

WELCOME

Spectroscopic Analysis of Phytopharmaceuticals

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Spectroscopy

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Mass spectrometry

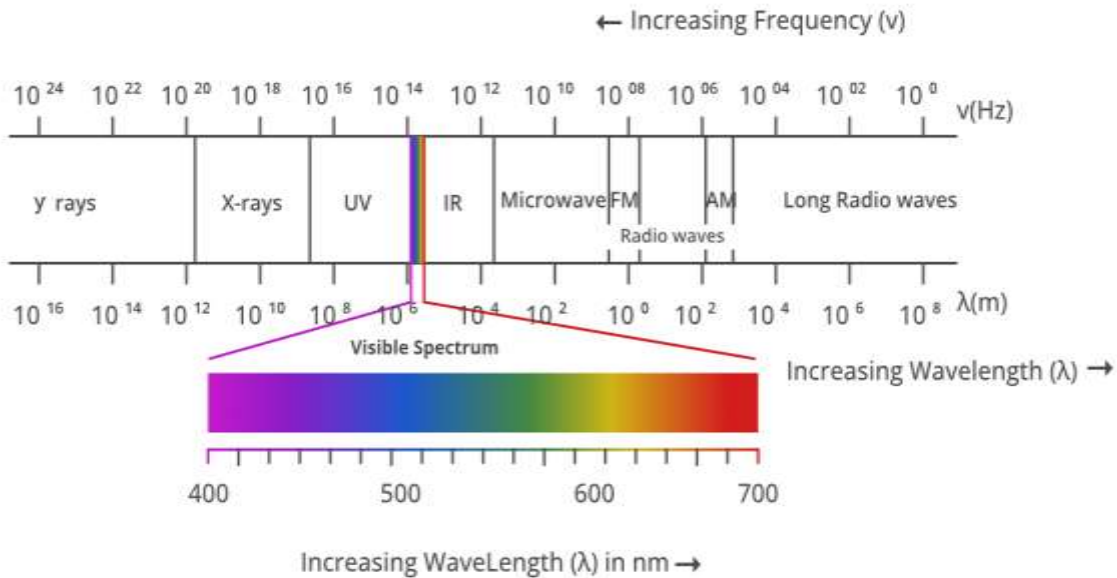
Nuclear Magnetic Resonance (NMR)
spectroscopy

INTRODUCTION

- ✓ *Phytopharmaceuticals* are pharmaceutical products composed of natural compounds derived from botanicals.
- ✓ As phytopharmaceuticals are a complex mixture of many components, it is not easy to do an analysis of each component.
- ✓ Thus for proper analysis, it is essential to understand the chemistry of phytochemicals present in herbal products.
- ✓ **Isolation, structural identification, and quantification** need to be planned accordingly.
- ✓ Hence, first need to look at **chromatographic techniques** isolating various components.
- ✓ Once the components are separated they should be analyzed by **spectroscopic methods** for identification and quantification.

- **Spectroscopy** is the analytical technique that uses the interaction of electromagnetic radiation (EMR) with matter to perform analysis. Matter may be ions, molecules, or atoms.
- **Spectrophotometer**- An instrument that measures the interaction between EMR and matter.
- Spectrophotometer measures the **% transmittance of light radiation** when light of certain intensity and frequency range is passed through the sample.
- Instrument compares the intensity of transmitted light with that of incident light.

❖ ELECTROMAGNETIC SPECTRUM



Type of Radiation	Frequency Range (Hz)	Wavelength Range
Gamma-rays	10^{20} - 10^{24}	$<10^{-12}$ m
X-rays	10^{17} - 10^{20}	1 nm-1 pm
Ultraviolet	10^{15} - 10^{17}	400 nm-1 nm
Visible	4 - 7.5×10^{14}	750 nm-400 nm
Near-infrared	1×10^{14} - 4×10^{14}	2.5 μm -750 nm
Infrared	10^{13} - 10^{14}	25 μm -2.5 μm
Microwaves	3×10^{11} - 10^{13}	1 mm-25 μm
Radio waves	$<3 \times 10^{11}$	>1 mm

❖ Types of spectrophotometry

- **ABSORPTION SPECTROSCOPY:** An analytical technique that concerns with the measurement of absorption of electromagnetic radiation. e.g. UV (200 - 400 nm)/ Visible (400 - 800 nm) Spectroscopy, IR Spectroscopy (0.76 - 15 μm).
- **EMISSION SPECTROSCOPY:** An analytical technique in which emission (of a particle or radiation) is dispersed according to some emission property & the amount of dispersion is measured. e.g. Mass Spectroscopy.

Spectroscopic Methods Used in Phytopharmaceutical Analysis

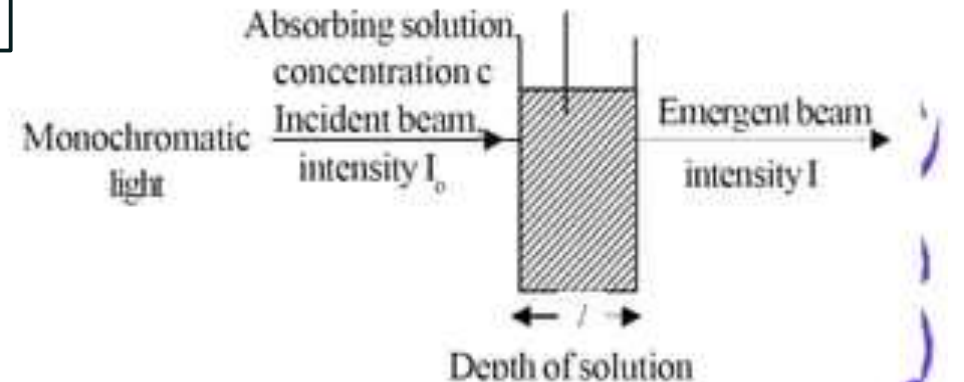
1. **UV-Visible Spectroscopy:** To find out whether the system is conjugated.
2. **IR Spectroscopy:** To identify the functional groups that are present in the compound
3. **NMR Spectroscopy:** It gives an idea about the structural backbone of the compound.
 - **^{13}C -NMR:** To identify how many types of carbon atoms are present in the compound.
 - **^1H -NMR:** To determine how many types of hydrogen atoms are present in the compound and how the hydrogen atoms are connected.
4. **Mass Spectroscopy:** To determine the compound's molecular structure and molecular weight.

❖ Fundamental laws

- ▶ **BEER'S LAW:** absorbance of the incident light is directly proportional to the concentration of the solution.
- ▶ **LAMBERT'S LAW:** absorbance of the incident light is directly proportional to the length of the light path.

$$\text{▶ } A = \log_{10} \frac{I_0}{I} = \epsilon lc$$

- ▶ A = absorbance
- ▶ ϵ = molar absorptivity
- ▶ b = length of light path
- ▶ c = concentration



UV-VISIBLE SPECTROSCOPY

- UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis.
- It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution.
- Instrument: Ultraviolet-Visible spectrophotometers.
- UV/Vis spectroscopy is in its simplest form, a sample is placed between a light source and a photo detector, and the intensity of a beam of light is measured before and after passing through the sample.

- The absorption spectra of plant components can be measured in a very dilute solution against a solvent blank using a spectrophotometer.
- For colourless compounds, measurements are made in the range of 200-400 nm; for coloured compounds, the range is 200-700nm.
- The wavelength of the maxima and minima of the absorption spectrum so obtained are recorded (in nm) and also the intensity of the absorbance (optical density) at the particular maxima and minima.
- When the matter absorbs the light, it undergoes excitation and de-excitation, resulting in the production of a spectrum.

❖ Principle of UV-Vis Spectroscopy

- When ultraviolet radiations are absorbed, this results in the excitation of the electrons from the ground state towards a higher energy state.
- Molecules containing π -electrons or non-bonding electrons (n-electrons) can absorb energy in the form of ultraviolet light to excite these electrons to higher anti-bonding molecular orbitals.
- The more easily excited the electrons, the longer the wavelength of light it can absorb. There are four possible types of transitions ($\pi-\pi^*$, $n-\pi^*$, $\sigma-\sigma^*$, and $n-\sigma^*$), and they can be ordered as follows: $\sigma-\sigma^* > n-\sigma^* > \pi-\pi^* > n-\pi^*$.

APPLICATIONS

❖ **Detection of Impurities**

UV absorption spectroscopy is one of the best methods to determine the impurities in organic molecules. Additional peaks can be observed due to impurities in the sample and can be compared with standard raw material. The impurities can be detected by measuring the absorbance at specific wavelength.

❖ **Quantitative analysis**

UV absorption spectroscopy can be used for the quantitative determination of compounds that absorb UV radiation

❖ **Qualitative analysis**

UV absorption spectroscopy can characterize those types of compounds which absorbs UV radiation. Identification is done by comparing the absorption spectrum with the spectra of known compounds.

❖ **Chemical kinetics**

Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the absorbance changes can be observed.

❖ **Detection of functional groups**

This technique detects the presence or absence of functional groups in the compound. Absence of a band at particular wavelength regarded as evidence for the absence of a particular group

❖ **Structure elucidation of organic compounds**

UV spectroscopy is useful in the structure elucidation of organic molecules, the presence or absence of unsaturation, and the presence of hetero atoms. From the location of peaks and combination of peaks, it can be concluded that whether the compound is saturated or unsaturated, hetero atoms are present or not etc.

❖ **Molecular weight determination**

The molecular weights of compounds can be measured spectrophotometrically by preparing the suitable derivatives of these compounds. For example, if we want to determine the molecular weight of amine then it is converted in to amine picrate. Then known concentration of amine picrate is dissolved in a liter of solution and its optical density is measured at λ_{\max} 380 nm.

Instrumentation of UV-vis Spectroscopy

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- Light Source
- Monochromator
- Sample and reference cells
- Detector
- Amplifier

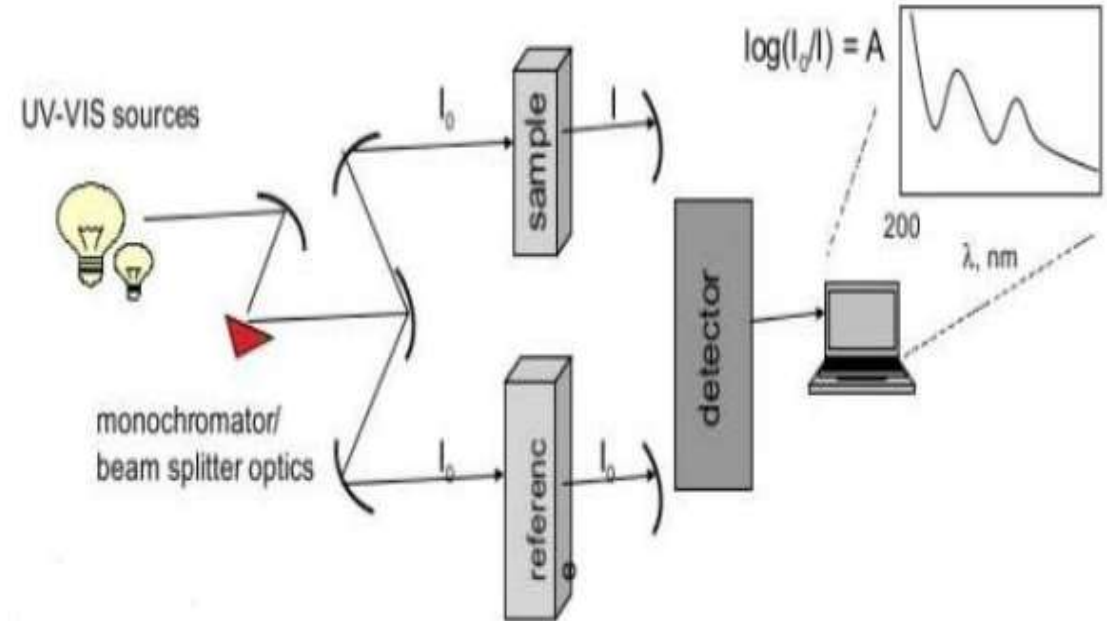


Fig. 05. Instrumentation of dual beam UV-VIS Spectrophotometer

INFRARED (IR) SPECTROSCOPY

IR SPECTROSCOPY

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- IR spectroscopy (which is short for infrared spectroscopy) deals with the **infrared region of the electromagnetic spectrum**.
- **IR rays**: light having a longer wavelength and a lower frequency than visible light.
- IR spectroscopy: analysis of the **interaction of a molecule with infrared light**.
- The major use of infrared spectroscopy is to **determine the functional groups of molecules**, relevant to both organic and inorganic chemistry.
- The method or technique of infrared spectroscopy is conducted with an instrument called an **infrared spectrometer** (or spectrophotometer) to produce an **infrared spectrum**.

