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Headspace SPME method development for the analysis of volatile polar residual solvents by GC-MS

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Abstract

A solid-phase microextraction (SPME) method has been developed and optimized for the polar residual solvent determination in pharmaceutical products. Five different polymer-coated fibers were investigated and the Carboxen/polydimethylsiloxane was found to be the most sensitive for all components. Two Headspace SPME methods were developed and optimized: one for the extraction from aqueous solutions, and the other for the extraction from organic solutions (*N*,*N*-dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO). The optimum equilibration time for all components and all systems was 30 min. It was found that the sample headspace volume has an important effect on method sensitivity and precision. At low headspace volumes (less than one-third of the vial volume), sensitivity improves but at the same time, precision worsens. For 10 ml headspace vials, the optimum headspace volume was found to be 3 ml. The total volatile organic content in the sample also has an important effect on method sensitivity and precision. At low organic content, sensitivity increases but precision drops significantly. Over 0.5% volatile organic content in the sample, the system becomes unstable due to stationary phase swelling by the organic components, and also the sensitivity of the method is drastically reduced. The optimum range for total volatile organic content was found to be between 0.01 and 0.1%. The added Na_2SO_4 quantity increases the extraction yield. It was found that slightly pressurizing the headspace vial improves the sensitivity of the method by a factor of 2. For the organic system, it was found that the addition of 100 μl DMSO or DMF to 50 mg drug substance and slightly pressurizing the headspace vial gives good results in terms of sensitivity and reproducibility. The measured detection limits were between 0.4 and 200 ng/ml, and the relative standard deviation data were between 2 and 9%. The Headspace SPME from aqueous solutions was found to be ten times more sensitive than Immersion SPME and Headspace SPME from organic solutions. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Extraction methods; Solid-phase microextraction; Residual solvent analysis

1. Introduction

The residual solvent determination in drug substances, excipients or drug products is known to be one of the most difficult and demanding ana-

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lytical tasks in the pharmaceutical industry. Furthermore, the determination of polar residual solvents from pharmaceutical preparations continues to present an analytical challenge mainly because these compounds are quite difficult to remove from water or other polar solvents.

In the past few years, solid-phase microextraction (SPME) has gained acceptance for solventless extraction of water samples. In SPME, the analytes are extracted into a stationary phase that is attached to a length of fused silica fiber [1]. The fiber is contained into a microsyringe for protection and ease of sampling. In SPME, an exhaustive extraction does not occur, but an equilibrium is established, as analytes partition between the stationary phase and the aqueous phase, or its headspace phase occurs. By sampling from the headspace above the sample matrix, SPME can extract a wide range of organic compounds from various matrices [2–12].

The present work describes an approach for Headspace SPME method development for the polar residual solvent analysis from pharmaceutical preparations [13–15]. The most important step for successful residual solvent analysis is the development of a stable, selective, sensitive and precise method of analysis of compounds with different volatility and polarities. The gas chromatography-mass spectrometry (GC-MS) technique provides the required selectivity. Furthermore, the development of a Headspace SPME method requires careful and extensive optimization of the main experimental parameters involved in the extraction and desorbtion process.

Table 1
The concentrations of standard solutions

Component	Stock solution (mg/ml)
2-Propanol	3.6
1-Propanol	10.0
Ethylmethylketone	4.8
t-Butanol	3.9
Ethylacetate	5.9
Methylacetate	5.6
Diisopropylether	2.2
t-Butylmethylether	2.2
Acetone	3.6

Earlier [14], we found that extraction time, headspace volume and total organic content have a critical influence on the extraction yield needing to be extensively optimized every time a SPME method is developed. At the same time, the chromatographic conditions, the injector desorbtion temperature and the injection depth also have significant influence on the reliability of the analytical data but, once optimized, the found optimum parameters can be employed for other SPME analytical methods.

We also investigated the residual solvent analysis for drug substances that are not soluble in water, and we developed a Headspace SPME method that uses organic solvents instead of water.

The Headspace SPME method was compared with an Immersion SPME method. At the same time, we were interested in the possibility of employing the Headspace SPME sample preparation method from organic solutions from the point of view of suitability for the residual solvent determination method in pharmaceutical products.

2. Experimental

2.1. Samples and standards

Individual organic solvents (1-propanol, 2-propanol, *t*-butanol, diisopropylether, *t*-butylmethylether, acetone, ethylmethylketone, methylacetate and ethylacetate) were obtained from Merck (E. Merck, 64271 Darmstadt, Germany) and were of 99.5% purity. A single standard of the nine organic solvents was prepared in Merck Uvasol spectroscopic grade methanol (E. Merck) and was used for this study as a primary stock solution at the concentrations presented in Table 1. Working standards were prepared by dilution of 1 ml stock solution in 10 ml (first dilution); respectively, 1 ml from the first dilution was added in 10 ml methanol (second dilution). Headspace aqueous standard solutions were prepared in 10 ml headspace amber vials by mixing 6.8 ml deionized water with 1 g natrium sulphate. Aqueous sample solutions were also prepared by mixing 6.8 ml deionized water with 1 g natrium

sulphate and with 50 mg proprietary drug substance. Organic solvents from the working standards were spiked into the vials to produce the desired concentration.

Immersion standard solutions were prepared in 10 ml headspace amber vials by spiking the working standards in 9 ml deionized water.

Dimethyl sulfoxide and *N*,*N*-dimethylformamide were obtained from Merck (E. Merck) and were of 99.5% purity. Organic standard solutions were prepared in 10 ml headspace amber vials by adding 100–150 μl organic solvent and then spiking the components from the working standards into the vials to produce the desired concentration. Organic sample solutions were prepared in 10 ml headspace amber vials by mixing 100–150 μl organic solvent with 50 mg proprietary drug substance, and then spiking the components from the working standards into the vials to produce the desired concentration.

When the sample concentration was calculated, the weight of the natrium sulphate was excluded because it was used as a matrix modifier to achieve a salting-out effect.

