

Article

# Determination of Aflatoxin B<sub>1</sub> and B<sub>2</sub> in Vegetable Oils Using Fe<sub>3</sub>O<sub>4</sub>/rGO Magnetic Solid Phase Extraction Coupled with High-Performance Liquid Chromatography Fluorescence with Post-Column Photochemical Derivatization

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**Abstract:** In this study, magnetic graphene nanocomposite Fe<sub>3</sub>O<sub>4</sub>/rGO was synthesized by facile one-pot solvothermal method. The nanocomposite was successfully used as magnetic solid phase extraction (MSPE) adsorbents for the determination of aflatoxins in edible vegetable oils through the  $\pi$ - $\pi$  stacking interactions. MSPE parameters including the amount of adsorbents, extraction and desorption time, washing conditions, and the type and volume of desorption solvent were optimized. Under optimal conditions, good linear relationships were achieved. Limits of detection of this method were as low as 0.02  $\mu\text{g}/\text{kg}$  and 0.01  $\mu\text{g}/\text{kg}$  for aflatoxin B<sub>1</sub> and B<sub>2</sub>, respectively. Finally, the magnetic graphene nanocomposite was successfully applied to aflatoxin analysis in vegetable oils. The results indicated that the recoveries of the B-group aflatoxins ranged from 80.4% to 106.0%, whereas the relative standard deviations (RSDs) were less than 8.1%. Owing to the simplicity, rapidity and efficiency, Fe<sub>3</sub>O<sub>4</sub>/rGO magnetic solid phase extraction coupled with high-performance liquid chromatography fluorescence with post-column photochemical derivatization (Fe<sub>3</sub>O<sub>4</sub>/rGO MSPE-HPLC-PCD-FLD) is a promising analytical method for routine and accurate determination of aflatoxins in lipid matrices.

**Keywords:** aflatoxin; magnetic solid phase extraction; graphene; high-performance liquid chromatography fluorescence; vegetable oil

**Key Contribution:** Magnetic graphene nanocomposite was synthesized to extract aflatoxins from vegetable oils with excellent extraction efficiency and low limit of quantification without tedious steps.

## 1. Introduction

Recently, edible vegetable oils have gained immense popularity over animal-based fats, which is attributed to their nutritional and health-promoting characteristic [1]. Vegetable oils meet dietary

demands by providing energy and by transporting fat-soluble vitamins and antioxidant compounds which are widely used in home cooking and the food industry. However, the majority of the edible oilseeds, such as peanut, soybean and maize are easily attacked by *Aspergillus* strains, namely *A. flavus* and *A. parasiticus*. The secondary metabolites of those fungi are mainly aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and B<sub>2</sub> (AFB<sub>2</sub>), which can cause carcinogenic, teratogenic, mutagenic, immune-suppressive and estrogenic effects that are harmful to the human health [2,3]. The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) has classified AFB<sub>1</sub> as carcinogenic to humans in 1993 [4].

To avoid hazardous symptoms to humans and animals, various countries have established specific regulations and prevention guidelines for aflatoxin management. The European Union has set strict standards for aflatoxins in groundnuts and other oilseed, in which the maximum levels (MLs) are 2 µg/kg for AFB<sub>1</sub> and 4 µg/kg for the total aflatoxin concentration (AFTs). In Japan, the ML for AFB<sub>1</sub> is 10 µg/kg in all foods, whereas the ML for peanuts and nuts is 10 µg/kg in Korea. In China, the MLs of AFB<sub>1</sub> are set at 20 µg/kg for peanut and maize oils, and at 10 µg/kg for the other vegetable oils [5–8]. Owing to the current regulations and the survey requirements in edible oils, it is important to develop simple and sensitive methods for the detection of aflatoxins in complex matrices.

Various methods have been developed for the determination of AFTs in different matrices, including thin layer chromatography (TLC) [9], enzyme-linked immunosorbent assays (ELISA) [10] and high-performance liquid chromatography coupled with fluorescence (FLD)/mass spectrometry (MS) detection [11,12]. According to the “gold standard” for trace amount AFT detection, HPLC-FLD is the main method used for routine quantification of this type of compounds in a complex matrix. To maintain the natural fluorescence of AFTs, chemical and photochemical derivatization (PCD) has been used to avoid the emission quenching in the aqueous mobile phase. The PCD method dramatically increases the fluorescence signal of AFTs by the UV irradiation of the hydroxyl radical, which is in compliance with the guidelines of the green preparation chemistry including the improvement of the automatic manipulator, the absence of derivatizing reagents and the lower requirement for detection maintenance.

