Application of gas chromatography time-of-flight mass spectrometry for target and non-target analysis of pesticide residues in fruits and vegetables

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ABSTRACT

In this work, the capability of gas chromatography coupled to time-of-flight mass spectrometry (GC-TOF MS) for quantitative analysis of pesticide residues has been evaluated. A multiclass method for rapid screening of pesticides (insecticides, acaricides, herbicides and fungicides) in fruit and vegetable matrices has been developed and validated, including detection, identification and quantification of the analytes. To this aim, several food matrices were selected: high water content (apples, tomatoes and carrots), high acid content (oranges) and high oil content (olives) samples. The well known QuEChERS procedure was applied for extraction of pesticides, and matrix-matched calibration using relative responses versus internal standard was used for quantification. The sample extracts were analyzed by GC-TOF MS. Up to five ions using narrow window (0.02 Da)-extracted ion chromatograms at the expected retention time were monitored using a target processing method. The most abundant ion was used for quantification while the remaining ones were used for confirmation of the analyte identity. Method validation was carried out for 55 analytes in the five sample matrices tested at three concentrations (0.01, 0.05 and 0.5 mg/kg). Most recoveries were between 70 % and 120 % with relative standard deviations (RSDs) lower than 20 % at 0.05 and 0.5 mg/kg . At 0.01 mg/kg, roughly half of the pesticides could be satisfactorily validated due to sensitivity limitations of GC-TOF MS, which probably affected the ion ratios used for confirmation of identity. In the case of olive samples, results were not satisfactory due to the high complexity of the matrix. An advantage of TOF MS is the possibility to perform a non-target investigation in the samples by application of a deconvolution software, without any additional injection being required. Accurate-mass full-spectrum acquisition in TOF MS provides useful information for analytes

identification, and has made feasible in this work the discovery of non-target imazalil, fluoranthene and pyrene in some of the samples analyzed.

Key words

Fruits and vegetables, pesticides, QuEChERS, GC-TOF MS, target and non-target, quantitative analysis

1. INTRODUCTION

The importance of food quality control is widely recognized nowadays to assure the compliance of regulation of these products and guarantee consumer health. The presence of pesticide residues in food is a matter of concern. For this reason, strict legislation exists at the EU level that establishes maximum residue levels (MRL), i.e. the upper legal concentration allowed for a pesticide residue in or on food or feed [1].

Keeping in mind the large number of pesticides applied worldwide, multiresidue methods are commonly used for monitoring pesticide residues in food. Both gas chromatography (GC) and liquid chromatography (LC) have been widely applied coupled with mass spectrometry (MS) using different analyzers. As regards GC-MS, single quadrupole [2-5], ion trap (ITD) [6-8] or triple quadrupole (QqQ) [3, 9-13], have been frequently used. ITD and QqQ analyzers are normally applied under tandem mass spectrometry (MS/MS) mode, offering notable advantages in sensitivity and selectivity. The information acquired in target MS/MS method is analyte-specific (e.g. characteristic ions/transitions monitored). Therefore, other pesticides that might be present in the samples would not be detected if they are not included in the scope of the method.

The recent progress in instrumentation has increased the use of time-of-flight (TOF) mass analyzers coupled to GC for analyzing pesticides in food [14-18]. The main advantage of TOF MS comes from the full spectrum acquisition, with better sensitivity than conventional scanning instruments (e.g. quadrupole) [19]. There are two commercially available approaches for the time-of-flight analyzers using gas chromatography: high-speed (HS) and high-resolution (HR). HS instruments allow acquiring at 100-500 spectra/s but only provide unit resolution. HS-TOF instruments are

suitable for detection of very narrow chromatographic peaks generated by fast and ultra-fast GC or by GCxGC and most applications reported are focused on quantification. On the other hand, HR instruments have normally 5000-10000 FWHM (full width at half-maximum) resolution and moderate scan speed (up to 20 Hz). HR-TOF has the possibility of resolving matrix components yielding ions with the same nominal mass as that of the target analyte, reducing background interferences and improving the analyte identification [20].

