www.pharmrecord.com

Unit- IV

Industrial production, estimation and utilization of the following phytoconstituents:-

Forskolin, Sennoside, Artemisinin, Diosgenin, Digoxin, Atropine, Podophyllotoxin, Caffeine, Taxol, Vincristine and Vinblastine

Presented By:-

Samarpan Mishra

Contents

In this Unit We can study

1. **Introduction**
2. **Industrial production**
3. **Estimation**
4. **Utilization**

of the following phytoconstituents:

1. **Forskolin,**
2. **Sennoside,**
3. **Artemisinin,**
4. **Diosgenin,**
5. **Digoxin,**
6. **Atropine,**
7. **Podophyllotoxin,**
8. **Caffeine,**
9. **Taxol,**
10. **Vincristine**
11. **Vinblastine**

1. Forskolin

1. Source and Plant Profile of Sennoside

- **Biological source:** Dried leaflets of *Senna alexandrina* (syn. *Cassia angustifolia*) and *Senna occidentalis*.
- **Family:** Fabaceae (Leguminosae).
- **Common name:** Senna, Indian senna, Tinnevelly senna.
- **Part used:** Dried leaflets and pods (leaflets contain higher sennoside content 2–4%).
- **Active constituents:** Sennosides (A and B), dianthrones (sennidins), flavonoids, mucilage.
- **Geographical distribution:** Native to North Africa (Egypt, Sudan) and widely cultivated in India, Middle East and other tropical regions.

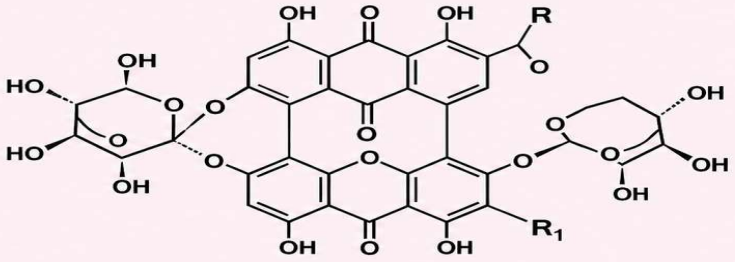


Senna alexandrina (Whole plant)



Dried leaflets **Pods**

2. Chemical Structure of Sennoside



Sennoside
(Sennoside A and B)

Molecular formula	:	$C_{42}H_{38}O_{20}$
Molecular weight	:	862.74 g/mol
Chemical class	:	Anthraquinone glycoside (dianthrone derivative)
IUPAC name (Sennoside A)	:	8,8′-bianthracene-1,1′,8,8′-tetraol 10,10′-dione 8-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside
R groups	:	Sennoside A: R = COOH, R_1 = CH_2OH Sennoside B: R = CH_2OH, R_1 = COOH

www.pharmrecord.com

Industrial Production of Forskolin

Raw Material (*Coleus forskohlii roots*)



Coleus forskohlii plant



Dried roots (10–20% forskolin)

- Select good quality *Coleus forskohlii* roots containing high forskolin (10–20%).
- Clean to remove soil, stones and foreign matter.
- Wash and dry if needed.

Cleaning, Drying and Size Reduction





- Dry the roots to reduce moisture (≤ 10%).
- Grind to suitable particle size (coarse powder) to increase surface area for extraction.

Extraction



Solvent extraction (Ethanol / Methanol / Hydroalcoholic solvent 50–90%) (60–80°C, 2–4 hours)

- Powdered roots are extracted with ethanol, methanol or hydroalcoholic solvent (50–90%).
- Extraction is carried out at 60–80°C for 2–4 hours with stirring or percolation.
- Forskolin is soluble in organic solvents.

Filtration





Forskolin containing extract

- Separate the plant residue by filtration.
- Filtrate contains forskolin along with other extractives (resins, lipids, pigments, etc.).

Concentration





- Filtrate is concentrated under reduced pressure (vacuum evaporation) to remove excess solvent and obtain a concentrated extract.

Purification (Chromatography)



Column chromatography (Silica gel / Sephadex / Preparative HPLC)

- Concentrated extract is purified by column chromatography using suitable solvent system (e.g., hexane: ethyl acetate or chloroform: methanol).
- Removes impurities such as resins, fatty matter, pigments and other diterpenes.

Crystallization





- Purified fraction is concentrated and kept at low temperature to crystallize forskolin.
- Crystallization can be induced by addition of a non-solvent (e.g., water or hexane).

Filtration and Washing





- Forskolin crystals are separated by filtration.
- Washed with cold solvent (e.g., ethanol/hexane) to remove adhering impurities.

Drying





- The washed crystals are dried in a tray dryer or vacuum oven at 40–60°C to obtain pure forskolin (moisture < 0.5%).

Pure Forskolin (Final Product)



- White to off-white crystalline powder of forskolin.
- Purity: ≥ 98% (by HPLC).
- Packed in airtight, light-resistant containers.
- Quality control: HPLC, UV, IR, melting point, etc.

www.pharmrecord.com

Estimation of Forskolin

1. Principle

- Forskolin is quantified mainly by HPLC or spectrophotometric methods.
- In UV spectrophotometry, forskolin shows maximum absorbance (λmax) around 225–230 nm in methanol.
- The amount of forskolin in the sample is calculated by comparing its absorbance with a standard forskolin solution.

2. Chemical structure of Forskolin



Forskolin
($C_{22}H_{34}O_7$)

3. Plant Source



Coleus forskohlii
(Family: Lamiaceae)

4. Methods of Estimation

1. **UV Spectrophotometry** (simple and rapid)
2. **HPLC** (most accurate and widely used)
3. **HPTLC**
4. **LC–MS**
5. **TLC–Densitometry**

A. UV Spectrophotometric Method

1 Preparation of standard solution



2 Preparation of sample solution

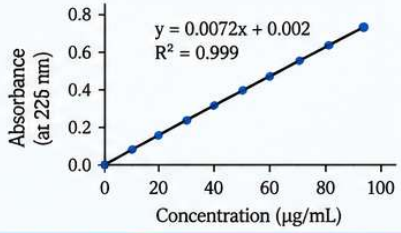


3 Measurement of absorbance



Measure absorbance
at 225–230 nm
against methanol as blank

4 Calibration curve



5. Procedure (UV Spectrophotometry)

1. Prepare standard forskolin solution (e.g., 10, 20, 40, 60, 80, 100 µg/mL) from stock solution (1000 µg/mL) by suitable dilutions.
2. Prepare sample solution by extracting powdered drug with methanol, filter and make up to known volume.
3. Measure the absorbance of standard and sample solutions at 225–230 nm using methanol as blank.
4. Plot calibration curve (absorbance vs. concentration) using standard solutions.
5. Calculate the amount of forskolin in the sample from the regression equation or directly from the calibration curve.

6. Calculation

Amount of forskolin in sample is calculated using the formula:

$$\text{Forskolin content (\% w/w)} = \frac{C \times V \times D \times 100}{W \times 10^4}$$

Where,
- **C** = Concentration from calibration curve (µg/mL)
- **V** = Final volume of sample extract (mL)
- **D** = Dilution factor (if any)
- **W** = Weight of sample (mg)
- **10^4** = Conversion factor (µg to g and % calculation)

7. Advantages and Limitations

Advantages
- Simple, rapid and economical
- Requires only UV spectrophotometer
- Suitable for routine analysis
- Good sensitivity and reproducibility

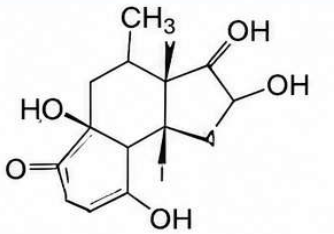
Limitations
- Less specific (interference from other extractives)
- Not as accurate as HPLC
- Requires pure standard for calibration

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

www.pharmrecord.com

Utilization of Forskolin

Forskolin



$(C_{22}H_{34}O_7)$



Coleus forskohlii
(Indian Coleus)

Cardiovascular uses

- Positive inotropic and vasodilatory agent
- Increases cAMP levels in cardiac and vascular smooth muscle
- Useful in congestive heart failure and hypertension (adjuvant therapy)



Respiratory uses

- Bronchodilator (relaxes bronchial smooth muscle)
- Useful in asthma, chronic bronchitis and other obstructive respiratory disorders



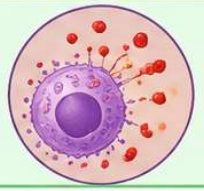
Anti-obesity and metabolic uses

- Increases cAMP levels → stimulates lipolysis
- Helps in weight management and reduction of body fat
- Often used in dietary supplements and nutraceuticals



Anti-inflammatory and anti-allergic uses

- Inhibits release of inflammatory mediators (e.g., histamine)
- Useful in inflammatory and allergic conditions
- Studied for potential use in glaucoma (reduces intraocular pressure)



Other potential uses

- Neuroprotective and cognitive enhancing effects (under study)
- Anticancer potential (via cAMP mediated pathways)
- Research in dermatology and skin disorders (e.g., psoriasis)



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

2. Sennoside

1. Source and Plant Profile of Sennoside

- **Biological source:** Dried leaflets of *Senna alexandrina* (syn. *Cassia angustifolia*) and *Senna occidentalis*.
- **Family:** Fabaceae (Leguminosae).
- **Common name:** Senna, Indian senna, Tinnevelly senna.
- **Part used:** Dried leaflets and pods (leaflets contain higher sennoside content 2–4%).
- **Active constituents:** Sennosides (A and B), dianthrones (sennidins), flavonoids, mucilage.
- **Geographical distribution:** Native to North Africa (Egypt, Sudan) and widely cultivated in India, Middle East and other tropical regions.

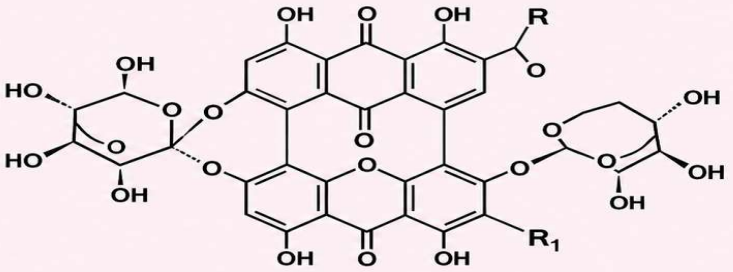


Senna alexandrina
(Whole plant)



Dried leaflets **Pods**

2. Chemical Structure of Sennoside



Sennoside
(Sennoside A and B)

Molecular formula	:	$C_{42}H_{38}O_{20}$
Molecular weight	:	862.74 g/mol
Chemical class	:	Anthraquinone glycoside (dianthrone derivative)
IUPAC name (Sennoside A)	:	8,8′-bianthracene-1,1′,8,8′-tetraol 10,10′-dione 8-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside
R groups	:	Sennoside A: R = COOH, $R_1 = CH_2OH$ Sennoside B: R = CH_2OH, R_1 = COOH

www.pharmrecord.com

Industrial Production of Sennoside

Raw Material (Senna leaves) *(Cassia angustifolia / Cassia acutifolia)*	 Senna plant  Dried senna leaves (2–4% sennosides)	• Select good quality senna leaves containing sennosides (2–4%). • Remove foreign matter (stems, soil, stones, dust). • Wash and dry if required.
Cleaning, Drying and Size Reduction	 	• Dry the leaves to reduce moisture (≤ 10%). • Remove foreign matter. • Grind to suitable particle size (coarse powder) to increase surface area for extraction.
Extraction	 Solvent extraction (Water / Hydroalcoholic solvent, e.g., ethanol 50–70%) (60–80°C, 1–3 hours)	• Powdered leaves are extracted with water or hydroalcoholic solvent (ethanol 50–70%) at 60–80°C for 1–3 hours with stirring or percolation. • Sennosides are soluble in hot water and alcohol.
Filtration	  **Sennoside containing extract**	• Separate the plant residue by filtration. • Filtrate contains sennosides along with other constituents (phenolics, sugars, tannins, etc.).
Concentration	 	• Filtrate is concentrated under reduced pressure (vacuum evaporation) to remove excess solvent and obtain a concentrated extract.
Purification (Chromatography)	 Column chromatography (Silica gel / Sephadex) Elution with suitable solvent (e.g., ethyl acetate / methanol : water)	• Concentrated extract is purified by column chromatography using suitable solvent system (e.g., ethyl acetate or methanol : water). • Removes impurities such as sugars, tannins, pigments and other anthraquinone derivatives.
Crystallization	 	• Purified fraction is concentrated and cooled to crystallize sennosides (A & B). • Crystallization can be induced by slow evaporation or addition of a non-solvent (e.g., water, ether, or hexane).
Filtration and Washing	 	• Sennoside crystals are separated by filtration. • Washed with cold solvent (e.g., ethanol/ether) to remove adhering impurities.
Drying	 	• The washed crystals are dried in a tray dryer or vacuum oven at 40–60°C to obtain pure sennosides (moisture < 0.5%).
Pure Sennosides (Final Product)		• Light yellow to yellow crystalline powder. • Purity: ≥ 95% (by HPLC). • Packed in airtight, light-resistant containers. • Quality control: HPLC, UV, melting point, etc.

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

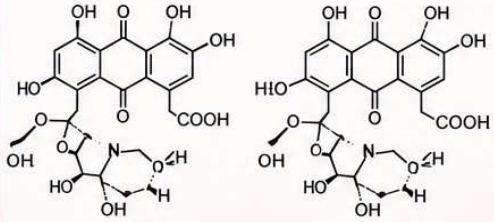
www.pharmrecord.com

Estimation of Sennoside

1. Introduction

- Sennosides are anthraquinone glycosides present in *Senna* leaves (*Cassia* species).
- Mainly Sennoside A and Sennoside B are responsible for laxative activity.
- They are estimated mainly by HPLC, HPTLC or UV spectrophotometry.

2. Chemical structures



Sennoside A ($C_{42}H_{38}O_{20}$)

Sennoside B ($C_{42}H_{38}O_{20}$)

3. Plant source



Senna alexandrina
(Family: Fabaceae)
Dried leaflets contain 2–4% sennosides (A and B)

4. Methods of Estimation

1. **HPLC** (most accurate and widely used)
2. **HPTLC**
3. **UV–Visible Spectrophotometry** (after hydrolysis)
4. **LC–MS** (for detailed analysis)
5. **TLC** (for qualitative estimation)

A. HPLC Method (Standard Method)

1 Preparation of standard solution



Accurately weigh 10 mg of Sennoside A and/or Sennoside B (standard) and dissolve in methanol. Make up to 10 mL to get 1000 µg/mL. Further dilute to get working solutions (10–100 µg/mL).

2. Preparation of sample solution



Powdered senna leaves (1 g)

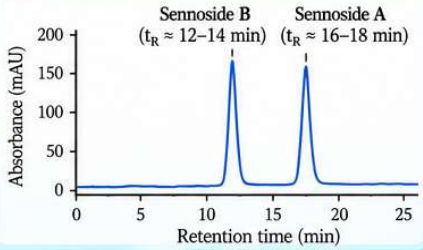


Extract with methanol (or methanol:water, 70:30) using ultrasonication/ reflux, filter and make up to known volume (e.g., 10 mL).

