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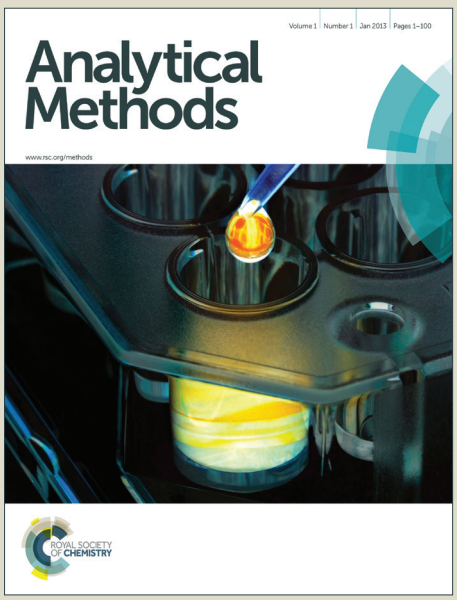

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"Analysis of pesticide residues in fruits and vegetables using gas chromatography – high resolution time-of-flight mass spectrometry"

Noelia Belmonte Valles[1], Samanta Uclés[1], Natalia Besil[1,2], Milagros Mezcua[1], and Amadeo R. Fernández-Alba[1].

[1]University of Almería, Agrifood Campus of International Excellence (ceiA3) Pesticide Residue Research Group, Department of Chemistry and Physic, University of Almería, 04120 La Cañada de San Urbano, Almería, Spain.

[2]Polo Agroalimentario y Agroindustrial. Departamento de Química Del Litoral, Centro Universitario Paysandú, Universidad de la República, Km 363, Ruta 3, 60000 Paysandú, Uruguay

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Abstract

This work reports a study of the operational parameters and development of a rapid automatic method for determining pesticide residues in fruit and vegetables using gas chromatography full spectra mass adquisition time-of-flight accurate mass spectrometry (GC-TOF-MS) in electron ionization mode (EI) based on the use of an "in house" accurate-mass database. The database contains 110 GC amenable compounds, with their main fragment ions obtained under electron ionization at 70 eV. In addition, it includes the retention times of each pesticide working at constant flow. This customized database was linked to commercial software which extracted all the potential compounds of interest from the GC–TOF MS raw data of each sample and matched them against the database to search for targeted compounds in the sample. Ethyl acetate extracts spiked at 10, 20, 50 and 100 µg/kg levels in tomato, orange and spring onion were tested using automatic detection of target pesticides; at 10 µg/kg a 100 % of pesticides were detected in tomato, 97.8 % of pesticides in spring onion and 95.6 % in orange, these results were obtained under an acquisition rate of 4GHz (12000 FWHM) and a mass error tolerance of 5 ppm. The retention time window for detection and identification was ± 0.2 min. Adequate linear responses in the 10-100 µg/kg range were obtained for all compounds in all matrices although saturation effects were observed in some cases. The developed method was applied to real samples, being the qualitative and quantitative results comparable to those obtained using GC-QqQ-MS/MS.

Keywords: GC-TOF-MS, fruits and vegetables, target method, non-target method.

Introduction

For a long time, electron ionization gas chromatography coupled with mass spectrometry and a single quadrupole analyser has been the technical of choice for routine laboratories for analysis of pesticides in fruits and vegetables [1-5]. The main advantage of this technology is its capability in detecting or identifying a great variety of compounds using well established mass spectral libraries at 70 eV as well as open post run analytical evaluation[6, 7]. However, significant selectivity and sensitivity

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limitations make this technique inadequate with the current strict regulations on pesticide residues in food[8, 9].

The application of time-of-flight mass spectrometers (TOF-MS) provided two complementary approaches: (i) instruments that feature unit mass resolution at high acquisition speeds (up to 500 spectra per second), which predetermines their use as detectors coupled to fast and ultra-fast GC or comprehensive two-dimensional GC (GC×GC); and (ii) instruments with a moderate acquisition speed (e.g. 20 spectra per second) but which have high mass resolution (>7000 FWHM), allowing a greater ability to resolve the analytes from the matrix components. A common characteristic of both TOF techniques is simultaneous sampling and analysis of all the ions across the whole mass range; TOF-MS provides greater sensitivity in full-spectrum acquisition mode compared to conventional GC-q-MS instruments in electron ionization mode, principally due to its high mass-analyzer efficiency; GC-TOF-MS can screen hundreds of compounds at high sensitivity within one run. The high mass-resolving power and mass accuracy provided by GC-TOF-MS makes it possible to obtain extracted ion chromatograms using narrow mass windows, thus excluding a large proportion of the chemical background and isobaric interferences, significantly improving signal-to-noise ratios. Under these conditions, pesticide identification capability is improved. Applications employing GC–TOF-MS (high-speed instruments), have shown it to be a powerful and highly effective analytical tool in food and environmental contaminant analysis (*e.g.*pesticide residues)[10-12]. Recently other ionization sources such as atmospheric chemical ionization have been coupled to GC-TOF-MS[13]. This soft ionization mode can allow the screening of a wide variety of contaminants and pesticides by tracing the protonated molecule along the chromatogram. In this case very little or no molecule fragmentation is observed. As widely known, EI has been the most frequently used ionization source in GC-MS methods, and rather strong fragmentation of the molecule typically occurs during ionisation. As a result, the molecular ion is often lost. In contrast, EI source can provide a more robust identification criterion, considering both target and non-target analytes, and others diagnostics ions can be selected from the full scan spectra.

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The aim of this work has been to develop a method for the detection of 110 pesticides and the identification of 45 pesticides in fruits and vegetables using GC-EI-TOF-MS combined with an in house accurate mass library for the automatic detection/identification.

The 45 target pesticides are those fully validated and the 110 non-target pesticides are those for which only match with the library in a diagnostic ion for this reason these pesticides are considered as detected but not identified.

Experimental

Chemicals and reagents

All pesticide analytical standards used in this study were purchased from Dr. Ehrenstorfer (Ausburg, Germany) and Sigma Aldrich (Steinheim, Germany) at analytical grade (purity > 95 %). A mixture standard solution containing all the pesticides studied was prepared at 10 µg/mL in ethyl acetate and stored at -20 °C. Ethyl acetate was obtained from Fluka Analytical Pestanal. Anhydrous magnesium sulphate ($MgSO_4$) was obtained from Pancreac Química S.A. and sodium chloride (NaCl) was from J.T Baker.

Gas chromatography

The separation of the pesticides from the whole fruit or vegetable extracts was carried out using a gas chromatography system (Agilent 7890A); two Agilent Ultra Inert GC column (HP-5MS UI 15 m x 0.25 mm x 0.25 µm) connected through a capillary flow technology (CFT) were used to provide analytical separation.

The samples were injected using a multimode inlet; injections were done in splitless mode with an ultra-inert liner, with a glass wool frit, from Agilent. The injection volume was 2 µL and the injector temperature was held at 280 °C during all the run time. Helium (99.999 % purity) was used as carrier gas. The oven temperature programme was as follows: 60 °C for 1 min, 120 °C at 40 °C/min then up to 310 °C at 5 °C/min. The analytical separation was performed under retention time locking conditions, using chlorpyrifos methyl as the locking compound at a retention time of 18.11 min. The instrument worked at constant flow (1.225 mL/min in the first column and 1.425 mL/min in the second column). The total run time was 40.5 min with 2 additional minutes for backflushing.

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Backflushing was employed to shorten the analysis time and reduce system maintenance avoiding the arrival of undesirable compounds of the matrix to the detector.

