# Determination of Veterinary Drug Residues in Milk by GC/MS and GC/ECD

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# Overview

**Purpose:** Develop a rapid, sensitive method for determining amitraz and its metabolites in milk.

**Methods:** Samples were hydrolyzed in acidic condition and extracted with hexane. After derivatization, the samples were analyzed by GC-ECD or directly by GC-MS.

**Results:** The results show that GC-MS determination of amitraz and its metabolites is rapid and sensitive.

# Introduction

Amitraz, N´-(2,4-dimethylphenyl)-N-[(2,4-dimethylphenyl)-imino]methyl-N-methyl- methanimidamide, is an acaricide used to control the infections produced by the mites on cattle. Its use can cause the presence of residues in milk. For this reason, the analysis of amitraz residues in milk has received special attention in the scientific bibliography as opposed to milk analysis. On the other hand, amitraz is a very labile pesticide whose degradation products contain the 2,4-dimethylaniline moiety[1-3]. Thus, the analysis of all the residues containing this substructure is advisable. In this context, the aim of this work has been to develop a method to determine the amitraz total residues in milk.

# Methods

### Sample Preparation

Milk samples were bought from the supermarket. Sample preparation is listed in Figure 1.

### Gas Chromatography

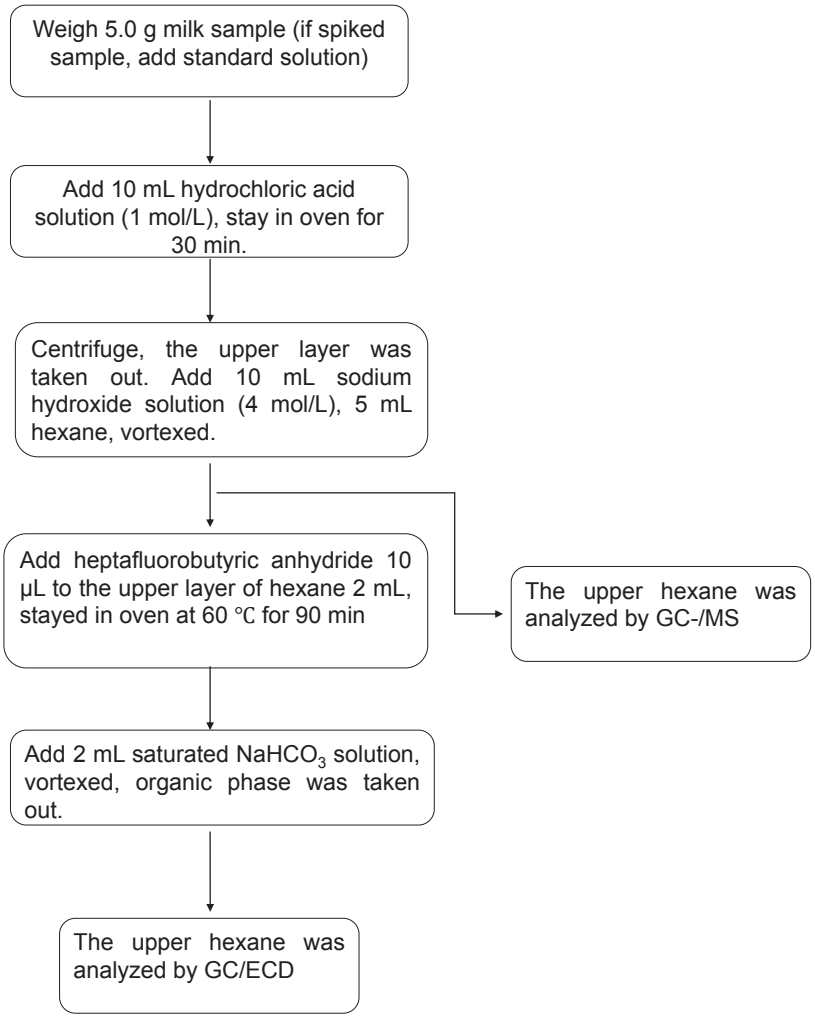
Thermo Scientific™ TRACE™1310 Gas Chromatograph
Column: Thermo Scientific™TraceGOLD TG-5MS (30 m × 0.25 mm × 0.25 µm);
Column temperature for MS detection: 50 ℃ (1 min), 20 ℃ / min to 250 ℃ (10 min);
Column temperature for ECD detection: 50 ℃ (1 min), 10 ℃ / min to 250 ℃ (10 min);
Injection mode: splitless, splitless time of 1 min; injection volume: 1 µL;
Inlet temperature: 260℃;
Carrier gas: helium (99.999%), constant flow mode, 1 mL / min;
ECD parameter: temperature, 300 ℃; makeup gas, nitrogen, 15 mL/min

## Data Analysis

Data is processed with Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System on GC and Thermo Scientific™ Xcalibur ™software on GCMS.

