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A BRIEF REVIEW ON PRINCIPLE, INSTRUMENTATION AND ANALYTICAL PARAMETER OF UV SPECTROSCOPY

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ABSTRACT

The processes required to ascertain the "identification, strength, quality, and purity" of such chemicals are included in the pharmaceutical analysis. It also covers the study of starting materials and intermediates used in the production of pharmaceuticals. Spectrophotometry is typically chosen by small-scale enterprises because the equipment is less expensive and has fewer maintenance issues. The analysis approach is based on evaluating the monochromatic light's absorption by colorless substances in the near ultraviolet path of the spectrum (200-380nm). The Beer-Lambert law, Beer's which states that a solution's absorbance is directly proportionate to its analyte concentration forms the basis for photometric methods of analysis. The essential working principle of a UV spectrophotometer is that light with a specific wavelength range passes through a solvent-filled cell and strikes a photoelectric cell, which converts the radiant energy into electrical energy that can be measured by a galvanometer. To get the absorbance spectra of a substance in solution or as a solid, ultraviolet-visible spectroscopy is used.

KEYWORDS: Beer-Lambert law, analytical chemistry, and UV-visible spectroscopy.

INTRODUCTION

Ultraviolet Spectroscopy is A method of optical spectroscopy that utilizes light in the visible, ultraviolet, and near infrared wavelengths known as ultraviolet (UV) spectroscopy. According to the Beer-Lambert equation, the concentration of the absorbing species in the solution and the Path length is exactly proportional to the absorbance of a solution. Thus, the concentration of the absorber in a solution can be determined using UV/VIS spectroscopy for a constant Path length. It is important to understand how quickly absorbance changes with concentration.

ANALYTICAL CHEMISTRY

The science of analytical chemistry looks for constantly better ways to determine the chemical makeup of both natural and manmade materials. The full picture (composition) of a substance at the chemical scale is known as its chemical composition, and it includes geometric aspects like species distributions and molecular morphologies, as well as one-dimensional parameters like percent composition and species identity (1-3). Analyzing samples effectively and efficiently involves knowledge of:

1. The chemistry that can occur in a sample.
2. Techniques for handling samples and conducting analyses for several issues (the tools-of-the trade).

3. The method's accuracy and precision.
4. Accurate data analysis and documentation.

The following are the key steps in an analytical process: (Figure 1).

The processes required to ascertain the "identification, strength, quality, and purity" of such chemicals are included in the pharmaceutical analysis. It also covers the study of starting materials and intermediates used in the production of pharmaceuticals.

TYPES OF ANALYTICAL CHEMISTRY QUALITATIVE ANALYSIS

Establishing the existence of a specific element or inorganic compound in a sample is the aim of qualitative inorganic analysis.

Establishing the existence of a specific functional group or organic molecule in a sample is the goal of qualitative organic analysis.

QUANTITATIVE ANALYSIS

The goal of quantitative analysis is to determine the concentration of a specific element (or compound) in a sample.

Methods for identifying analyses

1. Physical properties include mass, color, refractive index, and thermal conductivity.
2. Using Electromagnetic Radiation (Spectroscopy) Absorption, Emission, and Scattering.
3. Using electric charge, mass spectrometry, and electrochemistry.

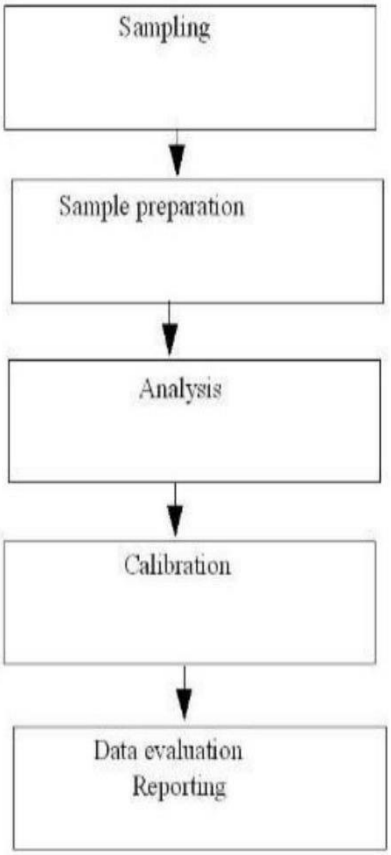

figure 1. steps in analytical cycle

ULTRAVIOLET ABSORPTION SPECTROSCOPY

Spectrophotometry is typically chosen, particularly by small-scale enterprises, as the equipment is less expensive and has fewer maintenance issues. The analysis approach is based on evaluating the monochromatic light's absorption by colorless substances in the near ultraviolet path of the spectrum (200-380nm). The foundation for the photometric analysis techniques is According to the Beer-Lambert law, Beer's solution's

absorbance is inversely proportional to its analyte concentration. The spectrophotometer's basic mode of operation for the UV region involves light of a specific wavelength passing through a cell filled with solvent before hitting a photoelectric cell, which converts the radiant energy into electrical energy that can be measured by a galvanometer.

