Using Interactive System Suitability in Empower 2

Summary

New features and functionality for Interactive System Suitability were implemented in Empower 2 Build 2154. For example, users can calculate user-defined summary statistics from multiple results and test the values against System Suitability conditions in real-time. This document clarifies the use of those features and presents important considerations for users implementing automated system suitability testing and reporting.

The following recommendations emerge from the discussion in this document on the use of interactive system suitability with intersample summary fields in Empower 2:

1. If using the Reinject on fault suitability mode in Run Samples, create a summary custom field that will only report the data for the most recent injections. This document provides an example of how to achieve that functionality.

2. For user defined limits specified in the Processing Method, in the Limits tab, the selection in the Check Limits field should always be set to During Custom Summary to ensure that custom field summary results are tested against System Suitability limits at the time of custom field processing only.

3. If you use intersample calculations that include a Summary type intersample calculation, the sample set must have a Summarize Custom Fields line. The placement of this Summarize Custom Fields line impacts the timing of custom field processing. This line is typically placed at the end of the sample set so that all the previous injections can be used in the calculations. In the example used in this technical note the Summarize Custom Fields line is at the end of suitability injections because we want the system to stop if a suitability failure occurs.

4. Reporting should be done manually if a suitability failure halts the sample set before Empower can execute the Report function in Run Samples.

These recommendations should be considered as complementary to existing Waters documentation available on the Waters Connections Elite website and in Empower 2 online help.

Introduction

A common requirement for HPLC/UPLC™ analyses in many regulated environments is the assurance that the instrument is suitable for the test it is about to perform. This System Suitability is commonly demonstrated at the beginning of every series of analyses before the results of the samples in that test can be considered valid. In HPLC/UPLC terms this requirement is often met by running a series of standards in replicate and testing the precision of the measurement by checking the deviation in the area counts for the peak(s) of interest. This involves calculating and checking the area count %RSD for the peak(s) in the System Suitability runs. Such test runs always precede the samples in the analysis. When the test injections fail to pass the suitability criteria, analysts would typically halt the sample set in order to avoid unnecessary consumption of precious samples and solvent by a system that is not performing satisfactorily.
Empower 2 offers the ability to enforce System Suitability requirements in an automated, unattended way. An example of this automated System Suitability feature is presented in this document along with a detailed description of the Empower 2 setup necessary to make it happen. The steps involved include:

1. Defining the calculated result to be tested (e.g., Area %RSD).
2. Setting limits on the test (e.g., Area %RSD < 2%)
3. Setting up the sample set to
   a. Identify the System Suitability injections,
   b. Calculate the summary result
   c. Define how you wish the system to respond to a failed test
   d. Generate reports (optional)

In the following pages, we will illustrate these steps and clarify how different selections in the processing methods, sample set and Run Samples window can affect the reporting of System Suitability testing results.

This document is intended to complement existing Waters documentation available on the Waters website and in Empower 2 online help, and assumes that the reader is familiar with creating and using custom fields, creating reports and processing data in Empower.

**Automating System Suitability**

**Step 1: Define the Intersample Calculations**

In order to test for the %RSD of Area for our peaks of interest, a Peak Level custom field must be created for the Project where these calculations take place. From the project’s properties in Configuration Manager of Empower 2, create a new Peak Level custom field. The following format applies when specifying intersample custom calculations:

```
Label.Injection.Channel.SUMMARY_FUNCTION(Statement)
```

For the purpose of this discussion, we will use the calculated custom fields shown in Figure 1. In addition to specifying the correct formula for the custom calculation, it is also important to set the proper Search Order when creating a custom field. The Search Order specified will be Result Set Only so that inter-sample calculations are performed only within the current result set.

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1 Refer to the Empower 2 Help topic “Creating intersample summary calculations for peaks and results”, and related help topics for more guidelines and examples for custom calculations.
The Peak type custom field Latest_Pcnt_RSD is defined for comparison with the built-in summary values for results in Empower. A Result type field MaxResultID is also defined.

Latest_Pcnt_RSD explicitly references only the latest result IDs for a given sample set, as determined by the Result type field MaxResultID. Under certain conditions, such as when faulted suitability injections are reinjected, this custom field would otherwise return values different than those reported by default. This issue is discussed in more detail toward the end of the document.

In the example Peak type custom field’s formula:

- The ss% portion of the formula is the label that is used to identify which injections in the sample set are the System Suitability runs.
- The % sign denoting injections specifies all injections of a sample line.
- No channel is specified. So Empower uses the most recent result (including the current result, if it matches) with the label ss and all injections with the same channel as the result being processed.

