Thermo Scientific Chromeleon Chromatography Data System

Performing automated system suitability testing and intelligent run control

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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, Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) includes automated system suitability testing and intelligent run control (IRC) as part of sequence acquisition and processing. Using Chromeleon CDS all SST calculations are automatically performed by the software and with IRC the system can react to changing conditions during the analysis. Based on user-specified criteria, the software can determine if the system is suitable and, if desired, even stop, pause or carry out additional tests during an analytical run if any of the tests fail. While an SST can be used in conjunction with IRC, the two features can also be employed separately. With IRC it is therefore possible to create an unconditional case that does not check any conditions and the defined actions are always executed at the end of the injection.

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 software to perform automated SST together with IRC, 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.

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FDA guidelines on system suitability testing

The FDA (CDER)[1] guidelines on Validation of Chromatographic Methods recommend the following tests and 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 (Rs)	Rs > 2
Tailing Factor (T)	T ≤ 2
Number of Theoretical Plates (N)	N > 2000

Table 1: FDA Recommended SST Specifications

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 CDS

Setting up automated system suitability testing in the software is easy and requires very little training. 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 the Chromeleon software 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 Valerophenone peak versus the next peak is > 2. When acquiring a sequence of samples using this processing method, the "Fail-Action" column details that if the test fails to meet this criteria, then a re-injection action will be performed before the sequence acquisition is aborted. The "Result" column in the method panel shows the post-acquisition results of each test. Note that the capacity factor for Acetanilide has failed specification.
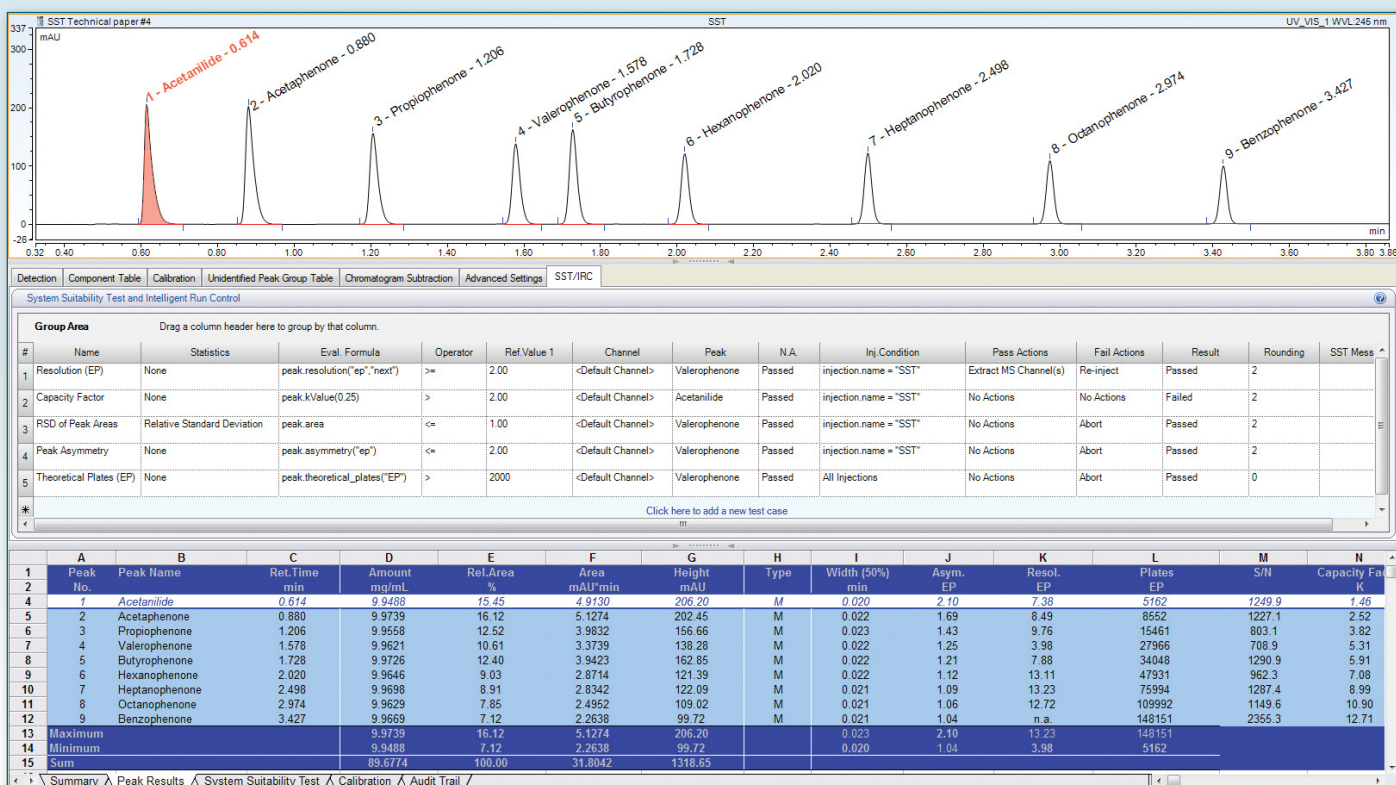

