Chem. Anal. (Warsaw), **49**, 869 (2004)

Determination of C3–C10 Aliphatic Aldehydes Using PFBHA Derivatization and Solid Phase Microextraction (SPME). Application to the Analysis of Beer

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Key words: aldehydes, derivatization methods, PFBHA, SPME, beer analysis

In the present study the SPME on-fibre derivatization of C3–C10 aldehydes with the use of PFBHA is presented. Various procedures for adsorption of PFBHA on SPME fibre were studied. Finally, headspace adsorption mode at 20°C was adopted. After saturation with PFBHA, the SPME fibre was exposed to aldehydes in the headspace, and extraction was performed for 20 min. This approach was compared to the in-solution derivatization with PFBHA and syringe injection of the solution of oximes. The former, however, occurred to be much more sensitive and allowed the aldehydes to be extracted from complex matrices. The developed method was applied to the analysis of C3–C10 aldehydes in beer. They were determined at the level: 0.07–83.20 μg L^{-1}, depending on the type of aldehyde and the brand of beer.

Przedmiotem pracy była derywatyzacja aldehydów alifatycznych C3–C10 na włóknie SPME, z uprzednio zaadsorbowanym PFBHA. Zbadano sposoby adsorpcji PFBHA. Optymalna metoda adsorpcji przebiegała z par roztworu PFBHA w temperaturze 20°C. Po wysyceniu włókien parami PFBHA, przeprowadzono adsorpcję aldehydów z ich par, po optymalnym czasie ekstrakcji 20 min. Rezultaty porównano z wynikami derywatyzacji przeprowadzonej przy użyciu PFBHA w roztworze i z nastrzykiem analizowanego roztworu. Metoda z użyciem

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SPME charakteryzuje się wyższą czułością i umożliwia analizę aldehydów w złożonej matrycy. Metodę wykorzystano do analizy aldehydów C3–C10 w piwie. Ich stężenie wahało się od 0.07 do 83.20 μg L^{-1} w zależności od rodzaju aldehydu i marki piwa.

Volatile aldehydes can affect the flavour of food products, even when present at low concentrations. This is due to the low odour thresholds of many of them [1]. The methods of their identification and quantitation should correctly operate at low concentrations (μg L^{-1}) of the analytes. High polarity and reactivity of carbonyl compounds in aqueous matrices imposes the need for their derivatization, since derivatives are less polar, more volatile and can be detected using selective detectors. Derivatization of aldehydes allows one to lower their detection limits. For some of them, *e.g.* formaldehyde, the isolation from the matrix by trapping as less volatile derivatives is indispensable. PFBHA (O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine) is often used for this purpose. Yamada and Somiya [2] developed a method utilising PFBHA for derivatisation of carbonyl compounds. PFBHA is widely used for the determination of aldehydes in disinfected and bottled water [3,4,5]. Adsorption on solid-phase extraction (SPE) cartridges facilitates the concentration of analytes [6]. Though oximes are eluted from the SPE cartridge with non-polar solvents, such as hexane, some other matrix constituents can be still present and thus co-eluted with the analyzed compounds.

Compared to water, food products represent much more complex matrices. Therefore, to isolate volatile compounds, headspace analysis is preferred over extraction with liquids. PFBHA derivatization of aldehydes in the solution with the subsequent SPME extraction of oximes was described by Wardencki *et al*. [7]. This procedure was applied to the determination of aldehydes in distilled alcoholic drinks. Solid phase microextraction without derivatization step was utilized for the analysis of alcohols and esters in beer [8].

Beer represents a very complex matrix comprising proteins, sugars, and over 500 flavour compounds [9], among which aldehydes are the important group. The most abundant one – acetaldehyde, is partially responsible for the „green" flavour of beer. It is produced during fermentation at the concentration of up to 40 mg L^{-1}, which is relatively high. Thus, acetaldehyde is usually determined by the static headspace method. Other aldehydes (of 3–10 carbon atoms in their molecules) are formed at much lower concentrations as a result of Strecker degradation of amino acids, oxidative degradation of iso-humulones, aldol condensation in the presence of aminoacids, autooxidation of fatty acids or enzymatic degradation of lipids [10]. These aldehydes, due to their low odour thresholds (μg L^{-1} range) and unpleasant smell can undesirably influence the quality of beer and cause stale, rancid and „cardboard" flavours.

