QbD in Analytical Method Development: The Analytical Method Lifecycle

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With acknowledgement to, Sophie Bailes, Claire Elliot and Aled Williams

JMP Users Group Meeting

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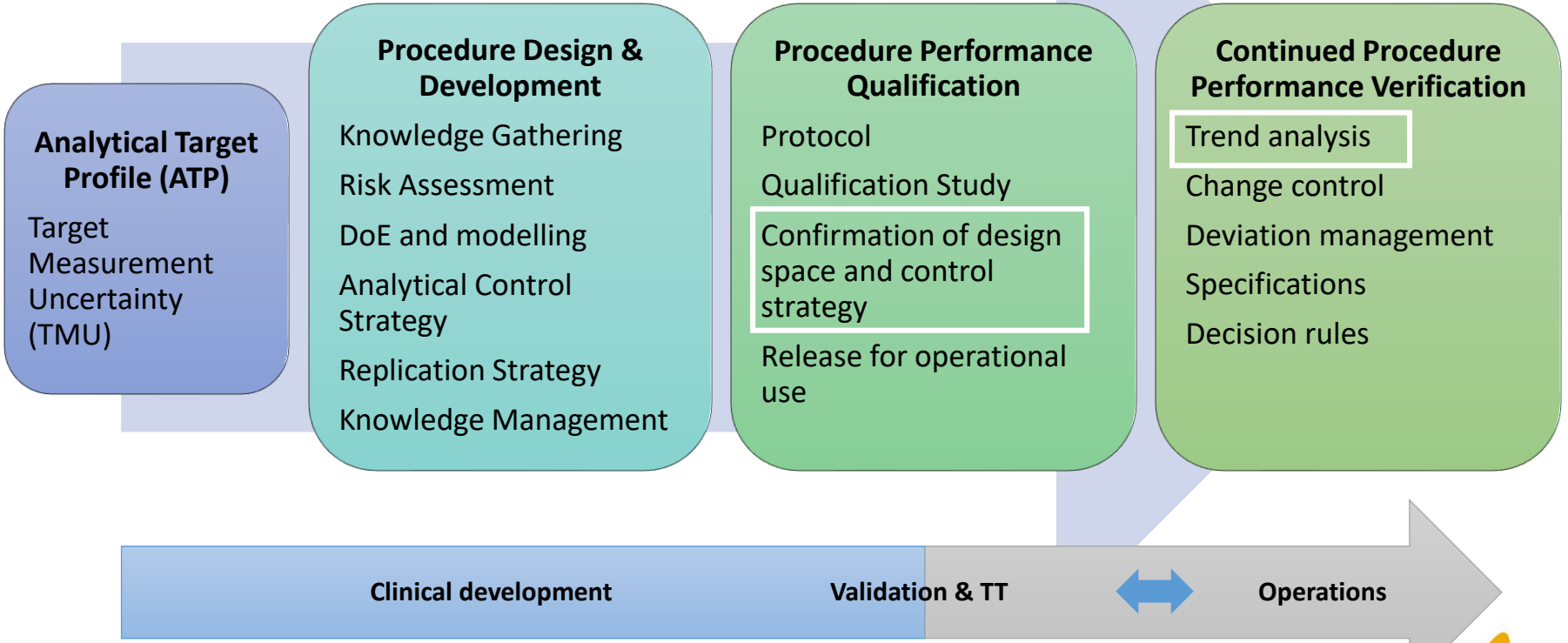
Background

- About me:
  - Principal Scientist, Statistics, AstraZeneca
  - Worked with ICI, Zeneca, AstraZeneca for 30 years
  - Variety of delivery and leadership roles in Drug Discovery, Operations, Pharmaceutical Development

- Analytical methods in Product and Chemical Development:
  - Develop chemical routes of manufacture drug substance (active ingredient)
  - Develop formulation of drug product
  - Strive for stable, robust and reproducible products and manufacturing processes
    - *Equally applies to analytical methods*

Key aspects of Analytical Method Lifecycle



Stimuli article: Proposed New General Chapter: The Analytical Procedure Lifecycle {1220}