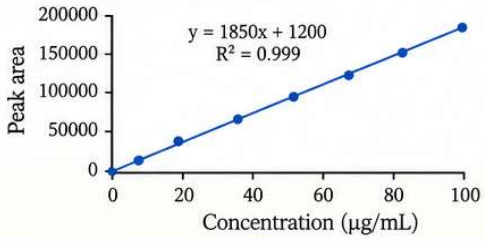
3 HPLC conditions (Typical)

Column	C18 (250 mm × 4.6 mm, 5 µm)
Mobile phase	Acetonitrile : 0.1% formic acid in water (gradient)
Flow rate	1.0 mL/min
Detection	UV at 270 nm
Injection volume	20 µL
Column temp.	30 °C
Run time	20–30 min

4 HPLC chromatogram



5. Calibration curve



6. Calculation

Amount of sennoside in sample is calculated using the regression equation from calibration curve.

$$\text{Sennoside content (\% w/w)} = \frac{C \times V \times D \times 100}{W \times 10^4}$$

Where,

- **C** = Concentration obtained from calibration curve (µg/mL)
- **V** = Final volume of sample extract (mL)
- **D** = Dilution factor (if any)
- **W** = Weight of sample (mg)
- 10^4 = Conversion factor (µg to g and % calculation)

7. Advantages

- Highly accurate and precise
- Can separate and quantify Sennoside A and B individually
- Suitable for routine quality control
- Sensitive and reproducible
- Can be applied to crude drugs and formulations



8. Limitations

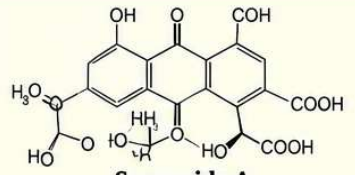
- Requires HPLC instrument and standard sennosides
- Time consuming compared to UV method
- Costly solvents and columns
- Interference from other anthraquinone glycosides in crude extracts

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

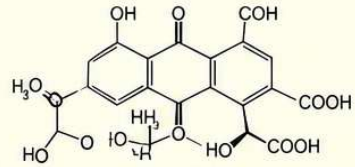
www.pharmrecord.com

Utilization of Sennoside

Sennoside



Sennoside A



Sennoside B

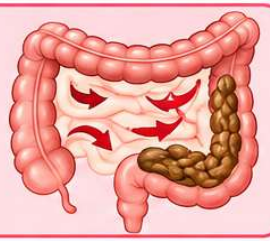


Senna alexandrina
(Family: Fabaceae)

Dried leaves contain Sennosides (A and B)

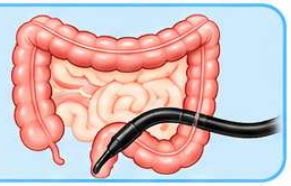
Laxative use (Main utilization)

- Natural stimulant laxative used for short-term relief of constipation
- Sennosides reach the large intestine and are converted to active anthrones (rhein, anthrone) by gut bacteria
- Stimulate intestinal peristalsis and increase secretion of water and electrolytes into the colon



Bowel cleansing (pre-operative use)

- Used before surgical procedures, colonoscopy and diagnostic tests to cleanse the bowel
- Helps in clearing the colon and improves visibility



Adjunct in weight management

- Sometimes used in combination with other herbs in slimming formulations (short-term use only)
- Promotes bowel movement and helps in reducing body weight by relieving constipation



Formulations

- Available as dried leaf, powder, tablets, capsules, granules and syrup
- Often combined with other laxative drugs (e.g., with docusate sodium)
- Used in over-the-counter (OTC) herbal laxative products



Other potential uses

- Used in traditional medicine for temporary relief of constipation
- Studied for potential use in bowel preparation and detoxification
- Investigated for possible use in metabolic health and skin care (formulations under study)



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

3. Artemisinin

1. Source and Plant Profile of Artemisinin

- **Biological source:** Dried leaves and aerial parts of *Artemisia annua* (syn. *Artemisia annual* L.).
- **Family:** Asteraceae (Compositae).
- **Common name:** Sweet wormwood.
- **Part used:** Dried leaves and flowering tops (aerial parts).
- **Active constituent:** Artemisinin (sesquiterpene lactone).
- **Geographical distribution:** Native to China, widely cultivated in Asia (China, India, Vietnam), Africa and other tropical regions.



Artemisia annua
(Whole plant)

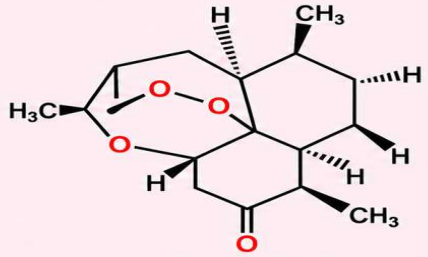


Leaves



Flowering tops

2. Chemical Structure of Artemisinin



Artemisinin
($C_{15}H_{22}O_5$)

Molecular formula	:	$C_{15}H_{22}O_5$
Molecular weight	:	282.33 g/mol
Chemical class	:	Sesquiterpene lactone (endoperoxide)
IUPAC name	:	(3R,5aS,6R,8aS,9R,12R,12aR)-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin-10(3H)-one
Key structural feature	:	Endoperoxide bridge (–O–O–) and lactone ring

www.pharmrecord.com

Industrial Production of Artemisinin



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

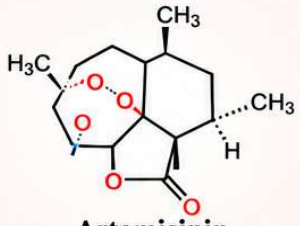
www.pharmrecord.com

Estimation of Artemisinin

1. Introduction

- Artemisinin is a sesquiterpene lactone (endoperoxide) isolated from *Artemisia annua*.
- It is a potent antimalarial agent used for treatment of *Plasmodium falciparum* malaria.
- Artemisinin content in crude drug and formulations is estimated mainly by HPLC, HPTLC or UV spectrophotometry.

2. Chemical structure of Artemisinin



Artemisinin
($C_{15}H_{22}O_5$)
Molecular weight: 282.33 g/mol

3. Plant source



Artemisia annua
(Family: Asteraceae)
Dried aerial parts contain 0.01–1.5% artemisinin (depending on variety and growing conditions).

4. Methods of Estimation

1. **HPLC** (most accurate and widely used)
2. **HPTLC** (simple and reliable)
3. **UV–Visible Spectrophotometry** (less specific)
4. **GC–MS** (for detailed analysis)
5. **LC–MS** (highly sensitive)

A. HPLC Method (Standard Method)

1 Preparation of standard solution



Artemisinin (standard)
10 mg

Accurately weigh 10 mg of artemisinin and dissolve in methanol to make up to 10 mL (1000 µg/mL). Further dilute to get working standard solutions (10–100 µg/mL).

2 Preparation of sample solution



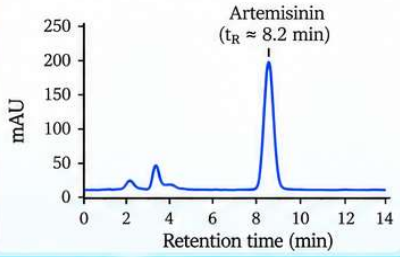
Dried aerial parts of *Artemisia annua* (1 g)

Extract with methanol (or methanol:water 70:30) using sonication or reflux, filter and make up to known volume (e.g., 10 mL).

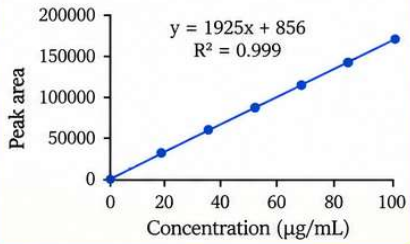
3 HPLC conditions (Typical)

Column	C18 (250 mm × 4.6 mm, 5 µm)
Mobile phase	Acetonitrile : Water (60:40, v/v)
Flow rate	1.0 mL/min
Detection	UV at 210–220 nm (210 nm commonly used)
Injection volume	20 µL
Column temp.	30 °C
Run time	15–25 min
Retention time (typical)	Artemisinin: 6–10 min (depending on system)

4 HPLC chromatogram



5. Calibration curve



6. Calculation

Amount of artemisinin in sample is calculated using the regression equation from calibration curve.

$$\text{Artemisinin content (\% w/w)} = \frac{C \times V \times D \times 100}{W \times 10^4}$$

Where,

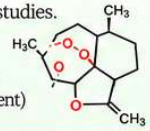
- **C** = Concentration obtained from calibration curve (µg/mL)
- **V** = Final volume of sample extract (mL)
- **D** = Dilution factor (if any)
- **W** = Weight of sample (mg)
- **10^4** = Conversion factor (µg to g and % calculation)

7. Advantages

- Highly accurate, specific and sensitive.
- Can separate artemisinin from other constituents.
- Suitable for quality control of crude drug and formulations.
- Can be used for stability studies.



Artemisinin (antimalarial agent)



8. Limitations

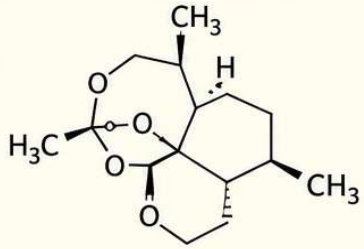
- Requires HPLC instrument and standard artemisinin.
- Time-consuming compared to UV method.
- Sensitive to extraction method and sample handling.
- Co-elution with similar compounds may occur (incorrect estimation if method not optimized).

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

www.pharmrecord.com

Utilization of Artemisinin

Artemisinin



$(C_{15}H_{22}O_5)$



Artemisia annua
(Family: Asteraceae)

Leaves contain Artemisinin

Antimalarial use (Main utilization)

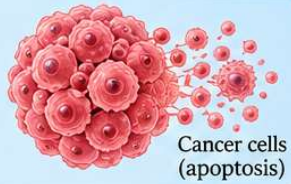
- Artemisinin and its derivatives (artesunate, artemether, dihydroartemisinin) are highly effective antimalarial drugs.
- Used for treatment of Plasmodium falciparum and multi-drug resistant malaria.
- Recommended by WHO in Artemisinin-based Combination Therapy (ACT).
- Act rapidly and reduce parasite load by generating free radicals.



Plasmodium falciparum

Anticancer potential

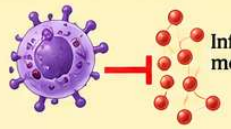
- Artemisinin derivatives show selective cytotoxicity against cancer cells (e.g., breast, lung, liver and colon cancer).
- Induce apoptosis and inhibit tumor cell growth.
- Research is ongoing; used in combination with other anticancer agents.



Cancer cells (apoptosis)

Anti-inflammatory and immunomodulatory use

- Inhibits release of inflammatory mediators (e.g., NF-κB pathway).
- Useful in inflammatory and autoimmune disorders (under study).
- May help in reducing oxidative stress.



Inflammatory mediators

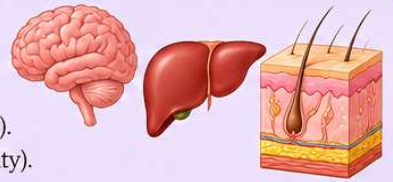
Antiviral and antimicrobial use

- Shows activity against certain viruses (e.g., Hepatitis B, SARS-CoV-2 – under study).
- Exhibits activity against bacteria, fungi and parasites.
- Used in research for new antimicrobial drug development.



Other potential uses

- Neuroprotective effects (useful in neurodegenerative diseases – under study).
- Anti-fibrotic activity (may help in liver fibrosis).
- Potential use in treatment of schistosomiasis (parasitic infection – under study).
- Investigated for use in skincare and anti-aging formulations (antioxidant activity).



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

4. Diosgenin

1. Source and Plant Profile of Diosgenin

- **Biological source:** Dried tubers (rhizomes) of *Dioscorea deltoidea* and *Dioscorea floribunda* (syn. *Dioscorea* spp.).
- **Family:** Dioscoreaceae.
- **Common name:** Wild yam, Chinese yam, Indian yam.
- **Part used:** Underground tubers (rhizomes) containing diosgenin.
- **Active constituent:** Diosgenin (steroidal sapogenin).
- **Geographical distribution:** Native to tropical and subtropical regions, widely distributed in India, China, Africa, Mexico and South America.



Dioscorea deltoidea (Whole plant)

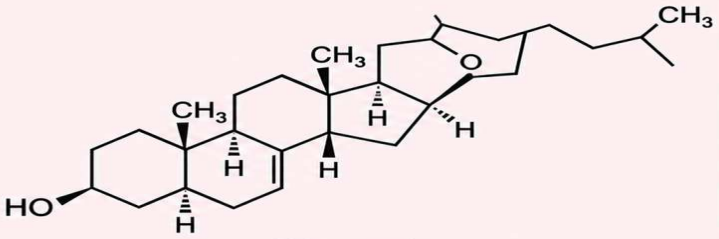


Tubers (rhizomes)



Leaves

2. Chemical Structure of Diosgenin

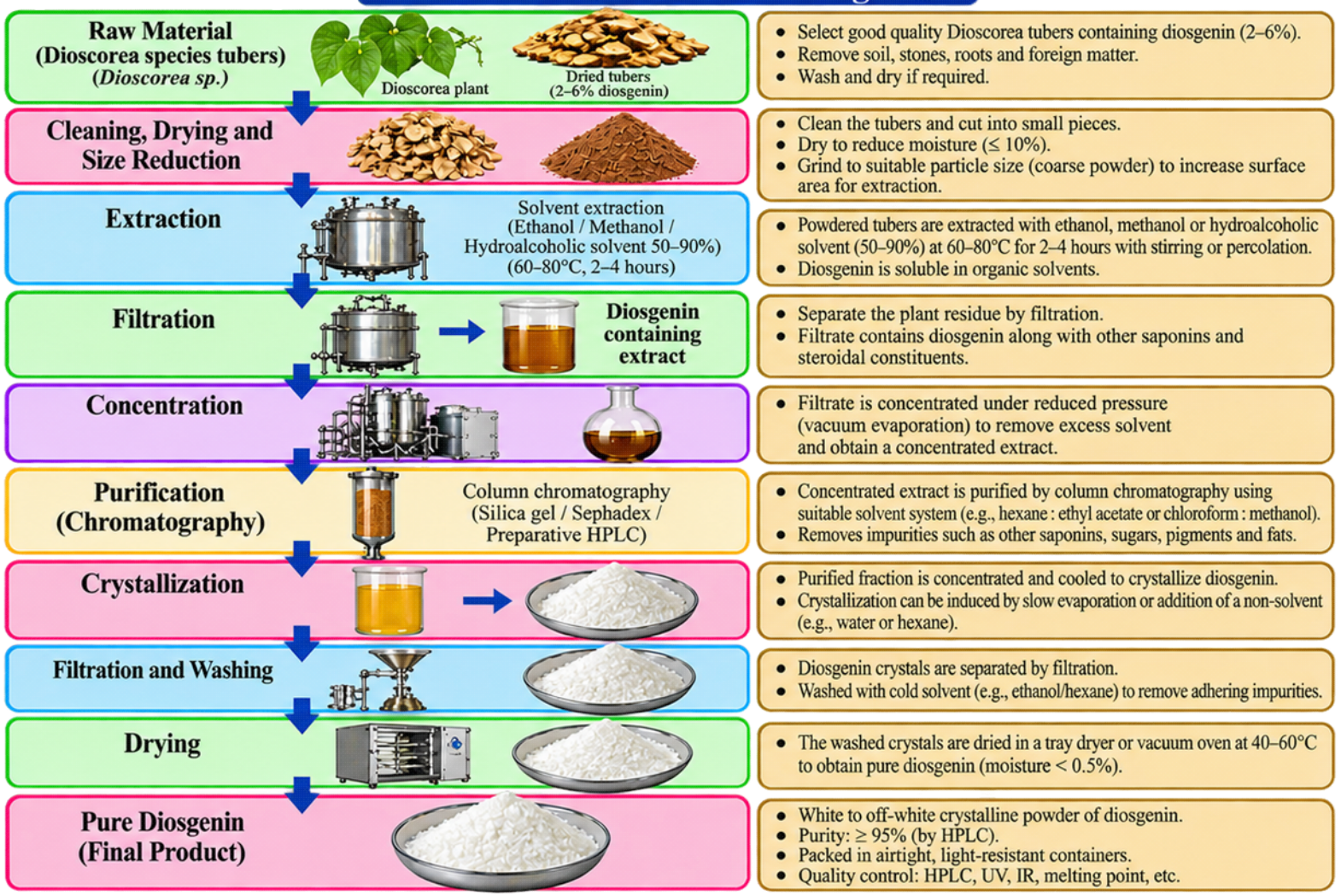


Diosgenin ($C_{27}H_{42}O_3$)

Molecular formula	:	$C_{27}H_{42}O_3$
Molecular weight	:	414.62 g/mol
Chemical class	:	Steroidal sapogenin (spirostane type)
IUPAC name	:	(3β,5α,25R)-spirost-5-en-3-ol
Key structural features	:	Steroid nucleus (spirostane skeleton) with a spiroketal side chain at C-17 and a hydroxyl group at C-3.

www.pharmrecord.com

Industrial Production of Diosgenin



- Select good quality Dioscorea tubers containing diosgenin (2–6%).
- Remove soil, stones, roots and foreign matter.
- Wash and dry if required.

- Clean the tubers and cut into small pieces.
- Dry to reduce moisture (≤ 10%).
- Grind to suitable particle size (coarse powder) to increase surface area for extraction.

- Powdered tubers are extracted with ethanol, methanol or hydroalcoholic solvent (50–90%) at 60–80°C for 2–4 hours with stirring or percolation.
- Diosgenin is soluble in organic solvents.

