AUTOMATED DETERMINATION OF ALKALINITY USING THE PC-TITRATE INSTRUMENTATION

1. SCOPE AND APPLICATION

1.1. The method is applicable to drinking, surface, ground, and saline waters as well as industrial and domestic waste. The method detection limit (MDL) is 0.65 mg $CaCO_3$/L and the practical quantitation limit (PQL) is 2.5 mg $CaCO_3$/L.

1.2. The test covered by this SOP is ALKALINITY.

2. SUMMARY OF THE METHOD

2.1. This method, based on Standard Methods 2320 B-2011, is used for determining the alkalinity of water samples with an automatic titration instrument – PC-Titrate™.

2.2. A fully automatic titration procedure is employed to titrate aqueous samples electrometrically to an end point of 4.5 (or 4.5 and 4.2 for alkalinity samples below 20 mg $CaCO_3$/L). The alkalinities are then calculated from the amount of the titrant (0.02 N H_2SO_4) used in the titration.

2.3. Interferences – Substances such as salts of weak organic and inorganic acids present in large amounts, may cause interference in the electrometric pH measurements. Oil and grease, by coating the pH electrode, may also interfere, causing sluggish response.

2.4. Sample Preservation and Holding Time – Samples for alkalinity determination are stored at ≤ 6 °C (not frozen) and must be analyzed within 14 days of collection to avoid qualification.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation: See NU-060 for the components of the PC-Titrate instrument.

3.2. Equipment:

Equipment	Manufacturer/Vendor[1]	Model/Catalog Number
pH Electrode	ManTech	PCE-80-PH1200D
Analytical Balance	Mettler Toledo	MS204TS/0
Adjustable Pipette, 10–100 µL	Eppendorf/Fisher	Research Plus, 3123000047
Adjustable Pipette, 100–1000 µL	Eppendorf/Fisher	Research Plus, 3123000063
Adjustable Pipette, 1–10 mL	Eppendorf/Fisher	Research Plus, 3123000080
Volumetric Flask, Class A, Assorted	Fisher (SR)	10-212B; 10-210-5D; 10-212E; 10-212-5L; 10-208M
Graduated Cylinder, Class A, Assorted	Fisher (SR)	08-553A; 08-548D
Glass Beaker, Assorted	Fisher (SR)	02-540E; 02-540G; 02-540K; 02-555-20E
Or Equivalent		
[1]**Note:** (SR) designates equipment is typically ordered through the in-house stockroom		

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3.3. Consumables and frequently replaced supplies:

Supplies/Consumables	Vendor[1]	Catalog Number
Electrode filling solution	Fisher	50-633-682
Lithium Grease	Fisher	NC0484446
Centrifuge Tubes, Polypropylene, 50 mL	Fisher (SR)	05-538-68, 05-53855A
Pipette tips, Eppendorf, 200 µL	Fisher (SR)	05-403-41
Pipette tips, Eppendorf, 1000 µL	Fisher (SR)	05-403-43
Pipette tips, Eppendorf, 10 mL	Fisher (SR)	05-403-116
Sample Beakers, Polypropylene, Assorted	Fisher (SR)	08-732-119; 08-732-120; 08-732-122
Syringe, Polypropylene	Fisher (SR)	B303134
Syringe Filters, Polypropylene, 25mm (0.45 µm pore size)	Fisher (SR)	05-713-404A
Weigh Dish, Hexagonal, Assorted	Fisher (SR)	02-202-102; 02-202-100
Weigh Dish, Aluminum, 110 mm	Fisher (SR)	08-732-100
Pipet, Transfer, Polyethylene, 7mL	Fisher (SR)	13-711-7M
Parafilm	Fisher (SR)	13-374-12
Or Equivalent [1]**Note:** (SR) designates supply is typically ordered through the in-house stockroom		

4. REAGENTS AND STANDARDS (SEE DEP SOPS NU-041 and NU-042)

Note: Before preparing reagents or standards, or running samples, please read the safety notes in the safety/hazardous waste section of this SOP and the MSDS for the chemicals used in this analysis.

