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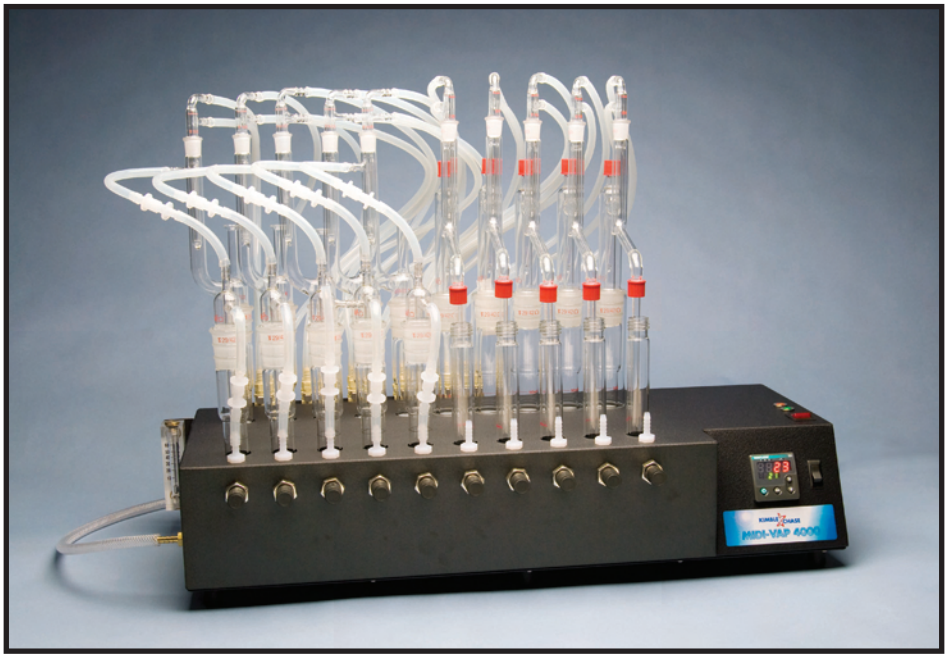


MIDI-VAP 4000
Distillation Systems

For 50mL Reduced Sample Volumes
USEPA / 600 Determination by Semi-Automated Colorimetry

Catalog No.	Method	Determination
479490-4000	Method 350.1	Midi-Ammonia
479400-4000	Method 335.4	Midi-Cyanide
479490-4000	Method 420.4	Midi-Phenols

Instruction Manual Number 9539240 (Rev. September 2011)

To register your Kimble Chase MIDI-VAP 4000 Distillation System, please call customer service within 10 days of receipt. Call toll-free: 1-888-546-2531 Ext. 1.

Kimble Chase Warranty
We warrant to the original user of this product that it is free from all defects in material and workmanship under normal use and service.

All mechanical and electronic components are guaranteed for a period of **1 year unless otherwise specified.** Should any part prove to be defective within this time as a result of faulty workmanship or material, Kimble Chase will, at its option, repair or replace it free of charge.

This guarantee is subject to the conditions set forth under "User's Responsibility." The term "Original User" as used in this guarantee shall be deemed to mean that person, firm, association, or corporation which has made the original purchase of this instrument for his or its own use; and this guarantee shall be void if this instrument is resold. EXCEPT AS SET FORTH HEREIN, NO WARRANTY OF MERCHANTABILITY OR OF FITNESS FOR PURPOSE SHALL APPLY. Kimble Chase assumes no responsibility for cost of handling, installation, etc., or for transport charges.

Liability
While all Kimble Chase products are manufactured to the highest standards, we cannot accept any claims for loss, damage, or injury due to failure to operate as intended. Our responsibility is limited to repair or replacement of defective material as covered by this guarantee.

IN NO EVENT SHALL THE COMPANY BE LIABLE FOR CONSEQUENTIAL OR SPECIAL DAMAGES. This guarantee is in lieu of all other guarantees, expressed or implied, and no person is authorized to assume for us any other obligation or liability in connection with this product.

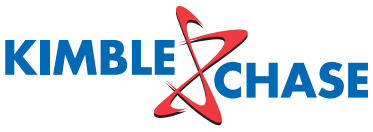
User's Responsibility - Guarantee Instructions
This product has been manufactured and tested with utmost care in order to provide the user with years of satisfactory service. To get the most satisfactory and economical results from this product, proper care should be taken of it as any other mechanism and periodic inspection should be provided.

The user should make certain that, in its installation, this product has been fully protected against improper electrical current, fluctuating voltages, or low voltages. Failures due to these conditions or any other condition not attributable to defective workmanship or materials are excluded from our guarantee.

The user is liable for any repairs or reworking due to the unit having been subjected to misapplication, misuse, damage or abusive tampering. Warranty repair units must be returned intact and are subject to inspection.

Installation of replacement parts other than standard Kimble Chase parts, or removal or defacement of the serial data plate, voids the entire guarantee.

If this product is not operating properly, call customer service 1-888-546-2531, ext. 1 for instructions on returned material authorization procedure. After receiving authorization to return your product, it should be carefully packed, using shock absorbing material, and insured, since Kimble Chase cannot assume responsibility for inadequate packing and damage in shipment.



NOTES:

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Please document your purchase here.

Catalog No. _____

Serial No. _____

Voltage of Heater Unit: _____

Printed in USA Instruction 9539240 Rev. 9/11

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Unpacking the Unit

The MIDI-VAP 4000 Distillation Systems are shipped in 3 separate cartons. One carton contains the heater and all required tubing. The other two cartons contain 5 each sets of either Ammonia / Phenols or Cyanide glassware depending upon the item ordered. Carefully unpack the cartons and check the contents to be sure that all parts are received. If you do not receive all of the items listed, please contact technical support at tech@kimble-chase.com. Please refer to page 16 for replacement part numbers.

The MIDI-VAP 4000 Manifold Heater [720440-4000 (115V), 720440-4220 (220-240V)] includes the following items:

1 each Manifold Heater, 115V or 1 each Manifold Heater, 220-240V

1 Complete Tubing Kit (Includes tubing and connectors for all tests.)

2 bags containing 5 ft. each of braided PVC "feed / drain" tubing for water manifold to water source or to drain

1 bag containing 5 ft. of clear Tygon® tubing for vacuum inlet

1 bag containing 20 each of silicone "condenser water manifold tubing" (17-inch length) with male quick disconnect fitting

1 bag containing 10 each of silicone "reaction flask to impinger" tubing (7-inch length) with slip fit connector - **for cyanide only**

1 bag containing 10 each of silicone "absorption flask to vacuum inlet" tubing (10-inch length) with slip fit connector - **for cyanide only**

The Cyanide Glassware (479460-0005) includes 5 sets each of the following items packed in foam in separate sleeves:

1 each cold finger condenser (282000-0000)

1 each distilling head (479461-0000)

1 each absorption impinger (479462-0023)

2 each 50mL reaction / absorption tubes (479455-0050)

The Ammonia / Phenols Glassware (479459-0005) includes 5 sets each of the following items packed in foam in separate sleeves:

1 each cold finger condenser (282000-0000)

1 each distilling head with 2 GL14 red caps, 1 silicone sealing ring and 1 PTFE / silicone septa (479456-4501)

1 each 50mL reaction tube with draft shield (479470-0050)

1 each short stem outlet tube for use in phenols procedure (479458-0000)

1 each long stem outlet tube for use in ammonia procedure (479458-0001)

1 each 50mL receiver tube with GL25 storage cap (479471-0050)

Safety Information

1. The MIDI-VAP 4000 Distillation System can be used for the analysis of ammonia, cyanide or phenols. The footprint is approximately 30" x 14.25". The approximate height, when assembled with glassware is 20". **For maximum safety of operation, the system should be used in a fume hood that is rated at 100 CFM and is suitable for the manipulation of caustic and corrosive substances.**

2. Boiling chips, beads or stones should be used in each sample tube to reduce bumping. The ammonia / phenols reaction tube is designed with a draft shield to optimize performance and reduce distillation time.

3. Do not attempt to move the components while they are hot, as sudden movement may result in bumping and / or sample loss.

4. To avoid bumping or boil over, allow system to cool 20 minutes before removing glassware.

5. **Installation of an in-line gas trap between the vacuum line and the vacuum pump is recommended to remove excess HCN vapor and to protect the vacuum pump when the unit is used for Midi-Cyanide analysis.**

6. **To avoid the possibility of shock hazard, unplug the unit prior to cleaning of exterior surfaces.**

7. Do not disconnect water or vacuum lines until the unit has completely cooled, as this may cause the samples to boil over.

8. The heater block temperature is factory preset to 126°C for cyanide, to 165°C for ammonia / phenols and has a maximum temperature of 190°C for other test protocols. Caution should be exercised when preparing or removing samples. **The heater should not be installed in locations where flammable materials (which have a flash point below 200°C) are present.**

9. While the case is PTFE-coated, it is not acid-proof. Spills should be wiped with a soft cloth and be followed by rinsing with distilled water. Holes are drilled through the bottom of the heater to allow spills to drain. It is recommended that small containers such as glass Petri dishes be placed under these holes to contain any spills.

