

you-try-it-04.xlsx
Step-by-Step Guide
ver. 8/5/2016

Abstract

This document provides step-by-step instructions for the Excel workbook you-try-it-04.xlsx (saved from Excel 2010). The worksheets contain data for practice exercises keyed to Chapter 4 of:

Basics of Analytical Chemistry and Chemical Equilibria by Brian M. Tissue
(John Wiley: New York, 2013).

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General Advice

It is often useful to work out or outline the first example on each worksheet on paper. After obtaining a result, compare your result to the answer in this guide. Next write formulas on the worksheet to do the calculations. If your formulas do not produce the same answer, try writing them step-by-step and compare intermediate results to calculations on paper to find errors. Try to write formulas to be general so that you can copy them to use for multiple cases.

Version History

7/29/2009	First posting.
9/4/2009	Typo and formatting corrections.
8/5/2016	Formatting revisions.

For updates visit the text support website:

<http://www.achem.org>

4.A conversions

This worksheet contains two sets of wavelength measurements and the accepted energy levels for atomic transitions. The exercise is to determine the vacuum wavelength from the experimental wavelengths measured in air and compare to the accepted transition energies.

Concepts	Concept Synopsis
electromagnetic radiation	We often use different wavelength, frequency, or energy descriptions for photons in different spectral regions. Useful equations are $c=\lambda\nu$ and $E=h\nu$.
spectroscopic transitions	Transitions occur when the photon energy equals, i.e., is resonant with, the difference between energy levels.

1. Convert wavelength measured in air to wavelength in vacuum.

Tables 4.A.1 and 4.A.2 give measured wavelengths for gas-phase Na and K, respectively. As you can see from the number of significant figures, these measurements can be made very precisely. The presumed upper and lower energy levels for the transitions are listed in columns H and I. The photon energy for a spectroscopic transition is the relative energy between the two levels. To remove experimental variables, the tabulated energy levels are given for vacuum conditions. The exercise is to convert the experimental data, measured in air, to vacuum energy and compare to the accepted energy levels. I will convert the air wavelength to vacuum wavelength and then to energy. Working backwards from the energy levels to air wavelength is another way to compare the data.

First convert the air wavelength to frequency. The frequency of a photon does not change as the photon travels through different media. The speed of light in air is given in cell H19. Using the first row in Table 4.A.1 as a working example, we calculate frequency with $c=\lambda\nu$:

$$\nu = \frac{c_{\text{air}}}{\lambda_{\text{air}}} = \frac{299704645 \text{ m/s}}{2852.810 \text{ \AA}} \cdot \frac{10^{10} \text{ \AA}}{1 \text{ m}} = 1.050559 \times 10^{15} \text{ s}^{-1}$$

Now dividing the speed of light in a vacuum by frequency gives the wavelength in vacuum.

$$\lambda_{\text{vac}} = \frac{c_{\text{vac}}}{\nu} = \frac{299792458 \text{ m/s}}{1.050559 \times 10^{15} \text{ s}^{-1}} \frac{10^{10} \text{ \AA}}{1 \text{ m}} = 2853.646 \text{ \AA}$$

Looking at the two expressions above, you can combine them to eliminate frequency and substitute the refractive index, n_{air} , for $c_{\text{vac}}/c_{\text{air}}$. I've done the calculation in two steps simply to show the concept of using the relationship between λ and ν .

To get these calculations into the worksheet, I've entered the conversion between angstrom, \AA , and meter, m, in cell D19, the speed of light in a vacuum in cell F19, and the refractive index of air in cell G19. The speed of light in air is the vacuum speed divided by n_{air} . In cell H19 I type the formula:

$$=F19/G19$$

To find frequency from the given wavelength, in cell D26 I type:

$$=(\$H\$19/C26)*\$D\$19$$

Now find the vacuum wavelength from the frequency. Now using the speed of light in a vacuum and the conversion between \AA and m, in cell E26 I type:

$$=(\$F\$19/D26)*\$D\$19$$

These two formulas can be copied to the other rows of the table, but I'll wait and do all of the empty cells after completing question 2. The result so far is shown in the following figure:

	A	B	C	D	E	F	G	H	I
17									
18		conversions/constants:		$\text{\AA}/\text{m}$	$\text{\AA}/\text{cm}$	$c_{\text{vac}} \text{ (m/s)}$	$n \text{ (air)}$	$c_{\text{air}} \text{ (m/s)}$	
19				1.0E+10	1.0E+08	299792458	1.000293	299704645	
20									
21									
22		Table 4.A.1							
23		Persistent Lines of Neutral Sodium (Na I)							
24			$\lambda \text{ (air)}$	Frequency	$\lambda \text{ (vac)}$	$E \text{ (meas)}$	$E \text{ (theor)}$	Energy Levels (cm^{-1})	
25		Intensity	(\AA)	(s^{-1})	(\AA)	(cm^{-1})	(cm^{-1})	lower	upper
26		5	2852.810	1.0506E+15	2853.646			0.000	35042.850

2. Convert the vacuum wavelength to energy in cm^{-1} .

Energy expressed in cm^{-1} is simply the inverse of wavelength. Find the vacuum energy by taking the inverse the vacuum wavelength and converting units to cm^{-1} (10^8 \AA per 1 cm):

$$\frac{1}{2853.646 \text{ \AA}} \frac{10^8 \text{ \AA}}{1 \text{ cm}} = 35042.89 \text{ cm}^{-1}$$

I've typed the conversion $1\text{E}8$ (\AA per cm) in cell E19. In cell F26 I enter the formula:

$$=(1/E26)*\$E\$19$$

This value can be compared directly to the value in cell I26 because the level in cell H26 is the ground state. In general, the transition energy is the difference between energy levels. In cell G26 I write the following formula for the energy difference between the two levels:

$$=I26-H26$$

Now to copy the formulas for the other cases, select cells D26:G26 and press Ctrl-C. Select cells D27:G31 and D37:G43 and press Ctrl-V. Adding formatting and highlights to the tables produces the following results:

	A	B	C	D	E	F	G	H	I
22	Table 4.A.1								
23	Persistent Lines of Neutral Sodium (Na I)								
24		λ (air)	Frequency	λ (vac)	E (meas)	E (theor)	Energy Levels (cm^{-1})		
25	Intensity	(\AA)	(s^{-1})	(\AA)	(cm^{-1})	(cm^{-1})	lower	upper	
26	5	2852.810	1.0506E+15	2853.646	35042.89	35042.850	0.000	35042.850	
27	2	2853.010	1.0505E+15	2853.846	35040.43	35040.380	0.000	35040.380	
28	15	3302.370	9.0754E+14	3303.338	30272.41	30272.580	0.000	30272.580	
29	8	3302.980	9.0738E+14	3303.948	30266.82	30266.990	0.000	30266.990	
30	1000	5889.950	5.0884E+14	5891.676	16973.10	16973.368	0.000	16973.368	
31	500	5895.924	5.0833E+14	5897.652	16955.90	16956.172	0.000	16956.172	
32									
33	Table 4.A.2								
34	Persistent Lines of Neutral Potassium (K I)								
35		λ (air)	Frequency	λ (vac)	E (meas)	E (theor)	Energy Levels (cm^{-1})		
36	Intensity	(\AA)	(s^{-1})	(\AA)	(cm^{-1})	(cm^{-1})	lower	upper	
37	700	4044.140	7.4108E+14	4045.325	24719.89	24720.139	0.000	24720.139	
38	700	4047.210	7.4052E+14	4048.396	24701.14	24701.382	0.000	24701.382	
39	1000	7664.899	3.9101E+14	7667.145	13042.66	13042.876	0.000	13042.876	
40	1000	7698.965	3.8928E+14	7701.220	12984.96	12985.170	0.000	12985.170	
41	700	11690.210	2.5637E+14	11693.635	8551.661	8551.818	12985.170	21536.988	
42	600	11769.620	2.5464E+14	11773.068	8493.962	8494.112	13042.876	21536.988	
43	700	11772.830	2.5457E+14	11776.279	8491.646	8491.804	13042.876	21534.680	

4.B absorbance

This worksheet contains light absorption measurements for a water sample from a swimming pool. Measurement of a standard solution provides calibration to determine the copper concentration in the unknown solution.

