

# Validation of a multiclass, multiresidue method and monitoring results for 210 pesticides in fruits and vegetables by gas chromatography-triple quadrupole mass spectrometry

Samanta Uclés<sup>a</sup>, Noelia Belmonte<sup>a</sup>, Milagros Mezcua<sup>a</sup>, Miguel Gamón<sup>b</sup>, Amadeo R. Fernández-Alba<sup>a</sup>

<sup>a</sup> University of Almería, Agrifood Campus of International Excellence (ceiA3). European Union Reference Laboratory for Pesticide Residues in Fruit & Vegetables, Ctra. Sacramento s/n, 04120 Almería, Spain; email - amadeo@ual.es

<sup>b</sup> Pesticide Residue Laboratory (Agro-Food Analysis Service) of the Generalitat Valenciana, Burjassot, Valencia, Spain

## ABSTRACT

A rapid, sensitive, accurate and reliable multiresidue method for the identification and quantification of 210 relevant pesticides in four representative fruit and vegetable commodities (tomato, potato, spring onion and orange) has been developed and validated by gas chromatography in tandem with triple quadrupole mass spectrometry. The method has been fully validated and applied to 292 samples from different countries.

Prior to instrumental analysis, an extraction procedure based on a sample extraction of multiclass analytes, using the ethyl acetate method was employed. Mass spectrometric conditions were individually optimized for each compound in the Selected Reaction Monitoring (SRM) mode to achieve maximum sensitivity.

The pesticides were separated in less than 25 min. This was followed by an exhaustive control of the retention times. The Retention Time Locking Method was applied, working at a constant pressure throughout the analysis. System maintenance was reduced by using a purged capillary flow device that provided backflush capabilities by reversing column flow immediately after elution of the last compound of interest.

Isotopically-labelled internal standards were employed to improve the quality of the analytical results.

In order to prove the effectiveness of the validated method and its suitability in routine analysis, it was applied to real samples as part of a survey. This work was applied to the analysis of 292 samples from different countries purchased in local markets, where iprodione, azoxystrobin, boscalid, pyriproxyfen and bifenthrin were the most commonly-found pesticides.

