Spectrophotometric Determination of Telithromycin in Tablet Dosage Form Based on Formation of Charge Transfer Complexes

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Abstract: A simple, accurate and sensitive spectrophotometric method for the determination of telithromycin in tablet dosage form has been developed and validated. The analysis was based on the formation of colored charge-transfer complex between telithromycin as n-electron donor and each of chloranilic acid (CLA) or 2, 3-dichloro-5, 6-dicyano-1,4-benzoquinone (DDQ) as electron acceptors. The formed complexes were measured spectrophotometrically at 588 and 518 nm for CLA and DDQ, respectively. Different variables affecting the reaction were carefully studied and optimized. Under the optimum reaction conditions, linear relationships with good correlation coefficients (0.9982 and 0.999) were found between the absorbance and the concentrations of the drug in the concentration ranges of 5-100 µg/ml for CLA and DDQ respectively with apparent molar absorptivities of 0.24×10^4 and 0.25×10^4 l mol^{-1} cm^{-1} respectively. The reaction stoichiometry in both methods was evaluated by Job's method of continuous variations and was found to be 1:1 (telithromycin: CLA or DDQ). The proposed methods were successfully applied to determination of telithromycin with good accuracy and precision. The results demonstrated that the methods are as accurate, precise and reproducible as the pharmacopieal method. The methods would be valuable for the routine application in quality control.
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1. Introduction

Telithromycin is: 3-De[(2,6-dideoxy-3-C-methyl- 11,12-dideoxy 6-O- α Lribohexoppyranosyl)oxy]-methyl-3-oxo-12,11-[oxycarbonyl [[4-[4-(3-pyridinil)-1H-imidazol-1-1-yl]butyl]imino]]erythromycin (Figure 1); is the first antibiotic belonging to a new class of 14-membered ring macrolides, named ketolides, to achieve clinical use (Merck Index 2001).

Ketolides, a novel family of semi-synthetic antimicrobial agents, were designed to overcome the increasing resistance of gram-positive cocci against macrolides (Bryskier 1999). The first member of ketolides introduced into clinical practice is telithromycin, a compound which was derived from the macrolide erythromycin-A by substitution of a keto group for L-cladinose at the three-position and addition of an N-substituted 11,12-carbamate side chain (Bryskier 1999).

Various methods cited in literature for its determinations in different matrices involved High performance liquid chromatography HPLC (Traunmüller et al 2005; Vaucher et al., 2009; Fatahalla et al 2001 and Vauche et al., 2010), HPLC-Mass spectrometry (MS) (Perret et al., 2002), spectrophotometry(Abou Attia et al., 2010 and Laurenet al 2011) and microbiology (Vaucher 2006). However, most of these methods involve time-consuming procedures, derivatization and/or sophisticated instruments that are not available in most quality control laboratories.

Spectrophotometry represents an attractive common technique adequate for solving many analytical problems, particularly when using the capabilities of modern instruments available nowadays. However, few spectrophotometric methods based on its oxidation have been reported for the analysis of telithromycin (Abou Attia et al 2010 and Laurenet al 2011).

The molecular interaction between electron donors and acceptors are generally associated with formation of charge-transfer complexes (Sharma et al. 2012; Refat et al. 2011; Alghanmi et al., 2013; Belfaragui 2013 and Ulu et al., 2010), which absorb radiation in both ultra-violet and visible region. The rapid formations of the charge - transfer complexes by the electron donating compound with various acceptors lead to their utility in the development of simple and convenient spectrophotometric methods for these compounds. The aim of the present study was directed to investigate the charge transfer reaction with telithromycin which has not yet been reported. The application of CLA and DDQ were selected due to their high electron affinities to achieve the spectrophotometric determination of telithromycin in its pure samples and in dosage form.

2. Experimental

2.1. Apparatus

A double-beam, ultraviolet-visible spectrophotometer (Thermo Scientific, England), equipped with 10 mm matched quartz cells.

2.2. Materials and reagents

Chloranilic acid (CLA), Fluka, Switzerland, and 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ), Merck-Schuchardt, Munich, Germany, 1 mg/ ml solution of each of them was prepared in acetonitrile the solutions were stable for 1 week at 4 °C. The solution was prepared fresh daily. Pure telithromycin was purchased from Sigma (St. Louis, Mo, USA), 1 mg/ml (in acetonitrile) of which was prepared freshly daily. Working solutions of telithromycin (100µg/ml) were obtained by suitable dilutions of the stock solution with acetonitrile. All solvents and other chemicals used were of analytical reagents grade.

