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A novel extraction method for three ochratoxins in human urine based on polystyrene/polyethersulfone electrospun nanofibers coated with copper nanoparticles†

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In this study, four types of nanofibers were prepared *via* electrospinning and characterized through SEM, TEM, EDS, FTIR, TG, XPS and water contact angle analyses, and the novel polystyrene/polyethersulfone nanofibers coated with copper nanoparticles (PS/PES-CuNPs nanofibers) were developed as an ideal adsorbent for the extraction of three ochratoxins from human urine. The solid-phase extractant of sample pretreatment displayed preferable sensitivity and an extraction effect, and the analytical method based on the novel packed-fiber solid-phase extraction strategy followed by high performance liquid chromatography-fluorescence detection (PFSPE-HPLC-FLD) achieved an exceedingly low limit of detection (LOD) and limit of quantification (LOQ) of 0.108–0.162 μg L^{-1} and 0.658–0.701 μg L^{-1}, respectively; a high spiked recovery of 71.3–92.0% and a lower adsorption time of 7 min, thus demonstrating excellent results compared with other reported adsorbents for ochratoxins from various samples. With the application of this method for the detection of ochratoxins in human urine samples, six in thirty samples were tested positive. This study confirmed that the PS/PES-CuNP nanofibers and PFSPE showed promising potential as a sensitive method for simultaneous extraction and detection of ochratoxins in complex samples.

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1 Introduction

As a highly toxic substance of the mycotoxin family, ochratoxins (OTs, including OTA, OTB and OTC) have been widely monitored since they were first reported in Nature in 1965.[1] Particularly, OTA was classified as a possible human carcinogen (Group 2B) in 1993 by the International Agency for Research on Cancer because of its strong nephrotoxicity, hepatotoxicity, immunotoxicity, teratogenicity, carcinogenicity, genotoxicity, and neurotoxicity.[2–4] Frequent exposure to OTs can lead to a range of health issues in animals and humans. Research has confirmed that ochratoxins have a causal relationship with Balkan endemic nephropathy (BEN).[5,6] Notably, ochratoxins are directly excreted in urine instead of being removed through glomerular filtration.[7] Therefore, the task of establishing a precise and effective analytical method for ochratoxin detection in human urine is imminent.

Owing to the complicated matrix of urine and the trace concentration of OTs, direct detection would affect the sensitivity and accuracy of analytical methods. Therefore, it is necessary to pretreat the samples before instrumental analysis. The purpose is to separate and enrich analytes and reduce matrix effects and interference in sample solution.[8] Currently, QuEChERS,[9] liquid–liquid extraction,[10,11] and immunoaffinity columns[12,13] have been reported to extract ochratoxins. However, selecting an appropriate pretreatment method is crucial for the enrichment of ochratoxins. Solid-phase extraction (SPE) is the best choice for sample pretreatment for its advantages of high selectivity, low cost, and simple preparation.[14] Generally, some nanomaterials with high structural stability, such as metal organic frameworks, molecular imprinting materials or carbon nanotubes, are utilized as SPE adsorbents. However, secondary pollution may arise in the extraction process because of their weak separation ability.[15,16] Therefore, the development of new adsorption materials for SPE has been one of the most challenging subject in analytical science.[17]

Polymer nanofibers stand out among other adsorbents as they exhibit superior characteristics, including exceptional

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mechanical and thermal stability.[18] Research has demonstrated that nanofibers are beneficial as adsorbents, since they possess a diameter size of submicrometers or even nanometers, resulting in superior specific surface area and porosity and exhibiting satisfactory adsorption capacity.[19,20] Furthermore, during the extraction process, nanofibers do not leak or accumulate in the SPE column and can be easily packed into the column.[21] Additionally, electrospinning is emerging as a preferable method for synthesizing nanofibers due to its simplicity of operation, convenience, and controllable process parameters. In addition, the functionalized polymer nanofibers can be promoted by chemical modification, developing optimal adsorbents with great selectivity for the targets.[22] It is reported that the detection of OTs by Au and Ag nanoclusters has been widely utilized, but copper nanoclusters have rarely been investigated.[23,24] Considering the adjacent positions and similar effects of copper, gold, and silver, it is also feasible that copper nanoparticles were introduced in electrospun nanofibers for the extraction of OTs.[25] Combining electrospun nanofibers with copper nanoparticles can effectively improve the adsorption performance of nanofibers, since more adsorption sites are introduced when the specific surface area of the adsorbent is enhanced.[26,27] Therefore, the proposed modification methods are expected to have satisfactory adsorption efficiency.

