

SPIKE RECOVERY AND DETERMINING A METHOD DETECTION LIMIT

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This experiment explores quality assurance practices which are commonly used to determine the limits, accuracy, and precision of an analytical method. The method detection limit (MDL) of the Ascorbic Acid Method for Phosphorus Determination will be determined and a spike recovery analysis will be performed on a lake water sample to determine whether the analytical signal (absorbance) depends on the matrix the analyte (phosphorus) is in.

BACKGROUND

The experiments you've done in this class and in your other lab courses are likely fairly well defined with the procedure clearly stated and the outcomes well known and explicitly defined. In fact these experiments were once research questions which experimentalists developed to measure specific aspects of a system with an acceptable level of precision and accuracy. Quality Assurance is the practice of checking whether the right answer is achieved, essentially insuring the user gets the right data, gets the data right, and KEEPS the data right every time a method is used.¹ Method validation exists as part of quality assurance and is a means to proving a method is appropriate for all intended purposes.

Imagine you work in an environmental testing lab, and your boss has just charged you with measuring phosphorus at various sites in Lake Mendota. How do you pick the best method to do the job? How do you know whether your measurements meet a minimum level of accuracy and precision? How can you prove there are no interfering substances in your sample matrix that shifts your measured value from the true value? Quality Assurance best practices are how you would navigate these questions to ultimately defend your experimental choices and resulting data.

In this experiment, you will practice two commonly used quality assurance methods. One is the recovery of a known addition, or spike, of analyte to a sample. This method is used to determine whether a systematic shift occurs in the analytical signal of an analyte due to matrix effects. To determine the percent recovery of a spike, the sample is split into two portions and a known amount of a standard solution of analyte is added to one portion. The concentration of the analyte is determined for both the spiked, F , and un-spiked portions, I , and the percent recovery, $\%R$, is calculated as

$$\%R = \frac{F-I}{A} * 100 \quad (1)$$

where A is the concentration of analyte added to the spiked portion.

The second quality assurance measure is to determine the method detection limit (MDL). The MDL is the smallest measurable concentration of analyte that is statistically different from the blank. The MDL is calculated as

$$MDL = t_{(n-1, 99\%)}(s) \quad (2)$$

where s is the standard deviation of seven or more replicate analyses and $t_{(n-1, 99\%)}$ is Students' t value for a

¹ Nancy W. Wentworth, U.S. Environmental Protection Agency

99% confidence level for a one-tailed distribution and $n-1$ degrees of freedom.²

In a few weeks, you will measure phosphorus concentrations in Lake Mendota at multiple inflow and outflow points around the lake. In this experiment, you will determine the MDL of the Ascorbic Acid Method for Phosphorus Determination, as well as determine whether the lake water matrix introduces any systematic error in absorbance measurements in the Ascorbic Acid Method for Phosphorus Determination.

Phosphorus occurs in lake water almost solely as phosphates, a chemical form in which each phosphorus atom is surrounded by four oxygen atoms. These are classified as orthophosphates, condensed phosphates, and organically bound phosphates. Orthophosphate, the simplest phosphate, has the chemical formula PO_4^{3-} . In natural waters, orthophosphate mostly exists as dihydrogen phosphate, H_2PO_4^- and hydrogen phosphate, HPO_4^{2-} . Condensed phosphates are polyphosphates such as pyrophosphate, $\text{P}_2\text{O}_7^{4-}$, and metaphosphate, $(\text{PO}_3^-)_n$. Total phosphorus is the total amount of phosphorus contained, regardless of its form. In order to measure total phosphorus, lake samples must be oxidized (digested) to convert organic phosphates and condensed phosphates to orthophosphate.

Lake water samples will be digested using the Persulfate Digestion Method from Standard Methods for the Examination of Water and Wastewater (4500-P.B.5.)³. Note that in the procedure, lake water samples are digested; however, standard phosphate solutions and blanks are not. You would be correct in pointing out that if we digest the lake water sample, it would be good laboratory practice to digest the standard phosphate solutions. In the interest of time, and based on previous experience, we assume that orthophosphate is the only form of phosphorus present in standard phosphorus solutions prepared from anhydrous potassium dihydrogen phosphate (KH_2PO_4).

