Journal of Pharmaceutical and Biomedical Analysis 75 (2013) 199–206

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Development and validation of a reversed phase liquid chromatographic method for analysis of oxytetracycline and related impurities

Getu Kahsay[a], Fairouz Shraim[a], Philippe Villatte[b], Jacques Rotger[c], Céline Cassus-Coussère[c], Ann Van Schepdael[a], Jos Hoogmartens[a], Erwin Adams[a,*]

[a] Laboratorium voor Farmaceutische Analyse, Faculteit Farmaceutische Wetenschappen, Katholieke Universiteit Leuven, O&N2, PB-923, Herestraat 49, B-3000 Leuven, Belgium
[b] Sanofi, Global Quality, 20 Avenue Raymond Aron, 92165 Antony Cedex, France
[c] Sanofi, Laboratory of Analytical Development Chemistry, Route d'Avignon, 30390 Aramon, France

ARTICLE INFO

Article history:
Received 29 October 2012
Received in revised form
26 November 2012
Accepted 27 November 2012
Available online 5 December 2012

Keywords:
Oxytetracycline
Analysis
Impurities
Method development
Reversed phase liquid chromatography

ABSTRACT

A simple, robust and fast high-performance liquid chromatographic method is described for the analysis of oxytetracycline and its related impurities. The principal peak and impurities are all baseline separated in 20 min using an Inertsil C_8 (150 mm × 4.6 mm, 5 μm) column kept at 50 °C. The mobile phase consists of a gradient mixture of mobile phases A (0.05% trifluoroacetic acid in water) and B (acetonitrile–methanol–tetrahydrofuran, 80:15:5, v/v/v) pumped at a flow rate of 1.3 ml/min. UV detection was performed at 254 nm. The developed method was validated for its robustness, sensitivity, precision and linearity in the range from limit of quantification (LOQ) to 120%. The limits of detection (LOD) and LOQ were found to be 0.08 μg/ml and 0.32 μg/ml, respectively. This method allows the separation of oxytetracycline from all known and 5 unknown impurities, which is better than previously reported in the literature. Moreover, the simple mobile phase composition devoid of non-volatile buffers made the method suitable to interface with mass spectrometry for further characterization of unknown impurities. The developed method has been applied for determination of related substances in oxytetracycline bulk samples available from four manufacturers. The validation results demonstrate that the method is reliable for quantification of oxytetracycline and its impurities.

1. Introduction

Tetracyclines (TCs) are broad-spectrum antibiotics that are active against both Gram-positive and Gram-negative bacteria. They are widely used for the treatment of infectious diseases in human and veterinary medicine, but also as additives in animal feeds to promote growth [1–4]. Oxytetracycline (OTC) is produced through fermentation by *Streptomyces rimosus* [5] and is being widely used, e.g. in farm animals to control intestinal and respiratory infections [6].

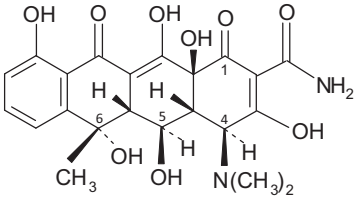
In aqueous solutions OTC epimerizes at position C-4 resulting in the formation of 4-epioxytetracycline (4-EOTC) [7]. Due to the presence of a secondary hydroxyl group at C-6, OTC is liable to acid degradation and forming the anhydrooxytetracycline derivative (AOTC). AOTC and its epimer EAOTC are quite unstable in acidic aqueous solutions due to the hydroxyl group at C-5 resulting in the scission of the ring and producing two aromatic isomers, α-apooxytetracycline (α-APOTC) and β-apooxytetracycline (β-APOTC) [8]. 2-Acetyl-2-decarboxamidooxytetracycline (2-ADOTC) and tetracycline (TC) are fermentation impurities of OTC [9,10]. 4-Epitetracycline (ETC) can be present due to epimerization of TC. The chemical structures of OTC and its related substances are shown in Fig. 1.

Khan et al. [9] described an LC method using a polymer column for the analysis of OTC and related substances. This method also served as basis for the related substances test in the current OTC monograph (OTC dihydrate and OTC hydrochloride) of the European Pharmacopoeia (Ph. Eur.) [11]. However, it is known that polymer columns give rather broad peaks and do not yield high efficiency. Using this method it is not possible to completely separate 2-ADOTC (impurity C) from OTC making quantification of this impurity difficult. The LC method presented by Diana et al. was able to separate most of the known impurities but in two separate isocratic runs, each taking 25 min [12]. LC–MS and LC–UV [13–15] methods were also reported for the analysis of OTC and its impurities. None of the above methods was able to separate all of the identified and known impurities of OTC using a simple mobile phase in a relatively short analysis time. Capillary electrophoresis (CE) methods [16,17] were also reported for the separation of OTC and its related substances. Separation of OTC and its impurities was satisfactory using the CE method described by Li et al. method [17].

