

## ROBUSTNESS TESTING

Robustness is the capacity of a method to remain unaffected by small, *deliberate* variations in method parameters; it is a measurement of the reliability of a method. In robustness, you challenge your method by varying certain method parameters, with the intention of determining which parameters, or factors, must be tightly controlled when running your method on a routine basis. Investing in a thorough and properly designed robustness study can help ensure successful method implementation and transfer down the road. Investing a little time up-front can save a lot of time, energy, and expense later.

In liquid chromatography, examples of typical robustness factors are:

- Mobile phase composition
- Number, type, and proportion of organic solvents
- Buffer composition and concentration
- pH of the mobile phase
- Temperature
- Flow rate
- Wavelength
- Gradient variations
- Hold times
- Slope
- Length

Factors that are varied as part of a robustness study are typically parameters that are specified in the method itself. If any of these factors cause variability in the resulting data, this information is typically documented in the method procedure so that the analyst knows to take measures to tightly control these factors, thereby ensuring that consistent results are obtained every time the analysis is run.

### Design of experiment (DOE)

DOE is the use of factorial experiments where multiple factor variations can be combined together in a single chromatographic run instead of the vary one-factor-at-a-time approach which has historically been popular. Performing experiments in the latter manner most likely resulted from being trained as scientists (one variable at a time) as opposed to a statistician, which also allows for simplistic data reduction. However, this approach is time consuming due to the necessity of large numbers of chromatographic runs. Additionally, possible interactions between factors such as pH changes, temperature, or ionic strength, remain undetected.

The DOE approach allows for the acquisition of a minimal amount of chromatographic runs, thus saving on the amount of sample, analyst time, instrument time, and solvent waste disposal. Furthermore, this approach allows for a full statistical data analysis, providing much more comprehensive information including the determination of factor interactions.

Method Validation Manager allows the use of the DOE approach to robustness without the necessity for a resident statistician or the use of a third-party statistical software which would need to be learned, validated, and maintained. Unlike third-party software, the Empower 2 CDS has the advantage that the data is traceable, secure, and audit trailed.

There are different types of experimental designs available. In a full factorial experiment, all possible combinations of factors are measured. A common full factorial design is one with all factors set at two levels each, a high and low value. If there are  $k$  factors, each at two levels, a full factorial design then has  $2^k$  runs. In other words, using four factors, there would be 24 or 16 design points or runs. To further illustrate the point, Figure 1 shows a full factorial design robustness study for four factors; pH, flow, wavelength, and percent organic in the mobile phase.

The screenshot shows a software window titled "Robustness Parameters" with three tabs: "Acquisition", "Experiment Design", and "Processing and Acceptance Criteria". The "Experiment Design" tab is active, displaying an "Experiment Design Table" with 16 rows and 5 columns. The columns are labeled "pH", "FlowRate", "Wavelength", and "PercentOrganic". The rows represent 16 different combinations of factor levels. At the bottom of the window, there are radio buttons for "Standard Experiment Plan:" (selected) and "(+) (-) 1 Experiment Plan:". Buttons for "OK", "Cancel", and "Help" are located at the bottom right.

	pH	FlowRate	Wavelength	PercentOrganic
1	9.3	.7	236	38
2	9.3	.7	236	42
3	9.3	.7	240	38
4	9.3	.7	240	42
5	9.3	.9	236	38
6	9.3	.9	236	42
7	9.3	.9	240	38
8	9.3	.9	240	42
9	9.7	.7	236	38
10	9.7	.7	236	42
11	9.7	.7	240	38
12	9.7	.7	240	42
13	9.7	.9	236	38
14	9.7	.9	236	42
15	9.7	.9	240	38
16	9.7	.9	240	42

Figure 1. Full factorial design of experiment using four factors.

Full factorial design runs can really start to add up when investigating large number of factors; for nine factors, 512 runs would be needed, without even taking into account replicate injections. In addition, the design presented in Figure 1 assumes linear responses between factors. In many cases, curvature is possible, necessitating center point runs (runs at the nominal conditions) further increasing the number of injections. For this reason, fractional factorial designs are commonly used for robustness studies incorporating more than five factors.

A fractional factorial DOE is a statistically chosen fraction or subset of the total factor combinations. In the example above, with nine factors resulting in 512 runs for a full factorial design, fractional factorial designs can accomplish the same evaluation in as little as 32 runs.

The available experimental design types in MVM are as follows: Full Factorial, 1/2 Factorial, 1/4 Factorial, 1/8 Factorial, 1/16 Factorial, 1/32 Factorial, and Plackett Burman. These designs provide main effect information as well as some 2-factor interaction effects. These designs allow for up to nine different factors to be varied in a robustness test with a maximum of only 32 experiments required.

