Original Article

Development And Validation Of Rp-Hplc Method For Determination Of Rifapentine And Moxifloxacin Hydrochloride In Bulk And Tablet Dosage Form

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Abstract

A novel, simple, precise, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous determination of Moxifloxacine hydrochloride and Rifapentine in bulk and pharmaceutical formulation. The assay involves the elution of Moxifloxacine hydrochloride and Rifapentine on Qualisil BDS C_{18} column (250 mm x 4.6 mm, 5 µ) using mobile phase composition of methanol: sodium phosphate dihydrate buffer with pH adjusted to 3 in the ratio of 70:30 (v/v). The wavelength of detection was 270 nm. The retention time of Moxifloxacine hydrochloride and Rifapentine were found to be 3.66 and 7.35 minutes respectively at a 1.0 ml/min flow rate. Linearity was studied in the concentration range of 4-24 µg per mL and 10-60 µg per mL for rifapentine and Moxifloxacine hydrochloride respectively, with a correlation coefficient of 0.999 and 0.998. The proposed method can be used for routine quality control in bulk and tablet dosage forms. The newly developed method was validated according to ICH guidelines with respect to linearity, recovery, precision, ruggedness, robustness, and sensitivity.

KEYWORDS: Chromatography, Estimation, ICH, RP-HPLC, Tuberculosis.

INTRODUCTION:

Since the introduction of widely and multidrug-resistant (MDR) strains of Mycobacterium tuberculosis, tuberculosis (TB) has remained a significant global issue, making it more difficult to manage. [1]

In addition to other drugs, rifapentine (RFP) is used to treat active tuberculosis. The Italian company that created rifampin first produced rifapentine in 1965, a cyclopentyl-substituted semi-synthetic form of rifamycin. The chemical structure of rifapentine is shown in Figure 1. Bacterial DNA-dependent RNA polymerase is inhibited by rifapentine. [2]

The fourth-generation fluoroquinolone moxifloxacin (MOXI), which is very effective against Mycobacterium tuberculosis, has a large C-7 side chain and a methoxy group in the C-8 position. In figure 2, the MOXI structure is shown. The important enzymes involved in bacterial DNA replication, transcription, repair, and recombination, DNA gyrase (topoisomerase II) and topoisomerase IV, are inhibited by moxifloxacin, which has bactericidal effect. The combination of RFP and MOXI is effective in treating tuberculosis with multidrug resistance. [3]

Current research work focuses on development of accurate, precise and robust RP-HPLC method for determination of RFP and MOXI in bulk as well as in tablet dosage form.

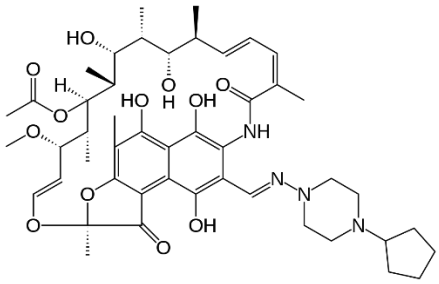

Figure 1: Structure of rifapentine

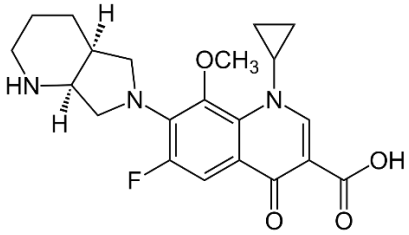

Figure 2: Structure of rifapentine

REAGENTS AND CHEMICALS:

RFP was supplied as a gift sample by Lupin Pharmaceuticals Ltd, Aurangabad, Maharashtra, India. MOXI was supplied as a gift sample by Cipla Pharmaceuticals Ltd, Baddi, India. All chemicals and reagents used were of analytical grade and were purchased from E. Merck Ltd, India. HPLC-grade water was obtained from Milli-Q water purification system (Millipore Co., Milford, MA. USA) and used throughout the study. All the solvents and solutions were filtered through a membrane filter (Millipore Millex® FH, filter units, Durapore-PVDF, Polyethylene, 0.45 µm pore size) and degassed before use. Tablets containing 150 mg of RFP and 400 mg of MOXI were prepared in-house.