Agenda

Illustration of the significance of analytical variability

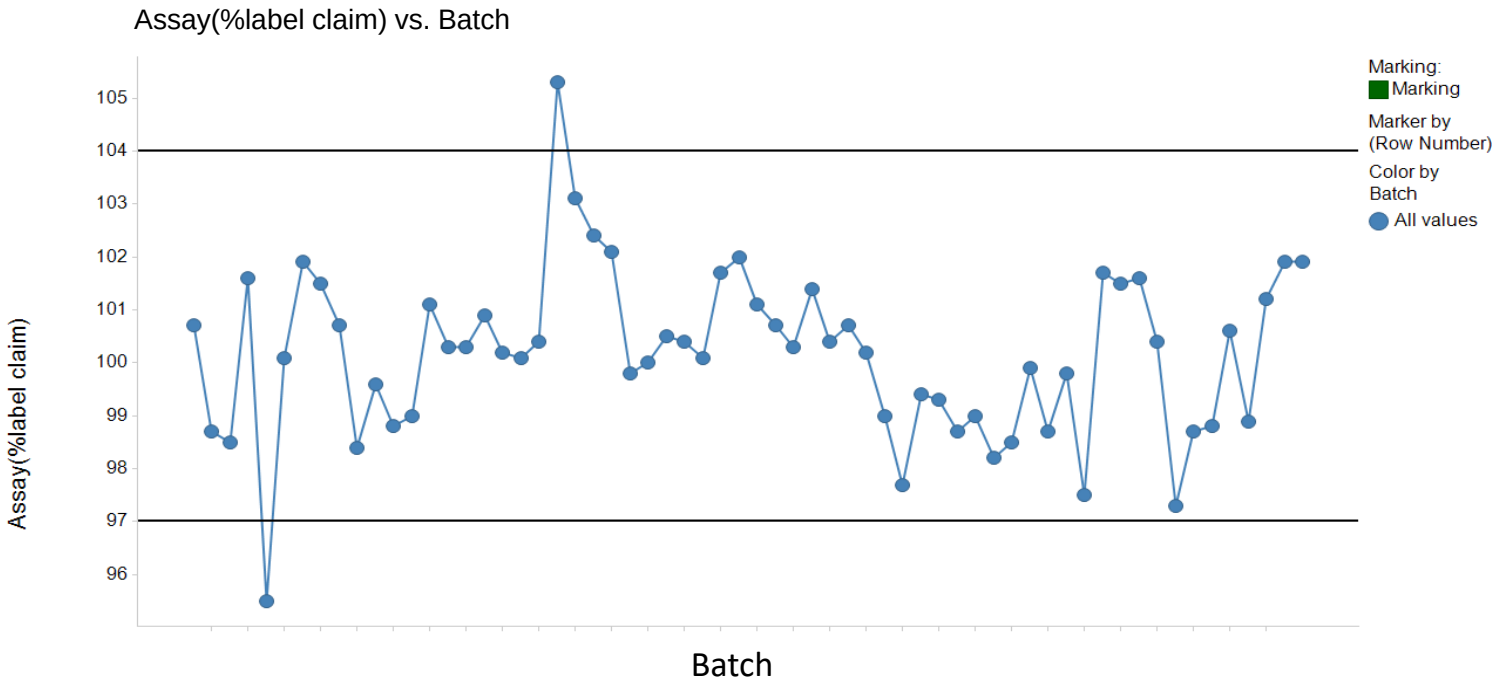
Robust analytical methods – timely application of Design of Experiments

- Effective DoE workflow
- Case study

Monitor method quality through lifecycle

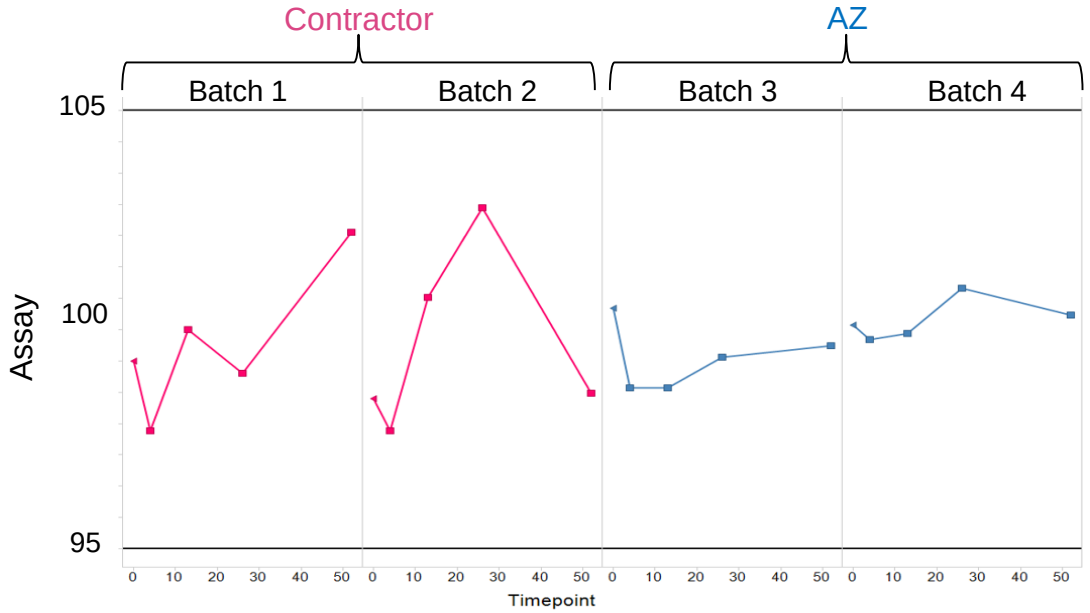
- Why continuous monitoring?
- Approaches to estimating method uncertainty
- Case study

Example


Assay(%label claim) vs. Batch
Assay(%label claim)
105
104
103
102
101
100
99
98
97
96
Batch
Marking:
Marking
Marker by
(Row Number)
Color by
Batch
All values

