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Standard Operating Procedure for
Determination of Aqueous Inorganic Carbon and calculated Carbonate Alkalinity
in waters of Fresh/Estuarine/Coastal Waters.

(References: ASTM D7573-09)

Document #: NASLDoc-015
Revision 2025-1
Replaces Revision 2024-1
Effective May 1, 2025

**I attest that I have reviewed this standard operating procedure and agree to comply
with all procedures outlined within this document.**

_____   _____   _____
Employee (Print)   Employee (Signature)   Date

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Employee (Print)   Employee (Signature)   Date

Revised by: _____ Date: _____

Reviewed by: _____ Date: _____

Laboratory Supervisor: _____ Date: _____

Changes affecting Revision 2025-1

Section 9.3.5: Removed all standards are analyzed to confirm the calibration.
Table: Added iDOC/DOC criteria

1. **SCOPE and APPLICATION**

    1.1 Aqueous inorganic carbon (TIC) is determined by wet chemical analysis where a measured sample is injected with a percentage of 1N hydrochloric acid. The carbonates are reduced to CO_2 and are detected using a non-dispersive infrared detector (NDIR) of an organic carbon analyzer. Carbonate alkalinity is calculated using the TIC concentration. The method is used to analyze all ranges of salinity.

    1.2 A Method Detection Limit (MDL) of 0.17 mg/L TIC was determined using the MDL method as specified in the EPA Federal Register 821-R-16-006, titled Definition and Procedure for the Determination of the Method Detection Limit, Revision 2.

    1.3 The quantitation limit/reporting limit for TIC was set at 1.00 mg/L C, which is equal to the lowest standard used.

    1.4 This procedure should be used by analysts experienced in the theory and application of inorganic carbon analysis. A three-month training period with an analyst experienced in the analysis using the organic carbon analyzer is required.

    1.5 This method can be used for all programs that require analysis of aqueous inorganic carbon.

    1.6 This procedure follows the procedures set forth within the operating manual of the Shimadzu TOC-L and references ASTM D7573-09.

2. **SUMMARY**

    2.1 The Shimadzu TOC-L is a high-temperature combustion instrument used to analyze aqueous samples for TIC, TOC, and non-purgeable organic carbon (NPOC).

    2.2 An aliquot of sample is pulled into the sampling syringe. A percentage of 1N hydrochloric acid (HCl) is injected into the syringe and the combined solution is bubbled for a measured time. The carbonates within the sample are reduced to carbon dioxide (CO_2). The CO_2 is carried by ultra-pure air to a non-dispersive infrared detector (NDIR) where CO_2 is detected.

    2.3 Carbonate alkalinity is then calculated after the concentration of inorganic carbon is determined.

3. **DEFINITIONS**

    3.1 Acceptance Criteria – Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)

    3.2 Accuracy – The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)

3.3   Aliquot – A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)

3.4   Analytical Range – 0-3000 mg/L using 5 ml syringe and 10-4500 μl (variable) injection volume, using regular sensitivity catalyst.

3.5   Batch – Environmental samples, which are prepared and /or analyzed together with the same process and personnel, using the same lot(s) of reagents. An **analytical batch** is composed of prepared environmental samples (extracts, digestates, or concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical batch can include samples originating from various environmental matrices and can exceed 20 samples. (NELAC/EPA)

3.6   Blank- A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)

3.7   Calibrate- To determine, by measurement or comparison with a standard, the correct value of each scale reading on a meter or other device, or the correct value for each setting of a control knob. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.8   Calibration – The set of operations that establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.9   Calibration Curve – The graphical relationship between known values, such as concentrations, or a series of calibration standards and their analytical response. (NELAC)

3.10   Calibration Method – A defined technical procedure for performing a calibration. (NELAC)

3.11   Calibration Standard – A substance or reference material used to calibrate an instrument. (QAMS)

   3.11.1 Initial Calibration Standard (STD) – A series of standard solutions used to initially establish instrument calibration responses and develop calibration curves for individual target analytes.

   3.11.2 Initial Calibration Verification (ICV) – An individual standard, which may be the same compound used as the calibrating standard, but not from the same vendor unless confirmed as different lots, analyzed initially, prior to any sample analysis, which verifies the acceptability of the calibration curve or previously established calibration curve.

3.11.3 Continuing Calibration Verification (CCV) – An individual standard which may be the same as the calibrating standard is analyzed after every 10 samples analysis.

3.12 Certified Reference Material (CRM) – A reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. (ISO 17025)

3.13 Combustion tube – Quartz tube filled with a platinum catalyst, heated to 680° C, into which the sample aliquot is injected.

3.14 Conditioning Blank – Reagent water (ASTM Type I) run before the calibration curve to decrease the instrument blank and stabilize the column conditions.

