

# Sensitive and selective analysis of fipronil residues in eggs using Thermo Scientific GC-MS/MS triple quadrupole technology

## Authors

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## Goal

To demonstrate the excellent sensitivity, selectivity, and linearity achieved for the analysis of fipronil and its metabolites in eggs using the Thermo Scientific™ TSQ™ 8000 Evo triple quadrupole GC-MS system.

## Introduction

Pesticides are chemicals used to protect crops and control a variety of pests such as weeds, fungi, rodents, and insects. Because of their extensive use, pesticides can be found in the air, soils, water, and ultimately in the food chain. Although their use is highly regulated, the misuse of pesticides can lead to unwanted contamination of food and possible health impacts on humans and the environment. An example of such events of pesticide misuse was recently reported in Europe where fipronil, an acaricide authorized to treat pets or selected plant seeds,<sup>1</sup> contaminated a large number of eggs and egg products. Recently, the European Commission revised the maximum residue levels for fipronil (the sum of fipronil and fipronil sulfone expresses as fipronil) and set it at 0.005 mg/kg in eggs.<sup>2</sup> This is a challenging quantification level and requires robust sample preparation and sensitive and selective analytical instrumentation.

## Keywords

Fipronil, fipronil sulfone, fipronil desulfinyl, eggs, food safety, sensitivity, selected reaction monitoring, TSQ 8000 Evo, triple quadrupole gas chromatography

In this study, the results obtained for the analysis of fipronil residues in eggs using a Thermo Scientific triple quadrupole GC-MS system are reported. System performance was tested using full scan acquisition and simple instrumental setup. The experiments performed focused on assessing the sensitivity, linearity, selectivity, and analytical precision for the analysis of fipronil and its metabolites in eggs.

## Experimental

In the experiments described here, a TSQ 8000 Evo triple quadrupole mass spectrometer was coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph for gas-phase separation of target compounds.

Injection of acetonitrile extracts of homogenized eggs was performed automatically using a Thermo Scientific™ TriPlus™ RSH autosampler. The GC-MS/MS system was tuned automatically using PFTBA and SRM transitions optimized using Thermo Scientific™ AutoSRM software. Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD TG-5SilMS GC, 30 m × 0.25 mm × 0.25 µm capillary column with 5 m integrated guard (P/N 26096-1425). Data was acquired using full-scan and timed selective reaction monitoring (t-SRM) and processed with Thermo Scientific™ TraceFinder™ software. Additional details regarding the GC and MS conditions as well as the SRM used are given in Table 1 and Table 2.

**Table 1A. Gas chromatography analytical parameters.**

### TRACE 1310 GC System Parameters

|                     |                                       |
|---------------------|---------------------------------------|
| Injection Volume:   | 1.0 µL                                |
| Liner:              | 6 baffled liner Siltek (P/N 453T2120) |
| Inlet:              | 70°C                                  |
| Transfer Rate:      | 5°C/s                                 |
| Transfer            |                                       |
| Final Temperature:  | 300°C                                 |
| Transfer Time:      | 2 min                                 |
| Inlet Module        |                                       |
| and Mode:           | PTV, splitless                        |
| Carrier Gas,        |                                       |
| Carrier Mode, Flow: | He, constant flow (1.2 mL/min)        |

### Oven Temperature Program

|                |          |
|----------------|----------|
| Temperature 1: | 40°C     |
| Hold Time:     | 1.5 min  |
| Temperature:   | 90°C     |
| Rate:          | 25°C/min |
| Hold Time:     | 1.5 min  |
| Temperature 3: | 280°C    |
| Rate:          | 5°C/min  |
| Hold Time:     | 0 min    |
| Temperature 4: | 300°C    |
| Rate:          | 10°C/min |
| Hold Time:     | 5 min    |

**Table 1B. Mass spectrometer analytical parameters.**

### TSQ 8000 Evo Mass Spectrometer Parameters

|                             |              |
|-----------------------------|--------------|
| Transfer Line:              | 250°C        |
| Ionization Type:            | EI           |
| Ion Source:                 | 300°C        |
| Electron Energy:            | 70 eV        |
| Acquisition Mode:           | FS and t-SRM |
| Q2 Gas Pressure<br>(Argon): | 60 psi       |
| Acquisition Mode:           | SRM          |
| Q1 Peak Width:              | 0.7 Da       |
| Q3 Peak Width:              | 0.7 Da       |

