International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 8 Issue 5, Sep-Oct 2024 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Simultaneous Estimation of Rifampicin and Isoniazide by Using UV Spectroscopy

Dr. S. N. Kapse, Rameshwar Sanjay Bahirat, Harshal Laxman Bhagat

Matoshri Collage of Pharmacy, Nashik, Maharashtra, India

ABSTRACT

A simple, accurate, and cost-effective UV spectroscopic method has been developed for the simultaneous estimation of rifampin in pharmaceutical formulations. Rifampin, a key anti-tuberculosis drug, exhibits characteristic absorption in the UV region, making it suitable for analysis via UV spectroscopy. The method involves determining the absorbance maxima of rifampin in a suitable solvent at specific wavelengths. The linearity of the drug was established in a given concentration range, and the method was validated according to ICH guidelines for parameters such as accuracy, precision, and reproducibility. This UV spectroscopic technique offers an efficient alternative to more complex analytical methods, providing reliable results for quality control and routine analysis in pharmaceutical environments.

KEYWORDS: *rifampin, isoniazid, uv- spectroscopy, simultaneous estimation method, method development*

How to cite this paper: Dr. S. N. Kapse | Rameshwar Sanjay Bahirat | Harshal Laxman Bhagat "Simultaneous Estimation of Rifampicin and Isoniazide by Using UV Spectroscopy" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-8 | Issue-5, October 2024, pp.861-873, URL: www.ijtsrd.com/papers/ijtsrd69462.pdf

IJTSRD69462

1. INTRODUCTION

Tuberculosis (TB) is a disease caused by Mycobacterium tuberculosis; the bacteria usually attack the lungs, but can also damage other parts of the body. Most people who are exposed to TB never develop symptoms because the bacteria can live in an inactive form in the body; but if the immune system weakens, TB bacteria can become active [1, 2].

INH (isonicotinic acid hydrazide) is an anti-mycobacterial agent which is bactericidal for both extracellular and intracellular organisms. It is the primary drug for the treatment of TB when the disease is caused by isoniazid-sensitive strains of the Mycobacterium tuberculosis. INH is a colourless, odourless, white crystalline powder slowly affected by exposure to air and to light; soluble in water, methanol and alcohol, slightly soluble in chloroform, very slightly soluble in ether [3, 4].

RIF (3-[[(4-methyl-1-piperazinyl)-imino]-methyl)]-rifamycin) is a semisynthetic derivative of rifamycin antibiotics which are produced by the fermentation of the strain Streptomyces mediterranei. The primary indications for RIF are for treatment of TB (pulmonary and extra-pulmonary lesions) and for leprosy. It is also useful for elimination of Neisseria meningococci in carriers and for Gram-positive (Staphylococcus aureus and S. epidermidis, Streptococcus pyogenes, S. viridans and S. pneumoniae) and Gram negative bacteria (Haemophilus influenzae type B). RIF is a red odourless powder; very slightly soluble in water, acetone, alcohol, ether, soluble in methanol and ethyl acetate, freely soluble in chloroform [3, 4].

Isoniazid (INH) and Rifampicin (RIF) are probably the most efficient antitubercular agents available in modern therapy; they are usually administered in combination, because of the high drug resistance shown by Mycobacterium tuberculosis, being a component of all combined antituberculosis chemo-therapy regimens recommended by WHO [5].

The combination of these drugs has high therapeutic advantages as it increases the treatment adherence and reduces the risk of resistance or relapses,

treatment costs and errors in drug administration; however, the combination of drugs brings new challenges to the pharmaceutical industry with respect to the development of new analytical methods for their simultaneous determination. Literature survey revealed high performance liquid chromatography (HPLC) [6, 7, 8], capillary electro-phoresis (CE) [9, 10], voltammetric [11], spectro-photometric analysis combined with multivariate regression [12, 13], derivative spectrophotometric [14] and visible spectrophotometric [14, 15] methods for the simultaneous determination of INH and RIF. The spectrophotometric methods for multicomponent sample analysis are based on the properties that the absorbance of a solution is the sum of absorbances of individual components and the measured absorbance is the difference between total absorbance of the solution and that of the blank solution. Various spectrophotometric methods can be used for estimation of drugs in combined dosage form including here simultaneous equation method and derivative spectrophotometry [16, 17].The aim of present work was to develop new simple, sensitive and rapid spectrophotometric methods and their validation, for the simultaneous determination of INH and RIF in combinations.

2. DRUG PROFILE:

2.1. RIFAMPICIN:

Summary: Rifampicin is indeed an antibiotic used to treat various mycobacterial infections, including

Mycobacterium avium complex and leprosy. It's also commonly used in combination with other anti bacterials to treat both latent and active tuberculosis.

Brand Names:- Isonarif, Rifadin, Rifamate, Rifater, Rofact.

Generic Name:- Rifampicin

Background: Rifampicin, also known as rifampin, is a semisynthetic antibiotic derived from Streptomyces mediterranei. It possesses a broad antibacterial spectrum, effective against various forms of Mycobacterium. In susceptible organisms, rifampicin works by inhibiting the activity of DNA-dependent RNA polymerase, forming a stable complex with the enzyme. This action suppresses the initiation of RNA synthesis. Rifampicin is bactericidal, meaning it kills bacteria, and it targets both intracellular and extracellular organisms.

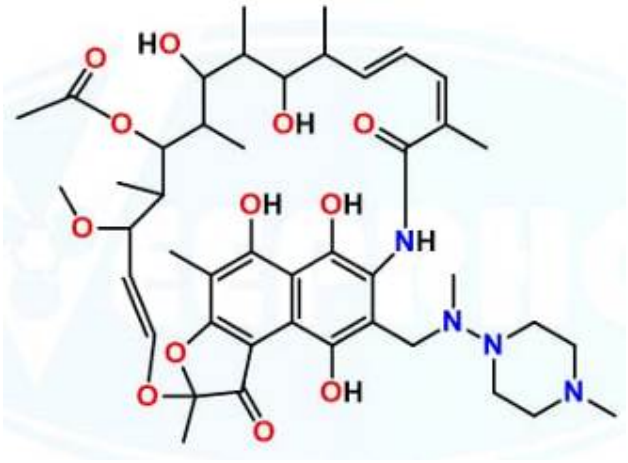


Fig no.1: RIFAMPICIN

Structure:-
Weight Average:- 822.9402
Monoisotopic:- 822.40512334
Chemical Formula:-C43H58N4O12
Synonyms:-Rifampicin, Rifampicina, Rifampicine, Rifampicinum, Rifampin.

