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RESEARCH PAPER

Negative chemical ionization gas chromatography coupled to hybrid quadrupole time-of-flight mass spectrometry and automated accurate mass data processing for determination of pesticides in fruit and vegetables

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Abstract Gas chromatography coupled to high resolution hybrid quadrupole time-of-flight mass spectrometry (GC-QTOF MS), operating in negative chemical ionization (NCI) mode and combining full scan with MSMS experiments using accurate mass analysis, has been explored for the automated determination of pesticide residues in fruit and vegetables. Seventy compounds were included in this approach where 50 % of them are not approved by the EU legislation. A global 76 % of the analytes could be identified at 1 μg kg^{-1}. Recovery studies were developed at three concentration levels (1, 5, and 10 μg kg^{-1}). Seventy-seven percent of the detected pesticides at the lowest level yielded recoveries within the 70 %–120 % range, whereas 94 % could be quantified at 5 μg kg^{-1}, and the 100 % were determined at 10 μg kg^{-1}. Good repeatability, expressed as relative standard deviation (RSD <20 %), was obtained for all compounds. The main drawback of the method was the limited dynamic range that was observed for some analytes that can be overcome either diluting the sample or lowering the injection volume. A home-made database was developed and applied to an automatic accurate mass data processing. Measured mass accuracies of the generated ions were mainly less than 5 ppm for at least one diagnostic ion. When only one ion was obtained in the single-stage NCI-MS, a representative product ion from MSMS experiments was used as identification criterion. A total of 30 real samples were analyzed and 67 % of the samples were positive for 12 different pesticides in the range 1.0–1321.3 μg kg^{-1}.

Keywords Gas chromatography · Quadrupole time-of-flight mass spectrometry · Negative chemical ionization · High resolution · Pesticides

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Introduction

Fruit and vegetables are the primary food most consumed throughout the world. To keep the produce free from pests and plagues, feed the population, as well as to have reasonable business productivity, farmers apply pesticides through the whole production chain. But pesticides are noxious to human health and the environment, forcing food authorities to keep a strict control on the occurrence of its residues on foodstuff. This control can be only pursued if regulatory and surveillance organisms have the appropriate analytical methodologies which, in an effective and confident way, are able to check the accomplishment of the ruling maximum residue limits (MRLs) for the almost 900 pesticides in the agrochemical market. As new toxicological information is gathered,

pesticide MRLs are continuously lowered [1, 2], forcing the development of more sensitive analytical methodology. Food for special groups of population like children and babies is specifically targeted [3], which bears the lowest MRLs and, in some cases, must be pesticides free, which is also the case for ecological products. For such cases, the limit of identification (LOI) and the limit of quantification (LOQ) of the analytical methods are never low enough. Sample preparation is a useful tool to reach high sensitivity but, nowadays, only coupled with the most advanced instrumental is it possible to determine trace amounts (mg kg^{-1}) of hundreds of pesticides simultaneously, either approved or not in different matrices.

Increasing sensitivity in pesticide residue analysis is fostered by mass spectrometry developments. Twenty years ago, it has been through the use of single quadrupole (MS) in SIM mode. More recently, the advent of tandem mass spectrometry (MS-MS), which only can achieve unit mass resolution (MS) or are limited to the selected transitions (MS-MS), allowed the sensitive determination of pesticide residues in most of the food matrices. Few years ago, the use of high-resolution mass spectrometry (HRMS) has shown to be a useful tool for the determination and elucidation of known and unknown compounds [4, 5], at trace level, due to its high resolution power to discriminate between different molecular formula with the same nominal mass. As the full mass spectrum is recorded, it is possible to look for specific compounds afterwards through retrospective analysis. Nowadays, the trends in food and environmental science are the use of HRMS for pesticide residue and their metabolites determination, which can be applied in combination of different ionization modes as electron ionization (EI) [6–8] atmospheric pressure chemical ionization (APCI) [9–11], negative and positive chemical ionization (NCI and PCI, respectively) [6, 12, 13]. Particularly, HRMS and NCI are an excellent combination to provide a selective-scope, based on the difference of electronegativity between the compounds that can be ionized. Compounds bearing halogenated, NO_2, or P ester groups are electron-acceptor and have enhanced response up to several orders of magnitude with NCI [14]. While EI commonly results in a more extensive fragmentation and covers a wide-scope of pesticides, NCI provides limited fragmentation and relevant M- ions in such spectra. Some reports show promising results using NCI in single stage-MS combined with μ-ECD [15] or with MSMS experiment at low resolution [16, 17], but there are few examples in the literature that report on the application of the coupling of NCI-QTOFMS, looking for accurate mass analysis with mass errors less than 5 ppm [6, 12]. The use of HRMS allows the use of libraries of exact masses for identification purposes and gives indications of possible analytes. The usefulness of this approach has been explored by our group either to identify emerging contaminants or their degradation products [18]. Furthermore, an automated mass accurate data processing method is an excellent tool to analyze huge amounts of samples in a short time, assuring the correct detection and determination of pesticide residues in ecological and pooled samples, where the absence of pesticide residues has to be assured.

The aim of this work was to evaluate the hybrid GC-QTOF instrumentation operating in negative chemical ionization mode, for the determination and quantification of 70 common pesticides (22.8 % of these are not included in the coordinated multi-annual control program of the European Union [19]). Using Mass Hunter software, we report on the evaluation of the automated accurate mass data processing method of the diagnostic ions generated by NCI mode in fruit and vegetables. The fitness of the method was checked through the analysis for pesticide residues of 30 real samples from the Almería area.

Experimental

Reagents

Acetone obtained from J.T. Baker (Deventer, The Netherlands), ethyl acetate (EtAc) from Fluka Analytical Pestanal (Steinheim, Germany), and acetonitrile (MeCN) from Sigma-Aldrich (Steinheim, Germany) were used throughout the work. All high purity reference standards were purchased from Dr. Ehrenstofer (Augsburg, Germany), Sigma-Aldrich (Steinheim, Germany), and Riedel-de Häen (Selze, Germany), and were stored at –30 °C. Individual pesticide stock solutions (1000–2000 μg mL^{-1}) were prepared by dissolving reference standards in the appropriate solvent and stored in amber screw-capped glass vials in the dark at –20 °C. Two working solutions at 10 μg mL^{-1}, used for the calibration, were prepared by diluting stock solutions in EtAc. Anhydrous magnesium sulphate ($MgSO_4$) was obtained from Panreac Quimica S.A. and sodium chloride (NaCl) was from J.T. Baker.

GC-MS/MS system and chromatographic conditions

An Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), equipped with an Agilent autosampler, was coupled to an accurate-mass quadrupole time-of-flight (Q-TOF) mass spectrometer, Agilent 7200, operating in NCI mode. Agilent MassHunter software GC-MS Acquisition ver. B07.01 and MassHunter Qualitative analysis B06.00 were used to acquire and to process data obtained, respectively.

The samples were injected using a multimode injector inlet in splitless mode. The injection volume was 2 μL and was carried out at 280 °C. Helium and nitrogen, both with a purity of 99.999 %, were used as carrier and collision gases, respectively. The GC separation was performed using two fused

silica HP-5MS UI capillary column of 15 m×0.25 mm inner diameter and a film thickness of 0.25 μm (Agilent, Palo Alto, CA, USA) connected by a capillary flow technology (CFT) union. The oven temperature was programmed as follows: 60 °C for 1 min; 40 °C min^{-1} to 120 °C, and finally up to 310 °C at 5 °C min^{-1}. The total run time was 40.5 min with two additional minutes for backflushing at 310 °C. Backflushing was used to shorten the analysis time and reduce system maintenance. The source and interface temperatures were set to 150 and 280 °C, respectively, and a solvent delay of 4 min was selected in order to prevent damage in the ion source filament. Retention time locked (RTL) setting was used to eliminate the need for adjusting retention times of the compounds, employing chlorpyrifos methyl as the locking compound at the retention time of 18.11 min. The instrument worked at constant flow.

Backflush system

The end of the chromatographic column is connected to the second column through a CFT union (used as purged capillary flow device), which allows system backflushing. 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 to –5.7 mL min^{-1}.

Mass spectrometer conditions: negative chemical ionization

The ion source and quadrupole temperatures were fixed at 150 °C. Methane was used as reagent gas (purity 99.995 %) and was set at a flow of 40 % (2 mL min^{-1}). The ionization energy was set at 70 eV and the emission current at 50 μA. Mass spectrometric grade perfluorotributylamine (PFTBA) was used daily as external calibrant in TOF mass calibration and, when a complete autotune was done, for quadrupole calibration. To ensure mass accuracy during analysis, a TOF mass calibration is carried out prior to each injection, being its eight *m/z* exact masses adjusted. This step can be programmed in the sequence table and when the internal mass calibration is not approved, the run is stopped automatically. Looking for the optimization of the MS parameters for each compound, all pesticides were monitored in full scan mode and TOF MS was registering at 2, 3, 4, 5, and 10 spectrum s^{-1}, acquiring in the 50–550*m/z* mass range. TOF MS resolution was about 12,000 (FWHM). For some pesticides, additional diagnostic ions can be obtained by fragmentation in the QTOF by performing acquisition in MS^2 mode. For its acquisition, the optimum collision energy for each precursor ion was selected after evaluating the fragment intensities at different collision energies (ranging from 3 to 30 eV). For MS^2 experiments, the quadrupole isolates the precursor ion at a medium MS resolution and the linear hexapole collision cell fragments it using nitrogen at 1.5 mL min^{-1}.

