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Accurate mass database for screening of pesticide residues in fruit and vegetables by GC-EI-HRMS

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ABSTRACT

The main objective of the presented work was to evaluate the capabilities of the GC-TOF-MS provided with electron ionization source for screening methods in fruit and vegetables by using an accurate homemade mass database. The compounds selected are focused especially on those pesticides no frequently detected and so, typically out of the main pesticide monitoring lists. Furthermore on compounds with very low ionization yield with electrospray sources, consequently being low LC-MS amenable.
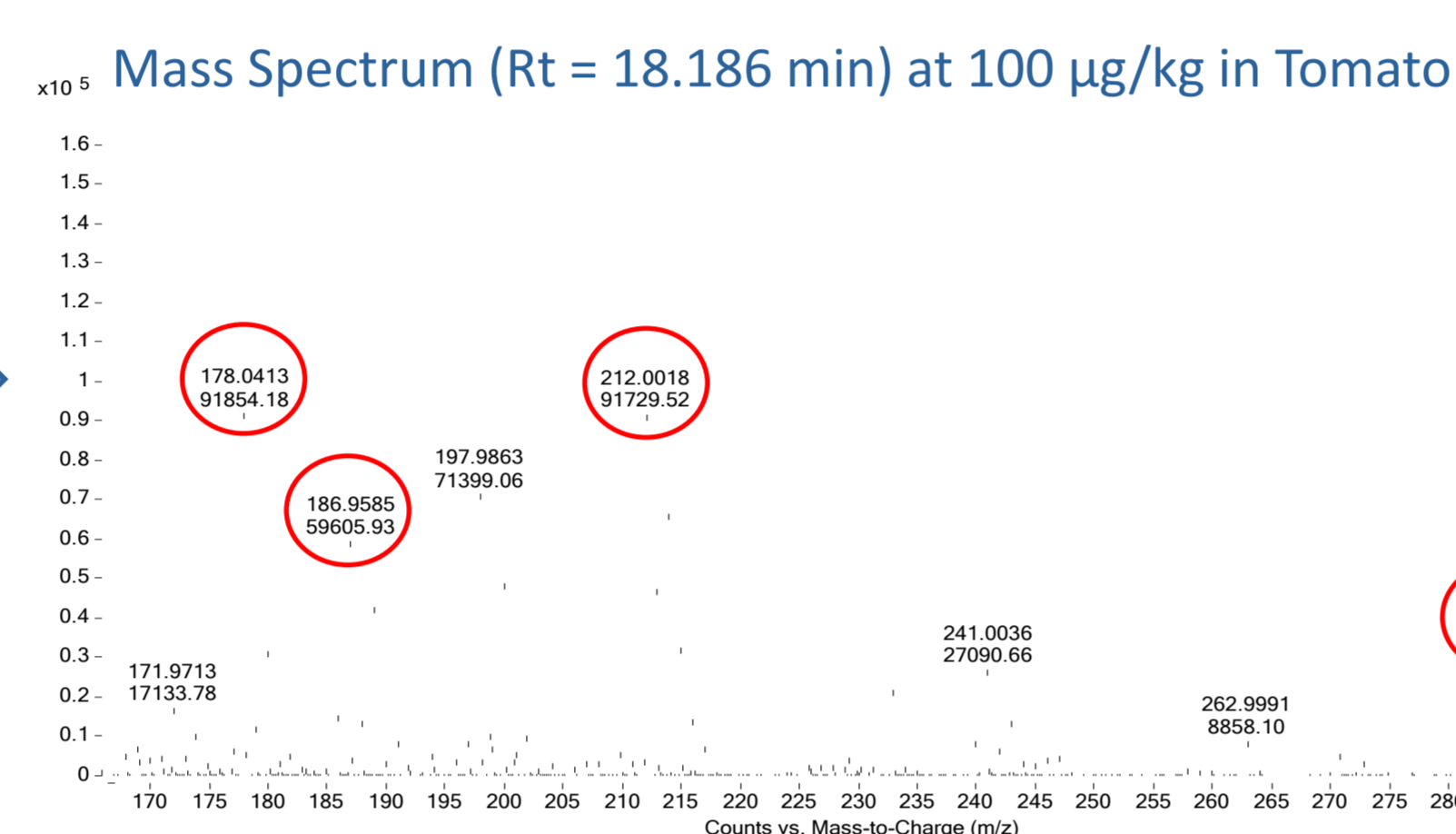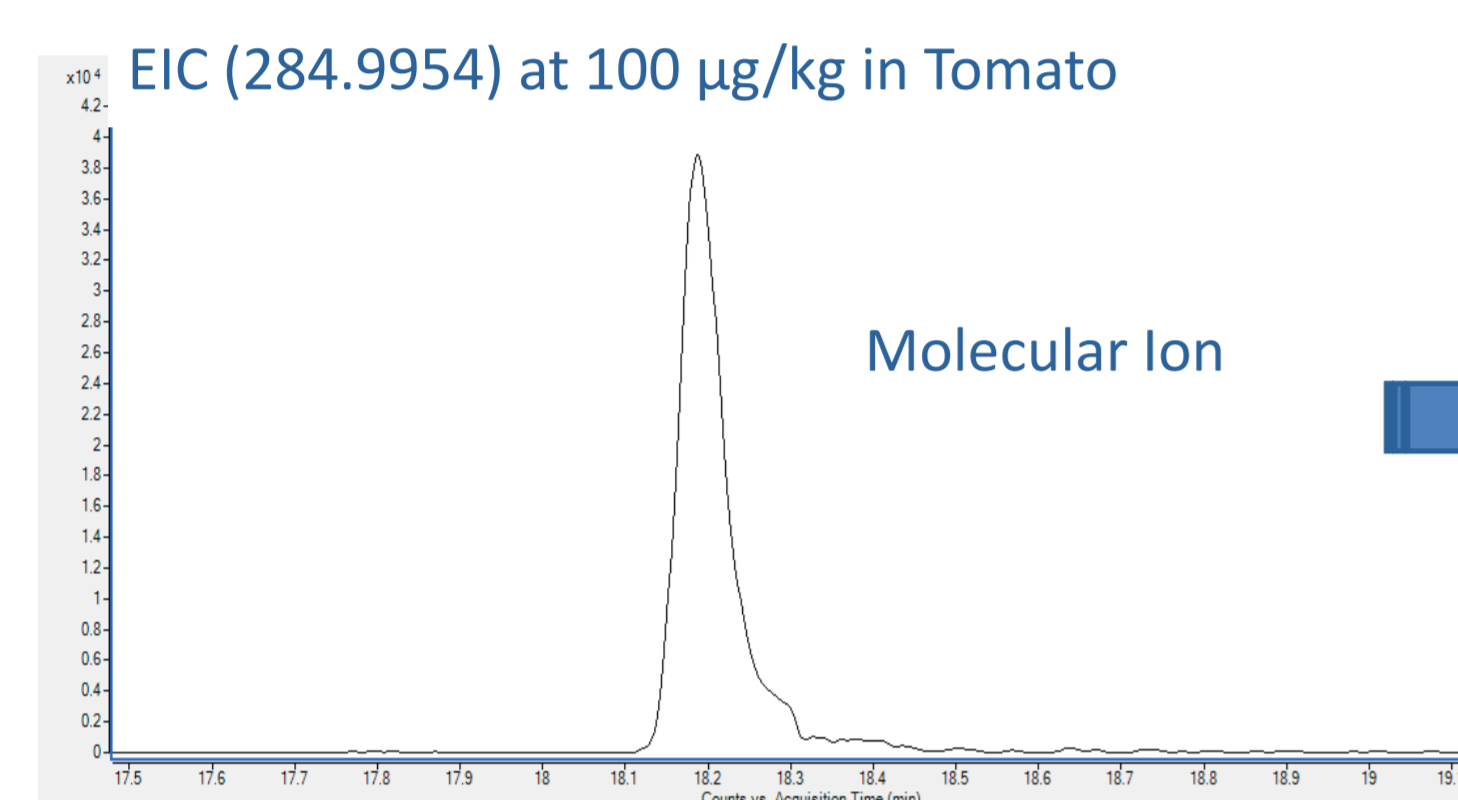
Analytical performance was tested in four different matrices: potato, tomato, spring onion and orange. The extraction technique applied to obtain the extract from the raw vegetables was miniaturized ethyl acetate with no clean-up.

