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Error Propagation in Calibration and Standard Addition
Spectrometric Assay of Vanillin in Commercial Vanilla Extract

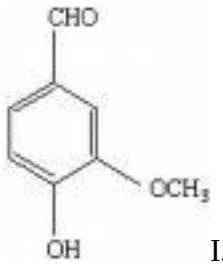
Introduction. Instrumental analysis involves a chemical measurement with a transducer. The transducer converts a chemical signal to an electrical signal, and the electrical signal is recorded for use. As with any transducer, it is necessary to calibrate the instrument if quantitative results are needed- that is, calibration establishes a relationship between concentration of the target species and the instrumental response of the transducer to that species.

There are two common ways of establishing the calibration. One method is to develop a calibration curve (usually a straight line). We get the line from measuring a series of solutions with very well know amounts of analyte, recording the responses, then performing least-squares regression on the response-concentration data to get a slope and intercept. This is calibration, and it is useful as long as the transducer is unchanged.

The other common way of calibrating the instrumental transducer recognizes that in certain situations, the response of the transducer is affected by both analyte concentration and by the sample matrix. So, a correction is needed to account for the fact that the analyte concentration that is present may not be the concentration actually "seen" by the transducer. That correction is done by the method of standard additions. In standard additions, the assumption is that the sample matrix affects the analyte signal, and that there is enough of whatever affects the signal to affect any added analyte. If so, then we can get the amount of unknown analyte in a solution by first measuring the unknown solution and recording the signal, then by adding small, known volumes of a standardized solution of analyte to the solution being analyzed, and noting the change in transducer signal that results. A plot of added amount of analyte versus the instrumental response is a series of points that follow a linear relation. Least squares analysis yields a line that best fits these points. The negative x–intercept of this line equals the amount of unknown present in the unknown solution. This method is essentially a "one-time" calibration for this specific sample and its specific conditions.

We will use these methods here on a "dry-lab" experiment to practice calculation of least-squares fits and the propagation of error though a calibration and a standards addition relationship. By the way, full detail on each step is not given here - or in the following labs. You'll need to read up on the methods, think about the experiment, and figure out for yourself what to do and how to do it from the general guidelines given.

Spectrometer measurements. The application we will use for the dry lab is the determination of vanillin (3-methoxy-4-hydroxy-benzaldehyde), represented by (I.) in commercial vanilla extract.



I.

vanillin

Vanillin is a compound found in the seeds of the vanillin plant and in the decomposition of lignin. Naturally-occurring vanilla is used for flavorings, mainly in the form of its ethanol extract from the seed pods. Vanillin is a weak acid, and the acid form (I.) is very soluble in chloroform. The phenolate base form is highly soluble in water. It also has a strong absorption from the phenolate group in the ultraviolet region (~300 nm) of the spectrum. We take advantage of these properties in this experiment.

PRE-LAB ASSIGNMENT

Write answers to the following questions in your lab notebook. Be prepared to show these to your TA at the beginning of your lab period. Also, be prepared to discuss the issues with your teammates and with the TA.

1. Does the estimate of uncertainty that you obtain in propagating error through a calibration measure random error, systematic error, or both? Why?
2. When a calibration is done, is it necessary to correct the responses for a blank response? Why or why not?
3. Suppose we did an accurate calibration, but because there was a power glitch in the building just as we finish the calibration, we have to replace the instrument 's detector. Should the calibration still give accurate results when applied to the unknown samples after we replace the detector with a new one? Why or why not?

EXPERIMENTAL

This work is to be done in groups during the first laboratory period. You will want to bring a calculator or a laptop with Excel to perform the linear regression calculations. If you don't have one, you can use Excel on the computers in the laboratory.

The two tasks below simulate 2 experiments, one calibration and one standards addition. The data are provided, so all that is necessary is for your team to work the data up and to report the unknown concentration and its uncertainty. This is what you will do in most of the other labs this semester. Unlike those, *you will perform the work-up in the laboratory* and can get help from the Teaching Assistant as needed. This laboratory will help you master the error propagation steps, which will save you time and trouble on subsequent laboratory reports.

