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An integrated solid phase extraction with ionic liquid-thiol-graphene oxide as adsorbent for rapid isolation of fipronil residual in chicken eggs

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ABSTRACT

A rapid and economic integrated solid-phase extraction (ISPE) method with ionic liquid-thiol-graphene oxide composite (IL-TGO) as a new adsorbent was established for the extraction of fipronil in chicken eggs. In this study, ISPE, a new form of solid-phase extraction, which combined the advantages of pipette-tip solid phase extraction (PT-SPE) and dispersive solid-phase extraction (DSPE), achieving rapid extraction of analyte with only a small amount of adsorbent. The three-dimensional porous IL-TGO was a new ideal adsorbent for fipronil due to the multiple adsorption interactions (π-π interaction, hydrophobic interaction and hydrogen bond), large adsorption capacity and fast adsorption rate. For this ISPE procedure, the analyte was adsorbed by IL-TGO through the pipette tip mode and then eluted through dispersive mode. Under the optimized condition, the proposed method performed following merits: good linearity (r = 0.9999), precision (RSD $\leq$ 6.3%), high recoveries (83.7% to 97.7%), rapid extraction (16 min), low consumption of adsorbent (3.00 mg of IL-TGO). The excellent recyclability of the ISPE device and the reusability of IL-TGO were observed and high recovery of fipronil (> 90%) was obtained after fifteen cycles. This work might provide promising strategy for the sensitive extraction and analysis of trace levels of fipronil from egg products.

1. Introduction

An efficient sample pretreatment procedure before instrumental analysis is essential to achieve accurate and sensitive result, on account of the complicated sample matrix and the trace levels of the analyte in the samples [1,2]. At present, various sample pretreatment techniques have been used in fipronil analysis, including solid-phase extraction (SPE) [3,4], and dispersive solid-phase extraction (DSPE) [5–8]. In these techniques, DSPE can quickly adsorb analyte by sufficient contact between the solution and adsorbent, and the separation was easily carried out through centrifugation or filtration, effectively avoiding the problem of high pressure or blockage generated in classical SPE system [9,10]. Pipette-tip solid-phase extraction (PT-SPE), as a derivation of SPE, proposes a tapered filter structure with small inner diameter which could efficiently extract the analyte with low consumption of reagents and adsorbents [11,12]. Recently, great efforts were made to develop new sample pretreatment methods by combing various pretreatment processes with different advantage to handle more complicated sample matrix, shorten pretreatment time, and achieve higher enrichment factor of analytes for trace analysis [13–15].

Among various forms of SPE, the selection of adsorbent is the core of efficient extraction and separation [16]. Different kinds of commercial adsorbents (CNT, HLB, SCX, SAX and PSA) have been developed for the cleanup and concentration of trace analytes [17]. However, due to the single adsorption interactions of the commercial adsorbents, in order to effectively remove interfering substances from the complicated matrix, many reported methods rely on lots of multiple adsorbents. Graphene oxide (GO), as a new kind of adsorbent, possesses large specific surface area and excellent chemical structure, and is suitable for the adsorption of the compounds containing conjugate structure [18,19]. During the last decades, ionic liquid (IL) has attracted extensive attention because it could be modified on the adsorbents to enrich their adsorption interactions and improve adsorption capacity [20–22].

The outbreak of “poisonous eggs” in Europe and China in 2017 and 2018, caused by the illegal use of fipronil in livestock and poultry breeding, has raised widespread concern about its food safety [23,24]. Fipronil residue in eggs could threaten human health, even

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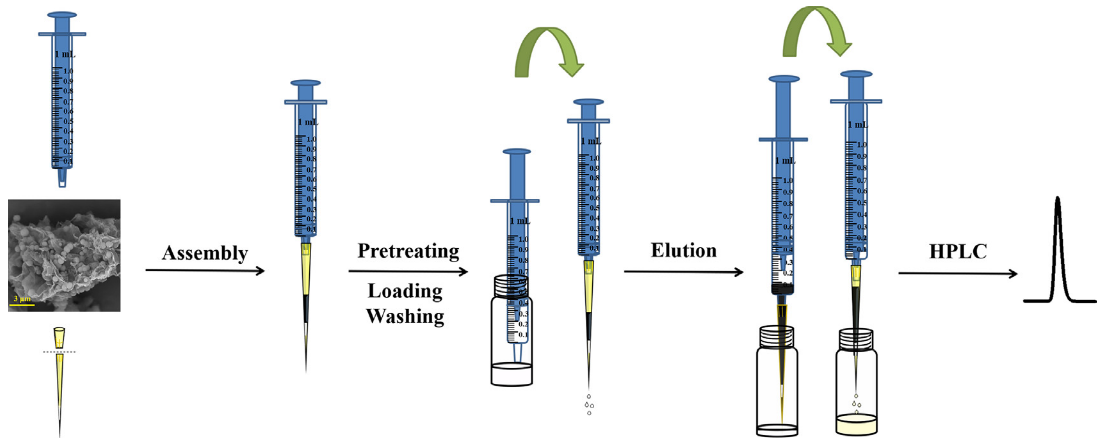

Fig. 1. Scheme of IL-TGO-ISPE procedure.