PRINCIPLE OF UV SPECTROSCOPY

The Principle of UV-Visible Spectroscopy is the idea that chemical compounds can absorb ultraviolet or visible light, creating distinct spectra in the process. The basis of spectroscopy is the interaction of light and matter. A spectrum is created when the substance absorbs the light through excitation and deexcitation processes.

The electrons existing in matter experience excitation when it absorbs UV energy. As a result, they move abruptly from their ground state (an energy condition with a negligible amount of energy) to their excited state (an energy state with a relatively large amount of energy associated with it). It is significant to remember that the amount of ultraviolet or visible radiation absorbed by an electron is always equal to the energy difference between its ground state and excited state.

The Beer-Lambert Law is the basic principle of absorbance spectroscopy. For a single wavelength, the following formulas are used: A = absorbance (unit less, commonly shown as arb. units or arbitrary units), a = molar absorptivity (M^{-1} cm^{-1}) b = path length of the cuvette or sample container (usually 1 cm), and c= concentration of the solution (M).

$A = a\ b\ c$

Where,

A: Absorbance a: Absorptivity b: path length c: Concentration

C: A /a b

ELECTRONIC TRANSITION

When radiation induces an electronic transition within a molecule or ion, the molecule or ion will display absorption in the visible or ultraviolet area. As a result, when a sample absorbs light in the ultraviolet or visible range, the molecules inside the sample experience a change in their electronic state. Electrons will be promoted from their ground state orbitals to higher energy excited state orbitals or antibonding orbitals by the light's energy. (14)

By absorbing ultraviolet and visible light, the following electronic transitions can take place:

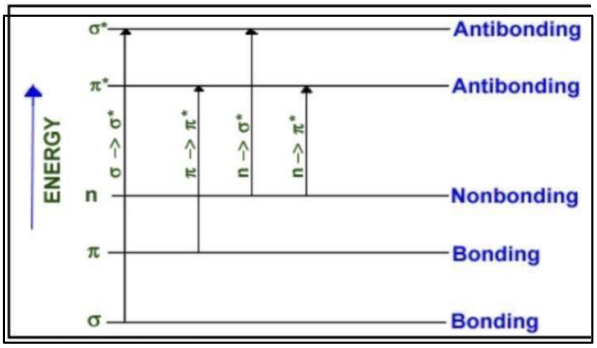

figure 2 electronic transition

1. **σ to σ* Transitions:** An electron is excited to the relevant antibonding orbital from a bonding orbital. There is a lot of energy needed. For example, the absorbance of methane, which only possesses C-H bonds and can only undergo σ to σ* transitions, peaks at 125 nm. In normal UV-Vis spectra, absorption maxima caused by to σ to σ* transitions are not visible (200 -700 nm) (15)

2. **n to σ* transitions:** These can occur in saturated compounds that have atoms with lone pairs (nonbonding electrons). Usually, these transfers need less energy than n to σ*. Transitions Light with a wavelength between 150 and 250 nm can start them.
3. **n to π* and π to π* Transitions:** The majority of organic compound absorption spectroscopy relies on n or π electron transitions to π*the excited state. This is due to the fact that the peaks of these transitions' absorption occur in a spectrum region that is appropriate for experiments (200 - 700 nm). In order to supply the electrons for these transitions, the molecule must have an unsaturated group. Molar absorptivity from π to π* transitions generally range from 1000 to 10,000 L mol-1 cm-1. Molar absorptivity from to π to π* transitions is quite low and range from 10 to 100 L mol-1 cm-1. (16)

INSTRUMENTATION

The following elements make up instruments used to measure the absorption of ultraviolet or visible light:

1. Source (UV and visible)
2. Monochromator or filter
3. Sample cells or containers
4. Detector

1. RADIATION SOURCE

It is crucial that the radiation source's power does not fluctuate drastically along the spectrum of its wavelengths. A continuous UV spectrum is produced when deuterium or hydrogen is electrically excited under low pressure. An excited molecular species is formed, and when it splits into two atomic species and an ultraviolet photon, this is the mechanism.

