AssureSST

- System Suitability Test

User Manual

Version 003

NMR

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1 Overview

AssureSST runs a user-selected set of experiments to monitor instrument performance. AssureSST is configured and controlled through the IconNMR automation software package which uses TopSpin to acquire and evaluate data. Upon completion of the SST, a PDF report is generated summarizing the specifications tested and the results obtained.

2 AssureSST Configuration

AssureSST parameters on the spectrometer are accessed through the IconNMR Configuration window (command "iconc" from the TopSpin command line). In the resulting window, select **AssureSST** in the pane on the left. The AssureSST section has three tabs (1) **System Suitability Test (SST)**, which allows the user to activate SST, schedule tests, and print reports (2) **SST Standard Tests**, which allows the user to select from the standard tests and set the specifications for SST, and (3) **SST User Tests**, which allows the user to set up the acquisition parameter set and analysis method for their own tests. Setup for AssureNMR is discussed in Chapter 7.

The SST (up to six individual standard experiments plus up to four user-defined experiments) as a whole must pass before any other samples queued in IconNMR can be acquired while under AssureNMR operation. The six standard tests are covered in the chapter *SST Standard Tests Tab [▷ 5]*. The user-defined tests are covered in section *SST User Tests [▷ 11]*. The user can modify this behavior through the IconNMR Configuration window, by selecting the **Fail Safe/Error Handling** window under Automation from the pane on the left. Unchecking the box beside 'Stop the run when 'Assure' System Suitability Test reports specification failure' allows queued samples to run even after the SST fails.

> **i**
> Any changes to the System Suitability Test (e.g. changing a specification or turning off one of the tests) immediately invalidates the previous suitability run. Thus, after a change, the test will be automatically queued when starting IconNMR even if the last successful SST was within the time specified.

It is possible to queue the SST at any time as NMR SuperUser (Chapter Running IconNMR: Supervisor).
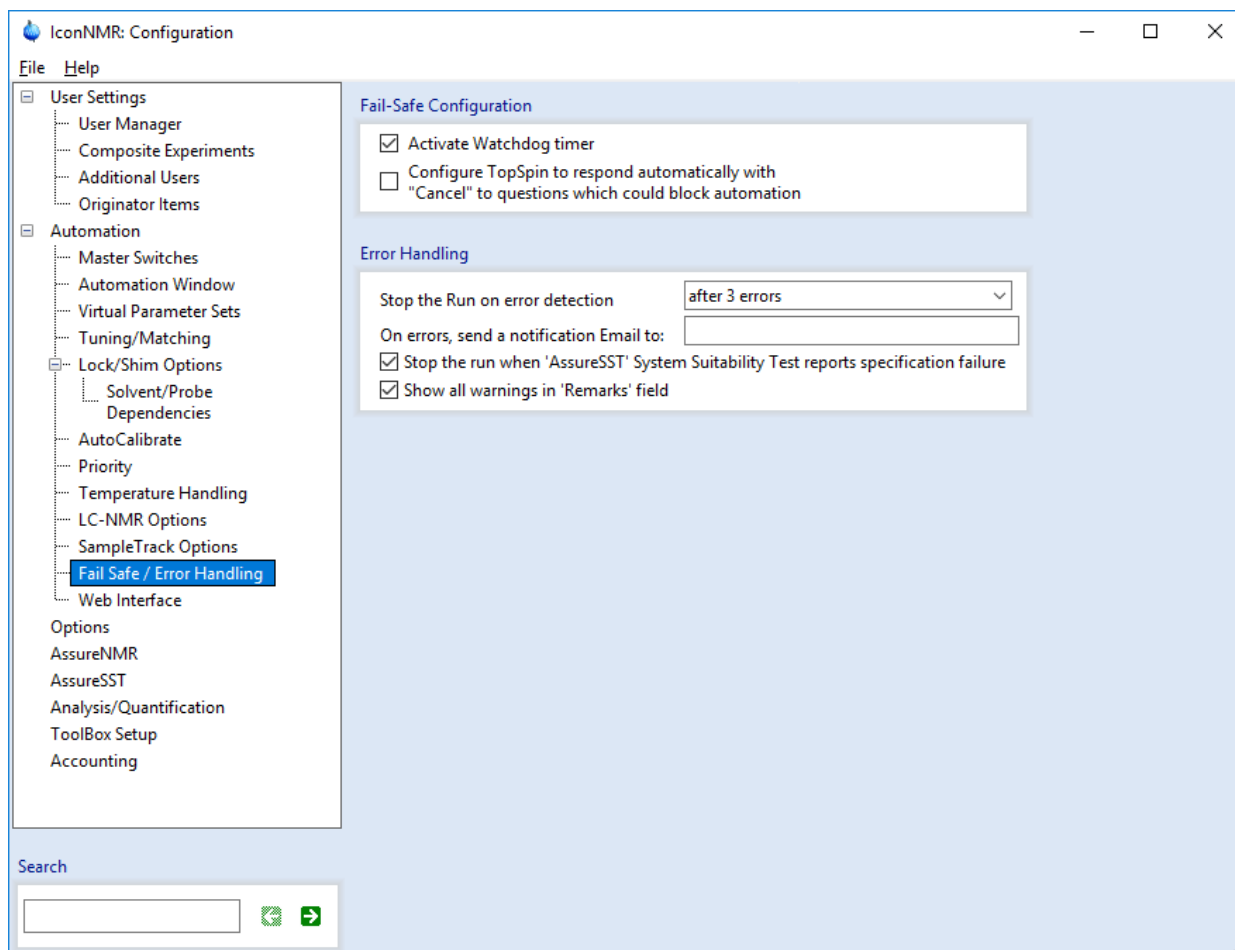
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Figure 2.1: Error Handling options that affect AssureSST.

