iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 13 **Issue:** V **Month of publication:** May 2025

DOI: https://doi.org/10.22214/ijraset.2025.70965

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 13 Issue V May 2025- Available at www.ijraset.com

HPLC and UV Spectroscopy in Pharmaceutical Analysis

Himanshi Aggarwal[1], Dr. Kalpana Singh[2]
Department of Quality Assurance, Llyod Institute of Management and Technology

Abstract: ***Ultraviolet (UV) spectroscopy and High-Performance Liquid Chromatography (HPLC) are two essential analytical methods that are frequently employed in pharmaceutical analysis for regulatory compliance, quality assurance, and medication development. Pharmaceutical substances, such as active pharmaceutical ingredients (APIs), contaminants, and degradation products, can be separated, identified, and quantified using high-performance liquid chromatography (HPLC), a very sensitive and accurate chromatographic technology. It is essential for pharmacokinetic research, stability testing, and bioequivalence evaluations. However, UV spectroscopy is a quick and inexpensive analytical method that helps with both qualitative and quantitative characterization of drug compounds by measuring how much ultraviolet light they absorb. It is widely used for pharmaceutical ingredient concentration determination, dissolving research, and purity testing.***

For frequent quality checks, UV spectroscopy offers a more straightforward and non-destructive method, even if HPLC gives better selectivity and resolution. Pharmaceutical analysis is more accurate and efficient when these two methods are combined. A popular technique for tracking drug formulations, identifying fake drugs, and guaranteeing batch-to-batch uniformity in production is HPLC combined with UV detection, or HPLC-UV. The concepts, procedures, and comparative uses of HPLC and UV spectroscopy in pharmaceutical analysis are examined in this paper. Their benefits, drawbacks, and most current developments in analytical technology are also covered. Researchers and pharmaceutical experts must be aware of these methods in order to guarantee the safety, effectiveness, and adherence to international regulatory requirements of drugs.

Keywords: Ultraviolet Spectroscopic Analysis, UV Spectroscopy, High-Performance Liquid Chromatography (HPLC), Spectrophotometric Methods, HPLC-UV.

I. INTRODUCTION

Pharmaceutical analysis is essential to quality assurance, medication development, and regulatory compliance. The effectiveness and safety of pharmaceutical goods are determined by the sensitivity, accuracy, and precision of analytical methods. Among the most used techniques for drug analysis are HPLC and UV spectroscopy because of their effectiveness and dependability.

Ultraviolet spectroscopy is the study of the absorption or reflectance of the ultraviolet and near-visible portions of the electromagnetic spectrum. It is also known as UV-visible spectrophotometry, or UV-Vis or UV/Vis. Because of its affordability and ease of use, this method is widely used in a variety of fundamental and practical applications [1,2]. The sample just has to absorb in the UV spectrum to be considered a chromophore. Absorption spectroscopy is used in addition to fluorescence spectroscopy. Absorbance (A), transmittance (%T), and reflectance (%R), along with their temporal changes, are the metrics of importance in addition to wavelength [3]. When white light enters a prism or slit, it will be dispersed into a spectrum. Higher frequency, more energetic purple light is at one end of the spectrum, while lower frequency, less energetic red light is at the other. Furthermore, the invisible electromagnetic radiation is found on this side. In addition to ultraviolet (UV) light, there are other kinds of high intensity invisible light [4]. UV radiation from standing in the sunlight can harm your skin. The following table provides a description of the sections and the wavelengths they have in compliance with ISO 21348:2007:

Table 1: The segments and their wavelengths are described in the table below in accordance with ISO 21348:2007 [5]

Ultraviolet Segment	Wavelength Region (10 nm - 400 nm)
Extreme Ultraviolet EUV	10 nm - 121 nm
Far Ultraviolet FUV	122 nm - 200 nm
Middle Ultraviolet MUV	200 nm - 300 nm
Near Ultraviolet NUV	300 nm - 400 nm

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 13 Issue V May 2025- Available at www.ijraset.com

A. Principle

The UV Visible Principle The distinctive spectra created when chemicals absorb visible or ultraviolet light form the basis of spectroscopy. The connection of light and matter is the foundation of spectroscopy. When matter absorbs light and experiences excitation and de-excitation phases, a spectrum is produced. When an electromagnetic wave strikes a substance, it can cause transmission, absorption, reflection, and scattering [6]. The observed spectrum illustrates the interactions of discrete-dimensional objects such as atoms, molecules, and macromolecules at various wavelengths. Absorption occurs when the energy difference between the excited and ground states of a molecule is equal to the frequency of the light that comes in [7]. This is the fundamental mechanism of molecular spectroscopy.

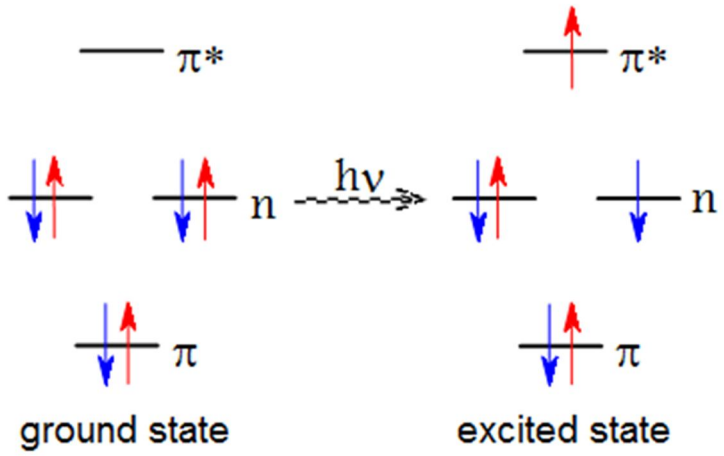


Fig 1: The process of energizing an electron to transition from its ground state to an excited one is depicted in this figure. This is molecular spectroscopy's fundamental concept. [8]

B. Beer-Lambert law:

This rule states that the absorbance of a solution (A) is directly proportional to the route length (b) and the concentration of the species that absorbs (c) in the solution.

Absorbance A = molar absorptivity constant x cell length x concentration

$$A = abc$$
$$C = A / a\,b$$

Where,

A = absorbance
A = molar absorptivity
b = path length
c = Concentration

C. Electronic Transition

Absorption in the visible or ultraviolet spectrum happens when light causes a change in electronics inside the framework of a molecule or ion. Therefore, the electrical states of a sample's molecules also change as it absorbs visible or ultraviolet light. The energy from the light will push electrons from their initial ground-state orbitals towards higher energy state of excitement orbitals or antibonding orbitals [9,10].

The various kinds of electronic transitions can be caused by absorption of UV and visible light:

1) σ to σ* Transitions:

An electron in the bonding's σ orbital is compelled to go to its corresponding antibonding orbital. It requires a great deal of energy. For example, methane, which only has C-H bonds and is able to undergo σ to σ* transitions, has an absorbance maximum at 125 nm. Because of σ to σ* transitions, absorption peaks are absent from typical UV-Vis spectra (200–700 nm) [11].

2) n to σ* Transitions:

Saturated compounds containing atoms with lone pairs, or non-bonding electrons, may undergo transitions from n to σ*. These adjustments often need less energy than σ to σ*. Attitude Shifts They can be initiated by light having a wavelength of 150–250 nm [12,13].

3) n to π* and π to π* Transitions:

Electron transitions from n or π to the π * excited state are the basis for most organic compound absorption spectroscopy. This is because these transitions' absorption maxima fall within an experimentally advantageous spectral range (between 200 and 700 nm). The π electrons needed for these transformations must originate from a molecule's unsaturated group. Molar absorptivity is rather modest, ranging from 10 to 100 L mol-1 cm-1 during the π to π* transitions. For π to π* transitions, the typical molar absorbtivities fall between 1000 and 10,000 L mol-1 cm-1 [14,15].

D. Limitation in Laws

- Scattering and reflection may cause the quoted absorption to vary.
- The solvent reacts by reducing the mean distance between ions at high concentrations, which alters the charge distribution and draws particles closer together [16].
- The presence of faulty lighting.

E. Types of UV-visible spectra:

Two types of absorbance devices are used to collect UV-visible spectra:

- Single beam spectrometer:
  Each of these instruments has a light source (who are frequently a deuterium or tungsten lamp), a detector, and a sample holding mechanism. On the other hand, some of them also have filters that let the user select a single wavelength. The single beam instrument (Figure 2) inserts a filter or monochromator between the beam's source and the specimen in order to analyze one wavelength at a time [17].

- Double beam spectrometer:
  This permits an extra accurate monochromator between the source and the sample; alternatively, it has a diode array detector that allows the device to measure the absorbance at all wavelengths at the same time. To deliver the beam to a reference specimen and the sample to be analyzed, the double beam apparatus (Figure 3) consists of a single source, a monochromator, a splitter, and a number of mirrors. Generally speaking, the concurrent instrument is faster and more efficient [18,19].

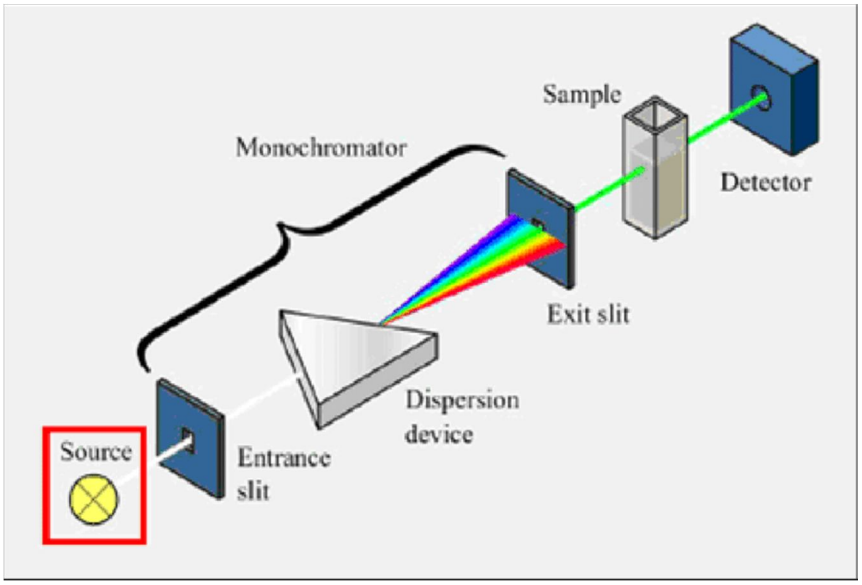


Fig 2: UV-Spectroscopy Single Beam [20]