The emerging packed-fiber solid-phase extraction (PFSPE) method is a solid-phase extraction technology that integrates extraction, enrichment, purification, and separation, greatly simplifying the steps of sample pretreatment.[28] In this study, new polystyrene/polyethersulfone nanofibers coated with copper nanoparticles were prepared as a selective adsorbent for extracting ochratoxins from human urine samples. An analytical method based on the novel PFSPE followed by high performance liquid chromatography-fluorescence detection (PFSPE-HPLC-FLD) was established for simultaneous determination of three ochratoxins in human urine.

2 Materials and methods

2.1 Materials and reagents

Ochratoxin A (OTA, $C_{20}H_{18}ClNO_6$, purity 98%, Mw = 403.81), Ochratoxin B (OTB, $C_{20}H_{19}NO_6$, purity 98%, Mw = 369.37), Ochratoxin C (OTC, $C_{22}H_{22}ClNO_6$, purity 98%, Mw = 431.87), analytically pure sodium borohydride, copper acetate, and sodium chloride were purchased from Shanghai Aladdin Company. Polystyrene (PS, $[CH_2CH(C_6H_5)]_n$, Mw = 18 500 Da) and polyethersulfone ($C_{24}H_{18}Cl_2O_6S_2$, purity ≥ 99%) were obtained from the Department of Applied Chemistry, Nanjing University of Technology. Polyacrylonitrile (PAN, $(C_3H_3N)_n$, Mw = 150 000) and analytically pure glacial acetic acid were purchased from Shanghai McLean Company. Polyvinylpyrrolidone (PVP) K30, *N*,*N*-dimethylformamide (DMF) and hydrochloric acid (analytically pure) were purchased from Shanghai National Pharmaceutical Group. Chromatographically pure methanol and acetonitrile were purchased from Tedia Corporation in the United States.

The preparation of artificial urine was based on previous research[29] and slightly modified. The detailed preparation plan can be found in Part 1 in ESI.†

2.2 Instruments and reagents

The following instruments were utilized: Fourier transform infrared spectrometer (FTIR 5700, Thero Electron Co., Waltham, USA), scanning electron microscope (SEM 8100, Tokyo, Japan), transmission electron microscope (TEM FEI Talos-S, Oregon, America), energy dispersive spectrometer (EDS, Oregon, America), Waters e4695 separation unit and Waters 2475 fluorescence detector (Waters Co., Milford, America), 4.6 × 150 mm 5 μm InertSustain C18 column (GL Sciences, Tokyo, Japan), syringe pump (Hawk Medical Instrument Co., Ltd, Shenzhen, China), and high voltage direct current power (Dongwen High Voltage Power Supply Co., Ltd, Tianjin, China).

2.3 Preparation of nanofibers

The nanofibers were prepared by electrospinning. Firstly, 0.5 g each of PS, PES, and copper acetate were dissolved in 8.5 mL of DMF. A uniform polymer solution for electrospinning was obtained by stirring the blend continuously for 10 h at room temperature. After, the solution was transferred into a 10 mL syringe with a 23 gauge stainless steel needle. The electrospinning parameters were set as follows: a receiving distance of 20 cm from needle tip to the collection screen, 19 kV of high voltage, and 1.5 mL h^{-1} flow rate of the spinning solution. The entire electrospinning process was carried out at 25 °C and 35% relative humidity environment. Then, 50 mg of PS/PES-Cu^{2+} composite nanofibers obtained on the collection screen were soaked in 20 mL of fresh aqueous solution of $NaBH_4$ (1.0 mol L^{-1}) for 48 hours at room temperature. The surface color of the nanofibers gradually changed from light blue to brown-black. After the reaction, the fibers were washed with ultrapure water three times and dried in the constant temperature drying oven at 50 °C for 20 min. Finally, 10% (w/v) PS/5% (w/v) PES-5% (w/v) CuNP nanofibers were obtained, and, similarly, 10% (w/v) PS-5% (w/v) CuNP nanofibers, 5% (w/v) PS/5% (w/v) PVP-5% (w/v) CuNP nanofibers, and 5% (w/v) PS/5% (w/v) PAN-5% (w/v) CuNP nanofibers were also prepared with PS, PVP, PAN, and copper acetate by the same operation.

