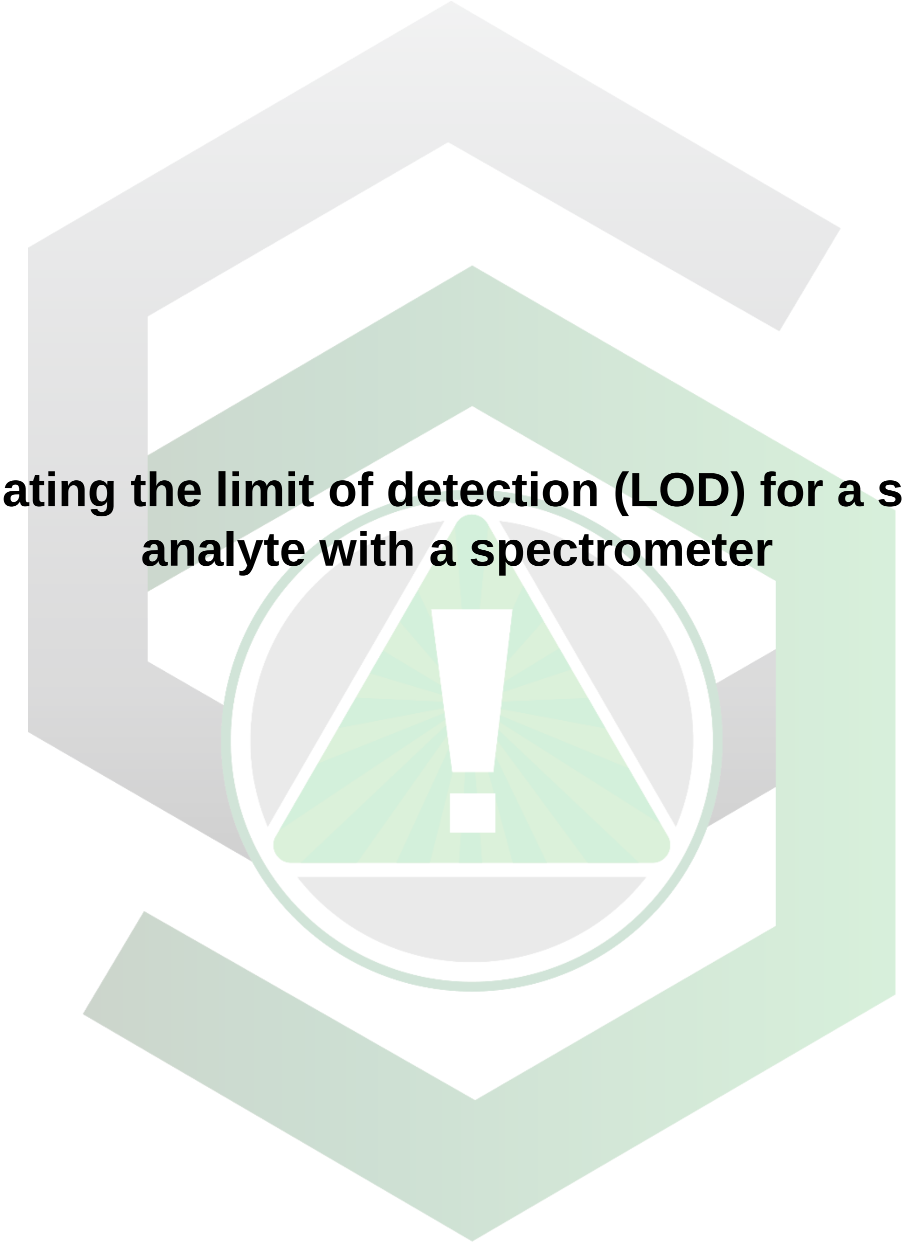

Calculating the limit of detection (LOD) for a specific analyte with a spectrometer

Created by: Francheska Colon, Naihomy Tirado, Edwin Caballero

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SOP-01	Francheska Colon Naihomy Tirado, Edwin Caballero		University of Puerto Rico at Mayagüez
Effectivity: July/10/2022	Calculating the limit of detection (LOD) for a specific analyte with a spectrometer		Revised by:
Revised:			Approved by:

This SOP uses the following:

- Equipment: Desired spectrometer.
- Instruments: Volumetric flask, pipettes, and sample container.
- Programs: Microsoft Excel**.**
- Reagents: Analyte and solvent of interest.

I. PLANNING INITIAL CONCENTRATION RANGE SCREENING

1. **Research** the LOD of the analyte of interest for the phenomenon and technique desired.

E.g., LOD for analyte “X” is 0.400 M.

2. **Determine** the upper limit (UL) by multiplying the LOD by 5.

$$E.g., \quad UL = LOD \cdot 5 \quad \rightarrow \quad UL = 0.400\ M \cdot 5 \quad \rightarrow \quad UL = 2.00\ M$$

3. **Determine** the increments by subtracting the UL from the lower limit (LL) and dividing by 4.

$$Increments = \frac{UL - LL}{4} \quad \rightarrow \quad Increments = \frac{(2.00 - 0.400)\ M}{4} \quad \rightarrow \quad Increments = 0.400\ M$$

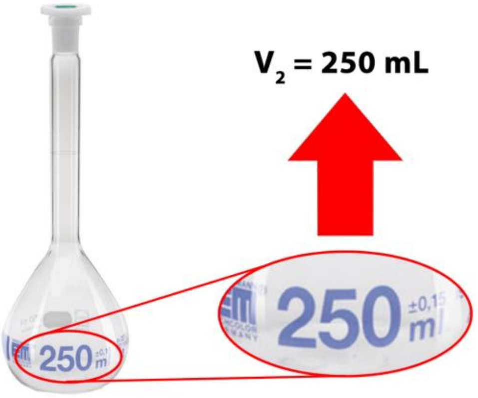
2.00 M	1.60 M	1.20 M	0.800 M	0.400 M

4. **(OPTIONAL)** IF LOD of the analyte of interest is not found, use the range from 50% to 10% at 10% increments.

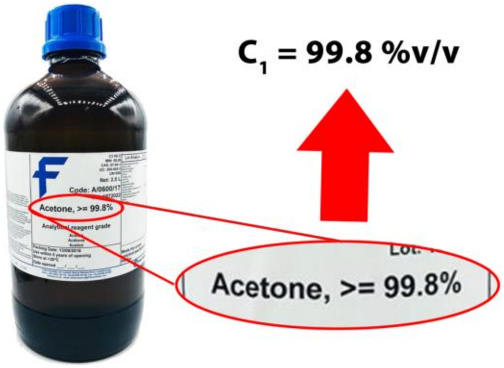
50%	40%	30%	20%	10%

5. **Determine** the volume of the available flasks and the amount per volume.

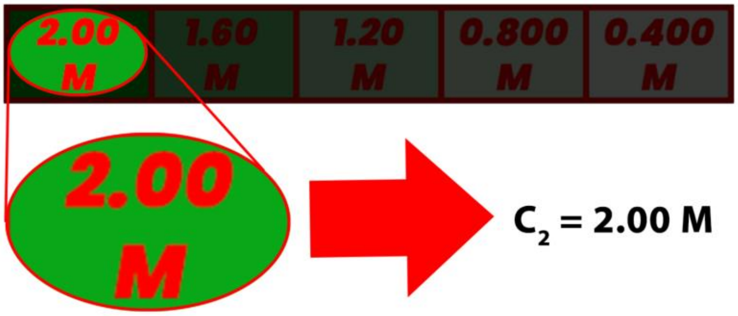
6. **Determine** the final volume of your solution (V2), i.e., the flask that will be used for the solution.