## Ethyl Acetate Extraction Method

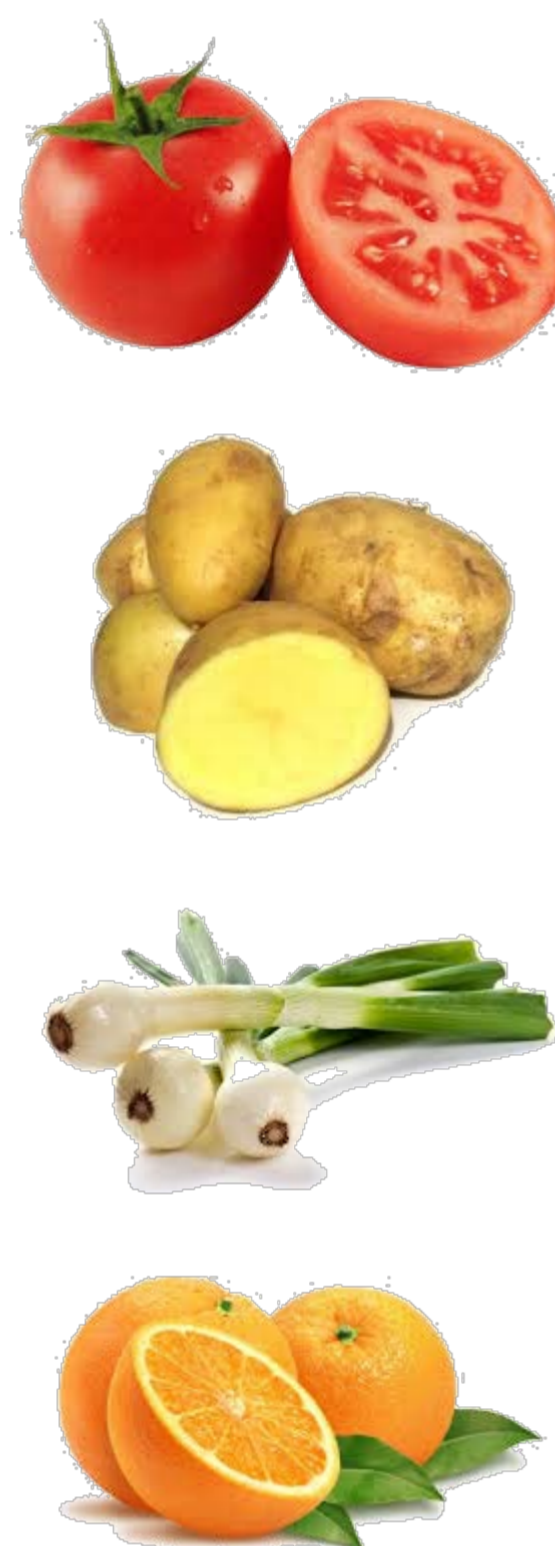
10 g of Sample + 10 ml of EtOAc

1.5 g of NaCl and 8 g of MgSO<sub>4</sub>

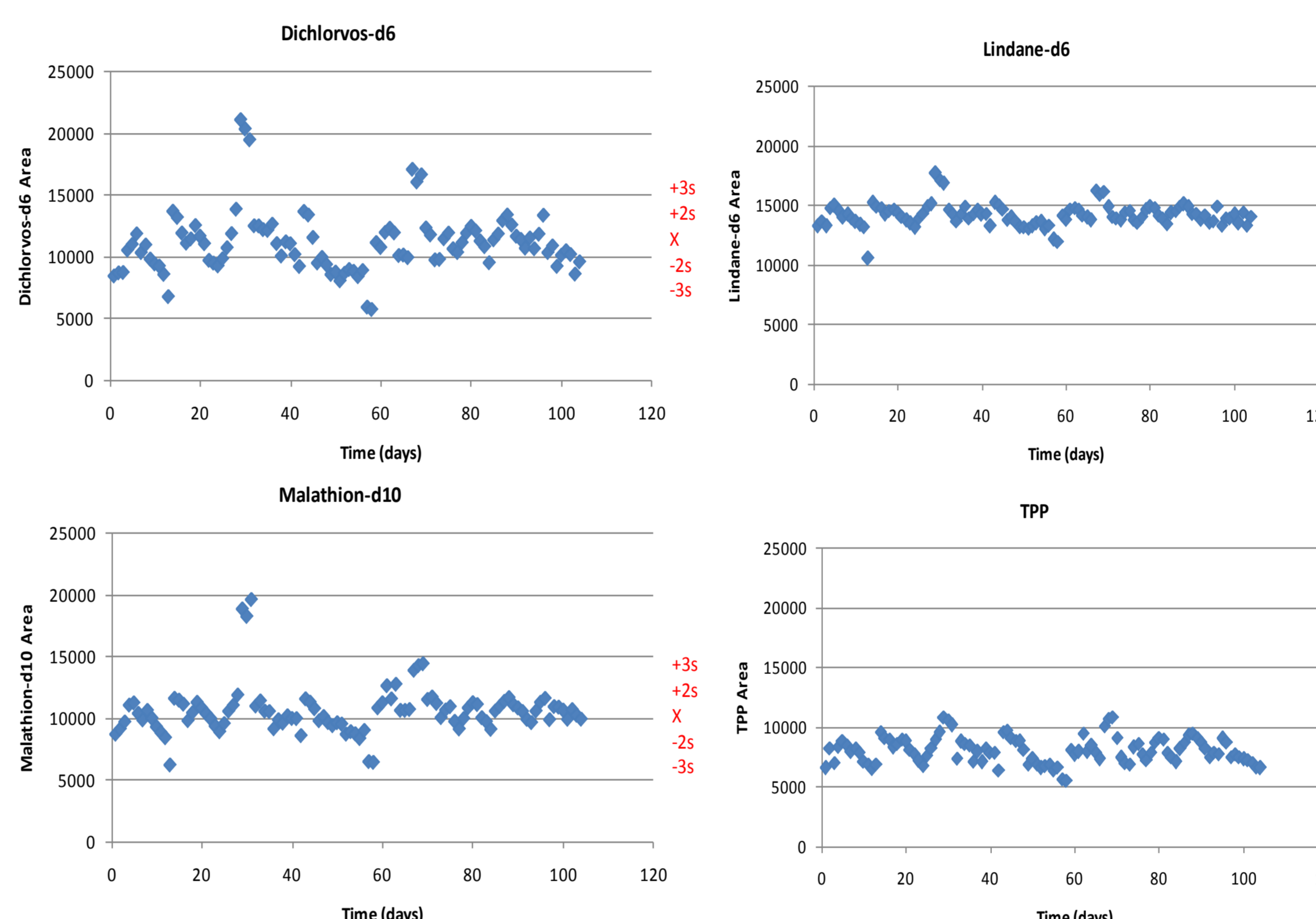
Shake 15 minutes automatically with "Agytax axial agitator"  
Amplitude: 100 mm  
Speed: 2.5 m/s  
Acceleration: 80 m/s<sup>2</sup>  
Abruptness: Level 6

