Food and Chemical Toxicology 104 (2017) 3–13

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Multi-residue method for the determination of antibiotics and some of their metabolites in seafood

Albert Serra-Compte [a], Diana Álvarez-Muñoz [a], Sara Rodríguez-Mozaz [a, *], Damià Barceló [a, b]

[a] ICRA-Catalan Institute for Water Research, H_2O Building, Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003 Girona, Spain

[b] Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

ARTICLE INFO

Article history:
Received 31 August 2016
Received in revised form
8 November 2016
Accepted 25 November 2016
Available online 28 November 2016

Keywords:
Antibiotics
Multi-residue
Seafood
UHPLC-MS/MS
Microbial growth inhibition test

ABSTRACT

The presence of antibiotics in seafood for human consumption may pose a risk for consumers. A methodology for the analysis of antibiotics in seafood based on QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction, followed by detection and quantification using liquid chromatography coupled to mass spectrometry was developed. The analytical method was evaluated for the determination of 23 antibiotics (including parent compounds and some metabolites) in fish, mussels and clams. Recoveries ranged between 30% and 70% for most of the compounds and method detection and quantification limits (MDLs and MQLs) were between 0.01 and 0.31 ng/g dry weigh (dw) and 0.02–1.03 ng/g (dw) respectively. Real seafood samples were analysed using this method. Nine antibiotics were found at levels above MDLs; however none of them exceed the maximum residue limits (MRL) established by the authorities. Tetracycline was the most ubiquitous compound, presenting also the highest concentration: 5.63 ng/g (dw) in fish from Netherlands. In addition, an alternative technique based on microbial growth inhibition was explored as semiquantitative detection method of antibiotics in seafood. This methodology could be applied as a fast screening technique for the detection of macrolides and β-lactams in seafood but further research is needed for other antibiotics families.

1. Introduction

Antibiotics usage in human and veterinary medicine has become a common therapeutic practice (Manzetti and Ghisi, 2014). This high antibiotic consumption, resulted in a gradual accumulation of antibiotics in the water bodies, being wastewater discharges, agricultural runoff and aquaculture the most important sources of this type of contamination into the environment (Loos et al., 2013; Nödler et al., 2014). It is well known that antibiotics pose a significant risk to environment, even at low concentrations (Kümmerer, 2009). For example antibiotics like bacitracin, flumequine, lincomycin and aminosidine showed to be harmful to aquatic organisms such as *Artemia* (Migliore et al., 1997), or metronidazole which showed a toxic effect to *Chlorella* spp and *Selenastrum capricornutum* (Lanzky and Halting-Sørensen, 1997). In addition, the occurrence of antibiotics in the natural aquatic systems may pose a risk for the wild organisms due to their bioaccumulative potential as for instance roxithormycin that showed a bioaccumulation factor higher than 600 L/Kg in different aquatic organisms (Xie et al., 2015). Furthermore, the bioaccumulation factor of some antibiotics in fish has been reported to be higher than 3000 L/Kg (Gao et al., 2012) in agreement with this, Chen et al. (2014) reported a bioaccumulation factor of 6488 L/Kg for trimethoprim in fish (*Lutjanus russelli*). Residues of these drugs can remain in fish tissues with the consequent potential risk of exposure for fish consumers (Cabello, 2006); especially when antibiotics are accumulated in seafood species highly consumed by the population. The use of antibiotics in food producing animals may provoke undesirable effects on consumer's health. If antibiotics are present at high enough concentrations in food producing animals then they may cause allergies or development of antibiotic resistant bacteria

Abbreviations: ACN, acetonitrile; CAFOs, confined animal feeding operations; dSPE, dispersive solid phase extraction; dw, dry weight; EU, european union; IS, internal standards; MDLs, method detection limits; MQLs, method quantification limits; MRLs, maximum residue limits; QuEChERS, quick, easy, cheap, effective, rugged, and safe; SPE, solid phase extraction; UHPLC-MS/MS, ultra high pressure liquid chromatography- tandem mass spectrometry; US, ultrasonic extraction; ww, wet weight.

* Corresponding author.
E-mail address: srodriguez@icra.cat (S. Rodríguez-Mozaz).

http://dx.doi.org/10.1016/j.fct.2016.11.031
0278-6915/

(Alderman and Hastings, 1998; Cañada-Cañada et al., 2009) causing treatment resistant illness, which can be a human health problem when treating infections (Heuer et al., 2009).

In order to protect human health and avoid the potential risks above mentioned, regulatory authorities like the European Union (EU) establishes Maximum Residue Limits (MRLs) for some pharmaceutical compounds, including antibiotics, in different foodstuffs from animal origin like fish and others seafood species (EU No 37/2010). Seafood for human consumption produced in aquaculture are likely to contain antibiotic residues since many antibiotics are commonly used in confined animal feeding operations (CAFOs) and aquaculture activities in order to treat or prevent bacterial infections (Stolker and Brinkman, 2005). Therefore, information regarding the presence of antibiotics in seafood is crucial for evaluating the fate, environmental effects, and human health risks of these substances. Most of the analytical methods developed so far have focused on one (Samanidou et al., 2008) or few (Evaggelopoulou and Samanidou, 2013) antibiotic families. Moreover, most of them were specific for one organism class like fish (Cháfer-Pericás et al., 2010a) or shrimps (Villar-Pulido et al., 2011). Analytical methods able to detect a broad spectrum of antibiotics are still scarce (Dasenaki and Thomaidis, 2010; Fedorova et al., 2013; Li et al., 2012). The limited number of analytical methods covering the detection of antibiotics belonging to several chemical families may be explained by the difficulty of the simultaneous extraction of antibiotics with different physicochemical properties. The extraction procedure technique and the solvents used are key issues for the simultaneous extraction of different antibiotics. Usually a compromise should be made between the extraction conditions and good performance of the method in terms of recovery, sensitivity, reproducibility, etc. Furthermore, most of the methods developed focus on pharmaceutical compounds administered to humans or animals, but few of them include antibiotics metabolites (Fernandez-Torres et al., 2011). The inclusion of antibiotics metabolites in multi-residue analytical methods is of great interest since they can be accumulated even at higher degree than the antibiotics themselves (Gros et al., 2013), and can be as bioactive or even more than the corresponding parent compound. As example, García-Galán et al. (2012) found that acetylated metabolites of some sulfonamides can be more toxic than the parent compound. According to this paper a risk classification ranked N_4-acetylsulfapyridine metabolite as toxic, whereas its parent compound, sulfapyridine, was classified as harmful (European Commission, 2002). However, other studies suggested that metabolites of antibiotics like sulfonamides may reduce their toxicity in microalgae (Eguchi et al., 2004).

