

Method ME355.01

DETERMINATION OF CYANIDE IN DRINKING WATER BY GC/MS HEADSPACE
ANALYSIS

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DETERMINATION OF CYANIDE IN DRINKING WATER BY GC/MS HEADSPACE ANALYSIS

1.0 **SCOPE AND APPLICATION**

This method provides for the determination of soluble cyanide salts in drinking water or source water. This method is applicable to all forms of cyanide that readily release HCN under acidic conditions (Cyanide amenable to chlorination), and meets or exceeds the level of detection needed for cyanide under the safe drinking water act. The MCL for cyanide is 200 ppb.

Analyte	MW	CAS Number
Cyanide Anion	26	57-12-5
Hydrogen Cyanide	27	74-90-8

The cyanide anion is converted to HCN in the presence of an acid and evolves as a gas, which is analyzed by headspace injection.

2.0 **SUMMARY OF METHOD**

A drinking water sample containing soluble cyanide salts is analyzed by direct headspace injection after acidification. The analyte is initially cryofocused on the head of the GC column using a liquid nitrogen cooled cryo trap. All the initial preparation steps are done using a Gerstel Auto injector/Prep station.

3.0 **DEFINITIONS**

- 3.1 Internal Standard (IS)—A pure analyte(s) added to a sample, extract or standard solution in a known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.
- 3.2 Laboratory Reagent Blank (LRB)—An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

- 3.3** Laboratory Fortified Blank (LFB)—An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.
- 3.4** Laboratory Fortified Matrix (LFM)—An aliquot of an environmental sample to which a known quantity of the method analyte is added. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.
- 3.5** Stock Standard Solution (SSS)—A concentrated solution containing the analyte prepared in the laboratory using assayed reference material or purchased from a reputable source.
- 3.6** Primary Dilution Standard Solution (PDS)—A solution of the analyte, prepared in the laboratory from stock standard solution and diluted as needed to prepare calibration solutions or other needed analyte solutions.
- 3.7** Calibration Standard (CAL)—A series of solutions prepared from the stock or primary dilution standard. A known amount of internal standard solution is added to each of these solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.8** Quality Control Sample (QCS)—A solution of the method analyte of known concentration, which is used to fortify an aliquot of the LRB or sample matrix. The QCS is obtained from a source external to the laboratory and is different from the source of the calibration standard. It is used to check laboratory performance with externally prepared test material.
- 3.9** Instrument Performance Check Solution (IPC)—A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.
- 3.10** Method Detection Limit (MDL)—The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.
- 3.11** Linear Calibration Range (LCR) -- The concentration range over which the instrument response is linear.

- 3.12** Minimum Reporting Level (MRL) – The minimum concentration that can be reported by a laboratory as a quantified value for the target analyte in a sample following analysis. This defined concentration must be no lower than the concentration of the lowest continuing calibration check standard for the target analyte.

4.0 INTERFERENCES

- 4.1** Care must be taken to insure all the analytical equipment is clean. The analysis of field and laboratory blanks provides information about the presence of contaminants.
- 4.2** Interfering contamination may occur when a sample containing a low concentration of the compound is analyzed immediately after a sample containing a relatively high concentration of the compound. Syringes and splitless injection port liners must be cleaned carefully or replaced as needed. After analysis of a sample containing high concentrations of the compound, a laboratory reagent blank should be analyzed to ensure that the accurate value is obtained for the next sample.

5.0 SAFETY

- 5.1** Cyanide is toxic in moderate concentrations. In the presence of acid it is liberated as a gas, which is easily inhaled. Care should be taken to ensure that acids used in the analysis do not come in contact with standards in the open. All solutions and sample preparation should be done under a hood for added protection.
- 5.2** Each laboratory is responsible for maintaining awareness of OSHA regulations regarding the safe handling of chemicals used in the method.

6.0 EQUIPMENT AND SUPPLIES

- 6.1** All glassware must be meticulously cleaned. This may be accomplished by washing with detergent and water, rinsing with water, distilled water, or solvents, air drying and heating (where appropriate) in a muffle furnace. Volumetric glassware should never be heated to the temperature obtained in a muffle furnace.
- 6.2** Sample containers – 40 ml glass vials with Teflon lined caps.
- 6.3** Volumetric Flasks-various.
- 6.4** Various volumetric pipettes.

6.5 GC Column—PLOT-Q column made by Aglient Technologies. Part #19091P-Q04 or equivalent.

6.6 Sample vials- 10 ml headspace with caps and seals. Microlier Analytical Supply part # 20-1000.

6.7 Gas Chromatography/Mass Spectrometer/Sample Prep/Data System

6.7.1. The GC system must be capable of temperature programming and be equipped for split/splitless injection. It must be able to control a liquid nitrogen cooled cryo trap fitted to the front of the GC column to allow for cryo focusing of the analyte.

6.7.2. The GC/MS interface should allow the capillary column or transfer line exit to be placed within a few mm of the ion source.

