Analysis of UV Absorbance

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As performed at the University of Massachusetts, Environmental Engineering Research Laboratory

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Standard Operating Procedures

Analysis of UV Absorbance

This guidance document was prepared to assist research assistants, post-docs and lab technicians in conducting analysis of UV absorbance and related light absorption methods in the UMass Environmental Engineering research laboratories. It aspires to outline our standard operating procedures, as they exist at the present time. It also emphasizes elements of quality control that are necessary to assure high quality data. Please help me keep this document current by alerting me to any errors or long-term changes in methodology or equipment. Special thanks to Guanghui Hua for help with preparation of version 1.

Dave Reckhow
Faculty QC officer for UV absorbance analysis

Scope

This method has been used in the UMass Environmental Engineering Laboratory for analysis of UV absorbance and related methods in clean water samples (i.e., drinking water, surface waters and uncontaminated groundwaters). It has been found to meet data quality criteria with all waters of these types for which it has been tested. This group of methods should not be used for other media without further validation.

Method Overview

Reproduced below is a simple, step-by-step outline of our TOC method for quick reference.

Table 1: Summary of Procedure for UV Absorbance Analysis

1. Filter samples if necessary
2. Turn on spectrophotometer and start log book record
3. Prepare **QC samples** (Table 2)
4. Load samples and read absorbance
5. Record sample identities in log book
6. Inspect data

Table 2. Typical Preparation of and testing of QC Samples

1. Prepare a 10 mg-C/L standard solution of Potassium Acid Phthalate (KHP). This can be the same standard used for calibrating the TOC analyzer.
2. Measure absorbance at 254 nm, and other wavelengths if desired
3. Absorbance should be 0.144 cm^{-1} at 254 nm.
4. Prepare any other QC samples as needed (see Table 5, page 25).

Detailed Procedures

Basis for Method

We use a protocol that is closely aligned with Standard Method 5910B, "Ultraviolet Absorption Method". Please refer to the latest version of this method (currently from the 20th edition, dated 1999; attached as Appendix 1) for all details. However, the analyst should keep in mind that we have occasionally made some specific modifications. Such modifications are itemized below in Table 3.

Table 3. UMass Protocol Specifics and Departures from Standard Method 5910B

§ from 5910B	Step or Apparatus	5910B protocol	UMass protocol
	Wavelength	253.7 nm	254 nm or full scan

UMass Detailed Procedures

Sample Preservation and Pretreatment

1. **Filter, unless sample is known to be free of particulate matter.**
   a) Assemble and clean the filtration apparatus
      - May be either glass Millipore-type or in-line syringe filter
   b) Pre-wash a Whatman GF/C glass fiber filter
      i. Place filter in housing
      ii. Rinse with 50 mL deionized water; repeat 5 times
      iii. If practical, final rinse with 10 mL of sample
   c) Groundwaters with high iron concentrations must be kept free of oxygen and analyzed immediately
      - This usually requires collecting samples under N_2, filling bottles headspace-free and transferring & filtering under N_2 (usually with syringe filters)
      - Separate Iron measurement must be made on the filtered sample so that the sample data can be adjusted for the UV absorbance of iron.

2. **If necessary, adjust pH to between 4 and 10 with one of the following buffers.**
   - Sufficient phosphate ($H_3PO_4/H_2PO_4^-/HPO_4^{-2}/PO_4^{-3}$) to bring the pH to between 4 and 10
     - Avoid high concentrations (i.e., above 100 mM)
     - Minimize dilution of the sample (
     - General buffer of choice, unless precipitate forms
   - A few drops of 6 N HCl or 40% NaOH
     - More dilute HCl or NaOH may be used
     - Use these reagents when:
       - waters are extremely alkaline/acidic, or
       - absorbance scans near the peak for phosphate are being conducted, or
       - use of phosphate causes formation of a precipitate

3. **Place aqueous samples in a refrigerator until analysis.**
   - Samples should be analyzed as soon after collection as possible, but under no circumstances should more than 7 days be allowed to elapse.

Selection of Spectrophotometer

In the environmental engineering laboratory (Elab II), we have many spectrophotometers, most of which are capable of measuring absorbance in the UV region. Ultraviolet absorbance cannot be measured on the Spectronic instruments, which do not have UV lamps and UV optics. Otherwise, the selection of an analyzer is largely a matter of convenience and availability

Those wishing to get a full spectrum will most likely use the HP 8453 diode array spectrophotometer. If this isn't needed, the Hach DR 4000 and the Genesys 10UV are also good candidates.