2.3. Procedure

Into 10-ml calibrated flasks were placed 0.05 - 1.0 ml aliquots of 1000 µg/ml of telithromycin in acetonitrile for the CLA and DDQ, respectively was added. 1 ml of CLA or DDQ reagent was added. The reaction mixture was allowed to stand for few second at 25°C. Then diluting to volume of 10 ml with acetonitrile the absorbance was measured at 588 and 518 nm for CLA and DDQ, respectively, against reagent blank prepared in the same manner

2.4. Procedure for dosage forms

The contents of ten tablets were weighed, and finely powdered. An accurately weighed quantity of the powder equivalent to 400 mg of the active ingredient was transferred into a100-mL calibrated flask, and dissolved in about 40 mL of acetonitrile. The contents of the flask were swirled, sonicated for 5 minutes, and then completed to volume with Acetonitrile. The contents were mixed well and filtered; the first portion of the filtrate was rejected. The filtered solution was diluted quantitatively with acetonitrile to obtain suitable concentrations for the analysis using the same procedure as described above. The nominal contents of the tablets were calculated using the corresponding regression equation.

2.5. Stoichiometric Relationship

Job's method of continuous variation (Job, 1936) was employed to establish the stoichiometry of the colored products. A 0.4 $\times 10^{-3}$M standard solution of telithromycin and reagent of CLA and DDQ were used. A series of solutions was prepared in which the total volume of telithromycin and reagent was constant (1.0 ml). The reagents were mixed in various proportions and diluted in a 10-ml calibrated flask with acetonitrile solvent. The absorbance was measured after treating each reagent at best time and conditions against a reagent blank following the above mentioned procedure.

3. Results and Discussion

Telithromycin is electron donating compound that was found to react with π-acceptors (CLA and DDQ) with formation of colored charge transfer complex. Under the described experimental conditions, the yellow adduct has a characteristic absorption spectrum with maximum absorbance at 588 and 518 nm (for CLA and DDQ, respectively) as shown in Figure 2.

The interaction of telithromycin with ChA and DDQ as π acceptors in non-polar solvent such as dichloroethane produced colored charge-transfer complexes with low molar absorpitivity values. In polar solvents such as acetonitrile, complete electron donner transfer from telithromycin (D), as an electron donor, to the acceptor moiety (A) take place with the formation of intensely colored radical ions with high molecular absorptivity values, according the following:

$$D + A \rightleftarrows (D\text{-}A) \rightleftarrows D^{\cdot +} + A^{\cdot -}$$

complex redicals ions

The dissociation of the donor-acceptor complex (D-A) was promoted by the high ionizing power of the polar solvent acetonitrile (Vogel, 1989). The predominant chromogens showing different absorption maxima at 588 and 518 nm for ChA and DDQ, respectively (Fig.2 and 4). The influence of different parameters on the color development was studied to determine optimum conditions for the assay procedures.

3.1. Effect of reagent concentration

When various concentrations of acceptors were added to a fixed concentration of telithromycin it was found that 1.0 ml of 0.1% solution of either CLA or DDQ were sufficient for the production of maximum and reproducible absorbance intensity. Higher concentrations of the reagents did not affect the absorbance intensity. Figure 2 show the absorption curves of different charge transfer reagents.

3.2. Effect of reaction time

The optimum reaction time, was determined by monitoring the color developed at room temperature (25 ± 5°C). Complete color development was attained instantaneously (with CLA and DDQ. The developed colors remained stable at room temperature for at least for more than one hour.

3.3. Stoichiometry of the reaction

Job's method of continuous variation (Job, 1936) was used for determining the molar ratio of telithromycin to each of the analytical reagents employed in the charge-transfer reactions. These ratios were 1:1 in all cases. This indicates that only one site is possible for the formation of the complex (Figure 3).

3.4. Effect of diluting solvent

Although charge transfer complexes are probably formed in many solvents, the high cut-off points of some solvents obscured the scanning of the shorter wavelengths and therefore clear-cut spectroscopic evidence for charge transfer formation could not be ascertained. Also the low solubility of telithromycin in some other solvents restricted this. In order to select the most appropriate solvent, the reaction were carried out in different solvent (namely: acetonitrile, acetone, methanol, chloroform and dichloromethane and 1,4-dioxane). Experimental indicated that, acetonitrile proved to be the most suitable diluting solvent because it affords an excellent solvating power for CLA and DDQ reagents and give high absorbance. Acetonitrile was considered as an ideal solvent for the other π – acceptors by offering maximum sensitivity, which is attributed to the high dielectric constant that promotes maximum yield of radical anions, and high solvation power for the acceptors (Foster 1969).

