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A review on the use of hormones in fish farming: Analytical methods to determine their residues

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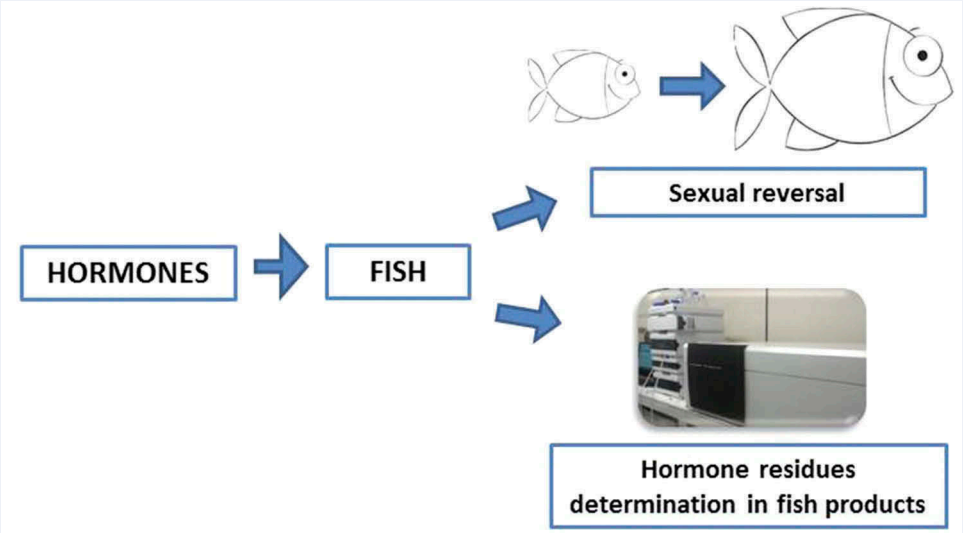
ABSTRACT

Hormones are used in fish farming to increase fish production when one sex of a species has the capacity to grow bigger and faster than the other sex. The technique to increase fish production based on sexual dimorphism mostly uses estrogens and androgens. These chemicals must be handled carefully to ensure environmental, biological and food safety, since they can contaminate the environment and promote changes in the endocrine system inducing adverse effects on the consumer health. Another important issue is the reliability of the analytical methods used to identify and/or quantify hormone residues in the meat of treated fish. Therefore, the aim of this review is to bring into view the use of hormones in fish farming, the possible impacts of this practice on humans and on the environment and to discuss the current methods of analysis for determining the hormone residues in food matrices, especially in fish.

Una revisión sobre el uso de hormonas en la piscicultura. Métodos analíticos para determinar sus residuos

RESUMEN

Las hormonas se utilizan en la piscicultura para aumentar la producción de pescado cuando un sexo de una especie tiene la capacidad de crecer más y más rápido que el otro sexo. La técnica para aumentar la producción de pescado con base en el dimorfismo sexual utiliza, principalmente, estrógenos y andrógenos. Estas substancias deben manipularse cuidadosamente para garantizar la seguridad ambiental, biológica y alimentaria, ya que pueden contaminar el medioambiente e inducir cambios en el sistema endocrino provocando efectos adversos en la salud del consumidor. Otro importante problema es la fiabilidad de los métodos analíticos utilizados para identificar y/o cuantificar los residuos de hormonas en la carne del pescado tratado. Así, el objetivo de esta revisión es presentar el uso de hormonas en piscicultura, los posibles impactos de esta práctica en seres humanos y en el medioambiente y discutir los métodos actuales de análisis para determinar los residuos de hormonas en varios alimentos, especialmente en el pescado.



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1. Introduction

The demand for fish has increased in recent years due to population growth and the constant search for a healthy diet. According to the Brazilian Institute of Geography and Statistics (IBGE, 2015), in 2015, fish farming increased to 1.5% when compared to the previous year, on the total fish

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production. The Brazilian aquaculture has been growing strongly in all regions of the country, mainly due to advances in the technical management of fish cultivation to cope with the increasing worldwide demand for fish as well as the economic and environmental sustainability of fish farming. In the Brazilian aquaculture scenario, tilapia (*Oreochromis niloticus*) and tambaqui (*Colossoma macropomum*) are the main farmed species.

Hormones are chemical messengers that are responsible for the communication between different types of cells that recognize their identity and function through receptors, which are protein structures specialized in molecular recognition. After the proximity and the hormone-receptor interaction, a series of biochemical reactions leading to specific biological responses occur (Reis-Filho, de Araújo, & Vieira, 2006).

Steroids are hormones produced from cholesterol and can be grouped into five subgroups based on their structural characteristics: estrogens, androgens, progestogens, glucocorticoids and mineral corticoids (Guedes-Alonso, Montesdeoca-Esponda, Sosa-Ferrera, & Santana-Rodríguez, 2014). The three former subgroups compose a group of sex steroids. The most important sex hormones used in fish farming are estrogens and androgens, which can be of natural origin (present in nature) or synthetic (synthesized or produced in laboratory).

Hormones in aquaculture are used for artificial reproduction and sex reversal. The first sustains the production chain with the constant production of seeds. The second is used when the growth rate and/or gain weight are different between the male and female. This difference between genders is very common in teleost fish and usually occurs during puberty (Taranger et al., 2010). Nevertheless, the use of hormonal products in fish farming can have harmful consequences, such as potential risks to human and environmental health related to hormone-dependent parameters. Moreover, its use outside of good animal husbandry practices may affect not only the fish farming production system itself but also the commercialization of this food commodity.

Currently, most issues related to food safety aim at residue control in foods due to the use of pesticides, veterinary drugs or accidents involving environmental contamination from chemicals. In relation to hormone residues in fish, Liu et al. (2011) studied the presence of steroidal hormones in crucian carp (*Carassius auratus*), carp (*Cyprinus carpio*) and silvery minnow (*Anabarilius alburnops*), which are fish species common in Dianchi Lake (China), as well as placed in the same position in the food chain. The authors conclude that steroidal hormones are likely ubiquitous contaminants in wild fish from that lake. The presence of steroid hormone residues was reported in fish tissue samples from two common fishes (*Boops boops* and *Sphoeroides marmoratus*) which are species that are at the bottom of the food chain and that were fished in the proximity of the outfall of the wastewater treatment plant of the city of Las Palmas de Gran Canaria (Spain) (Guedes-Alonso, Sosa-Ferrera, & Santana-Rodríguez, 2017). Steroid hormone residues were also reported in yellow croaker (*Larimichthys polyactis*) tissue samples collected from local supermarkets in Changchun (China) (Wang et al., 2012) and in tilapia (*Oreochromis mossambicus* and *Oreochromis niloticus*) purchased from supermarkets in Taipei City (Taiwan) (Chen, Wang, Gwo, & Chen, 2012). According to Epstein (1990 with Duarte, da Silva, & Meirelles, 2002), 3000 children in Puerto Rico had serious problems with premature sexual development and ovarian cysts due to ingestion of meat products with zeranol residues (xenobiotic anabolic agent with estrogenic activity promoting growth). Thus, eating foods contaminated with steroids can lead to endocrine disorders and may even result in the development of cancer (Bergman, Heindel, Jobling, Kidd, & Zoeller, 2013). Nevertheless, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an acceptable daily intake of 0–0.5 μg kg^{-1} body weight (bw) for zeranol (JECFA, 1988), 0–0.02 μg kg^{-1} bw for trenbolone acetate (JECFA, 1989), 0–0.05 μg kg^{-1} bw for 17β-estradiol, 0–30 μg kg^{-1} bw for progesterone, and 0–2 μg kg^{-1} bw for testosterone (JECFA, 2000).