2.2. SPME device

The SPME extractor and five fibers used in this study were purchased from Supelco (Supelco Park, Bellefonte PA, 16823-0048 USA). The silica fibers were coated with a 100 μm film of polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 65 μm carbowax/divinylbenzene (CW/DVB), 85 μm Polyacrilate (PA) and 75 μm Carboxen/polydimethylsiloxane (CX/PDMS).

All sampling was conducted at room temperature, while the aqueous/organic phase was under constant magnetic stirring of 900 rpm. The sampling time for both Headspace SPME and Immersion SPME was 30 min, based on the optimization of SPME extraction. During desorbtion, the temperature of the analytical column was kept at a low value (30°C) in order to achieve a focusing effect. Each day, a column blank was followed by a fiber blank and a water blank to determine the extent of any laboratory contamination. The SPME fibers were conditioned at their corresponding maximum operation temperature overnight. The adsorbed VOCs were desorbed in the injector at their optimum desorbtion temperature (for CX/PDMS, at 300°C; and for all other fibers, at 250°C) [14] for 1 min. The carryover was found to be less than 1% for all VOCs and was determined by running consecutive fiber blanks to determine the fraction of the original mass desorbed remaining on the fiber.

2.3. Gas chromatography-mass spectrometry

The GC-ion trap mass spectrometer (GC-MS) used in this study was a Finnigan MAT GCQ system (Finnigan MAT, Austin, TX, USA). The GC was equipped with a TPI injector. The GC was fitted with a 30 m × 0.25 mm i.d. SPB-1 column coated with a 1 μm film of stationary phase (Supelco, Supelco Park, Bellefonte PA, USA). The injector was equipped with a 1.5 mm i.d. liner (in order to obtain better peak shapes) and was operated in splitless/split mode with a splitless time of 1 min. Longer splitless time caused peak broadening. The column temperature was held at 30°C for 3 min after the injection, then programmed at 7°C/min to 60°C then at 40°C/min to 250°C, where it was held for 5 min. Even if all components elute under 8 min, a further heating of the column was necessary for the 'cleaning' of the SPME bleeding components. The GC-MS data acquisition started after the elution of methanol (used solvent). Helium was used as a carrier gas at a constant linear velocity of 35 cm/s.

The external electron ionization ion source was operated at an electron energy of 70 eV, and the filament emission was set at 200 mA. The ionization waveform was set 'on'. The ion trap was operated at a target value of 50, a trap offset of 10 V and at a sampling rate of 2 scans/s. The multiplier was set at a multiplier gain of 3.9×10^5. The system gave unit resolution. The ion trap manifold temperature was set at 180°C and the transfer line was set at a temperature of 200°C. The ion trap was calibrated automatically with FC-43 standard substance using m/z 69, 131, 264, 414 and 502 by the autotune routine of the GCQ software.

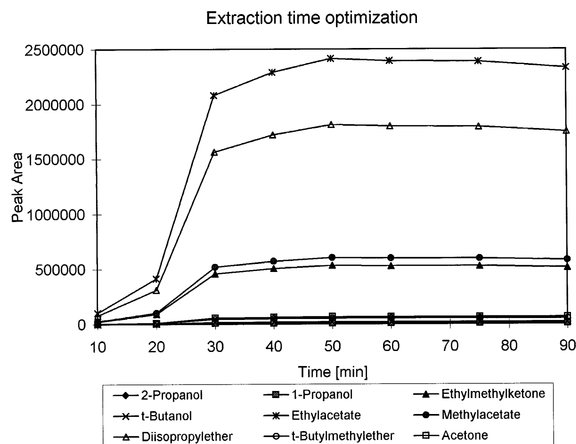

Fig. 1. Extraction time optimization for headspace SPME. For all other parameter optimum values, see Fig. 8.

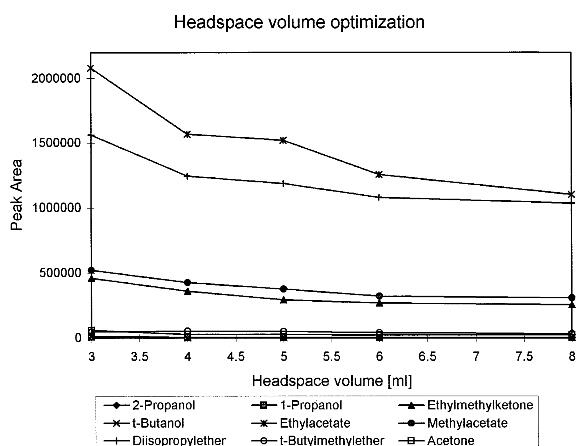

Fig. 2. Headspace volume optimization for headspace SPME. For all other parameter optimum values, see Fig. 8.

All data was acquired with GCQ MS/MS Version 2.0, March 1996 (Finnigan MAT, Austin, TX USA) validated software.

3. Results and discussion

3.1. Optimization of the Headspace SPME method

We have found that saturation of the aqueous phase with natrium sulphate increased the extraction efficiency of each analyte. The percent increase ranged from about 20% to almost 300%.

The optimized chromatographic conditions (low starting temperature of the column, 30°C; narrow bore injector liner, 1 min splitless time), together with the optimum desorbtion parameters (optimum injection depth into the injector of 2.5 cm; optimum desorbtion temperature for CX/PDMS of 300°C, and for all other fibers of 250°C) [14], are not influenced by the type of the extracted compounds and were kept constant during our study.

In order to maximize the sensitivity of this technique based on our previous experience [14], we used the 75 μm CX/PDMS-coated fiber as this fiber showed the best sensitivities for all investigated compounds.

The first step in our Headspace SPME method development was to establish the time required for all target analytes to reach a equilibrium. Fig. 1 shows that all analytes attained equilibrium after 30 min. The ethylacetate, methylacetate, diisopropylether, ethylmethylketone and acetone gave far the best sensitivities, mainly because their relative lower polarity compared with the other components, being much more easily to be extracted from the aqueous phase. The more polar compounds, like 1-propanol, 2-propanol and *t*-butanol have longer equilibration times and worse sensitivities, mainly because of their stronger affinity to the aqueous phase.