Concepts	Concept Synopsis
light absorption measurements	Transmittance, T , is the fraction of light power with and without an absorber: P/P_0 . Percent transmittance, % T , is $T \times 100$ %. Absorbance is $-\log(T)$.
Beer-Lambert law	Absorbance is directly proportional to absorber concentration.

1. Calculate T , % T , and A for the measurement.

Transmittance is the fraction of light that is measured when an absorber is placed in the light path. For our lux measurements that is:

$$T = \frac{211 \text{ lux}}{399 \text{ lux}} = 0.529$$

Being a ratio, transmittance is a unitless number. % T is this value times 100 %, or 52.9 %. Absorbance is $-\log(T)$:

$$A = -\log(0.529) = 0.277$$

Note that A is also a unitless number. In cells E23:E25 enter the labels $T =$, % $T =$, and $A =$. In cells F23:F25 I enter the formulas:

=C25/C24

=F23*100

=-log(F23)

The results should be the same values as above (see figure on next page).

2. Calculate the copper concentration in the pool water given the calibration data above.

The calibration data is an absorbance of 0.727 for a 1.0 ppm Cu²⁺ standard solution. Since absorbance is directly proportional to concentration, we can solve for the unknown concentration with a simple proportionality:

$$\frac{C_{\text{unk}}}{A_{\text{unk}}} = \frac{C_{\text{std}}}{A_{\text{std}}}$$

$$\frac{C_{\text{unk}}}{0.277} = \frac{1.0 \text{ ppm}}{0.727}$$

$$C_{\text{unk}} = 0.38 \text{ ppm Cu}^{2+}$$

The calibration data is embedded in text. Retype the values in cells C29 (0.727) and D29 (1.0). Add labels and in cell C30 enter the formula:

=F25

Calculate the unknown concentration in cell D30 with the formula:

= D29*C30/C29

Add some formatting to highlight the result and you are done:

	A	B	C	D	E	F	G
20							
21		Table 4.B.1					
22		sample	measurement (lux)				
23		nothing in the light path	450			T = 0.529	
24		test tube of pool water	399			% T = 52.9	%
25		pool water + reagent	211			A = 0.277	
26							
27							
28			Absorbance	Cu²⁺ conc			
29		standard	0.727	1.0	ppm		
30		unknown	0.277	0.38	ppm		
31							

3. Use the repetitive measurements to predict the LOD and LOQ for this method.

Recall from chapter 1 that LOD is the concentration that produces a signal 3×noise and LOQ is the concentration at 10×noise. The noise level is measured by the standard deviation of the blank, which in this case is the pool water before adding reagent. In cells C39 and C40 type:

=AVERAGE(C35:C38)

=STDEV(C35:C38)

The results are 397 ± 7 . Since we are measuring the decrease in light intensity, the light measurements for the LOD (blank – 3×std dev) and LOQ (blank – 10×std dev) are:

LOD: $397 - 21 = 375$

LOQ: $397 - 73 = 324$

(The 73 results from the standard deviation actually being 7.26.) Following the calculations from question 1, the corresponding transmittance, absorbance, and concentration values are:

	lux	T	A	conc (ppm)
LOD	375	0.945	0.024	0.034
LOQ	324	0.817	0.088	0.12

In cells B43 and B44 enter the labels LOD and LOQ. In cells C43 and C44 enter the formulas:

= $\$C\$39-3*\$C\40

= $\$C\$39-10*\$C\40

To calculate transmittance divide these values by the average of 397 lux. In col D enter:

= $C43/\$C\39

= $C44/\$C\39

Likewise for absorbance take the –log. In col E enter:

= $-\log(D43)$

= $-\log(D44)$

Now use the calibration data to convert the absorbance values to concentration, as we did for solving the concentration of the unknown solution. In col F enter:

= $E43*\$D\$29/\$C\29

$$= E44 * \$D\$29 / \$C\$29$$

The overall results will look something like:

	A	B	C	D	E	F	G
32							
33		Table 4.B.2 (pool water only)					
34		trial	measurement (lux)				
35		1	399				
36		2	389				
37		3	406				
38		4	394				
39		average	397.0				
40		stdev	7.3				
41							
42			lux	T	A	conc	
43		LOD	375	0.945	0.024	0.03	ppm
44		LOQ	324	0.817	0.088	0.12	ppm

These results show that we can measure copper in the water samples from approximately 0.1 to 1.0 ppm. Not bad for a home-built instrument.