The extraction and enrichment procedure used for edible oils plays important roles in the accurate quantification of AFTs due to their low concentration in the triacylglycerol matrix. The extraction methods have been optimized including liquid–liquid extraction (“dilute-and-shoot” method) [13], solid phase extraction (SPE) [14], QuEChERS [15], gel permeation chromatography (GPC) [16], matrix solid phase dispersion (MSPD) [17] and cloud point extraction (CPE) [18]. However, the majority of these extraction methods used for aflatoxins in oil samples require tedious and time-consuming procedures and large volumes of organic solvents, and result in limited cycles of interface phase between the analytes and the extract absorbents. Therefore, it is necessary to develop a simple, rapid and accurate method for the determination of aflatoxins in vegetable oil.

Recently, magnetic solid-phase extraction (MSPE) has attracted particular interest with regard to sample pretreatment. The magnetic adsorbents are uniformly dispersed and agglomerated in extract solutions by external magnetic field. The MSPE procedure could evidently improve the interface phase between the adsorbents and the extractant by increasing the mass transfer coefficients of the analytes [19]. The structure of MSPE adsorbents mainly consists of magnetic carriers and functional groups, which play key roles in the enrichment process and affect the performance of the detection method. The functional activity of the MSPE composites could be prepared by chemical modification including metal [20], oxidative metal [21], silica [22] and metal-organic frameworks [23], or the composite synthesis containing magnetic nanoparticles combined with antibodies [24], molecularly imprinted polymers [25], carbon nanotubes [26] and graphene (G) [27].

Among the various materials applied as MSPE adsorbents, G and its derivative possess the single-layer/few-layer of  $sp^2$  hybridized carbon motif in the honeycomb lattice, which contributes to ultra-high surface area and a large delocalized  $\pi$ -electronic carbon network. Owing to this unique characteristic, G-based magnetic composites have been widely applied in the separation and purification

of organic contaminants, biological macromolecules and heavy metals [28]. Reduced graphene oxide (rGO) possesses large amounts of polar groups with oxygen atoms, including hydroxyl, epoxy and carboxyl groups, which exhibit optimal adsorption capacity toward the oxygen and nitrogen functional groups of the organic pollutants by the interaction of dative bonds, cation- $\pi$  interactions, electrostatic interactions or hydrogen bonds compared with the corresponding adsorption capacity noted in non-polar and hydrophobic graphene adsorbents. Previous studies indicated that graphene oxide (GO) materials could be used as simple, rapid and cost-effective dSPE adsorbents to extract aflatoxins from peanut samples [29]. However, rGO materials are easily aggregated in extract solutions and difficult to retrieve from the suspension owing to ultra-light and hydrophilic properties. To prevent rGO aggregation and facilitate rGO-dSPE, the chemical fabrication of rGO and  $\text{Fe}_3\text{O}_4$  can be used to synthesize an rGO hybrid magnetite. This is a promising technological method used in the enrichment of AFTs from complex matrices with magnetic separation.

In the present study, magnetic graphene  $\text{Fe}_3\text{O}_4/\text{rGO}$  adsorbents were characterized and applied to extract  $\text{AFB}_1$  and  $\text{AFB}_2$  from vegetable oils. The adsorbents were coupled with high performance liquid chromatography fluorescence detection (HPLC-FLD) analysis.  $\text{Fe}_3\text{O}_4/\text{rGO}$  adsorbents were synthesized with high yield via the facile one-pot solvothermal method. The magnetic graphene-based adsorbents indicated optimal adsorption capacity toward AFTs due to the presence of  $\pi$ - $\pi$  interactions and hydrogen bonds. Furthermore, the adsorbents were washed with non-polar hexane to completely remove triglyceride matrix components. Following post-column derivatization (PCD) coupled with FLD detection, a rapid, simple and accurate  $\text{Fe}_3\text{O}_4/\text{rGO}$  MSPE-HPLC-PCD-FLD method was developed for the determination of  $\text{AFB}_1$  and  $\text{AFB}_2$  in vegetable oil samples.

## 2. Results and Discussion

### 2.1. Characterization of $\text{Fe}_3\text{O}_4/\text{rGO}$ Adsorbents

The synthesized adsorbent material was characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM). In Figure 1a, the characteristic peak of GO that was located at  $2\theta$  around  $11^\circ$  could not be observed, which was attributed to the reduction of GO. No distinct peak was observed in the range of  $2\theta$  from  $5^\circ$  to  $30^\circ$ , indicating that the resulting rGO was very poorly ordered along the stacking direction. The significant diffraction peaks of the sample ranged from  $30^\circ$  to  $70^\circ$  ( $2\theta$ ) and could be assigned to the crystal  $\text{Fe}_3\text{O}_4$  cubic structure (JCPDS 19-0629). As shown in Figure 1b, the surface of the rGO nanosheets was decorated with monodisperse nanoparticles, and the size of the  $\text{Fe}_3\text{O}_4$  nanoparticles was approximately 200 nm. Furthermore, rGO nanosheets containing several wrinkles and folds were observed, indicating that the stacking of rGO nanosheets was disordered due to the insertion of the  $\text{Fe}_3\text{O}_4$  nanoparticles. Figure 2 showed that the synthesized adsorbent material with good dispersibility can be easily collected by an external magnet field in the organic solvent.

