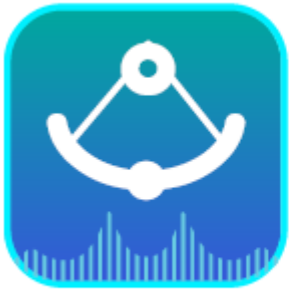


SMILEQ plugin

CONTENTS:

SMILEQ plugin

The SMILEQ plugin enables quantitative NMR analysis within JASON. It allows you to obtain the results of purity and molar concentration analysis of the analytes in a sample using an internal standard or external standard method. The plugin is compatible with JASON ver.3.2 or higher, and requires an additional SMILEQ license alongside the main JASON application. Also, the automatic work flow that is from measurement and generating report will realized to link with JEOL NMR software Delta (after ver. 6.1).

In Chapter 1, how to activate the SMILEQ plugin and each tool in SMILEQ plugin will be explained.

In Chapter 2, to be described about SMILEQ widgetl that set the analysis condition.

In Chapter 3, to be described about the preparation for qNMR analysis

In Chapter 4, to show the actual procedure about qNMR analysis that is realized by SMILEQ.

Finally, In Chapter 5, how to create the various report will be explained.

This document is intended to give the user an overview of the SMILEQ plugin for JASON. It is assumed that JASON is running under the Windows operating system.

1 Activate SMILEQ plugin

To get started with SMILEQ, you need to activate the plugin.

Activation is from starting to install and get the license for the plugin. Please have your SMILEQ plugin license key before the activation proceeding.

If you don't have license for SMILEQ, please start the procedure from the below process.

1. Copy the Computer ID by clicking on the 'Copy' box.
2. Visit https://www.jeoljason.com/ and select either a SMILEQ trial (https://www.jeoljason.com/try-our-software/) or purchase a SMILEQ plan (Academia: https://www.jeoljason.com/academia-plans/ or Industry https://www.jeoljason.com/industry-plans/)
3. Enter the Computer ID in the form on the trial page for SMILEQ trial or in the checkout form for a SMILEQ purchase.
4. You will receive an email, please verify your email address. Once this is completed, your License key will be emailed to you.

1.1 Start Plugin Manager

Select the Plugin Manager from the Main Menu, as shown in Figure 1.

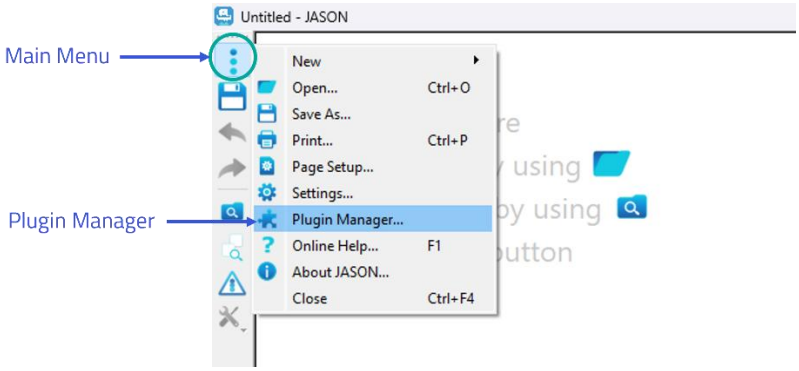


Figure 1: Start Plugin Manager

1.2 Install SMILEQ Plugin

Check the SMILEQ Plugin checkbox and click the OK button, as shown in Figure 2.

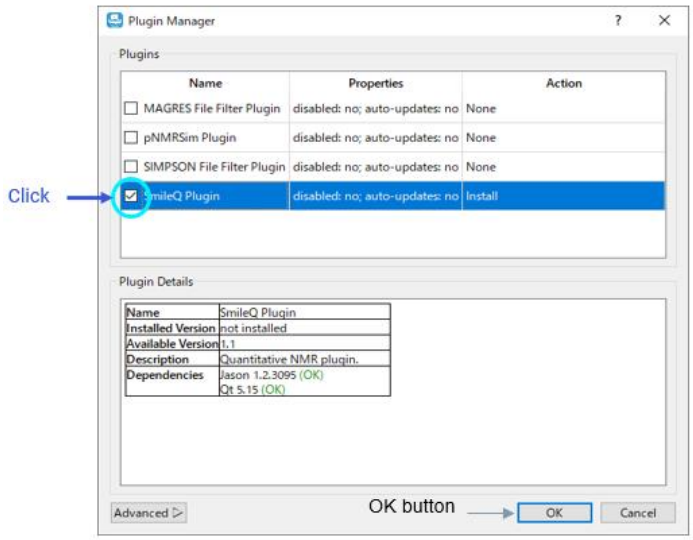


Figure 2: Start Plugin Manager

1.3 Accept Restart Dialog

After pressing the OK button, the dialog box shown below will appear. Click OK and then restart JASON.

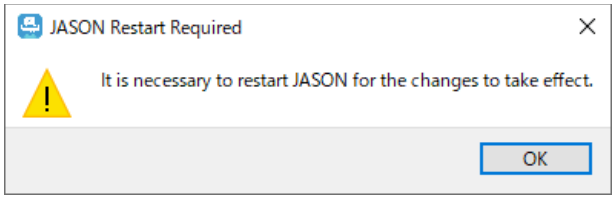


Figure 3: Restart Dialog

When you update to a new version of JASON, if necessary, you might need to turn the SMILEQ plug-in off in the plugin manager, restart the JASON and then turn the SMILEQ flag on again in the plugin manager.

1.4 Activate SMILEQ License

If you have not previously activated the SMILEQ plugin, the first time JASON is opened after installing the SMILEQ plugin, the JASON Plugin Licenses Dialog window will open, as shown in Figure 4.

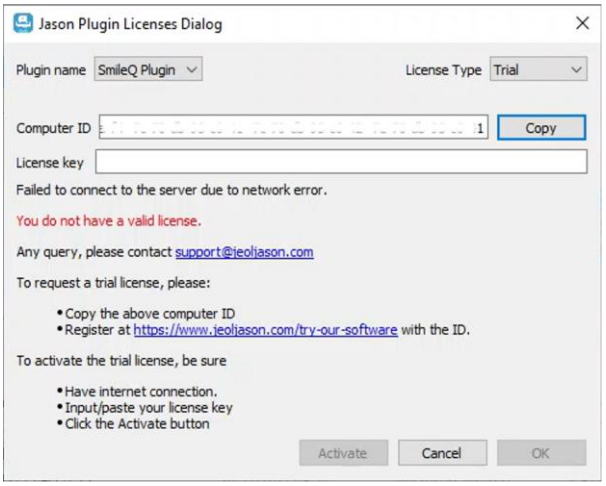
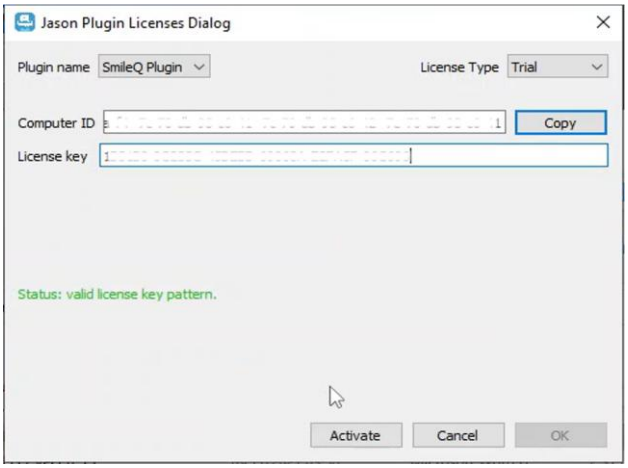


Figure 4: JASON Plugin Licenses Dialog (before the setting)

1. Copy and paste your SMILEQ license key into the license key box.
2. Select the Plugin name (SMILEQ Plugin) and the License Type (Commercial or Trial).
3. If you select Commercial for the License Type, select the appropriate item from the Company Type and Term pull-down menus. Finally, enter the license key in the box.



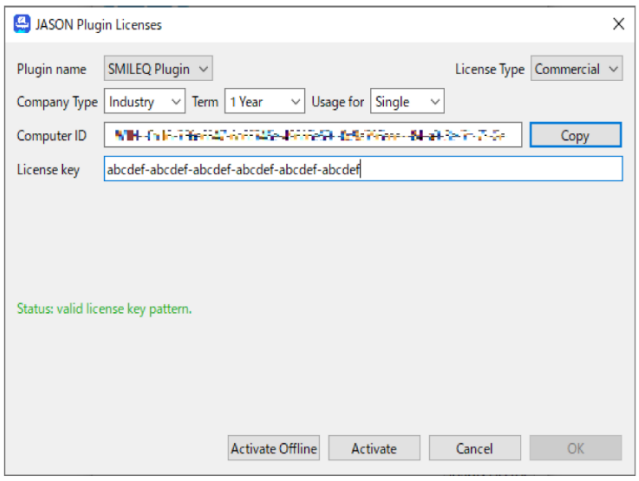


Figure 5: JASON Plugin License Dialog (after the setting)

- Copy and paste is recommended for the license key.
- If a valid license key is entered, [Activate] is activated.

4. Click the [Activate] button to activate the license key.

- If online activation is not possible, select 'Activate Offline' in the JASON Plug-in Licensing dialog, and follow the instructions on the next screen to create a 'Request Token'. Click the "Request Token" button and a file will be created, please send the following file (.dat format) found in C:\Users\<user name>\.jeollicense folder to support [support@jeoljason.com].

(Example email)

Subject: Offline Activation Request (SMILEQ)

Body: License Key and Request Token

- You will receive a 'Request Token' from JASON support team. Follow the instructions in the email to proceed with offline authentication

1.5 Re-open JASON

After successfully registering the SMILEQ plugin, the functionality can be accessed using one of the context panels on the left-hand edge of the frame.

Like the other NMR tool widgets (e.g., View and Processing), the SMILEQ panel requires NMR data to be loaded onto the canvas. The SMILEQ widget will appear automatically when appropriate data is selected.

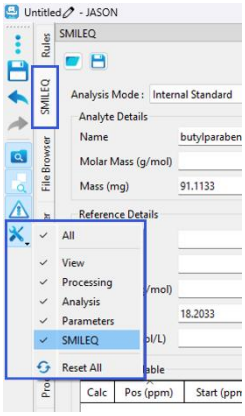


Figure 6: SMILEQ context menu and panel

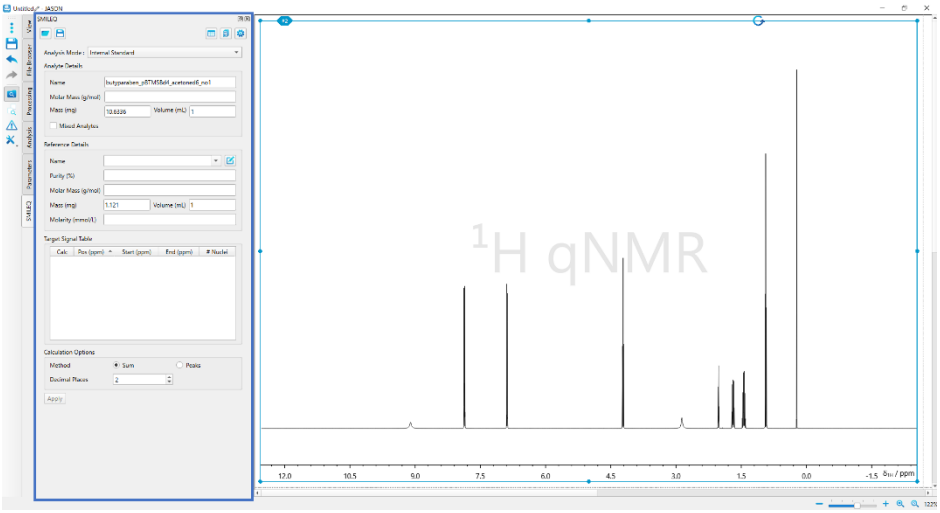


Figure 7: SMILEQ Widget

2 Overview of SMILEQ Widget

The SMILEQ widget is used to set the parameters for qNMR analysis, as is shown in *Figure* 8.

