Automate System Suitability Testing with Chromatography Software

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Key Words
Chromeleon Chromatography Data System, (CDS), System Suitability Testing, Chromatographic Suitability, SST

Goal

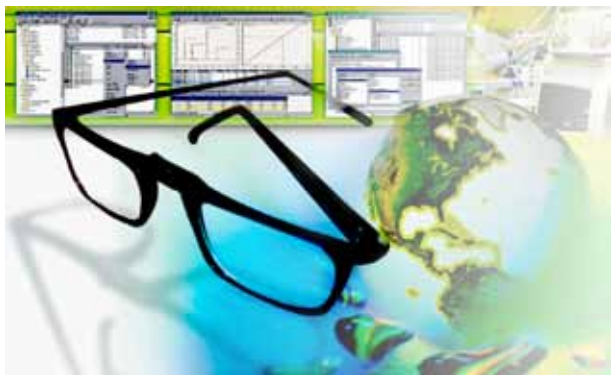
To illustrate and define the tools used by the Chromeleon Chromatography Data System (CDS) to perform Automated System Suitability Testing

Introduction

System Suitability Testing (SST) is commonly used by laboratories to ensure that the complete analytical system (including instrument, reagents, columns and analysts) is suitable for the intended application.

The United States Pharmacopeia (USP) Chromatography General Chapter states:

"System Suitability Tests are an integral part of gas and liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such."



The general goal of system suitability testing is to monitor chromatographic results to ensure chromatographic suitability (e.g. by testing tailing factor, column efficiency and resolution of critical peak pairs) and consistent system performance (e.g. by using replicate injections of test standards).

SST calculations are usually performed on standards before any samples are analyzed to ensure that potentially valuable samples are not injected into an unsuitable system. In a manual environment, this can require user interaction with every batch of samples and result in considerable throughput delays. Furthermore, if calculations are done manually, there is the potential for error and incorrect pass or fail results.

To overcome these problems, the Thermo Scientific Dionex Chromeleon 7 Chromatography Data System includes automated System Suitability Testing as part of sequence acquisition and processing. Using Chromeleon™, all SST calculations are automatically performed by the software.

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Based on user-specified criteria, Chromeleon can determine if the system is suitable and, if desired, even stop an analytical run if any of the tests fail.

System Suitability Testing is not limited to laboratories required to comply with guidelines set by the FDA and other regulatory bodies. Any laboratory can benefit from the ability of Chromeleon 7 to perform automated SST on hundreds of different types of calculated results. Possible test criteria include ensuring sample concentrations are within expected ranges, setting limits for detector signal-to-noise, limiting the acceptable levels of impurities and much more.

FDA Guidelines on System Suitability Testing

The FDA (CDER) 1 guidelines on Validation of Chromatographic Methods recommend the following tests and specifications:

Table 1: FDA Recommended SST Specifications

Test	Specification
Capacity Factor (k')	k' > 2
Precision/Injection Repeatability (RSD)	RSD ≤ 1% (n ≥ 5 is desirable)
Resolution of Analyte peak from closest peak (R^s)	Rs > 2
Tailing Factor (T)	T ≤ 2
Number of Theoretical Plates (N)	N > 2000

These specifications will vary depending on the actual conditions of the analytical method, but provide a good starting point. The FDA also recommends that at least 5 injections be made to determine repeatability. Commonly, all other tests are checked against these 5 injections as well, providing more confidence that the system is suitable for performing the analysis.

System Suitability Functionality in Chromeleon 7

Setting up Automated System Suitability Testing in Chromeleon 7 is easy. A wizard guides the user through selecting the test criteria, calculations are performed automatically and predefined report templates simplify reporting the results.

What Parameters Can Be Tested?

Any value that Chromeleon can calculate can be used in automated system suitability testing. This includes the key values recommended by the FDA, as well as many others such as relative retention time, area %, peak width and concentration.

Visualizing Tests and Results

Figure 1 shows the Chromeleon Chromatography Studio displaying a chromatogram, processing method (middle pane) and computed results (bottom pane).