7. **Find** the initial concentration (C1) of the analyte of interest.



8. **Determine** the desired concentration of your solution (C2).



9. **Determine** the volume needed for analyte with concentration C1 to reach the desired concentration (V1) with a final volume of V2.

$$V_1 = \frac{C_2 \cdot V_2}{C_1}$$

10. **(OPTIONAL IF AN INTEGER V1 VALUE IS NEEDED) Determine** the final concentration C2 for the analyte with an initial concentration C1 after adding a specific initial volume V1, close to the value obtained from step 6, and completing to a final volume of V2.

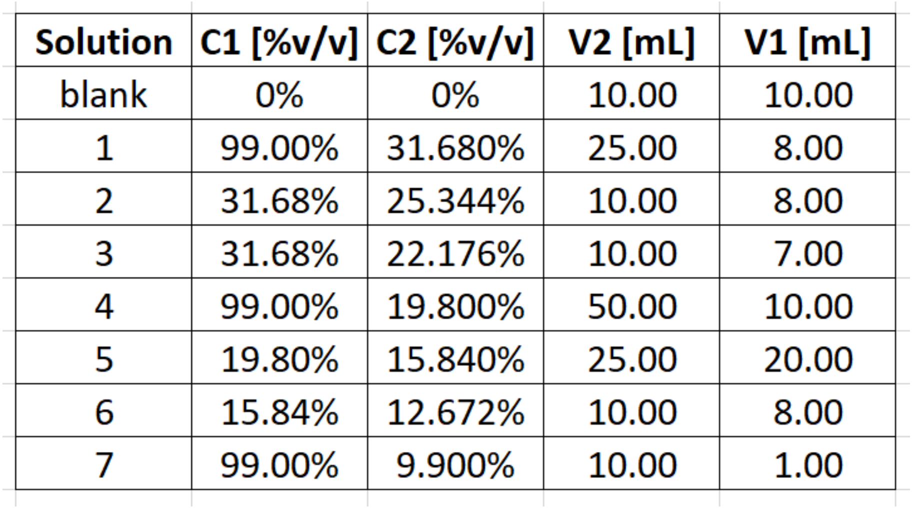
$$C_2 = \frac{C_1 \cdot V_1}{V_2}$$

11. **Repeat** steps 3-7 for the other solutions.

IF an integer value of V1 cannot be obtained for a solution with the initial C1 concentration of the analyte of interest, use the final concentration C2 of a solution as the initial concentration C1 to determine the volume.

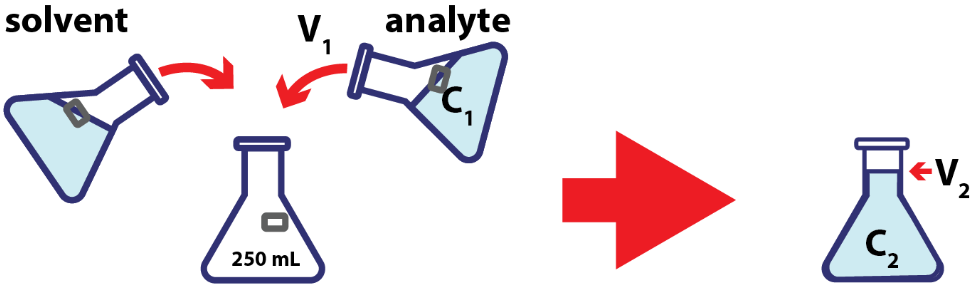
Solution used to create other solutions might be consumed, create more, or change V2 to a bigger value.

E.g.



Solution	C1 [%v/v]	C2 [%v/v]	V2 [mL]	V1 [mL]
blank	0%	0%	10.00	10.00
1	99.00%	31.680%	25.00	8.00
2	31.68%	25.344%	10.00	8.00
3	31.68%	22.176%	10.00	7.00
4	99.00%	19.800%	50.00	10.00
5	19.80%	15.840%	25.00	20.00
6	15.84%	12.672%	10.00	8.00
7	99.00%	9.900%	10.00	1.00

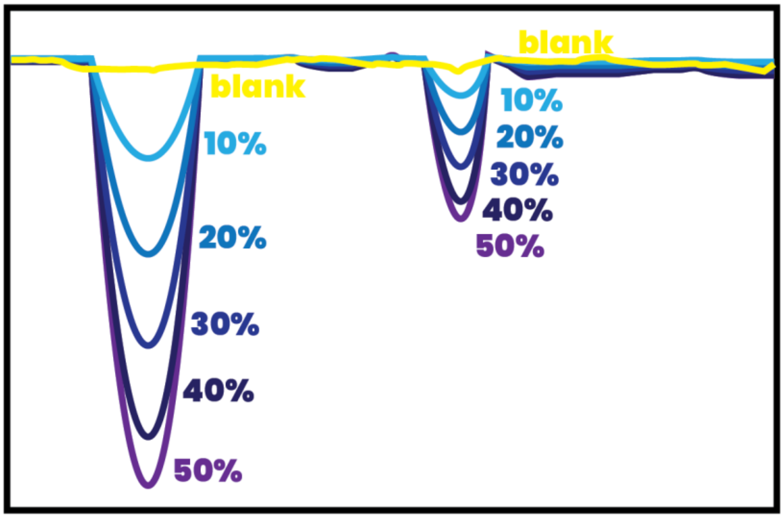
12. **Prepare** solutions by adding the initial volume of analyte (V1) of C1 concentration into the volumetric flask with the final volume (V2) to obtain the final concentration C2.



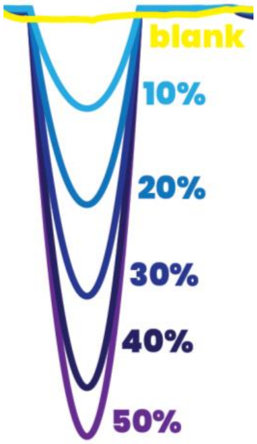
II. SCREENING LOD CONCENTRATION RANGE FOR ANALYTE

1. **Acquire** spectra for each concentration and blank.

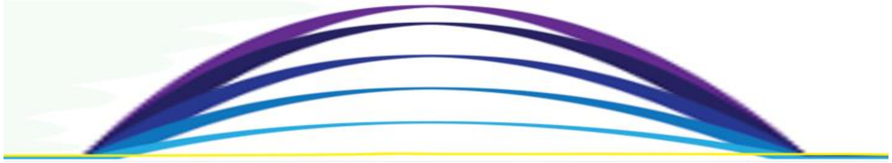
10 measurements per sample. Average the measurements.

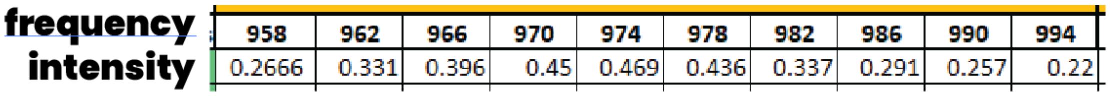


2. **Determine** the peak with intensity proportional to the analyte concentration.



3. **Limit** spectral data to the frequencies of the chosen spectral band.

