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# Synthesis of molecularly imprinted monolithic fibers for solid-phase microextraction of acetaldehyde from head-space of beverages stored in PET bottles

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ABSTRACT

Molecularly imprinted monolithic fibers were synthesized and evaluated for solid-phase microextraction (SPME) of acetaldehyde from head-space of beverages stored in poly(ethylene terephthalate) (PET) bottles. The fibers were prepared by co-polymerization of methacrylic acid and ethylene glycole diethacrylate as functional monomer and cross-linker, respectively. Acetaldehyde was used as template molecule during polymerization process, in order to leave specific cavities after leaching off from the co-polymer. Narrow bore (i.d. 100 μm) glass capillaries as molds were filled with polymerization mixture containing cross linker, functional monomer, template molecule, initiator and porogen solvent. This mixture is then polymerized to form a continuous porous monolith that conforms to the shape of the mold. The resulting monolithic fibers were employed by a micro-syringe to extract acetaldehyde from head-space of sample solutions. Gas chromatography/mass spectrometry (GC/MS) was used to analyze adsorbed acetaldehyde to the fibers. Parameters influencing adsorption of acetaldehyde from head-space of standard solutions to the fiber and subsequently desorption in the GC chamber were optimized. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.01 and 0.03 $\mu g\,mL^{-1}$, respectively. The intra-day and inter-day precisions of the peak areas for five replicates were 8 and 15%, respectively.

## 1. Introduction

Poly(ethylene terephthalate) (PET) polymer is one of the widely used materials for packaging purposes. Resistance to chemicals, low gas and water vapor permeability, strength, low weight, colorlessness and transparency are the main advantages of PET bottles. Hence, many beverages like carbonated drinks, fruit juices and especially mineral waters are packed in PET bottles.

One of the main drawbacks of PET polymers as packaging material is the migration of its degradation products to beverages in bottles. Acetaldehyde is the main degradation product of PET polymers which is generated during perform production [1–7]. After cooling down, acetaldehyde is trapped in the PET bottle wall and could contaminate the inside liquid [8]. The migration of acetaldehyde has great importance, because it has a distinct odor and taste even in low concentrations and in mineral water produce a fruity off flavor [9]. In addition, acetaldehyde is genotoxic in many biological systems and considered a possible human carcinogen by International Agency for Research on Cancer [10]. According to the European Commission Directive of 2002/72/EC specific migration limit for acetaldehyde is stated as 6 ppm [11]. Several authors have reported high concentration of acetaldehyde in drinks stored in PET bottles [12–17].

Among the several methods which have been reported to determine acetaldehyde in aqueous samples, solid-phase microextraction (SPME) has been the most preferred technique [18–20]. SPME is a versatile, simple and solvent-free technique for sample pretreatment prior to analysis [21]. The extracting fiber in SPME which is coated with an appropriate material could be directly inserted into the sample or into the head-space of the sample [22]. Owing to the high volatility and reactivity of low molecular weight aldehydes, a derivatization step prior to chromatographic techniques is inevitable to achieve satisfactory recovery and sensitivity. In-solution or on-fiber derivatizations with suitable derivatization reagents are the most performed procedures prior to detection of such compounds [18–20,23–29]. The use of complicated derivatization steps can be regarded as misleading, because the advantages of easy handling and fast sample preparation in SPME are abandoned. Hence, the development of reliable analytical approaches that do not need to additional steps such as derivatization seems worthwhile.

