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Determination of menthol and menthone in food and pharmaceutical products by solid-phase microextraction–gas chromatography

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Abstract

In the current contribution the optimization of menthol and menthone isolated from food and pharmaceutical samples by solid-phase microextraction (SPME) will be presented. Extraction efficiencies for commercial (polydimethylsiloxane) and laboratory-made (ethoxypolydimethylsiloxane) coated quartz fibers were compared. The results show, that SPME coupled with GC–flame ionisation detection is a reproducible method for isolation and for qualitative and quantitative determination of menthol and menthone at ppm as well as ppb levels. The described procedure can be recommended for routine food and pharmaceutical analyses. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Food analysis; Sample preparation; Menthol; Menthone

1. Introduction

Gas-phase sample preparation methods include static headspace (SHS) as well as purge-and-trap (PT) techniques [1,2]. The SHS method has been widely applied to analysis of volatile compounds because the extracting phase (air, helium or nitrogen) is compatible with gas chromatographs. It has been used to analyze volatile organic compounds (VOCs) in food, beverages, clinical and other samples [3,4]. SHS cannot achieve exhaustive extraction, except in the case of very volatile gases, and therefore requires careful calibration. On the other hand, the dynamic headspace (DHS) technique uses multiple processes and allows quantitative removal of VOCs. The DHS method for analysis of VOCs in water, has two steps [5,6]. In the first, an aqueous sample is purged with carrier gas to remove VOCs from the matrix. The second step is to quantitatively collect these compounds by using a cold or a sorbent trap. This technique has some drawbacks including foaming, carryover of analytes from a previous determination, and the fact that the stripping flow-rate is incompatible with the separation instrument [1].

An alternative to gas headspace analysis and adsorption of volatile analytes by adsorption surface is the solid-phase microextraction (SPME) technique developed by Pawliszyn and co-workers [7–10]. It uses a fused silica fiber, coated with an adsorbent that is mounted on a modified chromatographic syringe to extract samples and pass analytes directly into the gas chromatographic heated injector and/or high-performance liquid chromatographic interface with solvent. The fused silica fibers have a small diameter which allows convenient introduction into a chromatographic injector. These fibers coated with polymers such as polydimethylsiloxane (PDMS), polydimethylsiloxane with divinylbenzene, Car-

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bowax with divinylbenzene, Carbowax with template resin coating for surfactants, Carboxen with polydimethylsiloxane and polyacrylate are available commercially and can be used directly in extractions. The uncoated fiber can also be used or a variety of common chromatographic stationary phases can be attached to its surface. The coated fiber concentrates the organic analytes on its surface so they can be transferred to the gas chromatographic instrument and they can be thermally desorbed. This technique is utilized for the determination of organic micropollutants in water, which permits extraction without any solvent. It has been proven to be simple, time efficient and sensitive. By sampling from the headspace above sample matrixes, SPME can be used to extract target analytes from very complex matrices such as sludge, wastewater and soil [7]. SPME has been successfully used for analyzing VOCs, polycyclic aromatic hydrocarbons (PAHs) [8], polychlorinated biphenyls (PCBs) [8] as well as phenol and its derivatives in aqueous samples [7].

Menthol [5-methyl-2-(1-methylethyl)cyclohexanol] and menthone [5-methyl-2-(1-methylethyl)cyclohexanone] belong to the group of terpenes [11]. Usually, for the determination of these compounds, isolated from real samples by liquid–liquid and/or liquid–solid extraction, thin-layer chromatography (TLC) is applied [12]. At the present time SPME coupled with gas chromatography (GC) and/or high-performance liquid chromatography (HPLC) is successfully applied for isolation, enrichment and determination of individual analytes in food, drugs, cosmetics, sewage, etc [1]. Menthol is used in confectionery, perfumery as well as liqueurs, cigarettes, nasal inhalers and cough drop production. It is also used as a component of anaesthetic, antiseptic and gastric sedative drugs [11]. Menthone can be found in various volatile oils, such as pennyroyal, peppermint and geranium. It is used in perfume and flavor compositions [11]. Because of the wide range of applications of menthol and menthone in food and drugs production, the determination of these compounds is important not only for consumers but also for analytical chemists.

