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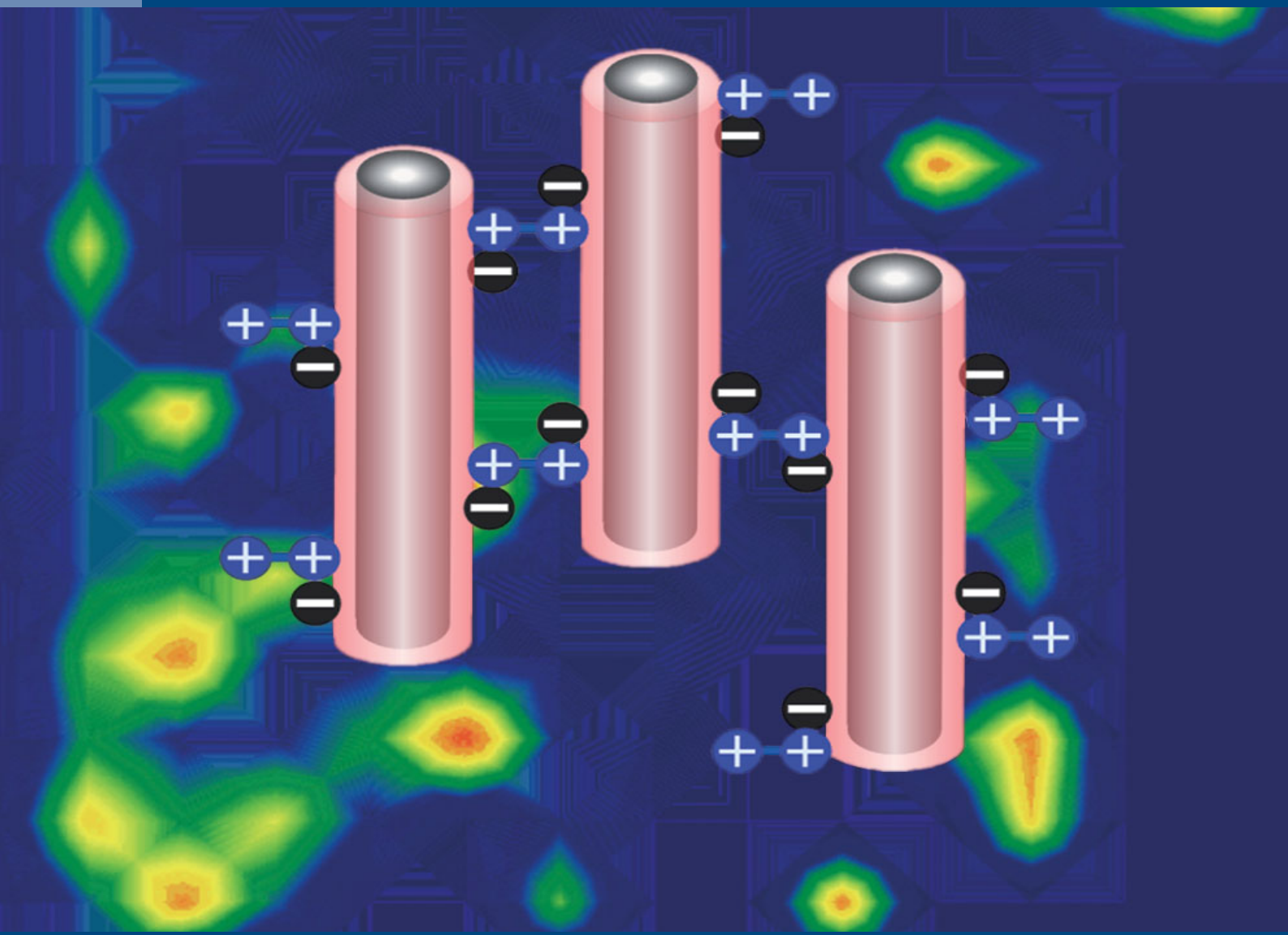

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Research Article

Rapid and sensitive detection of fipronil and its metabolites in edible oils by solid-phase extraction based on humic acid bonded silica combined with gas chromatography with electron capture detection

Solid-phase extraction based on humic acid bonded silica followed by gas chromatography with electron capture detection was developed to determine fipronil and its metabolites in edible oil. To achieve the best extraction performance, we systematically investigated a series of solid-phase extraction parameters. Under the optimized conditions, the method was validated according to linearity, recovery, and precision. Good linearities were obtained with R^2 more than 0.9996 for all analytes. The limits of detection were between 0.3 and 0.5 ng/g, and the recoveries ranged from 83.1 to 104.0% at three spiked concentrations with intra- and interday relative standard deviation values less than 8.7%. Finally, the proposed method was applied to determine fipronil and its metabolites in 11 edible oil samples taken from Wuhan markets. Fipronil was detectable in four samples with concentrations ranging from 3.0 to 5.2 ng/g. In China, the maximum residue limits of fipronil in some vegetables and maize are 20 and 100 ng/g (GB/T 2763-2014), respectively. The residues of fipronil and its metabolites in commercial edible oils might exhibit some potential threat to human health as a result of high consumption of edible oil as part of daily intake.

