



Comprehensive strategy for pesticide residue analysis through the production cycle of gilthead sea bream and Atlantic salmon



T. Portolés^a, M. Ibáñez^a, B. Garlito^a, J. Náchter-Mestre^{a, b, 2}, V. Karalazos^c, J. Silva^d, M. Alm^e, R. Serrano^a, J. Pérez-Sánchez^{b, 1, *}, F. Hernández^{a, 1, *}, M.H.G. Berntssen^{f, 1, *}

^a Research Institute for Pesticides and Water (IUPA), Avda. Sos Baynat, s/n, University Jaume I, 12071 Castellón, Spain

^b Institute of Aquaculture of Torre la Sal (IATS, CSIC), 12595 Ribera de Cabanes, Castellón, Spain

^c BioMar R&D, Grangemouth, FK3 8UL, UK

^d BioMar AS, N-7010 Trondheim, Norway

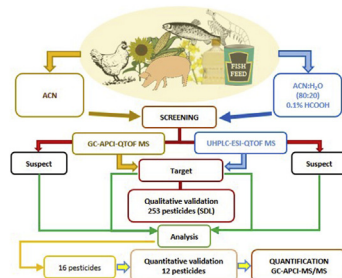
^e European Fat Processors and Renderers Association (EFPPA), Boulevard Baudouin, 1518, 4th Floor, BE - 1000, Brussels, Belgium

^f National Institute of Nutrition and Seafood Research, PO Box 2029 Nordnes, N-5817 Bergen, Norway

HIGHLIGHTS

- Complementary use of GC and UHPLC coupled to single QTOF MS platform.
- 800 pesticides were investigated in matrices from the marine food production chain.
- SDLs and LOQs at 0.01 and 0.005 mg/kg achieved by GC&LC-QTOF and GC-APCI-MS/MS.
- Plant material showed highest pesticide indices, with no feed-to-fillet transfer.
- Processed animal proteins had low pesticide indices.

GRAPHICAL ABSTRACT



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ABSTRACT

Plant ingredients and processed animal proteins are alternative feedstuffs for fish feeds in aquaculture. However, their use can introduce contaminants like pesticides that are not previously associated with marine Atlantic salmon and gilthead sea bream farming. This study covers the screening of around 800 pesticides by gas chromatography (GC) and liquid chromatography (LC) coupled to high resolution time-of-flight mass spectrometry in matrices throughout the entire marine food production chain. Prior to analysis of real-world samples, the screening methodology was validated for 252 pesticides to establish the screening detection limit. This was 0.01 mg kg⁻¹ for 113 pesticides (45%), 0.05 mg kg⁻¹ for 73 pesticides (29%) and >0.05 mg kg⁻¹ for 66 pesticides (26%). After that, a quantitative methodology based on GC coupled to tandem mass spectrometry with atmospheric pressure chemical ionization source (GC-APCI-MS/MS) was optimized for the pesticides found in the screening. Although several polar pesticides, of which pirimiphos methyl and chlorpyrifos-methyl were most dominant, were found in plant

* Corresponding authors.

E-mail addresses: jaime.perez.sanchez@csic.es (J. Pérez-Sánchez), felix.hernandez@uji.es (F. Hernández), Marc.Berntssen@nifes.no (M.H.G. Berntssen).

¹ Equally shared senior authorship.

² Current address: Nutrigenomics and Fish Growth Endocrinology Group, Institute of Aquaculture Torre la Sal, IATS-CSIC, Ribera de Cabanes, Castellón, Spain.

1. Introduction

The availability of wild fishery-derived raw materials is finite and the rapid and sustained growth rate of global aquaculture have forced the industry to explore alternative and more sustainable feed ingredients (Tacon and Metian, 2013). Much attention has been paid to plant ingredients and experimental evidence supports a successful replacement of marine feedstuffs at relatively high levels in most carnivorous farmed fish, such as Atlantic salmon (*Salmo salar*) and gilthead seabream (*Sparus aurata*) (Benedito-Palos et al., 2016; Ytrestrøy et al., 2015). Processed animal proteins (PAPs) from the rendering industry, re-authorized for use in aquafeeds in the European Union (EU) in 2013 (EC, 2013a), are another valuable source of feed ingredients for farmed marine fish (Hatlen et al., 2015).

