NOTESKARTS

www.noteskarts.com

PHARMACOGNOSY & PHYTOCHEMISTRY

UNIT – 4: INDUSTRIAL PRODUCTION, ESTIMATION & UTILIZATION

B. Pharmacy | 5th Semester |

- ★ Forskolin
- ★ Sennoside
- ★ Artemisinin
- ★ Diosgenin
- ★ Digoxin
- ★ Atropine
- ★ Podophyllotoxin
- ★ Caffeine
- ★ Taxol (Paclitaxel)
- ★ Vincristine
- ★ Vinblastine

INTRODUCTION

Industrial production of phytoconstituents involves large-scale extraction, purification, and processing of pharmacologically active compounds from plants.

The goal is to obtain pharmaceutical-grade material in commercially viable quantities with consistent quality and purity. This unit covers three aspects for each compound:

(1) Industrial Production – large-scale manufacturing processes.

(2) Estimation – quantitative analytical methods used in quality control.

(3) Utilization – therapeutic, commercial, and other applications.

CONCEPTS IN INDUSTRIAL PHYTOCHEMICAL PRODUCTION

- **SCALE-UP CHALLENGES:** Lab-scale isolation vs industrial-scale production differ in equipment, solvent recovery, energy efficiency, and yield optimization
- **GOOD MANUFACTURING PRACTICES (GMP):** All pharmaceutical-grade phytoconstituents must be produced under GMP (Schedule M, Drugs & Cosmetics Act in India; ICH Q7 globally)
- **STANDARDISATION:** Products standardised for content of marker compounds using validated HPLC or other pharmacopoeial methods
- **SUSTAINABILITY:** Over-exploitation of wild plant sources is a major concern; cultivation programs, plant cell/tissue culture, and semi-synthesis are alternative strategies
- **PLANT CELL CULTURE:** Used for Taxol (Taxus), Vinblastine/Vincristine (Catharanthus), Shikonin – when plant source is rare or slow-growing
- **SEMI-SYNTHESIS:** Chemical modification of readily available natural products to obtain rare/expensive ones (e.g., Diosgenin → Progesterone → oral contraceptives; 10-DAB → Paclitaxel)

FORSKOLIN

▶ A. Chemical Profile & Source

Source: Coleus forskohlii Briq. (Plectranthus barbatus); Family: Lamiaceae; Part: Tuberous roots (richest source ~0.1–1.5% Forskolin)

Chemical Class: Labdane-type diterpenoid; complex polycyclic diterpene with a unique bicyclo[3.2.1]octane core fused with a cyclohexane ring; C20 terpenoid

Chemical Formula: $C_{22}H_{34}O_7$; MW: 410.50; melting point 228–230°C; $[\alpha]D^{20} = -30°$ (in $CHCl_3$)

Imp Feature: Contains a unique cyclopentane ring with hydroxyl and acetoxy groups; only natural direct activator of adenylate cyclase (AC) enzyme; raises intracellular cAMP levels

Biosynthesis: MEP pathway → GGPP (C20) → Copalyl diphosphate → Manool → Forskolin via CYP450-mediated oxidations; biosynthetic genes cloned in C. forskohlii

▶ B. Industrial Production

INDUSTRIAL PRODUCTION PROCESS

Step 1: CULTIVATION: Coleus forskohlii cultivated in hot, dry climates; India major producer (Rajasthan, Gujarat, MP, Andhra Pradesh); tuberous roots harvested after 6–8 months

Step 2: HARVESTING & DRYING: Roots dug out mechanically; washed; sun-dried or dried in forced-air ovens at <50°C to preserve diterpene content; coarse powdering (20 mesh)

Step 3: EXTRACTION: Dried root powder extracted with non-polar solvents – DICHLOROMETHANE or ETHANOL (Soxhlet or Percolation tanks, 6–12 hours); Forskolin is moderately polar diterpene

Step 4: DEFATTING: Extract concentrated; suspended in water; partitioned with petroleum ether to remove waxes and chlorophyll; petroleum ether layer discarded

Step 5: LIQUID-LIQUID PARTITIONING: Aqueous suspension extracted with ETHYL ACETATE or n-BUTANOL; EtOAc layer enriched in Forskolin

Step 6: INDUSTRIAL COLUMN CHROMATOGRAPHY: Large-scale silica gel columns (up to 500 kg scale); gradient elution Hexane:Ethyl acetate (4:1 → 2:1 → 1:1); Forskolin elutes at ~40–50% EtOAc

Step 7: RECRYSTALLISATION: Fractions concentrated; recrystallised from ethanol/water or acetone/hexane; colourless prisms formed; filtration and drying at 50°C under vacuum

Step 8: STANDARDISED EXTRACT (Commercial): Industrial production often targets standardised extracts containing 10–40% Forskolin; tested by HPLC; packed under nitrogen to prevent oxidation

Step 9: YIELD: ~0.5–1.0% Forskolin from dry root; 10–40% standardised extract after concentration

▶ C. Estimation (Quality Control & Analysis)

Method	Details
HPLC (Primary, Official)	Reverse-phase C18 column (250×4.6 mm); Mobile: Acetonitrile:Water (55:45); UV detection 210 nm (no strong chromophore); Flow: 1 mL/min; External standard calibration; Purity NLT 95% (pharma grade)
HPLC-ELSD	Evaporative Light Scattering Detector – more sensitive for weakly UV-absorbing diterpenes; used for plant extract profiling
TLC-Densitometry	Silica gel 60 F_{254}; Hexane:EtOAc (6:4); Spray: Anisaldehyde-H_2SO_4; Densitometry at 520 nm; semi-quantitative; useful for screening
UV Spectrophotometry	Dissolved in EtOH; absorbance at 210–215 nm (very weak); less reliable for crude extracts
^{1}H-NMR Quantification	qNMR using dimethyl sulphone internal standard in $CDCl_3$; absolute quantification for reference standard certification

▶ D. Utilization

UTILIZATION & APPLICATIONS

- **CARDIOVASCULAR:** Direct adenylyl cyclase activator → ↑cAMP → vasodilation, positive inotrope; used in Cardiomyopathy (IV Colforsin Daropate Hydrochloride in Japan – Adehl®)
- **GLAUCOMA:** Topical Forskolin eye drops → ↑cAMP in ciliary epithelium → reduce intraocular pressure; alternative to beta-blockers
- **OBESITY/WEIGHT MANAGEMENT:** ↑cAMP → activates hormone-sensitive lipase → fat breakdown; Forskolin dietary supplements (Coleus forskohlii extract 10–20% Forskolin)
- **ASTHMA & RESPIRATORY:** ↑cAMP → bronchial smooth muscle relaxation; experimental use in COPD
- **RESEARCH TOOL:** Gold standard for cAMP pathway activation in cell biology research; used to study G-protein coupled receptor signalling
- **THYROID DISORDERS:** Stimulates thyroid hormone synthesis and release via ↑cAMP in thyroid cells
- **COSMETICS:** Used in skin firming products; ↑cAMP stimulates lipolysis in adipocytes.