Keywords: Bonded silica / Edible oils / Fipronil / Humic acid / Solid-phase extraction
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1 Introduction

Fipronil is a phenylpyrazole insecticide, which is commonly used to resist lepidopterous and orthopterous pests in vegetables and coleopterous larvae in soil [1]. The biochemical pathways of classical insecticides are cholinesterase inhibitors (organophosphates and carbamates) or sodium channel blockers (pyrethroids). However, some insects have developed resistance against those chemicals [2]. As the second generation of insecticides, fipronil acts on the γ-aminobutyric acid (GABA) receptor system as a noncompetitive blocker, which prevents the uptake of chloride ions resulting in excess of neuronal stimulation and death of the target insects [3]. However, it has been reported that fipronil exhibits high levels of toxicity against bees and many aquatic organisms [4]. Furthermore, fipronil can degrade to more toxic metabolites like desulfinyl, sulfide, and sulfone metabolites through photolysis, reduction, and oxidation processes, respectively, as shown in Fig. 1 [5]. Moreover, it has been reported that fipronil sulfone and fipronil desulfinyl are more active at the mammalian chloride channel than at insect chloride channels, resulting in the reduction in the selectivity between insects and humans, thus posting great threat to humans health [6]. Oil crops and their downstream commercial products like edible oil may contain residues of fipronil and its metabolites. Considering the high levels of edible oil consumption in the daily intake, it is significant to develop simple, efficient, and sensitive method for the monitoring of the concentration level of fipronil and its metabolites in edible oils.

Earlier, various methods, like GC with electron capture detection (ECD) and MS, HPLC–MS have been reported for the analysis of fipronil and its metabolites in different matrices, such as maize [5, 7], peanut [2], soil [8, 9], vegetables and fruits [1, 4, 10–12], rice [13], honey [14–16], pollen [17, 18], sugar [6], and plasma [19, 20]. However, earlier studies did not focus on development of a specific method for the analysis of fipronil and its metabolites in edible oils. Compared with the above mentioned sample matrices, the matrix of edible oil is much more complex due to the high concentration of endogenous compounds, such as triglyceride and tocopherol in the

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Abbreviations: ECD, electron capture detection; **HAS**, humic acid bonded silica

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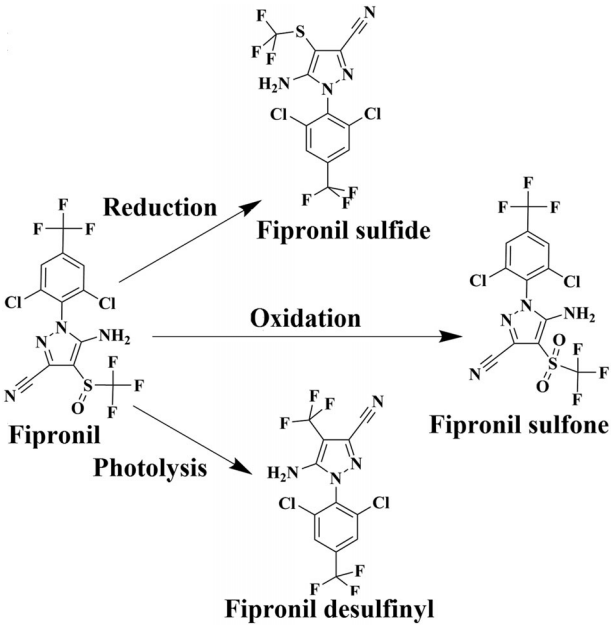

Figure 1. Chemical structure of fipronil and its metabolites.

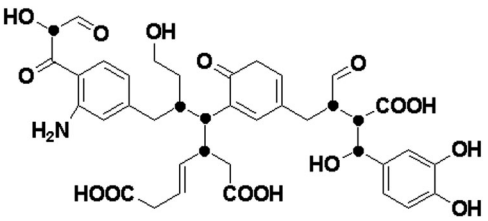

Figure 2. Proposed building block of humic acids.

matrix, which would rapidly decrease the column resolution efficiency and instrumental detection sensitivity [21–23]. As can be seen, analysis of fipronil and its metabolites in edible oils is challenging, and a highly efficient and rapid sample pretreatment method is badly needed for a satisfactory separation of fipronil and its metabolites from edible oil to avoid high amount of fat residues in the final solution.

SPE is a widely used sample pretreatment method, which has many advantages over traditional LLE in terms of selectivity, extracts, reproducibility, and avoidance of emulsion formation [24]. However, traditional SPE sorbents, such as C_{18} materials, show poor selectivity and only offer hydrophobic interaction for target compounds. Therefore, it is important to develop new and high selectivity materials as SPE sorbents to enrich and purify fipronil and its metabolites from edible oils. In our earlier studies, a humic acid bonded silica (HAS) was used as SPE sorbent to extract benzopyrene and abamectin from edible oils [25, 26]. Owing to its peculiar structure (shown in Fig. 2), HAS could provide multiple interactions with target analytes, such as chelation, charge-transfer interactions, hydrophobic interactions, dipole–dipole interactions, ion exchange reactions, and hydrogen bonding [27, 28]. As depicted in Fig. 1, the molecules of fipronil and its metabolites contain aromatic ring and some polar groups, thus those analytes could be captured through above interactions. In this paper, a GC–ECD coupled with SPE using HAS sorbent was proposed to determine fipronil and its metabolites in edible oils. Different parameters affecting the extraction process were studied and optimized in detail. To the best of our knowledge, this is the first time that the specific determination method of fipronil and its metabolites in edible oils is developed.

2 Materials and methods

2.1 Chemical and reagent

The humic acid bonded silica (50–74 μm) named as HiCapt Benzo was purchased from Weltech (Wuhan, China). The HAS SPE cartridges were packed as follows: A certain amount of sorbent was packed into a 6 mL polypropylene syringe, and the material was retained by two polyethylene frits. Then SPE was performed on a Supelco 12-port model SPE Vacuum Manifold (Bellefonte, PA, USA). Methanol, acetonitrile, *n*-hexane, acetone, dichloromethane, and ethyl acetate (HPLC grade) were all obtained from Fisher Scientific (USA).

