1.INTRODUCTION

The pharmaceutical analysis is a branch of chemistry, which involves the series of process for the identification, determination, quantitation, and purification. This is mainly used for the separation of the components from the mixture and for the determination of the structure of the compounds. The different pharmaceutical agents are as follows:

1). Plants
2). Microorganisms
3). Minerals
4). Synthetic compounds

Based upon the determination type, there are mainly two types of analytical methods. They are as follows:

1). Qualitative analysis: This method is used for the identification of the chemical compounds.
2). Quantitative analysis: This method is used for the determination of the amount of the sample.

UV-Visible spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. This property is influenced by the sample composition, potentially providing information on what is in the sample and at what concentration.

Light has a certain amount of energy which is inversely proportional to its wavelength. Thus, shorter wavelengths of light carry more energy and longer wavelengths carry less energy. A specific amount of energy is needed to promote electrons in a substance to a higher energy state which we can detect as absorption. This is why the absorption of light occurs for different wavelengths in different substances. Humans are able to see a spectrum of visible light, from approximately 380 nm, which we see as violet, to 780 nm, which we see as red. UV light has wavelengths shorter than that of visible light to approximately 100 nm. Therefore, light can be described by its wavelength, which can be useful in UV-Visible spectroscopy to analyse or identify different substances by locating the specific wavelengths corresponding to maximum absorbance.

Seeing is spectroscopy: we perceive the world via the interaction of visible light with the light receptors in our eyes. The light is emitted from the sun or from other light sources. It is then reflected from (or transmitted through) the objects in our surroundings. In these processes, the colour changes because some of the light is absorbed by the objects. The light not absorbed reaches our eyes. It carries the information of the molecular structure of our surroundings with it.

In our eyes its colour is analysed by 3 different types of photoreceptors which absorb different light in spectral regions. In this way we perform a spectroscopic experiment every time we

look at things. There is a light source, and object that reflects, transmits, scatters and absorbs light and a 2 Andreas Barth: Introduction to Spectroscopy wavelength dependent detector in our eyes. An apparatus for spectroscopic studies is called spectrometer and a plot of a particular property of matter against wavelength, frequency or energy of radiation is called spectrum. Not only light but also other types of electromagnetic radiation provide powerful information on biological systems. The study of the interaction of electromagnetic radiation with matter is called spectroscopy.

Electromagnetic spectrum

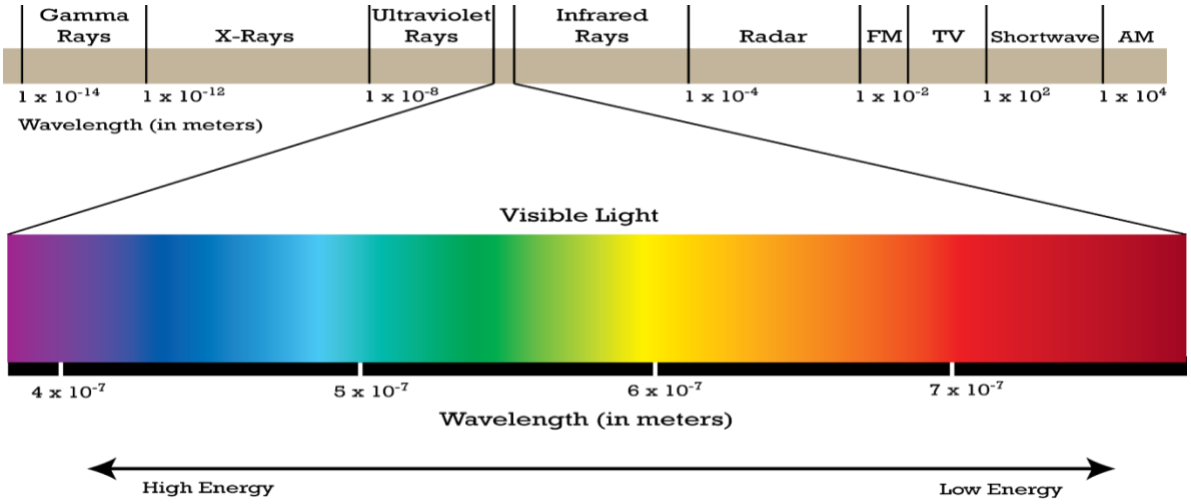

Fig. 1: Electromagnetic spectrum

Beer- Lambert's Law: The Beer -Lambert's law is a linear relationship between the concentration and absorbance, optical coefficient and molar absorption coefficient of a solution. Furthermore, the Beer Lambert law states that a linear relationship exists between the absorbance and concentration of the solution. Moreover, this relationship makes possible the calculation of the concentration of a solution by measuring its absorbance. The development of the law first took place by Pierre Bouguer before 1729. After its attribution to Johann Heinrich Lambert, the law included path length as a variable that had an effect on absorbance. Finally, an extension of the law took place by Beer in 1852 to include the concentration of solutions, and the law was named Beer-Lambert Law.

Derivation of the Formula of Beer Lambert Law

Beer-Lambert law derivation can take place from an approximation for the absorption coefficient for a molecule. This happens by carrying out an approximation of the molecule by an opaque disk whose cross-sectional area is representative of the effective area seen by a frequency w photon. Furthermore, the area would be approximately 0 in case the light's frequency is far from resonance. Moreover, the area is maximum if the w is close to resonance.

Let's take an infinitesimal slab, dz, of a particular sample. Io is the intensity which enters the sample at z=0. Furthermore, Iz is the intensity that enters the infinitesimal slab at z.

dI is the intensity whose absorption takes place in the slab, and I is the intensity of light that exits or leaves the sample. Afterwards, the total slab's opaque area because of the absorbers is * N * A * dz.

