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RP-HPLC method for the analysis of Roxithromycin in bulk and pharmaceutical dosage forms

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ABSTRACT

A rapid and sensitive reverse phase high performance liquid chromatographic method was developed for the determination of Roxithromycin present in bulk and pharmaceutical dosage forms. Roxithromycin was chromatographed on a reverse phase C18 column with a mobile phase consisting of acetonitrile and 0.05 M potassium dihydrogen phosphate buffer (pH 4.2) in the ratio of 70:30 v/v. The mobile phase was pumped at a flow rate of 1.5 ml/min. Valdecoxib was used as an internal standard and the eluents were monitored at 207 nm. The retention time of the drug was 4.0 min. With this method, linearity was observed in the range of 10-2000µg/ml. The LOD and LOQ were found to be 0.172µg/ml and 0.461µg/ml, respectively. The method was found to be applicable for analysis of drug in tablets. The results of the analysis were validated statistically.

KEYWORDS

Roxithromycin;
RPHPLC;
Acetonitrile;
Phosphate buffer.

INTRODUCTION

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-hexopy ranosyl]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**[4-8]**, HPLC**[9-12]** and microbiological methods**[13]**. These methods are too expensive and time consuming. The aim of this study is development of a simple, precise, rapid and accurate reverse phase HPLC method for the estimation of Roxithromycin in bulk and its pharmaceutical dosage forms.

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MATERIALS AND METHODS

Acetonitrile and water were of HPLC grade (Merck India Ltd, Mumbai). All other reagents were of AR grade. The determination of Roxithromycin was performed using an HPLC-UV analytical system, which consists of a binary gradient HPLC with Shimadzu LC10AT and LC10AT vp series HPLC pumps, with a 20µl sample loop (manual) and a variable wavelength UV-Visible detector set (SPD 10 A vp detector) at 207 nm. Peak areas were integrated using the Shimadzu CLASS-VP Version 6.12 SP1software. Chromatographic separation of Roxithromycin and IS was achieved using Hypersil ODS C-18 (250 × 4.6 mm, packed with 5 micron) column.

The mobile phase used was a mixture of acetonitrile and 0.05 M potassium dihydrogen phosphate buffer (pH 4.2) in the ratio of 70:30 v/v; it was filtered before use through a 0.45µm membrane filter and degassed for 30 min. The elution was carried out isocratically at the flow rate of 1.5 ml/min. Detection was carried out at 207 nm at ambient temperature.

Preparation of standard drug solutions

Standard stock solution of Roxithromycin (1 mg/ml) was prepared in mobile phase. To study the linearity range of the drugs, serial dilutions were made from standard stock solution in the range of 10-2000 µg/ml. In each of them, 20 µg/ml valdecoxib was added as an internal standard. Twenty microliters of each solution was injected into the HPLC system to obtain the chromatogram. From these chromatograms, the area under the peaks of the drug and the internal standard were noted. Using these values, the mean ratio of peak area of the drug to that of the internal standard for each dilution was calculated. The regression of the drug concentration over these ratios was computed.

Recovery of roxithromycin in tablets

Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 25 mg of Roxithromycin was extracted with mobile phase, sonicated for about 15 minutes and the volume was made upto 25 ml with mobile phase and then filtered through 0.45µm membrane filter. From the filtrate, different aliquots were taken in separate 10 ml volumetric flasks. These solutions were spiked with suitable volume of the internal standard solution, such that the concentration of each solution was 25µg/ml. The contents of the flasks was made up to the volume with the mobile phase and mixed well. Each of these solutions (20µl) was then injected 5 times into the column. The mean peak area ratio of the drug to the internal standard of 5 such determinations was calculated and the drug content in the tablets was quantified using the regression equation obtained for the pure sample.