Individual standard solution (1000 μg/mL) of fipronil was purchased from Agro-Environmental Protection Institute, Ministry of Agriculture (Tianjin, China). Three fipronil metabolites (99.99%) were purchased from Laboratories of Dr. Ehrenstorfer (Augsburg, Germany). The standard stock solution of fipronil and its metabolites (10 μg/mL) were prepared in methanol and stored at –18°C. The working standard solutions were prepared daily. The sample solutions were spiked to the desired concentrations for experiments.

2.2 Apparatus

GC–ECD analysis was performed using an Agilent 6890N system. The separation was achieved on a fused-silica capillary column (DB–1, 30 m × 0.32 mm id, film thickness 0.25 mm). The oven temperature was programmed at 150°C for 2.0 min, increased to 270°C at a rate of 6°C/min, and held for 5.0 min. The injection volume was 1.0 μL and splitless injection mode was used. Nitrogen (purity 99.999%) was used as the carrier gas at a flow rate of 2.0 mL/min. Temperatures at injection port and detector were 230 and 320°C, respectively.

2.3 Sample preparation

A 0.50 g of edible oil sample was exactly weighed into a 10 mL centrifuge tube, and then the test sample was spiked with known amount of analytes and incubated for 10 min at room temperature. The oil sample was diluted with 2 mL of *n*-hexane and vortexed for 1 min. Then, the mixture was loaded onto the HAS SPE cartridge which was sequentially preconditioned with 5 mL of acetone and 5 mL of *n*-hexane for condition. After the cartridge has been rinsed with 8 mL

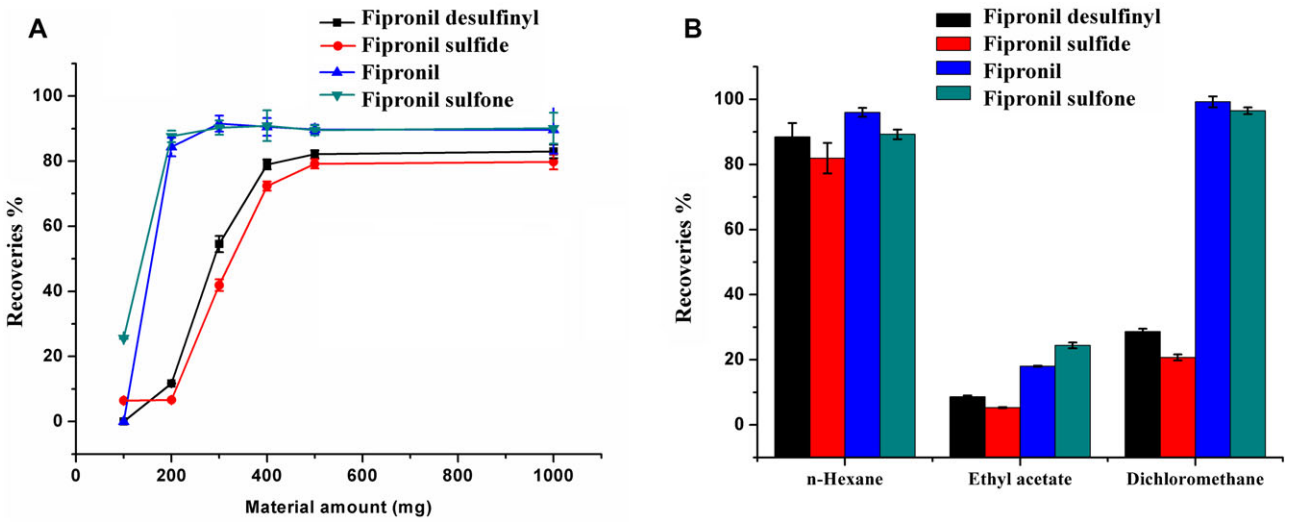

Figure 3. Effect of the amount of sorbent (A) and the types of loading solvent (B) on the recoveries of fipronil and its metabolites.

of ethyl acetate/*n*-hexane (30:70, v/v), 2 mL of acetone were used for elution (at about 1 mL/min) and the eluate was collected into a centrifuge tube. The collected fraction was evaporated to dryness under a mild nitrogen stream at room temperature. The residue was dissolved in 1 mL of *n*-hexane and the resulting solution was filtered through a disposable filter (0.45 μm pore size) for GC–ECD analysis.

2.4 Method validation

The analytical method was validated according to the single laboratory validation approach [29]. The performance of the method was evaluated according to linearity, LOD, LOQ, matrix effect, recovery, and repeatability. A blank oil sample was used to perform the whole method validation process.

3 Results and discussion

3.1 Optimization of the SPE conditions

The optimization of the SPE conditions was conducted on the blank oil samples spiked with 20 ng/g analytes. Various parameters such as amount of sorbent, loading solution, washing solution, desorption solution were investigated.

3.1.1 Optimization of the amount of sorbent

The sorbent is the core of SPE, and the amount of sorbent significantly affects the extraction efficiency of target analytes. Due to the complex matrices of edible oil, which is competitive with target analytes for adsorption sites in SPE process, cartridges packed with different amounts of HAS sorbents were evaluated according to the extraction efficiency of target analytes. As shown in Fig. 3A, with the amount of HAS ranging from 100 to 500 mg, the recoveries of four analytes demonstrated an upward trend; while the amount of material was further increased to 1000 mg, the recoveries did not change significantly. Therefore, 500 mg of HAS was selected in the following experiments.