CAUTION!

SOME REAGENTS USED IN THE DETERMINATION OF CYANIDE ARE CAUSTIC AND MAY CAUSE SKIN IRRITATION.

ALWAYS UNPLUG UNIT FOR CLEANING!

Instrument Set-Up

(see diagrams on page 5 and test protocols in Appendix)

1. Unpack the MIDI-VAP 4000 Distillation System heater, check the parts received against the listing on page 2 and immediately report any damages or shortages to Kimble Chase Customer Service (1-888-546-2531, info@kimble-chase.com). Retain the original packing materials in the event that the unit must be returned for repair.

2. Complete the flowmeter and water manifold tubing connections as follows:

   Connect the five-foot length of clear vacuum tubing to the brass hose barb (vacuum inlet) on the left side of the unit in front of the flowmeter. We recommend connecting the other end to a vacuum trap (vacuum filtration flask, Cat. No. 953760-series, filled with NaOH solution) and then to the vacuum source in order to best protect your pump.

   Connect one five-foot length of braided feed / drain tubing to the lower (water inlet) hose barb on the rear of the flowmeter. Connect the other end to a cold water source. **A chiller is recommended to maintain water temperature at 4°C and achieve best results.** Connect the second five-foot length of braided feed / drain tubing to the upper (water outlet) barb on the water manifold and place the other end into a drain.

   Refer to pages 10, 12, and 14 for glassware setup and standards preparation before proceeding to step 3.

3. Plug the 115V unit power cord into a suitable 3-wire grounded electrical outlet rated at 15 Amps. **The 220-240V model is supplied with an unterminated international standard cord.**

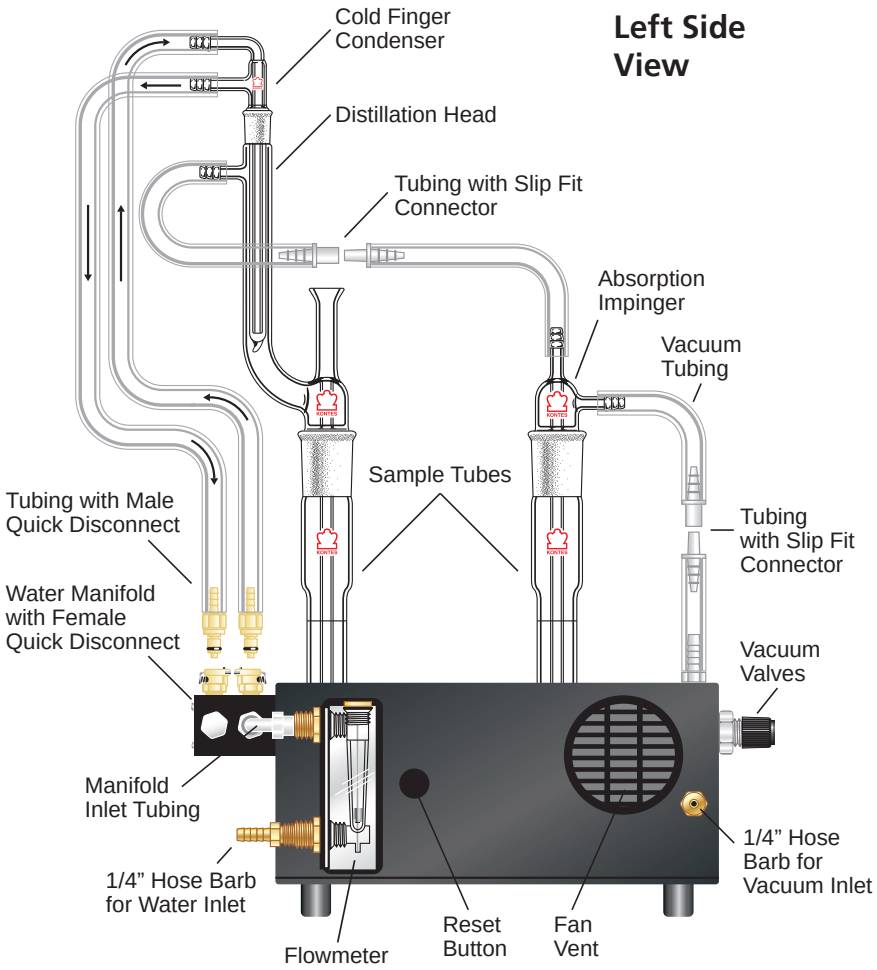
4. Turn the red, lighted power switch (on top of the unit) to the "ON" position. The green light indicates power to the unit. Turn the black, heater power switch (on the front of the unit) to the "ON" position and the amber light will come on indicating power to the heater. The controller display will show both ambient and set point temperatures. As the unit heats up, the temperature will rise until the set point is reached and the timing countdown sequence begins. The amber light will flicker when the set point is approached and then flash to indicate continued heating to the set point temperature. The timer will count down from 59 minutes to the end of the test at which time the controller will automatically shut off. Allow the unit to cool, keeping the water running for at least 15-20 minutes, and then turn off both switches. To view the time remaining at any time during the run, press the advance key 4 times. The time remaining will be displayed and then return to the original temperature display.

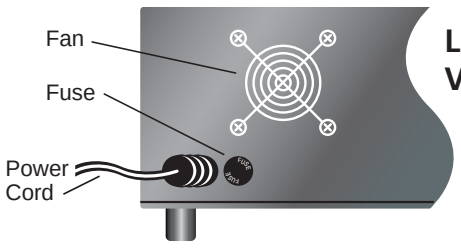
To initiate the temperature program for cyanide:	Press infinity key once.	→	Display shows FILE 1.
	Press advance key once.	→	Display shows STEP 1.
	Press infinity key a second time to lock temperature program and begin the sequence for cyanide distillation at 126°C.		

To initiate the temperature program for ammonia / phenols:	Press infinity key once.	→	Display shows FILE 1.
	Press the UP arrow once.	→	Display shows FILE 2.
	Press advance key once.	→	Display shows STEP 2.
	Press infinity a second time to lock temperature program and begin the sequence for ammonia / phenols distillation at 165°C.		

PLEASE NOTE: Any manual changing to the pre-set controller temperatures will automatically override (disengage) the timer function.

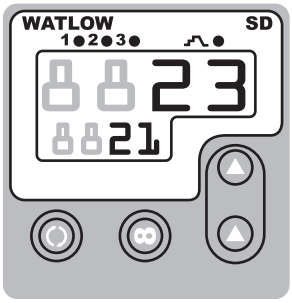
Heater Manifold Diagrams





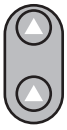
Digital Controller and Power Switches

Watlow SD Controller Detail



Advance Key
Advances the lower or right display through parameter prompts.

Infinity Key
Returns to the Home Page, adjusts the set point in the lower or right display.



Up and Down Keys
In the Home Screen, adjusts the set point in the lower or right display.

Instrument Controls

(refer to diagrams on page 5)

Red Power Switch
The lighted upper switch (power) indicates "system ON and ready" and is located on the right side top panel in front of the green / amber lights and is rated at 20 amps.

Black Heater Switch
The heater switch indicates "heater ON" and is located on the right side front panel next to the controller.

Watlow SD Controller
The Watlow SD controller is located on the front panel and is factory preset to 126°C for cyanide and to 165°C for ammonia/phenols. It is designed to control the operation of the heater block up to 190°C by automatically turning the power to the heating element on/off. The controller may be manually adjusted by pressing the "UP arrow" key until the desired temperature is reached.

Green Pilot Light
The green pilot light located on top of the unit indicates that the main power to the unit is on. Note: If the light fails to come on when the main switch is on, reset the unit by pressing the reset button that is located under the black grommet in front of the flowmeter on the left side of the heater manifold.

Amber Pilot Light
The amber pilot light located on top of the unit operates during the heat cycle. The flashing light indicates power to the heating element. The flashing stops when the heater reaches the preset temperature.

Flowmeter
The flowmeter measures the amount of cooling water entering the cold finger condensers. It is calibrated to indicate gallons per hour (GPH). Water through the flowmeter should be at 18GPH during all procedures.

Vacuum Valves
The vacuum valves are located on the front panel, one for each position. To adjust the valve rotate the black knob left or right. Rotating clockwise closes the valve and counterclockwise opens the valve. Do not over tighten the valve during shutoff as the needle may become damaged.

Main Fuse Holder
The main fuse holder is located on the left rear of the unit. A 15 amp fuse (8 amp for 220-240V) must be in place for the unit to operate. Note: Temperature controller and fan are fused separately.

Reset Switch
The reset switch is located on the side panel in front of the flowmeter under a black grommet. It is designed to protect the unit from burnout caused by failure of the temperature controller and / or power relay. To reset the unit, remove the rubber grommet, press the button and then replace the grommet.

Cooling Fan
Temperature controlled to run at 100°C and above.

