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# Packed-Nanofiber Solid-Phase Extraction Coupled with High-Performance Liquid Chromatography Fluorescence for Determining Gut Microbiota–Host Cometabolites and Indoleamines in Human Urine

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**Abstract:** Exercise reduces the risk of inflammatory diseases by modulating different tissue and cell types, including those within the gastrointestinal tract. Obtaining a more comprehensive understanding of pathophysiology requires monitoring of dynamic changes in cometabolites. This study aimed to develop a method for determining gut microbiota–host cometabolites and indoleamines in human urine. Four key gut microbiota–host cometabolites were chromatographically separated by isocratic elution, with a running time of 10 min. The linearity of this method was confirmed over different concentration ranges: 1.0–400 ng/mL for melatonin (MEL), indole-3-propionic acid (3-IPA), indole (IND), and skatole (SKT). This method was extremely sensitive and stable and hence could be successfully applied to characterize the changes in gut microbiota–host cometabolites in human before- and after-running urine. The concentrations of MEL, 3-IPA, IND, and SKT in after-running urine were 84.0 ± 9.69, 25.9 ± 3.39, 343.7 ± 36.8, and 14.6 ± 1.36 ng/mL, respectively. Moreover, the concentrations in before-running urine were 54.2 ± 5.10, 14.4 ± 1.30, 250.8 ± 14.1, and 9.43 ± 1.07 ng/mL, respectively, which showed significantly less difference in concentrations ($p < 0.05$) in before- than after-running urine. Overall, the established method could simultaneously monitor gut microbiota–host cometabolites and hence can be further applied to clinical and comprehensive pathophysiological studies.

**Keywords:** cometabolites; gut microbiota; HPLC-FLD; indoleamines; exercise



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## 1. Introduction

The gut microbiota is a large and complex population that maintains dynamic metabolic balance, participating in various physiological activities such as digestion, immunity, and metabolism in the human body. Its composition, structure, and proportion are closely related to the health status of the human body. Recent studies have shown that gut microbiota are not only related to intestinal, immune, and metabolic diseases but can even participate in regulating the gut–brain axis (also known as the brain–gut axis) function via various interconnected and interacting pathways such as the vagus nerve pathway, endocrine system, immune system, and metabolic system, affecting brain development and functional changes [1,2]. Dysfunction of intestinal flora is related to central nervous system diseases such as autism, Parkinson's disease, and emotional behavior disorder [3]. The gut microbiota participates in not only regulating physiological processes, such as host metabolism and immunity, but also functional responses of the brain–gut axis, and the mechanism of action is complex. Therefore, some researchers [2] proposed the concept of a "microbiota–gut–brain axis" to represent the complex network of communication between the gut, gut microbiota, and brain, which plays a significant role in regulating immune,

gastrointestinal, and central nervous system functions. The human gastrointestinal tract is inhabited by $1 \times 10^{13}$ to $1 \times 10^{14}$ microorganisms, which is more than 10 times that of the number of cells in our bodies and contains 150 times as many genes as our genome [4]; the gut microbiota is, therefore, often referred to as the forgotten organ [5]. In recent years, it has become more evident that our microbiome is essential to health, impacting nearly every aspect of host physiology [6,7].

Regular and appropriate exercise can reduce the incidence of metabolic and inflammatory diseases based on the study of human and animal models, and it is useful for muscle, adipose, brain, and other tissues [8]. Moreover, studies have shown that the gut and its associated gut microbiome in rodents can be influenced by exercise training [9–12]. Although the exact signaling mechanisms underlying the communication within the brain–gut microbiome (BGM) axis are yet to be completely understood, the microbial influence on tryptophan metabolism in humans has been studied intensively because it presumably affects several key physiological processes and metabolic pathways, thus playing a pivotal role in human health [13–15]. Indole is the main metabolite and a common component of human feces produced by gut bacteria from tryptophan, under the action of the enzyme tryptophanase, often detectable at concentrations up to 1100 μM [13]. Indole (IND) and its derivatives such as melatonin (MEL), indole-3-propionic acid (3-IPA), and skatole (SKT) are present in the blood, peripheral tissues, and urine [16–18], and play a significant role in many aspects, such as protecting against stress-induced lesions in the gastrointestinal tract, modulating expression of proteins and anti-inflammatory genes, and strengthening barrier properties [19,20]. Therefore, establishing a convenient method for determining the concentrations of urine indoleamines can be an essential task for monitoring the effect of stress from exercise on intestinal microflora metabolites. Moreover, it can provide insight into its clinical applications.