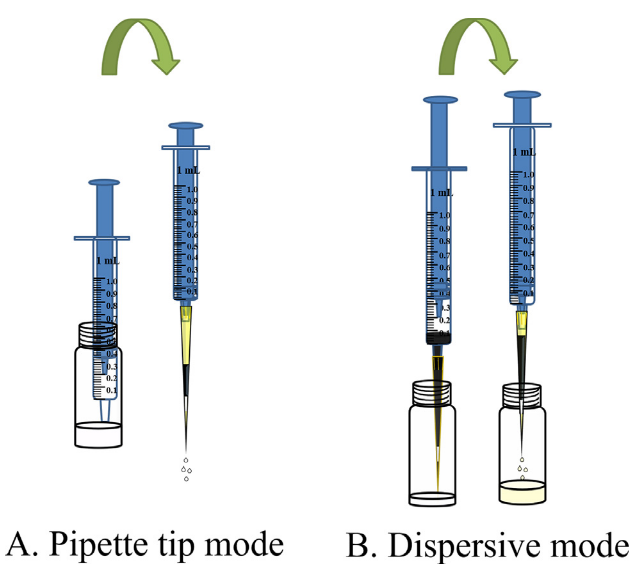

Fig. 2. Two modes for the operation of ISPE.

at low concentration, leading to series damage to liver to kidney and nervous system [25,26]. Owing to high toxicity and long duration, fipronil has been restricted or banned in many countries [27,28]. Hence, it is urgent to develop a rapid and practical method for the isolation and detection of fipronil in chicken eggs.

The combination of IL and GO provides a potential solution for the selective adsorption of fipronil. In this work, a rapid and economic integrated solid-phase extraction (ISPE) method combined the advantages of PT-SPE and DSPE was developed. To simplify the sample preparation process, integrated device was proposed as a solution to switch between different pretreatment modes of PT-SPE and DSPE. The analyte was adsorbed by ionic liquid-thiol-graphene oxide (IL-TGO) which was a new adsorbent for fipronil through the pipette tip mode and then eluted through dispersive mode. Different parameters influencing the extraction were optimized. The ISPE method based on IL-TGO was established and applied for the efficient extraction and rapid determination of fipronil from eggs.

2. Experimental

2.1. Chemicals and reagents

Fipronil, 1-allylimidazole (AIM), pentafluorobenzyl bromide (PFBBr), and (3-mercaptopropyl) trimethoxysilane (MPTMS) were obtained from Shanghai Aladdin Chemistry Co. Ltd. Methanol, anhydrous sodium sulfate ($NaSO_4$), 2,2′-azobis(2-methylpropionitrile) (AIBN), and N,N-dimethylformamide (DMF) were bought from Tianjin Kermel Chemical Reagent Co. Ltd. Acetonitrile (ACN) was purchased from the Shanghai Xingke Biochemistry Co. Ltd. Graphene oxide powder was obtained from Tangshan Jianhua Technology Co. Ltd. Ultrapure water was filtered with a 0.45-μm filter membrane.

2.2. Instruments and analytical conditions

The IL-TGO was freeze-dried using a GOLD-SIM freeze dryer (SIM International Group, USA). A Sorvall Biofuge stratos centrifuge (Thermo Fisher Scientific, USA) was used for the separation of the extraction solution. RE52AA rotary evaporator was purchased from Yarong Equipment Co., Ltd. (Shanghai, China). The morphology of adsorbent was observed by scanning electron microscopy (SEM), with a Phenom Pro SEM system (Phenom, Eindhoven, Netherland). Fourier transform infrared spectra (FT-IR) of the IL-TGO was obtained by a Vertex70 FTIR spectrometer, which was provided by Bruker, Karlsruhe, Germany. HPLC analysis was conducted by an LC-20A liquid chromatography system which was equipped with LC-20AT solvent delivery units and an SPD 20A UV-Vis detector and coupled with a Lab solution workstation as data acquisition system (Shimadzu, Kyoto, Japan) and the wavelength of detection was set at 280 nm. The Accucore XL C_{18} column (150 mm × 4.6 mm, 4 μm) (Thermo Fisher Scientific, USA) was used for HPLC separation. The mobile phase was acetonitrile-water (containing 3‰ TFA) (60:40, v/v) at a flow rate of 1.0 mL min^{-1}.

2.3. Synthesis of IL-TGO adsorbent

IL-TGO was prepared based on our previous work with some modifications [22]. 1-Allyl-3-pentafluorobenzyl imidazole bromide (PFBr) was obtained by mixing 0.45 mL of AIM and 0.47 mL of PFBBr.