The aim of this study was to investigate some factors influencing the analysis of aldehydes performed by their PFBHA derivatization and solid phase microextraction

(SPME). Our goal was to compare conventional method of derivatization of carbonyl compounds in the solution and subsequent liquid–liquid extraction with the SPME on-fiber derivatization. The latter approach eliminates matrix impurities co-extracted with the analyte, and lengthens the column lifetime, which is a key factor for such complex samples as beer.

EXPERIMENTAL

Methods and Materials

The following standards were used: propanal (97%, Aldrich), butanal (99%, Aldrich), pentanal (>98%, Fluka), hexanal (>98%, Fluka), heptanal (95%, Aldrich), octanal (99%, Aldrich), nonanal (95%, Aldrich), decanal (95%, Aldrich), and *trans*-2-nonenal (97%, Aldrich). PFBHA (o-2,3,4,5,6-pentafluorobenzylhydroxylamine) of >98% purity and hexane of >95% purity were purchased from Aldrich. Sulfuric acid and anhydrous sodium sulfate were of analytical grade, and were obtained from POCH (Polskie Odczynniki Chemiczne). Deionized water (18.2 MΩ) used for the experiments was obtained from MiliQ apparatus (Milipore). Six different bottles of beer (Pils type) were purchased in the local store and subjected to the analysis. Beer differed in the alcohol content (1.2 to 7.8% v/v) and in the extract content (11.1 to 15.1% m/v). Stock mixture of aldehydes was prepared as follows: approximately 100 mg of each standard was weighted and placed in a 10 mL volumetric flask partially filled with methanol. After that, the volume was made up to the mark. Working solutions were prepared by the appropriate dilution of methanolic stock solutions with water.

Direct derivatization of aldehydes in the sample

PFBHA solution was prepared by dissolving 10 mg of the reagent in 10 mL of water. 1 mL of 1 mg L^{-1} PFBHA solution was added to 20 mL of the sample, and the reaction vial was left at the room temperature for 1h. After that time the reaction was quenched by adding concentrated sulphuric acid (approximately four drops) and 1 mL of hexane to extract the generated oximes. The organic layer was washed with 3 mL of 0.1 n sulfuric acid, separated and dried over the anhydrous sodium sulfate (50 mg). 1 mL of the organic phase was injected into gas chromatograph.

On-fiber derivatization of aldehydes

SPME fibre was saturated with PFBHA using three different procedures, in order to find the one providing the highest peaks in the chromatogram: adsorption of PFBHA directly from its 0.1% solution at 20°C, 20 min adsorption of PFBHA from the headspace over its 0.1% aqueous solution at 20°C, or at 50°C. Fibre saturation was performed using 20 mL headspace vials filled with 10 mL of PFBHA solution. After the saturation, SPME fibre was used to extract aldehydes from 10 mL of their standard solutions. Extraction time ranged from 1 to 60 min. After that, oximes formed on the fibre were desorbed at 260°C in the injection port of gas chromatograph.

Equipment

SPME fiber coated with PDMS (Polydimethylsiloxane, Supelco, Inc.) was used throughout. The samples were analyzed on a Hewlett–Packard chromatograph, Model HP 6890 with split/splitless injection port and 0.75 mm liner and electron capture (ECD) detector. Injector and detector temperatures were kept at 260°C and 250°C, respectively. Helium (99.9995%) was used as a carrier gas (33 cm s^{-1}). Nitrogen (99.9995%) served as make up anode purge in ECD. Chromatographic separation was performed on a HP–5 column (30 m × 0.32 mm × 0.25 μm, Agilent Technologies, CA), applying the following temperature programme: 40°C for 2 min, 8°C min^{-1} up to 280°C, 280°C for 10 min. Injection port was set at the splitless mode. Data were collected and processed using a HP Chemstation 08.03.