In the current paper the results of qualitative and quantitative analyses of menthol and menthone contents in peppermint tea, menthol candies, peppermint chewing gum and gastric peppermint drops samples are presented. For this reason, the commercially available PDMS-coated fiber as reference and the new type of laboratory-made organic phase ethoxy-polydimethylsiloxane (PDES)-coated fiber were compared.

2. Experimental

2.1. Apparatus

The SPME apparatus (Supelco, Bellefonte, PA, USA) with PDMS (thickness 100 μm and 7 μm) coated fibers were used for the determination of analytes. For the comparison of extraction recoveries the syringe, after special modification, was used for testing the new type coated fiber PDES (thickness ca. 10 μm). Laboratory-made modification of Hamilton syringe 7000 series with chaney adapter (Hamilton, Reno, NV, USA) involved removing a portion just short of piston and coupling an 8 cm fiber of fused silica coated with 1 cm of stationary phase. The fiber was connected to the fused silica tube by polyimide resin or glue. This fused silica tube was connected to the piston by polyimide resin or glue (Fig. 1).

The chromatographic analysis was performed by GC system (Fisons 8160, Fisons Instruments, Milan, Italy). Carrier gas: helium (99.999%), pressure on the column head 15 kPa. The temperature of split–splitless injector was 200°C. The temperature of flame ionisation detection (FID) system was 250°C. The RTX 200 (Restek, Bellefonte, PA, USA) column (30 m×0.53 mm, 0.25 μm) was utilized.

The oven temperature programme was: 40°C hold for 2 min; heating rate of 10°C/min to 150°C (hold 4 min); heating rate of 20°C/min to 225°C (hold 2 min).

The acquisition of chromatographic data was performed by ChromCard computer software (Fisons Instruments).

2.2. Adsorption and desorption conditions

The spiked standards or determined analytes were isolated by static headspace from 2.01 ml of sample. The headspace vials (5 ml volume) were used in all experiments. The temperature of isolation (30±0.1°C) was obtained by a refrigerated circulator

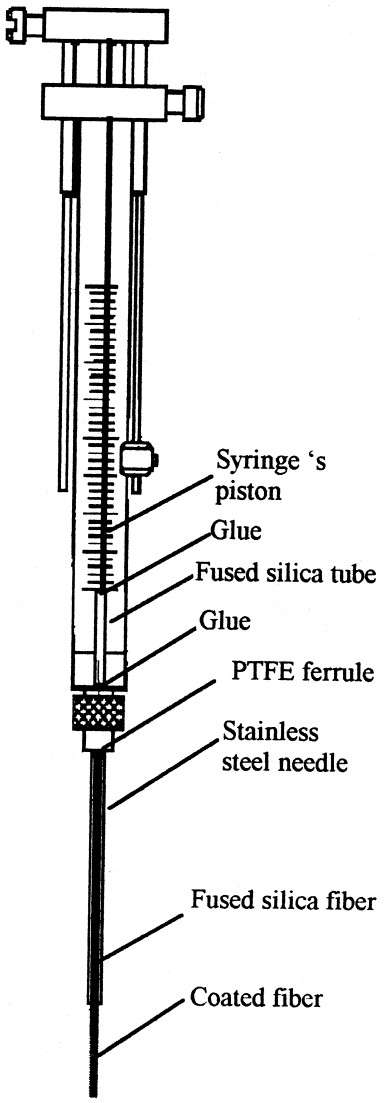

Fig. 1. Laboratory-made SPME device used in some experiments.

(Julabo Labortechnik, Seelbach, Germany). Stirring was not employed during the isolation.

The adsorption time was optimized. As results the adsorption profiles were evaluated. The optimum adsorption time of 15 min was applied.

The temperature of desorption was 200°C. The fiber was exposed in the injection port to desorbed volatiles for 2 min (injector position: splitless). After that, the oven temperature programme was started (position: split). The SPME fibers were preconditioned (temperature 200°C, time 10 min) before and after all analyses.

