Calibration Strategies and Dilution Practices

By Bill Hahn

Protocol/SPEX CertiPrep

- We manufacture inorganic and organic certified reference materials (CRM's)
- We also manufacture sample preparation equipment.
- We have an ISO 9001-2000 quality system
- We are NVLAP approved to manufacture Proficiency Testing Samples. We are Audited and accepted as contractors to various state and federal agencies.

Our Pedigree

- ISO 9001-2000 Registered with Underwriters Labs
- NIST/NVLAP Proficiency Testing Provider ISO-17025
- Extremely active in NELAC, INELA and CRMMA

Why talk about Calibrations and Dilutions at all?

- We have observed over the years that chemists use a variety of techniques to dilute solutions and calibrate instruments.
- We have also observed that many techniques in common practice contain an unacceptable amount of error and uncertainty.
- We wish to promote good laboratory practices and reduce the errors at the bench.
- The goal is to show calculations, uncertainties and lab practices that will help chemists make better measurements, or understand why they do so well.

Techniques

- This presentation pertains mostly to GC and GC/MS techniques
- The same calibration principles apply to AA, ICP, ICP-MS
- The dilution schemes are specific to GC practices

Calibration Strategies

- There are many ways to Calibrate an instrument.
- 1 point
- 2 point
- Multiple points
- External Standard
- Internal Standard

Do more points = more confidence?

- Generally yes, but there is a point of diminishing returns
- Calibrating below your working range skews your curves
- Calibrating above your linear range also skews your curves
- Too many points, over time, are meaningless and expensive

External vs Internal Standard

- External standard techniques are highly dependent on consistency in sample prep, dilutions and instrument conditions
- Internal standard techniques give you a relative response to quantitate and is generally much more accurate

Syringes and Pipettes

- Always use the same syringe for I/S additions
- Check your accuracy and precision
- Check the syringes, pipettes and volumetrics for accuracy and precision
- Make sure that all analysts are being consistent

Internal standard variations

Internal Std Variations at 40 ug/ml			
Cal Level	10 ul	20 ul	
Standard Error	2.72%	1.05%	
1	0.03	0.01	ug/ml
2	0.05	0.02	ug/ml
5	0.14	0.05	ug/ml
20	0.54	0.21	ug/ml
50	1.36	0.53	ug/ml
80	2.18	0.84	ug/ml
120	3.26	1.26	ug/ml
160	4.35	1.68	ug/ml
200	5.44	2.10	ug/ml
240	6.53	2.52	ug/ml

Typical linear calculations

- Average response factor
- Linear Regression

Average Response Factors

- Sum of all response factors / n
- Is a weighted way to evaluate a straight line
- Used in CLP, 8000, 500 and 600 series methods
- CLP Daily Cal range = 25 % rsd for 50 STD
- This means that from 37.5 to 62.5 passes
- NELAC is now requiring an estimate of Uncertainty

Linear Regressions

- $y=mx+b$
- m=slope
- b=intercept
- r=Correlation Coefficient
- r^2=The square of *r* is conventionally used as a measure of the strength of the association between *X* and *Y*. For example, if the coefficient is .90, then 81% of the variance of *Y* is said to be explained by the changes in *X* and the linear relation between *X* and *Y* ***within the range observed***.