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 13 Issue V May 2025- Available at www.ijraset.com

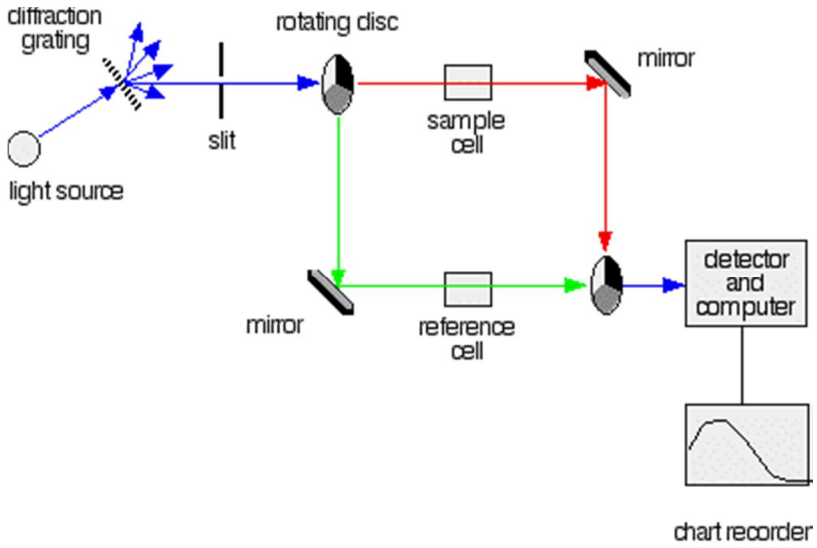


Fig 3: UV-Spectroscopy Double Beam [21]

II. INSTRUMENTATION

The components of equipment used to test UV or visible light absorption are listed below [22].

- Source
- Monochromator
- Sample cell
- Detector
- Readout system
    a) Amplifier
    b) Display
- Sources**:**

1) Sources of UV radiation

Over its wavelength range, the strength of the radiation source must not vary substantially. Whenever deuterium or hydrogen are electrically stimulated at low pressure, a continuous UV spectrum is created. This is due to the creation of an excited molecular species that breaks down into two atomic species and an ultraviolet photon [23]. Deuterium and hydrogen lamps generate light with wavelengths between 160 and 375 nm. Because glass absorbs light with wavelengths shorter than 350 nm, these lamps need quartz windows and quartz cuvettes [24].

D2 + electrical energy ♦ D2* ♦ D' + D'' + hv is one way to represent this.

2) Sources of visible radiation:

Tungsten filament lamps are commonly used to create visible light. This kind of light has a wavelength range of 350–2500 nm. The voltage at which a tungsten filament lamp works, increased to the fourth power, determines how much energy it produces. This suggests that for the quantity of energy generated to remain steady, the voltage applied to the lamp must be incredibly constant. Electronic regulators for voltage or constant-voltage transformers give this stability [25].

- Monochromator (Wavelength selector):

The following components are found in every monochromator:

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

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 13 Issue V May 2025- Available at www.ijraset.com

Polychromatic radiation, or radiation with many wavelengths, enters the monochromator through the input slit. Before the beam contacts the dispersion element at an angle, it is collimated. The beam's wavelengths are separated into their component parts by the prism or grating. Simply by altering the dispersing element or the exit slit may radiation of a certain wavelength leave the Monochromator by means of the exit slit [26,27].

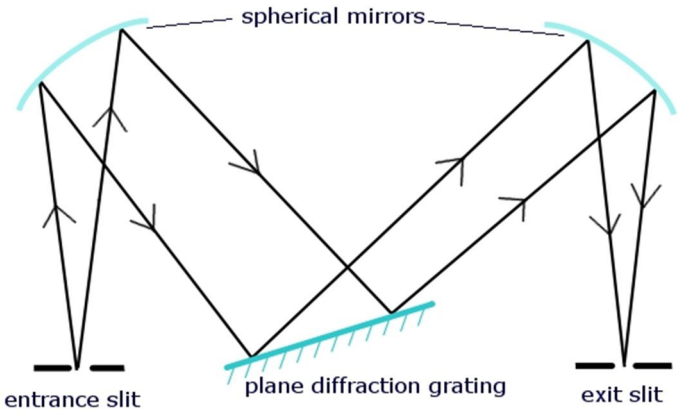


Fig 4: Diagrammatic Representation of Monochromator [28]

- Sample cell:

The radiation must be transparent to the particular radiation that will flow between the containers of the reference solution and the specimen. Fused silica or quartz cuvettes are required for UV spectroscopy. These cells are also transparent in the visible spectrum. Silicate glasses are commonly used to create cuvettes for use in the 350–2000 nm range [29].

- Detectors:

A detector converts a lighting signal into an electrical signal. It ought to respond linearly across a wide range of minimal noise and high sensitivity.

3) Photomultiplier tube detector

The photomultiplier tube is a commonly used detector in UV-Vis spectroscopy. It is composed of a photoemissive cathode that releases electrons when subjected to photons of radiation and an anode that releases multiple electrons for each electron that strikes it. Upon entering the tube, a photon of radiation contacts the cathode, causing the emission of many electrons. These electrons are driven by the initial anode, which is 90 volts higher in voltage than the cathode. Multiple electrons are released with each incident electron that hits the first electrode [30].

These electrons are further accelerated in the direction of the second anode to produce more electrons that are driven towards the anode. Electrons are collected at the anode. For each original photon, 106–107 electrons have been generated produced thus far. The current has since been identified and increased. Photomultipliers are particularly susceptible to ultraviolet and visible light. They respond quickly. Due of damage from bright light, photomultipliers can only detect low power radiation [31].

4) Photodiode detector

The photodiode detector is one kind of multichannel photon detector. All of the components of a dispersed radiation beam may be measured simultaneously by these detectors. A linear photodiode array is made up of several small silicon photodiodes grouped on one silicon chip. 64–4096 sensor components can be found on a single chip; the most common arrangement consists of 1024 photodiodes.Each diode also has a switch and a storage capacitor [32].Each diode-capacitor circuit may be gradually scanned one after the other. In use, the photodiode array is positioned so that the spectrum strikes it at the focal plane of the monochromator, which follows the dispersing element.

When recording the UV-Vis absorption spectra of substances that are rapidly passing through a sample flow cell, with the value in an HPLC detector, they can be especially useful [33].

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 13 Issue V May 2025- Available at www.ijraset.com

III. APPLICATION

A. Bacterial Culture

UV-Vis spectroscopy is often used for cultivating microorganisms. OD observations, particularly are used to estimate cell concentration and track growth, are often and quickly performed at a wavelength of 600 nm. 600 nm is commonly used and advised due to the optical properties of the bacterial growth circumstances beneath which they are grown and to avoid cell damage in circumstances where the cells are required for additional testing [34].

B. Beverage Analysis

Another common use of UV-Vis spectroscopy is the detection of certain compounds in beverages. The legal limitations for caffeine levels may be ascertained with the use of UV light. Certain families of colored chemicals, such anthocyanin, which is found in cherries, blueberries, raspberries, and blackberries, may be readily identified in wine using UV-Vis absorbance for quality control reasons by matching their recognized peak absorption wavelengths [35].

C. DNA and RNA Analysis

One common use is for quick assessments of the concentration and purity of RNA and DNA. Table 2 lists the wavelengths used in their investigation along with their indications. Before processing DNA or RNA samples for further purposes, including sequencing, it is sometimes necessary to ensure that they are free of contaminants, including as proteins or chemicals leftover from the procedure of isolation [36].

Table 2: An outline of the UV absorption that is crucial for figuring out the ratios of 260/280 and 260/230 [37]

The wavelength utilised in nanoscale absorbance analysis	What does the presence of this wavelength of UV absorption indicate?	Why is this wavelength of UV absorption occurring?
230	Protein	Protein Shape
260	DNA and RNA	Adenine, guanine, cytosine, thymine, uracil
280	Protein	Mostly tryptophan and tyrosine

D. Pharmaceutical Analysis

One of the sectors that uses UV-Vis spectroscopy most extensively is the pharmaceutical industry. For instance, by processing UV-Vis spectra using mathematical derivatives, overlapping absorbance peaks in the initially collected spectra can be separated to identify particular pharmaceuticals. For instance, in commercial veterinary powder formulations, the antibiotic chlortetracycline and the local anesthetic benzocaine may be identified simultaneously using the first analytical derivative on the absorbance spectra. It was possible to quantify both compounds simultaneously on a concentration range of micrograms per milliliter by developing calibration functions for every compound [38].

E. Other Applications:

This technique might possibly be used in many other fields. To ensure the safe distribution of power, for instance, monitoring transformer oil using a color index might be useful as a preventative measure [39]. Monitoring hemoglobin absorbance to determine hemoglobin concentrations may be useful for cancer research. UV-Vis spectroscopy may be used in kinetic and monitoring studies to ensure that certain dyes or dye intermediates have been completely and properly removed from wastewater through contrasting their spectra over time. It is also useful for monitoring air quality and determining the authenticity of food.

Additionally, UV-Vis spectroscopy is qualitatively advantageous in some extremely specialized investigations. Tracking variations in the wavelength that corresponds to the peak absorbance can be useful for both identifying the composition of batteries and examining specific structural protein changes. In more recent applications, such as the characterization of extremely small nanoparticles, variations in the wavelengths of peak absorbance are additionally useful. This approach has a vast array of almost infinite options [40].

IV. DERIVATIVE UV-SPECTROPHOTOMETRY

Derivative UV spectrophotometry is a popular analytical method for extracting supplementary quantitative as well as qualitative data in spectra which include unresolved regions. For either qualitative and quantitative analysis, absorbance's first or higher wavelength derivatives are employed. Derivative spectroscopy had a number of uses when it was initially developed in the 1950s. However, the method was not extensively used because of the challenge of producing derivative spectra employing UV-visible spectroscopy. Microcomputers, which produced derivative spectra in a more accurate, simple, rapid, and repeatable way, were able to overcome the vulnerability in the late 1970s [41].

The following are the goals of analytical chemistry's derivative methods:

- Differentiation of spectrum: As a qualitative method for identifying subtle variations in nearly similar spectra.
- Improvement of spectral resolution: All that is needed to resolve overlapping spectral bands is an estimate of their number and wavelengths.
- Analysis that is quantitative: It facilitates multicomponent analysis and corrects the unnecessary background absorption. The derivative spectroscopy technique is the first step in the process of distinguishing or resolving overlapping bands. The suppression of wide bands relative to sharp bands is one of the procedure's main characteristics [42].

