

## UV/Vis/NIR Spectroscopy

### Water and Environmental Analysis According to US EPA Regulations using the Lambda 25/35/45 UV/Vis Spectrophotometers



#### Introduction

UV/Vis spectroscopy is an important analytical technique used for the determination of many water constituents. Environmental regulations in many countries prescribe the use of UV/Vis spectroscopy. Depending on the method, UV/Vis determinations of metal and organic nonmetal analytes in water have a minimum detectability in the ppb-ppm range.

#### The Lambda UV/Vis Spectrophotometers



Figure 1: Lambda 25 UV/Vis Spectrophotometer

The Lambda 25 as well as the Lambda 35 and 45 UV/Vis spectrophotometers are ideal for environmental analysis. These instruments not only have high optical quality and excellent performance specifications, but offer a wide range of accessories commonly used for water analysis, including long pathlength cells and cell holders, flow through sipper systems, autosamplers, and Peltier temperature controlled cell changers. The Lambda 25/35/45 systems are completely computer controlled through the advanced UVWinlab V5 software, featuring full 32-bit Windows XP compatibility. The Lambda 25/35/45 spectrophotometers all feature a wide UV /Vis wavelength range (1100 nm to 190 nm), and are optically true double beam instruments, ensuring long term photometric stability and improved precision of assays. The Lambda 25 and 35 units utilize a single grating monochromator, whereas the Lambda 45 incorporates a double grating monochromator for reduced stray light and providing an extended dynamic range.

Additionally, the Lambda 25/35/45 spectrophotometers all feature high bandpass resolutions (as low as 0.5 nm), sufficient to fully resolve the absorbance peaks characteristic of all the environmental test procedures, including the narrow bandwidth chlorophylls. A summary of the technical specifications is shown in Figure 2.

	<b>Lambda 25</b>	<b>Lambda 35</b>	<b>Lambda 45</b>
<b>Wavelength Range</b>	190 – 1100 nm	190 – 1100 nm	190 – 1100 nm
<b>Bandwidth</b>	1 nm fixed	0.5, 1, 2, 4 nm	0.5, 1, 2, 4 nm
<b>Stray Light</b>			
220 nm (NaI)	<0.01%T	<0.01%T	<0.01%T
340 nm (NaNO <sub>2</sub> )	<0.01%T	<0.01%T	<0.005%T
370 nm (NaNO <sub>2</sub> )	<0.01%T	<0.01%T	<0.005%T
200 nm (KCl)	<1%T	<1%T	<1%T
<b>Wavelength Accuracy</b>			
At D2 peak (656.1 nm)	±0.1 nm	±0.1 nm	±0.1 nm
<b>Photometric Accuracy</b>			
At 1A (NIST 930D)	±0.001A	±0.001A	±0.001A
<b>Photometric Stability</b>			
1A, at 500 nm, 2 sec response	<0.00015	<0.00015	<0.00015
<b>Photometric Noise, 500 nm</b>			
0A RMS, Slit 1 nm	<0.00005A	<0.00005A	<0.00005A

**Figure 2. A summary of the technical specifications for the Lambda 25/35/45 UV/Vis spectrophotometers. These systems all feature the excellent optical performance expected from PerkinElmer UV/Vis spectrophotometers, ensuring confidence of results.**

### **Water and Environmental Methods**

A total of 60 preprogrammed water and environmental methods according to US EPA and/or US Standard Methods for the Examination of Water and Wastewater guidelines are available for the PerkinElmer Lambda 25/35/45 operating with

UVWinLab Version 5.1.5 or higher. These methods can be imported into existing UVWinlab installations for routine use. In addition to the method file, a custom report template is provided. Where possible, documentation is provided for the method developed on the Lambda spectrometers, from PE Handbook Part Number 0993-5033). The methods, report templates, and documentation are provided as a .zip file for extraction.

[1] "Methods for Chemical Analysis of Water and Waste", Environmental Monitoring Systems Laboratory (EMSL), Cincinnati, OH. 1983

[2] "Standard Methods for the Examination of Water and Wastewater" 18<sup>th</sup> Edition, American Public Health Association, 1992.

[3] "Water and Environmental Analysis According to US EPA Regulations", PerkinElmer Publication B2405, Part Number 0993-5033.

