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การพัฒนาวิธีสเปกโทรโฟโตเมตริกอย่างง่ายสำหรับการวิเคราะห์ปริมาณเตตราไซคลิน

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บทคัดย่อ

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บทนำ : เตตราไซคลิน (2-(amino-hydroxy-methylidene)-4-dimethylamino-6,10,11,12a-tetrahydroxy-6-methyl-4,4a,5,5a-tetrahydro tetracene-1,3,12-trione) เป็นยาปฏิชีวนะที่ออกฤทธิ์ยับยั้งเชื้อแบคทีเรีย และนำมาใช้โดยทั่วไปอย่างกว้างขวางเพื่อป้องกันและบำบัดอาการติดเชื้อทางด้านสัตวศาสตร์ โครงสร้างทางเคมีของเตตราไซคลินประกอบด้วยอะโรมาติก 4 วงเชื่อมติดกันและมีหมู่ฟังก์ชันต่างๆ ซึ่งสามารถเกิดสารประกอบเชิงซ้อนกับไอออนของโลหะได้หลายชนิด **วัสดุและวิธีการ :** เตรียมสารละลายเตตราไซคลินสำหรับการวิเคราะห์ด้วยวิธีการทางสเปกโทรโฟโตเมตริกให้มีความเข้มข้นอยู่ในช่วง 1.0 ถึง 100.0 ไมโครกรัมต่อมิลลิลิตรในขวดปริมาตรขนาด 25 มิลลิลิตร โดยการเจือจางสารละลาย เตตราไซคลินมาตรฐานเข้มข้นตามปริมาณที่เหมาะสมด้วยน้ำกลั่นปราศจากไอออน แล้วนำมาผสมกับน้ำแร่ซึ่งใช้เป็นรีเอเจนต์ในหลอดทดลอง จะได้สารละลายสีเหลืองอ่อนซึ่งเกิดจากปฏิกิริยาระหว่างเตตราไซคลินกับน้ำแร่โดยใช้เวลาในการเกิดสีของสารละลายเพียงสั้นๆ แล้วนำสารละลายเตตราไซคลินมาตรฐานและสารละลายตัวอย่างไปวัดค่าการดูดกลืนแสงด้วยเครื่องยูวี-วิสิเบิลสเปกโทรโฟโตมิเตอร์ **ผลการศึกษา :** วิธีการนี้อาศัยการตรวจวัดทางสเปกโทรโฟโตเมตริกของสารประกอบเชิงซ้อนสีเหลืองอ่อนซึ่งเกิดจากปฏิกิริยาระหว่างเตตราไซคลินและน้ำแร่ซึ่งใช้เป็นรีเอเจนต์ ช่วงความเป็นเส้นตรงอยู่ในช่วง 0.0 – 50.0 ไมโครกรัมต่อมิลลิลิตร ให้ความสัมพันธ์เป็นเส้นตรงแทนด้วยสมการ $y = 0.0230x + 0.0081$ มีค่าสัมประสิทธิ์สหสัมพันธ์ เท่ากับ 0.9998 ค่าการเบี่ยงเบนมาตรฐานสัมพัทธ์ของวิธีที่นำเสนอจากการวิเคราะห์เตตราไซคลินความเข้มข้น 1.0, 10.0 และ 20.0 ไมโครกรัมต่อมิลลิลิตร จำนวน n=12 มีค่าเท่ากับ 0.68, 0.21 และ 0.08% ตามลำดับ ร้อยละการคืนกลับจากการเติมตัวอย่าง 3 ความเข้มข้น คือ 5.0, 13.0 และ 35.0 ไมโครกรัมต่อมิลลิลิตร มีค่าอยู่ในช่วง 99.14 – 99.77% **สรุปผล :** วิธีการที่นำเสนอนี้ประสบความสำเร็จในการยืนยันวิธีการตรวจสอบสำหรับการวิเคราะห์เตตราไซคลินโดยเป็นไปตามข้อกำหนดของเภสัชตำรับประเทศสหรัฐอเมริกา ฉบับที่ 34 ค.ศ.2011 และวิธีการนี้ไม่มีการใช้สารเคมีเป็น รีเอเจนต์ในปฏิกิริยาซึ่งจะช่วยลดปริมาณของเสียจากสารเคมีที่เป็นพิษได้

คำสำคัญ : สเปกโทรโฟโตเมตริก, เตตราไซคลิน, น้ำแร่

Abstract

Development of simple spectrophotometric method for tetracycline determination

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Introduction : Tetracycline (2-(amino-hydroxy-methylidene)-4-dimethylamino-6,10,11,12a-tetrahydroxy-6-methyl-4,4a,5,5a-tetra hydrotetracene-1,3,12-trione) has a broad-spectrum antibiotic and commonly used in veterinary medicine for disease prevention and treatment. The chemical structure of tetracycline is having a fused ring, partially aromatic, 4-ring structure with