Typically, 200 mg GO was dispersed in 100 mL of ethanol-water solvent (4:1, v/v) with sonication for 3 h at room temperature. After adding 0.80 mL MPTMS, the mixture was ultraphonic for 0.5 h under the protection of nitrogen. Then 4.0 mL ammonia was dropwise added into the stirring mixture. After continuous stirring at 60°C for 6 h, the obtained precipitate was cooling and washed with ethanol for several times and then washed to neutral with water, and lyophilized for 12 h to obtain the thiol-functionalized graphene oxide (TGO).

TGO (100 mg) and AIBN (25.0 mg) was ultrasonically dissolved in DMF. Then the solution of PFBr (5.00 mg) in DMF was dropwise

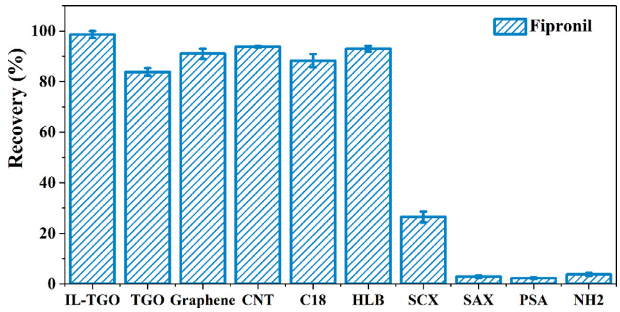

Fig. 3. Comparison of IL-TGO with other adsorbents.

added to the above mixture and the mixture was continuously stirred under a nitrogen atmosphere for 4 h at 70°C. Finally, IL-TGO was obtained after washing repeatedly with ethanol and water for removal of unreacted reagents, and then lyophilized overnight.

2.4. Preparation of eggs

The eggs (5.0 g) purchased from the local supermarkets of Baoding were homogenized and ultrasonic extraction with ACN (20 mL) for 5 min. After adding $NaSO_4$ (6 g) and NaCl (2 g), the mixture was vortexed for 1 min and centrifuged at 15000 r min^{-1} for 5 min. Then the supernatant was collected and ACN (10 mL) was added for re-extraction. The supernatant (30 mL) was combined and evaporated to dryness and dissolved in 5.0 mL of methanol-water (3:7, v/v) for ISPE process.

2.5. Assembling of the device and ISPE procedure

The scheme of IL-TGO-ISPE procedure has been present in Fig. 1. The ISPE device was assembled from a pipette tip, a thicker filter and a syringe. The filter (pore size 10 μm) was put into the tip of 200 μL pipette tip to prevent leakage of adsorbent, then cut off the top of pipette tip, the adsorbent IL-TGO (3.00 mg) was transferred to the pipette tip. The device has two modes as shown in Fig. 2. In the pipette tip mode (Fig. 2A), the syringe transferred solution from the vial and then was connected with a pipette tip. The solution flowed through the adsorbent from top to bottom by pushing the plunger of the syringe. In the dispersive mode (Fig. 2B), first connected the pipette tip and the syringe together, then inserted the ISPE device into the bottom of the solution vial, the solution flowed through the adsorbent from bottom to top by pulling the plunger of the syringe, during the pulling process, the solution and adsorbent entered the cavity formed by the pipette tip and the syringe together in a dispersed state. Subsequently, the adsorbent was intercepted by the filter through pushing the syringe to achieve the purpose of liquid-solid separation. The ISPE procedure was as follow:

First, the syringe was used to transfer methanol (1.0 mL) and water (1.0 mL) to activate the adsorbent. Later, egg sample solution (0.5 mL) was injected into ISPE device by the syringe, and washed with 0.30 mL of methanol-water (3:7, v/v), the loading and washing process were completed using a syringe to transfer different solvents into the pipette tip. During the elution process, first connected the sample-loaded pipette tip and the syringe together, and then used the syringe to pull 0.3 mL of methanol into the pipette tip, the methanol and the adsorbent were fully contacted, and then used the syringe to directly push the methanol eluent into clean vial and the pipette tip and syringe were always connected together during the entire elution process. In these processes, the flow rate could be controlled by adjusting the force of pressing the syringe. Finally, the obtained eluent was dried under nitrogen and reconstituted with 0.50 mL of mobile phase for HPLC analysis.

Table 1
Comparison of different modes for loading procedure.

The modes of addition solution	Loss rate (%)	RSD (%)
The pipette tip mode	0	0
The dispersive mode	2.7	13.5

Table 2
Comparison of different modes for elution procedure.

The modes of addition solution	Recovery (%)	RSD (%)	Time (min)
The pipette tip mode	96.8	1.2	15
The dispersive mode	97.0	1.7	5

3. Results and discussion

3.1. Design and fabrication of ISPE device

The ISPE device was proposed to speed up the procedure of extraction and reduce consumption of adsorbents. As shown in Fig. 2, a pipette tip and a syringe were used to set up the integrated device and IL-TGO was used as adsorbent. Pipette tip could accomplish the extraction process with a small number of adsorbents. Syringe not only provides positive pressure for solid-liquid separation, but also provides the space of dispersion. Therefore, for each stage (loading, washing and eluting) of SPE, there are two modes for solution and adsorbent that can be selected: the pipette tip mode (Fig. 2A) and dispersive mode (Fig. 2B).

