IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Analytical method development and validation of Isoniazid and Rifampicin by RP HPLC method

[1] Dr. Rajendra Wagh [2]Dr.Vilas L Badgujar [3]Mr.Pritesh S. Mahajan, ,
[1] Principle, at DCS's ARA College of Pharmacy, Nagaon Tal & Dist Dhule, Maharashtra
[2]HOD of Quality Assurance Department DCS's ARA College of Pharmacy, Nagaon Tal & Dist Dhule, Maharashtra
[3]M. Pharm Student of QA at DCS's ARA College of Pharmacy, Nagaon Tal & Dist Dhule, Maharashtra

ABSTRACT-

Isoniazid & Rifampicin both the drug use in combination for treatment of Tuberculosis (TB), Isoniazid is a first-line treatment for tuberculosis. It works by blocking the production of mycolic acid, which is a cell wall component in the tuberculosis bacterium while Rifampin acts via the inhibition of DNA-dependent RNA polymerase, leading to a suppression of RNA synthesis and cell death.

Here we use RP-HPLC as an Analytical method for the drug i.e. Isoniazid and Rifampicin, by using Analytical Technique we develop and Validate the Isoniazid and Rifampicin drug, here we take UV Spectroscopy, Chromatogram of isoniazid and Rifampicin, We also carried out the Intraday and Inter day Precision studies on RP- HPLC for Isoniazid and Rifampicin.

INTRODUCTION-

Isoniazid and Rifampicin are both used for the treatment of Tuberculosis, according to the World Health Organization, tuberculosis has been widely spread in the world for thousands of years and is a major problem in healthcare systems. In 2013, 9.0 million new cases (13% co-infected with HIV) and 1.5 million deaths were estimated. Tuberculosis is transmitted by a single agent, Mycobacterium tuberculosis (MTB), isolated by Robert Koch in 1882. Sixty-three years after the isolation of MTB, in 1945, the development of streptomycin made tuberculosis treatment possible. Before that, the only option was surgery. Until 1970, a combination of streptomycin, isoniazid (INH), and p-amino salicylic acid was used in tuberculosis treatment. Later, the inclusion of rifampicin (RIF) and pyrazinamide (PYZ) in the treatment substantially reduced the recurrence rate and treatment time. Combined treatment using various drugs is necessary for patient cure, without recrudescence, and for the prevention of drug-resistant mutants that may occur during treatment [(1-4)]

Here we use HPLC as Analytical Technique for Isoniazid and Rifampicin, the HPLC stand for the High-Performance Liquid Chromatography, which is a type of chromatography, Chromatography is analytical Technique which is firstly use for separating colors, later the technique has many advances, the latest advances is HPLC which has great ability to separate the substance Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase. The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights. [5-6]

Analytical chemistry may be defined as the science and art of determining the composition of materials in terms of the elements of composition contained. Pharmaceutical analysis is a bench of science that deals with the analytical procedures used to determine the purity, safety and quality of drugs and chemicals. It contains procedures to determine the identity , strength , quality and purity of new compounds . It also involves procedures for separating, identifying, and determining the relative amount of the components in sample of matter

Quality assurance plays a key role in finding the safety and efficiency of medicines. It has highly specific and sensitive analytical methods for the design , development, standardization and quality control of medicinal products . They are equally important for the pharmacokinetics and drug metabolism studies, both which are important for the assessment of bioavailability and clinical response Modern physical method of analysis are extremely sensitive even for small amount of samples of materials .It can be rapidly applied and can readily amenable to automation. So it is widely used in the product development and in the control of manufacture , formulation and also in monitoring the use of drugs and medicines.

The term pharmaceutical analysis includes both quantitave and qualitative analysis of Drugs and pharmaceutical substances starting from bulk drugs to finished dosage forms .So it is used as a diagnostic aids in the modern practice of medicine by the analysis of chemical constituents in the human body which may alter during the disease state.

