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Kinetic Spectrophotometric Method for the Quantitation of Telithromycin in Bulk and Drug Formulation

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ABSTRACT

A kinetic method for the accurate and sensitive determination of telithromycin has been described. The method is based on the oxidation of telithromycin with alkaline $KMnO_4$. At a fixed time of 24 min. the formed magnate ion is spectrophotometrically measured at 610 nm. The concentration of telithromycin is calculated using the calibration equation for the fixed time and initial rate methods. Beer´s law was obeyed from 3.24-12.9µg ml^{-1} The assays limits of detection were 0.2-0.70µg ml^{-1} and the R.S.D. (n= 5) was 0.6 %. Recovery was 99.8 and 100.83%. The analytical performance of the, in terms of accuracy and precision, were statistically validated, the results were satisfactory RSD $\leq$ 2%. The method has been applied successfully to commercial tablet dosage form. Statistical comparison of the result with the reference method showed excellent agreement and proved that no significant difference in the accuracy and precision.

Keywords: Telithromycin, Kinetics, Spectroscopic determination, $KMnO_4$.

INTRODUCTION

3 -De[(2,6-dideoxy-3-*C*- oxy] -11,12-dideoxy-6-*O*-methyl-3-oxo-12,11-[oxycarbonyl[4-[4-(3-pyridinil)-1*H*-imidazol-1-1-yl]butyl]imino] erythromycin; (Fig. 1) is the first antibiotic belonging to a new class of 14-membered ring Macrolide, named ketolides, to achieve clinical use. This new addition to the macrolide-lincosamide-streptogramin (MLSB) group was developed specifically for the treatment of community-acquired respiratory tract infections. [1] The discovery of ketolides, derived from erythromycin incorporating a C-3 ketone modification, revealed a class of compounds with excellent activity against some Macrolide-resistant bacteria, especially clinically important respiratory tract pathogens, such as *Streptococcus pneumoniae*. The positive results showed by the use of ketolides could be responsible for the increase of Macrolide antibiotics research in the pharmaceutical industry. [2] Assays reported in the literature for the determination of the TEL in biological fluids include high-performance liquid chromatography-mass spectrometry LC-MS, [3] LC with fluorescence detection [4] and microbiological assays. [5] Other researchers have described a sensitive method for the examination of 20 Microlide and ketolides in bulk, using an LC method with a volatile mobile phase, allowing the recovery and subsequent analysis of these antibiotics. [6] A microbiological assay in pharmaceutical dosage form applying cylinder-plate method was performed and described in a previous study. [6] The microbiological assay can reveal subtle changes not demonstrable by chemical methods. This assay makes it possible to evaluate the potency, which is very important in the analysis of antibiotics. [7] Kinetic spectrophotometric methods use in chemical and pharmaceutical analysis has gained special interest as they offer many advantages over regular analytical techniques. These advantages include improved selectivity and a decrease of interferences caused

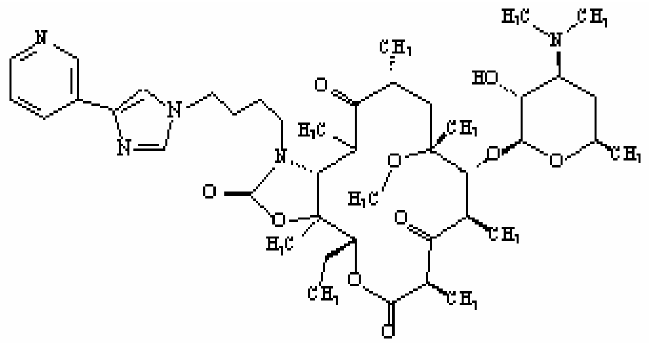

Fig. 1: Chemical Structural of Telithromycin

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by colored or turbid samples during testing. [8] However, no attempts were cited in the literature for the determination of TEL using kinetic spectrophotometric methods. The present work describes the development of simple and sensitive kinetic spectrophotometric methods for the determination of TEL in bulk and pharmaceutical formulations. These methods are based on the oxidation of TEL with alkaline $KMnO_4$. The initial rate and fixed time methods were adopted for determination of TEL in commercial formulations. These reactions were followed spectrophotometrically using the rate of color development at 610 nm.

