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Evaluation of headspace solid-phase microextraction for the analysis of volatile carbonyl compounds in spirits and alcoholic beverages

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Abstract

A method was developed for the determination of C_1–C_6 carbonyl compounds in alcoholic solutions using pentafluorobenzoxymation followed by headspace sampling solid-phase microextraction and subsequent analysis by GC with electron-capture detection. Experimental conditions—alcohol content, exposure time, temperature and sample agitation were optimised. In this method, a spirit or distilled alcoholic beverage is first adjusted to 20% (v/v) alcohol. Detection limits for particular aldehydes and ketone varied from 0.05 to 0.5 μg/l and relative standard deviation was between 2.3 and 20%. Generally, the method showed good linearity for the tested concentration range 8 μg/l–0.32 mg/l with regression coefficients ranging between 0.9434 and 0.9983. The method was applied to the analysis of real alcoholic beverages (vodkas).

Keywords: Food analysis; Derivatisation, GC; Carbonyl compounds; Aldehydes

1. Introduction

There is still a great demand for reliable and sensitive methodology for determination of individual carbonyl compounds (aldehydes and ketones) in different foodstuffs and drinks due to their potential adverse health effects [1,2].

In spirits and alcoholic beverages, low molecular mass carbonyls (C_1–C_6) are present as by-products of yeast fermentation, intermediates in the formation of fusel oil and as a result of alcohol oxidation at various stages of beverage production. Their presence is undesirable because some of them are responsible for unpleasant organoleptic properties of alcoholic drinks. Furthermore, they can bind in vivo to biological nucleophiles (proteins, DNA, cellular membranes, enzymes) resulting in toxic mutagenic and carcinogenic effects.

Problems with carbonyl compounds determinations are caused by: (a) their low concentrations and complex matrices (in alcoholic matrices up to 15 carbonyls may be present), (b) wide range of their concentration (from mg/l to ng/l levels) and (c) by their high reactivity, especially in the case of unsaturated constituents.

Carbonyl species can be determined by several methods (titrimetric, colorimetric, enzymatic) but HPLC and GC are the most convenient techniques. The application range of these techniques may be

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expanded by transformation of these compounds into appropriate derivatives and using selective detection. Four common derivatization agents, i.e. 2,4-dinitrophenylhydrazine (DNPH) [3,4], 2,4,6-trichlorophenylohydrazine (TCPH) [5], cysteamine (2-aminoethanethiol) [6,7] and *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxyamine (PFBHA) [8–12] have been found useful for this purpose. The derivatives formed (hydrazones, thiazolidines and oximes) prior to chromatographic analysis are usually extracted using appropriate solvents.

There are many papers on the determination of carbonyl compounds in aqueous solutions but little work has been done on their determination in alcoholic solutions. Recently, the procedure recommended for the determination of aldehydes and ketones, based on their derivatization with PFBHA and GC with electron capture detection (ECD) has been adapted for alcoholic solutions [13]. Liquid–liquid extraction (LLE) with subsequent GC–ECD permits to determine the C_1–C_6 carbonyls at the ng/ml level in different spirits and alcoholic beverages.

Solid–phase microextraction (SPME) constitutes a convenient alternative to commonly used extraction techniques (especially LLE) because is a simple, solvent-free, inexpensive, reliable and easily automated technique [14]. This technique has been successfully applied for the determination of a wide spectrum of analytes in a large variety of matrices [15–19].

In this work, the possibility of using headspace SPME sampling of oximes formed from corresponding carbonyl compounds in reaction with the PFBHA in alcoholic solutions to prior their chromatographic determination has been studied.

2. Experimental

2.1. Reagents and standards

The standards of methanal, ethanal, propanal, propenal (acrolein), butanal, isotutanal (isobutylraldehyde), pentanal (valeraldehyde), 2-butenal (crotonaldehyde), isopentanal (isovaleraldehyde) (all except methanal 97–99%) were purchased from Sigma–Aldrich. Methanal was provided in the form of 37% aqueous solution. Dimethylketone and hexanal were obtained from POCH (Gliwice, Poland). Stock standard solutions of each carbonyl compound were prepared in 10 ml of methanol (J.T. Baker, Deventer, Netherlands) and then diluted to the required concentration with neutral ethanol in the concentration range of 8 and 320 μg/l. The derivatizing reagent PFBHA (Sigma–Aldrich) was dissolved in doubly distilled water at a concentration of 10 mg/ml.