UMass Environmental Engineering: Vis and UV/Vis SPECTROPHOTOMETERS[a]

Characteristic	Bausch & Lomb to Milton Roy to:			ThermoSpectronic; ThermoElectron		Perkin-Elmer		Hewlett-Packard		Hach
	Spec 20	Spec 21D	Spec 70	Genesys 20	Genesys 10UV	Lambda 3A	Lambda 3B	HP 8452	HP 8453	DR/4000U
Location & condition[1]		Marst 24	3rd fl storage	Marston 24	304 Elab II 213 Elab II	308 Elab II 24 Marston	24 Marston	301 Elab II	308 Elab II	304 Elab II
Optical system	Single beam	Single beam	Single beam	Single beam	Split beam	DoubleBeam	Double Beam	Diode Array	Diode Array	Single Beam
Monochromator	Grating			Grating	Grating	Holographic Grating	Holographic Grating	Holographic Concave Grtn.		Seya-Namioka split-beam
Groove Density	600/mm			1200/mm	1200/mm	1440/mm	1440/mm			1200/mm
Detector	Phototube			Solid state		Photo-multiplier	Photo-multiplier	328 Photo-diodes		
Lamp(s)	Tungsten			Tungsten	Xenon	Tungsten-Br Deuterium	Tungsten-Br Deuterium	Deuterium[b]		Tungsten, Deuterium
Readout	Analog	Ditigal	Analog	Digital	Digital	Digital	Digital	Digital		Digital
Cell Holder	0.5-1" tube			1 cm & tubes		0.1-10cm	0.1-10cm	0.1-10cm		1-10 cm
Wavelength Range	340-625 nm*	Vis	Vis	325-1100nm	190-1100nm	190-900 nm	190-900nm	190-820nm		190-1100nm
Wavelength Accuracy	2.5 nm			2.0 nm	1 nm	0.5 nm	0.3 nm	2 nm		1 nm
Wavelength Precision	1.0 nm			0.5 nm	0.5 nm	0.2 nm	0.1 nm	0.05 nm		0.1 nm
Effective Bandwidth	20 nm			8 nm	5 nm	<2 nm	1 nm	2 nm		4 nm
Photometric Accuracy	2.5 %T			0.003 A; 1% (0.3A up)	0.5% T	0.3 %T[d], 0.005 A.	0.3 %T[d], 0.005 A	0.3 %T[d], 0.005 A		
Photometric Precision	1 %T					0.15 %T[d], 0.002 A	0.15 %T[d], 0.002 A			0.001 A
Stray Light	< 0.5%+			<0.1%T	<0.1%T	<0.05%	0.02%	<0.05%		<0.05%T
Baseline Flatness						0.005 A	0.002 A	0.001 A		
Noise				<0.002 A	<0.002 A	<0.0005 A	<0.0003 A	<0.0002 A		
Zero Abs Stability				<0.003A/hr	<0.001A/hr	<0.0005A/hr	<0.0005A/hr	<0.001A/hr		
Serial #										

NOTES:

[1] Green=good operating condition; blue=some operational problems; red=currently not operating

[a]Special Features
Lambda 3B - Buffer and Non-volatile Memory
Diode Array - Simultaneous spectra every 0.6 sec, microprocessor controlled, qualitative and quantitative functions, kinetics functions
[b]Plasma Discharge Deuterium lamp
[*]Accessory phototubes extend range to higher wavelengths (400-715 or 625-950)
[+]Requires optional stray light - second order filters (these fit directly in the filter holder), otherwise stray light may be higher
[d]at 1.0 Abs unit
All accuracies and precisions, as well as baseline flatness is - the indicated value

Startup of Spectrometers

HP 8453 Diode Array Spectrophotometer [2]

4. **Begin Entry in Log Book**
   - Record you name, date, number of samples, types of samples
   - Also record any problems or unusual performance noted
5. **Turn on the Computer and Spectrometer**
   a) Turn on Computer
      - log on as "user"; password is left blank
   b) Maximize CAG BootP Server screen
   c) Turn on Spectrophotometer
      - It then starts to go through checks and diagnostics; clicking of shutters etc.
   d) Watch CAG BootP Server screen as it establishes communication with the spec. Once it ends its documentation (last line should read "BootP request finished processing of outer most layer"), you can go on to the final step which is to:
   e) Launch ChemStation ("Instrument 1 online" icon).
      - Password is left blank also.