High Resolution (HR) mode was tested to establish the concentration range within an automatic identification was possible. For this automatic identification, different searching parameters concerning the retention time window and the mass error window were tested.

Additionally, the linear range was studied at the two resolution modes, even though this was not the main objective of the screening method. The matrix effects on identification and quantification was also studied between the four selected matrices.

The developed method was applied to real samples, comparing qualitative and quantitative results to those obtained by GC-QqQ-MS/MS. In light of the results, false positives were carefully investigated.

Building the Database: an example with Vinclozolin

EIC (284.9954) at 100 µg/kg in Tomato

Molecular Ion

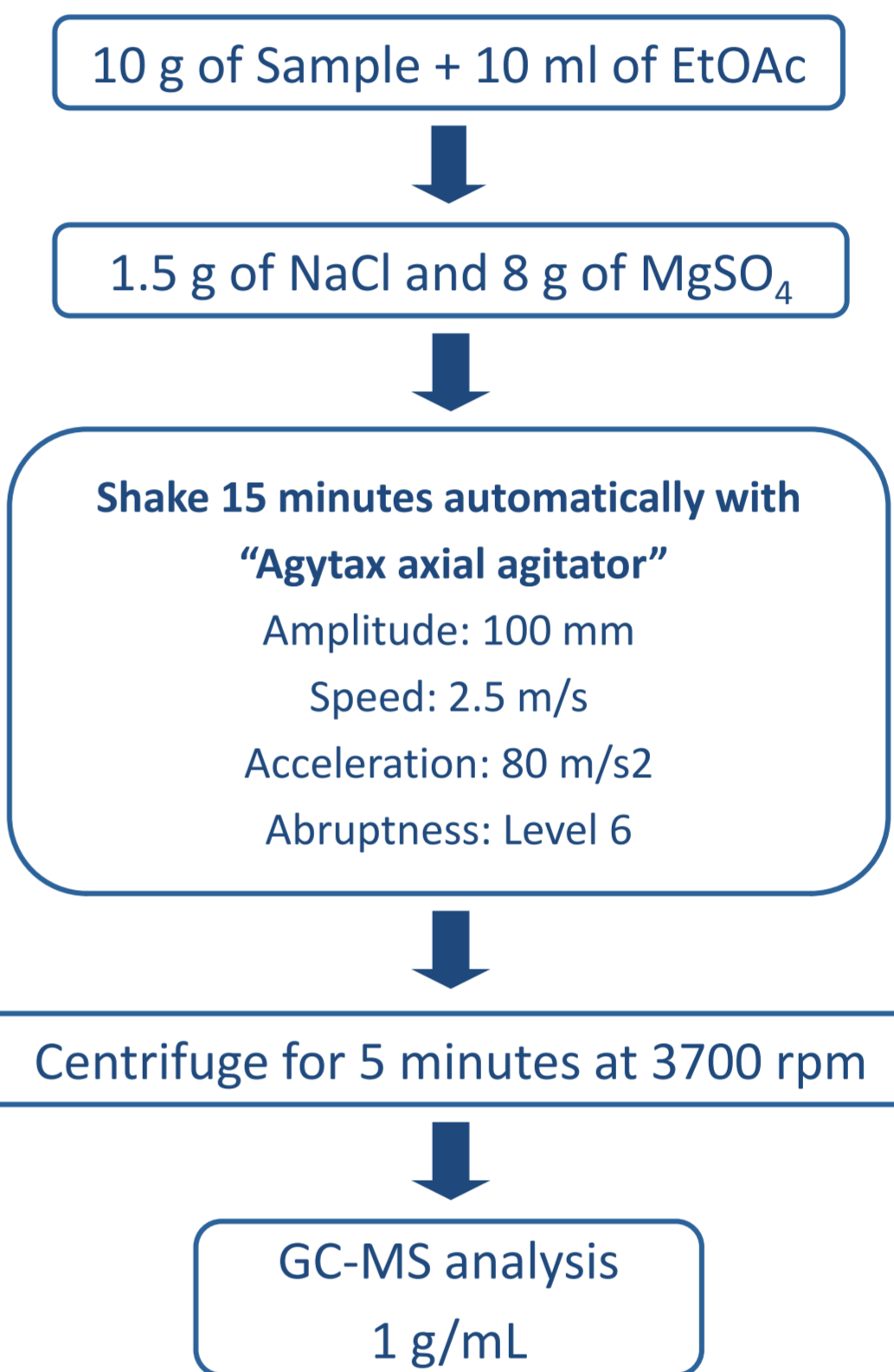Mass Spectrum (Rt = 18.186 min) at 100 µg/kg in Tomato