2. SENNOSIDE

▶ A. Chemical Profile & Source

Source: Leaves and pods of Cassia senna/C. angustifolia (Tinnevelly Senna, India) and C. acutifolia (Alexandrian Senna, Egypt); Family: Fabaceae

Chemical Class: Anthraquinone glycosides – specifically DIANTHRONE O-glycosides (Sennoside A & B are major; also C & D)

Chemical Formula: Sennoside A: $C_{42}H_{38}O_{20}$; MW: 862.7; Sennoside B: $C_{42}H_{38}O_{20}$; MW: 862.7 (meso-form stereoisomer of A)

Key Feature: Prodrugs: Sennosides hydrolysed by intestinal bacteria to Sennidins → Rhein-anthrone (active cathartic agent)

IP Standard: Senna leaf NLT 2.5% total sennosides (as sennoside B); Standardised senna extract: NLT 8.8% sennosides

▶ B. Industrial Production

INDUSTRIAL PRODUCTION PROCESS

Step 1: CULTIVATION: C. senna (angustifolia) cultivated in India (Tirunelveli/Tinnevelly, Tamil Nadu – world leader); semi-arid conditions; rain-fed or irrigated; harvested 2–3 times/year

Step 2: HARVESTING: Leaves hand-picked or mechanically stripped; pods harvested when dark brown; dried in shade or at 40°C; moisture content NMT 10%

Step 3: AQUEOUS EXTRACTION: Dried leaves/pods extracted with HOT WATER (60–70°C, not boiling to prevent degradation) or dilute ethanol (20–30%); counter-current extraction in industrial extractors

Step 4: CLARIFICATION & FILTRATION: Extract filtered through centrifuges or plate-and-frame filters; flocculants (bentonite) may be added to remove protein/pectin impurities

Step 5: CONCENTRATION: Filtered extract concentrated in multi-effect evaporators under vacuum (below 60°C) to a thick syrup or paste

Step 6: PRECIPITATION: Concentrated extract acidified to pH 2–3 with HCl or H_2SO_4 → Sennosides precipitate as insoluble free acids; alternatively, methanol/ethanol added (antisolvent precipitation) → crystallisation of sennosides

Step 7: FILTRATION & WASHING: Precipitate collected by vacuum/centrifugal filtration; washed with cold water; dried at 50–60°C under vacuum

Step 8: STANDARDISATION: Dried crude sennoside content determined by HPLC; blended if needed to achieve target specification (NLT 60% or 95% sennoside content depending on grade)

Step 9: SPRAY DRYING (for powder): Concentrated extract or standardised dispersion spray-dried with carriers (maltodextrin, lactose) to produce free-flowing standardised senna extract powder

Step 10: SCALE: India produces ~10,000+ metric tonnes senna leaf annually; Tirunelveli is the senna capital of the world

▶ C. Estimation (Quality Control & Analysis)

Method	Details
HPLC (IP/WHO – Gold Standard)	C18 reverse-phase column; Mobile: MeOH:Phosphate buffer pH 6.5 (45:55); UV 254 nm; Sennoside A and B resolved and quantified separately; NLT 2.5% in leaves; NLT 8.8% in standardised extract; linearity 50–500 µg/mL
SPECTROPHOTOMETRIC (Magnesium Acetate Method – IP)	Sennosides extracted and hydrolysed to anthraquinone aglycones; dissolved in MgOAc solution; pink/red colour measured at 515 nm; results expressed as sennoside B equivalent; suitable for quality control screening
TITRIMETRIC	Not commonly used – spectrophotometric and HPLC preferred
TLC	Silica gel; $CHCl_3$:MeOH:Water (70:30:4); Detection: NaOH spray → Red (anthraquinone glycosides); Rf Sennoside A ≈ 0.2, Sennoside B ≈ 0.3; used for identity confirmation

▶ D. Utilization

UTILIZATION & APPLICATIONS

- **STIMULANT LAXATIVE (CATHARTIC):** Sennoside A & B → Rhein-anthrone (via gut bacteria) → stimulates large intestinal peristalsis + inhibits Na^+/Cl^- absorption; acts in 6–12 hours
- **CONSTIPATION:** Most widely prescribed and OTC senna products (Senokot®, Ex-Lax Gentle Strength); short-term use recommended
- **BOWEL PREPARATION:** Pre-colonoscopy and pre-surgical bowel cleansing (combined with polyethylene glycol)
- **HEPATIC ENCEPHALOPATHY:** Sennosides reduce ammonia-producing intestinal bacteria; used with lactulose
- **HAEMORRHOIDS & ANAL FISSURES:** Soft stool facilitation reduces straining

- **PAEDIATRIC USE:** Sennoside syrup for children (age-appropriate doses)
- **EXPORT:** India exports substantial quantities of crude senna and standardised extracts globally (one of the largest phytochemical exports from India)

ARTEMISININ (Qinghaosu)

▶ A. Chemical Profile & Source

Source: Artemisia annua Linn. (Sweet Wormwood / Qinghao); Family: Asteraceae; Part: Leaves & flowering tops (0.01–0.8% Artemisinin)

Chemical Class: Sesquiterpene lactone endoperoxide; C15 terpenoid with a unique 1,2,4-trioxane (endoperoxide) ring

Chemical Formula: $C_{15}H_{22}O_5$; MW: 282.33; MP 152–157°C; $[\alpha]D^{20} = +66°$

Nobel Prize: Tu Youyou (Nobel Prize 2015) for discovering Artemisinin as antimalarial

Derivatives: Artesunate (water-soluble), Artemether (oil-soluble), Arteether – all semi-synthetic; Dihydroartemisinin (DHA) – key intermediate

▶ B. Industrial Production

INDUSTRIAL PRODUCTION PROCESS

Step 1: CULTIVATION: A. annua cultivated in China (Chongqing, Sichuan, Yunnan), Vietnam, East Africa (Tanzania, Kenya), India (Chhattisgarh, MP); high-yielding hybrid varieties (MEDIPLANT hybrids) developed; yields 1.5–2% artemisinin

Step 2: HARVESTING: Leaves + flowering tops harvested just before/at early flower stage (max. artemisinin); dried quickly at <40°C (endoperoxide heat-sensitive); moisture NMT 12%

Step 3: SOLVENT EXTRACTION (Industrial): Dried material extracted with n-HEXANE or PETROLEUM ETHER in stainless steel Soxhlet/counter-current extractors; Hexane selective for artemisinin; extract is green/yellow

Step 4: CONCENTRATION: Hexane extract evaporated using multi-stage falling-film evaporators under vacuum at <40°C; waxy green residue

Step 5: DEFATTING: Residue triturated with cold petroleum ether (40–60°C) or hexane to remove waxes; filtered; residue further processed

Step 6: COLUMN CHROMATOGRAPHY (Industrial scale): Large-scale silica gel columns (hundreds of kg); isocratic or gradient hexane:ethyl acetate; Artemisinin elutes at ~10–20% EtOAc

Step 7: CRYSTALLISATION: Column fractions concentrated; crystallised from acetone/hexane or ethyl acetate/hexane at 0–5°C; white prismatic crystals (MP 152–157°C); centrifuged and dried

Step 8: SEMI-SYNTHESIS TO DERIVATIVES: (a) Artemisinin + $NaBH_4$ (reduction) → Dihydroartemisinin (DHA); (b) DHA + Succinic anhydride → Artesunate (hemisuccinate ester, water-soluble); (c) DHA + Methanol + $BF_3 \cdot OEt_2$ → Artemether; Industrial API synthesis in dedicated pharmaceutical plants

Step 9: YIELD & SCALE: Global artemisinin production ~200–300 tonnes/year; single commercial plant processes 1,000+ tonnes of plant biomass

▶ C. Estimation (Quality Control & Analysis)

Method	Details
HPLC-UV (WHO/IP)	C18 column; Acetonitrile:0.01M H_3PO_4 (60:40); UV 210 nm (weak chromophore); Flow 1 mL/min; Retention time vs reference; NLT 98% purity (API); Linearity 50–500 µg/mL; sensitive baseline required
HPLC-ELSD	Better sensitivity than UV for artemisinin due to its minimal UV absorption; Evaporative Light Scattering Detector; used for plant extract quantification
$NaBH_4$ Colorimetric	Artemisinin reduced by $NaBH_4$ → DHA; treated with p-DMAB (p-dimethylaminobenzaldehyde) + HCl → Pink at 535 nm; used in field-deployable quality control
GC-MS (after derivatisation)	Trimethylsilyl (TMS) or acetyl derivative; FID detection; confirms molecular weight and fragmentation pattern m/z 282 (M^+)
qNMR	Quantitative ^{1}H-NMR; ^{1}H peak at δ 5.8 ppm (H-12 hemiacetal); internal standard dimethyl sulphone; reference standard characterisation

