Automating HPLC and GC Analytical Method Validation

Fusion AE™ Software Program White Paper

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Introduction

Chromatographic analytical method validation is both scientifically necessary and required by the regulatory agencies overseeing pharmaceutical drug development to establish that such methods are fit for their intended purposes. Method validation is time consuming and resource intensive, and is often required as changes are made to synthetic processes, formulations, or regulations governing drug manufacture. The method validation process involves a series of activities that are currently conducted in separate "technology islands" using available tools appropriate to each activity. However, until now, no overarching automated technological solution existed that combined all these individual activities under a single integrated-technology platform adapted to multiple instruments and data systems. This white paper describes Fusion AE, a software program developed by S-Matrix Corporation that provides an overarching automation technology for analytical method validation.

Acronym Definitions:

21 CFR 11 – Title 21, Part 11, of the Congressional Federal Register
CDS – chromatography data system
DOE – design of experiments (also DOX)
FDA – U.S. Food and Drug Administration
GC – Gas Chromatography
HPLC – high performance liquid chromatography
ICH – International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
PhRMA – Pharmaceutical Research and Manufacturers of America
SDK – Software Development Kit (third-party software development interface)
SOP – Standard Operating Procedure

Figures:

Bibliography:

1. FDA – CDER (CMC 3). Reviewer Guidance. Validation of Chromatographic Methods. November, 1994.

2. ICH-Q2A - Guideline for Industry. Text on Validation of Analytical Procedures. March, 1995.

3. ICH-Q2B - Guideline for Industry. Q2B Validation of Analytical Procedures: Methodology. November, 1996.

4. Method Validation by Phase of Development. An Acceptable Analytical Practice, *Pharmaceutical Technology*, November, 2004.

5. Torbeck, Lynn, (2002), Complying with ICH and FDA Requirements for Assay Validation, Suffield Press, Evanston, IL.

Analytical Method Validation Activities

Method validation activities encompass the planning and experimental work involved in verifying the fitness of an analytical method for its intended use. These activities are often captured in company Standard Operating Procedure (SOP) documents that usually incorporate FDA and ICH requirements and guidances[1-3]. Method validation SOP documents include a description of all aspects of the method validation work for each experiment type (e.g. Accuracy, 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 presented below.

- **Step 1 - Experimental Plan**
  - Included variables:
    - analyte concentrations.
    - instrument parameters.
    - environmental parameters.
  - Number of levels per variable.
  - Number of preparation replicates per sample.
  - Number of injections per preparation replicate.
  - Integration of standards.
  - Inclusion of system suitability injections.
  - Acceptance criteria.

- **Step 2 - Instrumental Procedures**

  Required transformations of the experiment plan into the native file or data format of the instrument's controlling CDS software (construction of Sample Sets and Method Sets or Sequence and Method files).

  - Number of injections (rows).
  - Specific type of each injection (e.g. sample, standard).
  - Required modifications to the analytical method (Robustness).