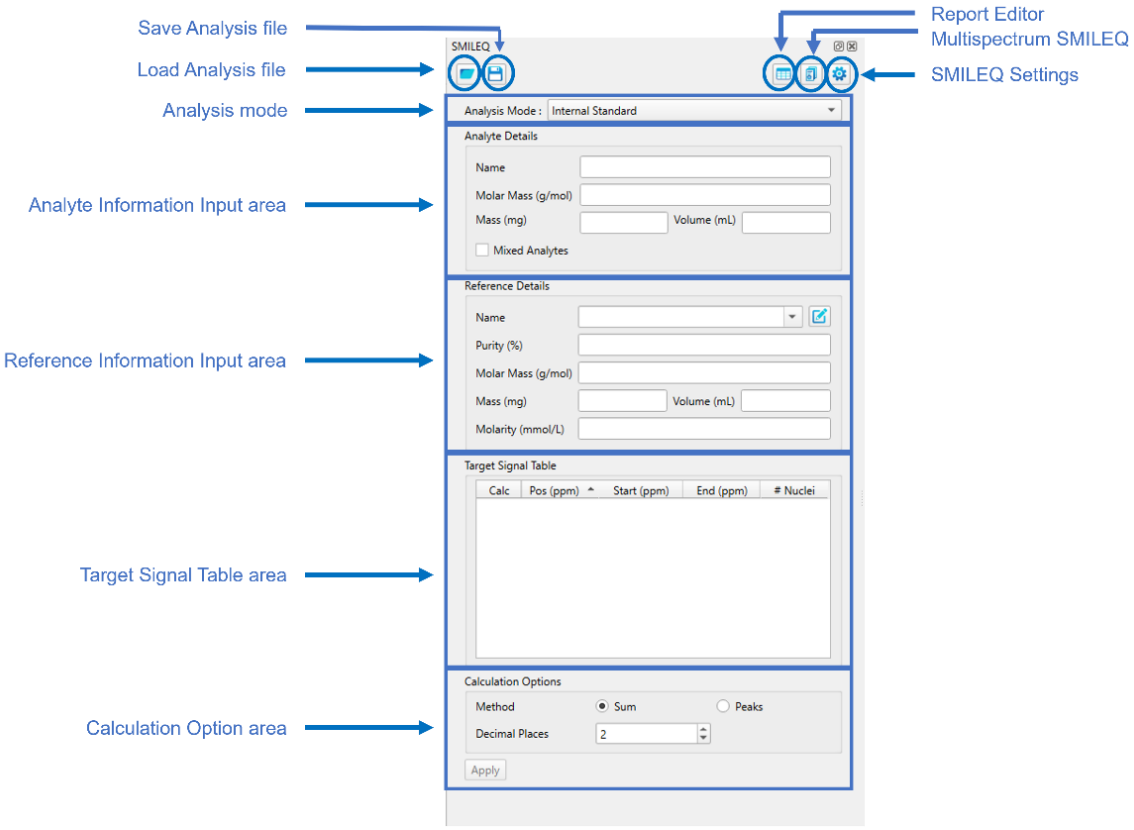


Figure 8: SMILEQ Widget

Analysis file Load/Save	Buttons to load/save analysis template files.
SMILEQ Setting	The SMILEQ settings tab allows the user to define the paths where various configurations files are stored, and adjust parameter mapping information. The details will be explained section 3.
Analysis Mode	Determines what information is entered into the SMILEQ panel depending on the type of analysis required.
Analyte Information Input Area	Area for defining the sample information required for quantitative analysis. This area is not shown for “External Standard – Reference” samples.
Reference Information Input Area	Area for defining the reference compound information required for quantitative analysis. This area is not shown for “External Standard – Analyte” samples.
Target Signal Table Area	Area for entering the number of resonances in the spectrum and whether they are should be used for quantitative analysis. These resonances can be defined as either Multiplets or Integrals. The multiplet and integral analysis tools available in JASON are used to generate the entries in this table.
MultiSpectrum SMILEQ	The MultiSpectrum button allows to perform the batch qNMR analysis across multiple analyte spectra.
Report Editor	The Report Editor button allows to perform generating various types report.
Mixed Analyte	To perform a mixture analysis, check the Mixed Analyte button.

2.1 Analysis Mode

The SMILEQ plug-in supports analysis using both the internal standard method and the external standard method.

In the analysis mode, select the mode appropriate for your analysis purpose from "Internal standard method", "External std - Analyte", or "External std - Reference". This must be set for each spectrum, so select the spectrum to be analyzed and choose the correct analysis mode.

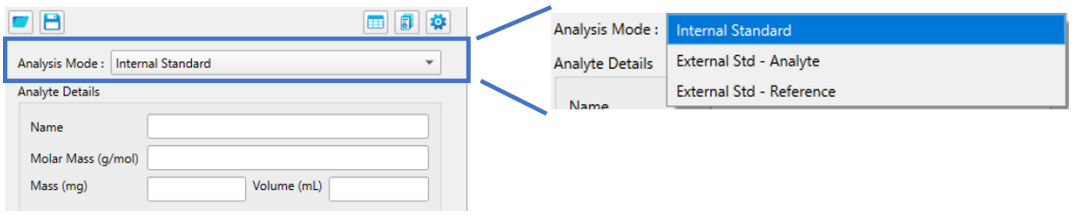


Figure 9 Analysis Mode

2.1.1 Internal Standard

Select the spectrum to be analyzed and set the analysis mode in the SMILEQ widget to "Internal Standard ", then you can set the analytical sample information, reference compound information, and analytical signal information in the SMILEQ widget.

2.1.1.1 Analyte Information Input Area

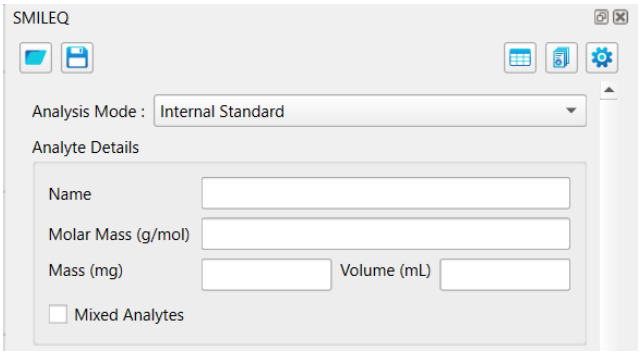


Figure 10: Setting of Analyte information Input Area

Name	Enter a sample identifier in the “Name” text field.
Molar Mass	Enter the molecular weight in the “Molar Mass” text field in units of g mol^{-1}.
Mass	Enter the weight of the sample in the “Mass” text field in units of mg.
Volume	Enter the volume of sample in the “Volume” text field in units of mL. This value is only required if molarity results are needed. The volume parameter entered here is the volume of solution in which the analyte sample was prepared, not the volume in the NMR tube.

The “**Name**”, “**Molar Mass**” and “**Mass**” or “**Volume**” fields must have values entered in order to perform the quantitative analysis. To obtain the molar concentration based on reference information, enter only the molar mass.
If the information is included in the NMR data (FID) that you read, it is possible to set these values automatically. Please refer to 3.2.3 Parameter Mapping.

2.1.1.2 Reference Information Input Area

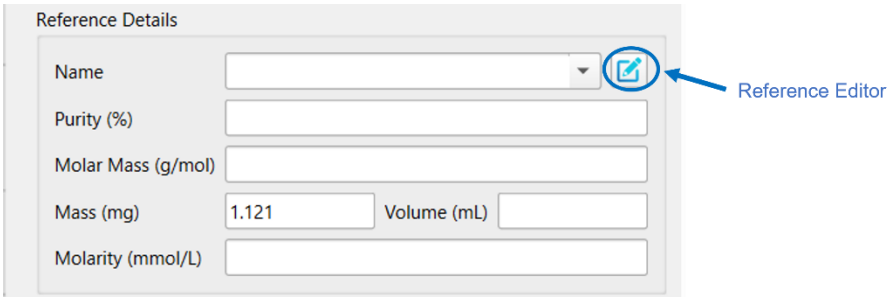


Figure 11: Setting of Reference Information Input Area

Name	Select a reference substance from the “Name” combo box. This drop-down list can be edited using the Reference Editor button. See section 2.2 Reference Editor for details. When a reference is selected, the values for purity (Purity (%)) of the reference compound and its molar mass (Molar Mass (g mol^{-1})) are automatically loaded.
Mass	Enter the weight value of the reference in the “Mass” text field in units of mg.
Volume	Enter the volume of sample in the “Volume” text field, if molarity results are required. this is the volume of sample prepared, not the volume of the NMR sample itself. For Internal Standard measurements this is the same volume as for the analyte, and will be populated automatically.

The **“Name”** and **“Mass”** fields must be completed in order to perform the analysis. To use the molar concentration, enter **“Molarity”** instead of **“Mass”** and **“Volume”**. If the information is included in the NMR data (FID) that you read, it is possible to set automatically. Please refer to 3.2.3 Parameter Mapping.

2.1.2 External Std - Analyte

Select the spectrum to be analyzed and set the analysis mode in the SMILEQ widget to "External Standard - Analyte", then you can set the analytical sample information, reference spectrum, and analytical signal information in the SMILEQ widget.

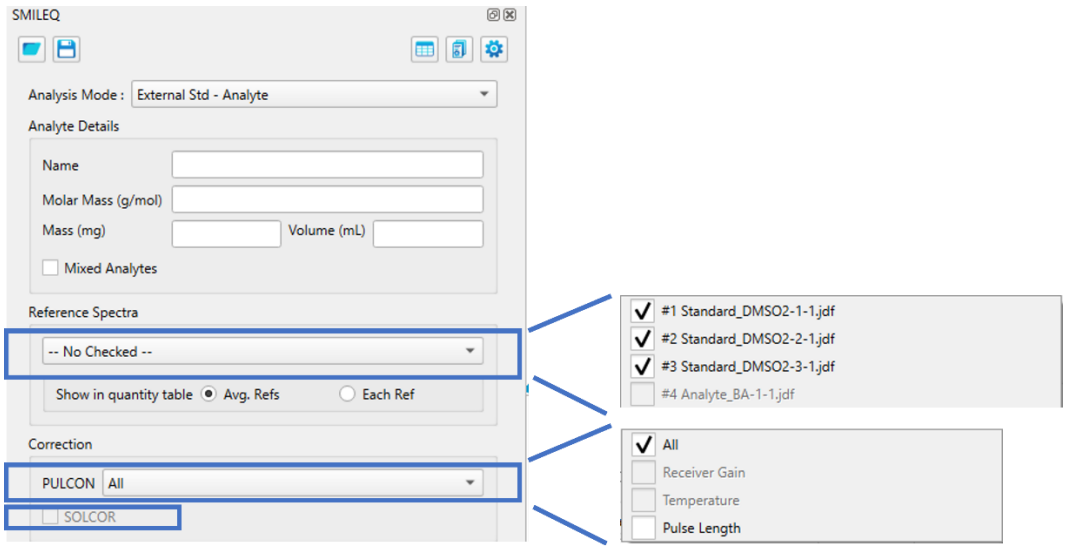


Figure 12: Setting of External Standard - Analyte information- Input Area

Name	Enter a sample identifier in the “Name” text field.
Molar Mass	Enter the molecular weight in the “Molar Mass” text field in units of g mol^{-1}.
Mass	Enter the weight of the sample in the “Mass” text field in units of mg.
Volume	Enter the volume of sample in the “Volume” text field in units of mL. This value is only required if molarity results are needed.
Reference Spectra	Select the spectrum to be used as the reference for the external standard method from the drop-down list. The data in the canvas is listed. If the data meets the reference conditions, the check box is enabled.
PULCON	Select the correction parameters to be used in PULCON from the drop-down list. If the parameters are different between the standard and the analyte, the check box for that parameter will be enabled.
SOLCOR	Enable correction by residual solvent signal. The checkbox is enabled only if the standard and analyte spectra each have a solvent signal defined (multiplet analysis, signal type set to solvent). This function is based on Ochoa *et al*. Magn. Reason. Chem. 62 (2024) 4-10.

