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# Preparation and evaluation of solid-phase microextraction fiber based on nano-structured copolymer of aniline and m-amino benzoic acid coating for the analysis of fatty acids in zooplanktons

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ABSTRACT

A novel nano-structured copolymer of aniline and m-amino benzoic acid (m-ABA) was introduced as a coating of solid phase microextraction (SPME) of saturated-fatty acids in zooplanktons by coupling to gas chromatography–mass spectrometry (GC–MS). The nano-structured coating was prepared using co-polymerization of m-aminobenzoic acid and aniline. Improved temperature resistance (up to 350 °C), relatively improved life time (more than 50 times) and satisfactory extraction efficiency were obtained by insertion of carboxylate groups into the framework of polyaniline. Different extraction parameters such as extraction temperature, extraction time, ionic strength, stirring rate and headspace volume were investigated and optimized. Single fiber repeatability and fiber-to-fiber reproducibility were <5.7% and <10.2%, respectively and the limits of detection varied from 0.01 (C14:0) to 6.07 μg $L^{-1}$ (C20:0). Correlation coefficients ($R^2$) of the calibration curves ranged from 0.992 (C20:0) to 0.998 (C18:0) with a linearity from 0.5 to 200 μg $mL^{-1}$. The recoveries obtained ranged from 83% (C16:0) to 115% (C14:0).



## 1. Introduction

Solid-phase microextraction (SPME), introduced by Pawliszyn and co-workers [1,2], is a fast, simple, easy to automize, inexpensive and solvent free extraction technique [3,4]. Different types of commercial SPME fibers are available [5]. However, these fibers still show some drawbacks such as low thermal and chemical stability, high cost, and poor reusability; hence their application is restricted to some extent [6]. Part of the drawbacks is related to the coating method and the brittle fused silica fiber used. Silica rods are mainly used as SPME support for these commercial coatings. Silica fibers are expensive, fragile, and can be easily broken [7]. To overcome these drawbacks an electrochemical coating approach was attempted by Wu and Pawliszyn [8,9]. The main advantages of electrochemical deposition rely on the low cost equipment, rigid control of the film thickness, and its uniform and fast deposition rate [10]. Conducting polymers are multifunctional materials with various interesting properties such as extraordinary stability, simplicity of synthesis and unique electrochemical properties [11,12]. These properties make them useful for SPME application [13]. Polypyrrole [8] and polyaniline (PANI) [14–16] were the most popular conductive polymers used for the SPME fibers. However, their application was limited by low thermal stability, i.e. 200 °C for polypyrrole [17] and 220 °C for common polyaniline. Unfortunately, using desorption temperatures higher than 220 °C led to lower fiber stability and unsatisfactory and irreproducible results [18]. The type of doping agent in the electrochemical polymerization process could be responsible for mechanical, thermal and solvent stability of those coated SPME fibers. Therefore, due to their low thermal and mechanical stability, several doping agents were proposed to enhance their stability, such as trifluoroacetate [19], dodecylsulfate [20] and polyphosphate [21].

One of the methods that can be used to enhance stability of polymers is to introduce some functional groups into the polymer backbone. Self-doped polyanilines (SPAN) are one of the polyaniline derivatives that can bear negatively charged functional groups. These novel materials have many properties different from those of the parent polyaniline, and some specific fields for their use are expected. In contrast to PANI, its self-doped derivatives contain an ionisable and negatively charged functional group in their structure which acts as an inner dopant anion bounded to the polymer backbone. Thus, no anion exchange between the polymer and surrounding takes place during oxidation or reduction. The presence of covalently bonded dopant groups in PANI structure has been claimed to increase the thermal stability of the polymer [22]. One of the most widely used techniques for obtaining thin

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films of self-doped polyanilines on the electrodes is electrochemical copolymerization of aniline with an aniline comonomer, bearing sulfonate or other anionogenic group [23–28].

Nanotechnology applications in SPME coating preparation have shown remarkable growth in recent years [29–34]. Compared with other materials used for SPME, nano-materials offer a significant higher surface area-to-volume ratio that promises much greater extraction capacity and efficiency [35]. Fibers with nano-structured coatings greatly can increase effective surface area, and consequently extraction capacity [36].

