IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

"A REVIEW ARTICLE ON MULTICOMPONENT ESTIMATION BY USING UV SPECTROSCOPY."

Vikas Gauswami[1], Kirti Kayasth[1], Prof. Divya Gupta[2], Prof. Mitali Dalwadi[2], Dr. Umesh Upadhyay[3]

Student[1], Professor[2], Principal[3]

Sigma Institute of Pharmacy, Bakrol, Vadodara, Gujarat, 390019, India.

ABSTRACT

Spectroscopy is a scientific discipline originating from Isaac Newton's experiments, expanding to study the interaction between matter and electromagnetic radiation. This interaction reveals energy quantization, observed across the electromagnetic spectrum. Experimental measurements yield energy level information, contributing to our understanding of matter's behavior.UV-Visible spectroscopy examines electromagnetic radiation interaction with atoms and molecules, focusing on absorption or emission profiles. Wavelength governs the visible color spectrum, measured by spectrophotometers. Absorbance follows Beer's Law, directly proportional to concentration and path length. Electronic transitions induce absorption bands, narrow for atoms and broader for molecules, broadening in solutions due to solvent-solute interactions.

[Keywords: UV Spectroscopy, Validation, GMP, Simultaneous Equation, Q-Ratio Absorbance, Derivative Spectroscopy, Difference Spectroscopy]

INTRODUCTION

SPECTROSCOPY: [1]

Spectroscopy, as a scientific discipline, originated with Isaac Newton's groundbreaking experiments involving the dispersion of visible light using a prism, initially referred to as optics. In its initial stages, spectroscopy focused on the study of visible light, specifically its properties such as color. However, as scientific advancements occurred, particularly through the work of James Clerk Maxwell, the scope of spectroscopy expanded to encompass the entire electromagnetic spectrum. In essence, spectroscopy became the branch of science dedicated to investigating the interaction between matter and electromagnetic radiation.

One of the fundamental outcomes of this interaction is the observation that energy is either absorbed or emitted by matter in discrete, quantized amounts known as quanta. These absorption and emission processes are observed across the entire electromagnetic spectrum, ranging from the gamma region, where phenomena like nuclear resonance absorption or the Mossbauer effect occur, to the radio region, where nuclear magnetic resonance is relevant.

When experimental measurements of radiation frequency (whether emitted or absorbed) are conducted, they yield values for the associated changes in energy. From these measurements, one can infer and draw

conclusions about the discrete energy levels that the matter can assume. The practice of spectroscopy encompasses both the experimental methods used to measure radiation frequency and the deduction of energy levels from these measurements. These combined efforts contribute to our understanding of the behavior of matter and its interaction with electromagnetic radiation across the electromagnetic spectrum.

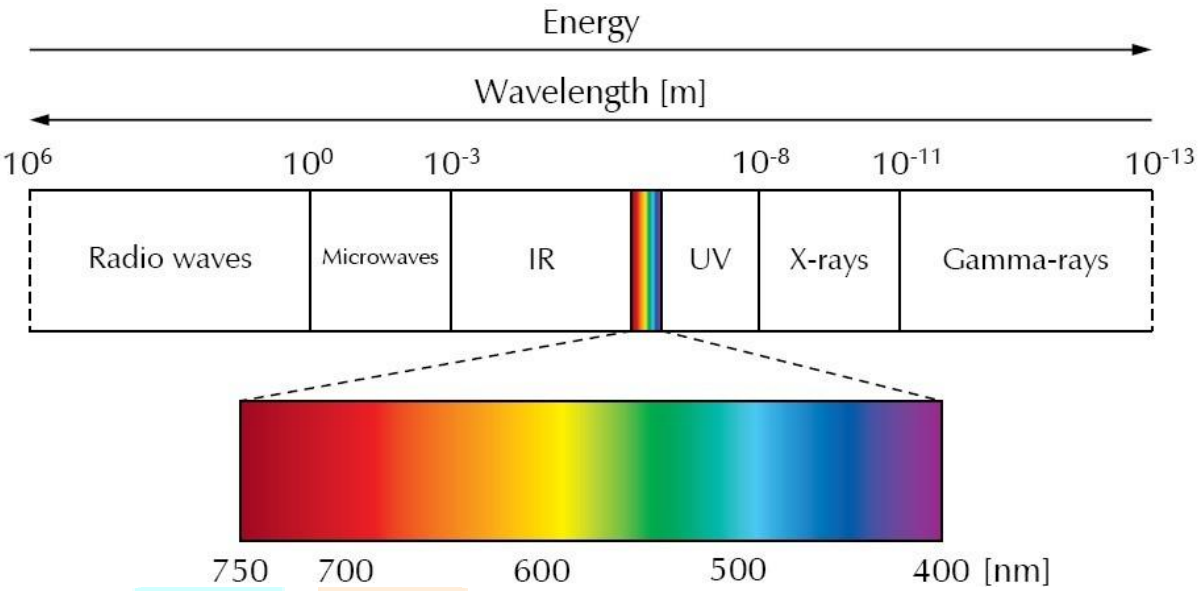

fig 1. spectroscopy [15]

UV SPECTROSCOPY [1,2,6]

Ultraviolet (UV) and visible radiation make up just a fraction of the electromagnetic spectrum. This spectrum encompasses various other types of radiation, including radio waves, infrared (IR), cosmic rays, and X-rays.

