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Simple and clean determination of tetracyclines by flow injection analysis

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ABSTRACT

An environmentally reliable analytical methodology was developed for direct quantification of tetracycline (TC) and oxytetracycline (OTC) using continuous flow injection analysis with spectrophotometric detection. The method is based on the diazo coupling reaction between the tetracyclines and diazotized sulfanilic acid in a basic medium, resulting in the formation of an intense orange azo compound that presents maximum absorption at 434 nm. Experimental design was used to optimize the analytical conditions. The proposed technique was validated over the concentration range of 1 to 40 μg mL^{-1}, and was successfully applied to samples of commercial veterinary pharmaceuticals. The detection (LOD) and quantification (LOQ) limits were 0.40 and 1.35 μg mL^{-1}, respectively. The samples were also analyzed by an HPLC method, and the results showed agreement with the proposed technique. The new flow injection method can be immediately used for quality control purposes in the pharmaceutical industry, facilitating monitoring in real time during the production processes of tetracycline formulations for veterinary use.

1. Introduction

Tetracyclines show antimicrobial activity against a wide range of gram-positive and gram-negative bacteria [1]. These low cost antibacterial compounds constitute one of the most widely used antibiotic groups, both in human medicine for the treatment of infectious diseases, as well as in the stockbreeding sector as preventive and curative drugs. They are also employed as additives in animal feed to promote rapid growth and weight gain of the animal [2].

Commercially available veterinary pharmaceuticals intended for the protection of animal health should meet the guidelines and regulations of the National Health Surveillance Agency (Portuguese acronym: ANVISA) [3]. Inappropriate dosages can cause allergic reactions and may increase the number of infections by resistant strains. Therefore, purity evaluation of intermediary products, as well as quality control of the final products, is vitally important at the industrial scale. This can be prejudicial to human and animal health, because conventional treatments may become ineffective [4].

Numerous analytical methods have been reported for the determination of tetracycline (TC) and oxytetracycline (OTC) in pharmaceuticals, including capillary electrophoresis [5], chemiluminescence [6], fluorimetry [7], near-infrared spectroscopy [8], and high performance liquid chromatography (HPLC) [9–11]. However, most of these methods require qualified personnel and specialist equipment, or have low sampling frequency.

The British Pharmacopeia [12], the United States Pharmacopeia [13], and the Association of Official Analytical Chemists (AOAC) [14] recommend the use of HPLC for the analysis of pharmaceuticals in their pure forms and in formulations. HPLC techniques are also widely used in the pharmaceutical industry for production monitoring, quality control of final products, stability testing, and evaluation of impurities [15]. An important disadvantage of this technique is the high cost of the equipment required.

Flow injection analysis (FIA) methodologies offer important advantages such as operational simplicity, low cost of analysis, high sampling rate, good precision, and the ability to investigate the kinetics of certain reactions. They can also be readily automated, have low susceptibility to contamination, and consume only modest amounts of reagents and samples [7,16]. They are environmentally safer [17,18], considering the substantial reductions in the amounts of waste generated, compared to other techniques [9–14].

Various flow analysis procedures have been described for the determination of TC and OTC in veterinary samples, using amperometric [19], chemiluminescent [20–22], and spectrophotometric detection [23–26]. However, employment of these methodologies involves the use of relatively high cost instrumentation [19], as well as expensive [20] and/or toxic [21,22] reagents. Other difficulties include the need for rigorous pH control [23], together with low sampling frequency [24], low sensitivity [25], and requirements for heating and cooling [26].

Several spectrophotometric methods based on colorimetric reactions have been described for the determination of tetracyclines in pharmaceutical preparations and biological fluids, using reagents such as ammonium vanadate [27], copper chloride [28], diphenyl-1-picrylhydrazyl [29], sodium molybdate [30], chloranilic acid [1],

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chloramine-T [31], p-N,N-dimethylphenylenediamine and sodium metaperiodate [32], zirconium (IV) [33], sodium tungstate [34], uranyl acetate [35], 4-aminophenazone and potassium iodate [36], as well as diazonium salts derived from p-nitroaniline [37] and 4-aminoantipyrine [38]. Nevertheless, disadvantages of the use of such reagents include low sensitivity [28,34], the need for heating [31] or cooling [37,38], and high reagent concentrations [28,30]. Furthermore, the presence of toxic substances [29,32,33,35,36] or large volumes of organic solvents [27] can result in harm to human health and the environment.

The aim of the present study was to develop an environmentally reliable analytical methodology for direct quantification of tetracycline (TC) and oxytetracycline (OTC) (Fig. 1) in veterinary pharmaceuticals, using continuous flow injection analysis with spectrophotometric detection. The proposed method is based on the reaction between tetracyclines and diazotized p-sulfanilic acid, in basic medium. This results in the formation of intense orange azo compounds with absorbance maxima at 434 nm, enabling spectrophotometric determination of the desired substances.