MATERIALS AND METHOD

Materials

All reagents and solvents used were of analytical reagent grade. Double distilled water was used to prepare all solutions.

Potassium permanganate ($KMnO_4$) (Merck, Germany): 6 $\times 10^{-3}$ M in distilled water.

Sodium hydroxide (NaOH) (El Nasr Chemical Co., Abu Zabel, Egypt): 1.0 M in distilled water.

Telithromycin reference standard (99.5%) and the pharmaceutical dosage form were kindly supplied by Aventis Pharma.

Shimadzu model UV-1800 recording spectrophotometer (p/n) equipped with kinetic accessory and temperature controlled cell (tec-240A). Recording range 0 to 1, wavelength 610 nm factor = 1, number of cell 1, reaction time 20 minutes, cycle time 0.1

Preparation of Standard stock solution

Into a 50 ml calibrated flask, an accurately weighed amount (100 mg) of the standard drug and was dissolved in 30 ml water. The resulting solution was completed to volume with water. The Stock solution of TEL containing (2 mg ml^{-1}) was diluted with distilled water to obtain working concentrations solution in the range of 3.24-12.9µg ml^{-1} (4×10^{-4} - 18×10^{-4} M) for TEL drug.

Preparation of sample solution of pharmaceutical tablets

Twenty tablets were weight and finely powdered. A quantity of the mixed powdered to 100 mg of TEL was transferred into a 50 ml calibrated flask ,dissolved in 25 ml water, swirled and sonicated for 5 min, completed to volume with water, shaken well for 10 min, and filtered. The first portion of the filtrate was rejected, and 25 ml of the filtrate was diluted with water to obtain working concentration in the range of 3.24- 12.9µg ml^{-1} for TEL drug.

Procedure

Aliquots of 1ml $KMnO_4$ solution (0.6×10^{-2} M) and 1.0 ml of 1 M NaOH solution are transferred to 10 ml calibrated flask. Accurate volume of working solution (8.12µg ml^{-1}) over the concentration range 3.24-12.9µg ml^{-1} for Telithromycin, are added, and the solution are mixed well and diluted to volume with water. At a fixed time of 25 min, the absorbance was measured directly 610 nm for TEL against an appropriate blank. The calibration graph was constructed by plotting the final concentration of the drug against absorbance values, measured at a fixed time of 24 min. alternatively; the corresponding regression equation was derived.

Data acquisition and processing

The kinetic data recorded for each method was manipulated using (Slide Write plus) software for curve fitting, regression and statistical analysis. The initial rate (ν) of the reaction at different concentrations was obtained from the slope of the tangent the absorbance–time curve. A calibration curve was constructed by plotting (log ν) of the reaction vs. (log C) for TEL As well; calibration curves were constructed by plotting the absorbance measured after a fixed time of (20 min) for $KMnO_4$.

RESULTS AND DISCUSSION

Kinetic and optimization of the reaction conditions

Oxidation- reduction reactions have been used as the basis for the development of simple and sensitive spectrophotometric methods for the determination of many pharmaceutical compounds. $KMnO_4$ is a strong oxidant, and its oxidation for the organic compounds is a pH dependent. The reaction between TEL and $KMnO_4$ in alkaline solution yields a green color as a result of magnate species, which absorbs at 610 nm as intensity of color increase with time (Fig. 3). It was deemed useful to elaborate a kinetically based method for the determination of Telithromycin. In order to come this conclusion, the reaction was investigated under various conditions of reagent concentration and alkalinity.

Optimization of reaction conditions

The factors affecting reaction condition (concentration of $KMnO_4$, alkalinity, temperature and the diluting solvent). Potassium permanganate, as a strong oxidizing agent, has been used in the oxidimetric-based analytical method for the determination of many compounds. During the course of the reaction, the valence of manganese changes and intermediate ions has been suggested participating oxidants. The main oxidizing species and the extent of oxidation reaction depend on the nature of the substrate, and the pH of the reaction medium. In alkaline medium, the heptavalent manganese changes to the green colored Mn (VI), while in neutral and acidic medium the permanganate was the basis of its use in the development of the spectrophotometric methods. TEL was found to be susceptible for oxidation with alkaline $KMnO_4$ producing a green color peaking at 610 nm (Fig. 3). At this wavelength, the various experimental parameters affecting the development and stability of the reaction product were carefully studied and optimized.