Centrifuge for 5 minutes at 3700 rpm

GC-MS analysis  
1 g/mL



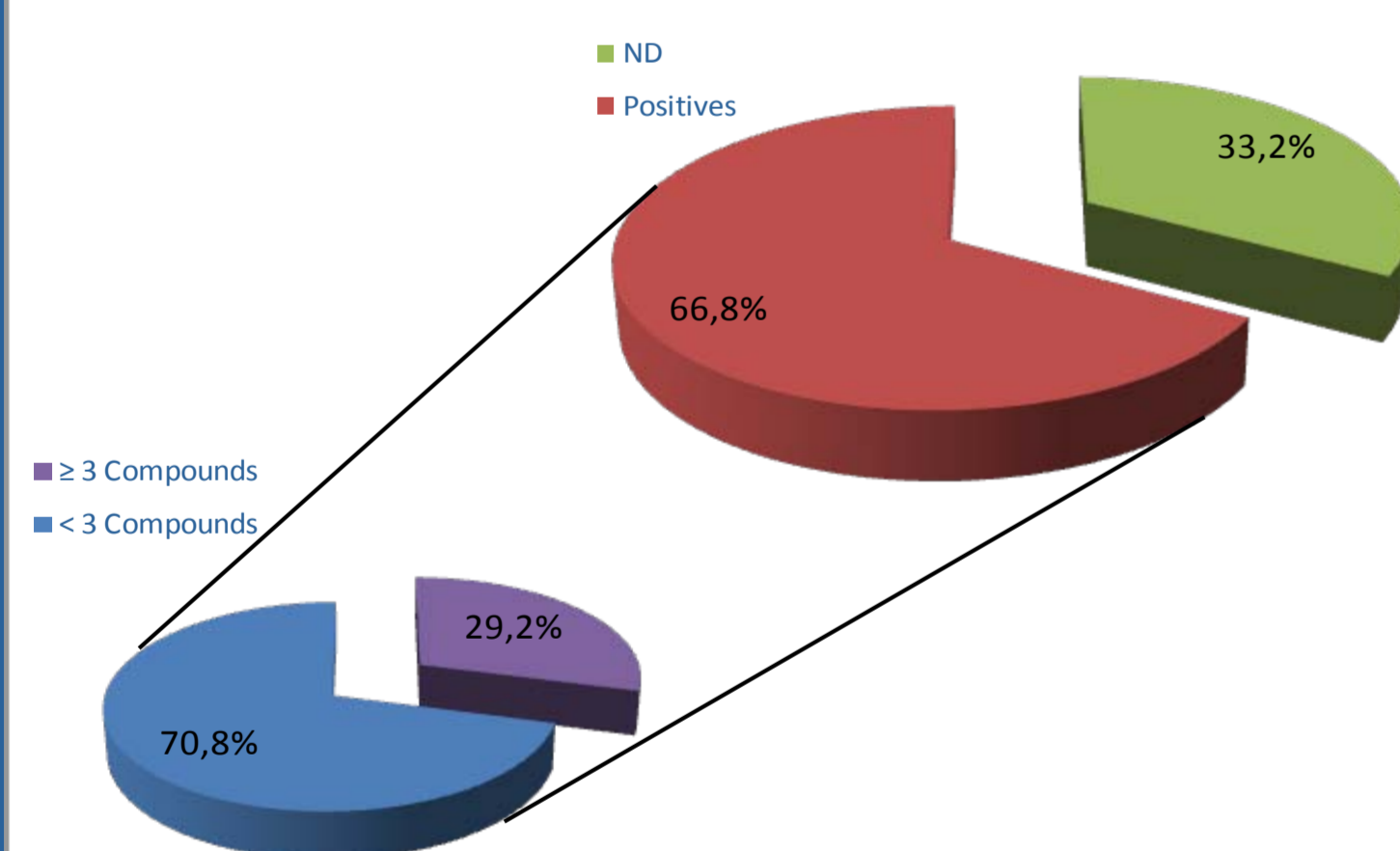
## Quality Control: Shewhart Charts



In order to control the stability of any method and guarantee the quality of the results, Shewhart charts were made, where the medium value (X) was obtained from an initial period of study (30 days). To establish the warning limit (WL) and action limit (AL),  $\pm 2s$  and  $\pm 3s$  (where  $s$  is the standard deviation for the period of study), respectively, were plotted on the chart. The internal standards (dichlorvos-d6, malathion-d10 and TPP) and injection standard (lindane-d6) were used to verify daily the suitability of the equipment. When a trend out of the AL was observed, maintenance operations were carried out.

## Pilot Survey (292 Real Samples)

Out of the 425 total pesticides detected, 46 (10.8%) were not approved in the European Union.