The “**Name**”, “**Molar Mass**” and “**Mass**” or “**Volume**” fields must have values entered in order to perform the quantitative analysis.

2.1.3 External Std – Reference-

Select the spectrum to be analyzed and set the analysis mode in the SMILEQ widget to "External Std - Reference", then you can only set the reference substance information in the SMILEQ widget

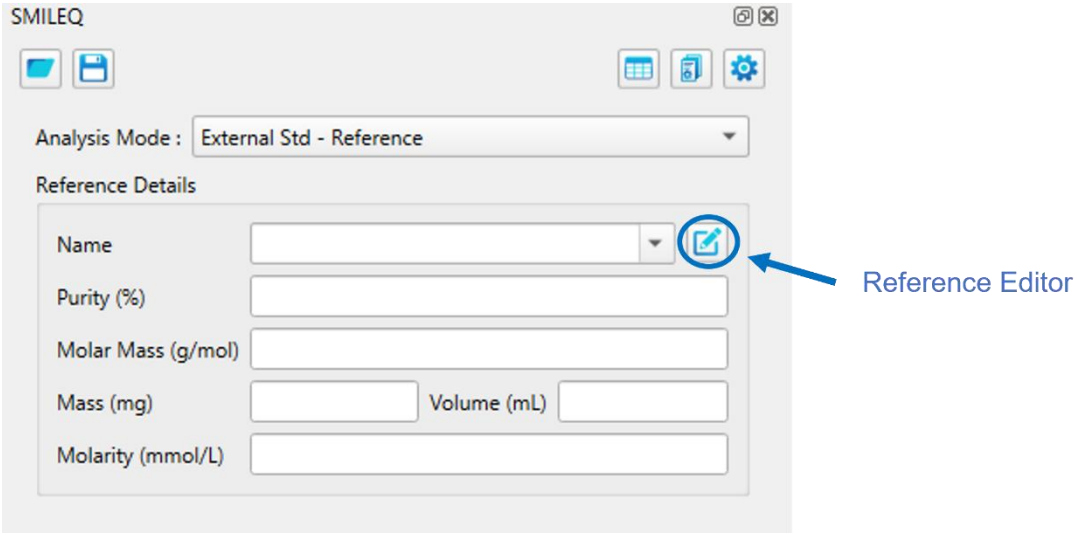


Figure 13: Setting of External Standard - Reference information- Input Area

Name	Select a reference substance from the “Name” combo box. This drop-down list can be edited using the Reference Editor button. See section 2.2 Reference Editor for details. When a reference is selected, the values for purity (Purity (%)) of the reference compound and its molar mass (Molar Mass (g mol^{-1})) are automatically loaded.
Mass	Enter the weight value of the reference in the “Mass” text field in units of mg.
Volume	Enter the volume of sample in the “Volume” text field, if molarity results are required.

The **“Name”** and “**Mass”** fields must be completed in order to perform the analysis. To use the molar concentration, enter “**Molarity**” instead of “**Mass”** and “**Volume”**.

2.2 Reference Editor

The information about the reference used in the analysis is saved as a reference file. This file can be registered and edited in the Reference Editor. The button to open the Reference Editor can be selected from the SMILEQ widget when "Internal Std" or "External Std - Reference" is selected in the analysis mode.

Clicking the Reference Editor button displays a table in which you can enter information about the particular reference substance used in the quantitative analysis.

It is also possible to load information from a selected spectrum on canvas or set up the use of multiple signals in one compound.

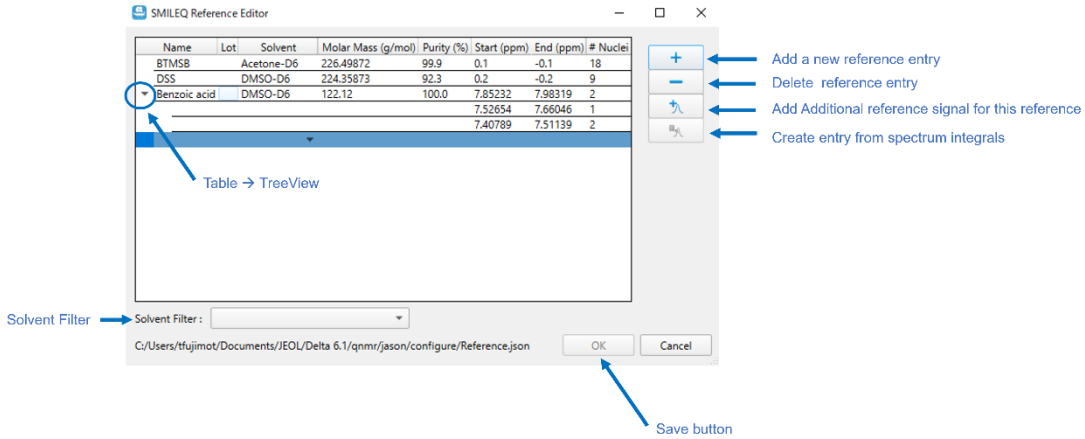


Figure 14: Editing of the Reference Editor Area

Name	Enter a sample identifier in the “Name” text field.
Lot	Information such as number of lot
Solvent	Solvents expected to be used
Molar Mass (g/mol)	Enter the molecular weight in the “Molar Mass” text field in units of g mol^{-1}.
Purity (%)	Reagent purity or certified value
Start, End(ppm)	Integration range
+,-	Click the “**+**”” button to add a reference entry to the table. Clicking the “**-**”” button will delete the reference entry or one of its signals.
Add additional signal for this reference	Clicking the “Add additional signal for this reference” button allows multiple signals to be defined for a given reference compound
Create entry from spectrum	Clicking the “Create entry from spectrum” button will populate the table from the integrals currently defined on the spectrum. This is useful to create an entry from an example spectrum of the reference compound.
Solvent Filter	The Solvent Filter allows the user to show reference compounds defined for specific solvents.
Table -> TreeView	If multiple signals are set, an expand icon will be displayed to the left of "Name". Click this icon to show or hide the information. You can enter, delete, or edit the information.
OK	Click the "OK" button to save the reference information to a local file.

- If any cells are blue then this means that the cell contents are invalid or missing information. The “OK” button cannot be pressed until these issues are rectified.
- Pressing the “OK” button will save the changes made to the file which is defined in the SMILEQ settings. The name and location of the current reference file s shown at the bottom of the dialog.

2.3 Target Signal Table Area

All multiplets or integrals defined on the spectrum will be shown in the Target Signal Table, except if they have been defined as solvent signals, see Figure 15. Target Signal Area is shown when selecting "Internal Std" or "External Std -Analyte" in SMILEQ widget.

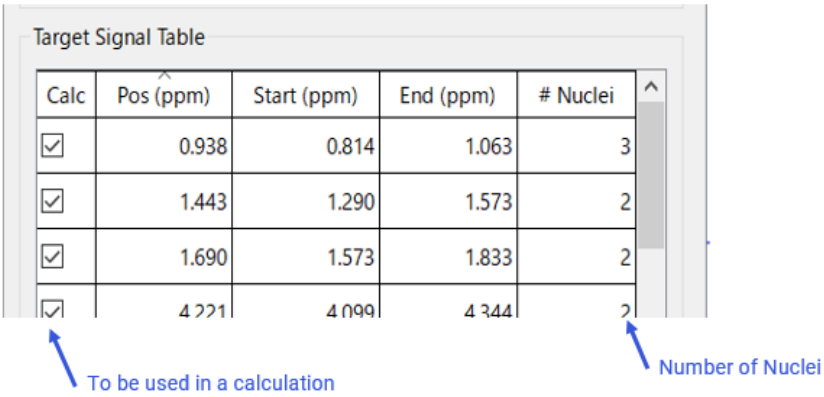


Figure 15: Editing of the Target Signal Table Area

Calc checkbox	The **Calc** checkbox is used to identify which multiplets or integral regions should be used in the quantification calculation.
Pos (ppm)	The position of the signal.
Start / End (ppm)	The position of the Integral region.
#Nuclei	Enter the number of identical nuclei associated with the multiplet or integral region in the column "# Nuclei". An estimate is populated here automatically, but this should be checked, and if necessary corrected, by the user.

The information (position, start, end, number of nuclei) is automatically filled in from the active spectrum (the spectrum selected on the canvas) where a multiplet analysis or integration region has been defined, or automatically filled in when a new one is defined.

2.4 Calculation Options Area

It is possible to select the quantitative information to be used in the calculation and set the number of decimal places. The calculation options area is displayed on the SMILEQ panel when "Internal Std or "External Std - analyte" is selected in the analysis mode.

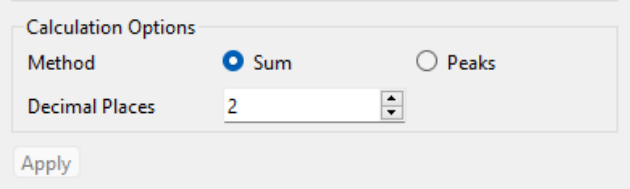


Figure 16: Setting of Calculation Options Area

Method	The purity and/or concentration of each analyte can be calculated using two slightly different approaches: **Sum**: This uses a point by point summation across the defined region to calculate the integral (recommended). **Peaks**: This uses the sum of the peak areas in the region as determined by the peak deconvolution algorithm.
Decimal Places	The number of decimal places shown in the results table is defined by the “Decimal Places” box in Table tool panel.
Apply	Click the “**Apply**” button to perform the quantitative analysis, and create the quantitative results table and quantitative parameters report on the canvas

If the analysis results are already available, the “**Apply**” button will change to the “**Update”** button, and the results will be updated when the button is pressed.

2.5 MultiSpectrum SMILEQ

MultiSpectrum SMILEQ allows the analysis of multiple datasets loaded onto the canvas. Once an analysis file directory has been defined in the main SMILEQ settings (from the cogwheel button), pressing the MultiSpectrum SMILEQ button opens dialog shown in Figure 17.

