MULTI-CLASS DETERMINATION OF UNDESIRABLES IN AQUACULTURE SAMPLES BY GAS CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY WITH ATMOSPHERIC PRESSURE CHEMICAL IONIZATION: A NOVEL APPROACH FOR POLYCYCLIC AROMATIC HYDROCARBONS

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ABSTRACT

In this work, a method for the analysis of 24 PAHs in 19 different matrices, including fish tissues, feeds and feed ingredients, has been developed using gas chromatography coupled to triple quadrupole tandem mass spectrometry with atmospheric pressure chemical ionization source (GC-APCI-MS/MS). The method is based on a modification of the unbuffered QuEChERS method, using freezing as an additional clean-up step and applying a 20-fold dilution factor to the final extract. The procedure was also tested for 15 pesticides and 7 polychlorinated biphenyl (PCB) congeners in order to widen the scope of the method.

The excellent sensitivity and selectivity provided by GC-APCI-MS/MS allowed the dilution of the sample extracts and quantification using calibration with standards in solvent for all the 19 matrices tested. The developed method was evaluated at 2, 5 and 50 $ng \cdot g^{-1}$ spiking levels. LOQs were 2 $ng \cdot g^{-1}$ for most compounds, and LODs ranged from 0.5 to 2 $ng \cdot g^{-1}$.

Analysis of real-world samples revealed the presence of naphthalene, fluorene, phenanthrene, fluoranthene and pyrene at concentration levels ranging from 4.8 to 187 $ng \cdot g^{-1}$. No PCBs, DDTs and pesticides were found in fillets from salmon and sea bream.

KEYWORDS

Polycyclic aromatic hydrocarbons; atmospheric pressure chemical ionization; gas chromatography; triple quadrupole; aquaculture; fish; feed.

INTRODUCTION

Farming of carnivorous marine fish species such as Atlantic salmon (*Salmo salar*) and gilthead sea bream (*Sparus aurata*) has traditionally relied on the use of marine feed ingredients such as fish oil and meal in their formulated feeds [1–3]. Limited access to fish meal and fish oil for the rapidly growing aquaculture industry has led to a need to develop more sustainable aquafeeds that rely less on marine ingredients from feral fish stocks [4,5]. Vegetable oils and proteins are the main alternative to marine feed ingredients in aquafeeds [2,5].

The development of new sustainable plant based feeds for marine fish farming introduces new challenges concerning contaminants that were previously not relevant when using traditional marine feed ingredients. Unrefined plant oils obtained from oilseeds such as soybeans, rapeseeds, olive seeds, and sunflower seeds are known to contain elevated levels of polyaromatic hydrocarbons (PAHs) [6–11]. Although fish oil also contains PAHs from environmental pollution [12], the use of plant oils causes increased PAH levels in plant-based salmon feeds compared to marine-based feeds [13]. In contrast, in a study on fish oil replacement in sea bream (*Sparus aurata*) feeds, plant oils did not have elevated PAH levels compared to fish oil, and it was concluded that feed PAH levels did not correlate with the amount of fish or plant oil used [14]. The genotoxic and carcinogenic "heavy" PAHs (>4-6 rings), such as benzo[a]pyrene (B[a]P), benzo[a]anthracene (B[a]A), chrysene (Chr), benzo[b]fluoranthene (B[b]F), have received special attention with regards to food safety [15]. Studies related to plant oil PAH contamination, however, mainly focus on light (2-4 rings) PAHs such as fluoranthene, naphthalene, anthracene, phenanthrene, as they are most dominantly present in unrefined plant oils. These light PAHs are also on the US EPA

(Environmental Protection Agency) list for environmental relevant PAHs but are mostly not carcinogenic and genotoxic [10,16].

The European union has set an upper limit for 4 PAHs (B[a]P +B[a]A+Chr+B[b]F) in food products to protect the consumer safety [15]. No upper limits for PAH in feed ingredients or animal feeds currently exist. The PAH levels of raw ingredients for feeds for farmed terrestrial animals such as cow, chicken, and pig have been reported in only few studies [11] and little is known on PAH levels in feeds for farmed fin fish species [17,18], especially for feed ingredients used in novel plant based fish feed [13,14,19].

Traditionally, the determination of PAHs in solid fatty matrices have involved time-consuming extractions prior to GC-MS with electron ionization (EI) analysis [20]. However, the sensitivity restrictions of the GC-EI-MS(/MS) systems [21,22] in addition to the inherent matrix effects make pre-concentration and time consuming clean-up steps, as gel permeation chromatography [23] or solid phase extraction [19,24], necessary. Recently, some methodologies have applied the QuEChERS procedure in aquaculture samples [25,26], reducing notably the time and cost of sample treatment. However, despite all efforts to minimize the presence of interferences, the still remaining matrix effects make necessary to use matrix-matched calibration for quantification purposes [22]. In the last years, the application of atmospheric pressure chemical ionization (APCI) source in combination with last generation triple quadrupole instruments has made possible to notably increase sensitivity and selectivity of the determination of organic contaminants in food safety, environment and doping control fields [27–30] in selected reaction monitoring (SRM) based methods. The improved performance of GC-APCI-MS/MS is more than welcome in the aquaculture field, where the analysis of a wide diversity of “difficult” matrices (oils, plant ingredients, marine ingredients, terrestrial animal ingredients, fish, etc.) with this

technique can be simplified by reducing the matrix content in the final extract by simple dilution which minimizes the matrix effect and avoids the need of using matrix-matched calibrations.

In this work, we explore the potential of the new GC-APCI-MS/MS technique for quantification and confirmation of PAHs, together with some selected pesticides and PCBs, in very different sample matrices from aquaculture field. Sensitivity of this new technique has been exploited in order to avoid the time-consuming and expensive purification steps commonly used in these complex matrices and with the aim of obtaining a cheap and efficient sample treatment. The developed methodology has been applied to the quantification of compounds that were previously found in wide-scope screening of aquaculture samples by combined use of GC&LC-QTOF MS. Up to our knowledge this is the first application of GC-APCI-MS/MS with triple quadrupole to the analysis of PAHs in samples from aquaculture field.

EXPERIMENTAL

Reagents

Benzo[j]fluoranthene (B[j]F), 5-methylchrysene (5-MC), benzo[c]fluorene (B[c]F), dibenzo[a,e]pyrene (D[a,e]P), dibenzo[a,h]pyrene (D[a,h]P), dibenzo[a,i]pyrene (D[a,i]P), dibenzo[a,l]pyrene (D[a,l]P), and cyclopenta[c,d]pyrene (C[c,d]P) individual standard solutions at 10 $mg \cdot L^{-1}$ (100 $mg \cdot L^{-1}$ for C[c,d]P), PAH MIX 9 containing 16 PAHs (10 $mg \cdot L^{-1}$ in cyclohexane) and PCB mix 3 (100 $ng \cdot \mu L^{-1}$ in isooctane) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Reference standards diphenylamine, ethoxyquin, p,p'-DDD, p,p'-DDT, tebuconazole, azoxystrobin, fluazinam and imazalil supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany); boscalid and malathion by Riedel-de Haën (Seelze, Germany); ethoxyquin dimer by

Synthetica AS (Oslo, Norway) and hexachlorobenzene (HCB), pirimiphos-methyl and chlopyrifos-methyl by Sigma-Aldrich (St Louis, MO, USA) with a purity 97-99.9% were used for standard preparation (see structures for all the compounds in **Table S1**). Stock standard solutions (around 500 $mg \cdot L^{-1}$) were prepared in acetone. Two mixtures of pesticide standards (individual concentration of each pesticide around 50 $mg \cdot L^{-1}$) were prepared by dilution of stock individual solutions in acetone. Working standard solutions containing all compounds were prepared by dilution of mixtures with acetone (for sample fortification in GC) and hexane (GC injection). Stock standard solutions and working solutions were stored in a freezer at -20 ºC.

