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Analytical Method Improvement

Yields Dramatic Decrease in Variation

for a Final Formulation Process

Midwest SAS Users Group – Indianapolis, Indiana
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Project Setting

- At an Elanco (Eli Lilly Subsidiary) Mfg Site in W. Indiana
- Large scale manufacturer of Animal Health Feed Additives
- Fermentation Based Industry
- Regulated Processes by Center for Veterinary Medicine (CVM)
- Products Beyond Patent Expiry – Cost Pressure Exists in this Industry
- Both Quality and Cost Drivers to Understand Process, Minimize Rework
- Presentation will focus one product.
- This product had quality investigations due to common cause variation

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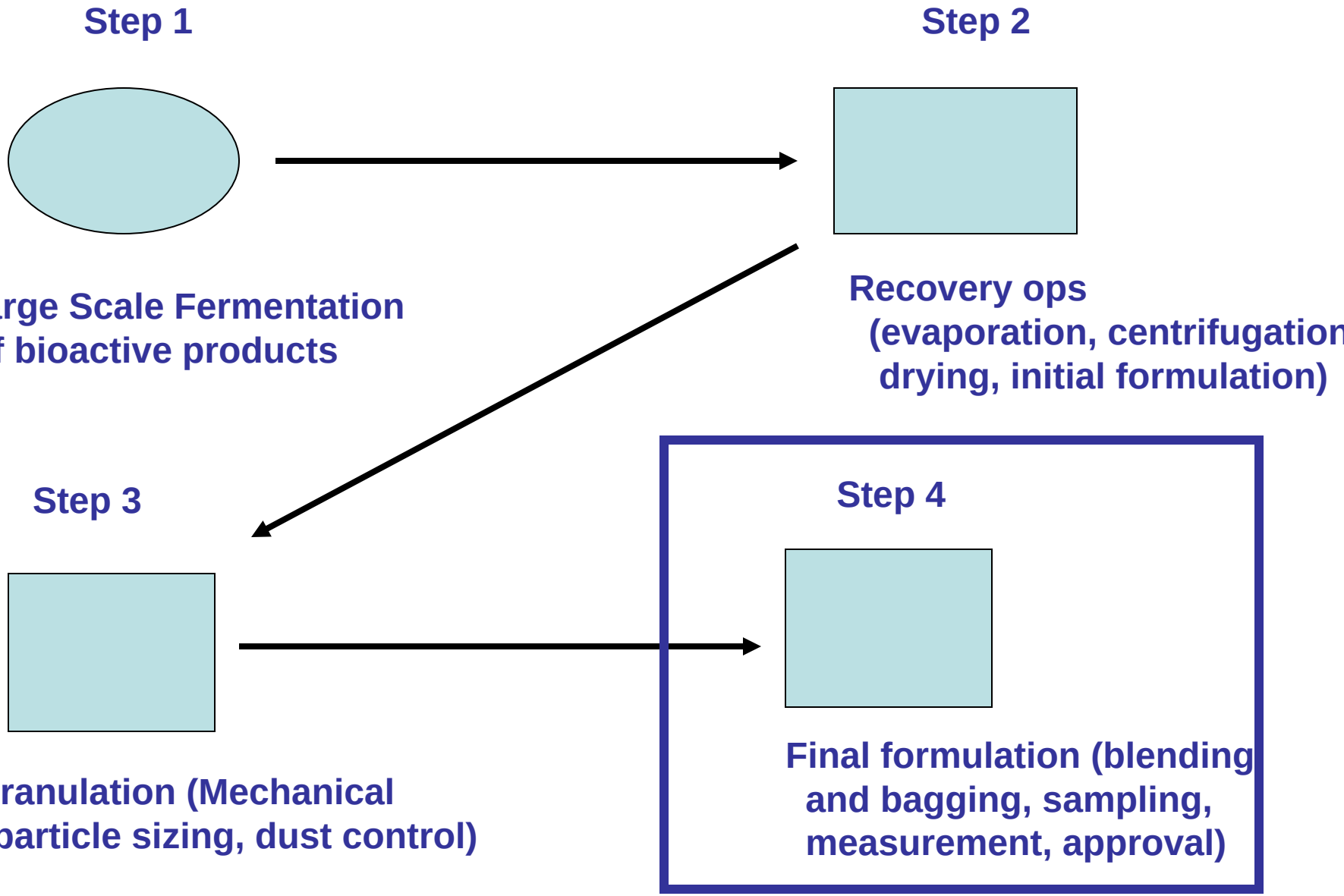
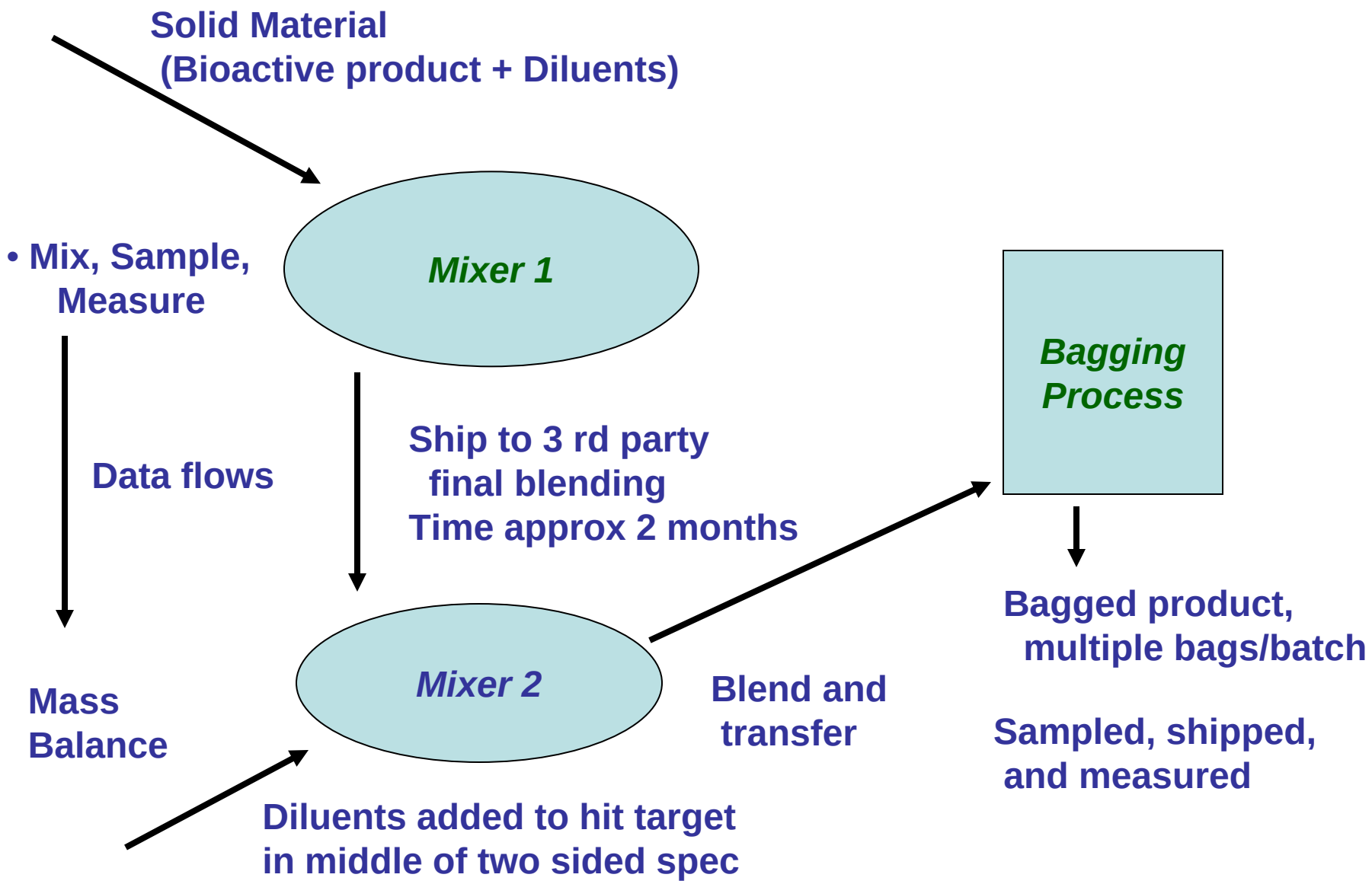
Process Overview