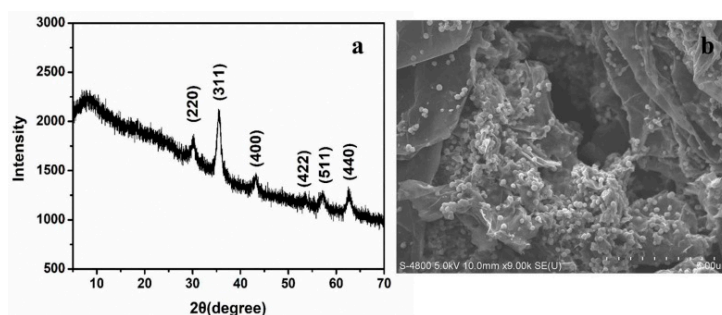
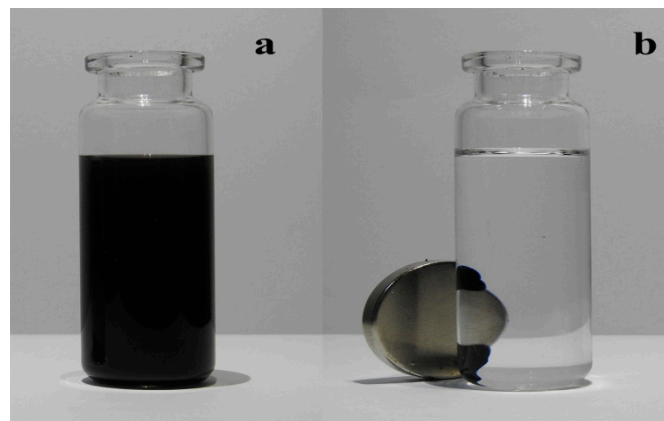


Figure 1. XRD spectrum (a) and SEM image (b) of  $\text{Fe}_3\text{O}_4/\text{rGO}$ .



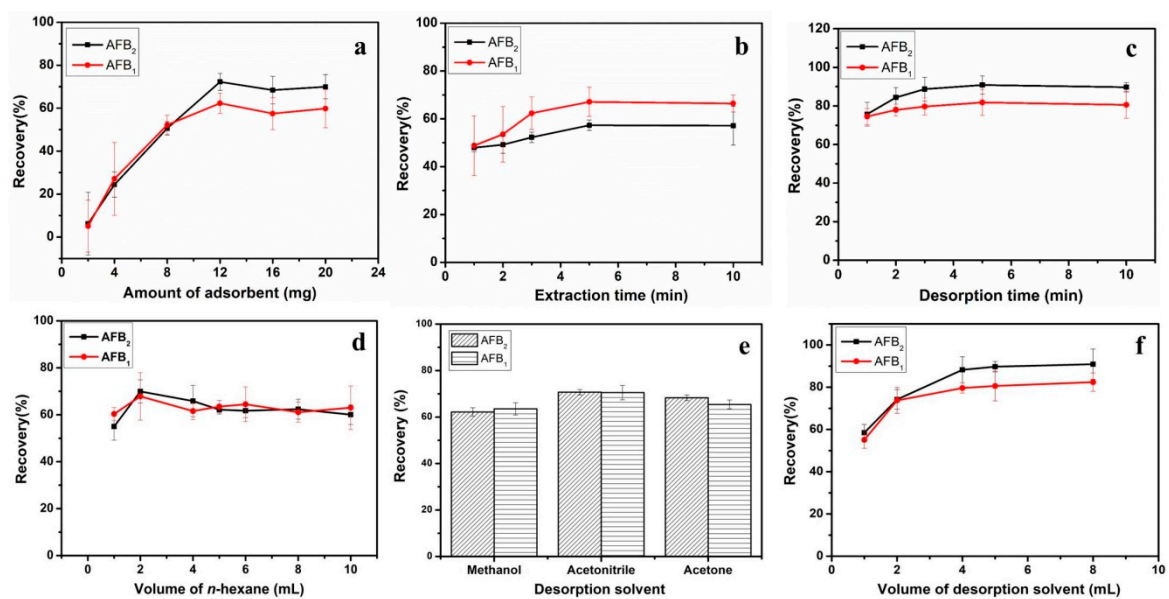
**Figure 2.** The dispersed and magnetic characteristic of the  $\text{Fe}_3\text{O}_4/\text{rGO}$  adsorbents in matrix solutions (a) and collected by external magnet field (b).