Principle of Infrared (IR) Spectroscopy

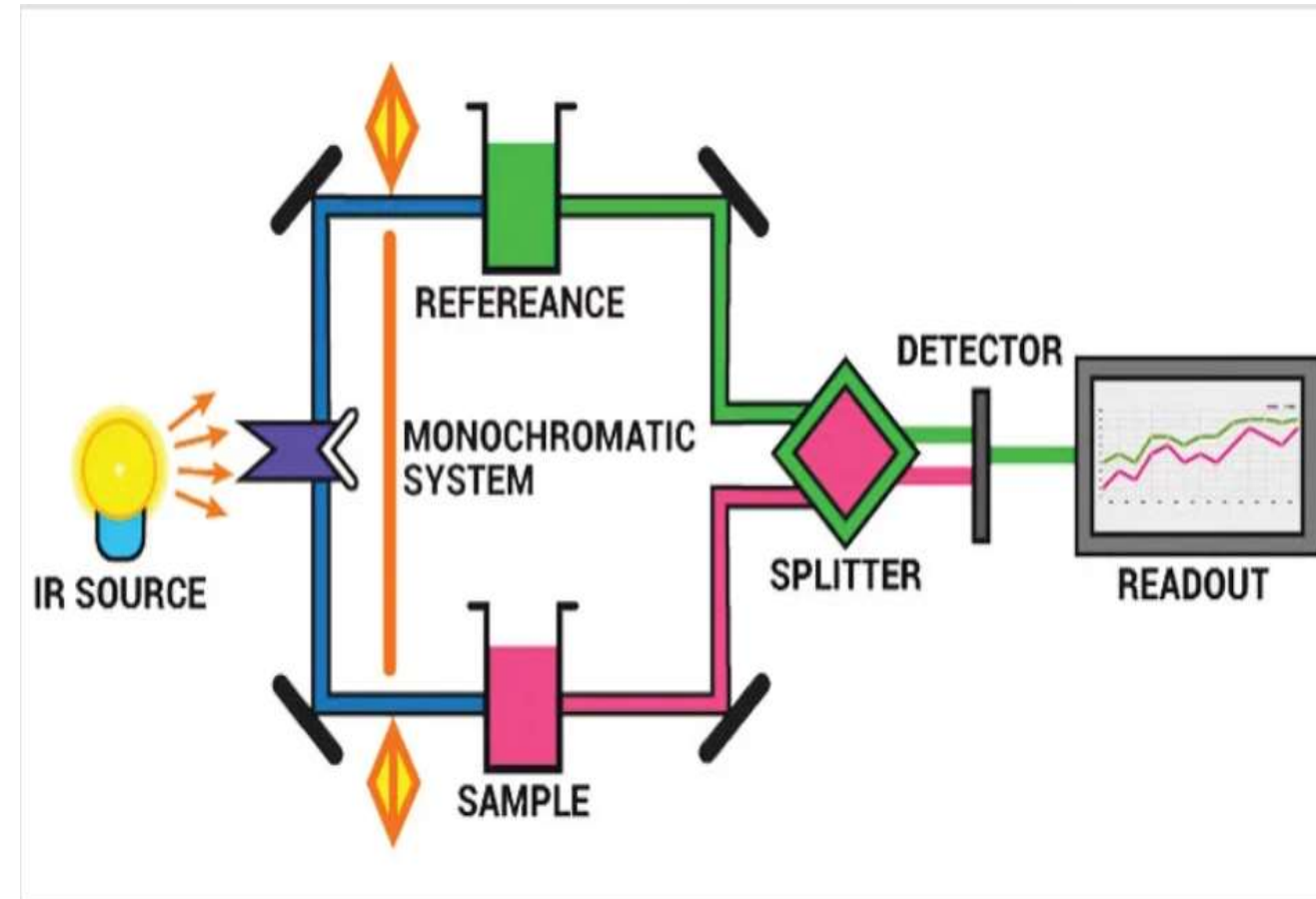
- IR spectroscopy is the analysis of infrared light interacting with a molecule.
- The portion of the infrared region most useful for analysis of organic compounds have a wavelength range from 2,500 to 16,000 nm.
- Photon energies associated with this part of the infrared are not large enough to excite electrons, but may **induce vibrational excitation** of covalently bonded atoms and groups.
- It is known that in addition to the rotation of groups, molecules experience a wide variety of vibrational motions, characteristic of their component atoms.
- When a molecule absorbs infrared radiation, its chemical bonds vibrate. The bonds can **stretch, contract, and bend**. This is why infrared spectroscopy is a **type of vibrational spectroscopy**.
- From IR spectrometers, there obtains an absorption spectrum of compounds that are a unique reflection of their molecular structure.
- The fundamental measurement obtained in infrared spectroscopy is an infrared spectrum, which is a plot of measured infrared intensity versus wavelength (or frequency) of light.
- IR Spectroscopy measures the vibrations of atoms, and based on this it is possible to determine the functional groups.

Instrumentation of Infrared (IR) Spectroscopy

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The main parts of the IR spectrometer are as follows:

- ✓ *Radiation source*
- ✓ *Sample cells and sampling of substances*
- ✓ *Monochromators*
- ✓ *Detectors*
- ✓ *Recorder*



Studying the progress of the reaction

Protein characterisation

Quantitative analysis

Identification of substances

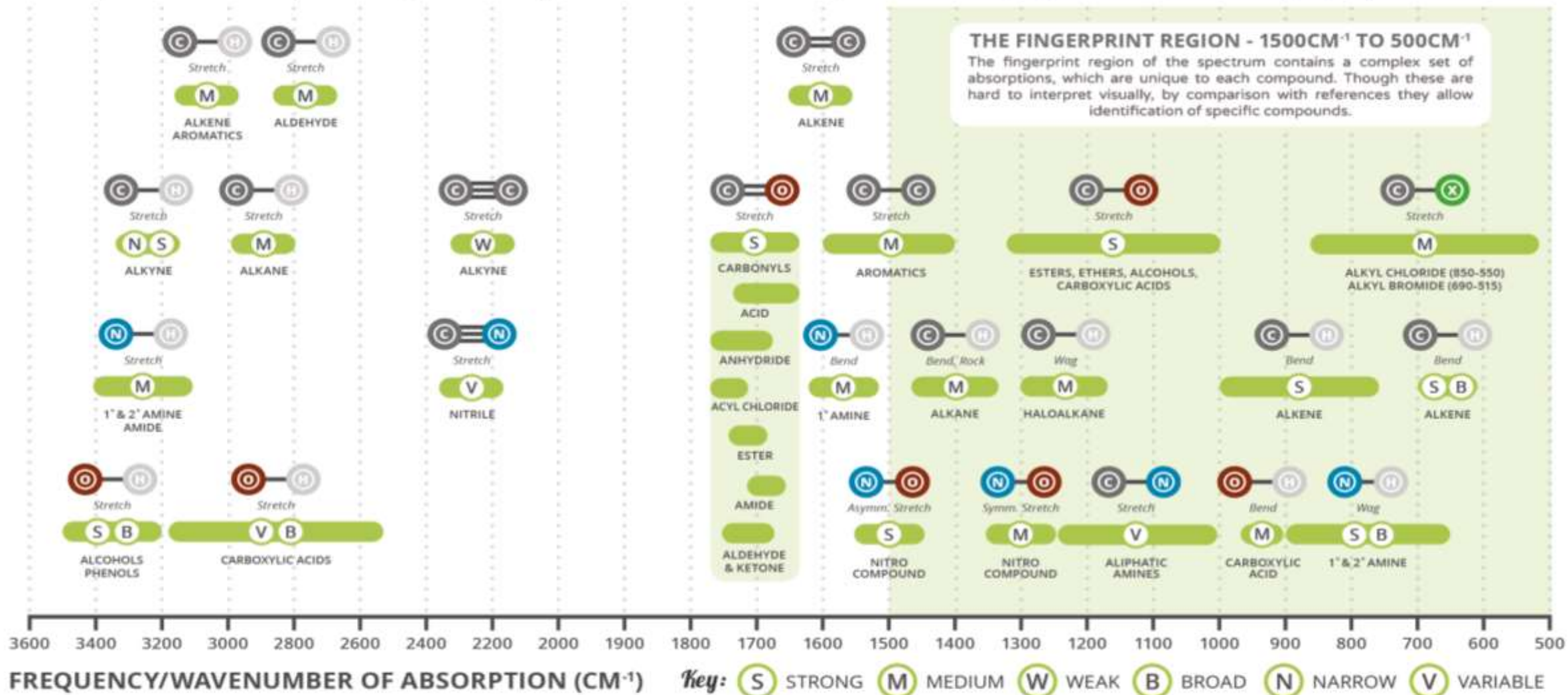


To know about interaction among molecules

Identification of functional group and structure elucidation

ANALYTICAL CHEMISTRY - INFRARED SPECTROSCOPY

Commonly referred to as IR spectroscopy, this technique allows chemists to identify characteristic groups of atoms (functional groups) present in molecules.

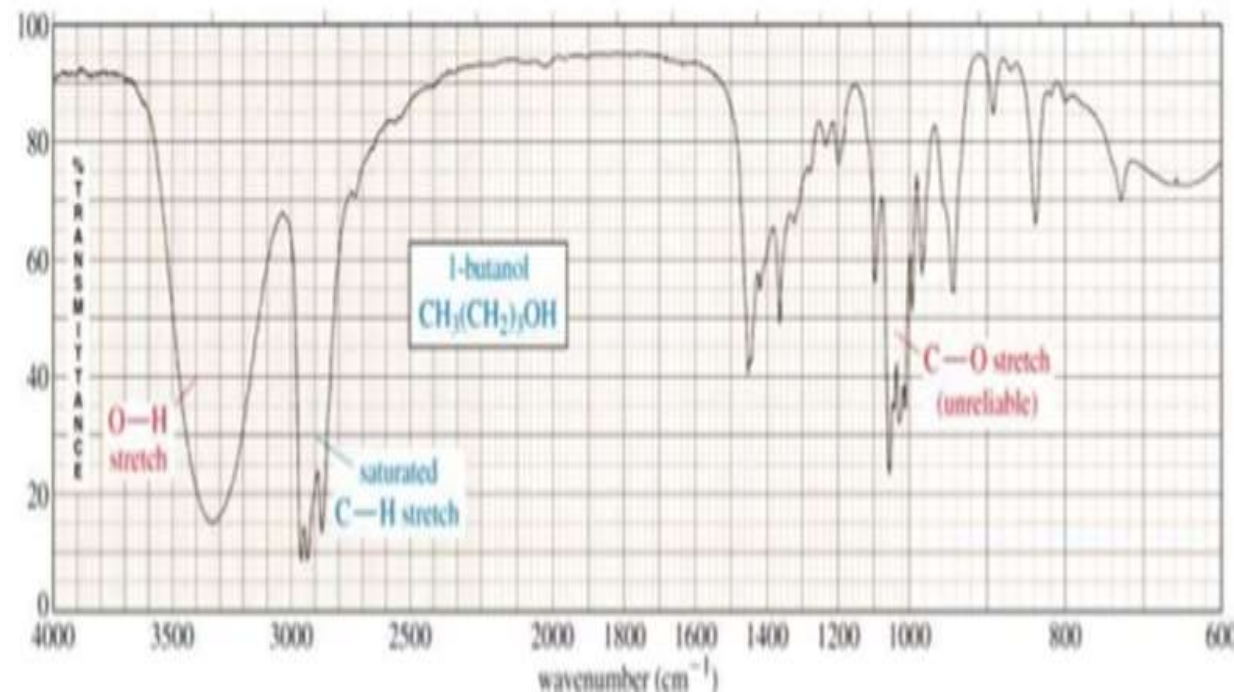


Infrared frequencies make up a portion of the electromagnetic spectrum. If a range of infrared frequencies are shone through an organic compound, some of the frequencies are absorbed by the chemical bonds within the compound. Different chemical bonds absorb different frequencies of infrared radiation. There are a number of characteristic absorptions which allow functional groups (the parts of a compound which give it its particular reactivity) to be identified. This graphic shows a number of these absorptions.

Functional Group	Absorption Location (cm^{-1})	Absorption Intensity
Alkane (C-H)	2,850–2,975	Medium to strong
Alcohol (O-H)	3,400–3,700	Strong, broad
Alkene (C=C)	1,640–1,680	Weak to medium
(C=C-H)	3,020–3,100	Medium
Alkyne (C≡C)	2,100–2,250	Medium
(C≡C-H)	3,300	Strong
Nitrile (C≡N)	2,200–2,250	Medium
Aromatics	1,650–2,000	Weak
Amines (N-H)	3,300–3,350	Medium
Carbonyls (C=O)		Strong
Aldehyde (CHO)	1,720–1,740	
Ketone (RCOR)	1,715	
Ester (RCOOR)	1,735–1,750	
Acid (RCOOH)	1,700–1,725	

IR SPECTRUM OF AN ALCOHOL

The most prominent band in alcohols is due to the **O-H bond**, and it appears as a strong, broad band covering the range of about **3000 - 3700 cm^{-1}** . The sheer size and broad shape of the band dominate the IR spectrum and make it hard to miss.

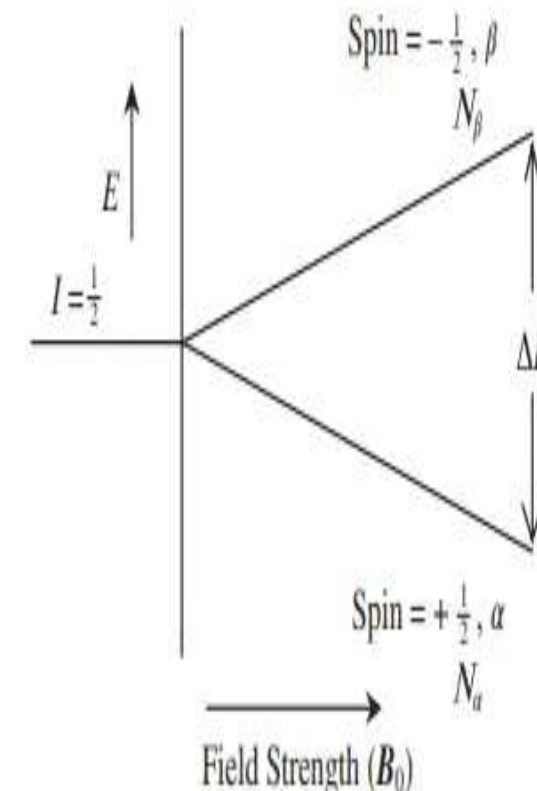


NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

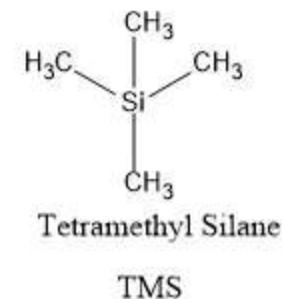
- Nuclear magnetic resonance (NMR) spectroscopy is the study of molecules by recording the interaction of radiofrequency (Rf) electromagnetic radiations with the nuclei of molecules placed in a strong magnetic field.
- It is a research technique that explores the magnetic properties of certain atomic nuclei.
- The NMR spectroscopy determines the physical and chemical properties of atoms or molecules.
- Nuclear magnetic resonance (NMR) spectroscopy is a crucial analytical tool for organic chemists.
- Not only can it provide information on the structure of the molecule, it can also determine the content and purity of the sample. Proton (^1H) NMR is one of the most widely used NMR methods by organic chemists.
- The protons present in the molecule will behave differently depending on the surrounding chemical environment, making it possible to elucidate their structure.