Stable isotopic labeled internal standards (SIL-IS) PCB 153-$^{13}C_{12}$ and PAH surrogate cocktail containing acenaphthylene-D_8, B[a]P-D_{12}, B(g,h,i)Pe-D_{12}, fluoranthene-D_{10}, naphthalene-D_8, phenanthrene-D_{10} and pyrene-D_{10} were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Hexachlorobenzene-$^{13}C_6$, tebuconazole-D_6 and p,p'-DDE-D_8 were also purchased from Dr. Ehrenstorfer.

HPLC-supragradient acetonitrile, acetone (pesticide residue analysis quality) and n-hexane (ultra-trace quality) were purchased from Scharlab (Barcelona, Spain). Anhydrous magnesium sulfate (extra pure) and anhydrous sodium acetate (reagent grade) were purchased from Scharlab. The QuEChERS commercial clean-up kits were purchased from Teknokroma (Barcelona, Spain). Each kit contains 50 mg of primary-secondary amine (PSA), 150 mg of anhydrous magnesium sulfate, and 50 mg of C_{18}, in 2 mL microcentrifuge tubes for d-SPE.

Samples

The sample material has been described before in detail [31]. A total of 76 samples (from 19 different matrices) were studied in this work. The list contains ingredients

from different origin (plant, terrestrial animals and marine), and also feeds based on these ingredients (PAPs not included), as well as fillets of Atlantic salmon and gilthead sea bream reared on these feeds. Fish individuals were fed for, respectively, 7 and 18 months, and fillet samples were taken for analysis at the end of the exposure trial and additionally for sea bream at 8 months (commercial size). The same feed compositions were provided throughout the feeding trial. The quantification was performed on feed ingredients, feeds produced from the same feed ingredients, and fish fillets of fish fed on these feeds. The feed samples were analysed at the beginning of the trial (additionally after 8 months for sea bream) and no stability assessment was made by analyzing the feed during storage (below 7ºC for salmon and sea bream feeds).

Commercially available plant and marine feed ingredients were provided by Biomar (Tech Center, Brande, Denmark) feed producer and processed animal proteins (PAPs) from non-ruminants were provided by the European Fat Processors and Renderers Association (EFPRA). The ingredients selected represent the novelties in fish feed compositions to reduce the inclusion of fish derivatives. Fish feeds for feeding trials were based on plant feed ingredients, and not PAPs, as higher levels of PAHs were found in plant feed ingredients. The feeds were produced by Biomar under commercial aquafeed production techniques based on high-temperature extrusion processes, which potentially could affect pesticide residue levels.

Sample treatment

Samples were thawed at room temperature and 1 g was accurately weighed and transferred to 15 mL falcon tubes and spiked with 0.2 mL of SIL-IS solution of 1 $\mu g \cdot mL^{-1}$. After 30 min, 2 mL of acetonitrile were added and the tube was vigorously shaken by vortex for 30 s. Then, 0.8 g of $MgSO_4$ were added and the tube was

immediately shaken for 30 s. Subsequently, the tube was centrifuged at 1893 rcf·g for 5 min and the upper layer of the extract was transferred to a 2 mL eppendorf tube and stored for at least two hours in a freezer to precipitate proteins and fix lipids to the tube walls (freezing cleanup). Then, a QuEChERS clean-up step was carried out prior injection in the GC-system [25]. Briefly, 1 mL of the extract was carefully transferred to the cleanup QuEChERS vial (50 mg of PSA + 150 mg of $MgSO_4$ + 50 mg of C18), and it was vigorously shaken for 30 s and centrifuged at 12557 rcf·g for 5 min. Then, 50 µL of the final acetonitrile extract was diluted with 300 µL of acetone and 650 µL of hexane in order to make the solution miscible (20-fold dilution factor).

Instrumentation

GC-APCI-MS/MS.

Data were acquired using a GC system (Agilent 7890A, Palo Alto, CA, USA) equipped with an autosampler (Agilent 7693) and coupled to a triple quadrupole (QqQ) mass spectrometer (Xevo TQ-S, Waters Corporation, Manchester, UK), operating in positive APCI mode. The GC separation was performed using a fused silica DB-17MS (50% phenyl-methylpolysiloxane) capillary column with a length of 30 m x 0.25 mm i.d. and a film thickness of 0.25 µm with a 1 m x 0.25 mm i.d deactivated post-column (J&W Scientific, Folson, CA, USA). The oven temperature was programmed as follows: 70 ºC (1 min); 20 ºC min^{-1} to 230 ºC; 5 ºC min^{-1} to 320 ºC (6 min). The injector was operated in pulsed splitless mode (50 psi), injecting 1 µL at 280 ºC. Helium was used as carrier gas at a ramped flow mode programmed as follows: 2 $mL \cdot min^{-1}$ (23 min); a ramp of 4 $mL \cdot min^{-1}$ to 6 $mL \cdot min^{-1}$ (9 min). In the SRM method, automatic dwell time (values ranging from 3 to 63 ms) was applied in order to obtain 15 points per peak. The interface and source temperatures were set to 320 ºC and 150 ºC respectively using N_2

as auxiliary gas at 275 $L \cdot h^{-1}$, as make-up gas at 300 $mL \cdot min^{-1}$ and as cone gas at 200 $L \cdot h^{-1}$. The APCI corona discharge pin was operated at 1.6 µA. The ionization process occurred within an enclosed ion volume, which enabled control over the protonation/charge transfer processes. In order to work under proton transfer conditions, an uncapped vial containing 1.25 mL of water was placed in a designed holder into the APCI source door to enhance protonation. Targetlynx (a module of MassLynx) was used to handle and process the acquired data.

Study of matrix effects

Nine out of nineteen different matrices (fish fillets, feed and PAPs) were selected for the study of matrix effects and different dilution factors were tested (crude extract, 5-fold, 10-fold and 20-fold dilution). To this aim, matrix-matched calibrations were prepared for each sample matrix by taking between 25-500 µL of the "blank" sample extract (for crude extract, 500 µL were evaporated to dryness) and adding 50 µL (125 µL for the 25 $ng \cdot mL^{-1}$ level) of the corresponding standard in hexane (between 10 and 100 $ng \cdot mL^{-1}$), resulting in final analyte concentrations between 1 and 25 $ng \cdot mL^{-1}$. The final volume of each calibration point was 500 µL in all cases (30% acetone, 5 % acetonitrile and 65% hexane). Matrix effect was evaluated by comparing the calibration graphs obtained with standards in solvent and in matrix (at the different dilution factors) [32]. The accepted relative error between the slopes of calibration in solvent and matrix-matched calibration was ±25%. Once the optimum dilution factor was selected, the absence of matrix effect was also tested for the remaining 10 matrices.