Quantitation was performed by external standard method according to peak area of quantitation ion. When confirmed, according to the fragment ions and their abundance ratio as a basis for positive discrimination.

**FIGURE 1. Sample preparation.**



# Results

## GC-ECD Determination Results

The chromatogram of 2,4-DMA derivatives is in Figure 2. The calibration curve is obtained with a series of standard solutions (Figure 3). Spiked experiments were done to validate the method (Figure 4). The results show that the average recovery was 84.1-87.3 %, RSD values of five parallel measurement ≤ 5.96%, the lower limit of the method for the determination is 2 ng / g.

**FIGURE 2. Chromatogram of 2.4-DMA derivative.**

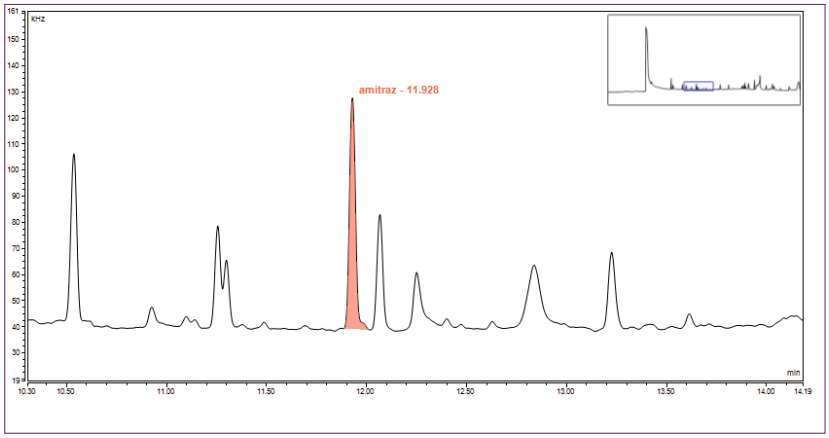



**FIGURE 3. Calibration curve of 2.4-DMA derivative.**

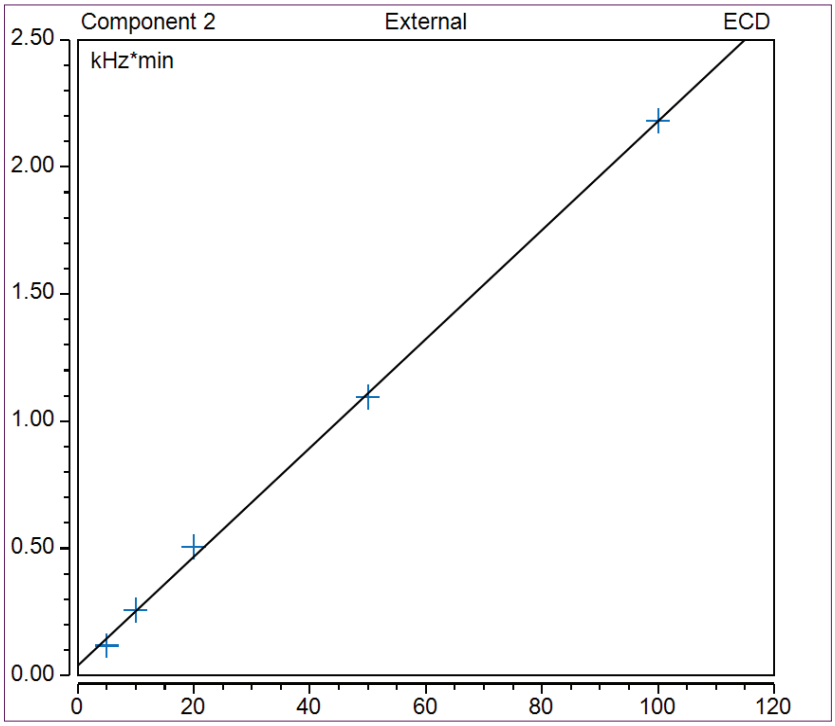
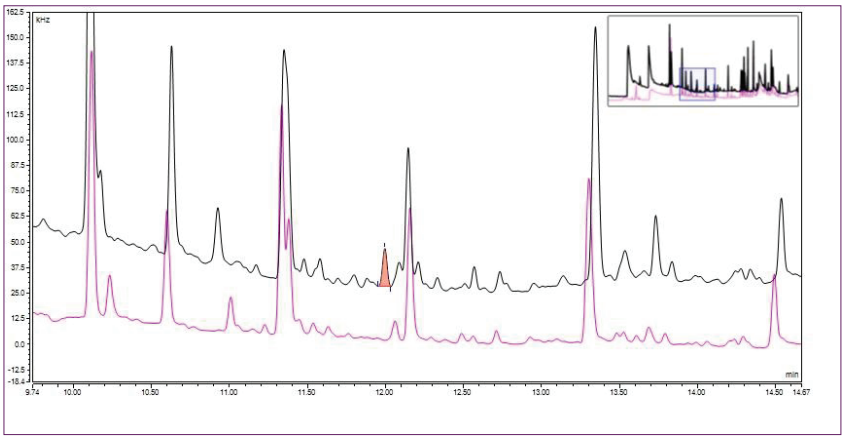


