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SIMULTANEOUS ESTIMATION OF ISONIAZIDE AND RIFAMPICINE BY ULTRA VIOLET VISIBLE SPECTROPHOTOMETRY

Shreyash Ravikant Pusadkar*1, Mahesh Ramesh Pawar*2, Anurag Vitthal Nimbalakar*3, Saurabh Devidas Nirmal*4, Sujal Dilip Patel*5, Parth Sushil Agrawal*6, Akshay Chandrashekhar Pawar*7, Prof. Shaebaz Deshmukh*8, Dr. R.H. Kale*9

*1,2,3,4,5,6,7,8,9PRMSS, Anuradha College Of Pharmacy, Chikhali, Buldhana, India.

ABSTRACT

Study or experiment involving the simultaneous spectrophotometric determination of rifampicin, isoniazid, and pyrazinamide using a generalized version of the net analyte signal standard addition method (GNASSAM). The study utilized UV spectra of these compounds and involved preparing a standard solution containing a mixture of all analytes, followed by the addition of this solution to the sample. The concentrations of analysts were determined based on the changes in net analyte signals observed in the UV spectra. The results demonstrated the acceptable performance of GNASSAM in determining the concentrations of these antituberculosis medications in synthetic mixtures.

Keywords: Simultaneous Determination, Chemometrics.

I. INTRODUCTION

Tuberculosis (TB) is a disease caused by Mycobacterium tuberculosis, primarily affecting the lungs but can also harm other parts of the body. While many exposed individuals don't show symptoms because the bacteria can remain inactive, weakened immune systems can activate TB. Isoniazid (INH) is a key drug in TB treatment, effective against both extracellular and intracellular TB bacteria. It's a white crystalline powder, soluble in water and alcohol. Despite declines in TB cases, there was a reversal in the trend in the U.S. during the mid-1980s to early 1990s, with an increase in cases and deaths. Rifampicin (RIF) is another important drug, derived from Rifamycin antibiotics. It's used to treat TB and leprosy, as well as eliminate certain bacteria. RIF is a red odorless powder, sparingly soluble in water but soluble in other solvents. INH and RIF are considered highly effective antitubercular agents and are often administered together due to drug resistance concerns.The aim of the present work was to develop simple, sensitive, and rapid spectrophotometric methods for the simultaneous determination of Isoniazid (INH) and Rifampicin (RIF) in combinations. These drugs, recommended by the WHO for TB treatment, offer therapeutic advantages by increasing treatment adherence and reducing resistance or relapses, treatment costs, and errors in drug administration. However, their combination presents new challenges for the pharmaceutical industry in developing new analytical methods for simultaneous determination.

INTRODUCTION TO DISEASE:

Tuberculosis (TB) is caused by Mycobacterium tuberculosis and other members of the M. tuberculosis complex, including M. bovis, M. africanum, and Microti. When infected droplet nuclei are inhaled, they are deposited on the bronchial mucosa and ingested by macrophages, which transport them into the pulmonary lymphatic system.

In most cases, the organisms replicate within macrophages and inhibit the phagocytic process, allowing them to replicate and spread before effective immune responses develop, typically within six to 12 weeks. However, in highly susceptible individuals, widespread seeding of TB can occur, especially in children and those who are immunosuppressed, leading to miliary TB, tuberculous meningitis, and TB septicaemia, which are life-threatening consequences. Historically, when previously unexposed populations were exposed to TB infection, high mortality rates occurred due to these severe forms of the disease.

PREVALENCE IN INDIA

There are 10 million (1 crore) people worldwide suffering from tuberculosis (TB), with 26.9 lakh (2.69 million) cases in India alone. TB can be fatal, especially in those with untreated HIV co-infection, and even with

