Illinois State Water Survey
Health and Environmental Applications Laboratory

Standard Operating Procedure
For
Measurement of UV absorbance
(based on SM5910 B 2013)

SOP Number: AN.HEAL.IN.UV254

Revision 0.5, effective 8 October 2024

Illinois State Water Survey
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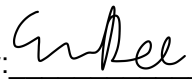
Prepared by:  Date: 12/17/2024
Evan Rea, HEAL Director

Approved by: Margarita Bargon Date: 12/19/2024
Rita Bargon, Quality Assurance Officer

The following individuals (in addition to those listed on the cover) have reviewed this SOP.

Name (printed)	Signature	Date
George Barton	George Barton	01/03/2025
Anthony Kilber	Anthony Kilber	01/03/2025

Beginning Revision	Ending Revision	Revision Date	Changes
-	0.1	10/5/23	SOP created
0.1	0.2	10/26/23	Incorporated comments and changed procedure to include either single wavelength or scan options. Added details on software use and data management.
0.2	0.3	1/19/24	Removed comments, corrected formatting and grammar. Added clarification regarding sample naming convention and QC limits.
0.3	0.4	7/29/24	Corrected QC values, absorbance cutoff values for reanalysis, and cuvette sizes.
0.4	0.5	10/8/24	Update title and procedure for checking over-range samples in section 11. Removed definition for duplicate.

Table of Contents

1.0 Scope and Application

1.1. This method covers the determination of UV absorbance at 254 nm in drinking, ground and surface waters. UV absorbance has strong correlations with organic carbon, color, and precursors of disinfection byproducts. Therefore, UV absorption can be used as an indicator of the presence of organic compounds. A scan between 220 to 600 nm may also be used.

1.2. The applicable absorbance range is approximately 0.005 to 1.3 cm^{-1}.

2.0 Summary of Method

2.1. A filtered sample is analyzed in duplicate using a spectrophotometer to measure UV absorbance at 253.7 nm (rounded to 254 nm). Other wavelengths and/or a scan of multiple wavelengths may also be measured.

2.2. The ideal sample pH is between 4 and 10. UV absorption by organic matter varies outside of this range. Report pH with absorbance results (not covered by this SOP).

3.0 Definitions

HEAL	Health and Applications Laboratory
ISWS	Illinois State Water Survey
MDL	Method Detection Limit
QAP	Quality Assurance Plan
Replicate	A sample that is measured multiple times, using separate aliquots, that are then averaged together for one reportable result.
Triplicate	The entire analytical process, including replicates, carried out on a sample three times, using separate aliquots, to give three reportable results.

4.0 Safety Warnings and Waste Management

4.1 Always wear eye protection in the laboratory.

4.2 Food, drinks, and smoking are not allowed in the laboratory.

4.3 Safety Data Sheets (SDS) applicable to this SOP can be found online by using the University of Illinois Division of Research Safety (DRS) website: https://www.drs.illinois.edu/Programs/SafetyDataSheets.

4.4 The Illinois State Water Survey Chemical Hygiene Plan covers the ISWS laboratory safety program, including, but not limited to, personal protective equipment used, control equipment inventory and operations (such as vented hoods), employee training programs, medical programs, and safety. The ISWS Chemical Hygiene Plan is available at https://go.illinois.edu/ISWS-Chemical-Hygiene-Plan.

4.5 The University of Illinois DRS has a laboratory safety plan available at https://www.drs.illinois.edu/site-documents/LaboratorySafetyGuide.pdf. The ISWS has their own laboratory safety manual, available at https://go.illinois.edu/ISWS-Laboratory-Safety-Manual.

4.6 The HEAL practices pollution prevention, which encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. The quantity of chemicals purchased should be based on the expected usage during its shelf life and disposal cost of unused material.

4.7 Laboratory waste management practices must be consistent with all applicable rules and regulations. Excess reagents and samples and method process wastes should be characterized and disposed of by DRS. It is the responsibility of the user of this method to comply with relevant disposal and waste regulations.

4.8 The HEAL has listed known health and safety warnings for this SOP, but this list should not be assumed to comprise all health and safety issues.

