DOXYCYCLINE HYCLATE: A REVIEW OF PROPERTIES, APPLICATIONS AND ANALYTICAL METHODS

ANA CAROLINA KOGAWA* AND HÉRIDA REGINA NUNES SALGADO

Departamento de Fármacos e Medicamentos, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Rodovia Araraquara-Jaú, km 1, 14801-902 Araraquara-SP, Brazil

ABSTRACT

The doxycycline hyclate is a broad-spectrum antibiotic oxytetracycline synthetic derivative used in several countries. It has been used to treat infectious diseases and as an additive in animal nutrition to facilitate growth. This drug is part of the list of medicines to the SUS (Unified Health System), a model of health care in Brazil, and it is free delivery in the public with a medical prescription. Thus, it is extremely important quality control of this medicine to be able to ensure their effectiveness and safety. Several existing analytical techniques, which offer flexible and broad-based methods of analysis and in some cases detection, have been discussed in this manuscript, focusing on bioanalytical and pharmaceutical quality control applications. This review will examine the published analytical methods reported for determination of doxycycline hyclate, discussing (a) separation methods such as thin layer chromatography and high performance liquid chromatography (HPLC) and (b) others such as, spectrophotometry and microbiological assay, from which it can be seen that HPLC methods have been used most extensively.

Key Words: doxycycline hyclate, analytical methods, quality control.

1. INTRODUCTION

The oxytetracycline (OTC) is a natural product produced by Streptomyces rimosus. Tetracycline (TC) is a semisynthetic derivative of chlortetracycline (CTC). Demeclocycline (DMC) is the product of a mutant strain of S. aureofaciens, while metacycline (MTC), doxycycline (DOX) and minocycline (MNC) are all semi-synthetic derivatives (Brunton LL et al. 2010).

The synthetic pathway of DOX involve MTC as an intermediate, during this process 6-epidoxycycline (EDOX) can be formed as a side product. DOX is a semisynthetic broad spectrum tetracycline antibiotic, widely used in veterinary medicine and as an animal feed supplement to prevent diseases (Fiori J et al. 2004).

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This drug is part of the list of medicines to the SUS (Unified Health System), a model of health care in Brazil, and it is free delivery in the public with a medical prescription. Thus, it is extremely important quality control of this medicine to be able to ensure their effectiveness and safety.

In Brazil this drug is marketed by pharmaceutical companies Apotex, EMS, Germed, Gross, Hexal, Legrand, Neo química, Neovita, Pfizer, Ranbaxy, Sanval, Teuto and União Química.

The significant increase in the number of drugs available, as well as the advancement and transformation of technologies used in the production of these have increased the interest of...
different sections of the society linked to health (regulatory agencies, governments, pharmacists) in the search for measures to ensure the integrity of the product available to the patient, taking the concepts of quality control outside the frame simply industrial but regulator.

The development of effective and reliable analytical methods for quality control of marketed drugs is extremely important and aims to provide reliable information about the nature and composition of the materials under analysis (La Roca MF et al. 2007). Validation is an important part of quality assurance program and aims to demonstrate that the analytical method is suitable for the intended proposal and it is safe to run (ICH 2005), and the procedures included in the standards of Good Manufacturing Practices (GMP) required by the U.S. FDA, and applied in pharmaceutical industries and should also occur according to good laboratory practice (GLP) (Shabir GA. 2003).

2. STRUCTURAL MODIFICATION

Tetracyclines are a group of broad-spectrum antimicrobial that has been used in the treatment of infectious diseases in humans and animals. Some derivatives of tetracycline, minocycline, oxytetracycline, metacycline, doxycycline, are frequently used in clinical practice and are based on the functionality of the molecular structure, which dominate the physical properties, appearance, and electronic and vibrational spectroscopic characteristics of the groups (Yasin A and Jefferies TM, 1988).

Some changes or maintenance in radical of the structure of tetracycline influence on antimicrobial activity.