▶ D. Utilization

UTILIZATION & APPLICATIONS

- **ANTIMALARIAL (PRIMARY USE):** WHO first-line treatment for Plasmodium falciparum (including drug-resistant strains) via ACTs (Artemisinin-based Combination Therapies)
- **WHO-RECOMMENDED ACTs:** Artemether + Lumefantrine (Coartem®/Riamet®) – most used globally; Artesunate + Amodiaquine; Artesunate + Mefloquine; Artesunate + Sulfadoxine-Pyrimethamine
- **SEVERE MALARIA:** Artesunate IV/IM (first-line WHO 2011) replaced Quinine for cerebral and severe malaria; faster parasite clearance
- **MECHANISM:** Endoperoxide reacts with Fe^{2+} (haem) → carbon-centred radicals → alkylate parasite proteins → parasite death; also targets cancer cells (high intracellular iron)
- **ANTICANCER (Emerging):** Artemisinin and DHA induce apoptosis in cancer cells via ROS generation; in clinical trials for breast, prostate, leukaemia
- **ANTI-SCHISTOSOMAL & ANTI-PARASITIC:** Active against Schistosoma, Toxoplasma, Leishmania
- **ANTI-INFLAMMATORY:** Inhibits NF-κB pathway; potential in autoimmune conditions
- **MARKET:** Global ACT market ~US$300–400 million annually; India manufactures and exports artesunate and artemether combinations

DIOSGENIN

▶ A. Chemical Profile & Source

Source: Wild yam tubers of Dioscorea deltoidea (Himalayan), D. floribunda & D. composita (Mexican); Family: Dioscoreaceae; Diosgenin = aglycone of Dioscin saponin

Chemical Class: Spirostane-type steroidal sapogenin; C27 steroid with a spiro-ring (F-ring, cyclopentane-tetrahydrofuran spiro); closely resembles steroid skeleton

Chemical Formula: $C_{27}H_{42}O_3$; MW: 414.62; MP 204–207°C; $[\alpha]D^{20} = -129°$ (in $CHCl_3$)

Significance: Starting material for Marker degradation → semi-synthesis of progesterone, cortisone, oral contraceptives, androgens; Russell Marker's discovery (1940s) revolutionised steroid hormone industry

Saponin Content: D. floribunda/D. composita rhizomes: 5–8% dioscin (yields 2–4% diosgenin after hydrolysis); D. deltoidea: 2–3%

▶ B. Industrial Production

INDUSTRIAL PRODUCTION PROCESS

Step 1: CULTIVATION & WILD HARVESTING: Mexico (D. composita, D. floribunda) – plantation and wild harvest; India (D. deltoidea – Himalayan yam, Uttarakhand, HP, J&K); 3–5 year growing cycle before harvest

Step 2: HARVESTING: Rhizomes/tubers dug by mechanical excavators or manually; washed; chipped or sliced to increase surface area; dried at 60°C

Step 3: ACID HYDROLYSIS (Key Industrial Step): Dried chips extracted directly with DILUTE SULPHURIC ACID (1–5% H_2SO_4) under pressure (autoclave, 120°C, 2–3 hours) – hydrolyses saponin glycosides → Diosgenin liberated from sugar moiety; alternatively: enzyme hydrolysis (cellulase + pectinase + β-glucosidase cocktail) at 50°C

Step 4: NEUTRALISATION: Hydrolysed slurry neutralised with $NaOH/Ca(OH)_2$; pH adjusted to 6.5–7.0

Step 5: SOLVENT EXTRACTION: Neutralised slurry extracted with PETROL ETHER or n-HEXANE (3–4 times); Diosgenin dissolved in organic layer; steroidal sapogenin is lipophilic

Step 6: WASHING & DECOLOURING: Hexane extract washed with NaOH (0.5%) to remove acidic impurities; then water; treated with activated carbon at 60°C; filtered

Step 7: EVAPORATION: Solvent recovered by distillation; crude diosgenin residue (waxy solid)

Step 8: RECRYSTALLISATION: Crude diosgenin dissolved in hot methanol or acetone; cooled to −10°C; white/off-white crystals; filtered; dried at 60°C under vacuum

Step 9: MARKER DEGRADATION (Industrial Semi-synthesis to Progesterone): Diosgenin → Diosone (C22 cleavage by oxidation) → Pregnane derivative → Progesterone (via 5 chemical steps, Russell Marker, 1940s); Modern plants perform this in dedicated synthesis suites

Step 10: SCALE: Mexico, India and China largest producers; world diosgenin production ~2,000–3,000 tonnes/year

▶ C. Estimation (Quality Control & Analysis)

Method	Details
HPLC (Primary)	C18 column; Mobile: MeOH:Water (95:5) or isocratic MeOH; UV 210 nm (weak absorption of steroid); external standard; Purity NLT 95–98% (industrial grade)

LIEBERMANN-BURCHARD COLORIMETRIC	Diosgenin dissolved in $CHCl_3$; add LB reagent ($Ac_2O + H_2SO_4$) → Blue-green → measure at 610–620 nm; calibration curve; used for crude extract screening
GC METHOD	After acetylation/TMS derivatisation; GC-FID or GC-MS; m/z 414 (M^+); identity and purity confirmation; used in regulatory submissions
GRAVIMETRIC	Crude plant extract hydrolysis; hexane extraction; evaporation; weigh diosgenin residue; rough purity estimate; used in agriculture/plant breeding
TLC Densitometry	Silica gel; $CHCl_3$:MeOH (95:5); LB reagent spray → blue-green; densitometry at 620 nm; batch screening

▶ D. Utilization

UTILIZATION & APPLICATIONS

- **PRECURSOR FOR STEROID HORMONE SEMI-SYNTHESIS (Primary Industrial Use):** Diosgenin → Progesterone (Marker degradation) → Pregnenolone; From Progesterone: Testosterone, Oestradiol, Norethindrone (oral contraceptive), Spironolactone, Hydrocortisone, Dexamethasone, Betamethasone – virtually all pharmaceutical steroids
- **ORAL CONTRACEPTIVES:** Norethindrone and norgestrel (first oral contraceptives) were derived from Diosgenin; 'The Pill' revolutionised women's reproductive health globally
- **ANTI-INFLAMMATORY CORTICOSTEROIDS:** Cortisone, Prednisolone, Dexamethasone – all originally synthesised from Diosgenin
- **ANDROGEN & ANABOLIC STEROIDS:** Testosterone, Nandrolone derived from diosgenin intermediate
- **DIRECT DIOSGENIN USES:** Antidiabetic (PPAR-γ agonist, adipogenesis regulation), Anticancer, Antifungal, Anti-inflammatory
- **COSMETICS:** Creams containing Dioscorea/wild yam extract marketed as 'natural progesterone creams' (though diosgenin is NOT converted to progesterone in the human body without chemical synthesis)

DIGOXIN

▶ A. Chemical Profile & Source

Source: Digitalis lanata Ehrh. (primary industrial source); D. purpurea Linn. (secondary); Family: Scrophulariaceae; Part: Dried leaves

Chemical Class: Cardenolide-type cardiac glycoside; Aglycone (Digoxigenin, $C_{23}H_{34}O_5$) + 3 × Digitoxose (2,6-dideoxy sugar); contains 5-membered α,β-unsaturated (butenolide) lactone ring

Chemical Formula: $C_{41}H_{64}O_{14}$; MW: 780.94; MP 235–240°C; $[\alpha]D^{20} = +9.5°$ (in pyridine)

Narrow TI: Therapeutic index is VERY NARROW; serum level: 0.8–2.0 ng/mL; toxicity above 2 ng/mL; TDM essential

IP Standard: Digoxin tablets: NLT 90.0% and NMT 110.0% of stated amount; IP purity: NLT 95.0%

▶ B. Industrial Production

INDUSTRIAL PRODUCTION PROCESS

Step 1: CULTIVATION: D. lanata cultivated on a large scale in European countries (Hungary, Germany, Netherlands, Austria) and India (Nilgiris in Tamil Nadu, Himachal Pradesh) under controlled conditions; 2-year biennial cycle

Step 2: HARVESTING: Leaves harvested in the second year (higher glycoside content); harvested in summer; dried quickly at 60°C to arrest enzymatic degradation (beta-glucosidase enzymes in plant can degrade glycosides if drying is slow)

Step 3: EXTRACTION: Dried coarsely powdered leaves extracted with 70–80% ETHANOL or METHANOL in industrial percolators or counter-current extractors; glycosides are polar and dissolve in aqueous alcohol