2.2. ISONIAZIDE:

Summary: Isoniazid is an antibiotic primarily employed in treating mycobacterial infections, frequently in combination with other ant mycobacterial agents. It is commonly used for both active and latent tuberculosis treatment.

Brand Names:-Isonarif, Isotamine, Isotamine B, Rifamate, Rifater

Generic Name:-Isoniazid

Background:-Antibacterial agent used primarily as a tuberculostatic. It remains the treatment of choice for tuberculosis.

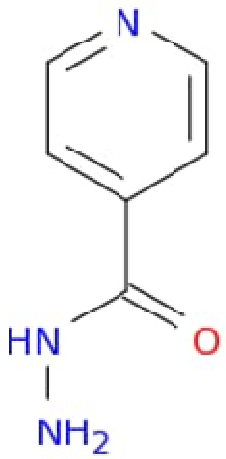


Fig no.2 ISONIAZID

Structure:-
Weight Average:- 137.1393
Monoisotopic:- 137.058911861
Chemical Formula:-C6H7N3O
Synonyms:-4-pyridinecarbohydrazide, Isoniazid, Isoniazida, Isonicotinic acid hydrazide, Isonicotinic

hydrazide, Isonicotinohydrazide, Isonicotinoylhydrazide, Isonicotinsa urehydrazid, Isonicotinylhydrazine.

3. UV SPECTROSCOPY:

UV spectroscopy:

The study of the interactions between light and electromagnetic radiation and matter is known as spectroscopy. The energy absorbed or emitted in discrete amounts known as quanta indicates this interaction. From radio waves to gamma rays, these absorption and emission processes reveal important details regarding the characteristics of the materials under study throughout the electromagnetic spectrum. UV spectroscopy is an analytical technique that utilizes light with wavelengths ranging from 200 to 800 nm, which falls within the ultraviolet (UV) or visible spectrum. Due to the technique's versatility, colorless chemicals in the UV range (200-400 nm) and colored substances in the visible range (400-800 nm) can both be analyzed. In essence, UV spectroscopy counts the precise UV or visible light wavelengths that a sample absorbs or transmits in comparison to a reference or blank. This measurement can provide information on the components and their concentrations within the sample because it is directly related to the composition of the sample.

Uv principle: The transition of electrons within a molecule or an ion from a lower to a higher energy level produces the UV absorption spectrum, while the opposite type of transition produces the UV emission spectrum. A molecule's or an ion's valence electrons can be stimulated or promoted by UV radiation to move from their ground state orbital to an excited stateorbital, or anti bonding, at a higher energy level. orbital, which exhibits absorption upon detection (45).

Chromophores: The presence of specific chromophores allows many organic compounds to absorb ultraviolet and visible light. operational unit. Chromophores are the real groupings that absorb the radiation. Statistically speaking, some electronic transitions are.

Auxochromes: The color of a molecule can be enhanced by certain groups known as auxochromes. These groups typically do not absorb light strongly within the 200-800 nm range but can influence the absorption spectrum of the chromophore they are attached to.

Solvents: The absorption spectrum of a compound can change depending on its chemical structure when it is dissolved in a solvent. Typically, non-polar solvents and molecules have minimal impact on the absorption spectrum. In contrast, polar molecules can experience significant changes when interacting with a polar solvent. This interaction often results in broadening of the absorption bands and a decrease in both structural resolution and the maximum molar absorptivity (ε max).

Potentially: there are three ground state orbitals involved. They are: σ (bonding) molecular orbital, π(bonding) molecular orbital and n (non bonding) atomic orbital

Instrumentation: There are two type of absorbance instruments to collect uv absorption spectra

- Single beam UV spectrophotometer
- Double beam UV spectrophotometer

A monochromator or filter is positioned between the light source and the sample in a single beam UV spectrophotometer. The device can measure one wavelength at a time with this configuration. UV Spectrophotometer with Dual Beam A double beam UV spectrophotometer, on the other hand, features a beam splitter and a number of mirrors in addition to a monochromator and light source that are similar. These elements aim the laser beam at the sample under analysis as well as at a reference sample. The measurements are more accurate in this configuration. In general, the double beam instrument is quicker and model (46)

CONTENTS:-

3.1. LIGHTSOURCE
3.2. SAMPLECELL
3.3.WAVELENGTHSELECTOR(MONOCHROMATOR)
3.4. DETECTORS
3.5. RECORDINGSYSTEM(DISPLAY)

3.1. LIGHTSOURCE:

Deuterium lamps are utilized for measurements in the ultraviolet range, while tungsten lamps are employed for measurements in the visible and near-infrared ranges in a spectrophotometer setup.

3.2. SAMPLE CELL:

Glass cells are typically used for measurements in the visible range, particularly for wavelengths greater than 340 nm, as light below this wavelength doesn't pass through them effectively. Quartz cells, on the other hand, allow light to pass through across the entire ultraviolet and visible ranges, but they are mainly utilized for ultraviolet measurements due to their higher cost.

In sample compartment spectroscopy, it's crucial for all materials in the beam path, except the analyte, to be as transparent to the radiation as possible. The geometry of all system components should aim to

maximize the signal and minimize scattered light. The choice of material for a sample cuvette dictates the optical window that can be utilized for measurement .

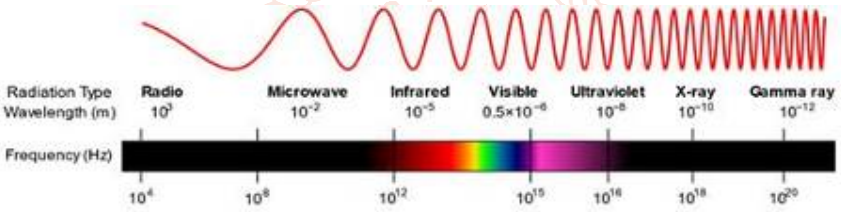
Fig no. 03: CAVETTE

3.3. WAVELENGTH SELECTOR (MONOCHROMATOR)

A wavelength selector is an instrument component that either selects and transmits a narrow band of wavelengths emitted from a broad-band optical source or transmits one or more lines from a discrete wavelength source.

A spectroscope plays a role in selecting monochromatic light from a light source, such as white light. Spectroscopes include filter type, prism type, and grating (diffraction grating) type. For grating-based monochromators used at small angles, the linear dispersion of wavelengths is constant, meaning the distance along the exit slit between where 300 nm light and 400 nm light strikes is the same as the distance between 600 nm to 700 nm.

