Assure

Assure Guide

System Suitability Test 1.2
Raw Material Screening 1.2

Version 1.1

NMR Systems

©; Bruker BioSpin GmbH
Printed in Federal Republic
of Germany 27-Oct-2011
Document Part No: B7150

Summary of Contents

Contents

Contents

1 Introduction

The Assure software package has two automation modules: Assure – System Suitability Test (SST) and a screening module named Assure - Raw Material Screening (RMS). The SST module is designed to monitor and maintain instrument performance. The RMS module assists in the quality control of materials, ingredients and components used in a wide range of products. Reference NMR spectral libraries are used by the screening software to identify the target material and known possible impurities. Additionally, the percent of unknown impurities relative to the target material are measured and reported. A reference spectral library and knowledgebase of common chemicals is included with the screening software. Users may add an unlimited number of compounds to user generated spectral libraries and knowledgebases. Reports designed for both the non-technical and advanced users are automatically generated to allow quick assessment of the results and to provide a permanent record of the material testing. To assure that the spectrometer is performing to user defined specifications a series of System Suitability Tests are run in addition to the screening software. The screening software may be used for pharmaceuticals, chemicals, polymers, food products, petroleum and many other materials. This software package allows for the testing and detection of any compounds having NMR active nuclei using standard NMR techniques and runs within the TopSpin software package. Assure-RMS software is designed for relatively pure samples or simple mixtures

Assure-SST and Assure-RMS were designed to be used in a production or research facility. As a result, many of the features incorporated allow use of this software by non-NMR spectroscopists and on NMR spectrometers in GLP environments.

1.1 Assure – System Suitability Test (SST)

Summary of Features

- Automated System Suitability Test includes acquisition and analysis of NMR standards ^{1}H lineshape, ^{1}H sensitivity, ^{13}C sensitivity, ^{19}F sensitivity, ^{31}P sensitivity, and temperature calibration.
- To reduce routine maintenance of the spectrometer shims, shim sets from successful ^{1}H lineshape experiments are stored and recalled as a starting shim set for queued raw material samples.
- Automated ‘Stop’ criteria to halt acquisition upon specification failure
- Automated PDF report generation of SST results

Software Design

The Assure-SST module was designed to work as a means of monitoring instrument performance on a regular basis. This is achieved via IconNMR which monitors the perfor-

mance and temperature of the system according to an interval selected by the user. Assure-SST module can work either in a standalone mode where the user can use the normal IconNMR submission interface or in parallel with the Assure-RMS module. If Assure-SST determines the system to be out of specifications then general sample submissions through IconNMR or Assure-RMS will halt until all specifications are achieved.

1.2 Assure - Raw Material Screening (RMS)

Summary of Features

- Utilization of Assure-SST for instrument performance check
- Automated data acquisition using IconNMR using user defined parameter sets
- Automated qualitative analysis of spectra using a supplied or generated NMR spectral database
- Handling of calculations requiring multiple spectra averages
- Absolute or Relative concentration determination
- Quantification on supported nuclei (^{1}H, ^{2}H, ^{13}C, ^{19}F, ^{31}P)
- Automated report generation including a summary Quality Control Report (QCReport.pdf) which reports a ‘pass’ or ‘fail’ report (or numerical) and a detailed Expert Report (ExpertReport.pdf) which outlines the total analysis
- Flexible report generation
- GLP compatible
- Customizable security features

Software Design

The Assure software utilizes four software components: (1) pre-existing components of TopSpin, (2) new features in IconNMR, (3) NMR spectral databases (SBASE), and (4) the Assure software as summarized in Figure 1.1. Successful completion of a System Suitability Test releases IconNMR for general sample submission. Submitted raw materials are collected with the acquisition and processing functions of TopSpin. The raw material spectrum (or spectra) is then passed to the Assure-RMS software for evaluation and generation of reports.

Figure 1.1: Assure-RMS Software Package Overview Diagram

Work Flow

The system is designed for sample submission by a novice user. The user should be able to prepare a sample and submit it via the Assure-RMS IconNMR interface. Figure 1.2 shows the progression of sample.

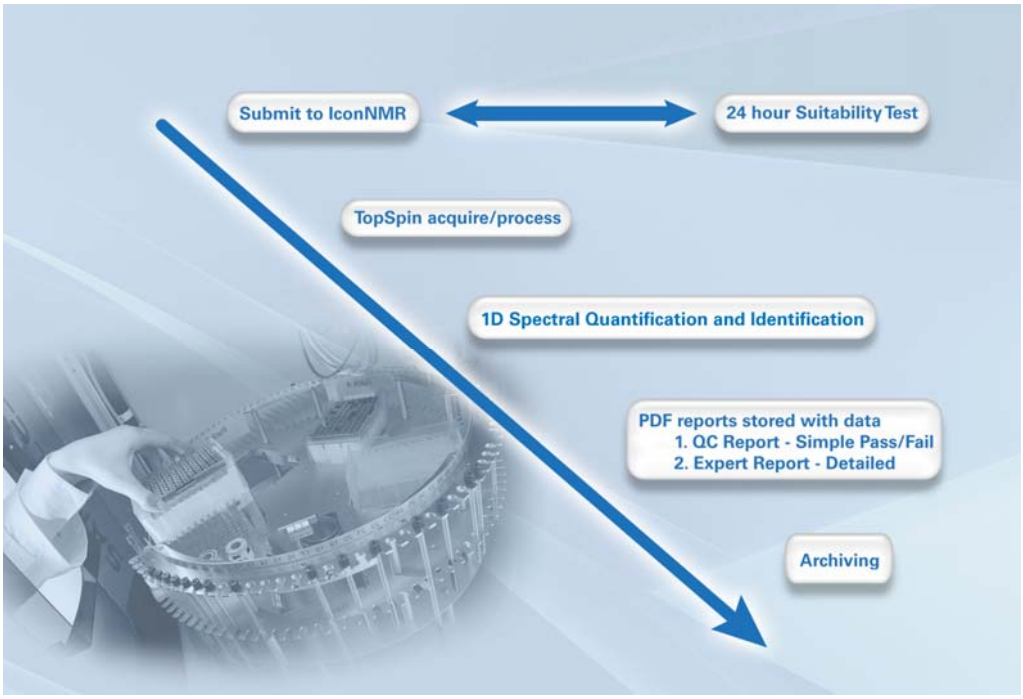


Figure 1.2: Workflow of Assure-RMS Software Diagram

User Interface

The access-limited user will observe the submission interface shown in Figure 1.3.

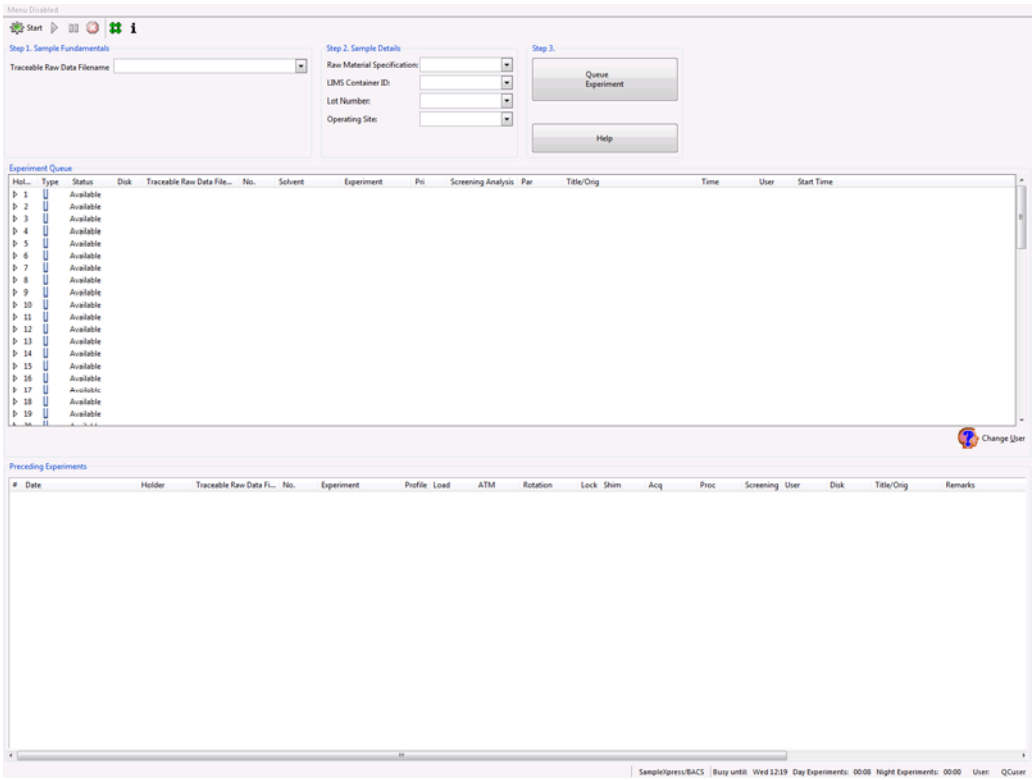


Figure 1.3: Raw Material Screening: IconNMR sample submission window for access-limited users.

1.3 Assure-SST and Assure-RMS Requirements

Computer and Operating System:

Operating System Requirements:

- Microsoft Operating System Windows 7 64-bit
- CentOS 5 64-bit

Minimum Software:

- TopSpin 3.1 pl2 or TopSpin 3.0 pl5
- IconNMR 4.6
- Assure-RMS 1.2
- AMIX 3.9.11

Software Licenses are required; licenses may be obtained by contacting license@bruker.de. Available licenses include:

- Assure-SST (standalone)
- Assure-RMS (includes Assure-SST)
- GLP (optional)

GLP license is not GLP Validation. GLP validation requires a separate visit by a GLP certified engineer.

Hardware Requirement:

- Spectrometer: Avance II console or later
- B(S)VT and B(S)CU-05 is required.
- High resolution probe equipped with ATMA
- Sample changer with position recognition (BACS-60, BACS-120 SampleJet, or SampleXpress)

NMR Reference Standards for Assure-SST

One set of NMR Reference standards are supplied with Assure-SST and Assure-RMS. Additional NMR standard reference samples may be purchased through the Bruker Online Store at www.bruker-biospin.com. 5mm NMR Standard Reference Samples to be used with the System Suitability Tests option are found in Table 1.1. Full list of Assure SST samples for alternate instrument configurations are listed in Chapter 10.

Test	Sample	Bruker P/N	Automation Position (default)

^{1}H Lineshape	1% Chloroform in acetone-d6	Z10248	1
^{1}H Sensitivity	0.1% Ethylbenzene in CDCL3	Z10901	2
^{13}C Sensitivity	10% Ethylbenzene in CDCL3	Z10153	3
^{19}F Sensitivity	0.05% Trifluorotoluene	Z10234	4
^{31}P Sensitivity	0.0485 M Triphenylphosphate	Z10201	5
Temperature calibration	99.8% methanol-d4	Z10627	6

Table 1.1: List of 5mm standard samples for System Suitability Tests including their sample type, reorder number and default sample position for the automation robot.

NMR Validation Standards for Assure-RMS

One set of Assure-RMS Validation Standards are supplied with Assure-RMS. Additional 5mm NMR Standard Validation Samples are found in Table 1.2 may be purchased through the Bruker Online Store.

Test	Sample	Bruker P/N
0% Adulterant	0:100% Lysine:Arginine in D2O	Z121840
4% Adulterant	4 (+/- 1.0):100% Lysine:Arginine in D2O	Z121839
5% Adulterant	5 (+/- 1.0):100% Lysine:Arginine in D2O	Z121838
6% Adulterant	6 (+/- 1.0):100% Lysine:Arginine in D2O	Z121837

Table 1.2: List of 5mm Validation Test samples including their sample type and reorder number.

1.4 Installation

TOPSPIN, IconNMR, AMIX, Assure-SST, Assure-RMS, and Assure-RMS SBASE

Proceed as described in the TopSpin Installation Guide. IconNMR, AMIX, Assure-RMS, and Assure-RMSSBASE (rmssbase) are selectable from the customized installation item during the TopSpin installation.

1.5 Default Home Directories

Assure and IconNMR have specific directories used for the Assure modules. The following directories are used for the noted information in accordance with Windows 7 and CentOS 5.

Windows 7

User Directory	%APPDATA%	c:\Users\<name>\AppData\Roaming
Shared Directory	%ALLUSERSPROFILE%	c:\ProgramData

CentOS

User Directory	/usr/Bruker
Shared Directory	/etc/opt/Bruker

Assure-RMS

Default settings	(%APPDATA%\Bruker or <user home dir>/.Bruker) \Raw Material Screening
Quant Methods	(%APPDATA%\Bruker or /usr/Bruker) \Raw Material Screening

Assure-RMS and IconNMR

Logfiles	(%APPDATA% or <user home dir>/.Bruker) \rawMaterialScreening.log
Released Quant Method	(%ALLUSERSPROFILE% or /opt/etc/) \Bruker\Raw Material Screening

1.6 Support

Software support is available from your local Bruker office or via E-mail from the following address:

nmr-software-support@bruker.de

The Bruker www servers www.bruker-biospin.de and www.bruker-biospin.com provide additional information such as downloadable upgrades for your Assure-SST, Assure-RMS, TopSpin and IconNMR installation.

2 Assure – System Suitability Test Software

2.1 General Features

Assure-SST is configured and controlled through the IconNMR automation software package which implements TopSpin to acquire and evaluate data. Upon completion of the SST standards, a PDF report is generated summarizing the specifications tested and observed results.

2.2 Assure-SST Configuration

Activation of the Assure system is found in the IconNMR Configuration window which contains a section for the ‘Assure System’. The ‘Assure System’ allows the system administrator to define (1) options for SST, (2) the individual system suitability tests, and (3) screening methods. Screening method configuration is discussed in Chapter 5.

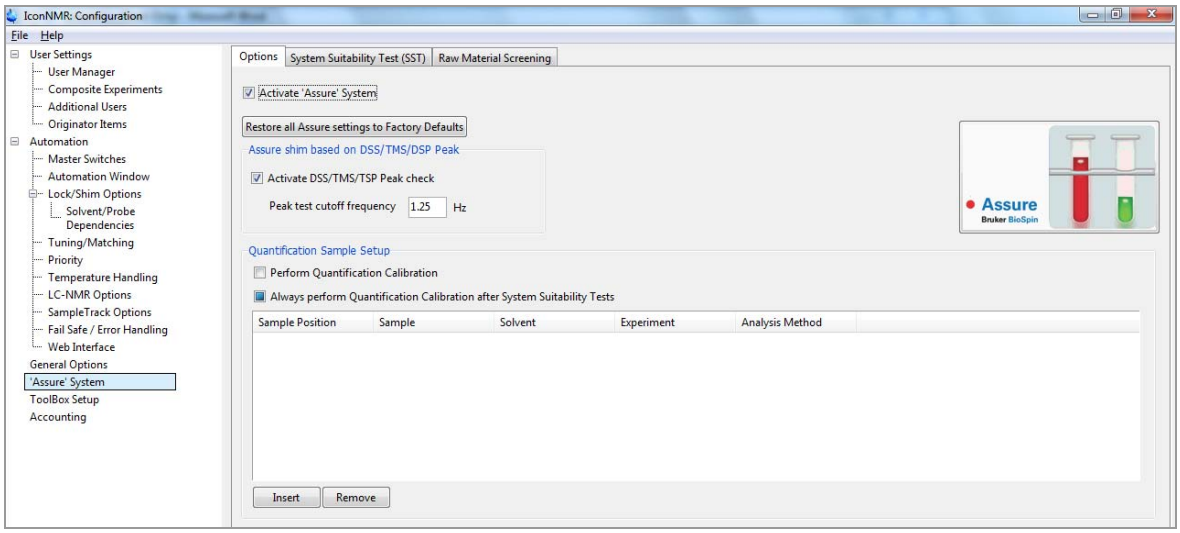


Figure 2.1: The Options tab in the Assure System.

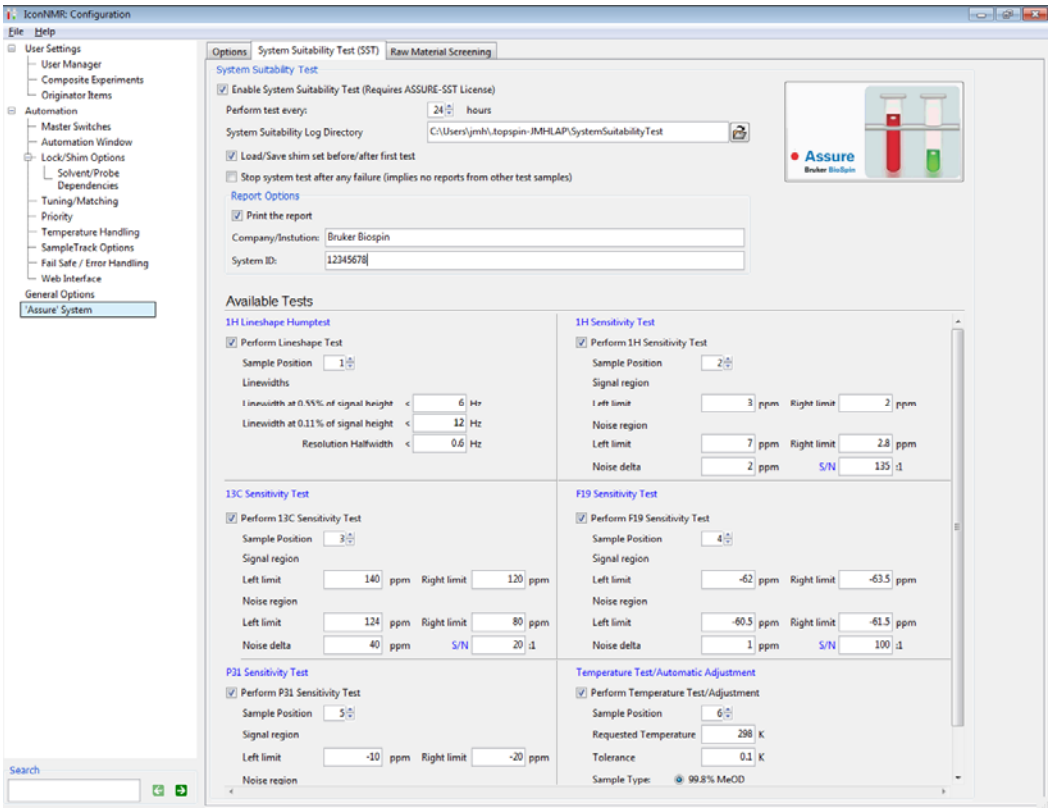
Options

Here the user can activate the Assure system and restore all the Assure settings in the Configuration to the factory defaults. Additional items that are built into Assure are a shim check option which evaluates shimming quality and an option to define a quantification standard available for Assure-RMS as covered in chapters 5.3.1 and 5.3.5.

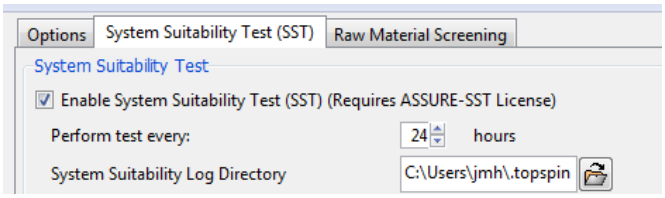
System Suitability Test

The System Suitability Tests will automatically be queued for acquisition as required to meet the time set in the entry for 'Perform test every: x hours'. If other samples are queued (e.g. for RMS) at the time when the System Suitability Test is called then the data acquisition of queued materials will halt and System Suitability Tests are performed before the acquisition of the samples is resumed.

The System Suitability Tests (up to six individual experiments) as a whole must 'pass' before the any other samples queued in IconNMR are acquired. The six tests are covered in sections 2.2.1.1 – 2.2.1.6 of this chapter.



The System Suitability Test can be set up for any set period in one hour increments. In the example below, the next System Suitability Test will start 24 hours after the completion of the previous test.



Strategies and flexibility in the System Suitability Test were designed to accommodate experiments which might exceed the clock. Experiments that run longer than the set time

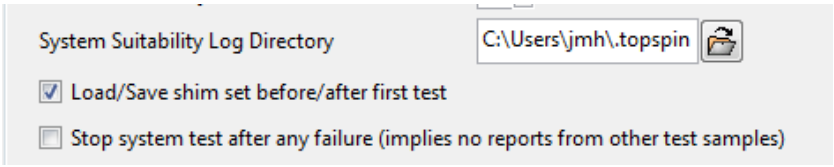
or experiments that would not finish before the next System Suitability Test would remain in the queue until sufficient time is present to run the sample.

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

It is possible to run the System Suitability Test at any time as NMR Superuser (Chapter 6.5).

Results from the System Suitability Tests are recorded in a log file written to the System Suitability Test directory selected in the QC Configuration window. Each of the experiments chosen in the configuration window will be run during the System Suitability Test. An example log file from a System Suitability Test is shown in chapter 7.1.

The Assure-SST system is designed to adjust to shim changes time. This is done by updating and using 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 written to 'IconNMRShimset.XX' where the XX extension correlates to the probe ID number on the system. For more information on probe identification type 'help edhead' in TopSpin. If a default shim set does not exist for the probe, then the current shims are used.



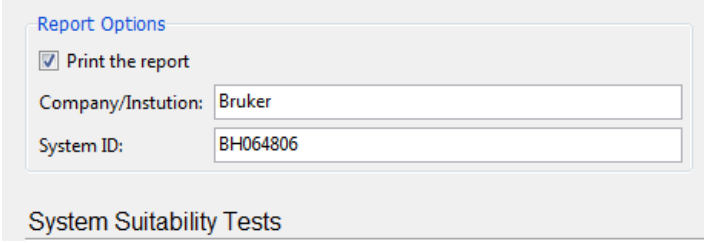
A checked box in the 'Load/Save shim set before/after first test' window overrides the 'Enable Shim File Loading" setting in the IconNMR Configuration, Solvent/Probe Dependencies Window (under 'Automation' and Lock/Shim Options). Therefore, the solvent dependent shim files in the Solvent/Probe Dependencies are not used.

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

The System Suitability Tests as a whole are considered the criteria for a properly functioning spectrometer for the purposes of the Raw Material Screening Software. As a result, a failed system suitability test is reported after the complete set of required tests is measured. For example, if the system administrator requires that four tests (^{1}H Lineshape, ^{1}H Sensitivity, ^{13}C sensitivity and Temperature) are required for the System Suitability Test, then all four tests will be completed before a final 'system pass' or 'system fail' result is obtained. Optionally, the user may select 'Stop system test after any failure' to immediately halt the acquisition after any failed test. If 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 with the failed test is resolved.

- Check ‘Stop system test after any failure’ to halt the System Suitability Tests after any failed test.

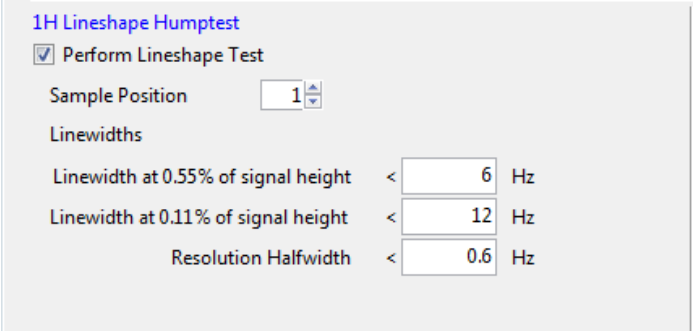
Automatic generation of a PDF report (section 2.3) for SST results are created when the ‘Print the report’ option is active. The administrator can include the Company/Institution and System ID in the report by filling in the corresponding fields.



2.2.1.1 ^{1}H 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 10 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 within the defined values.