Troubleshooting Guide

Electrical	Recommendation
No power to unit	1. Plug unit into working wall outlet 2. Turn both switches to "ON" position 3. Check / replace fuse located on back of unit 15A for 115V heater unit 8A for 220-240V heater unit 4. Reset breaker located under black grommet 5. Return unit for service (see page 8)
Controller error message	1. Return unit for service (see page 8)
Erratic controller temperature	1. Return unit for service (see page 8)
No heat / Samples will not boil	1. Turn the heater switch to 'ON' position 2. Check digital display for correct preset temperature 3. Check / replace fuse located on back of unit 15A for 115V heater unit 8A for 220-240V heater unit 4. Return unit for service (see page 8)
No power to controller	1. Turn the controller switch to 'ON' position 2. Return unit for service (see page 8)
Overheating	1. Check controller set point temperature 2. Return unit for service (see page 8)

Test Procedures	Recommendation
Poor recoveries	1. Adjust cooling water to 4˚C and 18GPH 2. Adjust vacuum to 3 bubbles / second 3. Verify that sample boils for 60 minutes 4. Check standard solutions 5. Check tubing connections for leaks
Condensation in tubing	1. Adjust cooling water to 4˚C and 18GPH 2. Adjust vacuum to 3 bubbles / second
Excessive foaming / boil over	1. Dilute sample to reduce concentration and adjust results

Return and Servicing Instructions

1. Return Authorization Number
Contact Kimble Chase Customer Service at 888-546-2531, ext. 1 and request a "Return Authorization" number. This "RA" number is required in order to return your unit for service and must appear on the outer carton shipping label. **Along with the RA, a certificate of decontamination will be issued which must be completed and returned with the heater manifold.** We will need the serial number and date of purchase to determine warranty status and may refer you to Technical Support for clarification of the reason for return. Repair turnaround time is 3-5 days from receipt of unit at Kimble Chase. Customers will be contacted by phone or email with repair estimates for authorization to perform out of warranty repairs prior to the work being started. Warranty units are repaired at no cost.

2. Loaner Unit Policy - Loaner units are available in the USA and Canada only.
For units under warranty, Kimble-Chase will provide a loaner unit at no charge for a period of up to 30 days. A service charge of $130.00 / month will apply to units kept after this 30-day period has expired. Contact customer service or technical service for details.

3. Packing the Unit
Clean and carefully pack the instrument in a large sturdy box (the original carton if possible). Please allow a minimum of 2 inches between any surface of the instrument and the box walls, including the bottom. Rigid packing materials will prevent the instrument from shifting during transport. It is strongly advised that the return shipment be insured against possible damage during transit.

4. Return Information
Please include with the instrument a note with the following information: Name, telephone number and email of the person to be contacted along with company name, return shipping address, and method of shipment. Please also include a brief description of the problem. This information is vital in order to provide prompt service.

5. Shipping the Unit
Ship the unit directly to Kimble Chase using the address provided by your customer service associate. Be sure to clearly indicate the RA # on the outside of the carton as well as your return address. Kimble Chase is not liable for lost or damaged equipment.

6. Repair / Return of the Unit
When the unit is returned to Kimble Chase, it will be evaluated and the contact person indicated will be notified of any repair costs. A purchase order or credit card number will be required before any repair work is done. Once repaired, your instrument will be returned to the address requested. Return freight charges will be prepaid and added to the repair invoice. Warranty repairs will be performed at no cost.

Parts List / General Information

The MIDI-VAP 4000 Distillation Systems for Cyanide and Ammonia / Phenols Reduced Sample Volumes are shipped complete with the 10-position heater block, water manifold, flowmeter, 10 complete sets of borosilicate glassware for model ordered, all tubing connections, and instruction manual.

- The 10-Position Heater Block features a Watlow SD temperature controller, 10-position vacuum manifold with needle valves, 10-position parallel feed water manifold system, reset button (located under black grommet) and flowmeter with scale indicating gallons per hour readout.
- The Glassware Sets are listed for specific model ordered. Refer to the diagram and the assembly set-up diagrams on pages 10, 12, and 14.
- The 10-Position Complete Tubing Set includes the items in the list following. Refer to the instrument setup instructions on page 4 and the diagram on page 5.

Tubing used for Cyanide, Ammonia and Phenols protocols:

2 each braided PVC "feed / drain" tubing, 5 ft. (water manifold to water source or to drain)

20 each of silicone "condenser to water manifold tubing" (17-inch length) with male quick disconnect fitting

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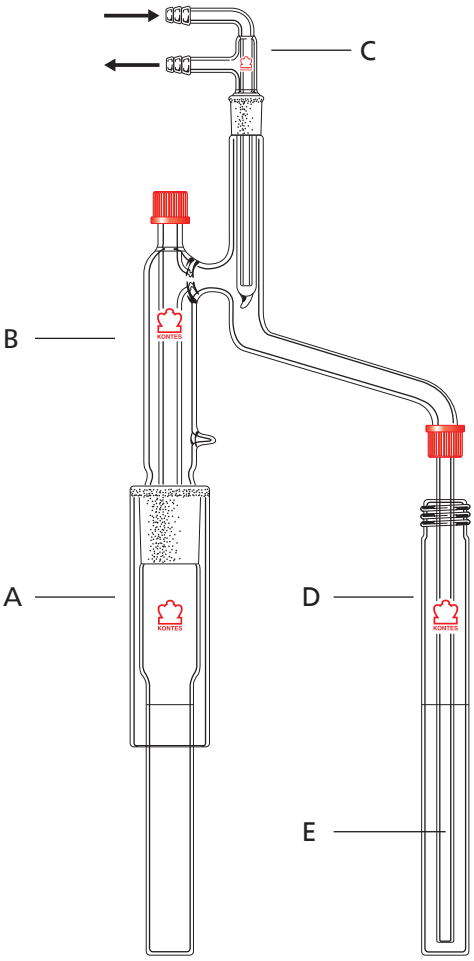
Additional tubing used with Midi-Cyanide protocol only:

1 ea. 5 ft. of clear Tygon® tubing for vacuum inlet

10 ea. silicone "reaction flask to impinger" tubing (7-inch length) with slip fit connector

10 ea. silicone "absorption flask to vacuum inlet" tubing (10-inch length) with slip fit connector

Midi-Ammonia Glassware Set-Up and Parts Diagram



Catalog No.	Description
479490-4000	MIDI-VAP 4000 Ammonia / Phenols Complete Includes:
	479459-0005 Midi-Vap Ammonia / Phenols Glassware set of 5 (**479490-4000** supplied with 2 sets of 5)
	720440-4000 MIDI-VAP 4000 Manifold Heater only, 115V w/ tubing kit
	720440-4220 MIDI-VAP 4000 Manifold Heater only, 220-240V w/ tubing kit

Glassware Component Parts

479470-0050	(A) 50mL Reaction Tube with Draft Shield for Ammonia / Phenols
479456-4501	(B) Distillation Head for Ammonia / Phenols
282000-0000	(C) Universal Cold Finger Condenser
479471-0050	(D) 50mL Receiver Tube for Ammonia / Phenols
479458-0001	(E) Long Stem PTFE Tube for Ammonia

Replacement Ammonia / Phenols Tubing Kit

479418-0043	Ammonia / Phenols Tubing Kit

Midi-Ammonia Assembly and System Operation

There are various forms of nitrogen reported in water analysis, typically as mg/L (parts per million).

Nitrate	NO3
Nitrite	NO2
Ammonia	NH3
Organic	N2
Total Kjeldahl	TKN

1. Organic nitrogen is calculated by subtracting the ammonia value from total Kjeldahl, which includes all forms of nitrogen, free and chemically bound. **Refer to EPA method 350.1 in appendix for test procedure.**

2. Place one 50mL sample tube containing standard or sample and assembled with distillation head and cold finger condenser in each rear hole of the heater as shown in the diagram on page 5 and adjust the pH to 9.5. Interference from residual chlorine is eliminated by adding sodium thiosulfate.

3. **Boiling chips, beads or stones should be added to each sample tube to reduce bumping.**

4. Attach the open ends of the 17" water manifold tubing to the top and bottom cold finger condenser inlet and outlet hose barbs so that the cooling water enters at the top and exits at the bottom. Attach other end to water manifold by snapping the quick disconnect fittings together. See diagram on page 5.

   Carefully introduce cooling water to the cold finger condensers and adjust for the number of positions as necessary. Check to ensure that all hoses are firmly in place and are not leaking. The recommended flow rate is 18GPH. To ensure maximum recoveries in samples where suspected CN levels are below 100 ppb, cold water temperature should be maintained at 4°C which will require installation of a chiller.