Then, the fraction of absorbed photons will be * N * A * dz / A so,

dI / Iz would be = – * N * dz

Integration of this equation can take place from z = 0 to z = b gives:

ln(I) – ln (Io) = – * N * b

Also, – ln (I / Io) would be = * N * b.

Furthermore, N (molecules/cm3) * (1 mole / 6.023×1023 molecules) * 1000 cm3 / litre = c (moles/litre) and also there would be 2.303 * log(x) = ln(x) then

– log (I / Io) would be = * (6.023×1020 / 2.303) * c * b

Also, – log (I / Io) would be = A = * b * c

here = * (6.023×1020 / 2.303) = * 2.61×1020

Instrumentation:
Instruments for measuring the absorption of U.V. or visible radiation are made up of the following components;
1. Sources (UV and visible)
2. filter or monochromator
3. Sample containers or sample cells
4. Detector

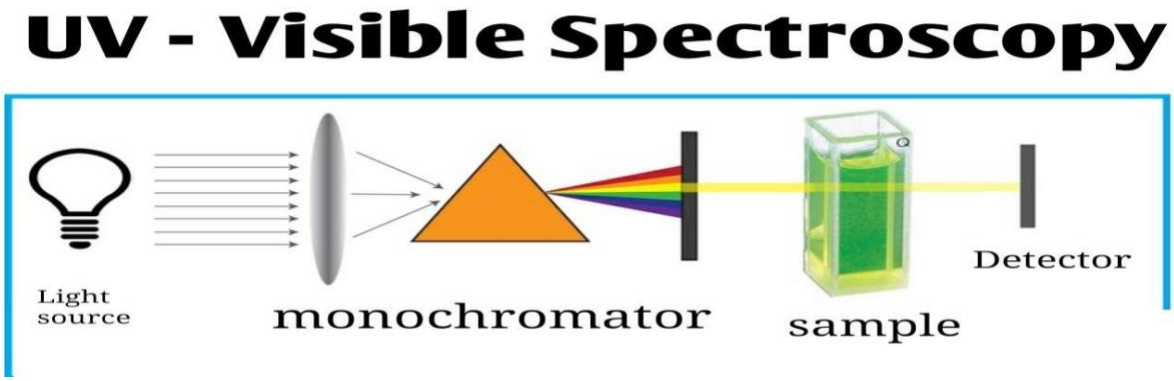

Fig. 2: Instrumentation of UV-Visible spectrophotometer

Radiation source

It is important that the power of the radiation source does not change abruptly over its wavelength range. The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum. The mechanism for this involves formation of an excited molecular species, which breaks up to give two atomic species and an ultraviolet photon

Both Deuterium and Hydrogen lamps emit radiation in the range 160 - 375 nm. Quartz windows must be used in these lamps, and quartz cuvettes must be used, because glass absorbs radiation of wavelengths less than 350 nm.

Various UV radiation sources are as follows
a. Deuterium lamp
b. Hydrogen lamp
c. Tungsten lamp
d. Xenon discharge lamp
e. Mercury arc lamp

Various Visible radiation sources are as follows
a. Tungsten lamp
b. Mercury vapour lamp
c. Carbonone lamp

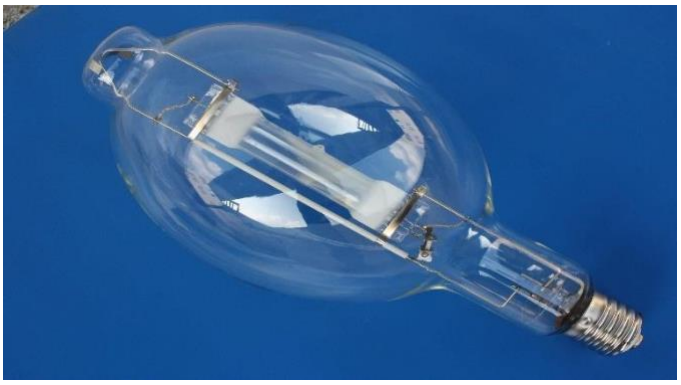

Fig. 3: Mercury lamp

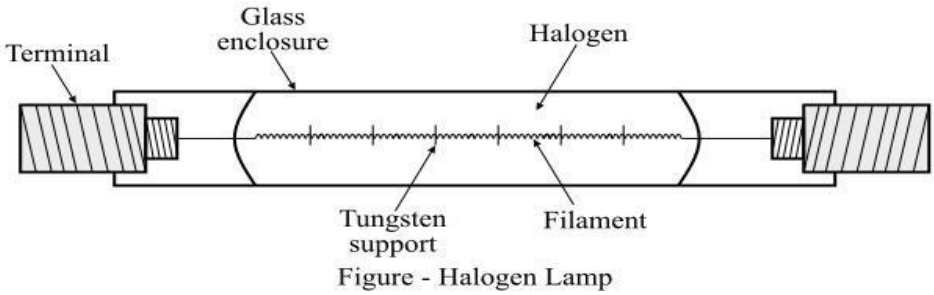

Fig. 4: Tungsten Halogen lamp

Filters or monochromators

All monochromators contain the following component parts;

- An entrance slit
- A collimating lens A
- A focusing lens
- An exit slit

Polychromatic radiation (radiation of more than one wavelength) enters the monochromator through the entrance slit. The beam is collimated, and then strikes the dispersing element at an angle. The beam is split into its component wavelengths by the grating or prism. By moving the dispersing element or the exit slit, radiation of only a particular wavelength leaves the monochromator through the exit slit.