The end of the chromatographic column is connected to the second column through a CFT (capillary flow technology) union, which allows system backflushing, eliminating unwanted heavy materials from the first column and prolonging column life. During the run time, the flow was set at 1.0 mL min-1 in the first column and 1.2 mL min-1 in the second column (with a difference of 0.2 mL min-1 over the flow in the first column). Once the analysis is finished, there is a 2 min post run time where a change in the flow is set: 6 mL min-1 in the second column and consequently the flow in the first column decreased until -5.8 mL min-1. The direction of flow inside the first column was changed, which allows remove all high weight molecules or compounds that are not easy to volatilize from the system. One added advantage of this device is that the first column can be changed without venting the mass spectrometer detector (MSD), as the CFT protects the MSD entrance when the column is disconnected by blanketing the connection with the backflush gas.

Time-of-flight Mass Spectrometer

The gas chromatography system was connected to a 7200 mass spectrometer time-of-flight instrument (Agilent Technologies, Santa Clara, USA), equipped with an electron ionization (EI) source. The ion source and quadrupole analyser temperatures were set at 280 and 150 °C, respectively. TOF MS was operated at two different acquisition rates 2GHz (7000 FWHM) and 4 GHz (12000 FWHM), acquiring in the *m/z* 45-550 mass range. Perfluorotributylamine (PFTBA) was used for daily MS calibration. The mass accuracy of the generated ions was controlled through an internal mass calibration performed before each injection. This calibration can be programmed in the worklist and, when the internal mass calibration done between samples shows mass errors higher than 5 ppm, the sequence is automatically stopped to ensure the accuracy of the masses.

Sample Treatment

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The studied matrices (tomato, spring onion and orange) were obtained from an ecological plantation in Almeria. The fruit and vegetable samples used for the application of the developed method were purchased from different local markets. For all matrices, the ethyl acetate extraction method[14] was employed, which is described below. A representative 10 g portion of previously homogenized sample was weighed in a 50 mL PTFE centrifuge tube. Then 10 mL of ethyl acetate and 50 µL of surrogate standard (triphenylphosphate) were added, and the tube was shaken vigorously for 3 seconds by hand. After that, 1.5 g of NaCl and 8 g of $MgSO_4$ were added and the tube was shaken automatically in an Agytax (Cirta Lab) for 15 min. The tube was then centrifuged (3500 rpm) for 5 min. Finally, the extract containing the equivalent of 1 g of sample per mL in 100 % ethyl acetate was directly injected into the GC-TOF MS and GC-QqQ MS systems.

Accurate mass database building

The experimental conditions described above were applied to create an accurate mass database containing 110 GC amenable pesticides typically found in fruit and vegetables. As it is well known, when EI source is used, the molecular ion is not present or the intensity is very low in the majority of the cases. Therefore, it was necessary a detailed investigation of each mass spectrum in order to establish the molecular formula with its theoretical exact mass, related to the experimental exact mass observed. The selected ions for this study were those ones that produce relative abundances higher than 20 % with respect to the base peak.

The created database includes two diagnostic ions for each pesticide, the molecular formula and molecular exact mass for each elucidated ion and the retention time for each compound (see table 1), table 1 shown all this information collected for the selected 110 pesticides. For its development, MS Interpreter was applied. MS Interpreter is a tool incorporated into NIST MS Search 2.0 database and it is combined with ChemDraw software. To facilitate the correct mass assignation of the ions the "generate formula from spectrum peak" tool, included on the Qualitative Mass Hunter software was used. The information containing retention time, molecular formula, exact mass and the name for every assigned ion was collected in an Excel file, which was converted into CSV format to be used as library and linked to the instrument software in order to perform an automatic search of pesticides presents. This library

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containing only 110 pesticides can be easily enlarged following the procedure explained above for each additional compound.

About the relative abundances of the selected ions, between all the ions present on the full scan spectrum of each pesticide, has been selected those which not present variations of over 20 % between the different concentration levels studied (10-100 µg/kg).

Results and discussion

Resolution power

Resolving power is one of the most critical parameters working with "difficult" matrices in HRMS. It was demonstrated that operating at a resolution power of 12000 FWHM, the number of detected pesticides with a mass error lower that 5 ppm is higher than that one obtained working at a resolution power of 7000 FWHM. The main advantage in accurate mass assignments is the ability of a mass spectrometer to resolve two peaks on the *m/z* scale, even when they are close together. When peaks are not (fully) resolved (this happens when the resolution power is not enough to distinguish between two close masses), the resulting measured mass profile will be the sum of the two individual mass profiles; and the top of the combined profile will lie somewhere between the exact masses of the two individual peaks. As a consequence, the mass assignment, which is based on a centroid algorithm of the detected profile, will result in an incorrect analyte mass. As sample complexity becomes greater (the number and intensity for matrix ions are higher than those ones for the analytes), the mass resolution can become a key parameter to the correct assignment of analyte masses.

Tomato, spring onion and orange matrix spiked with pesticides at different concentrations were analysed at 7000 FWHM and at 12000 FWHM resolutions, it was observed that the mass error were higher working at low resolution, especially at low concentration levels. Hence, for the development of this work, it was decided to operate at 12000 FWHM resolution power with the purpose to obtain the best identification reliability at low concentrations levels of pesticides.

Detection/identification study for target compounds

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The capabilities of the developed method were tested in three different matrices: tomato, spring onion and orange at four concentration levels: 10, 20, 50 and 100 µg/kg. Spiked samples were processed with the mass Hunter Software in which the created database was linked. The searching parameters set for detection were as follow; a retention time window of ± 0.2 min and a mass error tolerance of 5 ppm for the diagnostic ion (base peak of the mass spectrum). All selected pesticides included in the database were detected in tomato at 10 µg/kg concentration level. In the case of spring onion matrix all pesticides were detected, except chlorpropham; this pesticide was detected at 20 µg/kg. In orange matrix all pesticides were detected at 10 µg/kg with the exception of bupirimate and pirimicarb, these pesticides were detected at 20 µg/kg.

The criteria applied for identification was based on the presence of the mass of the base peak and the second more abundant ion selected (respect to the base peak) and the correct retention time. The retention time window was set at ± 0.2 min and the mass tolerance was fixed at 5 ppm. Almost all pesticides studied were identified at 10 µg/kg, except fenitrothion tomato matrix. In the case of spring onion matrix 91.3 % of pesticides included in the database were identified, the exceptions were chlorpropham, flutolanil, parathion and triphenylphosphate. In orange matrix, 86.9 % of the compounds were identified except bupirimate, butralin, flutolanil, parathion, pirimicarb and prometryn. It was observed different difficulties related with the capability of identification when the processing method linked to the accurate mass database was applied. In some cases it was due to software lacks, where some not automatically detected compounds could be manually detected. In figure 1 it is shown an example for prosulfocarb (128.1075 exact mass) in tomato matrix at 10 µg/kg. This compound was not automatically identified using the homemade database, but it was detected manually with a mass error lower than 5 ppm in all matrices

Mass accuracy study

The mass accuracy for each ion of the target pesticides was studied for all matrices assayed at 10, 20, 50 and 100 µg/kg, operating at 12000 FWHM. Setting 5 ppm as mass tolerance, at 10 µg/kg concentration level 100 % of the pesticides were detected in tomato matrix and 95.6 % of the pesticides were detected in spring onion and orange

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matrices; in addition 97.8 % of compounds were identified in tomato, 91.3 % in spring onion and 86.9 % in orange. At low concentration level (10 µg/kg) there were some cases were this tolerance mass value (5 ppm) was not enough for a correct identification due to low sensitivity level for some compounds: benalaxyl and chlorobenzilate (in tomato), chlorobenzilate (in spring onion), endrin and tolclofos methyl (in orange). In other way it was observed that the mass accuracy for at least one ion was below 5 ppm in the range between 10-100 µg/kg for all matrices studied, although at low concentration levels the mass accuracy was higher. To develop this work, a mass tolerance of 5 ppm was selected with the purpose to satisfy the requirements for identification according to European Procedures[15], assuming the drawback to obtain limits of identification higher than 10 µg/kg for some compounds.