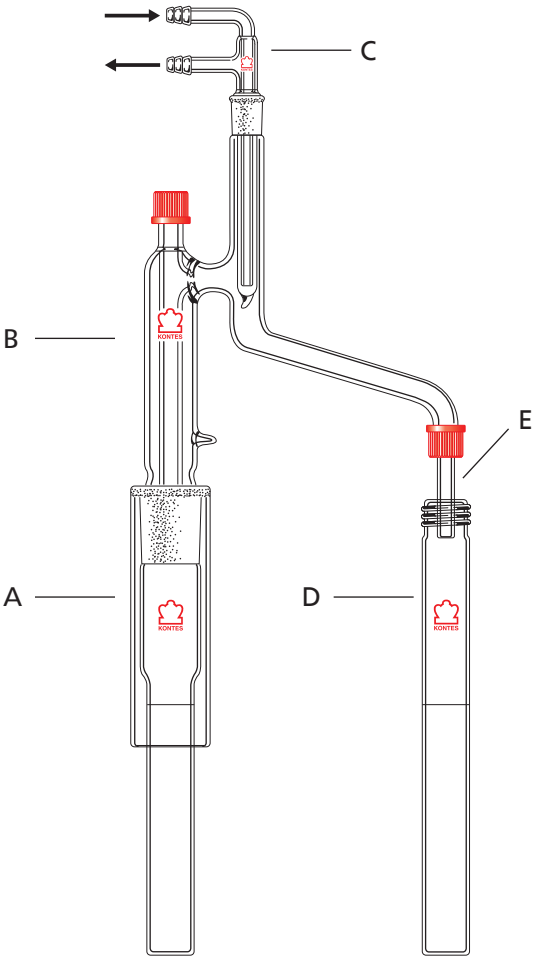
5. Ammonia liberated in the distilling step is captured under a layer of acid in the receiver tube. Place the 50mL receiver tubes in the front row of holes and assemble with the **long** stem glass tubes. Unscrew the red caps and remove the sealing rings, slide the tubes through the holes and reattach the caps and sealing rings to the distilling head arms.

6. The temperature for ammonia distillation is preset in FILE 2 to 165°C. See controller setup on page 4. It will take approximately 30 minutes for the unit to reach temperature at which time the test countdown will begin. The controller will automatically shut down the heater when the test is completed.

   NOTE: It is strongly recommended that the water temperature used during the distillation be at 4°C which will require the use of a chiller. The water should be left running for at least 20 minutes after unit shutdown to prevent boil over and as a safety precaution.

7. **Return to instrument setup "Step 3" on page 4.**

Midi-Phenols Glassware Set-Up and Parts Diagram



Catalog No.	Description
479490-4000	MIDI-VAP 4000 Ammonia / Phenols Complete Includes:
	479459-0005 Midi-Vap Ammonia / Phenols Glassware set of 5 (**479490-4000** supplied with 2 sets of 5)
	720440-4000 MIDI-VAP 4000 Manifold Heater only, 115V w/ tubing kit
	720440-4220 MIDI-VAP 4000 Manifold Heater only, 220-240V w/ tubing kit

Glassware Component Parts

479470-0050	(A) 50mL Reaction Tube with Draft Shield for Ammonia / Phenols
479456-4501	(B) Distillation Head for Ammonia / Phenols
282000-0000	(C) Universal Cold Finger Condenser
479471-0050	(D) 50mL Receiver Tube for Ammonia / Phenols
479458-0000	(E) Short Stem PTFE Tube for Phenols

Replacement Ammonia / Phenols Tubing Kit

479418-0043	Ammonia / Phenols Tubing Kit

Midi-Phenols Assembly and System Operation

Phenols refer to a group of all compounds which contain a hydroxy or hydroxyl (-OH) substituted group as a derivative of benzene. The reaction of the distillate with the 4-aminoantipyrine and alkaline ferricyanide forms a colored complex which is measured at 505 or 520 nm. **Refer to EPA method 420.4 in appendix for test procedure.**

1. Place one 50mL sample reaction tube containing standard or pH adjusted sample and assembled with distillation head and cold finger condenser into each rear hole of the heater as shown in the diagram on page 5. Interferences from sulfur compounds are eliminated by adjusting sample pH to 4.0.

2. **Boiling chips, beads or stones should be added to each sample tube to reduce bumping.**

3. Attach the open ends of the 17" water manifold tubing to the top and bottom cold finger condenser inlet and outlet hose barbs so that the cooling water enters at the top and exits at the bottom. Attach other end to water manifold by snapping the quick disconnect fittings together. See diagram on page 5.

   Carefully introduce cooling water to the cold finger condensers and adjust for the number of positions as necessary. Check to ensure that all hoses are firmly in place and are not leaking. The recommended flow rate is 18GPH. To ensure maximum recoveries in samples where suspected CN levels are below 100 ppb, cold water temperature should be maintained at 4°C which will require installation of a chiller.

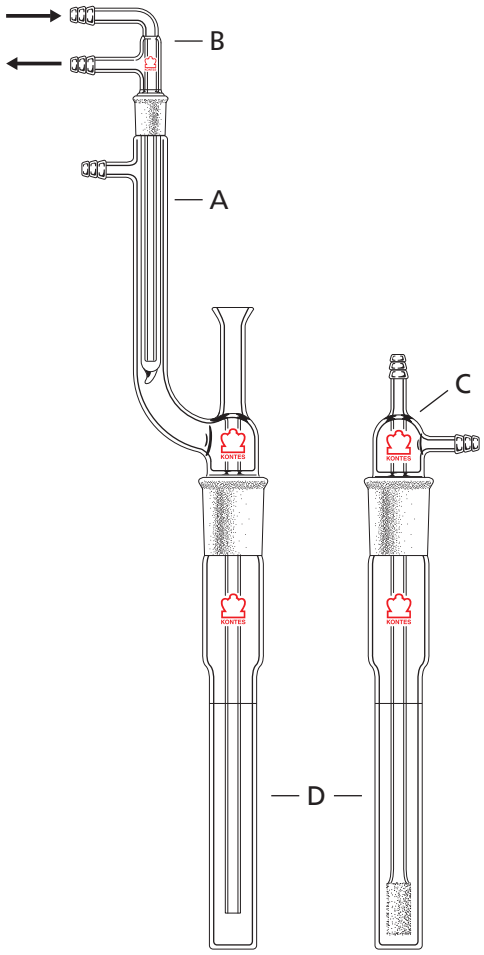
4. Place 50mL receiver tubes in front row of holes and assemble with **short** stem glass tube. Unscrew the red caps and remove the sealing rings, slide the tubes through the holes and reattach the caps and sealing rings to the distilling head arms.

5. The temperature for the phenols distillation is factory preset in FILE 2 to 165°C. See instrument setup instructions on page 4. It will take approximately 30 minutes for the unit to reach temperature at which time the test countdown begins. The controller will automatically shut down the heater when the test is completed.

   NOTE: It is strongly recommended that the water temperature used during the distillation be at 4°C which will require the use of a chiller. The water should be left running for at least 20 minutes after shutdown to prevent boil over and as a safety precaution.

6. **Return to Instrument Set-up "Step 3" on page 4.**

Midi-Cyanide Glassware Set-Up Diagram and Parts List



Catalog No.	Description
479400-4000	MIDI-VAP 4000 Cyanide Complete Includes:
	479460-0005 Midi-Cyanide Glassware set of 5 (**479400-4000** is supplied with 2 sets of 5)
	720440-4000 MIDI-VAP 4000 Manifold Heater only, 115V w/ tubing kit
	720440-4220 MIDI-VAP 4000 Manifold Heater only, 220-240V w/ tubing kit

Glassware Component Parts

479461-0000	(A) Distillation Head with Air Inlet Tube
282000-0000	(B) Universal Cold Finger Condenser
479462-0023	(C) Dispersion Tube with Coarse Porosity Frit
479455-0050	(D) 50mL Reaction / Absorber Tube (2 Supplied)

Replacement Cyanide Tubing Kit

479418-0046	Cyanide Tubing Kit

Midi-Cyanide Assembly and System Operation

1. This test is designed to reduce cyanide complexes to HCN gas. **For maximum safety of operation, the system should be used in a fume hood that is rated at 100 CFM velocity and which is suitable for manipulation of caustic and corrosive substances. Refer to method 335.4 in the Appendix for test procedure.** Use caution as some components are corrosive and may cause skin irritation.

2. Place one 50mL sample reaction tube assembled with distillation head and cold finger condenser into each rear hole of the heater. See diagram page 5. Silicone grease may be lightly applied to all joints to aid in preventing leaks.

3. Add 50mL of prepared NaOH solution to each absorber tube and assemble with a fritted dispersion head and place into each front row hole of the heater.

4. Make all tubing connections as per diagram found on page 5. There are two types of fittings used on the Cyanide Distillation System—quick disconnect fittings and slip fittings. The quick disconnect fittings are made of polypropylene with ethylene propylene o-rings (temperature range of -50°C to 145°C) for acid resistance. The metal tab on the female fitting (on the water manifold) should be depressed and held until the male fitting snaps into place to avoid damaging the o-ring and causing possible water leakage. The slip fit connections are polypropylene for acid resistance and are pressure fitted. When separating slip fit connections, it is important to twist and pull to loosen the connection and avoid distortion or damage to tubing or glassware.