![Chemical structure of tetracycline](image)

Any change in the oxygen of carbon 1 and in the hydroxyl of carbon 3 structure becomes the inactive structure. The group CONH$_2$ in the carbon 2 retains slight activity of the molecule. The N(CH$_3$)$_2$ on carbon 4 is essential for antimicrobial activity. The inclusion of OH-, CH$_3$CO-, RCOO- on carbon 5 retains the activity. The removal of OH-, CH$_2$- or both on the carbon 6 makes the compound more stable. The addition of Cl-, Br-, N(CH$_3$)$_2$- on carbon 7 retains activity, however, the presence of Cl- in the molecule present phototoxicity. The presence of Cl- or CH$_3$- on carbon 9 decreases the activity of the molecule.

3. STRUCTURAL FORMS

Doxycycline presents itself in three forms: hyclate, monohydrate and hydrochloride. From the doxycycline hyclate is possible to obtain other forms. The way hyclate dissolved in water and neutralized with sodium hydroxide becomes doxycycline monohydrate. This form with the addition of hydrochloric acid becomes doxycycline hydrochloride. Figure 2 illustrates the process above.
The percentage in the form of doxycycline hyclate is 86.6%, in the form hydrochloride is 89.1% and in the form of monohydrate is 96.1%.

4. **CHEMICAL STRUCTURE**

The doxycycline hyclate (Figure 3) is the form hemihydrate and hemiethanolate (Naidong W et al. 1990). His description is hygroscopic yellow crystalline powder, should be stored in airtight containers and protected from light (Reynolds JEF. 2007). It has the chemical name: 4 - (dimethylamine) -1,4,4\(^a\), 5,5\(^a\),6,11,12\(^a\)-octahidro-3, 5,10,12,12\(^a\) - pentahidroxi-6 - methyl-1,11-dioxo -2-naphthacene-carboxamide monohydrochloride monohydrate, combined with ethyl alcohol (Reynolds JEF. 2007).

This drug presents the molecular formula (C\(_{22}\)H\(_{24}\)N\(_2\)O\(_8\).HCl).\(_2\).C\(_2\)H\(_6\).H\(_2\).O, CAS 24390-14-5 and molecular weight 1025.89 g.mol\(^{-1}\) (Reynolds JEF. 2007). The doxycycline hyclate without burning fusion occurs in 201\(^\circ\)C (O’Neil MJ. 2006). Tetracycline pKas values are approximately 3-4, 7-8 e 9-10. Shariati and collaborators (2009) have the following pKa values for doxycycline hyclate: pK\(_{a1}\) 3.02 ± 0.3; pK\(_{a2}\) 7.97 ± 0.15; pK\(_{a3}\) 9.15 ± 0.3 (Figure 4). Doxycycline hyclate is sold in tablet dosage forms and lyophilized powder.

**Figure 2. Obtaining doxycycline monohydrate and doxycycline hydrochloride from the way hyclate.**

**Figure 3. Chemical structure of doxycycline hyclate (CAS 24390-14-5)**

**Figure 4. Functional groups corresponding to the pKa values.**
5. **MECHANISM OF ACTION**

Tetracyclines inhibit bacterial protein synthesis through their link to the bacterial 30S ribosome, impeding access of aminoacyl-tRNA acceptor site in the mRNA-ribosome complex (Brunton LL et al. 2010). However, DOX has been studied as an inhibitor of matrix metalloproteinases (intercellular substance), an action unrelated to its effects on bacterial protein synthesis (Skúlason S et al. 2003; Brunton LL et al. 2010).

6. **PHARMACOKINETICS**

Gastrointestinal irritation and perturbation of intestinal bacterial flora occur less frequently with doxycycline than with the more hydrophilic drugs, which must be given in higher doses for absorption (Aronson JK. 2006). As doxycycline is not interfered by food uptake is possible to improve the tolerability to administer the drug with food (Brunton LL et al. 2010).

Peak plasma concentration of 2.60 µg mL\(^{-1}\) was reported 2 hours after oral dose of 200 mg, falling to 1.45 µg mL\(^{-1}\) in 24 hours. After intravenous infusion of same dose the same peak plasma concentration was slightly higher, but became very similar to those observed after oral dosing in balance in the tissues (Martindale 2009).

