[APPLICATION NOTE]

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EMPOWER 2 METHOD VALIDATION MANAGER SOFTWARE: TOOL FOR RAPID METHOD VALIDATION

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INTRODUCTION

Within the compliant laboratory, the validation of analytical methods is a fact of life. Regulatory agencies must have documented evidence that the analytical methods employed by a laboratory yield accurate and reliable results. The laboratories, utilizing advanced planning and good scientific judgment, rely on validation as a means of assuring confidence in the results generated from their analytical methods. From both perspectives, there is no argument that analytical method validation is an important process and a permanent aspect of compliant laboratory operation.

Method validation is a demanding activity. It requires a large investment in personnel, materials, instruments, supervision, and, most of all, time. Some of the more time-consuming aspects of validation involve the creation of validation protocols and sample lists, tracking of the workflow from protocol to final reporting, the performance of calculations, and the intense need to organize and manage raw and processed data. The potential for errors in the many steps of the validation process is large and the time delay when errors occur can be costly.

Waters® Empower™ 2 Method Validation Manager (MVM) Software, coupled with the Waters ACQUITY UPLC® System, can dramatically address these time-consuming elements of analytical method validation. The advantages of using the ACQUITY UPLC System have been reported previously. MVM is designed to streamline the set-up, execution, calculation, and reporting of a method validation. It provides easy data tracking and complete organization of validation data and results monitored by the built-in oversight of automated error checking. MVM is a business-critical software that reduces the time and costs required to perform chromatographic method validation by as much as 80%. Because MVM 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.

To illustrate the straightforward operation and comprehensive functionality of MVM, a basic assay validation of the drug product acetaminophen will be summarized. Multiple screenshots from MVM are presented with the validation results to help demonstrate the application of this software to the validation process.

EXPERIMENTAL

Materials

Acetaminophen RS was purchased from Sigma-Aldrich Co. (St. Louis, MO). Methanol was acquired from Fisher (Fair Lawn, NJ). Water was purified with a MilliQ Gradient A10 System (Millipore, Billerica, MA).

UPLC conditions

The assay was performed on a Waters ACQUITY UPLC System consisting of a Binary Solvent Manager (BSM), Sample Manager (SM), and Tunable UV Detector (TUV). A Waters ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 X 50 mm Column was selected for the separation. All instruments were controlled, and data were collected and analyzed, using Empower 2 Method Validation Manager Software.

Assay conditions

Mobile phase:	90:10 water/methanol, mixed by pump
Flow:	0.65 mL/min
Temperature:	40 °C
Injection volume:	1.0 µL
Wavelength:	243 nm
Runtime:	2 min
Retention time:	0.7 min

METHOD

Solution preparation

The acetaminophen working standard was made from a 1:9 dilution of a 0.1 mg/mL acetaminophen stock standard. 10 mg of acetaminophen RS was weighed accurately into a 100 mL volumetric flask, diluted to mark, and mixed with mobile phase. A 1.0 mL aliquot was then transferred to a 10 mL volumetric flask, diluted to mark, and mixed with mobile phase. The final concentration of the working standard was 0.01 mg/mL acetaminophen RS.

Acetaminophen sample preparation

Sample preparations for this method were made using the following procedure:

Weigh and finely powder at least 20 tablets. Transfer an accurately weighed portion, equivalent to about 100 mg of acetaminophen, to a 200 mL volumetric flask. Add approximately 100 mL of mobile phase and shake the solution for 10 minutes, then sonicate for 5 minutes. Fill the flask to mark with mobile phase.

Transfer a 5.0 mL aliquot of the above solution to a 250 mL volumetric flask, dilute to mark with mobile phase, and mix. The final concentration of this preparation should be approximately 0.01 mg/mL acetaminophen.

Method system suitability criteria

The method system suitability criteria are listed in Table 1.

Parameter	Acceptance Criterion
%RSD RT min	≤ 1.0%
%RSD area acetaminophen in std	≤ 2.0%
USP tailing	≤ 1.5%
USP plates	≥ 1000

Table 1. Method suitability criteria.

Acetaminophen analysis with the ACQUITY UPLC System is shown in Figure 1.

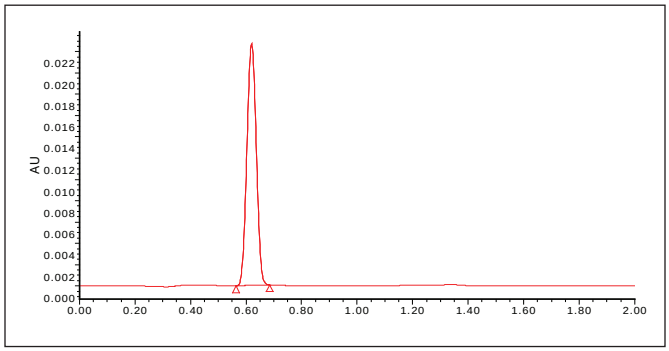


Figure 1. Analysis of acetaminophen.

Validation protocol and execution

The elements of the written validation protocol for this method were easily transferred into the validation protocol method template of MVM (Figure 2). The following validation tests were performed in this study:

- Robustness (for three factors)
- Repeatability
- Intermediate precision (different analyst)
- Linearity
- Accuracy
- Solution stability (24 hours)

