Page 118 to 119

Volume 1 • Issue 3 • 2017

Scientia Ricerca

Editorial

Chronicles of Pharmaceutical Science

ISSN: 2572-7761

UV – Visible Spectroscopic Studies- How to Overcome Interferences During Analysis?

Mohamad Taleuzzaman*

Department of Pharmaceutical Chemistry, Glocal School of Pharmacy, Glocal University, Mirzapur Pole, Saharanpur

***Corresponding Author:** Mohamad Taleuzzaman, Asst. Professor, Department of Pharmaceutical Chemistry, Glocal School of Pharmacy, Glocal University, Mirzapur Pole, Saharanpur.

Received: June 07, 2017; **Published:** June 13, 2017

Volume 1 Issue 3 June 2017

In UV-Visible spectroscopy studies, the source of energy for analysis is light which contain a quantized photons. It is found that over a certain range of wavelength which every chemical compound absorbs, transmits, or reflects light (electromagnetic radiation). Spectrophotometry is a measurement of how much a chemical substance absorbs or transmits and a spectrophotometer is an instrument that measures the amount of the intensity of light absorbed after it passes through sample solution. With the spectrophotometer, the concentrations of a substance (the amount of a known chemical substance) can also be determined by measuring the intensity of light detected. The measurement of quantized light intensity is according to Beer-Lambert Law. An UV/Vis spectrophotometer measures the intensity of light which passes through a sample. Then compares the intensity of light, after absorption and before it passes through the sample. It is expressed in absorbance (A) or transmittance (T). Practically as we know that a beam of light which source is visible and/or UV light source (colored red) is separated into its component wavelengths by a prism or diffraction grating. In spectrophotometer has half-mirrored device which split the monochromatic light into two equal intensity beams. From which one beam go to sample passes through a small transparent container (cuvette) containing a solution of the compound being studied in a transparent solvent. The other beam passes through the identical cuvette having only the solvent is called reference. After that the light which comes from reference cuvette the intensity is I0 and the light which comes from the sample cuvette the intensity defined as I. Both intensities of these light beams are then measured by electronic detectors and compared. Spectrophotometer automatically scans all the component wavelengths in over short period of time. The wavelength range of ultraviolet (UV) region scanned is normally from 200 to 400 nm, and the visible portion is from 400 to 800 nm.

Theoretically the absorbance spectrum of a solution containing a single analyte should be a single absorption band at the wavelength of maximum absorbance. However, in real samples the spectrum gets influenced by presence of other interfering species. Such interferences can be easily eliminated by adopting different approaches covered in the article.

In instrument analysis it is observed interference which is cause is either physical or chemical in nature both two factors is dominate in UV-Visible spectroscopy. Chemical interference is due to presence any compound that absorb wavelength in close to primary absorbing molecule. On the other hand physical interference cause is due to suspended solid impurities in sample which can lead to scattering. Scattering of light happed by presence of suspended impurities in the absorbing solution. Result observed that it reduce the absorbance of analyte of interest. Practically it can be minimise but it is not applicable for µl size sample. Other technical method that is applied to prevent the loss of absorbance due to scattering by reducing the gap between the sample and detector.

Iso-absorbance Measurements: This practical approach is applied, if in case an impurities present with a known characteristic absorbance with analyte. It is eliminated by selecting a wavelength where the impurities some absorbance as it does at analytical wavelength. On subtracting the absorbance at this wavelength from the absorbance at analytical wavelength the residual absorbance is the correct absorbance of the analyte.

Multicomponent Analysis: If it is found spectral overlap with the spectra of main analyte which is due to presence of more than one interference. The absorbance of interference is subtracted from the measured absorbance to get the true absorbance of the analyte of interest.

Three Point Correction: A three-point drop-line can be used to compensate for a background absorbance with a constant slope. In this method two wavelengths are selected close to the analytical wavelength but on either side of it. The interference of background can be accurately estimated using linear interpolation. The method is applicable particularly for non-linear background absorbance resulting from complex sample matrices.

Derivative Spectroscopy: By far the most convenient approach to background and noise correction is derivative spectroscopy. The inflexion point of the first derivative corresponds to the wavelength of maximum absorbance and the second derivative appears as the tip of the negative shaped peak. This helps differentiate between very closely spaced or overlapping absorbance peaks. The first derivative further eliminates baseline shifts, if any, and this helps improve the accuracy of quantitative analysis. In addition to baseline shift, derivative spectroscopy also helps overcome the effects of scattering from other unidentified interfering compounds.

A constant background absorbance over a large wavelength range can be eliminated using an internal reference or first order derivative.

References

1. Savitzky A and Golay M. "Smoothing and Differentiation of Data by Simplified Least Squares Procedures". *Analytical Chemistry* 36.8 (1964): 1627-1639.
2. Macadam DL. "Colour Measurement". *Springer: Berlin* (1981).
3. Chamberlain GJ and Chamberlain DG. "Colour, Its Measurement, Computation and Application". *Heyden*: London (1980).
4. Levine RL and Federici MM. "Quantitation of aromatic residues in proteins: model compounds for second-derivative spectroscopy". *Biochemistry* 21.11 (1982): 2600-2606.
5. Kisner HJ., *et al.* "Multiple analytical frequencies and standards for the least-squares spectrometric analysis of serum lipids". *Analytical Chemistry* 55.11 (1983): 1703.
6. Alford JS and Cline FL. "PMA's computer system validation committee, computer system validation-staying current: installation qualification". *Pharmaceutical Technology* 14 (1990): 88-104.
7. Standard Test Method for Estimating Stray Radiant Power Ratio of Spectrophotometers by the Opaque Filter Method, ASTM Standard E-387 (1990).