Accurate-mass full-spectrum data available in HR-TOF MS enable to obtain extracted ion chromatograms using narrow mass windows (nw-XICs). Reducing the mass window can notably improve the signal-to-noise due to exclusion of a large proportion of the chemical background and quasi-isobaric interferences. The potential of GC-TOF MS has been mainly explored in the qualitative field pursuing the detection and identification of GC-amenable organic contaminants [19, 21-24]. Up to five *m/z* ions of each target analyte are monitored, using as confirmation of identity criteria the presence of at least two *m/z* ions and the accomplishment of the intensity ratio within established tolerances [25]. Thus, wide-scope screening has been developed and validated from a qualitative point of view for around 150 organic micropollutants in water [24]. The elucidation of non-target compounds is also possible after MS data acquisition, without the need of reinjecting the sample, making use of powerful deconvolution software [19, 21-23, 26, 27]. Although there is wide consensus on the great qualitative potential of HR-TOF MS however its low dynamic range compared with conventionl MS instrumentation limits its quantitative applications and also affects mass accuracy, which can be deteriorated at certain concentration levels. The analog-to-digital converter (ADC) detector offers linear dynamic range of four orders of magnitude but, at low analyte signal intensities, noise becomes a limiting factor. The time-to-digital

converter (TDC) detector, on the contrary, is suitable for detection of weak signals, which is the case of analytes at ultra-traces levels, but it may present problems of saturation at high concentrations. New generations of HR-TOF MS typically use TDC for data acquisition, and allow dynamic range to be extended (DRE). The new DRE option overcomes the problems of saturation and makes quantification easier in HR-TOF MS instruments [15, 19, 20]. Even with some limitations, mainly as regards sensitivity, quantitative applications have been reported using GC-TOF MS in the food safety field [14-18, 28] but it has not been implemented for routine monitoring analysis yet, where GC-MS/MS remains the instrument of choice.

To take full advantage of the capabilities of GC-TOF MS for screening a large number of pesticides, a generic procedure with a wide scope is required. To this aim the QuEChERS method [12, 17, 18, 28-36], a rapid extraction procedure based on the use of acetonitrile as extractant, has been widely applied in food residue analysis. After the original method, developed in 2003 [29], several modifications have improved the scope of the method, like the use of acetate buffering during the extraction step (AOAC Official Method 2007.1) [30, 32] or citrate buffering (CEN Standard Method EN 15662) [33, 34]. The QuEChERS method has been tested for hundreds of pesticides using GC-MS and LC-MS for measurement, obtaining satisfactory results. Therefore, it seems appropriate to be used in combination with GC-TOF MS for a wide-scope screening [17, 18].

In this paper, a multi-residue method based on QuEChERS extraction and GC-TOF MS analysis has been developed for target and non-target analysis of pesticides in fruits and vegetables. The potential of GC-TOF for quantitative analysis has been investigated for 55 target analytes in different food commodities (orange, apple, carrot, tomato and

olive). The developed target methodology has been applied to the analysis of several samples containing incurred analytes. Additionally, taking advantage of the use of GC-TOF MS, the screening has been extended to non-target pesticides in the samples under study.

115 ## 2. EXPERIMENTAL

116 ### 2.1. Reagents

117 Individual reference standards were purchased from Dr. Ehrenstorfer (Augsburg,
118 Germany) with a purity >93-99 %. Stock standard solutions (around 500 mg/L) were
119 prepared in acetone and were stored in a freezer at -20 ºC. Eight mixtures of pesticide
120 standards (individual concentration of each pesticide around 50 mg/L) were prepared by
121 volume dilution of stock individual solutions in acetone. Two working standard
122 solutions containing all analytes at 5 mg/L were prepared by combining the eight
123 standard mixtures and diluting in hexane or in acetonitrile. Further dilutions were
124 prepared in hexane (for preparing matrix-matched calibration curves) or in acetonitrile
125 (for sample spiking purposes).

126 Triphenyl phosphate (TPP), purchased from Dr. Ehrenstorfer with a purity 99.5 %, was
127 used as internal standard. Stock standard solution (around 500 mg/L) was prepared by
128 dissolving reference standard in acetone. Then, a solution at 5 mg /L was prepared by
129 volume dilution in toluene.