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a wide variety of functional groups that form complexes with various metal ions. **Materials and methods :** Tetracycline solution for spectrophotometric analysis with concentrations ranging from 1.0 to 100.0 µg/mL were prepared in 25 mL volumetric flasks. The appropriate amounts of tetracycline stock solution were diluted with deionized distillated water which was mixed with the mineral water used as reagent in test tube. The light-yellow solution was occurred from the reaction between tetracycline and mineral water afterward leave it for a few minutes. The absorption signal of the working standard and sample solution were recorded using UV-Visible spectrophotometer. **Results :** The method is based on the spectrophotometric detection of the light-yellow complex formed by the reaction between tetracycline and mineral water used as green reagent. The linear regression equation over the range of 0.0 – 50.0 µg/mL was $y = 0.0230x + 0.0081$. The *correlation coefficient* (r^2) was found to be 0.9998. The relative standard deviation of the proposed method calculated from 12 replicate measurement of 1.0, 10.0 and 20.0 µg/mL tetracycline were 1.62, 0.21 and 0.08%, respectively. The percentage recoveries from spiked samples at three concentrations (5.0, 13.0 and 35.0 µg/mL) was ranged between 99.14 and 99.77%. **Conclusion :** The proposed method was successfully validated for tetracycline in compliance with requirements by The United States Pharmacopeia (USP 34) 2011. This method is not use chemical reagent in all the reactions that minimize the use of a toxic chemical wastes.

Keyword : Spectrophotometric, Tetracycline, Mineral water

Introduction

Tetracycline antibiotics (TCs) produced by *Streptomyces* are broad spectrum antibiotics ranging from gram-positive to negative bacterias, and are especially effective against *Staphylococcus*, *Streptococcus*, *Pneumococcus*, *Gonococcus*, *Cholera*, *Dysentery bacillis*, *Pertussis*, *Rickettsia*, *Chlamydia*, and *Mycoplasma*. TCs are actively transported into the cells of susceptible bacteria and exert a bacteriostatic effect by inhibiting protein biosynthesis after binding to the 30S ribosomal subparticle (Oka *et al.*, 2000). The chemical structure of tetracycline is show in Figure 1. There is having a fused ring, partially aromatic, 4-ring structure with a wide variety of functional groups that form complex with various metal ions in aqueous solution (Ruengsitagoon, 2008).

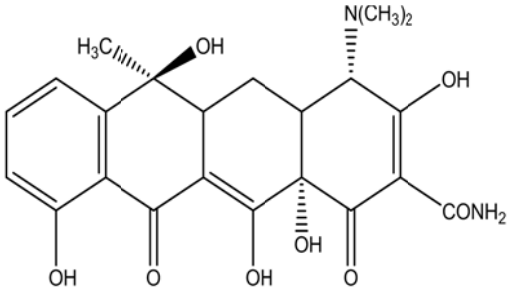

Figure 1. The chemical structure of tetracycline

Spectrophotometric methods are the most widely used for the determination of tetracycline in bulk and pharmaceutical products (Saha *et al.*, 1990; Emara *et al.*, 1991; Sultan *et al.*, 1988). They usually involve the formation of a coloured derivative compound with metals, such as iron(III) (Sultan *et al.*, 1988; Saha, 1987), copper(II) (Alwarthan *et al.*, 1991), magnesium(II) (Chang *et al.*, 1992) and some lanthanide ions. The reaction can be used to determine tetracycline concentrations assuming the colour intensity and absorbance is proportional to the tetracycline concentration, the complex is stable, and the reagent does not significantly react with other constituents thereby causing interferences.

A lot of previously studies involve the use of chemical reagents for tetracycline determination that cause contamination in the environment. For example, the waste from such studies are not treated, there foreign substances could contaminate soil or natural water. Green chemistry, also called sustainable chemistry, is a philosophy of chemical research and engineering that encourages the design of products and processes that minimize the use of hazardous substances (Williams, 2001; Anastas and Warner, 2000). It aims to explore the use of alternative

reagents or alternative synthetic methods that minimize the use of toxic chemicals.

In this study, the present paper describes a simple, rapid and low cost method for the quantitative determination of tetracycline based on the spectrophotometric detection of the light-yellow complex formed by the reaction between tetracycline and various metal ions in mineral water which was used as an alternative natural reagent. The resulting light-yellow complex is measured at the wavelength of 365 nm. To enhance the precision and accuracy of the method, the internal validation used was compliant with The United States Pharmacopeia (USP 34), 2011 (The United States Pharmacopoeial Convention, 2011).

Material and method

Chemical and reagents

All chemicals were of analytical reagent grade and were used without further purifications. All solutions were prepared with distilled deionized water and used through the experiment.

Standard of tetracycline was supplied from tetracycline (Sigma-Aldrich®, USA). Mineral water reagent from a local convenient store was supplied and filtered through Whatman No 42 filter paper and kept at room temperature for further use. Distilled deionized water was purified in a ultra pure water system (Milli-Q®, France).

