SACCHARUM

2020-2021 PHARMACOGNOSY-I PRACTICE

Pharmacopoeia: A book published usually under the jurisdiction of the government and containing a list of drugs, their formulas, and methods for making medicinal preparations, requirements and tests for their strength and purity, and other related information. (European Pharmacopoeia , Turkish Pharmacopoeia- Adaptadion of European Pharmacopoeia etc)

Monograph: The document which describes the physical characteristics of each plant and specifications of pharmaceutical raw materials and their active substance (ESCOP Monographs, Commission E Monographs, WHO Monographs, FFD Monographs)

Pharmacopoeia Analysis : It is an analysis to determine whether the active substances or excipients used in pharmaceutical preparations comply with the standards reported in the pharmacopoeia.

SACCHARUM

(TP 1974)

Saccharum is a diholoside composed of α-D glucopyranoside and β-D fructofuranosyl linked via semi-acetal bond.

TESTS

A) SOLUBILITY:

- Water
- Boiling water
- Ethanol (%95)
- Ether
- Chloroform

B) IDENTIFICATION REACTIONS

- Sucrose+ H_2SO_4 R —(heat)→ **Brown**, it is chared in time

- Aqueous-sucrose solution+ 0.1 N H_2SO_4 —(heat)→ NaOH TS (neutrolisation)

+

Cupric Potassium Tartrate TS

—(heat)→

Red Precipitate

H_2SO_4 R: 98% H_2SO_4
R: Reagent grade
TS: Test Solution

- Fehling A ($CuSO_4 + H_2SO_4 + H_2O$)
- Fehling B (Na, K tartrate + $NaOH + H_2O$)

Fehling Reaction

- Sucrose + H_2SO_4 $\xrightarrow{\text{heat}}$ Glucose + Fructose $\xrightarrow[\text{K, cuprictartrate}]{\text{+ NaOH}}$ $Cu_2O\downarrow$

$$CuSO_4 + 2\ NaOH \longrightarrow Cu(OH)_2 + Na_2SO_4$$

$$Cu(OH)_2 + R\text{-}CHO \longrightarrow Cu_2O\downarrow + RCOOH$$

Red

C) Determination of foreign matter and dyes

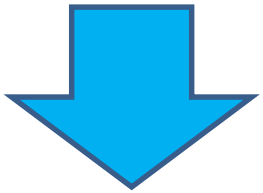
2 g sucrose+ 1ml water 

* soluble without residues
* unscented, sweet tasty syrup is obtained

D) Invert sugar and other reductor substances:

The product of sucrose hydrolysis is called invert sugar. Composed of glucose and fructose in equal amounts.