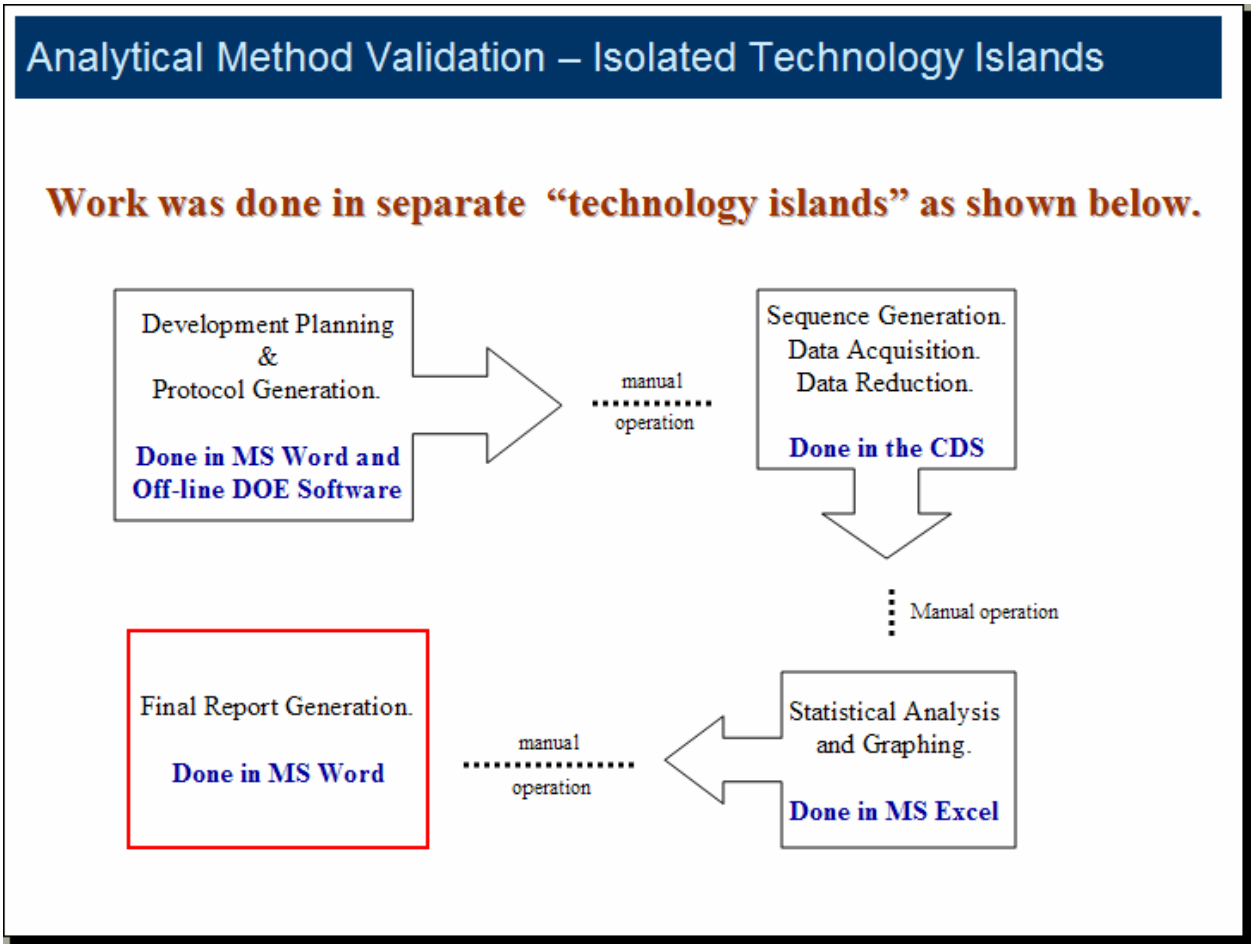
- **Step 3 - Analysis and Reporting of Results**
  - Analysis calculations and report content and format.
  - Comparisons to acceptance criteria (FDA & ICH Requirements).
  - Graphs or plots that should accompany the analysis.

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 is often an electronic document in MS Word format. The experimental plan (Step 1) within this SOP Guidance document has to be transferred to the HPLC or GC instrument for execution (Step 2) by manually re-keying the experiment into the instrument's controlling chromatography data system (CDS) software. In a few cases the statistical analysis of results (Step 3.a) can be done within the CDS, but it is most often done within a separate statistical analysis software package or spreadsheet program such as MS Excel. This also requires manually transferring the results data from the CDS to the statistical analysis software package. Reporting of results (Step 3.b) 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 presented below. The isolated technology islands are illustrated in Figure 1.

- **Step 1 - Experimental Plan**
  - Validation plan developed in MS Word.
  - Experimental design protocol developed in off-line DOE software.
- **Step 2 - Instrumental Procedures**
  - Manually build the Sequences or Sample Sets in the CDS.
  - Raw peak (x, y) data reduction calculations performed by the CDS (e.g. peak area, concentration).
- **Step 3.a - Statistical Analysis**
  - Calculated results manually transferred from the CDS to MS Excel.
  - Statistical analysis usually carried out manually in MS Excel.
  - Some graphs generated manually in MS Excel, some obtained from the CDS.
- **Step 3.b - Reporting of Results**
  - Reports manually constructed from template documents in MS Word.
  - Graphs and plots manually integrated into report document.

