PHG 322

Lab#

2

Quantitative Analysis of Glycosides

PHG 322 Practical course

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Background:

How a spectrophotometer works, and what is its principle?.

Objectives :

- To understand the methods of quantitative analysis of glycosides (e.g. Digitalis & senna)

- To understand the spectrophotometer and relationship between absorption of light and color

- To know some physical and chemical properties of digitalis and senna constituents.

- To learn some analytic laboratory skills.

1. Spectrophotometric determination of digitalis glycosides :

1.1 Principle:

Digitalis cardiac glycosides develop an orange-red color with baljet's reagent (picric acid in alkaline medium) . The intensity (absorbance) of color produced is proportional to the concentration of the glycosides.

1.2 Materials:

◈ Digitalis Tincture

◈ Baljet reagent freshly prepared (95 ml 1% picric acid + 5 ml 10% NaOH) mixed immediately before use and filtered through a sintered glass funnel.

1.3 Preparation of Tincture:

A 10% extract in 70% alcohol is prepared by shaking. 1 gm dried powder digitalis leaves + 20 ml 70% alcohol, left overnight with occasional shaking for 2 hrs then filtered.

1.4 Procedure:

A. Purification of the tincture:

1. Transfer 8ml of prepared digitalis extract or of tincture to a 100 ml volumetric flask . Add 60 ml distilled H_2O and 10 ml 12.5% lead acetate(to ppt resins, tannins and pigments). Shake well and complete to the volume (100ml) with distilled H_2O . Mix well and filter through a filter paper .

Technical Skill: Transferring a solution by pipette .

2. Pipette 50 ml of the filtrate into another 100 ml volumetric flask . Add 10 ml of 4.77% disodium hydrogen phosphate (Na_2HPO_4) solution (to ppt the excess Pb^{++} ions). Mix well , complete to volume with distilled water mix well again .
3. Filter , through a filter paper . If not clear , refilter through the same filter paper….Why?

B. Spectrophotometric Determination:

1. Transfer 10 ml of the purified and clear filtrate into a clean dry stoppered erlynmeyer flask . Add 10 ml Baljet's reagent .
2. Carry out a blank at the same time using:

3. Allow to stand for one hour (time necessary for maximum color development). Then dilute the solution with 20 ml distilled H_2O (by automatic buret) and mix .

Technical Skill: Reading a buret and meniscus.

4. Read the intensity of the color obtained against the blank provide , at 495 nm using a suitable spectrophotometer. The color is stamle for several hours.

   The different between Experiment and blnk (E-B) = original reading.

1.5 Calculation:

In the assay of tincture digitalis, 10 ml of the 10% tincture (=0.8 g powdered leaves) are diluted during purification to 200 ml, from which 10 ml (=0.04 g powdered leaves) are used in the assay, after being treated with 10 ml Baljt's reagent, and diluted with 20 ml of water (to a total of 40 ml). Assuming the absorbance of the tincture at 495 nm = A

The concentration (%) will be = $\frac{A \text{ g } \%}{170}$ which is the amount of glycosides present per 100 ml of the measured solution.

Thus percentage of glycosides: = $\frac{A \times 40 \times 100}{170 \times 100 \times 0.04}$ g% of total glycosides calculated as digitoxin

% of total glycosides = $\frac{A \times 100}{17}$ g% of total glycosides Calculated as digitoxin

N.B. In the assay, 70% alcohol is used for the extraction of cardiac glycosides of digitalis, since it prevents enzymatic hydrolysis and in the same time extracts the lowest amount of undesirable materials.

1.6 Preparation of the standard curve for digitoxin :

1. Prepare a 0.02% solution of digitoxin in chloroform-methanol (1:1 v/v) mixture (1 ml = 0.2 mg).
2. Transfer different volumes, viz. 1,2,3,4 and 5 ml (equivalent to 0.2, 0.4,0.6,0.8 and 1 mg of digitoxin respectively) each to a dry Erlynmeyer flasks.
3. Evaporate the solvent in each flask on a water bath and dissolve each residue in 0.35 ml 90 percent alcohol. Complete volume to 10 ml with distil-led water by means of graduated pipette.
4. Add 10 ml of freshly prepared Baljet's reagent to each flask. Set aside for one hour at room temperature, then dilute with 20 ml of distilled water and read the absorbance (A) of the developed orange-red color at 495 nm using a suitable spectrophotometer against a blank in which digitoxin solution is replaced by 10 ml of distilled water.
5. For each concentration, the mean of three determinations is taken for plotting the standard curve, depicting the concentration against absorbance (A).