Chromatography is a technique by which a mixture is separated into its components as a result of the relative ability of each component to be eluted along or through the stationary phase by mobile phase .The sample is placed on edge of the stationary phase (a solid or liquid) and a mobile phase is allowed to flow over the stationary phase to sweep the sample along the length of the stationary phase .Component which are strongly adsorbed to the stationary phase are swept less rapidly along the length of the stationary phase than those components that are less strongly adsorbed to stationary phase The word chromatography is derived from the Greek letters chromos meaning colour and the graphy means colour writing. The initial use of the terms is attributed to Tswett, who separated colour bands of plant pigments on a chromatography column that consist of an adsorbent powder that was washed with a liquid solvent termed as mobile phase . This is carried down the length of the tube that contains an immobile solid or liquid phase i.e. stationary phase

Experimental Data- System Suitability Studies-

The system suitability was evaluated by analyses of Isoniazid and Rifampicin mixture at concentration of 10-50 µg/ml for Isoniazid and 15-75 µg/ml for Rifampicin and measured at 260 nm and 313 nm . The column efficiency, resolution, and peak asymmetry were calculated for the standard solutions

Linearity and Range

The linearity response was determined by analyzing 6 independent levels of concentrations in the range of 10-50µg/ml for Isoniazid and 15- 75µg/ml Rifampicin.

Intra-day precision:

Sample solutions containing 10 mg of Isoniazid and 15 mg of Rifampicin three different concentration (20µg/ml, 30µg/ml, 40µg/ml Isoniazid and (30µg/ml, 45µg/ml, 60µg/ml) Rifampicin. Isoniazid and Rifampicin were analyzed two times on the same day and %R.S.D was calculated.

Inter-day precision:

Sample solutions containing 10 mg of Isoniazid and 15 mg of Rifampicin three different concentration (20µg/ml, 30µg/ml, 40µg/ml Isoniazid and (30µg/ml, 45µg/ml, 60µg/ml) Rifampicin. Isoniazid and Rifampicin were analyzed three times on the different day and
%R.S.D was calculated.

Accuracy

The accuracy was determined by Isoniazid and Rifampicin (equivalent to 10 mg of Isoniazid and 15 mg of Rifampicin (80 %, 100 % and 120 % of the label claimed, respectively) to quantity equivalent to average weight of marketed tablets. This powder mixture containing 10 mg of Isoniazid and 15 mg of Rifampicin were triturated and then subjected to chromatographic analysis using the described method. The resulting mixtures were analyzed in triplicates over three days. The % recovery of added drug was taken as a measure of accuracy.

Robustness:

The mobile phase composition was changed in (±1 ml/ min^{-1}**)** proportion of Methanol: 0.1%OPA (pH adjusted 4.2 with TEA)in the mobile phase composition and the flow rate was (±1ml/ min^{-1}**)** and the change in detection wavelength (±1 ml/ min^{-1}**)** and the effect of the results were examined it was performed using 30 µg/ml and 45µg/ml solution of Isoniazid and Rifampicin in duplicate.

Ruggedness

Ruggedness of an analytical technique is the capacity of a method to stay unaffected by extremely little but intentional changes in performance parameters, which in turn gives confidence that the produced approach is trustworthy to employ

RESULT AND DISCUSSION

UV Spectroscopy

UV absorption of 10 µg/mL solution of Isoniazid and Rifampicin in Methanol was generated, and absorbance was taken in the range of 200-400 nm. λmax of Isoniazid and Rifampicin in Methanol was found to be 260 and 313 nm respectively

