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Screening of Pesticides and Polycyclic Aromatic Hydrocarbons in Feeds and Fish Tissues by Gas Chromatography Coupled to High-Resolution Mass Spectrometry Using Atmospheric Pressure Chemical Ionization

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1 **Screening of pesticides and polycyclic aromatic hydrocarbons in feeds and fish tissues by**

2 **gas chromatography coupled to high-resolution mass spectrometry using atmospheric**

3 **pressure chemical ionization.**

4

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28 **Abstract**

29 This paper reports a wide-scope screening for detection and identification of pesticides and
30 polycyclic aromatic hydrocarbons (PAHs) in feeds and fish tissues. QuEChERS sample
31 treatment was applied, using freezing as an additional clean-up. Analysis was carried out by gas
32 chromatography coupled to hybrid quadrupole time-of-flight mass spectrometry with
33 atmospheric pressure chemical ionization (GC-(APCI) QTOF MS). The qualitative validation
34 was performed for over 133 representative pesticides and 24 PAHs at 0.01 and 0.05 mg/kg.
35 Subsequent application of the screening method to aquaculture samples made it possible to
36 detect several compounds from the target list, such as the chlorpyrifos-methyl, pirimiphos-
37 methyl, ethoxyquin, among others. Light PAHs (≤ 4 rings) were found in both animal and
38 vegetable samples. The reliable identification of the compounds was supported by accurate
39 mass measurements and the presence of at least two representative *m/z* ions in the spectrum
40 together with the retention time of the peak, in agreement with the reference standard.
41 Additionally, the search was widened to include other pesticides for which standards were not
42 available, thanks to the expected presence of the protonated molecule and/or molecular ion in
43 the APCI spectra. This could allow the detection and tentative identification of other pesticides
44 different from those included in the validated target list.

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52 **KEYWORDS:** gas chromatography, high resolution mass spectrometry, QuEChERS,
53 screening, organic contaminants, QTOF MS, feed, fish, qualitative validation

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55 **INTRODUCTION**

56 One of the goals of the aquaculture is the reduction of fish origin ingredients in feeds by using
57 new plant-based alternative feed ingredients in order that feed producers become less dependent
58 on fish meal and fish oil. There is a notable interest to know the impact of these substitutions on
59 the quality of farmed fish species and also on food safety of the final product. The use of
60 vegetable origin raw materials reduces the total load of potentially hazardous persistent organic
61 pollutants (POPs) among others but may load new undesirables, different from POPs. [1-3]
62 Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants that are widely
63 deposited in vegetable samples so their inclusion in priority lists becomes relevant.[4] In addition,
64 pesticides are among the most relevant contaminants when dealing with samples from vegetable
65 origin.

66 The analysis of organic undesirable compounds in fatty samples from aquaculture activities is
67 commonly conducted by gas chromatography coupled to mass spectrometry (GC-MS).
68 Generally, a time-consuming sample treatment is required to achieve low detection limits,
69 including one or more clean-up steps to eliminate matrix components that negatively affect
70 analysis (pigments, proteins, lipids…). [5-9] A widely used sample preparation approach is
71 QuEChERS, initially developed for determination of pesticides in fruits and vegetables.[10,11]
72 Modifications of this approach have been developed for different compounds and matrices
73 making this stage highly flexible depending on the sample matrix. [12-18] One of the most
74 distinguishing features of QuEChERS over previous sample preparation techniques is the use of
75 dispersive solid-phase extraction (d-SPE) for clean-up. Following quick and easy steps it is
76 possible to obtain clean extracts well-suited for both GC-MS and LC-MS analysis.

77 Large-scope screening approaches are becoming attractive in the last years, as conventional
78 target analysis offers a limited overview of a (normally) reduced number of organic compound
79 candidates. The combined use of GC-HRMS and LC-HRMS is currently one of the most
80 efficient strategies for wide-scope screening of organic pollutants.[19] The qualitative validation of

81 the screening method previous application to real samples is required to support that the method
82 fits properly at least for selected “model compounds”. In a wide screening of organic
83 contaminants, the number of targets investigated is, in principle, unlimited. Among the full
84 spectrum acquisition analyzers, the time-of-flight analyzer (TOF) is especially suited for this
85 purpose due to the high sensitivity and accurate mass data generated. [19-22] Additionally, in
86 modern GC-TOF MS methods using the recently revived atmospheric pressure chemical
87 ionization source (APCI) the investigation of target compounds is easier and more efficient.
88 This is due to the soft ionization that takes place under APCI in comparison with the highly
89 fragmentation observed in the widely used electron ionization (EI) source. Thus, working with
90 APCI, the molecular ion ($M^{+\cdot}$) or the protonated molecule ($[M+H]^+$) is commonly presented in
91 the mass spectrum (in most cases as base peak) which improves both selectivity and sensitivity
92 of the screening detection. [23] Also, the availability of a QTOF instrument allows performing
93 MS/MS and/or MS^E experiments to go further in the identification of compounds detected due
94 to the structure information given by the fragmentation pathways.

95 The aim of the present work is to complement a previous developed screening based on liquid
96 chromatography coupled to high-resolution mass spectrometry (LC-HRMS). [22] By combination
97 of GC-HRMS and LC-HRMS we pursue one of the main challenges in food safety and
98 toxicology: advancing towards the ideal “universal” screening where all type of analytes,
99 independently of their polarity and volatility, would be detected in the analysis. A QuEChERS-
100 based sample treatment has been applied, with some modifications. A critical stage was to
101 validate the GC-QTOF MS method for both pesticides and PAHs establishing the screening
102 detection limit (SDL) in complex aquaculture samples. The validated method has been applied
103 for screening pesticides and PAHs in commercially and experimentally available real samples.

104

105 **MATERIAL AND METHODS**

106 **Reagents and chemicals.** Individual pesticide reference standards were purchased from Dr.
107 Ehrenstorfer (Scharlab, Spain) with purity between 93-99%. Stock standard solutions (around
108 500 mg/L) were prepared in acetone and were stored in a freezer at -20 ºC. Nineteen mixtures of
109 pesticide standards (individual concentration of each pesticide around 50 mg/L) were prepared
110 by dilution of stock individual solutions in acetone. A working standard solution containing all
111 pesticides at 1 mg/L was prepared by dilution of mixtures with acetone. In our target list,
112 ethoxyquin is included in the pesticide list as a preservative. It is mainly considered as a
113 synthetic preservative but it is also used as pesticide (under commercial name as "Stop-Scald")
114 in order to prevent oxidation in vegetable and fruit samples.