RESULTS AND DISCUSSION

Direct derivatization

Derivatization was performed at 20°C for 1 h. Calibration plots were constructed in the concentration range of 1–500 μg L^{-1}. For all aldehydes R^2 equalled to 0.999 apart from octanal and decanal, for which $R^2 = 0.988$. Detection limits were lower than 1 μg L^{-1} for all the analytes. Repeatability studies were carried out with water samples spiked with 50 μg L^{-1} mixtures of aldehydes. The analysis was repeated 5 times, and RSD values ranged from 1.17% (for propanal) to 6.01% (*trans*-2-nonenal).

Though derivatized aldehydes form double peaks on the chromatogram due to the presence of syn- and anti- isomers, nonanal and decanal appeared as single peaks (Fig. 1). Similarly, Cancho *et al*. [11] did not observe double peaks of nonanal and decanal using DB 1701 column [11].

On-fiber derivatization. Extraction mode and extraction time

Elaboration of the on-fibre derivatization procedure required optimisation of several parameters: saturation of the fibre with PFBHA, the effect of time and temperature on the amount of extracted aldehydes.

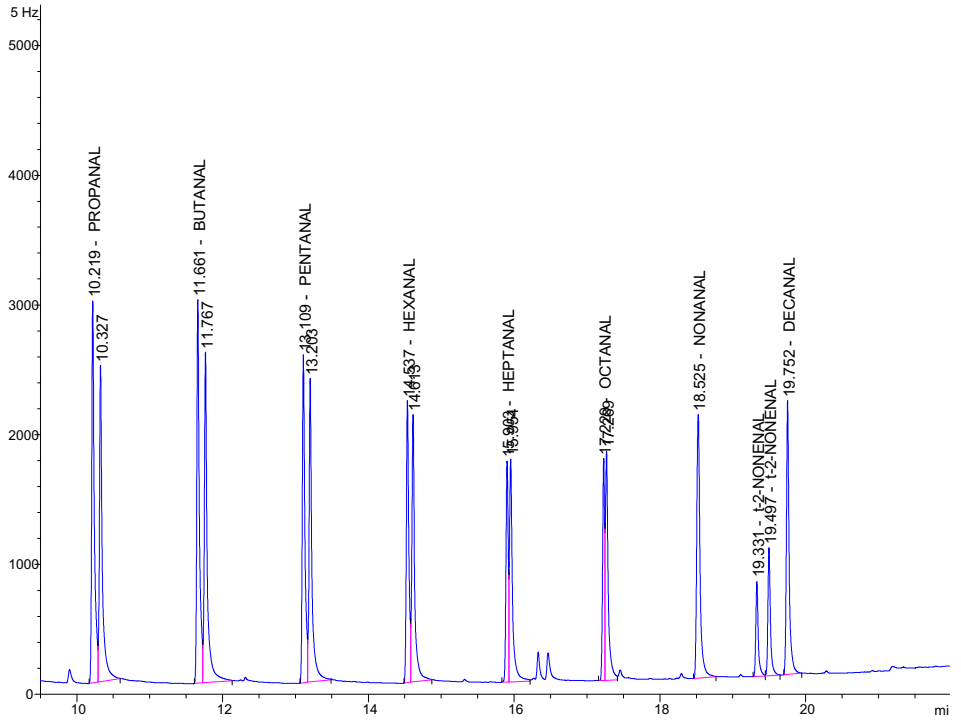

Figure 1. Chromatogram of a mixture of PFBHA derivatized aldehydes; the ECD signal is measured in Hertz and output display is the actual detector frequency divided by 5 in this model of detector. Retention times for hexanal – 14.537 min and 14.613 min, heptanal – 15.903 min and 15.954 min, octanal – 17.220 min and 17.269 min

