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Head-space solid phase microextraction for the GC-MS analysis of terpenoids in herb based formulations

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Abstract Head-Space Solid Phase Microextraction (HS-SPME) has been employed for sampling of volatile components and their volatile decomposition products occurring in herbal medicines and herb extracts with subsequent injection into a gas chromatographic column. The identification and quantification was performed by coupled gas chromatography – mass spectrometry (GC-MS) with classical splitless injection, electron impact ionization and a quadrupole mass analyzer. As fast and inexpensive technique for the isolation of organic analytes HS-SPME with GC-MS can be successfully employed for the quality control of herbal medicines and other formulations containing herb extracts. Analytical results with satisfying accuracy and precision are given.

1 Introduction

To determine selected analytes at lower and lower levels in a variety of samples (gases, liquids, solids) of increasingly complex matrices [1], techniques, like: Liquid–Liquid Extraction (LLE), Solid Phase Extraction (SPE), Gas Extraction (Static Head Space – HS, Purge & Trap – P&T, Closed Loop Stripping Analysis – CLSA, Thin Layer Head Space – TLHS) and Supercritical Fluid Extraction (SFE) have been employed widely. The advantages and limitations of these techniques are dealt with in detail in reviews [2, 3]. The necessity to use solvents (extraction medium in LLE, elution medium in SPE) is a self-evident disadvantage of some of the above techniques. The fact that solvents and gases used for purging and for thermal desorption must be of very high purity is an additional drawback.

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A new interesting and promising approach, named, Solid-Phase Microextraction, was developed by Pawliszyn. Although quite new, it has already experienced a number of applications reviewed in 1994 [4]. They include determinations of various classes of analytes in solid, liquid and gaseous samples of different composition and matrix complexity (Table 1).

In the case of liquid samples, the technique can be used in two different ways : by plunging the extraction fiber directly into a sample, or by placing it in the head-space being at equilibrium with a sample. Profound mathematical description of both approaches has already been given by Pawliszyn et al. in a number of papers [5, 9, 11, 12, 13].

The former approach is experimentally simpler and is applicable to a wider range of analytes while the latter (Head-Space Solid Phase Microextraction) is limited to volatile compounds but in a wide variety of sample matrices.

The sensitivities of both techniques are similar for typical analytes of medium volatility. However, HS-SPME has a number of advantages over direct SPME.

In general, matrices such as herbal medicines and other herb based formulations are rather complex what makes their analysis very difficult. Due to its characteristics, HS-SPME should prove very useful for the determination of volatile organic compounds in such samples. A procedure to determine β-pinene, β-myrcene, limonene and menthol in herbal medicines was developed using HS-SPME for sampling and injection and GC-MS for identification and quantification.

2 Experimental

2.1 Materials and standard solutions. Common herb pharmaceutical formulations subjected to the analysis are as follows:
- stomach drops (Herbapol, Kraków, Poland) containing *T-ra Valerianae, T-ra Amara, T-ra Menthae and T-ra Hyperici*

Table 1 Application of different fibers for solid-phase microextraction

Type of fiber for SPME	Application	Reference
– uncoated	– PCBs	5
	– BTEX (benzene, toluene, ethylbenzene, xylenes)	5
– coated with polyimide	– Volatile organohalogen compounds (1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene)	5
– coated with 85 μm polyacrylate[a]	– Phenol and derivatives	6, 7
	– N and P herbicides	8
– coated with liquid crystals	– PAH (Polynuclear Aromatic Hydrocarbons)	9
	– Dioxin congeners	
– coated with 7 μm polydimethylsiloxane[a]	– PAH	8
	– Phthalates	
	– Organochlorine pesticides	8
– coated with 100 μm polydimethylsiloxane[a]	– BTEX (benzene, toluene, ethylbenzene, xylenes)	9, 10, 11, 12, 13
	– Volatile organohalogen compounds (1,1,1-trichloroethane, dichloromethane, tetrachloromethane, 1,1,2,2-tetrachloroethane, 1,2- and 1,3-dichloropropanes, chloroform, bromodichloromethane, dibromochloromethane, bromoform, tri- and tetrachloroethylene)	14, 15
	– Flavour components	16
	– PAH	11, 17
	– S and P pesticides	18
– carbon fiber	– N and P pesticides	17

[a] Fibers commercially available

- peppermint drops (Herbapol, Kraków, Poland) containing *T-ra Menthae* and *Ol. Menthae*
- CRAVISOL (Herbapol, Klęce, Poland) containing extract *Crataegi fluidum, Intractum Melissae and Intractum Visci*
- peppermint oil (Herbapol, Lublin, Poland)
- anise oil (Herbapol, Lublin, Poland)

Standard solutions of β-pinene, β-myrcene, limonene and menthol used for quantitative analysis were prepared in ultra resi-analysed methanol (J.T. Baker Inc., Philipsburg, USA). For the quantitative analysis the standard addition method was employed.