About 80 to 95% of doxycycline in the circulation is linked to plasma proteins. The biological half-life ranges from 12 to 24 hours. Doxycycline is more lipid soluble than tetracycline. It is widely distributed in tissues and fluids. In patients with normal renal function about 40% of the dose is slowly excreted in the urine, although much will be excreted by this route if the urine is alkaline. However, most of the dose of doxycycline is excreted in the feces in the intestine after quelation. Although it was reported that doxycycline undergoes partial inactivation in the liver, many sources consider this doubtful, however, the kinetics of doxycycline has been reported altered in patients taking drugs that induce hepatic metabolism (Martindale 2009).

Doxycycline does not accumulate significantly in patients with kidney problems, presenting much fewer side effects (Brunton LL et al. 2010), although the excretion in the urine is impaired; larger amounts of doxycycline are excreted in the feces in these patients. However, it has been reported in patients with renal failure there is accumulation of doxycycline. The removal of doxycycline for hemodialysis is negligible (Martindale 2009).

7. **MICROBIOLOGICAL SPECTRUM**

Doxycycline is more active than tetracycline against many species of bacteria including *Streptococcus pyogenes*, enterococci, anaerobic, and various *Nocardia* spp. Cross-resistance is common, although some *Staphylococcus aureus* resistant to tetracycline respond to doxycycline.

Doxycycline is also more active against protozoa, particularly *Plasmodium* spp (Martindale 2009).

8. **CLINICAL APPLICATIONS**

Tetracycline has been widely used in the treatment of infectious diseases, and as additive in animal nutrition to facilitate growth (Brunton LL et al. 2010).

Often the choice is doxycycline over than other tetracyclines in the treatment of infections by their better absorption and long half-life, which allows fewer daily doses (Martindale 2009).

Doxycycline is useful for the treatment of respiratory tract infections because it provides a hedge against atypical micro-organisms, and since the respiratory pathogens are becoming increasingly resistant to other classes of drugs (Brunton LL et al. 2010). Another advantage is that it can also be administered to patients with kidney problems. However, relatively high doses to be administered in patients with urinary tract infection because of its low renal excretion (Martindale 2009).

It is often used to treat chronic prostatitis, sinusitis, syphilis, Chlamydia and pelvic inflammatory disease (Ramesh PJ et al. 2010). Doxycycline has action against protozoa and must be administered in combination with quinine in the management of chloroquine resistant *Plasmodium falciparum*.

Solutions of doxycycline are also used for malignant effusions (Yellin A. 1994) which
occurring when there is an increase in the amount of fluids, usually associated with malignancies and lymphomas of the lung, breast and ovary (Martindale 2009).

9. **ANALYTICAL METHODS**

9.1. **Spectrophotometric Method**
At British Pharmacopoeia (2010) the spectrophotometric method described uses methanol as solvent.

9.2. **Microbiological Method**
In abnormal conditions (heat, pH, moisture) tetracyclines undergo reversible epimerization at position C-4 and C-6 to form a mixture of degradation products. They are structurally very similar to doxycycline and between them (Monser L and Darghouth F, 2000). These products have very low antibiotic activity and many of them show some toxicity (Fiori J et al. 2004).

Kazemifard AG and Moore DE (1997) showed that the official methods of microbial analysis are time consuming and poor in terms of sensitivity and specificity, and another problem is the fact that the degradation products, such as 6-epidoxiciclina, which are present in varying amounts in raw materials and finished products may also have antimicrobial properties, 2-5 % of the activity of tetracycline.

Thus, the results of the bioassay would not necessarily be an accurate representation of antimicrobial potency.

9.3. **Thin Layer Chromatography (TLC)**
Naidong W and collaborators (1990) analyzed oxytetracycline and doxycycline by thin layer chromatography and compared with the high performance liquid chromatography. They obtained an excellent correlation ($r > 0.9999$) and relative standard deviation of the TLC for the analysis of the main component was < 2% and for HPLC < 1%. In comparison, the article says that it was observed that the different components are better separated by HPLC.

EDTA, as a chelating agent, binds in the possible metal layer of the silica and allow the elution of doxycycline, so it is essential in the analysis. Spraying of EDTA is the application faster and with better distribution. The alkaline pH preferably improves the chelating properties and prevents the formation of 4-epimers. With pH 9.0 all the impurities of oxytetracycline or doxycycline were well separated from the main compound and each other (Naidong W et al. 1990). The same authors also warn that the low water content in the mobile phase provides insufficient migration, while higher contents result in increased spread of the points.