Presently, the common methods for determining the concentrations of indoleamines include radioimmunoassay [21], thin-layer chromatography [22], gas chromatography-mass spectra (GC-MS) [23], and UPLC-MS [24]. Compared with chromatographic techniques, immunoassay has poor selectivity toward specific indoleamines. Although GC-MS detects indoleamines sensitively, sample derivatization is a must before analysis [25]. In ordinary circumstances, adjunct issues such as incomplete reaction, formation of the side products, and/or inefficient labeling at low analyte concentrations result from derivatization. However, analysis of these indoleamine markers has been difficult because of their tendency to decompose and the need to clean up the complex biological matrix in which they are present. The proposed method in this study had a drawback in that it could not determine all four analytes (MEL, 3-IPA, IND, and SKT) simultaneously in human urine samples. Also, only a standardized method exists for determining total IND and skatole levels in plasma [26]. Usually, only MEL is determined in human urine [27]. Besides, most of the methods are complex, expensive, and time-consuming. Additionally, the low concentration of indoleamines, their tendency to decompose, and the complex biological matrix in urine samples make their detection more difficult. Generally, solid-phase extraction (SPE) methods are usually applied to purify analytes before determining indoleamines in complex samples. Therefore, these analytical restrained conditions must be considered when establishing novel analytical methods, and we should ensure that the methods are sensitive.

As a powerful green extraction technique, packed-nanofiber SPE (PFSPE) has been successfully used to extract different classes of compounds because of its low consumption of organic solvents, simplicity, high rate of recovery, easy operation, and automation [28]. Recently, the features of unusual multi-interactions from conductive polymers, including ion-exchange properties, π–π interactions, hydrogen bonding, acid–base properties, polar functional groups, and electroactivity, resulted in high extraction efficiency for polar compounds, which have attracted the attention of scholars and researchers [29]. Owing to better environmental stability and facile synthesis of polypyrrole (PPy), it is especially promising for commercial applications compared with other conductive polymers [30]. In this

study, we combined PPy to analyze indoleamines. Additionally, 5-hydroxyindole-3-acetic acid (5-HIAA), which is a gut microbiota–host cometabolite from plasma samples, was successfully extracted using polystyrene–polypyrrole (PS–PPy) nanofiber sorbents [31].

In this study, PPy nanofibers were used as solid adsorbents and were combined with SPE technology to extract INDs in urine. This study not only detected INDs in urine but also determined other compounds involved in the L-tryptophan metabolism pathway in human urine. A highly selective, sensitive, and simple chromatographic method was established for the simultaneous determination of MEL, 3-IPA, IND, and SKT in human urine. The new method was successfully applied for detecting INDs in adult male urine before and after exercise to investigate whether the levels of INDs in urine changed after exercise.

## 2. Materials and Methods

### *2.1. Reagents and Chemicals*

All chemicals and reagents were of analytical grade. MEL, 3-IPA, IND, and SKT standards, internal standard of indoleamines (IS, 1-naphthol), sodium chloride (NaCl), calcium chloride ($CaCl_2$), urea, hydrochloric acid (HCl), sulfuric acid ($H_2SO_4$), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), methanol ($CH_3OH$, HPLC-grade), and sodium dihydrogen phosphate ($NaH_2PO_4$) were purchased from the Aladdin Company (Shenzhen, China; www.aladdin-e.com (accessed on 11 May 2022)). PPy nanofibers were obtained from Dong-qi Bio-Technology Co., Ltd. (Shenzhen, China; www.aladdin-e.com (accessed on 11 May 2022)).

Further, a 100 μg/mL stock solution was prepared by dissolving 1.0 mg of each indoleamine and internal standard (weighed in powder form) in 10 mL of distilled water at room temperature (25 °C) and stored in a brown flask at 4 °C for later use. The artificial urine was prepared using the method proposed by Zhao [32]. The artificial urine consisted of 19.4 mg/mL urea, 8.0 mg/mL NaCl, 1.1 mg/mL $MgSO_4 \cdot 7H_2O$, and 0.6 mg/mL $CaCl_2$, and the solution was adjusted to pH 4.0 with 6.0 mol/L HCl.

PPy was coated on electrospun PS by in situ polymerization with $FeCl_3$ as an oxidant, together with $Cl^-$ as a dopant. A piece of nanofiber mat was immersed in ethanol/water (1:1, *v*/*v*) solution. After rinsing, the mat was immersed in a 0.04 M aqueous solution of pyrrole. The polymerization of pyrrole and the deposition of PPy coatings on the PS nanofibers at room temperature were initiated by the addition of the same volume of aqueous solution of $FeCl_3$ (0.084 M). The mixture was shaken vigorously for 1 h. The PS nanofibers changed appearance from white to dark as a result of the coating of the black PPy during the polymerization. In the ultrasonic method, the mixture was put into an ultrasonic cleaning basin (40 KHz, output power 400 W) for 1 h. The fiber mats with the PPy coating were then washed with distilled water several times and were finally dried in a vacuum prior to use.