Validation procedure

In order to validate the method, parameters as percentage of recovery, linearity, limits of identification and quantification, and repeatability were studied. For the recovery studies, five replicates at three different concentration levels were done. For this, three representative portions of 60 g of homogenized sample were spiked with the mixture standard solution. Each portion was weighed and transferred to a beaker and fortified homogeneously with 60, 300, and 600 μL of a 1000 μg L^{-1} standard solution in ethyl acetate in order to obtain 1, 5, and 10 μg kg^{-1}, respectively. The mixtures were gently mixed in a beaker for 30 min and, following this, they were kept standing for another 30 min. After that, the ethyl acetate extraction procedure [20] described below was carried out. To determine linearity and detection limits, a volume of 50 μL of tomato blank extract was evaporated and reconstituted with an equal volume of the desired concentration of the mix in ethyl acetate in the range of 0.1–500 μg L^{-1}. Finally, repeatability of the instrumental method was evaluated by analyzing five replicates at two concentration levels: 5 and 20 μg kg^{-1} on the same day.

Real samples

A total of 30 samples of various kinds of fruits, vegetables, and fresh herbs (apple, asparagus, aubergine, bean, chive, dill, lettuce, mango, onion, parsley, papaya, pear, pepper, pineapple, plum, potato, rosemary, spinach, tomato) were analyzed. Fruits and vegetables were purchased from different local markets in Almeria (south-eastern Spain) and fresh herbs were from a local herbal store. All samples were stored in their original packaging under the recommended conditions until use.

The fresh matrices were chopped and triturated separately. After homogenization, a 10 g portion of sample was weighed and transferred into a 50 mL PTFE centrifugal tube. Following this, 10 mL of ethyl acetate was added and then the tube was shaken vigorously for 3 s by hand. Afterwards, 8 g of anhydrous magnesium sulphate and 1.5 g of sodium chloride were added and the samples were shaken in an automatic axial extractor (AGYTAX; Cirta Lab. S.L., Madrid, Spain) for 15 min. The extract was then centrifuged (3500 rpm) for 5 min, ending up with the equivalent of 1 g of sample per mL in 100 % ethyl acetate. The obtained final extract was injected into the GC-(NCI)-QTOF MS and GC-(EI)-QqQ MS systems.

Results and discussion

Optimization of the GC-(NCI)-QTOF MS acquisition method

The mass selective detector with high resolution quadrupole time-of-flight analyzer was operated in negative chemical ionization mode. A home-made database containing the ions generated in the ionization was developed. To build this database, a 50 μg kg^{-1} solution of standards was injected and all ions observed were matched to its molecular formula. The retention time and the ions monitored for each pesticide, used for identification and quantification, are presented in Table 1.

The ionization energy was evaluated by two trials using 70 and 140 eV. These values were chosen for different reasons: 140 eV value was setting for default after adjusting the masses of the calibrant (the best signal for PFTBA was obtained using 140 eV), and 70 eV is a common value used in different laboratories. The results showed no difference in the molecular ionization between both potentials and showed chromatograms with the same sensitivity, so that 70 eV was chosen as ionization energy value along all the work.

TOF MS was operated at 2, 3, 4, 5, and 10 spectrum s^{-1} acquiring in the 50–550 *m/z* mass range at 20, 50, and 100 μg kg^{-1}, the latter being the concentration where saturation phenomena were expected. Nevertheless, the more influencing parameter in detector saturation during method development was the speed of spectra acquisition (see Fig. 1). At lower acquisition speed, higher sensitivity was observed and, consequently, a greater number of compounds saturated the detector (see Fig. 1). At the same acquisition rate and higher concentrations, the effect was more remarkable, as expected. Similar saturation phenomena for the same pesticides were observed at higher acquisition speed at 50 and 100 μg kg^{-1}, respectively. The two concurrent phenomena registered at 2 spectrum s^{-1} compelled us to select a compromise acquisition speed of 3 spectrum s^{-1}.

As we have mentioned, saturated peaks could be seen in some analytes at different concentration levels: 20, 50, 100, 200, and 500 μg kg^{-1}. The detector saturation was evidenced especially for giving a double peak observed when the base peak (Q ion) at its exact mass was extracted with a narrow window of 10 ppm (see Fig. 1c). In some cases, no double peak was observed; nevertheless, the peaks had a plateau. In both cases, the experimental q/Q ratios (q: qualifier ion) using this saturated quantifier ion did not fit with the theoretical ones. Because of this phenomenon, the confirmation of analytes by evaluating the acceptable q/Q ion ratio could be a risk. To solve this problem, three different solutions can be useful: diluting samples, injecting less volume, and working with a higher number of spectrum s^{-1} lowering the sensitivity.

CID fragmentation (MS^2 approach)

MS^2 approach was employed as some pesticides presented only one fragment in full scan. For MSMS experiments, it was necessary to evaluate the speed of acquisition. Looking for the better conditions 20, 100, and 200 ms $spectrum^{-1}$ were evaluated. From the data obtained, it could be seen that at higher acquisition speed, lower intensity was observed. Finally, 200 ms $spectrum^{-1}$ was chosen to develop the method.

Precursor ion fragmentation was performed by collision induced dissociation with nitrogen, from which the best product ion was chosen. The collision gas flow was 1.5 mL min^{-1}. The optimum collision energy for each precursor ion was selected after evaluating the intensity of each fragment ion at different collision energies (CEs). The adequate CE for each transition was assayed in the range between 3 and 30 eV. The suitable CE is the one for which the selected precursor ion produces relative abundance approximately 25 % with respect to the selected product ion. The transitions obtained with the possible molecular formula, its neutral exact mass, and CEs chosen are shown in Table 2.

Data processing method

After optimizing the acquisition parameters for each compound, a target method to process full scan MS and MS^2 was developed. MassHunter Qualitative analysis was used to process data obtained from standards and samples in the analysis of target compounds. The processing method developed was linked to a home-made database of compounds to monitor the exact mass of each fragment ion using a narrow window of ±10 ppm and ±0.2 min over the established retention time. The home-made database contains two diagnostic ions from the full scan spectrum for each analyte with its molecular formula, exact neutral masses, retention times, and the names of the compounds. Detection is based both in the exact mass of each ion as in the retention time for each analyte according to the DG-SANCO [21]. The general methodology applied was based on the evaluation of the presence of the radical that was detected through its formula and searching for its accurate mass at the expected retention time. Two diagnostic ions were included for each pesticide in processing the screening method; in some cases, the two ions were from the MS spectrum and in others, one ion was from the MS spectrum and the other one was from the MS^2 spectrum. For identification purposes, the mass accuracy requirement must be less than 5 ppm for at least one diagnostic ion.

Optimization of automated searching parameters

The target screening method was developed aiming to identify pesticides with mass accuracy errors less than 5 ppm and not

Table 1 Identification parameters for each pesticide in single-stage MS mode: name, retention time (Rt, min), molecular formula, and the diagnostic ions with its molecular formula and exact neutral. F1: is used to differentiate the diagnostic ions during the data processing

