Setting standards in analytical science

EURACHEM Workshop May 2012

Ruggedness testing - and its contribution to MU evaluation

Overview

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- Quantifying uncertainty contributions
- Ruggedness tests
- What ruggedness tests tell us about uncertainty

Quantification methods

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- Published information
- Experience
- Calculation
- Random variation
- Systematic variation

Experimental studies

Random variation: Precision studies

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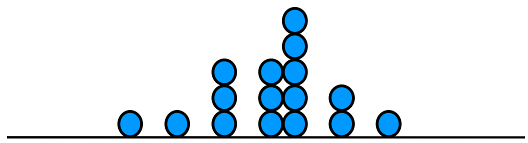
Simple Replication



$$s = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}}$$

Random variation: Precision studies

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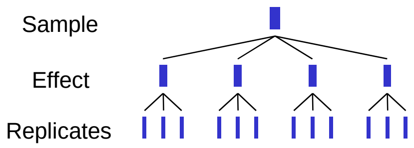
Nested design



Source of variation	SS	df	MS
Between groups	…	…	MS_B
Within groups	…	…	MS_W
Total	…	…	…

$$s_{within} = \sqrt{MS_W}$$

$$s_{between} = \sqrt{\frac{MS_B - MS_W}{n}}$$

Systematic variation: A simple uncertainty experiment

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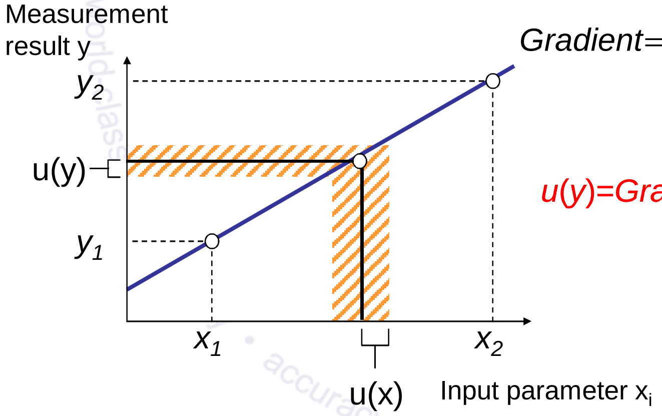

$$Gradient = \frac{(y_2 - y_1)}{(x_2 - x_1)}$$

$$u(y) = Gradient \times u(x)$$

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Ruggedness tests

Ruggedness - Definition

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"Intra-laboratory study to examine the behaviour of an analytical process when small changes in the environmental and/or operating conditions are made .... allows information to be obtained on effects of minor changes in a quick and systematic manner"

AOAC-PVMC

Also known as robustness testing

Ruggedness design

- Ruggedness tests generally use 'screening' designs
- Typically intended to be economical tests for significant effects in multiple factors
- Testing 'all at once' is far more economical than 'one at a time' for the same test power

Plackett-Burman experimental designs

Experimental parameter	Experiment number							
	1	2	3	4	5	6	7	8
A or a	A	A	A	A	a	a	a	a
B or b	B	B	b	b	B	B	b	b
C or c	C	c	C	c	C	c	C	c
D or d	D	D	d	d	d	d	D	D
E or e	E	e	E	e	e	E	e	E
F or f	F	f	f	F	F	f	f	F
G or g	G	g	g	G	g	G	G	g
Observed result	s	t	u	v	w	x	y	z

Plackett-Burman 7-factor experimental design

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- Identify up to 7 experimental parameters (A to G) for study
- Identify normal (A to G) and alternative (a to g) values for each parameter
- Carry out experiments on a representative sample or reference material

Choosing values for parameters

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- Choose normal and alternative values
  - e.g. normal extraction time is 30 minutes, alternative extraction time is 20 minutes

OR

- Set extremes of a range about the normal value
  - e.g. to investigate the effect of changing the normal extraction time of 30 minutes by ±10 minutes, set the "normal" extraction time to 20 minutes and the "alternative" to 40 minutes

Example: LC method for Tartrate in beverages

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- Primary interest: Measured tartrate or tartrate recovery
- Also of interest:
  - Is the chromatography likely to be stable?
  - Can we 'measure' chromatographic quality at the same time as tartrate?
- Solution: Monitor LC retention time and LC resolution (theoretical plate count) in the same experiment
  - We get the information essentially free

