

# Determination of pesticide residues in red wines from the Canary Islands and other regions of Spain by ultra-high performance liquid chromatography-single quadrupole-time of flight mass spectrometry

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## Introduction

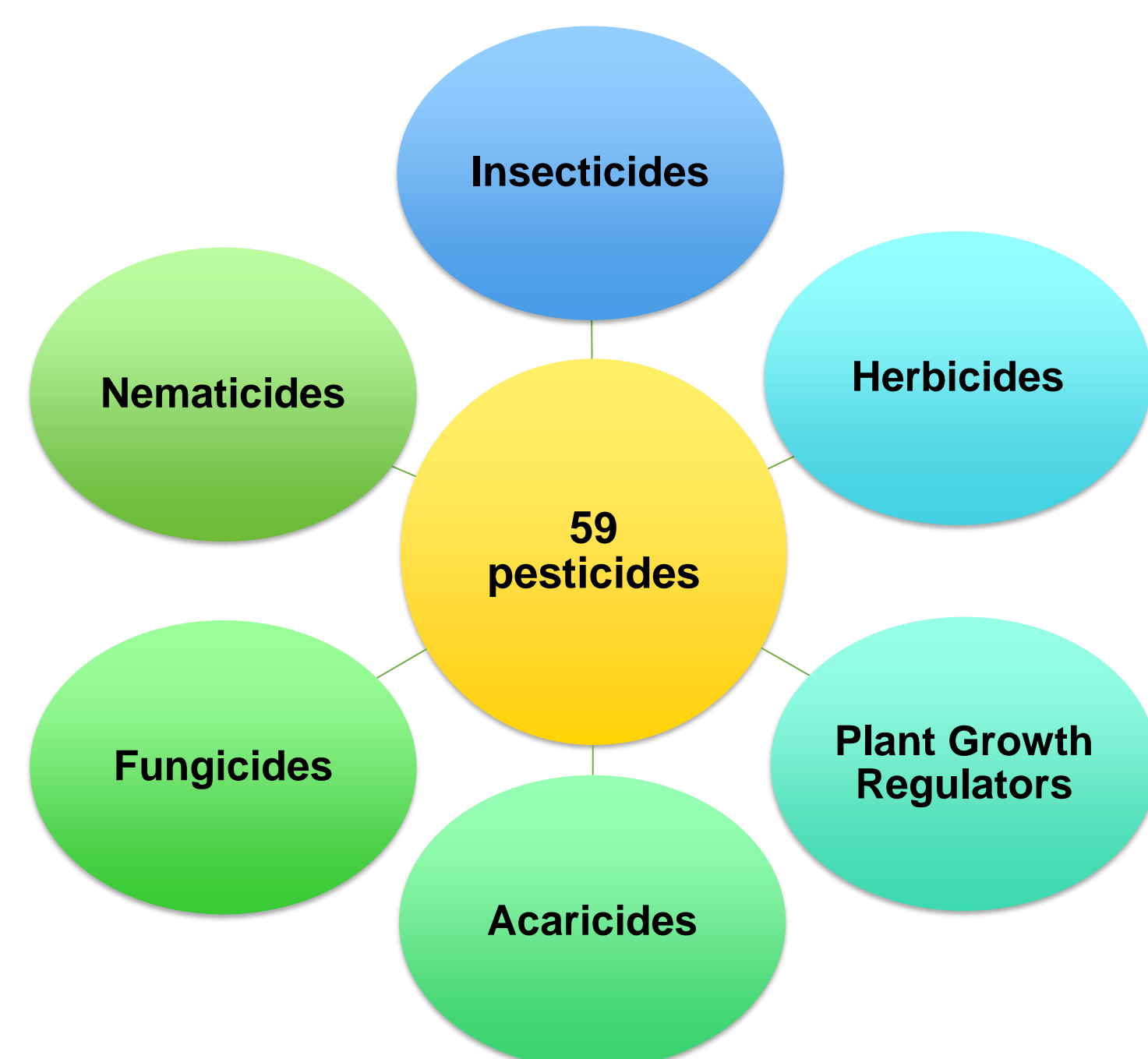
Wine is one of the most consumed alcoholic beverages in the world, being part of our culture for more than 6000 years. The agricultural practices used in different regions, especially those that use intensive agriculture as occurs in the Macaronesia Region make necessary to increase the control and monitoring of pesticide residues in these samples to ensure the safety of their consumption, particularly in those wines that have not been subjected to strict controls.