### *2.2. Characterization*

Three PPy and PS nanofibers samples were taken and treated with gold spraying; then, the morphological images of the PPy and PS nanofibers samples were obtained by using the scanning electron microscope (SEM, Hitachi S-3000N; www.hitachi.co.jp, accessed on 10 May 2017, Japan) at 20 kV and a transmission electron microscope (TEM, JEM-2010 microscope; www.jeol.co.jp, accessed on 12 May 2017, Japan).

### *2.3. Sample Preparation*

Forty-five patients were enrolled in the physical education institute in this study. All patients signed a written consent. Further, the ethics committee of the Zhongda Hospital affiliated to Southeast University, China approved this study. All recruited patients were men, aged 22 ± 3 years, who lived in similar living and training environments and provided urine before and after running. The urine samples were immediately frozen at −20 °C, brought to the laboratory, and stored at −80 °C.

### *2.4. Sample Pretreatment and Packed-Nanofiber SPE of Indoleamines*

When analyzing samples, the stored urine samples were thawed and centrifuged at 12,000 rpm for 3 min and the urine samples were acidified by adding HCl (HCl:urine, 1:1, *v*/*v*). Prior to the SPE procedure, the SPE columns were packed with 5.0 mg of nanofibers as the sorbents at the tips' ends. Further, 200 µL of methanol and 200 µL of water were used to activate the nanofibers in the extraction column. Also, 500 µL of the sample was added to the PFSPE column after being activated, and then the pressure handle of the PFSPE extraction array was rotated to push the fluid through the nanofibers (Figure 1). The analytes were eluted with 100 µL of the mixture comprising 95% methanol and 5.0% 0.2 mol/L $H_2SO_4$. The eluent was analyzed using HPLC-FLD.

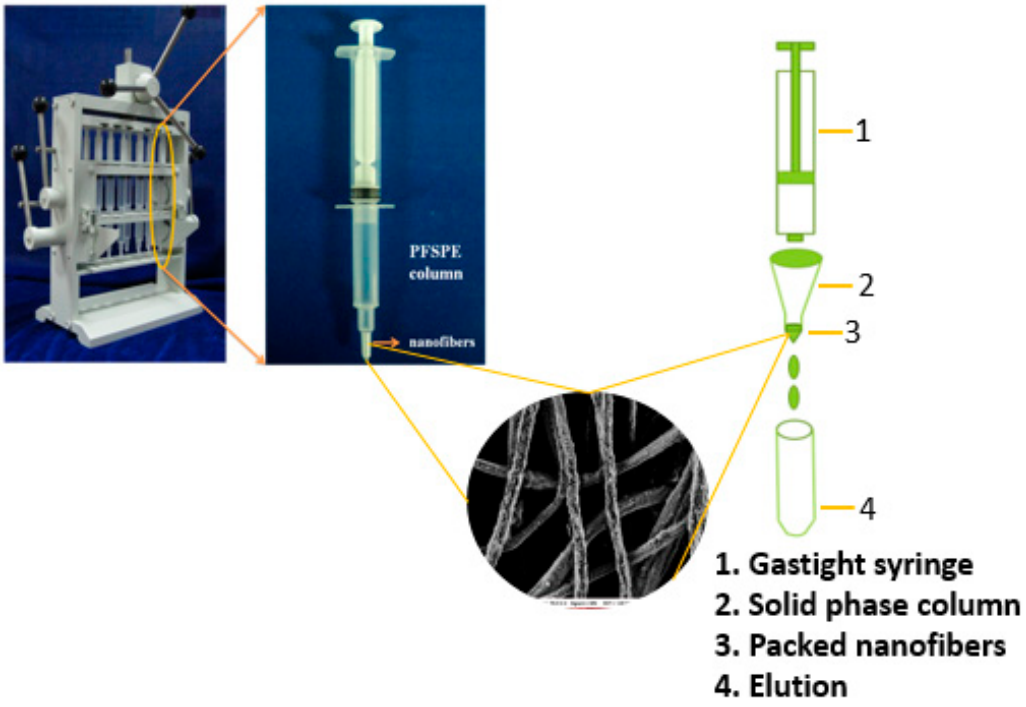


**Figure 1.** Schematic representation for the extraction solid-phase column and analysis of indoleamines in urine samples based on homemade PFSPE columns.

