# Rapid and simple determination of low level fipronil and amitraz in egg samples using the Bruker EVOQ™ LC-TQ Elite MS/MS system


## Abstract

We have developed a fast, sensitive, and reliable method to determine the presence of fipronil and amitraz in eggs using a simple extraction process and the Bruker EVOQ LC-TQ Elite mass spectrometry system. The proposed assay uses a liquid-liquid extraction (LLE) of a homogenized egg sample with acetonitrile (ACN). After filtering, the organic extract is injected into the system autosampler for unattended analysis. A method reporting limit (MRL) of 5 µg/kg (ppb) was established, meeting the quality criteria under ISO standard 17025 and the European validation procedures described in the SANTE Guidelines [1].

The exceptional sensitivity of this method achieves sub-ppb limits of detection (LOD) of both fipronil and amitraz.



*Keywords:*
*Fipronil, Amitraz,*
*Egg sample,*
*Food Safety Alert,*
*ISO 17025*
*SANTE Guidelines*



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## Introduction

In August 2017, the Netherlands Food and Consumer Product Safety Authority (known as the NVWA in Dutch) and various media outlets worldwide warned of unusually high levels of fipronil and amitraz in eggs distributed in at least 19 countries, primarily in Europe [2,3].

Fipronil is an insecticide that belongs to the phenylpyrazole family (CAS 120068-37-3) and is commonly used in pest management across Europe. It is banned in products destined for the food chain. Amitraz is an amidine used as an acaricide-insecticide (CAS 33089-61-1) [4]. The maximum residue limit established by the EU [5] for both compounds and their metabolites in eggs is 0.005 mg/kg forfipronil [6] and 0.01 mg/kg for amitraz [7].

The extraordinary sensitivity of the Bruker EVOQ LC-TQ Elite mass spectrometry system enables analysis with a minimal sample quantity and simple sample preparation, avoiding complex cleaning stages, to achieve reliable detection and quantitation below the established maximum residue limits.

The entire methodology was developed in accordance with the Quality Requirements for Testing and Calibration Laboratories (ISO standard 17025).

## Experimental

### Sample Preparation

2 g of homogenized egg (organic pesticide-free chicken eggs) are weighed in a 20 mL conical centrifuge tube and 2 mL of acetonitrile (ACN) are added to the sample. The mixture is shaken in an orbital shaker for 30 seconds. After this time, the samples are centrifuged at 4000 rpm and 1 mL of the organic supernatant is collected. The aliquot is then filtered (0.22 µm nylon filter) using a syringe and the extract is placed in the system autosampler vial for unattended analysis.

Working (stock) solutions of the target analytes were prepared in ACN using individual standards supplied by AccuStandard, Inc. (New Haven, CT, USA). The compounds analyzed are shown in Figure 1. Spiked egg samples were prepared with the addition of all target analytes (at the indicated concentrations) prior to the addition of the ACN. Triphenylphosphate (TPP) was added as an internal standard for quantitation calculations.

### Methodology

Instrument conditions are summarized in Table 1 and Figure 2. The MRM conditions for the analyzed compounds and their metabolites are shown in Table 2.

### Analytical method

The acquisition method is rapid and simple to create due to the intuitive method editor built into the Bruker MS Workstation data acquisition software.

Bruker's TASQ™ was used for the processing and statistical calculations of the quality parameters. This software allows for rapid and accurate review of results. Figure 3 provides an example of the detailed results display. Figure 4 shows the MRM chromatogram of an egg sample spiked with 5 µg/kg (ppb) of the compounds analyzed. Each com-