3.15 Corrective Action – Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent a recurrence. (ISO 8402)

3.16 Deficiency – An unauthorized deviation from acceptable procedures or practices. (ASQC)

3.17 Demonstration of Capability – A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)

3.18 Detection Limit – The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.

3.19 Duplicate Analysis – The analyses of measurements of the variable of interest performed identically on two subsamples (aliquots) of the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not the precision of sampling, preservation, or storage internal to the laboratory. (EPA-QAD)

3.20 External Standard (ES) – A pure analyte (sodium carbonate/sodium bicarbonate ($Na_2CO_3/NaHCO_3$)) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the unknown sample.

3.21 Field Duplicates (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 provide a measure of the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

3.22 Field Reagent Blank (FRB) – A aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.23 Furnace – Heats the combustion tube to the operating temperature of 680° C.

3.24 Holding time – The maximum time that samples may be held prior to analysis and still be considered valid. (40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.

3.25 Injection – The sample aliquot that is drawn into the syringe and injected into the combustion tube.

3.26 Instrument Detection Limit (IDL) – The minimum quantity of analyte of the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time absorbance line, etc.

3.27 Laboratory Duplicates (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.28 Laboratory Reagent Blank (LRB) – A matrix blank (i.e., reagent water) that is treated exactly like a sample including exposure to all glassware, equipment, solvents, and reagents 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 instrument.

3.29 Laboratory Control Sample (LCS) – A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes from a source independent of the calibration standard or a material containing known and verified amounts of analytes. The LCS is generally used to establish intra-laboratory or analyst-specific precision and bias or to assess the performance of all or a portion of the measurement system. (NELAC)

3.30 Limit of Detection (LOD) – The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank (ACS), also known as the MDL.

3.31 Limit of Quantitation (LOQ) – The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD, depending on the degree of confidence desired. Also known as the Quantitation Limit.

3.32 Linear Dynamic Range (LDR) – The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).

3.33 May – Denotes permitted action, but not required action. (NELAC)

3.34 Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured, and reported from the 99th percentile that the analyte concentration is greater than zero.

3.35 Must – Denotes a requirement that must be met. (Random House College Dictionary)

3.36 Non-Dispersive Infrared Detector (NDIR) – The detector found in the Shimadzu TOC-L analyzer. Carbon dioxide is detected.

3.37 Precision – The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance, or range, in either absolute or relative terms. (NELAC)

3.38 Preservation – Refrigeration, freezing, and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.

3.39 Quality Control Sample (QCS) – A sample of analytes of known and certified concentrations. The QCS is obtained from a source external to the laboratory and is different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials. Also known as CRM.

3.40 Run – One sample analysis from start to finish, including printout.

3.41 Run Cycle – Typically a day of operation – the entire analytical sequence of runs from the first run to the last run.

3.42 Safety Data Sheets (SDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.43 Sample Volume – Amount of sample injected into the combustion tube.

3.44 Sensitivity – The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.

3.45 Shall – Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)

3.46 Should – Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)

3.47 Standard Reference Material (SRM) – Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an indication of the accuracy of a specific analytical technique. Also known as CRM.

4. **INTERFERENCES**

4.1 Carbon dioxide is readily absorbed from the air into an aqueous sample. Care must be taken to avoid this. Sample collection bottles should be filled to the brim with no headspace. Standards should be prepared in small batches and used within 1-2 days of preparation. Sample vials should be covered when placed in the autosampler.

5.   SAFETY

5.1 Safety precautions must be taken when handling reagents, samples, and equipment in the laboratory. Protective clothing including lab coats, safety glasses, and enclosed shoes should be worn. In certain situations, it will be necessary to also use gloves and/or a face shield. If solutions come in contact with eyes, flush with water continuously for 15 minutes. If solutions come in contact with skin, wash thoroughly with soap and water. Contact Solomons Rescue Squad (911) if emergency treatment is needed and also inform the CBL Associate Director of Administration of the incident. Contact the CBL Associate Director of Administration if additional treatment is required.

5.2 The toxicity or carcinogenicity of each reagent used in this procedure may not have 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 hazardous materials and procedures.

5.3 Do not wear jewelry when troubleshooting electrical components. Even low voltage points are dangerous and can injure if allowed to short circuit.

5.4 The following hazard classifications are listed for the chemicals used in this procedure. Detailed information is provided on Material Safety Data Sheets (MSDS).