PRELABORATORY EXERCISES:

1. Estimate the MDL of the Ascorbic Acid Method for Phosphorus Determination as three times the standard deviation of a method blank. Absorbance values of a method blank and the equation of the calibration line are given in Table 1. Use this value as the estimated MDL needed to prepare your MDL Standard Phosphorus Solution. Concentration units are in $\mu\text{g P/L}$.

Blank Aliquot	Absorbance
1	0.007
2	0.016
3	0.018
4	0.027
5	0.009
6	0.024
7	0.013
Calibration Equation: $y = 0.00064x + 0.0029$	

² Electronic Code of Federal Regulations, Title 40: Protection of Environment Part 136: Guidelines for Establishing Test Procedures for the Analysis of Pollutants, Appendix B: Definition and Procedure for the Determination of the Method Detection Limit—rev. 1.11

³ Eaton, A.D.; Clesceri L.S.; Rice E.W.; Greenberg A.E.; Franson M.H., Eds., Standard Methods for the Examination of Water and Wastewater: 21st ed.; American Public Health Association: Washington, DC, Water Environment Federation: Alexandria, VA, and American Water Works Association: Denver, CO, 2005.

2. Determine the concentrations and calculate the volumes of *Standard Phosphate Solution* (~ 2.5 mg P/L) from the “Ascorbic Acid Method for Phosphorus Determination” lab needed to prepare the standard phosphorus solutions for your calibration curve. The concentrations of standards should range from the estimated MDL to 0.4 mg P/L. These calculations should be specific to the actual concentration of *Standard Phosphate Solution* you made. Make a table in your notebook to reflect how the standards will be prepared.
3. Calculate what the increase in phosphorus concentration will be when you add 2.00 mL of your *Standard Phosphate Solution* to 50.00 mL of lake water. Again, the calculation should be specific to your actual concentration of *Standard Phosphate Solution*.

BEFORE YOU BEGIN:

- Know what an MDL is and what information/data is needed to calculate it.
- Know what a spike recovery is and what information/data is needed to calculate it.
- Understand why it is necessary to determine the MDL and to perform a spike recovery for an analytical method.
- Be familiar with Beer’s law and how to determine concentrations using absorbance data.
- Know what matrix effects are.
- Be comfortable with the calculations and experimental process of making dilutions from stock solutions.

EXPERIMENTAL

Make sure to use acid washed glassware. For all glassware used, rinse with a small volume warmed 1 M HCl (at about 40°C) and then rinse numerous times with D.I. water. ***Start the acid digestion of the lake samples as soon as possible!***

MDL Standard Phosphorus Solution

Prepare 500 ml of a standard phosphorus solution with a concentration equal to the estimated MDL that you calculated in the Prelaboratory Exercises. Use your *Standard Phosphate Solution* (Reagent g) from the “Ascorbic Acid Method for Phosphorus Determination” to prepare this solution. Use distilled water as the blank. Determine the concentration of phosphorus in at least 7 aliquots of this standard solution using the Ascorbic Acid Method for Phosphorus Determination. ***You do not need to digest these samples.*** Use the standard curve you prepare today to calculate the concentrations of the solutions.

Phosphorus Standard Solutions for Calibration Curve

Prepare 6 standard phosphorus solutions ranging in concentration from the estimated MDL to 0.4 mg P/L. Explicitly plan out the concentrations of these solutions and the dilutions it will take to make these solutions in your laboratory notebook *BEFORE* the lab period begins. Prepare these solutions and measure their absorbance while your lake water samples are digesting. ***You do not need to digest these standards.***

Spiked Lake Water Sample

Take an aliquot of at least 100 mL of lake water from the carboy in the lab. Pipette 50 mL of this sample into two 250 mL Erlenmeyer flasks. One sample will be spiked and the other will not. Lake Mendota has a phosphorus concentration of approximately 0.1 mg P/L. The spike should increase the concentration by ~0.1 mg P/L (to make the concentration ~0.2 mg P/L). Add 2.00 mL of your *Standard Phosphate Solution* (~2.5 mg P/L) from the “Ascorbic Acid Method for Phosphorus Determination” lab to one of the 50 mL lake water samples. Accurately calculate the concentration of phosphorus added in the spike to the sample.

Combined Reagent

To prepare 100 mL of the combined reagent mix 50 mL 5N H₂SO₄, 5 mL antimony potassium tartrate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution. **Mix after addition of each reagent and mix in the order given.** If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The combined reagent is stable for ~4 hours.