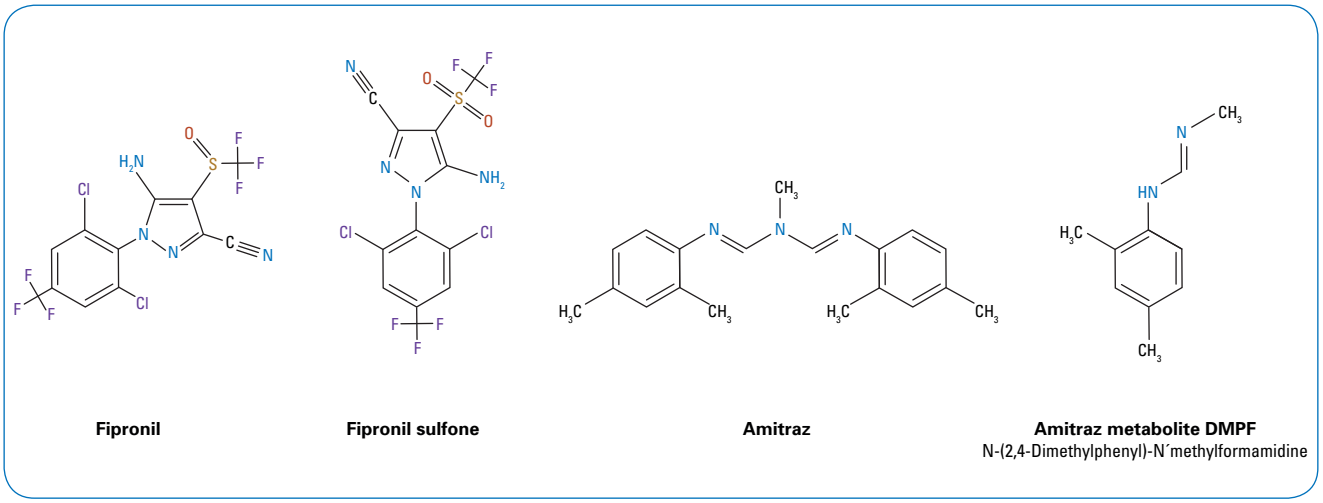


*Figure 1: Compounds analyzed. Left to right: fipronil, fipronil sulfone, amitraz, and N-(2,4-Dimethylphenyl)-N´methylformamidine [DMPF, an amitraz metabolite]*

*Table 1: Mass Spectrometry Method Conditions*

| Mass Spectrometer | Bruker EVOQ™ LC-TQ Elite MS/MS system |
|---|---|
| HESI | 4000 (pos. mode), 3800 (neg. mode) |
| Cone Temperature | 250 °C |
| Cone Gas Flow | 20 psi |
| Nebulizer Gas Flow | 60 psi |
| Heated Probe Temperature | 400 °C |
| Probe Gas Flow | 50 psi |
| CID Gas | Ar, 2.0 mtorr |
| Detector Mode | EDR |
| Polarity | Automatic polarity switching ( +/-) |
| **Liquid Chromatography** | **Bruker Elute™ UHPLC system** |
| LC Column | Bruker Intensity Solo C18 100 x 2.1 mm (P/N:BRKHSC18022100) |
| Mobile Phase A | Water + 0.05% formic acid + 2 mM ammonium formate |
| Mobile Phase B | Acetonitrile + 0.05% formic acid + 2 mM ammonium formate |
| Flow Rate | 400 µL/min |
| Injection Volume | 2 µL |
| Column Oven Temperature | 40 °C |
| Total Run Time | 14 min |
| Software | HyStar 4.1/Bruker MSWS 8.2.1/TASQ 1.4 processing software |

**LC Gradient**

**Time table**

| Line: | Time(min): | Flow (mL/min): | %A: | %B: |
|---|---|---|---|---|
| 01 | 0.00 | 0.400 | 98 | 2.0 |
| 02 | 0.40 | 0.400 | 98 | 2.0 |
| 03 | 0.50 | 0.400 | 65 | 35.0 |
| 04 | 7 | 0.400 | 2 | 98.0 |
| 05 | 11 | 0.400 | 2 | 98.0 |
| 06 | 11.1 | 0.400 | 98 | 2.0 |
| 07 | 13.5 | 0.400 | 98 | 2.0 |