Effect of potassium permanganate concentration

The reaction increases substantially with increasing the concentration of $KMnO_4$ in TEL (Fig. 3). Maximum absorbance was obtained when 2 ml of 6×10^{-3} M of $KMnO_4$ was used. Thus, the adoption of 2.5 ml of potassium permanganate in the final solution proved to be adequate for the maximum concentration of TEL used in determination process.

Effect of sodium hydroxide concentration

Maximum absorption was obtained when 1 ml of 1 M NaOH was used. Over this volume no change in absorbance could be detected obtained; 1.2 ml of 1 M of NaOH was used as an optimum value (Fig. 4).

Effect of temperature

At room temperature the reaction rate increase substantially with time, while heating the solution was found to increase the rate of the reaction however MnO_2 was precipitated, therefore room temperature was selected as the optimum temperature.

Kinetic of the reaction

Under the optimum conditions, the absorbance time curves of investigated TEL with $KMnO_4$ were constructed (Fig. 5). The initial rate of the reaction was determined from the slope of

Table 1: Analytical parameters for the initial rate method of the spectrophotometric method determination Telithromycin

Method	Linear range [M]	Least square equation (log k= log k´+ log n log C) [a]		Correlation Coefficient (r)	LOD (μg ml^{-1})	LOQ (μg ml^{-1})
		Intercept (log K)	Slope (n)			
$KMnO_4$	(4×10^{-4} – 18×10^{-4} M) (3.24- 12.9)[b]	0.76	1.054	0.9995	0.21	0.702

[a]ν is the reaction rate, K^1 is the conditional rate constant, n is the order of reaction, and C is the molar concentration of Telithromycin.

[b]Figures in parenthesis are the linear rang in μg ml^{-1}

Table 2: Analytical parameters for fixed time method of the kinetic spectrophotometric method for determination of Telithromycin with alkaline $KMnO_4$

Reaction time (min)	Linear range (μg ml^{-1})	Intercept (a)	Standard deviation	Slop (b)	Correlation coefficient (r)	LOD	LOQ
3	3.24 – 20	-0.047	0.00342	0.023	0.9992	0.211	0.702
5	3.24 – 16	0.0641	0.00319	0.0258	0.9995	0.156	0.5056
9	3.24 – 14.9	0.1361	0.00311	0.029	0.9996	0.0667	0.222
12	3.24 – 12.9	0.2451	0.00325	0.0232	0.9993	0.0402	0.134
15	3.24 – 12.9	0.277	0.0028	0.0225	0.9995	0.03	0.1
18	3.24 – 12.9	0.3121	0.00336	0.0212	0.9991	0.0315	0.1053
21	3.24 – 12.9	0.326	0.00313	0.0233	0.9993	0.0279	0.0949
24	3.24 – 12.9	0.345	0.00294	0.0237	0.9994	0.0259	0.0844

Table 3: Evaluation of the accuracy and precision of the initial rate and fixed time methods of the proposed kinetic spectrophotometric methods for determination of Telithromycin

Spectrophotometric	Amount taken (μg ml^{-1})	Recovery (%± RSD) [a]	
		Initial rate method	Fixed time method
$KMnO_4$	4.872	100.02 ± 0 .013	100.4 ± 0.12
	6.496	100.84 ± 0.015	100.2 ± 0.25
	8.12	101.059 ± 0.02	99.8 ± 0.3

[a] Recovery was calculate as the amount found /amount taken x 100. Values are mean ± R.S.D. for five determinations

Table 4: Determination of Telithromycin in their pharmaceutical dosage forms by the reference and proposed initial rate and fixed time spectrophotometric methods

Statistic	Proposed methods (n = 5) $KMnO_4$		Reported methods [a] (n = 5)
	Initial rate	Fixed time	
Mean recovery	100.83	99.8	100.31
± SD	0.63	0.7	0.69
N	5	5	5
F- test value [b]	1.346	1.15	-
t-test value [b]	0.853	0.8995	-

