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Analytical Methods

Multiresidue determination of tetracyclines, sulphonamides and chloramphenicol in bovine milk using HPLC-DAD

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ABSTRACT

A high-performance liquid chromatographic method for the simultaneous determination of tetracycline, oxytetracycline, chlortetracycline, sulfamethazine, sulfaquinoxaline, sulfamethoxazole and chloramphenicol in milk has been developed. The determination of these antimicrobials was carried out using HPLC-DAD with a C_{18} hybrid column and gradient elution with a mobile phase composed of an aqueous phase of 0.075 mol L^{-1} sodium acetate, 0.035 mol L^{-1} calcium chloride and 0.025 mol L^{-1}sodium EDTA, pH 7 and an organic phase of methanol:acetonitrile, 75:25 v/v. Sample preparation involved protein precipitation followed by solid-phase extraction using a polymeric cartridge. The method was validated and applied for the analysis of pasteurised milk samples commercialised in Brazil. The limits of quantitation for all antimicrobials, with the exception of choramphenicol, were below the maximum residue limit, which indicates that the method is appropriate for the determination of these antimicrobials in milk.

1. Introduction

Antimicrobial agents are widely used in food of animal origin for therapy to treat diseases, to control and prevent infection and for growth promotion and production efficiency.

The presence of residues of antimicrobial agents in food of animal origin in excess of the established maximum residue limits (MRLs) indicates that good veterinary practices were not respected and may contribute to the generation of resistance to bacteria in humans; nevertheless, current evidence suggests that the risk is low. In milk, however, their presence may cause allergic reactions in sensitive individuals and may interfere with starter cultures for cheese and other dairy products (Schenk & Callery, 1998).

The sulphonamides, among them, sulfamethazine (SMZ), sulfaquinoxaline (SQX) and sulfamethoxazole (SMX) play an important role as effective chemotherapeutics of bacterial and protozoal diseases in veterinary medicine. The Committee for Veterinary Medicinal Products considers that a tissue MRL of 100 μg kg^{-1} of the original drug substance should be applied to all substances of the sulphonamide group. In addition, the combined residues of all sulphonamides in bovine milk should not exceed 100 μg kg^{-1} (EMEA, 2007).

The Joint FAO/WHO Expert Committee of Food Additives and Contaminants (JECFA), at its 50th Meeting in 1998, established a group acceptable daily intake (ADI) of 0–0.03 mg kg^{-1} body weight for the tetracyclines (oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC)), alone or in combination. The committee also recommended MRLs in milk of 100 μg L^{-1}. Tetracyclines, in particular chlortetracycline, have been routinely employed to prevent and treat mastitis in lactating dairy cows (JECFA, 1998).

Chloramphenicol (CLP) is a broad-spectrum antibiotic that is an effective therapeutic agent for the treatment of mastitis in cattle. Historically, epidemiological data suggested that the treatment of humans with chloramphenicol was associated with induction of blood dyscrasias. Due to the potential risk to human health posed by its residues in food the use of CLP is prohibited in food-producing animals destined for the human consumption in many countries, among them, the USA, Australia, Europe and Brazil. Due to the lack of toxicological data available, no ADI was established for CLP and, in consequence, no MRL could be attributed (JECFA, 2004).

In many regions of the world antimicrobial drug residues are known to be present in food (Ekuttan, Kang'ethe, Kimani, & Randolph, 2007; Helena, Zdenka, Ksenija, Andrej, & Vesna, 2007; Husam et al., 2007). Lack of a control can be attributed to the costs of analyses. In many instances this is beyond the reach of most low income countries. Helena et al. (2007), reported the presence of CLP in some samples of raw milk taken in the autumn of 2002 in Slovenia. Studies conducted in Kenya showed samples of milk to contain tetracyclines at levels exceeding the established MRL (Ekuttan, Kang'ethe, Kimani, & Randolph, 2007).

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The governmental institution responsible for public health in Brazil (ANVISA, National Agency of Sanitary Surveillance) reported for the period 2004/2005 that 7% of the milk samples analyzed (312) showed to be positive in the screening test for the presence of tetracyclines, however none of them was confirmed by a chromatographic method. In addition, 7% of 306 milk samples analyzed indicated the presence of CLP, however the confirmatory test was not implemented at this time (ANVISA, 2006).

Thus, there is a need for countries to establish surveillance systems that allow for obtaining reliable data on veterinary antimicrobial usage. Therefore, it is necessary to establish simple, sensible and reliable analytical methods for the determination of multiresidues of different classes of antimicrobials in food and, in particular in milk.

The structures of the sulphonamides, tetracyclines and chloramphenicol used in veterinary medicine and evaluated in this work are shown in Fig. 1.

While rapid screening methods (immunological or microbial inhibition assays) are commonly used to detect the presence of antimicrobials in food, more accurate chromatographic methods are required by the governmental regulatory agencies to identify and confirm the presence of these compounds. Milk is consumed all over the world and its quality control is important to guarantee food safety.