Due to electron ionization is a very energetic ionization mode, we can obtain spectra with a lot of ions. Unfortunately tools like NIST are only available for unit mass, and not for exact mass. So we need the help provided by MS Interpreter or Generate Formula from Spectrum Peak (in Mass Hunter Qualitative software) to assign the molecular formula for the spectrum peak we are able to see.

NIST
MS Interpreter
Generate Formula from Spectrum Peak

Compound	Molecular Formula	Exact Mass
Vinclozolin	C12H9Cl2NO3	284.9954
Vinclozolin F1	C10H8Cl2N	212.0034
Vinclozolin F2	C10H9ClN	178.0424
Vinclozolin F3	C7H3Cl2NO	186.9592

Ethyl Acetate Extraction Method

10 g of Sample + 10 ml of EtOAc

1.5 g of NaCl and 8 g of $MgSO_4$

Shake 15 minutes automatically with "Agytax axial agitator"
Amplitude: 100 mm
Speed: 2.5 m/s
Acceleration: 80 m/s2
Abruptness: Level 6

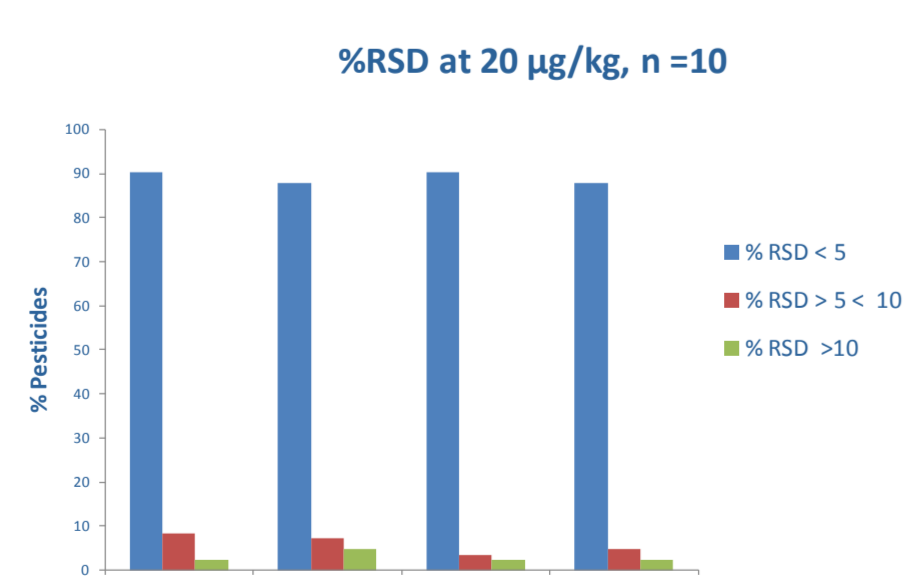Centrifuge for 5 minutes at 3700 rpm

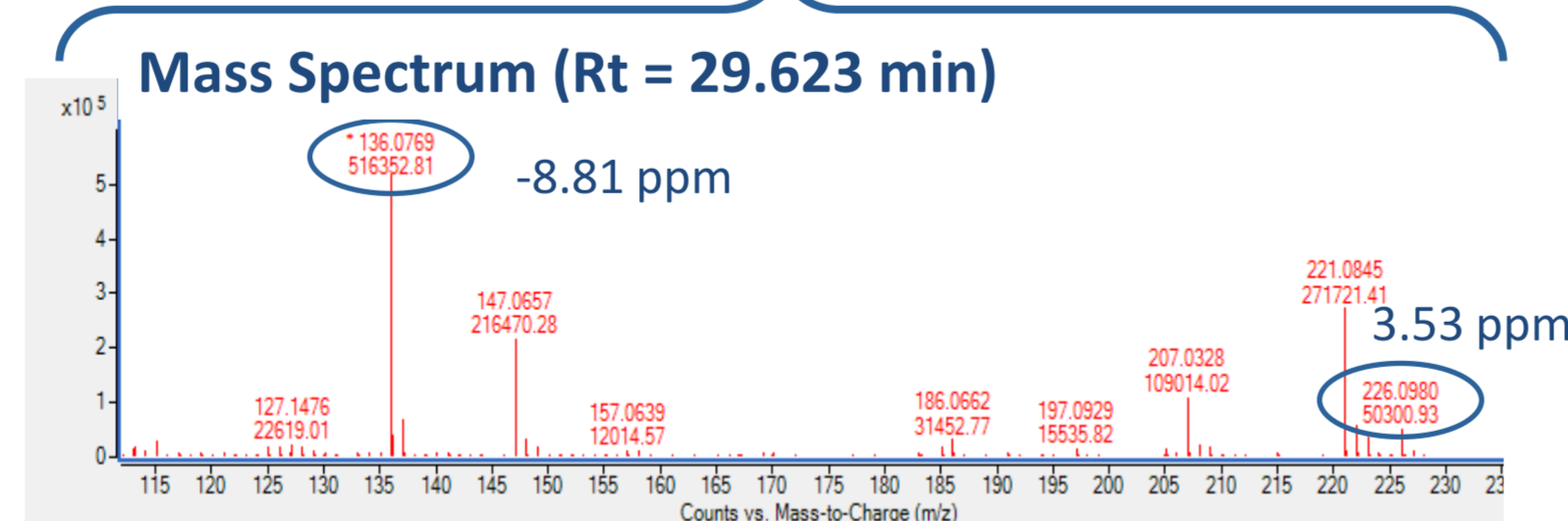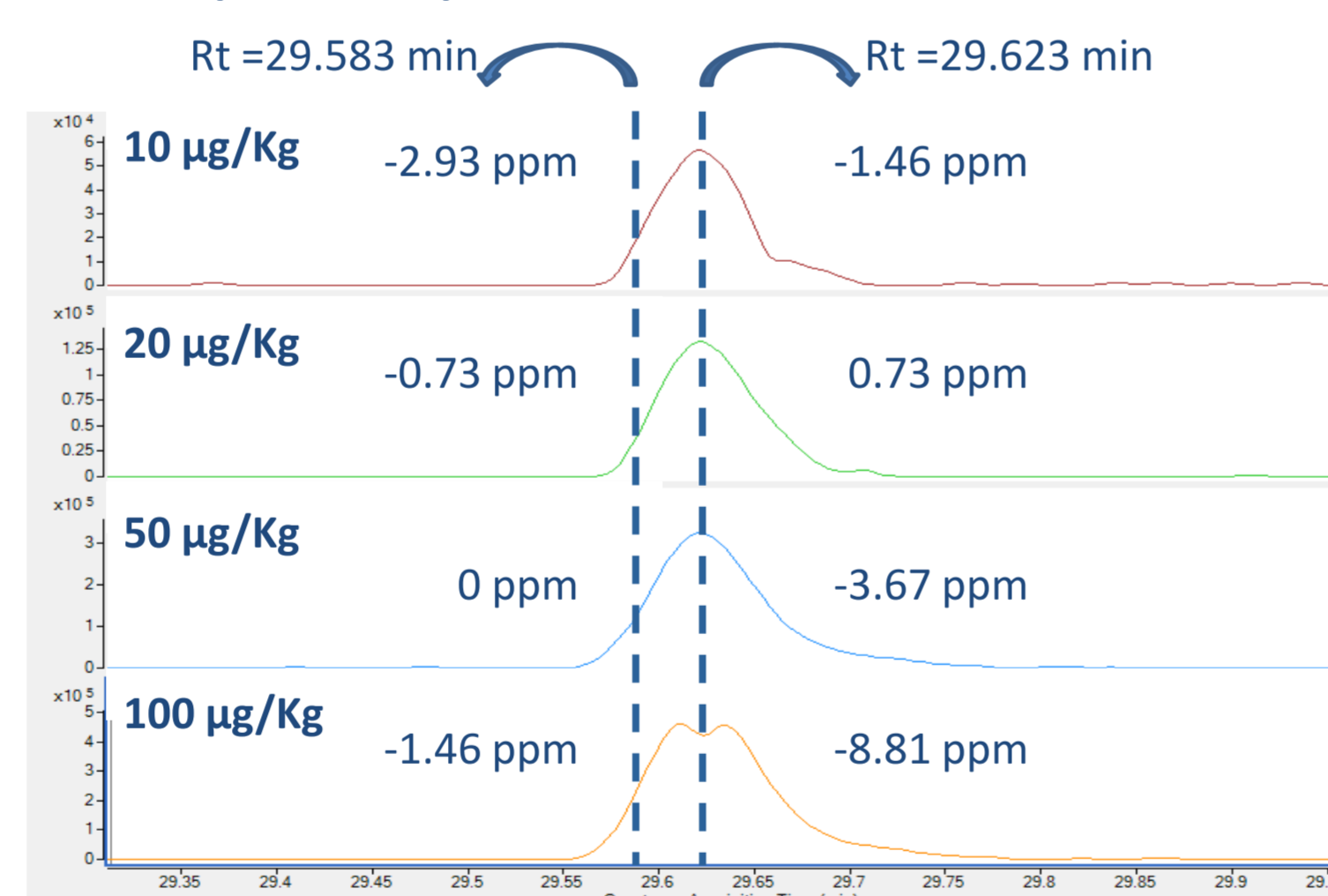GC-MS analysis
1 g/mL