Saturation of SPME fibre with PFBHA

Fibre saturation with PFBHA was performed either at 20°C, or 50°C from aqueous solution, or from the headspace over a 0.1% solution. Extraction lasted for 1, 5, 10, 20 and 30 min. PFBHA peak areas increased as the exposition time was increased. The smallest peak areas were observed for the fibres immersed in the solution, while the largest – for the fibres exposed in the headspace over the 0.1% PFBHA solution at 50°C. Figure 2 presents PFBHA absorption profiles under the applied conditions. RSD ranged from 3 to 23%, the latter observed for extraction at elevated temperatures. RSD values for SPME fibre immersed in the solution ranged from 3.29 to 9.22%, for the headspace sampling at 20°C from 3.24 to 15.43%, and for the headspace sampling at 50°C from 3.85 to 23.00%. The results of ANOVA analysis revealed no statistically significant differences between 20- and 30-minute extraction times. Thus, 20 min extraction time was chosen as the optimum one. To saturate the fibre, Staschenko *et al*. [12] used PFPH (pentafluorophenylhydrazine), which forms hydrazones with

carbonyl compounds [13,14]. They observed that SPME fiber coated with PDMS-DVB (polydimethylsiloxane-divinylbenzene) adsorbed 2.2 times more PFPH than PDMS did. Also the adsorption rate of PFPH increased between 10 and 80 min, but between 60 and 80 min the increase was not significant.

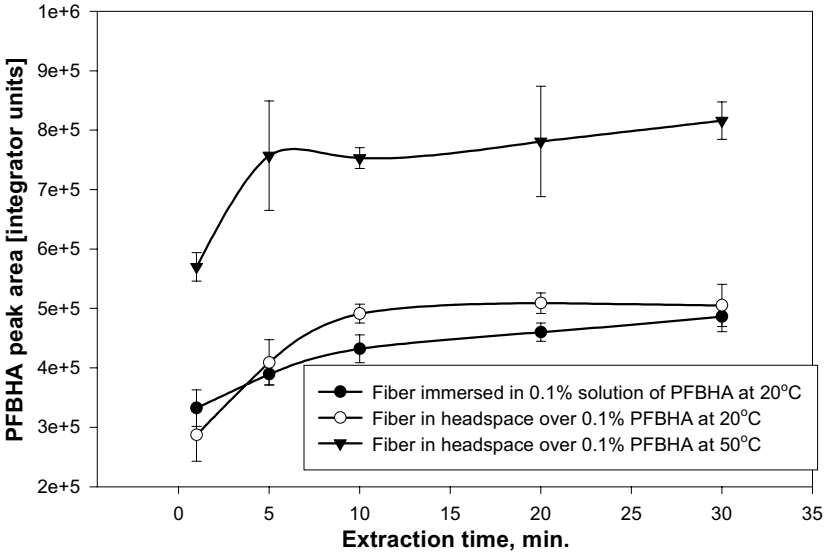

Figure 2. Extraction curves for PFBHA; peak areas correspond to PFBHA adsorbed on SPME fibre under different conditions; extraction time: 1–30 min

Extraction of aldehydes

The saturation of the fibre with PFBHA preceded the extraction of aldehydes. In the extraction procedure working standard solution containing each aldehyde at the concentration of 1 μg L^{-1} were used. 1, 5, 10, 20, 30 and 60 min extraction times were applied. Even 10-fold increase of the peak area was noted for exposition times between 1 and 60 min. Extraction profiles are shown in Figure 3. RSD values ranged from 2 to over 30%, the highest were obtained for 1 min extraction time. From the extraction curves it is evident that no equilibrium has been reached within examined extraction times. Considering the duration of chromatographic run, a 20 min extraction time (at 20°C) was chosen for further experiments.