Compound	Rt (min)	Molecular formula	Diagnostic ion (DI)	Molecular formula of DI	Exact mass of DI
Acrinathrin	30.653	C26H21F6NO5	M-C14H10O	C12H11F6O4	333.0562
Acrinathrin F1			M-C15H10O2	C11H11F6O3	305.0612
Aldrin	19.536	C12H8Cl6	M-C7H8Cl	C5Cl5	234.8443
Aldrin F1			M-C7H8Cl*	C5Cl4[37Cl]	236.8414
Azoxystrobin	37.006	C22H17N3O5	M-CH3OH	C21H13N3O4	371.0906
Azoxystrobin F1			M-C2H6OH	C20H10N3O4	356.0671
Bifenox	28.694	C14H9Cl2NO5	M$^-$	C14H9Cl2NO5	340.9858
Bifenox F1			M-C6H3Cl2	C8H6NO5	196.0246
Bifenthrin	28.270	C23H22ClF3O2	M-C14H14Cl	C9H8F3O2	205.0471
Bifenthrin F1			M-HCl	C23H21F3O2	386.1494
Bupirimate	23.970	C13H24N4O3S	M-C11H18N3	C2H6NO3S	124.0068
Bupirimate F1			M-C2H6NO2S	C11H18N3O	208.1450
Carbophenothion	25.837	C11H16ClO2PS3	M-C7H6CLS	C4H10O2PS2	184.9860
Carbophenothion F1			M-C5H12O2PS2	C6H4ClS	142.9722
Chinomethionat	21.845	C10H6N2OS2	M-CO	C9H6N2S2	205.9972
Chinomethionat isotopic peak			M-CO*	C9H6N2S[34S]	207.9930
Chlorfenapyr	24.407	C15H11BrClF3N2O	M-C3H6OH	C12H4BrClF3N2	346.9198
Chlorfenapyr isotopic peak			M-C3H6OH*	C12H4Br[37Cl]F3N2	348.9177
Chlorfenvinphos	21.506	C12H14Cl3O4P	M-C8H4Cl3	C4H10PO4	153.0317
Chlorfenvinphos F1			M-C10H8Cl3	C2H6O4P	125.0004
Chlorothalonil	16.645	C8Cl4N2	M$^-$	C8Cl4N2	263.8816
Chlorothalonil isotopic peak			M$^-$*	C8Cl3[37Cl]N2	265.8787
Chlorpyrifos	19.939	C9H11Cl3NO3PS	M-HCl	C9H10Cl2NO3PS	312.9496
Chlorpyrifos F1			M-C4H10O3P	C5HCl3NS	211.8895
Chlorpyrifos-Methyl	18.053	C7H7Cl3NO3PS	M-C2H6O3P	C5HCl3NS	211.8895
Chlorpyrifos-Methyl F1			M-C5HCl3N	C2H6O3PS	140.9775
Chlozolinate	21.354	C13H11Cl2NO5	M-CO2	C12H11Cl2NO3	287.0116
Chlozolinate isotopic peak			M-CO2*	C12H11Cl[37Cl]NO3	289.0089
Cypermethrin	33.315/33.516/ 33.652/33.729	C22H19Cl2NO3	M-C14H10NO	C8H9Cl2O2	206.9980
Cypermethrin F1			M-C14H11ClNO	C8H8ClO2	171.0213
Dichlofluanid	19.388	C9H11Cl2FN2O2S2	M-C3H6Cl2FNS	C6H5NO2S	155.0041
Dichlofluanid F1			M-CCl2FS	C8H11N2SO2	199.0541
Dicloran	14.706	C6H4Cl2N2O2	M$^-$	C6H4Cl2N2O2	205.9650
Dicloran isotopic peak			M$^-$*	C6H4Cl[37Cl]N2O2	207.9620
Dieldrin	23.332	C12H8Cl6O	M-C7H8ClO	C5Cl5	234.8443
Dieldrin isotopic peak			M-C7H8ClO*	C5Cl4[37Cl]	236.8414
Endosulfan alpha	22.380	C9H6Cl6O3S	M-CH2Cl2O3S	C8H4Cl4	239.9067
Endosulfan alpha F1			M$^-$	C9H6Cl6O3S	403.8169
Endosulfan beta	24.465	C9H6Cl6O3S	M$^-$	C9H6Cl6O3S	403.8169
Endosulfan beta F1			M-CH2Cl2O3S	C8H4Cl4	239.9067
Endosulfan Sulfate	26.027	C9H6Cl6O4S	M-HCl	C9H5Cl5O4S	383.8351
Endosulfan Sulfate F1			M$^-$	C9H6Cl6O4S	419.8118
Ethion	25.141	C9H22O4P2S4	M-C5H12O2S2	C4H10O2PS2	184.9860
Ethion isotopic peak			M-C5H12O2S2*	C4H10O2PS[34S]	186.9818
Fenarimol	30.266	C17H12Cl2N2O	M-HCl	C17H11ClN2O	294.0560
Fenarimol isotopic peak			M-HCl*	C17H11[37Cl]N2O	296.0530
Fenhexamid	26.132	C14H17Cl2NO2	M-HCl	C14H16ClNO2	265.0870

Table 1 (continued)

Compound	Rt (min)	Molecular formula	Diagnostic ion (DI)	Molecular formula of DI	Exact mass of DI
Fenhexamid isotopic peak			M-HCl*	C14H16[37Cl]NO2	267.0840
Fenitrothion	19.118	C9H12NO5PS	M-C2H6O3P	C7H6NO2S	168.0119
Fenitrothion F1			M^{-}	C9H12NO5PS	277.0174
Fenpropathrin	28.460	C22H23NO3	M-C14H10NO	C8H13O2	141.0916
Fipronil	21.593	C12H4Cl2F6N4OS	M-CHClF3	C11H3ClF3N4OS	330.9668
Fipronil F1			M-CHF3	C11H3Cl2F3N4OS	365.9357
Fipronil sulfone	23.889	C12H4Cl2F6N4O2S	M-HCl	C12H3ClF6N4O2S	415.9569
Fipronil sulfone F1			M-CF3	C11H4Cl2F3N4O2S	382.9384
Flucythrinate I	33.759	C26H23F2NO4	M-C15H10NO3	C11H13F2O	199.0934
Flucythrinate I F1			M-C14H10NO	C12H13F2O3	243.0833
Flucythrinate II	34.136	C26H23F2NO4	M-C15H10NO3	C11H13F2O	199.0934
Flucythrinate II F1			M-C14H10NO	C12H13F2O3	243.0833
Fluquinconazole	31.884	C16H8Cl2FN5O	M-HCl	C16H7ClFN5O	339.0323
Fluquinconazole F1			M-CHN	C15H7Cl2FN4O	347.9981
Fluvalinate-tau	35.484/35.604	C26H22ClF3N2O3	M-C14H10NO	C12H12ClF3NO2	294.0509
Fluvalinate-tau isotopic peak			M-C14H10NO*	C12H12[37Cl]F3NO2	296.0479
Fonofos	15.894	C10H15OPS2	M-C6H5	C4H10OPS2	168.9911
Fonofos F1			M-C4H10OPS	C6H5S	109.0112
Heptachlor	18.246	C10H5Cl7	M-HCl3	C10H4Cl4	263.9067
Heptachlor isotopic peak			M-HCl3*	C10H4Cl3[37Cl]	265.9038
Heptachlorepoxide I	21.038	C10H5Cl7O	M-C5H5Cl2O	C5Cl5	234.8443
Heptachlorepoxide I isotopic peak			M-C5H5Cl2O*	C5Cl4[37Cl]	236.8413
Heptachlorepoxide II	21.208	C10H5Cl7O	M-C5H5Cl2O	C5Cl5	234.8443
Heptachlorepoxide II isotopic peak			M-C5H5Cl2O*	C5Cl4[37Cl]	236.8413
Hexaconazole	22.972	C14H17Cl2N3O	M-C4H9Cl	C10H8ClN3O	221.0356
Hexaconazole F1			M-C4H8	C10H9Cl2N3O	257.0123
Iprodione	27.747	C13H13Cl2N3O3	M-CO	C12H13Cl2N3O2	301.0385
Iprodione isotopic peak			M-CO*	C12H13Cl[37Cl]N3O2	303.0355
Lambda-Cyhalothrin	30.222	C23H19ClF3NO3	M-C14H11ClNO	C9H8F3O2	205.0476
Lambda-Cyhalothrin F1			M-C14H10NO	C9H9ClF3O2	241.0243
Lindane (HCH-Gamma)	15.523	C6H6Cl6	M-Cl	C6H6Cl5	252.8912
Lindane (HCH-Gamma) isotopic peak			M-Cl*	C6H6Cl4[37Cl]	254.8883
Malaoxon	18.209	C10H19O7PS	M-C8H13O4	C2H6PO3S	140.9780
Malaoxon F1			M-C2H7O3PS	C8H12O4	172.0741
Malathion	19.604	C10H19O6PS2	M-C8H13O4	C2H6PO2S2	156.9547
Methidathion	22.044	C6H11N2O4PS3	M-C4H5N2O2S	C2H6PO2S2	156.9547
Myclobutanil	23.683	C15H17ClN4	M^{-}	C15H17ClN4	288.1142
Myclobutanil peak			M^{-*}	C15H17[37Cl]N4	290.1117
Nuarimol	26.714	C17H12ClFN2O	M-HCl	C17H11FN2O	278.0855
Nuarimol F1			M-H3ClO	C17H9FN2	260.0750
Ofurace	25.739	C14H16ClNO3	M-HCl	C14H15NO3	245.1052
Paraoxon-Methyl	16.458	C8H10NO6P	M^{-}	C8H10NO6P	247.0245
Paraoxon-Methyl F1			M-C6H4NO2	C2H6O4P	125.0004
Parathion	19.962	C10H14NO5PS	M-C6H4NO2	C4H10O3PS	169.0088
Parathion F1			M-C4H10O3P	C6H4NO2S	153.9963
Parathion-Methyl	18.039	C8H10NO5PS	M-C2H6O3P	C6H4NO2S	153.9963
Parathion-Methyl F1			M^{-}	C8H10NO5PS	263.0017

Table 1 (continued)

