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Development and Validation of HPLC method for simultaneous estimation of Rifampicin and Ofloxacin

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ABSTRACT

A very accurate, precise, and reliable isocratic HPLC technique has been developed and verified for the simultaneous quantification of Rifampicin and Ofloxacin. The chromatographic separation was performed using a Phenomenex C18 column with a mobile phase consisting of a combination of 0.05M KH_2PO_4 buffer at pH 4.0 and methanol in a ratio of 55:35. The separation was monitored at a wavelength of 230 nm. Ofloxacin had a retention time of 4.920 min, whereas Rifampicin had a retention time of 11.710 min. The calibration plots for Rifampicin and Ofloxacin were linear between the concentration ranges of 2–10 µg/ml and 4–20 µg/ml, respectively. The correlation coefficient for Rifampicin was 0.9999, while for Ofloxacin it was 0.9996. The Rifampicin had a limit of detection (LOD) of 0.2345 µg/ml, whereas the Ofloxacin had a LOD of 0.4625 µg/ml.The approach underwent validation to assess its linearity, accuracy, precision, and robustness. The percentage of recoveries was determined to be about 100% with little fluctuation. This approach can be used for regular analysis.

Keywords: Rifampicin, Ofloxacin and RP-HPLC.

Introduction:

TB treatment has grown more complicated due to the rise of multidrug-resistant (MDR) and extensively drug-resistant Mycobacterium tuberculosis strains, a worldwide challenge. In 2015, 10.4 million new cases of tuberculosis (TB) and 480,000 MDR-TB were recorded globally. TB killed nearly 1.4 million people globally in 2015 [1]. The prolonged period of therapy, hepatotoxicity, many side effects of conventional drugs, and early treatment termination make current tuberculosis treatment ineffective [2]. Thus, combination therapy reduces resistance by lowering treatment time, highlighting the need for new drug combinations. Streptomyces mediterranei produces rifampicin (RIF), a rifamycin antibiotic [3]. Complex semisynthetic macrocyclic chemical used to treat TB and other infectious disorders [4–7]. It is a key antituberculous agent. RNA production decreases and cell death occurs when RIF inhibits DNA-dependent RNA polymerase. This medicine only treats mycobacterial infections owing to the fast spread of drug-resistant microorganisms. Combining therapies delays resistance development in some circumstances. The drug also treats asymptomatic meningococcal carriers. Fluoroquinolones like ofloxacin (OFL) are now a common and promising drug-resistant therapy [9]. Clinical data suggests that fluoroquinolones may shorten treatment for drug-susceptible M. tuberculosis [10]. OfL is a human-made fluoroquinolone antibiotic. It prevents DNA replication by preventing bacterial DNA gyrase twisting [11].

Numerous studies have shown that adding OFL to the first rifampicin drug combination has a synergistic impact, improving therapy. This combination may work for drug-resistant and drug-susceptible isolates [12, 13]. A comprehensive literature review has documented several methods for measuring RIF [14–21] and OFX [22, 24] alone and in combination with other drugs. A high-performance liquid chromatographic (HPLC) method that concurrently measures RIF and OFX is unknown. This work aimed to create a selective, precise, and accurate reverse phase HPLC assay technique for parallel measurement of RIF and OFL in synthetic combinations. The suggested approach is validated under ICH Q2 (R1). To determine Ofloxacin and Rifampicin simultaneously using reverse phase high-performance liquid chromatography (HPLC), this study developed accurate and targeted methodologies.

Materials and Methods:

The drug substances used in this investigation include Rifampicin and Ofloxacin. The chemicals used for the analysis are Potassium dihydrogen orthophosphate (HPLC grade), Orthophosphoric acid (HPLC grade), Water (HPLC quality), and methanol (HPLC grade). The HPLC system used for the study is the Shimadzu-LC2010-CHT, manufactured in Kyoto, Japan.

Instrumentation:

An HPLC system, namely the Shimadzu-LC2010-CHT model from Kyoto, Japan, was used for the study. The system included an LC solutions data processing system, an SPD-M20A PDA detector, and an auto sampler. The data was collected using LC 2010 solutions software, specifically version 1.25. The investigation used an analytical balance (Shimadzu

AUW220 balance, Japan), a vacuum filtration assembly (TID 15, Mumbai, India), and an ultrasonic bath sonicator.