A. Measurement Techniques of the Derivative Spectroscopy

Distinguishing a zero order spectrum of a group of components can reveal the route to a derivative spectrum of any order. To distinguish a spectrum, a variety of methods are used, including numerical and analog approaches. Spectral distinction can be conceptualized digitally in a computer's memory or graphically on paper. The derivative spectra value is measured using three methods: numerical measurement, graphic measurement in order and the zero crossing technique [43].

1) Visual measurement: The derivative spectra are calculated on paper using the theoretical method of graphic measurement. Because the value that may be determined numerically can be removed or reduced indefinitely, the manual technique has the disadvantage of yielding inaccurate results.

2) Quantitative assessment: The method uses a number of locations to estimate the derivative values at a specific wavelength. It provides derivatives using spectral differentiation using a suitable numerical method [44].

3) Zero crossing methodology: The process analyzes the derivative spectra at a given wavelength when the derivative passes through the point at the zero line. One method for eliminating interference with one component's evaluation of another is the zero crossing technique.

B. Derivative Spectra

By expanding the distinction between spectra, derivative spectra are utilized in quantitative evaluation to distinguish between overlapping bands. The Savitzky-Golay digital algorithm technique is strongly advised for generating derivative spectra. The universal approach includes plotting the rate of change of the absorbance spectrum versus wavelength. Derivative spectra can be obtained experimentally in a variety of ways, and differentiating can be carried out numerically regardless of whether the electromagnetic spectrum was initially digitally recorded or in a computer-readable format. Wavelength modulation or calculating the spectrum's time derivative while scanning it steadily are two methods for getting real-time derivative spectra [45].

Derivative curves are often created using a computerized method. The derivative spectra are recorded using a wavelength modulation device, in which the wavelength of a light beam is changed by a little amount (1-2 nm), and the difference among the two readings is noted [46].

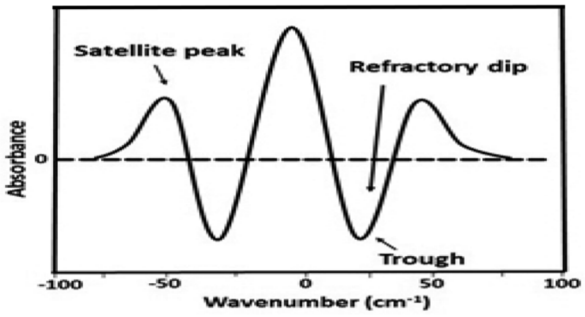


Fig 5: Derivative Spectra [47]

For second or fourth order derivative curves, the peak heights of long-wave or short-wave peak satellites are quantitatively quantified. Derivative spectra get more complicated when satellite peaks are included. The second derivative spectra show two different peaks and troughs. The solvents have a striking effect over peaks. The peaks and troughs shift to longer or shorter wavelengths according to the solvent's polarity [48].

C. The way of obtaining the derivative orders

Derivative spectroscopy is the process of converting a normal or zero order spectrum into a first, second, or higher derivative spectrum. It causes the derivative's shape to change significantly. It is possible to effectively separate overlapping signals by choosing the derivative order. It is anticipated that in order to meet parameters like signal height, breadth, and distance between maxima in the basic spectrum, higher orders will be needed for narrow spectral bands and lower orders for vast spectral bands. A Gaussian band is an optimal absorption band that clearly explains the transition occurring in the derivative spectra [49].

A graph showing a peak with maxima and minima, or the points of inflection that ought to travel below zero on the ordinate, is created when absorbance is plotted versus wavelength.

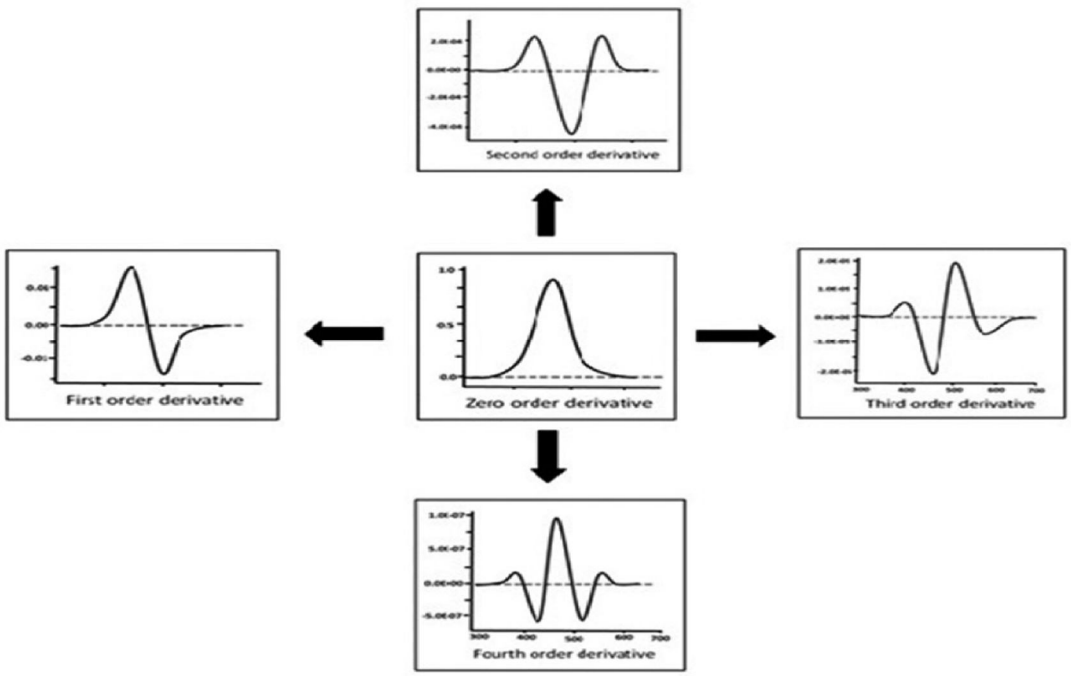


Fig 6: Oder of Derivative Spectra [50]

D. Zero order derivative spectrum

The zero order derivative is the initial stage in supplying more derivatives. That is, it is possible to derive the derivative of the nth order from infinity. One feature of the normal spectrum of absorption in derivative spectroscopy is the D0 spectrum, often known as zeroth order. The derivative of the spectra of the first, second, third, and fourth orders may be obtained directly from the zeroth order spectrum. As the order of derivatives increases, so does the sensitivity of determination. If the range is expressed as absorbance (A) as an indicator of wavelength (λ), the derivative spectrum is given as follows [51].

$$A = f(\lambda)$$

E. First order derivative spectrum

Spectra obtained by derivatizing the zero order spectrum once. This figure displays the absorbance change rate with wavelength or the absorbance change with wavelength against wavelength. The complexity is higher than that of the zero order spectrum, even when considering its derivatized version. In first order spectra, zero passes through the absorption band's λ max. The absorbance band of the first order derivative shows a positive and negative band containing peaks and minima. A dual-wavelength spectrophotometer scans the spectrum and obtains first-derivative spectra by measuring the distinction between two wavelengths [52].

$$dA/d\lambda = f'(\lambda)$$

F. Second order derivative spectrum

The absorbance spectrum is derivatized twice to get this type of spectrum. The curvature of the absorption spectra plotted against wavelength is seen here. The second derivative is immediately connected to the concentration since it is precisely proportionate. A high d2A/dλ2 ratio is required; the greater the ratio, the greater the sensitivity. The method is useful for obtaining atomic molecule and gas spectra [53].

$$d^2A/d\lambda^2 = f'(\lambda)$$

G. Third order derivative spectrum

Unlike the second order spectrum, the third derivative spectrum shows a dispersion function to the initial curve.

$$d^3A/d\lambda^3 = f''(\lambda)$$

H. Fourth-derivative spectrum

An inverted second-order spectrum with a sharper center peak than the initial band is called fourth order. Narrow bands are determined preferentially by the fourth derivative (UV-high pressure) [54].

$$d^4A/d\lambda^4 = f''(\lambda)$$

I. Polynomial degree

The polynomial degree has a significant impact on the total amount of polynomial points rather than the derivative's shape. A polynomial's range is limited; differentiation of half-width spectra is used for low-dimensional polynomials but for tiny half-width spectra associated with greater degree polynomials. The derivative spectrum is affected when the polynomial degree is incorrect. In multicomponent analysis, using different polynomial degrees may improve the selective selections and spectrum variations of the substances under examination [55].

J. Signal-to-noise ratio

It becomes difficult to use a derivative technique with more complex orders that provide a reduced signal-to-noise ratio. As a result, S/N falls with increasing orders. Noise produces the sharpest features in the spectrum. The low-noise capabilities of the spectrophotometer are particularly stressed since derivatization has a negative impact on S/N. If a spectrophotometer were to undergo an scan spectra and average a large number of them prior to derivatization, S/N may be raised. The optimal signal-to-noise ratio can be determined by subtracting the lowest minimum from the greatest maximum, however this makes the system more vulnerable to interference by other sources. The standard deviation, or σ, of a signal is a measure of its noise [56].

The standard deviation the noise that occurs in the normal spectrum of the blank absorbance is expressed by σ0, whereas the standard deviation σn shows the nth order derivative that may be obtained by σ0 [57].

K. Smoothing of spectra

To lessen high-frequency noise or to mitigate the effects of an increase in the signal-to-noise ratio, a method called low-pass filtering or smoothing is employed. Smoothing is a process that is applied to each row of data on an individual spectrum and works on neighboring variables. Much less noise may be produced by closely spaced variables with similar information in the data matrix without compromising the signal of interest. Because a high degree of smoothing may alter the derivative spectrum, caution must be used. The smoothing frequency (a) and the smoothing ratio (b), which is the width of the smoother peak divided by the total number M of data points, are the two main factors that affect the smoothing effect [58].

L. Advantages and Disadvantages of Derivative UV-Spectrophotometry

Advantages: UV Derivative Spectroscopy has improved in both sensitivity and selectivity. Identifying organic and inorganic compounds, identifying traces in a matrix, analyzing proteins and amino acids, identifying single components and numerous components in a mixture at once, and identifying traces in the environment are just a few of its many advantages. Some specific benefits of derivative spectral analysis are as follows [59]:

- When more than one overlapping peaks are present, absorbance bands can be identified even in a small wavelength range.
- When high and sharp absorbance peaks are present, weak and microscopic absorbance peaks can be identified.
- The quantitative analysis may be carried out even in the presence of background absorption since the derivative values and concentration levels have a linear connection; a broad absorbance spectrum gives a clear comprehension of the wavelength at that maximum spectrum [60].