The use of these alternative feed ingredients can introduce contaminants that were previously not associated with marine salmon and gilthead sea bream farming. One example are pesticides that are world-wide pre and post harvest used on crops or as anti-parasite agent in farming of terrestrial animals. Well known organochlorine pesticides (OCP) such as DDT and HCB have been mostly banned for agricultural use and are associated with fish oil (Berntssen et al., 2010; Friesen et al., 2008; Náchter-Mestre et al., 2009). These OCP pesticides have been replaced by less persistent and more water soluble pesticides (Seiber, 2002). EU Maximum Residue Level (MRL) legislation for non-OCP pesticides comprises most food commodities (EC, 2005), but for feed ingredients and fish, specific harmonized EU MRLs are not yet established (EC, 2013b). This emphasizes the need for data on the occurrence of pesticides in feed ingredients and the edible part of fish farmed on plant-based feeds. Extensive EU surveillance programmes exist on pesticide residues in food (EFSA, 2013). Several surveillance studies report on pesticides in terrestrial animals feed (i.e. (Gómez-Pérez et al., 2015)), as well as potential transfer of the pesticides to edible part of animals such as meat milk and eggs (Kan and Meijer, 2007; Leeman et al., 2007). For farmed fish, occurrence and feed-to-fillet transfer data on most (non POPs) pesticides, is limited (Lovell et al., 1996; Náchter-Mestre et al., 2014).

In addition to the above findings, the different physico-chemical characteristics of pesticides, together with the low concentration levels established by current legislation and the complexity of the matrices make necessary the use of last generation analytical techniques. Multi-residue methods (MRM) are applied with a clear tendency to liquid chromatography-mass spectrometry (LC-MS), although gas chromatography-MS (GC-MS) is still required to widen the number of compounds investigated. Thus, the combined use of GC and LC with tandem MS with last generation triple quadrupole (QqQ) instruments is one of the best options to get the sensitivity and selectivity required (Golge and Kabak, 2015; Hernández et al., 2012, 2013). Additionally, accurate-mass full-spectrum data obtained by high resolution MS opens the possibility to provide a complete overview of pesticide pollution, and not only those compounds initially targeted can be investigated. LC coupled to quadrupole-time of flight (QTOF) MS with electrospray (ESI) source has been widely explored for the screening of polar contaminants, their metabolites and transformation products (TPs) (Hernández et al., 2014, 2015a; Náchter-

Mestre et al., 2013). In relation to GC-MS, the soft ionization obtained from atmospheric pressure chemical ionization (APCI) source in GC-MS instruments has offered attractive features for screening purposes (Portolés et al., 2010, 2014). All this, thereby, opens fascinating perspectives in the analytical field (Hernández et al., 2015b; Pitarch et al., 2016) towards the screening of thousands of contaminants without standards (Castillo et al., 2016; Hernández et al., 2015b; Krauss et al., 2010).

The present work is based on our previous research on screening of pesticide residues (Náchter-Mestre et al., 2013, 2014) in farmed fish. A comprehensive strategy is presented for screening, identification and quantification of around 800 pesticides in commercially available plant and novel PAP feed ingredients and their transfer to the edible part of farmed Atlantic salmon and gilthead sea bream (two main species of the European aquaculture). The screening considers an initial qualitative validation of 252 pesticides using GC-(APCI)QTOF MS and UHPLC-(ESI)QTOF MS, followed by a target quantitative assessment by GC-(APCI)MS/MS QqQ for those pesticides identified in the qualitative validation.

2. Materials and methods

2.1. Chemicals and reagents

All pesticides and isotopically labelled reference standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and Sigma Aldrich (St Louis, MO, USA). Isotopically labelled internal standards (ILIS) Hexachlorobenzene-¹³C₆, Tebuconazole-D₆ and 4,4'-DDE-D₈ were also purchased from Dr. Ehrenstorfer. All standards had purities higher than 95%. Stock standard solutions (around 500 mg L⁻¹) were prepared in acetone and were stored at -20 °C. Twenty-two mixtures of pesticide standards (individual concentration of each pesticide around 50 mg L⁻¹) were prepared by dilution of stock individual solutions in acetone. Working standard solutions containing all pesticides were prepared by dilution of mixtures with acetone (for sample fortification in GC), hexane (GC injection), methanol (for sample fortification in LC) and water (instrument injection in LC). Stock standard solutions were stored at -20 °C, whereas working solutions were stored at 4 °C.

HPLC-grade water was obtained from a MilliQ water purification system (Millipore Ltd., Bedford, MA, USA). HPLC-grade methanol, HPLC-supragradient acetonitrile, acetone (pesticide residue analysis quality) and n-hexane (all ultra-trace quality) were purchased from Scharlab (Barcelona, Spain). Formic acid (HCOOH, content > 98%), sodium hydroxide (NaOH, reagent grade) and ammonium acetate (NH₄Ac, reagent grade) were supplied by Scharlab. Anhydrous magnesium sulfate (extra pure) and anhydrous sodium acetate (reagent grade) were purchased from Scharlab. Leucine enkephalin (used as lock mass in LC) and heptacosyl (for GC calibration) were purchased from Sigma Aldrich.

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 C₁₈, in 2 mL microcentrifuge tubes for d-SPE.

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