Contribution of Analytical Variability

Example: Stability Data

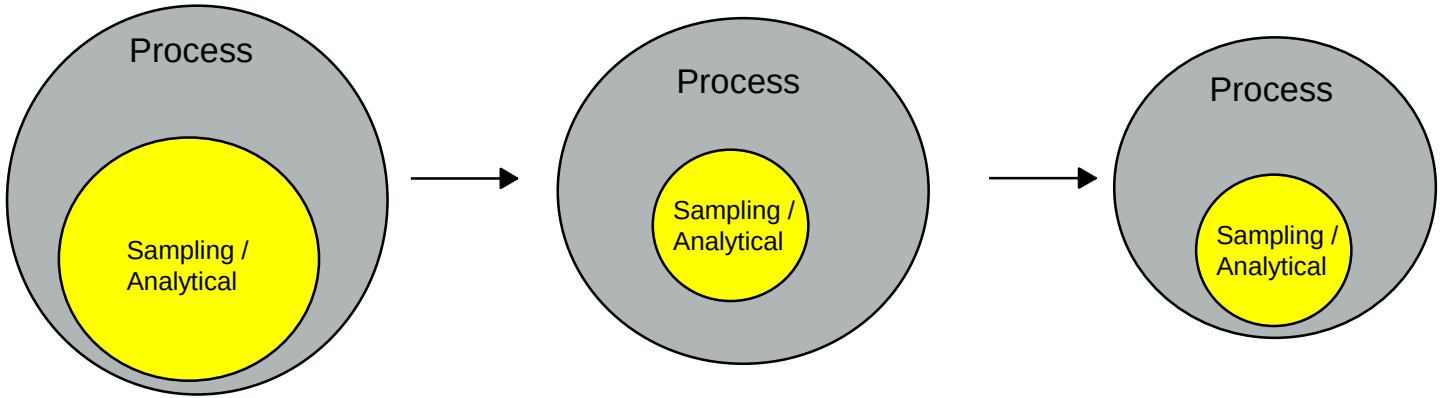


- Contractor more variable than AZ
- Analysis methodology diverged through development
  - Smaller extraction volume used at CMO resulted in poor solubilisation and dispersion.
  - Therefore incomplete extraction and more variability in results.

General Comments / Learning:

- Manufacturing variability comprises different components
- High analytical variability can…
  - …lead to misleading conclusions about product quality
  - …mask desired improvement to processes.

Components of Variability



Variance is proportional to area of circle with radius equal to standard deviation

Total variance = analytical variation + sampling variation + process variation = A + S + P

If analytical variation + sampling variation is high then this will obscure any change made to the manufacturing process.

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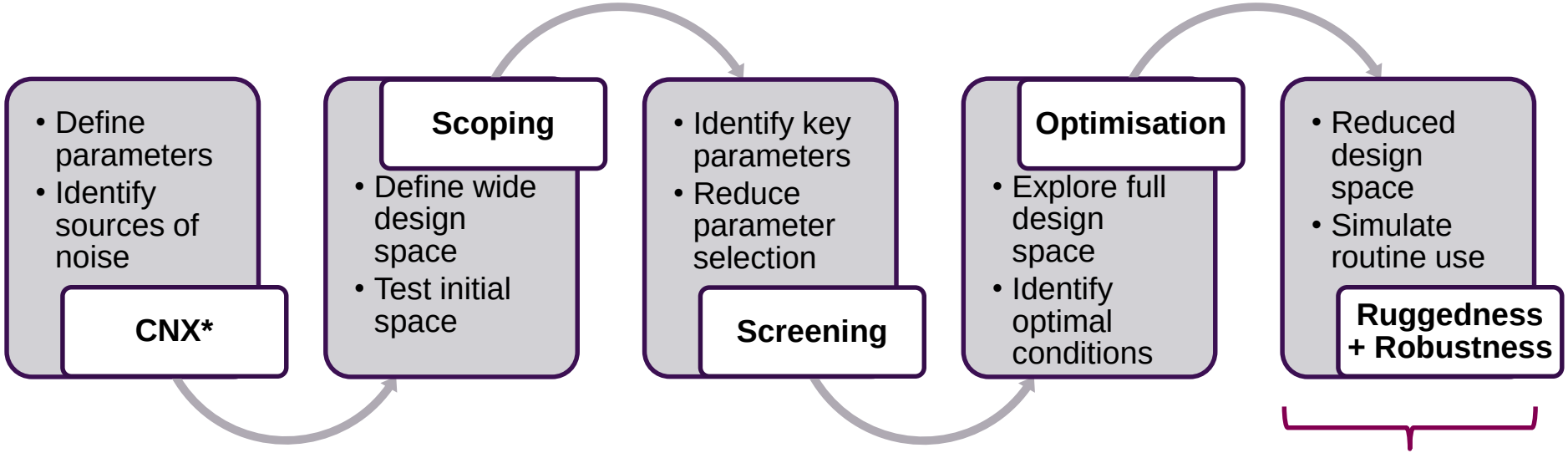
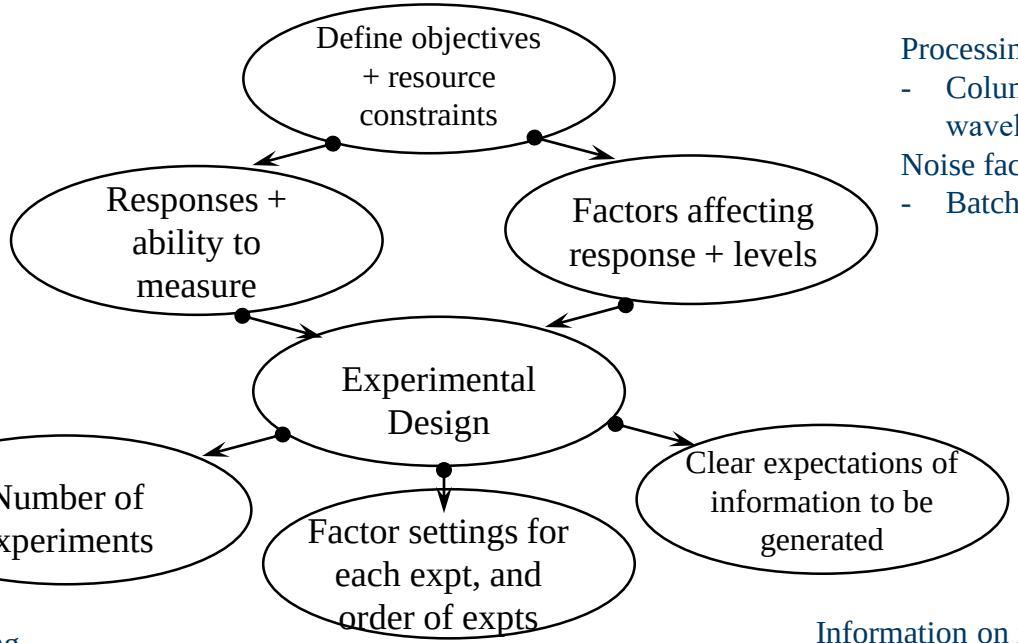
Design of Experiments (DoE) Workflow