5. Close all vacuum valves by turning the knobs on the front panel of the heater clockwise.

6. Carefully introduce cooling water to the cold finger condensers and adjust for the number of positions as necessary. Check to ensure that all hoses are firmly in place and are not leaking. The recommended flow rate is 18GPH. To ensure maximum recoveries in samples where suspected CN levels are below 100 ppb, cold water temperature should be maintained at 4°C which will require installation of a chiller.

7. Turn on the vacuum source and adjust the needle valve at each sample position so that a rate of 3 bubbles / second is observed in the sample distillation tube. Allow vacuum to draw for 5 minutes while observing the bubbles and then adjust the vacuum as necessary.

8. **Return to instrument setup "Step 3" on page 4.** Adjust vacuum as necessary to maintain a rate of 3 bubbles / second in the sample receiver tube.

9. The timer will automatically stop the heating process after the 60 minute set time. Allow distillation tubes to cool with water running for 15-20 minutes before removing samples for determination of CN.

10. Clean the frits in the dispersion heads as soon as possible after use by rinsing from the reverse side with water under pressure not to exceed 15lbs / sq. inch. Clogged frits may be cleaned by soaking in [HCL] for 20 minutes, followed by thorough rinsing.

Component Parts

MIDI-VAP 4000 Ammonia / Phenols Parts

Catalog No.	Description
479490-4000	**MIDI-VAP 4000, Ammonia / Phenols Complete**
282000-0000	Universal Cold Finger Condenser
479456-4501	Distilling Head for Ammonia / Phenols
479458-0001	Long Stem Tubing for Ammonia Test
479458-0000	Short Stem Tubing for Phenols Test
479459-0000	Ammonia / Phenols Glassware, Set of 1
479459-0005	Ammonia / Phenols Glassware, Set of 5
479470-0050	Ammonia / Phenols Reaction Tube, 50mL
479471-0050	Ammonia / Phenols Receiver Tube with Cap, 50mL
410479-0014	High Temperature Red PBT GL14 Open Top Cap, Pkg. / 10
410479-0025	High Temperature Red PBT GL25 Solid Cap, Pkg. / 10
410480-0014	Silicone Sealing Ring for GL14 Cap, Pkg. / 10
410481-0014	PTFE / Silicone Septum for GL14 Cap, Pkg. / 10
479400-0014	Quick Disconnect Fitting, Outer Female for Water Manifold, Pkg. / 5
479400-0018	Quick Disconnect Fitting, Inner Male for Water Manifold, Pkg. / 5
479400-0022	Quick Disconnect Fitting for Vacuum Tubing, Pkg. / 5
479418-0043	Ammonia / Phenols Tubing Kit

MIDI-VAP 4000 Cyanide Parts

Catalog No.	Description
479400-4000	**MIDI-VAP 4000, Cyanide Complete**
282000-0000	Universal Cold Finger Condenser
479455-0050	Sample Reaction / Absorber Tube for Cyanide, 50mL
479460-0000	Cyanide Glassware, Set of 1
479460-0005	Cyanide Glassware, Set of 5
479461-0000	Cyanide Distillation Head
479462-0023	Cyanide Gas Dispersion Tube with Coarse Porosity Frit
479418-0046	Cyanide Tubing Kit
479400-0014	Quick Disconnect Fitting, Outer Female for Water Manifold, Pkg. / 5
479400-0018	Quick Disconnect Fitting, Inner Male for Water Manifold, Pkg. / 5
479400-0022	Quick Disconnect Fitting for Vacuum Tubing, Pkg. / 5

MIDI-VAP 4000 Manifold Heater with Complete Tubing Kit

Catalog No.	Description
720440-4000	**MIDI-VAP 4000 Manifold Heater with Complete Tubing Kit, 115V**
720440-4220	**MIDI-VAP 4000 Manifold Heater with Complete Tubing Kit, 220-240V**

METHOD 350.1

DETERMINATION OF AMMONIA NITROGEN BY SEMI-AUTOMATED COLORIMETRY

Edited by James W. O'Dell
Inorganic Chemistry Branch
Chemistry Research Division

Revision 2.0
August 1993

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

METHOD 350.1

DETERMINATION OF AMMONIA NITROGEN BY SEMI-AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of ammonia in drinking, ground, surface, and saline waters, domestic and industrial wastes.

1.2 The applicable range is 0.01-2.0 mg/L NH_3 as N. Higher concentrations can be determined by sample dilution. Approximately 60 samples per hour can be analyzed.

1.3 This method is described for macro glassware; however, micro distillation equipment may also be used.

2.0 SUMMARY OF METHOD

2.1 The sample is buffered at a pH of 9.5 with a borate buffer in order to decrease hydrolysis of cyanates and organic nitrogen compounds, and is distilled into a solution of boric acid. Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside and measured colorimetrically.

2.3 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.

2.4 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 Cyanate, which may be encountered in certain industrial effluents, will hydrolyze to some extent even at the pH of 9.5 at which distillation is carried out.

4.2 Residual chorine must be removed by pretreatment of the sample with sodium thiosulfate or other reagents before distillation.

4.3 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 have 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 Sulfuric acid (Section 7.6)

5.3.2 Phenol (Section 7.7)

5.3.3 Sodium nitroprusside (Section 7.10)

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 An all-glass distilling apparatus with an 800-1000 mL flask.

6.4 Automated continuous flow analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.4.1 Sampling device (sampler)

6.4.2 Multichannel pump

6.4.3 Reaction unit or manifold

6.4.4 Colorimetric detector

6.4.5 Data recording device

7.0 REAGENTS AND STANDARDS

7.1 Reagent water - Ammonia free: Such water is best prepared by passage through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Regeneration of the column should be carried out according to the manufacturer's instructions.

Note: All solutions must be made with ammonia-free water.

7.2 Boric acid solution (20 g/L): Dissolve 20 g H_3BO_3 (CASRN 10043-35-3) in reagent water and dilute to 1 L.

7.3 Borate buffer: Add 88 mL of 0.1 N NaOH (CASRN 1310-73-2) solution to 500 mL of 0.025 M sodium tetraborate solution (5.0 g anhydrous $Na_2B_4O_7$ [CASRN 1330-43-4] or 9.5 g $Na_2B_4O_7 \cdot 10H_2O$ [CASRN 1303-96-4] per L) and dilute to 1 L with reagent water.

7.4 Sodium hydroxide, 1 N: Dissolve 40 g NaOH in reagent water and dilute to 1 L.

7.5 Dechlorinating reagents: A number of dechlorinating reagents may be used to remove residual chlorine prior to distillation. These include:

7.5.1 Sodium thiosulfate: Dissolve 3.5 g $Na_2S_2O_3 \cdot 5H_2O$ (CASRN 10102-17-7) in reagent water and dilute to 1 L. One mL of this solution will remove 1 mg/L of residual chlorine in 500 mL of sample.

7.5.2 Sodium sulfite: Dissolve 0.9 g Na2SO_3 (CASRN 7757-83-7) in reagent water and dilute to 1 L. One mL removes 1 mg/L Cl per 500 mL of sample.

7.6 Sulfuric acid 5 N: Air scrubber solution. Carefully add 139 mL of conc. sulfuric acid (CASRN 7664-93-9) to approximately 500 mL of reagent water. Cool to room temperature and dilute to 1 L with reagent water.

7.7 Sodium phenolate: Using a 1-L Erlenmeyer flask, dissolve 83 g phenol (CASRN 108-95-2) in 500 mL of distilled water. In small increments, cautiously add with agitation, 32 g of NaOH. Periodically cool flask under water faucet. When cool, dilute to 1 L with reagent water.

7.8 Sodium hypochlorite solution: Dilute 250 mL of a bleach solution containing 5.25% NaOCl (CASRN 7681-52-9) (such as "Clorox") to 500 mL with reagent

water. Available chlorine level should approximate 2-3%. Since "Clorox" is a proprietary product, its formulation is subject to change. The analyst must remain alert to detecting any variation in this product significant to its use in this procedure. Due to the instability of this product, storage over an extended period should be avoided.

7.9 Disodium ethylenediamine-tetraacetate (EDTA) (5%): Dissolve 50 g of EDTA (disodium salt) (CASRN 6381-92-6) and approximately six pellets of NaOH in 1 L of reagent water.

7.10 Sodium nitroprusside (0.05%): Dissolve 0.5 g of sodium nitroprusside (CASRN 14402-89-2) in 1 L of reagent water.

7.11 Stock solution: Dissolve 3.819 g of anhydrous ammonium chloride, NH_4Cl (CASRN 12125-02-9), dried at 105°C, in reagent water, and dilute to 1 L. 1.0 mL = 1.0 mg NH_3-N.

7.12 Standard Solution A: Dilute 10.0 mL of stock solution (Section 7.11) to 1 L with reagent water. 1.0 mL = 0.01 mg NH_3-N.

7.13 Standard Solution B: Dilute 10.0 mL of standard solution A (Section 7.12) to 100.0 mL with reagent water. 1.0 mL = 0.001 mg NH_3-N.