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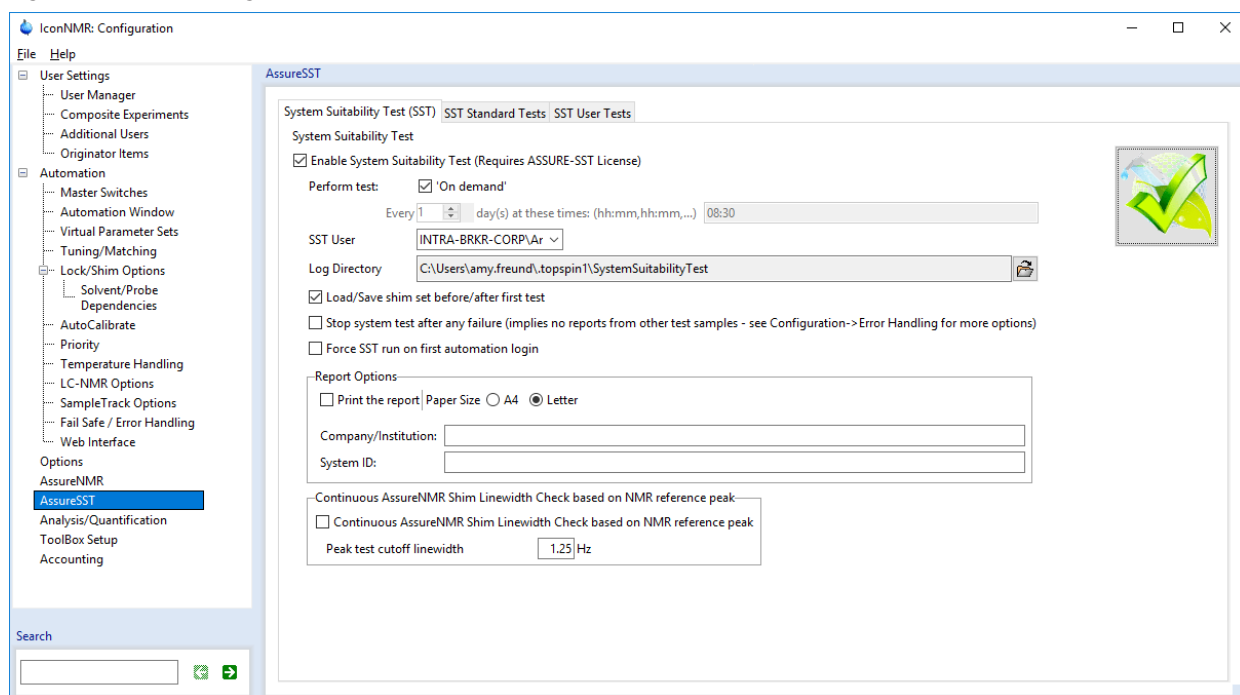H172419_3_003

2.1   System Suitability Test (SST) Tab

Figure 2.2: The System Suitability Test (SST) tab in the AssureSST section of the IconNMR Configuration window.

To activate AssureSST, check the first box on the **System Suitability Test (SST)** tab, 'Enable System Suitability Test (Requires ASSURE-SST License)'. Note that the AssureSST license is included with AssureNMR.

The System Suitability Test is automatically queued for acquisition as required to meet the time set in the entry for 'Perform test every: x day(s) at these times: (hh:mm,hh:mm,…)'. Note the times use a 24-hour format. If other samples are queued (e.g. for AssureNMR analysis) at the time when the SST is required, then the data acquisition of the queued samples will wait until the SST has been performed successfully. Strategies and flexibility in the SST were designed to accommodate experiments which might exceed the available time. Experiments that run past the set time for the next SST will remain in the queue until sufficient time is available to run the sample(s).

The option to 'Load/Save shim set before/after first test' provides a mechanism for AssureSST to adjust to shim changes over time. This is done through a default shim file that is used for all samples including the system suitability test samples. Once a ^{1}H Lineshape suitability test has been completed successfully, the shim set is updated by writing to 'IconNMRShimSet.probeName' where the probeName extension is the name from the edprobe table for the current probe. For more information on probe identification, type "help edprobe" in TopSpin.