3.5.Validity of the proposed method

3.5.1. Limit of quantification and limit of detection

The linear equation obtained by regression analysis was found to be: y = a× ± b, where *y* is equals the absorbance, × is equal the concentration, *a* is the slope, and *b* is the intercept and *r* is the correlation coefficient. The results are shown in Table 1. Beer's law displays a linear response over the concentration range of 5-100 ppm for ChA and DDQ respectively.

The limit of detection (LOD) and limits of quantification (LOQ) were determined (The united state pharmacopeia, 2000) using the formula: LOD or LOQ = K.S.D. a/b where K= 3 for LOD and 10 for LOQ. S.D.a is the standard deviation of the intercept, and *b* is the slope. Based on the basis of six replicate measurements, the limit of quantification was 5.0 ppm for both ChA and DDQ, and limit of detection were 1.5 ppm for iodine, CLA and DDQ.

3.5.2. Interferences study

Interferences studies were carried out in order to investigate the effect of common encountered compounds that present in telithromycin dosage form. It was found that, the proposed methods could be applied to determine telithromycin in dosage form. On the other hand, tablets excipients such as starch, lactose and glucose did not interfere in the proposed method. The good percentage recoveries revealed that there was no interference from the excipients present in the tablets form.

3.5.3. Precision and accuracy of the method

The precision and the accuracy of the method were investigated by inter-day (repeatability) by the analysis of telithromycin, six replicates at the limit of quantification range. The precision and the accuracy of the method are expressed as RSD and recovery of the measured concentration, respectively. Also reproducibility (Day to Day or intraday) was investigated. The results obtained (Table 2&3) are within the acceptance range of less than 3.0 % (precision) and 2.0 (accuracy).

3.5.4. Ruggedness

The ruggedness of the spectrophotometric method was evaluated by carrying out the analysis using two different analyst (operator) and different instruments on different days. The RSD of less than 3.0% were observed for repetitive measurements in three different day time periods using two different instruments and two operators. The results of the ruggedness indicated that the method is capable of producing results with high precision.

3.5.5. Robustness

The robustness of the method was demonstrated by the versatility of the experimental factors that is affecting the potential response. Preliminary inspection of the results (reagent concentration, reaction time, and stability of the resulting charge complex) under these various conditions suggested that the method is fairly robust, at the optimum conditions.

3.6. Applications

Reliability of the proposed methods for determination of the drug in the dosage forms was first assessed for its determination in pure solution. Determinations of telithromycin in solutions (five replicate) by spectrophotometric method gave an average recovery values of 98.1 – 100.0% and 98.4-100% with relative standard deviation of 1.7 - 3.1 % and 1.7- 3.1% for CLA and DDQ, respectively (results are shown in Table 4. This indicates high model accuracy of the proposed methods.

Results obtained for the analysis of telithromycin in its formulation by both the proposed spectrophotometric methods and the validated method (Lingerfelt et al., 1999) are given in Table 4. These data suggest that the proposed method can be carried out on real products with equal confidence and accuracy compared with reported method.

Conclusion

The suggested procedures using π acceptors confirm their suitability for the spectrophotometric analysis of telithromycin in its formulation. The methods have the advantage of being simple, accurate, sensitive and suitable for routine quality control of telithromycin in dosage form without of interference caused by excipients.

Table 1. Quantification parameters of the formed CT-complexes.

Parameters	CLA	DDQ
Wavelength, nm Beer`s law limits, µg/ ml	588 5 -100	518 5-100
Lower limit of quantification (LLQ), µg/ml	1.5	1.5
Lower limit of detection (LLD), µg/ ml	5.0	5.0
Intercept (*a*)	-0.0055	0.004
Slope (*b*)	0.003	0.0031
Correlation coefficient, r^2	0.9982	0.999
Molar absorptivity, l mol^{-1} cm^{-1}	0.24×10^4	0.25×10^4
Sandell`s sensitivity(µg cm^{-2})	0.338	0.324

* A = b × ± a where *a* is intercept, *b* slope and × concentration µg/ ml

Table 2. Evaluation of intra-day accuracy and precision of the proposed method.

CT Reagent	Drug taken (µg/ ml)	Found* (µg/ ml)	R, %	RSD, %	Error, %
CLA	15.0	15.33	100.2	2.75	+0.2
		14.66	97.7		-2.3
		14.6	97.3		-2.7
	30.0	29.33	97.7	2.19	-2.3
		30.2	100.6		+0.6
		30.6	102.0		+2.0
	70.0	67.46	96.38	2.48	+3.62
		70.36	100.5		+0.5
		70.6	100.8		+0.8
DDQ	15.0	15.96	106.4	2.51	+6.4
		15.45	103.0		+3.0
		15.19	101.29		+1.29
	30.0	31.06	103.5	1.9	+3.5
		31.0	103.0		+3.0
		30.38	101.0		+1.0
	70.0	69.48	99.25	2.37	-0.75
		70.22	100.3		+0.3
		66.83	95.84		-4.16

*Average of five determinations

Table 3. Evaluation of inter-day accuracy and precision of the proposed method.