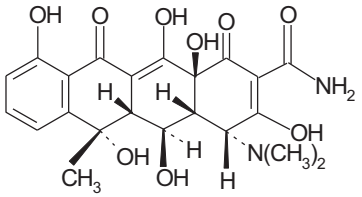
The present work describes a fast, simple and robust LC–UV method for analysis of OTC and its related impurities. The simple

* Corresponding author. Tel.: +32 16323444; fax: +32 16323448.
E-mail address: Erwin.Adams@pharm.kuleuven.be (E. Adams).

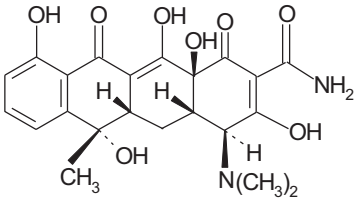
0731-7085/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jpba.2012.11.042



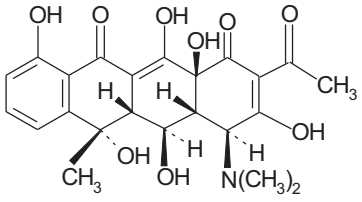
Oxytetracycline (OTC)



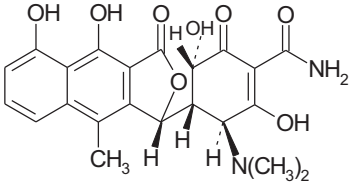
4-Epioxytetracycline (4-EOTC), Imp. A*



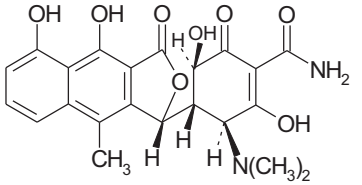
Tetracycline (TC), Imp. B*



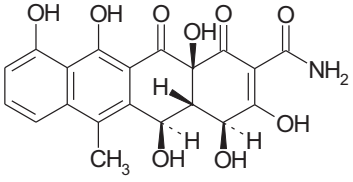
2-Acetyl-2-decarboxamidooxytetracycline (2-ADOTC), Imp. C*



α-Apo-oxytetracycline (α-APOTC), Imp. D*



β-Apo-oxytetracycline (β -APOTC), Imp. E*



Anhydro-oxytetracycline (AOTC), Imp. F*

Fig. 1. Chemical structure of OTC and related impurities. *Nomenclature according to the Ph. Eur.

mobile phase composition devoid of non-volatile constituents makes the method suitable for LC–MS.

2. Experimental

2.1. Chemicals and reagents

HPLC gradient grade acetonitrile (ACN) was procured from Fisher Scientific (Leicestershire, UK), HPLC grade methanol (MeOH) was obtained from Biosolve Ltd. (Valkenswaard, The Netherlands), trifluoroacetic acid (TFA, 99%) was obtained from Acros Organics (Geel, Belgium), stabilized tetrahydrofuran (THF, 99.8%) was obtained from Merck (Darmstadt, Germany) and hydrochloric acid (HCl) was obtained from J.T. Baker (Mallinckrodt, The Netherlands). A Milli-Q water purification system from Millipore-Bedford (MA, USA) was used to purify further the demineralized water.

2.2. Samples and reference substances

OTC hydrochloride bulk samples, OTC dihydrate bulk samples and OTC chemical reference substances (CRS) used in this study were obtained from the European Directorate for the Quality of Medicines and HealthCare (EDQM), Strasbourg, France. House standards of 4-EOTC, TC, 2-ADOTC, AOTC, α-APOTC and β-APOTC were available in the laboratory. These six compounds are mentioned as impurities in the respective Ph. Eur. monographs. Solutions were prepared by dissolving the samples in a 0.01 M HCl solution, hereafter named the solvent. A freshly prepared 0.8 mg/ml test solution was used for the determination of related substances. A 0.5% dilution of this solution was used as reference for the quantification of impurities. For method optimization, precision and robustness studies a 0.8 mg/ml (100%) solution of OTC spiked with 0.2–1% of the available impurities was prepared using the same solvent. For linearity a series of 7 solutions were prepared in the concentration range from 0.32 μg/ml to 0.96 mg/ml, corresponding to the LOQ and 120%, respectively. Each solution was injected in triplicate.

2.3. Instrumentation and chromatographic conditions

2.3.1. Chromatography

LC analyses were performed on an apparatus from Dionex Softron GmbH (Germering, Germany) equipped with a high-pressure pump (P680ALPG), autosampler (ASI-100T) and UV/vis photodiode array detector (UVD170U). For data processing and acquisition, Chromeleon software version 6.80 from Dionex (Sunnyvale, CA, USA) was used. An ultrasonicator from Branson Ultrasonics Corporation (Dabury, CT, USA) and a pH meter from Metrohm (Herisau, Switzerland) were used.

Chromatographic separations were achieved on an Inertsil C_8 (150 mm × 4.6 mm, 5 μm) column from Interchim (Montluçon