3.1.2 Optimization of the sample loading conditions

Since the viscosity of oil samples is very large, it is necessary to select an appropriate solvent to dilute oil samples for extraction of target analytes by HAS-based SPE method. Dichloromethane, *n*-hexane and ethyl acetate were investigated in this study. The results are demonstrated in Fig. 3B. Ethyl acetate was not suitable as loading solvent due to its high polarity and the recoveries of all analytes were below 30%. When dichloromethane was used as loading solvent, the recoveries of fipronil and fipronil sulfone were about 90% while the recoveries of fipronil desulfinyl and fipronil sulfide were also below 30%. As to *n*-hexane, the recoveries of all analytes were shown to be greater than 80%. Therefore, *n*-hexane was selected for further experiment.

3.1.3 Optimization of the washing conditions

After sample loading, it came to the washing procedure which was essential, especially for the oil samples with complex sample matrices, because it would seriously disturb the detection of target analytes. The washing step should meet the demands that the matrix interferences should be removed to the maximum extent while the loss of target analytes should be controlled as much as possible. Therefore, different proportions of ethyl acetate to *n*-hexane were tested as the cleanup solution. The results are shown in Fig 4A. With the content of ethyl acetate increasing from 10% to 30%, the recoveries of four analytes decreased gradually; when the content of ethyl acetate was higher than 30%, the recoveries decreased rapidly, especially for fipronil desulfinyl and fipronil sulfide. As shown in Fig. 1, compared with fipronil and fipronil sulfone, the

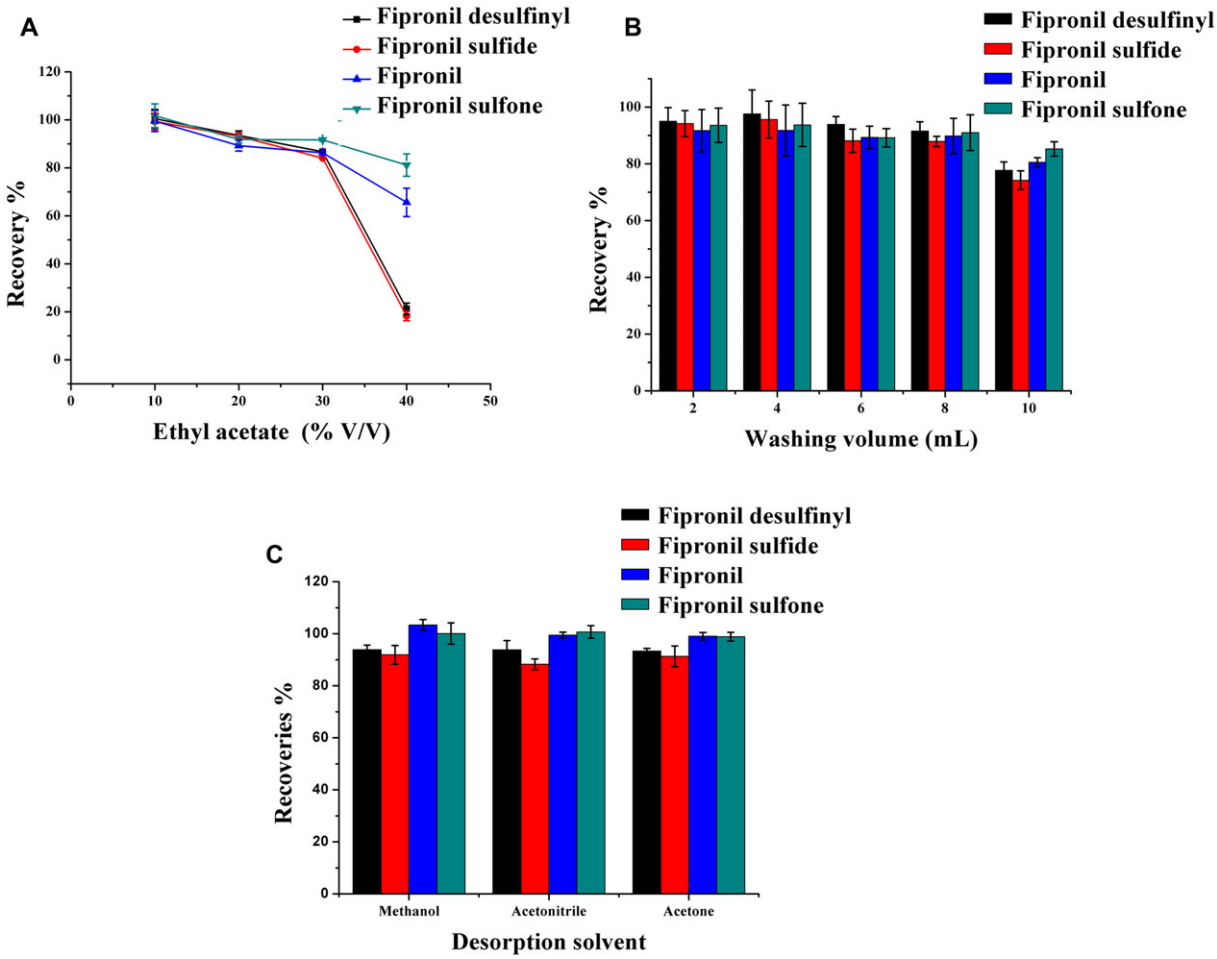

Figure 4. Effect of the proportion of washing solvent (the content of ethyl acetate, v/v) (A), the volume of washing solvent (B) and the types of desorption solvent (C) on the recoveries of fipronil and its metabolites.

