

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

CYANIDE

by

NPD GAS CHROMATOGRAPHY

PRINCIPLE

This method is used to quantitate cyanide following a positive Cyantesmo test paper result. The method uses a minimal amount of sample and acetonitrile as an internal standard.

A sample of the headspace is removed from a sealed sample vial, using a gas-tight syringe, and is injected into a gas chromatograph where it is detected by a nitrogen-phosphorus detector (NPD).

A five-point calibration curve is used for analysis along with a negative control (blank) and two positive controls.

SAFETY

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

REAGENTS AND MATERIALS

All reagents must be ACS grade or better.

1. Deionized water
2. Sodium cyanide (NaCN) stock solution – 500 mg/L in H₂O

Dissolve 96.2 mg of NaCN in 100 mL H₂O

Prepare separate stock solution for calibrators and controls. Label with lot number, preparer initials, date prepared, and expiration date.

Note: Cyanide is very toxic. Avoid breathing vapors or contact with skin. Use a fume hood where appropriate.

3. Acetonitrile

Internal standard (*prepare fresh each day of use*)

Dissolve 5 μL acetonitrile in 100 mL H_2O

4. 1-Octanol
5. Glacial acetic acid
6. Syringes
 - Hamilton 100 μL syringe
 - Hamilton 50 μL syringe
 - Hamilton 10 μL syringe
 - Hamilton 100 μL gas tight syringe
7. 2 mL injection vials (with 11 mm Teflon lined caps)
8. Certified negative blood, used for preparation of calibrators, positive control and negative control.

SAMPLE PREPARATION AND EXTRACTION PROCEDURE

1. Pipet 5.0 mL of a matching, negative matrix into each of the blank and calibrator tubes (the negative matrix used for gastric, urine and vitreous humor samples is deionized water). Cover the tubes with a cap to avoid possible contamination from other specimens. Fortify the tubes with various solutions as indicated.

For the Calibrators:

0.125 mg/L CN - add 1.25 μL of 500 mg/L CN to 5 mL blood or H_2O

0.25 mg/L CN - add 2.5 μL of 500 mg/L CN to 5 mL blood or H_2O

0.50 mg/L CN - add 5.0 μL of 500 mg/L CN to 5 mL blood or H_2O

1.0 mg/L CN - add 10.0 μL of 500 mg/L CN to 5 mL blood or H_2O

5.0 mg/L CN - add 50.0 μL of 500 mg/L CN to 5 mL blood or H_2O

For the Controls:

0.60 mg/L CN - add 6.0 μL of 500 mg/L CN to 5 mL blood or H_2O

1.2 mg/L CN - add 12.0 μL of 500 mg/L CN to 5 mL blood or H_2O

1. Aliquot 0.5 mL of each reference solution into 2 mL un-recycled vials. For the blank, pipet 0.5 mL of un-spiked negative matrix directly into the appropriate vial.
2. Using two separate syringes first add 100 μL of internal standard then add 5 μL of 1-octanol to each vial.
3. Seal vials with aluminum cap with Teflon lined rubber septum.
4. Under a fume hood, add 50 μL of glacial acetic acid into each vial by using a syringe.
5. Vortex for 5 seconds and centrifuged at ~ 3000 rpm for 5 minutes.
6. Allow vials to equilibrate at room temperature for at least 4 hours. If possible vials can be left overnight in a warm environment to equilibrate.

INSTRUMENTATION & METHOD

Agilent 6890 gas chromatograph equipped with an NPD detector and HP-17 (Crosslinked 50% Ph Me Silicone) column with dimensions of 10 m x 0.53 mm x 2.0 μ m film thickness. The method name is GCXCNQ.M (where "X" is the instrument number), and a copy of the current method parameters for each instrument is stored in the instrument maintenance log. Verify that the method loaded on the chemstation matches the printout of the current method. A summary of the major method parameters is listed below.

The GC conditions for cyanide vary significantly from normal operating conditions. Verify that they are correct. The method is setup to run in **manual** injection mode, only one injector is required.

