

METHOD 335.4

DETERMINATION OF TOTAL CYANIDE BY SEMI-AUTOMATED COLORIMETRY

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METHOD 335.4

335.4-1

DETERMINATION OF TOTAL CYANIDE BY SEMI-AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

- 1.1 This method covers the determination of cyanide in drinking, ground, surface, and saline waters, domestic and industrial wastes.
- 1.2 The applicable range is 5 to 500 µg/L.

2.0 SUMMARY OF METHOD

- 2.1 The cyanide as hydrocyanic acid (HCN) is released from cyanide complexes by means of a manual reflux-distillation operation and absorbed in a scrubber containing sodium hydroxide solution. The cyanide ion in the absorbing solution is converted to cyanogen chloride by reactions with chloramine-T, that subsequently reacts with pyridine and barbituric acid to give a red-colored complex.
- 2.2 Reduced volume versions of this method that use the same reagents and molar ratios are acceptable provided they meet the quality control and performance requirements stated in the method.
- 2.2 Limited performance-based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Section 9.0, Quality Control.

3.0 DEFINITIONS

- 3.1 **Calibration Blank (CB)** -- A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes, internal standards, or surrogate analytes.
- 3.2 **Calibration Standard (CAL)** -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.3 **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.
- 3.4 **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.

- 3.5 **Laboratory Fortified Sample Matrix (LFM)** -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. 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.
- 3.6 **Laboratory Reagent Blank (LRB)** -- An aliquot of reagent water or other blank matrices that are 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.
- 3.7 **Linear Calibration Range (LCR)** -- The concentration range over which the instrument response is linear.
- 3.8 **Material Safety Data Sheet (MSDS)** -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.
- 3.9 **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.
- 3.10 **Quality Control Sample (QCS)** -- A solution of method analytes of known concentrations that is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.
- 3.11 **Stock Standard Solution (SSS)** -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4.0 **INTERFERENCES**

- 4.1 Several interferences are encountered with this method. Some of the known interferences are aldehydes, nitrate-nitrite, oxidizing agents, such as chlorine, thiocyanate, thiosulfate and sulfide. Multiple interferences may require the analysis of a series of laboratory fortified sample matrices (LFM) to verify the suitability of the chosen treatment. Some interferences are eliminated or reduced by the distillation.
- 4.2 Sulfides adversely affect the procedure by producing hydrogen sulfide during distillation. If a drop of the sample on lead acetate test paper indicates the presence of sulfide, treat 25 mL more of the stabilized sample (pH \geq 12) than

that required for the cyanide determination with powdered cadmium carbonate. Yellow cadmium sulfide precipitates if the sample contains sulfide. Repeat this operation until a drop of the treated sample solution does not darken the lead acetate test paper. Filter the solution through a dry filter paper into a dry beaker, and from the filtrate, measure the sample to be used for analysis. Avoid a large excess of cadmium and a long contact time in order to minimize a loss by complexation or occlusion of cyanide on the precipitated material.

- 4.3 High results may be obtained for samples that contain nitrate and/or nitrite. During the distillation nitrate and nitrite will form nitrous acid that will react with some organic compounds to form oximes. These oximes will decompose under test conditions to generate HCN. The interference of nitrate and nitrite is eliminated by pretreatment with sulfamic acid.
- 4.4 Oxidizing agents, such as chlorine, decompose most of the cyanides. Test a drop of the sample with potassium iodide-starch paper (KI-starch paper) at time of collection; a blue color indicates the need for treatment. Add ascorbic acid, a few crystals at a time, until a drop of sample produces no color on the indicator paper; then add an additional 0.06 g of ascorbic acid for each liter of sample volume. Sodium arsenite has also been employed to remove oxidizing agents.
- 4.5 Other compatible procedures for the removal or suppression of interferences may be employed provided they do not adversely effect the overall performance of the method.
- 4.6 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

5.0 **SAFETY**

- 5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.
- 5.2 Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.
- 5.3 The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1 Hydrochloric acid (Section 7.5)

- 5.3.2 Silver nitrate (Section 7.9)
- 5.3.3 Potassium cyanide (Section 7.10)
- 5.3.4 Sulfuric acid (Section 7.14)
- 5.4 Because of the toxicity of evolved hydrogen cyanide (HCN), distillation should be performed in a well vented hood.