Validation and recovery experiments

Eleven SIL-IS were added at the initial stage of the procedure as quality control (surrogates) in order to correct for possible losses during the overall procedure and instrumental deviations. Linearity of relative response of analytes was established by analyzing standard solutions injected in triplicate in the range of 0.01-25 $ng \cdot mL^{-1}$ for all compounds except for C[c,d]P (0.005-12.5 $ng \cdot mL^{-1}$). Linearity was assumed when the correlation coefficient (R^2) was higher than 0.99 with residuals lower than 30%.

A full validation of the method was carried out for two sample matrices, salmon and sea bream fillets. Accuracy (estimated by means of recovery experiments) was evaluated by analyzing six replicates spiked at 2 $ng \cdot g^{-1}$ (1 $ng \cdot g^{-1}$ for C[c,d]P) and 50 $ng \cdot g^{-1}$ (25 $ng \cdot g^{-1}$ for C[c,d]P) for the two matrices tested. Precision was expressed as repeatability in terms of relative standard deviation (RSD, %) (n = 6) calculated for each fortification level. The limit of quantification (LOQ) objective, for salmon and sea bream fillets, was defined as the lowest concentration level validated following SANTE/11945/2015 guide criteria [33] (recovery 70-120% and RSDs below 20%). For those compounds that could not be validated due to the high concentration in the "blank" sample a statistically calculated LOQ was estimated as the analyte concentration that produced a peak signal of ten times the background noise in the chromatogram at the lowest fortification level tested for each compound. The limit of detection (LOD) was estimated for all compounds considering a signal-to-noise (S/N) ratio of three.

The methodology was validated for the remaining 17 sample matrices by analysis of quality control (QC) samples, i.e. "blank" samples spiked at 5 and 50 $ng \cdot g^{-1}$ injected in each batch. A total of 48 QCs prepared from 24 "blank" samples (corresponding to 17 different matrices) were analyzed. Recoveries between 60 and 140% for each individual sample were considered satisfactory following the acceptance criteria for routine recoveries according to SANTE/11945/2015 guide criteria [33].

The selectivity of the method was assured by choosing selective SRM transitions using $M^{+\bullet}$ or $[M+H]^+$ as precursor ion, when possible. Specificity was evaluated by verification of the absence of interfering peaks at the retention time of each compound in blank samples for the acquired SRM transitions.

The q_i/Q ratio, defined as the ratio between the intensity of the confirmation ion (q_i) and the quantification ion (Q), was used to confirm peak identity in real and spiked samples. The experimental q_i/Q value for each compound was calculated as the mean value obtained from three standard solutions injected in triplicate (RSD below 15% in all cases) Confirmation of analytes detected in samples was considered positive when the q_i/Q ratio was within acceptable tolerances (30% of the experimental q_i/Q value calculated from standards) according to SANTE/11945/2015 guide criteria [33].

RESULTS AND DISCUSSION

Ionization and fragmentation behaviour of PAHs in GC-APCI

In this work, the ionization behavior of the APCI interface was tested using PAH standards in solvent. Two mechanisms of ionization were simultaneously observed: i) charge transfer in which the nitrogen plasma created by the corona discharge needle promotes the formation of $M^{+\bullet}$, and ii) proton transfer, where the presence of water vapor traces in the source favors the formation of the $[M+H]^+$ ion.

The PAHs studied showed a mixture of two ions, $M^{+\bullet}$ and $[M+H]^+$, as base peak of the spectrum. In the case of acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, B[c]F and C[c,d]P, the $[M+H]^+$ intensity was slightly higher than $M^{+\bullet}$ (among 1.2 and 1.5 times higher). In contrast, the intensity of $M^{+\bullet}$ was around 1.1 and 1.2 times higher than $[M+H]^+$ for naphthalene, I[1,2,3-cd]P, B[g,h,i]Pe, D[a,h]A, D[a,h]P, D[a,e]P, D[a,i]P and D[a,l]P. For the rest of PAHs, the abundance of $M^{+\bullet}$ and

$[M+H]^+$ was similar. **Figure 1 (up)** shows the APCI spectrum of D[a,l]P where $M^{+\bullet}$ and $[M+H]^+$ can be observed as base peak of the spectrum. After that, the fragmentation of the PAHs in the collision cell was studied. $M^{+\bullet}$ and $[M+H]^+$ were selected as precursor ions for all PAHs. Fragmentation was performed at collision energies between 10-60 eV. The losses of $H^\bullet$ and H_2 from both $M^{+\bullet}$ and $[M+H]^+$ were the most abundant and common to all PAHs studied. Losses as $CH_3^\bullet$ or C_2H_2 among others were also commonly observed. The use of water as modifier favored the formation of the $[M+H]^+$ and the $M^{+\bullet}$ decreased in all cases (**see Figure 1, down**). However, $M^{+\bullet}$ still appeared in the spectra in a percentage among 35-80%, depending on the PAH.

Sensitivity and repeatability. Effect of different modifiers

Once the SRM transitions were optimized, the sensitivity and repeatability of the GC-MS/MS signals were evaluated for all the transitions selected (those coming from $M^{+\bullet}$ as well as from $[M+H]^+$). As explained above, both $[M+H]^+$ and $M^{+\bullet}$ were observed in the APCI spectra of all PAHs even under "dry" conditions. Thus, we investigated the effect of different modifiers in order to promote the formation of the protonated molecules and to increase the sensitivity of SRM transitions coming from $[M+H]^+$. Water, 0.5% formic acid, methanol and isopropanol were added separately in an uncapped vial, which was located within a specially designed holder placed in the source door. The sensitivity and repeatability of the GC-MS/MS signals for PAHs were evaluated under the different conditions (with and without the use of modifiers).

As can be seen in **Figure 2 (up)**, under charge transfer conditions (without adding a modifier), SRM transitions coming from $M^{+\bullet}$ were more sensitive than those coming from $[M+H]^+$ for acenaphthene, fluorene, B(c)F, C[c,d]P, chrysene, 5-MC, B(b)F and B(j)F (among 2 and 4 times higher). In contrast, the sensitivity of SRM transitions

coming from $[M+H]^+$ was higher than $M^{+\bullet}$ (among 3 and 14 times) for naphthalene, acenaphthylene, phenanthrene, anthracene, fluoranthene, pyrene and B[g,h,i]Pe. For the rest of PAHs, they were mostly similar. When adding water as modifier, the sensitivity of transitions coming from the $M^{+\bullet}$ decreased between 1.5 to 3 times for all compounds, except for D[a,h]A which was rather similar; and consequently, the intensity for $[M+H]^+$ transitions improved 1.5 to 2 times for high molecular weight PAHs (HMW-PAHs, with four or more aromatic rings).

When adding HCOOH 0.5%, the sensitivity of $[M+H]^+$ transitions remained almost the same as with only water **(Figure 2 (down))**. Similar results were obtained using MeOH as modifier, except for some compounds, such as naphthalene, fluorene, phenanthrene, fluoranthene, chrysene and B(b)F, among others, whose responses decreased considerably. Regarding isopropanol, its use was not beneficial because sensitivity extremely decreased for most of the compounds. Thus, the modifiers MeOH and isopropanol were discarded for further optimization.

