

# UVA UV254 Measurement of Waters by Spectrophotometer

Water Analysis Instruments, Thermo Fisher Scientific

## Key Words

Drinking water, source water, UV-absorbing organic constituents, UVA, UV254, 254nm wavelength, UV-vis spectrophotometer, EPA Method 415.3, Standard Methods 5910B, Orion AQ8000, Orion AquaMate, SAC 254, SUVA, Log 137

## Goal

This application note describes how to measure the absorbance of waters, such as drinking water and source water, at 254nm wavelength using a Thermo Scientific™ Orion™ AquaMate™ 8000 UV-vis spectrophotometer.



## Introduction

This method utilizes a spectrophotometer to measure the absorbance of waters, such as drinking water and source water, at 254nm wavelength. These results may be correlated to organic carbon, color, and/or disinfection byproduct precursors. Results can also indicate the efficacy of treatment processes that remove organic carbon, or may be used with a corresponding total organic carbon (TOC) result to calculate the Specific UV Absorbance (SUVA) value for a water sample<sup>1</sup>.

## Equipment

- Orion AquaMate UV-vis spectrophotometer (Cat. No. AQ8000)
- Filtration apparatus
- 0.45- $\mu$ m filters, 47 mm
- Vacuum source – aspirator, air flow or water flow, handoperated or low pressure electric vacuum pump
- UV disposable cuvettes, 1cm, or quartz sample cell, 1 cm, 5 cm, or 10 cm.
- Carousel for 10 cm cell, if needed (Cat. No. AQ100C)
- Orion pH meter and electrode

## Solutions

- Organic-free deionized water (DI)
- Reagents for pH adjustment - Sodium hydroxide, 0.1N, Hydrochloric acid, 0.1N, Orion pH 4.01 and 7.00 Buffers (Orion 910104, Orion 910107)
- Spectrophotometer Check Solution (SCS), **Optional:** Organic Carbon, KHP in pH 7 phosphate buffer, prepared<sup>2</sup> or purchased

## Sample Cell Storage and Cleaning

To help obtain reproducible results, clean and store sample cells per instructions in the Orion AquaMate User Guide.

## Meter Setup

1. Turn on the spectrophotometer.
2. Choose a suitable cell size and method for the organic carbon content expected in your samples. See chart (below).
3. Select the desired cell holder position (1 or 5 cm) by pressing the cell position key.
4. For 10 cm cell, install the carousel with 10 cm holder.
5. Access the USB memory stick using a computer.
6. Copy the desired preprogrammed method from the Orion folder to the **Thermo** folder<sup>3</sup>.
7. Remove the USB memory stick from the computer and insert it into the USB port on the front of the AquaMate spectrophotometer.
8. Select the method and load it.
9. Press “Run Test” to start the analysis. See below for choosing the method.

Sample Concentration	Organic Carbon > 0.5 mg/L	Organic Carbon > 0.1 mg/L	Organic Carbon > 0.05 mg/L
Quartz Cell Size*	1 cm (10 mm)	5 cm (50 mm)	10 cm (100 mm)
Method Name	UV254_1	UV254_5	UV254_10

\*Alternately, use disposable cuvettes formulated for UV measurements, available in the 1 cm (10 mm) cell path.

## Zero the Meter. Spectrophotometer Check

1. Touching only the frosted sides of the cell, rinse a clean cell three times with DI water. Then fill with DI water. Use a lint-free wiper to remove water on the outside.
2. Open the sample compartment and insert the sample cell containing DI water (the blank) into the sample holder, with the clear sides facing front and back. If sample cell is not in the light path, press the correct sample position key. Close the lid, then press the Measure Blank key.

3. If required, test a Spectrophotometer Check Solution (SCS). Using the same cell, empty and fill with the prepared SCS, wipe dry, and insert into the sample holder. Close the lid, and press the Measure Sample key. Record the displayed result.
4. The reading for the SCS should be within the desired criteria, per your QA plan. See Results section for examples.

## Sample Storage

Samples are not preserved. Analyze as soon as possible after collection. Samples may be stored for up to 48 hours at <6°C prior to analysis. See EPA Method 415.3 for SUVA storage<sup>1</sup>.

## Sample Preparation: pH Adjust and/or Sample Filtration

For non-SUVA: If the pH is not between 4 and 10, adjust pH per steps in “pH Adjustment of Sample” (see below). Note: Do not adjust pH for a SUVA determination.