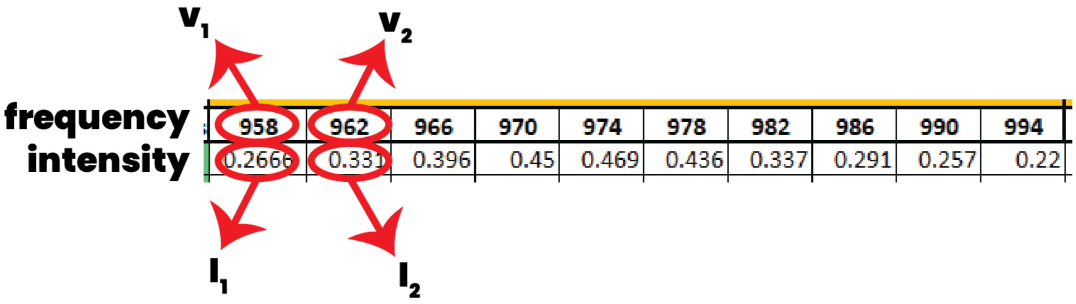




frequency	958	962	966	970	974	978	982	986	990	994
intensity	0.2666	0.331	0.396	0.45	0.469	0.436	0.337	0.291	0.257	0.22

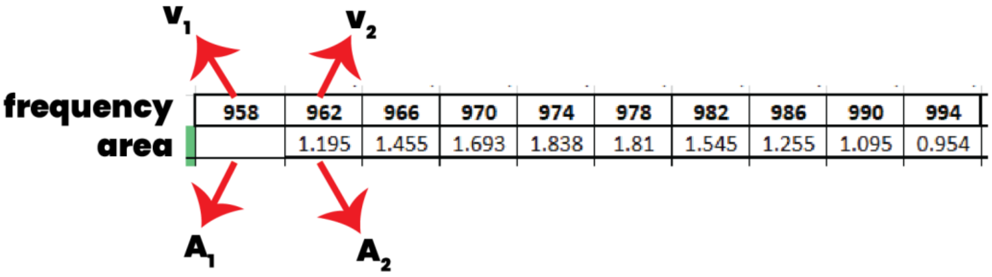
4. **Calculate** the area of the chosen peak with the first frequency (ν_1) and intensity (I_1) as well as the second frequency (ν_2) and intensity (I_2) with the following equation.

$$A = (\nu_2 - \nu_1) \cdot \left(\frac{I_1 + I_2}{2}\right)$$



frequency	958	962	966	970	974	978	982	986	990	994
intensity	0.2666	0.331	0.396	0.45	0.469	0.436	0.337	0.291	0.257	0.22

5. **Repeat** step 4 until the area for each frequency is calculated (first frequency does not contain area).



frequency	958	962	966	970	974	978	982	986	990	994
area		1.195	1.455	1.693	1.838	1.81	1.545	1.255	1.095	0.954

6. **Add** the area for each frequency to obtain the area of the peak (PA).

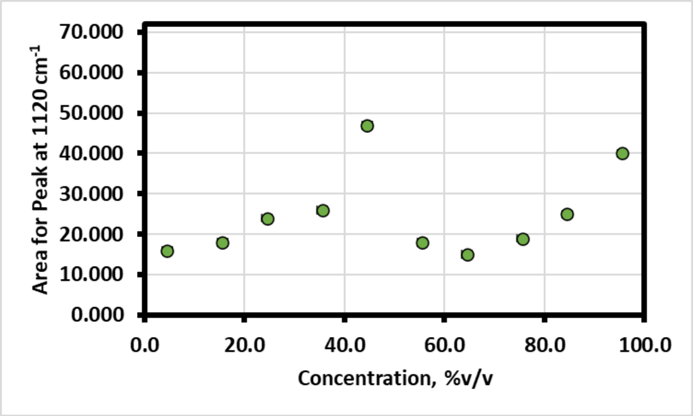
$$PA = \sum_{i=1}^{n} [A_i]$$

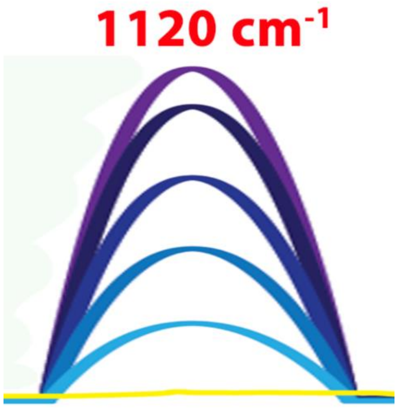
$$\rightarrow \quad PA = 0 + 1.195 + 1.455 + 1.693 + 1.838 + 1.810 + 1.545 + 1.255 + 1.095 + 0.954$$

$$\rightarrow \quad PA = 12.841$$

7. **Repeat** steps 4-6 for all samples.

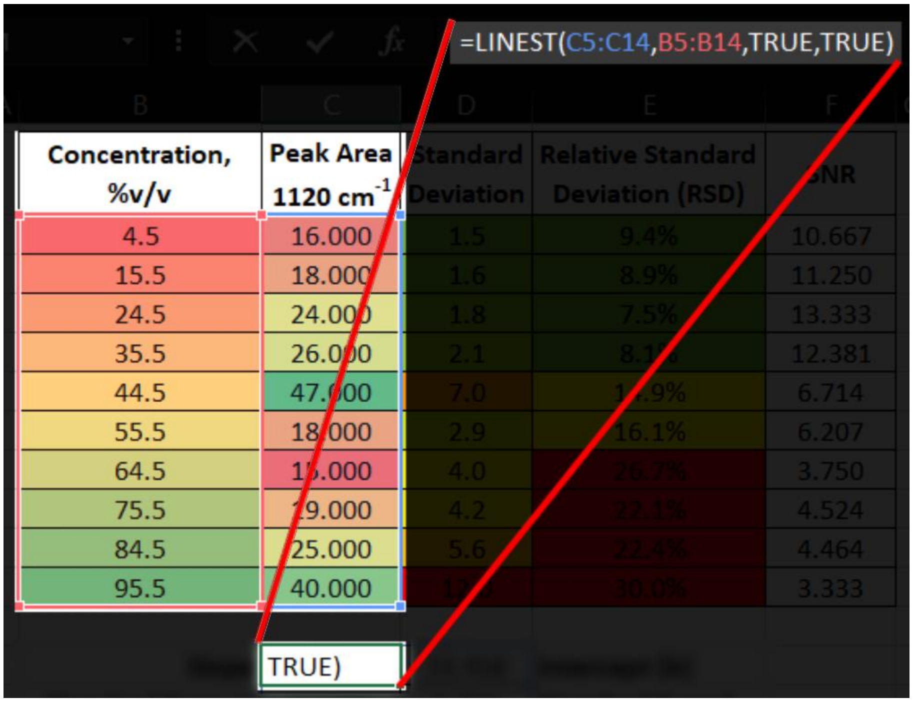
8. **Plot** peak area vs concentration.





9. **Calculate** slope and intercept error by using the $= LINEST$ function.

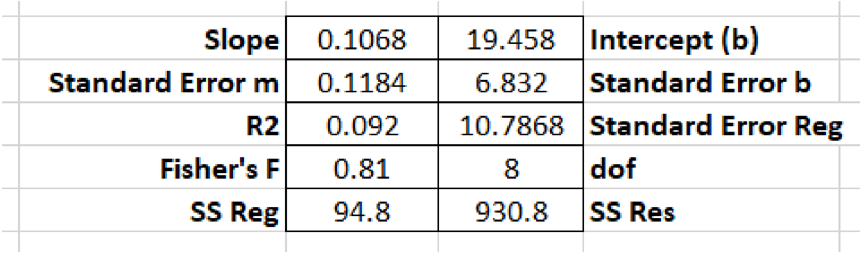
Select the 'y' values, then the 'x' values, write "TRUE" twice, and click enter. A range of values will appear below (2 columns and 5 rows).



