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A Critical Review of Properties and Analytical Methods for the Determination of Oxytetracyline in Biological and Pharmaceutical Matrices

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ABSTRACT

Antibiotics have an unquestionable importance in the treatment of many infections. Oxytetracycline is an antibiotic belonging to the class of tetracyclines, available for use in human and veterinary medicine. Development of analytical methods that prove the quality and efficacy of these drugs is fundamentally important to the pharmaceutical industry. In this context, the research presents an overview of the analytical profile of oxytetracycline, describing its chemical and pharmacological properties, and analytical methods for quantification of this drug in biological samples and pharmaceutical products. Oxytetracycline can be analyzed in these matrices by many types of methodologies. However, high-performance liquid chromatography is the most widely used, being recommended by official compendia. This kind of study can be useful to support the development of new efficient and sustainable analytical methods that may be utilized in the quality control routine of oxytetracycline in pharmaceutical products and pharmacokinetic monitoring in biological samples.

KEYWORDS

Analytical methods; liquid chromatography; oxytetracycline; pharmacological properties; quality control

Introduction

Production and marketing of drugs are complex processes that result in exorbitant profits to the pharmaceutical industry, an industry that is continuously seeking innovations in order to stand out in the market and obtain customer satisfaction and trust. Quality control associated with good production practices is the most important step in the pharmaceutical manufacturing, since this step uses sensitive, precise, accurate, and robust analytical methods to ensure both effectiveness and safety of these products (Rocha and Galende, 2014).

Antimicrobials are one of the most important drug classes in the pharmaceutical industry, generating approximately US$8.5 billion per year, worldwide (Petković et al., 2006). Between 2000 and 2010, consumption of antibiotic drugs increased by 36%. The five rapidly growing countries (Brazil, Russia, India, China, and South Africa) known as the BRICS had the greatest upsurge in antibiotic use accounting for 76% of this increase. Consumption of antibiotic in the BRICS is expected to double by 2030. The countries consuming the most antibiotics overall in 2010 were India (US$13 billion), China (US$10 billion), and the United States (US$7 billion). However, in per capita terms among these countries, the United States led in 2010 with US$22 per person, compared with US$11 in India and US$7 in China (Van Boeckel et al., 2015; Van Boeckel et al., 2014).

Since the discovery of penicillin, just over 80 years, various antimicrobial agents have been described in the literature, but only a few have been used in humans or animals (Petković et al., 2006). The indiscriminate use of these drugs on human health, animal, and food production has limited their effectiveness due to the emergence of bacterial resistance. Thus, encouraging the rational use of antimicrobials is very important for the continuity of their use in clinical practice safely and effectively (Chopra and Roberts, 2001).

Most of the antimicrobials are synthesized through the natural fermentation of different species of the bacteria *Streptomyces* (Bérdy, 2005; Watve et al., 2001). Antibiotics belonging to the tetracycline (TCs) class represent some of the commercial products and the most clinically important products derived from *Streptomyces* sp. This class of antibiotics is characterized by having a broad spectrum of action, and therefore, it has been widely used in human and veterinary medicine as well as in agriculture for the control of certain bacterial diseases that affect high-value fruits (Petković et al., 2006; McManus et al., 2002).

The worldwide production of TCs is estimated to be thousands of tonnes per year. In the United States, the amount of TCs used in the treatment of animals in 2001 was estimated to be 3200 tonnes, and it grossed about US$500 million in 2009 (Animal Health Institute, 2002; WHO, 2011). In 2010, TCs corresponded to the third most prescribed class of antibiotics (15%) in the United States (Van Boeckel et al., 2014).

Ten different TC derivatives have been marketed (Table 1). All ten can be given orally, and four (oxytetracycline,

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Table 1. Tetracyclines available on the market.

Tetracycline	Chemical name	Trade name	Year of discovery	Source	Therapeutic administration
Chlortetracycline	*7-chlortetracycline*	Aureomicyn	1948	N	Oral
Oxytetracycline	*5-hydroxytetracycline*	Terramicyn	1951	N	Oral and parenteral
Tetracycline	*[4S-(4α,4aα,5aα,6β,12aα)]—4-(Dimethylamino)-1,4,4a,5,5a, 6-11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-napthacenecarboxamide*	Achromicyn	1953	N	Oral
Demethyl-chlortetracycline	*6-demethyl-7-chlortetracycline*	Declomycin	1957	N	Oral
Rolitetracycline	*N-(1-pyrrolidinomethyl)tetracycline*	Reverin	1958	SS	Oral
Limecycline	*N-lysinomethyl-tetracycline*	Tetralysal	1961	SS	Oral and parenteral
Clomocycline	*N-methylol-7-chlortetracycline*	Megaclor	1963	SS	Oral
Methacycline	*6-methylene-5-hydroxytetracycline*	Rondomycin	1965	SS	Oral
Doxycycline	*α-6-deoxy-5-hydroxytetracycline monohydrate*	Doxirobe	1967	SS	Oral and parenteral
Minocycline	*7-dimethylamino-6-demethyl-6-deoxytetracycline*	Minocin	1972	SS	Oral and parenteral

N, natural; SS, semi-synthetic. Source: adapted from Chopra and Roberts (2001).

limecycline, doxycycline, and minocycline) can be also given parentally. In general, doxycycline and minocycline are the most commonly prescribed TCs for infections in humans (Chopra and Roberts, 2001), and oxytetracycline (OTC) for infections in animals intended for human consumption (Prado and Machinski-Júnior, 2011). Moreover, pharmaceutical combinations of OTC with drugs have been proposed for human use, such as hydrocortisone, polymyxin B, or nystatin (Lykkeberg et al., 2004; Smyrniotakis and Archontaki, 2007).

OTC stands out among the other TCs owing to its broad spectrum of action and affordability. These features have resulted in the indiscriminate use of OTC, especially in countries such as Brazil, where drugs-containing OTC are sold for veterinary use without special control by the Ministry of Agriculture, Livestock and Food Supply, and National Health Surveillance Agency. Unfortunately, this uncontrolled use of OTC in animals may result not only in microbial resistance, but also in environmental contamination, as approximately 20% of the drug is eliminated in its original form in faeces and urine; soil, rivers, and lakes are often their final destination (Halling-Sørensen et al., 2003).

Considering the importance of development and validation of analytical method for quality control of pharmaceutical products and the clinical relevance of OTC, mainly in veterinary medicine, this work provides an overview of relevant published literature and a discussion of data about the properties of OTC, as well as, the analytical methods described in the literature for determination of OTC and its related impurities in biological samples and pharmaceutical formulations. An extensive review was carried out using the databases SciFinder, ScienceDirect, and PubMed, searching for terms like determination of TCs, spectrophotometric determination of OTC, HPLC determination of OTC, and biological methods for OTC. The articles published in the period 1971–2016 were considered; moreover, searches were conducted in the official compendia.

Oxytetracycline

OTC was discovered in 1950 by Finlay and colleagues in soil samples containing an actinomycete called *Streptomyces rimosu* (Finlay et al., 1950). In 1953, Robert Woodward studied the chemical structure of OTC, enabling the pharmaceutical company Pfizer to produce the drug on a large-scale under the trade name Terramicina®. Several members of the family of TCs were developed in the period from 1950 to 1980, and during this period, they were among the most popular antibiotics in the United States (Shlaes, 2006; Zhanel et al., 2004; Speer et al., 1992).

OTC (Figure 1) belongs chemically to the polyketides. The rings are lettered A to D from right to left, and the numbers begin at the top of the ring A. OTC is chemically known as [4S-(4α,4aα,5α,5aα,6β,12aα)]octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacene-carboxyamide or 5-Hydroxytetracycline, occurs as hygroscopic yellow crystals, has a bitter taste, is odorless, and decomposes at 180°C. It is soluble in water, slightly soluble in ethanol, and insoluble in ether and chloroform (ONeil et al., 2006; Moffat et al., 2011). OTC is available as OTC freebase ($C_{22}H_{24}N_2O_9$; MW 460.4 gmol^{-1}), OTC hydrochloride ($C_{22}H_{24}N_2O_9$.HCl; MW 496.9 gmol^{-1}), OTC dihydrate ($C_{22}H_{24}N_2O_9.2H_2O$; MW 496.4 gmol^{-1}), and OTC calcium ($[C_{22}H_{24}N_2O_9]_2$.Ca), MW 958.9 gmol^{-1}) (Yuwono and Indrayan, 2005).