115 Benzo[*j*]fluoranthene, 5-methylchrysene, benzo[*c*]fluorene, dibenzo[*a,e*]pyrene,
116 dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene and cyclopenta[*cd*]pyrene
117 individual standard solutions and mixture PAH MIX 9 containing naphthalene, acenaphthylene,
118 acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene,
119 chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[*1,2,3-cd*]pyrene,
120 dibenzo[*a,h*]anthracene and benzo[*g,h,i*]perylene at10 mg/L were purchased from Dr.
121 Ehrenstorfer. A working standard solution containing all compounds at 1 mg/L except for
122 cyclopenta[*c,d*]pyrene which was at 0.5 mg/L, were prepared by combining the standard
123 mixtures and diluting in n-hexane.

124 Acetone (pesticide residue analysis quality), n-hexane (ultra-trace quality), acetonitrile
125 (reagent grade), toluene (for GC residue analysis) and glacial acetic acid were purchased from
126 Scharlab (Barcelona, Spain). Anhydrous magnesium sulphate (extra pure) and anhydrous
127 sodium acetate (reagent grade) were purchased from Scharlab. The QuEChERS commercial
128 products composed by 2 mL microcentrifuge tubes for d-SPE containing 50 mg primary
129 secondary amine (PSA), 150 mg anhydrous $MgSO_4$ and 50 mg C18, were purchased from
130 Teknokroma (Barcelona, Spain). This was the kit selected in our recommended procedure.
131 Moreover, another QuEChERS kit with the same composition together graphitized carbon black

132 (GCB, 50 mg) was also purchased from Teknokroma. It was also studied in the optimization of
133 the method (Scharlab, Barcelona, Spain).

134

135 **Samples.** Feed ingredients that are either used or tested and aquafeeds were directly purchased
136 or provided from manufacturers. Protein feed ingredients were pea protein (2 samples), pea (1),
137 wheat (3), wheat gluten (4), corn gluten (3), soya protein (4), sunflower meal (1), rapeseed cake
138 (1), fish meal (2), krill meal (1) and fish protein (1). Oil ingredients such as rapeseed oil (5),
139 palm oil (2), linseed oil (1) and fish oil (2) were also studied. As regards feed, five different
140 aquafeeds were analysed that had different composition of marine ingredients and plant
141 ingredients. With regard to fish, three fish species (atlantic salmon (*salmo salar*), sea bass
142 (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) were directly purchased from
143 supermarkets. Sea bream fillets (3) and one fish liver from other growing experiments were also
144 collected from IATS facilities.

145

146 **GC-QTOF MS instrumentation.** GC system (Agilent 7890A, Palo Alto, CA, USA) was
147 equipped with an autosampler (Agilent 7693) and coupled to a hybrid quadrupole-orthogonal
148 acceleration-TOF mass spectrometer (XEVO G2 QTOF, Waters Micromass, Manchester, UK),
149 using an APCI source (APGC® by Waters Corporation). A fused silica DB-5MS capillary
150 column (30 m long × 0.25 mm I.D. × 0.25 μm df) (J&W Scientific, Folson, CA, USA) was used
151 for GC separation. Injector was operated in splitless mode, injecting 1 μL at 280 ºC. The oven
152 temperature was programmed as follows: 90 ºC (1 min), 5 ºC/min to 315 ºC (5 min). Helium
153 was used as carrier gas at 2 mL/min. The interface temperature was set to 280 ºC using N_2 (from
154 liquid N_2) as auxiliary gas at 150 L/h and as cone gas at 16 L/h, and N_2 (from gas cylinder
155 quality 99.9990%) as make-up gas at 320 mL/min. The APCI corona pin was operated at 1.8 μA
156 and the cone voltage was set to 20V. The ionization process occurred within a closed ion
157 volume, which enabled control over the protonation/charge transfer processes. The water, used
158 as modifier when working under proton-transfer conditions, was placed in an uncapped vial,

159 which was located within a specially designed holder placed in the source door. In these
160 conditions, the most critical separation was between benzo[*b*]fluoranthene,
161 benzo[*j*]fluoranthene and benzo[*k*]fluoranthene, by one side, and between dibenzo[*a,i*]pyrene
162 and dibenzo[*a,h*]pyrene, by other side, whose results should be treated as primary data.

163 For MS^E experiments, two acquisition functions with different collision energies were
164 generated. The low energy function (LE), selecting a collision energy of 4 eV, and the high
165 energy (HE) function, with a collision energy ramp ranging from 10 to 40 eV in order to obtain
166 a greater range of fragment ions. [23] It should be noted that all the exact masses shown in this
167 work have a deviation of 0.55 mDa from the 'true' value, as the calculation performed by the
168 MassLynx software uses the mass of hydrogen instead of a proton when calculating $[M + H]^+$
169 exact mass. However, as this deviation is also applied during mass axis calibration, there is no
170 negative impact on the mass errors presented in this article. MS data were acquired in centroid
171 mode and were processed by the ChromaLynx XS application manager (within MassLynx v4.1;
172 Waters Corporation).

173

174 **Recommended analytical procedure.** Before analysis, ingredients and feed samples were
175 thawed at room temperature and ground using a Super JS mill from Moulinex (Bagnolet Cedex,
176 France). Fish tissues were also thawed at room temperature and processed in a crushing
177 machine (Thermomix, Vorwerk España M.S.L., S.C., Madrid). As a result, homogenized
178 samples were obtained in both cases. 5 g of sample was accurately weighed (precision 0.1 mg)
179 into centrifuge tubes (50 mL), and mixed in a Vortex with 10 mL of acetonitrile (Figure 1).
180 Then, 4 g of $MgSO_4$ was added and it was again shaken in a Vortex during 30 s. Following,
181 extract is centrifuged at 4500 rpm for 5 min (Consul centrifuge, Orto-Alresa, Madrid, Spain)
182 and the upper layer of the extract was transferred to a new centrifuge tube (15 mL) and stored
183 overnight in a freezer to precipitate proteins and fix lipids to the tube walls (freezing clean-up).
184 Afterwards, 1 mL of the extract was carefully transferred to the clean-up QuEChERS vial (50
185 mg PSA + 150 mg $MgSO_4$ + 50 mg C18) and it was shaken 30 s and centrifuged at 12000 rpm

186 for 5 min. After this clean-up, 0.5 mL were transferred to a new Eppendorf vial adding 0.1 mL
187 of hexane. The extract was concentrated to dryness at 30 ºC (to remove acetonitrile) under a
188 gentle stream of nitrogen, reconstituted with 0.2 mL of n-hexane and finally transferred to a vial
189 for GC injection. The samples were run twice, using water as modifier to favour in-source
190 protonation and without adding water for those compounds for which no protonation was
191 observed.