Figure 1. A processing method is shown above, configured with FDA recommended suitability tests

In the example in figure 1, the IRC actions are identified by the SST pass actions column showing MS Channel extraction and the SST fail actions column showing a re-injection (Figure 1). Valerophenone and Butyrophenone are a critical pair where resolution between the two peaks shows the greatest variation. It is possible that if the system or column had not properly equilibrated then resolution would fail, so to compensate for this scenario a re-injection of the sample can be performed to double check the result before aborting the sequence. If the SST passes specification then the extraction of the MS channels will be executed. The creation of an unconditional test case (Figure 2) employs IRC without the need of setting up any SST criteria. This means that the prescribed actions will always be executed during analysis as they will not be reliant on the outcomes of an SST result. This process essentially starts at Figure 2, and skips to the actions page in the wizard, where you will be presented with setting up the unconditional actions with associated error handling. An example could be to extract specific MS or UV channels from 3D data for alternative analysis.

Furthermore IRC removes the need for manual user intervention, since the CDS automatically reacts to changing conditions, saving time and resources (e.g. eluent waste or sample re-extraction) and minimizing human error. This can ensure more right first time analyses and allows for greater flexibility.

Reporting results

Chromeleon CDS 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 3 shows a customized system suitability injection report that includes the SST results, along with an overall pass/fail determination.
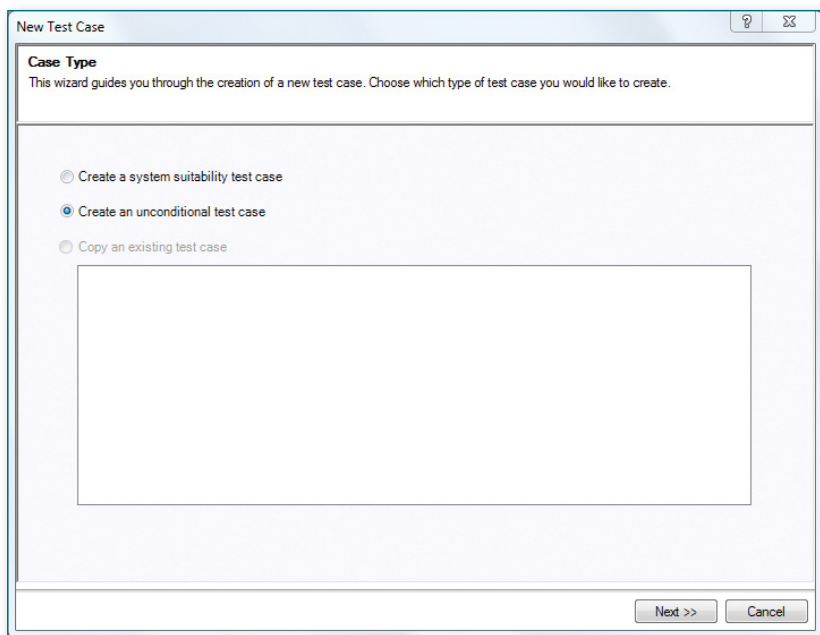