Once these modifiers were discarded, the repeatability of the response (n=10 at 10 $ng \cdot mL^{-1}$) was also evaluated under the remaining working conditions (without water, with water and with HCOOH 0.5%) (**Figure S1**). Our data showed a slightly poorer repeatability for $M^{+\bullet}$ and $[M+H]^+$ transitions working under dry conditions (relative standard deviations, RSD, between 20-30% for most compounds) being a bit better (RSD 10-17%) for fluoranthene, pyrene and low molecular weight PAHs (LMW-PAHs, 2-3 aromatic rings). This parameter was improved under wet conditions using water as modifier for $M^{+\bullet}$ (RSD 7-20%) and specially for $[M+H]^+$ transitions (RSD 2-8%), but dramatically get worst when adding HCOOH 0.5%, especially for M+· transitions (RSD 14-39%).

Overall, the use of water as modifier was selected for further experiments, selecting the transitions from $[M+H]^+$ for most of PAHs. In some particular cases, the most sensitive transitions using $M^{+\bullet}$ as precursor ion were used for confirmation purposes (except for b[j]F which was used for quantification) (**Table 1**).

In a later stage, the optimum conditions selected for PAHs were tested for the PCBs and pesticides in order to widen the applicability of the methodology to other relevant pollutants included in the method. In previous works, it was reported that sensitivity for halogenated hydrocarbons without any other heteroatoms, e.g. PCBs and many organochlorine pesticides (such as hexachlorobutadiene, pentachlorobenzene, hexachlorobenzene, HCHs, DDTs, trans-chlordane, mirex) was negatively affected by the introduction of water in the source [34,35]. Despite this fact, the sensitivity and repeatability of PCBs, p,p'-DDT, p,p'-DDD, p,p'-DDE and HCB was still enough to reach the maximum residue level regulated. Regarding the other pesticides included in the method, ionization efficiency was favored under proton-transfer conditions, i.e. selecting $[M+H]^+$ as precursor ion, except for ethoxyquin and ethoxyquin dimer that were scarcely affected.

Selectivity of the SRM transitions for PAHs

Selectivity of the selected SRM transitions, especially in cases where coelution occurred, was also studied during the optimization process. In such cases, the possibility of selecting different precursor ions for the same compound ($M^{+\bullet}$ or $[M+H]^+$) was of great advance in terms of specificity. This was the case of the determination of cyclopenta(c,d)pyrene ($C_{18}H_{10}$, MW=226), which nearly coelutes with chrysene ($C_{18}H_{12}$, MW=228) (see **Figure S2**). Chrysene shows 226 as an in-source fragment (coming from $M^{+\bullet}$ 228). Thus, when monitoring C(c,d)P with the transition coming

from its $M^{+\bullet}$ 226, an interference from chrysene (coming from an in-source fragment) is observed in the chromatogram, which makes the peak area integration troublesome, especially when the concentration of chrysene is higher than cyclopenta(c,d)pyrene. However, quantification and confirmation transitions for C(c,d)P coming from $[M+H]^+$ 227 did not suffer from this interference and showed higher specificity, which is commonly observed on EI-based methods [36]. A similar case occurred for the determination of indeno(1,2,3-cd)pyrene ($C_{22}H_{12}$, MW=276) due to the nearly coelution of dibenzo(a,h)anthracene ($C_{22}H_{14}$, MW=278). D(a,h)A shows an in-source fragment at m/z 276, so the transition coming from $M^{+\bullet}$ 276 for I(1,2,3,cd)P was interfered by D(a,h)A, while those coming from $[M+H]^+$ 277 were much cleaner.

Study of matrix effects

Matrix effects observed under GC-APCI-MS come from the GC liner (normally signal enhancement) and from the APCI source (normally signal suppression) [27]. As a summary of the matrix effect study for 9 out of 19 matrices (where different dilution factors were tested: crude extract, 5-fold, 10-fold and 20-fold dilution), signal enhancement/suppression was observed for: i) 36% of the combinations matrix/PAHs, 58% for matrix/pesticides and 52% for matrix/PCBs (crude extract); ii) 35% for matrix/PAHs, 46% for matrix/pesticides and 17% for matrix/PCBs (5-fold dilution factor); iii) 26% of matrix/PAHs, 33% for matrix/pesticides and 14% for matrix/PCBs (10-fold dilution factor); and iv) 11% for matrix/pesticides (20-fold dilution factor). As an illustrative example, **Figure 3** shows how the matrix effect for PAHs in sea bream fillet decreases when the different dilution factors studied are applied. Thus, 20-fold dilution of the final extract was finally selected for further experiments.

As a summary, for a 20-fold dilution, signal enhancement was observed only for ethoxyquin and ethoxyquin-dimer, whereas signal suppression was observed for boscalid and azoxystrobin (mainly in plant and marine ingredients). No significant matrix effect was observed for PAHs, PCBs and the rest of pesticides (**Figure S3**). According to our data, 20-fold dilution of the final sample extract eliminated matrix effect for most analyte/matrix combinations, and this allowed us to perform the quantification in the analysis of the 19 aquaculture matrices using calibration curve in solvent. The remaining matrix effects for boscalid and azoxystrobin could be corrected with the use of SIL-IS. Matrix effect for ethoxyquin and ethoxyquin-dimer could not be properly evaluated in feed, krill meal and fishmeal matrices because the analyte concentration in these samples was typically higher than the spiking levels assayed.

Analytical parameters

Validation of the analytical procedure was carried out in salmon and sea bream fillets considering the parameters of linearity, accuracy, precision, LODs and LOQs. Relative responses to the selected SIL-IS were used (see **Table 1**).

The study of the linearity revealed that correlation coefficients (R^2) were higher than 0.99, with residuals lower than 30% for most compounds in the range 0.01-25 ng·mL^{-1}. Some exceptions were: 0.0125-12.5 ng·mL^{-1} (C[c,d]P); 0.025-25 ng·mL^{-1} (naphthalene, acenaphthylene, acenaphthene, anthracene, B[c]F, B[a]A, chrysene, B[b]F, B[k]F, B[j]F, D[a,h]A, PCB 153 and 180, diphenylamine, tebuconazole, ethoxyquin dimer, boscalid and azoxystrobin); 0.1-25 ng·mL^{-1} (D[a,l]P and D[a,e]P, HCB and p,p'-DDE); 0.5-25 ng·mL^{-1} (D[a,i]P, D[a,h]P, ethoxyquin, fluazinam, imazalil, p,p'-DDD and p,p'-DDT).

Trueness and precision data for salmon and sea bream fillets are shown in **Table 2** which shows satisfactory recoveries and precision for the wide majority of compounds at the two concentrations studied, 2 and 50 $ng \cdot g^{-1}$. An LOQ objective of 2 $ng \cdot g^{-1}$ was empirically demonstrated for most compounds in both matrices, using samples spiked at this level and subjected to the overall analytical procedure. A statistical LOQ of 5 $ng \cdot g^{-1}$ (S/N = 10) was estimated for naphthalene, fluorene, fluoranthene and pyrene, as the high concentrations present in the "blank" samples did not allow the calculation of recoveries and precision below that concentration. Dibenzopyrenes have poor response, and therefore the LOQ was established in 50 $ng \cdot g^{-1}$. The same occurred for HCB, fluazinam, imazalil, p,p'-DDE, p,p'-DDD and p,p'-DDT which could not be detected at the low level of 2 $ng \cdot g^{-1}$ being 50 $ng \cdot g^{-1}$ still the normally applied MRL for these compounds (as commented above, these compounds were negatively affected by the introduction of water as modifier in the source). Similarly to some light PAHs, ETQ and ETQ-D were found at quite high concentrations in the samples impeding validation at 2 $ng \cdot g^{-1}$ level. Thus, LOQs were statistically calculated for S/N=10. LODs were found to be between 0.5-2 $ng \cdot g^{-1}$ for 80 % of the PAHs, all the PCBs and 50% of the pesticides studied. The rest of compounds showed LODs between 5 and 50 $ng \cdot g^{-1}$.