2.4 Sample collection

The morning urine samples of 30 participants, including 15 females and 15 males, all aged between 24 and 25 years old, living in Nanjing (China), were collected. Consent for sampling was obtained from all participants.

The sample processing method was based on previous research[29] slightly modified. The collected samples were stored at −20 °C for future use. Before analysis, the sample was thawed, then frozen and thawed again until it reached room temperature. It was centrifuged at a speed of 12 000 rpm min^{-1} for 15 min and the supernatant was retained for subsequent PFSPE.

2.5 Nanofiber solid-phase extraction

In the SPE experiment, 5.0 mg of nanofibers were weighed and installed into a specially designed SPE column using a steel rod (1.0 mm diameter) to prepare PFSPE columns. The PFSPE column was assembled onto a semi-automatic SPE processor which can pretreat 12 samples simultaneously.

After that, the fibers in PFSPE columns were activated by 150 μL each of methanol and water, separately. For extraction, 500 μL of standard solution or sample supernatant was added to the PFSPE column and uniformly pushed out of the column at a speed of 4–5 seconds per drop by rotating the pressure rod to drive the booster. The targets were captured by the filled adsorbent when the solution passed through the PFSPE column. After the adsorption process completed, 100 μL of MeOH/ACN/HAc/H_2O (40 : 50 : 5 : 5, v/v/v/v) was used to elute the target adsorbed on the nanofibers. Finally, the eluate was treated with a 0.22 μm filter membrane and then injected into the HPLC for further analysis. The process of PFSPE of the three ochratoxins is shown in Fig. 1.

2.6 HPLC analysis conditions

The analysis of the targets was accomplished on a Waters 2695 high-performance liquid chromatograph equipped with a Waters 2475 fluorescence detector. The separation of the target substance was performed on an InsertSustain C18 column (150 mm × 4.6 mm, 5 μm). HPLC operation conditions were set as follows: mobile phase: 1% acetic acid water : acetonitrile (45 : 55, v/v); elution method: isocratic elution; flow rate: 1.0 mL min; column temperature: 40 °C; injection amount: 20 μL. The optimal fluorescence responses of the three ochratoxins were obtained by setting excitation wavelength and emission wavelength to 333 nm and 460 nm, respectively.

2.7 Method validation

2.7.1 Linearity, LOD, and LOQ. The series standard working solutions were made by adding three ochratoxins to blank artificial urine and were used to construct standard curves. Each point on the calibration curve corresponds to the average value of three independent repeated experiments. The detection limit (LOD) and quantification limit (LOQ) of each target were defined as the concentrations corresponding to signal-to-noise ratios of 3 and 10,[30] respectively.

2.7.2 Recovery. The recovery and intra-day and inter day precision were tested by the blank sample spiking method. The intra-day precision was evaluated by the relative standard deviation of the high, medium, and low concentration levels for 1 day, and the inter day precision was evaluated by the relative standard deviation of the results obtained from continuous measurements for 5 days.

The extraction recovery was evaluated by comparing the total targets of the elution solution and that of the sample solution before PFSPE.[14] The equation of recovery was as follows,

$$\text{Recovery}(\%) = \frac{C_f \times V_f}{C_i \times V_i} \times 100\% \quad (1)$$

where C_i and V_i are the added concentration (μg L^{-1}) of OTs and the volume of spiked solution before PFSPE treatment, respectively, and C_f and V_f are the obtained concentration (μg L^{-1}) of OTs and the volume of eluent after PFSPE treatment, respectively.