**Table 2.** SRM transitions with corresponding compound retention time and collision energies used for fipronil, fipronil sulfone, and fipronil desulfinyl. The quantitation SRM transitions are in bold text.

| Compound Name           | RT (min)     | SRM transition (Precursor m/z → Product m/z) | Collision Energy (eV) |
|-------------------------|--------------|--|-----------------------|
| Fipronil desulfinyl     | 14.12        | <b>388 → 333</b>                             | 12                    |
| Fipronil desulfinyl     | 14.12        | 333 → 231                                    | 20                    |
| Fipronil desulfinyl     | 14.12        | 333 → 281                                    | 12                    |
| Fipronil desulfinyl     | 14.12        | 388 → 281                                    | 26                    |
| Fipronil desulfinyl     | 14.12        | 390 → 333                                    | 12                    |
| Fipronil desulfinyl     | 14.12        | 390 → 335                                    | 12                    |
| <b>Fipronil</b>         | <b>16.49</b> | <b>367 → 213</b>                             | <b>28</b>             |
| Fipronil                | 16.49        | 351 → 255                                    | 14                    |
| Fipronil                | 16.49        | 369 → 215                                    | 30                    |
| Fipronil                | 16.49        | 255 → 157                                    | 34                    |
| Fipronil                | 16.49        | 255 → 228                                    | 14                    |
| Fipronil                | 16.49        | 367 → 245                                    | 20                    |
| <b>Fipronil sulfone</b> | <b>18.30</b> | <b>383 → 255</b>                             | <b>15</b>             |
| Fipronil sulfone        | 18.30        | 255 → 228                                    | 10                    |
| Fipronil sulfone        | 18.30        | 335 → 255                                    | 10                    |
| Fipronil sulfone        | 18.30        | 385 → 257                                    | 15                    |
| Fipronil sulfone        | 18.30        | 452 → 241                                    | 20                    |
| Fipronil sulfone        | 18.30        | 452 → 255                                    | 25                    |

## Sample preparation

Organic egg samples were purchased from a local store and were subjected to QuEChERS extraction and dispersive mode (dSPE) clean-up using acetonitrile as final solvent.<sup>3</sup> For this 5 g of homogenized eggs were used. The clean-up procedure used dSPE sorbent with 600 mg anhydrous MgSO<sub>4</sub> and 500 mg C18 in the dSPE.<sup>3</sup> Fipronil and fipronil metabolites standards were purchased from LGC Ltd., (United Kingdom). To test the limit of detection (LOD)/limit of quantitation (LOQ)