6. **Wait for lamp output to stabilize**
   a) Usually takes ~20 minutes
      - You can run repeat scans on the same sample to watch it stabilize

Hach DR 4000 Spectrophometer[3]

7. **Begin Entry in Log Book**
   - Record your name, date, number of samples, types of samples
   - Also record any problems or unusual performance noted
8. **Turn on the Spectrometer**
   b) Turn on Spectrometer
   c) The initialization of the Spectrometer takes about 5 mins.
   d) After the spectrometer is initialized, select "single λ" option, then select " go to λ", enter 254.
   -

Genesys 10UV Spectrophotometer

1. **Begin Entry in Log Book**
   - Record your name, date, number of samples, types of samples
   - Also record any problems or unusual performance noted

[2] This must be done at least 1 hour before starting sample analysis
[3] This must be done at least 1 hour before starting sample analysis

2. **Turn on the Spectrometer**
   a) Turn on Spectrometer
   b) The initialization of the Spectrometer takes about 5 mins.
   c) After the spectrometer is initialized, select "set nm" option, enter 254.

Analysis of samples/standards[4]

HP 8453 Diode Array Spectrophotometer

3. **Zero the Spectrophotometer**
   a) Fill cuvette with lab reagent water
   b) Click on "zero" with mouse
   c) Replace contents of cuvette with another volume of reagent water
   d) Click on "scan"
   e) If absorbance at the wavelength of interest (e.g., 254 nm) is within 0.001 of zero, proceed to sample measurement
   f) If absorbance is greater than 0.001 or less than -0.001, re-zero the spec with this volume of water and repeat these steps starting with (c).
4. **Measure the first sample**
   a) Fill cuvette with sample
   b) Click on "scan" with mouse
   c) Record absorbance
      iv. Write number in lab notebook, or
      v. Make sure scans are saved and not overwritten
   d) Scanned data can be stored as "wav" files.
      - The "wav" files are importable to MS Excel as comma delimited data; one column of absorbance numbers for each nm of wavelength, and one column of standard deviations
5. **Write down in the log book, sample number and sample identity**
   -
6. **Inspect the first few scans**
   - make your first QC report by email (see "Data Analysis" below).
7. **Finish the absorbance run and Process data when analysis is complete.**

[4] typically requires 10 hours of analyzer time for a run of 30 samples

Hach DR4000 Spectrophotometer

1. Zero the Spectrophotometer

e) Fill cuvette with lab reagent water
f) Select "zero" to zero the spectrophotometer
g) Replace contents of cuvette with another volume of reagent water
h) Record the absorbance
i) If absorbance at the wavelength of interest (e.g., 254 nm) is within 0.001 of zero, proceed to sample measurement
j) If absorbance is greater than 0.001 or less than -0.001, re-zero the spec with this volume of water and repeat these steps starting with (c).

2. Measure the first sample

k) Fill cuvette with sample
l) Record absorbance
vi. Write number in lab notebook

8. Write down in the log book, sample number and sample identity

9. Inspect the first few scans

- make your first QC report by email (see "Data Analysis" below).

10. Finish the absorbance run and Process data when analysis is complete.

Genesys 10UV Spectrophotometer

1. Zero the Spectrophotometer

a) Fill cuvette with lab reagent water
b) Select "measure blank" to zero the spectrophotometer
c) Replace contents of cuvette with another volume of reagent water
d) Record the absorbance
e) If absorbance at the wavelength of interest (e.g., 254 nm) is within 0.001 of zero, proceed to sample measurement
f) If absorbance is greater than 0.001 or less than -0.001, re-zero the spec with this volume of water and repeat these steps starting with (c).

2. Measure the first sample

a) Fill cuvette with sample
b) Record absorbance
i. Write number in lab notebook

11. Write down in the log book, sample number and sample identity

12. Inspect the first few scans

- make your first QC report by email (see "Data Analysis" below).

13. Finish the absorbance run and Process data when analysis is complete.

Spectrophotometer Shut-down procedure

HP 8453 Spectrophotometer

1. **Turn off Analyzer**
   a) As soon as the day's run is complete turn off lamp with the computer software
      - Its important to safe lamp life
   b) Turn off switch on spectrophotometer
2. **Turn off Computer.**
      - Computer can be left on for longer periods of time
      - If you choose to shut down right away, do the following:
   a) End ChemStation program
   b) Shut down computer.