For UVA, UV254: Set up the filtration apparatus with a 0.45µm filter. Wash the filter with 50 mL DI and discard the rinse water. Filter 50 mL of the sample. Test the filtrate.

## Sample Measurement

Ensure the meter has been zeroed properly. Touching only the frosted sides of the cell, rinse the clean cell with a portion of the filtered sample, then fill with the filtered sample. Wipe dry. Insert the sample cell into the holder, close the lid, and press the Measure Sample key. Record the displayed result.

The results can be saved to the USB stick, if desired. If the reading is >0.900 absorbance, dilute and retest. Multiply the reading by the dilution factor. If the results are <0.010 absorbance, consider using a larger cell. Load the appropriate method, and re-zero the meter.



## Quality Control (QC)

Run an SCS and duplicate samples with each batch, or run QC samples per your QA plan. For SUVA testing, follow requirements of EPA Method 415.3<sup>1</sup>.

## pH Adjustment of Sample

**Note:** Do not adjust the pH of a sample which will be used for a SUVA calculation. Proceed to the filtration step, noted above.

1. Calibrate the pH probe in pH 4.01 and 7.00 buffers.
2. Warm the sample up to room temperature.
3. Shake the sample to insure homogeneity.
4. Measure 50 mL of the sample to a 100-mL beaker using a graduated cylinder.
5. Immerse the pH probe in the sample and record the initial pH.
6. Adjust the sample into the range of pH 4 to 10, by adding dropwise 0.1N sodium hydroxide to raise the pH or by adding dropwise 0.1N hydrochloric acid to lower the pH. Different strength acid or base can be used, if needed.
7. Note that the overall volume change should not be greater than 1% (0.5 mL). Discard and re-prepare with stronger acid or base if the volume changes more than 1%.
8. Record the adjusted pH. Proceed to the filtration step.

## Results of SCS Testing on the Orion AQ8000 – 25.0 mg/L Organic Carbon (KHP)

Bias Method UV254_1	Expected (per SM 5910B)	Result (AQ8000)	Difference	Evaluation
Absorbance	0.358 cm <sup>-1</sup>	0.360 cm <sup>-1</sup>	0.002 cm <sup>-1</sup> (0.6%)	Good
Organic Carbon Concentration	25.0 mg/L	24.9 mg/L	0.01 mg/L (0.4%)	Good