=LINEST(C5:C14,B5:B14,TRUE,TRUE)

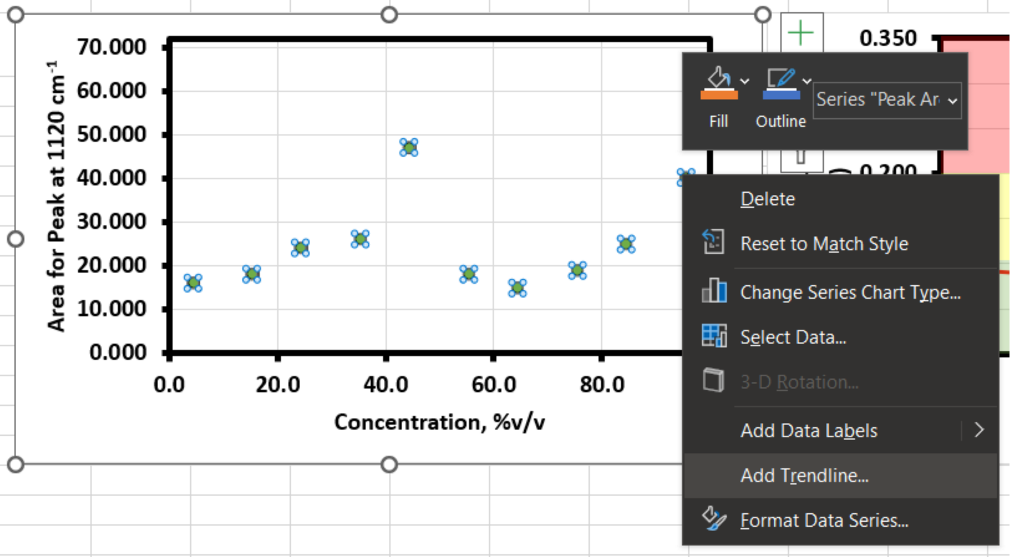
Concentration, %v/v	Peak Area 1120 cm^{-1}	Standard Deviation	Relative Standard Deviation (RSD)	SNR
4.5	16.000	1.5	9.4%	10.667
15.5	18.000	1.6	8.9%	11.250
24.5	24.000	1.8	7.5%	13.333
35.5	26.000	2.1	8.1%	12.381
44.5	47.000	7.0	14.9%	6.714
55.5	18.000	2.9	16.1%	6.207
64.5	15.000	4.0	26.7%	3.750
75.5	19.000	4.2	22.1%	4.524
84.5	25.000	5.6	22.4%	4.464
95.5	40.000	12.0	30.0%	3.333
	TRUE)			

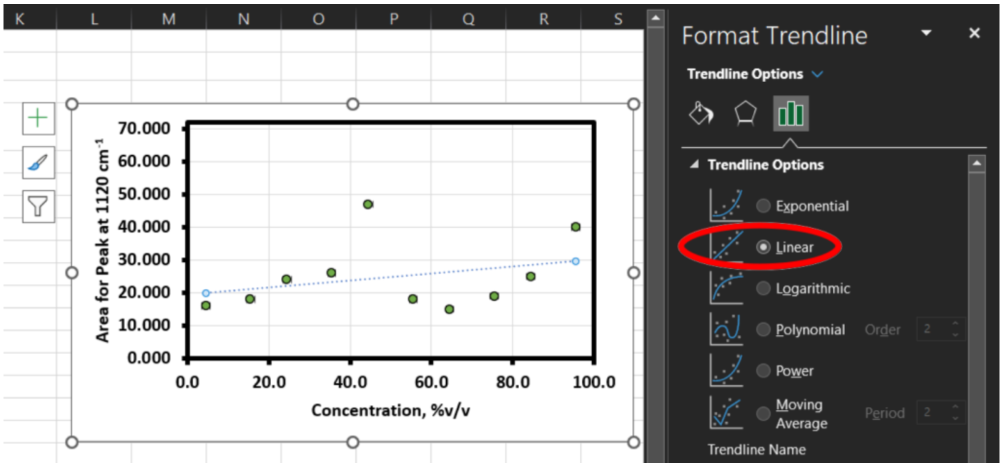
Names will not appear.



Slope	0.1068	19.458	**Intercept (b)**
Standard Error m	0.1184	6.832	**Standard Error b**
R2	0.092	10.7868	**Standard Error Reg**
Fisher's F	0.81	8	**dof**
SS Reg	94.8	930.8	**SS Res**

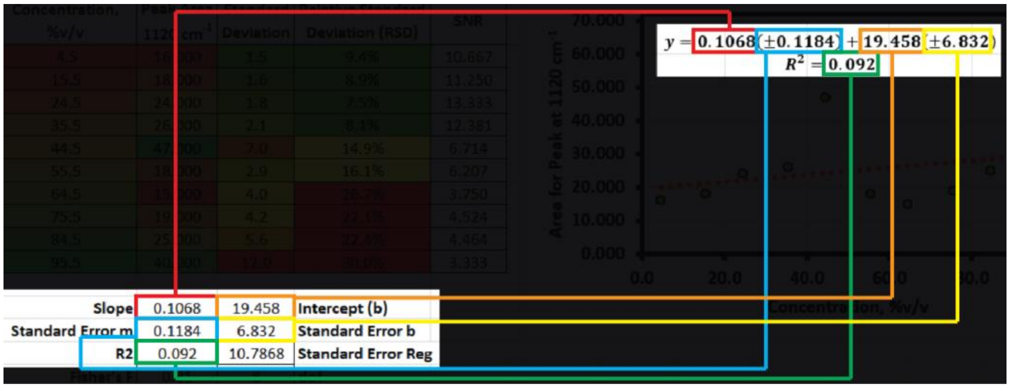
10. Add trendline by selecting the data points, right click, and choose “Add Trendline...”.





11. Write linear equation with R^2 and errors.

$$y = slope(\pm standard\ error\ m) + intercept(\pm standard\ error\ b)$$
$$R^2$$



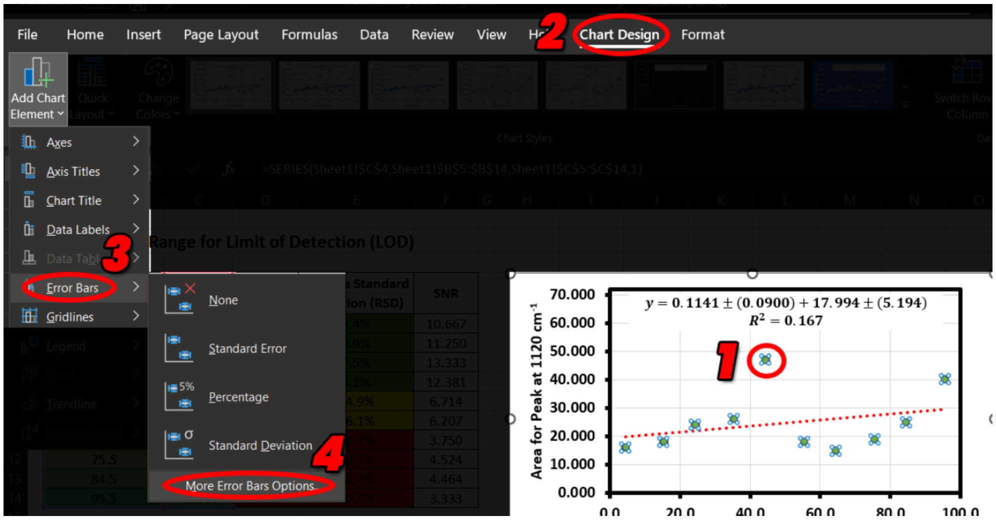
$$y = 0.1068(\pm 0.1184) + 19.458(\pm 6.832)$$
$$R^2 = 0.092$$