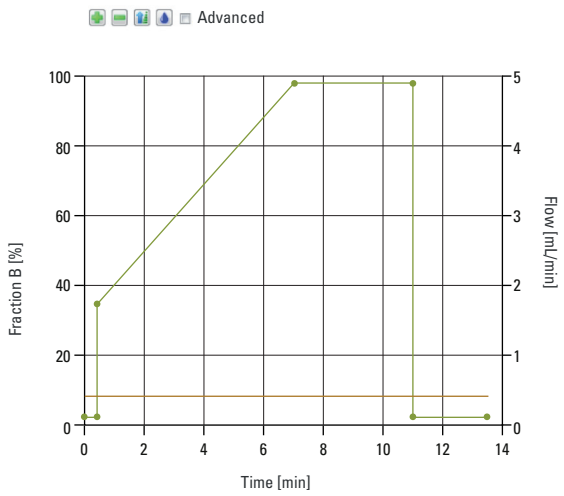


*Figure 2: LC gradient overview, as shown in the Elute series plug-in for the HyStar control software*

pound presents two transitions (MRM) in order to meet the validation criteria.

## Results and discussion

For validation of the method, a control and quality assurance procedure was applied using samples from organic, pesticide-free eggs spiked with the compounds to be analyzed and covering a wide range of concentrations.

*Table 2: MRM transitions for the compounds analyzed*

| Compounds | RT (min.) | Precursor ion | Polarity | Quantitation ion | CE (V) | Confirmation ion | CE (V) |
|---|---|---|---|---|---|---|---|
| Amitraz metabolite DMPF | 1.73 | 163 | Positive | 107 | -26 | 122 | -17 |
| Fipronil | 4.68 | 435 | Negative | 330 | +12 | 250 | +25 |
| Fipronil Sulfone | 5.10 | 451 | Negative | 282 | +27 | 415 | +14 |
| Amitraz | 6.48 | 294.3 | Positive | 163 | -12 | 122 | -28 |
| TPP (IS) | 4.90 | 327 | Positive | 152 | -29 | 215 | -20 |

The analytical quality criteria included evaluations of linearity, precision and accuracy.

## Linearity

The linearity of the method was demonstrated using a calibration curve with egg samples spiked across the concentration range under study. Four solutions were prepared with the following concentrations: 5 ppb, 10 ppb, 25 ppb and 100 ppb.

The applied quality criteria for linearity are: $R^2 \geq 0.990$ with a variation (RSD %) of the curve's response factor ≤ 30% (σRF) for the entire concentration range under study.

Figure 5 shows the calibration curves for all compounds analyzed. A summary of the calibration results is shown in Table 3, which demonstrates that all of the compounds analyzed present an $R^2 > 0.99$ and RSD < 15%, securely meeting the pre-established quality criteria.

## Precision

To determine precision, expressed as repeatability, the RSD was calculated for five replicates (n=5) of the egg samples spiked at corresponding low concentration levels of 5 µg/kg (ppb) and 10 µg/kg (ppb).

