

# Identification and Quantification of Pesticides in Fruit and Vegetables with Accurate Mass Database using Gas Chromatography Coupled to High Resolution Orbitrap Mass Spectrometry

Samanta Uclés, Víctor Cutillas, Amadeo R. Fernández-Alba

Agrifood Campus of International Excellence (ceiA3), European Union Reference Laboratory for Pesticide Residues in Fruits and Vegetables. University of Almería (Spain); e-mail: [samantaucles@ual.es](mailto:samantaucles@ual.es)

## ABSTRACT

To protect and prevent damages in fruit and vegetables crops, the addition of some pesticides may be needed, thus pesticide residues can be present in the final product. For this reason, to assess the food safety for consumers in fruit and vegetables is an important issue in laboratories.

Typically food samples are analyzed by using high performance or ultra high performance liquid chromatography (HPLC or UHPLC) and/or gas chromatography (GC) coupled to mass spectrometry (MS) to identify and quantify target compounds. The most common MS systems in routine analysis are based on triple quadrupole (QqQ) analyzers. But, even reaching low detection limits, these techniques have some limitations consequence of their unit mass resolution. That limitation can drive to analytical difficulties consequence of interferences from the matrix. That can represent an incorrect identification and quantification. This limitation can be overcome by applying high resolution accurate mass spectrometers (HRAMS) instruments such as Orbitrap-MS.

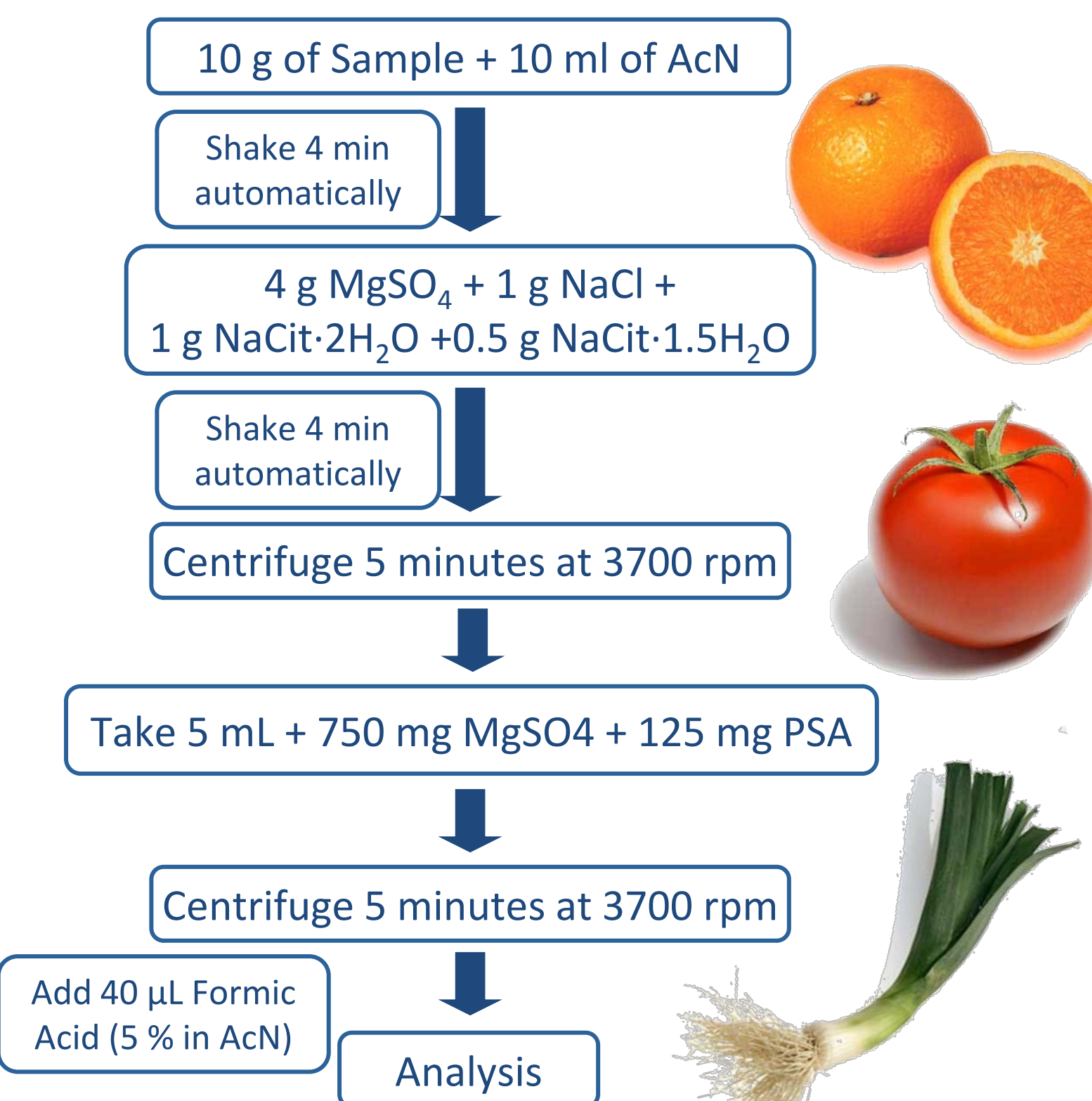
The present work is focused in the evaluation of GC-Orbitrap-MS, which has a high resolving power (working routinely at 60000 resolution FWHM at m/z 200, but able to achieve up to 120000) with mass accuracies lower than 5 ppm, according to European Union analytical quality control (EU AQC) procedures, but they were usually < 1 ppm. This evaluation was done by analyzing different representative commodities of fruits and vegetables spiked with 50 different pesticides.

The GC-Orbitrap-MS obtained very low limits of identification, below 5 µg/kg in all cases, good linearity (residuals < 20 % for every calibration level and R<sup>2</sup> > 0.99) and extremely good mass accuracy (< 1 ppm for around 90 %) for almost all combinations compound/matrix. Repeatability also met criteria described in EU AQC procedures (RSD < 20 %).

