EXPERIMENT NO: 3

ISOLATION AND IDENTIFICATION OF BARBITURATES WITH LIQUID-LIQUID EXTRACTION

A) General Information:

Barbiturates are sedative-hypnotic drugs; is an important group of drugs that cause poisoning as a result of accidental ingestion by suicidal or children. They dissipate in all tissue and body fluids; Inactive metabolites, mainly excreted in urine.

Non-volatile poisons can be divided into three groups according to the extraction conditions in which they can pass to the organic phase:

a) Acid exudates:

They pass from acidic aqueous medium to ether or chlorophore. Example: Acetyl salicylic acid, barbiturates, oxalic acid

b) Poisons which can be extracted in basic environment:

They pass from basic aqueous medium to ether or chlorophore. Example: Alkoloids, synthetic nitrogenous substances

c) Organic poisons extractable in ammonia medium:

They pass from ammonia to organic solvents. Example: Morphin

B) Experiment Preparation: (Isolation and identification of Barbiturates from biological material)

25 mL of urine is taken, controlled by indicator paper and adjusted to pH3 with 1N HCl. After collection into the separating funnel, it is extracted 3 times with 10 mL of ether. The ether phase is filtered through dry filter paper, carrying anhydrous sodium sulfate. The ether phases are combined and evaporated in an electric water bath under vacuum. The residue is dissolved in 1 mL of chloroform. The following experiments are carried out on the chloroform solution.

1) **Parri experiment**: 1 drop of extract is put into a capsule. A drop of 1% cobalt nitrate and 1 drop of 5% isopropylamine (in methanol) is added dropwise. Viole color indicates the presence of barbiturate.

2) **Mercury nitrate assay:** 2-3 drops extracted, put into the capsule. When 1 drop of mercury nitrate reagent is dropped, a white precipitate is formed in the presence of barbiturate.

3) **Recognition by TLC:** The chloroform solution is applied to the adsorbent layer prepared with Silicagel G together with standard barbiturate. The plate is placed in a previously saturated tank with a mixture of chloroform: acetone (9: 1). Once the developing solution has been drained sufficiently, the plate is removed from the tank and dried. First, 2% $HgNO_3$ (1% HNO_3) followed by 2% $HgNO_3$ and 0.1% Rodamine B (in ethyl alcohol) mixture is sprayed to determine the spots. Pink viole colors indicate the presence of barbiturates.