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

TRICHLOROETHANOL (TCE) by SPECTROPHOTOMETRY

PRINCIPLE

Chloral hydrate is rapidly metabolized to trichloroethanol (TCE) which is extracted into ether from whole blood or tissue homogenates. Pyridine and sodium hydroxide are added to the ether extract. The mixture is heated to form a reaction product with an absorption maximum at 368 nm. The absorbance at 368 nm is used for quantitation.

SAFETY

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

EQUIPMENT

Model 8453 Agilent Diode Array Spectrophotometer (ultraviolet-visible)

REAGENTS

1. Absolute diethyl ether; ACS grade (Fisher Scientific Company) or equivalent
2. Pyridine; ACS grade (Fisher Scientific Company) or equivalent
3. Trichloroethanol; ACS grade (Fisher Scientific Company) or equivalent
4. 0.5 N sodium hydroxide

   Dissolve 2 g of sodium hydroxide ACS grade (Fisher Scientific Company) or equivalent in water and dilute to 100 mL with water.

   Caution! This is an exothermic reaction!

STOCK SOLUTIONS

10,000 mg/L trichloroethanol in methanol

The density of trichloroethanol is 1.55. Add 645 µL of trichloroethanol to 100 mL of methanol to prepare a 10,000 mg/L stock solution. Transfer into properly labeled container (include concentration, lot number, date prepared, expiration date, initials of analyst).

Calibrators and controls are prepared from separate stock solutions of 10,000 mg/L (10,000 mg/L calibrator and 10,000 mg/L control, respectively).

CALIBRATOR WORKING SOLUTIONS

1. Transfer 1 mL of the 10,000 calibrator stock solution to a 30mL glass beaker. Add 19mL of distilled water. The concentration of the solution is 500 mg/L. Refrigerate at 2^0 - 8^0 C. Stable for one week.
2. Transfer 10 mL of the 500 mg/L calibrator stock solution to a 30mL glass beaker. Add 10mL of distilled water. The concentration of the solution is 250 mg/L. Refrigerate at 2^0 - 8^0 C. Stable for one week.
3. Transfer 2 mL of the 500 mg/L calibrator working solution to a 30mL glass beaker. Add 8mL of distilled water. The concentration of the solution is 100 mg/L. Refrigerate at 2^0 - 8^0 C. Stable for one week.
4. Transfer 1 mL of the 500 mg/L calibrator working solution to a 30mL glass beaker. Add 9mL of distilled water. The concentration of the solution is 50 mg/L. Refrigerate at 2^0 - 8^0 C. Stable for one week.

CALIBRATORS

Four calibrators are prepared in a validated negative matrix on the day the test is performed.

Add 0.4 mL of the 500 mg/L calibrator working solution to 3.6 mL drug free blood to make a 50 mg/L solution.

Add 0.4 mL of the 250 mg/L calibrator working solution to 3.6 mL drug free blood to make a 25 mg/L solution.

Add 0.4 mL of the 100 mg/L calibrator working solution to 3.6 mL drug free blood to make a 10 mg/L solution.

Add 0.4 mL of the 50 mg/L calibrator working solution to 3.6 mL drug free blood to make a 5 mg/L solution.

CONTROL WORKING SOLUTIONS

1. Transfer 1 mL of the control stock solution to a 50 mL glass beaker. Add 24 mL of distilled water. The concentration of the solution is 400 mg/L. Refrigerate at 2^0 - 8^0 C. Stable for one week.
2. Transfer 1 mL of the control stock solution to a 100mL glass beaker. Add 49mL of distilled water. The concentration of the solution is 200 mg/L. Refrigerate at 2^0 - 8^0 C. Stable for one week.

CONTROLS

Two positive controls are prepared in drug free matrix on the day the test is performed.

1. Add 0.4 ml of the 400 mg/L control working solution to 3.6 mL drug free blood to make a 40 mg/L solution.

2. Add 0.4 ml of the 200 mg/L control working solution to 3.6 mL drug free blood to make a 20 mg/L solution.

PROCEDURE

1. Add 4 mL drug free matrix (i.e., blood, water) to a 15 mL test tube. Label as negative control (blank).
2. Add 4 mL of each corresponding calibration solution to tubes labeled 50, 25, 10 and 5 mg/L.
3. Add 4 mL of each corresponding control solution to tubes labeled 40 and 20 mg/L.
4. Add 4 mL of the sample to be analyzed to a 15 mL test tube. Do in duplicate.
5. Add 4 mL of anhydrous ether to each tube and shake for 3 minutes.
6. Centrifuge at ≈ 3000 rpm for 10 minutes.
7. Remove 1.5 mL of the ether phase and transfer to 15 mL centrifuge tube.
8. Add a glass bead to each tube, and then add 2.25 mL pyridine and 1.5 mL NaOH. Gently mix by hand.
9. Loosely cap each tube.
10. Place each tube in a boiling water bath for 4 minutes.
11. There should be no color change in the solution.
12. Pour the contents into a 10 mL volumetric flask and dilute to volume with water.