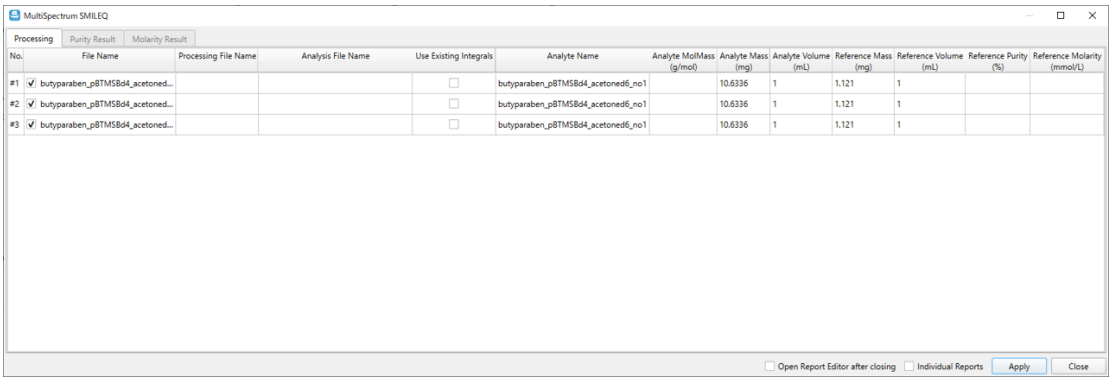


Figure 17: MultiSpectrum SMILEQ

File Name	
Processing File Name	Entries in the “Processing File Name” and “Analysis File Name” columns can be selected from the dropdown button which appears when you click on the appropriate cell in the table. You can then select the appropriate file from the list provided.
Analysis File Name	
Use Existing Integrals	The analysis is conducted by using the integration ranges present on the spectrum on the canvas.
Analyte Name	Other parameters in the table can be entered as necessary. If they can be obtained from the spectra on the canvas, they will be automatically entered. These can then be adjusted as needed. If the blank, the information is needed to set.
Analyte Molar Mass	
Analyte Mass	
Analyte Volume	
Reference Mass	
Reference Volume	
Reference Purity	It will be set automatically from Reference file, but it can be edited if necessary.
Reference Molarity	It will be shown the calculated value.
Individual report	Checking the “Individual Reports” box will generate the quantitative results table and quantitative parameters report for each individual spectrum selected for analysis.
Open Report Editor after closing	This checkbox allows “Report Editor” launches when Multispectrum SMILEQ is closed.
Apply	Click “Apply” to run the quantitation calculation across the selected spectra. The results will be displayed on the Purity Result tab and/or Molarity Result as appropriate.

2.6 Report Editor

The Report Editor allows you to generate several types of statistical report using multiple data sets.

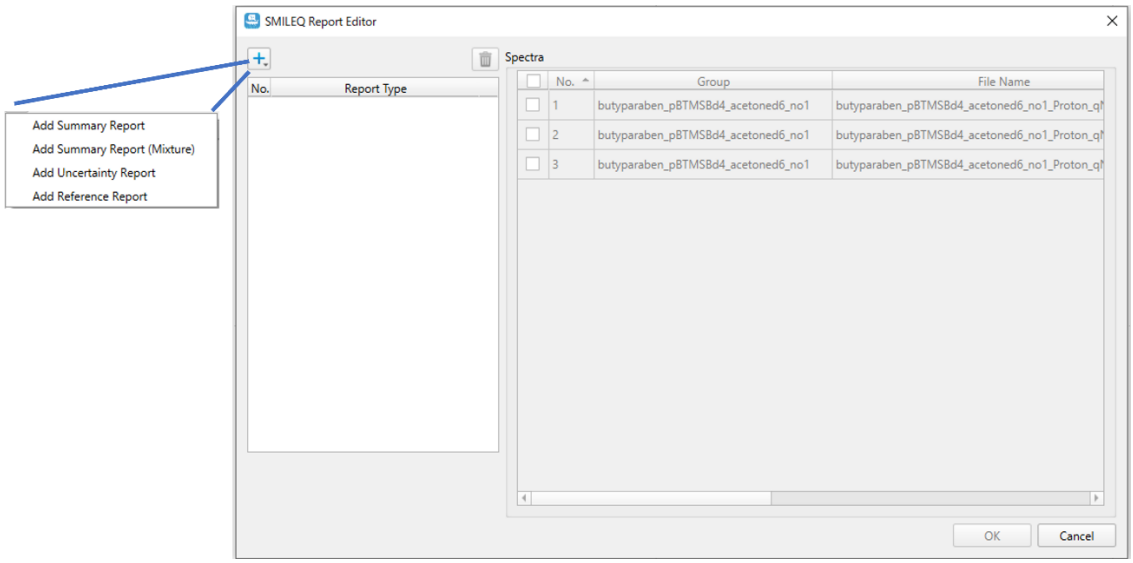


Figure 18: Report Editor

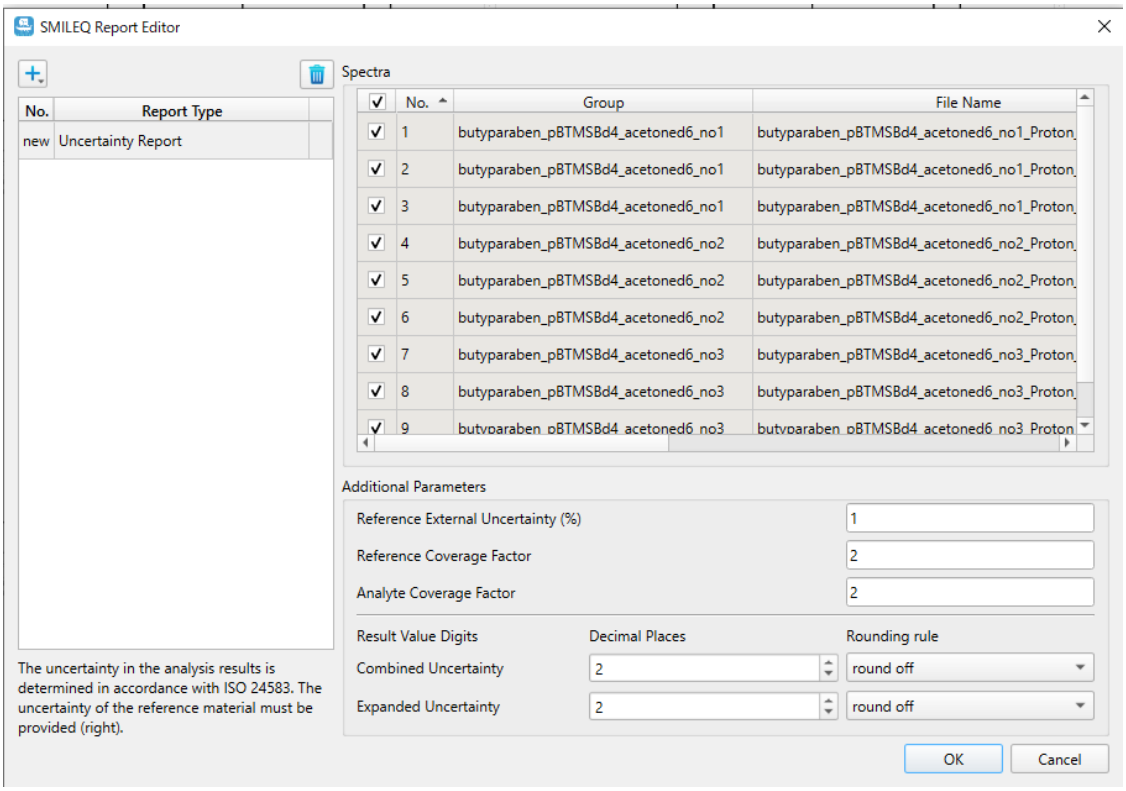


Figure 19: Report Editor (Uncertainty Report)

Several different reports are available, depending on the analysis to be performed and the information required. Summary Report will generate reports on the canvas mirroring the information on the Purity Results tab and Molarity Results tab. The Reference Report

option is used to provide the results of the analysis of External Standard reference samples.

The Uncertainty budget option creates an additional report in which the uncertain budget is analyzed in accordance with ISO 24583. Additional parameters are available for the calculation as shown Figure 19.

2.7 Mixed Analytes - Mixed Analyte Editor

When conducting mixture analysis, registration of the names and molar mass of the compounds to be analyzed is needed. This editor allows to save this information.

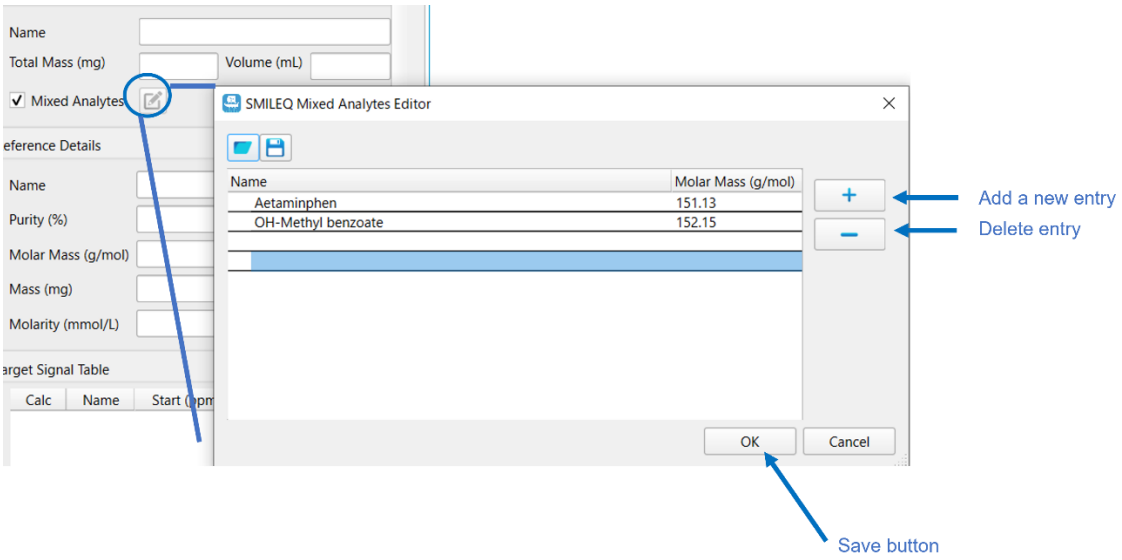


Figure 20 : SMILEQ Mixed Analyte Editor

Name	Enter a sample identifier in the “Name” text field.
Molar Mass (g/mol)	Enter the molecular weight in the “Molar Mass” text field in units of g mol^{-1}.
+,-	Click the “**+**”” button to add an entry to the table. Clicking the “**-**”” button will delete the entry.
Open the file	Open the list as file
Save the file	Save the list as file
OK	Click the "OK" button to save the reference information to a local file.

2.8 Others

Setting

This defines the path where the files used by the SMILEQ plug-in can save, file definitions, etc. For details, see 3.2 SMILEQ Settings.

Save and Load Analysis File

The parameters set in the SMILEQ panel as a file is possible to save and load. For details, please refer to 3.1.2 Creating an analysis file. The main parameters saved in the analysis file are as follow.

Analyte sample information	Name, Molar Mass, Volume
Reference information	Name, Molar Mass, Volume, Name of reference data (only in case of external std method)
Integration, etc	Region, umber of nuclei
Analysis related information	Analysis mode, options for calculation

3 Preparing for qNMR analysis

The SMILEQ plugin can be used in two ways. Firstly, to perform a manual quantification calculation and report the results for a given spectrum. The results will be purity and/or molarity. Secondly, using the relevant configurations files, automated quantification of a number of spectra can be performed. These procedures will be explained chapter 4 **Error! Reference source not found.**.

This chapter describes how the various configuration files for SMILEQ are prepared in advance of the analysis.

3.1 Necessary Files

To use the automated analysis, SMILEQ needs a reference file and an analysis file before starting the analysis.

To perform a manual quantification calculation, it is necessary to prepare for the reference file only (not necessary to create the analysis file).