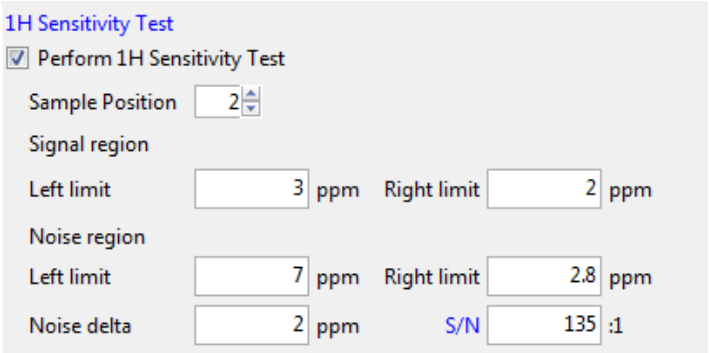
- Check the ‘Perform Lineshape Test’ box to require this experiment to run as part of the System Suitability Test.
- Set the ‘Sample Position’ to the holder position of this sample in the sample changer (BACS, SampleJet, SampleXpress)
- Enter the desired values for an acceptable lineshape test for the (1) lineshape at 0.55% of signal height, (2) linewidth at 0.11% of sample height and (3) the resolution at halfwidth.



2.2.1.2 ^{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 System Suitability Test.
- Set the 'Sample Position' to the holder position of this sample in the sample changer (e.g., SampleJet, SampleXpress)
- Enter the desired Signal Region (left and right limit) to be used for the signal peak.
- Enter the desired Noise Region (left and right limit).
- Enter the Noise delta (width of the noise range) in ppm.
- Enter the S/N requirement for a successful test.

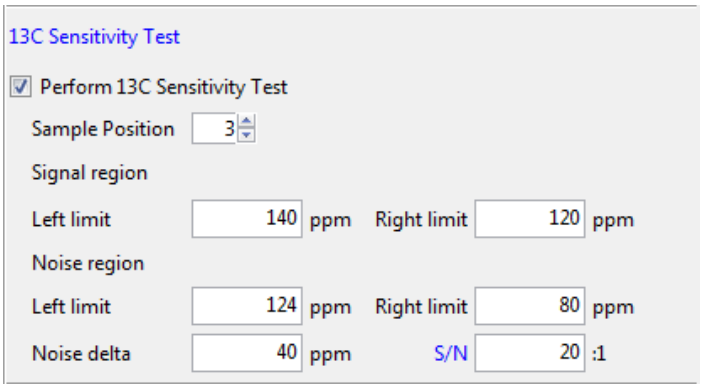


2.2.1.3 ^{13}C Sensitivity

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 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 ^{13}C Sensitivity Test' box to require this experiment to run as part of the System Suitability Test.
- Set the 'Sample Position' to the holder position of this sample in the sample changer (e.g., SampleJet, SampleXpress)
- Enter the desired Signal Region (left and right limit) to be used for the signal peak.
- Enter the desired Noise Region (left and right limit).
- Enter the Noise delta (width of the noise range) in ppm.

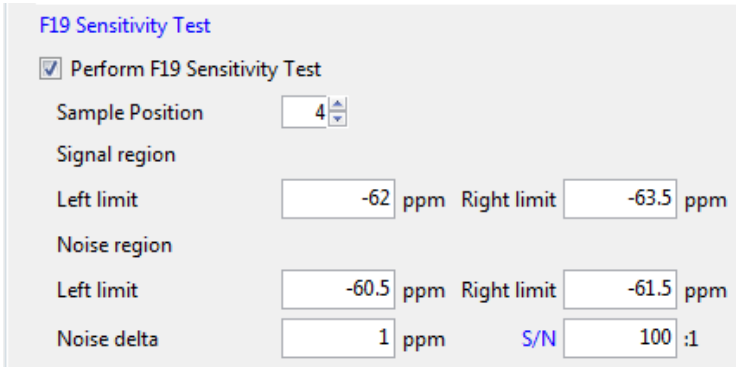
- Enter the S/N requirement for a successful test.



2.2.1.4 ^{19}FSensitivity

This test automatically measures and determines the ^{19}F Sensitivity. The typical sample used for the 13C 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.

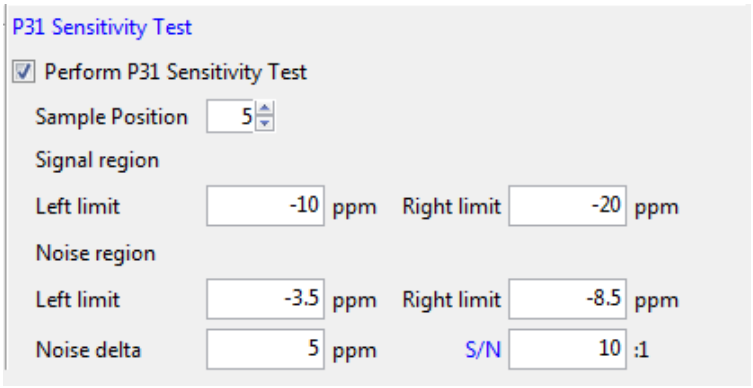
- Check the 'Perform ^{19}F Sensitivity Test' box to require this experiment to run as part of the System Suitability Test.
- Set the 'Sample Position' to the holder position of this sample in the sample changer (e.g., SampleJet, SampleXpress)
- Enter the desired Signal Region (left and right limit) to be used for the signal peak.
- Enter the desired Noise Region (left and right limit).
- Enter the Noise delta (width of the noise range) in ppm.
- Enter the S/N requirement for a successful test.



2.2.1.5 ^{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 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.

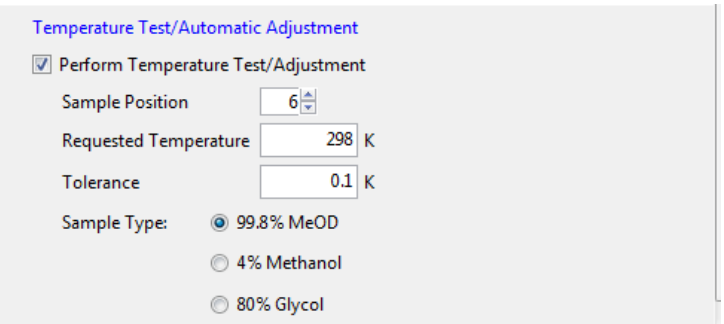
- Check the 'Perform ^{31}P Sensitivity Test' box to require this experiment to run as part of the System Suitability Test.
- Set the 'Sample Position' to the holder position of this sample in the sample changer (e.g., SampleJet, SampleXpress)
- Enter the desired Signal Region (left and right limit) to be used for the signal peak.
- Enter the desired Noise Region (left and right limit).
- Enter the Noise delta (width of the noise range) in ppm.
- Enter the S/N requirement for a successful test.



2.2.1.6 Temperature Test with Automatic Adjustment

This test automatically measures and, if necessary, adjusts the temperature to the temperature set for 'Requested Temperature'. The experiment is designed to run after the first three specific tests. Here, the 'Requested temperature' is set 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 System Suitability Test.
- Set the 'Sample Position' to the holder position of this sample in the sample changer (e.g., SampleJet, SampleXpress).
- Enter the Requested Temperature and sample.



2.3 Data Organization and Final PDF Report

Assure-SST automatically organizes the SST data into the first directory available to the user in the ‚User Manager' of IconNMR configuration. The generated data directory format 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 last experiment, SST generates a PDF report, exemplified in chapter 7.2, which summarizes the test specifications run, the criteria, and the results of each test including a pass or fail notation.

3 Quick Start: Assure-RMS

The quick start will guide you through the Assure-RMS screening method. This will provide a basic understanding of what is performed during automation. Specific items covered are sample submission, spectrum quantification and identification, and the generated PDF reports.

3.1 IconNMR Submission Interface

The starting of IconNMR automatically queues Assure-SST, which will confirm the instrument performance. Successful completion of the SST will allow samples queued to be run in automation either for basic automation submission or for Assure-RMS analysis as described below.

Submitting samples for Assure - Raw Material Screening are done through the IconNMR user interface (Figure 3.1). The user interface allows for minimal user interaction to prevent the alteration of key parameters of the instrumental setup and/or acquisition parameters. Sample submission is achieved by filling in each of the active fields:

- Traceable Raw Data Filename – parent directory where data is stored
- Raw Material Specification – defined acquisition parameters and quantification method to be used on sample
- LIMs Container ID – originator item for tracking, user filled
- Lot number – originator item for tracking, user filled
- Operating Site – indicate facility where NMR spectrum was acquired

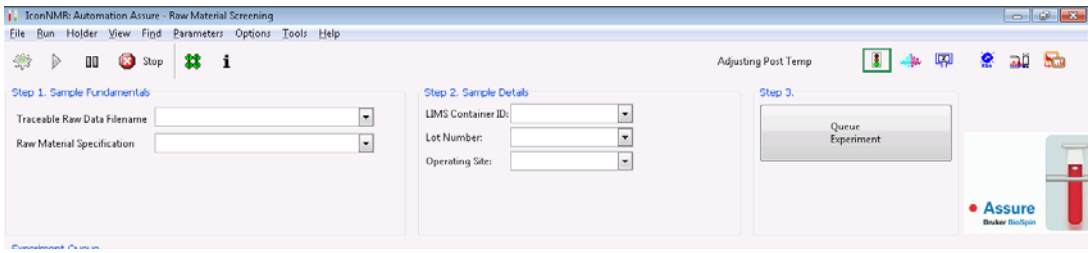


Figure 3.1: IconNMR easy dialog submission interface

Once all of the fields have been populated, selecting the ‘Queue Experiment’ button will result in a window which prompts the user to place the sample in the identified sample position, seen in Figure 3.2. Once the sample is in the automation robot at the designated position the user must select the ‘submit’ icon at the bottom of the page to release the sample to automation control.

Figure 3.2: AutoSampler Position window identifies the sample position of a queued sample.

While the data is acquired and processed in TopSpin and the evaluation is generated by Assure - Raw Material Screening, IconNMR is overseeing all of the processes and commands to be sure that the evaluation is complete from sample submission to spectral analysis.

3.2 Identification and Quantification of Spectral Components

Assure – Raw Material Screening evaluates the data with two parallel evaluations of the spectrum. Figure 3. illustrates the duality in of the spectral analysis, which involves the identification of compound(s) by a spectral match to a spectral base (SBASE) and a quantitative evaluation of the integral proportions of known and unknown signals.

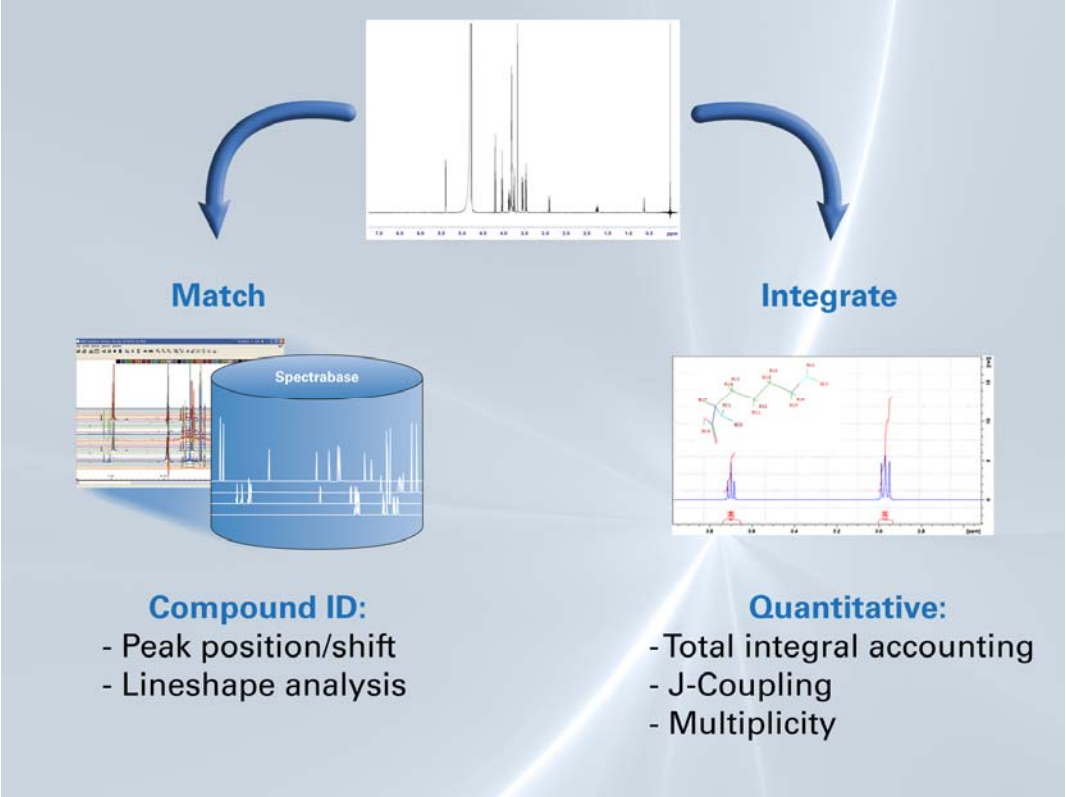


Figure 3.3: Summary of the parallel, complimentary spectral analysis techniques used in the Assure-RMS software package. Spectra are evaluated based on qualitative (left side) and quantitative (right side) properties of the constituents.

3.3 Access to Final Reports

Assure - Raw Material Screening software generates basic (QCReport.PDF) and detailed (ExpertReport.PDF) reports of the evaluation which is stored along with the experiment. Both reports contain information on the instrument, original spectrum, analysis results, and time. The reports can be accessed by right-clicking the spectral line in the 'Preceding Experiments' viewer of IconNMR (Figure 3.2).

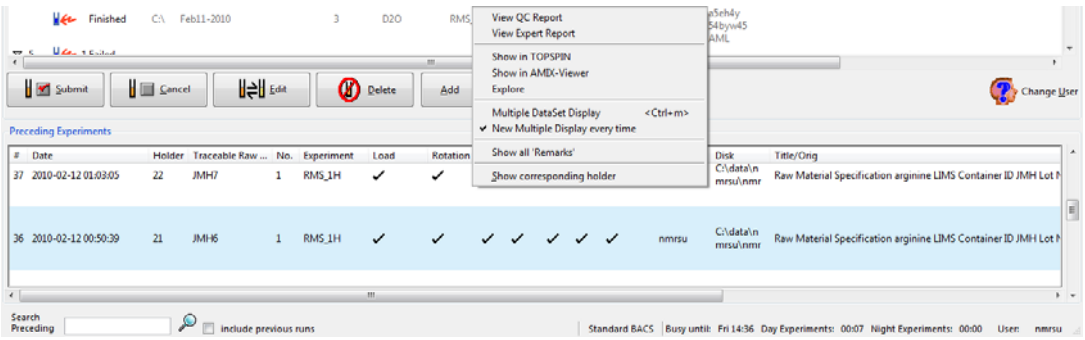


Figure 3.2 Access to the reports via IconNMR Preceding Experiments window.

The QC report shows a simple pass or fail result of the spectrum based on the criteria defined in the quant method. The more detailed Expert report contains information on all of the steps of the analysis. A sample of each of these reports can be found in Chapter 7.2.

4 AMIX: SBASE

Assure-RMS software utilizes an SBASE and KBASE for the identification of components within a material. SBASE's contain the pure spectra and KBASE's contain quantitative information on each individual compound.

Each facility screens different materials than those provided with Assure will need to establish an SBASE and KBASE appropriate to their facility. Bruker Analytical Services is available to provide this service if needed.

As of this manual version, the generation and registration of SBASE can be done with the Assure-RMS software. However, it is necessary that AMIX version 3.9.11 or greater is installed (no license required). Assure-RMS software invokes the 'Preparation' module of AMIX as in Figure 4.1.

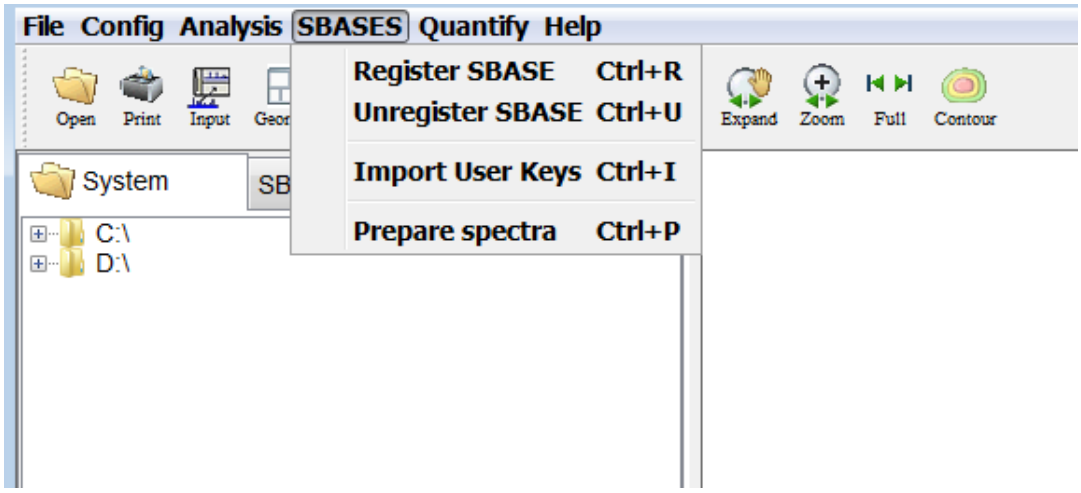


Figure 4.1: Access to the SBASE preparation module of AMIX from Assure-RMS is accomplished by clicking 'Prepare spectra.'

4.1 SBASE Registering and Requirements

Assure–RMS is delivered with an SBASE. In order to utilize the SBASE, it must be registered by the RMS software. It may also be the case that an SBASE is transferred from one location to another and needs to be registered on the local machine. By using the SBASES dropdown (Figure 4.1) and selecting 'Register SBASE' [Ctrl + R] navigate to the SBASE parent directory. For the RMS SBASE this is

Windows 7: C:\Bruker\Databases\SBASE\rmssbase

GNU/Linux: /opt/bruker/Databases/SBASE/rmssbase

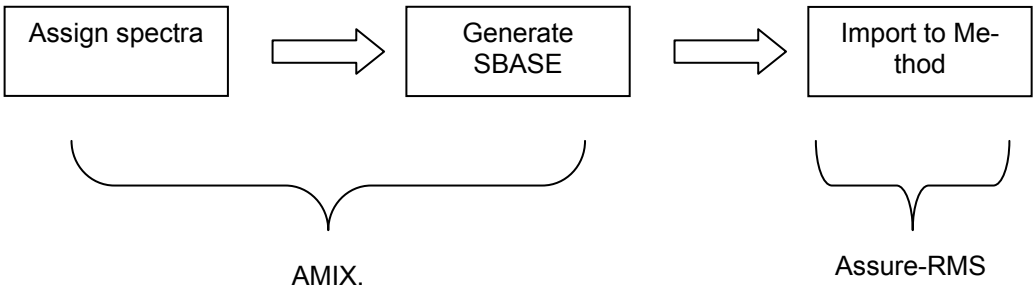
SBASE's registered with AMIX versions 3.9.4 or previous must be unregistered via AMIX and re-registered with Assure-RMS or AMIX version 3.9.7 or greater.

i In the event that an SBASE is updated in the same directory as the existing SBASE (more compounds, more data, changes in 'keys', or changes in nomenclature), it is necessary to unregister the previous SBASE from within the RMS window before proceeding to register the updates.

The first step in generating an SBASE is to make assignments and correlate them to the chemical structure of the compound. Once the assignments are made, AMIX stores the information with the spectrum allowing the information to be transferred to the knowledgebase.

There three necessary spectral notation are covered in section 4.2, 4.3, and 4.4.

In order to quickly import data and maintain proper spectral characterizations across data types, the import method has been designed to simplify and maintain data consistency among the databases. The flow of data into the database can be summarized in the schematic below. This flow is designed to maximize the speed of importing the data and maintaining the consistency across all databases.



There are three spectral notations required when assigning peaks in order to properly import a compound which can be automatically generated with the following data:

1. 1D proton spectrum - the minimal amount of data required for proton only screening can be limited to the 1D proton experiment. It is in this spectrum where all of the multiplicity and coupling assignments are made. Peak annotation is important for importing atom count for quantification.
2. 2D-HSQC spectrum - screening for carbon requires the 2D-HSQC experiment. This can be a standard HSQC or multiplicity edited HSQC (Ed-HSQC).
3. Molecular structure file - can be used for peak annotation, but is not absolutely necessary for annotation. The benefit when annotating in accordance with the molecular structure file is that the reported concentration assignments made via the quantification method coincide with the numerical atom assignments from the structure file.

The example below demonstrates the importation of lysine into a new SBASE. More detailed information on all the tools of generating an SBASE can be found in the AMIX User's manual.

4.2 Assigning Peaks

The tools for assigning peaks within a spectrum is found in the ‘Analysis’ menu. Peaks can be quickly picked with the drop down function ‘Auto Peak Pick’ as shown in Figure 4.2. The resulting picked peaks will be identified with tick marks above the peaks.

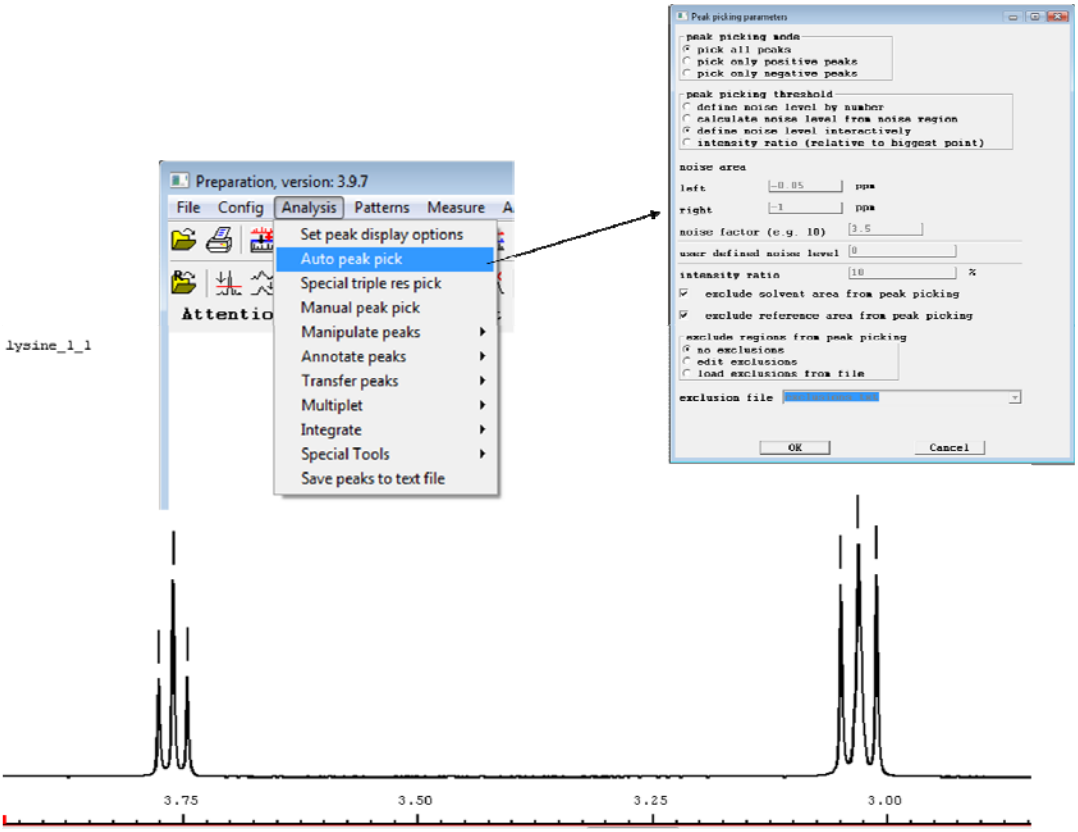


Figure 4.2: Action and window arguments for automated picking peaks in AMIX. The resulting peaks which have been picked are shown with tick marks on the Hα (ca. 3.75 ppm) and Hε (ca. 3.05 ppm) resonances.

