

APPLICATIONS

Fipronil, Amitraz and Metabolites from Egg Albumin using QuEChERS and LC-MS/MS

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Overview/Summary

Fipronil and Amitraz although effective as an ingredient to control pests such as fleas and as a field pest control for corn - is prohibited for use on food source animals, including chickens and livestock. However, it is still widely used and is commonly included in typical multi-residue analyses; and the recent contamination of Fipronil in chicken eggs in Europe and South Korea has raised immediate attention.

Here we describe a rapid, robust and sensitive method, utilizing a powerful SCIEX QTRAP 6500+ with IonDrive Pos/Neg switching, an ultra high performing Kinetex[®] core-shell particle morphology with a selective Polar C18 column chemistry, and a modified QuEChERS clean-up to reach below the 5 µg/kg MRL level.

Sample Preparation

Modified Optimized QuEChERS Protocol

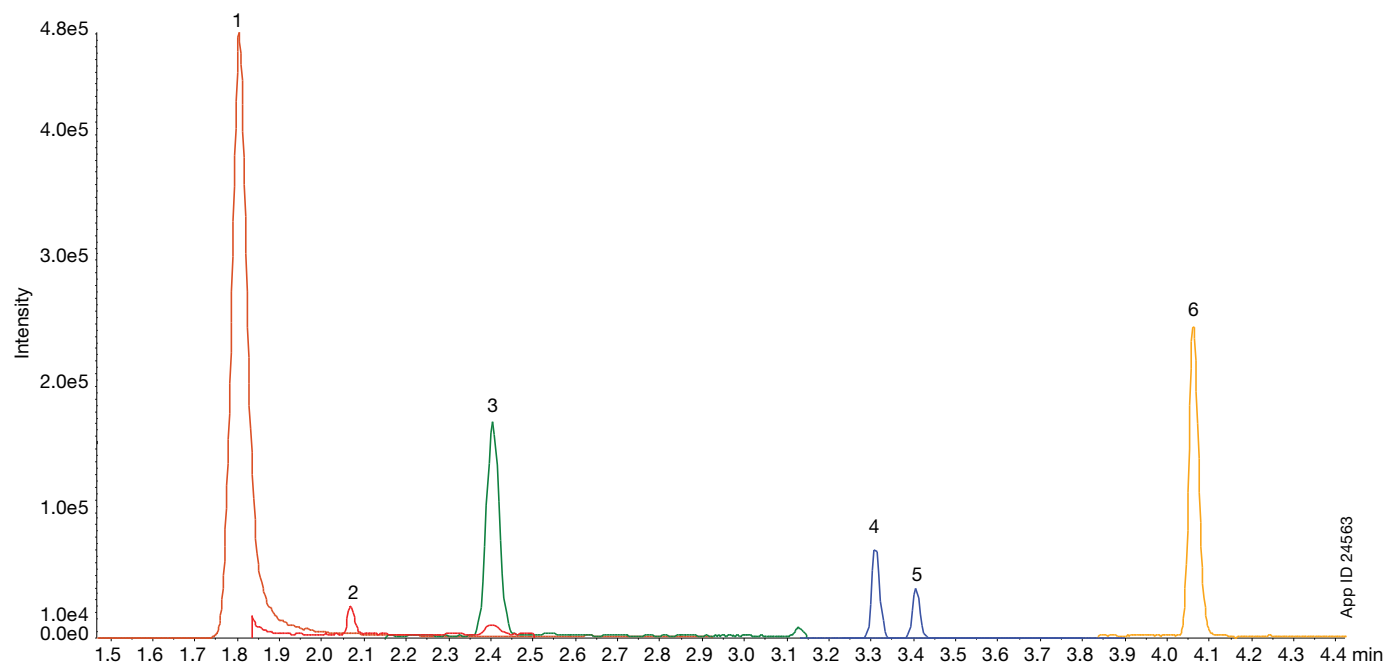
- Step 1:** Take 5 g of homogenized egg (no shell)
- Step 2:** Add 5 mL of water and shake for 10 min
- Step 3:** Add 10 mL of MeCN and shake for 10 min
- Step 4:** Add roQ salts (4.0 g MgSO₄, 1.0 g NaCl, 1.0 SCTD and 0.5 g SCDS) and shake for 10 min (P/N KS0-8909)
- Step 5:** Centrifuge at 2500 g for 5 min
- Step 6:** Take 4 mL of the MeCN layer, add 0.6 g of calcium chloride and shake for 10 min
- Step 7:** Centrifuge at 2500 g for 5 min
- Step 8:** Take 1 mL of the MeCN layer, transfer to an HPLC vial and add 1 mL of water

