https://doi.org/10.48047/AFJBS.6.14.2024.3737-3749

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Development and Validation of HPLC Method for the Simultaneous Estimation of Rifampicin and Isoniazid in Bulk and Tablet Dosage Form

Patil Shivam Ravindra * , Dr.Umair Ahmed, Dr.Bakhshi Abdurrahman ,Shabib Hassan,Ambreen Kausar, Dr.Kaveri Wagh ,Dr.Rashid Akhtar

Address for correspondence*
Royal College of Pharmaceutical Education and Research ,
Sayne Khurd ,Malegaon, Nashik, Maharashtra, 423203, India
Email id: shivampatil2020@gmail.com

Volume 6, Issue 14, Aug 2024

Received: 09 June 2024

Accepted: 19 July 2024

Published: 08 Aug 2024

doi: 10.48047/AFJBS.6.14.2024.3737-3749

ABSTRACT:
The HPLC method was developed and validated for simultaneous estimation of Rifampicin and Isoniazid. The mobile phase consisted of pH 3.5 phosphate: Methanol: water (45:30:25). The linearity range of Rifampicin was found to be 9-45 µg/ml and Isoniazid 6-30 µg/ml. The calibration curve was plotted, and regression equation of Rifampicin was found to be y = 78,303.72x - 48,524.90 with correlation coefficient (r2) of 0.9992 and Isoniazid y = 47,353.45x + 51,031.70 with correlation coefficient (r2) of 0.9993. Detection was done at 239 nm and the retention time of Rifampicin was found to be 2.8 min and Isoniazid 3.7 min with the flow rate of 0.8 ml/min. From accuracy study % recovery of Rifampicin was found in the range of 98.72-100.98% and Isoniazid is 98.77-101.45% which is in the limits according to the ICH guidelines. The limit of detection and limit of Quantitation of Rifampicin is 0.24µg/ml – 0.72µg/ml and Isoniazid is 0.06 µg/ml – 0.19 µg/ml respectively. The method was found to be simple, linear, rapid, accurate, precise, reproducible and robust. The % RSD was found within the limit as per ICH guidelines. All the analyzed validation parameters showed acceptable data with satisfactory correlation co-efficient and lower % RSD as per the ICH guidelines. The developed method can be utilized by industry for quantitative simultaneous estimation of Rifampicin and Isoniazid as bulk and in tablet dosage form.

KEYWORDS: Isoniazid, Rifampicin, HPLC method, ICH Guidelines

INTRODUCTION:

Rifampicin is an antibiotic used to treat numerous types of mycobacterial infections including Mycobacterium avium complex, leprosy, and in conjunction with other antibacterials to treat latent or active tuberculosis. A semisynthetic antibiotic generated from Streptomyces mediterranei. It has a broad antibacterial range, including effectiveness against numerous types of Mycobacterium. In sensitive organisms it inhibits DNA-dependent RNA polymerase activity by creating a stable

compound with the enzyme. It thereby suppresses the beginning of RNA synthesis. Rifampin is antibacterial, and operates on both intracellular and extracellular organisms.

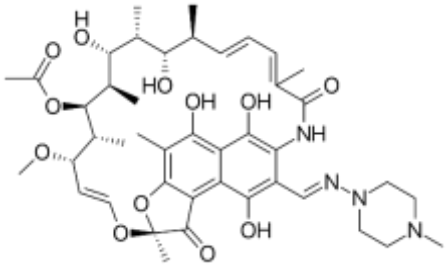

Fig 1: Structure of Rifampicin

Isoniazid is an antibiotic used to treat mycobacterial infections; most typically usage in combination with other antimycobacterial medicines for the treatment of active or latent tuberculosis. Antibacterial agent used largely as a tuberculostatic. It remains the therapy of choice for tuberculosis.

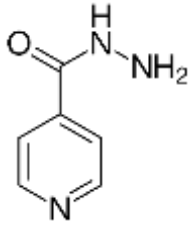

Fig 2: Structure of Isoniazid

MATERIALS AND METHOD:

Instruments:

The chromatographic method was performed Analytical Technologies HPLC system coordinated with a variable wavelength programmable UV identifier and a Rheodyne injector outfitted with 20µl fixed circle. An opposite stage Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron) was utilized. Wenser High Precision Balance Model: PGB 100 electronic equilibrium were utilized for Spectrophotometric judgments and gauging purposes individually.

