Analytical Methods

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Simultaneous determination of fipronil and its major metabolites in corn and soil by ultra-performance liquid chromatography-tandem mass spectrometry

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A simple, quick, effective method was developed for determination of fipronil and its three metabolites in soil and corn by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Samples were extracted with acetonitrile and cleaned-up with C18, C18/PSA, PSA/GCB for soil, corn grain, and corn straw, respectively. The MS/MS parameters optimization was performed in multi-reaction monitoring (MRM) mode, and electrospray ionization (ESI) in negative mode was selected. The mean recoveries of the four compounds in soil, corn grain, and corn straw matrices at four fortification levels were in the rang of 82.4%-104.6%; the intra-day and inter-day RSDs ranged from 1.2% to 7.7% and 2.4% to 9.4%, respectively. The LODs of fipronil and its three metabolites in soil, corn grain, and corn straw matrices were estimated to be 0.5-2.5 μg•kg^{-1}, the LOQs were 5 μg•kg^{-1} for soil and corn grain, 10 μg•kg^{-1} for corn straw. The developed method was also applied for studying fipronil dissipation in soil and corn. The result further confirmed the reliability and efficacy of the proposed method for routine pesticide residue monitoring in soil and corn samples.

Introduction

Fipronil, a broad spectrum systemic phenylpyrazole insecticide with excellent effectiveness against piercing-sucking and chewing pests, had been widely used for controlling many species of soil and foliar insects on various crops such as corn, sunflower, rice, vegetables and fruits. In addition, it had also been used in non-agricultural areas such as wood preservation and sanitizer.[1] However, acute toxicity studies show that fipronil is highly toxic to many aquatic organisms and bees.[2] Also, fipronil could be degradated to some more toxic metabolites in environment (fig. 1).[3] Fipronil sulfone, an oxidative producte, was found to be 6.6 times more toxic to freshwater invertebrates and 6.3 times more toxic to rainbow trout than the parent compound;[3, 4] Fipronil desulfinyl, another main degradation product formed by photolysis, is extremely stable and generally more toxic to a variety of animals than the parent molecule;[5] fipronil sulfide, a reductive metabolite, is 1.9 times more toxic to freshwater invertebrates.[6]

Thus, there has been a great concern about fipronil and its toxic metabolites. At present, fipronil is only allowed to be used for corn or other upland crops seeds treating (mainly for corn seeds coating) in China.[7] Moreover, in many European

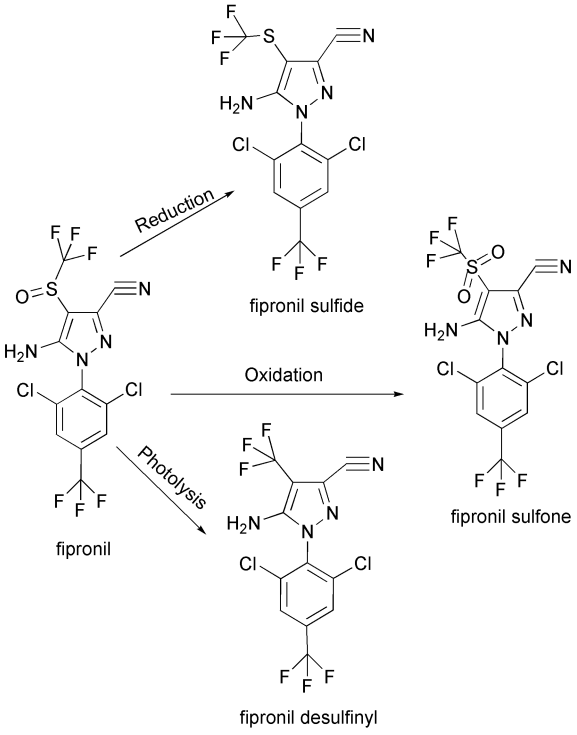

Fig. 1 The degradation routes of fipronil to three toxic metabolites.

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States, the use of fipronil as seeds tanning was temporarily suspended in recent years.[8] Therefore, it is of the utmost importance to develop high sensitivity, selectivity, accuracy and precision analytical methods of simultaneous determination of fipronil and its toxic metabolites in corn and soil for accurate evaluation the risks posed by these pesticides to environment.

To date, some methods have been reported for the analysis of fipronil, occasionally together with its metabolites in different matrices. But most of them were commonly performed by gas chromatography coupled with electron capture detection (ECD),[9-12] or mass spectrometry (MS) detection.[13-18] As for the determination of fipronil by LC-MS/MS, Sabatino et al. proposed a method for simultaneous determination of 7 neonicotinoids and fipronil in corn seeds,[8] however, the method was not involved in the determination of fipronil metabolites, and its analysis time was 32 min, indicating that the technique required large volumes of toxic solvent and was normally time-consuming. Similar literatures about determination of fipronil in water,[19, 20] soya grain,[21] bamboo,[22] apple, strawberry, tomato, spinach,[23] bovine milk[24] matrices by LC-MS/MS was also reported, but none of them related to the metabolites of fipronil except for the determination in pollen by A. Kadar et al.[25] and in rat plasma by M. Lacroix etal.[26]

In the present study, we have developed and validated a method for simultaneous determination of fipronil and its major metabolites fipronil sulfone, fipronil sulfide and fipronil desulfinyl in corn and soil samples by QuEChERS (quick, easy, cheap, effective, rugged and safe) using UPLC-MS/MS. The method's suitability was evaluated by applying the method in the determination of fipronil and its metabolites in corn and soil samples from our residual trial field to study the degradation of fipronil.