Figure 1. Method Validation – Isolated Technology Islands



Fusion AE – Technology Development Goals

The primary Fusion AE development goal was to fully automate the analytical method validation work. This required integrating the isolated technology islands identified above. The development team clearly understood that successful adoption of the final software program into standard use also required reducing or automating as many of the laborious routine and repetitive tasks associated with method validation as possible. Therefore, successful technology transfer hinged on fully realizing the automation goal. The two most critical and challenging technical elements of the automation effort were:

1. Automating data exchange between the off-line DOE software and the CDS.
2. Making the data exchange technology generic and extensible to multiple instruments and instrument data systems.

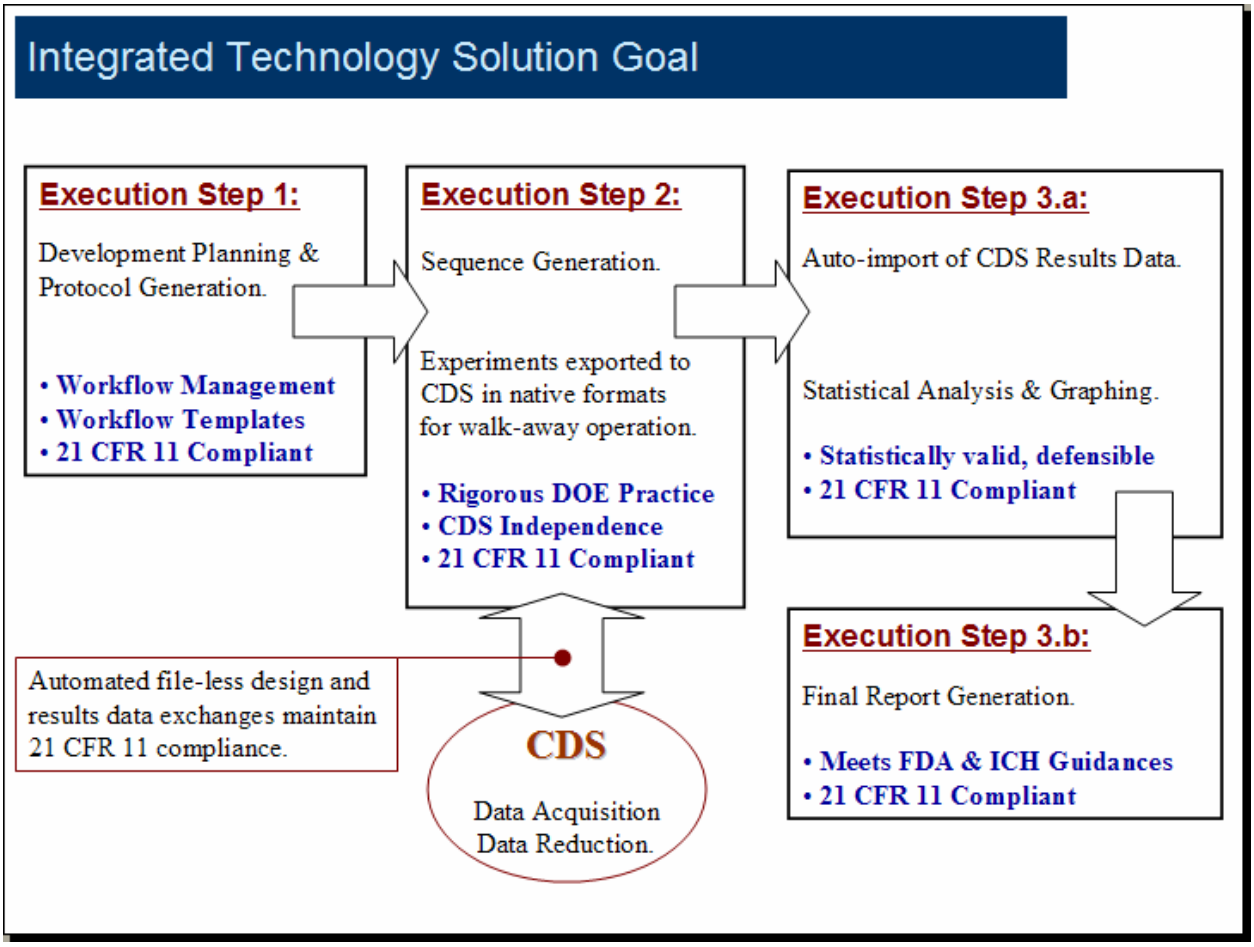
   Many instrument data systems can control multiple instruments from different vendors. Each instrument (e.g. HPLC System) often has different configurations and different levels of control for its complement of modules. Also, Each CDS has a different data architecture and different functionality within its respective SDK. Without a generalized interface technology, data exchange would potentially be limited to one or a few instruments controlled by a single instrument data system. SOPs therefore would not be able to automatically address instrument configuration differences in order to allow for creation and dissemination of workflow automation templates.

