Journal of Food Composition and Analysis 56 (2017) 140–146

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original research article

Determination of fluoroquinolones in fishes using microwave-assisted extraction combined with ultra-high performance liquid chromatography and fluorescence detection

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ARTICLE INFO

Article history:
Received 7 July 2016
Received in revised form 5 December 2016
Accepted 12 December 2016
Available online 12 December 2016

Chemical compounds studied in this article:
Norfloxacin (PubChem CID:4539)
Ciporfloxacin (PubChem CID:2764)
Enrofloxacin (PubChem CID:71188)
Danofloxacin (PubChem CID:71335)
Sarafloxacin (PubChem CID:56208)

Keywords:
Food analysis
Food composition
Microwave-assisted extraction
Fluoroquinolones
Solid-phase extraction
Ultra-high performance liquid chromatography
UHPLC
MAE
SPE
Gilthead seabream
Fishes

ABSTRACT

A new analytical methodology based on ultra-high performance liquid chromatography with fluorescence detection (UHPLC-FD) has been developed for the determination of five fluoroquinolone antibiotics (FQs) used in aquaculture (norfloxacin, ciprofloxacin, enrofloxacin, danofloxacin and sarafloxacin) in fish muscles (e.g. gilthead seabream (*Sparus aurata*)). The extraction and determination process was carried out by combining microwave-assisted extraction (MAE) along with a clean-up and pre-concentration step with solid phase extraction (SPE) followed by UHPLC-FD detection. The effects of different variables on MAE-SPE were studied and optimized using an experimental design. Validation was made in accordance with Decision 2002/657/EC. CC_α and CC_β were determined according to the maximum residue limit (MRL). The method detection limit (MDL) of the entire process ranged between 0.1 and 6.0 ng g^{-1}. The recoveries obtained at two spiked concentrations levels (25 and 250 ng g^{-1}) were greater than 90%, and the relative standard deviation was less than 8.7%. The developed methodology was successfully applied for the evaluation of the presence of FQs in fish muscle samples from aquaculture farms bought in various commercial shops in Gran Canaria Island (Spain).

1. Introduction

Before aquaculture began, fish species were traditionally reared in lagoons and coastal areas. Over the last few decades, global aquaculture production has grown dramatically (Rabasso and Hernández, 2015). This fact has caused undesirable effects, like ingestion of contaminants or escaped fishes, on ecosystems for feed waste, faeces, medicines and pesticides emissions (Read and Fernandes, 2003).

Aquaculture fish species are susceptible to diseases. The factors that contribute to the morbidity and mortality of farmed fish include stress and the poor water quality as well as disease-causing organism, i.e., bacteria, fungi, parasites and viruses (Banerjee et al., 2014; Cañada-Cañada et al., 2009; Quesada et al., 2013; Shao, 2001).

Antimicrobial compounds are used in aquaculture production with the objectives of inhibiting the growth of microorganisms as well as the treatment and prevention of diseases (Banerjee et al., 2014; Quesada et al., 2013). Their residues may affect the microbial community and also accumulate in the tissue of fishes, causing potential health risks for consumers (Brooks et al., 2005; Mimeault et al., 2005; Schwaiger et al., 2004). Of the drugs approved for

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http://dx.doi.org/10.1016/j.jfca.2016.12.012
0889-1575/

agriculture, antibiotics are among the most widely administered for animal health and management, especially for aquaculture. Administration methods include water treatment, incorporation in feed and direct injection (Shao, 2001).

Fluoroquinolones (FQs) are a group of synthetic antimicrobials developed to prevent and treat infections. Some of them were developed directly for veterinary treatment such enrofloxacin (ENRO) or sarafloxacin (SARA). They are also used for the promotion of growth in fish farms because they are approved for aquaculture treatment. These antibiotics are added directly into the water in fish farms leading to high concentrations in the water and sediment (Rusu et al., 2015). Ciprofloxacin (CIPRO) is one of the most prescribed antibiotics, and it is a major and active metabolite of enrofloxacin in organisms (Martínez-Carballo et al., 2007).