Slope	0.1068	19.458	Intercept (b)
Standard Error m	0.1184	6.832	Standard Error b
R2	0.092	10.7868	Standard Error Reg

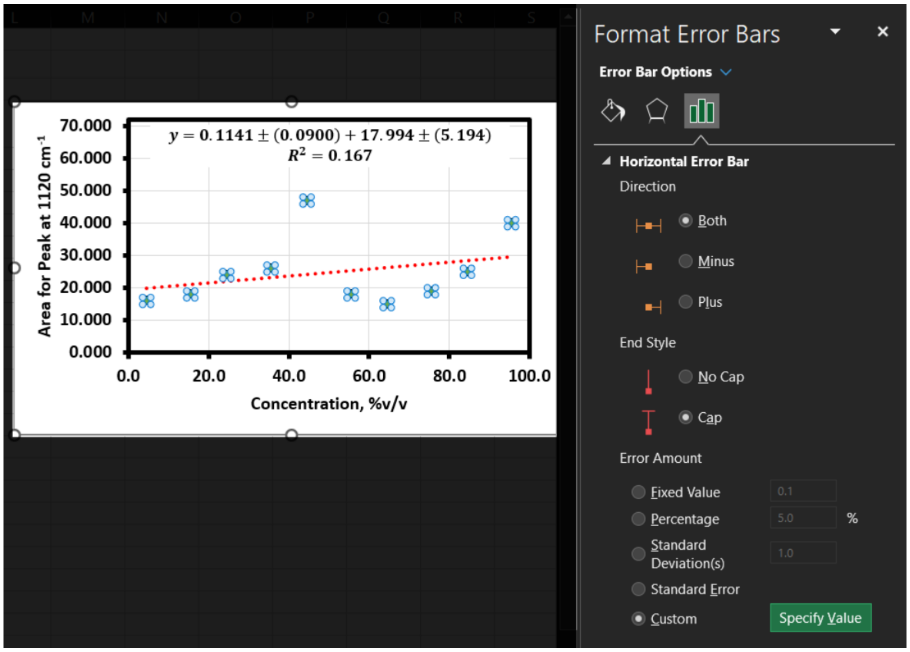
12. Calculate standard deviation (s) for each concentration.

$$= STDEV(Range\ of\ values)$$

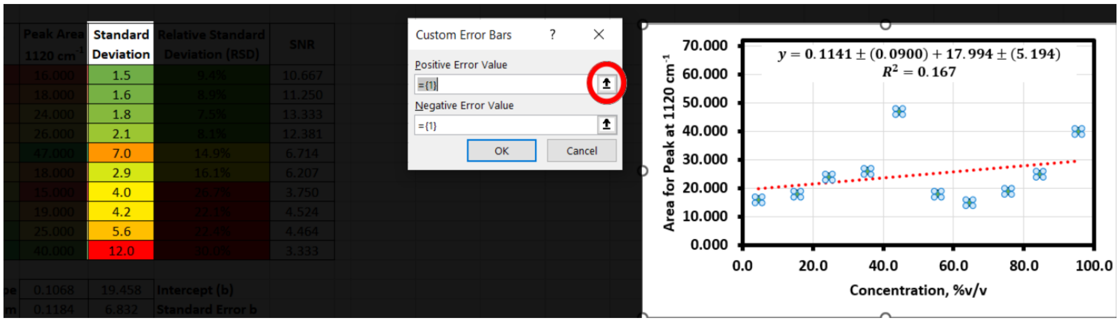
13. Add error bars by selecting the data points, selecting the “Chart Design” tab, “Add Chart Element” button, “Error Bars” list element, and “More Error Bars Options” option.



14. Use standard deviation by going to “Custom” in Error Amount and “Specify Value”.



15. Select standard deviation values in both positive and negative error values.

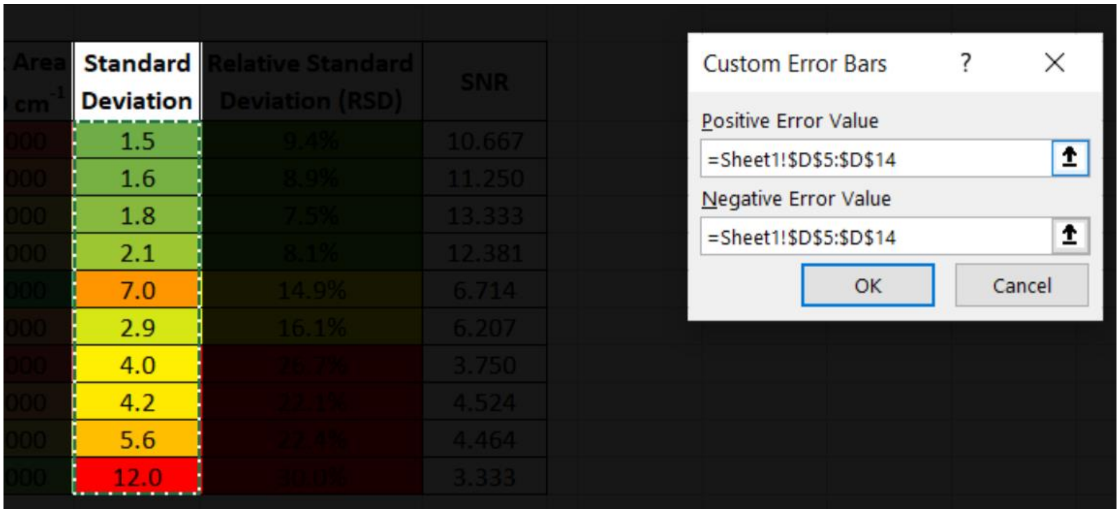


	Peak Area 1120 cm^{-1}	Standard Deviation	Relative Standard Deviation (RSD)	SNR
	16.000	1.5	9.4%	10.667
	18.000	1.6	8.9%	11.250
	24.000	1.8	7.5%	13.333
	26.000	2.1	8.1%	12.381
	47.000	7.0	14.9%	6.714
	18.000	2.9	16.1%	6.207
	15.000	4.0	26.7%	3.750
	19.000	4.2	22.1%	4.524
	25.000	5.6	22.4%	4.464
	40.000	12.0	30.0%	3.333
pe	0.1068	19.458	Intercept (b)	
m	0.1184	6.832	Standard Error b	

