



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

Ultraviolet segment	Wavelength region (10–400 nm)
Extreme ultraviolet	10–121 nm
Far ultraviolet	122–200 nm
Middle ultraviolet	200–300 nm
Near ultraviolet	300–400 nm

these shifts need a smaller amount of energy than  $\sigma$  to  $\sigma^*$ . Changes in attitude light with a wavelength between 150 and 250 nm can start them [12,13].

### 3. $n$ to $\pi^*$ and $\pi$ to $\pi^*$ Transitions

The majority of organic compound absorption spectroscopy relies on electron transitions from  $n$  or  $\pi$  to the  $\pi^*$  excited state. This is due to the fact that the absorption maxima for these transitions lie in a spectral range (between 200 and 700 nm) that is favorable for experimentation. The  $\pi$  electrons required for these transitions must come from an unsaturated group in the molecule. Molar absorptivity ranges from 10 to 100 L/mol/cm during the  $\pi$  to  $\pi^*$  transitions, which is a comparatively low value. Normal molar absorptivities for  $\pi$  to  $\pi^*$  transitions range from 1000 to 10,000 L/mol/cm [14,15].

### Limitation in laws

- The reported absorption may change as a result of scattering and reflection.
- The way the solvent reacts when a concentration is high, the average distance between ions decreases, affecting the charge distribution and bringing particles closer to one another [16].
- The existence of errant light.

### TYPES OF UV-VISIBLE SPECTRA

To gather UV-visible spectra, two different kinds of absorbance equipment are utilized:

#### Single beam spectrometer

All of these devices have a sample holding, a detector, and a light source (often a tungsten or deuterium lamp). However, some of them also feature filters that allow the user to choose one wavelength at a time. To analyze one wavelength at a time, the single beam instrument (Fig. 2) places a filter or monochromator between the source and the sample [17].

#### Double beam spectrometer

This allows for an additional precise monochromator among the sample and the source; alternatively, it features a diode array detector that enables the instrument to simultaneously detect the absorbance at all wavelengths. The double beam instrument (Fig. 3) has a single source, a monochromator, and then a splitter and a series of mirrors to deliver the beam to a reference sample and the sample to be analyzed. The concurrent instrument is typically more quicker and more effective [18,19].

### INSTRUMENTATION

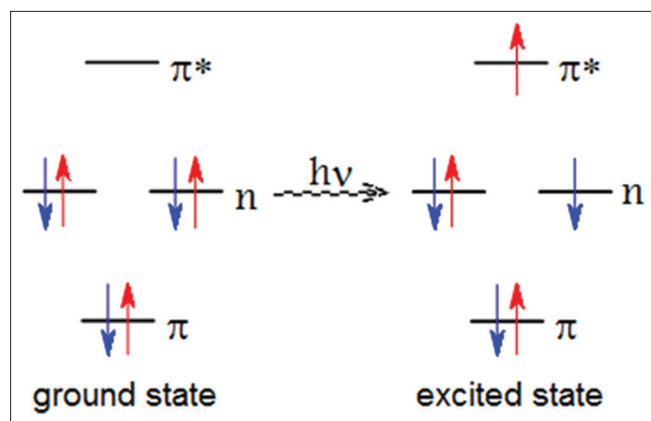
The following list of parts makes up instruments used to measure the absorption of UV or visible light [22].