130 Acetone (pesticide residue analysis quality), hexane (ultra trace quality), acetonitrile
131 (reagent grade), toluene (for GC residue analysis) and glacial acetic acid were
132 purchased from Scharlab (Barcelona, Spain). Anhydrous magnesium sulphate (extra
133 pure) and anhydrous sodium acetate (reagent grade) were purchased from Scharlab. The
134 QuEChERS commercial products, 2 mL micro-centrifuge tubes for d-SPE containing 50
135 mg primary secondary amine (PSA) and 150 mg anhydrous $MgSO_4$ or containing
136 additionally 50 mg C_{18} were purchased from Teknokroma (Barcelona, Spain).

137 ### 2.2. GC-TOF instrumentation

The GC instrumentation used consisted in an Agilent 6890N GC system (Palo Alto, CA, USA), equipped with an Agilent 7683 autosampler, coupled to a TOF mass spectrometer, GCT, 1.0 GHz TDC (Waters Corporation, Manchester, UK), operating in electron ionization (EI) mode. The GC separation was performed using a fused silica HP-5MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness) J&W Scientific (Folson, CA, USA). The oven temperature was programmed as follows: 90 ºC (hold 1 min); 5 ºC/min to 300 ºC (hold 2 min). The cycle time was 45 min. The temperature program was designed to get an optimum chromatographic separation between analytes and matrix components. Additionally, this improved chromatographic separation is expected to allow better non-target detection of unknown compounds, avoiding coelutions. Splitless injections of 1 μL of sample extracts were carried out with an injector temperature of 300 °C and with a splitless time of 1 min. Helium 99.999 % (Praxair, Valencia, Spain) was used as carrier gas at a constant flow of 1 mL/min.

The interface and ion source temperatures were set to 260 ºC and 250 ºC, respectively. A solvent delay of 4 min was used to prevent damage in the ion source filament. TOF MS was operated at an scan time of 0.95 s in the mass range *m/z* 50-650 and using a multi-channel plate voltage of 2850 V. As the GC-TOF instrument used in this work did not have the dynamic range enhancement (DRE) mode available, for high analyte concentrations (i.e highest validation level), the scan time was reduced to 0.65 s to avoid problems of detector saturation, as a consequence of the low dynamic range of TOF MS. For sample analysis, a scan time of 0.95 s was selected. If under these conditions a positive finding led to detector saturation, a second injection of the sample extract at 0.65 s would be required to obtain a suitable quantification. TOF MS resolution was about 6700 (FWHM) at *m/z* 264. Mass spectrometric grade PFTBA (Perfluorotri-n-butylamine), used for the daily mass calibration/verification as well as

for lock mass, was injected via syringe (~ 1 μL) in the reference reservoir at 30 ºC. The *m/z* monitored was 218.9856. The application manager TargetLynx, a module of Masslynx 4.0 software, was used to process data obtained for target compounds in samples extracts. The application manager Chromalynx was used to investigate the presence of non-target (unknown) compounds in sample extracts. Library searching was performed using the commercial NIST library.

2.3. Samples

Sample matrices used in this work were chosen to cover different commodity groups as classified in Annex 1 of SANCO/10684/2009 [25]. Apples, tomatoes and carrots were selected as high water content products; oranges were chosen due to their high acid content; and finally, olives were taken as high oil content products.

For each commodity, a blank sample (for validation purposes) was acquired from ecological agriculture. In addition, samples of four different varieties were obtained from local markets and/or particular crops from several areas of Spain.