Table 1:

Chemical	Health Hazard	Fire Hazard	Instability Hazard	Specific Hazard	
Sodium Carbonate, Anhydrous	2	0	1	Irritant	
Sodium Bicarbonate	1	0	0		
Hydrochloric Acid	3	0	2	ACID, COR	
Sodium Hydroxide	3	0	1	ALK, COR	
Platinum Catalyst on Alumina Beads	1	0	0		
Soda Lime	3	0	0	COR	

On a scale of 0 to 4 the substance is rated on four hazard categories: health, flammability, reactivity, and contact. (0 is non-hazardous and 4 is extremely hazardous)

HAZARD RATING
Health Hazard - Blue: 4 – deadly, 3 – extreme danger, 2 – hazardous, 1 – slightly
hazardous, 0 – normal material

Fire Hazard - Red: Flash Points: 4 – below 73° F, 3 – below 100° F, 2 – below 200°
F, 1 – above 200° F, 0 – will not burn

Instability Hazard - Yellow: 4 – may detonate, 3 – Shock and heat may detonate, 2 –
violent chemical change, 1 – unstable is heated, 0 - stable

Specific Hazard - White: Acid = ACID, Alkali = ALK, Corrosive = COR, Oxidizer =
OXY

6. EQUIPMENT AND SUPPLIES

6.1 A Total Organic Carbon Analyzer capable of maintaining a combustion
temperature of 680° C and analyzing for organic and inorganic carbon. The
Shimadzu TOC-L is used in this laboratory.
6.2 Refrigerator, capable of maintaining +4 ± 2° C.
6.3 Lab ware – All reusable labware (glass, Teflon, plastic, etc) should be
sufficiently clean for the task objectives. This laboratory soaks all labware
related to this method in a 10% HCl (v/v) acid bath overnight and rinses
copiously with reagent (ASTM Type I) water. Then the glassware is baked at
400° C for at least 1 hour. Clean check blanks are analyzed with every run.

7. REAGENTS AND STANDARDS

7.1 Purity of Water – Unless otherwise indicated, references to water shall be
understood to mean reagent water conforming to ASTM Specification D
1193, Type I. Freshly prepared water should be used for making the
standards intended for calibration. The detection limits of this method will
be limited by the purity of the water and reagents used to make the
standards.
7.2 Purity of Reagents – Reagent grade chemicals shall be used in all tests.
Unless otherwise indicated, it is intended that all reagents shall conform to
the specifications of the Committee on Analytical Reagents of the
American Chemical Society, where such specifications are available.
Other grades may be used, provided it is first ascertained that the reagent
is of sufficiently high purity to permit its use without compromising the
accuracy of the determination
7.3 Sodium Hydrogen Carbonate ($NaHCO_3$) and Sodium Carbonate (Na_2CO_3)
– primary standard for inorganic carbon.

Inorganic Carbon Stock Standard: Sodium Hydrogen Carbonate/ Sodium
Carbonate ($NaHCO_3/Na_2CO_3$) Standard,   1000 mg/l
Sodium Hydrogen Carbonate ($NaHCO_3$)   0.875 g

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Sodium Carbonate, Anhydrous (Na_2CO_3)   1.1025 g
Reagent water   250 ml

In a 250 ml volumetric flask, dissolve 0.875 g $NaHCO_3$ and 1.1025 g Na_2CO_3 in ~150 ml reagent water. Dilute to 250 ml with reagent water. Make fresh within 4 months. Store at 4° C.

7.4 Hydrochloric Acid – 1N
Hydrochloric Acid (HCl), concentrated,   86 ml
Reagent water, q.s.   1000 ml

In a 1000 ml volumetric flask, add 86 ml of concentrated hydrochloric acid to ~ 500 ml of reagent water. Dilute to 1000 ml with reagent water.
7.5

8   SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Water collected for TIC is unfiltered whole water and should not be acidified. The sample container may be any container which has been adequately cleaned. If frozen, freshwater samples should be stored in Teflon or plastic to prevent breakage.

8.2 The holding time for frozen TIC samples is 28 days. It has been shown that frozen QCS samples up to a year old still fall well within the control limits, therefore if the frozen sample is stored for longer than the holding time, there is minimal degradation.

8.3 TIC samples stored at ≤ 6° C should be analyzed within 28 days.

8.4 Sample containers should be filled to the brim with no headspace if refrigerated. If frozen, enough space for expansion should be left at the top of the container to prevent breakage.

9   QUALITY CONTROL

9.1 The laboratory 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 continued analysis of laboratory instrument blanks and calibration standard material, analyzed as samples, as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

9.2 Initial Demonstration of Capability

9.2.1   The initial demonstration of capability (iDOC) – is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.

9.2.2   Linear Dynamic Range (LDR) – The linear dynamic range for TIC should be established using a blank and four or more appropriate standards for the calibration curve.

9.2.3   Quality Control Sample (QCS/CRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 10% of the certified 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 analyses.

9.2.4   Method Detection Limits (MDL) – An MDL should be established for TIC using a spiked water sample (MDL_S), typically two to 10 times the estimated MDL and no more than ten times higher than the estimated MDL or reagent blanks (MDL_b). Process a minimum of seven spiked samples and seven blank samples through all steps of the method. The samples used for the MDL must be prepared and analyzed in at least three batches on three separate calendar dates. If multiple instruments are used for this analysis, MDLs must include data/calculations from all instruments. The higher of the two values will be the new MDL.