UV-Visible (UV-Vis) spectroscopy is a critical scientific technique that relies on the discrete interaction of electromagnetic radiation with atoms and molecules, leading to distinct absorption or emission profiles. This phenomenon is fundamental to the practice of spectroscopy. The wavelength of electromagnetic radiation governs the visible color spectrum, with the visible portion of the electromagnetic spectrum being what the human eye can perceive, spanning from approximately 400 to 800 nm.

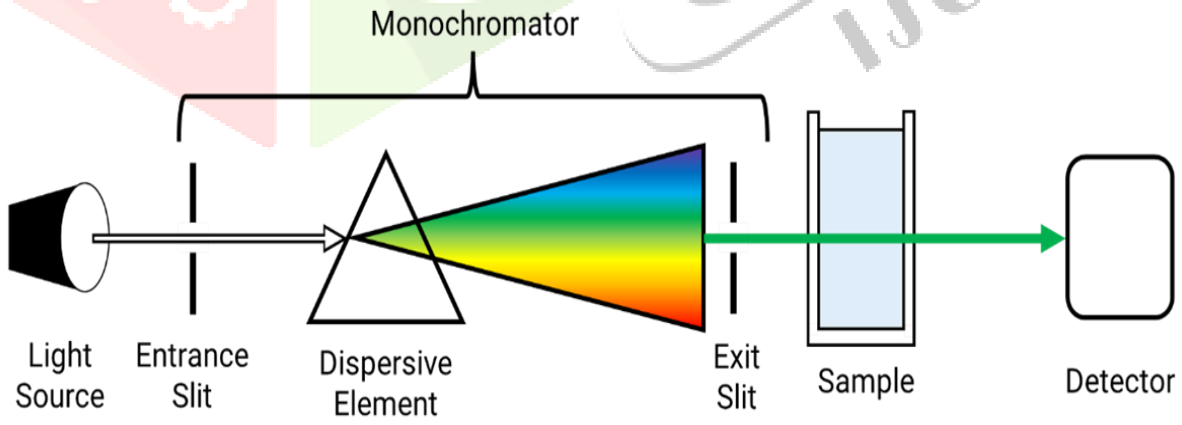

fig 2. uv spectroscopy [16]

When electromagnetic radiation with specific wavelengths or colors passes through a sample, it can be measured using spectrophotometers in terms of its optical density. Substances in the sample can absorb this light, causing it to vanish and become invisible. The relationship between the absorbed wavelengths and the transmitted wavelengths is complementary. For example, a blue substance would strongly absorb the complementary color, orange.

Beer's Law is a fundamental principle used to quantitatively describe how radiant energy is absorbed by materials. According to Beer's Law, the Absorbance [A] or transmission of radiation through a medium is inversely related to the Concentration [C] of the absorbing substance and the path length [b] of the radiation.

$$A = \varepsilon bc = \log (I/I_0)$$

Here,

A =Absorbance

C = Concentration

I = The emerging radiation intensity

I_0 = The incident radiation intensity

ε = The molar absorptivity (a constant), and

b = The path length.

The molar absorptivity (ε) remains constant and is unaffected by changes in concentration or path length.

The energy levels of atoms and molecules are discrete, and when electromagnetic radiation interacts with them, transitions between these energy levels can occur. In UV-Visible spectroscopy, the energy associated with the absorbed light is sufficient to cause electronic transitions, moving electrons from lower energy levels to higher ones. This results in characteristic absorption bands in the UV-Vis spectra, with the wavelength of light absorbed corresponding to the energy required for these transitions.

For atoms, the absorption bands are narrow and well-defined, while for molecules, the absorption bands are broader due to the superimposition of vibrational and rotational energy levels on electronic energy levels. This broadening is further increased in solutions due to solvent-solute interactions.

UV-Visible spectroscopy relies on the measurement of transmittance and absorbance. Transmittance is the fraction of incident light that passes through a sample, while absorbance is a measure of the amount of light absorbed and is defined as log (I/I_0) Absorbance values are linearly related to concentration and path length.

UV spectroscopy is based on Beer-Lambert's law, which states that the absorbance of a solution is directly proportional to the concentration of the absorbing species and the path length. This technique is widely used in laboratories for its simplicity, versatility, accuracy, speed, and cost-effectiveness.

INSTRUMENTATION OF UV [1,6]

1. SOURCE

The ideal light source for UV-visible spectrophotometers would emit a consistent intensity across all wavelengths, exhibit minimal noise, and maintain long-term stability. Unfortunately, such an ideal source does not exist. However, two commonly used light sources in UV-visible spectrophotometers are the deuterium arc lamp and the tungsten-halogen lamp.

1. **Deuterium Arc Lamp:** The deuterium arc lamp is known for producing a reliable intensity continuum in the UV (ultraviolet) region and also provides useful intensity in the visible spectrum. Modern deuterium arc lamps have low noise, but the lamp itself can be a limiting factor in overall instrument noise performance. Over time, the intensity of light emitted by a deuterium arc lamp decreases steadily, with a typical half-life of around 1,000 hours.

2. **Tungsten-Halogen Lamp:** Tungsten-halogen lamps are another common light source. They offer good intensity across part of the UV spectrum and the entire visible range. These lamps have low noise, minimal drift, and a longer useful life of approximately 10,000 hours.