Experimental

Reagents and chemicals

Fipronil standard (99.7% purity) and its metabolites, fipronil sulfone (99.7% purity), fipronil sulfide (97.1% purity) fipronil desulfinyl (97.8% purity) were obtained from Rhone-Poulenc Agro, Lyon, France. Chromatography grade acetonitrile, methanol and formic acid were purchased from Honeywell International (New Jersey, USA). Ultra-pure water was prepared by using Milli-Q water purification system (Bedford, MA, USA). Analytic grade anhydrous magnesium sulfate, sodium chloride, ammonium acetate and acetonitrile were purchased from Sinopharm Chemical Reagent Beijing Company (Beijing, China). Primary secondary amine (PSA, 40 μm), octadecylsilane (C18, 40 μm), and graphitized carbon black (GCB, 40 μm) sorbents were purchased from Agela Technologies, Inc. (Beijing, China).

Standard stock solution of fipronil (100mg•L^{-1}) and its three major metabolites were prepared in acetonitrile, the pure solvent solutions required for standard curve (5-1000 μg•L^{-1}) were prepared from the stock solution by serial dilution to 5, 10, 50, 100, 500, 1000μg•L^{-1} with acetonitrile. Correspondingly, matrix-matched standard solutions were prepared (5, 10, 50, 100, 500, 1000μg•L^{-1}) by adding blank sample extract (soil, corn grain, and corn straw) to each serially diluted standard solution. All solutions were protected against light with aluminum foil and stored at -20 ℃ prior to use.

Instrumentation and LC-MS/MS Analytical Conditions

Chromatographic separation of fipronil and its three major metabolites was performed on a Waters Acquity UPLC system, which included a Waters Acquity UPLC binary solvent manager, an Acquity UPLC sample manager, and Acquity cartridge heater equipped with a Waters Acquity UPLC BEH (bridged ethylene hybrid) Shield RP18 column (100 mm×2.1 mm, 1.7-μm particle size) (Milford, MA, USA). The mobile phase consisting of acetonitrile (A) and Milli-Q Ultra-pure water (B) was pumped at a flow rate of 0.3 mL min^{-1} during the analysis process. The gradient elution program was adopted (0.0 min, 30% A; 1.0 min, 70% A; 3.5 min, 95% A; 3.6 min, 30% A; and 5.0 min, 30% A). The four compounds were eluted within 3.0 min. The injection volume was 1 μL. The temperature of column oven was set at 40 ℃±5 ℃ for decreasing the viscosity, and the temperature of sample room was maintained at 5 ℃.

Analysis of the four compounds was conducted on a triple-quadrupole mass spectrometer (TQD, Waters Corp.) equipped with an electrospray ionization (ESI) source. The nebulizer gas was 99.95% nitrogen, and the collision was 99.999% argon with a pressure of 2×10^{-3} mbar in the T-wave cell. MS/MS detection was performed in negative ion mode and the monitoring conditions optimized for target compounds. The conditions were typically as follows: the capillary voltage was set at 3.0 KV, and the cone voltage was 30 V; the source temperature and desolvation temperature were held at 150 ℃ and 400 ℃, respectively, 50 L•h^{-1} cone gas flow and 1000 L•h^{-1} desolvation gas flow were used. Multi-reaction monitoring (MRM) was used for the detection of all pesticides with a dwell time of 0.072 s. Infusion experiments of each compound were conducted to optimize the intensity in both positive and negative ionization modes. All other ESI and MS parameters were optimized individually for each target compound and were listed in Table 1. The Masslynx software (version 4.1) was used to collect and analyze the data obtained.

Sample Preparation

Soil, corn grain and corn straw samples were collected from our experimental plots located in Shandong Province, China. Blank matrices were not applied and contaminated by fipronil and its metabolites, soil samples were passed through a 2-mm sieve. Approximately 500 g of chopped corn straw or corn grain samples were smashed in a food processor. An aliquot sample (10 g of soil, 10 g of corn grain, or 5 g of corn straw) was weighed in a 50-mL polypropylene centrifuge tube with screw caps. Appropriate volumes of working standard solution was added to blank samples for recovery study, and the tubes containing the targeted samples were vortexed by an XW-80A Vortex (Kirin Medical Instrument, China) for 30 s and allowed to stand for 2 hour at room temperature to distribute the pesticide evenly and to ensure complete interaction with the sample matrix. 5 mL ultra-pure water (10 mL for corn straw) and 10 mL acetonitrile was then added. After screwing the tube caps, the mixtures were vortexed vigorously for 5 min. Thereafter, 4 g anhydrous magnesium sulfate and 1.0 g sodium chloride[27] were added, the tubes were capped and immediately vortexed intensively for 3 min and then centrifuged with a TG16-WS centrifuge (Xiangyi Centrifuge Machines, China) for 5 min at relative centrifugal force (RCF) 2811 ×g (4000rpm). Next, A volume of 1.5 mL of prepared aliquot was sampled from the upper layer (acetonitrile) into a 5 mL single-use centrifuge tube containing an amount of sorbent (30mg C18 for soil, 30 mg PSA and 20 mg C18 for corn grain, 20 mg GCB and 50 mg PSA for corn straw) and 150 mg anhydrous magnesium sulfate. The samples were vortexed again for 1 min and then centrifuged (using a centrifuge, model Sigma 1-15, Germany) for 5 min at 3,600 rpm. The resulting supernatants were filtered with a 0.22-μm nylon syringe filter into an auto-sampler vial for UPLC-MS/MS injection.