In order to investigate the effect of different device on extraction of fipronil, a study was performed with different modes for loading procedure and elution procedure. And the results were shown in Tables 1 and 2. The loss rate and recovery were calculated by Eqs. (1) and (2):

$$\text{Loss rate} = \frac{C_1V_1}{C_0V_0} \times 100\% \quad (1)$$

$$Recovery = \frac{C_2V_2}{C_0V_0} \times 100\% \quad (2)$$

Where C_0 represents the concentration of analyte in standard solution before loading, V_0 represents the volume of the standard sample, C_1 represents the concentration of analyte after loading, V_1 represents the volume of the sample after loading(V_1 equals to V_0), C_2 represents the concentration of analyte after elution and V_2 represents the volume of elution solvent.

As shown in Table 1, the dispersive mode had a large loss of analyte during sample loading, while the pipette tip mode had no loss of analyte, so pipette tip mode was selected for the loading procedure. Then the recoveries of analyte under different elution modes were investigated. The results were shown in Table 2, the recovery of the pipette tip mode (96.8%, RSD 1.2%) was very close to that of the dispersive mode (97.0%, RSD 1.7%), but the pipette tip mode required a longer elution time (15 min) because of high back pressure during elution process, while the dispersive mode contributes to the adsorbent sufficiently dispersed in the eluent, can quickly achieve sufficiently contact between the adsorbent and the eluent, and achieve a balance of desorption in a shorter time (5 min), so the dispersive mode was selected for the elution procedure.

3.2. The choice of adsorbent

The choice of adsorbent was a crucial parameter in the efficient extraction of the analyte. Graphene as adsorbent has large specific