2.3. Determination of adsorption profiles

Adsorption–time profile studies were carried out to determine equilibration times for menthol at 100 μg/ml with 100 μm PDMS, 7 μm PDMS and ca. 10 μm PDES coated fibers. The temperature of isolation was 30±0.1°C. Stirring was not employed during the analyses. For other conditions see Section 2.2.

The analyses were performed within an extraction time of 5–30 min.

2.4. Samples and standards preparation

(i) 18.11 mg of peppermint tea was mixed with 10 μl of methanol and diluted with 2.0 ml of redistilled water in a headspace vial.

(ii) 46. 6 mg of menthol candies was mixed with 10 μl of methanol and diluted with 2.0 ml of redistilled water in a headspace vial.

(iii) 49.7 mg of peppermint chewing gum was mixed with 10 μl of methanol and diluted with 2.0 ml of redistilled water in a headspace vial.

(iv) 22.74 mg of gastric peppermint drops was mixed with 10 μl methanol and diluted with 2.0 ml of redistilled water in a headspace vial.

For the calibration procedure, five mixtures of menthol (POCh, Gliwice, Poland) in methanol, each containing of menthol at a concentration from 5.198 to 17.141 mg/ml, were prepared. After that, 10 μl of each standard was diluted with 2 ml of redistilled water (Milli-Q system, El Paso, TX, USA). The final concentrations of menthol in standard solutions were 25.86, 30.76, 53.94, 75.52 and 85.279 μg/ml. The calibration curves for determined menthol in various samples were evaluated.

The calibration method of menthone concentration in standard samples was performed by standard addition (1 μg/ml in the sample). The concentrations of menthone in real samples were found by comparison of their peak areas before and after standard addition.

The calibration procedure and determination of menthol and menthone in real samples was carried out under the same analysis conditions. The calibration data are listed in Table 1.

Table 1
Calibration parameters of standard mixtures for 100 μm PDMS, ca. 10 μm PDES, 7 μm PDMS

No.	Compound	Method	t_{av} [a] (n=10) (min)	R_A^2 [b]	Calibration equation	Detection limit (μg/ml)
1	Menthol	External standard	10.084	0.9995	y=593 668x+48891	0.05
	Menthone	Standard additions	11.423	–	–	0.06
2	Menthol	External standard	9.987	0.9986	y=134 961x+505 342	0.10
	Menthone	Standard additions	11.427	–	–	0.12
3	Menthol	External standard	9.818	0.9954	y=15 713x−64 197	0.40
	Menthone	Standard additions	11.350	–	–	0.45

[a] t_{av}=Average of the peak retention time.
[b] R_A^2=Correlation coefficient of the calibration curve using peak area.

2.5. Determination of detection limits

For determination of menthol and menthone detection limits the menthol candies sample was prepared according to Section 2.4 was diluted with defined water volumes. The analytes were isolated by 100 μm PDMS, 7 μm PDMS and ca. 10 μm PDES coated fibers. The successive solutions were prepared until there was no FID response. External standard method (for menthol) and comparison of FID response for menthone standard solutions evaluated concentrations of menthol and menthone in every solution. The detection limits are presented in Table 1.

3. Results and discussion

For quantitative analyses of menthol and menthone the external standard method and the internal standard method, respectively, were performed. The detection limits for determined menthol and menthone at the ppb level are observed. These values are in the range from 0.05 to 0.40 μg/ml for menthol and in the range from 0.06 to 0.45 μg/ml for menthone. The detection limits are different for the various coated fibers used. All calibration data such as average of the peak retention time, correlation coefficient of the calibration curve using peak area, calibration equations, detector response and detection limits are presented in Table 1.

Typical chromatograms obtained from menthol candies, peppermint tea, peppermint chewing gum, gastric peppermint drops, where the ca. 10 μm PDES coated fiber was used, are presented in Figs. 2–5. This PDES-coated fiber is selective not only for determined menthol and menthone, but also for other VOCs in these samples.