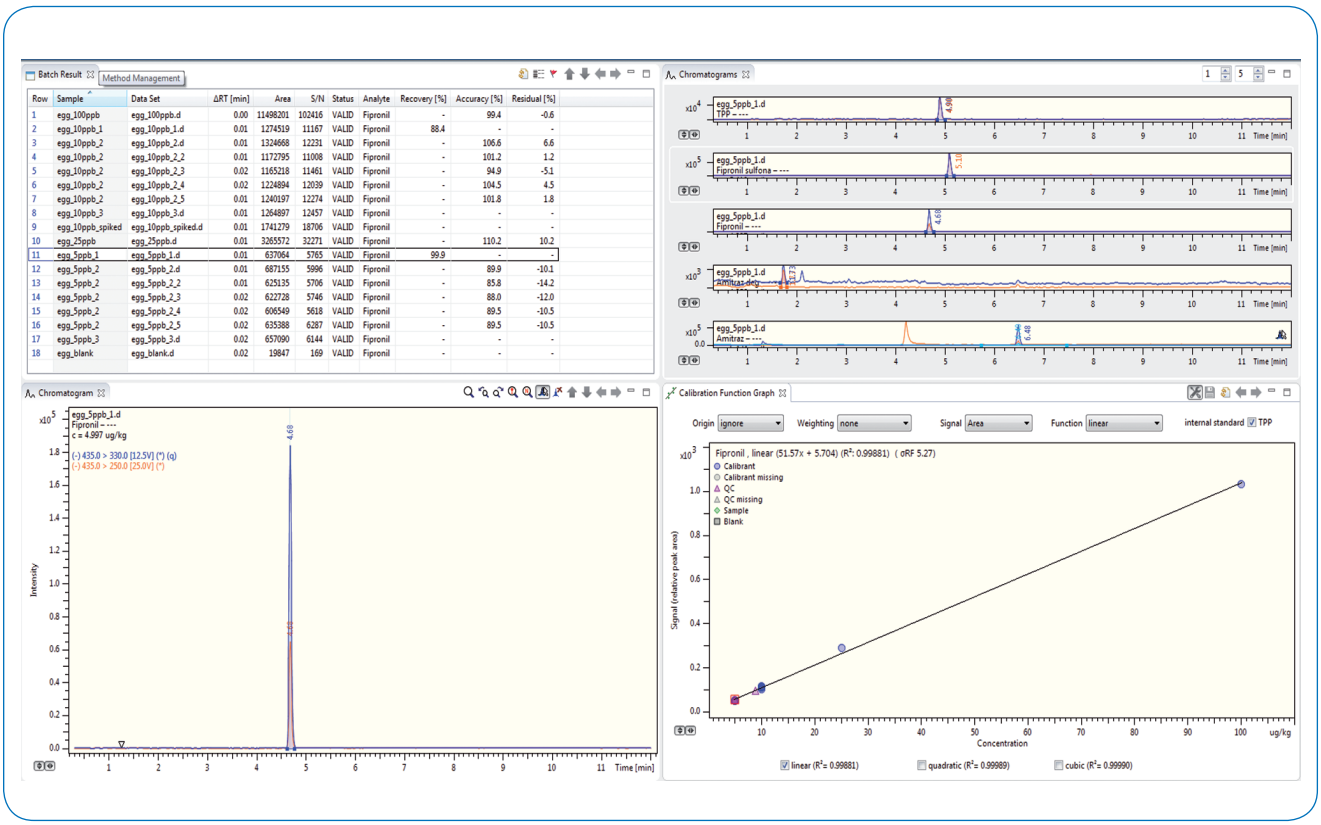


*Figure 3: Display of results by TASQ software*

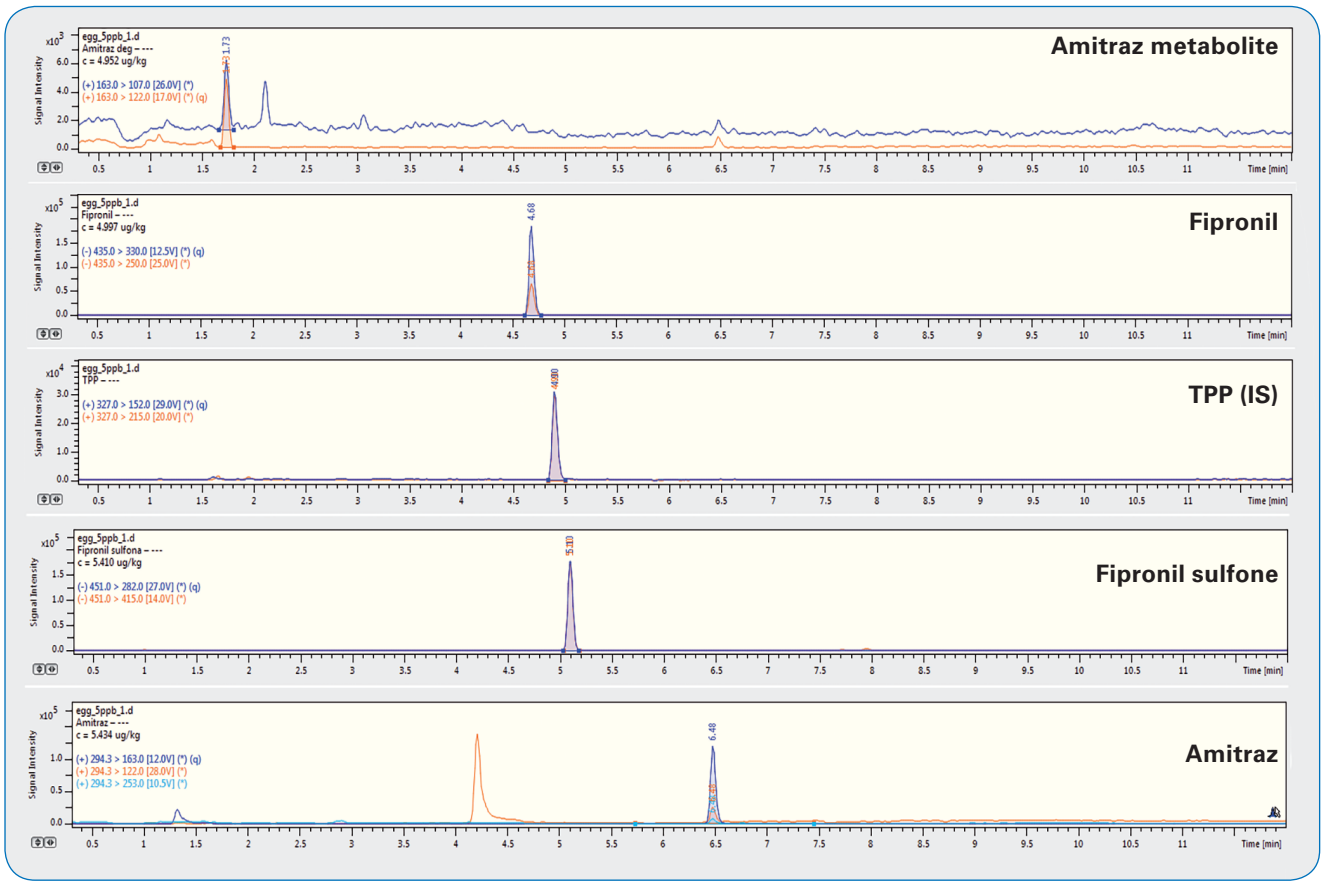


*Figure 4: MRM chromatogram of an egg sample spiked with 5 µg/kg (ppb) of the analyzed compounds, as shown in the TASQ software*

