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Analytical Methods

Development and validation of an HPLC method for the determination of six penicillin and three amphenicol antibiotics in gilthead seabream (*Sparus Aurata*) tissue according to the European Union Decision 2002/657/EC

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ABSTRACT

A confirmatory high performance liquid chromatography method for the determination of six penicillin antibiotics and three amphenicol antibiotics in gilthead seabream (*Sparus Aurata*) tissue was developed. Ampicillin (AMP), penicillin G (PG), penicillin V (PV), oxacillin (OXA), cloxacillin (CLO), dicloxacillin (DICLO), thiamphenicol (TAP), florfenicol (FFC) and chloramphenicol (CAP) were separated on an Inertsil, C_8 (250 × 4 mm, 5 μm) column by gradient elution with a mobile phase consisting of ammonium acetate 0.05 M and acetonitrile at 25 °C. Diode array detection with monitoring at 225 nm (for the determination of AMP, PG, PV, TAP and FFC), 240 nm (for OXA, CLO and DICLO) and 278 nm (for CAP) was applied. Examined antibiotics were isolated from gilthead seabream tissue by liquid–liquid extraction and further clean-up was performed by solid phase extraction using Oasis HLB (200 mg/6 mL) cartridges. The developed method was fully validated in terms of selectivity, linearity, accuracy, precision, stability and sensitivity according to the European Union Decision 2002/657/EC.

1. Introduction

Antibiotics are chemical substances produced by microorganisms that either destroy (bactericidal) or inhibit the growth of other microorganisms (bacteriostatic). Antibiotics can be either broad spectrum, active against a wide range of microorganisms or narrow spectrum active against a specific group of microorganisms being able to interfere with a metabolic process specific to those organisms. In general, antibiotics work by: (i) preventing the synthesis of bacterial cell wall components (e.g. penicillins); (ii) damaging the bacterial cytoplasmic membrane; (iii) interfering with protein or nucleic acid synthesis (Samanidou & Evaggelopoulou, 2007; Samanidou, Evaggelopoulou, & Papadoyannis, 2006). In aquaculture, antibiotics have been used mainly for therapeutic purposes and as prophylactic agents. Their extensive administration to fish, destinated for human consumption, has become a serious problem because their residues can persist in edible animal tissues. Antibiotics may be directly toxic or be the source of resistant human pathogens representing a possible risk to human health. They can produce allergic hypersensitivity reactions or toxic effects. For these reasons regulatory agencies have enacted decisions that keep these substances under control (Samanidou & Evaggelopoulou, 2007; Samanidou et al., 2006).

Penicillins are beta (β)-lactam antimicrobial agents used against various organisms by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. The peptidoglycan layer is important for cell wall structural integrity. Broad-spectrum penicillins include ampicillin (AMP), penicillin G (PG), penicillin V (PV), oxacillin (OXA), cloxacillin (CLO) and dicloxacillin (DICLO). Their chemical structures are quite similar as shown in Fig. 1. These are bactericidal in action, effective against Gram-positive and Gram-negative bacteria, but not very effective against *Pseudomonas* (Samanidou & Evaggelopoulou, 2007; Samanidou et al., 2006).

Amphenicols, such as thiamphenicol (TAP), florfenicol (FFC) and chloramphenicol (CAP) are synthetic broad-spectrum antibiotics. Their use in food animals is illegal in most countries. Their chemical structures are shown in Fig. 2.

Chloramphenicol acts primarily by binding reversibly to the 50S ribosomal subunit and also can inhibit mitochondrial protein synthesis in mammalian cells. Additionally, chloramphenicol is a broad-spectrum antibiotic exhibiting activity against both Gram-positive and Gram-negative bacteria as well as other groups of micro-organisms. It exerts its action through protein inhibition and is effective in the treatment of several infectious diseases. This, together with its low cost and ready availability, has made it extensively used since the 1950s in the treatment of food-producing fish all over the world. However, CAP is, in certain susceptible individuals, associated with serious toxic effects in humans in the form of bone marrow depression, particularly severe in the form of fatal