Control Information

Sample Inlet: GC
Injection Source: Manual

Oven Parameters

Initial Temp: 32 °C (Isothermal) Maximum temp: 280 °C
Initial Time: 4.00 min Equilibration Time: 1.00 min
Ramps: off
Post Temp: 32 °C
Post Time: 2.00 min
Run Time: 4.00 min

Front Inlet

Mode: Splitless
Initial Temp: 200 °C
Pressure: 0.81 psi (on)
Purge Flow: 30.3 mL/min
Purge Time: 2.00 min
Total Flow: 36.1 °C
Gas Saver: off
Gas Type: Helium

Column 1

Capillary Column
Model Number: Agilent 19095L-121
HP-50+ 50% Phenyl Methyl Siloxane
Max Temp: 310°C
Nominal Length: 10.0 m
Nominal Diameter: 530.0 μ m
Nominal Film Thickness: 2.00 μ m

Mode: Constant Flow
Pressure: 3.2 mL/min
Nominal Initial Flow: 0.81 psi
Average Velocity: 24 cm/sec
Inlet: Front Inlet
Outlet: Front Detector
Outlet Pressure: Ambient

Front Detector (NPD)

Temperature: 300°C (On)
Hydrogen Flow: 3.5 mL/min (On)
Air Flow: 98.0 mL/min (On)
Mode: Constant Column + Makeup Flow
Combined Flow: 30.0 mL/min
Makeup Flow: On
Makeup Gas Type: Helium
Adjust Offset: 30.00
Electrometer: On
Equilibration Time: 0.00

Signal 1

Data range: 20 Hz
Type: Front Detector
Save Data: On
Start Time: 0.04 min
Stop Time: 4.00 min
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

SEQUENCE

After loading the method, i.e. GCXCNQ.M, where “X” refers to the instrument number, verify that all the method parameters are correct.

Prepare a sequence using the following steps.

1. Create a folder to store the collected data.
 - a. Right click on “**Start**” on the Taskbar, select “**Explore**”. The local drive (C:) will open, select msdchem, select the instrument 1 or 2 and click on “**Data**”. While in the “**Data**” folder right click, select “**New**”, then “**Folder**”.
 - b. Name the new folder GCXMMDDYYx, where “X” is the instrument’s number and “x” is the letter designated to the sequence.

- c. Close the “Explore” window
2. Go back to the Instrument’s Control Window and click on Sample Name (to locate look for the green arrow)
 - a. Double check that the word Manual is entered in the “Current Method Injection Style” field.
 - b. Next to the “Data Path” field, click on Browse to locate and load the new folder just created.
 - c. Enter analyst’s initials in the “Operator Name” field.
 - d. Under Sample, the “Data File Name” for the first injection/data file should read “GCXMMDDx001”, where “X” is the instrument’s number and “x” is the letter designated to the sequence (the last three digits “001” will automatically increase by one with every injection).
 - e. Enter “Sample Name” and any “Misc Info”.
 - f. Enter “Sample Amount”; i.e. 100 to indicate a 100 μ L injection of the vial’s headspace.
 - g. Enter the dilution factor in the “Multiplier” field when injecting a diluted sample.
 - h. Click on “Ok and Run Method” to accept all changes and start the sequence.
 3. Log-in to ECM.
 4. A pre-run will automatically begin. Follow the instructions in the “Acquisition – Prepare To Inject” pop-up window.
 - a. Inject 100 μ L of headspace into the gas chromatograph using a gas-tight syringe.
 - b. After each injection the syringe is flushed many times with air to ensure that no residual cyanide carries over. Applying heat to the syringe may also help flush out any residual gas. In addition, blank air injections are made between sample runs to ensure that carryover is not observed.
Keep a hand-written log of the samples injected with their corresponding filename.
 5. Repeat steps 2 - 4 for each injection until all samples have been injected.

DATA PROCESSING

DATA ANALYSIS

Retrieving Method from Data Acquiring Instrument

1. Open the GC’s Data Analysis from the PC’s desktop.
2. Browse the left hand side menu to locate the batch on the C: Drive. Verify that all data files are in the appropriate folder(s).
3. Click on **Method**, then select the method for the batch (i.e., GC8CNQ.M); right click → **Load**
4. Save method to batch by clicking ECM on the tool bar, then select **Save Method To ECM**. Locate the batch, select ok to save a copy of the method into the batch to be processed.
5. A copy of the method has now been saved to ECM and will accompany the batch when retrieved from ECM on any Data Analysis Processing Station.