[4] "Colorimetric Determination of Nonmetals", Chemical Analysis Volume 8, David F. Boltz and James A. Howell, John Wiley & Sons, 1978

### **Installing the Methods**

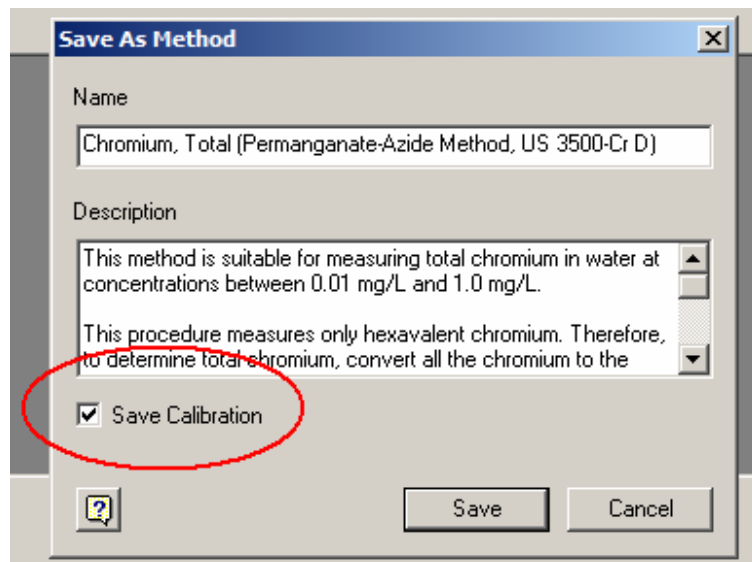
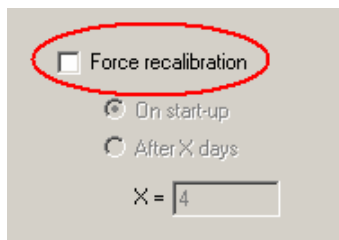
1. Copy the .zip file to a common area of you PC attached to the Lambda (i.e., C:/Temp), and extract the files. The environmental methods will extract by their names into individual folders.
2. Open UVWinlab.
3. From File, select Import.
4. Browse to the folder containing the environmental methods, and double-click on the desired folder. Click on the method name and then click OK. The method will be imported and added to the method list.
5. Once the method has been imported, the report template for that method needs to then be imported. From UVWinlab Explorer, click on the Report Templates menu.
6. Click on File...Import.
7. Browse to the same folder where the method file was imported, and click on the report template – usually the report template will be named EPA Quant, or will have the same or similar name as the method. Click OK.
8. Repeat this process for all remaining desired methods. Note that the common report template EPA Quant needs only to be imported once.

### **Running a Method**

9. Double-click to launch a method. Note that the majority of the water and environmental methods are of the type Wavelength Quant, and will require the user to prepare standards of known concentration. Most of the methods have a predefined standards table with concentrations the same as those published in PerkinElmer Publication 0993-5033. Where available, the PerkinElmer procedure is included for some of the environmental methods.

10. If required, adjust the standards table for the sensitivity of the assay.
11. The default is to always have the user to run fresh standards when the method is closed and re-opened. However, this can be changed so that the standard curve can be saved with the method. Under the Beer's Law Quant settings, make sure the "Force Recalibration" is unchecked. After the standard curve has been completed, the method is resaved, and the "Save Calibration" option is checked. When the method is reopened, the stored standard curve will be used to calculate concentrations.

To save a standard curve with the method for re-use, make sure the Force Recalibration option is unchecked under the Beers Law Quant menu, and re-save the method with the Save Calibration option checked, as shown.