Fusion AE development was also required to address the following related Analytical R&D technology development goals:

- Easy setup of DOE-based experiments – facilitate statistically rigorous practice.
- 21 CFR 11 compliance support toolset – help maintain compliance across integrated platforms.
- Method connectivity – early methods developed manually or using other software tools should be able to be optimized and validated using the new software.
- Simple documentation review – easy to defend and communicate.
- 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 2 illustrates the technology integration goals and related technology development goals.

Figure 2. Integrating the Technology Islands

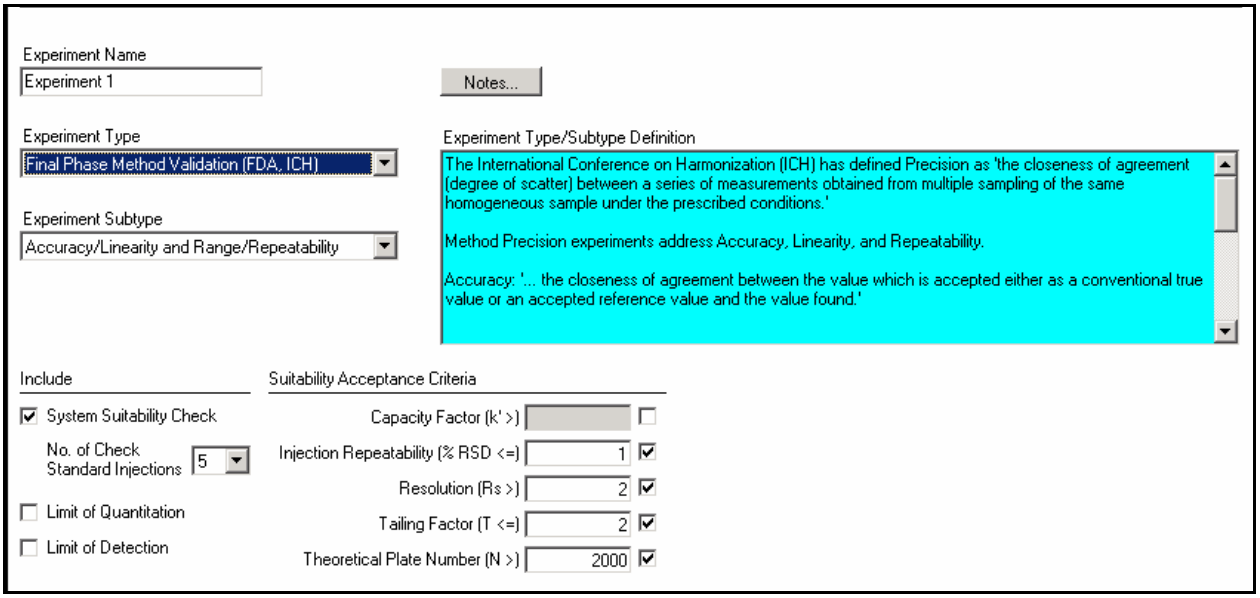


The ultimate Fusion AE development goal was creation of a central software environment for all analytical method validation work. To facilitate acceptance and widespread use, the resulting software platform was required to include four specific feature sets: a custom user interface specific to method 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. These feature sets are described below.