In addition, although a variety of fiber coatings for SPME are now commercially available, but their use is limited by the lack of selectivity. In the recent years, molecularly imprinted polymers

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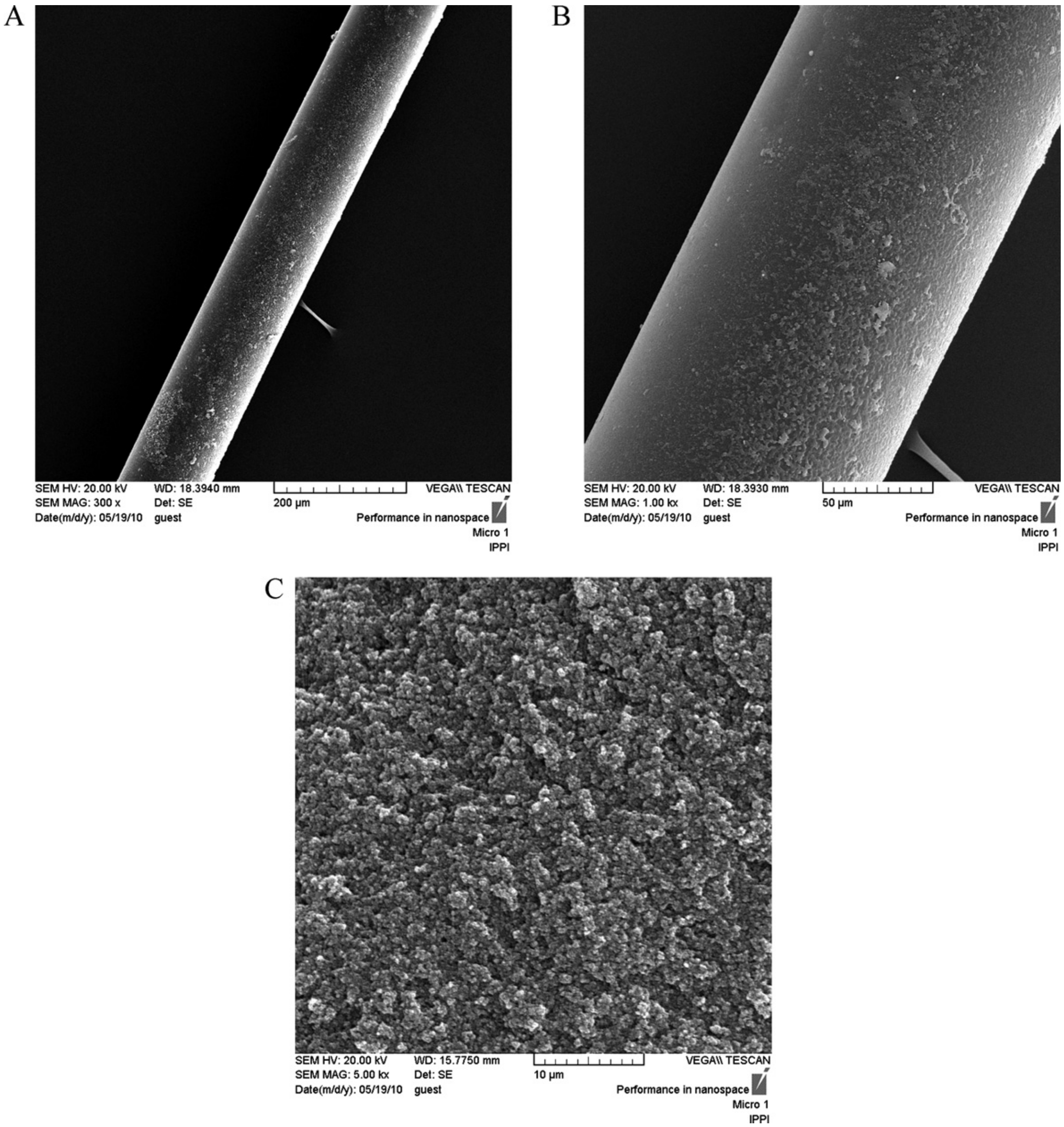

**Fig. 1.** Scanning electron images of monolithic fiber at different magnifications: (A) 300; (B) 1000; (C) 5000.

(MIPs) have been used in several areas of analytical chemistry. MIPs are cross-linked synthetic polymers prepared by co-polymerizing a functional monomer with a cross-linking monomer in the presence of a template molecule. After polymerization, the template molecules are removed from the porous network and leaving cavities in the polymeric matrix that are complementary in size, shape, and chemical functionality to the template. Thus, the MIPs are able to rebind selectively the template molecules [30,31]. The inherent selectivity associated with MIPs has made them efficient materials to be used in SPME technique. This combination has been successfully employed for extraction and pre-concentration of various analytes from different samples [32–35].