The linear dispersion from a prism-based monochromator is not constant. The resolving power or resolution for a monochromator is the ability to separate images.



Spectral Region	Technique	Transition	Units
Gamma Ray	Mixed Gamma	Nuclear	MeV/keV
X-ray	Absorption/Fluorescence	Core Electrons	keV/eV
UV-VIS	Absorption/Fluorescence	Valence Electrons	nm/
Infrared	Absorption	Vibrations	cm^{-1}
Microwave	Absorption	Rotations	MHz/GHz/THz/cm^{-1}

3.4. DETECTORS

A detector plays a crucial role in converting the light transmitted from a sample into an electric signal. These detectors are also referred to as photometric detectors. They are essential components in spectrophotometry, where they measure the intensity of light passing through a sample at different wavelengths, allowing for the analysis of substances based on their absorption or emission properties.

3.5. RECORDING SYSTEM:

Fig no. 4: UV SPECTROSCOPY MACHINE

3.6. PRINCIPAL:

Simultaneous equation are the set of algebraic equation that shares variables and are solved simultaneously Simultaneous equation method is used where a sample contain two absorbing drugs (X and Y) each of this absorbs at the λmax of eachother i.e.λ1 and λ2, it may be possible to determine both the drugs by the technique of simultaneous equation method provided that certain criteria apply. The information required is absorptivity of X at λ1 and λ2 and ax1 and ax2 respectively, absorptivity of Y at λ 1an day 1 an day 2 respectively, Absorbance of the diluted sample at λ1 and λ2, A1 and A2 respectively. Let Cx and Cy be the concentration of X and Y respectively in the diluted sample. Two equations are constructed based upon the fact that at λ1 and λ2 the absorbance of the mixture is the sum of the individual absorbance of X and Y.

Atλ1A1 = ax1 bcx + ay1 bcy ……(18)

Atλ2A2 =ax2bcx+ay2 bcy (19)

Rearrange eq. (19)

cy=A2-ax2cx/ay2

Substituting for cy in eq.(19),and rearranging gives cx=A2ay1–A1ay2/ax2ay1–ax1ay2 and

cy=A1ax2–A2ax1/ax2ay1 –ax1ay

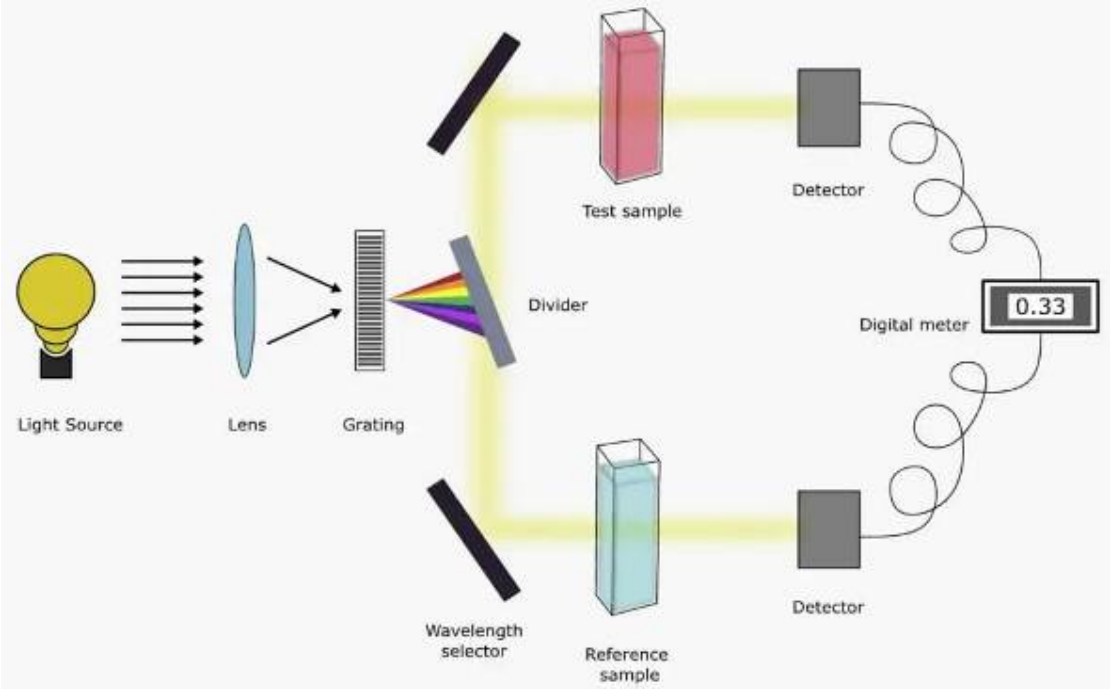


Fig no. 5

4. MATERIALS AND METHODS

4.1. Instruments

Lab India double beam UV visible spectrophotometer (UV 3092) with 1 cm matched quartz cells were used for all absorbance measurements with UV WIN Software. Shimadzu AX 200 balance was used for weighing the samples.

4.2. Materials

- Rifampicin
- Isoniazid
- methanol

4.3. Selection of common solvent

Ethanol of analytical reagent grade was selected as common solvent for developing spectral characteristics of drug. The selection was made after assessing the solubility of both the drugs in different solvents.

4.4. Preparation of Standard Stock Solution

The standard stock solution containing Rifampicin and Isoniazid were prepared by dissolving 100 mg of Rifampicin and 100mg of Isoniazid separately in 20 ml of ethanol. It was then sonicated for 10 minutes and the

final volume of both the solutions were made up to 100 ml with ethanol to get stock solutions containing 1000 µg/ ml each of Rifampicin and Isoniazid in two different 100 ml volumetric flask

4.5. Procedure for Determining the Sampling Wavelength for simultaneous analysis

By appropriate dilution of two standard drug solutions with and 10 µg/ml of rifampicin and 10ug/ml of isoniazid were scanned separately in the ranges of 400- 200 nm to determine the wavelength of maximum absorption for both the drug. rifampicin and isoniazid showed absorbance maxima at 337 nm and 263 nm respectively as shown in fig.

