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Method 351.2, Revision 2.0: Determination of Total Kjeldahl Nitrogen by Semi-Automated Colorimetry

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DETERMINATION OF TOTAL KJELDAHL NITROGEN BY SEMI-AUTOMATEDCOLORIMETRY

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METHOD 351.2

DETERMINATION OF TOTAL KJELDAHL NITROGEN BY SEMI-AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of total Kjeldahl nitrogen in drinking, ground, and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.

1.2 The applicable range is 0.1-20 mg/L TKN. The range may be extended with sample dilution.

2.0 SUMMARY OF METHOD

2.1 The sample is heated in the presence of sulfuric acid, H_2SO_4 for two and one half hours. The residue is cooled, diluted to 25 mL and analyzed for ammonia. This digested sample may also be used for phosphorus determination.

2.2 Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described.

2.3 Organic Kjeldahl nitrogen is the difference obtained by subtracting the free-ammonia value from the total Kjeldahl nitrogen value.

2.4 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.

2.5 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 High nitrate concentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

4.2 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 Mercury (Sections 7.2 and 7.3)

5.3.2 Sulfuric acid (Sections 7.2, 7.3, and 7.4)

5.3.3 Sodium nitroprusside (Section 7.9)

6.0 EQUIPMENT AND SUPPLIES

6.1 Balance - Analytical, capable of accurately weighing to the nearest 0.000l g.

6.2 Glassware - Class A volumetric flasks and pipets as required.

6.3 Block digestor with tubes.

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 distilled or deionized water, free of the analyte of interest. ASTM Type II or equivalent.

7.2 Mercuric sulfate: Dissolve 8 g red mercuric oxide (HgO) (CASRN 21908-53-2) in 50 mL of 1:4 sulfuric acid (10 mL conc. H_2SO_4: [CASRN 7664-93-9] 40 mL reagent water) and dilute to 100 mL with reagent water.

7.3 Digestion solution: (Sulfuric acid-mercuric sulfate-potassium sulfate solution): Dissolve 133 g of K_2SO_4 (CASRN 7778-80-5) in 700 mL of reagent water and 200 mL of conc. H_2SO_4. Add 25 mL of mercuric sulfate solution (Section 7.1) and dilute to 1 L.

Note 1: An alternate mercury-free digestion solution can be prepared by dissolving 134 g K_2SO_4 and 7.3 g $CuSO_4$ in 800 mL reagent water and then adding 134 mL conc. H_2SO_4 and diluting to 1 L. Use 10 mL solution per 25 mL of sample.

7.4 Sulfuric Acid solution (4%): Add 40 mL of conc. sulfuric acid to 800 mL of reagent water, cool and dilute to 1 L.

Note 2: If alternate mercury-free digestion solution is used, adjust the above solution to equal the acid concentration of the digested sample (Section 11.6).

7.5 Stock Sodium Hydroxide (20%): Dissolve 200 g of sodium hydroxide (CASRN 1310-73-2) in 900 mL of reagent water and dilute to 1 L.

7.6 Stock Sodium Potassium Tartrate solution (20%): Dissolve 200 g sodium potassium tartrate (CASRN 6381-59-5) in about 800 mL of reagent water and dilute to 1 L.

7.7 Stock Buffer solution: Dissolve 134.0 g of sodium phosphate, dibasic (Na_2HPO_4) (CASRN 7558-79-4) in about 800 mL of reagent water. Add 20 g of sodium hydroxide and dilute to 1 L.

7.8 Working Buffer solution: Combine the reagents in the stated order, add 250 mL of stock sodium potassium tartrate solution (Section 7.6) to 200 mL of stock buffer solution (Section 7.7) and mix. Add xx mL sodium hydroxide solution

(Section 7.5) and dilute to 1 L. See concentration ranges, Table 2, for composition of working buffer.

7.9 Sodium Salicylate/Sodium Nitroprusside solution: Dissolve 150 g of sodium salicylate (CASRN 54-21-7) and 0.3 g of sodium nitroprusside (CASRN 13755-38-9 or 14402-89-2) in about 600 mL of reagent water and dilute to 1 L.

7.10 Sodium Hypochlorite solution: Dilute 6.0 mL sodium hypochlorite solution (CASRN 7681-52-9) (Clorox) to 100 mL with reagent water.