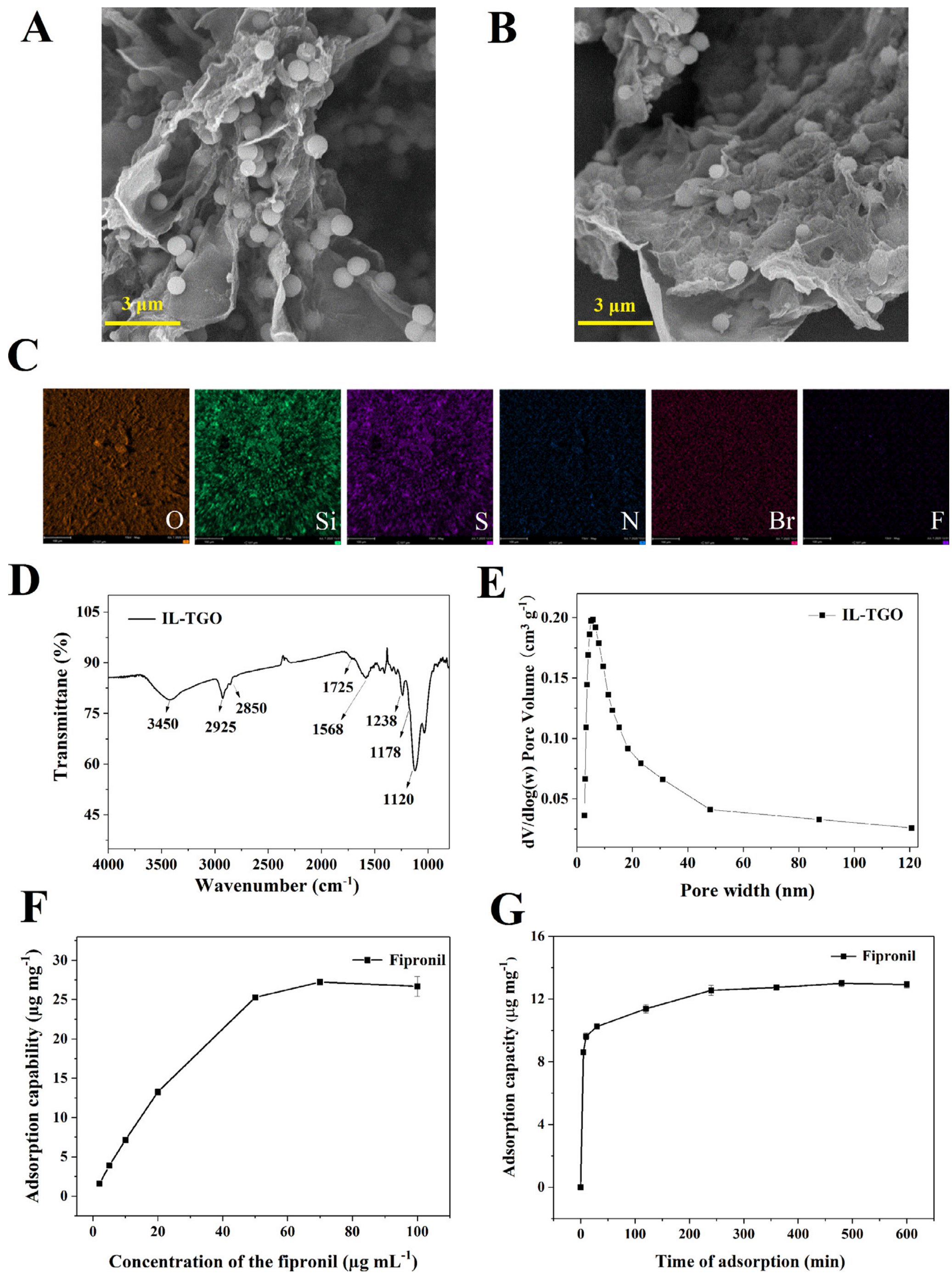

Fig. 4. SEM image of TGO (A) and IL-TGO (B); EDS map images (C), FTIR spectra (D), pore size distribution (E), static adsorption (F) and dynamic adsorption (G) of IL-TGO.

surface area and conjugated structure. The modification with ionic liquid could introduce new interaction sites and enriched the interactions of IL-TGO with the analyte. In order to evaluate the extraction performance of IL-TGO, its adsorption capacity to fipronil was compared with TGO and eight kinds of commercial adsorbents (Graphene, CNT, HLB, C_{18}, SCX, SAX, PSA, NH_2) referring to reported methods [17,27,29]. The results (Fig. 3) showed that IL-TGO had the highest recovery, HLB, graphene, CNT and C_{18} had relatively high recoveries, while ion exchange adsorbents SCX, SAX, PSA and NH_2 had low recoveries. The recovery of IL-TGO to fipronil (98.6%) was higher than that of TGO to fipronil (83.8%), which indicated that the hydrogen bond between the ionic liquid and fipronil contributes to the extraction. For the main adsorption interaction of these commercial adsorbents, graphene and CNT have π-π interaction and hydrophobic interaction with fipronil, C_{18} and HLB have hydrophobic interaction with fipronil, SCX and SAX are strong ion exchange adsorbents, PSA and NH_2 are weak ion exchange adsorbents, the low recoveries for ion exchange adsorbents indicated that ion exchange interaction between fipronil and the adsorbent was weak. The adsorption interaction between IL-TGO and fipronil could be explained by the interaction between functional groups. Fipronil has relatively high hydrophobicity and contains benzene ring, pyrazole ring and amino group [30]. And IL-TGO contains benzene ring, hydroxyl groups and fluorine atoms. The benzene ring and pyrazole ring of fipronil could interact with the benzene ring of IL-TGO by π-π interactions and hydrophobic interactions. The

