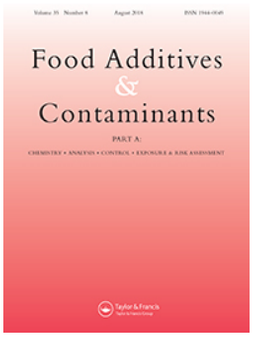

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Determination of fipronil and its metabolites in chicken egg, muscle and cake by a modified QuEChERS method coupled with LC-MS/MS

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ABSTRACT

An easy-to-use method for determining the levels of fipronil and its metabolites (fipronil-desulfinyl, fipronil-sulfone and fipronil-sulfide) in chicken egg, muscle and cake was developed and validated using a modified quick, easy, cheap, effective, rugged and safe (QuEChERS) approach coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. The samples were extracted using acetonitrile, salted out with sodium chloride at −20°C, and then purified by combined PSA and C18 phases and anhydrous magnesium sulphate. The recoveries were 80.4–119% with relative standard deviations (RSDs) < 10% for the different matrixes. The validated method was used to analyse the target compounds in 214 real samples collected in Beijing. The metabolite fipronil-sulfone was detected in most of the samples and was identified as the main residue in the egg and cake. The method was validated using a proficiency test for fipronil in products of animal origin published by Wageningen University & Research in 2017.

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Fipronil and its metabolites; chicken egg; muscle; cake; ultra-performance liquid chromatography-tandem mass spectrometry

Introduction

As an insecticide, fipronil can impact γ-aminobutyric acid (GABA) reception in nerve transmission and effectively block GABA-regulated chloride channels in the nervous system, paralysing or killing the target organism. Fipronil is widely used to control a variety of crop pests (Alain et al. 1997), tick and flea populations on household pets (dogs and cats (Zhang et al. 2016)), and lice and mites on domestic animals (Colin et al. 2003).

Animal metabolism studies using rats demonstrated that the fipronil residue content was highest in fat, and moderate levels were found in the adrenal glands, pancreas, skin, liver, kidneys, muscle, and thyroid as well as the ovaries and uterus in females (Powles 1992). Depending on the conditions, fipronil can degrade into a number of different metabolites. The animal metabolism of fipronil involves reduction to the sulfide, oxidation to the sulfone, and hydrolysis to amide RPA 200,766. Sulfone is the main metabolite in faeces, all tissues, milk and eggs (FAO/WHO 2000). In simple aquatic ecosystems and soil, fipronil is mainly metabolised to fipronil-desulfinyl, sulfone, sulfide and low-polarity products (Colin CDT et al. 2003). Fipronil does not accumulate in abiotic environments, but the metabolite studies showed that bioaccumulation of fipronil and fipronil-sulfone can occur in fatty tissues (FAO/WHO 2000). Fipronil is genotoxic (Yildirim and Agar 2016), and fipronil-desulfinyl is 10 times more toxic to mammals than fipronil itself. The metabolites fipronil-sulfone and fipronil-sulfide are more toxic to freshwater invertebrates than the parent compound (Kaur et al. 2015). Considering its relatively toxic metabolites, fipronil is tightly regulated within the European Union (2007/52/EC directive) due to its potential effects on the environment and human health. In addition, in 2009, announcement No. 1157 from the Ministry of Agriculture of P. R. China forbids the sale and use of fipronil-containing pesticide formulations in agriculture except for use in hygiene, on corn, and as a seed treatment agent. To ensure the health of human beings, the Food and

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Agriculture Organization (FAO) defines the maximum residue limit (MRL) values for fipronil in different tissues, and the lowest MRL is 0.02 mg/kg for eggs and poultry muscle (FAO/WHO 2000). The MRL in China is consistent with the MRL set by the FAO; however, the European Food Safety Authority (EFSA) set a more stringent limit of 0.005 mg/kg in poultry muscle and eggs. Recently, fipronil was illegally used to kill ectoparasites in breeder hens in Belgium (Belgium accuses Netherlands of tainted eggs cover-up 2017), and similar activities have also been reported (NOS 2017). These reports have attracted the attention of consumers, the governments of many European countries, and the whole world.