The analytical techniques more employed for the analysis of these compounds include both gas chromatography and liquid chromatography coupled to mass spectrometry in combination with different extraction techniques. Among them, the QuEChERS method [1] (*quick, easy, cheap, effective, rugged, safe*) is the most commonly applied, using good practice criteria for their determination as it is indicated by the SANTE/12682/2019 guidance [2].

In this work, a method based on QuEChERS sample preparation procedure and ultra-high performance liquid chromatography (UHPLC)-single quadrupole-time of flight (Q-ToF) analysis has been applied for the simultaneous separation and determination of 59 pesticides residues in red wine samples. The methodology was validated obtaining recovery values in the range 61-108% (with relative standard deviations lower than 20% in all cases) and limits of quantification between 2.60 µg/kg and 21.38 µg/kg. Finally, the analysis of red wine samples from different origins has been carried out.

## Experimental

### PESTICIDE RESIDUES STUDIED



### LC conditions

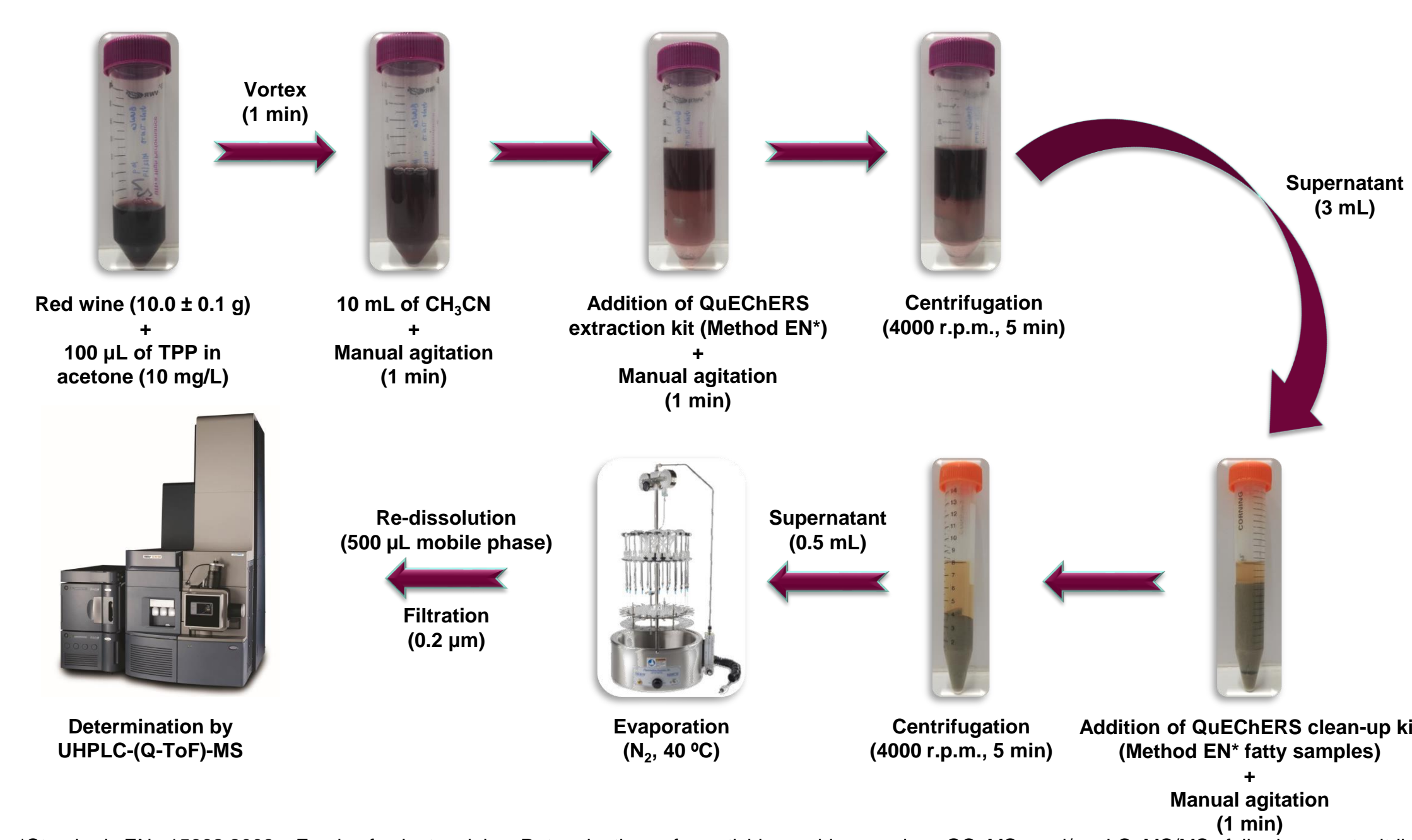
Time (min)	% A	% B
Initial	2	98
12.25	99	1
14.00	99	1
14.50	2	98
17.00	2	98

A: MeOH 10 mM ammonium acetate.  
B: H<sub>2</sub>O 10 mM ammonium acetate.  
Flow rate: 0.45 mL/min.