Compound	Rt (min)	Molecular formula	Diagnostic ion (DI)	Molecular formula of DI	Exact mass of DI
Pendimethalin	21.137	C13H19N3O4	M^-	C13H19N3O4	281.1376
Phosalone	29.335	C12H15ClNO4PS2	M-C8H5ClNO2	C4H10O2PS2	184.9860
Phosalone isotopic peak			M-C8H5ClNO2*	C4H10O2PS[34S]	186.9818
Phosmet	27.920	C11H12NO4PS2	M-C9H6NO2	C2H6O2PS2	156.9547
Phosmet F1			M-C2HO2PS2	C9H7NO2	161.0477
Phosmet-oxon	26.190	C11H12NO5PS	M-C9H6NO2	C2H6O3PS	140.9775
Phosmet-oxon F1			M-C2H5O3PS	C9H7NO2	161.0477
Propiconazole	26.348	C15H17Cl2N3O2	M-C5H9O	C10H8Cl2N3O	256.0044
Propiconazole isotopic peak			M-C5H9O*	C10H8Cl[37Cl]N3O	258.0015
Propyzamide	15.934	C12H11Cl2NO	M^-	C12H11Cl2NO	255.0218
Propyzamide F1			M-C5H7	C7H4Cl2NO	187.9670
Prothiofos	23.139	C11H15Cl2O2PS2	M-C4H12OP	C7H3Cl2OS2	236.9002
Prothiofos F1			M-C3H7	C8H8Cl2O2PS2	300.9080
Pyrazophos	30.609	C14H20N3O5PS	M-C10H10N3O2	C4H10O3PS	169.0088
Pyrazophos F1			M^-	C14H20N3O5PS	373.0861
Pyridaben	31.740	C19H25ClN2OS	M-C11H15	C8H10ClN2OS	217.0202
Pyridaben isotopic peak			M-C11H15*	C8H10[37Cl]N2OS	219.0173
Pyrifenox I	21.299	C14H12Cl2N2O	M-CH5ClO	C13H7ClN2	226.0298
Pyrifenox I isotopic peak			M-CH5ClO*	C13H7[37Cl]N2	228.0272
Pyrifenox II	22.251	C14H12Cl2N2O	M-CH5ClO	C13H7ClN2	226.0298
Pyrifenox II isotopic peak			M-CH5ClO*	C13H7[37Cl]N2	228.0272
Quinoxyfen	25.983	C15H8Cl2FNO	M-HCl	C15H7ClFNO	271.0200
Quinoxyfen isotopic peak			M-HCl*	C15H7[37Cl]FNO	273.0171
Tefluthrin	16.831	C17H14ClF7O2	M-C8H5F4	C9H9O2ClF3	241.0243
Tefluthrin F1			M-C8H6ClF4	C9H8F3O2	205.0476
Tetrachlorvinphos	22.488	C10H9Cl4O4P	M-C8H3Cl4	C2H6O4P	125.0004
Tetraconazole	20.320	C13H11Cl2F4N3O	M-C11H10Cl2N3	C2HF4O	116.9964
Tetradifon	28.971	C12H6Cl4O2S	M-HCl	C12H5Cl3O2S	317.9076
Tetradifon isotopic peak			M-HCl*	C12H5Cl2[37Cl]O2S	319.9046
Tolclofos-Methyl	18.216	C9H11Cl2O3PS	M-CH3Cl	C8H8ClO3PS	249.9620
Tolclofos-Methyl F1			M-HCl	C9H10ClO3PS	263.9777
Tolylfluanid	21.322	C10H13Cl2FN2O2S2	M-CHCl2FS	C9H12N2O2S	212.0619
Trifloxystrobin	26.433	C20H19F3N2O4	M-C10H11F3NO	C10H8NO3	190.0504
Trifluralin	13.888	C13H16F3N3O4	M^-	C13H16F3N3O4	335.1093
Vinclozolin	18.063	C12H9Cl2NO3	M-CO2	C11H9Cl2NO	241.0061
Vinclozolin isotopic peak			M-CO2*	C11H9Cl[37Cl]NO	243.0033

detecting false positives or negatives automatically. Pursuing this objective, it was necessary to consider how the MassHunter software determines mass errors. It is important to note that in the database, neutral mass must be used, as the calculation performed by the software takes into account the mass of the electron when the ion is generated. If the mass accuracy is determined manually is necessary to add an electron mass at the theoretical neutral mass. In the software, some parameters are available to be used, such as the average scans that can be taken at different percentages of peak height. In our work, we evaluated three strategies for average scans: > 0 %, 35 %, and 50 % of peak height (see Fig. 2). Using these strategies, it was possible to see that highest errors were obtained at the beginning of the peak, and also in the tail. In light of these results, the error was estimated by calculating the average at the 50 % upper part of the peak. Also, as the error increased in the region of saturation, the criterion of excluding 10 % of the peak was

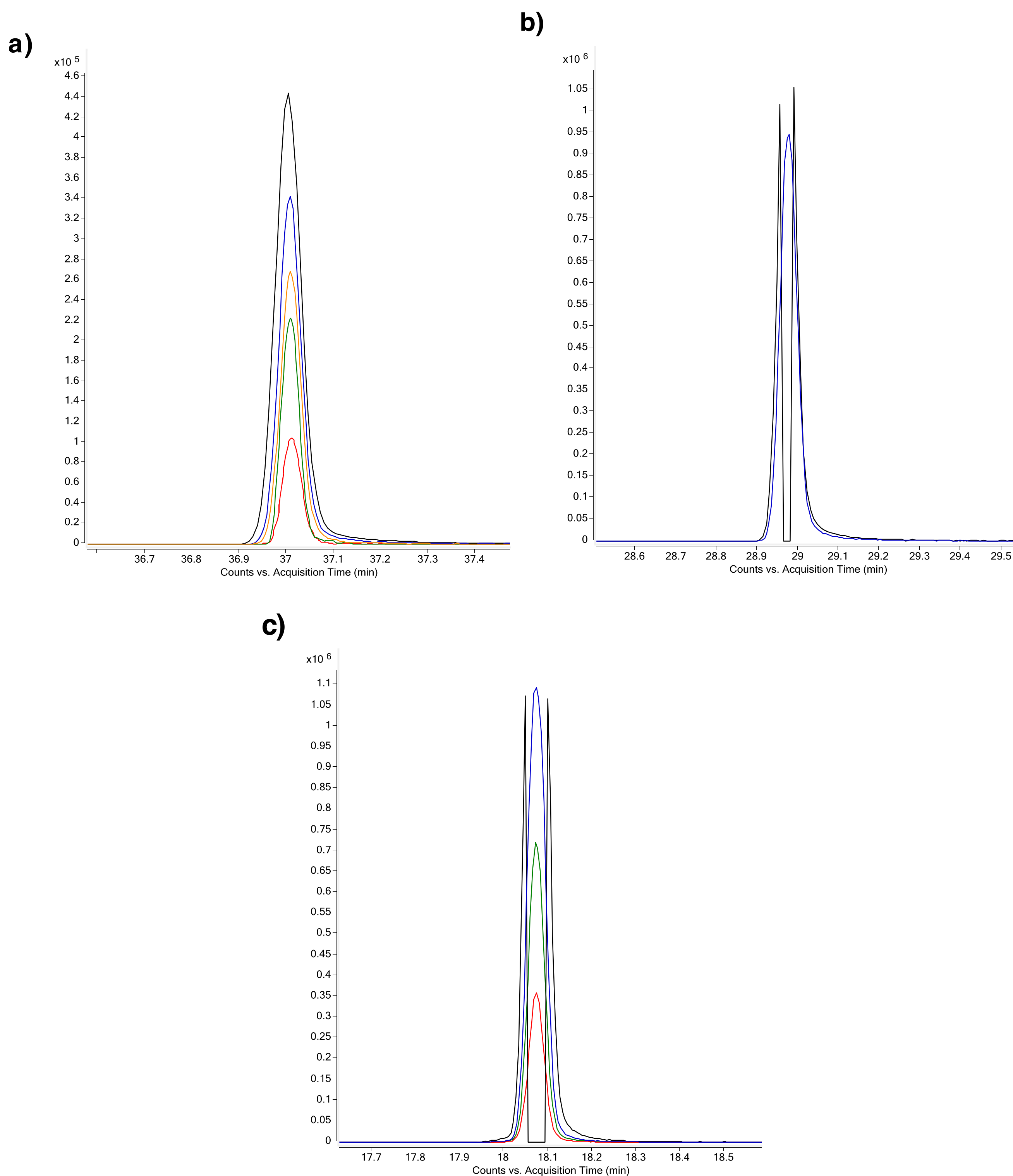

Fig. 1 (**a**) Extracted ion chromatogram for azoxystrobin ion (*m/z* 371.0911) at different acquisition speed (spectrum s^{-1}): black, blue, orange, green, and reed (2, 3, 4, 5, and 10 spectrum s^{-1}, respectively); (**b**) saturation effect observed for tetradifon ion (*m/z* 317.9081) with the acquisition speed, comparing 2 and 3 spectrum s^{-1} (black and blue, respectively); (**c**) Saturation effect observed for vinclozolin ion (*m/z* 241.0066) at the selected acquisition speed (3 spectrum s^{-1}) with various concentration levels: 5, 10, 20, and 50 μg kg^{-1} (red, green, blue, and black, respectively)

set up. Other parameters for obtaining the extracted ion chromatogram (EIC), the cleaned spectrum for each analyte detected and extracting MS/MS spectrum per CE with precursor tolerance ±10 ppm can be set.

Table 2 Identification parameters for each pesticide in MSMS experiment: name, retention time (Rt, min), precursor ion, product ion, molecular formula, and collision energy (CE, eV). Also mass accuracy of product ion at 10 μg kg^{-1} is presented

Compound	Rt (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Molecular formula	CE (eV)	Error (ppm) at 10 μg kg^{-1}
Fenpropathrin	28.460	141.0916	97.1022	C7H13	10	0.0
Malathion	19.604	156.9547	141.9317	CH3O2PS2	20	8.5
Methidathion	22.044	156.9547	141.9317	CH3O2PS2	20	3.5
Ofurace	25.739	245.1052	126.0191	C5H4NO3	10	7.9
Pendimethalin	21.137	281.1376	251.1396	C13H19N2O3	8	6.8
Tetrachlorvinphos	22.488	125.0004	78.9585	O3P	20	0.0
Tetraconazole	20.320	116.9964	96.9901	C2F3O	5	7.2
Tolylfluanid	21.322	212.0624	168.0119	C7H6NO2S	10	4.8
Trifloxystrobin	26.433	190.0509	158.0242	C9H4NO2	8	1.9
Trifluralin	13.888	335.1093	305.1113	C13H16F3N2O3	8	1.3

Application of automated home-made accurate mass data processing

Mass accuracy was evaluated in several injections at different concentration levels. Matrix matched calibrations were analyzed by the processing method looking for false negative detection while real samples previously analyzed in GC-QqQ MS served as a source of possible false positive detection. Retention time shifts were lower than 0.2 min for all the studied compounds and typical errors obtained from automated processed results of each chromatogram at 1, 5, and 10 μg kg^{-1} are shown in Table 3. For at least one ion of the total 70 analyzed pesticides, the accuracy of the masses measured was less than 5 ppm in almost 100 % at the three concentration levels. In the case of the second ion, the accuracy of the masses measured was less than 10 ppm, except for chinomethionate isotopic peak (C9H6N2S[34S]) and fenhexamid isotopic peak (C14H16[37Cl]NO2) that presented larger errors consistently. Several factors can affect the measure of mass accuracy when TOFMS is used. The principal one is due to saturation of the detector, but also variables such as temperature changes, data acquisition rate, and resolving power also play a role [6]. As five replicates at two concentration levels (5 and 20 μg kg^{-1}) were injected to check the repeatability of the area for validation parameters, we were able to evaluate the repeatability of the automatic application of the home-made accurate mass database. No false negatives were obtained using the database and all the ions were detected in each analysis, under the parameters previously described.