CHROMATOGRAPHIC PARAMETERS:

Selection of Chromatographic Mode:

The reversed-phase HPLC was used for method development.

Selection of Stationary Phase:

Based on reversed-phase HPLC mode and the number of carbon present in the molecule (analyte) stationary phase with C-18 bonded phase i.e., Qualisil BDS C_{18} (250 mm x 4.6 mm) with particle size 5 µm was selected.

Selection of Mobile Phase:

The selection was made on the basis of literature survey. After assessing the solubility of drug in different solvents, methanol and sodium phosphate buffer were selected as the first choice. With a view to separate both the drugs simultaneously, various mobile phases consisting of methanol and water were tried, but tailing and low resolution of the chromatogram were observed. Hence the combination of mobile phase was selected such that no tailing is observed as well as both these drugs were resolved properly. [4]

Selection of Detection wavelength

From the overlay spectra, 270 nm was selected for the estimation of both these drugs simultaneously.

Preparation of Stock Standard Solution

The stock standard solution was prepared by dissolving 10 mg, of RFP and MOXI in 100 ml methanol that gives a concentration of 100 µg/mL.

Optimization of Chromatographic Parameters:

In HPLC, optimization is the process of identifying a set of parameters that effectively separate and permit the quantification of the analytes from the endogenous material with acceptable accuracy, precision, sensitivity, and specificity, as well as with cost, simplicity, and speed. [5]

LINEARITY STUDIES [6]:

From RFP stock solution aliquots of 0.4, 0.8, 1.2, 1.6. 2, and 2.4 ml were taken in 10 ml volumetric flasks and diluted up to the mark with a mobile phase such that the final concentration of RFP in the range 4-24 µg per mL. From MOXI stock solution aliquots of 1, 2, 3, 4, 5, and 6 ml were taken in 10 ml volumetric flasks and diluted up to the mark with a mobile phase such that the final concentration of MOXI in the range of 10-60 µg/mL. A constant volume of 20 uL of each sample was injected with the help of a Hamilton Syringe. All measurements were repeated five times for each concentration and a calibration curve was constructed by plotting the peak area versus the drug concentration. Calibration curves are shown in Figure 4.

ANALYSIS OF BULK MATERIAL:

Accurately weighed quantities of 400 mg (MOXI) and 150 mg (RFP) were transferred to 100 ml, volumetric flask containing 50 mL, methanol and volume was adjusted up to mark. It was further diluted to get concentration 41.6 µg/mL of MOXI and 16 µg/m1 of RFP, constant volume 20 uL was injected into column and peak area was recorded. The concentrations of both these drugs were determined from their respective linearity curves. The procedure was repeated for six times; results are shown in chromatogram Figure 5.

ANALYSIS OF TABLET FORMULATION [7]:

To determine the content of MOXI and RFP in tablet formulation; twenty in house tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 400 mg of MOXI and 150 mg of RFP was weighed and transferred into 100 mL volumetric flask containing about 50 mL methanol and volume was adjusted up to mark. The solution was filtered through 0.45 µm membrane filter paper. The solution was further diluted with mobile phase to obtain concentration 41.6 µg/mL (MOXI) and 16 µg/mL (RFP). Six injections of the sample solutions were made into the column. The linearity curve of these two medicines was used to compute their concentrations. Results are shown in chromatogram Figure 6.

VALIDATION [8]:

The method was validated as per the ICH guidelines in terms of accuracy, and precision. ruggedness, robustness and sensitivity.

Accuracy

Accuracy is determined by applying the method to the samples in which 80%, 100% and 120% of the standard have been added. The accuracy studies were carried out 3 times. The results of accuracy study are indicated in table 2.

