



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L311

Dissolved Organic Carbon in Water – Shimadzu TOC-L (NPOC Mode)

Standard Operating Procedure

SOP Number	SOP-L311
Revision	0.2
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Date	2026-06-26
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1. Purpose

This procedure describes the determination of dissolved organic carbon (DOC) in natural water samples (surface water, groundwater, stormwater, treated effluent) by the non-purgeable organic carbon (NPOC) method on the Shimadzu TOC-L analyzer. DOC is a fundamental water quality parameter that influences aquatic ecosystem productivity, light penetration, metal complexation, disinfection byproduct formation, and nutrient cycling (Thurman, 1985; Wetzel, 2001).

The NPOC method acidifies the sample and sparges with carrier gas to remove inorganic carbon (CO_2 , HCO_3^- , CO_3^{2-}) before combustion. The remaining carbon is measured as NPOC, which for most natural waters is operationally equivalent to DOC when the sample has been filtered through 0.45 μm (APHA et al., 2012, Section 5310B).

For instrument operation, startup, and shutdown, see [SOP-L204 Shimadzu TOC-L](#). For water-extractable DOC from soils, see [SOP-L418](#).

2. Scope

- **Applies to:** Filtered (0.45 μm) water samples from surface water, groundwater, stormwater, effluent, and precipitation, analyzed for DOC in NPOC mode on the Shimadzu TOC-L
- **Does not apply to:** Total organic carbon in unfiltered water (use TOC mode on the TOC-L); particulate organic carbon (requires separate filtration and combustion); soil carbon by SSM (see [SOP-L407](#)); soil extract DOC (see [SOP-L418](#)); microbial biomass carbon (see [SOP-L409](#))
- **Location:** EA Program analytical laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve protocol, ensure training compliance
Instrument Manager	Maintain the TOC-L per SOP-L204
Trained Personnel	Prepare samples and standards, run analysis, export data
New Students	Complete training before independent use

4. Definitions

- **Calibration blank:** Deionized water ($18.2 \text{ M}\Omega \cdot \text{cm}$) of verified DOC less than 0.05 mg C/L; used as the zero point for calibration and as the system blank to verify background carbon from the instrument and reagents.

- **CCV (continuing calibration verification):** A mid-range KHP standard analyzed at regular intervals during an analytical run to confirm calibration stability; acceptance criterion is 90–110% recovery; if outside this range, re-calibrate and re-run affected samples (APHA et al., 2012).
- **Chromophoric dissolved organic matter (CDOM):** The fraction of DOC that absorbs UV/visible light; derived primarily from aromatic humic and fulvic acids; a surrogate indicator of DOC concentration in optically characterized waters (Thurman, 1985).
- **Dissolved organic carbon (DOC):** Organic carbon that passes through a 0.45 μm filter; in natural waters, DOC includes humic substances, fulvic acids, amino acids, carbohydrates, and other soluble organic compounds; the operationally defined quantity measured as NPOC by the Shimadzu TOC-L (APHA et al., 2012; Thurman, 1985).
- **Field blank:** A sample of deionized water processed through the field collection equipment (bottles, filters, cooler) and returned to the laboratory for analysis; used to detect contamination introduced during sample collection and transport (Sharp, 1993).
- **Humic substances:** High-molecular-weight, polydisperse aromatic compounds derived from the decomposition of plant and microbial material; typically comprise 40–80% of DOC in natural fresh waters (Thurman, 1985).
- **IC (inorganic carbon):** Carbon present as dissolved CO_2 , bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}); removed from water samples by acidification and sparging before NPOC measurement.
- **KHP (potassium hydrogen phthalate, $\text{KC}_8\text{H}_5\text{O}_4$):** The primary calibration standard for TOC analysis; a pure, non-hygroscopic organic acid weighed accurately to prepare standards of known carbon concentration; $1.0000 \text{ g KHP} = 0.4711 \text{ g C}$.
- **Method blank:** Deionized water acidified and processed identically to field samples, including any filtration and preservation steps; used to detect contamination from reagents and equipment; must read less than 0.2 mg C/L (APHA et al., 2012).
- **NPOC (non-purgeable organic carbon):** The organic carbon remaining in a sample after acidification (typically with HCl or H_3PO_4) and sparging with carrier gas to remove IC and volatile organic carbon (VOC); the quantity reported as DOC by the Shimadzu TOC-L (APHA et al., 2012, Section 5310B).
- **Particulate organic carbon (POC):** Organic carbon retained on a 0.45 μm filter; not measured by this SOP; requires separate filter collection and combustion analysis.
- **Pre-combusted glass vial:** A TOC autosampler vial heated to 450 $^\circ\text{C}$ for at least 4 hours to destroy residual organic carbon from the glass or manufacturing process; used to prevent contamination of samples from the container.
- **PVDF (polyvinylidene fluoride) filter:** A 0.45 μm syringe filter with low organic carbon leaching; preferred for DOC sample filtration; cellulose acetate filters must not be used because they leach DOC (Kaplan, 1994).
- **TOC (total organic carbon):** The sum of DOC and POC; in this SOP, only DOC (the filtered, NPOC fraction) is measured.

