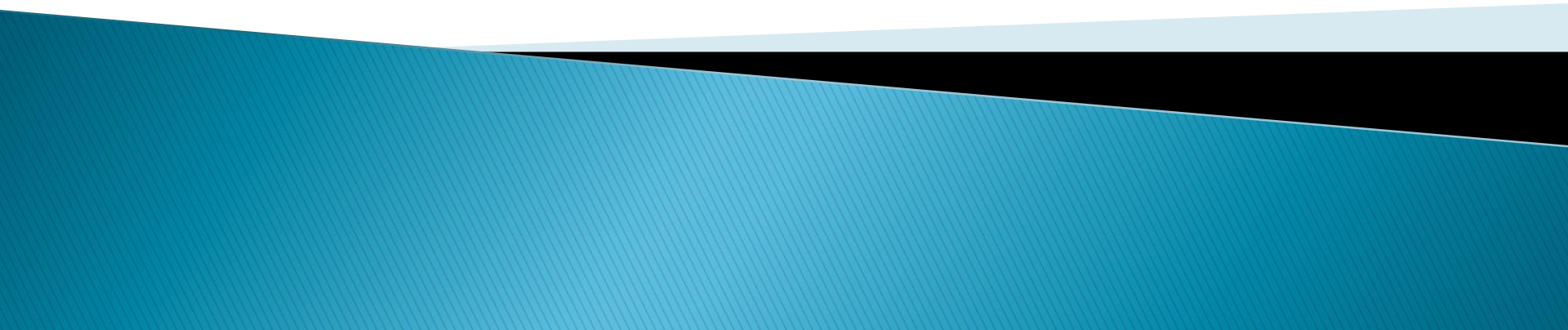


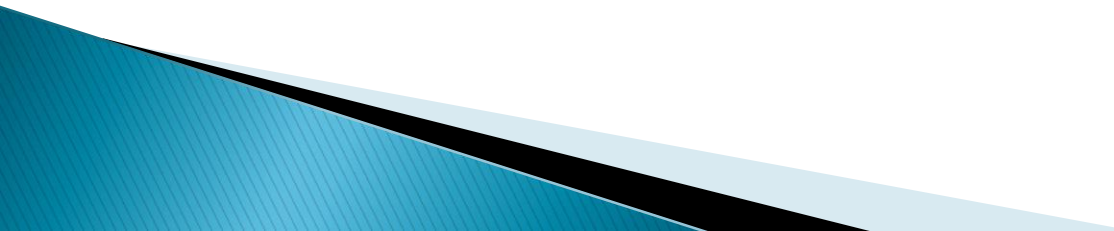
Determination of Total Alkaloid in Cortex Chinae
by Spectrophotometric Method
(European Pharmacopoeia)



Spectroscopic methods

•Spectrophotometry is a discipline that investigate the transmittance (permeability)/absorbance properties of a material as a function of wavelength.

➤ **Most commonly used spectroscopic methods:**

- Spectrophotometry (UV-Visible, IR, X-ray)
 - Colorimetry
 - Mass Spectroscopy
 - NMR Spectroscopy
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- Method: Spectrophotometry
 - Device: Spectrophotometer
- 

▶ Spectrophotometry is an analysis method based on the absorption of light energy.

• Wavelength;

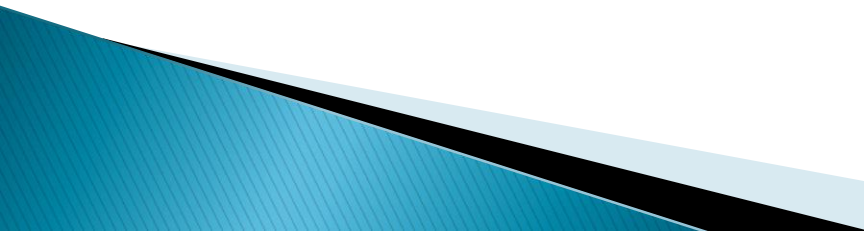
- 200–400 nm: Ultraviolet (UV = ultraviolet);
- 400 – 750 nm: Visible

Spectrophotometer

Deuterium (D2) lamp : For UV light,

Tungsten (W) lamp : Used for visible light.

- The ray emitted by the lamp is converted into a single wavelength ray(monochromatic ray).
- This beam is passed through from sample.
- The intensity of the light passing through the sample is detected by the detector and sent to the recorder or printer.