Zooplanktons play a vital role in marine ecosystem. Owing to the importance of zooplankton in marine food webs, a number of studies have been carried out on the biochemical composition including lipids of zooplankton [37–42]. Earlier studies have revealed that the important energy stores in zooplankton are lipids [38,41,43]. In addition to total lipid content and lipid class composition, fatty acid compositions can provide extensive information about the origin and fate of the lipids in zooplanktons and their physiological status [42].

This paper reports application of novel nano-structured SPAN as a SPME fiber coating. The proposed novel PANI fiber is expected to improve the thermal and mechanical stability. Then, it is proposed as a superior SPME coating for the analysis of the fatty acids in zooplanktons for the first time.

## 2. Experimental

### 2.1. Apparatus

The SPME holder for manual sampling was obtained from Azar Electrode Co. (Urmia, Iran). A magnetic stirrer (IKA-Werke, Staufen, Germany) was employed for temperature control and stirring during derivatization and extraction. Electrochemical measurements were carried out in a conventional three-electrode cell powered by a Potentiostat/Galvanostat, μ-Autolab, type III (The Netherland). Freeze drying was performed using an Operon Freeze dryer model FDB 55023 (Kimpo, Korea). The scanning electron microscopy images were obtained by S4160 Hitachi (Tokyo, Japan).

The gas chromatography–mass spectrometry (GC–MS) analysis were performed using a model 6890N network GC system (Agilent, USA) equipped with a 5973 mass selective detector (Agilent, USA) and a MSD chemstation software on a HP-5 fused silica capillary column (30 m × 0.25 mm I.D.) with a film thickness of 0.33 μm. The temperature program used was: 60 °C for 1 min increased at a rate of 20 °C $min^{-1}$ to 130 °C and held for 4.5 min, and at a rate of 6 °C $min^{-1}$ then reached to the final temperature of 280 °C. The injector and auxiliary temperature were 250 °C and 280 °C, respectively. Helium (purity, 99.999%) was used as carrier gas at a constant flow of 1.3 mL $min^{-1}$. Mass spectra were obtained by electron impact ionization (EI) at 70 ev.

### 2.2. Reagents and materials

Standard fatty acids {Myristic acid (C14:0), Palmitic acid (C16:0), Heptadecanoic acid (C17:0), Stearic acid (C18:0) and Eicosanoic acid (C20:0)} were purchased from Sigma–Aldrich (Steinheim, Germany). Standard solutions of fatty acids were prepared in hexane at concentration of 1.0 mg $L^{-1}$. Hexane, sodium chloride, $BF_3$–MeOH (14%, v/v), aniline, m-aminobenzoic acid and sulphuric acid were obtained from Merck (Darmstadt, Germany). Aniline was distilled under nitrogen atmosphere at reduced pressure, kept in the dark, and used within 3 days. All the chemicals were of analytical pure grade and the water used was HPLC grade.

### 2.3. Collection of zooplankton samples

Zooplanktons were collected from Chabahr gulf of Iran (Lat. N 25° 24′ 00.0″ and Long. E 060° 34′ 00.0″) at March 2010. The samples were collected using a Bongo net (300 μm) horizontally (~1 m from the surface of water). The zooplanktons were then retained on a 100 mesh size filter cup. The bulk sample was freeze-dried and stored in −80 °C for later analysis of the fatty acids.

### 2.4. Electrodeposition

The polymerization method published by Thiemann and Brett [44] was modified to prepare the self-doped PANI SPME fiber. The rate of polymer film growth resulting from electropolymerization of aminobenzoic acids is much lower for the polymerization of substituted anilines than for aniline. Therefore, for increasing the polymerization rate, mixed of m-aminobenzoic acid and aniline was used. Electrosynthesis of polyaniline was performed by potential cycling between 0.0 and 0.8 V vs. Ag/AgCl. To oxidize m-ABA, a slightly higher potential than that used to oxidize aniline is necessary. Therefore, a potential limit of 0.9 V was used. The higher potential value increases degradation products of aniline [44]. An electrochemical cell was assembled in three electrode configuration for electrodeposition of SPAN onto Pt wires in which the counter, reference and working electrodes were a platinum rode, a saturated Ag/AgCl electrode and a platinum wire (250 μm diameter and 4 cm length), respectively.