3.1.1 Creating a Reference File

The SMILEQ plugin allows the user to save reference substance information in the reference file.

1. Select the "**Reference Editor**" button in the reference information input area.
2. Add the new line and enter the following parameters:

Name:	name of the reference material
Lot:	lot number of reagents if available
Solvent:	Select solvent from the combo box
Molar Mass:	Molar Mass of reference substance (in g/mol)
Purity (%):	purity of the reagent
Start, End (ppm):	the integration range for the location of the reference signal
Nuclides:	number of nuclides corresponding to the reference signal

Additional signals for a given reference compound can be added by pressing the "Add an additional signal" button. If a spectrum of reference compound is available, with suitable integrals or multiplets defined, then the "Create entry from Spectrum Integrals" button can be used to populate an entry in the reference editor.

3. Press the OK button.
   Click the "OK" button to save the reference information to a local file.

   For details to see 2.2 Reference Editor

3.1.2 Creating an Analysis File

The SMILEQ plugin also allows the user to create an analysis file for efficient, automated, qNMR analysis. This saves the parameters used in the calculation as a JASON Quantitative Analysis File (.jqaf). To create the file, first the desired data is prepared and then the analysis is conducted.

The parameters for analysis are then saved, except that for the "**Mass**" of the sample. The saved file can then be used to perform quantification on other NMR data acquired under the same conditions as those used when the template was created.

The procedure for analysis can be found in 4.1 Internal Standard Method and 4.2 External Standard Method.

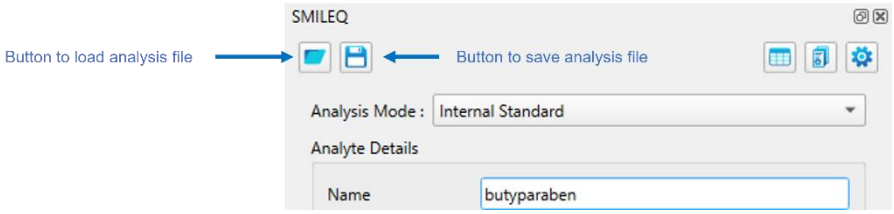


Figure 21: Button to save and load analysis template file

3.2 Necessary Setting

The SMILEQ settings tab allows the user to define the paths where various configurations files are stored.

3.2.1 Settings (General)

These settings are related to quantification with SMILEQ plugin.

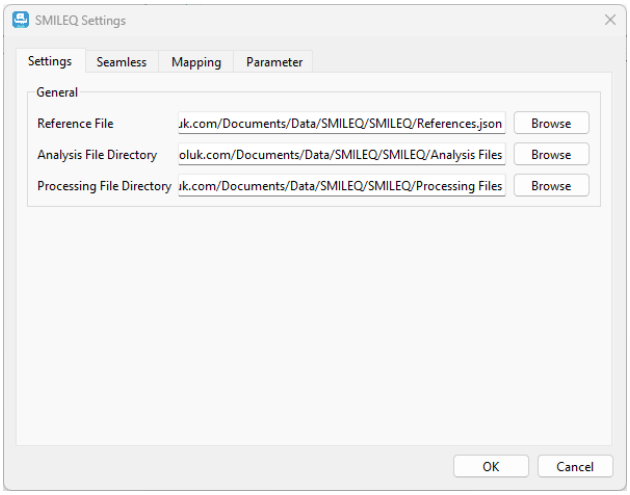


Figure 22: General Settings

Reference File: Location where reference file is saved. By default, this location is set to C:/Users/<user name>/.jason/plugins/smileq

Analysis File Directory: Location where you want to store analysis files JASON Quantitative Analysis File (.jqaf).

Processing File Directory: Location where you store processing template files (JASON Processing Lists, .jjp files)

3.2.2 Seamless

Seamless is an automatic data acquisition, processing and reporting workflow for quantitative NMR (qNMR), which combines Delta (JEOL NMR standard software) and JASON in a single work flow. JASON receives the NMR data (FID) acquired using Delta and then conducts data processing, quantitation analysis and produces reports of the results automatically using the SMILEQ plugin.

This section describes settings related to SMILEQ for Seamless. If you do not need to conduct analysis using Seamless, these settings are not necessary.

In the case of Seamless, the details of the calculations can be configured using the JASON SMILEQ plugin and the details saved as a Jason Quantitative Configuration File (.jqcf) file. Using the tool below, found on the Seamless tab in the SMILEQ settings, click the Save button to create the configuration file used by SMILEQ.

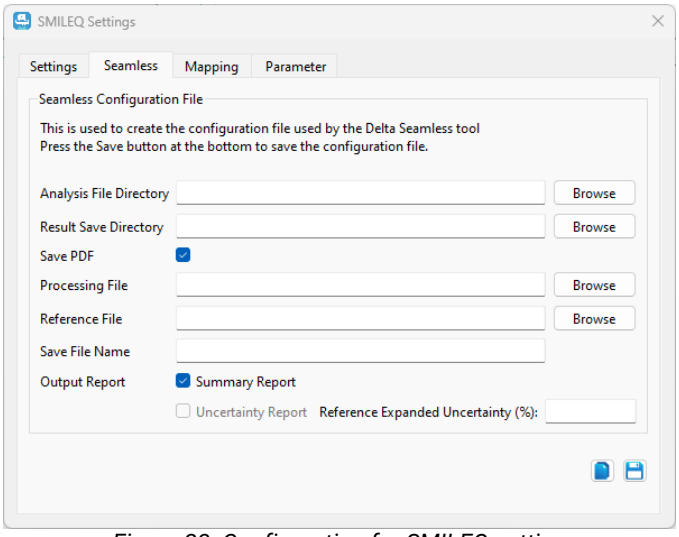


Figure 23: Configuration for SMILEQ setting

Analysis File Directory:	The directory where to store analysis file that is Jason Quantitative Analysis File (.jqaf) will be used SMILEQ.
Result Save Directory:	The directory where the results documents of SMILEQ will be saved.
Save PDF:	Option to create a PDF as results document.
Processing File:	The JASON processing file which contains the complete list of processing steps to be applied to the NMR data prior to the qNMR analysis.
Reference File:	Reference file which contains the list of reference substance information for analysis.
Save File Name:	Name to use for the results document.
Output Report:	These check boxes determine which reports will be automatically created by Seamless.

- If “**Processing File**” is not set, the default processing list will be applied.
- If "**Save File Name**" is not set, the analysis document will be saved with the name "result".

The name of the final output document will have the date and time appended to it.

- Delta V6.1 or greater is required to perform automatic qNMR analysis by using SMILEQ.
- For more detailed information, please refer to the Delta user’s manual “JASON User's Manual / SMILEQ SETUP GUIDE”

3.2.3 Parameter Mapping

SMILEQ can read certain parameters from the original data to speed up the qNMR workflow. The mapping of parameters from the raw data to SMILEQ can be set on the mapping tab of the SMILEQ settings, as shown in Figure 24.

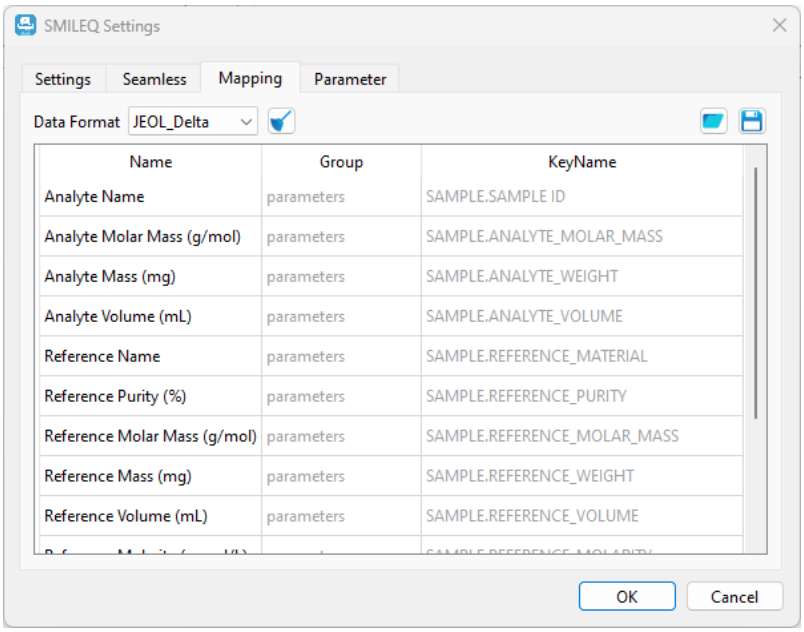


Figure 24 SMILEQ Setting-Mapping Dialog

Default values are supplied for JEOL data (qNMR Seamless), and can be modified. The vendor is specified from the drop-down list at the top, while the Group specifies in which file the parameter can be found. Mappings can be saved and loaded using the icons in the upper right.

3.2.4 Parameter

The parameters that are displayed in the results table of individual reports is able to select.

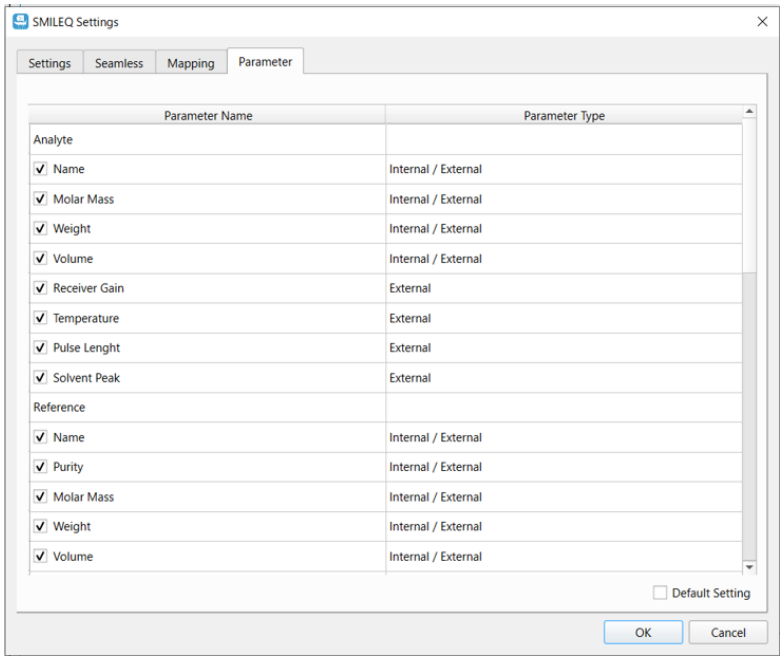


Figure 25: SMILEQ Setting-Parameter Dialog

When you press the OK button, the settings will be reflected not only in the JASON settings, but also in the results selected on the canvas. Also, if you check "Default Settings" and press the OK button, the settings will be saved as the default.

3.3 Integration and Baseline correction

The guidelines relating to qNMR state that accurate phase- and baseline correction are necessary for high quality qNMR analysis. To ensure reproducible results, the baseline correction processing item should be included in the processing list, and the auto baseline setting in the Multiplets/Integrals section of JASON's global settings should be switched off. This option can be found under the settings tab, shown in Figure 26.