To control pesticide abuse and ensure the safety of animal-derived foods, an easy-to-use method for determining fipronil and its metabolites in chicken egg, muscle and cake must be developed. Previous studies monitoring fipronil and/or its metabolites in different matrixes have typically focused on vegetables (Kaur et al. 2015), pollen (Kadar and Faucon 2006), ovine plasma (Bichon et al. 2008), tea (Zhou et al. 2011), water (Araujo et al. 2013), sugarcane juice, jaggery and sugar (Ramasubramanian et al. 2014), maize (Wang et al. 2014), peanut and soil (Li et al. 2015), and cauliflower (Duhan et al. 2015). Extraction and cleanup methods including liquid-liquid extraction, liquid-solid solvent extraction (Kadar and Faucon 2006), solid-phase extraction (SPE) (Bichon et al. 2008; Zhang et al. 2016), solid-phase microextraction (Vilchez et al. 2001; Zhou et al. 2011), single-drop microextraction (Araujo et al. 2013) and QuEChERS (quick, easy, cheap, effective, rugged, and safe) (Duhan et al. 2015; Kaur et al. 2015; Li et al. 2015) have been used to extract fipronil and/or its metabolites from complicated matrixes under optimised conditions. The detection methods used for the determination of fipronil and its metabolites include glassy carbon electrodes (Montes et al. 2015), GC (Zhou et al. 2011; Wang et al. 2014), GC-MS (Vilchez et al. 2001; Bichon et al. 2008; Ramasubramanian et al. 2014; Kaur et al. 2015; Shen et al. 2017), HPLC (Hafeez et al. 2016) and LC-MS/MS (Kadar and Faucon 2006; Li 2017, Li et al. 2015; Zhang et al. 2016) or time-of-flight mass spectrometry (Guo et al. 2017). Less time is generally required for LC analyses than for GC methods, and LC-MS methods have higher performance.

The simultaneous analysis of fipronil and its metabolites in animal-derived food samples has been reported. Li J et al. (Li et al. 2017) used dispersive solid-phase extraction coupled with LC-MS/MS to detect fipronil in the bird egg, but they did not examine egg-derived food samples. Guo et al. (Guo et al. 2017) established a rapid screening method for egg and egg-products. The objective of this work was to develop an easy-to-use sample preparation and accurate analytical method for the simultaneous determination of fipronil and its metabolites in the chicken egg, muscle and cake.

Materials and methods

Chemicals and materials

The standard solutions (100 μg/mL) of fipronil, fipronil-desulfinyl, fipronil-sulfide and fipronil-sulfone (all compounds were from AccuStandard, New Haven, USA) in methanol (MeOH) were provided by the China National Center for Food Safety Risk Assessment, China. HPLC-grade acetonitrile (ACN) and MeOH were obtained from J.T. Baker (Deventer, The Netherlands). Formic acid (FA) (99% purity) was obtained from Acros Organics (Morris Plains, NJ, USA). Sodium chloride (NaCl) of analytical purity was acquired from the Beijing Chemical Reagent Company (Beijing, China). Commercial ProElut QuEChERS tubes with five different sorbent mixtures ((1) 50 mg C18/150 mg $MgSO_4$, (2) 50 mg PSA/100 mg C18/100 mg $MgSO_4$, (3) 50 mg PSA/50 mg C18/150 mg $MgSO_4$, (4) 250 mg PSA/100 mg C18/200 mg $MgSO_4$, (5) 50 mg PSA/50 mg C18/50 mg Carb/150 mg $MgSO_4$) were purchased from Dikma Technologies Inc. (Tianjin, China). Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Milford, MA, USA).

Sample preparation

The samples (chicken egg, muscle and cake) were randomly purchased from a local supermarket (China). The cake was the egg foam type, and the egg white was used to make egg foam. The

yolk of the egg was mixed with flour to make the cake. Twenty chicken eggs were cracked into a stainless container and mixed well with an egg beater. The chicken muscle (approximately 500 g) and the cake (approximately 200 g) were homogenised using a laboratory mill. All samples were stored at −20°C.