6.7.3. The mass spectrometer must be capable of electron ionization at a nominal electron energy of 70 eV to produce positive ions. The spectrometer must be capable of scanning in the selective ion mode. Tuning must achieve a count of > 500,000 counts for mass 69 (using PFTBA) with a peak width of $.5 \pm .01$.

6.7.4. An interface data system is required to acquire, store, reduce, and output mass spectral data. The computer software must have the capability of processing stored GC/MS data by recognizing a GC peak within any given retention time window. The software must allow integration of the ion abundance of a specific ion between specified times or scan number limits, calculation of response factors (or construction of a linear regression calibration curve), and calculation of concentration of analytes using the calibration curve.

6.8 Gastight headspace syringe for use in sample injection.

6.9 Use of a Gerstel MSP prep station or other similar device greatly improves the speed and reproducibility of the method.

7.0 REAGENTS AND STANDARDS

7.1 Helium Carrier Gas—As contaminant free as possible.

7.2 Phosphoric acid 50 %-(Phosphoric Acid 85-87%) Add 59 mls of o-Phosphoric Acid to 41 mls of HPLC grade water for a total volume of 100 mls. Mix well.

- 7.3 Ascorbic Acid- (L-(+) Ascorbic Acid powder CAS# 50-81-70) Add 5 grams of ascorbic acid to 100 mls of HPLC grade water for final solution.
- 7.4 O-tolidine-(O-tolidine CAS# 119-93-7) Make a saturated solution by adding 1.5 g of the solid O-tolidine to 50 mls of water. (Solubility in water is 1.5 g/100 mls).
- 7.5 Internal Standard—Add 14.4 mg $K^{13}C^{15}N$ and 2 mls of .1 N NaOH to a 10 ml volumetric. Dissolve $K^{13}C^{15}N$ and dilute to volume with reagent grade water. Pipette 1 ml aliquot into a 100 ml volumetric flask containing 20 mls of .1 N NaOH and dilute with reagent grade water. This will yield 6 ppm of $[^{13}C^{15}N]^-$ solution.
- 7.6 Stock Standard (PDS)—Transfer 100ul of 1000ug/ml standard solution of KCN (Ultra ICC-008 1000mg/l free cyanide standard or other appropriate source) to a 100 ml volumetric containing 20 mls of .1 N NaOH. Dilute to volume with reagent grade water to yield a solution that has concentration of 1 ug/ml of cyanide.
- 7.7 Calibration curve is made from stock standard by taking 8 10 ml headspace vials and adding 1 ml of reagent grade water to each one. Remove the following volumes and replace with same amount of stock standard to yield listed concentrations. Cap with crimp caps.

Final concentration in ppb of cyanide	Amount Removed/added
0	0
25	25 ul
50	50 ul
100	100 ul
200	200 ul
300	300 ul
500	500 ul
750	750 ul

The MCL for cyanide in drinking water is (EPA) 200 ppb. This curve will bracket the MCL. The zero standard does not replace a LRB in the analytical sequence

8.0 **SAMPLE COLLECTION AND PRESERVATION**

- 8.1 Sample Collection. – Samples should be collected in 40 ml brown glass vials containing 1 ml of 1 N NaOH for preservation. In collecting a water sample from a tap, allow the system to flush until the water temperature

has stabilized (2 to 3 minutes should be sufficient). Collect the sample. Also supply one LFB made from reagent water placed in collection vials.

- 8.2** Maintain the sample at 4 degrees C in the dark until time for analysis.
- 8.3** Check samples prior to analysis by adding 2 drops of the O-tolidine solution to 1 ml of the sample. A yellow color indicated residual chlorine is present and the sample should be rejected.
- 8.4** Samples should be analyzed within seven days of collection.

9.0 QUALITY CONTROL

- 9.1** Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, the periodic analysis of laboratory reagent blanks, fortified blanks, and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

- 9.2.1** The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.
- 9.2.2** Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every six months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by $\pm 20\%$, linearity must be reestablished. If any portion of the range is shown to be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.
- 9.2.3** Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within $\pm 20\%$ of the stated

values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding on with the initial determination of MDLs or continuing with on-going analyses.

- 9.2.4** Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit.(4) To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$\text{MDL} = (t) \times (s)$$

Where: t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates].

s = standard deviation of the replicate analyses.

MDLs should be determined every six months, when a new operator begins work or whenever there is a significant change in the background or instrument response.

9.3 ASSESSING LABORATORY PERFORMANCE

- 9.3.1** Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed 1/3 the MRL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.
- 9.3.2** Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Section 9.4.2). If the recovery of any analyte falls outside the required control limits of 80-120%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.
- 9.3.3** The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 80-120%. When sufficient internal performance data becomes available (usually a

minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (x) and the standard deviation (s) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = x + 3s$$

$$\text{LOWER CONTROL LIMIT} = x - 3s$$

The optional control limits must be equal to or better than the required control limits of 80-120%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (s) data should be used to establish an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4 Instrument Performance Check Solution (IPC) -- For all determinations, the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required), and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within $\pm 20\%$ of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within $\pm 20\%$. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of analyte to a minimum of 10% of the routine samples. In each case, the LFM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

9.4.2 Internal Standard (IS) – The analyst must monitor the peak area of the internal standard in all injections during each analysis day. The

IS response (as indicated by peak area) for any chromatographic run must not deviate by more than ± 50 percent of the average IS area measured during the initial calibration. A poor injection could cause the IS area to exceed these criteria. Inject a second aliquot of the suspect sample to determine whether the failure is due to poor injection or instrument response drift.