1. Source
2. Monochromator
3. Sample cell
4. Detector
5. Readout system.
  - a. Amplifier
  - b. Display.

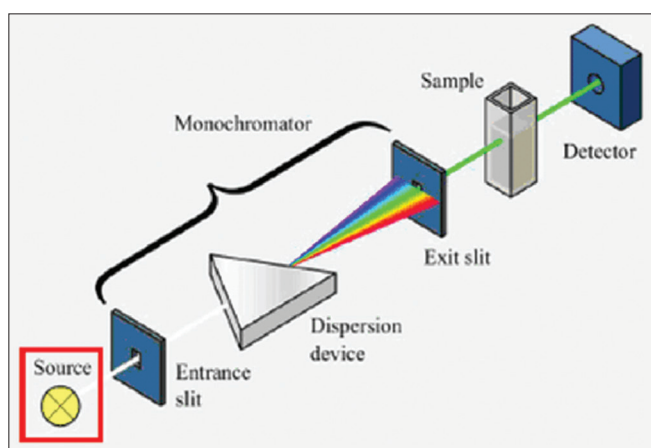
#### Sources

- a. Sources of UV radiation

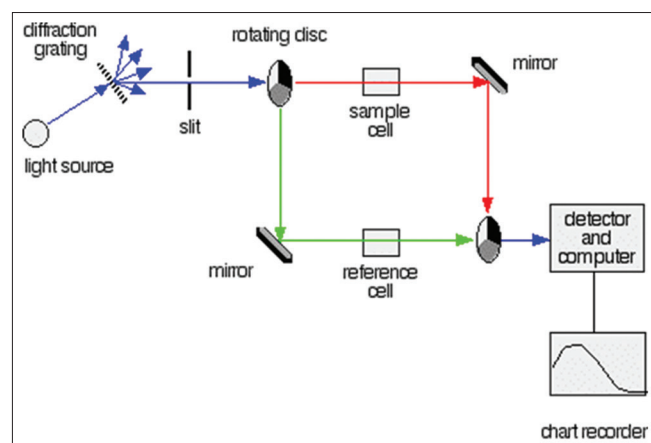
It is crucial that the radiation source's power does not fluctuate significantly over its wavelength range. A continuous UV spectrum is produced when deuterium or hydrogen is electrically excited at



**Fig. 1: This figure explains how an electron is energized to go from its ground state to an excited one. This is the basic idea of molecular spectroscopy [8]**



**Fig. 2: Ultraviolet-spectroscopy single beam [20]**



**Fig. 3: Ultraviolet-spectroscopy double beam [21]**

low pressure. The production of an excited molecular species, which disintegrates into two atomic species and an UV photon, is the process behind this [23].

$D_2 + \text{electrical energy} \rightarrow D_2^* \rightarrow D + D^* + h\nu$  is one way to represent this.

The wavelength range for radiation emitted by deuterium and hydrogen lamps is 160–375 nm. These lamps require quartz windows and quartz cuvettes since glass absorbs light with wavelengths below 350 nm [24].

#### b. Sources of visible radiation

Visible light is frequently provided by tungsten filament lamps. The wavelength range for this kind of light is 350–2500 nm. A tungsten filament lamp's energy output is proportional to the voltage at which it operates, raised to the fourth power. This implies that the voltage to the lamp needs to be extremely consistent for the amount of energy produced to be stable. This stability is provided by constant-voltage transformers or electronic voltage regulators [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

Through the entry slit, polychromatic radiation – radiation with many wavelengths – enters the monochromator. The beam is collimated before making an angle contact with the dispersion element. The prism or grating divides the beam's wavelengths into their individual components. Radiation of a specific wavelength may only depart the monochromator through the exit slit by changing the dispersing element or the exit slit [26,27].

#### Sample cell

The radiation that will pass between the sample and reference solution's containers has to be transparent to the radiation. For UV spectroscopy, quartz or fused silica cuvettes are necessary. In the visible spectrum, these cells are likewise transparent. Cuvettes for usage in the 350–2000 nm range can be made using silicate glasses [29].

#### Detectors

An electrical signal is created from a lighting signal by a detector. Over a broad range of low noise and high sensitivity, it should respond linearly.

##### a. Photomultiplier tube detector

In UV-Vis spectroscopy, the photomultiplier tube is a frequently employed detector. It is made up of an anode, which releases several electrons for every electron that strikes it, and a photoemissive cathode, which releases electrons when exposed to photons of radiation. When a photon of radiation enters the tube, it strikes the cathode, which releases several electrons. The initial anode, which is 90 volts more positive than the cathode, is where these electrons are propelled. For every incident electron that strikes the first electrode, multiple electrons are emitted [30].