## 2.2. Optimization of MSPE Conditions

In order to improve the extraction efficiency, several parameters were investigated including the adsorbent amount, the extraction time, the volume of washing solvent, the desorption time, the desorption solvent and its volume. The optimization of the MSPE conditions was performed using 2.0 g non-contaminated oil samples spiked with 5  $\mu\text{g}/\text{kg}$  for  $\text{AFB}_1$  and  $\text{AFB}_2$ , respectively. The recoveries were calculated by the ratios of the chromatographic peak of the analytes to those of the AFT standards.

### 2.2.1. Amount of $\text{Fe}_3\text{O}_4/\text{rGO}$ Adsorbents

To achieve optimal recovery efficiency toward aflatoxins, different amounts of  $\text{Fe}_3\text{O}_4/\text{rGO}$  adsorbents (2, 4, 8, 12, 16 and 20 mg) were added to the extract and the analytes were purified from the lipid samples. As shown in Figure 3a, the recoveries of  $\text{AFB}_1$  and  $\text{AFB}_2$  increased dramatically following an increase in the amount of adsorbent from 2 to 12 mg, and subsequently remained constant regardless of the further increase in the concentration of the  $\text{Fe}_3\text{O}_4/\text{rGO}$  MSPE adsorbents. Ultimately, 12 mg was applied as the amount of the adsorbent for the following experiments.



**Figure 3.** Effects of the key parameters on the recovery of aflatoxins ( $n = 3$ ), including the amount of  $\text{Fe}_3\text{O}_4/\text{rGO}$  (a), extraction time (b), desorption time (c), volume of *n*-hexane (d), desorption solvent (e), and volume of elution solvent (f).

### 2.2.2. Extraction and Desorption Time

The extraction and desorption time ranged from 1 to 10 min, respectively. In Figure 3b, the increase in the extraction time from 1 to 5 min caused a gradual increase in the extraction efficiency. Moreover, the dynamic process for the adsorption of AFTs was equilibrated synchronously. Therefore, the extraction time was set at 5 min for the rapid and efficient purification of AFTs owing to the high interfacial surface between the adsorbents and the diluted oil sample, and due to the increase in the mass transfer coefficients during the extraction procedure. In Figure 3c, the same variation tendency of desorption efficiency was noted, and the time period of 3 min was set to for the elution of the retained AFTs from the Fe<sub>3</sub>O<sub>4</sub>/rGO adsorbents.

### 2.2.3. Washing Conditions

When the analytes were loaded on MSPE adsorbents, the matrix interferences were eliminated by washing step notably for the complex lipophilic samples [30]. The polarity of the washing solvent requires optimal miscibility with the matrix component triglycerides in order to avoid the loss of AFTs. The double bond of the terminal furan ring, the phenyl, and the carbonyl moiety are the hydrophobic and hydrophilic structures of AFTs, which could be easily adsorbed by Fe<sub>3</sub>O<sub>4</sub>/rGO adsorbents via the  $\pi$ - $\pi$  interactions and the formation of hydrogen bonds with the MSPE adsorbents. The non-polar solvent *n*-hexane was selected and applied to the washing step to remove the triglycerides from the matrix, due to its low polarity and its ability to disrupt hydrophilic interactions. The influence of *n*-hexane volume on the recovery of AFTs was optimized in the range of 1 to 10 mL. As shown in Figure 3d, no significant changes in the recoveries were found following 2 mL of *n*-hexane use for purification. Therefore, 2 mL of *n*-hexane was selected in the washing procedure.

### 2.2.4. Desorption Conditions

It is vital to increase the efficiency of desorption conditions in order to disrupt the interactions between the extracted AFTs and the surface of the Fe<sub>3</sub>O<sub>4</sub>/rGO adsorbents. Therefore, it is essential to optimize the type and volume of the desorption solvent for optimal analytical performance. The recoveries of methanol, acetonitrile and acetone as desorption solvents were present in Figure 3e. The high polarity of the acetonitrile solvent was more efficient in disrupting the  $\pi$ - $\pi$  interactions and the hydrogen bonds with the MSPE adsorbents compared with that noted in the methanol and acetone solvents. In addition, the effects of the desorption volume range (1–8 mL) were evaluated. In Figure 3f, the recoveries of AFTs increased dramatically when the volume was increased from 1 to 4 mL, and no significant changes were noted afterwards. Therefore, 4 mL of acetonitrile was selected in the desorption process.

## 2.3. Method Validation

### 2.3.1. Matrix Effect

The matrix of the lipid samples could enhance or diminish the FLD intensity of the analytes and affect accuracy and reproducibility of the analysis method. The post-extraction spiked method was used to assess the matrix effects by comparing the calibration slopes between the lipid matrix and the pure standard solvent. The calibration curves were constructed by plotting the area against the concentration of the analytes. The matrix effect of Fe<sub>3</sub>O<sub>4</sub>/rGO MSPE-HPLC-PCD-FLD was evaluated by the following Equation (1):

$$\text{Matrix Effect (\%)} = \frac{\text{Slope in solvent} - \text{Slope in matrix}}{\text{Slope in solvent}} \times 100\% \quad (1)$$

The matrix effect of AFB<sub>1</sub> and AFB<sub>2</sub> were 14.9% and 12.5%, respectively. The results indicated that the MSPE purification step could not remove the lipid matrix completely. Therefore, the matrix-matched calibration curve was applied for the accurate quantification of the AFTs in oil samples.