In the example Result Type custom field formula:

- “SAME” in the label and injection position of the syntax references the previous injections in the sample set that have the same label and injection as the current injection.
Step 2: Define the System Suitability Limits

The next step is to enter the desired limits in the Limits Tab of the Processing Method. The Limits tab is used to specify the error and warning limits that can be set individually so that peak values can be monitored as part of System Suitability testing. Figure 2 shows the Limits tab of the Processing Method. After the components have been entered for the method they will appear on this tab. With the peak of interest selected, you can select the custom field to be checked for limits on the lower table. In this example the upper limit for the custom field, Latest_Pcnt_RSD, is set to 0.1%.

![Figure 2 -- Specifying the Error and Warning Limits for Individual Components in the Limits Tab](image)

In Figure 2, a new selection called During Custom Summary appears in the Check Limits column. By selecting During Custom Summary the user instructs the software to test for the specified limits during data acquisition at the point that the special function Summarize Custom Fields is executed in the Sample table of the Run Samples window. If Always is selected, then the results for each injection would be tested for the specified limits immediately following that injection. If During Custom Summary is selected, then the summarized results for all the System Suitability injections are tested for the specified limits. So, if the user selects Always instead of During Custom Summary, the intersample custom calculations would generate null values for the corresponding custom fields in the results, because the intersample custom fields would not yet have been calculated.

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2 If you use intersample calculations that include a Summary type intersample calculation, the sample set must have a Summarize Custom Fields line. Summarize Custom Fields calculates all intersample summary custom fields when in Run and Process, and Run and Report modes. The placement of this Summarize Custom Fields line impacts the timing of result calculations. Typically, this line is at the end of the sample set.

Using Interactive System Suitability in Empower 2
Step 3: Set Up and Start the Sample Set

Once the custom fields are defined, the limits specified, and the Processing Method included in the Method Set, it is possible to run a sample set which tests in real-time the System Suitability summary results against the specified limits.

In practice, Empower users may choose to either have replicate suitability injections defined on a single line in the sample set method, or to assign separate lines for each injection. The example custom fields presented in Figure 1 are applicable in both of those scenarios. The representative screenshots in Figure 3a and Figure 3b both show the Run Samples screen of Empower 2 with the sample set stopped following a suitability failure. In Figure 3a, replicate suitability injections are defined on a single line in the sample set method. In Figure 3b, each injection has a separate line.

Figure 3a – Example Run Sample Window in Empower 2 with Sample Set Stopped After Row 3 Due to Suitability Failure. Six Replicate Suitability Injections Are Defined for a Single Line With Label ss
Failure on row 8

Figure 3b – Example Run Sample Screen in Empower 2 with Sample Set Stopped After Row 8 Due to Suitability Failure. Each of the Six Suitability Injections Is On a Separate Line With a Unique Label

The System Suitability replicate injections are marked by a label to match the label in the custom field formula. In Figure 3a, the row with six System Suitability replicate injections is marked by a label ss. In Figure 3b, each of the six System Suitability injection rows is marked by a unique label, ss1, ss2, ss3, etc.

**NOTE:** The label is case sensitive.

In both figures, Run and Process is shown selected for the Run Mode which instructs Empower 2 to produce results as it acquires each injection. For the Suitability Mode Reinject on Fault is selected, so that Empower will reinject the suitability samples if the limits for %RSD for Area exceeded the limit set in the Processing Method.
Since the response to a fault (the Suitability Mode) is Reinject on fault, the Label Reference for Summarize Custom Fields function points to the line in the sample set (e.g., ss or ss1) where the reinjections would start.3

The Report function is optional. It is inserted in our example to produce a Peak Summary Report at the end of the six System Suitability injections. The Label Reference column for the Report function also contains the value ss* as a means of instructing Empower 2 to summarize only the suitability injections into the Peak Summary Report.

The order of functions Report and Summarize Custom Fields can be important in Run Samples. Since the Report function is placed after the Summarize Custom Fields function, the results are only reported if the sample set does not stop due to a fault. However, if a suitability fault occurs, then the software does not proceed to execute the Report line and reports are not automatically created. The user could then view the results for the last six injections in Review and generate reports manually.

With the sample set ready to run, click Start to produce a dialog box. Here the user specifies the sample set name, selects the run mode, selects the suitability mode, selects a printer for the reports from this run and, finally, selects a Shutdown Method, if necessary.

