ISOLATION, IDENTIFICATION AND ANALYSIS OF ALKALOIDS (RESERPINE AND CAFFEINE)

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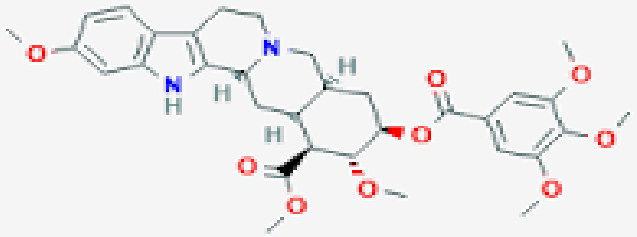
RESERPINE

- Biological source – Reserpine is an indole alkaloid obtained from the roots of *Rauwolfia serpentina*
- Family – Apocyanaceae
- It is a white or pale buff to slightly yellow, odourless, crystalline powder
- It is soluble in alcohol, acetone and chloroform.
- Reserpine is an antihypertensive and antipsychotic agent



EXTRACTION AND ISOLATION

- Rauwolfia root powder is exhaustively extracted with 90% alcohol by percolation
- The alcoholic extract is concentrated and dried under reduced pressure below 60°c to yield Rauwolfia dry extract.
- Rauwolfia dry extract is extracted with Ether-chloroform-90%alcohol (20:8:2.5)
- Collect the extract and add little dilute ammonia with intermittent shaking. Add water and allow the drug to settle after vigorous shaking.

- Filter off the solution and extract the residue with 4 volumes of 0.5N Ammonium sulphate in separating funnel. Combine all the extracts.
- The extract is made alkaline with dilute ammonia to liberate alkaloid. Finally it is extracted with chloroform.
- Collect the chloroform extract , concentrate and evaporate on water bath to yield total rauwolfia alkaloids.(30 different components)
- Residue is subjected to column chromatographic fraction for the separation of reserpine

Identification and Analysis

T.L.C Method

Sample preparation – Dissolved 1mg of Reserpine in 1ml of methanol or chloroform

Standard sample - Reserpine

Stationary phase - Silica gel – G

Mobile phase – Chloroform: acetone :diethyl amine (50:40:10)

Detecting agent – Dragendroffs reagent

RF Value – 0.72

Identification test & Analysis

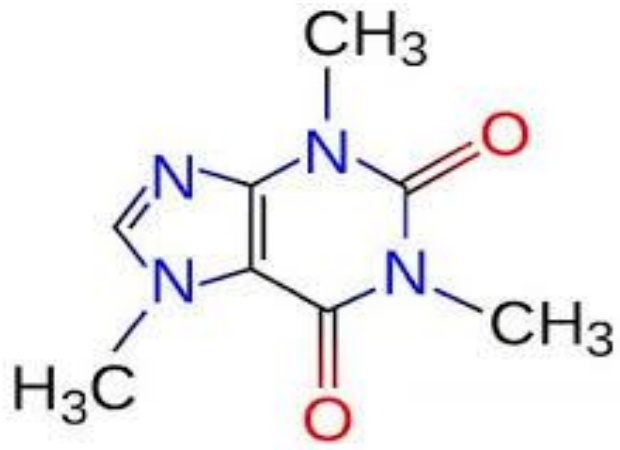
- 1) Sol. + Vanillin in acetic acid = violet red color
- 2) by colorimetry: Acidic sol. of drug + sodium nitrite

- **Analysis: HPLC**
- **Mobile Phase:** sol A (water & sol. B (acetonitrile)
- Both solvents filter with PTFE 0.45 µm membrane.
- Flow rate of mobile phase is 1ml/1min.
- Injection volume: 20µl at 25°C

- **UV detection:** 268 nm.

CAFFEINE

- Caffeine is a purine alkaloid obtained from Tea leaves, Coffee seeds, cocoa, and other species
- Biological source -It consists of dried leaves of plant known as *Thea sinensis*
- Family – Theaceae
- It is chemically 1,3,7, trimethyl xanthine. It is isolated from tea and coffee seeds during decaffeination process.
- Tea leaves contains 1-4% of caffeine and coffee contains 1-2% of caffeine
- It is white powder or white ,glistering needles, odour less, bitter in taste, Soluble in hot water.
- Caffeine is a CNS stimulant and Diuretic



EXTRACTION AND ISOLATION

- The powder tea leaves is extracted with boiling water and the aqueous extract is filtered while hot.
- The warm extract is treated with lead acetate to precipitate tannins and filtered.
- The filtrate is treated with excess of dilute sulphuric acid to precipitate lead in the form of lead sulphate.

- Filter and collect the filtrate
- The filtrate is boiled with Activated charcoal to remove colouring matter, if any and filtered to remove charcoal
- The filtered decolourized solution is extracted with chloroform successively .
- Combined the chloroform extracts evaporate on water bath to yield caffeine (white powder)
- It is recrystallized with alcohol

Isolation of Caffeine

- Ext. 50 gm pwd. Tea with 200 ml of ethanol for 6 hrs. (soxhlet app)
- Transfer to ext. in porcelain dish containing MgO suspended in 100 ml of water
- Mixture is evaporated to dryness on waterbath
- In residue add water & make paste. Add more water & make suspension. & filter & collect the residue.
- In this filtrate add 10% H2SO4 boil it for 30 min.
- While hot, add 25 ml CHCl3
- Add NaOH/KOH in it.(for decolorization)
- Add equal volume of water. Separate CHCl3 layer.
- Evaporat to dry. Add water for purification
- Finnaly get needle shape crystals.

Identification and Analysis

- Chemical test –
- Murexide test – To the caffeine add hydrochloric acid and potassium chlorate, heated to dryness. A purple colour is obtained by exposing the residue to vapours of dilute ammonia.
- Thin layer chromatography (TLC)

T.L.C Method

Sample preparation – Dissolved 1mg of caffeine in 1ml of methanol or chloroform

Stationary phase - Silica gel –G

Standard sample - Caffeine

Mobile phase – Ethyl acetate: methanol : acetic acid (80:10:10)

Detecting agent – Expose to vapors of iodine

RF Value – 0.41


THANK YOU