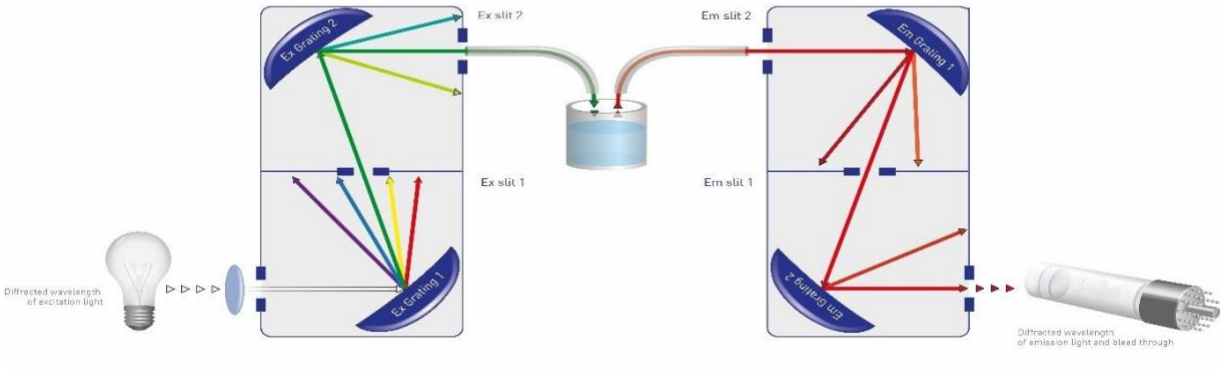

Fig. 5: Monochromator

Sample containers or sample cells

A variety of sample cells available for UV region. The choice of sample cell is based on

a) the path length, shape, size

b) the transmission characteristics at the desired wavelength

c) the relative expense

The cell holding the sample should be transparent to the wavelength region to be recorded. Quartz or fused silica cuvettes are required for spectroscopy in the UV region. Silicate glasses can be used for the manufacture of cuvettes for use between 350 and 2000 nm.The thickness of the cell is generally 1 cm.cells may be rectangular in shape or cylindrical with flat ends.

Detectors

In order to detect radiation, three types of photosensitive devices are

a. photovoltaic cells or barrier- layer cell

b. phototubes or photo emissive tubes

c. photomultiplier tubes

Photovoltaic cells: is also known as barrier layer or photonic cell. It consists of a metallic base plate like iron or aluminium which acts as one electrode. On its surface, a thin layer of a semiconductor metal like selenium is deposited. Then the surface of selenium is covered by a very thin layer of silver or gold which acts as a second collector tube.When the radiation incident upon the surface of selenium, electrons are generated at the selenium- silver surface and the electrons are collected by the silver. This accumulation at the silver surface creates an electric voltage difference between the silver surface and the basis of the cell.

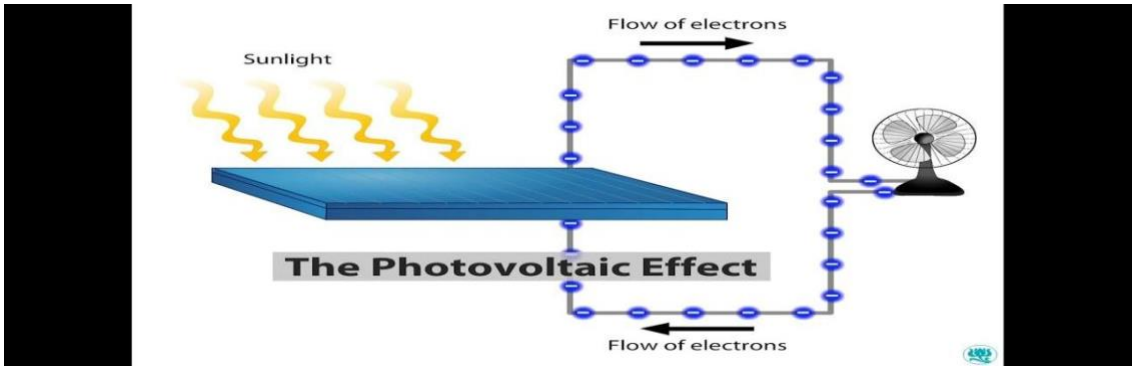

Fig. 6: Photovoltaic cell

Phototubes: are also known as photo emissive cells. A phototube consists of an evacuated glass bulb. There is light sensitive cathode inside it. The inner surface of cathode is coated with light sensitive layer such as potassium oxide and silver oxide.

When radiation is incident upon a cathode, photoelectrons are emitted. These are collected by an anode. Then these are returned via external circuit. And by this process current is amplified and recorded.

The photomultiplier tube is a commonly used detector in UV spectroscopy. It consists of a photo emissive cathode (a cathode which emits electrons when struck by photons of radiation), several dynodes (which emit several electrons for each electron striking them) and an anode.

A photon of radiation entering the tube strikes the cathode, causing the emission of several electrons. These electrons are accelerated towards the first dynode (which is 90V more positive than the cathode). These electrons are then accelerated towards the second dynode, to produce more electrons which are accelerated towards dynode three and so on. Eventually, the electrons are collected at the anode. By this time, each original photon has produced 106 - 107 electrons. The resulting current is amplified and measured.

Photomultipliers are very sensitive to UV and visible radiation. They have fast response times. Intense light damages photomultipliers; they are limited to measuring low power radiation.

Recording devices

- a). Most of the time amplifier is coupled to a pen recorder which is connected to the computer.
- b). Computer stores all the data generated and produces the spectrum of the desired compound.

Types of UV- visible spectrophotometers:

1. Single beam UV- visible spectrophotometer
2. Double beam UV- visible spectrophotometer

Single Beam Spectrophotometer

Single beam spectrophotometers determine colour by measuring the intensity of the light sources before versus after a test sample is inserted. This light source is modulated (turned on and off) to differentiate the light coming from the light source versus the light coming from the flame. The single light beam passes through the sample, and the single beam spectrophotometer measures the intensity of the light reflected from the reference to measure the sample.

Double Beam Spectrophotometer

Double beam spectrophotometers measure colour in a sample by using two, or "double," beams. One beam passes through the sample side, and the other beam passes through the reference side, so the reference and the sample can be read simultaneously with no need to recalibrate the instrument. These beams are recombined before they go to the monochromator to mitigate mechanical fluctuations, lamp intensity and other effects.