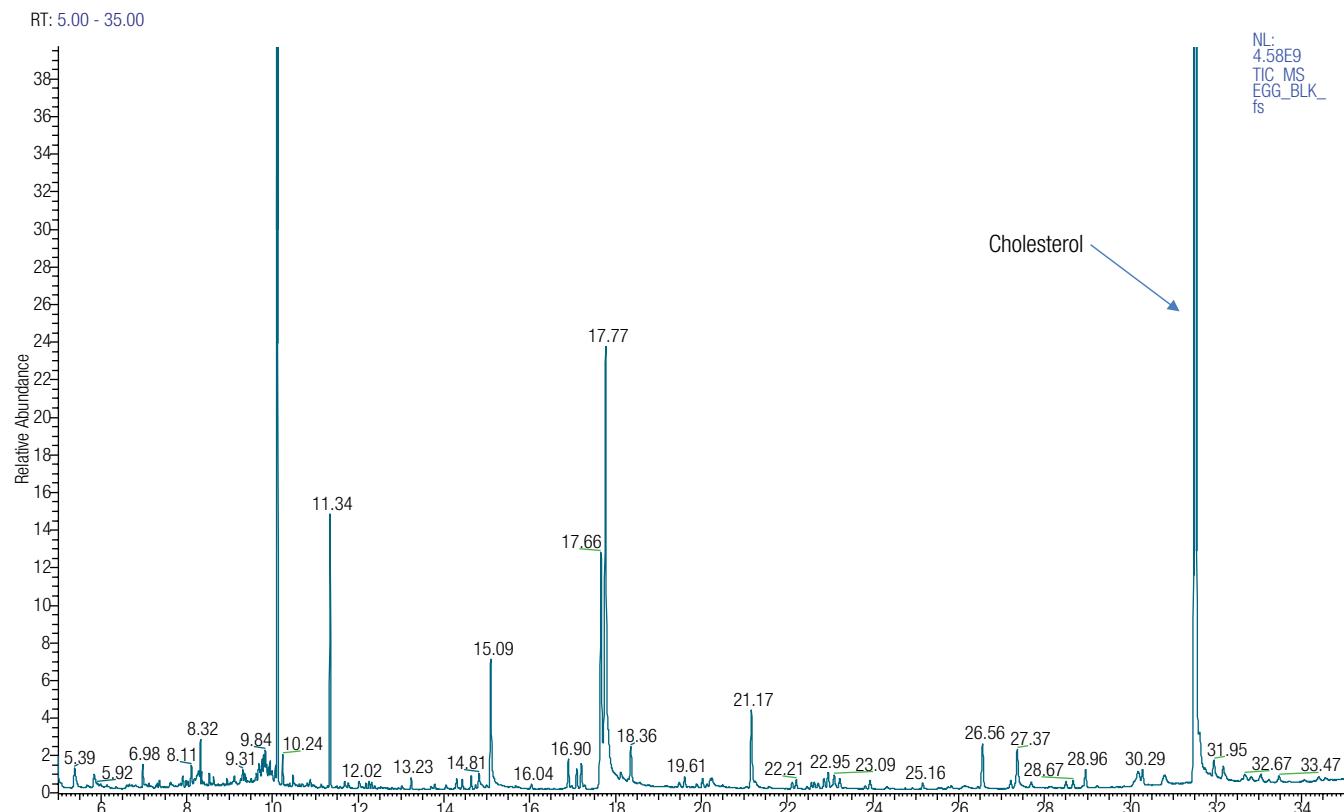
and assess the linearity of fipronil, fipronil desulfinyl, and fipronil sulfone, matrix-matched calibration solutions were prepared by spiking blank egg extracts with calibration solutions prepared in acetonitrile. Calibration levels for each of the three compounds were: 0.1, 0.5, 1, 5, 10, 50, and 100 ng/mL (corresponding to 0.2–200 µg/kg in eggs). Method performance (calculation of recoveries) was tested using egg samples spiked before the extraction with each analyte at 0.5, 1, and 5 µg/kg.