When 'Load/Save shim set before/after first test' is checked, it loads the shims saved as 'IconNMRShimSet.probeName' before shimming. In this case, the solvent dependent shim files in the **Solvent/Probe Dependencies** are not used.

- To use this feature, check the box 'Load/Save shim set before/after first test'.
- Save the desired starting shims by typing "wsh IconNMRShimSet.probeName" from the TopSpin command line.

The SST as a whole is the criterion for a properly functioning spectrometer for the purposes of the AssureNMR software. As a result, failure of any component test is reported after the complete set of required tests is measured. For example, if the system administrator requires four tests (^{1}H Lineshape, ^{1}H Sensitivity, ^{13}C sensitivity and Temperature) for the SST, then all four tests must be completed before a final 'system pass' or 'system fail' result is obtained. Optionally, the user may select 'Stop system test

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after any failure' to immediately halt the acquisition after any test fails. When this box is checked then the tests will stop after the first failed system suitability test and all subsequent system suitability tests will be cancelled until the issue is resolved and AssureSST is restarted.

• Check 'Stop system test after any failure' to halt the SST after any failed test.

Automatic generation of a PDF report (see example in Chapter Reports) for SST results occurs when the 'Print the report' option is active. The administrator can customize the 'Company/Institution' and 'System ID' in the report by filling in the corresponding fields.
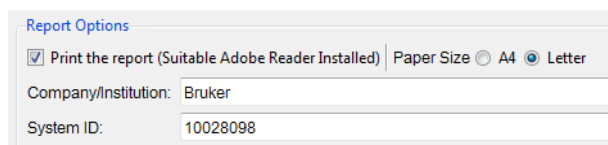

Figure 2.3: Report Options for AssureSST

Results from the SST are recorded in a log file written to the SST directory selected in the IconNMR Configuration window. Each of the experiments chosen on the **SST Standard Tests** tab and the **SST User Tests** tab will be run during the SST. An example log file from an SST is shown in Chapter Log File from the System Suitability Test.

The 'Activate Single Peak Linewidth Check' enables continuous monitoring of lineshape for every sample acquired with IconNMR for spectral quality control. When used with the proper processing AU (proc_assureshim) and a sample that has an NMR reference signal at 0 ppm, the system uses the halfwidth of the reference to determine whether the spectrum is of a high enough quality to be passed on for analysis. The threshold cutoff for half height should be at or below the value specified as the 'Peak test cutoff frequency' (in Hertz). A sample with a larger half width will be re-shimmed and re-acquired. Two consecutive sample failures results in automatic queuing of the System Suitability Test.

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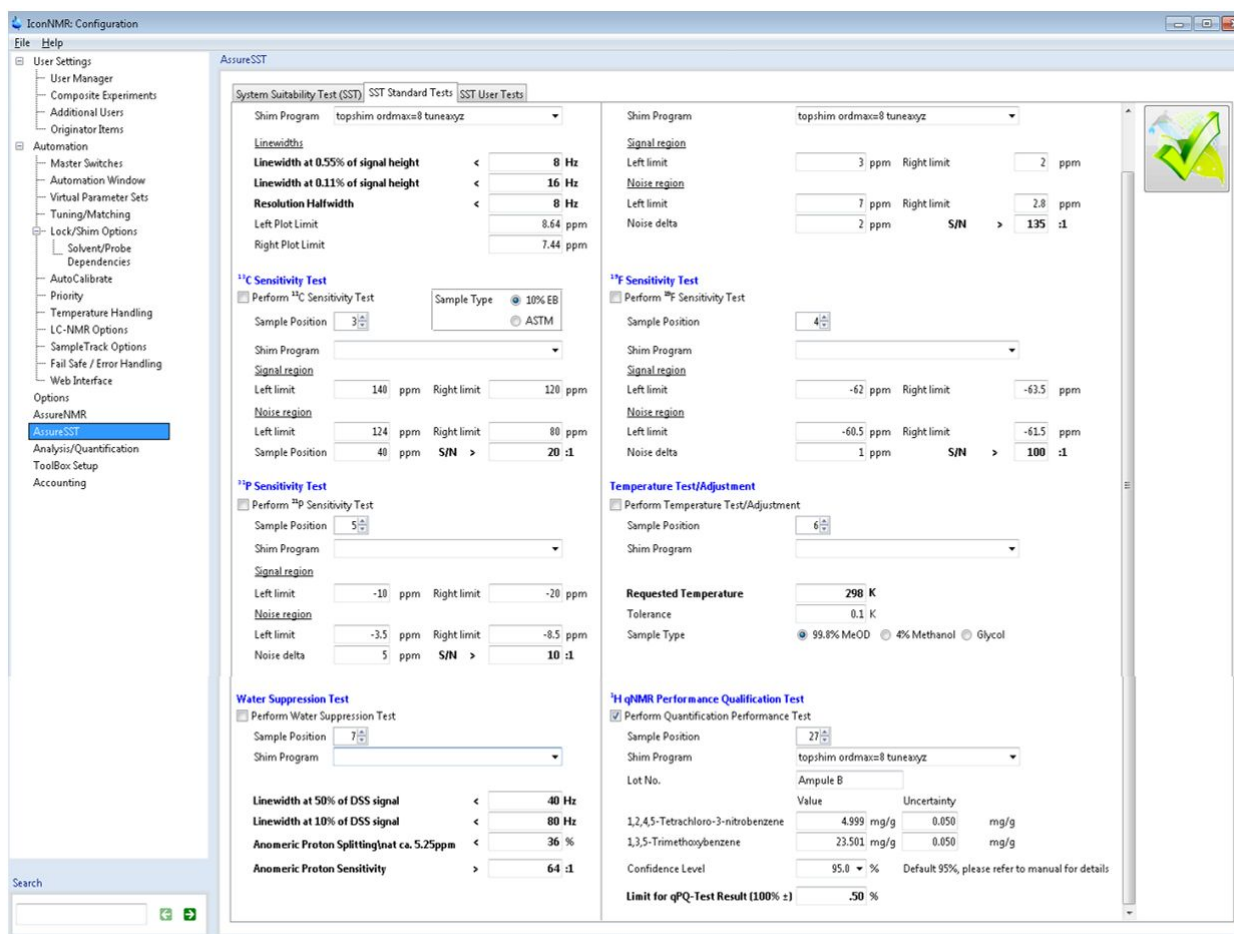## 2.2 SST Standard Tests Tab