9.2.4.1 Make all computations as specified in the analytical method and express the final results in the method-specified reporting units.

9.2.4.2 Calculate the sample standard deviation (S) of the replicate spiked sample measurements and the sample standard deviation of the replicate method blank measurements from all instruments to which the MDL will be applied.

9.2.4.3 Compute the MDL_S (the MDL based on spiked samples) as follows:

$$MDL_S = t_{(n-1,\ 1-\alpha=0.99)}S_S$$

where:
MDL_S = the method detection limit based on spiked samples
$t_{(n-1,\ 1-\alpha=0.99)}$ = the Student's t-value appropriate for a single-tailed 99th percentile
t statistic and a standard deviation estimate with n-1 degrees of freedom.
S_S = sample standard deviation of the replicate spiked sample analyses.

9.2.4.4 Compute the MDL_b (the MDL based on method blanks) as follows:

If none of the method blanks give numerical results for an individual analyte, the MDL_b does not apply. A numerical result includes both positive and negative results, including results below the current MDL, but not results of "ND" (not detected) commonly observed when a peak is not present in chromatographic analysis.

If some (but not all) of the method blanks for an individual analyte give numerical results, set the MDL_b equal to the highest method blank result. If more than 100 method blanks are available, set MDL_b to the level that is no less than the 99th percentile of the method blank results. For "n" method blanks where n ≥ 100, sort the method blanks in rank order. The (n * 0.99) ranked method blank result (round to the nearest whole number) is the MDL_b. For example, to find MDL_b from a set of 164 method blanks where the highest-ranked method blank results are ... 1.5, 1.7, 1.9, 5.0, and 10, then 164 x 0.99 = 162.36 which rounds to the 162nd method blank result.

Therefore, MDL_b is 1.9 for n =164 (10 is the 164th result, 5.0 is the 163rd result, and 1.9 is the 162nd result). Alternatively, you may use spreadsheet algorithms to calculate the 99th percentile to interpolate between the ranks more precisely.

If all of the method blanks for an individual analyte give numerical results, then calculate the MDL_b as:

$$MDL_b = \bar{X} + t_{(n-1, 1-\alpha = 0.99)} S_b$$

where:
MDL_b = the MDL based on method blanks
$\bar{X}$ = mean of the method blank results (use zero in place of the mean if the mean is negative)

$t_{(n-1, 1-\alpha = 0.99)}$ = the Student's t-value appropriate for the single-tailed 99th percentile
t statistic and a standard deviation estimate with n-1 degrees of freedom.
S_b = sample standard deviation of the replicate method blank sample analyses.

9.2.4.5 The verified MDL is the greater of the MDL_S or MDL_b. If the verified MDL is within 0.5 to 2.0 times the existing MDL, and fewer than 3% of the method blank results (for the individual analyte) have numerical results above the existing MDL, then the existing MDL may optionally be left unchanged. Otherwise, adjust the MDL to the new verification MDL. (The range of 0.5 to 2.0 approximates the 95th percentile confidence interval for the Initial MDL determination with six degrees of freedom.)

9.2.4.6 Method Detection Limits should be determined annually, whenever there is a significant change in instrumental response or a significant change in instrument configuration. Data for annual MDL calculation and verification is analyzed at least quarterly, throughout the year.

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. The LRB consists of reagent water treated the same as the samples. LRB data are used to assess contamination from the laboratory environment.

9.3.2 Quality Control Sample (QCS)/ Certified Reference Material (CRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning of the run, after every 10 samples, and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within $\pm 3\sigma$ of the certified values, performance of the determinative step of the method is unacceptable. As this analysis runs overnight, it is not possible to correct and reanalyze the ICVs/CRM before sample analysis. If there is a problem noted with the run the next morning, all trends including blanks, ICVs, CCVs, and CRMs must be scrutinized before accepting the data. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with the analyses. The results of these samples shall be used to determine batch acceptance.

9.3.3 The QCS/CRM will be obtained from a source external to the laboratory and different from the source of calibration standards.