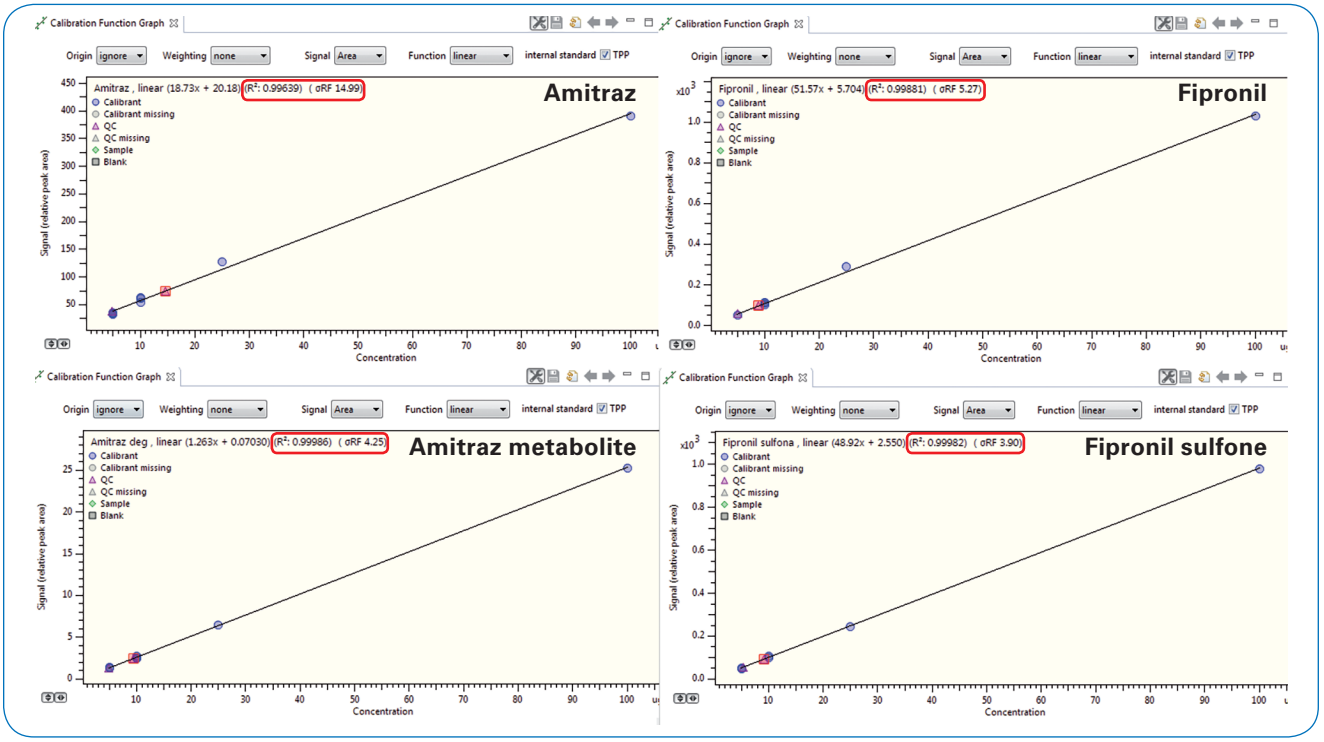


*Figure 5: Calibration curves for all compounds analyzed showing results for $R^2$ and RSD, as shown in the TASQ software*

*Table 3: Summary of linearity results for compounds analyzed*

| Summary of linearity results | | |
|---|---|---|
| **Compounds** | **$R^2$** | **RSD %** |
| Amitraz metabolite | 0.99986 | 4.25 |
| Fipronil | 0.99881 | 5.27 |
| Fipronil sulfone | 0.99982 | 3.90 |
| Amitraz | 0.99639 | 14.99 |

*Table 4: Summary of repeatability results for egg samples spiked with the targeted analytes*

| Summary of repeatability results | | |
|---|---|---|
| **Compounds** | **RSD %** | |
| | **Sample spiked with 5 ppb** | **Sample spiked with 10 ppb** |
| Amitraz metabolite | 2.06% | 2.59% |
| Fipronil | 4.84% | 5.23% |
| Fipronil sulfone | 4.28% | 3.61% |
| Amitraz | 5.64% | 6.60% |

The TASQ software automatically performs the statistical calculation, as can be seen in Figure 6 for fipronil.

A summary of the repeatability results is shown in Table 4, demonstrating that all of the compounds analyzed present repeatability of 2-7% for low concentration levels.

## Accuracy

Recoveries (in %) were calculated on the basis of five independent extractions (n=5) from egg samples spiked with all analytes at 5 µg/kg and 10 µg/kg concentration levels. All of the results are provided in Figure 7: the "Quantity" column indicates the measured concentration value and the "Accuracy" column shows the recovery percentage automatically calculated by the TASQ software.

Recoveries at 5 and 10 µg/kg concentration levels are between 80-102% for all analytes.

