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"A Complete Review on UV-Visible Spectroscopic Technique "

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ABSTRACT
Ultraviolet spectroscopy is an important and advanced analytical tool in the pharmaceutical industry: a cell with solvent and falls on the photoelectric cell, which converts radiant energy into electrical energy, which is measured by a galvanometer. Ultraviolet-visible spectroscopy is used to obtain the absorption spectra of a compound in solution or as a solid. UV-VIS spectrometers are simple for industry and have been used for 35 years. (200-400nm).UV-Vis provides details based on the absorbance or transmittance of a light beam with different wavelength and the different reactions of samples. Pharmaceutical analysis encompasses the procedures necessary to determine the "identity, potency, quality, and purity" of these compounds. It also includes the analysis of raw materials and intermediates during the manufacturing process of pharmaceuticals. Metal and metal oxide nanoparticles are typically characterized with wavelengths between 200 And 700 nm. The basic working principle of the spectrophotometer covering the UV range is that light of a defined wavelength range passes through use and handling. Both qualitative and quantitative analyzes can make use of it.

Keywords: Uv-vis spectroscopy, photodiode array, photomultiplier, Transducer,Photoemmissive cathode, monochromator.

INTRODUCTION

SPECTROSCOPY-is the study of the properties of matter through its interaction with various types of radiation (mainly electromagnetic radiation) of the electromagnetic spectrum.[1]

SPECTROMETRIC TECHNIQUE-are a large group of analytical methods that are based onatomic and molecular spectroscopy.Spectrometry and spectrometric methods refer to the measurement of the intensity ofradiation with a photoelectric transducer or other types of electronicdevice.[1]

UV-VIS SPECTROSCOPY- UV-Vis 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.[2]

Ultraviolet and visible absorption spectroscopy is the measurement of the absorptionofmonochromatic radiation by solution of chemical substances in the range of 185nm-380nmand 380nm-780nm of the spectrum spectroscopy.[3]

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Uv-visible spectrophotometers [4]

Principle

The principle of UV-Vis spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, producing different spectra. Spectroscopy is based on the interaction between light and matter. When matter absorbs light, it becomes excited and de-excited, resulting in the production of a spectrum.

When matter absorbs ultraviolet radiation, the electrons it contains become excited. This causes them to jump from a ground state (an energy state with an associated amount of energy) to an excited state (an energy state with a relatively large amount of energy associated).It is important to note that the difference in the energies of the electron's ground state and excited state is always equal to the amount of ultraviolet or visible radiation that it absorbs.[5]

Three types of ground state orbitals can be involved.

1) Σ (bonding) molecular orbital
2) Π (bonding) molecular orbital
3) N (bonding) atomic orbital

Additionally, two types of antibonding orbitals can be involved in the transition.

1) Σ* orbital (Sigma star)
2) 2) Π* orbital (Pi star)

There is no n* antibonding orbital since n electrons do not form bonds). Therefore, the following electronic transitions can occur through the absorption of ultraviolet and visible light.

1) Σ to σ*
2) N to σ*
3) N to π*
4) Π to π*

The transitions from σ to σ* and n to σ* take place in the far ultraviolet region or sporadically in the UV region due to their high energy requirements. Range from 180 to 240 nm. Therefore, the saturated groups do not show high absorption in the usual UV range. In contrast to transitions to the π* antibonding orbital, transitions from it to the π* type and to the π* type occur in molecules with unsaturated centers. They require less energy and take place at longer wavelengths. It soon becomes clear that the structure of the molecule controls both the maximum wavelength of absorption and its intensity. If one changes the chemical structure of a molecule, transitions into the antibonding π* orbital can occur in the visible Range, which normally take place in the UV range. Many inorganic compounds in solution also show absorption in the visible region. These include salts

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of elements with an incomplete inner electron shell (mainly transition metals) whose ions are complexed by hydration. Such absorptions result from a charge transfer process in which electrons move from one part of the system to another by the energy of visible light.[6]

Absorbance Laws

There are two absorption laws related to the principle of UV-Vis spectroscopy, namely Beer's law and Lambert's law.

Beer-Lambert Law

The statement of the Beer-Lambert law can be written as follows: When a solution containing a substance that absorbs monochromatic light strikes it, the speed at which the intensity of the beam increases over the length of the the thickness of the beam from solution is directly proportional to the concentration of the absorbing substance in the Solution and is also directly proportional to the intensity of the incident monochromatic radiation.