Fig.3.Absorption spectrum of Rifampicin in ethanol (10 µg/ml) showing λ max 337 nm

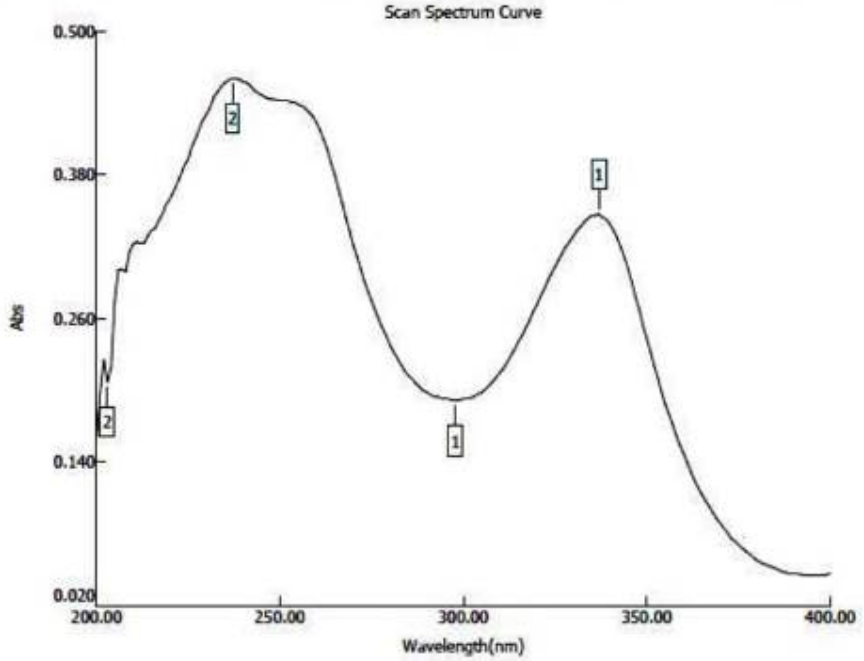
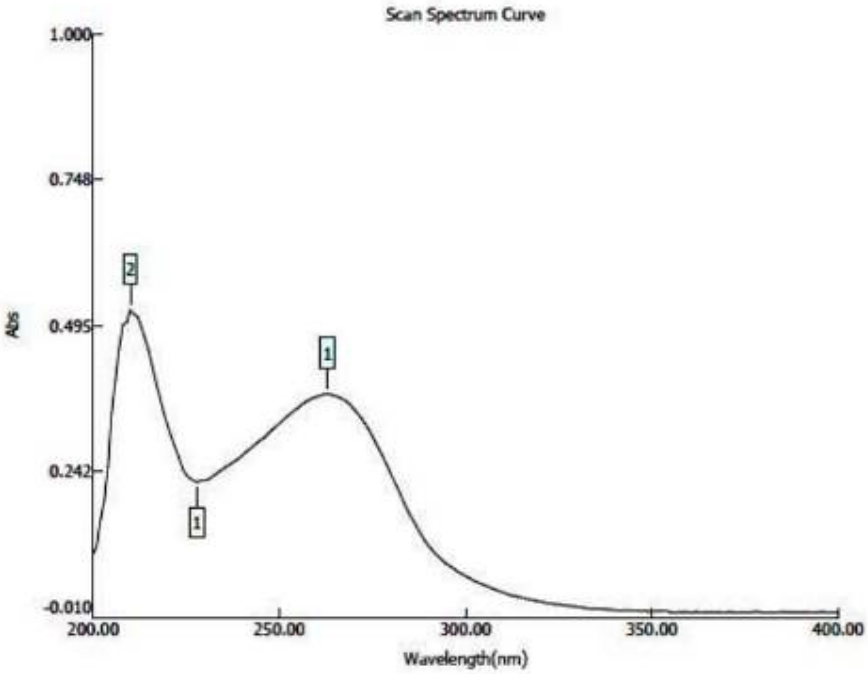
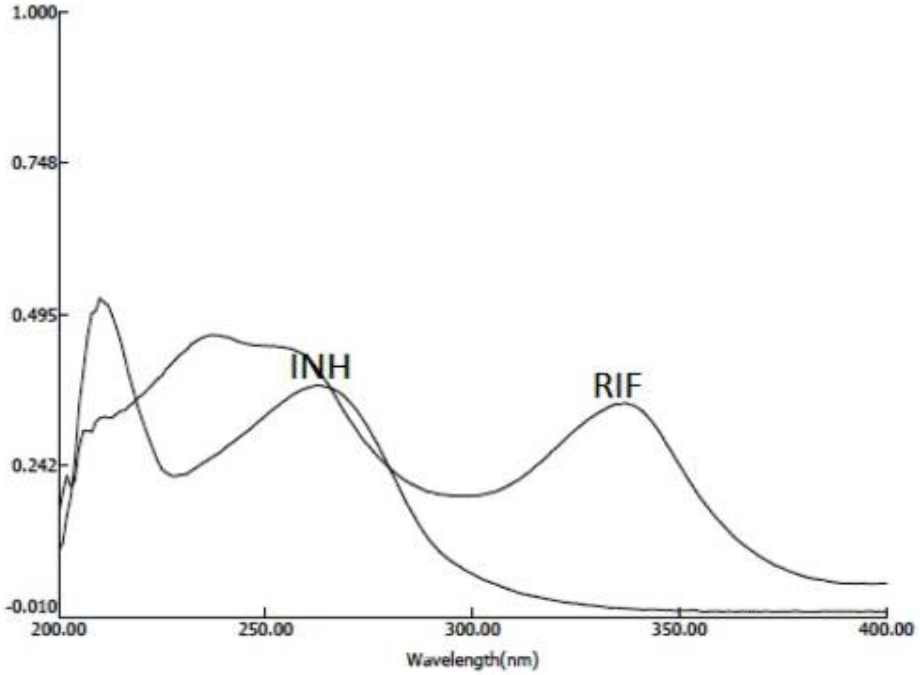


Fig.4. Absorption spectrum of Isoniazid in ethanol (10 µg/ml) showing λ max 263 nm





4.6. Selection of Method and Wavelength

For estimation of Rifampicin, simultaneous equation method employing 337 nm as analytical wavelength was used. For estimation of Isoniazid, 263 nm was selected as the analytical wavelength. In the simultaneous equation method developed for simultaneous estimation of Rifampicin and Isoniazid, the wavelengths were selected from the overlain spectra as shown in Fig. 5

4.7. Procedure for plotting calibration curve

Rifampicin and isoniazid showed linearity with absorbance with a range of 5 to 35 ug/ml and 5 to 25 ug/ml at their respective wavelength maxima, which were validated by least square regression method. coefficients of correlation were found to be 0.9991 for rifampicin and 0.9998 for isoniazid. for simultaneous estimation of rifampicin and isoniazid, a series of linearity solution were prepared by diluiting appropriate volume of standard stock solution of rifampicin and isoniazid were carried out in the ranges of 400 – 200nm against ethanol as blank. absorbance of series of linearity solution were recorded at selected wavelength 337nm and 263nm as shown in fig