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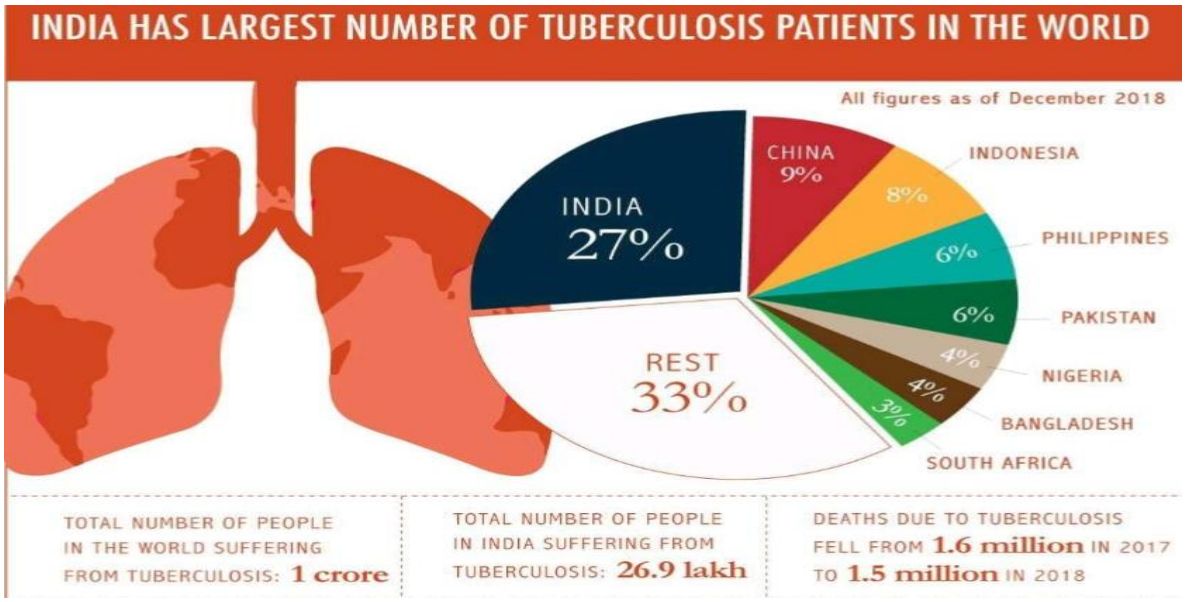
antiretroviral treatment for HIV, death rates are increased. However, there has been a decrease in deaths due to TB from 1.6 million in 2017 to 1.5 million in 2018.



DRUG PROFILE

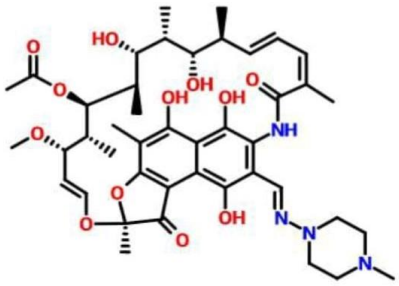
1. RIFAMPICIN:

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.



Structure:-

Weight Average:- 822.9402

Monoisotopic:- 822.40512334

Chemical Formula :-C43H58N4O12

Synonyms :-Rifampicin, Rifampicina, Rifampicine, Rifampicinum, Rifampin.

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.

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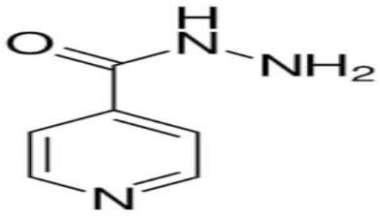
Brand Names :-Isonarif, Isotamine, Isotamine B, Rifamate, Rifater

Generic Name :-Isoniazid

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

Structure :-



Weight Average :- 137.1393

Monoisotopic :- 137.058911861

Chemical Formula :-C6H7N3O

Synonyms :-4-pyridinecarbohydrazide, Isoniazid, Isoniazida, Isonicotinic acid hydrazide, Isonicotinic hydrazide, Isonicotinohydrazide, Isonicotinoylhydrazide, Isonicotinsäurehydrazid, Isonicotinylhydrazine.

Instrumentation:-

SPECTROPHOTOMETER

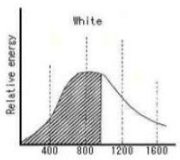
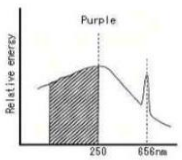
CONTENTS:-

1. LIGHTSOURCE
2. SAMPLECELL
3. WAVELENGTHSELECTOR(MONOCHROMATOR)
4. DETECTORS
5. RECORDINGSYSTEM(DISPLAY)

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.

Types and properties of light source:

Type	Tungsten	Deuterium
Sign	W WI	D_2
Property	A continuous spectrum of 300 - 3,000 nm is emitted.	A continuous spectrum of 168 - 500 nm, with maximum energy at 250 nm, is emitted.
Wavelength range	340 - 1,100 nm	185 - 360 nm
Spectrum energy		