To create extra electrons that are propelled toward the anode, these electrons are then accelerated in the direction of the second anode. At the anode, electrons are gathered. By now, 106–107 electrons have been created for every initial photon. After then, the current has been determined and amplified. Photomultipliers are extremely vulnerable to visible and UV light. Their reaction times are quick. Photomultipliers are only capable of monitoring low-power radiation due to damage from intense light [31].

##### b. Photodiode detector

One type of multichannel photon detector is the photodiode detector. These detectors can measure every component of a scattered radiation beam at the same time. Numerous tiny silicon photodiodes arranged

on a single silicon chip make up a linear photodiode array. A single chip can include 64–4096 sensor components; 1024 photodiodes are the most frequent configuration. A switch and a storage capacitor are also present for every diode [32]. One can progressively scan each diode-capacitor circuit in turn. When in utilize, the photodiode array is arranged so that the spectrum hits it at the monochromator's focal plane, which comes after the dispersing element.

They can particularly handy when capturing the UV-Vis absorption spectra of materials that are moving quickly through a sample flow cell, such as in an HPLC detector [33].

#### APPLICATION

##### Bacterial culture

When growing bacteria, UV-Vis spectroscopy is frequently utilized. A wavelength of 600 nm is often and rapidly used for OD measurements, which are used to assess cell concentration and monitor growth. Due to the optical characteristics of the bacterial growth conditions under which they are cultivated and to prevent cell damage in situations where the cells are necessary for further testing, 600 nm is frequently utilized and recommended [34].

##### Beverage analysis

The identification of certain substances in drinks is another typical use for UV-Vis spectroscopy. UV light can assist in determining the legal limits for caffeine levels. By matching their known peak absorption wavelengths, specific classes of colored substances – such as anthocyanin, which is present in cherries, blueberries, raspberries, and blackberries – can be easily detected in wine using UV-Vis absorbance for quality control purposes [35].

##### DNA and RNA analysis

One frequent use is for speedy RNA and DNA concentration and purity measurements. The wavelengths employed in their study and their indications are compiled in Table 2. It is commonly required to make sure that DNA or RNA samples are free of contaminants, such as proteins or chemicals left over from the isolation process, before preparing them for downstream uses like sequencing [36].

##### Pharmaceutical analysis

The pharmaceutical business is among the industries that utilize UV-Vis spectroscopy the most frequently. For example, overlapping absorbance peaks in the original spectra can be resolved to identify specific medicinal drugs by processing UV-Vis spectra utilizing mathematical derivatives. For example, using the first mathematical derivative on the absorbance spectra, it is possible to identify the antibiotic chlortetracycline and the local anesthetic benzocaine concurrently in commercial veterinary powder formulations. Through the creation of calibration functions for each chemical, simultaneous measurement of both compounds was achievable on a microgram per milliliter concentration range [38].

##### Other applications

Numerous other sectors might potentially make use of this technology. For example, evaluating a color index can be helpful in keeping an eye on transformer oil as a preventative precaution to guarantee the safe delivery of electricity [39]. Research on cancer may benefit from monitoring hemoglobin absorbance to ascertain hemoglobin concentrations. By comparing their spectra over time, UV-Vis

**Table 2: An overview of the UV absorbance that is important for calculating the 260/280 and 260/230 absorbance ratios [37]**

The wavelength utilized 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

UV: Ultraviolet

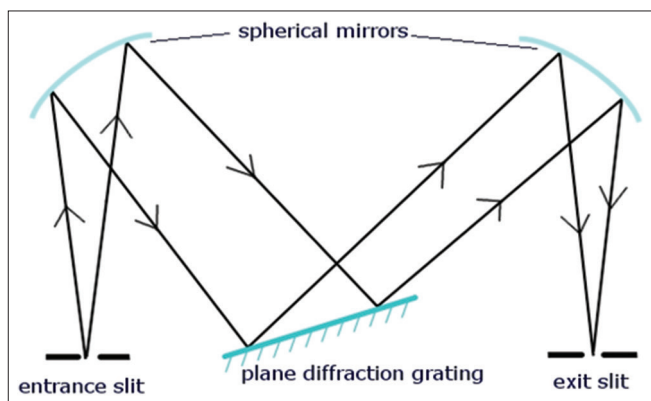


Fig. 4: Diagrammatic representation of monochromator [28]