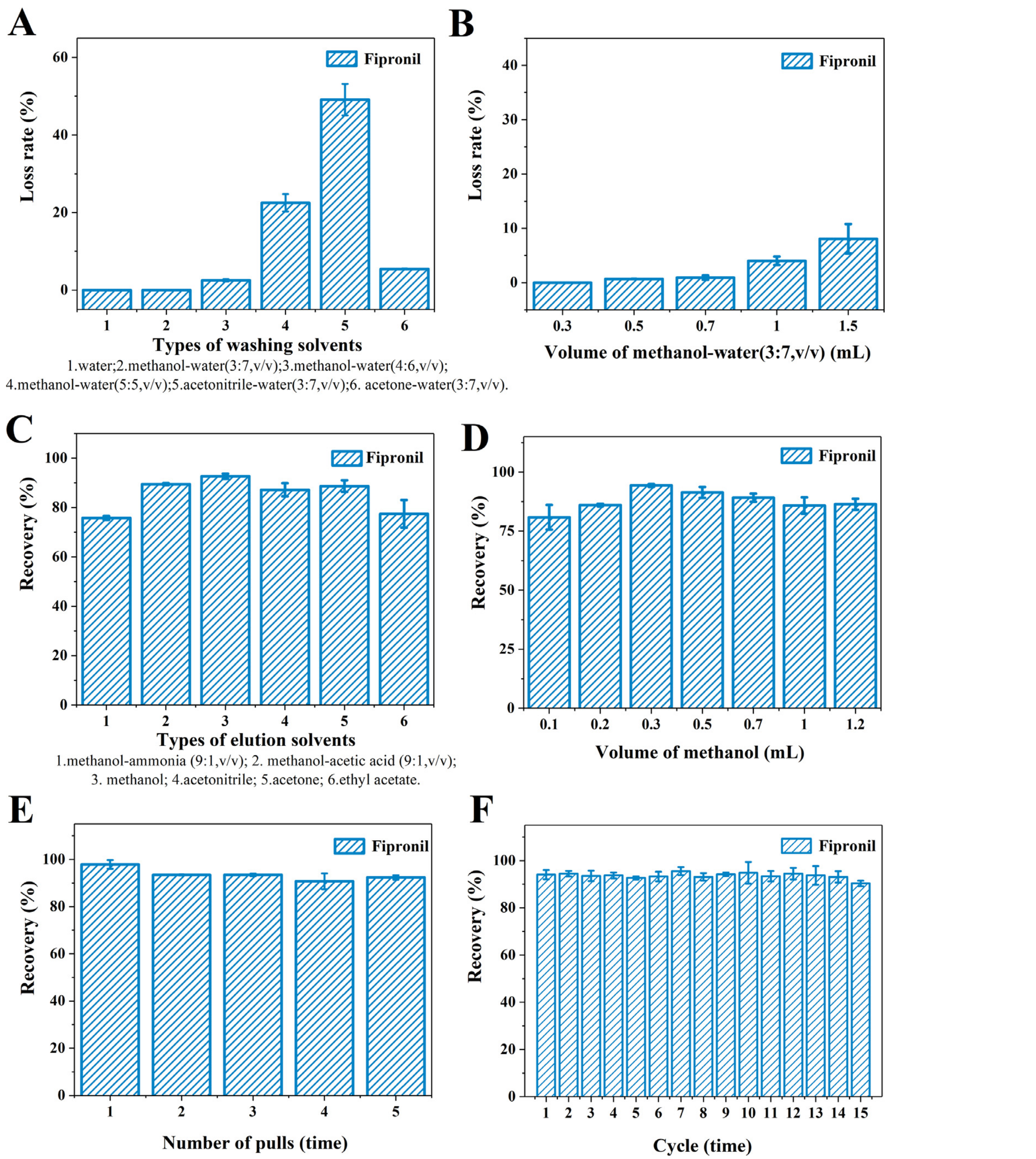

Fig. 5. Optimization of IL-TGO-ISPE procedure: (A) types of washing solvents, (B)volume of washing solvent, (C) types of elution solvents, (D) volume of elution solvent, (E) number of pulls, (F) number of adsorption-desorption cycles.

hydrogen bonds are generated between the amino group of fipronil and hydroxyl groups and fluorine atoms of IL-TGO. IL-TGO exhibited the best recovery among the above adsorbents. Therefore, IL-TGO was chosen as the adsorbent for fipronil extraction.

3.3. Characterization of IL-TGO

The SEM images in Fig. 4A and Fig. 4B indicated that TGO showed a fluffy three-dimensional structure and IL-TGO had a rough and porous surface morphology, which was beneficial for the high adsorption capacity to fipronil. During the functionalization of sulfhydryl groups, some small balls formed by self-polymerization of MPTMS were inserted into the sheet layers of GO and played a physical support between GO sheets. As presented in Fig. 4C, the elements Si and S from MPTMS and the elements N, Br and F from ionic liquid (PFBr) were uniformly distributed on the surface of the GO. The EDS map images and the FT-IR spectra confirmed that the abundant functional groups on the surface of IL-TGO play an important role in fipronil adsorption. As shown in Fig. 4D, the absorption bands at 3450, 1725 and 1238 cm^{-1} are ascribed to -OH, C=O and C-O-C stretching vibration [31]. The presence of Si-O-Si (1120 cm^{-1}) illustrates the hydrolyzed MPTMS was covalently bonded to the hydroxyl groups on GO. The vibration of the imidazole ring skeleton (1568 cm^{-1}) and Ar-F of benzene ring (1178 cm^{-1}) were due to the graft of ionic liquid [32,33]. The specific surface area of IL-TGO was 37.8 m^2 g^{-1}. The pore size distribution (Fig. 4E) showed that the pores of IL-TGO are mainly mesoporous. As shown in Fig. 4F, the adsorption capacity increased as the con-

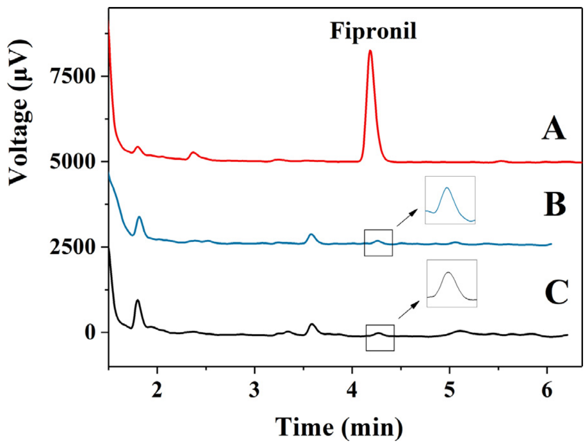

Fig. 6. Chromatograms of spiked sample at 1.00 μg g^{-1} (A), at 0.02 μg g^{-1} (B) and egg sample (C).

centration of fipronil increased and when the concentration further increased to 70 μg mL^{-1}, the maximum adsorption capacity was eventually reached to 27.2 μg mg^{-1}. For the dynamic adsorption experiment (Fig. 4G), in the initial 10 min, the adsorption capacity increased rapidly and 74.0% of the total adsorption capacity could be reached, and then slowly increased until 250 min, basically reached adsorption equilibrium. The results illustrated that IL-TGO could be used to efficient extraction of fipronil.

3.4. Optimization of the ISPE procedure

The parameters influencing the extraction and separation process were investigated, including washing solvent, elution solvent and elution times. In the optimization process, 1.00 μg g^{-1} of spiked egg sample was selected.