Selection of Wavelength:

The chromatographic conditions were optimized in order to establish a high-performance liquid chromatography (HPLC) approach for simultaneously determining RIF and OFL in both bulk and medicinal dose forms. To determine the appropriate wavelength, 10 µg/ml standard solutions of RIF and OFX were analyzed using methanol as a reference in the spectrum mode, scanning between 200 and 400 nm. Both medicines exhibited significant absorption at a wavelength of 230 nm, which was chosen as the wavelength for detection (Figure 1).

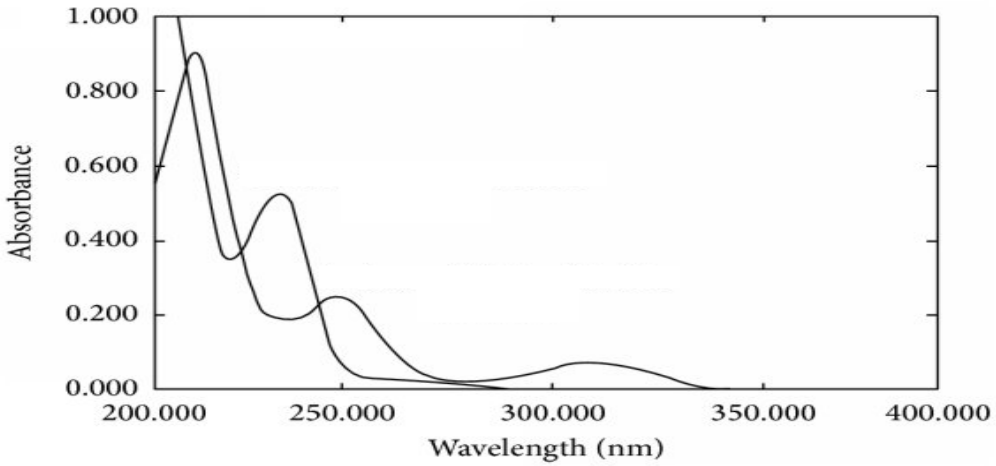

Fig.No. 01: Zero order overlay spectra of RIF and OFL.

Preparation of mobile phase:

The mobile phase was prepared by mixing Potassium dihydrogen orthophosphate, acetonitrile, and methanol at a ratio of 65:35. Subsequently, the pH of the buffer was adjusted to 4.0, and the resultant solution was filtered using a 0.45µ membrane.

Preparation of standard stock solution:

10.0 mg of Rifampicin and 20.0 mg of Ofloxacin were quantified using a digital microbalance and then transferred into a 10 millilitre volumetric flask. After adding seven millilitres of diluent, the mixture underwent sonication to aid in dissolving. Afterwards, the solution was mixed with the diluent until it reached its maximum volume, and eventually, it was further diluted to get the necessary final volume by adding additional diluent.

Chromatographic conditions:

High Performance Liquid Chromatography equipped with PDA detector.

For Rifampicin and Ofloxacin (isocratic)

Column	:	Phenomenex C18 150 x 4.6 mm, 5m analytical column
Wavelength	:	230 nm
Injection Volume	:	20µl
Column Temperature	:	Ambient

Flow rate : 1.0 ml/min

The RIF peak was seen at a retention time of 4.920 minutes. The displayed area measured 847,645 units, with a tailing factor of 1.23. Figure 2 and Table 1 indicate that the OFL peak was seen during a retention time of 11.710 minutes. The peak area was measured to be 6748754, with a tailing factor of 1.16 and a resolution of 5.35. This trial was deemed optimal due to its excellent outcomes and shorter retention period. The retention period of RIF is around 4.920 minutes, whereas the retention length of OFL is 11.710 minutes.