For this experiment, there is no written report. Only your 2 plots, your reported values, and your uncertainties are graded and so it is very important that you perform the calculations and that you document your calculations in the laboratory notebook.

I suggest always using the left side of the notebook for calculations and the right side for data. That way, you can check calculations and correct any errors.

Part I. A calibration was performed using an ultraviolet (UV) spectrometer. The following data were obtained from a series of solutions with known concentrations of vanillin in 0.1M NaOH, using a 1.00 cm cell:

Table 1 Calibration of UV Spectrometer at 347 nm

Vanillin (μg/mL)	A (347 nm)
1.05	0.16
2.06	0.26
3.08	0.34
4.13	0.44
5.18	0.57

After the calibration was performed, a volumetric pipette was used to dispense 1.00 mL of an unknown commercial vanilla solution containing vanillin. Extraction of the unknown with $CHCl_3$ was followed by a back extraction into 0.1M NaOH. The extract was diluted to 250.0 mL in a volumetric flask with 0.1M NaOH. Then, a 1.00 mL sample of the extract solution was diluted to 10.00 mL in a volumetric flask. The absorbance of this solution was measured in a 1.00 cm cell. The following replicate readings were obtained:

Absorbance of unknown vanillin solution, after extraction and dilution:
0.24, 0.25, 0.22, 0.22

1. Make a plot of the data in the Table above. Label your axes.
2. Use Beer's law to calibrate the absorbance measurement. Perform a linear regression to find the intercept and absorptivity for the calibration. Add the regression line to your plot of the data.
3. Use the calibration to find the average concentration of vanillin in the unknown *as measured*, ignoring the dilution.
4. Use the spreadsheet to propagate the error though the calibration and determine the uncertainty in the vanillin as measured.
5. Use the dilution information to determine the unknown concentration *as provided* (before dilution).
6. Now use propagation of error to determine the uncertainty in the vanillin concentration *as provided*. You'll need to look up uncertainties for volumetric pipettes and flasks used here.

Report the plot you made, the average vanillin concentration in the unknown as provided and your estimate of its uncertainty.

Part II. An unknown vanilla sample believed to contain vanillin was obtained. This sample was not the same as that analyzed above, and a calibration was not possible because the extraction could not be performed for safety reasons ($CHCl_3$ is a carcinogen) and a matrix match was not possible because the composition of the sample matrix was not known. Standard additions were therefore performed on a diluted sample of unknown.

A 1.00 mL sample of the unknown vanilla was added to a 250.00 mL volumetric flask, which was then diluted to the mark with 0.1M NaOH. A 1.00 mL sample of the analyte mixture from this flask was pipetted into each of five 10.00 mL volumetric flasks. To each flask, an amount of the standard was added, as indicated in the Table below, then the analyte (plus any standard) was diluted to 10.00 mL with 0.1M NaOH. Those five 10.00 mL flasks with solutions were used to perform standard additions measurements. As before, a 1.00 cm cell was used with a spectrometer set to measure the absorption at 347 nm.

The data are given below:

Table 1. Standard Additions for Vanillin Analysis

Total volume of vanillin standard added (mL)	A(347 nm)
0.00	0.24
1.00	0.32
2.00	0.39
3.00	0.47
4.00	0.54

The vanillin standard used had a concentration of $1.070 \pm 0.002 \times 10^{-6}$ g/mL and was made in 0.1M NaOH.

1. Plot the data given above. Label the axes.
2. Perform linear least squares regression to obtain a slope *m* and intercept *b*. Add the regression line to the plot of the data.
3. Find the x intercept of this relationship, and using the relationship given above, find the vanillin concentration in the unknown sample *as measured*.
4. From the uncertainties in *b* and *m*, find the uncertainty in the intercept. Use this uncertainty and the uncertainty in volume and concentration to determine the uncertainty in the unknown concentration *as measured*.
5. Now, considering dilutions, determine the concentration and the uncertainty of the vanillin in the unknown *as provided*, before the dilution. You'll need to look up uncertainties for volumetric pipettes used here and propagate the error though the dilution step.

Report the plot you made, the vanillin concentration in the unknown as provided and your estimate of its uncertainty.