- It is based on the fact that the nuclei of most of the atoms show spin and all nuclei are electrically charged.
- NMR-spectroscopy is based on the absorption of electromagnetic radiation in the radiofrequency region 3kHz-300 GHz.
- Nuclei of atoms have spin and electrical charge, so they generate magnetic fields. In the presence of an external magnetic field, nuclei of atoms align themselves either in the direction of the external magnetic field or in the opposite direction of the external magnetic field.
- In the presence of an external magnetic field, energy transfer takes place between the ground state to the excited state.
- It takes place at a wavelength that matches with radio frequencies and when the electron returns from an excited state to ground state, it emits the radio wave of the same frequency.
- This emitted radio frequency gives NMR spectrum.

- If a nucleus is aligned with the magnetic field, it is said to occupy the **α -spin state** and if it is aligned against the field, it is said to occupy the **β -spin state**.
- The external magnetic field establishes an **energy gap** between spin states.
- When the energy between the **two energy states lies within the ranges of the radiofrequency radiation** then the nucleus is said to be in nuclear magnetic resonance.
- Due to the induced magnetic field established by the **circulating electron density**, the nuclei/proton experiences a slightly smaller net magnetic field than an actual magnetic field. This effect is called the **shielding effect**.
- Therefore, **protons/nuclei in the different chemical environments** have different energy gaps between the α -spin state and the β -spin state and hence, absorb different frequencies of radiofrequency radiation.

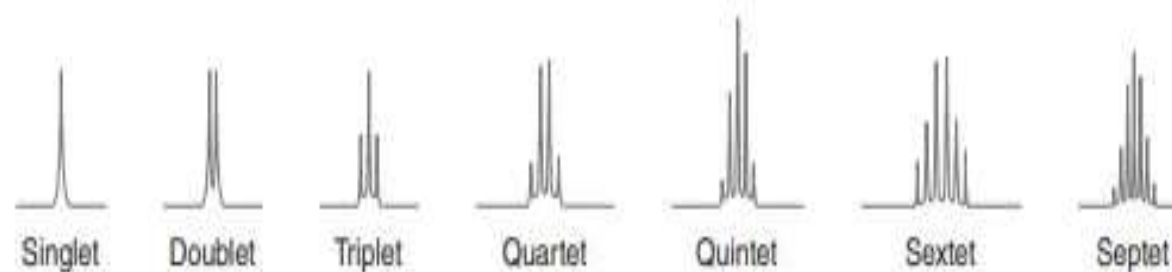


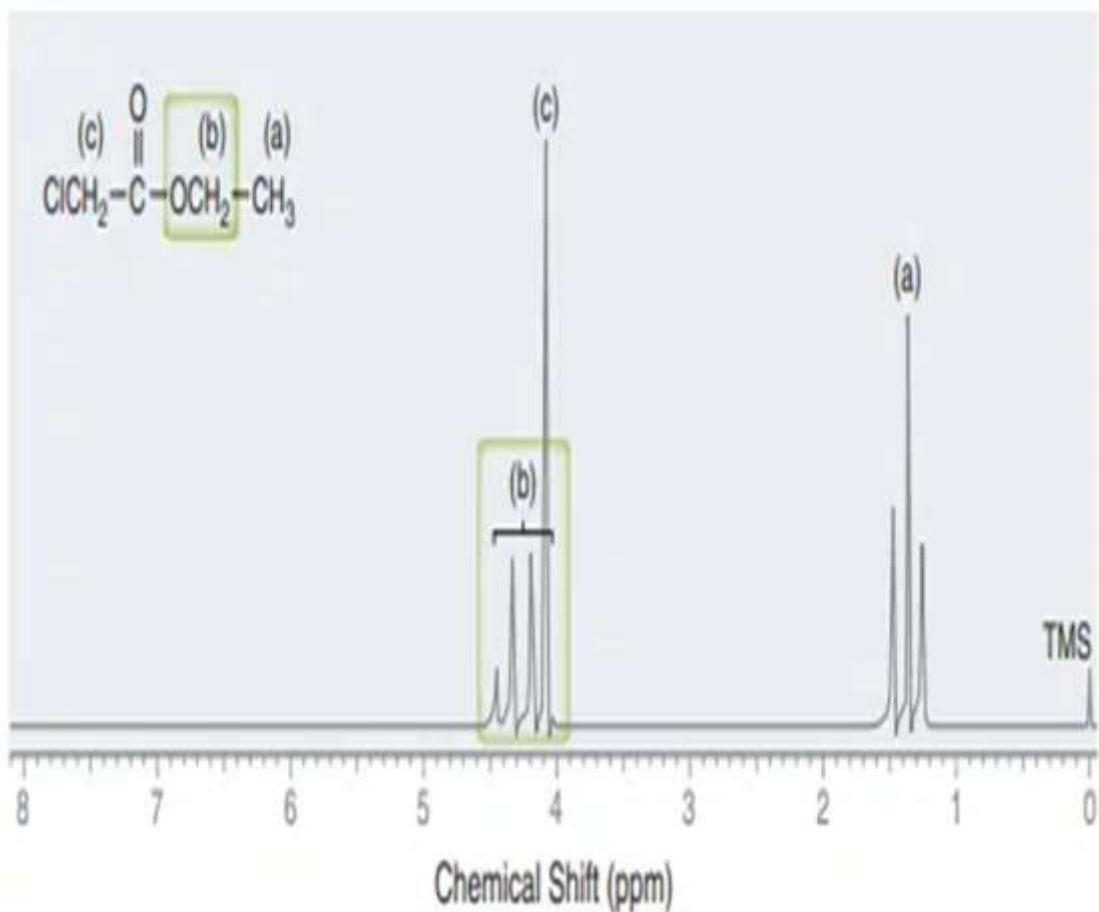
- ✓ A plot of the **frequencies** of the absorption peaks versus **peak intensities** constitutes an NMR spectrum.
- ✓ The signals on the left side of the spectrum (downfield) are high-frequency signals and the signals on the right side of the spectrum (up field) are low frequencies signals.
- ✓ **Chemical shift** is defined as the difference in parts per million (ppm) between the resonance frequency of the observed proton and that of the reference compound, **tetramethylsilane (TMS)**.
- ✓ TMS is a universally accepted reference compound because of the following facts.
 - ✓ TMS is chemically inert, symmetrical, volatile, and soluble in most organic solvents.
 - ✓ TMS gives a signal, intense, sharp NMR peak, and its protons are more shielded than almost all other protons in the organic compounds.



Coupling constant (spin-spin splitting in NMR spectroscopy)

- The splitting of a signal occurs due to the effects of neighboring protons (**n+1 rule**).
- The neighboring proton with a different chemical shift can influence the signal of the other.
- If the protons are in the same chemical environment, such protons are said to be equivalent and absorb the same magnetic field strength and they behave as one proton.
- If the protons are in different chemical environments, such protons are said to be non-equivalent protons and absorb different magnetic field strengths. Therefore, the splitting of the peak takes place.



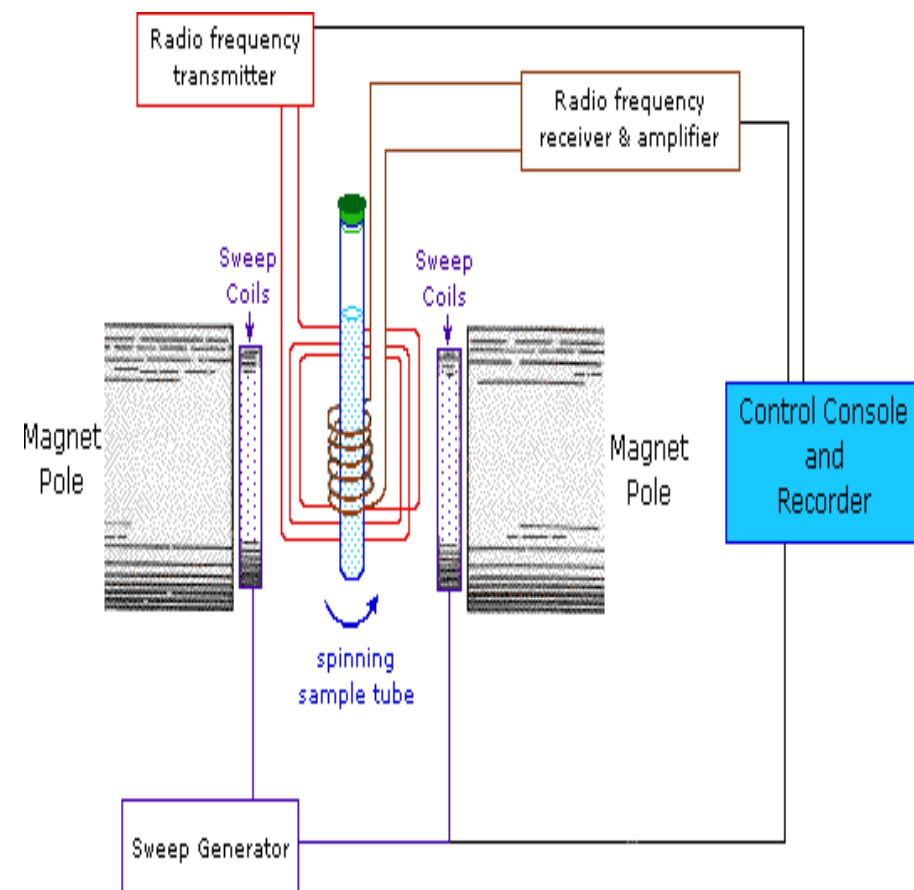


TYPE OF PROTON	CHEMICAL SHIFT (δ)	TYPE OF PROTON	CHEMICAL SHIFT (δ)
Methyl	R-CH_3 ~ 0.9	Alkyl halide	R-C(H)(X)R 2-4
Methylene	>CH_2 ~ 1.2	Alcohol	R-O-H 2-5
Methine	-CH ~ 1.7	Vinylic	=CH 4.5-6.5
Allylic	$\text{CH}_2\text{-CH=}$ ~ 2	Aryl	C_6H_5 6.5-8
Alkynyl	$\text{R-C}\equiv\text{C-H}$ ~ 2.5	Aldehyde	R-C(=O)H ~ 10
Aromatic methyl	$\text{C}_6\text{H}_5\text{-CH}_3$ ~ 2.5	Carboxylic acid	R-C(=O)OH ~ 12

INSTRUMENTATION

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- **Sample holder:** It is a glass tube which is 8.5 cm long and 0.3 cm in diameter.
- **Magnetic coils:** Magnetic coil generates a magnetic field whenever current flows through it
- **Permanent magnet:** It helps in providing a homogenous magnetic field at 60-100 MHz
- **Sweep generator:** Modifies the strength of the magnetic field which is already applied.
- **Radiofrequency transmitter:** It produces a powerful but short pulse of the radio waves.
- **Radiofrequency receiver:** It helps in detecting receiver radio frequencies.
- **RF detector:** It helps in determining unabsorbed radio frequencies.
- **Recorder:** It records the NMR signals which are received by the RF detector.
- **Readout system:** A computer that records the data.



APPLICATIONS

- **Identification of functional groups:** Every functional group has a characteristic signal in the NMR spectrum. By studying the chemical shift of the compound, it is possible to identify the functional group in the compound.
- **Structure elucidation of unknown compound:** By observing the chemical shift values and splitting of the signal of protons under different environments, it is possible to place hydrogen at a suitable place in the formula and hence the structure of a given compound can be established.
- **Comparison of two compounds and determination of the molecular weight:** By comparing the peak of the unknown compound with that of an unknown compound, the molecular weight can be determined.
- **Distinction between cis-trans isomer and conformers:** Protons have different values of chemical as well as coupling constants in cis and trans isomer. Therefore, it is easy to identify cis and trans isomers.

- **NMR fingerprinting**
- **NMR-based metabolomics:** Metabolite fingerprinting by NMR is a fast, convenient, and effective tool for discriminating between groups of related samples and it identifies the most important regions of the spectrum for further analysis.
- It is used in **food science**, study of **drugs**, **study of biofluids**, **cells and nucleic acids**.
- The coupling of high-performance liquid chromatography with nuclear magnetic resonance spectroscopy (**LC-NMR**) is one of the most powerful methods for the separation and structural elucidation of unknown compounds in mixtures.