Reagents and chemicals

Rifampicin and Isoniazid was procured from PharmaTech Solutions. HPLC grade Acetonitrile and water were acquired from Merck specialities private restricted, Mumbai.

Chromatographic conditions

Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron) was utilized for the chromatographic method at wavelength of 239 nm. pH 3.5 Phosphate buffer: Methanol: Water (45:30:25) was chosen as mobile phase for elution and the same solvent was utilized in the preparation of standard

and sample solutions. The elution was checked by infusing the 20µl and the flow rate was changed in accordance with 0.8 ml/min.

Preparation of Standard Stock solutions

Accurately Weighed and transferred 9 mg of Rifampicin and 6 mg of Isoniazid working Standards into a 100ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. and the final concentration of Rifampicin is 90 µg/mL and 60 µg/mL is of Isoniazid. The working standard solutions of these drugs were obtained by appropriate dilution of the respective stock solution with mobile phase.

Preparation of Mobile Phase A (pH 3.5 Phosphate buffer):

Dissolve 68.0 g of potassium dihydrogen phosphate in water and dilute to 1000.0 ml with the same solvent.

Adjust the pH 3.5 with o-phosphoric Acid.

Mobile phase was filtered through 0.45µm membrane filter and degassed by sonication for 20 min.

Preparation of Mobile Phase B: 100 % Methanol

Selection of mobile phase

Standard solutions of Rifampicin (45µg/mL) and Isoniazid (30µg/mL) were injected into the RP-HPLC system and run in different solvent systems. Different mobile phases systems like Phosphate buffer and methanol were initially tried in the isocratic mode in order to determine the best conditions.

HPLC Method Development

Optimisation of RP-HPLC method

The HPLC technique was designed for the simultaneous measurements of Rifampicin and Isoniazid. Different mobile phases were gone after for the process optimisation, nevertheless adequate retention periods, hypothetical plates and high resolution were found with pH 3.5 Phosphate buffer: Methanol: Water (45:30:25) utilizing Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron) using gradient technique.

Table 1: Optimized Chromatographic Conditions

Mobile phase	pH 3.5 Phosphate buffer: Methanol: Water (45:30:25)
Selection of column	Cosmosil C18 (250mm x 4.6mm ID, Particle size: 5 µm)
Injection volume	20 µL
Flow rate	0.8 ml/min
Column temperature	Room Temperature

Detection wavelength	239 nm
Run Time	6.0 minutes
Retention time	Rifampicin (2.8 min) and Isoniazid (3.7 min)

Validation of RP-HPLC method

Validation of the optimized RP-HPLC method was performed in accordance with the ICH Q2 (R1) guidelines.

Linearity

Test solutions of different concentration were injected separately, and the chromatograms were recorded. A series of test preparations of Rifampicin and Isoniazid were prepared by taking 1 ml - 5 ml from the stock solution containing Rifampicin (450µg/mL) and Isoniazid (300µg/mL) respectively in five 10 ml volumetric flask and final volume make up to the mark with mobile phase. A 20 µl volume of each concentration was injected into HPLC, three times under the optimized chromatographic conditions.

Accuracy

Samples are prepared normally covering 50 % to 150 % of the nominal sample preparation concentration. These samples are analyzed and the recoveries of each are calculated.

Precision

Intraday precision study was carried out by preparing test solution of same concentration and analyzing it at three different times in a day. The same procedure was followed for two different days to determine interday precision. The result was reported as % RSD.

Limit of Quantitation (LOQ) & Limit of Detection (LOD)

The LOD and LOQ were analysed from the slope(s) of the calibration curve and the standard deviation (SD) of the peak areas using the formula LOD = 3.3 s/s and LOQ = 10 s/s.

Robustness

Robustness was assessed by modifying the chromatographic conditions such as mobile phase composition, detection wavelength, flow rate, and so on, and the % RSD should be supplied. Small alterations were tolerated under the ideal circumstances, and the method's resilience was established. Individual variations of ± 2 nm in detecting wavelength and ± 0.1 ml/min in flow rate were tried. In triplicate, solutions of 100% test concentration with the required adjustments in the optimal circumstances were injected into the system.