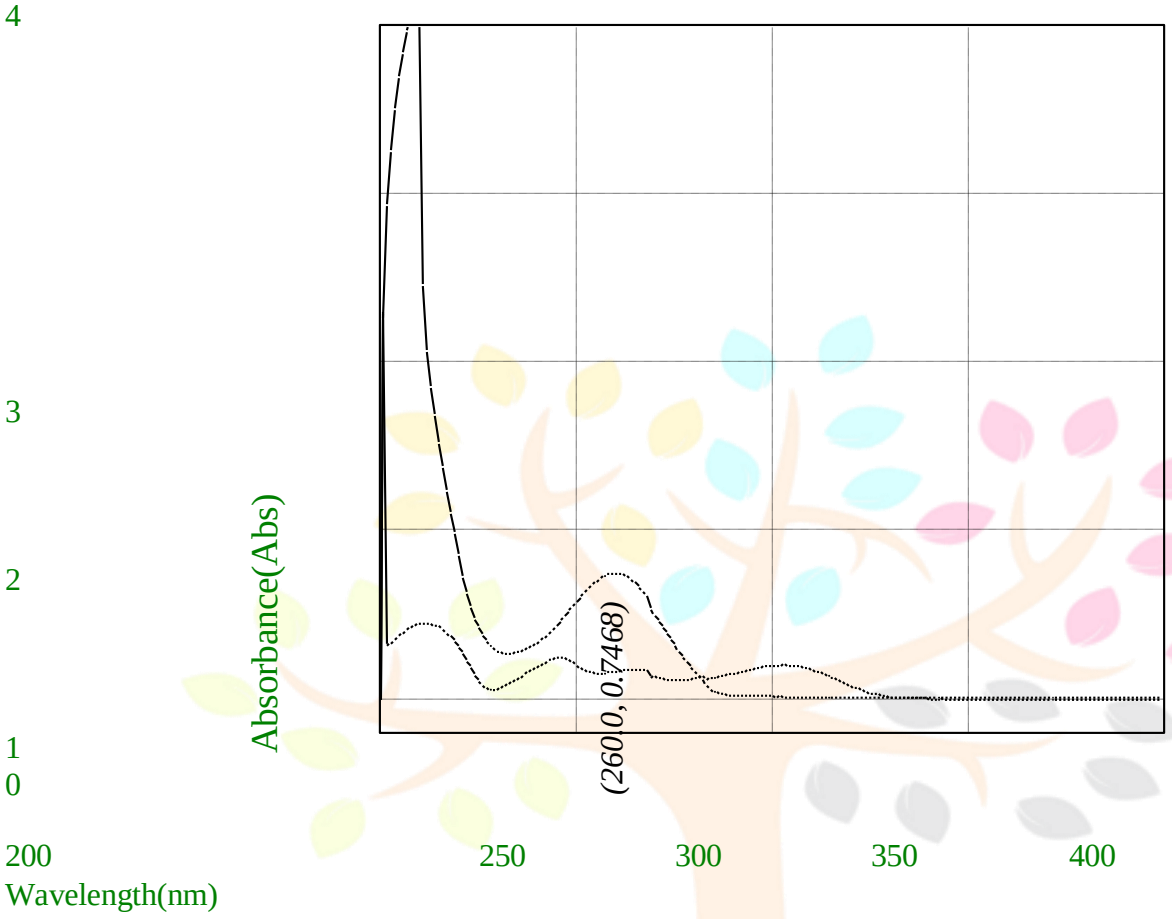

Fig.No:01 Iso-absorptive point of Isoniazid and Rifampicin

Chromatographic behavior of Isoniazid and Rifampicin mobile phase of various compositions

Thus, from the above, it has been observed that, using mobile phase of Methanol + Water (0.1%OPA) PH 4.2 with TEA (72+28% v/v) 283 nm, 1 ml/ min, gave adequate retention time at 5.667 min and 8.002 min. with good peak shape (Theoretical plates of 5517 of Isoniazid & 6537 of Rifampicin).

