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Carbon fibers modified with polypyrrole for headspace solid phase microextraction of trace amounts of 2-pentyl furan from breath samples

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ABSTRACT

This study introduces micrometric carbon strands as a suitable fiber for headspace solid phase microextraction. Compared to previous supports, carbon fibers have mechanical flexibility, wide thermal expansion, and a large surface area, which is an important factor in headspace solid phase microextraction. The electrophoretic technique was applied to modify the surface of stainless steel and carbon fibers with polypyrrole. Modified carbon fibers were used for extraction of 2-pentylfuran (2-PF) as a model analyte from patients' breath and coffee samples. 2-PF belongs to the furan family, which was suggested as a biomarker for *Aspergillus fumigatus* and was classified as a possible carcinogen. 2-PF can be found in many heat-processed foods and drinks. The separation and detection of the analyte was performed by gas chromatography coupled to mass spectrometry. The effective factors in the extraction performance of the analyte by carbon fiber supports were investigated and optimized. Under optimized extraction conditions (temperature, 20 °C; time, 15 min; desorption temperature, 200 °C; desorption time, 2 min; salt concentration, 10% w/v; and stirring rate, 700 rpm), the limit of detection was calculated as 0.05 ng mL^{-1}, whereas repeatability and fiber-to-fiber reproducibility (RSD %) was found to be in the range of 3.2–4.1%. The experimental results showed that the proposed fiber had greater extraction performance for 2-pentylfuran.

1. Introduction

Zhang and Pawliszyn introduced the headspace solid phase microextraction (HS-SPME) method in 1993 [1]. Since then, many scientists have presented different research works on the HS-SPME method [2]. The HS-SPME technique is a routine preconcentration method for volatile and semi-volatile compounds, which can be easily accomplished in the laboratory [2–6]. This technique has some advantages, such as simplicity, being time-saving, avoidance of organic solvents, many highly oriented commercial fibers, and good reproducibility [7]. However, fibers used in HS-SPME method are fragile and have low thermal expansion and chemical stability [8–10].

Therefore, in the past two decades, in order to overcome the mentioned problems, new supports such as metal wires including stainless steel (SS) [11], platinum [12], anodized aluminum [13], pencil lead [14], and copper coated with copper chloride anodized zinc were introduced [15,16]. It seems that the efficiency of the previous fibers can still be improved by new supports [2].

Carbon fibers (CFs) have been attracting great research interest because of their mechanical flexibility, good chemical resistance, larger surface areas, high conductivity, low weight, and thermal expansion [17]. They have been widely used in various fields of industry and science as substrates [18–25].

In addition, CFs or modified CFs have been used in order to adsorb materials for extraction. Therefore, some modifications of the surface, such as coating, etching, and thermal treatment were applied to CFs [24–28]. For example, Lee's group in 2008, for the first time, used graphite fiber as a sorbent for extraction of polycyclic aromatic hydrocarbons [19]. Similarly, in 2017, Feng et al. reported a simple electrophoretic deposition method for coating of graphene oxide onto CFs in order to use it for the in-tube-SPME method. It showed better extraction efficiency than bare CFs [18].

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Polypyrrole (PPy), as a conductive polymer, has been considered a highly potential material and has increasingly attracted attention owing to its unique properties (low cost, easy synthesis, and good adsorption). The surface cavities and interstitial spaces of the PPy structure are great enough to allow molecules to adsorb [29–31]. Polypyrrole with different structures, such as cauliflower and wire, has been applied as an efficient sorbent on a different substrate in the SPME method [29–32].

One way to encounter incurable diseases is early diagnosis before they spread in the body. Recently, different methods, such as different analytical methods or even use of animals, have been developed to detect diseases [33]. A similar approach that may be employed for early diagnosis of fungal infection diseases is measuring the volatile organic compounds. These studies are based on the fact that these compounds are produced from pathogens, microorganisms, and disease agents. Therefore, they may be applied as biomarkers for early detection of diseases in extremely low levels (parts per billion and parts per trillion ranges). *A. fumigatus* is a leading cause of pneumonia and invasive disseminated diseases and results in high mortality [34].

2-pentylfuran (2-PF) has been identified as a potential biomarker of *A. fumigatus*, which is considered an opportunistic pathogenic fungus [35–37]. In addition, IARC (International Agency for Research on Cancers) has classified furan derivatives as Group 2B, a possibly carcinogenic chemical to humans. 2-PF can be found in different foods and drinks, such as fruit juices and beverages [38,39]. Thermal degradation of food products is the most likely way of 2-PF formation.

In this research, for the first time, CFs were used as a support for the HS-SPME method. To attain such a goal, a suitable holder was fabricated for facile transfer of CFs into the injection port without deterioration of CF supports. Moreover, polypyrrole was immobilized on the fibers via the electrodeposition technique. The prepared composite can be used as a sorbent for separation and quantification of 2-PF as a model analyte from patients' breath and coffee samples. Under optimum conditions, the CFs@PPy efficiency was compared with that of the SS@PPy method for extraction of the trace amounts of 2-PF as a model analyte in real breath and coffee samples.

2. Experimental section

2.1. Instrumentation

The surface morphology of the CFs and CFs@PPy were examined using a MIRA3TESCAN-XMU field emission scanning electron microscopy (FESEM) (Brno, Czech). The chemical analysis of the CFs@PPy was performed using FESEM equipped with energy dispersive X-ray spectroscopy (EDXS). Fourier Transform infrared (FT-IR) spectroscopy (Model: Thermo scientific NICOLET IR100 (Madison, WI, USA)) was employed in the wave number range of 400–4000 cm^{-1} to analyze the chemical composition of the polypyrrole. The thermal stability of the composite was analyzed using NETZSCH TG 209 F1 Iris thermo gravimetric analysis (TGA) (Selb, Germany) at a heating rate of 20 °C min^{-1} in nitrogen media.