Hach DR4000 Spectrophotometer

1. **Turn off Analyzer**
   a) As soon as the day's run is complete turn off switch on the back of the spectrophotometer
      - It is important to safe lamp life

Genesys 10UV Spectrophotometer

1. **Turn off Analyzer**
   b) As soon as the day's run is complete turn off the switch on the back of the spectrophotometer
      - It is important to safe lamp life

Data Analysis & QC Reporting

1. **Assessment of KHP Standards**
   - Prepare a 10 mg-C/L standard solution of Potassium Acid Phthalate (KHP). This can be the same standard used for calibrating the TOC analyzer.
   - Measure absorbance at 254 nm, and other wavelengths if desired
   - Absorbance should be 0.144 cm^{-1} at 254 nm

2. **Replicate measurement**
   - Use at least two portions of filtered sample.

3. **Check baseline absorbance**
   - Lab reagent water is used to check the baseline absorbance every 10 samples. A non zero absorbance reading for the blank may indicate need for cell cleaning and problems associated with variation in the spectrometer response.

4. **Transfer sample absorbance data to spreadsheets**

   The UV spectral data can be imported and parsed as follows:

   a) Launch MS Excel with a blank worksheet
   b) Open the wav file from within Excel
   c) The text import wizard will appear; in step 1 allow it to be delimited by characters (probably the default)
   d) Go to next screen (step 2) and check the "comma" box for the delimiter
   e) Click on next screen, and then "finish"
   f) You'll see that the data have been inserted into two vertical columns starting on row #9. The left column has the absorbance values, and the right column has standard deviations (SD). The first 8 rows contain information that you'll probably want to delete. The 8th row indicates the starting wavelength and the ending wavelength. You should note what these are. In most cases they will be 190 and 1100.
   g) Insert a column to the left for the wavelength numbers. Type 190 (or whatever the starting wavelength is from the 8th row) in the cell of this new column that contains the first row of absorbance/SD data. You can have Excel automatically fill in the rest of the wavelength numbers by going to Edit, Fill, Series" (with the cursor on the cell with 190 in it). Select "series in *columns*", keep the "series type" as linear, keep the "step value" as 1, and type in 1100 (or whatever the end wavelength is in row 8) as the "stop value".

Clean Up

- All glassware used for UV absorbance measurement must be "organic-free"

    - All the vials and any glassware should be rinsed with tap water, let them soak in soapy water overnight and then rinse with RO water and leave them in the acid bath overnight. Then take them out, rinse them with distilled water (3 times) and once with super Q water. Dry them in the oven (The graduated glassware should be placed in the cooler oven)
- Pipette tips may be rinsed with water and super Q water, and let them dry
- Acid baths must be cleaned and refreshed on a weekly basis

Standard Solutions, Solvents and Supplies

Preparation of Calibration Standards[5]

5. **Prepare Stock Primary Standard.**
   a) Dry about 0.75 gm of Potassium Hydrogen Phthalate in oven at 103-110°C for 30 min. Cool in desiccator for 20-30 min.
   b) Weigh exactly 0.5314 gm using analytical balance. Add to a 250 mL volumetric flask and fill to mark with Super-Q water.
   c) Result is stock primary standard (1000 mg/L).
   d) Store in an brown glass bottle. Label with your name, the date and "1000 mg/L KHP standard." Note: 1 mL = 1 mg TOC.
   e) Store in refrigerator. Discard after 2-3 weeks.

6. **Prepare intermediate standard.**
   a) Prepare on the day KHP samples will be analyzed.
   b) Make a volumetric dilution of the Stock Primary Standard. Transfer exactly 1 mL of the stock primary standard with a volumetric pipette to a 100 mL volumetric flask half filled with Lab DI water. Fill to mark with DI water.
   c) Intermediate stock concentration is 10 mg-C/L. Store in refrigerator. Discard after 2 days.