In this work, an acetaldehyde-imprinted polymer as a monolithic fiber was synthesized in non-covalent approach and employed by a SPME device based on the Hamilton$^{TM}$ 7000 series microsyringe to extract acetaldehyde from head-space of beverages stored in PET bottles. Adsorption of volatile acetaldehyde during SPME procedure inside the cavities of MIP via hydrogen bindings could stabilize it and hence the derivatization step could be eliminated. After establishing equilibrium between headspace of sample and the fiber, it was directly inserted into a gas chromatography/mass spectrometry (GC/MS) injection port for thermal desorption of acetaldehyde and determination.

## 2. Materials and methods

### 2.1. Chemicals

All chemicals and solvents were purchased from Merck (Darmstadt, Germany). Methacrylic acid (MAA) and ethylene glycol dimethacrylate (EGDMA) were distilled under reduced pressure immediately before use. 2,2′-Azobis-isobutyronitrile (AIBN) was purchased from Acros organic (Geel, Belgium) and used as received.

Acetaldehyde stock solution (100 $\mu$g mL$^{-1}$) was prepared in cold de-ionized water. Prior to the addition of acetaldehyde and in order to remove dissolved oxygen, water was degassed with $N_2$ for 5 min.

**Table 1**
Operational conditions of GC/MS.

| | |
|---|---|
| Column | 30 m length × 0.25 mm i.d. and 0.25 μm film thickness fused silica capillary column DB-WAX (J&W Scientific, Folsom, CA, USA) |
| Oven temperature | Initial: 35 °C for 5 min, increased by 30 °C min$^{-1}$ to 150 °C for 2 min |
| Carrier gas | Helium |
| Average gas velocity | 25.57 cm s$^{-1}$ |
| Injection mode | Splitless |
| Injection temperature | 90 °C |
| Ionization voltage | 70 eV |
| Ion source temperature | 220 °C |

Working standard solution (10 μg mL$^{-1}$) was prepared by diluting stock solution in cold and degassed de-ionized water.

### 2.2. Instrumentation

Acetaldehyde analysis was performed on an Agilent 7890 A GC equipped with a 5975C mass selective detector (Agilent Technologies, USA). The GC/MS parameters are summarized in Table 1. Quantitative analysis was performed by using selected ion monitoring (SIM) with the characteristic ion at 29 *m*/*z* for acetaldehyde detection. The surface characteristic study of the fiber was monitored by scanning electron micrography with a VEGA-II scanning electron microscope (TESCAN, USA). The infrared absorption spectrum of coating between 400 and 4000 cm$^{-1}$ was obtained in a Spectrum 100 FTIR spectrometer (PerkinElmer, USA). The thermogravimetric analysis was performed in a Pyris 1 thermogravimetric analyzer (PerkinElmer, USA) under inert atmosphere (Ar), over the range of 20–620 °C (heating rate of 10 °C min$^{-1}$). Surface areas analyses were performed at 77 K by Brunauer–Emmett–Teller (BET) on an ASAP 2020 surface area and porosity analyser (Micromeritics Instrument Corporation, Creil, France). Fifty milligrams of MIP and NIP crushed fibers were degassed overnight at 100 °C to remove adsorbed gases and moisture.