9.3.4 Control Charts – The CRM data are graphed, and the slope, y-intercept, and correlation coefficient data, as well as the blank, ICV, and CCV data, are compiled and tracked. The CRM concentrations should fall within +/- 3σ of the expected value. The Accuracy Control Chart for QCS/CRM samples is constructed from the average and standard deviation of the QCS/CRM measurements per batch. The accuracy chart includes upper and lower control levels (CL=±3σ). These values are derived from the stated values of the QCS/CRM. The standard deviation (σ) is specified relative to statistical confidence levels of 99% for CLs. Enter QCS/CRM results on the chart each time the sample is analyzed

9.3.5 Calibration Verification – Initial Calibration Verification (ICV) - Immediately following the calibration curve. The ICVs are any standards not used in the curve and that fall within the middle of the curve. They are made from a $Na_2CO_3/NaHCO_3$ solution that is purchased from a separate vendor than the calibration stock solution or is confirmed to be a separate lot if from the same vendor. Continuing Calibration Verification (CCV) - Following every 10 samples, one or two CCVs are analyzed to assess instrument performance. The CCVs are made from the same material as calibration standards ($Na_2CO_3/NaHCO_3$), and are to be within ± 10% of the expected value. As this analysis runs overnight, it is not possible to correct and reanalyze the ICVs/CRM before sample analysis. If there is a problem noted with the run the next morning, all trends including blanks, ICVs, CCVs, and CRMs must be scrutinized before accepting the data. Failure to meet the criteria constitutes correcting the problem and reanalyzing the samples. If not enough sample exists, the data must be qualified if reported.

9.4 Assessing Analyte Recovery
    9.4.1 Matrix spikes are performed every 10 samples.
    9.4.2 1.0 ml of the highest carbonate standard in the curve is added to 10.0 ml of sample for a total volume of 11.0 ml.
    9.4.3 1.0 ml standard $1.0/11.0 = 0.09$
    9.4.4 0.09 X STD conc.
    9.4.5 10.0 ml sample $10.0/11.0 = 0.91$
    9.4.6 (original sample conc. X 0.91) + (0.09 x std conc.) = (expected conc.) mg/L
    9.4.7 Percent Recovery for each spiked sample should fall within 90-110%. Where:

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%SR = ((Actual Value-Original value)/ (Spike Concentration added)) x 100

    9.4.8   Relative Percent Difference (RPD) of duplicated samples should be less than 10%. Where:
RPD = difference of duplicates x 100
           Average of duplicates

    9.4.9   Assess whether the analytical result for the CRM/QCS sample confirms the calibration when calculated as follows
        % Recovery = AMC/CRM x 100
Where:
        AMC = Average measured concentration of the CRM sample
        CRM = Certified value of the CRM
The analytical result must fall with the range of 90-110%

9.5 Data Assessment and Acceptance Criteria for Quality Control Measures
    9.5.1   The Acceptance Criteria for TIC is 0.995. If the correlation coefficient is less than acceptable, all blanks and standards analyzed during the run may be averaged into the curve.
9.6 Corrective Actions for Out of Control Data
    9.6.1   If the acceptance criteria are still not met, the samples are to be rerun.

Table 2:

QC Indicator	Acceptance/ Action Limits	Action	Frequency (Batch)
Correlation Coefficient	≥ 0.995	If <0.995, rerun curve	1 per batch if acceptable.
Quality Control Sample (QCS)/ Certified Reference Material (CRM)	± 10%	If the QCS value falls between ±10-20%, blanks and stds must fall within range before accepting data. If QCS value is outside ± 20% of the target value reject the run, correct the problem and rerun samples.	Beginning of run following the ICV, after every 10 samples, and at end bracketed by final CCVs.
Initial Calibration Verification (ICV)	± 10%	Recalibrate if outside acceptance limits.	Beginning of run following standard curve.
Continuing Calibration	± 10%	If outside 10%, review the trend of subsequent CCVs,	After every 10 samples and at

		blanks, and CRMs. If all trend greater than ±10%, rerun all samples following the last in-control CCV.	end of batch.
Verification (CCV)			
Method Blank/Laboratory Reagent Blank (LRB)	≤ Method Quantitation Limit	If the LRB exceeds the quantitation limit, results are suspect. Rerun the LRB. If the concentration still exceeds the quantitation limit, reject or qualify the data, or raise the quantitation limit.	Following the ICV, after every 10 samples following the CCV and at the end of the run.
Laboratory Fortified Blank (LFB)	± 10%	If the % Recovery of the added analyte falls outside ±10%, take corrective action, including re-analysis of associated samples if required.	Beginning of run following the LRB.
Laboratory Fortified Sample Matrix Spike	± 10%	If the recovery of any analyte falls outside the designated acceptance limits and the QCS is in control, the recovery problem is judged matrix induced. Repeat the LFM and if the sample results are again outside the acceptable recovery range, the sample should be reported with a "matrix induced bias" qualifier.	1/10
Laboratory Duplicate	± 10%	If the RPD fails to meet the acceptance limits, the samples should be reanalyzed. If the RPD again fails to meet the acceptance limits, the sample must be reported with a qualifier identifying the sample analysis result as not having acceptable RPD for duplicate analysis.	1/20
iDOC/DOC Mean Percent Recovery	± 10%	If outside of range, correct the problem and repeat. Cannot analyze samples until passing.	Annual
iDOC/DOC Standard Deviation	≥2	If outside of range, correct the problem and repeat. Cannot analyze samples until passing.	Annual

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10 CALIBRATION AND STANDARDIZATION

10.1   Calibration – Daily calibration must be performed before sample analysis may begin. A minimum four-point calibration is used with the Shimadzu TOC-L.