Supplies

Table 4. Summary of Supplies for UV Absorbance Analysis

Item	Catalog #	Approx. Price	Approx # used/run[6]
Pasteur Pipettes	Fisher: 13-678-20A	720/ $46.10	10
Cuvettes (1-cm)			
Ink Cartridges	Fisher: 07-684-61	$15.20 each	
DIUF Water	Fisher: W2-20	$32.29	Not normally used
H_2SO_4	UMass Stockroom		34 mL
Potassium Acid Phthalate			

[5] typically requires 20 minutes
[6] Assuming about 30 samples analyzed

Maintenance and Troubleshooting

Regular Maintenance Activities

- Clean cuvette windows with detergent and DI water. Acid rinse may be needed for obvious residue

Troubleshooting

Past difficulties have resulted from:

- Loss of signal due to bad or exhausted lamp
    - Replace lamp

For further assistance:

- Consult spectrophotometer manuals
- Call manufacturer's technicians (800-829-4444)

Quality Assurance/Quality Control

General Approach

Quality assurance is an essential and integral part of a research study. The purpose of any QA plan is to insure that valid and reliable procedures are used in collecting and processing research data. The procedures outlined are designed to eliminate or reduce errors in experiments, sample preparation and handling, and analytical methods. Emphasis must be given throughout one's lab work to incorporate the plan into the research project by all research personnel.

Any equipment and experimental procedures that are used to provide numerical data must be calibrated to the accuracy requirements for its use. Records are to be kept of all calibrations. Calibration schedules are generally established for all aspects of physical and chemical measurements and these must be strictly followed. Physical standards and measuring devices must have currently valid calibrations, traceable to national standards. Most chemical standards are acquired from commercial suppliers, and they should be of the highest purity available. When necessary, unavailable standards should be synthesized using the best methodology available.

As a general rule, experiments should be replicated to assure reproducibility. All data reported should include a statement of its uncertainty, and the means for the determination and assignment of such limits. Standard reference materials are used for this purpose where possible. Statistically established confidence limits and an analysis of sources of systematic error are to be used in the absence of experimental demonstration of limits of inaccuracy.

All data will be subject to review by the faculty QC officer before release. The analysts involved will certify reports as well as all who review them. All analysts and QC officers must attest that the data and associated information contained in the report are believed to be correct and that all quality assurance requirements have been fulfilled, unless exceptions are approved and noted. Careful and detailed laboratory records will be maintained by each analyst, including source of reagents, meticulously detailed procedures (referring to an SOP, and any departures or clarifications), instrument and conditions of analysis, failed experiments, etc. Data output will be archived.

Regular meetings will be held to review the results and project progress, and to plan further experiments. The results will be analyzed promptly and summarized by means of internal reports or formal reports for external review. The experimental and analytical procedures will be reviewed for their performances and changes will be made as necessary. The quality assurance program as described in this document must be strictly observed.

Quality Assurance Objectives

The precision, accuracy and method detection limits will be evaluated, and where there are existing methods, held within the control limits set forth in the accepted references (e.g., APHA et al., 1999; USEPA-EMSL, 1990; ASTM, 1994). In addition to the analysis of sample replicates, a minimum of 10 percent of the time is typically involved in analytical determinations that are devoted to quality control. The precision of each test is determined through analysis of sample replicates. These are commonly presented in the form of control charts (e.g. Section 1020B of APHA et al., 1999).

The accuracy of each analysis will be determined by measuring spike recoveries in the matrix of interest. The relative errors will be calculated and will be considered acceptable if they fall within the control limits determined for the particular test. For new methods developed at UMass or for modifications of existing methods, we will have to establish criteria on acceptable control limits. In general, a test will not be deemed useful if its precision or accuracy is found to be equal to or greater than 20% of the highest values observed. Where possible, external performance standards will also be run. This serves as a measure of accuracy both for the analysis and for standard preparation.

Data generated by the QA program will be incorporated into a Quality Control (QC) archive that is used to monitor the fluctuations in precision and accuracy so that chance or assignable causes of error can be determined. Control charts such as X-charts for simple successive samples or cumulative sum techniques may be employed to record both precision and accuracy data (Taylor, 1987).

General Procedures

General sample collection and handling will be in accordance with the guidelines of Section 1060 of Standard Methods (APHA et al., 1999). All previously established analytical methods used in laboratory research will follow approved methods in the standard compilations (e.g., ,APHA et al., 1999; USEPA-EMSL, 1990, or ASTM, 1994).

Reagent grade chemicals or higher quality when needed will be used throughout the research. Super-Q water (purified by reverse osmosis, deionization, and carbon adsorption) will be used for preparation of reagents, sample blanks, and dilution water. Where necessary, this water will be further purified using batch UV irradiation. All glassware used in the experiments and in analytical analyses will be thoroughly cleaned with a chromium-free sequence of detergent, oxidant and acid to prevent interferences from trace contaminants.