Be sure to check the referencing of all spectra before picking peaks, This can be performed by the ‘Simple Calibration’ tool under the Measure drop down menu.

4.3 Annotating Peaks

Peaks can be quickly annotated from the molecular structure. This is with both the molecular structure and the spectrum opened in AMIX, and then selecting an atom from the molecular structure and selecting the corresponding signal on the spectrum as seen in Figure 4.3. When using the annotate feature from the molecular structure tool, it is important to remember these key points:

It is not necessary to annotate the peaks from the molecule.

It is absolutely necessary to annotate the correct number of atoms for quantification of two protons by filling the field with: **H,H**

- Annotating with multiple atoms can be done by selecting all atoms, then the peak
- It is only necessary to select one peak within a multiplet when annotating
- Simple annotation or changes can be made with ‘Annotate peaks’ tool

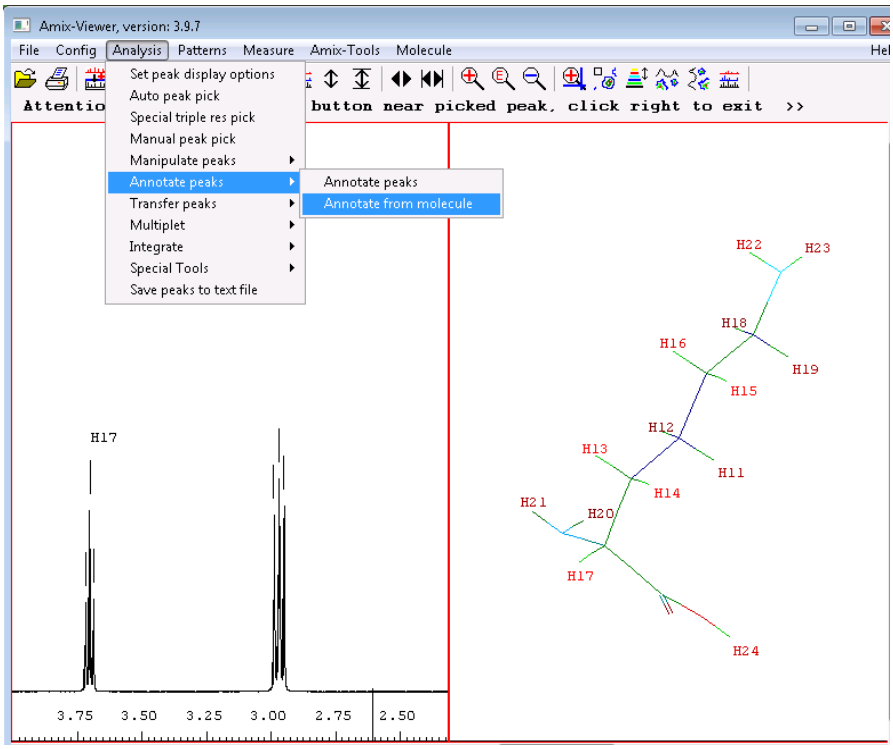


Figure 4.3: An example of the annotate feature from molecule and the resulting annotation in lysine proton H17 (Hα).

4.4 Assigning Multiplicity

Defining multiplicity is done by grouping picked peaks. It is therefore necessary that all of the peaks in the multiplet are picked in order for the multiplicity to be properly assigned. Figure 4.4 shows the assignment of a multiplicity of the lysine triplet using the drag-n-drop tool in the multiplicity editor. Note that AMIX will measure the coupling constants and allow the user to define the multiplicity once the peaks have been defined.

Correct multiplicity assignment is critical to the success of your quantification routine.

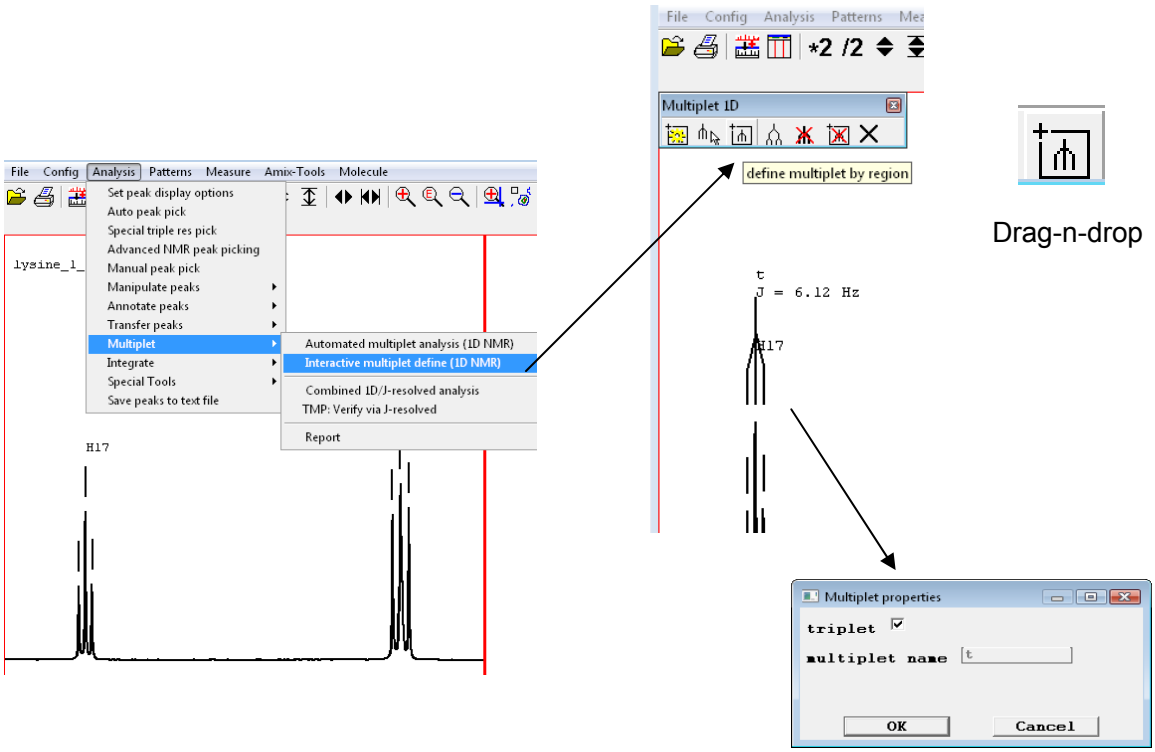


Figure 4.4: Multiplicity tool used to quickly assign multiplets and set coupling constants.

Notes on spectral assignments:

- Once all of peaks have been picked, annotated and correctly defined by multiplicity they are ready to be saved into the SBASE.
- In the case of 13C data it is only necessary to peak pick and annotate the HSQC. Annotations should be made for both 1H and 13C on each peak. AMIX can identify either normal or multiplicity edited HSQC.
- The peaks, annotations, and multiplicities are saved into the spectrum directory when the spectrum is closed. The spectral assignments will reopen with the spectrum.
- It is critical to utilize similar experimental conditions when acquiring data for the SBASE for each reference material. Conditions such as solvent, pulse sequence, and temperature must be identical between the reference library/knowledgebase and the material data.

4.5 SBASE

Data preparation from assigned NMR spectra is done to reduce the SBASE file size and to eliminate any signals that are not part of the target molecule. Data preparation for SBASE is also done under the 'Prepare data' module. All of the data carried from the previous spectral assignment sections is carried into the SBASE.

1. Create new/Register/Delete SBASE
    - Create new – define a new SBASE directory
    - Register – allows the user to register SBASEs that already exist so that it can be used (such as the RawMaterial_demo SBASE provided with the Assure-RMS installation disk)
    - Remove – used to remove SBASE from the registry

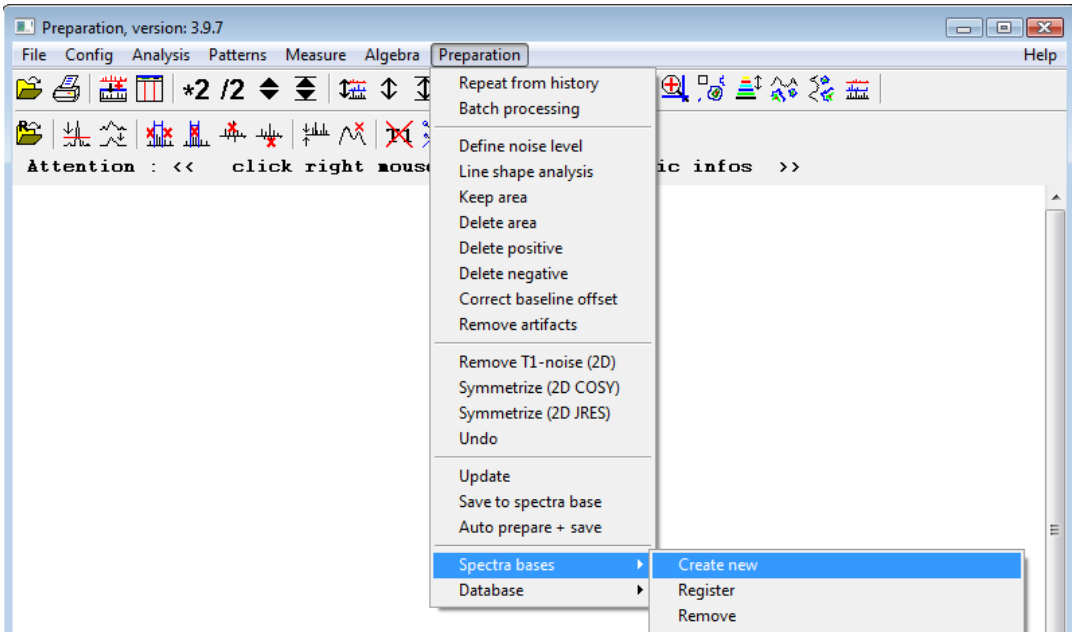


Figure 4.5: Tools of the Preparation module. The Preparation dropdown has all of the tools necessary to import a spectrum into an SBASE.

2. Import spectrum to SBASE – a large number of tools exist within the import option. The minimal steps to import a spectrum to an SBASE is as follows:
    - Open assigned spectrum into AMIX viewer, then under the 'Preparation' dropdown:
    - Define noise level – sets threshold for level at which peaks are kept or removed from the SBASE spectrum
    - Line shape analysis – removes everything below noise level
    - Delete area – drag and drop tool which allows removal of signal not associated with the constituent (e.g. water, TSP, etc.)
    - Save to spectral base

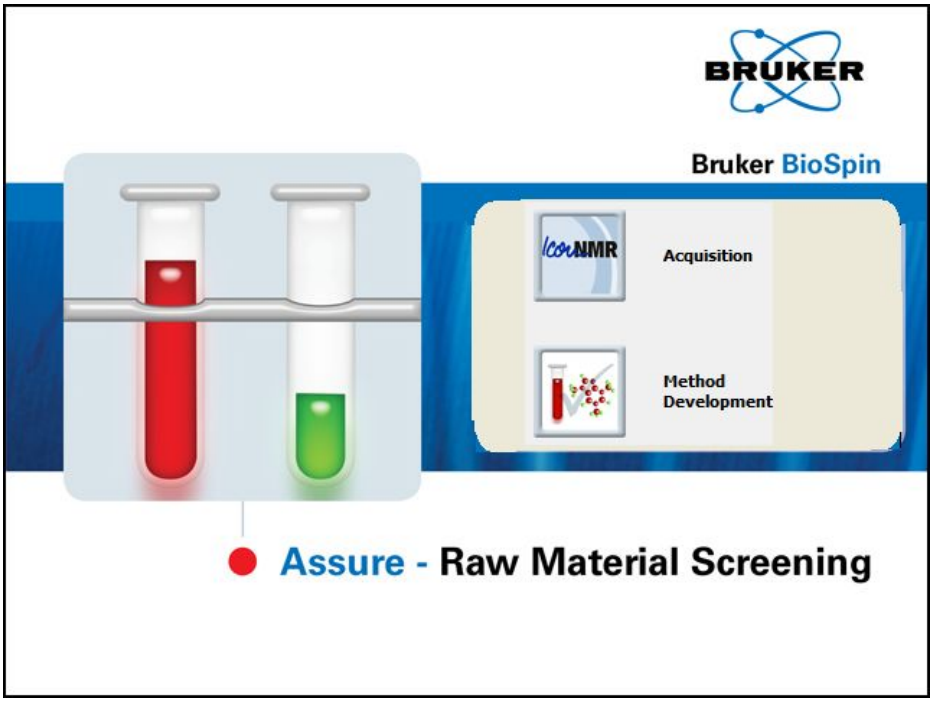
5 Assure - Raw Material Analysis Software

5.1 General Features

The Raw Material analysis software is called Assure-RMS which is the implementation of Java platform using many of the tools and features of AMIX. Specifically, the screening module uses matching algorithms and knowledgebase for identification and quantification of constituents, respectively. The analysis is printed out in a flexible reporting format including summary reports (QCreport.pdf), extended reports (ExpertReport.PDF) and EXCEL output. For further information on matching algorithms and knowledgebase refer to the AMIX manual.

Assure-RMS is launched with the following Icon:

The first window allows the user to go directly to TopSpin/IconNMR (Acquisition) or RMS user interface (Method Development):



The Acquisition option in the splash screen allows the user to launch the package directly into automation when used in combination with the instructions in section 6.1.

---

i Activation and configuration of Assure-RMS for automated analysis are covered in Chapter 6. This chapter only covers the stand alone Raw Material Screening analysis software and it's usage.

The RMS user interface shows several drop-down menus, Icons, a data browser toolbar, and a viewer window (Figure 5.1).

The data browser on the left allows the user to navigate to raw data and an SBASE browser can be opened or closed from the 'File' drop down menu. Figure 5.1 shows the viewer with the SBASEs which are displayed next to the System navigator.

Data can be opened by navigating through the directories or by simply dragging and dropping the experiment into the viewer window.

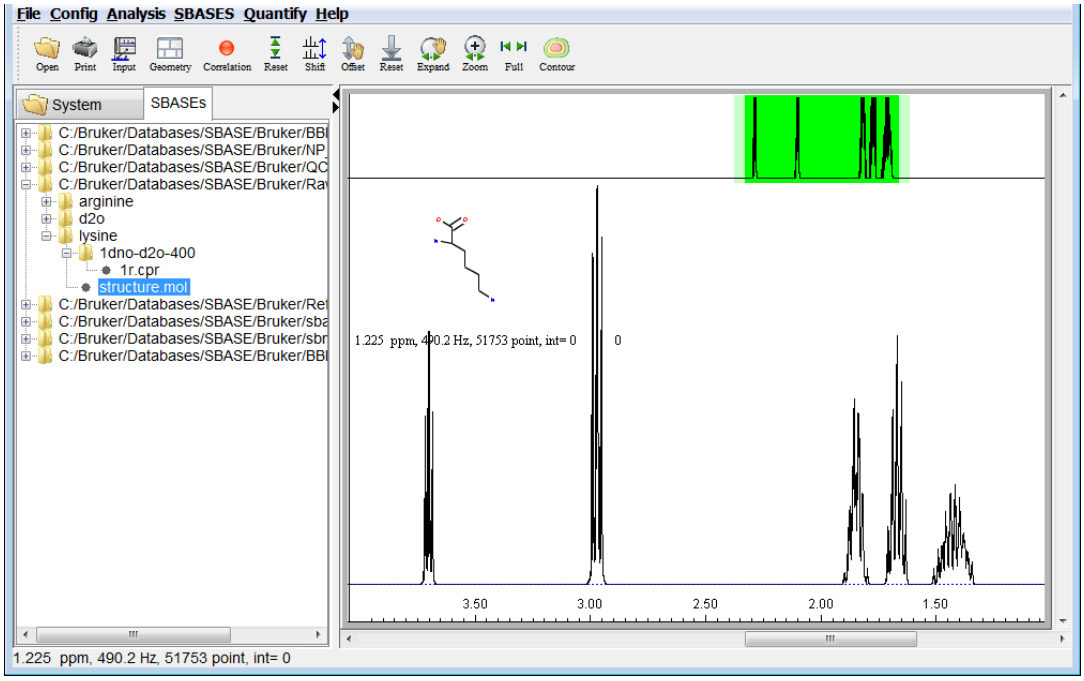
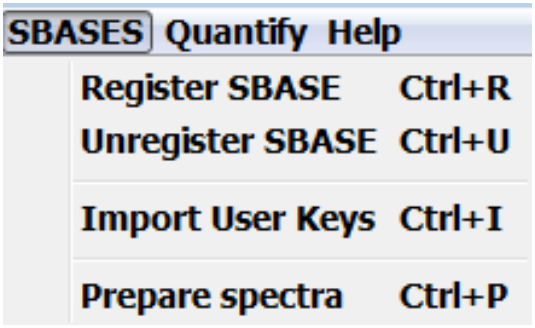


Figure 5.1: Assure - Raw Material Screening Analysis viewer window layout with SBASE browser open. The SBASE spectrum and molecular structure file from the provided SBASE are shown.

5.2 SBASES Dropdown

Within the SBASES dropdown window are the options for registering and unregistering SBASE, importing user defined keys, and an option to open the preparation modules. User defined keys are described in the AMIX manual. The Preparation module is a component of AMIX and is covered in chapter 3 of this manual with further details in the AMIX manual.

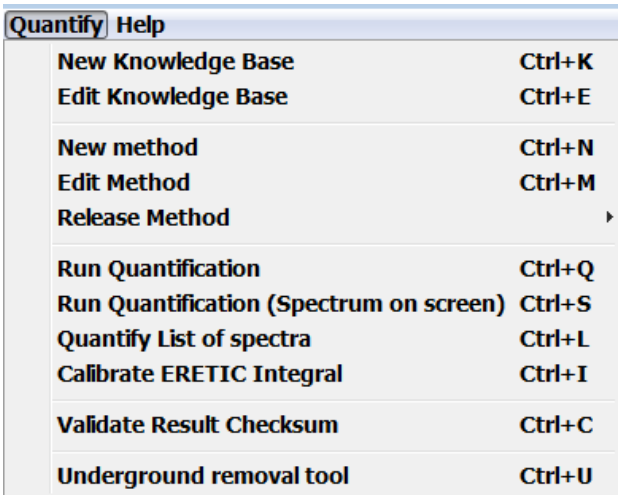


Registering the SBASE delivered with Assure-RMS (default directory installation) can be done by browsing to the parent directory C:\Bruker\Databases\SBASE\rmssbase.

The ability to open and close the SBASE browser (Figure 5.1) is found in the 'File' dropdown menu

5.3 Quantify Dropdown

All of the tools for building quantification methods and running analysis manually can be found in the 'Quantify' drop down menu.



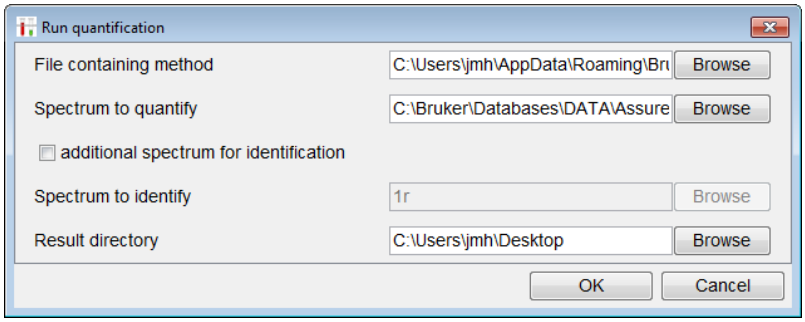
The Assure-RMS screening software in combination with SBASE analysis utilizes an NMR Knowledgebase which is a collection of compounds and their respective chemical shift assignments and coupling patterns. This Knowledgebase may be stored in any directory convenient to the user. Select the appropriate Knowledgebase by selecting 'Edit Knowledge Base' under the Quantify menu item.

---

i The examples in this section illustrate ^{1}H and ^{13}C examples, but Assure-RMS also supports ^{2}H, ^{19}F and ^{31}P.

Methods are subsets of the knowledgebase where the user selects a few compounds which are to be analyzed in the NMR spectrum.

Once methods and SBASEs have been properly organized, it is possible to evaluate the analysis method using the 'Run Quantification' item where the data, method and output results should be indicated.



Similarly, the user can directly analyze the spectrum on the screen using the 'Run Quantification (Spectrum on screen)' feature.

Batch analysis of data can be run using the 'Quantify List of spectra' option.

Calibration of a standard integral to be used for absolute quantification can be done using the 'Calibrate ERETIC Integral' function. Here the user defines the molar concentration of a known spectrum to generate a standard integral which can be used to quantify constituents in spectra which were collected on the same instrument. The calculated concentration implements the PULCON principle

5.3.1 Quantification and Identification of Spectral Components

Processed data from TopSpin is passed to the Assure - Raw Material Screening software that utilizes a spectral base and knowledgebase for identification and quantification of individual components of the sample. Evaluation is based on two criteria: quantification and spectral matching. Positive identification from both of these methods gives confidence in the identification of a constituent and is compiled into a quant method. The quant method also sets the level of a contaminant allowed in a passing sample or thresholds which results in a failed sample.

Matching

Spectral matching is done by projecting the raw material spectra onto a previously acquired spectrum of a known, pure sample stored within the SBASE. The criteria for spectral matching are that the mixture and the pure reference must both have intensity at the same location. Subroutines to determine the same multiplicity and lineshape are then used to ensure that the signals observed in the raw material spectrum are consistent with the structural properties reflected in the purified, reference spectrum. The search region is defined as +/- 0.02 ppm.

Quantification

Quantification of each component observed in a spectrum requires a compiled list of specific chemical shifts detailing the atom count at each of these signal regions. The presence of a compound will be confirmed when the all of the signal regions possess integrals and the integrals are in proportion to their respective atom count. Figure 5.2 illustrates in lysine how the areas of non-exchangeable peaks maintain a relative atom integral balance. All intensity is accounted for and there is no +/- threshold for expected integral.

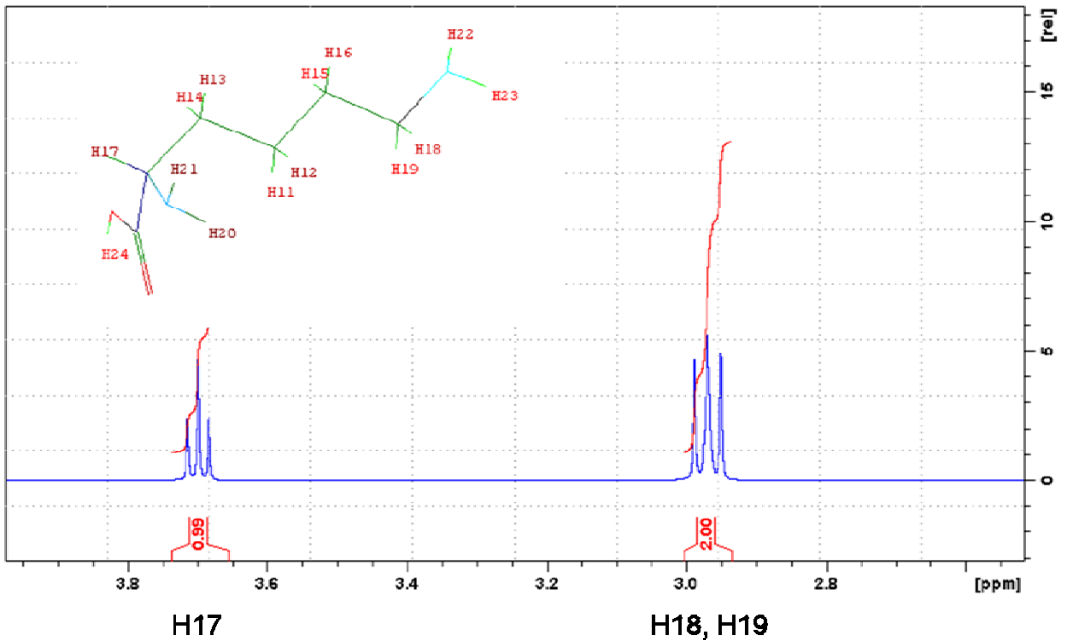


Figure 5.2: Atom integral balance of lysine. Spectrum shows Hα (H17 – ca. 3.7 ppm) and Hε (H18, H19 – ca. 2.95 ppm). Integrals of relative intensity are indicated.