Persulfate Digestion of Lake Water Samples

Phosphorus in lake water occurs in many forms. The ascorbic acid method only measures orthophosphate. Phosphorus in the lake samples is converted to orthophosphate using the Persulfate Digestion Method from Standards Methods for the Examination of Water and Wastewater (4500-P.B.5.).

- All reagents in the Persulfate Digestion Method will be prepared for you.

5. Persulfate Digestion Method

a. Apparatus:

- 1) *Hot plate:* A 30- × 50-cm heating surface is adequate.
- 2) *Autoclave:* An autoclave or pressure cooker capable of developing 98 to 137 kPa may be used instead of a hot plate.
- 3) *Glass scoop,* to hold required amounts of persulfate crystals.

b. Reagents:

- 1) *Phenolphthalein indicator aqueous solution.*
- 2) *Sulfuric acid solution:* Carefully add 300 mL conc H₂SO₄ to approximately 600 mL distilled water and dilute to 1 L with distilled water.
- 3) *Ammonium persulfate,* (NH₄)₂S₂O₈, solid, or potassium persulfate, K₂S₂O₈, solid.
- 4) *Sodium hydroxide,* NaOH, 1N.

c. *Procedure:* Use 50 mL or a suitable portion of thoroughly mixed sample. Add 0.05 mL (1 drop) phenolphthalein indicator solution. If a red color develops, add H₂SO₄ solution dropwise to just discharge the color. Then add 1 mL H₂SO₄ and either 0.4 g solid (NH₄)₂S₂O₈ or 0.5 g solid K₂S₂O₈.

Boil gently on a preheated hot plate for 30 to 40 min or until a final volume of 10 mL is reached. Organophosphorus compounds such as AMP may require as much as 1.5 to 2 h for complete digestion. Cool, dilute to 30 mL with distilled water, add 0.05 mL (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH. Alternately, heat for 30 min in an autoclave or pressure cooker at 98 to 137 kPa. Cool, add 0.05 mL (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH. Make up to 100 mL with distilled water. In some samples, a precipitate may form at this stage, but do not filter. For any subsequent dividing of the sample, shake well. The precipitate (which is possibly a calcium phosphate) redissolves under the acid conditions of the colorimetric reactive phosphorus test. Determine phosphorus by method C, D, or E, for which a separate calibration curve has been constructed by carrying standards through the persulfate digestion procedure.

- Digest your samples using a hot plate. While digesting, the samples should boil *GENTLY*. Monitor samples during the digestion and add distilled water as necessary to keep the solution volumes approximately the same. This practice avoids over-drying and creating noxious fumes.
- After digestion, bring the volume of the samples back up to 50 mL to match the volume of the original sample.
- Thirty minutes of digestion is sufficient for the lake water samples.

Analyze Standards and Samples for Phosphorus

Combine each 50 mL of each sample with 8 mL of the combined reagent and mix thoroughly. After at least 10 minutes and no more than 30 minutes, measure the absorbance of each solution at 880 nm.

CALCULATIONS AND RESULTS

1. Using EXCEL, prepare a calibration curve using the absorbance values of the calibration standards ranging from the estimated MDL to 0.4 mg P/L. Turn in a copy of your calibration curve along with your notebook and answer sheet.
2. Calculate the MDL Standard Phosphorus Solution concentrations using the calibration curve and determine the method detection limit.
3. Use the calibration curve to determine the concentration of phosphorus in the spiked and unspiked lake water samples and calculate the percent recovery of the spike.

SPIKE RECOVERY AND MDL PRACTICE

Name _____ Course _____ Section _____ Date _____

Concentration of Standard Phosphorus Solution (mg P/L) _____

Calibration Curve

	Concentration ($\mu\text{g P/L}$)	Average Absorbance
Standard 1		
Standard 2		
Standard 3		
Standard 4		
Standard 5		
Standard 6		
Equation from Excel:		

MDL

	Absorbance	Concentration ($\mu\text{g P/L}$)
Aliquot 1		
Aliquot 2		
Aliquot 3		
Aliquot 4		
Aliquot 5		
Aliquot 6		
Aliquot 7		

Standard Deviation of Replicate Aliquots ($\mu\text{g P/L}$) _____

MDL ($\mu\text{g P/L}$) _____

Spike Recovery

Concentration increase from spike ($\mu\text{g P/L}$) _____

Measured P in lake water ($\mu\text{g P/L}$) _____

Measured P in spiked lake water ($\mu\text{g P/L}$) _____

% Recovery _____

STAFF NOTES

Overview

Having familiarity with the Ascorbic Acid method for measuring phosphorus in an aqueous sample, students will now explore some aspects of how a method is validated. They will explore the method detection limit and do a spike recovery analysis, which determines whether the matrix contributes to the absorbance measurement.