2.4. Analytical procedure

The extraction procedure was carried out following the modified acetate-buffered version of the QuEChERS method [30]. The samples were chopped and homogenised in Homogeniser Thermomix TM30 (Vorwerk, Madrid, Spain) at room temperature during 2 min. 15 g of chopped and homogenised sample were weighed in a 50-mL Falcon conical tube and 15 mL of 1 % acetic acid (HAc) in acetonitrile (MeCN) (v/v) were added. After shaking for 30 s, 6 g of anhydrous $MgSO_4$ and 1.5 g of anhydrous NaAc were added and immediately shaken vigorously for 1 min. The tubes were centrifuged at 3000 rpm for 2 min and 1 mL of the upper layer of the extract was transferred to the dispersive-SPE tubes containing 50 mg of PSA and 150 mg of anhydrous $MgSO_4$ (for

187 orange and olive samples, SPE-tubes also contained 50 mg of C_{18}). The extracts were
188 vortexed for 30 s and then centrifuged at 3000 rpm for 2 min. 500 μL of the extract
189 were transferred into an evaporation graduated tube, containing 1 mL of toluene and 50
190 μL of the internal standard TPP at 5 mg/L. This extract was evaporated to
191 approximately 300 μL under a gentle nitrogen stream at 50 ºC. The extracts were
192 adjusted to a final volume of 500 μL with toluene prior to injection into GC-TOF MS.

193 For analyte quantification, matrix-matched calibration curves were prepared for every
194 matrix as follows: 500 μL of acetonitrile sample blank extract was transferred into an
195 evaporation tube containing 1 mL of toluene. The mixture was evaporated to
196 approximately 300 μL under a gentle nitrogen stream at 50 ºC. Then, 50 μL of 5 mg/L
197 TPP and 50 μL of hexanic pesticide standard solution of adequate concentration were
198 added, adjusting the final volume to 500 μL with toluene.

199 **2.5. Validation study**

200 Linearity of the method was studied by analyzing matrix-matched standards in duplicate
201 at concentrations ranging from 5 to 1000 μg/L. Linearity was assumed when regression
202 coefficient, r, was higher than 0.99 with residuals lower than 20 %.

203 Accuracy was estimated by means of recovery experiments, analyzing orange, apple,
204 carrot, tomato and olive samples spiked at three concentrations (0.01, 0.05 and 0.5
205 mg/kg). Experiments were performed by sextuplicate at each concentration. The spiking
206 of samples was made by adding the appropriate volume of the mixed pesticide standard
207 solution in acetonitrile to 15 g of homogenised fresh sample before extraction with
208 acetonitrile.

209 Based on SANCO/825/00 guideline [37], recoveries were considered satisfactory in the
210 range of 70-120 % at 0.05 and 0.5 mg/kg spiked concentrations, and from 60 to 120 %
211 at 0.01 mg/kg.

212 Intraday precision was estimated from recovery experiments (n=6). It was expressed as
213 repeatability of the method in terms of relative standard deviation (RSD). RSD values
214 below 20 %, at 0.05 and 0.5 mg/kg spiked concentrations, and below 30 % at 0.01
215 mg/kg were considered satisfactory [37].

216 Selectivity, considered as the ability of the method to discriminate between the analyte
217 peak and other chromatographic peaks, was tested by determining every analyte in the
218 presence of the rest of compounds included in the screening. It was based on the
219 monitoring of characteristics *m/z* ions, measured at accurate mass in the EI spectrum for
220 each compound.

221 The limit of quantification (LOQ) objective was established as the lowest concentration
222 that was validated with satisfactory recovery and precision in spiked samples.

223 The confirmation of identity criterion of positive findings in samples was the presence
224 of, at least, two *m/z* ions in the spectrum of the chromatographic peak at the expected
225 retention time, measured at accurate mass in the respective narrow window-extracted
226 ion chromatograms, nw-XIC (0.02 Da). The ion intensity ratio was evaluated in order to
227 know whether it fitted within the tolerances established by SANCO/10684/2009
228 guideline [25].

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3. RESULTS AND DISCUSSION

3.1. QuEChERS extraction and GC-TOF MS analysis

In this work, we have applied the QuEChERS AOAC Official Method 2007.01 [30], without any additional optimization. This version uses strong acetate buffering at pH 4.8 and gives better recoveries for some problematic pesticides than other QuEChERS versions [35]. In this method, the acetonitrile extract is directly injected in a PTV injector, which seems more adequate than split-splitless due to the large expansion volume of MeCN during vaporization [30, 38]. In our work, a split/splitless injector was used, as the PTV was unavailable in our GC-TOF instrument. So, it was necessary to perform a solvent exchange before GC-injection. Toluene was chosen due to the advantages reported [38]: miscibility with MeCN, high response for some polar-GC amenable pesticides and higher boiling point with the possibility of increasing the initial temperature in the GC oven.