Note: Standard preparations must be documented in the Laboratory Information Management System (LIMS) 'Standard Preparation Tracker' module. Unique ID numbers for each standard are generated in the module and documented in the reagent and standard logsheet (NU-RGTSTD) submitted with each analysis folder. The reagent and standard logsheet (NU-RGTSTD) template is located at \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Auto-T\Forms\. Follow preparation and labelling instructions in DEP SOP NU-042. A Certificate of Analysis for each purchased reagent and standard is maintained in the CertificatesofAnalysis subfolder within the Auto-T folder on the Nutrients Drive. All containers holding standards or reagents must be labeled with their unique ID and expiration date.

Note: The volumes prepared for the reagents and standards may be altered by the analyst as necessary. The final concentrations of the constituents of a standard or reagent must not be changed.

4.1. Chemicals:

	Chemical	Alias	Manufacturer[1]	Catalog Number	Purity	Expiration	Storage
4.1.1.	Sodium Carbonate Anhydrous	Na_2CO_3	Fisher Chemical	S263-500	ACS, ≥95%	See CoA	Room Temp
4.1.2.	Sulfuric Acid (0.02N (N/50)/Certified)	H_2SO_4	Fisher Chemical	SA226-1	ACS, ≥99%	See CoA	Room Temp

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4.1.3.	Sulfuric Acid	H_2SO_4	Fisher Chemical (SR)	A300S-212	Concentrated, ACS Plus ≥95%	See CoA	Room Temp
4.1.4.	Minerals QC CRM	ALTQC	NSI Lab Solutions	QCI-136	See CoA	See CoA	Fridge after opening
4.1.5.	pH 4.00 Buffer	4 Buffer	Fisher Chemical (SR)	SB101-500	N/A	See CoA	Room Temp
4.1.6.	pH 7.00 Buffer	7 Buffer	Fisher Chemical (SR)	SB107-500	N/A	See CoA	Room Temp
4.1.7.	pH 10.00 Buffer	10 Buffer	Fisher Chemical (SR)	SB115-500	N/A	See CoA	Room Temp
4.1.8.	DI Water	H_2O	In house	N/A	N/A	Daily	N/A
Or Equivalent [1]**Note:** (SR) designates chemical is typically ordered through the in-house stockroom							

4.2. Reagents:

4.2.1. 0.02 N Certified Sulfuric Acid Standard – purchased from Fisher Scientific (4.1.2.). Replace after one month of use. If unavailable, prepare and standardize the acid manually according to the instructions in 4.2.2 through 4.2.3.

4.2.2. 0.020 N Sodium Carbonate Solution (only used for standardization of prepared acid (4.2.3.)) –

Na_2CO_3 (4.1.1.)	1.0606 g
DI water (4.1.8.)	to 1000 mL

Add 1.0606 g (to the nearest +/- 0.05 mg) Na_2CO_3 (dried at 105 °C overnight and cooled in a desiccator) to a 1 L volumetric flask and dilute to the mark with deionized (DI) water. Invert flask at least 8 times to mix.

Store refrigerated. Holding time, 30 days.

4.2.3. 0.02 N Prepared Sulfuric Acid Standard – If the certified standard is not available, use the following procedure:

H_2SO_4 (4.1.3)	555.6 µL
DI water (4.1.8)	to 1000 mL

To a 1L volumetric flask add approximately 500 mL DI water. Pipette 555.6 µL of conc. H_2SO_4 into the flask. Dilute to the mark. Invert flask at least 8 times to mix. Titrate against 0.020 N sodium carbonate solution (see 4.2.1.) to standardize.

Store at room temperature. Titrate against 0.020 N sodium carbonate solution (see 4.2.1.) to standardize after 1 month. For standardization procedure, reference 5.3.1 and Standard Methods 2320 B-2011 section 3*b*.

4.3. Calibration Standards and Quality Control Standards:

4.3.1. 0.20 N (10,000 mg $CaCO_3$/L) Sodium Carbonate Spiking Solution –

Na_2CO_3 (4.1.1.)	1.0606 g
DI water (4.1.8.)	to 100 mL

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Add 1.0606 g (to the nearest +/- 0.05 mg) Na_2CO_3 (dried at 105 °C overnight and cooled in a desiccator) to a 100 mL volumetric flask and dilute to the mark with DI water. Invert flask at least 8 times to mix.

Molecular weight factor: 0.945

Store refrigerated. Holding time, 30 days.

4.3.2. A certified quality control sample – ALTQC (4.1.4.)

Prepared following the supplier's instructions, if needed. Standard can be stored at room temp, but is generally stored in the refrigerator. The concentration is provided by the manufacturer. There is not a recommended holding time by the manufacturer, but standard is given a 28-day holding time upon opening.

