ESS Method 130.1:
General Auto Analyzer Procedures

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1.0 Scope and Application

The continuous flow analysis method may be used to determine many chemical constituents in drinking and surface waters and wastes.

2.0 Apparatus and Summary of Method

2.1 The Auto Analyzer II system is comprised of five separate modules interconnected by tubing and electrical cables. The typical system includes: 1) Sampler; 2) Proportioning Pump; 3) Manifold; 4) Colorimeter; and 5) Printer/Plotter.

2.2 The Proportioning Pump uses flow-rated tubing to proportion the flow of samples and reagents through the system. Samples are separated by segments of wash solution. Segments of air are introduced at two second intervals to help separate samples, mix reagents, and cleanse tubing. Each parameter has a unique Manifold for introducing reagents, mixing, heating and diluting as needed. The Printer/Plotter is used to record the concentrations of constituents determined by the Colorimeter response.

3.0 Sample Handling, Preservation and Pretreatment

3.1 Samples are collected in specified containers and preserved according to Method 100.1 - Sample Preservation and Holding Times.

3.2 Samples must be free of particulate matter when introduced into the system. To accomplish this, samples are filtered according to Method 100.2. Total phosphorus and total Kjeldahl nitrogen samples should be centrifuged or held overnight after digestion to allow particulates to settle.

4.0 General Operating Procedures

4.1 Check maintenance log for any needed instrument care.

4.2 Turn on Colorimeter lamps at beginning of a work week and leave on until the end of the week. Allow to warm up for 30 minutes.

4.3 Check heating baths' temperatures. Clean platen with alcohol and install. Start Proportioning Pump.

4.4 Hydraulic Check

4.4.1 Pump Milli-Q water with appropriate wetting agent through the system. TKN(NH_3) and sulfate washes require Brij-35. Dissolved phosphorus and silica washes need sodium lauryl sulfate.

4.4.2 Check for leaks and pinched lines.

4.4.3 Establish a good bubble pattern.

4.5 Prepare any needed reagents and standards.

4.6 Check colorimeter output for each channel used.

4.6.1 On Colorimeter, turn Display Rotary Switch to *Zero*. Using a screwdriver, adjust *Zero* control to obtain zero on the voltmeter.

4.6.2 Turn Display Rotary Switch to *Full Scale*. Using a screwdriver, adjust *Full Scale* control to obtain full range (5.00 volts on the voltmeter).

4.7 Baseline Checks

4.7.1 On Colorimeter, turn Display Rotary Switch to *Damp* 1, set *Std Cal* at 1.0, and set reversing switch to "D".

4.7.2 When system is thoroughly washed with water and wetting agent, adjust *Baseline* control to obtain zero on the voltmeter. Check for straight baseline.

4.7.3 Introduce appropriate reagents into the system as directed in different methods and allow reagents to flow until a straight baseline is obtained. Using *Baseline* control, reset meter to *Zero*. This is correcting for background contamination in reagents.

4.8 Calibration Procedures

4.8.1 Load sample tray with standards specified in various method Tray Protocols. Glass dispo culture tubes (10 mL) are used for dissolved P, TKN, TOT-P, and low level TOT-P. Polystyrene dispo beakers (4 mL) are used for dissolved Silica and Sulfates. Fill remaining cups with unknown samples, duplicates, spikes, and mid-range standard checks according to Tray Protocol.

4.8.2 Place red peg at last cup. When an analysis requires use of the B wash solution receptacle, the red peg is placed at the second to last cup.

4.8.3 For each channel used, set *Std Cal* control on the Colorimeter to an approximate value expected (the approximate value can be obtained from previous day's run as recorded on the chart for Baseline and *Std Cal* settings). Raise baseline about 10% (approximately .14 on voltmeter).

4.8.4 Start Sampler.

4.8.5 When the first standard (primer) comes through, adjust the *Std Cal* control on the Colorimeter so that the top of the peak registers about 95% of full range (approximately 4.80 on voltmeter). Record *Std Cal* values on appropriate Baseline and *Std Cal* Settings chart.

4.9 Shutdown Procedures

4.9.1 After last cup has been sampled, turn off Sampler.

4.9.2 Check the return to 10% baseline after last sample value has been printed, to check on baseline drift.

4.9.3 Connect reagent tubes to wash bottles and flush at high speed, if available. Continue to wash with water and wetting agents until system is rinsed completely. Reset *Std Cal* to 1.0, and adjust baseline to zero.

4.9.4 Remove chart from Printer/Plotter, Initial, record *Std Cal*, & calculate correlation coefficient(r).

4.9.5 Shut off pump. Lift platen off and store upside down. Adjust pump so that the air bar is up. Disconnect wash water tubing from containers. *Caution*: Wash solution will siphon onto the laboratory bench if the wash water tubing is not removed from the containers.

4.9.6 Discard all used sample cups.

5.0 Quality Assurance Procedures

5.1 Baseline and *Std Cal* Settings Chart.

5.1.1 The *Std Cal* values for each nutrient and each range used are recorded.

5.1.2 Included in this chart are the date and analyst's initials.

5.2 Pump Tubing Chart

The lot numbers of all flow-rated pump tubing and date the packages are opened are recorded.