Custom User Interface

Figure 3 illustrates the custom user interface required for method validation software. The figure shows an Experiment Setup window that contains controls for incorporating System Suitability Check Standard injections into the experiment design and defining acceptance criteria for evaluating suitability results such as Peak Capacity Factor (k') and Peak Resolution (Rs).

Figure 3. Example of Custom Software Interface



Phased Approach to Method Validation

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 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 prior to the point at which final validation is usually conducted.

Fusion AE software development addressed the requirement for such a phased approach in terms of both experiment organization and 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, etc. will result in smaller experiments with reduced 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 Fusion AE is listed below by phase.

Early Phase Method Validation (Characterization)

- System Suitability
- Filter Validation
- Accuracy
- Linearity and Range
- Repeatability* (intra-assay precision)
- Sample Solution Stability (stability for a given time period under prescribed conditions)

Final Phase Method Validation (FDA and ICH Submittal Quality)

- System Suitability
- Accuracy/Linearity and Range/Repeatability – Combined Design

  (*ICH-Q2A states that Accuracy, Linearity, and Repeatability can be done together as a single combined experiment*).
- Robustness
- Ruggedness (Intermediate precision and Reproducibility)
- Specificity

** - 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.*

Full Complement of Automation Support Features

As a central software environment, Fusion AE required many custom support features to fully enable and automate the method validation experiment design suite just discussed. The required support features naturally group into five feature sets: Assay Types, Compounds, Analysis and Reporting, Acceptance Criteria Testing, and Workflow Management. The Assay Types feature set encompasses the four main assays routinely addressed in method validation. The Compounds feature set allows multiple compounds (active ingredients or impurities) to be included in the same experiment. The Analysis and Reporting feature set provides the statistical analysis and graphing results reports required by the FDA and ICH guidances. The Acceptance Criteria Testing feature set enables the Analysis and Reporting feature set to automatically compare actual results with pre-defined "Pass/Fail" acceptance criteria and report the results of the comparisons. The Workflow Management feature set enables construction of work templates and software-based administration and control of the work. These five feature sets and their component features are presented below.

- **Assay Types**
  - Potency (Drug Content).
  - Content Uniformity.
  - Dissolution.
  - Determination of Impurities.
- **Compounds**
  - Active Ingredients – up to 10 required.
  - Impurities – up to 10 required.
- **Analysis and Reporting**
  - Automated analysis – one button click.
  - Automated graphics – created as part of automated analysis.
  - Automated report construction – must meet all FDA and ICH guidances.
- **Acceptance Criteria Testing [user defined value = X]**
  - Filter Validation: % Bias Limits (+/-**X**).
  - Accuracy: % Bias (<**X**).
  - Linearity and Range: % Bias (<**X**).
  - Repeatability: % RSD (<=**X**)
  - Sample Solution Stability: % Recovery Limits (+/-**X**).
  - Robustness: % Effect (<**X**).
  - Ruggedness:
    - % Effect (<**X**)
    - Intermediate Precision % RSD (<=**X**)
    - Reproducibility % RSD (<=**X**)
  - Specificity: Difference of Practical Significance (<=**X**).

- **Workflow Management**
  - Ability to create and distribute workflow templates.
  - Ability to control feature access with user permissions and authorities settings.
  - Ability to control workflow with Review and Approve e-signing control loops.

Fusion AE Development – Final Results

Fusion AE software benchmarking involved conducting "live" method validation experiments in the walk-away mode with full instrument control and automated data exchange with the CDS. 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:

- **Central software environment for all analytical method validation work.**
- **Flexibility to support "method validatability" 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 first target data systems:**
  - PerkinElmer® TotalChrom™.
  - Varian® Galaxie®.
  - Waters® Millennium®[32] and Empower™.