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 Samples must be preserved with H_2SO_4 to a pH <2 and cooled to 4°C at the time of collection.

8.3 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 28 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, and 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 ± 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 ±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 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.[9] 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:

$$MDL = (t) \times (S)$$

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 become 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 90-110%. After each five to 10 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 established 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 10th 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 ±10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within ±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 calculate 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 diluting suitable volumes of standard solutions (Sections 7.12 and 7.13) to 100 mL with reagent water.

10.2 Process standards and blanks as described in Section 11.0, Procedure.

10.3 Set up manifold as shown in Figure 1.

10.4 Prepare flow system as described in Section 11.0, Procedure.

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 ±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 Preparation of equipment: Add 500 mL of reagent water to an 800 mL Kjeldahl flask. The addition of boiling chips that have been previously treated with dilute NaOH will prevent bumping. Steam out the distillation apparatus until the distillate shows no trace of ammonia.

11.2 Sample preparation: Remove the residual chorine in the sample by adding dechlorinating agent (Section 7.5) equivalent to the chlorine residual. To 400 mL of sample add 1 N NaOH (Section 7.4), until the pH is 9.5, check the pH during addition with a pH meter or by use of a short range pH paper.

11.3 Distillation: Transfer the sample, the pH of which has been adjusted to 9.5, to an 800 mL Kjeldahl flask and add 25 mL of the borate buffer (Section 7.3). Distill 300 mL at the rate of 6-10 mL/min. into 50 mL of 2% boric acid (Section 7.2) contained in a 500 mL Erlenmeyer flask.

Note: The condenser tip or an extension of the condenser tip must extend below the level of the boric acid solution.

11.4 Since the intensity of the color used to quantify the concentration is pH dependent, the acid concentration of the wash water and the standard ammonia solutions should approximate that of the samples.

11.5 Allow analysis system to warm up as required. Feed wash water through sample line.

11.6 Arrange ammonia standards in sampler in order of decreasing concentration of nitrogen. Complete loading of sampler tray with unknown samples.

11.7 Switch sample line from reagent water to sampler 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 NH_3-N/L.

13.0 METHOD PERFORMANCE

13.1 In a single laboratory (EMSL-Cincinnati), using surface water samples at concentrations of 1.41, 0.77, 0.59, and 0.43 mg NH_3-N/L, the standard deviation was ±0.005.

13.2 In a single laboratory (EMSL-Cincinnati), using surface water samples at concentrations of 0.16 and 1.44 mg NH_3-N/L, recoveries were 107% and 99%, respectively.

13.3 The interlaboratory precision and accuracy data in Table 1 were developed using a reagent water matrix. Values are in mg NH_3-N/L.

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 EPA 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 be conducted 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

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17.0 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
134	0.270	0.2670	-0.0011	0.0342	0.0015
157	0.692	0.6972	0.0059	0.0476	-0.0070
136	1.20	1.2008	0.0001	0.0698	-0.0112
195	1.60	1.6095	0.0076	0.1023	0.0006
142	3.00	3.0128	0.0069	0.1677	-0.0067
159	3.50	3.4991	-0.0083	0.2168	0.0165
156	3.60	3.5955	-0.0122	0.1821	-0.0234
200	4.20	4.2271	0.0177	0.2855	0.0488
196	8.76	8.7257	-0.0568	0.4606	-0.0127
156	11.0	11.0747	0.0457	0.5401	-0.0495
142	13.0	12.9883	-0.0465	0.6961	0.0027
199	18.0	17.9727	-0.0765	1.1635	0.2106

REGRESSIONS: $X = 1.003T - 0.003$, $S = 0.052T + 0.019$

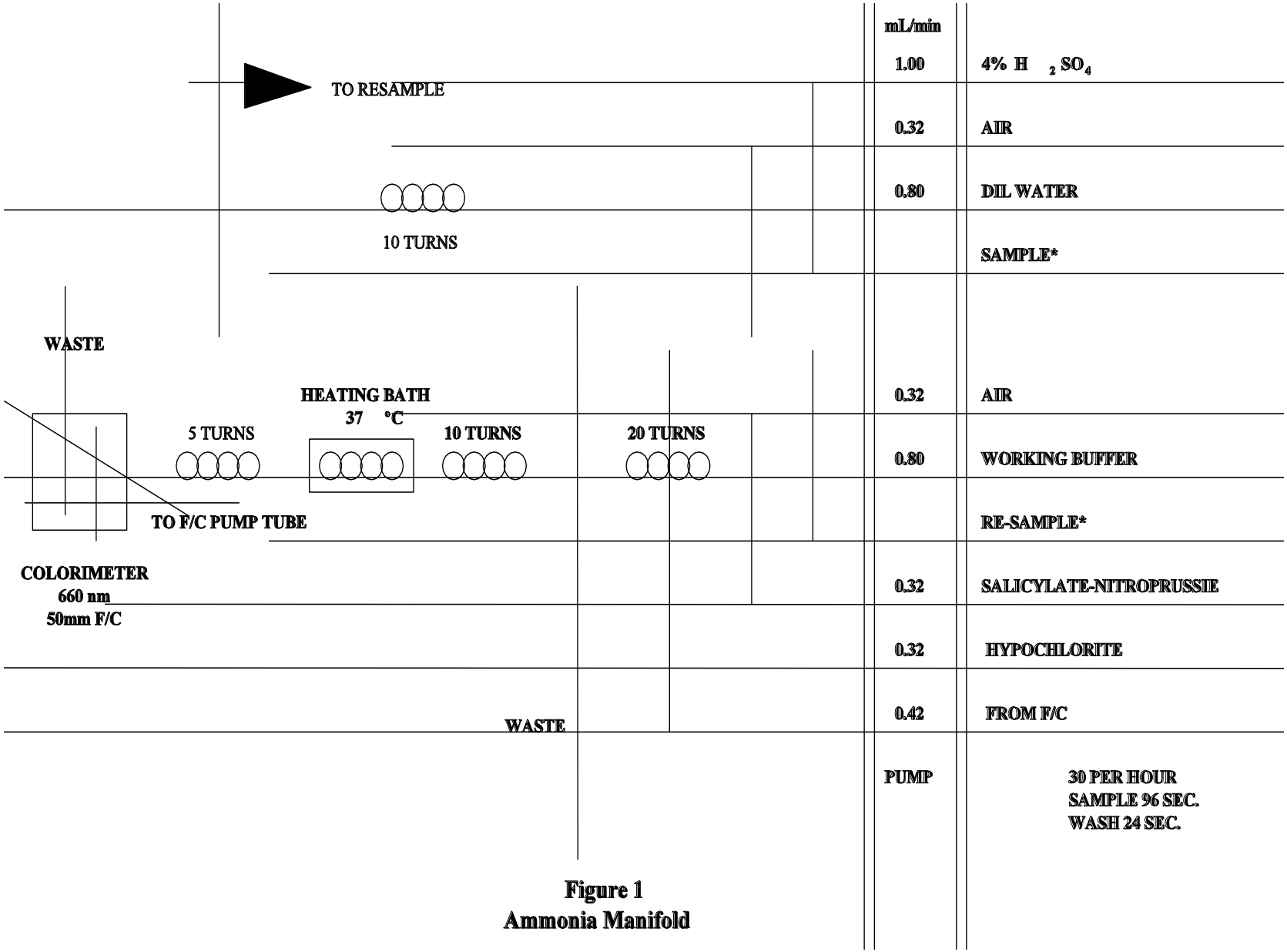

Figure 1
Ammonia Manifold

METHOD 335.4

DETERMINATION OF TOTAL CYANIDE BY SEMI-AUTOMATED COLORIMETRY

Edited by James W. O'Dell
Inorganic Chemistry Branch
Chemistry Research Division

Revision 1.0
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ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
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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 to500 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 ≥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 $MgCl_2 \bullet 6H_2O$ (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 $NaH_2PO_4 \bullet H_2O$ (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 ±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 ±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:

$$MDL = (t) \times (S)$$

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 (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:

UPPER CONTROL LIMIT = x + 3S
LOWER CONTROL LIMIT = x - 3S

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 ±10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within ±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 ±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 ±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.l 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.

l2.3 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.

l4.0 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: $X = 0.959T - 0.001$, $S = 0.128T + 0.003$