4.3.3. Laboratory Fortified Blank (LFB) –

Na_2CO_3 Spiking Solution (4.3.1.)	200 µL
DI water (4.1.8.)	40 mL

Add 200 µL of the 10,000 mg $CaCO_3$/L Na_2CO_3 solution to a 40 mL aliquot of DI water for a 50 mg $CaCO_3$/L spike.

Prepared daily. (Spike code _N2.)

4.3.4. Continuing Calibration Verification (CCV):

4.3.4.1. CHCK1 (100 mg $CaCO_3$/L check standard) –

Na_2CO_3 Spiking Solution (4.3.1.)	400 µL
DI water (4.1.8.)	40 mL

Add 400 µL of 10,000 mg $CaCO_3$/L Na_2CO_3 solution to a 40 mL aliquot of DI water.

Prepared daily.

4.3.4.2. CHCK3 (50 mg $CaCO_3$/L check standard) –

Na_2CO_3 Spiking Solution (4.3.1.)	200 µL
DI water (4.1.8.)	40 mL

Add 200 µL of 10,000 mg $CaCO_3$/L Na_2CO_3 solution to a 40 mL aliquot of DI water.

Prepared daily.

4.3.5. Buffer solutions at pH 4.00, 7.00 and 10.00 (4.1.5., 4.1.6., 4.1.7.) are used as calibration standards.

Store at room temperature. The expiration date is provided by the manufacturer.

5. SAMPLE ANALYSIS/QUANTITATION (SEE DEP SOP NU-033)

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Note: This SOP should be used in conjunction with DEP SOP NU-060 – Automated PC Titrate Instrument Operating Procedure, which gives a more detailed description of the instrumentation.

IMPORTANT – All sample tubes must have their caps removed prior to operation of the instrument or the probe will be damaged.

Note: Allow the samples to come to room temperature prior to measuring the alkalinity.

5.1. Calibration:

5.1.1. Pour approximately 45 mL of buffers 4.00, 7.00 and 10.00 (4.1.5., 4.1.6., 4.1.7.) into three separate PP centrifuge tubes appropriately labeled.

5.1.2. A multi-level calibration with pH 4.00, 7.00, 10.00 buffers may be carried out either independently on a daily basis or together with samples in each run.

5.1.3. If the calibration does not comply with the manufacturers specifications the instrument will not continue to perform sample pH analysis.5.1.4. Following the calibration and before samples are analyzed, a repeat measurement of the three calibration standards is taken. This is used to evaluate the relative error of the calibration.

5.2. Analysis of samples:

5.2.1. Start-up of the PC-Titrate Software – Log in to the PC-Titrate software; choose the appropriate method (alkalinity alone or in combination with other tests). Set up the run sequence corresponding to the timetable of PC-Titrate software (see DEP SOP NU-060 for details).

5.2.2. Loading samples – Pour or filter 45-47 mL of sample or standard into a 50 mL PP centrifuge tube. Load the sample tubes onto the autosampler in a sequence determined in section 5.2.1. It is recommended to run a couple of DI water cups at the very beginning as a rinse to clean up the system and condition the probe.

Note: Samples are not required to be filtered by the method but should be filtered when necessary to prevent contamination. Samples should be filtered if they are turbid or contain obvious particulates. **Filtered samples are identified on the backlog or bench sheet with an F.**

Example Run Sequence:

Rinse – Tap water
Rinse – DI water
pH CAL – 4.00, 7.00, and 10.00 pH buffers (5.1.)
pH CAL Check – 10.00, 7.00, 4.00 pH buffers (reverse)
Rinse – DI water
MDL – (blank: DI water, optional)
ALTQC (4.3.2., filtered)
CHCK1 or **CHCK 3** (4.3.4.)

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Blank – DI water
MBLK – Method blank: Filtered DI water (one per batch)
LFB_N2 (one per batch, 4.3.3.)
Batched samples: one sample run in duplicate (sample ID_D) for each batch of max. 20 samples.
Blank – DI water (before every CHCK)
CHCK 3 or **CHCK1** – alternate CCV CHCK every 20 cups
Blank – DI water

Click on the START button to start the run.

