PHARMACOGNOSY-I PRACTICE

Identification of Monosaccharides

Identification of Monosaccharides

- **Monosaccharides, composed of C, H, O, are frequently seen in nature in different forms. Two or more –OH groups can be present in the structure of these molecules.**
- **Monosaccharides, are often termed as sugar, monomeric sugar compounds or oses.**

- **Aldose: If the main group of sugar is Aldehyde,**
- **Ketose: If the main group of sugar is Ketone.**

Identification of Monosaccharides

- **We aim to conduct some simple tests for the determination of the monosaccharides.**
- Identification of Monosaccharides

  1) Molisch's Test
  2) Fehling's Test
  3) Seliwanoff's Test
  4) Osazone Test

Sample Name: *Radix Liquiritae*

Test Solutions should be prepared to examine monosaccharides with Molisch's, Fehling's and Seliwanoff's tests.

Preparation of Test Solution-I

- **2 spoon of dried and powdered sample (R. Liquiritiae) is crushed with 10 ml water in a beaker.**

BEAKER

SPOON

- … mixed vigorously by using a glass rod.

GLASS ROD

- …filtered through a fluted filter paper using a funnel.

FLUTED FILTER PAPER

!

If filtrate is going to used fluted filter paper should be used

In case the precipitate is going to taken plain filter paper should be chosen.

FUNNEL

How to Fold Fluted Filter Paper?

Start with a filter paper of the appropriate size. Fold the filter paper in half and crease. Fold in half again and crease. Then round the edge of the filter paper using scissors. And then fold in half again and crease. And then fold in half again and crease. Finally open it. Place it in the funnel.

Preparation of Test Solution-II

- Extraction process is repeated 2 more times and collected filtrates are combined to yield 30 ml total filtrate.
- Total filtrate is concentrated on bunsen burner until 10 ml of the solution is left.

BUNSEN BURNER

10 g lead is dissolved in 100 ml acteic acid

- 10% lead acetate solution (5 ml) is added into the concentrated filtrate (to precipitate unwanted matter in the mixture)
- …and filtered through the fluted filter paper.
- Precipitate is discarded.
- Few drops of saturated Na_2HPO_4 solution is added to the filtrate (to precipitate the excess Pb) and filtered (through a fluted filter paper).

Carbohydrates (diholosides and heterosides) are hydrolysed and the monosaccharides occur.

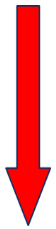
Preparation of Test Solution-III

- The filtrate is treated with dilute HCL (2-3 ml) and boiled gently for 5-10 minutes.
- Final mixture is transferred to a volumetric flask (25 ml) and filled with distilled water up to 25 ml to obtain the test solution.

VOLUMETRIC FLASK

1.Fehling's Test

- **This test is utilized to determination of reductor monosaccharides. Free aldehyde or ketone groups of monosaccharides can reduce the various metallic ions in alkaline medium. When monosaccharides are exposed to heat, they reduce deep blue solution of copper (II) ions to red precipitate of insoluble copper oxide.**



CU_2O
brownish-red precipitate

1.Fehling's Test

- **Fehling’s reagent is freshly prepared by mixing equal volumes of two stock solutions A and B.**

- **FEHLING A is copper sulphate solution (CUSO4 + H2SO4 + H2O)**
- **FEHLING B is potassium sodium tartrate (NA, K Tartrate + NAOH + H2O**

1.Fehling's Test

- Equal volumes (2ml) of Fehling A & Fehling B are put onto the 1 ml of the test solution in a test tube and tube is placed in a boiling water bath for a few minutes.
- Brownish-red precipitate (Copper oxide) and color change are observed in case presence of reductor monosaccharides in the sample.

1.Fehling's Test

- The deep blue ingredient in Fehling's solution is the bis(tartrate) complex of Cu_2^+

- In the process, the copper(II) ions of the complex are reduced to copper(I) ions. Red copper(I) oxide precipitates, which indicates a positive result.

