Whiskey analysis Interpretation – Step by Step guide

Theory

Internal standard

One of the most common ways industry calculates concentrations in samples is using the internal standard method. The internal standard method uses an extra compound that is not present in the sample to correct for errors made in the procedure (i.e. machine and user errors). For this method an Internal Standard (ISTD) is added to each sample in a known and constant concentration. This means that the concentration of ISTD should be the same in each sample. The measured value for the standard is compared to the measured value of the analyte (the compound we want to measure) resulting in a response factor (Rf). This Rf is compared to the calibration curve to give the concentration present in the sample.

Calculating Response factor

In this class we will use a GC fitted with an FID detector to measure concentrations. The machine will give a chromatogram as its result. This is simply said a graph that maps signal over time.

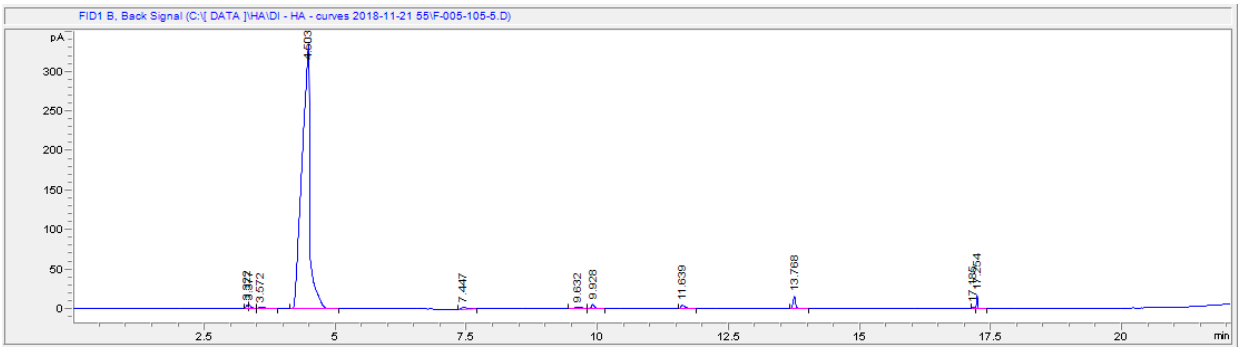

Figure 1: An example of a chromatogram

The area under one of the peaks correlates directly to the concentration. The GC will return areas directly. To compare the ISTD to a sample we simply divide the peak areas.

$$Rf = \frac{A_{compound}}{A_{ISTD}}$$

Equation 1: Rf calculation

Calculating calibration curves

For each compound a separate calibration curve is calculated. A curve is made by measuring external standards (STD). External standards differ from internal standards by the fact that they are not in your sample. This means that the external samples are in themselves a sample. For this class the external samples are prepared and measured in advance and only the results are supplied in Appendix 1: Calibration curve results.

From Table 3: Calibration Curve data. We can get the areas and concentrations ([ ]) for both the compound and the ISTD. Using Equation 1: Rf calculation we can calculate the Rf for each point (level) if we plot these Rf values verses the concentration ratio we get a calibration curve if we fit these values with a linear trendline we get the angle of the curve. Please note that instead of the real concentration of the standard (417 ng/ul we used the value of 1. This is done to make the curves show concentrations instead of ratios. This is allowed because the concentration of ISTD does not change)

