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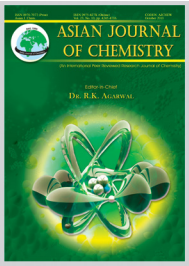

Simple Spectrophotometric Methods for Determination of Clarithromycin in Pure State and Tablets

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Three simple and sensitive procedures (methods **A**, **B** and **C**) for the assay of clarithromycin in pure form and formulations are described. Method **A** based on the condensation of clarithromycin with *o*-nitrobenzaldehyde in presence of 3.6 N HCl then in acetic acid media (method **A** λ_{max} 480 nm). Method **B** is based on the red ox reaction of clarithromycin with Fe(III) salt in glacial acetic acid. It is known that the macrolide ring (in clarithromycin) when treated with glacial acetic acid can under go dehydration forming the 8,9 anhydroclarithromycin hemiketol. The hydroxy group β to the double bond in this hemiketol reacts with Fe^{3+} to give the coloured product (λ_{max} 480 nm). Method **C** is based on formation of coloured radical anion on treating clarithromycin with 2,3 dichloro, 5,6-dicyno-1,4-benzoquinone (λ_{max} 450 nm). The valuable parameters in all these methods have been optimized. The results were statistically validated.

Key Words: Clarithromycin, DDQ, Clarithromycin, Nitro benzaldehyde.

INTRODUCTION

Clarithromycin [2R,3S,4S,5R,6R,8R,11R,12S,13R)-3-(2,6-dideoxy-3c,3-O-dimethyl-α-L-ribohexo pyranosoxy]-11,12-dihydroxy-6-methoxy-2,4,6,8,10,12-hexa-9-oxo-5-(3,4,6-trideoxy-3-dimethylamino-β. D-xylohexopyranosyloxy)-pentadecan-13-olide) is a macrolide antibacterial hydroxylated macro cyclic lactones containing 12 to 20 carbon atoms in the primary ring bind to the 50s sub units of bacterial ribosomes indicated to treat infections caused by bacteria. It is official in, USP[1], Merck index[2], Martindale's extra pharmacopoeia[3], Remington[4], PDR[5]. Existing analytical methods reveal that relatively little attention was paid in developing visible spectrophotometric methods for its determination. This paper describes three visible spectrophotometric procedures involving clarithromycin with reagents such as o-nitrobenzaldehyde in HCl and acetic acid media (method A), Fe(III) salt in glacial acetic acid (method **B**) and 2,3-dichloro, 5,6-dicyno, 1,4-benzoquinone (DDQ) (method **C**) by exploiting its structural features, tertiary amine, tertiary hydroxyl, secondary hydroxyl carbonyl groups.

EXPERIMENTAL

Drug solution: Acetic acid solution of *o*-nitrobenzalde-hyde (0.4 % Loba) method **A** was prepared by dissolving in glacial acetic acid. Fe(III) (0.0540 of BDH) solution was prepared by dissolving 0.08 g of anhydrous ferric chloride initially in 2 mL of conc. H_2SO_4 and 2 mL of triply distilled water and made up to 100 mL with glacial acetic acid (method **B**). DDQ (0.1 % Otto-kemi; method **C**) in chloroform was prepared.

A standard solution containing 1 mg/mL of clarithromycin was prepared by dissolving 100 mg of pure clarithromycin in 100 mL of glacial acetic acid. It was further diluted to get 10 mg/mL (method **A**) and 50 mg/mL method **B**) with glacial acetic acid. A standard solution contains 1 mg/mL of clarithromycin was prepared by dissolving 50 mg of clarithromycin in 50 mL of chloroform. A portion of the chloroform solution was evaporated to dryness and dissolved in methanol to obtain 1 mg/mL^{-1} stock solution. It was further diluted to get-1000 mg/mL (method **C**).

An accurately weighed amount of tablet powder, equivalent to 100 mg of clarithromycin was extracted 5 × 5 mL portion of methanol and the combined extract was diluted to 100 mL with the same solvent to obtain 1 mg/mL clarithromycin methanolic stock solution. Portions of this stock solution was evaporated on boiling water bath and dissolved in appropriate solvents (glacial acetic acid for methods **A** and **B** and methanol for method **C**) to the requisite concentration as under procedures described for bulk samples.

Method A: Method A aliquots of (0.5-3.0 mL, 10 mg/mL of the standard drug solution were transferred in to a series of 10 mL calibrated tubes. Two mL of *o*-nitrobenzaldhyde (2.64×10^{-4} M) solution and 3 mL of conc. HCl were added. The solutions were shaken thoroughly and kept aside for 15 min. The volumes were adjusted to 10 mL with glacial acetic acid and absorbance was measured at 480 nm against similarly prepared reagent blank. The amount of the drug was calculated from the calibration curve.

TABLE 1
OPTICAL AND REGRESSION CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS FOR CLARITHROMYCIN

Parameter	M_1 (TPOOO)	M_2 (WFB)	M_3 (picric acid)
λ_{max} (nm)	480	580	350
Beer's Law limits (μg mL^{-1})	1.75-50	2.5-15	5-30
Detection limit (μg mL^{-1})	8.769×10^{-2}	3.902×10^{-2}	0.2737
Molar absorptivity ($mol^{-1} cm^{-1}$)	2.16×10^{4}	2.10891×10^{4}	1.353×10^{4}
Sandell's sensitivity (μg cm^{-2}/0.01 absorbance unit)	3.44×10^{-2}	2.762×10^{-2}	5.52×10^{-2}
Optimum photometric rang (μg mL^{-1})			
Regression equation (y = a + bc)			
Slope (b)	0.02882	3.617×10^{-2}	1.829×10^{-2}
Standard deviation on slope (S_b)	8.653×10^{-5}	4.833×10^{-3}	8.571×10^{-5}
Intercept (a)	4.666×10^{-4}	6.666×10^{-4}	-1.2666×10^{-3}
Standard deviation in intercepts (S_a)	8.4246×10^{-4}	4.705×10^{-5}	1.668×10^{-3}
Standard error of estimation (S_e)	9.05×10^{-4}	6.346×10^{-4}	1.7928×10^{-3}
Correlation coefficient (r)	0.9998	0.9999	0.9999
Relative standard deviation (%)*	0.1776	0.5066	0.4034
% Rang of error (confidence limits)*			
0.05 level	0.1864	0.5316	0.4234
0.01 level	0.2923	0.8341	0.6641
% Error in bulk samples**	-0.1034	0.2592	0.2739

