Analytical Method Improvement

Yields Dramatic Decrease in Variation

for a Final Formulation Process

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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
Process Overview

Step 1

Large Scale Fermentation of bioactive products

Step 2

Recovery ops (evaporation, centrifugation, drying, initial formulation)

Step 3

Granulation (Mechanical particle sizing, dust control)

Step 4

Final formulation (blending and bagging, sampling, measurement, approval)
Double Click on Step 4

Solid Material (Bioactive product + Diluents)

- Mix, Sample, Measure

Mixer 1

Data flows

Ship to 3rd party final blending
Time approx 2 months

Mixer 2

Blend and transfer

Bagging Process

Bagged product, multiple bags/batch

Sampled, shipped, and measured

Diluents added to hit target in middle of two sided spec

Mass Balance
Defining the Need for Improvement

**Process**

**Individual Measurement of Active Measurement**

- **Mean (Avg):** 155.04
- **Control Limits:**
  - Lower Control Limit (LCL): Not shown
  - Upper Control Limit (UCL): Not shown

**Individual Measurement of Active Concentration**

- **Mean (Avg):** 209.11
- **Control Limits:**
  - Lower Control Limit (LCL): 204.35
  - Upper Control Limit (UCL): 213.87

**Analytical Method**
Effect of Analytical Variation

Solid Material
(Bioactive product + Diluents)

- Mix, Sample, Measure

Data flows

Ship to 3rd party final blending
Time approx 2 months

70% Of Total

Diluents added to hit target in middle of two sided spec

Blend and transfer

Bagged product, multiple bags/batch

Sampled, shipped, and measured
Lab Flow – Steps to Prepare a Result

Sample Prep
- Extract product
  - Dilute extract
    - Organic chemical reaction
      - HPLC

Equipment

Chemical reagents

People

Raw Materials

Detection and quantification

Results
Six Sigma Process Summary Slides

Six Sigma Tools

Complex analytical method

Process Maps

Cause and Effect Diagrams (100s of Variables)

FMEA Matrix

About 15 likely, larger factors

- Historical analysis
- DOE
- Models

Improved control systems
The Factors

- Mobile Phase Composition
- Column Temperature
- Column Age
- Mobile Phase Flow

- Reaction Temperature
- Vanillin Flow
- Glacial Acetic Acid

Roger

- Autodilutor
- Vanillin Makeup
- Caps
- Vanillin Condition

Bill S

LeRoy F

- Tech to Tech Differences
  - Autodilutor
  - Repipette
  - Standard Solutions
  - Vanillin
  - Chromatography

- Evaporation (uncapped)
- Repipetters

- 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
Is the instability important?

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)
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
Details of the Design – What We Were Thinking

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- 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

- 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.

Min Design, 8 runs...moving past 14-15 produced nice stability in Prediction Variance!
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.
**Experimental Results – A Well Understood Output**

### 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                    | -1.44124  | 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|

### 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

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- **Scientists expected this set of factors on this output to be significant**
- **Iterative process to model resolution**
- **Main effects, quadratics and interactions significant**
- **Factors 3 and 7 related and dominant**
Experimental Results – A Surprising Result

| Term                  | Estimate | Std Error | t Ratio | Prob>|t| |
|-----------------------|----------|-----------|---------| Holdings| |
| 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   |

Parameter Estimates

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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

**Control Chart**

**Individual Measurement of Product Active Concentration**

- **Before**
  - Avg = 160.02
  - LCL = 158.24
  - UCL = 161.79

- **Transition**

- **After**
  - LCL = 158.24

**Production**

**Control Chart**

**Individual Measurement of Active Concentration**

- **Old Control System**
  - Avg = 209.18
  - LCL = 204.80
  - UCL = 213.57

- **New Control System**

- **State**
  - LCL = 204.80
  - UCL = 213.57

**Analytical Method**

- Avg = 209.18
- LCL = 204.80
- UCL = 213.57
Results – Long Term Look at the Analytical Method

Levey Jennings of Control Sample Potency (mg/g)

Approximate 1 year break in data