- Separate the plant residue by filtration.
- Filtrate contains diosgenin along with other saponins and steroidal constituents.

- Filtrate is concentrated under reduced pressure (vacuum evaporation) to remove excess solvent and obtain a concentrated extract.

- Concentrated extract is purified by column chromatography using suitable solvent system (e.g., hexane : ethyl acetate or chloroform : methanol).
- Removes impurities such as other saponins, sugars, pigments and fats.

- Purified fraction is concentrated and cooled to crystallize diosgenin.
- Crystallization can be induced by slow evaporation or addition of a non-solvent (e.g., water or hexane).

- Diosgenin crystals are separated by filtration.
- Washed with cold solvent (e.g., ethanol/hexane) to remove adhering impurities.

- The washed crystals are dried in a tray dryer or vacuum oven at 40–60°C to obtain pure diosgenin (moisture < 0.5%).

- White to off-white crystalline powder of diosgenin.
- Purity: ≥ 95% (by HPLC).
- Packed in airtight, light-resistant containers.
- Quality control: HPLC, UV, IR, melting point, etc.

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

www.pharmrecord.com

Estimation of Diosgenin

1. Introduction

- Diosgenin is a steroidal sapogenin obtained from plants such as *Dioscorea* spp. (wild yam).
- It is an important precursor for the synthesis of steroidal drugs (e.g., corticosteroids, oral contraceptives, sex hormones).
- Diosgenin content in crude drug and formulations is estimated mainly by HPLC, HPTLC or UV spectrophotometry after hydrolysis of diosgenin glycosides.

2. Chemical structure of Diosgenin



Diosgenin
($C_{27}H_{42}O_3$)
Molecular weight: 414.62 g/mol

3. Plant source



Dioscorea deltoidea / Dioscorea bulbifera / Dioscorea composita
(Family: Dioscoreaceae)
Tuber contains 1–5% diosgenin (as glycosides)

4. Methods of Estimation

1. **HPLC** (most widely used, highly accurate)
2. **HPTLC** (simple and cost-effective)
3. **UV–Visible Spectrophotometry** (after color reaction)
4. **GC–MS** (for detailed analysis)
5. **TLC–Densitometry** (semi-quantitative)

A. HPLC Method (Standard Method)

1 Preparation of standard solution



Diosgenin (standard)
10 mg

Accurately weigh 10 mg of diosgenin and dissolve in methanol to make up to 10 mL (1000 µg/mL). Further dilute to get working standard solutions (10–100 µg/mL).

2 Preparation of sample solution



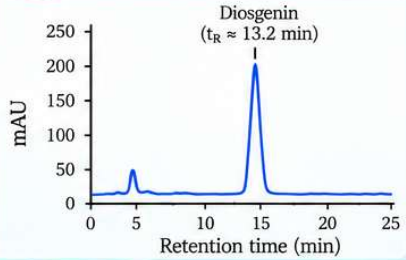
Powdered tuber of *Dioscorea* spp. (1 g)

Extract with methanol (or methanol:water 80:20) using ultrasonication/ reflux, filter and make up to known volume (e.g., 10 mL).

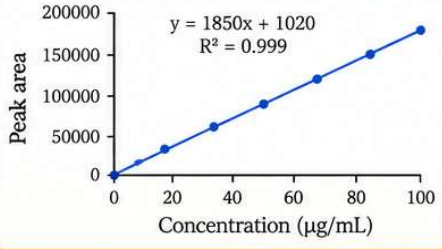
3 HPLC conditions (Typical)

Column	C18 (250 mm × 4.6 mm, 5 µm)
Mobile phase	Acetonitrile : Water (80:20, v/v)
Flow rate	1.0 mL/min
Detection	UV at 203–210 nm
Injection volume	20 µL
Column temp.	30 °C
Run time	15–25 min
Retention time (typical)	Diosgenin: ~12–15 min (depending on system)

4 HPLC chromatogram



5. Calibration curve



6. Calculation

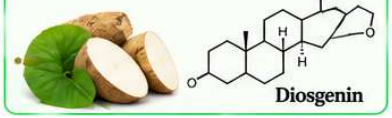
Amount of diosgenin in sample is calculated using the regression equation from calibration curve.

$$\text{Diosgenin content (\% w/w)} = \frac{C \times V \times D \times 100}{W \times 10^4}$$

Where,
- **C** = Concentration obtained from calibration curve (µg/mL)
- **V** = Final volume of sample extract (mL)
- **D** = Dilution factor (if any)
- **W** = Weight of sample (mg)
- **10^4** = Conversion factor (µg to g and % calculation)

7. Advantages

- Highly accurate, specific and sensitive.
- Can distinguish diosgenin from other steroidal compounds.
- Suitable for routine quality control of crude drug and formulations.
- Reproducible and precise.



Diosgenin

8. Limitations

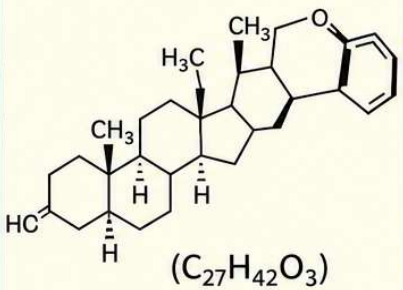
- Requires HPLC instrument and standard diosgenin.
- Time-consuming compared to UV method.
- Sensitive to extraction method and sample preparation.
- Co-elution with similar steroidal compounds may occur (method optimization required).

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

www.pharmrecord.com

Utilization of Diosgenin

Diosgenin



$(C_{27}H_{42}O_3)$



Dioscorea spp.
(Family: Dioscoreaceae)

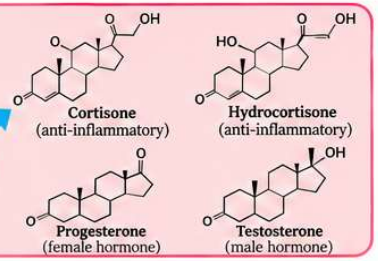
Mainly from *Dioscorea* species (e.g., *D. deltoidea*, *D. floribunda*, *D. composita*)

Tuber/rhizome contain diosgenin (steroidal saponin)

Precursor for steroid hormone synthesis (Main utilization)

- Diosgenin is an important raw material for the semi-synthesis of steroid hormones.
- Used for the industrial production of corticosteroids, sex hormones and other steroidal drugs.
- Widely used in pharmaceutical industry as a starting material.





Contraceptive use

- Diosgenin is converted to progesterone and other progestins, which are used in oral contraceptives.
- Helps in prevention of unwanted pregnancy.



Treatment of menopausal disorders

- Used as a source for synthesis of estrogenic and progestogenic drugs for hormone replacement therapy (HRT).
- Helps in relieving symptoms of menopause such as hot flashes, mood changes and osteoporosis.



Anti-inflammatory and analgesic potential

- Diosgenin exhibits anti-inflammatory, analgesic and immunomodulatory activities (under study).
- May help in conditions like arthritis and other inflammatory disorders.



Anti-diabetic and anti-obesity potential

- Shows potential in improving glucose metabolism and lipid profile (under research).
- May help in weight management and prevention of metabolic disorders.



Cosmetic and nutraceutical use

- Used in skincare formulations for anti-aging and skin protection (due to steroidal structure).
- Included in nutraceutical products for general health and wellness.



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

5. Digoxin

1. Source and Plant Profile of Digoxin

- **Biological source:** Dried leaves of *Digitalis lanata* and *Digitalis purpurea*.
- **Family:** Plantaginaceae (Scrophulariaceae).
- **Common name:** Foxglove (*D. lanata* – Grecian foxglove, *D. purpurea* – Purple foxglove).
- **Part used:** Dried leaves (containing cardiac glycosides such as digoxin).
- **Active constituent:** Digoxin (cardiac glycoside).
- **Geographical distribution:** Native to Mediterranean region, widely cultivated in Europe, North America, India (temperate regions) and other parts of the world.



Digitalis purpurea
(Whole plant)

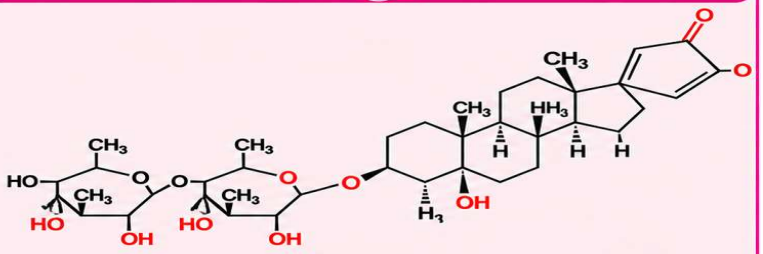


Leaves
(of Digitalis)



Flowers
(of Digitalis)

2. Chemical Structure of Digoxin

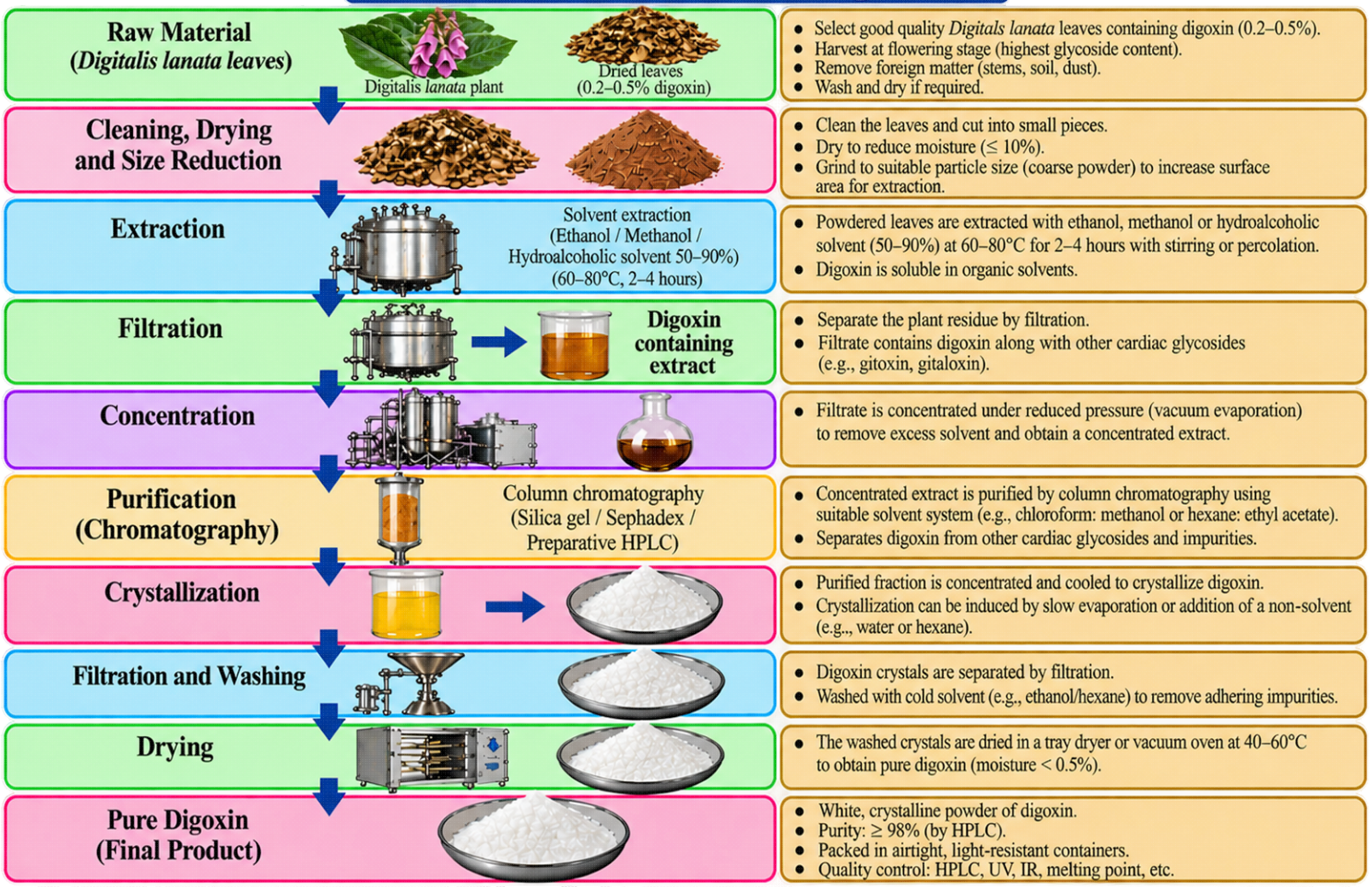


Digoxin
($C_{41}H_{64}O_{14}$)

Molecular formula	:	$C_{41}H_{64}O_{14}$
Molecular weight	:	780.94 g/mol
Chemical class	:	Cardiac glycoside (Cardienolide type)
IUPAC name	:	(3β,5β,14β)-3-[(2,6-dideoxy-3-O-methyl-α-L-ribo-hexopyranosyl)-(1→4)-[2,6-dideoxy-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-14-hydroxycard-20(22)-enolide
Key structural features	:	Steroidal nucleus (cardenolide) with an α,β-unsaturated lactone ring at C-17 and three digitoxose sugar units at C-3.

www.pharmrecord.com

Industrial Production of Digoxin



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

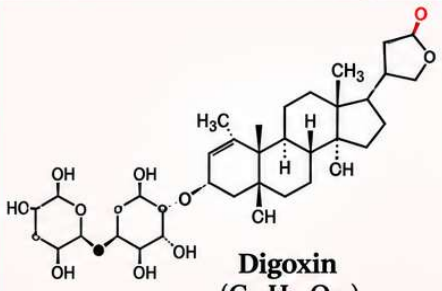
www.pharmrecord.com

Estimation of Digoxin

1. Introduction

- Digoxin is a cardiac glycoside containing a steroid nucleus with an unsaturated lactone ring.
- It is obtained from *Digitalis* leaves (*Digitalis purpurea* and *Digitalis lanata*).
- Digoxin is used for the treatment of congestive heart failure and certain cardiac arrhythmias.
- Digoxin content in crude drug and formulations is estimated mainly by HPLC, HPTLC or UV spectrophotometry after suitable extraction and purification.

2. Chemical structure of Digoxin



Digoxin
($C_{41}H_{64}O_{14}$)
Molecular weight: 780.94 g/mol

3. Plant source



Digitalis purpurea
(Family: Plantaginaceae)
Dried leaves contain 0.2–1.0% cardiac glycosides (mainly digoxin)

4. Methods of Estimation

1. **HPLC** (most accurate and widely used)
2. **HPTLC** (simple and cost-effective)
3. **UV–Visible Spectrophotometry** (after color reaction with Kedde reagent)
4. **LC–MS** (for detailed analysis)
5. **TLC–Densitometry** (semi-quantitative)

A. HPLC Method (Standard Method)

1 Preparation of standard solution



Digoxin (standard)
1 mg

Accurately weigh 1 mg of digoxin and dissolve in methanol. Make up to 10 mL (100 µg/mL). Further dilute to get working standard solutions (0.5–10 µg/mL).

2 Preparation of sample solution



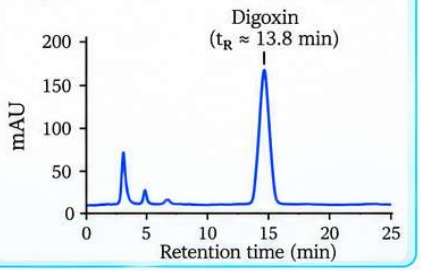
Powdered leaves of *Digitalis purpurea* (1 g)

Extract with methanol (or methanol:water 80:20) using ultrasonication/ reflux, filter and make up to known volume (e.g., 10 mL).