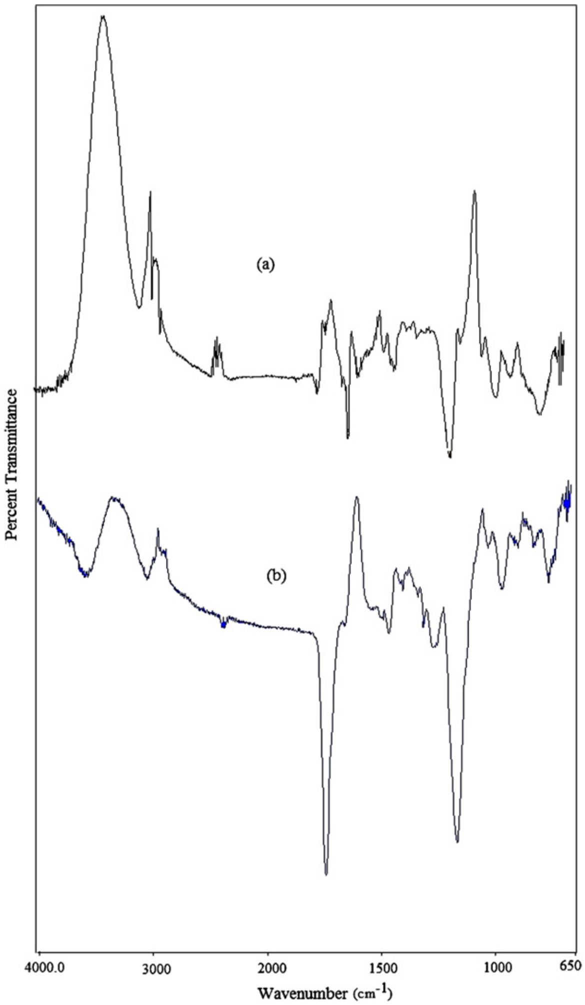

**Fig. 2.** HATR-IR spectra of the monolithic fiber before (a) and after (b) absorption of acetaldehyde.

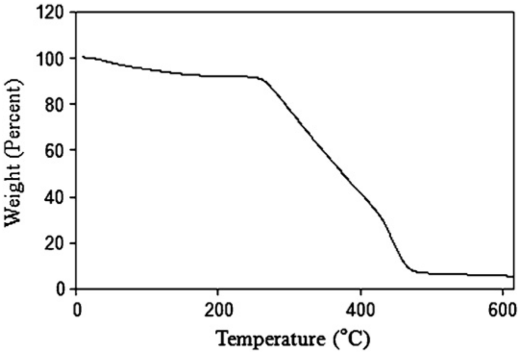

**Fig. 3.** TGA diagram of the synthesized monolithic fiber.

### 2.3. Acetaldehyde-imprinted monolithic fiber preparation

The preparation of the monolithic fiber was based on the procedure described by Turiel et al. [35]. Fifty six microliters (1 mmol) of acetaldehyde and 336 μL (4 mmol) MAA monomer were dissolved in 2 mL cold toluene as porogen solvent in a 10 mL glass tube. The mixture was kept at 5 °C for 5 h. Then, 3780 μL (20 mmol) EGDM cross-linker monomer and 40 mg AIBN as polymerization initiator were added.

In order to fabricate narrow bore capillaries as molds of fiber monoliths, the center part of a universal fused silica capillary was heated by a small flame. After a short time, two sides of capillary were pulled in opposite directions to make the desired capillary. The average thicknesses of the prepared capillaries were 120 μm.