Similar to other TCs, OTC is a strong chelating agent, and both its antibacterial and pharmacokinetic properties are influenced by the chelation of metal ions present in food and in the biological environment. OTC forms chelates at several positions in its molecule owing to the presence of various electron-donor groups. Coordination sites include oxygen atoms at the C-10, C-11, and C-12 positions, enolic oxygen at the C-3 position, and nitrogen atoms at the C-4 position, and at the carboxamide group in the A-ring. OTC possesses three dissociable protons: one in the hydroxyl group at the C-3 position, one in the keto-enol system at the C-10, C-11, and C-12 positions, and one in the dimethylamino group at the C-4 position with pKa values of 3.5, 7.7, and 9.3, respectively (Chartone-Souza

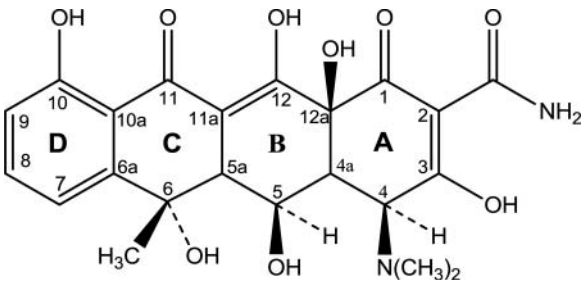

Figure 1. Chemical structure of oxytetracycline (CAS 79-57-2 – as freebase). Source: Moffat et al. (2011).

et al., 2005; Duarte et al., 1999). It is important to know the interactions between OTC and metals, as this knowledge helps us to understand specific physiological mechanisms, and thus design more effective and rapid assays that can favorably compete with the official analytical methods (Pereira-Maia et al., 2010; Couto et al., 2000).

Pharmacological properties

Pharmacokinetics

OTC, due to its water solubility, shows poor absorption orally (~58%), mainly after food intake. The majority of drug absorption occurs in the stomach and upper small intestine, and the percentage of drug which is not absorbed increases together with the increase in dose. OTC has relatively slow distribution (volume of distribution: 128 L), and 27–35% of the drug is bound to plasma proteins (Pereira-Maia et al., 2010; Agwuh and MacGowan, 2006). It shows half-life in the body ($t_{1/2}$) of 9.2 hours, being classified as short-acting TC. The drug has a peak concentration (C_{max}) of the 2 mgL^{-1}, time to peak concentration (t_{max}) of the 2–4 hours, and 50% of elimination via faecal route (Agwuh and MacGowan, 2006).

Spectrum of action and clinical applications

OTC shows activity against a wide range of Gram-positive and Gram-negative bacteria, spirochaetae, *Leptospira*, chlamydiae, mycoplasmas, rickettsiae, and protozoan parasites (Udalova et al., 2015; Chopra and Roberts, 2001).

Due to pharmacokinetic parameters being not very favorable and resistance by certain bacterial strains, OTC has not been prescribed for the treatment of humans infections in different countries (Chopra and Roberts, 2001).

In 1953, OTC was approved by Food and Drug Administration as feed additive, which meant that OTC can be used for therapeutic treatment of a large number of poultry on commercial farms and the antibiotic can be added directly to feed or water or can be administered in aerosols (Chopra et al., 1992). OTC has applications in the treatment of infections in poultry, cattle, sheep, and swine and it is also used to treat foulbrood disease of the honeybee, which is caused by either *Bacillus larvae* or *Streptococcus pluton* (Levy, 1992).

Moreover, due to its broad antibacterial spectrum and high potency, OTC is a commonly used antibiotic to combat bacterial infections in fish farming and in farm-raised shrimp for the treatment of vibriosis and necrotizing hepatopancreatitis infections (Reed et al., 2004).

Mechanism of action

OTC is a bacteriostatic antibiotic which inhibits bacterial protein synthesis by binding to the ribosomal complex (Chopra and Roberts, 2001). In Gram-negative bacteria, OTC move through membranes via porin channels and accumulate in the periplasmic space. Once inside the bacterial cell, the OTC molecules bind reversibly with the prokaryotic 30S ribosomal subunit, preventing the association of aminoacyl-tRNA with the bacterial ribosome and stopping protein synthesis (Figure 2).

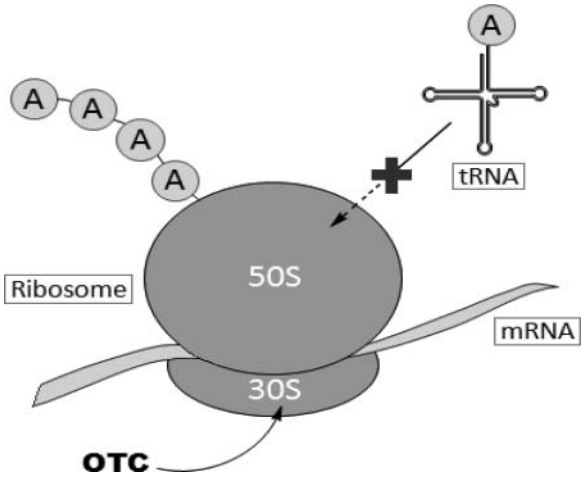

Figure 2. Mechanism of action of oxytetracycline. OTC, oxytetracycline; A, aminoacid. Source: Author's own figure.

OTC binds with the 70S ribosomes found in mitochondria and can also inhibit protein synthesis in mitochondria. OTC interacts weakly with the 80S ribosome of eukaryotic cells, creating a relatively weak inhibition of protein synthesis, which is hypothesized as the reason for the selective antimicrobial properties of the TCs, with limited side effects in humans (Roberts, 2003).

Degradation products and stability

OTC degradation products are formed under different environmentally relevant conditions based on knowledge of the chemical fate of the TCs at a wide pH range in buffer solutions (Halling-Sørensen et al., 2003; Halling-Sørensen et al., 2002).

The chemical structures and degradation pathway of OTC degradation products is showed in Figure 3

As described by Kahsay and coworkers (2013), in acidic aqueous solutions (pH 2–6) OTC can be reversibly epimerized at position C-4, resulting in the formation of 4-epioxytetracycline (4-EOTC). Kaplan and colleagues (1957) reported that epimerization is markedly inhibited by the presence of calcium and magnesium at pH levels above 6. OTC epimerize less willingly than other TCs because the hydroxyl group at C-5 position may form hydrogen bonding with the dimethylamino group (Hussar et al, 1968).

OTC is liable to acid degradation because of the presence of a secondary hydroxyl group at C-6, 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) (Kahsay et al., 2013). Under vigorous acidic conditions, α-APOTC and β-APOTC may be further degraded to terrinolidine (TL) in an irreversible process (Hochstein et al., 1953). During the synthesis of OTC, the fermentation process by Streptomyces rimosus produces two impurities: 2-Acetyl-2-decarboxamidooxytetracycline (2-ADOTC) and tetracycline (TC) (Khan et al., 1987). Under acid conditions, TC can undergo epimerization at C-4 and formed the 4-Epitetracycline (ETC). AOTC, α-APOTC,