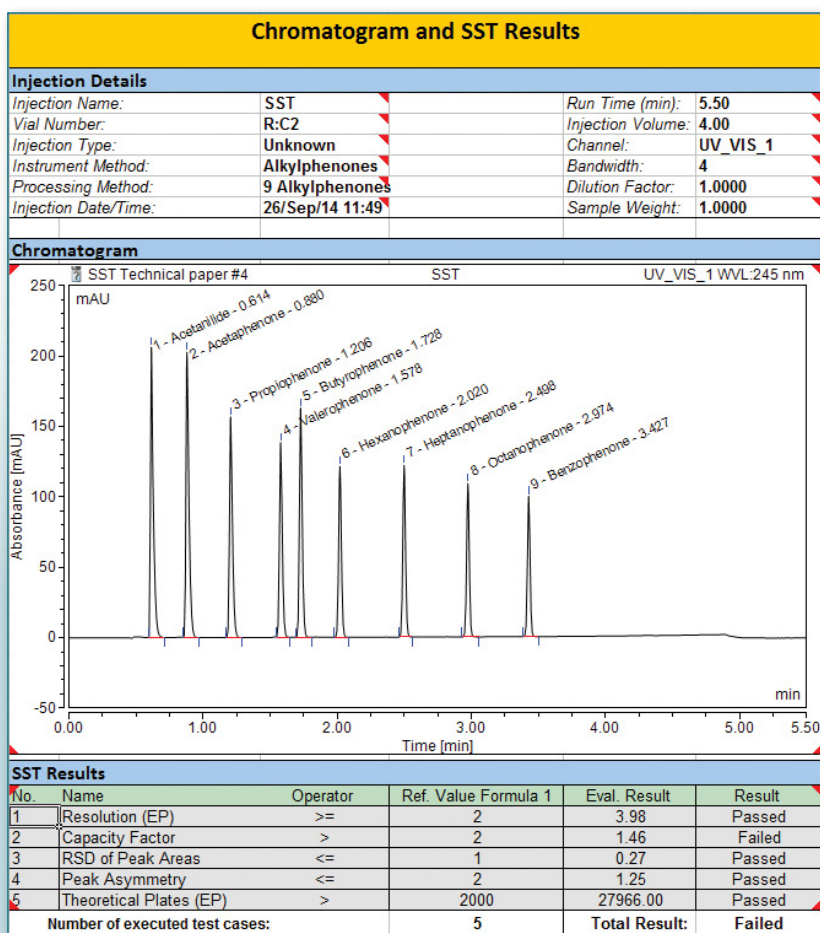Figure 2. The option of creating an unconditional test case

Figure 3. An example of an SST report is shown above.

Injection Details

Injection Name:	SST		Run Time (min):	5.50
Vial Number:	R:C2		Injection Volume:	4.00
Injection Type:	Unknown		Channel:	UV_VIS_1
Instrument Method:	Alkylphenones		Bandwidth:	4
Processing Method:	9 Alkylphenones		Dilution Factor:	1.0000
Injection Date/Time:	26/Sep/14 11:49		Sample Weight:	1.0000

SST Results

No.	Name	Operator	Ref. Value Formula 1	Eval. Result	Result
1	Resolution (EP)	>=	2	3.98	Passed
2	Capacity Factor	>	2	1.46	Failed
3	RSD of Peak Areas	<=	1	0.27	Passed
4	Peak Asymmetry	<=	2	1.25	Passed
5	Theoretical Plates (EP)	>	2000	27966.00	Passed
	Number of executed test cases:		5	Total Result:	Failed

System suitability testing during acquisition

During sequence acquisition, Chromeleon CDS automatically computes system suitability results at the completion of each injection. The automated system suitability testing can be configured such that IRC actions can be taken including aborting the sequence 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 4 shows details logged in the audit trail describing the tests performed and the actions performed.

#		Date	Time	Value	Message
1		7/16/2018	4:08:28 PM +01:00		Stopped the sequence queue run.
2		7/16/2018	4:08:28 PM +01:00		End of sequence "SST_Abort".
3		7/16/2018	4:08:28 PM +01:00		End of injection "SST".
4		7/16/2018	4:08:28 PM +01:00		An abort error occurred during the post acquisition operations.
5		7/16/2018	4:08:28 PM +01:00		Abort queue requested
6		7/16/2018	4:08:28 PM +01:00		System Suitability Test: "Theoretical Plates (EP)", Failed
7		7/16/2018	4:08:28 PM +01:00	1.219	OK to terminate instrument method chrom://ukhem-gmr0fh2/ChromeleonLocal/SST_Abort.seq/SST Test.instmeth.

Figure 4. This table depicts an Instrument Audit Report showing a sequence abort.