* Parameter classification:
Control / Nuisance / eXperimental

Design of Experiments (DoE) Workflow

"To identify optimum conditions for an HPLC method and demonstrate robustness around these conditions."



Key responses:
Resolution between key peaks and retention times

Processing factors:
- Column temp, detector wavelength, buffer, …

Noise factors:
- Batch of column, operator, day

19 experiments including 3 centre points

Experiments in blocks to account for day-to-day variation.

Information on individual factor effects / interactions.

Blocking Example – Head Space GC DoE

- Generic headspace method
- 5 runs per day
- Centre point condition run on each day
- All factors balanced within each day
- Runs randomised within day
- Eliminate risk of bias between days

Day	Run	Equil Temp(deg C)	Equil Time(mins)	Loop/Valve Temp(deg C)	Transfer Line Temp(deg C)	Vial Equil Time(mins)	Injection Time(secs)	Shaking(mins)
Day 1	1	75	30	170	185	30	30	3
Day 1	2	65	33	180	175	27	33	5
Day 1	3	85	27	160	195	33	27	1
Day 1	4	85	27	160	175	33	33	5
Day 1	5	65	33	180	195	27	27	1
Day 2	6	85	33	160	195	27	33	1
Day 2	7	75	30	170	185	30	30	3
Day 2	8	65	27	180	195	33	33	1
Day 2	9	85	33	160	175	27	27	5
Day 2	10	65	27	180	175	33	27	5
Day 3	11	75	30	170	185	30	30	3
Day 3	12	85	27	180	195	27	27	5
Day 3	13	65	33	160	175	33	33	1
Day 3	14	65	33	160	195	33	27	5
Day 3	15	85	27	180	175	27	33	1
Day 4	16	85	33	180	195	33	33	5
Day 4	17	85	33	180	175	33	27	1
Day 4	18	65	27	160	175	27	27	1
Day 4	19	75	30	170	185	30	30	3
Day 4	20	65	27	160	195	27	33	5

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Parameters

Ruggedness			
iSET as 1100	Column 1	1290 (no iSET)	Column 1
	Column 2		Column 2

Robustness	Low Point	Mid Point	High Point	Adj ±
Buffer Concentration (mM)	16	20	24	20%
Flow rate (ml/min)	0.4	0.5	0.6	0.1 mL/min
Temp (°C)	40	45	50	10%
Wavelength (nm)	277	280	283	3 nm
Gradient end time (min)	8.1	9	9.9	10%
	11.25	12.5	13.75	10%
	16.2	18	19.8	10%
	20.25	22.5	24.75	10%
% B (iscratic hold)	21.33	23.7	26.07	10%

Statistical Design Approach

- *Ruggedness* is the degree of reproducibility of results at set point conditions under typical variability of laboratories, instruments, analysts, etc, i.e. noise factors.