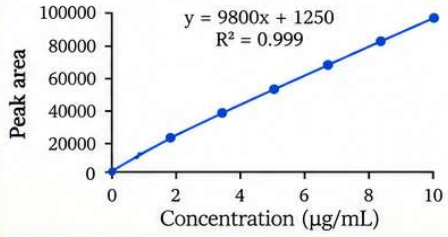
3 HPLC conditions (Typical)

Column	C18 (250 mm × 4.6 mm, 5 µm)
Mobile phase	Acetonitrile : Water (35:65, v/v) or with 0.1% phosphoric acid
Flow rate	1.0 mL/min
Detection	UV at 220–225 nm
Injection volume	20 µL
Column temp.	30 °C
Run time	15–25 min
Retention time (typical)	Digoxin: ~12–16 min (depending on system)

4 HPLC chromatogram



5. Calibration curve



6. Calibration

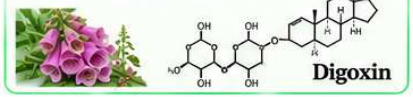
Amount of digoxin in sample is calculated using the regression equation from calibration curve.

$$\text{Digoxin content (\% w/w)} = \frac{C \times V \times D \times 100}{W \times 10^4}$$

Where,
- **C** = Concentration obtained from calibration curve (µg/mL)
- **V** = Final volume of sample extract (mL)
- **D** = Dilution factor (if any)
- **W** = Weight of sample (mg)
- **10^4** = Conversion factor (µg to g and % calculation)

7. Advantages

- Highly accurate, specific and sensitive.
- Can separate digoxin from other cardiac glycosides.
- Suitable for routine quality control of crude drug and formulations.
- Good reproducibility and precision.
- Useful for stability studies.



Digoxin

8. Limitations

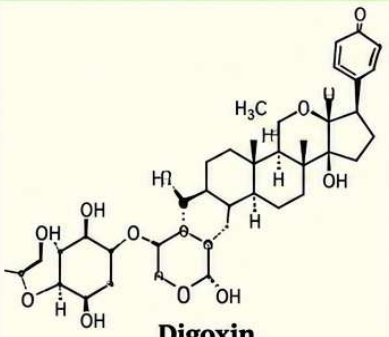
- Requires HPLC instrument and standard digoxin.
- Time-consuming compared to UV method.
- Expensive solvents and column.
- Sensitive to extraction method and sample preparation.
- Co-elution with other cardiac glycosides may occur (method optimization required).

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

www.pharmrecord.com

Utilization of Digoxin

Digoxin



Digoxin
$(C_{41}H_{64}O_{14})$



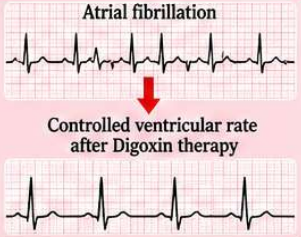
Digitalis purpurea
(Family: Plantaginaceae)

Dried leaves contain cardiac glycosides (mainly digoxin)

Cardiovascular use (Main utilization)

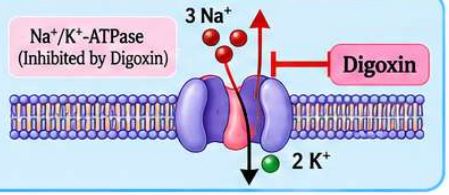
- Used in the treatment of congestive heart failure (CHF).
- Increases the force of myocardial contraction (positive inotropic effect).
- Helps in management of atrial fibrillation (controls ventricular rate).





Mechanism-based therapeutic use

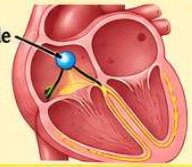
- Inhibits Na^+/K^+-ATPase in cardiac muscle.
- Increases intracellular Ca^{2+} → enhances myocardial contractility.
- Increases vagal tone → decreases heart rate and conduction through AV node (useful in atrial fibrillation).



Use in arrhythmias (Atrial fibrillation)

- Slows conduction through the AV node.
- Helps in controlling ventricular rate in atrial fibrillation and atrial flutter.





Clinical formulations

- Available as tablets, capsules, oral solution and injection.
- Used in standard doses under medical supervision.
- Narrow therapeutic index – dose adjustment required.



Other potential uses (under study / limited)

- Investigated for anti-cancer activity (induces apoptosis in some cancer cells).
- Studied for anti-viral activity (e.g., against certain viruses).
- Potential neuroprotective effects (under research).



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

6. Atropine

1. Source and Plant Profile of Atropine

- **Biological source:** Dried leaves and flowering tops of *Atropa belladonna*, *Datura stramonium* and *Hyoscyamus niger*.
- **Family:** Solanaceae (nightshade family).
- **Common name:** Belladonna, Deadly nightshade (*Atropa belladonna*); Thorn-apple (*Datura stramonium*); Henbane (*Hyoscyamus niger*).
- **Part used:** Dried leaves and flowering tops.
- **Active constituent:** Atropine (tropane alkaloid).
- **Geographical distribution:** Native to Europe, Western Asia and North Africa; widely cultivated in temperate regions of the world including India, Europe and North America.



Atropa belladonna
(Whole plant)



Leaves

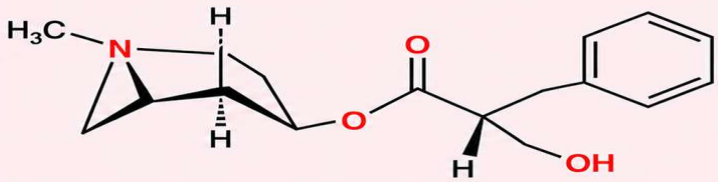


Flowers



Fruits (berries)

2. Chemical Structure of Atropine

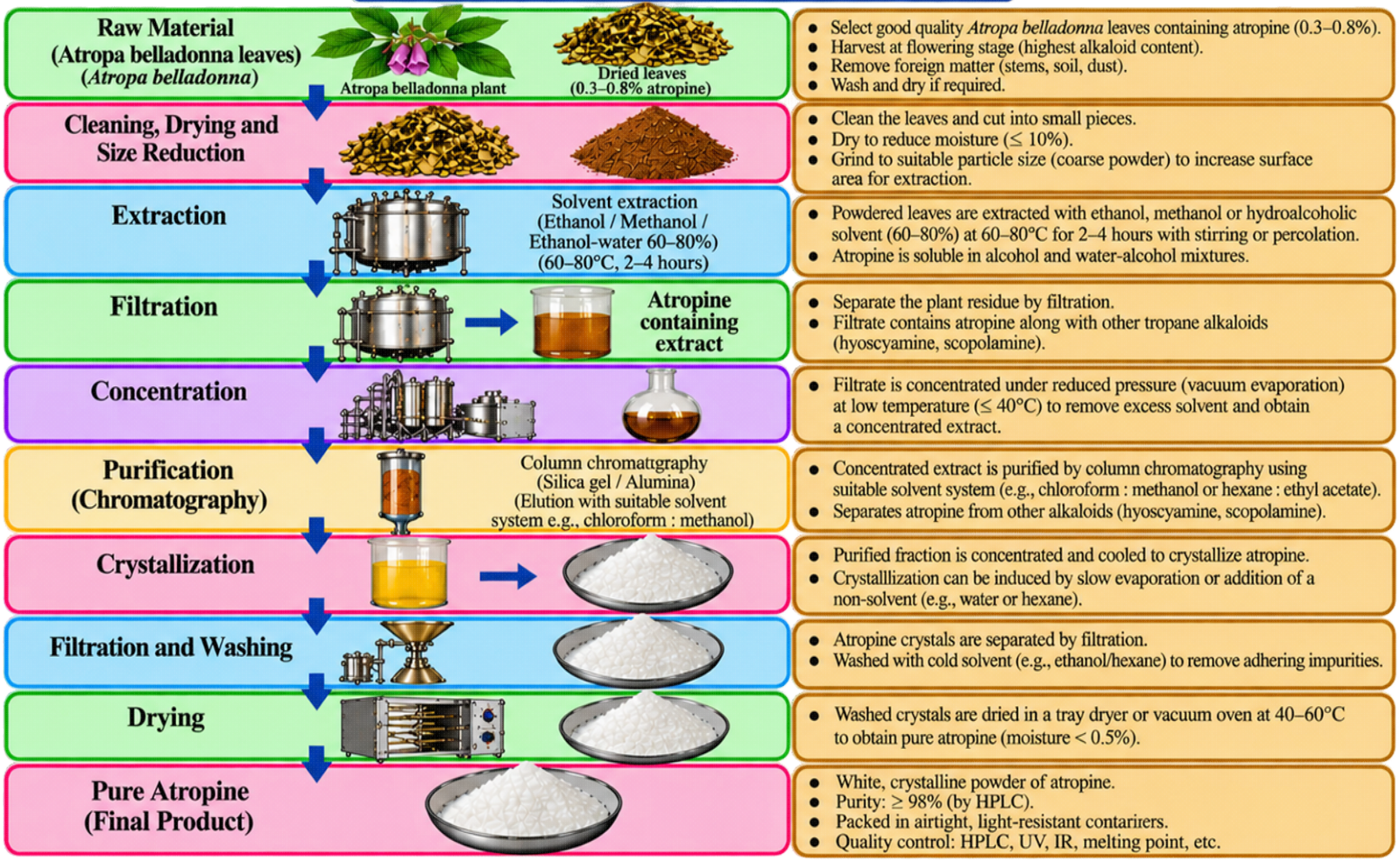


Atropine
($C_{17}H_{23}NO_3$)

Molecular formula	:	$C_{17}H_{23}NO_3$
Molecular weight	:	289.37 g/mol
Chemical class	:	Tropane alkaloid (ester of tropine and tropic acid)
IUPAC name	:	(±)-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl (2RS)-3-hydroxy-2-phenylpropanoate
Key structural features	:	Tropane nucleus (8-azabicyclo[3.2.1]octane), ester linkage with tropic acid, hydroxyl group and phenyl ring.

www.pharmrecord.com

Industrial Production of Atropine



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

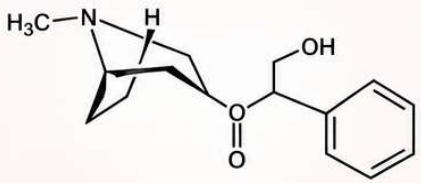
www.pharmrecord.com

Estimation of Atropine

1. Introduction

- Atropine is a tropane alkaloid occurring in plants of *Solanaceae* family, mainly in *Atropa belladonna*, *Datura* spp. and *Hyoscyamus niger*.
- It is a racemic mixture of hyoscyamine (l-isomer) and atropine (dl-isomer).
- Atropine is used as an antimuscarinic agent in the treatment of bradycardia, spasm, ophthalmic examination etc.
- Atropine content in crude drug and formulations is estimated mainly by HPLC, HPTLC, GC or UV spectrophotometry after derivatization.

2. Chemical structure of Atropine



Atropine
$(C_{17}H_{23}NO_3)$
Molecular weight: 289.37 g/mol

3. Plant source



Atropa belladonna
(Family: Solanaceae)
Dried leaves contain 0.3–1.0% total tropane alkaloids (primarily hyoscyamine and atropine).

4. Methods of Estimation

1. **HPLC** (most accurate and widely used)
2. **HPTLC** (simple and cost-effective)
3. **UV–Visible Spectrophotometry** (after derivatization, e.g., with orthophosphoric acid)
4. **GC–MS** (for detailed analysis)
5. **TLC** (for qualitative estimation)

A. HPLC Method (Standard Method)

1 Preparation of standard solution



Accurately weigh 10 mg of atropine and dissolve in methanol. Make up to 10 mL (1000 µg/mL). Further dilute to get working standard solutions (10–100 µg/mL).

2 Preparation of sample solution

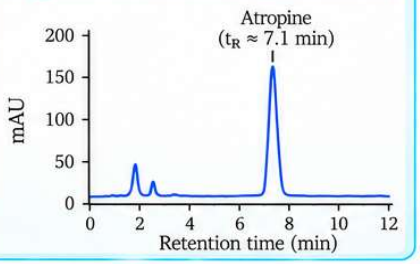


Extract with methanol (or methanol:water 80:20) using ultrasonication/ reflux, filter and make up to known volume (e.g., 10 mL).

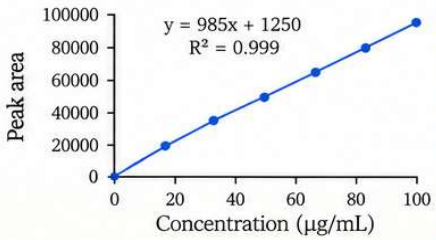
3 HPLC conditions (Typical)

Column	C18 (250 mm × 4.6 mm, 5 µm)
Mobile phase	Acetonitrile : 0.1% formic acid in water (40:60, v/v)
Flow rate	1.0 mL/min
Detection	UV at 210–220 nm (215 nm commonly used)
Injection volume	20 µL
Column temp.	30 °C
Run time	10–15 min
Retention time (typical)	Atropine: ~6–8 min (depends on system)

4 HPLC chromatogram



5. Calibration curve



6. Calculation

Amount of atropine in sample is calculated using the regression equation from calibration curve.

$$\text{Atropine content (\% w/w)} = \frac{C \times V \times D \times 100}{W \times 10^4}$$

Where,
- **C** = Concentration obtained from calibration curve (µg/mL)
- **V** = Final volume of sample extract (mL)
- **D** = Dilution factor (if any)
- **W** = Weight of sample (mg)
- **10^4** = Conversion factor (µg to g and % calculation)

7. Advantages

- Highly accurate, specific and sensitive.
- Can separate atropine from other tropane alkaloids (e.g., hyoscyamine, scopolamine).
- Suitable for routine quality control of crude drugs and formulations.
- Good reproducibility and precision.
- Useful for stability studies.



8. Limitations

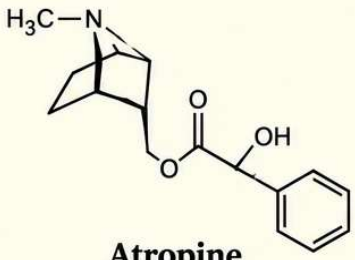
- Requires HPLC instrument and standard atropine.
- Time-consuming compared to UV method.
- Expensive solvents and column.
- Sensitive to extraction method and sample preparation.
- Co-elution with other tropane alkaloids may occur (method optimization required).

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

www.pharmrecord.com

Utilization of Atropine

Atropine



Atropine
($C_{17}H_{23}NO_3$)

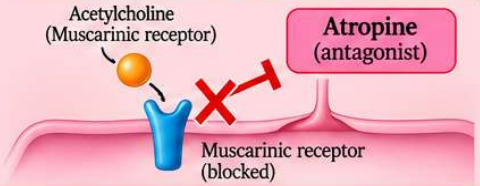


Atropa belladonna
(Family: Solanaceae)

Dried leaves and roots contain atropine (tropane alkaloid)

Antimuscarinic use (Main utillization)

- Atropine is a competitive antagonist at muscarinic acetylcholine receptors.
- Blocks parasympathetic actions of acetylcholine.
- Used to reduce excessive secretions and smooth muscle spasm.



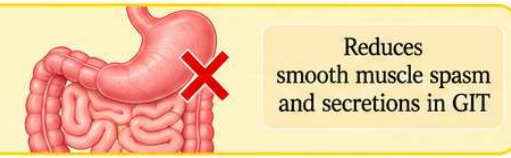
Ophthalmic use

- Produces mydriasis (dilation of pupil) by blocking muscarinic receptors in the eye.
- Used in eye examination (fundus examination).
- Also used in treatment of uveitis and to prevent synechiae.



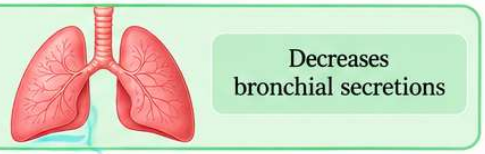
Gastrointestinal use

- Reduces gastrointestinal motility and secretions.
- Used in treatment of intestinal colic, diarrhea and peptic ulcer (adjunct).



Respiratory use

- Reduces bronchial secretions.
- Used as pre-anesthetic medication to dry respiratory secretions and prevent vagal bradycardia.



Cardiovascular use

- Increases heart rate by blocking vagal action on SA node (used in bradycardia).
- Used in atropine test to assess vagal tone.



Antidote use

- Used as an antidote in organophosphorus and carbamate poisoning (reverses muscarinic effects).
- Reduces excess salivation, bronchorrhea and bronchospasm.



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

7. Podophyllotoxin

1. Source and Plant Profile of Podophyllotoxin

- **Biological source:** Dried rhizomes and roots of *Podophyllum hexandrum* (Indian podophyllum) and *Podophyllum peltatum* (American mayapple).
- **Family:** Berberidaceae.
- **Common name:** Indian mayapple, Himalayan podophyllum (*P. hexandrum*); Mayapple (*P. peltatum*).
- **Part used:** Dried rhizomes and roots (contain podophyllotoxin ~4–8%).
- **Active constituent:** Podophyllotoxin (lignan).
- **Geographical distribution:** Native to Himalayan region of India (Jammu & Kashmir, Himachal Pradesh, Uttarakhand); also native to North America (*P. peltatum*). Grows in cool, temperate forests at high altitudes.