If you wish you can replace the single 399 lux value used in part 1 with the 397 lux average to see if it makes a difference in the final result for question 2. The formulas should all be correct so that changing any of the data values should not require rewriting formulas.

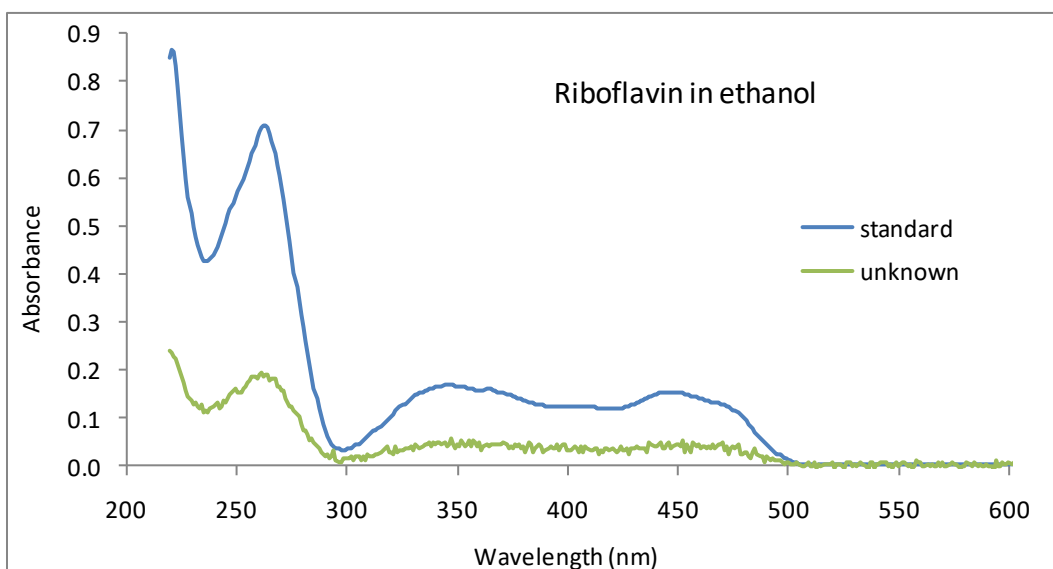
4.C Beers-law

This worksheet contains the absorption spectrum of riboflavin. We will use this reference spectral data to extract calibration information to then determine an unknown concentration.

Concepts	Concept Synopsis
Beer-Lambert law	The Beer-Lambert law is $A = \epsilon bc$.

1. Insert a scatter chart to plot both data sets and adjust axes and formatting.

Select the standard data in Table 4.C.1 and insert a scatter chart. Add a chart title, axis labels, and adjust the axes scales. Next select the unknown data in Table 4.C.2 (A data only), press Ctrl-C, click on the existing chart, and press Ctrl-V to paste the unknown plot into the chart. The result will be something like the following figure. I adjusted the x axis maximum in the figure to better show the useful peaks in the spectra.



The main utility of plotting the spectral data is to check that the overall shape of the curves are the same. Additional peaks in the unknown spectrum could indicate the presence of interferences. Many substances absorb in the UV region, and in this case the strongest riboflavin absorption is near 260 nm.

To check that the unknown spectrum matches the standard spectrum, right-click on the unknown plot, choose Format Data Series..., select the Secondary Axis button, and click the Close button. You can change the scale of the secondary axis to make sure that the unknown curve truly matches the standard. When you are done, undo the changes to reset both plots to the same y scale. In the event that another absorber overlaps with the peak in the UV, but not in the visible, the peak near 450 nm can be used for quantitative measurements. The disadvantage of using the weaker peak near 450 nm is that it provides a lower sensitivity.

- Determine the maximum absorbance values for the peaks near 260 and 450 nm. Calculate the molar absorptivity for each peak wavelength.

Because the peaks for molecules in solution are usually broad, it is not always obvious where the true peak maximum occurs. However, the broad peaks are advantageous in that the change in absorbance near the maximum is small. The slight dependence on wavelength makes the measurements less error-prone when selecting the exact peak position.