Figure 2.4: The SST Standard Tests tab in the AssureSST section of the IconNMR Configuration window.

The **SST Standard Tests** tab gives the user access to a set of six preset tests. These tests can be turned on and off, the sample positions can be specified, details for the analyses can be input, and criteria for passing the tests can be set from this tab. The details for each test are in the following sections.

2.2.1 ¹H Lineshape

Also referred to as the humptest, this test automatically measures and determines the ^{1}H lineshape using the GLP ^{1}H lineshape standard sample, chloroform in acetone. (See Chapter 13 for the correct sample.) The width of the chloroform line at 0.55% height and 0.11% height is calculated with a double exponential fit along the left and right side of the signal. The resolution test is also performed and evaluates the width of the chloroform signal at half height. These values are compared with the specifications set in this window. The test is passed if the results are less than or equal to the defined values.

- Check the 'Perform Lineshape Test' box to require this experiment to run as part of the SST.
- Set the 'Sample Position' to the holder position of the standard sample in the sample changer (SampleCase, SampleJet, SampleXpress, BACS).
- Enter the desired values for an acceptable lineshape test for the (1) 'Linewidth at 0.55% of signal height', (2) 'Linewidth at 0.11% of sample height' and (3) the 'Resolution Halfwidth' (linewidth at 50% of signal height).

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> The lineshape and sensitivity values are specific to the probe. When the probe is changed, the values must be updated to correspond to the current probe in the SST Standard Tests tab of the IconNMR Configuration window. If the probe has been used previously, stored parameters will be loaded for that probe.
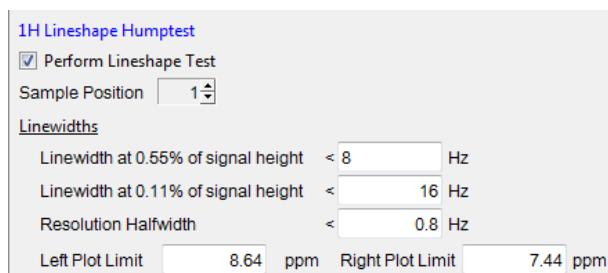

Figure 2.5: ^{1}H Lineshape Humptest parameters on the SST Standard Tests tab.

2.2.2 ^{1}H Sensitivity

This test automatically measures and determines the ^{1}H sensitivity. The ^{1}H sensitivity standard sample is 0.1% ethylbenzene in chloroform-d for all probes. The height of the biggest signal between the signal limits is calculated. A noise window of width 'Noise delta' in ppm is shifted in 25 steps along the spectrum between the noise limits. Each time, the noise value is determined and the signal-to-noise (S/N) ratio is calculated with respect to the height of the biggest signal within the signal limits. The best value must meet the specification defined in the S/N box.