TPP was used as internal standard (IS) in order to improve quantification by compensating the variations of the system. TPP was chosen on the basis of its use in most QuEChERS procedures, as it gives sharp peaks and intense signal [29].

Once pesticides were extracted from fruits and vegetables, their determination was performed by GC-TOF MS, taking the advantage of the accurate mass full-spectrum acquisition. First, an identification and quantification of target analytes was performed in the method validation. Later, the method was applied to different fruit and vegetable samples where the target analytes were determined. A non-target analysis was also carried out, without the need of reanalyzing samples, in order to detect the presence of other compounds not included in the target method.

A total of 55 target analytes were selected, including organophosphate, organochlorine and pyrethroid insecticides, as well as several herbicides, fungicides and acaricides. The detection of target analytes in the samples was carried out by obtaining a minimum of two (up to five, when possible) nw-XICs, with a mass window of 0.02 Da, at selected *m/z* analyte ions. The selected *m/z* ions were optimized in a previous work performed at our laboratory [24]. Quantification ion (*Q*) was used for quantification purposes (showed in **Table 1)**, while the rest of ions (q_i) were used for confirmatory of identity analysis (see reference [24] for more detailed information on the confirmatory ions selected).

Initially, the base peak of TPP spectrum, *m/z* 326.0708, was selected to calculate relative responses for each analyte. Unexpectedly, a reduction in the intensity of all spectra was observed along the sample sequence, which led to unsatisfactory IS correction. This reduction was more noticeable for low *m/z* ions and was mainly produced by the matrix. The voltage applied into the beam stearing, a half plate lens located between the ion chamber and the focus lenses, was optimized every day in order to get an adequate PFTBA spectrum (intensity and ion ratios). During a sequence, and mainly due to the effect of the matrix, this lens gets contaminated. Consequently, the optimized voltage at the beginning of the sequence did not remain sufficient to maintain a satisfactory sensitivity, specially at low *m/z* values. To improve the IS correction, the following strategy was applied: three *m/z* ions of TPP were selected (m/z_1 170.0732, m/z_2 233.0368 and m/z_3 326.0708) in order to calculate relative responses for analytes depending on the characteristic monitored ions. TPP m/z_1 was used for analyte ions < *m/z* 190, m/z_2 for those between *m/z* 190 and 250, and m/z_3 for those ions > *m/z* 250. **Table 1** shows the TPP *m/z* ion selected for each analyte.

In order to perform non-target analysis, a deconvolution package ChromaLynx Application Manager was used to automatically process the MS data acquired [21].

3.2. Method validation

Validation of the multi-residue method was carried out using orange, apple, carrot, tomato and olive in terms of linearity, accuracy, precision, selectivity and LOQ. Matrix-matched calibration curves using relative areas versus IS were used for quantification in spiked and non-spiked samples.

Linearity was tested in the general range of concentrations from 5 to 1000 µg/L. For more accurate quantification, the calibration set was split into the three ranges, adjusted to the concentration present in the spiked samples: 5-100 µg/L (for the lowest concentration, where 10 µg/L corresponds to 0.01 mg/kg in sample), 10-250 µg/L (for intermediate concentration, where 50 µg/L corresponds to 0.05 mg/kg), and 100-1000 µg/L (for the highest concentration, where 500 µg/L corresponds to 0.5 mg/kg). Correlation coefficients were higher than 0.99 and randomly distributed residuals were lower than 20 %.

Table 2 shows the validation results for oranges, apples, carrots and tomatoes. Data on olives are not shown, because they were not satisfactory for most pesticides. The matrix interferences caused by high concentrations of co-extractives in olives meant that TPP could not correct these deviations. Despite the use of additional clean-up, the high oil content in olives caused the contamination of the syringe, inlet, column and MS ion source making necessary extra maintenance [36, 39].