Double Click on Step 4

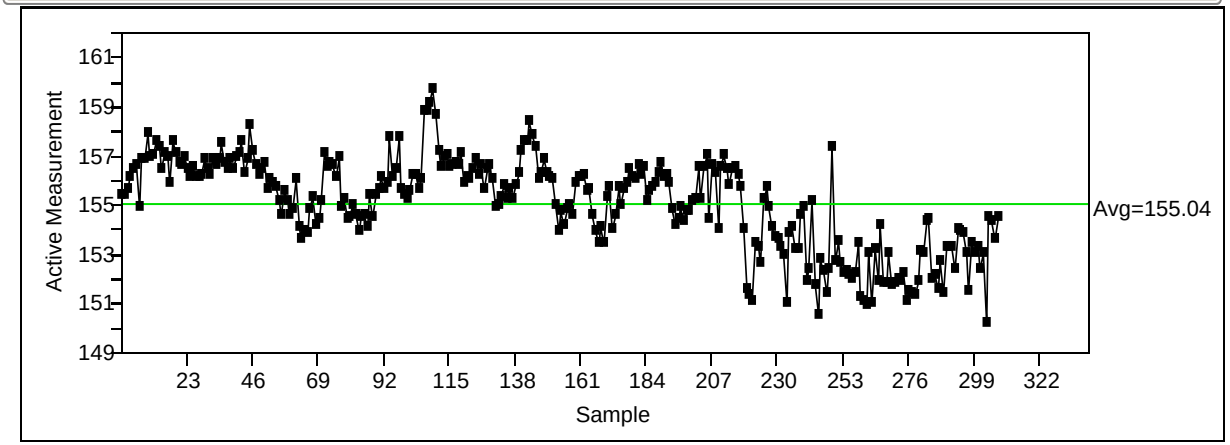


Defining the Need for Improvement

Process

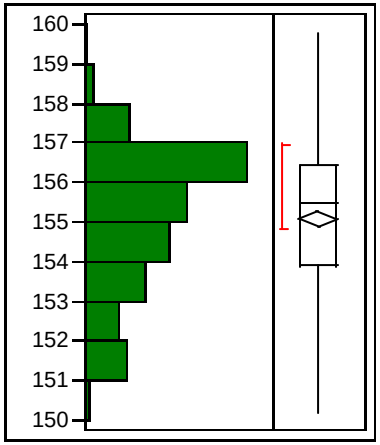
Control Chart

Individual Measurement of Active Measurement



Distributions

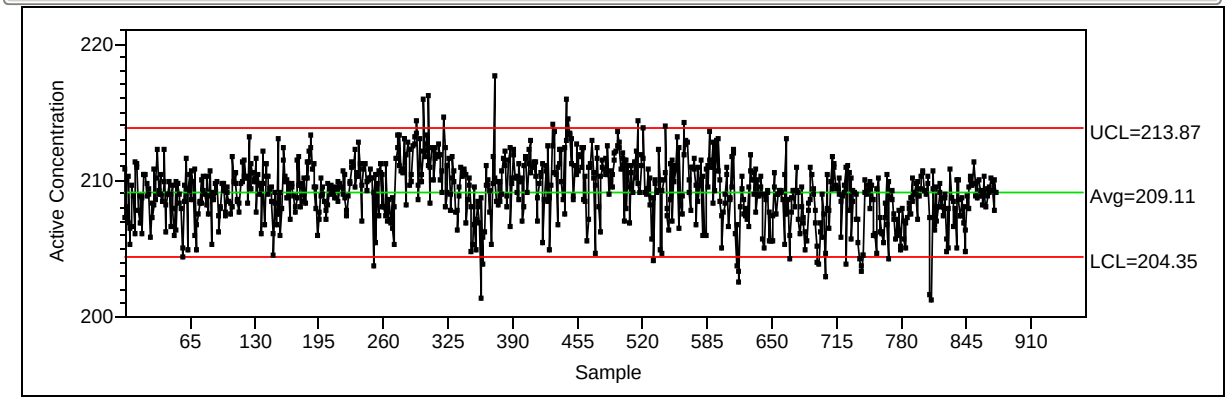
Active Measurement



Analytical Method

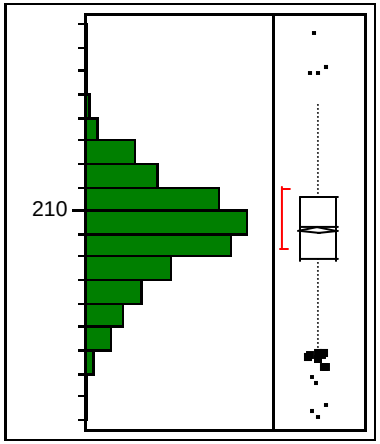
Control Chart

Individual Measurement of Active Concentration

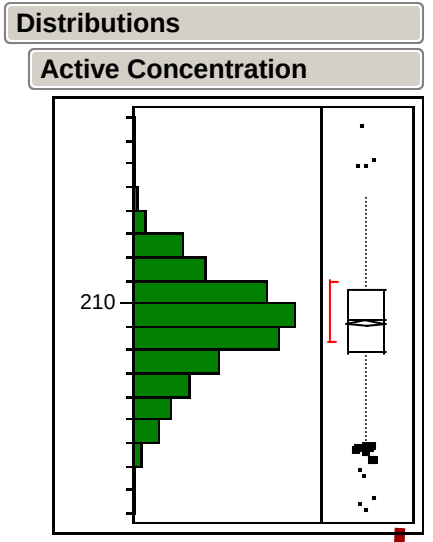


Distributions

Active Concentration



Effect of Analytical Variation



Solid Material
(Bioactive product + Diluents)