## Sensitivity and detection limits

The high sensitivity of the method can be seen in Figure 8, which shows the MRM chromatograms for all compounds analyzed for the first calibration level of 5 µg/kg in the extracted egg samples.

It has been demonstrated that both quantitation and confirmation ions have an excellent signal-to-noise (S/N) ratio at 5 ppb for all compounds analyzed, leading to the conclusion that sub-ppb limits of detection (LOD) can be easily achieved for all compounds analyzed in the egg matrix.

RSD Statistics Graph | RSD Statistics Table

| Row | Concentration | rel.Std.Dev | Mean | Median | Std.Dev. | Minimum | Maximum |
|---|---|---|---|---|---|---|---|
| 1 | 10.0 | 5.23 | 1225554 | 1224894 | 64153 | 1165218 | 1324668 |
| 2 | 5.0 | 4.84 | 635391 | 625135 | 30729 | 606549 | 687155 |

*Figure 6: Evaluation of precision of fipronil replicate quantitation at 5 and 10 ppb, as shown in the TASQ software*

| Row | Data Set | Analyte | Residual [%] | ΔRT [min] | Area | Quantity [ug... | Height | RT [min] exp. | S/N | Quantity | Accuracy [%] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | egg_5ppb_2_5 | Amitraz | -18.6 | 0.02 | 434273 | 4.1 | 122444 | 6.47 | 1813 | 4.1 ug/kg | 81.4 |
| 2 | egg_5ppb_2_5 | Amitraz deg | -0.7 | 0.01 | 16235 | 5.0 | 5351 | 1.73 | 152 | 5.0 ug/kg | 99.3 |
| 3 | egg_5ppb_2_5 | Fipronil | -10.5 | 0.02 | 635388 | 4.5 | 186299 | 4.67 | 6287 | 4.5 ug/kg | 89.5 |
| 4 | egg_5ppb_2_5 | Fipronil sulfona | -4.3 | 0.02 | 605302 | 4.8 | 180162 | 5.08 | 7395 | 4.8 ug/kg | 95.7 |
| Row | Data Set | Analyte | Residual [%] | ΔRT [min] | Area | Quantity [ug... | Height | RT [min] exp. | S/N | Quantity | Accuracy [%] |
| 1 | egg_10ppb_2_5 | Amitraz | -9.0 | 0.01 | 607831 | 9.1 | 173780 | 6.47 | 2874 | 9.1 ug/kg | 91.0 |
| 2 | egg_10ppb_2_5 | Amitraz deg | -2.5 | 0.01 | 28379 | 9.8 | 9254 | 1.73 | 281 | 9.8 ug/kg | 97.5 |
| 3 | egg_10ppb_2_5 | Fipronil | 1.8 | 0.01 | 1240197 | 10.2 | 356130 | 4.67 | 12274 | 10.2 ug/kg | 101.8 |
| 4 | egg_10ppb_2_5 | Fipronil sulfona | -0.6 | 0.02 | 1118584 | 9.9 | 329380 | 5.08 | 15467 | 9.9 ug/kg | 99.4 |

*Figure 7: Recovery for egg samples spiked with all compounds at 5 µg/kg and 10 µg/kg concentration levels, as shown in the TASQ software*