Approximately 2.0 g (±0.02 g) of the homogeneous cake and 5.0 g (±0.05 g) of the homogeneous chicken egg or muscle was weighed into a 50-mL polypropylene centrifuge tube. An additional 3 mL of ultrapure water was added to the centrifuge tube containing the cake. Then, 10 mL of ACN was added into the polypropylene centrifuge tubes, and the mixtures were vortexed for 15 s. The samples were then extracted by ultrasonication for 30 min and centrifuged for 5 min at 9000 rpm at a temperature below 4°C. Later, the supernatants were transferred to another set of tubes that contained 1 g of NaCl. The salt solutions were mixed for 30 s and placed in a freezer at −20°C for approximately 30 min to separate the lipids from the solvent. Subsequently, 1.5 mL of the supernatant from each sample was transferred to a commercial QuEChERS tube with different sorbent mixtures for cleanup. Then, the mixture was shaken vigorously for 30 s and centrifuged for 5 min at 9000 rpm below 4°C. After that, the upper layer was transferred to the autosampler vials for LC-MS/MS analysis.

LC-MS/MS analysis

Chromatographic separation was performed using a reversed-phase BEH C18 column (100 mm × 2.1 mm i.d., 1.7-µm particle size) (Waters, Milford, MA, USA) at 40°C. The flow rate was 0.3 mL/min, and the injection volume was 5 µL. The initial composition of the mobile phase was 40% water (A) and 60% ACN (B). The B phase was held for 0.5 min, increased to 100% by 3.0 min, and held at 100% until 3.5 min. Finally, the mobile phase was returned to the initial composition in 0.1 min, and the column was equilibrated for 2 min before the next injection. For mass spectrometric analysis, a Shimadzu LC-30A instrument coupled with a tandem mass spectrometer (LC/MS-8060) was used, and the data were processed by Lab Solutions Ver. 5.86 (chromatography workstation). Multiple reaction monitoring (MRM) was conducted in the negative ESI mode. The MS parameters are listed in Table 1.

Table 1. The MS parameters for the experiment.

MS parameters
Ion source: ESI(-)
Interface Voltage: 3.0 kV
Desolution Line Temperature: 250°C
Interface Temperature: 300°C
Heat Block: 400°C
Nebulizing gas: N_2 3.0 L/min
Heating gas: N_2 10.0 L/min
Drying Gas: N_2 10.0 L/min
Dwell Time: 59 ms
Collison gas: Ar
Analysis type: MRM

Method validation

The method was validated by determining the matrix effect (ME), limit of detection (LOD), limit of quantitation (LOQ), linearity of the calibration, recoveries and repeatability (% relative standard deviation (RSD)).

Results and discussion

Optimization of the MS/MS parameters

The triple quadrupole mass spectrometry parameters were optimised in the ESI negative mode using standards (0.1 µg/mL) of the individual compounds via auto-optimisation with direct injection. Fipronil, fipronil-desulfinyl, fipronil-sulfone and fipronil-sulfide were easily deprotonated to form $[M\text{-}H]^-$ ions of m/z 435.0, 387.0, 451.0 and 419.0, respectively, which were selected as the precursor ions. In the MRM mode, the two product ions with the highest sensitivity and optimal selectivity were selected to confirm each analyte. The ion with the higher response was used for quantification, and the less intense ion signal and the retention times were used for qualification. The optimised MS/MS parameters are shown in Table 2.

Optimisation of the LC conditions

The mobile phase composition for the chromatographic separation of fipronil and its metabolites was optimised using ACN-water, MeOH-water,

Table 2. MS analysis parameters for fipronil and its metabolites.