The use of hormones in food producing animals faces different legal regulations in different countries. The European Union prohibits the use of substances with hormonal or thyreostatic action and β-agonists in food producing animals, including aquaculture (EC, 1996, 2003, 2008). Other countries such as Canada, Australia, New Zealand, Argentina and the United States allow for the use of natural steroid hormones, such as testosterone, progesterone and 17β-estradiol, as well as the synthetics zeranol and trenbolone acetate. In those countries, only residues from synthetic compounds with maximum residue levels (MRLs) are controlled because they are considered to be the most potent endocrine compounds (Duarte et al., 2002). In Brazil, since 1991, the importation, production, marketing, and use of natural or artificial substances for animal growth purposes and/or weight gains have been prohibited. Some substances (i.e. testosterone, progesterone, estradiol, zeranol, trenbolone acetate), natural or synthetic, with estrogen or progestogen action are allowed for therapeutic purposes, estrous cycle synchronization, and preparation of donors and recipients for embryo transfer (MARA, 1991). Codex Alimentarius Commission (Codex) established that residues resulting from the use of 17β-estradiol, progesterone, and testosterone, as a production aid in cattle, in accordance with good animal husbandry practice are unlikely to pose a hazard to human health. Therefore, considering the establishment of an MRL (in muscle, liver, kidney, fat) is unnecessary. Thus, it is worth mentioning that the use of hormones for the purpose of sexual reversion is not established by the legislative framework of any country, nor in any recommendations of regional bodies (European Commission) nor international (JECFA or Codex) that deal with food safety.

In relation to the methods of analysis, the determination of hormone residues in foods of animal origin is difficult because of the complexity of the matrix and the low concentrations to be quantified (in the order of ng g^{-1}) (Guedes-Alonso et al., 2014). In this regard, analytical methods for detecting hormone residues are generally immunoassays, such as radioimmunoassays (RIA) and enzyme-linked immunosorbent assays (ELISA) (Zanardi, 2007). For quantitative determination of hormone residues in fishery products, analytical methods using liquid chromatography (LC) coupled to a tandem mass spectrometry (LC–MS/MS) system is currently the recommended analytical technique, which has become popular and widely used in food analysis due to its high specificity and detectability in the determination of residue levels of contaminants in complex matrices (Reis-Filho et al., 2006; Zanardi, 2007). Nevertheless, the determination of hormone residues in fish matrices by LC–MS/MS has been seldom reported (Guedes-Alonso et al., 2017; Jakimska et al., 2013; Wang et al., 2012; Xu, Chu, Peng, Jin, & Wang, 2006).

The main goal of this article is to review the literature available on the use of hormones in fish farming, as well as the qualitative and quantitative analytical methods developed

for the determination of hormone residues in food from aquaculture. The aim is to provide advice to the readers on the advantages and disadvantages on the use of hormones in fish farming. Challenges and perspectives on future trends of analytical methodologies in determining hormones mainly in fish products are also discussed.

2. The use of hormones in fish farming

2.1. Sex reversal

Sex reversal can be achieved with hormonal treatment during sex differentiation. Fish sex may be separated into genotype, which is determined by the genes responsible for gonads formation, and phenotype, which is the appearance of the ovary or testis. Phenotype differentiation occurs naturally, generally earlier in female than in males, during the ontogeny of the fish larvae (Piferrer, 2001). This process is very complex, but it can be manipulated using androgen and estrogen hormones.

The use of hormones in fish farming for sex reversal aims at the production of monosex population to increase growth rate or weight gain. Commercially, it is advantageous to rear individuals of the most profitable gender, thereby achieving more uniform lots and controlling undesirable breeding (Singh, 2013; Taranger et al., 2010). There are two methods for producing monosex fish populations: (i) direct, by which the fish are treated with hormones for the purpose of developing the desired sex, and (ii) indirect, by which parents or breeders are treated with hormones to obtain neomale (XX♂), neofemale (XY♀, ZZ♀), or supermale (YY♂) populations, and with them getting the entire batch of larvae of the same sex (Piferrer, 2001).

Protocols for the production of monosex female stocks in 35 different species of teleosts using a variety of estrogens were reported by Piferrer (2001). Those protocols included the type of estrogen, route of administration, dose, duration of treatment and timing. The author emphasizes the convenience of using the natural estrogen 17β-estradiol rather than synthetic estrogens. Among androgens, 17α-methyl testosterone (synthetic) has been widely employed to reverse females into males, being commonly used in tilapia. Both steroids have the advantage of being easily metabolized after the period of hormonal treatment (Mainardes-Pinto, Fenerich-Verani, de Campos, & da Silva, 2000; Zanoni, Leal, Filho, de Oliveira, & Ribeiro, 2013). Table 1 shows hormones used for sex reversal and differentiation in different species of fish for both male and female induction.

The administration of hormones for sex reversal treatment can be done using systemic (direct injection and silastic implantation), immersion, or dietary supplementation (hormone incorporated in fish feed) (Pandian & Sheela, 1995). Commercially, the most successful treatments use immersion and diet, as both methods reach a large number of fish, while the systemic transfer method is expensive and requires technical ability to be applied on the fish. In the immersion technique, the dose administered not only affects the efficiency of hormonal treatments but also other parameters, such as the type of hormone, water temperature and exposure time. The addition of hormones in the feed is more efficient because it is easily controlled, and allows optimum steroid dose to induce the complete sex reversal of all individuals (Pandian & Sheela, 1995; Piferrer, 2001).

Most authors mention the use of 17β-estradiol, estradiol valerate, 17α-methyl testosterone, or 17α-methyl dihydrotestosterone (by immersion and diet technique) to produce an all-female population by the direct and indirect feminization. On the other hand, androgen is used to masculinize females, producing neomales (Piferrer, 2001). Females are produced due to the common problems associated with precocious male sexual maturity and the advantage associated with the fact that females grow faster than males. Several authors reported great results on the effects of hormones on the proportions of sexual types (de Bem, 2009; Haffray et al., 2009; Lin, Benfey, & Martin-Robichaud, 2012). Moreover, the authors who used the implant method to test different doses of the hormone, verify that all fishes died and showed liver injury at high doses of 17β-estradiol (higher than 8 mg kg^{-1}) (Passini, 2013).

For production of an all-male population, tamoxifen (compound that blocks the estrogen receptor), fadrozole and letrozole (nonsteroidal aromatase inhibitor), 17α-methyl testosterone, 17α-ethynyl testosterone and 17α-ethynyl estradiol by direct and indirect methods have been used in species where males grow faster than females and reach a larger size (Garcia et al., 2013; Liao et al., 2014; Zanoni et al., 2013).