Aqueous-sucrose solution + Cupric Potassium Tartrate TS

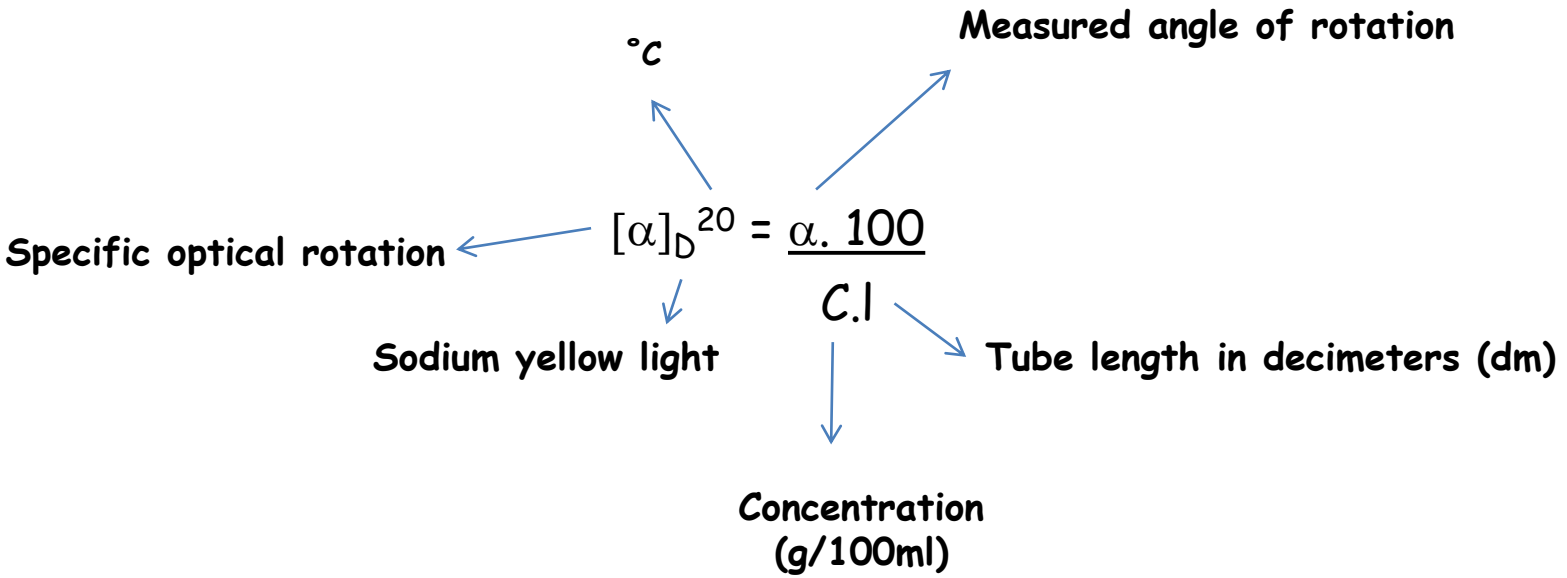


boiling

No color change

E) SPECIFIC OPTICAL ROTATION

Angle of spesific optical rotation ($[\alpha]_D^{20}$) **of a substance is the angle of rotation which occurs as polarized sodium D-line passes through a 10 cm (1 dm) long tube full of a solution containing 1 g optically active substance in 1 ml, at 20-25°C.**



- 20% *m/v* solution is prepared (5 g sucrose + 25 ml water in volumetric flask)
- Optical rotation is measured using polarimeter
- Specific optical rotation is calculated
- Between +66 - + 66.7 according to TP1974

POLARIMETRY

- Polarized light is the wave of light which oscillates on a plane.
- Plane polarized light beam is obtained using Nicol prism.
- An optically active compound makes linearly polarized light rotate as it passes through it. The degree of this rotation is specific for diferent compounds.
- Typical optically active substances contain at least one asymmetric atom in their molecule structure. A sample that contains only one enantiomer of a chiral molecule is said to be optically pure.
- Molecules that shift the angle clockwise are known as dextrarotatory ("right-rotating"), d or (+), while those that shift the angle counter-clockwise are called levarotatory ("left-rotating"), l, or (-)
- The method which provides the determination of concentration or structure elucidation of a compound depending on the principle of specific rotation is called polarimetry.

The factors of affecting the angle of rotation:

- Temperature
- The wavelength of the light
- The length of the path taken by the light in the sample
- Structure of the compound
- Concentration of compound in the solution

!!!! Pay attention while using polarimeter !!!!

-The solution should be fresh prepared and the mesurement should be done immediately after the preparation of solution.

-Solution must be clear.

-Polarimeter tube should be closed without air stuck inside.

Identification of Some Active Compounds

1. CARDIAC GLYCOSIDES

- Cardiac glycosides; group of steroidal glycosides act as cardiotonic agent. They increase tone, excitability and contractility of cardiac muscles.
- The aglycons of cardiac glycosides have steroidal structure and composed of cyclopentano phenanthrene ring and 5 or 6 membered unsaturated lactone structures linked to this ring.
- According to the type of lactone ring Cardiac Glycosides are classified into

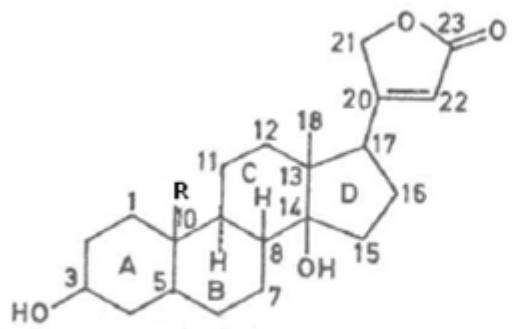
  -Cardinolides: They are C-23 containing 5-membered unsaturated lactone ring

  -Bufadienolides: They are C-24 containing 6-membered unsaturated lactone ring

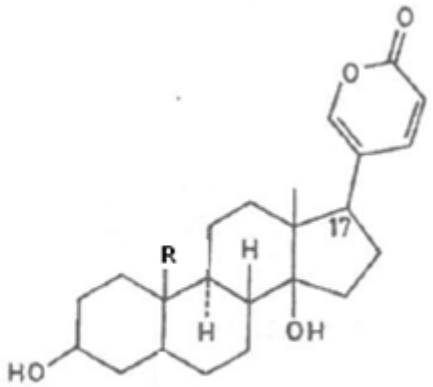
- Cardioactive glycosides contains 2 or more monosaccharides . Apart from monosaccharides, they may contain deoxyose, especially 2-deoxyose.