Podophyllum hexandrum (Whole plant)



Dried rhizomes



Leaves



Flower

2. Chemical Structure of Podophyllotoxin

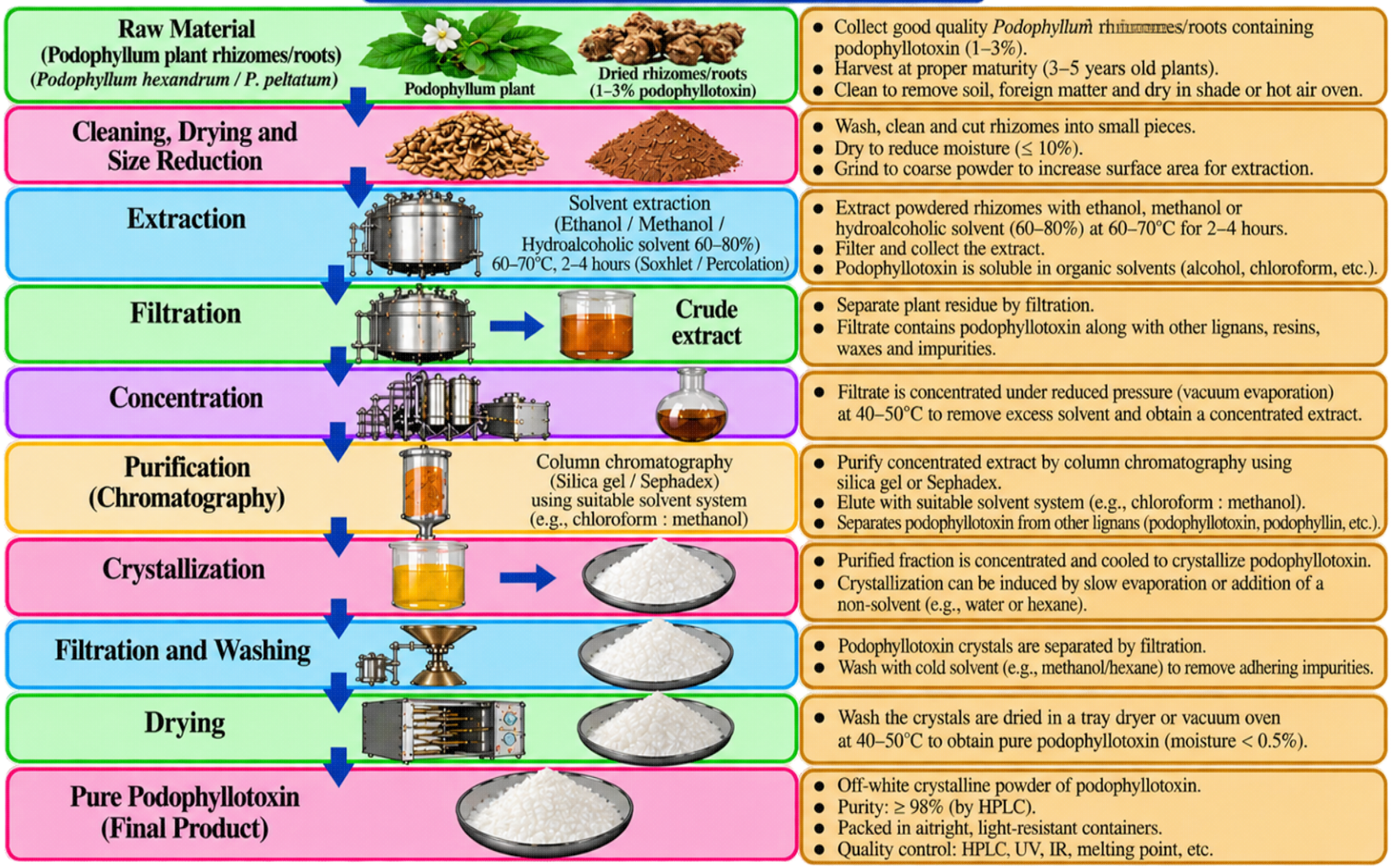


Podophyllotoxin ($C_{22}H_{22}O_8$)

Molecular formula	:	$C_{22}H_{22}O_8$
Molecular weight	:	414.40 g/mol
Chemical class	:	Lignan (aryltetralin lignan), cyclolignan
IUPAC name	:	(5*R*,5a*R*,8a*R*)-5-(3,4,5-trimethoxyphenyl)-5,5a,8,8a-tetrahydro-[1,3]dioxolo[4,5-g] isochromen-6-one
Key structural features	:	Aryltetralin lignan with a lactone ring, four methoxy groups (–OCH_3) and stereocenters at C-5, 5a and 8a.

www.pharmrecord.com

Industrial Production of Podophyllotoxin



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

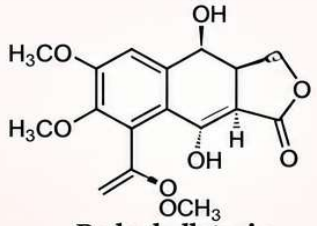
www.pharmrecord.com

Estimation of Podophyllotoxin

1. Introduction

- Podophyllotoxin is a lignan isolated from plants of the genus *Podophyllum*, mainly *Podophyllum hexandrum* (Indian mayapple).
- It is a cytotoxic compound used as a precursor for semi-synthetic anticancer drugs such as etoposide, teniposide and etoophos.
- Podophyllotoxin content in crude drug and formulations is estimated mainly by HPLC, HPTLC or UV spectrophotometry.

2. Chemical structure of Podophyllotoxin



Podophyllotoxin
($C_{22}H_{22}O_8$)
Molecular weight: 414.41 g/mol

3. Plant source



Podophyllum hexandrum
(Family: Berberidaceae)
Rhizomes and roots contain 0.5–4.0% podophyllotoxin (depending on source, age and growing conditions).

4. Methods of Estimation

1. **HPLC** (most accurate and widely used)
2. **HPTLC**
3. **UV–Visible Spectrophotometry** (after derivatization)
4. **LC–MS** (for detailed analysis)
5. **TLC** (for qualitative estimation)

A. HPLC Method (Standard Method)

1 Preparation of standard solution



Accurately weigh 10 mg of podophyllotoxin and dissolve in methanol. Make up to 10 mL (1000 µg/mL). Further dilute to get working standard solutions (10–100 µg/mL).

2 Preparation of sample solution

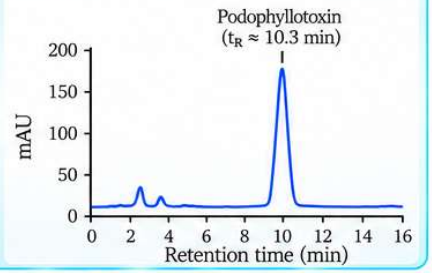


Extract with methanol (or methanol:water 80:20) using ultrasonication/ reflux, filter and make up to known volume (e.g., 10 mL).

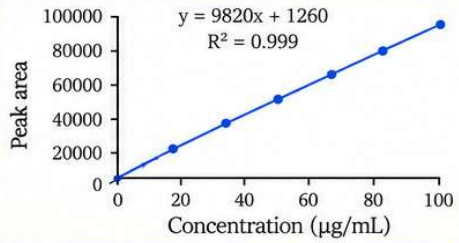
3 HPLC conditions (Typical)

Column	C18 (250 mm × 4.6 mm, 5 µm)
Mobile phase	Acetonitrile : Water (60:40, v/v) or Acetonitrile : 0.1% Formic acid (50:50, v/v)
Flow rate	1.0 mL/min
Detection	UV at 291 nm (λ_{max} of podophyllotoxin)
Injection volume	20 µL
Column temp.	30 °C
Run time	10–20 min
Retention time (typical)	Podophyllotoxin: ~9–12 min (depending on system)

4 HPLC chromatogram



5. Calibration curve



6. Calculation

Amount of podophyllotoxin in sample is calculated using the regression equation from calibration curve.

$$\text{Podophyllotoxin content (\% w/w)} = \frac{C \times V \times D \times 100}{W \times 10^4}$$

Where,

- **C** = Concentration obtained from calibration curve (µg/mL)
- **V** = Final volume of sample extract (mL)
- **D** = Dilution factor (if any)
- **W** = Weight of sample (mg)
- **10^4** = Conversion factor (µg to g and % calculation)

7. Advantages

- Highly accurate, specific and sensitive.
- Can separate podophyllotoxin from other lignans and impurities.
- Suitable for routine quality control of crude drug and formulations.
- Reproducible and precise.
- Useful for standardization and stability studies.



8. Limitations

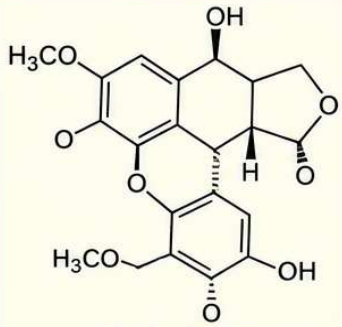
- Requires HPLC instrument and standard podophyllotoxin.
- Time-consuming compared to UV method.
- Expensive solvents and column.
- Sensitive to extraction method and sample preparation.
- Co-elution with other lignans may occur (method optimization required).

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

www.pharmrecord.com

Utilization of Podophyllotoxin

Podophyllotoxin



Podophyllotoxin
($C_{22}H_{22}O_8$)

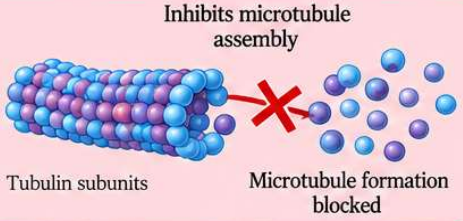


Podophyllum peltatum
(Family: Berberidaceae)

Rhizome and roots contain podophyllotoxin (lignan)

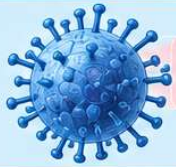
Anticancer use (Main utilization)

- Podophyllotoxin and its semi-synthetic derivatives (etoposide and teniposide) are important anticancer drugs.
- Used in treatment of various cancers such as testicular cancer, small cell lung cancer, lymphoma and leukemias.
- Inhibit microtubule assembly by binding to tubulin → prevents cell division (mitotic arrest).



Antiviral use

- Shows activity against certain viruses (e.g., HPV, herpes simplex virus) – under research.
- Inhibits viral replication by interfering with microtubule function and cell division.



Topical use (Warts and skin lesions)

- Podophyllotoxin (10–25% solution/cream) is used topically for treatment of genital warts and other viral skin lesions (e.g., caused by HPV).
- Causes local cytotoxic effect and inhibits mitotic division of infected cells.



Semisynthetic derivatives (Clinical use)

- Podophyllotoxin itself is highly toxic, so semisynthetic derivatives are used clinically.
- Important derivatives: Etoposide and Teniposide.
- Used in combination chemotherapy regimens for cancer treatment.



Other potential uses (Under study)

- Investigated for antifungal activity.
- Studied for potential use in Alzheimer's disease (inhibits microtubule disruption).
- Explored in combination therapies and targeted drug delivery systems.


Antifungal potential


Neuroprotective potential (under study)

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

8. Caffeine

1. Source and Plant Profile of Caffeine

- **Biological source:** Dried seeds of *Coffea arabica* (Coffee), dried leaves of *Camellia sinensis* (Tea), dried leaves of *Ilex paraguariensis* (Yerba mate), and seeds of *Theobroma cacao* (Cocoa).
- **Family:** Rubiaceae (Coffee), Theaceae (Tea), Aquifoliaceae (Yerba mate), Malvaceae (Cocoa).
- **Common name:** Coffee, Tea, Cocoa, Yerba mate.
- **Part used:** Seeds (coffee, cocoa) and leaves (tea, yerba mate).
- **Active constituent:** Caffeine (methylxanthine alkaloid).
- **Geographical distribution:** Native to tropical and subtropical regions. Coffee – Ethiopia; Tea – China and India; Cocoa – Central and South America; Yerba mate – South America. Widely cultivated worldwide.



Coffea arabica (Coffee plant)



Camellia sinensis (Tea plant)

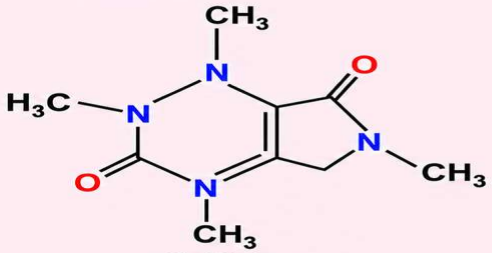


Theobroma cacao (Cocoa plant)



Ilex paraguariensis (Yerba mate)

2. Chemical Structure of Caffeine

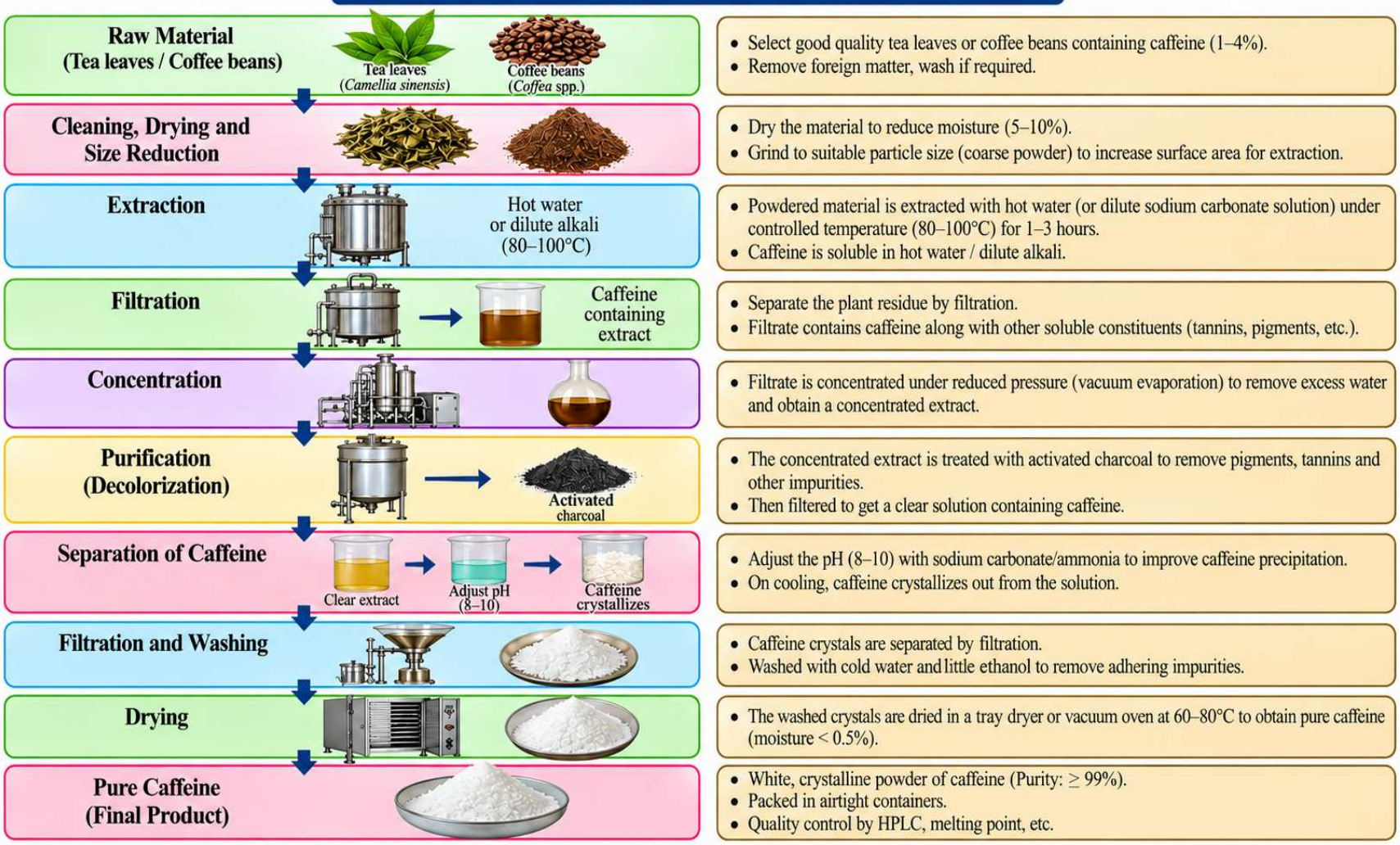


Caffeine ($C_8H_{10}N_4O_2$)

Molecular formula	:	$C_8H_{10}N_4O_2$
Molecular weight	:	194.19 g/mol
Chemical class	:	Methylxanthine alkaloid
IUPAC name	:	1,3,7-Trimethylpurine-2,6-dione
Key structural features	:	Purine (xanthine) nucleus with two carbonyl groups at C-2 and C-6 and three methyl groups at N-1, N-3 and N-7.

www.pharmrecord.com

Industrial Production of Caffeine



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

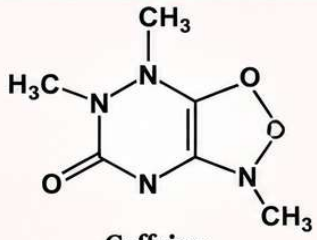
www.pharmrecord.com

Estimation of Caffeine

1. Introduction

- Caffeine (1,3,7-trimethylxanthine) is a methylxanthine alkaloid occurring in various plants such as tea, coffee, cocoa, kola nut, guaraná and mate.
- It is a central nervous system stimulant used to reduce fatigue and increase alertness.
- Caffeine content in crude drug and formulations is estimated mainly by HPLC, HPTLC, UV spectrophotometry or titrimetry after extraction and purification.