5.0 Cautions

5.1 The HEAL has listed known cautions for this SOP, but this list should not be assumed to cover all issues.

6.0 Interferences

6.1 Turbidity and UV absorbing inorganics will interfere, such as iron, nitrate, nitrite, and bromide. Some oxidants and reducing agents can also interfere, such as ozone, chlorate, chlorite, chloramines and thiosulfate.

6.2 Take care to handle the quartz sample cell to avoid any fingerprints and water spots. Clean and wipe the cuvette to remove markings that may interfere with absorption.

6.3 Check the cell for air bubbles. Tap the cell lightly on a padded surface to dislodge any trapped air bubbles.

6.4 Handle cuvettes carefully to avoid scratching their surfaces. If a cuvette develops a scratch, notify the Laboratory Director and use a different cuvette.

7.0 Personnel Qualifications

Analysts in training must complete at least five days of training, and a satisfactory demonstration of capability before analyzing routine samples.

8.0 Equipment and Supplies

8.1 Equipment

8.1.1 0.45 µm filtration device and 45mm 0.45 µm polyethersulfone (PES) filters. A glass fiber filter (934AH or equivalent) may also be used as a pre-filter.

8.1.2 Thermo Scientific Genesys 10S UV-Vis Spectrophotometer.

8.1.3 Sample cuvettes – 1cm and 5cm quartz cells.

8.1.4 Computer, running the VisionLite Software (Version 5.0).

8.1.5 Syringe barrels, 25 to 50 mL, to fit filtration device

8.2 Reagents and Standards

8.2.1 Method blank - Reagent water must be ASTM II or equivalent and free of organics. The HEAL uses 0.2 µm filtration on all deionized water during the treatment process. Additional purification is done using a benchtop water purification unit ('polisher'). Acceptable reagent water for use in this method should read < 0.010 cm^{-1} with an ideal absorbance of < 0.0045 cm^{-1} at 254 nm.

8.2.2 Organic carbon stock solution (1,000 mg/L) – dissolve 2.1254g anhydrous primary standard grade potassium hydrogen phthalate (KHP) ($C_8H_5KO_4$). Alternatively, a commercially prepared solution can be purchased. Stock purchased from Lab Chem, Catalog Number LC129101.

8.2.2.1 0.5 mg/L KHP working solution/ ICV – Pipette 0.50 mL of 1,000 mg/L KHP stock solution into a clean 1L volumetric flask containing ~300 mL of DI water. Then, dilute to the 1L mark with DI.

9.0 Instrument Setup and Calibration

9.1 Turn on the computer and spectrophotometer.

9.2 Navigate to the computer directory where the results will be saved: UV folder, then select the current year's folder.

9.3 Make a new folder inside the 'year' folder with the name "YYMMDD", which should be the analysis date. This is where the results will be saved.

9.4 Open VisionLite software and select the scan option to scan a range of wavelengths, or select the fixed option to measure at one specific wavelength.

9.5 Select the preferences tab and choose the newly made folder with the analysis date. In this same screen, ensure that the 'methods' directory is correctly navigating to the location of the desired method file. The method file needs to match the mode that was

selected (e.g., a scan method for a scan analysis). The most current scan template file should be used, which is for the wavelengths 220 to 600nm.

9.6 Fill cuvette with fresh DI and measure the baseline.

9.7 Then select the measure button and then check the 'measure blank' box. This will enable a zero/ blank reading to be measured first. Use fresh deionized water for the blank.

Sample names should include the XLIMS sample ID with identifiers added in the name to designate the sample type and run number. Use the "InstSeqGen" spreadsheet to generate the sample IDs. IDs should follow this convention:

For CCBs: 011824-020-CCB-01(1)
For CCVs: 011824-020-CCV-01(1)
For samples: 2401041-002(1)
For first duplicate: 2401041-002 DUP(1)
For second duplicate (triplicate): 2401041-002 DUP(2)

Additionally, each sample needs to have an identifier to distinguish between the two replicates. Copy/paste the sample ID for the sample to be measured from the spreadsheet into the 'measure' window, then type a space then a "1" or "2" to designate the first or second replicate. For example:

First replicate: 2401041-002(1) **1**
Second replicate: 2401041-002(1) **2**

9.8 Take measurements of a blank and confirm the mean blank absorbance should be less than 0.010 cm^{-1} at 254 nm. If it is not, then retest the blank again. If the blank is still high, then see section 12 for troubleshooting steps.

