SUPPORTING INFORMATION

High enrichment and ultra trace analysis of aflatoxins in edible oil by a modified hollow fiber liquid phase microextraction technique

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Experimental Methods

Chemicals and Materials.

The AFTs including AFB_1, AFB_2, AFG_1 and AFG_2 were purchased from J & K Scientific (Beijing, China). The individual stock standard solution (100 mg kg^{-1}) was prepared in acetonitrile and stored under -20 °C condition. Anti-AFTs antibody was obtained from Shanghai Medicine'nest Pharmaceutical (Shanghai, China). The antibody classified as IgG was purified using caprylic acid-ammonium sulfate from the abdominal water of immunized mouse, and stored in the 0.02 M pH 7.4 PBS buffer solution at -20 ºC. 3-Hydroxytyramine hydrochloride (dopamine hydrochloride) was purchased from Sigma-Aldrich (Shanghai, China). Tris hydroxymethyl amino methane (tris), polyethyleneimine (PEI, Mw¼ 70000, 50% in water), tert-amyl alcohol and bromoethane were obtained from Aladdin (Shanghai, China). The edible oil samples including blend oil, peanut oil and maize oil were bought from the local markets. Polyvinylidene fluoride fiber membranes (PVDF, 200 μm wall thickness, 1.2 mm i.d., 0.2 μm pore size, porosity 85%) were supplied from Tianjin University of Science and Technology (Tianjin, China). Deionized (DI) water was obtained using Millipore Direct-Q 3 UV water purification system (Merck, German).

Instrumentation.

The modification reaction of the PVDF hollow fibers was carried out with an electro-thermostatic water bath (Shanghai, China). The quantification experiments of AFTs

were carried out using a liquid chromatography/tandem mass spectrometry system (LC-MS/MS). Liquid chromatography (LC) was performed with an Agilent 1100 system (Agilent Technologies, USA). The separations were conducted using a ZORBAX SB-C18 column (2.1 mm × 150 mm, 3.5 μm, Agilent Technologies, USA). And the LC system was interfaced to an AB Sciex Qtrap 5500 (AB Sciex, Shanghai, China) equipped with a Turbo Ion Spray® source. A MultiPurpose Sampler (Gerstel, Germany) was utilized for the sample incubation and agitation.

Operation parameters of LC-MS/MS.

LC separation: Elution was completed using a 17-min gradient program operating with a 0.30 mL min^{-1} flow rate with a 10 μL injection volume. The oven temperature was 40 °C. Mobile phase A was pure water containing 0.1% formic acid, and mobile phase B was acetonitrile. The gradient elution program was applied as follows: 5% B for 1 min, linear gradient to 100% B between 1 and 2 min, held at 100% B until 12 min, and then returned to 5% B in 0.1 min.

MS/MS detection: Because the electron-donating property of C=O group, which the structures of AFTs were depicted in Figure S3, the mass spectrometer was operated in multiple reaction monitoring (MRM) mode with positive mode acquisition (ESI^+). The ion spray voltage was set to 4500 V. The turbo heater was maintained at 600 °C. Nitrogen was used as the curtain gas and collision gas. The curtain gas, collision gas, ion source gas 1, and ion source gas 2 were set to pressures of 20, 9, 50 and 50 psi,

respectively. The precursor and product ions of each analyte, along with the analyte-specific parameters, were listed in Table S1. The instrumental control and data processing utilities included the use of Analyst 1.5.2 software. The chromatograms and retention time of AFB_1, AFB_2, AFG_1 and AFG_2 were depicted in Figure S4.

A calibration curve (1-100 μg kg^{-1}) which used the acetonitrile as dissolved solvent was evaluated in every LC-MS/MS usage for supervising and adjusting the instrumental response.

Safety.

Aflatoxins are powerful hepatotoxins and carcinogens, so great care should be taken to avoid personal exposure and potential laboratory contamination. Gloves and other protective clothing were worn as safety precaution during the experiments. All items coming in contact with aflatoxins (glass vial, septa, microsyringe, etc.) were immersed in methanol and cleaned by ultrasonic for more than 30 min before they were discarded or for the afterward usage. And the liquid and solid wastes were collected and retreated uniformly.

Modification of the PVDF Hollow Fibers.