Analyte	Precursor ion (m/z)	Product ions (m/z)	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Fipronil	435.0	250.0	16	28	15
		330.0*	21	17	10
Fipronil-desulfinyl	387.0	351.1*	11	15	11
		282.1	14	31	12
Fipronil-sulfone	451.0	415.0*	12	17	13
		282.1	17	28	17
Fipronil-sulfide	419.0	262.0	16	28	15
		383.0*	21	17	10

*Note: The quantitation ion transitions are marked by **

and ACN-water with 0.1% FA and 5 mmol/L ammonium acetate. ACN-water with 0.1% FA and 5 mmol/L ammonium acetate provided satisfactory separation of fipronil and its metabolites under isocratic elution with 75% ACN, but ACN-water by itself provided significantly higher sensitivity (a 10-fold higher response than that of ACN-water containing 0.1% FA and 5 mmol/L ammonium acetate), although fipronil-sulfide and fipronil-sulfone were not baseline-separated (Figure S1). The temperature of the column (30°C, 35°C, 40°C, and 45°C) and the flow rate of the mobile phase (0.2, 0.25, 0.3, and 0.35 mL/min) were also optimised (chromatographs are not shown). Changes in the temperature and flow rate did not have noticeable impacts on the chromatographs. ACN-water was ultimately selected as the mobile phase for better sensitivity. The flow rate was 0.3 mL/min, and the column temperature was 40°C.

Optimisation of the sample preparation procedure

Extraction

Amount of water added to the cake. ACN, a commonly used solvent in QuEChERS methods, was selected to precipitate the protein and extract the target compounds. The original QuEChERS method was designed for samples with water content between 25% and 80% (Anastassiades et al. 2003), and the extraction solutions for the QuEChERS approach must contain high percentages of water (approximately 90%) (BS :2008). Because dry samples have low percentages of water (Faraji et al. 2018; Han et al. 2018), a small amount of water must be added to the dry samples at the beginning of the process to increase the extraction efficiency (Rodriguez-Carrasco et al. 2014). The water content of the cake in this study was approximately 20%; thus, the extraction efficiencies achieved by adding different amounts of water (0, 1, 3, 5 and 7 mL) were estimated from cake spiked with 10 ng/g of analyte. Although satisfactory recoveries (Figure 1) of the analytes were obtained when different amounts of water were added, a portion of the ACN extract was adsorbed by the matrix when 0 or 1 mL of water was added, and more significant MEs were observed when greater than 3 mL of water was added. In this work, the addition of 3 mL of ultrapure water made the 2 g of cake swell completely. Consequently, in this study, 3 mL of water per 2 g of cake was added before extraction to improve extraction efficiency.

Ultrasonic extraction time. Ultrasonic extraction times of 10, 20, 30, 40, and 50 min were evaluated. The correlation between the response value and the ultrasonic extraction time is shown in Figure 2. These data suggested that the ultrasonic extraction time has a slight effect on the extraction yields of the analytes up to 20 min, and the response did not increase further with extraction times over 30 min. Therefore, 30 min was selected as the ultrasonic extraction time for all extractions in this study.

Amount of nacl. NaCl was added to the extraction solution to remove the lipids and separate the ACN and water layers, and the amount of salt (0.5, 1, 2, and 3 g) was optimised. The responses of the analytes decreased when 3 g of NaCl was added, which may be because the electrolyte can reduce the extraction efficiency of the analytes. To ensure sufficient ion intensity of NaCl in the different matrixes, which varies due to the different water content in the matrixes, while not reducing the