According to the Beer-Lambert law, the greater the number of absorbing molecules (capable of absorbing light of a given wavelength), the greater the degree of radiation absorption. The Beer-Lambert law is the principle of absorption spectroscopy.[5]

A=a bc

Where,

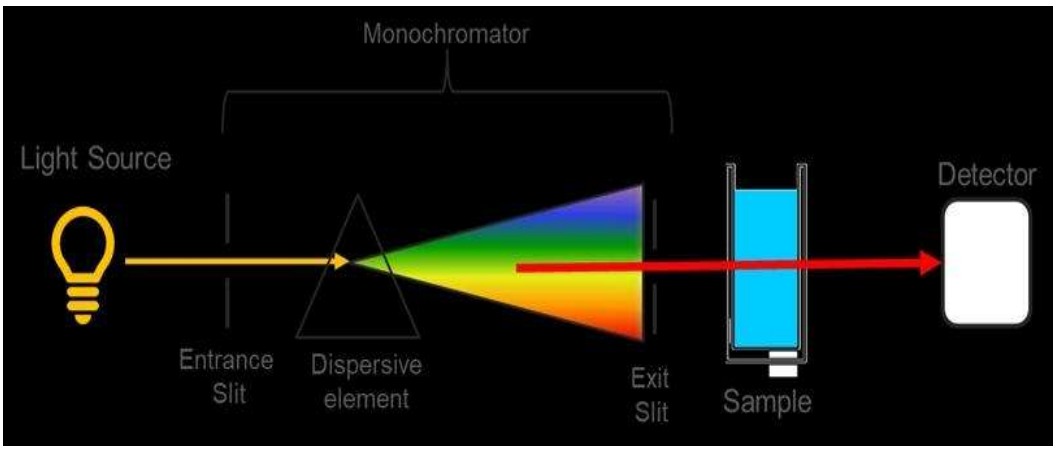
A = Absorbance

a = absorptivity

b = path length

c = concentration.

Instrumentation:



At this schematic diagram of a double-beam UV-Visible Spectrophotometer.[7]

Instruments for measuring the absorption of U.V. or visible radiation are made up of the following components.[6]

1.Source
2 .Monochromator
3.Sample cell
4.Detector
5.Readout system
6.Amplifier
7.Display

1. Sources

a)Sources of UV radiation

It is essential that the electricity of the radiation supply does now no longer Change all at once over its wavelength range. The electric excitation of deuterium or hydrogen at low strain produces a non-stop UV Spectrum. The mechanism for this entails the formation of an excited molecular species, which breaks up to offer atomic species and an ultraviolet photon.

b)Sources of visible radiation

The tungsten filament lamp is commonly used as a visible light source. This type of lamp is used in the wavelength range from 350 to 2500 nm**.** The energy emitted by a tungsten filament lamp is proportional to the fourth power of the operatingvoltage**.** This means that the lamp voltage must be very stable for the output power to be stable. Electronic voltage regulators or constant voltage transformers are used to ensure this stability.

2. Monochromator (Wavelength selector)

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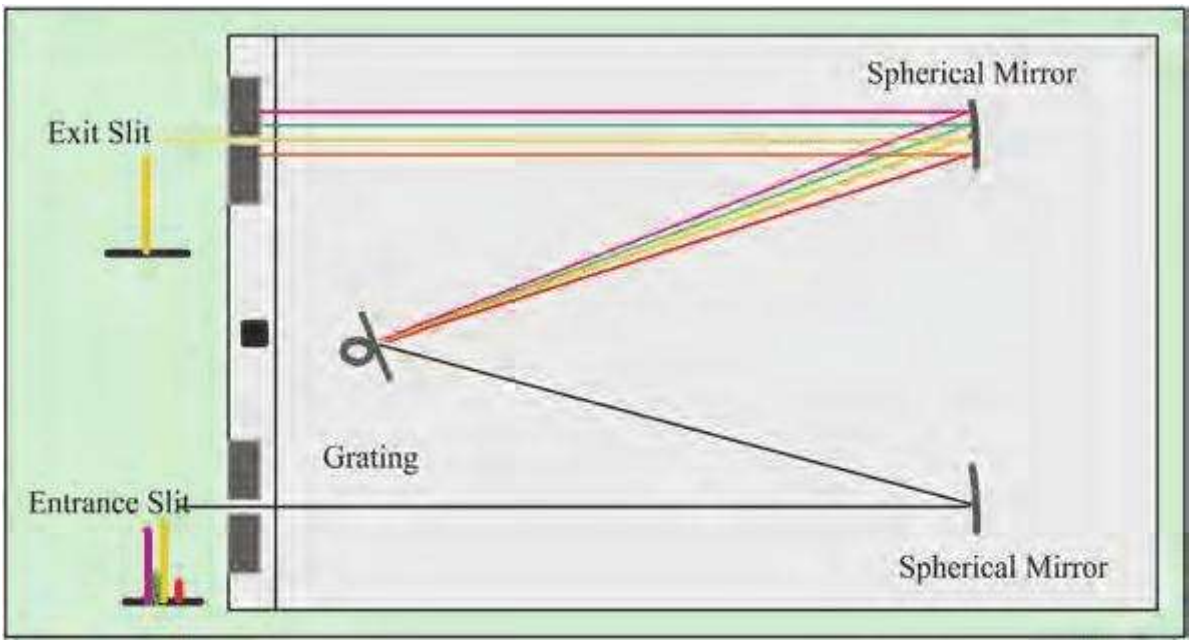
All monochromators include the following componentsStability.