Chromatographic conditions

To achieve sharp peaks with good resolution, under isocratic conditions, mixtures of acetonitrole and phosphate buffer in different combinations were tested as mobile phase on a C18 stationary phase. A binary mixture of acetonitrile and 0.05 M potassium dihydrogen phosphate buffer (pH 4.2) in 70:30 v/v proportions was proved to be the most suitable of all combination, since the chromatographic peaks were better defined, resolved and free from tailing with this system. Under the above-mentioned chromatographic conditions, the retention time obtained for Roxithromycin and the internal standard were 4.0 and 2.4 min, respectively. Each of the samples was injected 5 times and almost same retention times were observed in all cases.

RESULTS

From the linearity table (TABLE 1) of the proposed method it was found that the drug obeys linearity range

TABLE 1: Linearity table

Concentration (µg/ml)	Peak area ratio (drug/IS)	Statistical analysis
10	0.013	
20	0.027	Slope (a) = 0.0013
30	0.040	
40	0.053	
50	0.067	
100	0.133	
200	0.267	Intercept (b) = -0.0068
400	0.534	
500	0.669	
750	1.004	
1000	1.341	
1250	1.676	Correlation coefficient =
1500	2.010	0.9996
1750	2.347	
2000	2.685	

TABLE 2: Analysis of commercial forumlations

S. no.	Formulations	Labeled amount (mg)	Amount obtained (mg) by proposed method*
1	Roxid Tab	150	149.77±0.089
2	Roxiwin Tab	150	149.53 ±0.094

*Each value is average of five determinations ± standard deviation

TABLE 3: System suitability parameters

S. no.	Parameters	Obtained values
1	Theoretical plates (N)	3810
2	Resolution (R)	2.1
3	Tailing factor (T)	1.8
4	LOD ((µg/ml)	0.172
5	LOQ ((µg/ml)	0.461

within the concentration of 10-2000µg/ml. From the results of analysis of commercial formulations shown in TABLE 2, the amount obtained by the proposed method was found to be in good agreement with the labeled claim. The system suitability parameters shown in TABLE 3 were within the specified limits and which refers the commonly used excipeints and additives present in the pharmaceutical formulations did not interfere in the proposed method.

CONCLUSION

The proposed method was found to be simple, precise accurate and rapid for the determination of Roxithromycin from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Roxithromycin in pure form and its dosage forms and can also be used for dissolution or similar studies.

REFERENCES

[1] 'The Merck Index', 12th Edn., Merk Research Lab. Publication, Rahway, N.J., 8430 **(1996)**.

[2] 'British Pharmacopoeia', Addendum, Published by HMSO Electronical publication sales, London, 533 **(1999)**.

[3] 'European Pharmacopoeia', 3rd Edn., Published by Council of Europe, Strasbourg, 807 **(1997)**.

[4] T.K.Ravi, M.Gandhimathi; Eastern Pharmacist, **509**, 42, 121 **(2002)**.

[5] C.S.P.Shastary, K.R.Prasad; Mikrochim Acta, **122**, 77 **(1996)**.

[6] C.P.S.Shastary, S.G.Rao, K.Ramasrinivas; Indian Drugs, **35**, 594 **(1992)**.

[7] M.N.Reddy, T.K.Murthy, G.V.Raju, J.Muralikrishna, K.Seshukumar, D.G.Sankar, Indian J.Pharm.Sci., **64**, 73 **(2002)**.

[8] B.N.Suhagia, S.A.Shah, I.S.Rathod, H.M.Patel, K.R.Doshi, V.K.Parmar; Indian J.Pharm.Sci., **68**, 543-6 **(2006)**.

[9] R.T.Sane, V.D.Kulkarani, M.K.Patel, V.B.Tirodkar; Indian Drugs, **29**, 658 **(1992)**.

[10] V.De Oliveira, A.Bergold, E.S.Schapoval; Anal. Lett., **29**, 2377 **(1996)**.

[11] Q.C.Li, F.R.Li; Yaowu Fenxi Zahi, **19**, 317 **(1999)**.

[12] M.Demotes, G.A.Vincon, C.H.Jarry, H.C.Albin; J.Chromatogr.Biomed.Appl., **490**, 115 **(1989)**.

[13] J.Chanton, A.Bryskier, J.C.Gasc; J.Antibiotics, **39**, 660 **(1986)**.