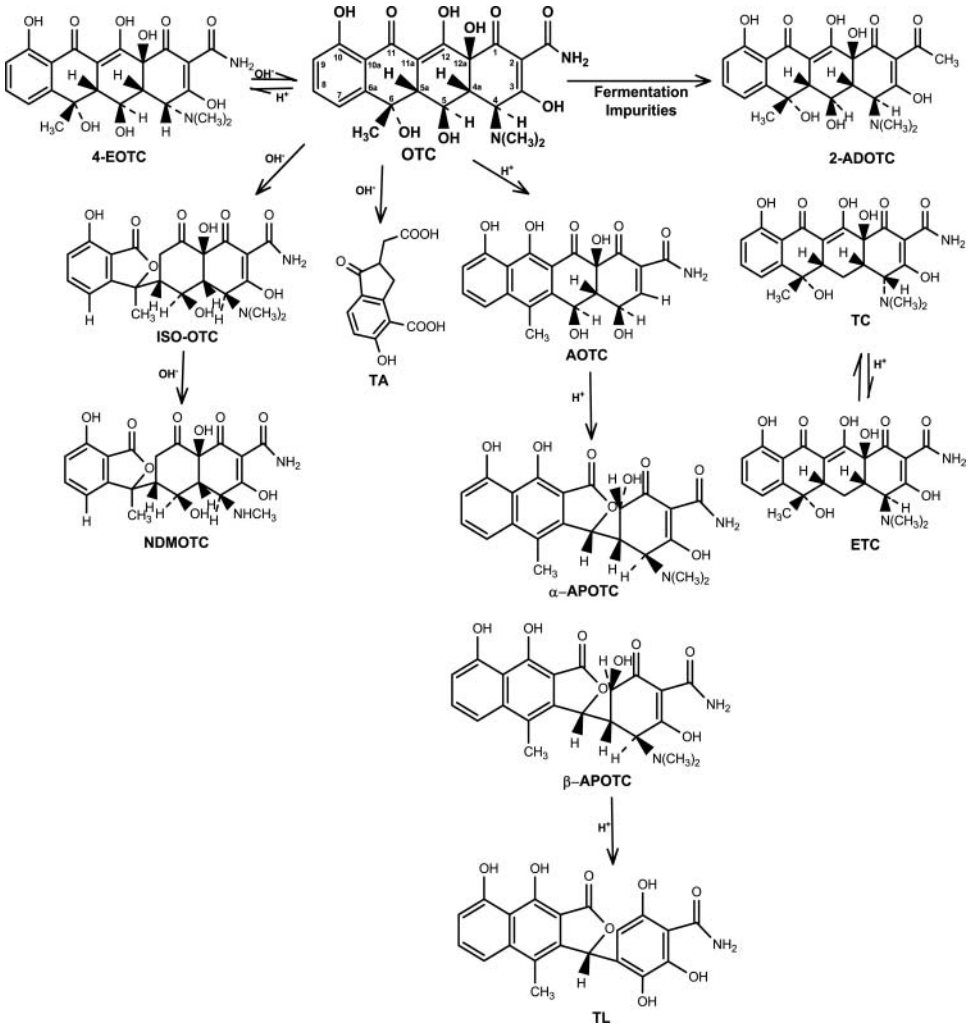

Figure 3. Chemical structures and degradation pathway of oxytetracycline degradation products. OTC, oxytetracycline; 4-EOTC, 4-epioxytetracycline; 2-ADOTC, 2-acetyl-2-decarboxamidooxytetracycline; TC, tetracycline; ETC, 4-epitetracycline; ISO-OTC, iso-oxytetracycline; NDMOTC, N-desmethyl-OTC; AOTC, anhydrooxytetracycline; α-APOTC, α-apooxytetracycline; β-APOTC, β-apooxytetracycline; TL, terrinolidine; TA, terranoic acid. Source: adapted from Halling-Sørensen et al. (2002).

β-APOTC, and TL are formed by manufacturing OTC as the hydrochloride salt. These impurities are not present in OTC base (Diana et al., 2002).

Profound changes in chemical structure of OTC may occur during the treatment with alkali (Halling-Sørensen et al., 2002). The main product of OTC in aqueous sodium hydroxide solution is terranoic acid (TA) (Hochstein et al., 1953). Due to hydroxyl group at C-6, OTC cleaves readily to iso-oxytetracycline (ISO-OTC). N-desmethyl-OTC (NDMOTC), an analog of the ISO-OTC, may be formed under alkaline conditions if oxygen is present (Mitscher 1978; Waller et al., 1952).

These impurities are therapeutically inactive, except for TC, and their content in commercial bulk OTC is restricted (Diana et al., 2002). The European Pharmacopoeia sets the limits for some impurities, such as 4-EOTC to be not more than 0.5% and any other not more than 2% (European Pharmacopoeia, 2012).

Moreover, all TCs have photosensitivity, but the drug stability is maintained for up to 30 days if the storage is realized out of reach of light (Al-Momani and Kanan, 2008). Besides the photostability, these antibiotics are also sensitive to high temperature and humidity (Moreno-Cerezo et al., 2001). OTC solutions, when exposed to intense sunlight or temperatures above 90°C and humidity, become dark without significant loss of the potency. However, the drug quickly loses the antimicrobial activity in acidic and basic solutions (Harvey, 1990). The hydrochloride crystals (OTC. HCl) show, approximately, 5% inactivation at 56°C, and their aqueous solutions are stable at pH 1.0 to 2.5 for at least 30 days at 30°C (ONeil et al., 2006).

Khan and coworkers (1987) developed a study that monitored the stability of OTC.HCl in the solid state during the storage for about 6 years at temperatures of 37, 50, and 70°C. The samples were stored in screw-capped and in rubber-stoppered vials. At 37 and 50°C, no significant decomposition was observed. Therefore, at 70°C a decrease in OTC content was observed and it was poorly compensated by an increase in AOTC and α or β-APOTC. In addition, in the rubber-stoppered vials the decomposition was much faster. This can probably be explained by the fact that the samples in screw-capped vials remain completely dry and the small amounts of water, liberated upon formation of AOTC, were eliminated by evaporation. It may be concluded that in order to maintain the stability of OTC, it is necessary to have adequate storage conditions, like temperature at 20°C and low humidity.

Analytical methods

Interest in the determination of TC antibiotics and other antibiotics in water, soils, wastewater, manure, and food products has quickened in the past decade (Udalova et al., 2015).Several studies in the literature report the use of high-performance liquid chromatography (HPLC) coupled with different detectors for the analysis of OTC residues in food (milk, honey, meat, and eggs) and in the environmental samples (Dzomba et al.,2015; Emiri et al., 2014; Olatunde et al., 2014; Grupta et al., 2014; Khossrokhavar et al., 2011; Prado and Machinski-Júnior, 2011; Li et al., 2008; Biswas et al., 2007; Pena et al., 2005; Senyuv et al., 2000; Omija, 1991). However, the present study focused on performing a comprehensive review of the analytical methods used to determine OTC and/or its degradation products in pharmaceutical formulations and biological matrices. For this purpose, the demand for developing the analytical methods has grown in recent years and there is a clear interest in simple, rapid, and sustainable new methods that can be used in either routine quality control or in hospital environment for dose adjustments (Fernandez-Torres et al., 2010).

HPLC, thin-layer chromatography (TLC), spectrophotometry, capillary electrophoresis (CE), chemiluminescence (CL), flow injection analysis (FIA), and microbiological assays are among those analytical methods that are described in the literature to determine OTC and/or its related impurities in pharmaceutical products and biological samples. Although, chromatographic methods, mainly reversed phase version of HPLC, are the most frequently used for the determination of TC antibiotics (Udalova et al., 2015).

High-performance liquid chromatography

HPLC is the method of choice for the determination of OTC in pharmaceutical products. It is the official technique recommended by both the British Pharmacopoeia and US Pharmacopoeia for the determination of OTC and its related impurities in bulk material, injections, tablets, capsules, ointments, oral suspensions, and veterinary oral powder (United States Pharmacopeia, 2014; British Pharmacopeia, 2013; European Pharmacopoeia, 2012). The use of HPLC as an official method can be justified by its high sensitivity, selectivity, and versatility, as it can simultaneously determine OTC and its degradation products and impurities eventually formed during the fermentation process (Fernandez-Torres et al., 2010; Smyrniotakis and Archontaki, 2007). Moreover, it is the most commonly used technique in the pharmaceutical industry for monitoring production, evaluating stability, and quality control of finished products. However, the high operating cost is the major disadvantage of this method (Rodríguez et al., 2016).

Most HPLC methods used to detect OTC in pharmaceutical products and biological matrices use silica-based stationary phases, and an organic modifier (methanol, acetonitrile, or propanol) is often added to the aqueous mobile phase (Knox and Jurand, 1979).The results obtained by Knox and Jurand (1975) indicate that acetonitrile is the best organic modifier for separating different TCs, as it provides symmetrical peaks with a high number of theoretical plates.