With the help of a syringe, the prepared capillaries were filled with the pre-polymerization mixture and both ends were closed by burning in flame. Then, the filled capillaries were kept in an oven at 60 °C for 24 h to complete polymerization. Finally, capillaries were cut and immersed in concentrate hydrofluoric acid for 2 h until silica walls being etched away. After repeated washing with methanol and de-ionized water, fibers were introduced in an oven at 90 °C for 2 h to complete removal of acetaldehyde and other

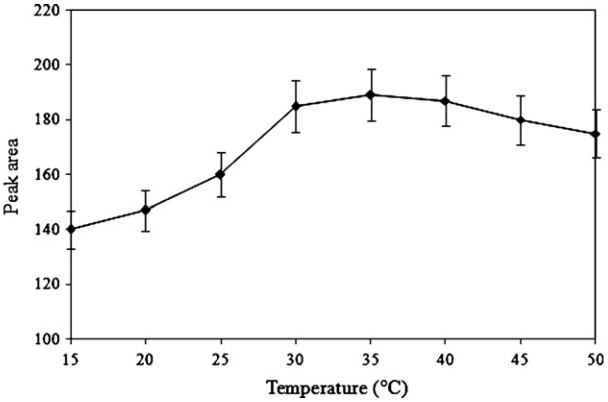

**Fig. 4.** Effect of extraction temperature on extraction recovery of acetaldehyde.

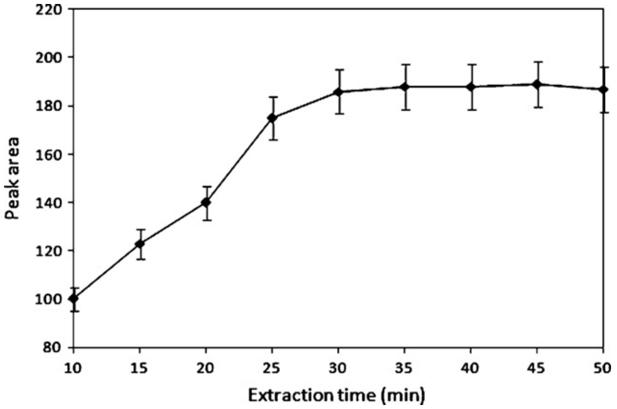

**Fig. 5.** Effect of exposure time on the extraction recovery of acetaldehyde.

volatile compounds. The resulting fibers were flexible rods with promising mechanical stability.

Also, a non-imprinted monolithic fiber that did not contain acetaldehyde was prepared simultaneously using the same protocol.

### 2.4. Head-space-SPME

Monolithic fibers were cut into 1.5-cm long and fixed in a SPME device based on the Hamilton™ 7000 series microsyringe. A fiber was inserted into the microtubing of the microsyringe and mounted by high temperature epoxy glue. Movement of the microsyringe plunger allowed exposure of the fiber during extraction and desorption in GC chamber. Then the fiber was conditioned with gradually rising temperature from 30 to 150 °C in the GC injection port under $N_2$ gas for 2 h.

Ten milliliters of standard (0.025–10 $\mu$g mL$^{-1}$) and sample solutions in 20 mL glass vials equipped with a Teflon-coated silicone rubber septum were stirred with a 10 mm × 4 mm magnetic bar at 500. The syringe needle of the SPME assembly penetrated the septum of the vial and the fiber was exposed in the head-space of the solutions for 15 min at 35 °C.

After extraction, the fiber was withdrawn into the needle and immediately inserted into the GC injector for desorption and analysis. The fiber was remained in desorption chamber until the end of run.

## 3. Results and discussion

### 3.1. Morphological study of the monolithic fiber

The morphological characteristics of the prepared fibers were investigated by scanning electron microscopy (SEM). The fibers were coated with a gold vapor layer prior to analysis by SEM. As shown in Fig. 1A and B, the synthesized fibers were homogeneous and dense monoliths with estimated thickness of around 100 $\mu$m. Several studies have shown that the extraction efficiency is enhanced for porous SPME fibers relative to non-porous ones. Fig. 1C shows that the synthesized fibers had very good porosity which made them favorable phases for efficient extraction of acetaldehyde.