- **Mix, Sample, Measure**

Analytical

Data flows

Ship to 3 rd party
final blending
Time approx 2 months

70% Of Total

Mass Balance

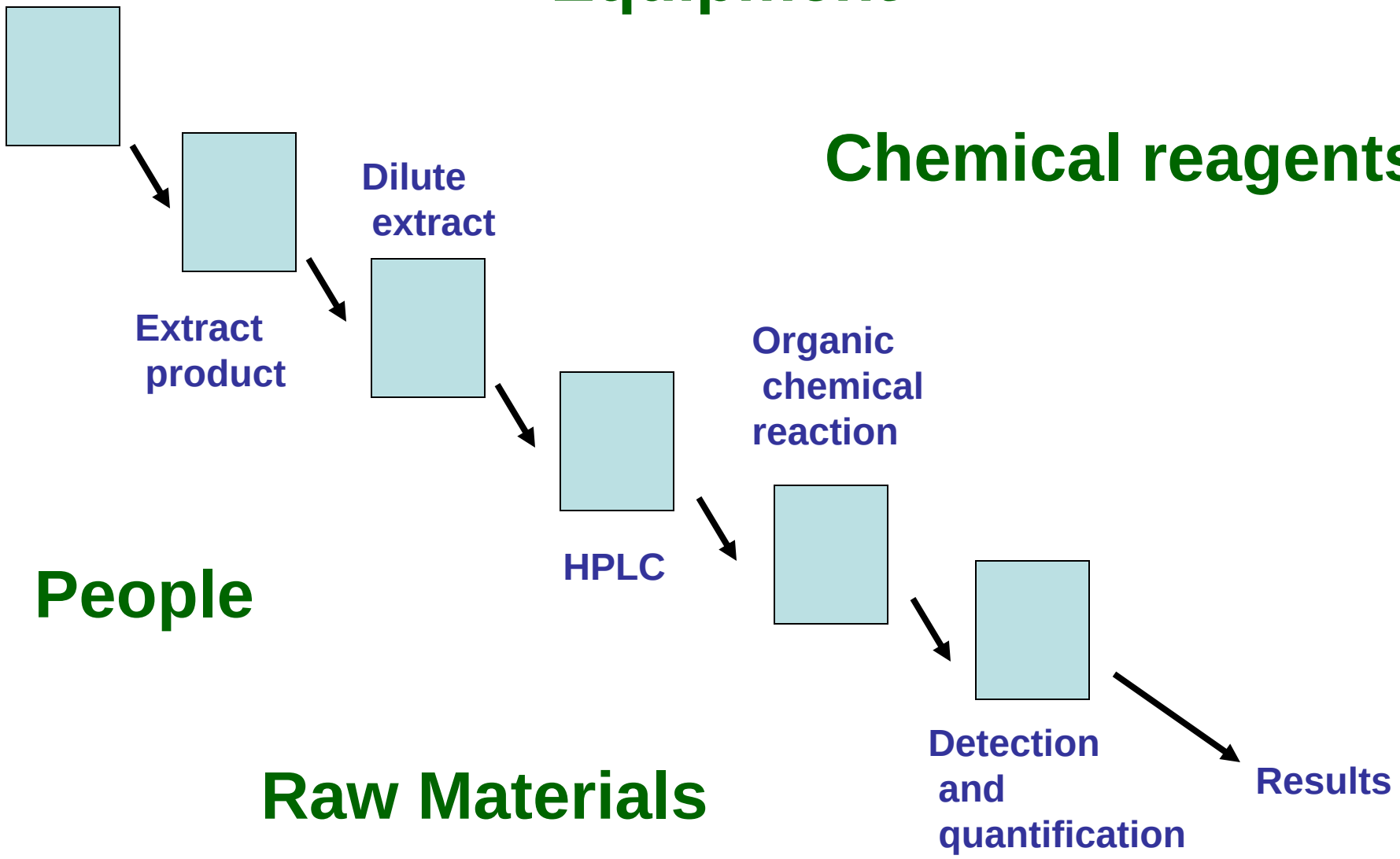
Blend and transfer

Bagged product, multiple bags/batch

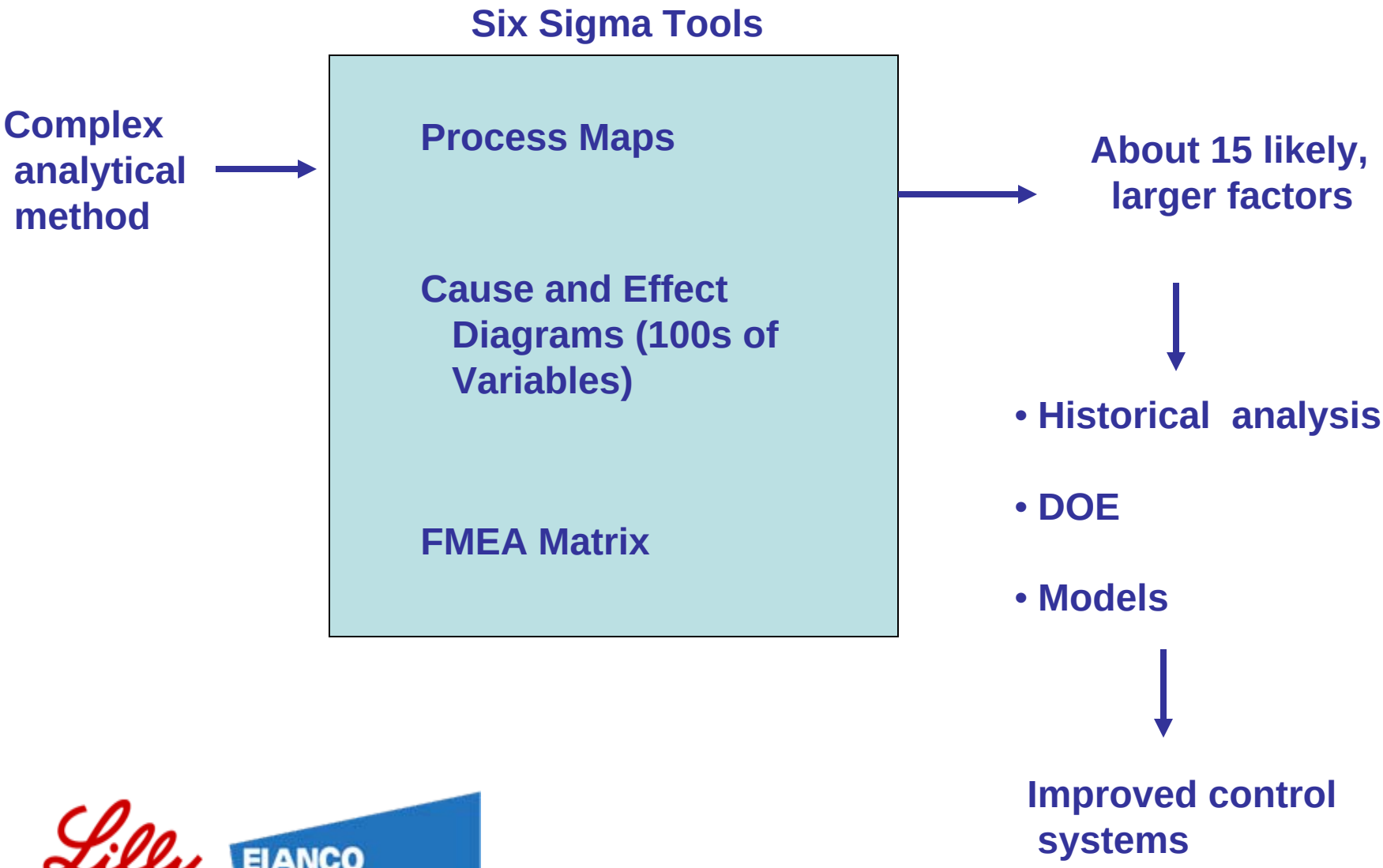
Sampled, shipped, and measured

Diluents added to hit target
in middle of two sided spec

Lab Flow – Steps to Prepare a Result



Six Sigma Process Summary Slides



Answers That Matter.

The Factors

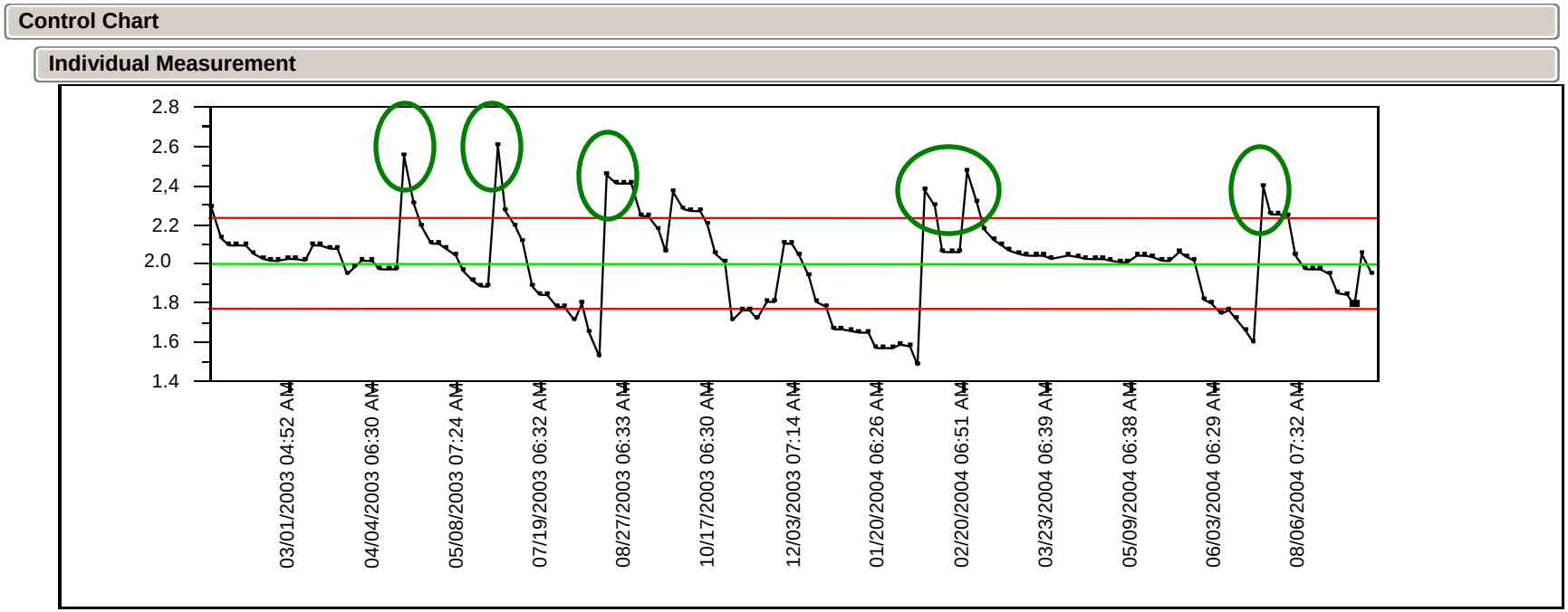
- Mobile Phase Composition
- Column Temperature
- Column Age
- Mobile Phase Flow
- Reaction Temperature
- Vanillin Flow
- Glacial Acetic Acid

Roger

- Autodilutor ← Bill S
- Vanillin Makeup
- Caps ← LeRoy F
- Vanillin Condition

- Tech to Tech Differences
  - Autodilutor
  - Repipette
  - Standard Solutions
  - Vanillin
  - Chromatography
- Evaporation (uncapped)
- Repipetter
- Column to Column Variation