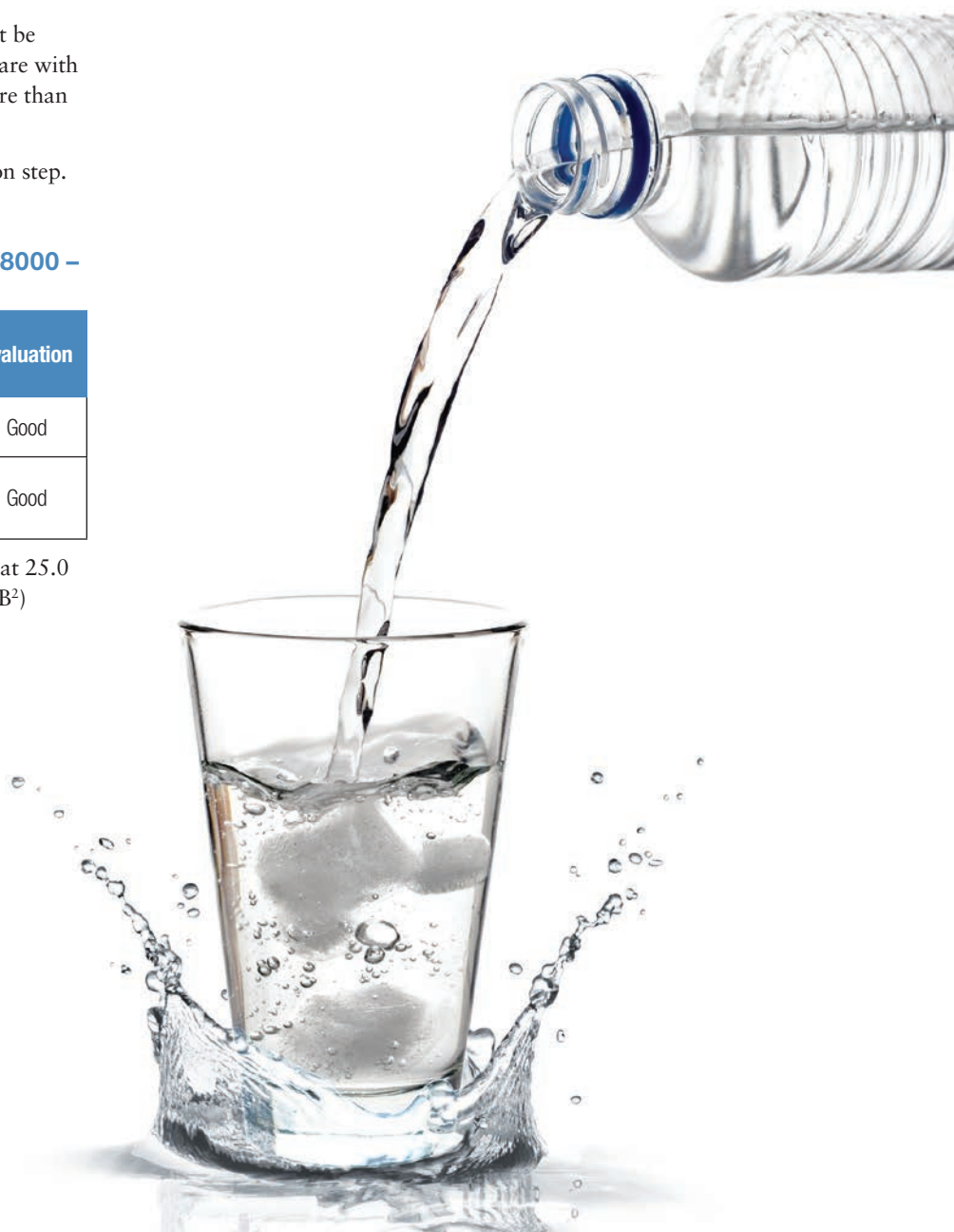
**Bias:** readings of a KHP organic carbon standard at 25.0 mg/L in phosphate buffer (prepared per SM 5910B<sup>2</sup>) tested in a 1 cm cell demonstrate good accuracy:

- The average AQ8000 absorbance result is within 0.002 absorbance units of the average reading expected per SM 5910B, 0.6% difference from the expected absorbance.
- The average AQ8000 organic carbon concentration result (calculated per SM 5910B) is within 0.1 mg/L of the expected value; 0.4% difference (99.6% recovery) from the expected value of 25.0 mg/L organic carbon.

Precision Method UV254_1	# of Samples Tested	Maximum %RSD (per SM 5910B)	Result (AQ8000)	Evaluation
Absorbance	14	< 10.7% RSD	0.3% RSD	Good

**Precision:** readings of a KHP organic carbon standard at 25.0 mg/L in phosphate buffer (prepared per SM 5910B) tested in a 1cm cell demonstrate good precision:

- The relative standard deviation (RSD) of 14 test results on the AQ8000 is 0.3% RSD, well within the maximum 10.7% limit expected per SM 5910B<sup>2</sup>.



## References and Footnotes

<sup>1</sup>EPA Method 415.3 Rev 1.1. UV254 for SUVA. <http://www.epa.gov/microbes/ordmeth.htm>.

<sup>2</sup>Standard Methods 5910B, UV-Absorbing Organic Constituents. [www.standardmethods.org](http://www.standardmethods.org).

<sup>3</sup>If the preprogrammed method is not on the memory stick, download the method file from the WAI Online Library at [www.thermoscientific.com/waterlibrary](http://www.thermoscientific.com/waterlibrary) or contact your local Technical Service group.

To purchase Thermo Scientific laboratory products, please contact your local equipment distributor and reference the part numbers listed below:

Product	Description	Part Number
Instruments	Thermo Scientific Orion AquaMate 8000 UV-visible spectrophotometer	AQ8000
	Thermo Scientific Orion pH meter	Multiple
	Thermo Scientific Orion pH electrode	Multiple
Solutions	Thermo Scientific Orion pH buffers 4.01	910104
	Thermo Scientific Orion pH buffers 7.00	910107
	Unity Lab Services Spectrophotometer Check Solution (SCS), wav photo accessory 0.25A + blank	222-234700
Deionized Water	Thermo Scientific Barnstead water purification systems	Multiple

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