molecules of fipronil desulfinyl and fipronil sulfide have not S=O groups, resulting in lower hydrogen bonding interactions between HAS adsorbent and two fipronil metabolites. It is easier to elute fipronil desulfinyl and fipronil sulfide from HAS adsorbent by ethyl acetate than fipronil and fipronil sulfone. Thus, ethyl acetate/*n*-hexane (30:70, v/v) was chosen as washing solution. We further optimized the volume of washing solution from 2 to 10 mL. As shown in Fig. 4B, the recoveries of fipronil and its metabolites have no obvious change with the volume of washing solution increasing from 2 to 8 mL. And when the volume of washing solution was 10 mL, there was an obvious reduction in the recoveries of fipronil and its metabolites. Therefore, 8 mL ethyl acetate/*n*-hexane (30:70, v/v) was considered to be the ideal washing solution for the high recoveries of fipronil and its metabolites and for the removal of interferences to the greatest extent.

3.1.4 Optimization of eluting solvents

The eluting conditions were further optimized. Different solvents such as methanol, acetonitrile and acetone were tested as eluents for the investigation of eluting efficiencies of fipronil and its metabolites. As shown in Fig. 4C, about 100% recoveries of fipronil and its metabolites could be achieved using those three solvents as eluents. Considering that acetone was much easier to concentrate for next analysis, acetone was selected as the eluting solvent. The effects of eluent volumes on the recoveries were also investigated. We found that 2.0 mL acetone was enough for the effective elution of fipronil and its metabolites from HAS cartridge.

To sum up, the optimal SPE conditions were as follows: 500 mg amount of HAS, *n*-hexane as loading solvent, 8 mL ethyl acetate/*n*-hexane (30:70, v/v) as washing solution, 2.0 mL acetone as eluting solvent. Figure 5 shows the typical chromatograms of fipronil and its metabolites for blank and spiked oil samples and standard solution under the optimized SPE conditions. No interferences from sample matrices were observed after SPE pretreatment.

Furthermore, the lot-to-lot reproducibility of HAS SPE sorbents was investigated to access the feasibility of the method for routine analysis. Three batches of the HAS sorbents were used to extract fipronil under the optimized

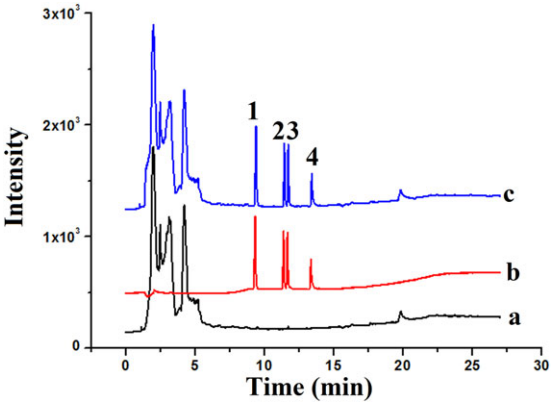

Figure 5. GC–ECD chromatograms of a blank oil sample (A), a 20 ng/g standard solution of fipronil and its metabolites (B) as well as a blank sample spiked with fipronil and its metabolites at the concentration of 20 ng/g (c) under the optimized SPE conditions. Peaks: 1. fipronil desulfinyl; 2. fipronil sulfide; 3. Fipronil; 4. fipronil sulfone.

conditions. The extraction recoveries of three batches of the HAS sorbents were 104.0, 98.3, and 106.2%, indicating the good batch reproducibility of HAS sorbents.

3.2 Method validation

3.2.1 Matrix effect

Matrix effect was always observed in GC analysis due to the adsorption of the analytes or matrix components on the active sites in the injection port. Matrix effect could be determined by the following equation: matrix effect (%) = (peak area of matrix standard–peak area of solvent standard) × 100/peak area of solvent standard. As shown in Table 1, the matrix effects of fipronil and its desulfinyl, sulfide, and sulfone metabolites were 1.3, 1.2, 3.0, and 0.4%, respectively. The result indicated that matrix effects of all analytes are very weak, and SPE process can effectively remove the matrices of edible oil samples.

3.2.2 Linearities and sensitivity

In current study, matrix-matched calibration solutions spiking in blank sample solutions at six concentration levels from 1 to 500 ng/mL were prepared to avoid the matrix effects. The calibration curves were established by plotting the peak areas of the analytes versus the concentrations of analytes. LODs and LOQs were calculated as the concentrations corresponding to a signal of three and ten times the SD of the baseline noise, respectively. As listed in Table 1, fipronil and its metabolites showed good linearity with R^2 above 0.9996. The LODs and LOQs were found to be in the range of 0.3–0.5 and 1.0–1.6 ng/g, respectively.

3.2.3 Recoveries and repeatability

Recoveries were investigated in spiked oil samples at three different concentration levels of 10.0, 50.0, and 200.0 ng/g. The recoveries were determined by comparing the calculated amounts of analytes in the samples (using calibration curves) with the spiking amounts. The repeatability of the method was assessed by determining the intra- and interday RSDs at three concentration levels. As listed in Tables 2 and 3, the recoveries at three different concentration levels of 10.0, 50.0, and 200.0 ng/g ranged from 83.1 to 106.0%, and intraday and interday RSDs were all below 8.7%. Those results indicated that the proposed method was suitable for routine analysis.