As it can be seen in **Table 2**, at the medium and high concentrations (0.05 and 0.5 mg/kg), most compounds presented satisfactory recoveries, ranging from 70 to 120 %. A few exceptions were observed with values slightly lower (between 60 and 70 %):

304 hexachlorobenzene (HCB) (orange); malathion and chlorfenvinphos (apple); *p-p'*-DDD
305 (tomato). Recoveries lower than 60 % were only found for chlorpropham (orange), and
306 chlorpyriphos ethyl and parathion ethyl (apple). However, in two out of these three
307 cases precision was satisfactory, with RSD below 20 %. Finally, only three recoveries
308 were slightly higher than 120 %, with the highest value of 129 % corresponding to
309 phorate in tomato at 0.5 mg/kg.

310 At the lowest spiked concentration (0.01 mg/kg), around 50 % of target analytes were
311 satisfactorily validated. A notable number of analytes (54 % in orange, 60 % in apple,
312 38 % in carrot and 32 % in tomato) could not be detected, due to the lack of sensitivity.
313 Recoveries lower than 60 % were found in orange for chlorpropham, trifluralin, HCB,
314 β-HCH + lindane, heptachlor, aldrin and dieldrin.

315 A few compounds were particularly problematic, including chlorothalonil, probably due
316 to its degradation during sample preparation or in the hot inlet during GC-injection as
317 reported by some authors [32, 40]; the pyrethroid fenvalerate showed poor sensitivity
318 [31]; and β-HCH and lindane were very close in retention time, making difficult their
319 individual determination, so the results for these two compounds were expressed as the
320 sum of both responses.

321 Intraday precision was satisfactory for most of pesticides at 0.05 and 0.5 mg/kg, with
322 RSDs below 20 %. Only in three cases, RSDs were higher than 25 % (fenvalerate in
323 orange and α– and β-endosulfan in apple, all at 0.05 mg/kg). At the lowest
324 concentration assayed (0.01 mg/kg), only in five cases RSD were higher than 30 %
325 (trifluralin, pirimiphos methyl, chlorpyrifos ethyl and dieldrin in orange; pirimiphos
326 methyl in apple) surely because of the poor sensitivity.

The LOQ objective was 0.01 mg/kg, and was achieved for around 50 % of the compounds investigated. Obviously, the statistical LOQ estimated for a signal-to-noise ratio of 10 from the chromatograms at the lowest spiked concentration was substantially lower than 0.01 mg/kg for the majority of pesticides.

Ion intensity ratios were evaluated for all compounds in every matrix, updating the reference values in each sequence of analysis. Values of reference corresponded to the matrix-matched calibration standard at the same spiked concentration. Maximum tolerances, established by SANCO/10684/2009 guideline, were: ± 10 % when *Q/q* intensity ratio was lower than 2, ± 15 % for *Q/q* between 2-5, ± 20 % for *Q/q* 5-10 and ± 50 % for *Q/q* ratio higher than 10 [25]. In the validation experiments, most analytes had ion ratios within the acceptance intervals. However, some exceptions were observed, indicating that accomplishment of the ion ratios within the maximum deviations admitted (between 10 % and 50 % depending on the relative signals) is a problematic issue, especially at low analyte concentrations. Thus, at 0.01 mg/kg several analytes could be quantified with satisfactory recovery, but the *Q/q* ratio was out of the tolerance as a consequence of the poor sensitivity for the confirmatory transition. These compounds are highlighted in **Table 2**. Further investigations are being made in our group on this relevant matter, as the non-accomplishment of the ion ratio can lead to report an actual positive as negative.

As an illustrative example, **Figure 1** shows the nw-XICs obtained (mass window of 0.02 Da) for tetradifon in all matrices studied at the lowest concentration validated. Three ions were monitored in this case. The narrow mass window used allowed a notable improvement in selectivity, also decreasing the background noise of the chromatogram.

3.3. Real sample analysis

352 **3.3.1. Target analysis**

353 The GC-TOF MS procedure was applied to 16 samples (four different varieties of the
354 four sample matrices validated: orange, apple, carrot and tomato) collected from several
355 areas of Spain. The results obtained are shown in **Table 3**.