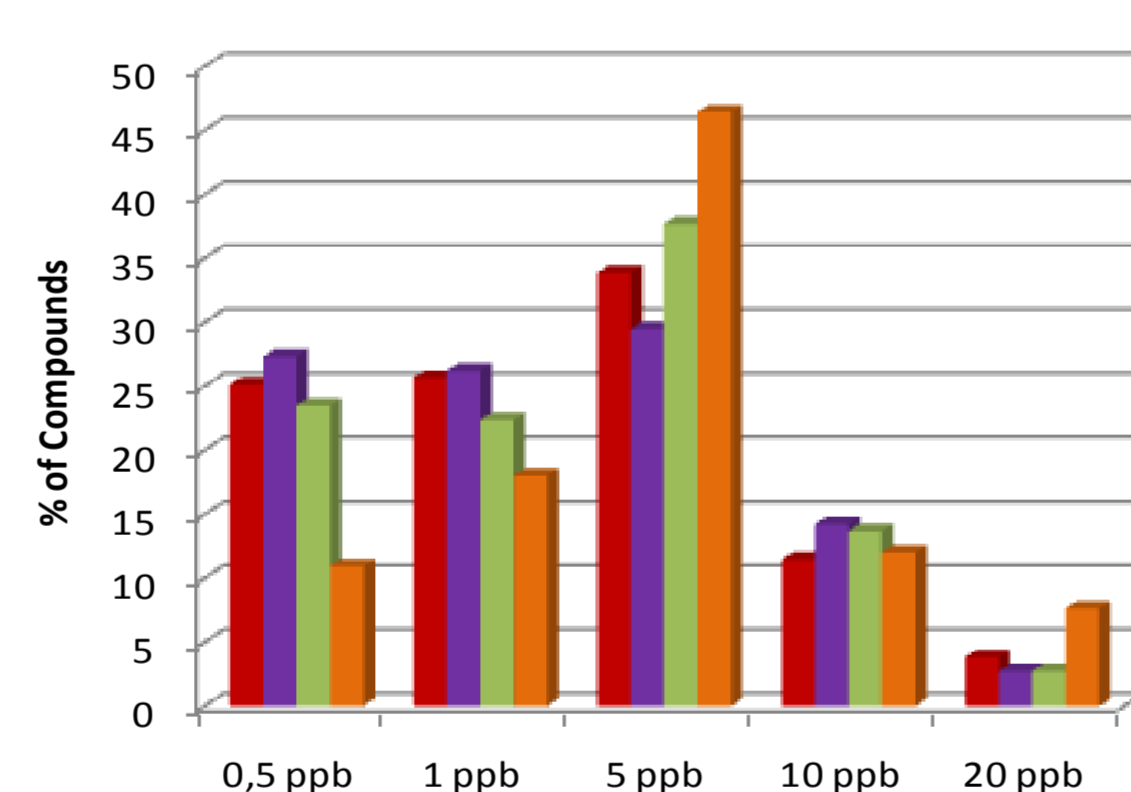


Samples analyzed: apples, aubergines, beans, brussel sprouts, coconuts, corn, dates, grapefruits, grapes, kakis, kiwis, lemons, mandarins, mangos, melons, oranges, papayas, pears, peppers, tomatoes, yuccas and zucchinis.