The results of qualitative and quantitative analyses of menthol and menthone in various samples utilizing the 100 μm PDMS and 7 μm PDMS commercial coated fibers are presented in Tables 2 and 3. The results of the experiments when the ca. 10 μm PDES coated fiber was used are presented in Tables 4 and 5. The highest concentrations of menthol are observed for gastric peppermint drops and peppermint chewing gum samples. The highest concentration of menthone are observed for gastric peppermint drops and peppermint tea samples. Relative standard deviations (RSDs) for menthol in the range from 1.53 to 8.81% and for menthone in the range from 2.58 to 8.79% showed good repeatability of method and good reproducibility of results.

The characteristic of the ca. 10 μm PEDS coated fiber, used in all experiments, was described in detail by Buszewski et al. [13]. The PDES-coated fiber contains $-OC_2H_5$ groups, in contrast to the PDMS-coated fiber which contains only $-CH_3$ groups. The structure of PDES coating considerably influenced its affinity to determined compounds. The higher affinity of menthol and menthone to the PDES-coated fiber than to the commercial PDMS-coated fiber (both fibers have the ca. similar thickness of coating) is observed. The polarity of determined compounds and stationary phase influenced the results of the analyses. The commercial PDMS coated fiber has a higher polarity than the laboratory-made PDES-coated fiber. Hence the higher affinity of menthol

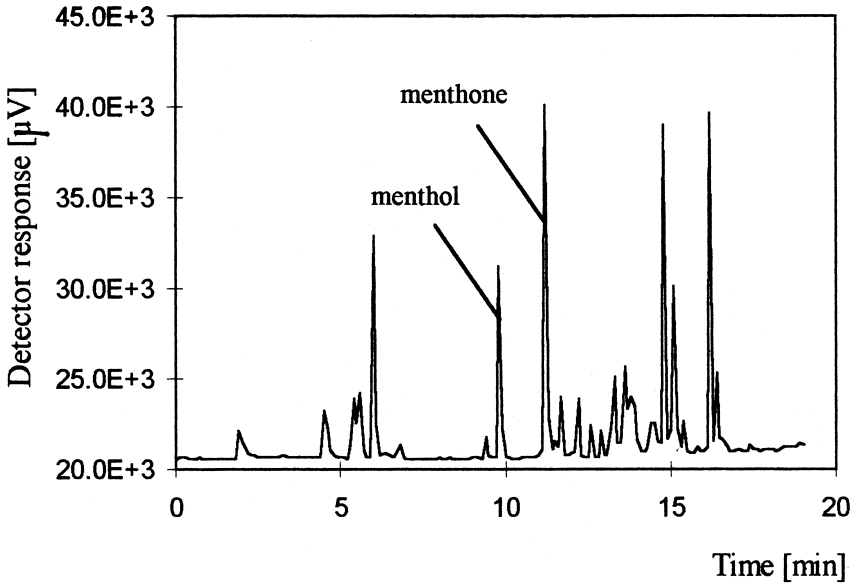

Fig. 2. Typical chromatogram obtained from menthol candies sample after ca. 10 μm PDES coated fiber use.

and menthone to PDES stationary phase is observed. These results are presented in Fig. 6. The commercial PDMS-coated fiber was used as a reference material for the laboratory-made PDES-coated fiber.

Even though the structures of menthol and menthone are somewhat different (menthol has a hydroxyl group and menthone has a ketone group), the affinity to PDES and PDMS stationary phases is similar. The