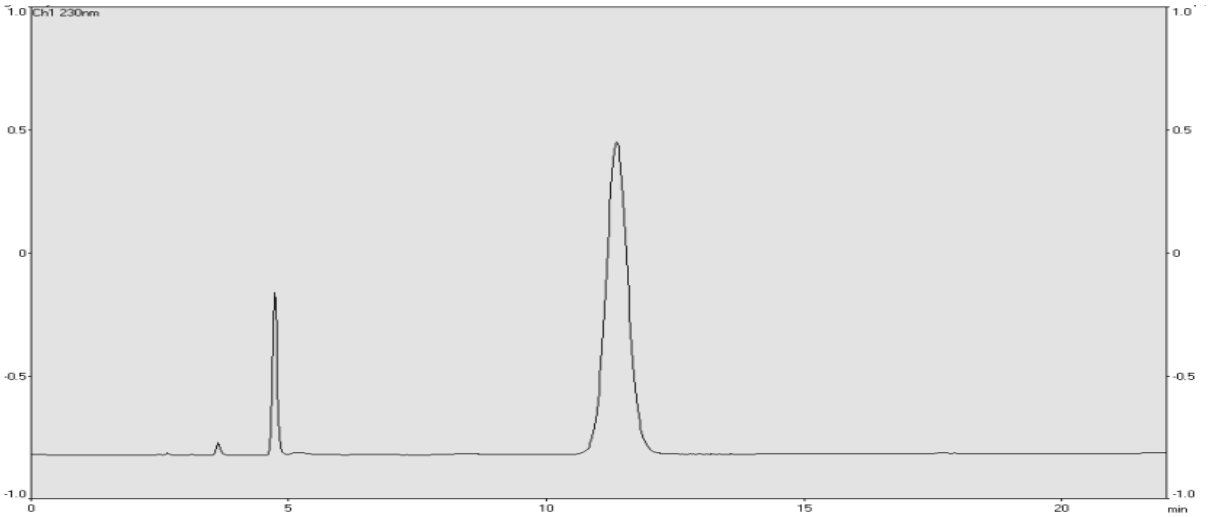

Fig.No. 02 : HPLC chromatogram for linearity of RIF and OFL at 230 nm

Table 1: System suitability parameters

S.No.	Name of the Peak	Retention Time (Mins)	Peak Area	Tailing Factor	Resolution	Plate Count
01	**Rifampicin**	4.920	847645	1.23	---	6978
02	**Ofloxacin**	11.710	6748754	1.16	5.35	7547

Preparation of sample solution:

A total of 10.0 mg of Rifampicin and 20.0 mg of Ofloxacin were accurately measured and transferred into a 10 milliliter volumetric flask. Afterward, 7 milliliters of diluent were added. Afterwards, the combination underwent sonication to dissolve the material, and was subsequently diluted with a diluent until the necessary volume was achieved. The solution was further decreased to a volume of 10 ml by introducing the diluent and then filtered using a 0.45µ Nylon syringe filter.

Procedure:

Six injections, each with a volume of 20 µl, were delivered using active standard solutions of RIF and OFL. Chromatograms were obtained and the peak responses were evaluated. The suitability of the system was evaluated by examining its parameters. The quantification of the quantities of RIF and OFL in the sample was achieved by examining the peak responses.

Method Validation:

The present analysis evaluated many criteria to verify the precision of the HPLC method in quantifying the amounts of RIF and OFL, in accordance with the authorized procedure. This demonstrates that the methodology is suitable for its designated objective. The validation criteria were implemented in compliance with the requirements set by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).

Linearity and Range:

The concentrations of RIF and OFL that showed a direct link with peak area were between 2 and 10 µg/ml for RIF and between 4 and 20 µg/ml for OFL. The findings are shown in Figures 3 and 4, Tables 2 and 3, and the accuracy of the calibration curve is confirmed by the high correlation coefficient of the regression equation.

Table 2: Linearity data of RIF

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	2	424822
3	4	847645
4	6	1282467
5	8	1695290
6	10	2119455
Slope		**37621**
Intercept		**2501.1**
Regression		**0.9997**

