

Research Article

Separation and Determination of Paraquat and Diquat in Human Plasma and Urine by Magnetic Dispersive Solid Phase Extraction Coupled with High-Performance Liquid Chromatography

Ou Sha,^{1,2} Bowen Cui,¹ Xiaobing Chen ,³ Hua Liu,¹ Jiawei Yao,¹ and Yuqing Zhu¹

¹Department of Chemical Engineering, Jiangsu Ocean University, Lianyungang 222005, China

²Analysis and Test Center of Jiangsu Marine Resources Development Research Institute, Jiangsu, Lianyungang 222005, China

³The First People's Hospital of Lianyungang, Lianyungang 222005, China

Correspondence should be addressed to Xiaobing Chen; jxm7901@163.com

Received 17 January 2020; Revised 22 March 2020; Accepted 15 June 2020; Published 16 July 2020

Academic Editor: Serban C. Moldoveanu

Copyright © 2020 Ou Sha et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

A magnetic dispersive solid phase extraction method coupled with high-performance liquid chromatography was proposed for the simultaneous separation and determination of paraquat (PQ) and diquat (DQ) in human plasma and urine samples. Based on the reduction of PQ and DQ to a blue radical and yellow-green radical by sodium dithionite in an alkaline medium, a fast colorimetric method was also developed for the fast detection of PQ or DQ. In this paper, $\text{CoFe}_2\text{O}_4/\text{SiO}_2$ magnetic nanoparticles were used as the adsorbent for the magnetic dispersive solid phase extraction of paraquat and diquat, and these two analytes were found to be eluted directly from the adsorbent by NaOH solution. The main factors affecting the extraction efficiency including amount of extractant, extraction time, sample volume, sample solution pH, and elution volume were optimized. Under the optimized experimental conditions, the calibration curve was linear at a concentration range of 28.5–570.2 $\mu\text{g/L}$, and the correlation coefficient of paraquat and diquat was 0.9986 and 0.9980, respectively. The limits of detection of paraquat and diquat were 4.5 $\mu\text{g/L}$ and 4.3 $\mu\text{g/L}$. The proposed MSPE-HPLC method was successfully applied to the detection of the paraquat and diquat in human plasma and urine with satisfied recoveries of PQ and DQ in the range of 87.5%–98.7%.

1. Introduction

Paraquat (PQ) and diquat (DQ) are nonselective contact herbicides, which have been widely used in the world for controlling the growth of weeds and grass in order to achieve high agricultural productivity (Figure 1) [1, 2]. However, PQ is highly toxic to human, and an exposure to paraquat via accidental or suicidal ingestion can cause acute renal toxicity and be frequently fatal in small doses. Compared with PQ, DQ causes severe toxic effect on the central nervous system [3]. Although PQ has been banned by the European Union and China and will be banned in Malaysia and other developing countries in the future, it is ubiquitous and can still be obtained in the developing countries for the characteristics of lower price and the most cost-effective control of weed [4, 5]. Findings are reported

by Mai that more than 300 PQ-poisoned cases reported per year in Vietnam [6].

Usually the concentrations of PQ or DQ in plasma and urine are applied to diagnose the severity of poisoning and help doctors to rescue patients [7]. Different approaches to measuring PQ or DQ concentration are available including high-performance liquid chromatography (HPLC) [8], high-performance liquid chromatography-mass spectrometry (HPLC-MS) [9], gas chromatography-mass spectrometry (GC-MS) [3], square wave voltammetry [10], and the spectrophotometric method [11]. Another option reported in the literature is the application of surface-enhanced Raman spectroscopy (SERS) [12]. Compared with other methods, HPLC is most commonly used for determination of PQ and DQ because the instrument is commonly available in laboratories. And HPLC has high sensitivity and separation efficiency.

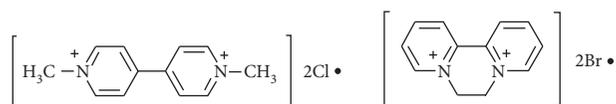


FIGURE 1: The structure of PQ and DQ.

Since PQ and DQ are both highly soluble in water and have extremely poor affinity for nonpolar solvents, making the separation and preconcentration before the determination of PQ or DQ is often problematic. The complexity, time consumption, and equipment demand of most approaches makes them impractical, especially in resource-poor environments. Improved sample preparation approaches for the extraction of PQ or DQ from plasma and urine samples, such as solid phase extraction [13], liquid phase extraction [14], and so on, have been developed [15]. However, the complex procedure including protein precipitation [16] by acetonitrile or protein removal by ultrafiltration membrane could reduce the sensitivity of the detection method and increase the analysis time.

Magnetic dispersive solid phase extraction (MDSPE) is a promising sample separation and pretreatment technique. In MDSPE, the magnetic adsorbent is dispersed in sample solution containing the target analytes. The higher the interfacial area between the extractant and sample, the faster the mass transfer, thus equilibrium is reached sooner. After extraction, an external magnetic field was applied to separate the enriched magnetic sorbent, and the sorbent phases with magnetic properties enable assisted magnetic separation of the aqueous sample. This approach enables the sorbent to interact equally with all the sorbent particles, achieving greater capacity per amount of sorbent and avoiding channeling or blocking of cartridges or disks, as it occurs in traditional SPE [17, 18].

CoFe_2O_4 , as an excellent soft magnetic material, has good superparamagnetism with high saturation magnetization and anisotropy constant, and it can be assembled into tiny and multifunctional biocompatible devices [19]. Aygar et al. synthesized $\text{CoFe}_2\text{O}_4@SiO_2$ nanoparticles for the purification of histidine-tagged proteins [20]. Abdolmohammad-Zadeh et al. prepared CoFe_2O_4 nanoparticles functionalized with 8-hydroxyquinoline for dispersive solid-phase microextraction and direct fluorometric monitoring of aluminum in human serum [21].

Depending on the urgent needs for developing rapid and high selective methods to determine PQ and DQ with lower detection limits in plasma and urine samples, magnetic dispersive solid phase extraction coupled with HPLC-UV, $\text{CoFe}_2\text{O}_4@SiO_2$ nanoparticles selected as the adsorbent, was developed for the separation and determination of PQ and DQ for the first time. After a series of optimization of extraction conditions, the proposed analytical method with notably reduced matrix effects was successfully applied for determination of PQ and DQ. It was contributed to reduce the matrix effects with the procedure of no need of protein precipitation and protein removal. Meanwhile, based on the reduction of PQ and DQ to form a blue radical and yellow-green radical by

sodium dithionite in alkaline medium, respectively, a rapid and simple method was established to colorimetrically detect PQ or DQ in human plasma and urine samples with naked eyes. This method proposed in this paper could offer a fast assessment of patient outcome based on the experimental data and helped the doctor to decide treatment strategies.

2. Experimental

2.1. Materials and Apparatus. Paraquat and diquat were purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Iron nitrate nonahydrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$), cobaltous nitrate hexahydrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$), sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$), tetramethyl orthosilicate (TEOS), polyethylene glycol 6000 (PEG 6000), phosphoric acid (H_3PO_4), ethanol, and triethylamine were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The stock solution of PQ or DQ (100.0 mg/L) was prepared by dissolving the appropriate amount of reagent in deionized water and storing at 4°C in the refrigerator until used. Solutions with lower paraquat or diquat concentrations were made by appropriate dilution of the standard solution. Britton–Robinson (B–R buffer) solution is configured with 0.04 mol/L mixed acid (H_3PO_4 , HAc, and H_3BO_3) and 0.2 mol/L NaOH solution. All reagents were of analytical grade.

2.2. Instrument. The HPLC analysis was performed by LC-20AD chromatograph (Shimadzu Corporation, Japan). The pH value was adjusted by a PHS-25B pH meter (Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China). X-ray diffraction (XRD) pattern was collected by a X'Pert Pro diffractometer (PANalytical, Netherlands). The Fourier transform infrared spectra (FT-IR) were recorded on a Bruker Vector 22 spectrometer (Bruker company, Germany). Magnetization measurement was studied by an MPMS-7 vibrating sample magnetometer (VSM) (Quantum Design, USA).