Example: Tartrate. SPE/LC parameters

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Exp No	Run Order	Sample size	SPE Flow rate	Additional SPE cleanup	LC Flow rate	Column temp	Buffer pH
1	3	2	Nominal - 1/s	YES	1	30	3.2
2	2	5	Nominal - 1/s	YES	0.7	20	3.2
3	6	2	High - 5/sec	YES	0.7	30	2.9
4	5	5	High - 5/sec	YES	1	20	2.9
5	1	2	Nominal - 1/s	NO	1	20	2.9
6	4	5	Nominal - 1/s	NO	0.7	30	2.9
7	7	2	High - 5/sec	NO	0.7	20	3.2
8	8	5	High - 5/sec	NO	1	30	3.2

Practical problems

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- The basic AOAC design leaves no degrees of freedom
  - and the tartrate design only one
- LC Temperature and buffer pH cannot be changed randomly during a run
  - These four combinations must be in different runs
- “Quick” answer:
  - Four runs allows replication of SPE experiments and leaves a degree of freedom for the LC factors after allowing for run effects

Results - Recovery

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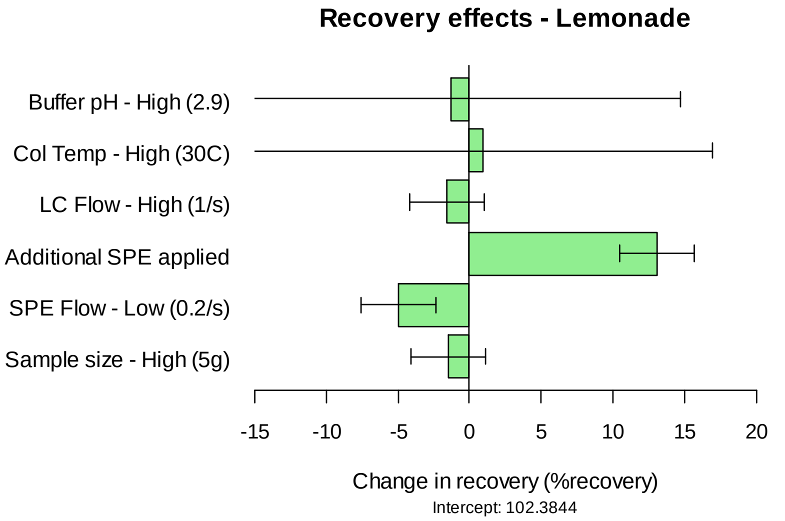
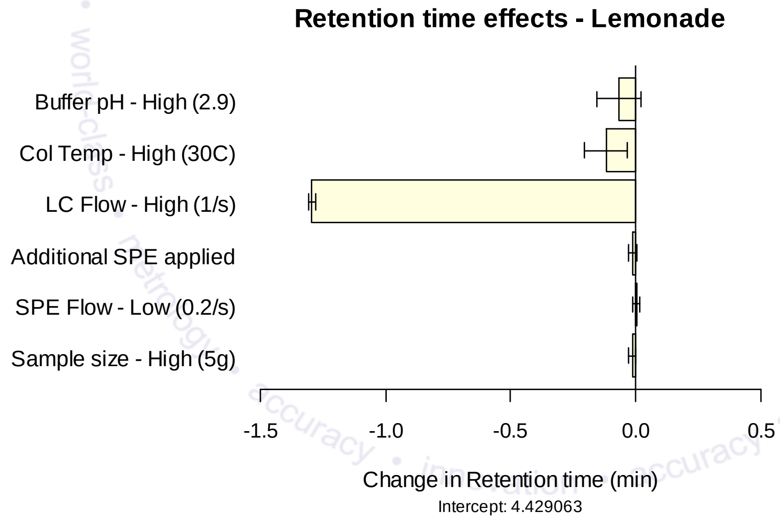