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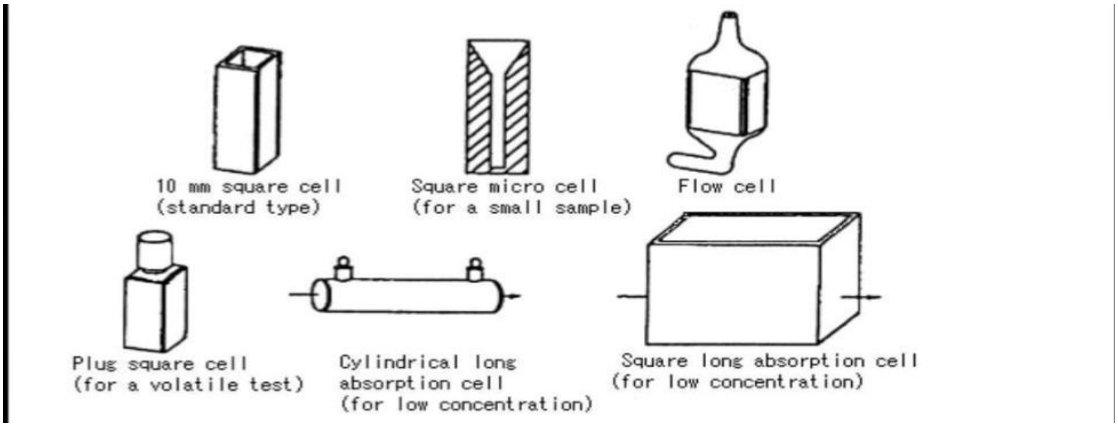
2. SAMPLE CELL:

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 measurements.



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.

- **Typesandpropertiesofdispersion**

Type	Filter	Prism	Grating (diffraction grating)
Properties	A single wavelength can be extracted with a filter. A filter is also used in combination with diffraction grating for filtering out stray light.	A spectrum of 175 - 2,700 nm can be dispersed. The degree of dispersion varies with the wavelength.	Dispersion is homogeneous at the entire wavelength, and a wide-range wavelength can be obtained with a diffraction grating. In addition, a constant spectrum featuring a constant slit breadth can be obtained.
Types and materials			
	Colored glass filter Interference filter	Crystal or fused quartz	Plane diffraction grating Concave diffraction grating

Types and properties of dispersion:

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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.

Optical semiconductor	Photomultiplier
High speed, high sensitivity, and a low noise are features over the wavelength from Ultraviolet to Near infrared. A photo cell sensitive only in the visible range is used. A Si photodiode is a representative one. 	This combines a photoelectric tube and an amplifier (approximately 10-fold amplification) sensitive in both the ultraviolet and visible regions, and the sensitivity can be changed sharply by increasing the applied voltage. 

RECORDING SYSTEM(DISPLAY):

PRICIPLE:

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 (XandY) each of this absorb sattheλmaxofeachotheri.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 Xatλ1 and λ2 and ax1 and ax2 respectively, absorptivity of Yat λ 1an day1 anday 2 respectively, Absorbance of the diluted sample at λ1 and λ2, A1 and A2 respectively. Let Cx and Cybe the concentration of X and Y respectively in the diluted sample. Two equation sare 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 .....(1)Atλ2A2 =ax2bcx+ay2 bcy (2)

Rearrangeeq. (2)

cy=A2-ax2cx/ay2

Substitutingforcyineq.(1),andrearranginggivescx=A2ay1–A1ay2/ax2ay1–ax1ay2

and

cy=A1ax2–A2ax1/ax2ay1 –ax1ay

II. MATERIAL

Chemicals and Reagents:

Pharmaceutical grade Isoniazid and Rifampicin were used in the experiment. All reagents were of analytical grade quality: hydrochloric acid acid, sodium hydroxide , methanol .Ultrapure water often from distillation

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process. Commercial pharmaceutical preparation Macox® plus (MACLEODS, INDIA) containing 150 mg INH and300mgRIFwaspurchasedfromalocalpharmacy

Requirements:-

Instruments:- A Spector 210 UV-VI spectrophotometer using matched 1 cm quartz cells andWinAspect7.01. Preparation of standard solutions

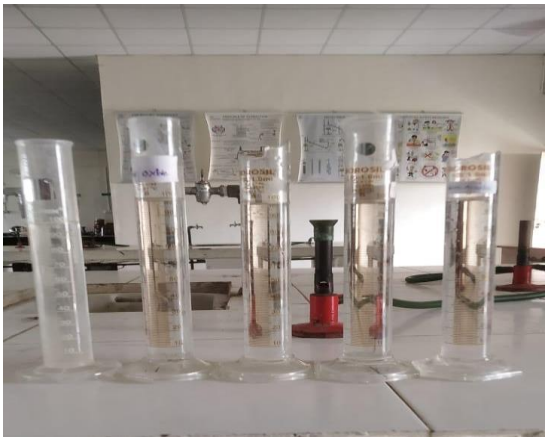
PROCEDURE:-

Preparation of standard solutions:-

100mg of INH and RIF were dissolved separately in different solvents(water,methanol,0.1N HCl, 0.1 N NaOH), the solution were sonicated for 5 minutes, diluted to a concentration of 25 µg/mL and scanned separately between 200 – 400 nm against a blank, in order to determine the maximum absorbtion wavelength of both drugs