Many UV-visible spectrophotometers incorporate both types of lamps. These instruments may use a source selector to switch between the lamps as needed, or they may combine the light from both sources to create a single broadband source.

Additionally, there is an alternate light source, the xenon lamp, which provides a consistent continuum across the entire UV and visible spectrum. However, xenon lamps tend to have higher noise levels compared to deuterium and tungsten lamps, making them suitable primarily for applications where high intensity is the primary concern, such as diffuse reflectance measurements.

2. MONOCHROMATORS:

A monochromator is an optical instrument used to create monochromatic (single-wavelength) light by filtering out unwanted wavelengths from a polychromatic light source.

Types of Monochromators:

i) Prism Monochromator: This type of monochromator uses a prism as the dispersion element. Prisms are capable of bending different wavelengths of light by different amounts, allowing for separation and selection of a specific wavelength.
ii) Grating Monochromator: Grating monochromators utilize diffraction gratings as the dispersing element. Diffraction gratings consist of closely spaced lines or rulings, which cause light to be diffracted at different angles depending on its wavelength. This enables the separation of wavelengths.

Components of a Monochromator: Every monochromator consists of the following key components:

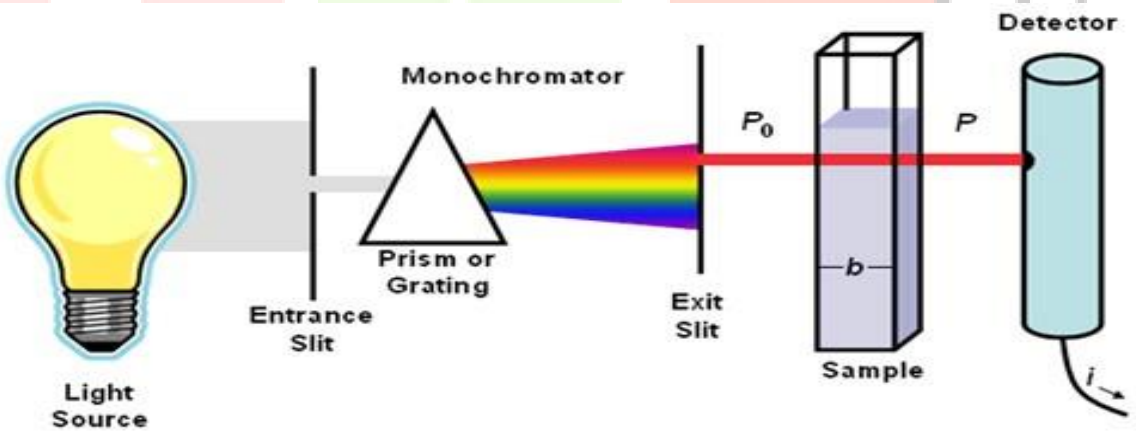

Fig 3. Components of Monochromator [17]

- **Entrance Slit:** This is the opening through which the incoming polychromatic light enters the monochromator.
- **Collimating Lens:** After entering the monochromator, the light is focused and converted into a parallel, collimated beam.
- **Dispersing Device:** This can be either a prism or a grating and is responsible for separating the various wavelengths present in the collimated beam.
- **Focusing Lens:** This lens helps to focus the selected wavelength onto the exit slit.
- **Exit Slit:** The exit slit is the final opening through which only the radiation of the desired wavelength passes. Adjusting either the dispersing element or the exit slit allows for the selection of specific wavelengths.

3. SAMPLE CONTAINERS (CUVETTE):

Cuvettes are sample containers that are transparent to all wavelengths of light flowing through them and are used to hold samples for spectroscopic measurements. The cuvette is composed of quartz, is square in shape, has a 1 cm route length, and may be utilized for wavelengths between 190 and 200 nm.

4. DETECTOR

A detector plays a critical role in spectrophotometry by converting a light signal into an electrical signal. Ideally, it should provide a linear response across a wide range, have low noise, and high sensitivity. Spectrophotometers commonly utilize either a photomultiplier tube detector or a photodiode detector.

1. **Photomultiplier Tube Detector:** This type of detector integrates signal conversion with multiple stages of amplification within the tube itself. The spectral sensitivity of the photomultiplier tube depends on the nature of the cathode material. A single photomultiplier tube offers excellent sensitivity across the entire UV-visible range and is particularly useful for low light levels. However, in analytical spectroscopy applications, high sensitivity often corresponds to low concentrations, resulting in low absorbances and high-intensity levels. To accurately detect small differences between blank and sample measurements, the detector must exhibit low noise at high intensity levels.
2. **Photodiode Detector:** Photodiode detectors are increasingly used in modern spectrophotometers. They offer a broader dynamic range and are more robust than photomultiplier tube detectors because they are solid-state devices. In a photodiode, light incident on the semiconductor material generates electron flow, depleting the charge in a connected capacitor. The amount of charge required to recharge the capacitor at regular intervals is proportional to the light's intensity. While earlier photodiodes had limited sensitivity in the low UV range, modern detectors have corrected this issue. Typically, silicon-based detectors have detection limits in the range of approximately 170 to 1100 nm.