10.1.1 Reagent water is used as the "zero point" in the calibration. The standards are calculated by the following equation:

$$\text{mg TIC/L} = (A_{STD} - \|\text{y intercept}\|)/\ m$$

Where: A_{STD} = Area of the standard
$\|\text{y intercept}\|$ = absolute value of the y intercept
m = slope of the regression line

TIC sample concentration is calculated using the following equation:
$$\text{mg TIC/L} = (A_S - \|y\|\ /\ m$$

Where: A_S = area of the sample,
$\|y\|$ = absolute value of the y intercept
m = slope of the regression line

Carbonate Alkalinity sample concentration is calculated using the following equation:

$$\text{mg } CO_3/L = (\text{mg TIC/L}) * (1 \text{ moles C}/12 \text{ g C}) *(60 \text{ g } CO_3/\ 1 \text{ mole})$$

example: 17.0 mg TIC/L = (17.0 * 60)/12 = 85.0 mg CO_3/L

11.0   References

Instrument Manual Total Organic Carbon Analyzer Model TOC-L. Shimadzu Scientific Corporation. 7102 Riverwood Drive, Columbia, Maryland 21046-2502. Phone: 410 381-1227.

ASTM D7573-09, Standard Test Method for Total Carbon and Organic Carbon in Water by High Temperature Catalytic Combustion and Infrared Detection, ASTM International, West Conshohocken, PA, 2009, www.astm.org

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Appendix I

PROCEDURE

Daily Operations

Refer to the original "Running the TOC-L" on p.20 to start the instrument.

1N HCl is used in the analysis of inorganic carbon and carbonate alkalinity. Switch the 1N HCl to the location next to the dilution H_2O. Move the 9N H_2SO_4 bottle to the empty slot. Insert acid sample line.

Continue to follow the steps to start the TOC-L.

Replace the reagent water with fresh daily. The standards are good for 2 consecutive runs only. Make fresh each time if not running the next day. It is ok to leave the dilution water for several days. It is not used for the standards.

Loading samples: Fill all sample vials to the shoulder, cover with septum and cap. Water readily picks up CO_2 from the air, therefore it is best to leave as little headspace as possible. A spiked sample is the only sample with less volume.

Because of this, plan several small runs, instead of one large run. It is better to run fewer than 20 samples in addition to blanks and standards per run.

One CRM vial filled to the shoulder is all that is needed. The software allows the one vial to be sampled a maximum of 3 times (this depends on the injection volume and # of washes). The CRM should be analyzed after the ICVs and blanks, after the next blank and low CCV, and again at the end bracketed within the final standards.

In creating a new method, select IC instead of NPOC. From there, the method setup is the same.

"Multiple injections" is not an option for IC analysis.

Include several conditioning blanks (minimum of 3) after switching from sulfuric acid to HCl. This replaces the acid for the other before the standard curve. Visually check that the conditioning blanks reach a steady state before running the standard curve. The area of the blank should be below 4.0.

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Running the TOC-L

Make sure the 2nd stage of the regulator on the air tank (Air Gas Ultra Zero Grade Air, size A) is set at no higher than 30 psi. Replace the tank when the tank pressure falls below 500 psi.

To turn on instrument, push the on/off switch on right side of instrument to on, and then push button located on front of instrument. The front indicator light will cycle through the colors ending with orange, which means the instrument is in a not-ready state. The indicator light turns green when the instrument is up to temperature and all parameters are OK. The light will be blue while the instrument is running samples. If the indicator light is red, refer to the software and the manual to determine the problem. If necessary, call Shimadzu (1-800-477-1227) for tech support.

Open software by clicking on the TOC-L sample table icon. There is no password. Just hit enter when password screen appears.

Open a new sample table by clicking on NEW in the toolbar. Click OK. Then hit CONNECT located in the tool bar. A sample table must be open to connect the instrument. The furnace automatically turns on.

At this time, refill the dilution, reagent blank, and rinse water bottles. The reagent blank water is in the 500 ml Teflon bottle or brown glass TOC reagent water bottle beside the instrument. The rinse water bottle is located behind the autosampler. The dilution bottle is located on the left side of the instrument along with the 9 N H_2SO_4 bottle, 1 N HCl bottle, and the drain bottle. Sulfuric acid is used in the NPOC analysis, and the HCl is used in the TIC analysis. Check the volume of the acid bottle in use, and the drain bottle. The liquid level of the drain bottle should be just below the arm. 250 mls of 9 N H_2SO_4 or 1 N HCl is plenty for several weeks of analysis. Unless the dilution water is being used in serial dilutions of the standard curve, it is not necessary to change daily. Replace weekly regardless.