The Headspace SPME from organic solvents and the Immersion SPME from aqueous solutions sample preparation methods showed the same behavior.

After establishing the equilibration time, we were interested to examine the effect of headspace volume on the sensitivity of analyzed components. Fig. 2 shows that low headspace volumes improve sensitivity, probably because reducing headspace volume shortens the diffusion path in the gaseous phase. The best sensitivity is given for a headspace volume of 3 ml with respect to a vial volume of 10 ml. At the same time, the use of low headspace volumes (under 4 ml) increased the relative standard deviation (R.S.D. (%)) of the measured peak areas by around 1.5 times. In cases when sensitivity is not critical, headspace volumes of 5 ml can

be used without significant loss in sensitivity and with a gain in precision. In this case, as the extraction efficiency of the investigated compounds was quite low, a low headspace volume was chosen in order to maximize the extraction efficiency.

The most important parameter regarding the extraction efficiency was found to be the total volatile organic content of the aqueous phase. As can be seen from Fig. 3, the sensitivity decreases drastically with the increase in volatile organic content. When the working aqueous solutions contain more than 0.5% volatile organics, the extraction efficiency is very low, or even no extraction (in the case of alcohols) occurs. The optimum working range for the total volatile content (TVC) was found to be between 0.01 and 0.1%. In this range, the extraction efficiency can be considered constant. When preparing standard and sample solutions with large differences in total volatile organic content, the best approach was to dilute the sample until it reached the optimum range and then to add methanol to the standards in order to bring the total organic content as close as possible to the sample TVC value. At the same time, the use of high boiling point solvents (dimethyl sulfoxide or *N*,*N*-dimethylformamide) is not generating such a large decrease in the extraction efficiency as methanol or ethanol. More, for Headspace SPME extraction from organic solutions, the optimum range was broader, from 0.01 to 0.5%.

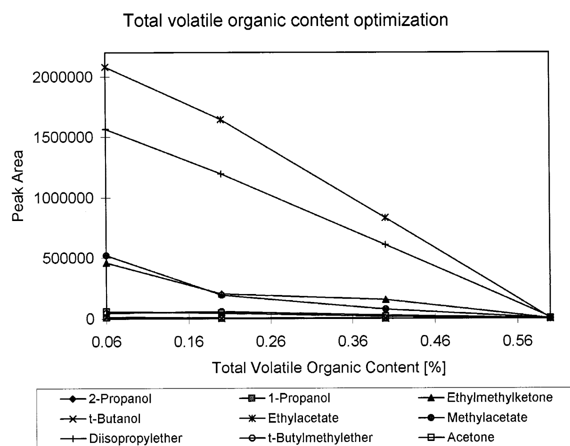

Fig. 3. Total volatile organic current optimization for headspace SPME. For all other parameter optimum values, see Fig. 8.

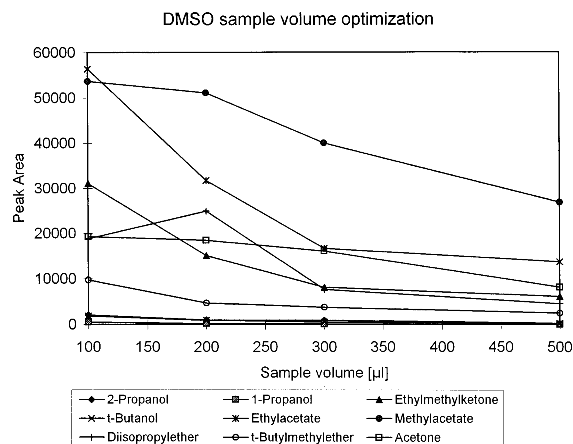

Fig. 4. Added dimethyl sulfoxide sample volume optimization for headspace SPME. For all other parameter optimum values, see Fig. 9.

We further investigated the possibility of extraction of polar solvents from high boiling point solvents like dimethyl sulfoxide (DMSO) and *N*,*N*-dimethylformamide (DMF). We found that the Carboxen/polydimethylsiloxane-coated fibers have low affinity for these solvents, allowing the extraction of volatile residual solvents. All other polymer-coated fibers are swelled by the organic solvent, which drastically shortens the fiber lifetime.

The extraction time was kept at 30 min. We investigated the influence of solvent volume on extraction efficiency. In the case of DMSO, it can be seen from Fig. 4 that increasing the added DMSO volume indeed reduced the extraction efficiency, but not to the extent experienced when increasing the TVC in aqueous phases. For low (around 100 µl DMSO) solvent volumes, the ethylacetate is better extracted than methylacetate and ethylmethylketone (similar to the aqueous system). At higher DMSO volumes, methylacetate is better extracted and ethylacetate shows a significant decrease in extraction efficiency. At the same time, diisopropylether starts to be better extracted than ethylmethylketone, and for DMSO volumes bigger than 300 µl, their extraction efficiencies become practically the same. The extraction effi-

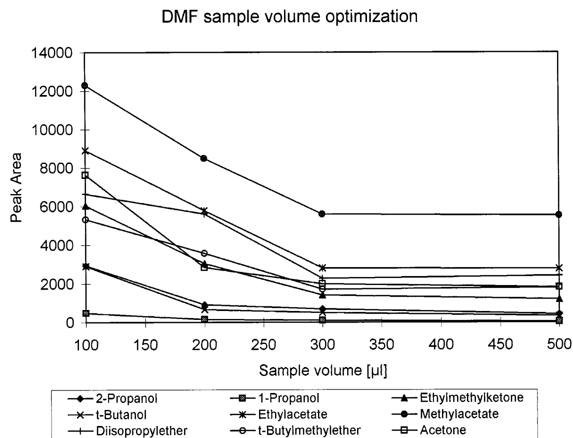

Fig. 5. Added *N*,*N*-dimethyl formamide sample volume optimization for headspace SPME. For all other parameter optimum values, see Fig. 9.