6.0 EQUIPMENT AND SUPPLIES

- 6.1 Balance -- Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 6.2 Glassware -- Class A volumetric flasks and pipets as required.
- 6.3 Midi reflux distillation apparatus including boiling flask condenser, and absorber as shown in Figure 1.
- 6.4 Heating mantel or heating block as required.
- 6.5 Automated continuous flow analysis equipment designed to deliver and react sample and reagents in the required order and ratios.
 - 6.5.1 Sampling device (sampler)
 - 6.5.2 Multichannel pump
 - 6.5.3 Reaction unit or manifold
 - 6.5.4 Colorimetric detector
 - 6.5.5 Data recording device

7.0 REAGENTS AND STANDARDS

- 7.1 Reagent water: Distilled or deionized water, free of the analyte of interest. ASTM Type II or equivalent.
- 7.2 Ascorbic acid: Crystal (CASRN-50-81-7)
- 7.3 Chloramine-T: Dissolve 2.0 g of chloramine-T (CASRN-127-65-1) in 500 mL of reagent water.
- 7.4 Magnesium Chloride Solution: Weigh 510 g of $MgCl_2 \cdot 6H_2O$ (CASRN-7786-30-3) into a 1000 mL flask, dissolve and dilute to 1 L with reagent water.
- 7.5 Pyridine Barbituric Acid Reagent: Place 15 g of barbituric acid (CASRN-67-52-7) in a 1 L beaker. Wash the sides of the beaker with about 100 mL of reagent

water. Add 75 mL of pyridine (CASRN-110-86-1) and mix. Add 15 mL of conc. HCl (CASRN-7647-01-0) and mix. Dilute to 900 mL with reagent water and mix until all the barbituric acid has dissolved. Transfer the solution to a 1 L flask and dilute to the mark.

- 7.6 Sodium dihydrogenphosphate buffer, 1 M: Dissolve 138 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (CASRN-10049-21-5) in 1 L of reagent water. Refrigerate this solution.
- 7.7 Sodium Hydroxide Solution, 1.25 N: Dissolve 50 g of NaOH (CASRN-1310-73-2) in reagent water, and dilute to 1 L with reagent water.
- 7.8 Sodium Hydroxide, 0.25 N: Dilute 200 mL of 1.25 N Sodium hydroxide solution (Section 7.7) to 1 L with reagent water.
- 7.9 Standard Silver Nitrate Solution, 0.0192 N: Prepare by crushing approximately 5 g AgNO_3 (CASRN-7761-88-8) crystals and drying to constant weight at 40°C. Weigh out 3.2647 g of dried AgNO_3 , dissolve in reagent water, and dilute to 1000 mL (1 mL = 1 mg CN).
- 7.10 Stock Cyanide Solution: Dissolve 2.51 g of KCN (CASRN-151-50-8) and 2 g KOH (CASRN-1310-58-3) in 900 mL of reagent water. Standardize with 0.0192 N AgNO_3 (Section 7.9). Dilute to appropriate concentration so that 1 mL = 1 mg CN.
- 7.11 Standard Cyanide Solution, intermediate: Dilute 10.0 mL of stock (1 mL = 1 mg CN) (Section 7.10) to 100.0 with reagent water (1 mL = 100.0 µg CN).
- 7.12 Working Standard Cyanide Solution: Prepare fresh daily by diluting 20.0 mL of intermediate cyanide solution (Section 7.11) to 200.0 mL with reagent water and store in a glass stoppered bottle. 1 mL = 10.0 µg CN.
- 7.13 Sulfamic Acid: (CASRN-212-57-3).
- 7.14 Sulfuric Acid, 18N: Slowly add 500 mL of concentrated H_2SO_4 (CASRN-5329-14-6) to 500 mL of reagent water.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

- 8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.
- 8.2 If the sample contains chlorine or hydrogen sulfide, see Section 4.0 for treatment.
- 8.3 Samples must be preserved with sodium hydroxide pH ≥ 12 and cooled to 4°C at the time of collection.