3 Results and discussion

3.1 Characterization of nanofibers

The morphology of the nanofibers was studied by SEM and TEM. As shown in Fig. 2, SEM images of all four nanofibers showed good fiber morphology at a submicron level. The copper nanoparticles synthesized on the surface of the nanofibers exhibited a uniform particle state without aggregation. This property of nanofibers was speculated have significant impact on the extraction efficiency of OTs.[25]

The elemental content of the composite nanofibers was measured by EDS spectroscopy analysis. As shown in Fig. 3, the EDS spectra of four fibers confirmed the successful synthesis of copper nanoparticles on the surface of the nanofibers. The EDS spectrum of PS/PES-CuNP nanofibers showed a higher presence of Cu element than the other nanofibers, which might be closely related to the high adsorption efficiency of this adsorbent.[25]

FT-IR spectroscopy also confirmed the successful incorporation of PVP, PAN and PES into the nanofibers, as shown in Fig. 4A. The four types of nanofibers exhibited spectral bands around 2960 and 1647 cm^{-1}, corresponding to the C–H stretching vibration and the C=O tensile vibration,[31] respectively, which is a characteristic of PS. For the PS/PES-CuNP nanofibers, the peaks at 1090 and 1150 cm^{-1} were attributed

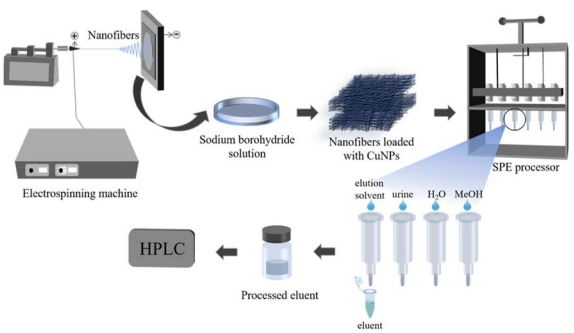

Fig. 1 Schematic illustration of PFSPE process and detection of OTs.

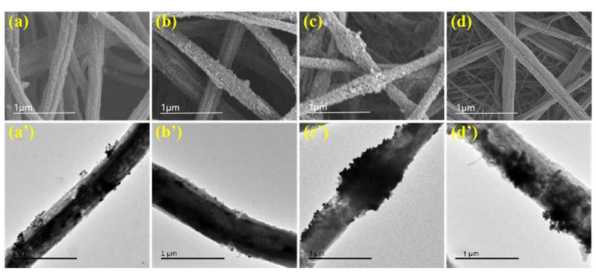

Fig. 2 SEM and TEM images of PS-CuNP nanofibers (a and a'), PS/PVP-CuNP nanofibers (b and b'), PS/PAN-CuNP nanofibers (c and c') and PS/PES-CuNP nanofibers (d and d').

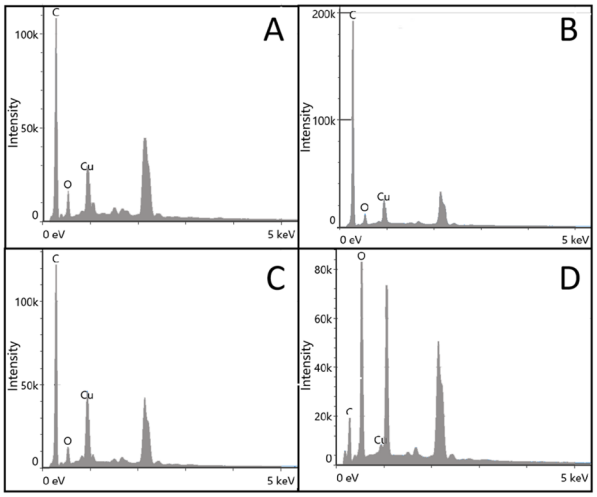

Fig. 3 EDS spectra of PS-CuNP nanofibers (A), PS/PVP-CuNP nanofibers (B), PS/PAN-CuNP nanofibers (C) and PS/PES-CuNP nanofibers (D).