### *2.5. Chromatographic Analysis*

The target indoleamines were processed by the Shimadzu LC-20A HPLC system (Shimadzu, Kyoto, Japan) equipped with a degassing device, a quaternary pump, a 100 µL sample loop, and a fluorescence detector (FLD, RF-10AXL, Kyoto, Japan). A C18 reversed-phase column (250 mm × 4.6 mm, 5 µm) (Shimadzu) was used for separating the analytes. A mixture of $CH_3OH$ and 15 mmol/L $NaH_2PO_4$ at a volume ratio of 60:40 was employed as the mobile phase at a flow rate of 1.0 mL/min. The excitation and emission wavelengths of the FLD were set at 274 and 340 nm, respectively.

## 3. Results

### *3.1. Morphology of PS and PPy Nanofibers*

The SEM images of three PS and PPy nanofiber samples are illustrated in Figure 2a,b, respectively. The quantity of the individual nanofibers is measured(35), and the average diameter of PPy nanofibers is rougher than that of PS nanofibers. Moreover, PPy nanofibers (Figure 3b) exhibited alternately dark and bright spots, depicting numerous nanopores, compared with PS nanofibers (Figure 3a). The porosity on the surface of composite nanofibers significantly improved the surface area, enhancing the extraction ability for targets.

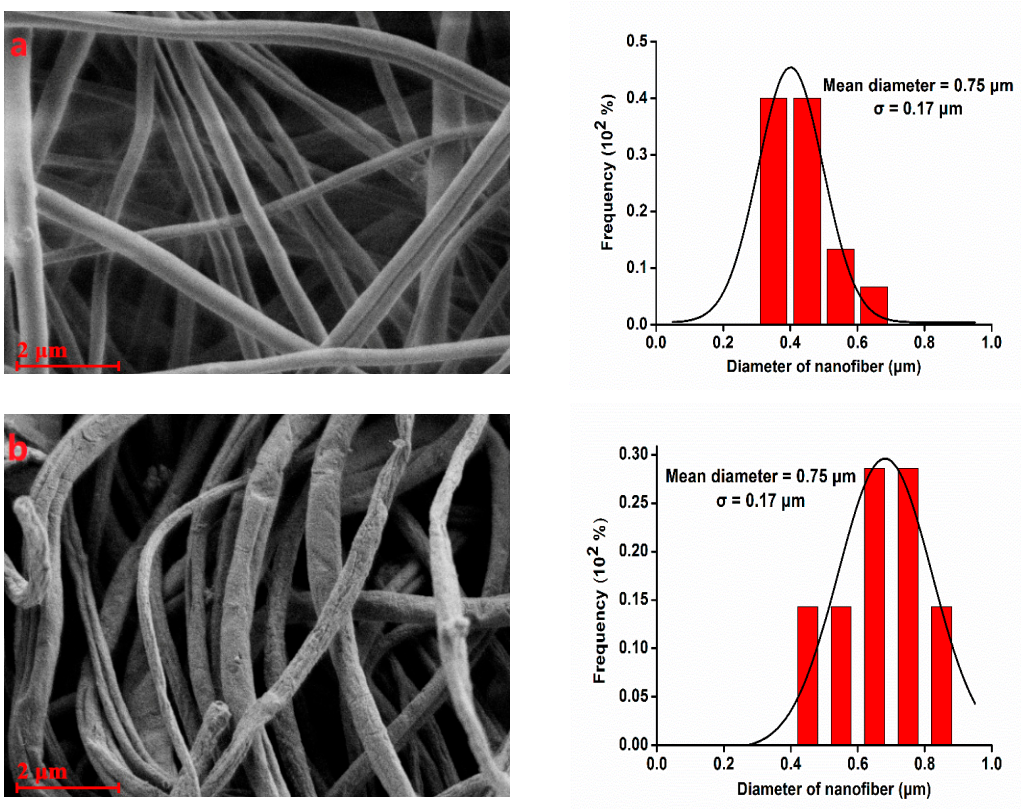


**Figure 2.** SEM images of (**a**) PS and (**b**) PPy electrospun nanofibers.

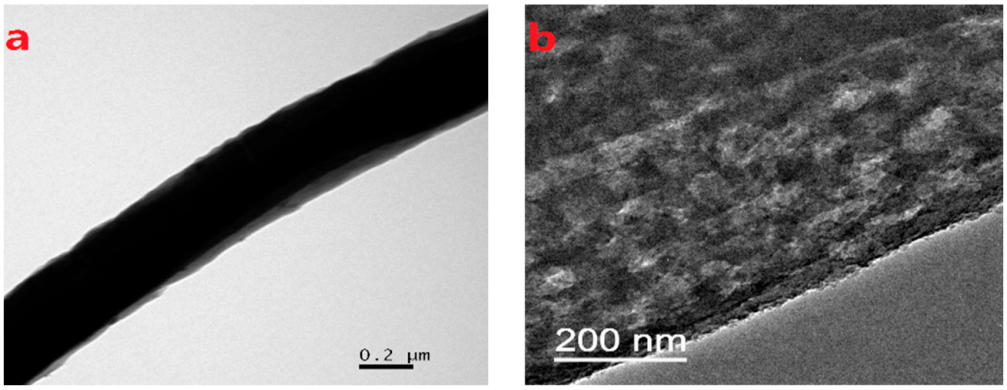