The processing method has been configured to perform the five system suitability tests recommended by the FDA. For example, Test 1 checks that the resolution of the acetophenone peak versus the next peak is > 2. When acquiring a sequence of samples using this processing

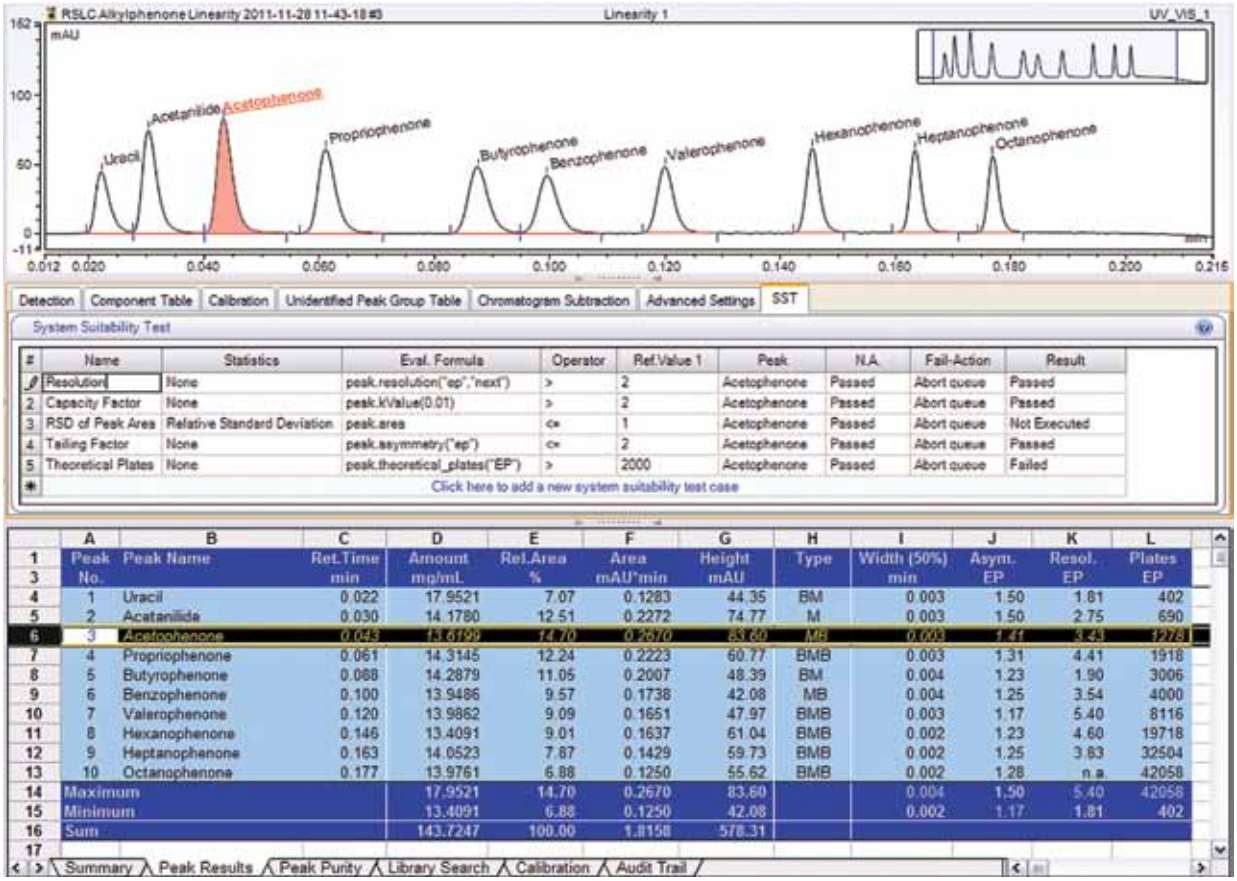


Figure 1. A Chromeleon processing method is shown above, configured with FDA recommended suitability tests

method, the "Fail-Action" column directs that if the test fails to meet this criterion, then sequence acquisition will be halted.