Analytical parameters evaluated

Method sensitivity: limits of detection, limits of quantification, and linearity of calibration curves

Based on the calibration curves, the LOIs of the method were determined. A matrix matched calibration curve in tomato was

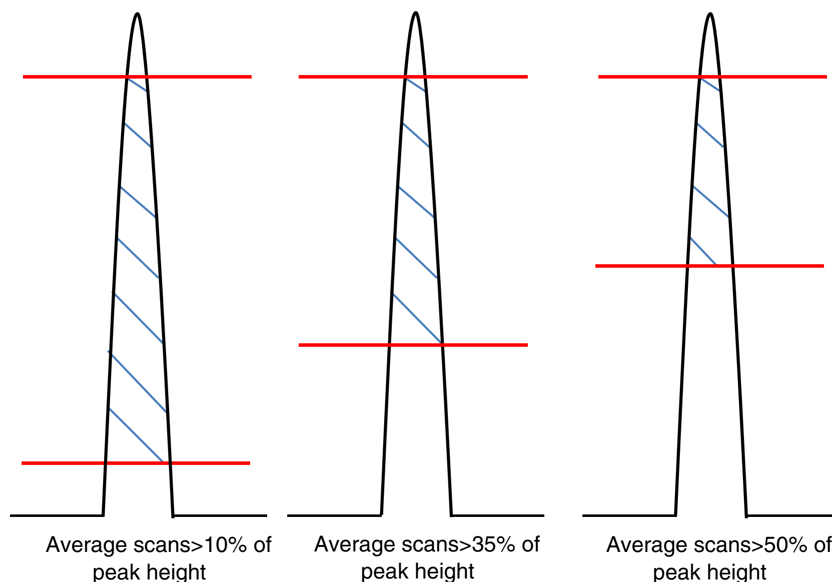

Fig. 2 Three different approaches evaluated in the automatic search, varying the height of the peak (>10 %, >35 %, and >50 %) where the spectrum is selected to make an average while the part of the peak eliminated to avoid saturation is kept constant in 10 %

Table 3 Typical mass errors at 1, 5, and 10 μg kg^{-1}

Compound	Rt (min)	Exact mass	Molecular formula	Mass error (ppm)		
				1 μg kg^{-1}	5 μg kg^{-1}	10 μg kg^{-1}
Acrinathrin	30.653	333.0562	C12H11F6O4	0.2	1.0	0.4
Acrinathrin F1		305.0612	C11H11F6O3	7.3	3.8	3.4
Aldrin	19.536	234.8443	C5Cl5	1.7	2.2	1.8
Aldrin F1		236.8414	C5Cl4[37Cl]	3.4	1.6	2.1
Azoxystrobin	37.006	371.0906	C21H13N3O4	6.7	1.2	0.7
Azoxystrobin F1		356.0671	C20H10N3O4	1.8	3.2	1.4
Bifenox	28.694	340.9858	C14H9Cl2NO5	2.1	1.0	0.5
Bifenox F1		196.0246	C8H6NO5	2.4	0.7	0.5
Bifenthrin	28.270	205.0471	C9H8F3O2	4.4	5.6	2.9
Bifenthrin F1		386.1494	C23H21F3O2	0.2	2.5	2.5
Bupirimate	23.970	124.0068	C2H6NO3S	9.5	0.2	2.2
Bupirimate F1		208.1450	C11H18N3O	7.6	7.2	3.1
Carbophenothion	25.837	184.9860	C4H10O2PS2	3.8	3.6	5.9
Carbophenothion F1		142.9722	C6H4ClS	9.2	0.1	1.0
Chinomethionat	21.845	205.9972	C9H6N2S2	0.6	5.2	0.8
Chinomethionat isotopic peak		207.9930	C9H6N2S[34S]	5.5	27.4	25.5
Chlorfenapyr	24.407	346.9198	C12H4BrClF3N2	0.0	1.8	1.1
Chlorfenapyr isotopic peak		348.9177	C12H4Br[37Cl]F3N2	0.7	1.3	1.8
Chlorfenvinphos	21.506	153.0317	C4H10PO4	1.3	1.3	3.7
Chlorfenvinphos F1		125.0004	C2H6O4P	2.7	1.9	3.8
Chlorothalonil	16.645	263.8816	C8Cl4N2	1.5	2.5	2.5
Chlorothalonil isotopic peak		265.8787	C8Cl3[37Cl]N2	1.4	2.3	2.5
Chlorpyriphos	19.939	312.9496	C9H10Cl2NO3PS	2.9	3.4	0.7
Chlorpyrifos F1		211.8895	C5HCl3NS	4.0	2.1	2.2
Chlorpyrifos-Methyl	18.053	211.8895	C5HCl3NS	2.0	4.0	2.4
Chlorpyrifos-Methyl F1		140.9775	C2H6O3PS	2.2	3.5	3.4
Chlozolinate	21.354	287.0116	C12H11Cl2NO3	0.7	3.3	0.8
Chlozolinate isotopic peak		289.0089	C12H11Cl[37Cl]NO3	0.3	3.3	0.4
Cypermethrin	33.315 33.516 33.652 33.729	206.9980	C8H9Cl2O2	nd	4.7	0.3
Cypermethrin F1		171.0213	C8H8ClO2	nd	1.1	2.5
Dichlofluanid	19.388	155.0041	C6H5NO2S	0.5	1.9	3.0
Dichlofluanid F1		199.0541	C8H11N2SO2	2.5	0.6	4.3
Dicloran	14.706	205.9650	C6H4Cl2N2O2	0.7	1.3	4.4
Dicloran isotopic peak		207.9620	C6H4Cl[37Cl]N2O2	1.6	1.7	3.5
Dieldrin	23.332	234.8443	C5Cl5	0.3	2.0	1.4
Dieldrin isotopic peak		236.8414	C5Cl4[37Cl]	0.6	1.4	1.3
Endosulfan alpha	22.380	239.9067	C8H4Cl4	0.3	3.5	1.0
Endosulfan alpha F1		403.8169	C9H6Cl6O3S	3.5	1.3	3.3
Endosulfan beta	24.465	403.8169	C9H6Cl6O3S	0.9	1.2	1.4
Endosulfan beta F1		239.9067	C8H4Cl4	2.2	3.9	1.4
Endosulfan Sulfate	26.027	383.8351	C9H5Cl5O4S	1.2	3.0	0.4
Endosulfan Sulfate F1		419.8118	C9H6Cl6O4S	7.5	2.2	1.9
Ethion	25.141	184.9860	C4H10O2PS2	7.0	0.1	0.3
Ethion isotopic peak		186.9818	C4H10O2PS[34S]	1.0	3.0	2.2

Table 3 (continued)