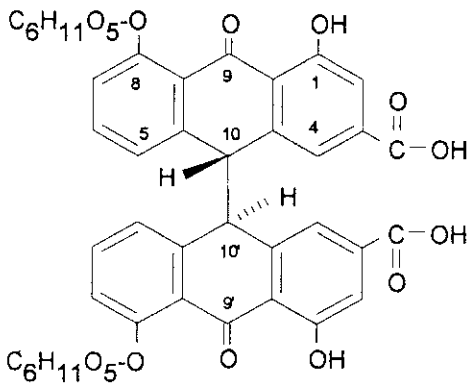
2. Spectrophotometric Assay of Sennosides in Pharmaceutical Formulation (Senna tablets e.g Pursennid® tablets)

2.1 Principle:

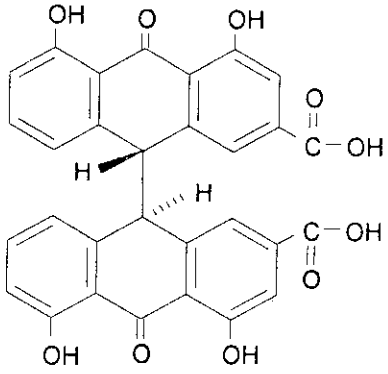
a- Senna contains mainly combined anthraquinones (sennosides) with only small amount of free anthraquinones. Since senna is assayed for the content of sennosides, removal of free anthraquinones sould be done first by extraction with an organic solvent.

b- Hydrolisis of anthraquinone glycoside into free anthraquinone (**rhein**)

c- Addition of standard alkali e.g. 1N KOH (Borntrager's test) gives a rose red color which is measured colorimetrically at 500 nm.



Sennoside A (laevo) and B (meso)



Sennidin (A and B)

2.2 Procedure:

1. Triturate 2 tablets, in a mortar, with 20 ml of warm dist. Water, then transfer the mixture quantitatively (using 20ml dist. Water) into a conical flask.

2. Digest the mixture on a boiling water bath for 15 minutes and filter while hot into a 250 ml separatory funnel. Wash the flask with 20 ml dist. H_2O and add washings to the separator.

3. Cool the mixture, add 2 drops HCI, and shake gently with 2 quantities, each of 15 ml CHCI3; discard the $CHC1_3$ 1ayer .

4. Filter the aqueous layer through a piece of cotton into a100 ml volumetric flask. Complete with H_2O to the mark.

5. Pipette 10 ml of the filtrate into a 250 ml round bottom flask (or conical flask) .

6. Adjust PH of the solution to pH 7-8 with few drops of 5%w/v solution of Na_2CO_3 (acout 5 g)

7. Add mixture of (8ml of 15% $Fecl_3$ + 12 ml H_2O) and mix.

8. Heat on a boiling water bath under reflux for 20 mins.

9. Add 1.3 ml conc. HC1 to render acidic and continue reflux for a further 20 minutes, shaking fre quently until ppt. is dissolved.

10. Cool, transfer the mixture quantitatively to a separator and extract with 3 quantities each of 25 ml of ether, passing each ether fraction over anhydrous Na2S0 4 (about 5g) placed in a small funnel, receiving the ether extract into a 100 ml volumetric flask; complete to volume with ether (100 ml).

11. Pipette 10 ml of etherial extract into a beaker, evaporate just to dryness on a water bath and dissolve the residue in 10 ml IN KOH accurately measured; filtering in case of any turbidity.

12. Without delay measure t}le absorbance of the color of the resulting solution at 500 nm, using 1 cm cuvette.

13. Determine the content of total combined anthraquinones in Senna, calculated as *sennoside A* or *B,* taking "200" as the value of E (1 %, 1cm). Each 1 mg of Rhein, is equivalent to 1.8 mg of Sennoside A or B.

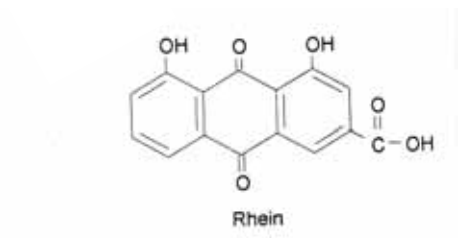
NOTES:

i. The method of assay depends on Borntrager's Test which is based on the reaction of anthraquinones with alkali to give a red color.

ii. Anthraquinone aglycones unlike the glycosides are soluble in organic solvents. Being phenolic, they are also soluble in alkali, giving a characteristic red color.

iii. Sennoside A is insoluble in H_2O, while B is more soluble but both sennosides A and B are soluble in $NaHCO_3$. Such solubility in $NaHCO_3$ may be utilized in separating such gIycosides containing carboxylic groups, from other phenolic glycosides.

iv. The role of dil. HCI is for hydrolysis of sennosides to give sennidins. However, the role of $FeCl_3$ at pH 7-8, under reflux, is to make cleavage at $C_{10} - C_{10}'$ and oxidation to give 1,8-dihydroxyanthraquinone 3-carboxylic acid-8-gIucoside, which can be hydrolyzed by heating with dil. HCI.



Rhein

2..3 Calculation

$$\text{Sennoside Content of each tablet} = \frac{A}{170} \times \frac{100}{10} \times \frac{100}{10} \times \frac{100}{2}$$

$$= \frac{A \times 1.8 \times 100}{4} \text{ mg}$$