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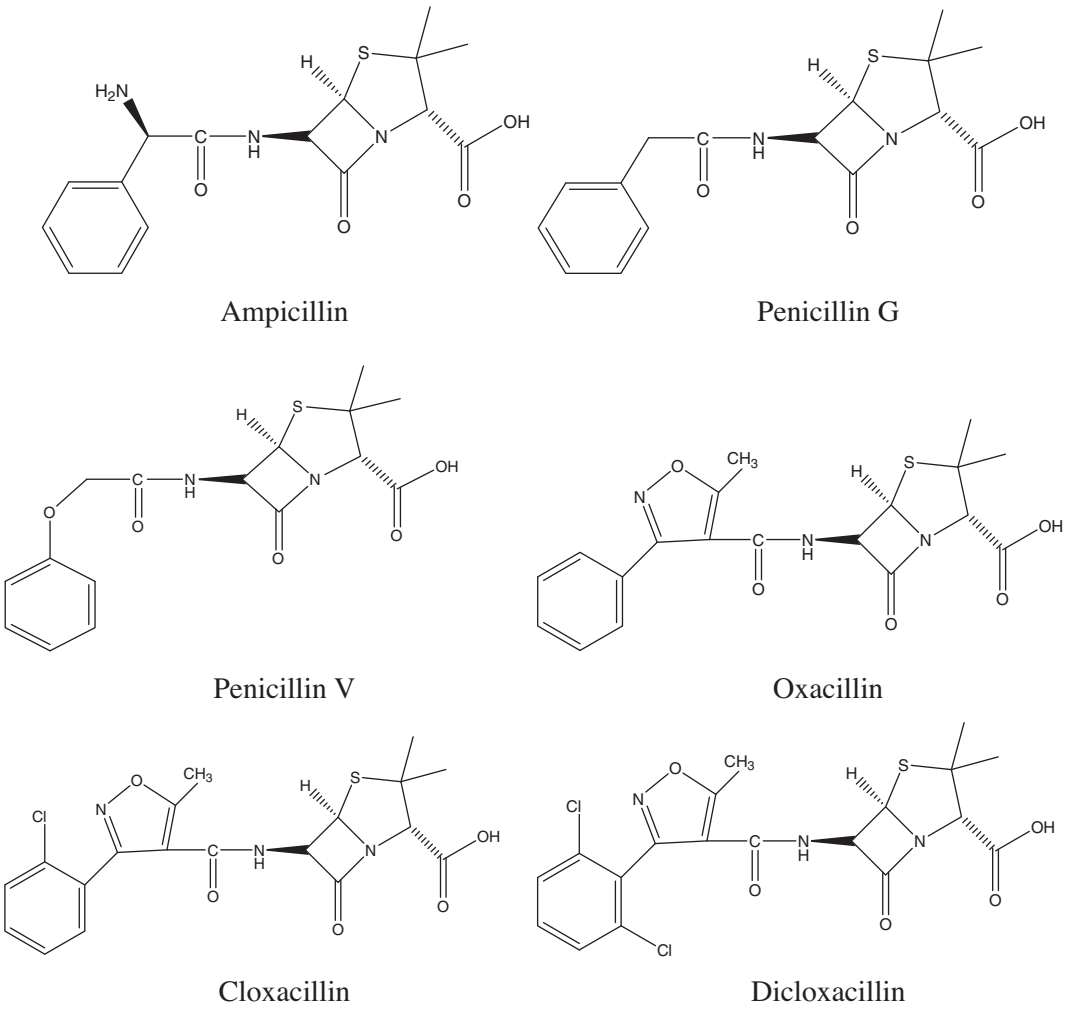

Fig. 1. Chemical structures of ampicillin, penicillin G, penicillin V, oxacilllin, cloxacillin and dicloxacillin.

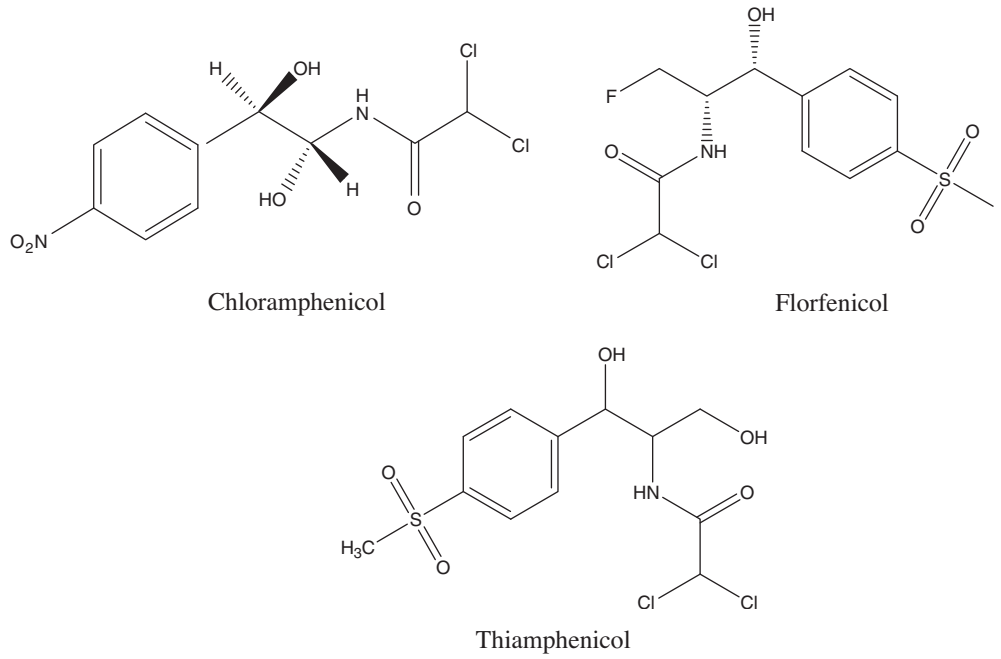

Fig. 2. Chemical structures of chloramphenicol, thiamphenicol and florfenicol.