A Comparison of Variance Components Analysis Methods for the Quantitation of Intermediate Precision for the Validation of Bioassays

Introduction

In the manufacturing of complex, large molecule pharmaceutical products the quality of the active pharmaceutical ingredient and its potency may be hard to control. Each production batch needs to be tested for its potency and compared to a reference standard. The relative potency of the batch is then used to adjust the administered dose by dilution, concentration or packaging size. The potency test is done by administering the dug product (DP) or drug substance (DS) in various concentrations to animals (in-vivo) or tissue/cell cultures (in-vitro) and observing a response. This is called an activity or biological assay (bioassay). Bioassays measure biological activity such as expression of an antibody for a vaccine, expression of IFNg or toxicity of a neurotoxin.

The U.S. Pharmacopeia (http://www.usp.org) define standards that aid in the design (USP <1032>, <1033>, <1034>) validation and analysis of bioassays. USP <1033> Biological Assay Validation provides guidance on the validation of a bioassay. Validation is the process of making sure that the bioassay is fit for use and fit for purpose. Fit for purpose refers to the use of the bioassay, such as in-process control or release testing for DS or DP. Fit for use means accuracy, specificity, repeatability, intermediate precision, and range. Validation of the bioassay is mandated by the EMA and FDA.

One of the key elements of validation is the measure of intermediate precision (IP). Precision contains three variance components; 1) between factor variation (inter-assay error), 2) repeatability (intra-assay error), 3) reproducibility (ICH Q2). Intermediate precision contains the first two variance components. Variance components are a quantitation of the variance resulting from changes in factors related to the execution of a bioassay. Typical examples of bioassay related factors are analyst, instrument, and day. These factors are characterized using a structured design of experiments (DOE) and the resulting data is analyzed using variance component calculation methods.

For the analysis of variance components, USP <1033> recommends the use of restricted maximum likelihood (REML). REML is a method that approximates the observed variation and is used to make estimates of the population variation. REML does not evaluate factors that influence the intra-assay error and is restricted to categorical factors only. There is another method for calculating variance components called partition of variation (POV) (Little, 1993). POV is based on ANOVA and sequential sum of squares and is an exact method that explains all observed variation. POV also quantitates the within factor variation effects and can quantitate the influence of both continuous and categorical factors.

REML allows for the isolation of the between day, between analyst, between instrument effect and repeatability. POV calculates the between day, between analyst, between instrument, within day,

within analyst, within instrument and common variation. Repeatability is the sum of the within components and common variation. Intermediate precision is the sum of all variation components.

USP recommends the use of precision to tolerance ratio for the setting of acceptance criteria for intermediate precision. This will be discussed in conjunction with variance components analysis in this paper.

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Study Questions

The following questions are the specific topics and analysis that will be discussed in this paper:

1. How does REM compare to POV for the quantitation of the between factor variance components?
2. How does REML compare to POV for the quantitation of the within factor variance components?
3. How does REML compare to POV for the quantitation of repeatability?
4. How does POV utilize ANOVA sums of squares calculations to determine variance components?
5. Which method is best for determining the variance components of a bioassay?
6. What is the best method of setting acceptance criteria for the intermediate precision and repeatability of a bioassay?

Variance components

Introduction to variance components

Single factor

Assume a response that looks like this:

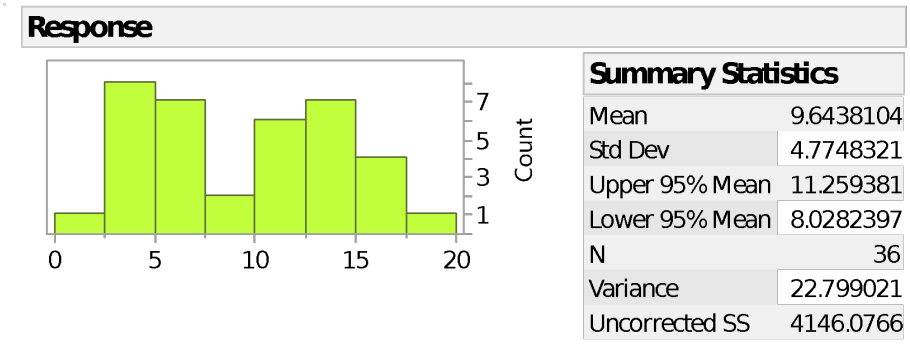
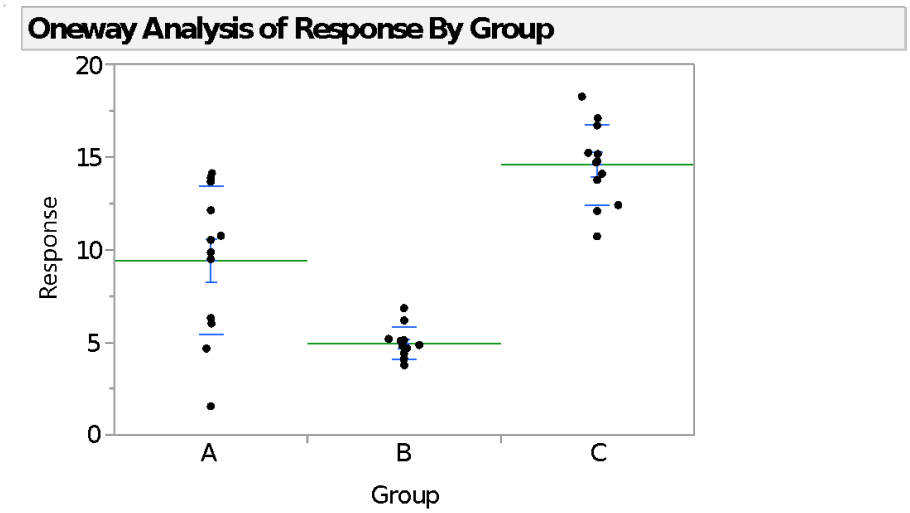


Figure 1 Distribution of example response

In variance components analysis the effect of one or more factors on the response is measured. The response has a standard deviation of 4.77 or a variance of 22.799. Since the calculation of standard deviation involves a square root, all math is done using variance and converted to a standard deviation only for the final answer. The factor 'group' is introduced and analyzed using ANOVA:



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
A	12	9.4202246	4.007876	1.1569741	6.8737417	11.966707
B	12	4.925584	0.8789061	0.2537183	4.3671537	5.4840143
C	12	14.585623	2.1737446	0.627506	13.204491	15.966754

Figure 2 Visualizing variance components

As the level of the factor 'group' changes, both the response mean and standard deviations change (are variable). The variation in the means and the variation of the standard deviations are two

components of the total variation. The first is the between variation and the second is the within variation.

There is a minimum amount of variation present in all groups (the level of B) and there is more variation at other levels of 'group'. This minimum amount of variation that is present in all groups is called the common variation since it is common to all groups.

Mathematically the variance components in this example are:

$$SD_{Overall} = \sqrt{SD^2_{Between\ Group} + SD^2_{Within\ Group} + SD^2_{Common}}$$

Multiple factors

In the multivariate case, the factor structure becomes important. There are three possibilities for the factor structure:

Nested

Nested factors are factors where some levels for one factor can only occur in combination with a specific level of another factor. Nested happens in manufacturing when product that comes from Machine A can only go to certain downstream machines and Machine B goes to other downstream machines. In this case the downstream machines are nested in the upstream machines. An example is analytical equipment (metrology) nested within laboratories.

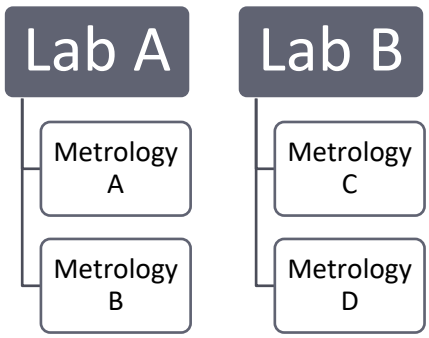


Figure 3 Nested factor structure

Metrology A and B only exist in Lab A and will never be combined with product from Lab B. In the case of nested data the variance components that can be calculated are:

$$SD_{Overall} = \sqrt{SD^2_{Between\ Lab} + SD^2_{Between\ Metrology[Lab]} + SD^2_{Within\ Lab} + SD^2_{Within\ Metrology[Lab]} + SD^2_{Common}}$$

Crossed

Crossed factors are structured experiments where all levels of factor 1 have been tested at all levels of factor 2. This structure allows us to see if the effect of factor 2 on the response depends on the level of factor 1, this is called a combination or interaction effect. These can only be calculated when factor combinations have been run correctly. Interaction effects are mathematically notated in the form: factor 1 * factor 2.

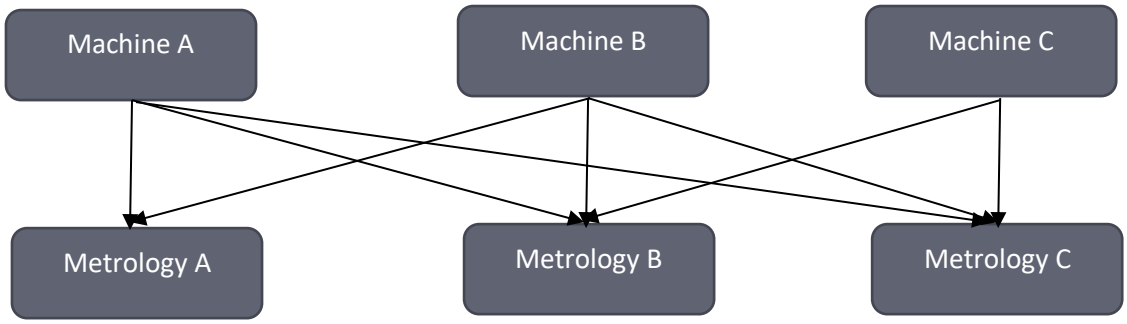


Figure 4 Crossed factor structure

In the case of a 2 factor crossed study the variance components are:

$$SD_{Overall} = \sqrt{SD^2_{Between\ Machine} + SD^2_{Between\ Metrology} + SD^2_{Between\ Machine*Metrology} + SD^2_{Within\ Machine} + SD^2_{Within\ Metrology} + SD^2_{Within\ Machine*Metrology} + SD^2_{Common}}$$

Mixed

When there is no clear factor structure, only the main effects can be calculated.

Variance components estimation methods

Platform

Data analysis is done using JMP Pro 15.1.0. running on Mac OSX 10.15.6. Variance components using REML are calculated using the variability plots platform. Variance components using POV are calculated using version 200617 of the POV and MSA script available from the TLC add-in found on https://thomasalittleconsulting.com/.

Repeatability is the sum of the within variance components and intermediate precision is the sum of the between and within components.

The 95% confidence intervals are calculated using $UCL = \sigma\sqrt{\frac{n-1}{\chi^2_{0.05/2,n-1}}}$ and $LCL = \sigma\sqrt{\frac{n-1}{\chi^2_{0.975,n-1}}}$

Restricted maximum likelihood (REML)

The current standard for calculating variance components as advised by USP is REML. It was invented in 1937 by M.S. Bartlett and has been refined in 1971 for unbalanced data sets. The restricted (or residual, or reduced) maximum likelihood approach is a particular form of maximum likelihood estimation that uses a likelihood function based on a contrast matrix. The likelihood function is calculated from the probability distribution of these contrasts, according to the model for the complete data set. Instead of the likelihood of y, the likelihood of Ky is used, where the matrix K is such that E[Ky]=0

REML can analyze multi factorial data in balanced and unbalanced designs but it only provides a measurement of the between variance components and the total of the within components. REML is

a predictive technique that tries to describe the population with multiple variance components, the errors expand and it predicts more variation than the sample contains.

Partition of Variation (POV)

POV was invented by Thomas A. Little in 1993 for the analysis of semiconductor data for hard drive manufacturing. In 2015 Thomas A. Little and Paul Deen collaborated on expanding the functionality of the POV engine with a full suite of Measurement System Analysis (MSA) tools. The POV engine is currently publicly available as a JSL script for use in JMP statistical software from SAS and can be found on the website https://thomasalittleconsulting.com.

POV is an exact method because it uses sums of squares to precisely quantify the sample variance components.

The data used to detail the POV method contains one response and the factors Machine and Metrology. A quick view of the data is provided in three graphs:

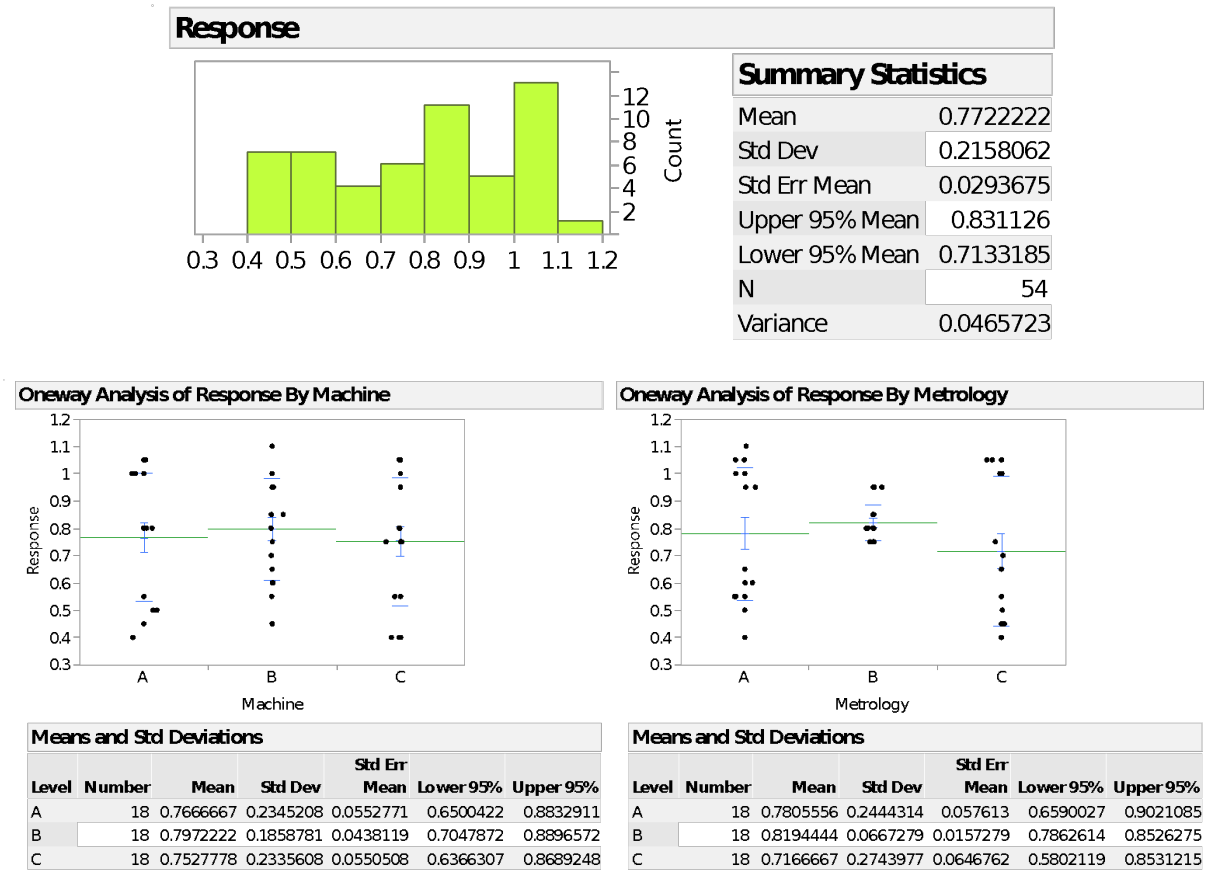


Figure 5 Three part data overview

POV uses generalized linear regression, using the fit model platform, to calculate the sum of squares for the model and the error.

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	0.1741667	0.021771	0.4270
Error	45	2.2941667	0.050981	Prob > F
C. Total	53	2.4683333		0.8986

Table 1 Total ANOVA

$$Var_{Between\ total} = \frac{SS_{Model}}{SS_{Total}} * Var_{Total}\frac{N-1}{N} = \frac{0.1741667}{2.4683334} * 0.04571 = 0.003225$$

$$Var_{Within\ total} = \frac{SS_{Error}}{SS_{Total}} * Var_{Total}\frac{N-1}{N} = \frac{2.294167}{2.4683334} * 0.04571 = 0.042485$$

Then the sequential sum of squares are used to calculate the between factor effects as a fraction of the total between variance. Sequential sum of squares is a variant of sum of squares calculation where each new factor is evaluated against the model with all of the factors before it already inserted. The sum of squares of the between components are:

Sequential (Type 1) Tests					
Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Machine	2	2	0.01861111	0.1825	0.8338
Metrology	2	2	0.09694444	0.9508	0.3941
Machine*Metrology	4	4	0.05861111	0.2874	0.8846

Table 2 Between ANOVA

The ratio of the sequential sums of squares to the total sum of squares, multiplied by the total between variance gives the between variance components:

$$Var_{Between\ Machine} = \frac{SS_{Machine}}{SS_{Total}} * Var_{Between\ Total} = \frac{0.01861111}{0.17416666} * 0.003225 = 0.000345$$

$$Var_{Between\ Metrology} = \frac{SS_{Metrology}}{SS_{Total}} * Var_{Between\ Total} = \frac{0.09694444}{0.174167} * 0.003225 = 0.001795$$

$$Var_{Between\ Machine*Metrology} = \frac{SS_{Machine*Metrology}}{SS_{Total}} * Var_{Between\ Total}$$
$$= \frac{0.05861111}{0.174167} * 0.003225 = 0.001085$$

Then the response is summarized into the variance, grouped by the factors. Because JMP always reports the sample variance, this is upscaled to the population variance by multiplying by (N-1)/N.

Machine	Metrology	N Rows	Variance(Response)	Population Variance
A	A	6	0.088	0.073
A	B	6	0.000	0.000
A	C	6	0.097	0.081
B	A	6	0.051	0.042
B	B	6	0.004	0.004
B	C	6	0.036	0.030
C	A	6	0.062	0.051
C	B	6	0.001	0.001
C	C	6	0.121	0.101

Table 3 Variance table

The common variance is equal to 0 as defined by the combination of Machine A, and Metrology B.

Using generalized regression to fit the population variance produces another set of sequential sum of squares for the within variance components. Using the nr of rows as a frequency allows POV to handle unbalanced data sets.

Sequential (Type 1) Tests					
Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Machine	2	2	0.00134353	.	.
Metrology	2	2	0.00791538	.	.
Machine*Metrology	4	4	0.00184781	.	.