There are six possible means of reporting the integration results. The first four correspond to relative internal quantification to the main component as summarized in Table 5.1

	Internal Composition	
	Absolute	**Relative**
% Molar	$\frac{\frac{\int \text{Lysine}}{\#\text{of Protons}}}{\left(\frac{\int \text{Lysine}}{\#\text{of Protons}} - \frac{\int \text{Arginine}}{\#\text{of Protons}}\right)}$	$\frac{\frac{\int \text{Lysine}}{\#\text{of Protons}}}{\frac{\int \text{Arginine}}{\#\text{of Protons}}}$
% Gram	$\frac{\frac{\int \text{Lysine} * MW}{\#\text{of Protons}}}{\left(\frac{\int \text{Lysine} * MW}{\#\text{of Protons}} + \frac{\int \text{Arginine} * MW}{\#\text{of Protons}}\right)}$	$\frac{\frac{\int \text{Lysine} * MW}{\#\text{of Protons}}}{\frac{\int \text{Arginine} * MW}{\#\text{of Protons}}}$

Table 5.1 Summary of quantifications methods available based on the relative concentration to the main component as exemplified with lysine as the adulterant and arginine as the main component.

The methods of absolute quantification available are seen below in Table 5.2. These can use either an inserted known standard such as TSP, DSS, or an inserted reference integral file such as ERETIC II.

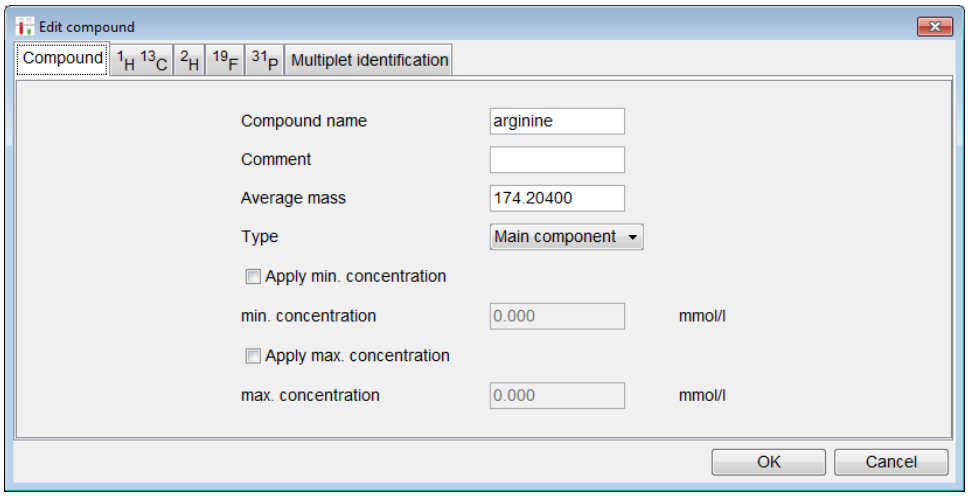
Absolute Composition	
Internal Reference	External Reference
Formulation	ERETIC, ERETIC II, PULCON

Table 5.2 Summary of absolute quantification methods

5.3.2 Quantify: Knowledgebase

Knowledgebase is a collection of all the definitions of the spectral properties of all of the compounds that are to be screened. It is different from a quantification method in that the definitions of which specific compounds and their adulterant thresholds are not necessary. The purpose of the Knowledgebase is to create a comprehensive table of all compounds from which identification and quantification methods could be easily generated.

The relevant fields in a Knowledgebase include the following details and rules to ensure a successful screening method:

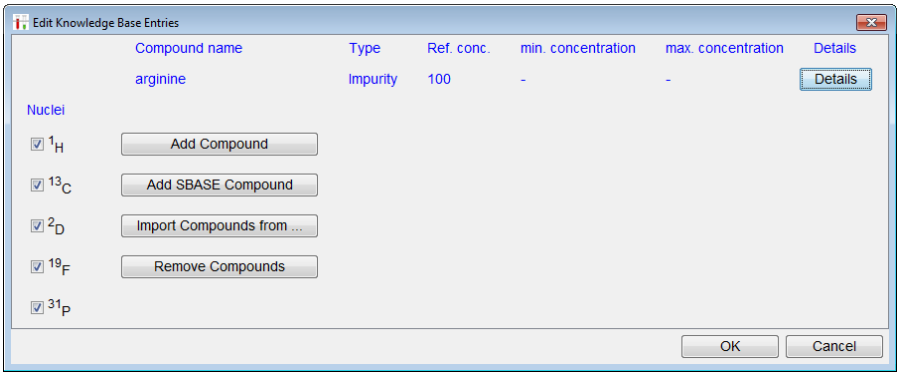


- Compound
    - Compound name – the same name must be used in the knowledgebase as is used in the SBASE for match results
    - Molecular weight – used in the calculation of concentration

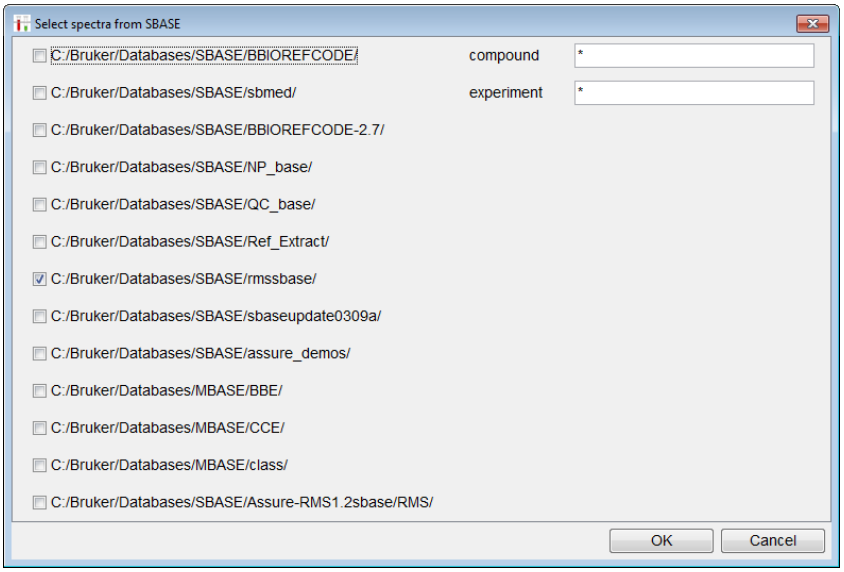
- 1H (^{13}C, ^{2}H, ^{19}F, or ^{31}P nuclei)
    - Area where signal is expected in the NMR spectrum and the number of atoms at the area
    - The multiplicity, J-coupling, and define which signal(s) are used for integration for each signal area
- Multiplet identification – set the plus/minus tolerance of J-coupling and point at which peaks are no longer picked.

Using the example SBASE provided, a knowledgebase can be made as follows:

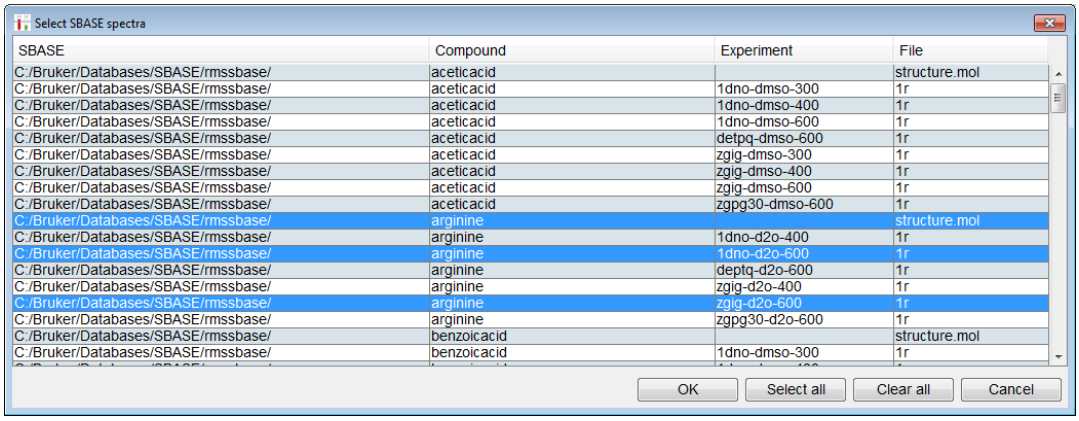
1. New/Edit Knowledgebase – opens the screen below to import a new compound from an SBASE or an alternative knowledgebase.



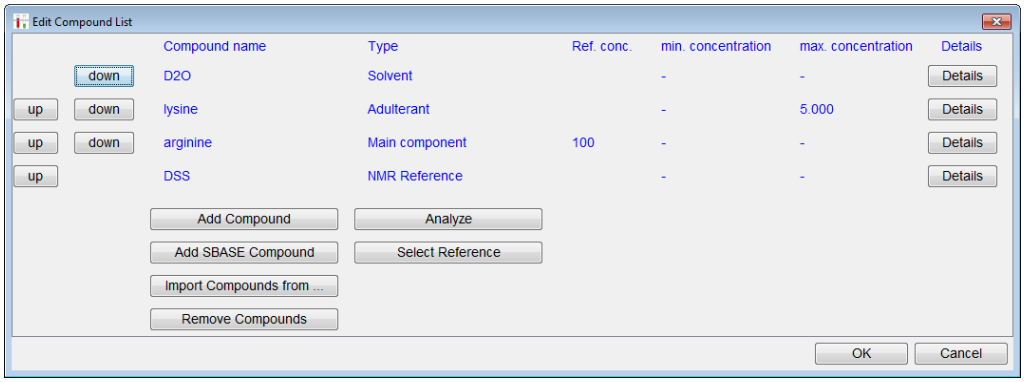
2. Select ‘Compound from SBASE’ to identify the molecular structure file (molfile) and spectra to associate with this compound and to assign the compound to a main component, adulterant, impurity, solvent or NMR reference signal.



3. SBASE selection - Choose the needed spectra and molfiles for the compound by holding shift key and selecting the desired files.



4. The sample is populated into knowledgebase once the spectra and molfile are selected and named according to the SBASE entry as shown here with arginine.



Selecting the ‘Details’ button at the right of the Knowledgebase Entries window allows the user to inspect the quantitative details such as molecular weight, integral regions, number of atoms, and multiplicity of the imported compound. The tabbed dialogs within the resulting ‘Edit Compound’ dialog is pre-filled to match when imported from an SBASE entry containing all of the spectral definitions of the compound. The definition and description of each tab is covered in the following ‘Quantify: Methods’ section.

5.3.3 Quantify: Method

The quantification method defines how a spectrum is evaluated by defining what the parent compounds are, what the adulterants are, which signals are irrelevant (e.g. solvent), and how the final report should discriminate between a sample that passes or fails the adulterant threshold requirements. It includes the definitions in the Knowledgebase plus some additional settings for limits on adulterant levels.

Selecting the New Method (ctrl + n) opens a dialog as shown in Figure 5.3. The dialog is similar to the dialog when editing an existing quantification method. The ‚General’ tab contains the options (as exemplified with the delivered arginine.quantmethod):

- Method Name – name of the method

- Edit Compounds – table which corresponds to the knowledgebase and is used to set the compound definitions of known constituents.
- Report Format – report a ‘Pass/Fail’ or numerical results
    - Precision – becomes active with ‘Number’ format
- Custom Report – choose to use default PDF report formats, edit a format template, or remove a format template
- Integrate by – choose whether the method should integrate by peak fitting or use a general region integration routine.
- Concentration – defines the quantification calculation that will be used
- Minimum reported threshold – the level of integration relative to the main component at which any signal NOT defined in ‘Edit Compound List’ is reported
- Failure threshold – level of integration relative to the main component at which any signal NOT defined in ‘Edit Compound List’ is reported and gives a FAILURE in the final report
- Noise factor – number of standard deviations (sd) above noise to define real peaks for any signal.
- Import Eretic reference – here the user defines the calibrated file used for quantification. Note: only active when ‘Concentration’ is set to one of the two ERETIC methods.
- Error confidence limit – used with the multi-sample acquisition tool. Here the limit is used to calculate the error for quantifying a set of spectra. The error is calculated from the standard deviation and T-test using this confidence limit.

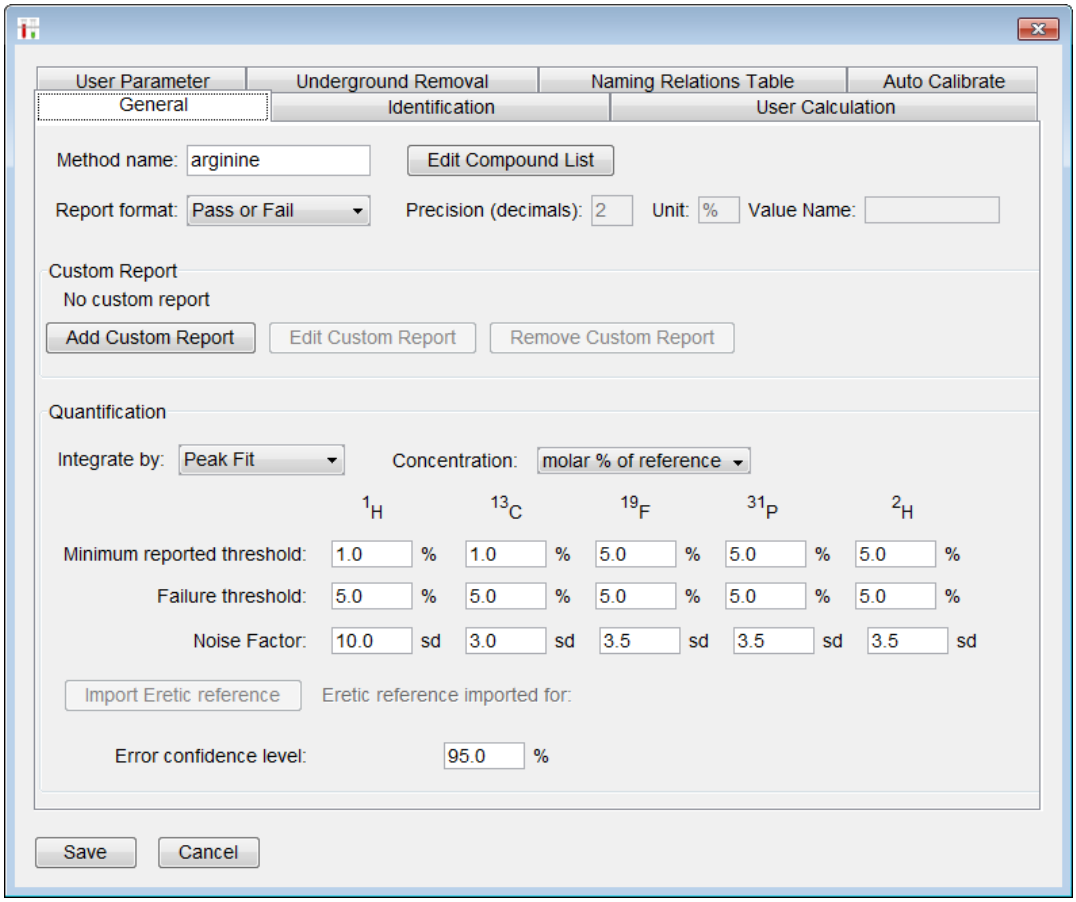


Figure 5.3: Shows the first window, first tab when editing or starting a new method. This dialog shows the arginine quant method provided with the release software

Figure 5.4 shows the 'Identification' tab of the method. This tab contains the options:

- Spectra base – SBASE which is used to match after quantification. It will determine if any compounds in the SBASE which are not in the 'Edit Compound List' field are in the spectrum.
- Experiment type - name of the experiment type which is defined in the SBASE
- Min match factor – the minimum level of confidence from a match at which the presence of a compound from SBASE is reported.
- Max. shift – plus and minus search region for SBASE matching

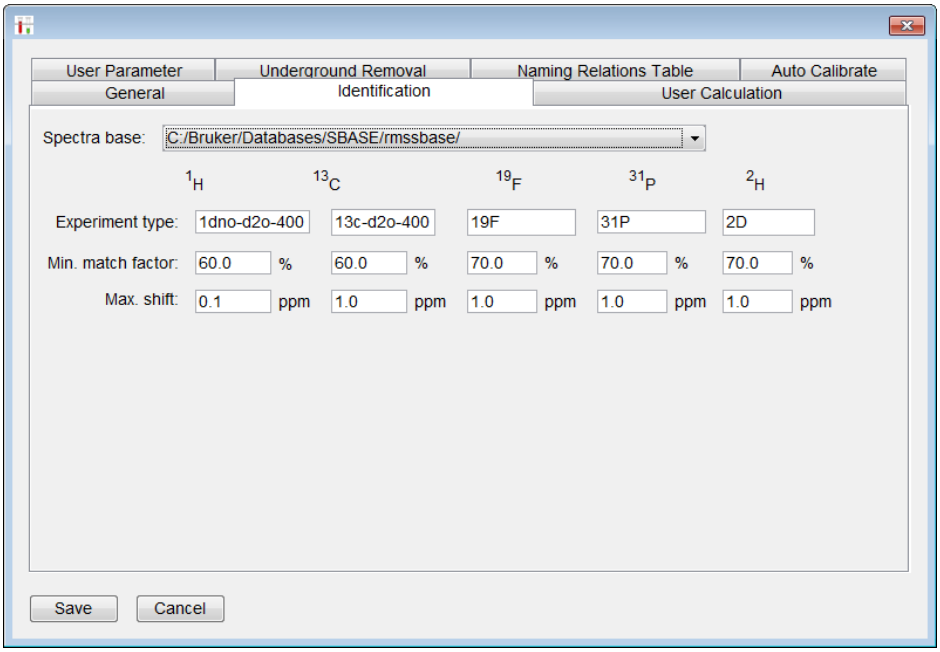


Figure 5.4: Shows the tab for Identification which uses the SBASE to identify and confirm compounds.

In the case that the integration routines available are not sufficient, it is possible for the user to define their own integration routine (Fig. 5.5). An example of the integration routine for tire rubber analysis is presented in chapter 7.3.

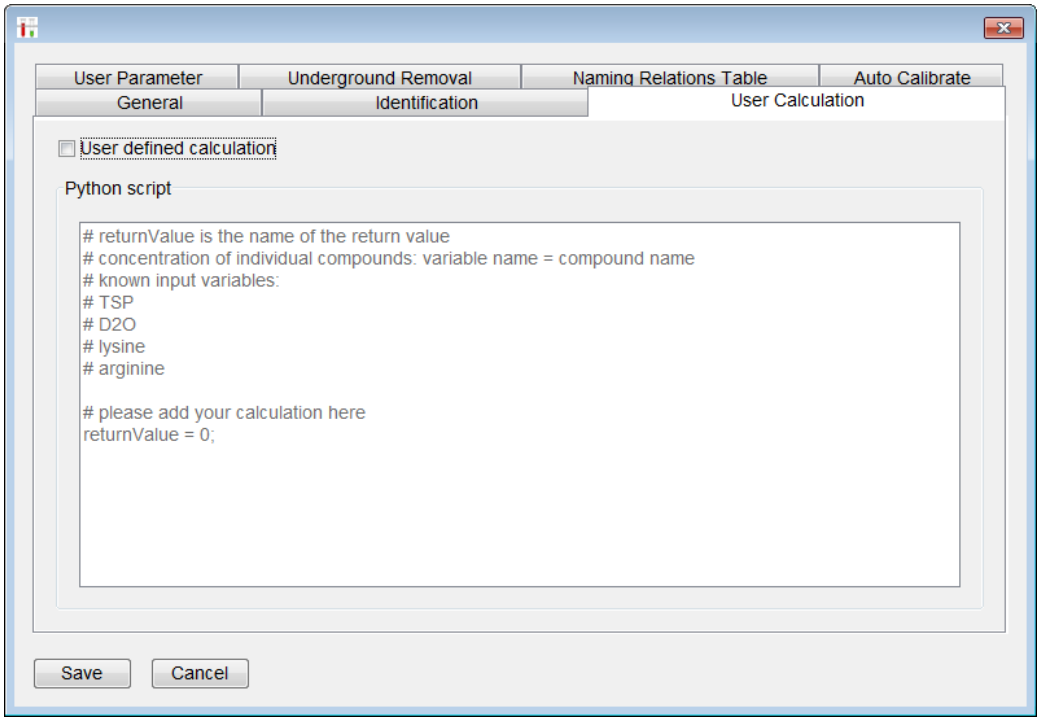


Figure 5.5: Dialog box for user defined integration routines

Figure 5.6 shows User Parameter where the user can adapt their own defined parameters for inputting values for the ‘User Calculation’ required at IconNMR submission. When

this box is active a dialog will open during IconNMR submission for the user to input a value to define input and output variables:

- New Integer – create a user defined integer
- New Decimal – create a user defined decimal

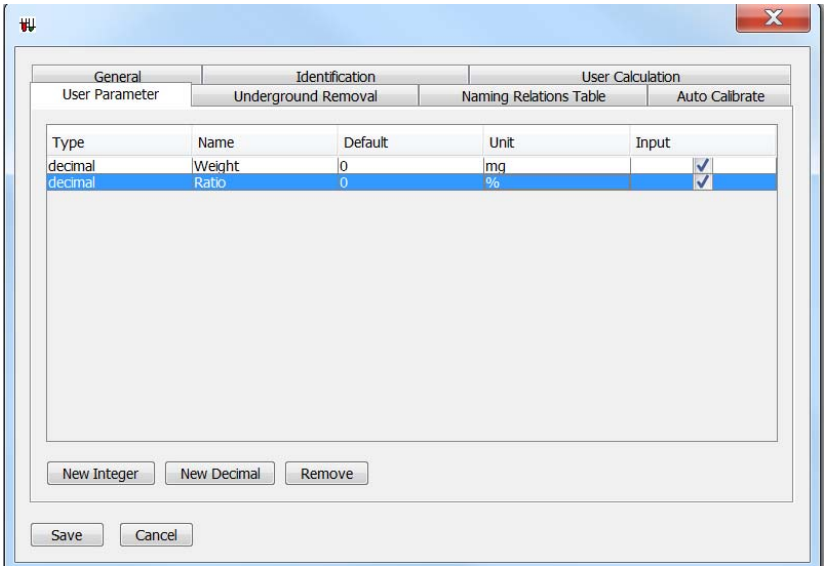


Figure 5.6: Dialog box for User Parameter

The naming relations table (Figure 5.7) allows the user to alter the name reported on the final QCReport or ExpertReport.