Figure 4. Automated Software Solution

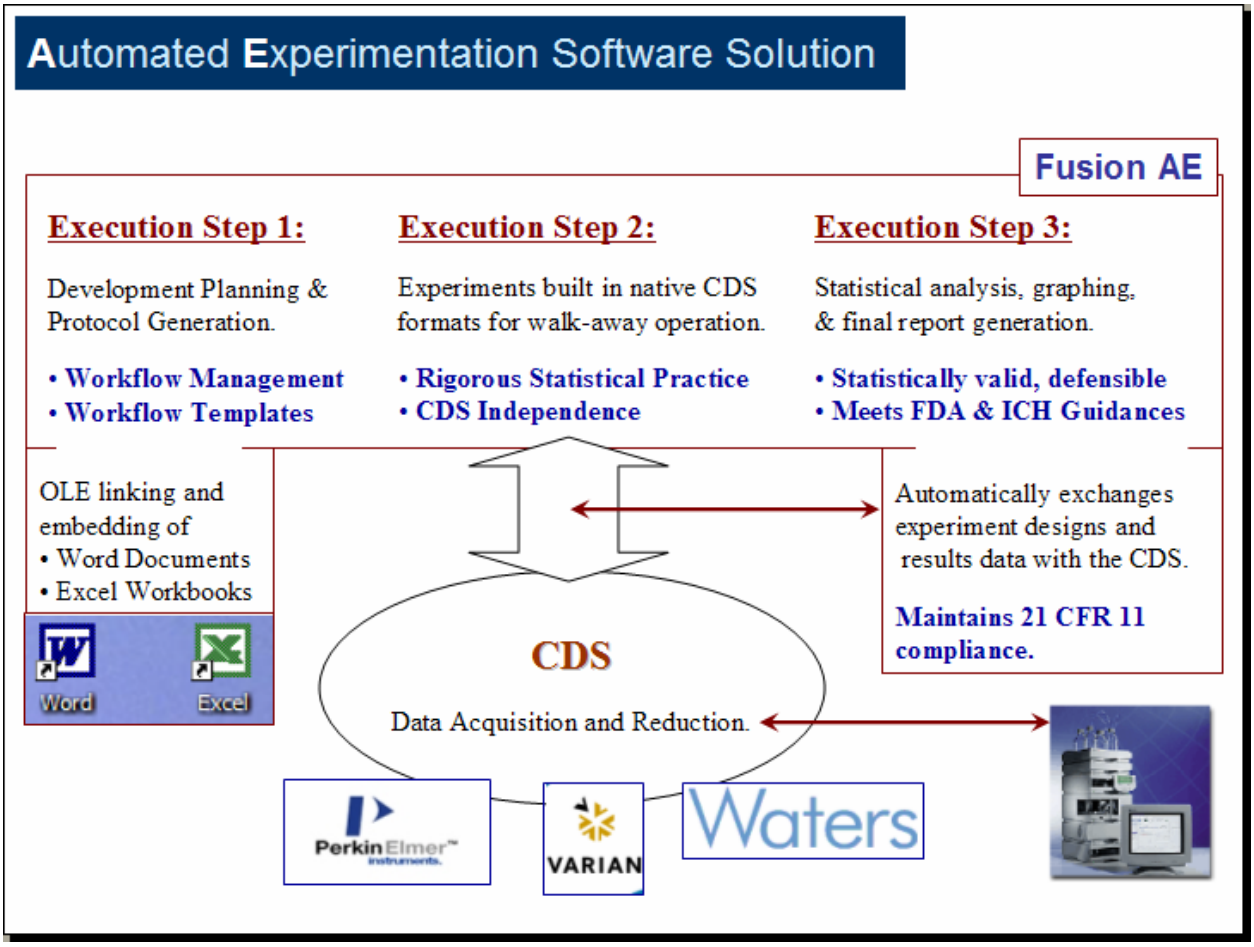