Table 4 Within ANOVA

The common variance is subtracted from the total within and the remainder is used for the within components using the same calculation that produced the between variance components.

$$Var_{Within\ Machine} = \frac{SS_{Machine}}{SS_{Total}} * (Var_{Within\ Total} - Var_{Common}) = \frac{0.00134353}{0.01110672} * 0.042485 = 0.005139$$

$$Var_{Within\ Metrology} = \frac{SS_{Metrology}}{SS_{Total}} * (Var_{Within\ Total} - Var_{Common}) = \frac{0.00791538}{0.01110672} * 0.042485 = 0.030277$$

$$Var_{Within\ Machine*Metrology} = \frac{SS_{Machine*Metrology}}{SS_{Total}} * (Var_{Within\ Total} - Var_{Common}) = \frac{0.00184781}{0.01110672} * 0.042485 = 0.007068$$

The complete set of variance components are:

	Variance	% of total	StdDev
Between Total	0.003225	7.06	0.056792
Between Machine	0.000345	0.75	0.018565
Between Metrology	0.001795	3.93	0.042371
Between Machine*Metrology	0.001085	2.37	0.032945

Within Total	0.042485	92.94	0.206118
Within Machine	0.005139	11.24	0.071688
Within Metrology	0.030277	66.24	0.174004
Within Machine*Metrology	0.007068	15.46	0.084072
Common	0	0	0
Total	0.04571	100	0.213799

Table 5 Variance components

Study question: How does POV compare to ANOVA sums of squares calculations?

POV uses (sequential) sums of squares to break observed variation down into its constituent components. It does this by splitting the total variance into the total between and the total within and using sequential sum of squares to split the between total into the between components and the within total into the within components (excluding the common variation).

Sample data description

The data set used to compare variance component analysis using REML and POV for the quantitation of intermediate precision is based on a structured experiment designed using the custom design tool in JMP. It is 132 run experiment using three categorical main effects: analyst, day and instrument. The experiment is ran at three different concentrations and has 6 repeats built in. Analyst has two levels, Day has three levels, Instrument has 2 levels, concentration is at 50, 100 and 150%.

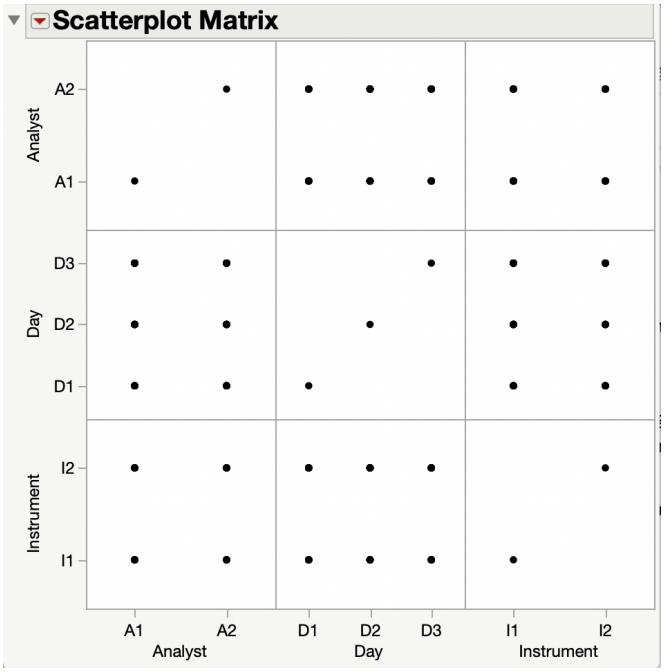


Figure 6 Experiment structure

	Concentration	Analyst	Day	Instrument	Response Sample 1	Response Sample 2	Response Sample 3
1	150	A1	D3	I2	145.98	153.28	154.86
2	150	A1	D3	I2	148.76	161.02	156.56
3	150	A1	D3	I2	147.03	153.10	146.77
4	150	A1	D3	I2	148.32	157.70	153.12
5	150	A1	D3	I2	153.41	151.39	156.66
6	150	A1	D3	I2	150.80	158.31	139.80
7	100	A1	D2	I1	105.55	110.25	106.85
8	100	A1	D2	I1	103.52	108.49	111.48
9	100	A1	D2	I1	109.69	106.00	108.90
10	100	A1	D2	I1	109.09	105.84	104.97
11	100	A1	D2	I1	114.78	109.30	112.76
12	100	A1	D2	I1	112.79	111.80	112.85
13	150	A2	D2	I1	171.31	160.44	164.19
14	150	A2	D2	I1	160.24	158.53	162.72
15	150	A2	D2	I1	160.34	163.95	160.14
16	150	A2	D2	I1	162.52	168.99	164.80
17	150	A2	D2	I1	163.26	163.84	167.26
18	150	A2	D2	I1	172.33	152.42	168.24
19	100	A2	D1	I1	109.73	112.55	106.08
20	100	A2	D1	I1	107.70	115.97	107.26
21	100	A2	D1	I1	109.54	108.29	100.43

Table 6 Sample of dataset

The sample responses are based on simulation, the simulation equations used are:

Sample 1 focuses on strong between effects and weak within effects.

```
Match( :Concentration,
      "50", Random Normal( 50, 0.5 ),
      "100", Random Normal( 100, 2 ),
      "150", Random Normal( 150, 4 )
) + Match( :Analyst, "A1", Random Normal( 0, 1 ), "A2", Random Normal( 5,
1 ) )
+Match( :Day,
      "D1", Random Normal( 0, 1 ),
      "D2", Random Normal( 5, 1 ),
      "D3", Random Normal( 0, 1 )
) + Match( :Instrument, "I1", Random Normal( 4, 1 ), "I2", Random Normal(
0, 1 ) )
+Random Normal( 0, 1 )
```

Sample 2 has weak between effects and strong within effects.

```
Match( :Concentration,
      "50", Random Normal( 50, 0.5 ),
      "100", Random Normal( 100, 2 ),
      "150", Random Normal( 150, 4 )
) + Match( :Analyst, "A1", Random Normal( 1, 2 ), "A2", Random Normal( 5,
1 ) )
+Match( :Day,
      "D1", Random Normal( 2, 3 ),
      "D2", Random Normal( 5, 2 ),
      "D3", Random Normal( 2, 1 )
) + Match( :Instrument, "I1", Random Normal( 4, 1 ), "I2", Random Normal(
2, 3 ) )
+Random Normal( 0, 1 )
```

Sample 3 has a mixed balance of between and within effects.

```
Match( :Concentration,
      "50", Random Normal( 50, 0.5 ),
      "100", Random Normal( 100, 2 ),
      "150", Random Normal( 150, 4 )
) + Match( :Analyst, "A1", Random Normal( 0, 1 ), "A2", Random Normal( 5,
2.5 ) )
+Match( :Day,
      "D1", Random Normal( 0, 2 ),
      "D2", Random Normal( 5, 1 ),
      "D3", Random Normal( 0, 0.5 )
) + Match( :Instrument, "I1", Random Normal( 4, 1 ), "I2", Random Normal(
0, 1 ) )
+Random Normal( 0, 1 )
```

Comparison

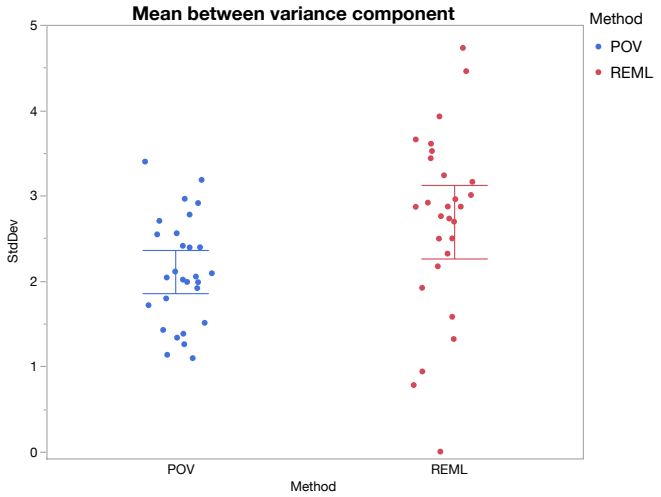
The full analytical reports for REML and POV are found in the appendix. The variance components are presented here for evaluation.

Between components

Study question: How does POV compare to REML for the quantitation of the between factor variance components?

		Concentration	N	Between Analyst	Between Day	Between Instrument
Sample 1	POV	50	42	2.962	2.394	1.715
		100	42	2.051	2.704	2.040
		150	48	3.398	1.381	2.777
	REML	50	42	4.457	2.917	2.495
		100	42	2.871	2.758	2.958
		150	48	4.730	0.939	3.927
Sample 2	POV	50	42	1.985	1.335	1.426
		100	42	2.411	1.258	1.509
		150	48	1.795	1.095	1.915
	REML	50	42	3.006	1.319	1.919
		100	42	3.608	0.780	2.171
		150	48	2.320	0.000	2.870
Sample 3	POV	50	42	2.558	2.912	1.988
		100	42	2.090	3.184	1.135
		150	48	2.392	2.545	2.015
	REML	50	42	3.657	3.237	2.868
		100	42	2.498	3.522	1.580
		150	48	3.437	3.161	2.731

Table 7 Between variance components



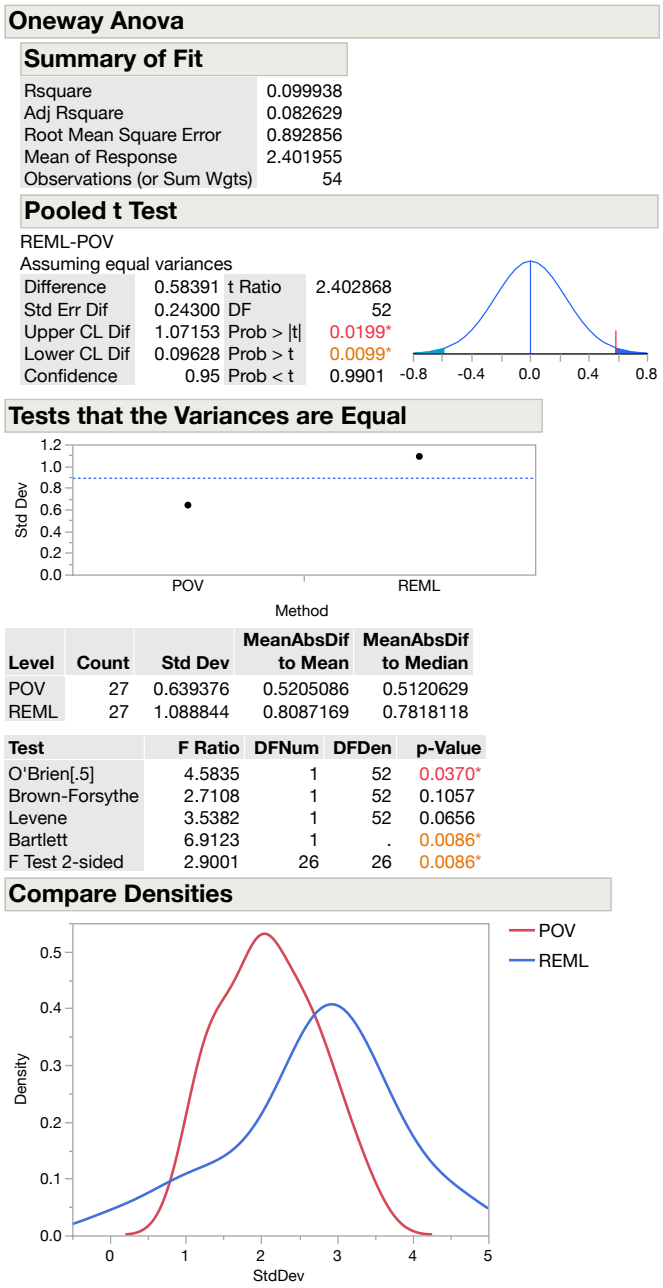


Figure 7 Test of means for sum of between components

It can be seen that the between variance components are on average bigger when using the REML method versus the POV method. ANOVA with a pooled t-test tells us this is a significant difference where REML is on average 0.58 bigger (95%CI 0.096-1.072) than POV. Since REML components do not follow a normal distribution we use Levene test for unequal variation and conclude that the variation is equal.

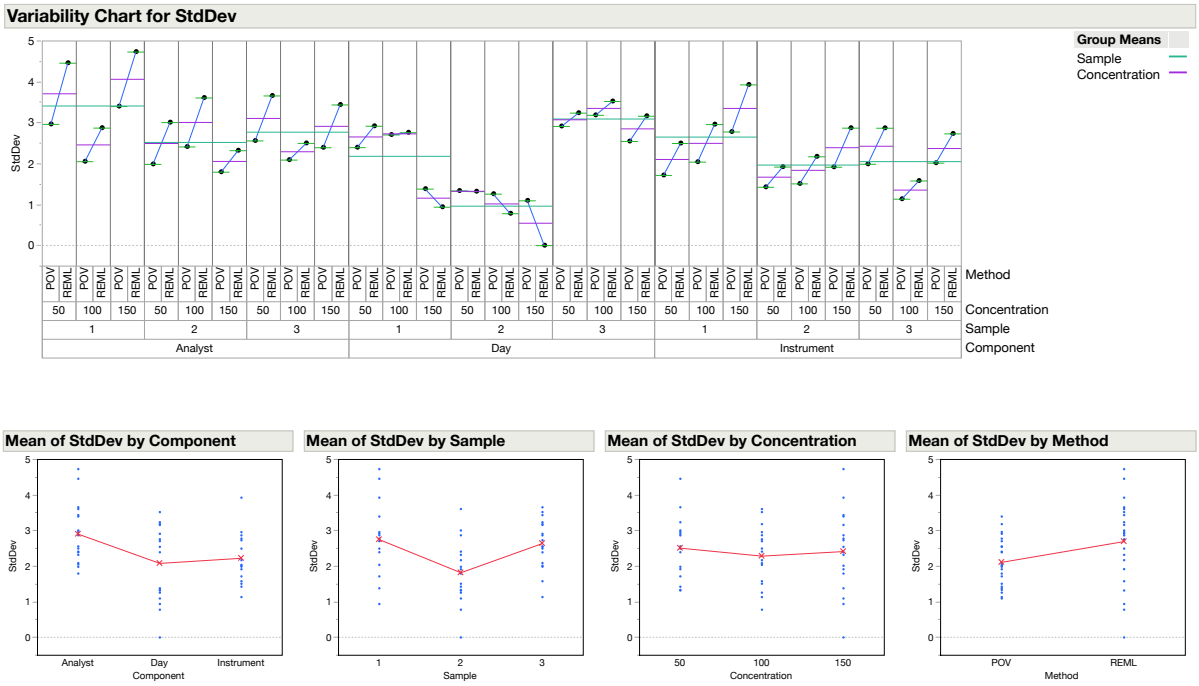
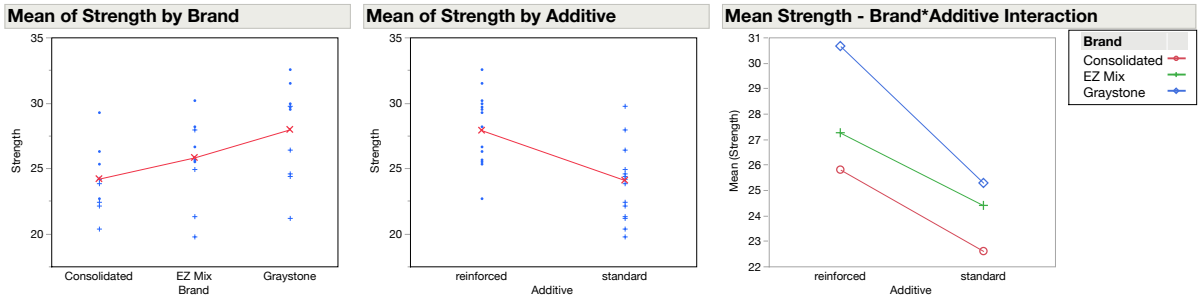


Figure 8 Visualizing the between components at different factor combinations

If the variation components are broken down by method and concentration, the same pattern emerges. Mostly REML components are bigger than POV, sometimes it is the other way around, very rarely are they matched.

When the components are comparatively large, REML overestimates the variance as compared to POV. When the effect is comparatively small REML underestimates compared to POV. The most blatant example of this is the Component=Day, Sample=2, Concentration=150 where REML produces a zero result which does not match the simulation equations. POV gets it right in that case.

To better visualize the wrongness of the zero problem another data set called cement is presented:



REML Variance Component Estimates

Random Effect	Var Component	Std Error	95% Lower	95% Upper	Pct of Total
Brand	2.9584828	3.5742118	0.6791237	509.05634	18.564
Additive	6.8607711	10.279934	1.2955219	15855.731	43.051
Brand*Additive	0	0	0	0	0.000
Residual	6.1170024	1.6965512	3.7936554	11.488237	38.384
Total	15.936256	10.973116	5.8356522	121.29335	100.000

-2 LogLikelihood = 144.62952257

Figure 9 cement REML variance components

Notice that the between Brand*Additive interaction component using REML is zero, but looking at interaction graph it is clear that those lines are not parallel and thus the interaction term cannot be zero. The zero is an artifact of how REML calculates and is a misleading result.

Within components and repeatability

Study question: How does POV compare to REML for the quantitation of the within factor variance components?

Study question: How does POV compare to REML for the quantitation of the repeatability?