2.2 Instrumentation. For the isolation of analytes an SPME holder for manual sampling (SUPELCO Inc., Bellefonte, PA, USA) with a 10 mm fiber coated with a 100 μm layer of polydimethylsiloxane as stationary phase (SUPELCO Inc., Bellefonte, PA, USA) was used. Before use, the fiber was conditioned by baking at 260 °C under a flow of purified nitrogen for 3 h.

The final analysis was performed by GC-MS on a Fisons GC-8000 gas chromatograph (Milano, Italy) coupled with a Fisons MD-800 quadrupole mass spectrometer (Milano, Italy). Chromatographic and mass spectrometric conditions are given in Table 2.

2.3 Analytical procedure. Solid phase microextraction was performed by placing the fiber (by piercing the septum of a sample vial with the SPME apparatus needle) in the head-space of an analyzed sample (3 ml) in a closed vial of 4 ml volume kept at a temperature of 20 ± 0.2 °C. Different extraction times from the 30 s to 40 min were applied to optimize the extraction time.

After the extraction, the SPME apparatus was quickly transferred to a GC splitless injector maintained at 250 °C and immediately, after the needle penetrated the injector septum, the fiber was extended inside the injection port and injection started. The desorption time was varied within 2 s and 5 min. The chromatographic column was thermostatted at 30 °C during desorption and for an additional 2 min after pulling out the needle from the injection port followed by the temperature programming (see Table 2).

Table 2 GC-MS parameters

GC conditions	
Type of GC	GC-8000 (FISONS Instruments, Milano, Italy)
Injection port	splitless (liner 1.2 mm I.D.)
Temperature of injector	250 °C
Desorption time	from 10 to 300 s
Precolumn	2 m × 0.32 mm I.D. phenyl-methyl deactivated (RESTEC, Bellefonte, PA, USA)
Analytical column	30 m × 0.32 mm I.D. DB-5ms 0.5 μm fim thickness (J&W, Folsom, Canada)
Temperature program	30 °C (2 min hold) to 120 °C at 2 °C/min to 220 °C at 5 °C/min
Temperature of GC-MS interface	270 °C (direct connection GC-MS)
Carrier gas	He (22cm/s) 99.999%
MS conditions	
Type of MS	quadrupole mass detector MD-800 (FISONS Instruments, Milano, Italy)
Ionisation mode	electron impact (70 eV)
Scan mode	full scan monitoring (from 35 to 350 m/z)
Scan time	1 s
Data acquisition	LAB-BASE-2, NBS Library, (FISONS Instruments, Milano, Italy)

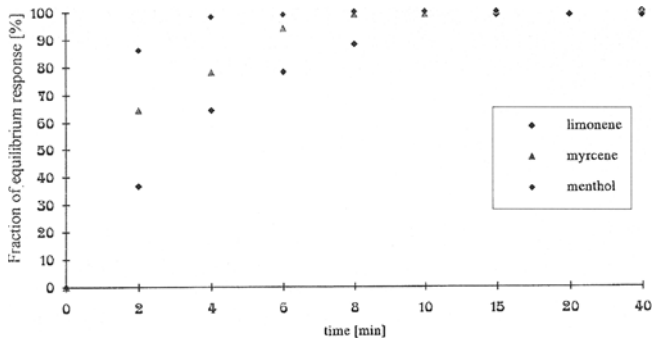

Fig. 1 Fraction of equilibrium response [%] for selected compounds (limonene, myrcene and menthol) in a sample of stomach drops (each point is average of three measurements)

3 Results and discussion

First, the time necessary to quantitatively desorb analytes from a fiber stationary phase in the injection port of the chromatograph was studied. All the analytes of interest desorbed quantitatively at 250 °C in 30 s. A desorption time of 60 s was selected for further experiments.