Chromatographic methods require elaborate sample preparation procedures before quantitation in order to eliminate interferents from the food matrix and concentrate the analyte. The extent of sample preparation depends on the detection device of the chromatographic system. Conventional sample treatment protocols involve protein precipitation, centrifugation and analyte extraction, followed by clean-up of the extract over solid-phase cartridges and eluate concentration under a nitrogen flow. Alternatively, liquid–liquid extraction, combined or not with solid-phase extraction and matrix solid-phase extraction has been employed (Andersen et al., 2005; Bogialli et al., 2005; Furusawa, 2003; Schenk & Callery, 1998).

Many liquid chromatographic methods have been published for the determination of tetracyclines (Oka, Ito, & Matsumoto, 2000; Andersen et al., 2005), sulphonamides (Furusawa, 2000; Van Rhijn, Lasaroms, Berendsen, & Brinkman, 2002) and chloramphenicol (Long et al., 1999; Perez et al., 2002) in milk. However, almost all of them aim at the determination of antimicrobials of a single group. Simultaneous determination of multiresidues of antimicrobials of different groups has been carried out using liquid chromatography coupled to tandem mass spectrometry (Koesukwiwat, Jayanta, & Leepipatpiboon, 2007). Samanidou and Nisyriou (2008) presented a review of the state of the art in the analytical strategies concerning the multiresidue analysis of antibiotics in milk.

This paper focuses on the development and validation of a simple HPLC-DAD method for the simultaneous multiresidue determination of TC, CTC, OTC and SMZ, SQX, SMX and CLP in milk which could be applied to quality control in the routine analysis.

2. Materials and methods

2.1. Equipment

The HPLC-DAD measurements were carried out using a binary gradient chromatographic system from Waters (USA), model 1525, coupled to a Waters photodiode array detector (PDA) model 2996 and a Rheodyne injector, model 7725 (sample loop of 50 μL). Data acquisitions were performed by the Millenium^{32} 4.0 software.

For the solid-phase extraction procedures a 12 port vacuum manifold from Alltech (USA) was employed.

Measurements of pH were made with a DM-20 pH-meter from Digimed (Brazil), using a combined glass electrode.

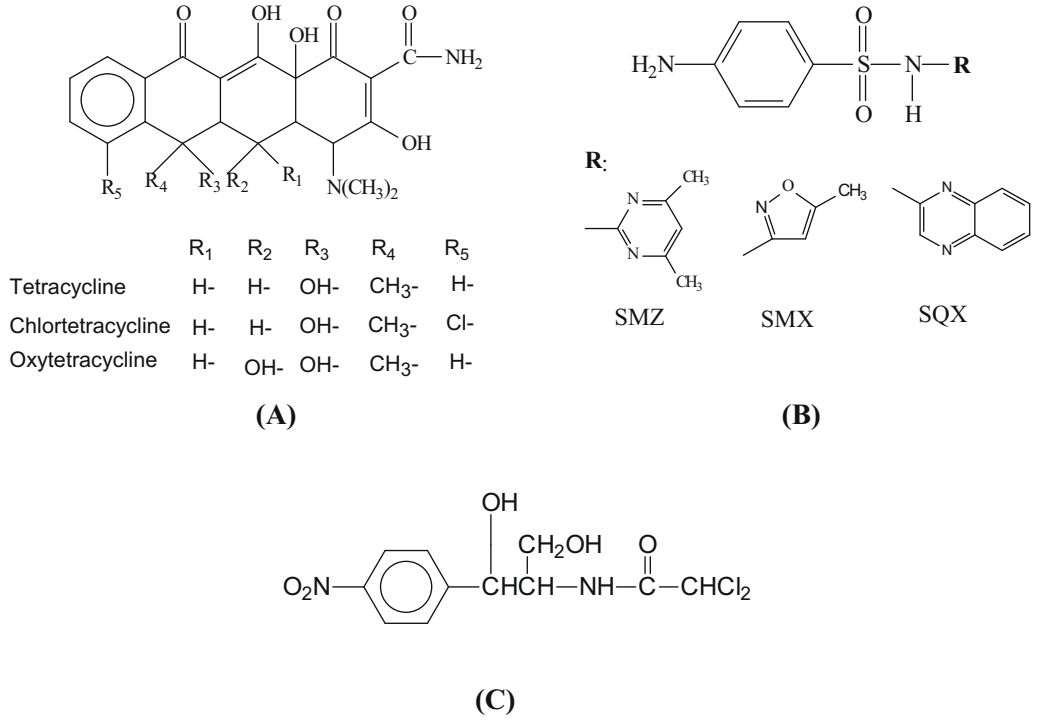

Fig. 1. Structures of (A) tetracyclines, (B) sulphonamides and (C) chloramphenicol.