M. Disadvantages

Even though it is a delicate method, it is however quite susceptible to a number of conditions. The process is limited to a particular system and has few applications due to its poor reproducibility. The method comes in second when the existing instrumental methodology, that evaluates signal, is not accessible. It is less accurate when measuring zero-crossing spectra. Due to the comparable nature of the derivative and zero order spectra, minor modifications to the basic spectrum can have a substantial impact on the derivative spectrum.

Poor repeatability may have an impact on the results when many spectrophotometers employed to obtain zero order spectra provide similar results but their derivatization shows different outcomes [61].

N. Applications

1) Single component analysis: Derivative spectrophotometry examines both the Area under the Curve and a single component in a pharmaceutical product.
2) Multicomponent analysis: Derivative spectrophotometry is used in pharmaceutical analysis to determine two or more compounds simultaneously by studying a number of elements in the presence of additional components. Spectral derivatization can remove the preponderance caused by the spectra of unwanted compounds.
3) Bioanalytical application: Beyond pharmaceutical analysis, derivative spectrophotometry has additional applications. characterization of the compounds found in various biological samples, such as urine, serum, plasma, and brain tissue. Human plasma has been shown to contain the amphotericine and diazepam derivative combination [62].
4) Forensic toxicology: Amphetamine, ephedrine, meperidine, and diazepam are among the illicit compounds that may be studied by derivative spectroscopy, which can be used in mixes.
5) Trace analysis: When assessing trace amounts of substances in the presence of high concentrations of potentially interfering molecules, the derivative signal processing approach is commonly used in real-world analytical jobs. This interference causes analytical signals to become weak, noisy, and superimposed over strong background signals. In addition to non-specific broadband interfering absorption, non-reproducible cuvette location, dirt or fingerprints on the cuvette walls, incorrect cuvette transmission matching, and solution turbidity, sample-to-sample baseline fluctuations can contribute to decreased measurement accuracy. Baseline shifts may result from practical errors, which can be either wavelength independent (massive suspended particles or bubbles blocking light) or weakly wavelength dependent (small particle turbidity) [62].

As a result, relevant absorption must be distinguished from the previously described baseline shift sources. Differentiation is expected to reduce sample-to-sample variability in background amplitude by attenuating the vast background. This frequently results in improved measurement precision and accuracy, especially when the analyte signal is weak compared to the background and the background is full of uncontrolled variability [63].

V. HPLC (HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY)

One of the most effective analytical methods for separating, identifying, and quantifying substances in a mixture is High-Performance Liquid Chromatography (HPLC). Pharmaceuticals, biotechnology, food safety, environmental studies, and many other scientific domains make extensive use of it. The basis of HPLC's operation is liquid chromatography, in which mixture constituents are separated according to how they interact with a stationary phase and a liquid mobile phase under high pressure [64].

A. Basic Principle of HPLC

Differential migration, the fundamental idea behind HPLC, is the process by which analytes in a sample mixture move down a chromatographic column at various rates because of their varied solubilities in the mobile phase and affinities for the stationary phase. The process of separation entails:

- The sample is carried along the column by the mobile phase, which is a liquid solvent or combination of solvents.
- Stationary Phase: A stationary phase within the column that causes separation by interacting with the analytes.
- Chemical Interactions: Analytes' polarity, hydrophobicity, charge, and molecular size all affect how they interact with the stationary and mobile phases [65].

Retention periods vary depending on how each mixture component interacts with the stationary and mobile phases. Compounds that are more firmly held elute more slowly than those that are poorly retained.

B. HPLC System Components

1) Mobile Phase Reservoirs: These hold the liquid mobile phase, which may consist of a combination or a single solvent. The kind of analytes and stationary phase determine which mobile phase is best.
2) Pump: Provides a constant flow rate by creating high pressure to force the mobile phase throughout the system [66].
3) Injector: Typically, an autosampler or manual injection port is used to introduce the sample into the mobile phase of the stream.
4) Column: The central element where separation takes place. It contains a stationary phase, like things made of silica. Various kinds of columns are employed according to the requirements for separation.
5) Detector: This device measures physical or chemical characteristics (such as UV absorption, fluorescence, and refractive index) to identify and quantify separated analytes [67].
6) Data System: Creates chromatograms with peak characteristics corresponding to various chemicals by recording and analyzing chromatographic findings.

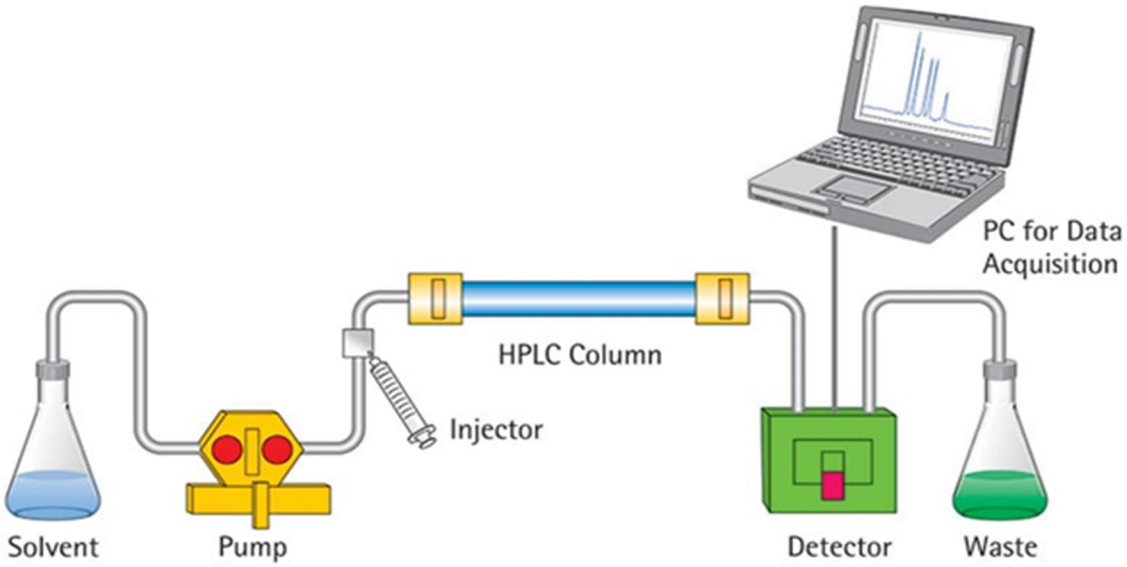


Fig 7: HPLC System Components [68]

C. Mechanism of Separation in HPLC

Analyte differential migration between a stationary phase with a liquid mobile phase is the basis for HPLC separation. Numerous physical and chemical interactions that occur between analytes and the stationary/mobile phases cause the separation to happen. These interactions eventually lead to the resolution of individual components within a mixture by determining the retention time, or the amount of time it takes for an analyte to move through the column.A number of variables affect separation efficiency, such as sample characteristics, temperature, flow rate, stationary phase selection, and mobile phase composition. The following are the main mechanisms that control separation in HPLC [69]:

1) Adsorption Chromatography (Normal-Phase HPLC, NP-HPLC)

Principle: The basic idea behind adsorption chromatography is the differential interaction of analytes with a polar stationary phase and an inversely polar mobile phase. This method is known as Normal-Phase HPLC (NP-HPLC) because it adheres to the conventional polarity principle, which states that non-polar compounds elute more quickly while more polar substances are retained more time because of their more powerful interactions with the stationary phase of the process [70].

Stationary Phase: Usually composed of modified silica containing polar functional groups (such as amino, cyano, or diol groups) or bare silica. Non-polar organic solvents like hexane, chloroform, ethyl acetate, or combinations of these make up the mobile phase.

The procedure of separation involves the adsorption of polar analytes into the stationary phase by van der Waals forces, hydrogen bonds, or dipole-dipole interactions. Stronger contacts result in longer retention times, while non-polar analytes elute more quickly due to their weaker interactions with the phase that is stationary.

Applications: Effective in lipid profiling and natural product examination, it is also used to separate polar substances such vitamins, lipids, carbohydrates, and some pharmaceutical medications [71].

2) Partition Chromatography (Reverse-Phase HPLC, RP-HPLC)

Principle: The basis of partition chromatography is the difference in analyte solubility between a chemically bound stationary phase and the mobile phase. The most commonly employed HPLC mode is reverse-phase HPLC (RP-HPLC), in which the mobile phase is comparatively polar and the stationary phase is non-polar.

Stationary Phase: Water-soluble silica with a C18 or C8 bond (octadecylsilane or octylsilane groups). Non-polar analytes have a stronger interaction with non-polar stationary phases.

Mobile Phase: A polar solvent or combination of solvents, often acetonitrile, methanol, water, or buffers (such as formic acid or phosphate buffer) [72].

Separation Mechanism: Because of their hydrophobic interactions with the stationary phase, non-polar analytes are more firmly held. By changing the polarity of the mobile phase, analytes' retention times may be modified. Polar analytes elute more quickly and interact with the stationary phase less strongly.

Applications: The most frequently encountered for the separation of medications, peptides, proteins, and metabolites; widely utilized in pharmaceutical analysis, safety in food testing, bioanalytical investigations, and surveillance of the environment [73].

3) Ion Exchange Chromatography (IEX-HPLC)

Principle: Charged analytes are separated by ion exchange chromatography according to how they interact with opposite one another charged groups on the stationary phase.The intensity of ionic interactions determines the separation, which may be controlled by changing the buffer's composition, pH, or ionic strength.

Stationary Phase: Has fixed charged functional groups found in ion-exchange resins: To maintain positively charged analytes (cations), cation-exchange chromatography (CEX) employs negatively charged groups (such as carboxylate and sulfonic acid). To maintain negatively charged analytes (anions), anion-exchange chromatography (AEX) employs positively charged groups, such as quaternary ammonium [74].

Mobile Phase: Salts (like potassium chloride and sodium chloride) or buffers (like phosphate and acetate) are used to regulate elution and alter ionic interactions in aqueous buffer systems with different pH and ionic strengths.

Separation Mechanism: Analyte elution results from competition between analyte and mobile phase ions caused by increasing the ionic strength of the mobile phase; charged analytes bind with the oppositely charged stationary phase; and the electric charge density and interacting resilience of each analyte determine the order of elution.

Applications: Utilized in water quality testing, peptide analysis, protein purification, and nucleic acid separation; crucial for biotechnology, pharmaceutical, and environmental purposes [75].