Ruggedness:

Ruggedness is the research of the effect of external circumstances on the approach. To evaluate the robustness of the offered strategy, elements were purposely varied. These influences included system variance, diverse analysis, and atmospheric changes. Two different analysts prepared the test solution according to the test method and injected three doses of test solution into the HPLC system at a flow rate of 0.8 ml/min.

Assay of marketed formulation

20 tablets of marketed formulation (AKT-2) of Lupin Pharmaceutical were taken, weighed individually, and crushed into fine powder. Average weight of tablet sample was weighed and transferred to 1000 mL volumetric flask & diluent was added to make up the volume. Sonicate for 10 min with occasional swirling. The above solution was filtered through 0.45µm membrane filter, The prepared stock solution is of 450 µg/ml of Rifampicin and 300 µg/ml of Isoniazid. For Analysis 3 ml solution was withdrawn and diluted up to 10 ml and injected into system.

System suitability

To verify the system, procedure, and column performance, system suitability features were studied. Six times a standard solution of Rifampicin and Isoniazid was injected into the system, and system suitability properties were analyzed.

RESULT AND DISCUSSION

Linearity

It was clarified from the analytical method linearity as the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a specific range. The peak area obtained from the HPLC chromatograph was plotted against corresponding concentrations to obtain the calibration graph. Isoniazid was found to be linear in the concentration range of 6-30 µg/ml and Rifampicin is in the range of 9-45 µg/ml.

Table 2: Summary of results of Linearity

Sr. No.	Rifampicin		Isoniazid	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	9	689341	6	320145
2	18	1325580	12	633982
3	27	2038458	18	910452
4	36	2798401	24	1189301
5	45	3476598	30	1463089