### MS conditions

Parameter	Value
Capillary voltage	1.00 kV
Source temperature	120 °C
Desolvation temperature	500 °C
Cone gas (N <sub>2</sub> ) Flow rate	20 L/h
Desolvation gas (N <sub>2</sub> ) flow	1000 L/h

### QuEChERS-UHPLC-(Q-ToF)-MS PROCEDURE



\*Standard EN 15662:2008. Food of plant origin. Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dSPE. QuEChERS Method [3]. TPP: triphenyl phosphate.

## Results and discussion

### MATRIX-MATCHED CALIBRATION

Table 1.- Matrix-matched calibration results of the main detected pesticides.

Pesticide	Linear range studied (µg/L)	Calibration data (n = 7)		
		$b \pm S_{b_0} \cdot t_{(0.05; 5)}$	$a \pm S_{a_0} \cdot t_{(0.05; 5)}$	R <sup>2</sup>
Azoxystrobin	10-150	$1.12 \cdot 10^{-2} \pm 7.70 \cdot 10^{-4}$	$-5.92 \cdot 10^{-2} \pm 5.22 \cdot 10^{-2}$	0.9950
Carbendazim	10-150	$5.91 \cdot 10^{-3} \pm 3.13 \cdot 10^{-4}$	$-2.37 \cdot 10^{-2} \pm 2.12 \cdot 10^{-2}$	0.9970
Dimethomorph	10-150	$3.94 \cdot 10^{-3} \pm 2.81 \cdot 10^{-4}$	$-1.43 \cdot 10^{-4} \pm 2.27 \cdot 10^{-2}$	0.9946
Fenhexamide	10-150	$2.38 \cdot 10^{-3} \pm 8.35 \cdot 10^{-5}$	$-6.79 \cdot 10^{-3} \pm 6.00 \cdot 10^{-3}$	0.9989
Imidacloprid	10-150	$1.09 \cdot 10^{-3} \pm 6.62 \cdot 10^{-5}$	$-5.92 \cdot 10^{-3} \pm 4.49 \cdot 10^{-3}$	0.9961
Thiophanate-methyl	10-150	$4.35 \cdot 10^{-3} \pm 1.78 \cdot 10^{-4}$	$-1.80 \cdot 10^{-2} \pm 1.21 \cdot 10^{-2}$	0.9982

R<sup>2</sup>: determination coefficient; b: slope; S<sub>b</sub>: slope standard deviation; a: intercept; S<sub>a</sub>: intercept standard deviation.

### RECOVERY STUDY

Table 2.- Results of recovery values and LOQs of the method for the main detected pesticides.

Pesticide	Relative recovery (RSD, %) <sup>a</sup>	LOQ <sub>method</sub> <sup>b</sup> (mg/kg)
Azoxystrobin	94 (13)	0.01
Carbendazim	89 (14)	0.01
Dimethomorph	81 (16)	0.01
Fenhexamide	88 (12)	0.01
Imidacloprid	80 (15)	0.01
Thiophanate-methyl	75 (12)	0.02

a) Average of the results obtained in the recovery study (n = 5) at a concentration of 0.075 mg/kg. b) Defined as the lowest matrix-matched calibration concentration which provides a signal-to-noise ratio higher than 10 for the quantification transition and at least 3 for the confirmation transition, taking into account the recovery values.