- An entrance slit
- A collimating lens
- A dispersing device (commonly a prism or grating)
- A focusing lens
- An exit slit

Polychromatic radiation (radiation with more than one wavelength) enters the monochromator through the entrance slit. The beam is collimated and then strikes the diffuser at an angle. The beam split into its components Wavelength by the grating or Prism. By moving the exit slit or dispersing elements, only radiation of a certain wavelength leaves the monochromator through the exit slit.

Fig-Turner grating monochromator [8]



3. Sample cell

The sample and reference solution containers must be transparent to the radiation passing through them. Cuvettes made of quartz or quartz glass are required for spectroscopy in the ultraviolet range. These cells are also transparent in the visible range. Silicate glasses can be used to produce cuvettes for use between 350 and 2000 nm.[9]

3. **Detector**

A detector converts a light signal into an electrical signal**.** It should provide a linear response over a wide range with low noise and high sensitivity**.**

1. Photomultiplier Tube Detector
2. Photodiode Detector

- **Photomultiplier Tube Detector**

The photomultiplier tube is a commonly used detector in UV-Vis spectroscopy**.** It consists of a photo-emitting cathode (a cathode that emits electrons when struck by radiationphotons**),** anodes (which emit multiple electrons for each electron struck).A photon of radiation entering the tube strikes the cathodeandcauses several electronstobeemitted**.** These electrons are accelerated toward the first anode (which is 90V more positive than the cathode**).** Electronshit first

Anode causing multiple electrons tobeemitted for each incident electron**.** These electrons are then accelerated toward the second anode to create more electrons**,** which are accelerated toward the anode**.**

Electrons are collected at the anode**.** At this point**,** each original photon has produced 106-107 electrons**.**The resulting current is amplified and measured**.** Photomultipliers are very sensitive to UV-visibleradiation**.** You have quick response times**.** Intense light damages the photomultipliers; They are limited to measuring low-power radiation.

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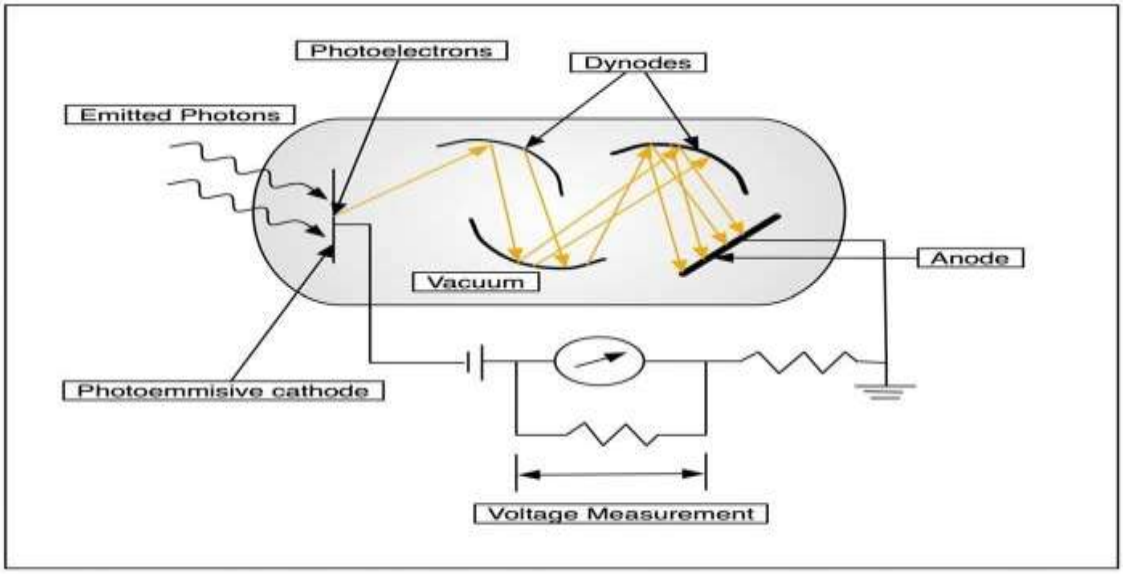

Fig-Photomultiplier tube[10]

- **The Photodiode detector**

This is an example of a multi-channel photon detector. These detectors are able to measure all elements of a scattered beam simultaneously. A photodiode linear array includes many small silicon photodiodes formed on a single silicon chip. There can be between 64 and 4096 sensor elements on a chip, with 1024 photodiodes being the most common. There is also a storage capacitor and a switch for each diode.