$$\frac{[compound]}{[ISTD]} = \frac{[compound]}{1} = [compound]$$

Equation 2: Concentration ratio

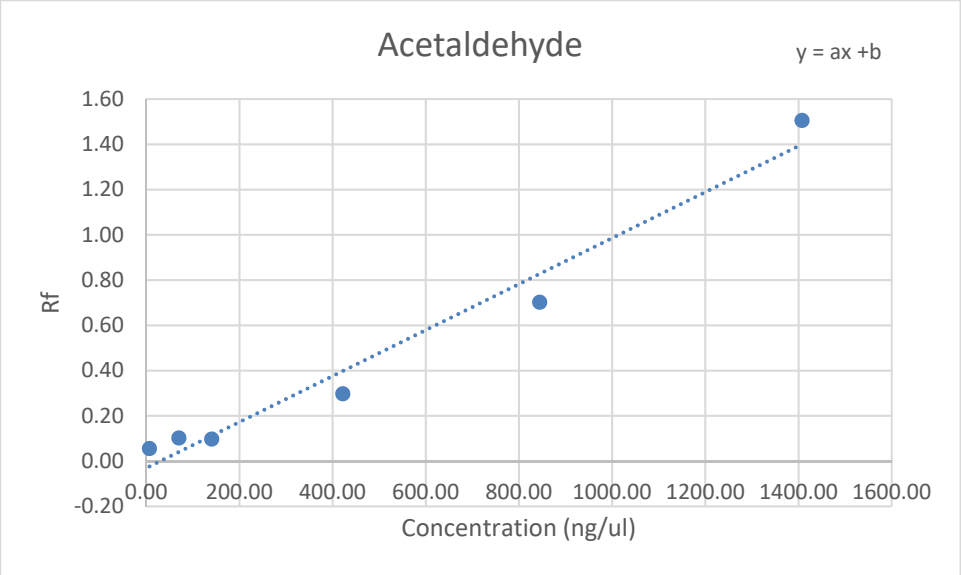

Figure 2: Calibration curve of Acetaldehyde

Calculating the concentration of your samples

In Figure 2: Calibration curve of Acetaldehyde we see an example of the data given, using the equation for the trend line we can calculate the concentration of our sample.

$$y = ax + b$$

Equation 3: The equation of the trendline.

Since our calibration curve plots Rf vs concentration we know that y=Rf and x=concentration ratio= concentration. Thus this equation can be rearranged to:

$$Rf = a[compound] + b$$

$$[compound] = \frac{Rf - b}{a}$$

Equation 4: Rearranged equation

Into $Rf = a[compound] + b$

$$[compound] = \frac{Rf - b}{a}$$

Equation 4: Rearranged equation we can fill in the Rf we get from our measurement to give us the concentration of acetaldehyde in our sample.

Example

In this example we are going to make the calibration curve for acetaldehyde and use an area of 12.459 for acetaldehyde and 30.584 for pentanol (ISTD). I suggest doing these calculations in excel or a similar program. Using a spreadsheet program makes sure that no number is rounded off until all calculations have been finished and fitting a trend line to your graphs is a lot faster than doing it manual with pen and paper.

Step 1: Calibration curve

To make the calibration curve we take the data from Appendix 1: Calibration curve results for acetaldehyde and pentanol.

Table 1: Calibration Curve data.

		Lvl 1		Lvl 2		Lvl 3	
Name	Rt (Min)	[ ]	Area	[ ]	Area	[ ]	Area
Acetaldehyde	2.089	7.00	1.73	70.38	3.04	140.76	2.88
Pentanol (ISTD)	14.579	417.00	31.39	417.00	30.00	417.00	29.87

		Lvl 4		Lvl 5		Lvl 6	
Name	Rt (Min)	[ ]	Area	[ ]	Area	[ ]	Area
Acetaldehyde	2.089	422.30	10.54	844.60	17.01	1408.00	42.58
Pentanol (ISTD)	14.579	417.00	35.48	417.00	24.24	417.00	28.30

Using Equation 1: Rf calculation we can calculate the Rf for each of the levels (use the corresponding ISTD area; ie. Compare the area for level 1 acetaldehyde with level 1 ISTD) with some rearranging we get the following table.

For level 1 this results in the following

$$Rf = \frac{1.73}{31.39} = 0.06$$

Equation 5: Rf calculations for Acetaldehyde calibration curve lvl 1

Doing this for all 6 levels results in the following table.

Table 2:concentrations and Rf values for the concentration curve

Level	Concentration	Rf
1	7.00	0.06
2	70.38	0.10
3	140.76	0.10
4	422.30	0.30
5	844.60	0.70
6	1408.00	1.50

The data in Table 2:concentrations and Rf values for the concentration curve can be plotted as a Scatter graph. Be careful that you use the concentrations as X axis values and Rf values as Y axis values.