Method validation

The developed method was validated to evaluate its performance by a conventional validation procedure which including the following parameters: specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), matrix effect, accuracy, precision, and stability. Blank samples (soil, corn grain, and corn straw) were analyzed to determine the absence of interfering substances around the retention time of analyte. The linearity of the method was evaluated by linear regression analysis of both standard solution and matrix-matched calibration curves. The LOD was considered to be the analyte's concentration that produced signal-to-noise (S/N) ratio of 3. And it was estimated from the chromatogram corresponding to the lowest point used in the matrix-matched calibration. The LOQ was defined as the lowest spiked level with satisfactory values of recovery (70%-120%) and RSD (⩽20%). Matrix effect could be calculated as follows: matrix effect (ME, %) =(slope of calibration curves in matrix - slope of calibration curves in solvent)/slope of calibration curves in solvent ×100%.

The recovery assays were carried out to investigate the accuracy and precision of the method. Five replicates of spiked samples (soil, corn grain, and corn straw) at four levels (5 or 10, 10 or 20, 100 and 500 $\mu g \cdot kg^{-1}$) were prepared on three different days with the same instrument by different operators. The precision in these conditions for repeatability and reproducibility was expressed as the intra-day and inter-day relative standard deviation (RSD_r and RSD_R respectively).

Stability of the stock solutions was tested monthly by injection of a newly prepared working solution. Soil, corn grain, and corn straw matrix-matched standards of 100 $\mu g \cdot L^{-1}$ were analyzed monthly, and all stable samples were stored at -20 ℃.

Dissipation dynamics study

The developed method was applied in a field trial of fipronil dissipation; the experiment were conducted in Shandong province in 2012. Experiment field consisted of three replicate plots with an area of 30 m^2 and a control plot (without fipronil for control samples). A buffer area was used to separate the plots with different treatments in the same field. The trials were conducted from June 10 to August 26. To investigate fipronil dissipation in corn plants, seeds were coated with fipronil commercial formulation (50 $g \cdot L^{-1}$ flowable concentrate for seed treatment (FS), provided by Shandong United Pesticide Industry Co., Ltd.) at the dosage of 1:83.3 (weight ratio of pesticide to seed) and then sowed. On June 28, when corn plants' mean height was about 15 cm, corn straw were collected randomly and taken as the 0-day samples. Subsequently, corn straws of 1, 3, 7, 14, 21 and 28 days were also sampled. For the dissipation study in soil, bald soil were sprayed using 50 $g \cdot L^{-1}$ fipronil FS at a rate of 24 g active ingredient hm^{-2}. Soil samples were collected at a depth of 0-10 cm and at various time intervals:0 (2 h after spraying), 1, 3, 7, 14, 21, 28, 45 and 60 days. All samples were put into polyethylene bags and transported to the laboratory. The subsamples were stored in the dark at less than -20 ℃ until analyses. When analyzed, all calculations for the analyses of the field samples were based on dry weight.

Results and Discussion

Optimization of MS/MS

The analysis of fipronil and its metabolites was performed in MRM mode, 500 $\mu g \cdot L^{-1}$ working solutions were infused to optimize the MS/MS parameters and to select two appropriate transitions for each compound by IntelliStart software in ESI positive and negative modes. The results demonstrated that responses of the four

analytes is higher in negative mode than that in positive mode. Thus, ESI in negative mode was selected for subsequent experiments. The infusion process was carried out under the same chromatographic conditions as those used during analysis. All compounds showed abundant $[M-H]^-$ ions, which were usually selected as precursor ions. Identification was conducted based on the retention time, on the two selected ion transitions and on their relative abundance. Table 1 shows the chemical formulas, molecular weights, precursor ions, cone voltages, and corresponding collision voltages.

Table 1 Experimental parameters and UPLC-MS/MS conditions of fipronil and its three metabolites in ESI^- mode.

Compound	Molecular formula	Molecular weight	t_R (min)	CV (V)	Quantification ion transition	CE 1 (eV)	Confirmatory ion transition	CE 2 (eV)	Ion ratio
fipronil	$C_{12}H_4Cl_2F_6N_4OS$	435.94	2.44	34	434.91→330.01	16	434.91→250.03	22	3.0
fipronil sulfone	$C_{12}H_4Cl_2F_6N_4O_2S$	451.93	2.65	36	450.97→414.97	26	450.97→282.03	26	1.2
fipronil sulfide	$C_{12}H_4Cl_2F_6N_4S$	419.94	2.69	36	418.98→382.99	10	418.98→262	26	8.0
fipronil desulfinyl	$C_{12}H_4Cl_2F_6N_4$	387.97	2.56	34	387→351.01	14	387→282.09	28	1.1

CV: cone voltage; CE: collision energy; ion ratio = area of quantification ion/area of confirmatory ion.