2.2. Instrumentation

Gas chromatographic analyses were carried out with a Perkin-Elmer Auto System XL GC instrument equipped with a ^{63}Ni ECD system and a split/splitless injector. The column used was a Rtx-5 capillary column (30 m×0.32 I.D., 3 μm film thickness). The split/splitless injector and detector temperatures were set at 280 and 250 °C, respectively. The initial oven temperature was kept at 100 °C for 1 min, which was increased to 160 °C at 3 °C/min, then raised to 220 °C at 5 °C/min and finally to 280 °C at 20 °C/min. The total time run was 36 min.

2.3. Derivatisation procedure

PFBHA reacts with the carbonyl species (R_1COR_2) to produce two oxime isomers (*Z*, *E*) when the alkyl groups R_1 and R_2 are different. Usually, the two isomers can be chromatographically separated [13]. The derivatisation process was carried out in ethanol–water solutions. First, 10 ml of an alcoholic solution of carbonyl compounds was placed in a 16-ml vial and then 0.1 ml of PFBHA solution (10 mg/ml) was added. The vial was capped with PTFE-faced silicone membrane and heated using a water bath at 45 °C for 1.5 h. After derivatisation, the sample was cooled to room temperature and two drops of concentrated sulfuric acid were added to adjust to pH 2. After shaking, the sample was ready for SPME experiments. The relatively long time needed for derivatisation influences the total time for analysis. Recently, a new derivatisation methodology with PFBHA using a microwave oven was proposed reducing the reaction time by a factor of 50–100 [20].

2.4. SPME procedure

A SPME holder for manual use and 100 μm PDMS (polydimethylsiloxane) was purchased from Supelco. This fiber was selected on the basis of previous experiments with direct SPME of oximes from alcoholic solutions [13]. During extraction, the sample was agitated using a magnetic stirrer. Immediately after extraction, the fiber was introduced into the GC injector for 5 min in split mode (1:20). By exposing the fiber to the carrier gas stream, the analytes were thermally desorbed at 250 °C and transferred onto the GC column. Optimal desorption time was found to be 5 min. This time was enough to ensure total desorption and no memory effects were observed which was confirmed by desorbing the same fiber a second time after the initial desorption.

3. Results and discussion

The effects of the main parameters that can affect the SPME process from headspace, i.e. temperature, extraction time, agitation and ionic strength were evaluated. In the case of a solution containing alcohols, it was also necessary to check the effect of alcohol content for extraction efficiency.

3.1. Selection of optimal concentration of alcohol

The presence of alcohol (methanol or ethanol) in the investigated solutions may act as a co-solvent for partitioning of carbonyl compounds in the phases involved [21,22]. Therefore, the first step was to check the effect of alcohol concentration on extraction efficiency. The exposure was carried out for the samples with the same amount of carbonyl compounds (60 μg/l) but with different alcohol content (10, 20, 40 and 70%, v/v). Each experiment was carried out three times and results were averaged. Fig. 1 presents the peak area of oximes versus ethanol concentration. It may be seen that the efficiency decreases with an increase in alcohol content. This suggests that alcohol competes with absorption in PDMS film which was suggested earlier [23–25]. Furthermore, higher concentrations of alcohol in solution favour the conditions for forming acetals and hemiacetals. On the other hand, it should be noted that high dilution may decrease the limit of detection for particular aldehydes and acetone. Similar results were obtained when methanol was used [26]. The direct immersion of the fiber in alcoholic solutions also confirmed this conclusion [26]. As a compromise between efficiency and limit of detection, an ethanol–water (20:80) solution was assumed to be optimal.