Individual diode-capacitor circuits can be scanned sequentially. In use, the photodiode array is placed at the focal plane of the monochromator (after the scattering element) so that the spectrum falls on the diode array. They are useful for recording UV-Vis absorbance spectra of samples that are rapidly passed through a sample flow cell, such as in an HPLC detector.

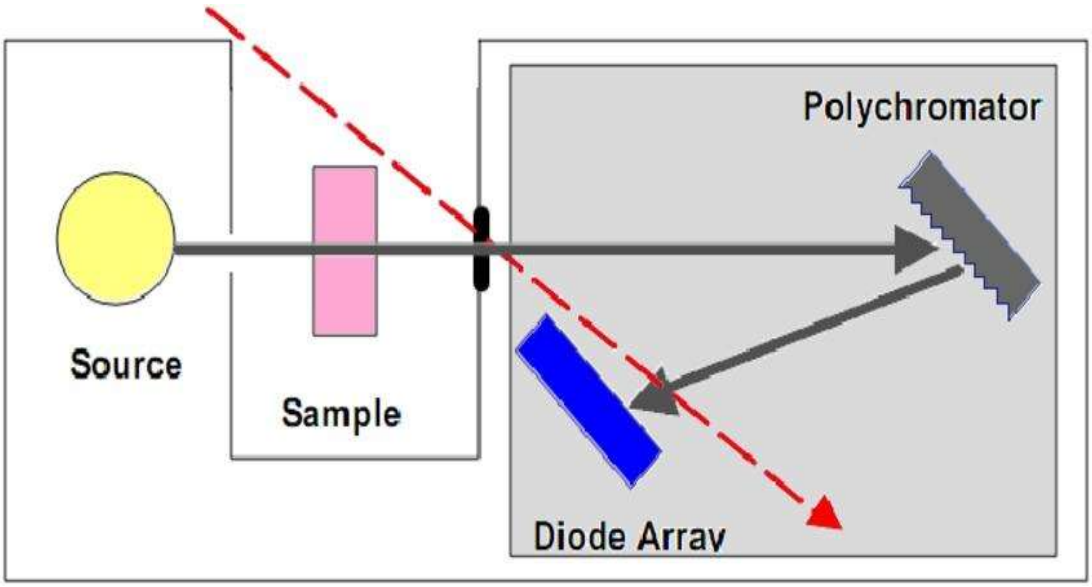

Fig-Photodiode Array[11]

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Charge-Coupled Devices (CCDs)

CCDs are just like diode array detectors, howeverin preference to diodes, they encompass an array .[6]

Colour Absorbed	Colour Absorbed	Absorbed Radiation
Violet	Yellow-green	400-435
Blue.	Yellow	435-480
Green-blue	Orange	480-490
Blue-green	Red	490-500
Green	Purple	500-560
Yellow-green	Violet	560-580
Yellow	Blue	580-595
Orange	Green-blue	595-605
Red	Blue-green	605-750

Table-Relationship between light and absorption and radiation(nm)

TYPES OF UV SPECTROSCOPIC ANALYTICAL TECHNIQUES:

The following are the various UV spectroscopic analysis techniques: Simultaneous Equation Method, Difference Spectrophotometry, Derivative Spectrophotometry, Absorbance Ratio Spectra, Dissipation Ratio Spectra, Response Ratio - Derived Spectra, Absorbance Ratio Q Method, Absorbance Factor Q Method, Dual Wavelength Method, Absorbance Factor Method , multivariate chemometric methods and isosbestic point method.

1. Simultaneous equation method

The simultaneous equations method is useful to determine drugs that are absorbed at λmax by others in the binary or ternary mixture.

Consider

• the absorption values of X at λ1 and λ2, ax1 and ax2, respectively.

• the absorption values of Y at λ1 and λ2, ay1 and ay2, respectively.

• the absorbance of the diluted sample at λ1 and λ2, A1 and A2,

However.

• X has concentration cx and Y has concentration c in the diluted sample. Consequently, the concentration of the mixture is the sum of the individual concentrations of X and Y.

So atλ1A1 = ax1bcx+ay1bcy (1)

In λ2 A2 = ax2bcx+ay2bcy (2)

If the cell is 1 cm, b = 1 Equation 2 becomes

cy= (A-ax2cx)/ay2

Replace the value of cy in Equation (1) with the following

ax1bcx= A-ay1cycx = (A2ay1-A1ay2)/(ax2ay1-ax1ay2) (3)

Similar for cy

cy= (A1ax2-A2ax1)/(ax2ay1-ax1ay2) (4)

The "Glenn" criterion of maximum precision was proposed based on the extinction ratio limiting the relative concentration of the component in the mix. The criteria for this ratio must be outside the range of 0.1 to 2 for an X or Y determination.

Conditions to meet this criterion:

• λmax of two components must be reasonably different

• Two components must not chemically interact, which reduces the initial assumption of absorbance.