- *Robustness* is the capacity of the method to remain unaffected by small changes to set point conditions e.g. flow rate, column temperature, etc.

Column	Instrument	TFA content Mobile Phase (%)	Flow rate (ml/min)	Temp (°C)	Wavelength (nm)	Gradient Time (min)
1	1	M	M	M	M	M
1	2	M	M	M	M	M
2	1	M	M	M	M	M
2	2	M	M	M	M	M

Ruggedness

1	1	L	H	L	H	H
1	1	L	L	L	L	L
1	1	H	H	H	L	L
1	1	H	L	H	H	H
1	2	L	H	H	L	H
1	2	H	H	L	H	L
1	2	L	L	H	H	L
1	2	H	L	L	L	H
2	1	H	L	L	H	L
2	1	L	H	H	H	L
2	1	L	L	H	L	H
2	1	H	H	L	L	H
2	2	L	H	L	L	L
2	2	H	L	H	L	L
2	2	L	L	L	H	H
2	2	H	H	H	H	H

Robustness

1	1	M	M	M	M	M
1	2	M	M	M	M	M
2	1	M	M	M	M	M
2	2	M	M	M	M	M

Ruggedness (repeatability / sample stability)

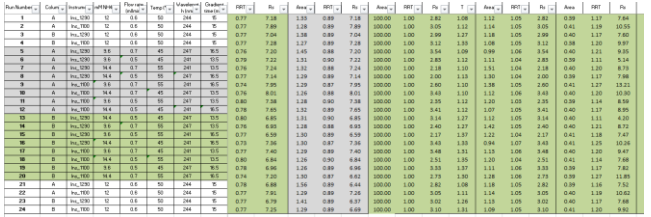
Ruggedness and Robustness Process

1) Program system with 24 methods

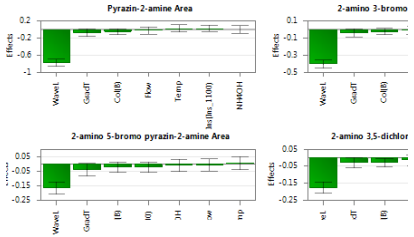
Run Number	Colum	Instrume	mM NH4	Flow rate (ml/mi	Temp (°	Wavelengt h (nm	Gradient time (mi
1	A	Ins_1290	12	0.6	50	244	15
2	A	Ins_1100	12	0.6	50	244	15
3	B	Ins_1290	12	0.6	50	244	15
4	B	Ins_1100	12	0.6	50	244	15
5	A	Ins_1290	9.6	0.7	45	247	16.5
6	A	Ins_1290	9.6	0.5	45	241	13.5
7	A	Ins_1290	14.4	0.7	55	241	13.5
8	A	Ins_1290	14.4	0.5	55	247	16.5
9	A	Ins_1100	9.6	0.7	55	241	16.5
10	A	Ins_1100	14.4	0.7	45	247	13.5
11	A	Ins_1100	9.6	0.5	55	247	13.5
12	A	Ins_1100	14.4	0.5	45	241	16.5
13	B	Ins_1290	14.4	0.5	45	247	13.5
14	B	Ins_1290	9.6	0.7	55	247	13.5
15	B	Ins_1290	9.6	0.5	55	241	16.5
16	B	Ins_1290	14.4	0.7	45	241	16.5
17	B	Ins_1100	9.6	0.7	45	241	13.5
18	B	Ins_1100	14.4	0.5	55	241	13.5
19	B	Ins_1100	9.6	0.5	45	247	16.5
20	B	Ins_1100	14.4	0.7	55	247	16.5
21	A	Ins_1290	12	0.6	50	244	15
22	A	Ins_1100	12	0.6	50	244	15
23	B	Ins_1290	12	0.6	50	244	15
24	B	Ins_1100	12	0.6	50	244	15

2) Run methods

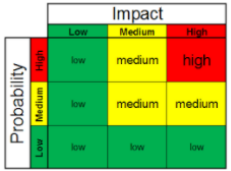
3) Process results and add data into spreadsheet



4) Statistical analysis



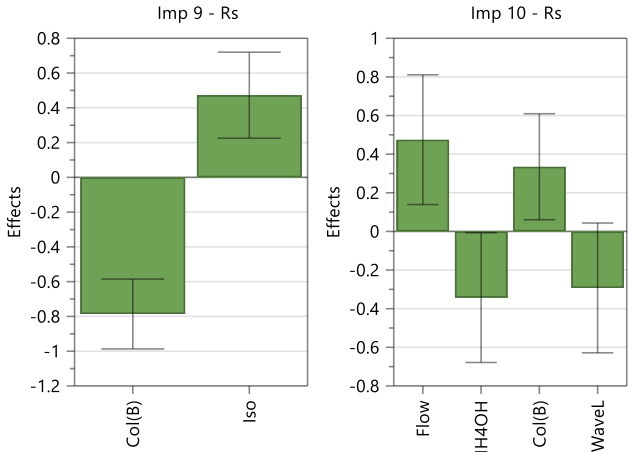
5) Interpret stats data and assess method risks. Identify suitable SST criteria.