Compound	Rt (min)	Exact mass	Molecular formula	Mass error (ppm)		
				1 μg kg^{-1}	5 μg kg^{-1}	10 μg kg^{-1}
Fenarimol	30.266	294.0560	C17H11ClN2O	1.4	2.4	0.2
Fenarimol isotopic peak		296.0530	C17H11[37Cl]N2O	0.5	3.0	3.1
Fenhexamid	26.132	265.0870	C14H16ClNO2	nd	5.4	0.8
Fenhexamid isotopic peak		267.0840	C14H16[37Cl]NO2	nd	15.0	7.9
Fenitrothion	19.118	168.0119	C7H6NO2S	3.2	2.1	2.8
Fenitrothion F1		277.0174	C9H12NO5PS	3.8	2.7	2.3
Fenpropathrin	28.460	141.0916	C8H13O2	nd	1.3	0.6
Fipronil	21.593	330.9668	C11H3ClF3N4OS	7.1	3.0	1.0
Fipronil F1		365.9357	C11H3Cl2F3N4OS	3.4	2.5	0.1
Fipronil sulfone	23.889	415.9569	C12H3ClF6N4O2S	nd	0.0	1.6
Fipronil sulfone F1		382.9384	C11H4Cl2F3N4O2S	nd	2.1	2.5
Flucythrinate I	33.759	199.0934	C11H13F2O	4.9	0.1	0.3
Flucythrinate I F1		243.0833	C12H13F2O3	5.3	0.6	0.6
Flucythrinate II	34.136	199.0934	C11H13F2O	2.0	0.5	0.6
Flucythrinate II F1		243.0833	C12H13F2O3	8.5	1.0	0.8
Fluquinconazole	31.884	339.0323	C16H7ClFN5O	1.0	0.6	0.4
Fluquinconazole F1		347.9981	C15H7Cl2FN4O	1.7	5.8	8.1
Fluvalinate-tau	35.484 35.604	294.0509	C12H12ClF3NO2	0.1	0.2	0.1
Fluvalinate-tau isotopic peak		296.0479	C12H12[37Cl]F3NO2	0.7	0.8	1.4
Fonofos	15.894	168.9911	C4H10OPS2	3.1	1.4	4.9
Fonofos F1		109.0112	C6H5S	1.5	5.8	2.7
Heptachlor	18.246	263.9067	C10H4Cl4	0.3	3.8	0.3
Heptachlor isotopic peak		265.9038	C10H4Cl3[37Cl]	1.2	4.5	0.1
Heptachlorepoxide I	21.038	234.8443	C5Cl5	nd	2.0	2.3
Heptachlorepoxide I isotopic peak	21.038	236.8413	C5Cl4[37Cl]	nd	1.4	3.5
Heptachlorepoxide II	21.208	234.8443	C5Cl5	nd	2.2	2.6
Heptachlorepoxide II isotopic peak	21.208	236.8413	C5Cl4[37Cl]	nd	1.9	2.4
Hexaconazole	22.972	221.0356	C10H8ClN3O	4.5	3.3	2.5
Hexaconazole F1		257.0123	C10H9Cl2N3O	6.3	1.0	0.9
Iprodione	27.747	301.0385	C12H13Cl2N3O2	2.1	0.9	0.6
Iprodione isotopic peak		303.0355	C12H13Cl[37Cl]N3O2	1.8	1.6	1.7
Lambda-Cyhalothrin	30.222	205.0476	C9H8F3O2	3.2	1.5	0.7
Lambda-Cyhalothrin F1		241.0243	C9H9ClF3O2	1.1	1.7	0.5
Lindane (HCH-Gamma)	15.523	252.8912	C6H6Cl5	4.9	0.0	5.4
Lindane (HCH-Gamma) isotopic peak		254.8883	C6H6Cl4[37Cl]	10.2	0.9	4.3
Malaoxon	18.209	140.9780	C2H6PO3S	nd	3.1	2.8
Malaoxon F1		172.0741	C8H12O4	nd	2.1	1.9
Malathion	19.604	156.9547	C2H6PO2S2	nd	0.4	5.5
Methidathion	22.044	156.9547	C2H6PO2S2	2.4	1.1	4.1
Myclobutanil	23.683	288.1142	C15H17ClN4	nd	nd	0.3
Myclobutanil isotopic peak		290.1117	C15H17[37Cl]N4	nd	nd	0.3
Nuarimol	26.714	278.0855	C17H11FN2O	7.6	2.8	0.5
Nuarimol F1		260.0750	C17H9FN2	0.2	2.8	0.3
Ofurace	25.739	245.1052	C14H15NO3	nd	3.4	3.7
Paraoxon-Methyl	16.458	247.0245	C8H10NO6P	nd	0.4	1.5
Paraoxon-Methyl F1		125.0004	C2H6O4P	nd	1.1	2.3

Table 3 (continued)

Compound	Rt (min)	Exact mass	Molecular formula	Mass error (ppm)		
				1 μg kg^{-1}	5 μg kg^{-1}	10 μg kg^{-1}
Parathion	19.962	169.0088	C4H10O3PS	0.3	1.6	3.3
Parathion F1		153.9963	C6H4NO2S	1.2	3.0	2.2
Parathion-Methyl	18.039	153.9963	C6H4NO2S	3.4	2.8	3.7
Parathion-Methyl F1		263.0017	C8H10NO5PS	2.9	1.5	3.8
Pendimethalin	21.137	281.1376	C13H19N3O4	nd	3.4	1.0
Phosalone	29.335	184.9860	C4H10O2PS2	4.2	0.8	4.7
Phosalone isotopic peak		186.9818	C4H10O2PS[34S]	5.7	2.1	1.4
Phosmet	27.920	156.9547	C2H6O2PS2	6.7	0.2	1.7
Phosmet F1		161.0477	C9H7NO2	0.3	1.5	0.0
Phosmet-oxon	26.190	140.9775	C2H6O3PS	nd	1.1	2.3
Phosmet-oxon F1		161.0477	C9H7NO2	nd	0.9	1.6
Propiconazole	26.348	256.0044	C10H8Cl2N3O	nd	0.9	2.1
Propiconazole isotopic peak		258.0015	C10H8Cl[37Cl]N3O	nd	1.9	2.6
Propyzamide	15.934	255.0218	C12H11Cl2NO	1.2	1.1	2.5
Propyzamide F1		187.9670	C7H4Cl2NO	0.9	0.7	0.1
Prothiofos	23.139	236.9002	C7H3Cl2OS2	1.2	3.5	0.3
Prothiofos F1		300.9080	C8H8Cl2O2PS2	2.3	3.0	0.3
Pyrazophos	30.609	169.0088	C4H10O3PS	1.8	1.4	2.2
Pyrazophos F1		373.0861	C14H20N3O5PS	2.1	0.5	4.1
Pyridaben	31.740	217.0202	C8H10ClN2OS	0.7	3.3	0.1
Pyridaben isotopic peak		219.0173	C8H10[37Cl]N2OS	17.1	0.1	0.0
Pyrifenox I	21.299	226.0298	C13H7ClN2	nd	2.4	2.4
Pyrifenox I isotopic peak		228.0272	C13H7[37Cl]N2	nd	4.0	1.9
Pyrifenox II	22.251	226.0298	C13H7ClN2	nd	2.4	2.2
Pyrifenox II isotopic peak		228.0272	C13H7[37Cl]N2	nd	5.9	2.7
Quinoxyfen	25.983	271.0200	C15H7ClFNO	4.8	3.7	0.6
Quinoxyfen isotopic peak		273.0171	C15H7[37Cl]FNO	10.0	7.3	4.7
Tefluthrin	16.831	241.0243	C9H9O2ClF3	0.5	3.3	2.5
Tefluthrin F1		205.0476	C9H8F3O2	0.3	5.0	1.0
Tetrachlorvinphos	22.488	125.0004	C2H6O4P	0.3	2.7	3.8
Tetraconazole	20.320	116.9964	C2HF4O	3.8	4.0	3.1
Tetradifon	28.971	317.9076	C12H5Cl3O2S	2.4	2.2	0.0
Tetradifon isotopic peak		319.9046	C12H5Cl2[37Cl]O2S	3.5	3.1	0.9
Tolclofos-Methyl	18.216	249.9620	C8H8ClO3PS	0.4	3.4	1.5
Tolclofos-Methyl F1		263.9777	C9H10ClO3PS	5.0	3.6	2.1
Tolylfluanid	21.322	212.0619	C9H12N2O2S	5.0	0.7	0.1
Trifloxystrobin	26.433	190.0504	C10H8NO3	nd	0.2	1.0
Trifluralin	13.888	335.1093	C13H16F3N3O4	4.1	1.2	4.1
Vinclozolin	18.063	241.0061	C11H9Cl2NO	1.3	4.9	1.4
Vinclozolin isotopic peak		243.0033	C11H9Cl[37Cl]NO	0.2	5.2	1.1

injected, ranging from 0.1 to 500 μg kg^{-1}. To establish the LOI for each pesticide, the two selected ions (in the case of pesticides fully identified with full scan) had to be present, at least one diagnostic ion with mass accuracy less than 5 ppm and a signal-to-noise ratio higher than 3. For pesticides in MS^2, this criterion is needed for the precursor ion in full scan, whereas for the MS^2 experiment only the presence of the transition is needed. MSMS experiment was developed for each case and at least one transition as the second parameter for a complete identification. The LOIs for

most of compounds (75.7 %) were between 0.1 and 1 μg kg^{-1}. However, around 23 % of the compounds had LOIs between 2 and 5 μg kg^{-1} and only myclobutanil had a LOI of 10 μg kg^{-1}. The LOIs and LOQs obtained for the studied pesticides are presented in Table 4.

According to the DG-SANCO [21], LOQ is the lowest level where a full validation has been carried out accomplishing acceptability criteria (mean recoveries in the 70 %–120 % range with RSD ≤20 %). Recovery studies were developed at three different concentration levels (1, 5, and 10 μg kg^{-1}) with five replicates for each concentration. The LOQ for each compound was also obtained from these experiments. Around 57 % of the studied analytes showed a 1 μg kg^{-1} LOQ, whereas less than 36 % presented 5 μg kg^{-1} LOQ and only 7 % of pesticides had 10 μg kg^{-1} of LOQ (aldrin, heptachlor, myclobutanil, propiconazole, and trifloxystrobin). In the case of propiconazole and trifloxystrobin, the RSDs obtained were 22 % and 26 %, respectively, at 10 μg kg^{-1}. The linearity was evaluated in the 1–500 μg kg^{-1} range for all pesticides spiked in tomato. Some pesticides showed a narrow lineal range of calibration curves because of the saturation phenomena. In Table 4 is presented the linear range for each compound and its correlation coefficient (R^2) values. For chinomethionate, the linear range is delimited to 1–10 μg kg^{-1}, whereas for tetracholvinphos and tolyfluanid their quantification is only possible in the 5–10 μg kg^{-1} range, owing to a combined saturation problem of the diagnostic ions employed in single-stage MS and the low sensitivity achieved for the selected transition in the MSMS experiment.