Excel should give you something that looks like

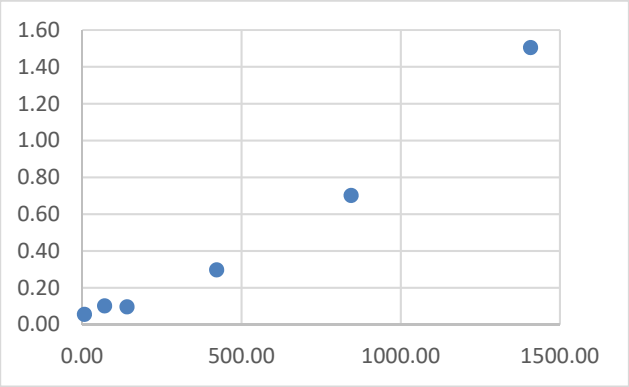

Figure 3: plot of calibration data of acetaldehyde

If you select your data points (left click once on one of the blue dots) and then right click on one of your data points (highlighted blue dots; cursor should look like a fat white + sign) you should be able to select add trendline. When you select the add trendline option you should get the next screen.

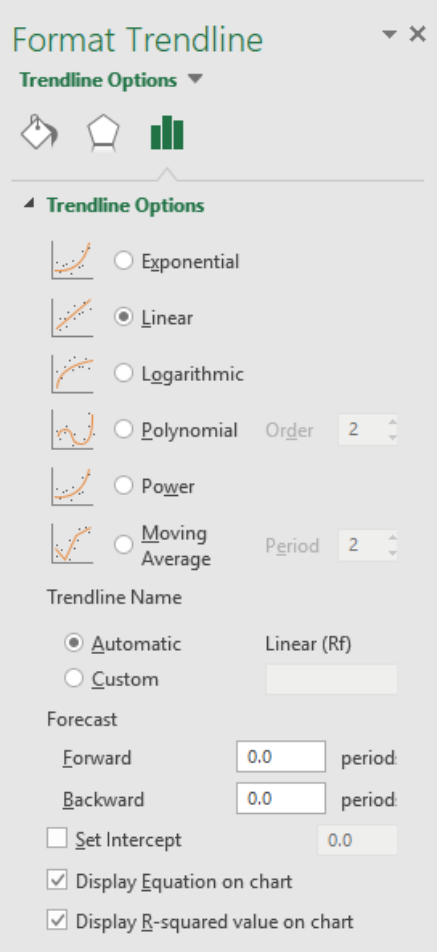

Figure 4: trend line options

In this screen make sure you select “Linear” and “Display Equation on screen”

You should end up with something like the following

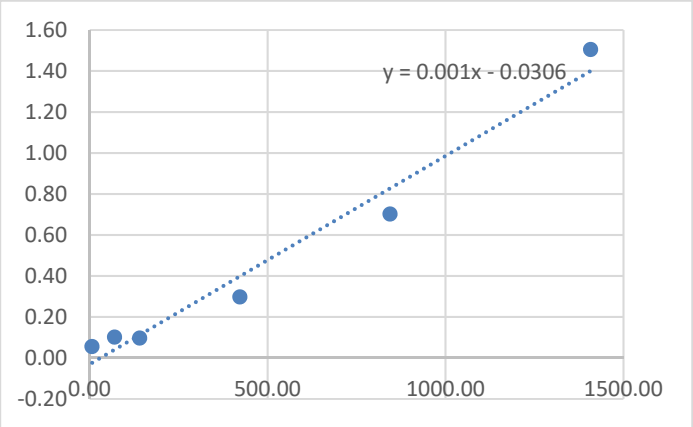

Figure 5: calibration curve with trendline

By selecting and right clicking the equation we can change the way excel shows us our numbers. Left click to select the equation then right click in the box around the equation and click on "format trend line label"; change the category to scientific with 3 decimal places. This should give you the following.

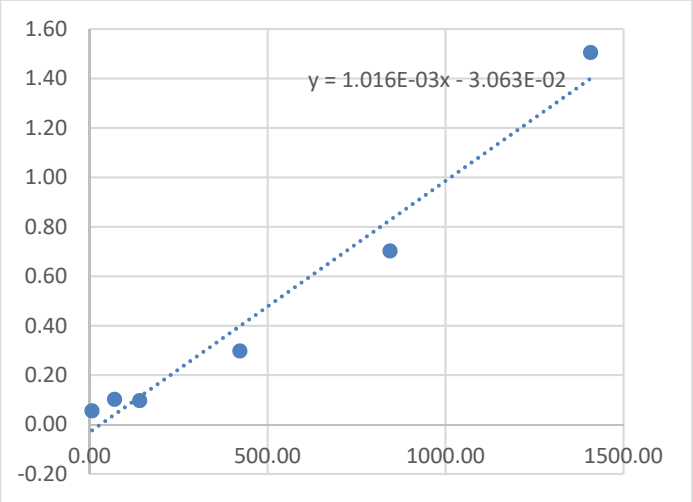

Figure 6: finished calibration curve

More experienced excel users will be able to make much prettier graphs off course, but this is the bare minimum needed.