Quantification

A quantitative method has been developed with at least two ions. As the relative abundance of the ions keeps constant with concentration, we set up a 20% of tolerance in relative abundance between standards and samples.

Compound Name	Retention Time (min)	Quantifier Ion Theoretical Mass	Qualifier Ion Theoretical Mass	Relative Abundance
Ametryn	18.469	227.1199	212.0964	≤20%
Benalaxyl	26.003	176.0706	148.1121	≤20%
Bifenthrin	28.334	181.1012	166.0988	≤20%
Bromopropylate	28.123	338.9015	182.9440	≤20%
Bromuconazole	27.921	292.9130	172.9555	≤20%
Bupirimate	24.017	208.1444	273.1016	≤20%
Cadusafos	14.085	213.0167	158.9698	≤20%
Chinomethionat	21.920	233.9916	205.9967	≤20%
Continue….				

With the help of this method, the response for linearity and reproducibility has been evaluated. Values for R^2 coefficient were higher than 0.99; and for reproducibility, around 90% of compounds have a Relative Standard Deviation (RSD) lower than 5%, for all matrices.
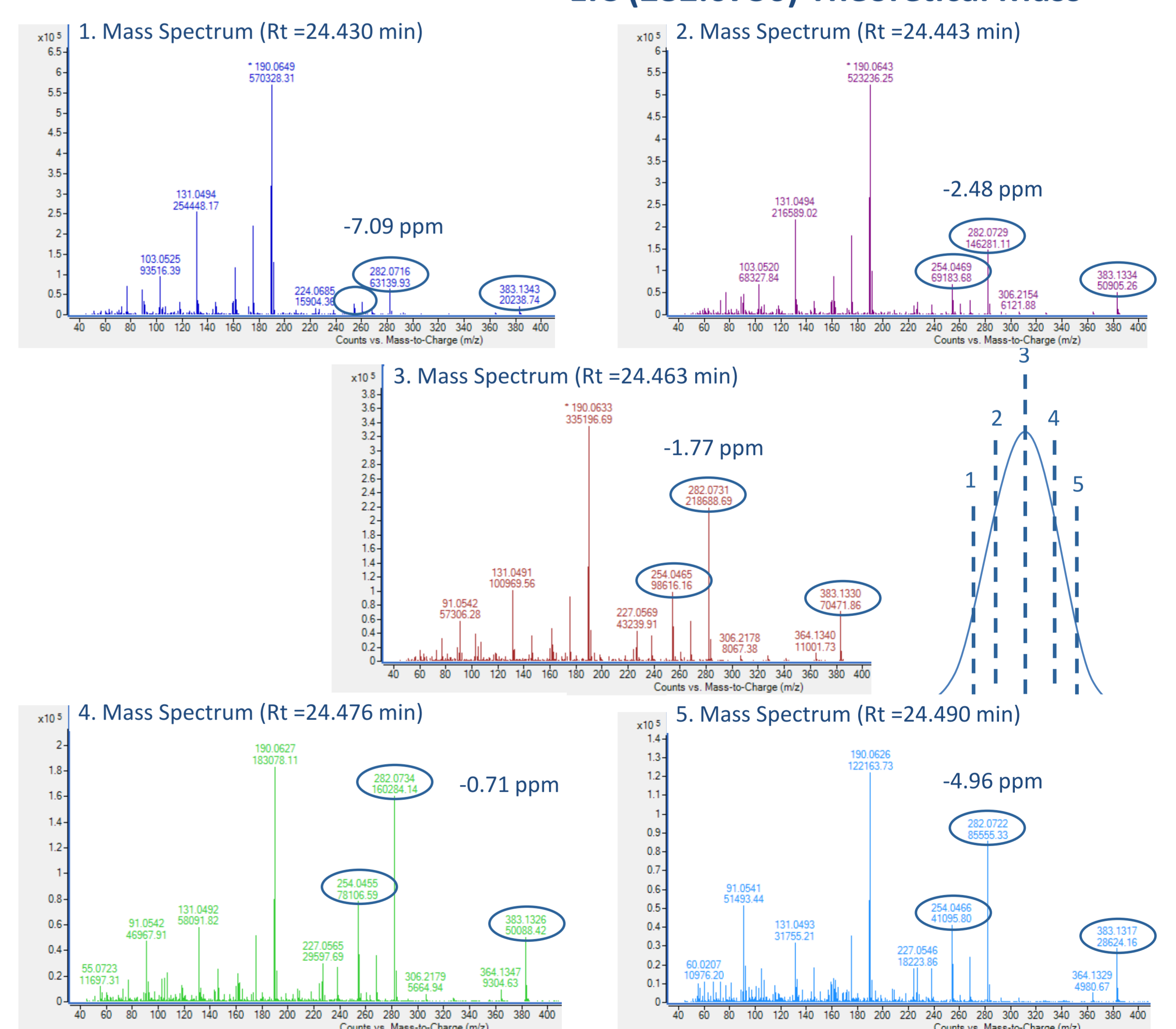
%RSD at 20 µg/kg, n =10