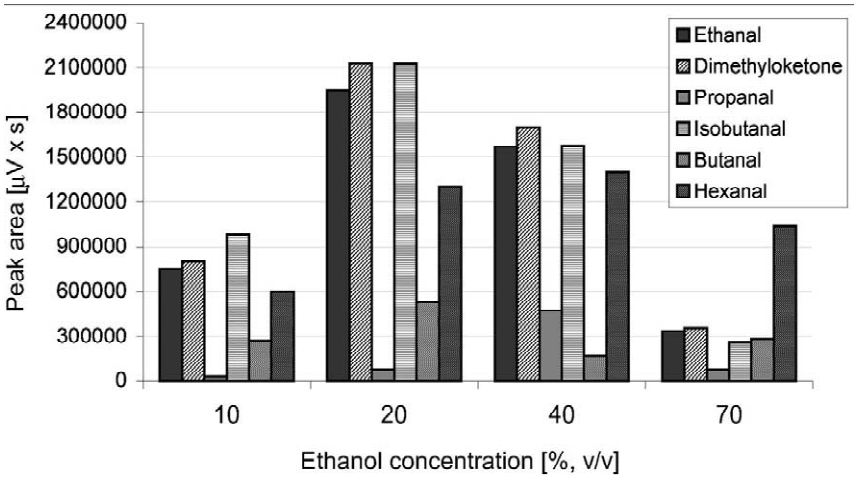

Fig. 1. Effect of ethanol concentration on extraction efficiency of the oximes at 35 °C from headspace SPME of ethanol–water solutions (100 μm PDMS fiber, 30 min exposure time, desorption time 5 min at 250 °C).

3.2. Effect of temperature

Extraction temperature controls the diffusion rate of analytes into the coating. An increase in extraction temperature causes an increase in the extraction rate and a simultaneous decrease in the distribution constant between the analytes and the fiber. Optimisation of extraction temperature is generally more important with headspace SPME than when working with direct immersion of the fiber in the liquid sample. Bao et al. [8] and Cancho et al. [12] used headspace extraction of oximes from aqueous solutions at room temperature. Room temperature is also recommended in the method proposed by the US Environmental Protection Agency (EPA) [9].

The effect of temperature in the extraction was investigated varying the temperature between 25 and 45 °C with a constant extraction time. As is shown in Fig. 2 an increase in temperature generally improves the mobility of carbonyl species through liquid and gas phase and better efficiencies were obtained. In the case of acrolein, a decrease in the extraction yield was observed. This may be caused by instability of this unsaturated aldehyde at elevated temperature. Considering the influence of temperature on the extraction efficiency (ambient temperature is usually variable in the range of 5 °C) to keep the vials at constant temperature, all SPME experiments were conducted at 35 °C.

3.3. Effect of extraction time and agitation

It is not required to reach the equilibrium by the SPME analysis. But exposure time strongly influences the extraction efficiency and eventually affects the detection limit. Usually the exposure time is a compromise between the analysis time and required detection limit. Absorption–time profiles on PDMS 100-μm fiber in headspace extraction mode were generated for each carbonyl compound and are presented in Fig. 3. Each data point is the average of three independent measurements. For methanal, ethanal, propanal and isobutanal, equilibrium was reached in 30 min. Higher-chain aldehydes and acetone needed longer exposure times. Considering this conclusion and duration time for analysis, an extraction time of 30 min was chosen for subsequent analyses.

Agitation is an important parameter that affects the time profiles. For the analytes that are less volatile, the extraction efficiency is usually notably enhanced by stirring because the transfer of the compounds from liquid solutions to headspace could conceivably be speeded up by agitation. This was confirmed by

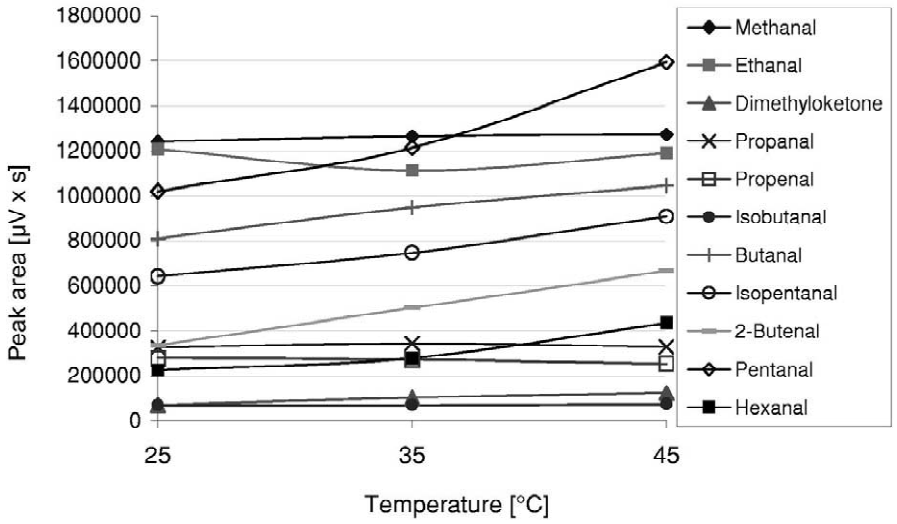

Fig. 2. Influence of temperature on detector response area of the oximes formed from carbonyl compounds (concentration level 90 μg/l) and headspace SPME extraction (30 min) with the 100 μm PDMS fiber from ethanol–water (20:80) solutions.