Linear Regression

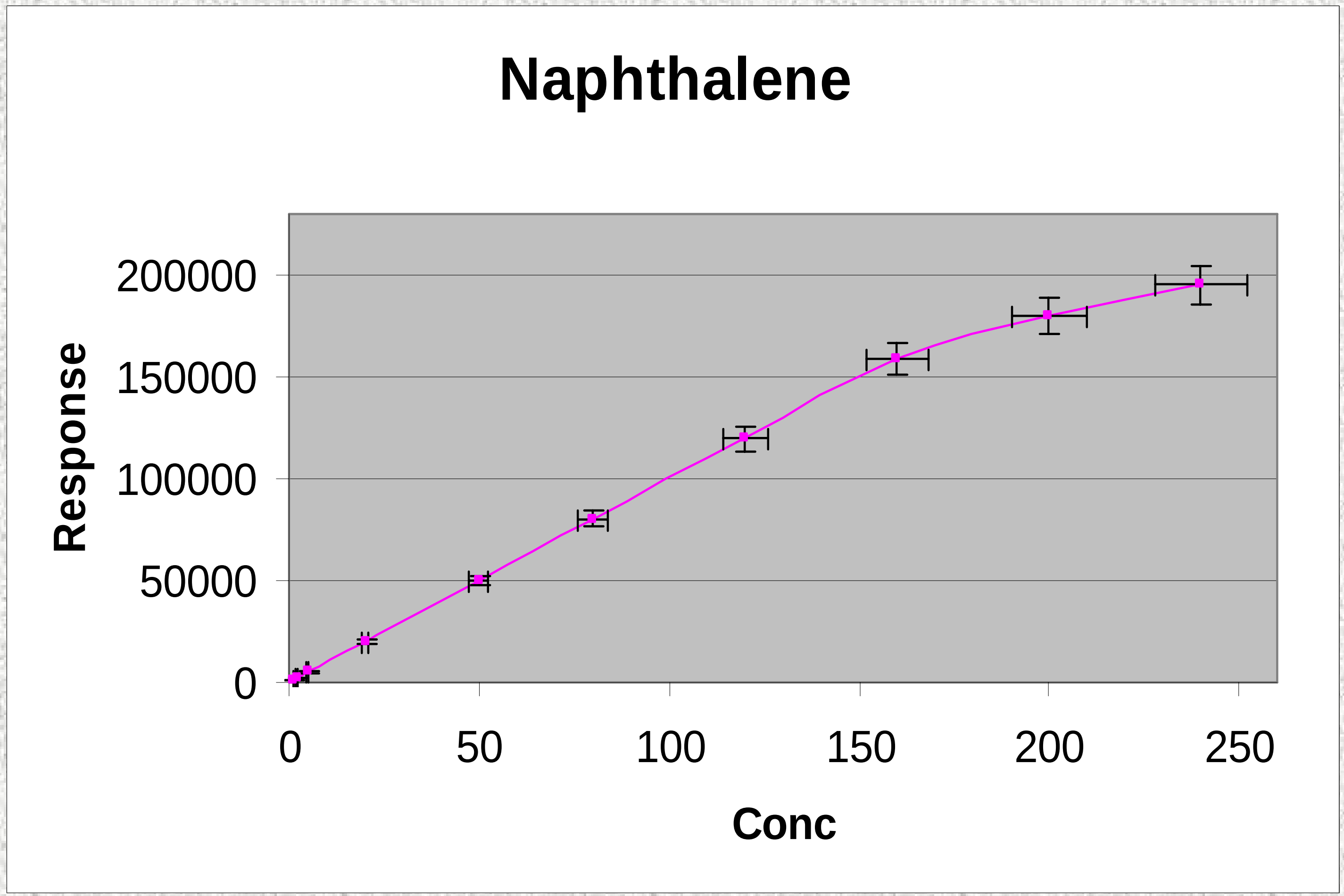
- Correlation Coefficient

$$r = \frac{1}{n-1} \sum \left(\frac{x - \overline{x}}{s_x}\right)\left(\frac{y - \overline{y}}{s_y}\right)$$

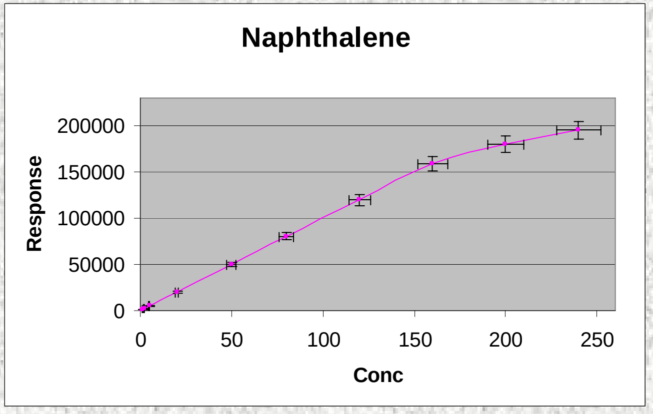
Typical Naphthalene Curve

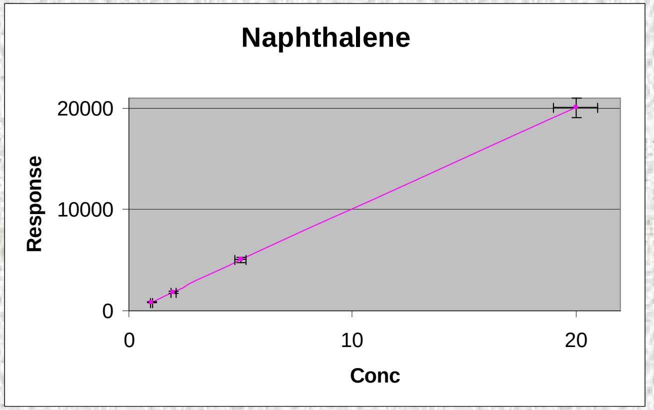
Cal Level	Response Factor
1	861
2	1792
5	5038
20	20048
50	50021
80	80118
120	119582
160	158733
200	180338
240	195025