3.4.1. Type and volume of the washing solvent

The washing solvent was of great significance in eliminating the interferences of the egg matrix without disrupting the interaction between the analyte and IL-TGO adsorbent.

After loading 0.50 mL spiked sample, six types of washing solvents (0.30 mL) including water, methanol-water (3:7, v/v), methanol-water (4:6, v/v), methanol-water (5:5, v/v), acetonitrile-water (3:7, v/v), and acetone-water (3:7, v/v) were evaluated. Fig. 5A illustrated that low loss rate of the analyte was observed by water and methanol-water (3:7, v/v). Methanol-water (3:7, v/v) had better purification efficiency compared with water. Moreover, the flow rate of methanol-water was faster due to its lower viscosity. Fig. 5B showed the loss rate was increased as increasing the washing solvent volume from 0.30 to 1.5 mL. Therefore, 0.30 mL methanol-water (3:7, v/v) was selected as washing solvent.

3.4.2. Type and volume of the elution solvent

The elution solvent was crucial for the desorption of fipronil from IL-TGO. Methanol-ammonia (9:1, v/v), methanol-acetic acid (9:1, v/v), methanol, acetonitrile, acetone, and ethyl acetate were investigated as eluent. As shown in Fig. 5C, the eluting efficiencies of different solvents were close. That was because fipronil had high solubility (> 1 mg mL^{-1}) in different solvents (methanol, acetonitrile, acetone, ethyl acetate), while the content of fipronil in eggs was low, so most solvents could elute it from the adsorbent, and methanol had the highest recovery of fipronil, so methanol was chosen as elution solvent. Subsequently, various volumes (0.10, 0.20, 0.30, 0.50, 0.70, 1.0, 1.2 mL) of methanol were investigated. The results in Fig. 5D indicated that the recovery of fipronil increased as the methanol volume increased from 0.10 mL to 0.30 mL, and then remained basically stable. Hence, 0.30 mL of methanol was used for further study.

3.4.3. The push-pull times of syringe

The push-pull times of syringe was an important parameter that affected the elution efficiency. The effect of push-pull times on the extraction of fipronil was investigated by plotting the recovery of fipronil versus the number of push-pull times of syringe (1-5 times). As shown in Fig. 5E, one time was enough to complete desorption of fipronil from IL-TGO. Therefore, one time was chosen in the push-pull times of syringe in the later experiments.

3.5. The extraction performance of IL-TGO-ISPE

The comparative experiments on PT-SPE, DSPE and ISPE were designed to evaluate the advantages of ISPE. The amount of adsorbent, the amount of sample loaded, the type and volume of the washing solvent and the elution solvent were the same in these three methods. And the recoveries of fipronil and the time of the pretreatment process were compared. As shown in Table 3, compared with other methods, the proposed ISPE method had the fastest extraction speed and a higher recovery. For PT-SPE, the IL-TGO adsorbent was pressed tightly and increased the resistance of the solution to pass through. In the ISPE, only a filter with a larger pore size (10 μm) was installed under the tip of the tube, and then the adsorbent was added without pressing hard, which made the flow resistance of the solution smaller. Therefore, the extraction time in PT-SPE was much longer than that of ISPE. The recovery of fipronil in ISPE was higher than that of DSPE. This was due to the light weight and strong hydrophobicity of the IL-TGO, which was prone to partial loss during the solid-liquid separation process, resulting in low recovery in DSPE.

3.6. Validation of IL-TGO-ISPE-HPLC method

The IL-TGO-ISPE-HPLC method was validated in the aspect of the linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision. As shown in Table 4, the board linear range (0.02-10.0 μg mL^{-1}) for fipronil was obtained with the correlation coefficient (r) of 0.9999. The LOD and LOQ were calculated with equation LOD/LOQ = ks_b/S, where k is the coefficients related to the confidence, s_b is the standard deviation of the blank samples; S is the slope of calibration curve [34]. Based on k of 3 and 10, the LOD and LOQ of this method were 4.76 μg kg^{-1} and 15.9 μg kg^{-1}, respectively. The LOD was equivalent to the national food safety standard of China, which proposed a method by using LC-MS/MS to detected fipronil in eggs. The precision of this method was evaluated by determining different levels of spiked samples (1.00 μg g^{-1}) three times a day on three consecutive days. The developed method featured good intra-day repeatability (RSD = 3.7%) and inter-day repeatability (RSD = 4.3%). The spiked samples at three levels (0.02, 0.50, 1.00 μg g^{-1}) of fipronil were used to test the accuracy of this method. As shown in Table 5, the recoveries ranged from 83.7% to 97.7% with RSDs ≤ 6.3%, which demonstrated that this method was feasible and applicable for the determination of fipronil in eggs.

3.7. Analysis of egg samples

The developed method was applied to determine fipronil from five kinds of chicken egg samples which were randomly purchased from the local market of Baoding city. As shown in Fig. 6, the chromatogram of spiked sample demonstrated that the interference peaks near fipronil had been effectively eliminated, which indicated that the interferences originating from the egg matrix were removed by the IL-TGO-ISPE procedure. At the same time, fipronil residue was found in one of these samples at the level of 20.2 ng g^{-1}, which was slightly higher than the minimum residue limit

Table 3
Comparison of ISPE with other pretreatment methods.