4) Size-Exclusion Chromatography (SEC-HPLC)

Principle: Also known as Gel Filtration Chromatography (GFC) or Gel Permeation Chromatography (GPC), separation depends on the molecular dimensions of analytes as opposed to the nature of their chemical interactions.

Stationary Phase: A porous substance having regulated pore sizes, including silica-based gels, agarose, polyacrylamide, or cross-linked dextran [76].

Mobile Phase: Organic or aqueous solvents, according on the kind of material (tetrahydrofuran for polymers, water for proteins, etc.).

Separation Mechanism: Molecules that are smaller enter the tiny openings and require longer to pass through, eluting later, whereas larger molecules cannot pass through the stationary phase's pores and move more quickly, eluting first.

Applications: Molecular weight analysis is crucial in the pharmaceutical, biomedical, and polymer sectors. It is frequently used for the examination of polymers, proteins, peptides, and polysaccharides [77].

5) Affinity Chromatography (Affinity-HPLC)

Principle: This method is very selective as it captures target molecules through antigen-antibody, enzyme-substrate, receptor-ligand, or metal-protein interactions. It also takes advantage of certain interactions between organisms between an analyte and a ligand mounted on the phase that is stationary.

Stationary Phase: Ligand-functionalized resins, including nickel-coated surfaces for His-tagged proteins, protein A, concanavalin A, or heparin [78].

Mobile Phase: A binding buffer to help the analyte and stationary phase interact.

To break connections and liberate analytes, use an elution buffer containing competing molecules or one with a changed pH or ion concentration.

Separation Mechanism: The ligand on the stationary phase is preferentially bound by the target analyte, and unbound components are removed by washing. The residual analyte is eluted when the mobile phase parameters (pH, salt concentration, and competitive binding) change.

Applications: Important in biotechnology, diagnostics, and pharmaceutical research, it is used for protein purification, antibody isolation, and the synthesis of recombinant proteins [79].

VI. FACTORS AFFECTING HPLC SEPARATION

Numerous factors pertaining to the stationary and mobile phases, in addition to experimental settings, affect the HPLC separation's resolution and efficiency. Gaining repeatable findings, increasing sensitivity, and optimizing separation all depend on an understanding of these variables. The following are the main variables affecting HPLC separation:

A. Mobile Phase Composition

Analyte retention, resolution, and peak form are all significantly influenced by the mobile phase. By modifying the buffer's composition, polarity, pH, and ionic strength, the mobile phase's composition may be tuned [80].

1) Polarity of the Mobile Phase

- The degree to which analytes interact with the stationary phase is influenced by the polarity of the mobile phase.
- Polar analytes are held longer in Normal-Phase HPLC (NP-HPLC) because to their interaction with the polar stationary phase (silica) and the application of a non-polar mobile phase (such as hexane or chloroform).
- In Reverse-Phase HPLC (RP-HPLC), non-polar analytes are held for a longer period of time because of hydrophobic interactions through the non-polar stationary phase (C18 or C8) and a polar mobile phase (such as water, methanol, or acetonitrile) [81].

2) pH of the Mobile Phase

- The ionization of analytes is strongly influenced by the pH of the mobile phase, which also affects peak form and retention.
- Since little variations in pH can result in significant changes in retention durations, pH management is essential for the separation of weak acids and bases.
- In RP-HPLC, a lower pH (below the pKa) for acidic analytes increases retention by suppressing ionization.
- A higher pH (above the pKa) for basic analytes increases retention by suppressing ionization.
- To keep the pH steady, buffers (such as phosphate, acetate, and ammonium formate) are frequently added [82].

3) Ionic Strength of the Mobile Phase

- Ionic strength controls how charged analytes interact with the stationary phase in ion-exchange chromatography (IEX-HPLC).
- Adding salts (such as KCl or NaCl) to increase the ionic strength might cause elution by competing with the analyte ions.
- For ionizable chemicals, a higher ion concentration can enhance resolution and lessen peak tailing [83].

B. Stationary Phase Type

Selectivity, retention duration, and separation efficiency are all impacted by the stationary phase's composition. The interaction between analytes and the phase, which affects peak form and resolution, is determined by the column packing composition. A greater accuracy is achieved with smaller particle sizes (1.7 µm vs. 5 µm), but higher back pressure is needed. For SEC-HPLC, choosing the right pore size is crucial since bigger holes enable the study of bigger molecules like proteins and polymers [84].

C. Flow Rate

Analysis duration, resolution, and sensitivity are all impacted by the mobile phase's flow rate. Greater Flow Rates: o Shorten retention periods (elution occurs more quickly). Inadequate contact time with the stationary phase results in a decrease in resolution. Could result in overlapping peaks and poor separation [85]. Reduced Flow Rates: Increase resolution by extending the duration of analyte interaction with the stationary phase. Lengthen retention periods by increasing analytical time. Conventional HPLC flow rates for ordinary columns (4.6 mm ID) are between 0.5 and 1.5 mL/min. Smaller particles (sub-2 µm) are used in Ultra-High-Performance Liquid Chromatography (UHPLC), which necessitates greater flow rates (1–2 mL/min) under elevated pressures.

D. Temperature

Reproducibility, peak shape, and retention duration are all impacted by temperature management in HPLC. Raising the temperature lowers column back pressure by decreasing solvent viscosity. Sharper peaks result from improved mass transfer across the stationary and mobile phases. Shortens retention periods for a variety of analytes [86].

Reproducibility and column longevity can be enhanced. Heat-sensitive analytes, such as proteins and peptides, may be degraded by an excessively high temperature. Modify column selectivity, which will impact repeatability. The majority of separations take place between 25 and 40°C. Compounds that are temperature-sensitive might need to be cooled (biomolecules, for example, need to be at 4°C).

E. Injection Volume

In order to avoid carryover effects, resolution loss, and peak broadening, the injection volume needs to be adjusted. Excessive Injection Volume: o Causes overloading, which results in peak widening and tailing. Decreases separation performance and column efficiency. Insufficient Injection Volume: This lowers detection sensitivity by producing a weak signal. 5–20 µL for standard columns (4.6 mm ID) in analytical HPLC. For UHPLC (lower column diameters), 1–5 µL. Preparative HPLC: o Depending on the size of the column, injection quantities can range from 100 µL to several millilitres [87].

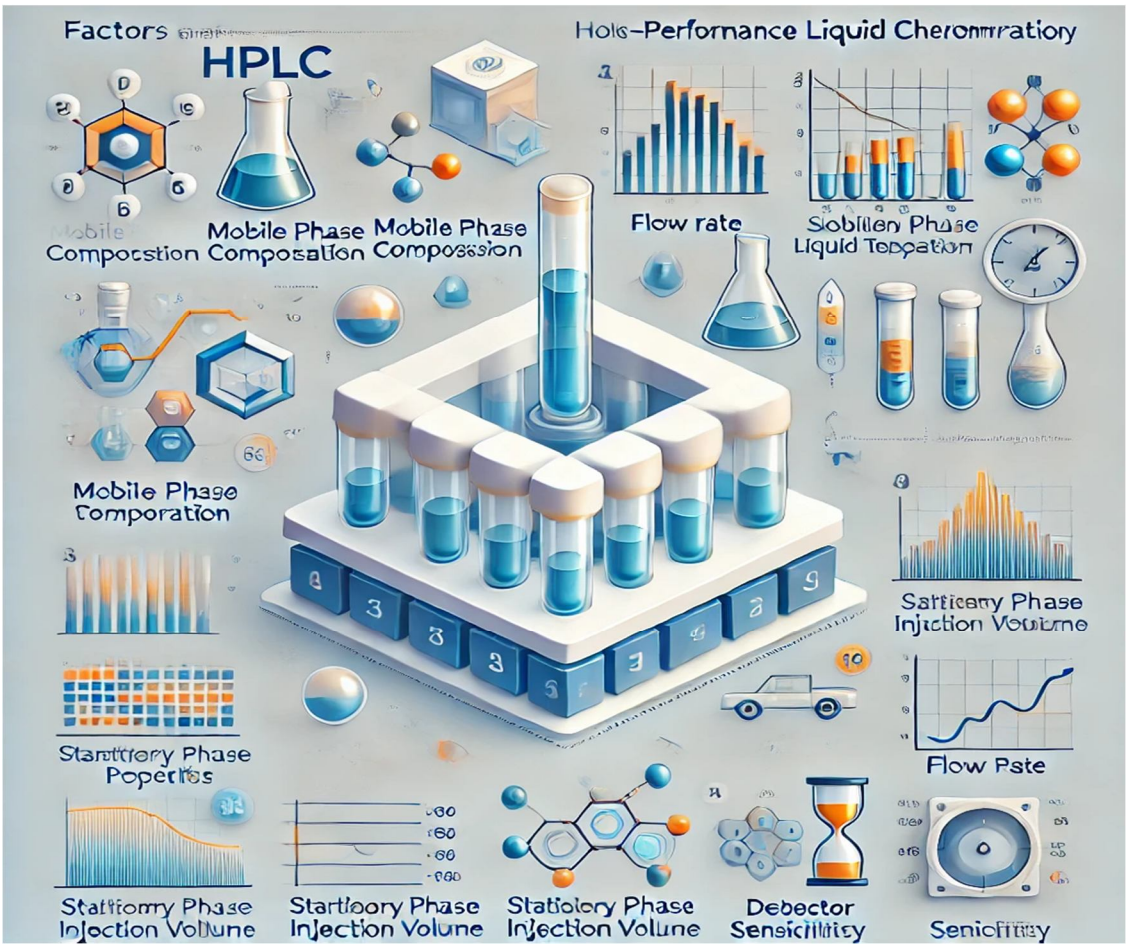


Fig 8: Factors Affecting HPLC Separation [88]

F. Applications of HPLC in the Pharmaceutical Sector

1) Drug Development and Formulation

The pharmaceutical sector uses HPLC extensively for the discovery and formulation of new drugs. It aids in evaluating the stability of medications under various circumstances and describing their chemical makeup.The method guarantees that the finished pharmaceutical product will remain safe and effective for the duration of its shelf life [89].

2) Quality Control and Regulatory Compliance

Strict quality control inspections are required by regulatory bodies like the EMA and the U.S. FDA, and HPLC is the recommended technique to guarantee compliance. It assists in identifying changes in medication formulations, degradation products, and contaminants. By guaranteeing batch-to-batch uniformity, HPLC keeps fake medications off the market [90].