ESTIMATION METHOD USED IN UV

1. Simultaneous Equation Method
2. Q-Absorbance Ratio Method
3. Derivative Spectroscopy
4. Difference Spectroscopy

1. SIMULTANEOUS EQUATION METHOD [5,13,18,25]

Principle:

If a sample contains two absorbing substances, X and Y, each of which absorbs at the λ max (maximum absorption wavelength) of the other, it is possible to determine both substances' concentrations if the following criteria are satisfied:

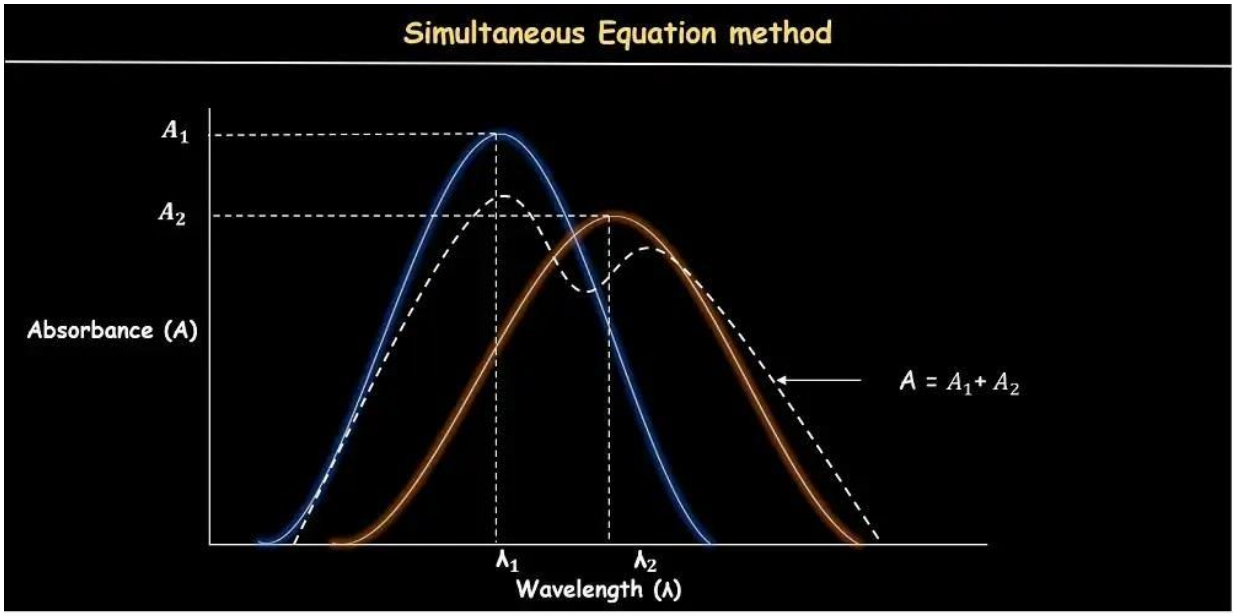

fig 4. simultaneous equation method [18]

- The absorptivities (molar absorptivities or extinction coefficients) of X at λ1 and λ2, denoted as ax1 and ax2, respectively.
- The absorptivities of Y at λ1 and λ2, denoted as ay1 and ay2, respectively.
- The absorbance of the diluted sample at λ1 and λ2, denoted as A1 and A2, respectively.

Two equations are constructed based on the fact that at λ1, the absorbance of the mixture is the sum of the individual absorbances of X and Y:

Equation 1: A1 = (ax1 * Cx) + (ay1 * Cy)

Equation 2: A2 = (ax2 * Cx) + (ay2 * Cy)

Where:

- A1 and A2 are the absorbances at λ1 and λ2, respectively.
- Cx and Cy are the concentrations of X and Y, respectively.
- ax1, ax2, ay1, and ay2 are the absorptivities of X and Y at the respective wavelengths.

Derivation: For measurements in a 1 cm cell (b = 1 cm), Eq. (2) can be rearranged as follows:

Equation 3: Cy = (A2 - ax2 * Cx) / ay2

Substituting the expression for Cy from Eq. (3) into Eq. (1) and rearranging gives:

Equation 4: A1 = (ax1 * Cx) + (ay1 * [(A2 - ax2 * Cx) / ay2]) Solving Eq. (4) for Cx

yields:

Equation 5: Cx = (A1 * ay2 - A2 * ay1) / (ax1 * ay2 - ax2 * ay1)

Criteria for Precision: To obtain maximum precision in this method, certain criteria based on absorbance ratios are suggested. These criteria specify that the ratios A1/A2 and ax1/ay1 should lie outside the range of 0.1 to 2.0 for the precise determination of Y and X, respectively. These criteria are met when the λ max values of the two components are reasonably dissimilar, and if the two components do not chemically interact, which would invalidate the assumption that the total absorbance is the sum of the individual absorbances.

2. Q-ABSORBANCE RATIO METHOD [7,14,19]

The 8-Q-absorbance ratio method, also known as the absorption ratio method, is a variation of the simultaneous equation method used for quantitative analysis. This method relies on the principle that for a substance obeying Beer's law, the ratio of absorbance at two wavelengths is a constant value regardless of concentration and path length. This constant is referred to as "Hufner's Quotient" or Q-value. The technique involves measuring absorbance at two specific wavelengths: one corresponds to the λ max of one of the components (λ2), and the other wavelength where both components have equal absorptivity, known as the iso-absorptive point (λ1).