192

193 **Method Validation.** Validation of the screening method was performed for qualitative purposes
194 on the basis of European analytical guidelines.[24-25] 20 different samples (details in samples
195 section) were spiked with over 133 pesticides and 24 PAHs at two levels, 0.01 and 0.05 mg/kg
196 (0.005 and 0.025 mg/kg for cyclopenta[*c,d*]pyrene), and analysed together with their non-spiked
197 samples ("blanks"). Additionally, two method blanks were analysed to ensure that no laboratory
198 contamination was introduced along the procedure. The SDL was set-up as the main validation
199 parameter to estimate the threshold concentration at which detection becomes reliable. SDL was
200 established as the lowest concentration tested at which a compound was detected in at least 95%
201 of 20 spiked samples under study (i.e. detected in at least 19 samples at each concentration
202 level) independently of its recovery and precision. The detection was made by using the most
203 abundant ion measured at its accurate mass (typically the protonated molecule). This means
204 that, at least, one peak (SDL) had to be observed in the respective narrow-window eXtracted Ion
205 Chromatogram (nw-XIC), at the same retention time (tolerance of ±0.5% with respect to
206 standard) and measured at accurate mass (mass error < 5 ppm). Table 1 and 2 show the results
207 obtained at each spiked level.

208

209 **RESULTS AND DISCUSSION**

210

211 Feed ingredients, feed compositions and fish tissues are complex matrices that contain a large
212 number of interferences that may hamper detection and identification of undesirable

213 compounds. In order to investigate the presence of any GC-amenable organic contaminant in
214 this kind of samples, clean-up steps are normally applied to improve sensitivity and selectivity.
215 [4,7,12-15,18] However, when the screening is focused on different chemical families of compounds,
216 the situation is more problematic since analytes have rather different chemical and physical
217 properties, and the analytical strategy should be suitable for all of them. [22,26] In this work, the
218 screening was focused on many different pesticides and PAHs in a single analysis. As the
219 objective was the detection and subsequent identification of the compounds detected in samples,
220 no recoveries and precisions were calculated in this work.
221

222 **Sample treatment optimization.** One of the goals of a wide screening method is to minimize
223 the possible analyte losses along sample treatment; so any restrictive step should be carefully
224 studied. Acetonitrile solvent was selected since it is not highly amenable with lipid content and
225 offers good recoveries for many GC-amenable compounds. In addition, in order to reduce the
226 amount of extracted interferences, different conditions were tested, like (1) the addition of
227 water, (2) addition of toluene and/or hexane together with acetonitrile for the extraction, and (3)
228 the addition of sodium acetate.

229 1- The addition of water did not offer better results than the only use of acetonitrile. Thus,
230 many compounds could not be detected at the lowest spiked level. Although some
231 authors reported that water incorporation to fatty samples improves the determination of
232 many pesticides, [27] in the samples under study the addition of water did not represent a
233 relevant improvement for pesticides.

234 2- The use of organic solvents such as hexane or toluene reduced the co-extractives in the
235 acetonitrile layer but also the presence of several non-polar compounds, like DDTs,
236 heptachlors, HCH-isomers in the sample extract, as they have more affinity to the
237 hexane or toluene layer. [27,28]

238 3- The addition of sodium acetate seemed not much favorable since it generated a turbid
239 extract. Although this situation does not represent a great disadvantage as reported by
240 other authors, [29] in our case, better results were obtained without using sodium acetate.

241 One of the easiest ways to reduce the amount of matrix interferences is to place the organic
242 extract stored in a freezer. Thus, the solution obtained is rather clean as most proteins and lipids
243 are fixed on the bottom and walls of the tube, respectively. As previously reported, the
244 application of low temperature before d-SPE cleanup substantially reduces the amount of co-
245 extractives.[13,14] Moreover, an improvement of chromatographic peak shapes, reduction of signal
246 suppression and minimization of retention time shifts were observed for some compounds, as
247 supported by the bibliography. [13,14] After the freezing, an aliquot can be easily taken and
248 centrifuged to improve the solid-liquid separation.

249 The d-SPE clean-up step was also studied by using two commercially available QuEChERS kits
250 (d-SPE with 150 mg Mg_2SO_4, 50 mg PSA and 50 mg C18; and d-SPE with 150 mg Mg_2SO_4, 50
251 mg PSA, 50 mg C18 and 50 mg GCB). The kit containing GCB was tested trying to improve
252 the removal of matrix that hampers the detection.[12,27] After using these clean-up cartridges,
253 several pesticides, as HCB and DDTs, were not detected at the spiked levels. GCB seems to
254 properly remove additional matrix components from vegetable extracts, but it also tends to
255 retain certain pesticides, such as terbufos, thiabendazole, HCB, and other planar-ring analytes.[12]
256 Finally, the QuEChERS kit without GCB was selected for sample clean-up.

257

258 **APCI ionization.** Ionization in GC-(APCI)MS occurs by charge-transfer and/or proton transfer
259 resulting in the formation of the molecular ion, protonated molecule, or both. By deliberately
260 introducing water in the source, this protonation can be promoted (details on this issue can be
261 found in recent publications). [23, 30] Thus, many compounds, including most pesticides and PAHs,
262 gave higher response when using water as modifier and measuring the protonated molecule. On
263 the contrary, halogenated hydrocarbons without any other heteroatoms, as some organochlorine
264 pesticides, showed better response under charge-transfer conditions, being the molecular ion the

265 diagnostic ion. In the latter case, better response was obtained without adding water in the
266 source. However, in some cases, this behavior was observed to show some irreproducibility
267 along the time. This might be due to the fact that humidity present in the atmosphere is an
268 uncontrolled parameter that may affect differently to those compounds ionized under charge-
269 transfer conditions. Also, for those compounds that show a tendency to protonation, ambient
270 humidity might be even better than the saturation conditions reached after the deliberate
271 introduction of water in the source. Under these circumstances, the fact of adding or not adding
272 water would affect mainly to the sensitivity, particularly for a few selected compounds.
273 Consequently, the samples were run twice (with and without intentional use of water as
274 modifier), which allowed to reach the optimum conditions for each compound.

275

276 **QTOF MS data processing.** The acquired data files from the GC-QTOF MS were processed
277 by using ChromaLynx software. A *txt* file with the list of the molecular formula for
278 representative ions was collected together with the retention time per compound. This
279 information was available when the reference standard was injected, and was used to search in
280 the recorded masses for each file. The software searches for $[M+H]^+$, $M^{+\cdot}$ and/or fragment ions
281 at a pre-fixed retention time (target approach).

282 When the reference standard was not available, the only information was that either the
283 molecular ion and/or the protonated molecule would be expected upon GC-(APCI)QTOF MS
284 analysis. In this case, both ions were included in the processing screening method, as the
285 behavior in the APCI source could not be previously evaluated for these compounds. Any
286 detection being made by this way would indicate potential presence of the compound and more
287 information would be required for further identification (e.g. MS/MS experiments,…).
288 Obviously, as no reference standard was injected, no experimental data on the behavior of the
289 compound along sample preparation and GC-MS sensitivity was available. The acquisition of
290 reference standard and injection in the GC-QTOF MS system would be needed for unequivocal
291 confirmation of the tentative identification.