### Robustness results

Creating robustness results in MVM is as easy as clicking a button. The resulting data is presented in a logical and interactive manner in both tabular and plot format. Effects plots and percent variance plots are particularly useful in robustness data assessment.

### Percent variance plot

Similar to a bar chart, or histogram, the Variance plot shows the factors and factor interactions on the Y axis plotted against percent variance on the X axis. The percent variance looks at the variability in your data due to each factor and factor combinations and compares this to the total variability in the data set, the sum of which is always 100%. An example percent variance plot is shown in Figure 2.

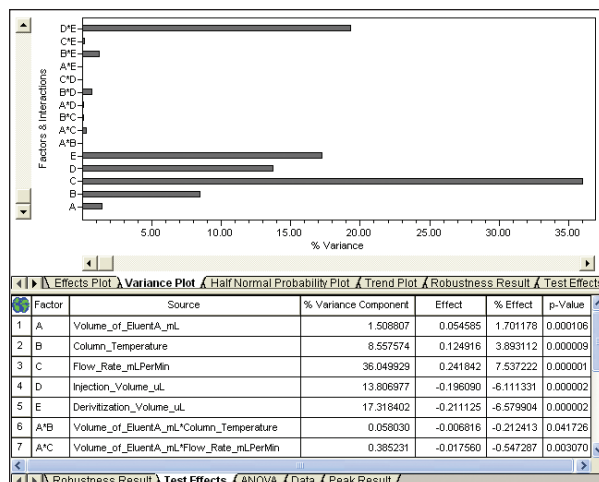


Figure 2. Percent variance plot.

### Effects plot

The effect is the change in a measured response due to the change of a factor. The effect value is one-half of the average response at the high level minus the average response at the low factor level. Like the percent variance plot, you can use this information to see which factors cause the greatest change or variation in your data. But rather than being based on a scale of 100, like the percent variance, the units of the effect value are the same as the value that you are measuring so it is easy to see the magnitude of the change in your data due to the change of a factor. Figure 3 displays an example effects plot.

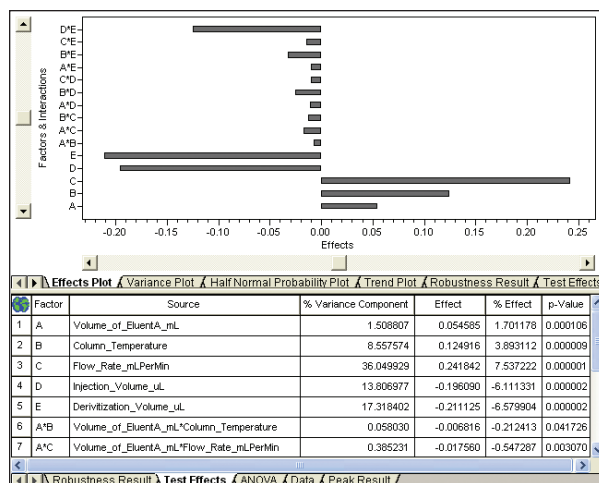


Figure 3. Robustness effects plot.

Effect data, provides an overview of not only what factors cause the greatest change in the response of your data, but also the magnitude of this change. If you are assessing retention time, the X axis for this plot would be in the units of minutes and you could easily determine how much your retention time changed relative to your factor changes. If you are assessing area, the X axis would be in area units and you could directly determine the area change in response to each factor and factor interaction change. The actual effect value is shown in the test effects table.

Empower 2 Method Validation Manager software provides an efficient, statistically sound approach to robustness testing. This capability is provided directly within the Empower 2 software, eliminating the need for third-party statistical software and the associated concerns while providing all of the security and compliance benefits of the Empower 2 CDS.

## CONCLUSION

The typical process used for validating chromatographic methods is time-consuming, error-prone, and riddled with compliance concerns; it can decrease laboratory productivity so much that the time and costs associated with bringing products to market are significantly increased. Method Validation Manager is a business-critical software that reduces the time and costs required to perform chromatographic method validation by as much as 80%.

Because Method Validation Manager allows the entire chromatographic method validation process to be efficiently performed within Empower 2, fewer software applications need be deployed, validated, and maintained. Software training and support is also minimized. When less software is required, the software that is business-essential can be deployed more quickly and efficiently. In addition, Method Validation Manager allows you to be fully compliant with governmental regulations by providing data security, a full set of user privileges, audit trails, and automatic data documentation; providing you with the necessary information and complete data traceability required for final reports and to pass audits and data reviews.

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