Other uses for automated system suitability test calculations

The SST functionality in Chromeleon CDS is not limited to the system performance values cited by the FDA. Any chromatographic value that can be calculated 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. 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 5 shows the Chromeleon Chromatography Studio displaying a chromatogram, the processing method with the 2 SST entries (middle panel) and the computed results (bottom panel).
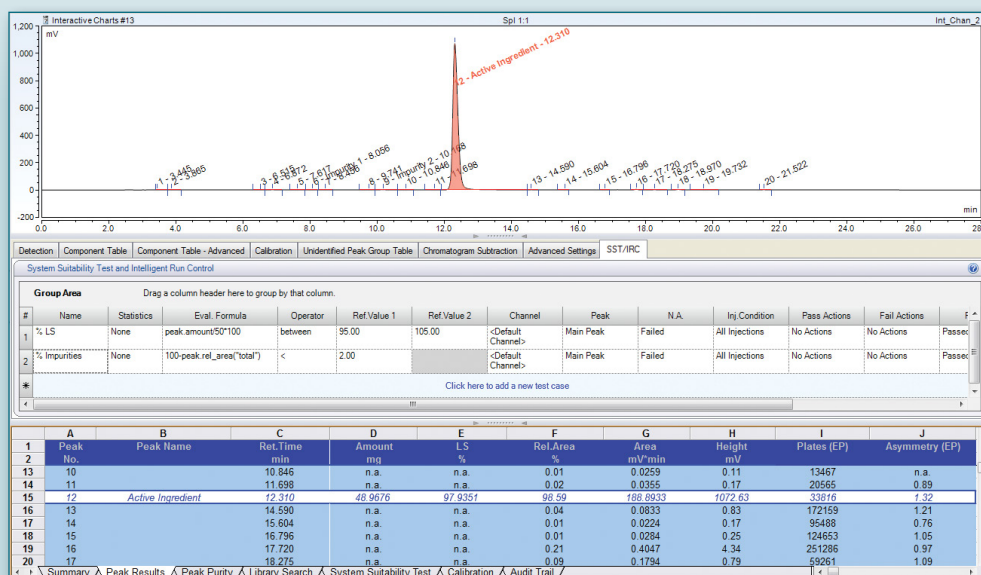

Figure 5. The SST may be used to test Tablet Purity and % Label Strength

Using control charts to monitor lab performance and data quality

When system suitability tests are used routinely, trends in SST results can be easily monitored. Chromeleon software 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.