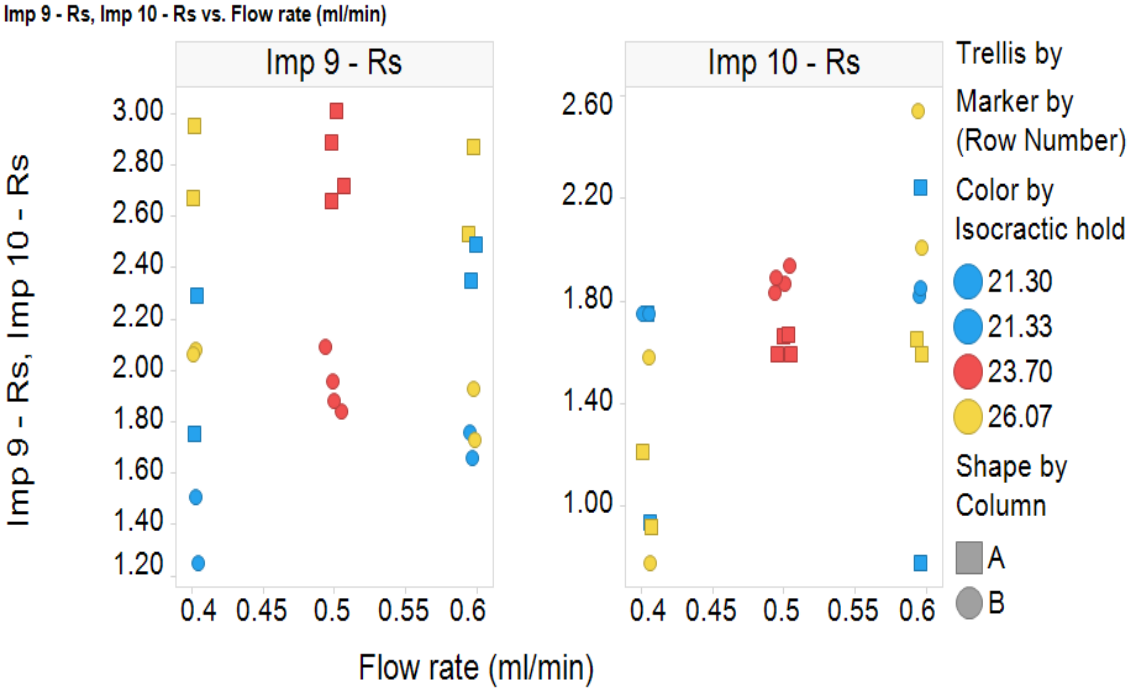
6) Update method if necessary and repeat using updated conditions

Case Study 1

Important factors identified affecting resolution:



Raw data plot:



Case Study 1 - Risk assessment

Improved understanding of parameters affecting assay and organic impurities method has led to appropriate SST controls and confidence in the method to safely transfer to new site.

Results & Impact

% Areas – Wavelength has the biggest effect on % area, however practically the difference observed is minimal.

Resolution – Three variables (flow, %B at isocratic hold and column) were found to impact the resolution of impurities 8, 9 and 10. Therefore resolution criteria have been included for these impurities in the method SST to mitigate the risk of impurity co-elution.

Relative Retention Time - Flow was found to impact the RRT of both the impurities and main component. To ensure that components are correctly identified by RRT during routine analysis the SST should incorporate those impurities affected.

Variable	%Area	Resolution	RRT
Wavelength (+/- 3nm)			
Column			
Flow (+/-0.1mL/min)			
ISet (1100 or 1200)			
%B at isocratic (+/- 10%)			
Ammonium hydroxide (+/- 2mM)			
Temperature (+/-5C)			

	Low risk
	Requires SST control

Design of Experiments – Key Points

- Apply structured approach to experimental planning
  - Best practice workflow
  - Able to meet specific objectives
  - Good use of randomisation and blocking
  - Overall approach a) leads to high quality data and decisions, b) is efficient
- Timely application of DoE – commercial late-stage vs application much earlier
- Important collaboration between the statistics group, separation science group and the project team
- Workshops given to scientists

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Why do we need continued verification?

The goal:

- Ruggedness and Robustness (through DoE) helps us understand how our method performs initially,
- Now we must ensure (**verify**) that it continues to perform.

How?

- Collect **relevant data** indicating performance during routine use.
- **Trend** and compare to validation criteria and Analytical Target Profile (ATP).
- Act on signals – identify **unusual behaviour** to drive improvement before Out-of-Specification or poor performance.
- Continuously **improve**. Use results to set future ATP and validation criteria.