Additivity of absorption should always be confirmed when developing a new application of this technique.[13]

2. Difference spectrometry

It Is a spectrophotometric technique for the quantitative determination of an analyte using as reference an equimolar solution of the same analyte but in a different physico-chemical environment due to their different spectral properties. In this spectroscopic technique isolation of an analyte from another component of the mixture or from another UV-active analyte present in the sample from the mixture is achieved. Altered physico-chemical conditions mainly include changes in pH , temperature. The technical requirement is that the analyte to be examined must be present in different chemical forms with different extinction values.The value is calculated as absorbance difference (amplitude difference in maxima and minima) and plotted against the concentration of the tested solution. Both the selectivity and the specificity of the analysis method are improved by difference spectroscopy, since the dosage form difference spectra of the pure drug are superimposed without interfering peaks from the excipients involved in the dosage form being present. Spectroscopy of drug

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substances in dosage forms. Difference spectroscopy with simultaneous determinations of many dosage forms. When determining binary mixtures, the wavelength is chosen such that the contribution of each component is zero at the wavelength where other components have maximum extinction.[13]

The selectivity and precision of spectrophotometric analysis of samples containing interfering absorbers can be significantly improved by using the differential spectrophotometric technique. The essential feature of this method is that the measured value is the absorbance difference (ΔA) between two equimolar solutions of the analyte in different chemical forms, which have different spectral characteristics.[12]

The criteria for applying differential spectrophotometry to the assay of the substance in the presence of another absorbing substance are the following:

1- Reproducible changes can be introduced in the spectrum of the analyte by adding one or more reagents.

2- The absorption of the interfering substances is not altered by this reagent. The simplest and most commonly used technique to change the spectral properties of the analyte is to adjust the pH to using aqueous solutions of acids, bases or buffers.[12]

Besides pharmaceutical assays, differential spectroscopy is also used in biopharmaceutical formulation development to characterize protein structure and study the structure's response to formulation composition. This application is based on the fact that stable protein conformations provide high real-time physical stability and difference spectra are used to characterize and quantify changes in protein structure.[13]

3.Derivative spectrophotometry

It Is a spectroscopic technique that differentiates spectra mainly in IR, UV-Vis absorption and fluorescence spectrometry.[19]

Derivative spectroscopy, as the name suggests, involves derivatives of the zero-order absorption, or simple absorption spectrum, with respect to wavelength. Derivative spectroscopy follows the principle of additivity, and absorbance also depends on concentration. Nowadays the derived spectra are obtained directly from spectrophotometers equipped with advanced software like As UV probe.This software eliminates the need for additional mathematical processing or changes to instrument parameters. Historically, derived spectra have been generated by optical methods (wavelength modulation technique) and electrical methods (analog resistance-capacitance device). Later, in 1974, a new mathematical technique called the Golay-Savitzky method was introduced, which became commercially popular and is now part of software.
Derivative spectroscopy is used to analyze a wide variety and complex origins such as: B. Pharmaceutical dosage forms, metal-containing inorganic samples, biological samples and food samples.

Derivative spectroscopy offers the following advantages:1.Resolving of overlapping peaks from complex samples such as ternary mixing.
2.Improving spectral quality by eliminating baseline shift and scattering.
3.Direct UV analysis of samples of complex origin without any treatment chemical pre-sample of biological origin.
4.Allow analysis of a sample with lower impurity profiling.

Derivative spectra increase the information content of fundamental zero-order spectra and are comparatively complicated. Derivative spectroscopy measurementtechnique: zero-crossing technique and peak-through technique. Is based on the fact that in the derived spectra the absorbance of one component shows no absorbance, in such a case the absorbance of the sample is equal to that of the other Components in the sample, which can be used to find their concentration .

In addition to pharmaceutical assays, derivative spectroscopy is also used in clinical studies, e.g. B. the quantitative determination of diazepam in human blood plasma without separating the drug from the biological matrix.

In addition, derivative spectroscopy was used for stability study purposes. Second derivative UV spectrophotometry, butamirate citrate and formoterol fumarate were determined by measuring the maximum amplitude at 260.4 and 261.8 nm, respectivelyUnaffected by its degradation product. With the derivatization of the spectra, the signal-to-noise ratio increases.

In addition, the reproducibility obtained with derivative spectroscopy is very poor. Anatov et al. reported the method of stepwise filtering method to improve the signal-to-noise ratio.Brown et al. reported a derived reprocessing method that achieved drift noise reduction for multivariate spectral data. Aside from the above methodology,

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few variants of derivative spectroscopy have been reported worldwide. The wavelet transform technique has also been usedsuccessfully for derivative spectroscopy.[13]

4.Ratio Derivative spectroscopy

The spectrophotometric determination of two or more compounds in the same sample without prior separation is required. In the last decade, ratio-derived spectroscopy has emerged as a good tool to accomplish this purpose, based on the work of Salinas et al, resolving binary mixtures when the spectra of the components overlap. It allows using the wavelength of the highest Value of analytical signals with different peaks and valleys, which allows the determination of an analyte in the presence of other compounds and excipients that may interfere with the analysis.