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 6 shows a control chart created automatically using the built-in query and interactive charting tools of Chromeleon CDS. This chart displays the theoretical plates obtained on a system over the duration of a sample sequence. However this could easily be extended to trend a number of batches over a longer period of time. In this example, though the system suitability checks pass, a period of sub-optimal chromatographic performance would easily be flagged. Clicking on any data point in the chart will open the source data, allowing further investigation where needed.
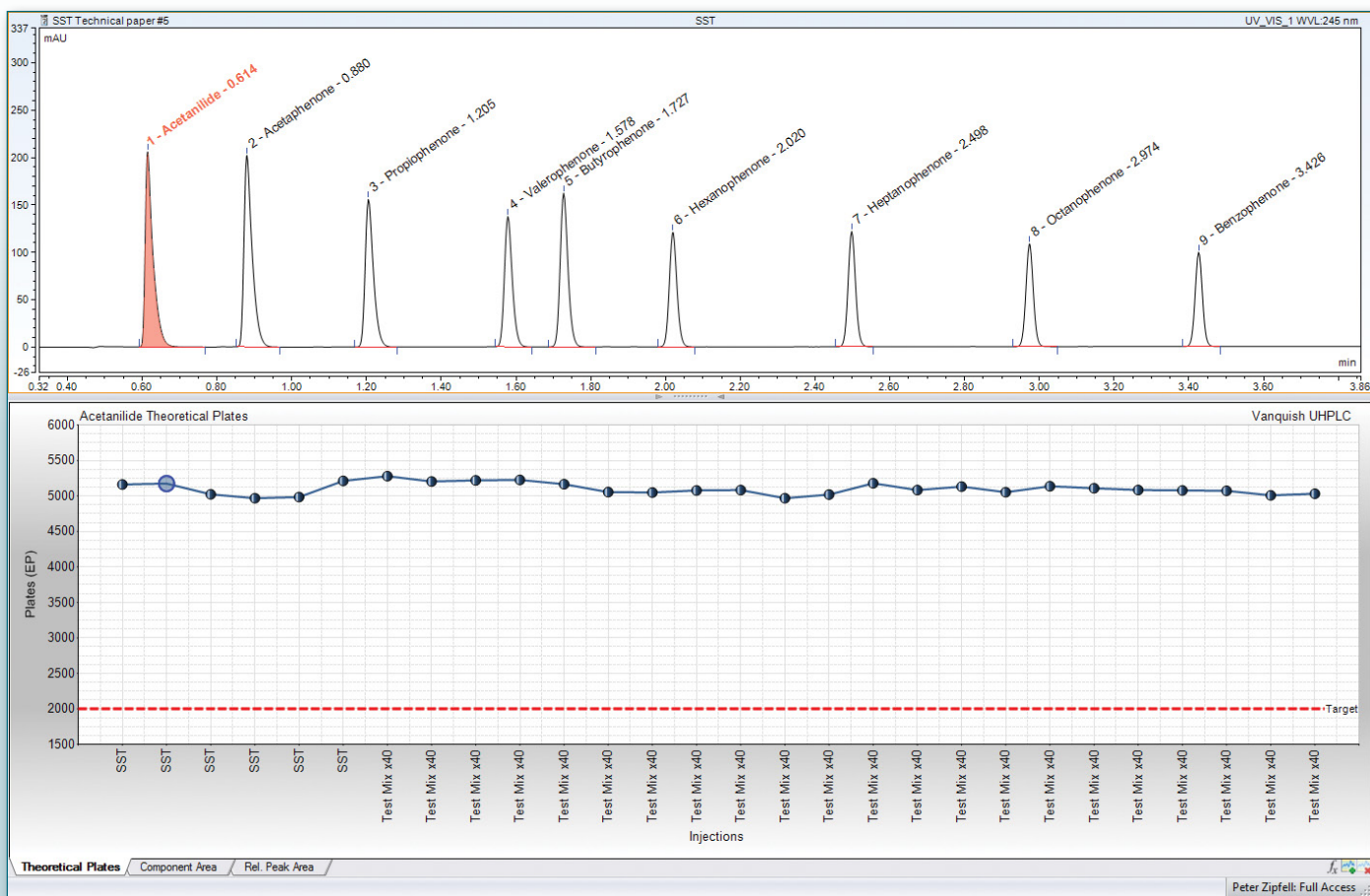

Figure 6. Control charts can be used to monitor theoretical plates over time.

Building an automated system suitability test

Chromeleon CDS 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 test is given a unique name. A series of predefined tests are available for common SST evaluations. These simplify test creation by preselecting the relevant settings on the subsequent wizard pages (see Figure 7). In addition to the commonly used system performance parameters, any chromatographic or calculated result may be specified using the <Custom Test Condition> 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 8). 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.

SST Wizard screen 3
Defining the test criteria

The evaluation page (Figure 9) defines how the pass/fail result will be determined. For tests on a single injection, 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 (Figure 10). This automatically delays test evaluation until the required number of injections have been processed. The user can also define what test result should be reported if the test cannot be evaluated.
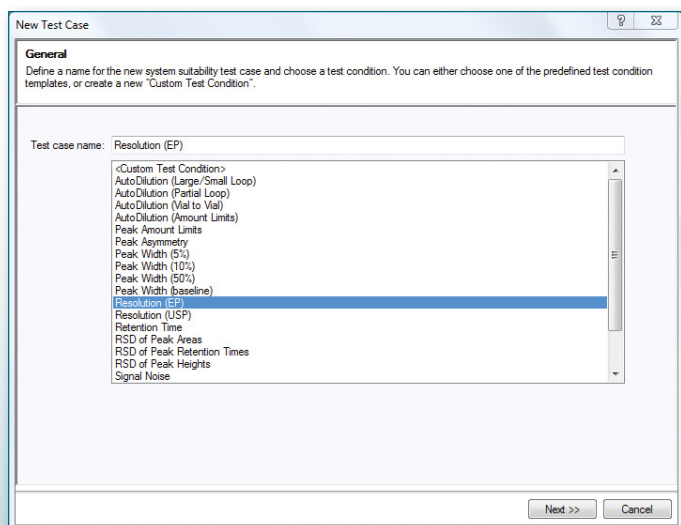