The "Result" column in the method panel shows the post acquisition results of each test. Note that the result for the %RSD test is "Not Executed," because the displayed injection is only the second line of the sequence, meaning that an insufficient number of samples (2 out of 5) have been analyzed. Note also that the Theoretical Plates test has failed, since the computed value (1278) is less than the test criterion (2000)

Reporting Results

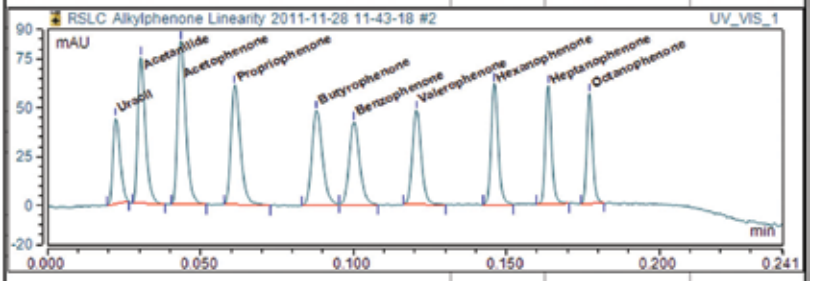
Chromeleon comes with a comprehensive set of default reports, which can be used as they are, or can be easily customized to meet the specialized needs of the lab. Predefined report tables that include system suitability related information can be inserted in any report. Figure 2 shows a customized system suitability injection report that includes the SST results, along with an overall pass/fail determination.

SST Injection Report

2 Linearity 1

Linearity 1 run 1.dad3000

Sample Name:	**Linearity 1**	*Injection Volume:*	**1.0**
Vial Number:	**RA2**	*Channel:*	**UV_VIS_1**
Sample Type:	**Calibration Standard**	*Wavelength:*	**n.a.**
Control Program:	**RSLC Linearity**	*Bandwidth:*	**n.a.**
Quantif. Method:	**RSLC Linearity**	*Dilution Factor:*	**1.0000**
Recording Time:	**11/28/2011 11:44**	*Sample Weight:*	**1.0000**
Run Time (min):	**0.24**	*Sample Amount:*	**1.0000**



System Suitability Test Results:

No.	Test Name	Operator	Ref. Value	Computed Result	Test Result
1	Resolution	>	2	3.45	Passed
2	Capacity Factor	>	2	3.35	Passed
3	RSD of Peak Areas	<=	1	n.a.	Not Executed
4	Tailing Factor	<=	2	1.46	Passed
5	Theoretical Plates (USP)	>	2000	1301.00	Failed
	Number of executed single tests:		**4**	**Total test result:**	**Failed**

Figure 2. An example of an SST report is shown above.

System Suitability Testing During Acquisition

During sequence acquisition, Chromeleon automatically computes system suitability results at the completion of each injection. The Automated System Suitability Testing can be configured such that Chromeleon can stop the sequence acquisition if a test fails. This ensures that samples are not injected onto a system that is not suitable for sample analysis, and that valuable samples and reagents are not wasted.

All acquisition-related system suitability events are recorded in the instrument audit trail.

Figure 3 shows the details logged in the audit trail describing the tests performed, the results obtained and the actions performed.

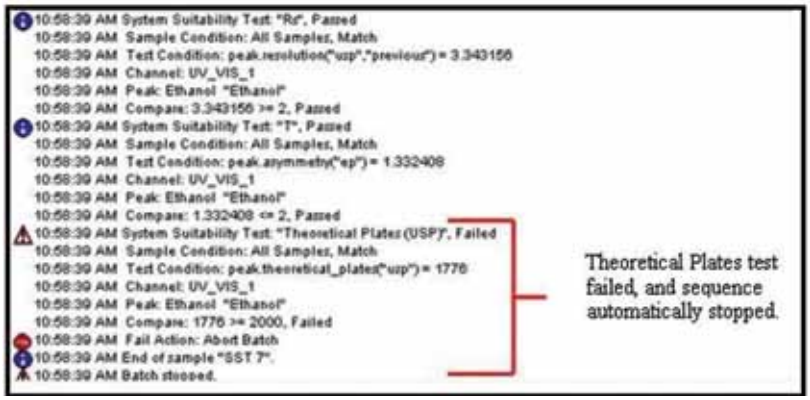


Figure 3. This table depicts an Instrument Audit Report showing a sequence abort.

Other Uses for Automated System Suitability Test Calculations

The System Suitability Testing functionality in Chromeleon is not limited to the system performance values cited by the FDA. Any chromatographic value that Chromeleon can calculate can be part of the test criteria. This means that Automated System Suitability Testing may be used for other purposes, such as testing product quality.

Comparing Results Against Specifications

In this example, tablets containing an active ingredient are analyzed for % Label Strength (%LS), which is defined as the amount of the active ingredient in the tablet as a percentage of the expected amount. The published label strength for the tablet is 50 mg ± 5%. In addition, the total amount of impurities (% Area of all other peaks in the chromatogram) must be less than 2%.

To implement these criteria, two tests are defined in Chromeleon. The first test checks that the %LS value for the active ingredient is between 95 and 105. The second test checks that the %Area of the active peak is greater than or equal to 98% (which is equivalent to testing that all the other peaks comprise less than 2% of the total peak area).

Figure 4 shows the *Chromeleon Chromatography Studio* displaying a chromatogram, the processing method with the 2 SST entries (middle panel) and the computed results (bottom panel).

Using Control Charts to Monitor Lab Performance and Data Quality

When Chromeleon System Suitability Tests are used routinely, trends in SST results can be easily monitored. Chromeleon includes tools that facilitate generating control charts to monitor chromatographic results over time. These charts can be used to identify trends or anomalies in the performance of systems, methods or users. Once such information is available, it is possible to determine the causes of the failure and appropriate corrective action, which will lead to higher data quality and greater laboratory productivity.

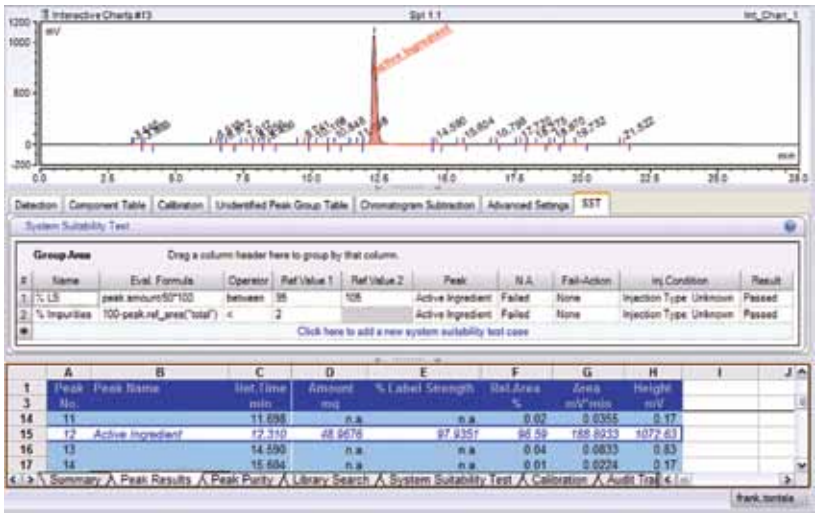


Figure 4. Chromeleon SST may be used to test Tablet Purity and % Label Strength

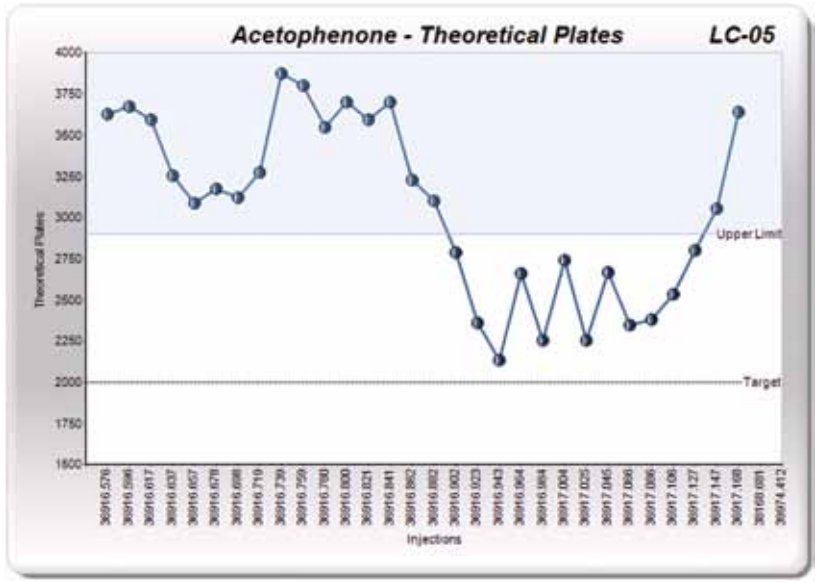


Figure 5. Chromeleon control charts can be used to monito theoretical plates over time.

Common reasons for inconsistent lab performance include:

- The instrument is not suitable for the analytical method. For example, instruments injecting samples onto a heated column that do not pre-heat the mobile phase can sometimes produce lower theoretical plates.
- The instrument needs to be serviced. For example, the detector lamp may be nearing the end of its rated life and need replacement.
- The analytical method is not optimized, resulting in marginal performance.
- The analyst is not trained sufficiently on either the analytical method or the instrument.

Figure 5 shows a control chart created automatically using the built-in query and interactive charting tools of Chromeleon. This chart displays the theoretical plates obtained on a system over time. In this example, though the system suitability checks routinely passed, clearly there was a period of sub-optimal chromatographic performance. Clicking on any data point in the chart will open the source data, allowing further investigation of the underlying cause.

Building an Automated System Suitability Test in Chromeleon

Chromeleon provides a wizard (the SST Wizard) to help users easily specify automatic system suitability tests. This wizard guides users through each step, as follows:

SST Wizard Screen 1 – Selecting the Test Parameter

On the first page of the wizard, the user selects the parameter to be tested (See Figure 6). In addition to the commonly used system performance parameters, any chromatographic or calculated result may be specified, using the <Custom Test> entry.

SST Wizard Screen 2 – Specifying the Applicable Injections

On the next wizard page, the user defines the injections to which the test will be applied. (Figure 7). For example, system suitability tests are not generally performed on blank injections (unless assessing signal noise) or samples. Rather, the tests are typically applied to the five or more specific System Suitability Test injections.

Alternately, injections may be defined by Injection Type or virtually any other property of the injection or its position in the sequence.

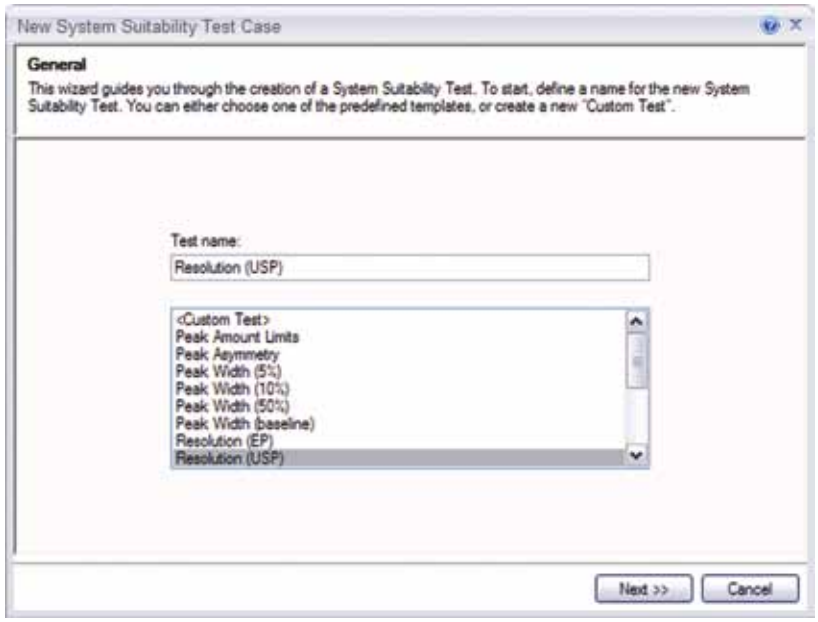


Figure 6. The System Suitability Test is selected.