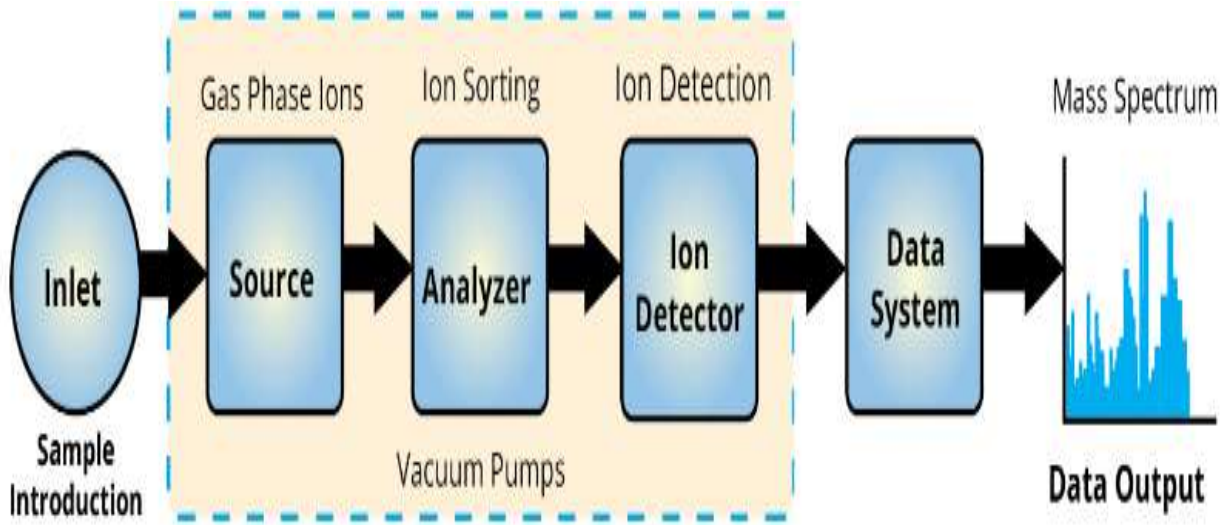
MASS SPECTROSCOPY

MASS SPECTROSCOPY

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- ✓ Mass spectrometry is an analytical technique to evaluate known materials and determine unknown compounds. It also helps to determine the structure and chemical properties of several molecules.
- ✓ powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample and to elucidate the structure and chemical properties of different molecules.
- ✓ It is a microanalytical technique requiring only a few nanomoles of the sample to obtain characteristic information pertaining to the structure and molecular weight of analyte.
- ✓ This technique basically studies the effect of ionizing energy on molecules.
- ✓ Nowadays mass spectrometry is used in many areas including pharmaceutical, clinical, geological, biotechnology and environmental.

- The principle involved in mass spectrometry is the **formation of several ions** from the sample.
- Further, these ions are separated according to their **mass-to-charge ratio**, which is also expressed as **m/z**, and then taking a record of the **relative abundance of each ion**.
- In the first step, **the sample compound is converted to ions in the gas phase** by the electron ionization method. After that, the molecular ions undergo **fragmentation**.
- Each ion is separated from the other in a mass spectrometer depending on their mass-to-charge ratio and identified according to their relative abundance.
- A **mass spectrum** is then formed, which shows the spectrum of ion abundance versus mass-to-charge ratio.
- The ions present give information about the structure and properties of the compound. In the spectrum, the molecule ion of the pure compound has the highest value of mass-to-charge ratio, followed by ions of heavier isotopes. By this, the **molecular weight of the compound is determined**.



Instrumentation of Mass Spectrometry

There are three major components present in mass spectrometry;

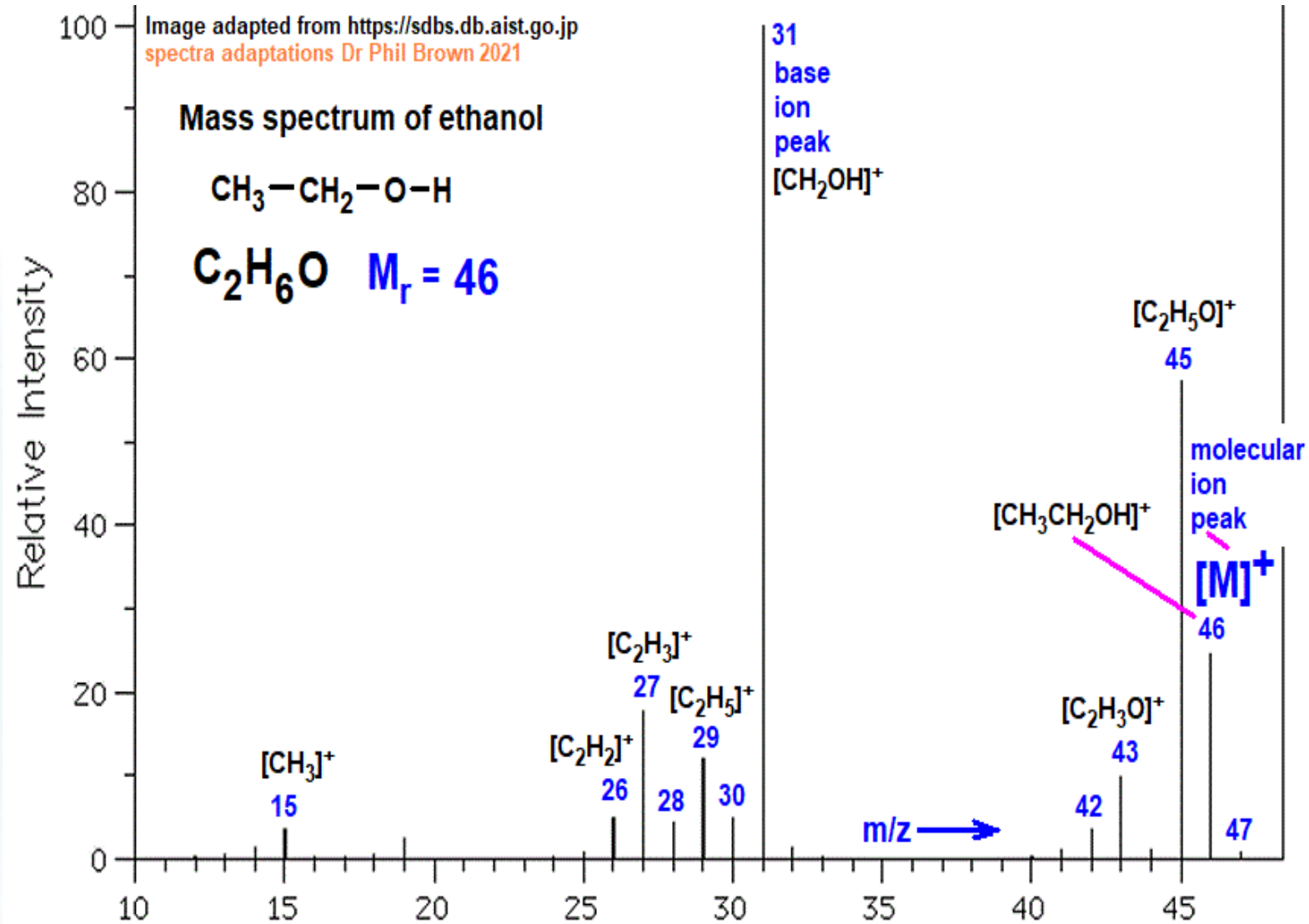
Ion Source: It produces gaseous ions from the given sample.

Analyzer: It is used to analyze and separate the ions into their characteristic mass according to their mass-to-charge ratio.

Detector System: Detectors in mass spectrometry detect the ions and maintain their relative abundance.

MASS SPECTRUM

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❖ *Phytochemical analysis*

A variety of analyte separation techniques like capillary electrophoresis, gas chromatography and high-performance liquid chromatography are united with mass spectroscopy for simultaneous separation and determination of analytes called CE-MS, GC-MS and HPLC-MS, respectively.

Analytical technique	Sample source	Analytes
HPLC-ESI-MS	<i>Leontopodium</i> species (Asteraceae)	Fatty acids, sucrose, diterpenes, sesquiterpene
GC × GC-MS	Essential oil of <i>Pelargonium graveolens</i>	α-Pinene, myrcene, limonene, citrinellal, geraniol
GC-MS	Methanolic fruit extract of <i>Momordica charantia</i>	Vitamin E, gentisic acid, 1-pentadecyne
GC-MS	Extracts of <i>Aerva lanata</i>	(R)-(+)- ζ -Valerolactone, 5,14-di (<i>N</i> -butyl)-octadecane, 9-octadecenoic acid, 2-propynoic acid
GC-MS	Ethanolic extract of <i>Azolla microphylla</i>	(R)-(+)- ζ -Valerolactone, 5,14-di (<i>N</i> -butyl)-octadecane, 9-octadecenoic acid, 2-propynoic acid
UHPLC-ESI-MS	<i>Rhizopus microsporus</i> var. <i>oryzae</i> challenged soya bean seedlings	Prenylated isoflavonoids and isoflavonoids like daidzein, genistein, glycitein
HPLC-ESI-MS/MS	<i>Radix astragali</i>	Calycosin, calycosin-7-O- β -D-glycoside, formononetin, formononetin-7-O-glycoside
HPLC-MS/MS	<i>Glycyrrhiza uralensis</i> Fisch. extract	Glycyrrhizic acid, liquorice saponin G2, liquiritin, licuraside, ononin, glycycomarin
LC/MS/MS	Dried plums	Hydroxycinnamics, including acids, esters and glycosides; hydroxybenzoic acids and one flavonoid
UHPLC-MS	<i>Licorice</i> root extract in 70% ethanol, ethanol and ethyl acetate	Prenylated flavonoids

❖ Structure elucidation

- ✓ Mass spectroscopy has major use in the structure elucidation of compounds. The mass spectrum is produced in the form of a bar graph which is interpreted by using base peak, Molecular ion peak, Isotopic peaks, Fragment ion peak, Metastable ion peak, Metastable ion.
- ✓ Eg: The structure of flavonoid monoglycosides like genistein-7-O-glucoside, genistein-4'-O-glucoside, 2'-hydroxy genistein-7-O-glucoside and apigenin, isolated from shoot of lupin (*Lupinus luteus* L.), was elucidated by using LSI-MS and EI-MS with double-focusing reversed geometry between mass spectrometer

- ❖ *Peptide and protein sequence/structure analysis*
- ❖ *Clinical studies*
- ❖ *Pharmaceutical analysis*
- ❖ *Forensic applications*
- ❖ *Metabolites analysis*
- ❖ *Applications of mass spectrometry in pharmaceutical analysis* - Drug discovery and absorption, distribution, metabolism, and elimination (ADME) studies, pharmacokinetic and pharmacodynamic analyses, metabolite screening, and preclinical development.
- ❖ *Applications of mass spectrometry in proteomics* - Characterization of proteins and protein complexes, sequencing of peptides, and identification of posttranslational modifications.

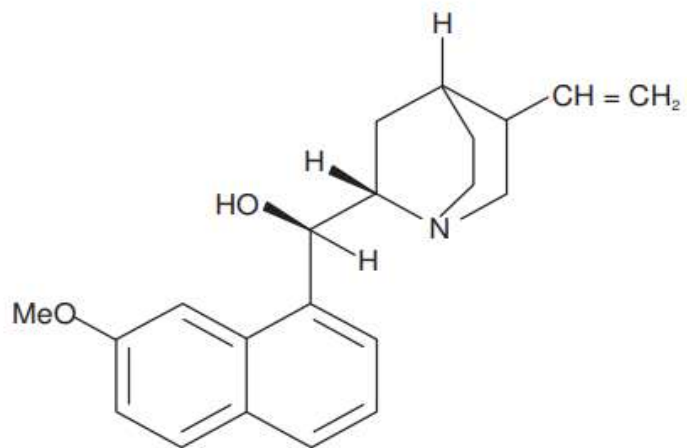
CINCHONA



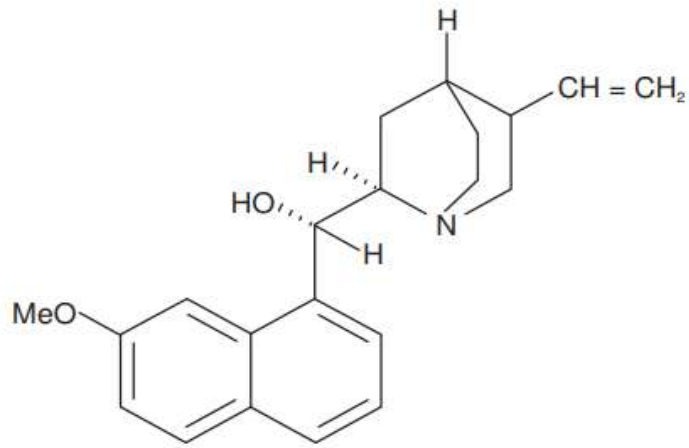
- **Synonyms:** Cortex Cinchonae, Countess, Peruvian or Jesuit's bark, Cinchona
- **Biological Source:** Cinchona is the dried bark of the stem or root of *Cinchona calisaya* Wedd., *Cinchona ledgeriana* Moens., *Cinchona officinalis* Linn., and *Cinchona succirubra* Pavon., belonging to family Rubiaceae.
- **Geographical Source:** Cinchona is a native of South America, occurring wild there. At present, it is mainly cultivated in Indonesia (Java), Zaire, India, Guatemala, Bolivia, Ceylon etc.

Chemical Constituents

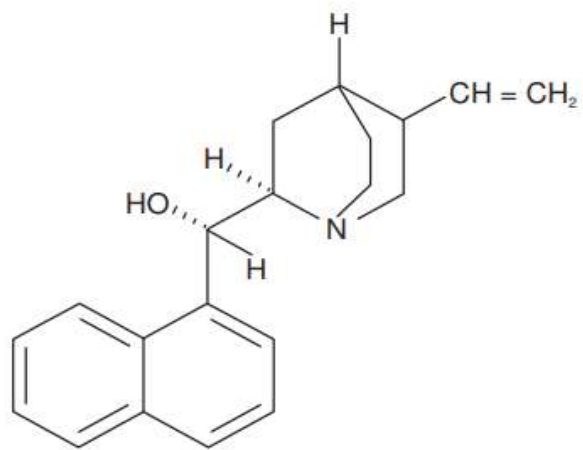
- More than 30 alkaloids have been reported in cinchona.
- The chiefly identified alkaloids are quinidine, quinine, cinchonine and cinchonidine.
- The other constituents available are quiniarnine, cinchotine, hydroquinine, hydrocinchonidine, cinchotannic acid, etc.
- Other than these it also consist of bitter glycoside, starch grains, calcium oxalate crystals and crystalline acid like quinic acid.



