is a lot of classical and unconventional methods for the determination of volatile compounds, residues of solvents, e.g. liquid–liquid

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extraction–GC or GC–olfactometry, multi-dimensional GC, purge and trap–GC [10,11], but for many kinds of foodstuffs and pharmaceuticals the normalized methods are still not available. In that case, sample preparation is necessary to isolate the desired components from complex matrices, because most analytical instruments cannot handle the matrix directly [12]. The most common, simple and recommended by the US Pharmacopeia is static headspace GC method (SHS-GC) [13].

In the authors' laboratory, universal, low time consuming, ecological and relatively cheap method, alternative to SHS-GC employing headspace analysis connected with solid phase microextraction and GC was developed.

2. Experimental

2.1. Materials

Fifteen cubic centimeter vials, phenolic caps and PTFE–silicone septa from Supelco were used in all analysis. Four fiber types from Supelco: 100 μm polydimethylsiloxane (PDMS; catalog no. 57300-U), 75 μm Carboxen (CAR)–PDMS (catalog no. 57318), 65 μm PDMS–divinylbenzene (DVB; catalog no. 57310-U) and 85 μm polyacrylate (PA; catalog no. 57304), with manual sampling holder (Supelco), were used. The samples were thermostated in a home-made heating block, connected with the stir plate (Supelco). Standard mixtures of selected solvents were prepared in refined rape oil (Olvit). The following solvents were used: hexane (Fluka, for UV spectroscopy), benzene (POCH, Lublin, Poland, for liquid chromatography), toluene (POCH, Lublin, pure for analysis), trichloromethane (POCH, Lublin, pure), 1,1,1-trichloroethane (POCH, Gliwice, Poland, pure), tetrachloromethane (POCH, Gliwice, pure for analysis), trichloroethene (POCH, Gliwice, pure), tetrachloroethene (Austranal Prepärate, pure). Methanol (POCH, Gliwice, pure) was used for cleaning the syringe (Hamilton) and other laboratory glass. Helium was used as the carrier gas, ultra-clean nitrogen as make-up gas for electron-capture detection (ECD) and argon for filling the vials.

2.2. Instrumentation

All GC experiments were performed, using a Perkin-Elmer Auto System XL GC coupled with a flame ionization detection (FID) system or ECD system, Perkin-Elmer. The Rtx-1 capillary column (30 m × 0.32 mm i.d., 5 μm film thickness; Restek) and Rtx-5 capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness; Restek) were used. The preconcentration step was performed using a Supelco manual holder with the selected fiber.

2.2.1. Temperature program for hydrocarbons (hexane, benzene, toluene)

The injector, working in splitless mode, was equipped with a glass liner with an inner diameter of 1 mm and was kept at 250 °C. An initial oven temperature of 60 °C was ramped at 20 °C min^{-1} to 150 °C and held for 4.5 min. Helium was used as the carrier gas with a flow rate of 1.35 mL min^{-1}. Gases for detector: hydrogen 45 mL min^{-1} and air 400 mL min^{-1}. The operating temperature of the FID system was 250 °C.

2.2.2. Temperature program for chlorinated hydrocarbons

The injector (100:1 split) equipped with a glass liner with an inner diameter of 1 mm was kept at 200 °C. The initial oven temperature was 45 °C and then ramped at 5 °C min^{-1} to 70 °C, not held and once again ramped at 15 °C min^{-1} to 120 °C and held for 0.5 min. Helium was used as the carrier gas with a flow rate of 1.5 mL min^{-1}. Nitrogen was used as make-up gas with a flow rate of 30 mL min^{-1}. The operating temperature of the ECD system was 280 °C.

2.3. Sample preparation

Standard mixtures, with concentration of 1 and 50 mg kg^{-1}, used to optimize the extraction process, were prepared by adding the exact amount of chosen solvents to the determined volume of refined oil. The optimization was made using the systematic repetition method. It means that by systematical change of the various operating parameters, such as temperature, time or phase ratio, the influence of these parameters on the extraction efficiency can be measured, by noting changes in peak areas [14]. In the result optimal operating parameters, assuring the equilibrium between compounds in a liquid sample and in a headspace, and between a headspace and a fiber coating were established: heating 15 cm^3 vials at 80 °C through 15 min, for hydrocarbons, and at 30 °C through 7 min for chlorinated hydrocarbons. To reduce the influence of the environment, the vials were filled with pure argon.