Higher values of mass tolerance yield large numbers of false positives detected and lower values can be the source of difficulties like undetected compounds preferably at low concentration levels. Retention time shifts were lower than 0.2 min in all cases studied.

By using the selected operational parameters, the database was employed as a library to evaluate the capability of detection/identification depending of the mass accuracy and the time window tolerance selected.

The mass error calculated is depending on the area of the selected peak. In the majority of the cases the best option was calculate the mass error in the apex of the peak, although in some cases this fact can be a problem. As example in figure 2 it was represented the characteristic fragment with m/z 136.0762 of pyriproxyfen compound at different concentration levels. At 100 µg/kg concentration, the shape of the peak was quite different than at low concentrations levels. For this compound it was observed a typical saturation effect at high concentration. When the mass error was calculated in the apex of the peak, the value obtained was higher (11.1 ppm), but this problem can be solved if the mass error was calculated on the average scan of 50 % of the peak high (-1.46 ppm).

In general, to minimize the mass error is preferable to calculate it in the 50 % upper part of the peak. In figure 3 it was showed the characteristic fragment 282.0742 (exact mass) from fluazifop-pbutyl compound in orange matrix at 50 µg/kg. The mass error was calculated at five different retention time, and it was observed that in points 1 and

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5 the mass error were higher than in points 2, 3 and 4; so it was concluded that it was preferable to select the region between points 2 and 4 to calculate the mass error, to obtain minimum mass error values.

Validation of the target method

The feasibility of the target method was evaluated in terms of linearity, reproducibility, repeatability and matrix effect.

Limits of detection and identification have been discussed above.

The recoveries of the selected compounds has been evaluated in a previous work[14], in all cases the percentage of recoveries are between 70 and 120 %

Linearity, repeatability and reproducibility

The linearity was studied working at 12000 FWHM resolution mode and in the range between 10 and 100 µg/kg in all matrices assayed. The detector response was considered linear if the coefficient of determination (R^2) was equal or higher than 0.99. Usually the detector response was linear across the whole investigated range, with very good linearity observed in this range showing correlation coefficients according to the establish criterion, except for bromuconazole and butralin in tomato; bifenthrin, flutolanil and pirimiphos methyl in spring onion and finally dicofol, flutolanil and trifluralin in orange (see table 2). In the mentioned cases, to be able to have a good linearity, the linear range was narrower (10-50 µg/kg) due to at 100 µg/kg concentration level it was appeared the saturation effect.

The selected operation mode for this work was High Resolution instead of extended dynamic range mode. The selected operation mode has limitations in quantification at high concentration levels. The higher level of the studied range is 100 ug/kg considering two reasons (i) the typical saturation effects at high concentration levels of this type of analyzers and (ii) the number MRLs present in the range of 10-100 ug/kg are around 70% in the EU regulation. Additionally the quantification of higher levels of concentration can be performed following different approaches such as; selecting ion fragments with lower abundance or injecting fewer amounts (e.g. 1 uL)

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The lowest level of the range (10 ppb) has been selected because it is the LMR default value (Regulation (EC) Nº 396/2005). Lower values could be included in the range for some compounds since the observed instrumental limit of identification is lower than 10 ppb in some cases. But these lower levels have not been deeply studied, for this reason no results has been presented in this paper.

For the base peak, the repeatability (inter-day) and reproducibility (inter-day) were evaluated, getting good results with RSDs of < 20 % for the 100 % of compounds studied in all matrices assayed. For this study, two spiked levels 10-50 µg/Kg were injected five times (repeatability) and over five consecutive days (reproducibility).

Matrix effect

For the matrix effect study the slope of all the matrices obtained from the linear curve in the range between 10 and 100 µg/kg were compared with the slope obtained in solvent.

All matrices showed a marked signal enhancement effect when compared with solvent. Matrix enhancement effects are frequently observed in GC/MS, however the differences in the slopes between matrices were very small. For this reason in this work we consider tomato matrix as a good reference of low matrix effects. While a positive value means higher sensitivity in the investigated matrix than in tomato, a negative value was equivalent to a lower sensitivity. Comparing ME (%) between tomato and spring onion the general tendency was not significant matrix effect. Only four compounds showed enhancement: bromuconazole (51.4 %), butralin (169.2 %), fenitrothion (117.1 %) and trifluralin (96.8 5). For orange matrix which is a more "difficult" matrix than tomato, only 3 compounds presented enhancement: butralin (72.5 %), fenitrothion (102.5 %) and parathion (61.8 %). Bupirimate (-61.8 %), flutolanil (-52.6 %) and trifluralin (-54.0 %) presented suppression of the signal; for the rest of the compounds studied as well as the other matrices investigated the differences between the slope of the matrix studied respect to the slope of the tomato matrix was in the range between ± 20 % so it was concluded that were not significant matrix effect.

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Analysis of real samples.

The developed method was employed in analysis of real samples, collected from a local market in Almeria (Spain). In the quantification method, the base peak, the second more intensive ion respect the base peak and the retention time, were selected as criteria of quantification per each compound. The base peak was used as quantifier ion and the second more intensive ion was used as qualifier ion. The relative abundance between both ions keeps constant at different concentration levels for standards and it cannot differ in more than 30 % for real samples.

Target compounds

The tomato matrix was selected as the quantification matrix due to all matrices analysed were belonging into the high water content within the classification for commodity groups in the Annex A of the European Procedure, moreover the differences observed in the slopes between matrices were very small as it was commented in the matrix effect study. The quantification was performed in tomato-matched calibration in the range 10-100 µg/kg. The obtained results showed that all of the samples analysed were positives with different residues of pesticides. The range of concentration detected was between 0.01-0.32 mg/kg. The pesticides found was as follow: bifenthrin, bupirimate, metalaxyl pirimicarb, pyriproxyfen, and p,p'-DDE. Bupirimate, bifenthrin and pyriproxifen were present in two different samples. The rest of target positives were found only once. Any pesticides detected exceed the limits maximum of residues permitted established by the European Union Legislation. The results obtained were compared with those obtained using GC-QqQ-MS system. The quantification differences with both systems are mostly within 50%.

In figure 4 was shown a positive of bifenthrin in tomato sample. The quantifier ion was *m/z* 181.1012 and the qualifier one was *m/z* 166.0788. The mass spectrum in tomato sample was compared with the mass spectrum for standard of bifenthrin. The relative abundance in tomato sample was 63,6 % whereas in the standard of bifenthrin in tomato matrix was 57.4 %, so the difference between both was below 30 %. The concentration found in tomato sample was 0.12 mg/kg.

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The number and distribution of interfering matrix components varies greatly depending on the particular vegetable matrix; even those included within the same commodity category according to EU procedures. The components present in the matrix often have similar masses than the target compounds, and when the co-elution is possible, this fact can drive to report false positive or false detections. False positives depend heavily on the matrix-the higher the complexity of the sample, the more false detects will appear. It was considered false positive in a real sample when the software reported the quantifier and qualifier ion with a relative abundance below 30% respect to the standard. The false positive could be discarded after confirmation analysis by GC-MS/MS, so it was not a problem although they represent a time-consuming task and, therefore, an important handicap to efficient laboratory workflow. The real samples analysed in this work and moreover seven blank of different samples was used to realize a previous study about false positives and negatives reported by automatic database.

