APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Detection of Fipronil and its Metabolites in Eggs by LC/MS/MS

Introduction

A recent recall of contaminated eggs in Europe has generated a serious health concern for fipronil poisoning[1, 2]. Fipronil is a highly toxic insecticide and it is used to protect crops. The U.S. EPA has classified fipronil as a group C carcinogen[3]. Therefore, it is not allowed anywhere near animals in the food production chain, specifically around chickens. Traditionally, fipronil is measured by GC/MS or HPLC, however, its metabolites are not included. According to (EU) No.1127/2014, the maximum residue allowance level of fipronil (including its metabolites) in egg is 5 µg/L[4]. In this study, we describe a fast and robust analytical method for identifying fipronil and its metabolites with the PerkinElmer QSight® 200 series LC/MS/MS triple quadrupole instrument. The results demonstrate excellent recovery, linearity and reproducibility and superior sensitivity for this method. The three-step sample preparation procedure is quick and easy, and the LC/MS/MS analysis time can be carried out within six minutes with great separation for all analytes.

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Experimental Conditions

Sample Preparation

5.00 g of homogenized egg was weighed and transferred to a 50 mL tube. Then the sample was extracted with 25 mL of acetonitrile. For better extraction, the samples were first vortexed for one minute, then sonicated for five minutes. Subsequently, 2 g of NaCl was added to the sample tube, votexed for one minute and then centrifuged at 6000 rpm for three minutes. The prepared sample was finally filtered through a 0.22µm filter then injected onto a PerkinElmer Brownlee® SPP C18 column and analyzed by a QSight 220 LC/MS/MS system.

LC Method Parameters

The starting conditions for the gradient flow were (60/40, A/B) with a 0.4 mL/min flow rate. Detailed LC conditions and time program are shown in the Table 1.

MS Method Parameters

MRMs for fipronil and its metabolites are optimized for the QSight 220 LC/MS/MS, CE values and source parameters are listed in Table 2 and 3, respectively.

Table 1. LC parameters.

Mobile Phase	Solvent A: Water Solvent B: Acetonitrile

	Time (min)	Flow Rate (mL/min)	% A	% B
1	0	0.4	60	40
2	2.8	0.4	5	95
3	4.0	0.4	5	95
4	4.1	0.4	60	40
5	6.0	0.4	60	40

Column	Brownlee SPP C18, 2.1x100 mm, 2.7µm
Oven	35 °C

Table 2. MS/MS parameters.

Compound	Precursor (m/z)	Fragment (m/z)	CE (eV)
Fipronil Desulfinyl	386.8	281.8	44
	386.8	350.8	20
Fipronil Sulfide	418.8	261.8	39
	418.8	382.8	21
Fipronil	434.8	249.8	36
	434.8	329.8	24
Fipronil Sulphone	450.8	243.8	66
	450.8	281.8	37

Table 3. MS source parameters.

Ionization Source	ESI negative mode
Source Voltage	– 4500 V
Dyring Gas Setting	100
Nebulizer Gas Setting	180
Source Temperature	450 °C

Results

Figure 1 shows example of chromatography for each analyte at 0.05 µg/L level. Excellent symmetric peak and good separation were observed.

This LC/MS/MS method showed excellent linear range over three orders of magnitude (0.05 – 10 µg/L) with good regression coefficient ($R^2 \geq 0.998$) for all analytes. Typical matrix-matched calibration curves for all four analytes are shown in Figure 2.

Recovery was also studied by matrix-spiked experiment at concentration level of 0.5 and 5.0 µg/L. The recovery obtained was between 92.7% – 114.5% with RSD < 5% for fipronil and its metabolites as shown in Table 4.