NOTE: when evaluating absorbance values (e.g., blanks and KHP standard), use the absorbance data for 254 nm.

9.9 Next, run the KHP working solution and confirm the reading is 0.009 ± 0.003 cm^{-1} at 254nm. If it is outside of this range, retest a new aliquot. If it is still out of range, then see section 12 for troubleshooting steps.

9.10 If the KHP solution passes, then proceed with analyzing samples (see section 11).

10.0 Sample Collection, Handling and Preservation

10.1 Sample Collection

10.1.1 Samples should be collected in amber glass bottles. At least 50mL sample volume should be collected to ensure a representative sample, allow for replicate analysis, and

minimize waste disposal.

10.1.2 To ensure clean, organic-free supplies, bottles should be acid washed then rinsed thoroughly with DI. Then they should be baked at 450°C for at least 2 h. Caps should be acid washed then rinsed with DI.

10.2 Sample Handling

10.2.1 Ensure samples are stored in amber glass bottles to avoid contamination from the container.

10.3 Sample Preservation and Holding Time

10.3.1 Samples should be filtered and stored at <6°C and analyzed within 48 h.

11.0 Sample Preparation and Analysis

11.1 Filter a sample directly into the cuvette, rinsing it once with filtrate before filling it.

Note: If a sample is filtered in the field, then it does not need to be filtered in the laboratory.

11.2 Place the cuvette into the holder in the spectrophotometer, then close the cover.

Note: Be sure there are no air bubbles inside the cuvette, water droplets on the outside, or scratches anywhere on the cuvette. See section 6.

11.3 Record the absorbance value from the spectrophotometer by pressing ‘measure’ in VisionLite.

In the window that comes up, type in a unique file name with the date of analysis. Type in a sample ID (see section 9.7 for sample naming convention) and press ‘measure’. Analyze each sample in duplicate, meaning that two portions of filtered sample are measured for each sample.

NOTE: If a sample series was created, then the sample name will be pre-populated, but it can still be changed. After measurement, the software will automatically increment to the next sample in the list.

11.3.1 Repeat 11.1 to 11.3 for each sample, being sure to measure each sample twice.

11.4 After every 10^{th} sample, measure a sample triplicate, a blank, and the KHP solution. Triplicate analysis means triplicating the entire sample analysis process (i.e., measure two filtered portions for the initial sample, and then measure two portions of the same sample again for a duplicate, then again for a triplicate).

11.5 After each scan using the 5cm cuvette, check the chart to see if the plotted line exceeds 1.3 abs at any wavelength. If so, then the sample will need to be re-analyzed using the 1cm cuvette. Make a note of which samples need to be re-analyzed and set them aside until all samples have been analyzed with the 5cm cuvette.

11.6 Once all samples have been measured with the 5cm cuvette, gather all the samples that were >1.3 abs and re-measure them using the 1cm cuvette. After each scan using the 1cm cuvette, check the absorbance at 254nm: if it exceeds 1.3 abs, then re-analyze the sample using a dilution factor to bring the absorbance value below 1.3 abs at 254nm.

11.7 Once the run is finished, select the file tab and select the save as option. Choose the .CSV file folder and change the file type to .csv. The folder will need to be reselected for each sample and QC run.

NOTE: If a sample series was used, then the graphical display should have a list of sample IDs in the legend. Click any sample name and a window will open that will allow data exporting. Check the 'for all' button and save the data as a CSV file. This will generate a CSV file for each sample with the sample name as the file name.

11.8 Copy the CSV files into the analytical batch report for UV254.

12.0 Troubleshooting

12.1 If the blank is reading >0.010 cm^{-1} at 254nm, then clean the cuvette and syringe barrel with HCL and rinse copiously with DI. Alternatively, discard the syringe barrel and filter(s) and use a new one. Collect a new aliquot of DI from a different water polisher in a clean vessel and retest. If values are still elevated, then discuss with the HEAL Director.