Open the front door of the instrument and check the liquid level of the humidifier located on the right-hand side. The level should be between the high and low marks. Add reagent water as needed by removing cap at top.

Check the level of liquid in the Type B Halogen Scrubber (the long tube next to the syringe which contains the rolled stainless mesh). Add 0.05 N HCl (40 ml reagent H_2O + 1 ml 1N HCl) so that the level is an inch or so above the level of the mesh screen. There is a small drain line attached to the 8-port valve at port 6 which is frequently pulled out of the drain when removing the cap of the Type B Halogen Scrubber. When recapping the scrubber, <u>ALWAYS check that the small tubing from port 6 on the 8-port valve is in the</u>

black capped drain port behind the scrubber. Replace the 0.05 N HCl each time the column is changed.

It is recommended that these next two steps should be performed before each run. An explanation of the Maintenance Menus can be found in the User's Manual, Chapter 7.6 p.302-308.

Before running blanks or beginning a sample run, from the program, select Instrument and Maintenance. Click on Residue Removal, then click start. Close when finished.

Next, under Instrument Maintenance, select Replace Flowline Content, and then click start. Close when finished.

If the instrument has been sitting unused, or if several runs of high salt samples have been analyzed, perform a TC Regeneration. Again, under Instrument Maintenance, select Regeneration of TC Catalyst, and then click start. This takes several minutes. Close when finished.

Keep track of pre-run checks by entering information on instrument pre-run check sheet and bench sheet.

Loading samples: Read Section in Full before proceeding.

The volume of the sample vial is 24 mls. Sample containers should be of adequate size to reduce volume limitations. Teflon bottles used in the lab hold 30 mls causing the analysis to be volume limited. Other types of containers (glass or PETG) have a larger volume. Fill the sample vial up to the shoulder. The absolute minimum volume to use in the sample vial is 10 mls. Choose a sample with maximum volume in the Teflon bottle ahead of time to be the QA sample for duplicates or to make a spike. Cover each sample vial with a septum (or foil square if not available) and secure with an open septum cap.

Standard Curve:

The reagent blank water is in the bottle beside the instrument. This bottle is considered Position 0 on the sample wheel.

Load the other standards in the curve in the first several slots of the wheel. There are 2 stock solutions for standards. One is marked for the calibration standards and CCVs, and the other is marked for the initial calibration verification (ICV) standards. The ICVs are not used in the curve, but are positioned within the middle of the curve and are analyzed before any samples are run.

Two standard curves are set up in the templates. The low curve ranges from 0-10 mg/L. The calibration standards used are 1.0, 2.0, 5.0, and 10.0 mg/L. The high curve ranges from 0.-30 mg/L. The calibration standards used are 5.0, 10.0, 15.0, 20.0, and 30.0 mg/L. The ICV standards are 1.5 and 12.0 mg/L. Both curves are used per each run.

QA/QC

Analyze a certified reference control sample (CRM) after every 10 samples along with a blank and CCV. With each batch of control samples, a method is created in the control sample folder. To insert a control sample, highlight the line in the sample table. Click on INSERT on the tool bar, and then click on Control Sample. Once the folder is open, click on the appropriate file. The control CRM will be inserted above the highlighted line.

 Analyze a blank, the lowest standard, and a CRM (or a mid-range standard) every 10 samples. The CRMs are in 30-ml bottles that are sealed in individual packets and kept in the freezer. Use 1 per run. Fill a 24 ml sample vial to the shoulder with CRM, cover with septum and cap. There is enough volume to sample the vial three times. When inserting the control sample in the sample table, assign the same vial position for each time. The autosampler is capable of returning to a particular vial site.

After the initial CRM, load a reagent blank in a vial. This is considered a cleanliness check of each batch of vials. A laboratory fortified blank (LFB) should be analyzed after the LRB. The concentration of the LFB is between 3 and not more than 10 times the MDL and is considered a control sample.

Because all samples vials are filled to the shoulder, select one sample as duplicate. Indicate on the bench sheet at the appropriate location that the duplicate is to be inserted at that spot. If sample volume is not an issue, two sample vials can be used instead.

For the sample chosen to be spiked, withdraw 10.0 mls of sample using a clean volumetric pipet per sample and add it to a sample vial. Then add 1.0 ml of the highest standard to the vial. Cover and cap, then gently shake to mix. Put the spiked sample in the proper location in the sample wheel.

Analyze spikes every 10 samples and duplicates every 20 samples.

End the run with blanks and CCVs, with the last CRM control sample inserted between the bracketing standards.

Sample Table:

To create a new calibration file, refer to the User's Manual, Chapter 4.1 pp. 89-93, and follow the Calibration Curve Wizard Setup. Several curve templates are set up and are overwritten with new curve data each time they are used.