292

293 **Qualitative validation of the screening method.** Firstly, different samples of each matrix were
294 injected in order to find the lowest contaminated matrix for spiking. In this previous analysis,
295 we found some matrices positives for several target compounds. It is noteworthy that
296 ethoxyquin and light PAHs were present in the wide majority of samples analyzed. The lowest
297 contaminated samples for each type of matrix were then spiked with a mixture of pesticides and
298 PAHs at a concentration of 0.01 and 0.05 mg/kg for each analyte (0.005 and 0.025 mg/kg for
299 cyclopenta[*c,d*]pyrene). 133 pesticides and 24 PAHs were selected in order to qualitatively
300 validate the screening by GC-QTOF MS. Twenty different samples of interest for marine
301 seafood were used for validation experiments. Table 1 and 2 show the number of
302 positive/negative findings for pesticides and PAHs, respectively, at each spiked level in the
303 samples studied. At 0.01 mg/kg, 76% of pesticides and 83% of PAHs were detected. At the
304 highest level validated (0.05 mg/kg), these values improved up to 91 % of detected pesticides
305 and up to 100 % of PAHs. Figure 2 shows the percentage of detections for the different matrices
306 studied. As it can be seen, oils were the most problematic matrices followed by feeds and
307 tissues while feed ingredients represented lower difficulty for detections. Regarding fish tissues,
308 liver was trickier than fish fillets.

309 Figure 3 shows different examples of the qualitative validation at the 0.01 mg/kg level. Four
310 groups are illustrated, attending at the samples studied: (A) ingredients, (B) oils, (C) feeds and
311 (D) fish tissues. The bottom of each figure shows the nw-XIC for the non-spiked sample and,
312 top shows the nw-XIC for the spiked sample at 0.01 mg/kg with the most abundant ion used for
313 detection, measured at accurate mass (mass error in ppm is also given). In the case of
314 ingredients (Figure 3A), HCH isomers were properly detected at 0.01 mg/kg in fish meal. These
315 compounds were satisfactorily validated in all samples at 0.01 mg/kg except for oils so, a SDL
316 at 0.05 mg/kg was finally proposed (Table 1). The group of dibenzo[*a,l*]pyrene,
317 dibenzo[*a,e*]pyrene, dibenzo[*a,i*]pyrene and dibenzo[*a,h*]pyrene were validated at 0.05 mg/kg
318 since they could not be detected in at least 95 % of samples at the lowest level, despite that in

319 corn gluten these isomers were detected at 0.01 mg/kg. The figure for oils (Figure 3B) illustrates
320 the validation for benzo[*a*]pyrene, a toxicity reference, at 0.01 mg/kg. The validation of
321 chlorpyrifos methyl was of relevance since it is widely used as an insecticide. In both cases, the
322 detection at 0.01 mg/kg was feasible in oils within low mass errors below 5 ppm. In the case of
323 feeds (Figure 3C), the widely known DDTs, included in the target list due to their common
324 presence in marine resources as part of the larger group of fat-soluble POPs that readily
325 accumulate along the marine food chain, were satisfactorily validated at 0.01 mg/kg for
326 ingredients, feeds and tissues but not for oils so, a SDL at 0.05 mg/kg was finally proposed. The
327 herbicide carfentrazone-ethyl is used in many crops such as wheat, corn or soya. Therefore, it
328 was included in the target list, and also because sub-products from these crops are commonly
329 incorporated in feed compositions (wheat gluten, corn gluten, soya protein). A SDL at 0.01
330 mg/kg was achieved in all samples studied for this compound. Finally, in fish tissues (Figure
331 3D), the nw-XICs illustrate the presence of phenanthrene and anthracene in the non-spiked
332 samples, a fact that was also observed in other types of samples, supporting the ubiquitous of
333 light PAHs in many environmental and food samples. The same occurred for ethoxyquin,
334 synthetic preservative widely used in fatty compositions to prevent lipids oxidation. The
335 presence of at least two representative ions for each compound at the expected retention time
336 with accepted mass errors (< 5ppm) allowed the reliable identification in positive samples.

337

338 **Screening of real samples.** The validated screening was applied to different types of samples,
339 searching for the target list of validated compounds. After the detection of any compound in the
340 samples, the reliable identification was required in order to avoid reporting false positives.
341 Although the presence of a *m/z* ion (commonly $[M+H]^+$), measured at accurate mass with low
342 mass error, and the agreement in retention time, gives confidence to the analysis, we followed
343 strict criteria for confirmation, which was based on the presence of, at least, another
344 representative *m/z* ion (commonly fragment ion) with low mass error.[23] This is feasible working

345 in the QTOF MS instrument that allows the use of MS^E mode (details in GC-TOF MS
346 instrumentation).

347 Figure 4 shows different positive findings in oils, proteins and feeds commonly used in animal
348 farming. As shown, at least two representative *m/z* ions were necessary to unequivocally
349 identify the presence of the compound in the sample, at the expected retention time (deviation ≤
350 ± 0.5 %, in comparison to standards) and measured at accurate mass within acceptable mass
351 deviation (≤ 5ppm). The main pesticides found were chlorpyrifos methyl and pirimiphos methyl
352 which were detected in several vegetable samples. Ethoxyquin, which use is currently
353 authorized in feed ingredients, was also found in feeds but, additionally, it was identified in the
354 edible part of several commercial fish samples. It seems that this synthetic preservative (and
355 possibly its transformation products) can arrive to consumers. Earlier studies also reported the
356 overall presence of synthetic antioxidants, such as ethoxyquin, in several commercially
357 important species of farmed fish, namely Atlantic salmon, halibut, cod and rainbow trout and
358 their aquafeeds, [31] as well as the carry-over from feed to fillet. [32] Therefore, quantitative
359 methods directed towards this compound and its derivatives will surely be necessary in the near
360 future.
361 As regards PAHs, “light” PAHs (e.g. phenanthrene, pyrene…) were in nearly all samples
362 analyzed. Although they are not the carcinogenic PAHs according to EFSA, they are
363 contaminants that can give (non-carcinogenic) toxic reactions in fish.[33] PAHs present poor MS-
364 fragmentation, a fact that makes their identification troublesome. In this work, after evaluating
365 the presence of the protonated molecule in the LE function, collision induced dissociation (CID)
366 fragments, or characteristic isotopic ions, were also evaluated for positive samples to achieve a
367 proper identification. As illustrative examples, at the bottom of Figure 4, positive findings for
368 light PAHs are presented for samples of rapeseed, linseed and wheat.