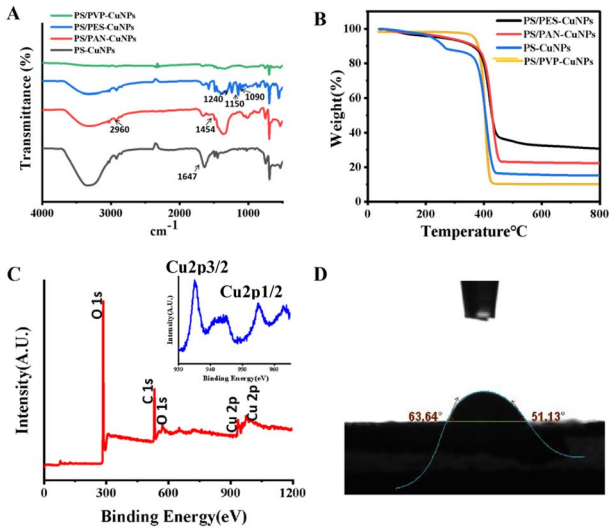

Fig. 4 (A) FTIR spectra of PS-CuNPs, PS/PVP-CuNPs, PS/PAN-CuNPs and PS/PES-CuNP nanofibers. (B) Thermal weight loss curves (TG) of PS-CuNPs, PS/PVP-CuNPs, PS/PAN-CuNPs and PS/PES-CuNP nanofibers. (C) X-ray photoelectron spectra (XPS) of PS/PES-CuNP nanofibers, and the inset is the Cu2p spectrum. (D) Contact angle measurement (CA) of water droplet on the surface of PS/PES-CuNP nanofibers.

to the absorption peaks of C=O=C and its related groups,[32] and the peak at 1240 cm^{-1} was attributed to the C–O vibration caused by the C–O–C related groups adjacent to the benzene ring.[33] In the spectrum of the PS/PAN-CuNP nanofibers, the characteristic absorption peak at 1454 cm^{-1} is attributed to the C–H stretching vibration.[34] The spectra of PS/PVP-CuNP nanofibers exhibited significant peaks at 1289 cm^{-1} caused by the vibration of the C–N bonds in the PVP material.[30]

As shown in Fig. 4B, with the increase of temperature, the weight loss of the four nanomaterials gradually increased as the coordinated water molecules were removed. The quick weight loss at 420 °C of the four nanofibers is attributed to the collapse of the CuNPs structure.[25] When the temperature was 800 °C, the weight loss rates of the PS-CuNPs, PS/PVP-CuNPs, PS/PAN-CuNPs and PS/PES-CuNP nanofibers reached 16.1%, 7.6%, 22.4% and 30.1%, respectively. As shown in Fig. 4C, the binding energies of 527.4 and 281.7 eV correspond to the photoelectron spectra of C1s and O1s,[14] respectively, and the core-level energy photoelectron peaks of Cu2p1/2 and 2p3/2 were exhibited at 754.1 and 735.5 eV, which implies the successful synthesis of CuNPs.[25]

3.2 Evaluation of PS/PES-CuNPs as an excellent adsorbent for ochratoxins

The impact of nanofibers with different compositions on the simultaneous adsorption of ochratoxins was researched by comparing the adsorption efficiencies of PS/PES-CuNPs and PS-CuNPs, PS/PVP-CuNPs, and PS/PAN-CuNP nanofibers. Specifically, 5 mg of nanofibers filled the SPE column and 500 μL of spiked artificial urine (5 μg L^{-1} for each type of ochratoxin) was treated with the method described in Section 1.4. The concentration of ochratoxins was determined by the HPLC method described in Section 1.5. As Fig. 5 shows, the adsorption recoveries of PS-CuNPs, PS/PVP-CuNPs, and PS/PAN-CuNP nanofibers for the three ochratoxins only achieved 20.1–67.4%, while the recovery of PS/PES-CuNPs significantly increased to 71.2–78.9%. Therefore, it can be confirmed that PES and Cu modification can contribute to significant improving the adsorption efficiency of the three ochratoxins.