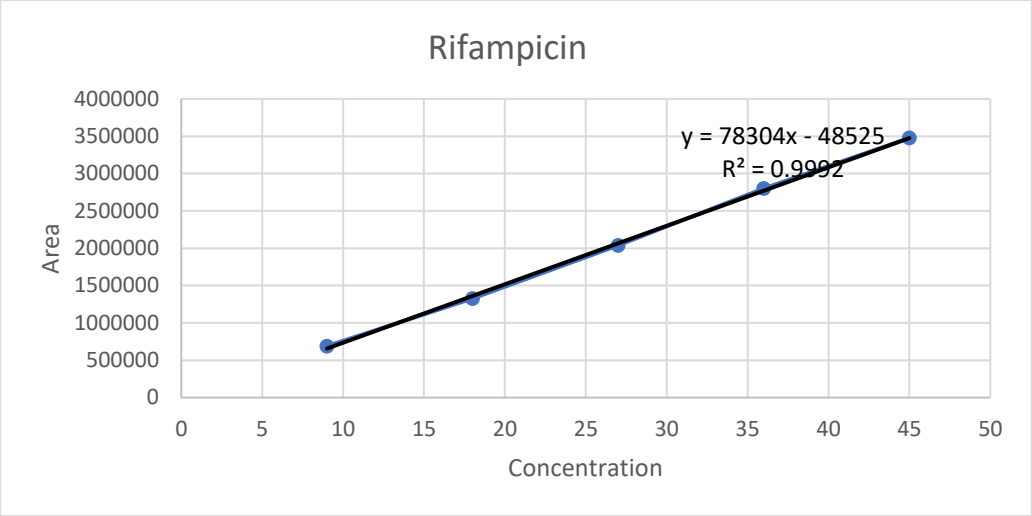

Fig 3: Calibration curve for Rifampicin

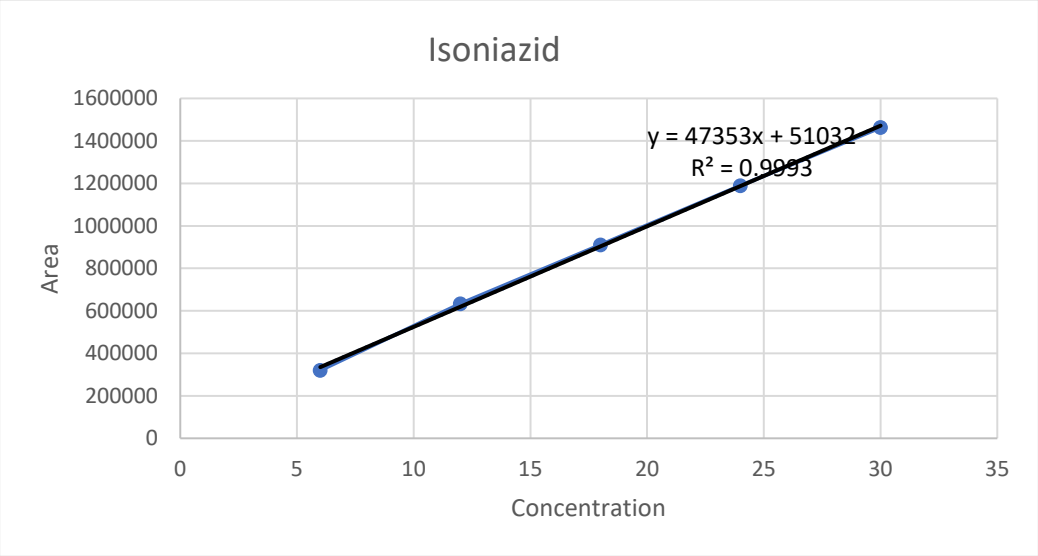

Fig. 4: Calibration curve for Isoniazid

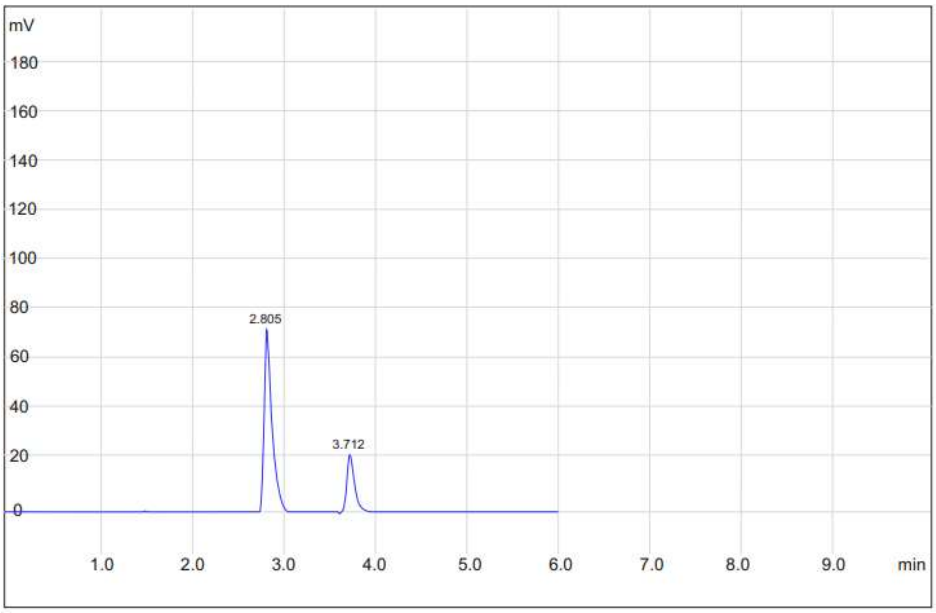

Fig 5: Chromatograph of Rifampicin and Isoniazid

Accuracy

The accuracy of the method determines the closeness of results obtained by that method to the true value. From the results of accuracy testing, it was showed that the method is accurate within the acceptable limits. The % RSD is calculated for the Rifampicin and Isoniazid and all the results are within limits. Acceptable accuracy was within the range and not more than 2.0% RSD.

Table 3: Statistical validation for accuracy of Rifampicin

Level of addition	% Mean recovery*	SD	% RSD
50%	99.18	0.65	0.65
100%	100.30	0.64	0.64
150%	99.59	0.70	0.70

Table 4: Statistical validation for accuracy of Isoniazid

Level of addition	% Mean recovery*	SD	% RSD
50%	100.09	0.49	0.49
100%	100.99	0.40	0.40
150%	99.79	1.15	1.15

Precision

Intraday and interday precision assures the repeatability of test results. The % RSD found was below 2 for both Rifampicin and Isoniazid.