Optimization of chromatography

Mobile phase composition plays an important role for peak shapes or retention behavior of the analyte. Therefore, modification of the mobile phase with additives is often performed to improve both LC separation and ionization efficiency. In this study, different mobile phase compositions (acetonitrile-water; acetonitrile-0.1% (v/v) formic acid aqueous solution; acetonitrile-0.2% (v/v) formic acid aqueous solution; acetonitrile-1.0% (v/v) formic acid aqueous solution; acetonitrile-2mmol ammonium acetate aqueous solution; acetonitrile-5mmol ammonium acetate aqueous solution; acetonitrile-5mmol ammonium acetate and 0.1% (v/v) formic acid aqueous solution; methanol-water) were tested in the gradient program with a 0.3mL min^{-1} flow rate. The data showed that the introduction of ammonium acetate or formic acid to the mobile phase could adversely affect peak shape and decrease the response values of the four target compounds. By contrast, when acetonitrile-water without any additives was used as the mobile phase, higher sensitivity and better peak shape could be obtained. The result may be related to the specific chemical properties of target analytes, or the sorts as well as the concentrations of the additives, however, this needs to be further investigated. Thus a solvent system consisting of acetonitrile and water was finally selected. Chromatographic conditions were optimized to achieve good resolution, increase the analyte signal and minimize analysis times. Typical UPLC-MS/MS chromatograms of standard, blank and fortified sample are shown in Fig. 2A-2D. There were no interference peaks around the retention times of the analytes, and the analysis time of the four compounds was less than 5.0 min. With the use of Acquity UPLC BEH Shield RP18 column in this study, analysis time was considerably reduced, much shorter than the previous study (32 min) [8].

Optimization of clean-up procedure

In this study, three common types of sorbent PSA, C18, and GCB were used to evaluate the effect on recovery in soil, corn grain and corn straw matrices. As we know, PSA has a weak anion exchange function and applies to extract polar compounds from the non-polar samples to remove matrix compounds like sugars and fatty acids. C18 is suitable to extract non-polar and moderately polar compounds from the polar samples, which are mainly used for reversed phase extraction. GCB, a weakly polar or nopolar sorbent, mainly used to remove hydrophobic interaction-based compounds, such as chlorophyll, carotenoids, sterols.[28, 29]

Considering some lipid compouds in corn grain and many types of chlorophyll in corn straw, C18/PSA and PSA/GCB combination were investigated for corn grain and corn straw, respectively. As shown in fig. 3, recoveries of fipronil and its three metabolites were all satisfied (79.6-109.6%) when 30 mg or 50 mg C18, 30 mg GCB, 50 mg PSA were used in soil cleanup. By contrast, the recoveries of the four compounds were very high (exceed 140%) or too low (lower than 60%) when using 10 mg GCB or 30 mg PSA for soil, this may be caused by insufficiently eliminating of interfering impurities. C18 is relatively cheaper than PSA or GCB, and good result using 30 mg C18 could be achieved. Furthermore, UPLC-MS full scan chromatograms (fig. 4) of soil blank matrices treated by 30 mg C18 showed the great decrease of signal intensity compared with that of unpurified blank matrices, which means some matrix components were removed to some extent. Taking these factors into consideration, 30 mg C18 was ultimately chosen as sorbent for soil. Similarly, the effect of some sorbent combination including 10 mg C18/30 mg PSA, 10 mg C18/50 mg PSA, 20 mg C18/30 mg PSA, 20 mg C18/50 mg PSA for corn grain and 20 mg GCB/30 mg PSA, 40 mg GCB/30 mg PSA, 20 mg GCB/50 mg PSA，40 mg

GCB/50 mg PSA for corn straw on recoveries were also evaluated. Satisfying recovery and RSD values were obtained and partial removal of some matrix components was observed by UPLC-MS full scan chromatograms when 20 mg C18/30 mg PSA for corn grain and 20 mg GCB/50 mg PSA for corn straw were used. Therefore, the two combinations were selected in final.