The modification (Figure 1) was based on the work reported by Shi et al.[1] Firstly, the PVDF hollow fibers were cutting into 3.0-cm segments, washed by methanol with ultrasonic for 15 min, and then dried in ambient conditions. Afterwards, the PVDF hollow fibers were inserted into the dopamine solution which was 1g L^{-1} in tris buffer

solution (15 mM, pH 8.5) in a beaker for dopamine polymerization onto the surface of hollow fibers. After 20 h, the fibers were taken out, washed by deionized water. Then PDA-modified fibers were placed in PEI solution with a concentration of 2 g L^{-1} in tris buffer solution (15 mM, pH 8.5) for 4 h at 60 °C. After that, the fibers were taken out and washed by deionized water to remove the unreacted PEI. The last step was put the fibers into a 4 g L^{-1} bromoethane in tert-amyl alcohol, reacting in 60 °C for 24 h. At last, the fibers were washed by ethanol absolute and deionized water, then dried in ambient condition.

Characterization of the Modified PVDF Hollow Fibers.

The morphologies of the membranes before and after modification were observed by field emission scanning electron microscope (FE-SEM, Quanta 400F, Netherlands) and high-revolution transmission electron microscope (HR-TEM, FEI Tecnai G2 Spirit, Netherlands). The hollow fibers were cut into a small square (approximately 2 mm × 2 mm), and pasted them on the sample plate with double-side tape to obtain the TEM images. On the other hand, to get the TEM images, the hollow fibers were cut into wire-shaped pieces, and treated by ultrasonic for 30 min, then picked them up onto the copper nets. The water contact angles were recorded by a water contact angle system (OCA, Dataphysics, Germany) to measure the change of the surface hydrophilicity of the membranes before and after modification. Similarly, to obtain the images of contact angles, the hollow fibers were cut into an approximately 1 cm × 1 cm square, and fixed them onto a glass plate.

Extraction Procedures.

The HF-LPME device based on the model designed by Ouyang et al.[2] was established for the extraction of AFTs in edible oil (Figure 4). Firstly, a 1.5-cm long pipet tip was used as a needle guide, and the 3.0-cm hollow fiber was fixed in the pipet tip with epoxy gel. The end of the hollow fiber was sealed by mechanical pressure. Secondly, the PVDF-PDA-QPEI hollow fiber was immersed in the pure water for 1 min to form the supporting liquid membrane (SLM). Then the fiber was immersed into the oil sample (8 g) with spiked AFTs standards in a 10-mL sample vial. Thirdly, a 30 μL volume of pH 7.4 PBS buffer solution containing anti-AFTs antibody used as extraction phase, was slowly injected into the lumen of the hollow fibers. Afterwards, the spiked sample was heated and stirred in the incubation for extraction using the MPS Sampler. Finally, 20 μL extraction phase was slowly withdrawn from the lumen and diluted with 20 μL acetonitrile, of which 10 μL mixture was used for LC-MS/MS analysis. The operation parameters of LC-MS/MS were described in detail above. The blend oil as sample matrix and a standard solution of AFTs at 125 μg kg^{-1} were used for the optimization of the extraction conditions.

Calculations.

In order to evaluate the extraction efficiency and the accuracy of the proposed method, the enrichment factor (EF) and the recovery (R) were determined as the following equations.

$$EF = \frac{PA_{extraction} * 2}{PA_{liquid}} \quad (1)$$

$$R = \frac{C_{found} - C_{real}}{C_{spiked}} \times 100\% \quad (2)$$

As for eq. 1, $PA_{extraction}$ means the peak area obtained by the proposed method, and PA_{liquid} is the peak area obtained by directly liquid injection of analyte at the same concentration level with the extraction procedure. Since the extraction phase that was ready for LC-MS/MS analysis was diluted twice with 20 acetonitrile, the $PA_{extraction}$ was multiplied by 2. And as for eq. 2, C_{found} is the concentration of AFTs in the real sample, which spiking a known amount of standard. C_{real} is the concentration of target analyte in real sample without spiked standard, and C_{spiked} is the concentration of analyte spiking into the real samples.