Chromatographic separation of TCs is a complex mechanism, due to the presence of different functional groups in their molecules that interact with silica. In addition, TCs form complexes with metal ions adsorbed on the reverse-phase columns, resulting in chromatographic peaks tailing. Mobile phases containing acids, such as phosphoric acid, citric acid, and oxalic acid are used to avoid the formation of these complexes (Pena et al., 1997).

Knox and Jurand (1979) concluded that chromatographic separations of TCs could be improved at pH values ranging from 1.0 to 2.5. In this pH range, the OTC molecule is predominantly in the zwitterionic form with the dimethylamino group protonated at C-4 and the hydroxyl group ionized at C-3 (Figure 1). Thus, the function of the acid in the mobile phase is to form ion pairs between the cationic form of the drug and the anion of the acid, preventing the strong adsorption on the stationary phase. One disadvantage of using strongly acidic mobile phases is the reduction in the useful life of the column, since most of the filler materials of reversed-phase columns are unstable at pH values below 3.0. To overcome this problem, it is recommended to use pre-columns and to wash the column with a neutral solvent such as acetonitrile:water (50:50, v/v) for 1 hour at the end of each day of work (Pena et al., 1997).

Another problem associated with the chromatographic separation of TCs is its strong adsorption onto the residual silanol groups of the filler material in reversed-phase columns, thus producing unresolved chromatographic peaks. The addition of silanol-blocking agents in the mobile phase, such as ethylenediaminetetraacetic acid (EDTA) and tetramethylammonium chloride can prevent this problem by improving the chromatographic resolution (Pena et al., 1997). Knox and Jurand (1979) have demonstrated that EDTA is insoluble in strongly acidic media (pH < 2), and that the pH range 3.0 to 5.0 is the ideal range, because in this pH range, EDTA forms ion pairs with the zwitterionic form of the drug, avoiding its irreversible adsorption onto silanol groups. However, EDTA must be used at a concentration of approximately 0.001 M, because its molecules can absorb UV light at higher concentrations, and thus interfere with the chromatographic analysis.

Khan and coworkers (1987) described an HPLC method using a polymer column for the analysis of OTC and related substances in pharmaceutical products. This method served as a base for testing related substances in the current OTC monograph in the European Pharmacopoeia (2012). However, it is known that polymer columns give broad peaks and do not result in highly efficient chromatographic separations. Therefore, using this method it is not likely to completely separate OTC from its impurities (Kahsay et al., 2013).

Despite all the problems associated with the analysis of TCs by HPLC, several studies in the literature have shown that this analytical method can be successfully used to determine OTC and/or its related impurities in biological matrices (Table 2) and pharmaceutical products (Table 3). It should be emphasized that biological samples cannot be analyzed directly, without pre-treatment, for quantifying an analyte of interest, but it is necessary to perform a previous step of sample clean-up and efficient extraction of the analyte from the biological matrix (Prabu and Suriyaprakash, 2012). For instance, TCs were determined in biological fluids only after the precipitation of proteins with trichloroacetic or perchloric acids, acetonitrile, or buffer solutions (Udalova et al., 2015). Due to these reasons,

Table 2. HPLC method used for the analysis of oxytetracycline in biological matrices.

Sample matrix	Column	Mobile phase	Detection	Flow rate (mL min^{-1})	Analysis time (min)	Reference
Cattle, sheep, and swine plasma and urine	μ Bondapak C18 packed with octadecylsilane bonded phase material Waters™ (300× 4.5 mm)	Isocratic: *Mobile phase A*: 0.01 M sodium dihydrogen phosphate monohydrate + 30% acetonitrile in distilled water pH 2.4. *Mobile phase B*: 0.01 M sodium dihydrogen phosphate monohydrate + 40% acetonitrile in water, pH 2.4.	UV 355 nm	1.0	~5.0	Sharma et al. (1977)
Plasma	Laboratory-prepared columns: copolymers of divinylbenzene with styrene: Amberlite™ XAD-2 and PRP-1. (100 × 3.0 mm)	Isocratic: *Mobile phase A*: acetonitrile: dichloromethane 0.2 M acetate buffer (pH 3.6) + 0.025 M EDTA (10:1.0:90, v/v). *Mobile phase B*: acetonitrile:dichloromethane 0.05 M: acetate buffer (pH 3.6) + 0.025 M EDTA (12.5:1.0: 87.5, v/v).	UV 268 nm and 357 nm	0.8 (Mobile phase A) 0.6 (Mobile phase B)	< 20.0	Reeuwijk and Tjaden (1986)
Rainbow trout plasma	Pre-column: Cyano Spheri-5 MPLC™ (30 mm × 4.6 mm, 5μm) Analytical column: Cyano Spheri-5 cartridge MPLC™ (100 × 4.6 mm, 5μm)	0.02 M oxalic acid (pH 2): methanol: N,N-dimethylformamide (950:50:50, v/v).	UV 350 nm	1.0	4.3	Iversen et al. (1989)
Bovine and salmon blood and plasma	Polystyrene-divinylbenzene copolymer – PLRP-S™ (150 × 4.6 mm, 5 μm)	acetonitrile: 0.005 M heptanesulphonic acid (sodium salt) with 0.02 M orthophosphoric acid (23:77, v/v).	UV 350 nm	0.70	< 10.0	Agasøster and Rasmussem (1991)
Salmon tissue	Capcell™ C18 type SG-20 (250× 4 6 mm, 5μm)	Gradient: *Mobile phase A*: 0.3 M acetate buffer (pH 6.5) containing 35 mM calcium chloride and 25 mM EDTA (buffer A). *Mobile phase B*: methanol 0–1.0 min: 67% A and 33% B 1–8.5 min: 45% A and 55% B 8.5–15 min: 67% A and 33% B.	Fluorescence 390 nm and 512 nm	1.0	~5.0	Iwaki et al. (1992)
Bovine plasma	Lichrosorb™ RP8 (125 × 4 mm, 5 μm)	0.01 M oxalic acid:methanol: acetonitrile (75:15:10, v/v).	UV 357 nm	1.0	~4.0	Nelis et al. (1992)
Chicken, swine, cattle and rainbow trout blood serum	Hisep™ shielded hydrophobic phase column, (150 × 4.6 mm, 5 μm) coupled with a guard column (20 × 4.6 mm) packed with the same material	methanol: 0.2 M oxalic acid pH 7.0 adjusted 28% aqueous ammonia (10:90, v/v).	UV 360 nm	1.0	~5.0	Ueno et al. (1992)
Human serum	Capcell™ C18 SG-120 column (250 × 4.6 mm, 5 μm);	methanol: 0.1 M sodium acetate buffer (pH 6.5) containing 35 mM calcium chloride and 25 mM EDTA (buffer A) (30:70, v/v).	Fluorescence 390 nm (excitation) and 512 nm (emission)	1.0	~5.0	Iwaki et al. (1993)
Sheep liver and cattle kidney tissues	Pre-column: Iminodiacetic-bonded hydrophilic polymeric support – Anagel-TSK-Chelate-SPW™ (100 × 6 mm,10 μm) Analytical column: polystyrene-divinylbenzene copolymer – PLRP-S™ (150 × 4.6 mm, 5μm) coupled with a guard-column PLRP-S™ (5 × 3 mm, 5μm)	Linear gradient: *Mobile phase A*: aqueous 0.1 M potassium dihydrogen phosphate + 0.01 M citric acid + 0.01M EDTA. *Mobile phase B*: 0.1 M potassium dihydrogen phosphate + 0.01 M citric acid + 0.01 M EDTA:methanol:acetonitrile (65:10:25, v/v/v).	UV 350 nm	*	<25.0	Stubbings et al. (1996)
Bovine liver tissue	TSK Gel Octyl™ (100 × 4.6 mm, 2 μm)	acetonitrile: 0.05% trifluoroacetic acid aqueous solution (20:80, vv).	MS–MS *m/z* 461	0.50	3.90	Oka et al. (1997)
Cattle, swine, and chicken muscle, kidney and liver tissue, cattle fat, egg, milk, and marine products (prawn and yellowtail)	YMC – Pack™ ODS (150 × 6 mm)	buffer pH 4 containing 0.01 M EDTA: methanol (77:23, v/v).	Fluorescence 380 nm (excitation) and 520 nm (emission)	1.0	<16.0	Fujita et al. (1997)
Swine plasma	Nucleosil™ C8 (250 × 4.6 mm, 5 μm) coupled with a guard-column Pellicular™ ODS, (37–53 μm)	Linear gradient: *Mobile phase A*: 0.01 M oxalic acid:acetonitrile (90/10, v/v). *Mobile phase B*: 0.01 M oxalic acid:acetonitrile (42:58, v/v).	UV 356 nm and 369 nm	0.80	*	Weimann et al. (1998)