The effect of the extraction time in SPME using fiber sorption of analytes was investigated for the head-space phase of stomach drops (Fig. 1; each point represents an average of three measurements). The investigations were carried out for extraction periods ranging from 30 s to 40 min. The plot (Fig. 1) of the mass of analytes sorbed on the fiber versus the extraction time reaches a plateau in less than 10 min. Therefore, equilibrium partition of these analytes among the liquid sample, the head-space and the fiber should be reached within this time. Since the stomach drops are the most complex matrices of all the formulations studied, the conclusion should be also valid for other samples in which the same or more volatile components are being determined.

Four different herbal medicines and herb extracts were analyzed using optimum extraction and desorption conditions, as given above. Exemplary chromatograms (chromatographic parameters in Table 2) are shown in Figs. 2 and 3.

The quantitative analysis was done by the standard addition method. The methanolic standard solution of analytes was added to samples and peak areas of such calibration solutions were compared with those of

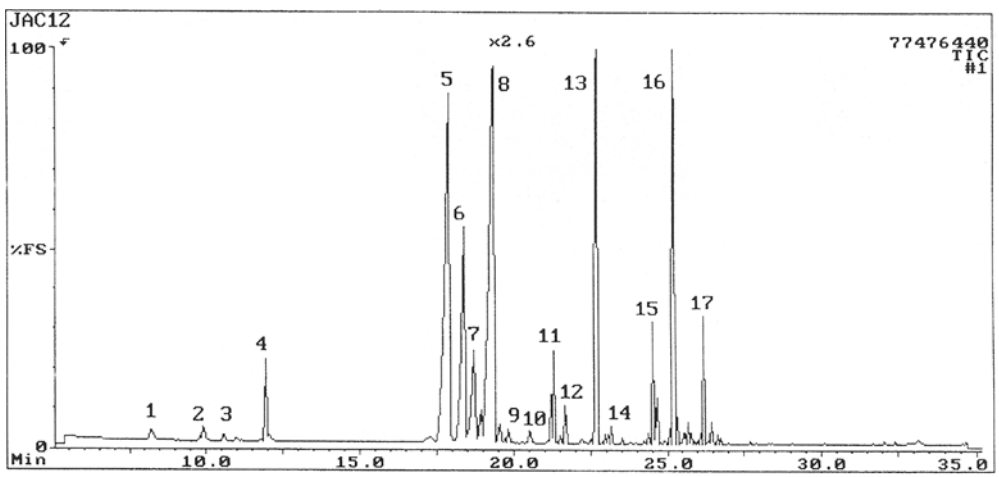

Fig. 2 HS-SPME chromatogram of stomach drops (reconstructed ion chromatogram). Identified compounds: *1* 4-carene, *2* β-pinene, *3* β-myrcene, *4* limonene, *5* 5-menthyl-2-(1-methylethyl) cyclohexanone (cis), *6* 5-menthyl-2-(1-methylethyl)-cyclohexanone (trans), *7* neomenthol, *8* menthol, *9* menthone, *10* menthyl acetate, *12* caryophyllene, *15* aminopyrine, *16* butylethyl phthalate, *17* butylpropyl phthalate

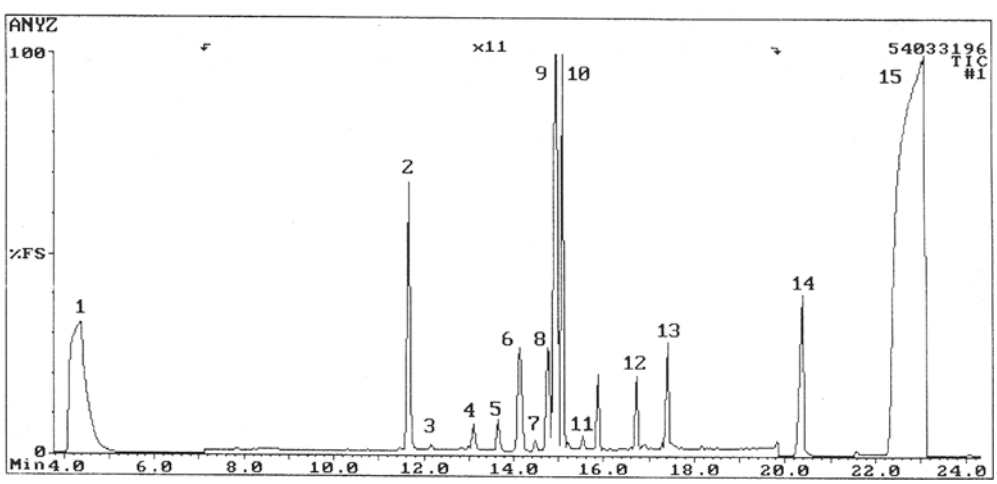

Fig. 3 HS-SPME chromatogram of anise drops (reconstructed ion chromatogram). Identified compounds: *1* chloronitromethane, *2* 4-carene, *3* camphene, *4* β-pinene, *5* β-myrcene, *9* limonene, *14* estragol, *15* trans-anethol