3.3 Optimization of conditions in the PFSPE process

3.3.1 Amount of adsorbent PS/PES-CuNPs. The adsorption mechanism of the nanofibers for the targets depends on the hydrophobic interaction and electrostatic interaction between PS/PES CuNP nanofibers and the carboxylic acid esters of the ochratoxins.[25] Therefore, the amount of nanofibers directly affected the adsorption efficiency of the PFSPE columns. As

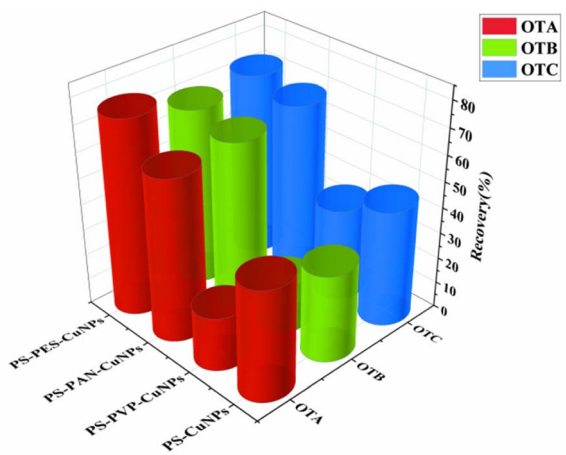

Fig. 5 Comparison of adsorption efficiencies for ochratoxins among four types of nanofibers.

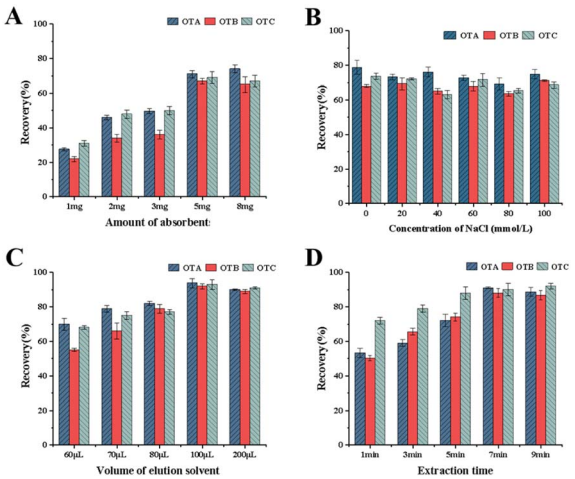

Fig. 6 Optimization of PFSPE ($n = 3$): (A) amount of absorbent, (B) ion species, (C) volume of elution solvent and (D) extraction time.

shown in Fig. 6A, when the dosage of adsorbent increased from 1 mg to 3 mg, the extraction recovery of ochratoxins significantly enhanced, indicating that a larger filling amount would improve adsorption efficiency. As the dosage of PS/PES-CuNP nanofibers increased to 5 mg and 8 mg, the recoveries of the three targets reached 69.5–73.2%, and a significant difference in adsorption efficiency between 5 mg and 8 mg of nanofibers was not seen. Based on the experimental results, 5 mg was chosen as the optimal filling amount for PS/PES CuNP nanofibers.

3.3.2 Effect of ionic strength. The ion effect in a sample solution is crucial to the adsorption efficiency. The extraction of the nanofibers to OTs could be affected by the salt concentration because of the salting-out and competition effects in sample solutions.[28] The adsorption performance of PS/PES-CuNP nanofibers was investigated with 0–100 mmol L^{-1} NaCl solution. As shown in Fig. 6B, apparent improvement of recovery for the three ochratoxins was not observed as the concentration of NaCl increased; this result revealed that PS/PES-CuNP nanofibers possess a great anti-interference property. Considering the achieved results, there is no NaCl added in the subsequent experiments.

3.3.3 Effect of desorbent volume. The volume of desorbent is an important factor in optimizing PFSPE, and can significantly affect the desorption efficiency of the targets from PS/PES-CuNP nanofibers. As shown in Fig. 6C, different levels of desorbent volume were examined (60 μL, 70 μL, 80 μL, 100 μL, and 200 μL). As the volume of desorbent increased within 60–80 μL, the extraction efficiency gradually improved. When the desorbent volume reached 100 μL, the best extraction efficiency was achieved, and the recovery of three ochratoxins was 92.1–94.7%. As the volume of the desorbent further increased, there was no significant improvement in the extraction efficiency. For environmental protection and cost saving considerations, 100 μL was chosen as the volume of desorbent for PFSPE.