### UHPLC-(Q-ToF)-MS SEPARATION

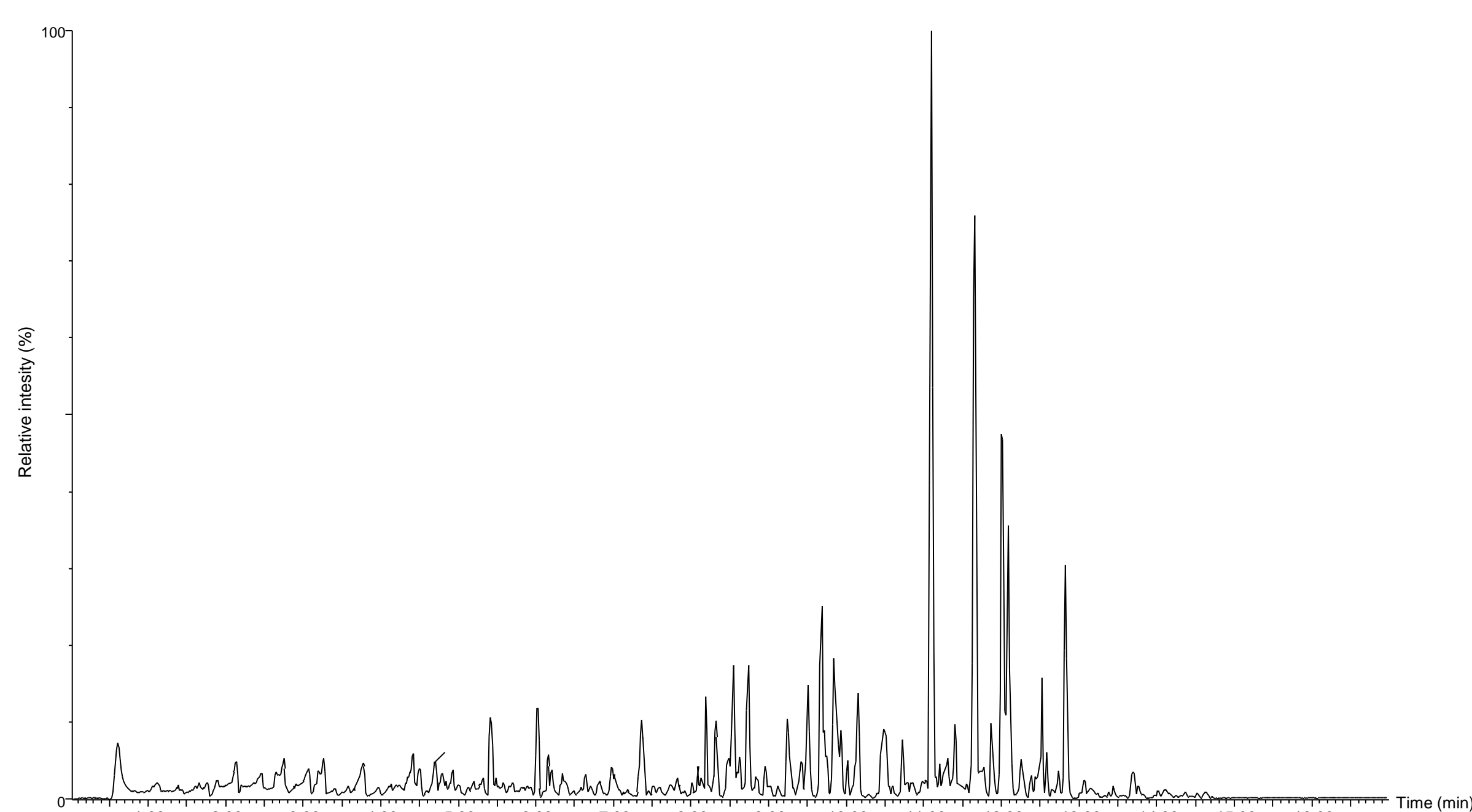


Figure 1.- UHPLC-(Q-ToF)-MS total ion chromatogram of a wine sample spiked with the pesticides and the internal standard (IS), TPP, after the QuEChERS method. Concentration of the target compounds and the IS: 0.075 mg/kg. Injection volume: 5 µL. Column temperature: 45 °C.

### SAMPLE ANALYSIS SUMMARY

Table 3.- Summary of the results obtained from the analysis of different red wine samples using the QuEChERS-UHPLC-(Q-ToF)-MS method.

Pesticide	Number of samples <sup>a</sup>	Concentration (mg/kg)
Carbendazim	40	0.01-0.80*
Carbofuran	2	0.004-0.007**
Dimethomorph	17	0.01-0.05
Fenhexamide	10	0.01-0.13
Imidacloprid	8	0.02-0.03
Methoxyfenozide	6	0.01-0.13
Thiophanate-methyl	23	0.02-1.29

a) Number of samples with pesticide residues at concentrations higher than their respective LOQs. \*Some samples exceed the MRL; \*\*Samples exceed the MRL twice.