### 2.3.2. Linearity, Accuracy and Precision of the Method

Linearity was evaluated through the matrix-matched calibration at six different concentration and the correlation coefficient ( $R^2$ ) was constructed by the linear regression equation. As listed in Table 1,  $R^2$  were higher than 0.9967 for AFTs; besides, the standard deviation of the residuals was less than 20%, indicating satisfactory linearity. The limit of detection (LOD) and the limit of quantification (LOQ) for AFTs were evaluated by the signal-to-noise ratio of the FLD chromatogram for oil samples ( $S/N = 3$  for LOD and  $S/N = 10$  for LOQ), respectively. The results showed that the LOD and LOQ were 0.02  $\mu\text{g}/\text{kg}$  and 0.10  $\mu\text{g}/\text{kg}$  for AFB<sub>1</sub>, and 0.01  $\mu\text{g}/\text{kg}$  and 0.10  $\mu\text{g}/\text{kg}$  for AFB<sub>2</sub> respectively, which could meet the strict regulatory levels set in vegetable oil by the National Criterion of China (10  $\mu\text{g}/\text{kg}$  for AFB<sub>1</sub>).

**Table 1.** Linear range and equation, correlation coefficient ( $R^2$ ), limits of detection (LOD), limits of quantification (LOQ) and precision for the determination of aflatoxins by Fe<sub>3</sub>O<sub>4</sub>/rGO magnetic solid phase extraction coupled with high-performance liquid chromatography fluorescence with post-column photochemical derivatization (Fe<sub>3</sub>O<sub>4</sub>/rGO MSPE-HPLC-PCD-FLD).

Analyte	Linear Range ( $\mu\text{g}/\text{kg}$ )	Linear Equation	$R^2$	LOD ( $\mu\text{g}/\text{kg}$ )	LOQ ( $\mu\text{g}/\text{kg}$ )	Intra-Day Precision (RSD %, $n = 6$ )					Inter-Day Precision (RSD %, $n = 4$ )				
						0.1	0.5	1.0	2.0	20	0.1	0.5	1.0	2.0	20
AFB <sub>1</sub>	0.10–25	$y = 130.686x$ ( $\pm 11.029$ ) – 2.31921 ( $\pm 1.14467$ )	0.9967	0.02	0.10	8.7	4.5	4.8	3.7	2.3	10.5	9.8	6.2	3.9	3.2
AFB <sub>2</sub>	0.10–20	$y = 480.364x$ ( $\pm 10.605$ ) + 1.55194 ( $\pm 0.43423$ )	0.9978	0.01	0.10	7.3	7.5	4.9	2.3	1.3	9.8	9.0	5.1	5.8	3.9

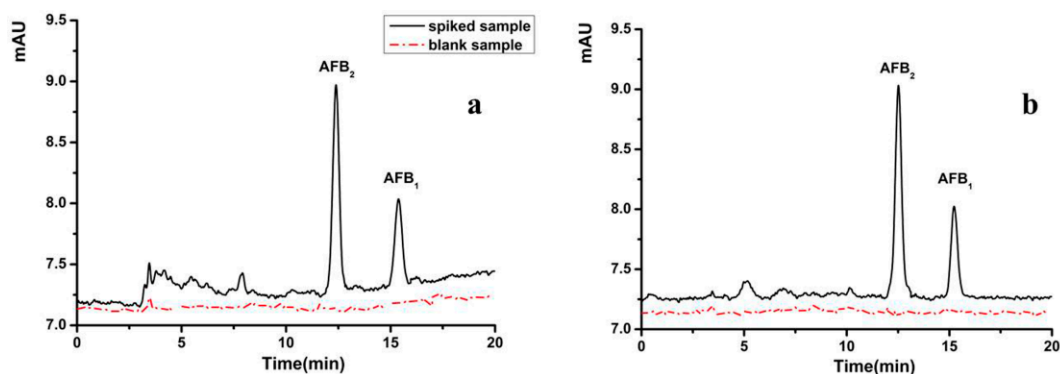
The reproducibility was evaluated by the intra-day and inter-day precision as relative standard deviation (RSD), which were validated by AFTs spiked at five different concentration (0.1, 0.5, 1.0, 2.0, 20  $\mu\text{g}/\text{kg}$ ) in blank peanut oil. Six parallel extractions of oil sample within one day were obtained the intra-day RSDs, and the inter-day RSDs were tested by extracting AFTs from spiked oil samples that were prepared independently in four individual days. The results showed that the intra- and inter-day RSDs were less than 8.7% and 10.5%, respectively. Therefore, the developed method had the acceptable repeatability for routine analysis.