2.3. Synthesis of $\text{CoFe}_2\text{O}_4@SiO_2$ MNPs. Synthesis of $\text{CoFe}_2\text{O}_4@SiO_2$ was carried out using the Stöber method [22]. 5.82 g $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 16.16 g $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, and 5.88 g $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ were dispersed in 100 mL of deionized water. Then, 30 mL of $\text{NH}_3 \cdot \text{H}_2\text{O}$ was quickly added into the solution to obtain the gels under stirring at 80°C for 2 h. The gels were thermally treated at 500°C for 3 h to obtain CoFe_2O_4 .

Then, 2.0 g of CoFe_2O_4 was dissolved in distilled water and placed in a three-neck flask in an ultrasonic bath for 10 min and dispersed in 40.0 mL of distilled water, 80 mL of ethanol, and 5.0 mL of ammonia. After that, 2.0 mL of TEOS was added slowly to suspension under mechanical stirring. The reaction was allowed to proceed at 60°C for 12 h. The obtained *c* was washed three times with deionized water and ethanol, respectively, and dried at 60°C for 6 h.

2.4. Sample Collection. This study was approved by the Ethics Committee of the Jiangsu Ocean University (Lianyungang, China). Human plasma samples were obtained from patients of PQ intoxication and healthy human with no known record of occupational exposure to PQ (as blank) from the First People's Hospital of Lianyungang. Written consent was signed by all participants. Plasma samples spiked with standard PQ stock solutions to indicate concentrations were used as positive controls. The venous blood was drawn from the forearm of subjects and rapidly transferred to vessels containing heparin as an anticoagulant. Plasma was separated by centrifugation of the venous blood at 14000 rpm for 10 min and stored at 4°C. Human urine samples were obtained from the First People's Hospital of Lianyungang and stored at 4°C. The urine samples were centrifuged for 30 min at 3000 rpm and filtered through 0.45 μm membrane filters, and no further treatment was needed.

2.5. Quantitative Process. Human plasma or urine samples were added into a 40 mL polyethylene tube and diluted to the scale line with deionized water. Then, 30.0 mg CoFe₂O₄@SiO₂ MNPs were put into this solution and dispersed by vortex for 10 min. The MNPs were collected by a Nd-Fe-B magnet, whereas the supernatant solution was discarded. The collected MNPs were washed three times by deionized water, then immersed into 1.0 mL of 2 mol/L NaOH, and vortexed for 1 min. Magnet was used to separate the MNPs again. Then, the eluent was adjusted to pH 7.0 with phosphoric acid and filtered through a 0.22 μm nylon syringe filter. A total 20 μL of elution was injected into the HPLC system for DQ or PQ determination. The complete process of MDSPE system combined with HPLC-UV for determination of PQ and DQ was shown in Figure 2. The extraction efficiency (1) and the amount of analyte adsorbed per unit mass of the adsorbent (adsorption capacity) (2) were calculated by the following equation:

$$\text{Extraction efficiency (\%)} = \frac{C_0 - C_t}{C_0} \times 100\%, \quad (1)$$

$$\text{Adsorption capacity} = \frac{C_0 - C_t}{m} \times V. \quad (2)$$

where C_t (μg/mL) was the concentration of PQ or DQ in the supernatant after adsorption; C_0 (μg/mL) was the initial concentration of PQ or DQ; V (L) was the volume of sample solution; and m (g) was the mass of adsorbents.

2.6. Colorimetric Detection Process. The colorimetric detection of paraquat or diquat concentration was performed by comparing the color against the standard colorimetric systems, which were made with known concentrations of PQ and DQ. After the MDSPE extraction procedure, as described in subsection 2.4, 25.0 mg/L of Na₂S₂O₄ solution (0.5 mL) was added to the eluent of PQ or DQ. Then, observation was done after 2 minutes shaking time and 1 minute stand-by time. The

colored solutions corresponding to different target concentrations were finally measured by naked eyes for the colorimetric detection.

2.7. HPLC Detection Conditions. Welch AQ-C18 column (4.6 mm × 250 mm, 5 μm, USA), an analytical column, was used for analyte. The mobile phase was a mixture of methanol and 0.2 mol/L sodium dihydrogen phosphate buffer (10 : 90, v/v) containing both 0.1 mol/L triethylamine and 12 mmol/L sodium 1-heptane sulfonate. The mobile phase was adjusted to pH 2.8 with phosphate acid at a flow rate of 0.5 mL·min⁻¹. The injection volume was 20 μL, and the detection wavelengths of PQ and DQ were set at 258 nm and 310 nm, respectively.

3. Results and Discussion

3.1. Characterization of CoFe₂O₄@SiO₂. The XRD patterns of CoFe₂O₄@SiO₂ (a) and CoFe₂O₄ (b) are shown in Figure 3. It can be observed that the XRD pattern of CoFe₂O₄ MNPs (curve (b)) is consistent with the ICPSD file of CoFe₂O₄ (No.22-1086) [23]. And the diffraction peaks of CoFe₂O₄@SiO₂ (curve (a)) are similar to those of CoFe₂O₄. Compared with these two curves, the broad peak at 20–28° found in CoFe₂O₄@SiO₂ correspond to the silica coated on the surface of CoFe₂O₄, which corresponds to the standard diffraction pattern of SiO₂ [24]. The result illustrated that the SiO₂ was successfully coated on the surface of CoFe₂O₄ MNPs.

The functional groups and chemical bonds in CoFe₂O₄@SiO₂ (curve (a)) and CoFe₂O₄ (curve (b)) were analyzed by means of FT-IR. The diffraction peaks of 465 cm⁻¹, 778 cm⁻¹, and 1130 cm⁻¹ attributed to the bending vibrations of the Si-O-Si are shown in Figure 4. The presence of Fe-O bonds in the magnetic particles was confirmed by the characteristic peaks appeared at 580 cm⁻¹ [25]. These results revealed the successful preparation of CoFe₂O₄@SiO₂ MNPs.

The magnetic properties of CoFe₂O₄@SiO₂ and CoFe₂O₄ were elucidated by the magnetization hysteresis curve. As shown in Figure 5, the saturation magnetization values of the CoFe₂O₄@SiO₂ and CoFe₂O₄ were 36 emu g⁻¹ and 42 emu g⁻¹, respectively. The saturation magnetization of CoFe₂O₄@SiO₂ was lower than CoFe₂O₄, which was attributed to the effect of the amorphous silica layer coated [26]. Although the saturation magnetization of CoFe₂O₄ decreased after coating SiO₂, the CoFe₂O₄@SiO₂ and CoFe₂O₄ could be rapidly separated from the solution by Nd-Fe-B magnet.

3.2. Optimization of PQ and DQ Extraction Conditions. To investigate the optimum conditions for the MDSPE process of PQ and DQ, 30.0 mg of CoFe₂O₄@SiO₂ was spiked with the mixture of PQ or DQ at a concentration of 500 μg/L. The relevant experimental conditions including pH value, amount of adsorbent, extraction time, sample volume, and elution volume were optimized. All the experiments were performed in triplicates.