		Concentration	Common	Within Analyst	Within Day	Within Instrument	Repeatability (Total within)
Sample 1	POV	50	1.051	1.024	0.728	0.793	1.820
		100	1.471	1.834	1.217	0.632	2.722
		150	2.458	1.056	3.361	1.373	4.510
	REML	50					1.939
		100					2.898
		150					4.772
Sample 2	POV	50	1.042	0.625	1.947	2.400	3.321
		100	2.154	1.088	2.421	1.288	3.653
		150	3.422	1.151	0.471	3.218	4.860
	REML	50					3.543
		100					3.881
		150					5.042
Sample 3	POV	50	1.014	2.342	0.666	0.725	2.735
		100	0.983	1.012	1.647	0.941	2.364
		150	2.706	0.397	3.314	1.815	4.664
	REML	50					2.914
		100					2.518
		150					4.933

Table 8 Within variance components

POV gives a full break down of the within components while REML does not provide any insight into the structure of the within variation and only provides the total within (repeatability). REML is a less insightful method for understanding the data. To better understand why REML not calculating the within variation components is a problem, look at Cement again but this time at the graphs belonging to the within components:

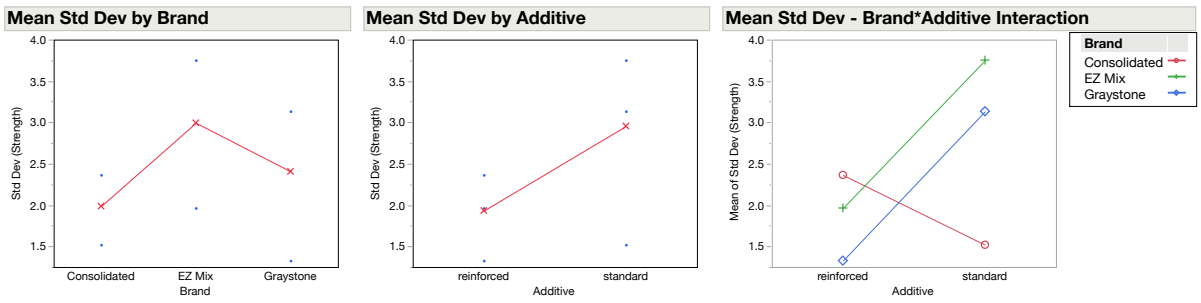


Figure 10 Cement within component graphs

And the corresponding POV analysis:

Partition of Variation (POV) Crossed analysis

2 Factor POV Analysis: Brand, Additive

Component	Pop Variance	% of Total	Sqrt(Var Comp)	F Ratio	Prob>F
Total Between	6.327	55.91	2.515	5.7246	0.0093*
Between Brand	2.380	21.03	1.543	5.7246	0.0093*
Between Additive	3.634	32.12	1.906	17.4821	0.0003*
Between Brand*Additive	0.312	2.76	0.559	0.7507	0.4828
	.	.	.		
Total Within	4.989	44.09	2.234		
Within Brand	0.796	7.03	0.892		
Within Additive	1.158	10.24	1.076		
Within Brand*Additive	1.625	14.36	1.275		
Common	1.410	12.46	1.188		
	.	.	.		
Grand Total	11.316	100.00	3.364		

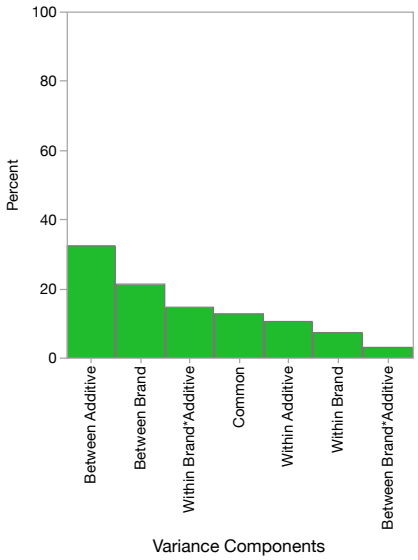
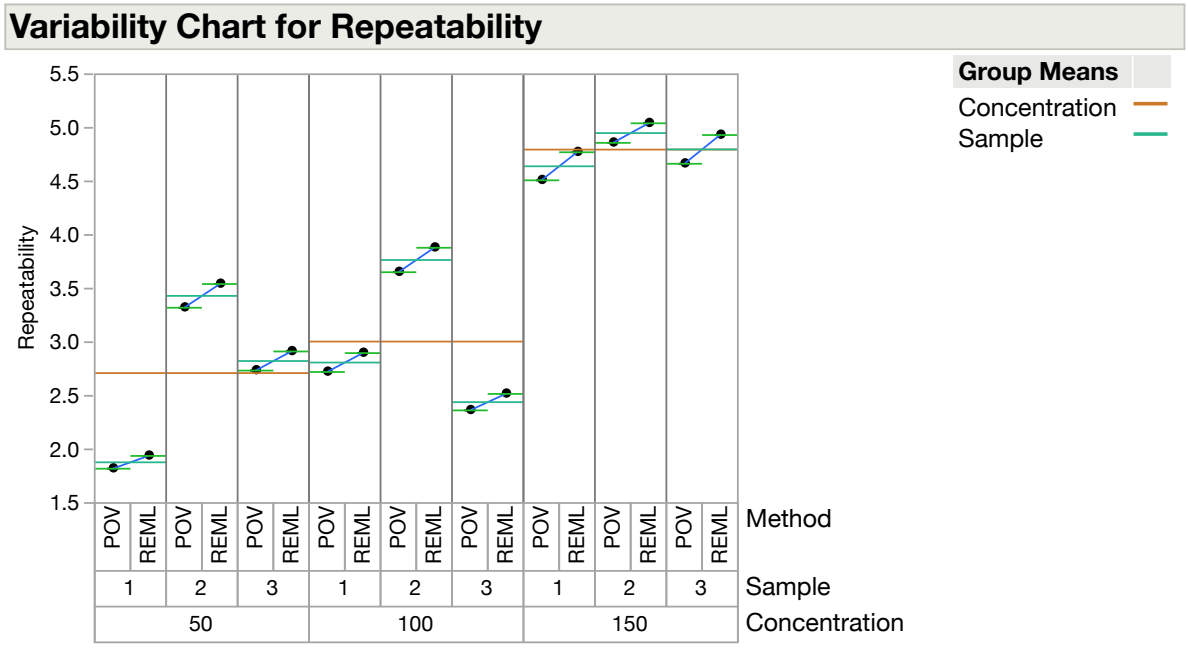


Figure 11 Cement POV result

The within components make up 44% of the total observed variation. Brand is the smallest component but the other ones are all about equally important.

The repeatability is the only part of the within components that can be compared between the two methods. The repeatability broken down by the factors looks like this:



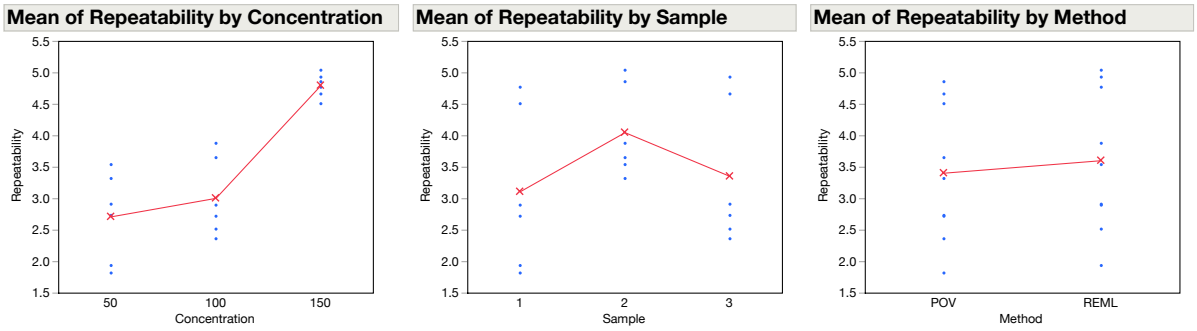


Figure 12 The repeatability broken down by Concentration, Sample and Method

It can be seen that the repeatability is always bigger when using the REML method versus the POV method.

Intermediate precision

Study question: Which method is best for determining the variance components of a bioassay?

		Concentration	Intermediate Precision (Total between and within)	IP % of tolerance	LCL 95% IP	UCL 95% IP
Sample 1	POV	50	4.556	39.1	3.7	5.8
		100	4.805	41.2	4	6.1
		150	6.443	55.3	5.4	8.1
	REML	50	6.194	53.2	5.1	7.9
		100	5.744	49.3	4.7	7.3
		150	7.839	67.3	6.5	9.8
	StdDev	50	4.556	39.1	3.7	5.8
		100	4.805	41.2	4	6.1
		150	6.443	55.3	5.4	8.1
Sample 2	POV	50	4.335	37.2	3.6	5.5
		100	4.798	41.2	3.9	6.1
		150	5.631	48.3	4.7	7.1
	REML	50	5.197	44.6	4.3	6.6
		100	5.779	49.6	4.8	7.4
		150	6.248	53.6	5.2	7.8
	StdDev	50	4.335	37.2	3.6	5.5
		100	4.798	41.2	3.9	6.1
		150	5.631	48.3	4.7	7.1
Sample 3	POV	50	5.144	44.2	4.2	6.6
		100	4.624	39.7	3.8	5.9
		150	6.166	52.9	5.1	7.7
	REML	50	6.369	54.7	5.2	8.1
		100	5.242	45	4.3	6.7
		150	7.321	62.8	6.1	9.2
	StdDev	50	5.144	44.2	4.2	6.6
		100	4.624	39.7	3.8	5.9
		150	6.166	52.9	5.1	7.7

Table 9 Intermediate precision

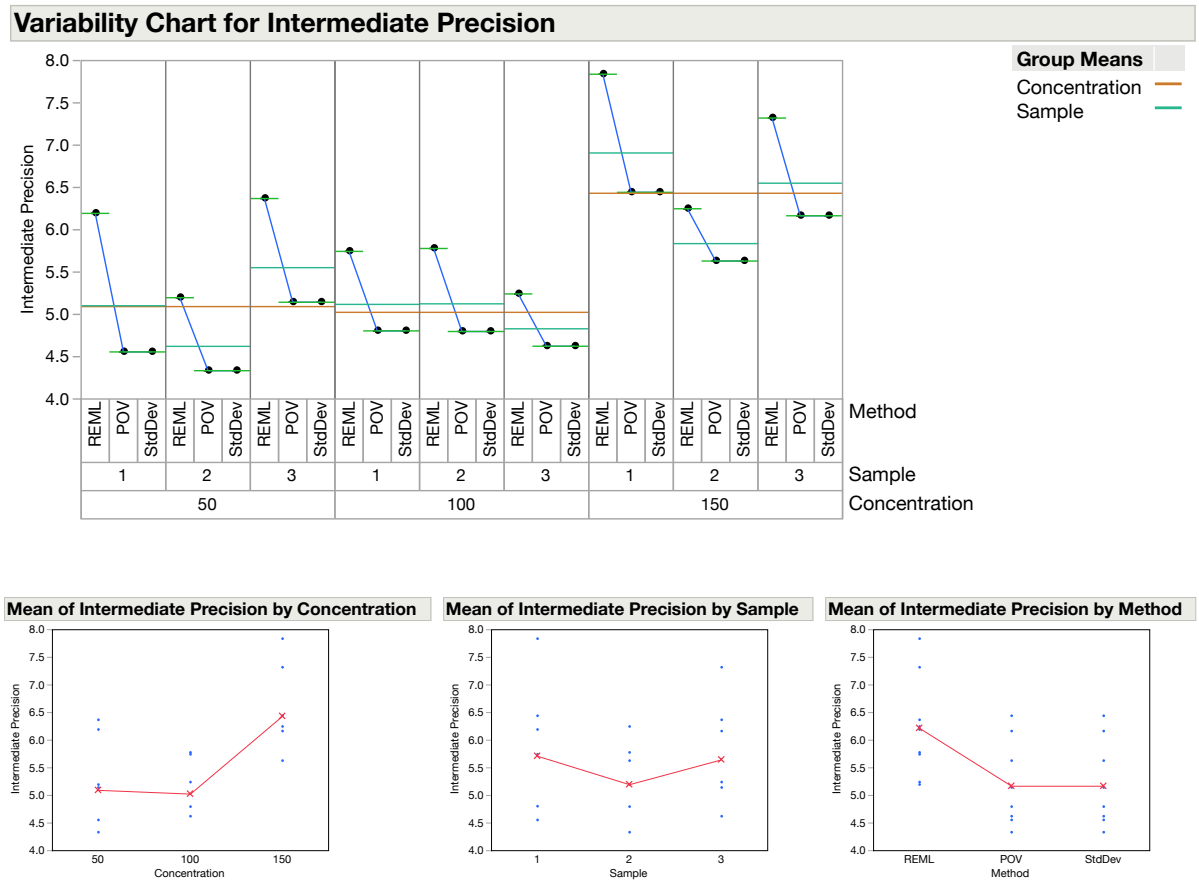


Figure 13 Visualization of intermediate precision

	Concentration	POV	REML	StdDev	POV - StdDev inflation	REML - StdDev inflation
Sample 1	50	4.556	6.194	4.556	0.00%	34.30%
	100	4.805	5.744	4.805	0.00%	18.10%
	150	6.443	7.839	6.443	0.00%	20.40%
Sample 2	50	4.335	5.197	4.335	0.00%	18.50%
	100	4.798	5.779	4.798	0.00%	19.00%
	150	5.631	6.248	5.631	0.00%	9.80%
Sample 3	50	5.144	6.369	5.144	0.00%	22.30%
	100	4.624	5.242	4.624	0.00%	12.00%
	150	6.166	7.321	6.166	0.00%	17.50%

Table 10 Inflation of intermediate precision by method

It can be observed that REML inflates the estimated total variance. POV is exact and predicts all the observed variation. Bigger variance components result in a bigger intermediate precision quantitation. For the same observed data and specification limits a REML based analysis will fail qualification more often than a POV based analysis. This can be remedied by opening the tolerance window wider but this will decrease the sensitivity of the bioassay validation making POV a better method to quantitate variance components for the use of bioassay qualification.

Acceptance criteria

How to set acceptance criteria for the intermediate precision and repeatability of a bioassay?

The standard method used for specifying acceptance criteria is using coefficient of variation:

$$CV = \frac{S}{\bar{X}} * 100$$

The method used in USP <1033> is based on Cpm:

$$Cpm = \frac{USL - LSL}{6\sqrt{\sigma^2_{Product} + Relative\ Bias^2 + IP^2}}$$

The better method is based on a precision to tolerance ratio, resembling the Cpm calculation:

$$\%\ of\ Tolerance = \frac{IP/\sqrt{n} * 5.15}{USL - LSL} * 100$$

Lets first see the problem with using CV:

Acceptance criteria settin…

USL	130
LSL	70
Number of determinations	3

	Mean potency	IP	CV (%)	% of Tol
1	175	17.5	10	86.72
2	130	13	10	64.42
3	100	10	10	49.56
4	70	7	10	34.69
5	50	5	10	24.78

Table 11 The problem with CV

Notice that in this example CV does not give us any information on what levels work and do not work for this assay. It does not relate to the specification limits for relative potency making it not very useful.

The Cpm calculation does not fair much better: why is product variation counted against the assay performance? If the assay is well controlled, the bias will be close to zero leaving just the Intermediate precision. Then Cpm is a dimensionless number which is not anchored in the understanding of the problem. Cpm is better than CV but should be avoided.

The better method is done by inverting the Cpm calculation. That answers the question: How big is the variation in relation to the tolerance? By introducing the square root of the number of determinations the intermediate precision is adjusted for repeated assay's. Lastly the 6 is swapped out for a 5.15. This is a multiplier to convert StdDev to width of the associated error distribution. 6 sigma covers 99.73% of the error distribution. 5.15 sigma covers 99% of the error distribution. For the extra 0.85 sigma only 0.73% more of the distribution is covered yet it fails significantly more assay's. ASQ recommends 5.15 be used as the multiplier for capability calculations.

The acceptance number for the P/T method comes from USP <1033>. In chapter 2.5 a Cpm of 1.3 is recommended. This is equivalent to shrinking the acceptance window on % of tolerance down from 100% to 77%. Then the product variation and the relative bias are extracted shrinking the window by another 25% resulting in a acceptance criteria of percent of tolerance < 60%.

Conclusion

1. How does POV compare to REML for the quantitation of the between factor variance components?
2. How does POV compare to REML for the quantitation of the within factor variance components?
3. How does POV compare to REML for the quantitation of the repeatability?
4. How does POV compare to ANOVA sums of squares calculations?
5. Which method is best for determining the variance components of a bioassay?
6. How to set acceptance criteria for intermediate precision and repeatability of a bioassay using both POV and REML?

As compared against the current standard advised by USP 1033, POV variance components analysis gives Intermediate Precision numbers that are closer to the group standard deviation and as such are a better description of the variance components. The between and within components are nescessarily closer to the true values as well, in fact the usage of the sequential sums of squares in the calculation of POV guarantees and exact description of the variance components. In sample 2 we see that REML produces a 0 variance components which we know is not correct based on the equations used for random data generation.

POV gives us more insight into the data by parsing out the within variation into its constituent components. REML does not break down the repeatability into its constituent components. If the need arises to improve the bioassay execution, understanding where the biggest sources of variation are is essential. By not parsing out the within variation, the ability to prioritize improvement efforts is lost.

Using POV there is less variance inflation making it a more reliable method in all three of the tested conditions. Using REML sample 1 and 3 would fail 'IP as a percentage of tolerance < 60%' for concentration 150 while using POV it does not fail.

Because of the exactness and completeness of the calculation, POV needs to become the standard for variance components estimation in bioassays and different applications. POV gives a more complete understanding of the sources of variation and a more complete characterization and validation of a bioassay design.

A better method for acceptance criteria is the percent of tolerance approach that relates the observed variation to the business case. By using percent of tolerance < 60% as the acceptance criteria for intermediate precision.