Reagents and Equipment

This lab uses the same reagents and equipment as what is listed for the Ascorbic Acid Method. Solid ammonium persulfate, with plastic spoons attached to the bottle, should be available to students. Stockroom staff should also put out large carboys of a lake water sample.

Discussion topics

Go over the details of the persulfate digestion, including safe handling and why its important. You can review the prelaboratory questions too. Here's a nicely worked out solution set, thanks to Amanda Buchberger:

1.) Estimate the MDL of the “Ascorbic Acid Method for Phosphorus Determination” as three times the standard deviation of a method blank. Absorbance values of a method blank and the equation of the calibration line are given in Table 1. Use this value as the estimated DML needed to prepare your MDL Standard Phosphorus Solution. Concentration units are in $\mu\text{g P/L}$.

Blank Aliquot	Absorbance
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Calibration Equation: $y=0.00064x + 0.0029$	

$$\text{Standard Deviation} = 0.00738725$$

$$3x \text{ Standard Deviation} = 3(0.00738725) = 0.02216174$$

$$\text{Absorbance} = 0.00064(\text{Concentration}) + 0.0029$$

$$\text{MDL} = \frac{((0.02216174) - 0.0029)}{0.00064} = 30.964736 \mu\text{g} \frac{\text{P}}{\text{L}} \cong \mathbf{30.1 \mu\text{g} \frac{\text{P}}{\text{L}}}$$

2.) Determine the concentrations and calculate the volumes of *Standard Phosphate Solution* (~2.5 mg P/L) from the “Ascorbic Acid Method for Phosphorus Determination” lab needed to prepare the standard phosphorus solutions for your calibration curve. The concentrations of standards should range from the estimated MDL to 0.4 mg P/L. These calculations should be specific to the actual concentration of the *Standard Phosphate Solution* you made. Make a table in your notebook to reflect how the standards will be prepared.

This should be student specific.

3.) Calculate the increase in phosphorus concentration will be when you add 2.00 mL of your *Standard Phosphate Solution* to 50.00 mL of lake water. Again, the calculation should be specific to your actual concentration of *Standard Phosphate Solution*.

$$M_1V_1 = M_2V_2$$

$$\left(2.5 \text{ mg } \frac{\text{P}}{\text{L}}\right) (2.00 \text{ mL}) = ((50.00 \text{ mL} + 2.00 \text{ mL})) \left(x \text{ mg } \frac{\text{P}}{\text{L}}\right)$$

$$x = 0.09615385 \text{ mg } \frac{\text{P}}{\text{L}} \cong \mathbf{0.096 \text{ mg } \frac{\text{P}}{\text{L}}}$$

Grading Rubric for the Answer Sheet

The five points for the answer sheet are distributed according to the following:

A. Calibration curve (1 point)—salient features include

- Correct units of $\mu\text{g/L}$
- A range of concentrations that span approximately to the MDL on the lower limit and about 400 $\mu\text{g/L}$ on the upper limit.
- The average absorbance scales accordingly (roughly linear with a maximum around 0.4 A for about 400 $\mu\text{g/L}$ P concentration)

B. MDL (2 points)—points should be divided between the data table and the MDL reporting:

- A values should be fairly small and show noise (remember they should be at the lower limit of detection).
- Units should continue to be reported in $\mu\text{g/L}$.
- While the reported MDL can vary quite a bit, typically students should be reporting an MDL in the range of 10-20 $\mu\text{g/mL}$. There should be no more than three significant figures to the MDL they report. This is limited by the noise in the A value.

C. Spike Recover (2 points)

- One point dedicated to the concentration increase from the spike, which should be around 100 $\mu\text{g/L}$.

- The other point is towards the reporting of % Recovery. Compare the difference between P measured in lake water versus spiked sample to the concentration of spike reported. The % recovery should be within 10% of 100. It might fall just outside of that range, which is ok. If they report way outside the range (say below 60 or above 120%) then there is likely a calculation error or a problem with the data or interpretation of these data.