- 8.4 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4°C and may be held for up to 14 days.

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 $\pm 10\%$, 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 $\pm 10\%$ 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 the MDL 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 90-110%, 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 90-110%. 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 (\bar{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:

$$\begin{aligned}\text{UPPER CONTROL LIMIT} &= \bar{x} + 3S \\ \text{LOWER CONTROL LIMIT} &= \bar{x} - 3S\end{aligned}$$

The optional control limits must be equal to or better than the required control limits of 90-110%. 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 $\pm 10\%$ of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within $\pm 10\%$. 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 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 90-110%. 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

9.4.3 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.

- 9.4.4 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 Prepare a series of at least three standards, covering the desired range, and a blank by pipetting suitable volumes of working standard solution (Section 7.12) into 100 mL volumetric flasks. To each standard (except those to be distilled) add 20 mL of 1.25 N sodium hydroxide and dilute to 100 mL with reagent water.
- 10.2 It is not imperative that all standards be distilled in the same manner as the samples. It is recommended that at least two standards (a high and low) and a blank be distilled and compared to similar values on the standard curve to insure that the distillation technique is reliable. If distilled standards do not agree within $\pm 10\%$ of the undistilled standards the analyst should find the cause of the apparent error before proceeding. Before distillation, standards should contain 4 mL 0.25N NaOH (Section 7.8) per 50 mL.
- 10.3 Set up the manifold as shown in Figure 2 in a hood or a well-ventilated area.
- 10.4 Allow the instrument to warm up as required. Pump all reagents, with 0.25N NaOH in the sample line, until a stable baseline is achieved.
- 10.5 Place appropriate standards in the sampler in order of decreasing concentration and perform analysis.
- 10.6 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solutions concentration/response data using computer or calculator based regression curve fitting techniques. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.
- 10.7 After the calibration has been established, it must be verified by the analysis of a suitable QCS. If measurements exceed $\pm 10\%$ of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

11.0 PROCEDURE

- 11.1 Pipet 50 mL of sample or an aliquot diluted to 50 mL into the MIDI distillation boiling flask. Add boiling chips as required. Pipet 50 mL of sodium

hydroxide 0.25 N (Section 7.8) into the absorbing tube. Connect the boiling flask, condenser, and absorber in the train as shown in Figure 1.

- 11.2 Start a slow stream of air entering the boiling flask by adjusting the vacuum source to maintain about three bubbles per minute.
- 11.3 If samples contain NO_3 and/or NO_2 , add 0.2 g of sulfamic acid (Section 7.13) after the air rate is set through the air inlet tube. Mix for three minutes prior to addition of H_2SO_4 .
- 11.4 Slowly add 5 mL 18 N sulfuric acid (Section 7.14) through the air inlet tube. Rinse the tube with distilled water and allow the airflow to mix the flask contents for three minutes. Pour 2 mL of magnesium chloride (Section 7.4) into the air inlet and wash down with a stream of water.
- 11.5 Heat the solution to boiling. Reflux for one and one half hours. Turn off heat and continue the airflow for at least 15 minutes. After cooling the boiling flask, disconnect absorber and close off the vacuum source and remove absorber tube.
- 11.6 Fill and connect reagent containers and start system. Allow the instrument to warm up as required. Pump all reagents, with 0.25N NaOH in the sample line, until a stable baseline is achieved.
- 11.7 Place standards, distilled standards and unknown samples (ALL in 0.25N NaOH) in sampler tray. Calibrate instrument and begin analysis.

12.0 DATA ANALYSIS AND CALCULATIONS

- 12.1 Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.
- 12.2 Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.
- 12.3 Report results in mg/L.

