Using Plackett Burman partial factorial designs for method robustness testing

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Reproducibility of a Method

- Ruggedness
- Robustness

Ruggedness of a Method

- “the degree of reproducibility of test results obtained by the analysis of the *same* samples under a variety *normal* test conditions” USP

Ruggedness test conditions

- Different
  - laboratories
  - analysts
  - instruments
  - reagent lots
  - analysis days
  - elapsed assay times
  - assay temperatures

- Factors are external to the method
- Should show a lack of influence
- ICH – “intermediate precision”

Robustness of a Method

"a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal use." USP, ICH

Factors are internal to the method

Should show a lack of influence

Typical robustness parameters

- HPLC
  - Mobile phase composition
  - Number, type, and proportion of organic solvents
  - Buffer composition and concentration
  - pH of the mobile phase
  - Different column lots (same brand and model)
  - Temperature
  - Flow rate
  - Wavelength
  - Gradient; slope and length

Experimental design

- The scientists approach
  - Univariate
    - Change a single variable at a time
      - Time consuming, inefficient
      - Interactions may not be detected

Experimental design 2

- The statisticians approach
  - Multivariate
    - Change many variables at a time
      - More efficient
      - May allow observation of interactions
      - Some main effects may be obscured

Multivariate approaches

- Comparative
  - Compare totally different methods e.g. solvent vs SPE extraction vs other methods
- Response surface modelling
  - Minimize or maximize a response
- Regression modelling
  - Quantify response variable to input variables
- Screening
  - Identify which factors are important or significant

Multivariate screening approaches

- Full factorial 2^k
- Fractional factorial
  - 2^{k-p}
  - Plackett Burman

Full factorial

- Each factor is set at two levels, high (+) or low (-).
- For k factors the number of experiments is 2^k
- The number of experiments increases rapidly
- Satisfactory for up to 5 factors

Factors k	Number of runs 2^k
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512

Full Factorial Design

- Full factorial
- Main effects
  - Effect A =

    $(y2 + y4 + y6 + y8)/4 - (y1 + y3 + y5 + y7)/4$
  - =differences of averages
  - = average y(+) – average y(-)
- All effects are ‘clear’ i.e no confounding by interactions.

	A	B	C	
1	-	-	-	Y1
2	+	-	-	Y2
3	-	+	-	Y3
4	+	+	-	Y4
5	-	-	+	Y5
6	+	-	+	Y6
7	-	+	+	Y7
8	+	+	+	Y8

Fractional factorial 2^{k-p}

- Same layout as full factorial
- Select $1/2^p$ of the experiments
- For p = 1 run half of experiments: 1,4,6,7.
- Effect = average y(+) – average y (-)
- Effect A = (y2 + y6)/2 –(y1 + y7)/2

- Main effects may be confounded by interactions

	A	B	C	
1	-	-	-	Y1
2	+	-	-	Y2
3	-	+	-	Y3
4	+	+	-	Y4
5	-	-	+	Y5
6	+	-	+	Y6
7	-	+	+	Y7
8	+	+	+	Y8

Box, G.E.P., Hunter, W.G., & Hunter, J.S. (1978) *Statistics for Experimenters. An introduction to Design, Data Analysis, and Model Building*, John Wiley and Sons, NY

Plackett-Burman Designs

- A two level fractional factorial design
- Experiments numbers n are in multiples of 4
- i.e. n = 8, 12, 20, 24, 28, 32 etc
- Factors k <= n – 1
- For k < n-1 use dummy factors
- Most commonly used are n=8 and n=12

- Plackett, R.L., & Burman, J.P. (1946) *Biometrika* **33**, 305-325

P-B usefulness

- Limitations
  - Main effects may be aliased by two way interactions
  - Choice of layout by Plackett and Burman was set to minimize these
- Thus
  - ‘these designs are very useful for economically detecting large main effects, *assuming all interactions are negligible* when compared with the few important main effects’