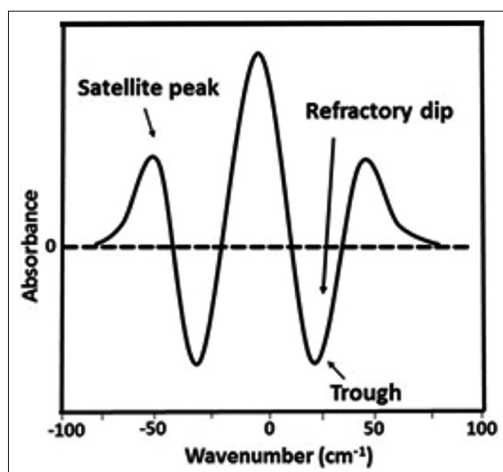


Fig. 5: Derivative spectra

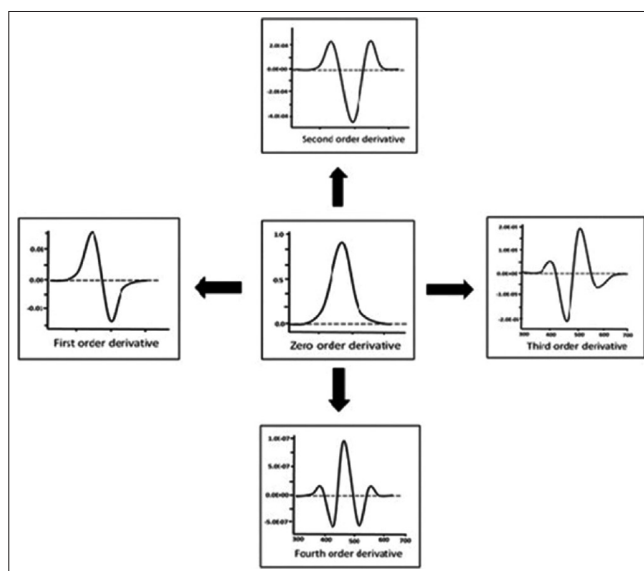


Fig. 6: Oder of derivative spectra

spectroscopy in the treatment of wastewater may be utilized in kinetic and monitoring studies to make sure that certain dyes or dye byproducts have been eliminated correctly. It is also helpful for keeping an eye on air quality and figuring out whether food is legitimate.

In addition, in certain highly specialized studies, UV-Vis spectroscopy is qualitatively beneficial. Determining the composition of batteries and

investigating particular structural protein changes can both benefit from tracking fluctuations in the wavelength that corresponds to the peak absorbance. Variations in the wavelengths of peak absorbance can also be helpful in more contemporary applications, such as the characterization of very tiny nanoparticles. There is a wide range of nearly limitless possibilities for this method.

#### DERIVATIVE UV-SPECTROPHOTOMETRY

For the purpose of obtaining complementary qualitative and quantitative information from spectra that contain unresolved bands, derivative UV spectrophotometry is a widely used analytical technique. First or higher derivatives of absorbance according to wavelength are used for both qualitative and quantitative analysis. When derivative spectroscopy was first introduced in the 1950s, it had several applications. However, due to the difficulty of obtaining derivative spectra using UV-visible spectroscopy, the approach was not widely used. The vulnerability was overcome in the 1970s by microcomputers, which provided derivative spectra in a more precise, straightforward, quick, and repeatable manner [40].

The objective with which derivative methods used in analytical chemistry are:

#### Spectral differentiation

As a qualitative technique that discerns minute differences in spectra that are almost identical.

#### Spectral resolution enhancement

Resolving overlapping spectral bands just requires estimating the quantity and wavelengths of the bands.