Historical Data Analysis – Instrument Component



- Instrument parameter
- Green ovals indicate maintenance
- Instability indicates deterioration
- Instability caused by chemistry
- Adjustments made to minimize impact
- Backbone of analytical process unstable
- Operated within registered conditions

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Is the instability important?

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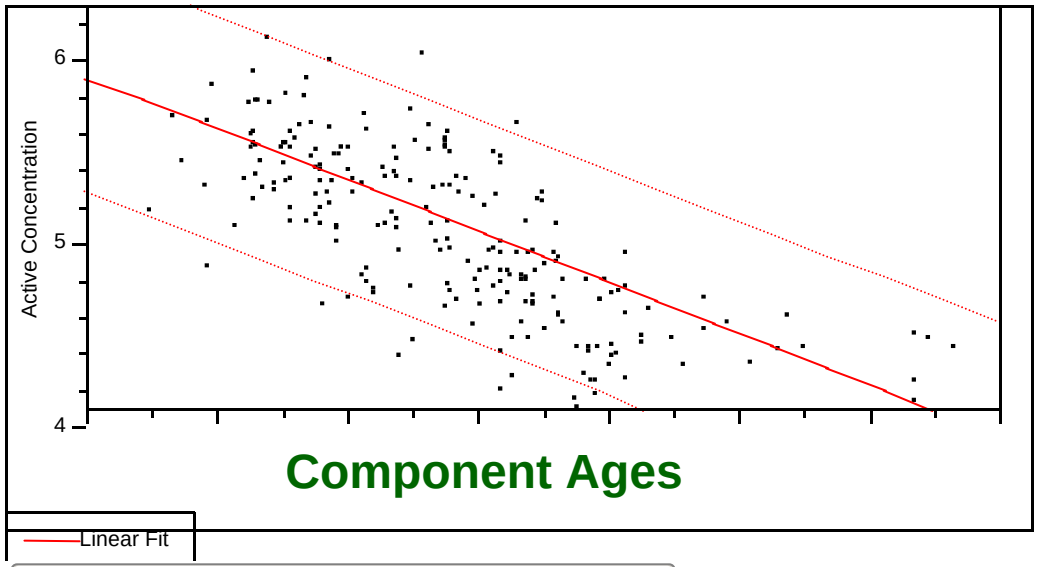
Active Concentration

Bivariate Fit of Control D&I By Ret. Time



Linear Fit

Control D&I = 10.551444 - 0.5579497 Ret. Time

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	10.551444	0.358918	29.40	<.0001
Ret. Time	-0.55795	0.036208	-15.41	<.0001

Controls:

Upgraded the technology of this equipment to be robust to withstand the chemistry. This change was properly registered with regulatory agencies. Essentially, the team made the backbone stable.