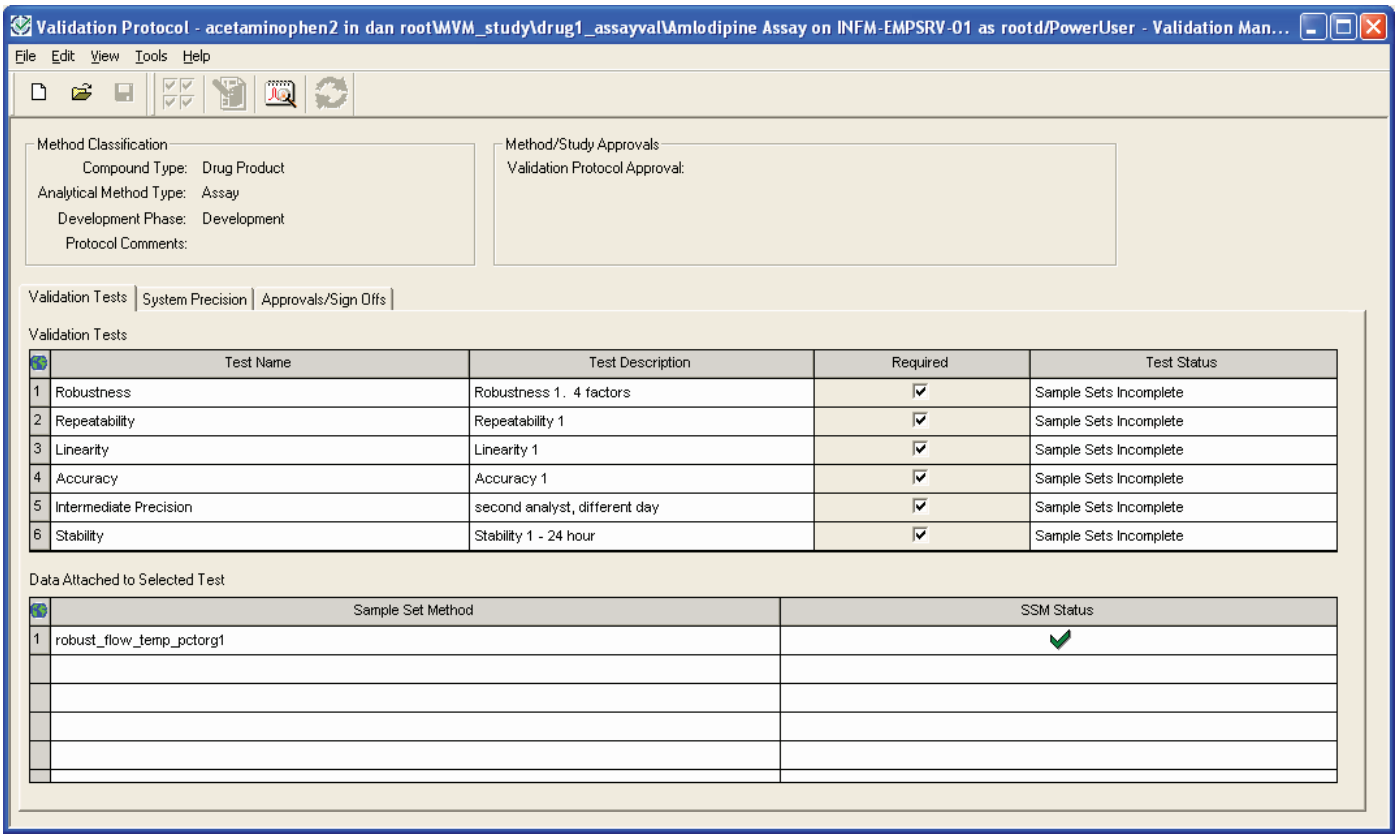


Figure 2. The written protocol can be easily transferred to Empower Method Validation Manager.

Individual tests and their associated acceptance criteria were configured, as shown in Figures 3 and 4.

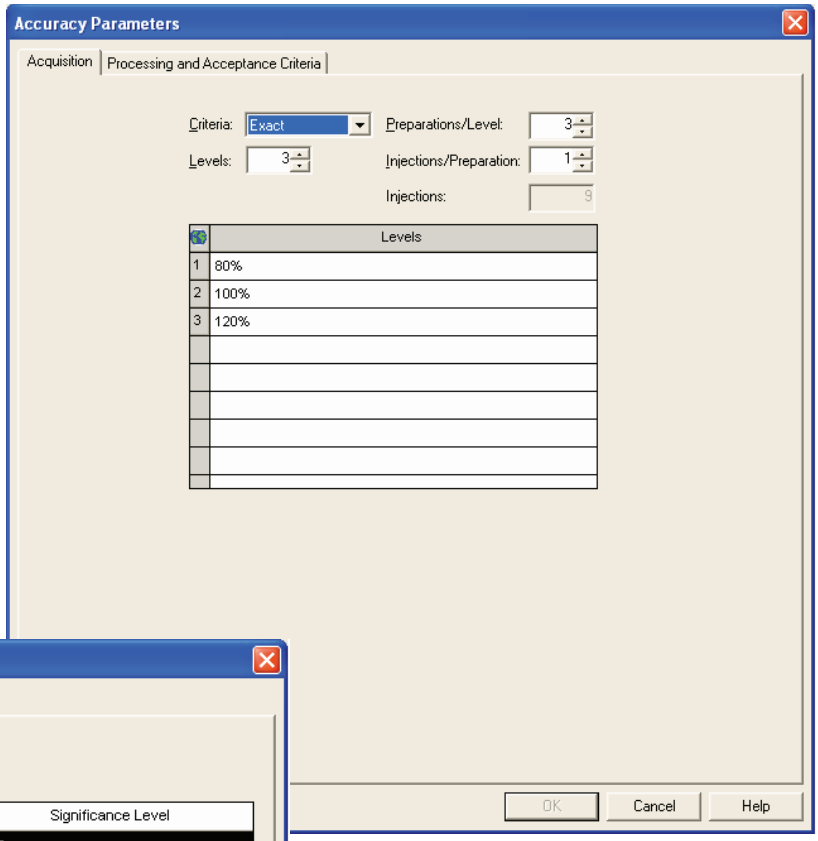


Figure 3. Accuracy test configuration. Three specified concentration levels with three individual preparations were injected one time. Levels were entered in the levels table. MVM then ensures that samples submitted for accuracy analysis match the defined parameters.

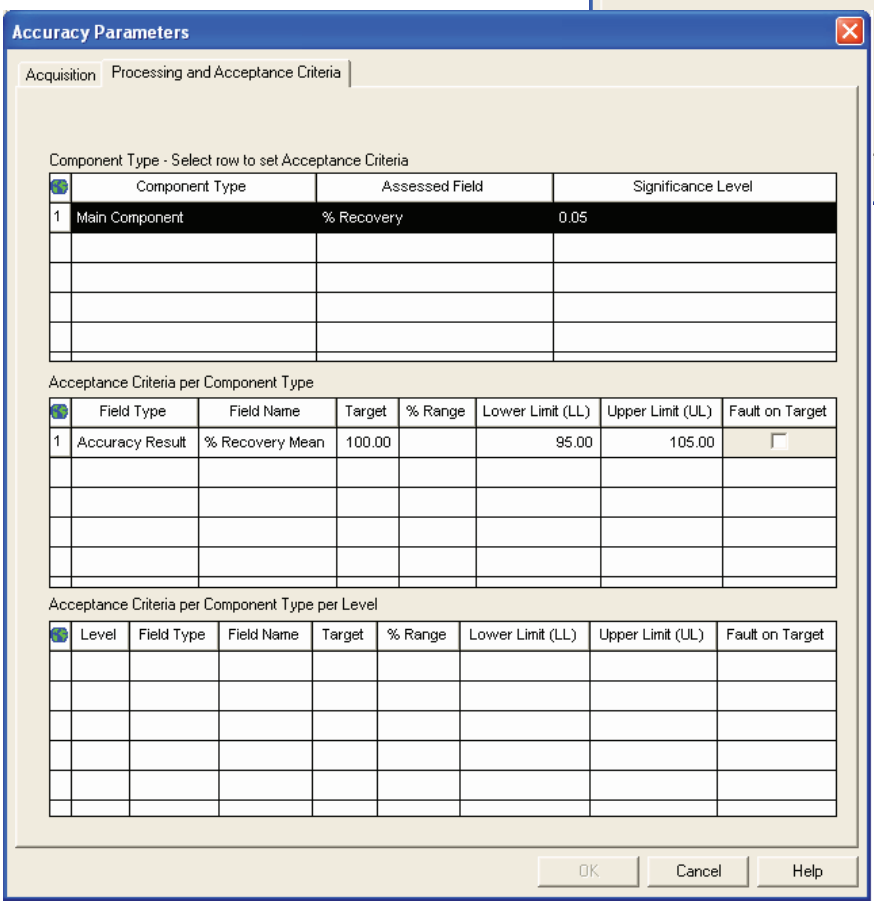


Figure 4. Acceptance criteria for the accuracy test. Percent recovery was the test result of interest. The acceptance range for the test is indicated as 95 to 105%.

Complete sample set methods were constructed and then saved as templates within the validation protocol method (Figure 5).

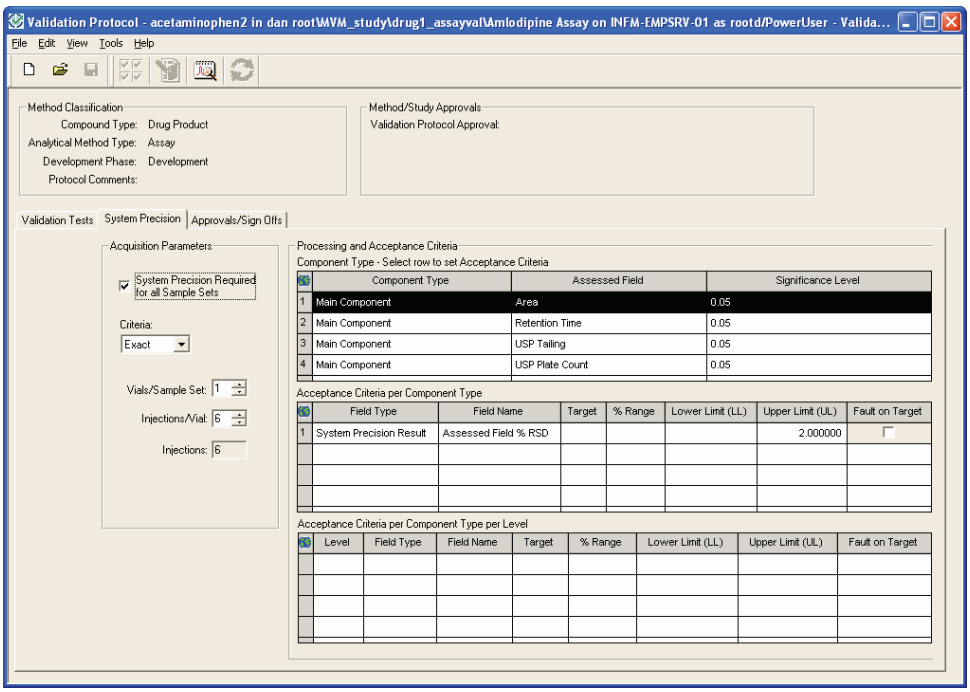
Figure 5. Sample set method.

robust_flow_temp_pctorg1 in dan root\MVM_study\drug1_assayval\Amlodipine Assay on INFM-EMPSRV-01 as rootd/PowerUser - Sample Set Method Editor

File Edit View Help

	Plate/Well	SampleName	Inj Vol (uL)	# of Injs	Function	Method Set / Report Method	Run Time (Minutes)	Level	Sample Preparation	Next Inj. Delay (Minutes)	analyst	Experiment Name	Robustness
1	1:a,1	test inj.	1.0	1	Inject Samples	aceta_robust1	2.00	0%		0.00			☐
2	1:a,2	sys prec. 100% std	1.0	6	Inject Standards	aceta_robust1	2.00	100%		0.00			☐
3	1:a,3	100% prep	1.0	1	Inject Samples	aceta_robust1	2.00	100%	Preparation 1	0.00		Experiment 1	☑
4	1:a,4	100% prep	1.0	1	Inject Samples	aceta_robust1	2.00	100%	Preparation 2	0.00		Experiment 1	☑
5	1:a,5	100% prep	1.0	1	Inject Samples	aceta_robust1	2.00	100%	Preparation 3	0.00		Experiment 1	☑
6	1:a,6	100% prep	1.0	1	Inject Samples	aceta_robust1	2.00	100%	Preparation 4	0.00		Experiment 1	☑
7	1:a,7	100% prep	1.0	1	Inject Samples	aceta_robust1	2.00	100%	Preparation 5	0.00		Experiment 1	☑
8	1:a,8	100% prep	1.0	1	Inject Samples	aceta_robust1	2.00	100%	Preparation 6	0.00		Experiment 1	☑
9					Clear Calibration	aceta_robust1							
10					Calibrate	aceta_robust1							
11					Quantitate	aceta_robust1							
12					Equilibrate	aceta_robust2	10.00			0.00			
13	1:a,1	test inj.	1.0	1	Inject Samples	aceta_robust2	2.00	0%		0.00			☐
14	1:a,2	ref. std. 100%	1.0	6	Inject Standards	aceta_robust2	2.00	100%		0.00		Experiment 3	☐
15	1:a,3	100% prep	1.0	1	Inject Samples	aceta_robust2	2.00	100%	Preparation 1	0.00		Experiment 3	☑
16	1:a,4	100% prep	1.0	1	Inject Samples	aceta_robust2	2.00	100%	Preparation 2	0.00		Experiment 3	☑
17	1:a,5	100% prep	1.0	1	Inject Samples	aceta_robust2	2.00	100%	Preparation 3	0.00		Experiment 3	☑
18	1:a,6	100% prep	1.0	1	Inject Samples	aceta_robust2	2.00	100%	Preparation 4	0.00		Experiment 3	☑
19	1:a,7	100% prep	1.0	1	Inject Samples	aceta_robust2	2.00	100%	Preparation 5	0.00		Experiment 3	☑
20	1:a,8	100% prep	1.0	1	Inject Samples	aceta_robust2	2.00	100%	Preparation 6	0.00		Experiment 3	☑
21					Clear Calibration	aceta_robust2							
22					Calibrate	aceta_robust2							
23					Quantitate	aceta_robust2							
24					Equilibrate	aceta_robust3	10.00			0.00			
25	1:a,1	test inj.	1.0	1	Inject Samples	aceta_robust3	2.00	0%		0.00			☐
26	1:a,2	ref. std. 100%	1.0	6	Inject Standards	aceta_robust3	2.00	100%		0.00		Experiment 2	☐
27	1:a,3	100% prep	1.0	1	Inject Samples	aceta_robust3	2.00	100%	Preparation 1	0.00		Experiment 2	☑
28	1:a,4	100% prep	1.0	1	Inject Samples	aceta_robust3	2.00	100%	Preparation 2	0.00		Experiment 2	☑
29	1:a,5	100% prep	1.0	1	Inject Samples	aceta_robust3	2.00	100%	Preparation 3	0.00		Experiment 2	☑
30	1:a,6	100% prep	1.0	1	Inject Samples	aceta_robust3	2.00	100%	Preparation 4	0.00		Experiment 2	☑
31	1:a,7	100% prep	1.0	1	Inject Samples	aceta_robust3	2.00	100%	Preparation 5	0.00		Experiment 2	☑
32	1:a,8	100% prep	1.0	1	Inject Samples	aceta_robust3	2.00	100%	Preparation 6	0.00		Experiment 2	☑
33					Clear Calibration	aceta_robust3							
34					Calibrate	aceta_robust3							
35					Quantitate	aceta_robust3							

Ready



Since the validation protocol called for method suitability parameters to be met by each analysis, system precision requirements were also configured (Figure 6).

Figure 6. System precision parameters. In this protocol, the % RSD of peak area must be no more than 2%.

During the process of test configuration and sample set method construction, errors were automatically caught by MVM, as indicated by a red X in the validation protocol window. Using the update status button and responding to error messages from the message center effectively guides all troubleshooting activity (Figure 7).

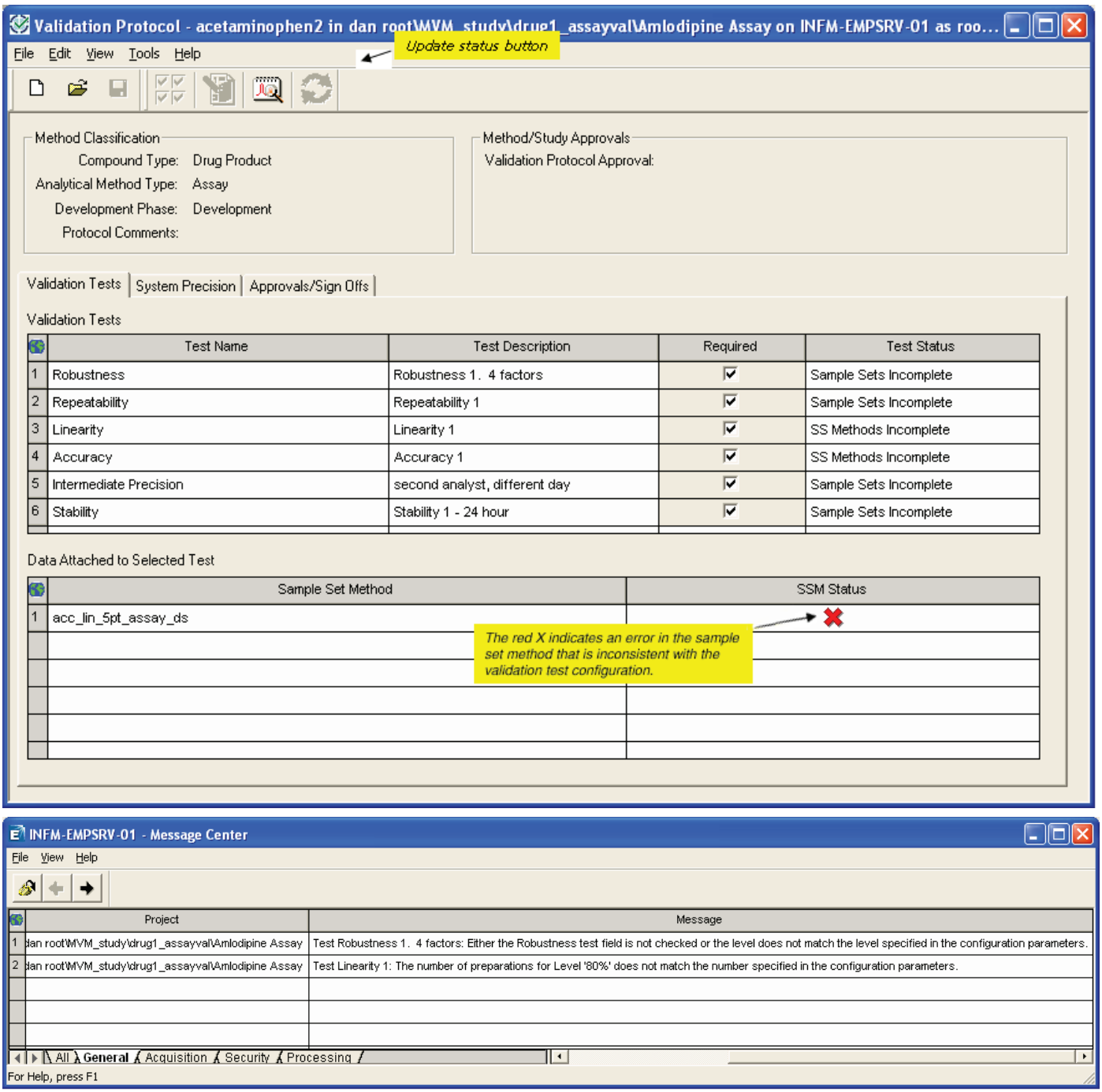


Figure 7. Linearity sample set method error caught by Empower 2 MVM Software. The message center indicated a problem, which was easily resolved. MVM ensured that all sample set methods were consistent with their respective test configurations. An earlier error for a robustness test configuration is also visible.

The validation protocol method was saved within a validation template project. Next, a validation working project was started and a new study was initiated based on the validation protocol method template.

The validation manager window lists the test configurations and acceptance criteria for the validation study. Additional functionality includes indicators that show test status and required approval (Figure 8). Since complete sample set methods are contained in the validation protocol method, the study can now be executed. Standards and samples were prepared then analyzed on the ACQUITY UPLC System as the previously established sample set methods.

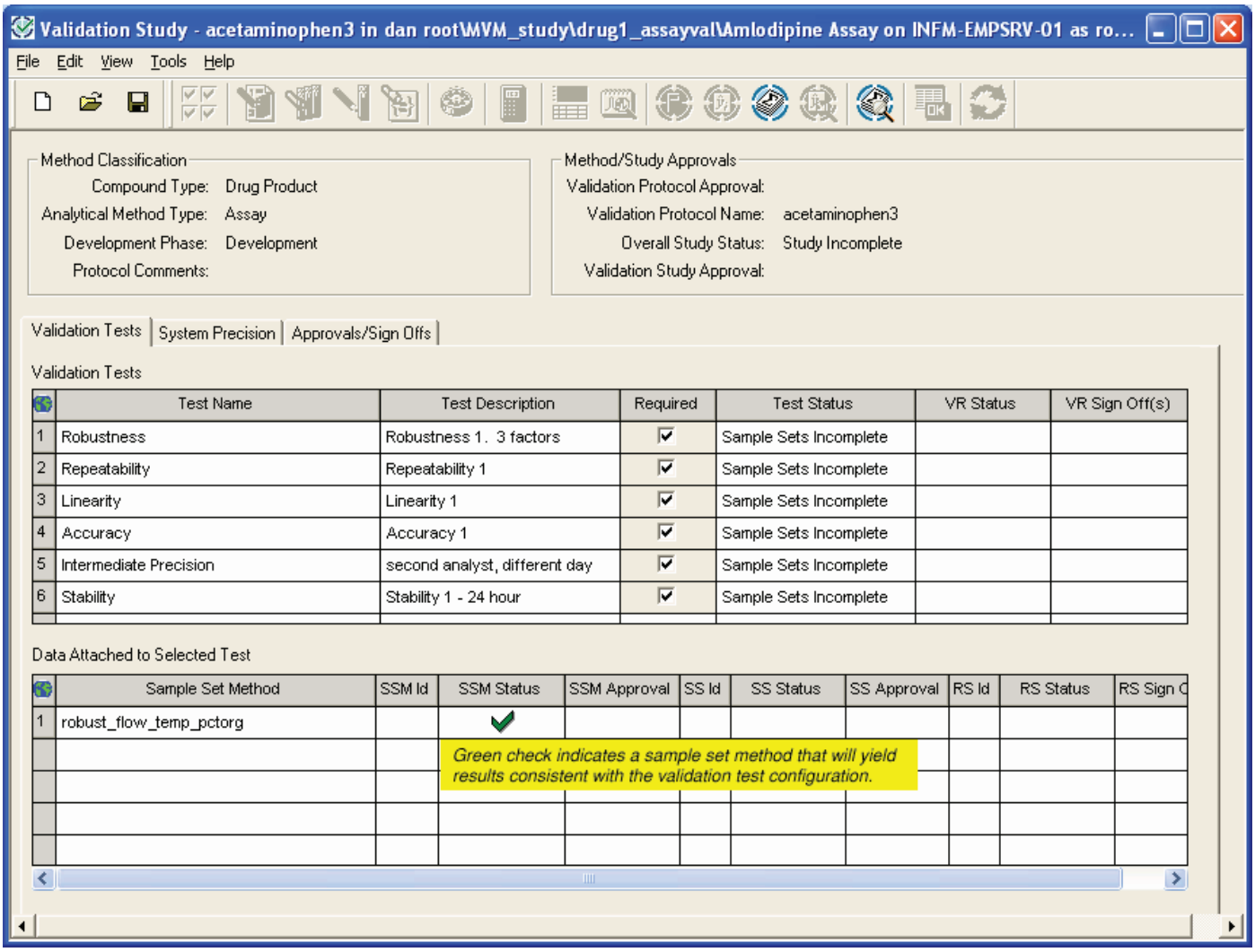


Figure 8. The green check mark in this validation manager window indicates that the sample set method is consistent with the user-configured test criteria.

RESULTS

Robustness

Robustness was evaluated using a 1/2 fractional factorial experimental design. The parameters assessed were flow rate, percent organic in the mobile phase, and column temperature (Table 2). Because the sample preparation procedure of this method is direct from the United States Pharmacopeia , only selected instrumental parameters were evaluated. The acceptance criteria for the test were:

1) The amount of acetaminophen determined must fall within 5% of the target value.
2) The %RSD of the amount must be no more than 3%.

A parameter that fails these criteria will need to be tightly controlled when performing the assay.

Experiment #	Column temperature °C	Percent Organic	Flow rate mL/min
1	37	8	0.750
2	43	8	0.550
3	37	12	0.550
4	43	12	0.750

Table 2. Experimental design of robustness from MVM.

The results of the robustness testing indicate that all three factors – percent organic, flow rate, and column temperature – had statistically significant effects on the determination of acetaminophen by this method. Referring to the effects plot in the validation result review window in Figure 9, varying the percent organic by ±2%, the temperature by ±3 °C, and the flow rate by ±0.1 mL/min, produced a 1%, 5%, and 10% effect on the assayed acetaminophen amount respectively.

In this case, robustness was evaluated for only the primary effects of the three factors with no consideration given to interaction. However, additional factors and the assessment of possible interactions between them, can all be performed easily and the results analyzed with MVM's powerful statistical techniques with a minimum of effort on the part of the validation analyst.

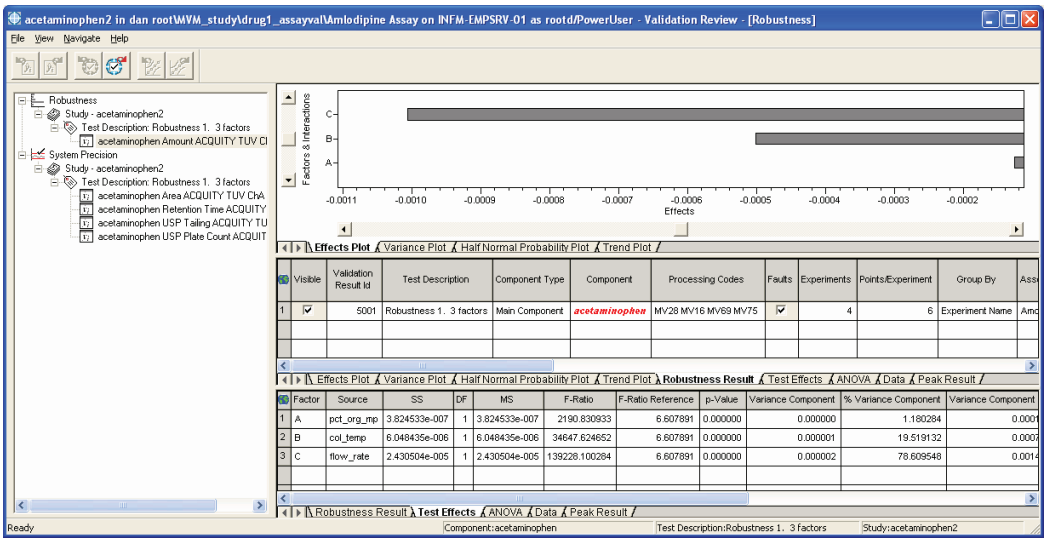


Figure 9. Validation test result review window. If the drug name appears in bold, red type, this indicates an out of specification result. Using the tabs of the review screen, the exact nature of the out-of-specification result can be quickly determined.

Repeatability

Repeatability (intra-assay precision) was tested by analyzing six individual sample preparations according to method conditions. The resulting 0.13% RSD for amount easily fell within the acceptance criterion of %RSD ≤2.0%, demonstrating that this assay is highly repeatable (Figure 10).

This repeatability result was used in the intermediate precision determination and as the initial time point for the solution stability test. MVM automatically consolidates test result calculations from separate sample set methods.

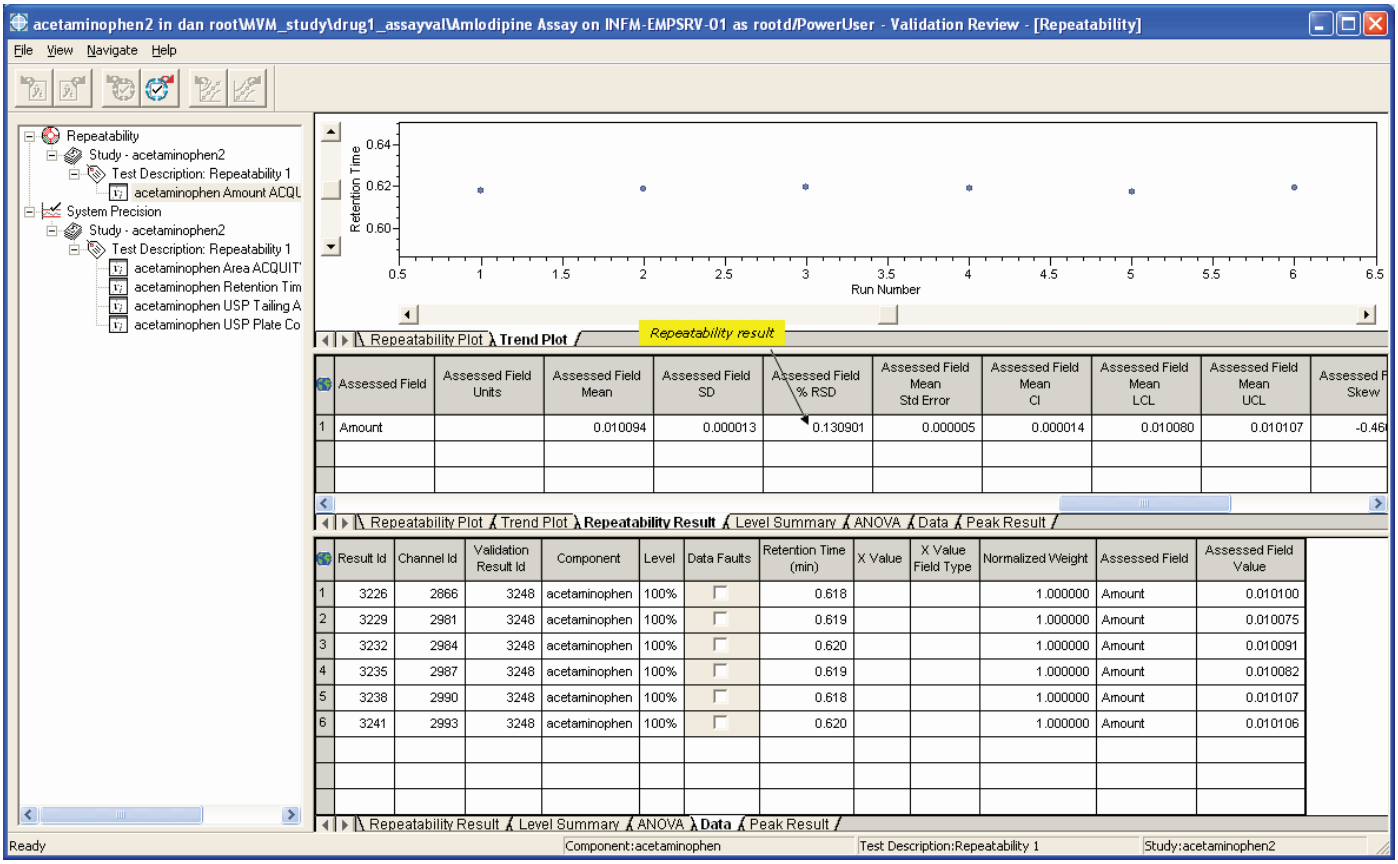


Figure 10. Repeatability test in the validation result review window.

Intermediate precision

Intermediate precision (ruggedness, inter-assay precision) was evaluated for a different analyst, on a different day, on a different instrument and column. Six individual sample preparations were analyzed according to method conditions. Results were compared with the repeatability determination. A difference of no more than 3.0% in the amount of acetaminophen between the two analysts was an acceptable result. The resulting 2.6% difference demonstrates the ruggedness of this assay (Figure 11).

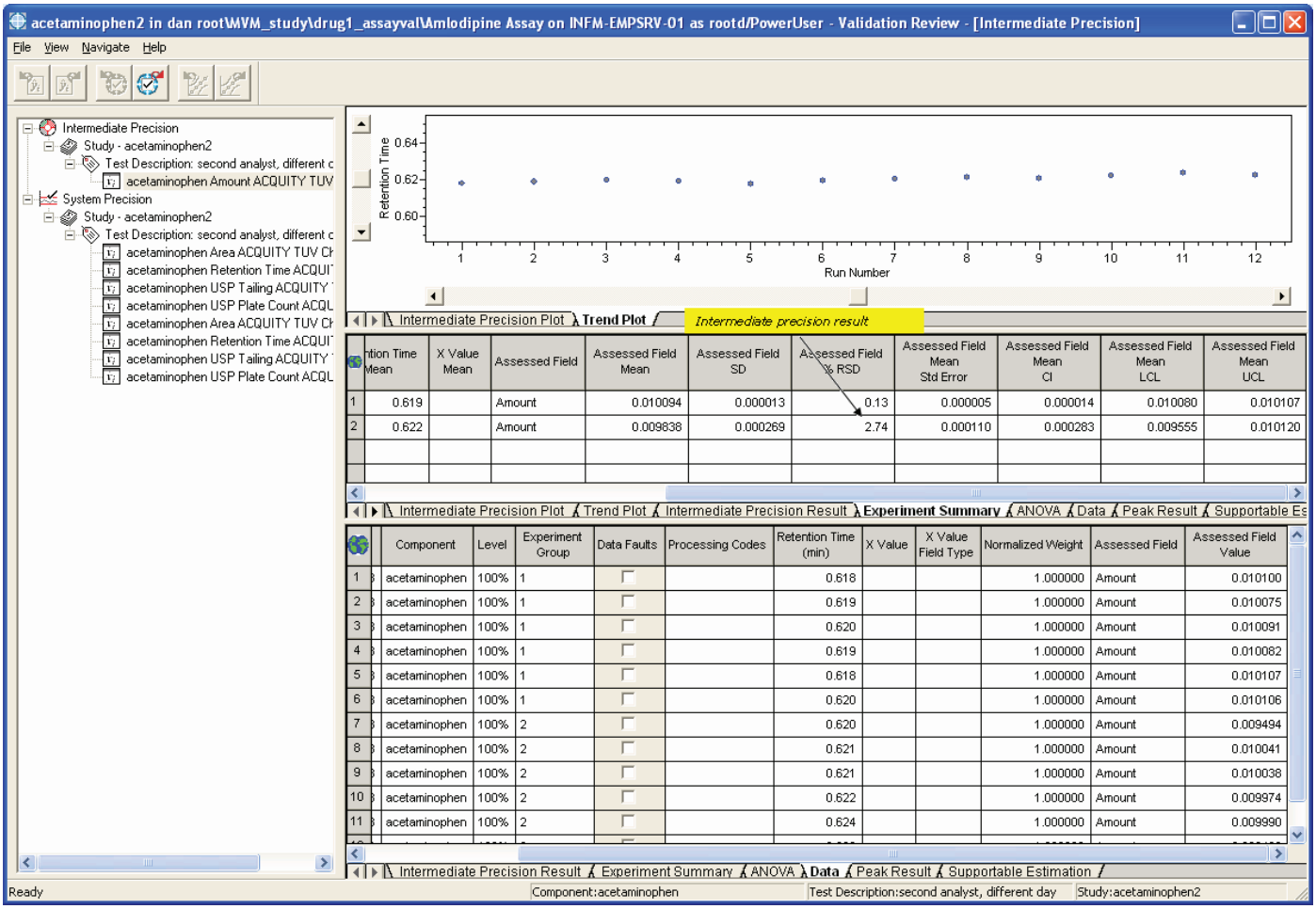


Figure 11. Intermediate precision result shown in the validation result review window.

Accuracy

Accuracy was assessed by analyzing triplicate preparations of mobile phase spiked with acetaminophen RS at 80, 90, 100, 110, and 120% of the target concentration of the method (0.01 mg/mL). The recovery result from the spiked acetaminophen ranged from 99 to 101% and fell within the 95 to 105% acceptance range. The method is very accurate for the range tested (Figure 12).

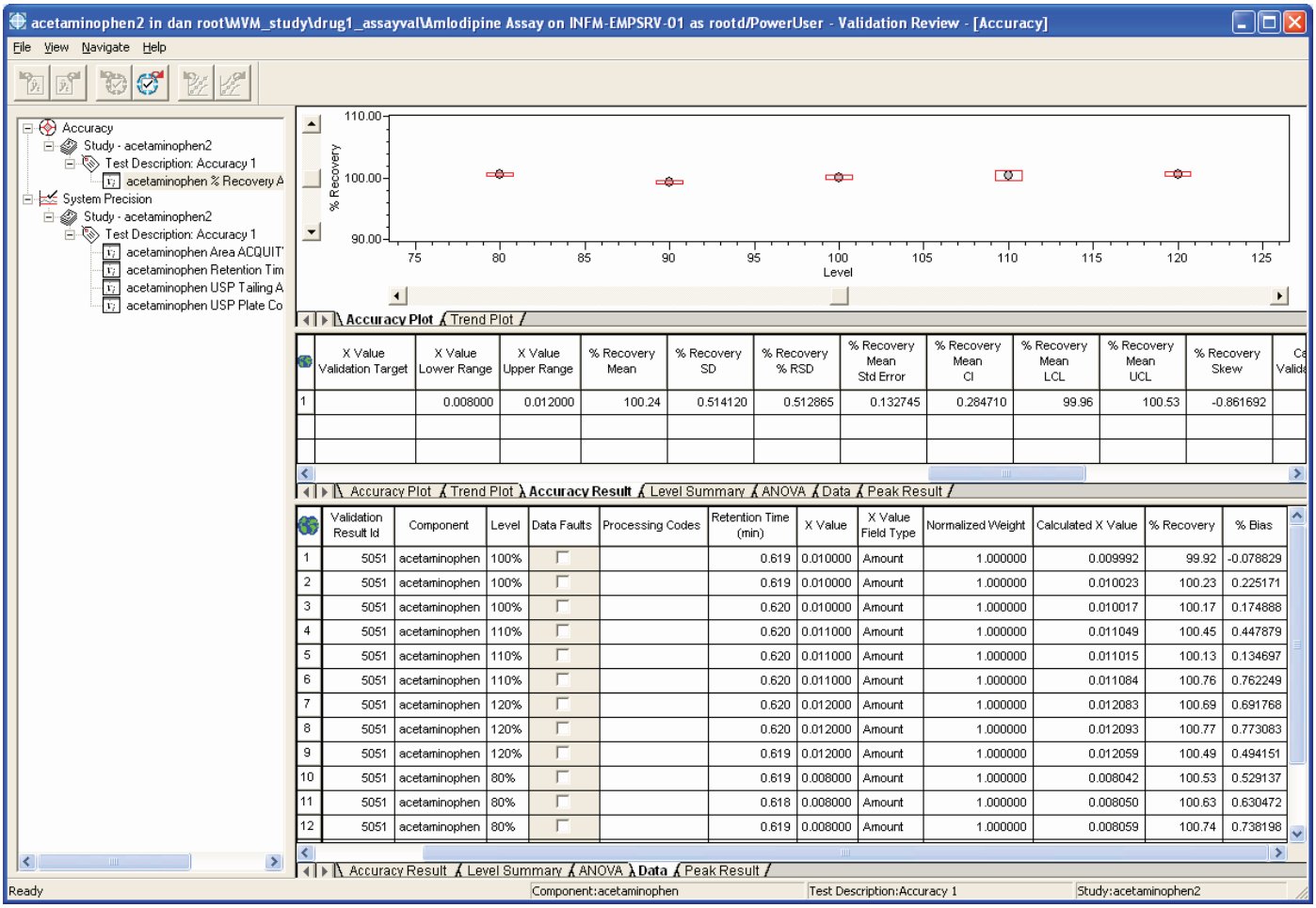


Figure 12. Accuracy validation result review window.

Linearity

Linearity was evaluated from the same experiment as the accuracy test. The results were linear with slope = 5.47 x 10^6, R^2 = 0.999, and a y-intercept of -720.3. The method is linear within the range tested (Figure 13).

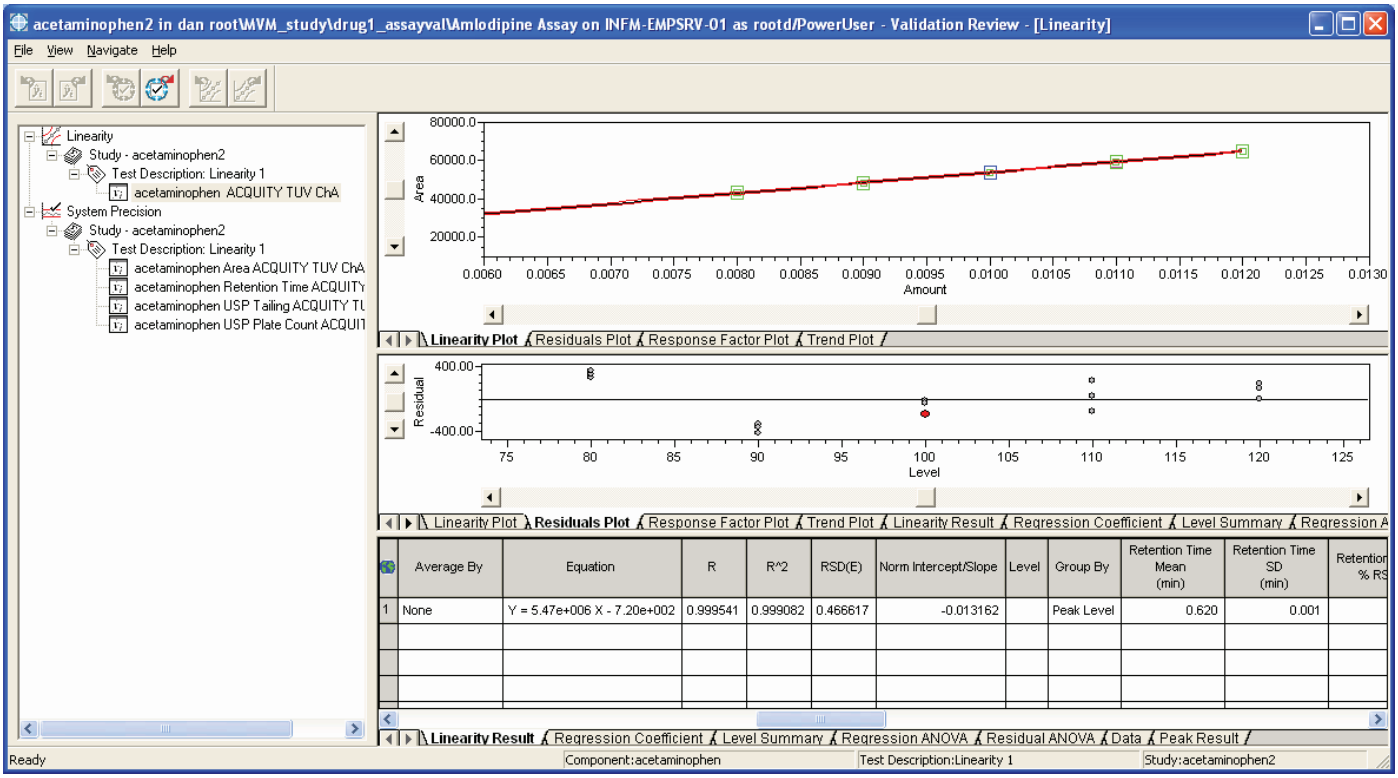


Figure 13. Linearity result shown in the validation result review window.

Stability

Stability was evaluated by the analysis of the repeatability sample preparations (N=6) after 24 hours at room temperature. The repeatability results were used as the time zero condition and were automatically used in the stability data processing. As shown in the validation results, acetaminophen sample preparations are stable for at least 24 hours (Figure 14).

