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UV-VISIBLE SPECTROSCOPY IN PHARMACEUTICAL ANALYSIS: PRINCIPLES, METHOD DEVELOPMENT AND RECENT APPLICATIONS

KAJA RESHMA [1*] | GOLLA BHARATH SAI [2]

[1] KAJA RESHMA, B.PHARM, M.PHARM, ASSOCIATE PROFESSOR, DEPARTMENT OF PHARMACEUTICAL ANALYSIS, NIMRA COLLEGE OF PHARMACY, IBRAHIMPATNAM, VIJAYAWADA - 521456, INDIA.

[2] DEPARTMENT OF PHARMACEUTICAL ANALYSIS, NIMRA COLLEGE OF PHARMACY, IBRAHIMPATNAM, VIJAYAWADA – 521456, INDIA.

ABSTRACT:

UV-Visible spectroscopy remains one of the most accessible optical techniques used in pharmaceutical laboratories for drug assay, dissolution testing, raw-material assessment and selected multicomponent measurements. Its analytical usefulness arises from absorption of ultraviolet or visible radiation by chromophores and from the quantitative relationship between absorbance and concentration described by the Beer-Lambert law. This review summarizes the fundamental basis of UV-Visible absorption, electronic transitions, instrumental components and variables that influence spectral response. Particular attention is given to pharmaceutical method development, including solvent and wavelength selection, calibration, selectivity, linearity, accuracy, precision and robustness under the current ICH Q2(R2) and Q14 framework. Recent literature also shows increasing use of derivative spectroscopy, photodiode-array detection, in-line measurement and multiwavelength data treatment. Although the technique is rapid and economical, dependable performance requires suitable sample preparation, adequate selectivity and control of chemical and instrumental interferences.

KEYWORDS:

UV-VISIBLE SPECTROSCOPY, PHARMACEUTICAL ANALYSIS, BEER-LAMBERT LAW, SPECTROPHOTOMETRIC METHOD, ANALYTICAL VALIDATION, QUALITY CONTROL.

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1. INTRODUCTION

Pharmaceutical analysis requires methods that can establish the identity, strength, purity and performance of drug substances and dosage forms. UV-Visible spectrophotometry is widely used because it combines comparatively simple instrumentation with rapid measurement and low operating cost. It is especially useful when an analyte contains an absorbing chromophore or can be converted to a measurable absorbing species. Recent reviews continue to describe its role in routine assay, formulation analysis, dissolution testing and selected bioanalytical or multicomponent applications [1,2].

The technique is not inherently selective for every pharmaceutical matrix. Excipients, degradation products, solvents and co-administered drugs may absorb in the same spectral region. Reliable results therefore depend on rational wavelength selection, appropriate sample preparation and evidence that the analytical procedure performs as intended. Current regulatory guidance places method development and validation within a lifecycle framework in which analytical performance is linked to the intended purpose [6,7].

This review connects the operating principles of UV-Visible spectroscopy with practical pharmaceutical analysis, emphasizing quantitative measurement, instrumentation, sources of analytical variability, method development, validation and recent applications [8,10].

2. REVIEW METHODOLOGY

A focused narrative approach was used. Recent peer-reviewed publications and current regulatory guidance were selected to represent five areas: fundamental UV-Visible theory, pharmaceutical applications, method-development practice, validation requirements and emerging analytical directions. Ten non-duplicate sources published between 2021 and 2025 were retained as the core references, including ICH Q2(R2) and Q14. The review is selective rather than systematic and is intended to summarize concepts most relevant to pharmaceutical analysis.

3. FUNDAMENTAL BASIS OF UV-VISIBLE ABSORPTION

UV-Visible spectroscopy measures the attenuation of electromagnetic radiation after it interacts with a sample.

Pharmaceutical measurements commonly use approximately 200-800 nm, depending on the instrument and solvent. Absorption occurs when incident radiation promotes valence electrons to higher-energy molecular orbitals. Chromophores are the structural units mainly responsible for absorption, while auxochromic groups such as hydroxyl or amino substituents can modify the wavelength and intensity of an absorption band [1,8].