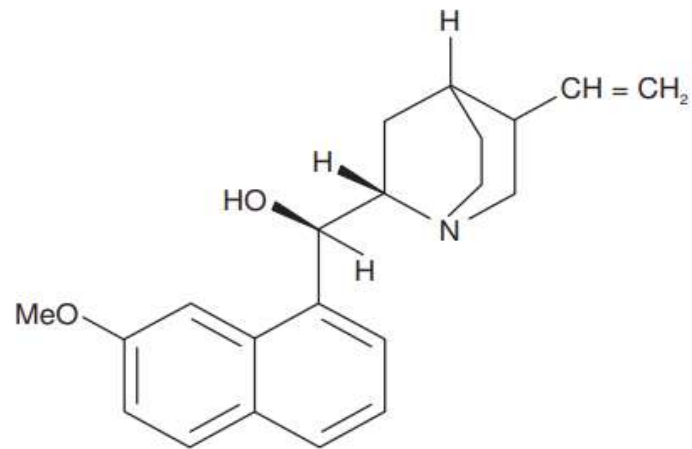
Quinine



Quinidine



Cinchonine



Cinchonidine

Characteristics

Colour	The outer surface is yellowish to brown, with short fractures and the inner surface varies in all the four species; like <i>Cinchona calisaya</i> and <i>Cinchona ledgeriana</i> is yellowish, <i>Cinchona officinalis</i> is slightly brown and <i>Cinchona succimbra</i> is reddish brown
Odour	Distinctive
Taste	Highly bitter and astringent.
Shape	Curved, quill or double quill.
Size	30 cm long and 2–7 mm thick.
Extra features	The outer surface consist of longitudinal and transverse cracks, fissures, ridges

Substitutes

Cuprea Bark (*Remijia pedunculato*); Family: Rubiaceae, it differs in its morphological character with cinchona but consist of constituents like Quinine, quinidine, cinchonine, cinchonamine, etc., the other species of Remijia, that is, R. purdieana (false Cuprea bark) does not contain quinine.

Chemical Test

- **Thalleioquin test:** To the extract of cinchona powder add one drop of dilute sulphuric acid and 1 ml of water. Add bromine water drop wise till the solution acquires a permanent yellow colour and add 1 ml of dilute ammonia solution, emerald green colour is produced.
- The powdered drug when heated with glacial acetic acid in dry test tube, evolves red fumes, which condense in the top portion of the tube.
- Cinchona bark, when moistened with sulphuric acid and observed under ultraviolet light shows a blue fluorescence due to the methoxy group of Quinine and quinidine.

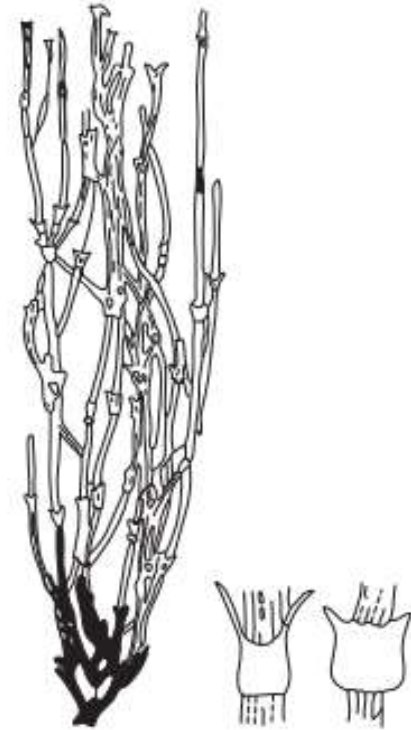
Uses

- It is mainly employed as antimalarial drug, but it is also used as analgesic, antipyretic, protoplasmic, bitter stomachic and tonic.
- Quinidine is cardiac depressant
- Cinchonidine is used in rheumatism and neuralgia.

Marketed products



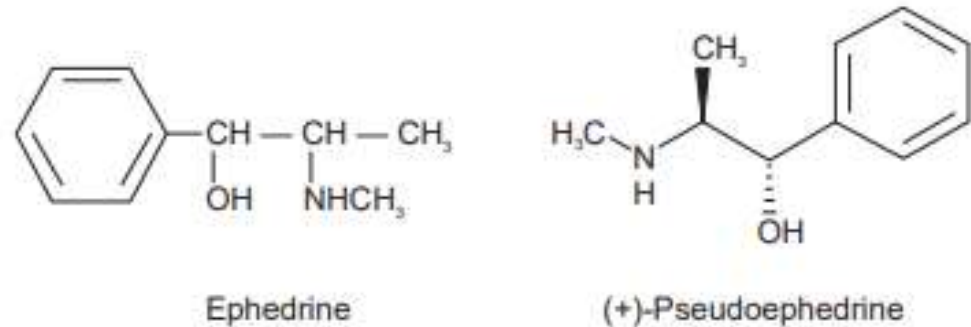
EPHEDRA



- **Synonym:** Ma Huang.
- **Biological Source:** Ephedra consists of the dried aerial parts of *Ephedra gerardiana* Wall, *Ephedra sinica* Stapf, *Ephedra equisetina* Bunge, *Ephedra nebrodensis* Tineo and other Ephedra species, belonging to family Ephedraceae.
- **Geographical Source:** It is mainly found in China, India, Nepal, Turkey, Pakistan and Bhutan.

Chemical Constituents

- Ephedra contains alkaloids **Ephedrine** (water-soluble salt of an alkaloid), Pseudoephedrine (analog of ephedrine), Nor pseudoephedrine (An analog of ephedrine).
- The leaves and stems of ephedra also contain many potentially active compounds, such as tannins, saponin, flavone and volatile oils.



Characteristics

- It consists of cylindrical woody stem that is grey or greenish in colour.
- Nodes, internodes, scaly leaves, and terminal buds are present in the stems.
- The distance between the internodes is 3–4 cm and the nodes bare the scaly leaves.
- They are bitter in taste.
- The plant has stamens and pistils on separate flowers.
- Fruit has two carpels with a single seed in each, branches slender and erect, small leaves, scale-like, articulated and joined at the base.

Chemical Test

- To the drug (10 mg) in water (1 ml) dilute HCl (0.2 ml), copper sulphate solution (0.1 ml) and sodium hydroxide solution (2 ml) are added; the **liquid turns violet**. On adding solvent ether (2 ml) and shaking vigorously, the ethereal layer turns purple and the aqueous layer becomes **blue**.

EXTRACTION

- Take weighed quantity of dried young stems of ephedra and powder to obtain coarse sized particles.
- Extract with petroleum ether.
- Filter and discard ether extract.
- Dry the marc and moisten with dilute ammonia.
- Extract this marc with aqueous alcohol.
- Filter and evaporate filtrate to obtained syrupy mass.
- Extract this mass with 3 portions of aqueous alcohol.
- Add sufficient quantity of sodium carbonate solution to make the extract alkaline.
- Filter and evaporate filtrate to obtained dry residue of ephedrine.

ESTIMATION: – TLC

- **Test solution:** Extract about 5g of freshly powdered drug with 50 ml methanol by shaking for 30 min at 500 in a conical flask. Filter and concentrate the filtrate under vacuum to about 5ml.
- **Standard solution:** Dissolve 5mg ephedrine in 10ml methanol
- **Solvent system:** Chloroform: Toluene : Ethanol[3:6:2]
- **Procedure:** Apply separately 10 μ l each of test and reference solution on a pre-coated silica gel 60 F₂₅₄ TLC plate of uniform thickness of 0.2mm. Develop the plate in the solvent system to a distance of 8cm and dry in a current of hot air.
- **Visualization spray** the air dried plate with ninhydrin reagent and heat at 110^o for 5min note the R_f value. R_f value : 0.15 color of band reddish purple (ephedrine)

Therapeutic uses

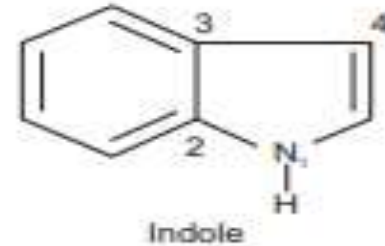
- Ephedrine is *antiallergenic, antiasthmatic, antispasmodic, decongestant, cough suppressant, stimulant and vasoconstrictor.*
- Pseudoephedrine is decongestant, cough suppressant and norpseudoephedrine is peripheral vasodilator used to treat angina.
- As a whole it is decongestant; it opens sinuses, increases sweating, dilates bronchioles (antiasthmatic use), diuretic, CNS stimulant, raises blood pressure, alleviates aches and rheumatism, alleviates hay fever/colds, etc.

Commercial applications

Ephedrine is available under the following different brand names: Akovaz, Emerphed and Corphedra.

INDOLE ALKALOIDS

- Indole (1-H-indole) is a benzopyrrole in which the benzene and pyrrole rings are through the 2, 3-positions of the pyrrole.



Indole alkaloids

Van Urk's test or Elrich's test: To an alkaloidal solution, add a few drops of para dimethyl aminobenzaldehyde (Elrich's reagent), 5% ferric chloride and sulfuric acid. A purple color is formed.

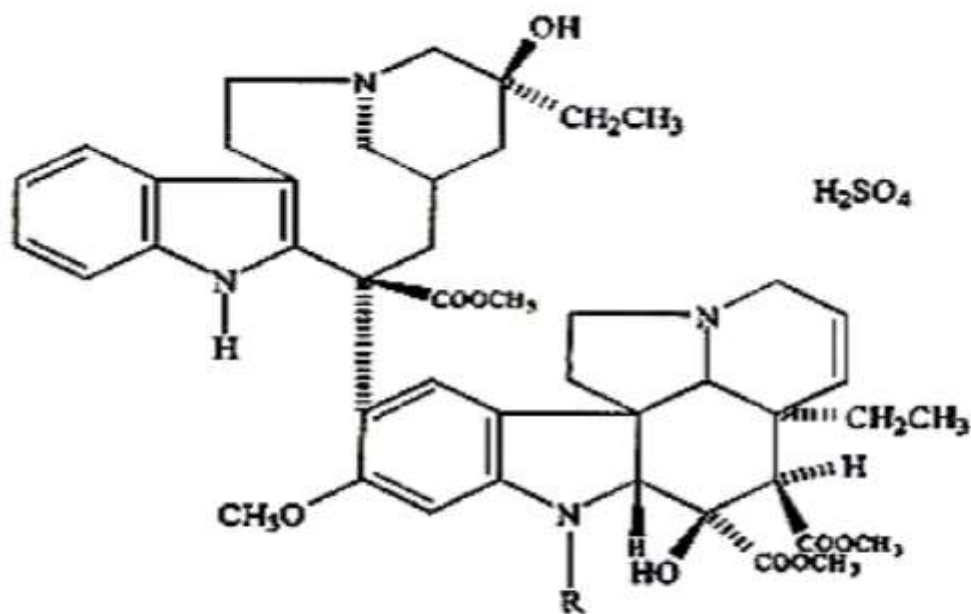
VINCA

- **Synonyms:** *Vinca rosea*, *Catharanthus*, Madagascar periwinkle, Barmasi.
- **Biological Source:** *Vinca* is the dried entire plant of *Catharanthus roseus* Linn., belonging to family Apocynaceae.
- **Geographical Source:** Madagascar, Tropical and subtropical countries especially in India, Australia, South Africa and North and South America.
- **Commercial varieties:** *Vinca difformis*, *Vinca erecta* *Vinca herbacea*, *Vinca ispartensis*, *Vinca major* L., *Vinca minor* L.



Chemical Constituents

- Alkaloids are present in entire shrub but leaves and roots contain more alkaloids.
- The important alkaloids in *Catharanthus* are the dimer indole indoline alkaloids **Vinblastine and Vincristine** and they possess definite anticancer activity.
- About 90 alkaloids have been isolated from *Vinca* from which some like **Ajmalicine, Serpentine and Tetrahydroalstonine** are known and are present in other species of Apocynaceae.
- **Vindoline and Catharanthine** are indole monomeric alkaloids. It also contains monoterpenes, sesquiterpene, indole and indoline glycoside.



R= CH₃ Vinblastine sulphate

R= CHO Vincristine sulphate

Vinca alkaloids are Dimeric alkaloids having Indole and Dihydroindole nuclei.

** Vincristine and Vinblastine are the major alkaloids in Vinca, they differ only in the substitution on the N- atom of the Dihydroindole nucleus.*

They differ in the presence of:
 N-methyl group in vinblastine.
 N-formyl group in vincristine.