## Results and discussion

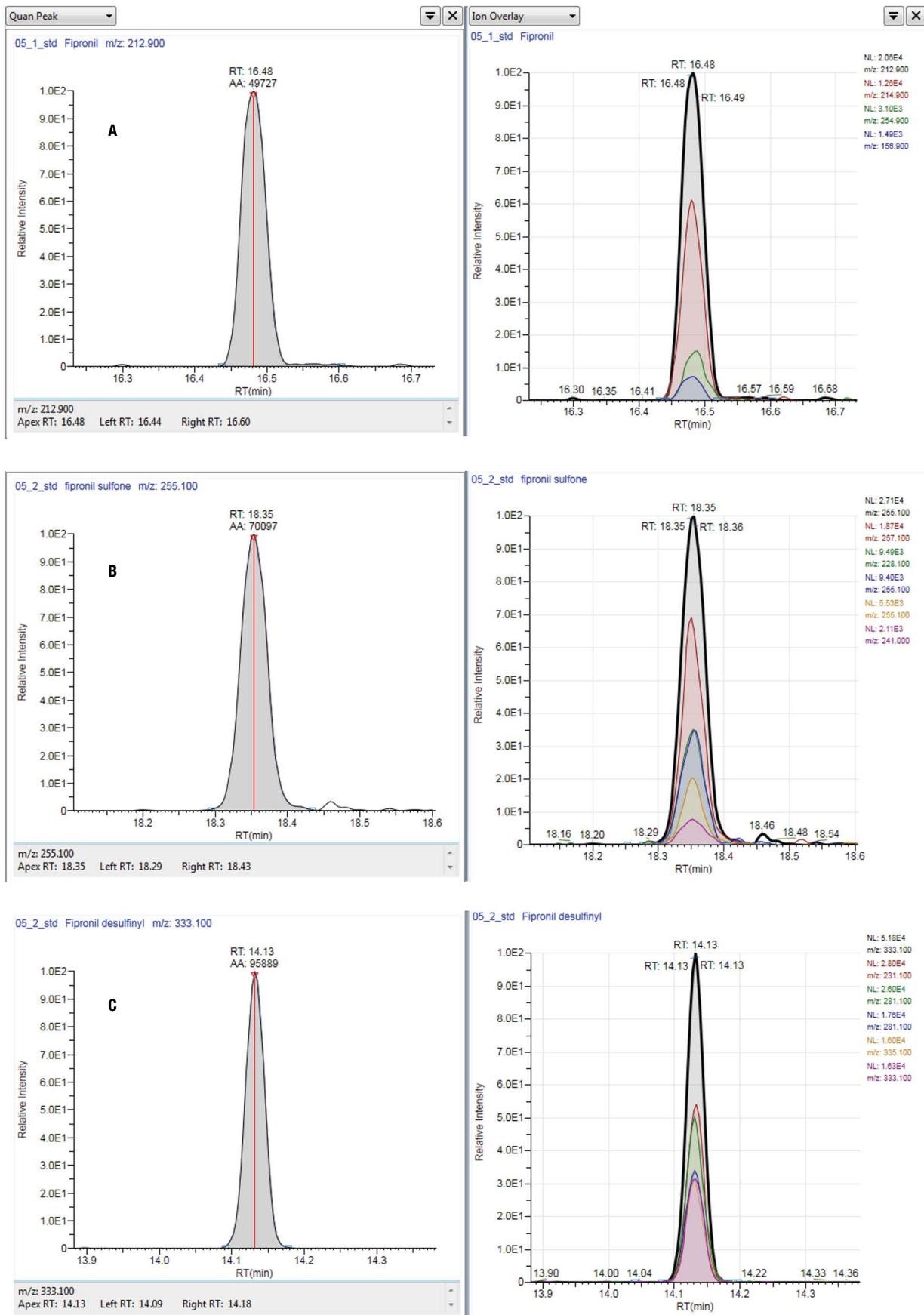
Analysis of pesticide residue in egg samples can be difficult due to the complexity of the matrix. An example of full scan data acquisition of an egg sample subjected to QuEChERS and dSPE clean-up is shown in Figure 1 with cholesterol as the dominant peak. Given the complexity of this chromatogram, analysis of fipronil and metabolites was carried out using selective reaction monitoring transitions (SRM).

## Selectivity and sensitivity - determination of LOD and LOQ

Using the SRM transitions, selective detection of fipronil and metabolites was achieved. Example of SRM chromatograms, including an ion overlay (quantitation ion and identification ions) at 0.5 ng/mL (1 µg/kg) level, are shown in Figure 2.



**Figure 1. Example of a total ion current (TIC) chromatogram of an egg sample acquired in full-scan using electron ionization (EI). Cholesterol (RT = 31.52 min) annotated as the base peak.**



**Figure 2. Selectivity of fipronil (A), fipronil sulfone (B), and fipronil desulfinyl (C) in egg samples at 0.5 ng/mL (corresponding to 1 µg/kg in eggs).** Both SRM quantitation ion (left) and overlaid quantitation and confirmation ions are shown.

The analytical methods for the determination of residues of fipronil sulfone metabolite in commodities of animal origin including eggs and egg products are expected to be able to reach a combined fipronil and fipronil sulfone LOQ of 5 µg/kg.<sup>1</sup> The lowest level at which these compounds were detected (LOD) was 0.2 µg/kg. At this level, absolute peak area reproducibility over n=6 consecutive injections was < 20% relative standard deviation (RSD).