Apparatus

A Double Beam UV-Visible Spectrophotometer (UV-1700, Shimadzu®, Japan) with 1-cm quartz cuvette was used for measuring an absorption of the standard and sample solution.

Standard solutions

Stock standard solution of tetracycline was prepared by dissolving 0.0100 g of tetracycline in distilled deionized water and diluting to 100 mL in a volumetric flask. The solutions are stable for more than 1 month when kept at 4°C and protected from light.

General Procedure

Standard tetracycline working solutions for spectrophotometric analysis with concentrations ranging from 1.0 to 100 µg mL^{-1} were prepared in 25 mL volumetric flasks by diluting the appropriate amounts of the tetracycline stock solution with distilled deionized water and mixed with the mineral water reagent in test tube. Formation of the light-yellow colour of the complex does not change for several hours and each test tube was mixed well and transferred into the 1-cm quartz cuvette which was measured the absorption with spectrophotometer.

Sample preparation

For the quantification of drugs from the commercial products, twenty randomly selected capsules of Achromycin® (tetracycline) 250 mg were weighed, powdered and mixed well. The sample was prepared from the amount of powdered sample corresponding to one capsule. The sample was dissolved in deionized water and sonicated for 5 minutes. After that the sample was filtered through Whatman No 42 filter paper and diluted with distilled deionized water to volume in order to obtain the appropriate concentration for analysis. This aliquot was analyzed using the proposed simple spectrophotometric method.

Results and discussion

The studies were proof for a simple, rapid and low-cost simple spectrophotometric method to determine tetracycline in pharmaceutical products. To assess the validity of the proposed methods, analytical performance characteristics for determination of tetracycline in pharmaceutical products was studied under optimum conditions (Table 1).

Table 1. Variables rang studied optimum conditions for determination of tetracycline

Parameters studied	Optimum value
Ratio for complexation (tetracycline : mineral water)	2:1
Mineral water reagent concentration (µg mL^{-1})	None dilution
Tested types of mineral water	Mineré®
Tested adding of acid	None added acid
Tested adding of buffer solution	None added buffer

Calibration graph

Linearity of response was studied using tetracycline standard solutions containing 1.0 to 100.0 μg mL^{-1}. By plotting absorbance for each solution versus its tetracycline concentration, the linear calibration curve over the range of 0.0 – 50.0 μg mL^{-1} which can be expressed by the regression equation $y = 0.0230x + 0.0081$ (r^2=0.9998) where y represents the absorbance and x is tetracycline concentration in μg mL^{-1} after subtraction of blank. The limit of detection was defined as the concentration of analyte that gave the signal that was different from the blank by an amount equal to three times the standard deviation of the blank signal (3σ). It was found to be 0.001 μg mL^{-1} tetracycline.

Reproducibility and Accuracy

To evaluate the precision of the methods, measurements were performed under conditions of repeatability. There was tested in order to show if the instrument response for a standard solution was always the same. This parameter considers only the error attributable to the operating system and not the error attributable to sample handling and preparation. The instrumental precision was calculated from twelve consecutive measurements of 1.0, 10.0 and 20.0 μg mL^{-1} tetracycline standard solution. The relative standard deviation (RSD, %) was shown as 1.62, 0.21 and 0.08% for 1.0, 10.0 and 20.0 μg mL^{-1} tetracycline standard solution, respectively.

In order to evaluate the recoveries of the analytical method in pharmaceutical products, the samples were prepared at three different concentrations (5.0, 13.0 and 35.0 μg mL^{-1}). The recoveries were obtained by spiking various amounts of tetracycline solution. It can be seen, good recoveries were found to be ranged between 99.14 and 99.77%. Accuracy and precision of the method were in compliance with The United States Pharmacopeia (USP 34), 2011.

Interferences

The specificity of the method should be defined in terms of the species analysed. This is covered to some extent by the assessment of accuracy, since any interference from excipients and ions will confer a systematic error on the method. Effects of some possible interfering both excipients and ions on the determination of tetracycline and different concentrations of excipients and ions at 50 and 100 μg mL^{-1} were investigated. All excipients and ions tested caused interference lesser than ±12%. However, the most serious interference from iron(III) ions was observed. The possible masking reagent for increasing the effect of iron(III) was can be complex formed better than the other cations in mineral water. Then, iron(III) reagent could be used for qualitative analysis and quantitative analysis of tetracycline by previously reported (Sultan *et al.*, 1988; Alwarthan *et al.*, 1991; Sultan *et al.*, 1992).

Conclusion

This work was to develop and to validate a simple, rapid and low-cost simple spectrophotometric method for determining tetracycline in pharmaceutical products. The linearity of the calibration graph is in the useful range for quantitative of tetracycline in pharmaceutical products. The procedure is very suitable for the routine quality control of tetracycline antibiotics in pharmaceutical products. This method is not require chemical reagent in all the procedure that minimize the use of a toxic chemical.

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