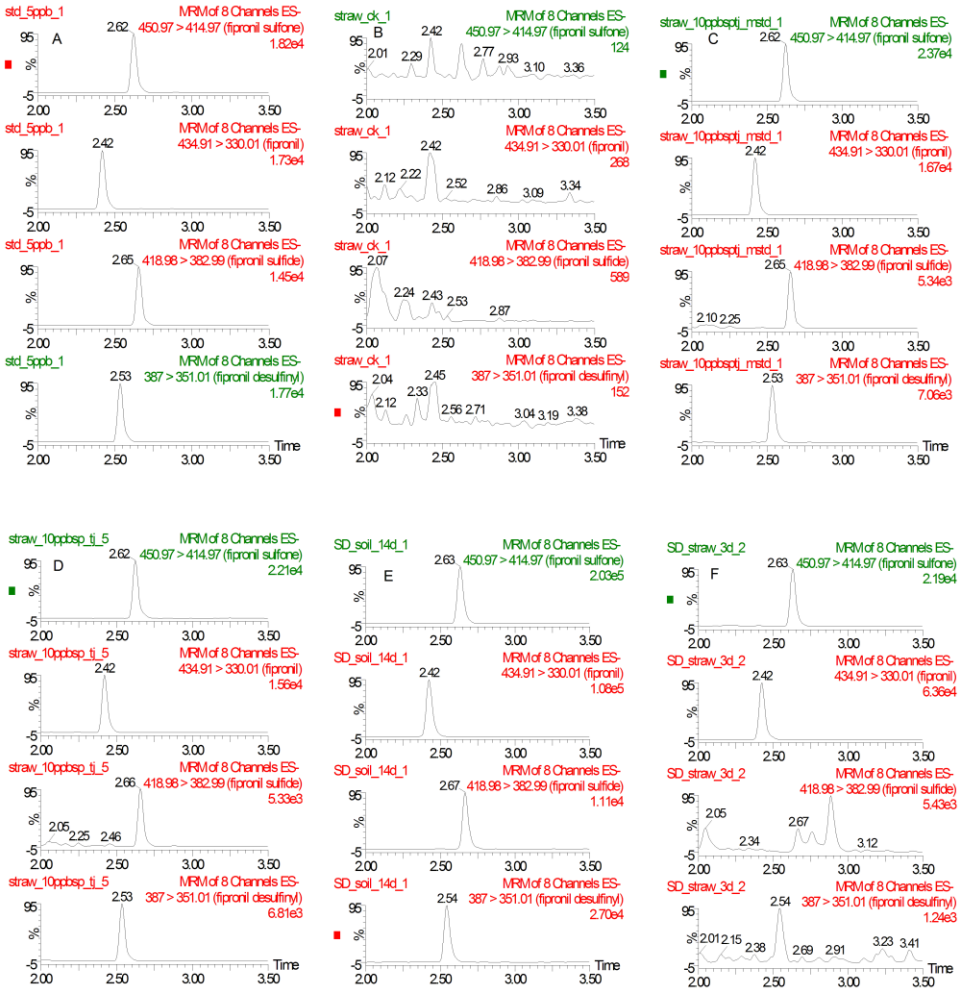

Fig. 2 Typical UPLC-MS/MS chromatograms of fipronil and its metabolites of : A. 5 μg•kg^{-1} standard in acetonitrile; B. blank corn straw sample; C. 5 μg•kg^{-1} matrix standard of corn straw (correspond to 10μg•kg^{-1} spiked level); D. spiked corn straw sample (10μg•kg^{-1});E. the 14th day soil samples; F. the 3rd day corn plant samples.

Method validation

Linearity, LOD and LOQ

The linearity and LOD were obtained using the peak areas of the product ion obtained through MS/MS mode. Linearity was evaluated by preparing different calibration curves (acetonitrile, and soil, corn grain, corn straw matrix) within the concentration range of 5-1000μg•L^{-1} for

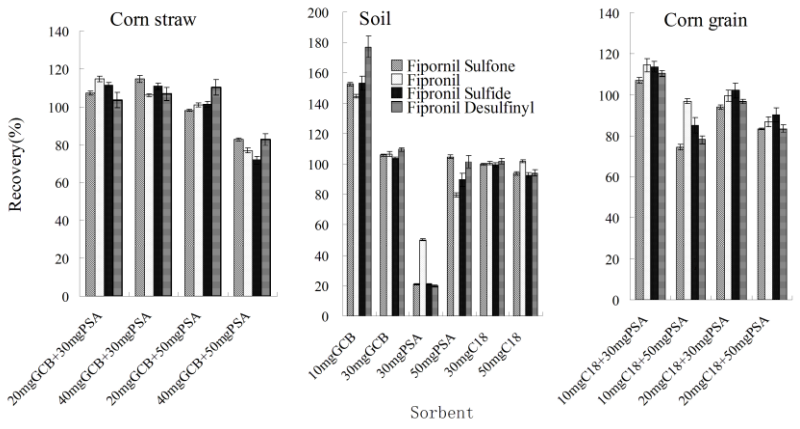

Fig. 3 Effect of different sorbents for targeted compounds in soil, corn grain, and corn straw at 0.01 mg kg^{-1} (n=5).

fipronil and its three metabolites. The linear regression results, LOD and LOQ of each pesticide in matrix were listed in Table 2. Excellent linearities were observed for the four compounds (R^2>0.998 in all cases), the LODs for the four pesticides were estimated to be 0.5-2.5 μg•kg^{-1}, based on five replicate extractions and analyses of spiked samples at low concentration levels. The LOQs were 5 μg•kg^{-1} in soil and corn grain, 10 μg•kg^{-1} in corn straw, these were lower than 20 μg•kg^{-1}, the maximum residue limits (MRLs) of fipronil in corn issued by Japan and the United States (http://www.mrldatabase.com/).μg•kg^{-1}