The data in Tables 4.C.1 and 4.C.2 near the 260-nm peak are reproduced below. For both data sets, we can see that the peak in the UV is at 263 nm.

260	0.6940		260	0.1903
261	0.7030		261	0.1933
262	0.7086		262	0.1846
263	0.7095		263	0.1916
264	0.7042		264	0.1882
265	0.6920		265	0.1760
266	0.6734		266	0.1806
267	0.6509		267	0.1806
268	0.6269		268	0.1667

Now looking at the data for the peak near 450 nm, the peak is more difficult to determine due to being broader. The highest absorbance value occurs at 447 nm for the standard solu-

442	0.1523		442	0.0492
443	0.1528		443	0.0368
444	0.1533		444	0.0369
445	0.1539		445	0.0433
446	0.1542		446	0.0479
447	0.1544		447	0.0349
448	0.1541		448	0.0421
449	0.1534		449	0.0463
450	0.1526		450	0.0437
451	0.1516		451	0.0417

tion, but the fluctuations in the unknown sample makes the peak less clear. We'll use the absorbance values at 447 nm and also take average values between 446 – 448 nm.

To get averages, enter the following formulas:

=AVERAGE(C275:C277)

=AVERAGE(F275:F277)

	A	B	C	D	E	F	G
274		445	0.1539		445	0.0433	
275		446	0.1542		446	0.0479	
276		447	0.1544	0.1542	447	0.0349	0.0416
277		448	0.1541		448	0.0421	
278		449	0.1534		449	0.0463	

I copy and paste the absorbance values of the standard and unknown solutions to a new table:

	A	B	C	D	E
32					
33		λ_{\max} (nm)	A_{std}		A_{unk}
34		263	0.7095		0.1916
35		447	0.1544		0.0349
36		447*	0.1542		0.0416
37		* average A values from 446-448 nm			

Table 4.C.1 contains the Beer-Lambert law information to calculate the molar absorptivity, ϵ , at each wavelength. Using the Beer-Lambert law, $A = \epsilon bc$, for a 1.00-cm path length and the known concentration of 2.00×10^{-5} M, the calculation for the molar absorptivity at 263 nm is:

$$0.7095 = \epsilon_{263}(1.00 \text{ cm})(2.00 \times 10^{-5} \text{ M})$$

$$\epsilon_{263} = 35475 \text{ M}^{-1} \text{ cm}^{-1}$$

In cell D33 I add a label for epsilon. In cell D34 I enter the formula:
 $=C34/(\$C\$44*\$C\$45)$

I copy this formula down two rows. The results are:

	A	B	C	D	E
32					
33		λ_{\max} (nm)	A_{std}	ϵ ($\text{M}^{-1} \text{ cm}^{-1}$)	A_{unk}
34		263	0.7095	35475	0.1916
35		447	0.1544	7721	0.0349
36		447*	0.1542	7711	0.0416
37		* average A values from 446-448 nm			

3. Determine the concentration of the unknown solution.

Using the strongest peak at 263 nm will provide the most sensitive measurement. I will also determine the concentration using the 447 nm peak as a check for any interfering absorbance at 263 nm. The calculation is similar to the previous one, but now I know ϵ and I solve for the unknown concentration. Entering the absorbance at 263 nm for the unknown solution and the molar absorptivity in the Beer-Lambert law:

$$0.1916 = (35475 \text{ M}^{-1} \text{ cm}^{-1})(1.00 \text{ cm}) c_{\text{unk}}$$

$$c_{\text{unk}} = 5.40 \times 10^{-6} \text{ M}$$

Looking at the two spectra and noting that the unknown peak is approximately 30 % of the intensity of the standard, this result makes sense compared to the concentration of the standard solution.