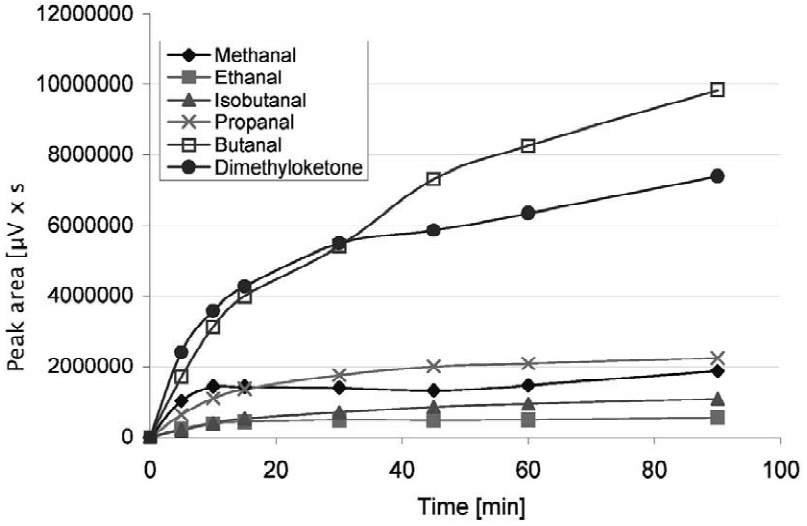

Fig. 3. Extraction-time profiles (magnetic stirring) for the oximes of carbonyl compounds in 20% ethanol–water solutions by headspace SPME (100 μm PDMS fiber, 60 μg/l carbonyl compounds concentration, 30 min exposure time, desorption time 5 min at 250 °C).

our experiments, both for direct [26] and headspace SPME. Comparing Figs. 3 and 4, it is clear that extraction efficiently was notably enhanced using a magnetic stirrer, especially for higher-chain components. In spite of the fact that ultrasonic agitation of the sample should be more effective, because the two phases are mixed, small differences between the two agitation systems were observed [26]. Therefore, a magnetic stirrer was used in the following experiments.

3.4. Salt effect

The addition of salting-out agents improved ex-

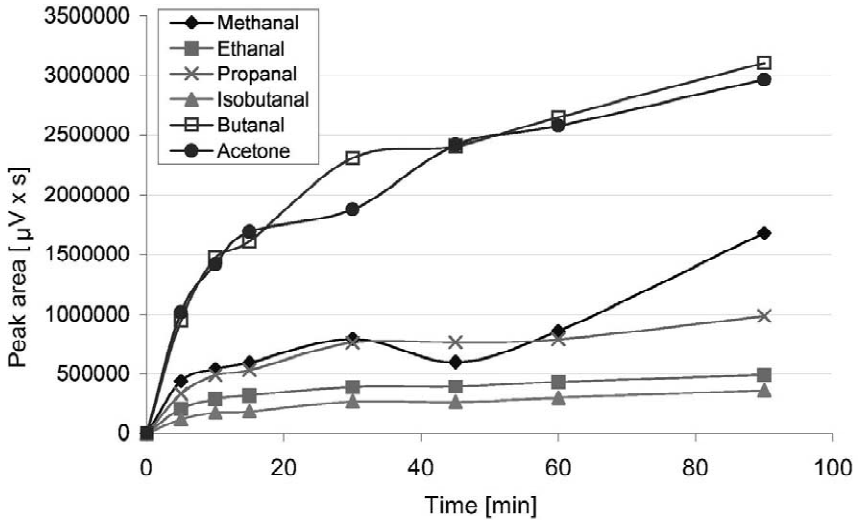

Fig. 4. Extraction-time profiles (without stirring) for the oximes of carbonyl compounds in ethanol–water (20:80) solutions by headspace SPME (100 μm PDMS fiber, 60 μg/l carbonyl compounds concentration, 30-min exposure time, desorption time 5 min at 250 °C).