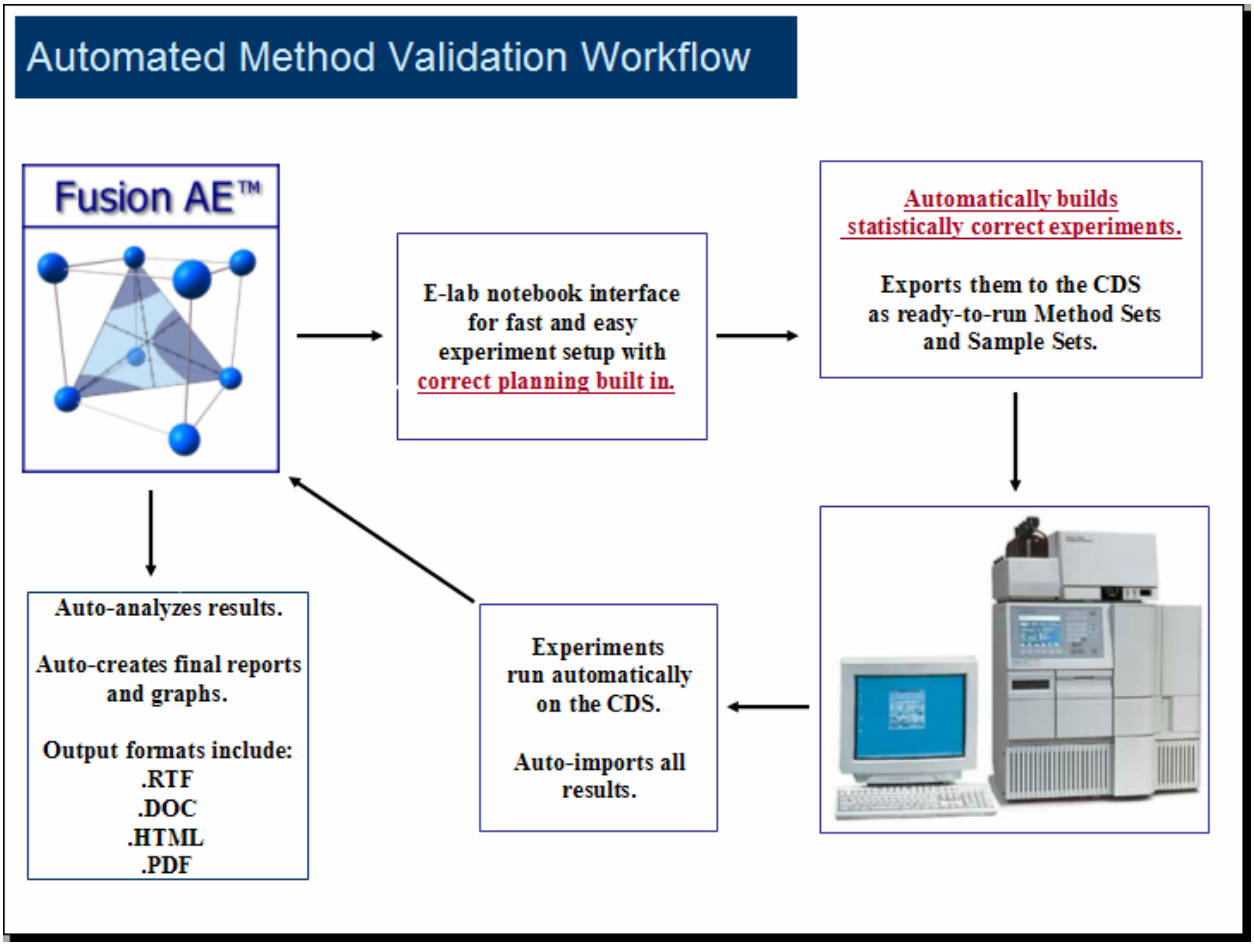