7.11 Ammonium chloride, stock solution: Dissolve 3.819 g NH_4Cl (CASRN 12125-02-9) in reagent water and bring to volume in a 1 L volumetric flask. 1 mL = 1.0 mg NH_3-N.

7.12 Teflon boiling chips.

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 linear calibration ranges 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.[6] 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 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 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 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 diluting suitable volumes of standard solution (Section 7.11) 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 and Table 2.

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 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 Pipet 25.0 mL of sample, standard or blank in the digestor tube.

11.2 Add 5 mL of digestion solution (Section 7.3) and mix with a vortex mixer (See Note 1).

11.3 Add four to eight Teflon boiling chips (Section 7.12). **CAUTION:** An excess of Teflon chips may cause the sample to boil over.

11.4 Place tubes in block digestor preheated to 160°C and maintain temperature for one hour.

11.5 Reset temperature to 380°C and continue to heat for one and one half hour.

(380°C MUST BE MAINTAINED FOR 30 MINUTES)

11.6 Remove digestion tubes, cool and dilute to 25 mL with reagent water.

11.7 Excluding the salicylate line, place all reagent lines in their respective containers, connect the sample probe to the sampler and start the pump.

11.8 Flush the sampler wash receptacle with about 25 mL of 4% sulfuric acid (Section 7.4) (See Note 2).

11.9 When reagents have been pumping for at least five minutes, place the salicylate line in its respective container and allow the system to equilibrate. If a precipitate forms after the addition of salicylate, the pH is too low. Immediately stop the proportioning pump and flush the coils with water using a syringe. Before restarting the system, check the concentration of the sulfuric acid solutions and/or the working buffer solution.

11.10 To prevent precipitation of sodium salicylate in the waste tray, which can clog the tray outlet, keep the nitrogen flowcell pump tube and the nitrogen Colorimeter "To Waste" tube separate from all other lines or keep tap water flowing in the waste tray.

11.11 After a stable baseline has been obtained, start the sampler and perform 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.

l2.3 Report results in mg N/L.

13.0 METHOD PERFORMANCE

13.1 In a single laboratory (EMSL-Cincinnati) using sewage samples at concentrations of 1.2, 2.6, and 1.7 mg N/L, the precision was ±0.07, ±0.03, and ±0.15, respectively.

13.2 In a single laboratory (EMSL-Cincinnati) using sewage samples at concentrations 4.7 and 8.74 mg N/L, the recoveries were 99% 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 N/L.

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 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 Section 14.3.

16.0 REFERENCES

1. McDaniel, W.H., Hemphill, R.N. and Donaldson, W.T., "Automatic Determination of total Kjeldahl Nitrogen in Estuarine Water", Technicon Symposia, pp. 362-367, Vol. 1, 1967.

2. Gales, M.E. and Booth, R.L., "Evaluation of Organic Nitrogen Methods", EPA Office of Research and Monitoring, June, 1972.

3. Gales, M.E. and Booth, R.L., "Simultaneous and Automated Determination of Total Phosphorus and Total Kjeldahl Nitrogen", Methods Development and Quality Assurance Research Laboratory, May 1974.

4. Technicon "Total Kjeldahl Nitrogen and Total Phosphorus BD-40 Digestion Procedure for Water", August 1974.

5. Gales, M.E., and Booth, R.L., "Evaluation of the Technicon Block Digestor System for the Measurement of Total Kjeldahl Nitrogen and Total Phosphorus", EPA-600/4-78-015, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, 1978.

6. 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
115	0.380	0.3891	-0.0091	0.0750	-0.0135
134	0.451	0.4807	0.0125	0.1181	0.0238
127	1.00	1.0095	-0.0000	0.1170	-0.0227
164	3.10	3.0992	0.0191	0.2821	-0.0310
138	3.50	3.4765	0.0020	0.3973	0.0512
115	5.71	5.6083	-0.0452	0.4869	-0.0417
175	7.00	6.9246	-0.0008	0.6623	0.0272
121	8.00	7.9991	0.0877	0.6283	-0.0894
120	15.0	15.0213	0.2080	1.2495	-0.0462
127	21.0	20.4355	-0.2937	1.7267	-0.0644
164	25.0	24.7157	0.0426	2.0147	-0.1067
175	26.9	26.1464	-0.4000	2.9743	0.6960