Non target compounds

Not target compounds are those ones which have not been validated and only the presence of one diagnostic ion (base peak, allowing a mass error lower than 5 ppm) with the correct retention time (± 0.2 min) was necessary for detection criterion. In all real samples evaluated the obtained results showed detected not target pesticides residues. The found pesticides were azoxystrobin, boscalid, iprodione, lambda-cyhalothrin, myclobutanil, penconazole and trifloxystribin. Iprodione was detected in eight various samples (tomatoes, green beans and grapes); azoxystrobin was detected in four samples (green beans, melon and tomato); lambda cyhalothrin and myclobutanil were detected in two samples each one (green beans). The rest of the not target positives were found only once (green bean, zucchini, cabbage).

Conclusions

The created database of 110 pesticides, which included the retention time and two ions per compound, was applied to automatic pesticide identification in tomato, spring onion and orange. This “in house” database is an excellent tool considering that commercial exact mass libraries are not available.

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The presented work allows the possibility to make target and non-target compounds, the target full scan method have been validated for 45 pesticides. For the detection or identification of non-target method the created library containing 110 pesticides can be used, this database can be enlarged easily.

The automatic identification was made and compared at two resolution powers, showing better results at high resolution mode. A rapid and automatic full scan method had been developed and a reliability of identification between 87-100 % at 10 µg/kg concentration level was demonstrated in all matrices assayed. Additionally, a quantification method was developed selecting two ions per compound, the retention time and a relative abundance ≤ 30 % between real samples and the standard, as criteria for identification. The base peak of the mass spectrum was used as quantifier ion and the more intensity respect to the base peak as qualifier ion. The method was applied for the analysis of real samples, and the obtained results were compared with those using GC-QqQ-MS, showing differences in the quantification results of less than 50 % which is the accepted value of uncertainty. Finally it is important to note that working in full scan acquisition mode a retrospective analysis is possible, allowing the search of compounds initially not included in the database.

Figure and table captions.

Table 1. Identification parameters containing in the "in home" database; name, retention time (Rt), exact mass and molecular formula for each fragment.

Table 2. Linear range and identification limits (LOIs) for each pesticide in the three matrices evaluated.

Figure 1. a) Overlaped extract ion chromatograms for prosulfocarb (m/z 128.1070, 251.1339) at 10 µg/kg in tomato matrix, with its signal-to-noise (S/N).

Figure 2. a) Extract ion chromatogram for pyriproxifen (m/z 136.0757) at different concentration levels in spring onion: black, red, green and pink (10, 20, 50 and 100 µg/kg, respectively). Mass errors have been calculated in two parts of the peak: in the apex and around 50 % of peak height. b) Mass accuracies of pyriproxifen ions in spring onion at 100 µg/kg, for 29.623 min retention time.

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Figure 3. a) Extract ion chromatogram for fluazifop p-butyl (m/z 282.0737) in orange at 50 µg/kg. b) Full scan spectrum obtained for each point decided in figure 3.a. of fluazifop p-butyl in orange at 50 µg/kg, with the mass accuracy for fluazifop p-butyl m/z 282.0737 ion.

Figure 4. a) Automatic identification of bifenthrin at 0.12 mg/kg in tomato sample. b) Automatic identification of bifenthrin standard in matrix matched in tomato at 0.10 mg/kg

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#	Compound	Rt (min)	Exact Mass	Molecular Formula
1	Ametryn	18.469	227.1205	C9H17N5S
	Ametryn F1		212.0970	C8H14N5S
2	Benalaxyl	26.003	148.1126	C10H14N
	Benalaxyl F1		176.1075	C11H14NO
3	Bifenthrin	28.334	181.1017	C14H13
	Bifenthrin F1		166.0783	C13H10
4	Bromopropylate	28.123	182.9446	C7H4BrO
	Bromopropylate F1		338.9020	C13H9Br2O
5	Bromuconazole	27.921	172.9561	C7H3Cl2O
	Bromuconazole F1		292.9136	C10H8BrCl2O
6	Bupirimate	24.017	208.1450	C11H18N3O
	Bupirimate F1		273.1021	C10H17N4O3S
7	Butralin	20.648	266.1141	C12H16N3O4
	Butralin F1		277.1426	C14H19N3O3
8	Cadusafos	14.085	158.9703	C2H8O2PS2
	Cadusafos F1		213.0173	C6H14O2PS2
9	Chinomethionate	21.921	205.9972	C9H6N2S2
	Chinomethionate F1		233.9922	C10H6N2OS2
10	Chlorobenzilate	24.638	138.9951	C7H4ClO
	Chlorobenzilate F1		251.0030	C13H9OCl2
11	Chlorpropham	13.335	127.0189	C6H6ClN
	Chlorpropham F1		171.0087	C7H6NO2Cl
12	Chlorpyrifos	19.999	196.9202	C5H2Cl3NO
	Chlorpyrifos F1		257.8948	C5H3NO3PSCl2
13	Chlorpyrifos Methyl	18.117	285.9261	C7H7NO3PSCl2
	Chlorpyrifos Methyl F1		124.9826	C2H6O2PS
14	Chlozolinate	21.418	258.9803	C10H7NO3Cl2
	Chlozolinate F1		186.9592	C7H3Cl2NO
15	DDE, p,p'-	23.420	246.0003	C14H8Cl2
	DDE, p,p'- F1		315.9380	C14H8Cl4
16	Diclorvos	6.163	109.0055	C2H6O3P1
	Diclorvos F1		184.9770	C4H7O4P1Cl1
17	Dicofol	26.670	138.9951	C7H4ClO
	Dicofol F1		110.9996	C6H4Cl
18	Endrin	24.170	260.8599	C7H2Cl5
	Endrin F1		242.9535	C11H6Cl3
19	Etoprophos	13.007	157.9625	C2H7O2PS2
	Etoprophos F1		113.9363	H3OPS2
20	Etrimphos	16.984	292.0647	C10H17N2O4PS
	Etrimphos F1		277.0412	C9H14N2O4PS
21	Fenitrotion	19.187	260.0146	C9H11NO4PS
	Fenitrotion F1		277.0174	C9H12NO5PS
22	Fluazifop-p-butyl	24.472	282.0742	C14H11F3NO2
	Fluazifop-p-butyl F1		383.1344	C19H20F3NO4