The specific surface areas for all fibers were measured via the Micrometrics TriStar 3000 Analyzer (Norcress, USA) using the Brunauer–Emmett–Teller (BET) method. An Agilent gas chromatograph instrument model 7890A (Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and Agilent 7890B GC equipped with an Agilent 5977B MS were used for separation and detection of the analyte. A split/splitless injection port was employed for injection of the samples into the GC-FID and GC–MS instruments in the splitless mode. The GC capillary column (30 m × 0.32 mm, i.d. 0.32 µm film thickness) was held at 40 °C for 1 min; it was then increased at 10 °C/min to temperatures as high as 190 °C and was held for 10 min. The high-purity He and N_2 (purity 99.999%) were used as the carrier gas and makeup gas at the flow rate of 2 mL min^{-1} and 25 mL min^{-1}, respectively. GC–MS separations were performed using an HP-5MS capillary column (30 m × 0.25 mm × i.d. 0.25 µm film thickness). The mass spectrometer was operated in the electron impact ionization mode at the electron energy of 70 eV. The ion source and interface temperatures were both set at 250 °C. The extracted analytes were desorbed and directly injected into a GC injector using an SPME holder (designed in our laboratory). The column temperature was held at 40 °C for 3 min and then was increased to the final temperature of 190 °C at 10 °C/min. An MR-3001K heater-magnetic stirrer from Heidolph (Kelheim, Germany) was used to control the extraction temperature and stirring rate.

2.2. Chemicals and reagents

All chemicals used were of analytical reagent grade. The CFs were purchased from Ircomposite Company (Tehran, Iran), with the average diameter of 6 µm. Tedlar bags (2 L, SKC Inc., Valley View Road, USA) and PTFE syringe filter membrane (pore size 0.45 μm) were used for real sample preparation. Pyrrole, sodium dodecyl benzene sulfonate (SDBS), and 2-PF were prepared from Sigma-Aldrich (Darmstadt, Germany). Analytical-grade acetone and methanol were obtained from Daejong (Seoul, Korea). The stock solution of furan derivation (200 mg L^{-1}) was prepared in methanol and stored in a freezer at −5 °C for later use. The working standard solutions were prepared daily by diluting the stock standard solution to the required concentration with water.

2.3. Synthesis of PPy on fiber

CFs and stainless steel (SS) wire (length of 30.0 mm, diameter of 0.2 mm) were used to fabricate the SPME fibers. The electrophoretic technique was applied to modify the surface of SS and CFs fibers with PPy. Prior to the coating of PPy, the fibers were washed sequentially with acetone, ethanol, and deionized water. Then, they were dried at 100 °C for 120 min. After that, the PPy nanostructures were directly prepared on the surface of the fibers using 20 mL distortion water solution containing 0.05 M of SDBS and 0.1 M of pyrrole monomer by applying a constant deposition potential of +1.7 V for the CFs@PPy and +0.8 V for the SS@PPy fibers for 20 min at room temperature. After the deposition process, the modified fibers were dried at 150 °C for 3 h.

2.4. Breath samples

Sixteen exhaled breath samples were obtained from respiratory patients and normal humans and stored into 2 L Tedlar bags. Almost all the participants were surveyed at a convenient time with common air from a conditioning system. The guidelines for research ethics for sampling were observed. After sampling, the sample bags were closed with a valve and carried immediately to the laboratory for further analysis. The conditioned breath samples were retained in the collection Tedlar bags for about 24 h and then were extracted by CFs@PPy. The extraction time and temperature of 2-PF from the Tedlar bags were 1 h and 40 °C, respectively. Afterwards, the CFs@PPy fiber was transferred directly into the injection port of the GC–MS instrument very quickly.

2.5. Coffee samples

Four types of coffee powder samples (coffee A, B, C, and D) were prepared from a local market in March 2018 (Tehran, Iran). At first, 0.5 g of the each powdered sample was accurately weighted into a beaker and 10 mL of boiling water was poured on it. Then,

that was allowed to stand for a few minutes. After filtering the samples by a membrane filter, they were kept in glass bottles at 3 °C prior to the analysis. The CFs@PPy-HS-SPME procedure was done under optimum conditions to extract the analyte.

2.6. CFs@PPy-HS-SPME procedure

Ten mL of the aqueous solution containing 50 μg L^{-1} of the analyte and 1.0 g of NaCl (10% w/v) were transferred into a 20-mL glass vial and were stirred with the rate of 700 rpm for 20 min at 20 °C (An ice bath was used to set the extraction temperature). Then, the HS-SPME holder was used to extract the analyte from the headspace of the sample. After completion of the extraction, it was withdrawn from the vial and was inserted into the injection port immediately and was held for 2 min at 200 °C for desorption of the analyte.

3. Results and discussion

3.1. Characterization of CFs and CFs@PPy

The most important character of CFs is huge increases in the side surface area. As shown in Fig. 1, 963 cylindrical wire with the outer diameter of 6 μm can be placed inside a wider cylindrical tube with the inner diameter of 200 μm (with the help of the Packomania website: "www.packomania.com"). The side surface area of a cylindrical fiber with the length of 30 mm and the diameter of 200 μm is 18.9 mm^2. However, a series of CFs of 30 mm long and with a 6 μm diameter (the side surface area for each fiber is 1.13 mm^2) has a 1089 mm^2 side surface area, which shows an area 57 times higher than a single 200 μm fiber. This surface area can effectively increase the extraction efficiency of the SPME fiber.

The digital images of the SPME holder are shown in Fig. 2(a). The image of the CFs after being placed on the holder needle shows that it has a large number of branches. The synthesized CFs@PPy was investigated by the FESEM and EDX analyses to evaluate their morphology and chemical composition, respectively. The FESEM image in Fig. 2(b) shows the surface morphologies of the bare CFs and the PPy composed on the surface of the CFs in the form of a cauliflower (which is the usual structure of electrodeposited PPy) [40]. The FESEM images with different resolutions are shown in Fig. 2(c) and (d). The EDX analysis of the CFs@PPy is shown in Fig. 3, which confirms the existence of nitrogen and carbon elements. The results are in good agreement with the structures of PPy. However, sodium dodecyl benzene sulfonate could act as a dopant for the PPy chains, and this role of SDBS could lead to observation of S and O elements in the EDX analysis. Furthermore, the other source of the oxygen element could be due to the air contamination of the samples. The thermogravimetric curves of the bare CFs and the polypyrrole coted on the CFs are shown in Fig. 3(a). The thermograms were recorded by heating the samples at the temperature range of 25 700 °C under the nitrogen atmosphere (60 mL min^{-1}). As can be found from this figure, the CFs demonstrated high thermal stability without significant weight loss over the entire range of the investigated temperatures. The thermograms of the CFs@PPy indicated a very small weight change around 234 °C. This weight loss can be related to the thermal degradation of the polymer backbone.