The accuracy and reliability of the methods were evaluated by spiking AFB<sub>1</sub> and AFB<sub>2</sub> in the blank oil samples. The recovery was measured by comparing the concentration of the analytes calculated from the matrix-matched calibration curve with the spiked concentration accordingly. The typical chromatographs of the HPLC-PCD-FLD for AFB<sub>1</sub> and AFB<sub>2</sub> are shown in Figure 4, and the recovery of analyte detection in the variety of the vegetable oils is summarized in Table 2. The recovery of the analytes were in the range of 80.4–106.0% and the RSDs were less than 8.1%, illustrating optimal accuracy and reliability of the method.

**Table 2.** Recovery and precision of AFB<sub>1</sub> and AFB<sub>2</sub> in vegetable oil samples <sup>a</sup>.

Analyte	Recovery (% , $n = 3$ ) <sup>b</sup>							
	Corn Oil	Soybean Oil	Rapeseed Oil	Rice Oil	Almond Oil	Peanut Oil I	Peanut Oil II	Peanut Oil III
AFB <sub>1</sub>	86.3 (5.3)	88.7 (5.0)	80.4 (4.6)	82.1 (5.6)	96.6 (7.7)	88.7 (6.7)	94.1 (6.4)	93.2 (8.1)
AFB <sub>2</sub>	105.8 (6.3)	102.6 (6.7)	98.1 (2.8)	100.6 (3.2)	103.5 (4.2)	106.0 (4.4)	103.2 (3.5)	95.3 (6.1)

<sup>a</sup> The concentration of AFB<sub>1</sub> and AFB<sub>2</sub> were spiked at 1  $\mu\text{g}/\text{kg}$  and 0.5  $\mu\text{g}/\text{kg}$ , respectively. <sup>b</sup> The analyzed data were the mean  $\pm$  standard deviation.



**Figure 4.** Typical chromatograms of aflatoxin analysis in peanut (a) and almond (b) oils. (AFB<sub>1</sub> and AFB<sub>2</sub> spiked at 1.0 µg/kg and 0.5 µg/kg, respectively.).

### 2.3.3. Analysis of Real Samples

The Fe<sub>3</sub>O<sub>4</sub>/rGO MSPE-HPLC-PCD-FLD method was applied to analyze the concentration of AFB<sub>1</sub> and AFB<sub>2</sub> in 82 vegetable oils from the supermarkets in Wuhan (China), including 15 corn oils, 15 peanut oils, 12 soybean oils, 12 rapeseed oils, 12 rice oils, 8 walnut oils and 8 almond oils. The results indicated the absence of positive samples. A trace amount of AFB<sub>1</sub> 0.7 µg/kg was found in only one peanut oil sample.

A comparative study of this proposed method was performed for the determination of AFB<sub>1</sub> and AFB<sub>2</sub> in vegetable oils and the results were shown in Table 3. The proposed method possessed optimal accuracy and recovery and excellent reproducibility. The complete Fe<sub>3</sub>O<sub>4</sub>/rGO MSPE steps could be achieved in 15 min and were directly analyzed, which could avoid laborious purification steps and time-consuming chemical derivatization. The sensitivity and selectivity of the proposed PCD-HPLC-FLD method were comparable with the sophisticated methodology using LC-MS/MS for aflatoxins. In addition, this was the first study that examined the application of the Fe<sub>3</sub>O<sub>4</sub>/rGO MSPE adsorbents for the extraction of AFB<sub>1</sub> and AFB<sub>2</sub> from vegetable oils. The method demonstrated high potential for simple, rapid and environmentally friendly pretreatment in complex fatty matrix.

**Table 3.** Comparison of pretreatment procedures, LOQ and recovery for the determination of AFB<sub>1</sub> and AFB<sub>2</sub> in edible oils. Magnetic solid-phase extraction, MSPE.