Fig.6. Overlay spectrum of Rifampicin in ethanol from 5-35 µg/ml

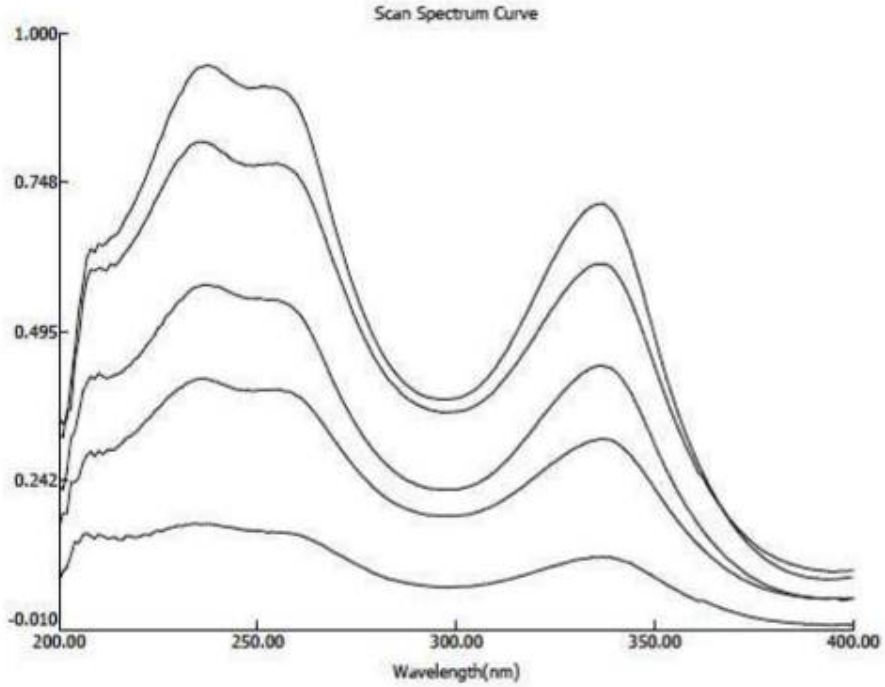
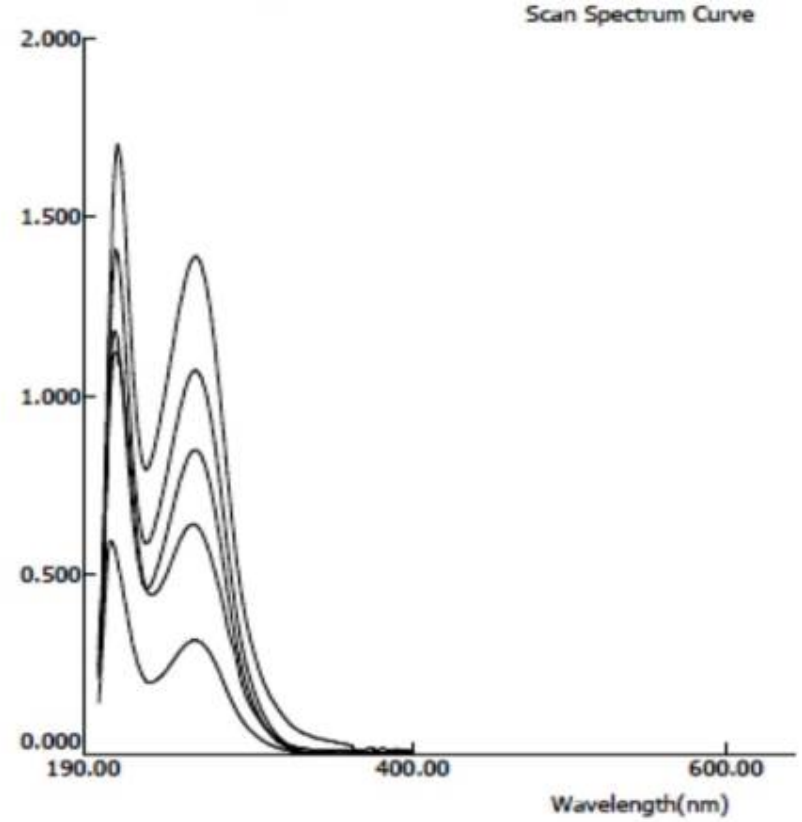


Fig.7. Overlay spectrum of Isoniazid from 5-25 µg/ml



4.8. Analysis of Capsule Formulation

The average weights of twenty capsule contents were determined and a quantity equivalent to 100 mg of Rifampicin was transferred to a 100 ml volumetric flask. The contents were dissolved by using 70 ml of ethanol, filtered through whatman filter paper no. 41 and then made up to volume with the same. The solution was further diluted with ethanol to give concentrations of 10 µg/ml of Rifampicin and 10 µg/ml of Isoniazid. The solution was scanned in the range 200-400 nm as shown in Fig. 8. Absorbance of these solutions was measured at 337 nm and 263 nm as Al and A2 respectively and concentrations of these two drugs in the sample were calculated using Simultaneous equation method. Results of analysis of the capsule formulations were reported in Table 1.

Fig.8. Overlain spectra of Isoniazid and Rifampicin in ethanol (10 µg/ml) showing λ max 263 nm and 337 nm in Formulation