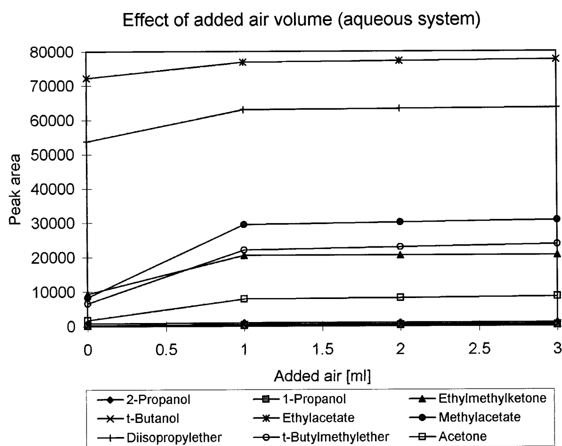

Fig. 6. Added air volume optimization for headspace SPME from aqueous solutions. For all other parameter optimum values, see Fig. 8.

ciency for more polar components like 1-propanol, 2-propanol, *t*-butanol, *t*-butylmethylether and acetone is not significantly influenced by the DMSO volume. The optimum DMSO volume is around 100 µl, which, as a matter of fact, is quite enough to dissolve 50 mg of some water-insoluble substances.

In the case when the solvent is DMF, the overall extractions efficiencies are much less than in the previous case. As can be seen from Fig. 5, the extraction efficiencies decrease when the DMF volume increases, and for DMF volumes larger than 300 µl, the system reaches saturation. At low DMF volumes (around 100 µl), acetone and *t*-butylmethylether have good extraction efficiency, which decreases significantly with DMF volume. The methylacetate has the best extraction efficiency. The optimum DMF volume is around 100 µl.

During our study, we observed the fact that slightly pressurizing the headspace vial improves the overall extraction efficiencies for aqueous and organic systems. We added, with a gas-tight syringe, different volumes of air in the headspace vial and extracted the pressurized test solutions. The maximum added air volume was 3 ml for aqueous test solutions and 5 ml for DMSO test solutions. As can be seen from Fig. 6 for aqueous systems, the pressurization have an insignificant influence on the extractions efficiencies of 2-propanol, ethylacetate and diisopropylether, the gain being around 1.2 times. For 1-propanol, ethylmethylketone and *t*-butanol, the gain in sensitivity was about 2.5 times. A significant effect on the extraction efficiencies was observed in the case of methylacetate and *t*-butylmethylether when the observed sensitivity gain was around four times and, respectively, in the case of acetone when the gain was around five times. We observed also the fact that slightly pressurizing the headspace vials is enough to generate the reported sensitivity increase, and that because after adding more than 2 ml of air, no further increase can be observed. The optimum air volume to be added to a headspace vial with a headspace volume of 3 ml was 2 ml air.

The situation was quite different for the DMSO test solutions, when the continuous increase in sensitivity was observed with the increase in pressure inside the headspace vial (Fig. 7). For ethylmethylketone, this effect was not observed and the gain in sensitivity for was around 1.6 times. For all other components, the sensitivity gain was almost constant, around 2.5 times. For practical purposes, the optimum volume of air to be added to a headspace vial with DMSO or DMF standard and sample solutions was 5 ml air.

Fig. 8 presents the chromatogram of the optimized CX/PDMS Headspace SPME of the

aqueous spiked test substance. It can be seen from the chromatogram that the peak shape is quite good, and even if acetone and 2-propanol are not resolved, the inherent selectivity of the GC-MS does compensate for that partial coellution.

In Fig. 9 is presented the chromatogram of the optimized CX/PDMS Headspace SPME of organic (DMF) spiked test substance. It can be seen from the chromatogram that peak shapes are much better than in aqueous systems, as the tailing of the late eluting components was substantially improved.

3.2. The Headspace SPME method evaluation

The first step in method evaluation was the determination of detection limits (DL) for all investigated compounds and systems, and the comparison with the detection limits of other polymer-coated fibers. Detection limits were investigated by extracting spiked aqueous standard and organic solutions as described in Section 2. The detection limit was performed by comparing measured signals from the selected ion chromatogram of samples with known low concentration of analyte with those of blank samples. The acceptance criteria was a signal/noise ratio of minimum 3:1.

Five different fibers with different coatings, polydimethylsiloxane/divinylbenzene (PDMS/DVB), polydimethylsiloxane (PDMS), carbowax/divinylbenzene (CW/DVB) polyacrylate (PA) and Carboxen/polydimethylsiloxane (CX/PDMS), were compared under same experimental parameters in order to find the most sensitive coating for all analytes under study. Table 2 shows the method detection limits for the Headspace SPME method for all investigated fibers and also for the two organic systems. The reported DLs for acetone and 2-propanol are consistent with the data from the literature [13].

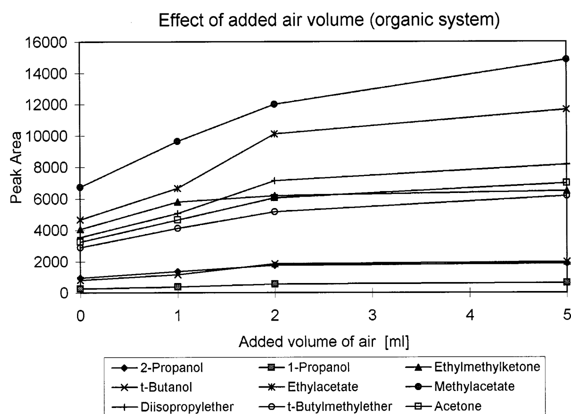

Fig. 7. Added air volume optimization for headspace SPME from organic solutions. For all other parameter optimum values, see Fig. 9.