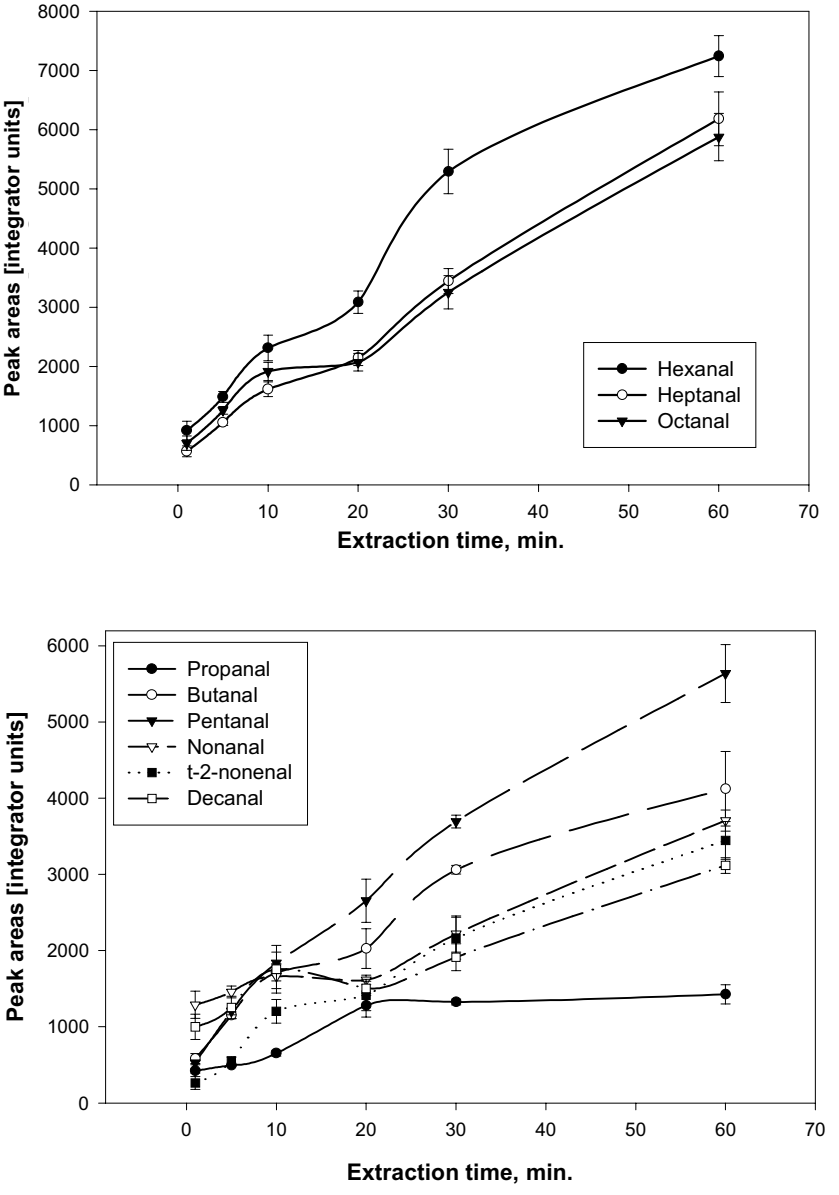

Figure 3. Extraction curves for C3–C10 aldehydes; aldehydes were adsorbed on SPME fibre after its previous saturation with PFBHA vapors; extraction time: 1–60 min

For the PFBHA–HS–SPME–GC–ECD extraction method calibration plots for all aldehydes were constructed. Linearity was observed in the concentration range of 1 to 100 μg L^{-1}. Correlation coefficients were 0.995 for acetaldehyde, 0.999 for

propanal, butanal and pentanal, 0.998 for hexanal and heptanal, 0.996 for octanal, 0.994 for nonanal, 0.982 for *trans*-2-nonenal and 0.989 for decanal. Detection limits were lower than for the in-solution (direct) derivatisation, especially for aldehydes with 4–8 carbon atoms in their molecules. Standard mixture of the same concentration (50 μg L^1) was analyzed by these two methods. The results are compared in Figure 4. Much larger peak areas and, in consequence, lower detection limits obtained in SPME methods was a result of the enrichment process, which proceeded in the SPME fibre coating. Repeatability ranged from 0.6 to 6% (50 μg L^1 concentration, 5 replicates), except from decanal, for which RSD = 15%.