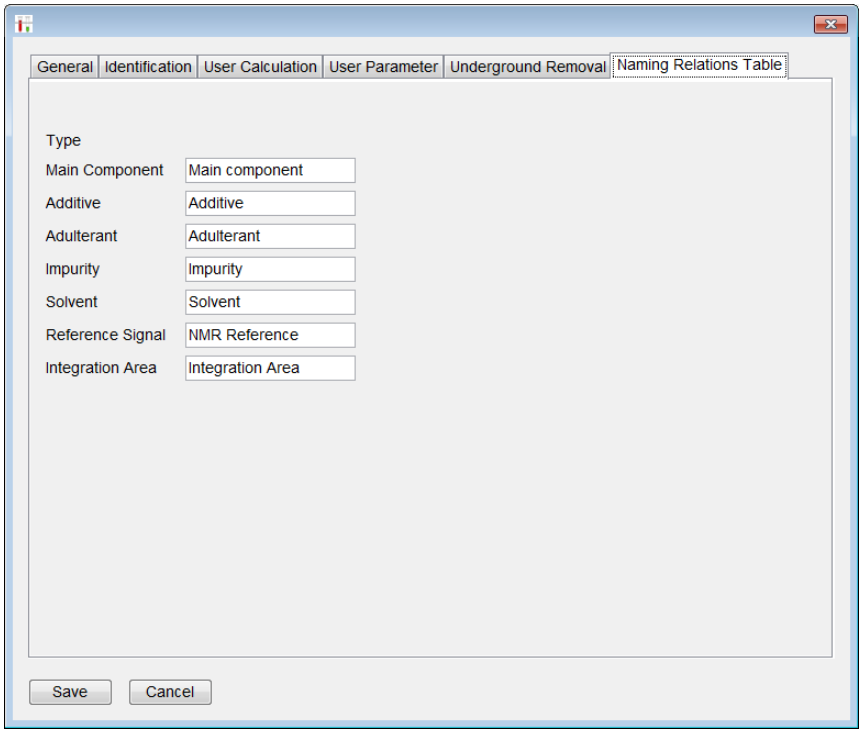


Figure 5.7: Dialog box for User Parameter

Configuring the system to generate a quantification standard is found in the Auto Calibrate dialog (Figure 5.8) where the user defines which compound from the ‘Edit Compounds’ list (and known concentration) should be used to generate a reference concentration integral. The resulting output in the directory of the analyzed data produces a reference integral file which is recognized by both RMS and TopSpin.

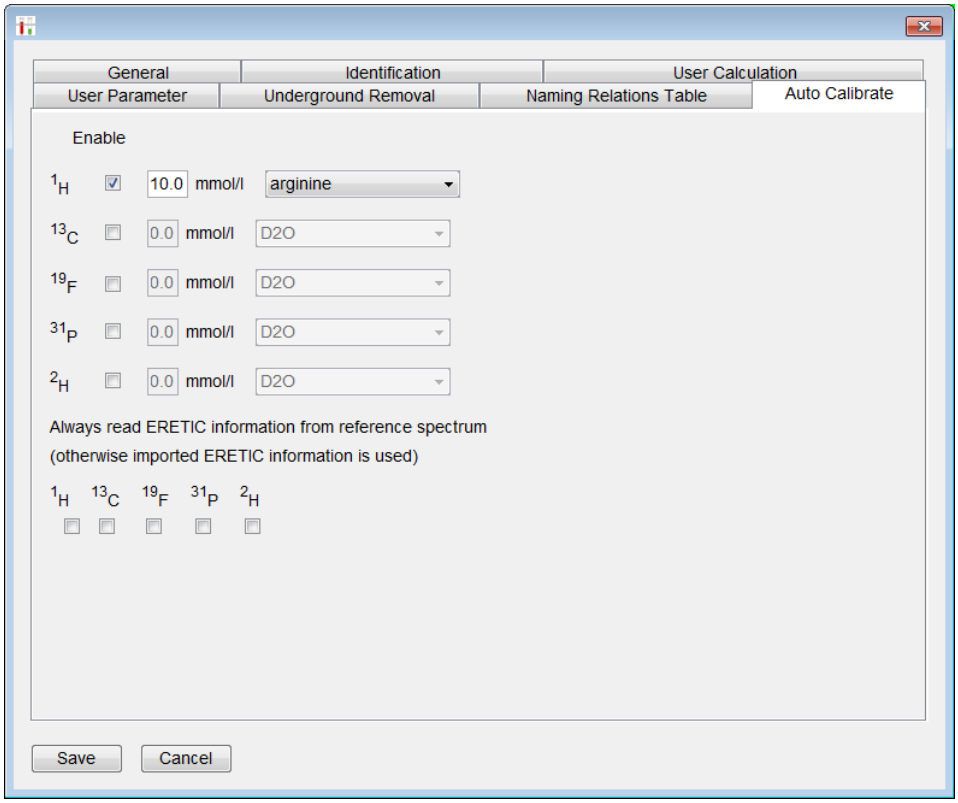


Figure 5.8: Dialog box for Auto Calibration of eretic integral reference file

The generation of the quantification reference file is done by using the ‘Calibrate ERETIC integral’ (Chapter 5.3.5).

Utilization of the calibrated reference file to quantify constituents in other spectra when using the stand-alone RMS software is done by selecting the path of the eretic file within the quant method. The dialog ‘Import ERETIC reference’ (Figure 5.9) in the main method interface become active when one of the two methods for quantification are selected from the ‘Concentration:’ drop-down menu.

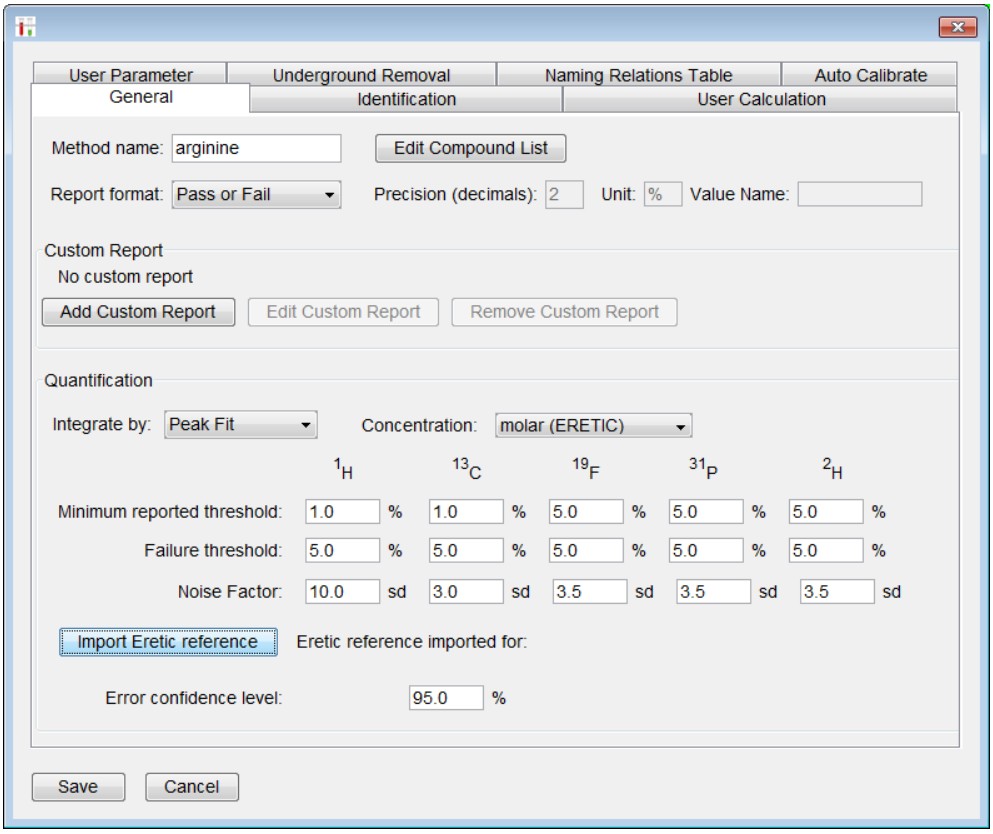


Figure 5.9: Activation of the 'Import Eretic reference' dialog box when a Concentration determination scheme is used which utilizes ERETIC.

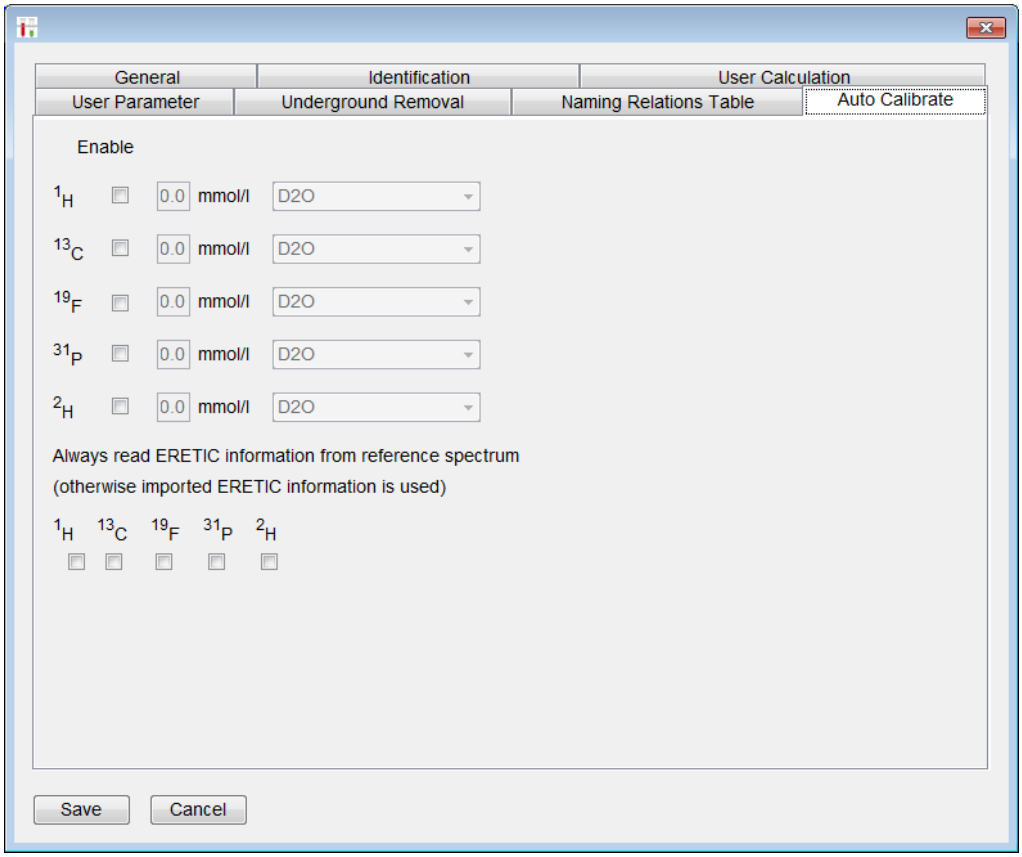
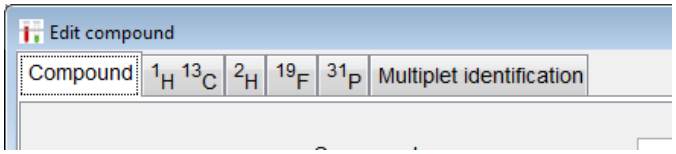


Figure 5.10: Dialog box for Auto Calibrate to define the nucleus, concentration and sample to use to generate the reference integral.

Edit Compounds

Within the 'Edit compounds' of the 'General' tab is the detailed description of the compounds and how they will be integrated according to the Concentration method. Like the Knowledgebase, it is possible to import more compounds manually, from an SBASE, or from a Knowledgebase. The entries list shows a summary of the method, and detailed definitions are edited or viewed by selecting the compounds 'Details' button.

Different tabs when checking compound 'Details':



- Compound – overview of compound
  - Name – name of compound. Must be same as SBASE name
  - Type – defines compound designation and how the compound is integrated. Table 5.3 summarizes the evaluation of the following compound types:
    - Main Component – main constituent of sample
    - Additive – constituent to be monitored

        - Adulterant – same as ‘Additive’, but evaluated after the presence or absence of ‘Additive’ has been evaluated
        - Impurity – known impurities in the sample
        - Solvent – solution(s) found within the sample that are used to solvate the constituents
        - NMR Reference – referencing standard
    - Apply ‘min. concentration’ – use if screening by a minimum detectable level is desired.
    - Apply ‘max. concentration’ – use if screening by a maximum detectable level is desired.
- ^{1}H (^{13}C, ^{2}H, ^{19}F, or ^{31}P)
    - Define expected signal regions for given nuclei
    - Set the number of atoms within each defined region
    - Set the annotation on how the region is labeled in the final report
    - Define the type of shape of the peak within the defined region. **NOTE: It is particularly important to accurately define the peak shape**.

      Defined Coupling Patterns:
        - S – singlet
        - D – doublet
        - T – triplet
        - Q – quartet
        - Quin – quintet
        - Sept – septet
        - DD – doublet of doublets
        - DT – doublet of triplets
        - DQ – doublet of quartets
        - AB
        - ABX (AB)

      Special Patterns:
        - Artificial – allows for the users to define coupling patterns explicitly such as those in lysine in the example SBASE which are not true triplets, but exhibit strong coupling.
        - M – multiplet – defines any set of peaks that can’t be described as a defined coupling pattern or by ‘Artificial’. In the case that another pattern from another compound exists in the same region, the defined multiplet will be extracted first and the remaining signal will be integrated.
        - R – region – integrates a region. It is only to be used when there is never any signals other than the one defined in the knowledgebase.

    - Coupling – measured coupling of line splitting

- Quant – whether or not the peak should be used for quantification
- Keep ratios – quantitation via deconvolution is done by maintaining the peak ratio within the splitting pattern. Ratio is defined from the spectrum of the purified compound.
- Details – active for artificial lineshape only. Allows definition of ratios

- Multiplet Identification
  - Set tolerances on multiplet ratios (when to stop picking peaks relative to main peak in multiplet) and J-coupling ranges.

		Failure on Quantify		Failure on Match	
	Full spectral integral	1H	13C	1H	13C
Main component	Yes	Yes	No	Yes	Yes
Additive	Yes	Threshold	Threshold	Threshold	Threshold
Adulterant	Yes	Threshold	Threshold	Threshold	Threshold
Impurity	Yes	No	No	No	No
Solvent	No	No	No	No	No
NMR Reference	No	No	No	No	No

Table 5.3. Summary of integration and failure triggers for defined compound types

A feature of the quantification building interface is the ability to evaluate the probability of success in identifying and quantifying the compounds in the method. By selecting the 'Analyze' button, a report is generated which evaluates the provided data and reports any conflicts that may hinder the success of the method. Figure 5.11 shows the results of the analysis of the arginine quantification method.

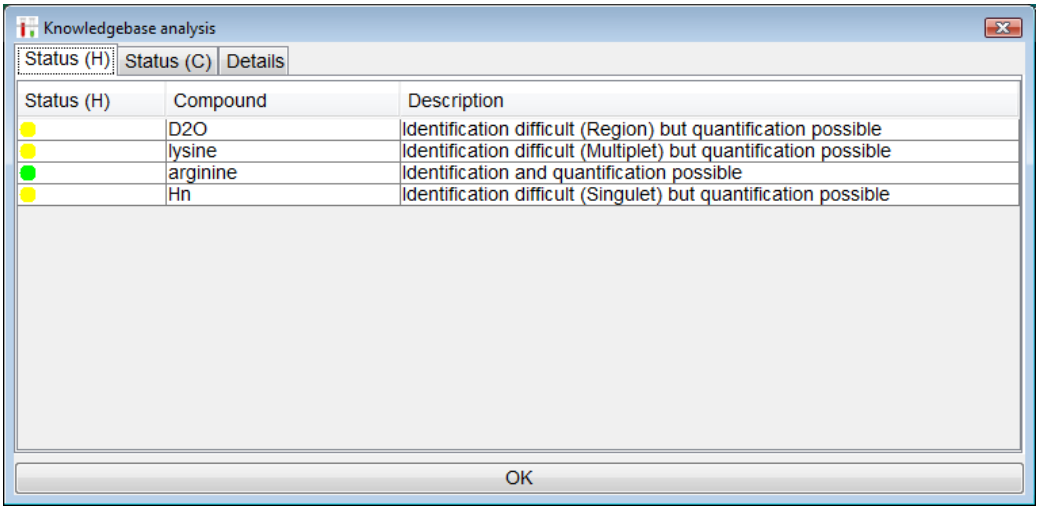


Figure 5.11: Analysis results of the arginine quantification method. Tabs are available for 1H, 13C and detailed reports. A green status ball suggests that the compound will be suitable and the yellow status ball suggests the compound may have difficulties for the identification and quantification as defined.

5.3.4 Quantification: Run Quantification

It is possible to run individual quantification reports manually. This can either be done on a single spectrum or on a series of spectra. To run a quantification method manually, choose 'Run Quantification' and fill in the dialog in Figure 5.10 as follows:

1. Quantification method (*.quantMethod)
2. 1H Spectrum – The full path to the 1r file must be used. For example, using the standard Bruker format the Windows 7 directory is:

<drive>:/Bruker/TOPSPIN/data/<user>/nmr/<filename>/<expno>/pdata/<procno>/1r

3. Confirmatory match on a second spectrum is possible if the full path of the data is provided
4. Result directory – this will print out the expert report and the QC report in the selected directory. Assure-RMS will not allow overwriting of other reports so it is necessary to remove old versions or select new directories before executing the quantification. When using 'run quantification' from Assure-RMS, the reports will be opened directly using the computer's default PDF viewer.

It is possible to simply run the currently displayed spectrum on the screen by selecting the 'Run Quantification (spectrum on screen)'.

Manual quantification of a series of spectra is possible and very similar to single manual runs. For batch analysis, a file is needed that contains the list of spectra to be analyzed. This list can be generated in TopSpin with the command 'serial' (see the TopSpin help menu for further information.

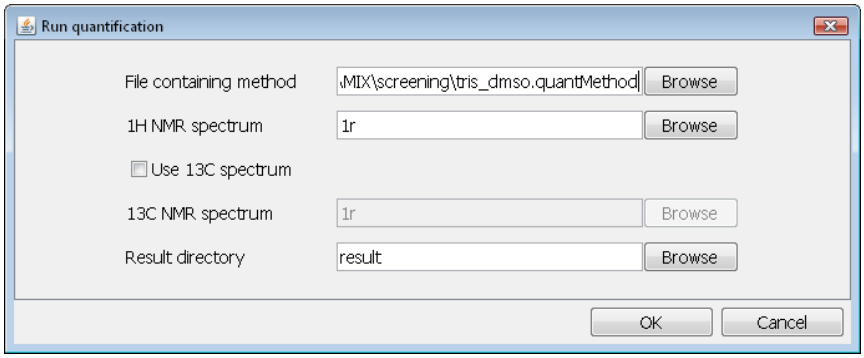


Figure 5.12: Dialog window for running quantification of a spectrum manually.

5.3.4.1 Details of Integration: Quantification and Detection

The Assure-RMS package identifies noise by dividing the spectrum into 16 equidistant regions, or a minimum of 512 points are used. The region fulfilling the following criteria is then used:

- Mean and median are similar
- Skewness is close to zero
- No real peaks in the region – no peaks above noise
- Region with most Gaussian-like distribution

Noise is then defined as the mean plus a factor (F) times the standard deviation

$$\text{Noise} = \text{Avg} + (F * \text{STD})$$

Peak identification is then based on the criteria of which F is used and the user can select LOQ (Level of Quantification) and LOD (Level of Detection) for the appropriate spectral evaluation scheme. In a composite experiment, the user would define the LOQ for the 1H spectrum and the LOD for the 13C spectrum. For example, setting of LOQ (STD = 3.0) on 1H and LOD (STD = 10) on 13C.

5.3.5 Quantify: Calibrate ERETIC Integral

Calibration of the reference integral with a known concentration is done for absolute quantification of unknown samples. The ERETIC integral utilizes the same format at ERETIC in TopSpin.

To generate an ERETIC integral, the quant method requires use of the ‘Auto Calibrate’ tab. Higher accuracy can be achieved using the ‘lineshape’ option.

The resulting analysis creates an ERETIC file in the reference experiments PROCNO. Use of the ERETIC file can be used to quantify constituents in unknown samples. The resulting

analysis copies the ERETIC file from the reference standard into the directory of the unknown sample. If there is a pre-existing ERETIC file, then that pre-existing file is incremented by 1 (e.g. ‘eretic_1’) instead of being deleted.

This function is used in combination with IconNMR to generate reference files in automation (Chapter 6.3 and 6.5).

6 TOPSPIN, IconNMR, and Assure in Automation

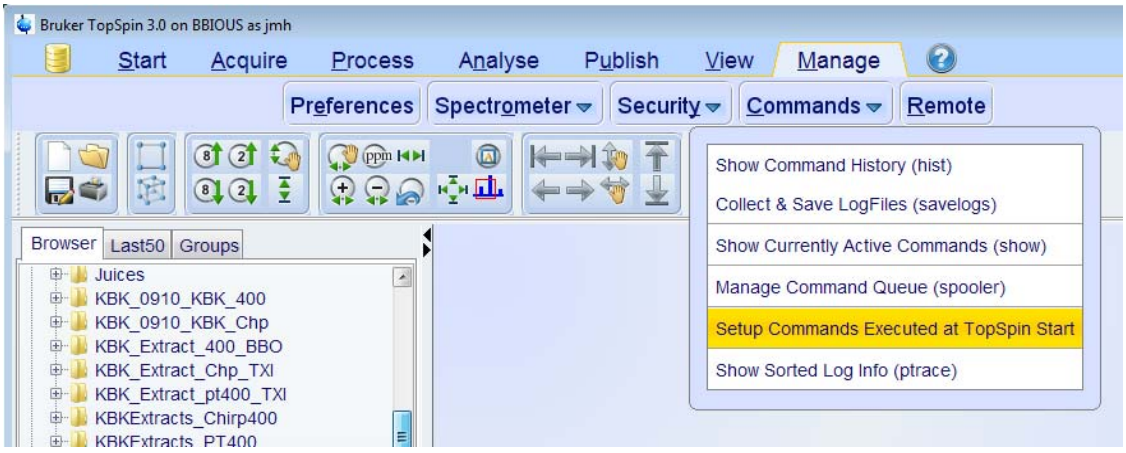
There are three levels of user interface to TopSpin configuration and commands: NMR Superuser, NMR User, and access-restricted accounts. The first two users and groups are set at the TopSpin install. In order to activate the access-limited user, a default user should be created on the computer. The examples illustrated here use the user 'QCuser' as the access-limited user.

The Assure-RMS software is designed to be used in a production facility with primary operators who are unfamiliar with the operation of a NMR spectrometer. For this reason, the software is designed to utilize two levels of users (1) QCUser and (2) NMR Superuser and/or NMR User. The QCuser may be configured to have minimal access to software functions while the NMR Superuser has broad access to NMR software functions. Details on additional files and directories where the QCuser should not have access are found in chapter 7.5.

6.1 Starting TOPSPIN

TopSpin configuration must be done as NMR Superuser. TopSpin is started using the desktop IconNMR.

In TopSpin 3.0 and above it is possible to launch directly into IconNMR without opening the TopSpin interface. This is beneficial on open access instruments when it is important to limit user access. Direct launching of IconNMR from the TOPSPIN desktop Icon can be done by opening TopSpin as QCuser. Under the menu flow item 'Manage', the item list 'Commands' has a selection for 'Setup Commands Executed at TopSpin Start'.



This will open a text file where the line ‘icona’ can be added. Upon closing TopSpin and then double-clicking the TopSpin Icon, TopSpin will open in the background and IconNMR will open in the foreground.

6.2 GLP Requirement

GLP software package is not required to be installed for the Assure software. Additional GLP configuration of the report layout can be done. Instructions can be found in the GLP manual or by typing ‘help glp’ in the TopSpin command line.

Although components of the GLP package are used, the default installation and a successful System Suitability Test is not a *de facto* GLP validation. In order for the instrument to be brought to full GLP compliance, contact Bruker to arrange for instrument validation by a certified GLP engineer.

6.3 IconNMR Configuration

The purpose of this section is to cover only Assure-RMS relevant settings within IconNMR. More detailed instructions on all of the tools within IconNMR are covered in the automation Setup or Reference Manuals and can be found in the IconNMR help menu.

IconNMR configuration can only be accessed with the NMR Superuser’s password. This is a security feature which prevents users from altering instrument functions. The configuration window can be accessed by typing ‘iconc’ into the TopSpin command prompt line.