The CX/PDMS-coated fiber has very good sensitivity toward all investigated components. For 2-propanol and *t*-butanol, the CX/PDMS fiber was greater than 30 times more sensitive, for acetone and 1-propanol it was greater than 50 times more sensitive, and for ethylacetate, methylacetate and ethylmethylketone it was greater than 100 times more sensitive than all other fibers. For *t*-butylmethylether, the CX/PDMS-coated fiber gave similar results to PDMS/DVB- and PDMS-coated fibers.

In the case of the CX/PDMS over DMSO, the sensitivities were lower than in the case of aqueous system, but still better than the best results from all other fibers. For methylacetate and acetone in the CX/PDMS over DMSO system, the sensitivity was almost 50 times more; for 1-propanol, 2-propanol and ethylmethylketone, the sensitivity was almost 10 times more than all other fibers. For all other components, the sensitivities were slightly better than all other fibers.

In the case of the CX/PDMS over DMF, the 1-propanol, 2-propanol, *t*-butanol and acetone detection limits were ten times greater than the best values from all other fibers. The rest of the compounds had similar detection limits as the best values from all other fibers.

All five fibers were also compared using the Immersion SPME sample preparation technique, as described in Section 2. The optimized parameters were kept constant during their evaluation. The detection limits for Immersion SPME sampling are presented in Table 3. The Immersion SPME sampling gave better results only in the case of 2-propanol, all other compounds being comparable with the Headspace CX/PDMS over DMSO SPME sampling method.

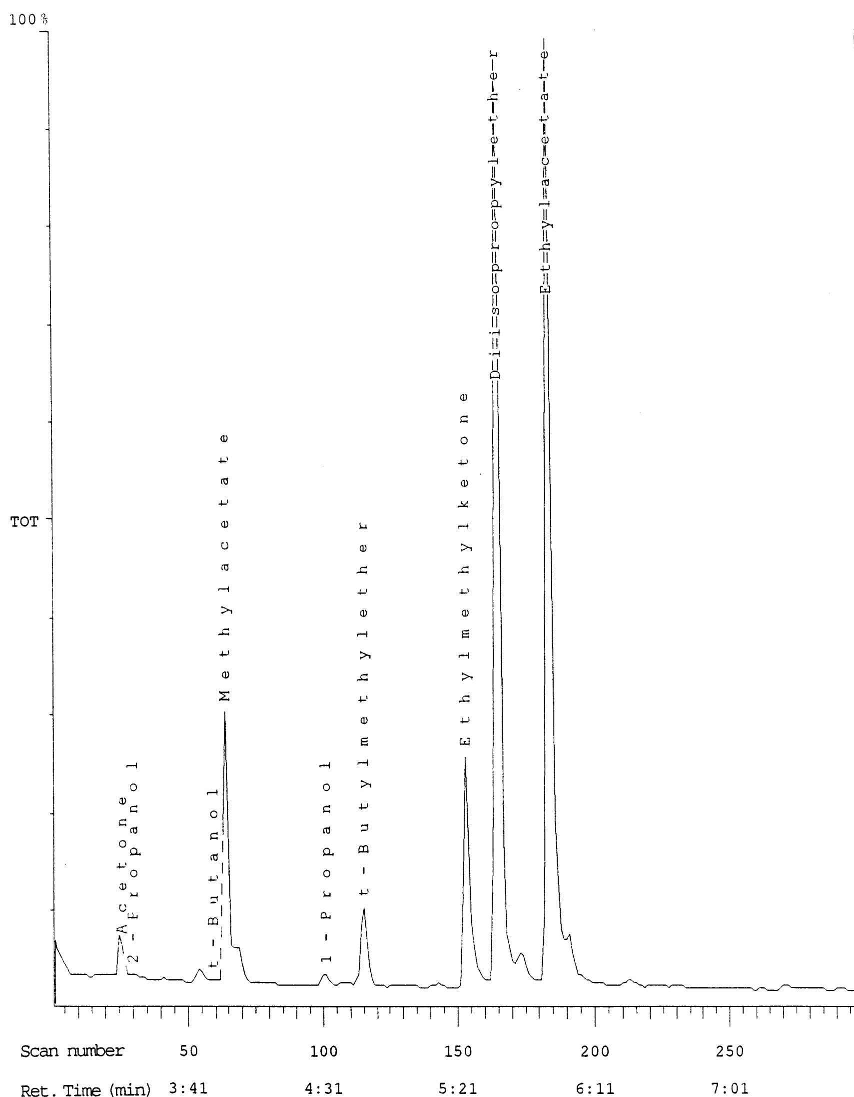

Fig. 8. Total ion chromatogram of aqueous standard solution under optimum conditions. The optimum parameter values were as follows: extraction time, 30 min; headspace volume, 3 ml; total organic content; 0.1%; added salt weight, 1 g Na_2SO_4; added air volume, 3 ml; injector temperature, 300°C; injection depth, 2.5 cm.

The repeatability of the method was investigated by extracting spiked aqueous solutions with concentrations given in the Table 4. The repeatability data were calculated from the analyte peak areas of five replicates, within one day and by one analyst. The repeatability of the developed methods was investigated for three systems, two aqueous with a total volatile organic content of 0.06% and 0.1%, and one organic in which the solvent was 100 μl DMSO. Acceptable R.S.D. values of peak areas were obtained for all sample preparation techniques used. It can be seen that for low volatile organic content, the repeatability of the analytical method was much worse than for higher volatile organic content. Between the investigated components, 1-propanol and *t*-butanol gave the biggest R.S.D. values, close to 9%, the method being the most precise for ethylacetate,

methylacetate and acetone with a R.S.D. around 5%. Using higher volatile organic contents, close to 0.1%, the sensitivity of the method will be reduced by almost 20% but the repeatability of the analytical method will be improved by 50%. For identification purposes, when the sensitivity is the most important parameter, low volatile organic environments should be used. For routine/quantitative purposes, a 0.1% volatile organic content should be used, because in this case the analytical method is more precise.