## Extraction Method: Citrate QuEChERS

## Experimental

## GC-MS Conditions



**System:**  
TRACE 1300 Series GC coupled to a QExactive GC Orbitrap MS (Thermo)

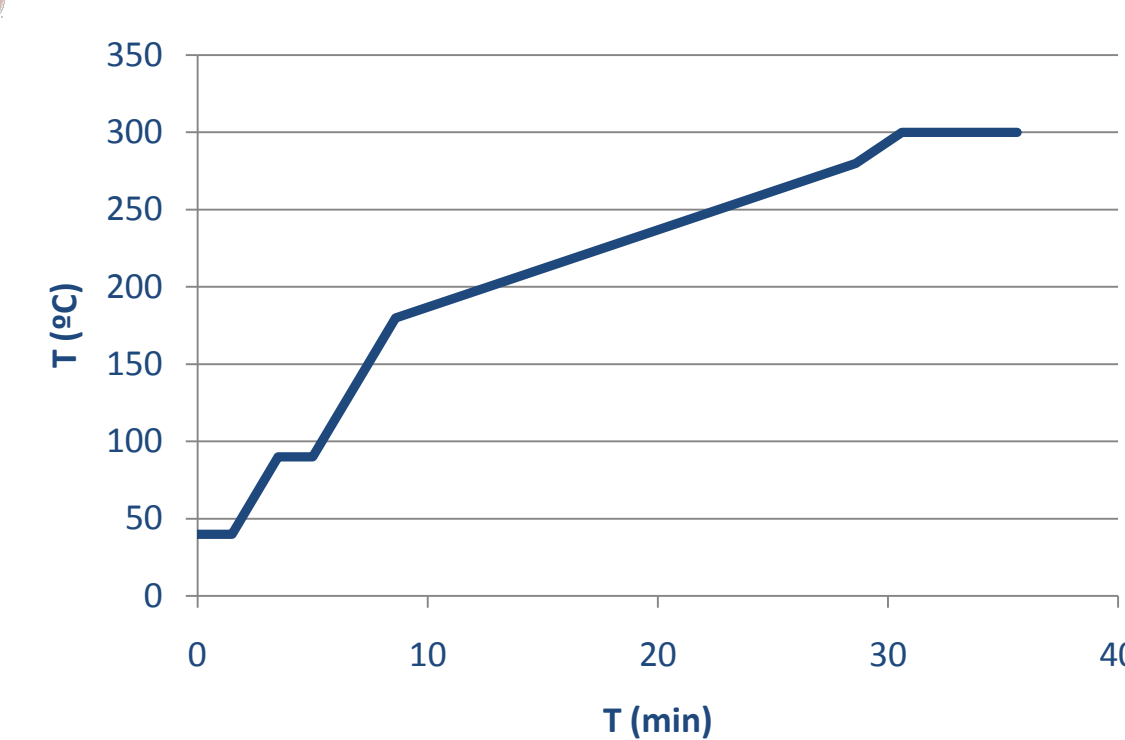
**GC Parameters:**

- Injection volume: 1 µL
- Splitless mode
- Column: TG-5SILMS with 5 m Safeward (30 m x 0.25 mm x 0.25 µm)
- Carrier gas: Helium
- Constant flow: 1.2 mL/min
- Total run time: 35.6 min
- Oven temperature program:



**MS Parameters:**

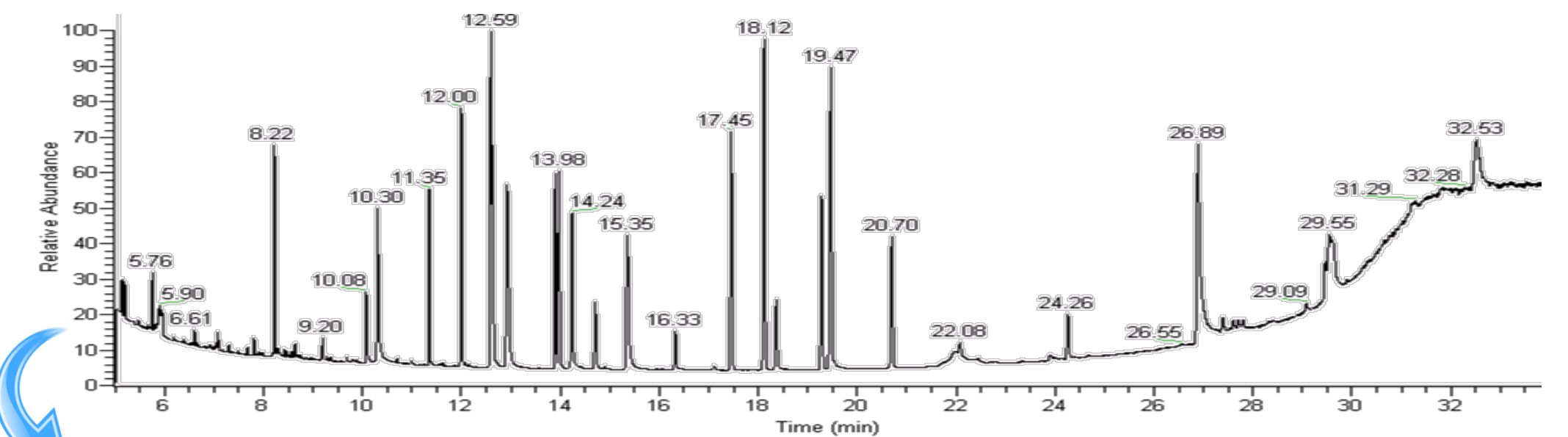
- Full Scan
- Positive mode
- Electron Impact
- Resolution: 60000 FWHM
- AGC: 1·10<sup>6</sup>
- Scan Range: 50-550 m/z