Prior to electrochemical deposition, the Pt wires were cleaned by acetone and HPLC grade water in an ultrasonic bath for 30 min. Then it was held at a potential of −1 V vs. Ag/AgCl in sulphuric acid electrolyte for 30 min. Electrochemical polymerization was carried out by cyclic potential sweep in electrolyte solution containing the 1:1-mixtures (total concentration of 0.1 M) of the m-aminobenzoic acid with aniline in sulphuric acid (0.5 M) between −0.3 and 0.9 V vs. Ag/AgCl at a scan rate of 20 mV $s^{-1}$ for 30 cycles. The fiber was washed by HPLC grade water to remove the unwanted chemicals such as monomers and the supporting electrolyte after deposition. The Pt coated wire was dried at room temperature in vertical position for 2 h.

### 2.5. Derivatization and headspace-SPME (HS-SPME) procedure

The fibers were conditioned prior to the first extraction by leaving in injection port of GC for 30 min at 300 °C. 100 μL of the working standard solutions of fatty acids and a 10 mm × 3 mm magnetic stirring bar were placed into a 20 mL glass vial which had been silicanized before use, for each SPME analysis. $BF_3$–MeOH (14%, v/v) was added to the sample. Then, the vial was tightly capped with a butyl rubber stopper wrapped with PTFE sealing tape and an aluminum cap. The vial was put in a thermostatic bath in order to derivatization under constant stirring speed. Afterwards, the vial was uncapped and a certain amount of NaCl salt was added. Then, the vial was tightly capped again and put in a thermo stable bath with magnetic stirring. The stainless steel needle, where the fiber is housed, was pushed through the vial septum, and the fiber pushed out of the housing and exposed to the headspace above the sample for extraction. Having the extraction been finished, the fiber was pulled back and inserted in the GC injection port for desorption and analysis. Analyses were performed in selected ion monitoring (SIM) mode and the quantifications achieved using peak area calculations of SIM runs. Each analysis was undertaken in triplicate and the average of their quantification results was reported.

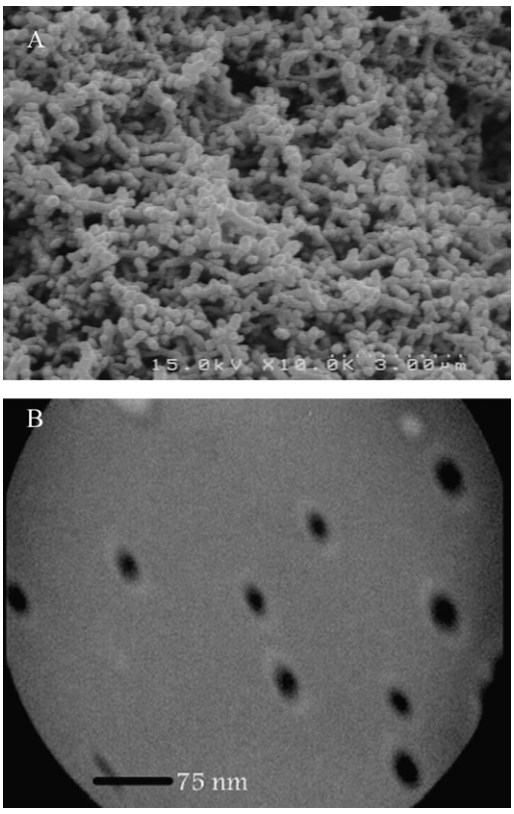


**Fig. 1.** Scanning (A) and transmission (B) electron micrographs of self-doped PANI.

### 2.6. Procedure for the analysis of fatty acids in zooplanktons

One milligram of the freeze-dried sample was added into a glass vial which has been formerly silicanized. Adding 200 μl of $BF_3$–$CH_3OH$ (14%, v/v) solution and a magnetic stirring bar, the vial was then tightly capped and put in a water bath of 60 °C for 30 min at a constant stirring speed. HS-SPME was carried out at 80 °C for 20 min with a constant stirring speed, after adding 1.0 mL of NaCl aqueous solution (15%, w/v) into the solution. Afterwards, GC–MS analysis was performed under the same conditions as standard solutions. The relative retention times as well as mass spectra were used for the peak identification. Quantitative analysis was carried out using external standard method based on the peak areas of selected ions.