According to Pandian and Sheela (1995), the advantages of hormonal treatment ensure maximum growth, eliminate early maturation in males and allow broodstock management. The disadvantages of this technique are related to the possible presence of carcinogenic steroid residues that can affect consumer health. Hormonal induction of sex reversal can become a stressful process, resulting in low survival rates, delayed sexual maturity and reduction of fish fertility, while high doses can lead to sterility, paradoxical sexual reversal and growth suppression. On a large scale, sexual reversal may become a technique to pollute the environment because more than 99% of hormones are metabolized and released within hours or days into the water.

Treatments for sex reversal purposes are conducted at the initial stage of fish development, before or during the process of gonadal sexual differentiation, such as during the last few months before being marketed (Almeida, 2013; de Bem, 2009). The residue of 17β-estradiol, for instance, disappears in less than a month after the end of the treatment, meaning that there is no risk of meat contamination by hormones at the time of slaughter. According to Specker and Chandlee (2003), summer flounder (*Paralichthys dentatus*) larvae and juvenile whole-body estradiol levels were not significantly different compared to the untreated controls after 24 h. In addition to being a natural steroid, which is quickly metabolized and excreted, 17β-estradiol is also considered the hormone with the least impact on the environment (Hendry, Martin-Robichaud, & Benfey, 2003; Piferrer, 2001; Specker & Chandlee, 2003). While treating Rainbow trout (*Salmo gairdneri*), Adeel, Song, Wang, Francis, Yang. (2017) reported that steroidal estrogens decrease significantly in the aqueous phase, presenting short half-lives. In fact, the half-lives of estrogenic steroids (17β-estradiol, estradiol and ethinyl estradiol) were estimated in 2–6 days at 20°C, in water and sediment, with ethinyl estradiol being more stable than two other estrogens (Williams, Jürgens, & Johnson, 1999). Subsequently, Jürgens et al. (2002) reported that 17β-estradiol was much more resistant than ethinyl estradiol to the biodegradation in the water of English rivers.

Table 1. Androgens, estrogens and nonsteroidal agents used for sex reversal and differentiation in different species of fish.

Tabla 1. Andrógenos, estrógenos y agentes no esteroides utilizados para la reversión sexual y la diferenciación en diferentes especies de peces.

Hormone	Fish species	References	Region/country
17β-Estradiol	Rainbow trout (*Salmogairdneri*)	Johnstone, Simpson, & Youngson, 1978	Aberdeen/Great Britain
	Atlantic salmon (*Salmosalar*)	Johnstone et al., 1978	
	Atlantic halibut (*Hippoglossus hippoglossus L.*)	Hendry et al., 2003	New Brunswick/Canada
	Summer flounder (*Paralichthys dentatus*)	Specker & Chandlee, 2003	Narragansett/USA
	Bagrid catfish (*Pseudo bagrus fulvidraco*)	Park, Kim, Cho, & Kim, 2004	Busan/South Korea
	Atlantic cod (*Gadusmorhua*)	Lin et al., 2012	St. Andrews/Canada
	Common snook (*Centropomus undecimalis*)	Passini, 2013	Florianópolis/Brazil
17α-Ethynylestradiol	Tilapia (*Oreochromis aureus*)	Melard, 1995	Tihange/Belgium
Estradiol valerate	Tetra (*Astyanax altiparanae*)	de Bem, 2009	Rio Claro/Brazil
Tamoxifen	Bagrid catfish (*Pseudo bagrus fulvidraco*)	Park et al., 2004	Busan/South Korea
Letrozole	Protogynous dusky grouper (*Epinephelus marginatus*)	Garcia et al., 2013	São Paulo/Brazil
	Medaka fish (*Oryzlas latipes*)	Liao et al., 2014	Taipei/Taiwan
Fadrozole	Atlantic halibut (*Hippoglossus hippoglossus L.*)	Babiak et al., 2012	Nordland/Norway
	Protogynous wrasse (*Halichoeres trimaculatus*)	Nozu, Kojima, & Nakamura, 2009	Okinawa/Japan
17α-Methyltestosterone	Brook trout (*Salvelinus fontinalis*)	Haffray et al., 2009	Rennes/France
	Nile tilapia (*Oreochromis niloticus*)	Curtis et al., 1991	Cowallis/USA
		Mainardes-Pinto et al., 2000	Nova Odessa/Brazil
		Toyama, Corrente, & Cyrino, 2000	Piracicaba/Brazil
		Hayashi, Boscolo, Soares, & Meurer, 2002	Maringá/Brazil
		Zanardi et al., 2011	Jaboticabal/Brazil
		El-Sayed, Abdel-Aziz, & Abdel-Ghani, 2012	Alexandria/Egypt
		Zanoni et al., 2013	Londrina/Brazil
	Rainbow trout (*Oncorhynchus mykiss*)	Tabata, Rigolino, & Tsukamoto, 1999	São Paulo/Brazil
	Tilapia (*Oreochromis Mossambicus*)	Johnstone, Macintosh, & Wright, 1983	Stirling/Great Britain
	Rainbow trout (*Salmo gairdneri*)	Johnstone et al., 1978; 1983	Aberdeen and Stirling,/ Great Britain
	Atlantic salmon (*Salmosalar*)	Johnstone et al., 1978	Aberdeen,/Great Britain
17α-Methyldihydrotestosterone	Atlantic halibut (*Hippoglossus hippoglossus L.*).	Hendry et al., 2003	New Brunswick/Canada
	Atlantic cod (*Gadusmorhua*)	Lin et al., 2012	St. Andrews/Canada
17α-Ethynyltestosterone	Tilapia hybrids (*Oreochromis niloticus* x *O. aureus*)	Rothbard et al., 1990	Tel Aviv/Israel

2.2. Artificial reproduction

Another use of hormones in aquaculture is in seed production, i.e. artificial reproduction, which is important for manipulating the final maturation and ovulation in the gonad.

Artificial reproduction has endless applications, it can be controlled by environmental parameters for natural reproductive performance and by exogenous hormones to induce/advance or delay/arrest fish maturation, and in making spawning occur a few months earlier or later than normal. By advancing the period of reproduction, the fish farmers have a greater flexibility in the marketing of the larvae and young fish. By restricting spawning to a certain period, this maximizes time and resources, allowing for the higher throughput and turnover of nursery ponds. By synchronizing spawning males and females, the farmer obtains fingerlings in periods when profitability is higher (Mylonas, Fostier, & Zanuy, 2010; Venturieri & Bernardino, 1999). In addition, this technique is very important for the reproduction of fish that need external stimuli, since they are not suitable for production in captivity.

Hormonal techniques for fish breeding are based on intramuscular or intraperitoneal injection in broodstocks. The oldest and still the most used hormone is the crude extract of the pituitary gland (PE) of mature fish (carp and salmon). Another largely used hormone is the gonadotropin-releasing hormone (GnRH) of both mammalians (mGnRH) and salmon (sGnRH). Gonadotropin-releasing hormone analogs (GnRHa), human gonadotropin (GtHs), dopamine antagonists (pimozide, domperidone, metoclopramide and reserpine) and synthetic hormones similar to gonadotropins (Ovopel) have been tested in several species to induce or block sexual maturation (Almeida, 2013; Araújo et al., 2014; Zohar & Mylonas, 2001). Zohar and Mylonas (2001) summarize the use of GnRHa and GtHs used to induce maturation and spawning in more than 30 species of fish.