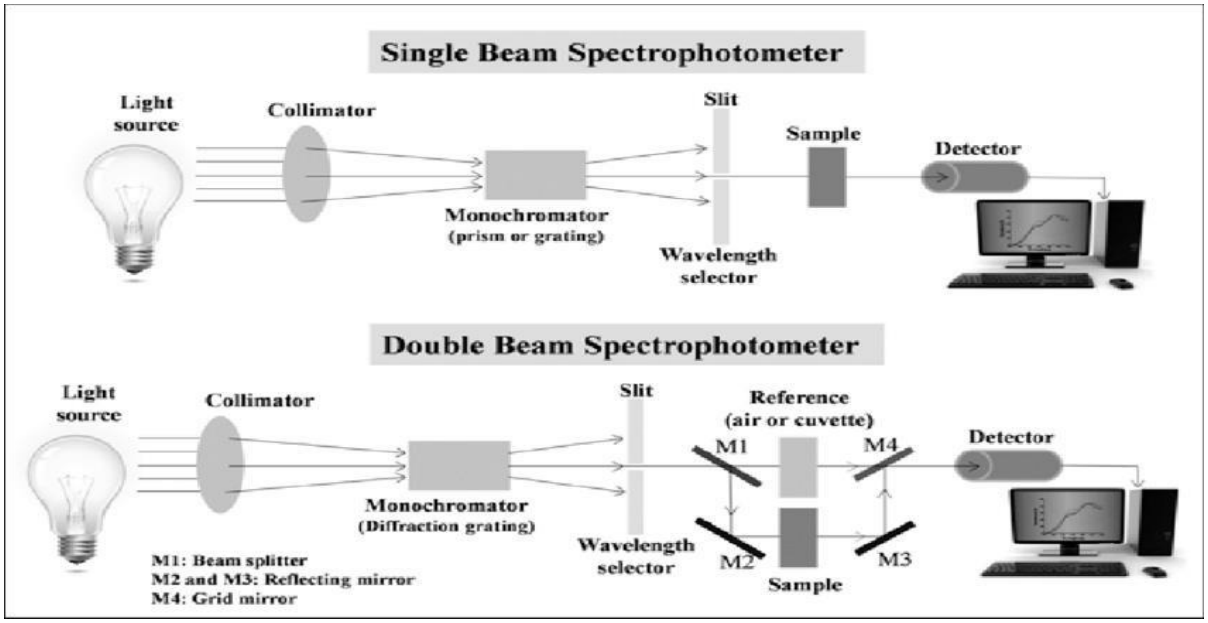

Fig. 7: Types of beams

Applications of UV-Vis spectroscopy

UV-Vis has found itself applied to many uses and situations including but not limited to:

- DNA and RNA analysis.
- Pharmaceutical Analysis.
- Bacterial culture analysis.
- Beverage analysis.

2. METHOD DEVELOPMENT OF UV SPECTROSCOPY

1. Selection of equipment
2. Equipment validation
3. Nature of sample
4. Selection of solvent
5. Selection of wavelength
6. Sample handling and measurement.

OPTIMIZATION OF UV SPECTROSCOPY

1). Wavelength
2). Solvent
3). Concentration
4). pH
5). Temperature

Method validation

Validation is establishing documented evidence, which provide a high degree of assurance that specific process will consistently produce a product meeting its specification and quality characteristics. The validation parameters are:

Accuracy:

It is the degree of agreement between an individual test generated by the true value. It is measured as the percentage of the analyte recovered by the assay. For the qualification of impurities, accuracy was determined by analysing samples with the known amount of impurities.

Precision:

It is the degree of agreement between an individual test results when the procedure is applied repeatedly to multiple samplings. It is normally expressed in relative standard variation. Precision is a measure of the degree of reproducibility or of the repeatability of the analytical method under normal operating circumstances.

Linearity:

The linearity of an analytical method is its ability to elicit test results that are directly (or by a well-defined mathematical transformation) proportional to the analyte concentration in samples within a given range. Linearity usually expressed in terms of the variance around the slope of regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte. The linear range of detectability that obeys beer's law is dependent on the compound analyzed.

Limit of detection:

The limit of detection (LOD) of an analytical procedure is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantified. It is a limit that specifies whether or not an analyte is above or below certain value. The LOD of detection of instrumental procedures is carried out by determining the signal-to noise by comparing test results from the samples with known concentrations of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted.

Limit of qualification:

limit of qualification (LOQ) is a parameter of quantitative assays for low levels of compounds in sample matrices such as impurities in bulk drugs and degradation products in finished pharmaceuticals. Like LOD, LOQ is expressed as concentrations, with the precision and accuracy of the measurement also reported. Sometimes a signal- to-noise ratio of 10 to1 is used to determine LOQ. It is measured by analysing samples containing known quantities of the analyte and determining the lowest level at which acceptable degrees of accuracy and precision are attainable.

Reproducibility:

Reproducibility means the precision of the procedure when it is carried out under different conditions-usually in different laboratories-on separate, putatively identical samples taken from the same homogenous batch of material. Comparisons of results obtained by different analysts, by the use of different equipment's, or by carrying out the analysis at different times can also provide valuable information.

Ruggedness:

Ruggedness is measure of reproducibility test results under the variation in conditions normally expected from laboratory to laboratory and from analyst to analyst. The Ruggedness of an analytical method is degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as; different laboratories, analyst, instruments, reagents, temperature, time etc. for the determination of ruggedness, the degree of reproducibility of test result is determined as function of the assay variable.