Precision:

By examining the method's repeatability, precision can be achieved. Studies on repeatability were conducted utilizing intra- and inter-day variation. Variations like different days and analysts lead to the intermediate precision. Three different concentrations covering the linearity range were used for the intra-day variation investigations on the same day. The developed method's inter-day fluctuations were evaluated by looking at three distinct concentrations on three different days. The determination of RFP and MOXI intra- and inter-day variation was performed at three distinct concentration levels of 8, 12, and 16 g per mL and 20.8, 31.2, and 41.6 g per mL, respectively. The results are shown in Table 3.

Ruggedness

Ruggedness of the method was performed by two different analysts keeping experimental and environmental conditions same. The concentrations used to perform ruggedness were 16 and 41.6 of RFP and MOXI respectively Table 4.

Robustness

The robustness of proposed method was done by small deliberate change in the chromatographic conditions such as change in pH (± 0.2), mobile phase composition (± 2) and flow rate (± 0.2). The results are shown in Table 5.

Sensitivity

The quantitation limit is a parameter of quantitative assay for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using following formul.

LOD = 3.3 (SD)/S;

LOQ = 10 (SD)/S;

Where,

SD = Standard Deviation of response,

S = the slope of the calibration curve.

System Suitability Test

The USP states that the system appropriateness test is an essential component of liquid chromatographic techniques. The assurance of the quality performance of the chromatographic system depends on system suitability testing. For system suitability testing, previously prepared solutions for chromatographic conditions were evaluated. Results are shown in Table 6.

RESULT AND DISCUSSION:

Optimization of chromatographic parameters:

After several trials, mobile phase for HPLC was optimized as methanol: sodium phosphate Dihydrate buffer in the ratio of 70:30 (v/v). Well-defined chromatograms were observed when the pH of the buffer was adjusted to 3 with Ortho-phthalaldehyde at a flow rate of 1 mL per minute; the retention time for MOXI and RFP was found to be 3.66±0.2 and 7.35 ±0.2 min respectively as shown in figure 3. The total time of analysis was less than 10 minutes. Finalized chromatographic conditions are indicated in Table 1.

