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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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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Abstract

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

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

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INTRODUCTION

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

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

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

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

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

MATERIAL AND METHODS

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

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

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

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

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

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

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

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

RESULTS AND DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Compound	m/z	positive/negative results (n=20) 0.01 mg/kg +/-	0.05 mg/kg +/-	SDL (mg/kg)	Compound	m/z	positive/negative results (n=20) 0.01 mg/kg +/-	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

523 All compounds were measured as $[M+H]^+$ after adding water in the APCI source, except for those marked in bold that were
524 measured as $M^{+\cdot}$ without adding water as modifier.
525 *These compounds were measured as fragment ions.
526 [a]The evaluation of the SDL was not feasible due to the presence of the analyte in several of the samples used for validation.

527

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

		positive/negative results (n=20)		
Compound[a]	**m/z**	**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.

533

534

535

Figure captions.

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

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

Figure 3. Validation. nw-XICs for the diagnostic *m/z* ion in samples spiked at 0.01 mg/kg (top) and non-spiked samples (bottom). Diagnostic ion corresponds to $[M+H]^+$ except for HCH isomers and DDTs where it corresponds to a fragment ion

(A) α, β, γ, δ-HCH isomers and dibenzo[*a,l*]pyrene, dibenzo[*a,e*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,h*]pyrene in feed ingredients

(B) benzo[*a*]pyrene and chlorpyrifos methyl in oils

(C) p,p'-DDD, p,p'-DDT and carfentrazone-ethyl in feeds

(D) phenanthrene, anthracene and ethoxyquin in fish tissues.

✓: accurate mass deviations within tolerance limits.

Figure 4. Real-world samples. nw-XICs for identified compounds in oils, proteins and feeds. For each matrix, the LE function (bottom) and HE (top) are shown to illustrate the presence of the protonated molecule (LE) and fragment ions (HE).

✓: accurate mass deviations within tolerance limits.

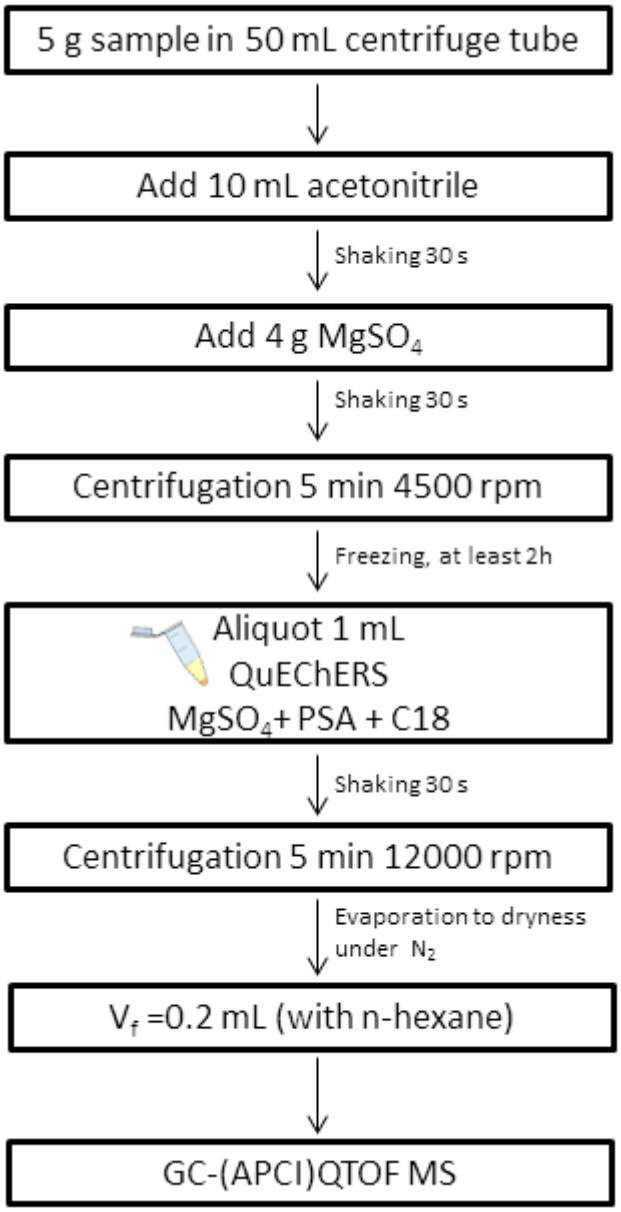

5 g sample in 50 mL centrifuge tube
Add 10 mL acetonitrile
Shaking 30 s
Add 4 g $MgSO_4$
Shaking 30 s
Centrifugation 5 min 4500 rpm
Freezing, at least 2h
Aliquot 1 mL
QuEChERS
$MgSO_4$+ PSA + C18
Shaking 30 s
Centrifugation 5 min 12000 rpm
Evaporation to dryness
under N_2
V_f =0.2 mL (with n-hexane)
GC-(APCI)QTOF MS

