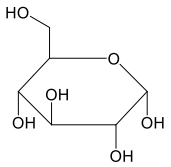
Practice No. 1

QUALITATIVE ANALYSIS OF DRUGS CONTAINING CARBOHYDRATES

Carbohydrates are a group of compounds that contain C, O, and H in their formula. They are classified into sugars and polysaccharides. Sugars are divided into two major groups, simple sugars and oligosaccharides.
Simple sugars consist of only one sugar unit and can't be hydrolyzed; they are subclassified according to the number of carbon atoms and they are divided into aldoses and ketoses.
Oligosaccharides are condensation products of two or more molecules of simple sugars and are subclassified according to the number of molecules of simples sugars yielded upon hydrolysis.

Simple sugars (monosaccharides):

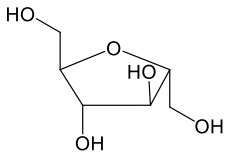
Glucose



(+)-D-glucopyranose

Other names: dextrose, grape sugar, blood sugar
This sugar is a frequent constituent of oligosaccharides. It is a hexose that occurs as white crystals that have a sweet taste.

Fructose

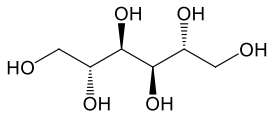


β-D-fructofuranose

Other names: levulose, fruit sugar
This sugar is a hexose that can found in many fruits.

Mannitol

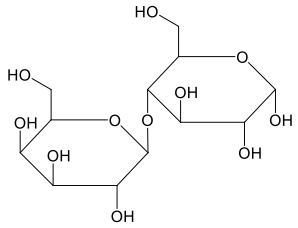
White crystalline sugar alcohol used as an osmotic diuretic agent and a weak renal vasodilator.



Mannitol

Oligosaccharides (disaccharides):

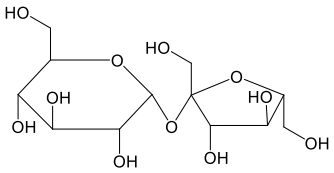
Lactose



lactose (*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose)

Milk sugar
Lactose is the principal sugar of mammalian milk, but appears to be absent in higher plants

Sucrose



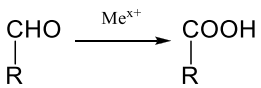
Sucrose (β-D-fructofuranosyl-α-D-glucopyranoside)

Sugar, cane sugar, beet sugar
Sucrose only occurs in plants that contain chlorophyll and hence are capable of photosynthesis.

Practical part:

Prepare 25 ml of 2% aqueous solution of the investigated sugar and perform the tests with the following reagents and solutions. Write down the resulting colors in tables.

1. Oxidation of sugars, metal ions



Fehling test (complex copper salt) for reducing sugars
2 ml of the reagent is heated with 2 ml of the sugar solution. In the presence of reducing carbohydrates, a yellow-orange color appears, red or green color

may also appear as a result of the precipitation of cuprous oxide. The color depends on the particle size of the carbon chain expelled.

Fehling reagent is prepared by mixing Fehling I and Fehling II in ratio 1:1 immediately before use!!!

Barfoed test (a solution of copper acetate) for monosaccharides
The reduction is more difficult than in the case of Fehling's solution
2 ml of the reagent is heated with 2 ml of the sugar solution. This reagent reduces only monosaccharides. It can be used to distinguish reducing monosaccharides from reducing disaccharides.

Tollens' test (ammonia complex salt of silver) for aldehydes
To 1 ml of 5% aqueous solution of silver nitrate, add a few drops of 10% ammonium hydroxide, the released silver oxide is dissolved by adding a few drops of 10% ammonium hydroxide, the reagent is thus prepared.
Add 1 ml of the sugar solution after shaking and allow it to stand for 10 minutes. If the solution does not release silver (which should form a metal mirror on the sides of the tube), warm the tube slightly. Non-exclusion of silver indicates a negative test.
After the reaction, pour the tube contents immediately!!!

Nylander's test (complex bismuth salt)
2 ml the test solution is boiled with 1 ml of the reagent. If the solution forms a dark or black precipitate (bismuth), it is a positive result.

Boettger's test
To 1 ml of the sugar, add a few crystals of alkali bismuth nitrate and a few drops of 10% sodium hydroxide. At first, white precipitate of bismuth hydroxide appears, which upon boiling is reduced and turns black (bismuth).

2. Color reaction of sugars

Reaction with lead acetate
2 ml of the sugar is heated with 2 ml of 10% lead acetate. A yellow color first appears. Lead hydroxide then precipitates upon the addition of ammonia

Molisch test (reaction with α-naphthol) for carbohydrates
To 1 ml of the sugar solution add 1-2 drops of methanolic solution of α-naphthol, then carefully sublayer 1 ml concentrated sulfuric acid. The presence of carbohydrates is indicated by the appearance of a purple ring at the interface

Seliwanoff test
To 1 ml of the sample add 1 ml of the reagent (a solution of resorcinol and HCl) and heat. Ketoses give red color

Anthrone test
To 1 ml of the sample, carefully add 1 ml of anthrone reagent to form two layers. If there is an interface of blue or blue-green color, the reaction is positive. Some deoxy sugars yield a red color.

3. Identification

For the following sugars, perform the identification tests and record the results in a table.

Glucose

1- 1 ml of glucose is heated with 1 ml of the alkaline solution of picric acid. A red color (picramic acid) appears (fructose and lactose give a positive test too)

Preparation of the alkaline solution of picric acid: 9.5 ml of 1% picric acid is mixed with 0.5 ml of 10% sodium hydroxide and diluted with 10 ml of methanol (the amount prepared is for the whole bench).