- Check the 'Perform ^{1}H Sensitivity Test' box to require this experiment to run as part of the SST.
- Set the 'Sample Position' to the holder position of the standard sample in the sample changer (SampleCase, SampleJet, SampleXpress, BACS).
- Enter the desired 'Signal region' (Left and Right limits) to be used for the signal peak.
- Enter the desired 'Noise region' (Left and Right limits).
- Enter the 'Noise delta' (width of the noise range) in ppm.
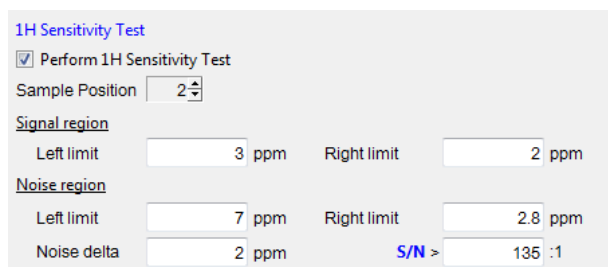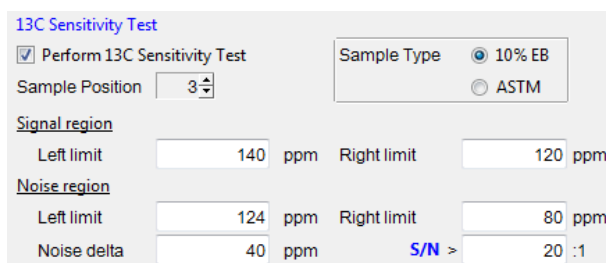- Enter the 'S/N' requirement for a successful test.

Figure 2.6: ^{1}H Sensitivity Test parameters on the System Suitability Test (SST) tab.

2.2.3 ^{13}C Sensitivity

This test automatically measures and determines the ^{13}C sensitivity. The typical sample used for the ^{13}C Sensitivity Test is 10% ethylbenzene in cholorform-d for all probes. The ASTM (American Society for Testing and Materials) sample (40% p-Dioxane in benzene-d6) may also be used. The height of the biggest signal within the signal limits is calculated. A noise window of 'Noise delta' ppm is shifted in 25 steps along the spectrum between the noise limits. Each time, the noise value is determined and the signal-to-noise ratio is calculated with respect to the height of the biggest signal. The best value must meet the specification defined in the S/N box.

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- Check the 'Perform ^{13}C Sensitivity Test' box to require this experiment to run as part of the SST.
- Set the 'Sample Position' to the holder position of the standard sample in the sample changer (SampleCase, SampleJet, SampleXpress, BACS)
- Select the Sample Type, either 10% ethylbenzene or the ASTM sample.
- Enter the desired 'Signal region' (Left and Right limits) to be used for the signal peak.
- Enter the desired 'Noise region' (Left and Right limits).
- Enter the 'Noise delta' (width of the noise range) in ppm.
- Enter the 'S/N' requirement for a successful test.
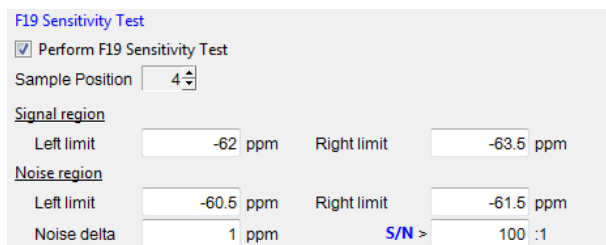
Figure 2.7: ^{13}C Sensitivity Test parameters on the SST Standard Tests tab.

2.2.4 ^{19}F Sensitivity

This test automatically measures and determines the ^{19}F Sensitivity. The typical sample used for the ^{19}F Sensitivity Test is 0.05% trifluorotoluene in chloroform-d for all probes. The height of the biggest signal between the signal limits is calculated. A noise window of 'Noise delta' ppm is shifted in 25 steps along the spectrum between the noise limits. Each time, the noise value is determined and the signal-to-noise ratio is calculated with respect to the height of the biggest signal. The best value must meet the specification defined in the S/N box.

- Check the 'Perform ^{19}F Sensitivity Test' box to require this experiment to run as part of the SST.
- Set the 'Sample Position' to the holder position of the standard sample in the sample changer (SampleCase, SampleJet, SampleXpress, BACS).
- Enter the desired 'Signal region' (Left and Right limits) to be used for the signal peak.
- Enter the desired 'Noise region' (Left and Right limits).
- Enter the 'Noise delta' (width of the noise range) in ppm.
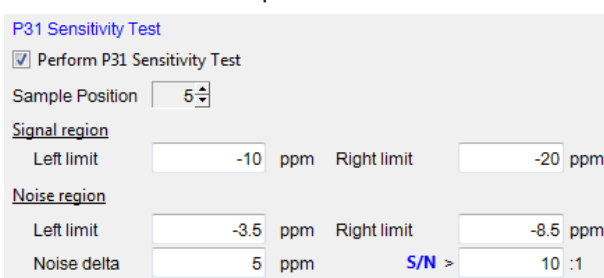- Enter the 'S/N' requirement for a successful test.

Figure 2.8: ^{19}F Sensitivity Test parameters on the SST Standard Tests tab.

2.2.5 ^{31}P Sensitivity

This test automatically measures and determines the ^{31}P Sensitivity. The typical sample used for the ^{31}P Sensitivity Test is 0.0485 M triphenylphosphate in acetone-d6 for all probes. The height of the biggest signal between the signal limits is calculated. A noise window of 'Noise delta' ppm is shifted in 25 steps

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along the spectrum between the noise limits. Each time, the noise value is determined and the signal-to-noise ratio is calculated with respect to the height of the biggest signal. The best value must meet the specification defined in the S/N box.