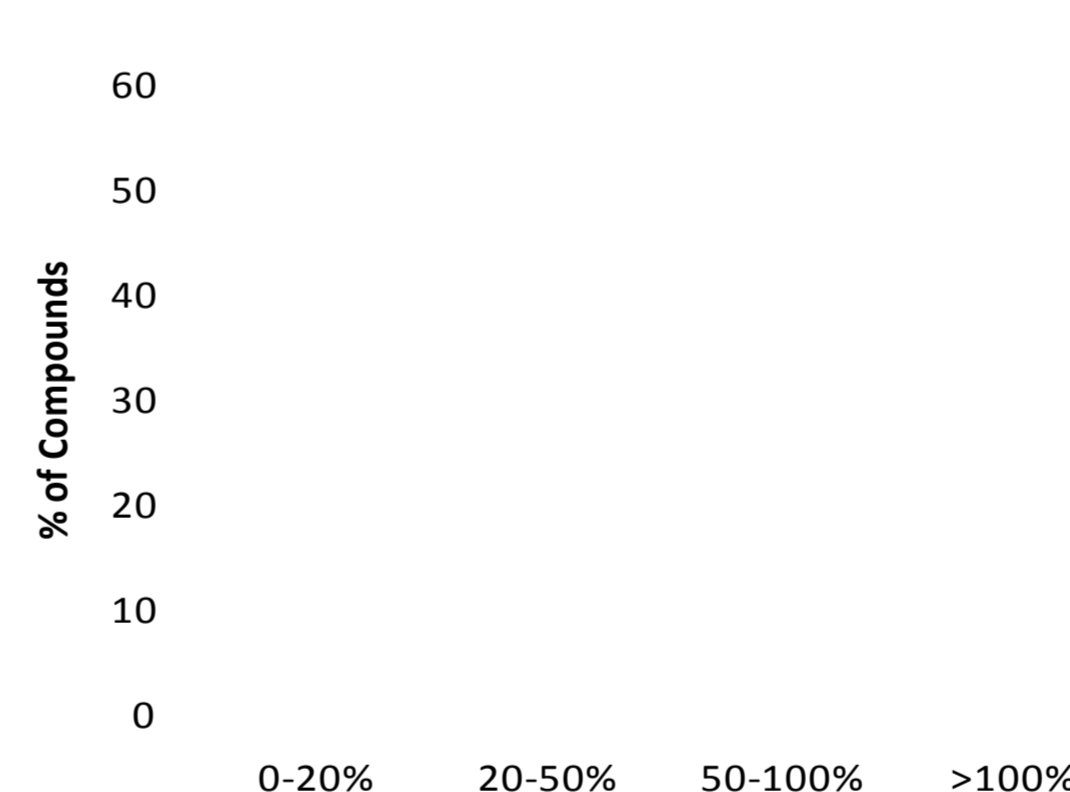
## Most Detected Compounds

Compound	% in Positive Samples	Category
Iprodione <small>Approved in EU</small>	35	Fungicide Nematicide
Azoxystrobin <small>Approved in EU</small>	34	Fungicide
Boscalid <small>Approved in EU</small>	29	Fungicide
Pyriproxyfen <small>Approved in EU</small>	12	Insecticide
Bifenthrin <small>Approved in EU</small>	10	Insecticide Acaricide

## Limits of Detection (LODs)



## Matrix Effects

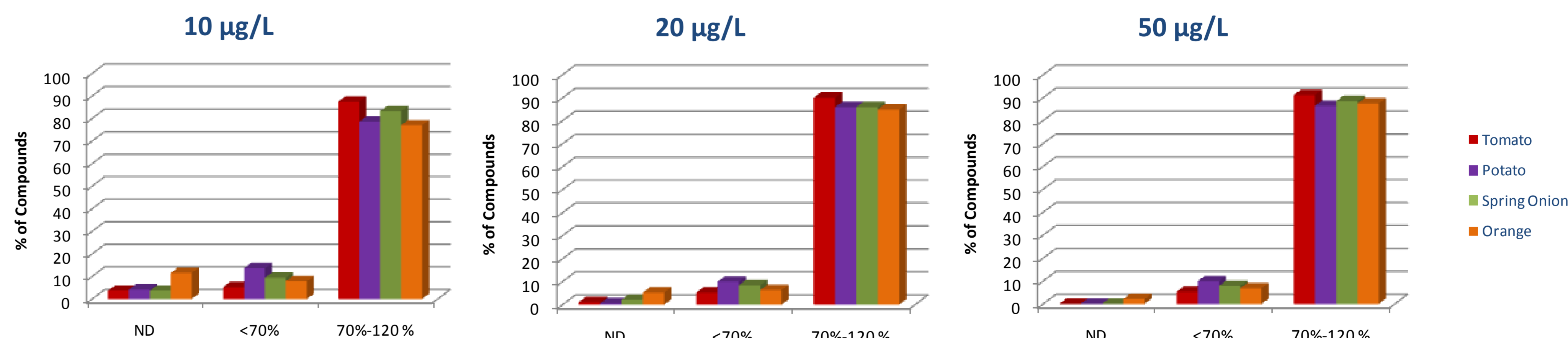


The linearity was studied with a matrix-matched calibration curve in the 0.5-1000 µg/kg range for all pesticides prepared in solvent and the matrix extracts of tomato, potato, spring onion and orange. Good linearity of calibration curves was achieved in the studied range, and the correlation coefficient (R<sup>2</sup>) values were higher than 0.99 for all pesticides.