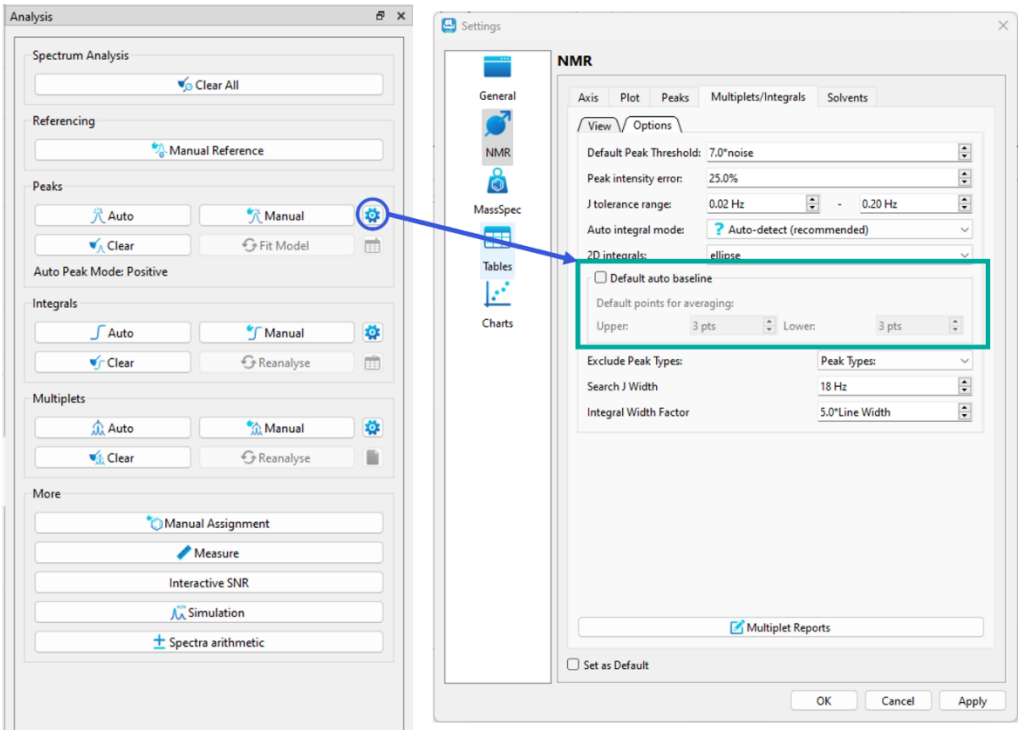


Figure 26 : Setting the auto baseline correction for integrals in JASON

If baseline correction is not included in the processing list, then ensure you active the auto baseline under the integration settings.

4 Analysis Procedure

The SMILEQ plugin provides several analysis procedures. In this chapter, each procedure will be described.

4.1 Internal Standard Method

👉 The movie is available URL: https://youtu.be/qrk803hcQJw

This procedure is used to perform a manual quantification analysis and report the results for a given spectrum loaded onto the canvas.

Before undertaking any quantitative analysis using SMILEQ, ensure that all standard NMR data processing steps have been performed, including apodization, zero filling, FFT, phase and baseline correction and chemical shift referencing. Details on how to perform these operations can be found in the main JASON manual, and on the JEOL JASON website and YouTube channel.

1. Using the tools on the analysis panel, multiplet or integral regions can be set, either automatically or manually as desired.

   👉 Signals such the reference substance, solvent(s) or impurities can be excluded at this stage. (The reference signal will be automatically integrated when the reference compound details are set later).

2. Select the SMILEQ widget and enter the required parameters in the sample information area (see section 2.1.1.1 for details of the necessary information and Figure 27).

   👉 Where the raw data (FID) has parameters such as mass or volume stored, these values will be set automatically in the appropriate fields on the SMILEQ widget.

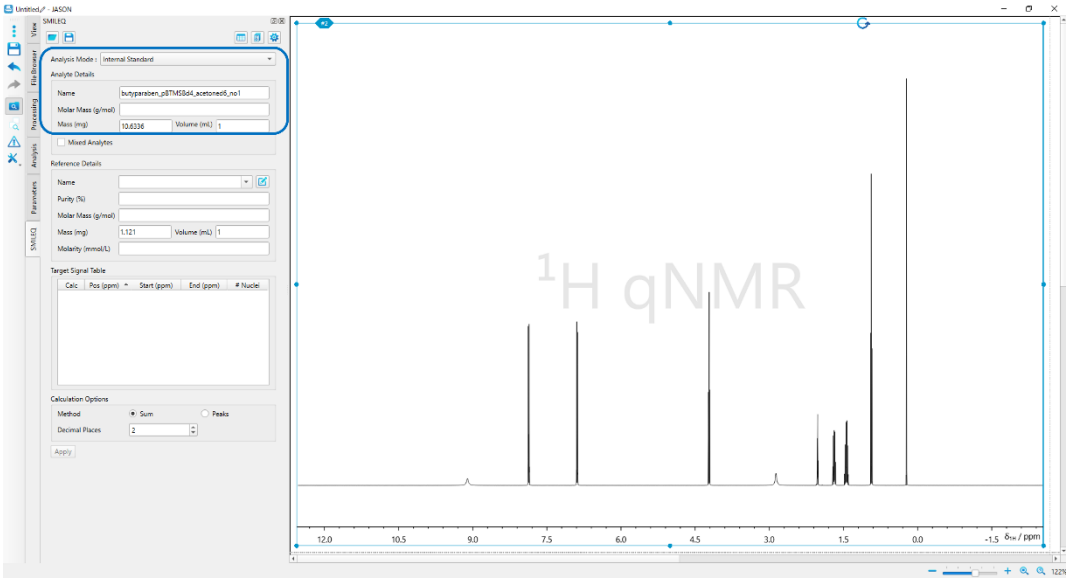


Figure 27: SMILEQ widget after conducting multiplet analysis

3. Fill in the box at reference information area. Select the desired reference from the "**Name**" and enter the reference parameters required for the analysis (see section 2.1.1.2 and Figure 28.)

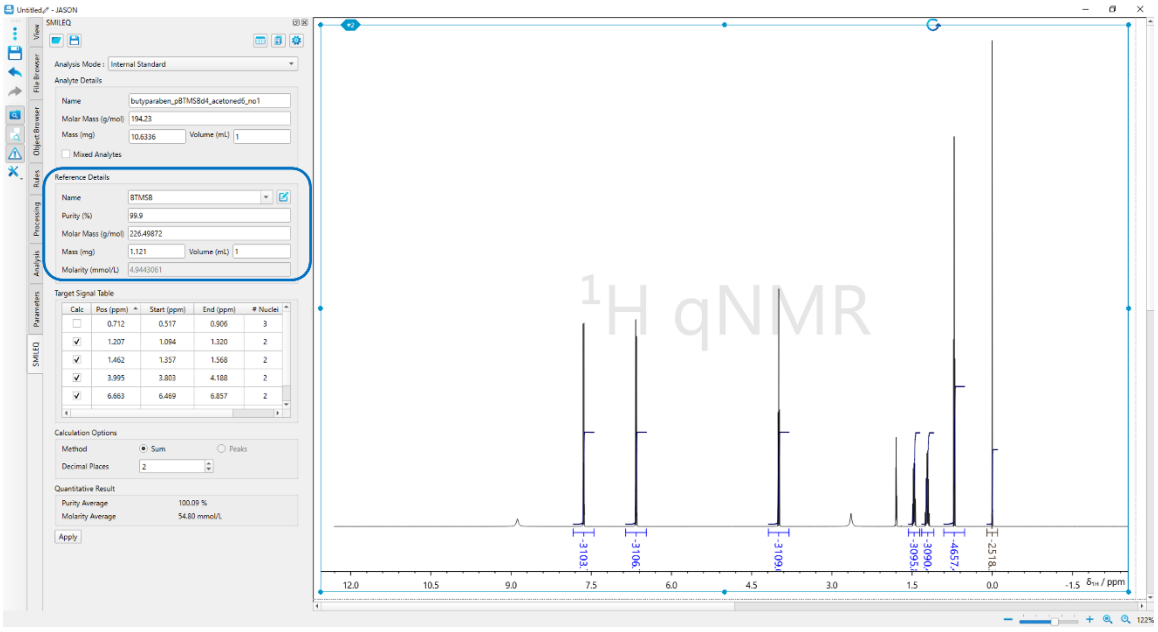


Figure 28: SMILEQ widget after selecting the reference

4. Confirm the "**Target Signal Table**" details. If necessary, manually (de)select the signal for the analyte or enter the nuclides information as shown in Figure 29. (see section 2.3 Target Signal Table Area)

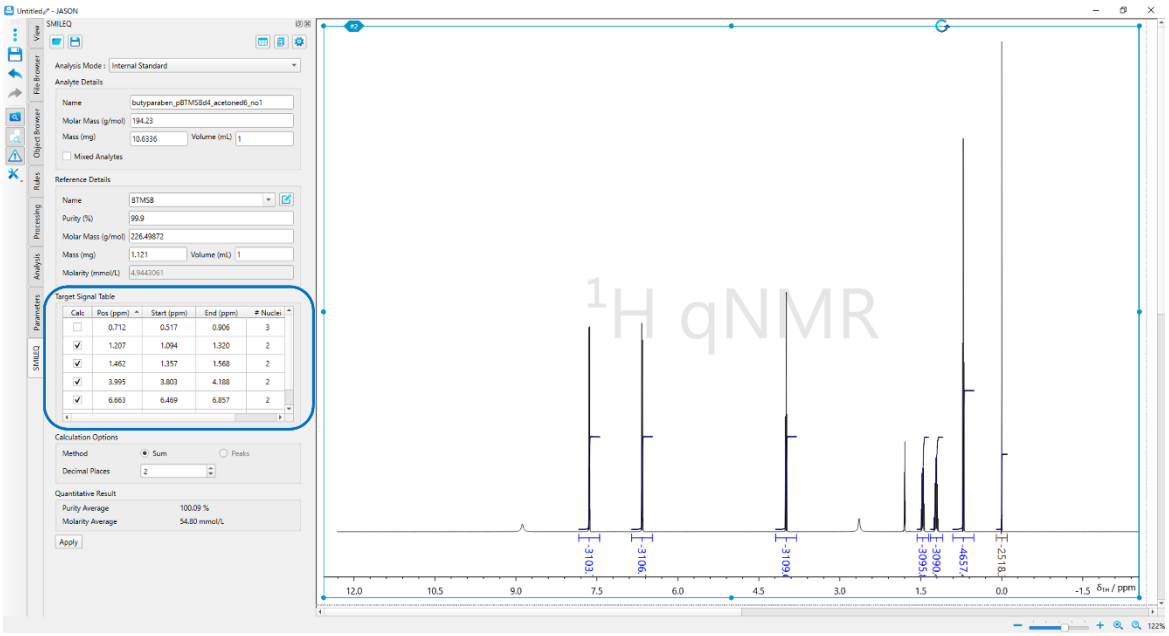


Figure 29: SMILEQ widget after confirming the target signal table

5. After setting the calculation option, press the "**Apply**" button to view the result.

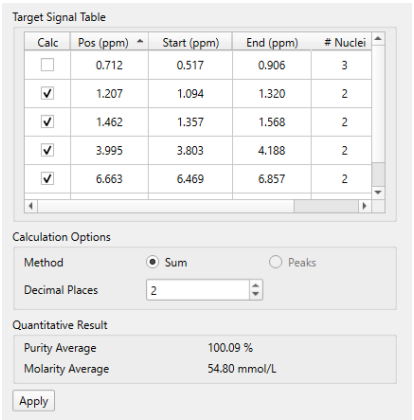


Figure 30: Calculation option on the SMILEQ widget

Figure 31 shows the result of a successful quantitative analysis, with the report and parameters tables being added to the canvas.

