Spectrophotometric Estimation of Roxithromycin in Tablet Dosage Forms

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A simple and sensitive spectrophotometric method has been developed for determination of roxithromycin in its pharmaceutical dosage forms. In the proposed method, roxithromycin is oxidized with potassium permanganate to liberate formaldehyde, which is determined *in situ* using acetyl acetone in the presence of ammonium acetate to give a yellow-coloured chromogen with absorption maxima at 412 nm. The method is found to be linear in the concentration range of 10-75 µg/ml with regression coefficient of 0.9987. No significant difference was found between the proposed method and the reported method when two-tailed t-tests are applied. Various reaction parameters, such as concentration of potassium permanganate and reagent, time required for oxidation and maximum colour intensity, were optimized. The method was validated and can be used successfully to assay roxithromycin in its pharmaceutical dosage form, viz, tablets.

Roxithromycin[1] is a macrolide antibiotic which acts on gram-positive bacteria and gram-negative bacteria. Chemically[1-2] it is (3*R*, 4*S*, 5*S*, 6*R*, 7*R*, 9*R*, 11*S*, 12*R*, 13*S*, 14*R*)-4-[(2, 6-dideoxy-3-*C*-methyl-3-*O*-methyl-a-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-10-[(*E*)-[(2-methoxy ethoxy)methoxy]imino]-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-b-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecan-2-one. It is used in respiratory tract infections[2] like pharyngitis, pneumonia, chronic bronchitis and bronchopneumonia. The recommended dosage for roxithromycin is 150-300 mg per day.

Roxithromycin is official in British Pharmacopoeia[2] and European Pharmacopoeia[3] and it is assayed by high-performance liquid chromatographic method. Literature survey reveals that roxithromycin is estimated in pharmaceuticals and biological fluids by spectrophotometric[4-7], HPLC[8-11] and microbiological methods[12]. These methods are too expensive and time consuming. The present work describes a simple, economical, accurate and reproducible spectrophotometric method for estimation of roxithromycin in pharmaceutical formulations. In the proposed method, roxithromycin is oxidized with potassium permanganate (excess potassium permanganate is decolourized with oxalic acid) to liberate formaldehyde, which is determined *in situ* using acetyl acetone in the presence of ammonium acetate to give a yellow-coloured chromogen with absorption maxima at 412 nm. The proposed method was successfully applied for determination of roxithromycin in its pharmaceutical formulations.

Double-beam Shimadzu 160A UV/Vis spectrophotometer having two matched quartz cells with 1 cm light path was employed for spectral measurement. Thermostatically controlled water bath (REMI Instruments, Mumbai) was used to control temperature of reaction mixture. Roxithromycin BP working standard was procured as a gift sample from Torrent Pharmaceuticals Ltd., Ahmedabad. Acetyl acetone (freshly distilled, ExcelaR), ammonium acetate, formaldehyde solution, glacial acetic acid and oxalic acid were purchased from S. D. Fine Chem. Pvt. Ltd., Mumbai. Double-distilled water was used in the study.

Ammonium acetate (30 g) was dissolved in water (50 ml). Freshly distilled acetyl acetone (1.0 ml) was added and the final volume was adjusted to 100 ml with water and stored in a refrigerator. Freshly prepared reagent was used in the study. Potassium permanganate (250 mg) was dissolved and diluted to 100 ml with water. Oxalic acid (10 g) was dissolved and diluted to100 ml with water. Roxithromycin (250 mg) was accurately weighed and transferred to a 100 ml volumetric flask. It was dissolved in glacial acetic acid (20 ml, 3 M) and diluted to 100 ml with distilled water. An aliquot (5.0 ml) was further diluted in water (50 ml) to obtain the final concentration of 250 µg/ml.

In a 10 ml volumetric flask, standard roxithromycin solution (2.0 ml) and glacial acetic acid solution (1.0 ml) were pipetted successively. The potassium permanganate solution (0.2 ml) was added. The reaction mixture was heated on water bath at 37° for 10 min. Excess of potassium permanganate was neutralized with oxalic acid. The reagent solution (2.0 ml) was added to it and mixed thoroughly. The reaction mixture was heated on water bath at 37° for 1 min and cooled and the volume was adjusted up to the mark with water. Absorbance of the coloured solution was scanned on Shimadzu UV-visible spectrophotometer from 600 nm to 200 nm against reagent blank. Maximum absorbance was obtained at 412 nm (fig. 1).

Standard solutions of roxithromycin (0.4, 0.8, 1.0, 2.0, 3.0 ml, 250 µg/ml) were pipetted out into a series of 10 ml volumetric flasks and analysed as above. Absorbance of the coloured solution was measured at 412 nm. It was found that the Beer's law is obeyed in the concentration

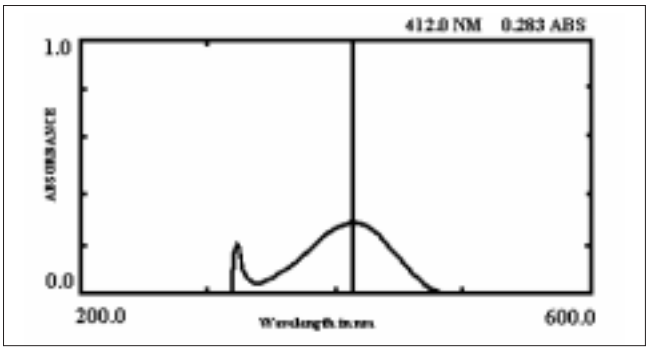

Fig. 1: Spectrum of yellow chromogen of roxithromycin

range of 10-75 µg/ml of roxithromycin (Table 1).

