

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

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8 **ABSTRACT**

9 In this work, the capability of gas chromatography coupled to time-of-flight mass
10 spectrometry (GC-TOF MS) for quantitative analysis of pesticide residues has been
11 evaluated. A multiclass method for rapid screening of pesticides (insecticides,
12 acaricides, herbicides and fungicides) in fruit and vegetable matrices has been
13 developed and validated, including detection, identification and quantification of the
14 analytes. To this aim, several food matrices were selected: high water content (apples,
15 tomatoes and carrots), high acid content (oranges) and high oil content (olives) samples.
16 The well known QuEChERS procedure was applied for extraction of pesticides, and
17 matrix-matched calibration using relative responses versus internal standard was used
18 for quantification. The sample extracts were analyzed by GC-TOF MS. Up to five ions
19 using narrow window (0.02 Da)-extracted ion chromatograms at the expected retention
20 time were monitored using a target processing method. The most abundant ion was used
21 for quantification while the remaining ones were used for confirmation of the analyte
22 identity. Method validation was carried out for 55 analytes in the five sample matrices
23 tested at three concentrations (0.01, 0.05 and 0.5 mg/kg). Most recoveries were between
24 70 % and 120 % with relative standard deviations (RSDs) lower than 20 % at 0.05 and
25 0.5 mg/kg . At 0.01 mg/kg, roughly half of the pesticides could be satisfactorily
26 validated due to sensitivity limitations of GC-TOF MS, which probably affected the ion
27 ratios used for confirmation of identity. In the case of olive samples, results were not
28 satisfactory due to the high complexity of the matrix. An advantage of TOF MS is the
29 possibility to perform a non-target investigation in the samples by application of a
30 deconvolution software, without any additional injection being required. Accurate-mass
31 full-spectrum acquisition in TOF MS provides useful information for analytes

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

34

35 **Key words**

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

38 1. INTRODUCTION

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

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

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

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

69 Accurate-mass full-spectrum data available in HR-TOF MS enable to obtain extracted
70 ion chromatograms using narrow mass windows (nw-XICs). Reducing the mass
71 window can notably improve the signal-to-noise due to exclusion of a large proportion
72 of the chemical background and quasi-isobaric interferences. The potential of GC-TOF
73 MS has been mainly explored in the qualitative field pursuing the detection and
74 identification of GC-amenable organic contaminants [19, 21-24]. Up to five m/z ions of
75 each target analyte are monitored, using as confirmation of identity criteria the presence
76 of at least two m/z ions and the accomplishment of the intensity ratio within established
77 tolerances [25]. Thus, wide-scope screening has been developed and validated from a
78 qualitative point of view for around 150 organic micropollutants in water [24]. The
79 elucidation of non-target compounds is also possible after MS data acquisition, without
80 the need of reinjecting the sample, making use of powerful deconvolution software [19,
81 21-23, 26, 27]. Although there is wide consensus on the great qualitative potential of
82 HR-TOF MS however its low dynamic range compared with conventional MS
83 instrumentation limits its quantitative applications and also affects mass accuracy,
84 which can be deteriorated at certain concentration levels. The analog-to-digital
85 converter (ADC) detector offers linear dynamic range of four orders of magnitude but,
86 at low analyte signal intensities, noise becomes a limiting factor. The time-to-digital

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

96 To take full advantage of the capabilities of GC-TOF MS for screening a large number
97 of pesticides, a generic procedure with a wide scope is required. To this aim the
98 QuEChERS method [12, 17, 18, 28-36], a rapid extraction procedure based on the use
99 of acetonitrile as extractant, has been widely applied in food residue analysis. After the
100 original method, developed in 2003 [29], several modifications have improved the
101 scope of the method, like the use of acetate buffering during the extraction step (AOAC
102 Official Method 2007.1) [30, 32] or citrate buffering (CEN Standard Method EN
103 15662) [33, 34]. The QuEChERS method has been tested for hundreds of pesticides
104 using GC-MS and LC-MS for measurement, obtaining satisfactory results. Therefore, it
105 seems appropriate to be used in combination with GC-TOF MS for a wide-scope
106 screening [17, 18].

107 In this paper, a multi-residue method based on QuEChERS extraction and GC-TOF MS
108 analysis has been developed for target and non-target analysis of pesticides in fruits and
109 vegetables. The potential of GC-TOF for quantitative analysis has been investigated for
110 55 target analytes in different food commodities (orange, apple, carrot, tomato and

111 olive). The developed target methodology has been applied to the analysis of several
112 samples containing incurred analytes. Additionally, taking advantage of the use of GC-
113 TOF MS, the screening has been extended to non-target pesticides in the samples under
114 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 °C. 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 MgSO₄ or containing
136 additionally 50 mg C₁₈ were purchased from Teknokroma (Barcelona, Spain).