Step 4: LEAD ACETATE PRECIPITATION: Ethanolic extract treated with lead acetate solution → precipitates tannins, proteins, resins; filtered (lead acetate precipitate discarded); lead ions removed from filtrate by H_2S or Na_2SO_4

Step 5: CONCENTRATION: Clarified extract evaporated under vacuum at 50°C; aqueous concentrate obtained

Step 6: CHLOROFORM EXTRACTION: Aqueous concentrate extracted with chloroform ($CHCl_3$) 3–4 times; $CHCl_3$ dissolves cardiac glycosides (moderate polarity); combined $CHCl_3$ layers

Step 7: ENZYMATIC PARTIAL HYDROLYSIS (Digilanidase): In industry, Lanatoside C (primary glycoside of D. lanata) treated with DIGILANIDASE enzyme (acetylesterase) → removes one acetyl glucose → yields DESLANIOSIDE; then mild acid hydrolysis (0.02M HCl, 60°C) → removes one more glucose → DIGOXIN; This enzymatic approach gives higher purity than direct chemical hydrolysis

Step 8: CHROMATOGRAPHIC PURIFICATION: Industrial preparative HPLC or large-scale silica gel column chromatography; $CHCl_3$:MeOH (9:1 → 85:15) elution; Digoxin fractions collected

Step 9: RECRYSTALLISATION: From dilute ethanol or methanol-water (9:1); white microcrystalline powder; filtered; dried at 60°C; MP 235–240°C confirms identity

▶ C. Estimation (Quality Control & Analysis)

Method	Details
HPLC (USP/BP – Official)	C18 column (250×4.6 mm); Mobile: MeOH:Acetonitrile:Water (30:10:60); UV 220 nm (cardenolide lactone chromophore); external standard; Digitoxin as related substance; Digoxin NLT 95%; Linearity 5–100 µg/mL

IMMUNOASSAY (FPIA/CLIA – For TDM)	Fluorescence Polarisation Immunoassay (FPIA) or Chemiluminescence Immunoassay (CLIA) for plasma Digoxin monitoring (0.8–2.0 ng/mL); high-throughput; automated analyser (Abbott, Roche)
UV Spectrophotometry	Raymond's reagent (m-dinitrobenzene + KOH) → violet-red colour specific for cardenolides; Absorbance at 480–490 nm; quantified against standard curve; used for crude extract screening
Kedde's Reagent Test	3,5-Dinitrobenzoic acid in NaOH → red-violet with cardenolide lactone; qualitative identification
TLC	Silica gel; $CHCl_3$:MeOH (9:1); Spray: Kedde's reagent → red-violet spots; $SbCl_3$ → coloured spots; Rf Digoxin ≈ 0.35–0.45

▶ D. Utilization

UTILIZATION & APPLICATIONS

- **CONGESTIVE HEART FAILURE (CHF):** Positive inotrope – inhibits Na^+/K^+-ATPase → intracellular Ca^{2+} ↑ → stronger cardiac contraction → improved cardiac output
- **ATRIAL FIBRILLATION (AF):** Rate control – slows AV nodal conduction (vagomimetic effect) → reduces ventricular rate in AF; used when beta-blockers contraindicated
- **ATRIAL FLUTTER:** Converts to AF or sinus rhythm; rate control
- SUPRAVENTRICULAR TACHYCARDIA (SVT): Digoxin slows conduction; used in neonatal SVT
- **DOSAGE FORMS:** Digoxin tablets (0.0625, 0.125, 0.25 mg), Paediatric elixir (0.05 mg/mL), IV injection (0.25 mg/mL); narrow TI requires precise dosing
- **ANTIDOTE:** DigiFab/Digibind (Digoxin-specific antibody Fab fragments) for acute digoxin toxicity
- **MARKET:** Generic digoxin; still widely used despite newer heart failure drugs; essential medicine (WHO EML)

ATROPINE

▶ A. Chemical Profile & Source

Source: Primarily from Atropa belladonna (roots and leaves) and Datura stramonium; also Hyoscyamus niger; Natural form: L-Hyoscyamine → Racemises to DL-Atropine during extraction; Family: Solanaceae

Chemical Class: Tropane alkaloid; ester of Tropine (bicyclic aminoalcohol) + Tropic acid; tertiary amine; pKa 9.7

Chemical Formula: Atropine: $C_{17}H_{23}NO_3$; MW: 289.37; Atropine Sulfate: $C_{34}H_{48}N_2O_6 \cdot H_2SO_4$; MW: 694.83; MP 115–118°C (free base)

Property: Competitive antagonist at all muscarinic acetylcholine receptors (M1–M5); selective for muscarinic over nicotinic receptors; crosses BBB (tertiary amine)

Biosynthesis: Ornithine → Putrescine → N-Methylputrescine → N-Methyl-Δ^1-pyrrolinium → Tropinone → Tropine + Tropic acid → L-Hyoscyamine → Atropine (racemisation during isolation)

▶ B. Industrial Production

INDUSTRIAL PRODUCTION PROCESS

Step 1: CULTIVATION: Atropa belladonna cultivated in Europe (UK, Germany, India – Himachal Pradesh, J&K at altitudes 800–2000 m); also Bulgaria, Hungary; 2–3 year perennial; leaves, stems, and roots harvested

Step 2: INDUSTRIAL EXTRACTION: Dried coarsely ground root/leaf extracted with METHANOL or DILUTE H_2SO_4 (pH 2–3) in stainless steel percolators; alkaloids extracted as salts

Step 3: LEAD SUBACETATE DEPROTEINISATION: Extract treated with lead subacetate → removes tannins/resins (precipitation); excess lead removed with H_2S; filtered

Step 4: BASIFICATION & SOLVENT EXTRACTION: Filtrate basified with NH_3 or NaOH (pH 10–11); free alkaloid bases extracted with CHLOROFORM or TOLUENE (3–4 times in industrial mixer-settlers)

Step 5: ACID STRIPPING (Purification): $CHCl_3$ extract shaken with 5% H_2SO_4 → alkaloids into aqueous acid layer; waxes and fats remain in $CHCl_3$; aqueous layer collected

Step 6: RE-BASIFICATION: Aqueous H_2SO_4 layer basified; re-extracted with $CHCl_3$ → purer alkaloid in organic phase

Step 7: CHROMATOGRAPHY: Industrial alumina or silica columns; $CHCl_3$:MeOH gradient; Atropine collected first (tertiary, less polar); Scopolamine elutes later

Step 8: SALT FORMATION: Free atropine base dissolved in ethanol; precise amount of dilute H_2SO_4 added (2 equivalents); Atropine sulfate crystallises from ethanol-ethyl acetate; filtered; dried

Step 9: ALSO: Atropine synthesised CHEMICALLY (total synthesis) on industrial scale using Tropine + Tropic acid (esterification) as route alternative to plant extraction; countries with restricted belladonna cultivation prefer synthetic route

▶ C. Estimation (Quality Control & Analysis)

Method	Details
HPLC (BP/IP Official)	C18; Mobile: Acetonitrile:0.01M KH_2PO_4 pH 3 (25:75); UV 210 nm; Scopolamine (related substance) separated; NLT 98.5% atropine sulfate; Linearity 10–200 µg/mL
NON-AQUEOUS TITRATION (BP/IP)	Atropine sulfate dissolved in glacial acetic acid + mercury(II) acetate; titrated with 0.1M $HClO_4$; Crystal violet indicator or potentiometric endpoint; 1 mL 0.1M $HClO_4$ = 17.42 mg atropine sulfate; direct, rapid, official
UV Spectrophotometry	In 0.1N H_2SO_4; λmax 251, 257, 262 nm (tropic acid phenyl ring); Less selective for crude extracts
Vitali-Morin Test (qualitative)	Evaporate with fuming HNO_3; dissolve in acetone + KOH → Violet colour; specific for tropane esters

GC-MS	After derivatisation; confirms identity and purity; used in forensic analysis and research