PROCESSING USING ENHANCED DATA ANALYSIS

On any processing station:

1. Click on the Processing Data Analysis icon on the desktop and Log-in by using: analyst's ocme login for the username and their password.
2. From the tool bar menu select ECM → Retrieve Entire Batch From ECM. Browse to locate and select the batch. Each datafile will be retrieved along with the method to be used for processing.
3. Once the retrieval is complete the batch folder is located in the C: \msdchem\ECM\retrieve folder.
4. Select the method under the batch by right clicking on it and Load the method.
5. Select NYC_OCME from the tool bar, and then click on Restore Master.
6. Select Calibrate from the tool bar, then Update, Global Update, Set Curve Fit Type, Linear Regression Force (0, 0), select OK. If a different type of curve fit is used in place of Linear regression force (0,0), this must be annotated on the front page of the master copy of calibrators and controls that is appended to each case chromatogram.
7. Edit the compounds for processing
 - a. Select **Calibrate** again, **Edit Compounds**; select **Name**, type in compound name in the space provided, click **Find Compound**.
 - b. Make sure Acetonitrile is the internal standard, and that Cyanide is a "T" compound
 - c. Click **OK** to save all changes to the compounds that have been made.
8. In order to ensure that only compounds that are found are printed in the report select **Quantitate** in the menu bar, then **Report Options**. In the lower right hand box labeled "All Reports" make sure that the box labeled "Omit Target Compounds that are Missed" is checked.
9. In the space provided type in QT 1, then click on **Execute**; this processes the data file.
10. Select the **View** from the top menu, select **Easy ID** (this function allows for Retention times to be assigned for the entire method and batch). Easy ID should only be used for your first calibrator level.
11. To select a compound from the list double click, use the right click to integrate a peak for the compound, use the left click to control zooming on the chromatogram.
12. Once all of the retention times have been assigned, click on **Exit** and click on **Yes** to "update retention times to the compound list".
13. Type in QT 1, then click on **Execute**.
14. Again select the **View** from the top menu, select **Q-Edit Quant Result** (this function to view the response and retention times for each compound). Cal 5.0 mg/L will not have an "x" next

to each compound name because it is the first calibrator to be processed. Click Exit when all compounds have been reviewed.

15. Assign the calibrator level:

- a. Select **Calibrate**, then **Update**, select **Update 1 Level**.
- b. Select **Add Level** and type in the concentration of the calibrator (i.e. 3.0, 1.0 etc.).
- c. Type-in the internal standard concentration (0.5 mg/L).
- d. On the right hand side of the window type-in the Level ID i.e. 5, 4, 3, 2, and 1; where the # is the calibrator level for the compound.
- e. Click on **Do Update** then select **OK**.

16. Load the next data file, Cal 1.0 mg/L. Type QT 1 then click on Execute.

17. Select the View, select Q-Edit Quant Result. Easy ID is not needed for this step, as retention times have already been set for this calibrator. Double check each of the compound's retention time and that each compound has a response. All auto-integrated compounds should have an "x" next to their name. Click Exit once when finished.

18. Assign the calibrator level.

19. Repeat steps 16-18 for the remaining levels.

20. Requant the calibrator:

- a. Go to **Tools, DOLIST**.
- b. Remove any options that are on the right hand side of the box.
- c. On the left hand menu select "**Summary Quant No Report**" which is **QT 2**, with arrows move this command to the empty space on the right then click **OK**.
- d. Select the files for the calibrator just processed and move them to the empty space on the right then click **Process**. Each file will be "requanted".
- e. Once again, review these files through **Q-Edit Quant Result** to verify quant results.

21. Process all the corresponding QC's:

- a. Go to **Tools, DOLIST**.
- b. Remove any options that are on the right hand side of the box.
- c. On the left hand menu select "**Quant No Report**" which is **QT 1**, with arrows move this command to the empty space on the right then click **OK**.
- d. Select the QC's to be processed and move them to the empty space on the right then click **Process**. Each file will be processed.

22. Review each QC by selecting **View**, select **Q-Edit Quant Result**.

23. Print the processed data files for the calibrators and QC's:

- a. Go to Tools, DOLIST.
- b. Remove any options that are on the right hand side of the box.
- c. On the left hand menu select "**Summary Quant w/o Calculations**" which is **QT 0, 1, "s"**, with arrows move this command to the empty space on the right then click **OK**.
- d. Only select all Cal levels and QC's just reviewed and quanted and move them to the empty space on the right then click **Process**. Each file will print.

