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A New Validated RP-HPLC Method for the Determination of Tinidazole and Roxithromycin in Its Bulk and Pharmaceutical Dosage Forms

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Abstract. To develop and validate a novel reverse-phase high-performance liquid chromatography determination of Tinidazole and Roxithromycin in its Bulk and Pharmaceutical Dosage Forms. Examination of simultaneous determination is centered around the advancement of novel RP-HPLC systematic technique for the assurance of medication substance in strong oral dose shapes and their approval. The optimized chromatographic condition was established for the estimation of Tinidazole and Roxithromycin by using Agilent C_{18} (4.6 X 250mm, 5 µm) column, sodium acetate buffer (pH 3) and Methanol (30:70% v/v) as mobile phase at a flow rate of 1.0 ml/min sustain an ambient temperature. The total analysis time was 10 minutes and the retention of Tinidazole and Roxithromycin was found to be 2.352 and 5.941 min with an injection volume of 20 µl. The system suitability parameters proved for optimized chromatographic conditions for Tinidazole and Roxithromycin were within limits. The developed method was showing good resolution and separation factors.

INTRODUCTION

Tinidazole is a nitroimidazole antitrichomonal agent effective against Trichomonas vaginalis, Entamoeba histolytica and Giardia lamblia infections. It also acts as a synthetic antiprotozoal agent. Tinidazole demonstrates activity both in vitro and in clinical infections against the following protozoa: Trichomonas vaginalis, Giardia duodenalis (also termed *G. lamblia*), and Entamoeba histolytica. Tinidazole does not appear to have activity against most strains of vaginal lactobacilli. Its Chemical formula is $C_8H_{13}N_3O_4S$ and the molecular weight of these compounds is 247.27. Chemically, it is called as 1-[2-(ethanesulfonyl)ethyl]-2-methyl-5-nitro-1*H*-imidazole [1-4] (Figure 1).

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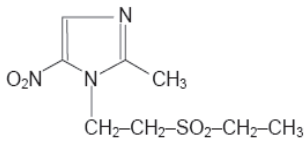

FIGURE 1. Chemical structure of Tinidazole

Roxithromycin is a semi-synthetic macrolide antibiotic. It is very similar in composition, chemical structure, and mechanism of action to erythromycin, azithromycin or clarithromycin. Roxithromycin prevents bacteria from growing by interfering with their protein synthesis. Roxithromycin binds to the subunit 50S of the bacterial ribosome and thus inhibits the translocation of peptides. Roxithromycin has similar antimicrobial spectrum as erythromycin, but is more effective against certain gram-negative bacteria, particularly Legionella pneumophila. It can treat respiratory tract, urinary and soft tissue infections. The molecular formula of this compound is C41H76N2O15and molecular weight of this compound is 837.04. Chemically, it is called as (3R,4S,5S,6R,7R,9R,11S,12R,13S,14R)-6-{[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy}-14-ethyl-7,12,13-trihydroxy-4-{[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy}-3,5,7,9,11,13-hexamethyl-10-(2,4,7-trioxa-1-azaoctan-1-ylidene)-1-oxacyclotetradecan-2-one. (Figure 2).

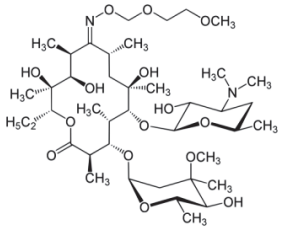

FIGURE 2. Chemical structure of Roxithromycin

An extensive literature survey was carried for the determination of Tinidazole and Roxithromycin in pure and pharmaceutical formulations. Several authors developed liquid chromatographic methods for the estimation of Tinidazole individually and combined with other drugs [1-10], spectroscopic methods [11,12,13] and liquid chromatographic technique for Roxithromycin individually or combined with other drugs [14-16], spectroscopic techniques [17-19] in pure and dosage form. No literature reported for simultaneous estimation of Tinidazole and Roxithromycin in pure and dosage form. Therefore, the present work aims to develop a fast, simple, precise reversed-phase high performance liquid chromatographic method for the estimation of Tinidazole and Roxithromycin in its Bulk and Pharmaceutical Dosage Forms. The developed chromatographic parameters were validated in accordance with ICH-Q2 (R1) guidelines.

EXPERIMENTAL SECTION

Potassium dihydrogen orthophosphate, Sodium perchlorate, Perchloric acid, orthophosphoric acid, Methanol, Acetonitrile, HPLC grade Water procured from Merck India. API was obtained from Bio Leo Labs Pvt Limited, Hyderabad.

Instrumentation

Waters (Alliance) HPLC system equipped with an autosampler and ultraviolet detector was used for the present investigation. The data acquisition was obtained from Empower-2 software.

