AVANCE IVDr

- IVDr Methods

User Manual

Version 003

Innovation with Integrity

NMR

Document Number: 10000057224

P/N: H161804

Contents

Contents

1 Introduction

This manual is intended to be a practical guide for the operators of IVDr systems. The main aspect of this manual is to enable new or experienced TopSpin™ users to work with B.I-Methods 2.0 (Bruker IVDr Methods 2.0), including calibration and validation of the QC samples, body fluids (urine, plasma/ serum and CSF) preparation and NMR-Screening.

Before starting any work, personnel must read the manual thoroughly and understand its contents. Compliance with all specified safety and operating instructions, as well as local work safety regulations, are vital to ensure safe operation**. Incompliance to Bruker's IVDr sample preparation and measurement SOPs may result in major errors determined by IVDr automatic data analysis.**

The figures shown in this manual are designed to be general and informative and may not represent the specific Bruker model, component or software/firmware version you are working with. Options and accessories may or may not be illustrated in each figure.

This manual can only be used for IVDr measurements when the B.I-Methods 2.0 are installed by a Bruker engineer.

1.1 Policy Statement

It is the policy of Bruker to improve products as new techniques and components become available. Bruker reserves the right to change specifications at any time.

Every effort has been made to avoid errors in text and figure presentation in this publication.

In order to produce useful and appropriate documentation, we welcome your comments on this publication.

1.2 Safety Regulations

In order to work safely in laboratories with NMR-spectrometers all users have to follow the safety regulations for magnetic, electrical, cryogenic, chemical and biological safety.

1.3 IVDr Philosophy

1.3.1 Standardization

The new AVANCE IVDr system, presently for research use only, is a complete, proven and standardized platform for NMR pre-clinical research and screening, as well as for IVD-by-NMR research.

Dedicated to NMR-based clinical screening and diagnostics research, the AVANCE-IVDr is optimized for ease-of-use and highest data quality and reliability. IVDr offers barcode analysis, control by a LIMS system, the high-throughput, temperature-controlled Autosampler SampleJet™, remote access, and automatic analysis and customizable analytical results reporting. Based on Bruker-validated SOPs, the AVANCE-IVDr platform enables the development of diagnostic tools for body-fluids, or even biopsy samples, that can address a variety of medical questions.

1.3.2 Automation

All the IVDr methods should be implemented under the same standard operating procedures (SOPs) and run as standard experiments in automation, therefore ICONNMR is a must-have for every IVDr user. SampleTrack® (Bruker Automated Sample Tracking System) is also the recommended software tool for the laboratory network.

1.3.3 Objectives

The SOPs guarantee the production of highly reproducible clinical data, enabling the sharing, exchanging, pooling and joint modeling of novel NMR assays between laboratories on a global basis. In a translational clinical research environment, the results produced by these NMR assays can easily be transferred into clinical screening and future IVD use.

This level of large-scale health-related NMR screening is paving the way for worldwide epidemiological studies as well as for pre-clinical in vitro research. The benefits have been significant: facilitated by the low cost per sample and the even lower cost per parameter as compared to established single parameter screening methods, novel NMR methods for determining the cause of disease, delivering individualized patient treatment and developing strategies for prevention are now available to many clinical researchers.

1.4 Font and Format Conventions

Type of Information	Font	Examples
Shell Command, Commands, "All that you can enter"	Arial bold	Type or enter **fromjdx zg**
Button, Tab, Pane and Menu Names "All that you can click"	Arial bold, initial letters capitalized	Use the **Export To File** button. Click **OK**. Click **Processing**…
Windows, Dialog Windows, Pop-up Windows Names	Arial, initial letters capitalized	The Stacked Plot Edit dialog will be displayed.
Path, File, Dataset and Experiment Names **Data Path Variables** **Table Column Names** **Field Names (within Dialog Windows)**	Arial Italics	*$tshome/exp/stan/nmr/lists* *expno, procno,*
Parameters	Arial in Capital Letters	VCLIST

Program Code
Pulse and AU Program Names
Macros
Functions
Arguments
Variables
	Courier	`go=2`
		`au_zgte`
		`edmac`
		`CalcExpTime()`
		`XAU(prog, arg)`
		`disk2, user2`

AU Macro	Courier in Capital Letters	`REXPNO`

Table 1.1: Font and Format Conventions

2 Getting Started

2.1 The IVDr System – Specifications Relevant for this Manual

- Standard AVANCE IVDr Configuration:
  - ASCEND 600 magnet
  - Gimbal vibration dampers
  - BOSS III shim system
  - 2 Channel console (AVANCE III HD or AVANCE NEO) with Windows workstation
  - 5 mm RT BBI probe with ATM and Z-grad
  - 2 x BCU-1 (1 x probe temperature regulation, 1 x SampleJet cooling)
  - SampleJet with cooling and pre-heating station, 5 mm shuttle (3 mm shuttle optional)
  - Windows spectrometer computer with TopSpin 3.6.1 or higher for AVANCE III HD, TopSpin 4.0.7 or higher for AVANCE NEO, validated for IVDr
  - Extra Windows workstation with SampleTrack Software
  - Barcode printer with ribbon and labels
  - AMIX
  - BBIOREFCODE
  - SampleTrack, including extra Windows workstation, barcode printer with ribbon and labels
- SOPs for body fluids such as Urine, Plasma/Serum, CSF and MeOH Extract
- Standard Sample Kit, including:
  - Quantification Reference Sample
  - Temperature Calibration Sample
  - Water-suppression test sample
  - FILCOR calibration sample
  - Pseudo Urine
  - Pseudo Plasma
  - Pseudo CSF
  - Pseudo MeOH Extract
- Consumables
  - SampleJet racks and NMR tubes for 5 mm/3 mm applications
  - Dedicated buffer solutions for urine and plasma/serum and CSF
- Options: Some features described in this manual may need additional accessories (for example for 3 mm applications).

2.2 IVDr Directory Structure

The IVDr directory structure is independent from the TopSpin version:

- Body fluid spectral data go into:

  C:\IVDrData\...
- Quality Control (QC) related spectral data go into:

  C:\Bruker\IVDr\RefData\nmr\...
- IVDr NMR methods and documents go into:

  C:\Bruker\IVDr\exp\au

  C:\Bruker\IVDr\exp\par

  C:\Bruker\IVDr\exp\pp

  C:\Bruker\IVDr\exp\py

  C:\Bruker\IVDr\conf

  C:\Bruker\IVDr\logfiles

  Now the TopSpin browser has direct access to the IVDr tools but not as default
- Add the above directories in **TopSpin Manage/Preferences/Directories/Manage source directories for edpul, edau, etc.** for the corresponding par, mac, au, pp and py as shown for “Parameter Sets” below:

  C:\Bruker\TopSpin3.5pl6\exp\stan\nmr\par\user

  C:\Bruker\TopSpin3.5pl6\exp\stan\nmr\par

  C:\Bruker\IVDr\exp\par

  Now the TopSpin browser has direct access to the IVDr tools but not as default (first line is default).

2.3 IconNMR

2.3.1 IconNMR Configuration

IconNMR™ allows fully automated acquisition and processing and is the ideal solution for labs with large numbers of samples or many users accessing the spectrometer.

IconNMR configuration for IVDr applications should be already set up by the Bruker engineer. However, users can always check the configuration settings later on by selecting **Configuration**.

Figure 2.1: IconNMR

This section shows the standard configuration settings.

User Settings

- User Manager
  - Assign experiments to users **nmrsu** and **SamTrack**.
  - Introduce new parameter sets.

    All the IVDr parameters are listed in the **Experiment List**.

    Standard installation is only for 5 mm applications. The parameters for 3 mm applications are optional.
  - The **Data Directory** should be **C:\IVDrData\data**

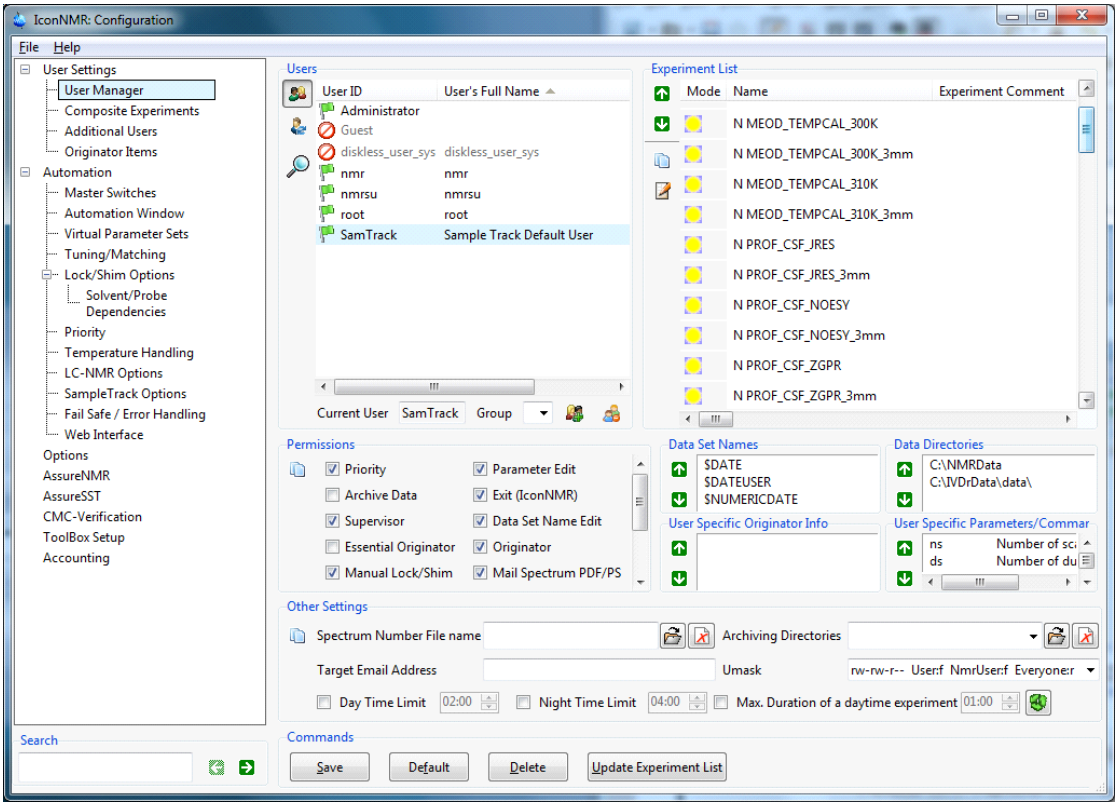


Figure 2.2: IconNMR Configuration: User Manager

Automation

- Master Switches
  - **Sample Changer/Automation Mode**: SampleJet/SampleTrack (SampleJet).
  - **Rack Sample Sequence** must be as shown in the picture to the left.
  - Check: **Enable Temperature Conditioning System**
  - Check: **Eject last sample in queue**
  - Check: **Never Rotate the Sample**
  - Check: **Save Sample DataMatrix/Barcode information to AUTOPOS status parameter**
  - Check: **Wait until processing completed before executing next experiment**
  - Check: **Eliminate 'data' and 'nmr' from the data set path**
  - Check: **Eliminate 'user' from the data set path**
  - The other details:

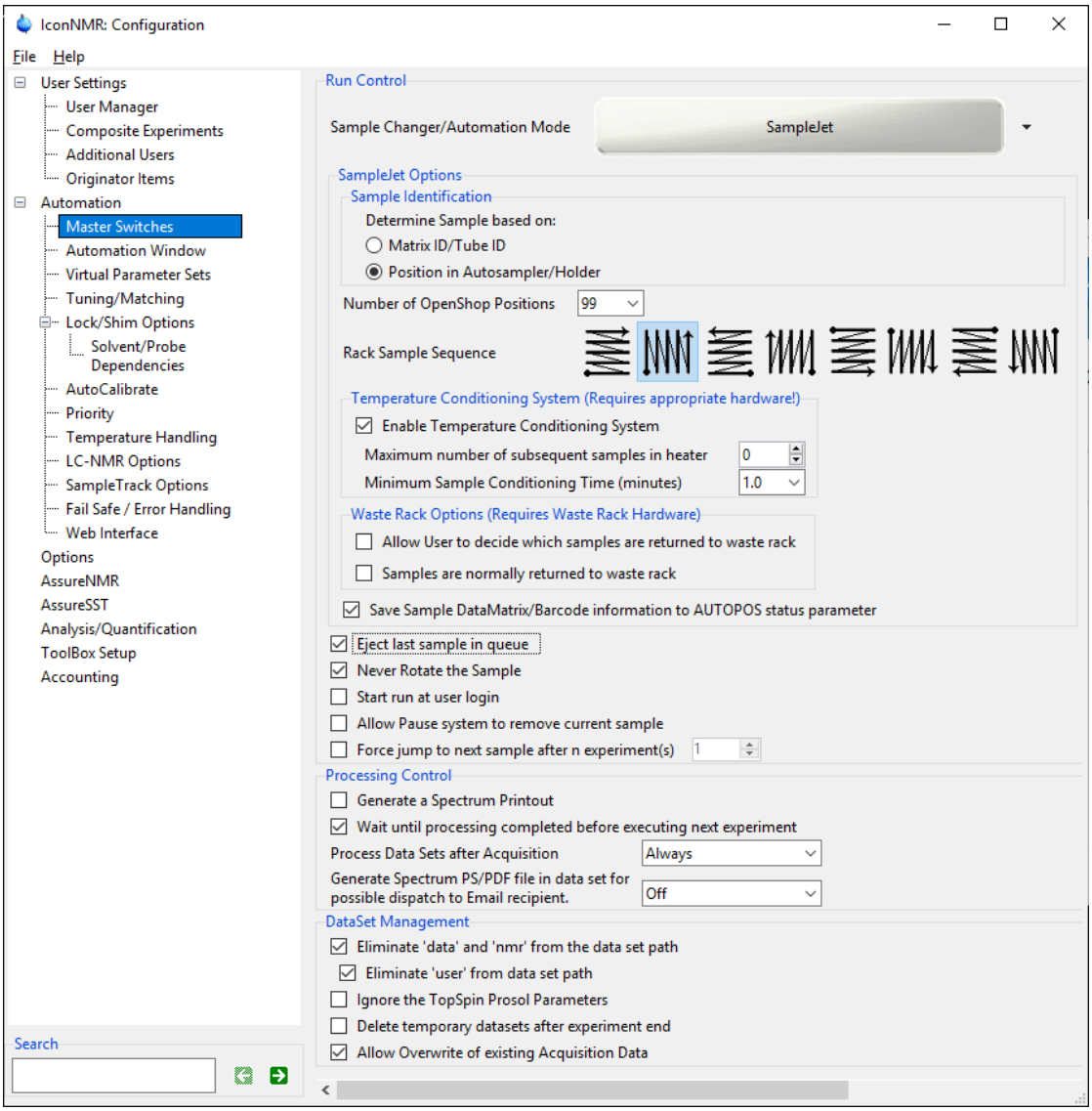


Figure 2.3: IconNMR Configuration: Master Switches (without SampleTrack)

- Tuning/Matching
  - Only on 1H Channel.
  - Only on all the NOESY1D experiments except the experiments for Sucrose, MeOD, MeOH Extracts and QuantRefC (refer to the following figures):

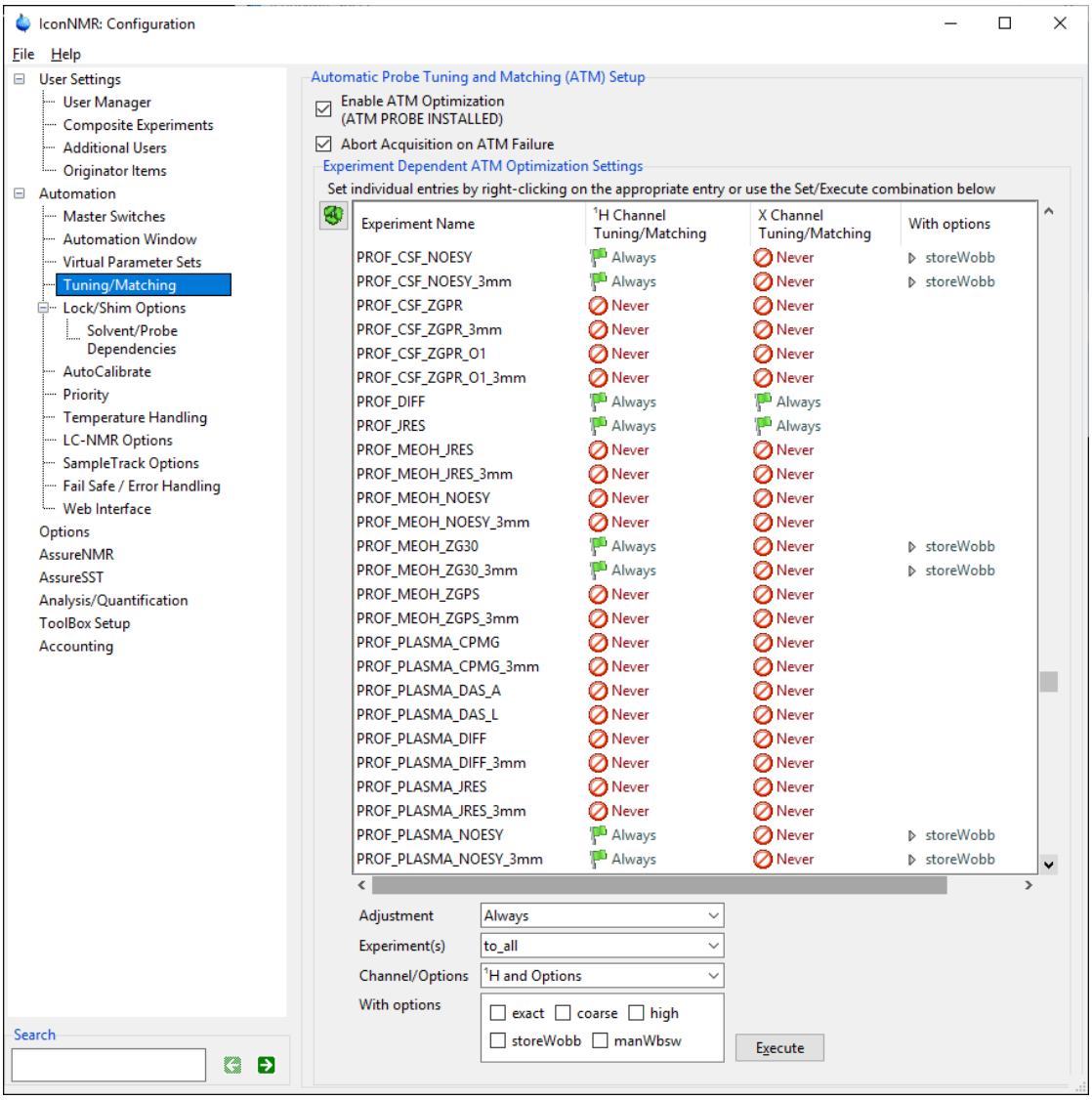


*Figure 2.4: IconNMR Configuration: Tuning/Matching – Body Fluids (Recommendation: Check **storeWobb**)*

Sucrose: Where Tuning/Matching on the O1-optimization tests:

Experiment Name	^{1}H Channel Tuning/Matching	X Channel Tuning/Matching	With options
SUC_GRADPROF	Never	Never	
SUC_GRADPROF_3mm	Never	Never	
SUC_NOESY	Never	Never	
SUC_NOESY_310K	Never	Never	
SUC_NOESY_310K_3mm	Never	Never	
SUC_NOESY_3mm	Never	Never	
SUC_ZGPR	Never	Never	
SUC_ZGPR2D	Never	Never	
SUC_ZGPR2D_310K	Never	Never	
SUC_ZGPR2D_310K_3mm	Never	Never	
SUC_ZGPR2D_3mm	Never	Never	
SUC_ZGPR_310K	Never	Never	
SUC_ZGPR_310K_3mm	Never	Never	
SUC_ZGPR_3mm	Never	Never	
SUC_ZGPR_O1	Always	Never	storeWobb
SUC_ZGPR_O1_310K	Always	Never	storeWobb
SUC_ZGPR_O1_310K_3mm	Always	Never	storeWobb
SUC_ZGPR_O1_3mm	Always	Never	storeWobb

*Figure 2.5: IconNMR Configuration: Tuning/Matching – Sucrose (Recommendation: Check **storeWobb**)*

MeOD: On all the experiments:

Experiment Name	^{1}H Channel Tuning/Matching	X Channel Tuning/Matching	With options
MEOD_TEMPCAL_300K	Always	Never	storeWobb
MEOD_TEMPCAL_300K_3mm	Always	Never	storeWobb
MEOD_TEMPCAL_310K	Always	Never	storeWobb
MEOD_TEMPCAL_310K_3mm	Always	Never	storeWobb

*Figure 2.6: IconNMR Configuration: Tuning/Matching – MeOD (Recommendation: Check **storeWobb**)*

QuantRefC: On all the experiments except the O1-optimization experiments:

Experiment Name	^{1}H Channel Tuning/Matching	X Channel Tuning/Matching	With options
QUANTREF600C_BA	Always	Never	storeWobb
QUANTREF600C_BA_310K	Always	Never	storeWobb
QUANTREF600C_BA_310K_3mm	Always	Never	storeWobb
QUANTREF600C_BA_3mm	Always	Never	storeWobb
QUANTREF600C_BA_O1	Never	Never	
QUANTREF600C_BA_O1_310K	Never	Never	
QUANTREF600C_BA_O1_310K_3mm	Never	Never	
QUANTREF600C_BA_O1_3mm	Never	Never	
QUANTREF600C_CS	Always	Never	storeWobb
QUANTREF600C_CS_3mm	Always	Never	storeWobb
QUANTREF600C_CS_O1	Never	Never	
QUANTREF600C_CS_O1_3mm	Never	Never	
QUANTREF600C_PS	Always	Never	storeWobb
QUANTREF600C_PS_3mm	Always	Never	storeWobb
QUANTREF600C_PS_O1	Never	Never	
QUANTREF600C_PS_O1_3mm	Never	Never	
QUANTREF600C_UR	Always	Never	storeWobb
QUANTREF600C_UR_3mm	Always	Never	storeWobb
QUANTREF600C_UR_O1	Never	Never	
QUANTREF600C_UR_O1_3mm	Never	Never	

*Figure 2.7: IconNMR Configuration: Tuning/Matching – QuanRefC (Recommendation: Check **storeWobb**)*

MeOH Extract: Only on the ZG30 experiments:

Experiment Name	^{1}H Channel Tuning/Matching	X Channel Tuning/Matching	With options
PROF_MEOH_JRES	Never	Never	
PROF_MEOH_JRES_3mm	Never	Never	
PROF_MEOH_NOESY	Never	Never	
PROF_MEOH_NOESY_3mm	Never	Never	
PROF_MEOH_ZG30	Always	Never	storeWobb
PROF_MEOH_ZG30_3mm	Always	Never	storeWobb
PROF_MEOH_ZGPS	Never	Never	
PROF_MEOH_ZGPS_3mm	Never	Never	

*Figure 2.8: IconNMR-Configuration: Tuning/Matching – MeOH Extract (Recommendation: Check **storeWobb**)*

- Lock/Shim Options
  - Solvent specific standard shims and shim routines.
  - Setup Shimming routine such as **TOPSHIM TUNEA**:

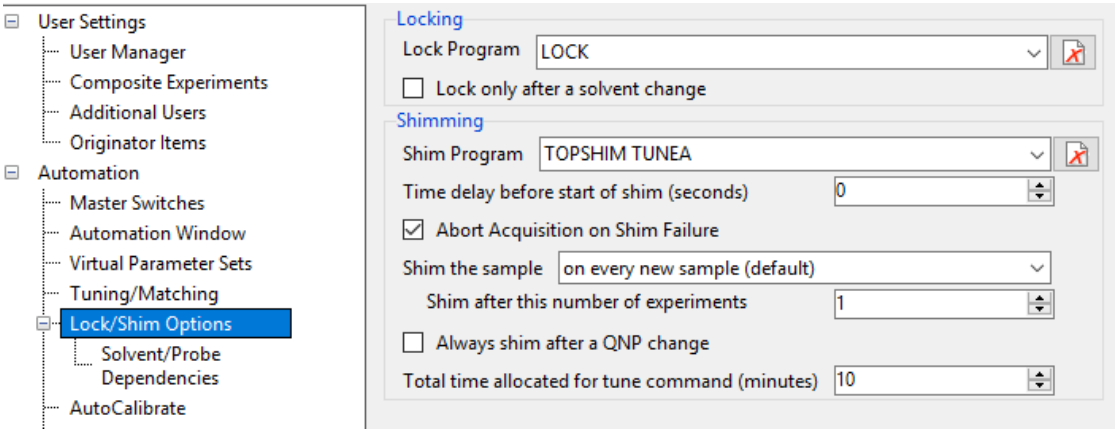


Figure 2.9: IconNMR Configuration: Lock/Shim Options

- Solvent/Probe Dependencies

  The names of the solvents are exactly fixed as well as the names of the dedicated shim-sets. The shim-sets are solvent specific and used as start values in automation. These shims need to be kept up to date:

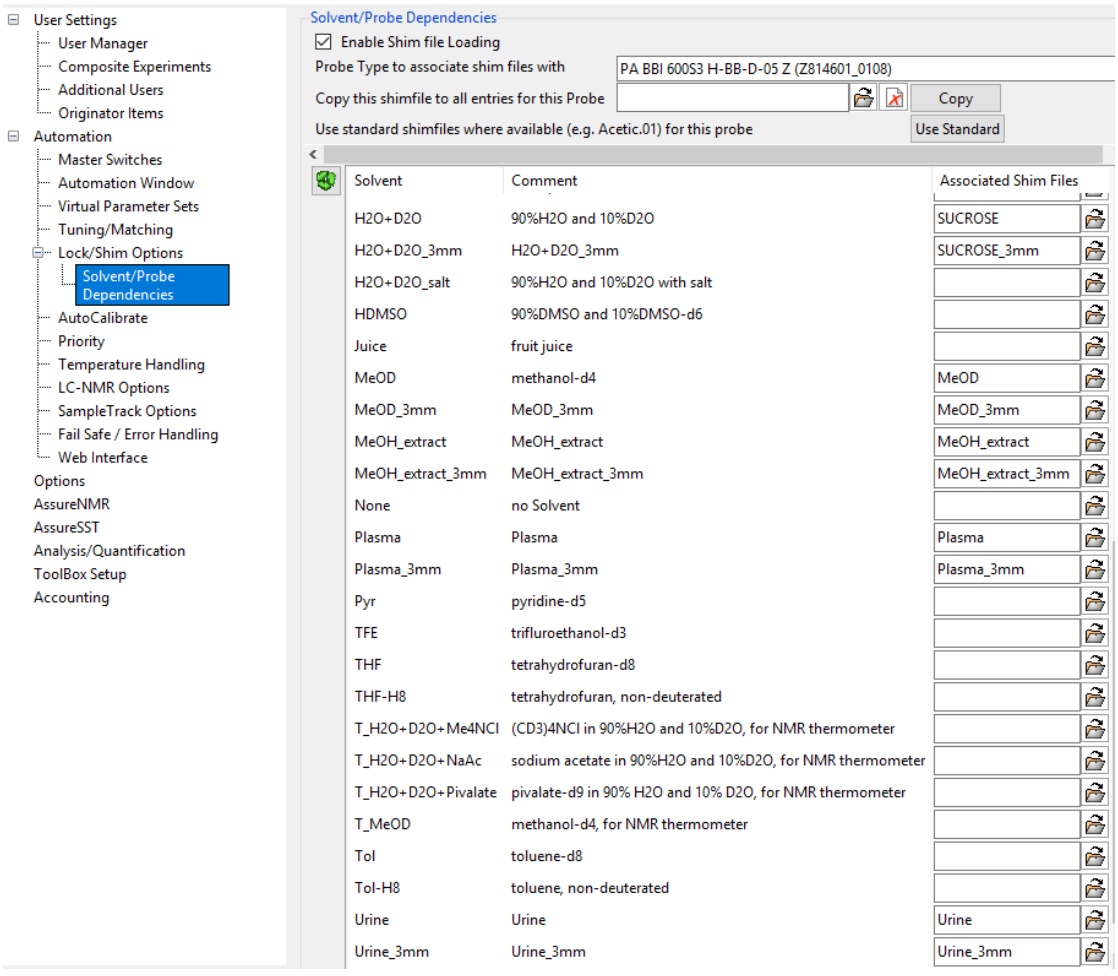


Figure 2.10: IconNMR Configuration: Lock/Shim Options - Solvent/Probe Dependencies

- Temperature Handling

  The settings should be “according to first experiments TE parameter” exactly like in the figure above. The temperature equilibrium time (default 300s) can be optimized according to the individual instrumental and environmental stability.

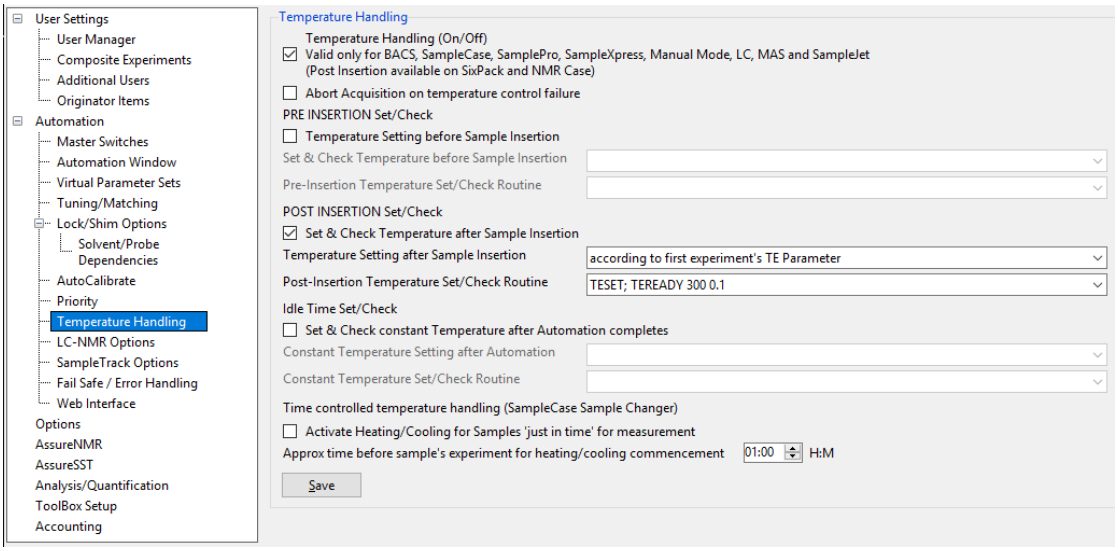


Figure 2.11: IconNMR Configuration: Temperature Handling

- SampleTrack Options
  - **Use Web Services (SOAP) Network Connection** should be activated when applicable:

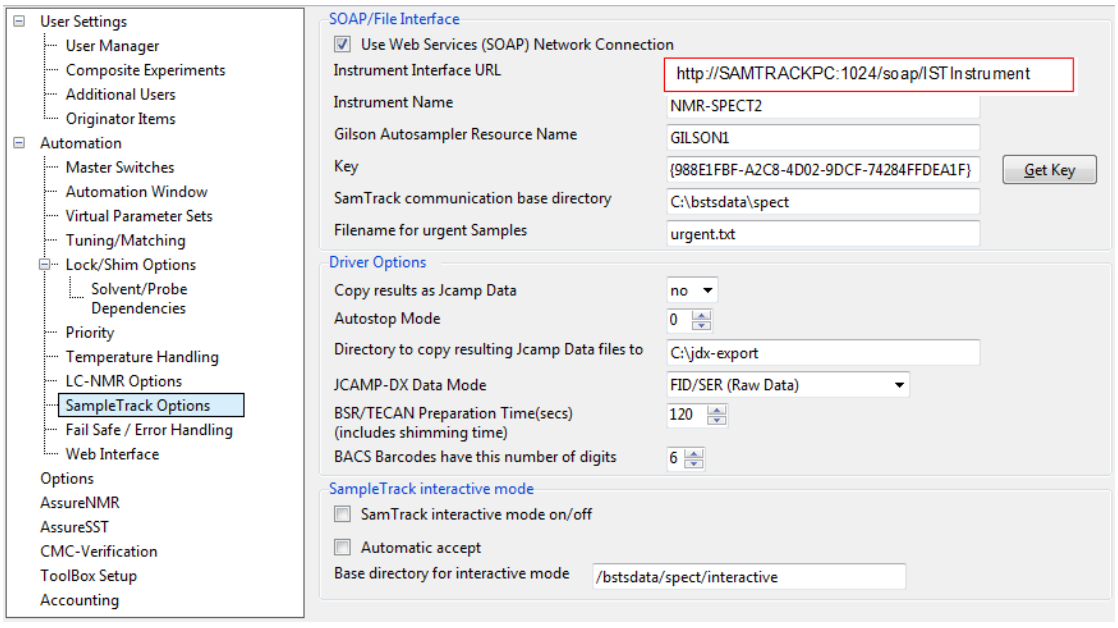


Figure 2.12: IconNMR Configuration for SampleTrack

2.3.2 IconNMR Automation

How to import an Excel spreadsheet:

- In ICON Automation | **File** | **Import Spreadsheet**

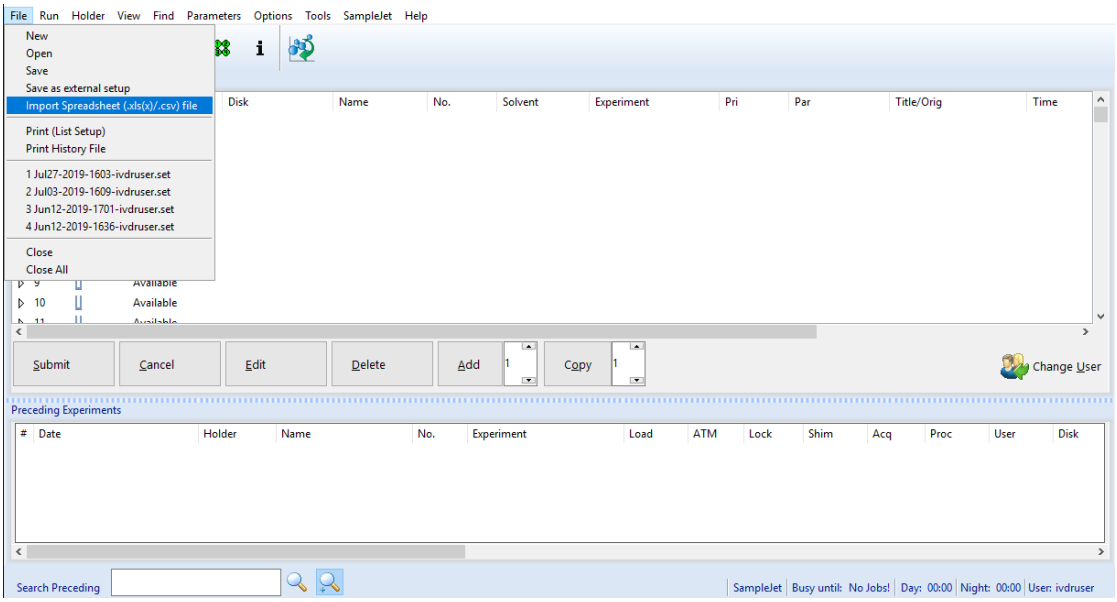


Figure 2.13: IconNMR Automation: Import Spreadsheet

- Load the Excel sheet (an Excel template is delivered with the installation).
- Choose the workspace:

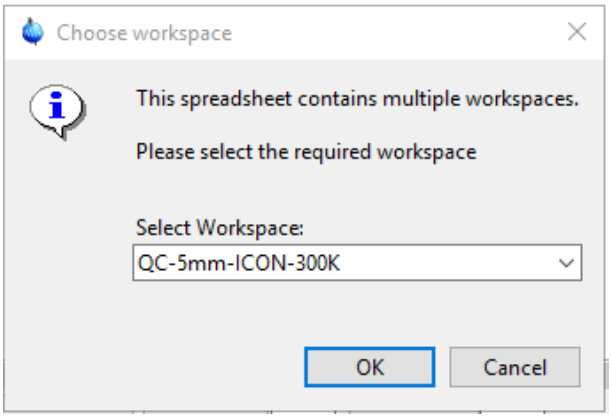


Figure 2.14: IconNMR Automation: Choose Workspace

- Load the Setup from Spreadsheet file and make sure that the all entries are correct.
- Click **Load into Setup Window**.

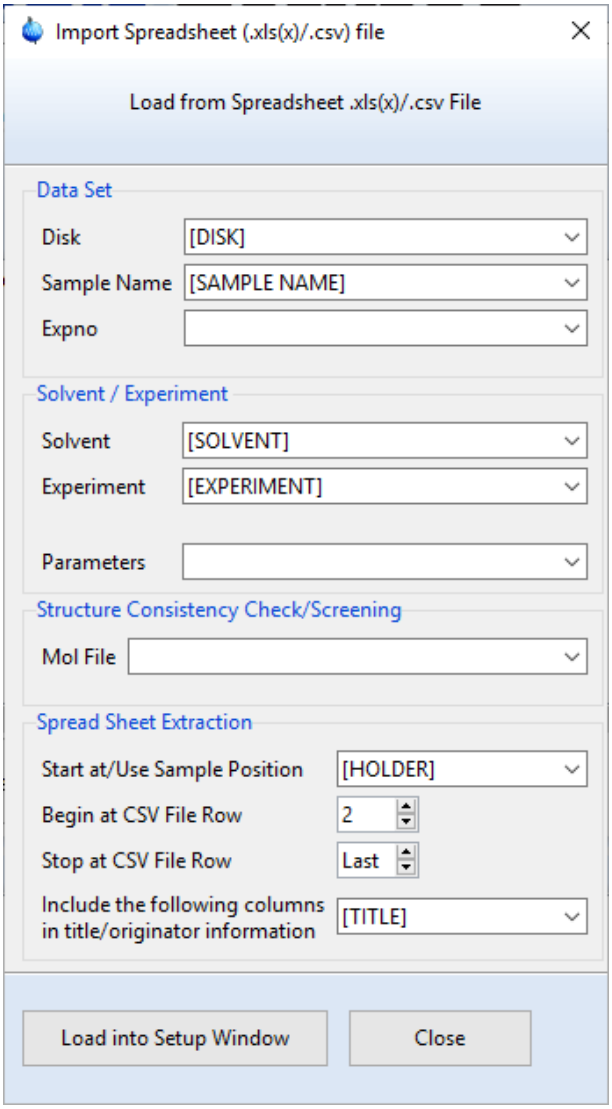


Figure 2.15: IconNMR Automation: Load Setup from Spreadsheet

2.4 SampleJet Configuration

Log into SampleJet as **User** and check if all the conditions are correct in **Basic Service**.

2.4.1 Basic Service / User Settings

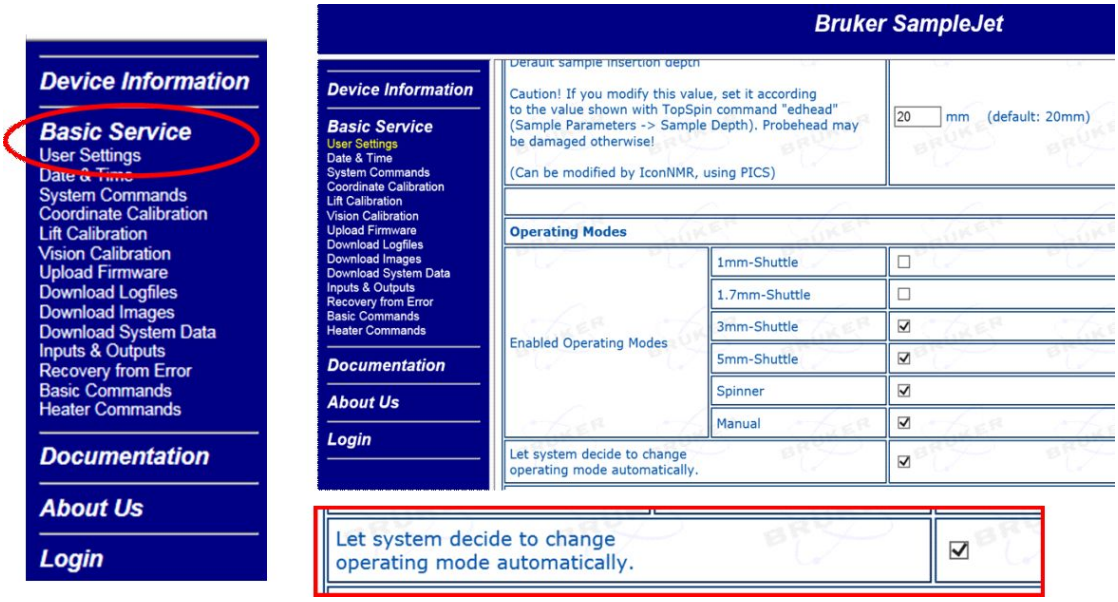


Figure 2.16: SampleJet- Basic Service: User Settings

2.4.2 Basic Service / Heater Commands

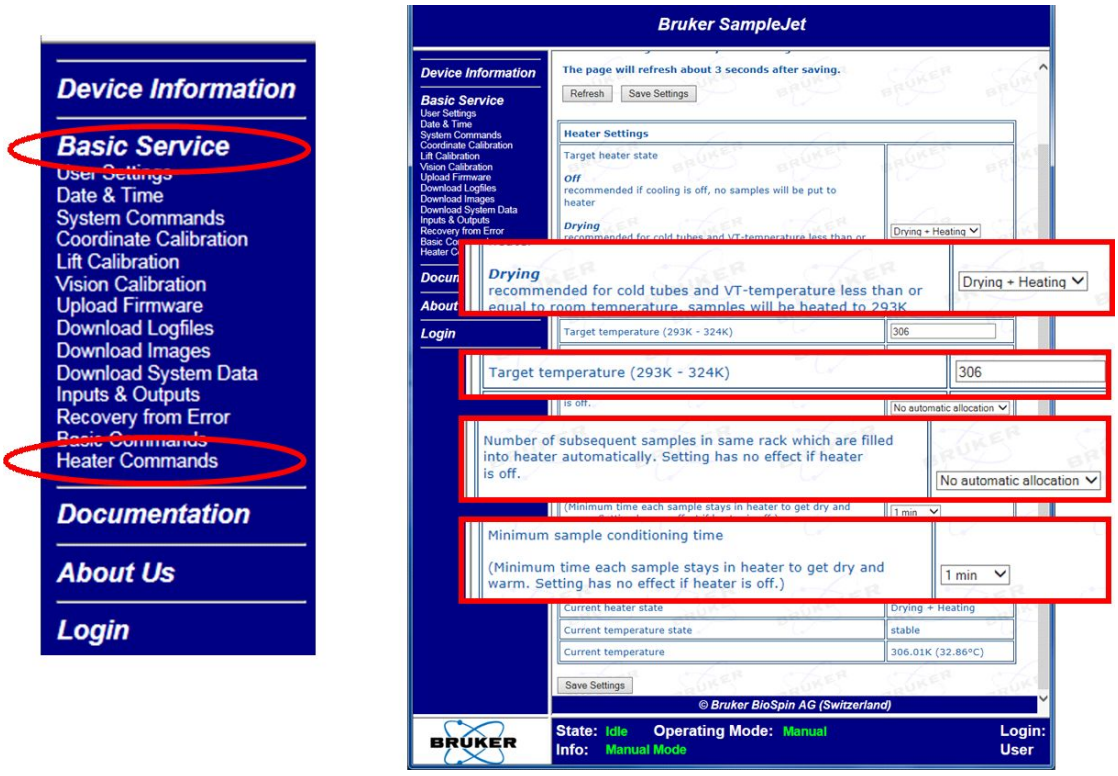


Figure 2.17: SampleJet- Basic Service: Heater Commands

The **Minimum sample conditioning time** setting must be identical in the SampleJet and IconNMR configurations. If not, the setting in IconNMR configuration will be used for the IconNMR automation.

2.5 SampleTrack

SampleTrack™ is a BRUKER laboratory automation and management system with a standardized interface for BRUKER spectrometers. As a software tool for the laboratory network, SampleTrack manages all automation process steps in an analytical environment. The order setup entry can be simultaneously derived from variable sources including spreadsheets, Web client, SampleTrack client, or through an external LIMS system. Consequently, all available instruments and preparation robots are fed with the generated orders. The successful installation of SampleTrack must be supported from the local IT-department.

2.5.1 IconNMR Configuration

IconNMR Configuration must be correct (see *Figure 2.12 [▶ 15]*).

2.5.2 Setup Samples

In Batch Mode

- Click on the symbol **Create new sample order**, select **Enter Samples in Batch Mode** on the **Profiler Order Setup Expert** window, and click **Next**:

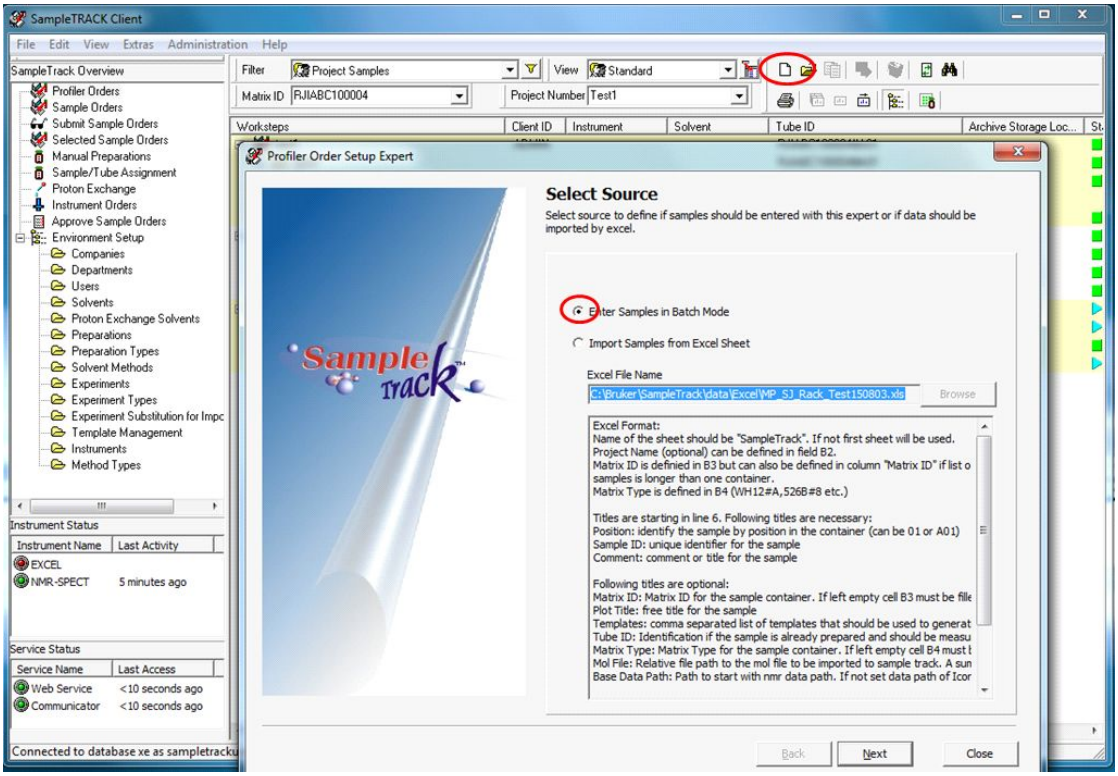


Figure 2.18: SampleTrack Sample - Setup: Batch Mode 1

- Enter the Project Name, then click **Next**:

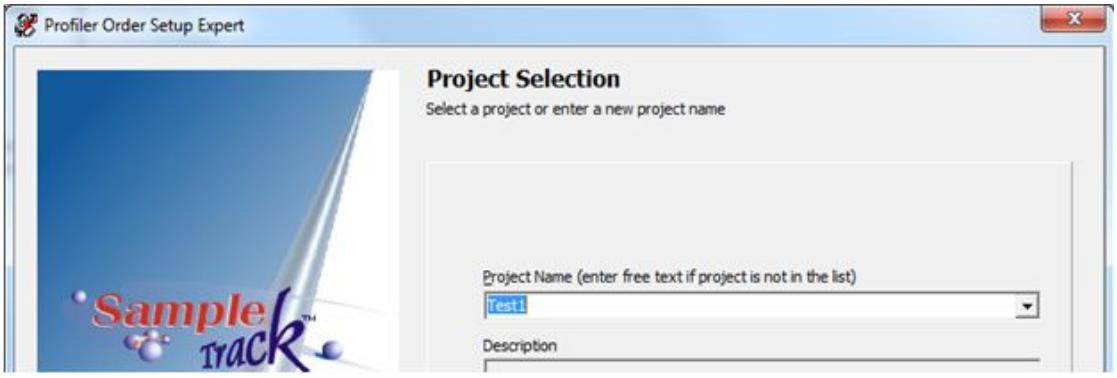


Figure 2.19: SampleTrack - Sample Setup: Batch Mode 2

- Select **User Following Source Container Information**, enter the Rack barcode ID (should start with Container Type ID ”RJI”), enter the Container Type (RJI = SampleJet Rack 5 mm, RJF = SampleJet Rack 3 mm), then click **Next**:

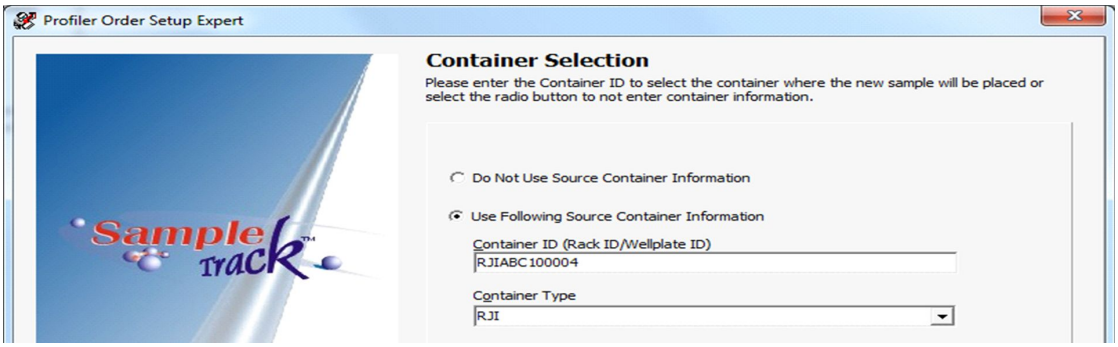


Figure 2.20: SampleTrack - Sample Setup: Batch Mode 3

- Enter the Sample ID for the selected sample position, then click **Next:**

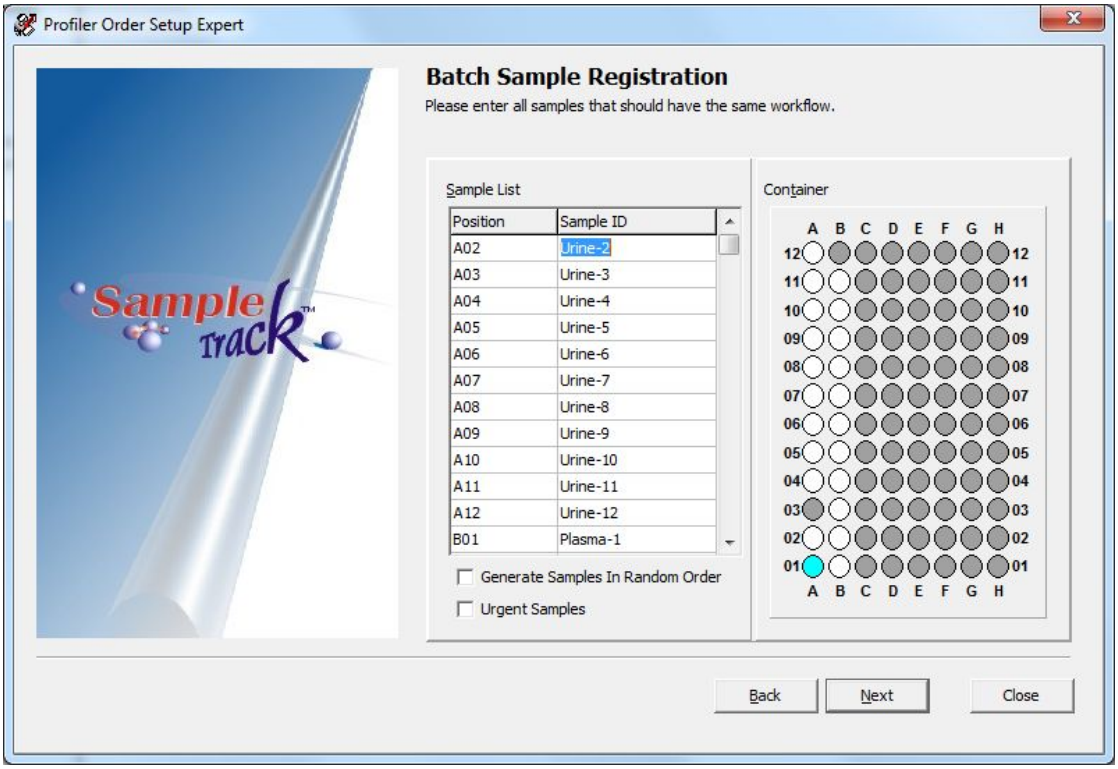


Figure 2.21: SampleTrack - Sample Setup: Batch Mode 4

- Select **Use Super Method for all further steps**, choose the Super method Name (e.g., IVDr Urine), check **Customize Super Method Details** (it is possible to choose a single experiment), and click **Next**:

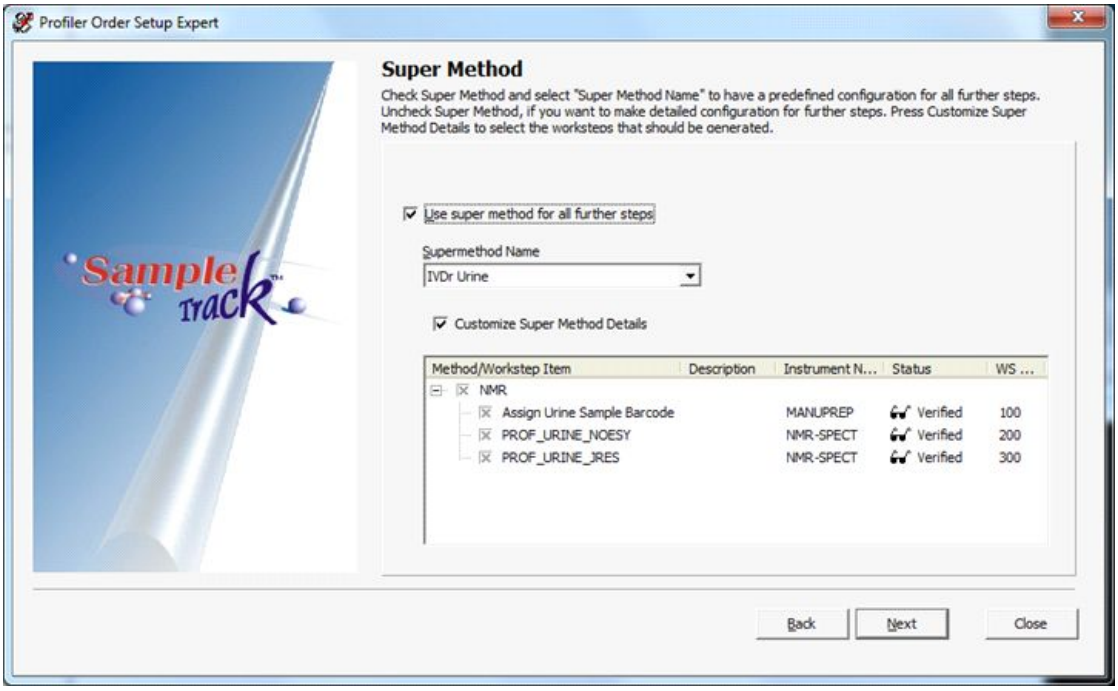


Figure 2.22: SampleTrack - Sample Setup: Batch Mode 5

- Setup Summary, click **Next**:

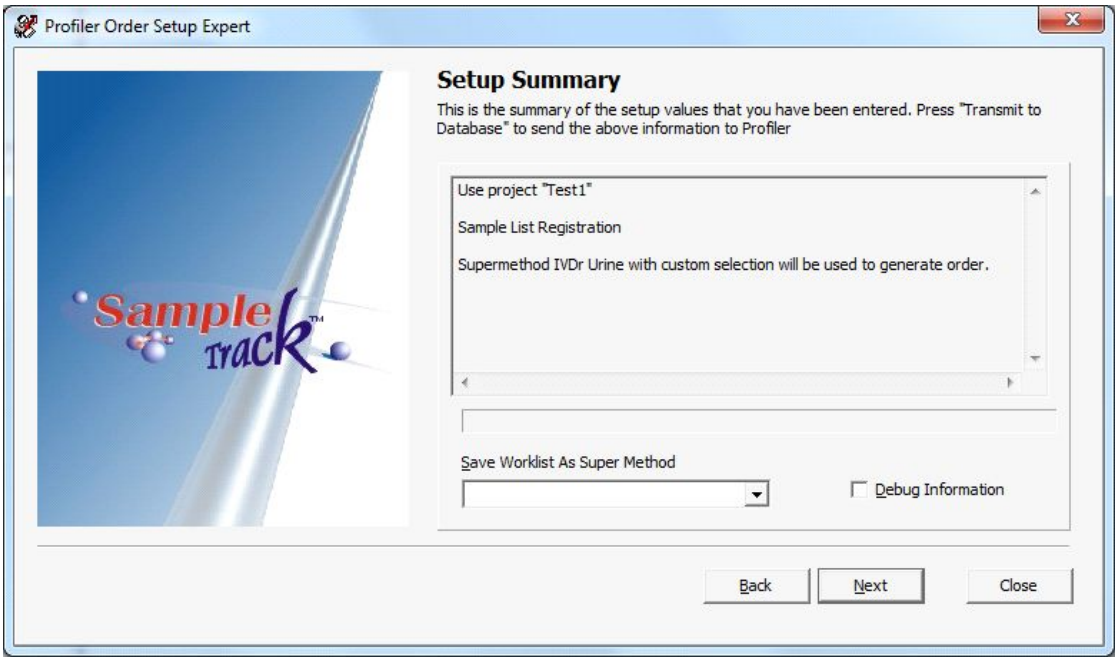


Figure 2.23: SampleTrack - Sample Setup: Batch Mode 6

- If another similar setup is going to be done, select **Start new setup with entered settings**; if a completely new setup is going to be done, select **Start new setup with default settings**; if the setup is finished, select **Finish this expert**, then click **Finish**:

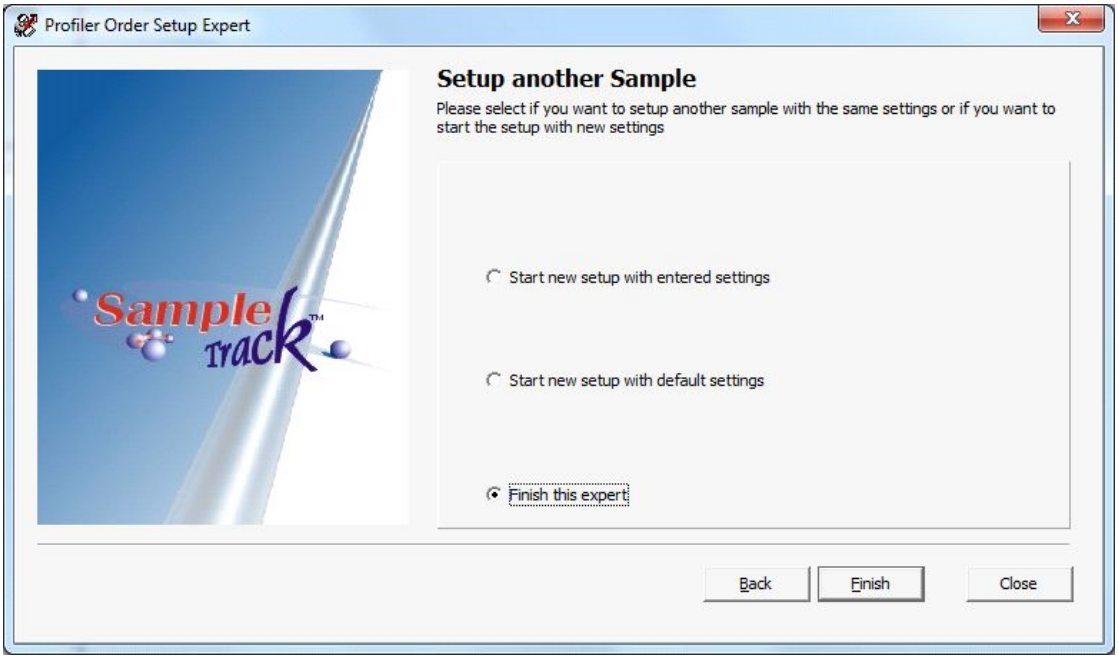


Figure 2.24: SampleTrack - Sample Setup: Batch Mode 7

With Excel-sheet

Note: Microsoft Office is needed on the SAMTRACK PC.

- Click on the symbol **Create new sample order**, select **Import Samples from Excel Sheet** on the Profiler Order Setup Expert window (an Excel Template is delivered with the installation), then click **Next**:

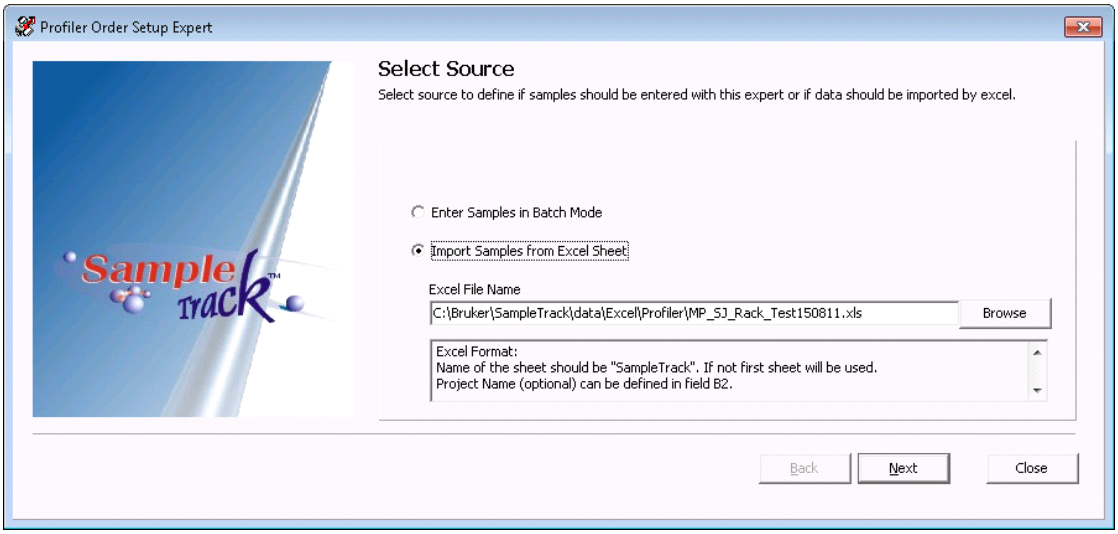


Figure 2.25: SampleTrack - Sample Setup: Excel Sheet 1

- Select the desired samples, then click **Next**:

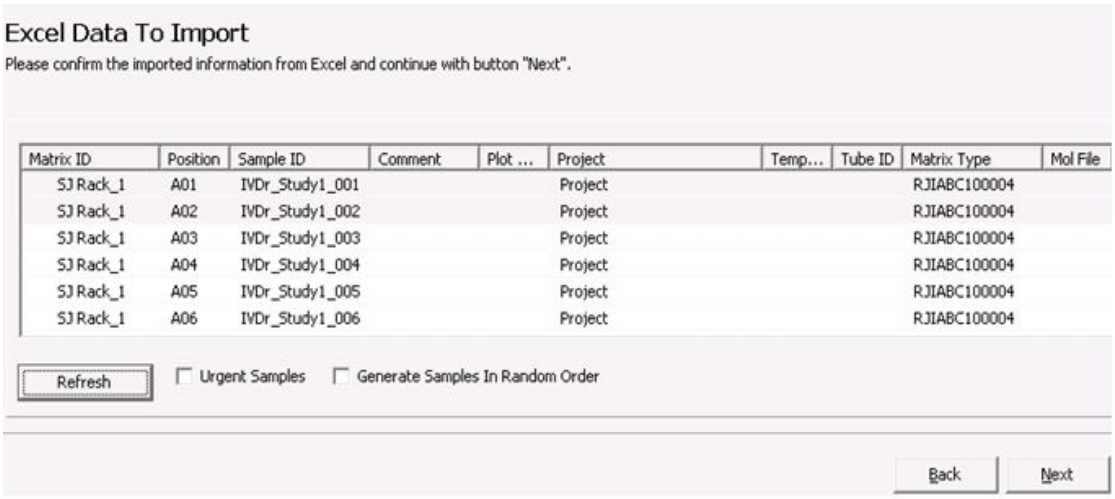


Figure 2.26: SampleTrack - Sample Setup: Excel Sheet 2

- Follow the workflow described in Batch Modes 5-7, see figures 2.19-2.21.

2.5.3 Start ICON

- Start ICON Run (make sure that **SampleTrack (SampleJet)** is selected).
- Click **Start**:

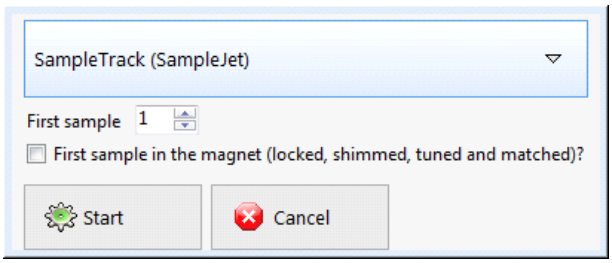


Figure 2.27: SampleTrack - Sample Setup: Start ICON

3 Sample Preparation

3.1 For 5 mm NMR Tubes

Urine

- Carefully thaw the samples, which have been stored at -80 °C, at room temperature (30 minutes for ≤ 2 mL volume sample).
- Centrifuge the samples for 5 to 10 minutes at ~2000 RCF.
- Pipette 100 µL of Bruker urine buffer into a Cryovial or Eppendorf container (of 1.5 mL or 2 mL volume).
- Add 900 µL of urine.
- Mix the buffered urine for 30 seconds on the Vortex mixer.
- Transfer 600 µL of well mixed sample into a 5 mm 7” NMR tube or a 5 mm SampleJet rack tube.

Plasma/Serum

- Carefully thaw the samples, which have been stored at -80 °C, at room temperature (30 minutes for ≤ 2 mL volume sample).
- Pipette 400 µL of Bruker plasma buffer into a Cryovial or Eppendorf container (of 1.5 mL or 2 mL volume).
- Add 400 µL of plasma/serum.
- Shake the mixture gently for 1 minute (do not use the Vortex mixer).
- Transfer 600 µL of well mixed sample into a 5 mm 7” NMR tube or a 5 mm SampleJet rack tube.

CSF (CSF = Cerebrospinal fluid)

- Carefully thaw the samples, which have been stored at -80 °C, at room temperature (30 minutes for ≤ 2 mL volume sample).
- Add 150 µL of Bruker CSF buffer into a Cryovial or Eppendorf container (of 1.5 mL or 2 mL volume). The CSF buffer = 60% Bruker urine buffer + 40% demineralized H_2O.
- Add 750 µL of CSF.
- Shake the mixture gently for 1 minute (do not use a Vortex mixer).
- Transfer 600 µL of well mixed sample into a 5 mm 7” NMR tube or a 5 mm SampleJet rack tube.

MeOH Extract

- Extract samples using a solvent of 4.76 % CD_3OD in MeOH (for instance, 50 mL Methanol-d4, ≥99.8 atom % D, in 1000 mL GC-MS grade Methanol), and 0.95 % w/v TSP (for instance, 100 mg 2,2,3,3-d(4)-3-(Trimethylsilyl)propionic acid sodium salt, ≥98 atom % D, in 1050 mL MeOH-CD_3OD mixture).
- Transfer the MeOH Extract using a pipette into a 5 mm 7” NMR tube or a 5 mm SampleJet rack tube. The target volume of MeOH Extract inside the tube must be 600 µL. Mind the material loss while transferring.

3.2 For 3 mm NMR Tubes

Urine

- Carefully thaw the samples, which have been stored at -80 °C, at room temperature (30 minutes for ≤ 2 mL volume sample).
- Centrifugate the samples for 5 to 10 minutes at ~2000 RCF.
- Pipette 25 µL of Bruker urine buffer into a Cryovial or Eppendorf container (of 1.5 mL or 2 mL volume).
- Add 225 µL of urine.
- Mix the buffered urine for 30 seconds on a Vortex mixer.
- Transfer 200 µL of well mixed sample into a 3 mm 7” NMR tube or a 3 mm SampleJet rack tube.

Plasma/Serum

- Carefully thaw the samples, which have been stored at -80 °C, at room temperature (30 minutes for ≤ 2 mL volume sample).
- Pipette 150 µL of Bruker plasma buffer into a Cryovial or Eppendorf container (of 1.5 mL or 2 mL volume).
- Add 150 µL of plasma/serum.
- Shake the mixture gently for 1 minute (do not use a Vortex mixer).
- Transfer 200 µL of well mixed sample into a 3 mm 7” NMR tube or a 3 mm SampleJet rack tube.

CSF (CSF = Cerebrospinal fluid)

- Carefully thaw the samples, which have been stored at -80 °C, at room temperature (30 minutes for ≤ 2 mL volume sample).
- Pipette 50 µL of Bruker CSF buffer into a Cryovial or Eppendorf container (of 1.5 mL or 2 mL volume). Note: The CSF buffer = 30% Bruker urine buffer + 70% demineralized H_2O.
- Add 250 µL of CSF.
- Shake the mixture gently for 1 minute.
- Transfer 200 µL of well mixed sample into a 3 mm 7” NMR tube or a 3 mm SampleJet rack tube.

MeOH Extract

- Extract samples using a solvent of 4.76 % CD_3OD in MeOH (for instance, 50 mL Methanol-d4, ≥99.8 atom % D, in 1000 mL GC-MS grade Methanol), and 0.95 % w/v TSP (for instance, 100 mg 2,2,3,3-d(4)-3-(Trimethylsilyl)propionic acid sodium salt, ≥98 atom % D, in 1050 mL MeOH-CD_3OD mixture).
- Transfer MeOH Extract using a pipette into a 5 mm 7” NMR tube or a 5 mm SampleJet rack tube. The target volume of MeOH Extract inside the tube must be 200 µL. Mind the material loss while transferring.

4 Quality Control

Quality Control (QC) ensures the spectral quality, as well as consistent, comparable and accurate results within specified limits of precision. Based on good performance QC, the NMR data generated can be quantified and analyzed correctly and be used for further interpretation and decision making. QC should be implemented on a daily basis.

Overview – 5 mm and 3 mm NMR Tubes

- MeOD
  - Calibrate the temperatures 300 K and 310 K and activate temperature correction.
  - Check if the calibrated temperature is still valid.
- Sucrose
  - At 300 K: Perform P1, O1, basic shim, GradProfile, water suppression test, grad performance, and the stability test.
  - At 310 K: Perform O1, basic shim, water suppression test, and the stability test.
- QuantRefC
  - At 300 K: Perform QuantRefC calibration for H_2O+D_2O, Urine, CSF.
  - At 310 K: Perform QuantRefC calibration for plasma and H_2O+D_2O at 310 K.
- FILCOR
  - Calibrate the Filcor value and update in EDSCON.
  - Check if the Filcor value is still valid.

IMPORTANT

- QC tests must be implemented, and the Control Charts should be checked on a daily basis.
- If any QC test is not successfully validated, no further tests should be started until all the QC results are within acceptance range:

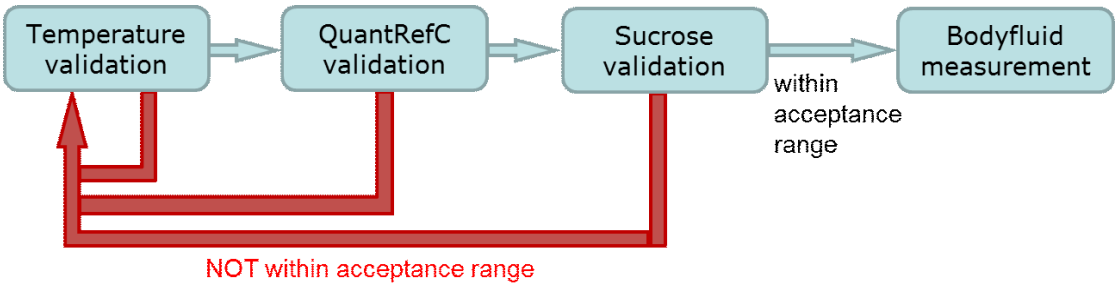


Figure 4.1: Flowchart of the IVDr Daily QC Tests

- QC experiments can be run in ICON automation ONLY when all the QC tubes use multi-use caps.

- The depth of all the QC tubes must be regularly checked:
  - Use the sample depth gauge to adjust the sample depth.
  - The sample tube should sit tightly inside the spinner.
  - Wipe the sample tube after the depth adjustment.

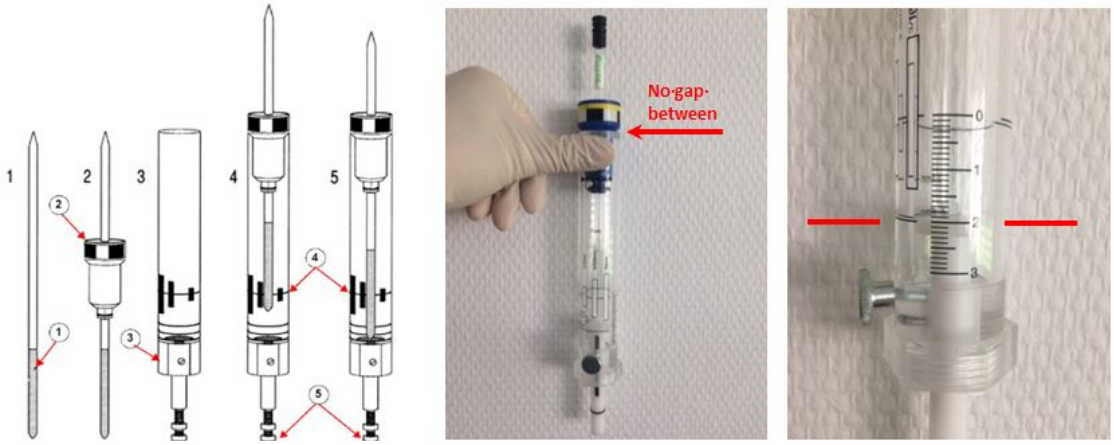


Figure 4.2: Basic Procedures for the Adjustment of the Sample Depth

1	Sample	4	Center Line
2	Spinner	5	Depth Adjustment Screw
3	Depth Gauge		
The bottom of the sample tube should be exactly at the label “2”.			

4.1 Temperature Calibration and Validation

Body fluid NMR results are extremely sensitive towards temperature, therefore temperature calibration/validation must be implemented as the first QC to ensure the correct absolute temperature for the corresponding body fluid experiments.

Sample	Solvent	Temperature	Experiment	Experiment Name
99.8 % MeOD Sample – 5 mm	MeOD	300 K	MEOD_TEMPCAL_300K	TEMPCAL-50WD-300K -<YYYY-MM-DD>
99.8 % MeOD Sample – 5 mm	MeOD	310 K	MEOD_TEMPCAL_310K	TEMPCAL-50WD-310K -<YYYY-MM-DD>
99. 8% MeOD Sample – 3 mm	MeOD_3mm	300 K	MEOD_TEMPCAL_300K_3mm	TEMPCAL-30WD-300K -<YYYY-MM-DD>
99.8 % MeOD Sample – 3 mm	MeOD_3mm	310 K	MEOD_TEMPCAL_310K_3mm	TEMPCAL-30WD-310K -<YYYY-MM-DD>

Table 4.1: Temperature Calibration and Validation (“50WD”: 5 mm NMR tube, “30WD”: 3 mm NMR tube)

4.1.1 Temperature Check 300 K for 5 mm Applications

- Set the TE to 300 K using the temperature correction. Wait at least 5 minutes after the target temperature is reached.
- Create a data set *C:\Bruker\IVDr\RefData\nmr\TEMPCAL-50WD-300K-<YYYY-MM-DD>*
- Read the parameter set: **rpar MEOD_TEMPCAL_300K all**.
- Enter **atma**.
- Enter **lock MeOD**.
- Enter **rsh MeOD**.
- Enter **topshim** (if needed, do manual shimming).
- Enter **xaua**.
- Enter **fp**.
- Enter **apk**.
- Shim Quality Check on MeOH multiplet at ~3.3 ppm, if needed go back to the step enter **rsh MeOD** and repeat the steps.
- After the experiment is finished enter **xaup**.
- Enter **wsh MeOD**.

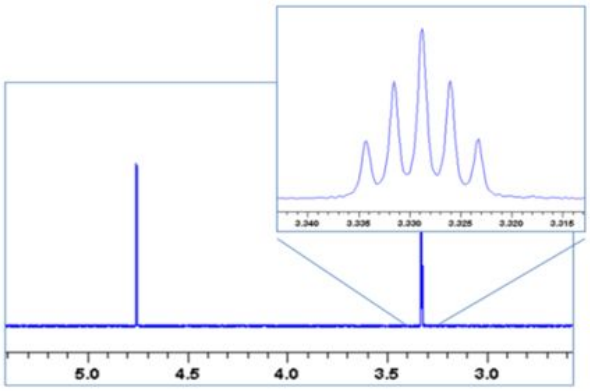
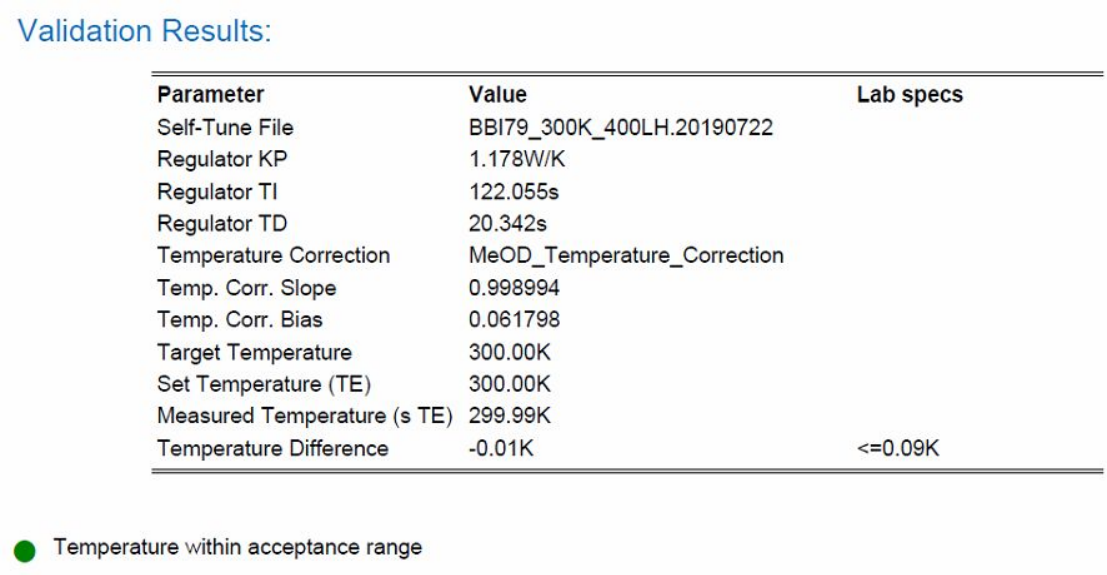


Figure 4.3: Shim Quality Check on MeOD Sample (without LB)

- Check the Validation Results of the PDF-report in the Temperature Validation data directory, i.e., *C:\Bruker\IVDr\RefData\ nmr\TEMPCAL-50WD-300K-<YYYY-MM-DD> \10\pdata\1\MEOD_TEMPCAL_300K_<YYYMMDD_HHMM>.pdf*
  - If the temperature is within the acceptance range, i.e., there is a green light, the procedure is finished (see figure below).
  - If the measured temperature is NOT within the acceptance range, calibrate the temperatures by running **ivdr_temperature_correction 5 mm**.



Validation Results:

Parameter	Value	Lab specs
Self-Tune File	BBI79_300K_400LH.20190722	
Regulator KP	1.178W/K	
Regulator TI	122.055s	
Regulator TD	20.342s	
Temperature Correction	MeOD_Temperature_Correction	
Temp. Corr. Slope	0.998994	
Temp. Corr. Bias	0.061798	
Target Temperature	300.00K	
Set Temperature (TE)	300.00K	
Measured Temperature (s TE)	299.99K	
Temperature Difference	-0.01K	<=0.09K

Temperature within acceptance range

Figure 4.4: Excerpt of the Validation Results of Temperature Calibration

Please note the temperature equilibration time after sample insertion or temperature change!

4.1.2 Temperature Check 300 K for 3 mm Applications

- Set the TE to 300 K using the temperature correction. Wait at least 5 minutes after the target temperature is reached.
- Create a data set *C:\Bruker\IVDr\RefData\nmr\ TEMPCAL-30WD-300K-<YYYY-MM-DD>*
- Read the parameter set: **rpar MEOD_TEMPCAL_300K_3mm all**.
- Enter **atma**.
- Enter **lock MeOD_3mm**.
- Enter **rsh MeOD_3mm**.
- Enter **topshim** (if needed, do manual shimming).
- Enter **xaua**.
- Enter **fp**.
- Enter **apk**.
- Shim Quality Check on MeOH multiplet at ~3.3 ppm, if needed go back to the step enter **rsh MeOD_3mm** and repeat the steps.
- After the experiment is finished, enter **xaup**.
- Enter **wsh MeOD_3mm**
- Check the **Validation Results** (refer to the step in *Temperature Check 300 K for 5 mm Applications [▶ 29]*).

4.1.3 Temperature Check 310 K for 5 mm Applications

- Set the TE to 310 K using the temperature correction. Wait at least 5 minutes after the target temperature is reached.
- Create a data set *C:\Bruker\IVDr\RefData\nmr\TEMPCAL-50WD-310K-<YYYY-MM-DD>*
- Read the parameter set: **rpar MEOD_TEMPCAL_310K all**.
- Enter **atma**.
- Enter **lock MeOD**.
- Enter **rsh MeOD_310K**.
- Enter **topshim** (if needed, do manual shimming).
- Enter **xaua**.
- Enter **fp**.
- Enter **apk**.
- Shim Quality Check on MeOH multiplet at ~3.3 ppm, if needed go back to the step enter **rsh MeOD_310K** and repeat the steps.
- After the experiment is finished, enter **xaup**.
- Enter **wsh MeOD_310K**.
- Check the **Validation Results** (refer to the last step in *Temperature Check 300 K for 5 mm Applications [▶ 29]*).

4.1.4 Temperature Check 310 K for 3 mm Applications

- Set the TE to 310 K using the temperature correction. Wait at least 5 minutes after the target temperature is reached.
- Create a data set *C:\Bruker\IVDr\RefData\nmr\TEMPCAL-30WD-310K-<YYYY-MM-DD>*
- Read the parameter set: **rpar MEOD_TEMPCAL_310K_3mm all**.
- Enter **atma**.
- Enter **lock MeOD_3mm**.
- Enter **rsh MeOD_310K_3mm**.
- Enter **topshim** (if needed, do manual shimming).
- Enter **xaua**.
- Enter **fp**.
- Enter **apk**.
- Shim Quality Check on MeOH multiplet at ~3.3 ppm, if needed go back to the step **Enter rsh MeOD_310K_3mm** and repeat the steps.
- After the experiment is finished, enter **xaup**.
- Enter **wsh MeOD_310K_3mm**.
- Check the **Validation Results** (refer to the last step in *Temperature Check 300 K for 5 mm Applications [▶ 29]*).

Temperature settings can also be optimized via the IVDr menu.

4.2 Shim Performance and Water Suppression Test

Sucrose tests must be valid prior to body fluid experiments to check shim performance, water-suppression, sensitivity, splitting, and gradient profile.

Sample	Solvent	Temperature	Experiment	Experiment Name
Sucrose 2 mM – 5 mm	H2O+D2O	300 K	SUC_ZGPR_O1 SUC_ZGPR SUC_GRADPROF SUC_NOESY	SUCROSE-50WD-300K-<YYYY-MM-DD>
Sucrose 2 mM – 5 mm	H2O+D2O	310 K	SUC_ZGPR_O1_310K SUC_ZGPR_310K SUC_GRADPROF SUC_NOESY_310K	SUCROSE-50WD-310K-<YYYY-MM-DD>
Sucrose 2 mM – 3 mm	H2O +D2O_3mm	300 K	SUC_ZGPR_O1_3mm SUC_ZGPR_3mm SUC_GRADPROF_3mm SUC_NOESY_3mm	SUCROSE-30WD-300K-<YYYY-MM-DD>
Sucrose 2 mM – 3 mm	H2O +D2O_3mm	310 K	SUC_ZGPR_O1_310K_3mm SUC_ZGPR_310K_3mm SUC_GRADPROF_3mm SUC_NOESY_310K_3mm	SUCROSE-30WD-310K-<YYYY-MM-DD>

Table 4.2: Shim Performance and Water Suppression Test

4.2.1 5 mm Sucrose at 300 K

EXPNO 1: Basic Shim, P1, O1

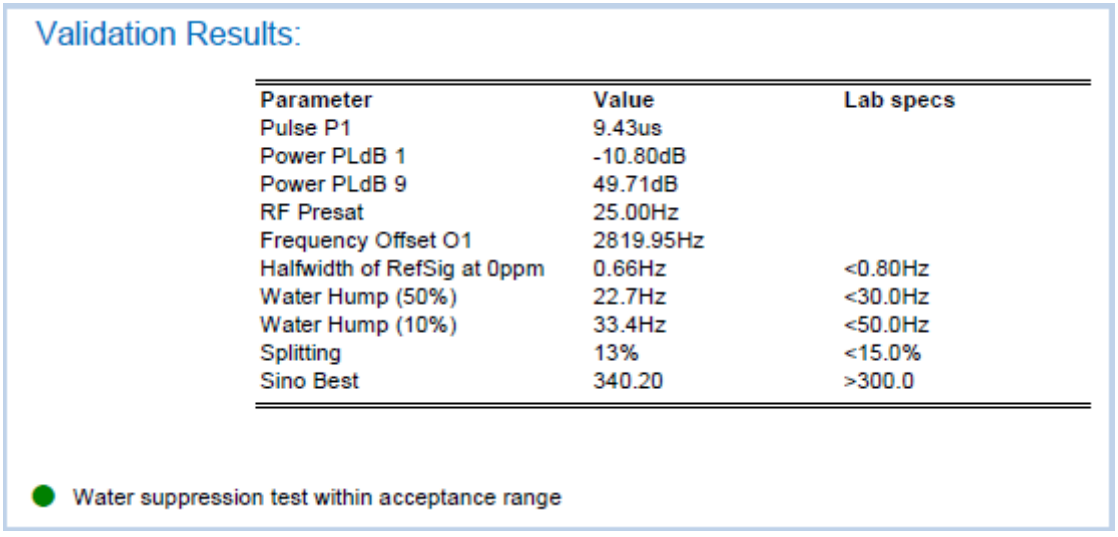
- Set the TE to 300 K using the temperature correction.
- Create a data set *C:\Bruker\IVDr\RefData\nmr\SUCROSE-50WD-300K-<YYYY-MM-DD>.*
- Enter **rpar SUC_ZGPR_O1 all**.
- Enter **atma**.
- Enter **lock H2O+D2O**.
- Enter **rsh Surose**.
- Enter **topshim**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Validate P1, PLdB9, O1 and shim quality from the PDF-report in the Reference-data directory, i.e., *C:\Bruker\IVDr\RefData\nmr\ SUCROSE-50WD-300K-<YYYY-MM-DD>> \1\pdata\1\SUC_ZGPR_O1_YYYYMMDD_HHMM.pdf.*
- If the shim quality is not acceptable, reenter **topshim** and repeat the steps following this.
- Enter **saveprofpars**.

EXPNO 2: Water-suppression Test

- Read parameter set: **rpar SUC_ZGPR all**.
- Enter **getprofpars**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
  - Validate the water suppression test results from the PDF report in the Reference-data directory, i.e., *C:\Bruker\IVDr\RefData\nmr\ SUCROSE-50WD-300K-<YYYY-MM-DD> \2\pdata\1\SUC_ZGPR _YYYYMMDD_HHMM.pdf*. If Validation Results show **Water suppression test within the acceptance range**, i.e., there is a green light, the procedure is finished (see figure below).
  - If the water suppression test is not within the acceptance range, the sucrose must be re-measured.

    If the half-width of RefSig at 0 ppm is NOT within the acceptance range (based on the individual labor specifications), or/and the Water Hump (50% or/and 10%) is NOT within the acceptance range, sucrose must be re-shimmed via **3D topshim**, followed by **1D topshim** with tuning afterwards.

    If the half-width of RefSig at 0 ppm is within the acceptance range, but the Water Hump (50 % or/and 10 %) is NOT within the acceptance range, the high ordered Z-shim must be optimized.
- Write shim: **wsh Sucrose**.



Validation Results:

Parameter	Value	Lab specs
Pulse P1	9.43us	
Power PLdB 1	-10.80dB	
Power PLdB 9	49.71dB	
RF Presat	25.00Hz	
Frequency Offset O1	2819.95Hz	
Halfwidth of RefSig at 0ppm	0.66Hz	<0.80Hz
Water Hump (50%)	22.7Hz	<30.0Hz
Water Hump (10%)	33.4Hz	<50.0Hz
Splitting	13%	<15.0%
Sino Best	340.20	>300.0

Water suppression test within acceptance range

Figure 4.5: Excerpt of the Validation Results of Water-suppression of Sucrose

EXPNO 3: Gradient profile reference data

- Enter **rpar SUC_GRADPROF all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Observe the spectrum, it should be smooth as shown in the following figure:

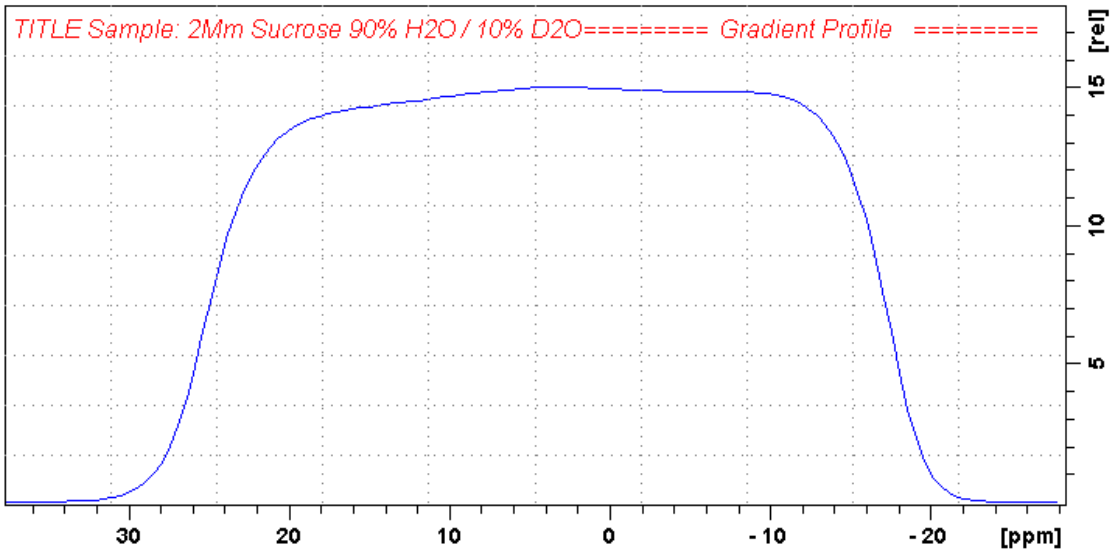


Figure 4.6: An Example of a Good Gradient Profile

The gradient profile should never show dips as observed in the following figure at -20ppm.

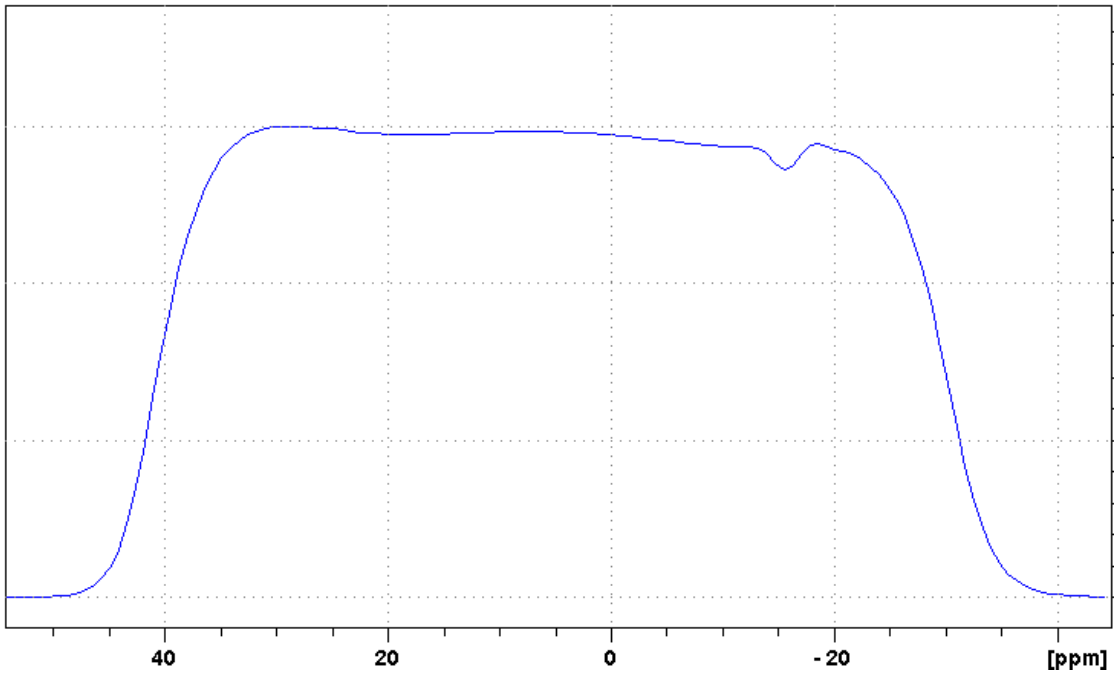


Figure 4.7: An Example of a Bad Gradient Profile

Contact Bruker for verification if this is observed.

EXPNO 4: Gradient performance reference data

- Enter **rpar SUC_NOESY all**.
- Enter **getprofpars**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.
- Compare the spectrum to the one without the gradient from EXPNO 2:

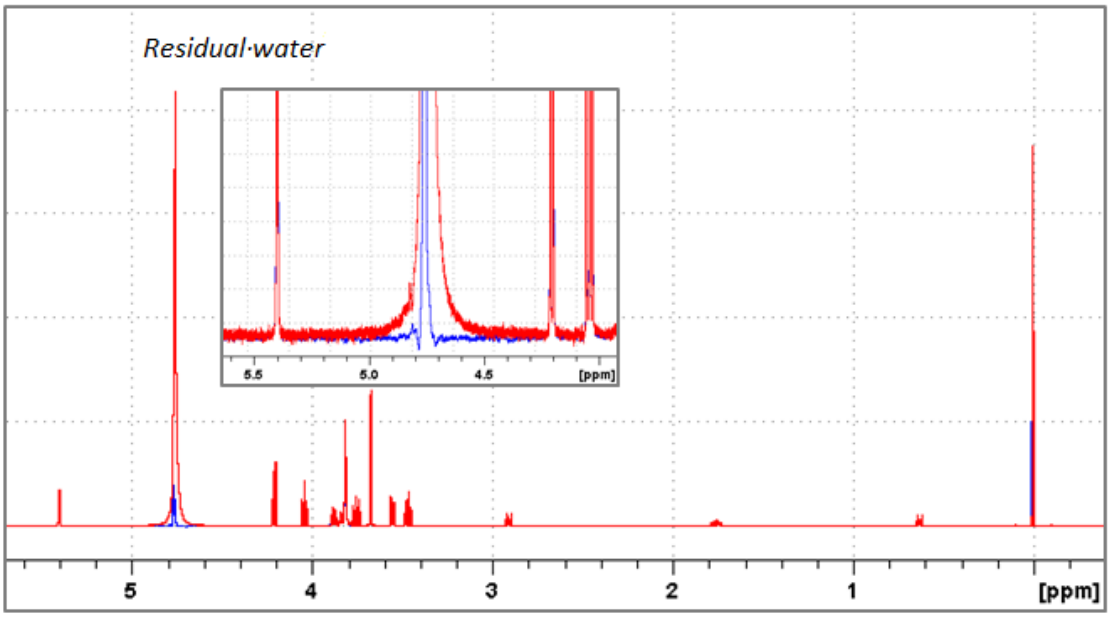


Figure 4.8: Gradient Profile of Sucrose: Spectral Comparison of SUC_NOESY to SUC_ZGPR

4.2.2 5 mm Sucrose at 310 K

EXPNO 1: Basic shim, P1, O1

- Set the TE to 310 K using the temperature correction. Wait at least 5 minutes after the target temperature is reached.
- Create a data set *C:\Bruker\IVDr\RefData\nmr\ SUCROSE-50WD-310K-<YYYY-MM-DD>*.
- Enter **rpar SUC_ZGPR_O1_310K all**.
- Enter **atma**.
- Enter **lock H2O+D2O**.
- Enter **rsh Surose_310K**.
- Enter **topshim**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Validate P1, PLdB9, O1 and shim quality from the PDF-report in the Reference-data directory, i.e., *C:\Bruker\IVDr\RefData\nmr\SUCROSE-50WD-310K-<YYYY-MM-DD>>\1\pdata\1\SUC_ZGPR_O1_YYYYMMDD_HHMM.pdf*.
- If the shim quality is not acceptable, reenter **topshim** and repeat the steps following this.
- Enter **wsh Surose_310K**.
- Enter **saveprofpars**.

EXPNO 2: Water-suppression Test

- Read the parameter set: **rpar SUC_ZGPR_310K all**.
- Enter **getprofpars**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Validate the water suppression test results from the PDF report in the Reference data directory, i.e., *C:\Bruker\IVDr\RefData\nmr\SUCROSE-50WD-310K-<YYYY-MM-DD>>\2\pdata\1\SUC_ZGPR_YYYYMMDD_HHMM.pdf*.

EXPNO 3: Gradient profile reference data

- Enter **rpar SUC_GRADPROF all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Observe the spectrum, it should be smooth (see *Figure 4.6 [▶ 34]*).

EXPNO 4: Gradient performance reference data

- Enter **rpar SUC_NOESY_310K all**.
- Enter **getprofpars**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

4.2.3 3 mm Sucrose at 300 K

EXPNO 1: Basic shim, P1, O1

- Set the TE to 300 K using the temperature correction. Wait at least 5 minutes after the target temperature is reached.
- Create a data set *C:\Bruker\IVDr\RefData\nmr\ SUCROSE-30WD-300K-<YYYY-MM-DD>*.
- Enter **rpar SUC_ZGPR_O1_3mm all**.
- Enter **atma**.
- Enter **lock H2O+D2O_3mm**.
- Enter **rsh Surose_3mm**.
- Enter **topshim**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Validate P1, PLdB9, O1 and shim quality from the PDF-report in the Reference-data directory, i.e., *C:\Bruker\IVDr\RefData\nmr\SUCROSE-30WD-300K-<YYYY-MM-DD>>\1\pdata\1\SUC_ZGPR_O1_YYYYMMDD_HHMM.pdf*.
- If the shim quality is not acceptable, reenter **topshim** and repeat the steps following this.
- Enter **wsh Surose_3mm**.
- Enter **saveprofpars**.

EXPNO 2: Water-suppression Test

- Read the parameter set: **rpar SUC_ZGPR_3mm all**.
- Enter **getprofpars**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Validate the water suppression test results from the PDF-report in the Reference data directory, i.e., *C:\Bruker\IVDr\RefData\nmr\SUCROSE-30WD-300K-<YYYY-MM-DD>>\2\pdata\1\SUC_ZGPR_YYYYMMDD_HHMM.pdf*

EXPNO 3: Gradient profile reference data

- Enter **rpar SUC_GRADPROF_3mm all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Observe the spectrum, it should be smooth (see *Figure 4.6 [▶ 34]*).

EXPNO 4: Gradient performance reference data

- Enter **rpar SUC_NOESY_3mm all**.
- Enter **getprofpars**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

4.2.4 3 mm Sucrose at 310 K

EXPNO 1: Basic shim, P1, O1

- Set the TE to 310 K using the temperature correction. Wait at least 5 minutes after the target temperature is reached.
- Create a data set *C:\Bruker\IVDr\RefData\nmr\ SUCROSE-30WD-310K-<YYYY-MM-DD>.*
- Enter **rpar SUC_ZGPR_O1_310K_3mm all**.
- Enter **atma**.
- Enter **lock H2O+D2O_3mm**.
- Enter **rsh Surose_310K_3mm**.
- Enter **topshim**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Validate P1, PLdB9, O1 and shim quality from the PDF-report in the Reference-data directory, i.e., *C:\Bruker\IVDr\RefData\nmr\SUCROSE-30WD-310K-<YYYY-MM-DD>>\1\pdata\1\SUC_ZGPR_O1_YYYYMMDD_HHMM.pdf*.
- If the shim quality is not acceptable, reenter **topshim** and repeat the steps following this.
- Enter **wsh Surose_310K_3mm**.
- Enter **saveprofpars**.

EXPNO 2: Water-suppression Test

- Read the parameter set: **rpar SUC_ZGPR_310K_3mm all**.
- Enter **getprofpars**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Validate the water suppression test results from the PDF-report in the Reference data directory, i.e., *C:\Bruker\IVDr\RefData\nmr\SUCROSE-30WD-310K-<YYYY-MM-DD>>\2\pdata\1\SUC_ZGPR_YYYYMMDD_HHMM.pdf*.

EXPNO 3: Gradient profile reference data

- Enter **rpar SUC_GRADPROF_3mm all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Observe the spectrum, it should be smooth (see *Figure 4.6 [▶ 34]*).

EXPNO 4: Gradient performance reference data

- Enter **rpar SUC_NOESY_310K_3mm all**.
- Enter **getprofpars**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**
- Update the parameter set: **ivdr_***wpar*

4.3 QuantRefC Calibration and Validation

QuantRefC is the key to the IVDr concept. It enables data exchanging, pooling and joint modeling across different NMR platforms and NMR labs. It is a key for any cooperation in large scale and long-term studies. If based on correctly calibrated and daily validated QuantRefC, the NMR data can be correctly quantified.

The QuantRefC should be calibrated in relation to the corresponding applications and validated prior to body fluid experiments for the correct data analyses. If the result is not within the accepted range, a new calibration must be implemented, followed by a new validation. All the calibration and validation PDF reports can be found in the Reference data directory in the subdirectory of the experiments, e.g.:

C:\Bruker\IVDr\RefData\nmr\QuantRefC-50WD-<ID>-BA-<date>\10\QuantValidation.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-50WD-<ID>-BA-310K-<date>\10\QuantValidation.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-50WD-<ID>-CS-<date>\10\QuantValidation.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-50WD-<ID>-UR-<date>\10\QuantValidation.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-50WD-<ID>-PS-<date>\10\QuantValidation.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-30WD-<ID>-BA-<date>\10\QuantValidation.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-30WD-<ID>-BA-310K-<date>\10\QuantValidation.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-30WD-<ID>-CS-<date>\10\QuantValidation.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-30WD-<ID>-UR-<date>\10\QuantValidation.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-30WD-<ID>-PS-<date>\10\QuantValidation.pdf

In addition, conventional Title PDF reports are available using the same layout as the temperature and water suppression reports. They are found in the same subdirectory and are intended to replace the reports given above on a longer term. Naming according to, e.g.:

C:\Bruker\IVDr\RefData\nmr\QuantRefC-50WD-<ID>-BA-<date>\10\QUANTREF600C_BA_<date>.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-50WD-<ID>-BA-310K-<date>\10\pdata\1\QUANTREF600C_BA_310K_<date>.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-50WD-<ID>-CS-<date>\10\pdata\1\QUANTREF600C_CS_<date>.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-50WD-<ID>-UR-<date>\10\pdata\1\QUANTREF600C_UR_<date>.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-50WD-<ID>-PS-<date>\10\pdata\1\QUANTREF600C_PS_<date>.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-30WD-<ID>-BA-<date>\10\pdata\1\QUANTREF600C_BA_3mm_<date>.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-30WD-<ID>-BA-310K-<date>\10\pdata\1\QUANTREF600C_BA_310K_3mm_<date>.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-30WD-<ID>-CS-<date>\10\pdata\1\QUANTREF600C_CS_3mm_<date>.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-30WD-<ID>-UR-<date>\10\pdata\1\QUANTREF600C_UR_3mm_<date>.pdf

C:\Bruker\IVDr\RefData\nmr\QuantRefC-30WD-<ID>-PS-<date>\10\pdata\1\QUANTREF600C_PS_3mm_<date>.pdf

Sample	Solvent	Temperature	Experiment	Experiment Name
QuantRefC – 5 mm	H_2O+D_2O	300 K	QUANTREF600C_BA	QuantRefC-50WD-<ID>-BA-<YYYY-MM-DD>
QuantRefC – 5 mm	H_2O+D_2O	310 K	QUANTREF600C_BA_310K	QuantRefC-50WD-<ID>-BA-310K<YYYY-MM-DD>
QuantRefC – 5 mm	CSF	300 K	QUANTREF600C_CS	QuantRefC-50WD-<ID>-CS-<YYYY-MM-DD>
QuantRefC – 5 mm	Urine	300 K	QUANTREF600C_UR	QuantRefC-50WD-< ID>-UR-<YYYY-MM-DD>
QuantRefC – 5 mm	Plasma	310 K	QUANTREF600C_PS	QuantRefC-50WD-< ID>-PS-<YYYY-MM-DD>
QuantRefC – 3 mm	H_2O +D_2O_3mm	300 K	QUANTREF600C_BA_3mm	QuantRefC-30WD-< ID>-BA-<YYYY-MM-DD>
QuantRefC – 3mm	H2O +D2O_3mm	310 K	QUANTREF600C_BA_310K_3mm	QuantRefC-30WD-<ID>-BA-310K-<YYYY-MM-DD>
QuantRefC – 3 mm	CSF_3mm	300 K	QUANTREF600C_CS_3mm	QuantRefC-30WD-< ID>-CS-<YYYY-MM-DD>
QuantRefC – 3 mm	Urine_3mm	300 K	QUANTREF600C_UR_3mm	QuantRefC-30WD-< ID>-UR-<YYYY-MM-DD>
QuantRefC – 3 mm	Plasma_3mm	310 K	QUANTREF600C_PS_3mm	QuantRefC-30WD-< ID>-PS-<YYYY-MM-DD>

Table 4.3: QuantRefC Calibration and Validation

4.3.1 5 mm QuantRefC Calibration/Validation for Basic

QuantRefC Basic is for **MeOH Extracts** and general application except Urine, Plasma/ Serum, and CSF.

- Set the TE to 300 K using the temperature correction.
- Create new data set via the TopSpin Main Bar **IVDr Menu** | **QuantNMR** | **Init Calibration/ Validation**:

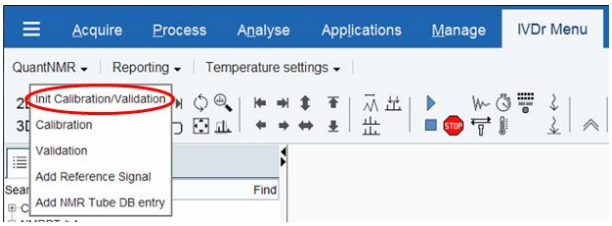


Figure 4.9: QuantNMR Menu Bar

- In the popup window, select the current QuantRefC sample:

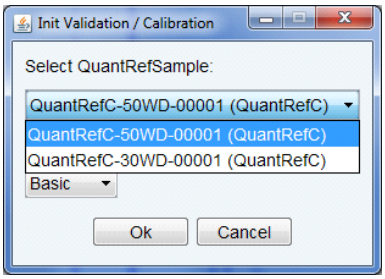


Figure 4.10: Init Validation/Calibration: Select QuantRefSample for 5 mm Applications

- Select method **Basic**:

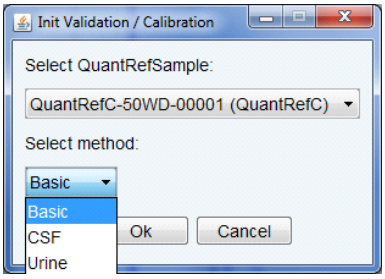


Figure 4.11: Init Validation/Calibration: Select Method for 5 mm Applications

- Enter **atma**.
- Enter **lock H2O+D2O**.
- Enter **rsh Sucrose**.
- Enter **topshim**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Do a Calibration/Validation via the TopSpin Main Bar **IVDr Menu** | **QuantNMR** | **Calibration/Validation**.

4.3.2 5 mm QuantRefC Calibration/Validation for Urine

- Set the TE to 300 K using the temperature correction.
- Create a new data set via TopSpin Main Bar **IVDr Menu** | **QuantNMR** | **Init Calibration/ Validation**.
- In the popup window, select **Urine**.
- Enter **atma**.
- Enter **lock Urine**.
- Enter **rsh Urine**.
- Enter **topshim**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.

4.3.3 5 mm QuantRefC Calibration/Validation for CSF

- Set the TE to 300 K using the temperature correction.
- Create a new data set via the TopSpin Main Bar **IVDr Menu** | **QuantNMR** | **Init Calibration/Validation**.
- In the popup window, select **CSF**.
- Enter **atma**.
- Enter **lock CSF**.
- Enter **rsh CSF**.
- Enter **topshim**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.

4.3.4 5 mm QuantRefC Calibration/Validation for Basic at 310 K

- Set the TE to 310 K using the temperature correction. Wait at least 5 minutes after the target temperature is reached.
- Create a new data set via TopSpin Main Bar **IVDr Menu** | **QuantNMR** | **Init Calibration/ Validation**.
- In the popup window, select **Basic_310K**.
- Enter **atma**.
- Enter **lock H2O+D2O**.
- Enter **rsh Sucrose_310K**.
- Enter **topshim**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.

4.3.5 5 mm QuantRefC Calibration/Validation for Plasma

- Set the TE to 310 K using the temperature correction.
- Create new data set via TopSpin Main Bar **IVDr Menu** | **QuantNMR** | **Init Calibration/ Validation**.
- In the popup window, select **Plasma**.
- Enter **atma**.
- Enter **lock Plasma**.
- Enter **rsh Plasma**.
- Enter **topshim**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Check the “Validation Results” in the PDF-report regardless the experiments.
  - If all the values are within the acceptance range, the procedure is finished.
  - If the *Quantitative Calibration* is NOT within the acceptance range, QuantRefC must be re-calibrated then validated (see section *Troubleshooting [▷ 63]* in the chapter Troubleshooting). If the *Maximum Internal Deviation* is NOT within the acceptance range, the QuantRefC is most likely degraded.

Validation Results:

Parameter	Value	Lab specs
Pulse P1	9.06us	
Power PLdB 1	-10.00dB	
Power PLdB 9	50.86dB	
RF Presat	25.00Hz	
Frequency Offset O1	2821.37Hz	
Halfwidth of RefSig at 0ppm	0.76Hz	<1.10Hz
Quantitative Calibration	102.85%	98.0-102.0%
Maximum Internal Deviation	4.43%	<4.0%

● Quantref validation result not within acceptance range

Figure 4.12: Excerpt of the QuantRefC Validation

4.3.6 3 mm QuantRefC Calibration/Validation

Similar to the 5 mm QuantRefC Calibration/Validation. Select the correct **QuantRefSample** and **method**:

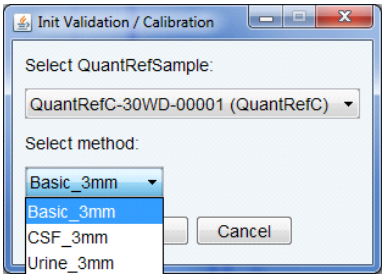


Figure 4.13: Init Validation/Calibration – Select QuantRefSample and Method for 3 mm Applications.

4.4 FILCOR Validation

Sample: FILCOR validation sample.

FILCOR, as a key parameter for validation of automated processing of IVDr data, is an NMR spectrometer parameter, which is not influenced by either temperatures or tube diameters. Therefore, there is only one FILCOR sample measured at 300 K. If the hardware is not changed, FILCOR only needs to be validated monthly. If the hardware is changed, FICOR must be validated.

- Set the TE to 300 K using the temperature correction. Wait at least 5 minutes after the target temperature is reached.
- Create a data set *C:\IVDrData\data\IVDr_INSTALL\nmr\FILCOR-50WD-<YYYY-MM-DD>*
- Read the parameter set: **rpar FILCOR_NOESY all**.
- Enter **atma**.
- Enter **lock H2O+D2O**.
- Enter **rsh Sucrose**.
- Enter **topshim**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Check the **Validation Results** of the PDF-report in the Reference-data directory, i.e., *C:\Bruker\IVDr\RefData\nmr\FILCOR-50WD-<YYYY-MM-DD>\10\pdata\1\FILCOR_NOESY_<YYYMMDD_HHmm.pdf*
  - If FILCOR is within the acceptance range, the procedure is finished.
  - If FILCOR is not within the acceptance range, a manual operation is needed: enter **edscon** and set FILCOR to the newly calibrated value FILCOR = FilCor in scon2 + FilCor Error. In this case FILCOR = 0.80 + (-0.31) = 0.49 µs. Click **Close** and **Save** to store the new value.

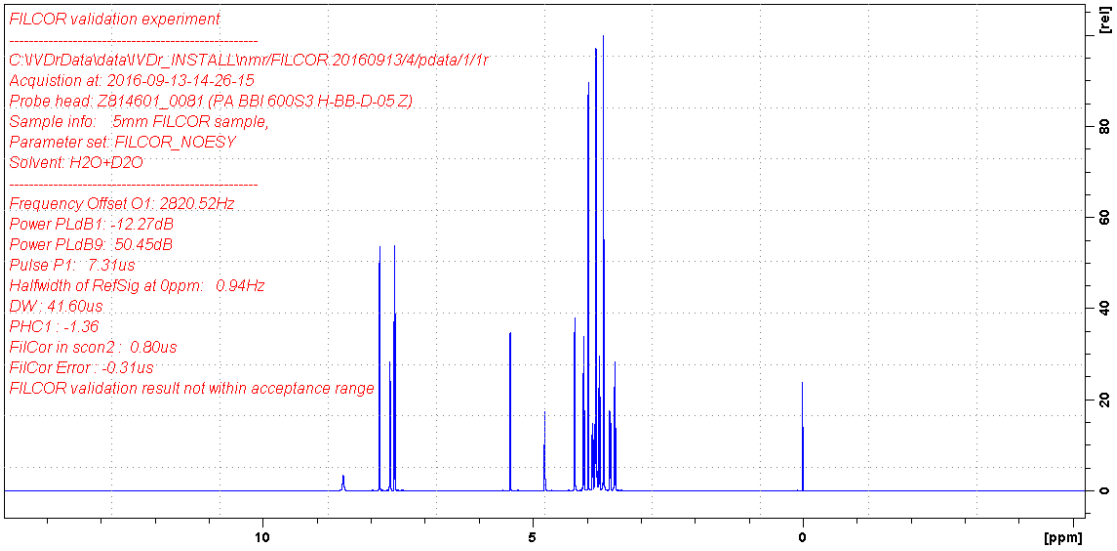


Figure 4.14: FILCOR Validation Experiment

Note: FILCOR values are typically between 0.5 µs and 1.3 µs.

4.5 Control Charts

The Control Charts is a set of documents of the instrumental performance over time, which are automatically generated, for all the QC experiments.

Location: *C:\Bruker\IVDr\RefData\controlcharts*

- Control Charts are generated automatically in automation or with **xaup** using the following parameter sets:
  - MEOD_TEMPCAL_300K, *_310K, *_300K_3mm, *_310K_3mm
  - WSTEST
  - FILCOR_NOESY
  - PROF_****_NOESY and their 3 mm versions
  - QUANTREF600C_BA, *_UR, *_PS, _*CS, and their 3 mm versions
- PDF-reports with graphical presentation of the data in the Control Charts can be generated via TopSpin Main Bar **IVDr Menu** | **Reporting** | **Control Chart PDF**. Select the type of Control Chart via the drop-down menu and enter start and end dates in the correct format YYYY-MM-DD.
- Click **OK**.

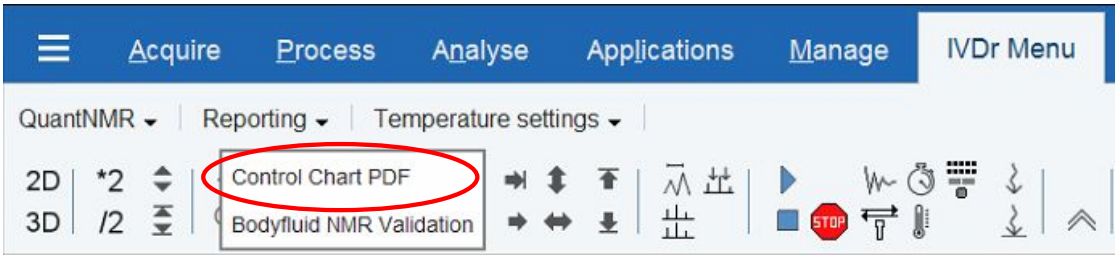


Figure 4.15: Reporting Control Chart PDF

Example:

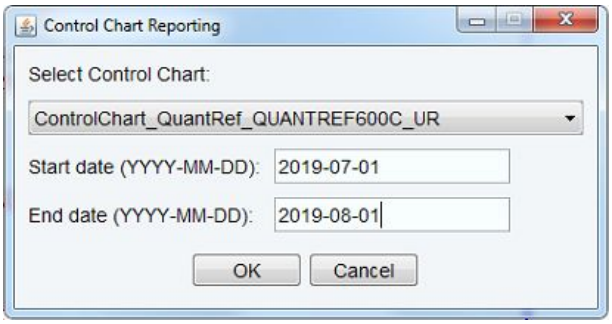


Figure 4.16: Reporting: An Example of Control Chart PDF Generating

- The report will be generated in the directory *C:\Bruker\IVDr\RefData\controlcharts\PDF*

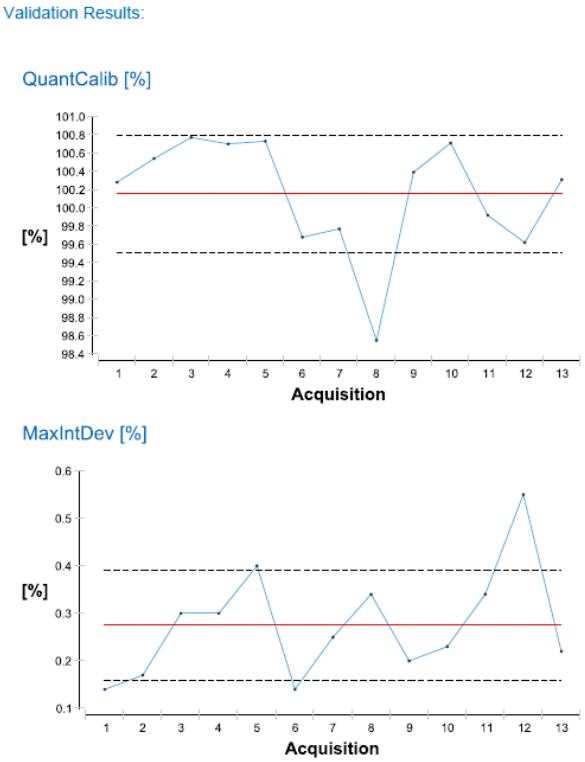


Figure 4.17: An Example of Control Chart for QuantRef

The report will be generated in the directory *C:\Bruker\IVDr\RefData\controlcharts\PDF*

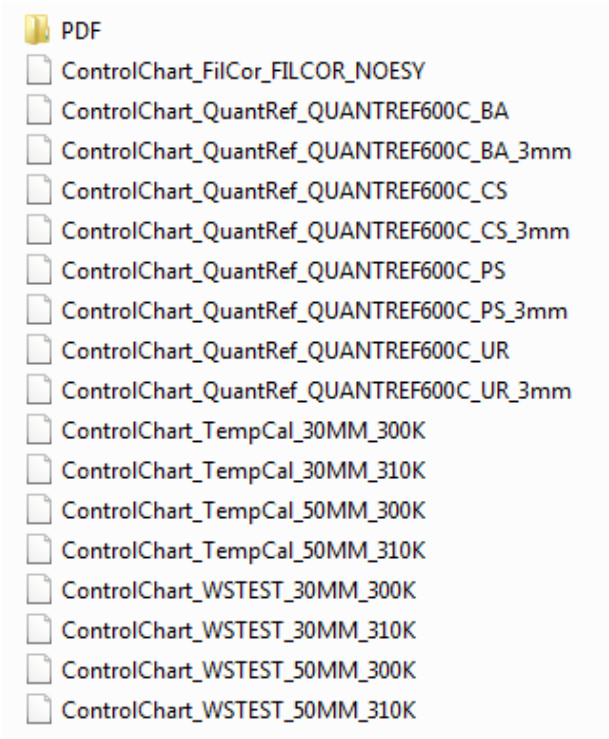


Figure 4.18: Control Charts List

5 NMR Measurements

The parameter sets should be optimized for the corresponding body fluid NMR measurements.

The optimization of NMR measurements in IVDr is an easy and fast procedure. All needed parameters are immediately available and base parameters already optimized. To ensure the NMR spectral quality, the regular check of the validity of the parameter settings is needed for O1-optimization* and solvent related shimming optimization. In IVDr methods, all the important parameters for acquisition and processing are fully controlled by au-programs.

***Note**: The B.I.Methods 2.0 also support automated parameter migration from O1 optimization experiments into all dependent parameter sets. This includes O1, P1 and all necessary power levels. Therefore, parameter set optimization and maintenance is simplified and can be done on demand during ICON automation as well. In principle, manual parameter set optimization and parameter migration is only needed if O1 optimization experiments are processed manually or O1 is optimized on-the-fly during other screening experiments.

Sample	Solvent	Temperature	Experiment
Urine test sample - 5 mm	Urine	300 K	PROF_URINE_NOESY, PROF_URINE_JRES
Urine test sample - 3 mm	Urine_3mm	300 K	PROF_URINE_NOESY_3mm, PROF_URINE_JRES_3mm
Plasma/Serum test sample - 5 mm	Plasma	310 K	PROF_PLASMA_NOESY, PLASMA_CPMG*, PLASMA_DIFF*, PLASMA_JRES* (* optional)
Plasma/Serum test sample - 3 mm	Plasma_3mm	310 K	PROF_PLASMA_NOESY_3mm, PLASMA_CPMG_3mm*, PLASMA_DIFF_3mm, PLASMA_JRES_3mm* (* optional)
CSF test sample - 5 mm	CSF	300 K	PROF_CSF_NOESY, PROF_CSF_JRES
CSF test sample - 3 mm	CSF_3mm	300 K	PROF_CSF_NOESY_3mm PROF_CSF_JRES_3mm
MeOH Extract test sample - 5 mm	MeOH_extract	300 K	PROF_MEOH_ZG30, PROF_MEOH_ZGPS, PROF_MEOH_NOESY, PROF_MEOH_JRES
MeOH Extract test sample - 3 mm	MeOH_extract_ 3mm	300 K	PROF_MEOH_ZG30_3mm, PROF_MEOH_ZGPS_3mm, PROF_MEOH_NOESY_3mm, PROF_MEOH_JRES_3mm

Table 5.1: Overview of NMR Measurements

5.1 Urine

5.1.1 Urine in 5 mm NMR Tube

Sample: Urine test sample – 5 mm.

Create data set *C:\IVDrData\data\<project name>\nmr\URINE.<date>*

EXPNO 1: Pulse, O1, Basic Shim

- Set the TE to 300 K using the temperature correction.
- Enter **rpar PROF_URINE_ZGPR_O1 all**.
- Enter **atma**.
- Enter **lock Urine**.
- Enter **rsh Urine**.
- Enter **topshim**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

Note: This experiment covers automatically the migration of O1, P1, PLdB1, and PLdB9 from EXPNO 1 to all dependent PROF_URINE_* experiments. It should run regularly to check O1 urine measurements.

EXPNO 2: Optimize 1D NOESY

- Enter **rpar PROF_URINE_NOESY all**.
- Enter **getprofpars**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- If all is ok – update the parameter set: **ivdr_wpar**.

Note: Manual processing must NOT be applied. Quality parameters include residual water linewidth and intensity, absolutely flat baseline and perfect phase, and a linewidth measured at TSP less than 1.3 Hz (including the standard LB value of 0.3 Hz into this calculation).

- If the spectral quality is good, write shim: **wsh Urine**:

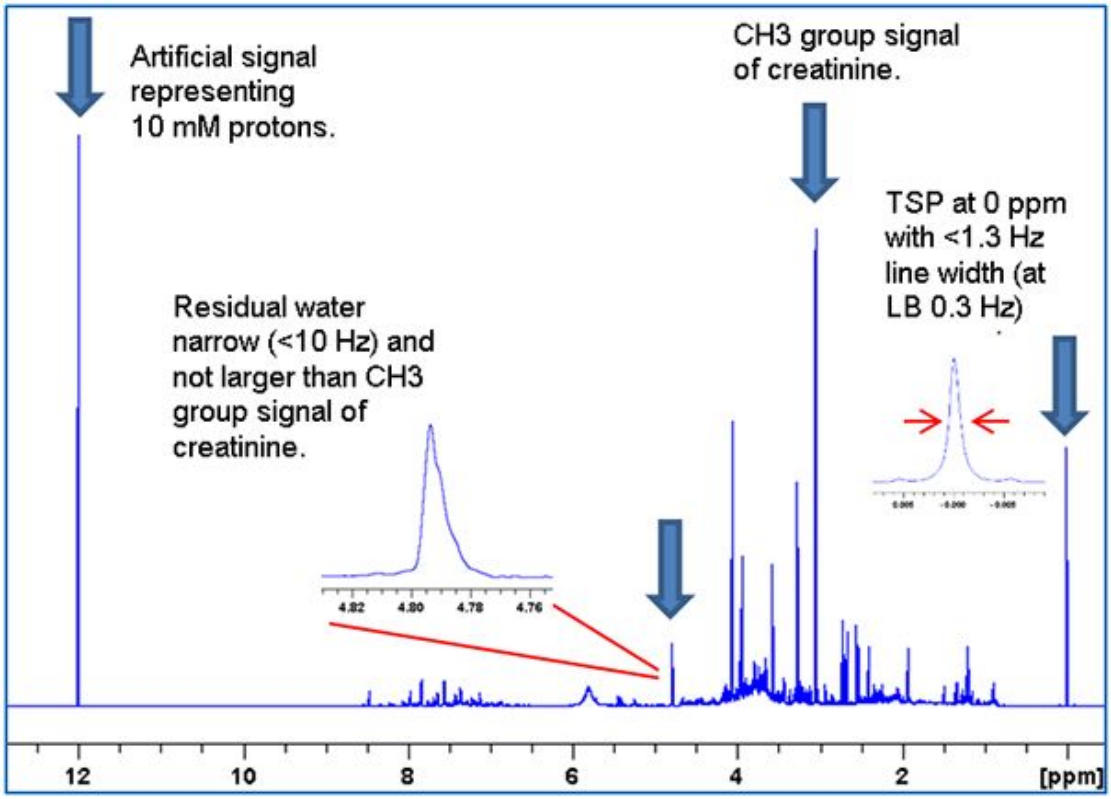


Figure 5.1: EXPNO 2: Optimize 1D NOESY of a Urine

EXPNO 3: Optimize 2D JRES

- Enter **rpar PROF_URINE_JRES all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

5.1.2 Urine in 3 mm NMR Tube

Sample: Urine test sample – 3 mm

Create a data set *C:\IVDrData\data\<project name>\nmr\URINE_3mm.<date>*

EXPNO 1: Pulse, O1, Basic Shim

- Set the TE to 300 K using the temperature correction.
- Enter **rpar PROF_URINE_ZGPR_O1_3mm all**.
- Enter **atma**.
- Enter **lock Urine_3mm**.
- Enter **rsh Urine_3mm**.
- Enter **topshim**.
- Enter **wsh Urine_3mm**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

Note: This experiment covers automatically the migration of O1, P1, PLdB1, and PLdB9 from EXPNO 1 to all dependent PROF_URINE_*_3mm experiments. It should run regularly to check O1 urine measurements.

EXPNO 2: Optimize 1D NOESY

- Enter **rpar PROF_URINE_NOESY_3mm all**.
- Enter **getprofpars**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.
- If the spectral quality is good (the same as for 5 mm), write shim: **wsh Urine _3mm**.

EXPNO 3: Optimize 2D JRES

- Enter **rpar PROF_URINE_JRES_3mm all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

5.2 Plasma

5.2.1 Plasma in 5 mm NMR Tube

Sample: Plasma test sample – 5 mm.

Create a data set *C:\IVDrData\data\<project name>\nmr\PLASMA.<date>*

EXPNO 1: Pulse, O1, Basic Shim

- Set the TE to 310 K using the temperature correction. Wait at least 5 minutes after the target temperature is reached.
- Enter **rpar PROF_ PLASMA _ZGPR_O1 all**.
- Enter **atma**.
- Enter **lock Plasma**.
- Enter **rsh Plasma**.
- Enter **topshim**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.
- Write the standard shim: **wsh Plasma**.

Note: This experiment covers automatically the migration of O1, P1, PLdB1, and PLdB9 from EXPNO 1 to all dependent PROF_PLASMA_* experiments. It should run regularly to check O1 urine measurements.

EXPNO 2: Optimize 1D NOESY

- Enter **rpar PROF_PLASMA_NOESY all**.
- Enter **getprofpars**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

  Note: Manual processing must NOT be applied. Quality parameters include residual water linewidth and intensity, absolutely flat baseline and perfect phase, and a linewidth measured at the Alanine doublet not exceeding 1.3 Hz (including the standard LB value of 0.3 Hz into this calculation).

- If the spectral quality is good, write shim: **wsh Plasma**:

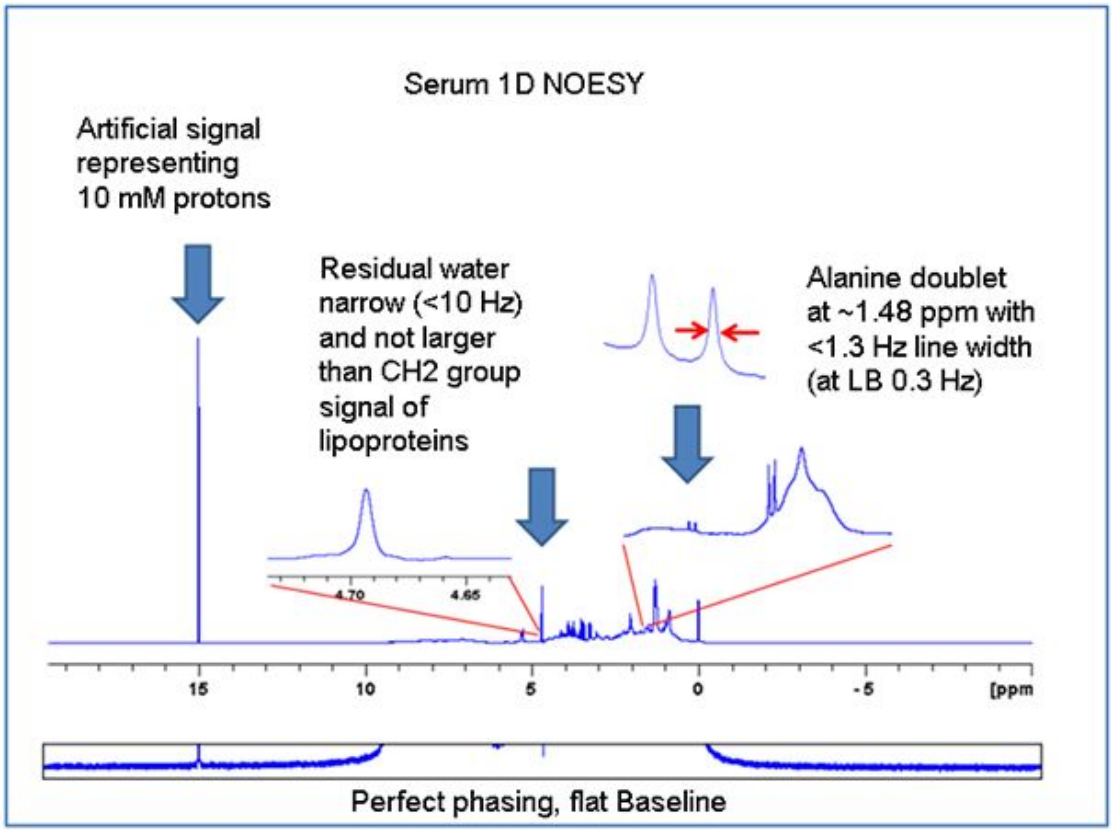


Figure 5.2: Optimize 1D NOESY of a Serum

EXPNO 3: Optimize 2D JRES

- Enter **rpar PROF_PLASMA_JRES all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

EXPNO 4: Optimize CPMG

- Enter **rpar PROF_PLASMA_CPMG all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

EXPNO 5: Optimize DIFF

- Enter **rpar PROF_PLASMA_DIFF all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

5.2.2 Plasma in 3 mm NMR Tube

Sample: Plasma test sample – 3 mm.

Create a data set *C:\IVDrData\data\<project name>\nmr\PLASMA_3mm.<date>*

EXPNO 1: Pulse, O1, Basic Shim

- Set the TE to 310 K using the temperature-correction. Wait at least 5 minutes after the target temperature is reached.
- Enter **rpar PROF_ PLASMA _ZGPR_O1_3mm all**.
- Enter **atma**.
- Enter **lock Plasma_3mm**.
- Enter **rsh Plasma_3mm**.
- Enter **topshim**.
- Enter **xaua**.
- When the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

Note: This experiment covers automatically the migration of O1, P1, PLdB1, and PLdB9 from EXPNO 1 to all dependent PROF_PLASMA_*_3mm experiments. It should run regularly to check O1 urine measurements.

EXPNO 2: Optimize 1D NOESY

- Enter **rpar PROF_PLASMA_NOESY_3mm all**.
- Enter **getprofpars**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.
- If the spectral quality is good (the same as for 5 mm), write shim: **wsh Plasma_3mm**.

EXPNO 3: Optimize 2D JRES

- Enter **rpar PROF_PLASMA_JRES_3mm all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

EXPNO 4: Optimize CPMG

- Enter **rpar PROF_PLASMA_CPMG_3mm all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

EXPNO 5: Optimize DIFF

- Enter **rpar PROF_PLASMA_DIFF_3mm all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

5.3 CSF

5.3.1 CSF in 5 mm NMR Tube

Sample: CSF test sample – 5 mm.

Create data set *C:\IVDrData\data\<project name>\nmr\CSF.<date>*

EXPNO 1: Pulse, O1, Basic Shim

- Set the TE to 300K using the temperature correction.
- Enter **rpar PROF_CSF_ZGPR_O1 all**.
- Enter **atma.**
- Enter **lock CSF**.
- Enter **rsh CSF**.
- Enter **topshim**.
- Enter **wsh CSF**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

Note: This experiment covers automatically the migration of O1, P1, PLdB1, and PLdB9 from EXPNO 1 to all dependent PROF_CSF_* experiments. It should run regularly to check O1 urine measurements.

EXPNO 2: Optimize 1D NOESY

- Enter **rpar PROF_ CSF _NOESY all**.
- Enter **getprofpars**.
- Enter **xaua**.
- When the experiment is finished, enter **xaup**.
- If all is ok – update the parameter set: **ivdr_wpar**.

  Note: Manual processing must NOT be applied. Quality parameters include residual water linewidth and intensity, absolutely flat baseline and perfect phase, and a linewidth measured at the Alanine doublet not exceeding 1.3 Hz or TSP less than 1.3 Hz (including the standard LB value of 0.3 Hz into this calculation).
- If the spectral quality is good, write shim: **wsh CSF**.

EXPNO 3: Optimize 2D JRES

- Enter **rpar PROF_ CSF _JRES all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

5.3.2 CSF in 3 mm NMR Tube

Sample: CSF test sample – 3 mm.

Create data set *C:\IVDrData\data\<project name>\nmr\CSF_3mm.<date>*

EXPNO 1: Pulse, O1, Basic Shim

- Set the TE to 300 K using the temperature correction.
- Enter **rpar PROF_CSF_ZGPR_O1_3mm all**.
- Enter **atma**.
- Enter **lock CSF_3mm**.
- Enter **rsh CSF_3mm**.
- Enter **topshim**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

Note: This experiment covers automatically the migration of O1, P1, PLdB1, and PLdB9 from EXPNO 1 to all dependent PROF_CSF_*_3mm experiments. It should run regularly to check O1 Urine measurements.

EXPNO 2: Optimize 1D NOESY

- Enter **rpar PROF_CSF_NOESY_3mm all**.
- Enter **getprofpars**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- If all is ok – Update the parameter set: **ivdr_wpar**.
- If the spectral quality is good (the same as for 5 mm), write shim: **wsh CSF_3mm**

EXPNO 3: Optimize 2D JRES

- Enter **rpar PROF_CSF_JRES_3mm all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

Note: Two parameters in CSF measurement have been modified compared to B.I.Method 1.0:

- D1 has been changed from 4 s to 10 µs
- PLdB9 (the pre-saturation power) has been changed from 50 Hz to 25 Hz.

5.4 MeOH Extract

5.4.1 MeOH Extract in 5 mm NMR Tube

Sample: MeOH_extract test sample – 5 mm

Create a data set *C:\IVDrData\data\<project name>\nmr\ MeOH_extract.<date>*

EXPNO 1: Navigator experiment 1) Two-fold solvent suppression shape

- Set the TE to 300 K using the temperature correction. Wait at least 5 minutes after the target temperature is reached.
- Enter **rpar PROF_MEOH_ZG30 all**.
- Enter **atma**.
- Enter **lock MeOD_extract**.
- Enter **rsh MeOD_extract**.
- Enter **topshim**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

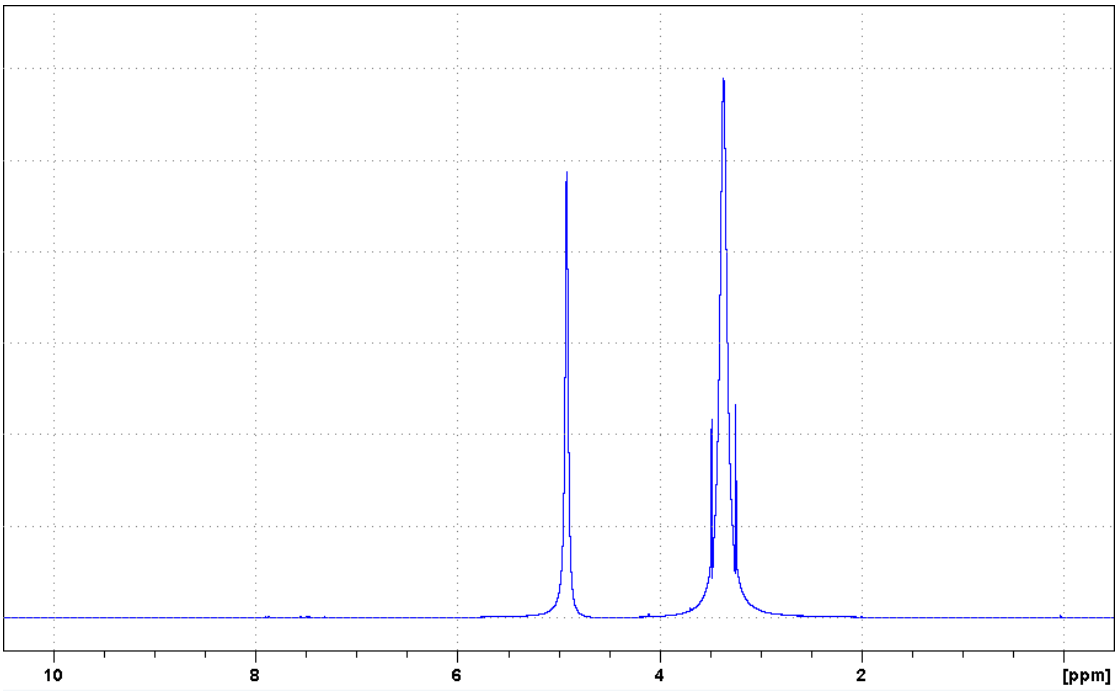


Figure 5.3: An Example of an MeOH_Extract PROF_MEOH_ZG30 Experiment

EXPNO 2: Navigator experiment 2) Pulse, O1, four-fold solvent suppression shape

- Enter **rpar PROF_MEOH_ZGPS all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

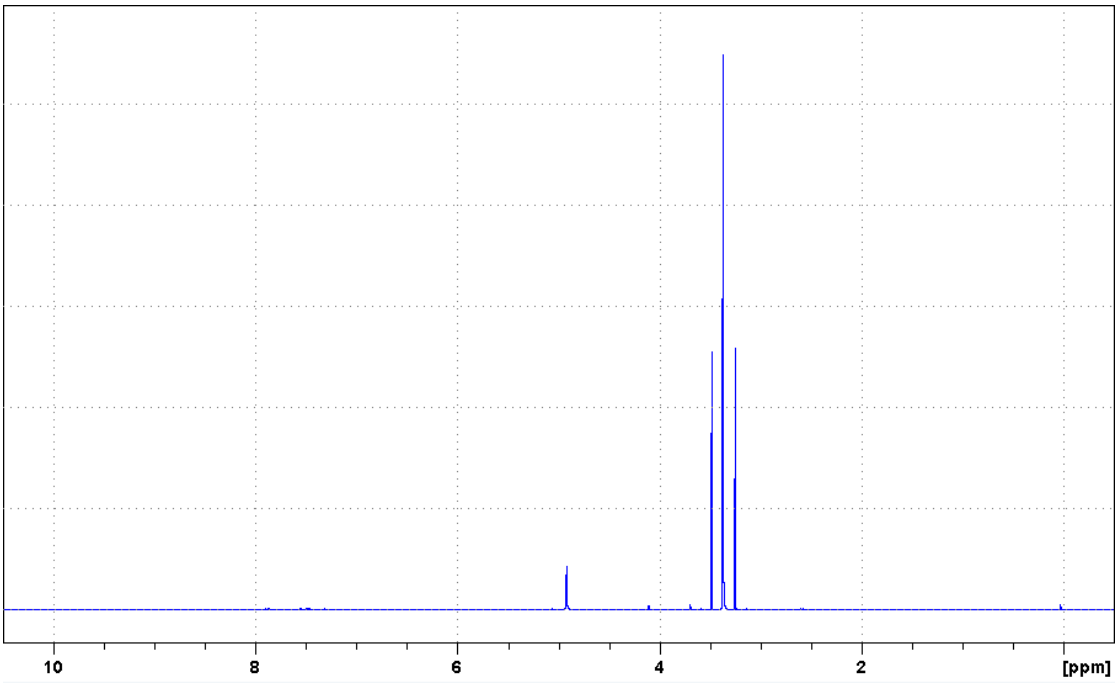


Figure 5.4: An Example of an MeOH_Extract PROF_MEOH_ZGPS Experiment

EXPNO 3: Optimize 1D NOESY

- Enter **rpar PROF_ MEOH_NOESY all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- If all is ok, update parameter set: **ivdr_wpar**.
- If the spectral quality is good, write shim: **wsh MeOH_extract**.

Note: **Manual processing must NOT be applied**. Quality parameters include residual water linewidth and intensity, absolutely flat baseline and perfect phase, and a linewidth measured at the Alanine doublet not exceeding 1.3 Hz or TSP less than 1.3 Hz (including the standard LB value of 0.3 Hz into this calculation).

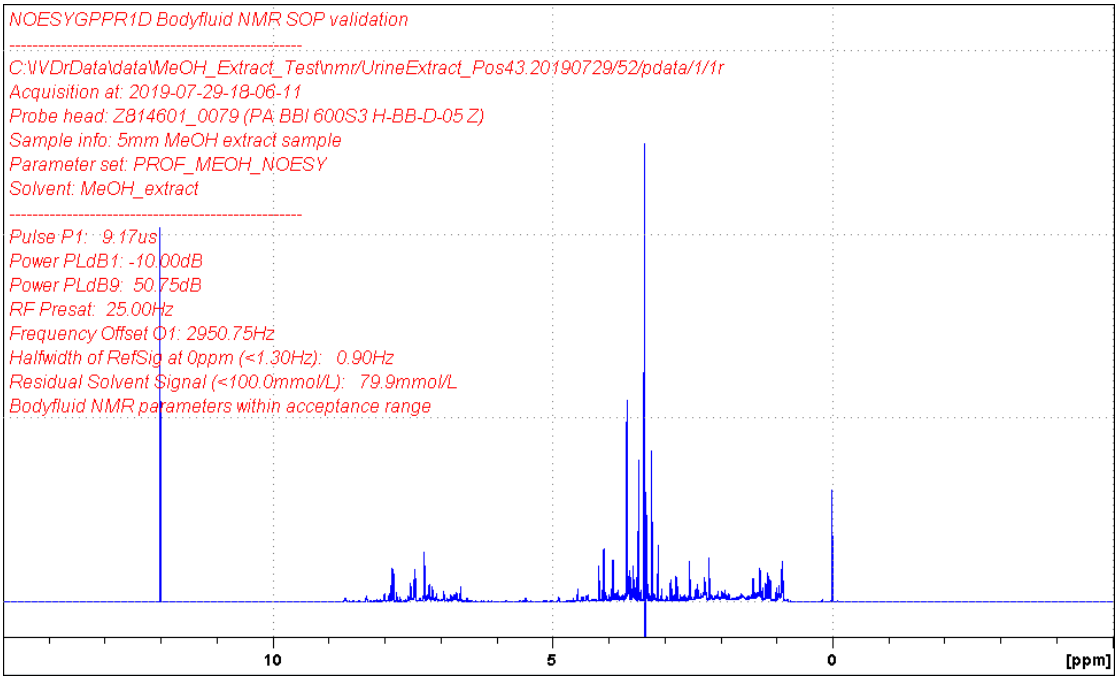


Figure 5.5: An Example of an MeOH_Extract PROF_MEOH_NOESY Experiment

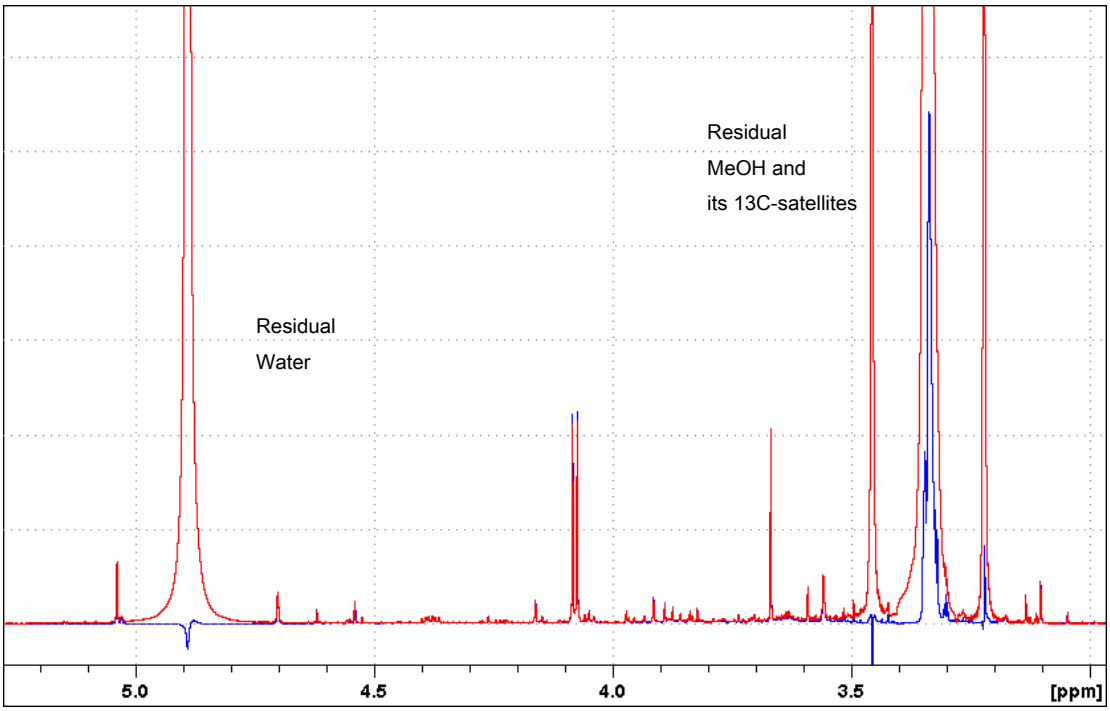


Figure 5.6: Comparison of PROF_MEOH_NOESY and PROF_MEOH_ZGPS with Multiple-Solvent Suppression

EXPNO 4: Optimize 2D JRES

- Enter **rpar PROF_MEOH_JRES all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

5.4.2 MeOH Extract in 3 mm NMR Tube

Sample: MeOH_extract test sample – 3 mm

Create a data set *C:\IVDrData\data\<project name>\nmr\ MeOH_extract_3mm.<date>*

EXPNO 1: Navigator experiment 1) Two-fold solvent suppression shape

- Set the TE to 300 K using the temperature correction. Wait at least 5 minutes after the target temperature is reached.
- Enter **rpar PROF_MEOH_ZG30_3mm all**.
- Enter **atma**.
- Enter **lock MeOH_extract_3mm**.
- Enter **rsh MeOH_extract_3mm**.
- Enter **topshim**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

EXPNO 2: Navigator experiment 2) Pulse, O1, four-fold solvent suppression shape

- Enter **rpar PROF_MEOH_ZGPS_3mm all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- Update the parameter set: **ivdr_wpar**.

EXPNO 3: Optimize 1D NOESY

- Enter **rpar PROF_MEOH_NOESY_3mm all**.
- Enter **xaua**.
- After the experiment is finished, enter **xaup**.
- If all is ok, update the parameter set: **ivdr_wpar**.
- If the spectral quality is good (the same as for 5 mm), write shim: **wsh MeOH_extract_3mm**.

EXPNO 4: Optimize 2D JRES

- Enter **rpar PROF_MEOH_JRES_3mm all**.
- Enter **xaua**.
- After experiment finished, enter **xaup**.
- Update parameter set: **ivdr_wpar**.

5.4.3 Body Fluid NMR Validation

The NMR quality of all PROF_****_NOESY experiments (and their 3 mm versions) is monitored during the screening workflow in order to verify optimal NMR parameter settings and observe the short and long-term behavior of the instrument. Control Charts will be generated in the directory *C:\Bruker\IVDr\RefData\controlcharts* which can be submitted to the Control Chart PDF report generation tool. In addition, XML-reports are generated in the individual dataset directories:

*C:\IVDrData\data\<MyProject>\nmr\<MySample>\10\pdata\1\<PROF_****_NOESY>.xml*

They can be used for result table generation via the IVDr DataBrowser or any third-party software. Title and PDF reports for individual spectra can be generated via TopSpin Main Bar **IVDr Menu** | **Reporting** | **Bodyfluid NMR Validation** or entering **ivdr_bodyfluid_validation full**.

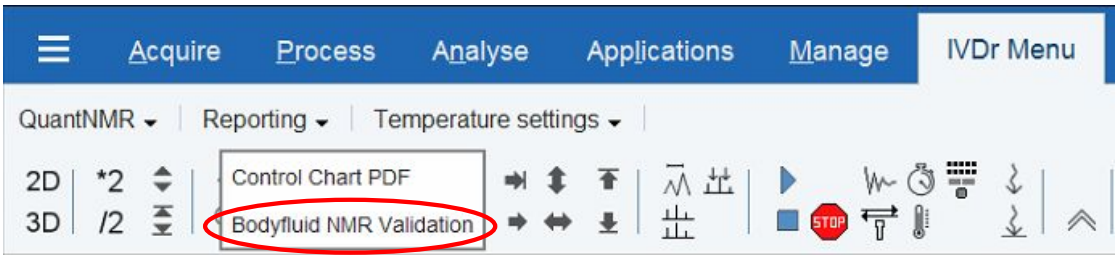


Figure 5.7: Reporting: Body Fluid NMR Validation

6 Troubleshooting

Temperature calibration

The temperature corrections, which have been originally optimized by the Bruker engineer, are maintained via TopSpin Main Bar **IVDr Menu** | **Temperature Settings**.

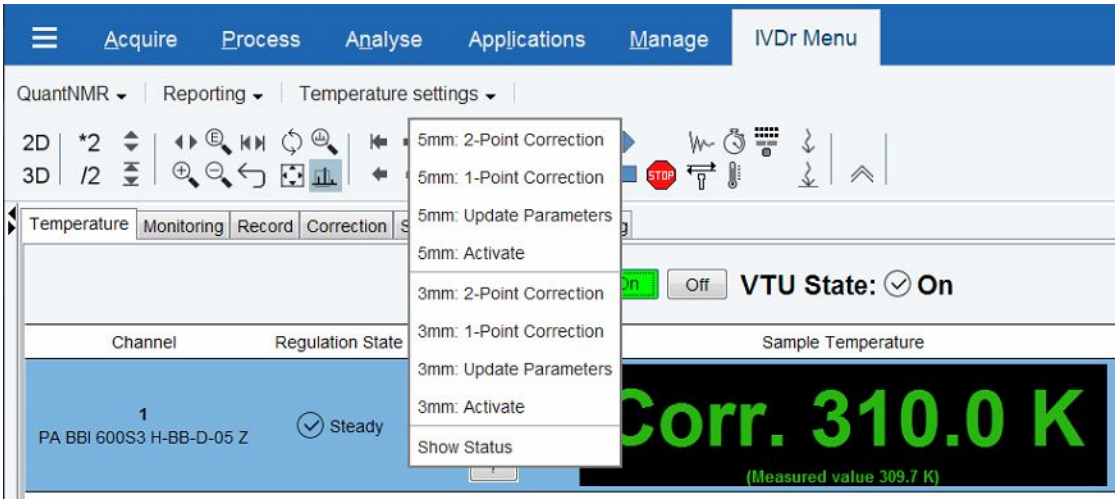


Figure 6.1: Reporting: Temperature Settings

Show Status can be used to print the current combination of self tune and temperature correction file to the screen and thus identify the current mode of operation (N/A indicates that the parameters are not yet set):

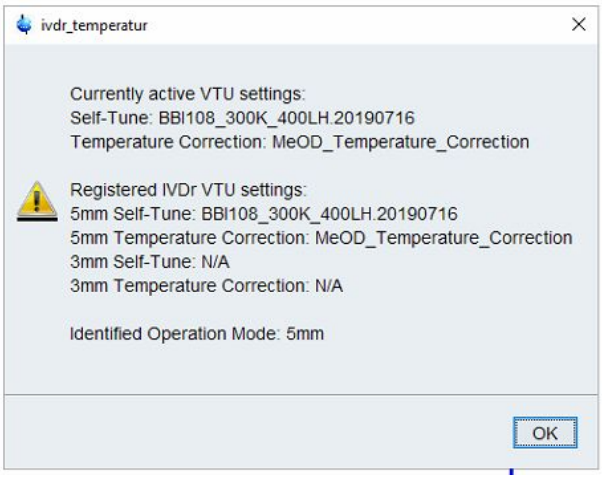


Figure 6.2: The Current Status of the Temperature Self-Tune and Correction

If the B.I. Methods are configured and optimized to work for both tube diameters, the mode of operation can be easily switched using the **Activate** buttons for the desired diameter.

If a temperature correction repeatedly yields failed temperature validations, it can be locally modified in a semi-automated fashion for the currently set temperature using the **1-point correction** buttons for the desired diameter.

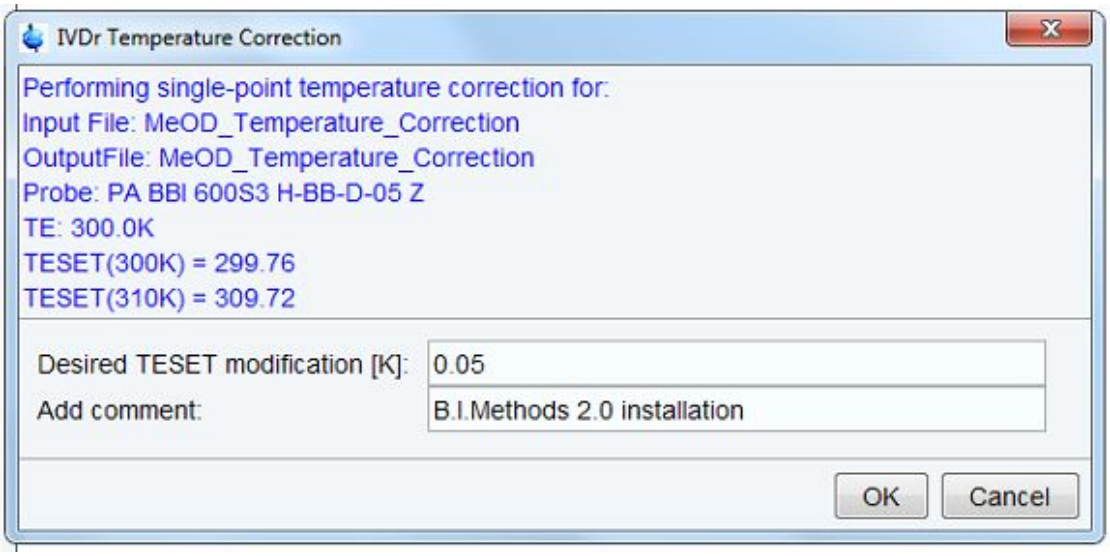


Figure 6.3: 1-Point Temperature Correction

Here, a modification to a single TESET for a given temperature can be given to account for an observed deviation of the ideal sample temperature. The temperature correction file is modified such, that only a local correction is applied. This is done such, that the resulting change of the temperature correction function does not influence the correction for the respective other body fluid screening temperature. That means for example, doing a local modification of the correction function by 0.05 K at 300 K will not influence the correction at 310 K.

If the change in TESET is too drastic, a 2-point correction procedure will be suggested. It is activated by the **2-point correction** buttons for the desired diameter.

If manual determination of the correction function is wanted, the **Slope** and **Offset** values need to be updated manually in the Edit temperature correction window (taking the equation TESET = slope * 300/310 + Offset). Activate Temperature correction. Now *Corr*. will be seen in EDTE window or in Acquisition Status Bar. Manual changes need to be introduced to the IVDr temperature configuration file via the **Update Parameters** buttons for the desired diameter.

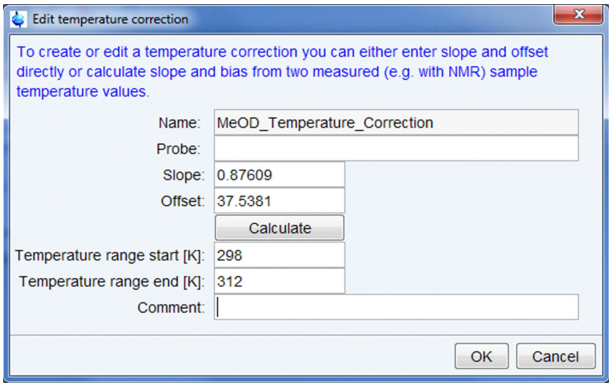


Figure 6.4: Edit Temperature Correction

Figure 6.5: Acquisition Status Bar/Sample Temperature

QuantRefC

If the *Quantitative calibration* is NOT within the acceptance range:

- Check the shim performance (for example, the line shape of TSP).
- Check the temperature.
- Check the solvent and the experiment.

Re-calibration of QuantRefC is performed ONLY when all the above suspects are excluded. To perfom a calibration, first follow the instructions in section *QuantRefC Calibration and Validation [38]* and then click on the TopSpin Main Bar **IVDr Menu** | **QuantNMR** | **Calibration**.

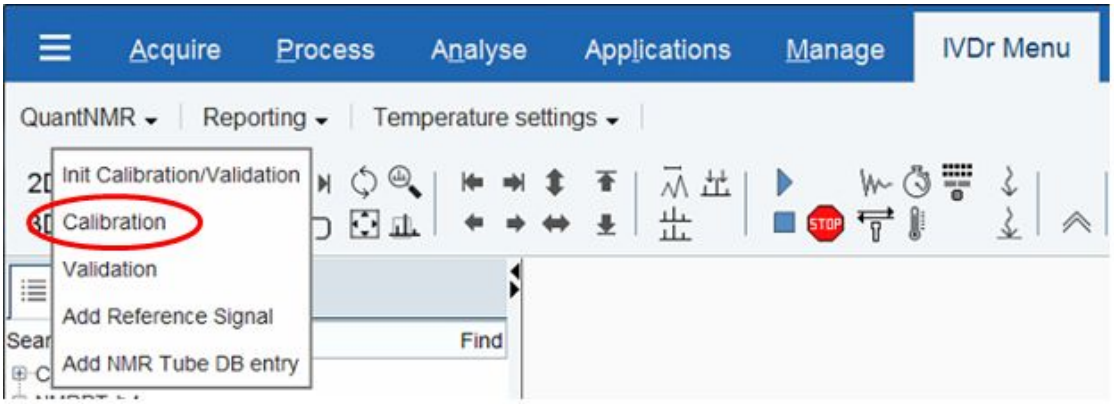


Figure 6.6: QuantNMR Calibration

If the *Quantitative calibration* is frequently NOT within the acceptance range, contact your local Bruker service.

SampleJet

Cooling status at racks: Regularly check the thermometers on the SampleJet.

IconNMR Configuration by SampleTrack

- Redo **get key** when communication has problems.

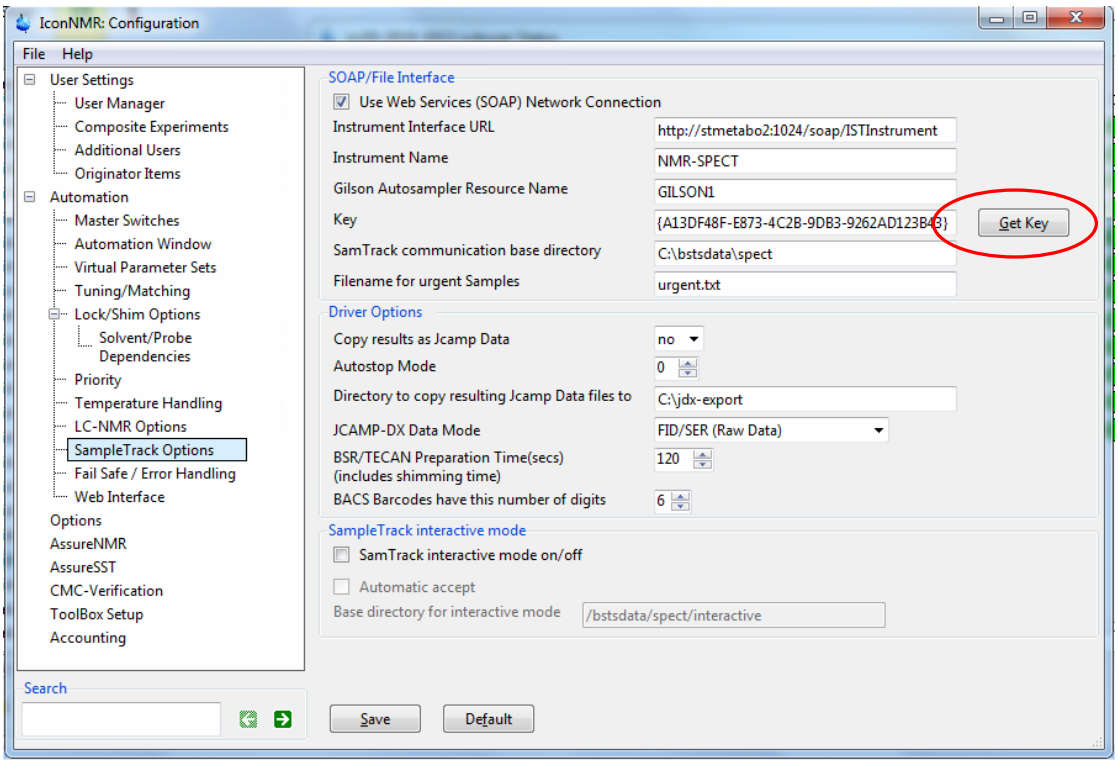


Figure 6.7: get key in IconNMR Configuration

- Load the configuration file and import the configuration which was optimized and saved by the Bruker engineer.

SampleTrack

If SampleTrack has a communication problem:

- Terminate and restart the Communicator, Webserver, and the SampleTrack client.
- Restart the SampleTrack host computer.

topshim or topshim tunea

This depends on the individual spectrometer optimization. Advice can be provided by the Bruker engineer or contact your Bruker service.

7 Contact

Manufacturer

Bruker BioSpin GmbH
Silberstreifen 4
D-76287 Rheinstetten
Germany

E-Mail: *nmr-support@bruker.com*
http://www.bruker.com
WEEE DE43181702

Bruker BioSpin Hotlines

Contact our Bruker BioSpin service centers.

Bruker BioSpin provides 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 service center or hotline you wish to contact from our list available at:

https://www.bruker.com/service/information-communication/helpdesk.html

Contact

List of Figures

List of Figures

List of Tables

List of Tables

Bruker Corporation

info@bruker.com
www.bruker.com

Order No: H161804