3) Pharmacokinetics and Bioavailability Studies

HPLC is used in clinical research to quantify medication concentrations in biological samples like urine and blood. In pharmacokinetic and bioequivalence investigations, it is essential for figuring out how drugs are absorbed, distributed, metabolized, and excreted. The method is essential for assessing how well generic medications work in comparison to branded versions.

4) Identification of Impurities and Degradation Products

HPLC plays a crucial role in pharmaceutical impurity profiling, guaranteeing the identification and removal of dangerous compounds. It aids in identifying degradation products that could arise as a result of environmental factors including moisture, light, or heat. This guarantees that medications are safe and effective for the duration of their lives [91].

5) Analysis of Complex Biological Molecules

Complex biological compounds, such as proteins, nucleic acids, and peptides, can be separated and quantified using HPLC. It is essential in biopharmaceuticals, where accurate biomolecule analysis is required to guarantee therapeutic efficacy. Developments like Ultra-High-Performance Liquid Chromatography (UHPLC) have increased these studies' resolution and speed.

6) Stability Testing of Pharmaceutical Products

Stability studies evaluate a drug's degradation over time under various storage circumstances. HPLC aids in monitoring chemical alterations and guarantees that medications do not become less effective or produce harmful byproducts. This data is essential for figuring out pharmaceutical product storage recommendations and shelf life [92].

7) Detection of Counterfeit and Substandard Drugs

By examining their chemical makeup, HPLC is a crucial method for detecting fake pharmaceuticals. By guaranteeing that pharmaceutical items fulfill the necessary requirements, it stops inferior or counterfeit medications from being sold. This strengthens public health protection and promotes patient safety [93-102].

VII. CONCLUSION

In pharmaceutical analysis, HPLC and UV spectroscopy are essential analytical methods that each have special benefits for guaranteeing the efficacy, safety, and quality of drugs. HPLC is crucial for identifying active pharmaceutical ingredients (APIs), detecting contaminants, and carrying out stability studies because of its great precision, sensitivity, and capacity to separate complicated mixtures. UV Spectroscopy, on the other hand, is frequently employed in regular quality control and formulation development processes since it offers a quick and affordable way to do quantitative analysis. The precision and dependability of pharmaceutical evaluations are improved by the combination of various methods, especially HPLC-UV.

Both approaches have drawbacks that need to be taken into account in pharmaceutical applications, notwithstanding their benefits. HPLC necessitates costly equipment, skilled workers, and lengthy processes, whereas UV spectroscopy is only applicable to substances that absorb UV light and does not have the same capacity for separation as HPLC. Recent developments have increased analytical efficiency and expanded their potential uses, such as diode-array detection (DAD) in UV spectroscopy and Ultra-High-Performance Liquid Chromatography (UHPLC). These methods' ongoing development guarantees their use in contemporary pharmaceutical manufacture and research.

All things considered, HPLC and UV spectroscopy together continue to be a vital component of pharmaceutical analysis, aiding in quality control, regulatory compliance, and medication discovery. They are vital instruments in the pharmaceutical sector because of their complementing functions in impurity detection, stability monitoring, and batch-to-batch consistency assurance. As technology develops, combining these approaches with new analytical methods like chemometrics and mass spectrometry can improve drug analysis even further, ultimately leading to better treatment results and patient safety.

1) Acknowledgement: The authors are thankful to Department of Pharmacy, LLYOD College, Greater Noida for providing kind guidance and excellent opportunity as well as necessary facilities for the research.

2) Conflicts of Interest: The authors confirm that the content of the article has no conflict of interest.

3) Data Availability: The original data that support the findings of this study are included in the Article.

4) Funding: This research paper received no external funding.

REFERENCES

[1] Picollo M, Aceto M, Vitorino T. UV-Vis spectroscopy. Physical sciences reviews. 2019 Mar 26;4(4):20180008.
[2] Verma G, Mishra M. Development and optimization of UV-Vis spectroscopy-a review. World J. Pharm. Res. 2018 Apr 19;7(11):1170-80.
[3] Brown JQ, Vishwanath K, Palmer GM, Ramanujam N. Advances in quantitative UV–visible spectroscopy for clinical and pre-clinical application in cancer. Current opinion in biotechnology. 2009 Feb 1;20(1):119-31.
[4] Förster H. UV/vis spectroscopy. Characterization I: -/-. 2004 Feb 11:337-426.
[5] Shinde G, Godage RK, Jadhav RS, Manoj B, Aniket B. A review on advances in UV spectroscopy. Research Journal of Science and Technology. 2020;12(1):47-51
[6] Rocha FS, Gomes AJ, Lunardi CN, Kaliaguine S, Patience GS. Experimental methods in chemical engineering: Ultraviolet visible spectroscopy—UV-Vis. The Canadian Journal of Chemical Engineering. 2018 Dec;96(12):2512-7.
[7] Van Den Broeke J, Langergraber G, Weingartner A. On-line and in-situ UV/vis spectroscopy for multi-parameter measurements: a brief review. Spectroscopy europe. 2006;18(4):15-8.
[8] Power AC, Chapman J, Chandra S, Cozzolino D. Ultraviolet-visible spectroscopy for food quality analysis. Evaluation technologies for food quality. 2019 Jan 1:91-104.
[9] Guo Y, Liu C, Ye R, Duan Q. Advances on water quality detection by uv-vis spectroscopy. Applied Sciences. 2020 Sep 30;10(19):6874.
[10] Begum R, Farooqi ZH, Naseem K, Ali F, Batool M, Xiao J, Irfan A. Applications of UV/Vis spectroscopy in characterization and catalytic activity of noble metal nanoparticles fabricated in responsive polymer microgels: a review. Critical reviews in analytical chemistry. 2018 Nov 2;48(6):503-16.
[11] Liauw MA, Baylor LC, O'Rourke PE. UV-Visible Spectroscopy for On-Line Analysis. Process analytical technology: Spectroscopic tools and implementation strategies for the chemical and pharmaceutical industries. 2010 Apr 16:81-106.
[12] Hou DB, Zhang J, Chen L, Huang PJ, Zhang GX. Water quality analysis by UV-Vis spectroscopy: A review of methodology and application. Spectroscopy and Spectral Analysis. 2013 Jul 15;33(7):1839-44.
[13] Schoonheydt RA. UV-VIS-NIR spectroscopy and microscopy of heterogeneous catalysts. Chemical Society Reviews. 2010;39(12):5051-66.
[14] Perkampus HH. UV-VIS Spectroscopy and its Applications. Springer Science & Business Media; 2013 Mar 8.
[15] Jentoft FC. Ultraviolet–visible–near infrared spectroscopy in catalysis: theory, experiment, analysis, and application under reaction conditions. Advances in catalysis. 2009 Jan 1;52:129-211.
[16] Uríčková V, Sádecká J. Determination of geographical origin of alcoholic beverages using ultraviolet, visible and infrared spectroscopy: A review. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015 Sep 5;148:131-7.
[17] Venkatachalam S. Ultraviolet and visible spectroscopy studies of nanofillers and their polymer nanocomposites. Spectroscopy of polymer nanocomposites. 2016 Jan 1:130-57.
[18] Ríos-Reina R, Azcarate SM. How chemometrics revives the UV-Vis spectroscopy applications as an analytical sensor for spectralprint (nontargeted) analysis. Chemosensors. 2022 Dec 22;11(1):8.
[19] Rojas FS, Ojeda CB. Recent development in derivative ultraviolet/visible absorption spectrophotometry: 2004–2008: A review. Analytica chimica acta. 2009 Mar 2;635(1):22-44.
[20] Lange R, Balny C. UV-visible derivative spectroscopy under high pressure. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology. 2002 Mar 25;1595(1-2):80-93.
[21] Sirajuddin M, Ali S, Badshah A. Drug–DNA interactions and their study by UV–Visible, fluorescence spectroscopies and cyclic voltametry. Journal of Photochemistry and Photobiology B: Biology. 2013 Jul 5;124:1-9.
[22] Yang Z, Zhu M. Case Studies: Ultraviolet-Visible (UV-Vis) Spectroscopy. InSpringer Handbook of Advanced Catalyst Characterization 2023 May 18 (pp. 265-283). Cham: Springer International Publishing.
[23] Chen J, Li JQ, Li T, Liu HG, Wang YZ. Application of UV-Vis and infrared spectroscopy on wild edible bolete mushrooms discrimination and evaluation: a review. Critical Reviews in Analytical Chemistry. 2023 May 19;53(4):852-68.
[24] Fedenko VS, Shemet SA, Landi M. UV–vis spectroscopy and colorimetric models for detecting anthocyanin-metal complexes in plants: An overview of in vitro and in vivo techniques. Journal of plant physiology. 2017 May 1;212:13-28.
[25] Lai Q, Zhu S, Luo X, Zou M, Huang S. Ultraviolet-visible spectroscopy of graphene oxides. AIP advances. 2012 Sep 1;2(3).
[26] Ojeda CB, Rojas FS. Recent developments in derivative ultraviolet/visible absorption spectrophotometry. Analytica Chimica Acta. 2004 Aug 2;518(1-2):1-24.
[27] Plane JM, Saiz-Lopez A. UV-Visible differential optical absorption spectroscopy (DOAS). Analytical techniques for atmospheric measurement. 2006 Apr 25:147-88.
[28] Atole DM, Rajput HH. Ultraviolet spectroscopy and its pharmaceutical applications-a brief review. Asian J pharm clin res. 2018;11(2):59-66.
[29] Cox H, Stace AJ. Recent advances in the visible and UV spectroscopy of metal dication complexes. International Reviews in Physical Chemistry. 2010 Oct 1;29(4):555-88.
[30] Imam SS. The future of non-invasive ways to treat cancer. Int J Pharm Sci & Res. 2021; 12(8): 4684-96.
[31] Imam SS, Agarwal S. A Pragmatic Approach To Treat Lung Cancer Through LoadingTheaflavin -3,3'-Digallate And Epigallocatechin Gallate In Spanlastic. Asian J Pharm Clin Res. 2021 Nov 7; 14(11): 1-8.
[32] Imam SS, Imam ST, Mdwasifathar, Kumar R, Ammar MY. Interaction Between Ace2 And Sars-Cov2, And Use Of EGCG And Theaflavin To Treat Covid 19 In Initial Phases. International Journal of Current Pharmaceutical Research. 2022 Mar; 14(2):5-10.
[33] Imam SS, Sharma R. Natural compounds promising way to treat Lung Cancer. International Journal of Pharmaceutical Research and Applications. 2023; 8(2): 552- 558.
[34] Imam SS, Sharma S, Kumari D, Khan S, Pathak P, Katiyar D. An Expedient Approach to Treat Asthma through Non-Steroidal, Natural Transferosomes Aerosol System. Innovare journal of medical sciences. 2022; 10(6): 7-11.
[35] Imam SS, Imam ST, Agarwal S, Kumar R, Ammar MY, Athar MW, Akthar A. Lung Cancer Therapy Using Naturally Occurring Products and Nanotechnology. Innovare journal of medical sciences. 2022; 10(4): 1-5.