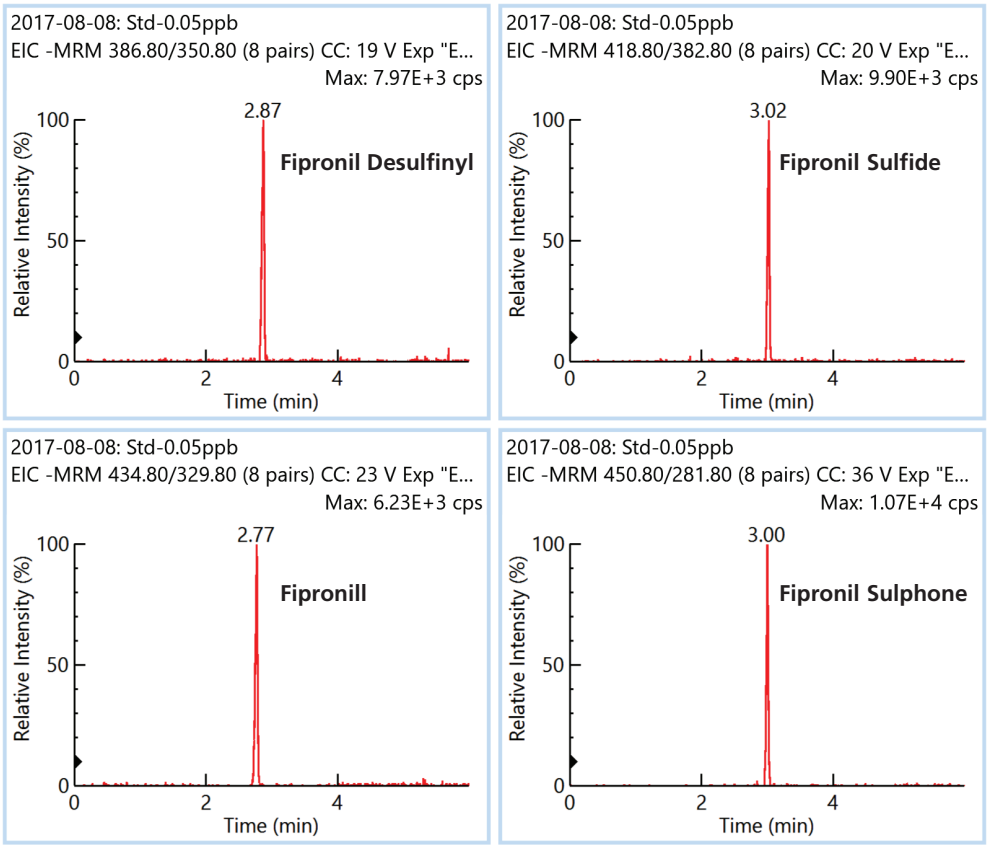

Figure 1. Matrix spiked chromatograms at 0.05 µg/L level for fipronil desulfinyl, fipronil sulfide, fipronil, and fipronil sulphone.

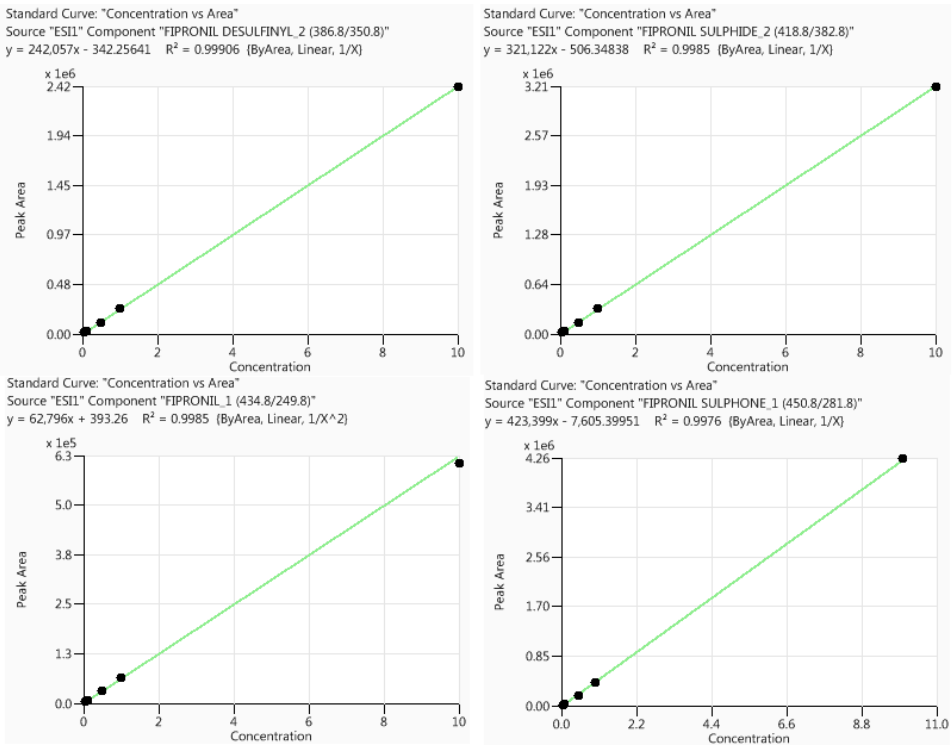

Figure 2. Selected matrix-matched calibration curves from 0.05 to 10 µg/L for fipronil desulfinyl, fipronil sulfide, fipronil, and fipronil sulphone.

Table 4. Recovery and RSD at spiked concentration level of 0.5 and 5.0 µg/L.

Compound	Spiked Level 0.5 µg/L		Spiked Level 5.0 µg/L	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Fipronil Desulfinyl	102.5	3.7	98.6	1.5
Fipronil Sulfide	106.8	4.3	103.9	3.0
Fipronil	107.5	3.4	113.8	2.6
Fipronil Sulphone	114.5	3.2	92.7	2.4

Conclusion

The LC/MS/MS methodology of detecting fipronil and its metabolites in eggs, namely, fipronil sulphone, fipronil sulfide, and fipronil desulfinyl was developed using a PerkinElmer QSight 220 LC/MS/MS triple quadrupole instrument. This methodology showed excellent linearity from 0.05 to 10 µg/L with good regression coefficient ($R^2 \geq 0.998$) for all analytes. The simple three-step sample preparation was demonstrated by excellent recovery of between 93 – 115% and RSD of less than 5% at both 0.5 and 5.0 µg/L spiked concentration levels. The method presented here provides excellent resolution and sensitivity (at least 100 times lower than that of EU MRL) for the quantification of fipronil and its metabolites in eggs and meets the analytical needs for food safety laboratories.

References

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