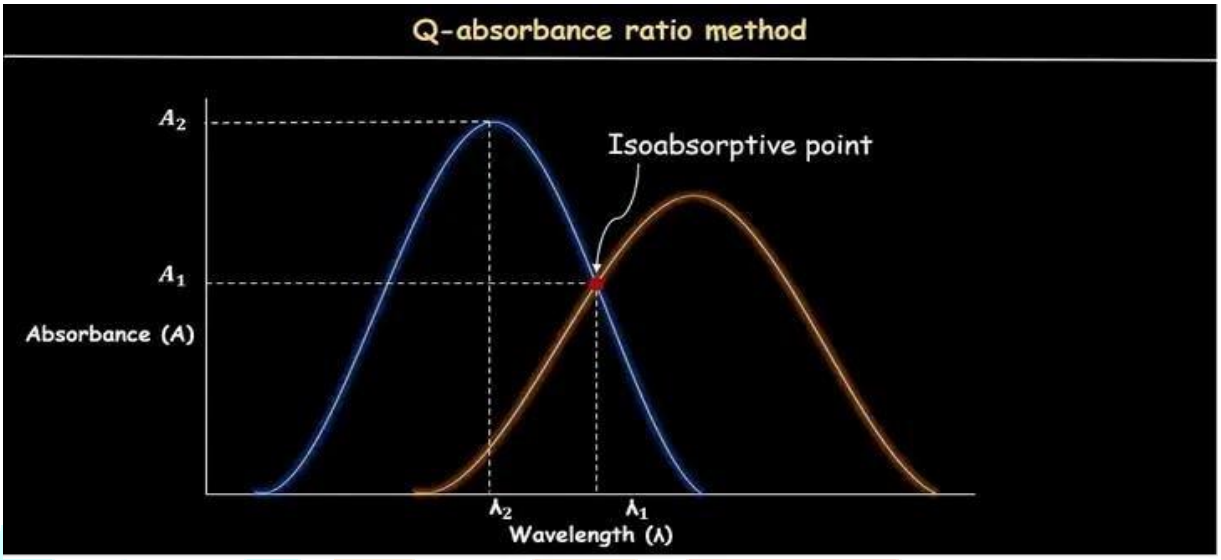

fig 5. q-absorbance ratio method [18]

To determine the concentrations of drugs X and Y using the Q-absorbance ratio method, the following equations are applied:

For drug X:

Cx = ((QM - Qy) / (Qx - Qy)) × (A1 / ax1) For drug Y:

Cy = ((QM - Qx) / (Qy - Qx)) × (A1 / ay1)

In these equations:

- Cx and Cy represent the concentrations of drugs X and Y, respectively.
- QX, QY, and QM are computed based on the average absorptivity values of the drugs at specific wavelengths.
- QX = ax2 / ax1
- QY = ay2 / ay1
- QM = A2 / A1

Where, ax1 and ax2 are the absorptivities of drug X at wavelengths λ1 and λ2.

ay1 and ay2 are the absorptivities of drug Y at wavelengths λ1 and λ2.

A1 and A2 indicate the absorbances of the sample solution at wavelengths λ1 and λ2.

3. DERIVATIVE SPECTROSCOPY [3,4,5]

This straightforward spectrophotometric method is based on generating ratio spectra to resolve binary mixtures. It enables the utilization of the wavelength with the highest analytical signal, which exhibits multiple maxima and minima. This feature provides an opportunity to determine active compounds even when other substances and excipients are present, potentially causing interference in the assay.