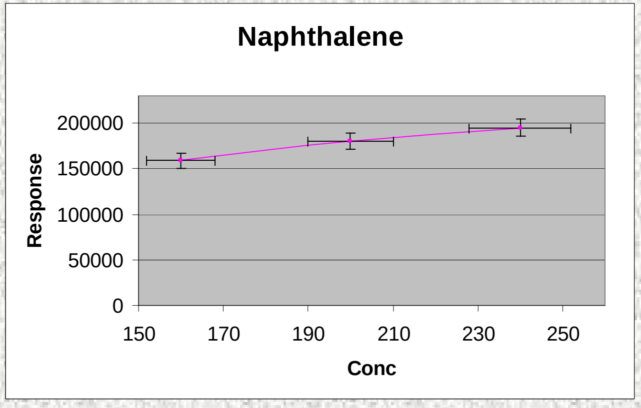
What does it look like?



A closer look…







Just the facts...

				Sample True Conc	
r2	r	used	slope	35 ug/L	100 ug/L
0.985524	0.992736	1-240	869.2124	34.7	109.0
0.999974	0.999987	20-160	861.6078	35.0	100.0
0.999899	0.999949	1-20	1010.35	29.8	93.8
0.988033	0.993998	160-240	453.65	66.5	208.9

Dilution schemes

- There are many ways to perform dilutions
- Using uncertainties, you can determine the best methods
- Always use dilutions appropriate to the scale of your application
- There is always the good, the bad and the ugly

Uncertainty in dilutions

- Without exploring the uncertainties of our measurements, we cannot have confidence in our calibrations.
- Most labs have ***<u>NEVER</u>*** analyzed their uncertainties.
- Analyzing your uncertainties can show you more accurate ways to dilute.

The Ugly…

- How many people create matter in sludge?
- Remember those 1,200,000mg/kg results?
- Huge dilutions = expanded error

The Bad

- Using a 10 ul syringe to dilute 2 ul to 1 L is bad
- The Expanded error at 2 ul = 23%
- A target of 2 ug/L from a 1000 ug/ml std using 2 ul = 1.77 to 2.23ug/L.

The Good

- Use dilutions that are within the 100x level of your working volumes
- Use at least 50% of your syringe volumes
- Use calibrated pipettes and glassware

An internal experiment in syringe use and uncertainty

- We had 7 people weigh various volumes with various size syringes.
- There were 7 observations at each volume.

Measure of liquid from syringes (1 of seven)

Chemist 1	H2O						
	1	2	3	4	5	6	7
2 ul from a 10 ul syringe	0.00205	0.00185	0.00182	0.00174	0.0019	0.00183	0.00191
5 ul from a 10 ul syringe	0.00508	0.00481	0.00465	0.00479	0.00494	0.00483	0.00463
10 ul from a 10 ul syringe	0.00967	0.00971	0.00969	0.0096	0.00989	0.00975	0.00969
5 ul from a 25 ul syringe	0.00489	0.00475	0.00485	0.00469	0.00459	0.0047	0.00461
10 ul from a 25 ul syringe	0.0096	0.00948	0.00959	0.00964	0.00963	0.00955	0.00958
25 ul from a 25 ul syringe	0.02427	0.02432	0.02415	0.02436	0.0246	0.02434	0.02431
10 ul from a 100 ul syringe	0.00984	0.00995	0.00971	0.00988	0.00992	0.01016	0.00973
25 ul from a 100 ul syringe	0.02447	0.02473	0.02469	0.02464	0.02461	0.02483	0.02481
50 ul from a 100 ul syringe	0.05008	0.04967	0.04964	0.05017	0.05002	0.0503	0.04975
250 ul from a 1000 ul syringe	0.25132	0.24409	0.2456	0.24615	0.24696	0.24694	0.24667
500 ul from a 1000 ul syringe	0.4991	0.49732	0.49673	0.49867	0.49795	0.497	0.4968
1000 ul from a 1000 ul syringe	0.99901	0.99883	0.99833	0.99801	0.99923	0.995	0.9974