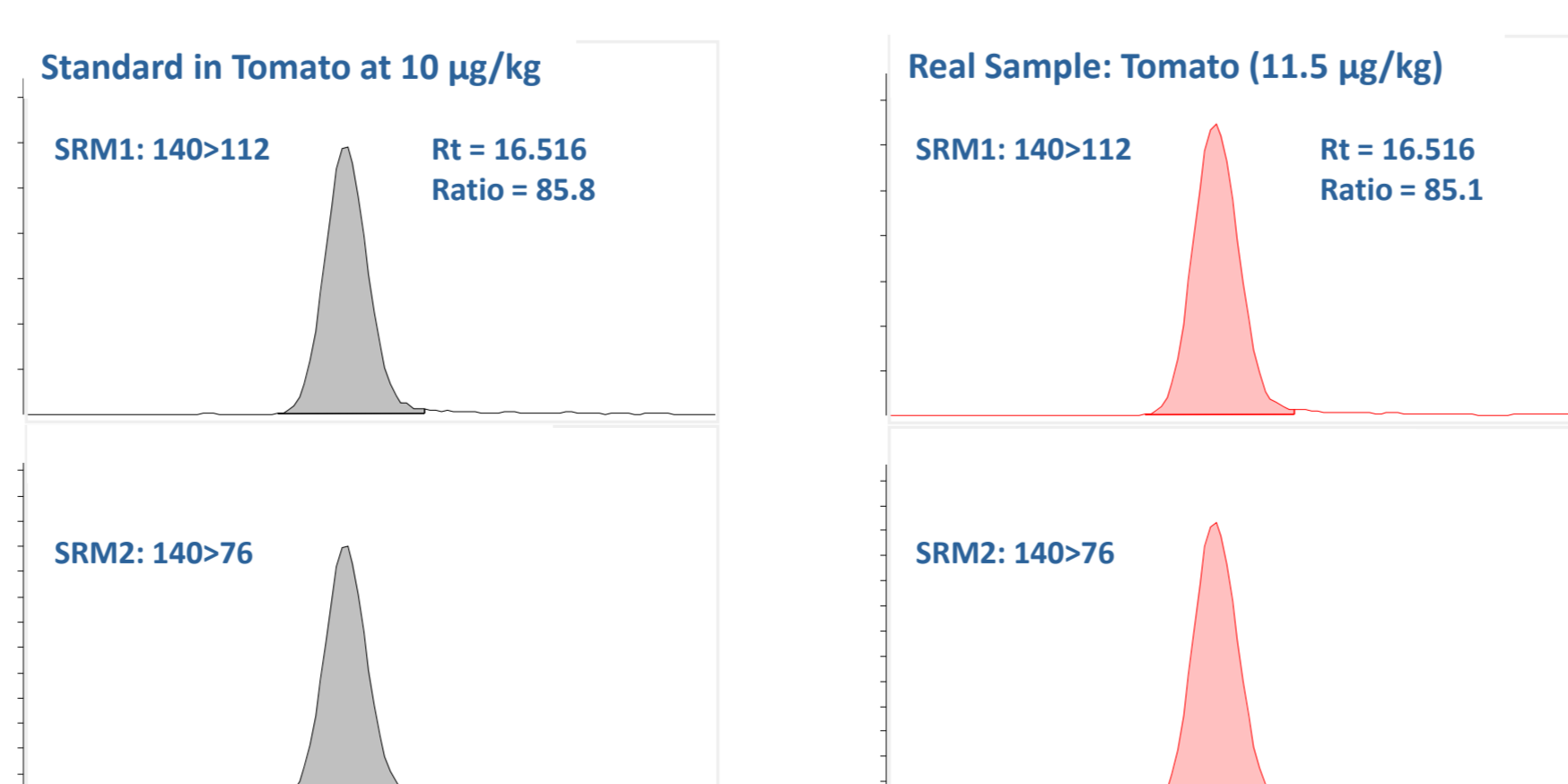
The slope obtained in each matrix was compared to that one in solvent, and matrix effect (%) was calculated.

Recoveries at three different concentration level were evaluated, with a Relative Standard Deviation (RSD, %) lower than 20%.

## Recoveries:



## Positive Result: Boscalid



## Conclusions

- ✓ GC-MS/MS in MRM mode is a powerful tool to identify and quantify a large number of pesticides.
- ✓ Sample extraction was carried out by miniaturized liquid-solid extraction with ethyl acetate. The method was fully validated and applied to 292 real samples.
- ✓ Regarding matrix effect, an enhancement response is produced due to the interaction with matrix components.
- ✓ The method is under control thanks to the use of procedure surrogates and injection surrogate.