

# Isolation, Identification and Analysis of Glycyrrhetic acid

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# Glycyrrhetic acid

- Biological source:
- Glycyrrhetic acid is a triterpenoid saponin glycoside obtained from the roots and stolons of *Glycyrrhiza glabra* belongs to family-Leguminosae.
- The chief constituent of liquorice is Glycyrrhizin (K & Ca salt of Glycyrrhizinic acid, a glucoside) which on hydrolysis yields Glycyrrhetic acid (Glycyrrhetic acid).
- Isolation:
- The isolation of glycyrrhizin from *Glycyrrhiza* is based on its solubility.

# Glycyrrhetic acid

- **Method 1:** Required quantity of coarse powder of Glycyrrhiza roots is extracted with boiling water, filtered and concentrate the extract to obtain a crude liquorice extract.
- Then this extract is again extracted in water and acidified with HCl to maintain pH 3-3.4 to precipitate Glycyrrhetic acid and filter.
- The residue is washed with water to yield Glycyrrhetic acid .

# Glycyrrhetic acid

- **Method 2:** In a conical flask transfer 20g powdered liquorice +50ml acetone +2 ml dil.  $\text{HNO}_3$  and mix the content and macerate for 2hrs with stirring.
- Filter, transfer the marc to stoppered conical flask & add 20 ml acetone, warm & filter.
- Combine both filtrate, conc. under vacuum,
- To the conc. Filterate, add dil  $\text{NH}_3$  sol. for ppt. of  $\text{NH}_4^+$  glycyrrhizinate
- Separate out ppt by filtration and wash it with 5ml acetone (2x) followed by drying & weigh the product

# Glycyrrhetic acid

- **Method 3:** Weigh accurate qty of liquorice powder +  $\text{CHCl}_3$  in soxhlet apparatus for 3hrs.
- Filter the content of flask & discard the filtrate.
- The residue left on filter paper is then extracted with 0.5M  $\text{H}_2\text{SO}_4$  for few hrs.
- Filter the content of flask. Transfer the filtrate in and extract with  $\text{CHCl}_3$
- Separate & combine the  $\text{CHCl}_3$  layer.
- The combined  $\text{CHCl}_3$  layer is evaporated to dryness to get glycyrrhetic acid

# Glycyrrhetic acid

- Properties:
- Appearance : White crystalline powder
- Odor : Characteristic
- Taste : Characteristic
- Solubility : In soluble in water\ but freely soluble in alcohol, chloroform, benzene, ether etc.

# Glycyrrhethinic acid

- Identification by chemical test:
- Libermann test: 3ml of extract and 3ml of acetic anhydride is heated and cooled. To this a drops of conc.  $\text{H}_2\text{SO}_4$  is added. Blue colour is observed which indicate the presence of triterpenoid.
- Libermann-Burchard test: In 3ml of extract, 2ml of chloroform, 1ml of acetic anhydride and one drop of conc.  $\text{H}_2\text{SO}_4$  is added. Blue-green to red orange color is observed which indicate the presence of triterpenoid or steroids

# Glycyrrhetic acid

- Estimation
- Reflux about 25 g of liquorice root powder+ 250ml water for 6hrs + NH<sub>3</sub> to adjust pH 6.5-7, keep aside for few hrs.
- Centrifuge extract & evaporate to 75% of original vol. Add H<sub>2</sub>SO<sub>4</sub> with stirring
- Decant supernatant liq. & suspend the ppt in 25ml H<sub>2</sub>O & adjust the pH 4 by Na<sub>2</sub>CO<sub>3</sub> & stir about 2-3 hr & then centrifuge
- Again suspend the residue in H<sub>2</sub>O, pH 6, followed by centrifugation, drying in oven.
- Calculate % on basis of air dried drug calculated as glycyrrhetic acid.

# Glycyrrhetic acid

- Analysis by TLC
- Sample preparation : 1mg of Glycyrrhetic acid is dissolved in 1ml of methanol:Chloroform (1:1)
- Standard sample : Glycyrrhetic acid
- Stationary phase : Silica gel –G
- Mobile phase : Toluene: Ethyl acetate: Glacial acetic acid (12.5:7.5:0.5)
- Detecting agent : 1% vanillin-sulphuric acid reagent or Anisaldehyde-sulphuric acid and heated for 10minutes at 110C
- RF Value : 0.41
- Colour spot : Purplish spot
- UV Blue fluorescence under at 365 nm.

# Glycyrrhetic acid

- **Analysis by colorimetric method**
- In the sample anisaldehyde and sulphuric acid is added, which shows purple color.
- The intensity of color is measured in colorimeter at 556nm.
- **Utilization:**
- It is used in Rheumatoid Arthritis, Inflammation and Addison's disease, ulcer, expectorants
- **Storage condition:**
- It should be stored in well closed and air-tight containers protected from light and in cool place.