Fructose

1- 1 ml of vanillin (in HCL) is mixed with 1 ml of the test sample and heated. In the case of fructose, the reaction gives a red color.

2- Mix 1 ml of fructose with 1 ml of the reagent Stanley - Benedict and heat in a water bath at 37 ° C for 30 minutes. Fructose reduces Cu^{2+} to the red-brown precipitate of Cu_2O (glucose and lactose do not react under these conditions).

Mannitol

1- To 5 ml of the sample, add 1 ml of 5% Na2CO3 solution and 3 ml of KMnO4. Heat the mixture for 2 minutes in the water bath, filter and add an equal volume of Fehling's reagent. The orange-red precipitate will disappear upon boiling.

2- 1.0 g of the sample is dissolved in distilled water free of CO_2 and diluted to 10 ml (solution A). To 3 ml of the freshly prepared solution of catechol (10 g / 100 ml under cooling in iced water), add 6 ml conc. sulfuric acid. To 3 ml of the cooled mixture, add 0.3 ml of solution A and heat gently over a flame about 30 seconds to form a pink color.

Lactose

1- Dissolve 0.25 g of lactose in 5 ml of water, and add 5 ml 17.5% ammonium hydroxide. The solution is then heated in a water bath at 80 ° C until a red color appears.

2- The oxidation of the sugar by concentrated nitric acid results in the formation of the poorly water-soluble galactaric acid.

3- 0.5 g of sodium acetate and 0.5 g phenylhydrazine are dissolved in 2.5 ml of water and heated long enough until complete dissolution. Add 0.2 g of lactose and heat in the water bath for about 30 minutes. After intense cooling,

crystals of phenylosazone appear. Phenylosazone forms upon the reaction of lactose with phenylhydrazine. Phenylosazone is soluble in hot water.

Sucrose

1- Add a few drops of concentrated sulfuric acid to 0.1 g of sucrose. The sugar becomes carbonized at low temperature (unlike glucose and lactose).

2- 3 ml of 10% sucrose solution is boiled for 3 minutes with a few drops of concentrated HCl. After neutralization to litmus and reaction with Fehling's reagent, a red precipitate occurs.

Mel - Honey

The product results from the flower nectar and the sweet juices of different plant organs by the enzymatic cleavage in the stomach of honeybees, *Apis mellifica L.*, *Apidae*. It contains primarily glucose, fructose and to a lesser extent sucrose and other sugars.

Identification
1- 20% aqueous solution of honey reacts slightly acidic to litmus (formic acid, etc.).
2- By adding a few drops of tannin solution to the aqueous solution of honey, it becomes cloudy. Immediately noticeable (honey proteins)

Purity test
5 g of honey are mixed with 14 ml ether for 1 minute in a plate, the ether extract is then filtered into a porcelain dish and dried in a warm water bath. To the dry residue, add a few drops of the freshly prepared 1% solution of resorcinol in conc. HCl. The color of the solution should not change permanently to red.
(Artificial honey, invert sugar added)

<u>Analysis of suar content using TLC</u>

Malti extractum - Malt extract

Dried, crushed malt (usually barley - *Hordeum vulgare, Poaceae*), pour water (52 ° C) in the ratio 1:5 and heat for 15 minutes at 70-75 ° C. Filter the suspension through cotton wool and use the filtrate to prove the presence of sugars.

Ceratoniae fructus - Locust beans

The uncracked semifleshed fruits of Ceratonia siliqua L. Caesapliniaceae. Contain up to 45% sugars.

Pour 20 ml of water-methanol (2:3) mixture on 3 g of the crushed berries in a

beaker, cover the beaker with a watch glass and macerate for 10 minutes. Filter the suspension through cotton wool and use filtrate for TLC.

Passulae minores - Raisins

Dried ripe berries of grapevine *Vitis vinifera, Vitaceae*.
Pour 20 ml of water-methanol (2:3) mixture on 3 g of the crushed berries in a beaker, cover the beaker with a watch glass and macerate for 10 minutes. Filter the suspension through cotton wool and use filtrate for TLC.

Cynosbati fructus – Rose hip

Dried fruit of *Rosa canina L., Rosaceae.*

The drug contains up to 2% L-ascorbic acid (a sugar derivative)

Ascorbic acid identity test: Dissolve 1.0 g of the sample in 20 ml water free of CO2. To 1 ml, add 0.2 ml of dilute nitric acid and 0.2 ml of silver nitrate to form a gray precipitate.

Test for sugars: pour 20 ml of water-methanol (2:3) mixture on 3 g of the crushed berries in a beaker, cover the beaker with a watch glass and macerate for 10 minutes. Filter the suspension through cotton wool and use filtrate for TLC.

Thin-layer chromatography (TLC)

Perform TLC on silica gel layer.
Eluting mixture: water : methanol : acetic acid : anhydrous dichloroethane (10:15:25:50)
Reference solution: glucose, fructose, lactose and sucrose in water-methanol mixture
Detection reagent: 0.5 g thymol in a mixture composed of 5 ml of sulfuric acid and 95 ml of 96% alcohol

2 μl of the sample and the standard solutions of glucose, fructose, lactose and sucrose are applied on the layer separately. Spots are dried, and developed until the mobile phase level reaches about 1 cm below the upper edge. After that it is dried in a stream of warm air and evenly sprayed with the reagent and heated for 10 min at 130 ° C.