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23	Fluopyram	21.640	173.0214	C8H4F3O
	Fluopyram F1		223.0250	C8H7ClF3N2
24	Flutolanil	23.150	173.0214	C8H4F3O
	Flutolanil F1		145.0265	C7H4F3
25	Hexaconazole	23.020	213.9939	C8H6Cl2N3
	Hexaconazole F1		256.0044	C10H8Cl2N3O
26	Metalaxyl	18.650	206.1181	C12H16NO2
	Metalaxyl F1		234.1130	C13H16NO3
27	Nuarimol	26.778	138.9951	C7H4ClO
	Nuarimol F1		235.0326	C13H9ClFO
28	Parathion	20.014	291.0330	C10H14NO5PS
	Parathion F1		139.0269	C6H5NO3
29	Picolinafen	28.295	238.0480	C12H7F3NO
	Picolinafen F1		376.0835	C19H12F4N2O2
30	Pirimicarb	17.387	166.0980	C8H12N3O
	Pirimicarb F1		238.1430	C11H18N4O2
31	Pirimiphos Methyl	19.314	290.0728	C10H17N3O3PS
	Pirimiphos Methyl F1		276.0572	C9H15N3O3PS
32	Profenophos	23.316	205.9134	C6H4BrClO
	Profenophos F1		336.9663	C11H15BrO3PS
33	Prometryn	18.627	241.1361	C10H19N5S
	Prometryn F1		226.1126	C9H16N5S
34	Propazine	15.496	214.0859	C8H13ClN5
	Propazine F1		172.0390	C5H7ClN5
35	Prosulfocarb	18.791	128.1075	C7H14NO
	Prosulfocarb F1		251.1344	C14H21NOS
36	Prothiophos	23.204	112.9285	H2OPS2
	Prothiophos F1		308.9940	C11H15ClO2PS2
37	Pyriproxyfen	29.624	136.0762	C8H10NO
	Pyriproxyfen F1		226.0994	C15H14O2
38	Quinoxyfen	26.062	272.0278	C15H8ClFNO
	Quinoxyfen F1		306.9967	C15H8Cl2FNO
39	Tebufenpyrad	28.644	171.0325	C7H8ClN2O
	Tebufenpyrad F1		318.1373	C17H21ClN3O
40	Tecnazene	12.430	200.8832	C5HCl4
	Tecnazene F1		212.8832	C6HCl4
41	Tetraconazole	20.372	336.0527	C13H11ClF4N3O
	Tetraconazole F1		170.9768	C8H5Cl2
42	Tetradifon	29.042	158.9671	C6H4ClOS
	Tetradifon F1		226.8892	C6H2Cl3OS
43	Tolclofos Methyl	18.281	264.9855	C9H11ClO3PS
	Tolclofos Methyl F1		249.9620	C8H8ClO3PS
44	TPP	27.052	326.0708	C18H15O4P
	TPP F1		215.0262	C12H8O2P
45	Trifluralin	13.947	264.0272	C13H5F3NO2
	Trifluralin F1		306.0702	C11H11F3N3O4

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Table 1. Identification parameters contained in the “in home” database: name, retention time (Rt), exact neutral mass and molecular formula for each fragment.

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#	Compound	Rt (min)	LOI (µg/Kg)			Linear Range (µg/Kg)		
			Tomato	Spring Onion	Orange	Tomato	Spring Onion	Orange
1	Ametryn	18.469	10	10	10	10-100	10-100	20-100
2	Benalaxyl	26.003	10	10	10	10-100	10-100	10-100
3	Bifenthrin	28.334	10	10	10	10-100	10-50	10-100
4	Bromopropylate	28.123	10	10	10	10-100	10-100	10-100
5	Bromuconazole	27.921	10	10	10	10-50	10-100	10-100
6	Bupirimate	24.017	10	10	20	10-100	10-100	20-100
7	Butralin	20.648	10	10	20	10-50	10-100	20-100
8	Cadusafos	14.085	10	10	10	10-100	10-100	10-100
9	Chinomethionate	21.921	10	10	10	10-100	10-100	10-100
10	Chlorobenzilate	24.638	10	10	10	10-100	10-100	10-100
11	Chlorpropham	13.335	10	n.i.	10	10-100	n.i.	10-100
12	Chlorpyrifos	19.999	10	10	10	10-100	10-100	10-100
13	Chlorpyrifos Methyl	18.117	10	10	10	10-100	10-100	10-100
14	Chlozolinate	21.418	10	10	10	10-100	10-100	10-100
15	DDE, p,p'-	23.420	10	10	10	10-100	10-100	10-100
16	Diclorvos	6.163	10	10	10	10-100	10-100	10-100
17	Dicofol	26.670	10	10	10	10-100	10-100	10-50
18	Endrin	24.170	10	10	10	10-100	10-100	10-100
19	Etoprophos	13.007	10	10	10	10-100	10-100	10-100
20	Etrimphos	16.984	10	10	10	10-100	10-100	10-100
21	Fenitrotion	19.187	20	10	10	20-100	10-100	10-100
22	Fluazifop-p-butyl	24.472	10	10	10	10-100	10-100	10-100
23	Fluopyram	21.640	10	10	10	10-100	10-100	10-100
24	Flutolanil	23.150	10	20	20	10-100	20-50	20-50
25	Hexaconazole	23.020	10	10	10	10-100	10-100	10-100
26	Metalaxyl	18.650	10	10	10	10-100	10-100	10-100

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#	Compound	Rt (min)	LOI (µg/Kg)			Linear Range (µg/Kg)		
			Tomato	Spring Onion	Orange	Tomato	Spring Onion	Orange
27	Nuarimol	26.778	10	10	10	10-100	10-100	10-100
28	Parathion	20.014	10	20	50	10-100	20-100	50-100
29	Picolinafen	28.295	10	10	10	10-100	10-100	10-100
30	Pirimicarb	17.387	10	10	20	10-100	10-100	20-100
31	Pirimiphos Methyl	19.314	10	10	10	10-100	10-50	10-100
32	Profenophos	23.316	10	10	10	10-100	10-100	10-100
33	Prometryn	18.627	10	10	20	10-100	10-100	20-100
34	Propazine	15.496	10	10	10	10-100	10-100	10-100
35	Prosulfocarb	18.791	10	10	10	10-100	10-100	10-100
36	Prothiophos	23.204	10	10	10	10-100	10-100	10-100
37	Pyriproxyfen	29.624	10	10	10	10-100	10-100	10-100
38	Quinoxyfen	26.062	10	10	10	10-100	10-100	10-100
39	Tebufenpyrad	28.644	10	10	10	10-100	10-100	10-100
40	Tecnazene	12.430	10	10	10	10-100	10-100	10-100
41	Tetraconazole	20.372	10	10	10	10-100	10-50	10-100
42	Tetradifon	29.042	10	10	10	10-100	10-50	10-100
43	Tolclofos Methyl	18.281	10	10	10	10-100	10-100	10-100
44	TPP	27.052	10	n.i.	10	10-100	n.i.	10-100
45	Trifluralin	13.947	10	10	10	10-100	10-100	10-50

Table 2. Linear range and identification limits (LOIs) for each pesticide in the three matrices evaluated (n.i. not identified)

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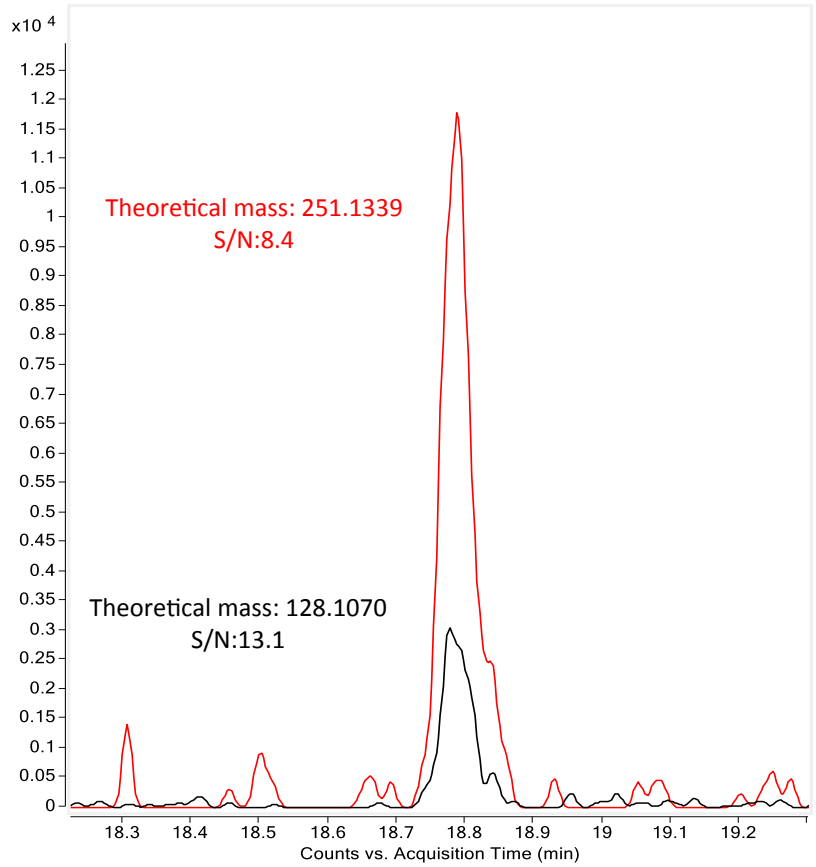

Figure 1. a) Extracted ion chromatograms overlap for prosulfocarb (*m/z* 128.1070, 251.1339) at 10 µg/kg in tomato matrix, with its signal-to-noise (S/N).