Trending analytical performance

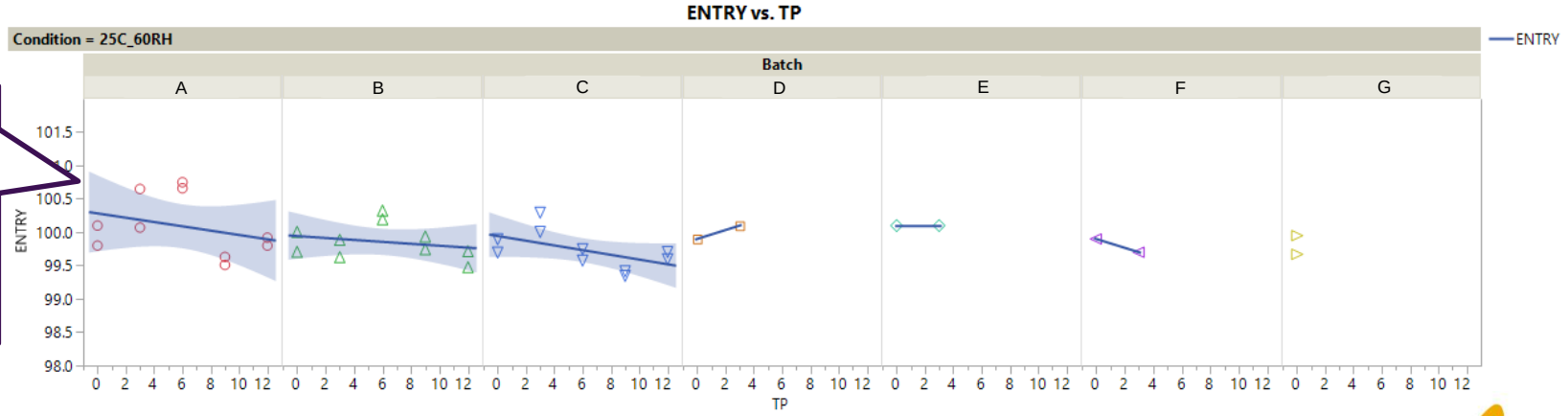
Where can we find estimates of our method performance during routine use?

- Intermediate precision
  - From validation, and gives us our first datapoint.
- Analytical Tech Transfers
  - Next data point when transferred to operations
- Duplicate/replicate testing
  - For many tests, at least 2 samples are analysed. Often more. The standard deviation of these can indicate variability.
- Stability Testing
  - Same batch analysed over many timepoints, often in duplicate.
  - More on next slide…
- Any further studies/transfers, partial revalidation, precision studies, etc.

Estimating uncertainty from stability data

- What if we don't do replicate preparations? How do we estimate the error in our single measurement?

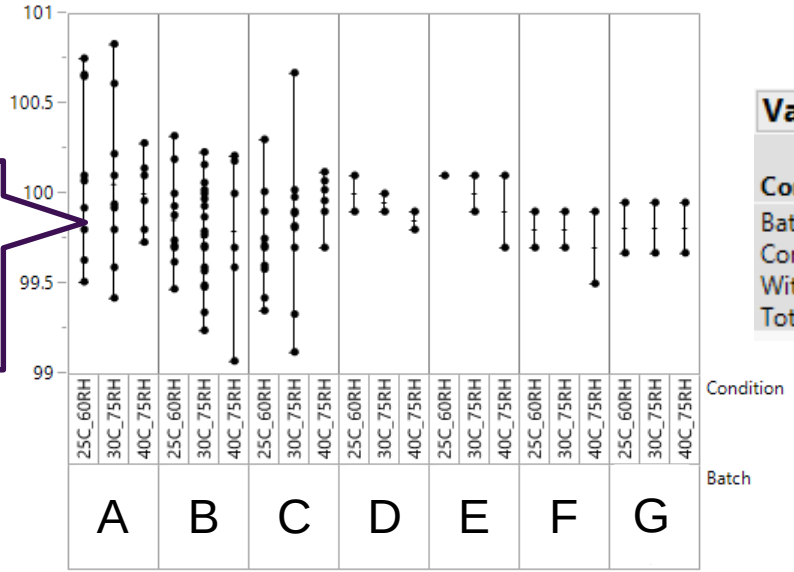
- Stability data can be pooled to look at method variability:
  - Fit a trend (if more then 3 time points and a significant one exists)
  - Residuals about that line can indicate method error



Confidence for slope spans 0, so trend not significant

Estimating uncertainty from stability data

- Using either residuals from fitted line, or raw data, can pool data to decouple batch-to-batch variation