9.4. **High Performance Liquid Chromatography (HPLC)**
The liquid chromatographic method for the determination of DOX is the choice of some Pharmacopoeias (F. Portuguesa, 2005; British Pharmacopoeia, 2010; USP 33, 2010). HPLC has also been applied for the determination of DOX and DOX-H in samples of liver and muscle tissue (Croubels S et al. 1998), plasma of turkey (Santos MDF et al. 1996), milk (Denobile M and Nascimento ES, 2004), medicated premixes for incorporation into medicated feed (Fiori J et al. 2004), pharmaceuticals (Monser L and Darghouth F, 2000) and bulk powders, tablets and human urine (Ramesh PJ et al. 2010). Both Monser L and Darghouth F (2000) and Ramesh and collaborators (2010) used in the mobile phase buffer, adjusted to pH 2 and 4, respectively.

Yasin A and Jefferies TM (1988) performed the analysis of tetracycline and its impurities by HPLC using column Hamilton PRP-1 polymer. The mobile phase was citrate phosphate buffer with 2-propanol, tetrahydrofuran and dichloromethane as organic modifier.

With the aim to reduce the analysis time, Hoogmartens and collaborators (1989) achieved mainly through the replacement of tetrahydrofuran by 2-methyl-2-propanol using polystyrene-divinylbenzene column.

Naidong W and collaborators (1990) describe another mobile phase for the analysis of doxycycline using PS-DVB column heated to 60°C. The mobile phase was 2-methyl-2-propanol: potassium phosphate buffer 2x10⁻¹ mol L⁻¹, pH 8.0: thiobarbituric acid (TBA) 2x10⁻² mol L⁻¹, pH 8.0: 1x10⁻² mol L⁻¹ EDTA, pH 8.0: water (5.8: 10: 5: 10: 69.2, v/v/v/v/v).

Bryan PD and Stewart JT (1993, 1994) proposed for separation of tetracyclines by HPLC mobile phase consisting of acetonitrile and 2x10⁻²
mol L$^{-1}$ sodium perchlorate, pH 2.0, and PS-DVB stationary phase.

Monser L and Darghouth F (2000) in a study by HPLC for the simultaneous determination of tetracycline and 6-epidoxiciclina powder in bulk and pharmaceutical preparations, used carbon packed column Hypercarb (porous graphitic carbon - PGC). The separation of oxytetracycline, metacyclina, chlortetracycline, doxycycline and 6-epidoxiciclina occurred in less than twelve minutes.

For the use of polymeric columns has been almost always in conjunction with the high temperature of the column, required the use of organic modifier viscous and alkaline mobile phase containing additives such as tetrabutylammonium ion or EDTA. In the study of Monser L and Darghouth F (2000) the pH 2.0 was selected to minimize the formation of isomeric analogues that occur rapidly in alkaline medium. In alkaline pH these compounds are deleted and their increased hydrophobicity and consequently these solutes can interact strongly with the hydrophobic surface of the PGC, the main reason for excessive retention. In acidic pH, these compounds are in ionized form, thus its hydrophobicity is decreased and so their retention. The best separation was achieved with potassium phosphate buffer (pH 2.0): acetonitrile (60:40, v / v), because with 50% acetonitrile the separation of oxytetracycline, metacyclina, chlortetracycline, doxycycline and 6-epi-doxycycline was not effective (Monser L and Darghouth F, 2000).

The PGC has advantages of physical and chemical stability and selectivity for diastereomeric and geometric isomers. Presents efficient separation of ionizable drugs and small molecules which are not retained on the column (ODS octadecilsiloxane). The PGC showed selectivity for separation of components, difficult or impossible to achieve in silica and polymeric (Monser L and Darghouth F, 2000).

In the development of a simple HPLC method for separation of doxycycline, Skúlason S and collaborators (2003) proposed the use of non-buffered mobile phase, but a mixture of acetonitrile: water: perchloric acid (26:74:0,25; v / v / v) adjusted to pH 2.5 with NaOH 5 M. The pH continued acidic (2.5) for two reasons: the ionized decreases the retention time and pH must be less than the pKa of doxycycline. The lifetime of the stationary phase is reduced at low pH, especially if the pH is below 2.0. The formation of isomeric analogues is decreased with low pH-value.

