

ASSAY VALIDATION

Ruggedness

and



Robustness

Ruggedness and robustness testing is applicable to pharmaceutical R&D and QC Laboratories, especially when methods are transferred and distinguishes the similarities and differences between the two analytical concepts and how they are applied in the laboratory.

Analytical methods are usually developed in an R&D laboratory and eventually transferred to the production quality control laboratory for routine product analysis. This process of technical data transfer from one lab to the other requires a clear demonstration that the methodology can be successfully transferred. How is it done?

There is an easy-to-use procedure to meet this regulatory and compendial requirement.

Ruggedness
is a USP
Requirement
Robustness
is not.

Ruggedness and Robustness.

The USP defines **ruggedness** as "the degree of reproducibility of test results obtained by the analysis of the *same* samples under a variety of *normal* test conditions such as:

- ◆ Different laboratories
- ◆ Different analysts
- ◆ Different instruments
- ◆ Different reagent lots
- ◆ Different analysis days

- ◆ Different elapsed assay times
- ◆ Different assay temperatures ..."

These factors are all *external* to the written analytical method and each parameter should show a lack or indeed absence of influence on the test results obtained.

But what about the *internal* factors of the written test method such as a change in the flow rate (mL/min) or the concentration of the organic acid in mobile phase (HPLC systems) or better still, a change from a Phenomenex Bondclone™ 10μ C-18 column to a Waters μ-Bondapak™ 10μ C-18 column? **T**hese small but deliberate internal variations in method parameters of the written analytical procedure should be evaluated to assess whether the analytical procedure remains unaffected by these slight changes.

Robustness is defined by both the USP and the ICH Tripartite guidelines as "*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* ". Robustness is defined both in the USP and ICH, but is **not** required.

Furthermore, **ruggedness** measures the lack of *external* influence on the test results whereas **robustness** measures the lack of *internal* influences on the test results. Internal and external variations are often mixed together in analytical validation packages erroneously, under the heading of one or the other. Internal and external analytical parameters should be separated

and appraised individually. A simple experimental design can evaluate both ruggedness and robustness, as separate distinguishable entities - together.

Table 1 Comparison Table.

Attribute.	Ruggedness.	Robustness.
USP Validation Requirement	☑	☒
ICH Validation Requirement	☒	☒
Internal change	☒	☑
External change	☑	-
Method Variations	-	☑
Environmental Variations	☑	-

Designing Analytical Experiments

Such a designed experiment can demonstrate that methodology and environmental factors may *or* may not influence the test results. It is hoped that the analytical method is both rugged and robust, however a well designed experiment may identify test conditions or specification limits that need to be closely controlled and tightened or even test parameters that need further investigation and optimization.

Advantages - Designing the Experiment

A designed experiment is a simple matrix design. The Plackett-Burman designs are most applicable to technical transfer of a validated analytical methods from development to quality control centres. For the most cost-effective design, the attributes of both R&D and QC laboratories should be incorporated into development validation protocol of the assay method.

The advantages of these designs are quite simple - the number of tests required is simply dramatically reduced. 56 assays (i.e. 7 x 8) are needed to evaluate seven internal and/or external variables, these can be reduced to eight quick assays using an eight-run Plackett-Burman design.

Even more dramatic, for eleven variables (11 x 12) a minimum of 132 one-factor-at-a-time data points would be required, but via matrix testing using an twelve-run design, only 12 HPLC assays are needed to produce the equivalent of 132 individual one-factor-at-time assays.

Few, if any HPLC assay analytical methods could have more than 12-15 significant environmental or method variations.

Table 1.