(*Continued on next page*)

Table 2. (*Continued*)

Sample matrix	Column	Mobile phase	Detection	Flow rate (mL min^{-1})	Analysis time (min)	Reference
Urine	Column switching Column 1 (extraction): ChromSpher 5 BioMatrix™ (150 × 4.6 mm) Column 2 (analytical) column: Brownlee™ RP-18, spheri-5 (30 × 2.1 mm, 5μm)	*HPLC 1 (extraction)*: Eluent 1A: 20 mM KH_2PO_4. *HPLC 2 (analytical)*: Eluent 2A: 0.1% trifluoroacetic acid and Eluent 2B: 80% Acetonitrile + 0.1% trifluoroacetic acid.	UV* MS/MS *m/z* 426	*HPLC 1*: 1.0 *HPLC 2*: 0.3–0.4	<5.0	Weimann and Bojesen (1999)
Sheep plasma	ODS C8*	acetonitrile: water containing KH_2PO_4 (0.04 M) with the pH 2.5 (10:90, v/v).	UV*	1.0	*	Kaya et al. (2001)
Calf muscle, liver, and kidney tissues	Polystyrene-divinylbenzene copolymer -PLRP-S™ (250 × 4.6 mm, 8 μm) coupled with a guard column of the same type (5 × 3 mm)	Gradient: *Mobile phase A*: 0.001 M oxalic acid + 0.5% (v/v) formic acid + 3% (v/v) tetrahydrofuran in water. *Mobile phase B*: tetrahydrofuran 0–22 min: 100% A 22.1–30 min: 90% A and 10% B 30.1–37 min: 100% A.	MS/MS *m/z* 443 ion	1.0	37	Cherlet et al. (2003)
Swine manure	XTerra™ RP18 column (150 × 3.0 mm, 3.5 μm) coupled with a guard column XTerra™ RP18 (20 × 3.0 mm, 3.5 μm)	5.5–13.0 min: 10% A and 90% B, 13.0–13.5 min: linear gradient to 96% A and 4% B.	MS–MS *m/z* 461 (OTC and EOTC) *m/z* 443 (α-APOTC and β-APOTC)	0.40	<12.0	Loke et al. (2003)
Swine plasma	OmniSpher™ C18 (250 × 4.6 mm, 5 μm) with Shimadzu™ C18 cartridge	acetonitrile:methanol:formic acid pH 2 (17.5:17.5:65, v/v/v).	UV 360 nm	1.4	2.93	Kowalski et al. (2006)
Swine plasma	Omni Spher™ C18 column (250 × 4.6 mm, 5 μm)	acetonitrile:methanol:formic acid pH 2 (17.5:17.5:65 v/v/v).	UV 360 nm	1.40	~3.0	Kowalski and Pomorska (2007)
Cattle tissue and plasma	LiChrocart™ RP18 analytical column (125 × 4.6 mm, 5 μm) coupled with coupled with a guard-column C18 (5 μm)	0.01 M citric acid + 0.01 M dipotassium orthophosphate: acetonitrile + 0.005 M tetramethylammonium chloride + 0.1 g L^{-1} EDTA (72:28, v/v).	UV 365 nm	2.0	*	Mestorino et al. (2007)
Chicken muscle	Zorbax Eclipse™ XDB-Phenyl column (150 × 3.0 mm, 3.5 μm), coupled with a guard-column Phenomenex C18 (2.0 mm)	Gradient: *Mobile phase A*: 0.1 M malonate + 50 mM Mg^{+2}, adjusted to pH 6.5 with concentrated NH_4OH). *Mobile phase B*: methanol 16% B (6 min), 16–40% B (4 min), 40% B (8 min), 40–80% B (3 min), 80% B (2 min), 80–16% B (3 min), 16% B (4 min).	Fluorescence 375 nm (excitation) and 535 nm (emission)	0.50	~5.0	Schneider et al. (2007)
Human blood plasma and urine	Novapak™ C18 column (250 × 4.0 mm, 5 μm)	acetonitrile:triethylamine:oxalate buffer adjusted to pH 2.5 with 0.5 M NaOH (29.5: 0.5: 70, v/v/v).	UV 355 nm	1.0	*	Al-Momani and Kanan (2008)
Bovine muscle	Chromolith™ Speed Rod RP 18 (50 × 4.6 mm)	Gradient: *Mobile phase A*: methanol. *Mobile phase B*: oxalic acid 0.01 M (pH 3.0) + 1.5% tetrahydrofuran 0–2.2 min: 100% B 2.2–3.0 min: 10% A and 90% B 3.0–6.5 min: 15% A and 85% B 6.5–10 min: 100%.	PDA 360 nm	4.0 and 4.5	6.64	Cristofani et al. (2009)
Human urine	Phenomenex™ Gemini C18 110 (150 × 4.6mm, 5 μm) coupled with a guard-column LiChroCART™ 4-4 LiChrospher™ 100 RP-18 (4 × 4 mm, 5 μm)	Gradient: *Mobile phase A*: 0.1% formic acid in water. *Mobile phase B*: acetonitrile 0–7.0 min: isocratic step at 99% A and 1% B 7.0–28 min: linear elution gradient from 99% to 70% A and from 1% to 30% B.	PDA 280 nm	0.70	18.54	Fernandez-Torres et al. (2010)

Chicken muscle	Pursuit™ C18 (100 × 2.0 mm, 5 μm) column coupled with a guard-column with a Polaris C18 (2.0 mm, 3 μm)	Gradient: *Mobile phase A*: 0.1% formic acid. *Mobile phase B*: acetonitrile + 0.1% formic acid 0–3.0 min: 90% A and 10% B 3.0–5.5 min: 25% A and 75% B 5.5–9.0 min: 90% A and 10% B.	MS–MS *m/z* 461	0.30	<12.0	Uekane et al. (2011)
Human urine	Trapping column: XBridge™ C18 column (30 × 2.1 mm, 10 μm) Analytical column: Acquity™ UPLC HSS T3 column, 100 × 2.1 mm, 1.8 μm)	*Pump A*: 0.2% formic acid water solution. *Pump B*: 0.2% formic acid water solution.	MS–MS *m/z* 461	*Pump A*: 0.50 *Pump B*: 1.50	5.17	Wang et al. (2014)
Bovine urine	Raptor™ biphenyl column (150 × 2.1 mm, 2.7 μm) coupled with a guard-column Raptor™ biphenyl (5 × 2.1 mm, 2.7 μm)	Gradient: *Mobile phase A*: aqueous formic acid 0.1% *Mobile phase B*: methanol 0–5.0 min: 98% A and 2% B 5.0–22 min: 50% A and 50% B 22–29 min: 5% A and 95%	MS–MS *m/z* 461	0–5 min: 0.10 Rest of the run: 0.20		Chiesa et al. (2015)
Swine manure	Kinetex C18 column (100 × 2.1 mm, 1.7 μm) with a Security Guard Ultra guard cartridge system	Gradient: *Mobile phase A*: water:acetonitrile (95:5, v/v) + 0.5% formic acid + 1% ammonium formate. *Mobile phase B*: acetonitrile:0.1% formic acid 0–1.0 min: 95% A and 5% B 1.0–3.0 min: 75% A and 25% B 3.0–7.0 min: 100% 7.0–9.0 min: 95% A and 5% B.	MS–MS *m/z* 461	0.40	3.21	Meersche et al. (2016)

*Data not available; *OTC, oxytetracycline; EOTC*, epioxytetracycline; α-APOTC, α-apooxytetracycline and β-APOTC, β-apooxytetracycline; PDA, photodiode array detector; MS/MS, mass spectrometer detector.