[a] References , respectively. Values are mean ± RSD of five determinations

[b] The tabulated values of t and F at 95 % confidence limit are t =2.306 and F = 6.38

tangents of the absorption time curves. The order of the reaction with respect to permanganate was determined by studying the reaction at different concentration of permanganate with fixed concentration of investigated TEL. The plot of initial rate (ΔA/Δt) against initial absorbance was linear passing through origin indicating that the initial order of the reaction with respect to permanganate was 1. The order with respect to investigated TEL was evaluated by measuring the rate of the reaction at several concentration of TEL at a fixed concentration of permanganate reagent. This was done by plotting the logarithm of initial rate of the reaction versus logarithm of molar concentration of investigated TEL and was found to be 1. However under the optimized experimental conditions, the concentration of TEL was determined using relative excess amount of $KMnO_4$ and sodium hydroxide solutions. Therefore pseudo-zero order condition was obtained with respect to their concentrations.

Quantization methods

Initial rate method

The initial rate of the reaction would follow pseudo-first order and were found to obey the flowing equation

$$V = \Delta A/\Delta t = k` C^n$$

Where V is the reaction rate, A is absorbance, t is the measuring time, K´ is the pseudo-first order rate constant, C is the molar concentration of TEL and n is the order of the reaction. The logarithmic form of the above equation is written as follows

$$\text{Log } v = \log \Delta A/\Delta t = \log K' + n \log C$$

Regression analysis using the method of least square was performed to evaluate the slopes, intercepts and correlation coefficient.

The analytical parameters and results of regression analysis are given in (Table 1). The value of n (≈1) in the regression equation confirmed that the reaction of TEL with $KMnO_4$ was pseudo first order with respect to TEL concentration. The limits of detection (LOD) were calculated and results obtained confirmed good sensitivity of the proposed method and consequently their capabilities to determine low amount of TEL.

Fixed time method

In this method, the absorbance of the reaction solution containing varying amount of TEL was measured at preselected fixed time. Calibration plots of absorbance versus the concentration of Telithromycin at fixed time were established for each investigated. The regression equation, correlation coefficients and detection limits are given in (Table 2). The lowest detection limit was obtained at fixed time of 24 min. However the fixed time of 3 min showed a wider concentration range for quantification.

According to the international conference of harmonization (ICH) guideline for validation of analytical procedures the detection limit is not required to be part of validation procedure for assay. [9] Therefore on the basis of wider