1.Fehling's Test

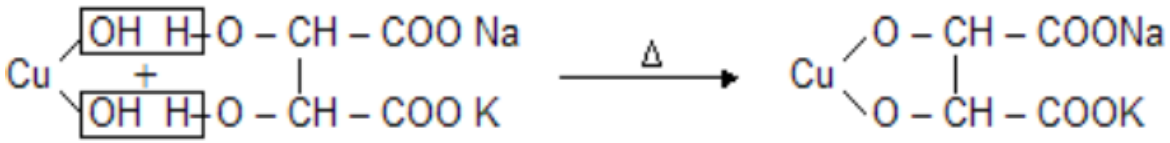
A : B

$CuSO_4$ solution : Alkaline solution of sodium potassium Tartarate

OH – CH – COONa
|
OH – CH – COOK
Rochelle salt

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

$Cu(OH)_2 \xrightarrow{\Delta} CuO + H_2O$



Deep blue colour complex (Fehling's solution)

Deep blue colour (CuO) $\xrightarrow{RCHO}$ Cu_2O + RCOOH
Red ppt.

2. Molisch Test

- **It is a test commonly used to detect monosaccharides.**
- **Molisch's reagent is 15% alcoholic solution of α-naphthol.**

- **The concentrated acids induce the formation of furfural and 5-hydroxymethyl furfural from pentoses and hexoses, respectively. These compounds react with α-naphthol which presents in the test reagent, and gives purple colour.**

2. Molisch Test's

- **1-2 drops of α-naphthol solution (Molisch's reagent) is added on to 2 ml of the test solution in a test tube.**
- **The tube is carefully inclined and concentrated H_2SO_4 is poured dropwise using a dropper, along the sides of the tube.**

2. Molisch Test’s

- **A violet circle is observed at the junction of two liquids in the test tube.**

2. Molisch Test’s

3. Seliwanoff's Test

- It is a color reaction to determine ketoses.
- The Seliwanoff reagent consists of resorcinol and concentrated hydrochloric acid.
- This test is used to discriminate aldoses (such as glucose) and ketoses (such as fructose). Acid dehydrates keto-hexoses and triggers occuring of 5-hydroxymethyl furfural.

3. Seliwanoff’s Test

- **5-hydroxymethylfurfural reacts with resorcinol in reagent then produce a red colour within two minutes. Aldo-hexoses reacts slowly and give the reddish colour after the ketoses.**

3. Seliwanoff's Test

- **1 ml of Seliwanoff's reagent is put on to 1 ml of test solution and heated to boiling in a water bath. A cherry-red condensation product is observed indicating the presence of ketoses in the test solution.**
- **1-micro-spatula sucrose and 3-5 ml water are mixed in a test tube and dissolved. Then 2 ml of Seliwanoff's reagent is added into the test tube, heated to boiling in a water bath. Aldo-hexoses give also the same reaction, but more slowly yielding yellow to faint pink color.**

3. Seliwanoff’s Test

Hydroxymethylfurfural

Fructose

Resorcinol

4) Osazone Test

- Sugar is heated with phenyl hydrazine hydrochloride, sodium acetate and acetic acid.
- Ketoses and aldoses react with phenylhydrazine to produce phenylhydrazone which further reacts with another two molecules of phenylhydrazine to yield osazone.
- Osazones are insoluble yellow compounds in water.
- These crystals have specific structures, precipitation times and melting points which help their identification.