Custom Error Bars

Positive Error Value: ={1}

Negative Error Value: ={1}

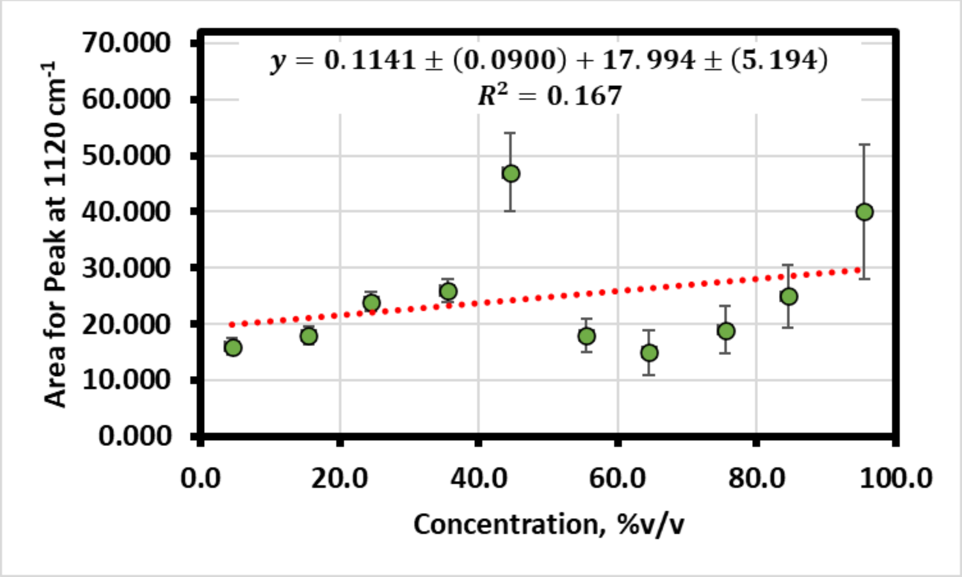


Area cm^{-1}	Standard Deviation	Relative Standard Deviation (RSD)	SNR
000	1.5	9.4%	10.667
000	1.6	8.9%	11.250
000	1.8	7.5%	13.333
000	2.1	8.1%	12.381
000	7.0	14.9%	6.714
000	2.9	16.1%	6.207
000	4.0	26.7%	3.750
000	4.2	22.1%	4.524
000	5.6	22.4%	4.464
000	12.0	30.0%	3.333

Custom Error Bars

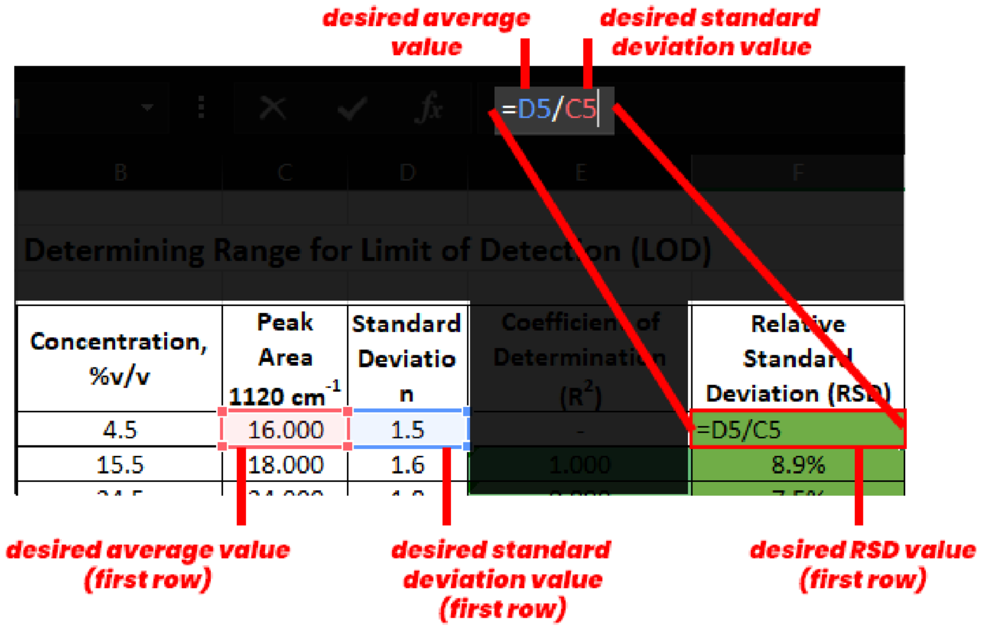
Positive Error Value: =Sheet1!$D$5:$D$14

Negative Error Value: =Sheet1!$D$5:$D$14



16. Calculate relative standard deviation (RSD) for the first row by dividing the average peak area with the standard deviation.

$$RSD = \frac{s}{\bar{x}} \times 100\%$$



desired average value

desired standard deviation value

=D5/C5

Determining Range for Limit of Detection (LOD)

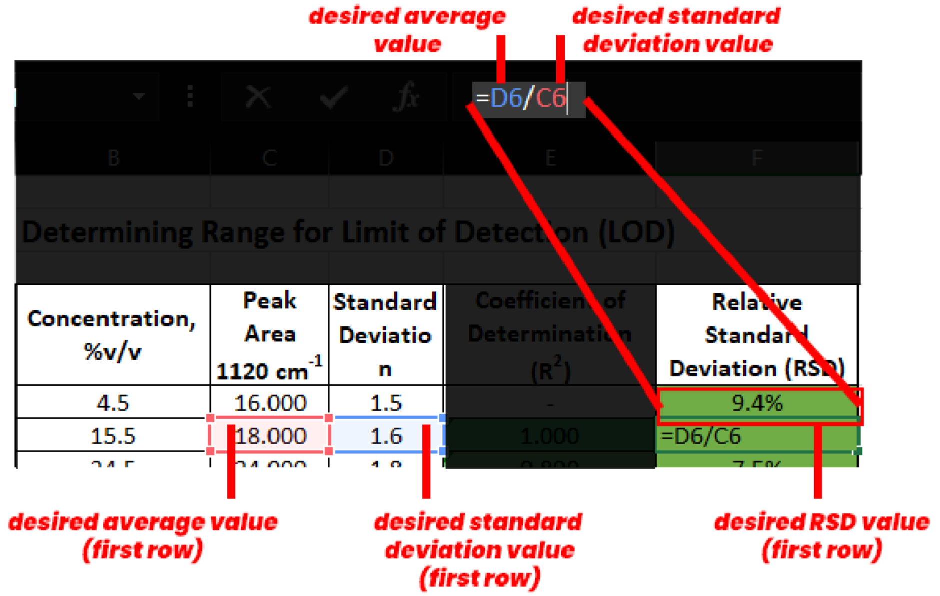
Concentration, %v/v	Peak Area 1120 cm^{-1}	Standard Deviation	Coefficient of Determination (R^2)	Relative Standard Deviation (RSD)
4.5	16.000	1.5	-	=D5/C5
15.5	18.000	1.6	1.000	8.9%

desired average value (first row)

desired standard deviation value (first row)

desired RSD value (first row)

17. Repeat step 9 for the next peak.



desired average value

desired standard deviation value

=D6/C6

Determining Range for Limit of Detection (LOD)