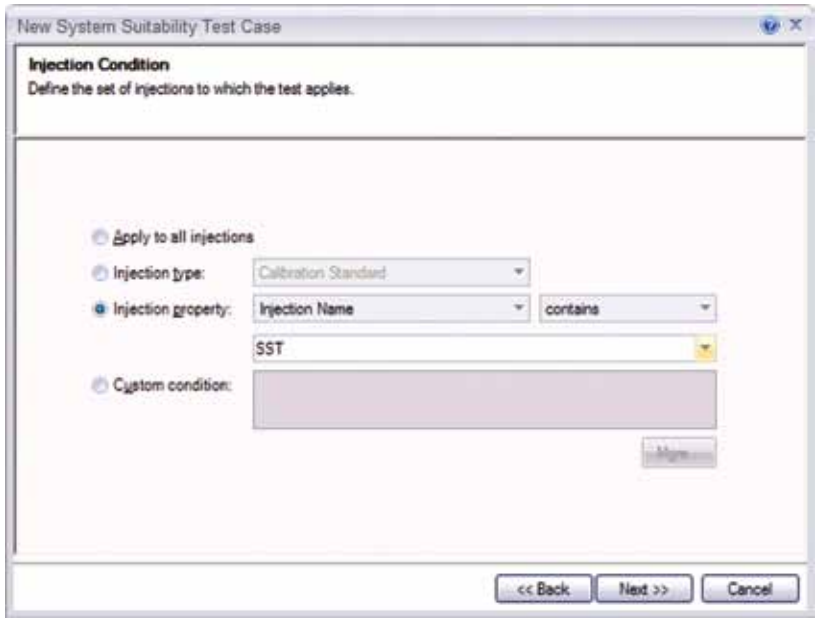


Figure 7. The set of injections for the test is specified.

SST Wizard Screen 3 – Defining the Test Criteria

The Evaluation page (Figure 8) defines how the pass/fail result will be determined. For tests on a single injection (Figure 9), the user simply specifies the operator and the threshold value. For tests that are based on multiple injections (e.g. %RSD of replicates) the type of statistical calculation and the required injection types and grouping of replicates are also specified. This automatically delays test evaluation until the required number of injections have been processed.

SST Wizard Screen 4 – Specifying the Peak and Channel Conditions

On the Peak/Channel page (Figure 10), the user defines the component(s) and channel to which the test will be applied. If the name of the peak is known, it can be selected directly. Otherwise, the peak can be selected by its position in the chromatogram (peak number) or by its properties (for example, the peak with the highest area or with the lowest width). Alternately, it is possible to have the test performed on all peaks.

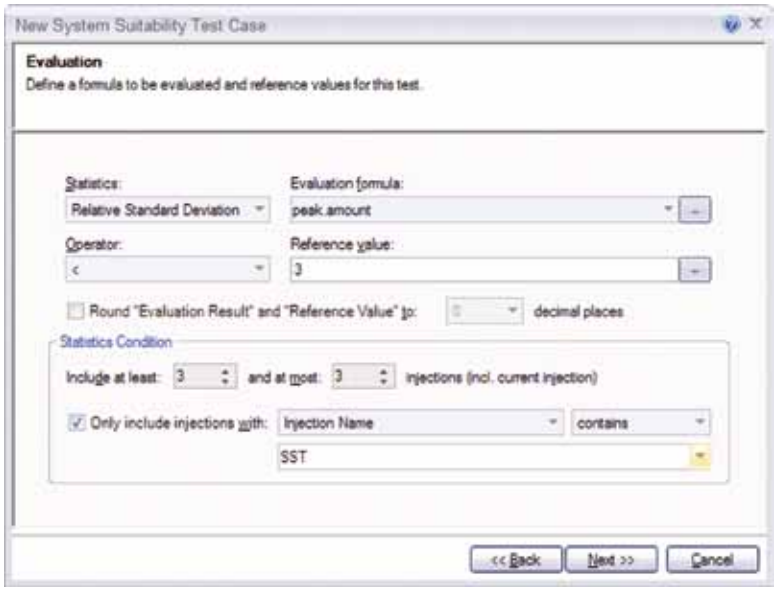


Figure 8. Evaluation criteria may be specified for a single-injection test.