3.2.4 Comparison with the previous methods

To the best of our knowledge, there was no reported special determination method of fipronil and its metabolites in edible oils. Due to the abundant lipid matrices in edible oils, the matrix of edible oil is much more complex compared with other agricultural products, which makes the sample preparation process of edible oil very difficult. The comparison in the terms of performance of our newly developed method with the previous reported methods in recent years for the determination of fipronil and its metabolites in food matrices are illustrated in Table 4. Recoveries, LODs, and RSDs of our method are satisfactory, and better than most other studies. Furthermore, an effective one-step process of extraction and cleanup of fipronil and its metabolites in edible oils was achieved with an HAS-based SPE in this work, and the required time for sample preparation of our method was only about 15 min, much less than that of reported methods, in which multi-step extraction and cleanup were necessary. The experimental and comparative results well indicated that our proposed method may be used to effectively monitor fipronil and its metabolites in edible oils.

Table 1. Linear dynamic range, R^2 value, limit of detection (LOD), limit of quantification (LOQ), and matrix effects of fipronil and its metabolites in edible oils

Analytes	Linear dynamic range (ng/g)	R^2 value	LODs (ng/g)	LOQs (ng/g)	Matrix effects (%)
Fipronil desulfinyl	1.0–500	0.9996	0.3	1.0	1.2
Fipronil sulfide	2.0–500	0.9997	0.5	1.6	3.0
Fipronil	1.0–500	0.9999	0.3	1.0	1.3
Fipronil sulfone	2.0—500	0.9999	0.5	1.6	0.4

Table 2. Average recoveries and RSDs of fipronil and its metabolites spiked in edible oils via GC-ECD analysis ($n = 4$)

Analytes	10 ng/g spiked level		50 ng/g spiked level		200 ng/g spiked level	
	Recoveries (%)	RSD (%)	Recoveries (%)	RSD (%)	Recoveries (%)	RSD (%)
Fipronil desulfinyl	94.9	8.5	94.7	9.9	94.6	5.4
Fipronil sulfide	86.3	1.7	83.1	11.2	83.4	5.8
Fipronil	106.0	11.4	104.0	19.7	100.6	9.3
Fipronil sulfone	85.1	7.1	90.8	8.6	96.2	7.9

Table 3. Method precisions at three different concentrations for determination of fipronil and its metabolites in edible oils

Analytes	Intraday precision (RSD, %; $n = 4$)			Interday precision (RSD, %; $n = 3$)		
	10 ng/g	50 ng/g	200 ng/g	10 ng/g	50 ng/g	200 ng/g
Fipronil desulfinyl	8.7	2.3	2.1	4.7	0.6	5.6
Fipronil sulfide	5.1	4.0	2.7	4.6	2.2	8.7
Fipronil	3.2	2.6	3.4	3.3	4.2	6.2
Fipronil sulfone	5.9	0.9	1.9	1.2	2.6	4.0

Table 4. Comparison of method performance with the previous method for detection of fipronil and its metabolites

Sample	Preparation methods	Detection techniques	LODs (ng/g)	Time for sample preparation (min)	Recoveries (%)	RSDs (%)	Reference
Green pepper	QuEChERS	UPLC-MS/MS	5	>30	80.2–112.0	3.6–8.2	[12]
Corn	QuEChERS	UPLC-MS/MS	0.5–2.5	>25	82.4–104.6	1.2–9.4	[5]
Vegetables and fruit	QuEChERS	GC-MS	10*	>25	86.0–112.0	<10.2	[1]
Sugar	QuEChERS	GC-MS	5*	>40	87.5–108.5	0.2–5.3	[6]
Peanut	QuEChERS	UPLC-MS/MS	0.3	>15	66.0–116.0	≤19.0	[2]
Maize	liquid–solid extraction, liquid–liquid partitioning, SPE cleanup	GC-ECD	0.3–0.5	>50	83.0–106.0	≤8.9	[7]
Rice	Extraction with acetone and dichloromethane, d-SPE cleanup	GC-ECD	3	>180	85.0–94.3	1.1–4.3	[13]
Okra	Extraction with EtOAc and d-SPE cleanup	UPLC-MS/MS	1	>20	80.0–107.0	Not mentioned	[10]
Oil	SPE	GC-ECD	0.3-0.5	About 15	83.1–106.0	0.9–8.7	This work

*LOQ value.

3.3 Application of the proposed method for determination of fipronil and its metabolites in commercial edible oil products

The proposed HAS-based SPE coupled with GC–ECD method was successfully applied to the trace analysis of fipronil and its metabolites in eleven kinds of vegetable oils from markets in Wuhan, including two rapeseed oils, two rice oils, two blend oils, two sesame oils, one peanut oil, one soybean oil, one corn oil. Fipronil ranging from 3.0 to 5.2 μg/kg was detectable in one rice oils, two blend oils and one corn oil samples. Multiple reaction monitoring (MRM) acquisition mode in GC–MS/MS system (Shimadzu GCMS-TQ 8030) was used for confirmation of structural identity of fipronil. Two confirmative ion pairs of fiprobil are 366.90>212.90 and 366.90>254.90. The residues of fipronil were confirmed in four samples by GC–MS/MS method. Fig. 6 showed typical chromatograms of a positive oil sample in GC–ECD and GC–MS/MS.