5. Safety and Hazards

All safety requirements from [SOP-L204](#) Section 4 apply.

Additional hazards specific to this method:

⚠ Hazards

- **Chemical:** Hydrochloric acid (2 M HCl) is used for sample acidification and preservation. Avoid skin and eye contact.
- **Glassware:** Pre-combusted glass vials are fragile. Handle with care to avoid cuts.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves

6. Required Materials**6.1. Equipment**

Item	Notes
Shimadzu TOC-L with ASI-L autosampler TNM-L (optional)	Operated per SOP-L204 If simultaneous TN analysis is desired
Vacuum filtration apparatus	Per SOP-L114
Analytical balance (0.001 g)	Per SOP-L200

6.2. Consumables

Item	Notes
Pre-combusted glass vials (40 mL, PTFE-lined caps)	Combust at 450 °C for 4 hours per SOP-L111
Autosampler vials (8 mL or 40 mL)	Pre-combusted or acid-washed
0.45 µm filters (PVDF or PES)	Do NOT use cellulose acetate (Kaplan, 1994)
Syringe filters (0.45 µm, 25 mm, PVDF)	Discard first 2–3 mL of filtrate

6.3. Reagents

Reagent	Notes
KHP stock standard (1000 mg C/L)	2.125 g KHP per 1 L DI water; prepare fresh monthly; store at 4 °C (APHA et al., 2012)
2 M HCl (Trace Metal grade)	For sample acidification and preservation

Reagent	Notes
Deionized water (18.2 MΩ · cm)	Verify DOC less than 0.05 mg/L before each session

! Important

Prepare the 1000 mg C/L KHP stock fresh monthly or verify against a certified reference material (CRM) before use. Degraded stock causes a systematic low bias across the calibration.

7. Sample Collection and Preservation

Proper sample handling is critical; contamination from fingers, plastic containers, and atmospheric CO₂ can bias results (Sharp, 1993).

- Collect samples in pre-combusted glass bottles. Do not use plastic bottles for DOC samples.
- Filter through 0.45 µm PVDF syringe filters within 4 hours of collection; discard the first 2–3 mL of filtrate.
- Acidify filtered samples to pH 2 with 2 M HCl (approximately 100 µL per 40 mL sample) to suppress microbial activity.
- Store acidified samples at 4 °C in the dark; analyze within 28 days (APHA et al., 2012).
- Cap vials immediately after filling to minimize atmospheric CO₂ contamination.

8. Procedure

8.1. Calibration

Prepare a 5-point calibration curve in the expected sample concentration range (APHA et al., 2012):

Level	Concentration (mg C/L)	Preparation
0	0.00	Acidified DI water blank
1	0.50	Dilute from 1000 mg/L KHP
2	1.00	Dilute from 1000 mg/L KHP
3	5.00	Dilute from 1000 mg/L KHP
4	10.00	Dilute from 1000 mg/L KHP
5	25.00	Dilute from 1000 mg/L KHP

Adjust the range to match expected sample concentrations. Load standards into pre-combusted autosampler vials and enter into the Sample Table in LabSolutions TOC.

8.2. Setting Up the Sample Table

1. Open LabSolutions TOC and start a new analysis.
2. Enter calibration standards (type = Standard), blanks (type = Blank), CCV standards, and samples.
3. Set the measurement mode to **NPOC** for all samples and blanks.
4. Set the number of injections per sample to 3; the software reports the mean and coefficient of variation (CV).
5. Enter dilution factors for any samples that have been diluted before the run.

8.3. Running the Analysis

1. Load all vials into the autosampler; verify rack positions match the Sample Table.
2. Confirm the ASI-L rinse reservoir is full.
3. Click **Start** and monitor the first few injections for normal peak shape and baseline stability.
4. The run proceeds unattended; the autosampler advances automatically.

8.4. Data Review and Export

1. After the run, open the Data Browser.
2. Check each result:
 - Replicate injection CV must be less than 2%.
 - Blank values must be less than 0.1 mg C/L.
 - CCV recovery must be 90–110%; re-calibrate and re-run affected samples if outside this range.
3. Dilute and re-run any sample exceeding the calibration range.
4. Export results to CSV per [SOP-L204](#) Section 7.9.
5. Record the run in the instrument logbook.