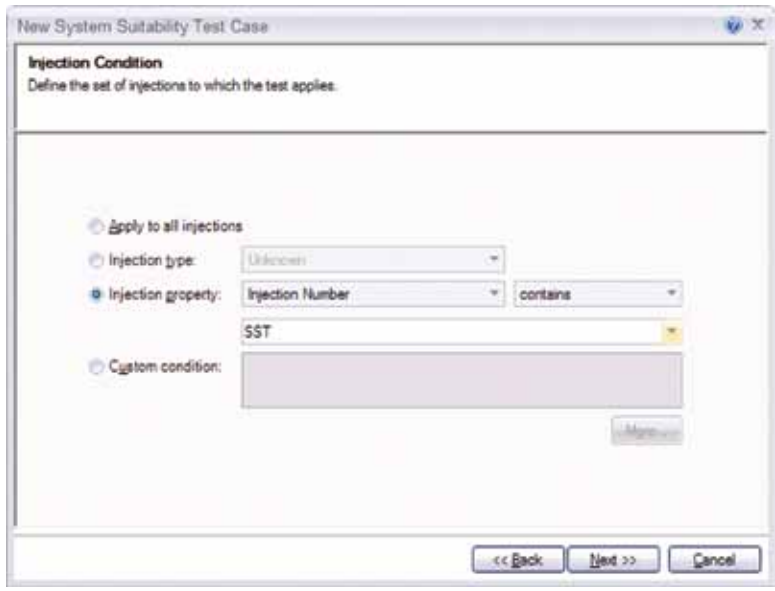


Figure 9. Evaluation criteria may be specified for a multiple-injection test.

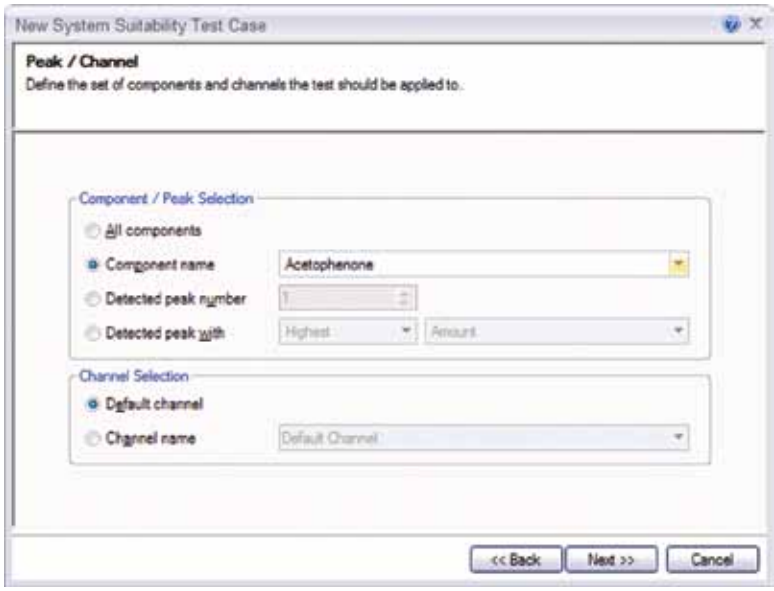


Figure 10. Tests may be applied to a specific peak and channel.

SST Wizard Screen 5 – Defining Failure Actions

The final wizard page (Figure 11) allows the user to define what test result should be reported if the test cannot be evaluated and, during sequence acquisition, what action Chromeleon should take if the test fails.

