

Aim of the experiment

Systematic extraction, isolation, purification and identification of **volatile poison** by various analytical techniques.

Requirements

- a) **Test sample:** Biological sample (divided in four parts) and spiked with acetone, chloroform, ethanol and acetaldehyde, respectively.
- b) **Materials:** Steam distillation apparatus (round bottom flask, condensers, rubber tubes), beakers, test-tubes, test-tube holder, test-tube stands, dropper, pipette, filter paper sheet.
- c) **Instrument:** Hot plate, heating mantle, water bath.
- d) **Chemicals:** Tartaric acid, sulfuric acid, resorcinol, sodium nitroprusside, sodium hydroxide, iodine solution, glacial acetic acid.

Principle

- a. The iodoform test reveals the presence of an aldehyde or ketone in which a methyl group is one of the groups immediately connected to the carbonyl carbon. A chemical reaction in which a methyl ketone is oxidized to a carboxylate by reaction with aqueous HO^- and I_2 .
- b. In the Legals test also known as the Rothera test, acetone reacts with an alkaline solution of sodium nitroprusside to form a purple-coloured complex.

Procedure

Extraction of volatile poison by steam distillation:

- a) Volatile substances can be separated or isolated from blood, urine, properly minced viscera or any matrix by steam distillation.
- b) Water is to be passed through steam distillation apparatus for cleaning of RBF and condensor. Further, water was boiled first to create the steam.
- c) Sample(s) is then added to RBF with tartaric acid such that steam passes through it.
- d) The steam is then further condensed and produced a mixture of both steam and matter.
- e) The mixture is then heated by passing additional steam, which proceeds to flow over the matter, evaporating the mixture. Owing to reduced vapour pressure, the necessary organic compounds evaporated as part of the mixture. Then distillate can be collected by condensation.
- f) The collected distillate is then further tested for volatile poison as ethanol, methanol, chloroform and acetone.

Reagent Preparation

Sodium hydroxide solution (10%): 10 gram of sodium hydroxide is added in volumetric flask and made up to 100 ml with distilled water.

Saturated solution of sodium nitroprusside: To the 10 ml of distilled water sodium nitroprusside (solute) is added using glass rod till no more solute can be dissolved at a given temperature.

Detection and Identification of volatile poison by colour test:

Test	Procedure	Observation	Inference
1	Iodoform Test: 1 ml. of the distillate is taken in a clean dry test tube. + 1ml of 1% iodine solution + A few drops of 10% sodium hydroxide are added drop wise until the brown colour of the iodine is discharged. Then mixture is gently heated in a water bath.	Yellow precipitate will form.	Indicates ethanol and acetaldehyde
2	Legal's Test: 1 ml. of the distillate is collected in a clean dry test tube + 2 drops of saturated solution of sodium nitroprusside (freshly prepared) + 1 drop of 10% sodium hydroxide + few drops of glacial acetic acid added to acidify the solution Subsequently on heating.	A red or yellowish red colour will appear. Changes the colour to carmine or purplish red. violet colour will appear	Indicates presence of acetone
3	1 ml. of the distillate was taken in a clean dry test tube + few drops of resorcinol were added. Shake. + few drops of concentrated sulfuric acid was added drop wise from the side of the test tube.	Red-violet colour ring will appear.	Indicates presence of chloroform

Conclusion: On the basis of observation, it can be concluded that the test sample contains _____ respectively.