Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 506–514
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

C_{18} columns for the simultaneous determination of oxytetracycline and its related substances by reversed-phase high performance liquid chromatography and UV detection

C.G. Smyrniotakis, Helen A. Archontaki *

Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Athens 15771, Greece

Received 1 March 2006; received in revised form 23 July 2006; accepted 27 July 2006
Available online 20 September 2006

Abstract

Simultaneous determination of oxytetracycline, 4-epioxytetracycline, α-apooxytetracycline, tetracycline and β-apooxytetracycline on C_{18} columns has been accomplished using a high performance liquid chromatographic method with UV detection. Separation was achieved on a Hypersil BDS RP-C_{18} column (250 mm × 4.6 mm) and on a Waters C_{18} Symmetry column (150 mm × 3.9 mm), 5 μm particle size each. These columns were equilibrated with mobile phases consisted of methanol–acetonitrile–0.1 M phosphate buffer pH 8.0 (12.5:12.5:75, v/v/v) and (15:15:70, v/v/v), respectively. The flow rate was 1.0 ml/min and the total elution time was 15 and 5 min, respectively. Both methods were applied to oxytetracycline raw material, human and veterinary formulations, where the excipients did not interfere. External standard calibration curves were linear for 4-epioxytetracycline, oxytetracycline, α-apooxytetracycline, tetracycline and β-apooxytetracycline in the concentration range of 0.27–200 μM, 0.05–200 μM, 0.03–200 μM, 0.35–200 μM and 0.20–200 μM on column A and 0.08–200 μM, 0.15–200 μM, 0.09–200 μM, 0.25–200 μM and 0.47–200 μM on column B, respectively. Day-to-day relative standard deviation of the determination for every component was less than 3%. Concerning the first column, limits of detection and quantification of the above compounds were in the concentration ranges of 10–106 nM and 30–352 nM, respectively, whereas on the second column these ranges became 27–144 nM and 81–475 nM, respectively. Recovery of the separated compounds was 95–105%.

Keywords: Oxytetracycline; Related substances; C_{18} columns; Reversed-phase HPLC and UV detection; 4-Epioxytetracycline; Tetracycline; α-Apooxytetracycline and β-apooxytetracycline

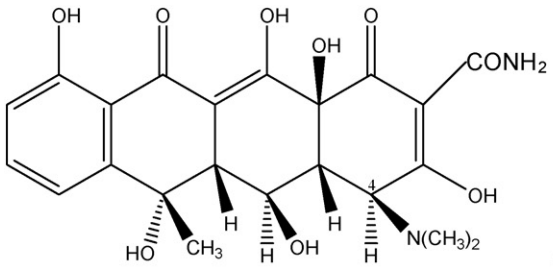
1. Introduction

Oxytetracycline (OTC) is a broad-spectrum antibiotic that is commonly used in veterinary medicine as inhibits the protein synthesis in gram-positive and gram-negative bacteria. The European community has approved the use of OTC in a wide range of animal species like cattle, sheep, goats and pigs. The major dosage forms of OTC, which are available for animal health are feed premixes, injectables, sprays, soluble powders and tablets. On the other hand, OTC is used for human treatment alone or in combination with hydrocortisone and polymyxin B in preparations for oral use, use on the skin and in the eye. However, oxytetracycline (Fig. 1) and oxytetracycline hydrochloride may contain several impurities that should not exceed certain levels in the raw material according to Pharmacopoeia [1].

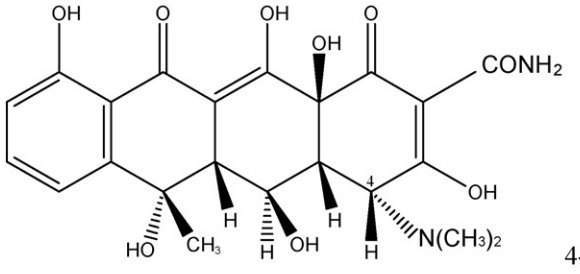
Although high-performance liquid chromatography (HPLC) on tetracyclines has been studied extensively, few articles have reported on the simultaneous separation of oxytetracycline, its degradation products and related substances, which may be formed during fermentation. The existing official method according to European and USP Pharmacopoeias [1,2] which is based on Khan's et al. work [3] includes an ion-pair RP-HPLC method at 60 °C, a complicated mobile phase, a copolymeric chromatographic column, which results in very wide peaks and a gradient elution with total elution time around 30 min. Moreover, Reenwijk et al. [4] have claimed that copolymeric columns are the only ones appropriate for such separations since silica-based materials present poor chromatographic efficiency and an apparently irreversible adsorption of tetracyclines.

* Corresponding author. Tel.: +30 210 7274756; fax: +30 210 7274750.
E-mail address: archontaki@chem.uoa.gr (H.A. Archontaki).

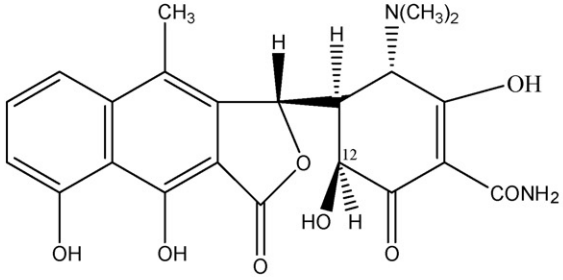
0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.07.048



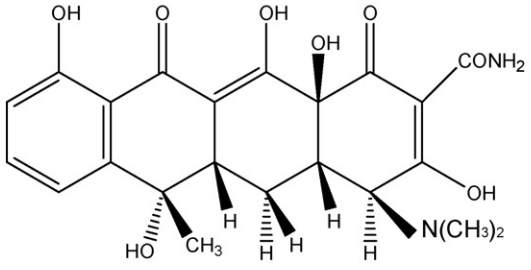
oxytetracycline



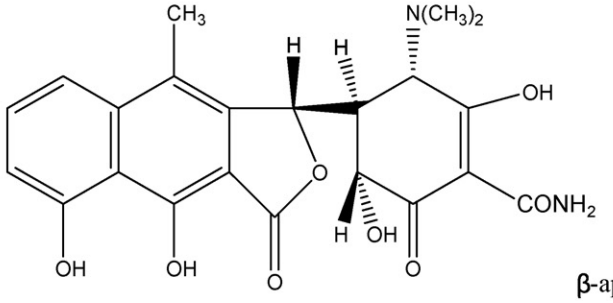
4-epioxytetracycline



a-apooxytetracycline



tetracycline



β-apooxytetracycline

Fig. 1. Structures of oxytetracycline, 4-epioxytetracycline, α-apooxytetracycline, tetracycline and β-apooxytetracycline.