## 3. Results and discussion

### 3.1. Properties of novel PANI coatings

#### 3.1.1. Scanning and transmission electron microscopy studies

The nano-structured SPAN was formed at potential of 0.9 V vs. Ag/AgCl. Scanning electron microscopy (SEM) was used to estimate the average thickness of each coating, which was found to be about 70 μm for nano-structured films. The surface morphology of nano-structured SPAN coatings was shown in Fig. 1A. As can be seen, the morphology of copolymer is fibrous. The nano-structured particles is observed in Fig. 1B with a diameter lower than 75 nm. The growth of the novel SPAN during the synthesis was much lower and steadier than that of common PANI, which resulted in long and intact fibers. Thus the film is more uniform and porous, and the effective surface area is larger, which is favorable for the adsorption/extraction of analytes. The high surface area will provide large stationary phase loading and high extraction capacity [45,46].

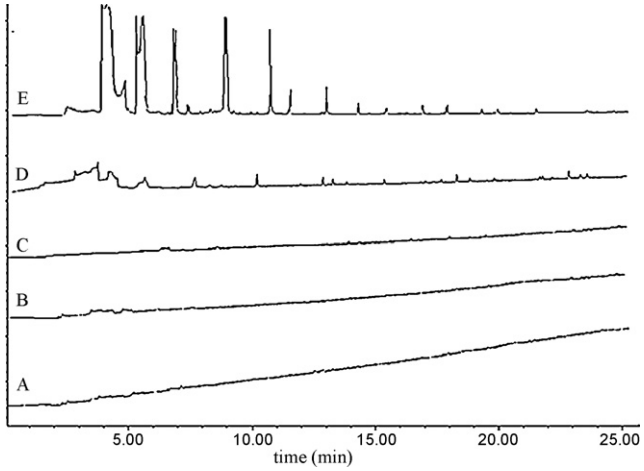


**Fig. 2.** Blank chromatograms for the self-doped PANI and PANI coated SPME fiber desorbed at different temperatures: (A) SPAN 250 °C, (B) SPAN 270 °C, (C) SPAN 300 °C, (D) SPAN 350 °C, and (E) PANI 250 °C.

#### 3.1.2. Thermal stability

Since the analytes extracted on the fiber are desorbed at an elevated temperature, the capability of resistance to high temperatures is very important in terms of practical application. The thermal stability of the SPAN fiber was compared with that of PANI fiber, and the result is shown in Fig. 2. The thermal stability of the novel fiber and PANI fiber was accessed by evaluating the performance of the fiber at 250, 270, 300 and 350 °C, successively. The results indicate that the SPAN fiber can be operated until 350 °C, while the recommended operating temperatures for PANI fibers are below 250 °C. Compared to the PANI fibers, the main feature of the developed coating was the excellent thermal stability (~350 °C), thus allowing the use of high desorption temperatures and consequently the reduction of carryover effects.

#### 3.1.3. Lifetime of the coating

The coating's lifetime is so much important for practical application. For most commercial SPME fibers, the extraction efficiency declines with extraction times increasing because the coating is prone to being damaged by high temperature, organic solvent, strong acidic or basic solution [47]. It was proved that there was no obvious decrease of the extraction efficiency after more than 50 times of adsorption/desorption cycle for the proposed coating (Fig. 3).

Although, the lifetime of common PANI prepared coatings is short, but specific PANI coatings with long lifetime was obtained with some modification of synthesis method [14,30,48]. In this work, we could prepare a coating with high thermal stability and relatively good lifetime.