The procedure includes the following steps:

• Acquisition of the spectra of the mixture of the samples to be examined.
• Split the mix spectra by a standard split spectrum.
•Directly indicates the concentration of one of the components in the mixture.[13]

5.Successive ratio derivative spectra method

This method is used to determine drugs in the ternary mixture without information about the concentration ratio of drugs in the mixture.[13]

6.Q-absorbance ratio method

The method is only applicable if Beers' law is followed for a specific combination of drugs. This method is based on the fact that the absorption ratio at any two wavelengths for a substance that obeys Beer's law is a constant value independent of concentration and path length. This constant is called the "Hufner ratio" or Q-value.

The method involves measuring the absorbance at two wavelengths, one of which is the maximum λ of one of the components (λ2) and the other Wavelength wave with equal absorbance of the two components (λ1), called the isoabsorption point.[12]

7.ABSORPTIVITY FACTOR METHOD

This method is a modification of the classic absorption method. In order to perform this method of spectroscopic analysis, the following conditions must be met.

• This method is applicable to the binary mixture.
• There should be a greater difference in the absorption capacity of both drugs.
• There should be no isoabsorption point.

Unlike the isoabsorption point method, the crossover does not occur at the same concentration, but it can occur at different drug concentrations. The ratio found is called the absorption factor (F) and the crossing point is called the absorption factor.

Ax = axBcx And
Ay = ayBcy

At the crossing point of the same absorbency with different active ingredient concentrations.

Ax= Ay
AxBcx = ayBcy
AxCx = ay
CyAx/ay = cy/cx = F
Ax/ay=F
Am = Ax+Ay = axBcx+ayBcy
Where b = 1
Am = axCx+ayCy
Ax = Fay
Am = FayCx+ayCy = ay (Fcx+cy)
Similarly, Am = ax (Fcy+cx)

Concentration of y drug may be decided the use of linear regression Equation among its awareness and absorbance at its wavelength Of most absorption in which interference because of different tablets is Null. Later from the awareness of y the awareness of x may be Determined the use of following equation.

Am = ay (Fcx+cy) = ax (Fcy+cx)
Ay (Fcx+cy) = ax (Fcy+cx)
Cx = [(Fcx+cy)-cy]/F [13]

8.Absorption factor method

The absorption factor method is another spectroscopic method applicable to the analysis of binary mixtures. In cases where overlapping spectra were observed, interference was observed in the absorbance maxima of one component while no interference was observed in the absorbance maxima of another compound.

Imagine a mixture of x and y that have a maximum wavelength at λx and Λy. Y shows interference at λx, but x shows no interference at λy. Range from 200 to 400 nm.The average absorbance factor value was calculated using the following equation:

(AY1λY1/AY2λY2)a+(AY1λY1/AY2λY2)b+(AY 1λY1/AY2λY2)c+(AY1λY1/AY2λY2)d =(AY1λY1/AY2λY2)avg

(AY1λY1/AY2λY2)Avg is the average value of the absorbance factor.

Since y alone in λ represents the extinction at this wavelength, the concentration of Y can be determined from which the concentration of x can be calculated according to the following formula:

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Ax Λx = A(X+Y)λ1-A(X+Y)λ2*(AY1λY1/AY2 λY2)avg
The equation above is the basis of the absorption factor method.[13]

9.MULTIVARIATE CHEMOMETRIC METHOD

It Is the processing of analytical data using mathematical techniques. Because of this correlation of physical properties with analytical data, it can also be defined as multiple measurements on the same sample. After chemometric methods, it is often better to measure many non-selective signals and then combine them in a multivariate model, where several variables are considered simultaneously.

Multivariate methods include:

1. Multiple linear regression (MLR) methods
a. Classical least squares or (K-matrix)
b. Inverse least squares or (P-matrix)
2. **Factor-based methods**
a. Principal component regression (PCR)
b. Partial least squares (PLS).[13]