Table 5: Data for intraday precision of Rifampicin

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	9	678554	688919.67	10058.99	1.46
2	9	689564			
3	9	698641			
4	27	2032657	2024537.00	17243.08	0.85
5	27	2025465			
6	27	2015489			
7	45	3457971	3460969.33	6151.41	0.18
8	45	3456892			
9	45	3468045			

Table 6: Data for interday precision of Rifampicin

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	9	698654	689855.33	8421.51	1.22
2	9	681870			
3	9	689042			
4	27	2035454	2029915.33	5657.00	0.28
5	27	2030145			
6	27	2024147			
7	45	3487645	3487133.00	8284.87	0.24
8	45	3478604			
9	45	3495150			

Table 7: Data for intraday precision of Isoniazid

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	0.5	987014	987260.67	8935.55	0.91
2	0.5	996317			
3	0.5	978451			
4	1.5	2681402	2672085.00	18394.86	0.69
5	1.5	2671841			
6	1.5	2663012			
7	2.5	4410215	4419068.33	7843.38	0.18
8	2.5	4425148			
9	2.5	4421842			

Table 8: Data for interday precision of Isoniazid

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	0.5	987452	988138.33	10079.04	1.02
2	0.5	978420			
3	0.5	998543			

4	1.5	2678131	2685191.00	9816.57	0.37
5	1.5	2681041			
6	1.5	2696401			
7	2.5	4412545	4415935.33	3925.48	0.09
8	2.5	4420236			
9	2.5	4415025			

Robustness

Robustness was studied by different deliberate variations in the chromatographic conditions i.e. Change in flow rate and wavelength. From robustness study % RSD was found to be within limit of 2 % for the Rifampicin and Isoniazid. Hence it is robust and complies per ICH guidelines.

Table 9: **Data for Robustness study of Rifampicin and Isoniazid**

Sr.No	Parameter	Condition	Rifampicin				Isoniazid			
			Area	Mean	SD	% RSD	Area	Mean	SD	%RSD
1	Change in Flow rate (ml/min)	0.7	2035641	2033745	6550	0.32	2686626	2682514	9686	0.36
2		0.8	2026456				2671450			
3		0.9	2039140				2689465			
1	Change in Wavelength (nm)	237	2036648	2033368	5966	0.29	916840	915847	4879	0.53
2		239	2026481				920154			
3		241	2036975				910548			

Ruggedness

Ruggedness was studied by different analysts. From robustness study % RSD was found to be within limit of 2 % for the Rifampicin and Isoniazid. Hence it is complying as per ICH guidelines.

Table 10: Data for ruggedness study of Rifampicin and Isoniazid

Sr.No	Analyst	Rifampicin				Isoniazid			
		Area	Mean area*	SD	% RSD	Area	Mean area*	SD	% RSD
1	Analyst-I	2035647	2031736	7057	0.35	913504	918101	4431	0.48
		2023589				922347			
		2035971				918452			
2	Analyst-II	2035641	2034333	5046	0.25	906647	913353	5809	0.64
		2028761				916845			
		2038597				916567			

Specificity

Excipients and impurities were not interacting with the standard drugs. Hence the method is specific.

Table 11: Data for specificity study of Rifampicin and Isoniazid

Drug	Drug conc. (µg/ml)	Excipients (µg/ml)	Total conc. (µg/ml)	Area	Mean	SD	%RSD
Rifampicin	9	18	27	681545	676969.67	6039.50	0.89
	9	18	27	670124			
	9	18	27	679240			
	18	18	36	1312507	1315993.67	8842.53	0.67
	18	18	36	1309426			
	18	18	36	1326048			
	27	18	45	2023564	2031627.33	6983.06	0.34
	27	18	45	2035670			
	27	18	45	2035648			
Isoniazid	6	12	18	326968	325851.00	4453.82	1.37
	6	12	18	320945			
	6	12	18	329640			
	12	12	24	640344	638168.00	2354.78	0.37
	12	12	24	635668			
	12	12	24	638492			
	18	12	30	908965	912150.67	4454.64	0.49
	18	12	30	910246			
	18	12	30	917241			

% Assay of Marketed formulation

The % Assay of (AKT-2) marketed formulation of Lupin Pharmaceutical was calculated.

