

Finding the Weakest Link in a Quantitative Analysis

A chemical analysis generally consists of three steps: obtaining a sample, preparing the sample for analysis, and making the appropriate measurements. Each step in the analysis is subject to random errors characterized by its standard deviation, s , or its variance, s^2 ; thus, s_{samp}^2 is the variance introduced when obtaining a sample, s_{prep}^2 is the variance introduced when preparing the sample for analysis, and s_{meas}^2 is the variance introduced when taking measurements on the sample. Sometimes we can partition one or more of these variances into smaller parts. For example, in a spectrophotometric analysis we can partition s_{meas}^2 into the variance due to the spectrometer's source, detector, and optics, σ_{spect}^2 , and the variance due to positioning the sample cell within the spectrometer, σ_{pos}^2 . Knowing the value of these variances is important if you wish to improve an analytical method as the step with the greatest variance is the method's weakest link. Improving the weakest link's variance improves the method's precision; improving the variance of other steps without improving the weakest link has little effect on the method's precision.

Accounting for these variances is not trivial. In a typical spectrophotometric analysis the total variance, s_{total}^2 , includes contributions from each step; thus, as suggested above

$$s_{\text{total}}^2 = s_{\text{samp}}^2 + s_{\text{prep}}^2 + s_{\text{spect}}^2 + s_{\text{pos}}^2$$

A simple determination of s_{total}^2 does not provide enough information to partition the overall variance into its component parts. One approach to estimating the individual variances is to use a nested design (see figure at end of this handout). A nested design consists of two or more levels, with the number of levels equal to the number of parameters we wish to evaluate. In this experiment we will determine the contribution of sampling, sample preparation, positioning of the sample cell, and the spectrometer to the analytical method's total variance; therefore, as shown in the figure, we will need four levels.

The first level consists of four samples collected randomly from the gross sample (identified using the Roman numerals I, II, III, and IV). Duplicate samples are obtained from each Level I sample and prepared for analysis; these eight samples represent the second level and are identified using the uppercase letters A and B. For the third level, each Level II sample is divided in half before measuring its absorbance; these 16 samples are identified using the numbers 1 and 2. Finally, each Level III sample is placed in the spectrometer and its absorbance measured twice without repositioning the sample, providing the 32 Level IV samples. Note that each Level I sample is divided into two Level II samples, that each Level II sample is divided into two Level III samples, and that each Level III sample is, in turn, divided into two Level IV samples.

The variance for the Level IV, s_{IV}^2 , is influenced by the spectrometer's variance only. Looking more closely at Level IV, note that the **difference** between any two samples obtained from the **same** Level III sample is influenced by indeterminate errors in the spectrometer only (be sure to convince yourself that this is true). The variance for Level IV, therefore, provides s_{spect}^2 . Because the variance is determined using differences, we cannot use the normal equation for variance. Instead, we calculate the variance using this equation

$$s_{\text{IV}}^2 = s_{\text{spect}}^2 = \frac{\sum_i (d_{\text{IV}})_i^2}{8n}$$

where d_{IV} is the difference between related Level IV samples (e.g. IA1a and IA1b) and n is the number of Level I samples (four in this case). The variance s_{IV}^2 has $4n$ degrees of freedom.

The variance for Level III, s_{III}^2 , includes contributions from the spectrometer and the positioning of the sample cell within the spectrometer; thus

$$s_{III}^2 = s_{\text{pos}}^2 + \frac{s_{\text{spect}}^2}{2} = \frac{\sum_i (d_{III})_i^2}{4n}$$

where d_{III} is the difference between related Level III samples (e.g. IA1 and IA2). The factor of 2 in the term for the spectrometer's variance accounts for our use of two Level IV samples to determine the result for each Level III sample. The variance s_{III}^2 has $2n$ degrees of freedom.

The variance for the Level II samples, s_{II}^2 , includes contributions from the spectrometer, the positioning of the sample cell, and sample preparation; thus

$$s_{II}^2 = s_{\text{prep}}^2 + \frac{s_{\text{pos}}^2}{2} + \frac{s_{\text{spect}}^2}{4} = \frac{\sum_i (d_{II})_i^2}{2n}$$

where d_{II} is the difference between related Level II samples (e.g. IA and IB). The factors of 2 and 4 in the term for the variances due to the sample cell's positioning and the spectrometer, respectively, account for our use of two Level III samples and four Level IV samples to determine the result for each Level II sample. The variance s_{II}^2 has n degrees of freedom.