2. Chemical structure of Caffeine



Caffeine
($C_8H_{10}N_4O_2$)
Molecular weight: 194.19 g/mol

3. Plant source



Coffee (*Coffea arabica*) | **Tea** (*Camellia sinensis*)

Cocoa (*Theobroma cacao*) | **Kola nut** (*Cola acuminata*)

4. Methods of Estimation

1. **HPLC** (most accurate and widely used)
2. **HPTLC** (simple and cost-effective)
3. **UV–Visible Spectrophotometry** (after appropriate extraction)
4. **GC–MS** (for detailed analysis)
5. **Titrimetry** (less common, based on alkaloidal nature)

A. HPLC Method (Standard Method)

1 Preparation of standard solution



Caffeine (standard)
10 mg

Accurately weigh 10 mg of caffeine and dissolve in methanol. Make up to 10 mL (1000 µg/mL). Further dilute to get working standard solutions (10–100 µg/mL).

2 Preparation of sample solution



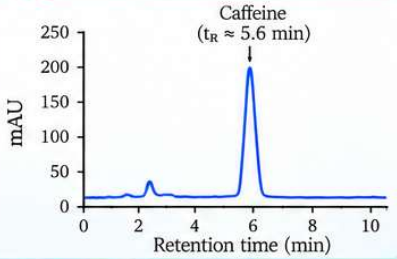
Powdered tea leaves (*or coffee powder*) (1 g)

Extract with methanol (or methanol:water 70:30) using ultrasonication/ reflux, filter and make up to known volume (e.g., 10 mL).

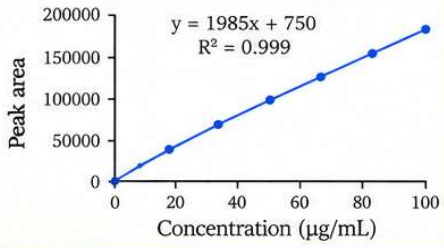
3 HPLC conditions (Typical)

Column	C18 (250 mm × 4.6 mm, 5 µm)
Mobile phase	Methanol : Water (30:70, v/v) or Acetonitrile : Water (20:80)
Flow rate	1.0 mL/min
Detection	UV at 272 nm (λ_{max} of caffeine)
Injection volume	20 µL
Column temp.	30 °C
Run time	8–12 min
Retention time (typical)	Caffeine: ~5–7 min (depending on system)

4 HPLC chromatogram



5. Calibration curve



6. Calculation

Amount of caffeine in sample is calculated using the regression equation from calibration curve.

$$\text{Caffeine content (\% w/w)} = \frac{C \times V \times D \times 100}{W \times 10^4}$$

Where,

- **C** = Concentration obtained from calibration curve (µg/mL)
- **V** = Final volume of sample extract (mL)
- **D** = Dilution factor (if any)
- **W** = Weight of sample (mg)
- **10^4** = Conversion factor (µg to g and % calculation)

7. Advantages

- Highly accurate, specific and sensitive.
- Can separate caffeine from other methylxanthines and impurities.
- Suitable for routine quality control of crude drugs and formulations.
- Reproducible results.
- Useful for stability studies.



8. Limitations

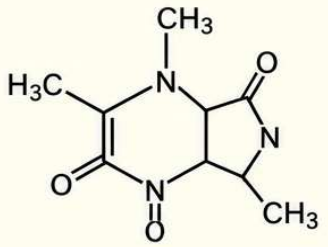
- Requires HPLC instrument and standard caffeine.
- Time-consuming compared to UV method.
- Expensive solvents and column.
- Sensitive to extraction method and sample preparation.
- Co-elution with structurally similar methylxanthines (e.g., theophylline, theobromine) may occur (method optimization required).

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

www.pharmrecord.com

Utilization of Caffeine

Caffeine



Caffeine

$(C_8H_{10}N_4O_2)$



Natural sources:

- Coffee beans (*Coffea arabica*)
- Tea leaves (*Camellia sinensis*)
- Cocoa seeds (*Theobroma cacao*)
- Guarana seeds (*Paullinia cupana*)

Family: Rubiaceae (Coffee)

Central nervous system (CNS) use (Main utilization)

- Acts as a CNS stimulant (antagonist of adenosine receptors).
- Increases alertness, attention, concentration and reduces fatigue.
- Used to improve mental performance and reduce drowsiness.
- Commonly used in beverages and over-the-counter products.



Increases alertness, concentration and reduces fatigue

Respiratory use

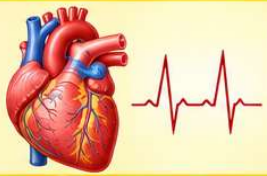
- Stimulates respiratory centre (mild respiratory stimulant).
- Used in treatment of apnea of prematurity (in combination with other drugs such as citric acid – caffeine).



Stimulates respiration (used in apnea of prematurity)

Cardiovascular use

- Mild positive inotropic and chronotropic effect.
- Can increase heart rate and cardiac output.
- Used in some formulations for mild hypotension and to counteract drug-induced bradycardia (e.g., in anesthesia).



Increases heart rate and cardiac output

Diuretic use

- Increases renal blood flow and glomerular filtration rate (mild diuretic).
- Promotes excretion of sodium, chloride and water.
- Helps in reduction of fluid retention.



Mild diuretic effect

Therapeutic and pharmaceutical uses

- Used in combination analgesic preparations for headache (e.g., with paracetamol/aspirin) – enhances analgesic effect.
- Used to counteract drowsiness and enhance physical performance.
- Included in weight management products (mild thermogenic effect).



Enhances analgesic effect and reduces drowsiness

Other potential uses

- Antioxidant activity (scavenges free radicals – under study).
- Potential role in neuroprotective effects (e.g., Parkinson's and Alzheimer's – under study).
- Used in skincare and cosmetic formulations (anti-cellulite and anti-aging – under study).



Antioxidant and cosmetic potential (under study)

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

9. Taxol

1. Source and Plant Profile of Taxol

- **Biological source:** Dried bark, leaves and needles of *Taxus brevifolia*, *Taxus baccata* and other species of *Taxus* (yew tree).
- **Family:** Taxaceae.
- **Common name:** Pacific yew (*Taxus brevifolia*), European yew (*Taxus baccata*).
- **Part used:** Bark (main source), also leaves and needles.
- **Active constituent:** Taxol (paclitaxel) – a diterpenoid (taxane) compound.
- **Geographical distribution:** Native to North America (*T. brevifolia*) and Europe, also found in East Asia. Cultivated in temperate regions worldwide for commercial production and semi-synthetic preparation.



Taxus brevifolia
(Whole plant)



Bark
(Source of Taxol)

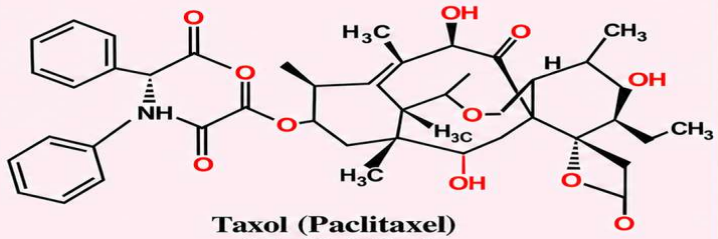


Leaves
(Needles)



Fruits (arils and seeds)

2. Chemical Structure of Taxol

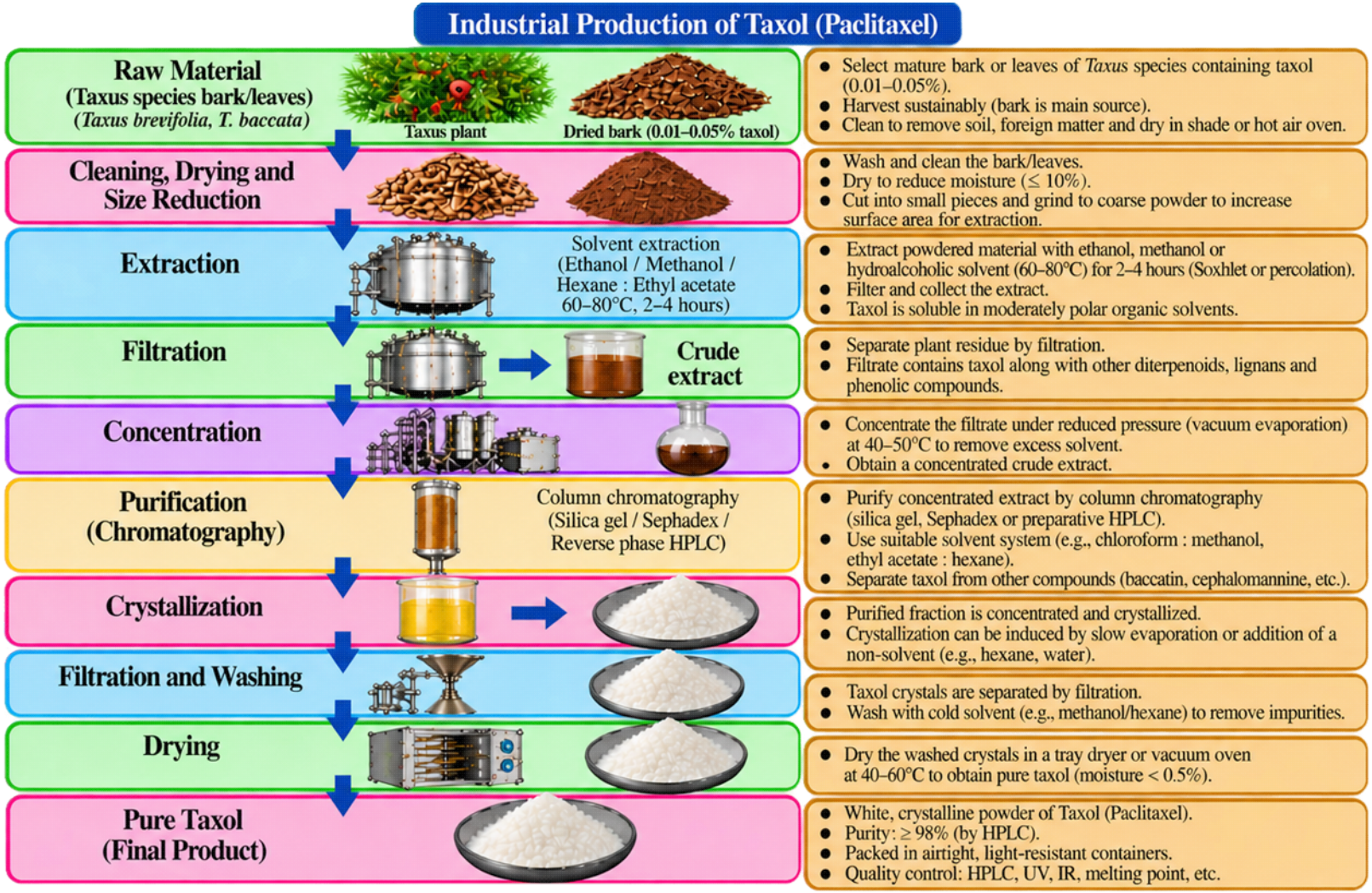


Taxol (Paclitaxel)
($C_{47}H_{51}NO_{14}$)

Molecular formula	:	$C_{47}H_{51}NO_{14}$
Molecular weight	:	853.91 g/mol
Chemical class	:	Diterpenoid (Taxane)
IUPAC name	:	(2α,4α,5β,7β,10β,13α)-4,10-Diacetoxy-7,13-dihydroxy-1,6α-epoxy-9-oxotax-11-en-2-yl benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine
Key structural features	:	Taxane skeleton (tricyclic diterpene) with an oxetane ring, multiple ester groups, hydroxyl groups and a C-13 side chain derived from N-benzoylphenylisoserine.

www.pharmrecord.com

Industrial Production of Taxol (Paclitaxel)



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

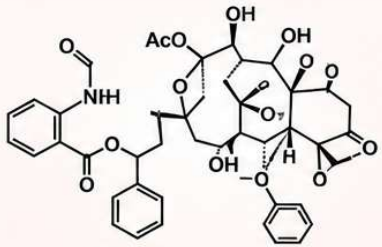
www.pharmrecord.com

Estimation of Taxol (Paclitaxel)

1. Introduction

- Taxol (Paclitaxel) is a diterpenoid compound obtained from the bark of *Taxus* species, mainly *Taxus brevifolia* (Pacific yew).
- It belongs to the class of taxanes and is a potent anticancer agent used in the treatment of breast cancer, ovarian cancer, lung cancer and other solid tumors.
- Taxol content in crude drug and formulations is estimated mainly by HPLC or HPTLC with UV detection after extraction and purification.

2. Chemical structure of Taxol



Taxol (Paclitaxel)

($C_{47}H_{51}NO_{14}$)

Molecular weight: 853.91 g/mol

3. Plant source



Taxus brevifolia

(Family: Taxaceae)

Bark contains 0.01–0.1% taxol (depending on species, age and growing conditions).

4. Methods of Estimation

1. **HPLC** (most accurate and widely used)
2. **HPTLC** (alternative method)
3. **UV–Visible Spectrophotometry** (less common, after suitable derivatization)
4. **LC–MS** (for detailed analysis and confirmation)
5. **TLC** (for qualitative estimation)

A. HPLC Method (Standard Method)

1 Preparation of standard solution



Taxol (standard)

10 mg



Accurately weigh 10 mg of taxol (paclitaxel) and dissolve in methanol to make up to 10 mL (1000 µg/mL).

Further dilute to get working standard solutions (1–100 µg/mL).

2 Preparation of sample solution



Powdered bark of *Taxus* spp. (1 g)

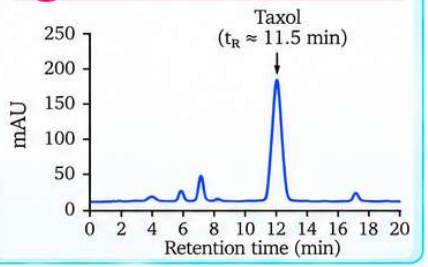


Extract with methanol (or methanol:water 80:20) using ultrasonication/ reflux, filter and make up to known volume (e.g., 10 mL). Further dilute to suitable concentration.

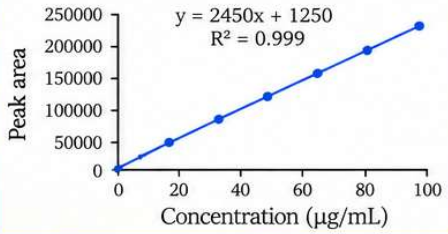
3 HPLC conditions (Typical)

Column	C18 (250 mm × 4.6 mm, 5 µm)
Mobile phase	Acetonitrile : Water (60:40, v/v) or Gradient elution
Flow rate	1.0 mL/min
Detection	UV at 227 nm (λ_{max} of taxol)
Injection volume	20 µL
Column temp.	30 °C
Run time	15–25 min
Retention time (typical)	Taxol: ~10–13 min (depending on system)

4 HPLC chromatogram



5. Calibration curve



6. Calculation

Amount of taxol in sample is calculated using the regression equation from calibration curve.

$$\text{Taxol content (\% w/w)} = \frac{C \times V \times D \times 100}{W \times 10^4}$$

Where,

- **C** = Concentration obtained from calibration curve (µg/mL)
- **V** = Final volume of sample extract (mL)
- **D** = Dilution factor (if any)
- **W** = Weight of sample (mg)
- **10^4** = Conversion factor (µg to g and % calculation)

7. Advantages

- Highly accurate, specific and sensitive.
- Can be used for routine quality control of crude drug and formulations (injections, tablets).
- Separates taxol from other taxanes and related impurities.
- Reproducible and precise.
- Suitable for stability studies.