Table 12. Data of % Assay of marketed formulation

Sr. NO.	Drug	Area of Sample	Area of Standard	% Assay
1	**Rifampicin**	2012546	2038458	**98.73**
2	**Isoniazid**	908248	910452	**99.76**

System Suitability Parameters:

System suitability parameters were measured to verify the system, method and column performance. Standard solution of Rifampicin and Isoniazid was injected into the system for six times and system suitability parameters were checked.

Table 13: System suitability parameter

Sr. No.	Isoniazid			Rifampicin		
	Retention Time (min)	Theoretical plates	Asymmetry Factor	Retention Time (min)	Theoretical plates	Asymmetry Factor
1	2.824	9055	1.07	3.902	10244	1.09

2	2.814	9562	1.08	3.809	11048	1.09
3	2.798	9922	1.08	3.964	10663	1.08
4	2.814	9823	1.07	4.075	11546	1.1
5	2.862	9716	1.08	3.811	10696	1.09
6	2.81	9736	1.07	3.904	10967	1.1
Mean			1.08			1.09
SD			0.01			0.01
%RSD			0.51			0.69

SUMMARY

The Rifampicin was found to be linear in the concentration range of 9-45 µg/ml and Isoniazid is 6-30 µg/ml. From Accuracy study % recovery of Rifampicin was found in the range of 98.72-100.98% and Isoniazid is 98.77-101.45% which is in the limits accordingly the ICH guidelines. Intraday and Interday precision assures that % RSD was within limits of ICH guidelines i.e., NMT 2 for both Rifampicin and Isoniazid. Limit of detection and limit of Quantitation of Rifampicin is 0.24µg/ml – 0.72µg/ml and Isoniazid is 0.06 µg/ml – 0.19 µg/ml respectively. Robustness was studied by deliberate variation i.e., change in Flow rate and change in Wavelength which was within 2 % of RSD as per ICH guidelines. The ruggedness study gives results within the limits of 2% in which variation in Analyst was studied. The % assay of AKT-2 was found to be Rifampicin (98.73%) and Isoniazid (98.76%).

CONCLUSION

The proposed chromatographic method for determining Rifampicin and Isoniazid from pure and dosage forms was found to be simple, precise, accurate, rapid, and specific. The mobile phase employed for method development is relatively easy to make and affordable likewise. The sample recoveries in the formulation were giving excellent results. This approach is inexpensive and run time is comparatively short which permits speedy analysis among all the created methods and consequently, all the investigated validation parameters provided acceptable results with appropriate correlation co-efficient and lower % RSD as per the ICH criteria. The discovered approach may be applied by industry for quantitative simultaneous measurement of Rifampicin and Isoniazid as bulk and in tablet dosage form.

REFERNCES

1. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1 Guideline.pdf
2. Chatwal G. R., Anand S. K., Instrumental Methods of Chemical Analysis, Fifth Edition, 2008, Himalaya Publishing House, 2.108-2.124.
3. Kasture A.V., Mahadik K. R., Wadodkar S. G., More H. N., Pharmaceutical Analysis, Vol.II, Seventh Edition, 2007, Nirali Publication, 28-30
4. Validation of Compendial Procedures, United State Pharmacopeia, USP 36 NF, 27 (2) (2010).
5. Kenkel J., Analytical Chemistry for Technicians, Third Edition, 2009, Published by CRC Publication, 2-4.