**Figure 3.** TEM images of (**a**) PS and (**b**) PPy electrospun nanofibers.

### *3.2. Optimization of Extraction Parameters*

The influence of salt concentration, different batches, the concentration of methanol in the eluent, and amounts of the PPy nanofibers on extraction were optimized, and the analytical characteristics of the developed PFSPE-HPLC-FLD method were evaluated.

#### 3.2.1. Effect of Salt Concentration on the Extraction

The influence of salt on the extraction efficiency of indoleamines using PFSPE was investigated by adding various amounts of NaCl (ranging from 0% to 36%, *w*/*v*). Figure 4a shows the extraction efficiency in terms of the NaCl concentrations and reveals that the addition of NaCl has an influence on the extraction efficiency of IND, and SKT, but there is no significant influence on the extraction efficiency of MEL and IPA.

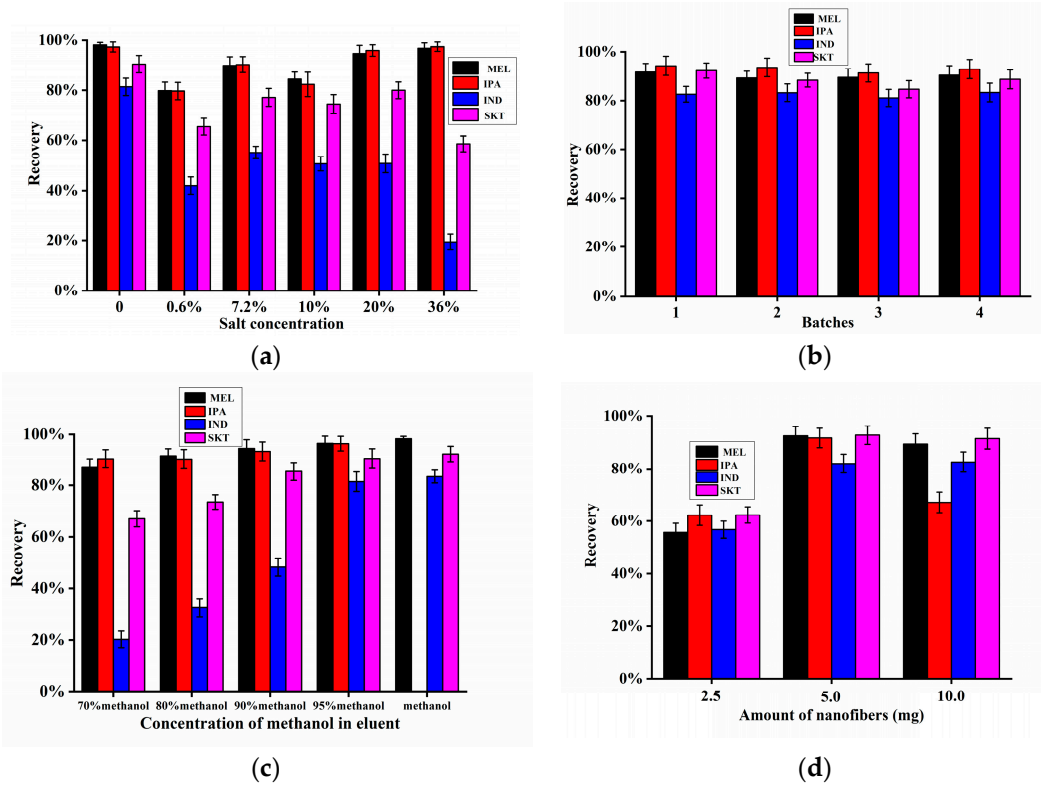


**Figure 4.** Influence of the different parameters on extraction recovery: (**a**) salt concentration, (**b**) different batches, (**c**) concentration of methanol in the eluent, and (**d**) amount of nanofibers (mixed standard solution containing 100 ng/mL of MEL, 3-IPA, IND, and SKT, and 2.5 µg/mL IS with pretreatment by PFSPE).

#### 3.2.2. Effect of Different Batches on the Extraction

The variations in the recoveries of analytes were studied with sorbents prepared in different batches. PPy nanofibers prepared in three independent batches exhibited almost reproducible recoveries (Figure 4b). It is clearly indicated that PPy nanofibers prepared in different batches were highly reproducible and maintained their extraction characteristics.