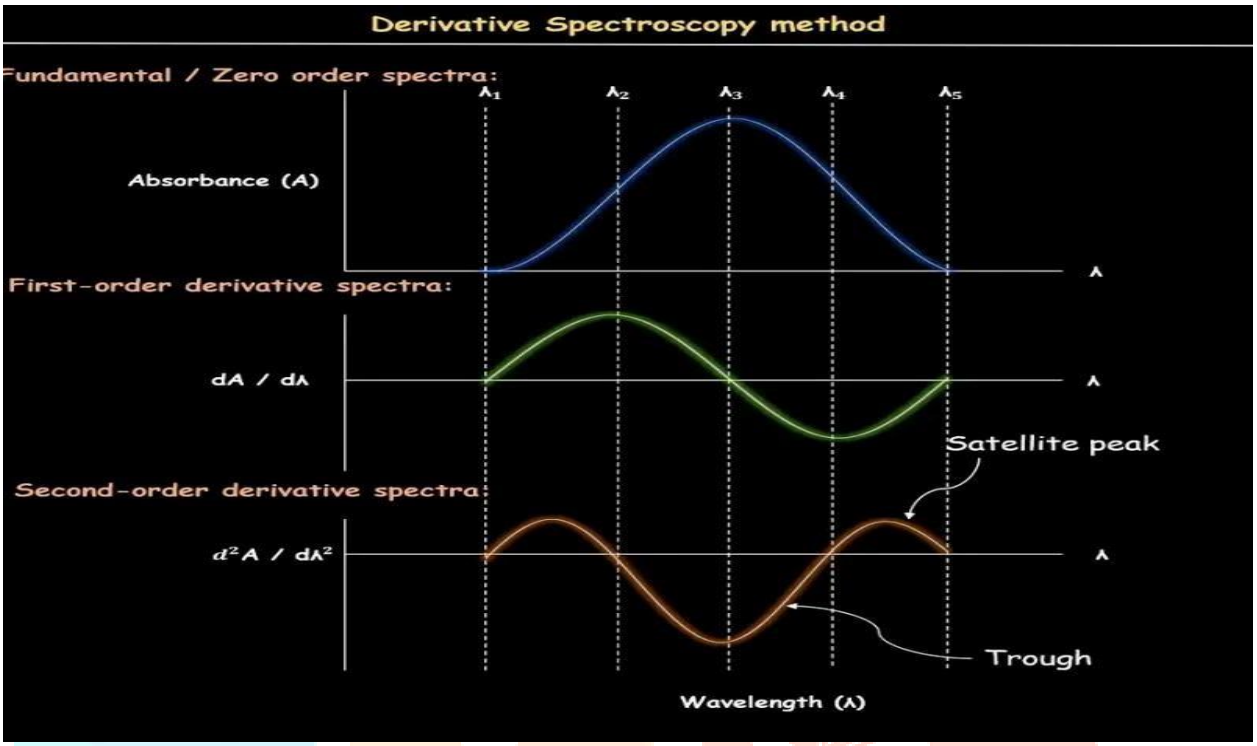

Fig 6. derivative spectroscopy method [18]

To facilitate the determination of the concentration of compound y without interference from drug x, one can calculate the first derivative. This operation effectively removes the constant value associated with compound x.

The observed difference between the two spectra arises from the constant interference value attributed to compound x. To eliminate this interference, you can either measure the difference in ratio spectra between two wavelengths or compute the derivative of the ratio spectra.

Additionally, employing the second derivative of the ratio spectra can enhance linearity, mean recoveries, and decrease relative standard deviation.

The derivative ratio spectra technique has been adapted for the determination of ternary mixtures using the derivative ratio spectra zero-crossing method. In this approach, amplitudes are measured at the zero-crossing points in the derivative ratio spectra.

4. DIFFRENCE SPECTROSCOPY [5]

Difference spectrophotometry is a valuable technique in spectrophotometric analysis when dealing with samples containing interfering substances. It can significantly enhance selectivity and accuracy by measuring the absorbance difference (ΔA) between two equimolar solutions of the analyte in different chemical forms that exhibit distinct spectral characteristics.

To apply difference spectrophotometry effectively in the presence of interfering substances, the following criteria should be met:

1. Reproducible Changes in Analyte Spectrum: It should be possible to induce consistent and reproducible changes in the spectrum of the analyte by adding one or more reagents. This is crucial for ensuring that the observed differences are due to the analyte and not random variations.
2. No Alteration in Interfering Substance Absorbance: The addition of reagents should not affect the absorbance of interfering substances. This is important because you want to isolate the changes in the analyte's spectrum without causing unwanted shifts in the interfering substance signals.

One commonly employed technique to alter the spectral properties of the analyte is adjusting the pH using aqueous solutions of acids, alkalies, or buffers. This pH adjustment can lead to distinct chemical forms of the analyte with varying spectral characteristics, allowing for the measurement of ΔA

VALIDATION [57,58]

Validation is a concept that originated in the United States in 1978 and has since evolved to encompass a wide range of activities. It is not mandated by regulatory requirements but is considered an essential part of current good manufacturing practice (cGMP). Validation is all about assessing the validity or effectiveness of a process or system. It's a collaborative effort involving people from various departments within an organization.

Validation is crucial in the following scenarios:

1. Completely new processes: When a new manufacturing process is introduced.
2. Latest equipment: When new equipment is used.
3. Adapted processes and equipment: When existing processes and equipment are modified to meet new requirements.
4. Processes with unreliable quality indicators: When the final product's quality cannot be easily determined through standard testing.

The pharmaceutical industry relies on expensive materials, advanced facilities, and highly skilled personnel. To reduce costs related to failures and improve productivity, detailed batch validation of the manufacturing process is necessary. Using equipment without knowing if it will produce the desired product or employing personnel without assurance of their capabilities can lead to significant problems.

Efficient resource utilization is critical for the industry's success. Costs associated with product failures, rejects, rework, recalls, and customer complaints make up a substantial portion of the total production cost. Ensuring product quality while reducing costs is the ultimate goal.

In simpler terms, validation is about making sure that new processes, equipment, or changes to existing ones work reliably and produce high-quality products. This is especially important in industries like pharmaceuticals, where errors can be costly and impact product safety. By thoroughly validating these processes, companies can minimize the risk of failures and save money in the long run.

MAJOR PHASES IN VALIDATION

The process of validation studies can be broken down into three main phases:

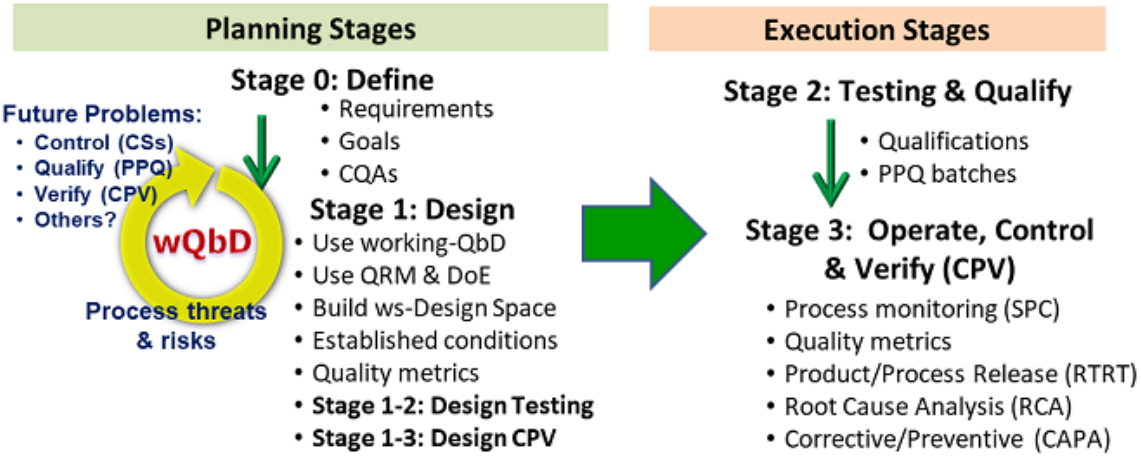

fig 7. phases of validation [19]

Phase 1: Pre-validation Qualification In this stage, we focus on activities related to product research and development, such as testing formulations in pilot batches, scaling up production, transferring technology to larger-scale operations, establishing stability conditions for products, and handling in-process and finished dosage forms. We also ensure that the equipment is qualified for its intended use, and we create master documents for the products. This phase is all about preparing and setting up the groundwork for the validation process.

Phase 2: Process Validation This phase is about validating the actual manufacturing process. We want to make sure that the critical process parameters are within acceptable limits and that even under challenging conditions, we can consistently produce good-quality products. It's like confirming that our production process is reliable and robust.

Phase 3: Validation Maintenance After the initial validation, we enter the maintenance phase. Here, we regularly review all documents related to the process, including audit reports, to ensure that nothing has changed or deviated from the validated process. We want to be certain that our standard operating procedures, including change control procedures, are followed consistently. The validation team, representing various departments, also checks for any changes or deviations that might require revalidation.

By carefully designing and validating our systems and process controls, we can have confidence that every batch we produce will meet the intended specifications. We assume that all manufacturing and control operations follow good manufacturing practices, especially when it comes to sterile product manufacturing.

In simpler terms, validation involves three key phases: preparing and setting up (Phase 1), ensuring our manufacturing process is reliable (Phase 2), and continuously monitoring and maintaining the process to prevent deviations (Phase 3). The goal is to consistently produce high-quality products while adhering to industry standards and best practices.

The recommended validation steps in GMP guidelines can be simplified as follows:

1. **Detailed Protocol**: Before starting any validation study, a detailed protocol or a set of protocols should be established. These protocols are subject to formal change control procedures, meaning any changes should be carefully documented and approved.
2. **Qualified Personnel**: The people conducting the studies and those operating the processes being studied must be properly trained and qualified. They should have the skills and competence needed for their assigned tasks.

3. **Data Review**: All data generated during the studies must go through a formal review process. It should be evaluated against predetermined criteria to ensure accuracy and compliance.
4. **Testing Facilities**: Adequate testing facilities, equipment, instruments, and testing methods should be available to conduct the validation studies effectively.
5. **Clean Room Standards**: Clean room facilities, both in the immediate working area and the surrounding environment, should meet specified cleanliness standards. Initial commissioning (qualification) and periodic re-testing should ensure that the clean room maintains its specified conditions. In addition, in-process equipment should be properly installed, qualified, and well-maintained.
6. **Process Simulation**: For aseptic processes, validation may involve "process simulation" studies. This means replicating the actual manufacturing process to ensure its effectiveness and sterility.
7. **Periodic Revalidation**: The validation process should be periodically rechecked to ensure that the validated process continues to meet the required standards and remains reliable over time.
8. **Comprehensive Documentation**: Thorough documentation is crucial throughout the validation process. This documentation defines, supports, and records all aspects of the validation, ensuring transparency and traceability.

Method validation aims to provide evidence that a method can perform accurately, reliably, and consistently. The key validation parameters according to ICH guidelines are as follows:

1) Accuracy: Accuracy measures how close the measured values are to the true or expected values. It can be determined by conducting at least nine determinations across a minimum of three concentration levels within the specified range. For example, accuracy testing for certain drugs showed recovery rates within an acceptable range, confirming the method's accuracy.

2) Precision: Precision assesses the agreement (or scatter) between measurements obtained from multiple samples of the same substance under specified conditions. Precision can be evaluated at three levels:

- **Repeatability**: Measures precision under the same conditions and over a short time interval.
- **Intermediate Precision**: Evaluates precision within a laboratory, over different days, by different analysts, or using different instruments.
- **Reproducibility**: Examines precision between different analytical laboratories.

3) Specificity: Specificity ensures that the analytical method can accurately assess the analyte of interest even in the presence of other expected components, including degradants, excipients, sample matrix, and sample blank peaks.

4) Limit of Detection (LOD): LOD is the lowest amount of an analyte that can be detected but not necessarily quantified with precision. It is typically calculated using the standard deviation of the response and the slope of the calibration curve.

5) Limit of Quantitation (LOQ): LOQ is the lowest amount of an analyte that can be quantified with precision. Like LOD, it is determined using the standard deviation of the response and the slope of the calibration curve.

6) Linearity: Linearity assesses the ability of the method to produce results directly proportional to the analyte's concentration within a specified range. Linearity is confirmed by preparing different concentrations of the analyte and measuring their absorbance or response.