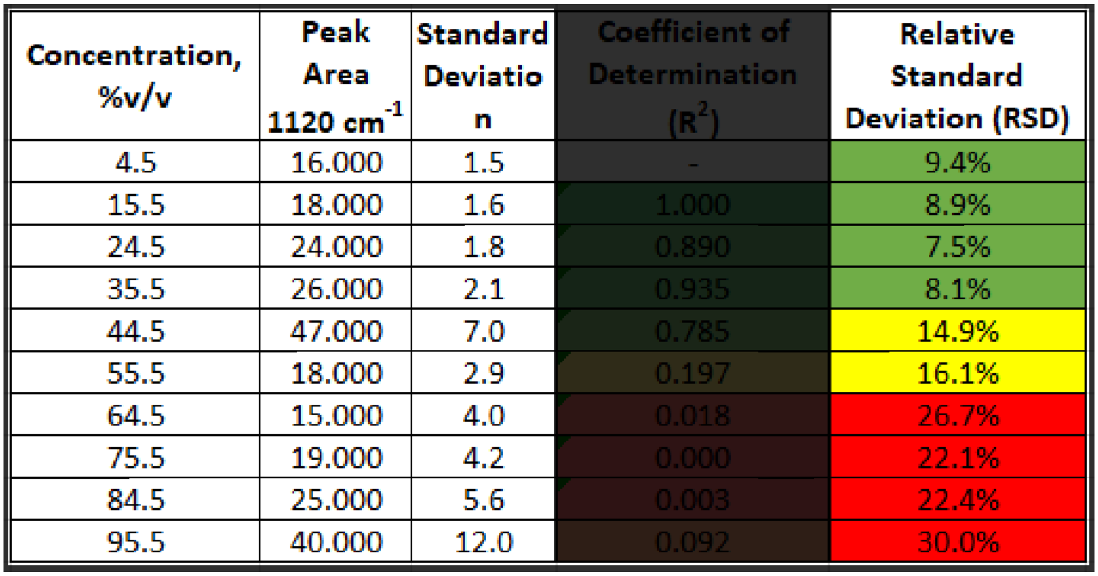
Concentration, %v/v	Peak Area 1120 cm^{-1}	Standard Deviation	Coefficient of Determination (R^2)	Relative Standard Deviation (RSD)
4.5	16.000	1.5	-	9.4%
15.5	18.000	1.6	1.000	=D6/C6

desired average value (first row)

desired standard deviation value (first row)

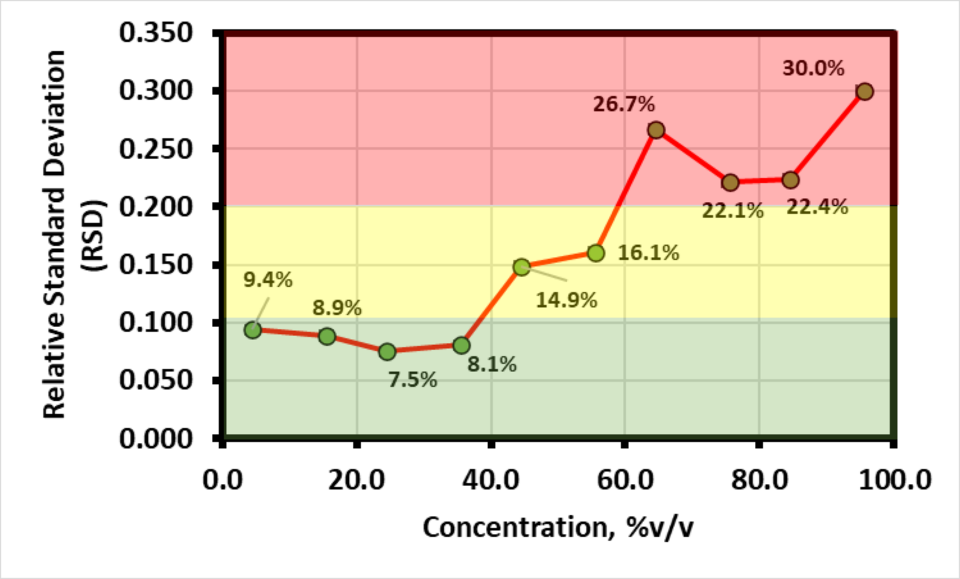
desired RSD value (first row)

18. Repeat step 10 for row with peak values.



Concentration, %v/v	Peak Area 1120 cm^{-1}	Standard Deviation	Coefficient of Determination (R^2)	Relative Standard Deviation (RSD)
4.5	16.000	1.5	-	9.4%
15.5	18.000	1.6	1.000	8.9%
24.5	24.000	1.8	0.890	7.5%
35.5	26.000	2.1	0.935	8.1%
44.5	47.000	7.0	0.785	14.9%
55.5	18.000	2.9	0.197	16.1%
64.5	15.000	4.0	0.018	26.7%
75.5	19.000	4.2	0.000	22.1%
84.5	25.000	5.6	0.003	22.4%
95.5	40.000	12.0	0.092	30.0%

19. Graph relative standard deviation (RSD) vs concentration.



20. Determine the concentration range by analyzing the RSD values.

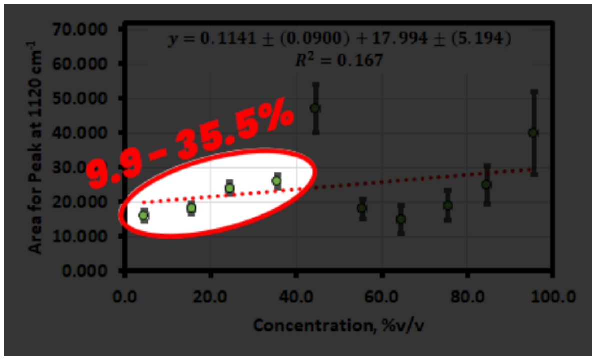
0%	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%	13%	14%	15%	16%	17%	18%	19%	20%	21%	22%	23%	24%	25%

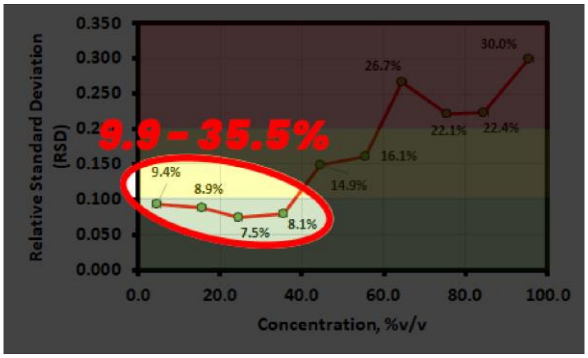
GREAT <10%

NEEDS WORK (10-20)%

BAD >20%

Values above 10% can be discarded from the desired LOD range. E.g., the concentration range for this data set is 9.9 – 35%v/v.



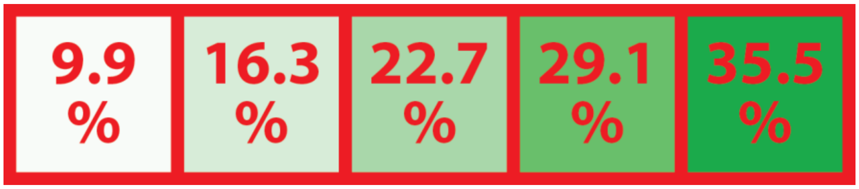


III. CALCULATING LOD FOR SPECIFIC ANALYTE

1. **Calculate** the increment concentrations for the range determined in the previous section (II).