Methods	Loading		Washing		Elution	
	Loss rate (%)	Time (min)	Loss rate (%)	Time (min)	Recovery (%)	Time (min)
PT-SPE	0	45	0	30	99.0	21
DSPE	6.7	11	4.3	10	79.5	10
ISPE	0	6	0	5	96.8	5

Table 4
Parameters of IL-TGO-ISPE-HPLC method.

Analyte	Regression equation	r	Linearity (μg mL^{-1})	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	RSD (%)	
						Intra-day	Inter-day
Fipronil	$y=2.1\times10^4x+3.5$	0.9999	0.02-10.0	4.76	15.9	3.7	4.3

Table 5
Recoveries of spiked samples by IL-TGO-ISPE-HPLC method (n = 3).

Analyte	0.02 μg g^{-1}		0.50 μg g^{-1}		1.00 μg g^{-1}	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Fipronil	83.7	6.3	97.7	4.7	96.7	4.8

Table 6
Comparison of the present method with reported methods.

Pretreatment method	Detection	Sample	Adsorbent	Linearity	Recovery (%)	LOQ	RSD (%)	Ref.
QuEChERS	LC-MS/MS	Chicken egg	250 mg	1-100 μg kg^{-1}	92.8-107.0	1.0 ng kg^{-1}	≤ 2.2	[7]
SPE	GC-EI-MS	Chicken egg	200 mg	1-200 μg kg^{-1}	84.7-103.6	1.0 μg kg^{-1}	1.93	[25]
QuEChERS	GC-NCI-MS	Chicken egg	300 mg	5-100 μg L^{-1}	87.7-95.9	0.10 μg kg^{-1}	≤ 11.2	[36]
QuEChERS	LC-MS/MS	Chicken egg	845 mg	0.05-20 μg L^{-1}	90.7-97.8	0.2 μg kg^{-1}	≤ 4.9	[37]
ISPE	HPLC-UV	Chicken egg	3 mg	20-10000 μg L^{-1}	83.7-97.7	15.9 μg kg^{-1}	≤ 6.3	This work

SPE: solid-phase extraction.
QuEChERS: quick, easy, cheap, effective, rugged and safe.
ISPE: integrated solid-phase extraction.

(MRL) of China (20.0 ng g^{-1}) [35]. Therefore, the presented IL-TGO-ISPE-HPLC method could be used for the extraction and analysis of fipronil from egg samples.

3.8. The reusability of IL-TGO-ISPE

For reducing the cost of the experiment and utilizing the resources sufficiently, the reusability of IL-TGO-ISPE was investigated. After accomplished each ISPE process, the adsorbent IL-TGO was washed with 1.0 mL of methanol and water, respectively. And then next ISPE process was carried out by loading spiked sample solution (0.50 mL, 1.00 μg g^{-1}). High recoveries of fipronil (> 90%) were observed until fifteenth cycle of ISPE, which indicating good reusability of IL-TGO-ISPE (Fig. 5F).

3.9. The comparison with other reported methods

The comparison between the IL-TGO-ISPE-HPLC-UV method and other reported methods for the determination of fipronil in egg sample were shown in Table 6. According to the literature, most of the methods currently used for the detection of fipronil in egg samples are based on GC-MS/MS or HPLC-MS/MS by QuEChERS or SPE pretreatment. However, QuEChERS and SPE pretreatment method generally require lots of multiple adsorbents. In the study, a simple, rapid IL-TGO-ISPE-HPLC-UV method was developed. Compared with these reported methods, the proposed method has low cost, fast analysis speed and low amount of adsorbent, and the device can be recycled 15 times, indicating good reusability of IL-TGO-ISPE. However, the limitation of this method was the relatively high LOD, which can be overcome by using a more sensitive detector (MS).

4. Conclusion

An integrated solid-phase extraction (ISPE) method based on IL-TGO as a new adsorbent have been developed and applied for the efficient extraction and rapid isolation of fipronil from chicken eggs. And this method has good accuracy and precision. Compared with traditional technologies, ISPE technology provided a new idea for sample preparation, which was largely attributed to its combination the advantages of the filler filling of PT-SPE and the dispersion and separation of DSPE. Compared with different kinds of commercial adsorbents, IL-TGO exhibited efficient extraction performance for fipronil, which was largely attributed to its combination of the advantages of GO (large adsorption capacity, conjugated structure) and IL (the multiple adsorption interaction). This sample pretreatment (IL-TGO-ISPE) method provides a promising strategy for rapid extraction and separation of fipronil in viscous egg products.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Mengyuan Li: Methodology, Writing - original draft. **Chunliu Yang:** Data curation, Validation. **Hongyuan Yan:** Writing - review & editing. **Yehong Han:** Software, Visualization. **Dandan Han:** Conceptualization, Supervision.

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