- Check the 'Perform ^{31}P Sensitivity Test' box to require this experiment to run as part of the SST.
- Set the 'Sample Position' to the holder position of the standard sample in the sample changer (SampleCase, SampleJet, SampleXpress, BACS).
- Enter the desired 'Signal region' (Left and Right limits) to be used for the signal peak.
- Enter the desired 'Noise region' (Left and Right limits).
- Enter the 'Noise delta' (width of the noise range) in ppm.
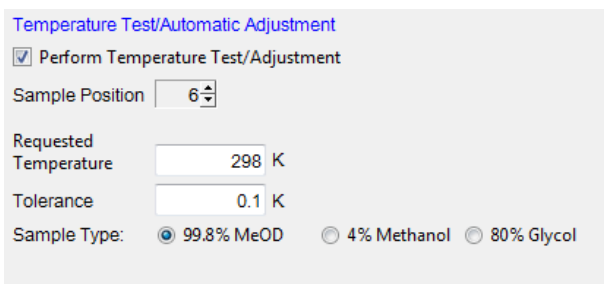- Enter the 'S/N' requirement for a successful test.

Figure 2.9: ^{31}P Sensitivity Test parameters on the SST Standard Tests tab.

2.2.6 Temperature Test with Automatic Adjustment

This test automatically measures and, if necessary, adjusts the temperature to the temperature specified in 'Requested Temperature'. The experiment is designed to run after the other components of the SST. In the example below, the temperature is measured with the 99.8% Methanol-d4 Temperature Calibration Standard which has a linear range from 282 K to 330 K. The method will attempt to adjust the temperature to the set point five times before failing. The final observed temperature after adjustment is recorded in the status parameters under the entry for USERA1.

- Check the 'Perform Temperature Test/Adjustment' box to require this experiment to run as part of the SST.
- Set the 'Sample Position' to the holder position of the desired temperature calibration standard in the sample changer (SampleCase, SampleJet, SampleXpress, BACS).
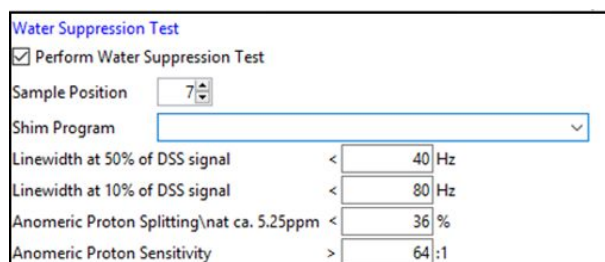- Enter the 'Requested Temperature' and 'Sample Type'.

Figure 2.10: Temperature Test parameters on the SST Standard Tests tab.

2.2.7 Water Suppression Test

This test will determine 2 factors important for the GLP Water Suppression standard sample, 2mM Sucrose + 0.5mM DSS in 90% H_2O + 10% D_2O; the suppression quality and the sensitivity level of the anomeric sucrose signal at 5.25ppm. A resolution test of the lineshape of DSS is also performed. The test is passed if the lineshape results are less than or equal to the defined values for lineshape and the

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percent splitting value of the anomeric proton splitting. The test also requires that the best value for proton sensitivity must be greater than or equal to the defined value for Anomeric Proton Sensitivity. Note that the test assumes the position of water to be 4.7ppm. There is no scout for the optimal water resonance.

- Check the 'Perform Water Suppression Test' box to require this experiment to run as part of the SST.
- Set the "Sample Position' to the holder position of the standard sample in the sample changer (SampleCase, SampleJet, SampleXpress, BACS.)
- Select the Shim program to use a shim program different from the default for IconNMR or default for the solvent.
- Enter the desired values for an acceptable lineshape test for the (1) 'Linewidth at 50% of DSS signal', (2) 'Linewidth at 10% of DSS Signal', (3) 'Anomeric Proton Splitting\nat ca. 5.25ppm'.
- Enter the 'Anomeric Proton Sensitivity' requirement for a successful test.

Water Suppression Test		
☑ Perform Water Suppression Test		
Sample Position	7	
Shim Program		
Linewidth at 50% of DSS signal	<	40 Hz
Linewidth at 10% of DSS signal	<	80 Hz
Anomeric Proton Splitting\nat ca. 5.25ppm	<	36 %
Anomeric Proton Sensitivity	>	64 :1

2.2.8 ¹H qNMR Performance Quantification (PQ) Test

This test will measure the potency of a certified quantification reference material (CRM). This CRM is available in ready-to-use sealed ampules from Merck KGaA[1] (formerly Sigma-Aldrich) as Bruker quantitative PQ (qPQ) Certified Reference Material (Product No. 42350). For exact conditions to follow, please refer directly to the prevailing SOP for this method and for your facility. However, the Bruker BioSpin recommendation is to use either freshly prepared samples of the quantification reference material, or a sealed NMR tube of same. Note that the sample must only be used within the validity period of the reference sample as given on the certificate.