The N_2 adsorption–desorption isotherm shows a curve type(IV) isotherm pattern with a sharp hysteresis loop at p/p_0 = 0.9–95, which indicates that CFs@PPy has a macroporous structure Fig. 4(a). The specific surface area and pore sizes of this material were determined by the BET method, which indicated a total specific surface of 11.1 m^2/g. The pore size distribution was calculated based on the Barrett–Joyner–Halenda method (BJH) plot. According to the BJH plot, it can be seen that the average pore sizes of the PPy@CFs were found to be 12.3 and 96 nm. The occurrence of these two peaks of meso-pores indicates that the synthesis martial had pore structures in mesoporous and macroporous regions.

The FT-IR spectrum of the described PPy in the transmission mode was obtained between (400 and 4000) cm^{-1}, as shown in Fig. 3(a). In this figure, the band at 3423 cm^{-1} refers to N–H stretching vibration, and the two minor bands at 2846 cm^{-1} and 2917 cm^{-1} belong to the C–H symmetric and antisymmetric in both CH_2 and CH_3 aliphatic. Moreover, C–H out of plane distortions were detected around 1633 cm^{-1} according to the literature [41]. The observed bands at 1544 cm^{-1} and 1452 cm^{-1} correspond to the C=C ring stretching. The bands at 1157 cm^{-1} belong to the S=O symmetric and antisymmetric in sulfonates, which was used in the synthesis of the PPy [41].

3.2. Injection method

We observed that the CFs were detached or broken while transferring the CFs into the injection port or the headspace of the sample with the commercial SPME holder. Therefore, we designed a suitable holder with three injection steps in order to solve the mentioned problem. Fig. 5 depicts the above steps schematically. In step 1, the holder needle was transferred into the headspace of

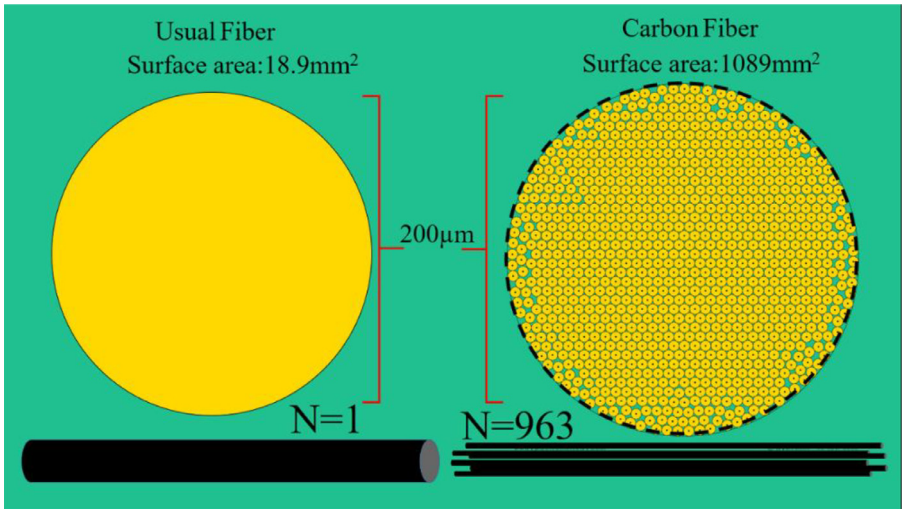

Fig. 1. A simple schematic difference in surface area and cross-section between CFs and the usual fiber.

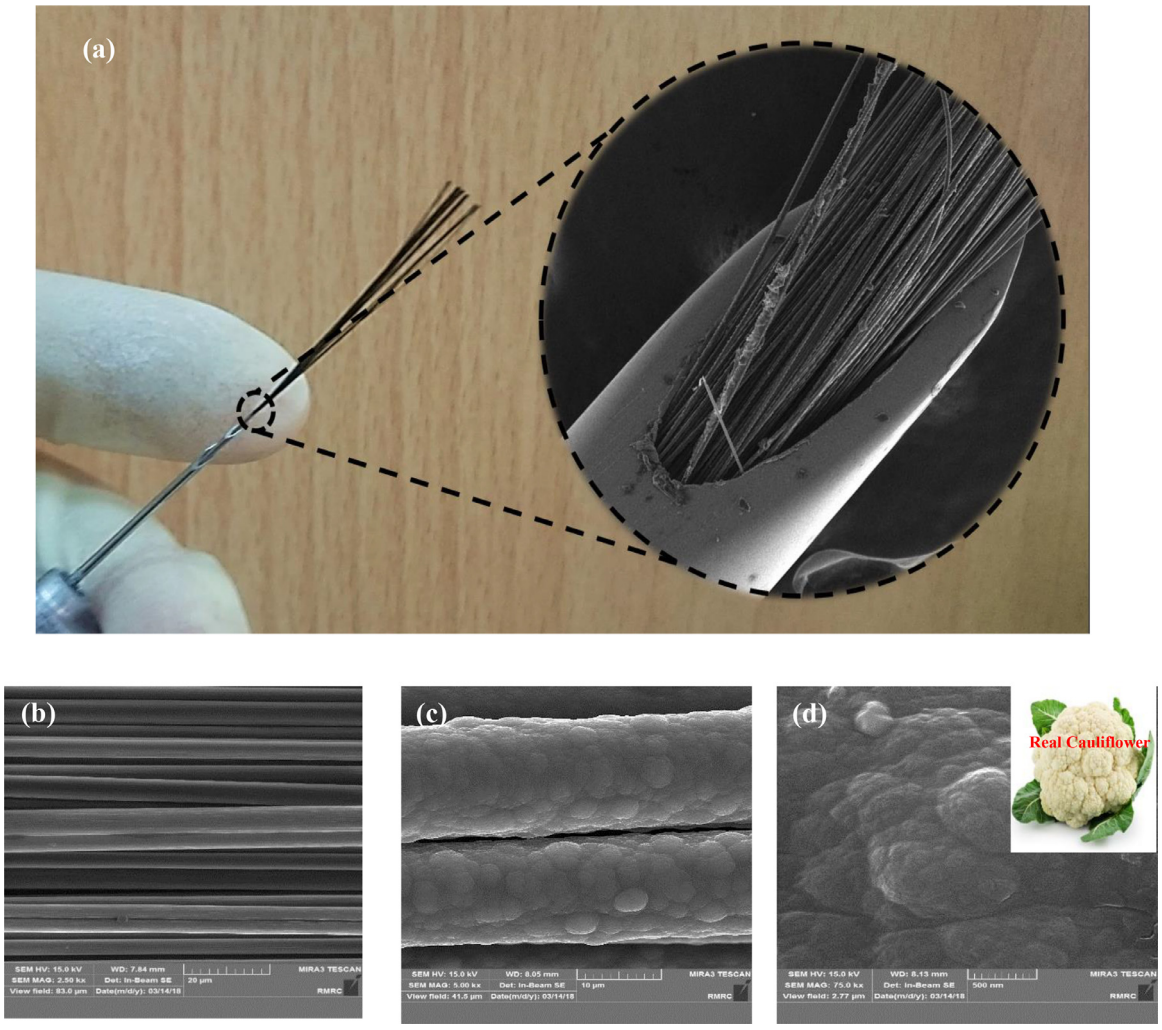

Fig. 2. (a) Schematic representation of an HS-SPME holder system and SEM of a needle holder, Field emission scanning electron microscope (FE-SEM) images of (b) bare carbon fiber and (c, d) PPy on a carbon fiber substrate.