The advantage of using dopamine antagonists is that they block the dopamine inhibitor mechanism, elevating GnRH secretion in the blood stream while inducing maturation. The most used and recommended dopamine antagonist is domperidone, as it is cheap, potent and does not cross the blood-brain barrier (Dasgupta, Sarkar, Sarangi, & Bhattacharya, 2009; Heyrati, Mostafavi, Toloee, & Dorafshan, 2007; Wang et al., 2009). When GnRH alone does not work properly, it is usually used in combination with dopamine antagonists (Almeida, 2013; Venturieri & Bernardino, 1999). The use of gonadotropin hormones to induce maturation and spawning in different fish species is shown in Table 2.

Regarding synthetic hormones, Ovopel is composed of a combination of the mGnRHa and dopamine antagonists (metoclopramide). It was used to induce the reproduction of wild mature asp (*Aspius aspius*) during out-of-season spawning (Targońska, Kucharczyk, Kujawa, Mamcarz, & Żarski, 2010), to induce spermiation of Amazon catfish (*Leiarius marmoratus*) (Araújo et al., 2014) and to induce ovulation of pacu (*piaractus mesopotamicus*) (Santos, De Santos, Salomão, & de Silva, 2012). Ovaprim is a synthetic hormone that combines sGnRHa and another dopamine antagonist (domperidone).

3. Impact of the hormone on the environment and consumer health

The 17β-estradiol residue in fish meat disappears in less than a month after finishing the sex reversal treatment, since this steroid is of natural origin, quickly metabolized and excreted. The residue of 17α-methyl testosterone is also rapidly metabolized after a period of hormonal treatment (Piferrer, 2001). Thus, theoretically, it should not be found at a high level in fish meat if the treatment was done in a correct manner and over the correct period.

Table 2. Hormones used to induce maturation and spawning in different species of fish.

Tabla 2. Hormonas usadas para inducir la maduración y el desove en diferentes especies de peces.

Hormone	Fish species	References	Region/country
Luteinizing hormone-releasing hormone analogous (LHRHa)	Milkfish (*Chanos chanos Forsskal*)	Lee et al., 1986	Waimanalo/USA
	Black sea bass (*Centropristis striata*)	Berlinsky, King, & Smith, 2005	Durham/USA
	Atlantic cod (*Gadus morhua*)	Garber, Fordham, Symonds, Trippel, & Berlinsky, 2009	New Brunswick/Canada
Carp pituitary extract	Nase (*Chondrostoma nasus*)	Szabó, Medgyasszay, & Horváth, 2002	Godollo/Hungary
	Kutum (*Rutilus frisii kutum*)	Heyrati et al., 2007	Tehran/Iran
	Wild loach (*Misgurnus anguillicaudatus*)	Wang et al., 2009	Hubei/China
	Dourado fish (*Salminus brasiliensis*)	Flora, Maschke, Ferreira, & De A Pedron, 2010	Santa Maria/Brazil
	Carp (*Cyprinus carpio carpio*)	Vazirzadeh et al., 2011	Karadj/Iran
	Amazon catfish (*Leiarius marmoratus*)	Araújo et al., 2014	Cuiabá/Brazil
	Tambaqui (*Colossoma macropomum*)	Filho, Brito, Cunha, Júnior, & Amorim, 2016	Recife/Brazil
Gonadotropin-releasing hormone analogous (GnRHa) + dopamine antagonists	Nase (*Chondrostoma nasus*)	Szabó et al., 2002	Godollo/Hungary
	Silver perch (*Bidyanus bidyanus*)	Levavi-Sivan, Vaiman, Sachs, & Tzchori, 2004	Rehovot/Israel
	Kutum (*Rutilus frisii kutum*)	Heyrati et al., 2007	Tehran/Iran
	Rohu (*Labeo rohita*)	Dasgupta et al., 2009	Bhubaneswar/India
	Wild loach (*Misgurnus anguillicaudatus*)	Wang et al., 2009	Hubei/China
	Carp (*Cyprinus carpio carpio*)	Vazirzadeh et al., 2011	Karadj/Iran
Gonadotropin-releasing hormone analogous (GnRHa)	Nase (*Chondrostoma nasus*)	Szabó et al., 2002	Godollo/Hungary
	Dusky groupe (*Epinephelus marginatus*)	Marino et al., 2003	Rome/Italy
	Greater amberjack (*Seriola dumerili*)	Mylonas, Papandroulakis, Smboukis, Papadaki, & Divanach, 2004	Crete/Greece
	Silver perch (*Bidyanus bidyanus*)	Levavi-Silvan et al., 2004	Rehovot/Israel
	Rose snapper (*Lutjanus guttatus*)	Ibarra-Castro & Duncan, 2007	Mazatlán/México
	Kutum (*Rutilus frisii kutum*)	Heyrati et al., 2007	Tehran/Iran
	Atlantic cod (*Gadus morhua*)	Garber et al., 2009	New Brunswick/Canada
	Wild loach (*Misgurnus anguillicaudatus*)	Wang et al., 2009	Hubei/China
	Honeycomb grouper (*Epinephelus merra*)	Kanemaru et al., 2012	Miyazaki/Japan
	Senegalese sole (*Solea senegalensis*)	Rasines et al., 2013	Santander/Spain
	Meagre (*Argyrosomus regius*)	Mylonas et al., 2013	Crete/Greece
	Longfin yellowtail (*Seriola rivoliana*)	Fernández-Palacios, Schuchardt, Roo, Hernández-Cruz, & Izquierdo, 2015	Las Palmas/Spain
Synthetic hormone similar to gonadotropin (Ovaprim)	Freshwater rotifer (*Brachionus calyciflorus*)	Sugumar & Munuswamy, 2006	Chennai/India
	Asp (*Aspius aspius*)	Targońska et al., 2010	Olsztyn/Poland
	Pinfish (*Lagodon rhomboids*)	DiMaggio, Broach, & Ohs, 2013	Gainesville/USA
Synthetic hormone similar to gonadotropin (Ovopel)	Asp (*Aspius aspius*)	Targońska et al., 2010	Olsztyn/Poland
	Amazon catfish (*Leiarius marmoratus*)	Araújo et al., 2014	Cuiabá/Brazil
	Pacu (*Piaractus mesopotamicus*)	Santos et al., 2012	Presidente Prudente/Brazil
Human chorionic gonadotropin (hCG)	Silver perch (*Bidyanus bidyanus*)	Levavi-Sivan et al., 2004	Rehovot/Israel
	Pinfish (*Lagodon rhomboids*)	DiMaggio et al., 2013	Gainesville/USA

According to Reis-Filho et al. (2006) natural estrogens are continuously introduced into the environment by human excretion, which follows the sewage disposal system, even after entering the environment at low concentrations (nanograms per liter), making them very persistent. These estrogens introduced into the environment could affect wildlife and human health by disrupting their normal endocrine systems, such as the feminization effects of males, altering the offspring sex ratios, infertility, reduced fertility, inhibition of the development of sexual organs and sexual reversal.