In relation to the remaining 17 matrices, a total of 48 QC samples, spiked at 5 $ng \cdot g^{-1}$ and 50 $ng \cdot g^{-1}$, were analyzed. **Figure 4** shows the box-plot diagrams representing the recovery values for PAHs in all matrices at the two concentrations assayed. Some recoveries could not be calculated (e.g. napthalene, fluorene, phenanthrene, pyrene, D[a,e]P, D[a,i]P and D[a,h]P) at 5 ng·g-1 spiking level due to the high analyte concentration present in "blank" sample. It can be emphasized that all QC recoveries were in the range 60 - 140%, with median values between 88 and 115 %, at 5 $ng \cdot g^{-1}$, and between 92 and 116% at 50 $ng \cdot g^{-1}$. Among the studied matrices, PAPs showed

higher complexity giving individual recoveries out of 70-120% range without a clear tendency for specific compounds. Data from above illustrate that the methodology applied was robust and satisfactory for the determination of very low PAH concentrations in different sample matrices, some of them of high complexity. The excellent sensitivity of GC-APCI-MS/MS allowed the extra dilution (x20) of the sample extracts, minimizing matrix effects but still reaching low LOQs, comparable and, in some cases, even lower than those reported in the literature [21,22] with the great advantage of using solvent calibration for quantification of the compounds in all matrices tested.

Analysis of samples

In order to demonstrate the applicability of the methodology developed in this paper, it was applied to the analysis of a notable number of samples from aquaculture production: 12 diets, 12 fish fillets, 19 terrestrial animal ingredients, 6 marine ingredients, 19 plant ingredients and 8 plant oils. A reagent blank and two spiked samples (5 and 50 ng·g-1) for each matrix analyzed were included in each batch. The results, in **Table S2,** are expressed in a wet weight basis. The q_i/Q ratios obtained for all positive samples were within the range of the tolerance accepted (30%) around the experimental q_i/Q value obtained from reference standards.

LMW-PAHs together with HMW-PAHs as fluoranthene and pyrene were found in most of the samples. Regarding diets, fish fillets, plant ingredients and PAPs, concentrations ranged from <5 $ng \cdot g^{-1}$ for acenaphthene to 126.6, 77.1, 88.6 and 177.5 $ng \cdot g^{-1}$ for naphthalene, respectively. In marine ingredients, they ranged from <5 $ng \cdot g^{-1}$ for acenaphthene and acenaphthylene to 129.0 $ng \cdot g^{-1}$ for phenanthrene. For plant oil ingredients, concentrations ranged from <5 $ng \cdot g^{-1}$ for acenaphthylene to 186.9 $ng \cdot g^{-1}$ for

phenanthrene. In the present study only two fish oils were analysed, and clearly a large variation exist in the environmental background levels of PAHs in fish oils [13,14]. **Figure S3** shows the identification of 5-MC in one fish meal. This PAH was below the LOQ in all samples analyzed. As can be seen, three transitions were acquired and q_i/Q ratios were in agreement with SANTE/11945/2015 identification criteria followed in the current research [33].

Regarding pesticides, the non-organochlorine pesticides that are currently used on crop such as chlorpiriphos-methyl and pirimiphos-methyl were only detected in the novel plant ingredients and not in marine feed ingredients such as fish oil and meal [25]. Malathion, imazalil and tebuconazole were only found in some ingredients while boscalid and azoxystrobin were indeed found in feed. It is worth to mention that studied PCBs, DDTs and pesticides were not found in fillets from salmon and sea bream. The anti-oxidant ethoxyquin and its main metabolite ethoxyquin dimer are deposited to marine feed ingredients such as fish meal to prevent lipid oxidation and spontaneous combustion during overseas transport and storage (Lundebye et al. 2010). The ethoxyquin levels were highest in fish meal and krill (32462 and 40076 $ng \cdot g^{-1}$, respectively), but surprisingly high levels were also found in fish oil (37137 $ng \cdot g^{-1}$) where normally other antioxidant such as BHT and BHA are used [37]. All other feed ingredients, including terrestrial and plant, also contained ethoxyquin, albeit at l00-150 fold lower levels than the marine ingredients (**Table S2**). PCB congeners were not detected in any sample.

CONCLUSIONS

The use of APCI has been evaluated for GC-MS/MS analysis of PAHs. The high sensitivity of this technique allowed the simultaneous quantification of 19 different

complex matrices (from aquaculture field) using solvent calibration. The key aspect for this aim was the elimination of matrix effect, without the need of time-consuming purification steps, only by a 20-fold dilution factor of the final QuEChERs extract, being this aspect a great contribution of the present work. Despite this, LOQs of the developed method were 2 $ng \cdot g^{-1}$ for most analytes, in the same order or better than those reported in previously published methods for similar matrices [21,22] showing higher efficiency [19]. Also, the use of both $M^{+\bullet}$ or $[M+H]^+$ gave an additional value to the selectivity in the determination/identification capabilities.

FIGURE CAPTIONS

Figure 1. APCI mass spectra of D[a,l]P under charge transfer conditions (up) and under proton transfer conditions with water as modifier (down).

Figure 2. Absolute sensitivity of SRM transitions coming from $M^{+\cdot}$ and $[M+H]^+$ for PAHs under charge transfer and proton transfer (with water) ionization conditions (up); absolute sensitivity of SRM transitions coming from $[M+H]^+$ under proton transfer conditions using different modifiers (bottom).

Figure 3. Matrix effect study for PAHs in sea bream fillet. Relative error between the slopes of calibration in solvent and matrix-matched calibration at different dilution factors.

Figure 4. Box plots that shows the recoveries of the 48 QCs (corresponding to 17 different matrices) spiked at 5 (up) and 50 $ng \cdot g^{-1}$ (down).

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Table 1. Experimental conditions of the optimized GC-APCI-MS/MS method using water as modifier. Quantifier (Q) and qualifier (q) transitions.