Mean syringe observations (H_2O) and Uncertainties

avg mean	u	u Exp	Exp Err	
2.15	0.2493	0.4986	23.15%	2 ul from a 10 ul syringe
5.04	0.2058	0.4116	8.16%	5 ul from a 10 ul syringe
9.90	0.1346	0.2692	2.72%	10 ul from a 10 ul syringe
2.19	0.0967	0.1934	8.82%	2 ul from a 25 ul syringe
5.06	0.1383	0.2766	5.47%	5 ul from a 25 ul syringe
9.94	0.1179	0.2358	2.37%	10 ul from a 25 ul syringe
20.00	0.1054	0.2108	1.05%	20 ul from a 25 ul syringe
24.70	0.1538	0.3077	1.25%	25 ul from a 25 ul syringe
10.18	0.3102	0.6205	6.09%	10 ul from a 100 ul syringe
25.06	0.2090	0.4181	1.67%	25 ul from a 100 ul syringe
50.19	0.1612	0.3224	0.64%	50 ul from a 100 ul syringe
100.43	0.3042	0.6083	0.61%	100 ul from a 100 ul syringe
250.82	1.3204	2.6409	1.05%	250 ul from a 1000 ul syringe
501.93	2.8685	5.7370	1.14%	500 ul from a 1000 ul syringe
996.95	2.3662	4.7325	0.47%	1000 ul from a 1000 ul syringe

Mean syringe observations and Uncertainties (Methanol)

avg mean	u	u Exp	Exp Err	
1.36	0.0973	0.1945	14.33%	2 ul from a 10 ul syringe
3.58	0.1100	0.2201	6.14%	5 ul from a 10 ul syringe
7.48	0.1690	0.3380	4.52%	10 ul from a 10 ul syringe
15.30	0.1339	0.2677	1.75%	20 ul from a 25 ul syringe
34.24	0.1967	0.3934	1.15%	25 ul from a 25 ul syringe
401.86	1.3142	2.6285	0.65%	500 ul from a 1000 ul syringe

Mean syringe observations and Uncertainties (Methylene Chloride)

avg mean	u	u Exp	Exp Err	
2.04	0.2262	0.4525	22.19%	2 ul from a 10 ul syringe
5.75	0.2553	0.5105	8.88%	5 ul from a 10 ul syringe
11.99	0.3788	0.7577	6.32%	10 ul from a 10 ul syringe
25.28	0.2246	0.4491	1.78%	20 ul from a 25 ul syringe
65.88	0.5345	1.0689	1.62%	50 ul from a 100 ul syringe
667.90	5.2251	10.4502	1.56%	500 ul from a 1000 ul syringe

A Frequently used dilution scheme

Conc	BN2	605-X	Phen	SV-X	SA5	SB5	I/S	Tot	Sol					
	2000	2000	2000	2000	1000	2000	4000			u -s	u i/s	u C	u E	OFF%
20	10	10	10	10	20	10	10	80	920	0.6205	0.2692	1.5436	3.0871	15.4%
50	25	25	25	25	50	25	10	185	815	0.4181	0.2692	1.0598	2.1178	4.24%
80	40	40	40	40	80	40	10	290	710	0.3224	0.2692	0.8343	1.6687	2.09%
120	60	60	60	60	120	60	10	430	570	0.4915	0.2692	1.2337	2.4673	2.06%
160	80	80	80	80	160	80	10	570	430	0.6083	0.2692	1.5141	3.0283	1.89%
											Total	Expanded	11.941	5.14%

Cumbersome……

- Without total isolation while performing these dilutions, errors are almost guaranteed
- Combining all of the uncertainties illustrates a 5.14% curve variability
- Add another 2.72% if the I/S was inclusive of the 1 ml volume = 7.86% curve variability
- This will cause a ~ 30% failures in PT's

Serial Dilutions

Conc	uL	Expected error
160	As before	1.89%
120	0.75	0.85%
80	0.5	1.14%
50	0.3125	1.08%
20	0.125	1.18%
	Average Error	1.23%

Serial Dilutions

- Offer much more accuracy (a factor of 6!)
- Combining all of the uncertainties illustrates a 1.23% curve variability
- Add another 1.05% if the I/S was inclusive of the 1 ml volume
- This will cause less failures (~2% in PT's) and more accurate results

Serial Dilutions (continued)

- Dilutions should not exceed 1000x at the 1Liter volume (error = 0.47%).
- The error is generally acceptable here.
- If you dilute in excess of 10,000x, then your analytical uncertainties become astronomical! (error = 4.7%)

Whatever your methods

- ***Validate***
- ***Validate***
- ***Validate***
- ***Validate***

References

- **References**
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- ILAC-G21-2000
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- EURACHEM/CITAC Guide, Second Edition
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- NIST Technical Note 1297
- Guide to the Expression of Uncertainty in Measurement (1995)

Thank You!
We Want You to Be Successful

Contact us for technical assistance!

Email to bhahn@prostds.com