3.3.4 Effect of adsorption time. The adsorption time is described as the required time for each active site on the adsorbent to be maximally occupied by the targets. The influence on extraction efficiency by adsorption time was investigated in this experiment. As shown in Fig. 6D, the extraction efficiency significantly improves with the extension of extraction time and achieved saturation at 7 min; the extraction recovery of the three ochratoxins reached 88.1–92.7%. The adsorption rate slightly changed but basically reached equilibrium as the extraction time was extended. This result indicated that the three ochratoxins can quickly accumulate on PS/PES CuNP nanofibers. Therefore, the optimal extraction time was determined to be 7 min for subsequent experiments.

3.4 Method validation

The calibration curve in a concentration range of 0.5–50 μg L^{-1} was obtained by diluting the standard stock solution of ochratoxins with artificial urine, as shown in Table 1. The standard curves of the three ochratoxins showed good linearity ($R^2 \geq 0.992$) within the specified concentration range. LOD and LOQ were 0.108–0.162 μg L^{-1} and 0.658–0.701 μg L^{-1}, respectively.

The recovery was verified by adding standards to artificial urine, and three concentration levels of low, medium, and high (1.0, 5.0, and 10.0 μg L^{-1}) for the targets were obtained. The absolute recovery was calculated as the ratio of the amounts of targets in the elution solution to that in the spiked solution before PFSPE.[12] As shown in Table 2, for the low, medium, and high concentrations of ochratoxins in spiked artificial urine samples, the recoveries were 71.3–92.0%, indicating that satisfactory extraction efficiency of ochratoxins was achieved in the urine sample. The intra-day and inter-day precision were both less than 7.1%. Overall, the detection method established in this experiment is sensitive and accurate and can be used for the detection of ochratoxins in human urine.

The chromatogram of the ochratoxins is shown in Fig. S1.† After PFSPE treatment, most of the interfering matrix in the urine sample was removed, and the response of the targets in urine was significantly enhanced. This result indicated that PS/PES CuNPs composite nanofibers have a good ability for impurity removal and selective adsorption for ochratoxins.

3.5 Comparison between PS/PES CuNP nanofibers and reported adsorbents

At present, there are several articles on methods for extracting OTs from food matrices. As displayed in Table S1,† the proposed method was comparable to most techniques in terms of adsorbent filled, organic solution usage, minimum detection

Table 1 Figures of merit for the PS/PES-CuNP nanofibers as sorbent for HPLC-FLD determination of OTs

Analyte	Linearity (μg L^{-1})	Linear equation	R^2	LOD (μg L^{-1})	LOQ (μg L^{-1})
OTA	0.5–50.0	$y = 4.3006x - 1.0130$	0.992	0.162	0.701
OTB	0.5–50.0	$y = 3.9075x - 1.4198$	0.992	0.108	0.658
OTC	0.5–50.0	$y = 11.1675x - 3.4206$	0.989	0.147	0.682

Table 2 Analytical parameters of the proposed method ($n = 5$)

Analyte	Spiked concentration ($\mu g\ L^{-1}$)	RSDs (%)		Recovery (%)
		Intra-day	Inter-day	
OTA	1.0	5.3	1.5	89.5–92.0
	5.0	2.9	4.2	88.9–90.1
	10.0	3.2	4.4	78.2–86.0
OTB	1.0	2.9	2.8	84.9–89.3
	5.0	5.1	5.6	85.7–91.1
	10.0	1.9	3.9	71.3–85.0
OTC	1.0	2.1	3.6	84.6–90.7
	5.0	3.8	2.8	87.3–89.9
	10.0	5.6	7.1	77.0–86.3

line, recovery, and a shorter extraction time (5–6 min), which was not mentioned in most articles. As seen from the main methodological parameters of each method listed in Table S1,† the proposed method has the advantages of simple preparation of adsorbent materials, high detection sensitivity, good recovery rate, and low detection limit. Moreover, three ochratoxins can be extracted simultaneously from complex matrices. Therefore, the detection method provided in this study shows great promise for application in the accurate quantitative analysis of OTs in complicated samples.