Lamps made of deuterium and hydrogen both produce radiation between 160 and 375 nm.

These lamps must have quartz cuvettes and windows since glass absorb light with wavelengths shorter than 350 nm. The following are many sources of UV radiation.

1. Deuterium lamp
2. Hydrogen lamp
3. Tungsten lamp
4. Xenon discharge lamp
5. Mercury arc lamp
6. Various Visible radiation sources are as follows
7. Tungsten lamp
8. Mercury vapor lamp
9. Carbonone lamp

2. FILTERS OR MONOCHROMATORS

All monochromators contain the following component parts;

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

Through the entrance slit, multi-wavelength polychromatic light enters the monochromator. Following collimation, the beam is directed at an angle toward the dispersion component. The grating or prism separates the beam's wavelengths into their individual components. Only radiation of a specific wavelength exits the monochromator through the exit slit when the dispersing element or the exit slit is moved.

3. SAMPLE CELLS OR CONTAINERS

There are numerous UV area sample cells available. The path length, shape, and size, the transmission properties at the chosen wavelength, and the relative cost all factor into the selection of the sample cell.

The sample-holding cell needs to be transparent to the wavelength range being measured. For UV spectroscopy, quartz or fused silica cuvettes are necessary. For use between 350 and 2000 nm, cuvettes can be made from silicate glasses. In most cases, a cell is 1 cm thick. Cells might be cylindrical with flat ends or rectangular in shape.

4. DETECTORS

Three different types of photosensitive devices are used to detect radiation.

a) Photovoltaic or barrier-layer cells
b) photo emissive tubes or phototubes
c) Photomultipliers Tube

Barrier layer or photronic cell are other names for photovoltaic cells. It includes An electrode is a metallic base plate made of iron or aluminium. A thin layer of a semiconductor metal, such as selenium, is deposited on the surface. Then A very thin film of silver or gold is present on the surface of selenium, which serves as an additional collector tube. Electrons are produced when radiation strikes the surface of selenium. formed at the surface of the selenium and silver, and the silver absorbs the electrons. Between the silver surface and the cell's foundation, this accumulation at the silver surface generates an electric voltage difference.

Phototube is also known as a photo emissive cell. A glass bulb that has been emptied makes up a phototube. It contains a light-sensitive cathode. A light-sensitive coating, such as potassium oxide or silver oxide, is applied to the cathode's inner surface. Photoelectrons are released when radiation strikes a cathode. By using an anode, they are gathered. These are then sent back via an external circuit. Additionally, this procedure amplifies the current and records it.

The Photomultiplier tube is A popular detector in UV spectroscopy. It is made up of an anode, a photo emissive cathode (a cathode that emits electrons when exposed to photons of light), many dynos (which emit several electrons for each photon of light that strikes them), and a photo emissive cathode. When a photon of radiation enters the tube and hits the cathode, it releases a number of electrons. These electrons are propelled in the direction of the first dynode (which is 90V more positive than the cathode). For each electron that strikes the initial dynode, several electrons are released into the environment. The second dynode receives these electrons, which are then accelerated there to create new electrons that are accelerated to the third dynode and so on. The anode is where the electrons are eventually collected. Each initial photon has now generated 106–107 electrons. Amplification and measurement are done on the generated current. UV and visible light are extremely sensitive to photomultipliers. Their reaction times are quick. Photomultipliers can only be used to measure low-power radiation since intense light damages them.

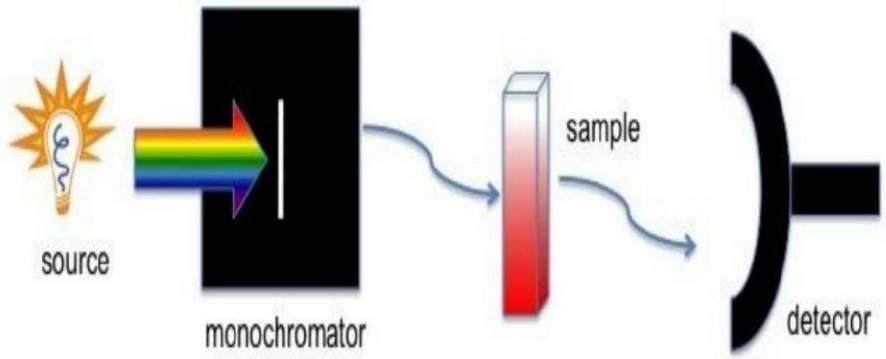

figure 3 instrumentation of uv – visible spectroscopy

TYPES OF UV – VISIBLE SPECTROPHOTOMETRY SINGLE BEAM SPECTROPHOTOMETER:

A single-beam spectrophotometer is a type of analytical instrument that allows all the light waves that are emitted by the light source to travel through the sample. As a result, measurements are made according to how much light is present before and after it passes through the sample. Compared to double-beam spectrophotometers, these single-beam instruments are smaller and have less complicated optical designs. Additionally, these devices cost less. Since it uses a non-split light beam, the sensitivity of detection of the light beam after it has passed through the sample is high (therefore, high energy exists throughout). There are single-beam spectrophotometers for use in visible and ultraviolet wavelength analysis. By measuring the amount of light, a single-beam spectrophotometer determines the concentration of an analyte in a sample.

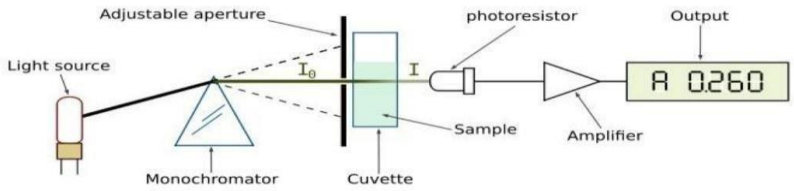

figure 4 single beam uv - spectrophotometer

Double Beam Spectrophotometer:

A double-beam spectrophotometer divides the light emitted by a light source into two portions, making it a useful analytical instrument. While the other fraction travels through the sample, one fraction serves as the reference (the reference beam) (sample beam). As a result, the sample is not penetrated by the reference beam. The sample beam can gauge the sample's absorbance. The absorption can be measured using the reference beam (the sample beam can be compared with the reference beam). As a result, the absorption is the ratio of the sample beam to a reference beam after the sample has been processed. The monochromator in a spectrophotometer separates the desired wavelengths from a light beam.

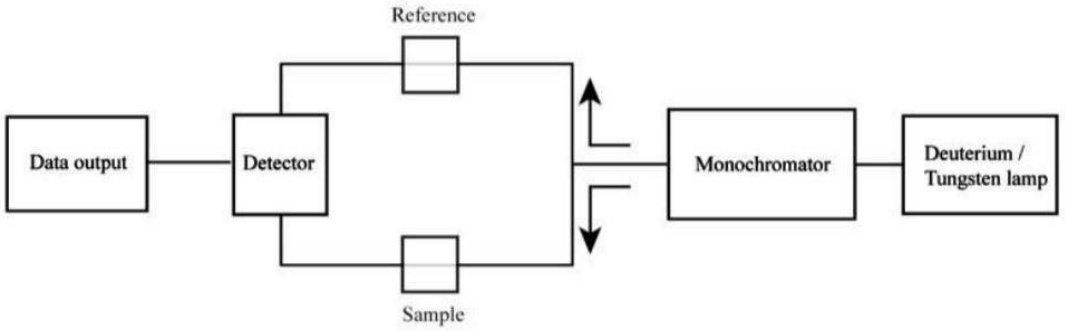

figure 5 double beam spectrophotometer

GENERAL RULE FOR PERFORMING UV SPECTROSCOPY

1. The Drug Must Be Totally Soluble in the Solvent
2. The drug must absorb UV visible light or radiation.
3. Drug and solvent must not interact
4. Cut-off wavelength must be taken into account when choosing a solvent.
5. At the measurement wavelength, the solvent must be UV transparent.
6. Stoppered cells should be used when employing volatile solvents to prevent evaporation from changing the concentration of the solution.
7. There must be linear absorption.
8. The Beer-Lambert law only applies to dilute solutions.
9. The calibration curve has to be straight.
10. For binary drugs, the two drugs must be soluble in the same solvent.
11. There must be no contamination in the drug or solvent. (20)

CUT-OFF OF SOLVENTS WAVELENGTH

The UV cut-off is the wavelength at which a solvent can also absorb light (UV or Visible). The measurement ought to be avoided there. It is challenging to tell whether the absorbance is caused by your solvent or your analyte. (17) Therefore, while selecting a solvent, be conscious of where the substance under examination is believed to absorb as well as the solvent's absorbance cut-off. Consider using a different solvent if they are near. Solvent cut-offs are illustrated in the following table. (18)

table 1: commonly used solvent and cut-off wavelengt(17)

Solvent	Cut-off (nm)
Acetone	325
Benzene	280
Water	180

Carbon Tetrachloride	290
Is-Octane	202
Cyclohexane	200
Ethyl Alcohol	205
Tetrachloroethylene	290

ANALYTICAL PARAMETER

A process of confirming or demonstrating through laboratory tests that a method, system, or analyst gives correct and reproducible results for an intended analytical application within a known and established range is known as validation.