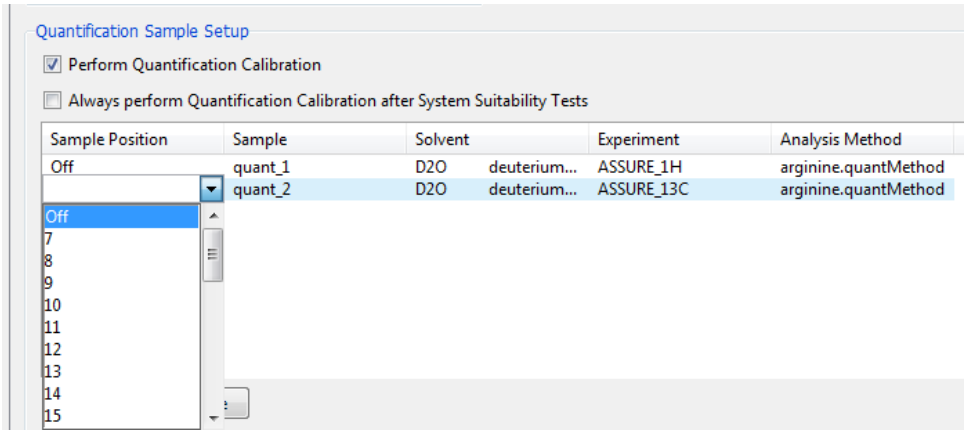
Options for Automation

When used with the proper processing AU (proc_assureshim) and a sample that has an NMR Reference signal, the system uses the halfwidth of the reference to determine if the spectrum is of a high enough quality to be passed on for spectral evaluation. The threshold cutoff for half height should be at or below the value set in the configuration window (in Hertz). A sample with a larger half width will be re-shimmed and re-acquired. Two consecutive sample failures result in automatic queuing of the System Suitability Test.

Quantification

A quantification standard of known concentration which will be periodically run is defined by it’s position in the rack where the user can activate different samples. The spectral name is defined as well as the solvent, parameter set, and quant method to be used to analyze the sample. It is possible to have the sample run after every SST.

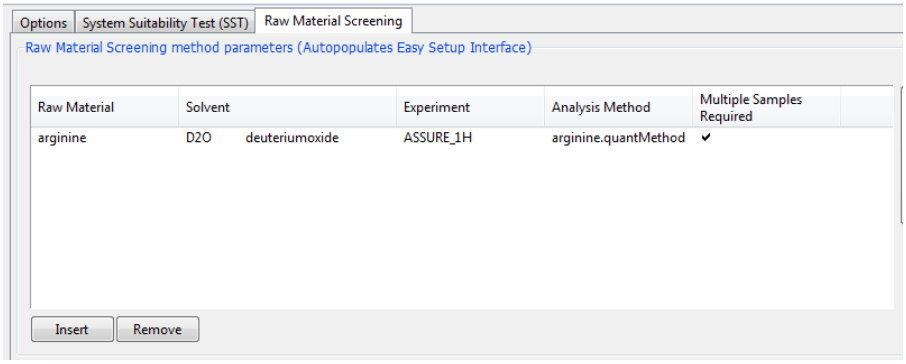
The quantification standard is used to generate an ‘eretic’ reference integral file. The file is used (and copied to the directory) of any subsequent spectrum where absolute quantification is used. Assure always uses the most recent ‘eretic’ file and accounts for the correct nuclei.



After running the quantification standard, IconNMR will copy the 'eretic' file (section XXX) generated by Assure-RMS to each subsequently acquired spectrum. IconNMR will continue to use the most recent 'eretic' file.

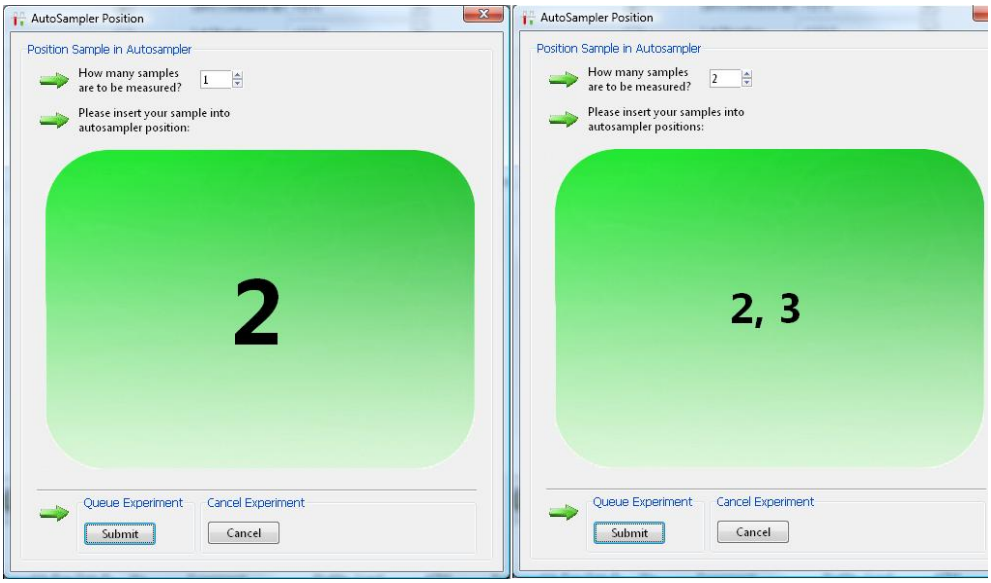
Raw Material Screening

Each raw material to be tested will be evaluated based on the parameters entered in the Raw Material Method Parameters. Here, IconNMR sets specifically the materials' solvent, which experiment(s), and the quant method used for analysis. The solvent selected may be any solvent listed in the solvent table. The experiment may be any experiment from the 'Experiment List' or 'Composite Experiment' listing in IconNMR. The Analysis Method may be chosen from any analysis method stored in the default method directory.



- Select 'Insert' to add a new raw material
    - Raw Material – arginine: Type in a name for the raw material in the first Column. This is the name that will appear in the IconNMR easy dialog
    - Solvent – D2O: Left click in the cell for a Solvent and select a solvent from the pull down list. The choices are automatically populated from the list of lock solvents from the local configuration
    - Experiment – Assure_1H: Left click in the cell for the Experiment and select an experiment from the pull down list. This list populates from local configuration

- Analysis Method – arginine.quantMethod: Left click in the cell for the Analysis Method and select an Analysis Method from the list. This list populates from the release folder of the RMS software
- Multiple Samples Required – by activating this box it is possible to analyze multiple spectra to find (e.g.) average quantification over multiple samplings. Submission of such into IconNMR opens a dialog where the



6.3.1 Additional IconNMR Configuration Elements

Beginning with the User Manager (Figure 6.1), the user configuration is where the supervisor can limit many of the actions of the user.

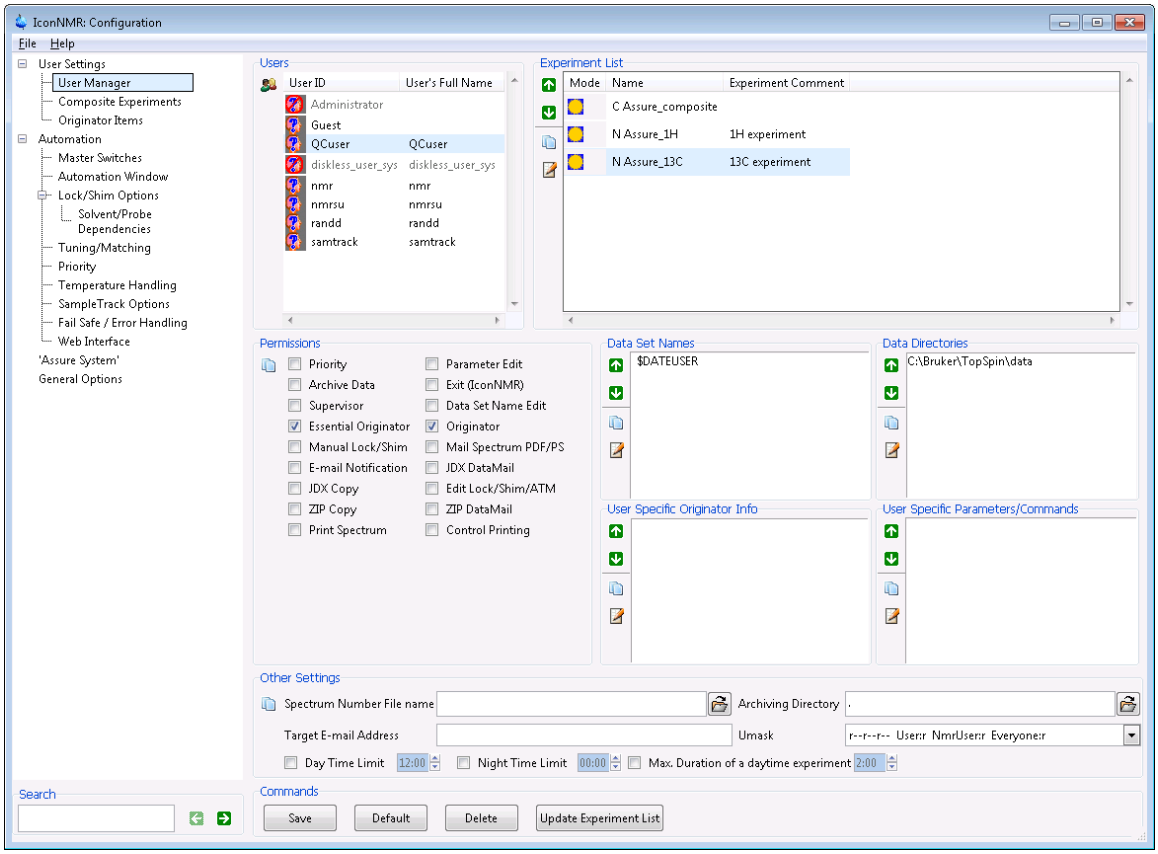


Figure 6.1: IconNMR screen showing the user manager interface and current settings for the access-limited QCuser.

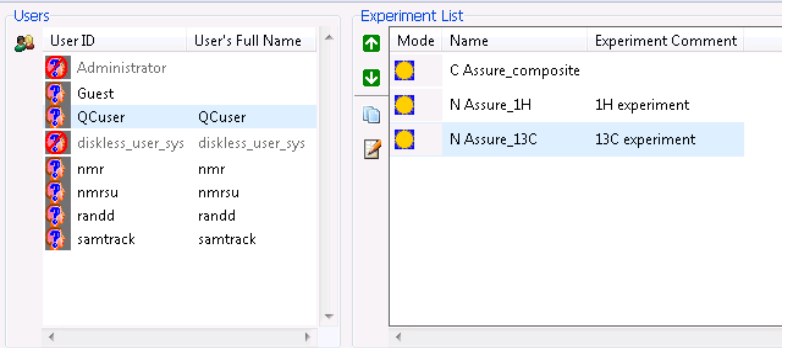
Default Permission

The easiest and fastest way to set permissions is to utilize the ‘restore defaults’ option under the ‘File’ drop down menu. This resets the selected user permissions to the access-limited settings.

Experiment List

Robust and reliable parameter sets are required for any experiments entailing a high degree of automation, reproducibility and precision. The parameter sets supplied for the Assure - Raw Material Screening software were designed and tested for this purpose and the use of these parameter sets is highly recommended.

Access to the parameter sets is granted through the ‘User manager’ panel, including any composite experiments that may be used. Parameter sets that are used for the screening mode are defined in the Assure - Raw Material Screening section of the IconNMR configuration.



User Permissions

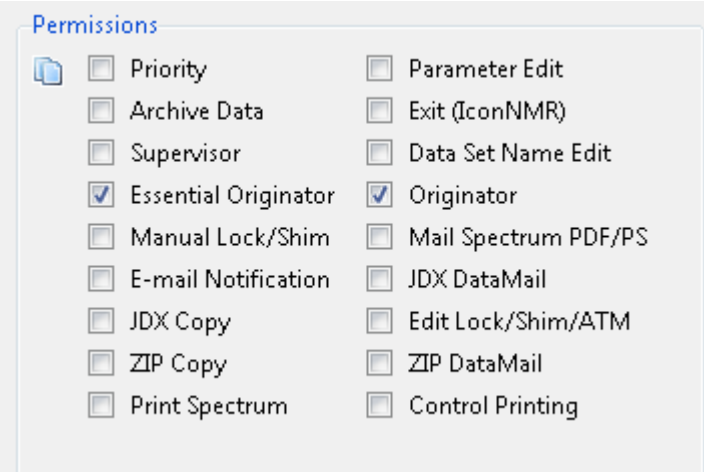
QCuser (or any user of Assure-RMS) must have permission for:

- 'Essential Originator'
- 'Originator'

Restrictions on QCuser are done by not allowing the following permissions:

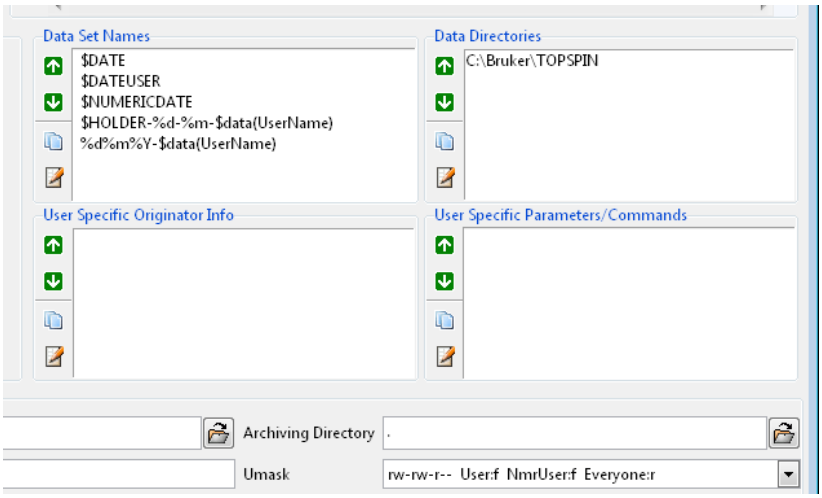
- Supervisor
- Exit (IconNMR)
- Parameter Edit
- Manual Lock/Shim
- Edit Lock/Shim/ATM

All other possibilities are defined by the NMR Superuser preferences.



Data Options and Archiving

QCuser should not have access to edit submission parameters of samples under the 'User Specific Parameters/Commands' so this box should normally be empty for such users. If archival of data is desired, check the box under 'Permissions' in the User Manager and designate an Archival Directory.



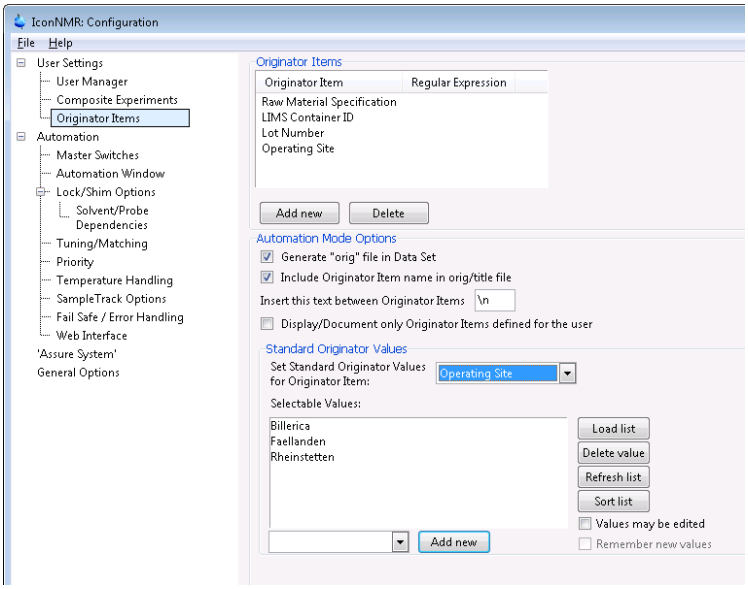
IconNMR Originator Items

IconNMR Originator items are used in the Raw Material Screening software for users to input tracking information regarding the samples specific details sample submission. These items are included with the spectrum and final report.

Originator items include 'Raw Material Specification', 'LIMS Container ID', 'Lot Number' and 'Operating Site'. It is essential that 'Raw Material Specification' should be the first Originator Item entry.

Other items include 'LIMS Container ID', 'Lot Number' and 'Operating Site' may be customized to reflect user preferences. The system administrator may add Standard Originator Values to any of these Originator Items by selecting the originator item and loading a list of selectable values or by using the 'Add new' button.

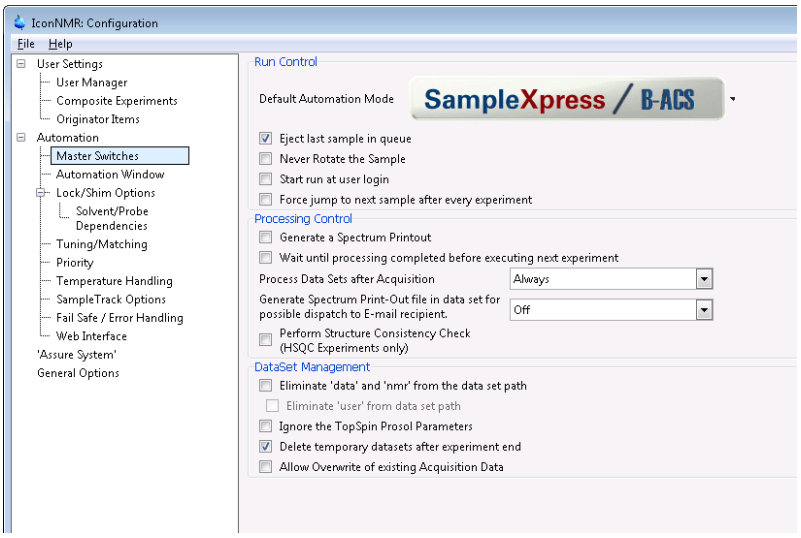
- Enter the 'Originator Items' window from the IconNMR Configuration window
- Select the desired Originator item in the window 'Set standard Originator Values for Originator Item' from the pull down button.
- A list of values may be loaded by left clicking on the 'Load List' button and selecting the desired text file.
- Alternatively, typing an entry into the type window at the bottom of the screen and click the 'Add new' button to add the new entry.



Configuring the Master Switches

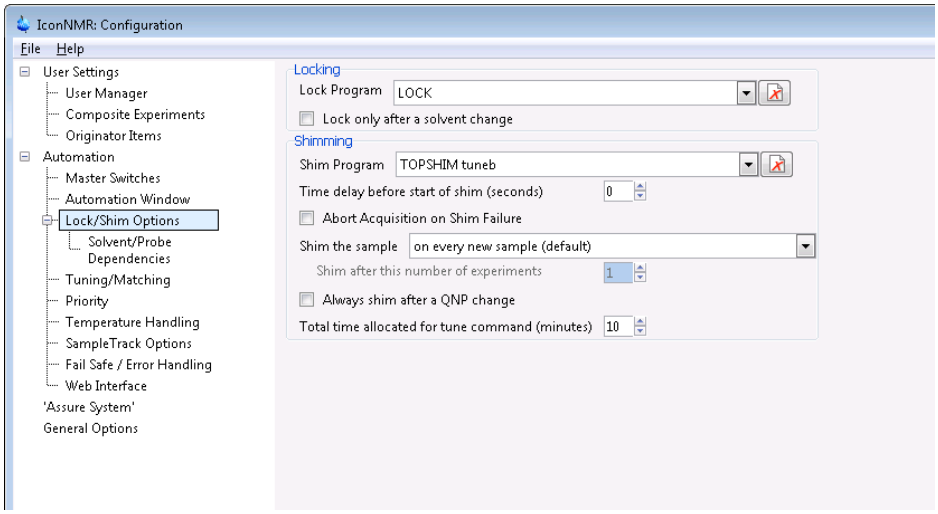
Additional configurations used in the Assure-RMS package are found in the Automation Window of IconNMR Configuration.

- Enter the IconNMR Configuration window
- Select ‘Master Switches’
- Turn on ‘Eject last sample in queue’.
- Turn off ‘Never Rotate the Sample’
- Turn on ‘Start run at user login’



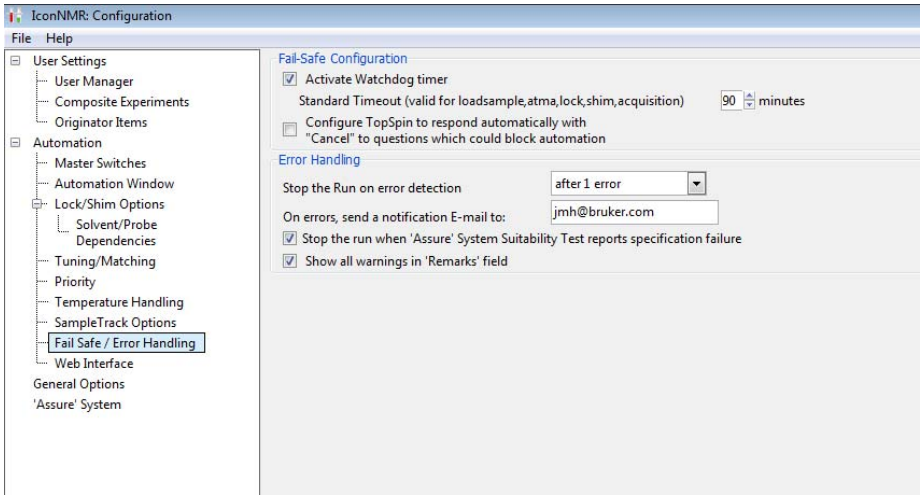
Shimming options

The recommended shimming routine of 'TOPSHIM tuneb (or tunebxyz)' is used to first adjust for possible solvent changes (tuneb) then carry out a 1d TopShim. For more help on shimming read the TopShim guide which can be found in the TopSpin help menu.



Fail Safe/Error Handling

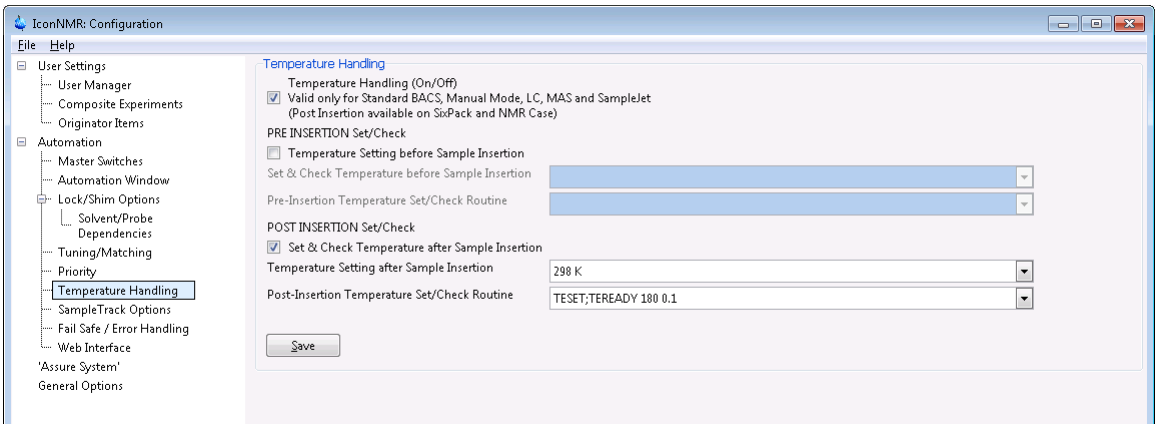
It is possible to have the system continue to run even if the Assure-SST produces a failure. Deactivating the 'Stop the run when 'Assure System Suitability Test' reports specification failure' will result in the system to continue running with an email alert sent to the defined E-mail alert address.



Temperature Handling

Optimal results are obtained only when temperature handling is engaged.

- Enter the IconNMR Configuration Window.
- Choose ‘Temperature Handling’ under the Automation Window.
- Check the ‘Temperature Handling’ box to turn the temperature handling on.
- Check the ‘Set & Check Temperature after Sample Insertion’ box.
- In the ‘Temperature Setting after Sample Insertion’ box choose ‘according to first experiment’s TE parameter’.
- In the ‘Post-Insertion Temperature Set/Check Routine’ box choose ‘TESET;TEREADY 180 0.1’ (probe values of the sample equilibration timer, represented here with 180, are site specific and must be determined individually)



6.4 Running IconNMR: Access-Limited User

Screening Samples using Assure-RMS Screening Software

When the QCuser starts TopSpin while logged onto the computer as QCuser the following user interface will appear. The System Suitability Tests automatically populates the Experiment Queue and are listed according to holder number. If the System Suitability Test is expired according to the settings in the QC configuration window then acquisition will begin immediately on the System Suitability Test. The QCuser may enter raw materials for testing without waiting for the System Suitability Test to finish.

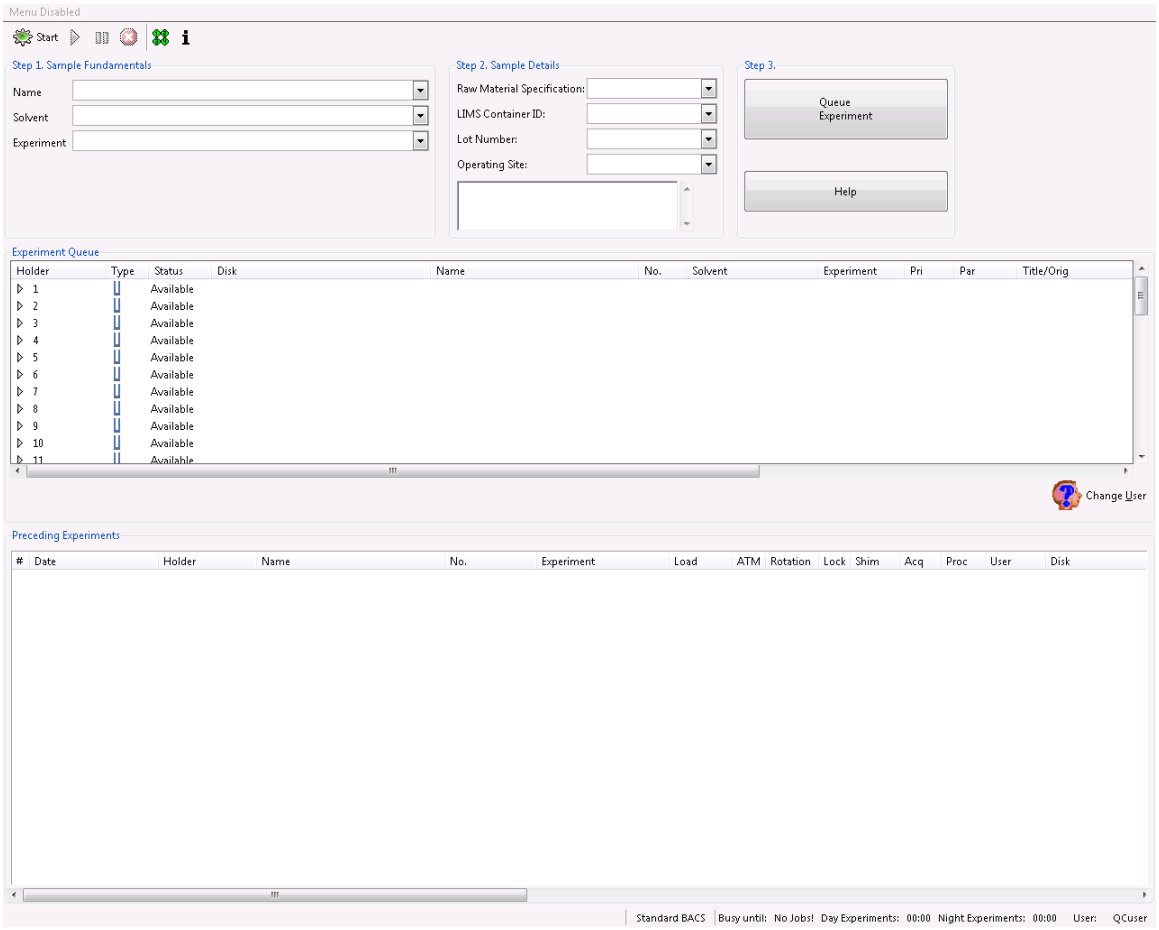


Figure 6.2: QCuser window interface.

Notice that several of the traditional choices of IconNMR are not accessible to the QCuser, such as stopping the system, canceling or deleting experiments. Details on setting the permissions for an access-limited user are covered in chapter 7.5.

The purpose of the QCuser is for submitting samples only. This is by design to keep the user from changing conditions important to the automation run in a GLP environment. Similarly, the QCuser has limited access to the functions in the TopSpin interface.

The only means to stop the automation is to use the 'Change User' IconNMR. This will allow the NMR Superuser to logon and change the run as needed.

Submitting a Sample

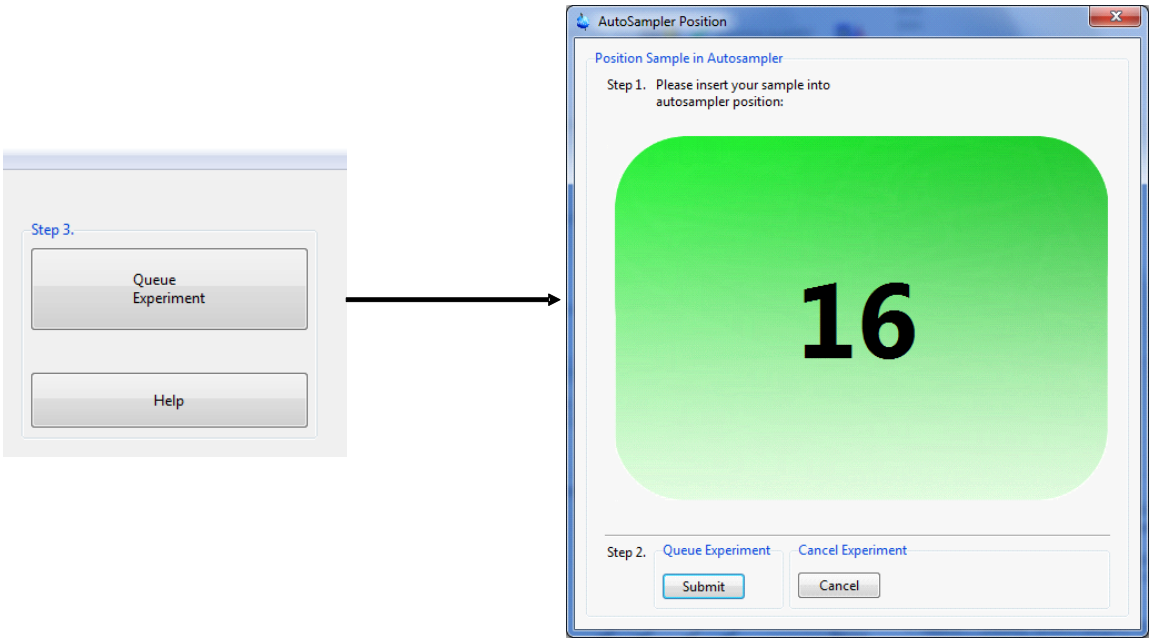
To submit samples, the QCuser only needs to complete Step 1-4 of the Assure-RMS Automation window. IconNMR will prompt the QCuser for which placeholder the sample will go into in step 4

Submit samples to queue

- Step 1. Sample Fundamentals: Select a 'Traceable Raw Data Filename' from the pull down menu
- Step 2. Sample Details:

1. Enter a ‘Raw Material Specification’ from the pull down menu
2. Enter a ‘LIMS Container ID’ number
3. Enter a ‘Lot Number’
4. Enter a ‘Operating Site’ from the pull down menu

- Step 3. Click on ‘Queue Experiment’
- Step 4. Place the sample in the holder as instructed by the software



Data is acquired and processed automatically on each sample in the queue. The data is analyzed in the background using Assure-RMS and once the analysis is complete two reports are generated including a (1) QC Report and a (2) Expert Report. Examples of each of these reports are in chapter 7.2. The reports indicate a ‘pass’ or ‘fail’ for the raw material screened.

These reports are stored in the data directory for each sample. For example, using the traditional Bruker data tree structure, the reports are found in the following directory:

C:\Bruker\TOPSPIN\data\[user]\nmr\[sample name]\[expno]

Access to these documents is also available by right clicking on the spectrum history in the “Preceding Experiments” as shown in Figure 6.3

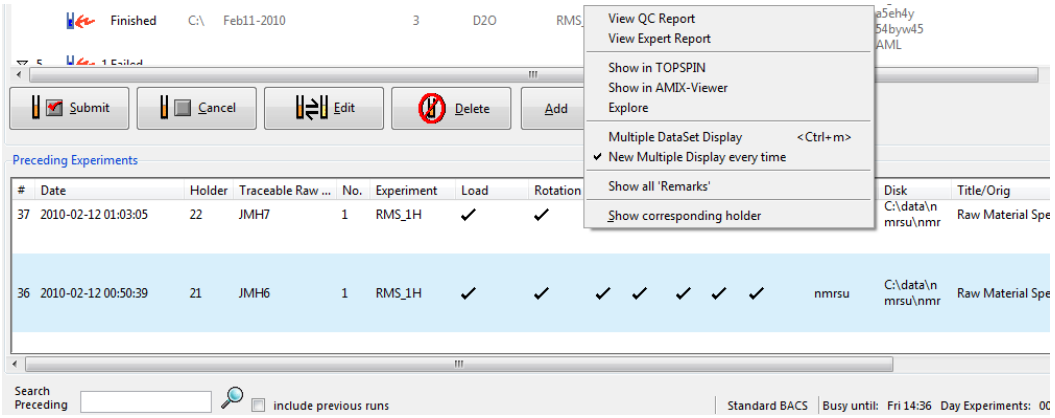
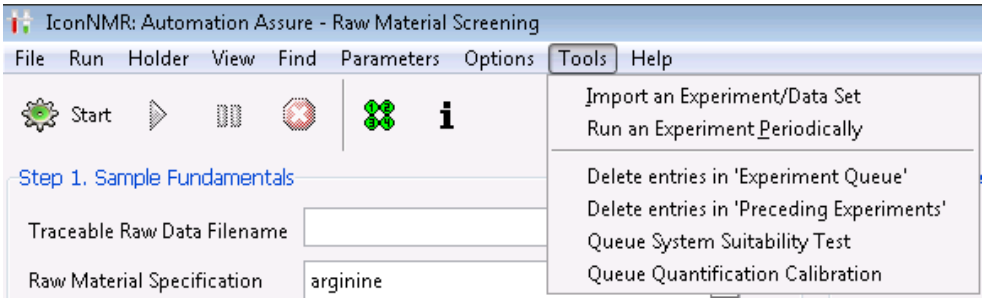


Figure 6.3: Access to reports from Assure-RMS QC-user window

6.5 Running IconNMR: Supervisor Options

The administrator (or users given ‘supervisor’ permission in IconNMR-User Manager configuration) has access to all of the tools of IconNMR with three additional features. The supervisor can:

1. Delete entries in Experiment Queue – removes all submitted samples
2. Delete entries in Preceding Experiments – clears the history
3. Queue System Suitability Test – sets the Suitability Test to start immediately or after the current experiment
4. Queue Quantification Calibration – sets the calibration standard to start immediately or after the current experiment



Batch Submission

Submitting samples from a spreadsheet in the application independent, comma separated files (csv) format can be done from the File drop down menu. Chapter 7.4 contains an example csv file for submission. The format for filling the dialogs is shown below:

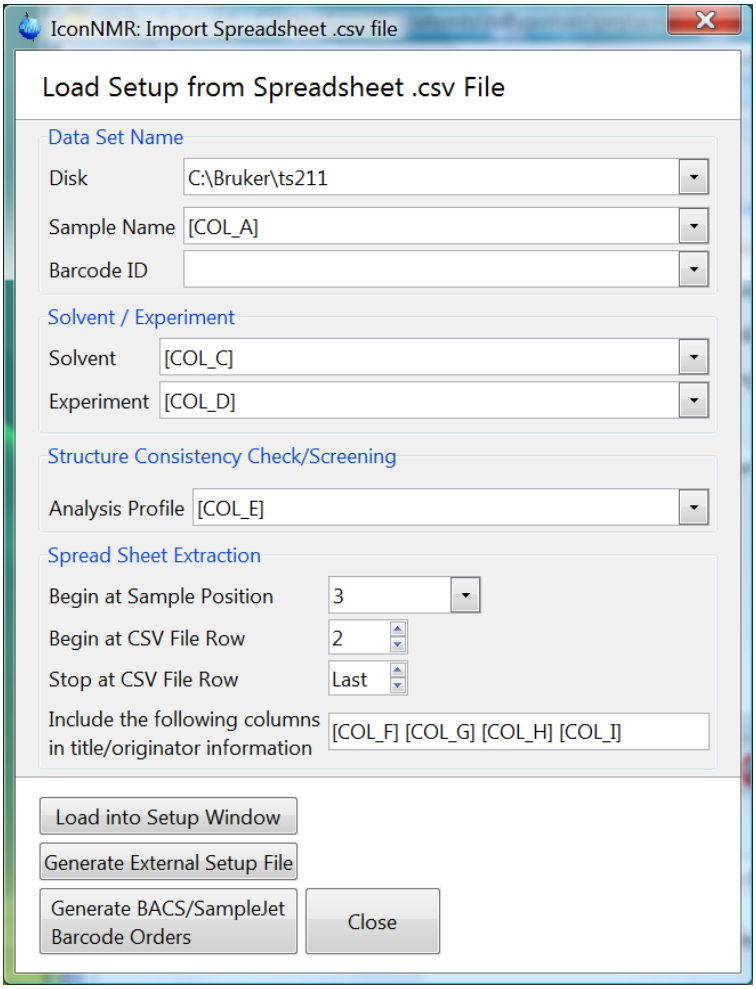


Figure 6.4: Loading setup from csv file

6.6 Visual Display of RMS Analysis and Remarks in IconNMR

Upon successful complettion of a screening test, IconNMR diplays the hard results of each step of the acquisition in the ‚Preceding Experiments' bar. Any error results in an X and a red bar. A failure is triggered by instrument errors, processing errors, or report generation errors. For samples which also fail the threshold limits as defined in a quant method, a failure error will also be generated.

Full details of the failure can be found in the remarks field in the ‚Preceding Experiments' or displayed in a new window by selecting ‚Show all Remarks' as shown in Figure 6.3

7 Example Log Files and Reports

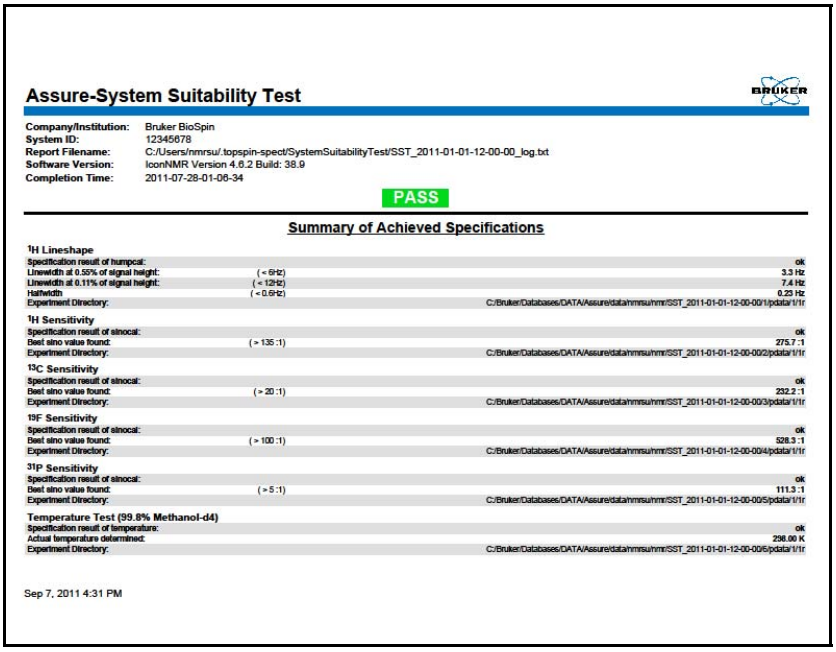
7.1 Example log file from System Suitability Tests

Filename: SystemTest_2010-01-7-15-16-36_log.txt

System Suitability Report
###
RESULT: PASSED
###
#
Output of System Tests Follows:
#
GLP procedure: This is GLP
Experiment: Dataset: SystemTest_2010-01-07-15-16-36 1 1
C:\Bruker\TOPSPIN_Screener
Specification result of humpcal: ok
Linewidth at 0.55% of signal height = 5.0 Hz (< 20Hz) ok
Linewidth at 0.11% of signal height = 10.8 Hz (< 30Hz) ok
Halfwidth = 0.54 Hz (< 5Hz) ok
#
GLP procedure: This is GLP
Experiment: Dataset: SystemTest_2010-01-07-15-16-36 2 1
C:\Bruker\TOPSPIN_Screener
Specification result of sinocal: ok
Best sino value found = 443.8 :1 (> 20 :1) ok
#
GLP procedure: This is GLP
Experiment: Dataset: SystemTest_2010-01-07-15-16-36 4 C:\Bruker\TOPSPIN_Screener
Specification result of sinocal: ok
Best sino value found = 25.8 :1 (> 20 :1) ok
#
Dataset: SystemTest_2010-01-07-15-16-36 3 C:\Bruker\TOPSPIN_Screener
Experiment: Experiment: Temperature test for Methanol-d4 99.8% atom%d
Specification result of temperature: ok
Actual temperature determined to be 300 on 2010-01-07-15-30-19 during system suitability test

7.2 Reports

SST Report



Assure-System Suitability Test

Company/Institution: Bruker BioSpin
System ID: 12345678
Report Filename: C:/Users/nmrsu/.topspin-spect/SystemSuitabilityTest/SST_2011-01-01-12-00-00_log.txt
Software Version: IconNMR Version 4.6.2 Build: 38.9
Completion Time: 2011-07-28-01-06-34

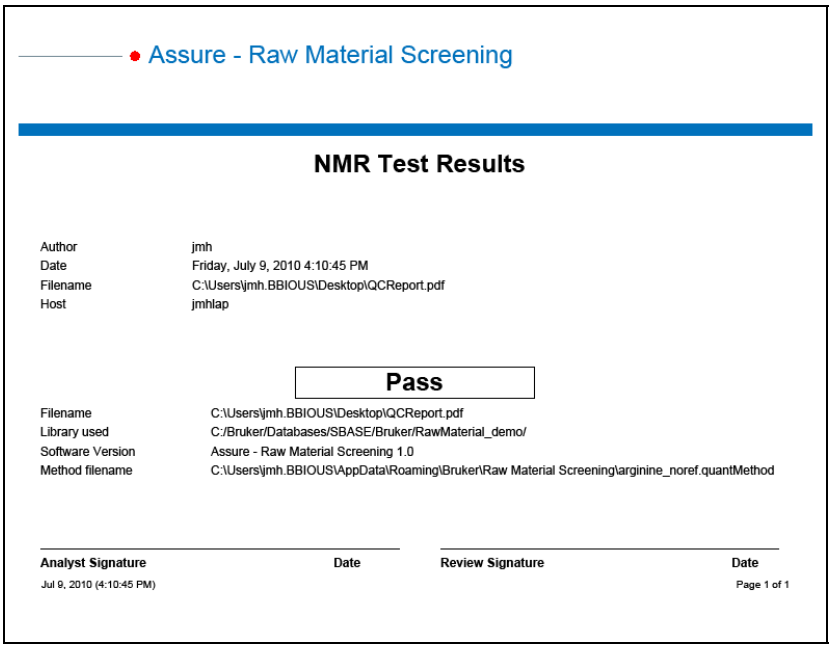
PASS

Summary of Achieved Specifications

^{1}H Lineshape		
Specification result of humpcal:		ok
Linewidth at 0.55% of signal height:	(< 6Hz)	3.3 Hz
Linewidth at 0.11% of signal height:	(< 12Hz)	7.4 Hz
Halfwidth	(< 0.6Hz)	0.23 Hz
Experiment Directory:		C:/Bruker/Databases/DATA/Assure/data/nmrsu/nmr/SST_2011-01-01-12-00-00/1/pdata/1/1r
^{1}H Sensitivity		
Specification result of sinocal:		ok
Best sino value found:	(> 135 :1)	275.7 :1
Experiment Directory:		C:/Bruker/Databases/DATA/Assure/data/nmrsu/nmr/SST_2011-01-01-12-00-00/2/pdata/1/1r
^{13}C Sensitivity		
Specification result of sinocal:		ok
Best sino value found:	(> 20 :1)	232.2 :1
Experiment Directory:		C:/Bruker/Databases/DATA/Assure/data/nmrsu/nmr/SST_2011-01-01-12-00-00/3/pdata/1/1r
^{19}F Sensitivity		
Specification result of sinocal:		ok
Best sino value found:	(> 100 :1)	528.3 :1
Experiment Directory:		C:/Bruker/Databases/DATA/Assure/data/nmrsu/nmr/SST_2011-01-01-12-00-00/4/pdata/1/1r
^{31}P Sensitivity		
Specification result of sinocal:		ok
Best sino value found:	(> 5 :1)	111.3 :1
Experiment Directory:		C:/Bruker/Databases/DATA/Assure/data/nmrsu/nmr/SST_2011-01-01-12-00-00/5/pdata/1/1r
Temperature Test (99.8% Methanol-d4)		
Specification result of temperature:		ok
Actual temperature determined:		298.00 K
Experiment Directory:		C:/Bruker/Databases/DATA/Assure/data/nmrsu/nmr/SST_2011-01-01-12-00-00/6/pdata/1/1r

Sep 7, 2011 4:31 PM

QC Report:



Assure - Raw Material Screening

NMR Test Results

Author	jmh
Date	Friday, July 9, 2010 4:10:45 PM
Filename	C:\Users\jmh.BBIOUS\Desktop\QCReport.pdf
Host	jmhlap

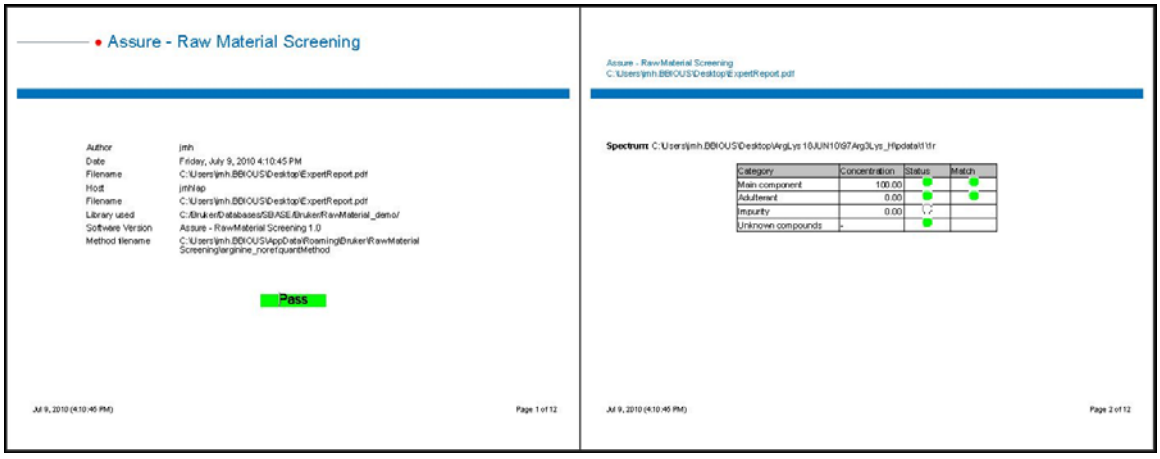
Pass

Filename	C:\Users\jmh.BBIOUS\Desktop\QCReport.pdf
Library used	C:/Bruker/Databases/SBASE/Bruker/RawMaterial_demo/
Software Version	Assure - Raw Material Screening 1.0
Method filename	C:\Users\jmh.BBIOUS\AppData\Roaming\Bruker\Raw Material Screening\arginine_noref.quantMethod