Raw data used here, as no significant trends observed

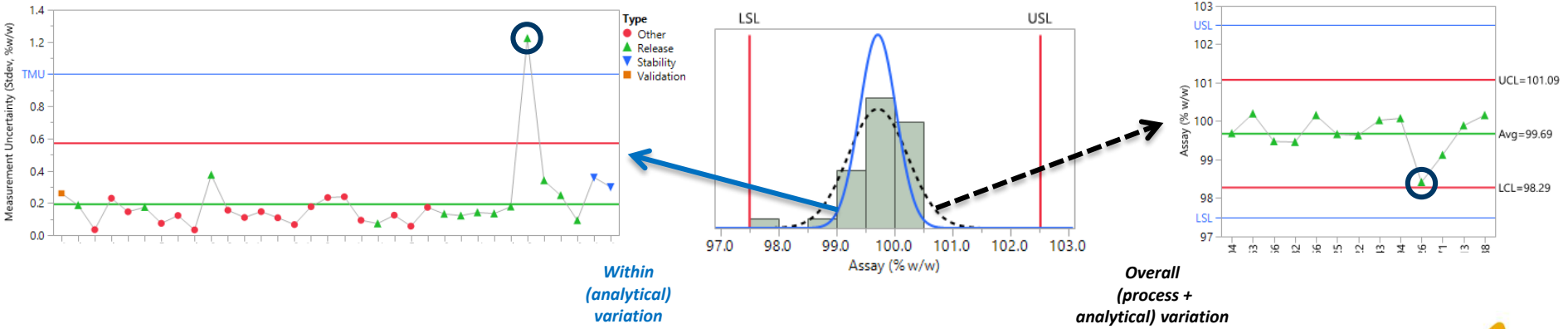
Variance Components

Component	Var Component	% of Total	20 40 60 80	Sqrt(Var Comp)
Batch	0.00576522	5.9		0.07593
Condition[Batch]	0.00281193	2.9		0.05303
Within	0.08960931	91.3		0.29935
Total	0.09818646	100.0		0.31335

"Within" variance is what's left after taking out condition and batch = analytical error

Trending

- So we have some estimates of uncertainty from stability and duplication… what now?
  - Trend against “Target Measurement Uncertainty” (e.g. set as per Analytical Target Profile, or a validation criteria of <1% Relative Standard Deviation)
  - Compare to trend of overall process (e.g. Assay)
  - Decouple analytical and process variance



EXAMPLE

Drug Substance (Assay – Water Free Basis)
97.5 to 102.5% w/w
API @ SiteName

Total capability stats

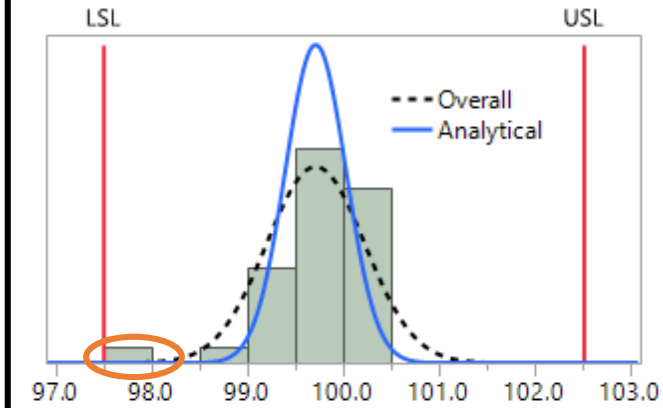
E.g.

Ppk	Predicted OOS	Actual OOS
1.4	0%	0%

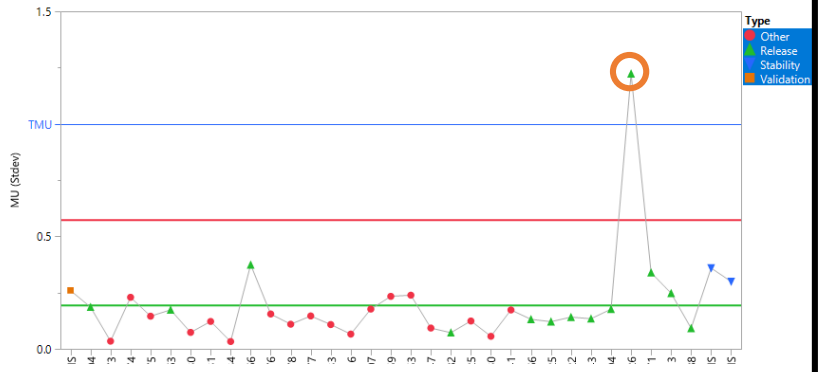
Analytical stats

E.g.

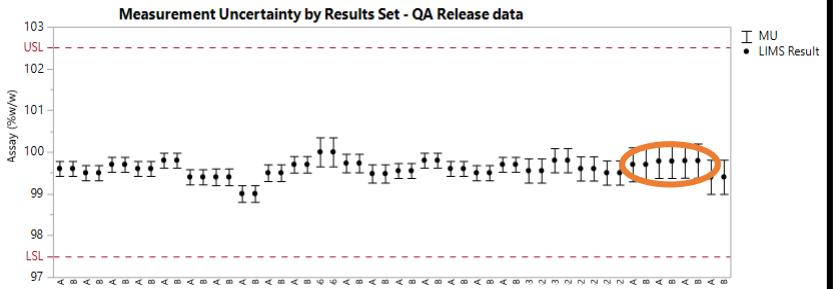
Cpk	Average MU	Number of MU>TMU	Analytical Deviations
2.3	0.196	1	0



Overall and Process Capability



Measurement Uncertainty



Instrument Uncertainty

Conclusions

- Starting with method definition – Analytical Target Profile
- Develop method
- Demonstrate robustness and ruggedness
- Continual verification of method performance

Use statistical tools, e.g. Design of Experiments, variability analysis, control charts