CT Reagent	Drug taken (µg/ ml)	Found* (µg/ ml)	R, %	RSD, %	Error, %
CLA	15.0	14.86	99.06	3.34	+0.94
		15.12	100.8		+0.8
		14.82	98.8		+1.2
	30.0	30.04	100.13	2.27	+0.13
		30.62	102.06		+0.06
		30.48	101.6		+1.6
	70.0	69.47	99.24	1.76	+.76
		72.96	104.2		+4.2
		72.10	103.0		+3.0
DDQ	15.0	15.58	103.8	3.09	+3.5
		15.53	103.5		+0.74
		14.74	98.26		+3.8
	30.0	30.8	102.6	1.92	+0.55
		29.53	98.43		+2.7
		30.87	102.9		+2.9
	70.0	68.8	98.34	1.65	+0.66
		70.38	100.5		+0.5
		70.34	100.48		+0.48

*Average of five determinations

Table 4. Direct determinations of telithromycin using the charge transfer complex methods.

Added (/ml)	Recovery, % ± RSD CLA	DDQ
5.0	98.1 ± 3.1	98.4 ± 3.1
10.0	98.3 ± 2.5	98.5 ± 2.4
20.0	98.5 ± 2.0	98.6 ± 2.0
40.0	98.5 ± 1.8	99.0 ± 1.8
60.0	99.0 ± 1.8	99.0 ± 1.8
80.0	99.5 ± 1.7	99.5 ± 1.7
100.0	100.0 ±1.7	100.0 ± 1.7

* Average of 5 measurements ± RSD.

Table 5. Determination of telithromycin in tablets using the official method and charge transfer complexes**.**

Parameters	Recovery, % ± RSD		
	CLA ± RSD	DDQ	Reported method
Label claim, mg/tablets 400	102.72 ± 2.79	100.86 ± 1.2	100.31 ± 0.69

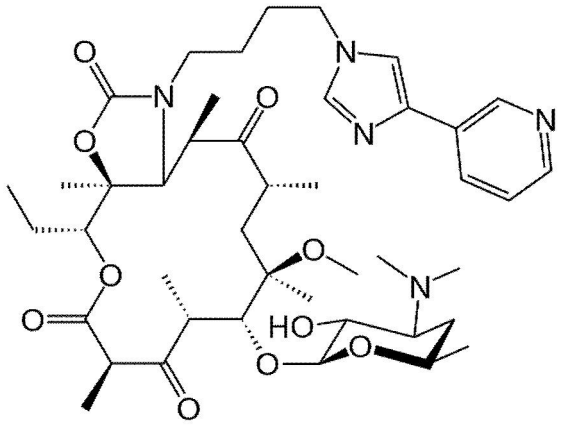

Fig. 1. Chemical structure of telithromycin.

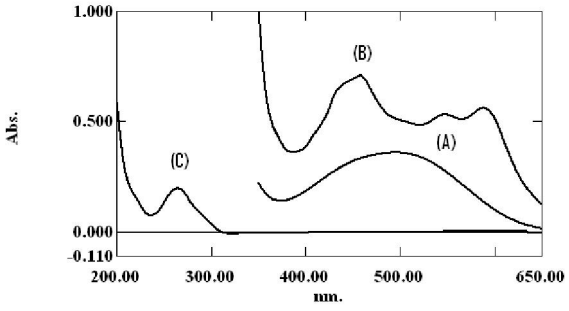

Fig. 2. Absorption spectrum of 40 µg/ml telithromycin solution: A, B, and C) of CLA, DDQ and pure drug, respectively.

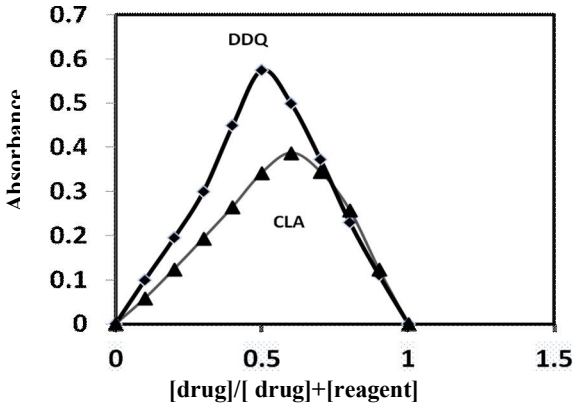

Fig. 3. Job`s plots of continuous varaiation method

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