Create a method by clicking on File/New/Method and follow the Method Wizard Setup. Refer to the User's Manual Chapter 4.1 pp.94-96. A new method is created with each run.

Use the drop-down box to select type of analysis (i.e.: IC). Leave default Sample Name and Default Sample ID empty.

Enter the file name, and then click Next.   (Example: dnr st martins041213)

The calibration curve is chosen on the next screen. Two curves (Low and High) are selected for TIC analysis. Click Next again. Confirm the injection parameters to match the low calibration curve. Click Next again.

Use default settings on the next page, and None for Pharmaceutical water testing on the last page.

Click Finish. The method is complete.

Editing the Sample Table:

Highlight the first line of the sample table to insert information. From the toolbar at the top, click on Insert.

Insert 3-4 conditioning blanks by clicking on Multiple Samples. Follow the wizard prompts. The water for conditioning blanks is the same as the reagent water in position 0.

Highlight the next available line to insert the calibration curve. Click on Insert/calibration curve. Choose the proper calibration file. Both the Low and High curves will be used.

Highlight the next available line to insert multiple samples. Follow the wizard prompts. Leave the Sample Name and Sample ID blank.

Once the sample table has been set up, enter the sample names and IDs.

It is easiest to insert Control samples after the sample names and IDs are in place. Highlight the line below where the control sample is to be inserted. Click on Insert and select Control Sample. Choose the proper file.

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When all sample and control information is entered into the table, enter the vial position numbers. Click on the carousel icon (looks like a birthday cake) in the sample table toolbar. The vial positions correspond to the numbered positions on the bench sheet. Be sure duplicate samples are numbered to match the original if sampling from the same vial. Click OK when finished.

Proof all entries and save the sample table. Click File/Save As to name the file. Example: 2013_05_09_dnr st martins 042213

Highlight the first line of the sample table.

Click START. The Measurement Start Window is displayed. Click on the procedure to be performed when the analysis is complete. The instrument is kept running except over weekends. If no samples are to be run the next day, select Keep Running in case samples go off-scale and need to be rerun. They can be inserted at the end of the sample table and run. Insert sample information and vial positions, then SAVE the file.

To open the Sample Window, click on the graph icon on the sample table to view peak information.

Accessing the data:

When the run has finished:

To save the file to another source (i.e. the shared network drive), (TOC-L-1) click File/Save As. Save the standard curve, highlight the standard curve in the sample table. Select Print: Print highlighted. Name file as a curve file. The file is saved to the shared network drive. Open the file as a PDF. Print to printer and save to the desktop.

To export data, click File/ASCII Export. Save the file in each form, Normal and Detailed. The Normal file contains only concentration information. The Detailed file includes all injection data. The ASCII files can now be opened in Excel. This works with both instruments.

Open TOC-L Sample Table on the desktop. Open the file from the shared network drive. Save the file to the desktop using the same file name.

Scheduled Maintenance

The column will last for about 10 runs. Usually the blanks and low standards start sharply rising during a run as the column crashes. It is best to change the column before that happens; therefore, due diligence is needed to determine the optimal life of the column.

During routine maintenance, replace the old column with a fresh column, ceramic plate, and new catalyst, new o-rings and syringe plunger tip. Replace the 0.05N HCl in the

Type B Halogen Scrubber. Rinse the cooling coil, syringe, ultrapure water trap, injection port and slider. The SO$_3$ mist scrubber and the copper Halogen scrubber need to be replaced about every 4-6 months. Record all information in the maintenance log.

Refer to the Shimadzu TOC-L Series Combustion Tube for High Salt Samples Instruction Manual for directions on packing the column (p.3) and making reagent acid (p.13).

Below is an example of the TIC bench sheet.

SHIMADZU DATA SHEET TIC curve		COLUMN RUN COUNTER		ANALYST: NLK OTHER:
TODAY'S DATE:		CRUISE:		
INSTRUMENT USED: TOC-L…1…2				
FILE NAME:				
SPIKE CONC.:10.0 mg/L….30 mg CO3/L				
STD CURVE USED: CIRCLE ONE		INJECTION VOLUME: 800 uL	**WORKING STDS MADE:**	
0,1,2,5,10	0,5,10,15,20,30	other uL	COMMENTS	
VIAL	SAMPLE ID	VIAL	SAMPLE ID	
0 CONDBLK		24		
0 CONDBLK		25		
0 CONDBLK		26		
1		27		
2		28		
3		29		
4		30		
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				LFB = 1.5 ppm CO3
16				Pre Run Checks
17				Mark All That Apply
18				Residue Removal (Daily) [ ]
19				Flowline Wash (Daily) [ ]
20				Rinse slider (Daily) [ ]
21				Regenerate Catalyst (as needed) [ ]
22				Rinse slider block (as needed) [ ]
23				New Column (as needed) [ ]

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