Interpreting System Suitability Failure Results

When the System Suitability injections are completed, the software calculates the %RSD for Area on all the System Suitability injections. Then the sample set produces the selected summary report. That summary value for each peak is stored in each of the System Suitability individual results. The software tests the summary value against the limits for that field as specified in the Processing Method (set to 0.1% in this example) and a suitability fault is generated for any peak which exceeds the limit. A suitability fault causes the software to resort to the action that was selected as the Suitability Mode when the Sample set was started. If the suitability mode is Stop on fault, then Empower stops acquisition immediately upon failure. If the suitability mode is Reinject on fault, then Empower reinjects the suitability samples as defined in the Label Reference column. If the reinjected samples fail, then acquisition stops. In figures 3a and 3b, the status bar at the bottom of the screen indicates that a Suitability Failure has occurred and the system is idle. The fault is due to the fact that peak 3 exceeded the defined limits in our example (see Figure 4 below).

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3 If the suitability mode was Stop on Fault which stops the Sample Set when a limit is exceeded, then the Label Reference in the Summarize Custom Fields line could be blank.
Figure 4 – Example Review Window In Empower 2, Showing Faulted Peak 3 With Custom Field Pcnt_RSD_for_Area Values Having Exceeded the 0.1% Limit Set in the Processing Method

Figure 5 shows a portion from a System Suitability Summary Report for a component Peak 3. Note that each of the six example suitability injections was reinjected once following the System Suitability failure and results were created for all twelve injections.

Note that different values are reported in Figure 5 for the built-in summary calculation, % RSD, and the user-defined custom calculation, Latest_Pcnt_RSD. The value for Latest_Pcnt_RSD, explicitly references only the latest result IDs for a given sample set, and it differs from the calculated value for % RSD for peak 3, because samples were reinjected on fault thus producing two sets of injections. The % RSD value for peak 3 in the initial injections of the six samples was 8.629. After reinjection, the % RSD for peak 3 was recalculated using all twelve Result IDs, thus producing the value 8.326. In contrast, the value for Latest_Pcnt_RSD, viz. 8.834, is identical to the built-in % RSD value for the six reinjected samples only.

Note that these discrepancies are only apparent when the System Suitability results are processed real-time and when Reinject on fault suitability mode is selected in Run Samples. This is because real-time processing of sample sets containing reinjected samples differs from background processing of the same sample set. When the same sample set is processed using background processing, only the reinjected injections are processed. Therefore, if the sample set were processed using background processing, then the values of both fields, % RSD and Latest_Pcnt_RSD, would be identical.

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4 This is a known defect: PCS #37930. It is being considered for a fix in a future Service Pack.
### System Suitability Summary Report

Reported by User: John Smith (SmithJ)

**Project Name:** systemtest\Stop on Fault Test

**Name:** Peak 3

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Name</th>
<th>Vial</th>
<th>Vial Id</th>
<th>RT</th>
<th>Area</th>
<th>Result Id</th>
<th>Max Result Id</th>
<th>Latest_Pcnt_RSD (latest results only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 System Suits 1</td>
<td>Peak 3</td>
<td>1:A,1</td>
<td>3334</td>
<td>0.788</td>
<td>43171</td>
<td>3337</td>
<td>3361</td>
<td>8.834</td>
</tr>
<tr>
<td>2 System Suits 2</td>
<td>Peak 3</td>
<td>1:A,2</td>
<td>3338</td>
<td>0.767</td>
<td>42758</td>
<td>3341</td>
<td>3365</td>
<td>8.834</td>
</tr>
<tr>
<td>3 System Suits 3</td>
<td>Peak 3</td>
<td>1:A,3</td>
<td>3342</td>
<td>0.766</td>
<td>34144</td>
<td>3345</td>
<td>3369</td>
<td>8.834</td>
</tr>
<tr>
<td>4 System Suits 4</td>
<td>Peak 3</td>
<td>1:A,4</td>
<td>3346</td>
<td>0.767</td>
<td>42877</td>
<td>3349</td>
<td>3373</td>
<td>8.834</td>
</tr>
<tr>
<td>5 System Suits 5</td>
<td>Peak 3</td>
<td>1:A,5</td>
<td>3350</td>
<td>0.767</td>
<td>42817</td>
<td>3353</td>
<td>3377</td>
<td>8.834</td>
</tr>
<tr>
<td>6 System Suits 6</td>
<td>Peak 3</td>
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<td>0.766</td>
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<td>3358</td>
<td>0.767</td>
<td>43432</td>
<td>3361</td>
<td>3381</td>
<td>8.834</td>
</tr>
<tr>
<td>8 System Suits 2</td>
<td>Peak 3</td>
<td>1:A,2</td>
<td>3362</td>
<td>0.766</td>
<td>42746</td>
<td>3365</td>
<td>3365</td>
<td>8.834</td>
</tr>
<tr>
<td>9 System Suits 3</td>
<td>Peak 3</td>
<td>1:A,3</td>
<td>3366</td>
<td>0.765</td>
<td>33949</td>
<td>3369</td>
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<td>8.834</td>
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<td>12 System Suits 6</td>
<td>Peak 3</td>
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<td>3378</td>
<td>0.767</td>
<td>42794</td>
<td>3381</td>
<td>3381</td>
<td>8.834</td>
</tr>
</tbody>
</table>