10.Isobestic point method

This technique can only be used when the spectra of the same concentration of the two drugs under study intersect at a point called the isosbestic or isoabsorption point. At the isosbestic point, both drugs have equal absorbencies and their mixture acts as a single component. And gives the same absorbance as the pure drug. The absorbance value at the isosbestic point (Aiso) was determined and the total concentration of both active ingredients was calculated. Since the concentration of one of them in this mixture can be measured by another spectroscopic (DS) method, the concentration of the other could be calculated by subtraction.
A linear correlation between the absorbance values and the corresponding drug concentrations was obtained. Imagine you have a mixture of two drugs x and y. The absorbance of any drug at any wavelength (λ) can be calculated from the equation.
A = abc
Therefore, for drug x: Ax= axbCx and
For drug y: Ay = aybCy
Where ax and ay are the absorptions of x and y, respectively; Cx and Cy are the concentrations of x and y, respectively; and are the absorbances when the path length (b) is 1 cm and the concentration is 1 g/100 ml for X and y, respectively.
If Cx = Cy, and ax = ay, this λ is called the isosbestic point, and
At this λ Ax = Ay
For a mixture of both drugs, the absorbance at this λ can be calculated from the equation
A = axCx+ayCy
A = ax (Cx+Cy)
Where A is the absorbance of your mixture at the isosbestic point and the concentrations of drugs x and y respectively in the mixture and CTM is the concentration of your mixture. From this we can conclude
A = axCTM
Thus the concentration of the second drug can be calculated by subtraction given the total concentration of both drugs when the concentration of one of them can be determined separately by another method.
This method is used to analyze the ternary mixture of chloramphenicol, dexamethasone sodium phosphate (DXM) and tetrizoline hydrochloride in eye drops.[13]

Calibration of UV visible spectroscopy

1.Limit of stray light

Stray light can be detected at a specific wavelength with a suitable filter or solution. For example, the absorbance of a 1.2% (w/v) potassium chloride solution in a 1 cm cuvette should be greater than 2.0 at approximately 200 nm compared to water as the reference liquid.[3]
Acceptance Criteria: Absorbance is greater than 2.
A UV and visible spectrometer, suitable for measurements in the ultraviolet and visible parts of the spectrum, consists of an optical system capable of producing monochromatic light in the range of 200-800 nm and a device used to measure the absorption is suitable.[14]

2.Resolutionpower

When specified in a monograph, record the spectrum of a 0.02% v/v solution of toluene in UV-hexane. The ratio of maximum absorbance at about 269 nm is not less than 1.5 unless otherwise noted in the monograph.[3]

3.Control of wavelength

Check the wavelength scale against the absorption maxima of the holmium percolate solution, the hydrogen-deuterium discharge lamp line, or the mercury vapor lines shown below. The permissible tolerance is ±1 nm for the range from 200 nm to 400 nm and ±3 nm for the range from 400 nm to 600 nm.[3]

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241.15 nm (Ho)	404.66 nm (Hg)
253.70 nm (Hg)	435.83 nm (Hg)
287.15 nm (Ho)	486.00 nm (Db)
302.25 nm (Hg)	486.10 nm (Hb)
313.16 nm (Hg)	536.30 nm (Ho)
334.15 nm (Hg)	546.07 nm (Hg)
361.50 nm (Ho)	576.96 nm (Hg)
365.48 nm (Hg)	579.07 nm (Hg)

Fig-Wavelength range [15]

4.Control of absorbance

Check the wavelength scale against the absorption maxima of the holmium percolate solution, the hydrogen-deuterium discharge lamp line, or the mercury vapor lines shown below. The permissible tolerance is ±1 nm for the range from 200 nm to 400 nm and ±3 nm for the range from 400 nm to 600 nm.[3]

Wavelength (nm)	Specific absorbance	Maximum Tolerance
235	124.5	122.9 to 126.2
257	144.5	142.8 to 146.2
313	48.6	47.0 to 50.3
350	107.3	105.6 to 109.0
430	15.9	15.7 to 16.1

Fig-Wavelength against absorption maxima[15]

5.Spectral of slit width

If the absorbance is measured at an absorption maximum, the width of the spectral gap should be small compared to half the width of the absorption band; otherwise an erroneously low absorbance will be measured. so further reduction does not result in a higher absorbance value.[3]

6.Cell

When measuring the absorbance of a solution at a specific wavelength, the absorbance of the reference cuvettes and their contents should not exceed 0.4 and preferably be less than 0.2 when the reference is measured in air at the same wavelength. The solvent in the reference cuvette must be from the same batch as that used to prepare the solution and must be non-fluorescent at the measurement wavelength.

Ethanol
Methanol
cyclohexane

used as a solvent must have an absorbance measured in a 1 cm cuvette at around 254 nm with respect to water and not exceeding 0.10.[3]

Solvents Cut –off Wavelength[16]

The UV limit is defined as the wavelength at which the solvent also absorbs light (UV or visible). Measurements should be avoided in this area. It is difficult to determine whether the absorbance is due to your analyte or your solvent. Therefore, when choosing a solvent, you should be

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aware of its absorption limit and where the compound under study is likely to absorb. If they are close, choose a different solvent.

The table below shows an example of solvent cut offs.

Table 1: Commonly used solvent and Cut –off Wavelength.