Adsorbents	Pretreatment	Pretreatment Time (min)	Derivatization Condition	Determination	Recovery (%)	LOQs (µg/kg)	Reference
-	Dispersive liquid-liquid micro-extraction after IAC clean up	<120	-	LC-FLD	96–109.9	2.8 × 10 <sup>3</sup> (AFB <sub>1</sub> ) 0.4 × 10 <sup>3</sup> (AFB <sub>2</sub> )	[31]
-	IAC clean up	<30	-	UPLC-MS/MS	90–105	0.12–0.15 (AFB <sub>1</sub> )	[32]
-	Supercritical fluid chromatography	15	-	UPC <sup>2</sup> -MS/MS	98, 104	0.05 (AFB <sub>1</sub> ) 0.08 (AFB <sub>2</sub> )	[33]
Humic acid-bonded silica	SPE	8–10	-	HPLC-MS/MS	82–106	0.044 (AFB <sub>1</sub> ) 0.057 (AFB <sub>2</sub> )	[14]
C <sub>18</sub> , PSA & neutral Al <sub>2</sub> O <sub>3</sub>	QuEChERS	38	-	HPLC-MS/MS	83–100.3	0.18 (AFB <sub>1</sub> ) 0.13 (AFB <sub>2</sub> )	[15]
-	Dispersive liquid-liquid micro-extraction	<20	In situ chemical derivatization	HPLC-FLD	91.8–121.5	0.10 (AFB <sub>1</sub> ) 0.017 (AFB <sub>2</sub> )	[34]
rGO-Fe <sub>3</sub> O <sub>4</sub>	MSPE	15	photochemical derivatization	HPLC-FLD	80.38–109.03	0.10 (AFB <sub>1</sub> ) 0.10 (AFB <sub>2</sub> )	This work



### 3. Conclusions

In the present study, Fe<sub>3</sub>O<sub>4</sub>/rGO MSPE adsorbents were synthesized and used for the extraction and purification of AFB<sub>1</sub> and AFB<sub>2</sub> from vegetable oils. The characterization data indicated that rGO nanosheets were coated with Fe<sub>3</sub>O<sub>4</sub> nanoparticles and possessed uniform size and shape. Owing to their unique features, the Fe<sub>3</sub>O<sub>4</sub>/rGO MSPE adsorbents were used for the enrichment and eliminating the presence of interfering substances in oils. Limits of detection of this method were as low as 0.02 µg/kg and 0.01 µg/kg for AFB<sub>1</sub> and AFB<sub>2</sub>, respectively. The recovery of the analytes was in the range of 80.4%–106.0% and the RSDs less than 8.1%, which suggested optimal accuracy and reliability for the routine determination of aflatoxins in a variety of vegetable oils. Therefore, the Fe<sub>3</sub>O<sub>4</sub>/rGO MSPE-HPLC-PCD-FLD could be applied as a promising analytical method for simple, rapid and accurate quantification of organic contaminants in complex matrices.

### 4. Materials and Methods

#### 4.1. Chemicals and Materials

Graphite flakes (~150 µm flakes), AFB<sub>1</sub> and AFB<sub>2</sub> standards were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). HPLC-grade methanol (MeOH), acetonitrile and acetone were supplied by Fisher Chemical Co. (Geel, Antwerp, Belgium). Potassium permanganate (KMnO<sub>4</sub>), hydrochloric acid (HCl, 37%), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>, 98%), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30%), phosphoric acid (H<sub>3</sub>PO<sub>4</sub>, 85%), ethylene glycol (EG), ethanol, ferric chloride (FeCl<sub>3</sub>), sodium acetate trihydrate (NaAc) and *n*-hexane were of analytical grade and were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Unless otherwise stated, all other inorganic chemicals and organic solvents were of analytical reagent grade or higher. The water used was purified with the Milli-Q system from Millipore Co. (Billerica, MA, USA).

A mixed stock solution was prepared with methanol and stored at −20 °C in the dark. A series of standard solutions were prepared by diluting the stock solution with methanol to appropriate concentrations. All the standard solutions were stored at 4 °C in the dark. The stability of AFB<sub>1</sub> and AFB<sub>2</sub> (0.10 µg/kg) were all evaluated as those standard solution kept in the auto-sampler at 4 °C (48 h) and the CV of six injections were 3.7% and 4.1%, respectively.

#### 4.2. Apparatus

An ultrasonic instrument KQ-800KDE (Kunshan Ultrasound Instrument Co., Kunshan, China) and a high-speed centrifuge CF16RXII (Hitachi Co., Tokyo, Japan) were used for GO preparation. Chromatographic analyses were performed on an Agilent 1100 HPLC-FLD system equipped with a photochemical post-column derivatization reactor (Pribolab Pte. Ltd., Singapore). The X-ray diffraction (XRD) experiment was performed on a X'Pert powder diffractometer (PANalytical Co., Almelo, The Netherlands) with a Cu Kα radiation (λ = 1.5418 Å) and a graphite monochromator. The diffraction data were recorded for 2θ between 0.5° and 70° with a resolution of 0.033°. The size and morphology of the magnetic nanoparticles were observed by a Hitachi S-4800 scanning electron microscope (Hitachi Ltd., Tokyo, Japan).