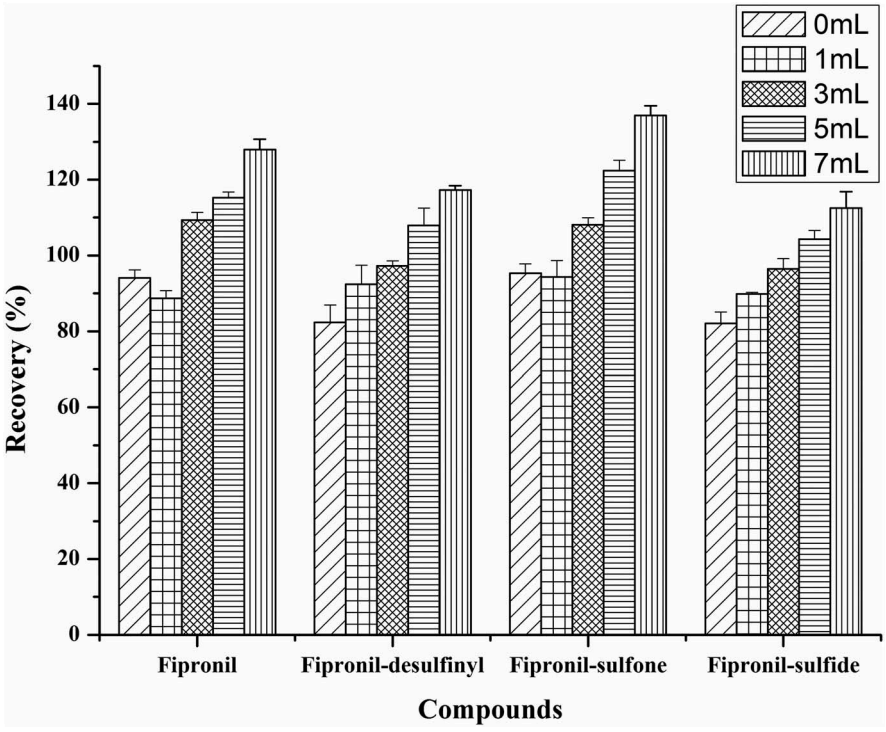

Figure 1. The recoveries of the analytes after adding different amounts of water to the cake (n = 6).

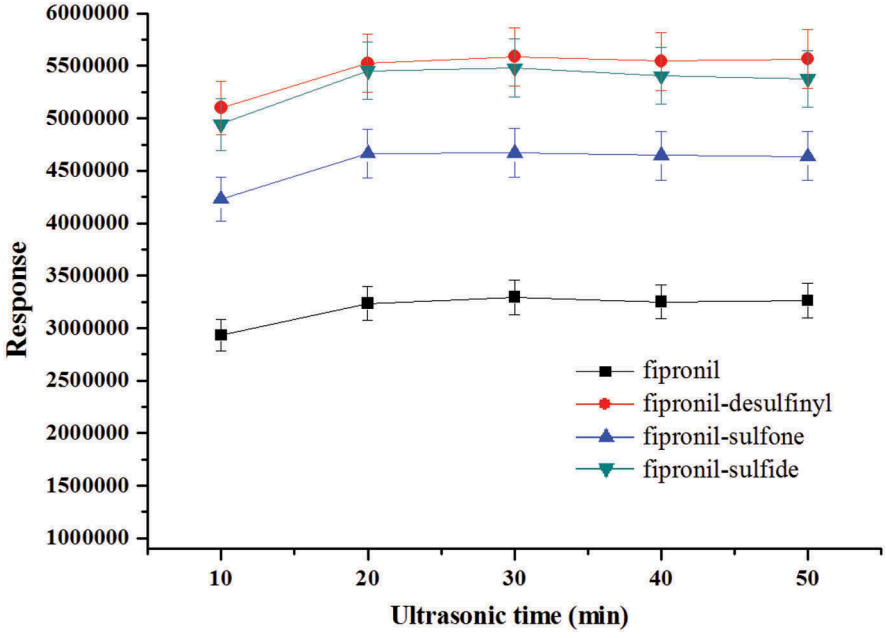

Figure 2. The effect of different ultrasonication times on extraction (n = 6).

extraction efficiency, 1 g of NaCl was added to the extraction solutions.

Freezing time. To determine how the freezing time affects the extraction efficiency of the analytes, we tested the following freezing times: 10, 20, 30, 40, and 50 min. The results indicated that increasing the freezing time had no noticeable impact on the analyte responses. However, the separation between the ACN and water layers increased, and the upper supernatant was more clarified with a freezing time of 30 min.

Ultimately, the final method was established as described in the Sample Preparation section.

Cleanup

PSA, C18 and GCB are recommended by the AOAC as sorbents for the QuEChERS procedures for determining pesticides (AOAC 2007). PSA tends to remove sugars, fatty acids and organic acids. C18 can remove lipids and nonpolar constituents, and GCB can effectively absorb pigments and sterols. The different sorbent mixtures in commercial QuEChERS kits (i.e. (1) 50 mg C18/150 mg $MgSO_4$, (2) 50 mg PSA/100 mg C18/100 mg $MgSO_4$, (3) 50 mg PSA/50 mg C18/150 mg $MgSO_4$, (4) 250 mg PSA/100 mg C18/200 mg $MgSO_4$, and (5) 50 mg PSA/50 mg C18/50 mg Carb/150 mg $MgSO_4$) were compared with respect to the analyte responses. Similar responses were obtained for the five sorbents for chicken muscle (Figure 3). Based on its ability to effectively remove impurities from the three different matrixes and to use the materials most economically, we chose (2) 50 mg PSA/100 mg C18/100 mg $MgSO_4$ as the cleanup material.