- 9.4.3** Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range 80-120%. Percent recovery may be calculated using the following equation:

$$R = \frac{C_s - C}{S} \times 100$$

Where: R = percent recovery

C_s = fortified sample concentration

C = sample background concentration

S = concentration equivalent of analyte added to sample.

- 9.4.4** If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control (Section 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related.

- 9.4.5** Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10.0 CALIBRATION AND STANDARDIZATION

- 10.1** Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed and is required intermittently throughout sample analysis as dictated by results of the CCC. After initial calibration is successful, a CCC is required at the beginning of each period in which analyses are performed not to exceed 12 hours. It is recommended that a CCC be performed at the end of each period of continuous instrument operation to bracket all the samples with a CCC.
- 10.2** Initial Calibration-- Calibrate the mass and abundance scales of the MS with calibration compounds and procedures prescribed by the manufacturer. The peak width at half height for the tuning ions of

PFTBA (69,219, 502) should be .50 and the abundance of ion 69 should be > 500,000.

- 10.3** Calibrate the system using the initial calibration solutions (section 7.5) Using the system software develop a linear regression calibration curve.
- 10.4** Internal standard and the target analyte should match the retention time window established by the calibration curve.
- 10.5** Analyze a CCC standard of an intermediate concentration. If the recovery is within 20% of the true value then the system is ready to analyze samples.
- 10.6** The following is a suggested GC oven program. Initial temperature is 110⁰ C with zero minutes hold. Initial ramp is 4⁰ C to a temperature of 130⁰ C then ramp at 99⁰ C to a final temp of 250⁰ C and hold for 1.79 minutes.
- 10.7** The cryogenic trap on the inlet needs to be set with an initial temperature of -10⁰ C with a hold time of 1.5 minutes followed by rapid heating to a maximum temperature of 220⁰ C.
- 10.8** The mass spectrometer is operated in the splitless mode with a constant flow of 1.1 ml per minute of helium carrier gas.
- 10.9** The mass spectrometer should be operated in the selected ion monitoring mode using ions 29 (internal standard) and ions 27 (target compound quantitation) and 26 (target compound confirmation).

11.0 PROCEDURE

- 11.1** Prepare the calibration standards (section 7.5) and QC standards. This can be done manually but preferably with the Gerstel automated sample system.
- 11.2** Take the samples to be analyzed and pipette 1 ml into a 10 ml headspace vial. (If you are not using the Gerstel to add internal standard, pipette 50 ul of internal standard into the headspace vial.).
- 11.3** Add 200 ul of ascorbic acid and the 200 ul of the phosphoric acid to the sample. (Manually or via Gerstel prep station).
- 11.4** Heat at 60 degrees Celsius for 4 minutes with agitation. (Manually or with the Gerstel automated sample system).
- 11.5** Analyze 1 ml of the headspace of the standards, QC samples and field samples under the same conditions used for the initial demonstration.

- 11.6 At the conclusion of data acquisition, use the same software program that was used to establish the calibration curve to quantify the samples.

12.0 DATA ANALYSIS AND CALCULATIONS

- 12.1 Calculate the analyte concentration using the multipoint calibration curve established from the standards analyzed at the beginning of the run.

13.0 METHOD PERFORMANCE

- 13.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with a 99% confidence that the value is above zero. MDL's are determined by each laboratory but should be below 1/3 the maximum allowed contamination level for cyanide in drinking water which is 200 ppb.
- 13.2 This method was tested by 3 laboratories using reagent water, high salt concentration water and drinking water samples high in TOC (total organic carbon) spiked at 50 ppb and at 200 ppb. All three laboratories reported results that were within the requirements of the method for reporting results.

14.0 POLLUTION PREVENTION

- 14.1 This method used small amounts of acid and labeled cyanide for the analysis. The small amount of chemicals used should minimize the potential hazard to both the environment and the analyst.

15.0 WASTE MANAGEMENT

- 15.1 It is the laboratory's responsibility to comply with all federal, state and local regulations governing waste management particularly the hazardous waste identification and land disposal restrictions. The laboratory using this method has the responsibility to protect the air, water and land by minimizing and controlling all releases from bench top and fume hood operations. Compliance is also required with any sewage discharge permits and regulations.

16.0 REFERENCES

1. This method was developed from the CDC method for the analysis of cyanide in whole blood. That method is part of the technology transfer group at CDC involved with chemical terrorism.