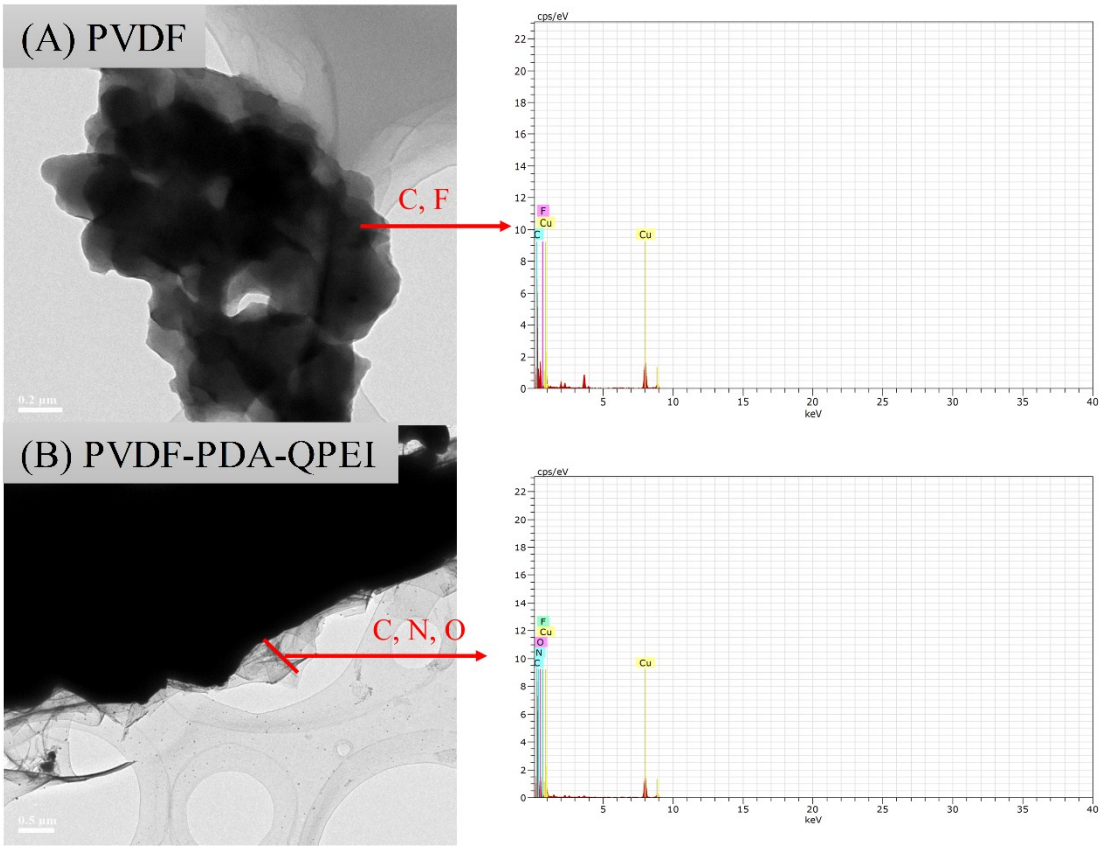

Figure S1. TEM characterization before and after modification of the PVDF hollow fibers. (A) PVDF hollow fiber; (B) PVDF-PDA-QPEI hollow fiber.