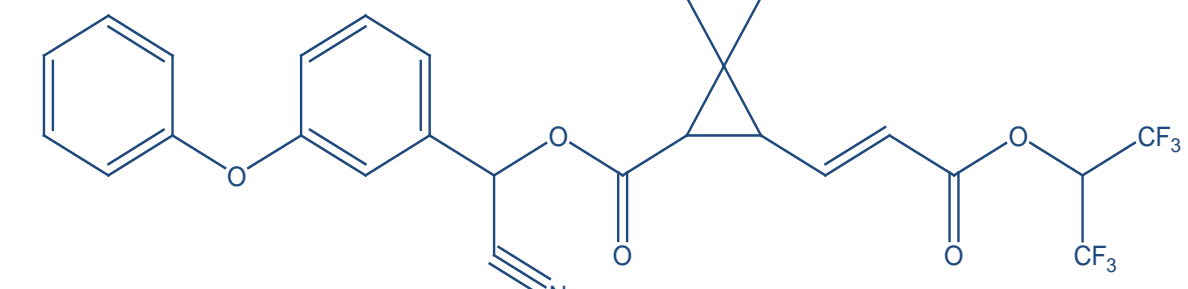
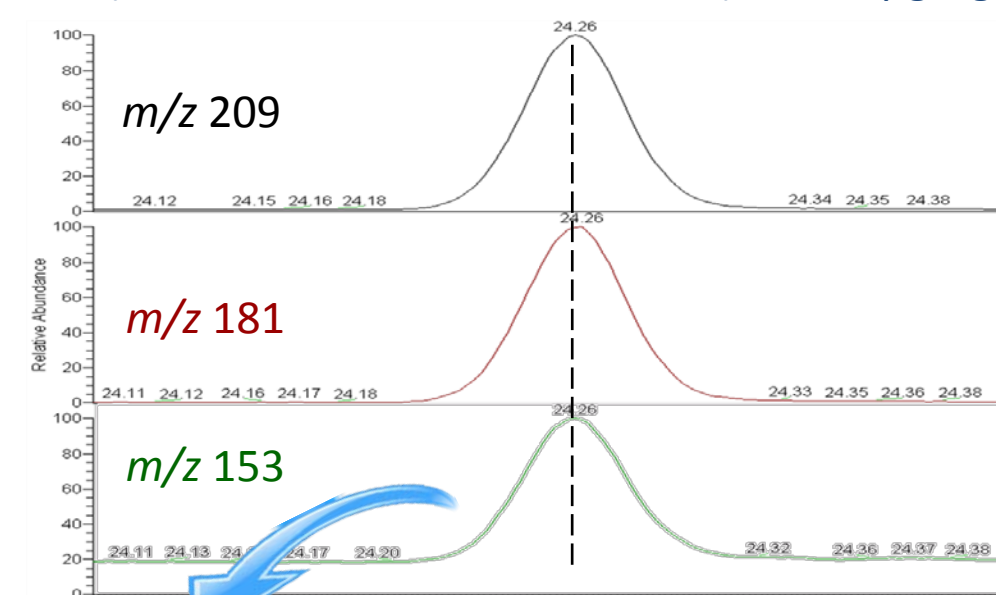
## Pesticide Database

The pesticide database employed for this study has been generated by two approaches: 1) obtaining the ions from high resolution NIST library, when it is present, or 2) by injecting standard solutions in pure solvent at a high concentration level (500 µg/kg), observing their generated experimental ions and calculating their theoretical mass ions with the help of the molecular formula.

TIC for Standard Mix in Solvent at 500 µg/kg

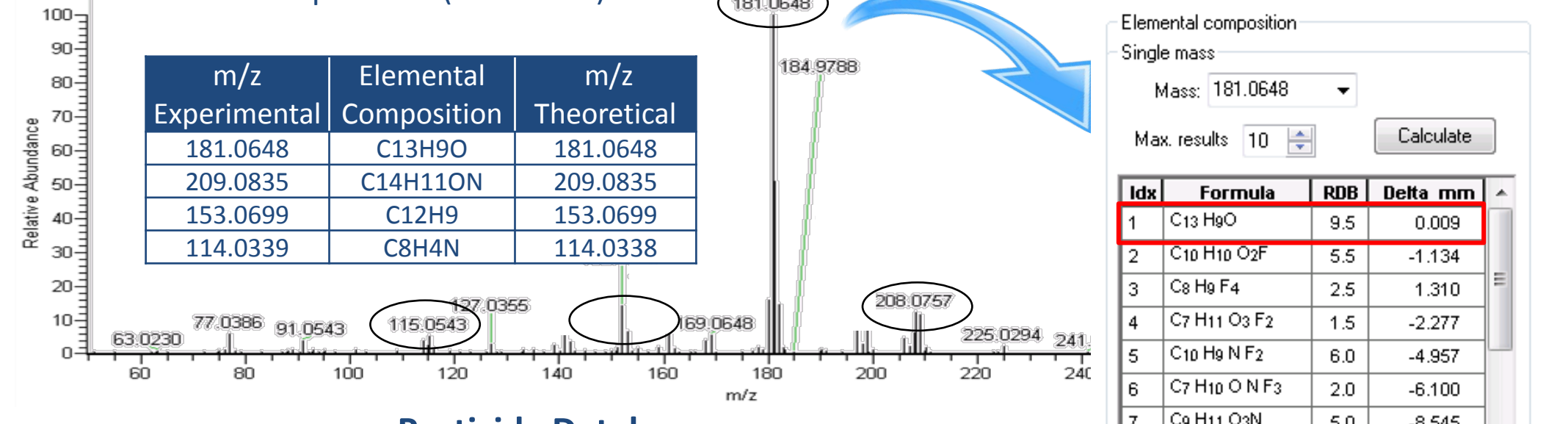


Extracted Ion Chromatogram for Acrinathrin (known ions with nominal mass) at 500 µg/kg



With Elemental Composition tool, present in Xcalibur Qual Browser software, introduce the exact mass observed in the spectrum and the maximum number of atoms for the molecule for the elemental composition calculation. Then we can estimate the theoretical ion to be included in the database

Subtracted spectrum (24.26 min)