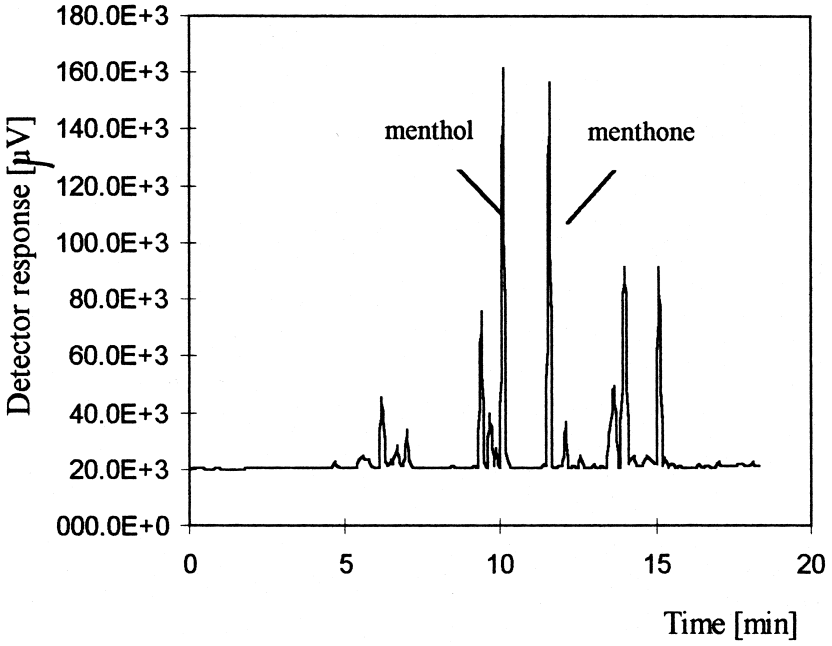

Fig. 3. Typical chromatogram obtained from peppermint chewing gum sample after ca. 10 μm PDES coated fiber use.

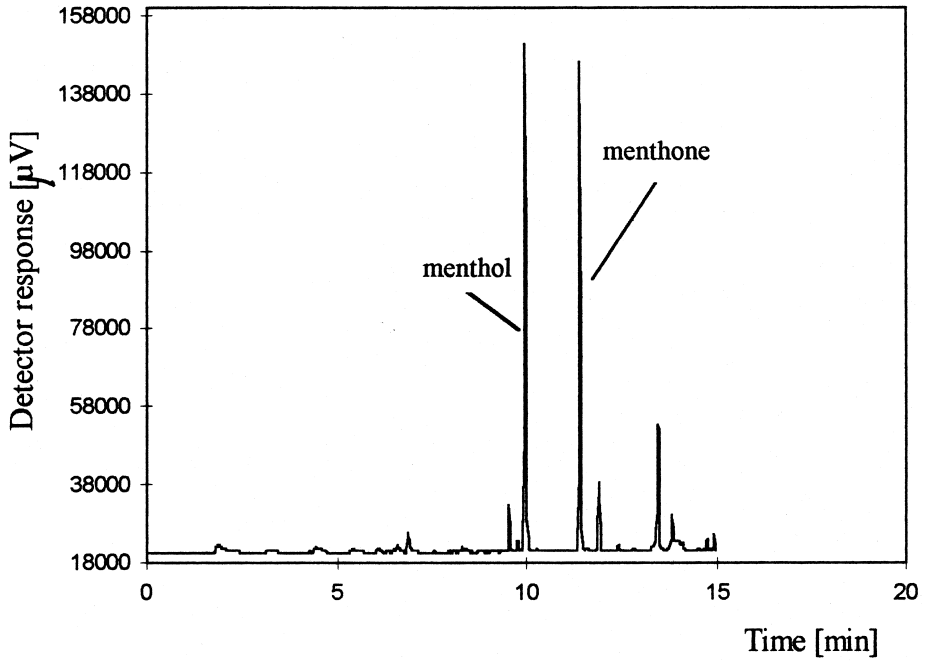

Fig. 4. Typical chromatogram obtained from peppermint tea sample after ca. 10 μm PDES coated fiber use.