Plant

Tabersonine, Voafrine, Vindolinine-N-oxide, Vincalokoblastine, Ajmalicine, Akuamicine, Calmodulin, Geraniol, Isovincoside, Leurocristine, Leurosine, Reserpine, Serpentine, Vindoline

Flower

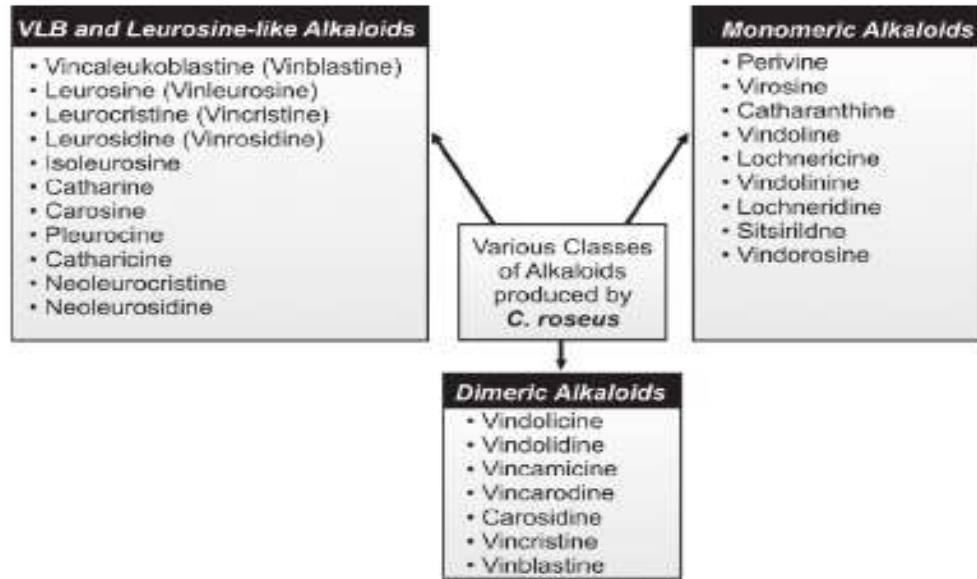
Catharicine, Hirsutidin, Indole-3-acetic acid, Malvidin, Vindolicine, Zeatin

Leaf

Vincalokoblastine, Vindoline-N-oxide, Vindolinine, Leurosine, Deoxyvincalokoblastine, Furfural, Quercetin, Syringic acid, Tannin, Vanillic acid, Vinaspine, Vincoline, Zeatin

Root

Hydroxy tabersonine, Ajmalicine, Alstonine, Carosidine, Bomesitol, Leurosivine, Vincaline, Vinosidine, Viosine, Zeatin



Adulterants and substitutes

- Vindoline type alkaloids are contained by the other catharanthus species such as *C. longifolius*, *C. trichophyllus*, and *C. lanceus*.

Macroscopic characters

- The leaves are green in colour.
- flowers are either violet, pinkish white or carmine red.
- roots are pale grey in colour.
- It has characteristic odour and bitter taste.
- The flowers are hermaphrodite (have both male and female organs) and are pollinated by bees.
- Leaves are petiolate, entire margin, ovate or oblong, glossy appearance and with acute apex.
- Fruit is follicles with numerous black seeds.



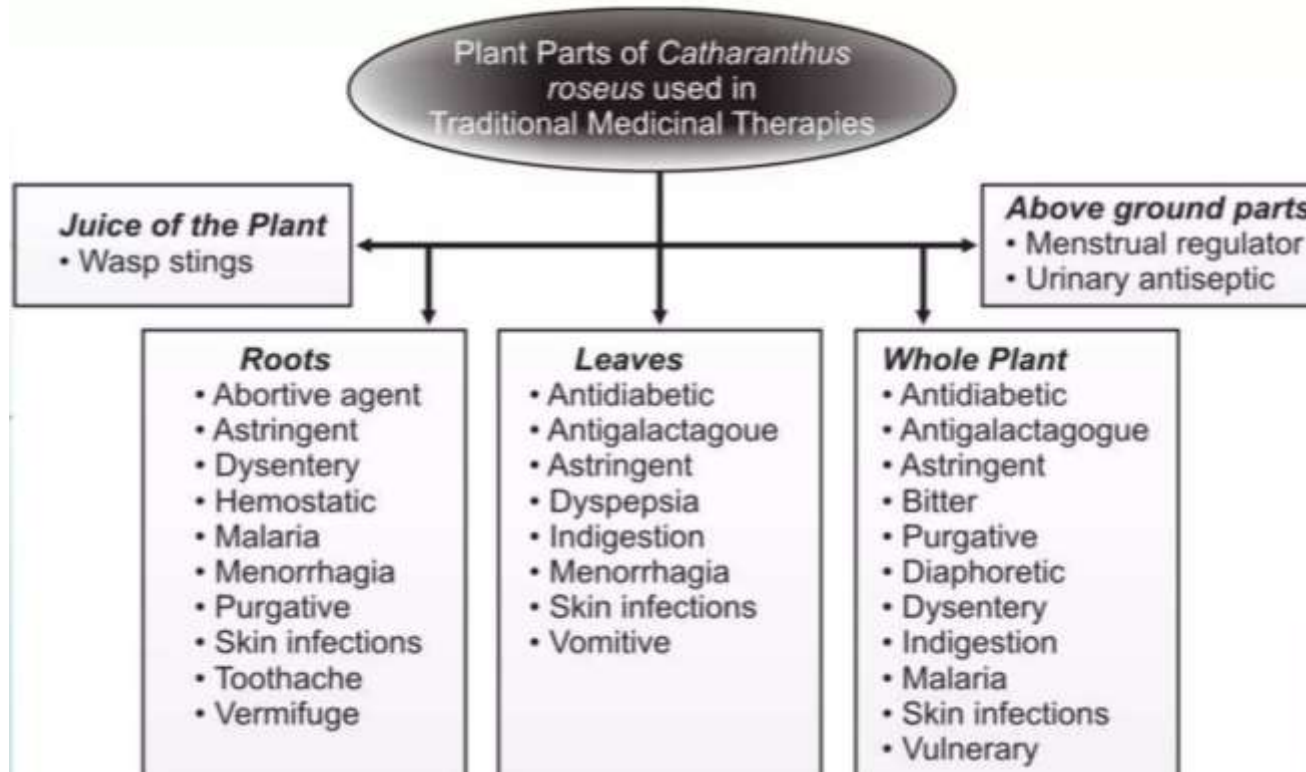
Extraction of Vinca alkaloids

- The dried leaf material is taken and is extracted with a solution of **hot ethanol–water–acetic acid** in a ratio of **9:1:1**.
- The solvent is removed and to the residue **hot hydrochloric acid solution of 2% is added**.
- The pH of the acidic extract is adjusted to 4, for the precipitation of the non- alkaloidal components, which can be separated by filtration.
- The pH of the aqueous acidic solution is now adjusted to 7 and then extracted with benzene.
- The benzene layer is evaporated to obtain vinblastine and other alkaloids.

Characterisation of vinca alkaloids

- **TLC**
- Vincristine dissolved in 25% water in methanol solution, spotted in Silica gel-G plate and developed using the solvent, acetonitrile: benzene (30:70).
- The dried plates are sprayed with 1% solution of ceric ammonium sulphate in 85% phosphoric acid.
- The R_f value of the appeared spot would be 0.39.

Therapeutic uses



Commercial applications

- ▶ NATURAL PRODUCTS
- ▶ 1. Etoposide (Ve Pesid)
- ▶ 2. Docetaxel (Taxoterel)
- ▶ 3. Paclitaxel (Taxol)
- ▶ 4. Vinblastine sulfate (Velban)
- ▶ 5. Vincristine sulfate (Oncovir)
- ▶ 6. Vinorelbine (Navelbine)

It is one of the ingredients of the preparation known as Cytocristin (Cipla).



RAUWOLFIA

- **Synonyms:** Sarpagandha, Chandrika; Chootachand; Indian snake root.
- **Biological Source:** Rauwolfia consists of dried roots of *Rauwolfia serpentina* Benth., belonging to family Apocynaceae.
- **Geographical Source:** native to the Orient and occurs from India to Sumatra. It is also found in Burma, Thailand, Philippines, Vietnam, Indonesia, Malaysia, Pakistan and Java.
- **Commercial varieties:** *Rauwolfia serpentine* L. Benth. ex Kurz., *Rauwolfia tetraphylla*.



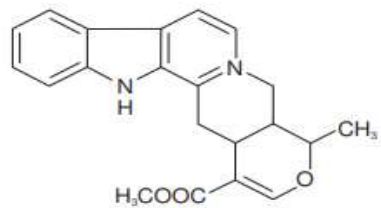
Cultivation and Collection

- Rauwolfia grows in tropical forests at an altitude of 1,200– 1,300 m at temperature 10–40°C.
- There should be enough rain or irrigation for its cultivation.
- The soil should be acidic (pH 4–6), clayey and manure is applied for better crop.
- Propagation is done by planting seeds, root cuttings or stem cuttings.
- Better drug is obtained when the propagation is carried out with fresh seeds.

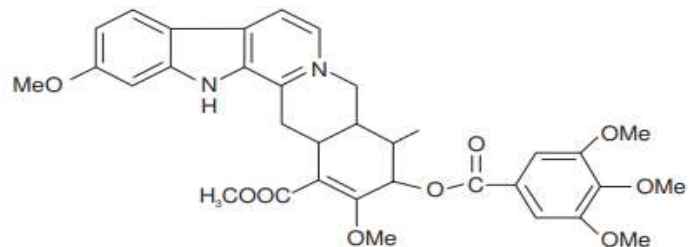
- The plants should be protected from nematodes, fungus and Mosaic virus.
- The drug is collected mainly from wild plants.
- Roots and rhizomes are dug out in October–November when the plant roots are two to four years old.
- The aerial parts and roots are separated.
- The roots are washed and dried in air.
- The roots containing moisture up to 12% should be protected from light.
- Rauwolfia should be packaged and stored in well-closed containers in a cool, dry place that is secure against insect attack.

Chemical constituents

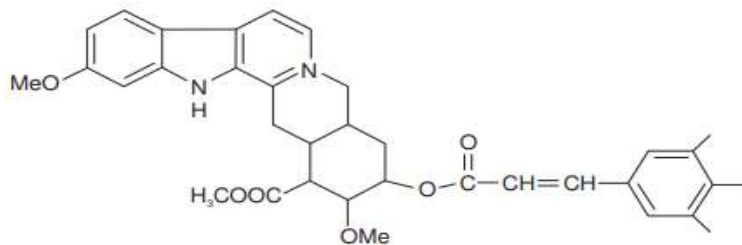
- Rauwolfia contains about 0.7–2.4% total alkaloidal bases from which more than 80 alkaloids have been isolated (indole alkaloids).
- The prominent alkaloids isolated from the drug are **reserpine, rescinnamine, ψ -reserpine, rescidine, raubescine and deserpidine.**
- The other alkaloidal components are ajmalinine, ajmaline, ajmalicine (8-yohimbine), serpentine, serpentinine, tetrahydroreserpine, raubasine, reserpinine, isoajamaline and yohambinine.
- The other substances present are phytosterols, fatty acids, unsaturated alcohols and sugars.



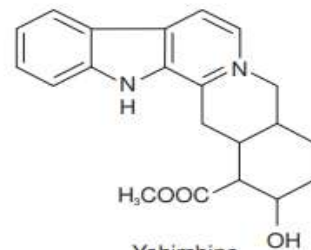
Serpentine



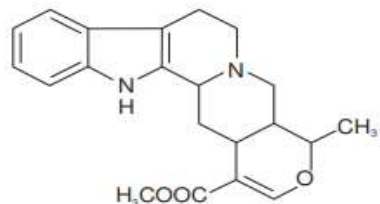
Reserpine



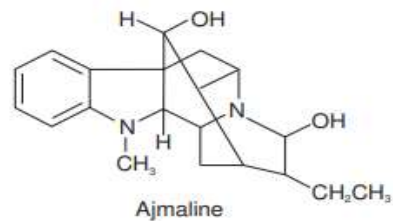
Rescinnamine



Yohimbine



Ajmalicine



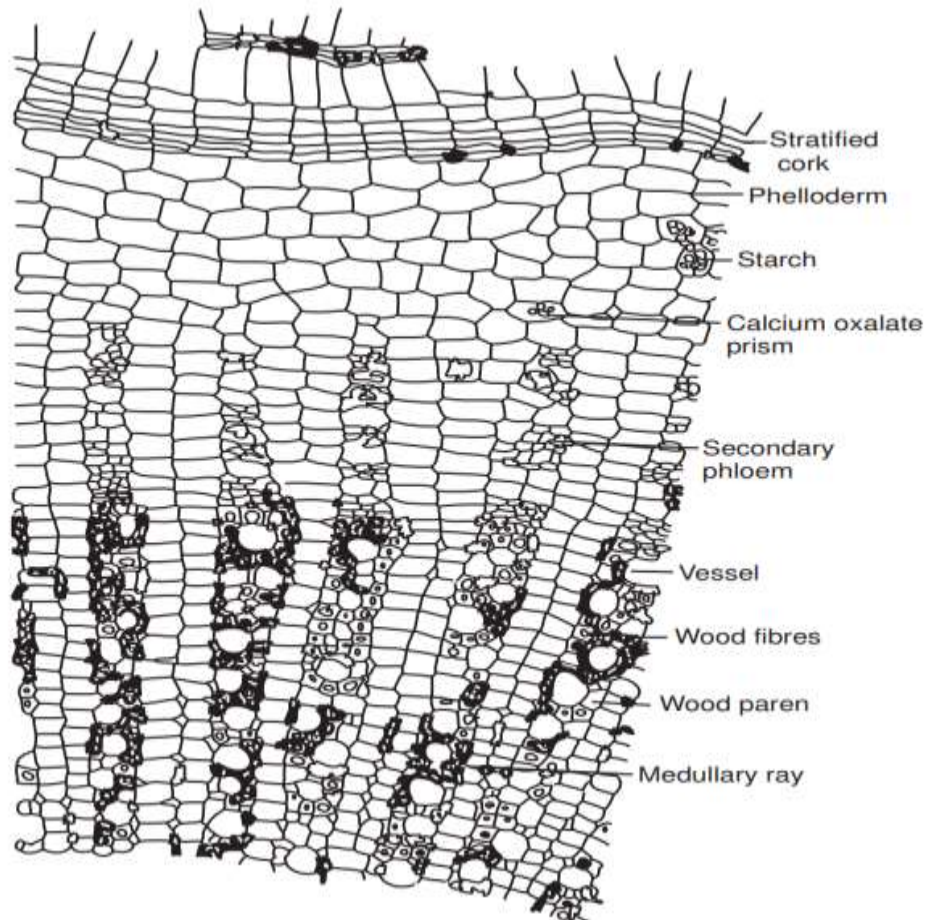
Ajmaline

Substitutes and adulterants

- *R. vomitoria*- this can be distinguished from the official drug on the basis of Sclereids which are present here in abundance.
- *R. canescens*: Here again the stone cells are present but the characteristic stratified cork is totally absent.
- *R. micrantha*: show both stratified cork and stone cells.
- Adulterants: *R. densitiflora* and *R. perakensis* do not contain reserpine. They do have stratified cork and stone cell.