The way to detect if the reproductive systems of aquatic organisms are affected by estrogen is by determining the level of vitellogenin (VTG) in the blood plasma of an organism. VTG is a protein that has an important role in the female reproductive system of oviparous vertebrates. It is synthesized in the liver, regulated by estrogen and transported via the blood to the ovaries, where it is incorporated into the developing eggs. If an increase is observed in the VTG plasma level of an organism, it means that it was exposed to substances with estrogenic activity. Although the concentrations of these hormones are very low (in the order of ng L^{-1} or µg L^{-1}), they can be sufficient to induce VTG synthesis even in male fish (Bila & Dezotti, 2003, 2007).

The other way that hormones are introduced into the environment is by the incorrect or illegal discard of water containing residues of these compounds used to treat fish. The contamination could have originated by fish excretion, as well as from the medicated feed that remains unconsumed by fish. According to some authors (Leonhardt, 1997; Specker & Chandlee, 2003), the liver metabolizes the hormones in water soluble compounds. Approximately 99% of the administered hormone through the diet during the sex reversal is metabolized and released into the water by bile and urine excretion within a few hours or days after treatment. Estrogens are then excreted mainly as inactive conjugates of sulfuric and glucuronic acids, which do not have direct biological activity, but can act as precursor hormone reservoirs that can be reconverted to free steroids with estrogenic activities by bacteria in the environment, such as the microorganisms in raw sewage and sewage treatment plants (Ying, Kookana, & Ru, 2002). In other words, the sex reversal technique can pollute the environment if the water in which fish were treated is not properly depurated.

Specific studies have demonstrated that the use of hormones does not result in residue accumulation in the tissues of the reversed fish. Rothbard et al. (1990) showed that in the muscle of tilapia treated for 11 weeks with a diet containing 60 mg kg^{-1} of 17α-ethynyl testosterone, a high concentration of the steroid was detected on day 0 (142.1 ng g^{-1}) and day 1 (168.6 ng g^{-1}) after hormonal exposure. The samples taken on days 3, 5 and 7 did not differ from the untreated controls (17α-ethynyl testosterone concentrations were below the detectable level of 50 ng g^{-1}). Curtis, Diren, Hurley, Seim, and Tubb (1991) fed Nile tilapia diets that contained 30 mg kg^{-1} of 17α-methyl testosterone for 30 days. The hormone concentration decreased logarithmically from day 1 to day 10 after treatment.

In humans, the exposure to hormones can cause endocrine disorders, such as early puberty in children, advances in bone age, as well as negative repercussions on growth, modification of sexual characteristics and cancer development (Bergman et al., 2013). These disorders especially occur in children because they are in the growing phase when puberty has not yet developed. Thus, Partsch & Sippell (2001 with Alves, Flores, Cerqueira, & Toralles, 2007) reported that precocious pseudo puberty could be induced in children by oral or dermal exposure to estrogen-containing foods or ointments, respectively, if they were chronically exposed to food (meat) derived from animals treated with high doses. In Milan, Bahrain, Puerto Rico and Jerusalem, there were reported cases of gynecomastia, larche, pubarche and precocious sexual development in children, which were induced by the consumption of poultry, meat, milk and cereals contaminated with estrogen, or its precursors, or by the contamination of drinking water with substances that had estrogenic activity (Alves et al., 2007).

4. Methods of analysis for determination of hormone residues in several matrices

Determining hormone residues in foods from animal origin is a difficult task, first because of the matrix complexity, and second because of the low concentrations that need to be quantified, at the order of μg kg^{-1} (or μg L^{-1}) and ng kg^{-1} (or ng L^{-1}) (Guedes-Alonso et al., 2014). In most analyses involving actual samples, a substantial clean-up of the extract and analyte concentration during sample preparation step is necessary to isolate the target compounds from the matrix and to achieve the required limits for detection and quantification.

To determine the hormone residues in samples from the environment and in foods, analytical methods have been developed to identify and quantify these substances in simple and complex matrices. In this regard, according to Zanardi (2007), analytical methods for detecting hormone residues are, generally, immunoassays, such as RIA and ELISA, which are routinely used. High-performance thin layer chromatography and gas chromatography–MS (GC–MS) can also be an important tool for steroid determination. Nonetheless, for quantitative determination, analytical methods using LC coupled with several types of detectors are the most widespread techniques. For hormone determination, the most common detectors coupled to LC are electrochemical, ultraviolet diode array (DAD), fluorescence, UV-Vis, MS and tandem mass spectrometry (MS/MS) (Reis-Filho et al., 2006). Table 3 summarizes the analytical techniques reported for the determination of hormones in several matrices.

GC–MS permits the simultaneous determination of many analytes with a detection limit in the order of ng kg^{-1} or ng L^{-1}, but the sample preparation is laborious and time-consuming. To turn the molecules volatile, the most used derivation technique has been silylation and acetylation (Zhou & Zhang, 2011). Hartmann, Lacorn, and Steinhart (1998) investigated 12 steroids in beef, veal, milk, milk products, pork, meat products, poultry, eggs, fish, plants, yeast and fermented alcoholic beverages using GC–MS. For each matrix, different sample preparations were studied by evaluating the recovery of the spiked samples. The authors reported residue levels between 0.03 and 1.41 μg kg^{-1} for 10 steroids, except estrogens that were not detectable. Long, Hong, and Jie (2007) reported a GC–MS method for the identification and quantitative determination of 17β-estradiol residues in the muscle of various fishery products. The authors reported extraction, clean-up and derivatization more able to enhance detection sensitivity. Great linearity and precision were obtained and the method was successfully applied to the analysis of samples from the retail market.

ELISA are simple and economical and are especially suitable for screening large numbers of hormonally active substances with great sensitivity. ELISA does not cause serious problems in discarding samples because it does not require the use of reagents that are dangerous to human and environmental health (Zhou & Zhang, 2011). Zanardi et al. (2011) analyzed tilapia carcass samples by ELISA using a commercial kit to determine testosterone at the residue concentration level of ng g^{-1}. The authors reported recovery values of 69.9%.

For the simultaneous separation of analytes, reversed-phase LC is performed (C18 columns). In general, for chromatographic separation, the mobile phases mainly consist of acetonitrile or methanol with water, and could contain a low level of an organic acid or base to help in the ionization of the compounds to be analyzed (Ardrey, 2003). Several spectroscopic techniques, such as ultraviolet (UV), fluorescence and diode-array (DAD), have been used in the LC determination of hormone residues. Nevertheless, the recommended and most used detectors are MS or MS/MS, which separate the analytes by the mass/charge (m/z) of the ions. To generate charged ionic analytes, the most used interface ionization techniques are electrospray (ESI) and atmospheric pressure chemical ionization (APCI), in the both negative and positive ion mode (Zhou & Zhang, 2011). The use of ESI or APCI will depend on the analyte that will be analyzed.

The MS/MS detection system uses two stages of mass spectrometry (MS1 and MS2). The first is used to isolate the ion of interest, and the second is used to make the relationship of this ion with the others from which it may have been generated, or which it may generate on decomposition (Ardrey, 2003). Recently, the LC–MS/MS system has become more popular and widely used in food analysis because it has a high specificity and detectability in the determination of the residue levels of contaminants in complex matrices. However, there is the disadvantage that the LC–MS/MS systems are not accessible to all laboratories for routine analysis due to the high cost of the equipment and the requirement of an experienced operator.