Appendix

Study design and case data:

Concentration	Analyst	Day	Instrument	Response Sample 1	Response Sample 2	Response Sample 3
150	A1	D3	I2	145.98	153.28	154.86
150	A1	D3	I2	148.76	161.02	156.56
150	A1	D3	I2	147.03	153.10	146.77
150	A1	D3	I2	148.32	157.70	153.12
150	A1	D3	I2	153.41	151.39	156.66
150	A1	D3	I2	150.80	158.31	139.80
100	A1	D2	I1	105.55	110.25	106.85
100	A1	D2	I1	103.52	108.49	111.48
100	A1	D2	I1	109.69	106.00	108.90
100	A1	D2	I1	109.09	105.84	104.97
100	A1	D2	I1	114.78	109.30	112.76
100	A1	D2	I1	112.79	111.80	112.85
150	A2	D2	I1	171.31	160.44	164.19
150	A2	D2	I1	160.24	158.53	162.72
150	A2	D2	I1	160.34	163.95	160.14
150	A2	D2	I1	162.52	168.99	164.80
150	A2	D2	I1	163.26	163.84	167.26
150	A2	D2	I1	172.33	152.42	168.24
100	A2	D1	I1	109.73	112.55	106.08
100	A2	D1	I1	107.70	115.97	107.26
100	A2	D1	I1	109.54	108.29	100.43
100	A2	D1	I1	110.78	104.47	106.53
100	A2	D1	I1	103.04	110.79	108.46
100	A2	D1	I1	109.10	113.60	107.44
100	A2	D2	I2	111.16	109.50	110.65
100	A2	D2	I2	112.48	106.18	113.12
100	A2	D2	I2	107.76	112.81	112.98
100	A2	D2	I2	109.43	110.43	108.69
100	A2	D2	I2	109.98	108.29	105.76
100	A2	D2	I2	110.74	111.91	109.56
50	A1	D2	I1	59.23	60.10	59.60
50	A1	D2	I1	58.61	63.92	58.56
50	A1	D2	I1	56.93	54.36	61.53
50	A1	D2	I1	56.72	64.06	59.01
50	A1	D2	I1	56.80	58.35	58.95
50	A1	D2	I1	58.81	62.79	60.40
150	A1	D3	I1	152.78	147.36	157.25
150	A1	D3	I1	157.84	162.66	152.11
150	A1	D3	I1	150.36	149.84	151.17
150	A1	D3	I1	152.84	161.64	158.80
150	A1	D3	I1	156.92	156.27	150.75
150	A1	D3	I1	157.37	163.08	158.30
150	A2	D1	I1	162.30	158.43	159.95
150	A2	D1	I1	164.80	168.37	151.73

150	A2	D1	I1	167.68	168.95	170.05
150	A2	D1	I1	164.53	164.62	158.01
150	A2	D1	I1	151.76	169.39	160.50
150	A2	D1	I1	155.77	167.65	161.66
50	A1	D3	I2	47.44	52.41	54.94
50	A1	D3	I2	50.39	60.07	49.45
50	A1	D3	I2	51.42	50.18	47.86
50	A1	D3	I2	50.41	58.23	50.23
50	A1	D3	I2	46.94	52.36	51.10
50	A1	D3	I2	47.02	53.96	50.03
100	A2	D2	I1	115.70	118.52	117.73
100	A2	D2	I1	112.16	111.14	109.92
100	A2	D2	I1	112.76	111.97	115.27
100	A2	D2	I1	114.50	118.38	114.42
100	A2	D2	I1	115.22	110.86	111.43
100	A2	D2	I1	110.83	116.41	108.93
100	A1	D1	I2	100.58	105.54	101.24
100	A1	D1	I2	101.58	110.52	98.38
100	A1	D1	I2	96.79	100.09	100.63
100	A1	D1	I2	102.17	103.91	100.05
100	A1	D1	I2	96.04	104.02	99.24
100	A1	D1	I2	100.55	96.59	100.82
150	A1	D2	I2	149.37	153.63	157.25
150	A1	D2	I2	157.83	160.46	153.21
150	A1	D2	I2	153.94	156.19	158.10
150	A1	D2	I2	153.18	157.20	160.54
150	A1	D2	I2	152.56	148.67	152.25
150	A1	D2	I2	145.86	159.80	156.75
50	A2	D3	I1	58.31	62.45	57.38
50	A2	D3	I1	56.42	61.10	61.98
50	A2	D3	I1	61.38	60.62	64.79
50	A2	D3	I1	59.81	60.93	62.59
50	A2	D3	I1	60.82	63.07	60.30
50	A2	D3	I1	57.51	63.18	56.69
150	A1	D1	I1	161.10	161.52	160.02
150	A1	D1	I1	150.99	156.20	151.60
150	A1	D1	I1	158.20	152.57	147.68
150	A1	D1	I1	154.34	153.30	149.02
150	A1	D1	I1	166.13	162.73	157.09
150	A1	D1	I1	147.33	165.48	148.05
100	A2	D3	I2	106.39	111.75	109.61
100	A2	D3	I2	101.46	97.70	103.75
100	A2	D3	I2	109.66	112.40	107.61
100	A2	D3	I2	109.14	114.11	104.79
100	A2	D3	I2	108.02	113.60	105.57
100	A2	D3	I2	101.90	111.27	104.89
50	A1	D1	I1	51.51	63.33	56.73
50	A1	D1	I1	54.52	56.59	48.42
50	A1	D1	I1	56.28	61.12	55.17
50	A1	D1	I1	53.18	58.02	56.37

50	A1	D1	I1	55.00	56.26	55.24
50	A1	D1	I1	56.08	59.79	53.59
50	A2	D1	I2	56.06	63.86	55.38
50	A2	D1	I2	55.55	57.81	48.78
50	A2	D1	I2	55.18	57.06	54.08
50	A2	D1	I2	58.69	52.78	57.15
50	A2	D1	I2	57.62	66.05	53.37
50	A2	D1	I2	54.45	63.69	56.99
150	A2	D3	I2	161.64	159.49	153.78
150	A2	D3	I2	155.04	163.01	153.30
150	A2	D3	I2	158.95	153.84	156.41
150	A2	D3	I2	160.14	159.46	154.93
150	A2	D3	I2	151.32	151.67	162.28
150	A2	D3	I2	160.60	158.77	151.58
50	A1	D1	I2	51.80	56.35	49.29
50	A1	D1	I2	48.40	54.89	52.52
50	A1	D1	I2	49.15	51.52	54.08
50	A1	D1	I2	52.83	55.82	51.11
50	A1	D1	I2	50.28	62.13	46.28
50	A1	D1	I2	51.32	54.26	49.54
100	A1	D3	I1	109.24	104.81	105.12
100	A1	D3	I1	106.48	106.48	102.11
100	A1	D3	I1	110.08	112.37	103.96
100	A1	D3	I1	102.80	108.89	102.45
100	A1	D3	I1	105.31	106.26	104.72
100	A1	D3	I1	110.25	105.84	105.61
150	A2	D1	I2	148.98	160.48	156.69
150	A2	D1	I2	155.54	147.48	143.96
150	A2	D1	I2	162.98	158.29	160.11
150	A2	D1	I2	151.38	158.55	159.96
150	A2	D1	I2	154.46	156.87	146.32
150	A2	D1	I2	158.47	156.08	153.97
50	A2	D2	I2	64.36	66.18	56.16
50	A2	D2	I2	57.34	59.28	66.09
50	A2	D2	I2	58.74	61.56	58.46
50	A2	D2	I2	64.58	57.91	66.37
50	A2	D2	I2	62.82	68.34	63.52
50	A2	D2	I2	61.40	62.01	60.45

Cement:

Strength	Brand	Additive	Humidity
32.6	Graystone	reinforced	56.4
31.5	Graystone	reinforced	44.1
30	Graystone	reinforced	50
29.8	Graystone	standard	52.5
29.7	Graystone	reinforced	46.7
29.5	Graystone	reinforced	53

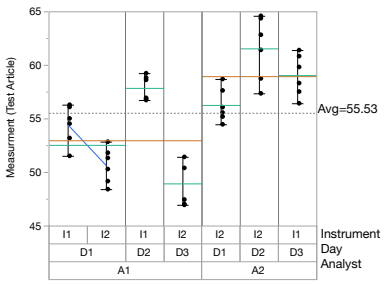
29.3	Consolidated	reinforced	44.6
30.2	EZ Mix	reinforced	42.4
28.2	EZ Mix	reinforced	45.2
28	EZ Mix	standard	40.7
28	EZ Mix	standard	47.2
26.7	EZ Mix	reinforced	50.7
26.4	Graystone	standard	48.9
26.3	Consolidated	reinforced	50.7
25.7	EZ Mix	reinforced	48
25.5	EZ Mix	reinforced	43
25.4	Consolidated	reinforced	46.4
25.3	Consolidated	reinforced	52.6
24.9	EZ Mix	standard	50.6
24.6	Graystone	standard	53.1
24.4	Graystone	standard	55.9
24.2	Consolidated	standard	47
23.9	Consolidated	standard	47.7
22.7	Consolidated	reinforced	51.8
22.4	Consolidated	standard	54.3
22.1	Consolidated	standard	59.9
21.3	EZ Mix	standard	58
21.2	Graystone	standard	68.7
20.4	Consolidated	standard	60
19.8	EZ Mix	standard	60.2

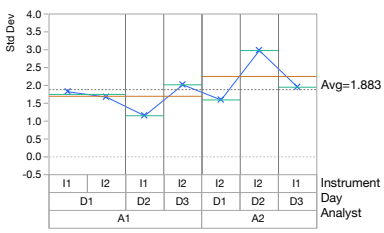
POV analysis reports

Sample 1:

Variability Gauge

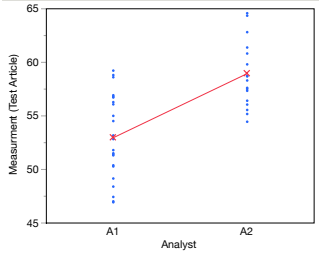
Variability Chart for Measurment (Test Article)



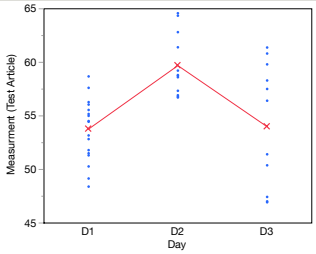


Gauge R&R Mean Plots

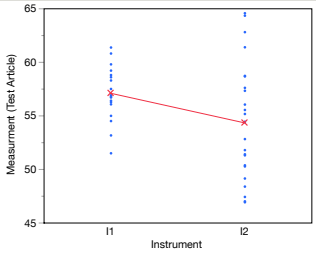
Mean of Measurment (Test Article) by Analyst



Mean of Measurment (Test Article) by Day

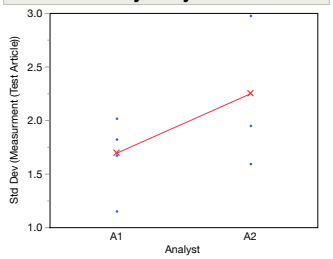


Mean of Measurment (Test Article) by Instrument

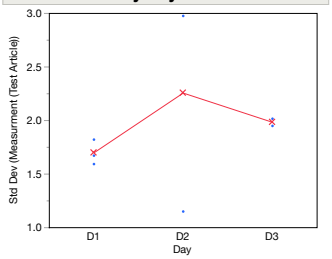


Gauge R&R Std Dev Plots

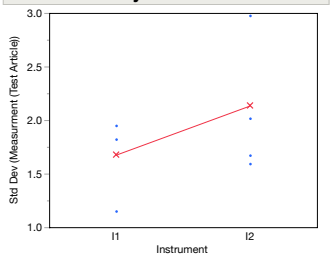
Mean Std Dev by Analyst



Mean Std Dev by Day



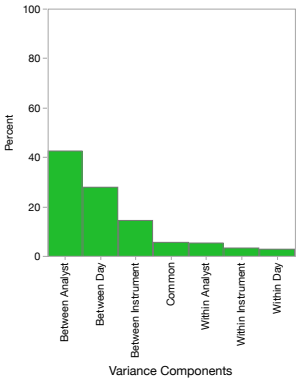
Mean Std Dev by Instrument



Partition of Variation (POV) Main Effect analysis

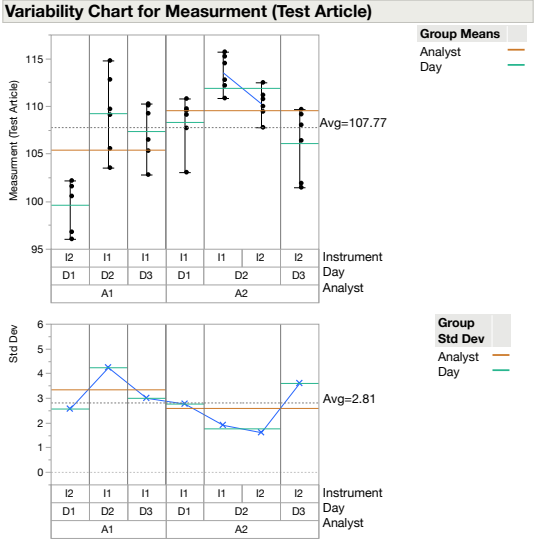
3 Factor POV Analysis: Analyst, Day, Instrument

Concentration	Component	Pop Variance	% of Total	Sqrt(Var Comp)	F Ratio	Prob>F
50	Total Between	17.449	84.05	4.177	98.0312	<.0001*
50	Between Analyst	8.774	42.26	2.962	98.0312	<.0001*
50	Between Day	5.732	27.61	2.394	32.0213	<.0001*
50	Between Instrument	2.943	14.17	1.715	32.8773	<.0001*
50	.	.	.	.		
50	Total Within	3.312	15.95	1.820		
50	Within Analyst	1.049	5.05	1.024		
50	Within Day	0.530	2.55	0.728		
50	Within Instrument	0.629	3.03	0.793		
50	Common	1.104	5.32	1.051		
50	.	.	.	.		
50	Grand Total	20.760	100.00	4.556		



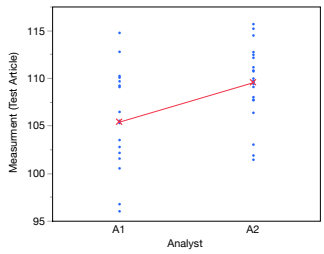
Variability Gauge

Variability Chart for Measurment (Test Article)

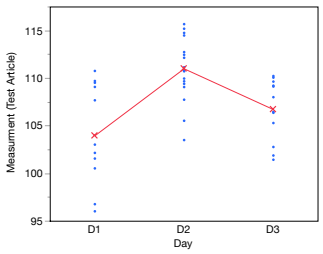


Gauge R&R Mean Plots

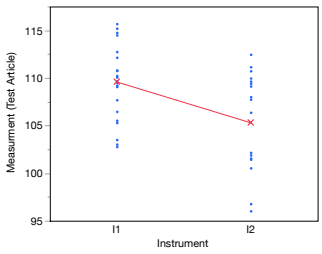
Mean of Measurment (Test Article) by Analyst



Mean of Measurment (Test Article) by Day

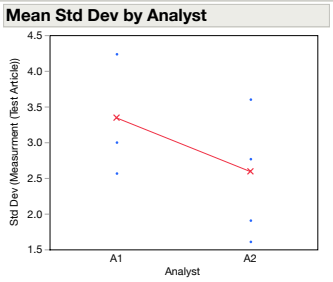


Mean of Measurment (Test Article) by Instrument

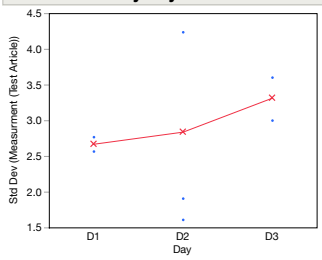


Gauge R&R Std Dev Plots

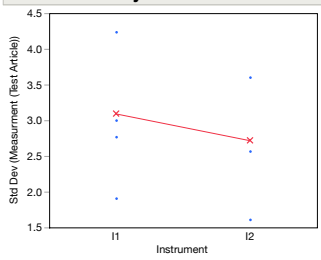
Mean Std Dev by Analyst



Mean Std Dev by Day



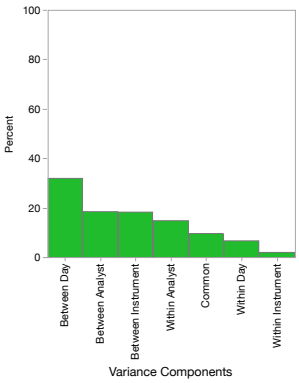
Mean Std Dev by Instrument



Partition of Variation (POV) Main Effect analysis

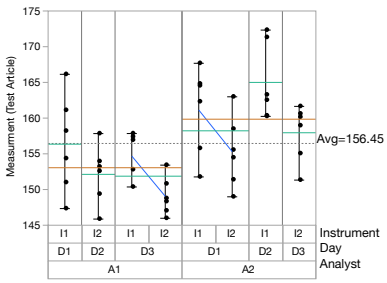
3 Factor POV Analysis: Analyst, Day, Instrument

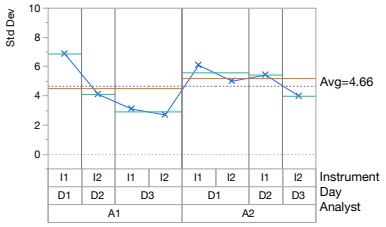
Concentration	Component	Pop Variance	% of Total	Sqrt(Var Comp)	F Ratio	Prob>F
100	Total Between	15.679	67.91	3.960	21.0124	<.0001*
100	Between Analyst	4.208	18.22	2.051	21.0124	<.0001*
100	Between Day	7.311	31.66	2.704	18.2543	<.0001*
100	Between Instrument	4.161	18.02	2.040	20.7801	<.0001*
100		.	.	.		
100	Total Within	7.409	32.09	2.722		
100	Within Analyst	3.365	14.57	1.834		
100	Within Day	1.481	6.42	1.217		
100	Within Instrument	0.399	1.73	0.632		
100	Common	2.164	9.37	1.471		
100		.	.	.		
100	Grand Total	23.089	100.00	4.805		



Variability Gauge

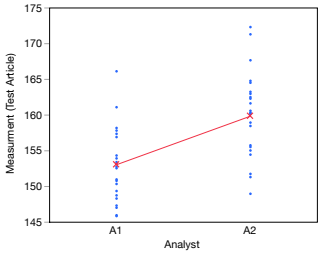
Variability Chart for Measurment (Test Article)



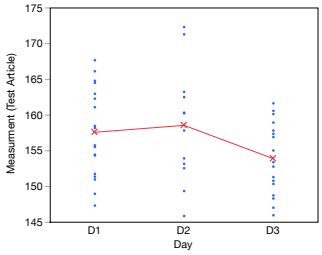


Gauge R&R Mean Plots

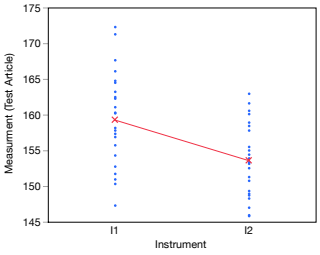
Mean of Measurment (Test Article) by Analyst