Macroscopical characters

- The roots and rhizomes are almost identical in external characters.
- The drug occurs in cylindrical or slightly tapering, tortuous pieces, 2–10 cm long, 5–22 mm in diameter.
- The outer surface is greyish-yellow, light brown or brown.
- Young pieces contain slight wrinkles while old pieces have longitudinal ridges.
- The fracture is short. A narrow, yellowish-brown bark and a dense pale yellow wood are present.
- Pieces of rhizome closely resemble the root but may be identified by a small central pith. They are attached to them with small pieces of aerial stem. Slight odour is felt in recently dried drug which decreases with age
- Taste is bitter.



Chemical tests

- **With Marqui's reagent:** It produces a brown color.
- **With Vitali's reagent:** It results in a grayish green color followed by brown.
- When treated with **ammonium molybdate solution:** It turns blue and then eventually brown.
- Freshly fractured surface with **Conc. Nitric acid**- red colour.
- **Vanillin in acetic acid** – Violet colour.
- Powder + **Sulphuric acid in p-dimethyl amino benzaldehyde** – violet to red colour (Indole alkaloids)
- Tincture of drug in **UV light** – blue fluorescence.

General extraction

- Uproot the plants when they are about 3 to 4 years old.
- Cut the roots properly and wash them with water to remove adhering impurities like sand or other earthy matter. Dry the roots in the air.
- Powder the leaves and subject them to hot extraction in a Soxhlet apparatus with methyl alcohol containing 1% concentrated HCl, until complete discoloration.
- Hydrolyze the crude extract with HCl.
- Extract the pulverized root with absolute methanol using cold maceration technique for 8 days with occasional shaking.
- Filter the extract and concentrate the residual solvent in the crude extract in-vacuo using rotary evaporator under reduced pressure to yield a reddish-brown crude extract.

Uses

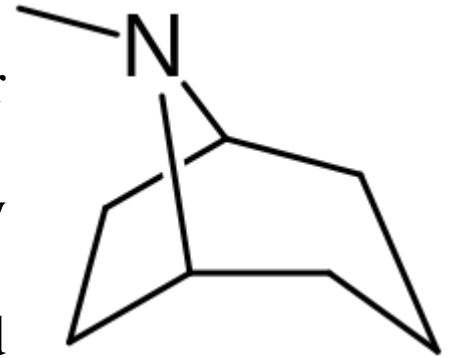
- Rauwolfia is used as hypnotic, sedative and antihypertensive.
- It is specific for insanity, reduces blood pressure and cures pain due to affections of the bowels.
- It is given in labors to increase uterine contractions and in certain neuropsychiatric disorders.
- Treatment of cardiac arrhythmias.
- Reserpine is an antihypertensive and tranquilizer.

Marketed Products

- It is one of the ingredients of the preparations known as **Confido, Lukol, Serpina** (Himalaya Drug Company) and **Sarpagandhan bati** (Baidyanath).

TROPANE ALKALOIDS

- Tropane is a bicyclic amine that has a pyrrolidine and a piperidine ring sharing a common nitrogen atom and 2 carbon atoms.
- Tropane does not occur naturally in free form rather it is found as part of esters in plant species.
- Esters of tropane are generally secondary metabolites of these plants.
- Tropane alkaloids are esters of hydroxytropans and various acids (tropic, tiglic)., are commonly found in plants of three families, the Solanaceae, Erythroxylaceae and Convolvulaceae families.



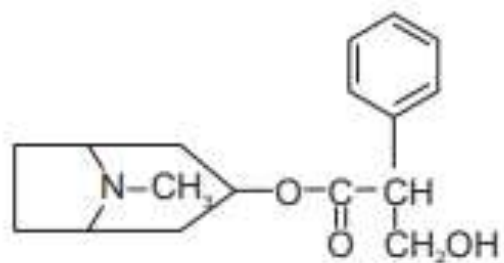
BELLADONNA

- **Synonyms:** Belladonna herb; Belladonna leaf; Deadly night shade leaves; Banewort; Death's herb, Dwale; Poison black cherry; Folia belladonnae.
- **Biological Source:** Belladonna consists of dried leaves and flowering tops of *Atropa belladonna* Linn. (European Belladonna), belonging to family Solanaceae.
- **Geographical Source:** *A. belladonna* is cultivated in United States, Canada, UK, Germany and India.

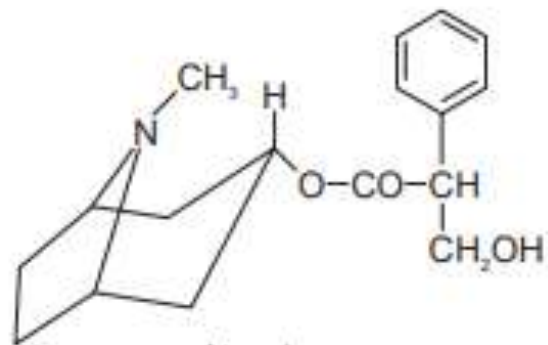


Chemical Constituents

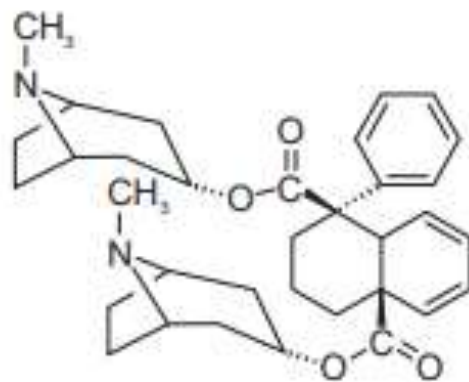
- Belladonna contains 0.3–1.0% total alkaloids, the prominent base is **l-hyoscyamine**
- Other components are **atropine**, apoatropine, choline, **belladonnine**, cuscohygrine, chrysotropic acid, volatile bases, such as atroscine, leucatropic acid; phytosterol, N-methylpyrroline, homatropine, hyoscyamine N-oxide, rutin, kaempferol-3-rhamnogalactoside and 7-glucoside, quercetin-7-glucoside, **scopoletin**, calcium oxalate, 14% acid soluble ash and 4% acid-insoluble ash.
- Addition of ammonia to the alcoholic solution of scopoletin shows blue fluorescence. This test is useful to detect Belladonna poisoning.



Hyoscyamine



Atropine



Belladonnine

Adulterants and substitutes

- *Phytolacca acinosa*
- *Ailanthus altissima* Roxb.

Macroscopic characters

- The drug contains leaves, smaller stems of about 5 mm diameter, flowers and fruits.
- Leaves are stalked, brittle, thin, entire, long-pointed, 5–25 cm long, 2.5–12 cm wide, colour dull-green or yellowish-green. The upper side is darker than the lower.
- A few flowers and fruits may be present.
- The flowers blooming in June are solitary, shortly stalked, drooping and about 2.5 cm long.
- The corolla is campanulate, five-lobed and of a dull purplish colour.
- The five-lobed calyx is persistent, remaining attached to the purplish-black berry.
- The fruit is bilocular, contains numerous seeds and is about the size of a cherry

Specific chemical tests

Tropane alkaloids

- ***Vitali–Morin’s test:*** The extract is treated with fuming nitric acid and is evaporated to dryness on a water bath. The residue is then treated with 3% caustic potash. A deep purple color is formed.
- ***Rathenasinkam’s test:*** In this test, nitric acid is used to effect the nitration of the benzene ring in atropine, hyoscyamine, and hyoscine. To the residue, add a few drops of ammonia and extract with chloroform. The chloroform extract is evaporated, and the residue is dissolved in acetone. A few drops of 10% sodium hydroxide is then added. A bluish-purple color is formed.

Uses

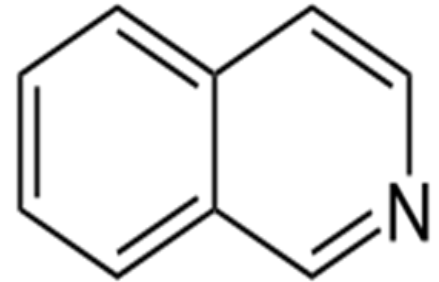
- The drug is used as adjunctive therapy in the treatment of **peptic ulcer; functional digestive disorders**, including spastic, mucous and ulcerative colitis. It relieves spasm of gut or respiratory tract.
- Due to anticholinergic property, it is used to **control excess motor activity** of the gastrointestinal tract and spasm of the urinary tract.
- Belladonna is **anticholinergic, narcotic, sedative, diuretic mydriatic and used as anodyne** and to check secretion.
- Consumption of Belladonna checks **excessive perspiration** of patients suffering from tuberculosis.
- Belladonna acts as a **parasympathetic depressant**.

Commercial applications

- It is one of the ingredients of the preparation known as **Belladonna plaster** (Surgi Pharma) for backache, stiffness of muscles and boil, swollen joints.

OPIUM

- Isoquinoline is a double carbon ring containing one nitrogen atom.
- The isoquinoline alkaloids are primarily formed in the plant families of Papavaraceae, Berberidaceae, Menispermaceae, Fumariaceae & Ranunculaceae



- **Synonyms:** Crude Opium; Raw Opium; Gum Opium; Afim; Post
- **Biological Source:** Opium is the air dried milky latex obtained by incision from the unripe capsules of *Papaver somniferum* Linn, or its variety *P. album* Decand., belonging to family Papaveraceae.
- **Geographical Source:** It is mainly found in Turkey, Russia, Yugoslavia, Tasmania, India, Pakistan, Iran, Afghanistan, China, Burma, Thailand and Laos.
- **Commercial varieties:** *Papaver somniferum* var. *paeoniflorum*, *P. somniferum* var. *laciniatum*.

Cultivation

- Opium is cultivated under license from the government.
- Its seeds are sown in October or March in alluvial soil. After germination of seeds snow falls.
- In spring the thin plant attains the height of 15 cm. Fertilizers are used for better crop.
- The poppy of first crop blossoms in April or May and the **capsule mature in June or July.**
- When the capsules are about 4 cm in diameter, the color **changes from green to yellow**; they are **incised with a knife** about 1 mm deep around the circumference **between midday and evening.**
- The knife, known as a ‘**nushtur**’ bears narrow iron spikes which are drawn down the capsule to produce several longitudinal cuts.
- The incision **must not penetrate into the interior of the capsule** otherwise latex will be lost.
- The latex tube opens into one another. The latex, which is white in the beginning, immediately coagulates and turns brown.

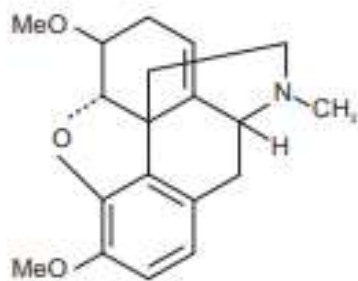


- Next morning it is removed by scrapping with a knife and transferred to a poppy leaf.
- Each capsule is cut several times at intervals of two or three days.
- After collection the latex is placed in a tilted vessel so that the dark fluid which is not required may drain off.
- By exposure to air the opium acquires a suitable consistency for packing.
- The dried latex is kneaded into balls, wrapped in poppy leaves and dried in shade.
- The principal commercial varieties of Opium are Turkish Opium, Indian Opium, Chinese Opium, Yugoslavian Opium and Persian Opium.

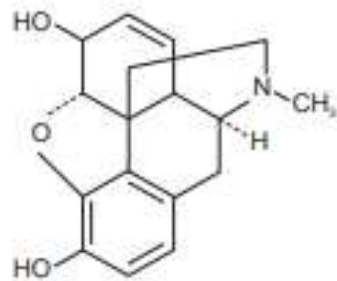


Chemical constituents

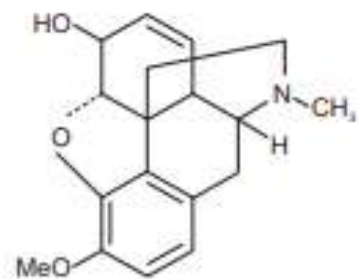
- Opium contains about 35 alkaloids among which **morphine** (10–16%) is the most important base. The alkaloids are combined with **meconic acid**. The other alkaloids isolated from the drug are **codeine** (0.8–2.5%), **narcotine**, **thebaine** (0.5–2%), **noscapine** (4–8%), **narceine** and **papaverine** (0.5–2.5%). Morphine contains a phenanthrene nucleus.
- **Morphine Type:** Morphine, codeine, neopine, pseudo or oxymorphine, thebaine and porphyroxine.
- **Phthalide Isoquinoline Type:** Hydrocotarnme, narcotoline, 1-narcotine, noscapine, oxynarcotine, narceine, and 5'-O-demethyl-narcotine.
- **Benzyl Isoquinoline Type:** Papaverine, dl-laudanine, laudanidine, codamine and laudanosine.
- **Cryptopine Type:** Protopine, cryptopine.
- **Unknown Constituents:** Aporeine, diodeadine, meconidine, papaveramine and lanthopine.