the sample. In step 2, the auxiliary needle removed the possible pieces of the septum from the holder needle. Finally, in step 3, the CFs was transferred into the headspace of the sample. In addition, the same method was performed to transfer the CFs into the injection port of GC or GC–MS.

3.3. Optimization of CFs@PPy preparation

Pyrrole was coated on the CFs via the electro deposition method under a fixed voltage. The effect of the deposition time on the CFs was investigated in the range of 5–30 min. Fig. 6(a) shows that the extraction efficiency was greatly influenced by deposition time. The extraction efficiency increased up to 20 min due to the increase in the coating of PPy on the CFs surface. When the electropolymerization time was more than 20 min, the extraction efficiency of CFs@PPy decreased rapidly. This might have been due to the decrease in the specific surface area and the pore size of the PPy nanostructure. By increasing the deposition time, the small pores were gradually filled and blocked by PPy.

3.4. Optimization of extraction conditions

Temperature is an important factor in extraction efficiency. At higher temperatures, due to the increase in the volatility of furan derivatives, the concentration of the analyte increased in the headspace and thus extraction efficiency rose too. The effect of temperature on the extraction efficiency of the analyte was studied in the range of 10–40 °C (Fig. 6(b)). The extraction efficiency increased by increasing the extraction temperature up to 20 °C. At temperatures higher than 20 °C, the amount of the extracted analyte decreased significantly. Therefore, 20 °C was selected as the best extraction temperature.

Moreover, the extraction time profiles of 2-PF were studied from 5 to 25 min under optimum extraction conditions (Fig. 6(c)). According to the results, after 15 min, no significant increase was found in the extraction efficiency, and so 15 min was selected as the optimum extraction time. Furthermore, the effect of salt addition on the extraction efficiency of 2-PF using CFs@PPy was evaluated by adding different amounts of NaCl (0–20% w/v) into the solution. The results showed that the best extraction efficiency was obtained by adjusting the NaCl concentration at 10% w/v. As shown in Fig. 6(d), the extraction efficiency of 2-PF increased rapidly by increasing the salt amount from 0% to 10% w/v, which was due to the salting out effect. The extraction efficiency then remained fairly stable in the range of 10–20% w/v. Thus, the subsequent experiments were conducted by adding 10% w/v NaCl into the solution. Finally, the effect of stirring rate on extraction efficiency was studied in the range of 0–1200 rpm (Fig. 7(a)). Stirring of the

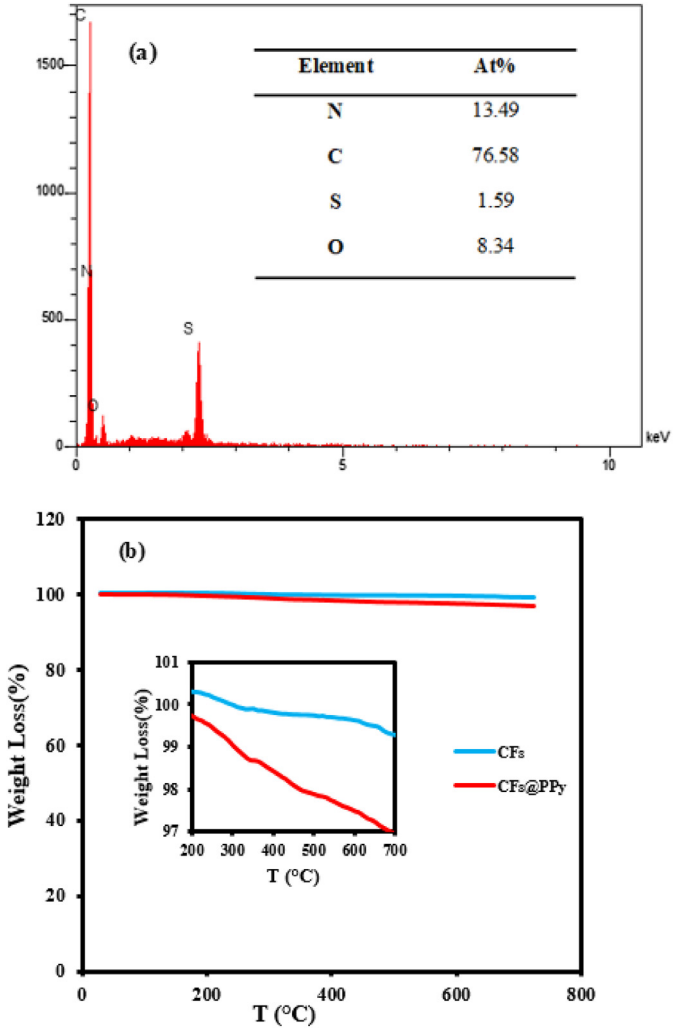

Fig. 3. (a) EDX spectrum analysis of the surface of the CFs@PPy sample along with corresponding atomic percentages (b) TGA thermograms of PPy and CFs@PPy.