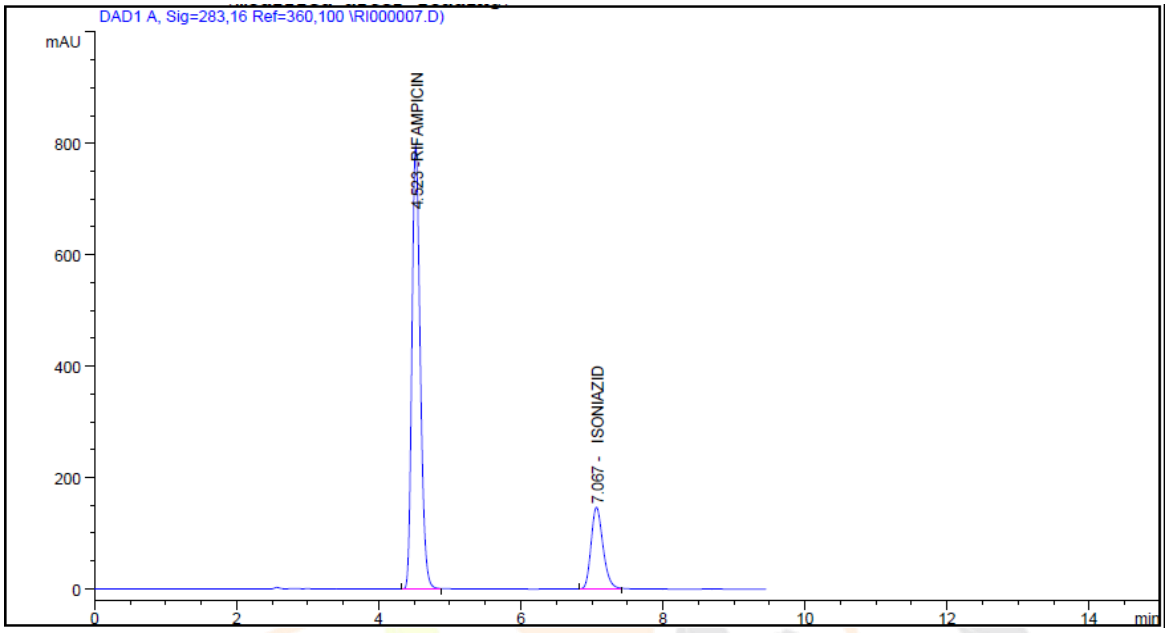

Fig.No: 02 Chromatogram of standard Combination of Isoniazid and Rifampicin

Analytical of Method Validation

Linearity:

From Isoniazid standard stock solution, different working standard solution (10- 50µg/ml) were prepared in mobile phase Likewise from Rifampicin standard stock solution different working standard solution (15-75µg/ml) were prepared in mobile phase 20 µl of sample solution was injected into the chromatographic system

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

The method was established by analyzing various replicates standards of Isoniazid and Rifampicin. All the solution was analyzed thrice in order to record any intra-day & inter- day variation in the result that concluded. The result obtained for intraday is shown in **(Table No. 01)** respectively.

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
30+20-01	4.546	3389.21997	7735	0.87	0.0000
	7.131	2148.8991	9621	0.83	10.37
45+30-01	4.548	4943.58984	7532	0.87	0.0000
	7.141	1287.41418	9166	0.82	10.19
60+40-01	4.546	6508.3447	7733	0.87	0.0000
	7.130	1704.0970	9375	0.83	10.29
Interday precision					
30+20-01	4.525	3410.4394	7707	0.75	0.0000
	7.021	888.35492	9638	0.75	10.12
45+30-01	4.522	4945.6665	7866	0.76	0.0000
	7.037	1297.16833	9492	0.75	10.19
60+40-01	4.510	6483.8657	7825	0.76	0.0000
	7.017	1725.0614	9564	0.75	10.20

Precision:-

Intraday and Inter day Precision studies on RP-HPLC for Isoniazid and Rifampicin which shows the high precision %amount in between 98% to 102% indicates to analytical method that concluded.

Robustness

Robustness Study of Isoniazid:

The changes were did flow rate (±1 ml/ min^{-1}), PH of mobile phase composition (±1 ml/ min^{-1}),and Wavelength (±1 ml/ min^{-1}) .%RSD for peak area was calculated which should be less than

Robustness Study of Rifampicin:

The changes were did flow rate (±1 ml/ min^{-1}), PH of mobile phase composition (±1 ml/ min^{-1}), and Wavelength (±1 ml/ min^{-1}). %RSD for peak area was calculated which should be less than 2%.

Limit of detection = 3.3 X 7.08/82.41= 0.2833(µg/mL)

Limit of Quantitation = 10 X 7.08/82.41= 0.8585 (µg/mL)

The LOD and LOQ of Isoniazid was found to be 0.2833 (µg/mL) and 0.8585(µg/mL), analytical method that concluded

Limit of Detection = 3.3X22.25/51.27=1.4321 (µg/mL)

Limit of Quantitation = 10 X22.25/ 51.27=4.3398 (µg/mL)

The LOD and LOQ of Rifampicin was found to be 1.4321 (µg/mL) and 4.3398 (µg/mL), analytical method that concluded.