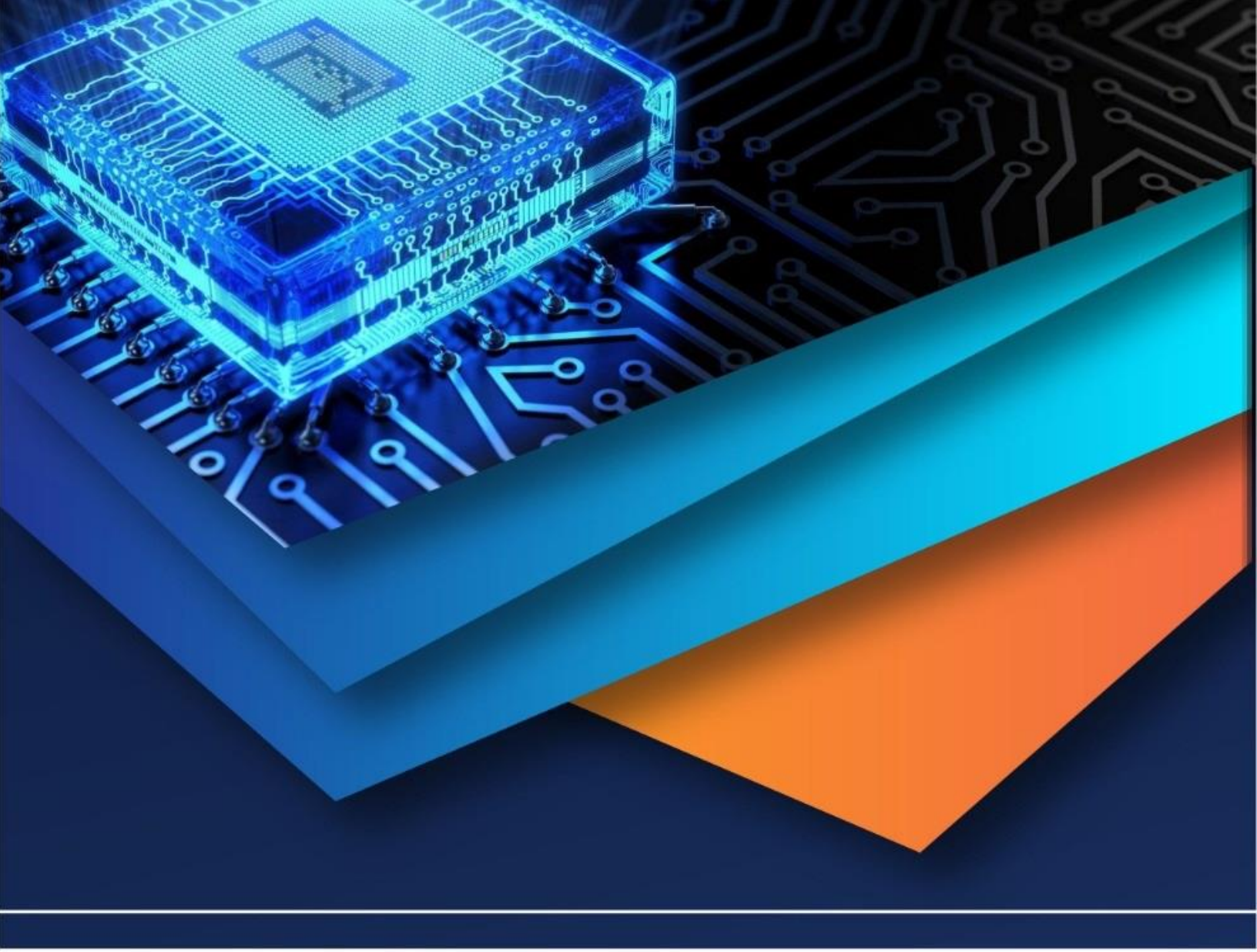
[36] Imam ST, Imam SS. The Cream which relieves the pain of Menstrual cramps without interfering with the Hormones or Period Cycle. Research Journal of Pharmacy and Technology. 2023; 16(3):1239-6.
[37] Imam SS. Topical Formulation Constituted with Transferosomes for the Treatment Of Non-Melanoma Skin Cancer. Asian J Pharm Clin Res. 2023 May 7;16(5):27-32.
[38] IMAM SS. NANOPARTICLES: THE FUTURE OF DRUG DELIVERY. Int J Curr Pharm Sci. 2023 Nov. 15;15(6):8-15.
[39] Imam SS, Mehdi S, Mansuri A. A COMPREHENSIVE REVIEW ON THE PHARMACOLOGICAL POTENTIAL OF RED GINSENG OIL, TEA TREE OIL AND HEMP SEED OIL. European Journal of Biomedical. 2024;11(4):167-77.
[40] Imam SS. Sublingual Tablets Amalgamated with Nano-particles and natural products to treat Oral Cancer. 2024; 17(5): 2056-62.
[41] Imam SS, Mehdi S. Factors Affecting Pharmaceutical Pricing In India. INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES. 2024;2(4):1367-1384. DOI: 10.5281/zenodo.11314004.
[42] Mehdi S, Tabrez UZ, Imam SS. Management of Green Pharmaceuticals. International Journal of Pharmaceutical Sciences And Research. 2024;15(7):1951-1962. DOI: 10.13040/IJPSR.0975-8232.15(7).1951-62.
[43] Roberts J, Power A, Chapman J, Chandra S, Cozzolino D. The use of UV-Vis spectroscopy in bioprocess and fermentation monitoring. Fermentation. 2018 Mar 13;4(1):18.
[44] Ahuja S. Overview: Handbook of pharmaceutical analysis by HPLC. Separation science and technology. 2005 Jan 1;6:1-7.
[45] Bulduk İ. Comparison of HPLC and UV spectrophotometric methods for quantification of favipiravir in pharmaceutical formulations. Iranian journal of pharmaceutical research: IJPR. 2021;20(3):57.
[46] Hosny NM, Huddersman K, El-Gizawy SM, Atia NN. New approach for simultaneous analysis of commonly used antigout drugs by HPLC/UV method; application in pharmaceutical and biological analysis. Microchemical Journal. 2019 Jun 1;147:717-28.
[47] Sarkar M, Khandavilli S, Panchagnula R. Development and validation of RP-HPLC and ultraviolet spectrophotometric methods of analysis for the quantitative estimation of antiretroviral drugs in pharmaceutical dosage forms. Journal of Chromatography B. 2006 Jan 18;830(2):349-54.
[48] Kepekci Tekkeli SE. Development of an HPLC-UV Method for the Analysis of Drugs Used for Combined Hypertension Therapy in Pharmaceutical Preparations and Human Plasma. Journal of analytical methods in chemistry. 2013;2013(1):179627.
[49] Bulduk I, Akbel E. A comparative study of HPLC and UV spectrophotometric methods for remdesivir quantification in pharmaceutical formulations. Journal of Taibah University for Science. 2021 Jan 1;15(1):507-13.
[50] Hoelke B, Gieringer S, Arlt M, Saal C. Comparison of nephelometric, UV-spectroscopic, and HPLC methods for high-throughput determination of aqueous drug solubility in microtiter plates. Analytical chemistry. 2009 Apr 15;81(8):3165-72.
[51] Siddiqui MR, AlOthman ZA, Rahman N. Analytical techniques in pharmaceutical analysis: A review. Arabian Journal of chemistry. 2017 Feb 1;10:S1409-21.
[52] Rossi C, Fardella G, Chiappini I, Perioli L, Vescovi C, Ricci M, Giovagnoli S, Scuota S. UV spectroscopy and reverse-phase HPLC as novel methods to determine Capreomycin of liposomal fomulations. Journal of pharmaceutical and biomedical analysis. 2004 Oct 29;36(2):249-55.
[53] Abdulwahab S, Ali EE, Hassan WS, Azab SM. Mathematically assisted UV-spectrophotometry as a greener alternative to HPLC-UV for quality control analysis of free-drug combinations. Microchemical Journal. 2021 Nov 1;170:106638.
[54] Bonfilio RB, De Araujo MB, Salgado HR. Recent applications of analytical techniques for quantitative pharmaceutical analysis: A review. WSEAS Trans. Biol. Biomed. 2010 Oct 1;7(4):316.
[55] Vieira DC, Salgado HR. Comparison of HPLC and UV spectrophotometric methods for the determination of cefuroxime sodium in pharmaceutical products. Journal of chromatographic science. 2011 Aug 1;49(7):508-11.
[56] Pourmand MR, Azar MS, Aghavalijamaat M. Development of validated UV spectrophotometric method for in vitro analysis of sumatriptan in pharmaceutical preparations in comparison with HPLC. Pharmaceutical Chemistry Journal. 2011 Jan;44(10):585-9.
[57] Mofavvaz S, Sohrabi MR, Heydari A. Application of UV/vis spectrophotometry based on using least squares support vector machine and continuous wavelet transform methods for the simultaneous analysis of antibiotics drugs in tablet formulation: Comparison with HPLC method. Optik. 2020 Oct 1;220:165246.
[58] Naveed S. Analytical determination of lisinopril using UV spectrophotometer and HPLC: An Overview. Mod Chem appl. 2014;2(137):2.
[59] Hadad GM, El-Gindy A, Mahmoud WM. HPLC and chemometrics-assisted UV-spectroscopy methods for the simultaneous determination of ambroxol and doxycycline in capsule. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2008 Aug 1;70(3):655-63.
[60] Cavrini V, Di Pietra AM, Gatti R. Analysis of miconazole and econazole in pharmaceutical formulations by derivative UV spectroscopy and liquid chromatography (HPLC). Journal of Pharmaceutical and Biomedical analysis. 1989 Jan 1;7(12):1535-43.
[61] Breier AR, Steppe M, Schapoval EE. Validation of UV spectrophotometric method for fexofenadine hydrochloride in pharmaceutical formulations and comparison with HPLC. Analytical letters. 2007 Oct 1;40(12):2329-37.
[62] Shokouhi S, Sohrabi MR, Mofavvaz S. Comparison between UV/Vis spectrophotometry based on intelligent systems and HPLC methods for simultaneous determination of anti-diabetic drugs in binary mixture. Optik. 2020 Mar 1;206:164304.
[63] da Costa Cesar I, Nogueira FH, Pianetti GA. Comparison of HPLC, UV spectrophotometry and potentiometric titration methods for the determination of lumefantrine in pharmaceutical products. Journal of pharmaceutical and biomedical analysis. 2008 Sep 10;48(1):223-6.
[64] Rodrigues AS, Lopes AR, Leão A, Couceiro A, Ribeiro AB, Ramos F, da Silveira MI, de Oliveira CR. Development of an analytical methodology for simultaneous determination of vincristine and doxorubicin in pharmaceutical preparations for oncology by HPLC—UV. Journal of chromatographic science. 2009 May 1;47(5):387-91.
[65] Marques GS, Leão WF, Lyra MA, Peixoto MS, Monteiro RP, Rolim LA, Xavier HS, Neto PJ, Soares LA. Comparative evaluation of UV/VIS and HPLC analytical methodologies applied for quantification of flavonoids from leaves of Bauhinia forficata. Revista Brasileira de Farmacognosia. 2013 Jan 1;23(1):51-7.
[66] Patrolecco L, Ademollo N, Grenni P, Tolomei A, Caracciolo AB, Capri S. Simultaneous determination of human pharmaceuticals in water samples by solid phase extraction and HPLC with UV-fluorescence detection. Microchemical Journal. 2013 Mar 1;107:165-71.
[67] Anbazhagan S, Indumathy N, Shanmugapandiyan P, Sridhar SK. Simultaneous quantification of stavudine, lamivudine and nevirapine by UV spectroscopy, reverse phase HPLC and HPTLC in tablets. Journal of pharmaceutical and biomedical analysis. 2005 Sep 15;39(3-4):801-4.
[68] McPolin O. An introduction to HPLC for pharmaceutical analysis. Lulu. com; 2009 Mar 1.