Figure 5. Automated Method Validation Workflow

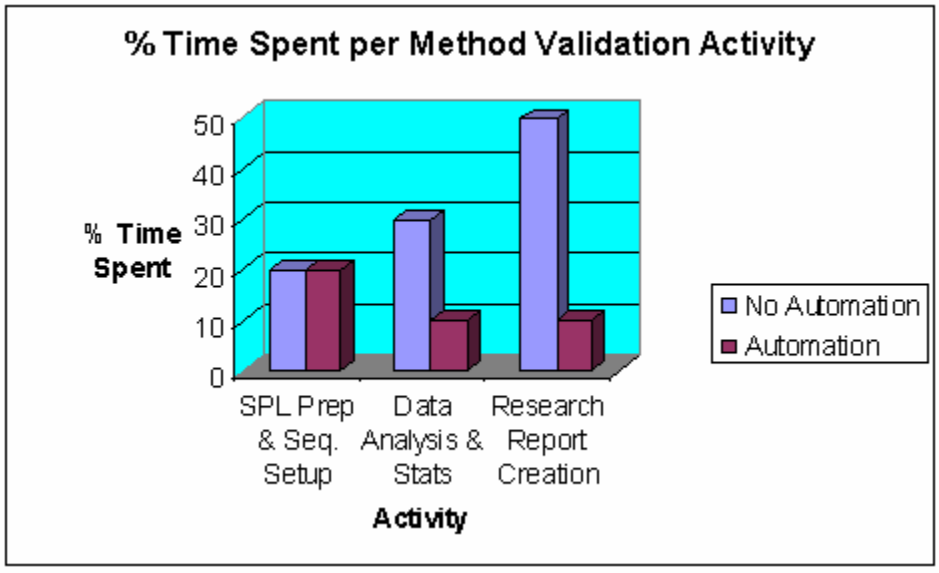


As final proof of concept, a senior analytical chemist at an international pharmaceutical customer facility used Fusion AE to carry out all Early Phase and Final Phase method validation experiments (except Robustness, which was done subsequently at a different lab) in the following seven workflow steps:

1. Prepare a series of HPLC injection samples and standards containing two active compounds.
2. Generate all experiment designs within Fusion AE.
3. 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.
4. Set up the HPLC (prepare the mobile phase reservoirs and load injection samples and standards into the autosampler).
5. Run the all nine experiment design sequences on the HPLC in walk-away mode.
6. Use the automated data exchange feature to import the results data sets from the CDS into Fusion AE.
7. 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. The entire method validation exercise took less than 12 hours of the analyst's time. Work records showed that on average, the same set of method validation tasks - from SOP planning and experiment design construction to final reporting - using the current manual "technology island" approach with existing tools required more than two weeks of analyst time. Thus this proof of concept exercise represented an 85% reduction in time and effort [(12 hrs/80 hrs)*100%]. Figure 6 illustrates a *minimum* expectation of the efficiency gain possible with the Fusion AE automated software solution in which only a few of the simpler experiments are performed and the time required for manually carrying out these automated operations (Steps 2, 3, 5, 6, and 7) is minimized. As the figure illustrates, under these circumstances the minimum efficiency gain is still at least 60% (20% gain in data analysis and 40% gain in report generation).

Figure 6. Efficiency Gained by Automation



Conclusions

A project of this magnitude and level of complexity presented several software development challenges in each of the four main program elements: User Interface, Experiment Design, Data Exchange, and Regulatory Compliance. The most critical project goal in each of these four main program elements is presented in Table 1. This table also presents the principal technical challenge associated with accomplishing each goal and the result achieved at the conclusion of Fusion AE development.

Table 1. 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 system	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 instruments
Data Exchange: Flexible data exchange adaptable to several target instruments	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 configuration
Regulatory Compliance: Maintaining 21 CFR 11 compliance support across multiple 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

Fusion AE enables the transformation of written SOPS, required for all analytical method validation experiments, into transferable, automated templates in a timely manner and with full cGMP compliance. It also allows harmonization of analytical validation tasks across multiple sites and can be extended to contract research organizations with full management control of all work. Additionally, the connectivity to multiple instruments from several different vendors connected to a CDS at a single site means that the work environment is transparent to the target instrument and configuration. This will enable greater flexibility in selecting instruments as needs change and technology improves.