11 Factor 12 experiment P-B layout

Factors	A	B	C	D	E	F	G	H	I	J	K	response
Experiment												
1	+	+	-	+	+	+	-	-	-	+	-	y1
2	-	+	+	-	+	+	+	-	-	-	+	y2
3	+	-	+	+	-	+	+	+	-	-	-	y3
4	-	+	-	+	+	-	+	+	+	-	-	y4
5	-	-	+	-	+	+	-	+	+	+	-	y5
6	-	-	-	+	-	+	+	-	+	+	+	y6
7	+	-	-	-	+	-	+	+	-	+	+	y7
8	+	+	-	-	-	+	-	+	+	-	+	y8
9	+	+	+	-	-	-	+	-	+	+	-	y9
10	-	+	+	+	-	-	-	+	-	+	+	y10
11	+	-	+	+	+	-	-	-	+	-	+	y11
12	-	-	-	-	-	-	-	-	-	-	-	y12
Weightings	0	-10	2	-8	-18	-28	-16	-4	8	-2	10	
Dummy					D2	D1	D3				D4	

Vander Heyden, Y., Nijhuis, A., Smeyers-Verbeke, J., Vandeginste, B.G., & Massart, D.L. (2001) J Pharm Biomed Anal 24, 723-753

Analysis of P-B results

- Youden test
  - Test for any overall significant effects
- Vander Heyden 1
  - Comparison of individual effects to Method Std Dev
- Vander Heyden 2
  - Comparison to the dummy factors
- Waters and Dovetoglou
  - Analysis of variance

Basic calculation - Differences

- From previous
- Factor A for 12 experiment P-B
- Also called standard errors

$$D_A = \frac{(Y_1 + Y_3 + Y_7 + Y_8 + Y_9 + Y_{11})}{6} - \frac{(Y_2 + Y_4 + Y_5 + Y_6 + Y_{10} + Y_{12})}{6}$$

Youden test

- Compare SD differences to within batch method precision
- SD replicates calculated from the Normal samples.
- Must be significantly larger than sqrt 2 SE

$$S_{Di} = \sqrt{2} \cdot \sqrt{\frac{\sum D_i^2}{n_i}}$$

$$> t.\sqrt{2} \cdot \frac{SD}{\sqrt{n_{normals}}}$$

Vander Heyden 1

- Individual differences are compared to the SE replicates

$$SE = \frac{SD}{\sqrt{n_{normals}}}$$

$$ABS\left|D_i\right| > t \cdot SE$$

See. Barwick, V.J., & Ellison, S.L.R. (2000) Development and Harmonization of Measurement Uncertainty Principles Part (d): Protocol for uncertainty evaluation from validation data. in VAM Technical Report No. LGC/VAM/1998/088 Eq 4.29

Vander Heyden 2

- Comparison of the differences of the factors to the differences of the dummy factors. NB ABS values again

$$D_i > t \cdot D_{dummy}$$

Waters and Dovetoglou

- Comparison of the Yi (+) to the Yi (-) using analysis of variance.
- Using NCSS calculated as multiple linear regression using the +1, -1 coefficients
- Also calculated in Excel following Spence et. al.

Spence, J.P., Cotton, J.W., Underwood, B.J., & Duncan, C.P. (1990) *Elementary Statistics*, Prentice Hall

Analysis of fluoroquinolones in egg: method summary

- 5g homogenized egg are spiked with standards, recovery spikes and IS and allowed to co-mingle 15 min
- 15 ml ACN containing 2% acetic acid added and shaken
- 2 g NaCL added
- Centrifuged 15 min at 3200 rcf and ACN poured off
- 10 mL hexane added to the ACN and shaken, and then aspirated
- Dried on N-Evap at 55 °C
- Redissolved in pH 3 buffer
- SPE Oasis conditioned with MeOH, water, 2% NaCL, pH3 phosphate
- Loaded
- Eashed with 30% MeOH inwater
- Eluted with ACN:MeOH = 80:20 (v/v)
- Dried
- Redissolved in 0.2% formic acid
- Filtered into vials
- Analysed by LC-MS-MS