#### 3.2.3. Effect of the Concentration of Methanol in Eluent on the Extraction

A high or low concentration of $H_2SO_4$ in a mixed solution is not suitable for elution. The ratio of $CH_3OH$ and $H_2SO_4$ was to be kept at a suitable level to increase the efficiency of elution. The results indicated that the extraction efficiency increased with the increase in $CH_3OH$ concentration and reached a maximum in the presence of 95% (Figure 4c), but 3-IPA could not be eluted using pure $CH_3OH$. Hence, 5% 0.2 mol/L $H_2SO_4$ in methanol aqueous solution was used for elution for all experiments in this study.

#### 3.2.4. Effect of the Amount of Nanofibers on the Extraction

The amount of PPy nanofibers is also an important parameter that affects the recovery rate of the target substance. Usually, the amount of the adsorbent should be kept large enough to extract as many analytes as possible. The optimization experiments were conducted with different amounts of fiber materials to determine the amount of nanofibers. When the nanofiber material increased from 2.5 to 10 mg, the extraction recovery rate also increased with the increase in solid adsorption dose (Figure 4d). As the amount of solid adsorption material increased from 5.0 to 10 mg, the extraction recovery rate decreased with the increase in solid adsorption material.

### 3.3. Method Validation

Under the optimum (no salt added, 5% 0.2 mol/L $H_2SO_4$ in $CH_3OH$ aqueous solution, 5.0 mg nanofibers as sorbent) conditions, figures of merit of the method including the LOD, the linear range, repeatability, limits of detection (LODs), limits of quantification (LOQs), and recoveries are received. Analytical data obtained are summarized in Table 1. The linearity of the method was confirmed over the concentration range of 1.0–400 ng/mL for MEL, 3-IPA, IND, and SKT, respectively. The LOD of melatonin, indole-3-propionic acid, indole, and 3-methylindole were 0.28 ng/mL, 0.59 ng/mL, 0.55 ng/mL, and 0.37 ng/mL, respectively. The standard recovery rates for low, neutral, and high concentrations of indole compounds range from 96.3% to 99.5%, 97.3% to 99/3%, 81.3% to 91.3%, and 89.3% to 93.8%. The correlation coefficients for all indoleamines are greater than 0.991, which shows a good linear correlation between concentration and peak areas. These figures of merit indicate that this method has excellent stability, reliability, and satisfactory sensitivity.

**Table 1.** Linear range, LOD, LOQ, recovery, and precision of indoleamines.

| Analytes | Calibration Range (ng/mL) | $R^2$ | LOD | LOQ | Recovery (%) ± RSD ($n$ = 5) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Within-day | | | Between-day | | |
| | | | | | Spiked Concentration (ng/mL) | | | | | |
| | | | | | 10 | 25 | 100 | 10 | 25 | 100 |
| MEL | 1–400 | 0.998 | 0.28 | 0.94 | 98.3 ± 4.4 | 99.3 ± 2.2 | 99.5 ± 1.8 | 96.3 ± 3.7 | 98.7 ± 3.1 | 99.1 ± 2.9 |
| 3-IPA | 1–400 | 0.991 | 0.59 | 1.98 | 97.7 ± 3.3 | 97.3 ± 3.1 | 98.2 ± 2.8 | 97.3 ± 2.3 | 98.2 ± 2.3 | 99.3 ± 2.1 |
| IND | 1–400 | 0.996 | 0.55 | 1.84 | 84.3 ± 4.2 | 87.3 ± 3.7 | 91.3 ± 3.2 | 81.3 ± 3.8 | 86.8 ± 3.7 | 89.9 ± 3.0 |
| SKT | 1–400 | 0.991 | 0.37 | 1.25 | 91.3 ± 3.8 | 92.3 ± 3.1 | 93.8 ± 2.9 | 89.3 ± 3.2 | 91.4 ± 2.4 | 92.6 ± 1.9 |

### 3.4. Sample Analysis

To evaluate the applicability of the method developed, real urine samples were analyzed. Under the optimized conditions, urine was dealt with using PS-PPy nanofibers. Figure 5c describes the chromatogram of the urine sample without any pretreatment, and Figure 5a,b depicts the chromatograms of the spiked urine and urine sample after the extraction with the PFSPE column packed with PS-PPy nanofibers. The results show that not only can good extraction effects for the indoleamines targets be obtained by the PFSPE column but it can also eliminate the interfering substances, reflecting a good characteristic of selective extraction of the targets. Furthermore, there is a good separation between the impurities and the targets under the chromatographic conditions in this experiment. The results suggest that the method is applicable to the quantitative analysis of indoleamines in human urine samples.