METHOD VALIDATION

To make sure that an analytical approach is precise, definite, repeatable, and robust over the defined range that an analyte will be evaluated, method validation is conducted. It is sometimes referred to as "the process of giving recorded evidence that the method achieves what it is meant to do" and the parameters are represented in the figure. Method validation ensures reliability throughout normal use.

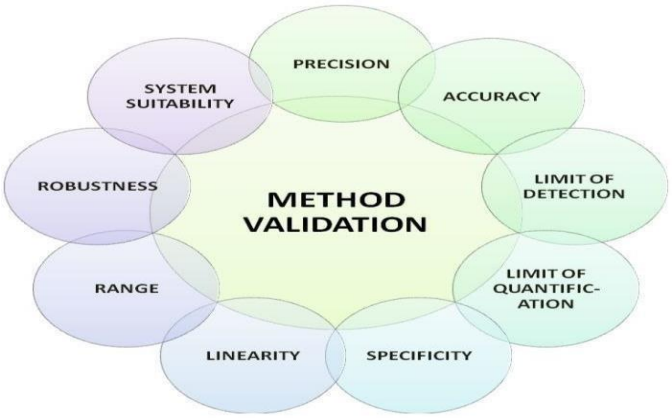

Figure 6 Method Validation

PRECISION

Precision is a measure of how repeatable an analytical procedure is when used routinely. It is typically stated as the percent relative standard deviation for a large enough sample size to be statistically significant. The ICH states that three different levels of accuracy should be used: repeatability, intermediate precision, and reproducibility. Repeatability is the outcome of the approach being used repeatedly under the same circumstances over a short period of time (interassay precision). It should be determined from a minimum of six determinations at 100% of the test or target concentration or from a minimum of nine determinations covering the procedure's stated range (for example, three levels, three repetitions each). The results of internal lab differences brought on by chance occurrences such as various days, analysts, equipment, etc. are referred to as intermediate precision. Experimental design should be used to determine intermediate precision so that the impacts (if any) of the different variables can be monitored. Results of cross-laboratory research are said to as reproducible. The standard deviation, relative standard deviation, coefficient of variation, and confidence interval are all important pieces of supporting documentation for precision investigations [10].

ACCURACY

Accuracy is a measure of how exact a method of analysis is, or how closely the value discovered agrees with a value that is acknowledged as either a conventional, actual value, or a reference value. It is determined by spiking samples in a blind trial and measuring the percentage of analyte recovered by assay. Accuracy measurements for the drug substance assay are acquired by contrasting the findings with the examination of standard reference material or by comparison to a different, thoroughly described method. The accuracy of the drug product assay is assessed by examining synthetic mixes that have been laced with known amounts of individual components. The accuracy of impurity quantification is assessed by examining samples (drug substance or drug product) spiked with known impurity concentrations. (If there are no impurities, refer to specificity).

SPECIFICITY

The ability to measure the target analyte precisely and specifically in the presence of other elements that could be anticipated to be present in the sample matrix is known as specificity. It serves to ensure that a peak reaction is exclusively caused by a single component by measuring the level of interference from things like other active components, excipients, contaminants, and degradation products. that there is no coelution. The resolution, plate count (efficiency), and tailing factor all contribute to the measurement and documentation of specificity in a separation. Modern photodiode array detectors that computationally compare the spectra recorded across a peak as a sign of peak homogeneity can also assess specificity. Specificity is a term used by ICH as well, and it is divided into two subcategories: identification and assay/impurity test.

LIMIT OF DETECTION

The lowest concentration of an analyte in a sample that can be identified but not quantitated is known as the limit of detection (LOD). It determines whether or not an analyte is above or below a specific value using a limit test.

It is stated as a concentration at a particular signal-to-noise ratio, typically a ratio of two or three to one. The ICH gives two additional options for determining LOD in addition to the signal-to-noise ratio convention: visual non-Instrumental techniques and ways to figure out the LOD of 0.211 g. The upper limit of the LOD value must not exceed 2 g when compared to standard references.