Step 2: calculating the concentration

As stated before in this example we will use areas of 12.459 and 30.584 for acetaldehyde and pentanol (ISTD) respectively. Using Equation 1: Rf calculation we can calculate the Rf for acetaldehyde.

$$Rf_{acetaldehyde} = \frac{A_{Acetaldehyde}}{A_{ISTD}} = \frac{12.459}{30.584} = 0.40737$$

Equation 6: Rf of acetaldehyde calculation

Using the above Rf value we can calculate the Concentration using the equation for the trendline from Figure 6: finished calibration curve. For this we first rearrange the equation into a more usable format and then fill in the Rf value.

$$y = ax + b = Rf = 1.016 \times 10^{-3}[Acetaldehyde] - 3.063 \times 10^{-2}$$

$$[Acetaldehyde] = \frac{Rf + 3.063 \times 10^{-2}}{1.016 \times 10^{-3}} = \frac{0.40737 + 3.063 \times 10^{-2}}{1.016 \times 10^{-3}} = 430.8\, ng/ul$$

Equation 7: Acetaldehyde concentration calculation

Appendix 1: Calibration curve results

Table 3: Calibration Curve data.

		Lvl 1		Lvl 2		Lvl 3	
Name	Rt (Min)	[ng/ul]	Area	[ng/ul]	Area	[ng/ul]	Area
Acetaldehyde	2.089	7.00	1.73	70.38	3.04	140.76	2.88
Ethyl acetate	3.359	6.58	5.16	66.17	1.96	132.33	4.00
Acetal	3.617	6.64	7.54	66.76	5.22	133.53	10.52
Methanol	3.848	4.95	47.60	49.78	64.04	99.55	79.40
Ethanol	4.553	Reference					
n-Propanol	7.617	6.15	0.23	61.81	2.27	123.61	4.72
i-Butanol	9.604	6.12	0.31	61.50	3.11	123.00	6.44
i-Pentylacetate	10.564	6.87	0.21	69.08	2.07	138.17	3.76
n-Butanol	12.354	6.10	0.32	61.27	3.22	122.54	6.16
i-Amylalcohol	13.651	14.51	0.92	145.86	7.83	291.73	15.29
Pentanol (ISTD)	14.579	417.00	31.39	417.00	30.00	417.00	29.87
Furfural	17.494	9.50	0.25	95.44	2.54	190.88	5.41

		Lvl 4		Lvl 5		Lvl 6	
Name	Rt (Min)	[ng/ul]	Area	[ng/ul]	Area	[ng/ul]	Area
Acetaldehyde	2.089	422.30	10.54	844.60	17.01	1408.00	42.58
Ethyl acetate	3.359	397.00	14.58	794.00	20.62	1323.00	38.20
Acetal	3.617	400.60	38.58	801.20	42.34	1335.00	42.41
Methanol	3.848	298.70	100.93	597.30	85.86	996.00	122.01
Ethanol	4.553	Reference					
n-Propanol	7.617	370.80	19.87	741.70	23.13	1236.00	44.96
i-Butanol	9.604	369.00	25.09	738.00	28.20	1230.00	55.28
i-Pentylacetate	10.564	414.50	19.43	829.00	25.99	1382.00	47.55
n-Butanol	12.354	367.60	24.93	735.20	27.95	1225.00	55.43
i-Amylalcohol	13.651	875.20	63.15	1750.40	71.52	2917.00	140.45
Pentanol (ISTD)	14.579	417.00	35.48	417.00	24.24	417.00	28.30
Furfural	17.494	572.60	14.99	1145.30	39.65	1909.00	72.55