Preparation of Solutions

Mobile Phase

24 gm of Sodium acetate into 1000ml volumetric flask dissolved in HPLC grated water and adjust pH 3 with orthophosphoric acid. A mixture of buffer and methanol in the ratio 30:70 v/v was taken, degassed in an ultrasonic water bath for five minutes at room temperature and then filtered through 4.5 µ filter under vacuum filtration. This was used as a mobile phase and diluent.

Standard Stock Solution

Standard stock solution was prepared by precisely 50 mg of Tinidazole and 30mg of Roxithromycin standards were weighed accurately and transferred into a clean 50 mL volumetric flask, dissolved in 10 mL of diluent, sonicated for five minutes at room temperature and made up to the mark with diluent. Further 0.8 ml were pipetted out into a 10ml volumetric flask and diluted up to mark with diluent to get a concentration of 80 µg/ml and 48µg/ml of Tinidazole and Roxithromycin.

Sample Stock Solution

Average weight of ten tablets was determined, ground well and an amount of the fine powder equivalent to 50 mg and 30mg of Tinidazole and Roxithromycin was accurately weighed and transferred into a clean 50 mL volumetric flask, dissolved in 10 mL of diluent, sonicated for ten minutes at room temperature, made up to the mark. Further 0.8 ml were pipetted out into a 10ml volumetric flask and diluted up to mark with diluent to get a concentration of 80 µg/ml and 48µg/ml of Tinidazole and Roxithromycin. Then the solution was filtered through 0.45 µ filter and made up to the mark.

General Assay Procedure

20 µL of working standard solutions in the concentration 80 µg/mL (Tinidazole) and 48 µg/mL (Roxithromycin) was injected into the HPLC system using the described chromatographic conditions, the chromatograms and peak area response at concentration were determined by using formulae.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

High-performance liquid chromatography is a novel technique used in the separation and assay of pharmaceutical formulations, especially in combined drugs. This technique is found to be very useful in the study of degradation. The development of a liquid chromatographic method was based on the Physico-chemical properties of selected drugs such as molecular weight, molecular formula, chemical structure, solubility, pKa value, UV absorption maxima, and inactive ingredients. Chromatographic separation was done by Agilent C_{18} (4.6 X 250mm, 5 µm) as column and mobile phase of sodium acetate buffer (pH 3) and Methanol (30:70% v/v). The optimum chromatographic conditions were established by testing different trials by changing one of the chromatographic conditions such as column, mobile phase, and its composition, the flow rate of the mobile phase, injection volume, run time, column temperature and detection wavelength keeping other constant. Finally, the desired separation was achieved by injecting 20 µL of standard solution into the Agilent C_{18} (4.6 X 250mm, 5 µm) column maintained at ambient temperature; elution was carried out by using mobile phase sodium acetate buffer (pH 3) and Methanol (30:70% v/v) at a flow rate of 1.2 mL/min, and the detection at wavelength of 212 nm. The optimized chromatogram is shown in Figure 3.

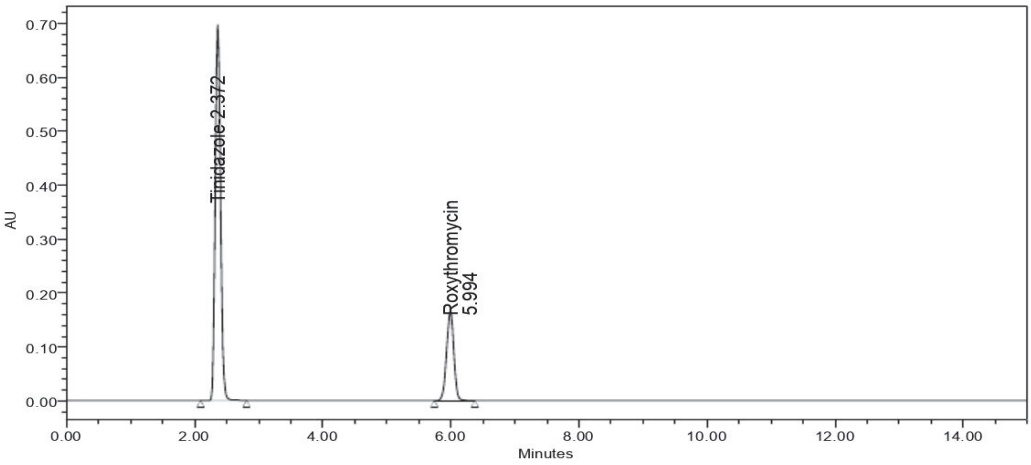

FIGURE 3. Optimized chromatograph of Tinidazole and Roxithromycin

Validation of assay method the method was validated in accordance with the International Conference on Harmonization recommended guidelines [20] for system suitability, linearity, specificity, sensitivity, accuracy, precision, and robustness.