4 Concluding remarks

In this study, the feasibility of SPE based on HAS sorbent coupled to GC–ECD for quantitative determination of fipronil

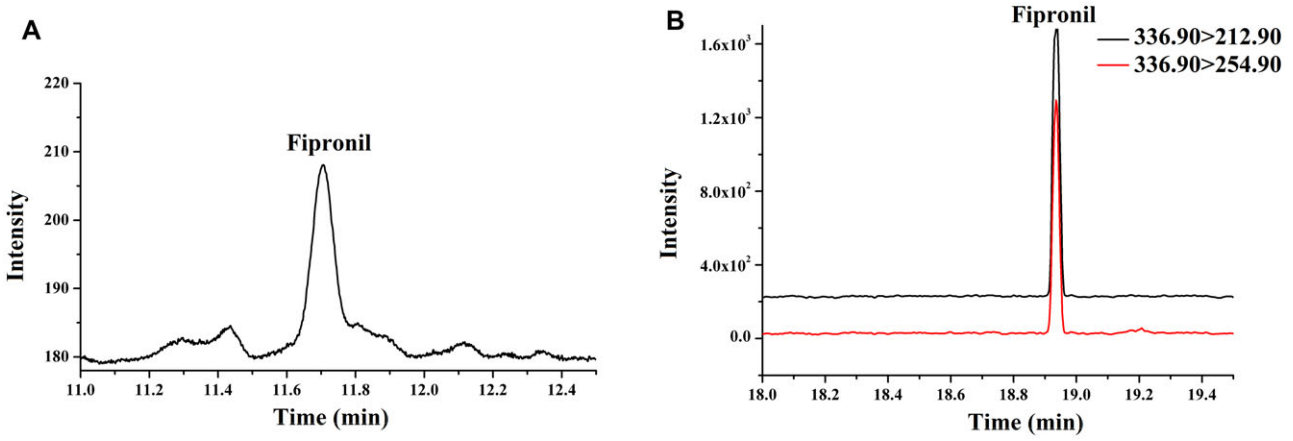

Figure 6. GC–ECD (A) and GC–MS/MS (B) Chromatograms of a positive edible oil sample.

and its metabolites in edible oils was demonstrated. The sampling, washing, and eluting conditions and the amounts of sorbent were optimized to remove the matrix interferences as much as possible and obtain high recoveries of target analytes. Under the optimized conditions, the proposed method was validated according to linearity, LOD, LOQ, matrix effect, recovery, and repeatability. Recoveries of analytes spiked at three different concentration levels were between 83.1 and 106.0% with intraday and interday RSDs below 8.7%. Then the proposed method was applied to determine fipronil and its metabolites in 11 kinds of vegetable oils and the detection results were confirmed by GC–MS/MS. Fipronil ranging from 3.0 to 5.2 μg/kg was detectable in four edible oil samples. In China, the maximum residue limit (MRL) of fipronil in some vegetables and maize is 20 and 100 ng/g (GB/T 2763-2014), respectively. Due to high consumption of edible oil by humans, the residue of fipronil and its metabolites in the commercial edible oils might has some potential threat to human health.

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The authors have declared no conflict of interest.

5 References

[1] Paramasivam, M., Chandrasekaran, S., Determination of fipronil and its major metabolites in vegetables, fruit and soil using QuEChERS and gas chromatography-mass spectrometry. *Int. J. Environ. Anal. Chem.* 2013, *93*, 1203–1211.

[2] Li, M., Li, P., Wang, L., Feng, M., Han, L., Determination and dissipation of fipronil and its metabolites in peanut and soil. *J. Agric.Food Chem.* 2015, *63*, 4435–4443.

[3] Vasylieva, N., Ahn, K. C., Barnych, B., Gee, S. J., Hammock, B. D., Development of an immunoassay for the detection of the phenylpyrazole insecticide fipronil. *Environ. Sci. Technol.* 2015, *49*, 10038–10047.

[4] Duhan, A., Kumari, B., Duhan, S., Determination of residues of fipronil and its metabolites in cauliflower by using gas chromatography-tandem mass spectrometry. *Bull. Environ. Contam. Toxicol.* 2015, *94*, 260–266.

[5] Cheng, Y. P., Dong, F. S., Liu, X. G., Xu, J., Meng, W., Liu, N., Chen, Z. L., Tao, Y., Zheng, Y. Q., Simultaneous determination of fipronil and its major metabolites in corn and soil by ultra-performance liquid chromatography-tandem mass spectrometry. *Anal. Methods* 2014, *6*, 1788–1795.

[6] Ramasubramanian, T., Paramasivam, M., Jayanthi, R., Chandrasekaran, S., A simple and sensitive single-step method for gas chromatography-mass spectrometric determination of fipronil and its metabolites in sugarcane juice, jaggery and sugar. *Food Chem.* 2014, *150*, 408–413.

[7] Wang, T., Hu, J., Liu, C., Simultaneous determination of insecticide fipronil and its metabolites in maize and soil by gas chromatography with electron capture detection. *Environ. Monit. Assess.* 2014, *186*, 2767–2774.

[8] Saini, S., Rani, M., Kumari, B., Persistence of fipronil and its metabolites in soil under field conditions. *Environ. Monit. Assess.* 2014, *186*, 69–75.

[9] Feng, X., He, Z. Y., Wang, L., Peng, Y., Luo, M., Liu, X. W., Multiresidue analysis of 36 pesticides in soil using a modified quick, easy, cheap, effective, rugged, and safe method by liquid chromatography with tandem quadruple linear ion trap mass spectrometry. *J. Sep. Sci.* 2015, *38*, 3047–3054.

[10] Hingmire, S., Oulkar, D. P., Utture, S. C., Shabeer, T. P. A., Banerjee, K., Residue analysis of fipronil and difenoconazole in okra by liquid chromatography tandem mass spectrometry and their food safety evaluation. *Food Chem.* 2015, *176*, 145–151.

[11] Kaur, R., Mandal, K., Kumar, R., Singh, B., Analytical method for determination of fipronil and its metabolites in vegetables using the QuEChERS method and gas chromatography/mass spectrometry. *J.AOAC Int.* 2015, *98*, 464–471.