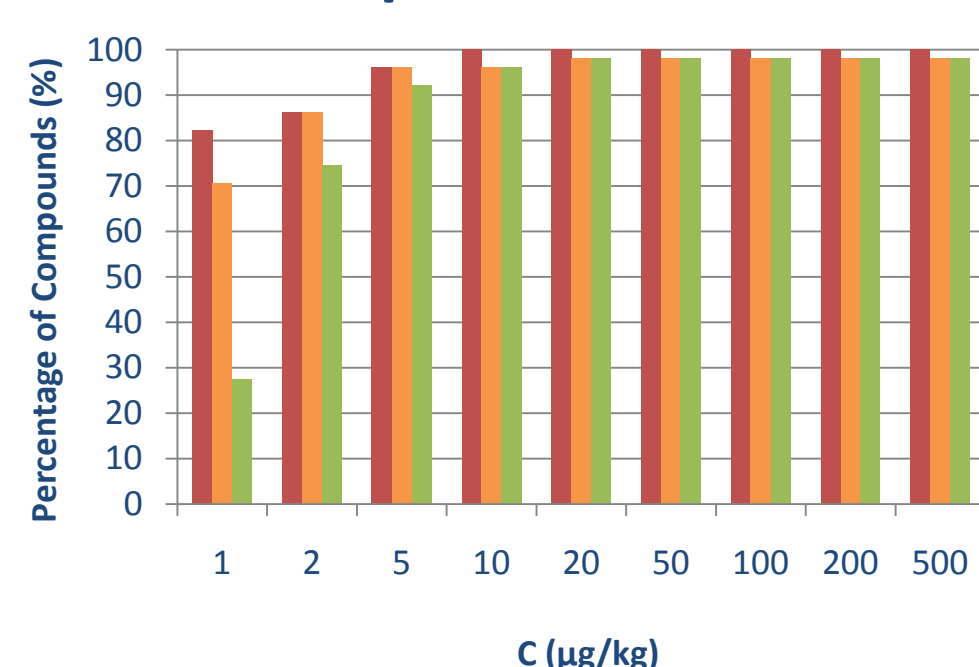


#	Compound Name	Peak Label	Peak Workflow	Associated Target Peak	Retention Time (min)
86	Diazinon	T1: 179.11789	TargetPeak	T1: 179.11789	12.76
87	Diazinon	T1C1: 304.1005	Confirming	T1: 179.11789	12.76
88	Diazinon	T1C2: 137.07094	Confirming	T1: 179.11789	12.76
89	Diazinon	T1C3: 199.05309	Confirming	T1: 179.11789	12.76
90	Dichlorvos	T1: 184.9765	TargetPeak	T1: 184.9765	8.25
91	Dichlorvos	T1C1: 109.05008	Confirming	T1: 184.9765	8.25
92	Dichlorvos	T1C2: 219.94535	Confirming	T1: 184.9765	8.25
93	Dichlorvos	T1C3: 127.01554	Confirming	T1: 184.9765	8.25
94	Dieldrin	T1: 262.85642	TargetPeak	T1: 262.85642	18.32
95	Dieldrin	T1C1: 276.8721	Confirming	T1: 262.85642	18.32
96	Dieldrin	T1C2: 344.89834	Confirming	T1: 262.85642	18.32
97	Dieldrin	T1C3: 377.87008	Confirming	T1: 262.85642	18.32
98	Dieldrin	T1C4: 79.05425	Confirming	T1: 262.85642	18.32

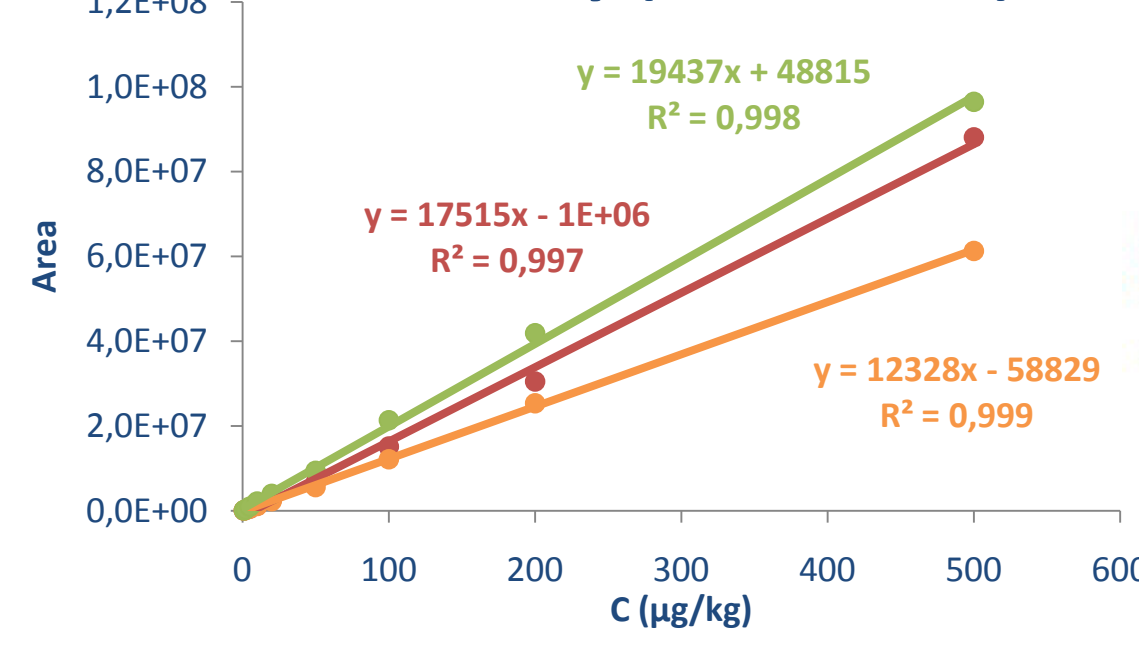
#	Rt (min)	Compound	#	Rt (min)	Compound
1	24.70	Acrinathrin	27	14.90	Fenpropiadin
2	30.69	Azoxystrobin (NIST)	28	15.36	Fenpropimorph (NIST)
3	22.43	Bifenthrin (NIST)	29	16.34	Fipronil (NIST)
4	9.26	Biphenyl	30	12.03	HCB
5	22.55	Bromopropylate (NIST)	31	11.92	HCH, alpha- (NIST)
6	18.39	Bupirimate (NIST)	32	12.46	HCH, beta- (NIST)
7	19.31	Chlorobenzilate (NIST)	33	12.62	HCH, gamma- (lindane) (NIST)
8	12.99	Chlorothalonil (NIST)	34	22.12	Iprodione
9	11.40	Chlorpropham (NIST)	35	18.42	Kresoxim-methyl (NIST)
10	15.16	Chlorpyrifos (NIST)	36	14.27	Metalaxyl (NIST)
11	13.93	Chlorpyrifos-methyl (NIST)	37	18.33	Myclobutanil (NIST)
12	24.30	Cyhalothrin, lambda- (NIST)	38	19.47	Oxadixyl (NIST)
13	27.64	Cypermethrin (NIST)	39	14.09	Parathion-methyl (NIST)
14	19.51	DDD pp'- (NIST)	40	16.09	Pendimethalin (NIST)
15	18.16	DDE pp'- (NIST)	41	10.40	Phenylphenol, 2- (NIST)
16	19.58	DDT op'- (NIST)	42	13.33	Pirimicarb (NIST)
17	20.75	DDT pp'- (NIST)	43	16.69	Procyimidone (NIST)
18	12.73	Diazinon (NIST)	44	12.41	Propazine (NIST)
19	8.23	Dichlorvos (NIST)	45	12.93	Pyrimethanil (NIST)
20	18.33	Dieldrin (NIST)	46	12.64	Terbutylazine (NIST)
21	17.50	Endosulfan, alpha- (NIST)	47	22.35	Tetramethrin (NIST)
22	19.32	Endosulfan, beta- (NIST)	48	14.11	Tolclofos-methyl (NIST)
23	20.59	Endosulfan, sulfate- (NIST)	49	21.35	TPP (NIST)
24	29.50	Esfenvalerate (SS, RR) (NIST)	50	11.37	Trifluralin (NIST)
25	28.03	Etofenprox (NIST)	51	14.01	Vinclozolin (NIST)
26	14.73	Fenitrothion (NIST)			