It can be seen that the organic system (CX/PDMS fiber over DMSO) is situated somewhere between the two previous cases as its repeatabilities vary from 2 to 6%. In this case, the worst repeatabilities were encountered in the case of 1-propanol, methylacetate and diisopropylether, and the best repeatabilities were given by ethylacetate and acetone.

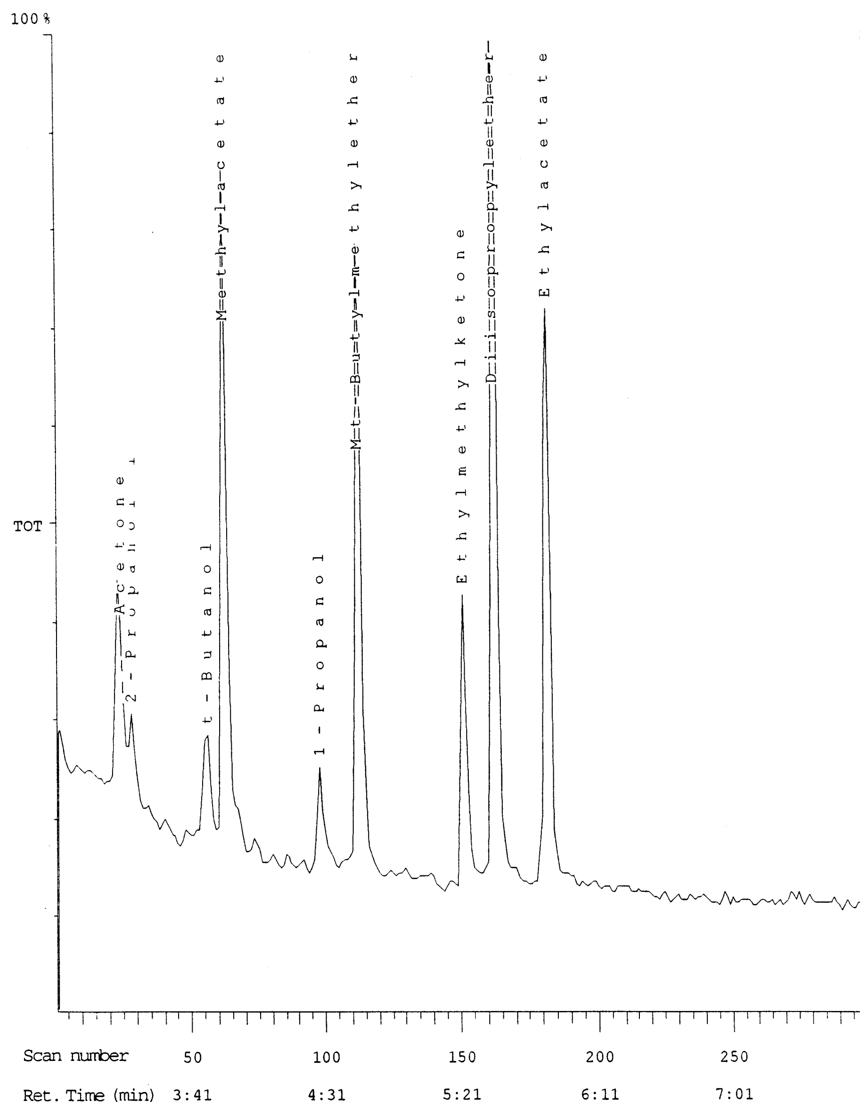

Fig. 9. Total ion chromatogram of organic (DMF) standard solution under optimum conditions. The optimum parameter values were as follows: extraction time, 30 min; total organic content; 0.1%; added air volume, 5 ml; injector temperature, 300°C; injection depth, 2.5 cm.

Table 2
Detection limits of the employed methods in the case of Headspace SPME sampling

Component	Detection limits for the employed methods (μg/ml)						
	DVB[a]	PDMS[b]	CW[c]	PA[d]	CX[e]	CX/O1[f]	CX/O2[g]
2-Propanol	0.8	1.5	0.8	0.5	0.01	0.05	0.06
1-Propanol	32.3	39.1	17.2	58.6	0.2	1.0	1.15
Ethylmethylketone	0.3	1.2	0.1	0.5	0.001	0.02	0.10
t-Butanol	1.3	4.5	1.1	1.8	0.03	0.2	0.10
Ethylacetate	0.1	0.4	0.05	0.2	0.0004	0.01	0.05
Methylacetate	0.3	1.1	0.2	0.4	0.001	0.005	0.03
Diisopropylether	0.02	0.02	0.02	0.06	0.0006	0.01	0.03
t-Butylmethylether	0.04	0.06	0.1	0.1	0.006	0.02	0.05
Acetone	0.5	1.6	0.3	0.3	0.005	0.01	0.02

[a] Polydimethylsiloxane/divinylbenzene fiber.
[b] Polydimethylsiloxane fiber.
[c] Carbowax/divinylbenzene fiber.
[d] Polyacrylate fiber.
[e] Carboxen/polydimethylsiloxane fiber over aqueous solutions.
[f] Carboxen/polydimethylsiloxane fiber over dimethylsulfoxide solutions.
[g] Carboxen/polydimethylsiloxane fiber over dimethylformamide solutions.

Table 3
Detection limits of the employed methods in the case of Immersion SPME sampling

Component	Detection limits for the employed methods (μg/ml)				
	DVB[a]	PMS[b]	CW[c]	PA[d]	CX[e]
2-Propanol	0.8	2.7	1.6	1.7	0.005
1-Propanol	18.2	53.8	44.5	47.8	0.6
Ethylmethylketone	1.0	3.4	0.6	6.2	0.01
t-Butanol	6.3	7.2	3.1	10.6	0.1
Ethylacetate	0.3	1.1	0.02	3.4	0.001
Methylacetate	1.2	3.0	0.7	3.4	0.006
Diisopropylether	0.04	0.1	0.03	0.8	0.007
t-Butylmethylether	0.15	0.2	0.2	2.6	0.02
Acetone	1.6	1.5	0.7	2.8	0.02

[a] Polydimethylsiloxane/divinylbenzene fiber.
[b] Polydimethylsiloxane fiber.
[c] Carbowax/divinylbenzene fiber.
[d] Polyacrylate fiber.
[e] Carboxen/polydimethylsiloxane fiber in aqueous solutions.

