STREAMLINE THE CHROMATOGRAPHIC METHOD VALIDATION PROCESS USING EMPOWER 2 METHOD VALIDATION MANAGER
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INTRODUCTION

Chromatographic method validation is a critical step in the workflow of pharmaceutical, food safety, chemical, and environmental laboratories that can adversely impact regulatory compliance, product development, and ultimately product release and availability. The current process of validating chromatographic methods is time-consuming and prone to errors, which can decrease laboratory productivity and increase the time and costs associated with bringing new and existing products to market.

Waters® Empower™ 2 Method Validation Manager (MVM) software not only automates the chromatographic method validation workflow but also addresses the limitations and bottlenecks faced with the typical chromatographic method validation process. By streamlining the method validation workflow, Method Validation Manager software offers up to an 80% reduction in the time and cost associated with the method validation process.

The typical validation process in use today requires the use of a variety of disparate software packages and is riddled with inefficiencies. MVM allows the entire chromatographic method validation process—from protocol planning through data acquisition, data processing, data review and approval, as well as final reporting—to be efficiently performed within Empower 2. So many of the manual and error prone steps present in the typical process—exporting data to additional software applications, checking for transcription errors, verifying data placement and calculation syntax, data review and approval, and final data reporting—are either streamlined or eliminated altogether when using MVM. Ultimately, this results in many significant business benefits, including a lower total cost of ownership.

- Fewer software applications need be deployed, validated, and maintained.
- The number of software applications on which users need to be trained is minimized.
- Ongoing software support is minimized.
- Software can be deployed more quickly and efficiently.
- Allows organization to reduce costs and focus the efforts of scientists on analyses.

Because Method Validation Manager is built on Empower 2, it is compliant-ready software and provides the tools that allow you to easily satisfy compliance requirements and efficiently locate information requested in an audit.

- MVM automatically tracks and manages your validation data for complete traceability and data mining.
- Validation calculations and statistics are structurally validated and performed within Empower.
- All data is securely stored in the Empower 2 database; no flat files are used.

This paper explores how the challenges encountered in the typical chromatographic method validation process are addressed by Empower 2 Method Validation Manager and how its advanced functionality translates to distinct operational advantages for the laboratories that employ the software.
USING EMPOWER 2 METHOD VALIDATION MANAGER TO OPTIMIZE YOUR CHROMATOGRAPHIC METHOD VALIDATION WORKFLOW

MVM allows you to streamline your chromatographic method validation process. When using Empower 2, the entire method validation process can be performed within the Validation Manager window.

Streamline the chromatographic method validation process

MVM provides all the tools that are required to perform and manage your method validation workflow completely from start to finish – from validation planning through data acquisition, processing and reporting in one comprehensive, automated application.

MVM clearly displays the status of each validation test in a validation study. Whether you need to acquire data, process data, or approve data, you can see at a glance what you need to do next. Hence, you don’t need to manually document and continually refer back to your laboratory notebook. Simply look at the Test Status field and the software tells you what you need to do next.

Throughout the entire method validation process, MVM continually monitors the data that you are using for each validation test and tells you whether your data adheres to your validation requirements. An ✔ or ✗ indicates that your data does or does not adhere to your validation requirement, respectively. A ⚠ indicates that your results are out of specification. With MVM, you have assurance that the data used for each validation test adheres to the requirements you have specified in your corporate validation SOP.

MVM allows you to quickly process your validation results – there is no complicated configuration at this step. Structurally validated results are calculated with the simple click of a button. This is an astronomical time savings when compared to the use of spreadsheets to perform these calculations.

Sign-offs and approvals can be required and provide you with checks in the method validation workflow. These show that someone has reviewed the data and then entered their username and password, as an indication of their approval or e-signature. This enforces the workflow because if a sign-off or approval is required, MVM will not allow the next step in the process to be performed until the sign-off or approval has taken place.

MVM provides the data management, documentation, and traceability so the analyst can work on other value added tasks.