## Results

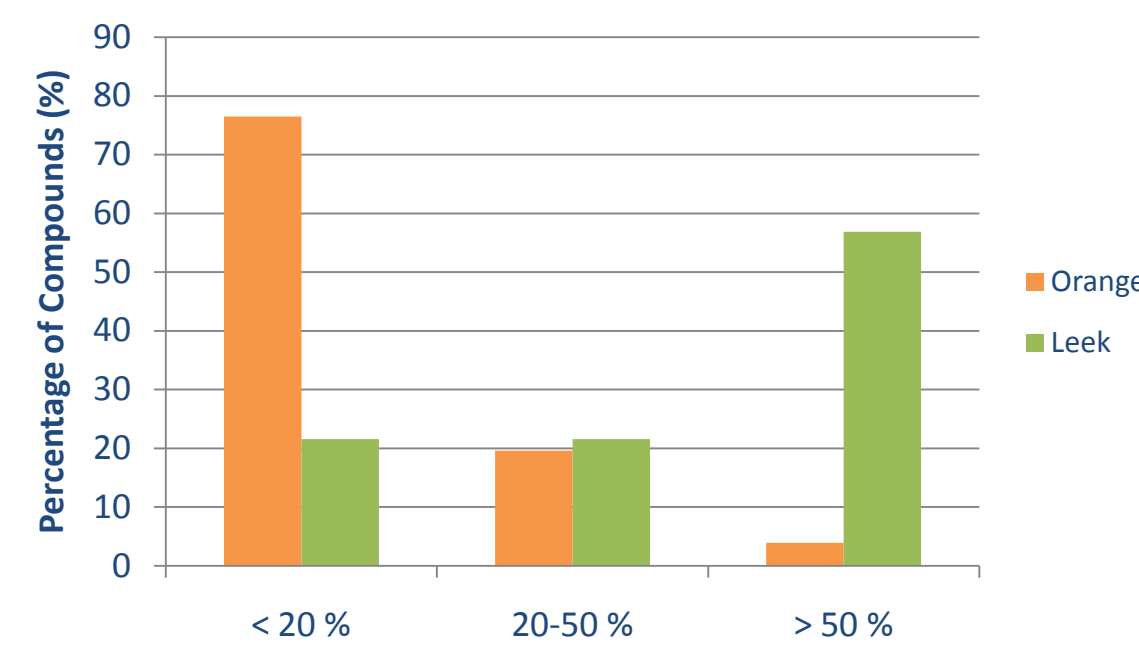
### Compounds Identified



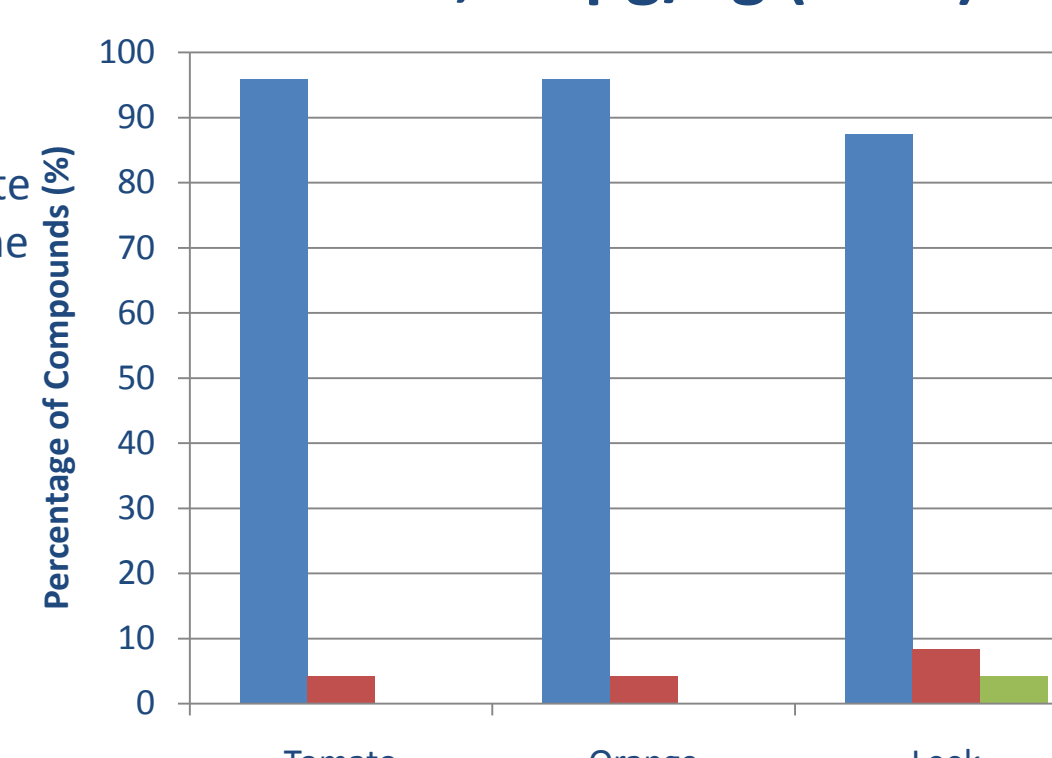
### Linearity (Vinclozolin)