▶ D. Utilization

UTILIZATION & APPLICATIONS

- **OPHTHALMIC:** Atropine 1% eye drops/ointment → Cycloplegia and Mydriasis for eye examination; retinoscopy; penalisation therapy for amblyopia (lazy eye) – Atropine 0.01% for myopia control
- **BRADYCARDIA & CARDIAC ARREST:** Atropine IV → Increases heart rate (blocks vagal tone); used in symptomatic sinus bradycardia, AV block (Mobitz I/II); ACLS protocol for bradycardia
- **ORGANOPHOSPHATE/CARBAMATE POISONING (Antidote):** Large doses of Atropine IV counteract excess ACh; used with pralidoxime (2-PAM); life-saving in OP insecticide poisoning
- **PRE-OPERATIVE MEDICATION:** Reduces secretions (saliva, bronchial) before anaesthesia; prevents vagal reflexes during surgery
- **ANTISPASMODIC:** Biliary colic, renal colic, intestinal hypermotility; hyoscine often preferred
- **ANTIDOTE FOR MUSHROOM POISONING (Muscarine type):** Clitocybe, Inocybe poisoning
- **BRONCHOSPASM:** Ipratropium bromide (quaternary analogue of atropine) – inhaled for COPD, asthma; Tiotropium (long-acting)
- **OVER-ACTIVE BLADDER:** Oxybutynin (synthetic semi-analogue) based on atropine pharmacophore.

PODOPHYLLOTOXIN

▶ A. Chemical Profile & Source

Source: Rhizomes and roots of P. emodi (Indian Himalayan Podophyllum – preferred, higher potency) and P. peltatum (American Mayapple); Family: Berberidaceae

Chemical Class: Aryltetralin cyclolignan; 4-stereocentre compound; γ-butyrolactone ring fused to aryltetrahydronaphthalene skeleton; phenylpropanoid dimer

Chemical Formula: $C_{22}H_{22}O_8$; MW: 414.41; MP 183–184°C; $[\alpha]D^{20} = -132°$ (in $CHCl_3$)

Conservation: P. emodi listed in Schedule VI of Wildlife Protection Act 1972 in India (restricted collection); also CITES Appendix II listed; cultivation programs needed

Derivatives: Etoposide (VP-16), Teniposide (VM-26): Semi-synthetic glycoside derivatives from Podophyllotoxin; actual marketed anticancer drugs

▶ B. Industrial Production

INDUSTRIAL PRODUCTION PROCESS

Step 1: WILD HARVESTING / CULTIVATION: P. emodi wild-harvested in Himalayan forests (HP, Uttarakhand, J&K, Nepal) under forest permits; cultivation in research stations and agroforestry; P. peltatum: Appalachian regions, USA

Step 2: RAW MATERIAL PREPARATION: Rhizomes dug in autumn (post-seeding); cleaned; sliced; air-dried or oven-dried at 40–50°C; coarsely powdered

Step 3: ALCOHOL EXTRACTION: Powdered rhizome extracted in ETHANOL (70–95%) using stainless steel percolators or Soxhlet (industrial); repeated 3×; combined extracts filtered

Step 4: CONCENTRATION: Ethanol extract concentrated under vacuum (50°C, multi-effect evaporators) to ~1/10 original volume

Step 5: CHLOROFORM EXTRACTION: Concentrated extract diluted with water; $CHCl_3$ extraction (3×) in industrial mixer-settlers; $CHCl_3$ layer retains podophyllotoxin and related lignans; aqueous layer (sugars, proteins) discarded

Step 6: CHROMATOGRAPHIC SEPARATION (Industrial): Large preparative silica columns or industrial MPLC (Medium Pressure Liquid Chromatography); Hexane:Ethyl acetate gradient (7:3 → 1:1); Podophyllotoxin elutes at 40–50% EtOAc fraction

Step 7: SEPARATION FROM RELATED LIGNANS: Industrial HPLC (preparative) separates: α-Peltatin, β-Peltatin, 4'-Demethylpodophyllotoxin from Podophyllotoxin; purity >98% required for pharmaceutical use

Step 8: RECRYSTALLISATION: From ethanol or $CHCl_3$:MeOH; white needle crystals; vacuum dried at 40°C; packed under nitrogen (light-stable but protect from moisture)

Step 9: SEMI-SYNTHESIS OF ETOPOSIDE: Podophyllotoxin + 4'-Demethylation (BBr_3) → 4'-Demethylpodophyllotoxin; + Glucosylation (Lewis acid catalysed) + Acetylation/Deacetylation steps → Etoposide (VP-16); entire synthesis done in dedicated pharmaceutical synthesis plant

▶ C. Estimation (Quality Control & Analysis)

Method	Details
HPLC (Primary – Industry Standard)	C18 (250×4.6 mm); Mobile: MeCN:Water (50:50); UV 290 nm; All related lignans resolved; NLT 98% podophyllotoxin (pharma grade); Quantify α-Peltatin, β-Peltatin as impurities; Linearity 10–200 µg/mL
UV Spectrophotometry	λmax 290 nm in methanol (aromatic/lactone chromophore); ε ≈ 4,100 L/mol/cm; Beer-Lambert quantification; used for quick batch assessment
TLC-Densitometry	Silica gel; Hexane:EtOAc (1:1); Anisaldehyde-H_2SO_4 → Blue-grey; densitometric scan; ≤ 2% related substances limit
Optical Rotation	$[\alpha]D^{20} = -132° \pm 5°$ in $CHCl_3$; confirms identity and stereochemical purity; important QC parameter
^{1}H-NMR Quantification	qNMR in $CDCl_3$; H-1 at δ 4.6 ppm (diagnostic); confirms identity and purity; reference standard certification

▶ D. Utilization

UTILIZATION & APPLICATIONS

- **PODOPHYLLIN RESIN (10–25% solution):** Topical antimitotic for condylomata acuminata (venereal warts) and papillomas; office application by physician; removed after 1–6 hours
- **CONDYLINE/WARTICON (0.5% podophyllotoxin solution):** Self-applied topical for genital warts; more purified, safer than crude podophyllin; European markets
- **ETOPOSIDE (VP-16):** Semi-synthetic derivative; inhibits Topoisomerase II → DNA strand breaks → apoptosis; used for Testicular cancer, Small-cell lung cancer (SCLC), Lymphomas, Acute leukaemia, Kaposi's sarcoma
- **TENIPOSIDE (VM-26):** Related semi-synthetic; Childhood leukaemia, Brain tumours, Neuroblastoma
- **MECHANISM (ETOPOSIDE):** Stabilises Topo II-DNA cleavage complex → prevents re-ligation → DNA double-strand breaks (different from podophyllotoxin itself which inhibits tubulin assembly)
- **RESEARCH:** Podophyllotoxin scaffold used in drug discovery for development of new anticancer leads
- **CONSERVATION CONCERN:** P. emodi critically endangered in Himalayas due to over-exploitation; tissue culture and agroforestry programs critical for sustainable supply

CAFFEINE

▶ A. Chemical Profile & Source

Sources: Tea leaves (Camellia sinensis, 1–4%); Coffee beans (Coffea arabica, 1–2%; C. robusta, 2–3%); Cola nuts (Cola nitida, 2–4%); Guarana seeds (Paullinia cupana, 3–5%); Cocoa beans (Theobroma cacao, 0.1–0.5%)

Chemical Class: Methylxanthine (Purine alkaloid); 1,3,7-Trimethylxanthine; structurally related to Theophylline (1,3-dimethylxanthine) and Theobromine (3,7-dimethylxanthine)

Chemical Formula: $C_8H_{10}N_4O_2$; MW: 194.19; MP 235–237°C; sublimes at ~180°C; [α]D = 0° (achiral)

Biosynthesis: Xanthosine → 7-Methylxanthosine → 7-Methylxanthine → Theobromine → Caffeine (via N-methyltransferases using SAM as methyl donor)

Production Scale: Global caffeine production: ~120,000–150,000 tonnes/year; largely from decaffeination of tea/coffee and synthetic production

▶ B. Industrial Production

INDUSTRIAL PRODUCTION PROCESS

Step 1: SOURCE 1 – NATURAL EXTRACTION FROM TEA (traditional industrial route):