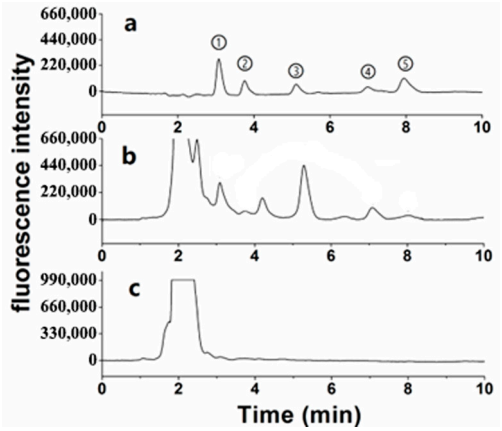


**Figure 5.** The chromatograms of artificial urine samples containing: ① MEL (50 ng/mL), ② 3-IPA (50 ng/mL), ③ IND (50 ng/mL), ④ IS (2.5 μg/mL), and ⑤ SKT (50 ng/mL) standard substances with pretreatment by PFSPE (**a**); urine with pretreatment by PFSPE (**b**); urine without pretreatment (**c**).

The concentrations of MEL, 3-IPA, IND, and SKT in the after-running urine are 84.0 ± 9.69 ng/mL, 25.9 ± 3.39 ng/mL, 343.7 ± 36.8 ng/mL, and 14.6 ± 1.36 ng/mL and 54.2 ± 5.10 ng/mL, 14.4 ± 1.30 ng/mL, 250.8 ± 14.1 ng/mL, and 9.43 ± 1.07 ng/mL in before-running urine. Hence, the concentrations of MEL, 3-IPA, IND, and SKT in the after-running urine are significantly ($p < 0.05$) higher than in the before-running urine. The significant increase in indoleamine concentration in the after-running urine, as shown in Figure 6, shows that there appears to be a change of indoleamine concentration in intestinal flora metabolites in the athletes' bodies after running.

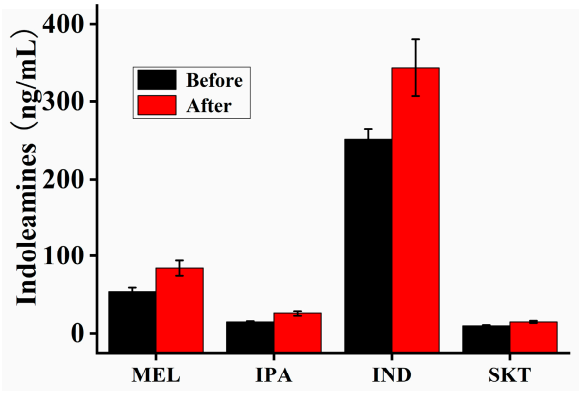


**Figure 6.** Mean indoleamine levels in before-running urine and after-running urine.

## 4. Discussion

As shown in the Figure 7, the target indole compounds to be tested contain benzene rings, hydroxyl groups, and carboxyl groups, with strong polarity and easy solubility in water, which increases the difficulty of extraction. PS has a benzene ring structure, and PPy is a five-membered heterocyclic compound containing a nitrogen heteroatom. Therefore, there are π-π and hydrogen-bonding interactions between the adsorbent component PS-PPy nanofibers and the target material; in addition, the PS component of nanofibers contain π bonds, which can also effectively adsorb benzene ring compounds through π-π interactions. As the benzene ring increases, the force will increase. In summary, it can be seen that the multiple interactions between the adsorbent and the target substance result in better extraction efficiency in sample pretreatment.

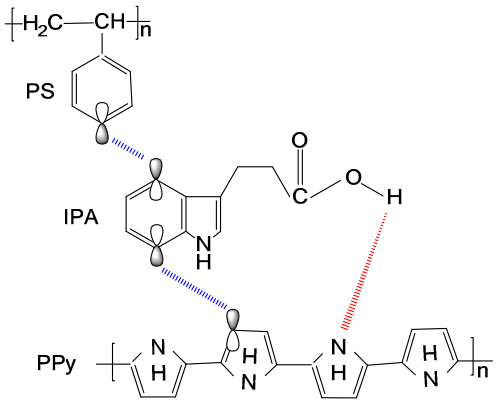


**Figure 7.** Diagram of the interaction between 3-IPA and PS-PPy nanofibers. The blue means H-H covalent bond and the red means N-H covalent bond.

In addition, composite nanofibers have a large specific surface area, and the pores on the surface of composite nanofibers can also provide a platform for increasing the interaction sites between the target substance and the adsorbent. Therefore, a small amount

of nanofibers can adsorb the target substance in the sample matrix, improving the extraction efficiency of nanofibers for the target substance.