13.0 METHOD PERFORMANCE

- 13.1 The interlaboratory precision and accuracy data in Table 1 were developed using a reagent water matrix. Values are in mg CN/L.
- 13.2 Single laboratory precision data can be estimated at 50-75% of the interlaboratory precision estimates.

14.0 POLLUTION PREVENTION

- 14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.
- 14.2 The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.
- 14.3 For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction", available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036, (202)872-4477.

15.0 WASTE MANAGEMENT

- 15.1 The U.S. Environmental Protection Agency requires that laboratory waste management practices conducted be consistent with all applicable rules and regulations. Excess Reagents, samples, and method process wastes should be characterized and disposed of in an acceptable manner. The Agency urges laboratories to protect the air, water and land by minimizing and controlling all releases from hoods, and bench operations, complying with the letter and spirit of any waste discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Section 14.3.

16.0 REFERENCES

1. Technicon AutoAnalyzer II Methodology, Industrial Method No. 315-74 WCUV digestion and distillation, Technicon Industrial Systems, Tarrytown, NY 10591, (1974).
2. Goulden, P.D., Afghan, B.K. and Brooksbank, P., Anal. 44, 1845 (1972).
3. USEPA Contract Laboratory Program, Document Number ILMO 1.0, Method for Total Cyanide Analysis by MIDI Distillation #335.2 CLP-M.
4. Code of Federal Regulations 40, Ch. 1, Pt. 136, Appendix B.

17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. INTERLABORATORY PRECISION AND ACCURACY DATA

Number of Values Reported	True Value (T)	Mean (X)	Residual for X	Standard Deviation (S)	Residual for S
126	0.020	0.0182	0.0002	0.0055	0.0000
94	0.055	0.0501	-0.0014	0.0092	-0.0007
158	0.090	0.0843	-0.0008	0.0171	0.0027
118	0.110	0.1045	0.0003	0.0165	-0.0004
148	0.180	0.1683	-0.0030	0.0236	-0.0023
92	0.270	0.2538	-0.0038	0.0275	-0.0099
132	0.530	0.5019	-0.0049	0.0775	0.0069
119	0.540	0.5262	0.0098	0.0679	-0.0039
148	0.610	0.5803	-0.0032	0.0851	0.0043
94	0.700	0.6803	0.0105	0.1082	0.0159
92	0.800	0.7726	0.0069	0.0880	-0.0170
158	0.970	0.9508	0.0222	0.1464	0.0197