- ▶ Light can be absorbed by atoms, ions and molecules. The absorbed energy causes to pass the electrons from lower energy orbitals (ground state) to the higher energy orbitals (excited state).
 - ▶ An electron in the molecule emerges from an orbit higher than its orbit. And, the absorption peak occurs.
 - ▶ Each functional group (eg C = C double bond) always absorbs in the same region.
- 

Absorption Spectrum

- ▶ A graph of amount of absorption against the wavelength of light is called the **absorption spectrum**.

Chromophore group in the functional group responsible for the absorption of light in a given wavelength range in any molecule; the wavelength of the light absorbed by the chromophoric groups and the groups that change the absorption coefficient are called the auxochrome group.

<u>Kromofor grup</u>	<u>Oksokrom grup</u>
>C=C<	CH_3
$\text{-C}\equiv\text{C-}$	I, Cl, Br
>C=O	OH
NO_2	OCH_3
$\text{-C}\equiv\text{N}$	SO_2NH_2
>C=C-C=C<	COOH
>C=C-C=O	NH_2
$\text{>C=C-C}\equiv\text{C-}$	CHO
$\text{N}\equiv\text{N}$	

Changes:

1) Bathochromic slip : Absorption band of a molecule is a longer wavelength shift.

2) Hypsochromic slip : It is a shorter wavelength shift of the absorption band of a molecule.

Increased severity of absorption band is called hyperchromic effect, reduction is called hypochromic effect.

- ▶ Monochromatic and light beam of I_0 ;
 - It is absorbed by any molecule in the solution in b cm thick and
 - leaves the tube at I intensity.
 - decrease in light intensity due to absorption by the molecule;
 - Explained by *Lambert-Beer* equation.

giren ışığın şiddeti

örnek kabının kalınlığı (cm)

$$\log \frac{I_0}{I} = \epsilon \cdot b \cdot C = A \Rightarrow \text{Absorbsiyon}$$

çıkan
ışığın
şiddeti

molar
absorbsiyon
katsayısı
(lt/mol.cm)

mol/lt

DENEYİN YAPILIŞI

Alkaloid kinotannik asite bağlı iken serbest hale geçer

0,2 g drog (C. Chinae) (**Rubiaceae**)

+
2 ml su + 1,4 ml dil HCl

15 dk su banyosunda ısıtılır,
sonra soğutulur

5 ml CHCl₃

+
10 ml eter

+
1 ml % 20'lik NaOH

20 dk hızla çalkalanır

0,6 g kitre zamkı

Berrak olana kadar çalkalanır

Süzüntü+4 ml
(1K Kloroform+2K Eter
karışımı)

Kuruğuğa kadar su banyosunda uçurulur

Artık

+
2 ml etanol

Çözülür

Çözeltiden 0,25 ml alınır

Su banyosunda
kuruğuğa kadar uçurulur

Artık 0,1 M HCL'de çözülüp, 0,1 M HCL ile 50 ml'ye tamamlanır

Preparation of Reference Solutions

15 mg quinine is dissolved in 0.1 M HCl to 50 ml.

15 mg of kinkonin dissolved in 0.1 M HCl to 50 ml.



$$x = \frac{[A_{316} \times A_{348c}] - [A_{316c} \times A_{348}]}{[A_{316q} \times A_{348c}] - [A_{316c} \times A_{348q}]} \times \frac{100}{M} \times \frac{2}{1000}$$

$$y = \frac{[A_{316} \times A_{348q}] - [A_{316q} \times A_{348}]}{[A_{316c} \times A_{348q}] - [A_{316q} \times A_{348c}]} \times \frac{100}{M} \times \frac{2}{1000}$$

c= cinchonine

q= quinine

- M = weight(g)
x = quinine type alkaloid percentage
y = cinchonine type alkaloid percentage
A316 = absorbance of test solution at 316 nm
A348 = absorbance of test solution at 348 nm
A316c = absorbance of the reference solution containing cinchonine at 316 nm
A348q = absorbance of the reference solution containing quinine at 348 nm
A316q = absorbance of the reference solution containing quinine at 316 nm
A348c = the absorbance of the reference solution containing cinchonine at 348 nm

$$\frac{100 x}{x + y} \quad (\text{Relative percentage of quinine alkaloids})$$