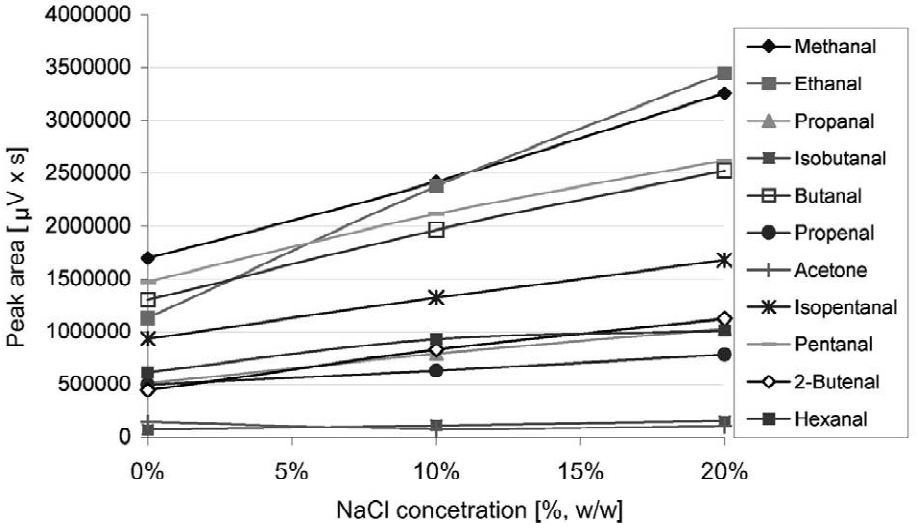

Fig. 5. Influence of sodium chloride content on detector response area of the oximes (100 μm PDMS fiber, 60 μg/l carbonyl compounds concentration, 30-min exposure time, desorption time 5 min at 250 °C).

traction efficiency for many analytes in different samples. The presence of dissociated ions decreases the solubility of analytes, which then partition more readily into the headspace. Furthermore, a salt alters the phase boundary enhancing the volatilisation into the headspace of analytes dissolved in liquid phase.

The effect of ionic strength on extraction efficiency was evaluated by analysing the amount of carbonyl compounds extracted in sample solutions containing 0, 10 and 20% (w/v) of sodium chloride (20% alcohol solutions). These results are shown in Fig. 5. As can be seen, the response of oximes increased in proportion to the added amount of NaCl.

3.5. Calibration

Calibration have to be carried out for each compound in order to achieve accurate quantitative results.

The linearity of the calibration graphs was tested with at least five calibration points over the expected concentration range of carbonyl species in alcoholic beverages. For each concentration level, five independent measurements were made. Table 1 presents the equations for calibration curves and correlation coefficients (r) and the relative standard deviations (RSDs) for the compounds tested. The weak

Table 1
Parameters of calibration curves for selected carbonyl compounds in the concentration range from 8 μg/l to 0.32 mg/l using headspace SPME extraction

Compound	Equation of calibration curve	Correlation coefficient	RSD (%, $n=3$)
Methanal	$y=14\,327\,282x-4394$	0.99825	10.5
Ethanal	$y=5\,743\,203x+5992$	0.99412	13.5
Dimethylketone	$y=2\,403\,352x+28\,727$	0.99448	6.6
Propanal	$y=11\,973\,054x+30\,960$	0.98917	15.4
Propenal	$y=9\,463\,557x+20\,842$	0.97991	5.5
Butanal	$y=24\,311\,503x-14\,208$	0.99761	9.8
Isopentanal	$y=41\,180\,319x-357\,586$	0.94337	13.7
2-Butenal	$y=6\,645\,899x+141\,035$	0.89896	11.1
Pentanal	$y=25\,679\,309x+206\,671$	0.96088	14.7
Hexanal	$y=17\,739\,371x-126\,481$	0.99401	11.1