Preparation of samples from pharmaceutical forms:-

Twenty tablets of Macox® Plus from the same batch were weighed accurately, average weight was calculated, the tablet swere finely powder edinamortarintoaho momentous powder; an amount of powder equivalent to the weight of one tablet was dissolved in 100 mL methanol by sonication for 5 minutes with intermittent shaking. The solution was filtered and the aliquot portion of filtrate was further diluted to get a final concentration of 12.5 µg/mL INH and 25µg/m LRIF. The content of NH and RIF in tablet dosage form was calculated using two framed simultaneous equations and derivative method.



Dilution chart :

- For isoniazid:- 50mg ----------→50ml--→1000g/ml
  1ml----------→100ml--→10micro gram /ml
- For rifampicin:-50mg----------→50ml--------→1000g/ml
  1ml----------→100ml-------→10micro gram/ml

Molecular formula:

1. Isoniazid:-

- Formula :C6H7N3O
- Molecular weight:137.139 g/mol.

2. Rifampicin:-

- Formula:C43H58C43H58N4O12
- Molecular weight: 822.95g/mol.

Calculation:

Weight of 20 tablet =16.06gm

Label claim:

Isoniazid =300 mg (0.300gm)

Rifampicin =450mg (0.450gm)

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Average weight =0.803gm

0.803gm of isoniazid tablet Powder contains 0.300 gm of isoniazid.

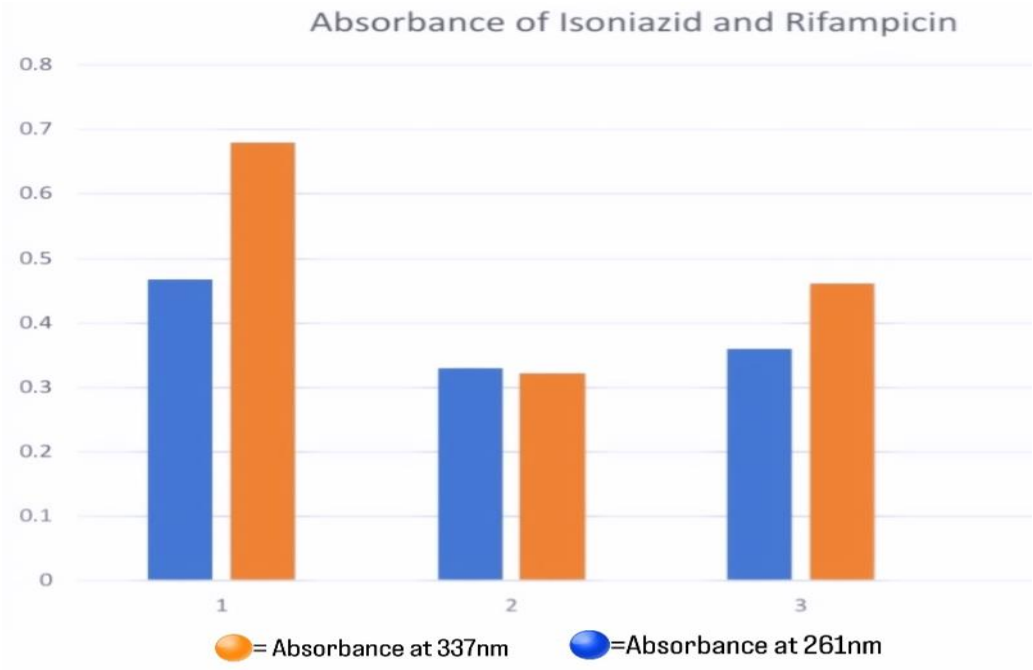
Xgm of isoniazid tablet powder contain 0.05gm of isoniazid.