**FIGURE 4. ECD Chromatogram of blank sample and spiked sample (upper is for spiked and lower for blank).**




## GCMS Determination Results

The TIC chromatogram of 2,4-DMA is in Figure 5. The mass spectrum of 2,4-DMA is shown in Figure 6. The calibration curve is obtained with a series of standard solutions (Figure 7). Spiked experiments were done to validate the method (Figure 8) .The results show that the average recovery was 73.8-81.7%, RSD values of five parallel measurement ≤ 8.28%, the lower limit of the method for the determination is 2 ng / g.

**FIGURE 5. Chromatogram of 2.4-DMA.**

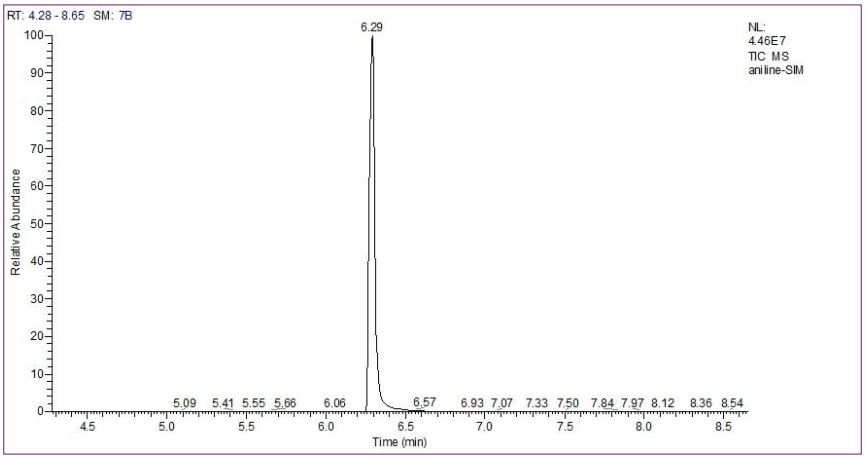


**FIGURE 6. Spectrum of 2.4-DMA.**

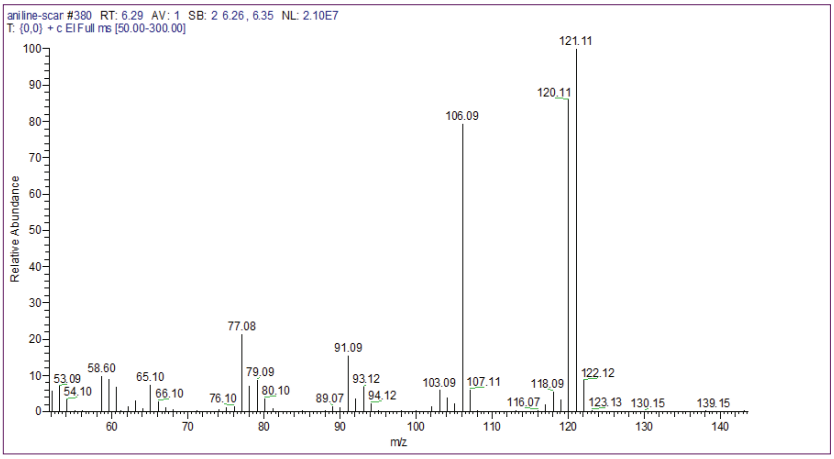



**FIGURE 7. Calibration curve of 2.4-DMA.**

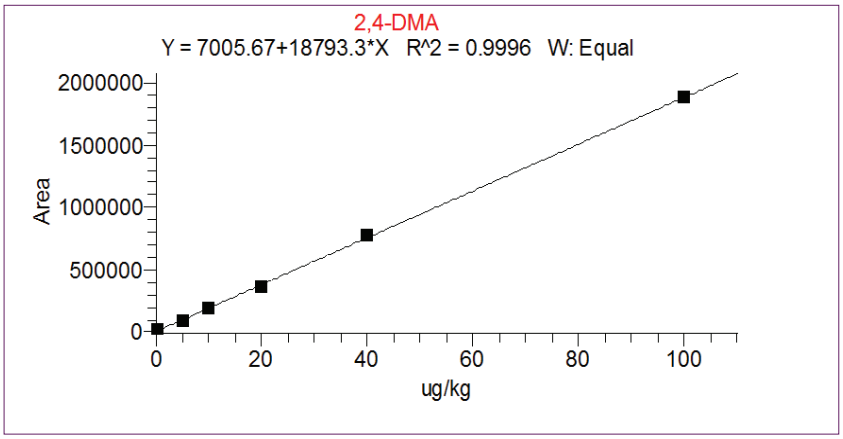
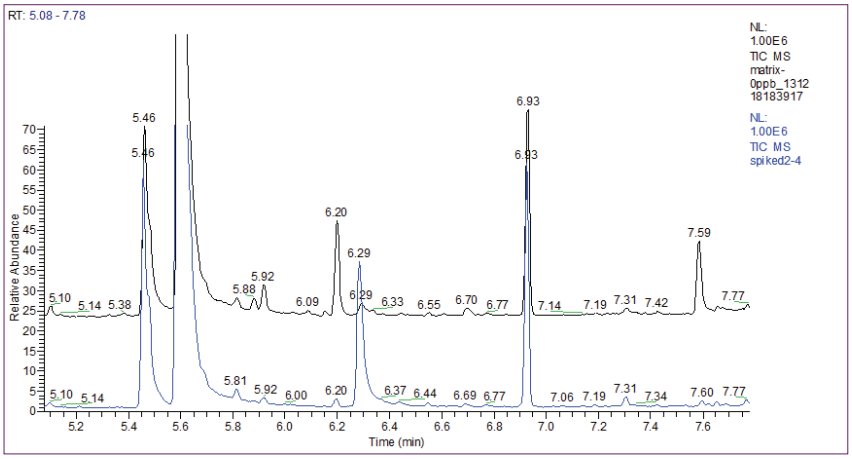



**FIGURE 8. GC-MS Chromatogram of blank sample and spiked sample (upper is for blank and lower for spiked, RT of 2,4-DMA is 6.29 min.)**



## Conclusion

Results showed that GC-MS determination of 2,4-DMA is a simple method for quantitation of amitraz in milk, which is free from interference, without need for derivatization, superior to GC-ECD analysis.

## References


[1] E. Korta, A. Bakkali, L.A. Berrueta, et. al. Determination of amitraz and other acaricide residues in beeswax. *Analytica Chimica Acta* 475 (2003) 97-103.

[2] J.J. Jimenez, J.L.Bernal, M.J. del Nozal, et. al. Extraction and clean-up methods for the determination of amitraz total residues in beeswax by gas chromatography with electron capture detection. *Analytica Chimica Acta* 524 (2004) 271-278.

[3] Jan Lenicek, Milan Sekyra, Andrea Rychtecka Novotna, et. al. Solid phase microextraction and gas chromatography with ion trap detector (GC-ITD) analysis of amitraz residues in beeswax after hydrolysis to 2,4-dimethylaniline. *Analytica Chimica Acta* 571 (2006) 40-44.





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