Most of the methods mentioned above for the analysis of antibiotics in seafood are based on detection with LC-MS/MS (i. e. Dasenaki and Thomaidis, 2010; Fedorova et al., 2013). However, alternative detection methodologies like immunoassay techniques or microbial growth inhibition tests have been tested for the analysis of antibiotics in seafood, but its applicability is still scarce. Immunoassays were applied for the detection of oxytetracycline (Cháfer-Pericás et al., 2010c) and sulfonamides (Cháfer-Pericás et al., 2010b) in fish samples. Some of them are commercially available, such as ELISA test kits for the specific detection of antibiotics like tetracyclines, β-lactams or chloramphenicol in seafood and meat (Randoxfood, 2016). A microbial growth inhibition test was applied for the analysis of three antibiotic families including quinolones, sulfonamides and tetracyclines in shrimps (Dang et al., 2010); whereas Barker, (1994) applied this methodology for the specific analysis of quinolones in fish. Some kits based on microbial growth inhibition are also commercially available i.e. PremiTest Antibiotic Test (Nelsonjameson, 2016), which provides a qualitative detection of a broad spectrum of antibiotics. Microbial growth inhibition tests are not as sensitive as LC-MS/MS methods and do not allow to distinguish between individual compounds. This type of test is rather intended as a screening methodology for the preliminary detection of some antibiotic residues and its metabolites with a similar mode of action in different types of food from animal origin. Furthermore, the application of this screening technique does not require the use of complex instrumentation. This would reduce the cost of the analysis and facilitate the implementation of this technique as routine method for the analysis of seafood in laboratories or aquaculture facilities.

The aim of this paper was to develop a fast methodology based on ultra high pressure liquid chromatography-triple quadrupole mass spectrometry (UHPLC-MS/MS) for the detection of antibiotics (from different chemical families), and some of their major metabolites, in several seafood matrices, especially in highly consumed species. Different extraction and clean-up procedures were tested in order to obtain a simple and fast method covering the maximum number of antibiotics possible. The method allowed the detection and identification of 23 individual compounds (including four of their major metabolites). After that, the method was applied for the analysis of real seafood samples of highly consumed species collected from aquaculture and natural environments. In addition, a method based on the inhibition of susceptible bacterium in the presence of antimicrobial residues was tested as an alternative technique for the detection of antibiotic families such as tetracyclines, quinolones, macrolides/β-lactams, amino-glycosides and sulfonamides.

2. Material and methods

2.1. Chemical and reagents

A list with the antibiotics included in the analysis based on UHPLC-MS/MS detection is presented on the supplementary material (Table S1). Antibiotic standards were of high purity grade (>90%). All antibiotic standards were purchased from Sigma-Aldrich except N-acetylsulfadiazine, N-acetylsulfamerazine and N-acetylsulfamethazine that were obtained from Toronto Research Chemicals (TRC), clarithromycin was purchased from Fluka and clindamycin from European Pharmacopeia (EP). Isotopically labelled compounds used as internal standards, azithromycin-d3, ampicilin-d5, erythromyicin-d13, ibuprofen-d3, lincomycin-d3 and sulfamethoxazole-d4 were obtained from TRC whereas ronidazole-d3, ofloxacin-d3 and ciprofloxacin-d8 were purchased from Sigma-Aldrich.

The cartridges used for solid phase extraction OASIS HLB (200 mg, 6 mL), the QuEChERS extract tubes (AOAC method), and the QuEChERS for dispersive solid phase extraction (dSPE) (15 mL, fatty acids tubes) were obtained from Water Corporation (Milford, MA, U.S.A.). PVDF filters (0.45 μm pore) were purchased from Merck Millipore Corporation (Darmstadt, Germany). HPLC grade methanol, water and acetonitrile were purchased from Merck (Darmstadt, Germany), whereas formic acid (98% purity), EDTA 0.01 mol/L, hydrochloric acid 0.1 mol/L and sodium hydroxide 1 mol/L were obtained from Sharlab (Barcelona, Spain).

Stock standards and isotopically labelled internal standards were prepared in methanol at a concentration of 1000 mg/L and stored at −20 °C. Working standard solutions containing all antibiotics and isotopically labelled internal standards (1 mg/L) were prepared in methanol/water (50/50, v/v) before each analytical run.

2.2. Sample collection and pre-treatment

Clams (*Chamelea gallina*) were the organisms selected to perform the different extraction procedures in order to find out