Skúlason S and collaborators (2003) They tested five columns. C18 ODS Hypersil (100 x 4.6 mm), Cosmosil C18 (150 and 250 x 4.6 mm) and Phenomenex Luna C8 (5 µm 150 and 250 x 4.6 mm). The best results were found using Phenomenex Luna C8 (5 µm 250 x 4.6 mm) with Phenomenex C8 guard column (4 x 10 mm). However, even with all the concern for reducing the time of analysis, the retention time with these parameters was 25.16 minutes.

Fiori J and collaborators (2004) proposed the method development for HPLC to analysis the doxycycline in a drug mixture for incorporation in food. For this, we tested two columns, Phenomenex Luna C18 3.5 µm (150 x 2.0 mm) and Phenomenex Synergi Polar RP-80A 4 µm (150 x 2.0 mm), with different mobile phases, oxalic acid (pH 2.5, 2x10$^{-2}$ mol L$^{-1}$): acetonitrile: methanol (75:17:8, v / v / v) and oxalic acid (pH 2.5, 2x10$^{-2}$ mol L$^{-1}$): acetonitrile (82:18, v / v), respectively. The mobile phase containing oxalic acid was able to improve the separation and symmetry of the peak.

The column Phenomenex Synergi Polar-RP 80A 4 µm (150 x 2.0 mm) was useful for analysis of tetracyclines and achieved the separation of metacyclina, 6-epidoxiciclina and doxycycline. A Phenomenex Luna C18 column 3.5 µm (150 x 2.0 mm) can be used to separate impurities doxycycline (metacyclina and 6-epidoxiciclina) when this is the main objective. Using the Luna C18 column retention time of doxycycline and impurities occurred in 12 and 9.5 minutes, respectively (Fiori J et al. 2004).

In Pharmacopoeia Portuguese (2005), British Pharmacopoeia (2010) and USP 33 (2010) the method of high performance liquid chromatography (HPLC) describes the use of buffer and column temperature controlled at 60°C.
**Table 1. Methods for determination of doxycycline in biological fluids and dosage forms**

<table>
<thead>
<tr>
<th>Method</th>
<th>Conditions</th>
<th>Detection system</th>
<th>Matrices</th>
<th>Reference</th>
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<tbody>
<tr>
<td>HPLC</td>
<td>Waters Nova-Pak column RP8 (150 mm x 3.9 mm). Mobile phase &quot;A&quot;: 1x10^{-2} mol L^{-1} oxalic acid: ACN: TEA (90:9:0:1, v/v/v); mobile phase &quot;B&quot;: ACN.</td>
<td>UV at 363 nm</td>
<td>Milk</td>
<td>Denobile M and Nascimento ES, 2004</td>
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<tr>
<td>HPLC</td>
<td>Nucleosil 100-5 C18 column (250 mm × 4.6 mm) and guard column (10 mm × 4.6 mm). Mobile phase: ACN: 1x10^{-2} mol L^{-1} hydrogenated sodium phosphate (20:80, v/v) and sodium salt octanesulfonic 5 mmol L^{-1} tetrabutylammonium hydrogenated sulfate 3 mmol L^{-1} and 0.01% EDTA, pH adjusted to 3.8 with phosphoric acid</td>
<td>UV in 370 nm</td>
<td>Sheep’s milk</td>
<td>Fletouris DJ et al. 2008</td>
</tr>
<tr>
<td>HPLC</td>
<td>Column Zorbax SB C18 (250 mm × 4.6 mm). Mobile phase: 10 mmol L^{-1} potassium phosphate buffer (pH 5.1): methanol (95:5, v/v)</td>
<td>UV in 230 nm</td>
<td>Standard tablets</td>
<td>Gaudiano MC et al. 2008</td>
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Table 1 (continuation). Methods for determination of DOX in biological fluids and dosage forms.

