Current application of UV-Spectroscopy

Analyze:

"What is it?

(Qualitative analysis)

"How much is there?

(Quantitative analysis)

Definition

Pharmaceutical analysis may be defined as a process or a sequences of processes to **identify and/or quantify a substance or drug**, the components of a pharmaceutical solution or mixture or the determination of the structures of chemical compounds used in the formulation of pharmaceutical product.

Classification

Pharmaceutical analysis can be classified in various ways.

All pharmaceutical analysis processes can be categorized into two groups—

- Qualitative Analysis
- Quantitative Analysis

Analytical Methods

Techniques employed in quantitative analysis are based upon—

- ✓ **Chemical Properties:**
- ✓ **Electrical Properties:**
- ✓ **Optical Properties:**
- ✓ **Thermal properties:**
- ✓ **Magnetic properties:**

UV-SPECTROSCOPY

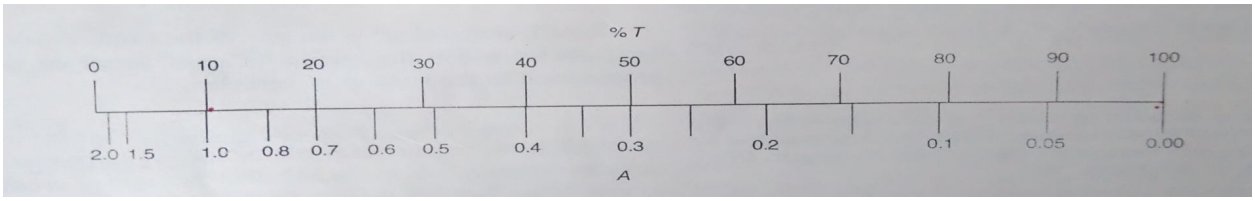
Beer's Law Limitation

Absorption spectroscopy from 160 nm to 780 nm Measurement of transmittance

Conversion to absorbance

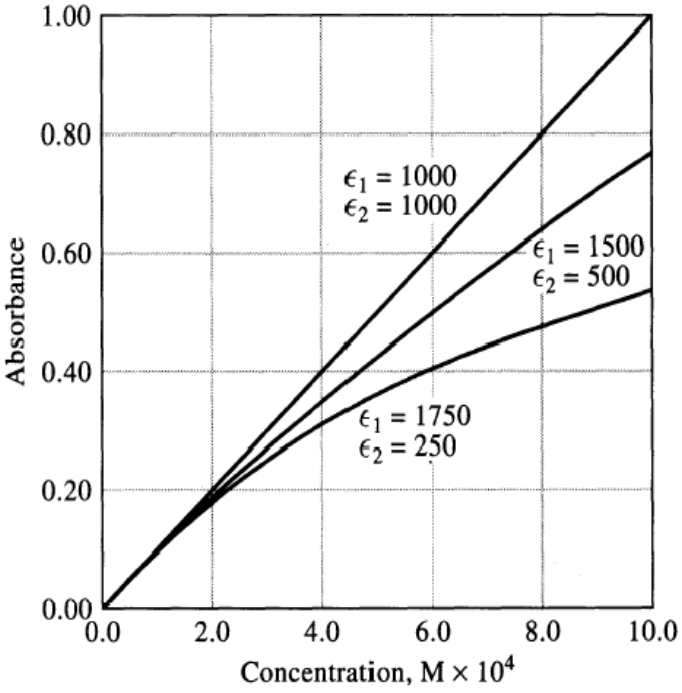
A=-logT=ebc

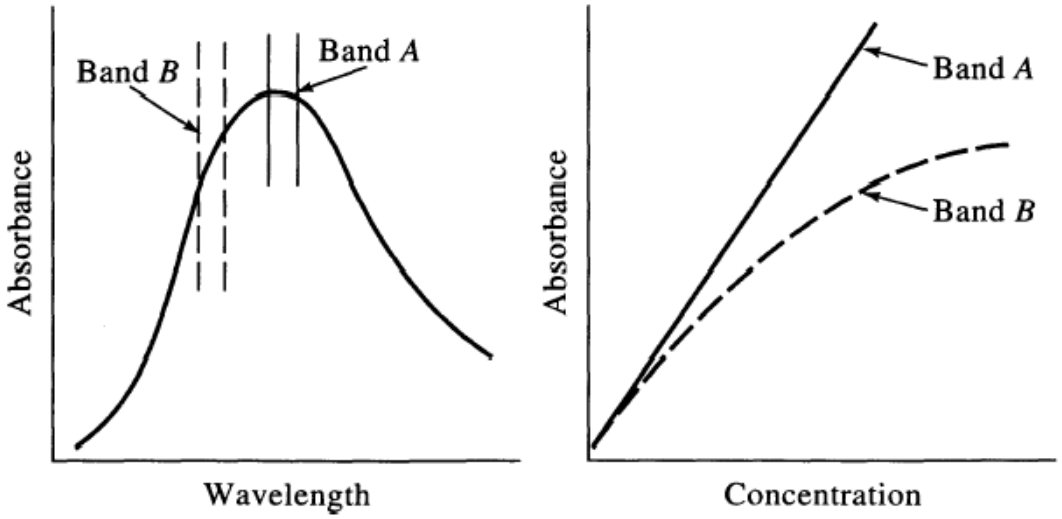
Measurement of transmittance and absorbance Beer's law



Polychromatic Light

More than one wavelength

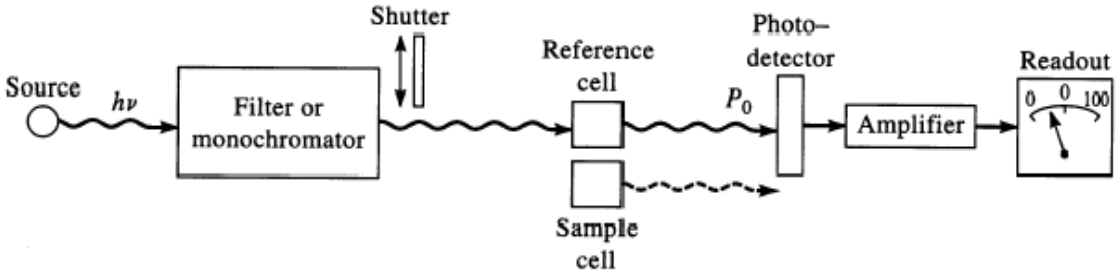


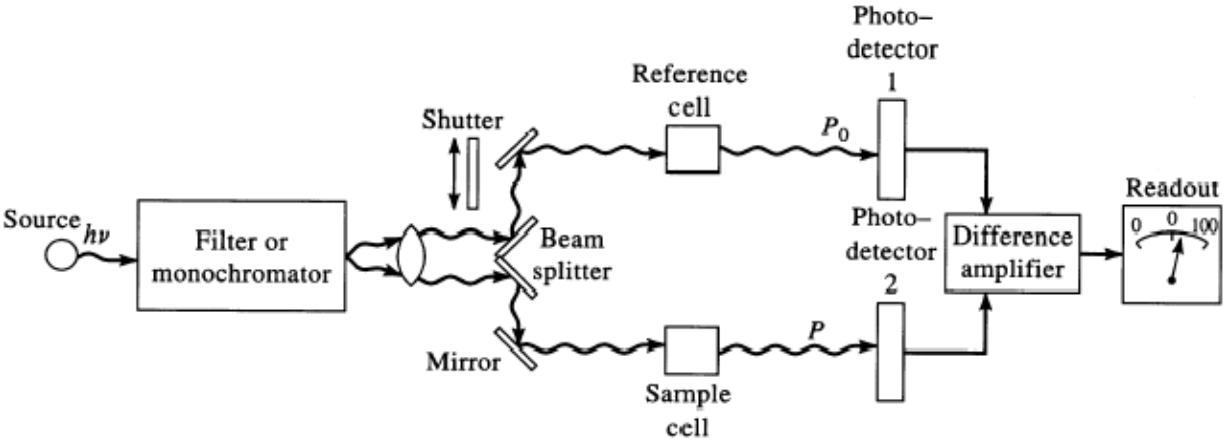


Instrumentation

- Light source
  - Deuterium and hydrogen lamps
  - W filament lamp
  - Xe arc lamps
- Sample containers
  - Cuvettes
    - Plastic
    - Glass
    - Quartz
- Monochromator
- Detector
    - Photo Voltaic Cell
    - Barrier Layer Cell
    - Photo Multiplier Tube