Validation of the proposed method

The sample preparation procedure with the best results was selected for validating the method. The analytical parameters, MEs, linearity, recovery, precision, detection limits (LODs) and quantitation limits (LOQs) were determined according to the guidelines of the Commission Decision 2002/657/EC.

Matrix effects

To evaluate the MEs, the slopes obtained by matrix-matched calibration curves were compared to those obtained by standard calibration curves. MEs were investigated by calculating the percentage of signal enhancement or suppression according to the equation ME% = Sm/Ss × 100, where Sm is the slope of the calibration plot with matrix-matched calibration solutions, and Ss is the slope of the calibration plot with the calibration solutions prepared in solvent. The results are shown in Table 3. When ME was equal to 100%±20%, no ME was present (Ferrer et al. 2011). Values over 120% and below 80% indicated ionisation enhancement and suppression, respectively (Kaczynski 2017). The ME data was in the range of 62.0 to 96.7%, which indicated ion suppression of fipronil-sulfone in muscle and egg as well as suppression of four target compounds in cake. To compensate for the MEs, matrix-matched calibration standards were used for accurate quantification of fipronil and its metabolites in all samples in this study.

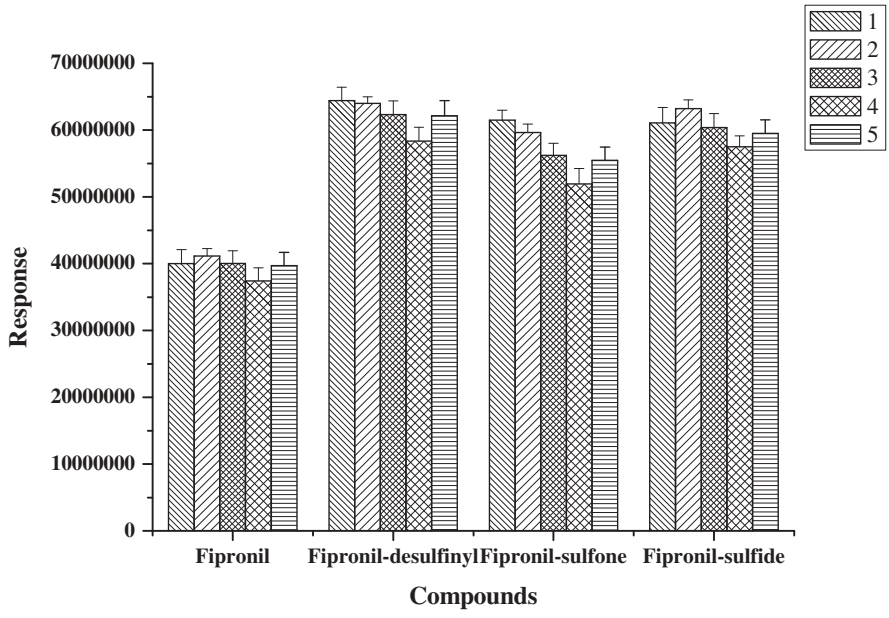

Figure 3. The different responses from the various combinations of cleanup materials for chicken muscle matrix (n = 6).

Note: The numbers in the legend have the same meaning in the main text: (1) 50 mg C18/150 mg $MgSO_4$, (2) 50 mg PSA/100 mg C18/100 mg $MgSO_4$, (3) 50 mg PSA/50 mg C18/150 mg $MgSO_4$, (4) 250 mg PSA/100 mg C18/200 mg $MgSO_4$, (5) 50 mg PSA/50 mg C18/50 mg Carb/150 mg $MgSO_4$).

Table 3. The matrix effects (MEs %) of the four compounds in different matrixes.