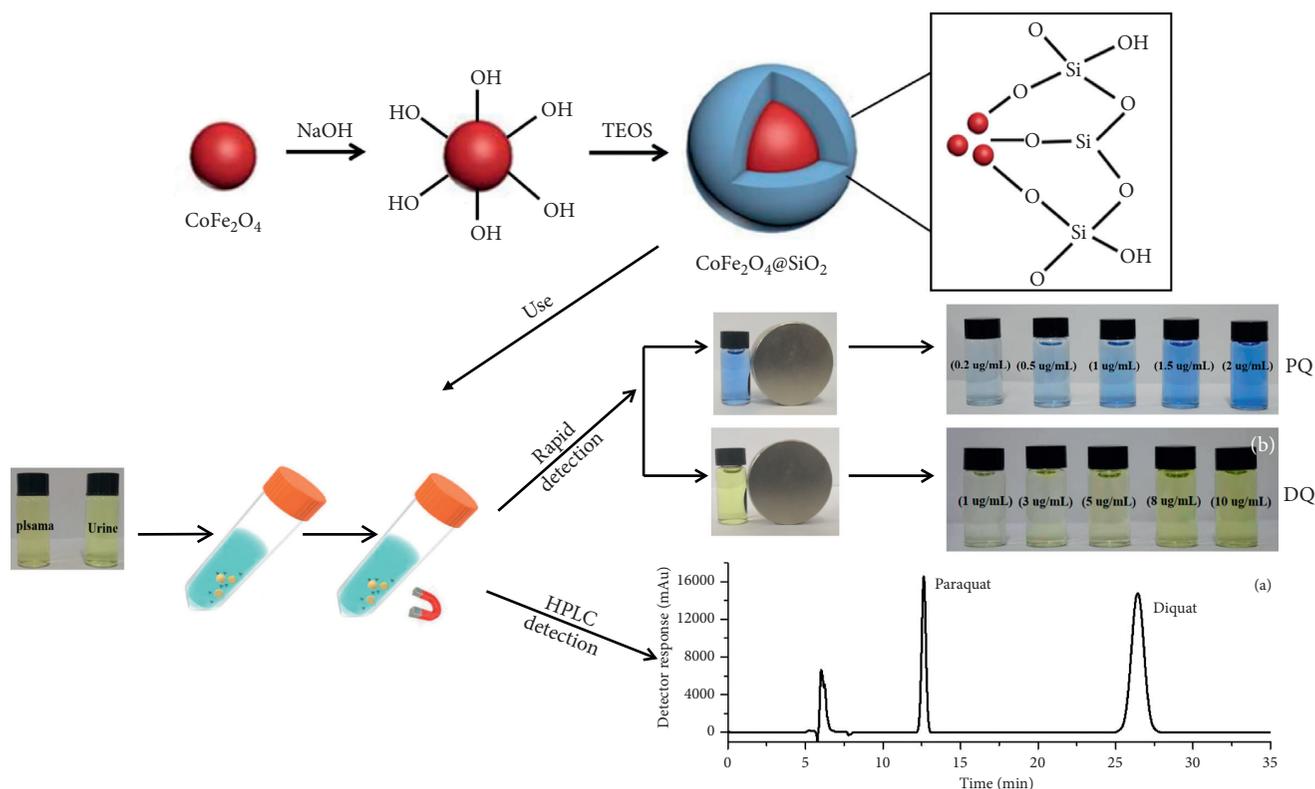


FIGURE 2: Preparation of CoFe₂O₄@SiO₂ and the MSPE procedure for paraquat/diquat in plasma and urine.

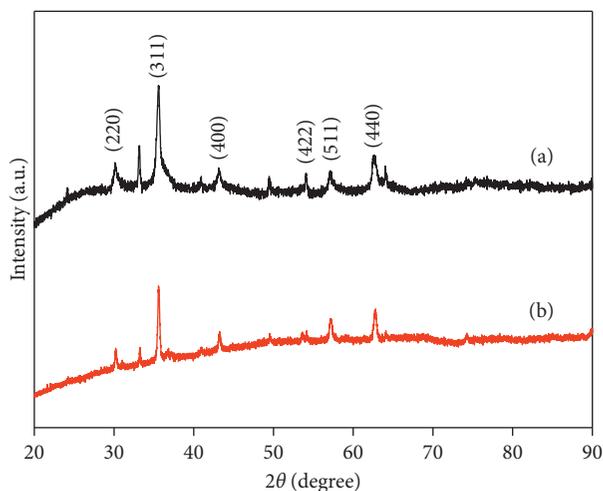


FIGURE 3: XRD patterns of CoFe₂O₄@SiO₂ (a) and CoFe₂O₄ (b).

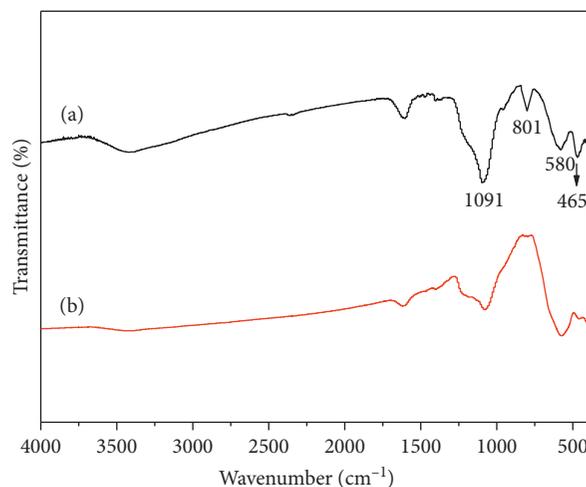


FIGURE 4: FT-IR spectra of CoFe₂O₄@SiO₂ (a) and CoFe₂O₄ (b).

3.2.1. Effect of pH. The pH of the sample solution plays an important role in the extraction of PQ and DQ by affecting both the existing forms of the target compounds and the adsorbent. In this study, the effect of sample pH ranging between 4.0 and 11.0 was investigated by adding appropriate volumes of B-R buffer solution. As shown in Figure 6, the extraction efficiencies of PQ and DQ were increased by the pH and kept stable in the pH range of 7.0–11.0. Generally, paraquat and diquat are used as the forms of dichlorides and dibromides. In aqueous solution, they are both positively charged form and are retained stable when the solution is

under weak acid or neutral conditions, whereas they are unstable in alkaline solution with a high pH value [27]. When CoFe₂O₄@SiO₂ nanoparticles were selected as the sorbent, it has been reported that the surface negative charge of CoFe₂O₄@SiO₂ magnetic nanoparticles could be changed with the pH of the solution. As the pH of the solution increases, the negative charge on the surface of the nanoparticles increases gradually. Therefore, the cationic herbicide could be adsorbed to the adsorbent surface by an electrostatic interaction with the CoFe₂O₄@SiO₂ magnetic nanoparticles [28].

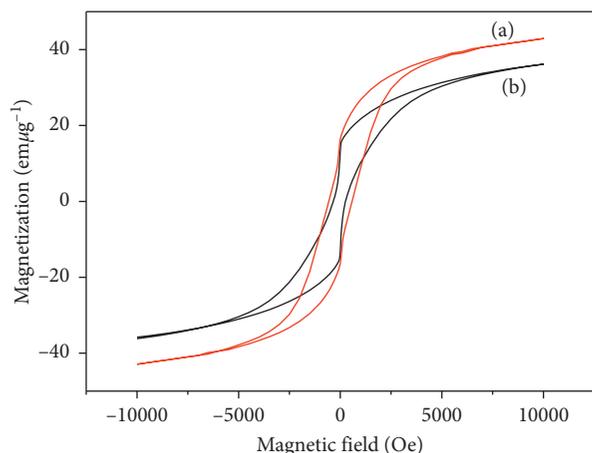


FIGURE 5: Magnetic hysteresis curves of CoFe_2O_4 (a) and $\text{CoFe}_2\text{O}_4@SiO_2$ (b).

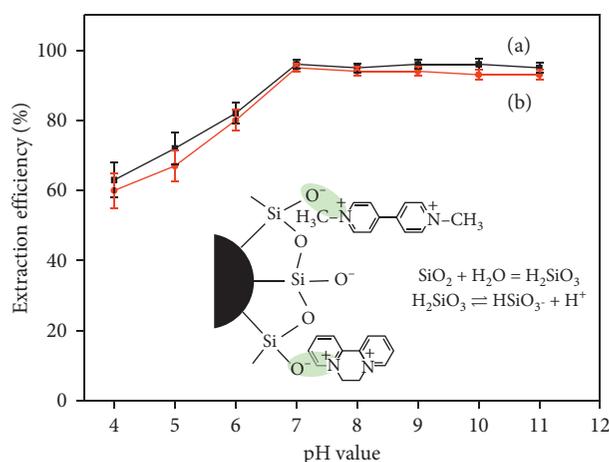


FIGURE 6: Effect of pH for the extraction efficiency of PQ (a) and DQ (b) and the adsorption mechanism.

Since the pH value of body fluids generally fluctuates between 6.8 and 7.5, pH 7.0 was selected for further experiments. The extraction efficiencies of PQ and DQ in different samples are shown in Figure 7. It was found that the matrix effects on the extraction efficiency of PQ and DQ could be neglected. Therefore, a standard solution was chosen for the optimization process.

3.2.2. Effect of the Amount of Extractant. As the amount of adsorbent increases, it can provide a larger adsorption specific surface area, thus increasing the extraction rate. To select the optimal amount of the magnetic extractant for extraction of the PQ/DQ, the different amount of $\text{CoFe}_2\text{O}_4@SiO_2$ MNPs was evaluated in the range of 1.0 mg–40.0 mg. As shown in Figure 8, the extraction efficiency of PQ/DQ increased significantly with the amount of $\text{CoFe}_2\text{O}_4@SiO_2$ MNPs in the range from 1.0 mg to 25.0 mg. Subsequently, the maximum extraction efficiency was achieved and kept stable while the amount of adsorbent was above 25.0 mg. Therefore, 30.0 mg of extractant was selected for further experiments.