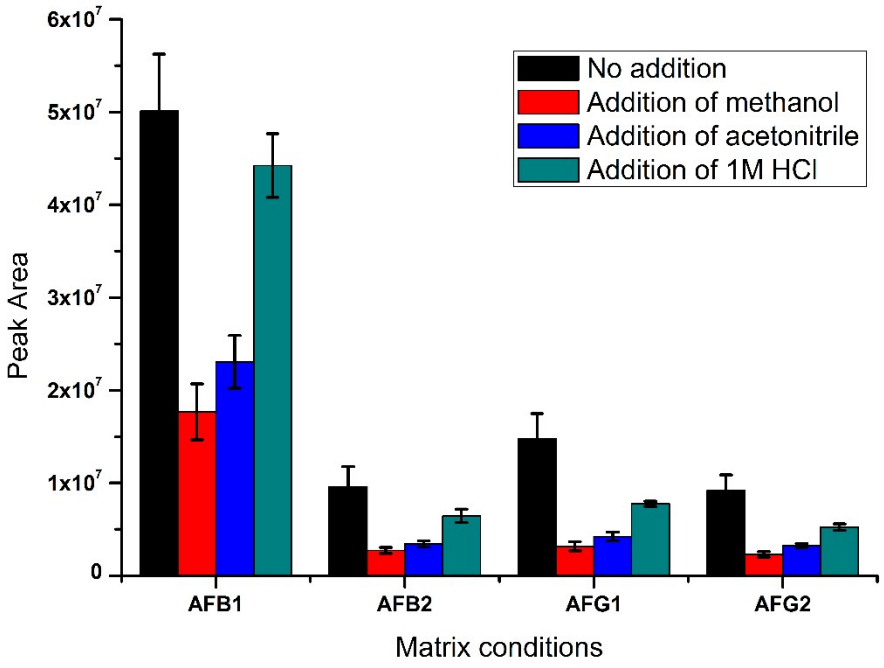

Figure S2. The evaluation of extraction efficiency in terms of the addition of methanol, acetonitrile, 1M HCl solution for the determination of AFB_1, AFB_2, AFG_1 and AFG_2 in blend oil samples. Extraction conditions: extraction temperature, 50 ˚C; extraction time, 20 min; agitation speed, 500 rpm; concentration of anti-AFTs antibody in PBS buffer solution, 30 mg L^{-1}.

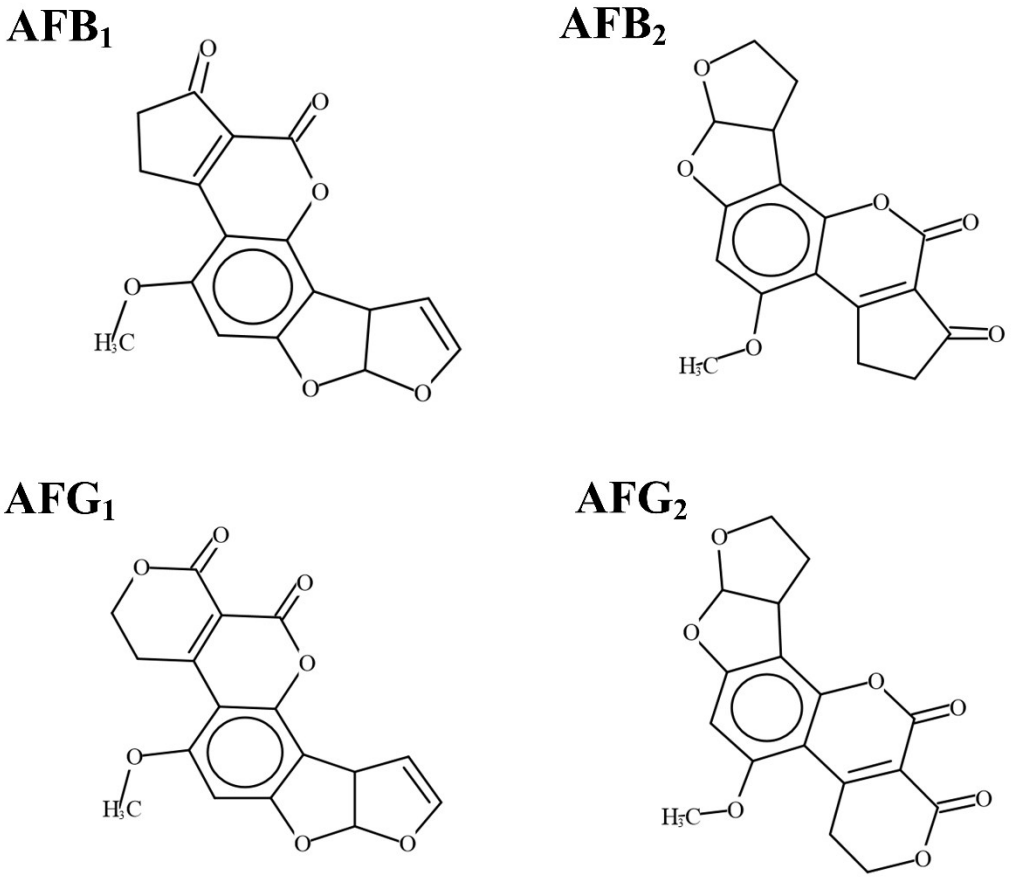

Figure S3. The structures of AFTs (AFB_1, AFB_2, AFG_1 and AFG_2).

