

Automating HPLC and GC Analytical Method Validation

Patrick Lukulay and Richard Verseput



DR. PATRICK LUKULAY, SR. PRINCIPAL SCIENTIST, PFIZER INC.

The chromatographic analytical method validation process involves a series of activities that are currently conducted in separate “technology islands” using tools that exist for each activity. **This article describes a software program that provides an overarching automation technology for analytical method validation and brings together the individual activities under one integrated-technology platform that is adapted to multiple instruments and data systems.**

Patrick Lukulay, PhD, is the Analytical R&D Group Leader at Pfizer Global R&D (Ann Arbor, MI). **Richard Verseput** is President of S-Matrix Corporation (Eureka, CA), tel. 707.441.0405, fax 707.441.0411, richard.verseput@smatrix.com.

Method validation activities encompass the planning and experimental work involved in verifying the fitness of an analytical method for its intended use. These activities often are captured in company standard operating procedure (SOP) documents that usually incorporate US Food and Drug Administration and International Conference on Harmonization (ICH) requirements and guidances (1–3). Method validation SOP documents describe all aspects of the method validation work for each experiment type (e.g., accuracy and linearity) within a framework of three general execution sequence steps: experimental plan, instrumental procedures, and analysis and reporting of results. The individual elements within these three general steps are detailed in the following paragraphs.

Step 1: experimental plan. An experimental plan should specify analyte concentrations, instrument parameters, and environmental parameters. The plan also should include the number of levels per variable, the number of preparation replicates per sample, and the number of injections per preparation replicate. An integration of standards, inclusion of system suitability injections, and the acceptance criteria also are part of the plan.

Step 2: instrumental procedures. The instrumental procedures step involves making the required transformations of the experiment plan into the native file or data formats of the instrument’s controlling chromatography data system (CDS) software (construction of sample sets and method sets or sequence and methods files). In addition, the following are specified: the number of injections (rows), the specific type of each injection (e.g., sample or standard), and the required modifications to the analytical method (robustness).

Step 3: analysis and reporting of results. This step includes analysis calculations, report content and format, comparisons to acceptance criteria (FDA and ICH requirements), and graphs or plots that should accompany the analysis.

Method validation technology platforms

The execution steps in method validation activities generally involve manual operations carried out on unconnected technology platforms. The method validation chemist works in what are essentially isolated technology islands with manual operations providing the only bridges.

To illustrate, an SOP guidance of ten is an electronic document in MS Word. The experimental plan (step 1) within the SOP guidance must be transferred to the high performance liquid chromatography (HPLC) or gas chromatography (GC) instrument for execution (step 2) by manually rekeying the experiment into the instrument's controlling CDS software. In a few cases, the statistical analysis of results (step 3a) can be conducted within the CDS, but it is most often performed within a separate statistical analysis software package or spreadsheet program such as MS Excel. This process also requires manually transferring the results data from the CDS to the analysis software package. Reporting of results (Step 3b) is usually carried out in MS Word and therefore requires the manual transfer of all results, tables, and graphs from the separate statistical analysis software package. The manual operations within the three general execution sequence steps are described in the following steps, and the isolated technology islands are illustrated in Figure 1.

Step 1: experimental plan. The validation plan is developed in MS Word, and the experimental design protocol is developed in off-line DOE software.

Step 2: instrumental procedures. Sequences or sample sets are manually built in the CDS and the raw peak (x, y) data reduction calculations are performed by the CDS (e.g., peak area and concentration).

Step 3a: statistical analysis. Calculated results are manually transferred from the CDS to MS Excel. Statistical analysis usually is carried out manually in MS Excel. Some graphs are generated manually in MS Excel, and some are obtained from the CDS.

Step 3b: Reporting results. Reports are manually constructed from templated documents in MS Word. Graphs and plots are manually integrated into a report document.

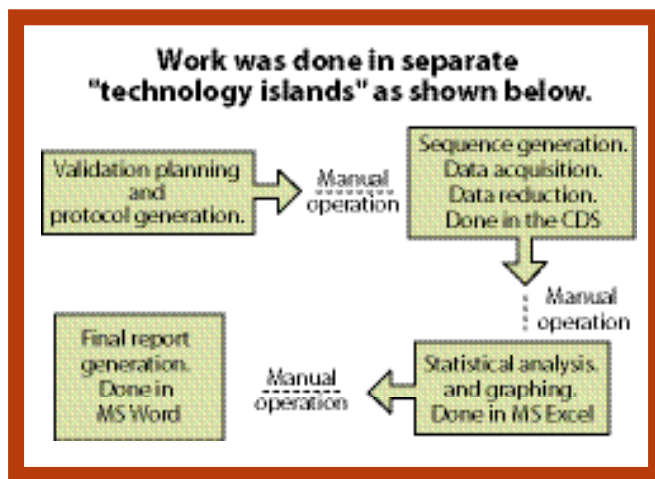


Figure 1: Methods validation: isolated technology islands.