369 After the first screening for which reference standards were available, we focused our screening
370 to find any other pesticides for which reference standard was not available in our lab. Although
371 tentative detection for some of these pesticides occurred, based on the presence of $[M+H]^+$

372 and/or $M^{+\cdot}$ in the LE function, further investigation of fragment ions, from the LE and/or HE
373 function, did not allow us to confirm its identity, so they could not be reported as positive
374 identifications.

375 With this work, we pursued the achievement of an essential tool in food safety and toxicology:
376 the use of wide-scope screening for detection of large number of compounds. The combination
377 of GC-HRMS and LC-HRMS is nowadays the closest approach to the ideal "universal"
378 screening. The GC-QTOF MS screening developed has been qualitatively validated for a
379 notable number of pesticides and PAHs in complex samples from aquaculture activities,
380 allowing the detection of these compounds in a rapid and efficient way at the low SDL
381 established. This method in combination with the LC-QTOF MS screening previously
382 developed allows searching of many undesirables of different polarity and volatility in distinct
383 sample matrices. [22,34,35]

384

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Table 1. Validation results for pesticides. Screening detection limit (SDL).

Compound	m/z	positive/negative results (n=20) 0.01 mg/kg +/-	positive/negative results (n=20) 0.05 mg/kg +/-	SDL (mg/kg)	Compound	m/z	positive/negative results (n=20) 0.01 mg/kg +/-	positive/negative results (n=20) 0.05 mg/kg +/-	SDL (mg/kg)
2-Phenylphenol	171.0810	20/0	20/0	0.01	Fipronil	436.9465	20/0	20/0	0.01
4-4'-Dichlorobenzophenone	251.0030	20/0	20/0	0.01	Flucythrinate*	412.1549	11/9	20/0	0.05
Alachlor*	238.0999	19/1	20/0	0.01	**Fludioxonil**	248.0397	17/3	19/1	0.05
Aldrin	362.8835	4/16	14/6	-	Folpet*	259.9340	19/1	20/0	0.01
alpha-endosulphan	404.8247	14/6	20/0	0.05	**gamma-HCH** *	180.9379	13/7	19/1	0.05
alpha-HCH*	180.9379	14/6	19/1	0.05	**HCB**	281.8131	13/7	19/1	0.05
Atrazine	216.1016	20/0	20/0	0.01	Heptachlor	370.8289	18/2	20/0	0.05
Atrazine desethyl	188.0703	20/0	20/0	0.01	Heptachlor epoxide A	386.8238	5/15	20/0	0.05
Atrazine desisopropyl	174.0546	20/0	20/0	0.01	Heptachlor epoxide B	386.8238	5/15	20/0	0.05
Azinphos methyl	318.0136	5/15	11/9	-	**Hexachlorobutadiene**	257.8131	10/10	16/4	-
Azoxystrobin	404.1246	20/0	20/0	0.05	Imazalil	297.0561	5/15	10/10	-
beta-endosulfan	404.8247	12/8	20/0	0.05	Iprodione	330.0412	20/0	20/0	0.01
beta-HCH*	180.9379	13/7	19/1	0.05	Isodrin	362.8835	4/16	14/6	-
Bifenthrin*	181.1017	20/0	20/0	0.01	lamba-Cyhalothrin	450.1084	7/13	20/0	0.05
Bromophos	392.8883	20/0	20/0	0.01	Leptophos	410.8778	19/1	20/0	0.01
Bromophos ethyl	364.8570	19/1	20/0	0.01	Malathion	331.0439	20/0	20/0	0.01
Bromopropilate*	156.9864	19/1	20/0	0.01	Metalaxyl	280.1549	20/0	20/0	0.01
Buprofezin	306.1640	19/1	20/0	0.01	Methamidophos	142.0092	15/5	19/1	0.05
Cadusafos	271.0955	18/2	20/0	0.05	Methidathion	302.9697	12/8	15/5	-
Captafol	347.9186	1/19	3/17	-	Methiocarb*	169.0687	20/0	20/0	0.01
Captan*	263.9653	4/16	4/16	-	Methoxychlor*	236.9641	20/0	20/0	0.01
Carbaryl*	145.0646	7/13	20/0	0.05	Metolachlor	284.1417	20/0	20/0	0.01
Carbofuran*	165.0916	8/12	20/0	0.05	Metribuzin	215.0967	20/0	20/0	0.01
Carbophenothion	342.9817	1/19	12/8	-	**Mirex***	269.8131	10/10	18/2	-
Carfentrazone ethyl	412.0443	19/1	20/0	0.01	Molinate	188.1109	20/0	20/0	0.01
Chinomethionat	235.0000	20/0	20/0	0.01	Oxadixyl	279.1345	13/7	19/1	0.05
Chlorfenapyr	406.9774	20/0	20/0	0.01	Oxyfluorfen	362.0407	20/0	20/0	0.01
Chlorfenson	302.9649	13/7	15/5	-	**p,p'-DDD***	235.0081	16/4	20/0	0.05
Chlorfenvinphos	358.9774	20/0	20/0	0.01	**p,p'-DDE**	315.9380	14/6	20/0	0.05
Chlorothalonil	264.8894	3/17	19/1	0.05	**p,p'-DDT***	235.0081	14/6	19/1	0.05
Chlorpropham*	172.0165	20/0	20/0	0.01	Parathion ethyl	292.0409	20/0	20/0	0.01
Chlorpyrifos ethyl	349.9341	20/0	20/0	0.01	Parathion methyl	264.0096	20/0	20/0	0.01
Chlorpyrifos methyl	321.9028	20/0	20/0	0.01	Pendimethalin	282.1454	9/11	14/6	-
Coumaphos	363.0223	20/0	20/0	0.01	**Pentachlorobenzene**	247.8521	14/6	20/0	0.05
Cyanazine	241.0968	18/2	20/0	0.05	Permethrin*	355.1101	2/18	20/0	0.05
Cyanophos	244.0197	20/0	20/0	0.01	Phorate	261.0207	12/8	19/1	0.05
Cyfluthrin	434.0726	1/19	3/17	-	Phosmet	318.0024	12/8	20/0	0.05
Cypermethrin	416.0820	0/20	3/17	-	Pirimicarb	239.1508	20/0	20/0	0.01
Cyprodinil	226.1344	20/0	20/0	0.01	Pirimiphos methyl	306.1041	20/0	20/0	0.01
delta-HCH*	180.9379	13/7	19/1	0.05	Procymidone	284.0245	20/0	20/0	0.01
Deltamethrin	503.9810	0/20	4/16	-	Propetamphos	282.0929	1/19	9/11	-
Diazinon	305.1089	20/0	20/0	0.01	Propham*	138.0550	20/0	20/0	0.01
Dichlofenthion	314.9778	20/0	20/0	0.01	Propiconazole	342.0776	20/0	20/0	0.01
Dichloran	206.9728	20/0	20/0	0.01	Propoxur	210.1130	10/10	20/0	0.05
Dichlorvos	220.9537	20/0	20/0	0.01	Propyzamide	256.0296	20/0	20/0	0.01
Dieldrin	378.8785	14/6	20/0	0.05	Pyriproxyfen	322.1443	20/0	20/0	0.01
Diflufenican	395.0819	20/0	20/0	0.01	Quinalphos	299.0619	20/0	20/0	0.01
Dimethoate	230.0074	15/5	19/1	0.05	Resmethrin	339.1960	20/0	20/0	0.01
Dioxathion*	271.0228	16/4	20/0	0.05	Simazine	202.0859	20/0	20/0	0.01
Diphenylamine	170.0970	20/0	20/0	0.01	tau-Fluvalinate	503.1349	1/19	12/8	-
Endosulfan ether	340.8628	20/0	20/0	0.01	Tefluthrin	419.0649	14/6	20/0	0.05
Endosulfan sulfate	420.8196	4/16	12/8	-	Terbacil*	161.0118	20/0	20/0	0.01
Endrin	378.8785	12/8	20/0	0.05	Terbumeton	226.1668	20/0	20/0	0.01
EPN	324.0459	20/0	20/0	0.01	Terbumeton desethyl	198.1355	19/1	20/0	0.01
Ethalfluralin	334.1015	20/0	20/0	0.01	Terbuthylazine	230.1172	18/2	20/0	0.05
Ethion	384.9954	12/8	20/0	0.05	Terbuthylazine desethyl	202.0859	20/0	20/0	0.01
Ethoxyquin	218.1545	20/0	20/0	-[a]	Terbutryn	242.1439	20/0	20/0	0.01
Etofenprox*	359.2011	19/1	20/0	0.01	Tetradifon	354.8921	19/1	19/1	0.01
Famphur	326.0286	20/0	20/0	0.01	Thiabendazole	202.0439	4/16	20/0	0.05
Fenamiphos	304.1136	17/3	20/0	0.05	Tolclofos methyl	300.9622	19/1	20/0	0.01
Fenarimol	331.0405	20/0	20/0	0.01	Tolyfluanid*	237.9660	10/10	12/8	-
Fenhexamid	302.0715	20/0	20/0	0.01	trans-Chlordane	405.7978	0/20	4/16	-
Fenitrothion	278.0252	20/0	20/0	0.01	Triadimefon	294.1009	20/0	20/0	0.01
Fenoxycarb	302.1392	19/1	20/0	0.01	Triflumizole	346.0934	18/2	20/0	0.05
Fenthion	279.0278	20/0	20/0	0.01	Trifluralin	336.1171	20/0	20/0	0.01
Fenvalerate	420.1366	0/20	5/15	-	Vinclozolin	286.0038	19/1	20/0	0.01

