

## Standard Operating Procedure

### Agilent 8453/4 UV-Vis (DAD) (Standard Mode)



1. Turn on the instrument. Allow 15 minutes warm-up time (30 min for sensitive work)
2. Log on to the computer with the appropriate username and password
3. Wait until the uv-vis has undergone the setup procedure and the light on the unit will show green.



4. Proceed to the start the program using the icon shown and ignore the login box by pressing cancel, you do not have authorization to save/change methods.

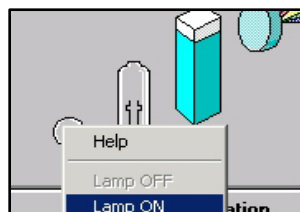
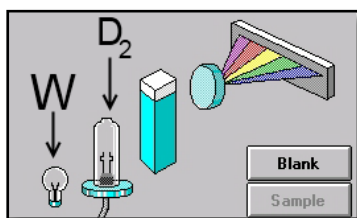
5. If your course or lab has an established method load using the Method button

6. Ensure that the mode is set to Standard in the pull-down menu

7. Chemstation UV software will give you several options in the TASK pull down menu

- a. Fixed Wavelengths –monitoring sample data at up to six different wavelengths
- b. Spectrum/Peaks –measuring the maxima &/or minima over a wavelength range
- c. Ratio/Equation -perform a user-definable equation based on measured data
- d. Quantification -single component analysis based on various types of calibration curves

8. At this point if the lamp(s) have not been lit, turn them on manually by right clicking on the lamp(s) needed



- a. The tungsten lamp (W) is needed if analyzing from 400-1100nm
- b. Deuterium lamp (D2) is required if analyzing from 190-400 nm

9. Go to the Setup button and determine the following parameters that are required

- a. Please select 'Prompt for sample information'
- b. Peak\Valley Find
- c. Data type
- d. Wavelength range

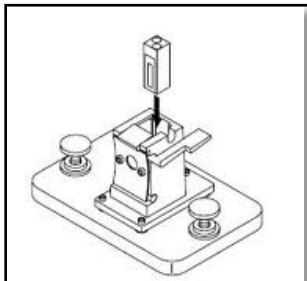
10. Autosave the data to a results file located in Options & Info under the Method tab

- a. UV Chemstation DOES NOT save data/results unless prompted

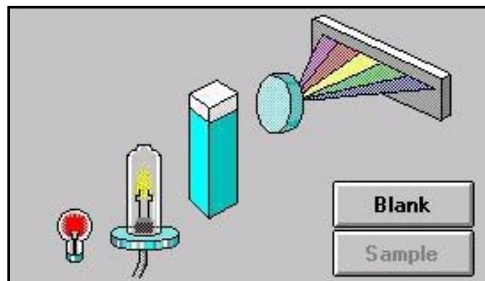
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11. Place a cuvette with the Blank solution in the sample holder and click Blank

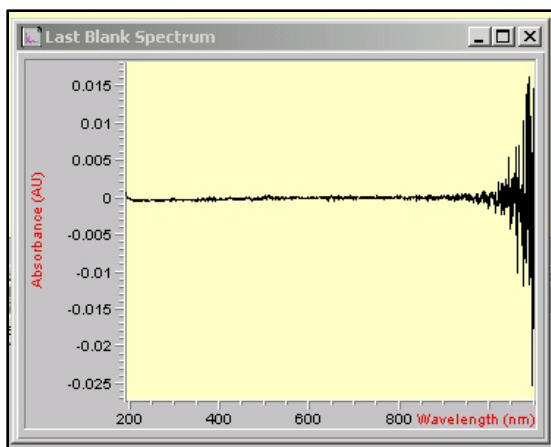


Standard Cell Holder

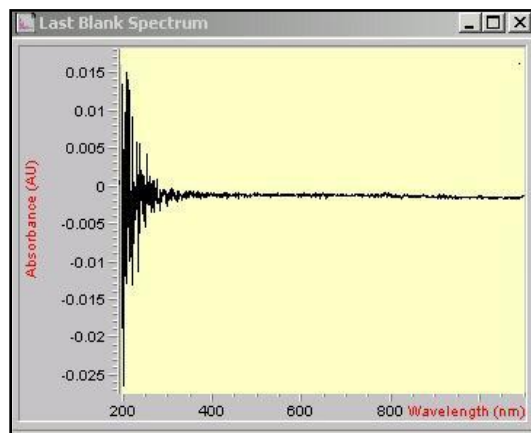


Click 'Blank' button

12. Correctly ran Blank spectra MUST appear as the following, depending on the cuvette



Quartz Cell



Plastic/Glass Cells

13. Place your sample in the Cell Holder and select the Sample button (it is no longer greyed out)



14. To Print the Method default report click on

15. To Print a refined or zoomed spectra click on File->Print->Current View

a. Ensure that the correct printer is selected (ask the TRACES Staff)



16. To Save the spectra file click on the

a. It is recommended to save ALL your results. To do so save the results click file→ Save Results As

b. Select the results name with a \*SR extension

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17. All spectral manipulation and evaluation is available under the MATH tab
  - a. If possible, attempt on data evaluation in the offline version.
  - b. Abs, %T, Subtraction, Derivatives
  - c. Please see TRACES Manager for Advanced Mode\*
  - d. The data set of interest MUST be highlighted/selected if any manipulation is desired
18. Upon completion of all experimental work
  - a. Clear all the sample/standard/blank data from this icon
  - b. Turn OFF the lamps by right clicking on lamps
  - c. Remove all standard, samples and blanks from the holders and the room
  - d. Clean the surrounding area and dispose of all waste in the appropriate container
19. BE ADVISED THAT THIS IS NOT A SECURE SYSTEM, ALL DATA AND INFORMATION IS VUNERALBE
  - a. If the data is valuable-save to an external drive or send the data via email or ftp servers\*
    - i. Data can be saved in various formats (i.e., jcamp, pdf, csv...)
  - b. If the data needs to be secure-DELETE from the Chem32/Data folder your data\*
20. Exit Chemstation software from the File tab
  - a. You will be prompted to save all data and results if you have not done so
  - b. Do not attempt to save configuration, this option is not available
21. Log off the computer

**\*The TRACES Manager will provide full details during hands-on training.**