Spectrometers





A

Single component Analysis

- Magnitude of molar absorptivities
    - Absorptivity
    - Molar Absorptivity
    - $E^{1\%}_{1Cm}$
- Calibration curve Methods
    - O order, 1,2 Derivative methods
    - Difference spectroscopy Method
    - Dual Wavelength spectroscopy
    - Area under curve method

Others methods

One Point assay

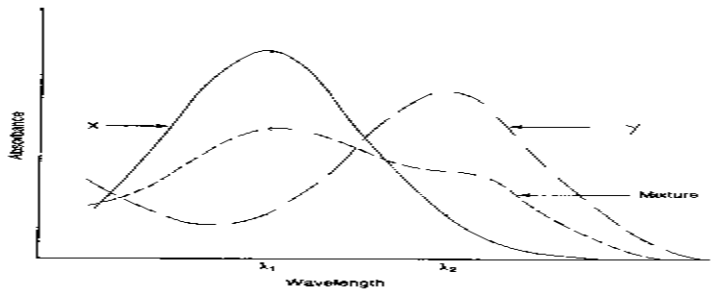
$$c_{test} = \frac{A_{test} \times c_{std}}{A_{std}}$$

Two Point Assay

$$c_{test} = \frac{(A_{test} - A_{std_1})(c_{std_1} - c_{std_2}) + c_{std_1}(A_{std_1} - A_{std_2})}{A_{std_1} - A_{std_2}}$$

Multicomponent assay

1. Simultaneous equation method



The individual absorption spectra of substances X and Y, showing the wavelengths for the assay of X and Y in admixture by the method of simultaneous equations.

Let c_x and c_y be the concentrations of X and Y respectively in the diluted sample.

Two equations are constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of the individual absorbance's of X and Y.

At λ_1

$$A_1 = a_{x1}\, b\, c_x + a_{y1}\, b\, c_y \qquad (1)$$

At λ_2

$$A_2 = a_{x2}\, b\, c_x + a_{y2}\, b\, c_y \qquad (2)$$

The absorption ratio method

OR

Q-Absorbance Ratio Method

The absorbance of the sample solution (CIL and METO) i.e. A1 and A2 were recorded at 231 nm (Iso-absorptive point) and 224 nm (λmax of METO) respectively, and ratios of absorbance were calculated, i.e. A2/A • Relative concentration of two drugs in the sample was calculated using following equations. 1

CX = [(QM-QY)/ (QX-QY)] × A1/aX1…….. (iii)

CY = [(QM-QX)/ (QY-QX)] × A1/aY1…….. (iv)

The Q-values and absorptivitie for both drugs were calculated as follows, QM = Absorbance of Sample solution at 224 nm (A2)/ Absorbance of Sample solution at 231 nm (A1) QX = Absorptivity of CIL at 224 nm (ax2)/ Absorptivity of CIL at 231nm (ax1) QY = Absorptivity of METO at 224 nm (ay2 231 nm (ay)/ Absorptivity of METO at 1 Where, A)

METHOD DEVELOPMENT AND ITS VALIDATION

Types of Analytical Procedures to be Validated

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

1. Identification tests.
2. Quantitative tests for impurities' content.
3. Limit tests for the control of impurities.
4. Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Typical validation characteristics which should be considered are:

- Accuracy
- Precision
- Repeatability
- Intermediate Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range

TABLE

Type of analytical procedure characteristics	IDENTIFICATION	TESTING FOR IMPURITIES quantitat.	 limit	ASSAY - dissolution (measurement only) - content/potency
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Interm.Precision	-	+ (1)	-	+ (1)
Specificity (2)	+	+	+	+
Detection Limit	-	- (3)	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- signifies that this characteristic is not normally evaluated

+ signifies that this characteristic is normally evaluated

(1) in cases where reproducibility (see glossary) has been performed, intermediate precision is not needed

(2) lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)

(3) may be needed in some cases