Table 3. HPLC method used for the analysis of oxytetracycline in pharmaceutical products

Pharmaceutical product	Column	Mobile phase	Detection	Flow rate (mL min^{-1})	Analysis time (min)	Reference
Bulk material and related impurities (EOTC and AOTC)	LiChrosorb™ RP-S (100 × 4.7 mm, 10 μm)	Isocratic: *Mobile phase A*: tetrabutylammonium hydrogen sulfate 0.5 g/L pH 2.6: acetonitrile (92:8, v/v) *Mobile phase B*: tetrabutylammonium hydrogen sulfate 0.5 g/L pH 2.6: acetonitrile (80:20, v/v)	UV 275 nm	1.0	<10.0	Mourot et al. (1980)
Bulk material, capsules, and tablets	Spherisorb™ S5 C8 (200 × 4.6 mm, 5 μm) coupled with a guard-column LiChrosorb™ RP-8 (30 × 4.5 mm, 10 μm).	0.2 M ammonium oxalate:0.1 M Na_2EDTA: dimethylformamide (55:20:25, v/v/v)	UV 280 nm	1.0	24.0	Barnes et al. (1985)
Bulk material and related impurities (AOTC, EOTC, TC, ADOTC, α-APOTC, and β-APOTC)	Styrene divinylbenzene copolymer PLRP-S™ 100 A (250 × 4.6 mm, 8 μm)	tert-butanol-0.2 M:phosphate buffer pH 8.0: 0.02 M tetrabutylammonium sulphate: pH 8: 0.0001 M EDTA pH 8: water (5.9:10:5:10:78.1, m/v/v/v/v).	UV 254 nm	1.0	<30.0	Khan et al. (1987)
Bulk material and capsules	Capcell™ C18 type SG- 120 (250 × 4.6 mm, 5 μm)	0.1 M acetate buffer (pH 6.5) containing 35 mM calcium chloride and 25 mM EDTA (buffer A): methanol (45:55, v/v)	Fluorescence 390 nm (excitation) and 512 nm (emission)	1.0	~5.0	Iwaki et al. (1992)
Bulk material, related impurities (TC, α-APOTC, and β-APOTC), injectable, and capsules	Column A: polystyrene-divinylbenzene copolymer Column PLRP-S™ (250 × 4.6 mm, 5 μm) Column B: polymethacrylate polymer with C18 ligands PMCIR™, (150 × 4.6 mm, 6 μm)	acetonitrile: 0.02 M sodium perchlorate pH 2.0 (20:80, v/v),	UV 280 nm	1.0	~30.0	Bryan and Stewart (1994)
Bulk material, tablets, and ointment	Carbon column packed with Hypercarb™ porous graphitic carbon – PGC (100 × 4.6 mm, 7 μm)	0.05 M potassium phosphate buffer (pH 2.0): acetonitrile (40:60, v/v)	UV 268 nm	1.0	< 5.0	Monser and Darghouth (2000)
Bulk material, ointments, powder, vaginal tablets, and capsules	Silasorb™ RP-8 (250 × 4 mm, 10 μm)	methanol:0.01 M oxalic acid, pH 3.0 (30:70, v/v)	UV 250 nm	0.95	~7.0	Papadoyannis et al. (2000)
Bulk material and related impurities (AOTC, EOTC, TC, ETC, ADOTC, α-APOTC, β-APOTC, and TL)	XTerra™ RP-18 column (250 × 4.6 mm, 5 μm)	Isocratic: *Mobile phase A*: acetonitrile:0.25 M tetrabutylammonium hydrogen sulfate pH 7.5: 0.25 M EDTA pH 7.5: water (11.5:36:16:36.5, v/v/v/v) *Mobile phase B*: acetonitrile: 0.25 M tetrabutylammonium hydrogen sulfate pH 7.5: 0.25 M EDTA pH 7.5: water (34:36:16:14, v/v/v/v).	UV 280 nm	1.0	<25.0	Diana et al. (2002)
Bulk material and related impurities (EOTC, TC, ETC, ADOTC, α-APOTC, and β-APOTC)	XTerra™ MS C18 (100 × 2.1 mm, 3.5 μm)	Gradient: *Mobile phase A*: methanol:water (5:95, v/v) with formic acid (0.08 M) *Mobile phase B*: methanol:water (95:5, v/v) with formic acid (0.08 M) 0–6 min: 89% A and 11% B 6–11 min: a linear gradient to 50% A and 50% B 11–20 min: 50% A and 50% B 20–25 min: linear gradient to 89% A and 11% B	MS–MS *m/z* 461 (OTC and EOTC) *m/z* 460 (ADOTC) *m/z* 445 (TC) *m/z* 426 (α-APOTC and β-APOTC)	0.25	30.0	Lykkeberg et al. (2004)
Bulk material, related impurities (EOTC, TC, α-APOTC, and β-APOTC), human tablets and powder; veterinary powder, ointment, and aerosol spray	Column A: Hypersil™ BDS RP-C18 column (250 × 4.6 mm, 5 μm) Column B: Waters™ C18 Symmetry (150 × 3.9 mm, 5 μm)	Isocratic: *Mobile phase A*: methanol:acetonitrile:0.1 M phosphate buffer pH 8.0 (12.5:12.5:75, v/v/v) *Mobile phase B*: methanol:acetonitrile:0.1 M phosphate buffer pH 8.0 (15:15:70, v/v/v)	UV 253 nm	1.0	Column A: 15.0 Column B: 5.0	Smyrniotakis and Archontaki (2007)

Bulk material and tablets	SynergiFusion™ RP18 column (150 × 4.6 mm; 4 μm)	Linear gradient: *Mobile phase A*: methanol *Mobile phase B*: acetonitrile *Mobile phase C*: 5 mM oxalic acid 0–3 min: 9.5% A, 14.5% B, and 76% C; 3–10 min: from initial conditions to 18% A, 30% B, 52% C.	UV 277 nm Resonance Rayleigh Scattering (RSS): 370 nm (excitation and emission)	0.80	<5.0	Wang et al. (2008)
Bulk material, related impurities, powder, and injectables	Agilent Eclipse™ Plus C18 column (50 × 4.6 mm id, 1.8 μm particle size)	Gradient: *Mobile phase A*: 0.3% aqueous trifluoroacetic acid (TFA) *Mobile phase B*: methanol 0–2 min: 93% A and 7% B 2–14 min: 31% A and 69% B 14–18 min: 100% B	MS/MS *m/z* 426, 410, and 242	0.40	<18.0	Vučićević-Prčetić et al. (2011)
Bulk material	Inertsil™ C4 WP300	100% water	PDA 282 nm	1.0	2.32	Furusawa (2013)
Bulk material, related impurities (EOTC, TC, α-APOTC, and β-APOTC), and veterinary ointment	Hypersil™ BDS RP-C18 column (250 × 4.6 mm, 5 μm)	acetonitrile:methanol:80 mM dipotassium phosphate pH 7.5 (17.5:17.5:65 v/v/v)	UV 254 nm	0.7	9.0	Giugiu (2013)
Bulk material and related impurities (AOTC, EOTC, ADOTC, α-APOTC, and β-APOTC)	Inertsil ™ C8 (150 × 4.6 mm, 5 μm)	Gradient: *Mobile phase A*: 0.05% trifluoroacetic acid pH 2.2 *Mobile phase B*: acetonitrile:methanol: tetrahydrofuran (80:15:5, v/v/v) 0–5 min: 90% A and 10% B 5–20 min: 65% A and 35% B 20–25 min: 90% A and 10% B	UV 254 nm	1.3	~17.0	Kahsay et al. (2013)
Veterinary powder	Nucleosil™ C8 (250 × 4.6 mm; 5 μm)	5 mM monobasic potassium phosphate pH 2.5: methanol:acetonitrile (70:18:12, v/v/v)	UV 254 nm	1.1	4.15	Tauber and Chiurciu (2014)

EDTA, ethylenediaminetetraacetic acid; EOTC, 4-epioxytetracycline; ADOTC, Acetyl-2-decarboxamidooxytetracycline; TC, tetracycline; AOTC, anhydrooxytetracycline; α-APOTC, α-apooxytetracycline; β-APOTC, β-apooxytetracycline; UV, ultraviolet; PDA, photodiode array detector; MS/MS, mass spectrometer detector.

analyzing OTC in biological samples by HPLC can be laborious and likely to introduce errors (Knox and Jurand, 1979).