#### Quantitative analysis

In addition to correcting the extraneous background absorption, it makes multicomponent analysis easier. The process of differentiating or resolving overlapping bands starts with the derivative spectroscopy approach. One of the key features of this procedure is the suppression of wide bands in comparison to sharp bands.

#### MEASUREMENT TECHNIQUES OF THE DERIVATIVE SPECTROSCOPY

The path to a derivative spectrum of any order may be found by differentiating a zero-order spectrum of a set of components. Many techniques are employed to discriminate a spectrum, such as the use of an analog or numerical approach. Spectral differentiation can be thought out either graphically on paper or digitally stored in a computer's memory. Three approaches are used to measure the derivative spectra value: The zero crossing technique, numerical measurement, and graphic measurement.

#### Visual measurement

The theoretical approach of graphic measurement is used to compute the derivative spectra on paper. The manual method has the drawback of producing erroneous findings since the value that can be obtained numerically can be eliminated or diminished indefinitely.

#### Quantitative assessment

The technique makes use of a series of sites where estimating the derivative value at a certain wavelength is done. It uses an appropriate numerical approach to provide derivatives by spectral differentiation.

#### Zero crossing methodology

At a specific wavelength, when the derivative crosses the point at the zero line, the procedure measures the derivative spectra. The zero-crossing approach can be used to remove interference from one component's assessment of another component.

#### DERIVATIVE SPECTRA

Derivative spectra are used in quantitative analysis to resolve overlapping bands by enlarging the difference between spectra. For

producing derivative spectra, the Savitzky-Golay digital algorithm approach is highly recommended. Plotting the absorbance spectrum's rate of change against wavelength is part of the universal technique. Numerous experimental methods may be used to acquire derivative spectra, and even if the spectrum was originally digitally captured or is in computer-readable form, differentiation can be performed numerically. Real-time derivative spectra can be obtained through wavelength modulation or by obtaining the time derivative of the spectrum while the spectrum is scanned at a constant rate.

A computerized approach is extensively used to create derivative curves. A wavelength modulation device is employed to record the derivative spectra, where a beam of light alters in wavelength by a minor change (1–2 nm) and the difference between the two readings is recorded [41].

Peak heights of long-wave peak satellites or short-wave peak satellites are measured quantitatively for second or fourth-order derivative curves. The inclusion of satellite peaks raises the complexity level of derivative spectra. Two distinct peaks and troughs are observed in the second derivative spectra. Over peaks, the solvents have a remarkable impact. Based on the polarity of the solvent, the peaks and troughs move to longer or shorter wavelengths.

#### THE WAY OF OBTAINING THE DERIVATIVE ORDERS

Converting a normal or zero-order spectrum to a first, second, or higher derivative spectrum is achieved by derivative spectroscopy. It results in significant modifications to the derivative's form. Selecting the derivative order appropriately allows overlapping signals to be usefully separated. It is predicted that higher orders will be used for narrow spectral bands and lower orders for large spectral bands to achieve criteria such as signals height, breadth, and distance between maxima in the fundamental spectrum. An ideal absorption band that provides a clear understanding of the transition taking place in the derivative spectra is represented by a Gaussian band.

When absorbance is plotted against wavelength, a graph is produced that displays a peak with maxima and minima, or points of inflection that should pass through zero on the ordinate.

#### ZERO ORDER DERIVATIVE SPECTRUM

The first step in providing additional derivatives is the zero-order derivative, that is, the derivative of the  $n$ th order may be obtained from infinity. The D0 spectrum, or zeroth order, in derivative spectroscopy, is a characteristic of the normal absorption spectrum. The zeroth-order spectrum may be used directly to derive the derivative spectra of the first, second, third, and fourth orders. The sensitivity of determination rises with an increase in the order of derivatives. The derivative spectrum is provided as follows if a spectrum is represented as absorbance (A) as a function of wavelength ( $\lambda$ ).

$$A = f(\lambda).$$

#### FIRST ORDER DERIVATIVE SPECTRUM

Spectra result from once derivatizing the zero-order spectrum. This is a plot showing the absorbance change with wavelength against wavelength, or the absorbance change rate with wavelength. Even in its derivatized form, the complexity surpasses that of the zero-order spectrum. The absorbance band's  $\lambda$  max is passed through by zero in first-order spectra. A positive and negative band with peaks and minima may be seen in the absorbance band of the first-order derivative. A dual-wavelength spectrophotometer measures the difference between two wavelengths and uses that difference to scan the spectrum and get first-derivative spectra.