3. Results and discussion

3.1. GC separation

Preliminary experiments with model solutions of hexane, benzene, toluene (10 mg kg^{-1} of each component) and of the trichloromethane, 1,1,2-trichloroethene, 1,1,1-tricholroethane, tetrachloromethane and tetrachloroethene (1 mg kg^{-1} each) in refined oil were performed to ensure complete separation of the selected solvents. In the real samples, compounds were identified by comparison of the measured retention values of a given solvent with the relevant values from the chromatograms of the standard solutions. The separation of all hydrocarbons was accomplished in 9 min and of chlorinated hydrocarbons in 10.80 min.

The splitless injection mode was applied to hydrocarbons, while for chlorinated hydrocarbons a 100:1 split was set. Figs. 1c and 2d show the chromatograms for a model mixture of selected solvents using the chosen fiber.

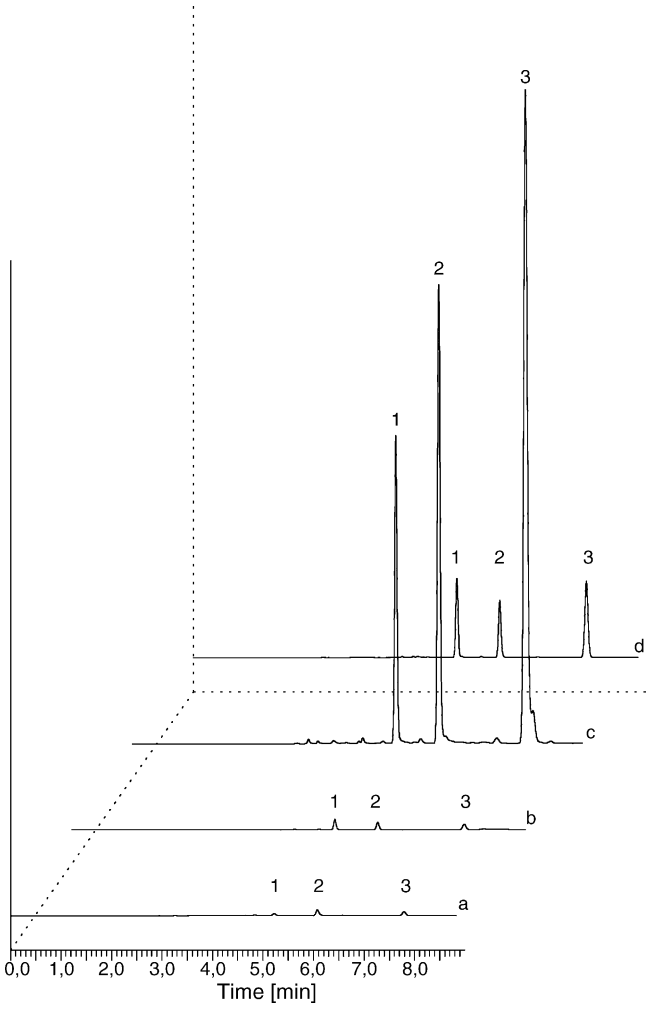

Fig. 1. Chromatogram of refined oil spiked with 10 mg kg^{-1} of each standard compound obtained using various fibers (a–d); (a) fiber PA (b) fiber PDMS; (c) fiber CAR–PDMS; (d) fiber PDMS–DVB. The intensity of the scale in all chromatograms is the same. *Extraction conditions*: temperature, 80 °C; time, 15 min; sample volume, 5 cm^3 in 15 cm^3 vial: (1) hexane, (2) benzene, (3) toluene.