Planar chromatographic methods

Paper chromatography was the first planar chromatographic method for determination of OTC in the presence of its degradation products (Sina et al., 1971). Difficulties with separations of TCs by TLC on silica gel were attributed to the formation of chelate complexes with metallic ions and to counter this, sequestering agents such as edetate, citrate, oxalate, or phosphate need to be added (Weng et al., 1990). Despite of these difficulties TLC and TLC-densitometry methods have been described for the qualitative identification and quantitative analysis of OTC in pharmaceuticals products (Krzek et al., 2000; Weng et al., 1990).

According to Yuwono and Indrayan (2005), a method using cellulose as stationary phase and isopropyl alcohol:acetone: water (75:15:15, v/v/v) as mobile phase was described for analyses of OTC in biological samples.

Recently, a normal silica gel and reversed phase (RP-18W plates) high performance thin-layer chromatography (HPTLC) method coupled with IR-MALDI-o-TOF-MS detection was described for the determination of OTC and other TCs in bulk material. The normal silica gel plates were developed in chloroform: methanol: 5% aqueous EDTA (13:4:1 or 10:4:1, v/v/v). A solvent system containing methanol:acetonitrile:0.5 M oxalic acid, pH 2.5 (1:1:4, v/v/v) was used for the development of RP-18W plates. HPTLC-separated TCs are detected and quantified by UV spectroscopy at 360 nm and identified directly on TLC plates by IR-MALDI-TOF-MS. The MS analysis of TCs from RP-18W HPTLC plates was found to be superior when compared to the spectra acquired from the silica gel plates. (Meisen et al., 2010).

Spectrophotometry

Spectrophotometric methods have been used for the determination of OTC in biological samples and pharmaceutical formulations. OTC has been determined in these products by spectrophometric methods based on derivative techniques (Toral et al., 2015; Toral et al., 2011; Gallego and Arroyo, 2002; Salinas et al., 1991; Salinas et al., 1989), derivative fluorimetry (Salinas et al., 1990), near infrared spectroscopy (Szlyk et al., 2007), multivariate calibration methods, (Gallego and Arroyo, 2002), and fluorescence absorption (Carlotti et al., 2010). However, these analytical methodologies for the analysis of this antibiotic are not very common. Often, chelating or oxidizing reagents that react specifically with the drug and produce or enhance the color of the solution are used (Priya and Radha, 2014).

Several spectrophotometric methods based on colorimetric reactions have been described for the determination of OTC in pharmaceuticals, such as: ammonium vanadate (Abdel-Khalek and Mahrous, 1983), sodium cobaltinitrite (Mahrous and Abdel, 1984), ammonium molybdate (Morelli and Peluso, 1985), aminoantypirine (Ayad et al, 1986), iron III ions (Sultan et al., 1988), sodium molybdate (Jelikić-Stankov et al., 1989), cupric chloride (Saha, 1989), uranyl acetate (Saha et al., 1990), diphenyl-1-picrylhydrazyl (Emara et al., 1991), uranium VI (Rao et al., 1996), chloranilic acid (Fahelelbom, 2008), and zirconium IV ions (Prasad and Rao, 2010). As result, colored complexes are formed and measured in the range of 290–650 nm. Nevertheless, disadvantages of the use of such reagents include low sensitivity, the need for heating or cooling, high reagent concentrations, and the presence of toxic substances and large volumes of organic solvents (Rodríguez et al., 2016).

The spectrophotometric methods reported in the literature for the analysis of OTC in various matrices are summarized in Table 4.

Flow injection analysis

FIA is a well-established automated technique with numerous applications in quantitative chemical analysis (Tzanavaras and Themelis, 2007). This analytical method is based on the injection of a sample into a non-segmented carrier stream, which in turn is carried through a modulator towards the detector (Waseema et al., 2013). FIA is generally coupled with other analytical techniques as a detection system, such as: CL and spectrophotometry.

FIA coupled with CL is a powerful analysis tool, which enjoy not only the unique features involving automation, miniaturization, versatility, and inexpensiveness, but also lower limit of detections and wide linear ranges (Waseema et al., 2013). The mentioned advantages of FI-CL have increased the use of this technique in the analysis and quality control of pharmaceuticals.

FIA-CL methods have been described for the determination OTC in veterinary pharmaceuticals products (Townshend et al., 2005; Zheng et al., 2001; Zhang et al., 1995; Halvatzis et al., 1993). Anastasopoulos and Timotheou-Potamia (2011) developed a FIA-CL method for the analysis of OTC and other TCs in commercial products for human use, which showed high sensitivity with a detection limit of 0.015 μg mL$-$1 for OTC. This method was based on the chemiexcitation of the Al (III)-OTC highly fluorescent complex by using the permanganate or cerium (IV)-sulphite chemiluminogenic reactions.

FIA coupled with UV spectrophotometry is also popular among analytical and pharmaceutical chemists (Tzanavaras and Themelis, 2007). However, when developing a FI method based on direct UV measurement, special attention should be paid on the choice of the carrier stream. In order to avoid matrix effects or even potential precipitation of the analytes and clogging of the flow channels, the carrier and the solvent of the sample must be as consistent as possible. For this reason, fractions of organic solvents, such as methanol or ethanol, are frequently used as carriers (Tzanavaras and Themelis, 2007; Can et al., 2006; Yeniceli et al., 2005).

The determination of OTC in pharmaceuticals by FIA coupled with spectrophotometry detection is also reported in the literature (Rufino et al., 2009; Al-Momani and Kanan, 2008; Medina et al., 2000; Karlíček and Solich, 1994; Alwarthan et al., 1991). A recent methodology for direct quantification of OTC in veterinary pharmaceuticals using continuous FIA with spectrophotometric detection was developed by Rodríguez and colleagues (2016). This method was based on the reaction between the drug and diazotized p-sulfanilic acid, in basic medium, resulting in the formation of intense orange azo compounds with absorbance maxima at 434 nm, enabling spectrophotometric determination of the desired substance. According to

Table 4. Spectrophotometric methods used for the analysis of oxytetracycline in various matrices.

Matrix	Technique	Solvent	Wavelength (nm)	Limit of detection	Correlation coefficient	Linear range	Recovery (%)	Reference
Bulk material and pharmaceutical formulation (capsule and powder)	VIS absorption: colorimetric reaction with ammonium vanadate	ammonium vanadate solution in sulphuric acid	750	*	*	20–100 μg mL^{-1}	*	Abdel and Mahrous (1983)
Pharmaceutical formulation (capsule and powder)	colorimetric reaction with sodium cobaltinitrite	sodium cobaltinitrite solution in aqueous acetic acid	294	*	0.9999	10–30 μg mL^{-1}	99–102	Mahrous and Abdel (1984)
Bulk material and pharmaceutical formulation (capsule)	VIS absorption: colorimetric reaction with ammonium molybdate	ammonium molybdate solution in 50% v/v sulphuric acid	670	0.38 μg mL^{-1}	0.9996	*	*	Morelli and Peluso (1985)
Pharmaceutical formulation (injectable)	VIS absorption: colorimetric reaction with aminoantipyrine	water, aminoantipyrine, potassium ferricyamide, and chloroform	440	*	*	40–160 μg mL^{-1}	98–101	Ayad et al (1986)
Bulk material and pharmaceutical formulation (capsule)	VIS absorption: complexation reaction with iron(III)	sulphuric acid and ferric ammonium sulphate solution	435	*	*	10–200 μg mL^{-1}	103	Sultan et al. (1988)
Pharmaceutical formulation (injectable and capsule), urine, and honey	first-derivative	water and sodium acetate – acetic acid buffer pH 3.8	*pharmaceutical formulation*: 343 and 376 *urine*: 376 *honey*: 352	*	0.9999 and 1.0000	*	*pharmaceutical formulation*: 105–107 *urine*: 100–110 *honey*: 103–109	Salinas et al. (1989)
Pharmaceutical formulation (capsule)	VIS absorption: colorimetric reaction with sodium molybdate	water, sodium molybdate, sodium nitrate, and acetate buffer pH 5.5	404	2.5 μg mL^{-1}	0.9997	2.50–34.78 μg mL^{-1}	*	Jlikić-Stankov et al. (1989)
Pharmaceutical formulation (powder, injectable, capsule, tablet, suspension, and ointment)	VIS absorption: colorimetric reaction with uranyl acetate	uranyl acetate in dimethylformamide solution	406	*	0.9998	0–120 μg mL^{-1}	98–103.5	Saha et al. (1990)
Pharmaceutical formulation (injectable)	first-derivative fluorimetry	chloroacetic acid: chloroacetate buffer pH 3.8 and aluminum chloride 0.8 M	λ_{ex}: 390 λ_{em}: 500	2.0×10^{-3} μg mL^{-1}	0.9997	0.05–0.6 μg mL^{-1}	99.5	Salinas et al. (1990)
Honey	fourth-order derivative	acetate buffer pH 3.8	363	*	0.9980	*	89–109	Salinas et al. (1991)
Pharmaceutical formulation (capsule)	VIS absorption: colorimetric reaction with DHP in MeOH	acetate buffer pH 6, DHP, and MeOH	520	*	0.9976	2.5–20 μg mL^{-1}	99.9	Emara et al. (1991)