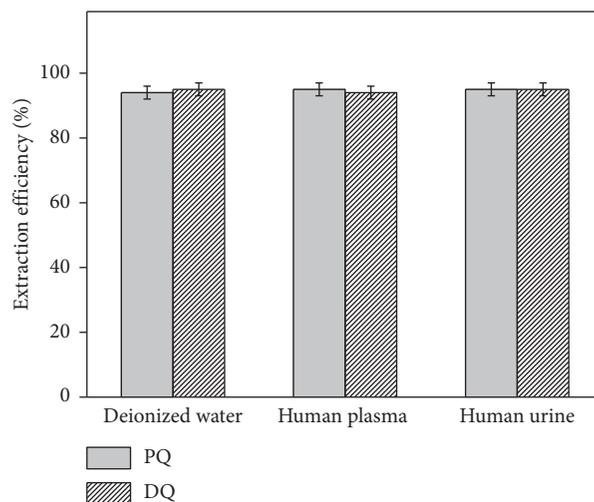


FIGURE 7: The extraction efficiencies of PQ and DQ in different samples.

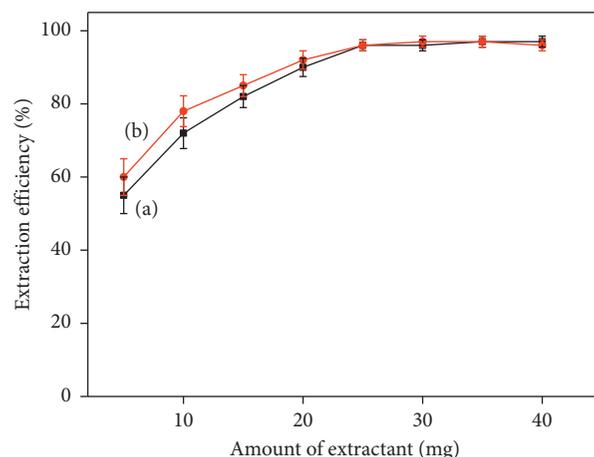


FIGURE 8: Effect of the amount of adsorbent for the extraction efficiency of PQ (a) and DQ (b).

3.2.3. Effect of Extraction Time. In the MDSPE process, the extraction time is one of the critical factors that influence the extraction efficiency of PQ and DQ. The effect of extraction time on the extraction efficiency was investigated in the range of 0–20 min. With the increased extraction time, the contact between the extractant and the analyte was more effective, and accordingly, the adsorption equilibrium was promoted. As shown in Figure 9, the maximum extraction efficiency was obtained and remained constant while extraction time exceeded 10 min. Therefore, the extraction time of 10 min was selected for further experiment.

3.2.4. Effect of Sample Volume. Preconcentration factor is a significant factor for the analysis method. The effect of sample volume on extraction efficiency was investigated by changing the sample volumes. As shown in Figure 10, the extraction efficiency remained stable ($E \geq 90\%$) when the sample volume

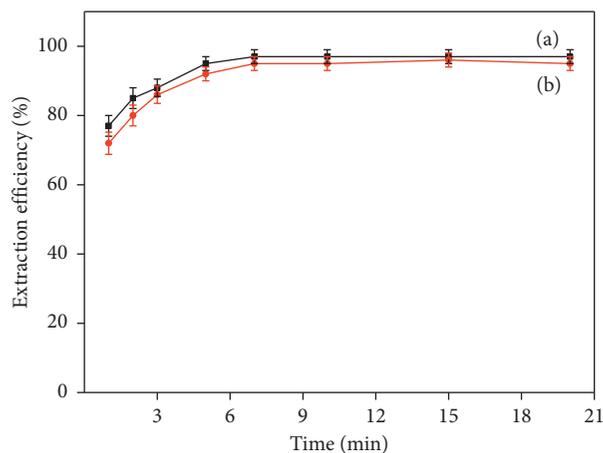


FIGURE 9: Effect of time for the extraction efficiency of PQ (a) and DQ (b).

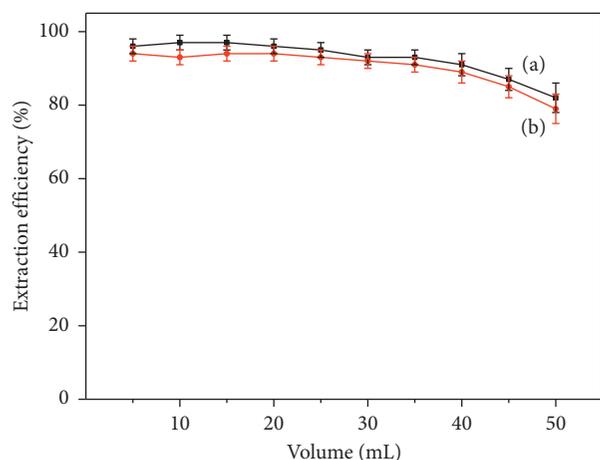


FIGURE 10: Effect of volume for the extraction efficiency of PQ (a) and DQ (b).

was less than 40.0 mL. Then, the extraction efficiency was reduced obviously. Since the extraction time and the amount of adsorbents were set at a certain value, the dispersion degree of adsorbents in sample solution decreased with the increase of sample volume, resulting in the decrease of effective contact probability between the adsorbent and the analytes. Therefore, the extraction efficiency reduced, whereas the pre-concentration factor of analytes increased by increasing the sample volume. Therefore, 40.0 mL was selected as the maximum sample volume to obtain a higher pre-concentration factor and the better extraction efficiency.

3.2.5. Adsorption Capacity. Adsorption capacity is a significant parameter for adsorption properties. In this work, the adsorption capacity of $\text{CoFe}_2\text{O}_4@/\text{SiO}_2$ MNPs for PQ and DQ was calculated by varying the concentration of PQ or DQ, while the amount of extractant was selected as 30.0 mg, and the sample volume was selected as 20.0 mL. As shown in Figure 11, the adsorption capacity for PQ and DQ reached 1.8 mg/g or 2.2 mg/g, while the concentration of PQ or DQ was 2.5 mg/L.

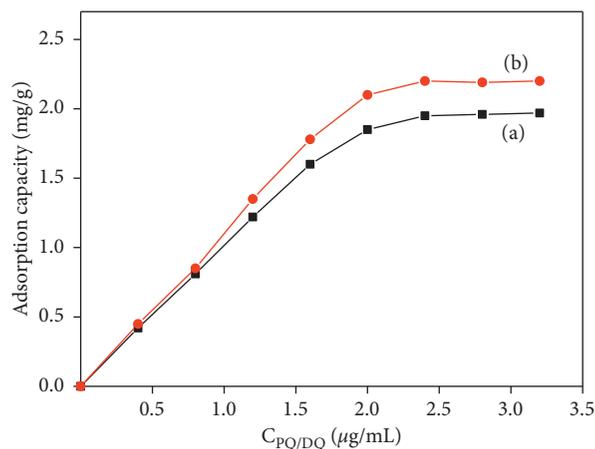


FIGURE 11: Adsorption capacity of $\text{CoFe}_2\text{O}_4@/\text{SiO}_2$ to PQ (a) and DQ (b).

3.2.6. Effect of Elution Volume. Although PQ and DQ could be absorbed by $\text{CoFe}_2\text{O}_4@/\text{SiO}_2$ MNPs under the pH of 7.0–12.0, the reduced product of PQ and DQ reacted with the strong alkaline (NaOH solution) could not be absorbed by $\text{CoFe}_2\text{O}_4@/\text{SiO}_2$ MNPs [29]. Therefore, 2.0 mol/L of NaOH solution was chosen as the eluent. The effect of the elution solvent volume was investigated in the range of 0.2–2.0 mL. When the volume of eluent increased to 1.0 mL, PQ and DQ could be desorbed completely. To obtain a higher pre-concentration factor, 1.0 mL of desorption solvent volume was chosen.

3.2.7. Reusability Test. The adsorbed $\text{CoFe}_2\text{O}_4@/\text{SiO}_2$ MNPs were washed with 2 mol/L NaOH solution and distilled water, respectively, and then, the regenerated MNPs were used for the adsorption of PQ or DQ again. As shown in Figure 12, the adsorption efficiencies of PQ and DQ are still maintaining 85% after six times adsorption/desorption procedures, which illustrated that $\text{CoFe}_2\text{O}_4@/\text{SiO}_2$ MNPs have a better recycling performance for extraction of PQ and DQ.