The principal electronic transitions are sigma to sigma-star, n to sigma-star, pi to pi-star and n to pi-star. Pi-related and n to pi-star transitions are especially important for many pharmaceutical molecules containing unsaturated or carbonyl groups. When absorbance is scanned over wavelength, the resulting spectrum may contain one or more bands, and the wavelength of maximum absorbance (lambda max) is commonly selected for quantitative measurements [1,5].

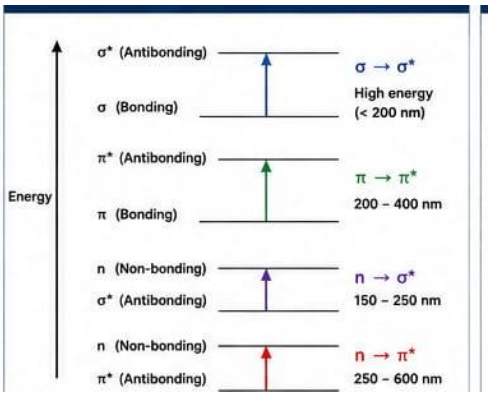

FIG. 1: SIMPLIFIED ELECTRONIC TRANSITIONS CONTRIBUTING TO UV-VISIBLE ABSORPTION.

Transmittance (T) is the fraction of incident radiation that passes through a sample, while absorbance (A) is expressed logarithmically as A = -log10(T). Quantitative methods commonly apply the Beer-Lambert relationship, A = epsilon bc, where epsilon is molar absorptivity, b is optical path length and c is concentration. At fixed path length and under appropriate conditions, absorbance is proportional to concentration. Deviations can arise from high concentration, chemical association or dissociation, pH changes, solvent effects, scattering, stray light and matrix absorption [1,4-6].

4. INSTRUMENTATION

A UV-Visible spectrophotometer contains a radiation source, wavelength-selection system, sample compartment, detector and electronic signal-processing components. Deuterium lamps are commonly used in the ultraviolet region, while tungsten or tungsten-halogen lamps provide visible radiation. A monochromator uses slits, optics and a dispersive element such as a diffraction grating to isolate a narrow wavelength band [1,8]. Quartz or fused-silica cuvettes are required for ultraviolet measurements, whereas glass cells can be suitable for many visible measurements. Photomultiplier tubes, photodiodes and photodiode-array detectors are common detection systems.

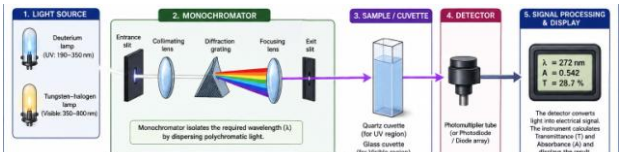

FIG. 2: MAIN COMPONENTS AND OPTICAL PATH OF A UV-VISIBLE SPECTROPHOTOMETER.

The major components can be summarized by function: the radiation source provides stable UV or visible energy; the monochromator selects the required wavelength band; the sample cell provides a defined optical path; the detector converts transmitted radiation into an electrical response; and the data system processes, displays and records absorbance or spectral information.

5. FACTORS AFFECTING ANALYTICAL RESPONSE

The observed spectrum reflects both the analyte and its measurement environment. The solvent should dissolve the analyte, contribute minimal absorbance at the analytical wavelength and maintain chemical stability. For ionizable compounds, pH can alter the relative amounts of protonated and unprotonated species and thereby change both lambda max and absorptivity. Concentration must remain inside the established working range; excessively concentrated solutions may deviate from Beer-Lambert behavior, while very dilute solutions can show poor signal-to-noise ratios [4,5].

Cuvette cleanliness, orientation, fingerprints, bubbles, suspended particles and turbidity affect the light reaching the detector. Instrumental factors including bandwidth, wavelength accuracy, stray light and baseline stability are also important, particularly for narrow absorption bands or low absorbance levels [1,6].