Robustness:

Robustness of analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provide an indication of its reliability during normal usage. The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provide an indication of its reliability during normal usage. The robustness of a method is evaluated by varying method parameters such as percent organic solvent, pH, ionic strength, temperature and determine the effect (if any) on the results of the method

3. LITERATURE REVIEW

1). Development and Validation of an HPLC Method for Simultaneous Determination of Rifampicin, Isoniazid, Pyrazinamide, and Ethambutol Hydrochloride in Phar Development and Validation of an HPLC Method for Simultaneous Determination of Rifampicin, Isoniazid, Pyrazinamide, and Ethambutol Hydrochloride in Pharmaceutical Formulations Paula R Chellini, Eduardo B Lages, Pedro H C Franco, Fernando H A Nogueira, Isabela C César, Gerson A Pianetti

Literature survey revealed numerous analytical techniques including HPLC (Smith et al., 1999; Calleja et al., 2004; Kumar et al., 2004; Tatarczak et al., 2005; Ali et al., 2007; Allanson et al., 2007; Hartkoorn et al., 2007; Liu et al., 2008; Bhusari et al., 2009; Sabitha et al., 2009; Wang et al., 2012; Mansuri et al., 2014; Moreno-Exebio and Grande-Ortiz, 2014) and UV spectroscopic methods (Rote and Sharma, 1997; Benetton et al., 1998; Khamar and Patel, 2012a,b; Tella et al., 2012; Begum et al., 2013; Priyanshu and Madhav, 2013) are available to assay rifampicin alone and in combination with other agents.. A simple, rapid, economic, accurate, and precise method for the estimation of rifampicin in a mixture of isoniazid and pyrazinamide by UV spectrophotometric technique (guided by the theoretical investigation of physicochemical properties) was developed and validated. Theoretical investigations revealed that isoniazid and pyrazinamide both were freely soluble in water and slightly soluble in ethyl acetate whereas rifampicin was practically insoluble in water but freely soluble in ethyl acetate. This indicates that ethyl acetate is an effective solvent for the extraction of rifampicin from a water mixture of isoniazid and pyrazinamide. Computational study indicated that pH range of 6.0–8.0 would favor the extraction of rifampicin. Rifampicin is separated from isoniazid and pyrazinamide at pH 7.4 ± 0.1 by extracting with ethyl acetate. The ethyl acetate was then analyzed at λ_{max} of 344.0 nm. The developed method was validated for linearity, accuracy and precision according to ICH guidelines. The proposed method exhibited good linearity over the concentration range of 2.5–35.0 μg/mL. The intraday and inter-day precision in terms of % RSD ranged from 1.09 to 1.70% and 1.63 to 2.99%, respectively. The accuracy (in terms of recovery) of the method varied from of 96.7 ± 0.9 to 101.1 ± 0.4%. The LOD and LOQ were found to be 0.83 and 2.52 μg/mL, respectively. In addition, the developed method was successfully applied to determine rifampicin combination (isoniazid and pyrazinamide) brands available in Bangladesh.

2). Development and validation of RP-HPLC method for simultaneous estimation of rifampicin, isoniazid and pyrazinamide in human plasma

B. Prasanthi, J. Vijaya Ratna & R. S. Ch. Phani Journal of Analytical Chemistry volume 70,

A rapid, simple, sensitive and cost-effective stability indicating high performance liquid chromatographic method for the simultaneous determination of rifampicin, isoniazid and pyrazinamide in human plasma was developed and validated in accordance to Food and Drug Administration (**FDA**) guidelines. The three drugs were eluted under isocratic mode using a 250 × 4.0 mm i.d., 5 μm Phenomenex ODS 2 C18 column. The mobile phase was composed of a mixture of acetonitrile, methanol and water in the ratio of 30: 5: 65 (v/v, pH adjusted to 5.2) at a flow rate of 1.0 mL/min. The limits of detection and quantification for rifampicin were 0.13 and 0.4 μg/mL, for isoniazid—0.6 and 1.8 μg/mL; and for pyrazinamide—0.5 and

1.6 μg/mL, respectively. The method can be successfully applied for pharmacokinetic, bioavailability or bioequivalence studies of rifampicin, isoniazid and pyrazinamide combination in human subjects.

3).Simultaneous Quantification of Linezolid, Rifampicin, Levofloxacin, and Moxifloxacin in Human Plasma Using High-Performance Liquid Chromatography with UV

This technique may also be used in many other industries. For example, measuring a color index useful for monitoring transformer oil as a preventative measure to ensure electric power is being delivered safely. Measuring the absorbance of hemoglobin to determine hemoglobin concentrations may be used in cancer research. In wastewater treatments, UV spectroscopy can be used in kinetic and monitoring studies to ensure certain dyes or dye by-products have been removed properly by comparing their spectra over time.It also finds great utility in food authenticity analysis and air quality monitoring.

4).Visible spectrophotometric and first-derivative UV spectrophotometric determination of rifampicin and isoniazid in pharmaceutical preparations

Author links open overlay panelS.A Benetton, E.R.M Kedor-Hackmann, M.I.R.M Santoro, V.M Borges Two methods are described for the determination of rifampicin and isoniazid in mixtures by visible spectrophotometry and first-derivative ultraviolet spectrophotometry. The absorbance at 475 nm in buffer solution pH 7.4 was employed to determine rifampicin after applying the three-point correction technique between 420 and 520 nm, while the amplitudeof the first-derivative spectrophotometric spectrum at 257 nm in HCl 0.012 M was selected.

