Bioguided Extraction and Isolation in search of New Pharmaceuticals

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Importance of Plants

- **Plants remain the most important source of natural drugs.**
- **More than 30% of prescription drugs are natural products.**
- **More than 60% of anticancer and anti-infective drugs are natural products.**

The main sources of drugs are as follows:

- ***Natural substances; Semisynthetic substances; Synthetic substances***

Retrieval of information

- ***Medical botany***
- ***Ethnobotany***
- ***Herbaria (herbarium)***
- ***Field exploration***
- ***Phytopharmacological surveys***

Steps in Phytochemical investigation

- 1- Selection of promising plant materials.
- 2- Proper collection of selected plants.
- 3- Authentication of plant material.
- 4- Drying of plant materials.
- 5- Grinding of the dried plants.
- 6- Garbling of the dried plants
- 7- Packing, storage and preservation
- 8- Extraction and fractionation of constituents.
- 9- Methods of separation and purification.
- 10- Methods of identification of isolated compounds (structure elucidation e.g. UV, IR, MS, H-NMR and C-NMR).

Collection, identification and storage of crude drugs

- **In proper collection**
- **Time, age, season of collection**

Precursors may not available in sufficient quantity

Plant may not prepare to synthesis the compounds

- **Part of collection – leaf, fruits, bark, flowers**

Leaves-Before vegetation, Fruits before dehiscent

- **Taxonomist**
- **Voucher specimen is must**
- **Proper drying depending on part of investigation**
- **Storage and packing material**

Drying

Drying is done in:

Shade and in sunlight (Natural drying).

Hot air drying or by freeze-drying (Artificial drying)

Uses of Drying

1. **Ease of transport.**
2. **Ease of grinding**
3. **Inhibit the growth of microorganisms.**
4. **Preservative of active constituents**

Drying may change

Size and Shape; Appearance; Nature of compound; Color; Odour and Taste

Extraction Techniques

There is no general (universal) method for the extraction of plant materials.

Extraction depends on

- **The texture of the plant material.**
- **The water content of the plant material.**
- **The type of substances to be extracted or nature of active constituents**
- **The chemical atmosphere the compound present**
- **Physical and chemical nature of constituent**
- **Identification method**

Extraction: The process of isolation of soluble material from an insoluble residue. Which may Solid –Liquid ; Liquid-Liquid.

OR

is the separation of medicinally active portion of plants or animal tissues through the use of selective solvent and suitable methods extraction

Basic principle:-

Diffusion of the solvent in to the solid

Dissolution of the active ingredient in side the solid particle

Diffusion of the solution from the solid particle to the surrounding liquid

MASS TRANSFER: it is an unit operation which involves the transfer of mass of soluble material from a solid to a liquid

It is continued till an equilibrium is set up between the solution in the cells and the free solution , so it reaches the K value

$$K = \frac{\text{Con. of extractive substance in the miscella}}{\text{Con. of extract substance in the drug residue}}$$

Equilibrium is determined by the properties of

Drug, Type, Quantity, Degree of communition; Moisture content; Solvent

Drug Extracts: These are the preparations obtained by extracting the herbal drug of certain particle size with suitable extraction medium

TYPES OF EXTRACTS:

- **AQUEOUS EXTRACTS- Decoction; Infusion; Digestion**
- **TINCTURES (alcoholic preparations)- Belladonna tincture**
- ❑ **LIQUID /FLUID EXTRACTS- more concentrated than tinctures**
- ❑ **THIN EXTRACTS-Liquid extracts having honey like consistency**
- ❑ **THICK EXTRACTS/ SOFT EXTRACTS- Glycyrrhiha extract U.S.P**
- ❑ **DRY EXTRACTS-: senna ,belladonna extract**
- **OILY DRUG EXTRACTS macerating the ground material in the oil.**
- **OLEO RESINS**
- **STANDARDIZED EXTRACTS: Extracts are standardized to active constituents.**

Extraction methods

1. **Maceration**
2. **Percolation**
3. **Vertical or turbo extraction**
4. **Counter current extraction**
5. **Hot continuous extraction**
6. **Infusion and decoction**
7. **Steam distillation**
8. **Ultra sound extraction**
9. **Extraction with gases**
10. **Super critical fluid extraction**
11. **Miscellaneous methods**

The choice of the method depends upon the characteristics of drug ,solvent ,temperature, advantages, disadvantages

Choice of solvent

- **As a general empirical rule:**

  Non polar solvents (petroleum ether and hexane) will dissolve non-polar compounds (fats and waxes).
- **While polar solvents (methanol, ethanol and water) dissolve polar compound (alkaloid salts and sugars) *(tha***
- **The affinity of the solute for the organic phase may be greatly increased by using mixture of solvents instead of single ones (*sometimes used mixtures of solvent to increase the solubility*).**

Maceration

- **Maceration**
- **Kinetic maceration**
- **Re maceration**
- **Digestion**

METHOD:

Modified maceration

Applications

Senna liquid extract; Squill tincture; Opium tincture

Advantages:-

1. **Widely used**
2. **Small samples can also prepared**
3. **Strong swelling drugs high mucus content drugs**
4. **Cheap and simple**

Disadvantages:-Don't exhaustively extract

Maceration

Powdered drug

Macerated with fresh juice of drug or several drugs

Kept till the fluid is soaked into the solid

Percolation

- Percolation :it is a continuous flow of the solvent through the bed of the crude drug
- Material is exhaustively extracted by fresh solvent

Procedure :-

1. Communition –shredding mills
2. Pre swelling :-dampening of the drug with menstrum, and allowed to stand for 15 minutes
3. Intermediate maceration:- macerated for 24 hours
4. Percolation

Applications – Homeopathic mother tincture

ADVANTAGES:-

1. Exhaustive extraction
2. Laboratory and industrial scale method
3. Continuous process
4. Too much technical skill is not required

DIS ADVANTAGES:-

1. Not applicable to fine powders, resins, swelling drugs
2. Large quantity of the solvent
3. Pre swelling is difficult in industries
4. Removal of fine particles from the extract is difficult

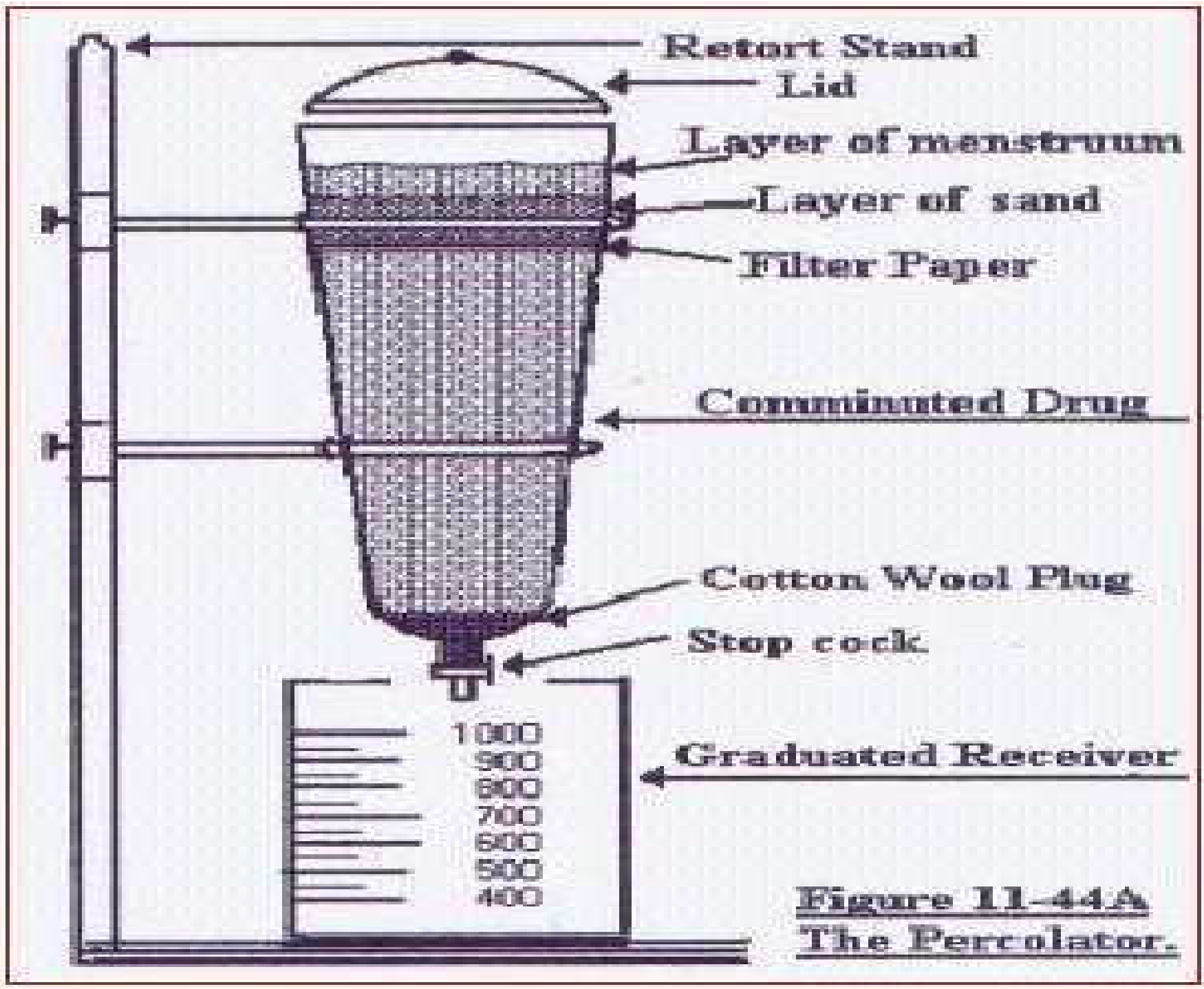
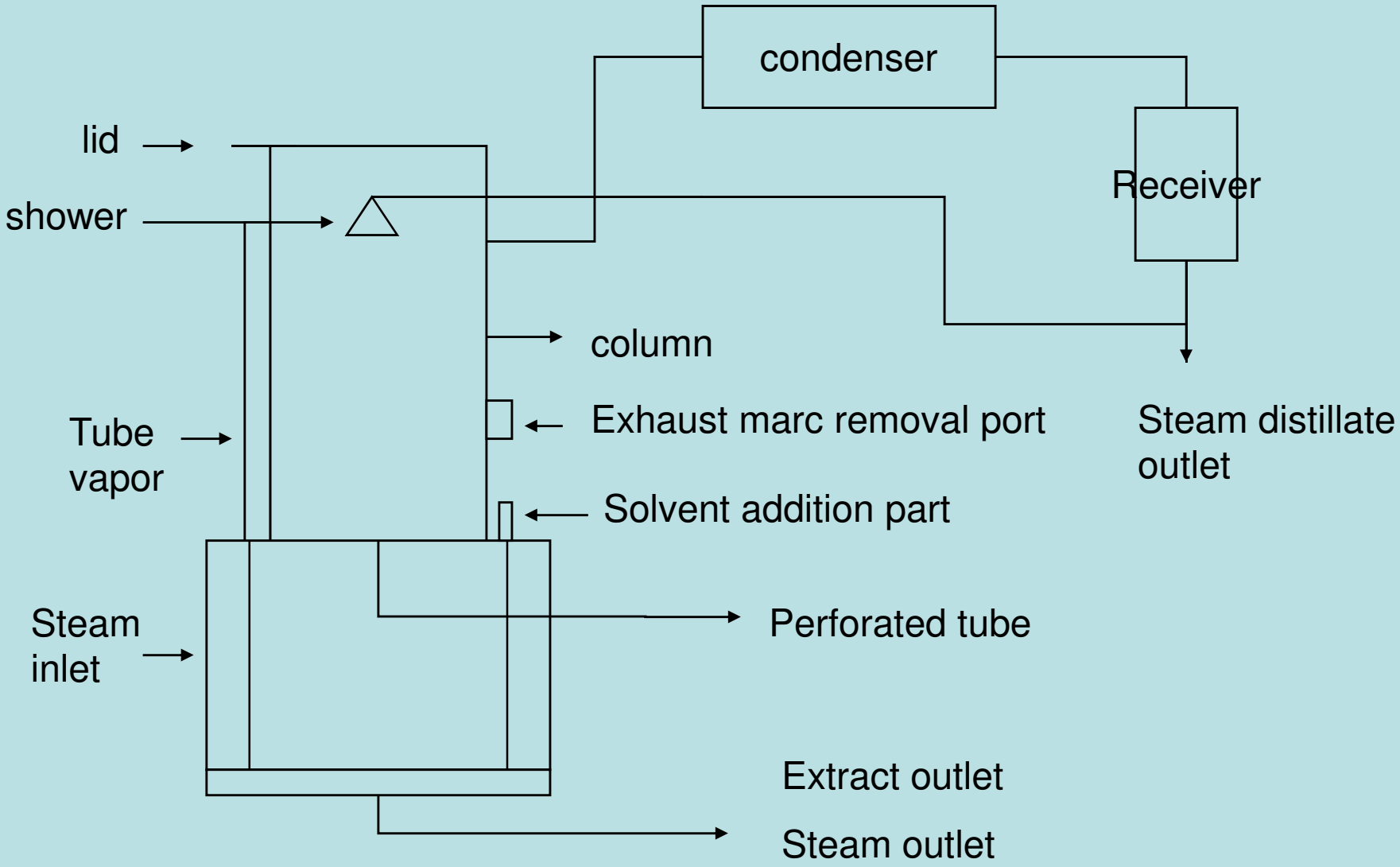


Figure 11-44A
The Percolator.

Block diagram of commercial percolator



Vertical /Turbo extraction

Plant material is stirred in the menstrua with a high speed mixer or homogenizer

PRINCPLE:-shredding and shearing forces – fragments the material

Faster establishment of the equillibrium

ADVANTAGES:-

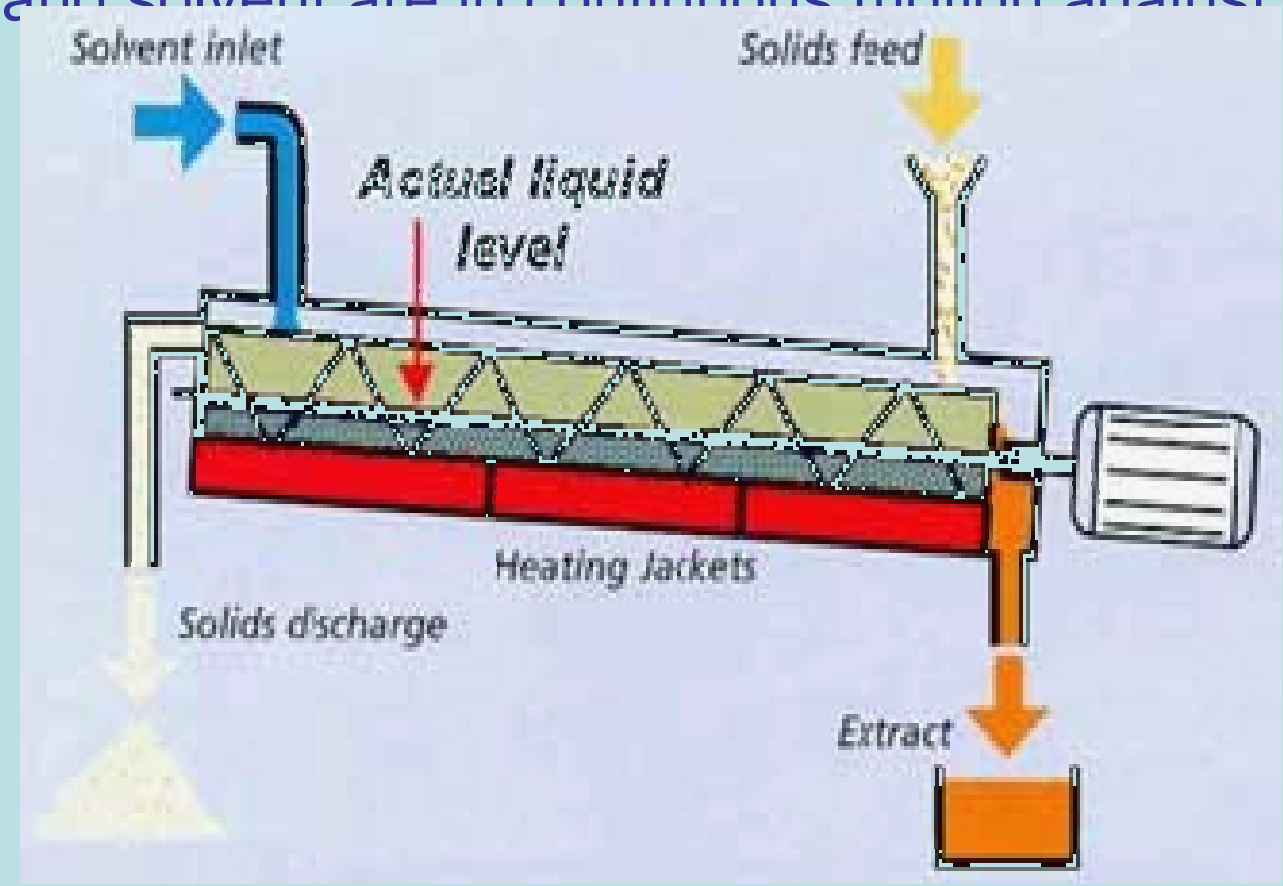
- High recovery of active constituent
- Less time

DIS ADVANTAGES:-

- Raise in temperature may decompose the active ingredient
- Further communition

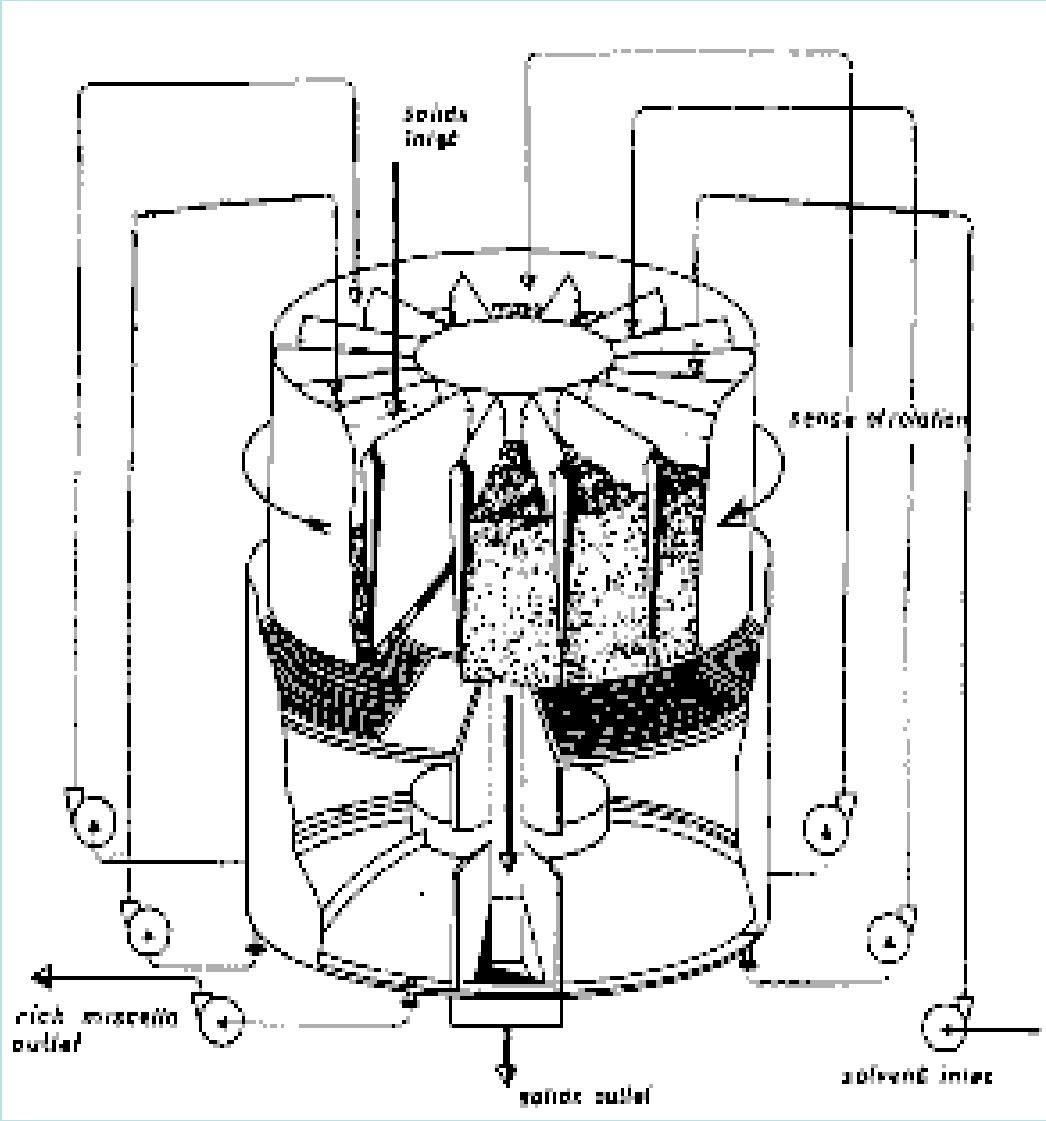
COUNTER CURRENT EXTRACTION

- Extraction material and solvent move againt to each other
- HELICAL COUNTER CURRENT EXTRACTOR
- Material and solvent are in continuous motion against one other



CAROUSEL EXTRACTION

- Material is moved against stationary solvent units
- Rotary extractors, Basket, Belt extractors



CONTINUOUS EXTRACTION

HOT CONTINUOUS EXTRACTION/SOXHLETION

- Same solvent can be circulated through out extractor for several times

METHOD:- material is placed in the thimble –cellulose or cloth Placed in soxhlet which is having a siphoning device and side arm

ADVANTAGES:-

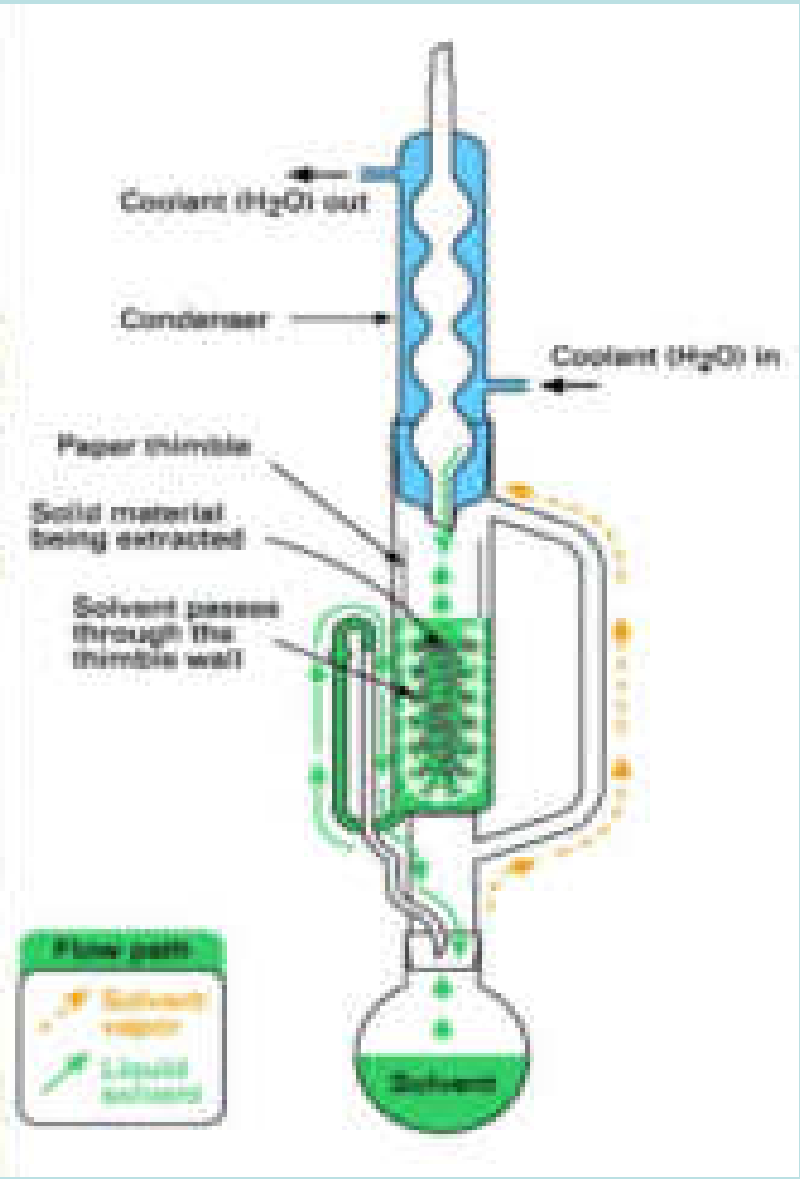
1. Complete exhaustion
2. Less solvent
3. Automatic continuous method
4. Less time

DIS ADVANTAGES:-

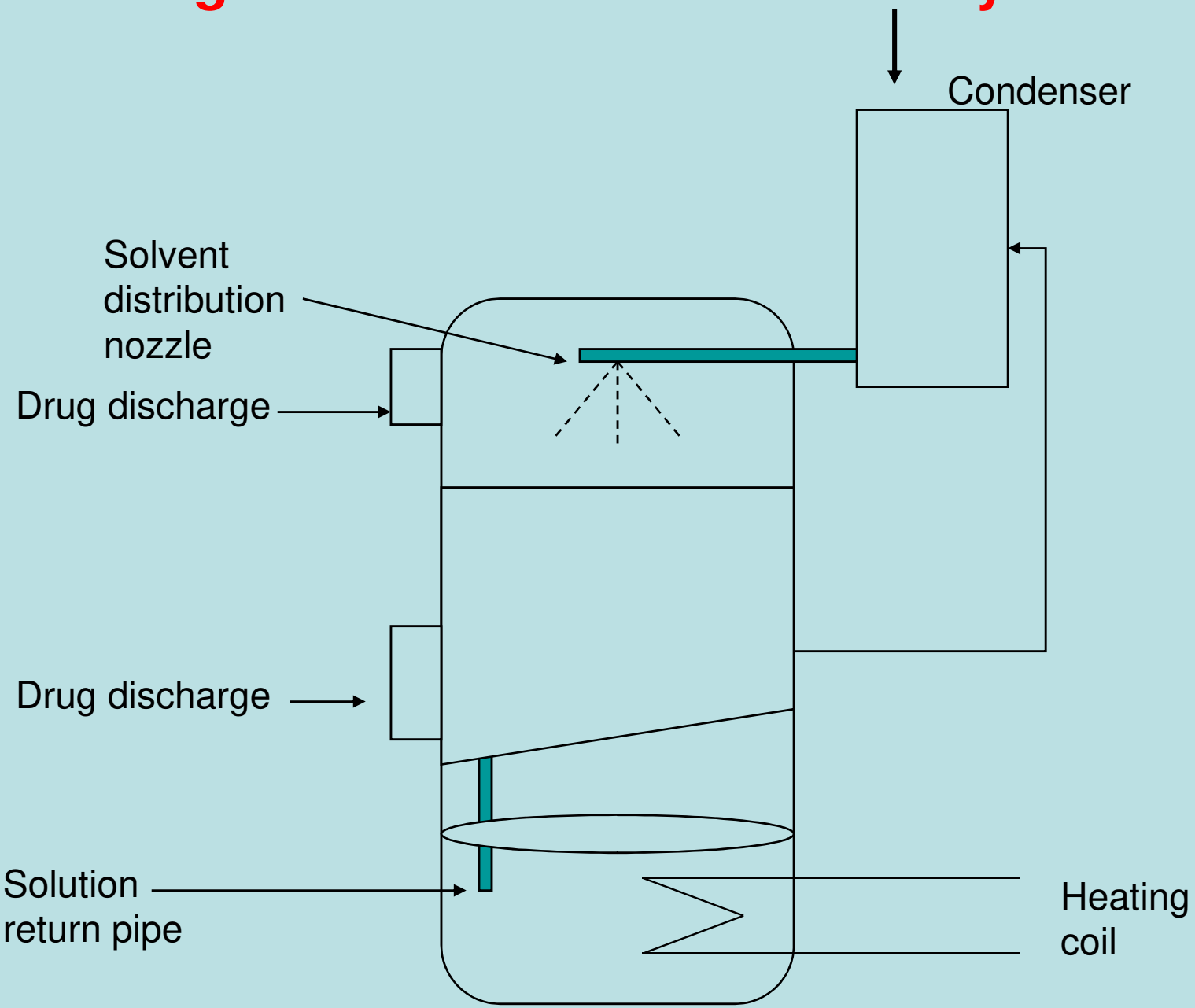
1. Thermal degradation
2. High boiling point solvents are not recommended
3. Use of binary and ternary mixtures is not advisable

APPLICATIONS:

Diosgenin ,berberine etc


Coolant (H2O) out
Condenser
Coolant (H2O) in
Paper thimble
Solid material being extracted
Solvent passes through the thimble wall
Flow path
Solvent vapor
Liquid solvent
Solvent

Large scale extraction assembly



ULTRA SOUND EXTRACTION

- **ULTRA SOUND WAVES = Frequencies above 20,000 Hz**
- **These sound waves are used as force to accelerate the extraction**
- **Increase the permeability of cell walls**
- **Cavitations are produced**

Production of ultra waves

Magneto strictive or Piezoelectric ultra sonic transmitters

Applications:-

Belladona herb extract; Rauwolfia root extract

Advantages:

Faster rate of extraction

Less time is required

DIS ADVANTAGES:

Costly method

Not applicable to large scale production

STEAM DISTILLATION

- **PRINCIPLE:-** Two immiscible liquid mixture

Total vapor pressure of boiling mixture is equal to the sum of the partial pressures

Boiling starts when total vapor pressure is equal to the atm.pressure

Boiling point is reached at low temperature

METHODS

- HYDRO DISTILLATION; WET DISTILLATION;DRY DISTILLATION

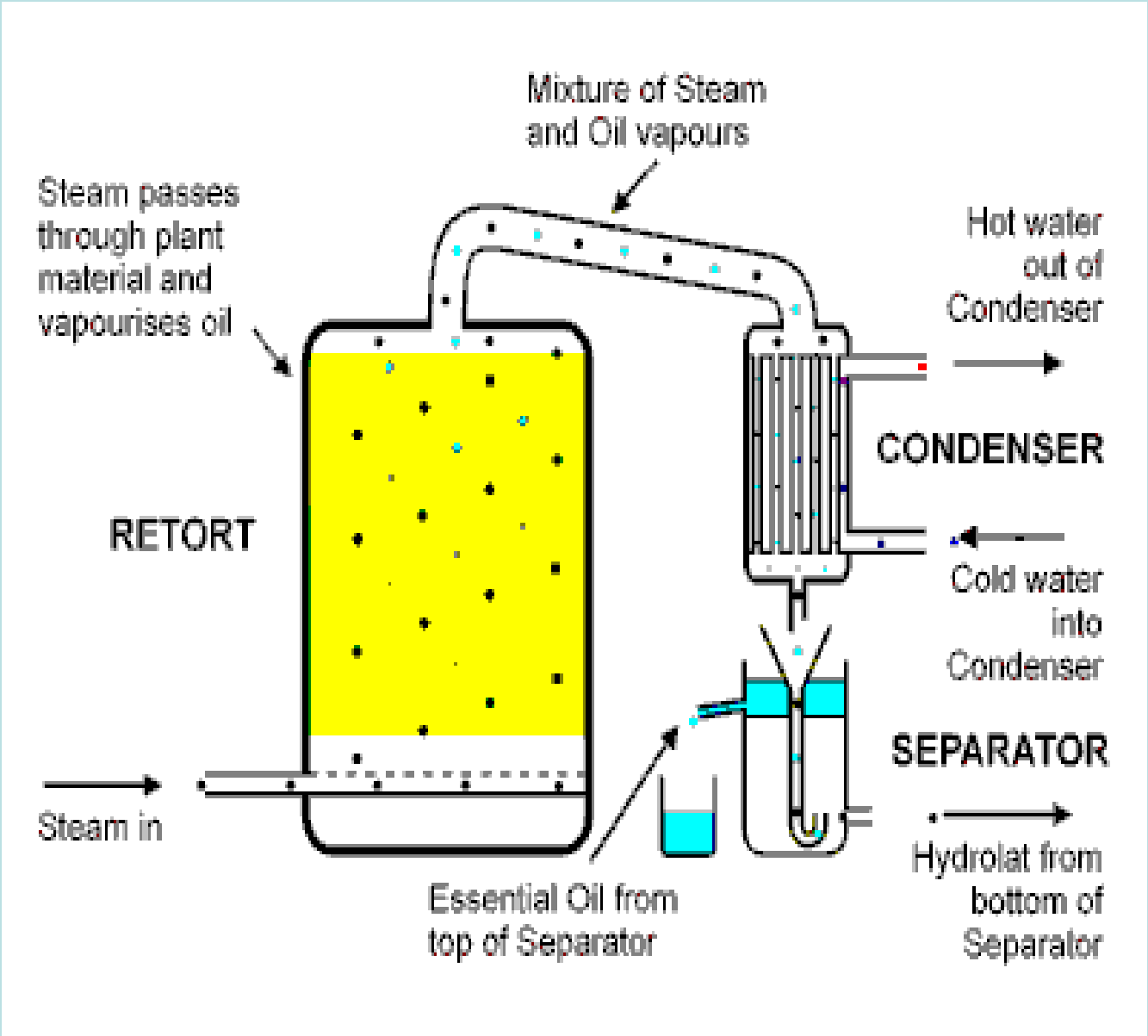
ADVANTAGES:- Separate filtration is not required

Simple equipment, Volatile oils are extracted

DIS ADVANTAGES:

APPLICATIONS

STEAM DISTILLATION



SUPER CRITICAL FLUID EXTRACTION

CRITICAL TEMPERATURE:- ***It is the highest temperature that a gas can be liquefied by only a change in pressure (Tc)***

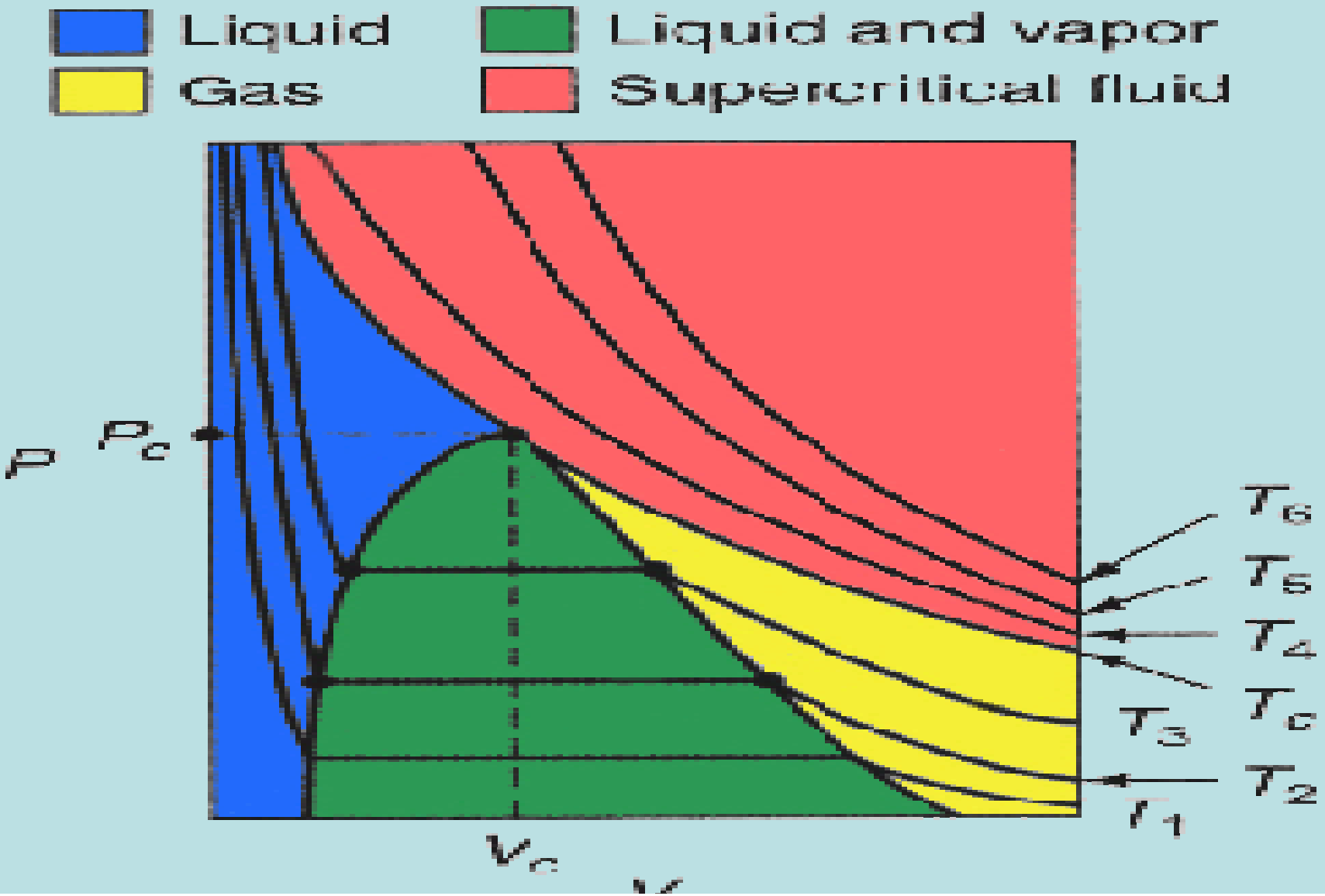
SUPER CRITICAL FLUID:-. ***when a substance is above Tc, it exists in a single phase which is neither liquid nor gas; this is a supercritical fluid.***

- ***Another way to look at it is that Tc is the highest temperature that a gas can be liquefied by only a change in pressure***

CHARACTERISTICS

1. ***Combination of vapor and liquid properties***
2. ***Density and viscosity is less than liquid***
3. ***Diffuse like gases***
4. ***Compressible and homogeneous***

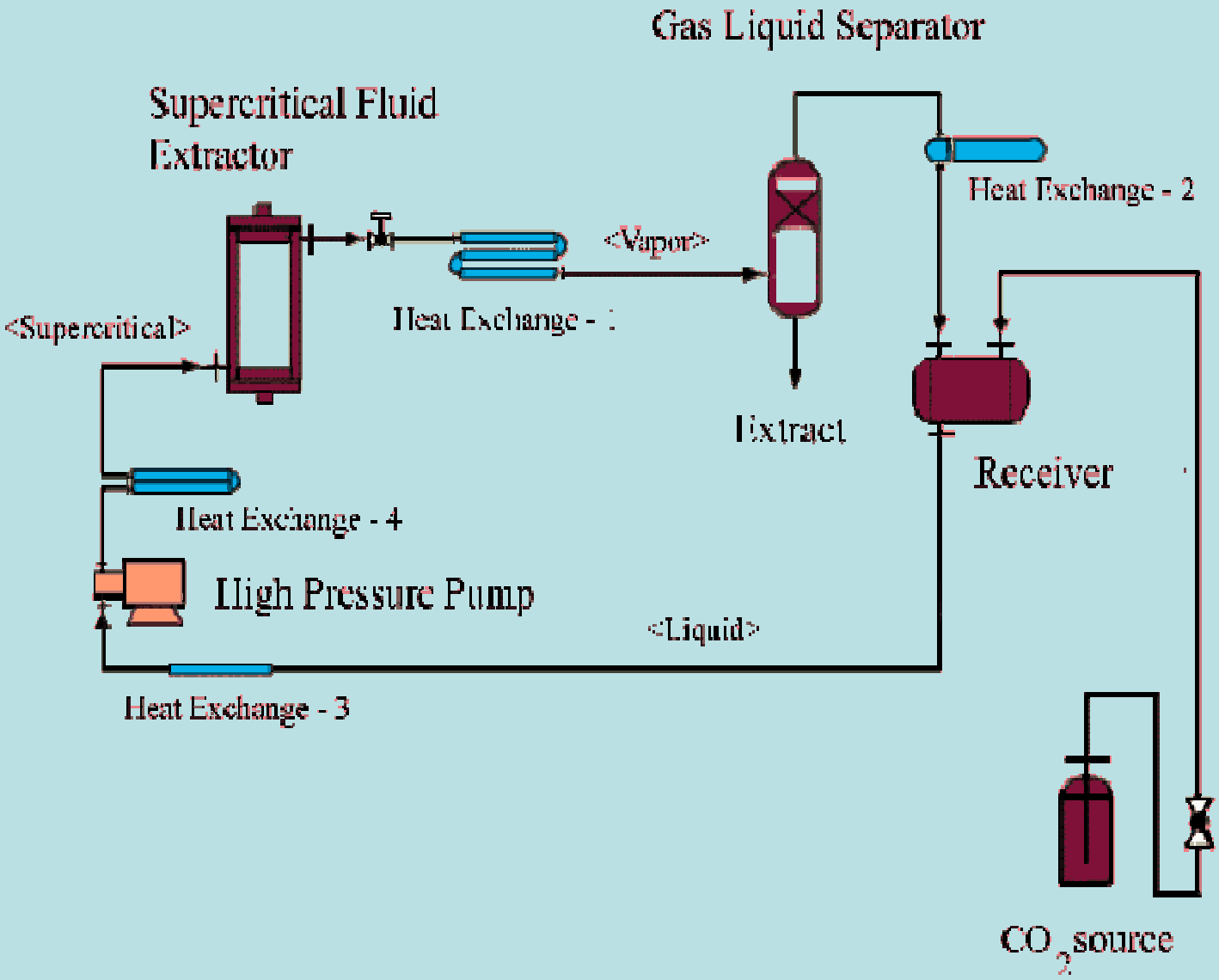
PHASE DIAGRAM



CRITICAL PARAMETERS

Substance	T_c (K)	P_c (atm)
CO_2	304	75
H_2O	647	224
C_2H_6	305	50
C_2H_4	282	51
C_3H_8	370	44
Xe	290	59
NH_3	406	116
N_2O	310	73

Apparatus


Gas Liquid Separator
Supercritical Fluid Extractor
Heat Exchange - 2
<Vapor>
<Supercritical>
Heat Exchange - 1
Extract
Receiver
Heat Exchange - 4
High Pressure Pump
<Liquid>
Heat Exchange - 3
CO_2 source

APPLICATIONS

There are many applications of supercritical fluids, but among the most important are

1. **Industrial extraction and purification.**
2. **The most commonly used supercritical fluid used in industry is carbon dioxide, due to it's convenient critical parameters, low cost, easy and non-toxic disposal, and safety.**
3. **The most well known application of supercritical fluids is Supercritical Fluid Extraction (SFE)**

1. **Extraction of pyrethrins**
2. **De caffecation of coffee**
3. **De odourisation of packed food products**

ADVANTAGES

1. **Low solvent usage**
2. **Less thermal degradation**
3. **Controllable selectivity**
4. **More efficient than other methods**

DIS ADVANTAGES:

Highly sophisticated method

Extraction by electric energy

Electrical energy is used in the form of an electric field

Eg: extraction of scopolamine from the seeds and capsules of Indian thorn apple

Infusion and decoction

Infusion: hot or cold water is added to the milled drug

Eg: Aloin

Decoction: sample is boiled with the water

Eg: caffeine

Extraction with gases

Phytosols = extracting fluids which consists of 1,1,1,2 tetra fluoro ethane

ADVANTAGES:

Better products

High yield

Low cost than steam distillation and scf

Miscellaneous methods

- Enfleurage
- Expression
- Microwave treatment
- Per vaporation
- sublimation

SOLVENTS

- **CHARACTERISTICS**
- **SELECTION OF THE SOLVENT**
- **POLAR SOLVENTS**
- **NON POLAR SOLVENTS**
- **SEMI POLAR SOLVENTS**
- **AZEOTROPIC MIXTURES:**

Combination of solvents of varying polarities

These mixtures up on concentration of extracts boils constantly and condensate can be re used

BINARY MIXTURES:

n-hexane:benzene 81:19 61.9 (b.p)

TERNARY MIXTURES

N-propanol:cyclohexane:water 18:54.8:26.9 65.4(b.p)

CHARACTERISTICS OF PHYTO CONSTITUENTS:

1. **Polarity; PH; Thermo stability**

DRYING OF EXTRACTS

- Spray dryer
- Vacuum tray dryer
- Vacuum rotary dryer

Bioguided Extraction

- **Drug will be extracted by series of solvents**
- **All extracts are tested for activity and tested for phytocomstituents.**
- **Active extract is tested by TLC and Column chromatography.**
- **All fractions are tested biologically.**
- **The active fraction is again subjected to column. The process is repeated till desired compound is obtained**
- **The isolated compound structure is determined**

RESULTS OF PRELIMINARY CHEMICAL INVESTIGATIONS

Helicteres isora :

5 Kg of dried root powder extracted with 80% Ethanol

↓

113g of yield (2.26%)

↓ Fractionation

Pet. Ether Ext. 0.04%	Chloroform Ext. 0.48%	Ethyl acetate Ext. 0.25%	Butanol Ext. 0.90%	Left over Aq. Ext. 0.55%

Phytoconstituents : Steroids, triterpenoids and their glycosides, tannins and carbohydrates

Caralluma Attenuata:

5 Kg of fresh whole plant extracted with Ethanol

↓

162g of yield (3.24%)

↓ Fractionation

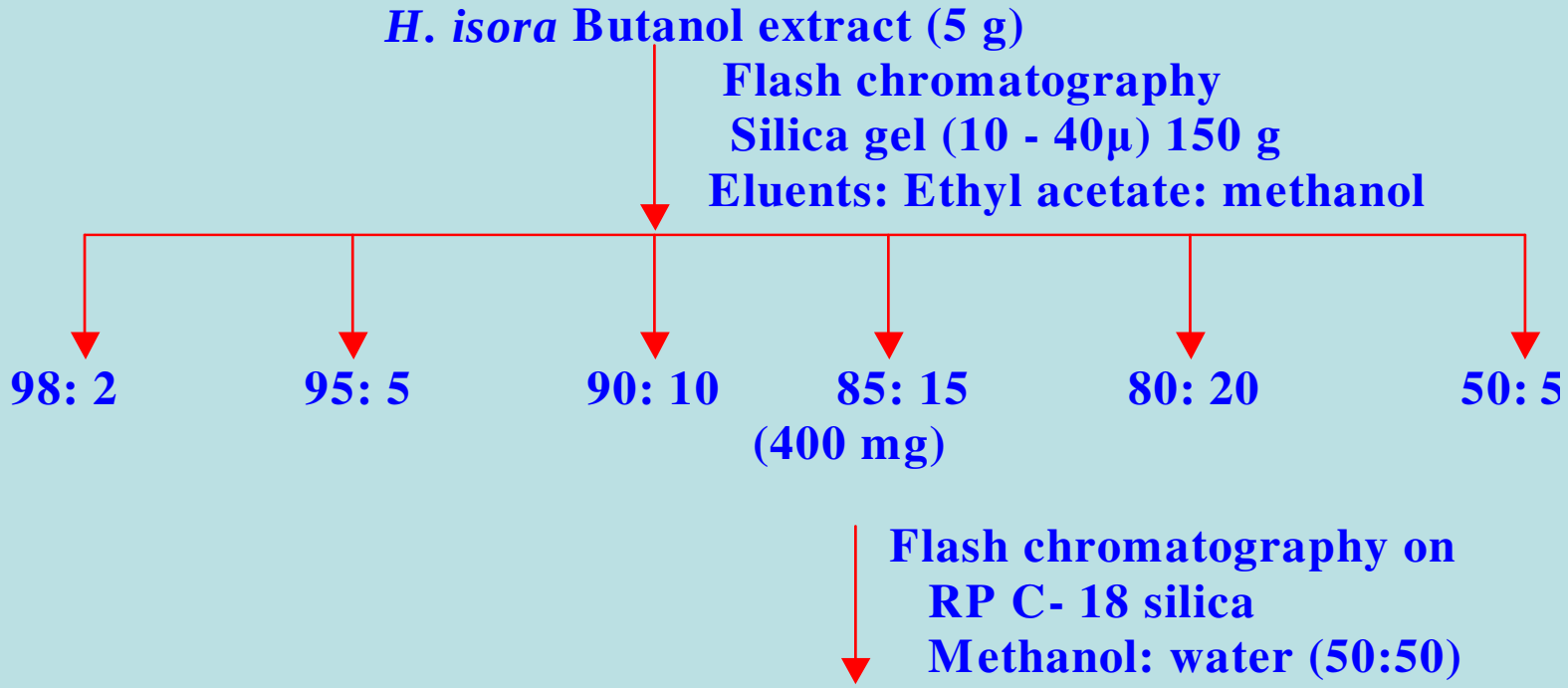
Pet. Ether Ext. 0.09%	Chloroform Ext. 0.60%	Ethyl acetate Ext. 0.15%	Butanol Ext. 0.37%	Left over Aq. Ext. 1.93%

Phytoconstituents : Steroids, triterpenoids, flavonoids and their glycosides, amino acids, proteins and carbohydrates

RESULTS AND DISCUSSION OF CHEMICAL INVESTIGATION

Column chromatography of *H. isora* butanol extract:

Scheme I



ISOLATION OF CA-C-1

Scheme - II

***C. attenuata* chloroform extract (3.5 g)**

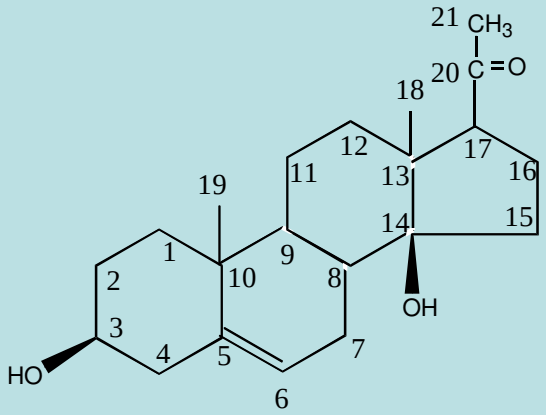
Flash chromatography,
Silica gel 120 g (10 – 40 µ)
Eluents:
2 L of pet ether: acetone:: 95: 5

- **1 – 20 prefractions of CA-C-1**
- **21 – 30 Pure CA-C-1 (120 mg)**
- **CA-C-1 along with minor compounds**
- **54 - 80 (mixture)**

- **Colorless, amorphous solid, mp. 227 –228°C**
- **Liebermann – Burchard test + ve**
- **Steroidal compound.**
- **Pet. Ether: Acetone (90: 10) – Rf: 0.3**
- **Pet. Ether: Acetone (95: 05) – Rf: 0.25**

^{13}C DEPT of CA-C-1

	Total	Carbon number
s (C)	5	5,10,13,14,20
d (CH)	5	3,6,8,9,17
t (CH_2)	8	1,2,4,7,11,12,15,16
q (CH_3)	3	18,19,21



3β,14β-dihydroxypregn-5-en-one

Isolation of CA-B-1:

Scheme III

***C. attenuata* butanol extract (6 g)**

Flash chromatography

Silica gel 150 g (10 – 40 µ)

Eluent: 2 L of ethyl acetate: methanol: wat

(81:11:8)

1-21	22-28	29-50	51-80

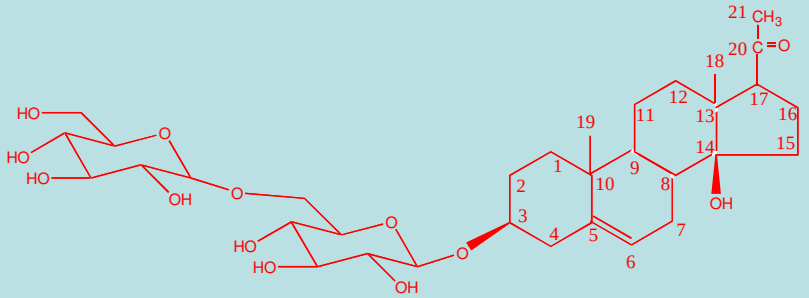
Pure CA-B-1

(90 mg)

- **Colorless, crystalline solid, mp. 256-258°C**
- **(+)ve with Liebermann – Burchard and Molish test.**
- **Steroidal glycoside**
- **Ethyl acetate: methanol: water – 81:11:8 – Rf – 0.22**
- **Chloroform : methanol – 80: 20 – Rf – 0.33**

^{13}C DEPT of CA-B-1

	Total	Carbon number
s (C)	5	5,10,13,14,20
d (CH)	15	3,6,8,9,17,1',2',3',4',5',1'',2'',3'',4'',5''
t (CH_2)	10	1,2,4,7,11,12,15,16,6',6''
q (CH_3)	3	18,19,21



3-O-β-D-glucopyranosyl-(1→6)- β-D-glucopyranosyl-3 β, 14 β-dihydroxypregn-5-en-20-one.

Bioguided Isolation:

This is a process where greater number of samples assayed in greater number of possible assays to find greater number of useful compounds.

The main objective of Bioguided isolation is targeted isolation of new bioactive plant products, i.e., lead substances with novel structures and novel mechanism of actions.

Hurdles faced during Bioguided isolation are

- Dereplication (the rapid identification of known compound from partially purified mixture)
- Purification of new compound by chromatographic methods
- Structural determination by spectroscopic methods
- scale-up of the production
- Optimization of lead compound

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