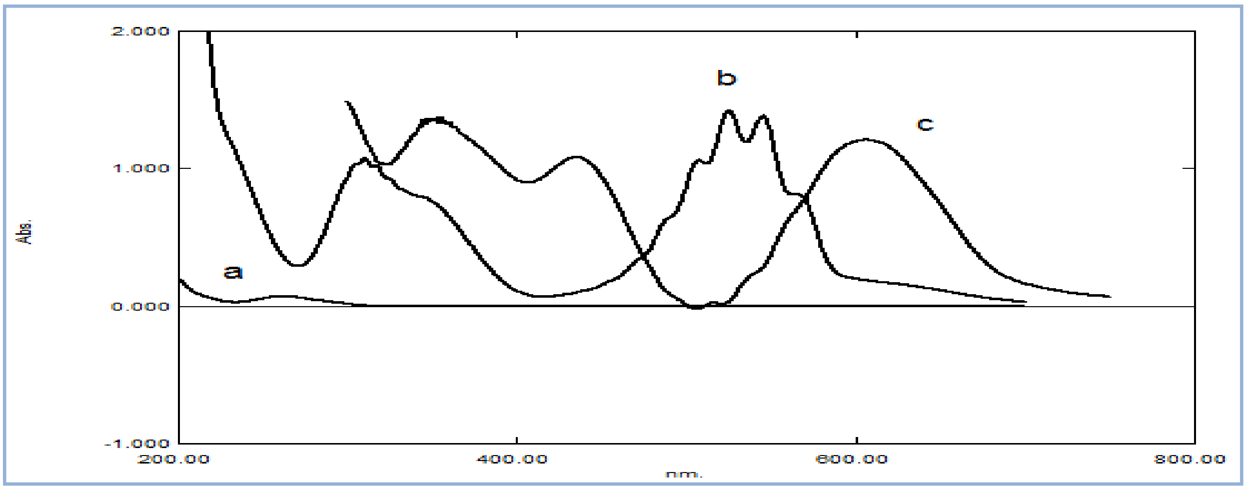

Fig. 2: Absorption spectra of (a) Telithromycin 8.12 µg ml^{-1}, (b) alkaline $KMnO_4$ (6×10^{-3} M) and (c) The concentration 0.2×10^{-3}, 1.6×10^{-3} M The reaction of $KMnO_4$ with Telithromycin was carried out at temperature (25±5 °C) for 24 min.

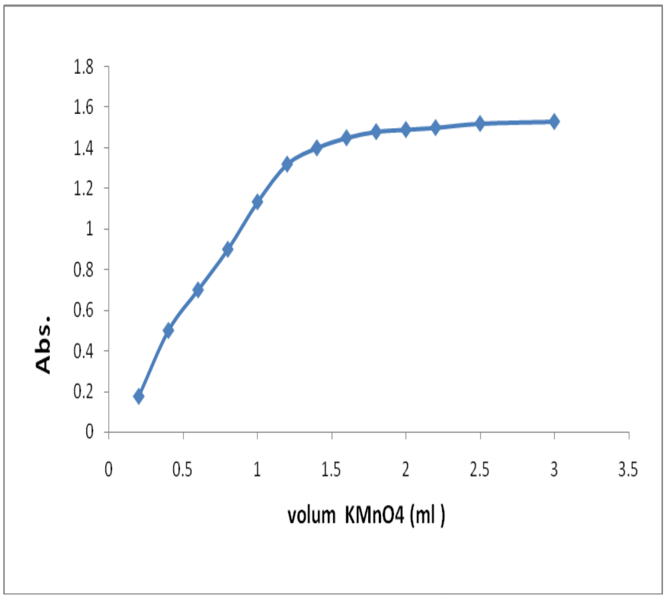

Fig. 3: Effect of potassium permanganate (6×10^{-3} M) volume on the reaction between the investigated Telithromycin (8.12µg ml^{-1}) and alkaline potassium permanganate.

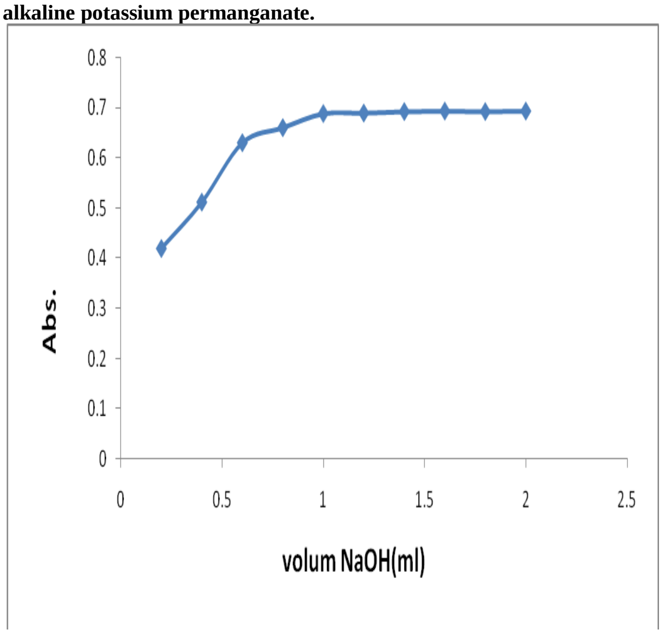

Fig. 4: Effect of sodium hydroxide concentration (1M) on the reaction between the investigated Telithromycin (8.12 µg ml^{-1}) and alkaline potassium permanganate.