Sample	Fipronil	Fipronil-desulfinyl	Fipronil-sulfone	Fipronil-sulfide
Chicken muscle	88.6	91.1	80.6	91.8
Cake	75.5	64.2	62.0	86.7
Chicken egg	89.3	86.9	79.5	96.0

Linearity

The linearity was validated using the matrix-matched calibration curves for each compound and was assayed by spiking samples with the target analytes at seven levels as follows: 0.02, 0.1, 0.2, 1, 2, 4, and 10 μg/kg for egg and chicken and 0.05, 0.25, 0.5, 2.5, 5, 10, and 25 μg/kg for cake. The MRL of fipronil is 0.02 mg/kg for eggs and poultry muscle (20 μg/kg based on the FAO regulation). Thus, to better match the concentrations present in the different matrixes, we prepared another matrix-matched standard curve at the following seven concentrations: 1, 2, 5, 10, 20, 50, and 100 μg/kg for egg and chicken and 2.5, 5, 12.5, 25, 50, 125, and 250 μg/kg for cake. The correlation coefficients (R^2) of all analytes were higher than 0.996.

Recovery and precision

The recovery study was conducted using five spiking levels (0.2, 2, 10, 20, and 40 μg/kg for egg and chicken and 0.5, 5, 25, 50, and 100 μg/kg for cake), and these levels included the concentrations equal to 0.5 MRL (10 μg/kg), MRL (20 μg/kg), and 2 MRL (40 μg/kg) for the different matrixes. For each spiking level, six replicates were analysed. As shown in Table 4, the recoveries ranged from 80.4% to 119%, and the precisions ranged from 0.05% to 8.07% and are expressed as the RSDs. The recovery and precision ranges satisfied the requirements for this experiment and the FAO regulations.

Lods and loqs

The LODs were determined from the lowest concentration (in spiked blank samples) to give a signal-to-noise (*S*/*N*) ratio equal to 3 at the retention time of the peak of interest, and the LOQs were calculated at an *S*/*N* equal to 10. This method had an LOQ range of 0.2–5 ng/kg for the four target compounds in the three different matrixes. The LODs of fipronil, fipronil-desulfinyl, fipronil-sulfone and fipronil-sulfide were 0.4, 1.0, 0.1, and 0.2 ng/kg, respectively, for chicken egg and muscle and 1.0, 2.5, 0.25, and 0.5 ng/kg, respectively, for cake. The LOQs of fipronil, fipronil-desulfinyl, fipronil-sulfone and fipronil-sulfide were 1.0, 2.0, 0.2, and 0.4 ng/kg, respectively, for chicken egg and muscle and 2.5, 5.0, 0.5, and 1.0, respectively, for cake. This method is more sensitive than methods reported previously.

Application to real samples

To estimate the reliability and practicality of the developed method, 94 chicken egg, 65 muscle and 55 cake samples from different brands were purchased from the local supermarket and analysed. The results are shown in the supporting information (Table S1), and the

Table 4. The recoveries and RSDs of the four compounds in different spike concentrations(n = 6).

		Fipronil		Fipronil-desulfinyl		Fipronil-sulfone		Fipronil-sulfide	
Matrix	Spiked concentration (μg/kg)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Chicken muscle	0.2	101	2.4	103	4.3	99.0	1.9	98.6	4.9
	2	96.9	3.6	103	7.3	99.8	2.1	96.8	3.1
	10	92.5	3.2	95.7	8.1	92.1	1.9	89.8	3.0
	20	102	0.8	101	0.4	104	0.1	101	0.5
	40	103	0.5	102	0.9	102	0.2	102	0.3
Chicken egg	0.2	107	0.7	118	2.2	104	2.6	93.6	3.7
	2	107	2.2	119	1.9	104	2.0	105	2.2
	10	94.9	2.2	106	2.1	92.7	2.2	94.4	1.8
	20	106	2.0	102	2.8	104	2.4	100	3.2
	40	92.8	1.6	89.6	2.8	95.9	1.0	97.4	2.8
Cake	0.5	87.7	1.2	91.5	1.7	94.3	0.9	101	1.4
	5	98.4	0.7	104	0.7	90.4	1.4	97.5	0.8
	25	92.8	0.7	80.4	0.7	96.3	1.9	99.8	1.7
	20	94.8	0.7	93.0	0.4	92.8	0.3	96.3	0.9
	40	93.4	0.1	92.4	0.5	91.7	0.8	94.8	0.2