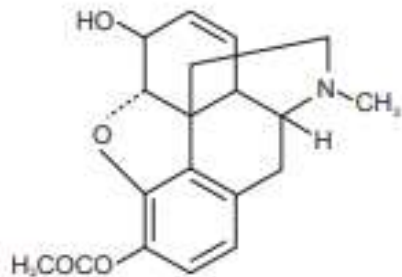
Thebaine



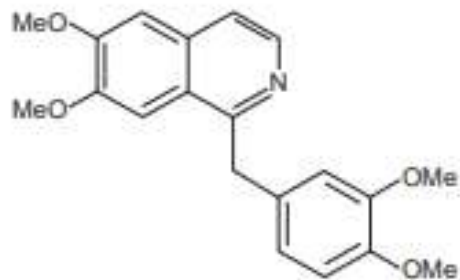
Morphine



Codeine



Heroin



Papaverine

Macroscopic features

- Opium occurs in **rounded or flattened mass** which is 8–15 cm in diameter and weighing from **300 g to 2 kg each**.
- The external surface is **pale or chocolate-brown**, texture is uniform and slightly granular.
- It is plastic like when fresh and turns hard and brittle after sometime.
- Fragment of poppy leaves are present on the upper surface.
- Internal surface is **coarsely granular, reddish-brown, lustrous; odour is characteristic; taste is bitter and distinct**.
- Opium is intended only as a starting material for the manufacture of galenical preparations and is not dispensed as such.

Chemical Tests

- **Aqueous extract** of Opium with **FeCl₃ solution** gives **deep reddish purple** colour which persists on addition of HCl. It indicates the presence of meconic acid.
- Morphine gives **dark violet colour** with **conc. H₂SO₄ and formaldehyde**.
- **Marquis' reagent**: Formaldehyde in concentrated sulfuric acid. **Purple red changing to purple** is observed.
- **Frshde's reagent**: Molybdate in concentrated sulfuric acid. **Violet, quickly changing to strong purplish red**, fading out to weaker brown or brownish, then developing green.
- **Mecke's (or Lafon's) reagent**: (Selenious acid in concentrated sulfuric acid). **Green, quickly greenish blue**, changing to blue, slowly to bluish green with a yellow–brown edge, then olivaceous green.

General methods of extraction

- Opium is dissolved in three times its weight of hot water and filtered.
- The residue is re-extracted and the two filtrates are combined.
- The total filtrate volume is reduced to half and then poured into a solution of boiling calcium hydroxide. Additional precipitants form and they are captured by filtration.
- The captured residues are re-extracted with three parts of water, and again filtered to obtain clean filtrate.
- The total combined filtrates are combined and the residues are removed from the process.
- The total combined filtrates are concentrated to a weight approximately twice that of the original opium and the resulting solution is again filtered with any captured residues being removed from the process.

Characterisation of morphine

- TLC
- Standard solution: morphine 1 mg/ml in methanol.
- Sample solution: dissolve 5 mg sample in each 1 ml of methanol, and place both a 1 μ l and 5 μ l.
- Solvent system: toluene: acetone: ethanol: conc. Ammonia (45:45:7:3).
- Visualization: UV light 254 nm/Dragendorff's spray reagent.
- Rf value: 0.19.

Uses

- Opium and morphine have **narcotic, analgesic and sedative action and used to relieve pain, diarrhea dysentery and cough.**
- Poppy capsules are astringent, somniferous, soporific, sedative and narcotic and used as anodyne and emollient.
- Codeine is mild sedative and is employed in cough mixtures.
- Noscapine is not narcotic and has cough suppressant action acting as a central antitussive drug.
- Papaverine has smooth muscle relaxant action and is used to cure muscle spasms.
- Opium, morphine and the diacetyl derivative heroin, cause drug addiction.

Commercial applications



- *Arymo*
- *Avinza*
- *Doloral*
- *Duramorph*
- *Embeda*
- *Infumorph*
- *Kadian*
- *M-ediat*

TANNINS

BLACK CATECHU

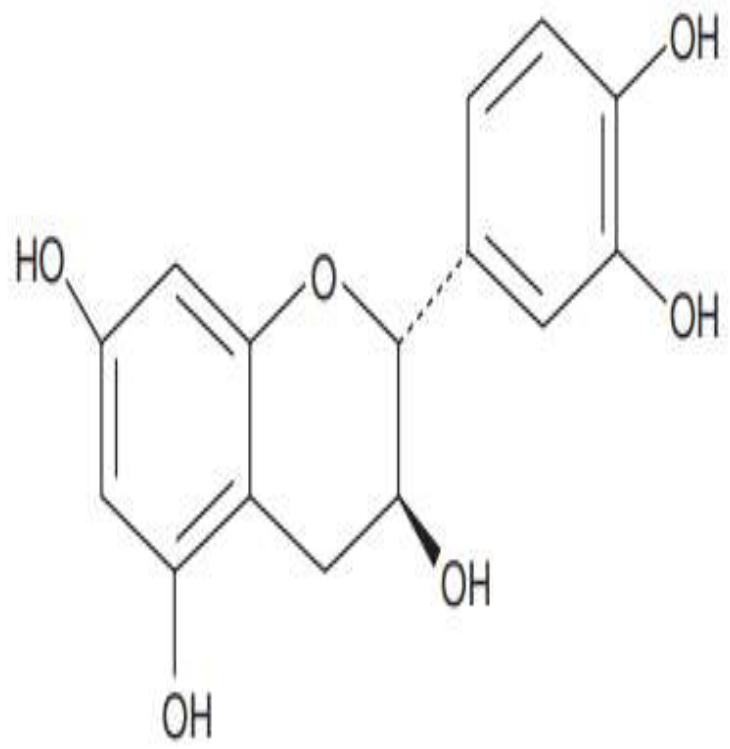
- **Synonym:** Cutch, black catechu, kattha.
- **Biological Source:** Black catechu is the dried aqueous extract prepared from the heartwood of *Acacia catechu* Willdenow, belonging to family, *Leguminosae*.
- **Geographical Source:** *A. catechu* is common throughout the tract from Punjab to Assam ascending to an altitude of 300 m. It is also quite common in drier regions of peninsula such as Madhya Pradesh, Maharashtra, Gujarat, Rajasthan, Bihar, and Tamil Nadu.

Morphology

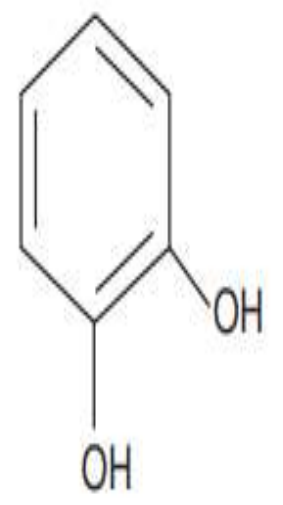
Colour	Black or brownish black mass
Odour	Odourless
Taste	Astringent and subsequently sweet taste
Size	Irregular mass
Extra features	Outer surface is firm and brittle. When broken the fractured surface appears glassy with small cavities

Chemical Constituents

- Cutch or black catechu resembles pale catechu or gambier in its composition.
- It contains about 2–12% of catechin and about 25 to 33% of phlobatannin catechutannic acid.
- The principle fraction of cutch has been identified as a mixture of catechin isomers which includes (-) epicatechin, acatechin, DL-acacatechin, L-acacatechin and D-isoacacatechin.
- It also contains 20–30% gummy matter, catechin red, quercetin and quercetin.
- It yields 2–3% of ash.



Catechin



Catechol

Chemical Tests

- Because of the presence of catechin, black catechu gives pink or red colour with vanillin and HCl.
- Catechin when treated with HCl produces phlorogucinol, which burns along with lignin to give purple or magenta colour. For this purpose, tannin extract is taken on match stick dipped in HCl and heated near the flame.
- Lime water when added to aqueous extract of black catechu gives brown colour, which turns to red precipitate on standing for some time.
- Green colour is produced when ferric ammonium sulphate is added to dilute solution of black catechu. By the addition of sodium hydroxide, the green colour turns to purple.

Uses

- Cutch is used in medicine as astringent.
- It cures troubles of mouth, diseases of the throat and diarrhoea.
- It also increases appetite.

Marketed Products

It is one of the ingredients of the preparation known as Koflet lozenge (Himalaya Drug Company) as cough expectorant, and Gum tone (Charak Pharma Pvt. Ltd.).

PALE CATECHU



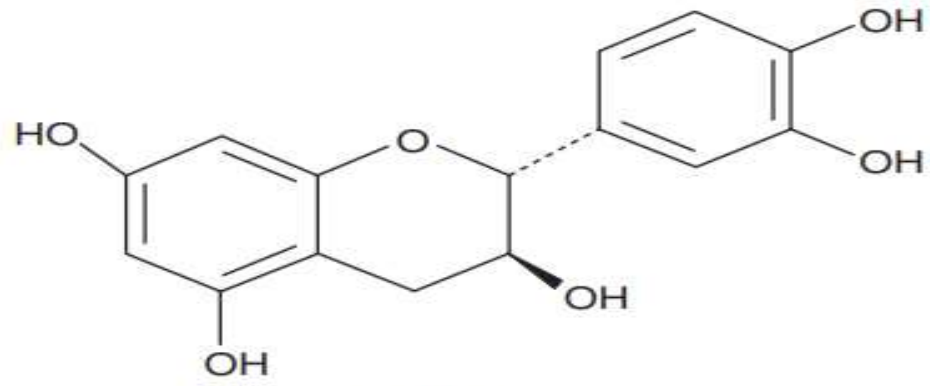
- **Synonyms:** Gambier, pale catechu, catechu
- **Biological Source:** Gambier or pale catechu is a dried aqueous extract produced from the leaves and young twigs of *Uncaria gambier* Roxburgh., belonging to family **Rubiaceae**.
- **Geographical Source:** U. gambier is a native of erstwhile Malaya. It is cultivated in Indonesia, Malaysia, Sumatra, Bornea, and Singapore at elevation up to 150 m. The plant is used mostly for the production of the drug, which is marketed through Singapore.

Morphology

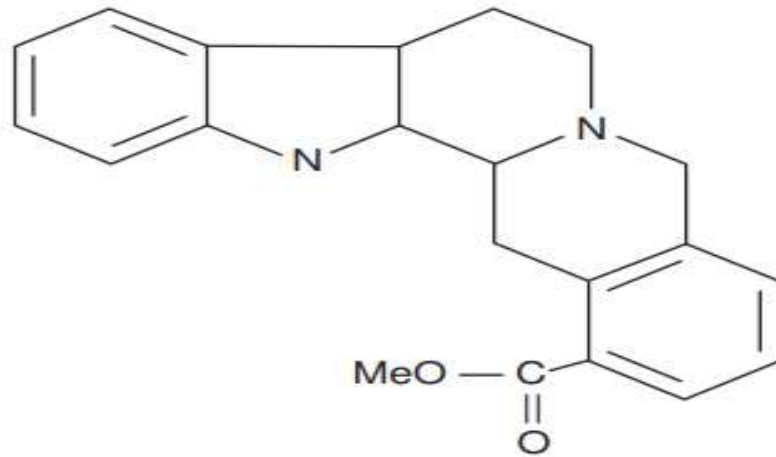
Colour	Dull reddish brown colour externally and pale brown to buff colour internally.
Odour	Odourless
Taste	At first it is bitter and astringent but later it is sweet.
Shape	Strips, flakes or coarse powder
Size	Pale catechu comes in the form of cubes or rectangular blocks of 2 to 4 cm length
Shape	Regular cubes or as rectangular blocks.

Chemical Constituents

- Pale catechu contain about 7 to 30% of pseudo tannin **catechin** and 22 to 55% of a phlobatannin **catechu tannic acid**.
- Both of the about component constitute over 60% of the drug.
- It also contains **catechu red, gambier fluorescin and quercetin**.
- It contains indole alkaloid up to 0.05%, which includes gambirtannin and its derivatives. Gambirtannin gives a strong fluorescence under UV light.
- Catechin forms white, needle like crystals, which dissolves in alcohol and hot water. Catechutannic acid gives green colour with ferric chloride.



Catechin



Gambirtannin

Chemical Tests

- ***Gambier fluorescin test:*** Gambier fluorescin present in pale catechu gives the fluorescence. To its alcohol extract, a little sodium hydroxide is added and shaken with petroleum ether. The petroleum ether layer shows green fluorescence. Black catechu gives negative test.
- ***Vanillin-hydrochloric acid test:*** Drug shows pink or red colour with a mixture of vanillin:alcohol:dilute HCl in the ratio 1:10:10. The reaction produces phloroglucinol which along with vanillin gives pink or red colour.
- A ***matchstick*** dipped in a decoction of Pale catechu is air dried and again dipped into concentrated HCl and warmed near the burner. Pink or purple colour is produced.
- Small quantity of powder is heated on water bath with 5 ml chloroform and filtered. The filtrate is evaporated in a white porcelain dish in a water bath. A greenish-yellow residue is produced due to the presence of chlorophyll in the drug. Black catechu gives this test negative due to the absence of chlorophyll.

General methods of extraction and analysis

- Areca catechu peel was chopped and dried using an oven at 60°C. Then the dried Areca catechu peel is milled by using grinding machine, then sieved with 10 mesh size sieve.
- Catechin was extracted from Areca catechu peel flour by maceration method using acetone distillate.

Estimation of catechin

Thin-layer chromatographic identification

- The crude drug of pale catechu (1 g) was macerated with 95% ethanol for 6 hr and then evaporated to dryness.
- The residue was dissolved in 1 mL of ethanol. Three microliters of the sample solution were applied onto a thin-layer chromatographic (TLC) plate coated with silica gel GF254.
- Mobile phase: chloroform, ethyl-acetate and formic acid (3:6:1 v/v/v).
- After development, the plate was removed and allowed to dry at room temperature and examined under UV light with 254 nm and 365 nm.
- Then, the plate was sprayed with vanillin reagent and heated in an oven at 105°C for 5 min.
- The R_f values of catechin: 0.22 ± 0.05

Uses

- Pale catechu is medicinally used as local astringent.
- It is largely used in various countries of east for chewing with betel leaf.
- Large proportion of gambier is used in dyeing and tanning industries.
- It is used for tanning of animal hides to convert it to leather.

Marketed products

Powder Acacia Catechu Khadir Chhal Extract