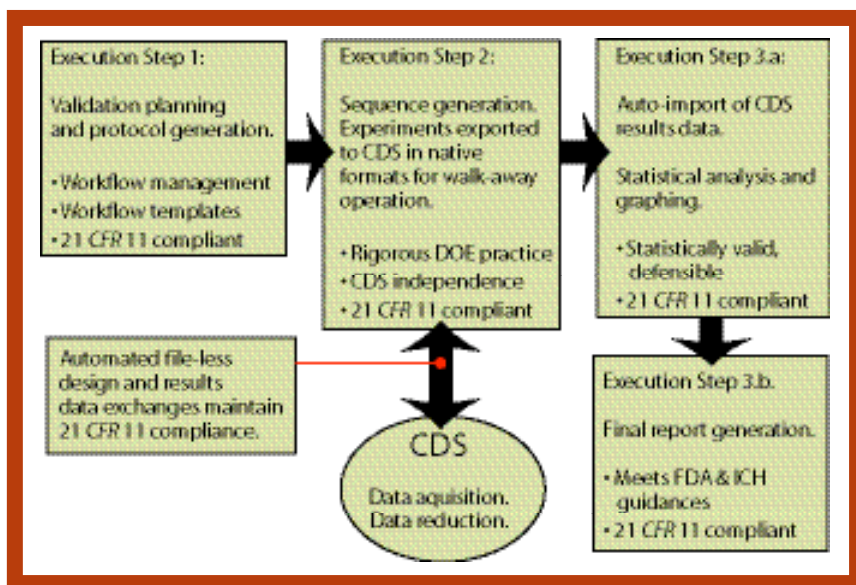


Figure 2: Integrating the technology islands.

Strategic Validation Technology Initiative program technology goals

The overall goal of the Strategic Validation Technology Initiative (SVTI) program was to fully automate the analytical method validation work, which required integrating the isolated technology islands. The SVTI team clearly understood that adopting the final software program into standard use required minimizing the drudge work. Therefore, successful technology transfer hinged on the automation goal. The two most critical and challenging technical elements of the automation effort were automating the data exchange between the off-line design of experiments (DOE) software and the CDS and making the data exchange technology generic and adaptable to the targeted data systems. Important target instrument platforms were controlled by various instrument data systems. Each target CDS had a different data architecture and different functionality within its respective software development kit (SDK, third-party software development interface). Without a generalized technology, data exchange would be limited to a single CDS. SOPs,

therefore, would have to be manually adapted to instruments controlled by different data systems. Also, the SVTI software would not be able to automatically address instrument configuration differences to allow for the creation and dissemination of workflow automation templates.

The SVTI project also was required to address the following related analytical R&D technology development goals:

- Implement easy setup of DOE-based experiments and facilitate statistically rigorous practice.
- Establish 21 CFR 11 compliance support toolset and help maintain compliance across integrated platforms;
- Develop method connectivity. Early methods developed manually or using other software tools should be able to be optimized and validated using the new software.
- Generate final reports that are simple to review, communicate, and defend.
- Establish standardized reporting. Report form and content should be independent of the specific instrument, CDS, and facility. Reports should meet all FDA and ICH guidelines.