The Headspace SPME equipped with a Carboxen/polydimethylsiloxane-coated fiber was chosen because of its better precision and sensitivity as a sample preparation method for the determination of residual solvents in two proprietary drug products of Gedeon Richter LTD by GC-MS. The first proprietary drug product (Drug 1) was a peptide compound, soluble in water, and the second (Drug 2) was a synthetic drug, insoluble in water.

From the chromatographic point of view, the organic system gave better peak shapes than the aqueous. Fig. 10 shows the CX/PDMS aqueous Headspace SPME chromatogram of spiked sam-

ple solution of Drug 1, with concentrations presented in Table 4. As can be seen from the chromatogram, even in a strong matrix (peptidic matrix, polar), the extraction could easily happen with recoveries (by assay of known added amounts of analyte in the sample) greater than 90%. Near the methylacetate peak, we also found an unknown, which was identified and quantitated. The identified compound was found to be methylene chloride, a solvent used during the synthetic route.

Fig. 11 shows the CX/PDMS organic (DMSO) Headspace SPME chromatogram of spiked sample solution of Drug 2, with concentrations presented in Table 4. It can be seen from the chromatogram that the peak shape was much better compared with the aqueous system. Around the methylacetate peak, we identified two unknown components, which were shown to be dimethyl sulfide and 1-propanethiol, two impurities of the solvent (DMSO). The identified impurities were also observed during the optimization of the method, but in much lower quantities. The better extraction of these impurities can be attributed to the matrix effect of Drug 2 substance. The extraction recoveries for all components (by assay of known added amount of analyte in the sample) were greater than 90%.

All mass spectra of unknowns were checked against the NIST mass spectral library, and the fit between measured and found spectra was greater than 94%.

4. Conclusions

It is evident from the presented data that extensive optimization is necessary each time a Headspace SPME method is developed. We found that the extraction time, total volatile content, headspace volume, pressure inside the headspace vial and, for organic systems, the added organic solvent quantity are very important parameters. These parameters need to be reoptimized each time a new component is added to the analytical method. At the same time, we found that chromatographic conditions (low starting temperature of the column, 30°C; narrow bore injector liner, 1 min splitless time), together with the optimum desorbtion parameters (optimum injection depth into the injector of 2.5 cm; optimum desorbtion temperature for CX/PDMS of 300°C, and for all other fibers of 250°C) [14] are not influenced by the type of the extracted compounds and do not need to be reoptimized. We also found that sensitivity and reproducibility are inversely related

Table 4
The repeatability of analytical data

Component	Concentrations (ng/ml)		Quantification mass (m/z)	Repeatability of peak areas (R.S.D. (%) of five replicates)		
	CX[a]	CX/O1[b]		CX/0.06[c]	CX/0.1[d]	CX/O1[b]
2-Propanol	152	254	45	7.3	4.9	3.1
1-Propanol	429	714	59	8.7	4.3	5.7
Ethylmethylketone	206	342	43	4.5	2.4	3.3
t-Butanol	165	275	59	9.1	4.6	4.2
Ethylacetate	251	418	61	4.8	3.2	2.2
Methylacetate	239	399	75	5.9	3.1	5.6
Diisopropylether	93	155	45	7.8	3.9	6.2
t-Butylmethylether	95	159	73	6.8	3.8	3.3
Acetone	152	254	58	4.5	2.5	2.5

[a] Carboxen/polydimethylsiloxane fiber over aqueous solutions.
[b] Carboxen/polydimethylsiloxane fiber over dimethylsulfoxide solutions.
[c] Carboxen/polydimethylsiloxane fiber over aqueous solutions with a total volatile content of 0.06%.
[d] Carboxen/polydimethylsiloxane fiber over aqueous solutions with a total volatile content of 0.1%.

