FORENSIC TOXICOLOGY LABORATORY
OFFICE OF CHIEF MEDICAL EXAMINER
CITY OF NEW YORK

ETHCHLORVYNOL (COLOR TEST)

PRINCIPLE

Ethchlorvynol reacts with diphenylamine to form a pink (fuchsia) color. The absorbance can be used for qualitative identification and quantitative measurement of the ethchlorvynol concentration in urine or protein-free filtrates of blood. Ethyl acetate will extract ethchlorvynol from biological fluids, and aliquots of the extract can be analyzed by gas chromatography.

SAFETY

The handling of all reagents, samples and equipment is performed within the guidelines which are detailed in the safety manual.

REAGENTS

All chemicals should be analytical reagent grade.

1. Trichloroacetic acid, Fisher ACS grade or equivalent.
2. Ethchlorvynol, Pfizer, or equivalent
3. Diphenylamine Sigma-Aldrich, 99% or equivalent
4. Concentrated sulfuric acid (H_2SO_4), Fisher ACS grade or equivalent.
5. Sodium chloride, Fisher ACS grade or equivalent.
6. Trichloroacetic acid, 20.0 g/100 mL
7. Dissolve 20.0 g of trichloroacetic acid and 1.0 g sodium chloride in water and dilute to 100 mL.
8. Ethchlorvynol stock solution, 100 mg/L

   Dissolve 10.0 mg of ethchlorvynol in approximately 50.0 mL of ethanol and dilute to 100 mL with water.
9. Ethchlorvynol positive control, 0.5 mg/L

   Dilute 0.1 mL of ethchlorvynol stock solution to 20 mL with water.
10. Deionized water. Used as blank matrix, and to make dilutions.

PROCEDURE

1. Precipitate proteins by adding 4.5 mL of the trichloroacetic acid solution to 1 mL of blood. Mix by Vortex, then centrifuge 5 to 10 minutes.
2. Pipet 2.0 mL of the supernatant from Step 1 (or 2.0 mL of urine or centrifuged gastric content), 2.0 mL of ethchlorvynol positive control solution and 2.0 mL of the water as a negative control into separate, correctly labeled tubes.
3. Carefully underlay with 1 mL of concentrated sulfuric acid.
4. Sprinkle a spatula tip full of diphenylamine on top at let stand for at least 10 minutes. The emergence of a pink (fuchsia) color indicates the presence of ethchlorvynol.

INTERPRETATION

No meaningful interpretation related to the subject's behavior or condition can be made from the procedure. A positive result simply indicates that the subject ingested ethchlorvynol, in which case this test should be followed by a quantitative procedure for ethchlorvynol.

ACCEPTANCE CRITERIA

1. Only specimens that have been analyzed with successful controls can be reported.
2. Negative control must not react with diphenylamine.
3. Positive control must produce a pink (fuchsia) color in the presence of diphenylamine.

REPORTING

1. Samples which do not cause a color reaction with diphenylamine will be reported as "ethchlorvynol not detected".
2. Samples which cause a color reaction with the diphenylamine will be reported as "ethchlorvynol detected".

Note: *The final toxicology report will indicate that positive results are unconfirmed and that confirmation is available upon request.*

REFERENCE

Irving Sunshine, ed., *Methodology for Analytical Toxicology.* CRC Press, Inc.; Boca Raton, FL, 1975.