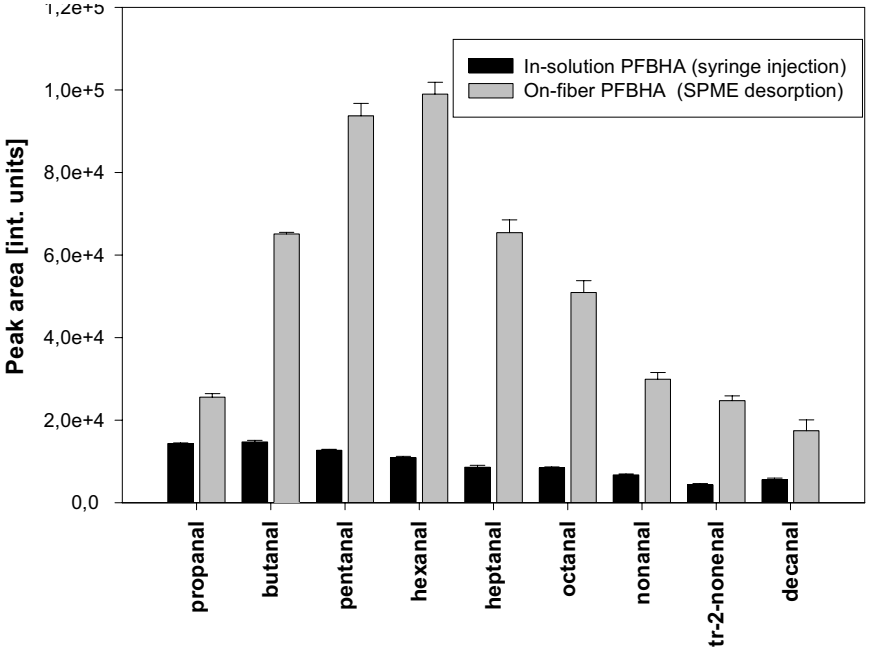

Figure 4. Comparison of the peak areas of C3–C10 aldehydes determined applying the in-solution (derivatized aldehydes injected with a syringe) and the on-fibre (desorption of derivatized aldehydes from the SPME fibre) methods. 50 μg L^{-1} standards solution was used for the comparison

The developed method was compared to the derivatization of the aldehydes in the solution. The generated oximes were adsorbed on the SPME fibre. For most of the analytes one obtained the peaks of *ca* 2-fold larger areas than previously (Fig. 5). Interestingly, the longer the carbon chain of the aldehyde, the more consistent were the results obtained by both methods. Moreover for nonanal, *trans*-2-nonenal and decanal the peak areas were higher when on-fibre derivatization was applied. These facts should be considered when analysing long-chain aldehydes.

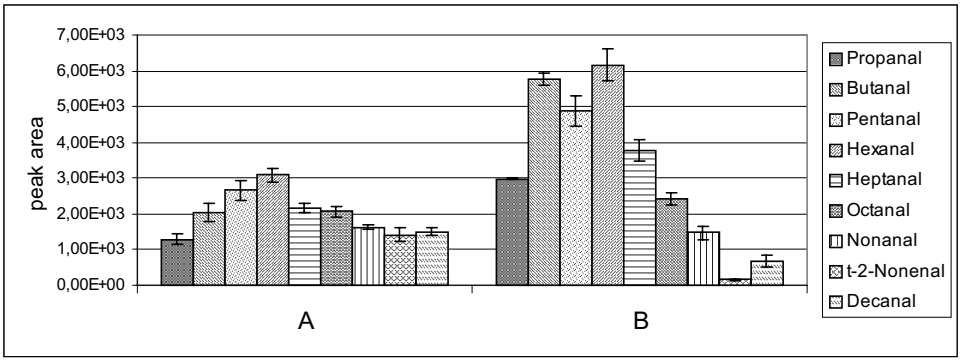

Figure 5. Comparison of the peak areas of aldehydes determined using two approaches: A – saturation of the fibre with PFBHA (for 20 min at 20°C, headspace), followed by adsorption of aldehydes (for 20 min at 20°C, headspace); B – adsorption of oximes generated during in-solution derivatisation with PFBHA