3.2.8. Adsorption and Desorption Mechanisms. In this study, the adsorption and desorption mechanisms of $\text{CoFe}_2\text{O}_4@/\text{SiO}_2$ toward PQ/DQ were investigated. As the result of zeta potential, the surface of $\text{CoFe}_2\text{O}_4@/\text{SiO}_2$ NPs had negative charge with a zeta potential of -8.1 mV, which generated the electrostatic attraction with cationic pesticides (PQ or DQ). Therefore, the adsorption of $\text{CoFe}_2\text{O}_4@/\text{SiO}_2$ to PQ/DQ could be ascribed to electrostatic attraction. Depending on previous reports, paraquat and diquat can be reduced to blue free radicals and green free radicals by $\text{Na}_2\text{S}_2\text{O}_4$ in an alkaline solution [30]. In order to prove that PQ and DQ can be desorbed by NaOH solution, $\text{Na}_2\text{S}_2\text{O}_4$ was added to the eluents of PQ and DQ, respectively. The UV spectrogram of PQ/DQ-NaOH- $\text{Na}_2\text{S}_2\text{O}_4$ and eluent of PQ/DQ- $\text{Na}_2\text{S}_2\text{O}_4$ are shown in Figure 13. No noticeable differences were found between these two spectral curves. The results revealed that PQ and DQ could be desorbed completely from the surface of $\text{CoFe}_2\text{O}_4@/\text{SiO}_2$ by NaOH solution.

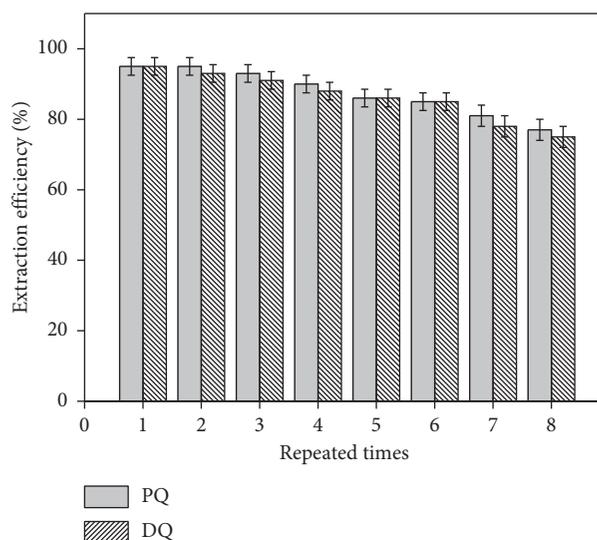


FIGURE 12: Reusability tests of $\text{CoFe}_2\text{O}_4@\text{SiO}_2$ MNPs for the extraction efficiency toward PQ/DQ.

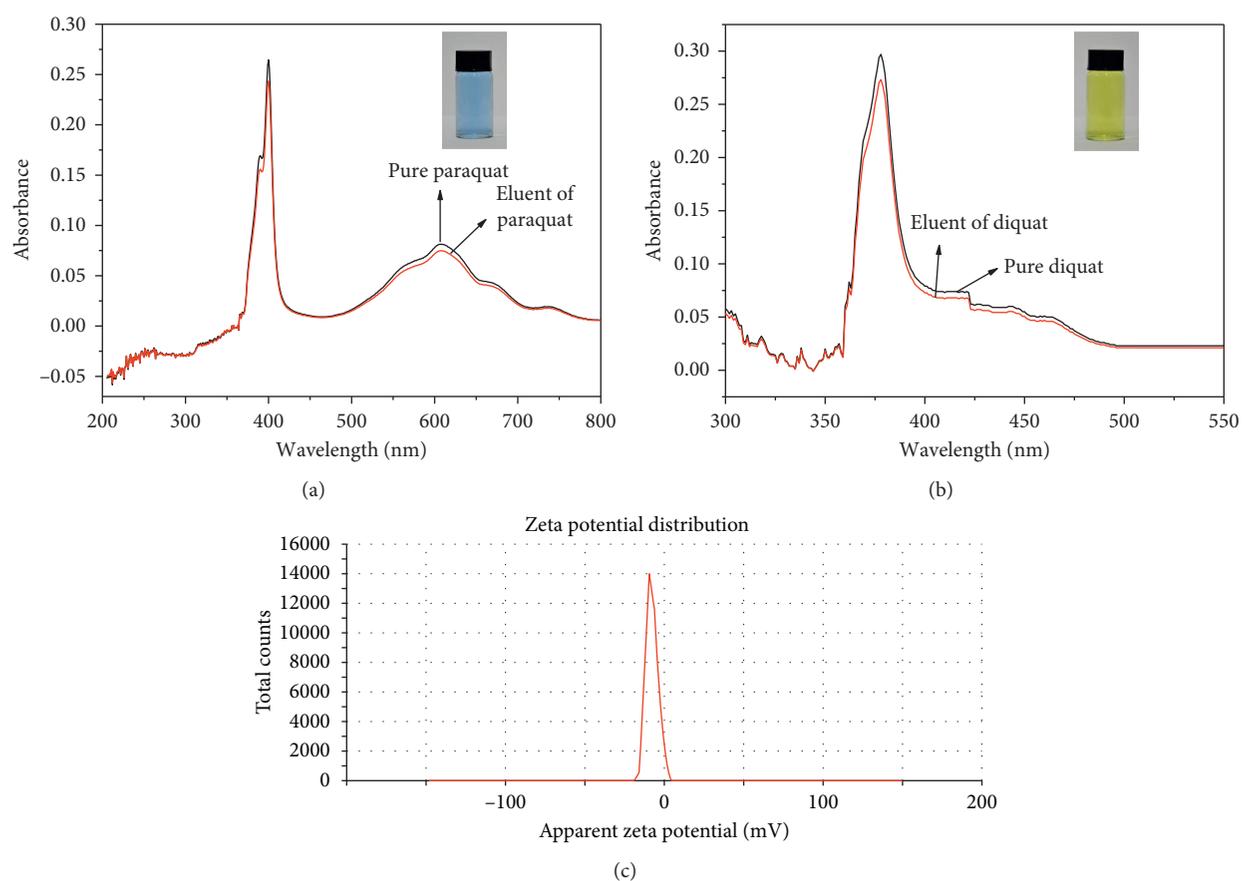


FIGURE 13: (a) The UV spectrogram of PQ-NaOH- $\text{Na}_2\text{S}_2\text{O}_4$ and eluent- $\text{Na}_2\text{S}_2\text{O}_4$; the insert image is blue free radicals of paraquat. (b) The UV spectrogram of DQ-NaOH- $\text{Na}_2\text{S}_2\text{O}_4$ and eluent- $\text{Na}_2\text{S}_2\text{O}_4$; the insert image is green free radicals of diquat. (c) The zeta potential of $\text{CoFe}_2\text{O}_4@\text{SiO}_2$ NPs.