The horizontal attenuated total reflectance (HATR) infrared spectra of the acetaldehyde-imprinted monolithic fiber before and after adsorption of acetaldehyde are shown in Fig. 2. The peak at around 1700 cm$^{-1}$ corresponding to stretching vibration of C=O on carbonyl groups was intensified after adsorption of acetaldehyde. The BET analysis showed surface areas of 289 and 302 m$^2$ g$^{-1}$ for MIP and NIP, respectively.

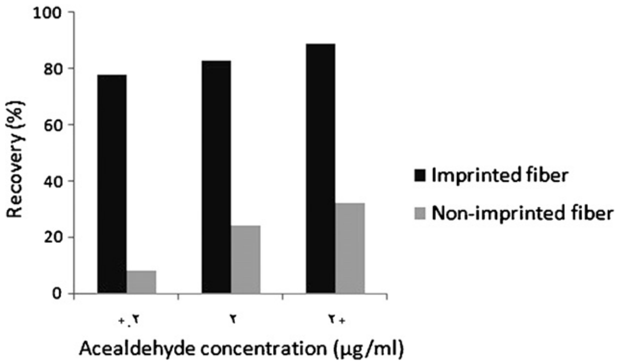

**Fig. 6.** Imprinting effect studies of acetaldehyde-imprinted monolithic fiber before and after of acetaldehyde adsorption.

### 3.2. Thermogravimetric analysis (TGA)

In order to study the thermal stability of the synthesized monolithic fiber at elevated temperatures in GC injection port, thermogravimetric analysis (TGA) was employed. Appropriate amounts of fibers were heated over the range of 20–620 °C and the weight changes were monitored. The resulting thermogram shows that the fiber could withstand without any weight loses up to 248 °C (Fig. 3). Hence, they could be successfully employed without the risk of fiber collapse in the GC injector during desorption step at 90 °C.

### 3.3. Head-space SPME optimization

In order to optimize the extraction step, the effects of main extraction parameters such as extraction temperature, time and stirring speed were examined. All data were the average of 3 replicates of each experiment. The effect of extraction temperature is shown in Fig. 4 by plotting acetaldehyde peak areas in total ion current chromatogram as a function of extraction temperature. As can be seen, the extraction ability of fiber increases with increasing temperature up to 35 °C. It could be due to the increasing distribution constant of acetaldehyde between the aqueous phase and head-space of the solution. Subsequent decrease of extraction efficiency after this temperature is probably due to decrease of the partition coefficient of acetaldehyde between head-space and fiber. Thus an adsorption temperature of 35 °C was identified as the optimal extraction temperature.

Fig. 5 shows the extraction time effect on the equilibrium of acetaldehyde between the fiber and head-space of sample. The results showed that increasing extraction time up to 30 min increased overall extraction yield of acetaldehyde. The transient profile shows that a plateau was reached after 30 min which was maintained up to 50 min. Hence, the extraction time for subsequent analyses was then fixed at 30 min.

Generally, stirring of the sample solution reduces the time required to reach equilibrium and extraction time by enhancing the diffusion of the analyte from solution towards the fiber. But in this work, by changing stirring rates ranging from 100 to 1000 rpm,

**Table 2**
Acetaldehyde concentration and recovery studies beverage samples using proposed procedure.

| Beverage | Found ($\mu$g mL$^{-1}$) | Added ($\mu$g mL$^{-1}$) | Recovery (%) |
|---|---|---|---|
| Mineral water I | N.D.[a] | 2.00 | 82.5 |
| Mineral water II | 10.80 | 4.00 | 90.0 |
| Coke | N.D. | 2.00 | 75.8 |
| Orange juice | 17.14 | 2.00 | 83.7 |
| Pineapple juice | 12.34 | 2.00 | 88.7 |

[a] N.D.: Not determined.