Rt (min)	Compound	Internal Standard	Precursor ion	Product ion	CE (eV)	q/Q ratio
5.53	Naphthalene-D_8		136	134.1	30	Q
5.55	Naphthalene	Naphthalene-D_8	129	128.1	20	Q
				126.8	20	0.273
				103	20	0.011
				79	20	0.002
			128	102.1	20	0.063
7.65	Acenaphthylene-D_8		160	158.3	30	Q
7.66	Acenaphthylene	Acenaphthylene-D_8	153	152	20	Q
				150.1	30	0.010
				125.9	30	0.018
			152	126.1	30	0.018
7.82	Acenaphthene	Acenaphthylene-D_8	155	154	20	0.539
				153	20	Q
				129.2	20	0.367
				128	30	0.317
			154	153	20	0.852
8.42	Fluorene	Acenaphthylene-D_8	167	166	20	0.349
				165.1	20	Q
				152	20	0.956
				115	30	0.064
			166	165.1	20	0.607
8.72	Diphenylamine	Tebuconazole-D_6	170	93	30	Q
				168	30	0.035
				153	20	0.086
				152	20	0.070
			169	168	20	0.506
8.87	HCB	HCB-$^{13}C_6$	282	247	30	Q
				212.1	30	0.718
				177	40	0.557
				142	50	0.435
8.86	HCB-$^{13}C_6$		292	222	40	Q
9.01	Ethoxyquin	Tebuconazole-D_6	218	202	20	0.576
				174	20	Q
				148	20	0.671
				160	30	0.510
			217	202	10	0.273
9.81	Phenanthrene-D_{10}		188	186.4	20	Q
9.85	Phenanthrene	Phenanthrene-D_{10}	179	178.1	20	Q
				176.7	30	0.025
				176.1	40	0.044
				152.1	30	0.083
			178	152	30	0.083
9.88	Anthracene	Phenanthrene-D_{10}	179	178	20	Q
				176.7	30	0.025
				176.1	50	0.026
				152.1	30	0.069
			178	152	30	0.112
9.98	PCB 28	4-4'-DDE D_8	256	221	20	0.060
				186	30	Q
				151	40	0.177
10.07	Fluazinam	Tebuconazole-D_6	465	372.9	20	Q
				337.7	40	0.087
				303.9	40	0.077
				268.9	50	0.045
10.25	Chlorpyrifos-methyl	Tebuconazole-D_6	322	290	10	0.161
				212	30	0.065
				177	40	0.021
				125	20	Q
10.39	PCB 52	4-4'-DDE D_8	290	220	30	Q
			292	257	20	0.605
				220	30	0.689
				150	50	0.334
10.41	Pirimiphos-methyl	Tebuconazole-D_6	306	164	20	Q
				108	40	0.505
				125	30	0.269
				95	20	0.145
10.71	Malathion	Tebuconazole-D_6	331	211	10	0.026
				143	30	0.017
				125	30	Q
				117	20	0.047
				99	10	0.732
11.69	PCB 101	4-4'-DDE D_8	324	254	40	Q
			326	291	20	0.938
				256	40	0.936
				184	50	0.081
12.17	Fluoranthene-D_{10}		212.2	210.3	30	Q
12.22	Fluoranthene	Fluoranthene-D_{10}	203	202.1	30	Q
				200	40	0.069
				175.9	50	0.007
				152	50	0.014
			202	200.8	30	0.053
12.30	4,4'-DDE-D_8		324	254	40	Q
13.98	B[c]F	Pyrene-D_{10}	217	215.9	20	0.129
				215	30	0.370
				202	30	Q
				200.1	40	0.048
			216	215	30	0.393
14.27	PCB 138	$^{13}C_{12}$-PCB 153	360	325	20	0.683
				290	40	Q
				218	60	0.559
			358	253	50	0.150
14.31	4,4'-DDT	4-4'-DDE D_8	235	165	20	Q
				115	50	0.026
				99	40	0.023
14.64	Tebuconazole-D_6		314	296	10	Q
14.70	Tebuconazole	Tebuconazole-D_6	308	290	10	Q
				165	20	0.430
				151	20	0.246
				125	40	0.865
15.67	PCB 180	$^{13}C_{12}$-PCB 153	392	322	40	0.557
			394	324	30	Q
			396	361	20	0.454
				254	60	0.448
16.80	B[a]A	Pyrene-D_{10}	229	228	40	Q
				226.9	30	0.262
				226	40	0.629
				202.2	30	0.148
			228	226	40	0.568
17.07	C[c,d]P	Pyrene-D_{10}	227	226	30	Q
				225	40	0.076
				200.8	40	0.002
			226	224.9	40	0.039
17.11	Chrysene	Pyrene-D_{10}	229	227.9	40	Q
				227	30	0.440
				226	40	0.842
				202.1	30	0.219
			228	226	40	0.579
18.75	5-MC	Pyrene-D_{10}	243	242	30	0.043
				228	30	Q
				226.1	40	0.261
				202.1	40	0.066
			242	241.1	20	0.227
19.96	Ethoxyquin dimer	Tebuconazole-D_6	433	418	10	0.076
				216	20	Q
				188	30	0.744
			432	417	20	0.307
				173	50	0.354
21.06	B[b]F	B[a]P-D_{12}	253	251.9	30	Q
				250.8	40	0.064
				250.1	50	0.349
				226	50	0.051
			252	250	40	0.188
21.16	B[k]F	B[a]P-D_{12}	253	251.9	30	Q
				250.8	40	0.046
				250.1	50	0.174
				226	50	0.011
			252	250	40	0.165
22.28	B[j]F	B[a]P-D_{12}	253	251.9	30	0.899
				248.6	60	0.020
				226	50	0.269
				224.1	60	0.120
			252	250	40	Q
21.97	Boscalid	Tebuconazole-D_6	343	307	20	0.336
				140	20	Q
				112	40	0.413
			342	140	20	0.039
22.75	B[a]P-D_{12}		264.3	262.3	30	Q
22.66	B[a]P	B[a]P-D_{12}	253	252.1	30	Q
				250.7	40	0.023
				250.2	50	0.201
				226	50	0.037
			252	250	40	0.146
24.97	Azoxystrobin	Tebuconazole-D_6	404	372	10	
				344	20	0.243
				329	30	0.414
				172	50	0.212
				156	50	0.211
25.19	I[1,2,3-cd]P	B[g,h,i]Pe -D_{12}	277	276.1	30	Q
				275.4	50	0.123
				274.3	50	0.192
25.22	D[a,h]~~P~~A	B[a]P-D_{12}	279	278.1	30	Q
				276.8	40	0.161
				275.9	40	0.333
				263.3	30	0.267

12.34	4,4'-DDE	4-4'-DDE D_8	316	246.1	30	Q
				210	40	0.083
				281	20	0.162
				176	50	0.483
12.51	Imazalil	Tebuconazole-D_6	297	254.8	10	0.031
				201.1	10	0.050
				176.3	20	0.334
				159	20	Q
				109	20	0.259
12.93	Pyrene-D_{10}		212.2	210.3	30	Q
12.97	Pyrene	Pyrene-D_{10}	203	202.2	30	Q
				200.8	40	0.067
				200	50	0.041
				176.1	50	0.003
			202	200.8	30	0.039
13.03	PCB 118	4-4'-DDE D_8	326	291	20	0.107
				256	40	Q
				184	50	0.470
			324	254	40	0.968
13.28	PCB 153	$^{13}C_{12}$-PCB 153	360	325	20	0.478
				290	40	Q
				218	60	0.551
			358	253	50	0.151
13.27	PCB 153-$^{13}C_{12}$		376	306	40	Q
13.65	4,4'-DDD	Tebuconazole-D_6	235	165	20	Q
				115	50	0.028
				99	40	0.029
25.87	B[g,h,i]Pe -D_{12}		288.2	286.3	30	Q
25.93	B[g,h,i]Pe	B[g,h,i]Pe -D_{12}	277	276.2	40	Q
				275.1	50	0.153
				274	50	0.154
				272.7	60	0.005
			276	275.1	50	0.053
28.86	D[a,l]P	B[g,h,i]Pe -D_{12}	303	302.1	30	Q
				300.8	30	0.067
				300	50	0.455
				276	40	0.057
			302	300	50	0.288
29.92	D[a,e]P	B[g,h,i]Pe -D_{12}	303	302.1	30	Q
				300.1	50	0.341
				298.6	60	0.008
				297.9	60	0.022
			302	300	50	0.183
30.59	D[a,i]P	B[g,h,i]Pe -D_{12}	303	302.1	30	Q
				300.1	50	0.245
				297.9	60	0.019
				276	50	0.033
			302	300	50	0.178
31.00	D[a,h]P	B[g,h,i]Pe -D_{12}	303	302.1	30	Q
				300.1	50	0.274
				297.9	60	0.019
				276	50	0.033
			302	300	50	0.219

Table 2. Mean recoveries (%) and precision (RSD, %) for PAHs, PCBs and pesticides after application of overall analytical procedure to salmon fillet and gilthead fillet (n = 6), LOQs objective of the method.