Data on the certificate delivered with each lot of the material (see an example in figure (qPQ.1) must be used to complete the information in the qPQ test box, a typical example of which is shown further below in figure qPQ.2.

- Check the 'Perform qPQ Test' box to require this experiment to run as part of the SST.
- Set the "Sample Position' to the holder position of the standard sample in the sample changer (SampleCase, SampleJet, SampleXpress, BACS.)
- Select the Shim program to use a shim program different from the default for IconNMR or default for the solvent.
- The following information will be found on the Certificate of Analysis delivered with the sample and must be manually transcribed into the qPQ test tab:
  - Lot. No.
  - 1,2,4,5-Tetrachloro-3-nitrobenzene certified value for mass and uncertainty
  - 1,3,5-Trimethoxybenzene certified value for mass and uncertainty

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[1] 1 The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

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SIGMA-ALDRICH®

TraceCERT®
Traceable Certified Reference Materials

Certificate of Analysis – Certified Reference Material

Bruker quantitative PQ (qPQ) Certified Reference Material

Produced in double accredited laboratory fulfilling ISO/IEC 17025 and ISO 17034

Product no.:	42350
Lot no.:	BCBZ9945
Description of CRM:	1,3,5-Trimethoxybenzene (TMXB) and 1,2,4,5-Tetrachloro-3-nitrobenzene (TCNB) in DMSO-d_6 (solution)
Expiry date:	DEC 2020
Storage:	20-25°C; storage under Argon
Chemical formula:	$C_9H_{12}O_3$ (TMXB); $C_6HCl_4NO_2$ (TCNB)
Molecular mass:	168.19 g/mol (TMXB); 260.89 g/mol (TCNB)

Constituents	Certified value	Expanded uncertainty, $U=k \cdot u$ ($k=2$) [1][2]
1,2,4,5-Tetrachloro-3-nitrobenzene	23.51 mg/g	0.18 mg/g
1,3,5-Trimethoxybenzene	5.00 mg/g	0.04 mg/g

Figure 2.11: Excerpt of a typical CoA for the PQ reference material.

- The field for 'Limit for qPQ-Test Result (100% ±)±)' defines the acceptance criterion for the ^{1}H qNMR Performance Quantification (PQ) Test. The value is calculated automatically from the given uncertainties of each component and the selected confidence level. The field 'Limit for qPQ-Test Result (100% ±)' cannot be edited. Choosing a different confidence level allows for adjustment of the acceptance criterion.
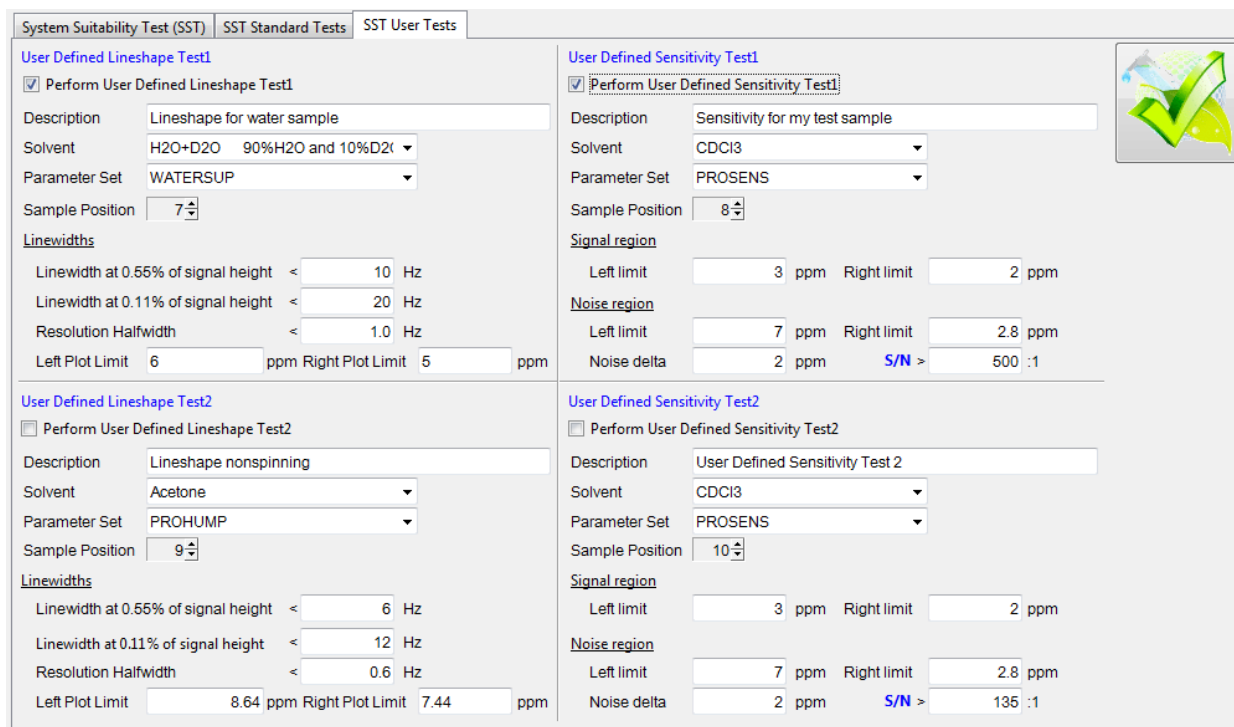
Figure 2.12: qPQ Test Parameters on the qPQ test tab.