REGRESSIONS: $X = 0.959T - 0.001$, $S = 0.128T + 0.003$

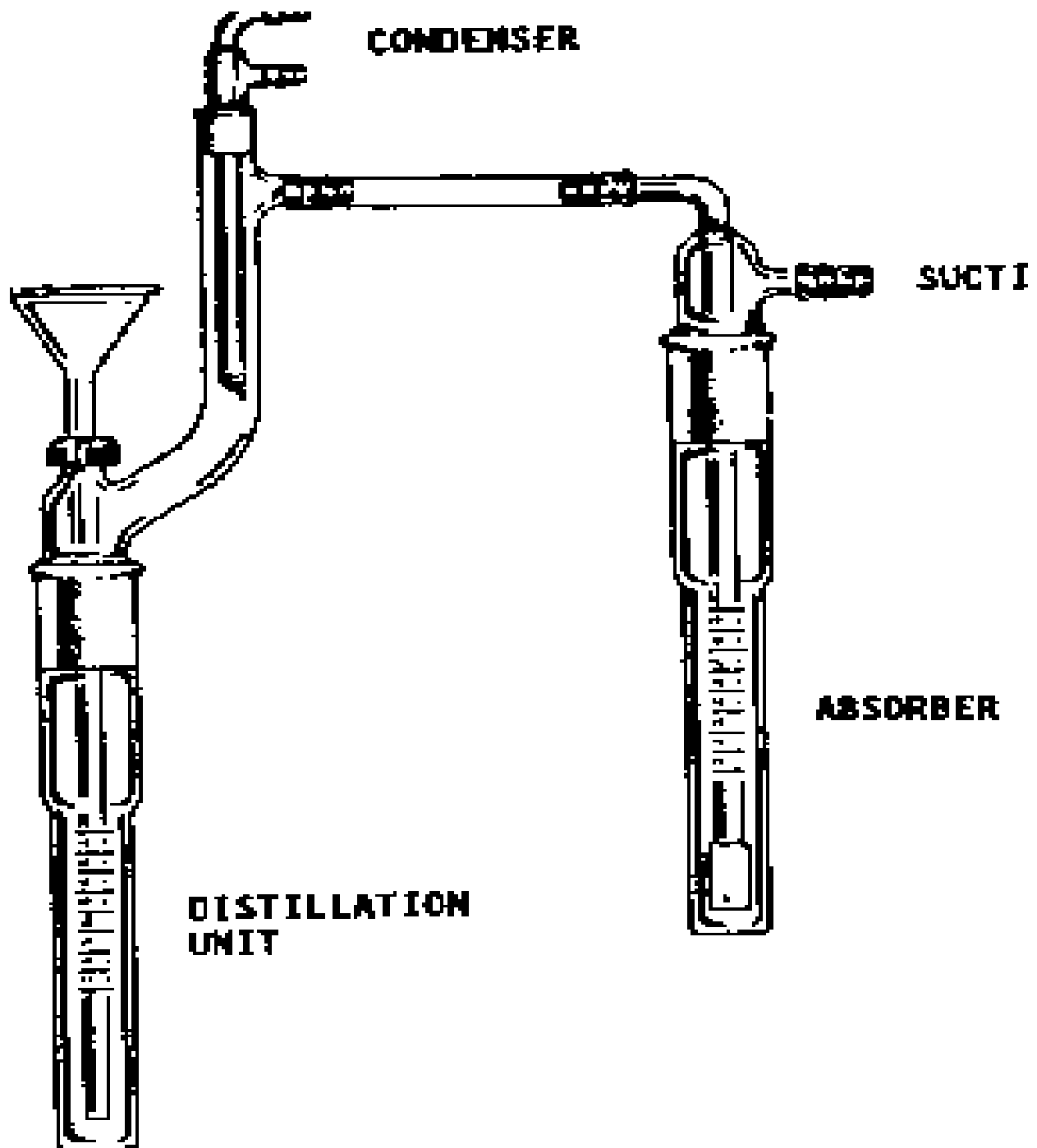


FIGURE 1. MIDI DISTILLATION APPARATUS

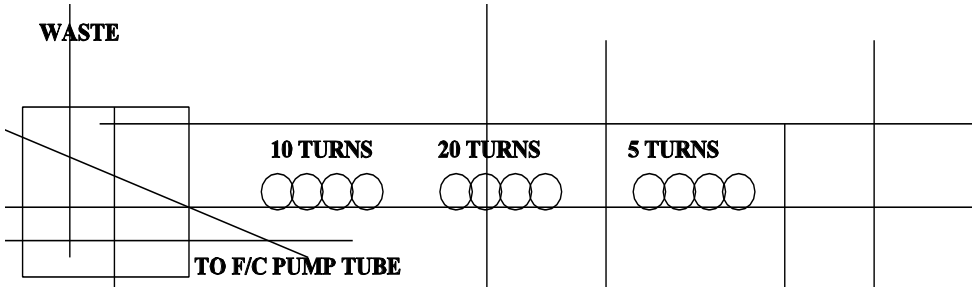

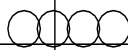

					mL/min	
					0.32	AIR
	10 TURNS 	20 TURNS 	5 TURNS 		1.00	SAMPLE
	TO F/C PUMP TUBE				0.42	BUFFER
COLORIMETER 570 nm 15mm F/C					0.10	CHLOROMINE-T
					1.00	PYRIDINE-BARBITURIC REAGENT
					1.00	FROM F/C
			WASTE		PUMP	
						30 PER HOUR SAMPLE 103 SEC. WASH 17 SEC.

Figure 2
Cyanide Manifold

335.4-16