Solvent	Cut-off (nm)
Is-octane	202
Ethyl alcohol	205
Cyclohexane	200
Acetone	325
Tetrachloroethylene	290
Benzene	280
Carbon tetrachloride	265
Water	180

ADVANTAGES[17]

1) The core advantage is the accuracy of the UV-VIS spectrophotometer.
2) The UV-VIS spectrometer is easy to handling and use
3) Provide robust operation.
4) UV-VIS spectroscopy is simple to operate.
5) Cost effective instrument.
6) Cover the entire of ultraviolet and visible.
7) It can be utilized in the qualitative and quantitative analysis.
8) The Derivative graph can be obtained by UV-VIS spectrophotometer.
9) It can be used in the degradation study of drug.
10) Only possible for the analytes which have a chromophore.

DISADVANTAGES[17]

1)Only molecules with chromophores are analyzed.
2) Absorbance results can be affected by pH, temperature, impurities and impurities.
3) Only liquid samples can be analyzed.
4) Takes time to prepare. use.
5) Handling of the cuvette can affect the sample reading.

APPLICATIONS[17]

UV –vis spectroscopy has many different application

1. Detection of impurities
2. Structural elucidation of organic compounds
3. Quantitative analysis
4. Qualitative analysis
5. Chemical analysis
6. Quantitative analysis of pharmaceutical substance
7. Dissociation constant of acids and bases
8. Molecular weight determination
9. As HPLC detector
10. Deviations from the Beer-Lambert law

Companies

- Avantes UV-visible spectrometers.
- Analytik Jena spectrometers.
- Avantes spectrometers.
- Oxford Instruments spectrometers.
- SAFAS spectrophotometers.
- SAFAS UV-visible spectrophotometers.

REFERENCE

[1]. https://www.researchgate.net/publication/337674152_UV-VISIBLE_SPECTROMETRY
[2]. https://www.technologynetworks.com/analysis/articles/uv-vis-spectroscopy-principle-strengths-and-limitations-and-applications-349865
[3]. Indian pharmacopoeia 2018,volume 1,8th edition effective from 1st january 2018,page no183-184
[4]. https://radiantinstruments.com.au/product/double-beam-spectrophotometer-2/
[5]. https://byjus.com/chemistry/principle-of-uv-visible-spectroscopy
[6]. Govinda Verma and Dr.Manish Mishra.World journal of pharmaceutical research.Development and optimization of UV spectroscopy.Volume 7,Issue 11,2018.
[7]. https://www.smacgigworld.com/blog/applications-of-uv-vis-spectrophotometry.php
[8]. https://www.researchgate.net/figure/Diagram-of-a-discrete-light-diffraction-in-a-Czerny-Turner-monochromator_fig5_221922577

[9]. Modern Chemical Techniques, Ultraviolet/visible Spectroscopy, The Royal Society of Chemistry, 102,103.
[10]. https://www.researchgate.net/figure/Photomultiplier-tube-PMT-schematic-The-emitted-photons-strike-the-photocathode-Due_fig4_34709753
[11]. https://www.researchgate.net/figure/Schematic-of-a-photodiode-array-spectrophotometer_fig3_221915678
[12]. Ref-Amira H. Kamal*, Samah F. El-Malla and Sherin F. Hammad.European journal of pharmaceutical and medical research.A review on UV spectrophotometric method for simultaneous multicomponent analysis,2016,page no 348-356.
[13]. Dipali M.Atole, Hrishikesh H.Rajput.Asian journal of pharmaceutical and clinical research. Ultraviolet spectroscopy and it's pharmaceutical application- A brief review.Vol 11 , Issue 2,2018.
[14]. Shital S.Patil,Poonam A.Salunke,et al.A textbook of advanced instrumentation techniques.Pee vee publication.
[15]. https://www.pharmaguideline.com/2011/08/ultraviolet-and-visible-absorption.html?m=1
[16]. Ganesh shinde,godage R.K,Dr.R.S.Jadhav,Barhate Manoj,Bhagwat Research journal of science and technology.A review on advances in UV Spectroscopy.Vol-12,Issue 1,2020.
[17]. Diya Patel,Diya Panchal,Kunj Patel,Prof. Mitali Dalwadi,Dr. UmeshUpadhyay.International Journal of Creative Research Thoughts (IJCRT).A Review on UV Visible Spectroscopy.Volume 10, Issue 10 October 2022,page no-b410.
[18]. https://www.medicalexpo.com/medical-manufacturer/uv-visible-spectrometer-31553.html
[19]. Ref -Vivekkumar K. Redasani,Priyanka R. Patel,Divya Y. Marathe,Suraj R. Chaudhari1,Atul A. Shirkhedkar,Sanjay J. Surana.Journal of the Chilean Chemical Society. A REVIEW ON DERIVATIVE UV-SPECTROPHOTOMETRY ANALYSIS OF DRUGS IN PHARMACEUTICAL FORMULATIONS AND BIOLOGICAL SAMPLES REVIEW. Vol.63 no.3 Concepcion 2018