Data Quality Indicators

A wide range of data quality indicators are normally calculated for the purpose of assessing method performance. Some of these are defined below.

Precision

Precision may be expressed as the relative percent difference (RPD) from duplicate measurements (C_1 and C_2) of the same sample:

$$RPD = \frac{|C_1 - C_2| x100\%}{(C_1 + C_2)/2}$$

When three or more replicates are available, the relative standard deviation (RSD) should be used:

$$RSD = \left(\frac{s}{\bar{y}}\right) x100\%$$

where the standard deviation (s) is determined from:

$$s = \sqrt{\sum_{i-1}^{n} \frac{(y_i - \bar{y})^2}{n-1}}$$

Accuracy

Accuracy is best assessed by analysis of a standard reference material (SRM) prepared in the matrix of interest. It is quite rare that such materials are available, so two possible compromises may be used instead. These are the laboratory-prepared matrix spikes, and the independent SRM prepared in a standard matrix. One or both may be analyzed and the percent recovery (%R) calculated as a measure of accuracy.

$$\%R = \left(\frac{S - U}{C_{sa}}\right) x100\%$$

where:

S = measured concentration in spiked aliquot

U = measured concentration in unspiked aliquot

C_{sa} = actual concentration of spike added

$$\%R = \left(\frac{C_m}{C_{srm}}\right) x100\%$$

and:

C_m = measured concentration of SRM

C_{srm} = actual concentration of SRM

Method Detection Limit (MDL)

The method detection limit (MDL) will be defined as:

$$MDL = s_7 \bullet t_{(n-1,1-\alpha=0.99)}$$

where:

s_7 = standard deviation of 7 replicate analyses where the mean is no more than 10 times the MDL

$t_{(n-1,1-\alpha=0.99)}$ = Students' t-value for a one-sided 99% confidence level and a standard deviation estimate with n-1 degrees of freedom.

Linearity

The calibration curve linearity (L) is defined as the ratio of the slope using the highest standards (S_U) divided by the slope determined from the lowest standards (S_L) as follows:

$$L = \frac{S_U}{S_L}$$

The highest standards shall be all those that fall within the top 50% of the calibration range including the 50% standard if it is used. If only one standard falls within that range, the S_U shall be calculated based on the top two standards. The lowest standards are all those that fall within the bottom 50% of the calibration range including the 50% standard if it is used. Least squares linear regression is used to determine slopes.

Sampling Custody

In most cases analyses will be performed immediately upon return from the field or after preparation of samples in the laboratory. Problems with sample custody are minimized, because the same people who receive (or sometimes, collect) the samples also analyze them. In general sample collection, handling, and preservation will be in accordance with the guidelines of Section 1060 of Standard Methods (APHA et al., 1999). All samples must be fully labeled with the sample identity, date, and name of researcher.

Sample Collection and Storage

Samples are collected and stored in clean borosilicate (e.g., Pyrex, Kimax) glass containers. Containers must be capped with either Teflon-lined septa or ground glass stoppers. Exceptions are made for large volume samples which may be stored and shipped in clean polyethylene carboys. Glass containers are cleaned with detergent, followed by 5% sulfuric acid soak, and final rinsing with reagent-grade water. These containers are dried in a 150ºC oven.

Samples for TOC analysis must be acidified and kept in a refrigerator from the time of collection to the start of analysis. Some organics are biodegradable, so care must be taken to minimize this type of loss.

Data Reduction, Validation and Reporting

To ensure the accuracy and permanency of collected data, all research data are recorded with permanent ink in bound notebooks and all QC data (precision, accuracy) are recorded in instrument log notebooks. Summary QC graphs and tables are reviewed at least quarterly by the Faculty QC officer to observe noteworthy trends or inconsistencies. These are maintained in loose leaf notebooks for subsequent use in preparing progress reports, final reports, and theses. Major concerns and conclusions are reported to the external Project Officer via the progress reports.

Pages from the laboratory data books are regularly duplicated so that a file copy of raw data can be placed in safe storage in the event that the book is lost or destroyed. At the end of the project, all bound data books and any loose leaf data will be stored by the project team for at least ten years. Summary data files will be put on magnetic media so that statistical analysis of the data can be done. Our laboratory has several personal computers that can be used for this purpose.