*Average of six determinations considered; **Average of three determinations

TABLE-2
ASSAY OF CLARITHROMYCIN IN PHARMACEUTICAL FORMULATIONS

Formulations*	Labelled amount (mg)	Amount found by proposed methods			Reference method	Recovery by proposed methods (%)		
		M_1 TPOOO	M_2 WFB	M_3 picric acid		M_1 TPOOO	M_2 WFB	M_3 picric acid
Tablets	125	123.99 ± 0.99 F = 1.13; t = 0.07	124.00 ± 1.030 F = 1.16; t = 0.03	123.47 ± 1.15 F = 1.43; t = 1.01	124.04 ± 0.96	99.19 ± 0.72	99.20 ± 0.83	98.78 ± 0.92
Tablets	250	248.65 ± 2.33 F = 1.10; t = 0.02	247.38 ± 1.61 F = 1.89; t = 0.61	248.70 ± 1.94 F = 1.30; t = 0.005	248.7 ± 2.22	99.46 ± 0.91	98.95 ± 0.64	99.48 ± 0.77
Tablets	250	247.98 ± 1.80 F = 1.23; t = 0.67	247.7 ± 2.43 F = 1.48; t = 0.27	248.17 ± 1.58 F = 1.54; t = 0.27	248.4 ± 2.00	99.19 ± 0.72	99.10 ± 0.97	99.26 ± 0.63
Tablets	500	492.69 ± 2.87 F = 1.67; t = 2.15	494.76 ± 3.22 F = 2.11; t = 1.28	496.18 ± 1.84 F = 1.44; t = 1.09	498.7 ± 2.22	98.53 ± 0.57	98.95 ± 0.64	99.23 ± 0.36

*Formulations from four different pharmaceutical companies; **Average ± standard deviation on six determinations, the t- and F – test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F= 5.05, t = 2.57; *** Recovery of 10 mg added to the pre-analyzed pharmaceutical formulations (average of three determinations).

Method B: In method B aliquots of (0.5-3 mL, 50 mg/mL) the standard clarithromycin solutions were transferred in to a series of 10 mL calibrated tubes containing 5 mL of (3.32×10^{-3} M) ferric ion stock solution and the total volume in each flask was brought to 10 mL with glacial acetic acid and they were allowed to reach at 50 ºC for 15 min and the absorbance measurements were taken at 480 nm against a reagent blank after cooling to ambient temperature. The amount of the drug was obtained from the Beer's law plot.

Method C : In method C, aliquots of (0.5 -3.0 mL, 400 mg/mL) the standard clarithromycin solutions were taken in a series of 10 mL calibrated tubes and the chloform in each tube was evaporated in a hot water bath to dryness. The residue was dissolved in 0.5 mL of methanol and 2 mL of DDQ (4.405×10^{-3} M) solutions was added, shaken well and made up to mark with dichloro methane. Reagent blank was simultaneously prepared and read at 450 NM. The amount of drug was computed from Beer's law plot.

The utility of each method was verified by a means of replicate estimation of tablets. The values obtained by the proposed and reference methods (UV) for pharmaceuticals formulations were compared (Table-1) and are in good agreement. These results were compared statistically by t and F tests and found not to differ significantly. The results of recovery experiments by the proposed methods are also listed in Table-2.

RESULTS AND DISCUSSION

The optimum conditions for the colour development of methods (**A**, **B** and **C**) were established by varying the parameters one at time, keeping the others fixed and observing the effect produced on the absorbance of the coloured species. The coloured solutions exhibited by λ_{max} at 540, 620 and 450 nm respectively for method **A**, **B** and **C**.

The optical characteristics such as Beer's law limits (mg/mL), molar absorbtivity ($mol^{-1}cm^{-1}$) and Sandell's sensitivity (mg cm^2 0.001 absorbance unit) for methods **A**, **B** and **C** found to be 0.5-3.0; 1.063×10^{5}; 4.016×10^{-3}; 0.5-3.0; 1.032×10^{5}; 2.267×10^{-3} and 5-30; 4.951×10^{3} and 6.622×10^{-2} respectively. The slopes, intercepts obtained by linear least squares treatment of the results for methods **A**, **B** and **C** were found to be 1.246×10^{-1}, 1.466×10^{-3}, 0.9999, 1.762×10^{-1}, $1.216 \times$

10^{-4}, 0.9999 and 1.512×10^{-2}, -2.666×10^{-4} and 0.9999, respectively.

Estimating six replicate samples of drugs with the Beer's law limits tested the precision and accuracy of the methods. The per cent relative standard deviation as well as the per cent range of error (95 % confidence limit) for methods **A**, **B** and **C** were found to be 0.3287, 0.5999 and 0.452 and 0.5413, 0.9878 and 0.7456, respectively.

The application of each method was verified by means of replicate estimations of commercial formulations clarithromycine (cream and tablets). The values obtains by proposed methods and the reference methods for formulations were compare statistically with t and F tests and were found not to differ significantly. The results are summarized in Table-2.

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