In addition, the LOQ was determined as the lowest concentration level of fipronil and metabolites with a peak area repeatability of < 20% RSD and ion ratio confirmation within < 20% of the average values calculated across a calibration curve ranging from 0.1 to 100 ng/mL (corresponding to 0.2–200 µg/kg in eggs). Based on these criteria, the estimated LOQ for all three compounds was 0.5 µg/kg. The results of these tests are summarized in Table 3 and Table 4.

### Linearity

Linearity of fipronil and its metabolites was determined in eggs spiked at concentrations of 0.1, 0.5, 1, 5, 10, 50, and 100 ng/mL (corresponding to 0.2–200 µg/kg in eggs). All compounds showed excellent linear responses with coefficients of determination  $R^2 > 0.999$  (Figure 3). Moreover, the average response factor values across this calibration range was 2.5% for fipronil desulfinyl, 6% for fipronil, and >10% for fipronil sulfone indication excellent linearity.

### Calculation of recoveries

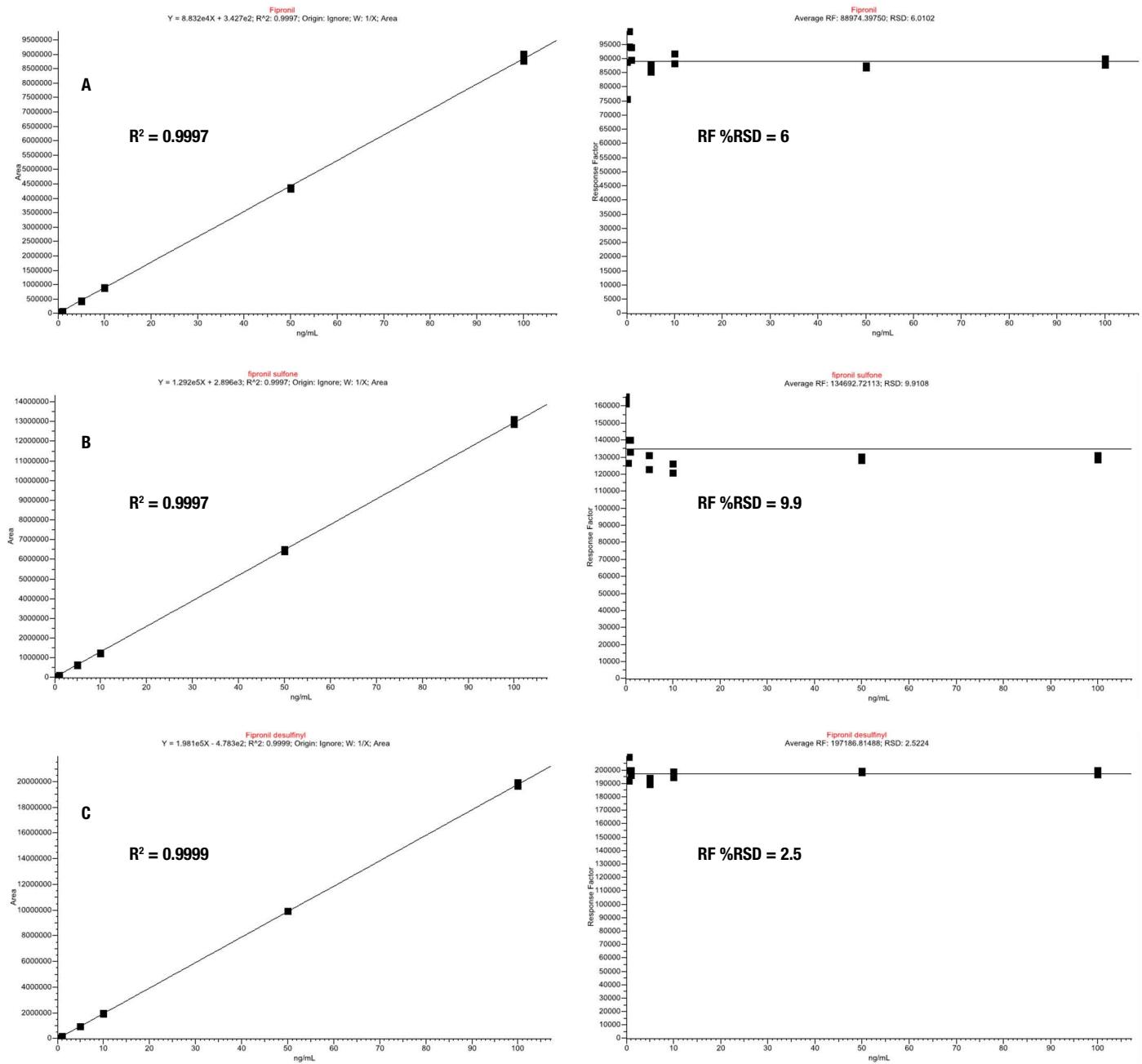
The recovery of the method was tested by analyzing egg samples spiked with fipronil and its metabolites prior to extraction at 0.5, 1, and 5 µg/kg. Four repeat injections were used at each level and the results show average recovery values for the spike levels tested between 99.4% and 100.7% (Table 5).