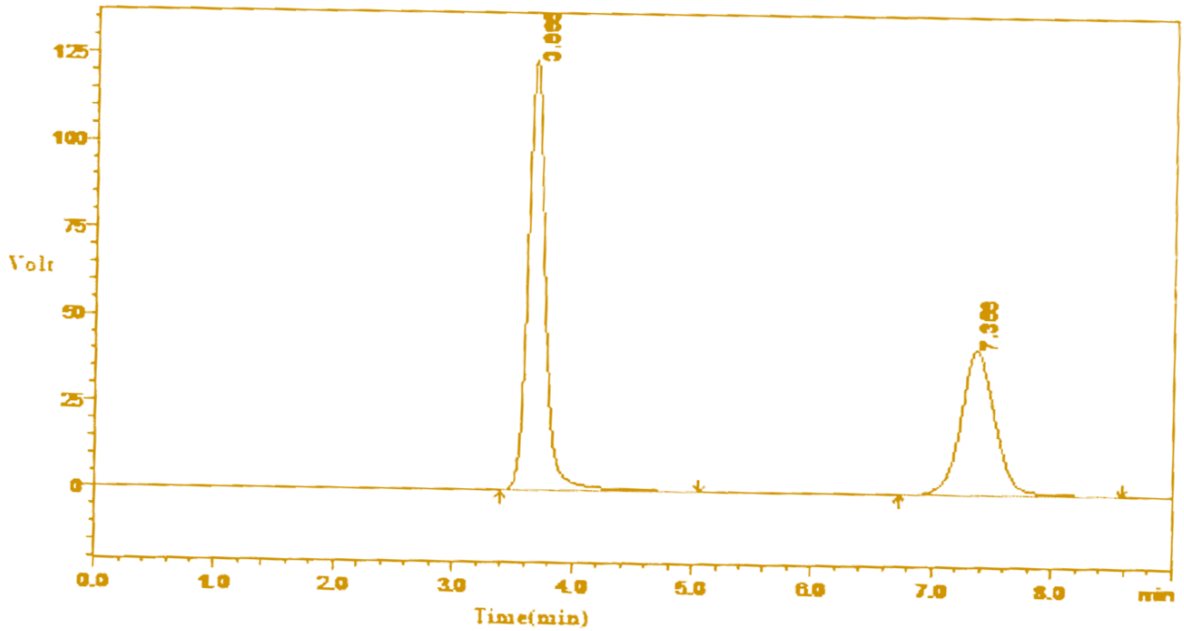

Figure 3: Chromatogram of MOXI and RFP in methanol and buffer (70:30)

Table 1: Final chromatographic conditions

Chromatographic mode	Chromatographic condition
HPLC system	LC-20 Shimadzu
Pump	Binary gradient
Detector	PDA
Data Processor	LC solution
Stationary phase	Qualisil BDS C_{18} (250 mm x 4.6 mm)
Mobile phase	Methanol: Sodium phosphate dihydrate buffer, 70:30 (v/v)
Detection wavelength	270 nm
Flow rate	1 ml/min
Sample size	20 µL

Linearity:

Linearity range of RFP was found to be 4-24 µg/ml and MOXI was found to be 10-60 µg/ml with correlation coefficient (r^2 value) of 0.999 and 0.998 for RFP and MOXI respectively which indicates a good correlation between concentration and peak area.

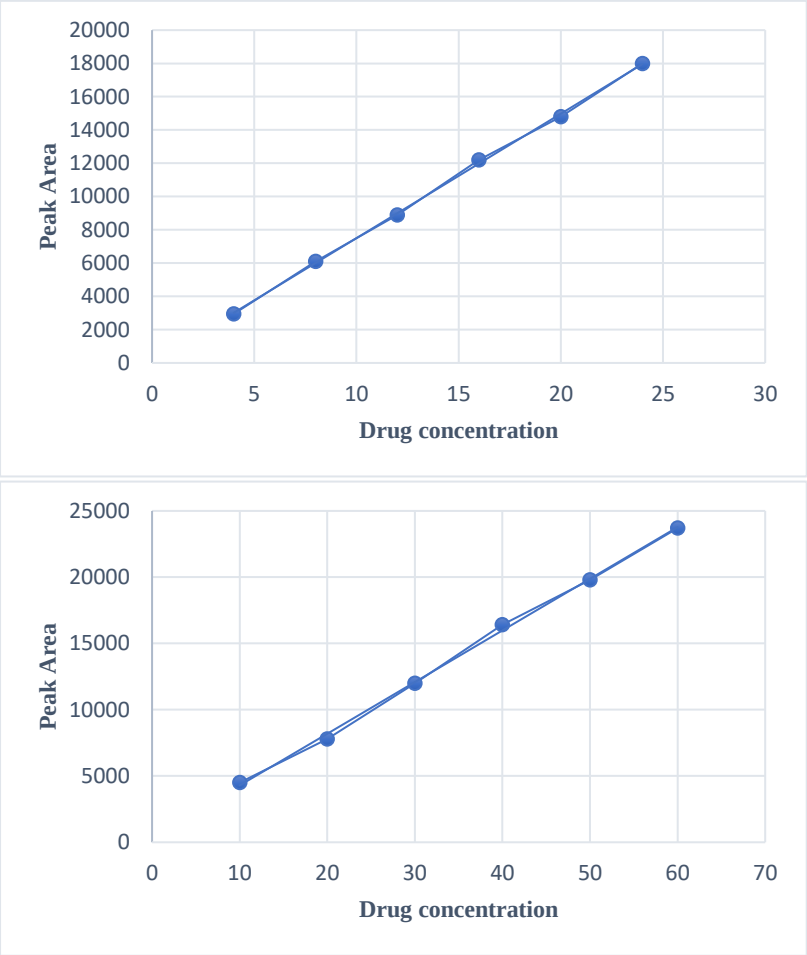

Figure 4: Calibration curves of RFP and MOXI respectively.