### Matrix Effect



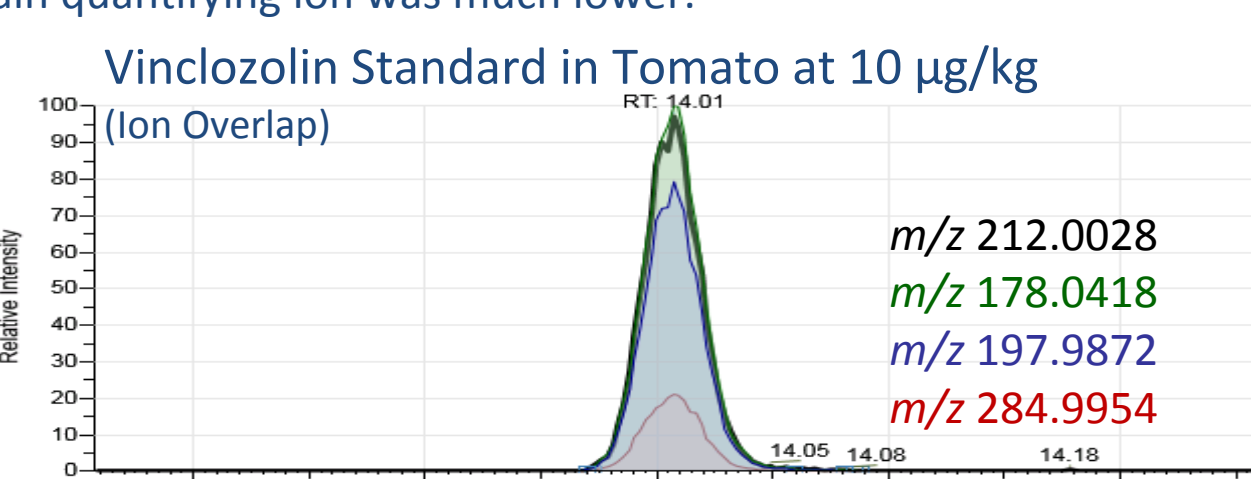
### RSDs, 10 µg/kg (n = 5)



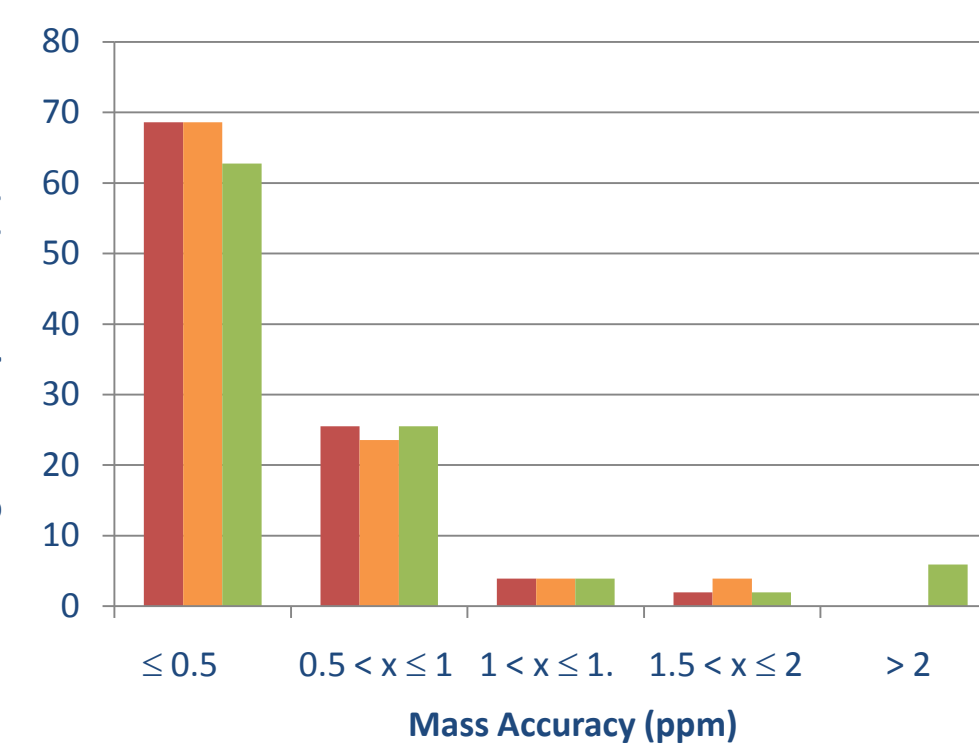
Results provided good linearity with residuals < 20 % for each calibration level and R<sup>2</sup> values > 0.99. This system does not saturate for concentrations/conditions tested.

Comparing obtained slopes to tomato matrix, orange presented around 75 % of pesticides with no matrix effect (ME < |20| %). However, leek showed the highest percentage of compounds in the strong matrix effect range (ME > |50| %).

Repeatability was also tested by injecting 5 replicates at 10 µg/kg concentration level and expressed as RSD (%). As average, the result was around 90 % of compounds with RSDs < 5 %.