Several studies have been reported in the literature that use LC–MS/MS to determine hormone residues in various matrices and to allow for the determination of a large number of compounds with a high precision and sensitivity (Guedes-Alonso et al., 2014, 2017). In this regard, a sample preparation step prior to the chromatographic separation is usually necessary and can include several steps, such as filtration, extraction, clean up, purification, evaporation, hydrolysis, and derivatization, among others (Reis-Filho et al., 2006). A summary of the advantages and disadvantages of the analytical technique used for determination of residues of hormones in several matrices is shown in Table 4.

Extracting hormones from water (including drinking water, ground water, surface water and effluents from sewage treatment plants, sewage, marine sediments, soil and biological sludge) is usually performed by solid phase extraction (SPE) disks or cartridges. Steroid hormones are excreted by humans and reach the aquatic environment through the sewage systems daily. Thus, several authors have stated that municipal wastewater is the main source of the contamination of aquatic

Table 3. Analytical techniques for the determination of hormone residues in several matrices.

Tabla 3. Técnicas analíticas para la determinación de residuos de hormonas en varias matrices.

Analytical technique	Matrix	Analyte	Reference
LC/UV-Vis	Fishery products	ETES	Rothbard et al., 1990
		E1, E2, E3, EE	Hu et al., 2010
	Animal foods	E2, E3, PRO, BOL, TES	Shi et al., 2011
	Sediment	METES	Falone, 2007
	Water	METES	Falone, 2007
		E2, EE	Fernandes et al., 2011
	Yogurt	E2, E3, PRO, BOL, TES	Shi et al., 2011
LC/fluorescence	Fishery products	E2, E3, DES	Jiang et al., 2009
	Water	E2	Lopes et al., 2010
LC/DAD	Water	E1, E2, E3, EE, MES, DES	de Alda & Barcelo, 2001
		E1, E2, E3, EE	Verbinnen, Nunes, & Vieira, 2010
		E1, E2, αE2, E3, EE, PRO, NORET, NORG, MES	Almeida & Nogueira, 2015
LC–MS/MS	Food of animal origin	E1, E2, E3, EE, DES, METES, TES, TBL, NAN, HEX, DIEN, STAN	Shao et al., 2005
		EPITES, NAN, METES, PTS, MEPRO, PRO, E1, E2, E3, EE	Xu et al., 2006
		AP, BOL, CMA, FMT, MPA, MGA, MEBOL, METES, NE, NORG, NORTES, PRO, 16-OH-STAN, TES, TBL	Blasco, Poucke, & Peteghem, 2007
		DES, E2, EE, BOL, NORTES, METES, TBL, TRIAM-ACE, DEXA, FMS, ZEL,ZAL, ZEA, MGA, MLA, MPA	Kaklamanos, Theodoridis, & Dabalis, 2009
		NAD, TBL, BOL, FMT, NAN, AED, MDROL, TES, DHEA, METES, AND, STAN, DHT, NET, 17-HPT, 21-HPT, NORG, MGA, MEPRO, CMA, PRO, MPA, TRIAM, PRD, PRNL, CORT, CORS, FMS, DEXA, FCN-ACE, MPRNL, TRIAM-ACE, FML, BDS, E1, E2, E3, EE, DES, HEX, DIEN, MEST, MEAND, DAN, MESOL, ALD, BEC, FLUA, PCL	Yang, Shao, Zhang, Wu, & Duan, 2009
		CMA, MPA, MGA, PTS, NORG, METES, NAN, Z, E1, E2, αE2, E3, EE, EV, DES	Wang et al., 2010
		AED, TES, PRO, STAN, 17-HPT, PRNL, PRD, HCORT, MGA, MPA	Fan et al., 2014
	Water	E1, E2, EE	Ingrand, Herry, Beausse, & De Roubin, 2003
		E1, E2, E3, EE, E1-3S, E1-3G, E2-3S, E2-3G, E2-17G, E2-3G17S, E2-3S17G, E2-3, 17DiS, E3-3S, E3-3G	Isobe et al., 2003
	Fishery products	EPITES, NAN, METES, PTS, MEPRO, PRO, E1, E2, E3, EE	Xu et al., 2006
		COR, E1, αE2, E3, MEPRO, PRO, 17-HPT, TES, NORTES	Wang et al., 2012
		PRO, NORG, E1, E2, E3, EE, E1-3S	Jakimska et al., 2013
		PRO, NORG, E1, E2, E3, EE, DES, TES, NAN, PRD, PRDNL, BOL, MGA, NORET, CORT	Guedes-Alonso et al., 2017
GC/MS	Foods, meats and vegetables	P5, ADD, 17-HPT, DHEA, DHT, AND, αE2, E3	Hartmann et al., 1998
	Bovine liver	DES, Z	Cardoso, Silva, & Santos, 1999
	Fishery products	E2	Long et al., 2007
RIA	Bovine liver	DES, Z	Cardoso et al., 1999
	Fishery products	E2	Specker & Chandlee, 2003
ELISA	Fishery products	METES	Zanardi et al., 2011

Abbreviations: 16-OH-stan (16-β-hydroxystanozolol); 17-HPT (17α-hydroxyprogesterone); 21-HPT (21α-hydroxyprogesterone); ADD (androstenedione); AED (4-androstene-3,17-dione); ALD (aldosterone); and (androsterone); AP (acetoxyprogesterone); BDS (budesonide); BEC (Beclomethasone); BOL (boldenone); CMA (chlormadinone acetate); COR (corticosterone); CORS (cortisol); CORT (cortisone); DAD (ultraviolet diode array); DAN (Danazol); DES (diethylstilbestrol); DEXA (dexamethasone); DHEA (dehydroepiandrosterone); DHT (dihydrotestosterone); DIEN (dienestrol); E1 (estrone); E1-3G (estrone-3-glucuronide); E1-3S (estrone-3-sulfate); E2 (17β-estradiol); E2-17G (estradiol-17-glucuronide); E2-3,17DiS (estradiol-3,17-disulfate); E2-3G (estradiol-3-glucuronide); E2-3G17S (estradiol-3-glucuronide-17-sulfate); E2-3S (estradiol-3-sulfate); E2-3S17G (estradiol-3-sulfate-17-glucuronide); E3 (estriol); E3-3G (estriol-3-glucuronide); E3-3S (estriol-3-sulfate); EE (17α-ethynylestradiol); ELISA (enzyme-linked immunosorbent assay); EPITES (epitestosterone); ETES (17α-ethynyltestosterone); EV (estradiol valerate); FCN-ACE (fluocinolone acetonide); FLUA (Fludrocortisone acetate); FML (fluorometholone); FMS (flumethasone); FMT (fluoxymesterone); GC–MS (gas chromatography–mass spectrometry); HCORT (hydrocortisone); HEX (hexestrol); LC (Liquid chromatography); MDROL (methandrostenolone); MEAND (Methylandrostenediol); Mebol (methylboldenone); MEPRO (medroxyprogesterone); MES (mestranol); MESOL (Mestanolone); MEST (Mesterolone); METES (17α-methyl-testosterone); MGA (megestrol acetate); MPA (medroxyprogesterone acetate); MLA (melengestrol acetate); MPRNL (methylprednisolone); MS/MS (tandem mass spectrometry); NAD (19-nor-4-Androstene-3,17-dione); NAN (nandrolone); NE (northandrolone); NET (19-norethindrone); NORET (19-norethisterone); NORG (levonorgestrel ou Norgestrel); NORTES (19-nortestosterone); P5 (pregnenolone); PCL (Clobetasol propionate); PRD (prednisone); PRNL (prednisolone); PRO (progesterone); PTS (testosterone 17-propionate); RIA (radioimmunoassay); STAN (stanzolol); TBL (trenbolone); TES (testosterone); TRIAM (triamcinolone); TRIAM-ACE (triamcinolone acetonide); UV-Vis (ultraviolet–visible spectrophotometry); ZAL (zearalanol); ZEA (zearalenone); ZEL (zearalenol); αE2 (17α-estradiol); Z (Zeranol).