All compounds were measured as $[M+H]^+$ after adding water in the APCI source, except for those marked in bold that were measured as $M^{+\cdot}$ without adding water as modifier.

*These compounds were measured as fragment ions.

[a]The evaluation of the SDL was not feasible due to the presence of the analyte in several of the samples used for validation.

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528 Table 2. Validation results for PAHs. Screening detection limit (SDL).

Compound[a]	m/z	positive/negative results (n=20) 0.01 mg/kg +/-	0.05 mg/kg +/-	SDL
Naphthalene	129.0704	20/0	20/0	-[b]
Acenaphthylene	153.0704	20/0	20/0	-[b]
Acenaphthene	155.0861	20/0	20/0	-[b]
Fluorene	167.0861	20/0	20/0	-[b]
Phenanthrene	179.0861	20/0	20/0	-[b]
Anthracene	179.0861	20/0	20/0	-[b]
Fluoranthene	203.0861	20/0	20/0	-[b]
Pyrene	203.0861	20/0	20/0	-[b]
Benzo[*c*]fluorene	217.1017	20/0	20/0	0.01
Cyclopenta[*c,d*]pyrene[c]	227.0861	20/0	20/0	0.005
Benzo[*a*]anthracene	229.1017	20/0	20/0	0.01
Chrysene	229.1017	20/0	20/0	0.01
5-Methylchrysene	243.1174	19/1	20/0	0.01
Benzo[*j*]fluoranthene	253.1017	19/1	20/0	0.01
Benzo[*b*]fluoranthene	253.1017	19/1	20/0	0.01
Benzo[*k*]fluoranthene	253.1017	19/1	20/0	0.01
Benzo[*a*]pyrene	253.1017	19/1	20/0	0.01
Indeno[*1,2,3,cd*]pyrene	277.1017	19/1	20/0	0.01
Dibenzo[*a,h*]anthracene	279.1174	19/1	20/0	0.01
Benzo[*g,h,i*]perylene	277.1017	19/1	20/0	0.01
Dibenzo[*a,l*]pyrene	303.1174	9/11	20/0	0.05
Dibenzo[*a,e*]pyrene	303.1174	9/11	20/0	0.05
Dibenzo[*a,i*]pyrene	303.1174	9/11	20/0	0.05
Dibenzo[*a,h*]pyrene	303.1174	9/11	20/0	0.05

529 [a] PAHs were measured as $[M+H]^+$ after adding water in the APCI source.

530 [b] The evaluation of the SDL was not feasible due to the presence of the analyte in the samples

531 used for validation.

532 [c] Cyclopenta[*c,d*]pyrene was spiked at 0.005 and 0.025 mg/kg, respectively.

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537 **Figure captions.**

538

539 **Figure 1**. Sample treatment applied in the GC (APCI)- QTOF MS screening method.

540

541 **Figure 2**. Validation results. Number of pesticides detected at 0.01 and 0.05 mg/kg in different
542 type of samples.

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544 **Figure 3**. Validation. nw-XICs for the diagnostic *m/z* ion in samples spiked at 0.01 mg/kg (top)
545 and non-spiked samples (bottom). Diagnostic ion corresponds to $[M+H]^+$ except for HCH
546 isomers and DDTs where it corresponds to a fragment ion
547 (A) α, β, γ, δ-HCH isomers and dibenzo[*a,l*]pyrene, dibenzo[*a,e*]pyrene, dibenzo[*a,i*]pyrene,
548 dibenzo[*a,h*]pyrene in feed ingredients
549 (B) benzo[*a*]pyrene and chlorpyrifos methyl in oils
550 (C) p,p'-DDD, p,p'-DDT and carfentrazone-ethyl in feeds
551 (D) phenanthrene, anthracene and ethoxyquin in fish tissues.
552 ✓: accurate mass deviations within tolerance limits.
553