Completed Wizard

Upon completion of the Wizard, the new test is added to the SST table of the method. Double clicking on any item in the test row will reopen the appropriate wizard page (Figure 12).

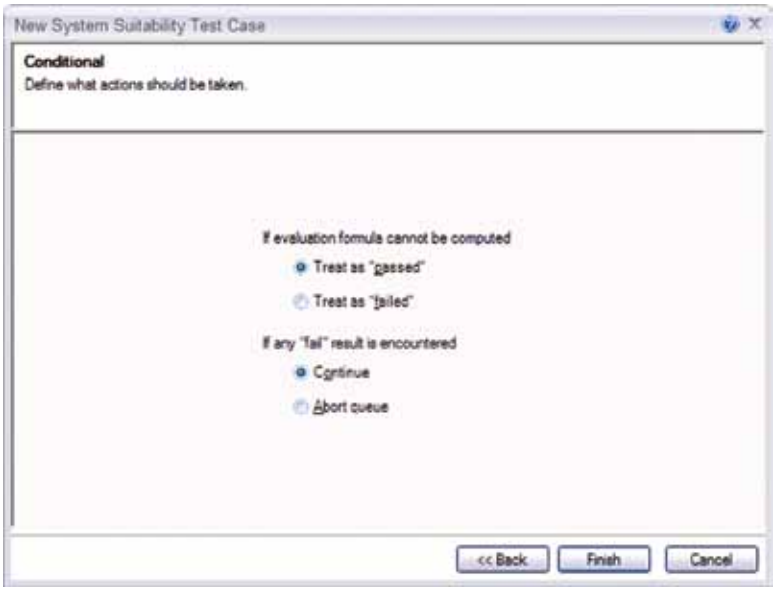


Figure 11. Conditional actions for the test results may be specified.

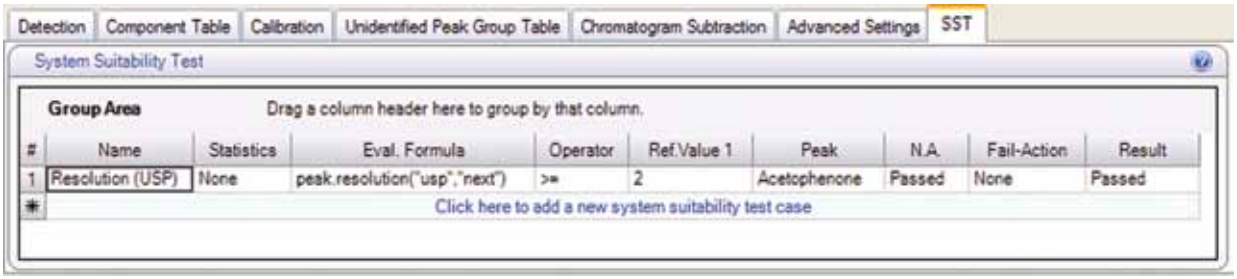


Figure 12. The new test is added to the SST table.

Conclusion

Chromeleon 7 offers many significant advantages for System Suitability Testing:

- Improved reliability of System Suitability Tests results. Chromeleon performs all calculations, eliminating any errors that can be caused by manual calculation steps.
- Faster generation of System Suitability Test results. As soon as the peak is detected, Chromeleon will automatically recalculate the system suitability results. This increases throughput by reducing delays created by having to manually recalculate the results.
- Reduced sample loss. Chromeleon can automatically stop sequences as soon as it detects a System Suitability failure. This prevents samples from being injected on to a system that is not suitable for the analytical method and eliminates sample loss.
- The automated SST functionality can be extended to perform other types of tests, such as comparing sample results against specifications.
- The functionality can also be used to generate control charts to monitor system performance in the laboratory and quickly identify systems that continually produce high levels of failure.
- By automating some of the most repetitive and time consuming steps found in laboratories, the Automated System Suitability Test functionality saves time and money and increases laboratory productivity.

References

1. Center for Drug Evaluation and Research (CDER) Reviewer Guidance Validation of Chromatographic Methods, November 1994.