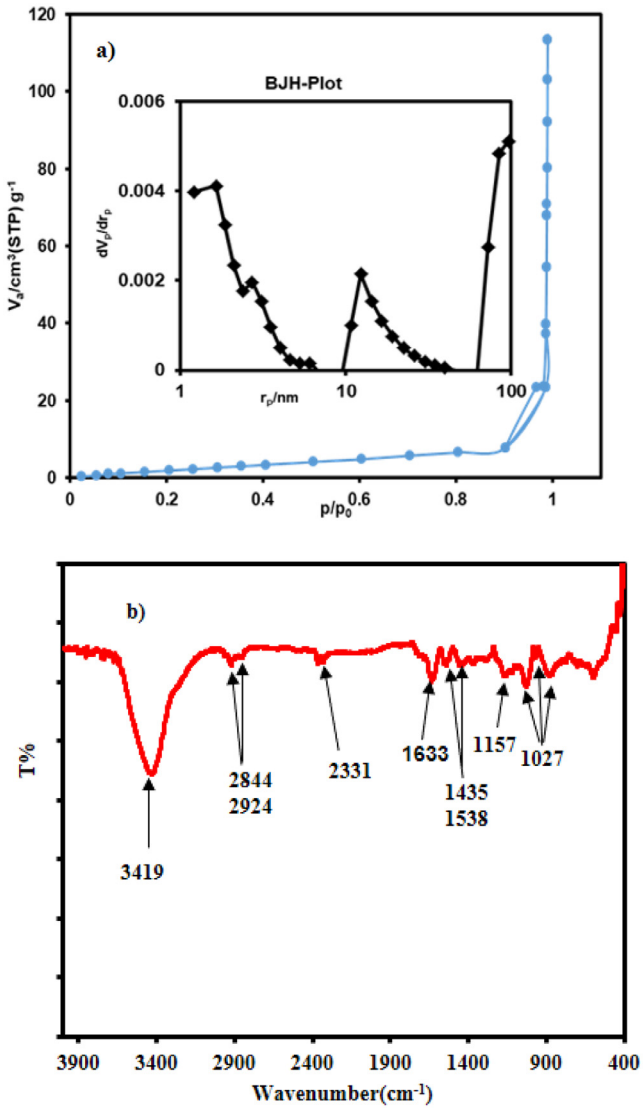

Fig. 4. a) BET nitrogen adsorption–desorption isotherms and BJH pore size distributions of CFs@PPy. b) Infrared transmission spectrum (FTIR) for the PPy on CFs, measured using KBr pellets.

sample accelerated mass transfer from the sample solution into the headspace phase and thus decreased the required time to reach equilibrium. The results indicated that the highest response for the analyte was obtained at the stirring speed of 700 rpm.

3.5. Investigating the effective factors in desorption of 2-PF from CFs@PPy

To obtain the optimum desorption time and injector temperature, 2-PF was desorbed from the fiber at various desorption times and temperatures. Time and temperature were adjusted in the range of 1–5 min and 100–300 °C, respectively. According to the results, the best desorption time was obtained at 2 min. In addition, no obvious difference in extraction efficiencies was witnessed at different desorption temperatures (Fig. 7(b) and (c)). The injection temperature was set at 200 °C in the subsequent experiments.

3.6. Effect of the CFs and PPy on extraction performance

One of the aims of this study was to determine the effect of the CFs on extraction performance. Fig. 8 compares the surface area and extraction abilities of all the fibers with maximum extraction efficiency. The results show that the extraction performance of 2-PF by CFs@PPy was 15 times more than SS@PPy. This could be only attributed to the larger surface area of the CFs. In addition, the extraction performance of CFs@PPy was better than that of the bare CFs. This could be due to the increase in the surface area and also the increase in the interactions between PPy and analytes such as hydrophobicity, acid–base character, $\pi - \pi$ interaction, polar functional groups, and hydrogen bonding [42]. Moreover, to study the extraction capacity of the fibers, the CFs@PPy and SS@PPy fibers were exposed to the headspace of the solutions of 2-PF with various concentrations under optimal conditions. Immediately after completion of extraction, the fibers were inserted into the injection port of the GC in the splitless mode for thermal desorption of the analyte. The peak area of the 2-PF was drawn as a function of its concentration in the solution (Fig. S1a, b). The maximum amounts of the extracted analyte were obtained from the plateaus part of the curves. The amount of the extracted analyte was quantified by GC-FID. A comparison of the chromatographic peak area with the results obtained from direct injection under the same chromatographic conditions reveals that the extraction capacity of the fibers were 28.2 nmol and 1.09 nmol for the CFs@PPy and SS@PPy fibers, respectively. These results indicate that the extraction capacity of the CFs@PPy fiber is higher than the SS@PPy fiber.

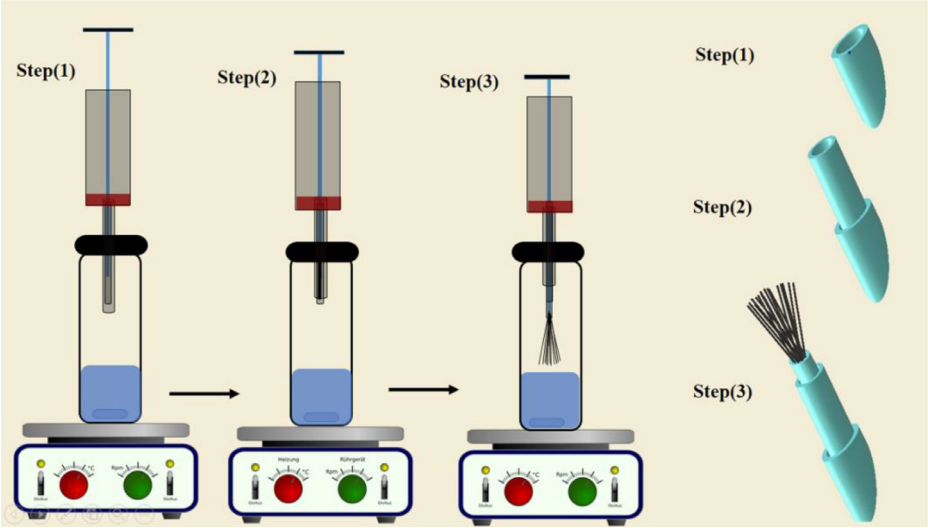

Fig. 5. HS-SPME holder used for transferring fibers in the injector port.