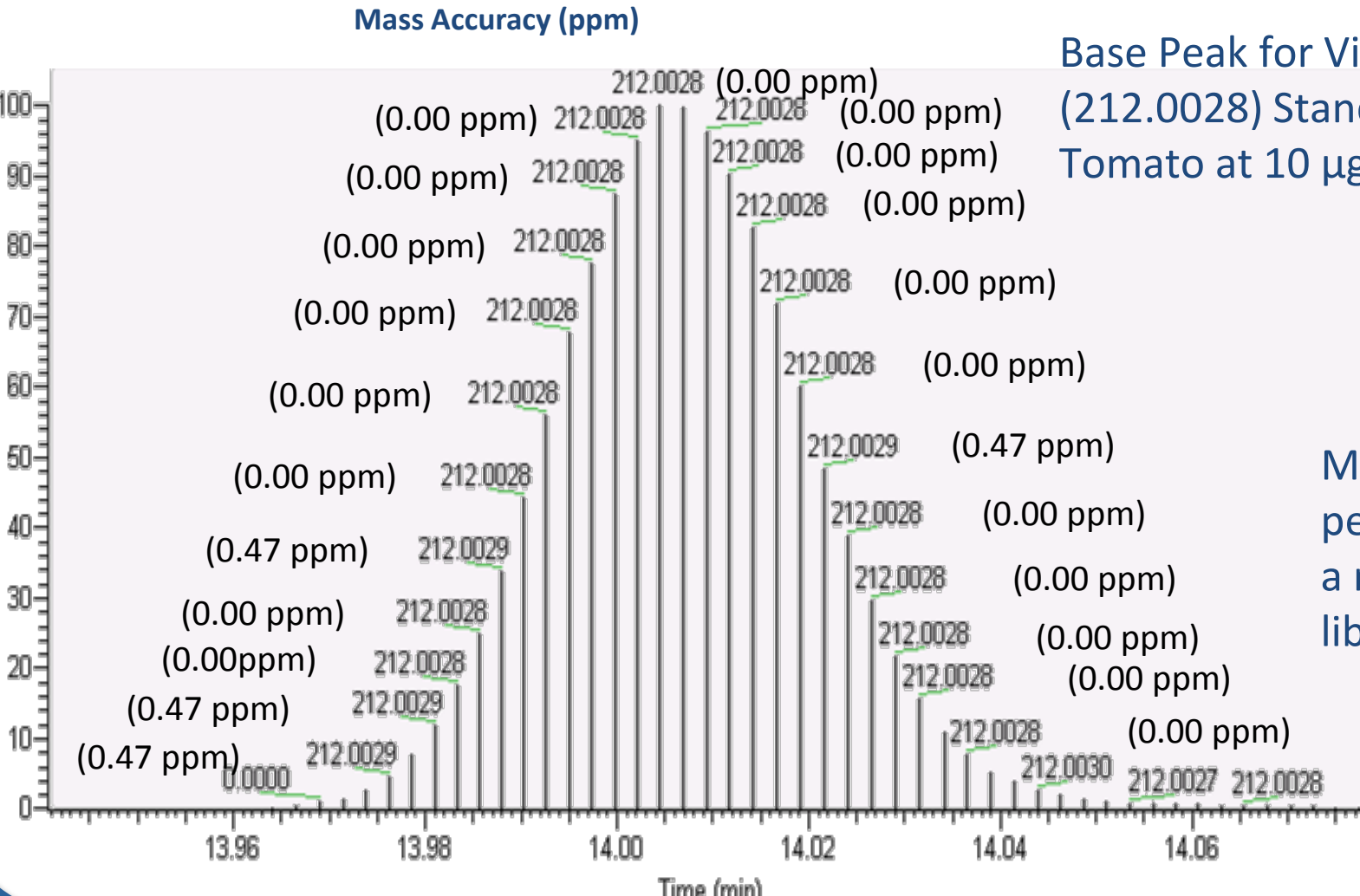


### Mass Accuracy at 10 µg/kg

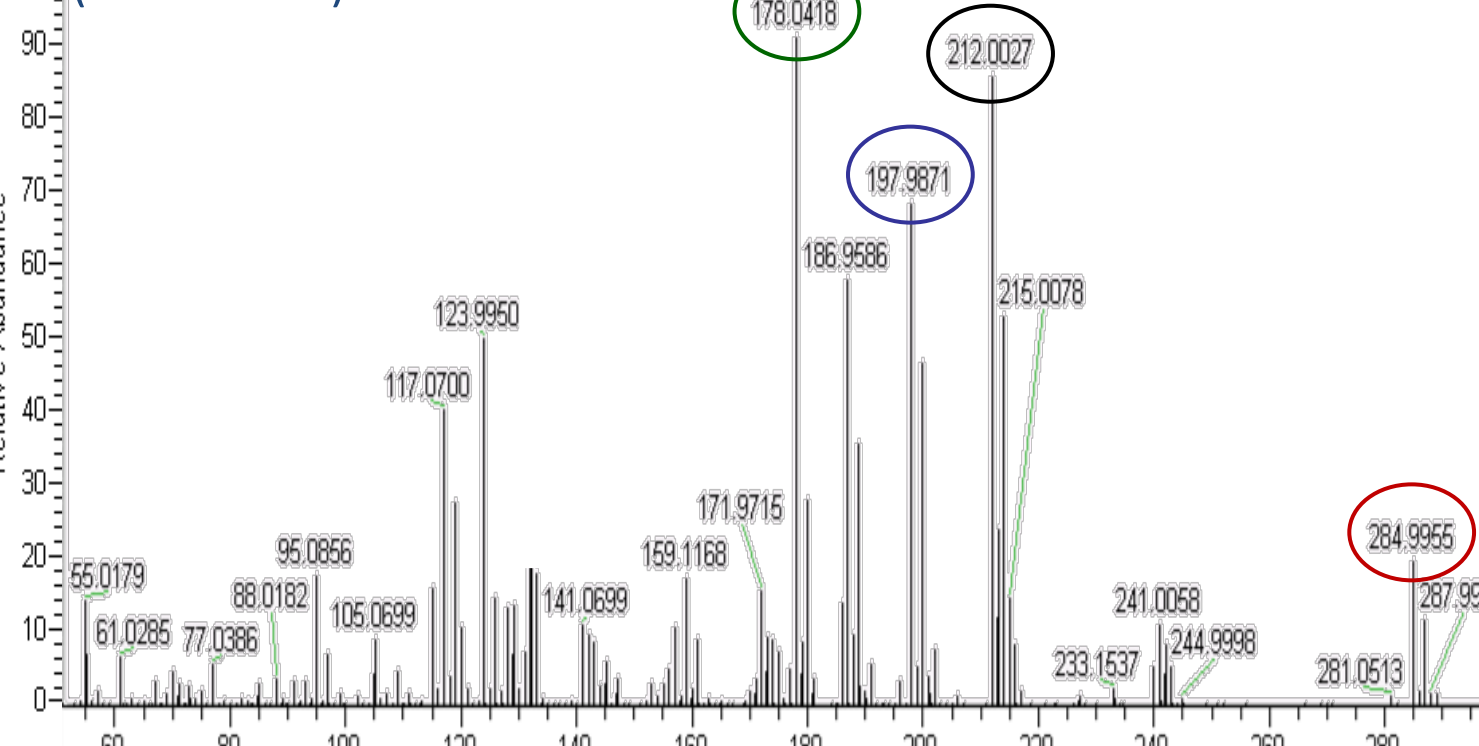


Mass accuracy for all pesticides/matrices was also evaluated. As can be seen for 90-95 % of the pesticides mass accuracies were ≤ 1 ppm. The software employed for data treatment was TraceFinder, which provides mass accuracies for each selected ion in the database in the apex of the peak.

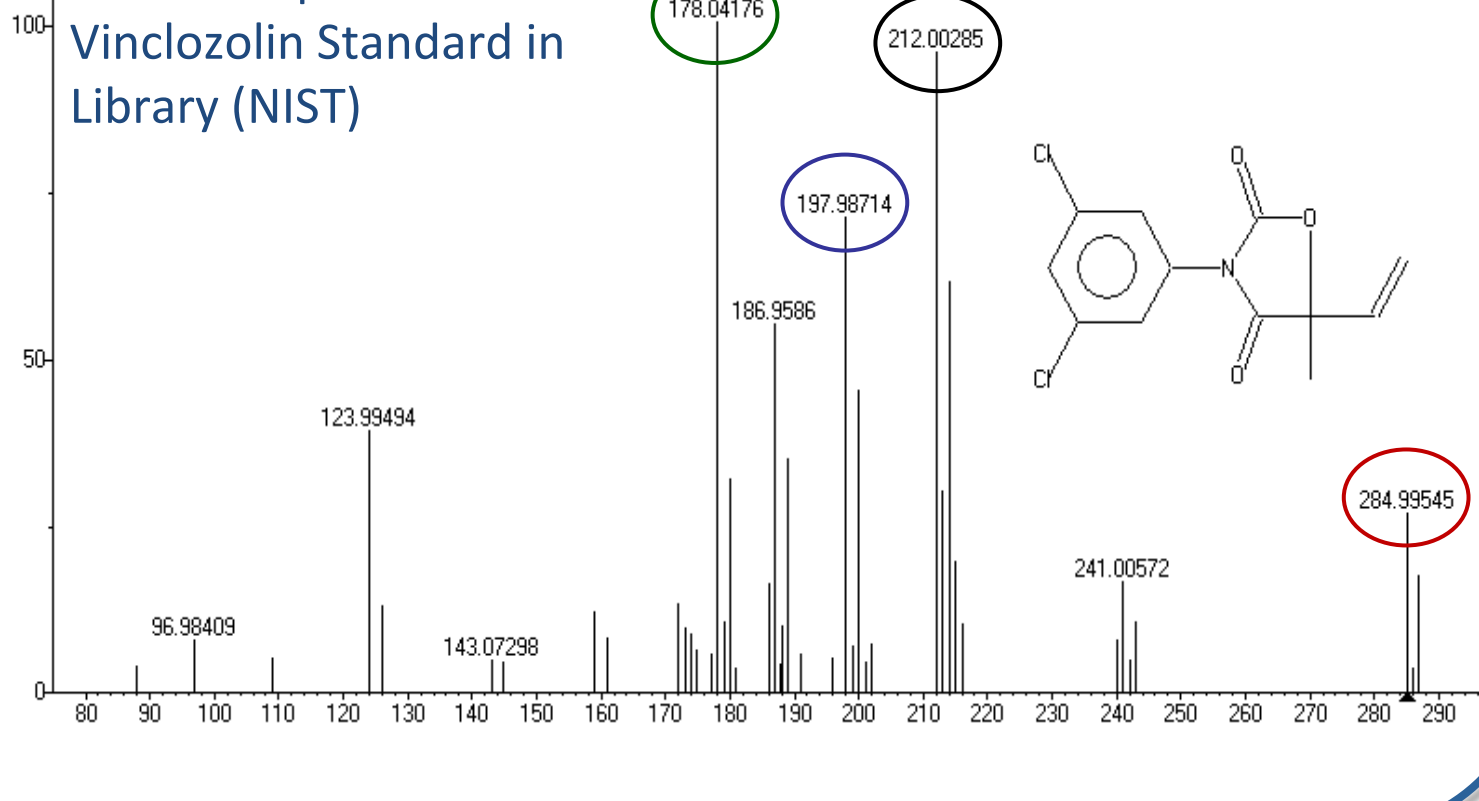
From base peak chromatogram for HCB (point to point) we can observe mass accuracy is rather constant along all the chromatographic peak.



### Full Scan Spectrum for Vinclozolin Standard in Tomato at 10 µg/kg (14.005 min)



### Full Scan Spectrum for Vinclozolin Standard in Library (NIST)



## Conclusions

- ✓ This first evaluation shows the identification power for pesticide residues in fruit and vegetables of this HRAMS platform, being able to evaluate all target selected compounds at 10 µg/kg, in agreement with the EU AQC procedures.
- ✓ Due to the wide linear range is possible to inject real samples with high concentration levels for pesticide residues with no saturation phenomenon, and so on to adequately quantify them.
- ✓ Mass accuracies are < 5 ppm (requirement in EU AQC procedures) and keep constant along chromatographic peak.
- ✓ This work is an starting point to increase the number of pesticides included in the database for being applied in a higher number of commodities.