3.6 Actual sample analysis

3.6.1 Matrix effect evaluation. The practical application of PS/PES CuNP nanofibers was evaluated by testing non-invasive human samples with the analytical method developed. Similar to urine samples, saliva contains many complex substances, such as proteins and fats, which may interfere or prevent the accurate detection of targets. The reserve solution was diluted with methanol, blank saliva, and blank artificial urine, separately. All solutions had internal standards added and were processed and analyzed according to procedures 2.5 and 2.6. The matrix effect (ME) of the sample solution was evaluated using the following equation,[29]

$$\mathrm{ME}\ (\%) = (S_1/S_2) \times 100 \quad (2)$$

where S_1 is the slope of the matrix matching the calibration curve and S_2 is that of the calibration standard in the standard solution (methanol).

As shown in Table 3, the results showed that the matrix effects of ochratoxins in artificial urine samples and saliva samples were 89.6–112.8% and 96.1–110.9%, respectively. A value of ME from 85.0–115.0% can be considered to have a negligible matrix effect,[35] indicating that the influences of saliva and urine matrices were well controlled.

3.6.2 Analysis of ochratoxins in actual human urine samples. The applicability of the PS/PES-CuNP nanofibers coupled with HPLC was investigated by analyzing 30 actual urine samples (15 males and 15 females). The test results are shown in Table S2 in ESI.† In the 15 male samples, 3 positive samples were detected to have been exposed OTA at different levels (0.96 $\mu g\ L^{-1}$, 1.64 $\mu g\ L^{-1}$, 0.52 $\mu g\ L^{-1}$), and one positive sample was detected of OTC at 0.31 $\mu g\ L^{-1}$. The detection rate in females is lower than that in males: only OTC was detected, with concentrations of 0.48 $\mu g\ L^{-1}$ and 0.33 $\mu g\ L^{-1}$ in two females' positive urine samples. Overall, PS/PES-CuNP nanofibers can achieve simultaneous adsorption of three ochratoxins in human urine samples and showed great potential on pretreatment for large amounts of human samples.

Table 3 Evaluation of matrix effect and analytical results of three OTs in samples

Analytes	Matrix	Slope (10^4)	Slope matrix/solvent	ME %
OTA	Methanol	3.8137		
	Urine	4.3006	1.128	112.8
	Saliva	3.6641	0.961	96.1
OTB	Methanol	3.9020		
	Urine	3.9075	1.002	100.2
	Saliva	4.3269	1.109	110.9
OTC	Methanol	12.4701		
	Urine	11.1675	0.896	89.6
	Saliva	13.4930	1.082	108.2

4 Conclusion

In this work, a new strategy was proposed that used polystyrene/polyethersulfone electrospun nanofibers coated with copper nanoparticles as an excellent adsorbent for the rapid and sensitive extraction of ochratoxins from human urine. The nanofibers were characterized by TEM, SEM, XPS, EDS, TG, XPS, FTIR and CA. The results proved that the copper nanoparticles with positive charges were successfully integrated onto the PS/PES nanofibers, which played a key role in the sample pretreatment for extracting ochratoxins. The nanofiber-packed solid-phase extraction (PFSPE) requires less time, less sorbent dosage, and less volumes of sample and eluent than classic solid-phase extraction techniques and has greater application potential. In addition, an analytical method based on PFSPE was proposed, and extraction factors, such as adsorbent amount, ionic strength, adsorption time and desorbent volume, were optimized. Under optimal conditions, the novel PFSPE-HPLC-FLD method was established and utilized to detected ochratoxins in actual human urine samples, and OTs were detected in 5 positive samples of 30 samples. The results indicated that the novel PFSPE-HPLC-FLD method is sensitive, simple, low-cost and time-saving and suitable to trace and monitor mycotoxins in complex matrix samples.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts of interest to declare.

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