System Suitability

System suitability study is used to make sure that the reproducibility of the HPLC system is sufficient for the analysis to be done. Parameters including plate count, resolution, tailing factor and relative standard deviation for peak area response and retention time of drugs were calculated using Tinidazole and Roxithromycin standard solution with concentration 80 and 48 µg/mL, respectively. The parameters required for the system suitability test of the method are in acceptable limits as presented in Table 1.

TABLE 1. System suitability of Tinidazole and Roxithromycin

Parameter	Tinidazole	Roxithromycin
Peak area % RSD	0.13	0.10
Retention time(min)	2.372	5.994
USP resolution	-	20.265
USP tailing factor	1.18	1.065
USP theoretical Plates	3877.03	13157.245

Linearity

Linearity was assessed by plotting the peak area response of drugs against the concentration of drug-using a simple least-squares regression. The calibration curves were constructed by plotting the peak area *versus* the corresponding concentrations of drug in the range of 20-120 µg/mL for Tinidazole and 12-72 µg/mL for Roxithromycin. The concentration of Tinidazole and Roxithromycin was calculated from the following regression equation: Tinidazole: y=50644x-56210 (R2 = 0.998) Roxithromycin: y=27948x-31599.(R2 = 0.999).

Sensitivity

The sensitivity of the method is assessed by determining the limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ of the analytes were calculated using the following equations: LOD = 3 s/m and LOQ = 10 s/m. where ‘s’ is the standard deviation of the peak area (five runs) of the standard drug, ‘m’ is the slope of the

calibration curve. The Calculated LOD was 0.34 and 0.16µg/ml and LOQ was 1.03 and 0.48 µg/ml for Tinidazole and Roxithromycin, respectively. The low values of LOD and LOQ indicated the sufficient sensitivity of the method for the assay of Tinidazole and Roxithromycin.

Precision

The precision of finite replicate measurements either in intermediate precision or method precision is expressed as a percent of relative standard deviation (%RSD) in statistical analysis and the acceptability should be %RSD ≤ 2.0. The results were shown in Table 2.

TABLE 2. Precision data of Tinidazole and Roxithromycin

Injection	Peak Area			
	Tinidazole		Roxithromycin	
	Method precision	Intermediate precision	Method precision	Intermediate precision
Inj-1	3991258	3982638	2974215	1296258
Inj -2	3962014	3982596	1298473	1298620
Inj -3	3982587	3982429	1299357	1298405
Inj -4	3971047	3987623	1298697	1297698
Inj -5	3982589	3987982	1295120	1295891
Inj -6	3975231	3984682	12963587	1296590
Mean	3977454	3984658	3521575	1297244
SD	10272.79	2574.54	4673969	1155.67
% RSD	0.25	0.064	132.7238	0.08

Recovery Study

The validity of the proposed method was assessed through recovery study by applying the standard addition technique. For this, standard Tinidazole and Roxithromycin were spiked to placebo at three different concentration levels (50, 100 and 150 %). The mean percent recovery of drug at each level was determined. Results given in Table-3 showed that the suggested method is valid and applicable for the analysis of Tinidazole and Roxithromycin with an acceptable percentage recovery. There was no interference from common excipients. The results were represented in table 3.

TABLE 3. Recovery result of Tinidazole and Roxithromycin

Tinidazole				
%Concentration (at specification Level) (n=3)	Peak area (n=3)	Amount Added (mg)	Amount Found (mg)	% Recovery
50%	3168376	40	39.94	99.86
100%	3963837	50	49.97	99.94
150%	4744206	60	59.81	99.68
Roxithromycin				
50%	1031014	24	23.99	99.96
100%	1286911	30	29.98	99.93
150%	1545293	36	35.96	99.88

Robustness

Method robustness was investigated to find out whether small variations in chromatographic conditions such as flow rate of mobile and mobile phase composition affected system suitability for the analysis of Tinidazole and Roxithromycin. Standard drug solution (Tinidazole 80 µg/mL and Roxithromycin 48 µg/mL) was evaluated under test conditions. The system suitability parameters were determined (Table-4). From the results (Table-4), it was observed that small changes in the flow rate and mobile phase composition had minimal effects on system suitability parameters. Hence the proposed method is robust.

TABLE 4. Robustness result of Tinidazole and Roxithromycin

Tinidazole					
	System Suitability Results		Change in Organic Composition in Mobile Phase	System Suitability Results	
Flow Rate (ml/min)	**USP Plate Count**	**USP Tailing**		**USP Plate Count**	**USP Tailing**
10% less flow rate	4402.81	1.11	10% less	3874.80	1.12
*Actual flow rate	4005.68	1.12	*Actual	4005.68	1.12
10% more flow rate	3548.56	1.09	10% more	4010.14	1.10
Roxithromycin					
10% less flow rate	13416.90	0.93	10% less	14260.08	0.96
*Actual flow rate	12025.96	0.94	*Actual	12025.96	0.94
10% more flow rate	11867.41	0.95	10% more	13126.95	0.94

Stability Studies

A study of forced degradation was carried out to evaluate the stability of the drugs in formulations. In the present investigation acid, base and peroxide degradation studies and degradation in presence of thermal energy or photo light were carried out, and the percent of degradation was calculated from the peak area of degradation standard and the degraded test solution. The results of the degradation and stability of drugs were presented in Table 5.