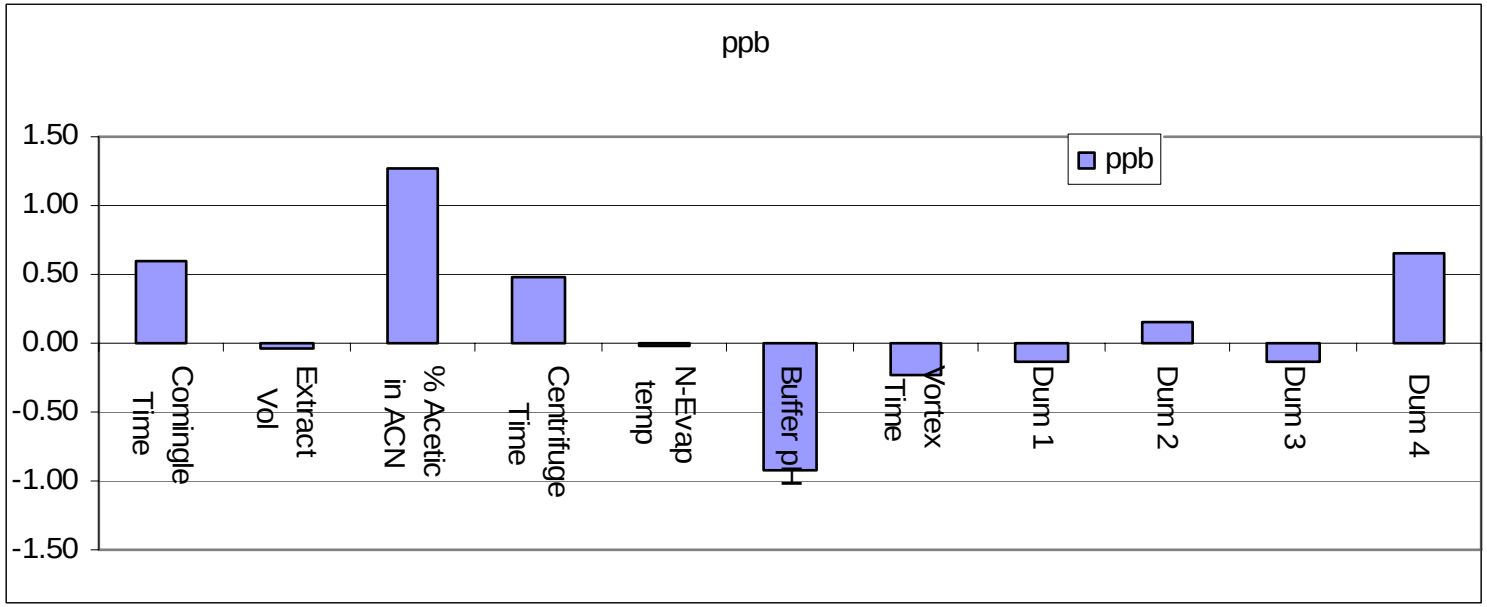
Fluoroquinolones Factors Exp 1

	Factor	+	Normal	-
A	Co-mingle time (min)	10	15	20
B	Extraction volume of ACN	14	15	16
C	% acetic acid in ACN	1.8	2.0	2.2
D	Centrifuge time (min)	10	15	20
E	N-Evap temperature (°C)	50	55	60
F	Buffer pH	2.8	3.0	3.2
G	Vortex time (x 15 sec)	1	2	3
H	Dum 1	-	-	-
I	Dum 2	-	-	-
J	Dum 3	-	-	-
K	Dum 4	-	-	-

Sample sequence for LC-MS-MS analysis

Method Blank + I.S. 1	Normal e	Normal 10 b
Method Blank + I.S. 2	Normal f	Normal 10 c
Method Blank + I.S. 3	Expt 2	Normal 10 d
Method Blank + I.S. 4	Expt 4	Normal 10 e
MMCC 0.2 ppb	Expt 5	Normal 10 f
MMCC 0.5 ppb	Expt 6	Method Blank + I.S. 1
MMCC 2 ppb	Expt 10	Method Blank + I.S. 2
MMCC 5 ppb	Expt 12	Method Blank + I.S. 3
MMCC 20 ppb	Expt 1	Method Blank + I.S. 4
MMCC 50 ppb	Expt 3	MMCC 0.2 ppb
Method Blank + I.S. 1	Expt 7	MMCC 0.5 ppb
Normal a	Expt 8	MMCC 2 ppb
Normal b	Expt 9	MMCC 5 ppb
Normal c	Expt 11	MMCC 20 ppb
Normal d	Normal 10 a	MMCC 50 ppb