INSTRUMENTATION

1. Turn on the spectrophotometer and let it warm up for at least 1 hour.
2. Turn on computer and printer.
3. Replace waste tubing if needed. Make sure the waste is going into a waste reservoir.
4. Run diagnostic tests on instrument (dark current, intensity, stability)
5. Click on instrument offline icon to bring up the offline screen.
6. Click on the word measure, then click on diagnostics.

7. Click on dark current, wait a minute until the test is completed. Click on print icon to print the dark current report.
8. Follow the same steps above for the intensity and stability tests.
9. Load trichloroethanol method. (TCE Quant.M)
10. Use distilled water to prime the instrument. Put some distilled water on a clean beaker, insert tubing and press the button next to it that will start the flow of water. Once there are no bubbles in the tubing you can stop priming.
11. Run a trichloroethanol standard. Absorbance of TCE is 368 nm.
12. Repeat step no. 10.
13. Prime instrument with an extracted blank (e.g. blood, water or liver blank).
14. Click on run calibrator. Run the highest calibrator. Fill out the name of the calibrator in the corresponding field. Click insert, and fill out TCE and "x" MG/L, where x is calibrator concentration (note upper case letters must be used), click enter.
15. Run a blank. Close window.
16. Run the next calibrator. Repeat steps 14 to 15 until all calibrators have been run.
17. Run a blank as a calibrator to establish a baseline (close to zero). Label as a blank. Click insert, type TCE, "0" MG/L.
18. Click on save as calibrators. Use the date on which you run the calibrators to save as file. (e.g. TCE030513C.s)
19. Run an extracted blank. Close window.
20. Click on run samples and run the first sample. Fill out the name of the sample in the corresponding field. Click insert.
21. Run the next sample. Repeat steps 19 to 20 until all samples and quality controls have been run.
22. Click on save as samples. Use the date on which you run the samples to save as file. (e.g. TCE030513S.s)
23. Click on process report (calibrators and samples). Print reports.

ACCEPTANCE CRITERIA

1. A sample is considered positive for trichloroethanol if there is an absorbance at 368 nm.
2. Calibrators and controls must be within $\pm$ 20% of target concentration for blood and water and ± 30% tissue.
3. Four calibrators (5 mg/L, 10 mg/L, 25 mg/L and 50 mg/L) are used to establish the calibration curve. One calibrator may be dropped if the acceptance criteria are not met. The calibration curve may be extended to 20% of the highest calibrator value.

4. Linear correlation coefficient (r^2) value must be equal to or greater than 0.98. If any of the above criteria are not met, results can be accepted as qualitative only. Repeat as necessary for quantitative analysis.
5. Samples with final concentrations greater than the 20% of the highest calibrator must be diluted and re-extracted along with the appropriate matrix control.

REPORTING

1. All results must be entered on the result summary form in the case file.
2. Copies of all the calibrators and controls along with a copy of the sequence worksheet must be attached to the original UV data of the case and placed in the case file folder.
3. All negative cases are reported on the toxicology report sheet as “trichloroethanol not detected”.
4. Concentrations of trichloroethanol lower than 5 mg/L are reported as “less than 5 mg/L”.
5. Concentrations greater than or equal to 5 mg/L are reported to the nearest tenth of one percent (e.g., trichloroethanol 10.76 mg/L is reported as 10.7 mg/L).

> ***Note:*** *When reporting gastric contents results, in addition to reporting mg/kg concentrations, the gastric content is also reported as total mg in the gastric content (concentration in mg/kg x weight in kg = total drug in mg).*

REFERENCE

A. J. McBay, V. R. Boling and P. C. Reynolds, “Spectrophotometric determination of trichloroethanol in chloral hydrate poisoning”, Journal of Analytical Toxicology, Vol.4, 99 - 101,1980.