LC-MS/MS Conditions

Column:	Kinetex 2.6µm Polar C18																		
Dimensions:	100 x 2.1 mm																		
Part No.:	00D-4759-AN																		
Mobile Phase:	A: 5mM Ammonium Formate in Water B: 5mM Ammonium Formate in Methanol + 0.05 % Formic Acid																		
Gradient:	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>% B</th> </tr> </thead> <tbody> <tr><td>0</td><td>5</td></tr> <tr><td>0.5</td><td>5</td></tr> <tr><td>1.5</td><td>40</td></tr> <tr><td>3.0</td><td>80</td></tr> <tr><td>5.0</td><td>87</td></tr> <tr><td>5.5</td><td>95</td></tr> <tr><td>5.6</td><td>5</td></tr> <tr><td>7.0</td><td>5</td></tr> </tbody> </table>	Time (min)	% B	0	5	0.5	5	1.5	40	3.0	80	5.0	87	5.5	95	5.6	5	7.0	5
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3.0	80																		
5.0	87																		
5.5	95																		
5.6	5																		
7.0	5																		
Injection Volume:	5 µl																		
Flow Rate:	0.9 mL/min																		
Temperature:	40 °C																		
Detection:	MS/MS																		
Detector:	SCIEX QTRAP [®] 6500+ with IonDrive Pos/Neg switching																		
System:	SCIEX ExionLC [™]																		
Analytes:	<table border="1"> <thead> <tr> <th>Analyte</th> <th>RT (min)</th> </tr> </thead> <tbody> <tr><td>1. DPMF</td><td>1.8</td></tr> <tr><td>2. DMA</td><td>2.08</td></tr> <tr><td>3. DMF</td><td>2.39</td></tr> <tr><td>4. Fipronil</td><td>3.30</td></tr> <tr><td>5. Fipronil Sulfone</td><td>3.39</td></tr> <tr><td>6. Amitraz</td><td>4.05</td></tr> </tbody> </table>	Analyte	RT (min)	1. DPMF	1.8	2. DMA	2.08	3. DMF	2.39	4. Fipronil	3.30	5. Fipronil Sulfone	3.39	6. Amitraz	4.05				
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*MS Conditions on reverse side

QuEChERS Modified Extractions – Pre Extraction Spiked Sample



5.03 g Egg spiked with 100µL of 500ng/mL Fip, 508 ppb DPMF, 272 ppb DMF, 301 ppb DMA, 299.1 ppb Ami – extracted via QuEChERS Method



APPLICATIONS

Kinetex[®] Ordering Information

SecurityGuard [™] ULTRA Cartridges [‡]				
2.6 µm Minibore Columns (mm)	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1
Polar C18	00A-4759-AN	00B-4759-AN	00D-4759-AN	00F-4759-AN
	AJ0-9532 for 2.1 mm ID			

SecurityGuard [™] ULTRA Cartridges [‡]				
2.6 µm MidBore [™] Columns (mm)	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
Polar C18	00B-4759-Y0	00D-4759-Y0	00F-4759-Y0	AJ0-9531
	for 3.0 mm ID			

SecurityGuard [™] ULTRA Cartridges [‡]				
2.6 µm Analytical Columns (mm)	50 x 4.6	100 x 4.6	150 x 4.6	3/pk
Phases	50 x 4.6	100 x 4.6	150 x 4.6	3/pk
Polar C18	00B-4759-E0	00D-4759-E0	00F-4759-E0	AJ0-9530
	for 4.6 mm ID			

[‡] SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000.

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MS Conditions

Positive Ion Mode

Name	Q1	Q3
Fipronil 1	437.0	368.0
Fipronil 2	437.0	289.9
Amitraz 1	294.2	163.2
Amitraz 2	294.2	253.2
DMA 1	122.1	107.0
DMA 2	122.1	76.9
DMF 1	150.1	107.0
DMF 2	150.1	123.1
DPMF 1	163.2	107.1
DPMF 2	163.2	122.1

Negative Ion Mode

Name	Q1	Q3
Fipronil Sulfone 1	450.8	282.1
Fipronil Sulfone 2	450.8	415.0

Ordering Information

roQ[™] Extraction Kits

Extraction Kits contain fifty easy-pour salt packets and fifty 50 mL stand-alone centrifuge tubes

Description	Unit	Part No.
EN 15662 Method Extraction Kits		
4.0 g MgSO ₄ , 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS	50/pk	KS0-8909*
AOAC 2007.01 Method Extraction Kits		
6.0 g MgSO ₄ , 1.5 g NaOAc	50/pk	KS0-8911*
Original Non-Buffered Method Extraction Kits		
4.0 g MgSO ₄ , 1.0 g NaCl	50/pk	KS0-8910
6.0 g MgSO ₄ , 1.5 g NaCl	50/pk	KS0-8912

*AOAC and EN Extraction Kits also available in traditional non-collared 50 mL centrifuge tubes, Part No.: KS0-8911-NC and KS0-8909-NC

roQ Extraction Salt Packets

Salt packets only. Centrifuge tubes not included.

Description	Unit	Part No.
AOAC 2007.01 Method Extraction Packets		
6.0 g MgSO ₄ , 1.5 g NaOAc	50/pk	AH0-9043
EN 15662 Method Extraction Packets		
4.0 g MgSO ₄ , 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS	50/pk	AH0-9041
Original Non-Buffered Method Extraction Packets		
4.0 g MgSO ₄ , 1.0 g NaCl	50/pk	AH0-9042
6.0 g MgSO ₄ , 1.5 g NaCl	50/pk	AH0-9044



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