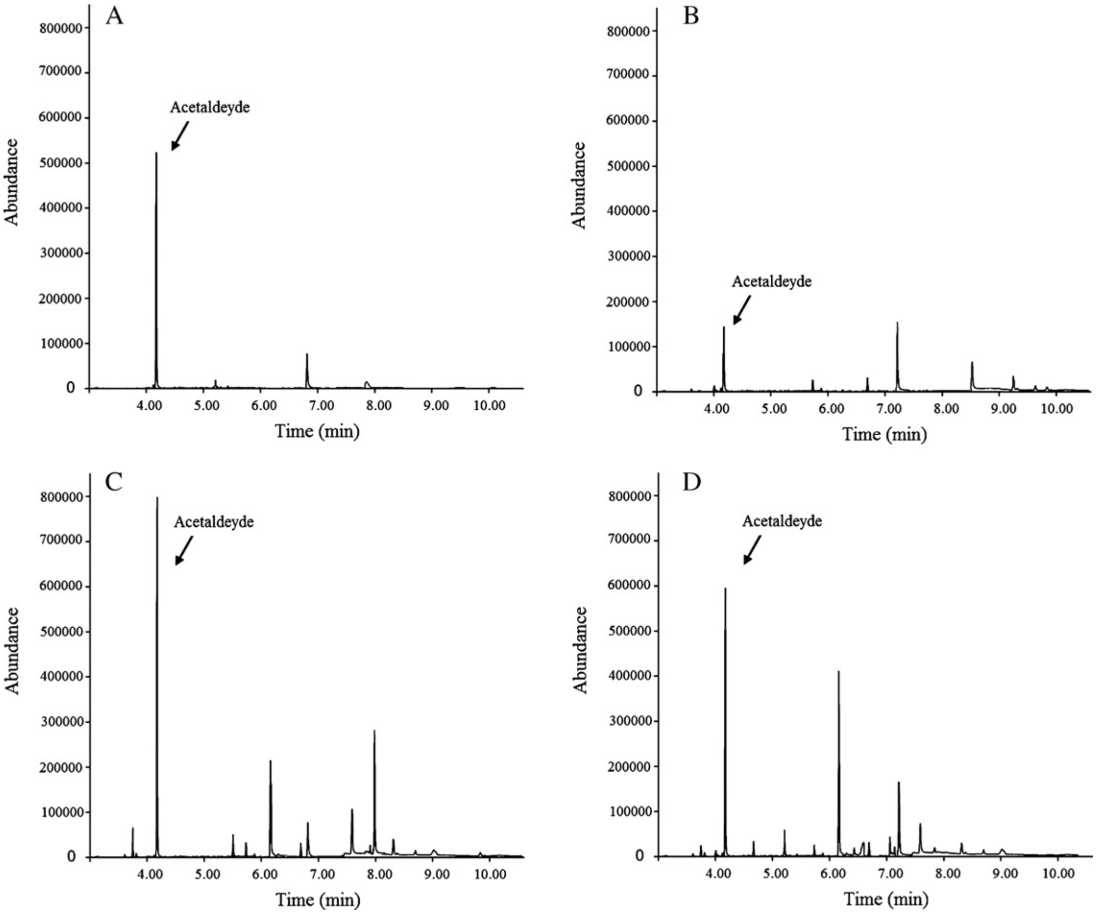

**Fig. 7.** Total ion chromatograms in SIM mode with the characteristic ion at 29 *m*/*z* for acetaldehyde detection obtained from spiked samples at 2 μg mL$^{-1}$. A: mineral water; B: coke; C: orange juice; D: pineapple juice.

the extraction efficiency did not show considerable changes. This observation could be due to the high volatility of acetaldehyde that established equilibrium rapidly even with low stirring rates. Hence, subsequent experiments were conducted at 500 rpm.

### 3.4. Imprinting effect

In order to evaluate the presence of imprinted sites on the acetaldehyde-imprinted monolithic fiber, SPME experiments were carried out working in parallel with the corresponding non-imprinted fiber. For this purpose, 10 mL portions of standard acetaldehyde solutions in three concentration levels (0.2, 2.0 and 20.0 μg mL$^{-1}$) were extracted by fibers according to the proposed procedure. As it can be seen from Fig. 6, significant imprinting effect could be observed by comparing the resulting recoveries from two fibers.