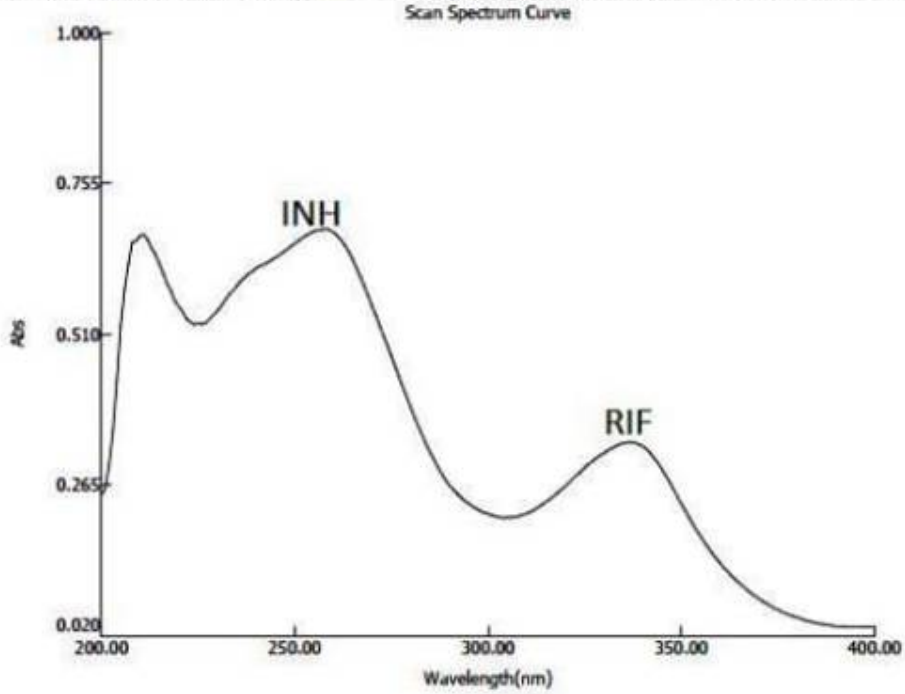


Table 1: Results of Analysis of Capsule Formulation

Drug	Label claim mg/cap	Amount found mg/cap	% Label claim*	% RSD*
RIF	450	452.26	100.50	0.691
INH	300	302.86	100.95	0.597

**Mean of six determinations*

4.9. Determination of absorptivity value

The solutions of each drug in triplicate were read against solvent blank at the selected wavelengths and A (1% 1 cm) value were calculated using below formula:

Absorptivity, A (1% 1 cm) = Absorbance at selected wavelengths/Concentration in g / 100 mL Simultaneous equation method by
Cx = A2 ay1-Al ay2/ax2 ayl - axl ay2
Cy = Al ax2-A2 ax1/ax2 ay1 - axl ay2

where:
ax1= The absorptivity of Rifampicin at 337.0 nm,
ax2= The absorptivity of Rifampicin at 263.0 nm ay1= The absorptivity of Isoniazid at 337.0 nm,
ay2= The absorptivity of Isoniazid at 263.0 nm
C x and C y are the concentrations of Rifampicin and Isoniazid
Al is the absorbance of mixture at 337 nm and A2 is the absorbance of mixture at 263 nm

4.10. Validation Parameters

The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte.

Linearity

The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of Rifampicin and Isoniazid. For simultaneous equation method, the Beer- Lambert's concentration range was found to be for 5-35µg/ml for Rifampicin and 5-25µg/ml for Isoniazid as shown in Fig. 9 and 10.

Fig.9. Calibration curve of Rifampicin in ethanol (5-35 µg/ml)

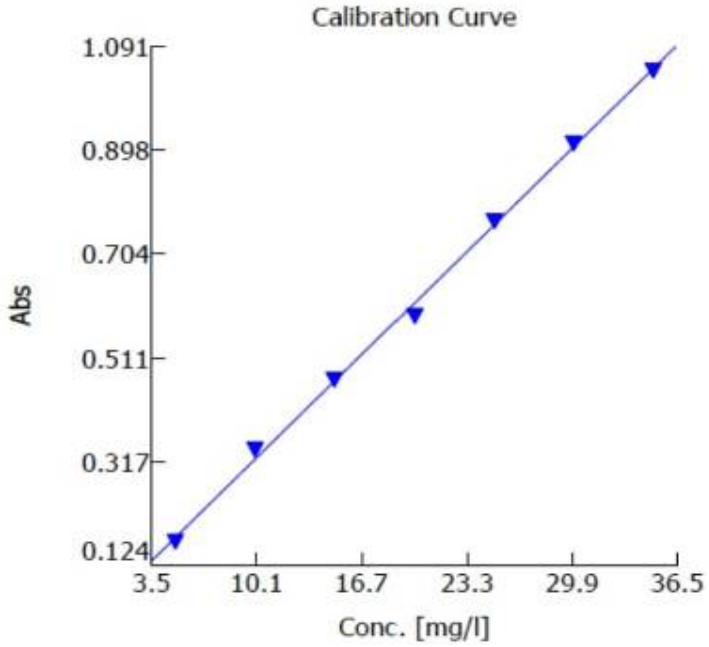
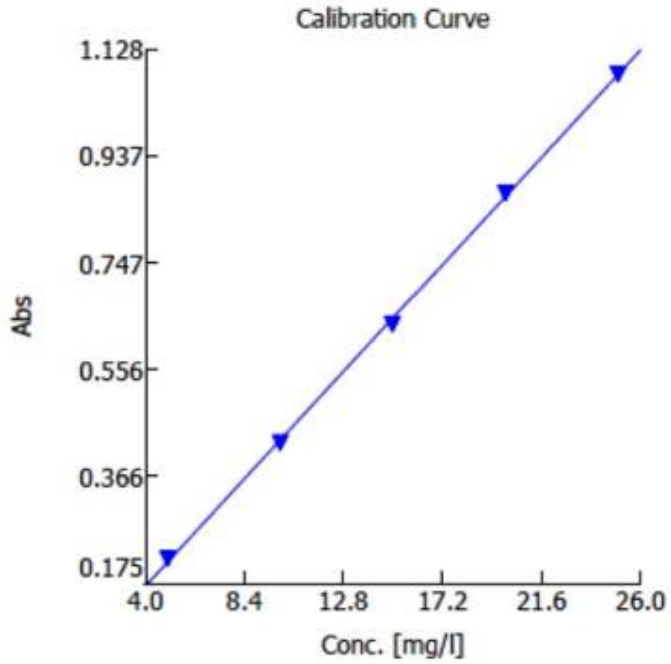


Fig.10. Calibration curve of Isoniazid in ethanol (5-25 µg/ml)



Sensitivity:

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the following equation LOD=3.30/s and LOQ=10 σ/s, Where o is standard deviation of y intercept of calibration curve (n=6) and s is slope of regression equation. The results of the same are shown in Table 4.

Precision:

The precision of the method was established by carrying out the analysis of the analytes (n=6) using the proposed developed method. The low value of relative standard deviation showed that the method was precise. The results are shown in Table 2.

Inter-day precision:

It was done by analyzing the solutions by same analyst on alternate days till 3rd day. The % RSD is shown in Table 2.

Intraday precision:

It was done by analyzing the solutions by same analyst three times within a day at intervals of 1 hr. The % RSD is shown in Table 2.