Different Annexes to Council Regulation No 2377/90 include different pharmacologically active substances to protect public health, on the basis of the scientific assessment of the safety of those substances (EEC, 1990). Maximum residue limits (MRLs) of veterinary medicinal products in foodstuffs of animal origin were established for these compounds. As specified in this regulation, MRLs indicate the maximum concentration of a residue resulting from the use of a veterinary medicinal product (expressed in $mg\,kg^{-1}$ or $pg\,kg^{-1}$ on a fresh weight basis) that may be accepted by the Community to be legally permitted or recognized as acceptable in or on a food. The Commission approved administrative MRLs for some FQ traces in animal tissues to 10–100 $\mu g\,kg^{-1}$ in Europe.

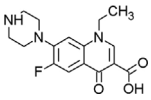
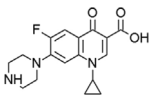
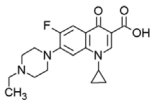
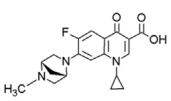
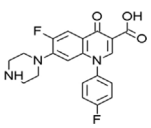
This regulation has been repealed and replaced by the Council Regulation No 470/2009 (EEC, 2009) and, recently, for the regulation No 37/2010 that includes these pharmacologically active substances and their classification regarding their MRLs in foodstuffs of animal origin (EEC, 2010). In this document, ENRO and CIPRO present MRL values of 100 $\mu g\,kg^{-1}$ in muscle, while SARA has a MRL value of 30 $\mu g\,kg^{-1}$ in salmon muscle because some of these are making their way into food chains and reaching the consumer (Tittlemier et al., 2007). These compounds together with norfloxacin (NOR) and danofloxacin (DANO) have been selected as representative members of the FQ family that were chosen for this study. Their structures and characteristics are shown in Table 1.

Different methods for the extraction, preconcentration and determination of FQs have been developed to determine the presence of these compounds in various matrices. In this sense, different FQs have been determined in different environmental samples such as waters (Montesdeoca-Esponda et al., 2012a; Seifrtova et al., 2010; Speltini et al., 2016) and wastewater (Lindberg et al., 2004), soil and sediment (Montesdeoca-Esponda et al., 2012b; Vázquez-Roig et al., 2010) or compost from sewage sludge (Dorival-García et al., 2015) between them.

However, the MRLs established in Europe require sensitive and specific methods to monitor and determine the FQ residues in aquaculture products because these data play an important role in guaranteeing the safety of food. Food-based matrices are very complex, and chromatography combined with mass spectrometry has been one of the most sensitive and selective analytical methodologies used (Dufresne et al., 2007; Hernando et al., 2006; Rezk et al., 2015; Romero-González et al., 2007; Samanidou et al., 2008; Storey et al., 2014). However, LC combined with fluorescence (Kirbis et al., 2005; Zhang et al., 2010) or photodiode array detection (Evaggelopoulou et al., 2014) has also been used to obtain similar results.

In all cases, solvent extraction combined with centrifugation and solid phase extraction (SPE) has been used as a preparation method in liquid samples. The extraction of analytes from solid samples is complicated, especially in environmental applications, because the solute biological matrix interactions are very difficult to predict and overcome (Camel, 2001; Montesdeoca-Esponda et al., 2012b). Conventional extraction methods required a high

Table 1
Selected fluoroquinolones in this work.

Compound	Abbreviation	Identification number	Retention time (min)	Structure	pK_a	log K_{ow}	CAS
Norfloxacin	NOR	1	2.93		6.3	−1.09	70458–96-7
Ciprofloxacin	CIPRO	2	3.40		5.9	0.4	85721–33-1
Enrofloxacin	ENRO	3	3.97		6.3	1.1	93106–60-6
Danofloxacin	DANO	4	4.28		6.04	−0.3	112398–08-0
Sarafloxacin	SARA	5	6.23		6.22	0.3	98105–99-8