554 **Figure 4**. Real-world samples. nw-XICs for identified compounds in oils, proteins and feeds.
555 For each matrix, the LE function (bottom) and HE (top) are shown to illustrate the presence of
556 the protonated molecule (LE) and fragment ions (HE).
557 ✓: accurate mass deviations within tolerance limits.
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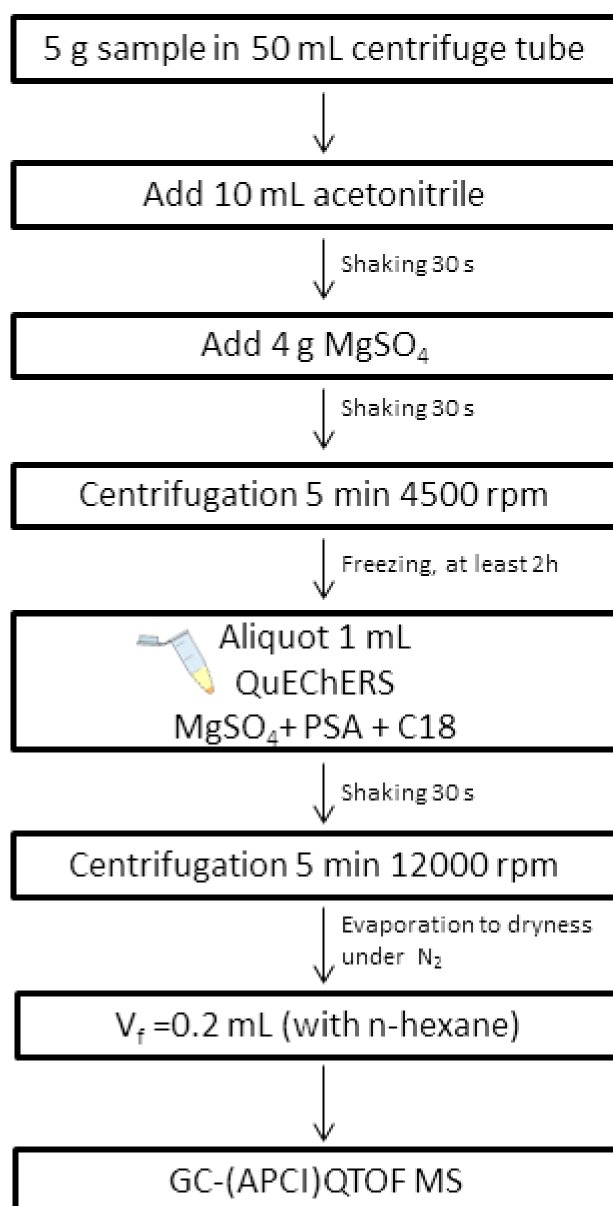

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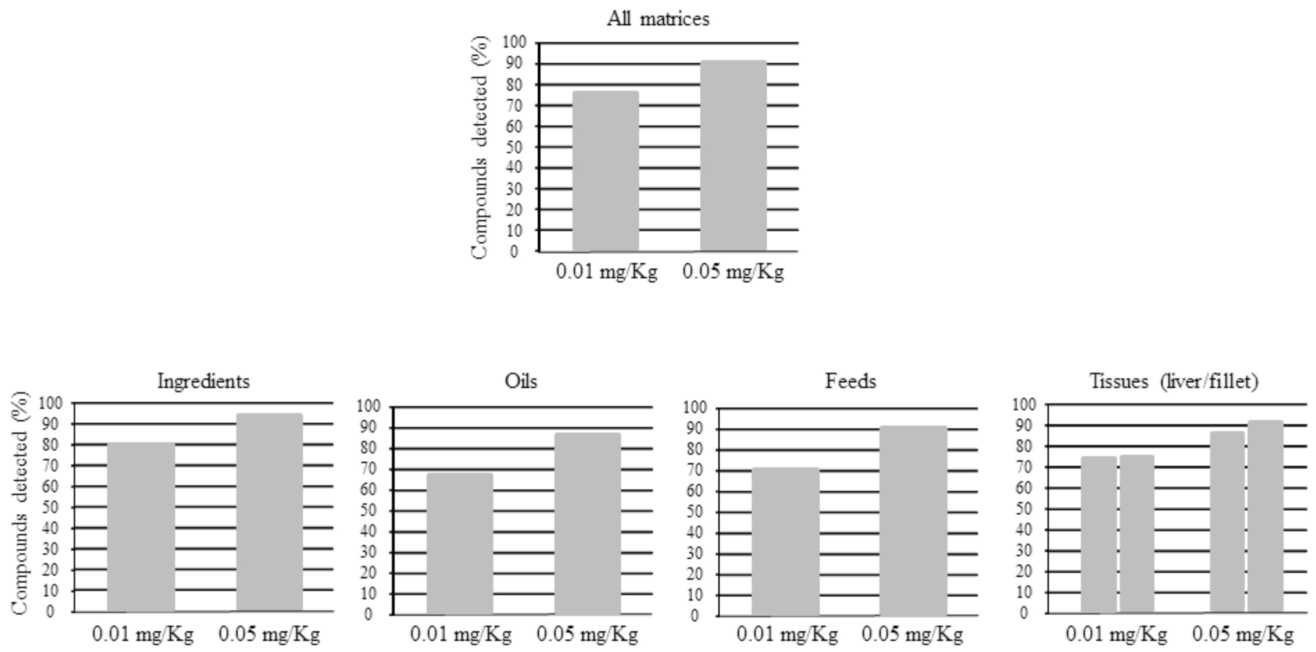