In cell F33 I add the label c_{unk} , and in cell F34 I enter the formula:
`=E34/(D34*F44)`

Again I copy this formula down two rows. As in the absorptivity calculation, I include the path length, $\$C\45 and $\$F\44 , but they could be omitted since the values are 1.00. After formatting the cells and adding unit labels, the result will look something like the following figure:

	A	B	C	D	E	F	G
32							
33		λ_{max} (nm)	A_{std}	ϵ ($\text{M}^{-1}\text{cm}^{-1}$)	A_{unk}	c_{unk} (M)	
34		263	0.7095	35475	0.1916	5.40E-06	M
35		447	0.1544	7721	0.0349	4.52E-06	M
36		447*	0.1542	7711	0.0416	5.40E-06	M
37		* average A values from 446-448 nm					

Note that averaging several values for the 447 nm data gives a result that agrees with the measurement at 263 nm. Using only the single value gives a different result. In looking at the data, the random noise

The software that controls spectrometers often has data analysis capability. The software works well, but it is up to the operator to set how much averaging or smoothing the software is doing to ensure reasonable results.

4.D fluorescence

This worksheet contains a table of fluorescence measurements for riboflavin standards and an unknown.

Concepts	Concept Synopsis
fluorescence	The fluorescence intensity will be directly proportional to an-alyte concentration at low concentration.

- Determine the mean and standard deviation of all repetitive measurements. Create a calibration curve of the fluorescence vs concentration.

In cells H22 and I22 type the labels 'average' and 'std dev,' respectively, and in H23 and I23 enter the formulas:

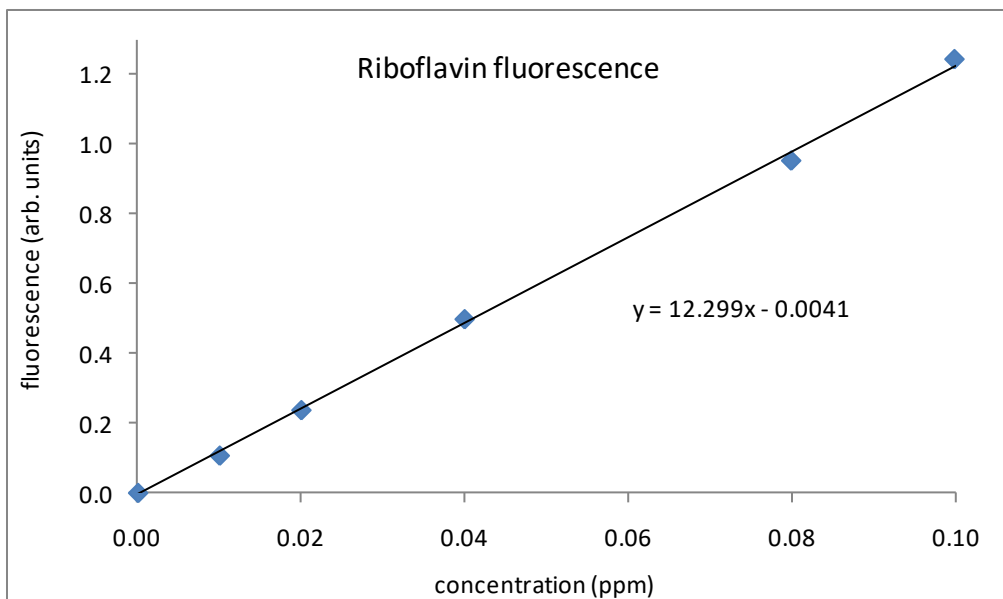
=AVERAGE(C23:F23)

=STDEV(C23:F23)

Copy these formulas down to row 29 and the result will be something like the following figure:

	A	B	C	D	E	F	G	H	I
20		Table 4.D.1 Fluorescence Measurements							
21									
22		conc (ppm)	1	2	3	4		average	std dev
23		0.00	0.01	0.00	0.00			0.003	0.006
24		0.01	0.11	0.11	0.11			0.110	0.000
25		0.02	0.24	0.24	0.24			0.240	0.000
26		0.04	0.50	0.50	0.50			0.500	0.000
27		0.08	0.96	0.97	0.93			0.953	0.021
28		0.10	1.25	1.24	1.24			1.243	0.006
29		unknown	0.75	0.74	0.77	0.77		0.758	0.015

Highlight the data in cells B23:B28 and in H23:H28. Insert a scatter chart and add axis labels and a trendline. In the following figure I've also added a chart label and displayed the trendline equation on the chart:



The main reason to plot the calibration data is to check that the measurement is linear over the concentration range of interest. In this case we can be sure that the instrument does respond linearly from zero to 0.10 ppm riboflavin.