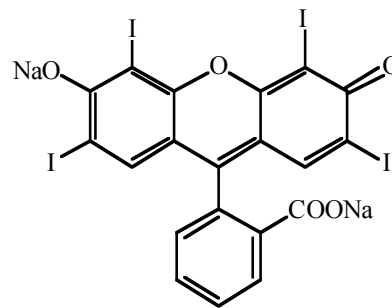
Finally, the variance for Level I, s_I^2 , is determined using the standard equation for variance, and includes contributions from sampling, sample preparation, the positioning of the sample cell, and the spectrometer; thus

$$s_I^2 = s_{\text{samp}}^2 + \frac{s_{\text{prep}}^2}{2} + \frac{s_{\text{pos}}^2}{4} + \frac{s_{\text{spect}}^2}{8} = \frac{\sum_i (X_i - \bar{X})^2}{n - 1}$$

where X_i is the result for each Level I sample and \bar{X} is the average result for all four Level I samples. The factors of 2, 4 and 8 in the terms for the variances due to sample preparation, the sample cell's positioning, and the spectrometer, respectively, account for our use of two Level II samples, four Level III samples, and eight Level IV samples to determine the result for each Level I sample. The variance s_I^2 has $n - 1$ degrees of freedom.

Directions

Erythrosin B, whose structure is shown here, is a dye that has several analytical uses, including as a biological stain for bacteria in soils, a plasma stain for nerve cells (when used in conjunction with methylene blue), as a phosphorescent probe for studying the diffusion of membrane proteins, and in the quantitative determination of phospholipids. It also is an acid-base indicator whose color is orange in strongly acidic solutions and red in aqueous solutions of pH greater than 3. Finally, Erythrosin B, which also is known as Acid Red 51 and FD&C red dye no. 3, is used as a food coloring in maraschino cherries.



The sample we are analyzing is a mixture of Erythrosin B and NaCl. Erythrosin B adheres to the surface of the salt crystals, imparting a pink color to the salt crystals (in fact, this is a common way to dispense indicators that are unstable in solution and cannot be dispensed in solution form). Pour the gross sample onto a piece of paper, shape it into a flattened cone, and divide it into quarters. Obtain an approximately 1-g sample from each quarter; these are your four Level I samples.

Transfer each Level I sample into a clean glass or agate mortar and pestle. Grind each sample for several minutes to reduce the particle size and further homogenize the sample. Divide each of these processed samples in half and obtain an approximately 0.25-g sample from each half. Quantitatively transfer each sample to a 50-mL volumetric flask and dilute to volume with distilled water. Be sure to thoroughly mix each solution. These are your eight Level II samples.

Divide each of your Level II samples approximately in half by transferring at least 10 mL into separate scintillation vials, beakers, or test tubes (the remaining Level II sample can be discarded). These are your 16 Level III samples.

Measure the absorbance of each sample at a wavelength of 526 nm using distilled water as a reference. Measure the absorbance, A , of each Level III sample twice, without removing the sample cell from the spectrometer between measurements. These 32 absorbances are the results for Level IV. Calculate the concentration, C , of Erythrosin B in each Level IV sample using Beer's law

$$A = abc$$

where a is Erythrosin B's absorptivity, which has a value of $0.0916 \text{ cm}^{-1} \text{ ppm}^{-1}$, and b is the pathlength, which is 1.00 cm. Convert these values to % w/w Erythrosin B by accounting for the sample's mass and its dilution. To determine the % w/w Erythrosin B for the Level III samples, average the associated results for Level IV. For example, the % w/w Erythrosin B for sample IA1 is the average of the results for samples IA1a and IA1b. The results for the Level II and Level I samples are found in a similar manner; thus, the % w/w Erythrosin B for sample IA is the average for samples IA1 and IA2, and the result for sample I is the average result for IA and IB.

Hazards

There are no specific health hazards associated with this experiment, although you should exercise appropriate caution when working with any chemicals.

Waste Disposal

All solutions may be discarded down the drain with water.

Report

Summarize your data in four tables. For the first table report results for your 32 Level IV samples using the following headings: sample ID, mass of sample, absorbance, and % w/w Erythrosin B. The remaining tables reports results for the Level III, Level II, and Level I samples and include the following headings: sample ID and % w/w Erythrosin B. Using your data, calculate values for S_{samp}^2 , S_{prep}^2 , S_{spect}^2 , S_{pos}^2 and S_{total}^2 . In addition, briefly answer the following questions:

1. Are the differences between S_{samp}^2 , S_{prep}^2 , S_{spect}^2 and S_{pos}^2 statistically significant?

2. Based on your results, which step is the weakest link in this analysis?
3. How might you go about improving the overall variance for this analysis?
4. Explain why the difference between the results for samples IA1a and IA2b are influenced by indeterminate errors only. Is the same true for the difference between samples IA1 and IA2? How about for samples IA and IB, or samples I and II?

Acknowledgments

This experiment is adapted from an experiment developed by Frank Settle and Michael Pleva at Washington and Lee University. Should you wish to consult their paper, the reference is *Anal. Chem.* **1999**, *71*, 538A-540A.