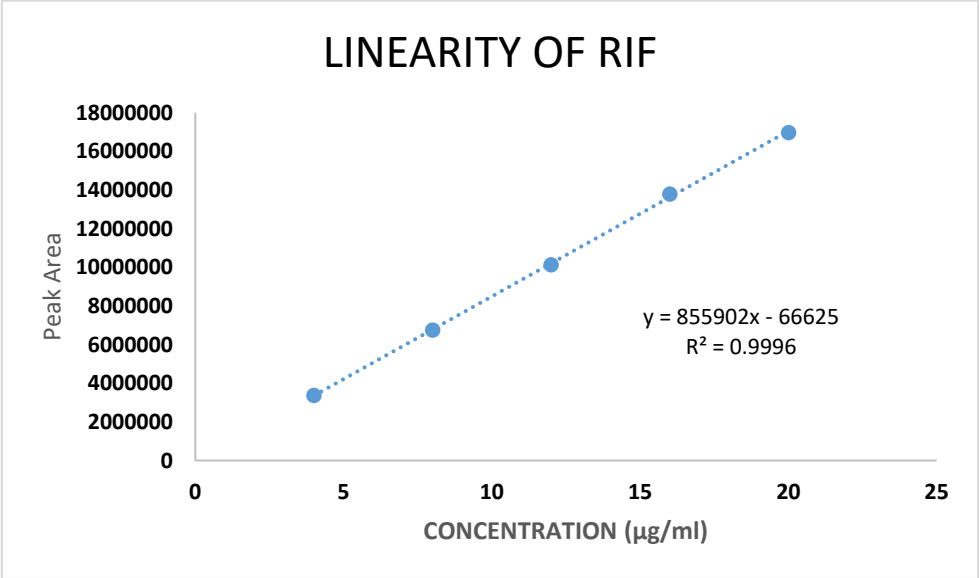

Fig.No. 03 : Linearity of Rifampicin

Table 3: Linearity data of OFL

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	4	3378378
3	8	6748754
4	12	10124156
5	16	13797811
6	20	16971885
Slope		**855902**
Intercept		**-66625**
Regression		**0.9996**

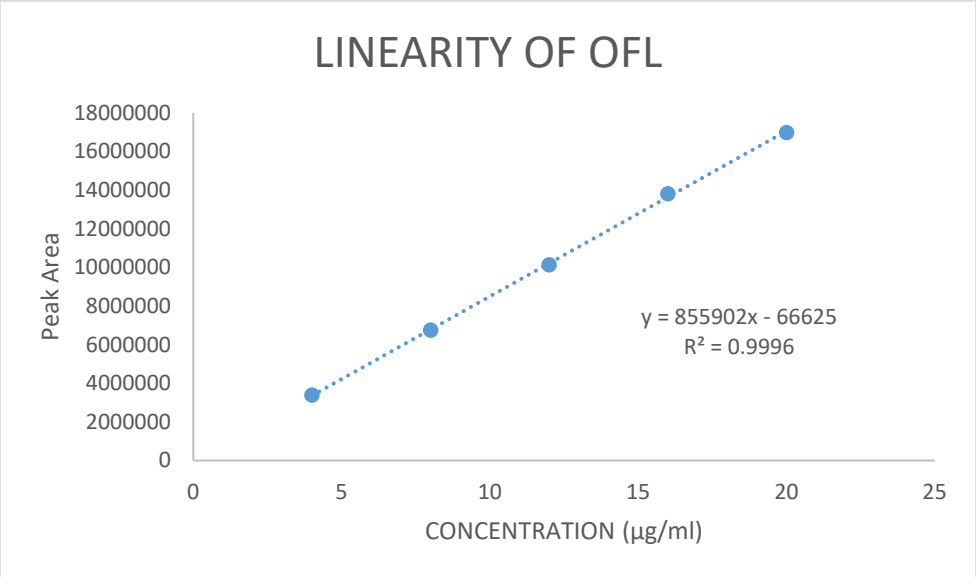

Fig.No. 04 : Linearity of Ofloxacin

Accuracy and Precision:

To evaluate the accuracy of recovery, we added extra Standard medication at three distinct concentration levels to a previously analyzed test solution. Our data indicate that the proposed technique is very accurate in predicting both RIF and OFL concurrently. The RSD was below 2.0%. In addition, we successfully obtained a recovery rate of 100.49% for RIF and 100.10% for OFL. The dependability of the Method is shown by its high level of repeatability and low values of relative standard deviation (RSD). The tables designated as 4 and 5.