Table 2: Results of Precision Studies

Precision**					
Concentration (µg/ml)		Inter-day		Intraday	
RIF	INH	RIF	INH	RIF	INH
20	20	0.784	1.374	0.578	0.673

***%RSD of six determinations*

Accuracy

To check the accuracy of the developed method and to study the interference of formulation excipients, analytical recovery experiments were carried out by using standard addition method at 50, 100 and 150% levels. From the total amount of drug found, the percentage recovery was calculated. The results revealed no interference of excipients.

The results of recovery studies were summarized in Table 3.

Table 3: Results of Accuracy of the method

Drug	% Amount added	Label claim (mg)	Amount Recovered (mg)	% Recovery	Mean % Recovery
RIF	50	450	676.1	99.61	99.23
	100		898.7	99.23	
	150		1125.6	98.86	
INH	50	300	450.9	99.89	99.80
	100		600.4	99.96	
	150		751.3	99.56	

5. RESULTS AND DISCUSSION

The proposed method for simultaneous estimation of Rifampicin and Isoniazid in combined sample solutions was found to be simple, accurate and reproducible. Beer's law was obeyed in the concentration range of 5-35 µg/ml for Rifampicin and 5-25 µg/ml for Isoniazid respectively. The correlation coefficients were found to be 0.9991 for Rifampicin and 0.9998 for Isoniazid which shows the good linear relationship for both drugs. The capsule assay results obtained by proposed method was very close to labeled claim and low value of relative standard deviation, suggesting that the developed method has high precision. In order to check the accuracy of the developed methods, known quantities of standard drugs of Rifampicin and Isoniazid in three different levels were added to its pre-analyzed capsule sample and analyzed by the developed methods. The mean percentage recoveries were found in the range of 99.0-100.0 and it showed the non interference of the excipients from the capsule formulation. The results of optical characteristics such as Beer's law limits, correlation coefficient, slope, intercept and molar absorptivity values were summarized in Table 4.

5.1. RESULTS AND DISCUSSION

The proposed method for simultaneous estimation of Rifampicin and Isoniazid in combined sample solutions was found to be simple, accurate and reproducible. Beer's law was obeyed in the concentration range of 5-35 µg/ml for Rifampicin and 5-25 µg/ml for Isoniazid respectively. The correlation coefficients were found to be 0.9991 for Rifampicin and 0.9998 for Isoniazid which shows the good linear relationship for both drugs. The capsule assay results obtained by proposed method was very close to labeled claim and low value of relative standard deviation, suggesting that the developed method has high precision. In order to check the accuracy of the developed methods, known quantities of standard drugs of Rifampicin and Isoniazid in three different levels were added to its pre-analyzed capsule sample and analyzed by the developed methods. The mean percentage recoveries were found in the range of 99.0-100.0 and it showed the non interference of the excipients from the capsule formulation. The results of optical characteristics such as Beer's law limits, correlation coefficient, slope, intercept and molar absorptivity values were summarized in Table 4.

Table 4: Optical Characteristics and Summary of Validation Parameters of the proposed method

S.No.	PARAMETER	RESULTS	
		RIF	INH
1	λ max (nm)	337	263
2	Beer's law limit (µg/ml)	5-35	5-25
3	Molar absorptivity (L mole^{-1} cm^{-1})	25349	9341
4	Corrélation coefficient (r^2)	0.9991	0.9998
5	Regression equation (y = a + bC) ** Slope (b) Intercept (a)	y = 0.029x + 0.030 0.029 0.030	y = 0.043x - 0.005 0.043 0.005
6	Inter-day Precision (% RSD) *	0.784	1.374
7	Intraday Precision (% RSD) *	0.578	0.673
8	Limit of detection (LOD) (µg/ml)	1.653	0.585
9	Limit of quantification (LOQ) (µg/ml)	5.007	1.772

**Average of six determinations (n=6)*

***y = a + bC where C is the concentration in µg/ml*

6. CONCLUSION:

The proposed synchronous UV Spectrophotometric technique was created and approved completely for quantitative assurance of Rifampicin and Isoniazid in joined container dose structure. The developed method was found to be straightforward, quick, accurate, precise, and cost-effective. It also yielded a satisfactory recovery of the analytes, making it suitable for routine quality control analysis of pharmaceutical capsule formulations containing Rifampicin and Isoniazid.

7. References:

[1] Daniel T., The history of tuberculosis. Resp Med., 2006; 100: 1862-1870.

[2] Soroceanu V., Rais C., Ștefănescu E., Brumărel M., Safta V., Adauji S., Priscu V., Taerel AE., Epidemiological and economic aspects of tubercolosis in children. A comparative analysis: Romania vs. The Republic of Moldova. Farmacia, 2016; 64(1): 152-158.

[3] European Pharmacopoeia, 8th ed. Strasbourg: Council of Europe, 2014.

[4] Martindale: The Complete Drug Reference, 38thed. London: Pharmaceutical Press, 2014.

[5] Rais C., Taerel A.E., Stefanescu E., Brumărel M., Safta V., Adauji S., Priscu V., Soroceanu V., Epidemiological aspects of tuberculosis in adults in Romania versus the Republic of Moldova. Farmacia, 2016; 64(4): 643-650.

[6] Smith P.J., van Dyk J., Fredericks A., Determination of rifampicin, isoniazid and pyrazinamide by high performance liquid chromatography after their simultaneous extraction from plasma. Int J. Tuberc. Lung. Dis., 1999; 3:S325-328.

[7] Fang P.H., Cai H.L., Li H.D., Zhu R.H., Tan Q. Y., Gao W., Xu P., Liu Y.P., Zhang W.Y., Chen Y.C., Zhang F., Simultaneous determination of isoniazid, rifampicin, levofloxacin in mouse tissues and plasma by high performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci., 2010; 878(24): 2286-2291.

[8] Zhou Z., Chen L., Liu P., Shen M., Zou F., Simultaneous determination of isoniazid, pyrazinamide, rifampicin and acetylisoniazid in human plasma by high-performance liquid chromatography. Anal. Sci., 2010; 26(11): 1133-1138.

[9] Acedo-Valenzuela M.I., Espinosa-Mansilla A., Munoz de la Pena A., Canada-Canada F., Determination of antitubercular drugs by micellar electrokinetic capillary chromatography. Anal. Bioanal Chem., 2002; 374: 432-436.

[10] Faria A.F., de Souza M.V.N., Bruns R.E., de Oliveira M.A.L., Simultaneous determination of first-line anti-tuberculosis drugs by capillary zone electrophoresis using direct UV detection. Talanta, 2010; 82: 333-339.