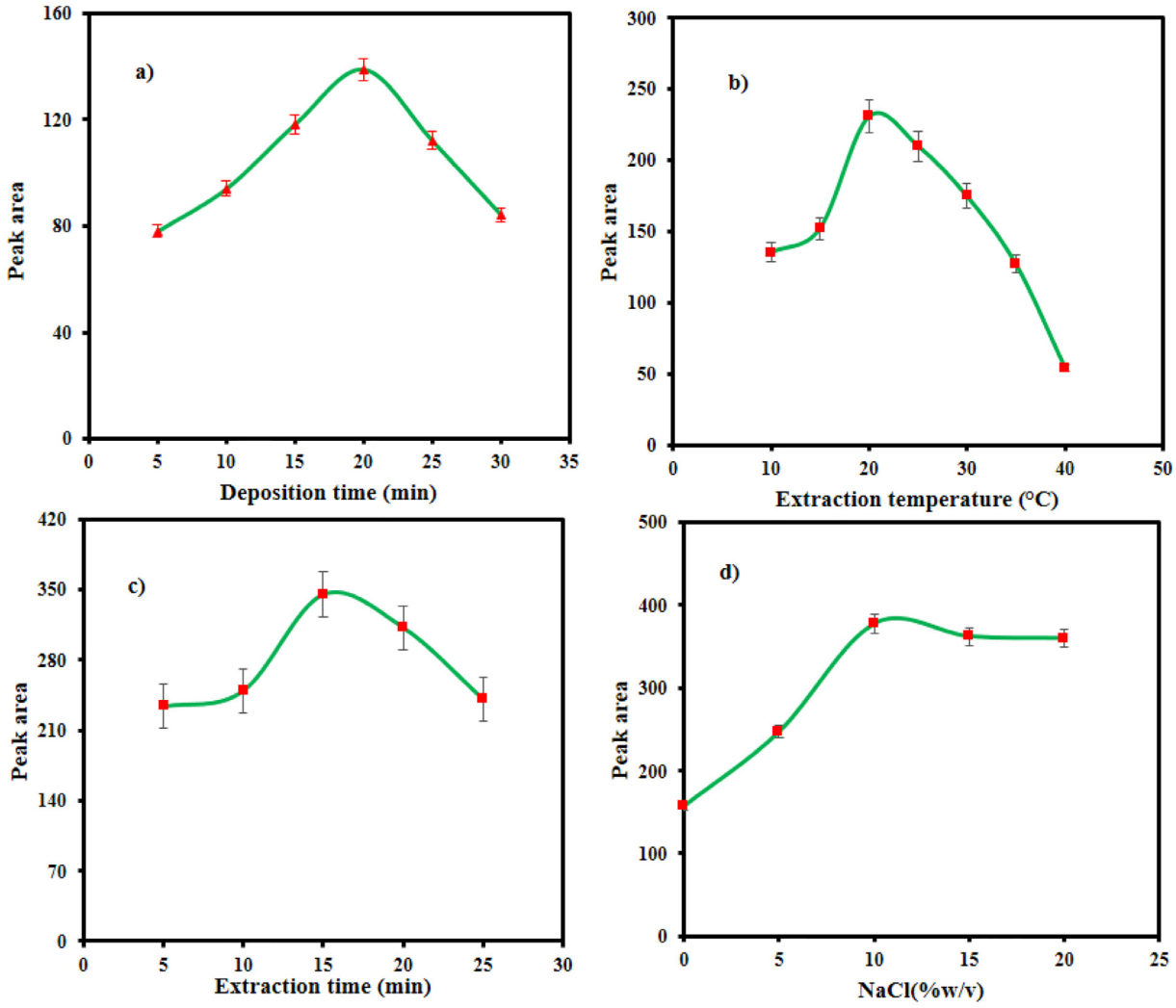

Fig. 6. Effects of a) deposition time b) extraction temperature c) extraction time and d) salt concentration on extraction efficiency of 2-PF by CFs@PPy-HS-SPME. Extraction conditions: analyte concentration: 100 ng mL^{-1}; sample volume: 10.0 mL; stirring rate: 700 rpm; deposition time: 2 min; desorption temperature: 250 °C.