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<tr>
<td>HPLC</td>
<td>Column Phenomenex Synergi Polar-RP 80-A (150 mm x 2 mm). Mobile phase: 2x10^{-2} mol L^{-1} oxalic acid (pH 2.5): ACN (82:18, v/v)</td>
<td>UV at 346 nm</td>
<td>Drug mixture for food</td>
<td>Fiori J et al. 2004</td>
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<tr>
<td>HPLC</td>
<td>Phenomenex Luna C18 column (150 mm x 4.5 mm) and guard column µBondapak C18. Mobile phase: ACN: 2.3x10^{-2} mol L^{-1} phosphate buffer (25:75, v/v) with pH adjusted to 3.0 with a 4x10^{-2} mol L^{-1} octanesulfonic acid</td>
<td>UV at 244 and 350 nm</td>
<td>Human serum</td>
<td>Li C et al. 2004</td>
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<tr>
<td>HPLC</td>
<td>PS-DVB column of poly (styrene-divinylbenzene) (250 mm x 4.6 mm). Mobile phase: 2-methyl-2-propanol: potassium phosphate buffer 2x10^{-1} mol L^{-1} (pH 8): hydrogenated tetrabutylammonium sulfate 2x10^{-2} mol L^{-1} (pH 8), 1x10^{-2} mol L^{-1} EDTA (pH 8): water (5.8: 10:5:10:69,2, v/v/v/v/v)</td>
<td>UV at 254 nm</td>
<td>Commercial samples and standards</td>
<td>Naidong W et al. 1990</td>
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<tr>
<td>HPLC</td>
<td>Symmetry Shield RP8 column (150 mm x 4.6 mm) and Brownlee RP-8 pre-column. Mobile phase: 1x10⁻² mol L⁻¹ potassium acid phosphate: methanol: ACN (72.1:20:5 v/v) 3x10⁻² mmol L⁻¹ sodium EDTA with 60% perchloric acid (2.9 mL), adjusted to pH 2.5 with potassium hydroxide</td>
<td>UV at 350 nm</td>
<td>Plasma of rat brain</td>
<td>Colovic M and Caccia S, 2003</td>
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<td>HPLC</td>
<td>C18 column PLRP-S (PSDVB) (250 mm × 4.6 mm, 5 µm) and PM-C18 column (150 mm x 4.6 mm). Mobile phase: ACN: 2x10⁻² mol L⁻¹ sodium perchlorate (pH 2) (15:85, v/v)</td>
<td>UV at 280 nm</td>
<td>Standard and bulk powder</td>
<td>Bryan PD and Stewart JT, 1993; Bryan PD and Stewart JT, 1994</td>
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<tr>
<td>HPLC</td>
<td>Rogel Column (RSL) (250 mm x 4.6 mm). Mobile phase: tetrahydrofuran:</td>
<td>UV at 280 nm</td>
<td>Standards</td>
<td>Hoogmartens J et al. 1989</td>
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<td></td>
<td>dimethylformamide: acetic acid: water: EDTA (72:10:16:2:0.0015, v/v/v/v/m)</td>
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<tr>
<td>HPLC</td>
<td>PGC column (100 mm x 4.6 mm). Mobile phase: potassium phosphate buffer 5x10^{-2} mol L^{-1} (pH 2): ACN (40:60, v/v)</td>
<td>UV at 268 nm</td>
<td>Bulk powder and tablets</td>
<td>Monser L and Darghouth F, 2000</td>
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<tr>
<td>HPLC</td>
<td>Pre-column and column PLRP-S and PRP-1 (Hamilton) (250 mm x 4.6 mm). Mobile phase: 2-methyl-2-propanol: 2x10^{-1} mol L^{-1} potassium phosphate buffer (pH 8): 2x10^{-2} mol L^{-1} tetrabutylammonium sulfate (pH 8), 1x10^{-1} mol L^{-1} EDTA (pH 8): water (6:10:5:1, 78, v/v/v/v/v)</td>
<td>UV at 254 nm</td>
<td>Standard</td>
<td>Hoogmartens J et al. 1989</td>
</tr>
<tr>
<td>HPLC</td>
<td>Column styrene-divinylbenzene copolymer (250 mm x 4.6) maintained at 60°C. Mobile phase: 2 – methyl – 2 - propanol, tetrabutylammonium hydrogen sulphate, sodium edetate, with pH adjusted to 8</td>
<td>UV at 254 nm</td>
<td>Standard</td>
<td>British Pharmacopoeia, 2010</td>
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<tr>
<td>HPLC</td>