Figure 1

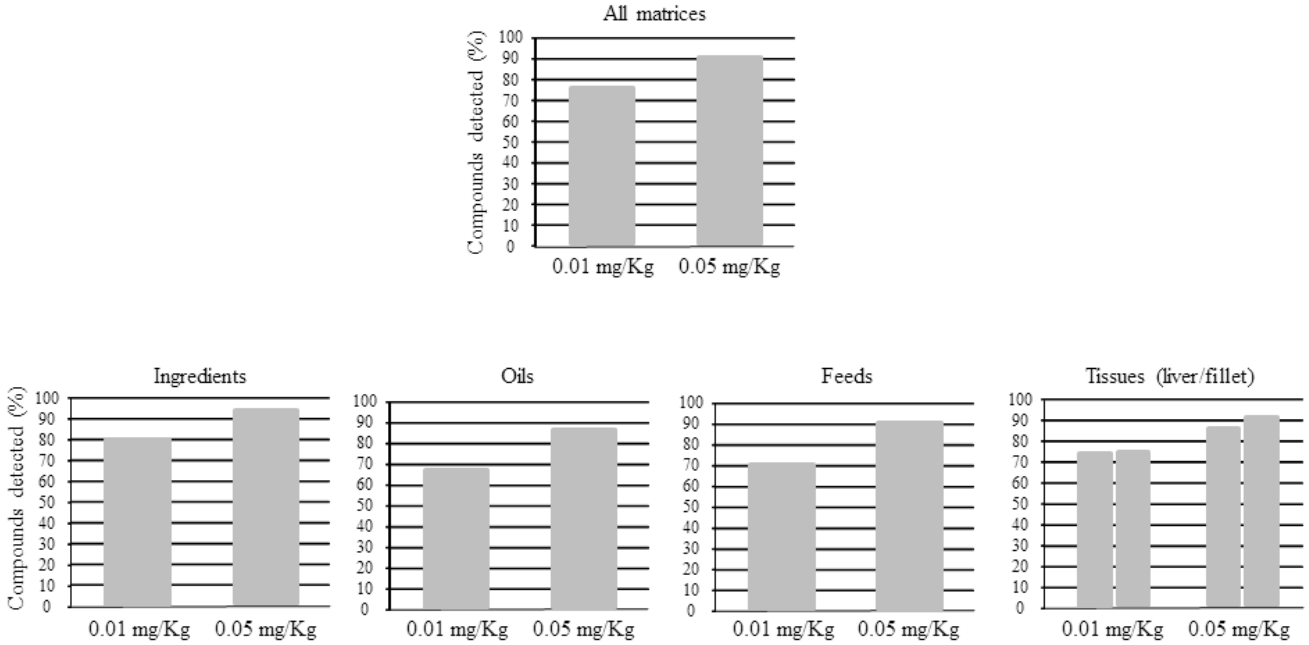

All matrices
Compounds detected (%)
100
90
80
70
60
50
40
30
20
10
0
0.01 mg/Kg
0.05 mg/Kg
Ingredients
Oils
Feeds
Tissues (liver/fillet)