Analysis of bulk material:

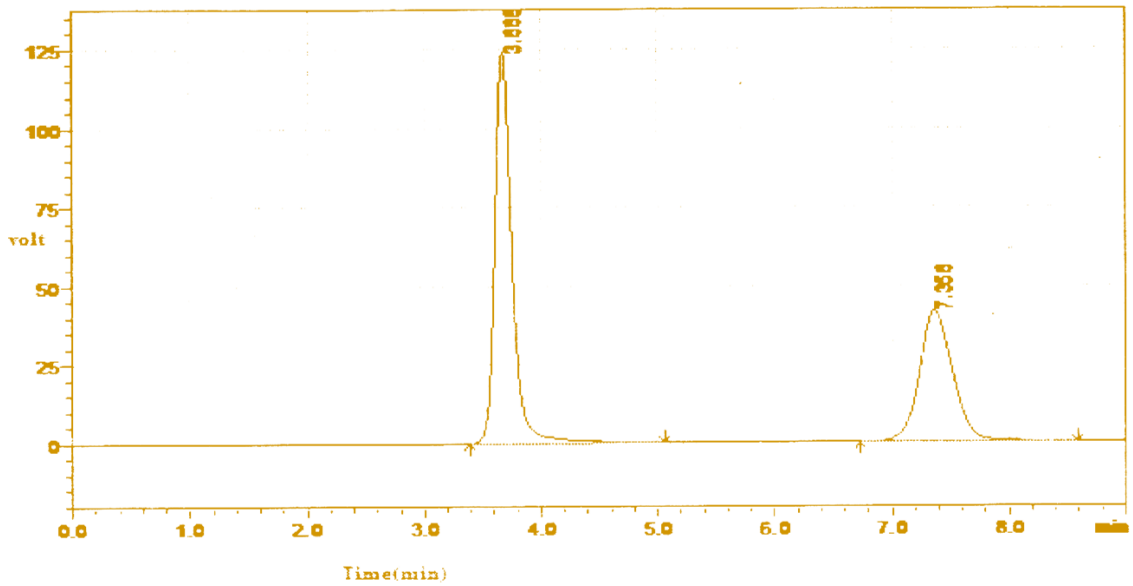

Figure 5: Chromatogram of standard MOXI (16 µg) and RFP (41.6 µg) in methanol:Buffer system (70:30 v/v pH adjusted to 3)

In analysis of bulk material amount of RFP taken was 16 mg and the amount found during analysis is 15.84±0.022 with % of Relative Standard Deviation (% RSD) of 0.139. Also, in case of MOXI amount taken for analysis was 41.6 mg and amount estimated after analysis was 41.38±0.681 with % RSD of 1.646. The values are mean of six estimations at each level.

Analysis of Tablet formulation:

Amount of RFP and MOXI in tablet formulation was 150 mg and 400 mg respectively. Proper dilution yielded concentration of 16 µg/mL of RFP and 41.6 µg/mL of MOXI. Analysis with developed method found amount of RFP and MOXI 15.82±0.057 µg/mL and 41.08±0.243 µg/mL respectively with % RSD of 0.363 and 0.591 which indicated suitability of method.