The dynamic limited range observed for some analytes is the major drawback of the GCQTOF MS system used. This is a common problem of the TOF MS systems attributable to fast saturation of the detector [22] hampering the analyte quantification at different levels depending of the sensitivity of the compound. However, this problem can be overcome by selecting characteristic ion fragments with lower abundance or istopes (e.g., Cl34, C13) because the stability of the isotopic pattern in TOF MS is very high. This approach, when necessary, does not represent a big negative impact in the workflow of the laboratory. Other approaches such as sample dilution or smaller volume injection are also applicable. But, in these last cases, duplicate analyses should be necessary.

Recoveries and repeatability

In order to assay the method trueness, recoveries trials were performed as described earlier. The precision was also evaluated and it was expressed in terms of relative standard deviation (RSD). In the lowest fortified level (1 μg kg^{-1}), recoveries ranging from 70 % to 120 % were accomplished in around 77 % of detected pesticides, whereas at 5 μg kg^{-1} 94 % were in the acceptable range see Table 4. At 10 μg kg^{-1}, all pesticides were detected and the recovery rates were in the range of 70 % to 120 %. The RSDs obtained in the recovery experiments were below 20 %, except for propiconazole (26 %), trifloxystrobin (22 %) at 10 μg kg^{-1}, and nuarimol (25 %) at 1 μg kg^{-1}.

The repeatability of the instrumental method was also evaluated at 5 and 20 μg kg^{-1} and was expressed in terms of RSDs. The results demonstrated that the method was repeatable, obtaining RSDs <15 % at 5 μg kg^{-1} and RSDs <20 % at 20 μg kg^{-1}.

Analysis of real samples

In order to check the positives found by GC-(NCI)-QTOF, a comparison was carried out with results obtained by GC-(EI)-QqQ MSMS using two transitions in the MRM mode. Table 5 shows the positives of some pesticides found in real samples. In GC-(NCI)-QTOF, the developed automatic method described above (±10 ppm extraction window and ±0.2 min over the established exact masses and retention times, respectively) for the application of the home-made database was employed to the evaluation of real samples. Positives were compared with the matrix matched calibration curve, both in the q/Q ratio and in the intensity, for quantification. A 30 % tolerance in the q/Q ratio was allowed. The method proposed in the present communication allowed the quantification of eight pesticides for which the concentration level was below the LOQ of the QqQ, as well as detecting 14 pesticides that the triple Quad could not detect.

A total of 30 real samples were analyzed using GC-(NCI)-QTOF, resulting in 33.3 % of the samples that could be considered free of the target pesticides and 66.7 % of the samples that contained at least one pesticide. The range of concentration was between 1–2936 μg kg^{-1} and no pesticide exceeded the EU MRLs. Of the positive samples, 80 % contained one or two pesticides, whereas the rest had three or more pesticides. A total of 12 different pesticides were detected in all the samples. The most common pesticides found were chlorpyrifos ethyl (in 16 samples), bupirimate (in six samples), and iprodione (in four samples). Only one pesticide not approved by the European Union was found (chlorfenapyr at LOQ), which could not be detected by GC-QqQ MSMS because of the LOI for this compound.

Conclusions

In the present work, a multiresidue acquisition procedure that can be coupled to an automatic data processing method has been implemented and validated for the simultaneous quantification of 70 pesticides in tomato using a home-made database. GC-QTOF operated in negative chemical ionization mode has proven to be a key tool for qualitative

Table 4 Linear range, detection limits (LODs), quantification limits (LOQs), and repeatability at 5 and 20 μg kg^{-1} for each studied pesticide, expressed in μg kg^{-1}. Average recovery (R, %), and relative standard deviation (RSD, %) for the GC-(NCI) QTOF applied to tomato matrix (n=5) at three spiked levels (1, 5, and 10 μg kg^{-1})

Compound	Rt (min)	LOD (μg/kg)	LOQ (μg/kg)	Linear range (μg/kg)	R^2	Repeatability, RSD (%)		1 μg kg^{-1}		5 μg kg^{-1}		10 μg kg^{-1}	
						5 μg kg^{-1}	20 μg kg^{-1}	R (%)	RSD (%)	R (%)	RSD (%)	R (%)	RSD (%)
Acrinathrin	30.653	0.5	1.0	1-20	0.9997	2.0	2.3	79.0	8.3	80.3	1.2	86.7	1.3
Aldrin	19.536	0.5	10.0	10-200	0.9971	3.4	0.4	61.4	11.2	66.0	4.2	71.1	2.0
Azoxystrobin	37.006	1.0	1.0	1-500	0.9994	2.3	1.5	76.1	17.4	81.0	5.7	77.4	3.6
Bifenox	28.694	1.0	1.0	1-50	0.9965	0.8	1.5	75.3	2.8	80.7	3.6	83.8	2.9
Bifenthrin	28.270	0.5	1.0	1-200	0.9998	3.0	3.8	82.2	7.8	85.6	2.8	87.6	2.9
Bupirimate	23.970	1.0	1.0	1-100	0.9995	2.9	4.3	70.9	6.9	75.7	5.5	85.3	6.1
Carbophenothion	25.837	1.0	1.0	1-20	0.9995	2.0	2.0	81.6	4.6	82.7	3.7	89.9	3.6
Chinomethionat	21.845	1.0	1.0	1-10	0.9995	0.8	*	91.0	6.2	103.0	4.7	107.5	1.1
Chlorfenapyr	24.407	0.1	1.0	1-20	0.9992	1.6	0.8	77.1	3.5	77.0	3.6	77.8	1.9
Chlorfenvinphos	21.506	1.0	5.0	5-20	0.9989	1.7	0.3	67.7	5.7	75.4	4.4	77.4	2.7
Chlorothalonil	16.645	0.1	1.0	1-10	0.9914	1.0	0.4	86.0	5.0	91.2	2.9	85.8	3.1
Chlorpyrifos	19.939	0.1	1.0	1-50	0.9998	1.1	1.8	80.3	3.7	77.7	2.7	79.7	1.8
Chlorpyrifos-Methyl	18.053	0.5	1.0	1-50	0.9999	1.2	1.2	73.3	4.4	73.9	2.4	74.1	2.8
Chlozolinate	21.354	0.1	5.0	5-20	0.9892	1.1	1.1	64.8	5.2	70.1	3.1	71.2	2.5
Cypermethrin	33.315 33.516 33.652 33.729	2.0	5.0	5-500	0.9990	3.2	3.6	nd	-	77.5	8.0	84.5	1.4
Dichlofluanid	19.388	1.0	5.0	5-200	0.9969	2.4	8.0	68.3	9.5	75.8	2.2	74.2	2.2
Dicloran	14.706	0.5	1.0	1-50	0.9973	3.6	0.8	84.7	9.6	84.3	2.0	85.8	1.4
Dieldrin	23.332	0.5	1.0	1-200	0.9966	1.3	1.6	77.0	4.9	77.4	2.9	79.6	1.7
Endosulfan alpha	22.380	1.0	1.0	1-200	0.9939	0.9	2.3	76.6	8.2	78.4	4.6	76.8	0.5
Endosulfan beta	24.465	0.5	1.0	1-500	0.9914	4.0	1.6	76.4	7.7	78.1	3.5	78.2	3.1
Endosulfan Sulfate	26.027	1.0	1.0	1-100	0.9988	1.5	2.2	70.6	4.1	75.4	5.0	74.3	1.1
Ethion	25.141	1.0	1.0	1-20	0.9996	4.9	1.9	76.3	2.6	77.3	3.4	82.7	3.7
Fenarimol	30.266	1.0	1.0	1-500	0.9999	3.3	2.3	70.2	1.6	79.1	2.2	78.8	2.6
Fenhexamid	26.132	5.0	5.0	5-500	0.9974	3.0	2.1	nd	-	102.4	5.1	86.4	2.9
Fenitrothion	19.118	0.5	1.0	1-100	0.9991	3.4	1.1	78.2	3.3	81.4	3.5	83.3	3.2
Fenpropathrin	28.460	2.0	5.0	5-50	0.9999	1.5	1.3	nd	-	80.6	3.3	83.9	1.9
Fipronil	21.593	0.5	1.0	1-100	0.9978	2.8	3.0	78.0	9.0	79.4	4.4	74.4	5.6
Fipronil sulfone	23.889	5.0	5.0	5-100	0.9996	2.3	2.1	nd	-	71.4	2.3	74.5	3.5
Flucythrinate I	33.759	0.5	1.0	1-200	0.9988	1.0	0.2	83.0	2.6	82.1	3.5	86.2	0.7
Flucythrinate II	34.136	0.5	1.0	1-200	0.9995	3.0	3.4	83.6	0.3	82.8	2.5	86.8	1.1
Fluquinconazole	31.884	0.5	1.0	1-200	0.9995	1.1	1.4	72.9	6.9	77.7	3.6	79.4	2.7
Fluvalinate-tau	35.484 35.604	0.5	1.0	1-100	0.9995	0.8	2.2	75.5	3.3	77.4	3.9	82.9	0.9
Fonofos	15.894	0.5	1.0	1-20	0.9992	3.6	0.7	74.9	4.3	80.2	2.7	82.6	2.7
Heptachlor	18.246	0.5	10.0	10-200	0.9958	2.5	1.2	53.2	7.2	68.3	2.5	70.5	1.5
Heptachlorepoxide I	21.038	5.0	5.0	5-200	0.9992	1.5	1.7	nd	-	74.8	3.2	76.5	4.2
Heptachlorepoxide II	21.208	5.0	5.0	5-200	0.9991	1.8	2.1	nd	-	77.9	2.6	76.5	2.8
Hexaconazole	22.972	0.5	1.0	1-100	0.9991	1.0	2.0	72.1	6.1	77.1	1.8	81.4	8.3
Iprodione	27.747	0.5	1.0	1-500	0.9963	7.9	1.7	87.4	5.7	80.0	2.3	77.6	5.8
Lambda-Cyhalothrin	30.222	0.5	1.0	1-50	0.9987	2.5	0.9	78.6	3.3	81.7	3.3	83.0	5.5
Lindane (HCH-Gamma)	15.523	5.0	5.0	5-500	0.9996	14.9	2.9	nd	-	85.3	3.7	77.8	7.7
Malaoxon	18.600	1.0	5.0	5-100	0.9997	1.8	1.5	68.7	12.5	75.4	3.2	78.7	1.5