X=(0.803×0.05)÷0.300

X=1338gm

Observation Table:

Sr. No.	Drug's	Absorbance	
		Λ1=261nm	Λ2 =337nm
1	Isoniazid	0.467.	0.680
2	Rifampicin	0.330.	0.322
3	Unknown	A1=0.360.	A2=0.462



Formula:

$$\text{Absorptivity of drug} = \frac{\text{Concentration of drug of } \Lambda}{\text{Concentration of drug}}$$

- For the isoniazid :

$$\text{Absorptivity of isoniazid at } \Lambda = \frac{\text{Concentration of isoniazid of } \Lambda 1.}{\text{Concentration of isoniazid Solution.}} = \frac{0.467}{10} = 0.0467\text{nm}$$

$$\text{Absorptivity of isoniazid at } \Lambda 2 = \frac{\text{Concentration of isoniazid of } \Lambda 2.}{\text{Concentration of isoniazid Solution.}} = \frac{0.680.}{10} = 0.0680\text{nm}$$

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- For rifampicin:

$$\text{Absorptivity of rifampicin at } \Lambda 1 = \frac{\text{Concentration of rifampicin at } \Lambda 1}{\text{Concentration of rifampicin Solution.}} = \frac{0.330}{10} = 0.0330 \text{ nm}$$

$$\text{Absorptivity of Rifampicin at } \Lambda 2 = \frac{\text{Concentration of rifampicin at } \Lambda 2}{\text{Concentration of rifampicin Solution}} = \frac{0.322}{10} = 0.0322 \text{ nm.}$$

For the sample:

The absorbance at 261 nm (A1) =0.360nm.

The absorbance at 337nm (A2) =0.462nm.

For the Cx = Concentration of isoniazid.

$$Cx = \frac{A2\,ay1 - A1ay2}{ax2ay1 - ax1ay2}$$

$$= \frac{0.462 \times 0.0330 - 0.360 \times 0.0322}{0.0680 \times 0.0330 - 0.0467 \times 0.0332}$$

$$= \frac{0.01524 - 0.01159}{0.00224 - 0.00148}$$

$$= \frac{0.00364}{0.00076}$$

$$Cx = 4.8$$

For the Cy=

Concentration of Rifampicin:

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$

$$= \frac{0.360 \times 0.0680 - 0.462 \times 0.0467}{0.0680 \times 0.0330 - 0.0467 \times 0.0322}$$

$$= \frac{0.02448 - 0.02157}{0.00224 - 0.00150}$$

$$Cy = 3.932$$

III. RESULT

The concentration of isoniazide and rifampacin present in Combination tablet formation by UV Spectroscopy using simultaneous equation was found to be Cx=4.8 and Cy=3.932

IV. DISCUSSION

The simultaneous determination of multiple drugs, like INH and RIF, in a sample presents a complex challenge, particularly when their absorption spectra overlap, leading to interference during quantification. Traditional

methods, such as simultaneous equations, may encounter limitations when dealing with such intricacies, as they rely on distinct absorption characteristics for accurate analysis. However, when interference occurs at the absorption maxima of these drugs, the reliability of simultaneous equations diminishes. In such scenarios, derivative spectrophotometry emerges as a sophisticated solution. This method operates by calculating the rate of change of absorbance with respect to wavelength, thereby accentuating subtle differences in spectral features. By transforming the original absorption spectrum into its derivative form, derivative spectrophotometry enhances resolution and sensitivity, making it adept at discerning individual components within complex mixtures. Moreover, derivative spectrophotometry effectively mitigates the influence of background interferences, enabling more precise quantification of each drug, even in the presence of other compounds or excipients. Its ability to emphasize minor spectral details makes it particularly valuable for quality control analysis, where accurate determination of drug concentrations is paramount. Furthermore, the simplicity and rapidity of derivative spectrophotometric methods contribute to their attractiveness in pharmaceutical analysis. These methods typically require minimal amounts of analytes and solvent, facilitating efficient and cost-effective analysis of pharmaceutical formulations. Consequently, derivative spectrophotometry represents a powerful tool for the simultaneous determination of INH and RIF in combined pharmaceutical forms, offering both accuracy and efficiency in complex analytical scenarios.

V. FUTURE PROSPECTS

Indeed, the combination of rifampin and isoniazid is a common treatment for tuberculosis (TB) infection. Rifampin, classified as an antibiotic, functions by killing or inhibiting the growth of bacteria responsible for TB. When used in combination with other TB medications, such as isoniazid, it becomes even more effective in treating the infection. This combination therapy is a cornerstone in the management of TB and is often included in treatment regimens recommended by health organizations worldwide.

VI. CONCLUSION

The technique of simultaneous equations can be used for the simultaneous estimation of two absorbing drugs like INH and RIF if they absorb at different maximum wavelengths. However, interference at their absorption maxima can occur due to absorption by the other drug, making simultaneous equations challenging. In such cases, the first derivative spectroscopic method offers an alternative. This method enhances the resolution of mixtures by emphasizing subtle spectral features and reducing the effect of background interferences. These methods are characterized by simplicity, rapidity, and accuracy, making them suitable for routine analysis in quality control of combined pharmaceutical forms containing both INH and RIF.

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