6. Gupta V., Jain A.D. K., Gill N.S., Gupta K., Development and validation of HPLC method - a review , Int. Res J Pharm. App Sci., 2(4) (2012) 17-25
7. Azim M.S., Mitra M., Bhasin P.S., HPLC method development and validation: A review, Int. Res. J. Pharm. 4(4) (2013) 39-46.
8. Jeffery G. H., Bassett J., Mendham J., Denny R. C., Vogel's Textbook of Quantitative chemical Analysis, Fifth Edition, 1991, Longman Scientific and Technical Publishers, 3-13.
9. Pavia D. L., Lampman G. M., Kriz G. S., Introduction to Spectroscopy, Third Edition, Thomson Learning publication, 356, 797-817.
10. Dong M.W., Modern Hplc for practicing scientists, John Wiley & Sons, New Jersey, 2006
11. Beckett A. H., Stenlake J. B., Practical Pharmaceutical Chemistry, Fourth Edition Part II, 2004, CBS Publishers and distributors, 284-300,162-163.
12. Furnis B. S., Vogel's Textbook of practical organic chemistry. Longman group UK Ltd 5th Edition Edition, 1989, UK, 384-386.
13. Prathap B., Rao G.H.S., Devdass G., Review on Stability Indicating HPLC Method Development, International Journal of Innovative Pharmaceutical Research.3(3) (2012) 229237.
14. Rashmin M., An introduction to analytical method development for pharmaceutical formulations. pharmainfo.net; 2008; 6 (4); 1-11
15. Wang Q., Ma D., Higgins J.P. Analytical method selection for drug product dissolution testing. Dissolution Technol. 2006; 24:6–13.
16. K. Kalyani, V. Anuradha, A Simple Stability Indicating Method Development and Validation for the Simultaneous Estimation of Naloxone Hydrochloride and Buprenorphine Hydrochloride in Pharmaceutical Dosage Forms by RP-HPLC, Ijppr.Human, 2016; Vol. 6 (3): 206-222.
17. Glass B.D., Kustrin S. A., Optimization of a Stability-Indicating HPLC Method for the Simultaneous Determination of Rifampicin, Isoniazid, and Pyrazinamide in a Fixed-Dose Combination using Artificial Neural Networks, Journal of Chromatographic Science, Vol. 45, January 2007.
18. Tilinca M., Hancu G., Simultaneous Determination Of Isoniazid And Rifampicin By Uv Spectrophotometry, Farmacia, 2017, Vol. 65, 2.
19. Giacobbe L C L, Guzman M. L., Validation of a simple isocratic HPLC-UV method for rifampicin and isoniazid quantification in human plasma, Journal of Applied Pharmaceutical Science Vol. 8(07), pp 093-099, July 2018
20. Mahjoub A. A., Khan A. H., Simultaneous determination of isoniazid and pyrazinamide in plasma by high performance liquid chromatography, Tropical Journal of Pharmaceutical Research November 2016; 15 (11): 2475-2481.
21. Dhal S. K. and Sharma R., Development and Validation of RPHPLC Method for Simultaneous Determination of Pyridoxine Hydrochloride, Isoniazid, Pyrazinamide and Rifampicin in pharmaceutical Formulation, Chem. Anal. (Warsaw), 54, 1487 (2009).
22. Khatak S., khatak M., Development and Validation of a RP-HPLC Method for Simultaneous

Estimation of Antitubercular Drugs in Solid Lipid Nanoparticles, Indian J Pharm Sci 2018;80(6):996-1002.

23. Hakkimane S. S., Guru B. R., Nano Formulation Analysis: Analytical Method Development Of Isoniazid And Simultaneous Estimation Of Antitubercular Drugs Isoniazid And Rifampicin By Reverse Phase High Pressure Liquid Chromatography, Asian J Pharm Clin Res, Vol 10, Issue 5, 2017, 330-335.
24. Zeynali K. A., Saeb E., Simultaneous Spectrophotometric Determination of Rifampicin, Isoniazid and Pyrazinamide in a Single Step, Iranian Journal of Pharmaceutical Research (2016), 15 (4): 713-723.
25. Prasanthia B., Vijaya Ratna B, J., Development and Validation of RPHPLC Method for Simultaneous Estimation of Rifampicin, Isoniazid and Pyrazinamide in Human Plasma, Journal of Analytical Chemistry, 2015, Vol. 70, No. 8, pp. 1015–1022.
26. Arifa SK, Basava R. D, Simultaneous estimation of rifampicin and isoniazid in combined dosage form by a simple UV spectrophotometric method, Der Pharmacia Lettre, 2013, 5 (3):419-426.
27. Available from: https://go.drugbank.com/drugs/DB01045
28. Available from: https://go.drugbank.com/drugs/DB00951
29. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Rifampicin
30. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Isoniazid