Mean of Measurment (Test Article) by Day

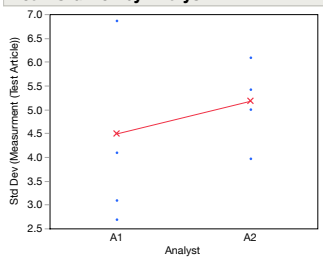


Mean of Measurment (Test Article) by Instrument

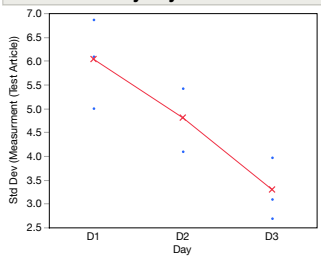


Gauge R&R Std Dev Plots

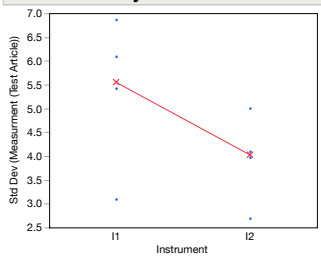
Mean Std Dev by Analyst



Mean Std Dev by Day



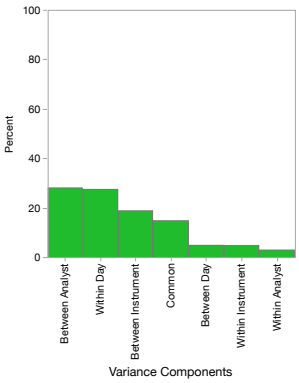
Mean Std Dev by Instrument



Partition of Variation (POV) Main Effect analysis

3 Factor POV Analysis: Analyst, Day, Instrument

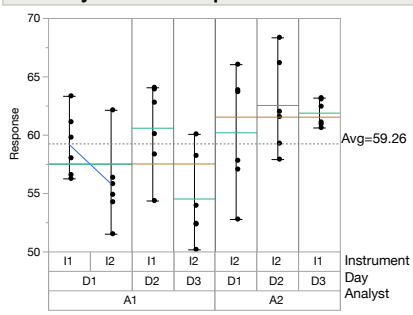
Concentration	Component	Pop Variance	% of Total	Sqrt(Var Comp)	F Ratio	Prob>F
150	Total Between	21.165	50.99	4.601	24.4055	<.0001*
150	Between Analyst	11.545	27.82	3.398	24.4055	<.0001*
150	Between Day	1.907	4.60	1.381	2.0161	0.1456
150	Between Instrument	7.712	18.58	2.777	16.3025	0.0002*
150		.	.	.		
150	Total Within	20.342	49.01	4.510		
150	Within Analyst	1.116	2.69	1.056		
150	Within Day	11.297	27.22	3.361		
150	Within Instrument	1.886	4.54	1.373		
150	Common	6.043	14.56	2.458		
150		.	.	.		
150	Grand Total	41.507	100.00	6.443		

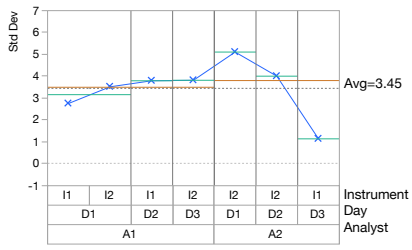


Sample 2:

Variability Gauge

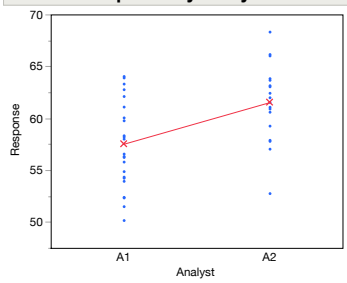
Variability Chart for Response



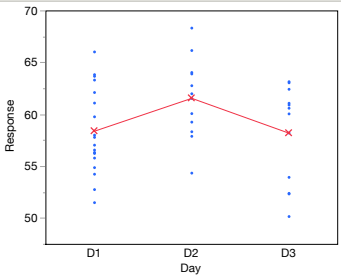


Gauge R&R Mean Plots

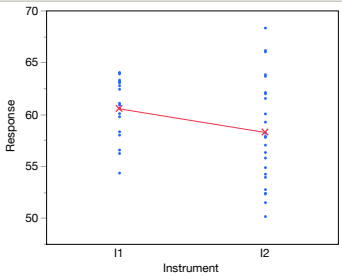
Mean of Response by Analyst



Mean of Response by Day

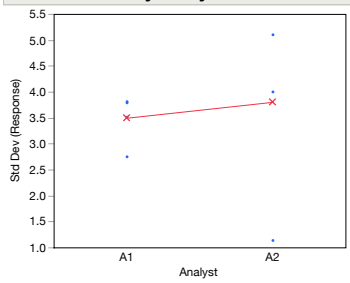


Mean of Response by Instrument

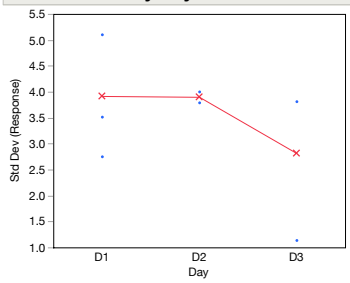


Gauge R&R Std Dev Plots

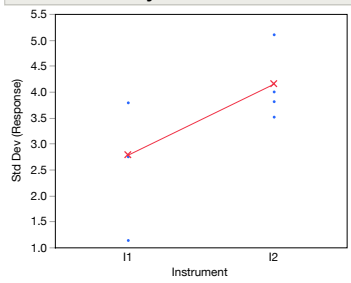
Mean Std Dev by Analyst



Mean Std Dev by Day



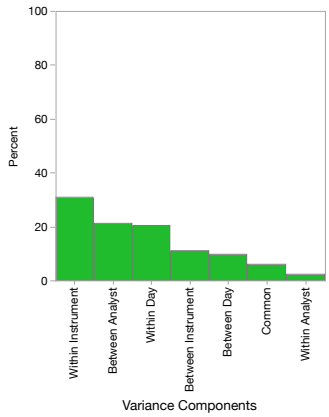
Mean Std Dev by Instrument



Partition of Variation (POV) Main Effect analysis

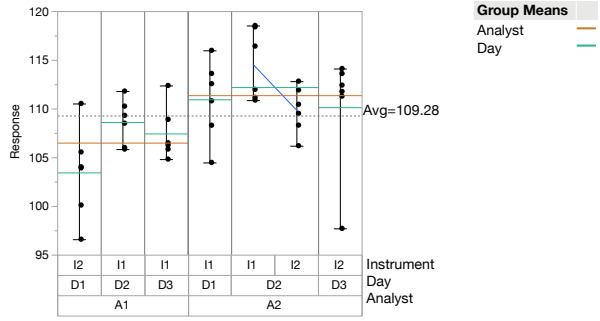
3 Factor POV Analysis: Analyst, Day, Instrument

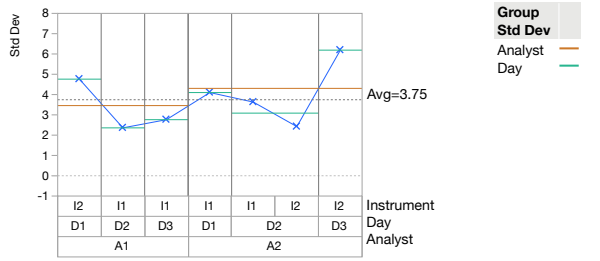
Concentration	Component	Pop Variance	% of Total	Sqrt(Var Comp)	F Ratio	Prob>F
50	Total Between	7.756	41.28	2.785	13.2186	0.0008*
50	Between Analyst	3.941	20.98	1.985	13.2186	0.0008*
50	Between Day	1.783	9.49	1.335	2.9896	0.0626
50	Between Instrument	2.033	10.82	1.426	6.8174	0.0130*
50		.	.	.		
50	Total Within	11.032	58.72	3.321		
50	Within Analyst	0.390	2.08	0.625		
50	Within Day	3.793	20.19	1.947		
50	Within Instrument	5.762	30.67	2.400		
50	Common	1.086	5.78	1.042		
50		.	.	.		
50	Grand Total	18.788	100.00	4.335		



Variability Gauge

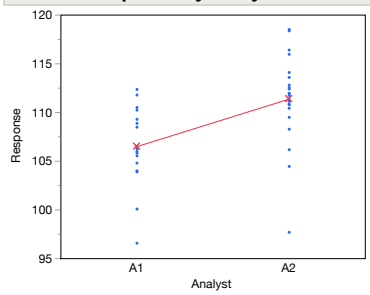
Variability Chart for Response



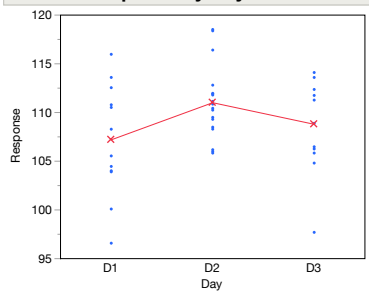


Gauge R&R Mean Plots

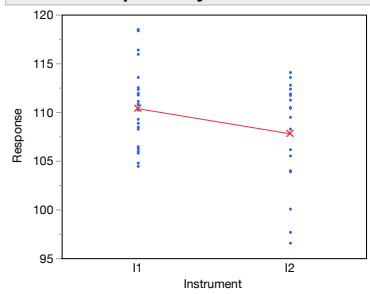
Mean of Response by Analyst



Mean of Response by Day

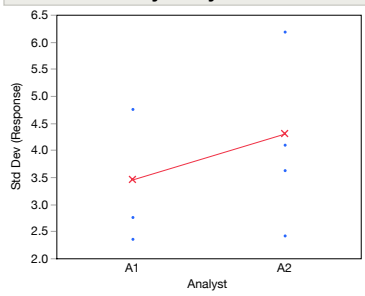


Mean of Response by Instrument

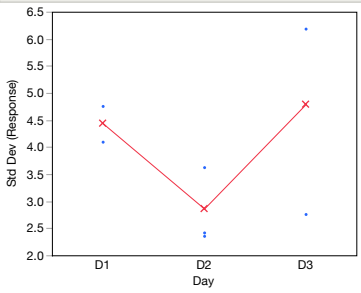


Gauge R&R Std Dev Plots

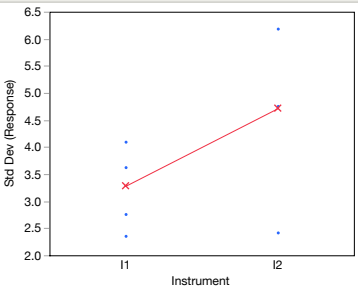
Mean Std Dev by Analyst



Mean Std Dev by Day



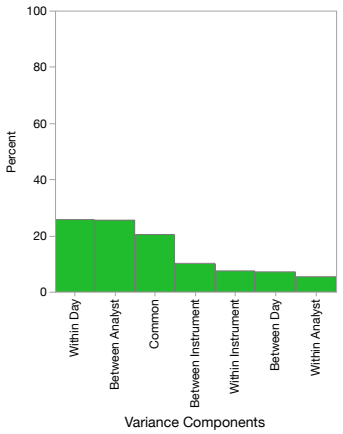
Mean Std Dev by Instrument



Partition of Variation (POV) Main Effect analysis

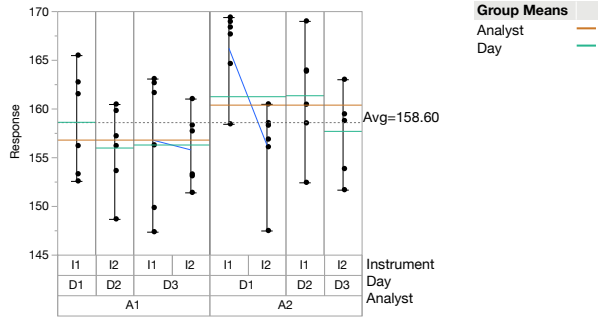
3 Factor POV Analysis: Analyst, Day, Instrument

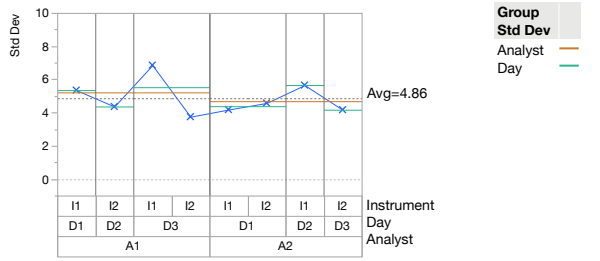
Concentration	Component	Pop Variance	% of Total	Sqrt(Var Comp)	F Ratio	Prob>F
100	Total Between	9.675	42.03	3.110	16.1233	0.0003*
100	Between Analyst	5.815	25.26	2.411	16.1233	0.0003*
100	Between Day	1.583	6.88	1.258	2.1950	0.1256
100	Between Instrument	2.277	9.89	1.509	6.3125	0.0165*
100		.	.	.		
100	Total Within	13.344	57.97	3.653		
100	Within Analyst	1.183	5.14	1.088		
100	Within Day	5.862	25.47	2.421		
100	Within Instrument	1.660	7.21	1.288		
100	Common	4.639	20.15	2.154		
100		.	.	.		
100	Grand Total	23.018	100.00	4.798		



Variability Gauge

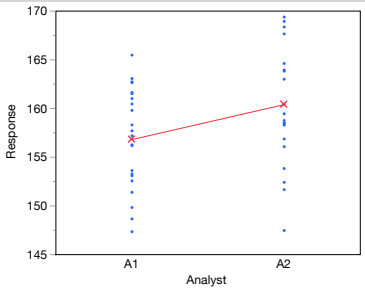
Variability Chart for Response



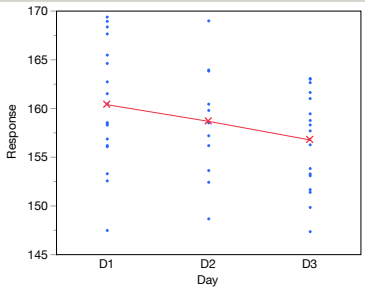


Gauge R&R Mean Plots

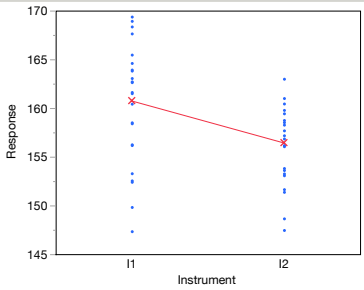
Mean of Response by Analyst



Mean of Response by Day

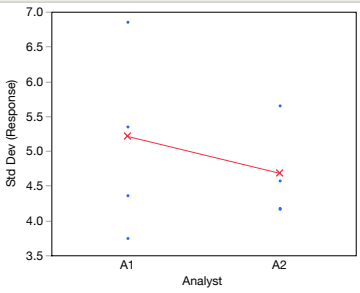


Mean of Response by Instrument

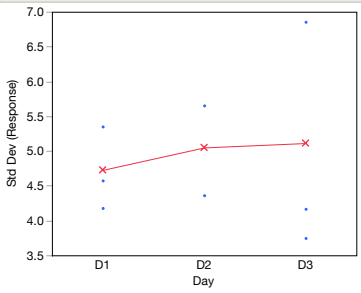


Gauge R&R Std Dev Plots

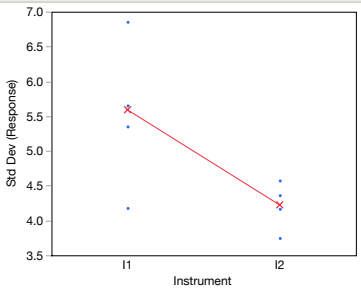
Mean Std Dev by Analyst



Mean Std Dev by Day



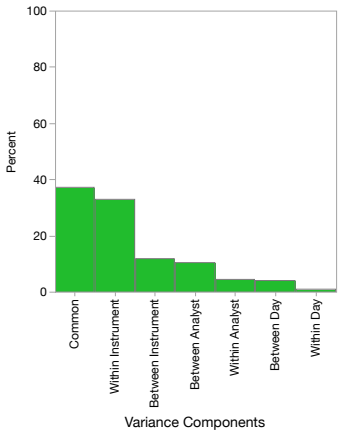
Mean Std Dev by Instrument



Partition of Variation (POV) Main Effect analysis

3 Factor POV Analysis: Analyst, Day, Instrument

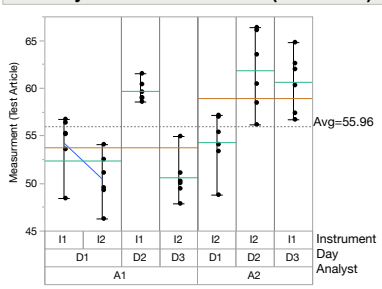
Concentration	Component	Pop Variance	% of Total	Sqrt(Var Comp)	F Ratio	Prob>F
150	Total Between	8.088	25.51	2.844	5.8636	0.0197*
150	Between Analyst	3.220	10.16	1.795	5.8636	0.0197*
150	Between Day	1.199	3.78	1.095	1.0917	0.3448
150	Between Instrument	3.668	11.57	1.915	6.6796	0.0132*
150		.	.	.		
150	Total Within	23.615	74.49	4.860		
150	Within Analyst	1.325	4.18	1.151		
150	Within Day	0.222	0.70	0.471		
150	Within Instrument	10.356	32.67	3.218		
150	Common	11.711	36.94	3.422		
150		.	.	.		
150	Grand Total	31.703	100.00	5.631		

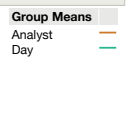


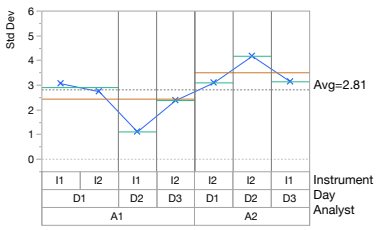
Sample 3:

Variability Gauge

Variability Chart for Measurment (Test Article)

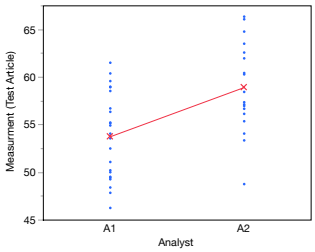




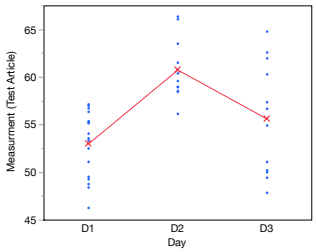


Gauge R&R Mean Plots

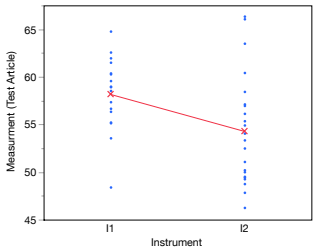
Mean of Measurment (Test Article) by Analyst