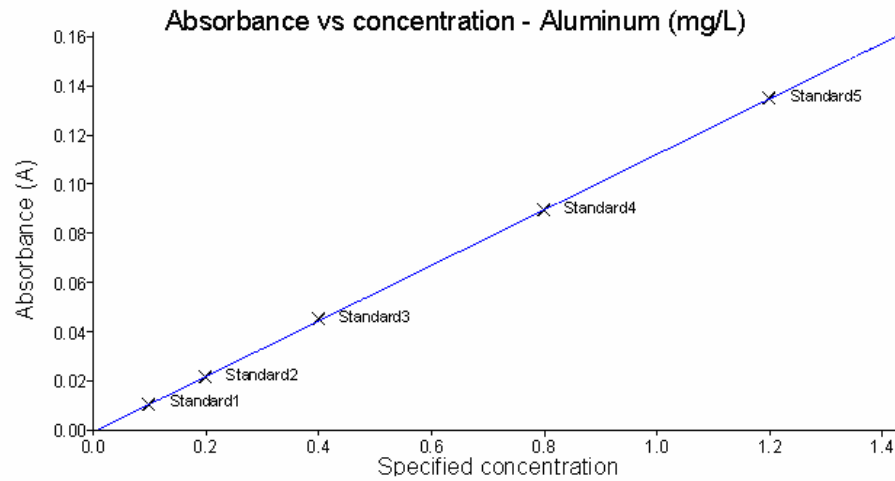


The complete methods list is provided in Figure 3. An example report for an aluminum standards assay is shown in Figure 4 and an example report for a trichromatic chlorophyll assay in Figure 5.

**Figure 3. Methods List**

Analyte	Method	Sensitivity
Aluminum	Eriochrome Cyanine	0 - 1.2 mg/L
Ammonia, Nitrogen	Nesslerization	0.01 - 2.5 mg/L
Ammonia, Nitrogen	Phenate	0.01 - 2.0 mg/L
Arsenic	Silver Diethylthiocarbamate	0.01 - 0.3 mg/L
Beryllium	Aluminon	0 to 2.0 mg/L
Boron	Curcumin	0 to 2.0 mg/L
Bromide	Phenol Red	0.1 to 10 mg/L
Cadmium	Dithizone	0 to 0.1 mg/L
Chemical Oxygen Demand (COD)	Closed Reflux	3 to 900 mg/L
Chloride	Mercuric Thiocyanate	0.0 to 20 mg/L
Chlorine, Free	DPD	0.1 to 7.5 mg/L
Chlorine, Free	Syringaldazine (FACTS)	0.1 to 10 mg/L
Chlorophyll	Jeffrey and Humphrey (1975)	< 0.01 mg/L
Chlorophyll	SCOR-UNESCO	< 0.01 mg/L
Chlorophyll a,b,c	US 10200 H - Trichromatic	< 0.01 mg/L
Chlorophyll a	US 10200 H - Acidification	< 0.01 mg/L
Chlorophyll a	Lorenzen (1965)	< 0.01 mg/L
Chromium, Hexavalent	Diphenylcarbazide	0.01 to 1.0 mg/L
Chromium, Total	Permanganate-Azide	0.01 to 1.0 mg/L
Color	Platinum-Cobalt	0 to 500 units
Copper	Neocuproine	0.01 to 8.0 mg/L
Copper	Bathocuproine	0.02 to 5.0 mg/L
Cyanide	Pyridine-Barbituric	0.02 to 1.0 mg/L
Cyanide	Thiocyanate	0.1 to 2.0 mg/L
Fluoride	SPADNS	0.1 to 1.4 mg/L
Hazen-APHA Color	Platinum-Cobalt	
Iodide	Leuco Crystal Violet	0 to 50 mg/L
Iodine	Leuco Crystal Violet	0 to 50 mg/L
Iron	Phenanthroline	0.05 to 5 mg/L
Lead	Dithizone	0 to 0.5 mg/l
Manganese	Persulfate	0 to 15 mg/L
Manganese	Formaloxime	0.2 to 30 mg/L
Mercury	Dithizone	0 to 5.0 mg/l
Nitrate	Brucine	0.1 to 10.0 mg/L
Nitrate	Ultraviolet Spectrophotometric Screening	0 to 11 mg/L
Nitrate	Cadmium Reduction	0.01 to 1.0 mg/L
Nitrite	Azo Dye	0.01 to 1.0 mg/L
NTA (Chelating Agents)	Zinc-Zincon	0.5 to 10 mg/L
Ozone	Indigo	0.0 to 3 mg/L
Phenols	4-Aminoantipyrene	0.05 to 10 mg/L
Phenols	MBTH	0.04 to 1.2 mg/L
Phosphorus (Single Reagent)	Molybdate-Ascorbic Acid	0.01 to 0.5 mg/L
Phosphorus (Two Reagent)	Molybdate-Ascorbic Acid	0.01 to 0.5 mg/L
Phosphorus	Vanadomolybdophosphoric Acid	4.0 to 18 mg/L
Phosphorus	Stannous Chloride	0.01 to 2.0 mg/L
Phosphorus in Soil	Vanadate Molybdate	2 to 50 mg
Phosphorus Total in Sludge	Vanadate Molybdate	2 to 100 grams
Selenium	2,3-Diaminonaphthalene	0.01 to 2 mg/L
Silica High	Molybdosilicate	2 to 25 mg/L
Silica Low	Heteropoly Blue	0.4 to 2 mg/L
Silver	Dithizone	0 to 5.0 mg/L
Sulfate	Barium Sulfate	5 to 40 mg/L
Sulfide	Methylene Blue	0.1 to 2 mg/L
Sulfite	Phenanthroline	0.01 to 10 mg/L
Surfactants	MBAS (Anionic Surfactants)	0 to 100 mg/L
Tannin-Lignin	Folin Phenol	0 to 9 mg/L
Turbidity	Formazin	0 to 40 NTU
UV 254nm Absorbance	Direct absorbance	
Vanadium	Gallic Acid	0 to 2.0 mg/L
Zinc	Dithizone I and II	0 to 5 mg/L