Analyst Signature **Date** **Review Signature** **Date**

Jul 9, 2010 (4:10:45 PM) Page 1 of 1

Expert Report:



Assure - Raw Material Screening

Author	jmh
Date	Friday, July 9, 2010 4:10:45 PM
Filename	C:\Users\jmh.BBIOUS\Desktop\ExpertReport.pdf
Host	jmhlap
Filename	C:\Users\jmh.BBIOUS\Desktop\ExpertReport.pdf
Library used	C:\Bruker\Databases\SBASE\BrukerRawMaterial_demo\
Software Version	Assure - RawMaterial Screening 1.0
Method filename	C:\Users\jmh.BBIOUS\AppData\Roaming\Bruker\RawMaterial Screening\arginine_noref.quantMethod

Pass

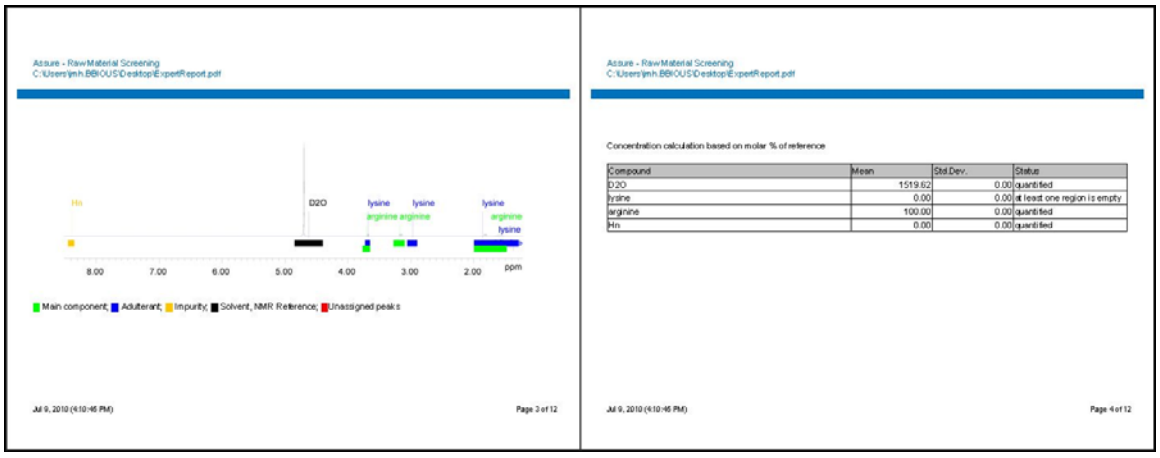
Jul 9, 2010 (4:10:45 PM) Page 1 of 12

Assure - RawMaterial Screening
C:\Users\jmh.BBIOUS\Desktop\ExpertReport.pdf

Spectrum: C:\Users\jmh.BBIOUS\Desktop\Arg\Lys 10JUN10\97\Arg3Lys_1\pdata\1\1r

Category	Concentration	Status	Match
Main component	100.00		
Adulterant	0.00		
Impurity	0.00		
Unknown compounds	-		

Jul 9, 2010 (4:10:45 PM) Page 2 of 12



Assure - RawMaterial Screening
C:\Users\jmh.BBIOUS\Desktop\ExpertReport.pdf

Main component; Adulterant; Impurity; Solvent, NMR Reference; Unassigned peaks

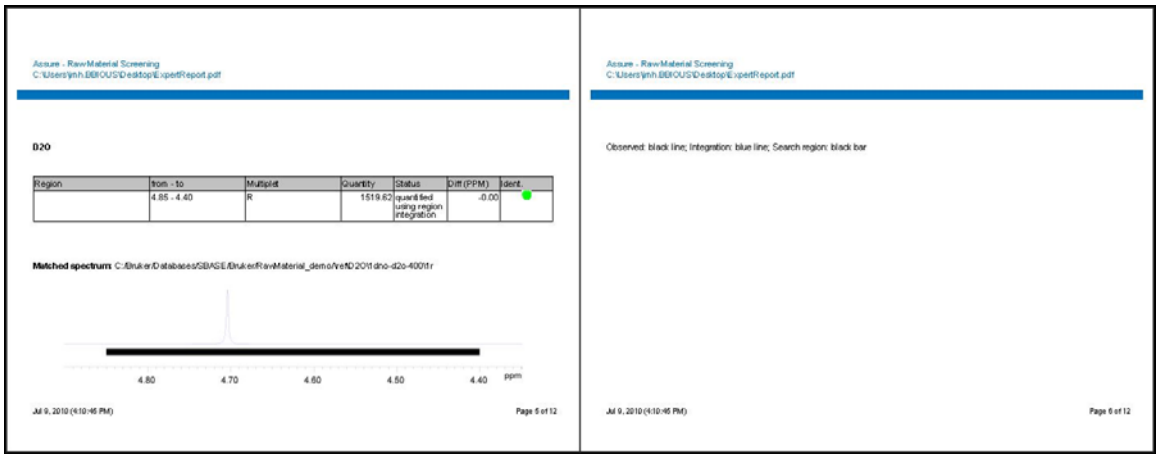
Jul 9, 2010 (4:10:45 PM) Page 3 of 12

Assure - RawMaterial Screening
C:\Users\jmh.BBIOUS\Desktop\ExpertReport.pdf

Concentration calculation based on molar % of reference

Compound	Mean	Std.Dev.	Status
D2O	1519.62	0.00	quantified
lysine	0.00	0.00	at least one region is empty
arginine	100.00	0.00	quantified
Hn	0.00	0.00	quantified

Jul 9, 2010 (4:10:45 PM) Page 4 of 12



Assure - RawMaterial Screening
C:\Users\jmh.BBIOUS\Desktop\ExpertReport.pdf

D2O

Region	from - to	Multiplet	Quantity	Status	Diff (PPM)	Ident.
	4.85 - 4.40	R	1519.62	quantified tuning region integration	-0.00	

Matched spectrum: C:\Bruker\Databases\SBASE\BrukerRawMaterial_demo\ref\D2O\1\dho-d2o-400\1r

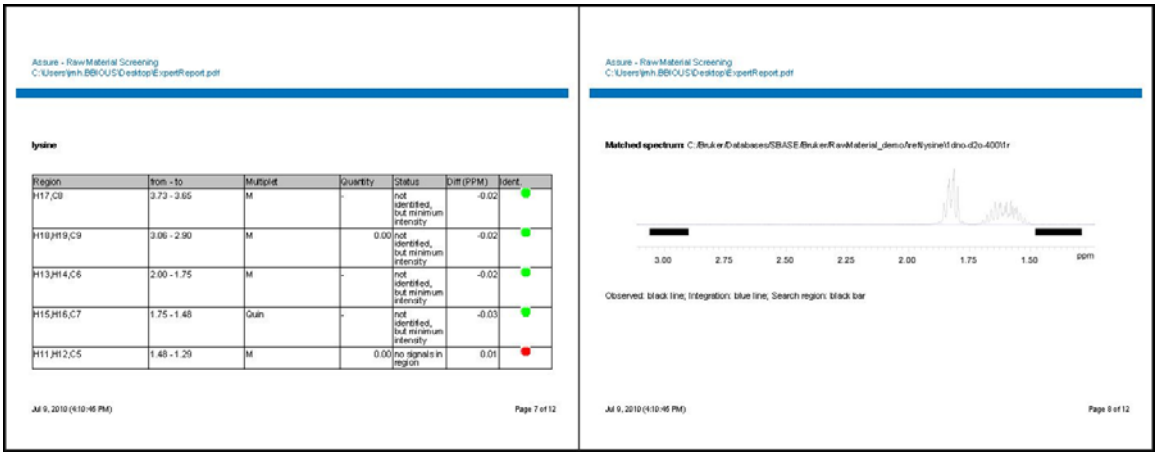
Jul 9, 2010 (4:10:45 PM) Page 5 of 12

Assure - RawMaterial Screening
C:\Users\jmh.BBIOUS\Desktop\ExpertReport.pdf

Observed: black line; Integration: blue line; Search region: black bar

Jul 9, 2010 (4:10:45 PM) Page 6 of 12

Example Log Files and Reports



Assure - RawMaterial Screening
C:\Users\jmh.BBIOUS\Desktop\ExpertReport.pdf

lysine

Region	from - to	Multiplet	Quantity	Status	Diff (PPM)	Ident.
H17,C9	3.73 - 3.65	M	-	not identified, but minimum intensity	-0.02	
H18,H19,C9	3.06 - 2.90	M	0.00	not identified, but minimum intensity	-0.02	
H13,H14,C6	2.00 - 1.75	M	-	not identified, but minimum intensity	-0.02	
H15,H16,C7	1.75 - 1.48	Quin	-	not identified, but minimum intensity	-0.03	
H11,H12,C5	1.48 - 1.29	M	0.00	no signals in region	0.01	

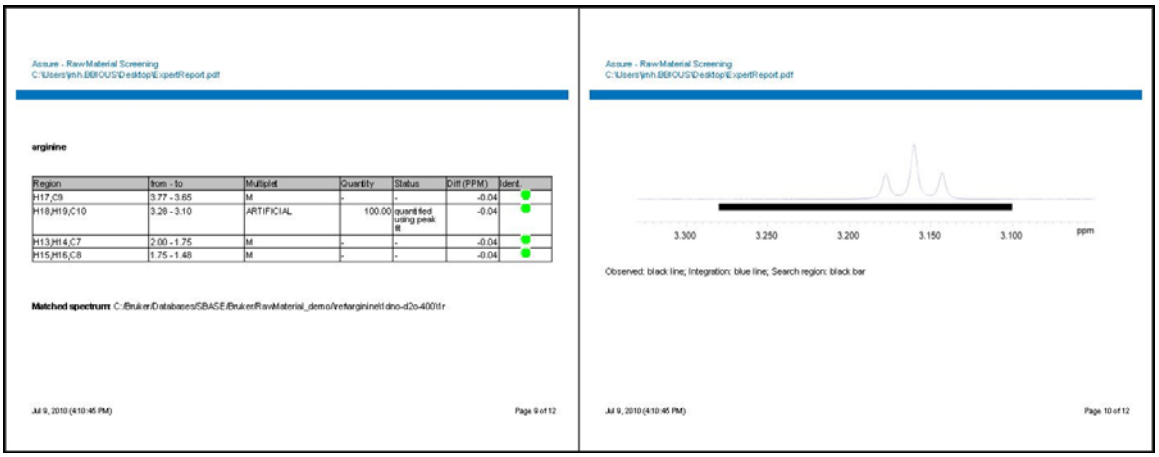
Jul 9, 2010 (4:10:46 PM) Page 7 of 12

Assure - RawMaterial Screening
C:\Users\jmh.BBIOUS\Desktop\ExpertReport.pdf

Matched spectrum: C:/Bruker/Databases/SBASE/BrukerRawMaterial_demo/ref/lysine/1/dino-d2o-400/1r

Observed: black line; Integration: blue line; Search region: black bar

Jul 9, 2010 (4:10:46 PM) Page 8 of 12



Assure - RawMaterial Screening
C:\Users\jmh.BBIOUS\Desktop\ExpertReport.pdf

arginine

Region	from - to	Multiplet	Quantity	Status	Diff (PPM)	Ident.
H17,C9	3.77 - 3.65	M	-	-	-0.04	
H18,H19,C10	3.28 - 3.10	ARTIFICIAL	100.00	quantified using peak fit	-0.04	
H13,H14,C7	2.00 - 1.75	M	-	-	-0.04	
H15,H16,C8	1.75 - 1.48	M	-	-	-0.04	

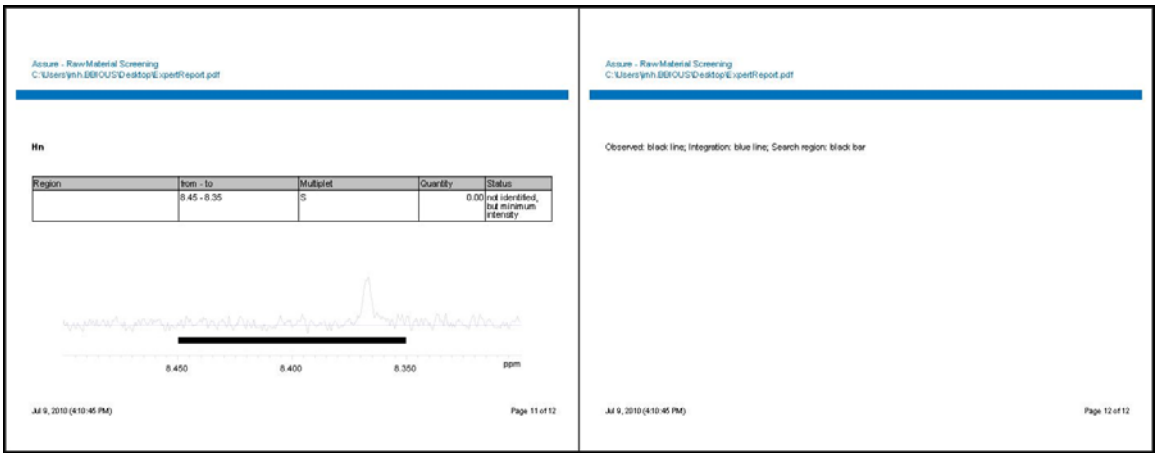
Matched spectrum: C:/Bruker/Databases/SBASE/BrukerRawMaterial_demo/ref/arginine/1/dino-d2o-400/1r

Jul 9, 2010 (4:10:46 PM) Page 9 of 12

Assure - RawMaterial Screening
C:\Users\jmh.BBIOUS\Desktop\ExpertReport.pdf

Observed: black line; Integration: blue line; Search region: black bar

Jul 9, 2010 (4:10:46 PM) Page 10 of 12



Assure - RawMaterial Screening
C:\Users\jmh.BBIOUS\Desktop\ExpertReport.pdf

Hn

Region	from - to	Multiplet	Quantity	Status
	8.45 - 8.35	S	0.00	not identified, but minimum intensity

Jul 9, 2010 (4:10:46 PM) Page 11 of 12

Assure - RawMaterial Screening
C:\Users\jmh.BBIOUS\Desktop\ExpertReport.pdf

Observed: black line; Integration: blue line; Search region: black bar

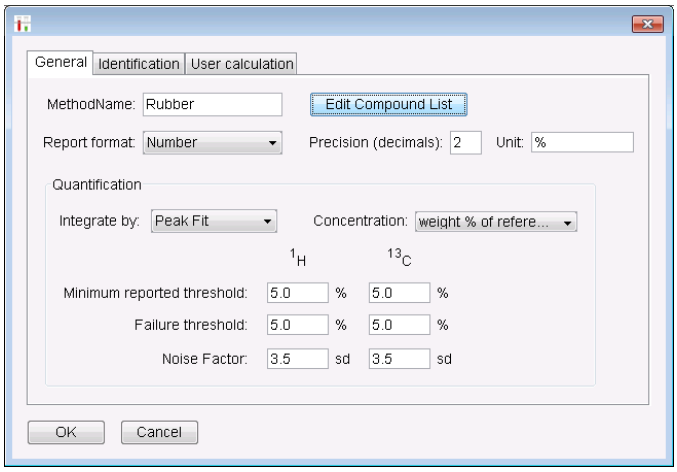
Jul 9, 2010 (4:10:46 PM) Page 12 of 12

7.3 User Defined Integration Routine

The images here illustrate the settings of the user defined integration routine. Here, the method reflects the screening of tire rubber set by ISO 21461. It determines the aromaticity of oil in rubber components according to the following equation:

$$\%H_{Bay} = \frac{I_{8.3-9.5\,ppm}}{\left(I_{6.0-9.5\,ppm} - I_{CHCl3}\right) + I_{0.2-5.8\,ppm}} \cdot 100$$

1. Method report format “Number” is selected.



2. Edit Compound List.

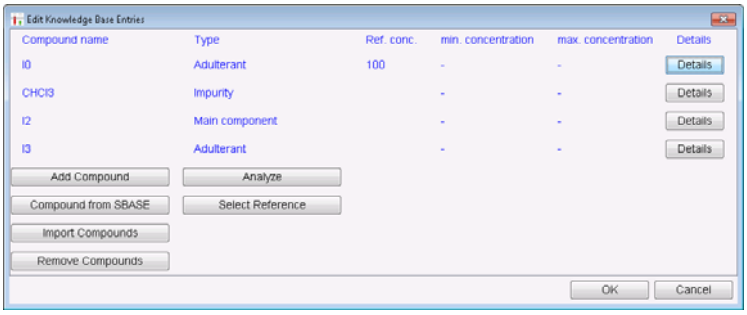
   Four compounds are defined:

   I0 : Region 9.5-6.0 ppm

   I2 : Region 9.5-8.3 ppm

   I3 : Region 5.8-0.2 ppm

   CHCl3 : Singlet at 7.2 ppm



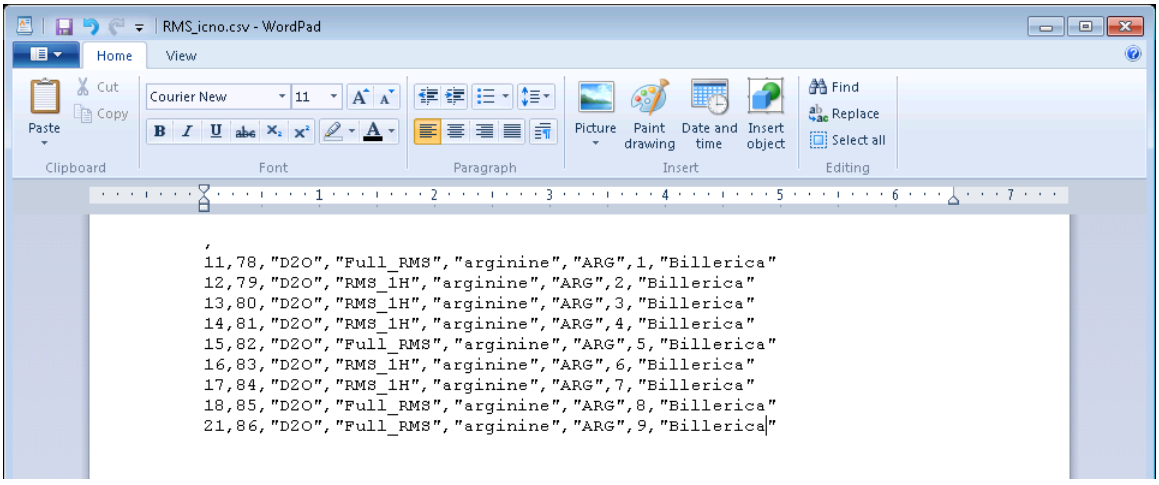
3. User Calculation is defined as a Python script. The integrals of all defined compounds are available by their compound name. “returnValue” is reported on the documents.

I1 = I0-CHCl3;

returnValue = 100 * I2 /(I1 + I3);

7.4 Example csv File for Batch Submission

Image of a csv file for batch submission of samples. Notice, the first line MUST have some character and cannot contain a sample order.



.

7.5 Computer Security

Before installing TopSpin on a PC, the PC administrator must decide which access rights PC users will have to the files of the TopSpin installation.

The policy of the TopSpin installer program is: During the installation process the installer program will inherit the existing access rights from the installation directory (Under Windows, this refers to the ACL: Access Control List security descriptors). In a second step, the installer program will give write or execute rights to a number of files that need these rights during program execution.

Therefore, it is recommended that the following installation procedure be performed by the PC administrator who must have Adminstrator/Superuser rights with respect to the operating system:

1. Create a PC user group "NMRUser". The accounts of all PC users who should be allowed to run TopSpin must be in this group.
2. Create the directory where you want to install TopSpin, e.g. c:\Bruker.
3. Assign the wished access rights to this directory, e.g. "read only" and "execute". As an aid, you may inspect the default access rights of the directory "c:\Program Files" under Windows 7. These are typical for any software and might also be suitable for you.
4. Install TopSpin in the chosen directory. All TopSpin files will inherit the previously set rights of the directory. At the end of the installation process, the TopSpin installer program will modify the access rights of some files and directories as required during TopSpin run time (write/execute access) for the "NMRUser" group. These are:
   - classes\lib\topspin_py
   - conf\global
   - conf\instr\spect
   - conf\instr\topshim\parameters\user
   - conf\instr\topshim\solvents\user
   - prog\au\bin
   - prog\curdir
   - prog\logfiles
   - savelogs
   - conf\instr\<Instrument>\inmrusers\.autoopts.

Except for the GNU subdirectory of TopSpin, which requires read/execute access for Everyone, solely the groups NMRUser and NMRSuperUser will be granted access to needed Topspin directories and files. So besides the users inherited from the installation directory, only users belonging to these two groups will have access to the Topspin installation.

Using these guidelines, your TopSpin installation will be well protected against any inadvertent damage.

Example Log Files and Reports

8 Definitions

GLP – Good Laboratory Practice

GMP – Good Manufacturing Practice

ICH – International Conference on Harmonisation (of Technical Requirements for Registration of Pharmaceuticals for Human Use)

Knowledgebase – composite library of compounds which contains detailed molecular structure information such as coupling constants, multiplicity and atom count

LOD – Level of detection

LOQ – Level of quantification

QA – Quality Analysis

QC – Quality Control

Quant Method – method which contains details of how the spectrum or spectra will be evaluated including limits for defining failure, quantification method, peak identification and limits and SBASE to be matched.

RMS – Raw Material Screening

SBASE – Spectral database of purified compounds to be used by match algorithm for identification of compounds in screening mode.

System Suitability Test – A set of four samples run periodically to evaluate the continued performance of the instrument.

9 Contact

Manufacturer:
Bruker BioSpin NMR
am Silberstreifen
D-76287 Rheinstetten
Germany
Phone: +49 721-5161-0
http://www.bruker-biospin.com

NMR Hotlines

Contact our NMR service centers.

Bruker BioSpin NMR provide dedicated hotlines and service centers, so that our specialists can respond as quickly as possible to all your service requests, applications questions, software or technical needs.

Please select the NMR service center or hotline you wish to contact from our list available at:

http://www.bruker-biospin.com/hotlines_nmr.html

10 Assure-SST reference standards

300 MHz / 5mm Room Temperature	
Description	Part No.
Lineshape - 3% Chloroform	Z10230
1H Sensitivity - 0.1% Ethylbenzene, 40mm filling	Z10901
13C Sensitivity - 10% Ethylbenzene	Z10153
NMR Thermometer 99.8% Methanol-d4	Z10627

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
NMR Thermometer 99.8% Methanol-d4	Z10627

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
NMR Thermometer 99.8% Methanol-d4	Z10627

600 – 900 MHz / 1 mm Room Temperature	
Description	Part No.
Lineshape - 1% Chloroform	-
1H Sensitivity - 0.1% Ethylbenzene	
13C Sensitivity - 10% Ethylbenzene	
NMR Thermometer 99.8% Methanol-d4	

600 – 900 MHz / 1.7mm CryoProbe	
Description	Part No.
Lineshape - 1% Chloroform	Z10717
1H Sensitivity - 0.1% Ethylbenzene	Z10718
13C Sensitivity - 10% Ethylbenzene	Z10723
NMR Thermometer 99.8% Methanol-d4	Z10734

A.1 Figures

Appendix

A.2 Tables