<td>C18 column (300 mm x 4.6 mm) and mobile phase: potassium acid phosphate 5x10^{-2} mol L^{-1} (pH 2.5): ACN (84:16, v/v)</td>
<td>Electrochemistry 1,2 V</td>
<td>Bulk powder, tablets and capsules</td>
<td>Kazemifard AG and Moore DE, 1997</td>
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<tr>
<td>HPLC</td>
<td>Hypersil BDS C8 column (250 mm x 4 mm). Mobile phase: buffer 1x10^{-2} mol L^{-1} potassium dihydrogen phosphate adjusted to pH 4 with 1x10^{-1} mol L^{-1} potassium hydroxide: ACN (60:40, v/v)</td>
<td>UV at 325 nm</td>
<td>Plasma of turkey</td>
<td>Ramesh PJ et al. 2010</td>
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<tr>
<td>HPLC</td>
<td>Supelcosil™ C18 column (150 mm x 4.6 mm). Mobile phase: 5x10^{-3} mol L^{-1} oxalic acid adjusted to pH 2.4: ACN: methanol (71:16:13, v/v)</td>
<td>UV at 360 nm</td>
<td>Bovine Milk, human plasma and water samples</td>
<td>Shariati S et al. 2009</td>
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<tr>
<td>HPLC</td>
<td>Phenomenex Luna C8 column (250 mm x 4.6 mm) with Phenomenex C8 guard column (4 mm x 10 mm). Mobile phase: ACN: water: perchloric acid (26: 74: 0.25, v/v/v) adjusted to pH 2.5 with 5 mol L^{-1} sodium hydroxide</td>
<td>UV at 350 nm</td>
<td>Standard</td>
<td>Skúlason S et al. 2003</td>
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<tr>
<td>HPLC</td>
<td>PLRP-S polymeric column and pre-column (250 mm x 4.6 mm and 5 mm x 3 mm, respectively). Mobile phase: 1x10^{-2} mol L^{-1} oxalic acid: ACN: methanol (80:15:5, v/v/v).</td>
<td>Fluorescence (excitation at 406 nm and emission at 515 nm)</td>
<td>Samples of liver and muscle tissue</td>
<td>Croubels S et al. 1998</td>
</tr>
<tr>
<td>HPLC</td>
<td>Column styrene-divinylbenzene copolymer (250 mm x 4.6) maintained at 60° C. Mobile phase: 2-methyl-2-propanol, tetrabutylammonium hydrogen sulphate, sodium edetate, with pH adjusted to 8</td>
<td>UV at 254 nm</td>
<td>Standard</td>
<td>F. Portuguesa, 2005</td>
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<tr>
<td>HPLC</td>
<td>Column containing C18 packing and maintained at 60° C. Mobile phase: 2.72 g of potassium phosphate monobasic, 0.74 g NaOH, 0.50 g of tetrabutylammonium hydrogen sulphate and 0.40 g of sodium edetate to 1000 mL of water. Addition of 60 g of tertiary butyl alcohol with the pH adjusted to 8 using 1 mol L^{-1} NaOH</td>
<td>UV at 270 nm</td>
<td>Standard</td>
<td>USP 33, 2010</td>
</tr>
</tbody>
</table>

_HPLC = high performance liquid chromatography, ACN = acetonitrile._
10. CONCLUSION

The advantages for antibiotics of the HPLC method in opposition the microbiological are specificity, speed and accuracy (Thomas AH. 1987). However, the lower antimicrobial activity of potential contaminants in a sample can be detected by bioassay, both in shape / size of the spread of antibiotic action in agar, using agar diffusion method, as the suspension of microorganisms by turbidity, using the turbidimetric method. The main advantage of this is the shortest analysis time (F. Bras. V, 2010).

The existence of bioassays with HPLC analysis provides an important approach in the quality of doxycycline hyclate. After all, the quality control is that the regulatory process by which measures actual quality performance, comparing it with the quality objectives and act on the difference (Juran JM and Gryna FM, 1991).

11. ACKNOWLEDGMENTS

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