A very hard question – Solved by an Innovative DOE

- Some factors did not have developmental data available
- Based on experience, talented scientists did not agree about factors
- Method features complex chemistry (non-linear, quadratics likely)
- Seven Factors Were Selected for a Structured DOE
- JMP 5.1 Custom Design Platform was Utilized to Design the Experiment
- RSM Platform was utilized with 3 levels per factor (curvature expected)
- Chose more than minimum runs to give additional DOF
- No blocking, conditions simulated daily execution
- Center points included for error estimation
- One of the Seven Factors was expected to not be significant (Conscience)

JMP Custom Designer Dialog Box

Answers That Matter.

Model

Main Effects | **Interactions** | **RSM**

Cross | **Powers** | **Remove Term**

Name	Estimability
X4	Necessary
X5	Necessary
X6	Necessary
X7	Necessary
X1*X1	If Possible
X1*X2	If Possible
X2*X2	If Possible

Design Generation

Number of Runs:	8
(•) Minimum	8
( ) Default	64
( ) Compromise	128
( ) Grid	2187
( ) User Specified	.

Make Design

Why RSM?

Many chemical factors nonlinear

- Reaction Kinetics
- Extraction
- Disolution
- Flow Characteristics
- Color based detection
- Interactions among variables likely

→ **User Selected Number of Runs**

Complex chemistry, but time and execution limits.

Special Thanks: Dr. Mark Johnson and Dr. Chris Nachtsheim
Full day course in Raleigh Durham at ASA Q&PR

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Details of the Design – What We Were Thinking

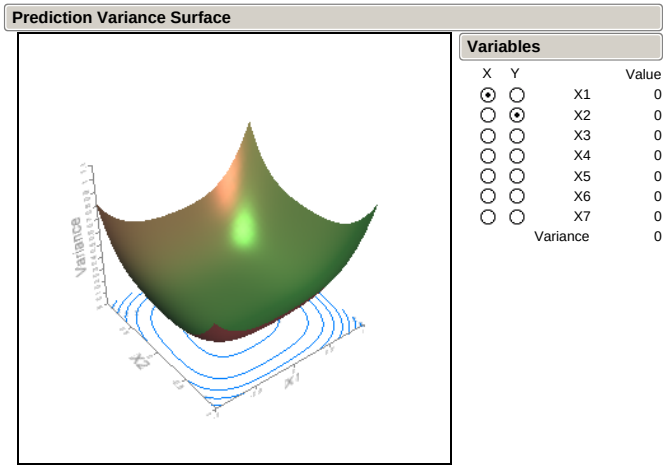
Design

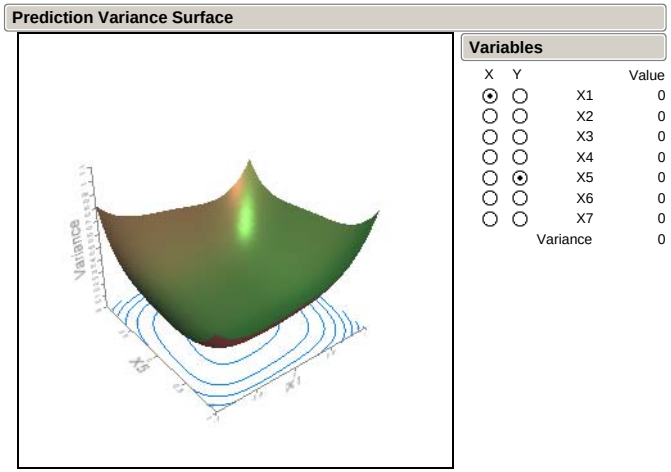
Run	X1	X2	X3	X4	X5	X6	X7	Y
1	0	0	-1	0	0	0	-1	.
2	1	-1	0	-1	-1	-1	-1	.
3	-1	0	1	1	0	-1	0	.
4	0	0	0	0	0	0	0	.
5	0	0	0	0	0	0	0	.
6	-1	-1	-1	-1	0	0	0	.
7	0	1	-1	1	1	1	-1	.
8	-1	0	0	0	1	-1	1	.
9	0	0	0	-1	0	1	1	.
10	0	1	1	0	-1	-1	0	.
11	0	0	0	-1	-1	0	0	.
12	1	0	1	-1	1	0	-1	.
13	0	-1	0	1	0	1	0	.
14	1	0	-1	0	0	1	0	.
15	0	0	0	0	0	0	0	.
16	0	1	-1	-1	0	-1	1	.
17	-1	1	0	0	0	0	-1	.
18	0	-1	-1	0	1	-1	0	.
19	1	1	0	1	1	0	1	.
20	0	-1	1	1	0	0	1	.
21	-1	-1	1	0	-1	1	-1	.
22	-1	0	-1	1	-1	0	1	.
23	0	0	0	1	0	-1	-1	.
24	0	0	0	0	0	0	0	.
25	0	0	0	0	0	0	0	.

- **Ranges selected to model reasonable level of variation**
- **Design was randomized**
- **5 Center Points – Model normal method execution**
- **No blocking – accurately simulate day to day ops**
- **One of the factors was in the controlled state (conscience)**

- **Design was executed over 6 days, 25 runs gave balance between statistical integrity, simulation of day to day operation and business drivers (cost-speed).**

Details of Design – The Balance





Min Design, 8 runs...moving past 14-15 produced nice stability in Prediction Variance!

- **Custom Designer offers a 'Simulate responses' dialog.**
- **Simulation was spiked with different number of runs to see where it would lose the ability to resolve variables**
- **N of 20 runs allowed for very good discernment of 6-7 equivalently sized responses**
- **Repeated center points across design yields a good look at uncontrolled factors**