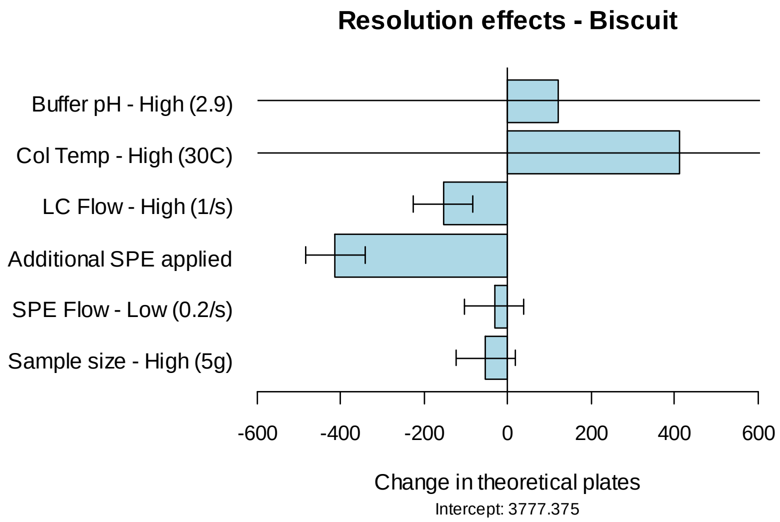
Results – Retention time

LGC *Setting standards in analytical science*



Results – LC Resolution

LGC *Setting standards in analytical science*



An unexpected bonus

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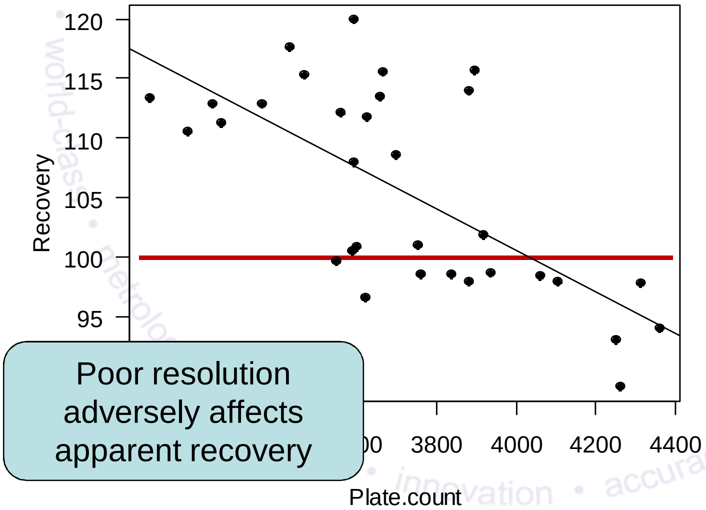

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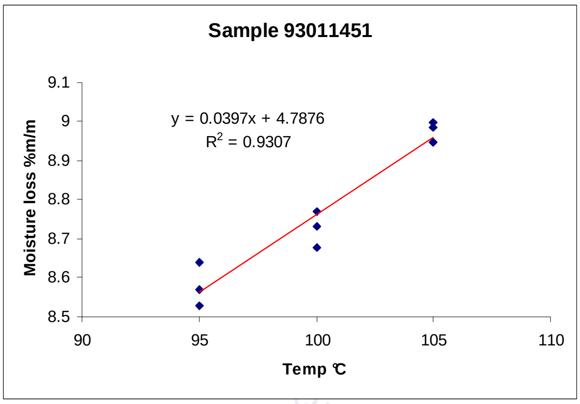
What does Ruggedness tell us about Uncertainty?

Example: Moisture determination



- Sample 1

$$\frac{\partial y}{\partial T} \approx 0.03$$

- Sample 2

$$\frac{\partial y}{\partial T} \approx 0.04$$

Uncertainties differ by sample

Example: Moisture determination

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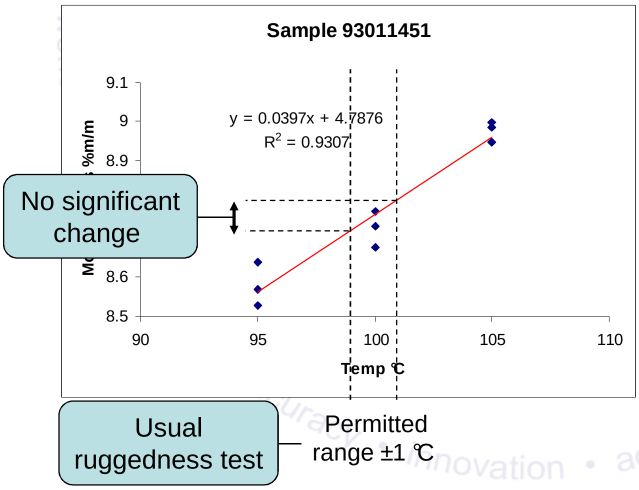

Implications of low test power

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- Ruggedness tests with small ranges will not give reliable gradients
  - ... and unreliable uncertainty estimates
- Most ruggedness tests should give insignificant effects over permitted range

Conclusion

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- Ruggedness tests are important checks for in-house methods
- Monitoring more than one response can add useful information at minimal cost
- ‘Standard’ Ruggedness tests examine changes which are expected to be insignificant
- “lack of statistical significance in ruggedness tests is better interpreted as [a] reason to leave an effect out of the uncertainty budget”*

**J R Cowles, S Daily, S L R Ellison, W A Hardcastle, C Williams; Accred. Qual. Assur. 6 368-71 (2001)*