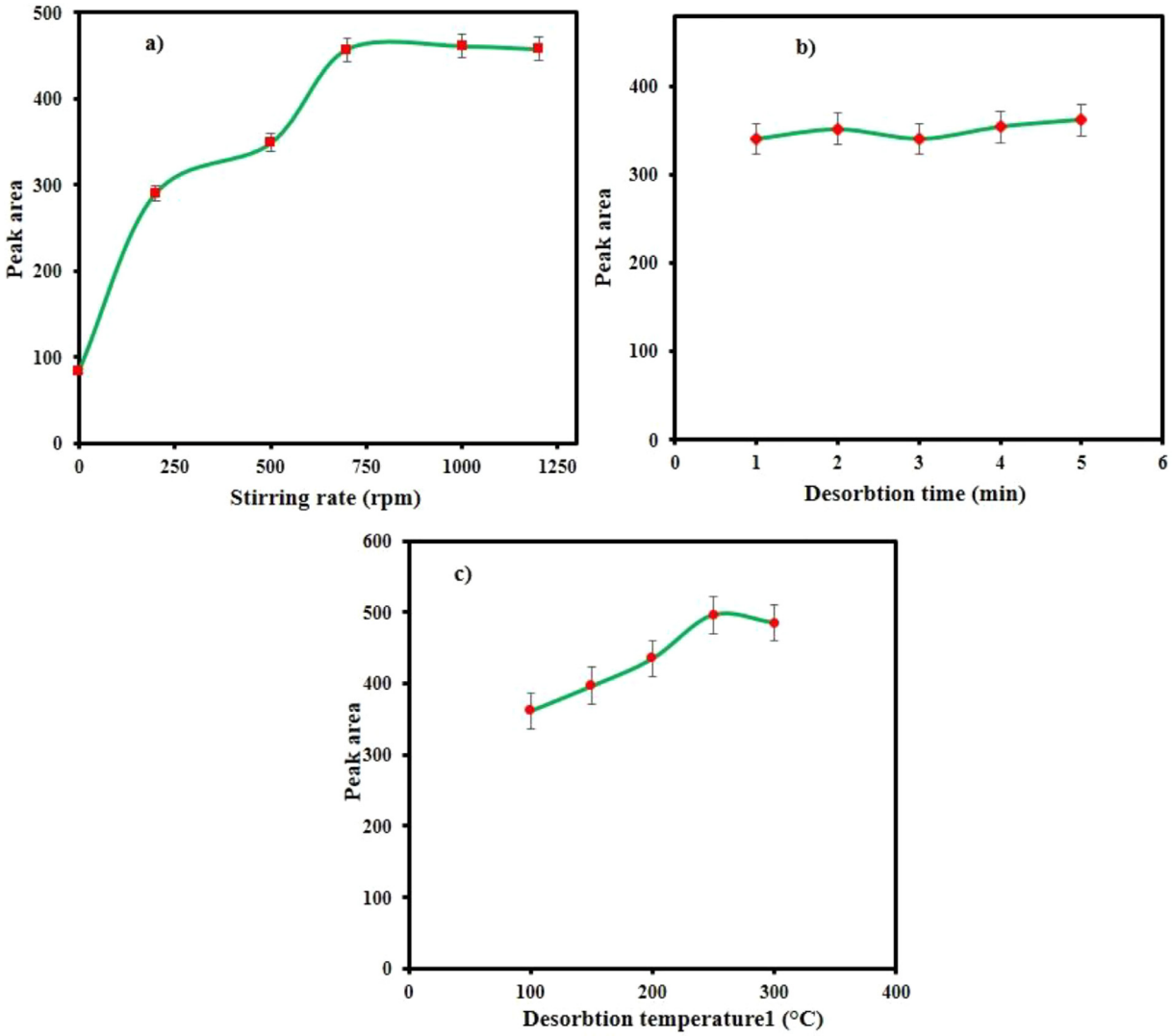

Fig. 7. Effects of a) stirring rate b) deposition time and c) desorption temperature on extraction efficiency of 2-PF by CFs@PPy-HS-SPME. Extraction conditions: analyte concentration: 100 ng mL^{-1}; sample volume: 10.0 mL; deposition time: 20 min; extraction temperature: 20 °C; extraction time: 15 min; salt concentration: 10% w/v.

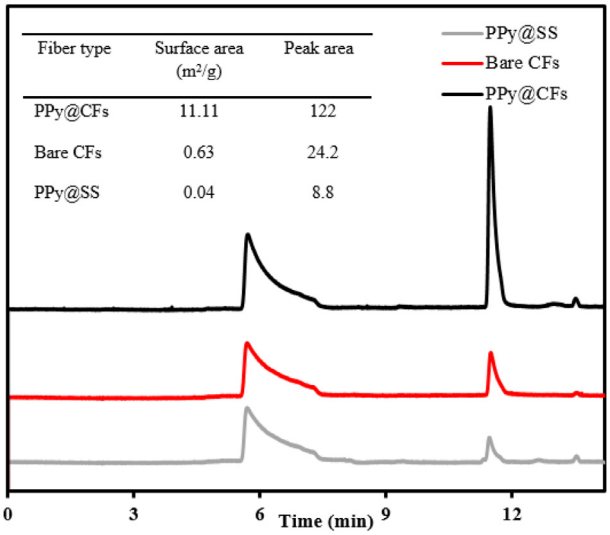

Fig. 8. GC-FID analysis chromatograms related to extraction of 2-PF by CFs@PPy, SS@PPy, and bare CFs.

3.7. Reusability of CFs@PPy

Fiber stability was investigated by several repetitions of the extraction processes of 2-PF by CFs@PPy. The experimental results showed that CFs@PPy could be applied more than 20 times without any considerable decrease in extraction performance. Furthermore, to test the long-time stability of CFs@PPy, it was stored at room temperature for 6 weeks. The results showed that the extraction performance was almost stable and was not affected by the passage of time. These advantages could make CFs@PPy appropriate for routine application in the HS-SPME method.

3.8. Method evaluation

The analytical performance of the CFs@PPy-HS-SPME method, including limit of detection (LOD), linear dynamic range (LDR), coefficient of determination (R^2), and relative standard deviation (RSD), was evaluated. The results are presented in Table 1. The calibration curves were obtained using ten standard solutions with various concentrations. According to the results, the dynamic linear ranges of 0.1–50 ng mL^{-1} with correlations of determination better than 0.997 were obtained. The LODs based on a signal-to-noise ratio of 3:1 (S/N = 3) were 0.05 ng mL^{-1}. Intra-day precision

Table 1
Linear range precision and detection limit data for 2-PF.

Analyte	LDR (ng mL^{-1})	R^2	EF	LOD (ng mL^{-1})	Precision[a] (RSD %, $n=5$)		
					Intra-day	Inter-day	Fiber-to-fiber
2-PF	0.1–50	0.997	4175	0.05	6.2	4.9	8.6

R^2, coefficient of determination; RSD, relative standard deviation.
[a] Were calculated based on the extraction of 2 ng mL^{-1} of the analytes in 10 mL initial solution; EF, Enrichment factor.

Table 2
Determination of 2-PF in the coffee samples using CFs@PPy-HS-SPME.

Sample	$C_{initial\ (ng\ mL^{-1})}$	$C_{added\ (ng\ mL^{-1})}$	$C_{found\ (ng\ mL^{-1})}$	RR%	(RSD % $n=5$)
Coffee A[a]	<LOD	25	22	88	2.9
		50	46	92	2.4
Coffee B[a]	2	25	27	96	3.3
		50	52	98	2.9
Coffee C[b]	8	25	29	84	4.1
		50	49	82	3.9
Coffee D[b]	6	25	29	92	3.2
		50	57	102	3.1

[a] Coffee purchased from local market.
[b] Expired coffee.

was obtained from five consecutive replicates and was expressed as percentage of relative standard deviation (RSD %) with the value of 6.2%. Inter-day RSD % was also obtained on five different days, with the value of 4.9%. Finally, the fiber-to-fiber RSD was 8.6% under the identical conditions.

3.9. Analysis of the real samples

Monitoring 2-PF in different matrices is important for human health. This method was applied to extract and determine 2-PF in coffee and breath samples. The obtained results were very encouraging and showed that CFs@PPy-HS-SPME has good potential for measuring 2-PF in different matrices.

3.10. Analysis of 2-PF in the coffee samples

In sample preparation and chromatographic methods, using internal standard (IS) can reduce sample matrix effects and thus improve precision of determination [43]. On the other hand, according to some reports, the use of the internal standard technique does not generally eliminate matrix effects in quantitative headspace analyses. This may create a serious problem with obtaining precise results using the HS-SPME method [44]. In the present work, *n*-propylbenzene, whose structure and retention time in GC is similar to that of 2-PF, was used as internal standard. Four types of fresh and expired coffee were analyzed to validate the practicability and accuracy of the CFs@PPy-HS-SPME method.