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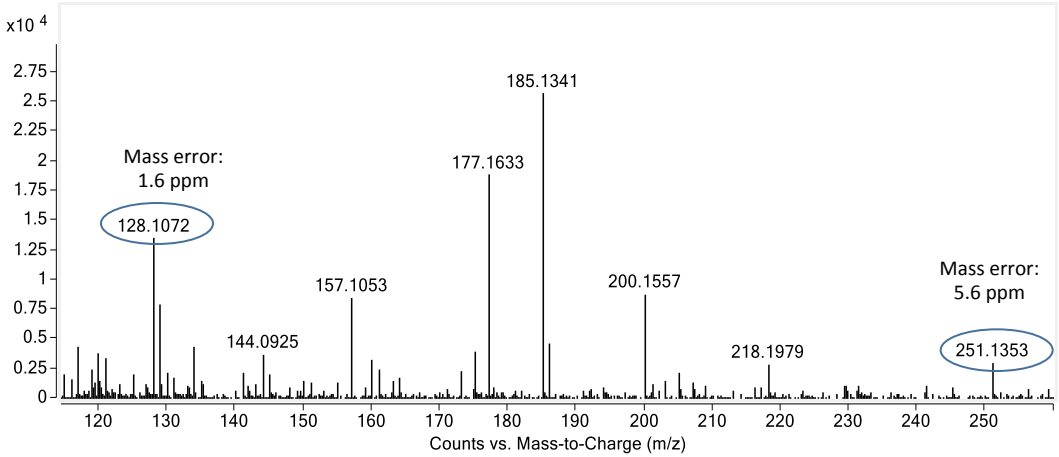

Figure 1. b) Mass accuracies for prosulfocarb ions in tomato at 10 µg/kg.

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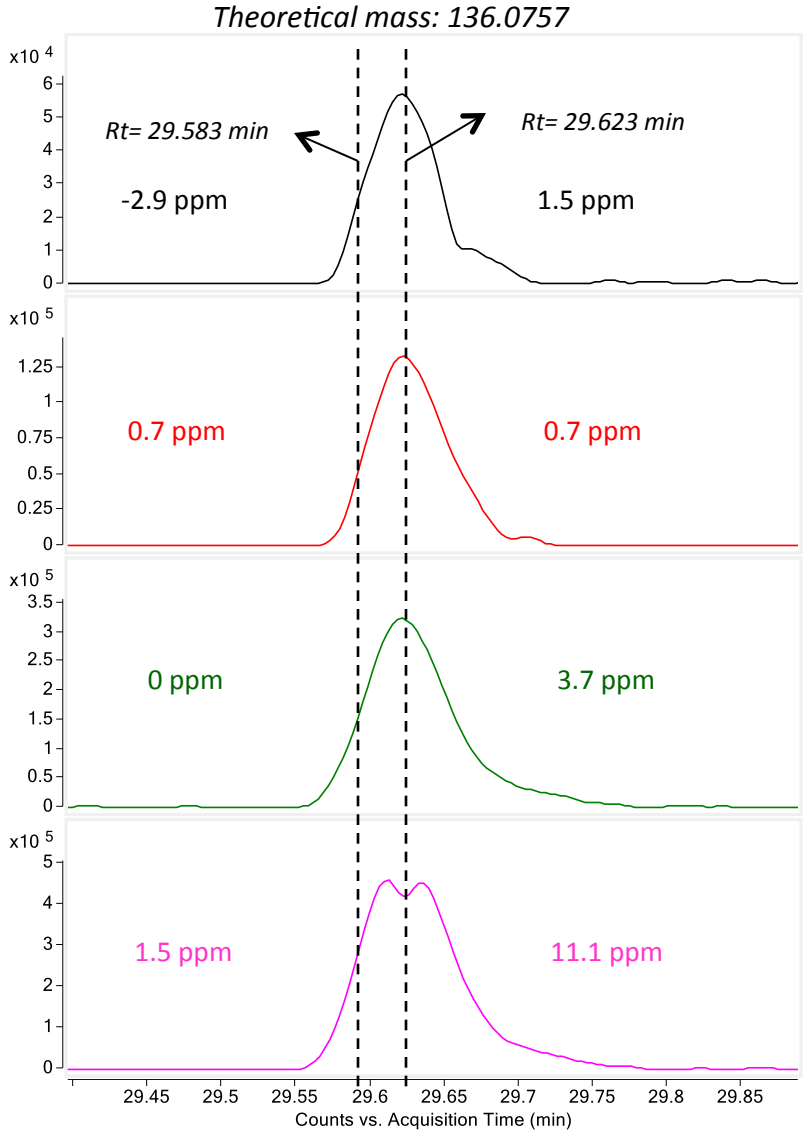

Figure 2. a) Extracted ion chromatogram for pyriproxyfen (*m/z* 136.0757) at different concentration levels in spring onion: black, red, green and pink (10, 20, 50 and 100 µg/kg, respectively). Mass errors have been calculated in two parts of the peak: in the apex and around 50 % of peak height.

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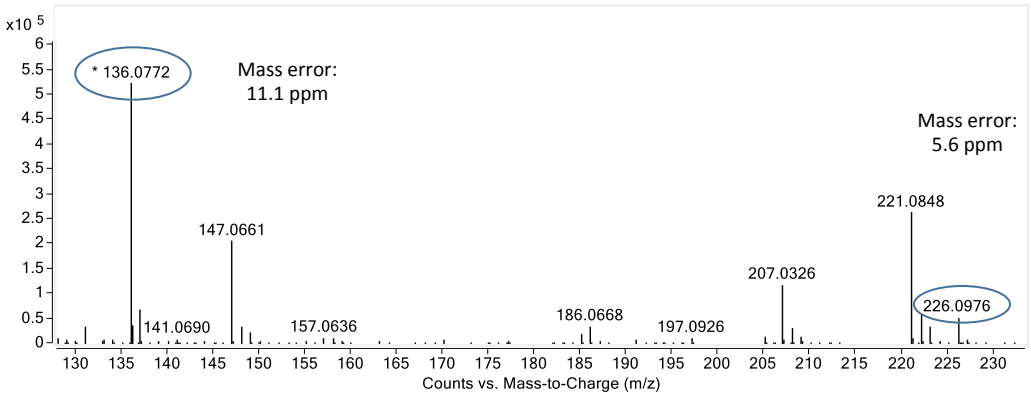

Figure 2. b) Mass accuracies for pyriproxifen ions in spring onion at 100 µg/kg and 29.623 min retention time.