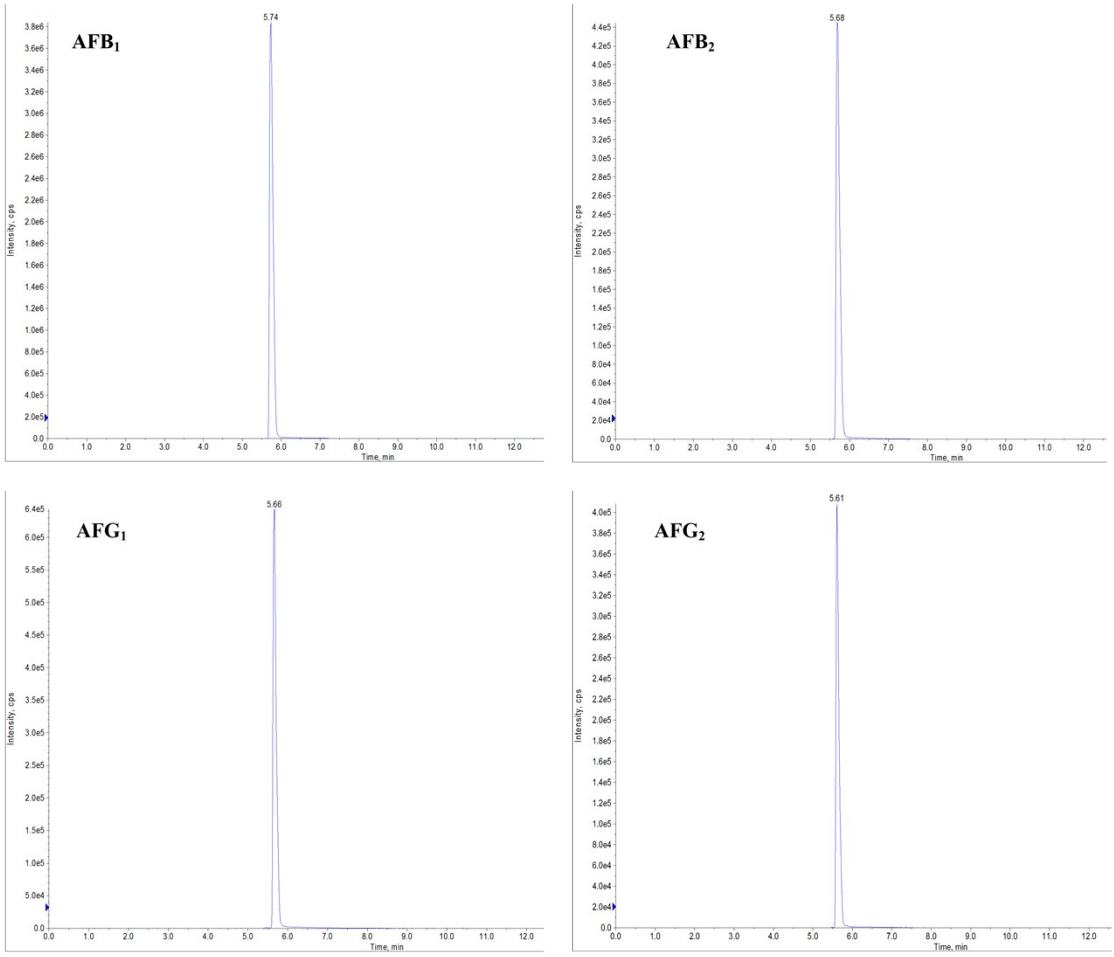

Figure S4. The chromatograms of AFB_1, AFB_2, AFG_1 and AFG_2.

Table S1. The analyte-specific parameters including precursor ion (Q1), product ion (Q3), declustering potential (DP), entrance potential (EP), collision energy (CE), collision cell exit potential (CXP) of AFB_1, AFB_2, AFG_1 and AFG_2 in this study.

Analyte	Q1	DP/V	EP/V	Q3	CE/eV	CXP/V
AFB_1	312.9	98	4	240.9	50	9
AFB_2	315.1	125	6	270.9	40	10
AFG_1	329.2	130	12	200.0	58	7
AFG_2	331.1	150	5	189.1	51	19

References:

(1) H. Shi, L. Xue, A. Gao, Y. Fu, Q. Zhou, L. Zhu, *J. Membrane Sci.* 2016, **498**, 39-47.

(2) G. Ouyang, J. Pawliszyn, *Anal. Chem.* 2006, **78**, 5783-5788.