Calibration Curve

Calibration curve is a procedure for determining the concentration of a compound in an unknown sample. ***CalibrationCurve.xls*** is an Excel workbook to perform a calibration calculation when using internal standards. This calculation is based on a conventional statistical procedure, described, for example, in D. A. Skoog, J. J. Leary: Principles of Instrumental Analysis, 4th edition, and utilizes the method of least squares for linear regression. In case of any question or problems with this file, please, contact Dr. Dominik Heger, hegerd@chemi.muni.cz.

The calibration curve production from the HPLC or GC data

1. Make at least two independently prepared (most concentrated or stock) solutions for each compound, i.e., an analyt, A (which is the compound you wish to determine in an unknown sample) and an internal standard, IS. Weigh the solid as well as liquid (non-volatile) compounds. Do not use volumetric glass for small amounts of liquids or for any amounts of viscous liquids.
2. Prepare at least 7 different solutions – mixtures of both A and IS. The concentration of the latter should be similar in all samples (including that of unknown samples). Make sure the A concentration range is broad enough. Mix diluted A and IS solutions randomly; the points of the calibration curve should be distributed unevenly. The chromatographic peaks must be well separated but not too far from each other. Inject each solution at least twice. If the A/IS areas ratio differs by more than 5%, repeat the measurement. Remove the outliers. All other data should be used for the calibration calculation. Thus, at least 14 values, obtained from the analysis of 7 solutions, help to avoid errors of rounding the averages and to make the standard deviation smaller.
3. Use a new ***calibr-empty*** sheet (and rename it as you like) or ***calibr-empty-Rev*** (will be discussed later) in the Excel file ***CalibrationCurve100310.xls***. This workbook also contains some of the already measured data that you may find useful for practicing: ***calibr-Krausci, calibr-Krausci-Rev***, ***A112*** (an example *a1-12* from D. A. Skoog, J. J. Leary: Principles of Instrmental Analysis, 4th edition; this worksheet utilizes the mathematics described there), ***119a*** (an example *19.1a* from J. H. Zar, Biostatistical Analysis, 5th edition, currently available in the Dominik Heger's office). The ***Calibration curve*** part, where you create you calibration curve, and the ***Reading from calibration curve*** part, where you can calculate the concentrations of A in your samples, are located in ***calibr-empty*** sheet.
    a. *Calibration curve*
    Fill in the A, B, C, D columns using the concentration values used and the peak areas measured belonging to both A and IS. Adjust the number of rows you are using. If you do not need them all, delete those not used in order to avoid seeing #DIV/0! Then you need to check the magenta cells whether they display the data in all columns. At this point your calibration curve is plotted and the linear regression is calculated. Check whether all your points are included in the graph and that the r^2 calculated in W9 is the same as that of the graph, i.e. whether you are using the same data for both the calculation and the plot itself. Now check the green cells. These values are the **slope *m*** and **intercept *b*** with its standard deviations (s_m, s_b), then the coefficient of correlation, ***r***, and its standard deviation, s_r, and the coefficient of determination, r^2. You can also check the range of concentrations of A and IS (**P12**, **13**).

b. Reading from the calibration curve

To obtain the concentrations of A from the calibration curve, enter the concentration of IS into the column B and the area of the measured peaks from the chromatogram to the columns C and D. Fill in the number of measurements you had done into the column E to determine these values of areas (preset value is 1). Then the Excel calculates the ratio of the areas (column F) and the ratio of concentrations (column G) and its standard deviation s_x (column G) from the calibration curve. The concentration of A is calculated from the concentration of internal standard (column I). The standard deviation *sc* of the concentration of A (column J) is calculated by error propagation. The percent relative standard deviation (*sr* (%)) is in the next column (K). In case of A, make at least two injections and then compare the results. The average of these two measurements is calculated on the right starting from the column M. Make sure that the references in the cells M and N refer to the correct rows in the column F. The average concentration of the analyt A can be found in the column Q and its standard deviation *sc* is in the column R.

The ratio of both the concentrations and areas is calculated in **calibr-empty** as [A]/[IS]. A reverse ratio is calculated in **calibr-empty-Rev**. Although there is only insignificant difference between both calculations; however, that with a larger coefficient of correlation *r* gives a smaller cumulative percent relative standard deviation *cprs* (as found in L23; make sure you are summing all the data you want). This approach is thus recommended. The calibration curve using the same [A]/[IS] gives better distribution of the points as well as a larger ***r***; see **calibr-Krausci.**