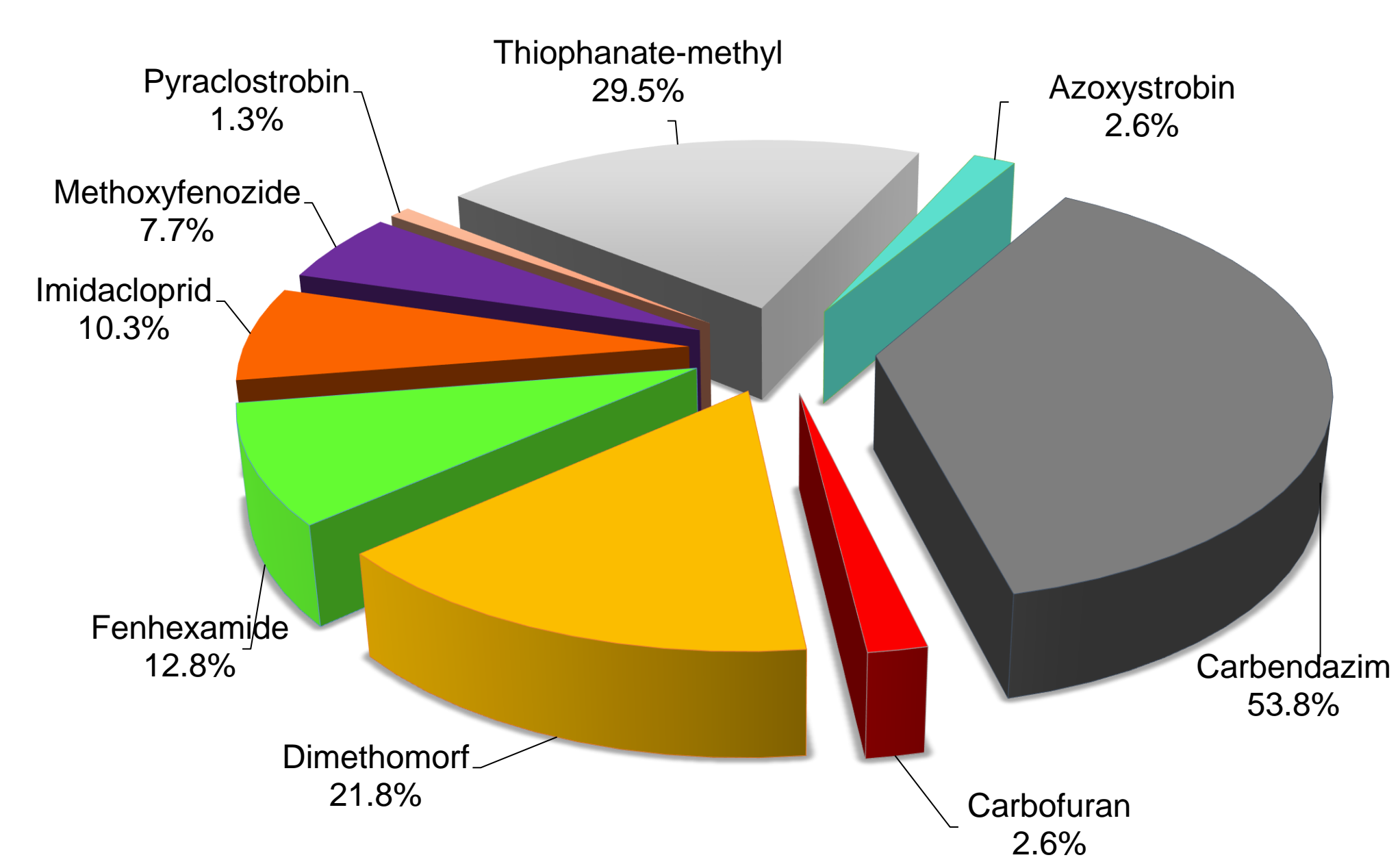


Figure 2.- Percentage of samples (n = 78) with analytes at concentrations higher than the LOQ.

## Conclusions

- In this work, the QuEChERS method combined with UHPLC-(Q-ToF)-MS has been applied for the analysis of 59 pesticide residues in 78 red wine samples from the Canary Islands and other regions of Spain.
- A percentage of 96.2% of the samples (75 samples) showed one or more pesticide residues, while 3.8% (3 samples) did not contain any of them.
- Carbendazim, thiophanate-methyl and dimethomorph were the most frequently found pesticides in the red wine samples analyzed in the present study.
- Four samples with violations of the MRLs, in wine grapes, have been found for not approved pesticides, carbendazim (0.5 mg/L) and carbofuran (0.002 mg/L).

## Acknowledgments

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1. Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. J AOAC Int. 2003;86:412-31.
2. Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. Document N° SANTE/12682/2019.
3. Standard EN 15662:2008. Food of plant origin. Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dSPE. QuEChERS Method.



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