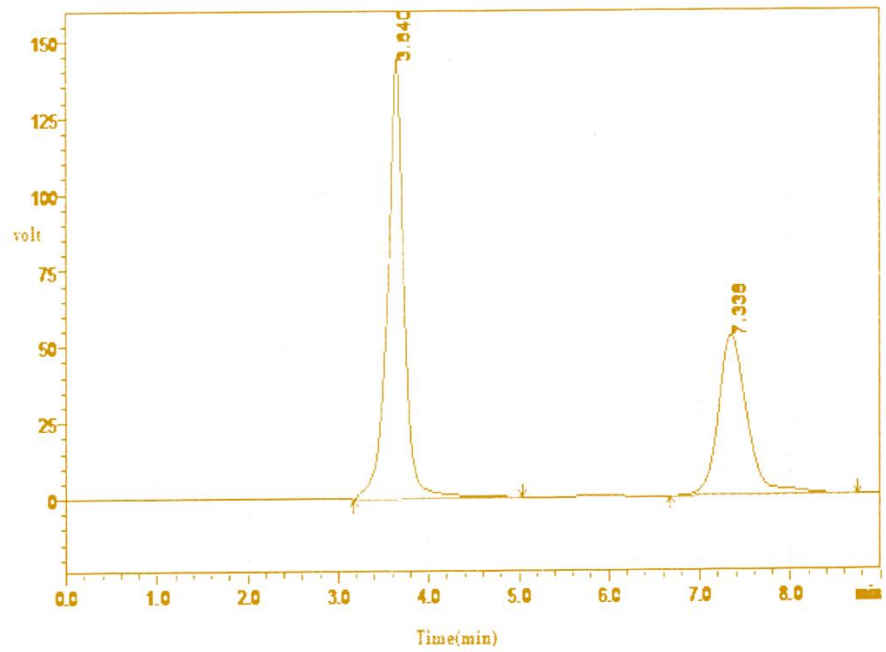

Figure 6: Chromatogram of standard MOXI (16 µg) and RFP (41.6 µg) extracted from tablet formulation

Validation:

Method validation was carried out to determine accuracy, precision, ruggedness, robustness and sensitivity of developed method.

Accuracy:

Table 2: Results of Accuracy study.

Drugs	Initial amount (µg/ml)	% Amount added	% Amount recovered	% RSD
		80	98.56	1.28
RFP	8	100	99.00	0.72
		120	99.86	0.78
		80	100.89	1.19
MOXI	20.8	100	99.45	0.70
		120	99.18	082

* Mean of three estimations at each level

The accuracy of method was determined by adding 80%, 100% and 120% standard drug. The accuracy is then calculated from the test results as a percentage of the analyte recovered by the assay. and the percent recovery and percent RSD was calculated. Results of accuracy study indicate that method suitably identified added amount of drug.

Precision:

The repeatability was evaluated by assaying six times of sample solution prepared for assay determination. The intra- and inter-day precision studies of RFP and MOXI were carried out by estimating different concentrations of RFP (8, 12, 16 µg per mL) and MOXI (20.8, 31.2, 41.6 µg per mL), three times on the same day and three different days (first, second, third), and the results are reported in terms of % RSD.

Table 3: Intra-day and Inter-day Repeatability studies.

Drug	Conc. (µg/mL)	Intra-Day Amount Found (%) (n=3)		Inter-day Amount Found (%) (n=3)	
		% Mean	% RSD	% Mean	% RSD
	8	101.46	0.17	101.73	0.24
RFP	12	99.38	0.75	99.47	0.19
	16	98.83	0.60	99.04	0.72
	20.8	99.19	0.22	99.19	0.24
MOXI	31.2	99.34	1.00	99.42	0.93
	41.6	98.83	0.59	100.51	0.56

* Mean of three estimations at each level

Repeatability studies indicated good fit of method for intra-day as well as for inter-day variation.

Ruggedness study:

Table 4: Results of ruggedness studies by change of analyst.

Drug	% Amount found (n=6)		% RSD	
	Analyst I	Analyst II	Analyst I	Analyst II
RFP	99.36	99.24	0.78	0.36
MOXI	99.44	98.06	1.22	0.66

* Mean of six estimations at each level

Ruggedness studies showed that change in analyst doesn't cause a change in results. Also, % RSD values indicated good fit of method though there is analyst change.