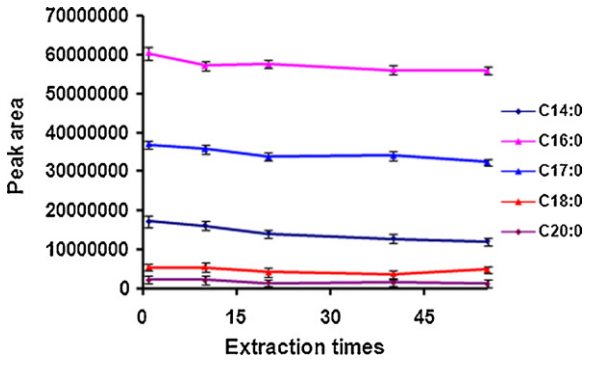


**Fig. 3.** Lifetime profile of SPAN fiber.

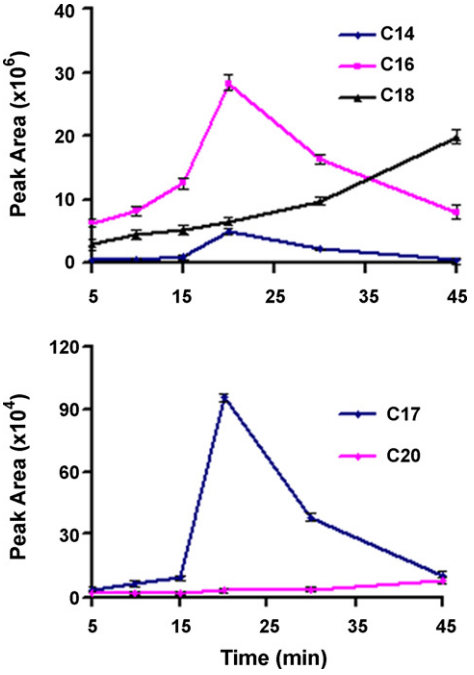


**Fig. 4.** Effect of the extraction time on the extraction efficiency of the fatty acids after derivatization. HS-SPME conditions: 1.0 mL $H_2O$, 80 °C, stirring rate 200 rpm, desorption time: 2 min.

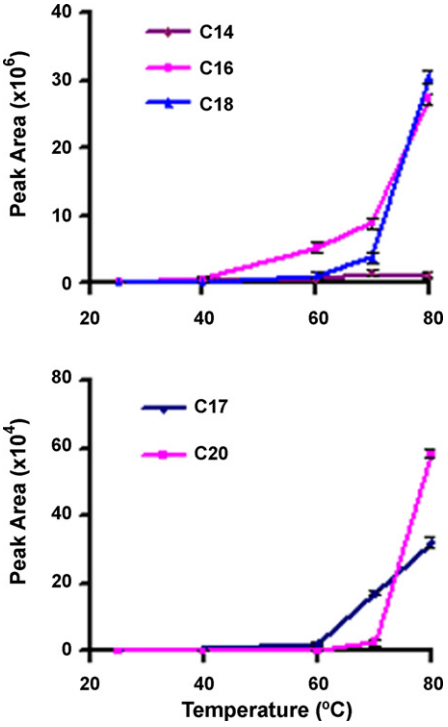


**Fig. 5.** The extraction temperature profile for fatty acids analysis after derivatization. Extraction time: 20 min. Other conditions are the same as in Fig. 4 except for the extraction temperature. The figure was obtained with three replicate measurements.

### 3.2. HS-SPME parameters optimization

Before the analysis of fatty acids, preliminary studies were performed to investigate the interaction between variables affecting the analytes responses and no significant interaction between factors on the response variable was observed. Therefore, optimization of the extraction SPME conditions was carried out using one at a time method. The variables include: extraction time and temperature, ionic strength, stirring rate and headspace volume.

The influence of time on the extraction efficiency of fatty acids after derivatization was shown in Fig. 4. The mentioned variable was studied between 5 and 45 min and it was shown that maximum peak areas were obtained for C14:0, C16:0 and C17:0 when the extraction time is 20 min and longer equilibrium time is needed for longer chain fatty acids (C18:0, C20:0). Observed decrement of peak areas for C14:0, C16:0 and C17:0 can be attributed to the loss of shorter chain fatty acids at higher extraction times than 20 min. Saving the time, 20 min was selected for all the fatty acids studied.

The influence of temperature on the extraction efficiency of fatty acids was shown in Fig. 5. It can be seen that the extraction yields of all fatty acids except C14:0 increase until 80 °C. Although the signal probably continues to increase until a temperature value higher than 80 °C and then drops, but for practical problems of working at these temperatures (such as adjustment of water bath temperature), the temperature of 80 °C was set in the following experiments for HS-SPME of fatty acids.

The influence of ionic strength was studied by adding different amounts of NaCl, ranging from 0 to 30% (w/v) to a spiked sample. As shown in Fig. 6, the maximum extraction efficiency in the concentration of 15% was obtained. Therefore, in the further experiments the concentration of NaCl was fixed at 15%.

Sampling agitation enhances extraction process and reduces extraction time because the equilibrium between the aqueous and vapor phases can be achieved more rapidly. Five different stirring rates were selected: 0 (static case), 200, 400, 600 and 800 rpm. The results show clearly that stirring produce causes an increase in the analytical signal compared to the stagnant case. As can be seen in Fig. 7, the peak areas of all analytes increase with increase in the stirring rates up to 800 rpm. Hence a stirring rate of 800 rpm was chosen for the further works.