Matrix effect

It is well known that the presence of matrix components may result in a sample matrix-induced enhancement or suppression effect. The matrix effect depends on the instrument, the type and amount of matrix, the sample pre-treatment procedure, the analytes and concentration of the analyte.[30] For mass spectrometry detector, this may be caused by the competition between the analyte and a coeluting component for the available charge, and for the access to the droplet surface for gas-phase emission.[31, 32] Thus, in the present study, the matrix effect

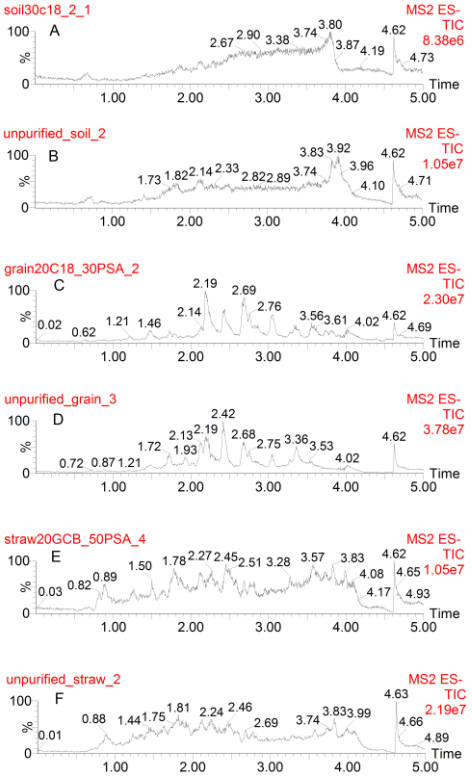

Fig. 4 Full scan chromatograms of blank matrices: A. soil purified by 30mg C18; B. unpurified soil; C. grain purified by 30 mg PSA plus 20 mg C18; D. unpurified grain; E. straw purified by 20 mg GCB plus 50 mg PSA; F. unpurified straw. Ionization mode: ES$^-$, scan duration: 0.2sec,mass (m/z): 50~650,cone voltage: 30v.

Table 2 Comparison of matrix-matched calibration and solvent calibration (5-1000 μg•kg^{-1})

Compound	Matrix	Regression equation	R^2	Matrix effect (%)	LOD(μg•kg^{-1})	LOQ(μg•kg^{-1})
fipronil	Acetonitrile	y = 64.2x + 5050.9	0.9997	-	-	-
	Soils	y = 60.5x + 4740.8	0.9996	-5.8	0.5	5
	Corn grain	y = 69.8x + 3459.7	0.9992	8.7	1.1	5
	Corn straw	y = 77.5x + 1844	0.9994	20.7	2.0	10
fipronil sulfone	Acetonitrile	y = 69.0x + 5964.2	0.9996	-	-	-
	Soils	y = 66.9x + 6197	0.9992	-3.0	0.7	5
	Corn grain	y = 53.5x + 2878.2	0.9995	-22.5	0.8	5
	Corn straw	y = 106.3x + 2641.7	0.9983	54.1	1.8	10
fipronil sulfide	Acetonitrile	y = 37.0x + 4058	0.9998	-	-	-
	Soils	y = 32.2x + 3748.5	0.9998	-13.0	1.5	5
	Corn grain	y = 29.0x + 2581.5	0.9988	-21.6	1.6	5
	Corn straw	y = 34.5x + 593.8	0.9995	-6.8	2.5	10
fipronil desulfinyl	Acetonitrile	y = 49.5x + 3715.7	0.9987	-	-	-
	Soils	y = 46.3x + 3524.5	0.9986	-6.5	1.4	5
	Corn grain	y = 41.4x + 2293	0.9982	-16.4	0.8	5
	Corn straw	y = 41.6x + 541.4	0.9993	-16.0	2.4	10

using the proposed method was investigated in corn grain, corn straw and soil by comparing the standards in the solvent with the matrix-matched standards. It was considered that, if the ME values were in the range of ±10%, the matrix effect could be ignored; when the ME values were between -20% and +20%, a mild signal suppression or enhancement effect occured; for ME values of ±50% and for ME values below -50% or above +50%, a medium effect and a strong effect appeared, respectively.[33] As shown in Table 2, obvious signal suppression or

Table 3 Recoveries (n=15, %), RSD_r[a] and RSD_R[b] (%) of target compounds in different matrices at four spiked levels.