Note: The ALTQC must be filtered if any samples in the analytical batch are filtered. The MBLK must be filtered if any samples in the prep batch are filtered. Filtered QC is identified on the bench sheet with an F.

5.3. Calculations:

5.3.1. Standardization/acid normality is given by (used if certified H_2SO_4 solution is unavailable) –

$$\frac{(\text{grams of } Na_2CO_3\text{/L solution}) \times (\text{mL of } Na_2CO_3 \text{ titrated})}{53.00 \times (\text{mL of acid used in standardization})}$$

5.3.2. Alkalinity of samples (performed by instrument software) –

$$\frac{(\text{Normality of acid}) \times (\text{mL of acid}) \times 50{,}000}{\text{Volume of sample (mL)}}$$

5.3.3. Alkalinity of low alkalinity samples (performed by instrument software) –

$$\frac{[(2A - B) \times \text{Normality of acid} \times 50{,}000]}{\text{Volume of sample (mL)}}$$

A = mL of acid to pH 4.5
B = mL of acid to pH 4.2

5.4. For general calculations including RPDs, LFB recoveries, and QC statistics (UCL and LCL for LFB's and RPD's) see Sections 5.9 of the FDEP Quality Manual.

6. TROUBLESHOOTING/MAINTENANCE

6.1. Problems with calibration may be due to instrument failure. Consult troubleshooting section of instrument manual.

6.2. Always keep the probe in water or buffer when not in use. For further details on maintenance of the instrument and the probe, refer to DEP SOP NU-060 and related hardware manual.

7. DATA ARCHIVAL

7.1. All analytical results are automatically saved in the database of the instrument software. Raw data is retrievable with the 'HISTORICAL DATA' function of PC-Titrate software. At the beginning of every week , the raw data is moved to the

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network drive by the analyst for archiving in the PCT BACKUP SYSTEM 1 (for PC-Titrate 1) or PCT BACKUP SYSTEM 2 (for PC-Titrate 2) folder located at: \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\Archive\PC-TITRATE\.

7.2. A tab delimited text file for each run must be exported to the network drive: \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\Automatic Titrators\PC-Titrate1\ or \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\Automatic Titrators\PC-Titrate2\ , depending on which instrument is used. The file is stored in the analysis year subfolder for the instrument used. The file name should use the format YYMMDDN-A# where YY is the year, MM is the month, DD is the day, N is the first letter of the test/s conducted, A is Auto-Titrator, and # is 1 (for PC-Titrate 1) or 2 (for PC-Titrate 2) which indicates what instrument was used (e.g., alkalinity, fluoride and conductivity run = 120206AFC-A1). This file is used by QC manager for data processing and uploading into LIMS.

7.3. The raw data is archived in paper format. A report folder is created for each analytical run. A folder may contain more than one analysis (e.g. Alkalinity, and Conductivity, and/or Fluoride) in it if the analyses are part of the same analytical run. The folder is labeled with the test (e.g., ALK, W-COND, and/or W-F), filename/s (YYMMDDN-A#), and initials of the analyst.

7.4. Each report folder should contain the following items – general results report, calibration report(s), running list report (bench sheet), reagent and standard logsheet(s), and a copy of the data and QC assessments uploaded from QC Manager to LIMS at the time of test authorization.

7.5. The report folders are on file with the nutrients and general chemistry supervisory personnel. Periodically, the files are moved to the State Records Center for long time storage according to DEP SOP LB-006.

7.6. Method validation data is on file with the supervisor. Digital copy is also stored on the network, under an appropriate sub-folder in \\floridadep\data\labs\Lab5\Chemistry\Inorganic\Nutrient\Validations\PC Titrate.

8. QUALITY CONTROL/DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES (SEE DEP SOP NU-038 AND NU-043)

8.1. A CCV standard (CHCK1 or CHCK3, 4.3.4.) and a QC standard (ALTQC, 4.3.2.) must be analyzed prior to every analytical run. Recoveries must be 90 to 110% for the CCV standards. Recovery for the ALTQC standard must be within the manufacturer's certified limit but not exceed 85 to 115%.

8.2. Analyze a blank and a CCV (CHCK1 or CHCK3) standard every 20 cups tested and at the end of the analyses.

8.3. Each sample batch begins with an MBLK and one LFB (4.3.3). One randomly selected sample is run in duplicate for every batch of 20 samples. Acceptance limits for the blanks and MBLKs are < 0.65 and > -0.65 mg $CaCO_3$/L.