- The identification reactions for cardiac glycosides are based either on the color reactions of aglycone or desoxyoses. According to this:

1. **Reaction of Keller-Kiliani:** Identification of 2-deoxyose
2. **Reaction of Baljet :** Identification of 5-membered unsaturated lactone ring



Cardenolide (C23)



Bufadienolide (C24)

- **Experimental Procedure:**

Drog: Folia Digitalis

- Powdered sample+ 70% EtOH → Boiling for 2 min. → Filter

Filtrate
+
Dilute to double of its volume with water
+
Pb-subasetat

To get rid of chlorophyll and other pigments

↓

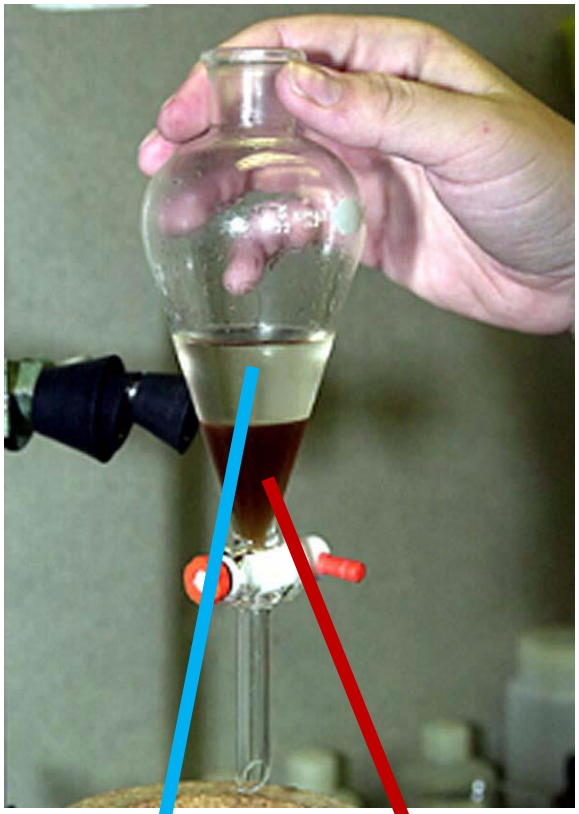
filter

↓

the filtrate is extracted with chloroform in a separation funnel

The chloroform phase

The chloroform phase is separated into 2 capsules. Evaporated in water-bath



Water phase

Chloroform phase

1. Apply Keller-kiliani R. in porcelain capsule

Residue in capsule

+

3.5% glacial acetic acid-$FeCl_3$ solution

↓

Concentrated H_2SO_4 is poured droppwise alongside the tube with

↓

On the contact surface of two liquids brunette ring;

↓

2-deoxyose

2. Apply Baljet R. in porcelain capsule

Residue in capsule

+

Baljet reagent

↓

Orange- Red color

↓

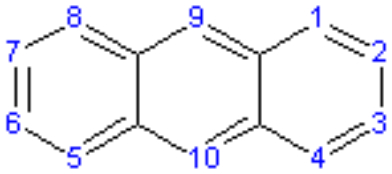
5-membered unsaturated lactone ring

Baljet reagent:

- **95 k %1 picric acid**
- **5 k % 10 NaOH**

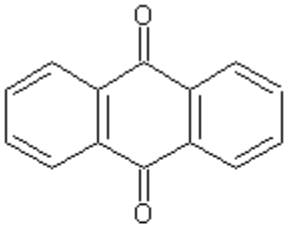
<u>The principle:</u> color change Due to the nitridation of 5 membered-unsaturated lactone rings in alkaline medium

2. ANTHRAQUINONES



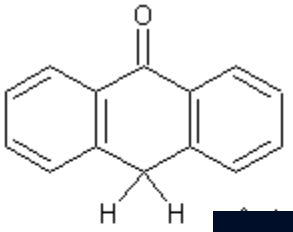
Anthracene

Their aglycone is anthracene derivative.



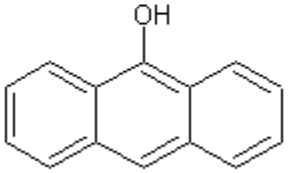
red

oxs



Anthraquinone

Anthrone



Anthranole

- Borntrager's reaction is carried out to identify free anthraquinone derivatives. As the result of this reaction red color is observed.
- This reaction is only positive for free anthraquinone derivatives.
- The reaction is conducted after preliminary hydrolysis for glycosides.
- For the reduced derivatives, namely anthrone and anthranole, oxidation reaction is needed prior to identification test.

Experimental Procedure:

Drug: Folia Sennae

Powdered sample + dil.H_2SO_4 —boiled→ Filter (hot)

Aglycones precipitate in cold solution

Hydrolysis is carried to get free anthraquinones from glycosides. Anthracene derivatives need to be hydrolyzed in general, since they occur in glycoside forms in the plant.