Robustness studies:

Robustness studies were carried out by deliberate change in chromatographic conditions like pH change, change in mobile phase composition and change in column temperature. Robustness studies indicated good fit of method for change in pH, change in mobile phase composition and change in temperature of the column.

Table 5: Results of robustness study

Parameters	RFP		MOXI	
	Tailing factor	Theoretical plate	Tailing factor	Theoretical plate
Change in pH of buffer				
3.2	1.243	3061	1.143	3249
2.8	1.123	3097.2	1.035	3317
Change in mobile phase composition				
Methanol: Buffer (68:32)	1.251	3117	1.139	3550
Methanol: Buffer (72:28)	1.135	3127	1.241	2763
Change flow rate				
0.8 ml	1.358	2101	1.312	3025
1.2 ml	1.176	3360	1.147	2911

* Mean of three estimations at each level

Sensitivity:

LOD and LOQ were found to be 0.29 µg/ml and 0.96 µg/ml for RFP and 1.2 µg/ml and 4.3 µg/ml for MOXI, respectively.

System suitability study:

Primary SST parameters are the resolution (R), Theoretical plate (N), and tailing factor (T). These parameters are most important as they indicate system specificity, precision, and column stability.

Table 6: System suitability factors.

Parameters	RFP	MOXI
Theoretical plate (N)	3061	3317
Tailing factor (T)	1.123	1.139
Resolution (R)	1.654	1.942

CONCLUSION:

The developed method gave good resolution between RFP and MOXI with a short analysis time (less than 10 minutes) and high efficiency and complies with the system suitability test specifications of ICH. The use of C_{18} column in the present work has shown better elution of analytes with good resolution, improved plate count, and capacity factor. So, the C_{18} column can be used to achieve high specificity in a shorter time of analysis of RFP and MOXI as per ICH guidelines. The method is found to be robust, rugged, sensitive, accurate, and precise.

REFERENCES:

1. Shah P, Pandya T, Gohel G, Thakkar V. Development and Validation of HPLC method for simultaneous estimation of Rifampicin and Ofloxacin using experimental design. J Taibah Univ Sci. 2012;13(1):146-154.
2. Sonal S, Kambili C, Shama DA. Rifapentine for the Treatment of Pulmonary Tuberculosis. Clin Infect Dis. 2006;43(11);:1468–1475.
3. Kaur V, Pawar P. Formulation and Evaluation of Moxifloxacin Hydrochloride Niosomes for Controlled Ophthalmic Drug Delivery. J. Pharm. Technol. Res Manag. 2015;3(1):11–28.
4. Ahuja BK, Jena SK, Paidi SK, Bagri S, Suresh S. Formulation, optimization and in vitro-in vivo evaluation of febuxostat nanosuspension. Int J Pharm. 2015;478(2):540-552.

5. Peraman R, Mallikarjuna S, Ammineni P, Kondreddy VK. RP-HPLC method development and validation for simultaneous estimation of atorvastatin calcium and pioglitazone hydrochloride in pharmaceutical dosage form. J Chromatogr Sci. 2014;52(9):1038-1042.
6. Akula G, Talari Y, Phanindra SS, Akula G. Method development and Validation for simultaneous estimation of Melatonin and Zolpidem tartrate by using RP-HPLC. Sch Acad J Pharm. 2015;4(4):240-244.
7. Sharma S, Bhandari A, Choudhary VR, Rajpurohit H, Khandelwal P. RP-HPLC Method for Simultaneous Estimation of Nitazoxanide and Ofloxacin in Tablets. Indian J Pharm Sci. 2011;73(1):84–88.
8. Pardhi V, Pant G, Flora SJS. RP-HPLC Method Development and Validation for Bedaquiline Fumarate to Evaluate its Forced Degradation Behavior and Stability in Official Dissolution Media. Future J Pharm Sci. 2020;6:42.