356 Regarding oranges, one of the samples analyzed was found to contain chlorpyriphos
357 ethyl (0.1 mg/kg) and other showed the presence of terbuthylazine (0.02 mg/kg) and
358 chlorpyriphos ethyl (0.16 mg/kg) **(Figure 2)**. In the case of apples, cyprodinil (0.03
359 mg/kg) and chlorpyriphos ethyl (< LOQ) were also found in one and two samples,
360 respectively. In carrots, unexpectedly *p,p'*-DDE was detected, although at low
361 concentration (0.02 mg/kg) in one of the samples. As already known, *p,p'-DDE* is the
362 DDT metabolite and is more frequently found in the environment (e.g. soil). Finally,
363 one tomato sample was positive to pyriproxyfen (0.05 mg/kg). All positive findings
364 were below the MRL established for each crop and were consistent with authorisations
365 of use with the crop they appeared. Identification of these analytes in the samples was
366 confirmed by means of the presence of at least two *m/z* ions at the expected retention
367 time with mass errors normally below 3 mDa with few exceptions. The high number of
368 co-extractives in QuEChERS extracts might lead to matrix-induced mass shifts.
369 However, in this work the mass errors observed in samples were similar to those
370 normally obtained with our instrument in other matrices, as water or more diluted food
371 extracts. New GC-TOF generations provide better sensitivity and improved mass
372 accuracy, leading to lower mass errors, compared with the GC-TOF used in this work.
373 The measured ion intensity ratios were also evaluated. Ion ratios were in good
374 accordance with those of calibration standards within the tolerances established [25].
375 However, as observed in the validation study, a few cases exceeded the maximum
376 tolerance, but a clear evidence existed that they were positive findings from the rest of

parameters evaluated (retention time, several ions present in the samples and accurate masses). This fact illustrates that maximum tolerances established in the current guidelines for *Q/q* ratio are a controversial issue, and may require revision, as we previously suggested for organic contaminants in water [24]. Concentrations found in samples were generally higher than the LOQ objective, except for chlorpyriphos ethyl in apple.

3.3.2. Non-target analysis

One advantage of GC-TOF MS is the possibility of investigating the presence of non-target compounds, others than those included in the initial target list of the method. This searching can be made in a post-target way, i.e. by obtaining XICs at certain characteristic/abundant ions of the additional pesticides investigated, or also in a non-target way, without any kind of selection of the compounds to be searched. Obviously, the non-target analytes would include not only pesticides but also other GC-MS amenable compounds that might be present in the samples, which could include organic pollutants or simply a common constituent of the sample. In this work, the non-target analysis allowed the detection of other compounds not included in the validated method. It was carried out by applying the Chromalynx Application Manager, which allowed the automated detection of sample components and their subsequent identification from the full-acquisition accurate-mass data obtained.

In this way, the post-harvest fungicide imazalil was detected in one orange and three apple samples. **Figure 3** shows the residue of imazalil in an apple sample, detected and identified in a non-target way. Accurate mass confirmation automatically performed for four representative ions and the library forward match (>700 used as criterion) led to the confirmation of the identity of imazalil with mass errors below 1.8 mDa for all ions. In

addition, the structures proposed for at least four fragments ions observed in the EI spectrum were compatible with the chemical structure of imazalil. The injection of reference standards allowed the presence of imazalil in the samples to be confirmed.

Two PAHs, fluoranthene and pyrene, were also found. Both compounds presented the same spectra, so standard solutions of these compounds were necessary for their discrimination from retention times. Fluoranthene was only detected in one carrot sample, whereas pyrene was detected in most of samples. **Figure 4** shows the detection of pyrene finding in carrot. Accurate mass confirmation automatically performed for four representative ions and library forward match (>700) suggested that the candidate compound detected was pyrene, with mass errors below 1.9 mDa for the ions shown. The structures proposed for at least four fragments ions observed in the EI spectrum were supported with the chemical structure of this compound. Retention time information obtained by injection of the reference standard provided further supporting evidence for the confirmatory of identity.