7) Range: Range defines the interval between the upper and lower quantities of the analyte that can be accurately measured. The range for an assay test should typically be 80% to 120% of the expected concentration.

8) Ruggedness: Ruggedness evaluates the method's reproducibility under varying conditions, such as different laboratories, analysts, equipment, environmental factors, etc.

9) Robustness: Robustness examines the method's ability to remain consistent when minor deliberate changes are made to method parameters, such as pH, flow rate, temperature, and mobile phase composition.

CONCLUSION:

Spectroscopy is a fundamental scientific discipline that has evolved to encompass the entire electromagnetic spectrum, playing a crucial role in understanding the interaction between matter and electromagnetic radiation. UV-Visible spectroscopy, a key technique, relies on the absorption of light by atoms and molecules to reduce energy levels and concentration. It utilizes principles such as Beer's Law and various methods like Simultaneous Equation, Q-Absorbance Ratio, Derivative Spectroscopy, and Difference Spectrophotometry to quantify analytes. The validation of processes and methods is paramount in industries like pharmaceuticals to ensure product quality and consistency, involving meticulous phases from preparation to ongoing maintenance. Validation parameters, including accuracy, precision, specificity, LOD, LOQ, linearity, range, ruggedness, and robustness, are essential to assess the reliability and accuracy of analytical methods.

REFRENCE

1. 1. Diya P, Diya P, et al. “A Review on UV Visible Spectroscopy.” *International Journal of Creative Research Thoughts.* **2022,** 10

2. Dhara P, Mitali D, et al. “Development and Validation of UV Spectroscopic and RP-HPLC Method for Simultaneous Estimation of Lamotrigine and Buproprion in Synthetic Mixture.” *world journal of pharmacy and pharmaceutical sciences.* **2023,** 12(6)

3. Abhi K, Kishan S, et al. “Application of Absorption Correction Method and First Order Derivative Spectrophotometry for Simultaneous Determination of Anti-hypertensive Combination (Amlodipine and Benazepril).” *Int J Pharm Sci.* **2013,** 5(3), 371-375

4. Mahesh A, Muhammad S, et al. “Simultaneous Estimation of Moxifloxacin and Flavoxate by RP-HPLC and Ecofriendly Derivative Spectrophotometry Methods in Formulations.” *Int. J. Res. Public health.* **2019,** 16

5. Amira H, Samah F, et al. “A Review on Spectrophotometric Methods for Simultaneous Multicomponent Analysis.” *European Journal of Pharmaceutical and Medical Research.* **2016,** 3(2), 348-360

6. Tony Owen, “Fundamentals of UV Visible Spectroscopy.” *Agilent Technologies,* **2000,** 5980-1397

7. Prasanth S, Atulya K, et al. “Q-Absorbance Ratio and Vierodt’s Spectrophotometric Method for The Simultaneous Estimation and Omeprazole.” *Int. J. Pharm. Drug. Anal.* **2023,** 11(1), 9-16.

8. Geeta R, Saranjit S, et al. “Simultaneous Estimation of Simvastatin and Fenofibrate from Their Combined Dosage form by UV-VIS Spectroscopy using Simultaneous Equation Method.” *Pharma spire,* **2021,** 13(3), 117-121.

9. Khalid Q, Abdul Q, et al. “Q-Absorbance Ratio, Derivative Spectrophotometric and H-Point Standard Addition Methods for Simultaneous Determination of Pridinol Mesylate, And Diclofenac Sodium in Pharmaceutical Formulation.” *Journal of Pure and Applied Sciences,* **2021,** 33(4), 43-60.

10. Leela K, Lakshmi D, et al "Q-Analysis and Simultaneous Equation Method for Estimation of Domperidone and Naproxen by UV Spectrophotometry in Bulk and Tablet Dosage Form." *Austin J Anal Pharm Chem* **2020,** 7(1)

11. Mahmoud M, Amr M, et al. "Q-Absorbance Method for Simultaneous Determination of Paracetamol and Orphenadrine Citrate in Their Combined Pharmaceutical Dosage Forms." *European Journal of Pharmaceutical and Medical Research,* **2020,** 7(1), 464-468.
12. Sanjay J, Maheshwari R, et al. "Simultaneous Estimation of Norfloxacin and Tindazole in Solid Dosage Form by UV Spectrophotometry Using Mixed Solvency Concept." *World Journal of Pharmaceutical and Medical Research,* **2017,** 4(1), 112-117.

13. Shivani S, Swapnil G, et al. "A Review on Analytical Method Development and Validation." *Int. J. App Pharm.* **2018,** 10(6), 8-15.

14. Harpreet K, Gurpreet S, et al. "Pharmaceutical Process Validation." *Journal of Drug & Therapeutics.* **2013,** 3(4), 189-194.

15. http://www.gamry.com

16. https://jascoinc.com/learning-center/theory/spectroscopy/uv-vis-spectroscopy/instrumentation/

17. https://dept.cord.edu/chemistry/analyticallabmanual/experiments/uvvis/intro.html

18. https://pharmagroww.com/multicomponent-analysis-by-uv-spectroscopy/

19. https://www.pharmaceuticalonline.com/doc/a-functional-history-of-process-validation-part-the-key-to-a-more-effective-future-0001