Sample	Spiked level (μg•kg^{-1})	fipronil			fipronil sulfone			fipronil sulfide			fipronil desulfinyl		
		Recovery	RSD_r	RSD_R	Recovery	RSD_r	RSD_R	Recovery	RSD_r	RSD_R	Recovery	RSD_r	RSD_R
Soils	5	100.1	1.9	3.3	92.2	1.5	3.5	92.6	3.4	3.8	93.8	6.0	5.3
	10	99.0	2.0	3.3	98.5	1.2	2.4	95.5	2.4	3.2	93.9	3.1	4.0
	100	97.0	1.6	3.5	101.7	1.9	3.4	99.8	3.8	5.3	103.6	5.2	6.5
	500	94.3	2.9	3.3	94.7	3.1	4.4	94.7	3.4	4.7	95.2	6.9	7.4
Corn grain	5	95.8	4.5	4.6	90.0	3.0	4.2	82.4	3.4	4.7	93.0	5.1	6.0
	10	94.2	3.6	5.0	95.0	3.3	4.9	97.2	5.0	6.0	94.2	4.5	5.3
	100	95.5	3.0	3.8	88.9	1.7	3.0	92.3	2.1	3.4	88.3	2.8	3.8
	500	98.9	1.2	4.7	102.7	1.8	3.7	93.1	3.2	4.6	99.0	0.7	5.0
Corn straw	10	103.1	6.8	6.0	93.4	4.4	7.7	92.3	2.1	8.4	91.0	4.6	9.2
	20	104.6	3.3	3.5	96.7	3.3	3.7	91.8	7.7	7.1	87.5	7.6	9.4
	100	95.5	2.5	3.0	95.2	2.7	3.1	95.2	3.3	4.9	94.8	3.5	3.5
	500	93.4	3.6	5.0	91.9	4.4	5.1	91.8	2.6	4.1	88.9	2.9	4.7

[a] RSD_r is Intra-day precision (n=5) and [b]RSD_R is Inter-day precision(n=15)

enhancement differences were observed for the four compounds in the three matrices at a range of -22.5% to 51.4%. Consequently, the external matrix-matched calibration standards were used for accurate quantification to obtain a more realistic determination in all samples in this study.

Accuracy and precision

Evaluation of the recoveries and RSDs of fipronil and its three metabolites was performed to validate the developed method. The blank samples (soil, corn straw and corn grain) were spiked at four different concentration levels (5, 10, 100, 500 μg•kg^{-1} for soil and corn grain; 10, 20, 100, 500 μg•kg^{-1} for corn straw) and analyzed in quintuplicate. Table 3 lists the results of the mean recoveries with RSD values of the four compounds in soil, corn straw and corn grain. Satisfactory accuracy and precision were achieved at the four fortified concentration levels, indicating that this proposed method was reliable. For fipronil, the mean recoveries ranged from 93.4% to 104.6% with 1.2%-6.8% intra-day RSD, and they were 88.9% to 102.7% with 1.2%-4.4% intra-day RSD for fipornil sulfone. The mean recoveries were 82.4%-99.8% with 2.1%-7.7% intra-day RSD for fipronil sulfide, and 88.3%-103.6% with 2.7%-7.6% intra-day RSD for fipronil desulfinyl. In general, the intra-day (n=5) and inter-day RSDs (n=15) for the UPLC-MS/MS method ranged from 1.2% to 7.7% and 2.4% to 9.4%, respectively.

Application to residue dissipation study

The effectiveness and applicability of the developed method in measuring trace levels of the target compounds were monitored by analyzing corn and soil samples collected from our residual study trial field. A gradual and continuous dissipation of fipronil residue in soil, corn straw was observed. The residues of fipronil in soil degraded from 85.7 to 5.2 μg•kg^{-1} over the experimental period of 60 days with the dissipation rate of 93.9%. In corn straw, the initial concentration of fipronil was 65.9 μg•kg^{-1}, which declined to 7.2 μg•kg^{-1} after 28 days with the dissipation rate of 89.1%. Simultaneously, the concentrations of fipronil metabolites in soil and corn straw was also monitored. In soil, the concentration of fipornil sulfone, fipronil sulfide and fipronil desulfinyl reached to 58.5, 4.8, 12.6 μg•kg^{-1} in the 14th day, respectively. However, in corn straw, neither fipronil desulfinyl nor fipronil sulfide was detectable, the concentration of fipornil sulfone was 7.8 μg•kg^{-1} in the 3rd day. Fig. 2E and Fig. 2F presents the UPLC-MS/MS chromatograms of fipronil and its metablites in the 14th day soil samples and the 3rd day corn straw samples.

Conclusions

A quick, easy, effective, rugged, reliable and accurate method was developed for determination of fipronil and its three metabolites in soil and corn by UPLC-MS/MS. The four compounds was separated within 5.0 min with good specificity. The sample clean-up procedure was optimized by comparing the effects of dispersive sorbents or their combination. Satisfactory validation parameters in terms of linearity, repeatability, accuracy, and precision were obtained. The mean recoveries of the four compounds in soil, corn grain, and corn straw matrices were in the rang of 82.4%-104.6%; the intra-day (n=5) and inter-day RSDs (n=15) for the proposed method ranged from 1.2% to 7.7% and 2.4% to 9.4%, respectively. The LOQs of fipronil and its three metabolites were 5 $\mu g \cdot kg^{-1}$ for soil and corn grain, 10 $\mu g \cdot kg^{-1}$ for corn straw. The MRLs value of fipronil in corn grain is 20 $\mu g \cdot kg^{-1}$ established by Japan and the United States. Therefore, the LOQs of the proposed analytical method were low enough to determine the residues at this level. Also, the developed method was used to study fipronil dissipation in soil and corn, which further confirmed the reliability and efficacy of the proposed method for routine pesticide residue monitoring in soil and corn samples.