Take to separation funnel

↓

Filtrate

+

extracted with benzene

↓

Not shaken strongly to prevent emulsion formation

the benzene layer becomes yellow color in case of the presence of anthraquinones

The upper phase (benzene phase) is transferred into a tube

↓

+ shake with 10% NH_3 → The lower layer (NH_3) becomes **RED**

3. CYANOGENIC GLYCOSIDES

- Glucosides which form hydrogen cyanide (HCN) when they are hydrolized. Aglycone moieties consist of HCN + aldehyde or HCN + ketone.

- The identification reaction is based on the formation of Na-iso purpurate.

Deneyin yapılışı:

Drog: Semen Amygdalae amarae

Powdered sample + moistened with water

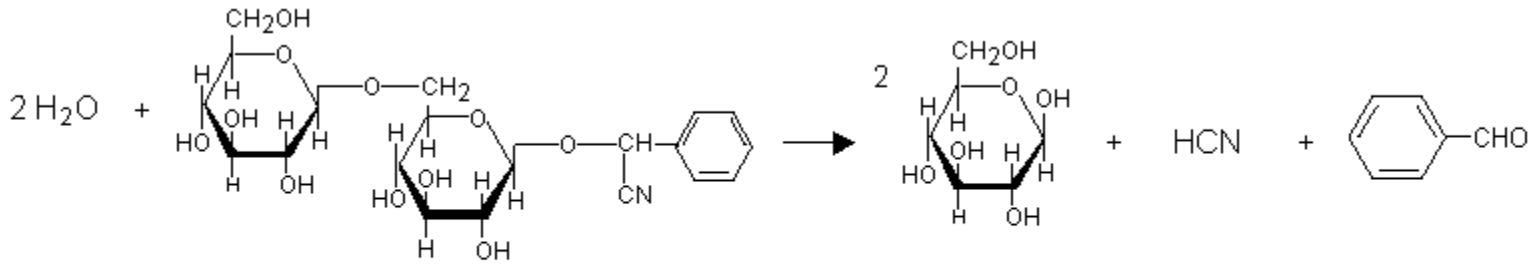
water is added to hydrolyze the glycosides

filter paper soaked with picric acid is steeped with Na_2CO_3
Filter paper is attached to the neck of flask using a plug

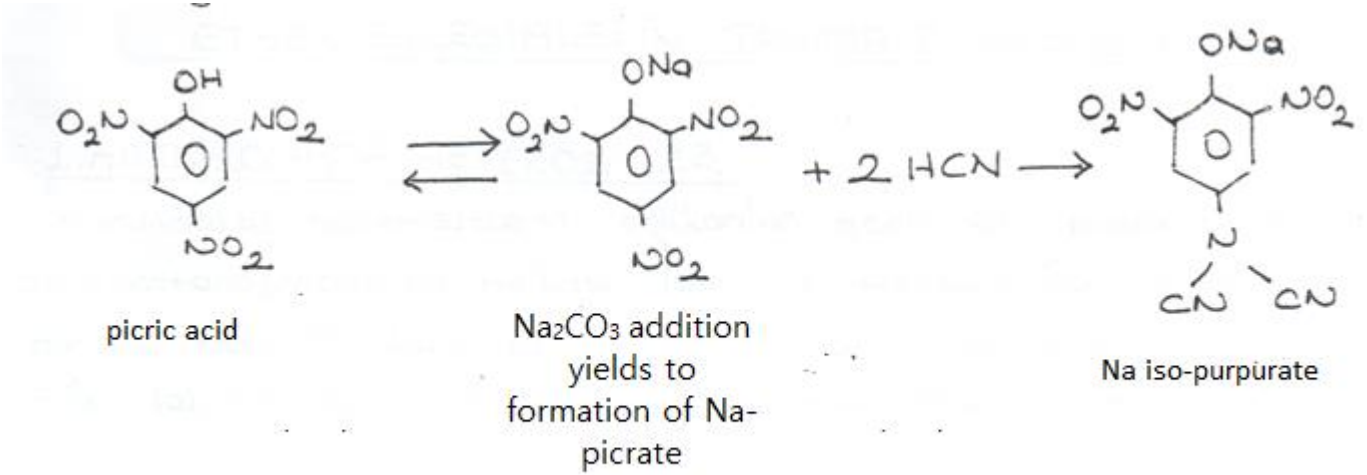
heat

Sodium picrate paper becomes brick red color in the presence of HCN

The principle of the experiment:



Water + amygdalin → 2 glucose + cyanide + benzaldehyde



picric acid

Na_2CO_3 addition yields to formation of Na-picrate

Na iso-purpurate