4. DRUG PROFILE:

Name of the drug: Rifampicin
Drug category: Anti-tubercular
Molecular structure:

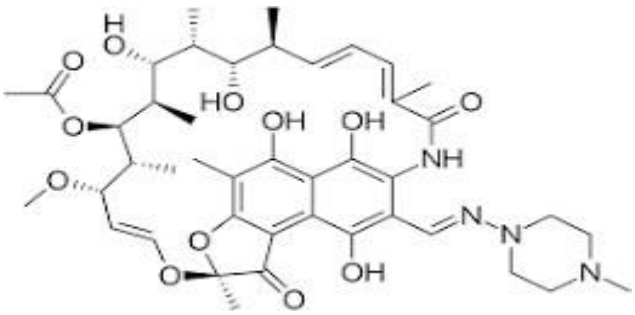

Fig. 8: Molecular structure of rifampicin

IUPAC Name: Rifampicin is 5,6,9,17,19,21-hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-[N-(4-methyl-1-piperazinyl)-formamidoyl]-2,7-(epoxypentadeca-1,11-13-trienimino)-naphtho-[2,1-b] furane-1,11(2H) dion-21 acetate
Chemical formula: $C_{43}H_{58}N_4O_{12}$
Molar mass: 822.94 g/mol.
Generic Name: Rifampicin.
Summary: Rifampicin is an antibiotic used to treat several types of mycobacterial infections including Mycobacterium avium complex, leprosy, and in combination with other antibacterials to treat latent or active tuberculosis.
Brand Name: Isonarif, Rifadin, Rifamate, Rifater, Rofact, RCin

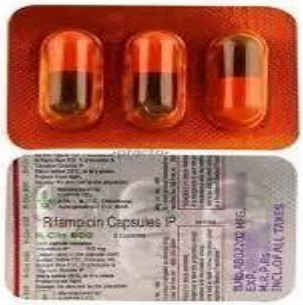

Fig. 9: RCin capsules 600mg

Mechanism of action:
Rifampicin inhibits bacterial DNA-dependent RNA synthesis by inhibiting bacterial DNA-dependent RNA polymerase.
Crystal structure data and biochemical data suggest that rifampicin binds to the pocket of the RNA polymerase β subunit within the DNA/RNA channel, but away from the active site. The inhibitor prevents RNA synthesis by physically blocking elongation, and thus preventing

synthesis of host bacterial proteins. By this "steric-occlusion" mechanism, rifampicin blocks synthesis of the second or third phosphodiester bond between the nucleotides in the RNA backbone, preventing elongation of the 5' end of the RNA transcript past more than 2 or 3 nucleotides.

In a recent study Rifampicin was shown to bind to cytochrome P450 reductase and alter its conformation as well as activity towards supporting metabolism of progesterone.

Pharmacokinetics:

Orally administered rifampicin results in peak plasma concentrations in about 2–4 hours. 4-Aminosalicylic acid (another antituberculosis drug) significantly reduces absorption of rifampicin, and peak concentrations may be lower. If these two drugs must be used concurrently, they must be given separately, with an interval of 8 to 12 hours between administrations.

Rifampicin is easily absorbed from the gastrointestinal (GI) tract; its ester functional group is quickly hydrolysed in bile, and it is catalysed by a high pH and substrate-specific esterase's. After about 6 hours, almost all of the drug is deacetylated. Even in this deacetylated form, rifampicin is still a potent antibiotic; however, it can no longer be reabsorbed by the intestines and is eliminated from the body. Only about 7% of the administered drug is excreted unchanged in urine, though urinary elimination accounts for only about 30% of the drug excretion. About 60% to 65% is excreted through faeces.

The half-life of rifampicin ranges from 1.5 to 5.0 hours, though hepatic impairment significantly increases it. Food consumption inhibits its absorption from the GI tract, and the drug is more quickly eliminated. When rifampicin is taken with a meal, its peak blood concentration falls by 36%. Antacids do not affect its absorption. The decrease in rifampicin absorption with food is sometimes enough to noticeably affect urine colour, which can be used as a marker for whether or not a dose of the drug has been effectively absorbed.

Distribution of the drug is high throughout the body, and reaches effective concentrations in many organs and body fluids, including the cerebrospinal fluid. Since the substance itself is red, this high distribution is the reason for the orange-red colour of the saliva, tears, sweat, urine, and faeces. About 60% to 90% of the drug is bound to plasma proteins.

Therapeutic uses:

Rifampicin is an antibiotic used to treat several types of mycobacterial infections including Mycobacterium avium complex, leprosy, and in combination with other antibacterials to treat latent or active tuberculosis.

Adverse effects:

The most serious adverse effect is hepatotoxicity, and people receiving it often undergo baseline and frequent liver function tests to detect early liver damage.

The more common side effects include fever, gastrointestinal disturbances, rashes, and immunological reactions. Taking rifampicin usually causes certain bodily fluids, such as urine, sweat, and tears, to become orange-red in colour, a benign side effect that nonetheless can be frightening if it is not expected. This may also be used to monitor effective absorption of the drug (if drug colour is not seen in the urine, the patient may wish to move the drug dose farther in time from food or milk intake). The decolourization of sweat and tears is not directly noticeable, but sweat may stain light clothing orange, and tears may permanently stain soft contact lenses. Since rifampicin may be excreted in breast milk, breastfeeding should be avoided while it is being taken.

Other adverse effects include:

- Liver toxicity—hepatitis, liver failure in severe cases
- Respiratory—breathlessness
- Cutaneous—flushing, pruritus, rash, hyperpigmentation, redness and watering ofeyes
- Abdominal — nausea, vomiting, abdominal cramps, diarrhoea
- Flu-like symptoms—chills, fever, headache, arthralgia, and malaise. Rifampicin has good penetration into the brain, and this may directly explain some malaise and dysphoria in a minority of users.
- Allergic reaction—rashes, itching, swelling of the tongue or throat, severe dizziness,and trouble breathing.

5. NEED FOR THE STUDY

Literature review reveals that there are few spectroscopic and chromatographic methods for rifampicin in bulk and tablet dosage forms were identified. The established methods are of longer run time and involve more organic solvent consumption. Hence there is need to develop most accurate, precise, and cost-effective method for rifampicin. So many methods are available in the market but they are not available for quantification. So my method is Help full for quantification.

