Standard Curve Basics

Purpose
Standard curves are needed for any colorimeter (UV-spec, LACHAT, AA, etc.) for you (or the computer) to calculate the concentration in your samples. Essentially, you pass a known concentration of an analyte (e.g. PO_4^{3-}, Ca^{2+}) through a detector and use the reported absorbance (or peak area, volts, etc) to develop an equation you will use to calculate the concentrations of your unknowns (samples).

Materials
- Pipette(s) that can span the necessary volume range
- 1 50mL beaker with DI water
- 1 empty (waste) beaker
- 1 mini weighing dish
- Analytical balance
- Standard stock solution (in a beaker)
- 5+ 100mL volumetric flasks
- DI water (or other diluent) to bring standards to volume

Calculating your curve
$C_1V_1 = C_2V_2$

Where C_1 is the concentration of your stock solution, V_1 is the volume of stock you will need (this is your "x"), C_2 is the final concentration of your standard, and V_2 is the final volume of your standard (size of the flask into which you are diluting the stock solution). You must prepare a curve that spans the expected range of your samples, and use no fewer than five standards in order to get an accurate standard curve.

Use the table below as a guide to making your standard curve:

Cal	C_1 (mg NO_3-N/L)	V_1 (mL)	C_2 (mg NO3-N/L)	V_2 (mL)
0	1000	0	0	100
1	1000	0.025	0.25	100
2	1000	0.05	0.5	100
3	1000	0.1	1.0	100
4	1000	0.15	1.5	100
5	1000	0.2	2.0	100
6	1000	0.5	5.0	100

Procedure
1. Set the pipette to the necessary volume and place the empty weigh dish on the balance
2. Close the balance and tare
3. Pipette the volume needed using DI water and expel it into the weigh dish. Close the balance.
4. Adjust the pipette as necessary until it reads the correct volume (±0.0002). Weighing each time you make an adjustment.
5. When you get the correct volume, **do not change the pipette tip**, but pipette the necessary volume of stock solution into the correct volumetric flask.
6. Replace the pipette tip and repeat the process for each standard.

7. Dilute with DI water (or other diluent) to the mark and mix well.
8. If pouring into a new tube (e.g. for the LACHAT), rinse the tube with a small amount of standard before pouring in the rest of the standard solution.

FAQ

How do I make an accurate standard curve?

- Check the date on the stock standard to make sure it is still good (most high concentration standards are good for at least a year).
- Check the accuracy of your pipette for every standard you make by weighing out the necessary amount with water on the scale.
- **Do not** push the pipette down all the way when you take the liquid.
- **Do** push the pipette down all the way when you want to expel the liquid.
- Avoid bubbles in the pipette tip.
- When adding DI water (or diluent), be **very careful** not to overshoot the mark on the volumetric flask. If you do, pour it out and start over.
- Thoroughly mix your completed standard solution.
- Discard low concentration standards after 2 days and make new ones.

What should my r^2 be when I run my curve?

Never accept a curve less than 0.995.

What should I do if my samples are CONSISTENTLY higher than my highest standard?

- If you are in the middle of a run on the LACHAT, you can easily add a higher calibration standard in Omnion.

What should I do if a few of my samples are MUCH higher than my highest standard?

- Look at your samples, are they murky, have precipitate, are not clear? If so, these samples may need to be filtered and re-run.
- Check the shape of the peak. Is it normal? Was there an air-spike?
- Consider diluting the sample to bring it into range if you think that the sample is actually high and not contaminated.