3.3. Colorimetric Detection Method. In aqueous systems, paraquat and diquat could be reduced to form blue radicals and yellow radicals by sodium dithionite in an

alkaline medium, respectively. Under the optimum conditions, the working range concentration was studied by using different concentrations of PQ and DQ from 0.2 to

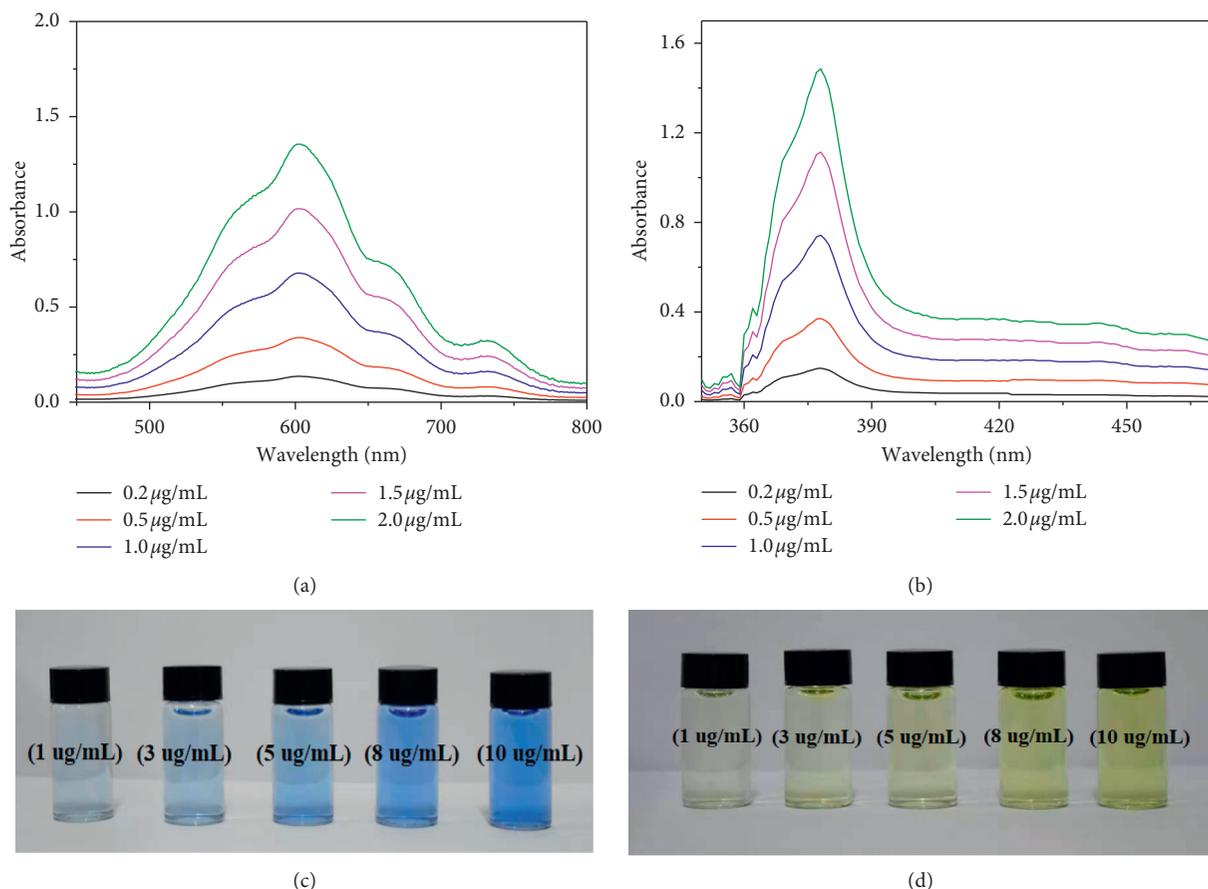


FIGURE 14: The UV spectra of PQ (a) and DQ (b) at different concentrations from 0.2 to 2.0 $\mu\text{g/mL}$. The colorimetric responses to different concentrations of PQ (c) and DQ (d) from 0.2–2.0 $\mu\text{g/mL}$.

2.0 $\mu\text{g/mL}$. The developed color of these two radical was observed by the naked eye. The color change depended on the amount of PQ and DQ as shown in Figure 14. The gradual change to the blue and the yellow color was observed by the naked eye with the increased concentration. At the concentration of 0.2 $\mu\text{g/mL}$ of PQ or DQ, the obtained color can be distinguished from blank by the naked eye. So the detection limits of this colorimetric detection method were both 0.2 $\mu\text{g/mL}$ for PQ and DQ in human plasma (5 mL) and both 0.02 $\mu\text{g/mL}$ in human urine sample (50 mL).

3.4. Analytical Properties. Under the optimal conditions, a series of quantitative parameters with regard to the linearity, limits of detection (LODs), limit of quantitation (LOQ), and the preconcentration factor were used to validate the proposed MDSPE–HPLC method. Linear regression analysis was performed using peak areas against the concentrations of PQ and DQ. The good linearity was obtained in the range of 28.5–570.2 $\mu\text{g/L}$ for PQ and DQ with good correlation coefficients (>0.9950). The LOD and LOQ defined as three times and ten times ratio of signal to noise for chromatograms were obtained from the blank, respectively [31]. The LOD of PQ and DQ was 4.5 $\mu\text{g/L}$ and 4.3 $\mu\text{g/L}$, respectively. The LOQ of PQ

and DQ was 15.2 $\mu\text{g/L}$ and 14.3 $\mu\text{g/L}$, respectively. The preconcentration factor, defined as the slope of the regression equations before the extraction and after the elution, was 30.8 fold. Table 1 presents a comparison of the performance of the proposed MDSPE method with other methods for determination of PQ in plasma and urine. The proposed method described herein provided a lower range of LOD and satisfactory recoveries compared to other reported methods.

3.5. Precision and Accuracy. Quantification of paraquat and diquat in human plasma and urine was evaluated for both intraday and interday precision and accuracy at three different concentration levels with relative standard deviation (RSD). Intraday precision and accuracy were evaluated with 6 replicate bench preparations at each concentration level analyzed on the same day. Interday precision and accuracy were evaluated with a total of 18 replicate bench preparations in three consecutive days. Table 2 indicated the recoveries of the spiked concentrations (10.0, 20.0, and 50.0 ng/mL) of PQ or DQ in plasma and urine samples. The intraday recovery ranged from 88.6% to 96.7% with the RSD varied from 2.7% to 5.8%. The interday recovery ranged from 84.9% to 92.7% with the RSD varied from 2.6% to 6.7%.

TABLE 1: Comparison of the developed method with other methods for determination of paraquat.

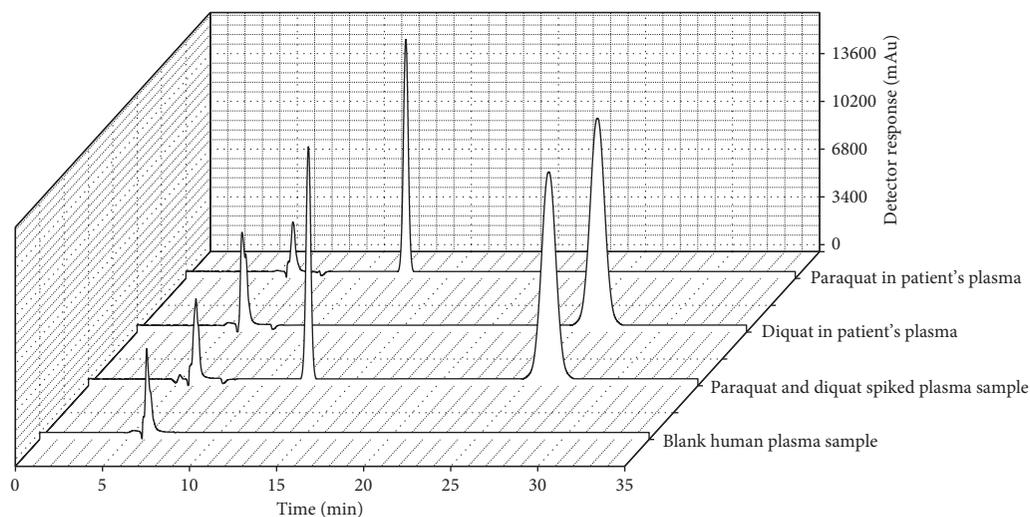
Sample	Method	Sample preparation	LOD ($\mu\text{g/mL}$)	Linear range ($\mu\text{g/mL}$)	RSD (%)	Recovery (%)	Ref.
Plasma and urine	GC-MS	RP-SPE ^a	0.05	0.1–5.0	—	98	[22]
Whole blood	HPLC/ UV	RP-SPE	0.03	0.3–30.0	3.3	88–107	[32]
Plasma and urine	GC-MS	SPME ^b	0.01	0.1–5.0	5.1	94–100	[33]
Plasma	HPLC/ UV	RP-SPE	0.01	0.02–1.0	3.6	98–107	[34]
Water, soil, and vegetables	HPLC/ UV	RP-DLL ME ^c	0.02	—	<5.5	88–96	[14]
Plasma and urine	HPLC/ UV	MSPE	0.004	0.028–0.57	2.3	90–108	This work

TABLE 2: The intraday and interday accuracy and precision of paraquat and diquat in human plasma and urine.