6. PHARMACEUTICAL APPLICATIONS

For drug assay, absorbance at a selected wavelength is related to concentration using a reference standard or calibration curve. Tablet and capsule analysis usually involves accurate sampling, extraction, filtration when necessary and dilution into the validated working range. UV-Visible measurement is also widely used for dissolution testing when the drug and dissolution medium provide adequate spectral selectivity [1,2].

Multicomponent analysis can be performed using measurements at selected wavelengths, derivative techniques or mathematical treatment of multiwavelength data. UV detection is also routinely coupled with HPLC, where chromatographic separation improves selectivity before optical detection. Other uses include raw-material screening, kinetic measurements, acid-base dissociation studies and selected stability investigations [2,8].

7. ANALYTICAL METHOD DEVELOPMENT AND VALIDATION

A fit-for-purpose UV-Visible method begins with a clearly defined analytical objective. Development should characterize the analyte spectrum and identify a solvent, pH, concentration range and sample-preparation

procedure that maintain chemical stability while minimizing blank and placebo absorption. Wavelength selection should consider both signal intensity and selectivity, not merely the highest absorbance value [5,8].

ICH Q14 describes science- and risk-based principles for analytical procedure development and links method understanding with a suitable control strategy [7]. For a UV method, development knowledge may include the effects of wavelength, solvent composition, pH, extraction time, sample stability and instrument settings. ICH Q2(R2) provides the current validation framework [6]. Depending on intended use, important characteristics include specificity/selectivity, accuracy, precision, reportable range, sensitivity where relevant and robustness.

Recent pharmaceutical studies illustrate these principles. Sharma et al. developed and validated UV-Visible methods for curcumin and tetrahydrocurcumin [4], while Gupta et al. reported a simultaneous UV method for teneligliptin and pioglitazone with assessment of specificity, linearity, precision, accuracy, sensitivity and robustness [9].

For a quantitative UV-Visible procedure, validation commonly demonstrates that relevant interferences do not materially affect the analyte response, that response is suitable across the intended concentration interval, that results are sufficiently accurate and precise, that low-level performance is adequate when relevant, and that small deliberate changes in method conditions do not cause unacceptable variation [6].

8. RECENT DEVELOPMENTS

Recent literature shows that pharmaceutical UV-Visible analysis is moving beyond isolated single-wavelength readings. Photodiode-array instruments can rapidly acquire full spectra and support spectral comparison, purity assessment and time-resolved monitoring. Derivative spectroscopy can improve resolution of overlapping signals and has been applied to in-line biopharmaceutical measurements [3]. Chemometric treatment of multiwavelength data further extends the value of UV-Visible spectra when direct measurement lacks sufficient selectivity [2,8].

A recent review of pharmaceutical studies published from 2015 to 2023 found that dosage-form analysis remained a major application area, showing that conventional UV-Visible procedures continue to have substantial practical relevance while newer applications increasingly use multivariate treatment, miniaturized optics and process-oriented measurements [10].

9. ADVANTAGES AND LIMITATIONS

Major advantages include operational simplicity, rapid analysis, modest sample requirements and comparatively low instrument and running costs. These features make UV-Visible spectroscopy useful for teaching, screening, routine quality control and formulation studies [1,2]. Its principal limitation is selectivity: different compounds may absorb at the same or nearby wavelengths, and the method is vulnerable to poor sample preparation, unsuitable blanks, turbidity, dirty cells and uncontrolled pH. When adequate selectivity cannot be demonstrated, a separation-based or otherwise more selective analytical procedure should be considered [6,7].

10. CONCLUSION

UV-Visible spectroscopy remains a practical and relevant technique in pharmaceutical analysis. Its quantitative basis is straightforward, but dependable performance requires control of sample chemistry, wavelength, concentration, optical cells and instrumental conditions. Modern method development should connect these variables with the intended analytical purpose and should be supported by validation consistent with ICH Q2(R2) and Q14. Recent applications demonstrate continued evolution through photodiode-array detection, derivative spectroscopy, in-line measurement and multiwavelength data processing. For suitable analytes and matrices, the technique offers an effective balance of speed, cost and analytical performance.

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