Table 4: Precision data of RIF and OFL

Injection Number	RIF				OFL			
	Retention Time	**Peak Area**	**Plate Count**	**Peak Symmetry**	**Retention Time**	**Peak Area**	**Plate Count**	**Peak Symmetry**
1	4.92	847711	6978	1.12	11.71	6748754	7567	1.22
2	4.91	847645	6999	1.23	11.65	6746734	7678	1.22
3	4.92	847723	6912	1.34	11.7	6749234	7689	1.34
4	4.9	847812	6923	1.25	11.45	6749467	7356	1.56
5	4.91	848657	6956	1.34	11.34	6749278	7547	1.1
6	4.92	849675	6979	1.45	11.34	6749678	7898	1.11
Average	**4.913**	**1403270**			**11.532**	**6748858**		
Standard Deviation	**0.008**	**813.59**			**0.176**	**1084.73**		
% RSD	**0.1662**	**0.06**			**1.52**	**0.02**		

Table 5: Accuracy data of RIF and OFL

Sample Preparation No.	RIF Assay (%)	OFL Assay (%)
1	100.12	99.78
2	100.23	99.9
3	101.34	99.89
4	99.99	100.55
5	100.83	100.23
6	100.34	100.25
Mean	**100.48**	**100.10**
SD	**0.5126**	**0.2927**
RSD (%)	**0.5102**	**0.2924**

Robustness:

The outcomes of the robustness assessment are shown in Table 6. Both components had similar tailing factors, elution orders, resolutions, relative standard deviations, and recoveries. The research indicated that the relative standard deviation (RSD) of the peak locations was much less than 2.0%.

Table 6: Robustness data of RIF and OFL

Condition	Rifampicin			Ofloxacin		
	% RSD	Tailing Factor	% Recovery	% RSD	Tailing Factor	% Recovery
1) Change in Flow rate						
Normal Condition (1.0 ml per minute)	0.18	1.11	100.01	0.11	1.21	100.21
Flow rate (0.8ml per minute)	0.19	1.12	100.21	0.12	1.22	100.12
Flow rate (1.2 ml per minute)	0.20	1.21	100.21	0.13	1.25	100.23
2) Change in minor component in the mobile phase						
Normal Condition (Potassium dihydrogen orthophosphate, methanol in a ratio of 65:35)	0.18	1.23	100.21	0.18	1.31	100.23
(Potassium dihydrogen orthophosphate, methanol in a ratio of 55:45)	0.12	1.31	100.02	0.14	1.28	99.32
(Potassium dihydrogen orthophosphate,	0.15	1.29	100.32	0.19	1.26	99.89

methanol in a ratio of 75:25)						
3) Change in Wave Length						
Normal:Wave Length 230 nm	0.14	1.31	100.42	0.18	1.26	100.41
Wave Length 235 nm	0.12	1.22	100.34	0.23	1.21	100.71
Wave Length 225 nm	0.19	1.12	100.71	0.19	1.28	100.12
4) Change in Ph						
Normal:pH 4.0	0.18	1.23	100.23	0.24	1.29	100.23
pH 4.5	0.19	1.25	100.34	0.21	1.19	100.34
pH 3.5	0.11	1.28	100.12	0.28	1.23	100.87

Ruggudness:

The mean peak areas of Rifampicin and Ofloxacin were 845,682 and 6,748,934, respectively, with relative standard deviations (RSD) of 0.12% and 0.21%, respectively.

SUMMARY:

The study presents a newly designed and validated reverse-phase high-performance liquid chromatography (RP-HPLC) technique for precise quantification of RIF and OFL in both bulk and pharmaceutical samples. The literature analysis revealed a deficiency in approaches for precisely determining RIF and OFL in substantial quantities. Consequently, there is an urgent want for a direct, cost-effective, and precise solution to address this issue.

The concentrations of RIF and OFL were determined by injecting a solution consisting of Potassium dihydrogen orthophosphate and methanol (in a ratio of 65:35) with a pH of 4.0 onto a Phenomenex C18 column with dimensions of 150 x 4.6 mm and a particle size of 5m. The flow rate was set at 1.0 mL/min, while the injection volume was 20 µL. The RIF peak had a retention duration of 4.920 minutes, whereas the OFL peak showed a retention period of 11.710 minutes.

After its enhancement, the technique underwent verification in accordance with ICH requirements to evaluate its compatibility with the system, linearity, sensitivity parameters, precision, accuracy, and durability. The findings of all validation parameters were within acceptable limits. The tests showed RSD values below 2, indicating a low level of variability. The recovery rate ranged from 99% to 101%.

CONCLUSION:

The recommended RP-HPLC technique offers a time-efficient and basic approach that is both simple and rapid. Additionally, it guarantees cost-efficiency. Therefore, it is a favored method for simultaneously measuring the amounts of Rifampicin and Ofloxacin. The implemented method was thoroughly verified to ensure adherence to ICH standards in every area.

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