8. Limitations

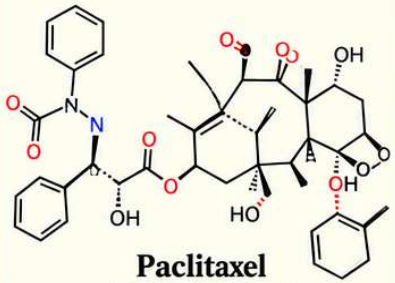
- Requires HPLC instrument and pure standard taxol.
- Time-consuming compared to UV method.
- Expensive solvents and column.
- Sensitive to extraction method and sample preparation.
- Co-elution with other taxanes (e.g., 10-deacetylbaccatin III) may occur (method optimization required).

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

www.pharmrecord.com

Utilization of Taxol (Paclitaxel)

Taxol (Paclitaxel)



Paclitaxel
($C_{47}H_{51}NO_{14}$)



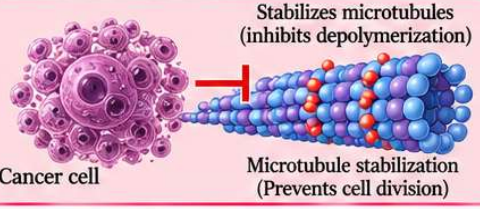
Taxus brevifolia
(Western Yew)
(Family: Taxaceae)



Bark contains paclitaxel
(diterpenoid, taxane)

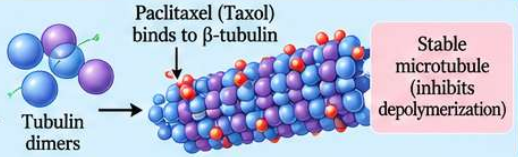
Anticancer use (Main utilization)

- Potent anticancer drug used in treatment of breast cancer, ovarian cancer, non-small cell lung cancer and Kaposi's sarcoma.
- Stabilizes microtubules and inhibits their depolymerization, leading to mitotic arrest and apoptosis of cancer cells.
- Used alone or in combination chemotherapy regimens.



Mechanism of action

- Binds to β-tubulin subunit of microtubules.
- Promotes microtubule assembly and prevents depolymerization.
- Blocks mitotic spindle function → inhibits mitosis (M phase arrest).
- Leads to apoptosis of rapidly dividing cancer cells.



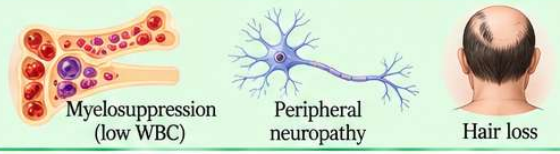
Clinical formulations and use

- Available as injection (paclitaxel in Cremophor EL or albumin-bound form: nab-paclitaxel).
- Used in combination with other anticancer drugs (e.g., carboplatin, doxorubicin, trastuzumab).
- Used for breast, ovarian, lung and other solid tumors.



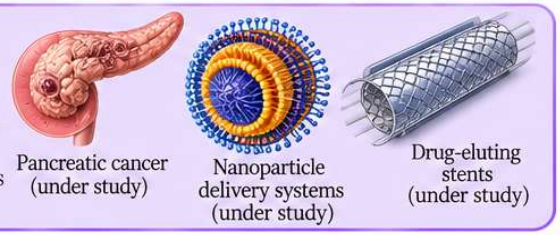
Side effects

- Myelosuppression (neutropenia), peripheral neuropathy.
- Hypersensitivity reactions (due to Cremophor EL).
- Hair loss, nausea, vomiting and fatigue.



Other potential uses (Under study)

- Investigated for treatment of other cancers (e.g., pancreatic, prostate, head and neck cancers).
- Studied for use in combination therapies and targeted drug delivery systems (e.g., nanoparticle formulations).
- Potential use in drug-eluting stents and other biomedical applications (under research).



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

10. Vincristine

1. Source and Plant Profile of Vincristine

- **Biological source:** Dried leaves and aerial parts of *Catharanthus roseus* (syn. *Vinca rosea*).
- **Family:** Apocynaceae.
- **Common name:** Madagascar periwinkle, Vinka, Sadabahar.
- **Part used:** Dried leaves (main source) and eerial parts.
- **Active constituent:** Vincristine (an indole alkaloid).
- **Geographical distribution:** Native to Madagascar; widely cultivated in tropical and subtropical regions including India, Sri Lanka, Southeast Asia, Africa and other parts of the world.



Catharanthus roseus
(Whole plant)



Leaves
(Source of Vincristine)

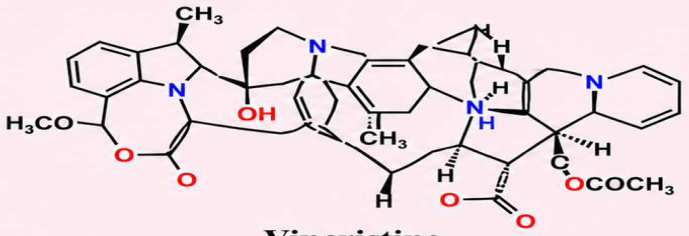


Flower



Fruits (Follicles)

2. Chemical Structure of Vincristine

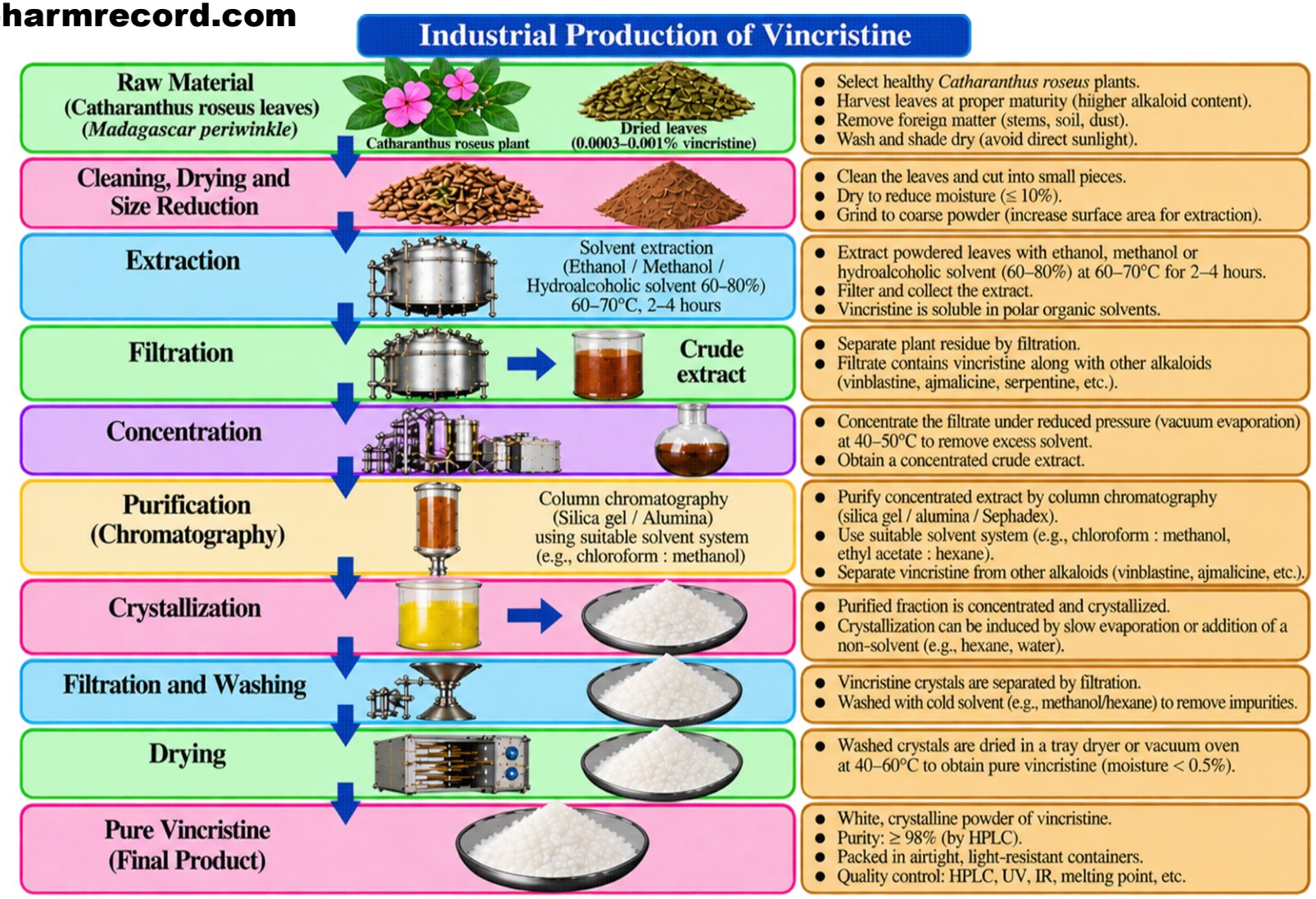


Vincristine
($C_{46}H_{56}N_4O_{10}$)

Molecular formula	:	$C_{46}H_{56}N_4O_{10}$
Molecular weight	:	824.96 g/mol
Chemical class	:	Indole alkaloid (*Vinca* alkaloid, dimeric alkaloid)
IUPAC name	:	Methyl (3β·16β)-16-((2*R*,3*S*)-3-(ethylcarbamoyl)-2-hydroxypropyl)-19,20-didehydro-3,4-dideoxy-vinblastine-3-carboxylate
Key structural features	:	Dimeric indole alkaloid composed of catharanthine and vindoline units with multiple chiral centers, ester and carbamate groups.

www.pharmrecord.com

Industrial Production of Vincristine



- Select healthy *Catharanthus roseus* plants.
- Harvest leaves at proper maturity (hiigher alkaloid content).
- Remove foreign matter (stems, soil, dust).
- Wash and shade dry (avoid direct sunlight).

- Clean the leaves and cut into small pieces.
- Dry to reduce moisture (≤ 10%).
- Grind to coarse powder (increase surface area for extraction).

- Extract powdered leaves with ethanol, methanol or hydroalcoholic solvent (60–80%) at 60–70°C for 2–4 hours.
- Filter and collect the extract.
- Vincristine is soluble in polar organic solvents.

- Separate plant residue by filtration.
- Filtrate contains vincristine along with other alkaloids (vinblastine, ajmalicine, serpentine, etc.).

- Concentrate the filtrate under reduced pressure (vacuum evaporation) at 40–50°C to remove excess solvent.
- Obtain a concentrated crude extract.

- Purify concentrated extract by column chromatography (silica gel / alumina / Sephadex).
- Use suitable solvent system (e.g., chloroform : methanol, ethyl acetate : hexane).
- Separate vincristine from other alkaloids (vinblastine, ajmalicine, etc.).

- Purified fraction is concentrated and crystallized.
- Crystallization can be induced by slow evaporation or addition of a non-solvent (e.g., hexane, water).

- Vincristine crystals are separated by filtration.
- Washed with cold solvent (e.g., methanol/hexane) to remove impurities.

- Washed crystals are dried in a tray dryer or vacuum oven at 40–60°C to obtain pure vincristine (moisture < 0.5%).

- White, crystalline powder of vincristine.
- Purity: ≥ 98% (by HPLC).
- Packed in airtight, light-resistant containers.
- Quality control: HPLC, UV, IR, melting point, etc.

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

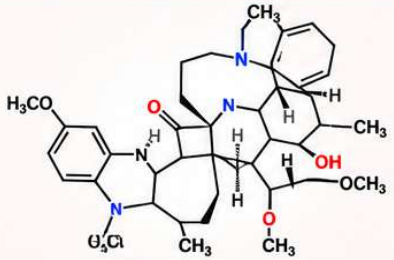
www.pharmrecord.com

Estimation of Vincristine

1. Introduction

- Vincristine is a vinca alkaloid isolated from the leaves of *Catharanthus roseus* (Madagascar periwinkle).
- It is used as an anticancer agent (mainly in leukemia, lymphoma and solid tumors).
- Vincristine content in crude drug and formulations is estimated mainly by HPLC, HPTLC or UV spectrophotometry after extraction and purification.
- Due to low natural abundance, highly sensitive and selective analytical methods are required.

2. Chemical structure of Vincristine



Vincristine
($C_{46}H_{56}N_4O_{10}$)
Molecular weight: 824.98 g/mol

3. Plant source



Catharanthus roseus
(Family: Apocynaceae)
Leaves contain 0.0003–0.001% vincristine (depends on variety, geographical location and growing conditions).

4. Methods of Estimation

1. **HPLC** (most accurate and widely used)
2. **HPTLC** (simple and cost-effective)
3. **LC–MS / LC–MS/MS** (for high sensitivity and confirmation)
4. **UV–Visible Spectrophotometry** (less common, due to low content and interference from other alkaloids)
5. **TLC–Densitometry** (for qualitative and semi-quantitative analysis)

A. HPLC Method (Standard Method)

1 Preparation of standard solution



Vincristine (standard)
1 mg



Accurately weigh 1 mg of vincristine and dissolve in methanol. Make up to 10 mL (100 µg/mL). Further dilute to get working standard solutions (0.1–10 µg/mL).

2 Preparation of sample solution



Dried leaves of *Catharanthus roseus* (1 g)

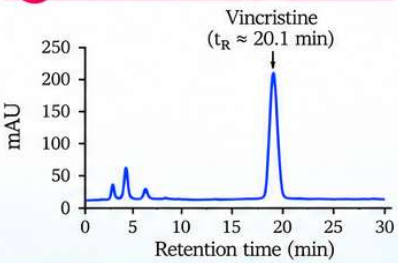


Extract with methanol (or methanol:water 80:20) using ultrasonication/ reflux, filter and make up to known volume (e.g., 10 mL). Further dilute to suitable concentration.

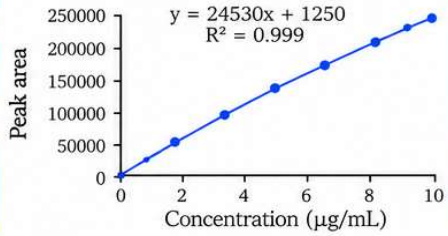
3 HPLC conditions (Typical)

Column	C18 (250 mm × 4.6 mm, 5 µm)
Mobile phase	Acetonitrile : 0.1% Formic acid in water (40:60, v/v) (or Gradient elution)
Flow rate	1.0 mL/min
Detection	UV at 254 nm (λ_{max} of vincristine)
Injection volume	20 µL
Column temp.	30 °C
Run time	20–30 min
Retention time (typical)	Vincristine: ~18–22 min (depending on system)

4 HPLC chromatogram



5. Calibration curve



6. Calibration

Amount of vincristine in sample is calculated using the regression equation from calibration curve.

$$\text{Vincristine content (µg/g)} = \frac{C \times V \times D}{W}$$

Where,
C = Concentration obtained from calibration curve (µg/mL)
V = Final volume of sample extract (mL)
D = Dilution factor (if any)
W = Weight of sample (g)

7. Advantages

- Highly accurate, specific and sensitive.
- Can separate vincristine from other vinca alkaloids (e.g., vinblastine).
- Suitable for routine quality control of crude drug and formulations (injections).
- Good reproducibility and precision.
- Useful for stability studies.



8. Limitations

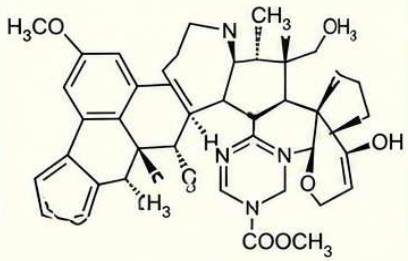
- Requires HPLC instrument and pure standard vincristine.
- Time-consuming compared to UV method.
- Expensive solvents and column.
- Sensitive to extraction method and sample preparation.
- Co-elution with other vinca alkaloids (e.g., vinblastine, vinorelbine) may occur (method optimization required).