In this work only PDMS fibre was used. Cancho *et al*. [11] found out that mixed SPME phases allow more compounds to be adsorbed than PDMS ones and the extraction efficiency depends on the number of carbon atoms and the structure of aldehydes. Staschenko *et al.* [12] observed higher recoveries of aldehydes after the on-fibre derivatization (87%) compared to the in-solution derivatization (only 61%). Bao *et al.* [15] used PFBHA for derivatization of carbonyl compounds in water, followed by the extraction with SPME from liquid or headspace. Derivatization was completed within 2 h at the room temperature. In these experiments absorption equilibrium for C2–C6 aldehydes was reached within 20–60 minutes, while for C7–C10 aldehydes equilibrium was still not reached within 120 min. Recently, Ochiai [16] has developed a method for the determination of carbonyl compounds in beer. The method utilized stir bar sorptive extraction (SBSE) with in-situ derivatization, and was followed by thermal desorption and GC–MS analysis.

Analysis of beer samples

Direct in-solution derivatisation with the syringe injection of oximes could be easily applied to the analysis of aldehydes in water, due to the relative simplicity of the matrix. In the case of beer, subsequent injections resulted in a dramatic increase of the initial detector signal, which indicated clogging of the column with co-extracted matrix impurities. After analysing 2–3 samples, the column had to be washed several times with 10 μL of hexane and methanol, followed by the heating at 290°C. For this reason, direct derivatization is not recommended for the analysis of beer aldehydes. The time required to complete derivatization in such matrix is also questionable: Olaja *et al.* [17] claim that 5 h is needed.

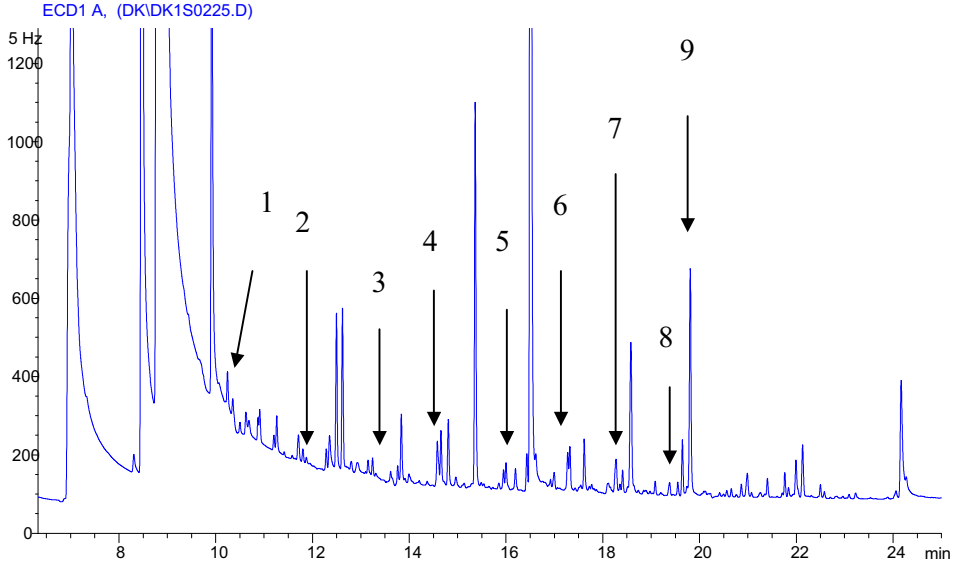

Figure 6. Chromatogram of beer sample analysed with the proposed method 1 – propanal; 2 – butanal; 3 – pentanal; 4 – hexanal; 5 – heptanal; 6 – octanal; 7 – nonanal; 8 – *trans*-2-nonenal; 9 – decanal

All beer samples were analyzed using PFBHA–HS–SPME method utilizing adsorption of PFBHA in the first stage and subsequent adsorption of aldehydes (Fig. 6). The results are presented in Table 1.