9. Calculations

The TOC-L software reports NPOC concentration directly in mg C/L, corrected for dilution factor if entered in the Sample Table. Report as DOC:

$$\text{DOC (mg C/L)} = \text{NPOC}_{\text{reported}} - \text{NPOC}_{\text{method blank}}$$

If samples were diluted before analysis, apply the dilution factor:

$$\text{DOC (mg C/L)} = (\text{NPOC}_{\text{reported}} - \text{NPOC}_{\text{blank}}) \times D$$

where D is the dilution factor.

9.1. R Code for Batch Processing

```
# SOP-L311: DOC (NPOC) -- batch calculation from TOC-L CSV export
raw <- read.csv("TOC-L_export.csv", stringsAsFactors = FALSE)

blanks <- raw[grepl("^Blank|^DI|^MQ", raw$SampleID, ignore.case = TRUE), ]
ccv    <- raw[grepl("^CCV|^CVS|^Check", raw$SampleID, ignore.case = TRUE), ]

mean_blank <- mean(blanks$NPOC_mg_L, na.rm = TRUE)
cat(sprintf("Mean method blank: %.3f mg C/L\n", mean_blank))

samples <- raw[!grepl("^Blank|^DI|^MQ|^CCV|^CVS|^Check|^Std",
                     raw$SampleID, ignore.case = TRUE), ]
samples$DOC_mg_L <- (samples$NPOC_mg_L - mean_blank) * samples$Dilution
samples$DOC_mg_L[samples$DOC_mg_L < 0] <- 0
samples$QC_flag <- ifelse(samples$CV_pct > 2, "CV>2%", "")

print(samples[, c("SampleID", "NPOC_mg_L", "Dilution", "DOC_mg_L", "QC_flag")],
      row.names = FALSE)
```

10. Quality Control

10.1. Instrument QC

Per [SOP-L204](#):

- System blank less than 0.1 mg C/L
- Calibration $r^2 \geq 0.999$
- Replicate injection CV less than 2%
- CCV recovery 90–110%

10.2. Method QC

QC Element	Frequency	Acceptance Criteria
Method blank	1 per batch	Less than 0.2 mg C/L
Field blank	1 per sampling event	Less than 0.5 mg C/L
Laboratory duplicate	1 per 10 samples	RPD less than 10%
CRM (when available)	1 per batch	Recovery 90–110%
Matrix spike	1 per 20 samples	Recovery 80–120%

10.3. Common Contamination Sources

Source	Prevention
Fingers touching vial interiors	Always wear gloves; handle by exterior only
Cellulose acetate filters	Use PVDF or PES filters exclusively (Kaplan, 1994)
Detergent residue on glassware	Pre-combust glass; acid-wash and rinse with DI
Atmospheric CO ₂ absorption	Minimize headspace; cap immediately
Degraded DI water	Verify DI DOC less than 0.05 mg/L before each session
Old or degraded KHP stock	Prepare fresh monthly; refrigerate

11. Waste Disposal

Waste Type	Disposal Method
Acidified sample waste	Neutralize and drain, or collect per SOP-L006
Calibration standard waste	Drain (dilute KHP, non-hazardous)
Used syringe filters	Standard trash
Pre-combusted glass vials	Rinse and reuse, or glass recycling

12. References

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- Kaplan, L. A. (1994). Dissolved organic carbon retention by anion exchange resin at alkaline pH and analysis of dissolved organic carbon. *Limnology and Oceanography*, 39(2), 467–471. <https://doi.org/10.4319/lo.1994.39.2.0467>
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- Wetzel, R. G. (2001). *Limnology: Lake and river ecosystems* (3rd ed.). Academic Press.

13. Related SOPs

SOP	Title	Relationship
L204	Shimadzu TOC-L Instrument	Instrument operation, startup, shutdown
L020	Reagent Preparation	KHP stock and standard preparation
L111	Muffle Furnace	Pre-combustion of glass vials
L114	Vacuum Filtration	Sample filtration
L407	Total Carbon (Soils)	SSM solid carbon companion
L409	Microbial Biomass Carbon	CFE extract DOC companion
L418	Water-Extractable DOC	Soil extract DOC companion
L802	Quality Control	QC requirements

SOP	Title	Relationship
F300	Surface Water Sampling	Sample collection and preservation

14. Training Record

Before performing this analysis independently, personnel must:

1. Read this SOP in full
2. Complete instrument training per [SOP-L204](#) Section 14
3. Demonstrate competence preparing KHP calibration standards
4. Demonstrate proper sample filtration technique (PVDF filter, first-rinse discard)
5. Run a complete batch (calibration, blanks, CCV, samples) under supervision with acceptable QC results
6. Be signed off by the PI or instrument manager