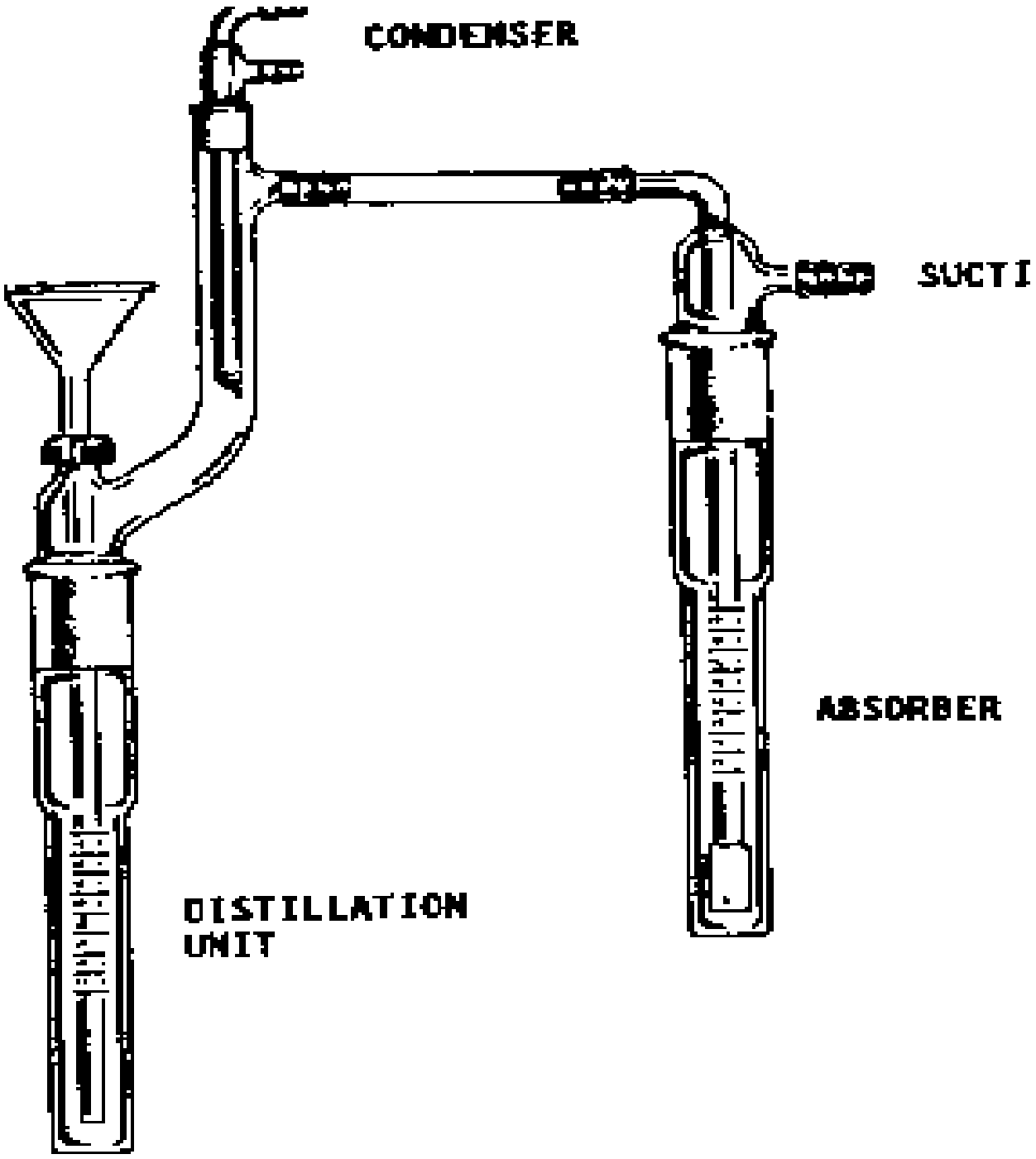

FIGURE 1. MIDI DISTILLATION APPARATUS

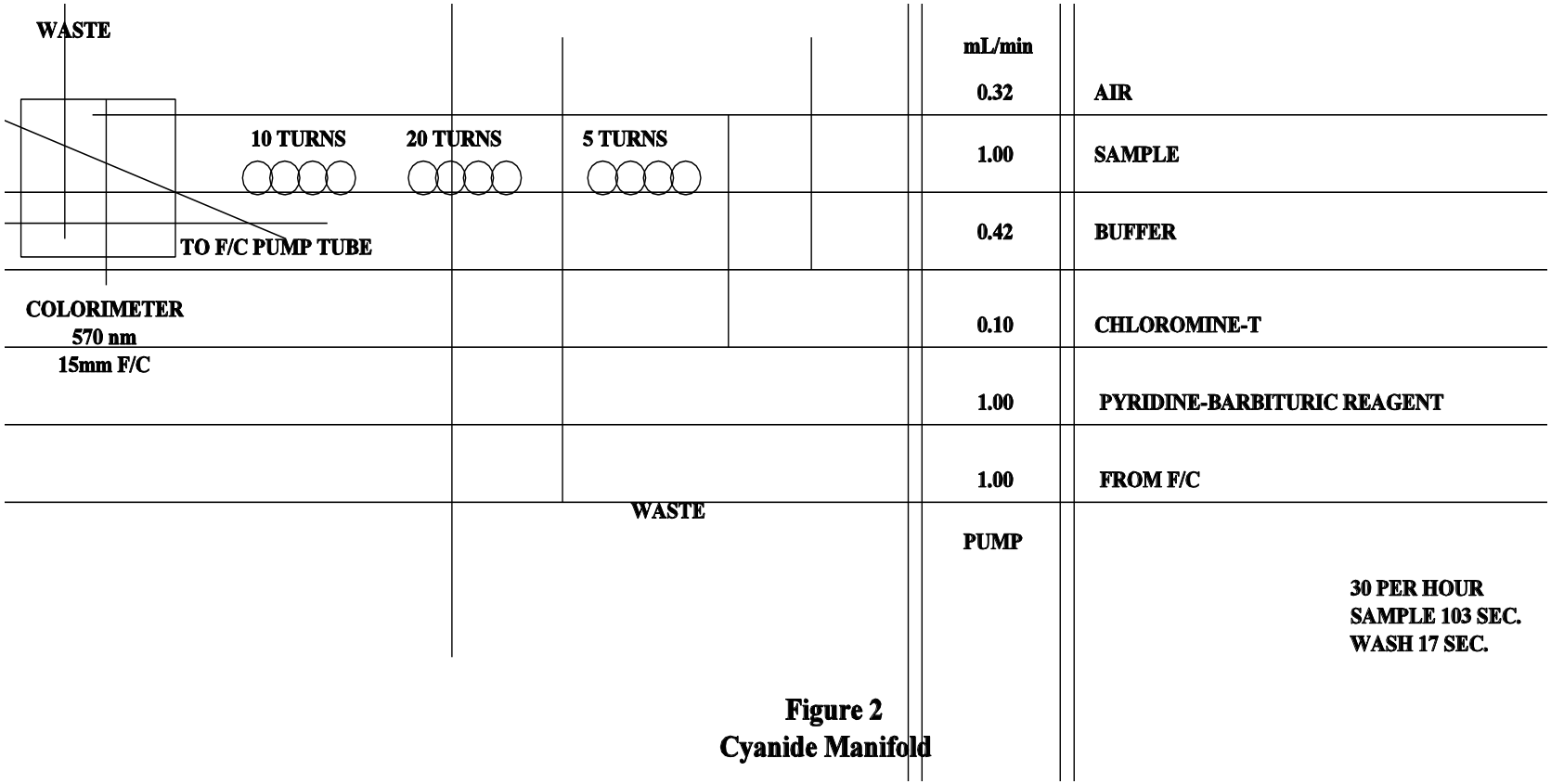

Figure 2
Cyanide Manifold

METHOD 420.4

DETERMINATION OF TOTAL RECOVERABLE PHENOLICS BY SEMI-AUTOMATED COLORIMETRY

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Revision 1.0
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METHOD 420.4

DETERMINATION OF TOTAL RECOVERABLE PHENOLICS BY SEMI-AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of phenolic materials in drinking, ground, surface, and saline waters, and domestic and industrial wastes.

1.2 The applicable range is from 2 to 500 µg/L. The working ranges are 2 to 200 µg/L and 10 to 500 µg/L.

2.0 SUMMARY OF METHOD

2.1 This semi-automated method is based on the distillation of phenol and subsequent reaction of the distillate with alkaline ferricyanide and 4-aminoantipyrine to form a red complex which is measured at 505 or 520 nm.

2.2 Color response of phenolic materials with 4-aminoantipyrine is not the same for all compounds. Because phenolic type wastes usually contain a variety of phenols, it is not possible to duplicate a mixture of phenols to be used as a standard. For this reason, phenol has been selected as a standard and any color produced by the reaction of other phenolic compounds is reported as phenol. This value will represent the minimum concentration of phenolic compounds present in the sample.

2.3 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.

2.4 Limited performance based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Sect. 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 Interferences from sulfur compounds are eliminated by acidifying the sample to a pH of 4.0 and aerating briefly by stirring.

4.2 Oxidizing agents such as chlorine, detected by the liberation of iodine upon acidification in the presence of potassium iodide, are removed immediately after sampling by the addition of an excess of ferrous ammonium sulfate (7.11). If chlorine is not removed, the phenolic compounds may be partially oxidized and the results may be low.

4.3 Background contamination from plastic tubing and sample containers is eliminated by filling the wash receptacle by siphon (using Kel-F tubing) and using glass tubes for the samples and standards.