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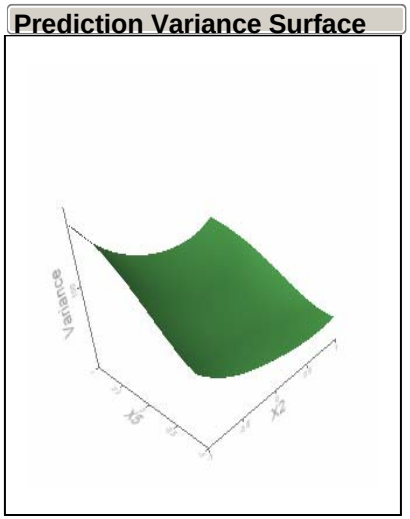
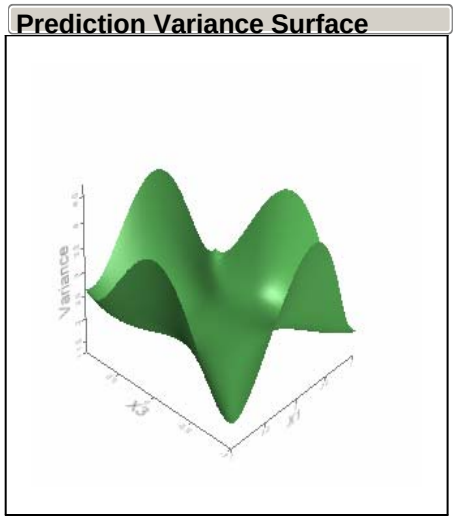
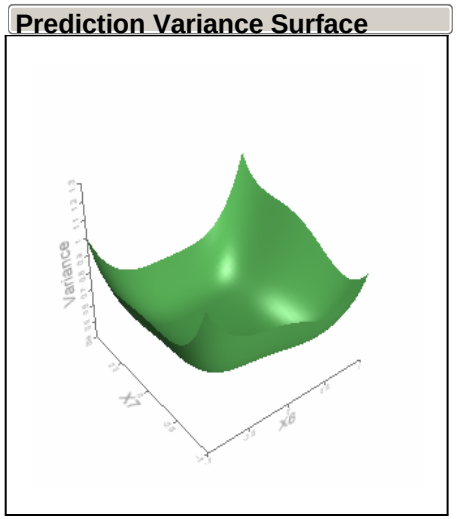
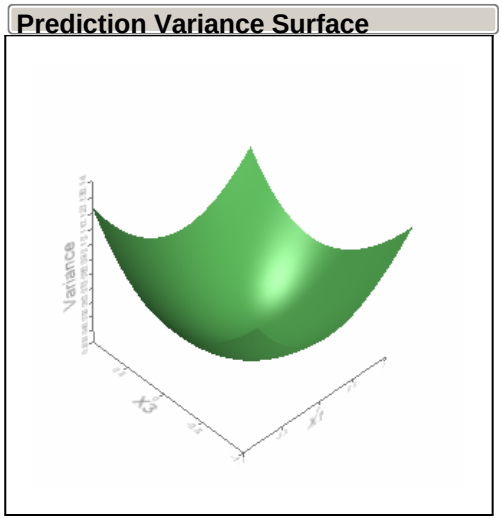
Why The Balance Became Important…



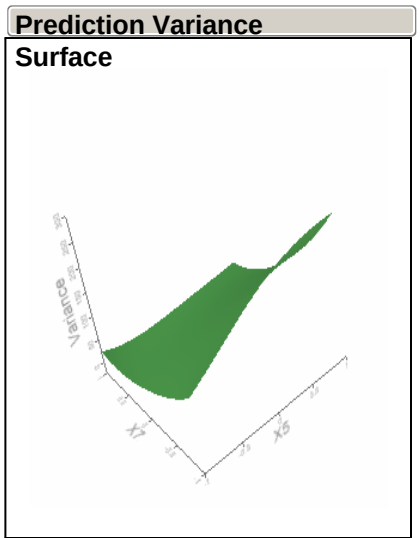




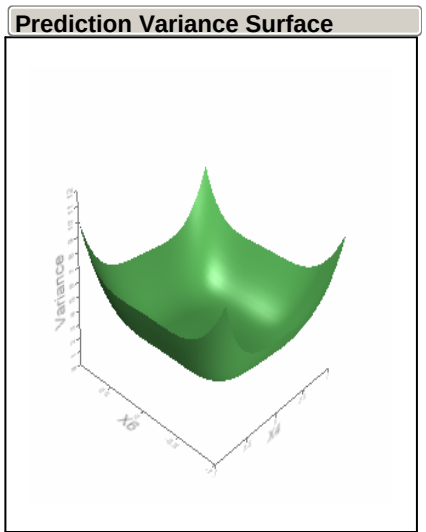


- **An unforeseen interaction caused one experimental setting to fail. Having more than min runs (8) and more than PVS stable min, produced good results.**

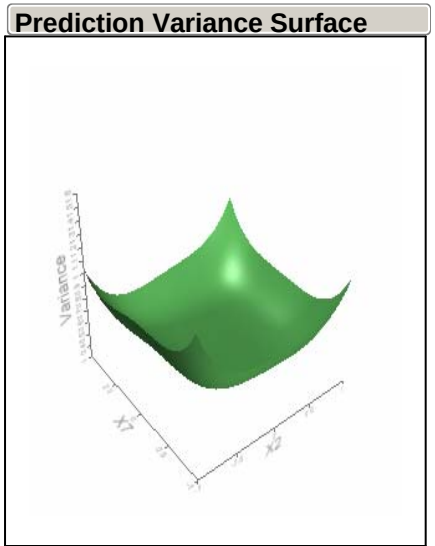
8 Runs



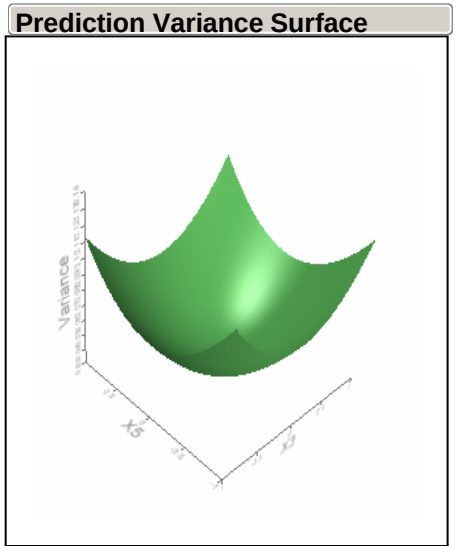
12 Runs



16 Runs



25 Runs

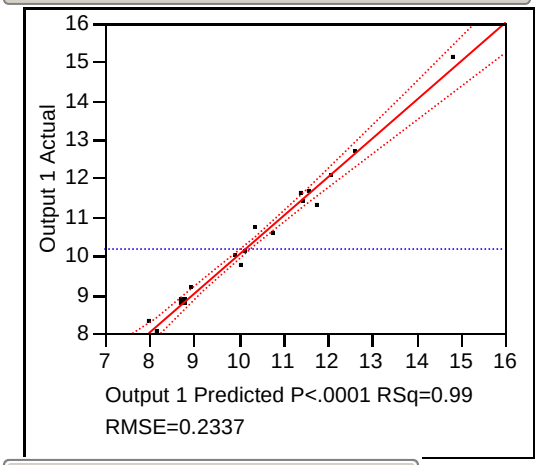


Experimental Results – A Well Understood Output

Response Output 1

Whole Model

Actual by Predicted Plot



Summary of Fit

RSquare	0.986582
RSquare Adj	0.980712
Root Mean Square Error	0.233665
Mean of Response	10.20106
Observations (or Sum Wgts)	24