### 3.5. Linear range and analytical precision

The linearity of the proposed method was obtained by constructing calibration curves in the corresponding concentrations. The stock and working standard solutions of acetaldehyde were added to the 10 mL mineral water sample in SMPE vial to obtain the desired concentration in the range from 0.05 to 20 μg mL$^{-1}$. Subsequently, the extraction and determination were processed according to the proposed method. The results showed a good linearity ($R^2 = 0.989$) between acetaldehyde peak areas and concentration. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated with the signal-to-noise ratio of 3 and 10, respectively. The LOD and LOQ of acetaldehyde were 0.01 and 0.03 μg mL$^{-1}$, respectively.

The reproducibility of the proposed method was expressed as the relative standard deviation (RSD) of peak areas. The intra-day and inter-day precisions of the peak areas for five replicates were 8 and 15%, respectively.

### 3.6. Durability of the monolithic fiber

The synthesized monolithic fiber was checked for its repetitive extracting capability. Fifty times utilization of fibers according to the recommended procedure did not show significant loss of extraction. Hence, it was showing that the fiber's extracting capability was not affected by high temperature employed for desorption in the injection port.

### 3.7. SPME of beverages stored in PET bottles

The developed head-space SPME method with monolithic fiber was applied to the analysis of acetaldehyde in five beverage samples (mineral water I, mineral water II, coke, orange juice and pineapple juice). SPME from real samples were performed under the same conditions as extractions of the standard solutions. All samples were purchased from local markets and stored at 40 °C for 20 h. Then, the samples were kept at 4 °C prior to the analysis. Total ion chromatograms in SIM mode of spiked samples are shown in Fig. 7. The results which are shown in Table 2 present that acetaldehyde was detected in mineral water II, orange and pineapple juices with concentrations higher than threshold levels.

**Table 3**
Comparison of SPME techniques for determination of acetaldehyde in beverages.

| Sample | Derivatization reagent | LOD ($\mu$g mL$^{-1}$) | Upper concentration limit ($\mu$g mL$^{-1}$) | Ref. |
|---|---|---|---|---|
| Tap water, mineral water | *o*-(2,3,4,5,6-Pentafluorobenzyl)-hydroxylamine | 0.0005 | 100 | Sugaya et al. [18] |
| Aqueous solution | *o*-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride | 0.011 | 0.2 | Beránek and Kubátová [20] |
| Wine | Pentafluorophenylhydrazine | 0.005 | 0.25 | Wang et al. [19] |
| | *o*-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride | 0.0005 | 0.25 | |
| Mineral water, coke, apple juice, pineapple juice | No reagent | 0.01 | 20 | This work |

In order to ensure the accuracy of the method, the recovery of spiked beverage samples was studied by comparing the extracted amounts of acetaldehyde with the total amounts added. As shown in Table 2, the recoveries were in the range from 75.8% to 90.0%.

### 3.8. Comparison with other works

A comparison of the proposed method with other reported SPME procedures for acetaldehyde determination in beverages is summarized in Table 3. Because the recommended procedure does not need to time consuming derivatization steps and additional reagents, it shows a simpler feature. Moreover, the proposed method has a long dynamic range and promising LOD in comparison with other procedures.

## 4. Conclusions

In this study, an acetaldehyde-imprinted monolithic fiber was developed to extract acetaldehyde from head-space of beverages in a SPME procedure. Promising mechanical and thermal stability, excellent durability, wide linear range and good extraction efficiency for acetaldehyde suggest that the developed fiber is an attractive tool for determination of acetaldehyde content in beverages. Compared with other techniques, the study shown here provided a simple and reliable method without a derivatization step for routine control of acetaldehyde in beverages stored in PET bottles.

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