Step 2: Tea fannings/dust (lowest grade) extracted with HOT WATER at 80–90°C in countercurrent extractors; CaO or $Ca(OH)_2$ added to precipitate tannins as calcium tannate; caffeinated aqueous extract collected

Step 3: Caffeinated extract extracted with organic solvent (DICHLOROMETHANE or CHLOROFORM or ETHYL ACETATE) in industrial mixer-settlers; caffeine partitions into organic layer

Step 4: Solvent evaporated; crude caffeine paste obtained; further purified by SUBLIMATION (industrial sublimation chambers at 180°C, vacuum) or RECRYSTALLISATION from hot water or methanol

Step 5: SOURCE 2 – BY-PRODUCT OF DECAFFEINATION (Largest industrial source):

Step 6: Coffee beans or tea subjected to decaffeination: (a) Organic solvent method – CH_2Cl_2 extracts caffeine selectively; caffeine recovered from solvent by evaporation; (b) Supercritical CO_2 decaffeination – SC-CO_2 at 300 bar, 60–70°C extracts caffeine; (c) Swiss Water Process – hot water extraction

Step 7: Caffeine-rich solvent extract purified by crystallisation or preparative chromatography; pharmaceutical-grade caffeine obtained

Step 8: SOURCE 3 – SYNTHETIC PRODUCTION: Uric acid → Xanthine → Theophylline → Caffeine (via methylation); or direct synthesis from dimethyl urea; synthetic caffeine now dominant for pharmaceutical use

Step 9: PHARMACEUTICAL-GRADE PROCESSING: Crude caffeine purified by recrystallisation from hot water; sublimation gives highest purity (>99%); tested by IP/USP methods; granulation for tablet manufacture

▶ C. Estimation (Quality Control & Analysis)

Method	Details
HPLC (BP/USP Official)	C18; Mobile: 0.05M KH_2PO_4 (pH 4.5):MeOH (80:20) or Acetonitrile:0.1% H_3PO_4 (12:88); UV 254 nm (purine ring); NLT 98.5% (anhydrous basis); Related substances: theophylline, theobromine; Linearity 50–500 µg/mL
UV SPECTROPHOTOMETRY (Quick, Official)	Dissolve in 0.1N H_2SO_4; λmax 272 nm; ε = 9,800 L/mol/cm; Beer-Lambert law A = εcl; linear 5–50 µg/mL; Official IP method; highly specific
NON-AQUEOUS TITRATION	Too weakly basic for direct $HClO_4$ titration (pKa ~14); NOT used; UV preferred
Murexide Test (qualitative ID)	Caffeine + HNO_3 (evaporate) → residue + NH_3 → Purplish-red (Murexide); specific for purine alkaloids
GC-MS	After derivatisation if needed; m/z 194 (M^+), 109, 55; confirms identity

▶ D. Utilization

UTILIZATION & APPLICATIONS

- **CNS STIMULANT:** Competitive antagonist of adenosine receptors (A1 and A2A) → Alertness, reduced fatigue, improved concentration; most widely consumed psychoactive substance in world
- **ANALGESIC ADJUVANT:** Caffeine (65–100 mg) enhances efficacy of paracetamol, aspirin, ibuprofen by ~40%; present in many OTC combination analgesics (Combiflam Plus, Saridon)
- **NEONATAL APNOEA:** Caffeine citrate (Peyona®) – treatment of apnoea of prematurity; stimulates respiratory centre
- **DIURETIC (Mild):** Increases GFR and reduces tubular reabsorption; effect weak compared to furosemide

- **ERGOGENIC AID:** Improves endurance exercise performance; legal sports supplement (WADA removed from prohibited list in 2004)
- **HEADACHE/MIGRAINE:** Caffeine enhances analgesic effect; vasoconstriction (reverses cerebral vasodilation of migraine)
- **BRONCHODILATOR:** Theophylline-like (structurally related) effect; used in asthma historically
- **BEVERAGES INDUSTRY:** Tea, coffee, cola drinks, energy drinks (Red Bull, Monster); biggest commercial use globally
- **COSMETICS:** Topical caffeine in eye creams, anti-cellulite creams (stimulates lipolysis in adipocytes)
- **WEIGHT-LOSS SUPPLEMENTS:** Thermogenic effect; caffeine + ephedrine combination (now restricted)

TAXOL (PACLITAXEL)

▶ A. Chemical Profile & Source

Source: Bark of Taxus brevifolia (Pacific Yew, USA – original source); Taxus baccata (European Yew); Taxus wallichiana (Himalayan Yew, India); Currently: Needles (renewable) of Taxus spp. as primary source of 10-DAB for semi-synthesis

Chemical Class: Taxane diterpenoid alkaloid; C20 terpenoid; complex tricyclic ring system (6-8-6 ring system); 11 stereocentres; taxane + benzoyloxy and hydroxyl substituents

Chemical Formula: Paclitaxel: $C_{47}H_{51}NO_{14}$; MW: 853.91; MP 213–217°C; $[\alpha]D^{25} = -49°$ (in MeOH)

Key Mechanism: UNIQUE: Stabilises (not destabilises) microtubules → prevents mitotic spindle depolymerisation → metaphase arrest → apoptosis; Opposite of Vinca alkaloids

Market: Paclitaxel + Docetaxel global market ~US$2–3 billion/year; major anticancer drugs; Bristol-Myers Squibb (Taxol®); generic paclitaxel available

▶ B. Industrial Production

INDUSTRIAL PRODUCTION PROCESS

Step 1: SOURCE EVOLUTION: 1971: Paclitaxel isolated from bark of T. brevifolia (bark contains 0.01–0.05% paclitaxel; 6 large trees needed for 1 patient's treatment → unsustainable); 1992: FDA approved; immediately caused supply crisis

Step 2: SEMI-SYNTHETIC ROUTE (Current industry standard – >95% of production):

Step 3: 10-Deacetylbaccatin III (10-DAB) extracted from Taxus baccata/yunnanensis NEEDLES (renewable – can harvest repeatedly without killing tree; needles contain 0.1–0.2% 10-DAB)

Step 4: Needle extraction: Dried Taxus needles extracted with METHANOL or ETHANOL; concentrated; 10-DAB-rich fraction obtained by liquid-liquid partitioning; purified by chromatography

Step 5: CHEMICAL SEMI-SYNTHESIS: 10-DAB + Protecting groups (Bn or TBS) → protected 10-DAB; + Side chain (β-lactam route – Holton synthesis or Ojima β-lactam method) → esterification at C13; deprotection → Paclitaxel; 10–14 chemical steps; done in GMP pharmaceutical synthesis facility

Step 6: PLANT CELL FERMENTATION (PCF – Biotechnology route): T. chinensis cells grown in 75,000-litre bioreactors (Phyton Biotech – subsidiary of DFB Pharmaceuticals; also Novascreen); produces paclitaxel ~0.01–0.1 g/L of culture; cells fed with phenylalanine and other precursors; paclitaxel extracted from culture medium and cells; purified by preparative HPLC

Step 7: CHEMICAL TOTAL SYNTHESIS: Holton total synthesis (1994), Nicolaou total synthesis (1994), Danishefsky (1996) – all academically important but NOT industrially viable (>50 steps, low yield)

Step 8: FORMULATION: Paclitaxel dissolved in Cremophor EL (polyethoxylated castor oil):Ethanol (1:1) → diluted for IV infusion; Albumin-bound paclitaxel (nab-paclitaxel, Abraxane®) avoids Cremophor

▶ C. Estimation (Quality Control & Analysis)

Method	Details
HPLC (USP/BP Official)	C18 (250×4.6 mm); Mobile: MeCN:Water (45:55) or MeOH:Water (65:35); UV 227 nm (taxane chromophore); Related substances: 10-DAB, Baccatin III, 7-Epi-paclitaxel; NLT 97% purity API; Quantitative with external standard; Linearity 20–200 µg/mL
HPLC-MS (LC-MS)	Used in bioanalytical method for plasma paclitaxel pharmacokinetics; ESI-MS $[M+NH_4]^+$ = 872; sensitive (LOQ 1–5 ng/mL plasma); research and clinical PK studies
UV Spectrophotometry	λmax 227 nm in methanol; ε = 29,000 L/mol/cm; quick purity estimate for formulation QC
Optical Rotation	$[\alpha]D^{25}$ = −49° ± 3° in MeOH; confirms stereochemical integrity; critical QC parameter
NMR (^{1}H-NMR/^{13}C-NMR)	Complete spectral characterisation of reference standard; diagnostic peaks at δ 8.1 (benzoyl H), δ 5.8 (H-2'), δ 4.9 (H-5); impurity profiling