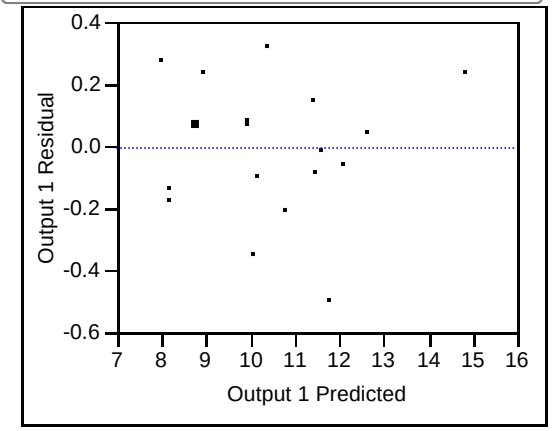
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	7	64.233736	9.17625	168.0657
Error	16	0.873587	0.05460	Prob > F
C. Total	23	65.107323		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	9.9216411	0.091212	108.78	<.0001
Factor 2	-0.189254	0.077787	-2.43	0.0271
Factor 3	-1.444124	0.068889	-20.96	<.0001
Factor 4	0.1590399	0.090533	1.76	0.0981
Factor 7	-1.54408	0.084805	-18.21	<.0001
(Factor 3-0.08333)*(Factor 3-0.08333)	0.184444	0.117899	1.56	0.1373
(Factor 7-0.08333)*(Factor 7-0.08333)	0.7918543	0.114981	6.89	<.0001
(Factor 3-0.08333)*(Factor 7-0.08333)	0.5076592	0.099467	5.10	0.0001

Residual by Predicted Plot



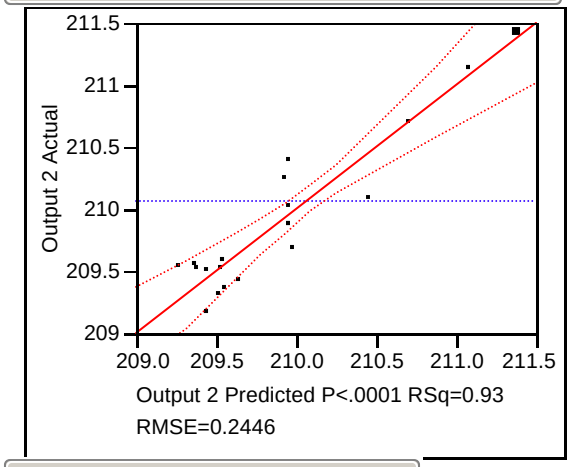
- Iterative process to model resolution
- Main effects, quadratics and interactions significant
- Scientists expected this set of factors on this output to be significant
- Factors 3 and 7 related and dominant

Experimental Results – A Surprising Result

Response Output 2

Whole Model

Actual by Predicted Plot



Summary of Fit

RSquare	0.928134
RSquare Adj	0.889805
Root Mean Square Error	0.244579
Mean of Response	210.0776
Observations (or Sum Wgts)	24

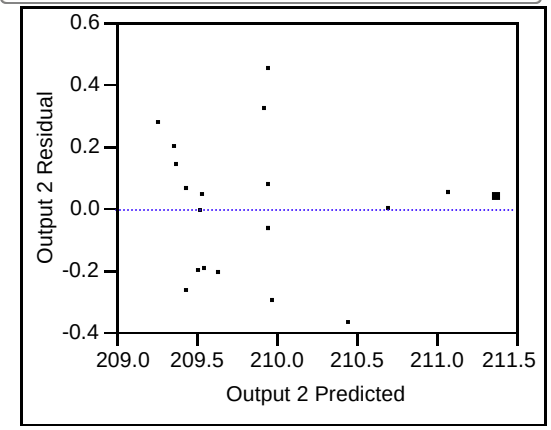
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	11.588180	1.44852	24.2152
Error	15	0.897283	0.05982	Prob > F
C. Total	23	12.485463		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	209.93824	0.085778	2447.5	<.0001
Factor 1	0.3707098	0.077783	4.77	0.0003
Factor 3	0.5138375	0.067743	7.59	<.0001
Factor 4	0.1860368	0.091468	2.03	0.0600
Factor 7	0.3089785	0.090022	3.43	0.0037
(Factor 7-0.08333)*(Factor 7-0.08333)	-0.189995	0.130174	-1.46	0.1650
(Factor 7-0.08333)*(Factor 3-0.08333)	0.2755249	0.104287	2.64	0.0185
(Factor 3-0.08333)*(Factor 1-0.29167)	0.3890686	0.082275	4.73	0.0003
(Factor 3-0.08333)*(Factor 4-0.125)	0.1815639	0.109261	1.66	0.1173

Residual by Predicted Plot



- **Scientist were divided and most did not believe factor 3 would dominate this output**
- **Iterative process to model resolution**
- **Main effects, quadratic and interactions significant**
- **Factor 3 became one of several targeted variables for rigorous control systems**

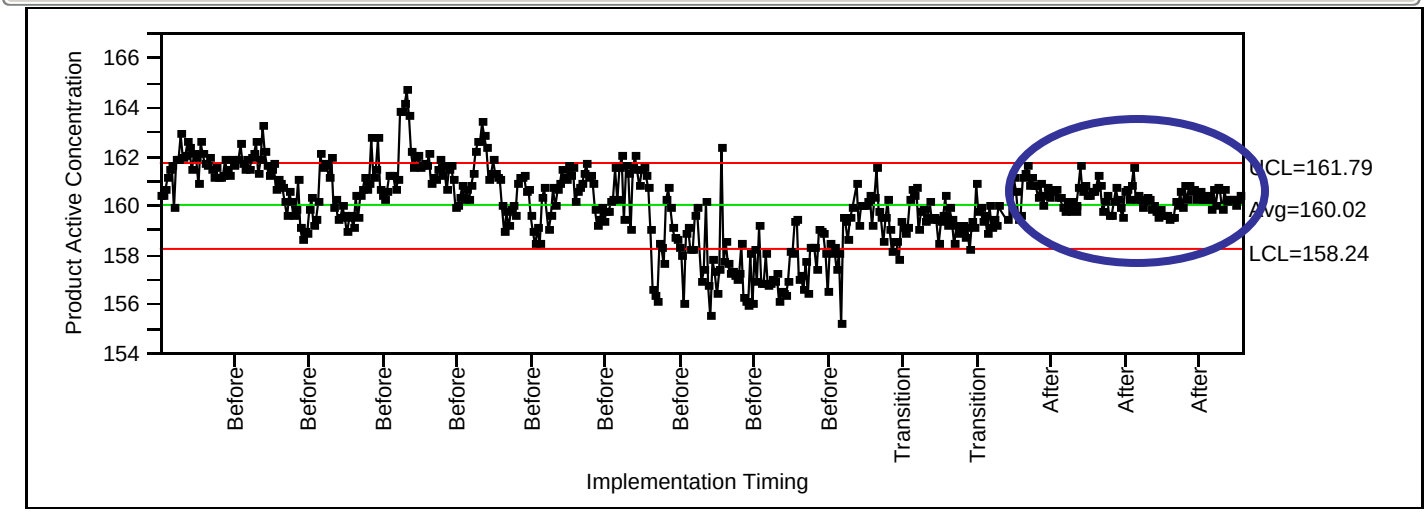
Results

Answers That Matter.