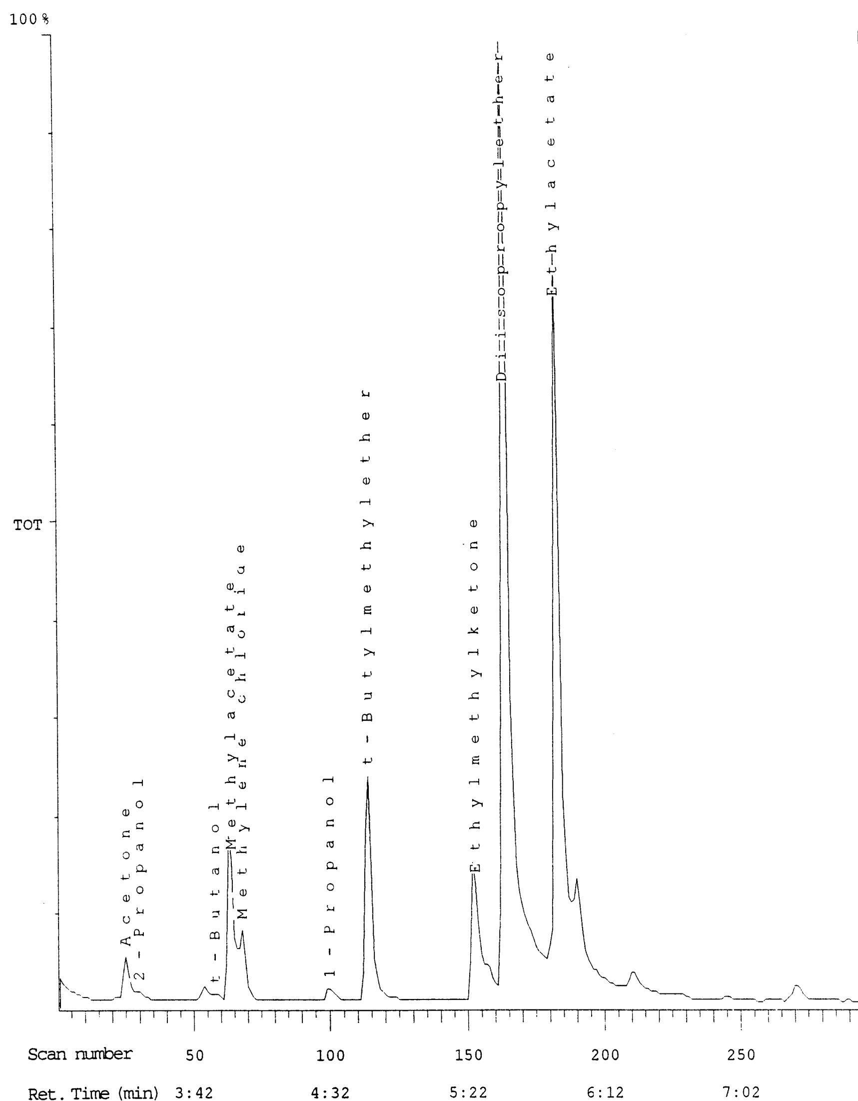

Fig. 10. Total ion chromatogram of aqueous spiked sample solution of Drug 1 with concentrations from Table 4.

parameters. When maximizing sensitivity (using low headspace volumes with low total volatile organic contents), the reproducibility worsens. For routine purposes, larger headspace volumes and higher (around 0.1%) total volatile contents should be used. At the same time, when routine measurements are done, care should be taken that sample solutions and test solutions have similar total volatile organic contents, in the range 0.01–0.1%.

Between the investigated polymeric films, the Carboxen/polydimethylsiloxane-coated fiber showed by far the best sensitivities for all compounds. The fiber was able to extract compounds with different polarity and volatility from aqueous and organic environments. The Carboxen/polydimethylsiloxane-coated fiber showed very good stability in organic media.

Between the investigated sample preparation techniques, Headspace SPME from aqueous sam-

ples proved to be more sensitive, and Headspace SPME from organic solutions proved to be more precise. The Immersion SPME technique gave similar sensitivities as Headspace SPME from organic solutions and can be replaced with the later one. At the same time, Headspace SPME from organic solutions gave better peak shapes than from aqueous solutions.

The SPME GC-MS proved to be a powerful technique in the identification and determination of unknown solvent residues in pharmaceutical products. With this technique, we were able to identify residual solvents in our proprietary pharmaceutical products. Even if SPME techniques are not yet accepted as sample preparation methods by Pharmacopoeias, taking into consideration their precision, accuracy and speed of analysis, we can state that they are suitable for qualitative/quantitative residual solvent determination in pharmaceutical products.

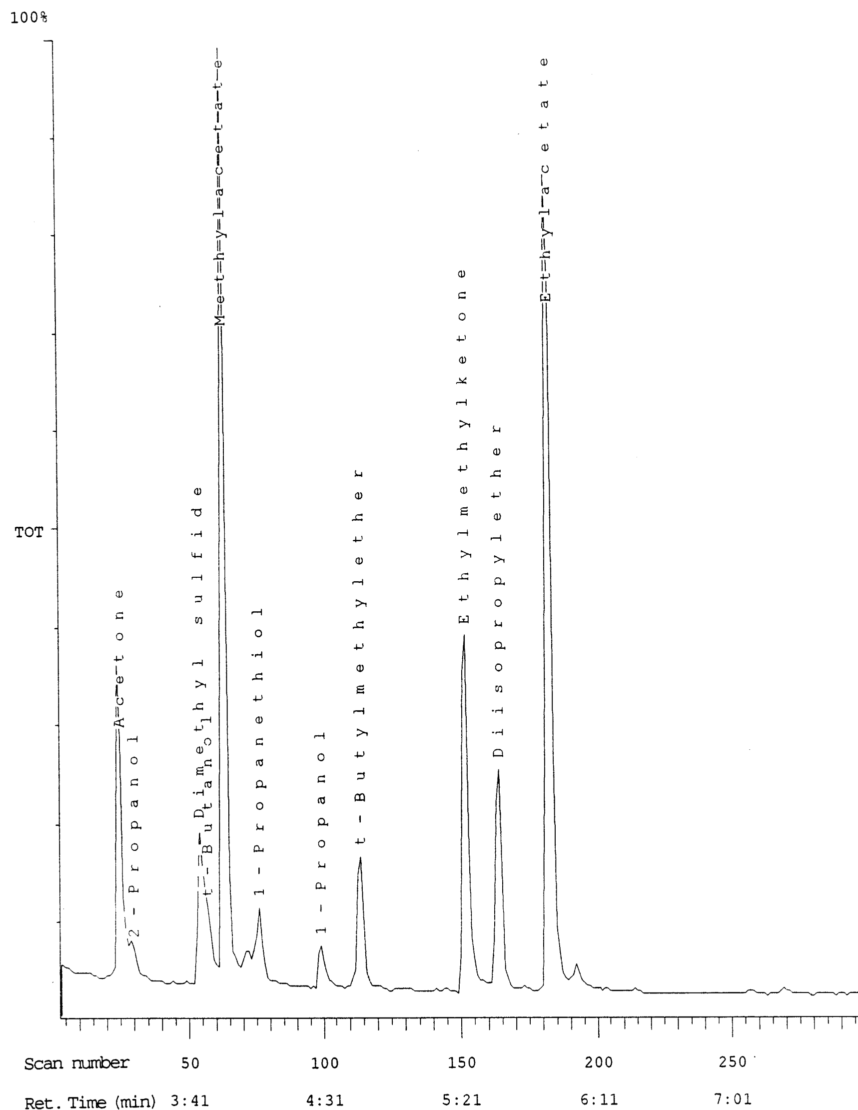

Fig. 11. Total ion chromatogram of organic (DMSO) spiked sample solution of Drug 2 with concentrations from Table 4.

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