6. AIM AND OBJECTIVE

Review of literature for rifampicin gave information regarding its, various analytical methods that were conducted alone and in combination with other drugs.

Literature survey reveals that certain chromatographic methods were reported for estimation of rifampicin.

In a view of the need for a suitable UV method for routine analysis of rifampicin in formulations, attempts were made to develop simple, precise and accurate analytical method for estimation of rifampicin and extended it for their determination in formulation.

Its main objective is to develop simple, precise validated method for rifampicin following ICH guidelines which includes the validation parameters and report it within the limits.

7. PLAN OF WORK

Based on our aim and objective our work is planned in the following manner.
Outline of our plan of work:

Selection of drug:

Based on public health relevance
Based on its need/scarcity in the market
Evidence on safety and Efficacy

Literature review:

To understand the existing research data
To improve our research methodology
To bring clarity and focus on research methodology

Method Development

Method Optimization

Method Validation:

Accuracy
Precision
Linearity
LOD
LOQ
Ruggedness

8. MATERIALS AND METHODS

METHOD DEVELOPMENT

S.NO	INSTRUMENTS
1.	UV-VIS Visible spectrophotometer
2.	Electronic Balance
3.	BIOTECHS Ultrasonicate
4.	pH meter
5.	Refrigerator

Table. 1: List of instruments

S.NO	CHEMICALS AND SOLVENTS
1.	Potassium phosphate
2.	Sodium hydroxide
3.	pH 7.4 Buffer
4.	HPLC Grade water
5.	Methanol

Table. 2: List of chemicals and solvents

DETERMINATION OF SOLUBILITY
Methanol – Freely soluble
Water – soluble
pH 7.4 Buffer - soluble

PREPARATION OF BUFFER SOLUTION:
Take 6.8grms of KH_2PO_4 and add 1 gram of NaOH and dissolve in1000ml distil water.

PREPARATION OF RIFAMPICIN STANDARD AND SAMPLE:
Standard stock solution was prepared by dissolving 100mg of rifampicin API into 100ml using mixture of methanol and pH 7.4 phosphate buffer in volumetric flask to get 1mg/ml concentrations. Pipette out 10ml from the above solutions was further diluted to 100ml. Transfer 1ml of above solution into a 10ml volumetric flask. 10µg/ml of the solution was scanned 400-800nm and absorption was found to be 475nm.

SELECTION OF SOLVENT
Rifampicin is freely soluble in methanol and soluble in water and pH 7.4 phosphate buffer. By using the mixture of solvents rifampicin can be solubilized i.e. methanol and pH 7.4 phosphate buffer and methanol (1:1) this method is validated and developed.

DETERMINATION OF ƛ MAX FOR RIFAMPICIN.
Scan the standard solution in 1cm square cell over the range of 400-800 using a solventmixture of methanol and pH 7.4 buffer (1:1) as blank.

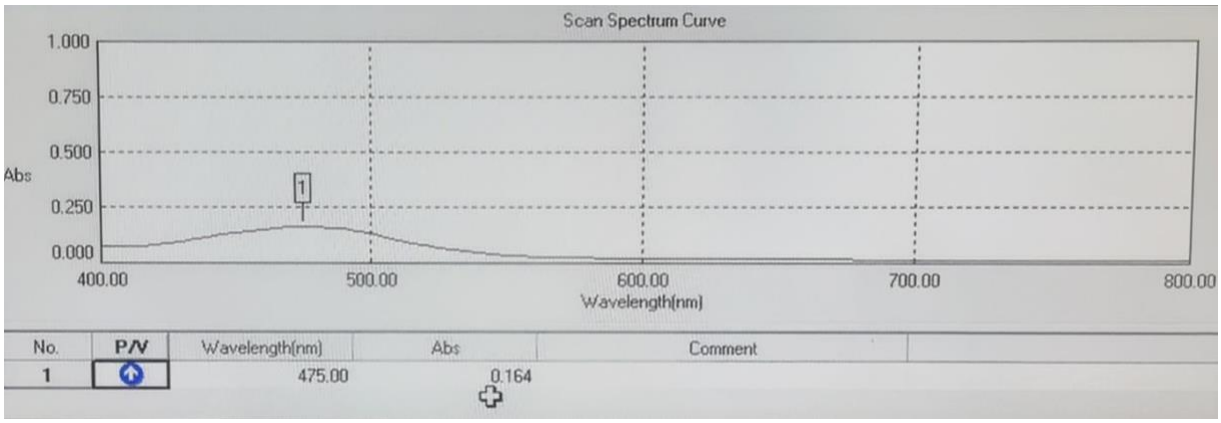

Fig. 10: Spectrum of Rifampicin

FOR RIFAMPICIN ASSAY: Weigh accurately about 0.1 g, dissolve in sufficient methanol; to produce 100.0 ml with phosphate buffer pH 7.4 and measure the absorbance at the wavelength of 475nm. For capsules weigh accurately quantity of the mixed contents of 20 capsules equivalent to 0.1 g of rifampicin, shake with 80 ml of methanol, add sufficient methanol to produce 100.0 ml and filter. Dilute 2.0 ml of the filtrate to 100.0 ml with phosphate buffer pH 7.4 and measure the absorbance of the resulting solution at the maximum about 475nm using the phosphate buffer as blank.

LINEARITY:

From the graph it was found that rifampicin obeys Beer's law and the linearity concentrations lies between 5-25 µg/ml. The linearity data calibration curve was shown in the figure. The correlation co-efficient, intercept and slope were calculated for rifampicin and the results were identified.

ACCURACY:

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (50%,100%,150%) of the pure drug to the pre analyzed formulation. The solutions were prepared in triplicates and the % recovery was calculated .

PRECISION:

Six replicates of three different concentrations were scanned within the entirelinearity range and %RSD were reported for repeatability (intraday) and intermediate precision (inter-day).