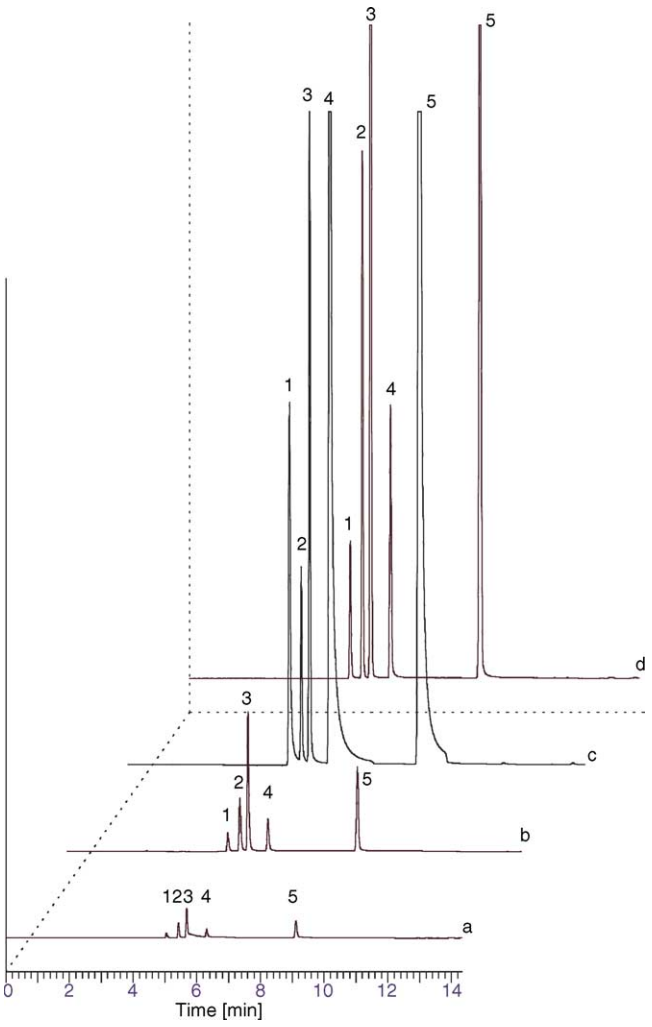

Fig. 2. Chromatogram of refined oil spiked with 1 mg kg^{-1} of each standard compound obtained using various fibers (a–d); (a) fiber PA; (b) fiber PDMS; (c) fiber CAR–PDMS; (d) fiber PDMS–DVB. The intensity of the scale in all chromatograms is the same. *Extraction conditions*: temperature, 30 °C; time, 7 min; sample volume, 6 cm^3 in 15 cm^3 vial: (1) trichloromethane; (2) 1,1,1-trichloroethane; (3) tetrachloromethane; (4) trichloroethene; (5) tetrachloroethene.

3.2. Isolation/preconcentration step

Because of a trace level of the analytes, an isolation and enrichment step is necessary before their analysis. Several parameters have to be optimized during development of the method. The yield and repeatability of the extraction process is affected by the type of fiber coating, the temperature of extraction, the time needed to achieve the equilibrium between the liquid sample and the headspace in the headspace vial (phase ratio—sample to headspace phase) [15,16], and between the analytes in headspace and in the polymer coating on the fused silica fiber.

3.2.1. Fiber choice

Four fiber types (Table 1) [17] were evaluated to choose the one able to absorb the greatest amount (expressed as peak areas) of hexane, benzene and toluene at 80 °C during 15 min extraction and trichloromethane, 1,1,1-trichloroethane, tetrachloromethane, trichloroethene, tetrachloroethene at 30 °C during 7 min. A standard mixture of hexane, benzene and toluene in oil at a concentration of 50 mg kg^{-1} each, was used. The concentration of chloroderivatives in standard mixture was 1 mg kg^{-1}. Each extraction was repeated three times.

Table 1
Tested fibers

Stationary phase	Polarity	Film thickness (μm)	Hub description	Maximum temperature (°C)
PDMS	Nonpolar	100	Red	280
PDMS–DVB	Semi-polar	65	Blue	270
PA	Polar	85	White	320
CAR–PDMS	Semi-polar	75	Black	320

For hydrocarbons two coatings, i.e. a PA and a PDMS showed small extraction efficiencies. Fiber coated with PDMS–DVB allowed all compounds to be detected, as did a CAR–PDMS fiber, but the latter provided much larger peak areas (Fig. 1), so the CAR–PDMS fiber was chosen for subsequent analysis. For chloroderivatives the PDMS–DVB fiber was chosen for extraction (Fig. 2) because of the highest extraction efficiencies.