2. Experimental

2.1. Apparatus

The continuous flow analysis system (Fig. 2) consisted of an ASIA analyzer (Ismatec, Zürich, Switzerland) equipped with a variable speed (1–50 rpm) four-channel peristaltic pump (Model 7610, Rheodyne, USA), Tygon tubing (2.06 and 1.85 mm i.d.) for fluid propulsion, an automatic sample injection valve (Model 5041, Rheodyne, USA), and an acrylic confluence point. The reaction coil (204 cm length), reagent lines, and sampling loop (120 cm length, 603 μL internal volume) were constructed from 0.8 mm i.d. polytetrafluoroethylene tubes. Measurement of absorbance at 434 nm employed a diode array spectrophotometer (Model HP 8453A, Hewlett Packard, USA) coupled to a quartz flow cell (10 mm optical path length, 80 μL internal volume). Data acquisition used UV–Visible ChemStation software (Agilent).

The HPLC analyses were performed using a Shimadzu Prominence system equipped with an LC-20AT quaternary gradient pump, a SIL-20A HT autosampler, an SPD-M20A diode array detector, a CTO-20A column oven, and a CBM-20A software interface. LCsolution software was used for data acquisition and processing. A C18 reversed phase analytical column (150 × 4.6 mm i.d., 5 μm) was employed for the chromatographic determinations.

2.2. Reagents and preparation of stock and working solutions

2.2.1. Chemicals

All the reagents used were analytical grade. Deionized water (18 MΩ cm, Milli-Q system, Millipore) and common laboratory glassware were used for preparation of the samples and working solutions. Hydrochloric acid (37% purity, 1.85 g cm^{-3}), sodium nitrite (99.0% purity), and p-sulfanilic acid (99.0% purity) were obtained from Merck. Sodium acetate (99.0% purity) and tetracycline standards (TC and OTC, 99.5% purity), were obtained from Sigma-Aldrich.

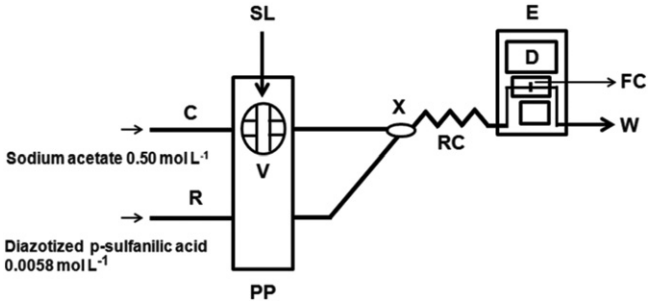

Fig. 2. Diagram of the proposed continuous flow procedure. C: carrier solution; R: chromogenic reagent; PP: peristaltic pump; V: injection valve; SL: sample loop (603 μL); X: confluence point; RC: reaction coil (204 cm); E: spectrophotometer; FC: flow cell; D: detector; W: waste.

2.2.2. Solutions

A stock solution of HCl (1.004 mol L^{-1}) was prepared by appropriate dilution of concentrated hydrochloric acid in deionized water in a 1 L volumetric flask, and was standardized by means of a volumetric procedure. A 0.50 mol L^{-1} stock solution of sodium nitrite was obtained by dissolving 3.45 g of the solid in deionized water in a 100 mL volumetric flask. An alkaline working solution of 0.50 mol L^{-1} sodium acetate was prepared by dissolving 10.25 g of the solid in deionized water in a 250 mL volumetric flask. Stock solutions of tetracycline and oxytetracycline, at concentrations of 100 μg mL^{-1}, were prepared daily by dissolving 0.0108 g of each antibiotic in deionized water in 100 mL volumetric flasks.

2.3. Preparation of the chromogenic reagent

An aqueous 0.0058 mol L^{-1} solution of p-diazotized sulfanilic acid (diazonium salt), used as the chromogenic reagent, was prepared by dissolving 0.25 g of p-sulfanilic acid in a 25 mL beaker and transferring quantitatively to a 250 mL volumetric flask. Finally, 2.5 mL of 1.004 mol L^{-1} HCl and 6 mL of 0.50 mol L^{-1} sodium nitrite were added, sequentially and with shaking, and the flask was completed with deionized water.

2.4. Preparation of samples

Six commercial veterinary pharmaceuticals containing tetracycline and oxytetracycline agents were obtained from local pharmacies in Araraquara city (São Paulo, Brazil) and included injectable solutions, tablets, and soluble powder. All were tested prior to the labeled expiry dates.

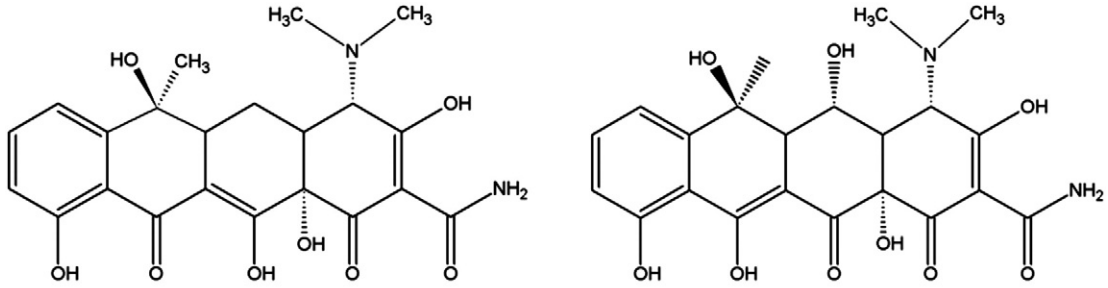

Fig. 1. Chemical structures of tetracyclines.