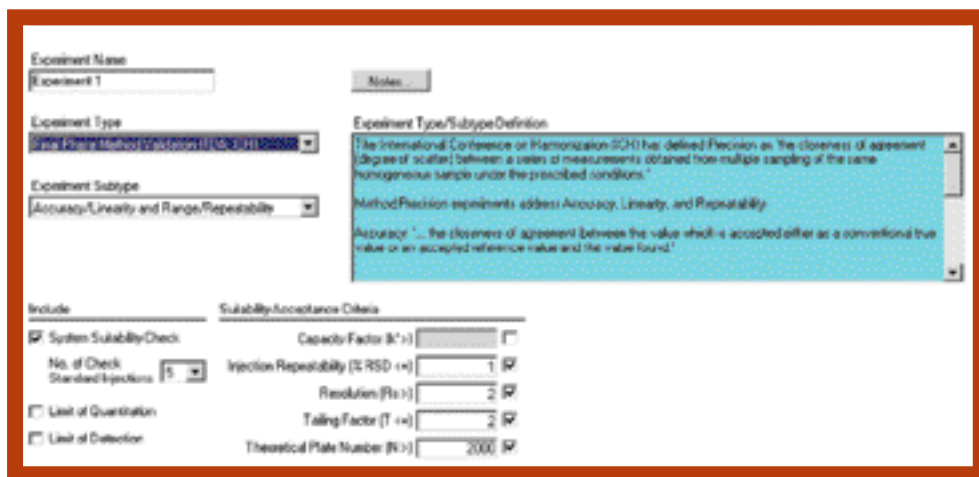


Figure 3: An example of custom software interface.

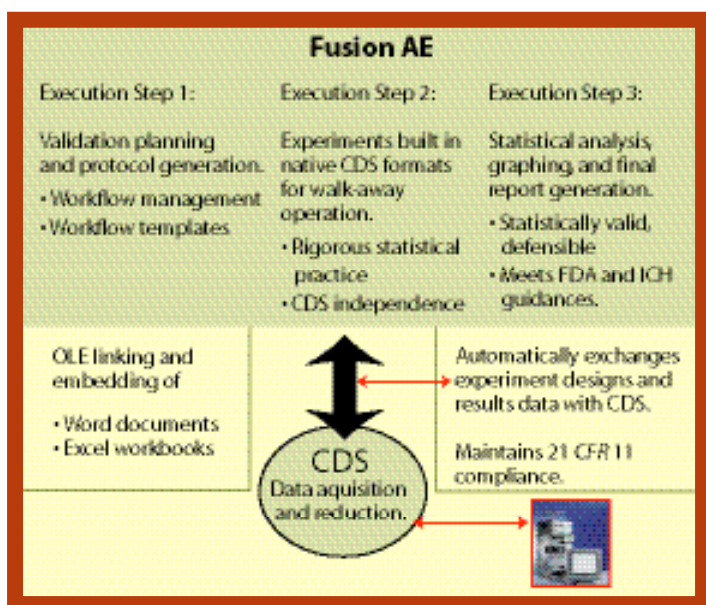


Figure 4: An automated software solution.

The main technology integration goals and the related technology development goals are illustrated in Figure 2.

SVTI program: product feature requirements

The SVTI program goal was the development of a central software environment for all analytical methods validation work. To facilitate acceptance and widespread use, the resulting software program was required to include four specific feature sets: a custom user interface specific to method validation experimentation, a phased approach to method validation, the FDA and ICH required complement of method validation experiments, and a full complement of automation support features.

Custom user interface. The custom user interface required of method validation software is illustrated in Figure 3. An experiment setup window contains controls for incorporating system suitability check standard injections into the design and defines acceptance criteria for evaluating suitability results such as peak capacity factor (k') and peak resolution (R_s).

Phased approach to method validation. The Pharmaceutical Re-

search and Manufacturers of America's (PhRMA's) analytical technical group recommends a phased approach to analytical method validation in which early-phase validation efforts are done upstream on a reduced set of validation elements appropriate to the stage of development(4). This process involves method performance characterization experiments to define the "validatability" of the current method. The need for this is obvious when one considers that analytical methods are being used in drug discovery

and development well before the point at which final validation is usually conducted.

SVTI software development addressed the need for and value of a phased approach in terms of both experiment organization and experiment structure. Method validation experiments were partitioned into early phase (characterization) and final phase (FDA and ICH submittal quality). Some experiments are contained within both phases (e.g., accuracy and linearity). In these cases, the software default settings in terms of number of sample preparation replicates, number of injections per preparation replicate, number of concentration levels, and so forth will result in smaller experiments with less time and resource burden in the early phase, while the final-phase counterpart has the defaults set to those defined in the FDA and ICH guidances.

Required complement of validation experiments. The complement of method validation experiments built into the Fusion AE software (S Matrix) is categorized as follows.

Early-phase method validation (characterization). Early-phase validation includes system suitability; filter validation; accuracy; linearity and range; repeatability (intra-assay precision); and sample solution stability (stability for a given time period under prescribed conditions). Repeatability is affected by both sample preparation error and instrument error (injection precision). Therefore, to demonstrate the repeatability of the method as documented, all repeatability experiments were required to include sample preparation replicates.