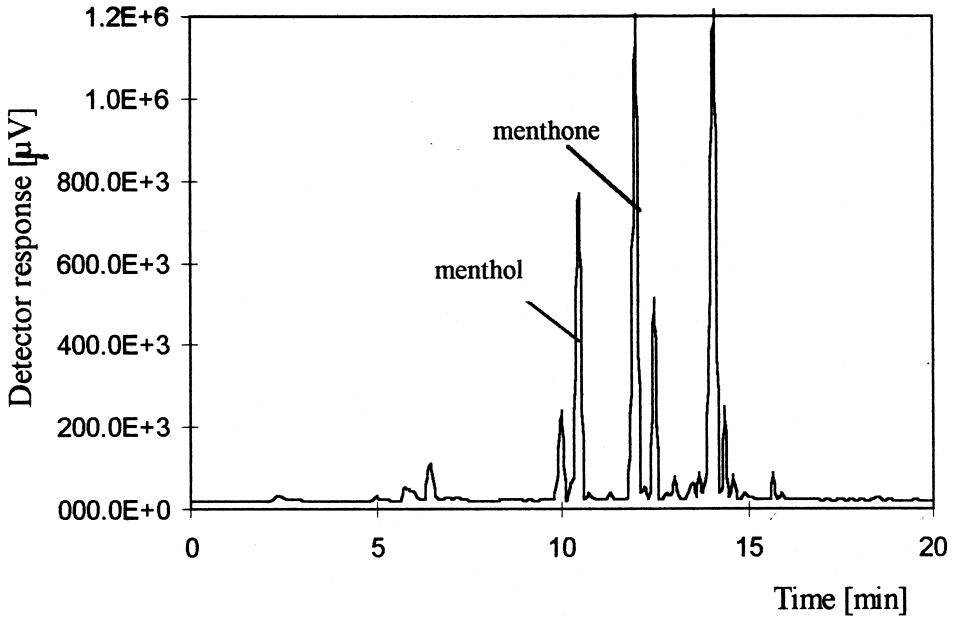

Fig. 5. Typical chromatogram obtained from gastric peppermint drops sample after ca. 10 μm PDES coated fiber use.

Table 2
Menthol content in food samples for $n=3$ using 100 μm PDMS (7 μm PDMS)

Sample	Sample mass (mg)	Determined concentration (μg/ml)	SD	RSD (%)	Concentration of original sample (%, w/w)
Menthol candies	46.60	5.56 (5.55)	0.49 (0.11)	8.81 (1.98)	0.022 (0.024)
Peppermint tea	18.11	8.73 (15.73)	0.63 (0.24)	7.22 (1.53)	0.010 (0.169)
Peppermint chewing gum	49.70	95.63 (54.09)	5.42 (0.95)	5.67 (1.76)	0.387 (0.220)
Gastric peppermint drops	22.74	433.35 (436.35)	9.49 (9.74)	2.18 (2.23)	38.304 (38.593)

Table 3
Menthone content in food samples for $n=3$ using 100 μm PDMS (7 μm PDMS)

Sample	Sample mass (mg)	Determined concentration (μg/ml)	SD	RSD (%)	Concentration of original sample (%, w/w)
Menthol candies	46.6	1.47 (5.01)	0.12 (0.24)	8.16 (4.79)	0.006 (0.022)
Peppermint tea	18.11	5.12 (15.41)	0.45 (0.98)	8.79 (6.36)	0.057 (0.171)
Peppermint chewing gum	49.7	31.88 (32.20)	0.56 (0.83)	1.76 (2.58)	0.129 (0.130)
Gastric peppermint drops	22.74	590.36 (592.37)	15.97 (19.32)	2.71 (3.26)	52.182 (52.359)

Table 4
Menthol content in food samples for $n=3$ using ca. 10 μm PDES

Sample	Sample mass (mg)	Determined concentration (μg/ml)	SD	RSD (%)	Concentration of original sample (%, w/w)
Menthol candies	47.25	5.45	3.94	8.34	0.023
Peppermint tea	18.69	13.73	1.10	7.96	0.148
Peppermint chewing gum	49.43	49.10	3.28	6.67	0.200
Gastric peppermint drops	22.74	468.40	19.47	4.16	41.400

detector responses for menthol and menthone are quite similar. The molecular mass of menthol differs from menthone's by one mass of hydrogen.