24. Once all calibrators and QC's are printed, process the all the blank injections and cases:
 - a. Go to **Tools, DOLIST**.
 - b. Remove any options that are on the right hand side of the box.
 - c. On the left hand menu select "**Quant No Report**" which is **QT 1**, with arrows move this command to the empty space on the right then click **OK**.
 - d. Select the data files to be processed and move them to the empty space on the right then click **Process**. Each file will be processed.
25. Review each blank and case through **Q-Edit Quant Result**:
 - a. Check the responses and retention times of the needed compounds.
 - b. If a compound is needed but not integrated, double click on the compound and integrate.
 - c. Click **Exit** once when finished.
26. If a compound is too low to be integrated or no peak is present include an overlay:
 - a. Go to **Tools, Overlay Chromatogram**.
 - b. Select the calibrator or standard to overlay on the case; multiple overlays can also be set.
 - c. Select **Process**.
 - d. Go to **File, Print** then select **Chromatogram**
27. Once all blanks and cases are processed, print as directed in step # 23.
28. Print an R² report by:
 - a. Go to **Calibrate, List** then select **Calibration Report** and **OK**.
 - b. Right click on the list and select **Print**, choose the correct printer.
29. Once everything is printed, save the Method and all the data files from the batch back to ECM:
 - a. Select **ECM** from the top tool bar, select **Save Multiple Data Files to ECM**.
 - b. Select all data files on the left and move them to the right empty space, the click **Process**.
 - c. Allow each data file to be successfully copied to ECM before exiting Data Analysis.
30. After uploading the files to ECM, delete the sequence from the C: Drive of the processing station:
 - a. Right click on the **Start** on the lower left hand corner and select **Explore**
 - b. Open the **MSDCHEM** folder on the **C: Drive**, then select **1 → ECM → Retrieve**
 - c. Locate the batch processed, right click on it and delete it.

REINJECTION CRITERIA

Occasionally, samples analyzed may need to be reinjected for a variety of reasons. The criteria for reinjection, other than poor chromatography or requests made by the appropriate supervisor, are listed below.

1. Analytes in a calibrator or control are not present in the chromatogram.
2. QC failure: reinject the blank, the calibrator of interest and the associated QC.

3. Examine peak shape for signs of overload. If peaks are still overloaded after reinjection, re-extract samples with overloaded peaks using appropriate dilutions. If a calibrator peak is still overloaded after re-injection, the batch must be re-extracted.

CARRYOVER

Two types of carryover are possible with the methods employed by GC. One is the “carryover” of late-eluting peaks from one injection into the next or even next two or three injections. This is usually apparent from the shape of the peak (broad).

The other possibility is “carryover” due to contamination from an excessive amount of drug in the previous sample, either through the syringe or by drug being retained in the injection port or column. Any discrepancy is resolved, usually by repeating the extraction. Any samples with large concentration of drug are rescheduled with dilutions, to reduce the amount to within the linear range of the method. If it appears that carryover of this type has occurred, the reviewer will reschedule the case after the overload to verify that there was no contamination.

Frequent air blank injections help to control this type of potential carryover.

DATA REVIEW

(Second level review)

1. Check chromatography of all injections. Examine the peak shape and note if any peaks show non-Gaussian shape. Identify unresolved peaks and peaks with shoulders on either side. Address any noted problems before proceeding with data review. Consult a supervisor about any unusual events.
2. Check that all significant peaks in the chromatogram are integrated. If the printout allows, check if the baseline used to integrate is appropriate. The analyst should consult with a supervisor about any unusual events, such as the presence of overload peaks
3. Check that all components for the calibrator are present and that each peak is properly assigned. Correct any errors. If any calibration compounds do not extract, or if unexpected multiple peaks are detected for a calibration compound, the analyst must consult with a supervisor.
4. Check the blank for significant peaks that might co-elute with a compound of interest. There is some room for judgment in this step, but the analyst should consult with a supervisor if there are ANY questions. If any significant peaks are present in a blank for the batch, the run must be rejected.
5. Review QC samples, determine if the controls meet all acceptance criteria and verify the concentration of the components.
6. A copy of the batch calibrators and controls are forwarded to the QC officer, who will enter the results of the QC sample in the QC database. If the batch fails, annotate sample chromatograms, listing the reason for failure.
7. Annotate the following information on the first calibrator’s chromatogram or sequence list:
 - a. Calibrators and blank reviewed and accepted (or not accepted). Any QC or calibrator failures are annotated on the proper chromatograms.