Figure 2

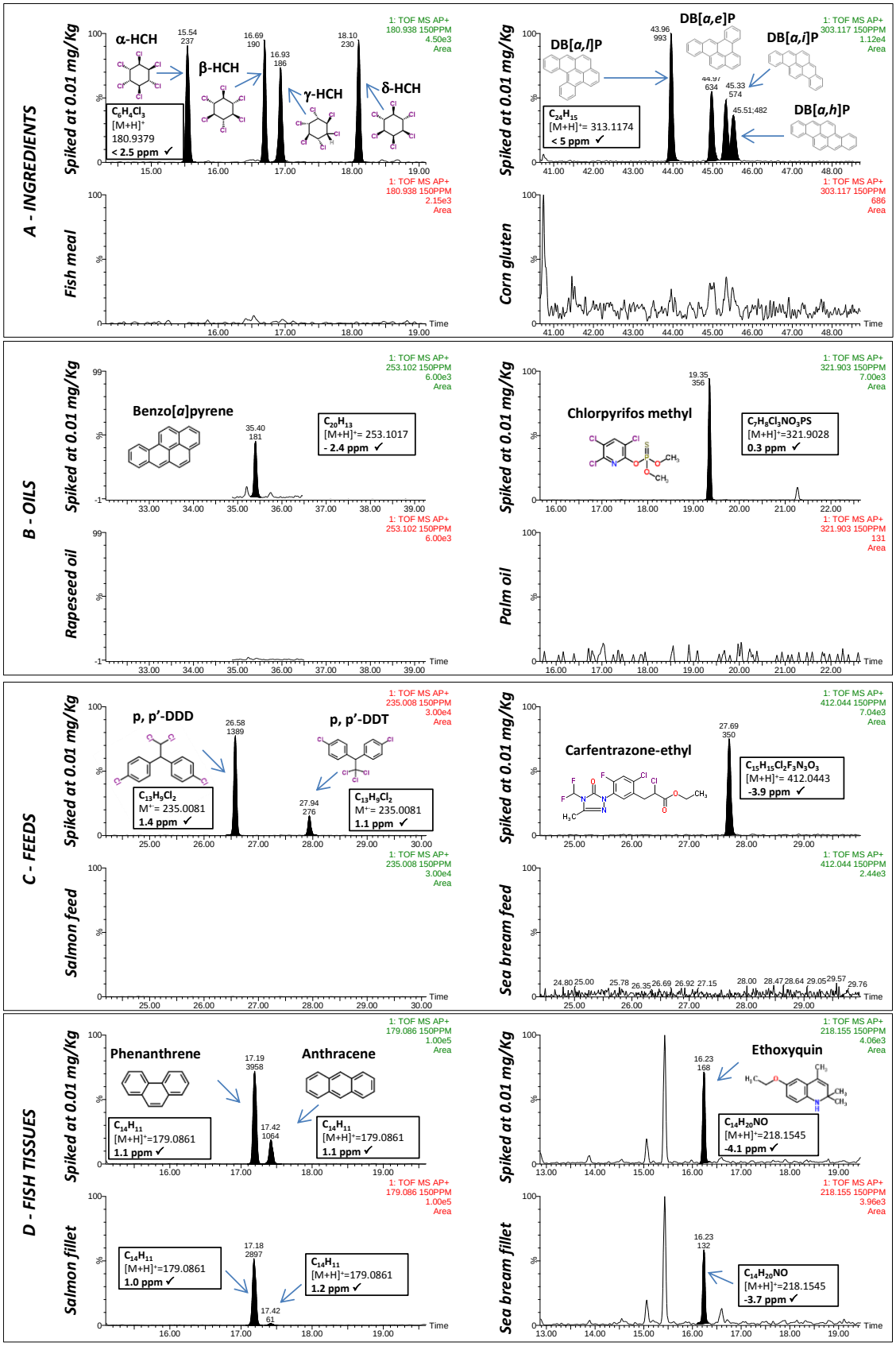

A - INGREDIENTS
Spiked at 0.01 mg/Kg
α-HCH
β-HCH
γ-HCH
δ-HCH
C6H4Cl3
[M+H]+
180.9379
< 2.5 ppm ✓
DB[a,l]P
DB[a,e]P
DB[a,i]P
DB[a,h]P
C24H15
[M+H]+= 313.1174
< 5 ppm ✓
Fish meal
Corn gluten
B - OILS
Benzo[a]pyrene
C20H13
[M+H]+= 253.1017
- 2.4 ppm ✓
Chlorpyrifos methyl
C7H8Cl3NO3PS
[M+H]+=321.9028
0.3 ppm ✓
Rapeseed oil
Palm oil
C - FEEDS
p, p'-DDD
C13H9Cl2
M+= 235.0081
1.4 ppm ✓
p, p'-DDT
C13H9Cl2
M+= 235.0081
1.1 ppm ✓
Carfentrazone-ethyl
C15H15Cl2F3N3O3
[M+H]+= 412.0443
-3.9 ppm ✓
Salmon feed
Sea bream feed
D - FISH TISSUES
Phenanthrene
Anthracene
C14H11
[M+H]+=179.0861
1.1 ppm ✓
Ethoxyquin
C14H20NO
[M+H]+=218.1545
-4.1 ppm ✓
Salmon fillet
C14H11
[M+H]+=179.0861
1.0 ppm ✓
C14H11
[M+H]+=179.0861
1.2 ppm ✓
Sea bream fillet
C14H20NO
[M+H]+=218.1545
-3.7 ppm ✓
Time