An Eight Run Design Template

Test No	External / Internal Changes / Variations							ASSAY TEST RESULT
	A	B	C	D	E	F	G	
1	+	+	+	-	+	-	-	99.3
2	-	+	+	+	-	+	-	101.5
3	-	-	+	+	+	-	+	100.4
4	+	-	-	+	+	+	-	97.9
5	-	+	-	-	+	+	+	98.5
6	+		+	-	-	+	+	99.0
7	+	+		+	-	-	+	97.9
8	-	-	-	-	-	-	-	100.9

The (+) or (-) signs are used as variables in the 8 run design. Assign (-) to Analyst I ; Day I; Column I and (+) to Analyst II; Day II; Column II and so on A to G are chosen as the external variations (ruggedness) anticipated to arise during use in the Development Lab.

Table 2. 8 RUN DESIGN

Template for Ranked Effect and Means

External & Internal changes/variations	Ranked Effects	M values
A - Analyst I & II	1.8	-1.35
F - Analyst III & IV		-0.76
G - Reagents I & II		-0.35
E - Week I & II		0
B - Week III & IV		+0.35
C - Column I & II		+0.76
D - HPLC No I & No II	0	+1.35

The M values are obtained from statistical design tables. The ranked Effects are calculated by simple addition of assay test results and then dividing by half the number of runs (i.e. 4 in a 8 run design).

Calculating the Ranked Effects For A

99.3	+
101.5	-
100.4	-
97.9	+
98.5	-
99.0	+
97.9	+
100.9	-
-7.2	Σ
-1.8	/4

Figure 1.

Sum the Assay values by assigning a positive or negative value obtained from the 8 run design

For D

99.3	-
101.5	+
100.4	+
97.9	+
98.5	-
99.0	-
97.9	+
100.9	-
0	Σ
0	/4

Figure 2.

Perform this addition for each of the eight variables and divide the sum by 4 (half the number of runs)

Results.

A linear-linear scale is used. Plotting the Ranked Effect on the X-axis vs. the M values on the Y-axis produce a normal probability plot of effects. If a value lies outside this straight line one can conclude that the method is not rugged / or robust, as classified, for that particular variable (e.g. [say] flow rate).

12 Run Designs.

A template for 12 run design is used (Tables 3 & 4), when more than seven factors are present. This design will give 11 factors for analysis. The M values are constant for any given design and are actually the means of the order statistics (3) for a sample size of eleven. As they always remain the same, the template can be used for any ruggedness / Robustness validation method protocol. Use a Eight Run for evaluating say, ruggedness only, and a Twelve Run design for both

ruggedness and robustness when transferring a method to another laboratory.

Table 3.

An Twelve Run Design Template

No	External / Internal Changes / Variations (11)											ASSAY TEST RESULT
	A	B	C	D	E	F	G	H	I	J	K	
1	+	+	-	+	+	+	-	-	-	+	-	99.3
2	-	+	+	-	+	+	+	-	-	-	+	101.5
3	+	-	+	+	-	+	+	+	-	-	-	100.4
4	-	+	-	+	+	-	+	+	+	-	-	97.9
5	-	-	+	-	+	+	-	+	+	+	-	98.5
6	-	-	-	+	-	+	+	-	+	+	+	99.0
7	+	-	-	-	+	-	+	+	-	+	+	98.8
8	+	+	-	-	-	+	-	+	+	-	+	99.9
9	+	+	+	-	-	-	+	-	+	+	-	100.6
10	-	+	+	+	-	-	-	+	-	+	+	98.9
11	+	-	+	+	+	-	-	-	+	-	+	97.9
12	-	-	-	-	-	-	-	-	-	-	-	100.9

The (+) or (-) signs are used as variables in the 12 run design. Assign (-) to Analyst I ; Day I; Column I and (+) to Analyst II; Day II; Column II and so on...

A to K are chosen as the external (ruggedness) / internal (robustness) variations anticipated during the transfer from the R&D to QC laboratory.

The assay results are entered into the table on completion of the 12 HPLC assay analyses.

Table 4.