Trans-limonene oxide was also identified in orange samples. This compound is present as a racemic mixture of cis and trans- limonene oxide, with a strong smell of orange-lemon. Other components detected in orange were sesquiterpenes (α-farnesene, α–humulene or copaene) and the flavour enhancer, maltol. The natural pesticide, falcarinol, was also detected in some carrot samples. When required, the unequivocal confirmation of these compounds could be carried out by injecting reference standards.

4. CONCLUSIONS

In this work, a multi-residue method has been developed for a total of 55 pesticides and metabolites in representative fruit and vegetable matrices. The use of QuEChERS in combination with GC-TOF MS allowed reliable analysis in orange, apple, carrot and tomato. Validation of the method for olives was hampered by the greater complexity of the matrix, even after dispersive SPE clean-up using C_{18} sorbent. In the case of olives, further clean-up, or an alternative extraction and clean-up is required to improved detectability of analytes by GC-TOF MS.

For these four matrices, recoveries and precision were acceptable at 0.05 mg/kg and 0.5 mg/kg. At 0.01 mg/kg spiked concentration, satisfactory data were obtained for approximately 50 % of the compounds, mainly due to insufficient sensitivity of our GC-TOF instrument. Particulary problematic was the accomplishment of the ion ratios due to the poor signal of the confirmatory ions in several analyte/matrix combinations.

The potential of GC-TOF MS has been proved both in target and non-target analysis. Target identification requires the presence of at least two *m/z* ions, measured at their accurate mass using narrow window-extracted ion chromatograms at the expected retention time. TPP was used as internal standard to minimize deviations in responses and to improve quantification. It is noteworthy that appropriate correction required to use different TPP *m/z* ions depending on the analyte *m/z* ion used for quantification. Matrix-matched standard calibration was applied in order to perform a correct quantification in orange, apple, carrot and tomato samples. Full-spectrum accurate-mass data acquired in GC-TOF MS has also allowed a non-target research of the samples analyzed. The analysis of samples from different origin and varieties has revealed the presence of several target analytes, included terbuthylazine, chlorpyrifos ethyl, cyprodinil, bifenthrin and pyriproxyfen, all at concentrations below 0.2 mg/kg, together

with other non-target compounds such as imazalil, fluoranthene or pyrene. New GC-TOF instruments provide improved sensitivity and dynamic linear range compared to the instrument employed in this work. Hopefully, we will see many qualitative and quantitative applications in the field of pesticide residue analysis in the near future based on GC-TOF MS. Continuous software developments will also facilitate the non-target analysis, which may become an interesting approach in GC-MS due to the commercial availability of standardized spectra libraries.

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552 **FIGURE CAPTIONS**

553 **Figure 1.** GC-TOF MS narrow window-extracted ion chromatogram (mass window of
554 0.02 Da) at different *m/z* (*Q*:158.9665, q_1: 226.8886, q_2: 353.8843) ions for tetradifon in
555 orange, apple, carrot and tomato spiked at 0.01 mg/kg.

556 **Figure 2.** GC-TOF MS narrow window-extracted ion chromatogram (mass window
557 0.02 Da) showing the detection of target chlorpyriphos ethyl in orange. Experimental EI
558 accurate mass spectrum and chemical structures proposed for the most abundant
559 fragment ions together with experimental mass errors (in mDa).

560 **Figure 3**. Identification of non-target imazalil in apple. (A) Extracted ion
561 chromatograms for four imazalil ions used for deconvolution. (B) Library mass
562 spectrum of imazalil at nominal masses (match 839). (C) Deconvoluted accurate mass
563 spectrum of imazalil from the sample and chemical structures proposed for four
564 representative EI fragment ions together with mass errors.

565 **Figure 4**. Identification of non-target pyrene in carrot. (A) Extracted ion
566 chromatograms for four pyrene ions used for deconvolution. (B) Library mass spectrum
567 of pyrene at nominal masses (match 872) (C) Deconvoluted accurate mass spectrum of
568 pyrene from the sample and chemical structures proposed for four abundant EI fragment
569 ions together with mass errors.