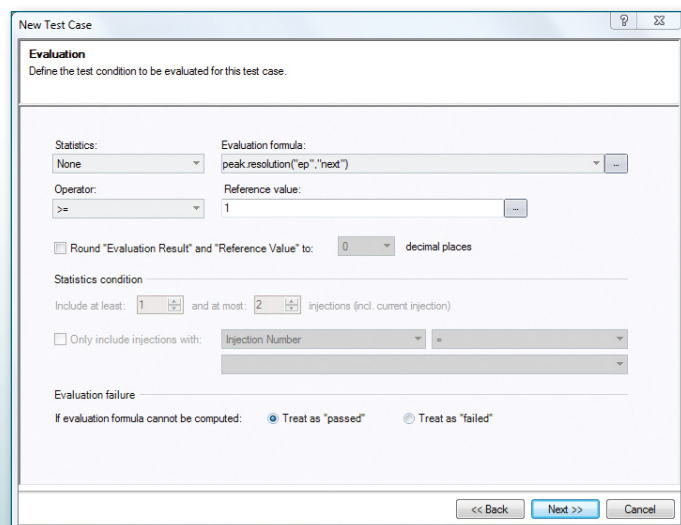Figure 7. The System Suitability Test is selected.

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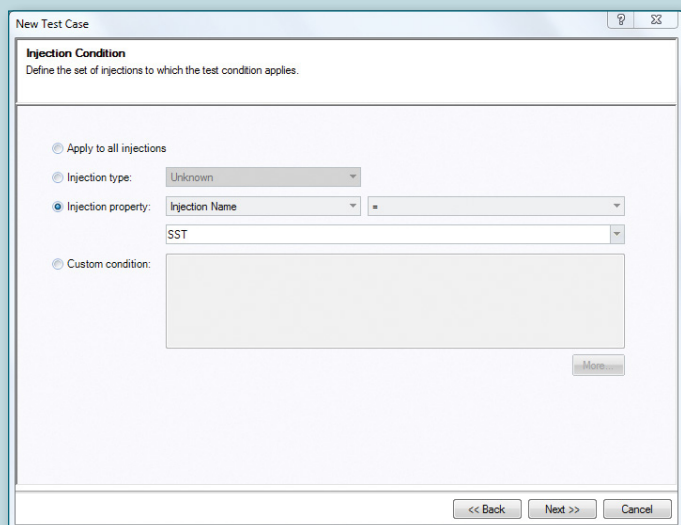

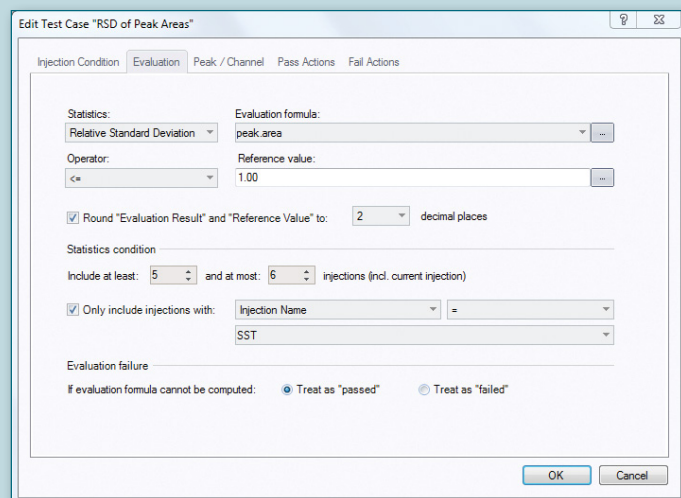Figure 8. The set of injections for the test is specified.

Figure 10. Evaluation criteria may be specified for a multiple-injection test.

SST Wizard screen 4
Specifying the peak and channel conditions

On the peak/channel page (Figure 11), 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.