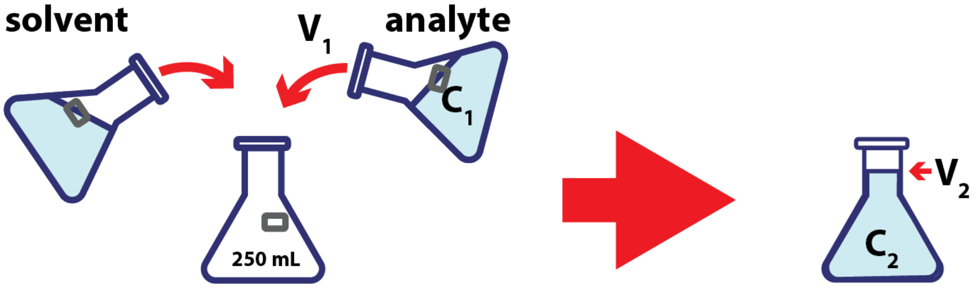
E.g., calculate the increments for the range 9.9 – 35.5%v/v for 5 samples.

$$Increments = \frac{UL - LL}{n - 1} \quad \rightarrow \quad Increments = \frac{(35.5 - 9.9)\ \%}{5 - 1} \quad \rightarrow \quad Increments = 6.4\ \%$$



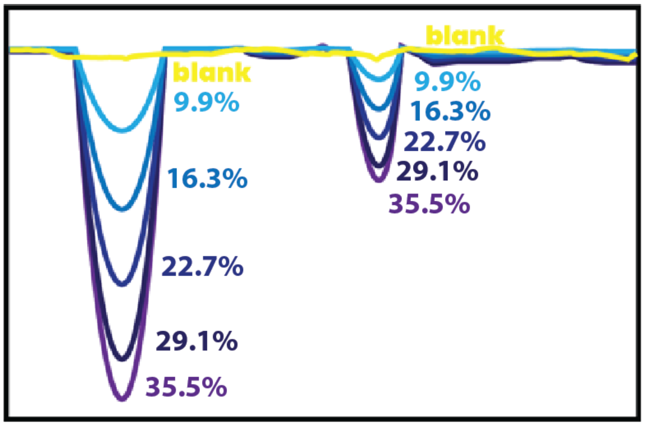
2. **Prepare** solutions as shown in section I.

$$V_1 = \frac{C_2 \cdot V_2}{C_1}$$



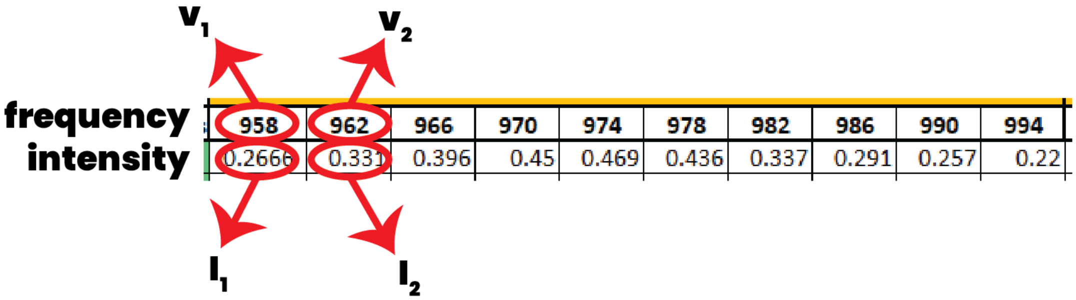
3. **Acquire** spectra for each sample.

4. **Repeat** step 3 until 10 measurements are acquired for each concentration and 20 measurements for the blank.



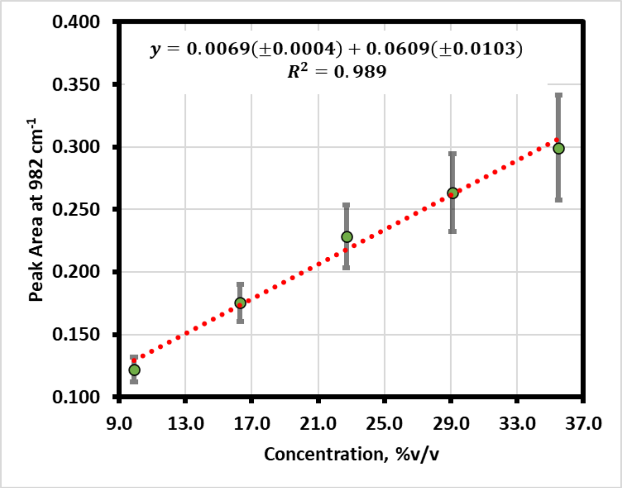
5. **Calculate** the area for the peak of interest for each concentration as in step II.

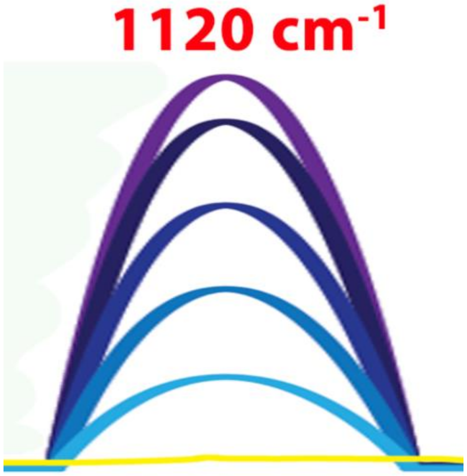
$$A = (\nu_2 - \nu_1) \cdot \left(\frac{I_1 + I_2}{2}\right)$$



frequency	958	962	966	970	974	978	982	986	990	994
intensity	0.2666	0.331	0.396	0.45	0.469	0.436	0.337	0.291	0.257	0.22

6. **Plot** peak area vs concentration.



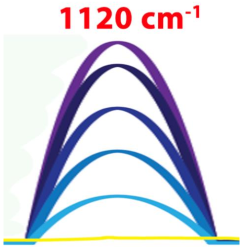


7. **Register** the slope (m) for the calibration curve.

$$y = 0.0069(\pm 0.0004) + 0.0609(\pm 0.0103) \quad \rightarrow \quad m = 0.0069$$

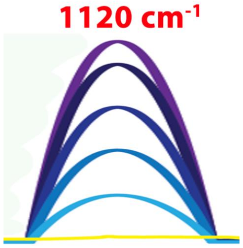
8. **Calculate** the average for all 20 peak area measurements for the blank sample.

$$\bar{S}_{bl} = \frac{\sum_{i=1}^{n}[PA_i]}{n}$$



9. **Calculate** the standard deviation for all 20 peak area measurements for the blank sample.

$$s_{bl} = \sqrt{\frac{\sum_{i=1}^{n}[(PA_i - \overline{PA})^2]}{n-1}}$$



10. **Calculate** the LOD with the average ($\bar{S}_{bl}$), standard deviation (s_{bl}), and regression slope (m).

$$LOD = \frac{(\bar{S}_{bl} + 3.3 \cdot s_{bl}) - \bar{S}_{bl}}{m} \quad \rightarrow \quad LOD = \frac{(0.018 + 3.3 \cdot 0.006) - 0.018}{0.0069} \quad \rightarrow \quad LOD = 2.9\%$$

11. **Calculate** the LOQ with the average ($\bar{S}_{bl}$), standard deviation (s_{bl}), and regression slope (m).

$$LOQ = \frac{(\bar{S}_{bl} + 10 \cdot s_{bl}) - \bar{S}_{bl}}{m} \quad \rightarrow \quad LOQ = \frac{(0.018 + 10 \cdot 0.006) - 0.018}{0.0069} \quad \rightarrow \quad LOQ = 8.7\%$$

Advisor Signature ______________________

Co-Advisor Signature ______________________