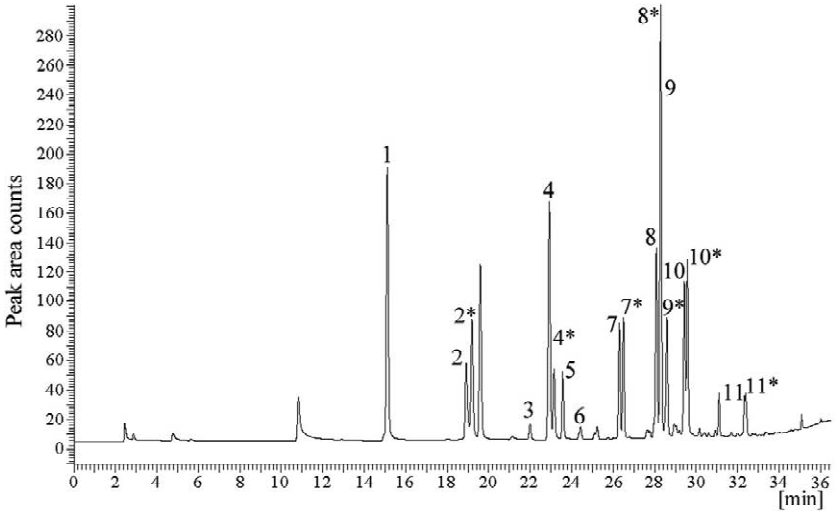

Fig. 6. GC–ECD chromatogram after headspace SPME extraction of oximes after derivatization of carbonyl compounds with PFBHA of real sample (sample No. 1, vodka, 40%) diluted to 20% ethanol. 1=Methanal; 2, 2*=ethanal; 3=dimethylketone; 4,4*=propanal; 5,5*=propenal; 6,6*=isobutanal; 7,7*=butanal; 8,8*=isopentanal; 9,9*=2-butenal; 10,10*=pentanal; 11,11*=hexanal. Numbers with and without asterisks: *E* and *Z* isomers.

correlation for 2-butenal and isopentanal may be explained by the overlapping of corresponding oxime isomers and using only one peak for quantification. The detection limits were estimated as three times the standard deviation of the baseline noise and ranged from 0.05 to 0.5 μg/l of 100% alcohol.

3.6. Real samples

The head space SPME method developed was applied to real alcoholic samples. Four samples of distilled alcoholic beverages (vodkas) were analysed. Fig. 6 shows a chromatogram of oximes extracted under optimised conditions described above from alcoholic beverages (40%). The initial sample was diluted to 20% ethanol before the derivatisation and extraction steps. The content of particular compounds was calculated for 1 liter of the 100% spirit and is given in Table 2. The results obtained showed that acetaldehyde was the most abundant aldehyde in all samples of vodkas, ranging from 0.1 to 0.9 mg/l. It confirms that ethanol oxidation is a dominant process in beverage production.

Table 2
Content of carbonyl compounds in distilled beverage (vodka, 40% ethanol) determined by the headspace SPME–GC–ECD procedure

Carbonyl compound	Concentration of carbonyl compounds in distilled alcoholic beverages (mg/l 100%, $n=3$)			
	No. 1	No. 2	No. 3	No. 4
Methanal	0.0100±0.002	0.0130±0.002	0.0100±0.002	0.022±0.004
Ethanal	0.1020±0.007	0.1830±0.013	0.0700±0.005	0.913±0.062
Dimethylketone	0.0060±0.0006	0.0140±0.001	0.0170±0.002	0.114±0.004
Propanal	0.0010±0.0002	0.0010±0.0002	–	0.084±0.008
Propenal	–	–	–	0.114±0.004
Butanal	0.0050±0.0005	0.0050±0.0005	0.0020±0.0002	0.0080±0.0007
Isopentanal	0.0150±0.002	0.0120±0.002	0.010±0.002	0.035±0.004
2-Butenal	0.0340±0.005	–	–	–
Pentanal	0.0060±0.0008	0.0060±0.0008	–	0.037±0.005
Hexanal	0.0270±0.001	0.0300±0.001	0.0190±0.001	0.072±0.012

4. Conclusions

The feasibility of SPME for the analysis of carbonyl compounds in different distilled alcoholic beverages is demonstrated. First, a sample of spirit or distilled alcoholic beverage is diluted to 20% (v/v) alcohol and carbonyls are derivatised with PFBHA. Next, the corresponding oximes are extracted using 100 μm PDMS fiber by direct immersion into solution at room temperature or from headspace at 35 °C. The analytical characteristic of the headspace SPME method: linearity, precision and limit of detection is comparable with direct SPME [26]. But it was proved that the headspace SPME sampling is less sensitive to the matrix effects and prolongs the life time of the fiber used. Total analysis time is about 2 h (1.5 h for derivatisation and 30 min for chromatographic analysis).

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