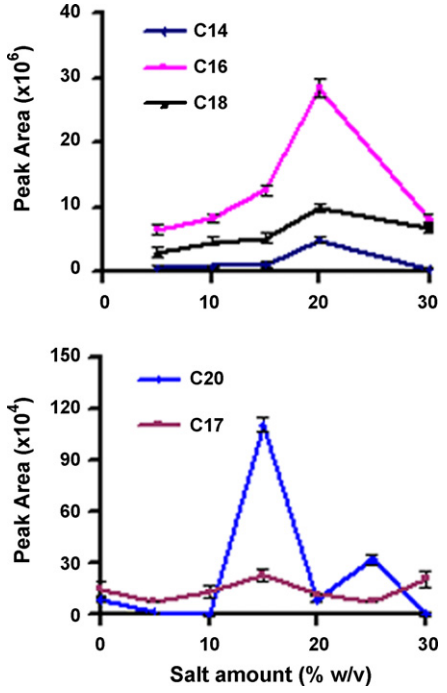


**Fig. 6.** The profile of ionic strength for fatty acids analysis after derivatization. Extraction time 20 min and extraction temperature 80 °C. Other conditions are the same as in Fig. 4 except for the ionic strength.

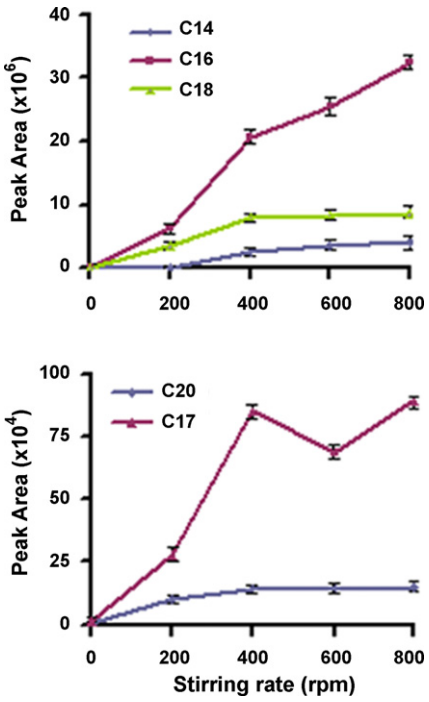


**Fig. 7.** The stirring rate profile for fatty acids analysis after derivatization. Extraction time 20 min, extraction temperature 80 °C and concentration of NaCl was fixed at 15%. Other conditions are the same as in Fig. 4 except for the stirring rate.

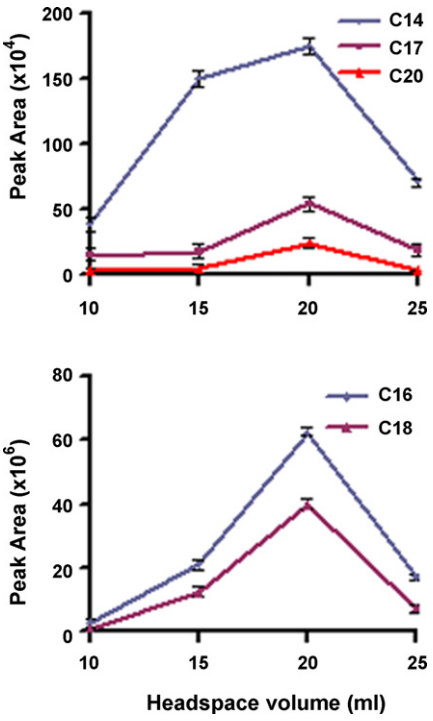


**Fig. 8.** The headspace volume profile for fatty acids analysis after derivatization.

It was necessary to check the effect of the headspace volume on the enrichment of volatile compounds in order to obtain the highest sensitivity for the procedure. Thus different headspace volumes included 10, 15, 20 and 25 mL were examined, and 20 mL headspace volume showed the highest extraction efficiency (Fig. 8).

### 3.3. Analytical performance of the method

Table 1 summarizes the figures of merit of the proposed HS-SPME method. The reproducibility was estimated by the relative standard deviation (RSD, $n = 5$) of the peak areas of the analytes. The RSD values for single fiber, shown in Table 1, ranged from 1.5% for C20:0 to 5.7% for C17:0. The fiber to fiber RSD values vary from 4.8% for C20:0 to 10.2% for C17:0. A linear regression analysis of peak area versus the analytes concentration was performed using standard solutions with the concentration range of 0.1–300 μg mL$^{-1}$ in fourteen points. The linear dynamic range was 0.5–200 μg mL$^{-1}$. The correlation coefficients for the calibration curves are presented in Table 2. The linearity is satisfactory with the correlation coefficients ($R^2$) ranging from 0.992 (C20:0) to 0.998 (C18:0) within a wide concentration range. The limits of detection varied from 0.01 (C14:0) to 6.07 μg L$^{-1}$ (C20:0).