▶ D. Utilization

UTILIZATION & APPLICATIONS

- OVARIAN CANCER: Paclitaxel + Carboplatin (first-line platinum doublet); also second-line therapy
- BREAST CANCER: HER2+ (with Trastuzumab), Triple-negative (with Gemcitabine or Carboplatin), Hormone-sensitive; Dose-dense regimens
- NON-SMALL CELL LUNG CANCER (NSCLC): Paclitaxel + Carboplatin or Gemcitabine; Nab-paclitaxel + Carboplatin
- KAPOSI'S SARCOMA: AIDS-related KS refractory to anthracyclines
- GASTRIC CANCER: Second-line therapy

• nab-PACLITAXEL (Abraxane®): Albumin-bound; no Cremophor needed → no premedication; better tolerability; approved for pancreatic cancer (with Gemcitabine), breast cancer, NSCLC

• DOCETAXEL (semi-synthetic from 10-DAB with different side chain): Prostate cancer (Docetaxel + Prednisone), Breast, Gastric, Head & neck, NSCLC

• DRUG-ELUTING STENTS: Paclitaxel-coated coronary stents (Taxus® Express) – prevents restenosis after angioplasty

• COMBINATION REGIMENS: TC, AC-T, TCH, Gem-Abraxane – standard chemotherapy backbones

VINCRISTINE & VINBLASTINE (Vinca Alkaloids)

▶ A. Chemical Profile & Source

Source: Catharanthus roseus (Linn.) G.Don (Syn: Vinca rosea); Madagascar Periwinkle; Family: Apocynaceae; Parts: Leaves (primary) and roots

Chemical Class: Bisindole monoterpenoid indole alkaloids (MIAs); formed by coupling of two monomeric indole alkaloids: CATHARANTHINE + VINDOLINE → Bisindole alkaloids via peroxidase reaction

Vincristine (VCR): $C_{46}H_{56}N_4O_{10}$; MW: 824.95; $[\alpha]D^{25}$ = +17° (in MeOH); Leurocristine; N-formyl group on vindoline nitrogen

Vinblastine (VLB): $C_{46}H_{58}N_4O_9$; MW: 810.99; $[\alpha]D^{25}$ = +42° (in MeOH); Vincaleukoblastine; N-methyl group on vindoline nitrogen

Key Structural Difference: Vincristine has N-formyl (–CHO) at N1' position; Vinblastine has N-methyl ($–CH_3$); This tiny difference results in DIFFERENT CLINICAL USES and TOXICITY PROFILES

Mechanism: Both bind β-tubulin → DESTABILISE microtubules (opposite of Taxol) → Inhibit mitotic spindle formation → Metaphase arrest → Cell death; Vincristine more potent tubulin binder

Concentration in Plant: Vinblastine: 0.001–0.01% of dry leaf; Vincristine: 0.0001–0.001% (10–100× lower than VLB – hence much more expensive)

▶ B. Industrial Production

INDUSTRIAL PRODUCTION OF VINCRISTINE & VINBLASTINE

Step 1: CULTIVATION: C. roseus cultivated extensively in Tamil Nadu, Andhra Pradesh, Karnataka, West Bengal (India); also Madagascar, USA (Texas), Australia; tropical climate; annual or perennial; leaves harvested at full bloom (max. alkaloid content)

Step 2: LEAF HARVESTING: Leaves machine-harvested 3–4 times per year; dried rapidly at 50–60°C (enzymatic degradation risk); moisture NMT 8%; stored in moisture-proof bags; dried leaf yield: 5–6 tonnes/hectare/year

Step 3: LARGE-SCALE EXTRACTION: Dried leaf powder (tonnes scale) extracted with DILUTE H_2SO_4 (pH 2–3) or dilute acetic acid; all alkaloids dissolve as salts; or methanol extraction on large-scale extraction tanks; repeated extraction 3× counter-currently

Step 4: BASIFICATION & SOLVENT EXTRACTION: Aqueous acid extract basified with NH_4OH or NaOH (pH 8–9); free alkaloid bases extracted with BENZENE (historically) or modern alternatives: ETHYL ACETATE, TOLUENE, or CHLOROFORM; industrial mixer-settlers

Step 5: PRELIMINARY FRACTIONATION: The crude mixed alkaloid extract contains >100 alkaloids; separated by ACID-BASE FRACTIONATION: (a) Extract shaken with dilute tartaric acid → quaternary & less basic alkaloids stay in organic layer; more basic alkaloids (VLB, VCR) extracted into tartrate solution; (b) Tartrate solution basified → VLB and VCR back into organic phase

Step 6: INDUSTRIAL COLUMN CHROMATOGRAPHY (Multi-stage): (a) ALUMINA COLUMNS (basic Al_2O_3): $CHCl_3$ → $CHCl_3$:MeOH gradient; Vinblastine elutes first (less polar); (b) SILICA GEL COLUMNS: Further fractionation; (c) ION-EXCHANGE CHROMATOGRAPHY: Amberlite IRC-50 or similar for further purification

Step 7: PREPARATIVE HPLC (Final Purification): Industrial preparative HPLC systems (hundreds of kg scale); C18 or phenyl column; Acetonitrile:buffer gradient; Vincristine and Vinblastine separated to pharmaceutical purity (>95%)

Step 8: VINCRISTINE vs VINBLASTINE SEPARATION: Critical step – VCR is 10–100× rarer; Preparative HPLC essential for separation; slight difference in polarity (VCR = more polar due to formyl group); VCR elutes slightly before VLB on reverse-phase

Step 9: SALT FORMATION: VLB + H_2SO_4 → Vinblastine Sulfate (water-soluble); VCR + H_2SO_4 → Vincristine Sulfate; lyophilised for injection vials; sterility testing; endotoxin testing

Step 10: BIOTECHNOLOGY ALTERNATIVE – PLANT CELL CULTURE: Catharanthus roseus cells in bioreactor (50–75,000 litre scale); elicitation with methyl jasmonate or chitosan to boost alkaloid production; yields still ~10–100× lower than plant; research active area

Step 11: SEMI-SYNTHESIS: Catharanthine and Vindoline (more abundant monomers) coupled chemically/enzymatically → VLB (3–5% yield); VLB then formylated (selectively) → VCR (patented processes)

▶ C. Estimation – Quality Control

Method	Details for Vincristine / Vinblastine
HPLC (USP/BP – Official, Primary)	C18 (250×4.6 mm); Mobile: MeCN:Phosphate buffer pH 7.0 (38:62) or MeCN:ammonium acetate buffer gradient; UV 254 nm or 215 nm; VLB and VCR separated clearly (RT difference ~2–3 min); Vincristine sulfate NLT 98%; Vinblastine sulfate NLT 97%; External standard; Related substances: Catharanthine, Leurosine, Desacetyl VLB
UV Spectrophotometry (Identification)	λmax in MeOH: VLB 214 nm (ε=46,000) and 262 nm; VCR 214 nm and 262 nm; Similar spectra – cannot distinguish by UV alone; HPLC needed for differentiation
Optical Rotation	VLB: $[\alpha]D^{25}$ = +42° ± 3° (in MeOH); VCR: $[\alpha]D^{25}$ = +17° ± 2° (in MeOH); confirms stereochemical purity; significant QC parameter

TLC (Identity test)	Silica gel; $CHCl_3$:MeOH:NH_3 (85:14:1); UV 254 nm → dark quenching; Spray: Dragendorff's → Orange-red spots; VLB Rf ≈ 0.55; VCR Rf ≈ 0.45; compare reference
LC-MS/MS (Bioanalytical)	For pharmacokinetic studies; VLB $[M+H]^+$ = 811; VCR $[M+H]^+$ = 825; MRM transitions used; LOQ ~0.5–1 ng/mL plasma; TDM not routine but used in research
Protein Binding Assay	Both VLB and VCR >95% protein bound; important for pharmacokinetic interpretation; ultrafiltration + HPLC for free fraction determination

