CONSTRUCTING A CALIBRATION CURVE

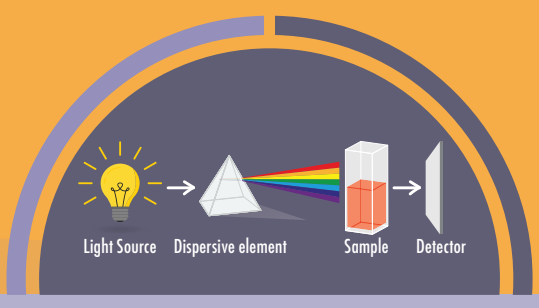
A STEP-BY-STEP GUIDE

WHAT IS A CALIBRATION CURVE?

Calibration curves are used to determine the concentration of an unknown sample, to calculate the limit of detection, and the limit of quantitation. The curve is created with a set of samples at a known range of concentrations. The data are then fit with a function to enable concentration prediction.

WHAT IS AN ULTRAVIOLET-VISIBLE (UV-VIS) SPECTROPHOTOMETER?

A UV-Vis spectrophotometer measures the transmission and absorption of light to determine the concentration of an analyte in solution. It consists of a light source, a wavelength selector, a detector, and a computer.

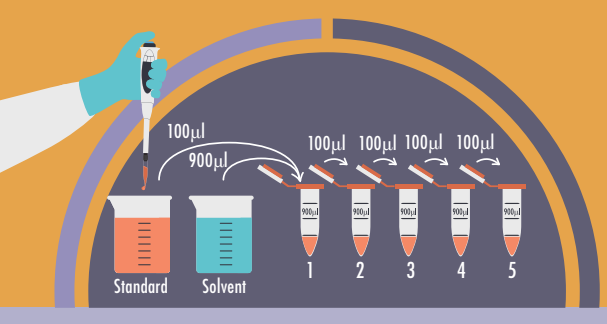


HOW DOES A UV-VIS SPECTROPHOTOMETER WORK?

The UV-Vis light passes through the sample to the detector. The transmittance is measured and used to calculate the absorbance.

STEP 1: MAKE A CONCENTRATED STOCK SOLUTION

Prepare a stock solution of the standard by mixing the solute with solvent.



STEP 2: MAKE THE STANDARDS FOR THE CALIBRATION CURVE

Perform a serial dilution

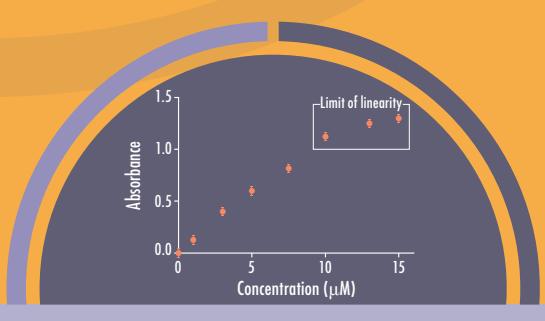
A minimum of five standards are recommended. Pipette the required volume of standard and solvent into the first flask or microtube, then mix. Repeat this process by pipetting from the previous solution to the new flask or microtube and adding solvent.

Prepare the samples

Transfer the standards and unknown samples to cuvettes. The unknown samples should have the same buffer and pH as the standards.

STEP 3: RUN THE STANDARDS AND SAMPLES IN THE SPECTROPHOTOMETER

Place each standard in the UV-Vis spectrophotometer and obtain three to five readings each. Repeat with the unknown samples.



STEP 4: PLOT THE DATA

Plot the data with absorbance on the y-axis and concentration on the x-axis. Determine the standard deviation and add error bars.

Examine the calibration curve

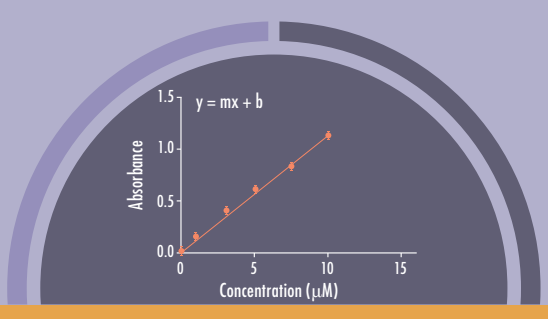
Examine the plot. It should look linear and have a non-linear section—the limit of linearity (LOL), a sign that instrumental detection is nearing saturation.

STEP 5: FIT THE DATA TO A LINEAR REGRESSION

Use statistical software to fit the data to a linear regression. The output is the equation y = mx + b, where m is slope (the units are absorbance/µm), and b is the y-intercept (the units are absorbance).

Obtain a coefficient of determination

The coefficient of determination (R^2) evaluates the goodness of fit. R^2 is typically between 0.0 and 1.0, with 1.0 being a perfect fit.



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