Table 4 (continued)

Compound	Rt (min)	LOD (μg/kg)	LOQ (μg/kg)	Linear range (μg/kg)	R^2	Repeatability, RSD (%)		1 μg kg^{-1}		5 μg kg^{-1}		10 μg kg^{-1}	
						5 μg kg^{-1}	20 μg kg^{-1}	R (%)	RSD (%)	R (%)	RSD (%)	R (%)	RSD (%)
Malathion	19.604	2.0	5.0	5-50	0.9992	2.1	3.0	nd	-	74.3	3.0	80.4	5.4
Methidathion	22.044	1.0	1.0	1-500	0.9949	3.5	4.0	77.7	8.5	80.2	4.4	82.5	2.7
Myclobutanil	23.683	10.0	10.0	10-500	0.9931	1.6	0.3	nd	-	nd	-	86.6	6.7
Nuarimol	26.714	1.0	5.0	5-500	0.9982	3.6	2.9	71.1	25.6	79.1	5.2	83.7	12.4
Ofurace	25.739	2.0	5.0	5-500	0.9998	2.5	3.3	nd		89.3	7.2	94.1	5.6
Paraoxon-Methyl	16.458	5.0	5.0	5-200	0.9997	2.4	2.7	nd	-	75.6	3.5	88.1	5.6
Parathion	19.962	0.5	1.0	1-200	0.9922	4.3	1.6	81.9	4.3	79.4	2.0	78.8	1.4
Parathion-Methyl	18.039	0.5	1.0	1-200	0.9967	3.2	2.4	79.3	5.8	82.4	1.1	84.8	2.0
Pendimethalin	21.137	5.0	5.0	5-200	0.9977	2.2	1.7	nd	-	82.4	4.1	84.7	2.2
Phosalone	29.335	0.5	1.0	1-20	0.9992	4.0	2.4	73.0	1.3	75.4	4.8	79.3	4.2
Phosmet	27.920	1.0	1.0	1-50	0.9991	1.2	2.6	72.0	5.8	77.2	3.1	78.2	3.5
Phosmet oxon	26.190	5.0	5.0	5-50	0.9989	3.5	1.9	nd	-	71.2	5.6	72.8	4.8
Propiconazole	26.348	2.0	10.0	10-500	0.9994	13.5	6.0	nd	-	57.8	22.8	75.6	25.9
Propyzamide	15.934	1.0	5.0	5-500	0.9998	1.7	2.1	63.5	10.7	82.3	2.9	86.6	3.4
Prothiofos	23.139	0.1	1.0	1-50	0.9999	2.2	2.0	75.6	4.2	78.2	1.9	81.4	2.2
Pyrazophos	30.609	2.0	5.0	5-500	0.9963	9.0	19.7	nd	-	72.5	12.0	77.4	2.1
Pyridaben	31.740	0.5	1.0	1-200	0.9995	2.7	2.7	73.0	9.2	79.7	4.2	82.9	1.9
Pyrifenox I	21.299	2.0	5.0	5-500	0.9998	2.3	5.9	nd	-	81.5	4.5	89.8	3.1
Pyrifenox II	22.251	2.0	5.0	5-500	0.9999	5.4	3.2	nd	-	86.3	3.1	87.3	3.7
Quinoxyfen	25.983	1.0	5.0	5-200	0.9995	0.9	3.0	64.3	18.3	80.7	7.8	84.0	2.6
Tefluthrin	16.831	0.5	5.0	5-20	0.9980	0.7	1.2	63.3	4.7	70.9	2.5	76.6	2.3
Tetrachlorvinphos	22.488	0.1	5.0	5-10	0.9992	0.7	*	66.1	7.9	76.9	6.0	78.9	12.5
Tetraconazole	20.320	0.5	1.0	1-100	0.9972	3.2	3.7	90.7	4.0	83.2	3.0	81.0	1.8
Tetradifon	28.971	0.1	1.0	1-20	0.9983	1.1	1.4	75.7	3.6	74.8	4.4	76.7	2.3
Tolclofos-Methyl	18.216	1.0	1.0	1-200	0.9940	1.7	1.9	77.3	7.7	76.5	3.3	79.7	2.6
Tolylfluanid	21.322	0.5	5.0	5-10	0.9998	0.1	1.9	69.2	5.9	71.9	2.6	75.2	3.4
Trifloxystrobin	26.433	2.0	10.0	10-200	0.9984	1.0	3.6	nd	68.0	64.0	5.9	78.5	21.8
Trifluralin	13.888	0.5	5.0	5-20	0.9996	2.0	0.6	62.3	6.3	72.3	2.4	75.8	1.5
Vinclozolin	18.063	0.1	1.0	1-20	0.9938	1.9	1.2	72.7	4.7	75.6	2.5	77.2	2.9

and quantitative determination of pesticides because of its high sensitivity and selectivity, providing very low LOIs (75.7 % of pesticides had a LOI ≤1 μg kg^{-1}). The high selectivity of the NCI makes matrix interferences limited. This is related to the kinds of pesticides that are able to be analyzed in NCI mode, where most of them are halogenated ones, which usually have, by large, the lowest acute reference dose (ARfD) values. The use of QTOF was useful allowing the MSMS experiment, providing valuable fragmentation information, and high-mass accuracy of product ions for their use as second diagnostic ion in the identification step.

Automated accurate mass analysis in NCI was achieved using QTOF mass spectrometer with MassHunter. Data obtained for radical anions generated by NCI afforded mass accuracies within 5 ppm for most ions generated with excellent repeatability, with no false positives or negatives informed. In addition, 30 samples from Almería were analyzed, where chlorpyrifos, bupiramate, and iprodione were the most commonly-found pesticides with mass accuracies consistently below 5 ppm in at least one diagnostic ion. The reporting and detection limits of the GC-NCI(Q)TOF method were lower than the GC-(EI)-QqQ, a standard procedure for pesticide residue analysis, which was used for comparison as it was able to quantify and detect 22 affectional pesticides. From the results obtained, the combined use of HRMS and NCI shows the potential of a useful tool to analyze samples containing pesticide residues at very low concentrations and to ensure their absence in case of organically cultivated samples or baby foods. It is important to note that commercial libraries combining high resolution and different ionization modes are not currently

Table 5 Pesticide concentrations (μg kg^{-1}) found in different real samples analyzed by GC-(NCI)-QTOF and GC-QqQ using the described approach

Real sample	Compound	C (μg/Kg)	
		GC-QqQ	GC-QTOF
Apple	Bupirimate	<LOQ	4.1
	Chlorpyrifos	nd	<LOQ
Pear	Chlorpyrifos	<LOQ	4.9
	Chlorpyrifos-methyl	12.2	16.7
	Iprodione	1274.0	1356.1
	Propiconazole	<LOQ	<LOQ
Mango	Chlorpyrifos	nd	<LOQ
	Iprodione	nd	2.6
Apple	Chlorpyrifos	12.8	17.0
	Lambda-cyhalothrin	<LOQ	4.5
	Pyridaben	10.9	31.5
Tomato	Chlorpyrifos	nd	<LOQ
	Fenhexamid	21.4	12.4
Plum	Chlorfenapyr	nd	<LOQ
	Iprodione	1185.7	1321.3
Onion	Chlorpyrifos	<LOQ	<LOQ
Lettuce	Chlorpyrifos	nd	<LOQ
Spinach	Chlorpyrifos	<LOQ	<LOQ
	Cypermethrin	191.5	269.9
Zucchini	Chlorpyrifos	nd	<LOQ
Beans	Azoxystrobin	15.1	16.1
Coliflower	Chlorpyrifos	<LOQ	<LOQ
Apple	Bupirimate	<LOQ	2.8
	Chlorpyrifos	nd	<LOQ
Papaya	Chlorfenapyr	nd	<LOQ
Tomato	Chlorpyrifos	nd	<LOQ
	Fenhexamid	19.6	13.7
Apple	Bupirimate	15.2	15.6
	Chlorpyrifos	nd	<LOQ
Cucumber	Chlorpyrifos	nd	<LOQ
Spring onion	Bupirimate	<LOQ	3.4
	Lambda-cyhalothrin	36.1	35.2
Dill	Azoxystrobin	11.6	8.3
	Bupirimate	<LOQ	3.4
	Chlorpyrifos	30.4	21.4
	Chlorpyrifos-methyl	nd	<LOQ
	Iprodione	191.1	125.1
Curly parsley	Bupirimate	<LOQ	2.7
	Chlorothalonil	nd	1.0
	Chlorpyrifos	<LOQ	<LOQ

nd: not detected

available. This report provides an easy way to obtain this information when working with this type of automatic configuration.

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