Table 4. Advantages and disadvantages of the analytical technique used for determination of residues of hormones in several matrices.

Tabla 4. Ventajas y desventajas de las técnicas analíticas utilizadas para la determinación de residuos de hormonas en diversas matrices.

Analytical technique	Advantages	Disadvantages	Reference
LC–UV/Vis	• Relatively low cost analytical technique; • Easy to operate and interpret the data.	• Low sensitivity and, consequently, high LOQ. For low LOQ's values, a more elaborate sample preparation step is needed to clean-up the extract and to concentrate the analyte (e.g. using SPE cartridges, which are expensive and make the method more laborious); • The UV/Vis detector can detect single wavelength at one time. • The molecule to be analyzed need to present a chromophore in their structure.	Rothbard et al., 1990 Hu et al., 2010 Shi et al., 2011 Falone, 2007 Fernandes et al., 2011
LC-fluorescence	• Relatively low cost analytical technique; • High specificity and selectivity; • Easy to operate and interpret the data.	• To reach low LOQ's values, as well as LC-UV-Vis, a more elaborate sample preparation step is needed (e.g. using SPE cartridges); • The molecule to be analyzed needs to have fluorescence.	Jiang et al., 2009 Lopes et al., 2010
LC-DAD	• Relatively low cost analytical technique; • Easy to operate and interpret the data; • DAD can scan a wavelength range 190–800 nm region.	• As well as LC-UV-Vis, it features low sensitivity and, consequently, high LOQ. For low LOQ's values, a more elaborate sample preparation step is needed (e.g. using SPE cartridges); • The molecule to be analyzed need to present a chromophore in their structure.	de Alda & Barcelo, 2001 Verbinnen et al., 2010 Almeida & Nogueira, 2015
LC–MS/MS	• Ability of determining several analytes (multiresidue method), without the need for elaborated chromatographic separation; • High specificity, selectivity and sensitivity, allowing to achieve low LOQ's values; • No elaborate sample preparation is needed, making the sample preparation step simpler, faster and easier; • There is no need for derivatization steps; • It is possible to analyze both, unconjugated (i.e. free) and conjugates steroid hormones.	• It is an expensive analytical technique; • Usually there is the suppression or increase of signal caused by the competitive ionization between endogenous molecules present in the matrix to be evaluated and the target analyte, called matrix effect.	Draisci, Palleschi, Ferretti, Lucentini, & Cammarata, 2000 Ferretti et al., 2008 Shao et al., 2005 Xu et al., 2006 Blasco et al., 2007 Kaklamanos et al., 2009 Yang et al., 2009 Wang et al., 2010 Fan et al., 2014 Ingrand et al., 2003 Isobe et al., 2003 Wang et al., 2012 Jakimska et al., 2013 Guedes-Alonso et al., 2017
GC–MS	• High sensitivity and specificity, reaching low LOQ's values; • Good chromatographic resolution.	• There is a needed for derivatization of steroids (using silylation, acylation or alkylation), in order to reduce their polarity and thermal instability, which make the method tedious and time-consuming; • Limits the target compounds to unconjugated (i.e. free) estrogens; • Inability to determine some steroids such as estriol.	Hartmann et al., 1998 Cardoso et al., 1999 Long et al., 2007
ELISA	• High selectivity and specificity; • Available as commercial kits; • Easy to perform the assay.	• It is not possible to simultaneously determine multi-residues of steroids in complex matrices; • Although the kits are commercially available, they are expensive; • False positives and negatives are possible.	Zanardi et al., 2011
RIA	• High selectivity and specificity.	• Radiation hazard: need to use radiolabeled reagent; • Requires specially trained person to handle radioactive material; • Laboratories require special licenses to handle radioactive material.	Specker & Chandlee, 2003 Cardoso et al., 1999

Abbreviations: LC–UV/Vis (liquid chromatography–ultraviolet/visible detector); LC-fluorescence (liquid chromatography-fluorescence detector); LC-DAD (liquid chromatography-diode array detector); LC–MS/MS (liquid chromatography–tandem mass spectrometry detector); GC–MS (gas chromatography–mass spectrometry detector); ELISA (enzyme-linked immunosorbent assay); RIA (radioimmunoassay); LOQ (limit of quantification); SPE (solid phase extraction).

environments (Alda, De, Díaz-Cruz, Petrovica, & Barcelo, 2003; Almeida & Nogueira, 2015; Guedes-Alonso et al., 2014).

Even though several articles have been published on the determination of steroid residues in matrices, such as water, sediments and food of animal origin, the determination of hormone residues in fish matrices have been seldom reported, even though fish farming is an activity of growing economic importance. In Table 5, analytical methods using LC and different detector systems to determine the hormone residues in fishery products are reported. Analysis procedures, including extraction, recovery, detection and quantification limits, and their references for the determination of hormone residues in fishery samples are presented.

Rothbard et al. (1990) were one of the first groups to report hormone residue determination in fish (tilapia muscle). The authors used liquid-solid extraction and obtained low recovery (56.1%) in comparison to that reported later (80.0–94.1%) by Hu, Wang, Chen, Hu, and Li (2010), which used the same detection

Table 5. Analytical method conditions for determination of hormone residues in fishery products by liquid chromatography using different detectors.

Tabla 5. Condiciones de métodos analíticos para la determinación de residuos de hormonas en productos pesqueros mediante cromatografía líquida utilizando diferentes detectores.