Compounds	Salmon fillet			Sea bream fillet		
	Spiking levels (ng·g-1)		LOQ objective (ng·g-1)	Spiking levels (ng·g-1)		LOQ objective (ng·g-1)
	2	50		2	50	
Naphthalene	[a]	98 (18)	5[b]	[a]	98 (17)	5[b]
Acenaphthylene	119 (11)	101 (7)	2	106 (8)	103 (2)	2
Acenaphthene	74 (19)	72 (6)	2	93 (12)	73 (3)	2
Fluorene	[a]	95 (11)	5[b]	[a]	108 (6)	5[b]
Phenanthrene	[a]	116 (9)	2[b]	[a]	113 (9)	2[b]
Anthracene	91 (8)	104 (5)	2	101 (8)	107 (6)	2
Fluoranthene	[a]	103 (7)	5[b]	[a]	96 (11)	5[b]
Pyrene	[a]	106 (7)	5[b]	[a]	99 (14)	5[b]
B(c)F	98 (11)	94 (8)	2	90 (3)	90 (2)	2
B(a)A	114 (5)	101 (5)	2	113 (9)	73 (2)	2
C(c,d)P*	108 (7)	115 (4)	1	112 (19)	112 (7)	1
Chrysene	107 (12)	101 (4)	2	116 (8)	76 (6)	2
5-MC	117 (5)	102 (5)	2	108 (7)	75 (3)	2
B(b)F	80 (13)	104 (7)	2	106 (20)	96 (4)	2
B(k)F	71 (8)	103 (6)	2	78 (7)	86 (4)	2
B(j)F	104 (17)	76 (4)	2	115 (18)	102 (5)	2
B(a)P	104 (3)	88 (7)	2	113 (14)	86 (5)	2
I(1,2,3,cd)P	83 (12)	104 (5)	2	112 (9)	102 (3)	2
D(a,h)A	113 (11)	100 (5)	2	109 (11)	81 (6)	2
B(g,h,i)Pe	84 (8)	101 (4)	2	98 (20)	101 (3)	2
D(a,l)P	-	97 (4)	50	-	77 (6)	50
D(a,e)P	-	95 (8)	50	-	72 (9)	50
D(a,i)P	-	86 (13)	50	-	95 (13)	50
D(a,h)P	-	81 (10)	50	-	73 (6)	50
Diphenylamine	119 (20)	100 (17)	2	-	98 (17)	50
HCB	-	112 (11)	50	-	116 (17)	50
Ethoxyquin	[a]	[a]	15[b]	[a]	[a]	75[b]
Fluazinam	-	76 (4)	50	-	107 (15)	50
Chlorpiriphos-methyl	77 (10)	100 (6)	2	113 (13)	80 (7)	2
Pirimiphos-methyl	92 (12)	82 (4)	2	115 (13)	110 (9)	2
Malathion	95 (20)	94 (13)	2	90 (11)	104 (10)	2
4,4'-DDE	-	95 (19)	50	-	76 (19)	50
Imazalil	-	87 (8)	50	-	112 (14)	50
4,4'-DDD	-	112 (9)	50	-	114 (18)	50
4,4'-DDT	-	109 (9)	50	-	115 (11)	50
Tebuconazole	103 (19)	109 (11)	2	99 (16)	113 (12)	2
Ethoxyquin dimer	[a]	[a]	5[b]	[a]	[a]	15[b]
Boscalid	115 (7)	103 (17)	2	103 (4)	106 (11)	2
Azoxystrobin	95 (16)	102 (14)	2	119 (10)	109 (9)	2
PCB-28	106 (13)	117 (7)	2	110 (11)	120 (8)	2
PCB-52	107 (19)	100 (12)	2	98 (20)	97 (12)	2
PCB-101	106 (17)	120 (3)	2	109 (15)	118 (3)	2
PCB-118	110 (21)	105 (3)	2	98 (19)	121 (3)	2
PCB-153	95 (20)	120 (14)	2	87 (15)	115 (16)	2
PCB-138	115 (6)	118 (13)	2	117 (8)	116 (13)	2
PCB-180	105 (11)	111 (13)	2	81 (18)	108 (3)	2

[a] Recoveries could not be calculated due to the high concentration in the blank samples

[b] LOQ was estimated as the analyte concentration that produced a peak signal of ten times the background noise in the chromatogram at the lowest fortification level studied for each compound

*C[c,d]P spiked at 1 ng·g-1 and 25 ng·g-1

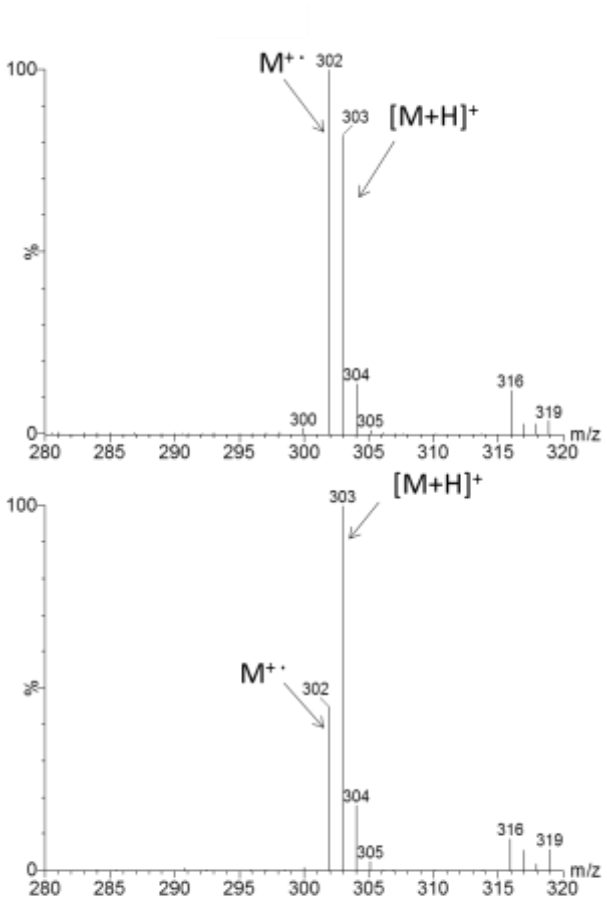

M+·
302
303
[M+H]+
100
%
0
304
316
319
300
305
m/z
280
285
290
295
300
305
310
315
320
[M+H]+
303
100
M+·
302
%
304
316
319
305
0
m/z
280
285
290
295
300
305
310
315
320

Figure 1.