Production

Control Chart

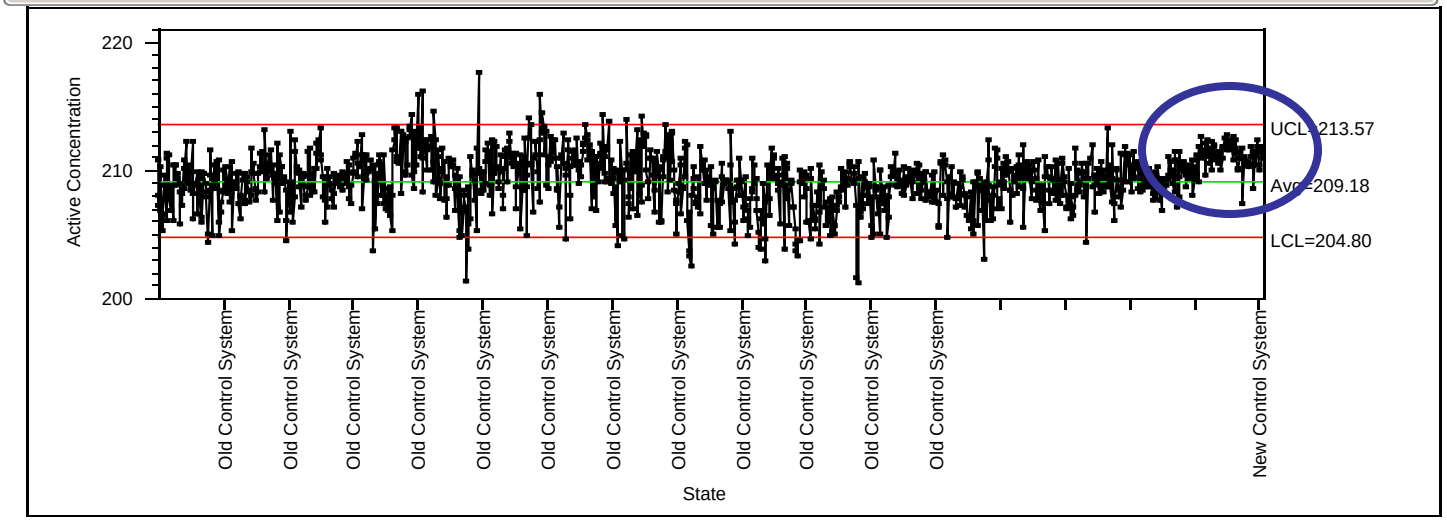
Individual Measurement of Product Active Concentration



Analytical Method

Control Chart

Individual Measurement of Active Concentration



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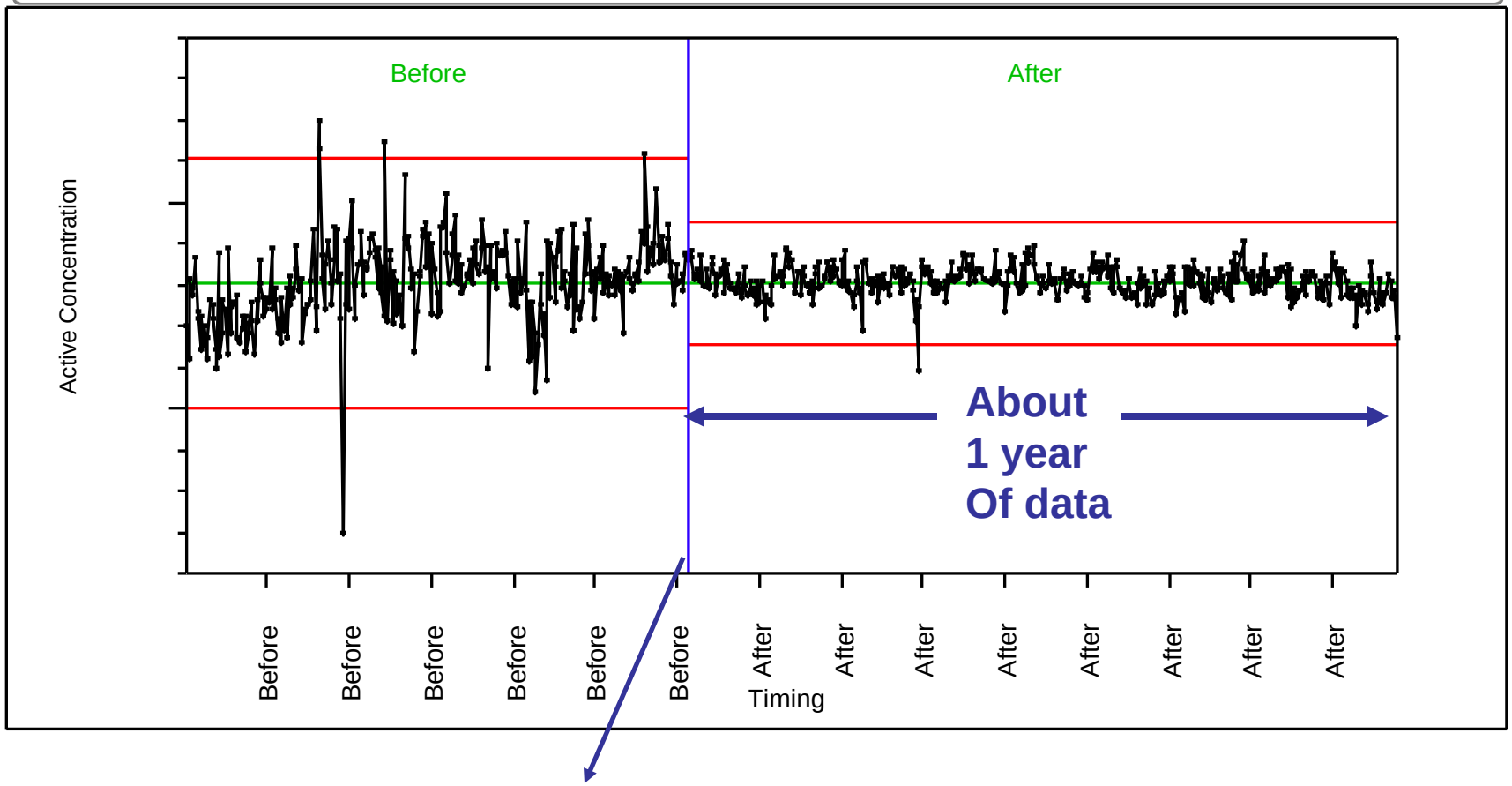
Results – Long Term Look at the Analytical Method

Control Chart

Levey Jennings of Control Sample Potency (mg/g)



Approximate 1 year break in data

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