Final-phase method validation (FDA and ICH submittal quality). Final-phase validation includes system suitability; accuracy–linearity and range–repeatability combined design (ICH Q2A states that accuracy, linearity, and repeatability can be performed together as a single combined experiment); robustness; ruggedness (intermediate precision and reproducibility); and specificity.

Full complement of automation support features. The central software environment developed under the SVTI program required many custom support features to fully enable and automate the method validation experiment design suite just described. The required support features naturally group into five feature sets: assay types, compounds, analysis and reporting, acceptance criteria testing, and workflow management.

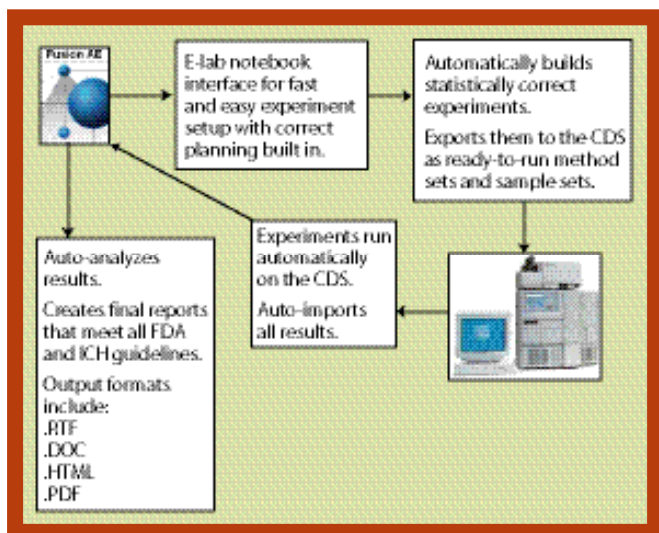


Figure 5: Automated method validation workflow.

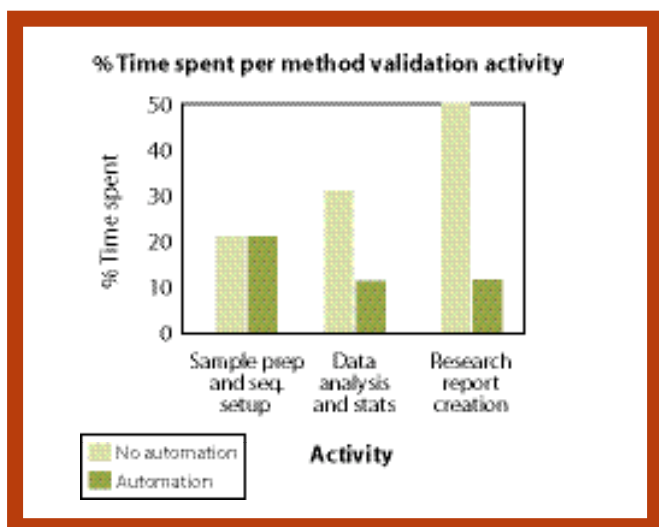


Figure 6: Efficiency gained by automation.

Assay types. The assay types feature set addressed the four main assays routinely addressed in method validation. Component features included potency (drug content), content uniformity, dissolution, and determination of impurities.

Compounds. The compounds feature set allowed multiple compounds (active ingredients or impurities) to be included in the same experiment (must accommodate as many as 10 active ingredients or impurities).

Analysis and reporting. The analysis and reporting feature set provided the statistical analysis and graphing results reports required by the FDA and ICH guidances. Component features included automated analysis (one-button click); a automated graphics created as part of a automated analysis; and automated report construction to meet all FDA and ICH guidances.

Acceptance criteria testing (user defined value = X). The acceptance criteria testing feature set enabled the analysis and reporting feature set to automatically compare actual results with predefined pass-fail acceptance criteria and report the results of the comparisons. Component features included

- filter validation: % bias limits ($\pm X$)
- accuracy: % bias ($< X$)
- linearity and range: % bias ($< X$)
- repeatability: %RSD ($\leq X$)
- sample solution stability: % recovery limits ($\pm X$)
- robustness: % effect ($< X$)
- ruggedness: % effect ($< X$); intermediate precision %RSD ($\leq X$); and reproducibility %RSD ($\leq X$)
- specificity: difference of practical significance ($\leq X$).

Workflow management. The workflow management feature set enabled construction of work templates, software-based administration, and control of the work. Component features included an ability to create and distribute workflow templates; an ability to control feature access with user permissions and a authorities settings; and an ability to control workflow with review and approve e-signing control loops.