Table 1. The contents of C3–C10 aliphatic aldehydes in beer. The analytes were extracted using PFBOA––HS–SPME–GC–ECD method

Aldehyde	Contents of aldehydes, μg L^{-1}					
	Brand A	Brand B	Brand C	Brand D	Brand E	Brand F
Propanal	1.13 ± 0.06	0.71 ± 0.03	0.94 ± 0.05	1.25 ± 0.03	1.15 ± 0.12	2.45 ± 0.10
Butanal	0.23 ± 0.01	0.16 ±0.01	0.17 ± 0.01	0.22 ± 0.02	0.16 ± 0.01	0.84 ± 0.11
Pentanal	0.10 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.15 ± 0.01	0.41 ± 0.14	0.23 ± 0.01
Hexanal	0.33 ± 0.03	0.25 ± 0.02	0.23 ± 0.02	0.76 ± 0.03	0.38 ± 0.03	1.11 ± 0.03
Heptanal	0.17 ± 0.01	0.17 ± 0.01	0.43 ± 0.01	0.83 ± 0.02	0.28 ± 0.02	1.32 ± 0.04
Octanal	0.36 ± 0.02	0.29 ± 0.04	0.29 ± 0.02	0.38 ± 0.03	1.79 ± 0.61	83.20 ± 0.67
Nonanal	1.74 ± 0.03	2.06 ± 0.33	1.63 ± 0.09	1.78 ± 0.09	11.94 ± 0.46	23.33 ± 0.10
Trans-2-nonenal	0.75 ± 0.07	0.46 ± 0.05	0.56 ± 0.11	0.47 ± 0.04	0.37 ± 0.17	1.02 ± 0.11
Decanal	3.23 ± 0.24	1.50 ± 0.12	2.52 ± 0.20	2.28 ± 0.22	2.27 ± 0.25	28.36 ± 0.57

The most interesting from the analytical point of view were medium-long chain aldehydes, especially *trans*-2-nonenal, that can cause off-flavour of beer. They could be quantified when present at the concentrations lower than 1 μg L^{-1}. In one brand of beer much higher concentrations of octanal, nonanal and decanal were detected, probably due to the presence of flavourings. Unsaturated aldehydes were analysed by Ochiai [16] using SBSE. The contents of *trans*-2-nonenal in normal beer samples were 0.065–0.072 μg L^{-1}, while in beer subjected to the accelerated ageing test (60°C for 1 day) *trans*-2-octenal, *trans,cis*,-2,6-nonadienal and *trans*-2-nonenal was also detected at the concentration of 0.069–0.44 μg L^{-1}.

CONCLUSIONS

It has been proved that the presented method for the determination of aldehydes, utilizing fibre saturation with PFBHA vapours and subsequent absorption of aldehydes is more advantageous than in-solution derivatization and syringe injection. The use of SPME fibre enables the sample enrichment, which improves detection limits. It also eliminates undesirable matrix effects on the method performance. The method is recommended for the determination of aliphatic aldehydes in water, as well as in more complex matrices, such as beer. In such matrices the presence of other compounds, mainly carbohydrates, proteins, colorants and lipids may produce artefacts and complicate the interpretation of chromatograms, especially when detection is performed without mass spectrometer. The presented method is based on the headspace analysis. It lengthens the column lifetime and does not require any solvent.

Acknowledgements

Financial support from the Polish Committee for Scientific Research (grant No KBN 3 P06T 065 22) is acknowledged.

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Received April 2004
Accepted May 2004