SST Wizard screen 5
Defining pass actions with IRC

On the pass actions page, you can define the actions to be executed when the system suitability test case passes. In the example shown in Figure 12, we are pre-selecting a number of extracted ion chromatograms. However they will only be extracted if the SST passes. Multiple pass actions can be added if required. At the bottom of the Figure 12 we can also see error handling.
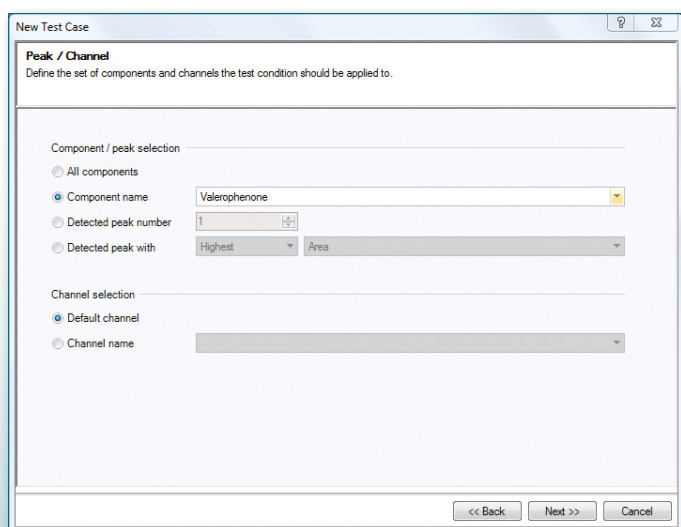
This is further illustrated in Figure 13 (exists for fail actions too), but in summary it defines whether the injection/sequence continues, pauses or aborts.

SST Wizard screen 6
Defining fail actions with IRC

Similarly with pass actions, fail actions can also be applied. Figure 14 shows the re-inject option, but any of the options can easily be highlighted and activated. Again it is possible to add multiple actions to the same test case, but if there is no requirement for either a pass or fail action then a user can skip to finish and just use the wizard for standard SST creation. However a Fail-abort action must be added if the sequence should be halted pending the outcome of the SST result.

Completed Wizard

Upon completion of the Wizard, the new test is added to the SST table of the processing method. Double clicking on any item in the test row will re-open the appropriate wizard page if further editing is needed (Figure 15).
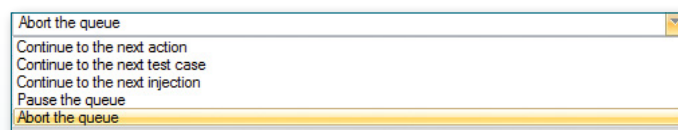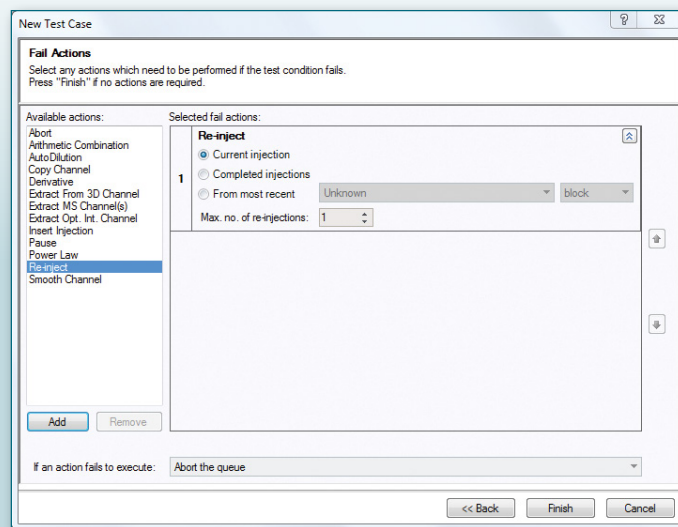

Figure 13. Error handling options.

Figure 11. Tests may be applied to a specific peak and channel.

Figure 14. Fail actions can be added to the test case.

Figure 12. Pass actions can be added to the test case.

Figure 15. The new test is added to the SST table.

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Conclusion

Chromeleon CDS offers many significant advantages for system suitability testing:

- Improved reliability of system suitability tests results. The software 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 CDS will automatically recalculate the system suitability results. This increases throughput by reducing delays created by having to manually recalculate the results.

- Reduced sample loss. The software 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.

- Intelligent run control can adapt to changing conditions during analysis and can make non-subjective decisions based on actual analytical results. This can reduce or even eliminate questioning during an audit or inspection, if used in accordance with standard operating procedures and technical controls.

- In addition IRC allows laboratory staff to focus on additional work and priorities and will also give an analytical run the best chance of completing successfully during any hours of un-attendance.

- Test cases can also be unconditional and do not have to be dependent on whether a run passes or fails. This could be used to collate or extract additional information for data analysis.

- The automated SST functionality can be extended to perform other types of tests, such as comparing sample results against known 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 and intelligent run control 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.

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