[12] Xavier, G., Chandran, M., George, T., Beevi, S. N., Mathew, T. B., Paul, A., Arimboor, R., Vijayasree, V.,

Pradeepkumar, G. T., Rajith, R., Persistence and effect of processing on reduction of fipronil and its metabolites in chilli pepper (Capsicum annum L.) fruits. Environ. *Monit. Assess.* 2014, *186*, 5429–5437.

[13] Kumar, R., Singh, B., Mandal, K., Kar, A., Sarao, P. S., Bioefficacy and fate of fipronil and its metabolites in basmati rice under sub-tropical climatic conditions. *Crop Prot.* 2013, *45*, 41–48.

[14] Garcia-Chao, M., Agruna, M. J., Calvete, G. F., Sakkas, V., Llompart, M., Dagnac, T., Validation of an off line solid phase extraction liquid chromatography-tandem mass spectrometry method for the determination of systemic insecticide residues in honey and pollen samples collected in apiaries from NW Spain. *Anal. Chim. Acta.* 2010, *672*, 107–113.

[15] Jimenez, J. J., Bernal, J. L., Del Nozal, M. J., Martin, M. T., Mayo, R., Sample preparation methods to analyze fipronil in honey by gas chromatography with electron-capture and mass spectrometric detection. *J. Chromatogr. A* 2008, *1187*, 40–45.

[16] Nakajima, T., Tsuruoka, Y., Kanda, M., Hayashi, H., Hashimoto, T., Matsushima, Y., Yoshikawa, S., Nagano, C., Okutomi, Y., Takano, I., Determination and surveillance of nine acaricides and one metabolite in honey by liquid chromatography-tandem mass spectrometry. *Food Addit. Contam.* 2015, *32*, 1099–1104.

[17] Jimenez, J. J., Bernal, J. L., Del Nozal, M. J., Martin, M. T., Mayo, R., Comparative study of sample preparation procedures to determine fipronil in pollen by gas chromatography with mass spectrometric and electron-capture detection. *J. Chromatogr. A* 2007, *1146*, 8–16.

[18] Kadar, A., Faucon, J.-P., Determination of traces of fipronil and its metabolites in pollen by liquid chromatography with electrospray ionization-tandem mass spectrometry. *J. Agric. Food Chem.* 2006, *54*, 9741–9746.

[19] Bichon, E., Richard, C. A., Le Bizec, B., Development and validation of a method for fipronil residue determination in ovine plasma using 96-well plate solid-phase extraction and gas chromatography-tandem mass spectrometry. *J. Chromatogr. A* 2008, *1201*, 91–99.

[20] Lacroix, M. Z., Puel, S., Toutain, P. L., Viguie, C., Quantification of fipronil and its metabolite fipronil sulfone in rat plasma over a wide range of concentrations by LC/UV/MS. *J. Chromatogr. B* 2010, *878*, 1934–1938.

[21] Chamkasem, N., Ollis, L. W., Harmon, T., Lee, S., Mercer, G., Analysis of 136 pesticides in avocado using a modified QuEChERS method with LC-MS/MS and GC-MS/MS. *J. Agric.Food Chem.* 2013, *61*, 2315–2329.

[22] Rajski, L., Lozano, A., Ucles, A., Ferrer, C., Fernandez-Alba, A. R., Determination of pesticide residues in high oil vegetal commodities by using various multi-residue methods and clean-ups followed by liquid chromatography tandem mass spectrometry. *J. Chromatogr. A* 2013, *1304*, 109–120.

[23] Farajzadeh, M. A., Feriduni, B., Mogaddam, M. R. A., Determination of triazole pesticide residues in edible oils using air-assisted liquid-liquid microextraction followed by gas chromatography with flame ionization detection. *J. Sep. Sci.* 2015, *38*, 1002–1009.

[24] Qi, D. J., Kang, X. J., Chen, L. Q., Zhang, Y. Y., Wei, H. M., Gu, Z. Z., Electrospun polymer nanofibers as a solid-phase extraction sorbent for the determination of trace pollutants in environmental water. *Anal. Bioanal.Chem.* 2008, *390*, 929–938.

[25] Luo, D., Yu, Q. W., Yin, H. R., Feng, Y. Q., Humic acid-bonded silica as a novel sorbent for solid-phase extraction of benzo[a]pyrene in edible oils. *Anal. Chim. Acta.* 2007, *588*, 261–267.

[26] Wu, J.Y., Zhang, Y., Peng, X. T., Yu, Q. W., Feng, Y. Q., Rapid and sensitive detection of abamectin in edible oils by solid phase extraction combined with ultra-high-pressure liquid chromatography-tandem mass spectrometry. *Anal.Methods* 2014, *6*, 8767–8772.

[27] Alvarez-Puebla, R. A., Valenzuela-Calahorro, C., Garrido, J. J., Retention of Co(II), Ni(II), and Cu(II) on a purified brown humic acid. *Langmuir* 2004, *20*, 3657–3664.

[28] Jansen, S. A., Malaty, M., Nwabara, S., Johnson, E., Ghabbour, E., Davies, G., Varnum, J. M., Structural modeling in humic acids. *Mater. Sci. Eng. C* 1996, *4*, 175–179.

[29] European Commission. (2010). Guidance document on pesticide residue analytical methods Document No. SANCO/825/00/rev. 8.1/16/11/2010. Available from:http://ec.europa.eu/food/plant/protection/resources/guide_doc_825-00_rev7_en.pdf>.