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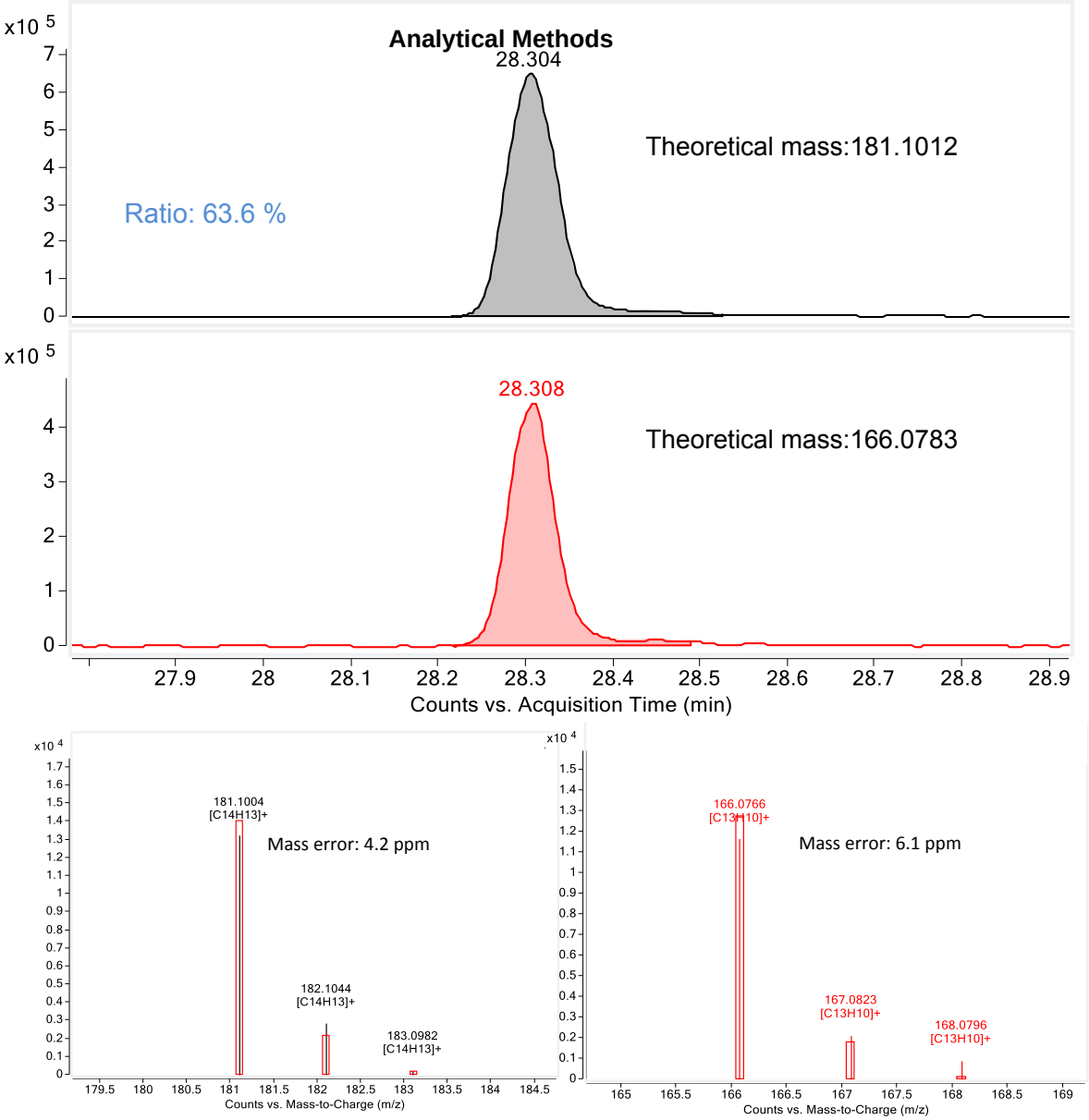

Figure 4. a) Automatic identification of bifenthrin at 0.12 mg/kg in tomato sample.

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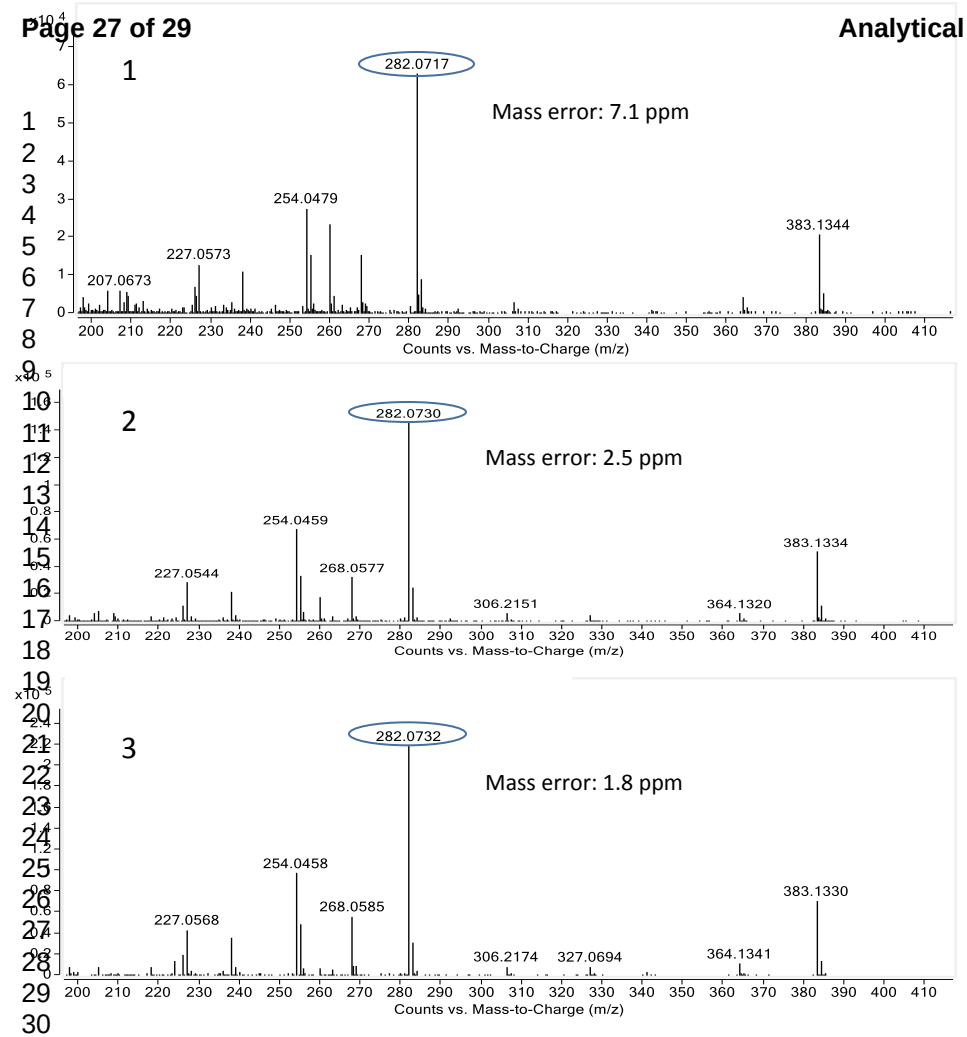

Figure 3. b) Full scan spectrum obtained for each point decided in figure 3.a. of fluazifop-p-butyl in orange at 50 µg/kg, with the mass accuracy for its *m/z* 282.0737 ion.