LINEARITY AND RANGE

The capacity of a method to produce test findings that are directly proportional to analyte concentration within a certain range is known as linearity and is represented by the variance. LODs obtained by processes like thin layer chromatography (TLC) or titrations may be used in visual non-instrumental procedures. LODs can also be computed using the SD of the response and the S of the calibration curve at values close to the LOD using the following formula:

$$LOD = 3.3(SD/S)$$

Based on the standard deviation of the blank, the residual standard deviation of the regression line, or the standard deviation of the y-intercepts of regression lines, the standard deviation of the answer can be calculated. To validate the level, a suitable number of samples should be evaluated at the limit according to the method used to determine LOD. LOD values are always individual to a certain set of experimental circumstances. The detection limits of a method will change if something alters the method's sensitivity, including the instrument, sample preparation, etc.

LIMIT OF QUANTIFICATION

The lowest concentration of an analyte in a sample that can be measured with respectable precision and accuracy under the method's specified operational circumstances is known as the Limit of Quantification (LOQ). Similar to LOD, LOQ is stated as a concentration with information about the measurement's precision and accuracy included. To calculate LOQ, a signal-to-noise ratio of ten to one is occasionally employed. This signal-to-noise ratio is a useful generalisation, but it's important to keep in mind that the LOQ is determined by striking a balance between the desired precision and accuracy, and attention. In other words, precision improves as the LOQ concentration level falls. A larger concentration must be recorded for LOQ if greater precision is needed.

The analytical technique and its intended application require this trade-off. The ICH has acknowledged a signal-to-noise ratio of ten to one as usual and, like LOD, specifies two more ways to measure LOQ: visual non-instrumental approaches and a method for computing the LOQ. Once more, the formula for the calculation is based on the standard deviation of the response (SD) and the slope of the calibration curve (S):

$$LOQ = 10(SD/S)$$

Once more, based on the standard deviation of the blank, the residual standard deviation of the regression line, or the standard deviation of y-intercepts of regression lines, the standard deviation of the response can be calculated. Nearly 10 times more value exists in LOQ than in the blank. The LOQ was discovered with concentrations of the slope of the regression line that was within limits. The range is the distance between the analyte's upper and lower (inclusive) levels that have been shown to be quantified using the method as written precisely, accurately, and linearly. Normal expression of the range uses the same units as the test results

attained using the procedure. In addition to some minimum stated ranges, the ICH standards stipulate a minimum of five concentration levels. The minimum specified range for the assay is between 80 and 120% of the target concentration. The minimal range for an impurity test is between the reporting level of each contaminant and 120% of the specification.

ROBUSTNESS

A method's robustness is its ability to withstand minor, purposeful changes to its input parameters. A method's robustness is assessed by changing the method's inputs, such as the percentage of organic matter, pH, ionic strength, temperature, etc., and observing the impact (if any) on the method's outputs. Robustness should be taken into consideration early on in the development of a method, as stated in the ICH standards. Additionally, method parameters should be sufficiently controlled and a warning statement should be included in the method description if the findings of a method or other measures are sensitive to changes in method parameters.

RECOVERY STUDIES

Studies on recovery were conducted utilizing the spiking method. The test sample used in this procedure has a concentration of 10 g/ml. The test solution is spiked by adding the standard medication to it. The sample solutions were spiked with 8, 10, and 12 g/ml, and the absorbances of the three spiking concentrations were measured. The amount of medication that can be recovered using the suggested method can be calculated from this absorbance [9–13].

UV-VIS SPECTROSCOPY APPLICATION (19)

1. Identifying Impurities
2. Clarification of Organic Compound's Structure
3. conjugation detection
4. discovering a functional group
5. Identifying a geometric isomer
6. Calculation of Molecular Weight 7. Cis-Trans Isomerism: A Distinction.

CONCLUSION

A frequently used technique in UV spectroscopy was derivative spectrophotometry, and recently a number of developments in statistical software allowed for the creation of a range of derivative spectrophotometric procedures. By incorporating the mathematical equations, new avenues for using UV-visible spectroscopy in the realm of analysis are revealed. These techniques are superior in terms of ease of use and measurement sensitivity when compared to the widely used UV-visible spectrophotometric methods for mixtures. These techniques don't call for complex experimental procedures or much care. The presented approaches clearly work in complicated mixtures with partially and severely overlapped spectra and have the distinct benefit of being separation methods. The developed methods exhibit good drug determination precision and accuracy.

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