Many factors such as: contact time between two phases, stirring and matrix effect influence the adsorption and desorption processes. These factors have considerable influence on the equilibrium between the concentration of analytes in the sample and the surface of the fiber [1,14]. Adsorption profiles were carried out to determine equilibration times for menthol using various coated fibers (Fig. 6). A higher adsorption profile for PDES ca. 10 μm than for 7 μm PDMS is observed.

All experiments confirmed that the thickness of the fiber coating exerts an influence on extraction recoveries. For the differing thickness of the fiber

Table 5
Menthone content in food samples for $n=3$ using ca. 10 μm PDES

Sample	Sample mass (mg)	Determined concentration (μg/ml)	SD	RSD (%)	Concentration of original sample (%, w/w)
Menthol candies	47.25	4.39	0.35	7.97	0.019
Peppermint tea	18.69	13.68	0.96	7.02	0.147
Peppermint chewing gum	49.43	26.68	1.01	3.78	0.108
Gastric peppermint drops	22.74	591.67	13.02	2.20	52.298

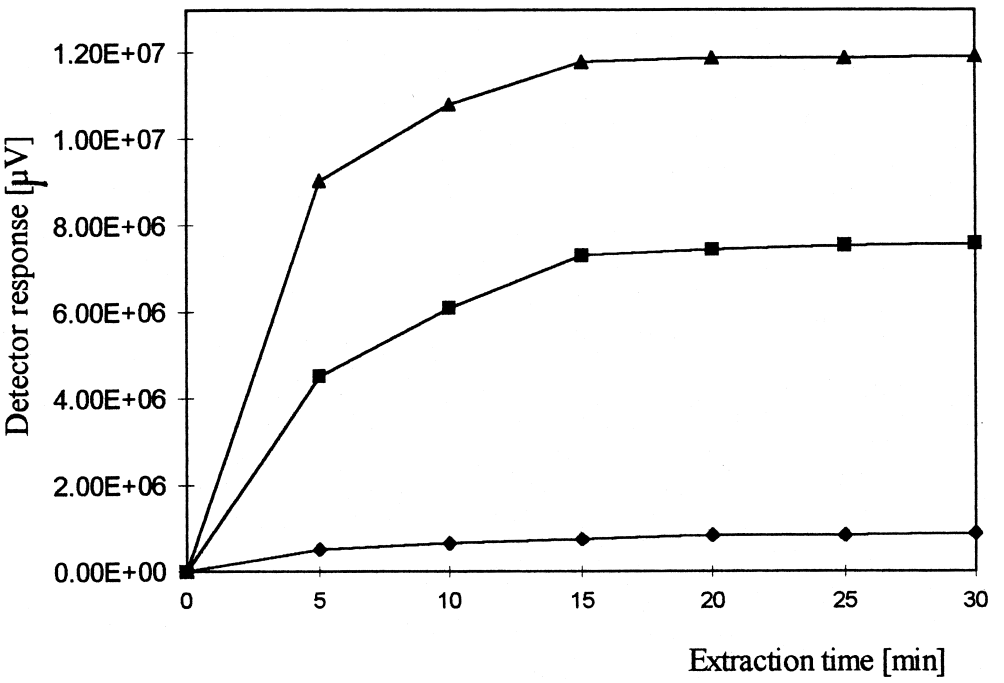

Fig. 6. The isotherm of adsorption profiles for menthol standard sample 26 μg/ml (diamonds=7 μm PDMS, squares=ca. 10 μm PDES, triangles=100 μm PDMS).

coatings (7 μm PDMS and ca. 10 μm PDES) higher extraction recoveries for 10 μm PDES (about 90% better) are observed.

The final results of determination of menthol and menthone in real samples suggest the possibility of application of SPME in food and pharmaceutical product laboratory tests.

4. Conclusions

SPME coupled with GC–FID is a good method for isolation and determination of menthol and menthone and can be recommended for routine food and pharmaceutical analyses. Compared with other extraction methods, SPME can be performed easily and is fast. It is inexpensive and environment-friendly (solvent-free).

Good selectivity of PDES makes it possible to search for new applications of PDES as coatings for SPME fibers.

Acknowledgements

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