3.2.2. Time and temperature optimization

The HS-SPME process conducted at room temperature, for hydrocarbons, resulted in poor extraction efficiencies of higher boiling analytes, such as benzene and toluene. It can be explained by relatively high partition coefficients (sample/gaseous phase and gaseous phase/film of the fiber). To enhance the transfer of high-boiling compounds into the headspace and then to the fiber coating, an extraction temperature of 80 °C was chosen after investigation. Moreover, at higher temperatures, the time needed to achieve equilibrium between the liquid sample and the gaseous phase, and between the gaseous phase and the CAR–PDMS fiber was significantly reduced. The optimum equilibrium time was determined by analyzing of 5 mL samples at different exposure times and finally, the 15-min period was found to be sufficient for hexane, benzene and toluene.

For the second group of compounds, an increase of temperature resulted in a decrease of peak areas for all compounds. The best efficiency of extraction was obtained at so called "room conditions", but instability of these conditions cause that repeatability of measurements was lower. In that case, a temperature of 30 °C was chosen for 7 min extraction of 6 cm^3 samples.

3.2.3. Phase ratio

To investigate the effect of the phase ratio on the concentrations of the selected solvents in the gas phase, and consequently in the fiber film, the 15 cm^3 headspace vials were filled with different volume of standard mixtures (2–9 cm^3). The samples were equilibrated for 15 min at 80 °C (for hydrocarbons) and for 7 min at 30 °C (for chlorinated hydrocarbons). It can be seen that for a 15 cm^3 vial the extracted amount slightly increases if the sample volume is 5 cm^3 for the first group of analytes and 6 cm^3 for the second.

3.2.4. Desorption process

After isolation and preconcentration on the fiber, the analytes are directly move to the hot injector port, where they are desorbed from the fiber.

The temperature of desorption was the same as the temperature of the GC injector, so it was 250 °C for hexane, benzene and toluene (CAR–PDMS fiber) and 200 °C for chlorinated hydrocarbons (PDMS–DVB fiber). The desorption times were optimized like the other parameters. It was found that 2 min (for hydrocarbons) and 1 min (for

Table 2
Equations of calibration curves and correlation coefficients for selected compounds (hexane, benzene, toluene, trichloromethane 1,1,1-trichloroethane, tetrachloromethane, trichloroethene, tetrachloroethene)

Compounds	Concentration range (mg kg^{-1})	Equation of calibration curves	Correlation coefficient
Hexane	0.008–0.5	$y = 70800x + 2240$	0.9966
	100–1000	$y = 83.0x + 15200$	0.9154
Benzene	0.008–0.5	$y = 110000x + 3580$	0.9863
	100–1000	$y = 351x + 452000$	0.9490
Toluene	0.008–0.5	$y = 145000x + 3450$	0.9991
	100–1000	$y = 750x + 2 \times 10^6$	0.9781
Trichloromethane	0.008–0.125	$y = 92700x - 33.1$	0.9999
	0.125–1	$y = 84100x + 1340$	0.9999
	1–8	$y = 61700x + 26800$	0.9987
1,1,1-Trichloroethane	0.004–0.06	$y = 152000x - 153$	0.9987
	0.06–1	$y = 150000x - 382$	0.9999
	1–8	$y = 126000x + 28300$	0.9999
Tetrachloromethane	0.002–0.016	$y = 218000x + 115$	0.9994
	0.016–0.5	$y = 242000x - 457$	0.9999
	0.5–2	$y = 301000x - 33300$	0.9997
	2–8	$y = 381000x - 224000$	0.9991
Trichloroethene	0.002–0.008	$y = 182000x - 72.5$	0.9994
	0.008–0.125	$y = 189000x - 207$	0.9999
	0.125–1	$y = 174000x + 2630$	0.9999
	1–8	$y = 128000x + 63000$	0.9998
Tetrachloroethene	0.0005–0.004	$y = 584000x + 39.0$	0.9998
	0.004–0.016	$y = 413000x + 122$	0.9992
	0.016–0.25	$y = 380000x + 672$	0.9997
	0.25–8	$y = 451000x - 17300$	0.9998