Twenty tablets were weighed accurately and powdered. The powder equivalent to 25 mg roxithromycin was dissolved in glacial acetic acid (20 ml, 3 M), sonicated for 15 min and filtered through Whatman No. 41 filter paper. The residues were washed thoroughly with distilled water. The filtrate and washing were combined in 100 ml volumetric flask and diluted to mark with the same solvent to produce the final concentration of 250 µg/ml. The solution (2.0 ml) was analysed as above. Amount of roxithromycin was computed from the calibration curve (Table 2).

It was known that α-amino alcohol, in which the amine group is primary or secondary, liberates formaldehyde on periodate oxidation[13]. The liberated formaldehyde is determined *in situ* using acetyl acetone in the presence of ammonia, which gives a yellow-coloured chromogen (3, 5- diacetyl-1, 4- dihydrolutidine) with absorption maximum at 412 nm[14].

In the proposed method, roxithromycin is oxidized with potassium permanganate (excess potassium permanganate is decolourized with oxalic acid) to liberate formaldehyde, which is determined *in situ* using acetyl acetone in the presence of ammonium acetate to give a yellow-coloured chromogen with absorption maximum at 412 nm. The colour is found to be stable for at least 2 h.

In the proposed method, various parameters, such as concentration of potassium permanganate and reagent, time required for oxidation and maximum colour intensity, were studied and optimized to obtain maximum colour intensity. The optical characteristics of roxithromycin such as Beer's law limit, Sandell's sensitivity and molar extinction coefficient were determined. The linear regression equation for determination of roxithromycin is $y = 0.0097x + 0.0258$ with correlation coefficient 0.9987. The RSD was found to be 0.14-2.86% (Table 3).

For recovery study, known amounts of pure drug were added to the previously analysed pharmaceutical preparations and the mixtures were analysed by the proposed method. The percent recovery was calculated, which was found to be 99.96-101.37% for roxithromycin. The analysis was carried out in triplicate – for three pharmaceutical dosage forms, i.e., tablets. The results of analysis of pharmaceutical dosage forms are shown in Table 2. Good recovery confirmed the accuracy and specificity of the proposed method and the lack of interference from the common excipients, film-coating materials and colorant used in the manufacture of tablets. The developed method was also compared with the reported method[10] and no significant difference was observed. This method is particularly useful for routine in-process quality control for its pharmaceutical preparations, i.e., tablets.

TABLE 1: REGRESSION ANALYSIS DATA OF THE CALIBRATION CURVES PREPARED BY THE PROPOSED METHOD

Concentration (µg/ml)	Absorbance at 412 nm ±S.D.*	% C.V.
10	0.106±0.0024	2.25
20	0.222±0.0041	1.86
25	0.279±0.0035	1.25
50	0.537±0.0037	0.68
75	0.740±0.0075	1.01

*Five replicate samples

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TABLE 2: ANALYSIS OF TABLET FORMULATIONS

Formulation	Label claim	% Amount found by		% recovery by
	Mg/tablet	Proposed method Mean ± S.D.[d]	Reported method[10] Mean ± S.D.[d]	proposed method[d]
Tablet -1	150	97.84±0.48	99.30±0.90	100.59±1.33
Tablet -2	150	92.28±0.45	90.95±1.20	100.44±1.35
Tablet -3	150	91.86±0.29	90.85±1.76	101.37±0.96
t-test (two-tailed)[e]	-	Tcal = 0.878	Ttab = 2.131	No significant difference

[d]Means three replicate samples. [e]Means t-test (two-tailed) for amount found by proposed method and that by reported method for tablets, which indicated there is no significant difference between the two methods. Tablets are dosage forms of roxithromycin which were procured from local market.

TABLE 3: OPTICAL CHARACTERISTICS OF THE PROPOSED METHOD

Parameters	Values
Wavelength for measurement (nm)	412
Beer's Law limit (µg/ml)	10-75
Molar absoptivity (l/mole/cm)	8.980×10^{3}
Sandell's sensitivity (µg/ml/cm²/0.001 abs. unit)	9.32×10^{-2}
Regression equation (Y[a])	
Slope (b)	0.0097
Intercept (a)	0.0258
Correlation coefficient (r)[b]	0.9987
Precision	
Intra day precision (%)[b]	0.79-2.75
Inter day precision (%)[b]	0.95-2.86
Relative standard deviation (%)[c]	0.1399
Recovery (%)	99.96-101.37

[a]Means Y=a+bC, where 'C' is concentration in µg/ml and Y is absorbance unit. [b]Means five replicate samples. [c]Means five replicate samples.

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