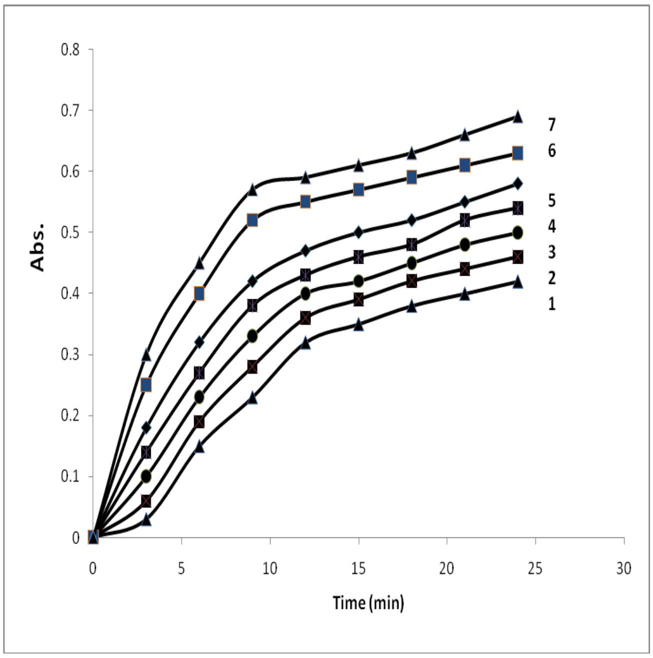

Fig. 5: Absorbance versus time graph for the reaction of Telithromycin and alkaline potassium permanganate at 25 °C showing the dependence of the reaction on Telithromycin concentration 1) 0.4×10^{-5} (2) 0.6×10^{-5} (3) 0.8×10^{-5} (4) 1×10^{-5} (5) 1.2×10^{-5} (6) 1.6×10^{-5} (7) 1.8×10^{-5} M, λ = 610 nm.

concentration range and less time of analysis, the fixed time of 3 min. was recommended for determination.

Validation of the proposed method

Accuracy and Precision

The accuracy and precision of the proposed kinetic spectrophotometric methods were determination at three concentration levels of TEL by analyzing five replicate sample of each concentration by both the initial rate and fixed time methods. The relative standard deviations (RSD) for the results did not exceed 2 % (Table 3), proving the high reproducibility of the results and the precision of the method. This good level of precision was suitable for quality control analysis of TEL of its pharmaceutical tablets.

Analytical recovery and interference liabilities

The accuracy of the proposed methods was also checked by performing recovery experiments using the standard addition method. Known amount of the pure TEL were added to preanalyzed TEL-containing pharmaceutical tablets, and the determined by the recommended procedures. The obtained mean recoveries and relative standard deviations were in the range 99.8-100.83 and 0.7-0.63% respectively (Table 4). These results prove the accuracy of the proposed methods and absence of interferences from the common excipients. It is worth noting that all the proposed kinetic spectrophotometric methods were performed in the visible region away from the UV-absorption region of the UV-absorbing interfering excipients materials that might be co-extracted from the TEL containing tablets. Although the method involving $KMnO_4$, being based on oxidation reaction, is not selective, but the obtained good recoveries ensured the suitability of the method for the analysis of TEL in its dosage forms without interference excipients. This was attributed to the great sensitivity of the method that necessitated the dilution of the sample, and consequently the excipients beyond their interference capability.

Application of the proposed method

The initial rate fixed time method of the proposed kinetic spectrophotometric methods for determining TEL has been tested on commercial pharmaceutical tablets. The concentration of TEL was computed from its corresponding regression equations. The results of the proposed methods (initial rate and fixed time were statistically compared with of the reported method [5], in respect to the accuracy and precision. The obtained mean recovery values of the labeled amounts were 100.83-99.8 ± 0.63-0.7% (Table 4). In the t- and F- tests, no significant difference were found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level. This indicated the similarities between the precision and accuracy of the proposed and the reference method for the determination of TEL in the dosage form.

The present study described, for the first time, a simple and sensitive kinetic spectrophotometric method for determination of TEL in their dosage forms. The proposed initial rate and fixed time methods can be easily applied as they do not require elaborate samples and /or tedious procedures for the analysis. As well, both methods are sensitive enough for analysis of lower amount of the drug. Furthermore, the proposed method does not require expensive instruments and /or critical analytical reagents. These advantages give the proposed methods a great value and encourage its application to the analysis of TEL in quality control laboratories.

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