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8.4. Acceptable limits for the recovery of the LFBs (4.3.3.) are established from the upper and lower control limits (UCL and LCL) calculated in the QC Statistics module in LIMS. The limits are evaluated regularly and updated at least annually. The QC Statistics Calculation Reports are archived annually with the PC-Titrate instrument validation data on the Chemistry drive: \\floridadep\data\labs\Lab5\Chemistry\Inorganic\Nutrient\Validations\PC Titrate\QC Stats Limits\.

8.5. Unless below the PQL, sample duplicate analyses should have an RPD of less than the upper control limit calculated in the QC statistics module. See Section 8.4. for evaluation and archival information.

8.6. For general method performance and acceptance criteria for QC measures and corrective action for out-of-control data and contingencies for handling out-of-control or unacceptable data, refer to DEP SOPs NU-038 and SOP NU-043.

8.7. Calibration – The calibration standards (4.3.5.) are 4.00, 7.00, and 10.00 pH buffers. Every day samples are analyzed a new calibration is performed at the beginning of the analysis. The acceptance criteria are set according to manufacturer's instructions and are documented on the Calibration Report. The instrument will not proceed with sample analysis if the criteria are not met.

8.8. Following the calibration and before samples are analyzed, a repeat measurement of the three calibration standards is taken. The measured values should be within 0.05 pH units of the buffer solution value. This will be used to evaluate the relative error of the calibration.

8.9. Annual MDL verification is performed using the LIMS statistics tracker module, (see DEP SOP NU-043). Method blank (MBLK) data is collected and utilized for the ongoing MDL verification.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT

9.1. Review the laboratory Health and Safety Plan (accessible at https://floridadep.sharepoint.com/dear/SitePages/Lab%20Health%20and%20Safety.aspx).

9.2. Safety glasses, gloves and lab coats should be worn at all times whilst handling samples and reagents.

9.3. Read the MSDS information for safe reagent and sample handling.

9.4. Buffers and leftover samples with a pH value of > 2 or < 12.5 units may be flushed down the sink with water.

10. REFERENCES/DEFINITIONS

For definitions see section 3 of the FDEP Quality Manual. All abbreviations used throughout the document are defined at least once in the document usually at the first time of use.

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10.1. Standard Methods Online, 2320 B-2011.

10.2. Automatic PC-Titrate Instrument Operations Manual.

10.3. DEP SOP NU-033, Summary of Sample Analysis Procedures for the General Chemistry and Nutrients Workgroups.

10.4. DEP SOP NU-038, Nutrients and General Chemistry: Quality Assurance and Quality Control.

10.5. DEP SOP NU-041, Reagent Preparation Procedure.

10.6. DEP SOP NU-042, Standard Preparation Procedure.

10.7. DEP SOP NU-043, Nutrients and General Chemistry Quality Assurance and Data Authorization Guidelines.

10.8. DEP SOP NU-060, Automated PC-Titrate Instrument Operating Procedure.

Quality Manual, Bureau of Laboratories, FDEP.

10.9. Laboratory Health and Safety Plan.

10.10. DEP SOP LB-006, Standard Operating Procedure for Records Maintenance and Storage.

Appendix of Significant Changes

04/28/2008 Reference method changed from EPA 310.1 to SM 2320 B.

04/14/2015 Data archival information was clarified in Section 7. Section 8 was modified and acceptance limits for CCV's, ALTQC, and RPD updated. Frequency of sample duplicate analysis was changed to one per batch.

02/04/2016 The test name was updated to ALKALINITY in Sections 1 and 7.3. File paths and web addresses were updated.

02/21/2018 Certificate of Analyses storage location updated to network drive.

12/10/2019 Updated equipment in Section 3. Section 4 was modified to include updated file paths and hold times for ALTQC and LFB. The run sequence was clarified in Section 5. Acceptance criteria for quality control measures were updated in Section 8.

12/28/2020 In Section 3, the instrumentation, equipment, and supplies were updated and inputted into table format. In Section 4, chemicals were updated and inputted into table format. Preparation procedures for reagents and standards were reformatted.

01/18/2022 The pH standards will be rerun following the calibration as a means of evaluating the relative error of the calibration.

07/14/2023 Updated data drive file path links. Clarified standardization procedure.

10/01/2024 Updated data drive file path links.

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