**Initial Samples Only**

**Component Summary For Area**

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Inj</th>
<th>Vial</th>
<th>Vial Id</th>
<th>Peak 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 System Suits 1</td>
<td>1</td>
<td>1:A,1</td>
<td>3334</td>
<td>43171</td>
</tr>
<tr>
<td>2 System Suits 2</td>
<td>1</td>
<td>1:A,2</td>
<td>3338</td>
<td>42758</td>
</tr>
<tr>
<td>3 System Suits 3</td>
<td>1</td>
<td>1:A,3</td>
<td>3342</td>
<td>41444</td>
</tr>
<tr>
<td>4 System Suits 4</td>
<td>1</td>
<td>1:A,4</td>
<td>3346</td>
<td>42877</td>
</tr>
<tr>
<td>5 System Suits 5</td>
<td>1</td>
<td>1:A,5</td>
<td>3350</td>
<td>42817</td>
</tr>
<tr>
<td>6 System Suits 6</td>
<td>1</td>
<td>1:A,6</td>
<td>3354</td>
<td>42851</td>
</tr>
</tbody>
</table>

**Mean**

<table>
<thead>
<tr>
<th>Mean</th>
<th>Std. Dev.</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>41436</td>
<td>3448</td>
<td>8.326</td>
</tr>
</tbody>
</table>

**Reinjected Samples Only**

**Component Summary For Area**

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Inj</th>
<th>Vial</th>
<th>Vial Id</th>
<th>Peak 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 System Suits 1</td>
<td>1</td>
<td>1:A,1</td>
<td>3358</td>
<td>43432</td>
</tr>
<tr>
<td>2 System Suits 2</td>
<td>1</td>
<td>1:A,2</td>
<td>3362</td>
<td>42746</td>
</tr>
<tr>
<td>3 System Suits 3</td>
<td>1</td>
<td>1:A,3</td>
<td>3366</td>
<td>43949</td>
</tr>
<tr>
<td>4 System Suits 4</td>
<td>1</td>
<td>1:A,4</td>
<td>3370</td>
<td>42701</td>
</tr>
<tr>
<td>5 System Suits 5</td>
<td>1</td>
<td>1:A,5</td>
<td>3374</td>
<td>42727</td>
</tr>
<tr>
<td>6 System Suits 6</td>
<td>1</td>
<td>1:A,6</td>
<td>3378</td>
<td>42794</td>
</tr>
</tbody>
</table>

**Mean**

<table>
<thead>
<tr>
<th>Mean</th>
<th>Std. Dev.</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>41391</td>
<td>3657</td>
<td>8.834</td>
</tr>
</tbody>
</table>

**Figure 5** – A Portion of the Example System Suitability Summary Report Showing Results Calculated Using Six System Suitability Injections and Six Reinjections that Occurred After the Suitability Failure

Using Interactive System Suitability in Empower 2
Recommendations

The following recommendations may be helpful in ensuring the effective usage of interactive System Suitability when used with intersample summary fields in Empower 2:

1. If using the Reinject on fault suitability mode in Run Samples, create a summary custom field that will only report the data for the most recent injections. This document provides an example of how to achieve that functionality.

2. For user defined limits specified in the Processing Method, in the Limits tab, the selection in the Check Limits field should always be set to During Custom Summary to ensure that custom field summary results are tested against System Suitability limits at the time of custom field processing only.

3. If you use intersample calculations that include a Summary type intersample calculation, the sample set must have a Summarize Custom Fields line. The placement of this Summarize Custom Fields line impacts the timing of custom field processing. This line is typically placed at the end of the sample set so that all the previous injections can be used in the calculations. In the example used in this technical note the Summarize Custom Fields line is at the end of suitability injections because we want the system to stop if a suitability failure occurs.

4. Reporting should be done manually if a suitability failure halts the sample set before Empower can execute the Report function in Run Samples.

These recommendations should be considered as complementary to existing Waters documentation available on the Waters Connections Elite website and in Empower 2 online help.

If you have any further questions, you can contact Waters Technical Support (dial 1-800-252-4752, option # 3 or create an e-ticket at http://www.waters.com/techassist).