- b. If the files did not transfer, do so, and notify supervisor.
 - c. Note any deviations from the SOP or any comments in a concise but detailed fashion.
 - d. Initial and date.
 - e. Regression coefficient (r^2) must be greater than or equal to 0.98.
8. Make sure that each control has the appropriate target concentration range label.

Note: *By initialing and dating this document, the analyst certifies that a complete and accurate review was done, to the best of the ability of the analyst.*

9. A supervisor or qualified staff reviews calibrators and controls, dates and initials the results of the review before photocopying.
10. When possible, all data should be photocopied using double sided mode. A master copy of the sequence list, calibration, control and any other data is prepared. Arrange this master copy as follows (from top to bottom): Calibrators, all QC samples and blanks, r^2 report, sequence list and the lot sheet form. It is the responsibility of the analyst to verify that the master copy is legible and is an accurate copy of the originals, with no information cut off at the margins. Photocopies are made from this master, one for each case in the batch. Attach a copy of the calibrator and control package to each original case chromatograph.

DATA REVIEW

(Third level review)

Quantitative Results

1. The reviewer should review the calibration, control, and batch information received after the photocopying of the batch. Use the same criteria as listed in batch review, with the additional criteria that the r^2 of the four-point calibration curve of each component present in any case in the batch must be equal to or greater than 0.98. Notify a supervisor about any errors and make sure they are corrected before proceeding.
2. Retrieve the case file for each sample in the batch and associate it with the chromatogram(s).
3. Review the case's chromatogram. Determine if the results are consistent with all the other applicable case data to date. For quantitation, the ratio of peak to internal standard for both chromatograms should be examined. Consult a supervisor about rescheduling the sample, or other appropriate corrective action.
4. If the chromatogram shows overloaded peaks, or poor recovery of the internal standard, then repeating the sample may be necessary. If the problem is an overload, re-schedule with appropriate dilutions. In cases of poor recovery, the sample, its control, blank, and a calibrator may be re-injected once. If the criteria as listed in batch review are still not met, the sample is re-scheduled. All chromatograms must be attached to the case file. Consult with the supervisor if there is any question.

Note: *Do not discard any sample paperwork.*

5. Cases where an overdose is suspected are scheduled for further testing. Liver and gastric contents are the specimens of choice. Consult with the supervisor regarding tissue analysis.

ACCEPTANCE CRITERIA

1. A standard curve must be established using a linear regression force (0,0). Five calibrators (0.125, 0.25, 0.50, 1.0, 5.0 mg/L) are used to establish the standard curve. One or a maximum of two calibrator points may be dropped from the curve if appropriate acceptance criteria are not met (e.g., if poor recovery of the drug is exhibited or the concentration is $\pm 20\%$ of the target concentration). If the highest or lowest calibrator point is dropped, the range of the calibration curve is adjusted to the range between the highest and lowest valid calibrator concentration for that batch of samples.
2. Retention times must be within $\pm 2\%$ of the established retention time.
3. All positive matrix controls must be within $\pm 20\%$ of the target concentration for blood batches and within $\pm 30\%$ for tissue batches.
4. All negative matrix controls (blanks) must be negative.
5. The linear regression correlation coefficient (r^2) must be equal to or higher than 0.98.

REPORTING

1. Evaluate and review data in accordance with guidelines in the Acceptance Criteria section.
2. Positive matrix control must be reviewed and accepted, signed and dated by reviewer. Any notations of failing values must be noted for the positive matrix control.
3. A double-sided photocopy of the approved master copy, which consists of all calibrators, controls and blanks injected along with the written "Manual Injection Sample Log" and the lot form sheet, must be attached to all individual case chromatograms from that batch.
4. Concentrations of CN below 0.125 mg/L are reported as "cyanide not detected".
5. Concentrations of CN below 0.25 mg/L and greater than 0.125 are reported as "cyanide less than 0.25 mg/L".
6. Concentrations greater than 0.25 mg/L are reported as cyanide, with the concentration reported in mg/L. All results are reported to two decimal points (e.g., 0.523 mg/L is reported as 0.52 mg/L; 0.578 mg/L is reported as 0.57 mg/L.)

REFERENCES

J. Zamecnik and J. Tam, "Cyanide in blood by gas chromatography with NP detector and acetonitrile as internal standard", Journal of Analytical Toxicology, Vol. 11, No. 1, pp 47-48, 1987.