Mean of Measurment (Test Article) by Day

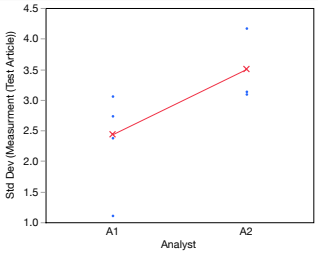


Mean of Measurment (Test Article) by Instrument

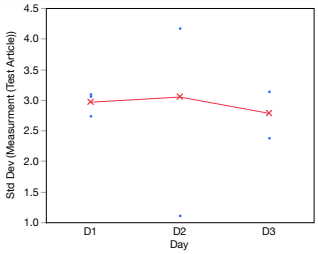


Gauge R&R Std Dev Plots

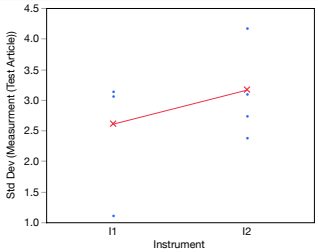
Mean Std Dev by Analyst



Mean Std Dev by Day



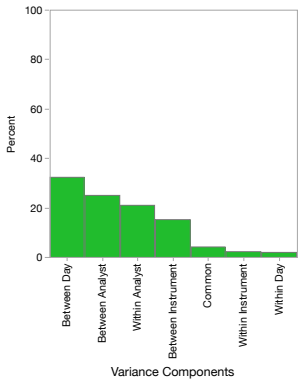
Mean Std Dev by Instrument



Partition of Variation (POV) Main Effect analysis

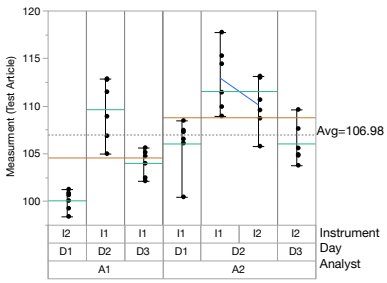
3 Factor POV Analysis: Analyst, Day, Instrument

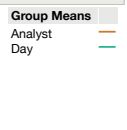
Concentration	Component	Pop Variance	% of Total	Sqrt(Var Comp)	F Ratio	Prob>F
50	Total Between	18.976	71.72	4.356	32.3625	<.0001*
50	Between Analyst	6.544	24.73	2.558	32.3625	<.0001*
50	Between Day	8.480	32.05	2.912	20.9703	<.0001*
50	Between Instrument	3.952	14.94	1.988	19.5473	<.0001*
50	.	.	.	.		
50	Total Within	7.481	28.28	2.735		
50	Within Analyst	5.483	20.72	2.342		
50	Within Day	0.444	1.68	0.666		
50	Within Instrument	0.526	1.99	0.725		
50	Common	1.029	3.89	1.014		
50	.	.	.	.		
50	Grand Total	26.458	100.00	5.144		

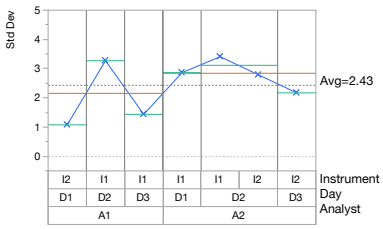


Variability Gauge

Variability Chart for Measurment (Test Article)

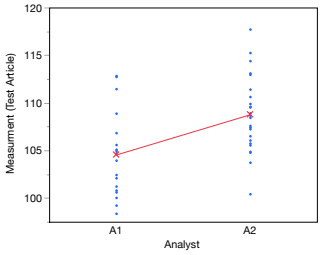




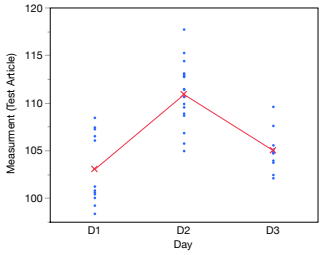


Gauge R&R Mean Plots

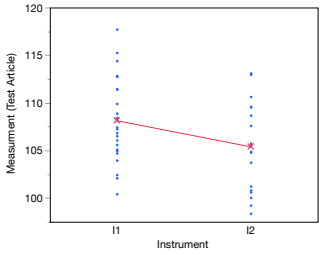
Mean of Measurment (Test Article) by Analyst



Mean of Measurment (Test Article) by Day

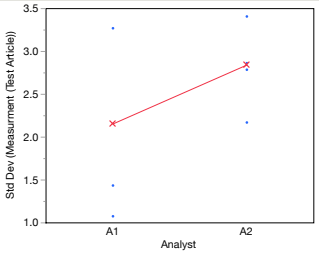


Mean of Measurment (Test Article) by Instrument

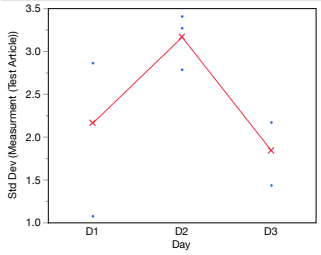


Gauge R&R Std Dev Plots

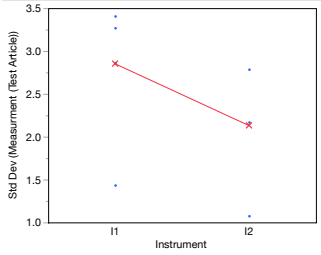
Mean Std Dev by Analyst



Mean Std Dev by Day



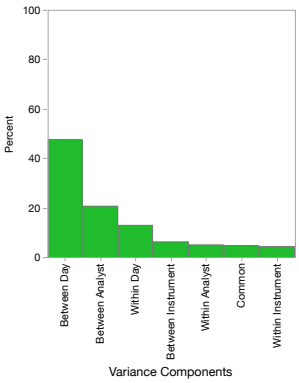
Mean Std Dev by Instrument



Partition of Variation (POV) Main Effect analysis

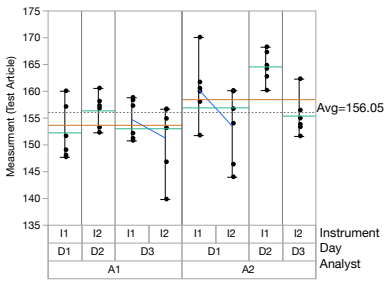
3 Factor POV Analysis: Analyst, Day, Instrument

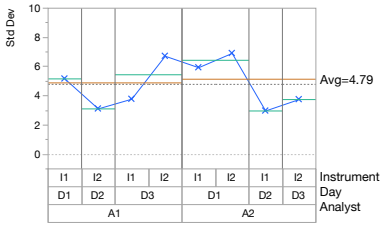
Concentration	Component	Pop Variance	% of Total	Sqrt(Var Comp)	F Ratio	Prob>F
100	Total Between	15.790	73.86	3.974	28.9165	<.0001*
100	Between Analyst	4.367	20.43	2.090	28.9165	<.0001*
100	Between Day	10.135	47.41	3.184	33.5548	<.0001*
100	Between Instrument	1.287	6.02	1.135	8.5233	0.0059*
100		.	.	.		
100	Total Within	5.588	26.14	2.364		
100	Within Analyst	1.025	4.79	1.012		
100	Within Day	2.712	12.69	1.647		
100	Within Instrument	0.885	4.14	0.941		
100	Common	0.966	4.52	0.983		
100		.	.	.		
100	Grand Total	21.378	100.00	4.624		



Variability Gauge

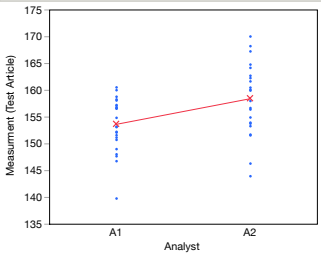
Variability Chart for Measurment (Test Article)



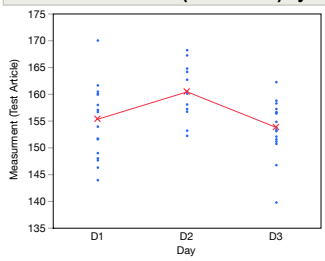


Gauge R&R Mean Plots

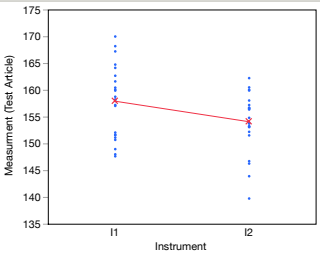
Mean of Measurment (Test Article) by Analyst



Mean of Measurment (Test Article) by Day

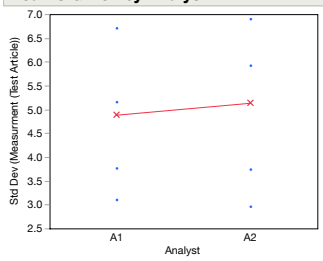


Mean of Measurment (Test Article) by Instrument

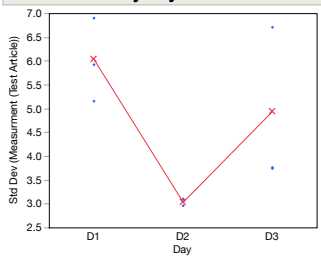


Gauge R&R Std Dev Plots

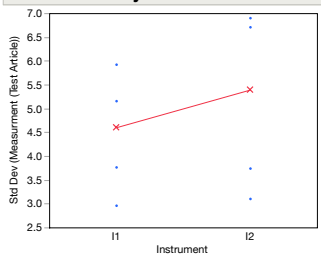
Mean Std Dev by Analyst



Mean Std Dev by Day



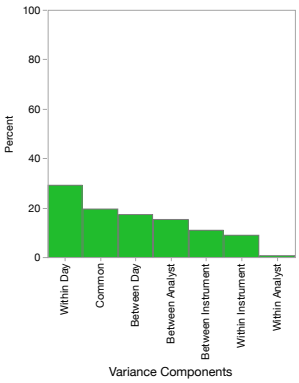
Mean Std Dev by Instrument



Partition of Variation (POV) Main Effect analysis

3 Factor POV Analysis: Analyst, Day, Instrument

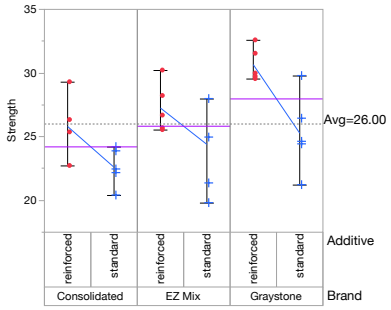
Concentration	Component	Pop Variance	% of Total	Sqrt(Var Comp)	F Ratio	Prob>F
150	Total Between	16.257	42.77	4.032	11.3067	0.0016*
150	Between Analyst	5.721	15.05	2.392	11.3067	0.0016*
150	Between Day	6.476	17.04	2.545	6.3995	0.0037*
150	Between Instrument	4.060	10.68	2.015	8.0241	0.0070*
150		.	.	.		
150	Total Within	21.757	57.23	4.664		
150	Within Analyst	0.157	0.41	0.397		
150	Within Day	10.980	28.88	3.314		
150	Within Instrument	3.296	8.67	1.815		
150	Common	7.325	19.27	2.706		
150		.	.	.		
150	Grand Total	38.015	100.00	6.166		

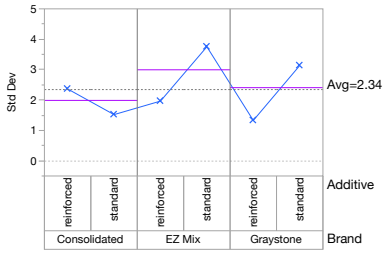


Cement:

Variability Gauge

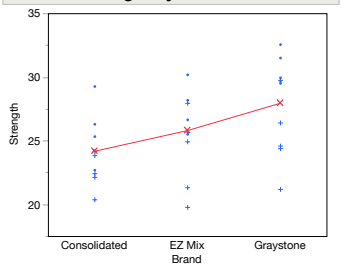
Variability Chart for Strength



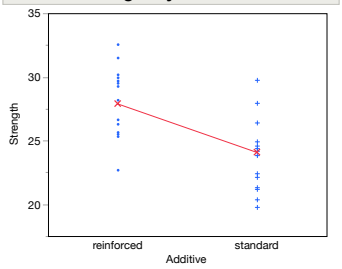


Gauge R&R Mean Plots

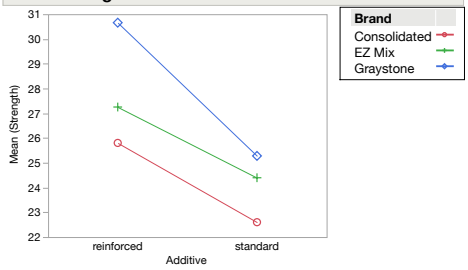
Mean of Strength by Brand



Mean of Strength by Additive

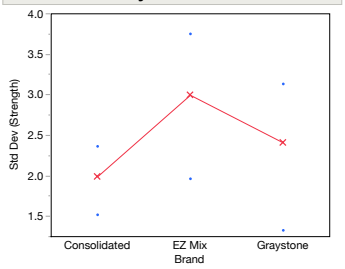


Mean Strength - Brand*Additive Interaction

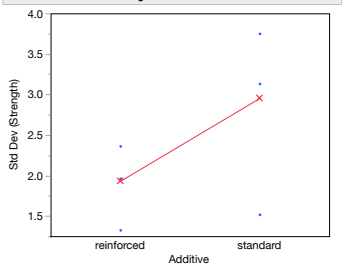


Gauge R&R Std Dev Plots

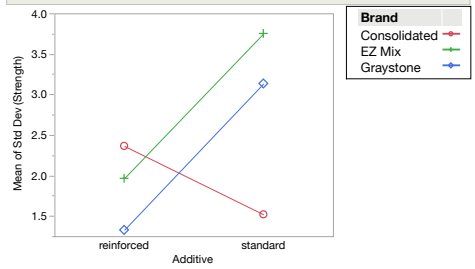
Mean Std Dev by Brand



Mean Std Dev by Additive



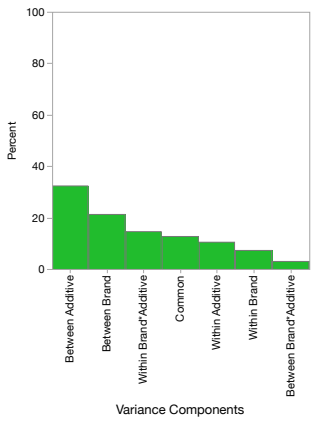
Mean Std Dev - Brand*Additive Interaction



Partition of Variation (POV) Crossed analysis

2 Factor POV Analysis: Brand, Additive

Component	Pop Variance	% of Total	Sqrt(Var Comp)	F Ratio	Prob>F
Total Between	6.327	55.91	2.515	5.7246	0.0093*
Between Brand	2.380	21.03	1.543	5.7246	0.0093*
Between Additive	3.634	32.12	1.906	17.4821	0.0003*
Between Brand*Additive	0.312	2.76	0.559	0.7507	0.4828
	.	.	.		
Total Within	4.989	44.09	2.234		
Within Brand	0.796	7.03	0.892		
Within Additive	1.158	10.24	1.076		
Within Brand*Additive	1.625	14.36	1.275		
Common	1.410	12.46	1.188		
	.	.	.		
Grand Total	11.316	100.00	3.364		

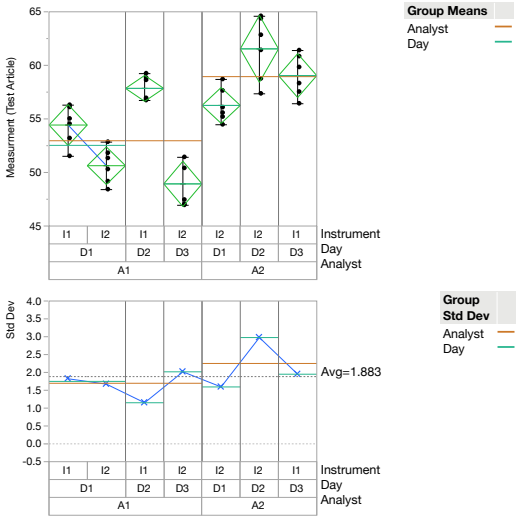


REML analysis reports

Sample 1:

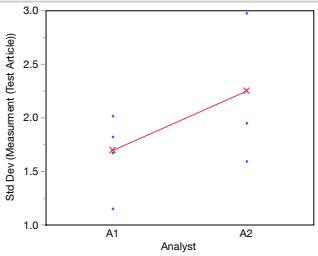
Variability Gauge Concentration=50

Variability Chart for Measurment (Test Article)

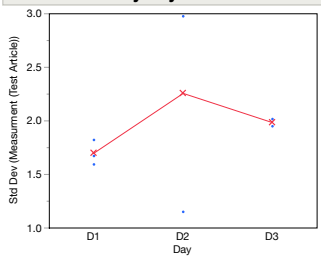


Gauge R&R Std Dev Plots

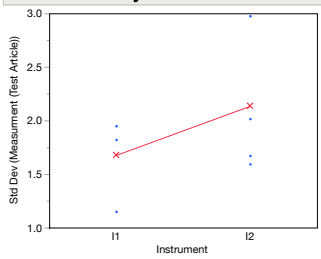
Mean Std Dev by Analyst



Mean Std Dev by Day

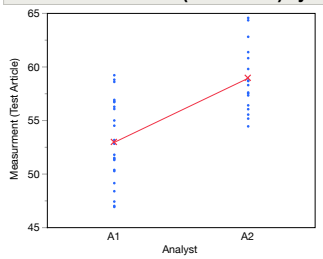


Mean Std Dev by Instrument

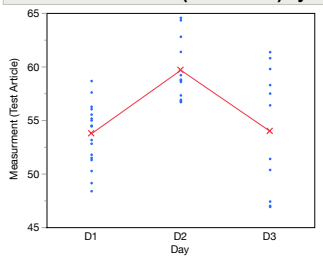


Gauge R&R Mean Plots

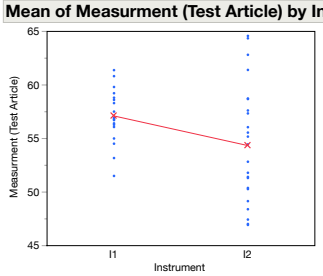
Mean of Measurment (Test Article) by Analyst



Mean of Measurment (Test Article) by Day



Mean of Measurment (Test Article) by Instrument



Data was unbalanced, so a REML Fit was performed.