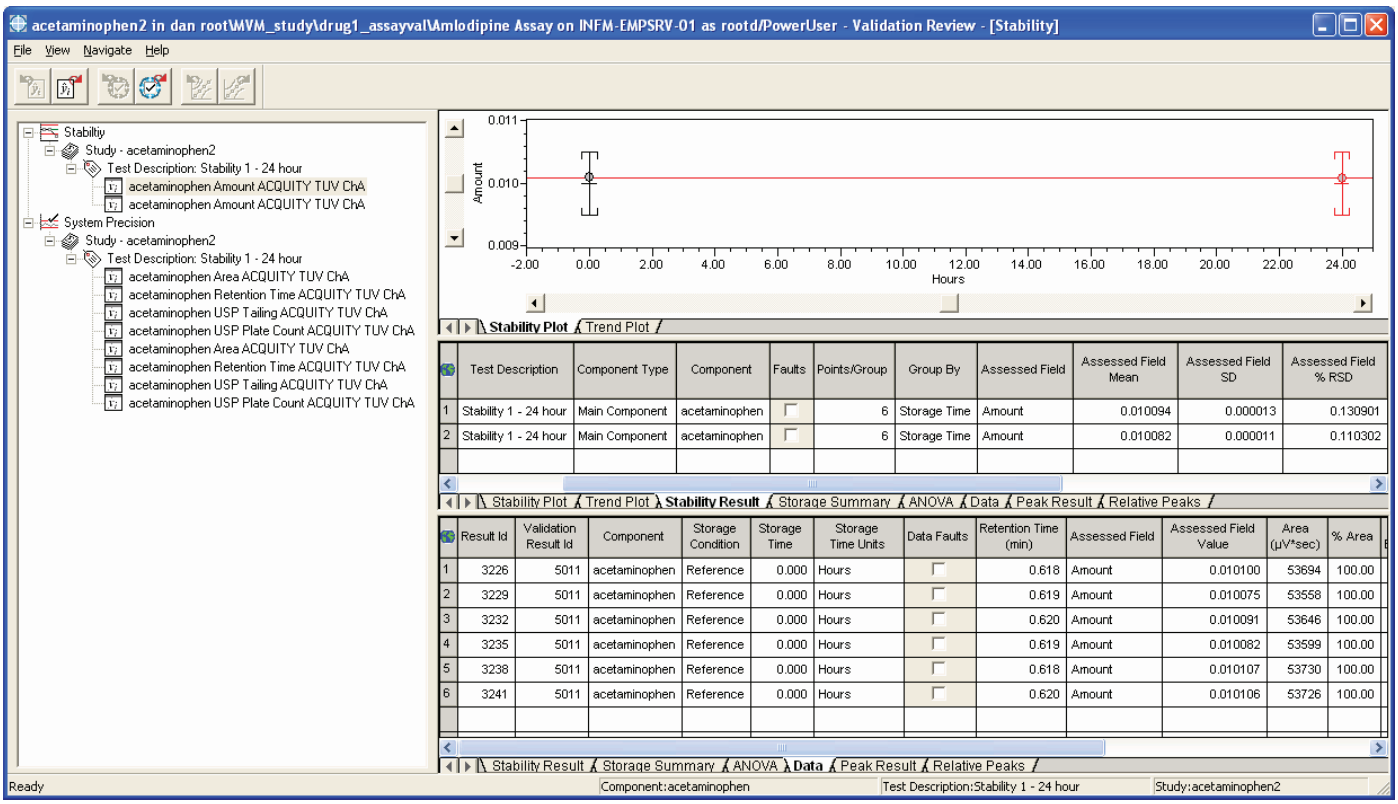


Figure 14. The consolidated results of two separate sample sets are presented in the stability validation test result review window.

Validation summary

The status and final results for each of the validation tests was clearly displayed in the validation manager window. The green checks indicated tests with acceptable validation results, while the yellow triangle flagged robustness test results that fell outside the acceptance range (Figure 15).

The method for the assay of acetaminophen was analyzed for robustness, repeatability, intermediate precision, accuracy, linearity, and solution stability. This assay was found to be linear, accurate, repeatable, and to be accurately and precisely performed by more than one analyst. Additionally, samples prepared following the method procedure were documented as stable for 24 hours. From the robustness testing, altering the column temperature and flow rate was found to significantly affect the accuracy and precision of the method. The method will be revised to clearly state the need to control these two factors.

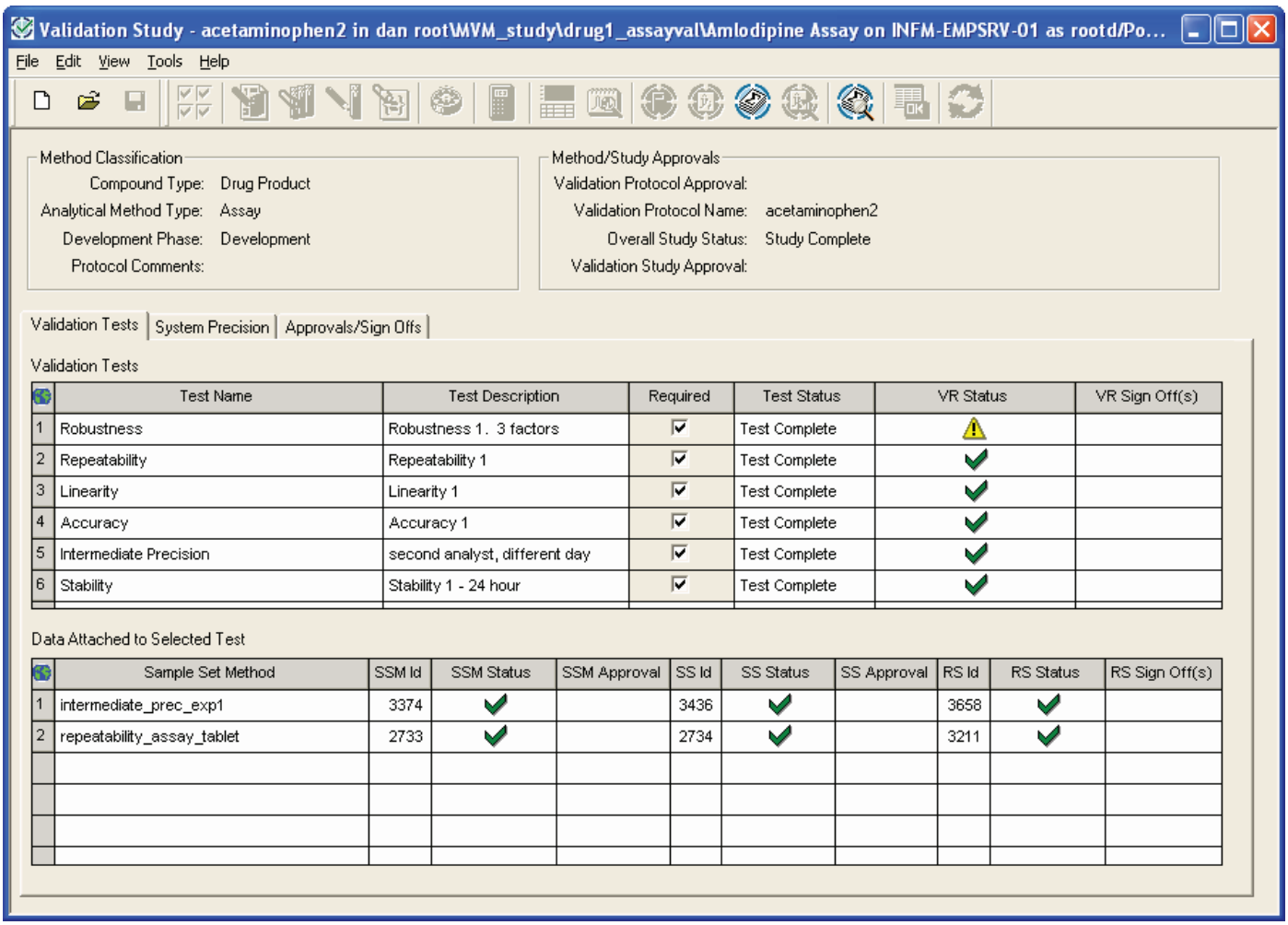


Figure 15. The validation manager window shows that validation is complete.

CONCLUSION

Empower 2 Method Validation Manager Software effectively streamlines the validation process and integrates smoothly into the validation workflow of the compliant laboratory.

Some of the benefits from the use of MVM are:

- **Regulatory compliance:** Empower 2 MVM Software easily meets all of the regulatory needs of the compliant laboratory.
- **Straight-forward validation troubleshooting:** The update tool/message center provides an application-directed, time-efficient troubleshooting process, reducing the time required to get the validation back on track.
- **Data traceability:** Out of specification results are clearly indicated and subsequent investigations are facilitated by the self-contained, completely traceable data management capability of the MVM.
- **Reduction of supervisory review:** The onus of supervisory review is reduced using MVM, enabling rapid progression in the validation workflow. Potentially error-prone steps such as processing, calculation, and overall data management are all eliminated with the automatic, self-contained design of MVM. The need for any additional third party software packages is also eliminated.
- **Validation consistency:** The ability to create project and sample set method templates ensures consistency of validation protocols with the guidance documents of the laboratory. This reduces errors in the execution of the protocols and increases confidence in the data acquired and the results obtained.

MVM not only effectively organizes and manages the performance of a method validation, it also delivers inarguable confidence in its results. Coupling Empower 2 Method Validation Manager Software to the ACQUITY UPLC System provides an unparalleled solution to the validation needs of a laboratory.

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