Figure 3

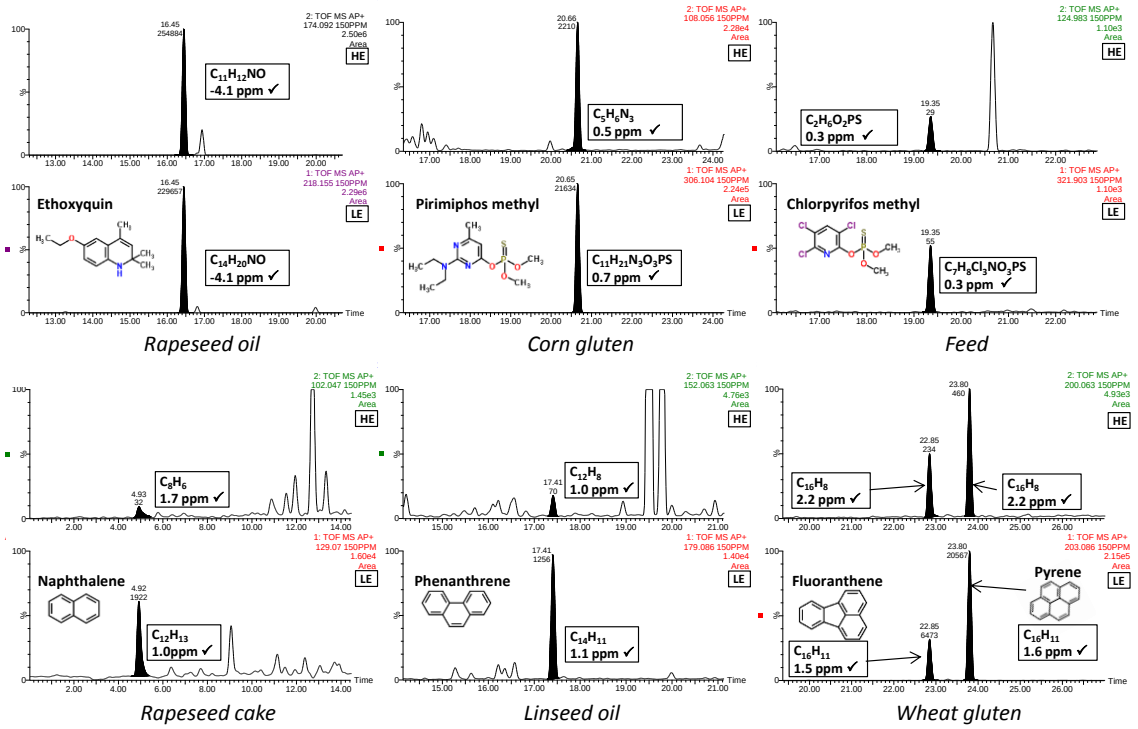
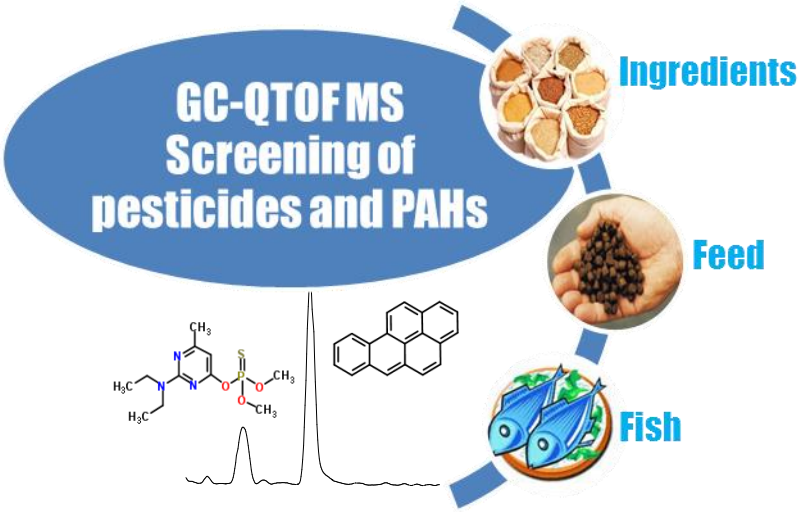

Ethoxyquin
$C_{14}H_{20}NO$
-4.1 ppm ✓
$C_{11}H_{12}NO$
-4.1 ppm ✓
Rapeseed oil
Pirimiphos methyl
$C_{11}H_{21}N_3O_3PS$
0.7 ppm ✓
$C_5H_6N_3$
0.5 ppm ✓
Corn gluten
Chlorpyrifos methyl
$C_7H_8Cl_3NO_3PS$
0.3 ppm ✓
$C_2H_6O_2PS$
0.3 ppm ✓
Feed
Naphthalene
$C_{12}H_{13}$
1.0ppm ✓
C_8H_6
1.7 ppm ✓
Rapeseed cake
Phenanthrene
$C_{14}H_{11}$
1.1 ppm ✓
$C_{12}H_8$
1.0 ppm ✓
Linseed oil
Fluoranthene
$C_{16}H_{11}$
1.5 ppm ✓
Pyrene
$C_{16}H_{11}$
1.6 ppm ✓
$C_{16}H_8$
2.2 ppm ✓
$C_{16}H_8$
2.2 ppm ✓
Wheat gluten

Figure 4


GC-QTOF MS
Screening of
pesticides and PAHs
Ingredients
Feed
Fish

TOC graphic