REML Variance Component Estimates

Random Effect	Var Component	Std Error	95% Lower	95% Upper	Pct of Total
Analyst	19.869209	28.376358	3.9197439	23087.925	51.793
Day	8.5063587	8.8158394	2.2358514	424.76474	22.174
Instrument	6.2274329	9.0838005	1.2050757	9711.5416	16.233
Residual	3.7595923	0.8741924	2.4987222	6.2931776	9.800
Total	38.362593	31.111887	12.377838	518.63898	100.000

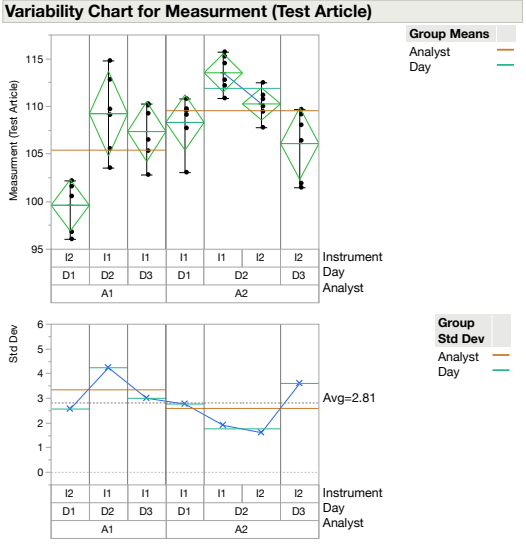
-2 LogLikelihood = 189.46875876

Variance Components

Component	Var Component	% of Total	20 40 60 80	Sqrt(Var Comp)
Analyst	19.869209	51.8		4.4575
Day	8.506359	22.2		2.9166
Instrument	6.227433	16.2		2.4955
Within	3.759592	9.8		1.9390
Total	38.362593	100.0		6.1938

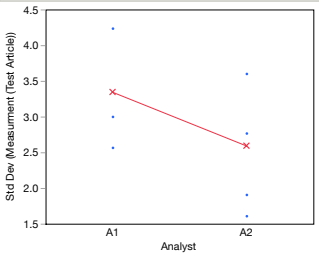
Variability Gauge Concentration=100

Variability Chart for Measurment (Test Article)

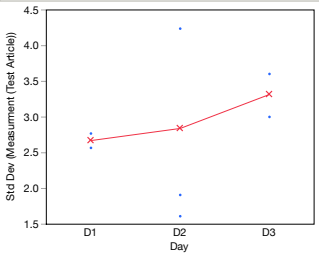


Gauge R&R Std Dev Plots

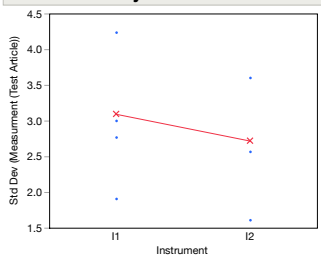
Mean Std Dev by Analyst



Mean Std Dev by Day

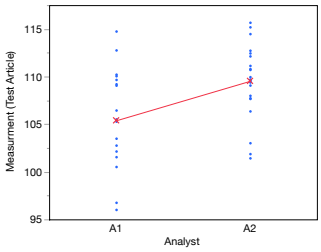


Mean Std Dev by Instrument

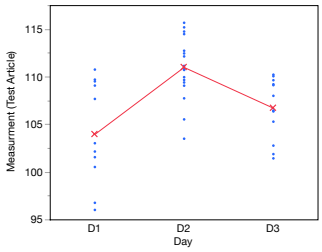


Gauge R&R Mean Plots

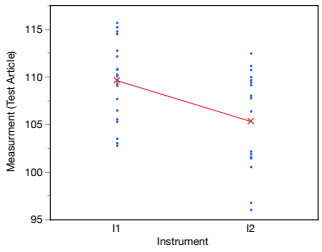
Mean of Measurment (Test Article) by Analyst



Mean of Measurment (Test Article) by Day



Mean of Measurment (Test Article) by Instrument



Data was unbalanced, so a REML Fit was performed.

REML Variance Component Estimates

Random Effect	Var Component	Std Error	95% Lower	95% Upper	Pct of Total
Analyst	8.243828	12.279678	1.5650746	17441.838	24.982
Day	7.6077027	8.2196986	1.928305	511.10971	23.054
Instrument	8.7476943	12.992313	1.6651513	17724.642	26.509
Residual	8.3999164	1.9505663	5.5855334	14.049683	25.455
Total	32.999141	19.69029	13.395727	172.2979	100.000

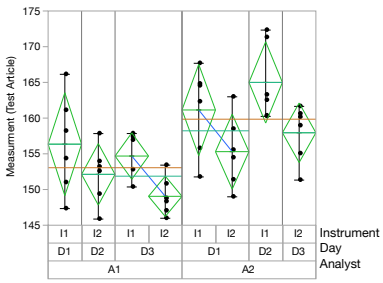
-2 LogLikelihood = 218.60522024

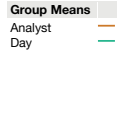
Variance Components

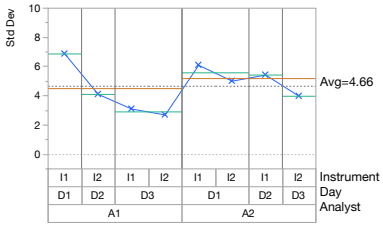
Component	Var Component	% of Total	20 40 60 80	Sqrt(Var Comp)
Analyst	8.243828	25.0		2.8712
Day	7.607703	23.1		2.7582
Instrument	8.747694	26.5		2.9577
Within	8.399916	25.5		2.8983
Total	32.999141	100.0		5.7445

Variability Gauge Concentration=150

Variability Chart for Measurment (Test Article)

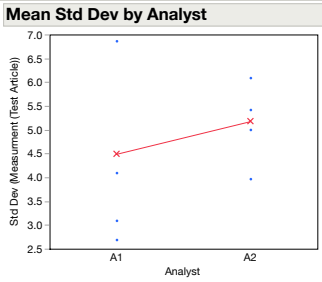




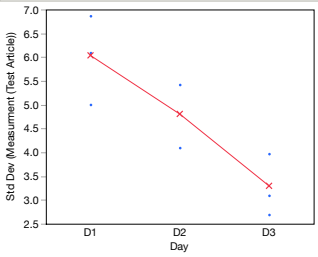


Gauge R&R Std Dev Plots

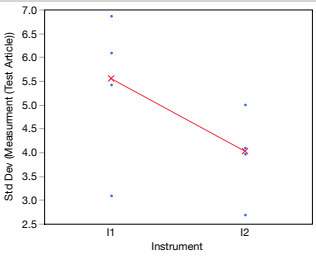
Mean Std Dev by Analyst



Mean Std Dev by Day

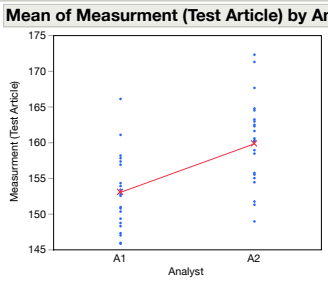


Mean Std Dev by Instrument

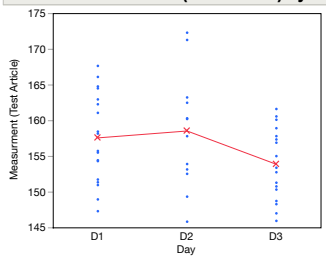


Gauge R&R Mean Plots

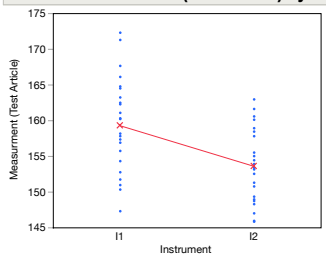
Mean of Measurment (Test Article) by Analyst



Mean of Measurment (Test Article) by Day



Mean of Measurment (Test Article) by Instrument



Data was unbalanced, so a REML Fit was performed.

REML Variance Component Estimates

Random Effect	Var Component	Std Error	95% Lower	95% Upper	Pct of Total
Analyst	22.368834	33.029872	4.2806564	41603.952	36.406
Day	0.8818032	2.6015466	0.0943463	1.451e+13	1.435
Instrument	15.420636	23.204192	2.9006128	38009.393	25.097
Residual	22.771975	4.9255864	15.529391	36.614163	37.062
Total	61.443248	40.752366	23.130921	419.69327	100.000

-2 LogLikelihood = 291.08314263

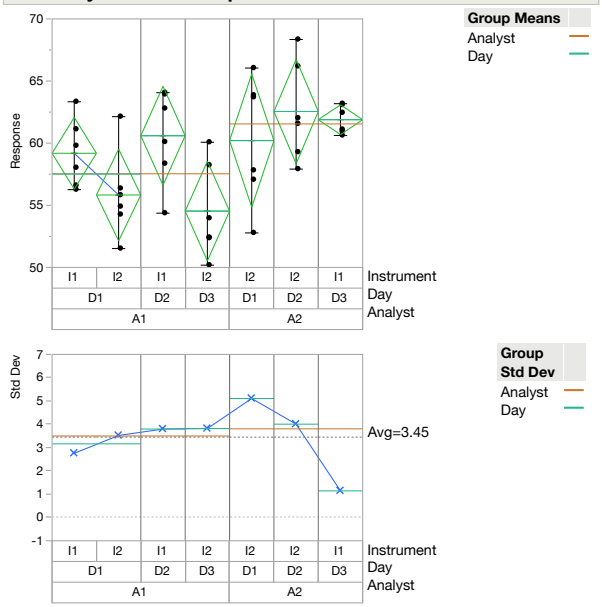
Variance Components

Component	Var Component	% of Total	20 40 60 80	Sqrt(Var Comp)
Analyst	22.368834	36.4		4.7296
Day	0.881803	1.4		0.9390
Instrument	15.420636	25.1		3.9269
Within	22.771975	37.1		4.7720
Total	61.443248	100.0		7.8386

Sample 2:

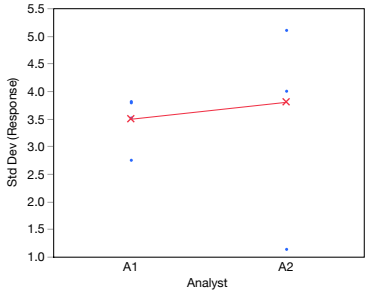
Variability Gauge Concentration=50

Variability Chart for Response

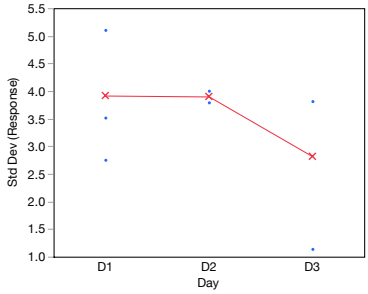


Gauge R&R Std Dev Plots

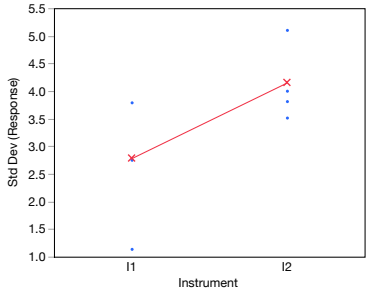
Mean Std Dev by Analyst



Mean Std Dev by Day

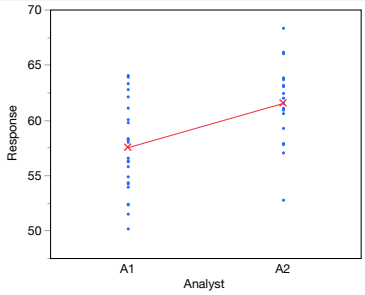


Mean Std Dev by Instrument

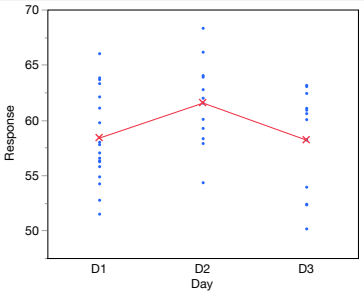


Gauge R&R Mean Plots

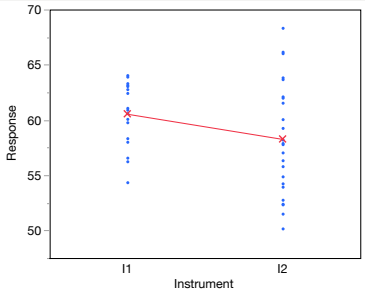
Mean of Response by Analyst



Mean of Response by Day



Mean of Response by Instrument



Data was unbalanced, so a REML Fit was performed.

REML Variance Component Estimates

Random Effect	Var Component	Std Error	95% Lower	95% Upper	Pct of Total
Analyst	9.037233	13.699817	1.6884601	24954.036	33.457
Day	1.7406274	2.7978723	0.3083058	12616.348	6.444
Instrument	3.6839029	6.1270156	0.6325899	49653.461	13.638
Residual	12.550061	2.924179	8.3348198	21.032695	46.461
Total	27.011824	15.603466	11.212459	131.1329	100.000

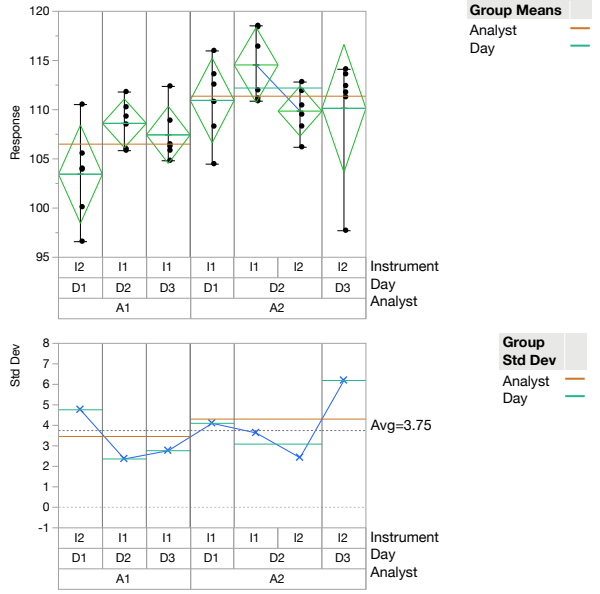
-2 LogLikelihood = 230.58242236

Variance Components

Component	Var Component	% of Total	20 40 60 80	Sqrt(Var Comp)
Analyst	9.037233	33.5		3.0062
Day	1.740627	6.4		1.3193
Instrument	3.683903	13.6		1.9193
Within	12.550061	46.5		3.5426
Total	27.011824	100.0		5.1973

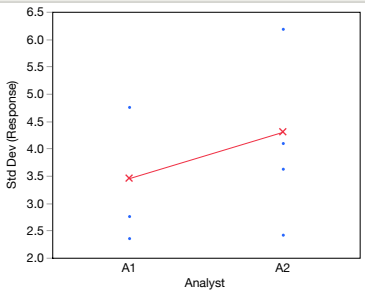
Variability Gauge Concentration=100

Variability Chart for Response

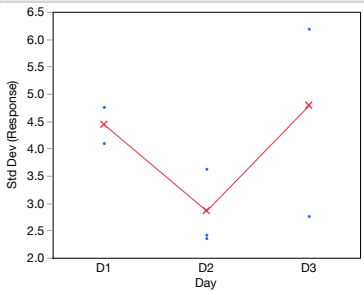


Gauge R&R Std Dev Plots

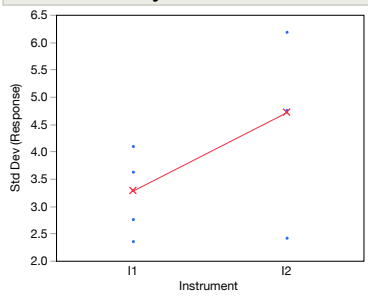
Mean Std Dev by Analyst



Mean Std Dev by Day

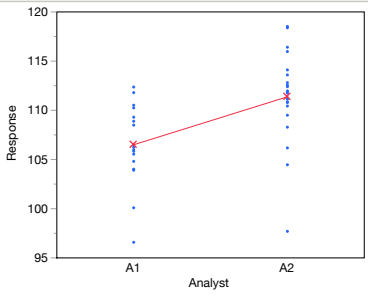


Mean Std Dev by Instrument

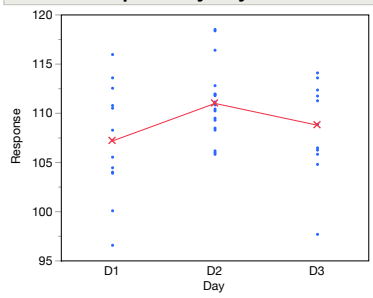


Gauge R&R Mean Plots

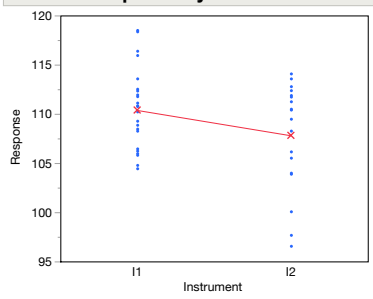
Mean of Response by Analyst



Mean of Response by Day



Mean of Response by Instrument



Data was unbalanced, so a REML Fit was performed.

REML Variance Component Estimates

Random Effect	Var Component	Std Error	95% Lower	95% Upper	Pct of Total
Analyst	13.014554	19.531551	2.4539843	30808.131	38.970
Day	0.6083354	1.7373418	0.0665683	1.408e+12	1.822
Instrument	4.7124962	7.7859224	0.8141038	56098.856	14.111
Residual	15.060837	3.482084	10.030695	25.127081	45.097
Total	33.396222	21.442227	12.872434	208.89784	100.000

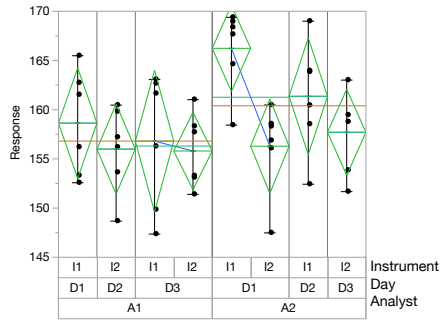
-2 LogLikelihood = 237.05923444

Variance Components

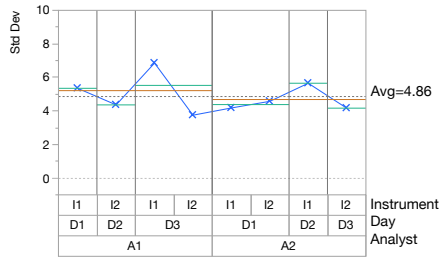
Component	Var Component	% of Total	20 40 60 80	Sqrt(Var Comp)
Analyst	13.014554	39.0		3.6076
Day	0.608335	1.8		0.7800
Instrument	4.712496	14.1		2.1708
Within	15.060837	45.1		3.8808
Total	33.396222	100.0		5.7789

Variability Gauge Concentration=150

Variability Chart for Response



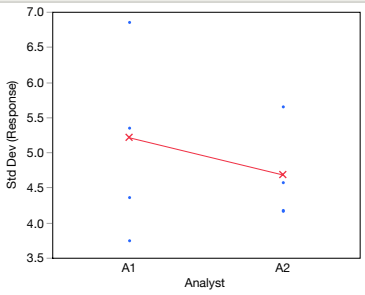
Group Means
Analyst
Day



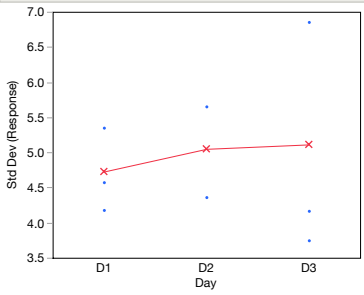
Group
Std Dev
Analyst
Day

Gauge R&R Std Dev Plots

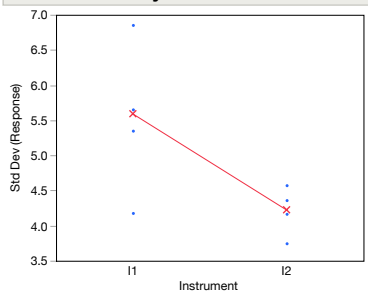
Mean Std Dev by Analyst



Mean Std Dev by Day

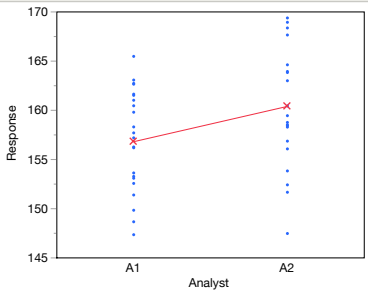


Mean Std Dev by Instrument

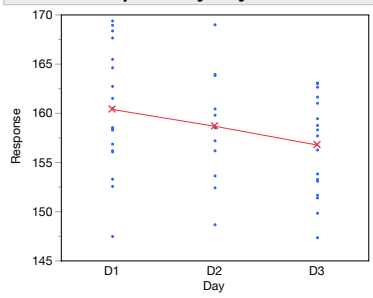


Gauge R&R Mean Plots

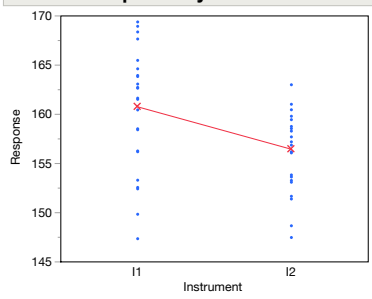
Mean of Response by Analyst



Mean of Response by Day



Mean of Response by Instrument



Data was unbalanced, so a REML Fit was performed.