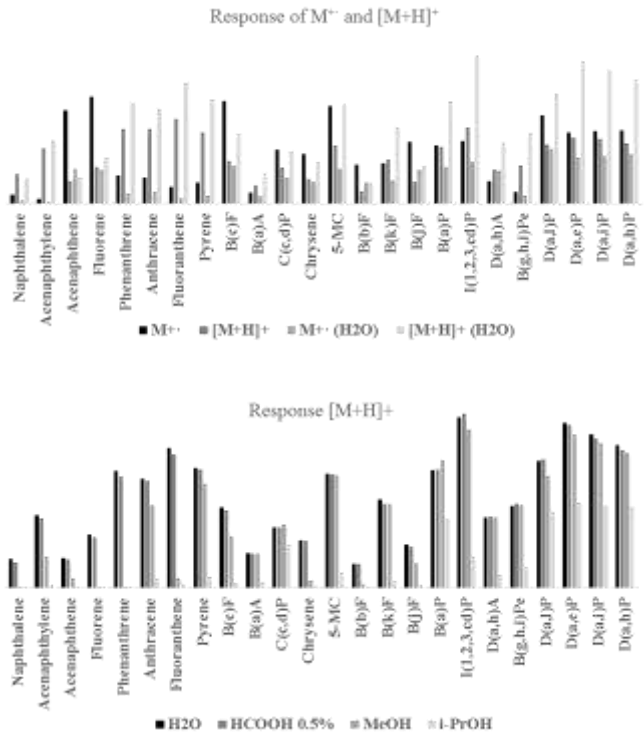

Response of M+· and [M+H]+
Naphthalene
Acenaphthylene
Acenaphthene
Fluorene
Phenanthrene
Anthracene
Fluoranthene
Pyrene
B(e)F
B(a)A
C(c,d)P
Chrysene
5-MC
B(b)F
B(k)F
B(j)F
B(a)P
I(1,2,3,cd)P
D(a,h)A
B(g,h,i)Pe
D(a,l)P
D(a,e)P
D(a,i)P
D(a,h)P
M+·
[M+H]+
M+· (H2O)
[M+H]+ (H2O)
Response [M+H]+
Naphthalene
Acenaphthylene
Acenaphthene
Fluorene
Phenanthrene
Anthracene
Fluoranthene
Pyrene
B(e)F
B(a)A
C(c,d)P
Chrysene
5-MC
B(b)F
B(k)F
B(j)F
B(a)P
I(1,2,3,cd)P
D(a,h)A
B(g,h,i)Pe
D(a,l)P
D(a,e)P
D(a,i)P
D(a,h)P
H2O
HCOOH 0.5%
MeOH
i-PrOH

Figure 2

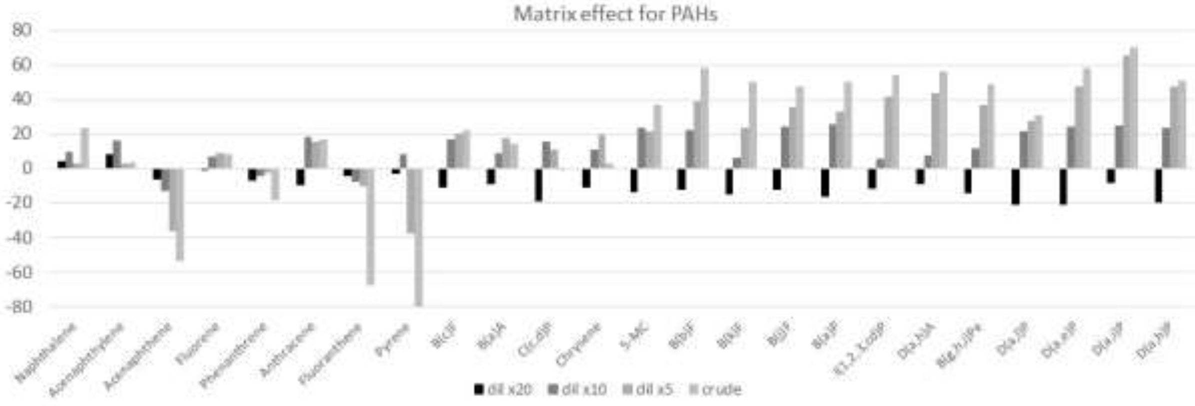
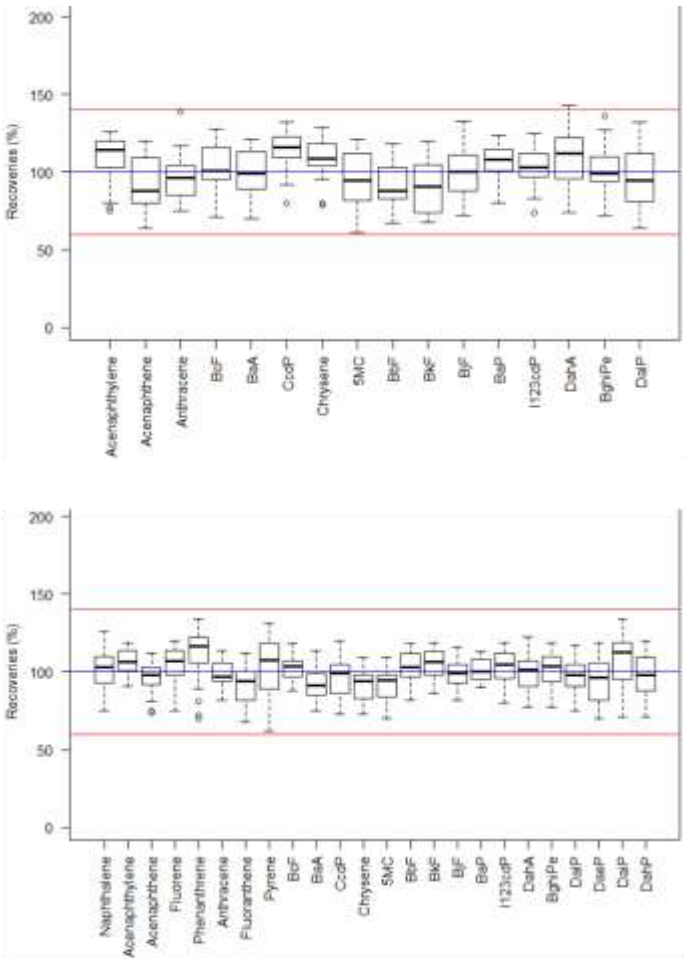

Matrix effect for PAHs
80
60
40
20
0
-20
-40
-60
-80
Naphthalene
Acenaphthylene
Acenaphthene
Fluorene
Phenanthrene
Anthracene
Fluoranthene
Pyrene
Chrysene
5-MC
dil x20
dil x10
dil x5
crude

Figure 3



* Recoveries for naphthalene, fluorene, phenanthrene, pyrene, D(a,e)P, D(a,i)P and D(a,h)P could not be calculated at 5 ng/g spiking level due to the high concentration present in blank sample

Figure 4

(not colour necessary)