**Calibration Curve**



**Calibration Details**

Calibrated on:	12/08/2006 03:31:02 PM Central Standard Time
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Wavelength (nm):	535	Type of Fit:	Linear	Component:	Aluminum (mg/L)	Units:	mg/L
Slope:	0.1128	Intercept:	-0.0006555	Corr. Coeff.	0.999976		

**Standards Table**

Standard ID	Concentration	Ordinate (A)	Residual
Standard1.Sample	0.1000	0.0106	0.0005000
Standard2.Sample	0.2000	0.0216	0.002400
Standard3.Sample	0.4000	0.0450	-0.005000
Standard4.Sample	0.8000	0.0893	0.002700
Standard5.Sample	1.200	0.1348	-0.0006000

**Results Table**

Sample ID	Description	Aluminum (mg/L)	Ordinate (A)
Sample1.Sample	CCR	0.9034	0.1013
Sample2.Sample	CCR	0.6767	0.0757
Sample3.Sample	CCR	0.7944	0.0890

**Figure 4. Example concentration report for an Aluminum assay.**

Determination of Chlorophyll (Trichromatic Method -US 10200 H)

Chlorophyll (Trichromatic Method, US 10200 H-2c) Wednesday, December 13, 2006 3:08 PM  
Central Standard Time

Chlorophyll concentrations in mg/L

Sample ID	Description	Extract	Filtered	A750	A664	A647	A630	Chl a	Chl b	Chl c
sample1.Sample	Cooks Bay	50.00	2.00	0.2007	0.3545	0.2692	0.2448	42.83	12.21	7.611
sample2.Sample	Cooks Bay	50.00	2.00	0.1926	0.2789	0.2318	0.2182	24.02	7.203	4.678
sample3.Sample	Cooks Bay	50.00	2.00	0.1951	0.3625	0.2699	0.2435	46.61	13.4	8.485
sample4.Sample	Cooks Bay	50.00	2.00	0.2143	0.4781	0.3302	0.2882	73.55	20.23	12.26



User: PerkinElmer Method Developer  
Template: Chlorophyll Trichromatic Method  
Date: 13 December 2006 03:19 PM Central Standard Time

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**Figure 5. Example report for a chlorophyll assay using the trichromatic method.**

### Conclusion

The PerkinElmer Lambda 25/35/45 UV/Vis spectrophotometers represent excellent choices for environmental analysis. Not only do these units have excellent performance, with double beam optics and high resolution, but the availability of a wide range of cell holders and accessories, including long path cell holders, sippers, autosamplers, and Peltier temperature controlled accessories, allows high sample throughput and enhanced assay precision. The 60 preprogrammed environmental methods provided for UVWinlab V5 eliminates the end user from having to “program” these methods, and ensures rapid implementation of these systems in water quality laboratories.