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References

1 P. Connelly, *Department of Pesticide Regulation, California Environmental Protection Agency*, 2001.
2 A. Decourtye, J. Devillers, E. Genecque, K. Le Menach, H. Budzinski, S. Cluzeau and M. Pham-Delegue, *Arch Environ Con Tox*, 2005, **48**, 242-250.
3 C. Tingle, J. Rother, C. Dewhurst, S. Lauer and W. King, *Briefing AI*, 2000, **1**.
4 E. W. Durham, M. E. Scharf and B. D. Siegfried, *Pestic Biochem Phys*, 2001, **71**, 97-106.
5 D. Hainzl, L. M. Cole and J. E. Casida, *Chem Res Toxicol*, 1998, **11**, 1529-1535.
6 A. B. Beeler, D. K. Schlenk and J. M. Rimoldi, *Tetrahedron Lett*, 2001, **42**, 5371-5372.
7 M. o. A. o. t. p. s. r. o. China, *Chin Plant Prot*, 2009, **29**, 43.
8 L. Sabatino, M. Scordino, V. Pantò, E. Chiappara, P. Traulo and G. Gagliano, *Food Addit Contam, Part B*, 2013, **6**, 11-16.
9 G.-G. Ying and R. S. Kookana, *J Environ Sci Health, Part B*, 2001, **36**, 545-558.
10 P. Zhou, Y. Lu, B. Liu and J. J. Gan, *Chemosphere*, 2004, **57**, 1691-1696.
11 M. H. S. Kurz, S. Martel, F. F. Gonçalves, O. D. Prestes, M. L. Martins, R. Zanella and M. B. Adaime, *J Brazil Chem Soc*, 2013.
12 A. Bobé, J. F. Cooper, C. M. Coste and M. A. Muller, *Pestic Sci*, 1998, **52**, 275-281.
13 D. Hainzl and J. E. Casida, *Proceedings of the National Academy of Sciences*, 1996, **93**, 12764-12767.
14 K. K. Ngim and D. G. Crosby, *Environ Toxicol Chem*, 2001, **20**, 972-977.
15 M. Raveton, A. Aajoud, J. Willison, M. Cherifi, M. Tissut and P. Ravanel, *Chemosphere*, 2007, **69**, 1124-1129.
16 M. Raveton, A. Aajoud, J. C. Willison, H. Aouadi, M. Tissut and P. Ravanel, *Environ Sc Technol*, 2006, **40**, 4151-4157.
17 M. Paramasivam and S. Chandrasekaran, *Int J Environ Anal Chem*, 2012, 1-9.
18 J. A. Harman-Fetcho, C. J. Hapeman, L. L. McConnell, T. L. Potter, C. P. Rice, A. M. Sadeghi, R. D. Smith, K. Bialek, K. A. Sefton and B. A. Schaffer, *J Agr Food Chem*, 2005, **53**, 6040-6048.
19 F. F. DonatoA, M. KemmerichA, J. d. F. FaccoA, C. d. A. FriggiA, O. D. PrestesB, M. B. AdaimeA and R. ZanellaA, *Braz J Anal Chem*, 2012, **7**, 331-340.
20 A. Demoliner, S. S. Caldas, F. P. Costa, F. F. Gonçalves, R. M. Clementin, M. R. Milani and E. G. Primel, *J Brazil Chem Soc*, 2010, **21**, 1-10.
21 I. R. Pizzutti, A. de Kok, R. Zanella, M. B. Adaime, M. Hiemstra, C. Wickert and O. D. Prestes, *J Chromatogr A*, 2007, **1142**, 123-136.
22 M. Ding, D. Zhong, F. Tang and W. Fang, *Chin J Chromatogr*, 2013, **31**, 117-121.
23 O. Lacina, J. Urbanova, J. Poustka and J. Hajslova, *J Chromatogr A*, 2010, **1217**, 648-659.
24 H. Tian, *Chemosphere*, 2011, **83**, 349-355.
25 A. Kadar and J.-P. Faucon, *J Agr Food Chem*, 2006, **54**, 9741-9746.
26 M. Lacroix, S. Puel, P. Toutain and C. Viguié, *J Chromatogr B*, 2010, **878**, 1934-1938.
27 M. Anastassiades, S. J. Lehotay, D. Stajnbaher and F. J. Schenck, *J Aoac Int*, 2003, **86**, 412-431.
28 F. Dong, X. Chen, X. Liu, J. Xu, Y. Li, W. Shan and Y. Zheng, *J Chromatogr A*, 2012, **1262**, 98-106.
29 X. Liu, J. Xu, F. Dong, Y. Li, W. Song and Y. Zheng, *Anal Bioanal Chem*, 2011, **401**, 1051-1058.
30 L. Polgár, B. Kmellár, J. F. García-Reyes and P. Fodor, *Anal Methods*, 2012, **4**, 1142-1148.
31 F. Dong, X. Liu, J. Li, L. Cheng, C. Zhang and Y. Zheng, *J Aoac Int*, 2010, **93**, 1013-1019.
32 P. Payá, M. Anastassiades, D. Mack, I. Sigalova, B. Tasdelen, J. Oliva and A. Barba, *Anal Bioanal Chem*, 2007, **389**, 1697-1714.
33 M. Li, X. Liu, F. Dong, J. Xu, Z. Kong, Y. Li and Y. Zheng, *J Chromatogr A*, 2013, **1300**, 95-103.