**Table 3. LOD, LOQ, and absolute peak area repeatability (as %RSD) in egg matrix for fipronil and metabolites determined from n=6 repeat injections of the lowest standards (0.1 µg/kg and 0.5 µg/kg).**

| Compound Name       | LOD (µg/kg) | LOQ (µg/kg) | Repeatability (%)<br>0.5 µg/kg Spike Level | Repeatability (%)<br>5 µg/kg Spike Level |
|---------------------|-------------|-------------|--|--|
| Fipronil            | 0.1         | 0.5         | 3.7  | 3.7                                      |
| Fipronil sulfone    | 0.1         | 0.5         | 3.8  | 1.7                                      |
| Fipronil desulfinyl | 0.1         | 0.5         | 3.4  | 3.5                                      |

**Table 4. Ion ratio stability for fipronil and metabolites (n=6 injections) at 0.5 µg/kg and at 5 µg/kg.**

| Compound Name       | Ion Ratio Repeatability (%)<br>0.5 µg/kg Spike Level | Ion Ratio Repeatability (%)<br>5 µg/kg Spike Level |
|---------------------|--|--|
| Fipronil            | 6.7  | 2.7  |
| Fipronil sulfone    | 1.9  | 0.6  |
| Fipronil desulfinyl | 3.7  | 1.3  |



**Figure 3.** Linearity of fipronil, fipronil sulfone, and fipronil desulfinyl assessed using a matrix-matched calibration curve ranging from 0.1 to 100 ng/mL (corresponding to 0.2–200 µg/kg in eggs).  $R^2$  values as well as average % RF are shown.

**Table 5.** Recoveries (%) calculated for fipronil, fipronil sulfone, and fipronil desulfinyl at three concentration levels (0.5, 1, 5 µg/kg) from n=4 repeat injections. Recovery %RSD is also shown.

| Compound Name       | Recovery (%)<br>0.5 µg/kg<br>Spike Level | %RSD<br>(n=4) | Recovery (%)<br>1 µg/kg<br>Spike Level | %RSD<br>(n=4) | Recovery (%)<br>5 µg/kg<br>Spike Level | %RSD<br>(n=4) |
|---------------------|--|---------------|--|---------------|--|---------------|
| Fipronil            | 100                                      | 7.1           | 100                                    | 0.9           | 100                                    | 3.1           |
| Fipronil sulfone    | 100                                      | 7.4           | 100                                    | 4.4           | 100                                    | 3.3           |
| Fipronil desulfinyl | 99                                       | 6             | 101                                    | 1.6           | 100                                    | 2.3           |

## Conclusions

The results of these experiments show that by using the Thermo Scientific TSQ 8000 Evo triple quadrupole GC-MS system excellent sensitivity, selectivity, and linearity were achieved for fipronil, fipronil sulfone, and fipronil desulfinyl. All compounds were detected at 0.2 µg/kg (LOD) and identified at 0.5 µg/kg (LOQ), which is five times below the EU statutory MRL for the sum of fipronil and fipronil sulfone. The results presented in this work are compliant with the SANTE 11945/2015 analytical quality control guidelines for pesticides<sup>4</sup> and demonstrate that the TSQ 8000 Evo GC-MS/MS system provides sensitive and confident detection and quantification of pesticide residues in difficult matrices.

## References

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