Differences e.g. ciprofloxacin



0.601	**-0.033**	**1.276**	**0.472**	**-0.022**	**-0.924**	**-0.240**	**-0.133**	**0.150**	**-0.140**	**0.653**

Youden tests for fluoroquinolones vs SE normals

Compound	Significance level
Cipro	
Dano 314	p<0.1
Dano340	p<0.1
Enro	p<0.1
Sara	
Nor	p<0.1
Lome	p<0.1

Vander Heyden 1
individual differences vs SE normals

Factor	A	B	C	D	E	F	G
	Co-mingle	Ext vol	% acetic	Centri fuge time	N-Evap temp	Buffer pH	Vortex time
Ciprofloxacin			**				
Danofloxacin			*				**
Enrofloxacin						**	
Sarafloxacin							
Norfloxacin			**			**	
Lomefloxacin						**	

* p<0.1, ** p<0.05

Vander Heyden 2 vs dummy factors

Factor	A	B	C	D	E	F	G
	Co-mingle	Ext vol	% acetic	Centri fuge time	N-Evap temp	Buffer pH	Vortex time
Ciprofloxacin			**			*	
Danofloxacin							
Enrofloxacin						**	
Sarafloxacin			**	**		**	
Norfloxacin			*			*	
Lomefloxacin						*	

* $p<0.1$, ** $p<0.05$

Waters and Dovetoglou by Anova

Factor	A	B	C	D	E	F	G
	Co-mingle	Ext vol	% acetic	Centri fuge time	N-Evap temp	Buffer pH	Vortex time
Ciprofloxacin			**			**	
Danofloxacin							
Enrofloxacin						**	
Sarafloxacin			**	**		**	
Norfloxacin							
Lomefloxacin							

* p<0.1, ** p<0.05

Summary of all methods – Exp 1

Factor	A	B	C	D	E	F	G
	Co-mingle	Ext vol	% acetic	Centri fuge time	N-Evap temp	Buffer pH	Vortex time
Ciprofloxacin			** (abc)			** (b)	
Danofloxacin			* (a)				** (a)
Enrofloxacin						** (abc)	
Sarafloxacin			** (bc)	** (bc)		** (bc)	
Norfloxacin			** (a) * (b)			**(a) *(b)	
Lomefloxacin						** (a) * (b)	

* p<0.1, ** p<0.05
a vs SD, b vs dummy, c by Anova

Conclusions of Exp 1

- Significant effects were:
  - caused by the % of acetic acid in the extraction solvent (ACN).
  - caused by the buffer pH
- But
  - The changes used were somewhat greater than one would expect in making solutions
- Therefore repeat with smaller changes
  - Add different other factors

Fluoroquinolones Factors - Exp 2

	Factor	+	Normal	-
A	Extraction volume of ACN	14	15	16
B	Percent acetic acid in ACN	1.95	2.00	2.05
C	Amount of NaCl	1.9	2.0	2.1
D	Volume of hexane (mL)	9	10	11
E	Dum 2	-	-	-
F	Dum 1	-	-	-
G	Dum 3	-	-	-
H	Buffer pH	2.9	3.0	3.1
I	Buffer volume (mL)	9	10	11
J	Wash volume (mL)	2x4.5	2x5.0	2x5.5
K	Elution volume (mL)	6	7	8

Summary of all methods – Exp 2

Factor	A	B	C	D	E	F	G
	Co-mingle	Ext vol	% acetic	Centri fuge time	N-Evap temp	Buffer pH	Vortex time
Ciprofloxacin							
Danofloxacin							
Enrofloxacin							
Sarafloxacin							
Norfloxacin							
Lomefloxacin							

No significant effects were observed

Conclusions

- All three methods of evaluating the Plackett–Burman design detect the main effects of robustness changes.
- A 12 experiment P-B layout is ideal for 7 to 8 factors as can include dummy factors
- A 12 experiment P-B layout is feasible to run in one day
- Total number of extractions is about 28-30

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Some references

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