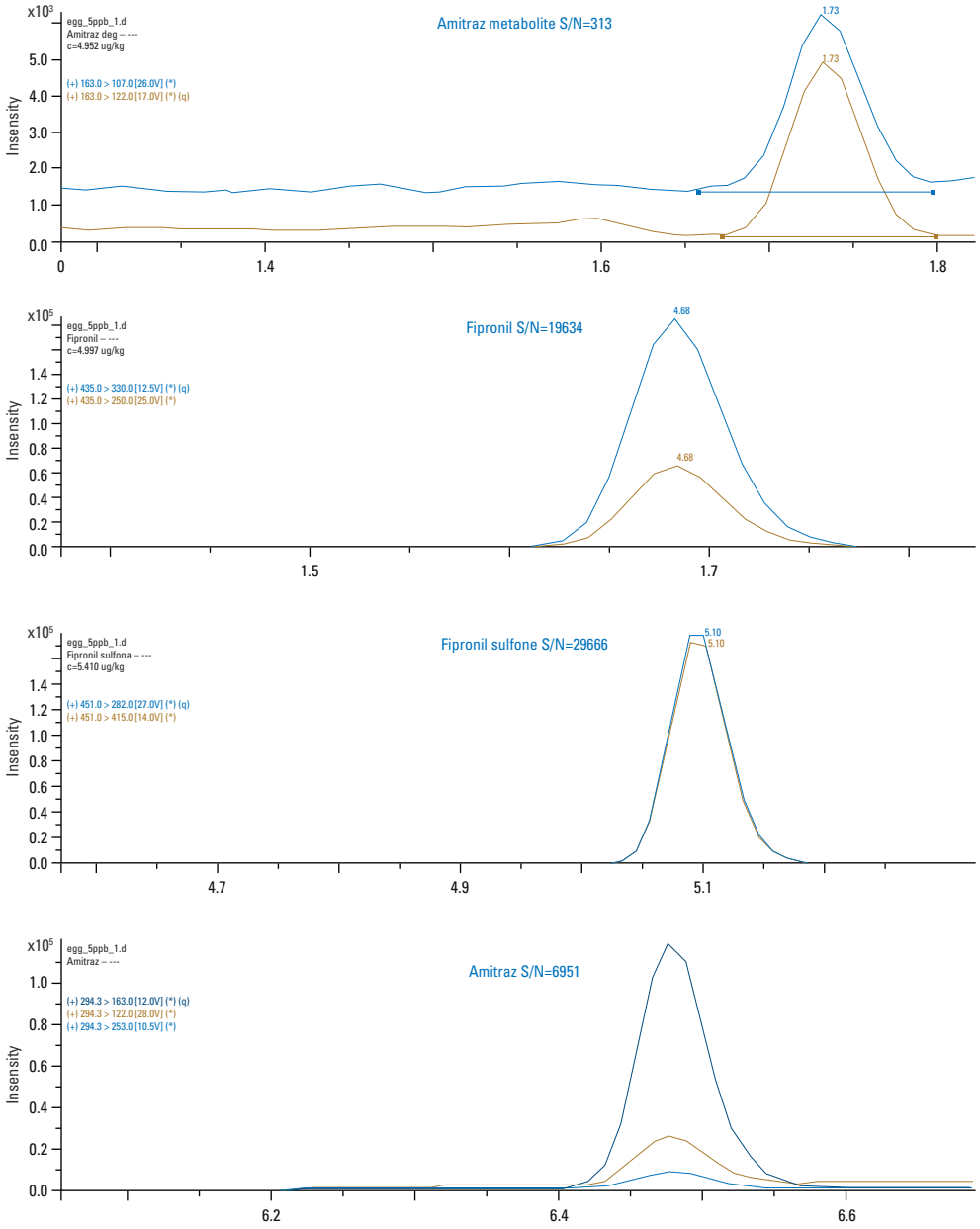


*Figure 8: MRM chromatogram of an egg sample spiked with all analytes at 5 µg/kg. Quantitation ions and confirmation ions are shown, as well as signal-to-noise (S/N), for each compound, as shown in the TASQ software.*

## Conclusion

A rapid and ultra-sensitive method with a very simple sample preparation process has been developed for the detection of fipronil and amitraz in egg samples using the Bruker EVOQ™ LC-TQ Elite mass spectrometry system. The method provides excellent analytical quality parameters for linearity, accuracy and precision in accordance with the ISO 17025 standard. This method has been validated and is ready for implementation in food quality control process laboratories.

# Learn More

You are looking for further Information? Check out the Link or scan the QR Code.

**www.bruker.com/evoq-lc**


**References**

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[6] Commission Regulation (EU) No 1127/2014

[7] Commission Regulation (EU) No 623/2017


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