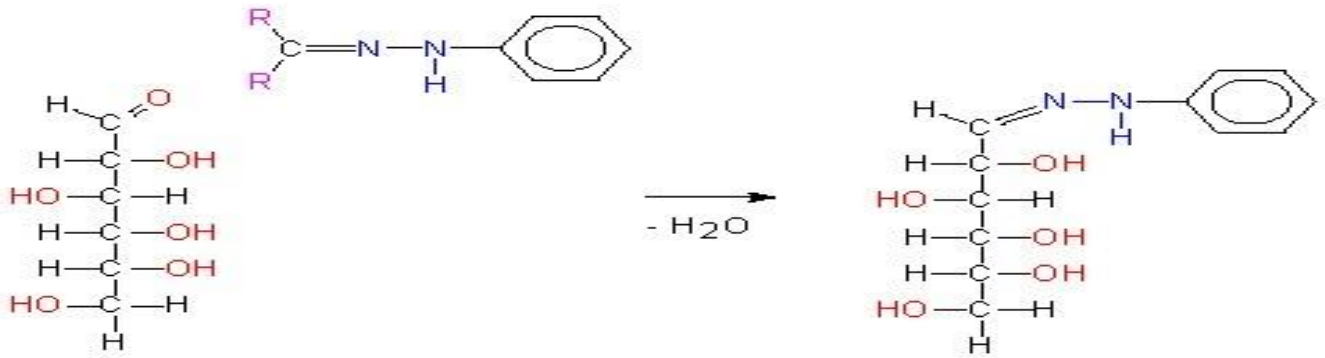
4) Osazone Test

- Depending on the time required for the formation of the insoluble yellow osazone, sugars can be identified as following:
- Mannose: 1-5 min
- Fructose: 2 min
- Glucose:5 min
- Xylose: 7 min
- Arabinose: 10 min
- Galactose: 20 min

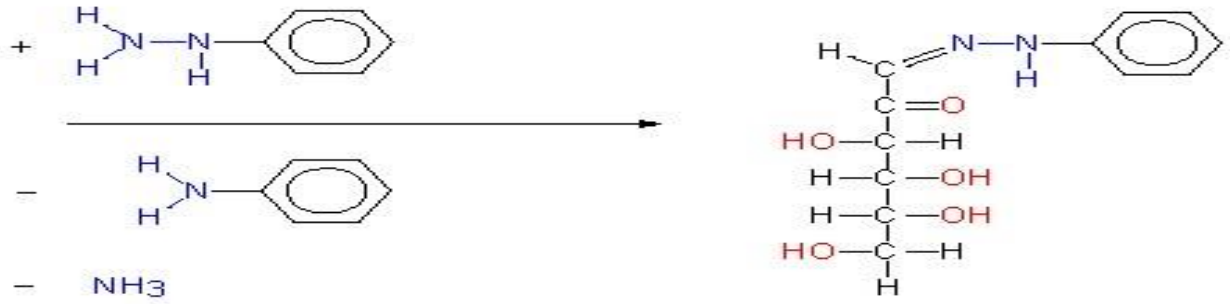
4) Osazone Test

- 1-micro-spatula unknown sugar sample is put in a test tube.
- 1-micro-spatula sodium acetate, 10-11 drops of phenylhydrazine hydrochloride and 6-7 drops of glacial acetic acid are added, mixed and heated in boiling water bath. Precipitation time of osazone cyristal is noted and evaluated to determine monosaccharide.

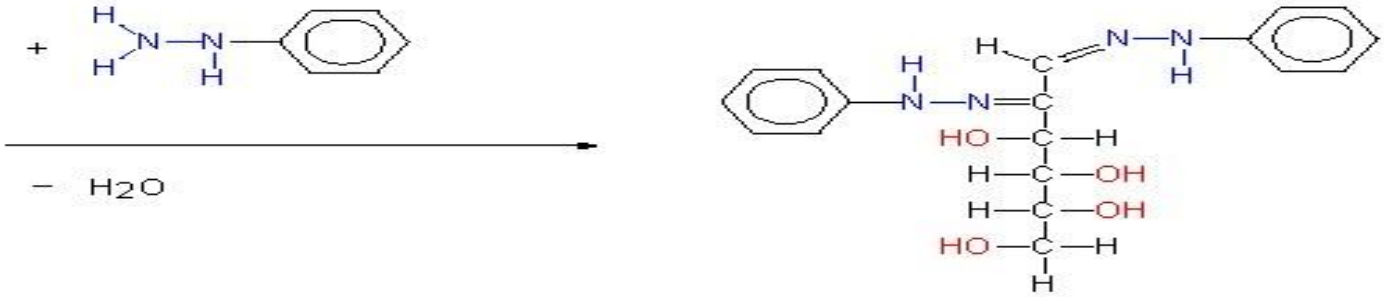
1. D - Glucose + Phenylhydrazin ⟶ Hydrazon