REGRESSIONS: $X = 0.986T + 0.024$, $S = 0.083T + 0.057$

TABLE 2. CONCENTRATION RANGES

Range mg/LN	Pump mL/min.		mL NaOH Buffer (Section 7.7)
	Sample	Resample	
0-1.5	0.80	0.32	250
0-5.0	0.16	0.32	120
0-10.0	0.16	0.16	80

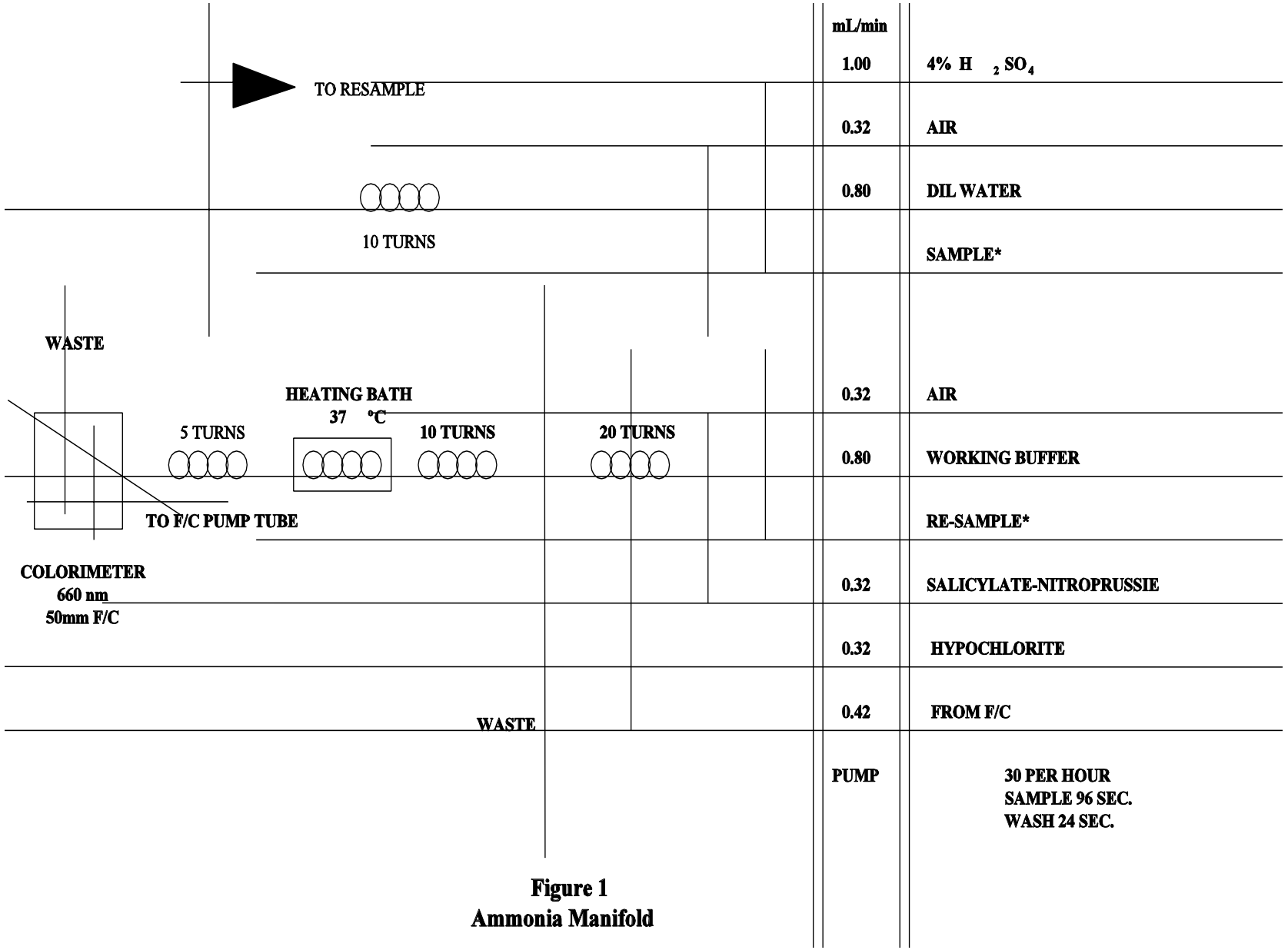

Figure 1
Ammonia Manifold