2. Determine the riboflavin concentration in the unknown solution.

Using LINEST to find the slope and intercept with their uncertainties. Select cells B31:C36, type:

```
=LINEST(H23:H28,B23:B28,TRUE,TRUE)
```

and press Ctrl-Shift-Enter. The results show that the uncertainty in the intercept is larger than the value, so we are justified to force the intercept through 0,0. With cells B31:C36 still highlighted, change the first TRUE parameter to FALSE in the LINEST formula and again press Ctrl-Shift-Enter:

```
=LINEST(H23:H28,B23:B28,FALSE,TRUE)
```

Add a title above the results and you'll have something like the figure to the right. Note that the slope is only slightly different compared to the trendline or the first LINEST results (12.299).

LINEST slope:	
12.243	0
0.120	#N/A
1.000	0.016
10357.116	5
2.773	0.001

The unknown concentration is found from the linear equation:

$$y = 12.243x + 0.0$$

Entering the unknown measurement of 0.758 for y we obtain:

$$0.758 = (12.243 \text{ ppm}^{-1}) x$$

$$x = 0.758 / (12.243 \text{ ppm}^{-1})$$

$$x = 0.0619 \text{ ppm riboflavin}$$

We can propagate the error for this calculation:

$$\frac{s_x}{x} = \frac{s_y}{y} + \frac{s_{\text{slope}}}{\text{slope}}$$

where s is standard deviation, and the x , y , and slope refer to the terms in the linear equation. Entering the values that we have:

$$\frac{s_x}{0.0619 \text{ ppm}} = \frac{0.015}{0.758} + \frac{0.120}{12.243}$$

$$s_x = 0.0018 \text{ ppm}$$

We can now report our unknown as (0.062 ± 0.002) ppm riboflavin. To perform this calculation in Excel, in cells F31 and H31 enter the labels 'c_{unk}' and 'std dev' and in cell I32 enter 'ppm.' In cell F32 find the unknown concentration with the formula: =H29/B32

In cell H32 enter the following formula to propagate error to find the standard deviation of the unknown concentration:

$$=(I29/H29+B33/B32)*F32$$

These formulas should produce the results as above (see figure below).

3. Determine the riboflavin concentration and uncertainty in the unknown dry sample.
The sample size of the dry powder was 1.080 g.

There are usually numerous ways to account for dilution in a sample preparation procedure. For this particular sample, a useful format will be to report the result as a weight of riboflavin per gram of formula. The measured result is the concentration in 50.0 mL of solution. The amount of riboflavin in this solution is:

$$\frac{0.0619 \text{ } \mu\text{g riboflavin}}{\text{mL solution}} \times 50.0 \text{ mL solution} = 3.10 \text{ } \mu\text{g riboflavin}$$

Since all of the sample was used to make the measured solution, this result represents the total amount of riboflavin in the dry powder. Since we started with 1.080 g of sample, the concentration is:

$$\frac{3.10 \mu\text{g riboflavin}}{1.080 \text{ g powder}} = 2.87 \mu\text{g riboflavin/g}$$

The relative uncertainty does not change compared to the measured solution, so the uncertainty in this result is

$$\frac{(0.0018 \text{ ppm})(50.0 \text{ mL solution})}{1.080 \text{ g powder}} = 0.083 \mu\text{g riboflavin/g}$$

The final result is $(2.9 \pm 0.1) \mu\text{g riboflavin/g formula}$.

In cell F34 I enter 1.080, the dry weight of infant formula. In cells F36 and H36 I enter the formulas:

=F32*50/\$F\$34

=H32*50/\$F\$34

Adding appropriate labels and formatting produces something similar to the following figure:

28		0.10	1.25	1.24	1.24			1.243	0.006
29		unknown	0.75	0.74	0.77	0.77		0.758	0.015
30									
31		LINEST slope:				c_{unk}		std dev	
32		12.243	0			0.0619	±	0.0018	ppm
33		0.120	#N/A						
34		1.000	0.016			1.080	g	dry infant formula	
35		10357.116	5			c_{unk}		std dev	
36		2.773	0.001			2.9	±	0.1	ppm
37									