SVTI program: results

Fusion AE software development was carried out at the S-Matrix facility in Eureka, California. Project management and benchmarking were carried out at Pfizer's facility in Ann Arbor, Michigan. Benchmarking involved using the software to conduct "live" method validation experiments in the walk-away mode with full instrument control and automated data exchange with the CDS. The work culminated in the delivery of a commercial release of the Fusion AE software program that met all SVTI project requirements. The Fusion AE software solution is illustrated in Figure 4. The corresponding automated method validation workflow is illustrated in Figure 5. Notable features of the software program include the following:

- a central software environment for all analytical method validation work
- flexibility to support "method validity" studies done as part of method development
- transferable electronic template generator for work standardization
- management workflow control
- rigorous DOE methods and practice integration
- automated data exchange between the target technology islands
- 21 CFR 11 compliance support across all technology islands
- data exchange with the target instrument data systems: PerkinElmer "TotalChrom"; Varian "Galaxie"; and Waters "Millennium³²" and "Empower."

As final proof of project, a senior analytical chemist at Pfizer used the Fusion AE system to carry out all early-phase and final-phase method validation experiments (except robustness, which was done subsequently at a different lab) in the following seven work steps:

- Prepare a series of HPLC injection samples and standards containing two active compounds.
- Generate all experiment designs within Fusion AE.
- Use the automated data exchange feature to export the designs to the CDS as ready-to-run methods and sequences in the native file format of the CDS.
- Set up the HPLC (prepare the mobile phase reservoirs and load injection samples and standards into the autosampler).

Table I: Key project goals, challenges, and results.

Project goal	Principal challenge	Final result
User interface: easy setup of DOE-based experiments	A unique complement of on-screen user settings controls is needed to generate each of the required validation experiment designs	An intuitive, DOE-transparent interface that displays required design settings in logical order and layout for each experiment design type
Experiment design: transform DOE software generated designs into file and data formats of the target data systems	Lack of standardized nomenclature and settings structure for run type designations such as suitability, standard, sample, or unknown between target data systems	Ability to set all required run types within DOE designs exported for automatic execution by each of the target data systems
Data exchange: flexible data exchange adaptable to several target instruments and data systems	Lack of standardized data formats and instrument control structures within and between instruments and data systems	A dynamically updatable instrument control driver set that adapts a generic data exchange engine to a target instrument and data system
Regulatory compliance: maintaining 21 CFR 11 compliance support across multiple instrument data system software platforms	The instrument data system software platforms provided little or no programmatic access to their internal 21 CFR 11 support features	Use of compliant data tracking values in all data exchanges to support 21 CFR 11 compliance (e.g., data identity and audit trail) across the different software platforms

- Run all nine experiment design sequences on the HPLC in walk-away mode.
- Use the automated data exchange feature to import the results data sets from the CDS into Fusion AE.
- Use the automated analysis, graphing and reporting features to generate submittal-quality reports for all nine experiment designs that meet all FDA and ICH guidances.

The analyst began the proof-of-project work on a Thursday morning at 9:00 am. All work was completed by noon of the following day. Ruggedness testing was limited to analyst and day. The work took less than 12 hours of the analyst's time. Work records showed that, on average, the same amount of work—from SOP planning and experiment design construction to final reports—using manual approaches and existing tools took more than two weeks of an analyst's time. Thus the proof-of-project work represented an 85% reduction in time and effort ($[(12 \text{ h} / 80 \text{ h}) \times 100\%]$). Figure 6 illustrates a minimum expectation of the efficiency gain possible with the automated software solution in which only a few of the simpler experiments are required and the time required for manually carrying out the automated

operations (steps 2, 3, 5, 6, and 7) is minimized. As the figures show, under these circumstances the minimum efficiency gain is still at least 60% (20% gain in data analysis and 40% gain in report generation).

Conclusion

A project of this complexity presented several software development challenges in each of the four main software program elements: user interface, experiment design, data exchange, and regulatory compliance. The most critical project goal in each of the four main program elements is presented in Table I. The table also presents the principal technical challenge associated with accomplishing the goal as well as the result achieved at the conclusion of the project.

The final Fusion AE deliverable enables the transformation of written SOPS for all required analytical method validation experiments into transferable automated templates in a timely manner and with full CGMP compliance. It also allows harmonization of the work across multiple sites and can be extended to contract research organizations with full management control of all work. Moreover, the connectivity to multiple instrument data systems means that the work environment is transparent to the instrument and the CDS. This will enable greater flexibility in selecting instrument platforms as needs change and technology improves.

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