Throughout the entire method validation process, Empower 2 Method Validation Manager monitors and tracks all of your method validation activity. MVM tells you what activity you need to do next. MVM tells you if your data adheres to your validation requirements. MVM tells you if your results are out of specification. All of this while providing you documentation and traceability in a compliant-ready environment.
When performing chromatographic method validation, the potential exists to minimize the number of chromatographic injections required and still maintain result quality and integrity while also providing automatic traceability of your validation data.

This can be readily accomplished with Method Validation Manager by using chromatographic injections in a sample set for multiple validation tests. For example, it is commonly desired to use some of the same injection data for linearity, accuracy and repeatability testing. The benefits of acquiring sample sets as validation test composites are obvious; efficient acquisition yields savings of the following:

- Amount of sample consumed
- Sample preparation time
- Instrument usage time
- Volume of solvent waste disposal

However, the method validation workflow in use today does not typically promote this efficiency because it results in difficulty of data documentation and traceability. Consider that the method validation workflow typically includes the use of third-party spreadsheets or statistical software. When this approach is used, the chromatographic result data must be transferred from the chromatographic data system (CDS) to a spreadsheet. Because each validation test; accuracy, linearity, robustness, specificity, and so on, requires a different set of validation calculations, the chromatographic data pertaining to each validation test is typically transferred individually to the proper spreadsheet independent from the other data for the other validation tests.

**Steps in method validation workflow**

1. Within the sample sequence(s), locate the correct chromatographic results pertaining to a particular validation test.
2. Accurately transfer the result data to the region of the spreadsheet that is appropriate for that validation test.
3. Confirm the data transfer and placement on the spreadsheet.
4. Document the source and location of raw data, result data, and metadata pertaining to the validation test.
5. Repeat steps 1-4 for each validation test.

**There is no longer a choice between efficient acquisition and efficient documentation and traceability**

It is much easier for the analyst to maintain discrete sample sequences for each validation test as opposed to the validation test composite approach because he can transfer each set of data in its entirety, rather than transfer a subset of the data for each validation test. Ultimately, acquisition efficiency is replaced in lieu of the efficiency of data transfer, data processing, and data documentation.

When using Empower 2 MVM, there is no need to make a choice between these trade-offs. During the creation of a sample set, the user has the ability to assign injections to each validation test.

In this manner, the software is aware of which injections pertain to which validation tests and not only automatically uses the appropriate data when performing the calculations specific to each validation test, but also maintains full traceability of this data.

Figure 1 displays an example of a validation test composite where injections from within one sample set will be used for a variety of validation tests.

**Automatic traceability**

The user is responsible for specifying which injections pertain to each validation test. Beyond that, MVM takes full control. MVM performs the required validation calculations while completely and automatically maintaining traceability of all related data so the user can easily trace a validation calculation back to its raw data (Could there be an outlier?), to its acquisition parameters (Was the column temperature set correctly?), to its system information (What system was used? Had it just been serviced? Was it due for service?), to its user information (Was the user that acquired the data properly trained on this procedure?), to custom information (What Batch Number was used?) and so on.
ELIMINATE THE USE OF SPREADSHEETS DURING METHOD VALIDATION USING
EMPOWER 2 METHOD VALIDATION MANAGER

Analytical method validation is the process of establishing through experimentation that a method is suitable for its intended use. It is an important regulatory requirement for pharmaceutical and other industries as it provides documented evidence and assurance that the methods in use are suitable for the determination of identity, quality, strength, purity, and potency of their products.

The validation process can be a time-consuming and repetitive task, consisting of several sequential steps. These steps include, planning (protocol generation), sample preparation and experiment setup, data acquisition, calculation of results (both chromatographic and validation results), and report generation. Of these, one of the most time-consuming, tedious, and error-prone steps is that of calculating validation results. Currently, this step is typically accomplished by importing the chromatographic results generated by the CDS to a third-party software package such as Microsoft Excel or SAS’s JMP Statistical software.