(*Continued on next page*)

Table 4. (*Continued*)

Matrix	Technique	Solvent	Wavelength (nm)	Limit of detection	Correlation coefficient	Linear range	Recovery (%)	Reference
Pharmaceutical formulation	VIS absorption: colorimetric with uranium (VI)	water, buffer solution pH 6, and 0.01 M uranium (VI)	413	*	*	*	99–102	Rao et al. (1996)
Bulk material and pharmaceutical formulation (ointment)	ratio spectrum zero-crossing derivative	water: ethanol: acetate buffer pH 4.5 (79:16:5, v/v/v)	*first-derivative order*: 253.4 *second-derivative order*: 286	*first-derivative order*: 1.85×10^{-1} mg L^{-1} *second-derivative order*: 2.16 mg L^{-1}	*first-derivative order*: 0.9996 *second-derivative order*: 0.9999	2.0–36 mg L^{-1}	95–105	Gallego and Arroyo (2002)
Bulk material	near infrared (NIR) combined with partial least-squares (PLS) regression method	water	**	0.20 mg mL^{-1}	*NIR*: 0.9984 *PLS*: 0.9678	1.0–6.1 mg mL^{-1}	95.47	Szlyk et al. (2007)
Bulk material and pharmaceutical formulation (capsule and injectable)	VIS absorption: colorimetric reaction with chloranilic acid	MeOH, borate buffer pH 9.0, chloranilic acid, and MeCN	540	0.50 μg mL^{-1}	0.9989	5.0–25 μg mL^{-1}	99–101	Fahelelbom (2008)
Bulk material, pharmaceutical formulation, and urine	VIS absorption: colorimetric reaction with zirconium (IV)	buffer solution pH 3, zirconium (IV) solution, and water	413.6	*	0.9994	1.0–33.5 μg mL^{-1}	98–102	Prasad and Rao (2010)
Bulk material	UV absorption and fluorescence	water, MeCN, DMSO, and EtOH	λ_{abs}: 269 and 354 (H_2O); 265 and 364 (MeCN); 265 and 360 (DMSO); 266 and 362 (EtOH) λ_{em}: 515 (only in H_2O)	*	*	*	*	Carlotti et al. (2010)
Fish feed	first-derivative	phosphate buffer pH 7.0 in presence of EDTA 0.1 M	382.2	5.77×10^{-6} mol L^{-1}	0.9992	1.88×10^{-5}–0.1×10^{-3} mol L^{-1}	89–123	Toral et al. (2011)
Salmon muscle and skin	second-derivative	oxalic acid in MeOH	393	271.0 μg kg^{-1}	> 0.997	8.0–40 μg kg^{-1}	90–105	Toral et al. (2015)

*Data not available **Wavenumber: 6200–5500 cm^{-1}; DHP, 2,2-diphenyl-1–picrylhydraxyl; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; EtOH, ethanol; MeCN, acetonitrile; MeOH, methanol; UV, ultraviolet; VIS, visible.

these researchers, the advantages of the proposed flow method include operational simplicity, high sampling frequency (52 samples per hour), and minimal generation of aqueous waste that can be easily treated.

It is worth to mention that the coupling of FIA with amperometric (Palaharn et al., 2003) and potentiometric (Couto et al., 1998) detection also was used for the determination of OTC in pharmaceutical formulation for human and veterinary use. These methods exhibited sensitivity and reproducibility for the analysis of OTC and other TC antibiotics.

Capillary electrophoresis

CE has recently become a very valuable tool for pharmaceutical analysis due to its high-resolution speed and the very small sample volume needed (Yuwono and Indrayan, 2005).

As mentioned by Mamani et al. (2006), many methods using CE for the determination of TCs and their impurities have been reported and several electrolytes like phosphate, phosphate and triton-X, phosphate and tris and methanol, sodium carbonate and EDTA, citric acid and alanine was employed (Tavares et al., 1994; Li et al., 1996; Van Schepdael et al., 1996; Pesek and Matyska, 1996; Li et al., 1997; Castellanos et al., 2000).

Tjørnelund and Hansen (1997) developed a simple method for the assay of OTC in ointments by CE. The drug was well separated from related impurities and degradation products using non-aqueous capillary electrophoretic technique with magnesium ion as metal chelate compound (Yuwono and Indrayan, 2005).

CE coupled with fast cyclic voltammetric detection was also used for the separation and determination of OTC and other TCs antibiotics, using boric acid–sodium tetraborate buffer as a complex (Zhou et al., 1999).

Mamani and coworkers (2006) validated an optimized capillary CE for the analysis of OTC and other TCs in pharmaceuticals products, using experimental planning to analyze the influence of fused-silica capillaries, the electrolyte composition, pH and concentration, as well as temperature and applied voltage. The optimal separation conditions found by the authors were 50 mmol L^{-1} sodium carbonate plus 1 mmol L^{-1} EDTA pH 10, voltage 13 kV, and temperature 23°C.

Chemiluminescence

In recent years, there has been increasing interest in the application of CL method for the determination of OTC in pharmaceutical matrix due to its low detection limits and wide linear working ranges (Han et al., 2000). Extra-weak CL generated during oxidation of OTC has been observed in the presence of H_2O_2 in basic and air-saturated solutions (Kruk et al., 1992).

Han et al. (2000) proposed a CL method using tris(2,2-bipyridine) ruthenium (II), a versatile base reagent of electrogenerated CL processes, for the quantification of OTC in commercial formulations. The method proved to be linear, sensitive with a low limit of detection (2.0×10^{-8} g mL^{-1}), and accurate.

Microbiological methods

As previously mentioned by Yuwono and Indrayan (2005), the United States Pharmacopoeia describes a microbiological method for the analysis of OTC and nystatin capsules, OTC and nystatin for oral suspension, OTC.HCl and hydrocortisone ointment, and OTC.HCl and polymyxin B sulfate ointment. Limpoka and co-workers (1987) also used this method for determination of OTC in elephant plasma after intravenous and intramuscular administration of Terramicina™ injectable solution.

These microbiological assays are based on the determination of the level of OTC by a microbiological response to a series of standard OTC concentrations by a strain of test microorganism. Although the microbiological techniques' are simple, versatile, and relatively cheap, they are time consuming and poor in terms of sensitivity and specificity (Yuwono and Indrayan, 2005).

Conclusion

This review describes OTC's chemical and pharmacological properties and also presents an overview of the analytical methods for quantification of this drug in biological matrices and pharmaceutical products. Despite the difficulties surrounding the chromatographic separation of TCs antibiotics, the HPLC is the technique most used for detection OTC and its related impurities in these matrices. This kind of study can serve as an updated source of research about the analytical profile of OTC, assisting in the development of more advantageous and sustainable methodologies that can be used in the quality control routine and in therapeutic drug monitoring.

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