4.4 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 have 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 Potassium ferricyanide (7.2)

5.3.2 Phenol (7.5)

5.3.3 Sulfuric acid (7.10)

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 Distillation apparatus, all glass consisting of a 1-L pyrex distilling apparatus with Graham condenser. Reduced volume apparatus also may be used.

6.4 pH meter with electrodes.

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 Buffered potassium ferricyanide: Dissolve 1.0 g potassium ferricyanide (CSRN 13746-66-2), 1.55 g boric acid (CASRN 10043-35-3), and 1.875 g potassium chloride (CASRN 7447-40-7) in 400 mL of reagent water. Adjust to pH of 10.3 with 1 N sodium hydroxide (CASRN 1310-73-2) (7.3) and dilute to 500 mL. Add 0.25 mL of Brij-35 (CASRN 9002-92-0). Prepare fresh weekly.

7.3 Sodium hydroxide (1N): Dissolve 20 g NaOH in 250 mL of reagent water, cool and dilute to 500 mL.

7.4 4-Aminoantipyrine: Dissolve 0.13 g of 4-aminoantipyrine (CASRN 83-07-8) in 150 mL of reagent water and dilute to 200 mL. Prepare fresh each day.

7.5 Stock phenol: Dissolve 0.50 g phenol (CASRN 108-95-2) in 500 mL of reagent water and dilute to 500 mL. Add 0.25 mL conc. H_2SO_4 (CASRN 7664-93-9) as preservative. 1.0 mL = 1.0 mg phenol.

7.6 Standard phenol solution A: Dilute 1.0 mL of stock phenol solution (7.5) to 100 mL with reagent water. 1.0 mL = 0.01 mg phenol.

7.7 Standard phenol solution B: Dilute 10.0 mL of standard phenol solution A (7.6) to 100 mL with reagent water. 1.0 mL = 0.001 mg phenol.

7.8 Standard solution C: Dilute 10.0 mL of standard phenol solution B (7.7) to 100 mL with reagent water. 1.0 mL = 0.0001 mg phenol.

7.9 Sodium hydroxide, 1+9: Dilute 10 mL of 1N NaOH (7.3) to 100 mL with reagent water.

7.10 Sulfuric acid, 1+9 : Slowly add 10 mL conc. H_2SO_4 (CASRN 7764-93-9) to 70 mL of reagent water. Cool and dilute to 100 mL with reagent water.

7.11 Ferrous ammonium sulfate: Dissolve 0.55 g ferrous ammonium sulfate in 250 mL reagent water containing 0.5 mL H_2SO_4 and dilute to 500 mL with freshly boiled and cooled reagent water.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in glass bottles only. All bottles must be thoroughly cleansed 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 Samples must be preserved at time of collection with H_2SO_4 to a pH of < 2 and cooled to 4°C.

8.3 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 up to 28 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, and 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 6 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 ± 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 ± 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 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:

$$MDL = (t) \times (S)$$

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 6 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 (Sect. 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 become 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 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 established 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 ± 10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within ± 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 (Sect. 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 3 standards, covering the desired range, and a blank by pipetting suitable volumes of working standard solutions (7.6, 7.7, 7.8) into 100-mL volumetric flasks. Suggested ranges include 1 to 5, 10 to 100, and 200 to 500 µg/L.

10.2 It is not imperative that all standards be distilled in the same manner as the samples. It is recommended that at least one standard 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 ± 10% of the undistilled standards, the analyst should find the cause of the apparent error before proceeding. Before distillation, standards should be adjusted to a pH of 4 with H_2SO_4.

10.3 Set up the manifold as shown in Figure 1 in a hood or a well-ventilated area.

10.4 Allow the instrument to warm up as required. Pump all reagents 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 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 quality control sample (QCS). If measurements exceed ± 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 Distillation

11.1.1 Measure 500 mL sample into a beaker. Adjust the pH to approximately 4 with 1+9 NAOH (7.9) or 1+9 H_2SO_4 (7.10), and transfer to the distillation apparatus.

11.1.2 Distill 450 mL of sample, stop the distillation, and when boiling ceases add 50 mL of warm reagent water to the flask and resume distillation until 500 mL have been collected.

11.1.3 If the distillate is turbid, filter through a prewashed membrane filter.

11.2 Set up the manifold as shown in Figure 1.

11.3 Fill the wash receptacle by siphon with reagent water. Use Kel-F tubing with a fast flow (1 L/h).

11.4 Allow the instrument to warm up as required. Run a baseline with all reagents, feeding reagent water through the sample line. Use polyethylene tubing for sample line. When new tubing is used, about 2 hours may be required to obtain a stable baseline. This two hour time period may be necessary to remove the residual phenol from the tubing.

11.5 Place appropriate phenol standards in sampler in order of decreasing concentration. Complete loading of sampler tray with unknown samples, using glass tubes.

11.6 Switch sample line from reagent water to sampler 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 μg/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 Phenol/L.

13.2 Single laboratory precision data can be estimated at 50 to 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 EPA 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 Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess Reagents and 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 Sect. 14.3.

16.0 REFERENCES

1. Technicon AutoAnalyzer II Methodology, Industrial Method No. 127-71W, AAII.

2. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p. 574, Method 510 (1975).

3. Gales, M.E. and Booth, R.L., "Automated 4 AAP Phenolic Method," AWWA 68, 540 (1976).

4. Code of Federal Regulations 40, Ch. 1, Pt. 136, Appendix B.

17.0 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
99	0.020	0.0149	0.0000	0.0074	0.0000
87	0.250	0.1443	-0.0052	0.0268	-0.0038
76	0.400	0.2352	-0.0021	0.0422	-0.0036
110	0.545	0.3364	0.0142	0.0681	0.0076
89	0.604	0.3610	0.0043	0.0625	-0.0039
107	0.660	0.3959	0.0064	0.0894	0.0173
86	0.800	0.4627	-0.0087	0.0806	-0.0057
62	0.817	0.4692	-0.0122	0.0776	-0.0104
76	0.970	0.5680	-0.0029	0.1017	-0.0017
89	2.96	1.7734	0.0377	0.3065	0.0018
61	4.18	2.3916	-0.0582	0.4044	-0.0237
110	4.54	2.7150	0.0545	0.5382	0.0737

REGRESSIONS: $X = 0.585T + 0.003$, $S = 0.101T + 0.005$

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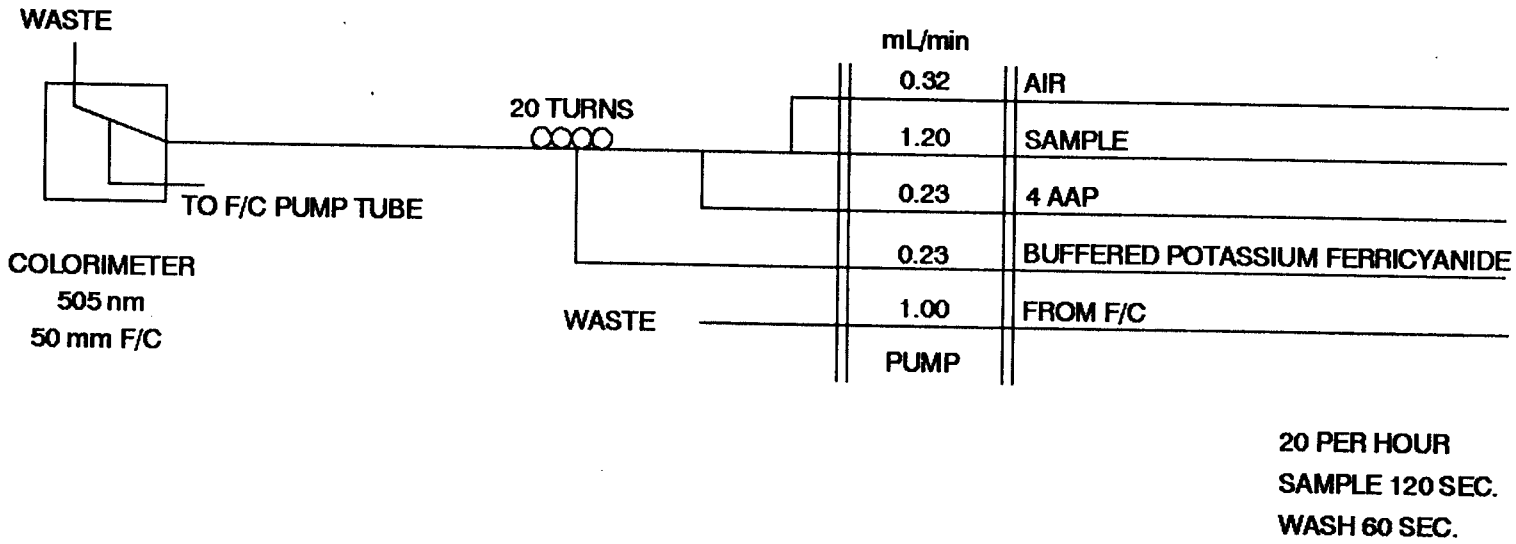

Figure 1
Phenol Manifold