12.2 If the KHP standard is outside of the acceptable range, then follow the steps in 12.1 except using the standard instead of DI. If this does not bring the readings within the limits, then remake the KHP standard and retest. Contact the HEAL Director for additional assistance.

12.3 If interferences are suspected, then a scan from 220 to 400 nm can be used to detect irregularities. Typical scans of natural organic matter are featureless curves of increasing absorbance with decreasing wavelength. Sharp peaks or irregularities may indicate inorganic interferences.

12.4 If the absorption of the blank decreases throughout the analysis, clean the cuvette and re-measure a blank. Then, re-analyze a sample from earlier in the analysis to compare values and see if the cleaned cuvette is giving different results. If so, the earlier samples will need to be re-analyzed.

12.5 Refer to the Genesys 10S UV-Vis Spectrophotometer User Manual for more

troubleshooting help.

13.0 Data and Record Management

13.1 Report mean UV absorption in cm^{-1} following the equation below:

$$UV_{\lambda}^{pH} = \left(\frac{\overline{A}}{b}\right) D$$

Where:

UV_{λ}^{pH} = mean UV absorption, cm-1, subscript denotes wavelength used (254 nm) and superscript denotes pH

$\overline{A}$ = mean replicate absorbance measured

b = cell path length, cm

D = dilution factor (D = final volume / initial volume)

13.2 % Relative standard deviation (RSD) formula:

$$\%\, RSD = \left(\frac{standard\ deviation}{mean}\right) * 100$$

13.3 Relative percent difference (RPD) formula, where D1 and D2 are the average replicate readings of each duplicate sample.

$$RPD = \frac{|D1-D2|}{\frac{D1+D2}{2}} * 100$$

13.4 Record and save all electronic data, and then upload files to LIMS.

13.5 Make any notes or comments in the laboratory notebook.

14.0 Quality Control and Quality Assurance

14.1 Each sample must be measured twice, using two separate portions.

14.2 At the beginning of the analysis, analyze the 0.5 mg/L KHP standard. It should read within the range 0.009 ± 0.003 cm^{-1} at 254nm. If it is outside of this range, retest a new aliquot. If it is still out of range, then see section 12 for troubleshooting steps.

14.3 After every 10th sample, analyze a triplicate sample. For UV values >0.045 cm^{-1}, the RPD limit is ≤10%. For UV values <0.045 cm^{-1}, the limit is ≤20%. Evaluate triplicate RPD at 254 nm and 280 nm.

14.4 After every 10th sample, analyze a blank. The mean blank absorbance should be <0.010 cm^{-1} at 254 nm. If it is not, then retest the blank again. If the blank is still high, then see section 12 for troubleshooting steps.

14.5 For each batch of filters, analyze a filter blank using the same amount of DI as you would use for a sample. The filter blank should read <0.010 cm^{-1} at 254 nm. If the blank reads >0.010 cm^{-1} at 254 nm, then increase the volume of DI filtered.

NOTE: this step is not needed if samples are filtered in the field.

14.6

Table 14-1. QC summary table.

Type	Frequency	Criteria @ 254 nm
Blank	At beginning of analysis and after every 10^{th} sample	± 0.010 cm^{-1}
ICV	Beginning of analysis	0.009 ± 0.003 cm^{-1}
Triplicate	One per batch of 20 samples	±10%

14.7 Corrective Action

14.7.1 Further corrective actions for nonconforming quality control samples are given the Corrective Action Policy (AD.HEAL.0.CorrectiveAction) and in the HEAL QAP.

15.0 References

Standard Methods for the Examination of Water and Wastewater, 23rd edition. (2017). Part 5910 UV-Absorbing Organic Constituents: 5910 B Ultraviolet Absorption Method (Approved by Standard Methods Committee, 2013). A.D. Eaton, R.B. Baird, E.W. Rice (Eds.). Washington, DC: American Public Health Association.

Rea, E. Quality Assurance Plan for the Health and Environmental Applications Laboratory (HEAL). 2023. Illinois State Water Survey, Champaign, IL.

AN.HEAL.IN.UV254.0.5

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