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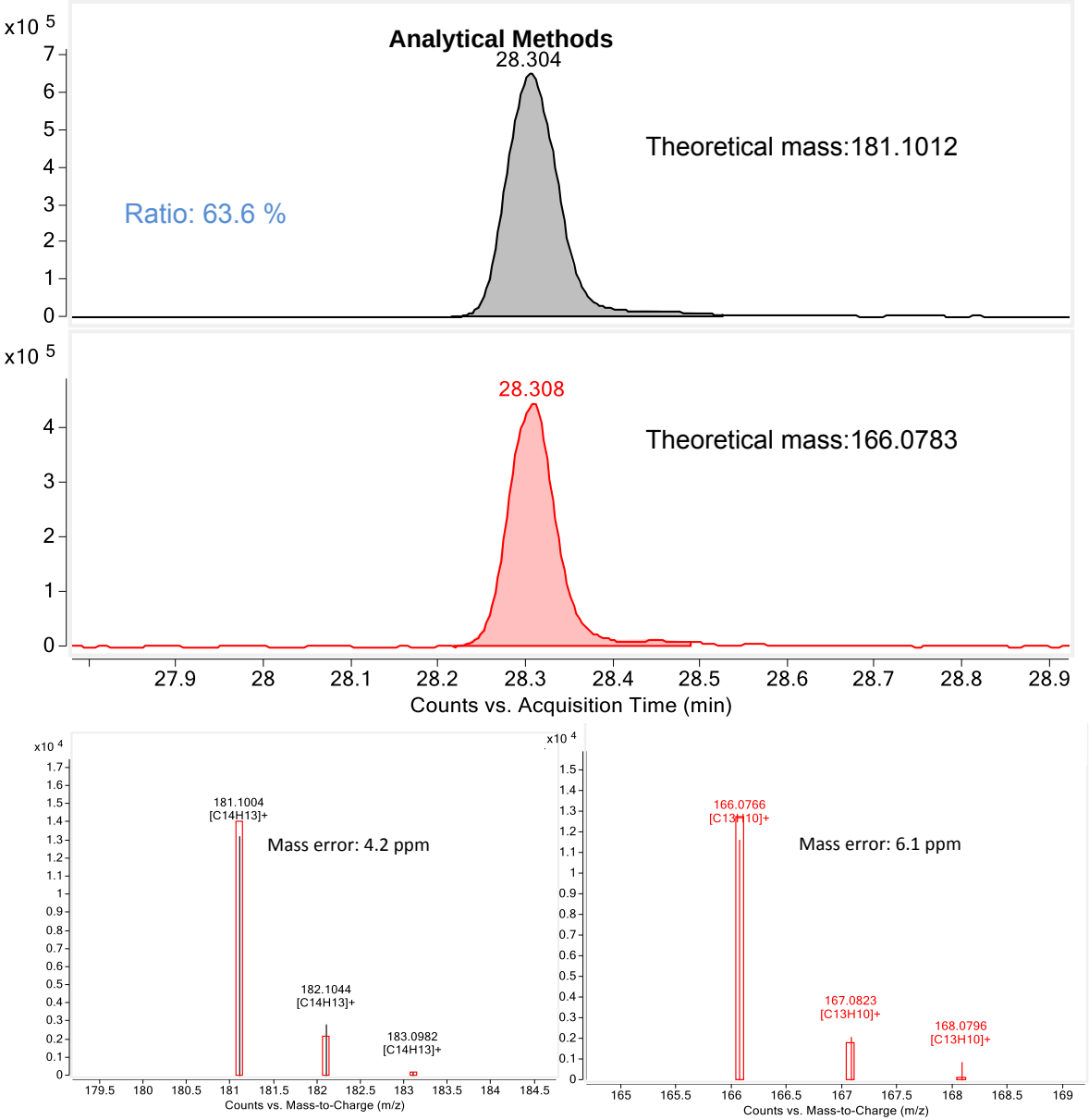

Figure 4. a) Automatic identification of bifenthrin at 0.12 mg/kg in tomato sample.

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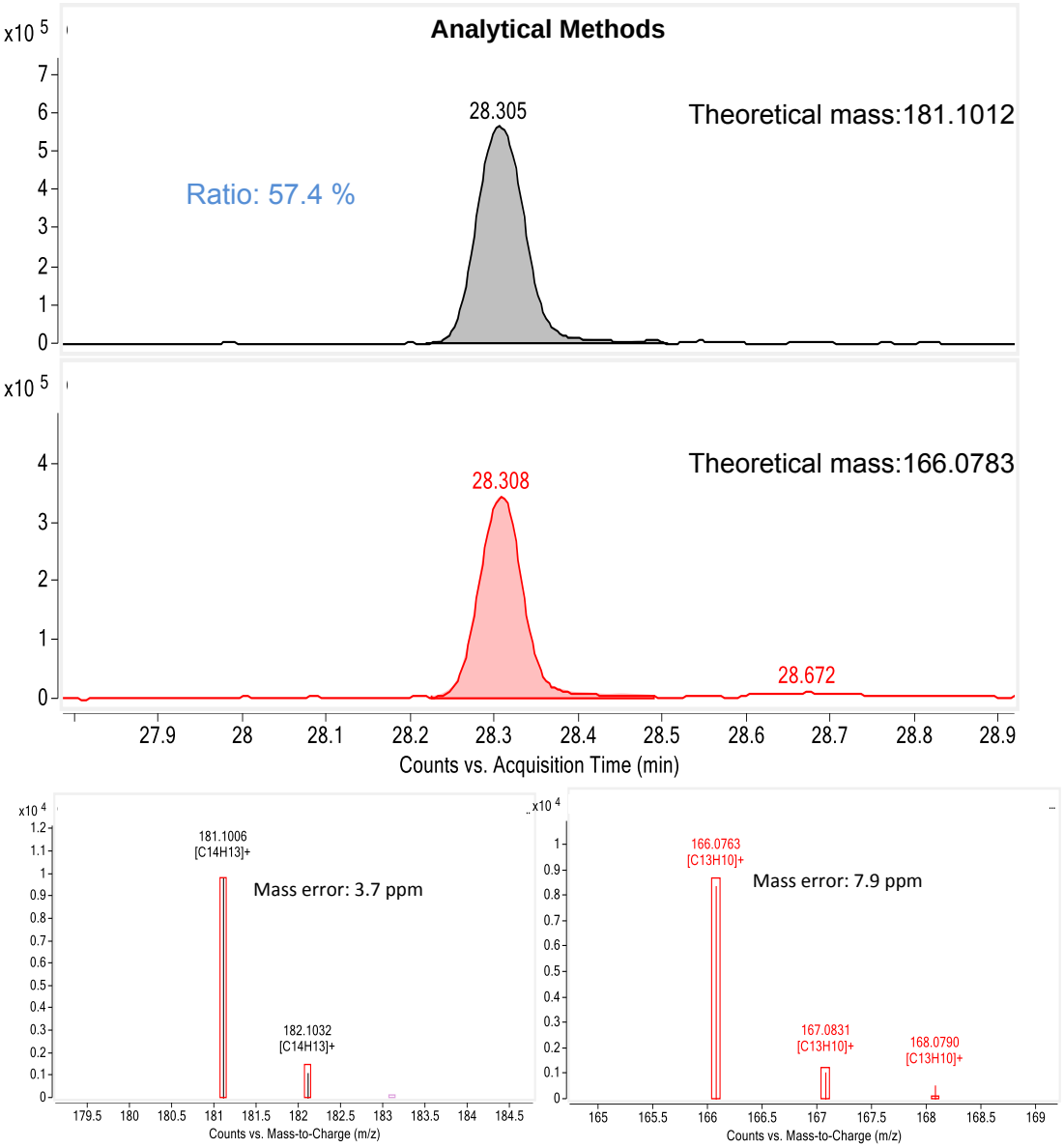

Figure 4. b) Automatic identification of bifenthrin standard in matrix matched in tomato at 0.10 mg/kg.

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