% RSD < 5
% RSD > 5 < 10
% RSD >10

Some Precautions

Saturation: Pyriproxyfen in Spring Onion
EIC (136.0757)

Rt =29.583 min
Rt =29.623 min

10 µg/Kg -2.93 ppm   -1.46 ppm
20 µg/Kg -0.73 ppm   0.73 ppm
50 µg/Kg 0 ppm   -3.67 ppm
100 µg/Kg -1.46 ppm   -8.81 ppm

Mass Spectrum (Rt = 29.623 min)
-8.81 ppm
3.53 ppm

In the case of saturated peaks, the error obtained for the desired ion is higher in the saturation region. In this way it is very important establish a different area where extract the mass spectrum and measure the error, and have an alternative ion with a lower intensity, which does not saturate
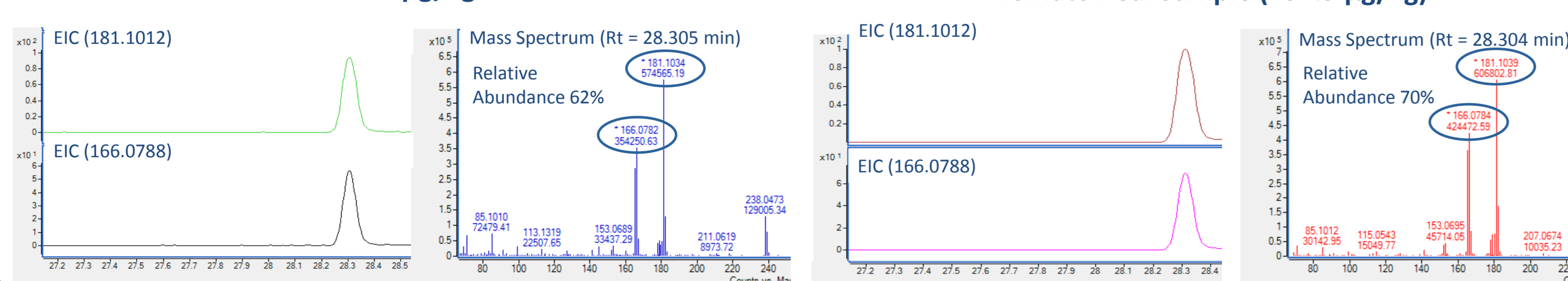
Error along chromatographic peak: Fluazifop-p-butyl at 50 µg/kg in Orange
EIC (282.0736) Theoretical Mass

1. Mass Spectrum (Rt =24.430 min)   -7.09 ppm
2. Mass Spectrum (Rt =24.443 min)   -2.48 ppm
3. Mass Spectrum (Rt =24.463 min)   -1.77 ppm
4. Mass Spectrum (Rt =24.476 min)   -0.71 ppm
5. Mass Spectrum (Rt =24.490 min)   -4.96 ppm

Positive Result: Bifenthrin

Standard at 100 µg/kg in Tomato
EIC (181.1012)
EIC (166.0788)
Mass Spectrum (Rt = 28.305 min)
Relative Abundance 62%

Tomato Real Sample (131.9 µg/kg)
EIC (181.1012)
EIC (166.0788)
Mass Spectrum (Rt = 28.304 min)
Relative Abundance 70%

Conclusions

✓ Easy to transfer methods from EI-simple quad to EI-QTOF taking the advantage of high resolution for discrimination of matrix.

✓ The way in the experimental exact mass is obtained is a critical parameter: a single point in chromatographic peak or as an average in a range of the peak, allowing a mass error lower than 5 ppm.