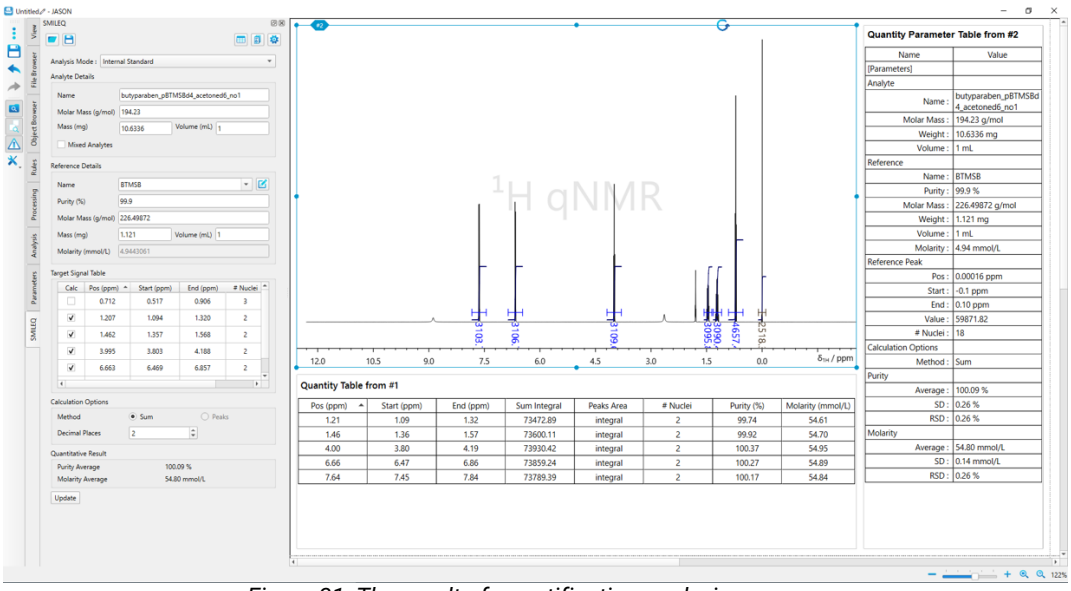


Figure 31: The result of quantification analysis

The details of the analysis conditions used can be saved as an analysis file (.jqaf file). See section 3.1.2 Creating an Analysis File. This analysis file is necessary for the procedure described below in Section 4.3 “Automated quantitative analysis”.

4.2 External Standard Method

This procedure is used to perform a manual quantification analysis and report the results for a given spectrum loaded onto the canvas.

This step optimizes parameters, performs quantitative analysis, and reports the results for a specific spectrum. Before proceeding with the analysis in the SMILEQ panel, ensure that all data processing steps have been completed (FFT, apodization, zerofilling, phase adjustment, baseline correction, chemical shift reference setting, etc.).

Load all analyte and standard substance spectra onto the canvas.

1. Select the standard substance spectrum, and in the SMILEQ panel select “External Std – Reference” from the Analysis Mode pull-down menu. This will set the SMILEQ panel to show only those fields relevant to the external standard. Select the reference compound from the Name drop down.

- If the data file contains information such as weight and sample volume, the corresponding fields will be automatically populated.
- Providing the sample volume is not needed if molarity calculations are not required.
- When using multiple spectra, make sure to specify the same setting for each reference spectrum.

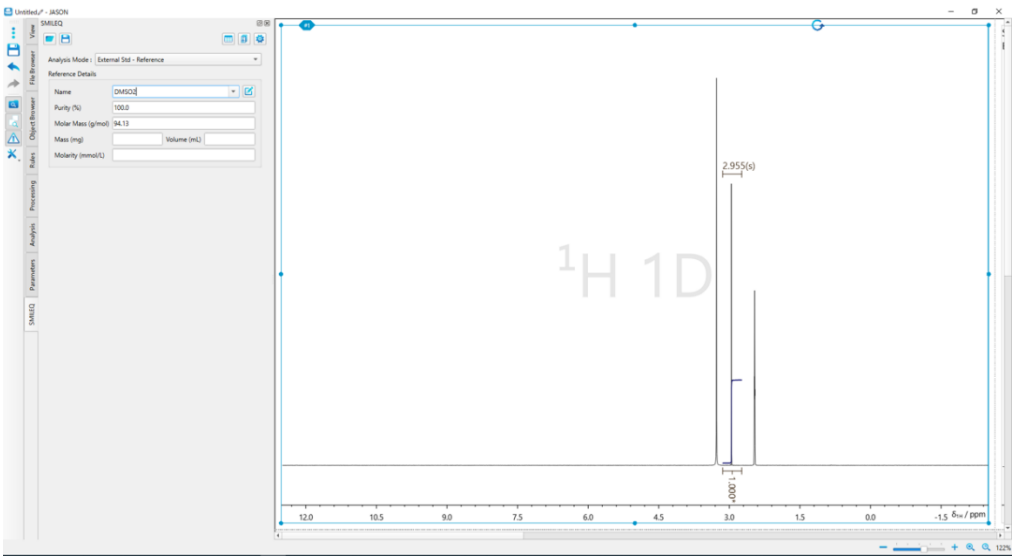


Figure 32 : SMILEQ panel External standard method (reference target setting screen)

2. Select the spectrum to be analyzed, and in the SMILEQ panel select "External Std – Analyte” " from the analysis mode pull-down menu. Fill in the information for the analyte, such as name, molar mass etc. If this information is contained in the data file, these parameters will be populated automatically.

- If the data file contains information such as weight and sample volume, the corresponding fields will be automatically populated
- Providing the sample volume is not needed if molarity calculations are not required

3. Select which signals are included from the “Target Signals Table” by selecting the appropriate check boxes. Quantitative information (integration) of a signal can be obtained by multiplet analysis or integral values using the standard tools in JASON).
   - Multiple analysis or integration results that are not the target of analysis, such as standard substances, solvents, or impurities should be unchecked in the Target Signals Table.
   - Check the details of the signal area to be analyzed. Add signals to be analyzed and change the integration range as necessary.
   - Adjust the area and check the number of nuclei, modifying if necessary.

4. Select which spectrum to use in the reference spectrum area.
   - If you use more than one, you can calculate the average value or individually. The results will be displayed in the Quantitative table.

5. Select either the average or individual option.

6. In the Correction area, select the calibration parameters to be used by PULCON.
   - If the parameters differ between the spectra of the standard material and the analytical sample, the checkbox for that parameter will become enabled. Ticking these checkboxes allows corrections for pulse width, temperature, receiver gain etc

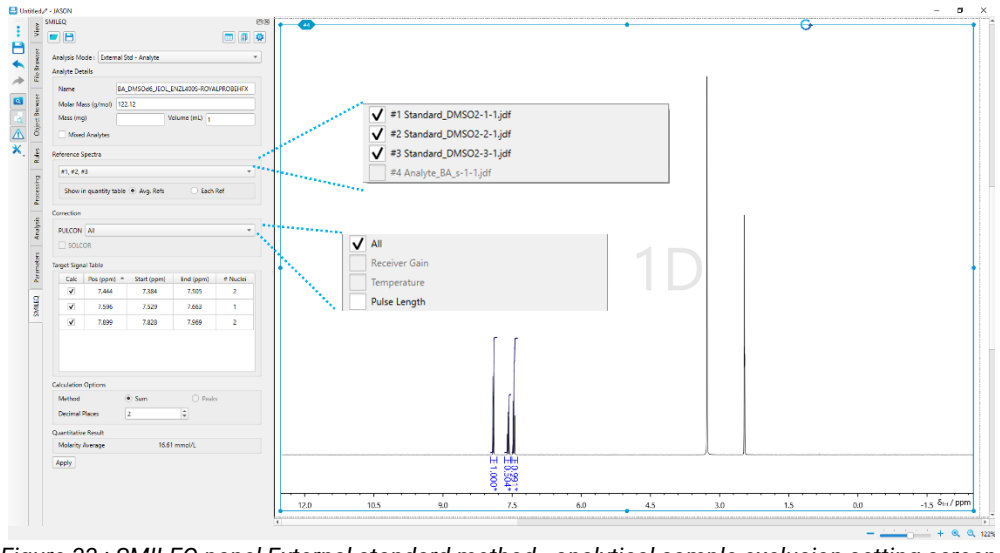


Figure 33 : SMILEQ panel External standard method - analytical sample exclusion setting screen.

7. Check the details of the signal area to be analysed. If necessary, add additional analysed signals, adjust the integration range, enter or modify the number of nuclei.

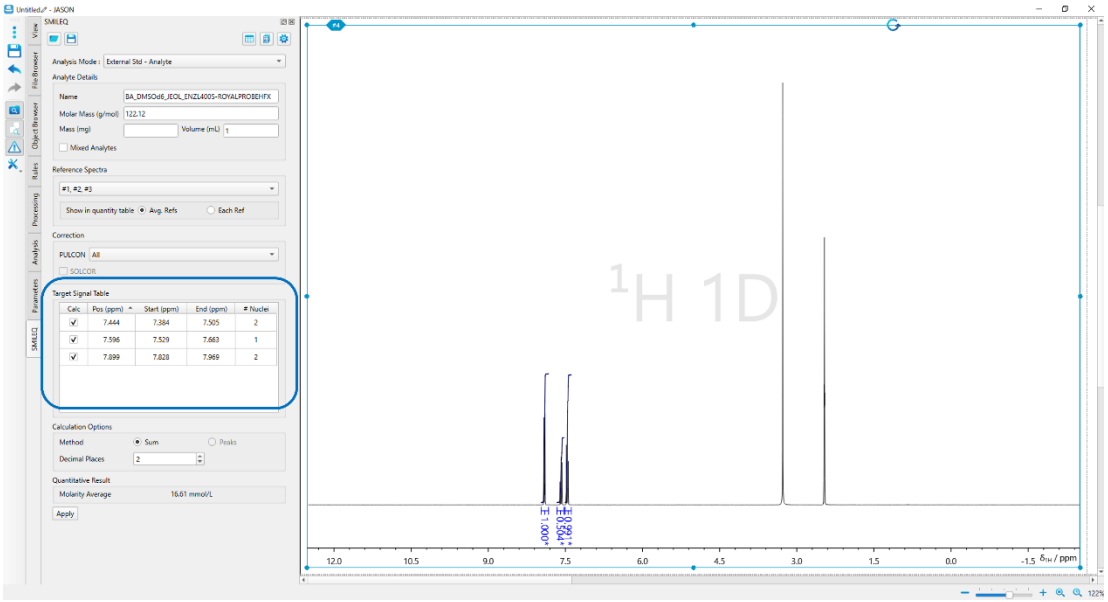
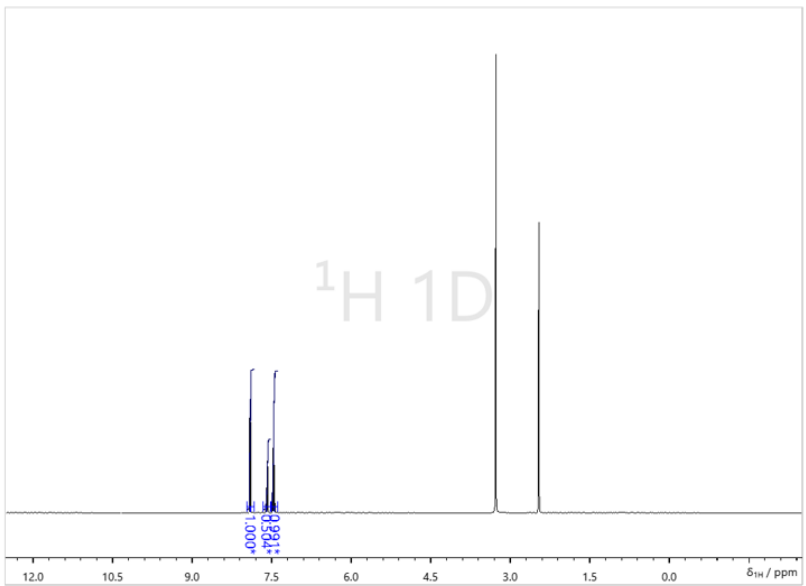