▶ D. Utilization

VINCRISTINE (VCR) – Uses	VINBLASTINE (VLB) – Uses
• **Acute Lymphoblastic Leukaemia (ALL):** DRUG OF CHOICE; cornerstone of ALL treatment protocols (VAMP, VAD regimens) • **Wilm's Tumour (nephroblastoma):** Standard of care with actinomycin-D • **Hodgkin's Lymphoma:** MOPP (Mustargen+VCR+Procarbazine+Pred) or ABVD alternatives • **Non-Hodgkin's Lymphoma:** CHOP regimen (Cyclophosphamide+Doxorubicin+VCR+Prednisone) • **Rhabdomyosarcoma & Neuroblastoma:** Paediatric solid tumours • **Brain Tumours:** Medulloblastoma protocols • Multiple Myeloma: VAD protocol (Vincristine+Adriamycin+Dexamethasone) • **TOXICITY:** Predominantly NEUROTOXIC (peripheral neuropathy, constipation, paralytic ileus, SIADH) – dose-limiting; less myelosuppression than VLB	• **Hodgkin's Lymphoma:** ABVD regimen (Adriamycin+Bleomycin+VLB+Dacarbazine) – STANDARD FIRST-LINE • **Testicular Cancer:** BEP (Bleomycin+Etoposide+Cisplatin) or VBP protocols; highly curative • **Breast Cancer:** CMF or MVAC-based regimens • **Kaposi's Sarcoma:** AIDS-related; antivascular effect • **Bladder Cancer:** MVAC (Methotrexate+VLB+Adriamycin+Cisplatin) • **Choriocarcinoma & Gestational Trophoblastic Tumours:** Combination regimens • **Histiocytosis X:** VLB effective • **TOXICITY:** Predominantly MYELOSUPPRESSIVE (neutropenia, thrombocytopenia) – dose-limiting; less neurotoxic than VCR; also alopecia, mucositis
SEMI-SYNTHETIC DERIVATIVES: Vinorelbine (Navelbine®) – 5'-nor-anhydro-VLB; non-small cell lung cancer, breast cancer; less neurotoxic; Vindesine (Eldisine®) – desacetyl VLB amide; leukaemia; Vinflunine (Javlor®) – fluorinated; bladder cancer	

EXAM-ORIENTED MCQs – PHARMACOGNOSY & PHYTOCHEMISTRY UNIT 4

Q1. Forskolin is a direct activator of which enzyme?

a) Phospholipase C b) Adenylate cyclase (Adenylyl cyclase) c) Protein kinase A d) Phosphodiesterase

✓ **Answer: b) Adenylate cyclase – Forskolin is the ONLY known direct natural activator of adenylyl cyclase → increases cAMP**

Q2. Industrial isolation of Sennoside uses which key step to precipitate them?

a) Basification to pH 10 b) Acidification to pH 2–3 c) Steam distillation d) Solvent fractionation with hexane

✓ **Answer: b) Acidification to pH 2–3 with HCl or H_2SO_4 → Sennosides precipitate as insoluble free acids**

Q3. The MOST SENSITIVE analytical method for Artemisinin estimation in plant extracts is:

a) UV spectrophotometry at 210 nm b) HPLC-ELSD c) GC-MS d) Titrimetry

✓ **Answer: b) HPLC-ELSD (Evaporative Light Scattering Detector) – most sensitive as artemisinin has minimal UV chromophore**

Q4. Diosgenin is converted to Progesterone industrially by which process?

a) Hydrolysis b) Oxidation c) Marker degradation d) Hydrogenation

✓ **Answer: c) Marker degradation – Russell Marker's 5-step chemical process converts Diosgenin → Progesterone**

Q5. Which enzyme is used in industrial production of Digoxin from Lanatoside C?

a) Beta-glucosidase b) Digilanidase (acetylesterase) c) Pectinase d) Lipase

✓ **Answer: b) Digilanidase (acetylesterase) – cleaves the acetyl glucose from Lanatoside C → Deslanioside → Digoxin**

Q6. The therapeutic index of Digoxin is:

a) Very wide (safe drug) b) Very narrow (0.8–2 ng/mL serum) c) Moderate d) Not required to monitor

✓ **Answer: b) Very narrow (0.8–2.0 ng/mL) – Therapeutic Drug Monitoring essential; toxicity above 2 ng/mL**

Q7. Industrial production of Paclitaxel (Taxol) currently uses which PRIMARY source material?

a) Taxus brevifolia bark (whole tree) b) Synthetic total synthesis c) 10-Deacetylbaccatin III (10-DAB) from Taxus needles for semi-synthesis d) Chemical synthesis from acetate

✓ **Answer: c) 10-DAB from renewable Taxus needles → semi-synthesis (Holton/Ojima method) → Paclitaxel; this is the industrial standard**

Q8. Paclitaxel's mechanism differs from Vinca alkaloids in that it:

a) Inhibits DNA synthesis b) Stabilises microtubules (prevents depolymerisation) c) Destabilises microtubules like Vinca alkaloids d) Inhibits Topoisomerase

✓ **Answer: b) Stabilises microtubules – prevents depolymerisation; Vinca alkaloids do the OPPOSITE (destabilise)**

Q9. Caffeine is industrially obtained as a by-product of which process?

a) Tea fermentation b) Coffee roasting c) Decaffeination of tea/coffee d) Green tea extraction

✓ **Answer: c) Decaffeination of tea/coffee – caffeine is recovered from the decaffeination solvent; also synthetic production**

Q10. Vincristine has N-FORMYL group while Vinblastine has N-METHYL group. This structural difference leads to:

a) Same clinical uses and toxicity b) VCR: mainly neurotoxic; VLB: mainly myelosuppressive c) VCR: myelosuppressive; VLB: neurotoxic d) No pharmacological difference

✓ **Answer: b) VCR: dose-limiting NEUROTOXICITY (peripheral neuropathy); VLB: dose-limiting MYELOSUPPRESSION (neutropenia)**

Q11. Which alkaloid is the DRUG OF CHOICE for Acute Lymphoblastic Leukaemia (ALL)?

a) Vinblastine b) Colchicine c) Vincristine d) Taxol

✓ **Answer: c) Vincristine (VCR) – cornerstone of ALL treatment protocols**

Q12. Etoposide (VP-16) is a semi-synthetic derivative of which natural compound?

a) Colchicine b) Vincristine c) Podophyllotoxin d) Taxol

✓ **Answer: c) Podophyllotoxin – Etoposide = glycoside derivative; inhibits Topoisomerase II (not tubulin like podophyllotoxin itself)**

Q13. The concentration of Vincristine in C. roseus leaves is approximately:

a) 0.1–0.5% b) 0.001–0.01% c) 0.0001–0.001% d) 1–2%

✓ **Answer: c) 0.0001–0.001% (10–100× lower than Vinblastine) – reason VCR is extremely expensive**

Q14. Which HPLC detector is preferred for Artemisinin quantification due to its minimal UV chromophore?

a) UV at 254 nm b) Fluorescence detector c) ELSD (Evaporative Light Scattering Detector) d) Conductivity detector

✓ **Answer: c) ELSD – Artemisinin does not have significant UV absorbance (no conjugated aromatic system)**

Q15. Atropine is the RACEMIC form. Which isomer occurs naturally in the plant?

a) D-Hyoscyamine (Dextro) b) L-Hyoscyamine (Laevo) which racemises during extraction c) Both in equal amounts d) Neither – atropine is fully synthetic

✓ **Answer: b) L-Hyoscyamine (laevo) is the natural form – more potent; racemises to DL-Atropine during extraction/isolation process**

END OF UNIT 4 NOTES | BEST OF LUCK FOR EXAMS!

Prepared by Noteskarts | www.noteskarts.com | YouTube: @Noteskarts