Sample	Analyte	Concentration (ng/mL)	Intraday			Interday		
			Mean (ng/mL)	Accuracy (%)	RSD (%)	Mean (ng/mL)	Accuracy (%)	RSD (%)
Plasma	Paraquat	100.0	90.6	90.6	5.7	84.9	84.9	4.7
		200.0	184.2	92.1	5.2	177.4	88.7	2.9
		500.0	482.0	96.4	3.4	463.5	92.7	2.6
	Diquat	100.0	88.9	88.9	5.4	84.5	84.5	4.6
		200.0	184.8	92.4	3.5	173.8	86.9	6.7
		500.0	485.5	96.7	3.8	465.5	92.7	5.2
Urine	Paraquat	100.0	89.5	89.5	4.7	85.4	85.4	6.3
		200.0	177.4	88.7	5.4	180.8	90.4	5.5
		500.0	467.2	93.4	2.7	450.5	90.1	3.9
	Diquat	100.0	88.6	88.6	5.8	84.6	84.6	5.1
		200.0	184.8	92.4	4.3	174.6	87.3	4.7
		500.0	477.5	95.5	4.6	456.5	91.2	3.8

TABLE 3: The recoveries and matrix effect of paraquat and diquat.

Sample	Analytes	Concentration (ng/mL)	Recoveries \pm RSD (%)	Matrix effect \pm RSD (%)
Patient's plasma	Paraquat	152.5	92.3 \pm 2.6	88.7 \pm 4.5
	Diquat	255.8	98.7 \pm 3.2	95.7 \pm 3.7
Patient's urine	Paraquat	210.6	87.5 \pm 4.7	91.7 \pm 2.5
	Diquat	356.8	93.5 \pm 3.8	103.8 \pm 4.3



(a)

FIGURE 15: Continued.

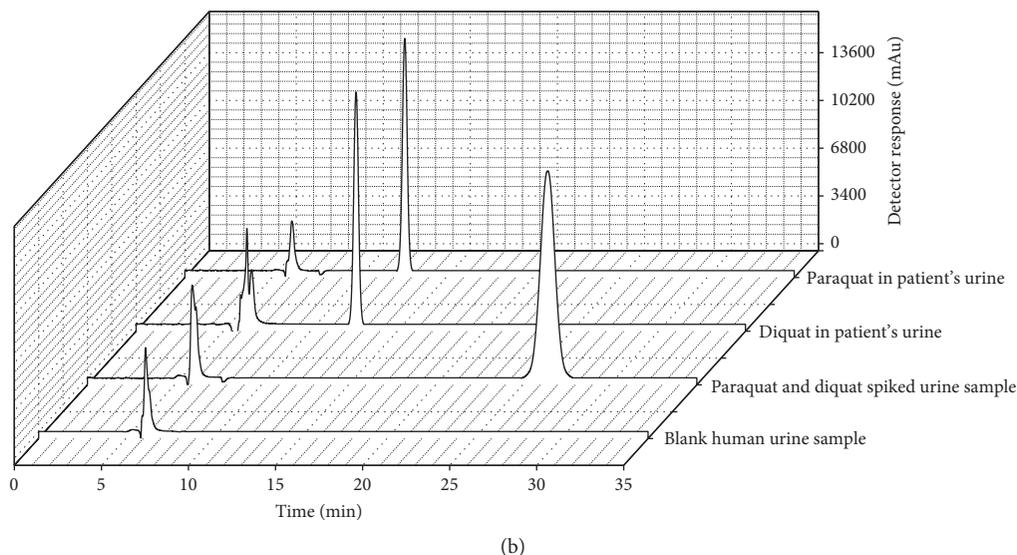


FIGURE 15: (a) The HPLC chromatogram of PQ and DQ in patient's plasma; PQ and DQ spiked into human plasma samples; and blank human plasma sample. (b) The HPLC chromatogram of PQ and DQ in patient's urine; PQ and DQ spiked into human urine samples; and blank human urine sample.

3.6. Application of Real Sample. The method was applied to determine the concentrations of paraquat and diquat in patient plasma and urine ($n = 6$). The recovery was evaluated by the response signals of analytes in patient's plasma and urine with the response signals of spiked analytes in human plasma and urine samples. The matrix effect was evaluated by comparing the response signals of analytes in plasma and urine samples with those in neat solutions. As shown in Table 3, the recoveries of paraquat and diquat ranged from 87.5% to 98.7% with RSD less than 4.7%. The matrix effect of nine analytes ranged from 88.7% to 103.8% with RSD less than 4.5%, which is acceptable according to the FDA guidelines [35]. The HPLC chromatogram of PQ and DQ in patient's plasma and urine, the HPLC chromatogram of PQ and DQ spiked into human plasma samples, and the HPLC chromatogram of blank human plasma and urine are shown in Figure 15. The results showed that the developed method could effectively eliminate interference in plasma and urine samples.

4. Conclusion

In this work, a high selective and sensitive method for separation and determination of paraquat and diquat in human plasma and urine by magnetic dispersive solid phase extraction coupled with high-performance liquid chromatography was developed. The whole sample preparation time before determining by HPLC is dramatically decreased by using MDSPE without protein precipitation pretreatment. Therefore, the loss of trace analytes with the precipitation of matrix proteins, which results in a weak purification effect and low detection sensitivity and reliability, could be avoided. Desorption can also be completed in a few minutes by using NaOH solution and make the operation quick and easy. Meanwhile, the colorimetric detection method does

not require high levels of financial investment or involve high instrument maintenance costs. This proposed that the naked-eye and quantitative detection methods have been successfully applied to detect PQ and DQ in real plasma and urine samples, demonstrating high sensitivity that allows for fast assessment of patient outcome based on routine clinical parameters following the history of PQ or DQ ingestion and thus facilitates the decision on treatment strategies.

Data Availability

The data used to support the findings of this study are included within the article and are also available from the first author upon request.

Disclosure

All authors of this manuscript have directly participated in planning, execution, and/or analysis of this study. The contents of this manuscript have not been copyrighted or published previously. The contents of this manuscript are not under consideration for publication elsewhere. There are no directly related manuscripts or abstracts, published or unpublished, by any authors of this manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This study was supported by the Postgraduate Research and Practice Innovation Program of Jiangsu Province (KYCX19_2272, KYCX18_2609, and KYCX18_2605); the funding of the Priority Academic Program Development of Jiangsu Higher Education Institutions; the funding of

University Students Practice Innovation Training Program Project of Jiangsu Province and Huaihai Institute of Technology (SY201911641104002, and Z201911641104003); the Haiyan Project of Lianyungang City (ZKK201820); the funding of Research Topic on New Technology and Method of Analysis and Test of Large Scientific Instrument and Equipment Sharing Service Platform in Jiangsu Province (201801); and Science and Technology Planning Project of Lianyungang High-Technology Industrial Development zone (ZD201925).