Procedures specific to Measurement of UV absorbance

General Analytical QC

Many types of QC procedures are required as indicated under standard method 5910B. The guidelines below are prepared assuming that samples are run in groups, whereby a "daily" frequency refers to once every day that the analytical method is being used.

Table 5. Summary of QC Elements as Applied to UV Absorbance

Types of Samples or Standards	§ in Std. Meth.	Purpose	Frequency	Timing	QC data
Laboratory Reagent Blank (LRB)		To provide a zero absorbance calibration	1 per day, and after every 20 samples	Beginning of day	Response
Field Reagent Blank (FRB)		Test all field conditions for interferents	1 per day, if sampling occurred outside of the lab	mid day	Response
Spiked sample, or Laboratory Fortified Sample Matrix (LFM)		To test recovery in the sample matrix	1 for every 20 samples[7]	Mixed throughout day	% recovery, mean and standard deviation
Unknowns or "samples"		This is what you really want to measure	As many as desired	Mixed throughout day	Relative standard deviations

Special QC Tests

The following are "special" tests that are not part of the normal QC protocol. They are used when first learning this analytical method (e.g., IDC), and they may be used when there are suspected problems or there is a need for method performance evaluation.

Assessment of linearity and light scattering

This is normally performed when high concentrated samples are measured and cannot be diluted for some reason

[7] These are generally done using the calibration compound (e.g., KHP). Spike recovery studies may also be done with a "challenge" compound.

Procedure

d) Prepare a set of KHP standards, including 0, 10, 50, 100, 200,300 and 400 mg-C/L
e) Measure UV absorbance on each
    - You may wish to look at other wavelengths, in which case KHP may not be the best standard
f) Plot UV absorbance versus concentration
g) Identify region of linearity

Performance

KHP standards are expected to obey Beer's law (linear response) up to an absorbance value (1.5-3 depending on the spectrophotometer) where stray light and other non-idealities cause a depressed signal. See criteria in Table 6.

QC Criteria

Quality control data must be analyzed as soon as possible. The best practice is to have the QC data tabulated and evaluated as the run is underway. However, it is recognized that there will be times when this is impossible. QC and calibration data must always be analyzed and reported within 24 hours of completion of a run (see section on

Data Analysis & QC Reporting, page 16). Quantitative criteria (Table 6) must be applied, and violations must be immediately reported to the faculty QC officer. The graduate and faculty QC officer along with the analyst will then work out a plan for returning the analysis to acceptable levels of QC. Table 6 lists some typical corrective action, however the actions taken may differ depending on the particular circumstances. Excursions from QC criteria can be quite complex, and many analytical characteristics and conditions must be considered before a decision can be made on the most effective steps to be taken.

In several cases, quantitative criteria are based on long term trends, and these must be monitored by means of appropriate control charts. Calibration check standards, spike recovery and blank water values are documented over time in this way. All summarized QC data (tabular and graphical) must be kept in a notebook in the Marcus Hall TOC room (Rm 5). A duplicate set must be deposited with the faculty QC officer (D. Reckhow).

Table 6: Quantitative Criteria for Judging Data Acceptability

Types of Samples or Standards	Frequency	Timing	QC data Acceptance Criteria	Typical Corrective Action
				❖
Calibration Check Standard	10 mg-C/L of KHP		Calibration = ±10% of literature value	❖ Run newly prepared standard ❖ Examine spectrophotometer for problems, needed maintenance
			KHP standard linearity must extend across full range of unknown samples (usually zero to 1.5 or as much as 3.0)	❖
Lab Reagent Blank	Daily		≤0.002 cm^{-1}	❖ Seek alternative reagent water ❖ Change spec cell
Unknowns or "samples"	As many as desired	Mixed throughout day	RSD or RFD for replicate analyses ≤5%	❖ Re-run samples and/or discard outliers[8] until precision can be brought under control
			Estimated concentration in unknowns must not exceed linear range	h) Re-run samples following dilution ❖ If within 150% of max linear range, and samples can't be re-run, measurements may be reported, but must be flagged as tentative. The non-linearity must be fully

[8] using Dixon's Q Test, or some logic test (e.g., monotonic increase with timed data series; unlikely sudden changes in natural system).

				characterized and applied to the sample data

Literature Cited

Appendix

Standard Method 5910B:

(20th edition, 1999)