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

#### SECOND ORDER DERIVATIVE SPECTRUM

This kind of spectrum is produced by twice derivatizing the absorbance spectrum. This is a plot of the absorption spectrum's curvature versus wavelength. The second derivative is exactly proportional to the concentration, meaning it is directly related.  $d^2A/d\lambda^2$  needs to be high; the higher the ratio, the higher the sensitivity. The technique is helpful for acquiring gas and atomic molecular spectra.

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

#### THIRD ORDER DERIVATIVE SPECTRUM

The third derivative spectrum displays a dispersal function to the original curve, in contrast to the second-order spectrum.

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

#### FOURTH-DERIVATIVE SPECTRUM

Fourth order is an inverted spectrum of second order with a center peak that is sharper than the original band. Fourth derivative (UV-high pressure) selectively determines narrow bands.

$$d^4A/d\lambda^4 = f^{(4)}(\lambda).$$

#### POLYNOMIAL DEGREE

Instead of determining the form of the derivative, the number of polynomial points is greatly influenced by the polynomial degree. The range of a polynomial is narrow; low-degree polynomials employ differentiation of half-width spectra, but higher-degree polynomials use it for tiny half-width spectra. Inappropriate polynomial degree results in distorted derivative spectrum. Using various polynomial degrees in multicomponent analysis might enhance the spectrum differences of the chemicals being tested and their selective selection.

#### SIGNAL-TO-NOISE RATIO

Using a derivative approach with higher orders that result in lower signal-to-noise becomes challenging.  $S/N$  decreases with higher orders as a result. The spectrum's sharpest characteristics are caused by noise. Due to derivatization's detrimental influence on  $S/N$ , the spectrophotometer's low-noise capabilities are under more pressure. Before derivatization,  $S/N$  can be increased if a spectrophotometer was to scan spectra and average many of them. The difference between the highest maximum and the lowest minimum can be used to calculate the best signal-to-noise ratio, although doing so increases the susceptibility to interference from other components. A signal's noise is represented by its standard deviation, or  $\sigma$ .

The standard deviation while standard deviation  $\sigma_n$  indicates the  $n$ th order derivative that may be derived by  $\sigma_0$ ,  $\sigma_0$  expresses the noise of the normal spectrum of the blank absorbance.

#### SMOOTHING OF SPECTRA

A technique known as low-pass filtering or smoothing is used to reduce the high-frequency noise or to alleviate the circumstances caused by an increase in the signal-to-noise ratio. Smoothing is an operation that works on neighboring variables and is carried out on individual spectrum for every row of the data. Closely spaced variables in the data matrix with equivalent information can have much less noise without sacrificing the signal of interest. Care must be exercised since a high degree of smoothing may change the derivative spectrum. The smoothing effect is primarily determined by two variables: (a) The smoothing frequency and (b) The smoothing ratio, or the breadth of the smoothed peak divided by the number  $M$  of data points.



## ADVANTAGES AND DISADVANTAGES OF DERIVATIVE UV-SPECTROPHOTOMETRY

### Advantages

The sensitivity and selectivity of UV derivative spectroscopy have been enhanced. Its many benefits include the ability to identify both organic and inorganic chemicals, determine traces in a matrix, analyze proteins and amino acids, identify single components and multiple components in a mixture simultaneously, and determine traces in the environment. The following are some particular advantages of derivative spectral analysis:

- Absorbance bands can be distinguished even in a narrow wavelength range when there are two or more overlapping peaks present
- Weak and tiny absorbance peaks can be distinguished when strong and sharp absorbance peaks are present
- A wide absorbance spectrum provides a precise understanding of the wavelength at that maximum spectrum
- Because the derivative values and concentration levels have a linear relationship, the quantitative analysis may be conducted even when background absorption is present.