Salt is not added to the solution in the subsequent experiments. The reason is attributed, perhaps, to the inhibition of the mass transfer effect by the increased viscosity of the sample, leading to changes in the physical properties of the diffusion layer near the organic film, which could reduce the diffusion rate of the target analyte into the membrane and sorbent. Consequently, salt is not added to the solution in the rest of the experiments. The amount of PS-PPy nanofibers played a vital role in the recovery of indoles. If there are insufficient nanofibers, the target analytes cannot be completely adsorbed; however, excessive nanofibers may lead to incomplete elution. MEL, IND, and SKT exist as molecules in an acidic medium that can be eluted from the surface of PS-PPy nanofibers using a $CH_3OH$-$H_2SO_4$ mixed solution. In this work, strong polar organic compounds such as indoles have strong interactions with PPy nanofibers, which may be caused by the anion exchange performance of the polypyrrole itself and the π-π bond interaction between polypyrrole and the target material. According to existing literature reports, methanol has weaker polarity compared to water, but it also has hydroxyl groups that can compete with polar target substances for adsorption sites on the adsorbent; thus, the target substance from the solid-phase adsorption material can be eluted. Because MEL, IND, and SKT exist in molecular form, although they have multiple interactions with PPy at this time, as the methanol ratio increases, they can still be eluted more from the adsorption material. For 3-IPA, the elution rate of pure methanol decreased sharply, suggesting that there is COOH in its molecule, which has a strong interaction with PPy in the form of ions under neutral conditions, while methanol cannot elute. However, it can exist in molecular form under acidic conditions, and the interaction with nanofibers is actually weakened. Therefore, it can be eluted off the surface of PPy nanofibers using an acidic methanol aqueous solution. Therefore, an aqueous solution of a mixture of 0.2 mol/L $H_2SO_4$ and 95% $CH_3OH$ was ultimately selected as the target eluent.

Indole, as the main metabolite of tryptophan, is produced by gut bacteria through the action of tryptophanase and is also a component of gut microbiota metabolites. Tryptophan metabolites such as melatonin (MEL), indole-3-propionic acid (3-IPA), indole (IND), and 3-methylindole (SKT) are present in the blood, peripheral tissue, and urine. These derivatives play an important role in many aspects, such as protecting the gastrointestinal tract from irritability damage and regulating protein expression and antitumor activity. Clark et al. [33], through their research on the exercise ability of horses, found that supplementing glucose during exercise or supplementing tryptophan before exercise can significantly prolong or shorten their average exhaustion time. However, there are almost no reports of changes in the other metabolites of tryptophan or indoles after exercise. In the study of this chapter, the results indicate that there is a change in the concentration of indoleamine, a metabolite of the gut microbiota, in athletes after running: the concentration of indoleamine in urine significantly increases after running. Based on the metabolic pathway of tryptophan, it is speculated that exercise promotes the metabolism of tryptophan to produce indoles.

## 5. Conclusions

In this work, a more convenient technique for sample pretreatment is proposed for the simultaneous extraction and detection of indoleamines by diminutive homemade PFSPE equipment. The linearity of the method is confirmed over the concentration range of 1.0–400 ng/mL for melatonin (MEL), indole-3-propionic acid (3-IPA), indole (IND), and skatole (SKT), respectively. This method is extremely sensitive and stable and it is successfully applied to characterize the changes of gut microbiota–host cometabolites in human urine, before and after running. The concentrations of MEL, 3-IPA, IND, and SKT in after-running urine are 84.0 ± 9.69 ng/mL, 25.9 ± 3.39 ng/mL, 343.7 ± 36.8 ng/mL, and 14.6 ± 1.36 ng/mL and 54.2 ± 5.10 ng/mL, 14.4 ± 1.30 ng/mL, 250.8 ± 14.1 ng/mL, and 9.43 ± 1.07 ng/mL in before-running urine, showing significantly ($p < 0.05$) lower

values in before-running urine than in after-. The PS–PPy nanofibers with comforting mechanical properties and chemical characteristics, such as good stability, strong adsorb ability, and environment-friendly, were synthesized by in situ polymerization of pyrrole on the surface of PS nanofibers, and the novel strategy based on PFSPE coupled with HPLC exhibited excellent analysis performances for indoleamines in urine. It was applied to the measurement of indoleamines in human urine samples; therefore, this method may be be applied to be a valuable tool for clinical researches and comprehensive studies of the pathophysiological roles.

**Author Contributions:** X.K. conceived the research. L.W. performed the research, analyzed the data, wrote the manuscript, and designed the experiments. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki, and approved by the ethics committee of Zhongda hospital with 2015ZDKYSB031.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** Data is contained within the article.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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