TABLE 5. Results of degradation studies

Stress condition	Tinidazole			Roxithromycin		
	Area	**% Assay**	**% Degraded**	**Area**	**% Assay**	**% Degraded**
Standard	3965878	100	0.0	1289171	100	0
Acid	2507362	63.22	36.78	811610	62.96	37.04
Base	3144548	79.29	20.71	1020585	79.17	20.83
Photo	5367570	92.68	7.32	1174903	91.14	8.86
Thermal	3263923	82.30	17.70	1059228	82.16	17.84

SUMMARY

An analytical method for the simultaneous estimation of Tinidazole and Roxithromycin based on RP-HPLC technique with an ultraviolet detector was developed. The developed method has done with the necessary validation procedures, following ICH guidelines, for reliable analysis of Tinidazole and Roxithromycin with adequate sensitivity, precision, and accuracy for the routine analysis. Also, the method proved to have suitable selectivity and robustness for the analysis.

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REFERENCES

1. D. B. Meshram, P. Mishra, S. D. Desai, M. R. Tajne, Der Chemica Sinica. **8**, 133-137 (2017).
2. B. Siddartha, I. Sudheer Babu, Ch. Ravichandra Gupta, C. Parthiban, World J. Pharm. Pharm. Res. **3**, 1138-1148 (2014).
3. B. Chiranjeevi, B. Swati, M. K. Obula Reddy, P. Shanmugasundaram, M. V. Aanandhi, Int. J. ChemTech Res. **3**, 1309-1317 (2011).
4. M. M. Baraka, E. Elsadek, L. M. Abdelaziz, S. S. Elbermawi, Int. J. Curr. Pharm. Res. Vol **6**, 48-53 (2014).
5. G. Sowjanya, T. Devi, V. S. Valli, V. Pratyusha, V. L. N. Seshagiri Rao, Int. Curr. Pharm. J. **1**, 317-321 (2012).
6. S. Murugan, V. Sunil kumar, P. V. Ruth Madhuri, M. Niranjan Babu, M. K. Kathiravan, Int. J. Novel Trends in Pharm. Sci. **4**, 130-139 (2014).
7. P. Swetha, D. Vijay Kumar, B. Rakesh, A. Ashok Kumar, Int. J. Pharm. Pharm. Sci. **7**, 30-35 (2015).
8. S. K. Patel, P. P. Kapupara, K. V. Shah, Int. J. Res. Develop. Pharm. Life Sci. **4**, 1635-1640 (2015).
9. M. K. Darwish, I. Salama, S. Mostafa, M. El-Sadek, J. Chromatogr. Sci. **51**, 566-576 (2013).
10. T. Sirisha, B. M. Gurupadayya, S. Sridhar, Trop. J. Pharm. Res. **13**, 981-987 (2014).
11. K. A. Umadevi, K. Vandana, D. Arun Kumar, T. Siva Kishore, L. Harika, P. Kishanta Kumar, Int. J. Pharm. Biol. Archives. **2**, 1152-1156 (2011).
12. V. Prathyusha, S. K. Abdul Rahaman, S. Revathi, G. Renuka, Int. J. Pharm. Ind. Res. **3**, 295-300 (2013).
13. S. A. Patel, J. V. Patel, Int. J. Pharm. Chem. Biol. Sci. **3**, 372-379 (2013).
14. M. E. K. Wahba, J. Chromatogr. Sci. **51**, 44-52 (2013).
15. K. Ravi Sankar, S. Prafulla Kumar, M. Mathrusri Annapurna, Anal. Chem. Indian J. **8**, 39-41 (2009).
16. K. K. Kumar, K. E. V. Nagoji , R. V. Nadh, Indian J. Pharm. Sci. **74**, 580-583 (2012).
17. N. Rahman Ahmed, Iraqi National J. Chem. 360-368 (2013).
18. C. M. Bhaskar Reddy, G. V. Subbareddy, J. Chem. Pharm. Res. **4**, 3684-3687 (2012).
19. R. Hari Babu, K. K. Rajasekhar, Asian J. Chem. **21**, 7419-7421 (2009).
20. ICH-Q2 (R1): Validation of Analytical Procedures: Text and Methodology, FDA, Vol 60, 1995: 11260.