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Analytical procedure for the determination of chlortetracycline and 4-epi-chlortetracycline in pig kidneys

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Abstract

A liquid chromatography-diode array detection (LC-DAD) procedure has been developed for assaying chlortetracycline (CTC) and its 4-epimer (4-epi-CTC) residues in pig kidneys. The procedure involved extraction with 0.1 M oxalic buffer followed by protein precipitation with trichloroacetic acid. Further solid-phase extraction (SPE) clean-up on a Strata X polymeric cartridge was allowed to obtain an extract suitable for LC analysis. Chromatographic separation was carried out on a C_8 analytical column, using isocratic elution with methanol–acetonitrile–0.01 M oxalic acid (15:15:70, v/v/v) at ambient temperature. The flow-rate was 1.2 ml/min and the eluate was analysed at 365 nm. The whole procedure was evaluated according to the requirements of the European Union regulation 2002/657/EC determining specificity, decision limit (CCα), detection capacity (CCβ), trueness, precision and robustness during validation process. The decision limit (CCα) was 674.8 μg/kg for CTC and 683.6 μg/kg for 4-epi-CTC. The detection capacity (CCβ) was 683.6 and 696.3 μg/kg for CTC and 4-epi-CTC, respectively. The recoveries of CTC and 4-epi-CTC from spiked samples at the levels of 300, 600 and 900 μg/kg (0.5 × MRL, 1 × MRL and 1.5 × MRL) were higher than 70%. This method has higher throughput than reported previously extraction method with oxalic acid and acetonitrile used for dechelation and deproteinization.

Keywords: Chlortetracycline; 4-epi-Chlortetracycline; Residues; Kidneys

1. Introduction

Chlortetracycline (CTC), the first member of the tetracycline family, is a broad-spectrum antibiotic widely used in veterinary practice for the treatment of bacterial infections [1,2]. CTC is active against aerobic and anaerobic Gram-positive and Gram-negative bacteria as well against some microorganisms that are resistant to cell-wall-inhibitor antibacterial agents. CTC is actively transported into the cells of susceptible bacteria and acts by inhibiting protein biosynthesis after binding to the 30S ribosome subparticle. CTC is licensed for the treatment of respiratory and systemic infections in pigs, poultry and other farm animals [3,4].

Because of common use, residual CTC may occur in tissues collected from slaughtered animals if the adequate withdrawal time has not been observed [5,6]. Therefore, edible tissue animal origin may be a potential hazard for the consumers. To protect consumers' health, the European Community (EC) legislation on veterinary drug residue has laid down maximum residue limits (MRL) for CTC, including its 4-epimer (4-epi-CTC) at 600 μg/kg in kidney, at 300 μg/kg in liver (and in skin + fat) and at 100 μg/kg in muscle, for all food-animal producing animals [5,6].

Under mildly acidic (pH 2–6) conditions, CTC reversibly epimerises to 4-epi-chlortetracycline (4-epi-CTC) which is an antibacterially inactive compound (Fig. 1). This requires the development of methods that are capable in separating CTC and 4-epi-CTC in tissues of animal origin.

A number of analytical procedures are currently available for the determination of CTC and 4-epi-CTC residues in animal tissues. A comprehensive review of these methods is included in article on the chromatographic analysis of tetracycline antibiotics in food [7]. Generally, analytical method described in literature show an extraction step with mild acid (pH 2–4) solvents followed by solid-phase extraction (SPE)

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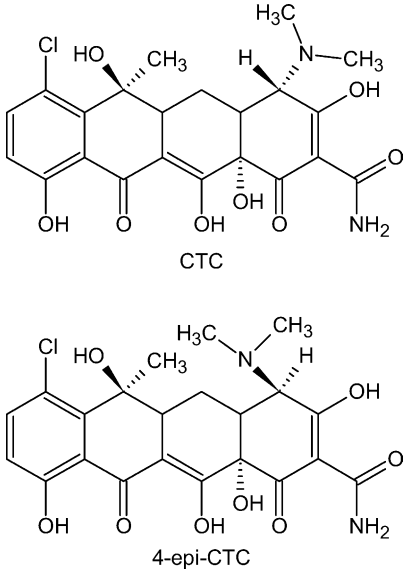

Fig. 1. Structure of CTC and 4-epi-CTC.

or metal chelate affinity chromatography (MCAC) as clean-up procedures [8–21]. However, some these procedures can be laborious or the use of some type disposable cartridges can lead to poor and inconsistent recoveries. The recovery variability problem has also been reported by other authors [10]. It is therefore recommended to use of SPE cartridges and LC analytical columns containing a high purity silica or polymer materials.