#### 4.3. Synthesis of Fe<sub>3</sub>O<sub>4</sub>/rGO Adsorbents

GO was prepared from graphite flakes using KMnO<sub>4</sub> and a 9:1 mixture of concentrated H<sub>2</sub>SO<sub>4</sub>/H<sub>3</sub>PO<sub>4</sub> as oxidizing agents by a method reported in our previous study [29]. The Fe<sub>3</sub>O<sub>4</sub>/rGO nanocomposite was synthesized via a facile one-pot solvothermal method [35]. In a typical process, 400 mg of GO was mixed in 60 mL of EG and was homogenized for 4 h under ultrasonic vibrations in order to produce a homogeneous solution. Subsequently, 0.65 g of FeCl<sub>3</sub> was dissolved in the GO containing solution. Homogenization was achieved by ultrasonic vibrations for 20 min. 2.6 g of NaAc was added into the aforementioned solution, which was vigorously stirred for 30 min to ensure that the precursor was dissolved in the solution completely. Finally, the mixed solution was transferred

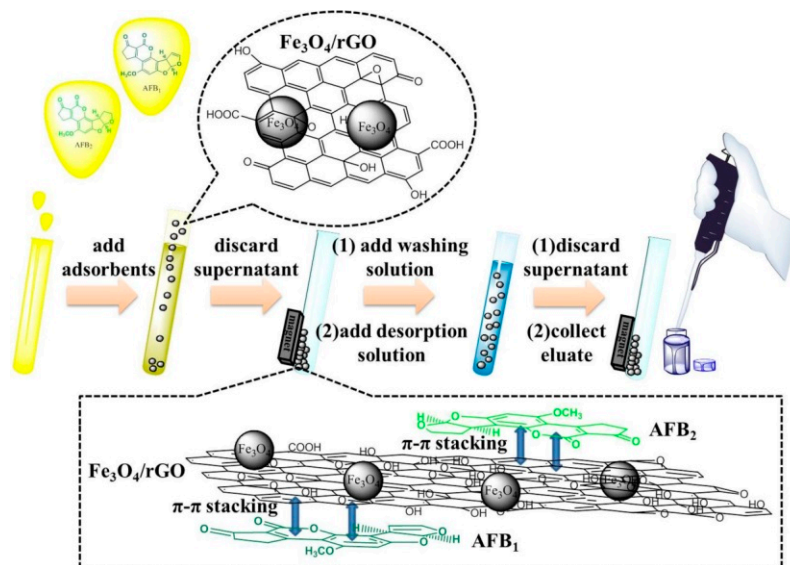
into a teflon-lined stainless-steel autoclave, maintaining a set temperature of 200 °C for 8 h. When the autoclave was cooled down to room temperature, the obtained black product was filtered, washed with ethanol for several times and dried in vacuum.

#### 4.4. Sample Preparation

Different types of edible vegetable oils, including rapeseed oil, peanut oil, corn oil, soybean oil, walnut oil, rice oil and almond oil, were purchased from local markets (Wuhan, China). All the oil samples were stored at room temperature.

#### 4.5. Magnetic Solid-Phase Extraction Procedure

The MSPE procedure of AFB<sub>1</sub> and AFB<sub>2</sub> from the oil samples was illustrated in Figure 5. Initially, 2.0 g ( $\pm 0.001$  g) of oil sample was weighed accurately and diluted with 10 mL of *n*-hexane. Subsequently, 12.0 mg of Fe<sub>3</sub>O<sub>4</sub>/rGO adsorbents were added to the mixture and vigorously vortexed for 5 min. A powerful magnet was applied to the bottom of the tube to attract and isolate the magnetic graphene adsorbent, and the supernatant was discarded. A total of 2 mL of *n*-hexane was used for washing in order to remove the interfering compounds in the lipid matrix by vortexing for 60 s. Finally, 4 mL of acetonitrile was used for the desorption and was added by ultrasonic agitation for 3 min. The desorption solution was evaporated under a mild stream of N<sub>2</sub> at 40 °C and reconstituted with 100  $\mu$ L H<sub>2</sub>O/MeOH (55:45, *v/v*).



**Figure 5.** The schematic illustration of MSPE based on the magnetic graphene nanocomposite.

#### 4.6. HPLC-PCD-FLD Analytical Conditions

The chromatographic separation was performed on a Kromasil C<sub>18</sub> column (150 mm  $\times$  4.6 mm, 5  $\mu$ m particle size) using a H<sub>2</sub>O/MeOH (55:45, *v/v*) mobile phase at a flow rate of 0.8 mL/min with a total running time of 20 min. The detection wavelengths were set at 360 nm and 440 nm for the excitation and emission, respectively. The column temperature was set at 30 °C and the injection volume was adjusted to 10  $\mu$ L.

#### 4.7. Statistical Analysis

All the vegetable oils were analyzed in triplicate, and the results were reported as average  $\pm$  standard deviation (SD). The statistical analyses were performed using the @Risk 5.5.1 software package from Palisade Co. (Australia, 2010). Significant differences were determined by the Student *t*-test at a significance level of 0.05 ( $p < 0.05$ ).

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