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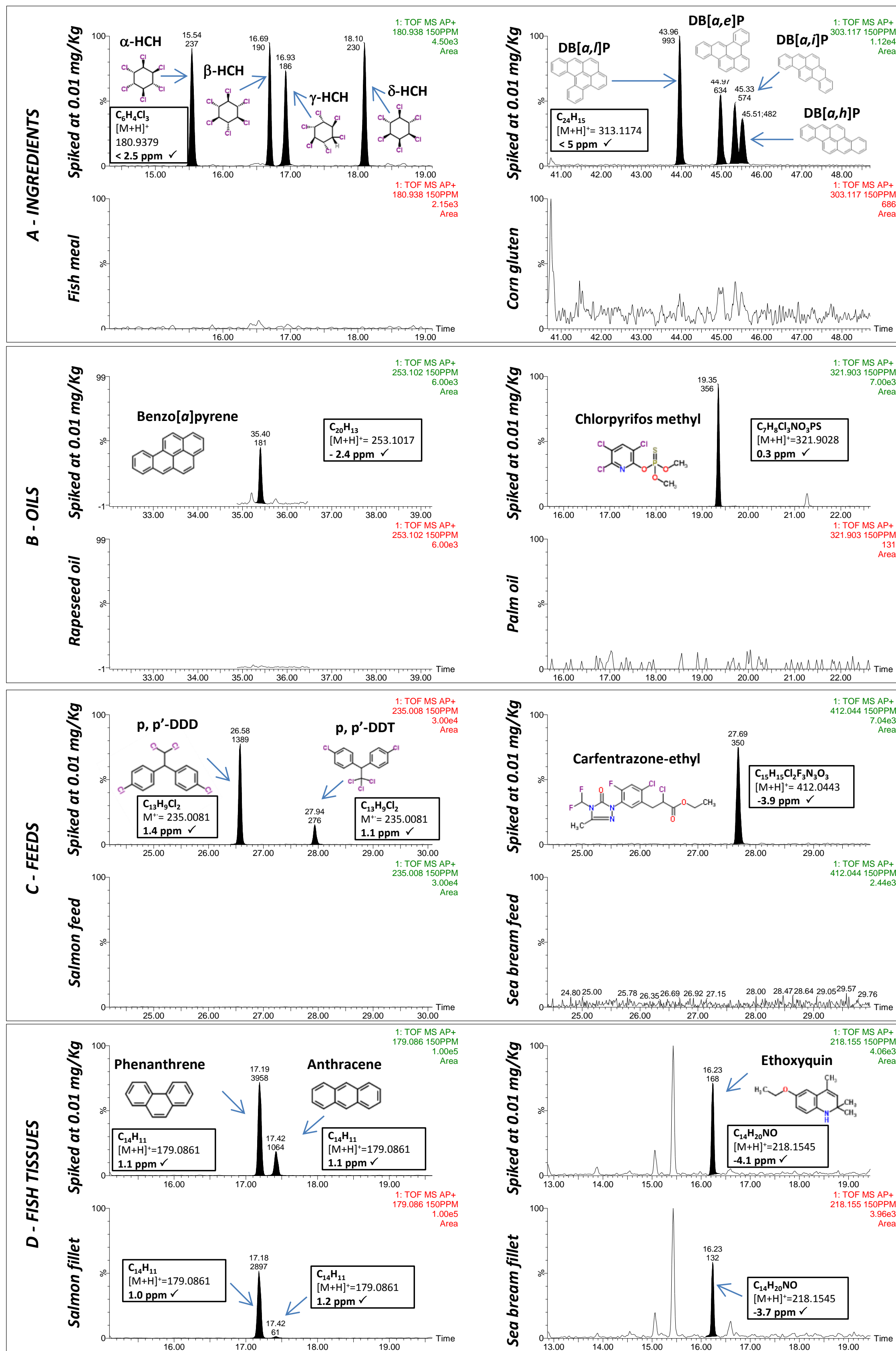

616 Figure 3

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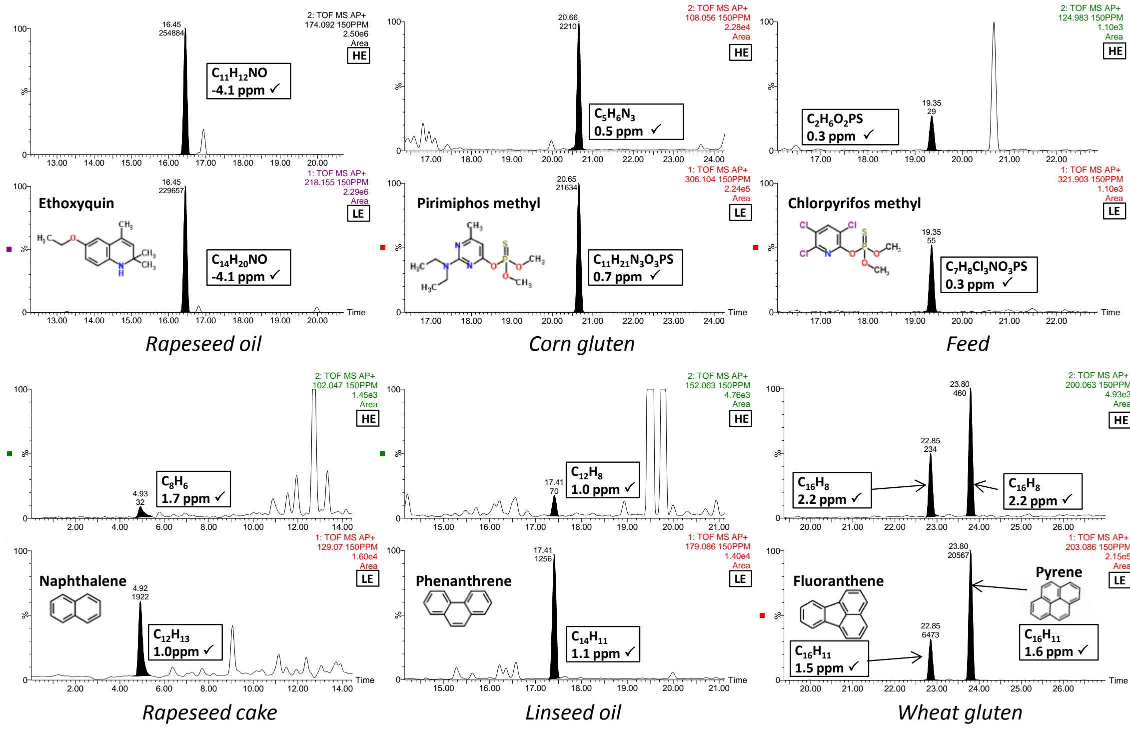

619 Figure 4

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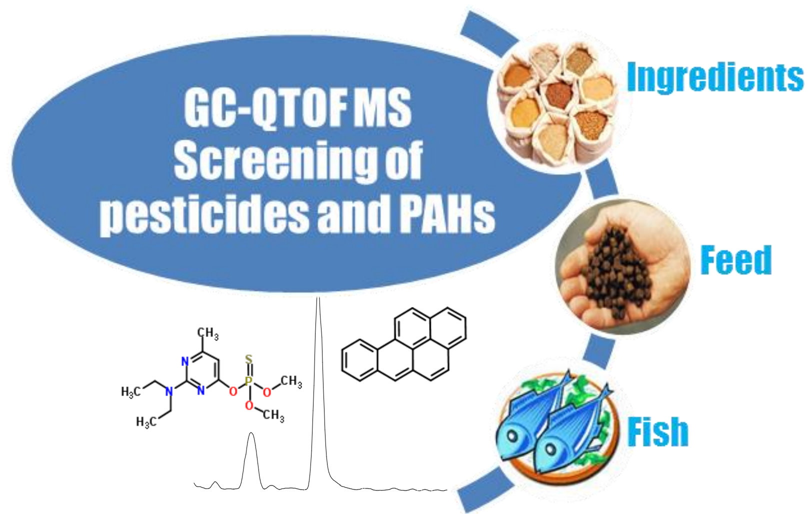

645 TOC graphic