Table 3 Results of determinations

Herbal Medicines	Concentration of compounds in herbal medicines [μg/cm^3] and RSD [%] for n = 7			
	β-myrcene	β-pinene	limonene	menthol
Anise drops	4.3 (2.9%)	7.6 (3.9%)	41.2 (3.1%)	0.21 (4.5%)
Peppermint drops	11.4 (3.0%)	13.5 (4.2%)	56.2 (3.2%)	29.3 (3.3%)
CRAVISOL	ND	ND	74.2 (3.7%)	2.5 (4.9%)
Stomach drops	7.5 (3.9%)	3.6 (3.2%)	23.5 (3.4%)	14.4 (5.4%)

ND = not detected (below 10 ppb)

corresponding samples. For each solution five injections were made. Identification was performed by library search. Reverse fit values ranging from 0.97 to 0.98 (1.00 means ideal fit) indicate that identification data are reliable.

The results of quantitative and qualitative analysis for all the samples are given in Table 3. The statistical evaluation of the quantitative data shows that the HS-SPME-GC-MS method developed enables the determination of volatile nonpolar or slightly polar components of herbal medicines with a relatively good precision. Relative standard deviations varied from 2.9% to 5.4% (number of measurements n = 7 in each case). The detection limits (concentration equivalent to a peak height 3 times of the baseline noise) of the procedure for the compounds analyzed were in the order of a few μg/kg. The method should be applicable to other formulations of this type, as well.

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References

1. Namieśnik J, Biziuk M, Chrzanowski W, Wardencki W, Zygmunt B (1995) Chem Anal (Warsaw) 40:115–141
2. Namieśnik J, Górecki T, Biziuk M, Torres L (1990) Anal Chim Acta 237:1–60
3. Pool SK, Dean TA, Oudsema JW, Pool CF (1990) Anal Chim Acta 236:3–42
4. Zhang Z, Yang MJ, Pawliszyn J (1994) Anal Chem 66:844A–853A
5. Arthur CL, Pawliszyn J (1990) Anal Chem 62:2145–2148
6. Buchholz KD, Pawliszyn J (1993) Environ Sci Technol 27:2844–2848
7. Buchholz KD, Pawliszyn J (1994) Anal Chem 66:160–167
8. Boyd-Boland AA, Chai M, Luo YZ, Zhang Z, Yang MJ, Pawliszyn J, Górecki T (1994) Environ Sci Technol 28:569A–574A
9. Arthur CL, Potter DW, Buchholz KD, Motlagh S, Pawliszyn J (1992) LC-GC Intern 5:8–14
10. Potter DW, Pawliszyn J (1992) J Chromatogr 625:247–255
11. Zhang Z, Pawliszyn J (1993) Anal Chem 65:1843–1852
12. Zhang Z, Pawliszyn J (1993) JHRC 16:689–692
13. MacGillivray B, Pawliszyn J, Fowlie P, Sagara C (1994) J Chromatogr Sci 32:317–322
14. Chai M, Arthur CL, Pawliszyn J, Belardi RP, Pratt KF (1993) Analyst:1501–1505
15. Czerwiński J, Wolska L, Biziuk M, Namieśnik J (1994) Proceedings of Conference "Organic Compounds in the Environment and Methods of their Determination", Jachranka, Poland, May 24–26, 1994, pp 143–144
16. Mani V, Woolley C (1994) Proceedings of Sixteenth International Symposium on Capillary Chromatography, September 27–30, 1994, Riva del Garda, Italy, vol II, pp 1176–1179
17. van der Kooi MME, Noji THM (1994) Proceedings of Sixteenth International Symposium on Capillary Chromatography, September 27–30, 1994, Riva del Garda, Italy, vol II, pp 1087–1098
18. Eisert R, Levsen K (1994) Proceedings of Sixteenth International Symposium on Capillary Chromatography, September 27–30, 1994, Riva del Garda, Italy, vol II, pp 1387–1395