Table 3
Validation parameters for hexane, benzene and toluene

Parameter	Compounds		
	Hexane	Benzene	Toluene
Linearity (mg kg^{-1})	0.008–0.5 100–1000	0.008–0.5 100–1000	0.008–0.5 100–1000
Dynamic range (mg kg^{-1})	0.002–10000	0.002–7000	0.002–5000
Precision[a]			
Repeatability (%)	4.7	3.6	5.1
Intermediate precision (%)	6.9	3.9	5.9
Detection limit (mg kg^{-1})	0.002	0.002	0.002
Quantification limit (mg kg^{-1})	0.006	0.006	0.006

[a] Precision for model solution, 1 mg kg^{-1}.

chlorinated hydrocarbons) was enough for complete desorption. In spite of long desorption times, causes by the thick-film coated fibers chosen to SPME, the peaks did not tailed. But to be sure, that desorption was complete the fibers were checking-cleaned (10 min at 200 °C in the injector) after each three consecutive injections to avoid sample carry-over.

3.3. Quantification

Due to the fact that HS-SPME analysis is an equilibrium method, even when a portion of analytes is injected to the GC system, it is strictly connected to the concentration of the analytes in the sample [18]. For quantitative analysis it is indispensable to perform a calibration step.

The determined calibration curves and determination coefficients, for selected hydrocarbons, in two concentration ranges (0.008–0.5 and 100–1000 mg kg^{-1}) and for chlorinated hydrocarbons (in three or four different ranges, depending on the compound) are presented in Table 2.

Finally, the worked out method was validated. The following parameters [19–21] were determined for all compounds:

(i) the linearity;
(ii) dynamic range;
(iii) precision;
(iv) repeatability;
(v) intermediate precision;
(vi) limit of detection (LOD); and
(vii) limit of quantification (LOQ).

The validation parameters are summarized in Tables 3 and 4.

4. Conclusions

A rapid, sensitive and precise analytical method employing HS-SPME and capillary GC has been developed for determination of the solvent residues in vegetable oils. The method allows the determination of hexane, benzene, and toluene from one sample using a SPME (CAR–PDMS)–GC–FID system and C_1–C_2 chloroderivatives using a SPME (PDMS–DVB)–GC–ECD system. The extraction conditions were optimized: 15 min exposition at 80 °C for hydrocarbons and 7 min at 30 °C for chlorinated hydrocarbons. Plotting the calibration curves made quantification possible. The limits of detection are as follows: 0.002 mg kg^{-1} for hexane, 0.002 mg kg^{-1} for benzene, 0.002 mg kg^{-1} for toluene, 0.001 mg kg^{-1} for trichloromethane, 0.003 mg kg^{-1} for 1,1,1-trichloroethane, 0.0002 mg kg^{-1} for tetrachloromethane, 0.0006 mg kg^{-1} for trichloroethene, 0.0006 mg kg^{-1} for tetrachloroethene.

The repeatability of the analysis (expressed as a standard deviation) for concentration of 1 mg kg^{-1} oscillate from 1.5% for tetrachloroethene and trichloroethene to 5.1% for toluene.

The developed method can be successfully applied for routine determination of solvent residues in real samples, for example in edible oils in a wide range of concentrations.

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Table 4
Validation parameters for trichloromethane, 1,1,1-trichloroethane, tetrachloromethane, trichloroethene, tetrachloroethene

Parameter	Compounds				
	$CHCl_3$	$C_2H_3Cl_3$	CCl_4	C_2HCl_3	C_2Cl_4
Linearity (mg kg^{-1})[a]	0.008–8	0.004–8	0.002–8	0.002–8	0.004–8
Dynamic range (mg kg^{-1})	0.001–45	0.003–30	0.0002–10	0.0006–35	0.0006–8
Precision[b]					
Repeatability (%)	2.7	3.7	1.9	1.9	3.5
Intermediate precision (%)	3.7	4.9	4.1	3.5	4.3
Detection limit (mg kg^{-1})	0.001	0.003	0.0002	0.0006	0.0006
Quantification limit (mg kg^{-1})	0.003	0.009	0.0006	0.0018	0.0018

[a] Linearity, details in Table 2.
[b] Precision for model solution, 1 mg kg^{-1}.

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