### 3.4. Determination of fatty acids in zooplanktons

The external calibration method was used to measure the concentration level of saturated fatty acid with even carbon number (C12–C20) in zooplanktons. The results show the concentration range of 6–119 μg g$^{-1}$ for the analyzed fatty acid in zooplankton of Chabahr gulf. Peak identification was accomplished using the relative retention times as well as mass spectra. Table 2 shows the retention times, recovery and selected ions for quantification and content of some saturated fatty acids in zooplankton of Chabahr gulf. The recovery values were obtained after spiking the zooplankton sample by 1 μg mL$^{-1}$ of standard solution and the values ranged

**Table 1**
Analytical performance data of HS-SPME–GC–MS method for the analysis of fatty acids.

| Fatty acids | LODs (μg L$^{-1}$) | Linear range (μg mL$^{-1}$) | Correlation coefficient ($R^2$) | RSD % ($n = 5$) | |
|---|---|---|---|---|---|
| | | | | Single fiber | Fiber-to-fiber |
| C14:0 | 0.01 | 0.5–200 | 0.995 | 4.9 | 8.4 |
| C16:0 | 0.04 | 0.5–200 | 0.992 | 5 | 9.1 |
| C17:0 | 0.15 | 0.5–200 | 0.997 | 5.7 | 10.2 |
| C18:0 | 0.04 | 0.5–200 | 0.998 | 5.4 | 10 |
| C20:0 | 6.07 | 0.5–200 | 0.992 | 1.5 | 4.8 |

**Table 2**
Retention times, selected ions and obtained values of some saturated fatty acids in zooplanktons of Chabahr gulf.

| Fatty acids | Ion selected for quantification (m/z) | Retention time (min) | Obtained values of fatty acids (μg g$^{-1}$) | Recovery (%) |
|---|---|---|---|---|
| C14 | 242 | 15.6 | 95 | 115 |
| C16 | 270 | 18.9 | 119 | 83 |
| C17 | 74 | 20.6 | 6 | 102 |
| C18 | 298 | 22.1 | 42 | 95 |
| C20 | 74 | 25.0 | 38 | 93 |

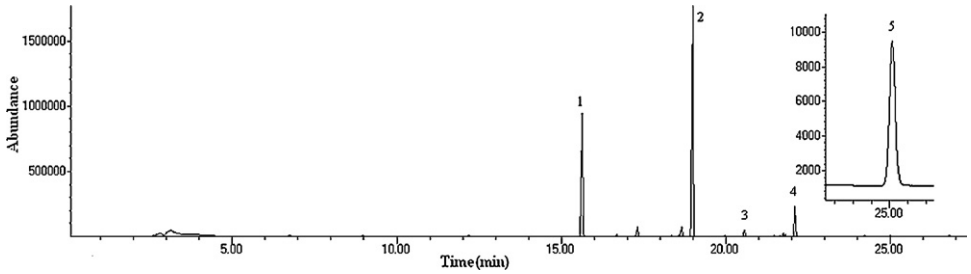


**Fig. 9.** The chromatogram obtained by HS-SPME–GC–MS under SIM mode for zooplankton sample: (1) C14:0; (2) C16:0; (3) C17:0; (4) C18:0; and (5) C20:0.

from 83% for C16:0 to 115.0% for C14:0 (Table 2), demonstrating satisfactory accuracy of the method. Fig. 9 illustrates selected ion chromatogram of zooplankton sample.

## 4. Conclusion

In this paper, a novel SPAN fiber was synthesized by a simple electrodeposition method using co-polymerisation of m-aminobenzoic acid and aniline. High temperature, chemical and mechanical resistance, reproducible synthesis, low cost and relatively long life span are the main advantages of this fiber. One of the main advantages of the proposed method is the elimination of organic extraction step, because the derivatized analyte needed no separation from the mixture of derivatizing agent and analyte. Therefore, it could be extracted immediately after derivatization. The method also showed satisfactory accuracy, linearity, precision and detection limits.

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