Shortcomings of typical approach

The approach described above is problematic for the following reasons:


- Method validation requires the calculation of many different validation results; thus the development of the many formulas on multiple spreadsheets is a daunting task.
- Formulas must be validated to ensure they are correctly created and produce correct results.
- Spreadsheets pose data integrity and security concerns in a compliant environment.
- The burden of spreadsheet lifecycle maintenance is upon the user.
- Every time a method is validated, it is required to verify that the data transfer from the CDS to the spreadsheets is free of transcription errors. This is typically done in duplicate by the user and an additional peer review.
- In producing the final report, data must be tediously configured and compiled using various software applications including MS Word, MS Excel, and the CDS.
- Data mining becomes unmanageable because there is no traceability between the different software applications used.

The routine use of spreadsheets for validation becomes a management headache. With Method Validation Manager software, all of these concerns and manual step are completely eliminated. MVM allows you to perform the chromatographic method validation process completely from protocol planning and data acquisition through reporting, all within Empower 2. All validation calculations are performed within the Empower 2 CDS and thus are structurally validated, secure, audit trailed, and traceable.

Data management

There are a total of eleven possible validation tests that may need to be assessed during method validation:

- Linearity and range
- Accuracy
- Specificity
- LOD and LOQ
- Repeatability
- Intermediate precision
- Reproducibility
- System precision
- Robustness
- Stability

Each of these tests requires a different number of sample preparations and injections. Many validation tests include the variation of factors such as analyst, column, day, laboratory, instrument, pH, temperature, flow rate, and so on. Some validation tests may require multiple sample sets to be acquired. Additionally, a subset of the injections used for one test may be also used for another test, either alone, or in conjunction with other injections. All of this adds up to a total of tens or even hundreds of injections that need to be managed and ultimately processed to determine the validation-specific calculations. Moreover, the calculations necessary for each validation test are unique. For example, the calculations for a linearity test are different than those necessary for a robustness or accuracy test.
A better way to search, confirm, and document your data

The analyst must exercise great care when transferring data from the chromatography data software to the spreadsheet. Assuming the spreadsheet calculations have been previously validated, the analyst needs an efficient way to:

- Find the correct chromatographic data for each validation test independent from the other validation test data.
- Accurately transfer each validation test’s data to the correct region of the appropriate spreadsheet.
- Confirm the data transfer and placement on the spreadsheet.
- Confirm whether results are within specification.
- Transfer data from spreadsheet to software application to be used for final report.
- Document the source and location of raw data, result data, and meta data pertaining to the validation test.

ASSURED REGULATORY COMPLIANCE WITH EMPower 2 METHOD VALIDATION MANAGER

Regulatory compliance challenges

The current chromatographic method validation process in use today is riddled with inefficiencies and regulatory compliance concerns. Using MVM provides confidence that your method validation process is streamlined and meets all compliance regulations.

Managing regulatory compliance and data security are some of your lab’s biggest challenges. Empower 2 software was engineered to maintain security, data integrity and compliance. Empower 2 is a compliant-ready software solution that allows you to configure the system to comply with GxP and 21 CFR Part 11 regulations. Multi-level security is provided for every job – from assigning user access privileges, automated log-outs due to inactivity, expired passwords, as well as and the ability to limit the number of password entry attempts to establishing audit trails and regulating project access. Empower 2 software ensures the utmost integrity for all of your data.

Method Validation Manager assurance

Since Method Validation Manager is powered by Empower 2, MVM contains the same regulatory and compliant-ready benefits:

- Complete traceability of all validation data – result, raw data, methods, and all meta data.
- All data is stored in the embedded Oracle relational database which provides security and data integrity.
- Audit trails capture all chromatographic and method validation activity.
- A full set of user privileges control both chromatographic and method validation activities, as well as data access.
- Corporate method validation requirements can be approved and locked.
- All calculations are performed within Empower 2 and are structurally validated by Waters.
- No flat files are used.
- No third-party software is required to manage, maintain, or validate.
- Data traceability is automatically maintained, so the user isn’t burdened with this error-prone task.
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.

![Figure 1. Full factorial design of experiment using four factors.](image)
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.

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.
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.