[11] Hammam E., Beltagi A.M., Ghoneim M.M., Voltammetric assay of rifampicin and isoniazid drugs, separately and combined in bulk, pharmaceutical formulations and human serum at a carbon paste electrode. Microchem. J., 2004; 77: 53-62.

[12] Goicoechea H.C., Olivieri A.C., Simultaneous determination of rifampicin, isoniazid and pyrazinamide in tablet preparations by multivariate spectrophotometric calibration. J. Pharm. Biomed. Anal., 1999; 20: 681-686.

[13] Li B., He Y., Lv J., Zhang Z., Simultaneous determination of rifampicin and isoniazid by continuous-flow chemiluminescence with artificial neural network calibration. Anal. Bioanal. Chem. 2005; 383: 817-824.

[14] Bennetton S.A., Kedor-Hackmann E.R.M., Santoro M.I.R.M., Borges V.M., Visible spectrophotometric and first-derivative UV spectrophotometric determination of rifampicin and isoniazid in pharmaceutical preparations. Talanta., 1998; 47: 639-647.

[15] Stets S., Tavares T.M., Peralta-Zamora P.G., Pessoa C.A., Nagata N., Simultaneous determination of rifampicin and isoniazid in urine and pharmaceutical formulations by multivariate visible spectrophotometry. J. Braz. Chem Soc., 2013; 7: 1199-1205.

[16] Chaudhary J., Jain A., Saini V., Simultaneous estimation of multicomponent formulations by UV-visible spectroscopy: an overview. Int. Res. J. Pharm., 2011; 2: 81-83.

[17] Bosch Ojeda C., Sanchez Rojas F., Recent developments in derivative ultraviolet/visible absorbtion spectrophotometry. Anal. Chim. Acta., 2004; 518: 1-24.

[18] Daniel T., The history of tuber culosis. Resp Med., 2006; 100:1862-1870.

[19] Soroceanu V., Rais C., Stefanescu E., Brumarel M., Safta V., Adauji S., Priscu V., Taerel AE., Epidemiological and economic aspects of Tubercolosis in children. A comparative

analysis: Romaniavs. The Republic of Moldova. Farmacia, 2016; 64(1): 152-158

[20] N Maggi; C R Pasqualucci; R Ballota; P Sensi. Chemotherapia, 1966, 11, 285.

[21] M Gallieni; P Braidotti; M Cozzolino; S Romagnoli; P Carpani; Int J Artificial Organs 1999, 22, 477.

[22] JO Maryadele Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals. 14th ed., Whitehouse station, New Jersey: Published by Merck Research Laboratories, Division of Merck and Co., Inc; 2006; pp. 1417.

[23] British Pharmacopoeia, Vol. II; The British Pharmacopoeia Commission, London; 2010; pp. 1844, 3063.

[24] 24.Indian Pharmacopoeia, Vol. III; The Controller Publication, Govt. of India, New Delhi; 2010; pp. 2054-2065.

[25] United States Pharmacopoeial Convention, Inc; 2005; pp. 3501 – 3507.

[26] R Panchagnula; A Sood; N Sharda; K Kaur; CL Kaul. Journal of Pharmaceutical and Biomedical Analysis, 1999, 18(6), 1013–1020.

[27] CJ Shishoo; SA Shah; IS Rathod; SS Savale; MJ Vora. International Journal of Pharmaceutics, 2001, 228(1-2), 53–67.

[28] TT Mariappan; KC Jindal; S Singh. Journal of Pharmaceutical and Biomedical Analysis, 2004, 36(3), 905– 908.

[29] MY Khuhawar; FMA Rind. Pakistan Journal of Pharmaceutical Sciences, 1998, 18(2), 49-54.

[30] E Calleri; ED Lorenzi; S Furlanetto; G Massolini; G Caccialanza. Journal of Pharmaceutical and Biomedical Analysis, 2002, 29(6), 1089–1096.

[31] A Manna; I Ghosh; S Datta; PK Ghosh; LK Ghosh; BK Gupta. Indian Journal of Pharmaceutical Science, 2000, 62(3), 185-186.

[32] J Ali; N Ali; Y Sultana; S Baboota; S Faiyaz. Acta chromatographica, 2007, 18, 168-179.

[33] RA Harvey, PC Champe. Pharmacology, 2nd ed., Lippincott Williams and Wilkins, 2000; pp. 331-335.

[34] Shinkich Shimizu et al. Ulmanns Encyclopedia of Industrial Chemistry, John Wiley and sons, 2007.

[35] A Safari; F Abbasitabar; MR Hormozi Nezhad. Chem Anal (Warsaw), 2007, 52, 835.

[36] S Yao; W Li; X Su; X Zuo; W Wei. Talanta, 1999, 50(3), 469.

[37] Tarek Wahdan. Chem Anal (Warsaw), 2005, 50, 457.

[38] MSM Quintino; L Angnes Journal of Pharmaceutical and Biomedical Analysis, 2006, 42(3), 400- 404.

[39] Ait Moussa et al. J. Chromatography B, 2005, 5, 181.

[40] A Carlina; N Gregory; J Simmonsc. Journal of Pharmaceutical and Biomedical Analysis, 1998, 17, 885.

[41] M Y Khuhawara; L A Zardari. Analytical Sciences, 2008, 24(11), 1493.

[42] M Tatarczak; J Flieger; H Sxumilo. Journal of Planar Chromatography, 2005, 18(103), 207-211.

[43] P Nagendra; H S Yathirajan; K N Mohona. J Indian Chem Soc, 2002, 79(1), 75.

[44] X Juan; S Bo'an; A Xinpingi; H Zhike. Journal of Pharmaceutical and Biomedical Analysis, 2004, 36(1), 237-241.

[45] Mandru, A., Mane, J. and Mandapati, R., 2023. A Review on UV-visible spectroscopy. Journal of Pharma Insights and Research, 1(2), pp.091-096

[46] Kamal, A.H., El-Malla, S.F. and Hammad, S.F., 2016. A review on UV spectrophotometric methods for simultaneous multicomponent analysis. Eur J Pharm Med Res, 3(2), pp.348-60.

[47] Skoog, DA West D.M Holler F.J & Crouch, SR (2013) fundamental Analytical chemistry (9thed)(2)

[48] Mishra, K., Soni, H., Nayak, G., Patel, S.S. and Singhai, A.K., 2011. Method development and validation of metformin hydrochloride in tablet dosage form. Journal of Chemistry, 8(3), pp.1309-1313