Since standard reference materials with a certified content of the target analyte were not accessible, add-found method was considered an alternative to the validation studies. To ensure the absence of matrix effects, the analyte with appropriate amounts was spiked into the real samples, and recoveries were calculated based on the spiked amounts. To investigate the relative recoveries, the coffee samples were spiked at two concentration levels of 25 and 50 ng mL^{-1} of 2-PF and the analyte was extracted under optimized conditions. The percentage of relative recovery (RR%) was calculated based on the following equation:

$$RR\% = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (1)$$

where C_{found}, C_{real}, and C_{added} are the concentrations of the analyte after the addition of a known amount of the standard into the real sample, the concentration of the analyte in the real sample, and the concentration of a known amount of the standard that was spiked into the real sample, respectively. Each experiment was conducted in triplicates, and the contents were calculated and are tabulated in Table 2. According to the table, recoveries in the range of 82–102% were obtained for samples. Based on the obtained RRs%, it can be concluded that the accuracy of the method is acceptable. Moreover, the results demonstrate that the different matrices of the four types of coffee did not show an effect on extraction efficiency. Finally, the quantification analysis of the samples was performed by GC–MS with the selected ion monitoring (SIM) mode after extraction by the CFs@PPy-HS-SPME procedure. In this work, for the qualification analysis, the mass fractions of 81, 82, and 138 were employed. The chromatograms related to the coffee samples before and after spiking with 25 and 50 μg L^{-1} of the 2-PF standard solutions are shown in Fig. 9. The obtained results showed that using internal standards can improve the

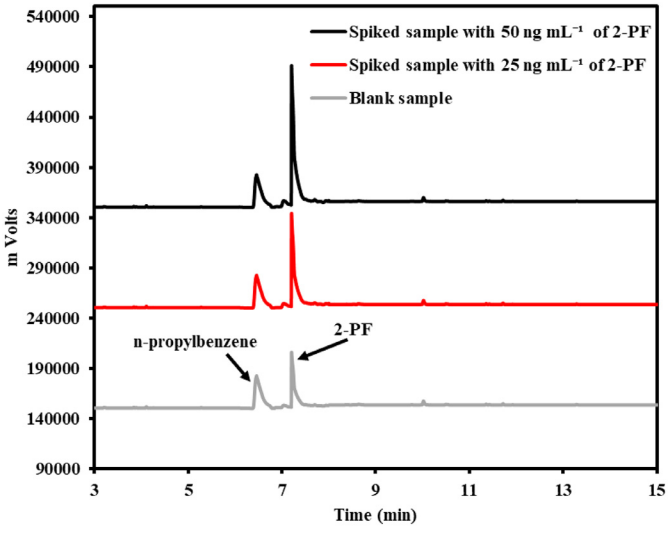

Fig. 9. GC–MS-SIM analysis chromatograms of coffee C sample obtained after CFs@PPy-HS-SPME under optimum conditions using CFs as SPME fiber; blank and spiked with 25 and 50 ng mL^{-1} of 2-PF.

Table 3
Demographic and microbiological data of patients collected in the study, and the results of testing breath samples by HS-SPME–GC–MS for detection of 2-PF with and without *A. fumigatus*.

Underlying disease	Age	Job	Sex	*Aspergillus*	2-PF
Cystic fibrosis	21	Student	Male	+	D
	22	–	Female	+	D
	15	Student	Male	+	N.D
	20	–	Female	+	D
Asthmatic atopic	20	Student	Male	+	D
	27	Taxi driver	Male	+	N.D
	18	–	Female	+	D
	14	Student	Male	+	N.D
Normal controls	18	Student	Male	*	N.D
	20	Taxi driver	Female	*	N.D
	25	Student	Male	*	N.D
	23	Taxi driver	Male	*	N.D

D: detected, N.D: not detected, (*): There was no sign of *A. Fumigatus*

Table 4
Comparison of the proposed method with other microextraction techniques.

LDR[a]	LOD[a]	Sorbent	RSD[a]	Ref.
1.13–50 (attograms)	0.56 (attograms)	DVD/CAR/PDMS	5.11	[36]
1–50 (nmol mol^{-1})	Not mentioned	DVB/Carboxen/PDMS	3.5	[37]
1.06–106 (ng mL^{-1})	0.23) ng mL^{-1})	CAR/PDMS	5.9	[39]
0.1–50 (ng mL^{-1})	0.050 (ng mL^{-1})	CFs@PPy	4.9	Proposed method

[a] LDR: linear dynamic range; LOD: limit of detection; RSD: relative standard deviation; NR: not report; DVB/CAR/PDMS: Divinyl benzene /Carboxen/Polydimethylsiloxane; CAR/PDMS: Carboxen/Polydimethylsiloxane.

precision of the CFs@PPy-HS-SPME procedure followed by GC–MS for extraction and determination of 2-PF in coffee samples (Table S1).

3.11. Analysis of A. fumigatus biomarker in the breath sample

CFs@PPy-HS-SPME was applied to detect 2-PF in 16 pooled breath samples. Subsequently, the breath samples were obtained from three separate groups and were analyzed by GC–MS. The results are presented in Table 3. Three groups are considered in this table. The first and the second groups (*A. fumigatus* positive) were Cystic Fibrosis and Asthmatic Atopic individuals, respectively. As the nutrition type and sampling time of patients can have an effect on 2-PF detection, 2-PF was detected in only some of them in the concentration range of LOD and LOQ. The third group included healthy people (*Aspergillus* negative) in which 2-PF was not found. The results indicated that the method could be a suitable way for early detection of these diseases by identifying and determining 2-PF in breath samples.

3.12. Comparison of the applied method with some other reported methods

A comparison between the present method (CFs@PPy-HS-SPME) and some of the reported methods for extraction and determination of furan derivatives is made in Table 4. In most cases, the figures of merit for the present method are better than or comparable with those for the other reported methods. For example, the LOD obtained in this study is comparable to or better than that of the other methods. In addition, the LDR of our method is better than that of the other reported methods. The analytical performance and sensitivity of the developed method are also acceptable.

4. Conclusions

Applicability of the CFs@PPy-SPME-GC–MS method was evaluated for extraction and determination of 2-PF in coffee and breath samples. A simple electrochemical approach was applied to the fabrication of the CFs coated with PPy as support for HS-SPME. CFs@PPy has better physicochemical properties (such as a large surface area, high efficiency, reusability, and reproducibility) in comparison with bare CFs and SS@PPy fibers. The modified fiber was used for extraction of 2-PF in the mentioned real samples. Due to the absence of 2-PF in the breath of healthy humans, it can be concluded that 2-PF might be indeed a suitable volatile biomarker in breath to recognize fungal infections of the lung. In addition, the proposed method exhibited analytical characteristics similar to or better than the previous extraction methods. Its linearity was acceptable and created suitable limit of detection and typical RSD. Finally, the proposed method is sufficiently attractive because of its simplicity, sensitivity, analytical precision, low consumption of organic solvents, low cost, and promising sample preparation techniques.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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