Reference-

1. WHO: Report 2014, Global Tuberculosis Control (2014) World Health Organization, Geneva, Switzerland
2. The Official Web Site of the Nobel Prize (2012) Nobelprize.org (accessed July 18, 2014)

3. Panchagnula, R., & Agrawal, S. (2004) Int. J. Pharm. 271, 1–4. http://dx.doi.org/10.1016/j.ijpharm.2003.11.031

4. Guidelines for Clinical and Operational Management of Drug-Resistant Tuberculosis (2013) International Union Against Tuberculosis and Lung Disease, Paris, France

5. . Hostettmann, K; Marston, A; Hostettmann, M (1998). Preparative Chromatography Techniques Applications in Natural Product Isolation (Second ed.). Berlin, Heidelberg: Springer Berlin Heidelberg.https://microbenotes.com/chromatography-principle-types-and-applications

6. Nandeesha Itigimatha, Kailash S Chadchan, D H Majunatha, "Simple and Sensitive RP HPLC and UV Spectroscopic Methods for the Determination of Rifampicin in Pure and Pharmaceutical Formulations." Turk. J. Pharm. Sci., 2021; 10(4): 5381-5386.

7. Shah Dimal A, Gondalia Ishita I, Patel Vandana B, Mahajan Ashok, Chhalotiya Usmangani K, "Stability indicating liquid chromatographic method for the estimation of isoniazid ." J. chem. Metro, 2020; 14(2): 125-132.

8. Nita Yadav, Anju Goyal, "Method development and validation of isoniazid in pharmaceutical dosage form by UV spectrophotometric methods." Int. J. Pharm. Chem. and Analysis, 2017; 4(3): 54-58.

9. Kshirsagar, S.A., Mane, S.B., Hanchate, Y.S., Katte, A.S., and Kulkarni, K.V., "UV Spectrophotometric method development and validation for determination of isoniazid in API and in pharmaceutical dosage form." Int. J. Pharma. Res. Sch., 2018; 7(1): 19-27.

10. Dr. Pradnya Lokhande, "Analytical Method Development and Validation of isoniazid and Rifampicin by using RP-HPLC with ICH Guidelines" Int. J. Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, 2019; 3(3): 259-263

11. Bansode Ashwini S, Devhadrao Nitin V, Shinde Ashwini C, Shinde Vishnu C, & Gaikwad D.D., "Analytical method development and validation of Rifampicine in pure form by HPLC." World J. Pharm. Sci., 2017; 5(10): 37–48

12. Gopal S. Irache et al. "RP-HPLC Method Development and Validation of isoniazid and Rifampicin in Pharmaceutical Dosage Forms" Int. Research J.Pharm., 2017; 8(8): 52- 55

13.Alfonso genera in Remington's Pharmaceutical series, 18th -Edn., Mack publishing company, 1990, page :648.

14.Amini et al., Determination of Ofloxacin in Plasma by HPLC with UV detection, Journal of Applied Sciences, 5(9), 2005, Pp. 1655, 1657.

15.Beckett, A.H., and Stenlake, J.B., Practical Pharmaceutical Chemistry, CBS Publishers and Distributors, Volume - II, 1997,page : 157

16.Berezkin, V.G and Horwood, E., (1990) Chromatographic Adsorption Analysis.

17.Bhusari KP et al., Spectrophotometric methods were developed for the estimation of ofloxacin and ornidazole simultaneously in tablet dosage form, Asian Journal of Research Chemistry, 2009, Pp. 60-62.

18.Dan Diaconu et al., Studies regarding the stability of pharmaceutical formulation released through association of beta lactamic antibiotic. Stability of an oral suspension powder containing cefadroxil and cefixime, Roumanian Society of Biological Sciences, Vol. 2, No. 3, 2006 Pp. 2773-2780.

19.David G. Watson, Pharmaceutical Analysis, A textbook for Pharmacy students and Pharmaceutical chemists, 2nd edition, Page: 286.

20.Dharuman J et al., RPHPLC method development and validation for the simultaneous estimation of ofloxacin and tinidazole in tablets, International Journal of Pharmatech Research, Vol. 1, April – June 2009, Pp. 121-124.

21.Douglas A. Skoog., Donald, M. West, and James Holler, F., Fundamentals of Analytical Chemistry. 7lh Edn., 1-3, 628-641.

22.Dragica Zendelovska et al., Developed in high-performance liquid chromatographic method for the determination of Ofloxacin and Lomefloxacin in human plasma, Journal of the Serbian Chemical Society, Vol 70 2005, Pp. 1451 – 1460

23.Elsevier ICH Topic Q2A, "Validation of Analytical Procedures": Methodology, 6th Nov 1996, www.ich.org