Analyte	Matrix	ES	FM	DS	LOD	LOQ	Ref.
ETES	Tilapia muscle	HPLC Bondapak C18 column (7.8 × 300 mm; 10 µm)	H_2O, ACN + 0.1% TFA 1 mL/min and 2 mL/min	UV-vis	50 ng/g	–	Rothbard et al., 1990
EPITES, NAN, METES, PTS, MEPRO, PRO, E1, E2, E3, EE	Fish muscle	Supelco DiscoveryC18 column (150 × 2.1 mm; 5 µm)	ACN, H_2O 0.3 mL/min	MS/MS	0.06–0.22 ng/g	0.12–0.54 ng/g	Xu et al., 2006
E2, E3, DES	Tilapia and prawn	ODS C18 (VP-ODS, 150 × 4.6 mm, 5 µm)	H_2O, MeOH 1.0 mL/min	FLD	0.023 mg/L	0.076 mg/L	Jiang et al., 2009
E1, E2, E3, EE	Fish and shrimp	Diamonsil C18 column (250 × 4.6 mm, 5 µm)	ACN, 0.12% acetate buffer 1.0 mL/min	UV-Vis	0.98–2.39 µg/L	–	Hu et al., 2010
COR, E1, αE2, E3, MEPRO, PRO, 17-HPT, TES, NORTES	Fish muscle	Zorbax Eclipse C18 column (150 × 4.6 mm, 3.5 µm)	ACN, H_2O 0.8 mL/min	MS/MS	0.03–0.15 ng/g	0.11–0.47 ng/g	Wang et al., 2012
PRO, NORG, E1, E2, E3, EE, E1-3S	Fish muscle	Acquity UPLC BEH C18 column (50 × 2.1 mm, 1.7 µm)	MeOH, H_2O (pH 9) 0.4 mL/min	MS/MS	0.002–3.09 ng/g	0.005–9.26 ng/g	Jakimska et al., 2013
PRO, NORG, E1, E2, E3, EE, DES, TES, NAN, PRD, PRDNL, BOL, MGA, NORET, COS	Fish muscle, skin, viscera	Acquity UPLC BEH C18 columm (50 × 2.1 mm, 1.7 µm)	MeOH, H_2O	MS/MS	0.14–49.0 ng/g	3.95 µg/g	Guedes-Alonso et al., 2017

Abbreviations: ES (stationary phase); MP (mobil phase); DS (detection system); LOD (limit of detection); LOQ (limit of quantification); FLD (fluorescence detector); 17-HPT (17α-hydroxyprogesterone); COR (corticosterone); DES (diethylstilbestrol); E1 (estrone); E1-3S (estrone-3-sulfate); E2 (17β-estradiol); E3 (estriol); EE (17α-ethynylestradiol); EPITES (epitestosterone); ETES (17α-ethynyltestosterone); MEPRO (medroxyprogesterone); METES (17α-methyl-testosterone); NAN (nandrolone); NORG (norgestreloulevonorgestrel); NORTES (19-nortestosterone); PRO (progesterone); PTS (testosterone 17-propionate); TES (testosterone; αE2 (17α-estradiol); NORET (norethisterone); MGA (megestrol acetate); BOL (boldenone); COS (cortisone); PRD (prednisone); PRDNL (prednisolone).

technique to determine 4 estrogen residues in fish and shrimp. Hu et al. (2010) used molecularly imprinted polymer (MIP)-coated fiber for the extraction of estrogens in the fishery samples and demonstrated a higher extraction efficiency than the three commercial SPME fibers they had investigated (polydimethylsiloxane, polydimethylsiloxane/divinylbenzene and carbowax/template resin fiber). The MIP-coated SPME fiber was very stable, and could be repeatedly used more than 100 times for standard solutions.

Molecularly imprinted polymer (MIP) is a synthetic polymer with specific binding sites of complementary size, shape, and functional groups to the template molecule, which involves a retention mechanism based on molecular recognition. The advantages of MIP (stable, ease of preparation, low cost, and reusability) have led to its wide application in chromatography, catalysis, chemical sensors, and solid-phase extraction. Jiang et al. (2009) used MIP as an SPE sorbent (MISPE) to selectively extract 17β-estradiol from fish and prawn tissues prior to HPLC analysis because MIP showed affinity for this hormone in acetonitrile solution, which was confirmed by absorption experiments. The MISPE method could eliminate all matrix interference simultaneously, and showed good recoveries (78.3–84.5%).

Xu et al. (2006) using LC–MS/MS reported a recovery in the range of 64–75% and low quantitation limits (0.12–0.54 ng g^{-1}) for 10 anabolic steroids. The authors describe a relatively simple sample preparation procedure using enzymolysis. Next, the sample was extracted with tert-butyl methyl ether and cleaned up through reverse solid-phase extraction.

Dynamic microwave-assisted extraction-salting-out liquid–liquid extraction (DMAE–SLLE) was an effective technique in reducing the sample preparation time and solvent consumption. DMAE has been developed to avoid the degradation or contamination of the analytes. SLLE is a classic homogeneous liquid–liquid extraction method using salt (ammonium sulfate, sodium chloride, ammonium acetate, sodium sulfate, and magnesium sulfate) to induce phase separation. Thus, Wang, Zhang, Pan, and Chen (2010) used DMAE–SLLE and ammonium acetate as a MS friendly salt and could obtain higher recoveries (75.3–95.4%).

Jakimska et al. (2013) selected three extraction methods for comparison based on their applicability in biota samples. The first extraction protocol consisted of pressurized liquid extraction (PLE) followed by gel permeation chromatography (GPC) clean-up. The second extraction method was a PLE extraction followed by a Florisil clean-up. The third approach was based on QuEChER approach, which involved two steps: liquid–liquid partitioning followed by the application of a specific salt, which was used for salting out water from the sample. The clean-up step with dispersive solid phase extraction (dSPE) was tested using four sorbent mixtures ($MgSO_4$ and PSA; $MgSO_4$, PSA and C18; $MgSO_4$, PSA, C18 and GPC; $MgSO_4$, PSA and GPC). PLE with GPC clean-up was considered an inefficient method because only five out of the 19 compounds had recoveries higher than 40%, and PLE with Florisil clean-up allowed the extraction of most of the compounds; however, the estrone metabolite (estrone-3-sulfate) was still not extracted from the matrix. Therefore, the QuEChERS approach allowed for the simultaneous extraction of all target compounds, provided satisfactory recoveries and was chosen as the most efficient procedure for sample preparation. The best results were obtained from the combination of the extraction salt composed of sodium

acetate and $MgSO_4$, and the dSPE sorbent composed of $MgSO_4$, PSA and C18. This combination gave the most satisfactory results since this sorbent mixture is dedicated to samples with high lipid content.

5. Conclusions

The use of hormones in fish farming aiming sex reversal and artificial reproduction generally improve the profitability of the fish industry. However, it is recommended that the use of hormones for the purpose of sexual reversion should be established by the legislative framework of the countries that use this technique, in considering human and environmental safety. The most common hormones for sex reversal in fish are 17β-estradiol and 17α-methyl testosterone, which are natural steroids and therefore rapidly excreted soon after the period of the hormonal treatment. On the other hand, breeding induction treatments vary much more in different fish species. Different compounds have been tested over the years to achieve production of high-quality and high-quantity eggs and fingerlings, especially out of season. However, extreme care must be taken to produce the least possible environmental impact and assure food and human health safety, rendering sustainability in the fish sector.

LC coupled to MS in tandem is one of the best alternatives to determine hormonal residues regardless of the matrix. This allows qualitative analysis on a large number of compounds and quantitative analysis of the residues of these compounds with high precision and sensitivity over a shorter analysis time. Currently, the analytical methods intended for the determination of hormone residues in foods, and fish in particular, are focused on the quantitation of several analyte residues simultaneously (multi-residue determination).

Disclosure statement

No potential conflict of interest was reported by the authors.

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