References

- [1] D. Oulkar, R. Shinde, Z. Khan, and K. Banerjee, "High throughput residue analysis of paraquat and diquat involving hydrophilic interaction liquid chromatographic separation and mass spectrometric determination," *Food Additives & Contaminants: Part A*, vol. 36, no. 1, pp. 120–130, 2019.
- [2] L. M. Niu, Y. Liu, K. Q. Lian, L. Ma, and W. J. Kang, "Characterization of a sensitive biosensor based on an unmodified DNA and gold nanoparticle composite and its application in diquat determination," *Arabian Journal of Chemistry*, vol. 11, no. 5, pp. 655–661, 2018.
- [3] T. Saito, T. Fukushima, Y. Yui, and A. Nakamoto, A. Namera and S. Inokuchi, "Monolithic spin column extraction and GC-MS for the simultaneous assay of diquat, paraquat, and fenitrothion in human serum and urine," *Analytical and Bioanalytical Chemistry*, vol. 400, no. 1, pp. 25–31, 2011.
- [4] M. A. Aramendía, V. Borau, F. Lafont et al., "Determination of diquat and paraquat in olive oil by ion-pair liquid chromatography–electrospray ionization mass spectrometry (MRM)," *Food Chemistry*, vol. 97, no. 1, pp. 181–188, 2006.
- [5] N. Elenga, C. Merlin, R. Le Guern et al., "Clinical features and prognosis of paraquat poisoning in French Guiana a review of 62 cases," *Medicine*, vol. 97, no. 15, Article ID e9621, 2018.
- [6] M. Q. Chui, L. Y. Thang, and H. H. See, "Integration of the free liquid membrane into electrokinetic supercharging-capillary electrophoresis for the determination of cationic herbicides in environmental water samples," *Journal of Chromatography A*, vol. 1481, pp. 145–151, 2017.
- [7] M. See, Y. Hori, M. Fujisawa et al., "Rapid analysis method for paraquat and diquat in the serum using ion-pair high-performance liquid chromatography," *Biological & Pharmaceutical Bulletin*, vol. 28, no. 4, pp. 725–728, 2005.
- [8] M. C. Carneiro, L. Puignou, and M. T. Galceran, "Comparison of capillary electrophoresis and reversed-phase ion-pair high-performance liquid chromatography for the determination of paraquat, diquat and difenzoquat," *Journal of Chromatography A*, vol. 669, no. 1-2, pp. 217–224, 1994.
- [9] R. D. Whitehead, M. A. Montesano, N. K. Jayatilaka et al., "Method for measurement of the quaternary amine compounds paraquat and diquat in human urine using high-performance liquid chromatography–tandem mass spectrometry," *Journal of Chromatography B*, vol. 878, no. 27, pp. 2548–2553, 2010.
- [10] M. Luque, A. Ríos, and M. Valcárcel, "Sensitive determination of paraquat and diquat at the sub-ng ml⁻¹ level by continuous amperometric flow methods," *The Analyst*, vol. 123, no. 11, pp. 2383–2387, 1998.
- [11] P. Shivhare and V. K. Gupta, "Spectrophotometric method for the determination of paraquat in water, grain, and plant materials," *The Analyst*, vol. 116, no. 4, pp. 391–393, 1991.
- [12] H. Luo, X. Wang, Y. Huang, K. Lai, B. A. Rasco, and Y. Fan, "Rapid and sensitive surface-enhanced Raman spectroscopy (SERS) method combined with gold nanoparticles for determination of paraquat in apple juice," *Journal of the Science of Food and Agriculture*, vol. 98, no. 10, pp. 3892–3898, 2018.
- [13] O. Sha, B. Cui, H. Liu et al., "A simple and rapid method for determination of paraquat in human urine and plasma by improved solid adsorption using equipment built in-house," *Journal of the Iranian Chemical Society*, vol. 16, no. 10, pp. 2071–2080, 2019.
- [14] M. Rashidipour, R. Heydari, A. Maleki, E. Mohammadi, and B. Davari, "Salt-assisted liquid-liquid extraction coupled with reversed-phase dispersive liquid-liquid microextraction for sensitive HPLC determination of paraquat in environmental and food samples," *Journal of Food Measurement and Characterization*, vol. 13, no. 1, pp. 269–276, 2019.
- [15] F. Latifeh, Y. Yamini, and S. Seidi, "Ionic liquid-modified silica-coated magnetic nanoparticles: promising adsorbents for ultra-fast extraction of paraquat from aqueous solution," *Environmental Science and Pollution Research*, vol. 23, no. 5, pp. 4411–4421, 2016.
- [16] X. H. Luo, J. Zhang, Z. G. Zhang et al., "Urine metabolic changes of acute paraquat poisoning rats after intervention with curcumin," *Latin American Journal of Pharmacy*, vol. 37, pp. 131–138, 2018.
- [17] Y. Wang, Y. Tong, X. Xu, and L. Zhang, "Developed magnetic multiporous 3D N-Co@C/HCF as efficient sorbent for the extraction of five trace phthalate esters," *Analytica Chimica Acta*, vol. 1054, pp. 176–183, 2019.
- [18] Y. Yu and Z. Fan, "Magnetic solid-phase extraction coupled with HPLC for the determination of allura red in food and beverage samples," *Food Additives & Contaminants: Part A*, vol. 33, no. 10, pp. 1527–1534, 2016.
- [19] D. Psimadas, G. Baldi, C. Ravagli et al., "Comparison of the magnetic, radiolabeling, hyperthermic, and biodistribution properties of hybrid nanoparticles bearing CoFe₂O₄ and Fe₃O₄ metal cores," *Nanotechnology*, vol. 25, no. 2, 2014.
- [20] G. Aygar, M. Kaya, N. Özkan, S. Kocabiynk, and M. Volkan, "Preparation of silica-coated cobalt ferrite magnetic nanoparticles for the purification of histidine-tagged proteins," *Journal of Physics and Chemistry of Solids*, vol. 87, pp. 64–71, 2015.
- [21] H. A. Zadeh and E. Rahimpour, "CoFe₂O₄ nanoparticles functionalized with 8-hydroxyquinoline for dispersive solid-phase microextraction and direct fluorometric monitoring of aluminum in human serum and water samples," *Analytica Chimica Acta*, vol. 881, pp. 54–64, 2015.
- [22] Y.-H. Deng, C.-C. Wang, J.-H. Hu, W.-L. Yang, and S.-K. Fu, "Investigation of formation of silica-coated magnetite nanoparticles via sol-gel approach," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 262, no. 1-3, pp. 87–93, 2005.
- [23] B. Viltuznik, A. Kosak, Y. L. Zub, and A. Lobnik, "Removal of Pb(II) ions from aqueous systems using thiol-functionalized cobalt-ferrite magnetic nanoparticles," *Journal of Sol-Gel Science and Technology*, vol. 68, no. 3, pp. 365–373, 2013.
- [24] C. Ren, X. Ding, H. Fu, C. Meng, W. Li, and H. Yang, "Preparation of amino-functionalized CoFe₂O₄@SiO₂ magnetic nanocomposites for potential application in absorbing heavy metal ions," *RSC Advances*, vol. 6, no. 76, pp. 72479–72486, 2016.
- [25] H. Zhu, Y. Shen, Q. Wang et al., "Highly promoted removal of Hg (ii) with magnetic CoFe₂O₄@SiO₂ core-shell

- nanoparticles modified by thiol groups," *RSC Advances*, vol. 7, no. 62, pp. 39204–39215, 2017.
- [26] H. Wang, J. Huang, L. Ding, D. Li, and Y. Han, "A facile synthesis of monodisperse $\text{CoFe}_2\text{O}_4/\text{SiO}_2$ nanoparticles," *Applied Surface Science*, vol. 257, no. 16, pp. 7107–7112, 2011.
- [27] R. Kesari, M. Rai, and V. K. Gupta, "Spectrophotometric method for determination of paraquat in food and biological samples," *Journal of AOAC International*, vol. 80, no. 2, pp. 388–391, 1997.
- [28] M. Takahashi and K. Takahashi, "Ionised silica in the estuary of a river as supply to seawater: identification and ionization efficiency of silica species by FAB-MS," *Estuarine, Coastal and Shelf Science*, vol. 121-122, pp. 1–7, 2013.
- [29] O. Sha, Y. Wang, X. Yin, X. Chen, L. Chen, and S. Wang, "Magnetic solid-phase extraction using $\text{Fe}_3\text{O}_4@\text{SiO}_2$ magnetic nanoparticles followed by UV-vis spectrometry for determination of paraquat in plasma and urine samples," *Journal of Anal Methods Chemistry*, vol. 2017, pp. 1–9, 2017.
- [30] S. L. Tompseit, "Rapid detection and determination of paraquat and diquat in urine and gastric aspirate," *Proceedings of the Association of Clinical Biochemists*, vol. 5, no. 3, pp. 75-76, 1968.
- [31] J. Vial and A. Jardy, "Experimental comparison of the different approaches to estimate LOD and LOQ of an HPLC method," *Analytical Chemistry*, vol. 71, no. 14, pp. 2672–2677, 1999.
- [32] W. Zhang, Y. Su, J. Shi et al., "Reversed-phase high performance liquid chromatography method for the determination of paraquat in whole blood," *Analytical Methods*, vol. 6, no. 16, pp. 6560–6564, 2014.
- [33] L. Gao, J. Liu, C. Wang et al., "Fast determination of paraquat in plasma and urine samples by solid-phase microextraction and gas chromatography-mass spectrometry," *Journal of Chromatography B*, vol. 944, pp. 136–140, 2014.
- [34] Y. Zou, Y. Shi, Y. Bai, J. Tang, Y. Chen, and L. Wang, "An improved approach for extraction and high-performance liquid chromatography analysis of paraquat in human plasma," *Journal of Chromatography B*, vol. 879, no. 20, pp. 1809–1812, 2011.
- [35] FDA, *Guidance for Bioanalytical Method Validation, Guidance for Industry: Bioanalytical Method Validation*, FDA, Washington, D.C, USA, 2001.