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

www.pharmrecord.com

Utilization of Vincristine

Vincristine



Vincristine
$(C_{46}H_{56}N_4O_{10})$

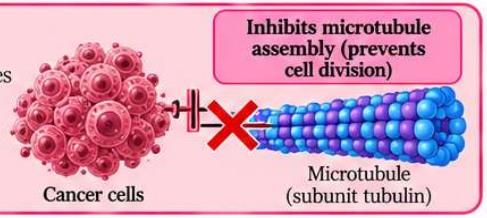


Catharanthus roseus
(Madagascar periwinkle)
(Family: Apocynaceae)

Leaves contain vincristine
(indole alkaloid)

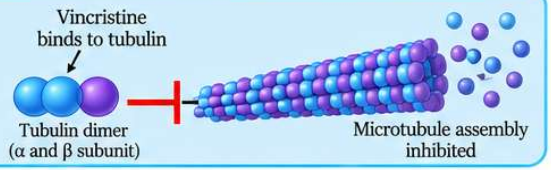
Anticancer use (Main utilization)

- Potent anticancer drug used in treatment of hematological malignancies and solid tumors.
- Commonly used in combination chemotherapy regimens (e.g., for leukemia, lymphoma, Wilms tumor, neuroblastoma).
- Effective against rapidly dividing cancer cells.



Mechanism of action

- Binds to β-tubulin subunit of microtubules.
- Inhibits microtubule assembly and prevents polymerization.
- Leads to mitotic arrest at metaphase and inhibits cell division (mitotic spindle formation is blocked).



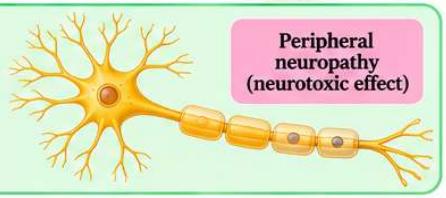
Clinical formulations and use

- Available as injection (vincristine sulfate).
- Used in combination chemotherapy regimens such as VCR (vincristine), VCR + prednisone, VCR + doxorubicin, etc.
- Mainly used for acute lymphoblastic leukemia (ALL), Hodgkin's and non-Hodgkin's lymphoma, Wilms tumor, neuroblastoma and other solid tumors.



Adverse effects (Side effects)

- Neurotoxicity (dose-limiting): peripheral neuropathy, sensory and motor neuropathy, paresthesia, loss of reflexes.
- Autonomic neuropathy: constipation, paralytic ileus, urinary retention.
- Mild myelosuppression (less compared to other vinca alkaloids).
- SIADH (syndrome of inappropriate antidiuretic hormone secretion).



Other potential uses (Under study)

- Investigated in other solid tumors (e.g., sarcomas, brain tumors).
- Studied for potential role in combination with targeted therapies.
- Research in nanoparticle delivery systems to reduce neurotoxicity.
- Explored for synergistic effects with other anticancer agents.



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

11. Vinblastine

1. Source and Plant Profile of Vinblastine

- **Biological source:** Dried leaves of *Catharanthus roseus* (syn. *Vinca rosea*).
- **Family:** Apocynaceae.
- **Common name:** Madagascar periwinkle, Vinka, Sadabahar.
- **Part used:** Dried leaves (main source).
- **Active constituent:** Vinblastine (a dimeric indole alkaloid).
- **Geographical distribution:** Native to Madagascar; widely cultivated in tropical and subtropical regions including India, Sri Lanka, Southeast Asia, Africa and other parts of the world for commercial production.



Catharanthus roseus
(Whole plant)



Leaves
(Source of Vinblastine)

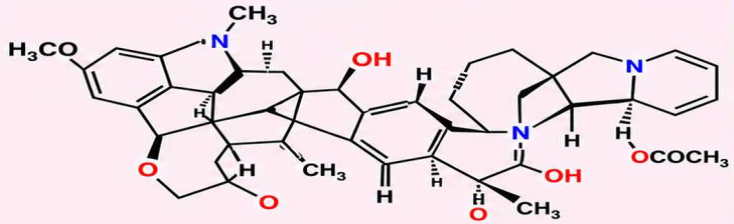


Flower



Fruits (Follicles)

2. Chemical Structure of Vinblastine

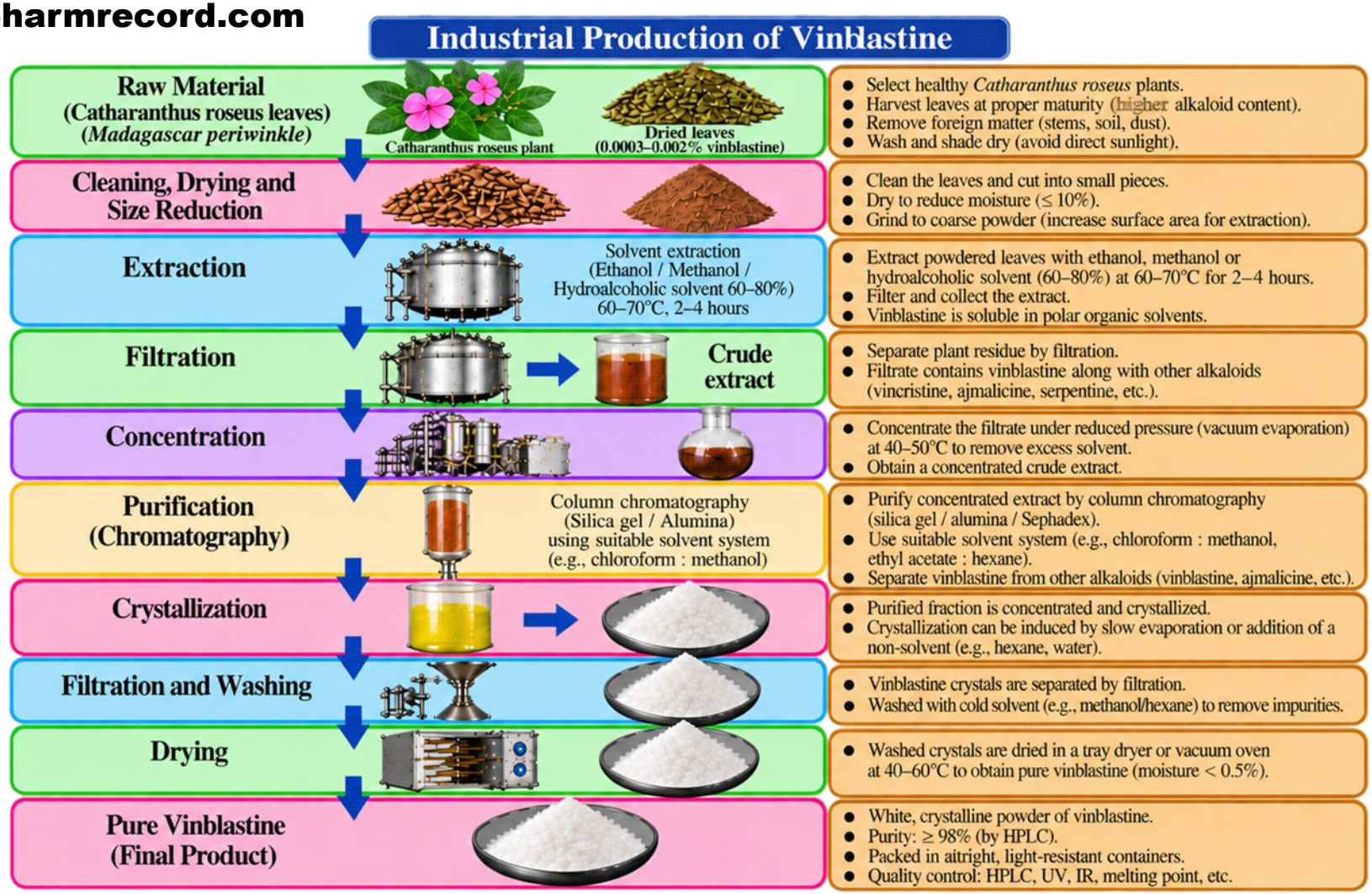


Vinblastine
($C_{46}H_{58}N_4O_9$)

Molecular formula	:	$C_{46}H_{58}N_4O_9$
Molecular weight	:	810.98 g/mol
Chemical class	:	Indole alkaloid (*Vinca* alkaloid, dimeric alkaloid)
IUPAC name	:	Methyl (3β,16β)-16-[(2*R*,3*S*)-3-(acetoxy)-2-hydroxypropyl]-19,20-didehydro-3,4-dideoxy-vinblastine-3-carboxylate
Key structural features	:	Dimeric indole alkaloid composed of catharanthine and vindoline units containing multiple chiral centers, ester and acetate groups.

www.pharmrecord.com

Industrial Production of Vinblastine



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

www.pharmrecord.com

Estimation of Vinblastine

1. Introduction

- Vinblastine is a vinca alkaloid isolated from the leaves of *Catharanthus roseus* (Madagascar periwinkle).
- It is used as an anticancer agent (mainly in Hodgkin's lymphoma, non-Hodgkin's lymphoma, testicular cancer, breast cancer and other solid tumors).
- Vinblastine content in crude drug and formulations is estimated mainly by HPLC, HPTLC or UV spectrophotometry after extraction and purification.
- Due to low natural abundance and presence of other vinca alkaloids, selective and sensitive analytical methods are required.

2. Chemical structure of Vinblastine



Vinblastine
$(C_{46}H_{58}N_4O_9)$
Molecular weight: 810.98 g/mol

3. Plant source



Catharanthus roseus
(Family: Apocynaceae)
Leaves contain 0.0003–0.001% vinblastine (varies with plant variety, geographical location and growing conditions).

4. Methods of Estimation

1. **HPLC** (most accurate and widely used)
2. **HPTLC** (simple and cost-effective)
3. **LC–MS / LC–MS/MS** (for high sensitivity and confirmation)
4. **UV–Visible Spectrophotometry** (less common, due to low content and interference from other alkaloids)
5. **TLC–Densitometry** (for qualitative and semi-quantitative analysis)

A. HPLC Method (Standard Method)

1 Preparation of standard solution



Vinblastine (standard) 1 mg

Accurately weigh 1 mg of vinblastine and dissolve in methanol. Make up to 10 mL (100 µg/mL). Further dilute to get working standard solutions (0.1–10 µg/mL).

2 Preparation of sample solution



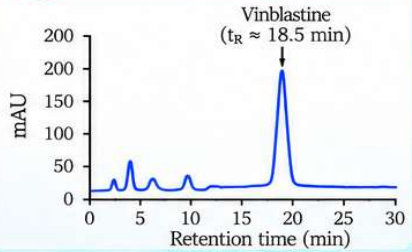
Dried leaves of *Catharanthus roseus* (1 g powdered drug)

Extract with methanol (or methanol:water 80:20) using ultrasonication/ reflux, filter and make up to known volume (e.g., 10 mL). Further dilute to suitable concentration.

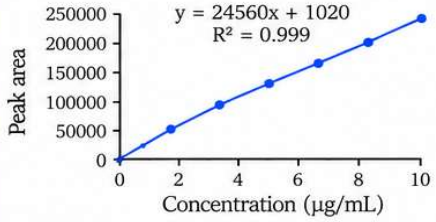
3 HPLC conditions (Typical)

Column	C18 (250 mm × 4.6 mm, 5 µm)
Mobile phase	Acetonitrile : 0.1% Formic acid in water (40:60, v/v) (or Gradient elution)
Flow rate	1.0 mL/min
Detection	UV at 254 nm (λ_{max} of vinblastine)
Injection volume	20 µL
Column temp.	30 °C
Run time	20–30 min
Retention time (typical)	Vinblastine: ~16–20 min (depending on system)

4 HPLC chromatogram



5. Calibration curve



6. Calculation

Amount of vinblastine in sample is calculated using the regression equation from calibration curve.

$$\text{Vinblastine content (µg/g)} = \frac{C \times V \times D}{W}$$

Where,
- **C** = Concentration obtained from calibration curve (µg/mL)
- **V** = Final volume of sample extract (mL)
- **D** = Dilution factor (if any)
- **W** = Weight of sample (g)

7. Advantages

- Highly accurate, specific and sensitive.
- Can separate vinblastine from other vinca alkaloids (e.g., vincristine).
- Suitable for routine quality control of crude drug and formulations (injections, tablets).
- Good reproducibility and precision.
- Useful for stability studies.

8. Limitations

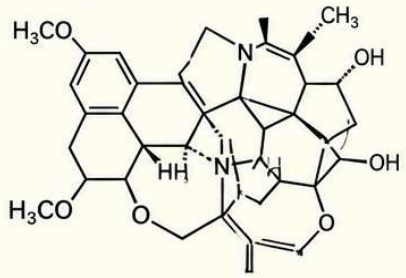
- Requires HPLC instrument and pure standard vinblastine.
- Time-consuming compared to UV method.
- Expensive solvents and column.
- Sensitive to extraction method and sample preparation.
- Co-elution with other vinca alkaloids (e.g., vincristine, vinorelbine) may occur (method optimization required).

Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

www.pharmrecord.com

Utilization of Vinblastine

Vinblastine



Vinblastine
($C_{46}H_{58}N_4O_9$)

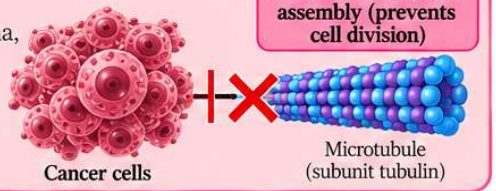


Catharanthus roseus
(Madagascar periwinkle)
(Family: Apocynaceae)

Leaves contain vinblastine
(vinca alkaloid)

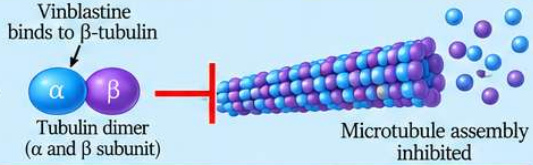
Anticancer use (Main utilization)

- Potent anticancer drug used in treatment of Hodgkin's lymphoma, non-Hodgkin's lymphoma, testicular cancer, breast cancer and other solid tumors.
- Used in combination chemotherapy regimens (e.g., MOPP, ABVD, CAP).
- Effective against rapidly dividing cancer cells.



Mechanism of action

- Binds to β-tubulin subunit of microtubules.
- Inhibits microtubule assembly and prevents polymerization.
- Causes mitotic arrest at metaphase and inhibits cell division.
- Leads to apoptosis of rapidly dividing cancer cells.



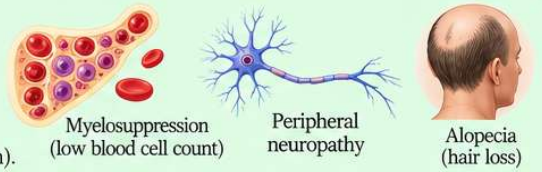
Clinical formulations and use

- Available as injection (vinblastine sulfate).
- Used in combination chemotherapy regimens such as MOPP (for Hodgkin's lymphoma) and other multi-drug regimens.
- Used for Hodgkin's and non-Hodgkin's lymphoma, testicular cancer, breast cancer, Kaposi's sarcoma and other solid tumors.



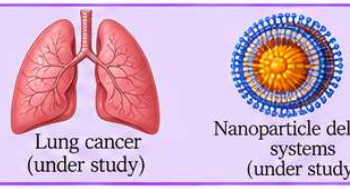
Adverse effects (Side effects)

- Myelosuppression (leukopenia, anemia, thrombocytopenia).
- Neurotoxicity: peripheral neuropathy (less than vincristine).
- Gastrointestinal effects: nausea, vomiting, constipation.
- Alopecia (hair loss).
- SIADH (syndrome of inappropriate antidiuretic hormone secretion).



Other potential uses (Under study)

- Investigated for use in other solid tumors (e.g., lung cancer, prostate cancer).
- Studied in combination with targeted therapies and immunotherapy.
- Explored for nanoparticle and liposomal delivery systems to reduce toxicity.
- Potential role in anti-angiogenic therapy (under study).



Mr. Samarpan Mishra (Assistant Professor) Specialization-Pharmaceutical Chemistry

Thank you

www.pharmrecord.com