REML Variance Component Estimates

Random Effect	Var Component	Std Error	95% Lower	95% Upper	Pct of Total
Analyst	5.3812407	9.1110351	0.9092406	102167.6	13.783
Day	0	0	0	0	0.000
Instrument	8.2381773	13.150517	1.468406	52928.028	21.100
Residual	25.423275	5.359697	17.490362	40.331427	65.117
Total	39.042693	16.732765	19.535872	113.32319	100.000

-2 LogLikelihood = 293.30481704

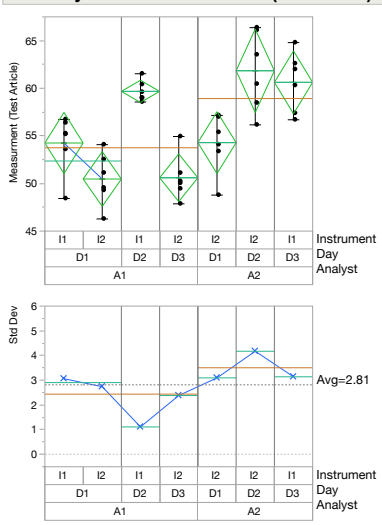
Variance Components

Component	Var Component	% of Total	20 40 60 80	Sqrt(Var Comp)
Analyst	5.381241	13.8		2.3198
Day	0.000000	0.0		0.0000
Instrument	8.238177	21.1		2.8702
Within	25.423275	65.1		5.0421
Total	39.042693	100.0		6.2484

Sample 3:

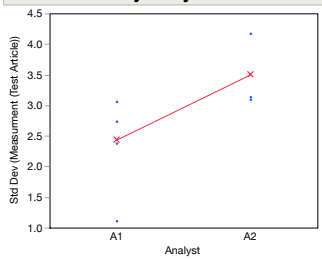
Variability Gauge Concentration=50

Variability Chart for Measurment (Test Article)

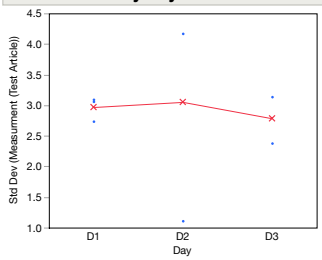


Gauge R&R Std Dev Plots

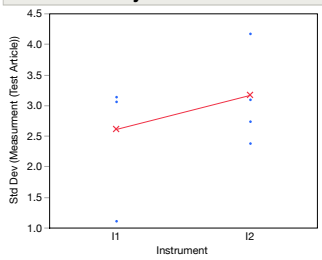
Mean Std Dev by Analyst



Mean Std Dev by Day

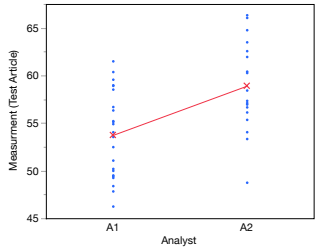


Mean Std Dev by Instrument

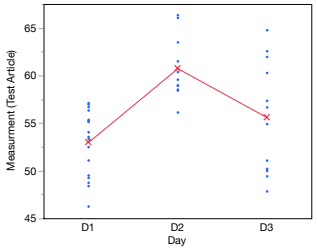


Gauge R&R Mean Plots

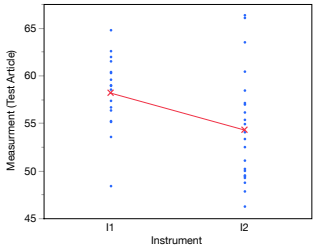
Mean of Measurment (Test Article) by Analyst



Mean of Measurment (Test Article) by Day



Mean of Measurment (Test Article) by Instrument



Data was unbalanced, so a REML Fit was performed.

REML Variance Component Estimates

Random Effect	Var Component	Std Error	95% Lower	95% Upper	Pct of Total
Analyst	13.375142	19.541917	2.5843775	21350.347	32.970
Day	10.477785	11.137904	2.6938794	624.87098	25.828
Instrument	8.2257766	12.259227	1.5608996	17540.374	20.277
Residual	8.4888611	1.972803	5.6430199	14.205079	20.925
Total	40.567564	25.658806	15.805852	244.06629	100.000

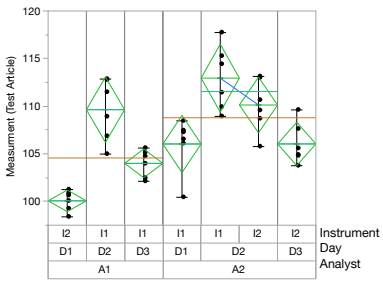
-2 LogLikelihood = 220.00021894

Variance Components

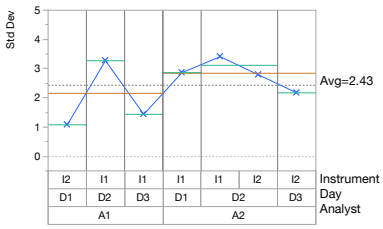
Component	Var Component	% of Total	20 40 60 80	Sqrt(Var Comp)
Analyst	13.375142	33.0		3.6572
Day	10.477785	25.8		3.2369
Instrument	8.225777	20.3		2.8681
Within	8.488861	20.9		2.9136
Total	40.567564	100.0		6.3693

Variability Gauge Concentration=100

Variability Chart for Measurment (Test Article)



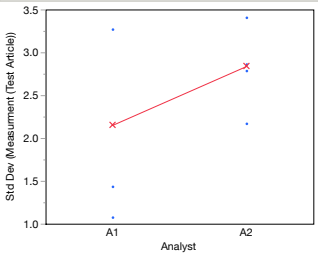
Group Means
Analyst
Day



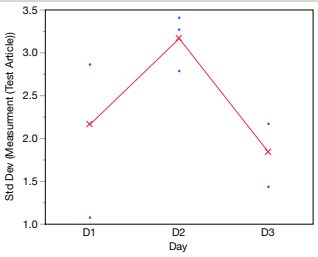
Group
Std Dev
Analyst
Day

Gauge R&R Std Dev Plots

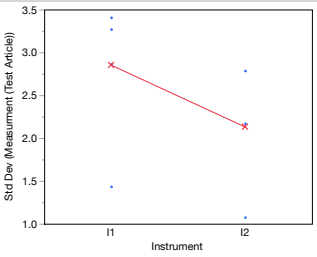
Mean Std Dev by Analyst



Mean Std Dev by Day

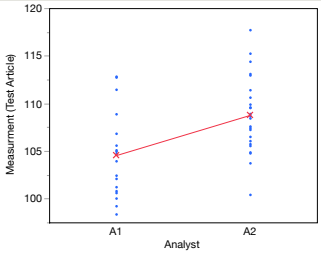


Mean Std Dev by Instrument

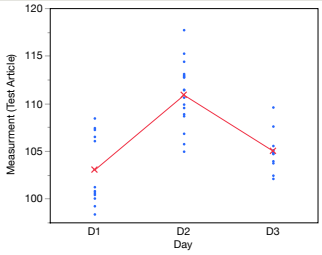


Gauge R&R Mean Plots

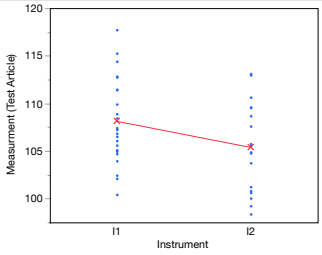
Mean of Measurment (Test Article) by Analyst



Mean of Measurment (Test Article) by Day



Mean of Measurment (Test Article) by Instrument



Data was unbalanced, so a REML Fit was performed.

REML Variance Component Estimates

Random Effect	Var Component	Std Error	95% Lower	95% Upper	Pct of Total
Analyst	6.2402349	9.295106	1.184708	13200.641	22.711
Day	12.403202	12.852718	3.2604994	618.78226	45.141
Instrument	2.4952174	3.9978169	0.4432664	17090.385	9.081
Residual	6.3378861	1.4723296	4.2137634	10.603217	23.067
Total	27.476541	16.283284	11.206801	141.22862	100.000

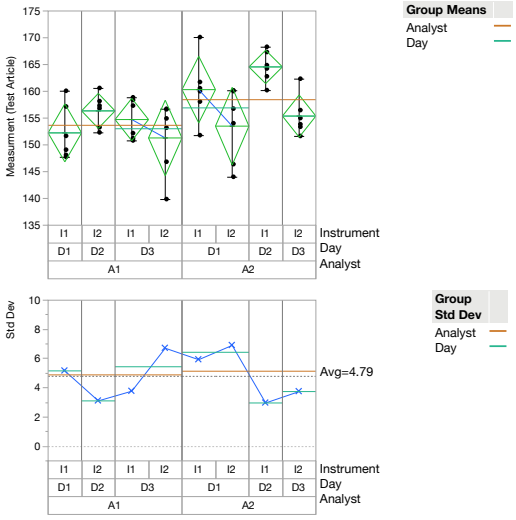
-2 LogLikelihood = 207.61777938

Variance Components

Component	Var Component	% of Total	20 40 60 80	Sqrt(Var Comp)
Analyst	6.240235	22.7		2.4980
Day	12.403202	45.1		3.5218
Instrument	2.495217	9.1		1.5796
Within	6.337886	23.1		2.5175
Total	27.476541	100.0		5.2418

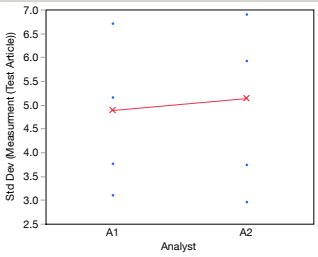
Variability Gauge Concentration=150

Variability Chart for Measurment (Test Article)

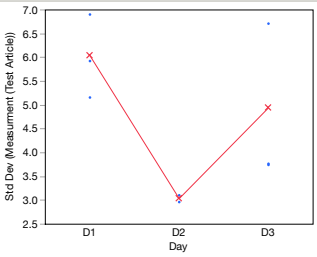


Gauge R&R Std Dev Plots

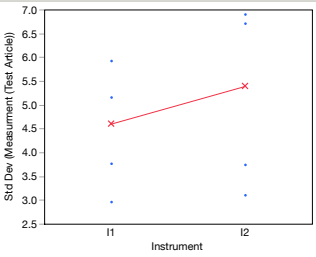
Mean Std Dev by Analyst



Mean Std Dev by Day

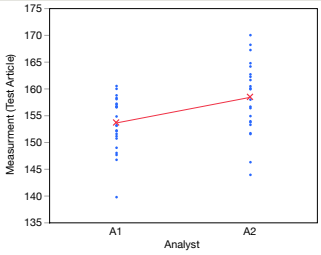


Mean Std Dev by Instrument

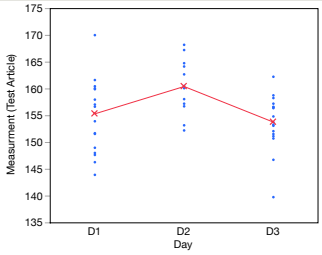


Gauge R&R Mean Plots

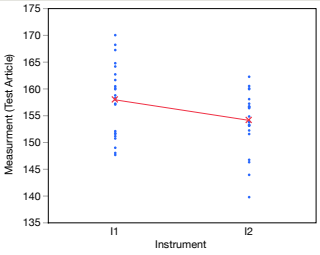
Mean of Measurment (Test Article) by Analyst



Mean of Measurment (Test Article) by Day



Mean of Measurment (Test Article) by Instrument



Data was unbalanced, so a REML Fit was performed.

REML Variance Component Estimates

Random Effect	Var Component	Std Error	95% Lower	95% Upper	Pct of Total
Analyst	11.813313	18.269572	2.1672826	44725.463	22.043
Day	9.9915791	11.79937	2.3407814	1392.9005	18.644
Instrument	7.4557431	12.107549	1.3083577	64781.518	13.912
Residual	24.331053	5.2567678	16.5992	39.097044	45.401
Total	53.591688	25.573803	25.167572	182.08625	100.000

-2 LogLikelihood = 295.78841987

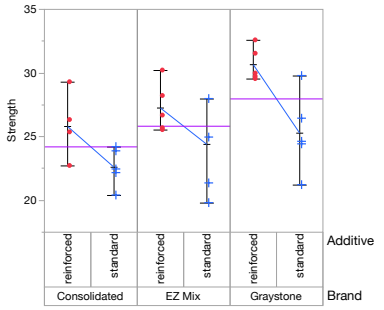
Variance Components

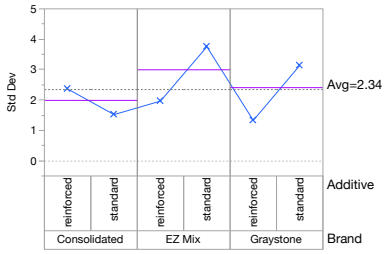
Component	Var Component	% of Total	20 40 60 80	Sqrt(Var Comp)
Analyst	11.813313	22.0		3.4371
Day	9.991579	18.6		3.1609
Instrument	7.455743	13.9		2.7305
Within	24.331053	45.4		4.9327
Total	53.591688	100.0		7.3206

Cement:

Variability Gauge

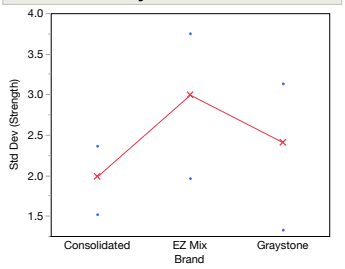
Variability Chart for Strength



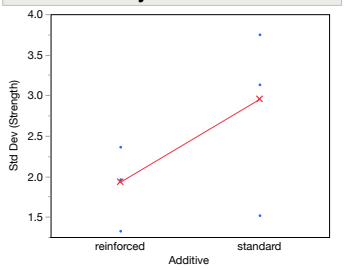


Gauge R&R Std Dev Plots

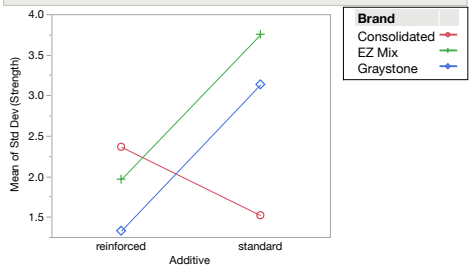
Mean Std Dev by Brand



Mean Std Dev by Additive

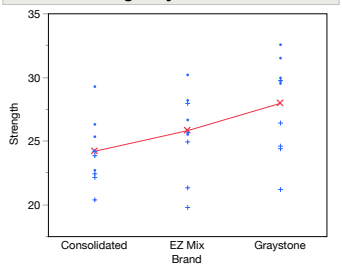


Mean Std Dev - Brand*Additive Interaction

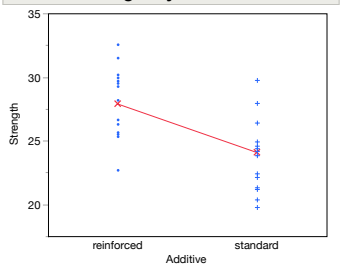


Gauge R&R Mean Plots

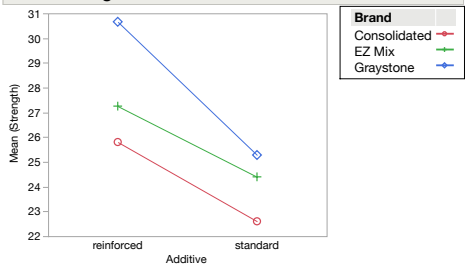
Mean of Strength by Brand



Mean of Strength by Additive



Mean Strength - Brand*Additive Interaction



REML Variance Component Estimates

Random Effect	Var Component	Std Error	95% Lower	95% Upper	Pct of Total
Brand	2.9584828	3.5742118	0.6791237	509.05634	18.564
Additive	6.8607711	10.279934	1.2955219	15855.731	43.051
Brand*Additive	0	0	0	0	0.000
Residual	6.1170024	1.6965512	3.7936554	11.488237	38.384
Total	15.936256	10.973116	5.8356522	121.29335	100.000

-2 LogLikelihood = 144.62952257

Variance Components

Component	Var Component	% of Total	20 40 60 80	Sqrt(Var Comp)
Brand	2.958483	18.6		1.7200
Additive	6.860771	43.1		2.6193
Brand*Additive	0.000000	0.0		0.0000
Within	6.117002	38.4		2.4733
Total	15.936256	100.0		3.9920