Template for Ranked Effect and Means

External & Internal changes/variations	Ranked Effects	M values
A- R&D & QC Lab		-1.59
F- Day I & II		-1.06
G- Analyst I & II		-0.73
E- Analyst III & IV		-0.46
B- Reagents I & II		-0.22
H- [Solvent] I & II		+0.00
C- Heating Rate I & II		+0.22
J - Column I & II		+0.46
K- Temperature I & II		+0.73
I - Flow rate I & II		+1.06
D- Elapsed time I & II		+1.59

The M values are obtained from statistical design tables. The Ranked Effects are calculated by simple addition of (-) (+) assay test results and then dividing by half the number of runs (i.e. divide by 6 in a 12 run design).

The effects from A - K were selected as the most significant variables between the two labs. Templates are available for up to 100 run variables (100 x 99).

Method Procedure.

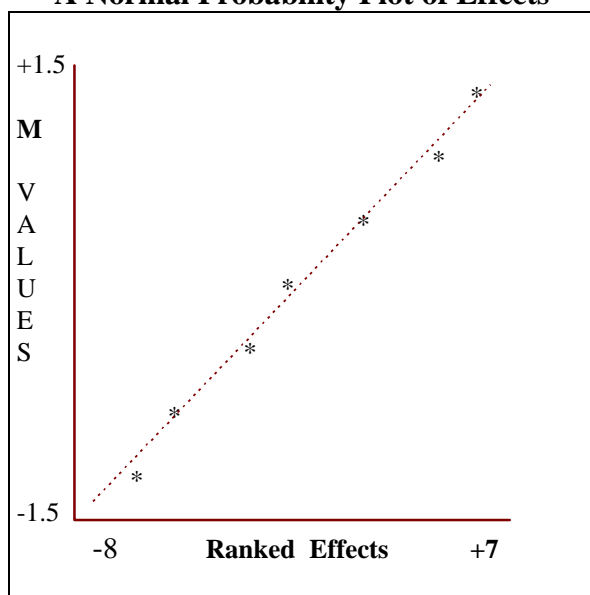
1. Choose the number of variables required and select a run design template.
2. Assigning the minus (-) or plus (+) values: These are arbitrary designations. As a standard rule assign a 'minus' (-) to **I** or a lower limit and a 'plus' (+) to **II** or an upper limit. Evaluate a range limit by assign (-) value for lower and (+) value for higher (i.e. Flow rate 1.2 mL/min assign (-) and 1.8 mL/min assign (+)). Likewise Day I assign (-) and Day II assign (+) and so on...
3. Perform the HPLC assays in a random order.
4. Tabulate the assay results in the template.
5. Calculate the Effects (Figures 1 and 2).
6. Rank the Effects from smallest to largest.
7. Plot the Effects against the M values.
8. Evaluate the plot.

Conclusion.

The results from the plot form a near straight line. It can be concluded that the analytical method is (a) rugged for the *external* factors over the tested range and (b) robust for the *internal* factors over the tested range in the 12 run design.

Figure 3.

A Normal Probability Plot of Effects



Process Qualification Stage.

The evaluation of ruggedness and robustness should be finalised at the end of the development phase - around the time of the process qualification lot manufacture.

The ruggedness/robustness evaluation should be developed with the commercial laboratory equipment in mind. It should show the reliability of an analysis with respect to deliberate variations in the method parameters.

Ruggedness/robustness determinations are essential when transferring analytical methods from the development laboratory to the commercial plant quality control laboratory. There may usually be a difference in columns or HPLC machine models used.

A consequence of ruggedness / robustness evaluation is that a series of system suitability parameters are established to ensure that the validity of the analytical procedure is maintained whenever used.

References:

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6. ASTM Standard Guide For Conducting Ruggedness Tests E1169 American Society for testing Materials Philadelphia 1989.
7. Kateman and L. Buydens, *The Ruggedness Test Quality Control in the Analytical chemistry* John Wiley and Sons NY 2nd Edition 1993, pp118 125.