ROBUSTNESS:

Robustness of the method was determined by carrying out the analysis at different wavelengths (±2nm). The respective absorbance was noted and the results were indicated by % RSD.

LIMIT OF DETECTION (LOD):

LOD= 3.3 ×S. D/SLOPE

LIMIT OF QUANTIFICATION (LOQ):

LOQ= 10 ×S. D/SLOPE

9. RESULTS AND DISCUSSION

LINEARITY

S.NO	CONCENTRATION	ABSORBANCE
1	5	0.080
2	10	0.164
3	15	0.241
4	20	0.327
5	25	0.411
	STDEV	0.13
	Slope	0.02
	R^2	0.9997

Table. 3: Linearity of Rifampicin

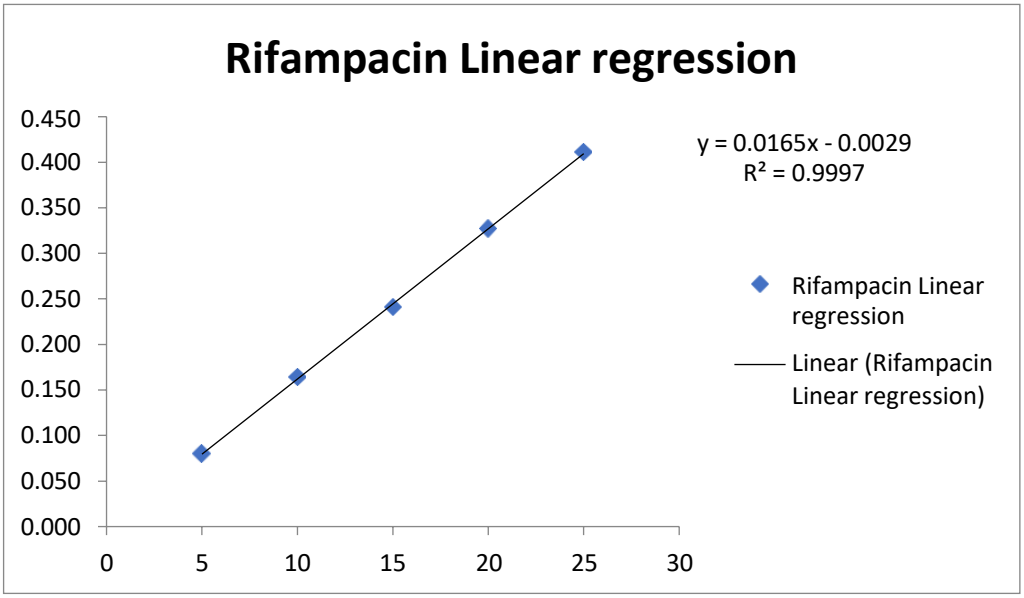

Fig. 11: Calibration curve of Rifampicin

ACCURACY:

SAMPLE (%level)	AMOUNT FOUND(µg/ml)	AMOUNT ADDED(µg/ml)	AMOUNT RECOVERED (%)	Recovery Mean (%)
50	4.98	5.00	99.7	99.8
50	5.00	5.01	99.8	
50	4.99	5.00	99.8	
100	9.91	10.01	99.0	99.3
100	9.95	10.01	99.4	
100	9.94	10.00	99.4	
150	14.98	15.00	99.8	99.4
150	14.88	15.02	99.1	
150	14.91	15.00	99.4	

Table.4: Accuracy of Rifampicin

PRECISION

System Precision

S.NO	CONCENTRATION	ABSORBANCE
1	10	0.165
2	10	0.164
3	10	0.166
4	10	0.165
5	10	0.162
6	10	0.160
	MEAN	0.164
	STDEV	0.002
	%RSD	1.38

Table.5: System precision of Rifampicin

Method Precision

S.NO	CONCENTRATION	ABSORBANCE
1	10	0.163
2	10	0.164
3	10	0.164
4	10	0.166
5	10	0.162
6	10	0.164
	MEAN	0.164
	STDEV	0.001
	%RSD	0.81

Table. 6: Method precision of Rifampicin

Intra-day Precision

S.NO	CONCENTRATION	ABSORBANCE
1	10	0.165
2	10	0.167
3	10	0.166
4	10	0.164
5	10	0.166
6	10	0.165
	MEAN	0.166
	STDEV	0.001
	%RSD	0.63

Table.7: Method precision of Rifampicin

Inter-day Precision

S.NO	CONCENTRATION	ABSORBANCE
1	10	0.164
2	10	0.163
3	10	0.163
4	10	0.162
5	10	0.164
6	10	0.160
	MEAN	0.163
	STDEV	0.002
	%RSD	0.93

Table.8: Inter-day precision of Rifampicin

ROBUSTNESS

S.NO	WAVELENGTH	ABSORBANCE
1	474	0.167
2	475	0.164
3	476	0.168

Table.9: Robustness of Rifampicin

LOD and LOQ

LOD	0.016 (1% from 10ppm)
LOQ	0.053 (3% from 10ppm)

Table.10: LOD and LOQ

SUMMARY REPORT

Characteristics	Acceptance criteria
Accuracy	Recovery 98-102.5 % (Individual)
Precision	%RSD < 2%
Repeatability	%RSD < 2%
Intermediate Precision	%RSD < 2%
LOD	13ppm
LOQ	40ppm
Linearity	Correlation coefficient (R^2) > 0.995
Range	2-10µg/ml

Table.11: Summary report

10. CONCLUSION

A simple spectroscopic method has been developed, validated and degradation studies of rifampicin in bulk and its dosage forms, the method was found to be specific along with qualitative and quantitative determination of rifampicin in pharmaceutical preparations. The proposed method was found to be accurate, economical so can be used for routine analysis of rifampicin in bulk and its dosage form.

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