137 **2.2. GC-TOF instrumentation**

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

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

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

169 **2.3. Samples**

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

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

177 **2.4. Analytical procedure**

178 The extraction procedure was carried out following the modified acetate-buffered
179 version of the QuEChERS method [30]. The samples were chopped and homogenised in
180 Homogeniser Thermomix TM30 (Vorwerk, Madrid, Spain) at room temperature during
181 2 min. 15 g of chopped and homogenised sample were weighed in a 50-mL Falcon
182 conical tube and 15 mL of 1 % acetic acid (HAc) in acetonitrile (MeCN) (v/v) were
183 added. After shaking for 30 s, 6 g of anhydrous $MgSO_4$ and 1.5 g of anhydrous NaAc
184 were added and immediately shaken vigorously for 1 min. The tubes were centrifuged at
185 3000 rpm for 2 min and 1 mL of the upper layer of the extract was transferred to the
186 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 C₁₈). 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 °C. 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 °C. 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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233 **3. RESULTS AND DISCUSSION**

234 **3.1. QuEChERS extraction and GC-TOF MS analysis**

235 In this work, we have applied the QuEChERS AOAC Official Method 2007.01 [30],
236 without any additional optimization. This version uses strong acetate buffering at pH
237 4.8 and gives better recoveries for some problematic pesticides than other QuEChERS
238 versions [35]. In this method, the acetonitrile extract is directly injected in a PTV
239 injector, which seems more adequate than split-splitless due to the large expansion
240 volume of MeCN during vaporization [30, 38]. In our work, a split/splitless injector was
241 used, as the PTV was unavailable in our GC-TOF instrument. So, it was necessary to
242 perform a solvent exchange before GC-injection. Toluene was chosen due to the
243 advantages reported [38]: miscibility with MeCN, high response for some polar-GC
244 amenable pesticides and higher boiling point with the possibility of increasing the initial
245 temperature in the GC oven.

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

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

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

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

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

282 **3.2. Method validation**

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

287 Linearity was tested in the general range of concentrations from 5 to 1000 $\mu\text{g/L}$. For
288 more accurate quantification, the calibration set was split into the three ranges, adjusted
289 to the concentration present in the spiked samples: 5-100 $\mu\text{g/L}$ (for the lowest
290 concentration, where 10 $\mu\text{g/L}$ corresponds to 0.01 mg/kg in sample), 10-250 $\mu\text{g/L}$ (for
291 intermediate concentration, where 50 $\mu\text{g/L}$ corresponds to 0.05 mg/kg), and 100-1000
292 $\mu\text{g/L}$ (for the highest concentration, where 500 $\mu\text{g/L}$ corresponds to 0.5 mg/kg).
293 Correlation coefficients were higher than 0.99 and randomly distributed residuals were
294 lower than 20 %.

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

301 As it can be seen in **Table 2**, at the medium and high concentrations (0.05 and 0.5
302 mg/kg), most compounds presented satisfactory recoveries, ranging from 70 to 120 %.
303 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.

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

331 Ion intensity ratios were evaluated for all compounds in every matrix, updating the
332 reference values in each sequence of analysis. Values of reference corresponded to the
333 matrix-matched calibration standard at the same spiked concentration. Maximum
334 tolerances, established by SANCO/10684/2009 guideline, were: $\pm 10\%$ when Q/q
335 intensity ratio was lower than 2, $\pm 15\%$ for Q/q between 2-5, $\pm 20\%$ for Q/q 5-10 and \pm
336 50% for Q/q ratio higher than 10 [25]. In the validation experiments, most analytes had
337 ion ratios within the acceptance intervals. However, some exceptions were observed,
338 indicating that accomplishment of the ion ratios within the maximum deviations
339 admitted (between 10 % and 50 % depending on the relative signals) is a problematic
340 issue, especially at low analyte concentrations. Thus, at 0.01 mg/kg several analytes
341 could be quantified with satisfactory recovery, but the Q/q ratio was out of the tolerance
342 as a consequence of the poor sensitivity for the confirmatory transition. These
343 compounds are highlighted in **Table 2**. Further investigations are being made in our
344 group on this relevant matter, as the non-accomplishment of the ion ratio can lead to
345 report an actual positive as negative.

346 As an illustrative example, **Figure 1** shows the nw-XICs obtained (mass window of
347 0.02 Da) for tetradifon in all matrices studied at the lowest concentration validated.
348 Three ions were monitored in this case. The narrow mass window used allowed a
349 notable improvement in selectivity, also decreasing the background noise of the
350 chromatogram.

351 **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 chlorpyrifos
357 ethyl (0.1 mg/kg) and other showed the presence of terbuthylazine (0.02 mg/kg) and
358 chlorpyrifos ethyl (0.16 mg/kg) (**Figure 2**). In the case of apples, cyprodinil (0.03
359 mg/kg) and chlorpyrifos 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

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

383 **3.3.2. Non-target analysis**

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

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

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

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

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

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424 4. CONCLUSIONS

425 In this work, a multi-residue method has been developed for a total of 55 pesticides and
426 metabolites in representative fruit and vegetable matrices. The use of QuEChERS in
427 combination with GC-TOF MS allowed reliable analysis in orange, apple, carrot and
428 tomato. Validation of the method for olives was hampered by the greater complexity of
429 the matrix, even after dispersive SPE clean-up using C₁₈ sorbent. In the case of olives,
430 further clean-up, or an alternative extraction and clean-up is required to improved
431 detectability of analytes by GC-TOF MS.

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

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

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