Upon completion of the test, the results of the qPQ test – Pass if the result lies within the calculated limit or Fail, if without, are automatically appended to the SST test report pdf.

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2.3 SST User Tests

SST also allows users to specify their own tests. For analysis purposes, the tests are classified as lineshape tests or sensitivity tests. The user must supply information about the solvent, the parameter set to use, and the sample position. In the parameter set, the acquisition au program (AUNM) must be au_zgglp and the processing au program (AUNMP) must be proc_1dglp. The user must also supply the information for the analysis – linewidths and plot region for the lineshape tests, signal and noise regions for the sensitivity tests – interactively on the **SST User Tests** tab.

Figure 2.13: SST User Tests tab for SST, with one lineshape test and one sensitivity test activated.

3 Data Organization and Final PDF Report

AssureSST automatically places the SST data in the first directory listed in the Data Directories window for the SST user in the **User Manager** of the IconNMR Configuration window. The generated data directory name uses the following date-stamped format *SST_{YYYY_MM_DD_HH_MM_SS}*. Each directory will contain all of the data for a queued System Suitability Test.

Within the data directory for the last experiment that makes up the SST, a PDF report (see the example in Chapter 9.2) is stored which summarizes which tests were run, the criteria, and the results of each test including a pass or fail notation.

4 Assure-SST Reference Standards

300 MHz / 5mm Room Temperature	
Description	**Part No.**
Lineshape - 3% Chloroform	Z10230
1H Sensitivity - 0.1% Ethylbenzene, 40mm filling	Z10901

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13C Sensitivity - 10% Ethylbenzene	Z10153
19F Sensitivity – 0.05% Trifluorotoluene	Z10234
31P Sensitivity – 0.0485 M Triphenylphosphate	Z10201
NMR Thermometer 99.8% Methanol-d4	Z10627
Water Suppression - 2mM sucrose	Z10246

400 – 900 MHz / 5mm Room Temperature	
Description	**Part No.**
Lineshape - 1% Chloroform	Z10248
1H Sensitivity - 0.1% Ethylbenzene, 40mm filling	Z10901
13C Sensitivity - 10% Ethylbenzene	Z10153
19F Sensitivity – 0.05% Trifluorotoluene	Z10234
31P Sensitivity – 0.0485 M Triphenylphosphate	Z10201
NMR Thermometer 99.8% Methanol-d4	Z10627
Water Suppression - 2mM sucrose	Z10246

400 – 900 MHz / 5mm CryoProbe	
Description	**Part No.**
Lineshape - 0.3% Chloroform, 40mm filling	Z10903
1H Sensitivity - 0.1% Ethylbenzene, 40mm filling	Z10901
13C Sensitivity - 10% Ethylbenzene	Z10153
19F Sensitivity – 0.05% Trifluorotoluene	Z10234
31P Sensitivity – 0.0485 M Triphenylphosphate	Z10201
NMR Thermometer 99.8% Methanol-d4	Z10627
Water Suppression - 2mM sucrose	Z10246

600 – 900 MHz / 1 mm Room Temperature	
Description	**Part No.**
Lineshape - 3% Chloroform	Z100926
1H Sensitivity - 0.1% Ethylbenzene	Z100927
13C Sensitivity - 10% Ethylbenzene	Z100928
31P Sensitivity – 0.0485 M Triphenylphosphate	Z100934
NMR Thermometer 4% Methanol	Z100935
Water Suppression - 2mM sucrose	Z10246

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600 – 900 MHz / 1.7mm CryoProbe	
Description	**Part No.**
Lineshape - 1% Chloroform	Z10717
1H Sensitivity - 0.1% Ethylbenzene	Z10718
13C Sensitivity - 10% Ethylbenzene	Z10723
19F Sensitivity – 0.05% Trifluorotoluene	Z10728
31P Sensitivity – 0.0485 M Triphenylphosphate	Z10722
NMR Thermometer 99.8% Methanol-d4	Z10734
Water Suppression - 2mM sucrose	Z10246

5 Contact

Manufacturer

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D-76287 Rheinstetten

Germany

E-Mail: *nmr-support@bruker.com*

http://www.bruker.com

WEEE DE43181702

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