The method, routinely used in our laboratory [20], prepared for assaying parent tetracycline agents in animal tissues. However, it was not assaying the suitability to the simultaneous determination of CTC and 4-epi-CTC in pig kidney.

This paper reports improvement of sample preparation approach by using the combination of oxalic buffer and trichloroacetic acid (TCA) solution for extraction step followed by suitable SPE cartridges, resulting in further increases in throughput similar to those achievable by other analytical protocols described for the determination of CTC and its 4-epimer [18,21,22]. The whole procedure was validated according to the requirements of the European Union regulation 2002/657/EC [23].

2. Experimental

2.1. Reagents and materials

Acetonitrile, methanol (HPLC grade), and oxalic acid were purchased from J.T. Baker (Deventer, the Netherlands). Sodium oxalate and trichloroacetic acid were obtained from (P.O.Ch., Gliwice, Poland). Deionised water was purified by a Mili-Qplus system from Millipore (Bedford, MA, USA). Analytical standards of CTC and 4-epi-CTC were supplied by Sigma (Poole, UK). The following SPE disposable cartridges were tested: Bakerbond octadecyl (C_{18}) 500 mg (catalogue No. 7020-03), Bakerbond octyl (C_8) 500 mg (7087-03), Bakerbond styrene-divinylbenzene (SDB1) (7519-02) from J.T. Baker (Devender, the Netherlands). Oasis HLB (Part No. 186000115) from Waters (Milford, MA, USA), and Strata X (8B-S100-ECH) from Phenomenex (Torrance, CA, USA).

2.2. Buffer and standard solutions

A pH 3.5 oxalic buffer (1000 ml) was prepared by combine 500 ml of 0.1 M sodium oxalate with 500 ml of 0.1 M oxalic acid. The solution was filtered through a 0.45 μm PTFE filter from Milipore (Bedford, MA, USA).

Individual stock solutions of CTC and 4-epi-CTC (1 mg/ml) were prepared by dissolving 10 mg of the analytical standards in 10 ml of methanol and stored in the dark at −20 °C (stable for at least 6 months). Combined stock solution (100 μg/ml) was prepared by diluting 1 ml of each individual stock solution with 8 ml of methanol and stored in the dark at 4 °C (stable for at least 3 month). Working standard aqueous solutions of the analytes were prepared by serial dilution of the combined stock solution with oxalic buffer at pH 3.5 and stored in the dark at 4 °C (stable for at least 2 weeks).

2.3. Sample preparation equipment

Centrifugation of the samples was performed in a Verifuge 3.0R a refrigerated centrifuge (Heraus, Germany). A vortex stirrer type IKA (IKA, Labortechnik, Stanfen, Germany) was used to mix samples during treatment. The pH of the buffer solution was adjusted with 780 pH Meter (Metrohm, Switzerland). Evaporation under nitrogen was conducted in a Reacti-Vap evaporator from Pierce (Rockfold, IL, USA). A Polytron, Kinematica (Littau-Luze, Switzerland) was used to homogenisation of the kidney samples with extraction solutions. A SPE 12G vacuum manifold (J.T. Baker, Devender, the Netherlands) was used for solid-phase extraction clean up.

2.4. Blank samples

Kidney samples were obtained from the healthy adult pigs that were not treated with any antibacterial compounds within the previous 4 weeks. The samples were minced, accurately weighed (2.5 g wet mass) and deep-frozen at −20 °C until the time of analysis.

2.5. Spiking procedure

In order to investigate the spiking solutions and time exposition on extraction procedure, the CTC and 4-epi-CTC working standard solutions were prepared in water, oxalic buffer and methanol, respectively. The samples were spiked with working standards at the concentrations corresponding to level of 600 μg/kg and were kept in the dark at ambient temperature. The samples were analysed as follow: (a) directly, (b) 15 min after spiking, and (c) 60 min after spiking.