[69] Kumar AH, Sudha V, Swaminathan S, Ramachandran G. Comparison of HPLC & spectrophotometric methods for estimation of antiretroviral drug content in pharmaceutical products. Indian Journal of Medical Research. 2010 Oct 1;132(4):390-4.
[70] Gülfen M, Canbaz Y, Özdemir A. Simultaneous determination of amoxicillin, lansoprazole, and levofloxacin in pharmaceuticals by HPLC with UV–Vis detector. Journal of Analysis and Testing. 2020 Jan;4:45-53.
[71] Kazakevich YV, Lobrutto R. HPLC for pharmaceutical scientists. John Wiley & Sons; 2006 Dec 13.
[72] Kumar Talluri MV, Bairwa MK, Theja Dugga HH, Srinivas R. Development and validation of RP-HPLC and ultraviolet spectrophotometric methods of analysis for simultaneous determination of paracetamol and lornoxicam in pharmaceutical dosage forms. Journal of liquid chromatography & related technologies. 2012 Jan 1;35(1):129-40.
[73] de Melo J, Kellermann Hurtado F, Weich Prado A, Jost e Souza M, Bueno Rolim CM. Validation of HPLC and UV spectrophotometric methods for the determination of bezafibrate in pharmaceutical formulations. Journal of liquid chromatography & related technologies. 2007 Nov 1;31(2):269-80.
[74] Görög S. The changing face of pharmaceutical analysis. TrAC Trends in Analytical Chemistry. 2007 Jan 1;26(1):12-7.
[75] Vu Dang H, Truong Thi Thu H, Dong Thi Ha L, Nguyen Mai H. RP-HPLC and UV spectrophotometric analysis of paracetamol, ibuprofen, and caffeine in solid pharmaceutical dosage forms by derivative, Fourier, and wavelet transforms: A comparison study. Journal of analytical methods in chemistry. 2020;2020(1):8107571.
[76] Patil PN, Jacob S. HPLC analysis of cephalosporins and study of different analytical parameters. International Journal of Pharmaceutical Sciences and Research. 2012 Jan 1;3(1):1.
[77] Zhang JL, Cui M, He Y, Yu HL, Guo DA. Chemical fingerprint and metabolic fingerprint analysis of Danshen injection by HPLC–UV and HPLC–MS methods. Journal of pharmaceutical and biomedical analysis. 2005 Jan 4;36(5):1029-35.
[78] Naveed S, Sultana N, Arayne MS. HPLC-UV method for the determination of enalapril in bulk, pharmaceutical formulations and serum. J Anal Bioanal Techniques. 2012;3(130):2.
[79] Ribela MT, Gout PW, de Oliveira JE, Bartolini P. HPLC analysis of human pituitary hormones for pharmaceutical applications. Current Pharmaceutical Analysis. 2006 May 1;2(2):103-26.
[80] Tubino M, Vila MM, Palumbo MN. Determination of nitrofurazone in topical pharmaceutical preparations: comparison of the UV-visible diffuse reflectance versus transmittance versus HPLC methods. Journal of the Brazilian Chemical Society. 2009;20:1901-7.
[81] Antonisamy JM, Eahamban K. UV—VIS spectroscopic and HPLC studies on Dictyota bartayresiana Lamour. Asian Pacific Journal of Tropical Biomedicine. 2012 Feb 1;2(2):S514-8.
[82] da Silva DC, Oliveira CC. Development of Micellar HPLC-UV Method for Determination of Pharmaceuticals in Water Samples. Journal of analytical methods in chemistry. 2018;2018(1):9143730.
[83] Singh DK, Verma R. Comparison of second derivative-spectrophotometric and reversed-phase HPLC methods for the determination of prednisolone in pharmaceutical formulations. Analytical sciences. 2007;23(10):1241-3.
[84] Gohel NR, Patel BK, Parmar VK. Chemometrics-assisted UV spectrophotometric and RP-HPLC methods for the simultaneous determination of tolperisone hydrochloride and diclofenac sodium in their combined pharmaceutical formulation. Scientia Pharmaceutica. 2013 Jul 14;81(4):983.
[85] Ebel S, Mueck W. Efficiency of four different techniques in coupled HPLC-UV/VIS to quantify overlapping peaks with known spectral features. Chromatographia. 1988 Dec;25:1039-48.
[86] Hemdan A, Tawakol SM. HPLC–UV chromatographic methods for detection and quantification of undeclared withdrawn synthetic medications in counterfeit herbal medicines with confirmation by HPLC–PDA and mass spectrometry. Chromatographia. 2018 May;81:777-83.
[87] Ryan TW. HPLC impurity profile analyses of pharmaceutical substances using UV photodiode array detection. Analytical letters. 1998 Feb 1;31(4):651-8.
[88] Al-Salman HN, Al-Jadaan S, Alnuaim M, Hussein HH. Estimation of lidocaine-HCl in pharmaceutical drugs by HPLC-UV System. Am J PharmTech Res. 2017;7(1):1-1.
[89] Dogan A, E. Bascı N. Development and validation of RP-HPLC and ultraviolet spectrophotometric methods of analysis for the quantitative determination of chlorhexidine gluconate and benzydamine hydrochloride in pharmaceutical dosage forms. Current Pharmaceutical Analysis. 2011 Aug 1;7(3):167-75.
[90] Matias R, Ribeiro PR, Sarraguça MC, Lopes JA. A UV spectrophotometric method for the determination of folic acid in pharmaceutical tablets and dissolution tests. Analytical methods. 2014;6(9):3065-71.
[91] Maleki H, Mashinchian O. Characterization of Streptomyces isolates with UV, FTIR spectroscopy and HPLC analyses. BioImpacts: BI. 2011 Jun 9;1(1):47.
[92] Hurtado FK, Nogueira DR, Bortolini F, da Silva LM, Zimmermann E, e Souza MJ, de Melo J, Bueno Rolim CM. Determination of levofloxacin in a pharmaceutical injectable formulation by using HPLC and UV spectrophotometric methods. Journal of liquid chromatography & related technologies. 2007 May 1;30(13):1981-9.
[93] Conkle JL, Lattao CV, White JR, Cook RL. Pharmaceutical analysis for environmental samples: individual and simultaneous determination of ciprofloxacin, ofloxacin and norfloxacin using an HPLC with fluorescence and UV detection with a wetland soil matrix. Analytical letters. 2009 Nov 13;42(18):2937-50.
[94] Sadasivudu P, Shastri N, Sadanandam M. Development and validation of RP-HPLC and UV methods of analysis for fluconazole in pharmaceutical solid dosage forms. International Journal of ChemTech Research. 2009 Oct;1(4):1131-6.
[95] Campíns-Falcó P, Sevillano-Cabeza A, Gallo-Martínez L, Bosch-Reig F, Monzó-Mansanet I. Comparative study on the determination of cephalexin in its dosage forms by spectrophotometry and HPLC with UV-vis detection. Microchimica Acta. 1997 Sep;126:207-15.
[96] Campíns-Falcó P, Sevillano-Cabeza A, Gallo-Martínez L, Bosch-Reig F, Monzó-Mansanet I. Comparative study on the determination of cephalexin in its dosage forms by spectrophotometry and HPLC with UV-vis detection. Microchimica Acta. 1997 Sep;126:207-15.
[97] Nikolin B, Imamović B, Medanhodžić-Vuk S, Sober M. High perfomance liquid chromatography in pharmaceutical analyses. Bosnian journal of basic medical sciences. 2004 May;4(2):5.
[98] Elliott SP, Hale KA. Applications of an HPLC-DAD drug-screening system based on retention indices and UV spectra. Journal of analytical toxicology. 1998 Jul 1;22(4):279-89.
[99] Gumustas M, Kurbanoglu S, Uslu B, Ozkan SA. UPLC versus HPLC on drug analysis: advantageous, applications and their validation parameters. Chromatographia. 2013 Nov;76:1365-427.

[100] Parmar VK, Desai SB, Vaja T. RP-HPLC and UV spectrophotometric methods for estimation of pirfenidone in pharmaceutical formulations. Indian journal of pharmaceutical sciences. 2014 May;76(3):225.

[101] Sharmin N, Shanta NS, Bachar SC. Spectrophotometric analysis of azithromycin and its pharmaceutical dosage forms: Comparison between spectrophotometry and HPLC. Dhaka University Journal of Pharmaceutical Sciences. 2013;12(2):171-9.

[102] Ashraful Islam SM, Md Abuzar S, Paul PK. Validation of UV-Spectrophotometric and RP-HPLC methods for the simultaneous analysis of Paracetamol and Aceclofenac in marketed tablets. International Journal of Pharmacy & Life Sciences. 2011 Dec 1;2(12).



10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY