Phytochemistry

Extraction, Isolation And Separation of Physiologically Active Natural Compounds

Extraction Techniques

- **Maceration**: Soaking of plant material in a suitable liquid.
- **Decoction**: Decoction is a method of extraction, by boiling, of dissolved chemicals, or herbal or plant material, which may include stems, roots, bark and rhizomes. Decoction involves first mashing, and then boiling in water to extract oils, volatile organic compounds, and other chemical substances.

Extraction Techniques

- **Infusion** is a very simple chemical process used with botanicals that are volatile and dissolve readily, or release their active ingredients easily, in water, oil or alcohol.
- An infusion is the outcome of steeping plants that have desired chemical compounds or flavors in a solvent such as water or oil or alcohol.

Extraction Techniques

- **Percolation** concerns the movement and filtering of fluids through porous materials.
- In Phytochemistry, it means the extraction of the soluble principles of a crude drug by the passage of a suitable liquid through it.

Extraction Techniques

- Spontaneous isolation without extraction.
- Usually necessary at least one extraction and one purification step for compound isolation.
- Not only separation of high molecular compounds, but also removal of low molecular impurities disturbing late stages of separation and purification.
- *Matrix effect.*

Extraction – **Fick´s Diffusion Law**

$$\Delta c/\Delta t = - (DA/h) \times (c_0 - c)$$

- **dc/dt** - diffusion speed
- **D** - coefficient of diffusion (depending on temperature and particle size)
- **A** - interface
- **h** – diffusion layer
- **(c_0 - c)** – concentration gradient

- **Extraction process depends on**:

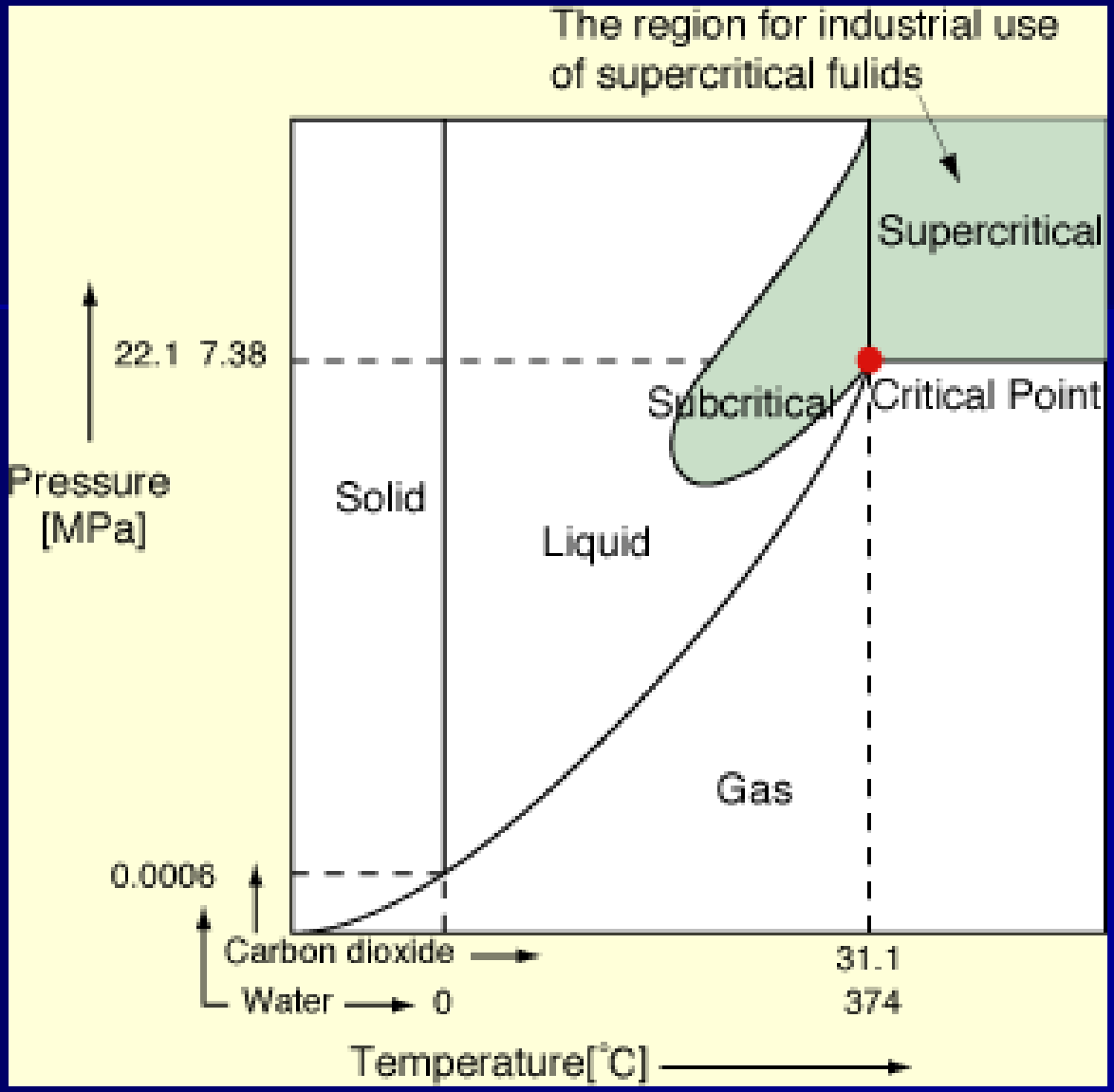
- Type of drug and its form (*Matrix effect*)
  - grade of disintegrations according to the diameters holes of sieve
  - grinding and mill apparatus
  - cutting apparatus

- Weight ratio of drug and extraction medium
- Humidity of the drug
- Extraction method

- Extraction medium and its composition

Supercritical Fluid Extraction (SFE)

- Extraction process is running with help of supercritical fluids.
- **Supercritical fluid**
  - Pressure and temperature crosses critical levels.
  - Its physical properties are on the cross between gases and liquids.
  - Density close to liquids → good solvation ability.
  - Diffusion constant close to gases → rapid transfer of mass.
  - Viscosity lower than liquids → advantage especially for supercritical fluid chromatography.
  - Low surface tension → easy material penetration.



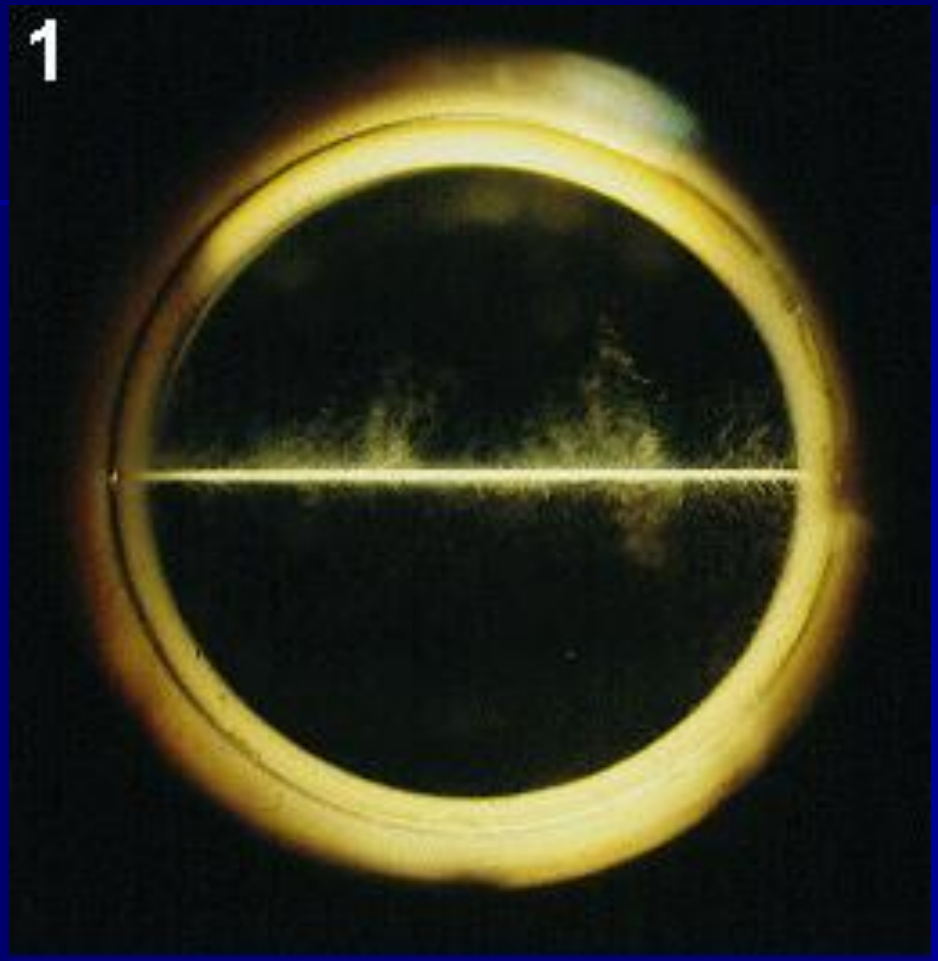
Phase diagram for water and carbon dioxide

Characteristics	Density (kg/m^3)	Viscosity (cP)	Diffusivity (mm^2/s)
Gas	1	0.01	1-10
SCF	100-800	0.05-0.1	0.01-0.1
Liquid	1000	0.5-1.0	0.001

Component	**P_c [bar]**	**T_c [^oC]**
CO_2	73.8	31.1
N_2O	72.4	36.4
NH_3	112.7	132.5
SO_2	78.8	157.6
SF_6	37.6	45.5
Xe	58.3	16.6

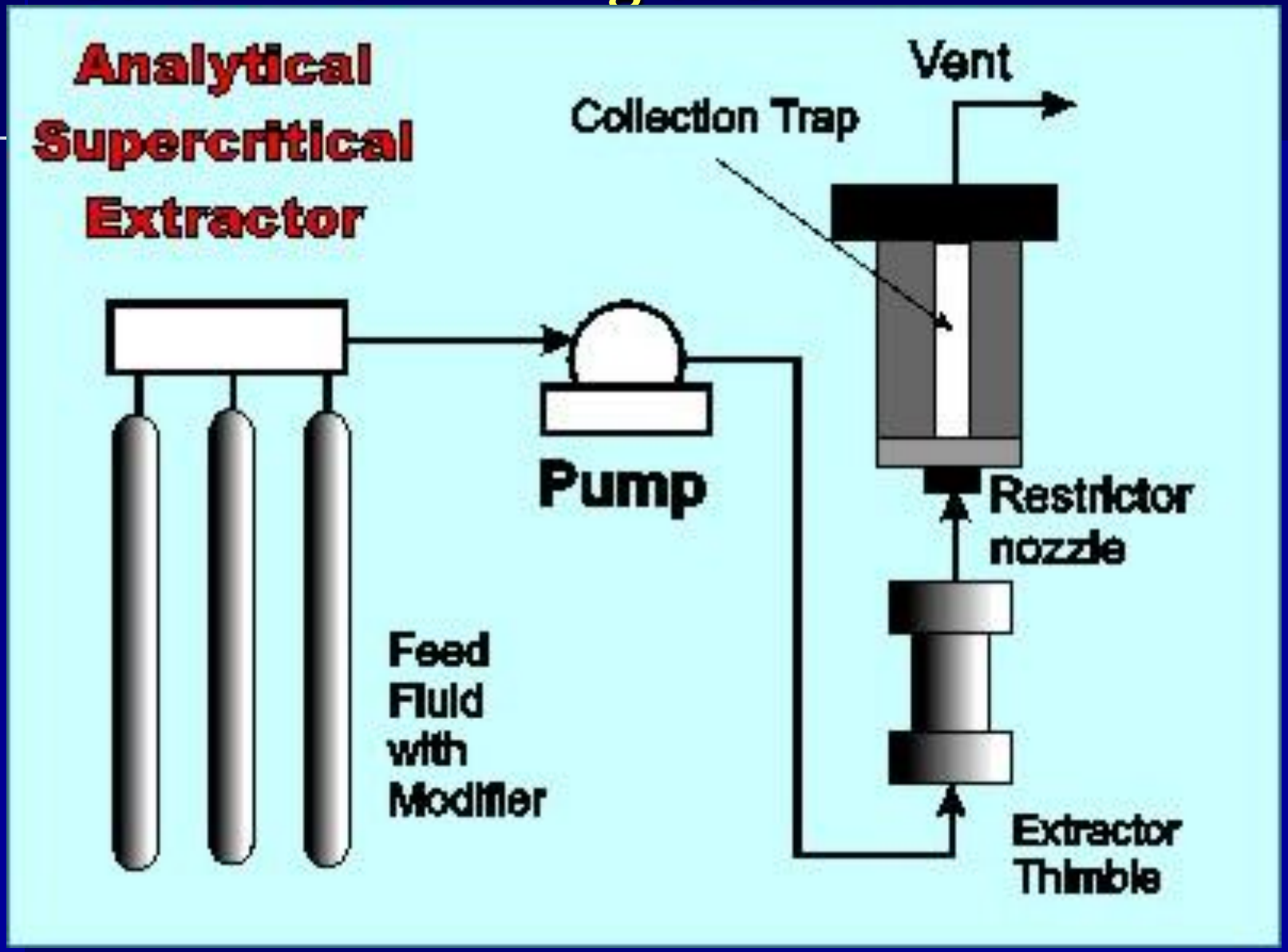
Liquid	Critical temperature (Kelvin)	Critical pressure (bar)
CO_2	304.1	73.8
Ethane	305.4	48.8
Ethylene	282.4	50.4
Propane	369.8	42.5
Propylene	364.9	46.0
Trifluoromethane	299.3	48.6
Chlorotrifluoromethane	302.0	38.7
Trichlorofluoromethane	471.2	44.1
NH_3	405.5	113.5
H_20	647.3	221.2
Cyclohexane	553.5	40.7
n-Pentan	469.7	33.7
Toluen	591.8	41.0

Generation of Supercritical Fluid



Below the critical parameters,
two distinct phases exist

Schematic Drawing of SFE



- SFE Advantages:
  - Fine technique.
  - In ideal case no need for organic solvents.
  - Ecological harmlessness.
  - Relatively cheap.
  - Rapid.
  - Possibility of automatization.
  - Solvation power affected by the changes in pressure.

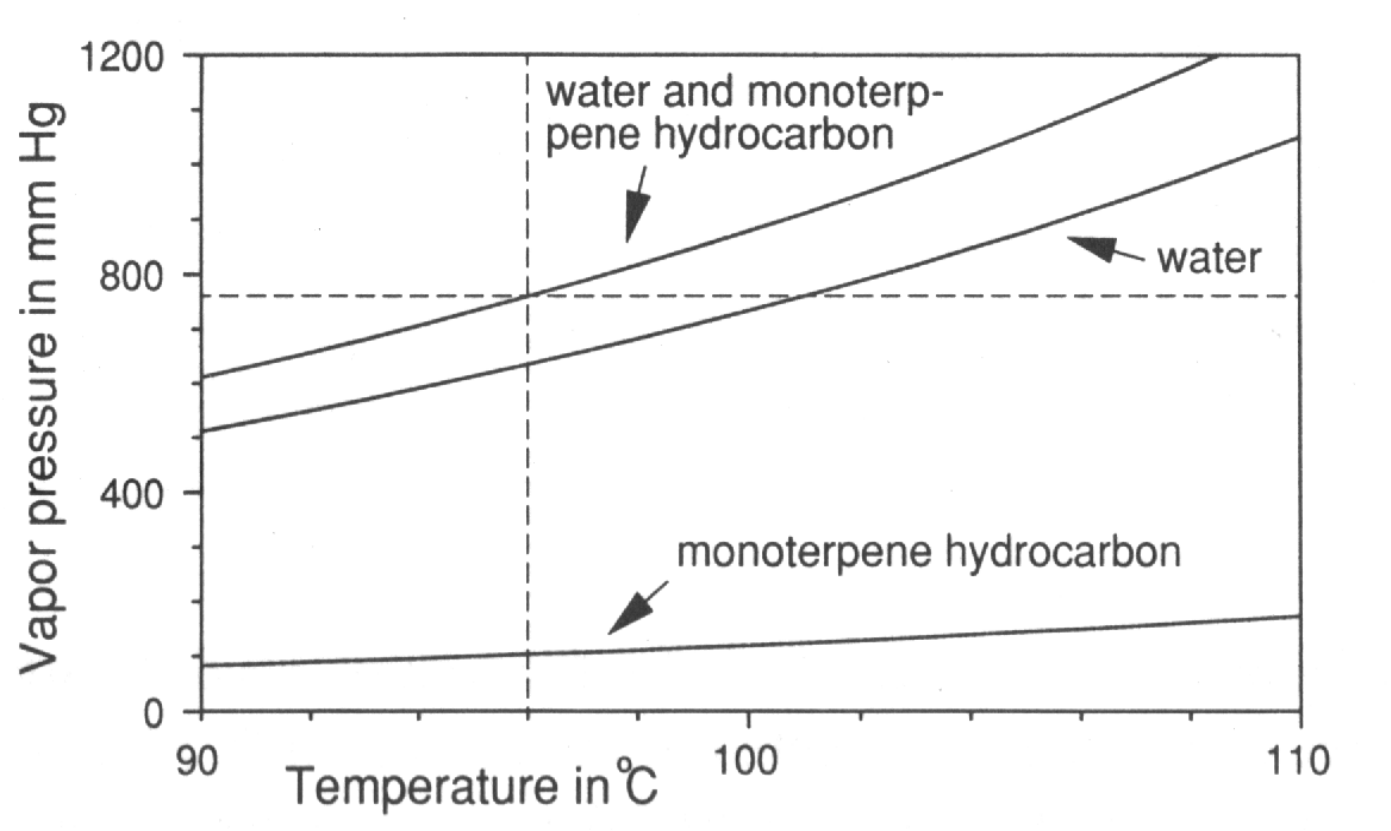
- SFE Disadvantages:
  - Less suitable for more polar compounds.
  - More demanding on equipment.
  - Need to use high pressures.
  - Less suitable for the extraction of plant leaves.
  - Problems with extraction tuning.
  - Difficult to extract fresh material (water content).

- **CO_2** – inflammable, non-explosive, widely available, cheap, ecological harmlessness, useful supercritical area, (T=31.1 ºC; P=7.28 MPa), suitable for extraction of less polar (hydrophobic) compounds (volatile oils, oils, waxes, carotenoids etc.)
  - **Used for:**
    - Hop extraction.
    - De-caffeinization of coffee (production of caffeine).
    - Extraction of taxol (Paclitaxel TM) from *Taxus brevifolia.*
    - Extraction of essential oils and spices.
    - Non-pharmaceutical purposes.

Distillation with Water Steam (Hydrodistillation)

- Method suitable for isolation of water insoluble volatile compounds.
- Common content of volatile compounds in plant material – > 1 % of weight – difficult SFE or solvent extraction.
- Selective.
- Simple.
- Pure.

Principles of Steam Water Distillation



- Advantages:
  - Selective for volatile compounds.
  - Simple apparatus.
  - Very cheap method.
  - Only water used as a „solvent“.
  - Useable in preparative scale.
  - Extract obtained very pure.

- Disadvantages:
  - Utilizable for non-polar (hydrophobic) volatile compounds.
  - Possibility of material decomposition (presence of water, high temperatures).
  - Improper for very low quantities of starting material.
  - Quantitative extraction time consuming.
  - Possibility of loss of polar compounds from the total bulk of compounds.

Principles of Water Steam Distillation

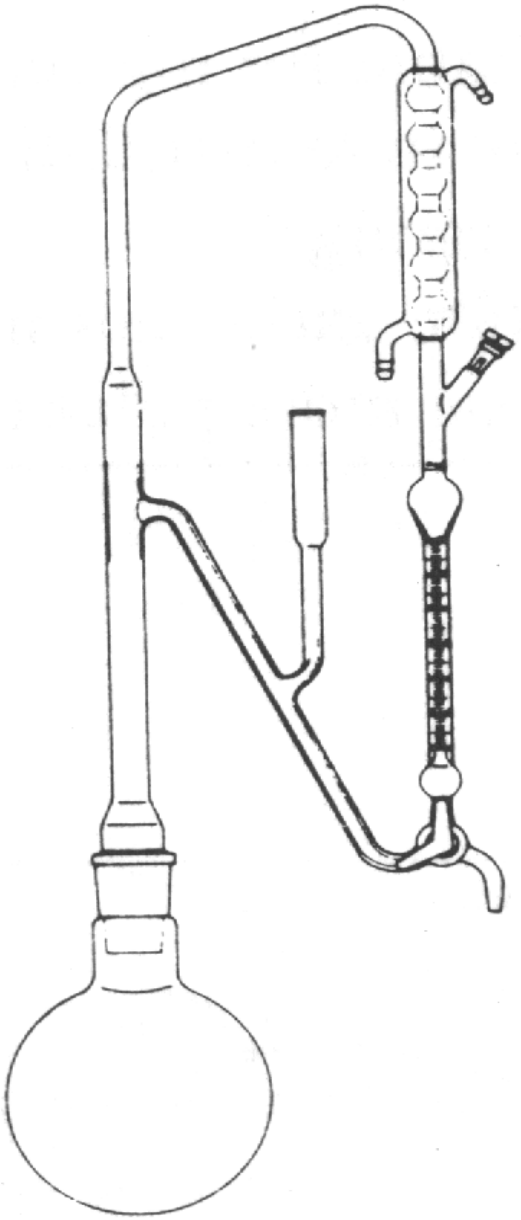
- Possible forms:
  - 1. „True“ water steam distillation.
  - 2. Hydrodistillation.

– True water steam distillation:

- Steam is generated separately in „steam generator“.
- Steam is forced down into material.
- Used in industry.

– Hydrodistillation:

- Plant material is suspended in boiling water.
- Water steam is generated *in situ*.
- Clevenger apparatus. Suitable for laboratory purposes.



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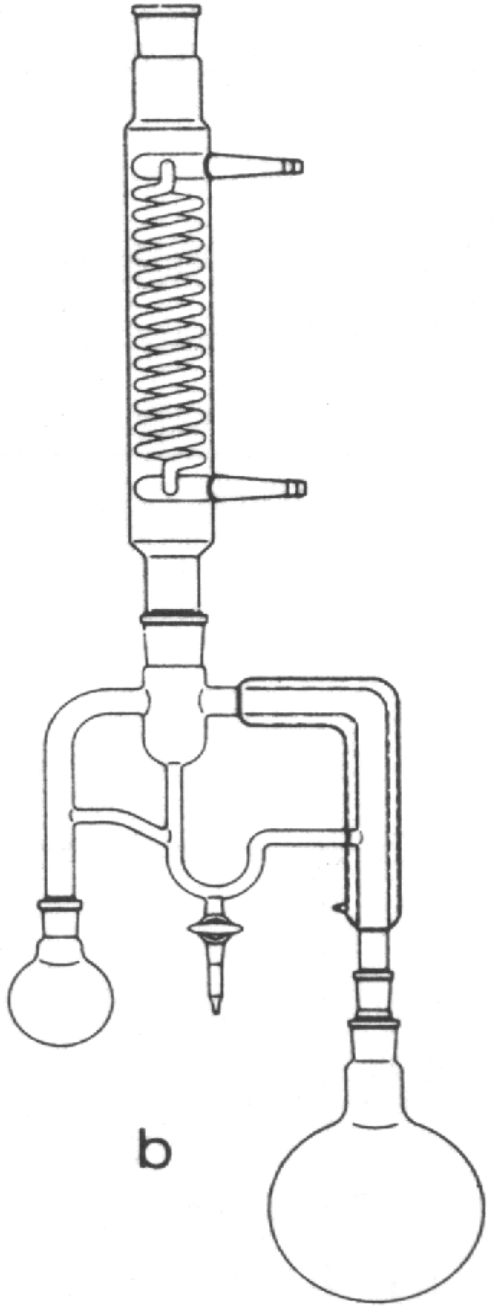

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- The matrix effect:
  - If the volatile compounds (like essential oils) are located on the surface of the plant material (trichomes, glandules…) – *matrix* effect is weak or not present, distillation is very rapid.
  - If the volatile compounds are present deeper inside the material, distillation is slower and it is affected by the matrix effect and diffusion.

- Special case – steam distillation extraction (SDE):
  - Does not use water steam only.
  - Mixtures diethyl ether/water 1:1, pentane/water 1:1.
  - Volatile non-polar compounds are dissolved in organic solvent, consequently two phases arise.

Separation and Purification Techniques

- Liquid-liquid extraction.
- Precipitation.
- Crystallization.
- Chromatography.

Liquid-liquid extraction.

- Following the extraction.
- Based on different solubility in immiscible or partially miscible solvents.
- Coarse separation of compounds into fractions with similar polarity, or creation of complexes and salts.
- Coarse extracts are usually not directly suitable for chromatographic separation:
  - Wide spectrum of compounds.
  - Very variant polarity.
  - Bad solubility in common chromatographic solvents.
- On analytical scale often replaced by SPE.

- Advantages:
  - Without necessity of special equipment.
  - Good capacity.
  - Possibility of fine tuning.
  - No irreversible adsorption.
  - Solvents could be chosen directly according to the compounds characteristics.

- Disadvantages:
  - Limited ability of separation.
  - Formation of problematic inter-phases and emulsions.
  - No possibility of automatization.

- Examples of common mixtures and their use:
  - Water: diethyl ether (petroleum ether)
  - Water: chloroform
  - Water: n-butanol
  - Methanol: hexane

  - Isolation of alkaloids.
  - De-fatting of extracts.
  - Separation of steroids.
  - Separation of glycosides from aglycones.

Precipitation

- Selective removal of group of target analytes or impurities.
- Followed by decantation, filtration and/or centrifugation.
- Employing the formation and dissolution of well defined precipitates.
- In present time:
  - Isolation of alkaloids (Mayer's reagent, Valser's reagent).
  - Separation of polyphenolic compounds (lead acetate, Polyvinylpolypyrrolidone PVPP).

Crystallization

- Final purification step.
- Possible in range from milligrams to tons.
- Suitable solvent – usually chosen by method (trial and error).
- Target compounds usually less soluble than impurities.
- In present time of lower importance
- Stays necessary for some identification methods (X-ray).