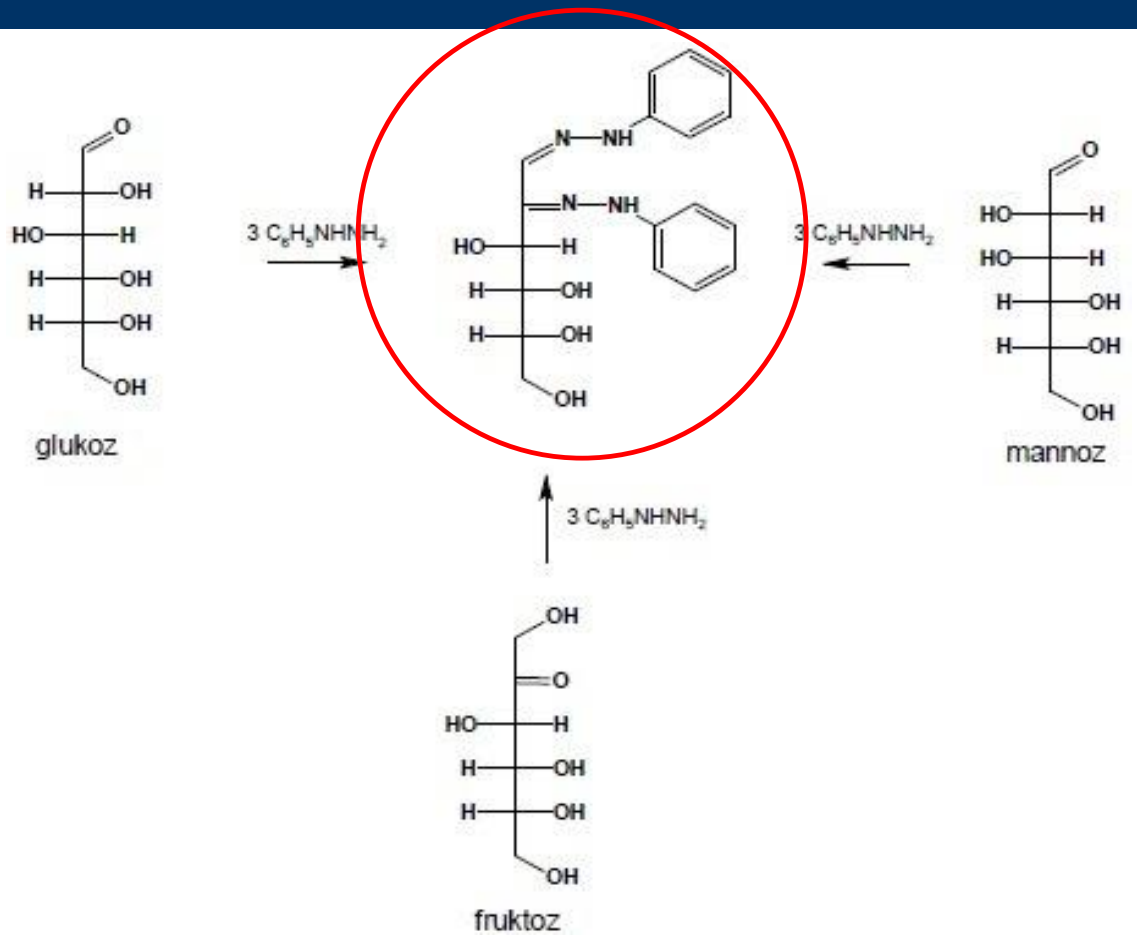
2. + Phenylhydrazin - Anilin - Ammoniak ⟶ Osonhydrazon



3. + Phenylhydrazin - Water ⟶ Osazon



4) Osazone Test



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