5.3 A calibration curve as described in each method is run at the beginning of each range to establish system linearity. Subsequently, mid-range standards are included after every 10-20 samples (depending on method) to verify the curve and at the end of the run.

5.4 Precision is checked every day by analyzing 10% of all samples in duplicate. When filtered samples are analyzed in duplicate, the sample and its duplicate must be filtered separately and be treated as independent samples. The absolute differences between duplicates are plotted on Shewhart Charts to verify that the analyses are within the quality control limits.

5.5 Accuracy is verified daily by analyzing a sample spiked with a standard solution.

5.5.1 For soluble chemical constituents, a spike sample is made by mixing an equal volume of sample with an equal volume of a standard solution of approximately the same concentration. The calculations are as follows:

$$\frac{\text{Spiked Sample Concentration} - \text{½ sample conc.}}{} \times 100 = \%\ \text{Recovery}$$

½ Standard conc.

5.5.2 For total phosphorus and total Kjeldahl nitrogen digested on the Block Digestor, a volume of sample (10 mL or less) is pipetted into the digestor tube and a volume of standard (10 mL or less) is added and run through the digestion procedure with samples and standards. The standard added can be Nicotinic Acid for TKN or AMP for TP separately or a combination of Glutamic Acid and KH_2PO_4 for a dual spike. The calculations are as follows:

$$\frac{\text{Spiked Sample Concentration - Sample Background Concentration}}{\text{Spike Concentration}} \times 100 = \%\ \text{Recovery}$$

5.5.3 The % Recoveries are plotted on Shewhart Charts to verify that the analyses are within the quality control limits.

5.6 Daily worksheets are stamped with "Q.C. Audit_____ Date_____" whereby another chemist can verify, initial and date that the analyses meet the Q.C. criteria designated for the lab and the particular parameter measured.

5.7 Reagents are dated and initialed when they are prepared.

6.0 Preventive Maintenance

6.1 A log is kept for dating maintenance procedures performed on any module. Figure 2.

6.2 Daily

6.2.1 Clean surfaces of entire system and area.

6.2.2 Check surface of pump platen and rollers. Clean with alcohol, if necessary.

6.3 Weekly

6.3.1 Remove, clean with alcohol, and lightly lubricate side rails with Semi-Fluid Lubricant.

6.3.2 Clean pump rollers and platen with alcohol.

6.4 Monthly

6.4.1 Change pump tubing monthly or when deemed necessary.

6.4.2 Adjust silicone tubing under air bar to a new position.

6.4.3 Oil air bar linkage with one drop Prolonged Service oil.

6.4.4 Oil two felt pads with two drops oil.

6.4.5 Oil needle bearings of main drive shaft by putting one drop oil in each of two holes.

6.5 Three Months

6.5.1 Replace sample tubing.

6.5.2 Clean sample probe with wire stylet.

6.5.3 Clean sampler pole with freon and oil lightly.

6.6 Six Months

6.6.1 Put one drop oil on each end of each pump roller and chain interface. Rotate rollers and wipe off excess oil with alcohol.

6.6.2 Clean Colorimeter flowcell and filters.

6.6.3 Clean Colorimeter lamp and socket controls.

6.7 Eighteen Months

Lubricate four spots on Sampler as directed in Instrument Manual.

7.0 Miscellaneous Maintenance

7.1 Clean heating bath with cleaning acid.

7.2 Clean dilution coils and debubblers with 50% HCl.

7.3 Change various tubing and connections and clean glass connections.

7.4 Clean sample splitter.

7.5 Clean color reagent line on phosphorus Auto Analyzer with 20% NaOH and H_2O_2. This is done as follows:

7.5.1 MQ line in MQ H_2O (No Levor) and Color Reagent line in 20% NaOH for 20 minutes.

7.5.2 MQ line in MQ H_2O (No Levor) and Color Reagent line in H_2O_2 for 10 minutes.

7.5.3 Both lines in MQ H_2O (No Levor) for 10 minutes.

7.5.4 Both lines in MQ H_2O with Levor (4 mL Levor/125 mL MQ) for 10 minutes.

7.5.5 Both lines in MQ Levor wash solution (3.0 mL/L MQ) until stable baseline is obtained.

8.0 Peaking FlowCell

Whenever any maintenance has been performed on the Colorimeter it is necessary to peak the flowcell as follows:

8.1 Turn Display Rotary Switch to *Normal*

8.2 Set Std Cal at 1.0.

8.3 Set reversing switch at "D".

8.4 Set *Baseline* control at mid-point. (Control has 10 complete turns, so set at 5 turns from either extreme.)

8.5 Set voltmeter at half scale (2.50) by using both sample and reference apertures.

8.6 Rotate the peaking screw on the sample phototube housing assembly to obtain minimum deflection on voltmeter.

8.7 Rotate the peaking screw on the *reference* phototube housing to obtain *maximum* deflection on the voltmeter.

8.8 Open both apertures completely clockwise.

8.9 Note voltmeter reading:

8.9.1 If value is *below zero*, more light is reaching the sample phototube than the reference. Correct by closing *sample* aperature (A) to adjust value to zero.

8.9.2 If value is *above zero*, less light is reaching the sample phototube than the reference. Correct by closing the *reference* aperature (B) to adjust to zero.

8.9.3 One aperature should be *Fully Open* at all times.

8.9.4 Fine adjust by using *Baseline* control.