### Disadvantages

Despite being a sensitive approach, it is nevertheless quite vulnerable to several factors. Due to its low repeatability, the procedure is restricted to a specific system and has limited uses. When the current instrumental technique – which measures signal – is unavailable, the approach comes in second. When measuring zero-crossing spectra, it is less precise. Since the derivative and zero-order spectra are similar in form, slight changes to the basic spectrum can significantly alter the derivative spectrum.

When multiple spectrophotometers used for zero-order spectra provide comparable findings but their derivatization displays different results, poor repeatability might affect the results in a way.

## APPLICATIONS

### Single component analysis

In pharmaceutical formulation, derivative spectrophotometry studies a single component in addition to the Area under the Curve.

### Multicomponent analysis

In pharmaceutical analysis, derivative spectrophotometry studies several components in the presence of other components, that is, determining two or more substances at the same time. The predominance brought on by the spectra of bothersome chemicals can be eliminated by spectral derivatization.

### Bioanalytical application

Derivative spectrophotometry has other uses outside of pharmaceutical analysis. Identification of the chemicals present in different biological samples, including brain tissue, plasma, serum, and urine. The sequence of derivatives of amphotericin and diazepam has been identified in human plasma.

### Forensic toxicology

Derivative spectroscopy may be applied in mixtures and is particularly useful in the study of illegal substances such as amphetamine, ephedrine, meperidine, and diazepam.

### Trace analysis

The derivative signal processing method is frequently employed in real-world analytical tasks when measuring trace levels of chemicals in the presence of significant concentrations of compounds that might interfere. Analytical signals become faint, noisy, and overlaid on massive background signals as a result of this interference. Degraded measurement accuracy is caused by sample-to-sample baseline variations and factors such as non-specific broadband interfering absorption, non-reproducible cuvette placement, dirt or fingerprints on the cuvette walls, improper cuvette transmission matching, and

solution turbidity. Practical errors can be the cause of baseline shifts; these mistakes can be weakly wavelength-dependent (small particle turbidity) or wavelength-independent (large suspended particles or bubbles obstructing light).

Therefore, it is necessary to distinguish pertinent absorption from the aforementioned baseline shift causes. It is anticipated that differentiation will attenuate the wide background to lessen sample-to-sample variability in background amplitude. In many cases, this leads to increased measurement accuracy and precision, particularly when the analyte signal is weak in relation to the background and there exists an abundance of uncontrolled variability in the background.

## CONCLUSION

A crucial technique for researching the optical characteristics of PMCs is UV-Vis spectroscopy. It looks at how nanofillers can improve the characteristics of nanocomposites and advances our understanding of how the matrix and nanofiller interact. UV-Vis spectroscopy is one of the most significant characterization methods for researching optical characteristics. In the direction of the creation of functional materials with important technological applications; it highlights the value of the UV-Vis spectroscopy technique in characterizing polymer nanocomposites with optically responsive nanofillers like metals, semiconductor nanocrystals, and nano oxides. A derivative these days, spectrophotometers that are controlled by software may do spectrophotometry. This facilitates analysts' ability to extract meaningful information from the spectra of the corresponding chemicals. The UV spectrum derivatives provide useful information for understanding the chemicals used in pharmaceutical formulation.

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## AUTHOR'S CONTRIBUTION

Both the authors Sheikh Wajiha Shabbir and Shilpi Chauhan have contributed equally in the paper.

## CONFLICTS OF INTEREST

The authors confirm that the content of the article has no conflicts of interest.

## DATA AVAILABILITY

The original data that support the findings of this study are included in the Article.

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#### Author Queries???

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AQ2: Kindly cite figures 1, 4-6 in the text part

AQ3: Kindly provide author initial