chromatogram of a real sample is shown in Figure S2. Among the tested samples, the concentrations for fipronil, fipronil-desulfinyl, fipronil-sulfone and fipronil-sulfide ranged from 0.002–0.706 μg/kg, 0.002–0.082 μg/kg, 0.002–4.17 μg/kg, and 0.002–0.004 μg/kg, respectively, which were all below the corresponding EU-MRLs. According to the FAO regulations, the metabolites should be expressed in terms of the total fipronil content. The total fipronil residues were 0.005–4.10 μg/kg with a median value of 0.05 μg/kg for cake, 0.01–4.88 μg/kg with a median value of 0.056 μg/kg for egg and 0.004–0.724 μg/kg with a median value of 0.035 μg/kg for muscle, which was also lower than the EU-MRLs in muscle and egg. Considering that fipronil is lipophilic, we chose the muscle and fat from the whole chicken and used our method to detect the total of fipronil level in the examined tissues that were expected to have higher levels of the fipronil residues, From the results (Table 5), we observed that the residue in fat was about three to five times that in muscle, which proved again that fipronil is lipophilic. The total fipronil level was 0.724 μg/kg in chicken muscle. The estimated total fipronil in fat was 3.62 μg/kg, which was five times that in chicken muscle. The sum of the total fipronil in muscle and fat was 4.344 μg/kg, which was also lower than 5 μg/kg.

Fipronil-sulfone was detected in most of the samples. The results of the metabolite fipronil-sulfone indicated that the residue of fipronil-sulfone was present in a higher concentration in egg compared to that muscle, which is consistent with the lipophilic nature of the compound and with previous reports that fipronil-sulfone can bioaccumulate in fatty tissues (Fipronil (202)).

To certify the accuracy of our method, we participated in a proficiency test for fipronil in products of animal origin from Wageningen University & Research in 2017 (Project number: 1,277,333,401-Fipronil 2017). This proficiency test focused on the levels of fipronil and fipronil-sulfone in chicken egg, chicken muscle and chicken fat. Our method was used to detect the samples, and the results are shown in Table 6. A z-score between +2 and −2 is considered a satisfactory performance; between +2 and +3 or −2 and −3 is considered a questionable performance; and anything outside of this range (> +3 or < −3) is considered unsatisfactory. The z-score in our study was between −1.05 and 0.25, which was considered satisfactory performance. All the results demonstrated that the present method could be used not only to detect fipronil in chicken egg and muscle but also to analyse the level of fipronil in chicken fat.

Table 5. The results on the distribution of fipronil amongst chichen muscle and fat.

Kinds	Muscle(μg/kg)	Fat(μg/kg)
Sanhuang chicken	0.026	0.078
Hemp rooster	0.029	0.136
Blackone chicken	0.074	0.282

Conclusions

In this study, a rapid and sensitive analytical method for the simultaneous determination of fipronil and its three metabolites in chicken egg, muscle and cake was established with a modified QuEChERS sample preparation method and LC-MS/MS analysis. The method was simple, convenient and fast and was shown to be valid for all tested matrixes and in the proficiency test for fipronil in products of animal origin.

Table 6. Occurrence of fipronil and its metabolitees in the proficiency test sample.

	Fipronil(μg/kg)			Fipronil-sulfone(μg/kg)			Sum fipronil+sulfone metabolite(μg/kg)		
Material	Result	Proficiency value	Z score	Result	Proficiency value	Z score	Result	Proficiency value	Z score
Chicken egg	4.94	5.0	−0.09	9.57	9.2	0.18	14.17	14	0.02
Chicken muscle	3.34	4.3	−1.05	17.36	17	0.00	20.1	21	−0.18
Chicken fat	8.99	9.1	−0.05	69.49	66	0.25	76.01	74	0.15

Disclosure statement

No potential conflict of interest was reported by the authors.

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