Figure 34: SMILEQ panel Signal tables for analysis / calculation options

8. After setting the calculation options, click the Apply button to view the results.



Quantity Table from #1

Pos (ppm)	Start (ppm)	End (ppm)	Sum Integral	Peaks Area	# Nuclei	Avg. Purity (%)	SD Purity (%)
7.44	7.38	7.50	16928.29	integral	2	98.77	3.05
7.60	7.53	7.66	8613.67	integral	1	100.52	3.10
7.90	7.83	7.97	17079.53	integral	2	99.66	3.08

Quantity Parameter Table from #4

Name	Value
[Parameters]	
Analyte	
Name :	BA_DMSOd6_J
Molar Mass :	122.12 g/mol
Weight :	2.04 mg
Volume :	1 mL
Reference	
Name :	DMSO2
Avg. Purity :	100.00 %
Avg. Molar Mass :	94.13 g/mol
Avg. Weight :	0.50 mg
Avg. Volume :	1.00 mL
Avg. Molarity :	5.31 mmol/L
QValue :	1.95e-03
SD of QValue :	6.02e-05
RSD of QValue :	3.09e+00
Reference Peak	
Pos :	2.94 ppm
Start :	2.74 ppm
End :	3.14 ppm
Avg. Value :	16359.49
# Nuclei :	6
Spectra :	#1, #2, #3
Calculation Options	
Method :	Sum
Purity	
Average :	99.65 %
SD :	0.87 %
RSD :	0.88 %
Molarity	
Average :	16.65 mmol/L
SD :	0.15 mmol/L
RSD :	0.88 %

Figure 35: Example of quantitative analysis results

Analysis conditions (integral range, standard material, molar mass, etc.) can be saved as an analysis file. This analysis file is described in 4.3 Automated Quantitative analysis

In the external standard method, separate analysis files are created for each analyte target and reference target.

4.3 Automated quantitative analysis

This procedure can be used to perform automatic analysis by using an analysis file that was configured for quantification analysis in advance.

Before initiating the analysis using the SMILEQ widget, ensure that all processing of the NMR data (apodization, zero filling, FFT, phase and baseline correction and chemical shift referencing) has been performed.

The automated quantitative analysis is then performed as follows:

1. Select the SMILEQ widget and load the .jqaf analysis file using the load button.

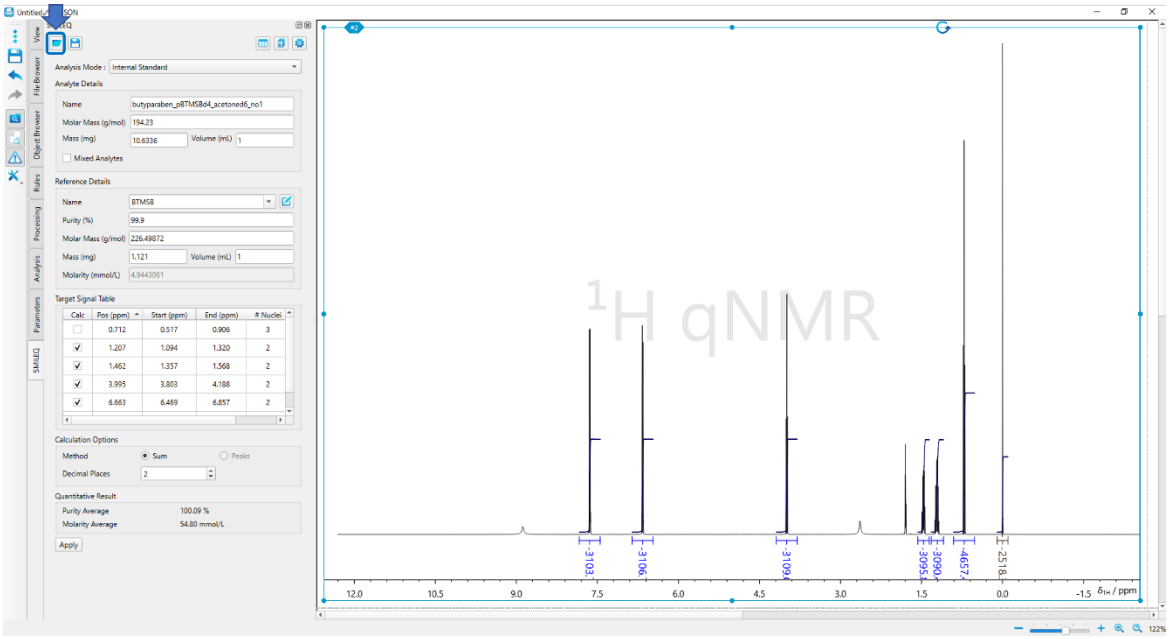


Figure 36: SMILEQ widget after loading the template file for analysis

2. Fill in any blank fields in the sample information area and reference information area if necessary.

3. Press the "**Apply**" button to view the result. The report and parameters tables will be added to the canvas.

In the external standard method, load analysis files are created for each analyte target and reference target.

The movie is available URL: https://youtu.be/ZDecamUawH4

4.4 Multispectrum Analysis

Multiple spectrum analysis means processing and analyzing multiple FIDs at once. To conduct it, you need to specify the file to be used, the directory to be referenced, and prepare the processing file and analysis file to be used in advance.

For details, see section 3. Preparation for qNMR analysis.

This procedure can be used to perform quantitative analysis across multiple spectra of the same analyte by using a processing file and an analysis file that was configured for quantification analysis in advance.

SMILEQ supports multiple spectrum analysis both the internal standard method and the external standard method.

4.4.1 Internal Standard Method

The movie is available URL: https://youtu.be/kjRLq-eO8Lw

1. Load the datasets into canvas.
2. For all loaded spectrum, to confirm that the analysis mode “Internal Standard” in the SMILEQ panel from the pull-down menu is selected.
3. Select one of the spectra on the canvas and click the Multispectrum SMILEQ button in the SMILEQ panel.
4. Multispectrum SMILEQ dialog is opened. The column of "Processing File Name" and "Analysis File Name" is dropdown button, it is able to select the desired setting condition. Setting of other parameters will be set if necessary.
5. Click “Apply” to run the calculation and will be display the list of result on Purity Result tab and/or Molarity Result.

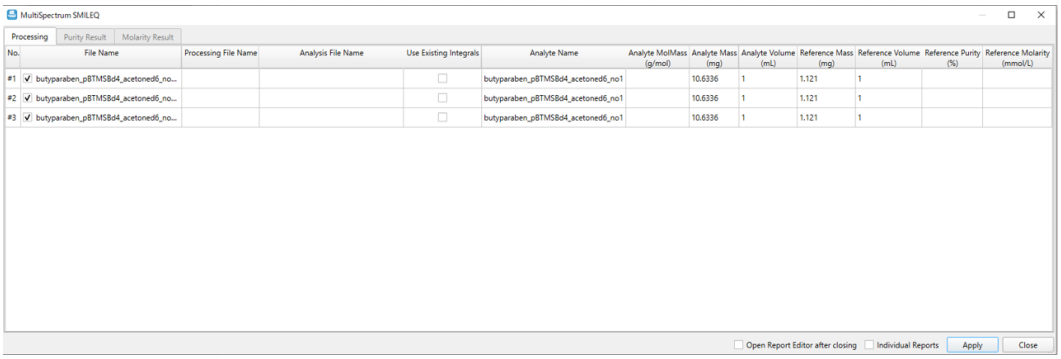


Figure 37: Multispectrum SMILEQ dialog (Internal Std)

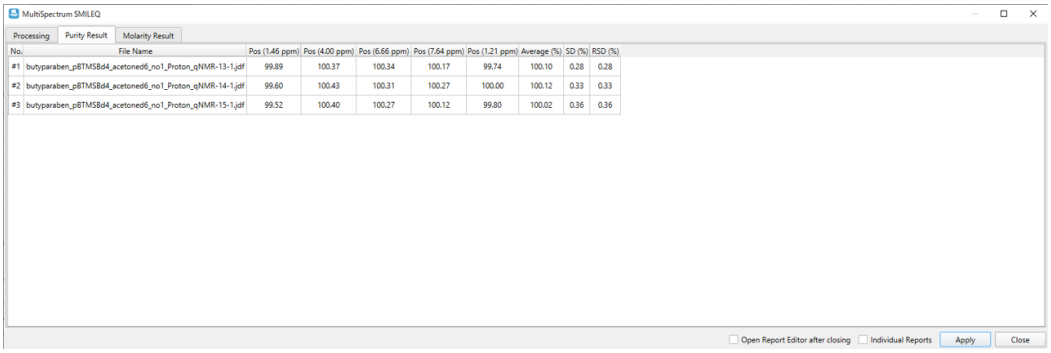


Figure 38: The table for Purity Result

6. When “Individual Reports” check box is ON and “**Apply**” button select, the individual report will be generated.
7. When the “Open Report Editor after closing” check box is ON , the Report Editor will be opened after closing Multispectrum SMILEQ dialog automatically.

4.4.2 External Standard Method

1. Load the datasets into canvas.
2. For each loaded spectrum, select it and choose the analysis mode in the SMILEQ panel from the pull-down menu.
3. Select one of the spectra on the canvas and click the Multispectrum SMILEQ button in the SMILEQ panel.
4. Multispectrum SMILEQ dialog is opened. The column of "Processing File Name" and "Analysis File Name" is dropdown button, it is able to select the desired setting condition. Setting of other parameters will be set if necessary.

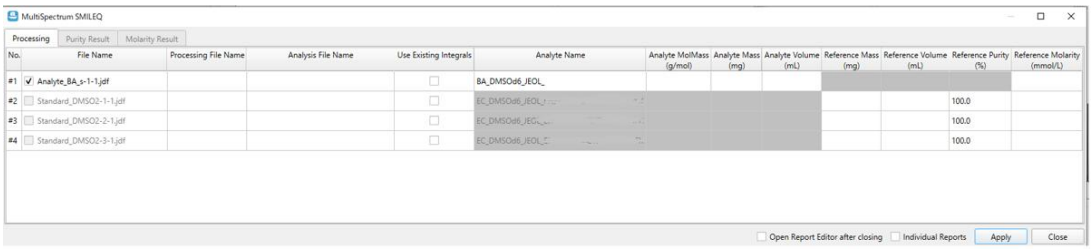


Figure 39:Multispectrum SMILEQ dialog (External Std)

4.5 Mixture analysis

Mixture analysis means processing and analyzing spectra containing several analytes in one sample.

This procedure is used to perform a manual quantification analysis and report the results for a given spectrum loaded onto the canvas.

Before undertaking any quantitative analysis using SMILEQ, ensure that all standard NMR data processing steps have been performed, including apodization, zero filling, FFT, phase and baseline correction and chemical shift referencing.

1. Using the tools on the analysis panel, multiplet or integral regions can be set, either automatically or manually as desired.

   Signals such the reference substance, solvent(s) or impurities can be excluded at this stage. (The reference signal will be automatically integrated when the reference compound details are set later).

2. At the SMILEQ widget, Mixed Analytes check box ON and open SMILEQ Mixed Analyte Editor. If you need to add the analyte information, (see section 2.7 Mixed Analytes-Mixed Analyte Editor for details and Figure 40)

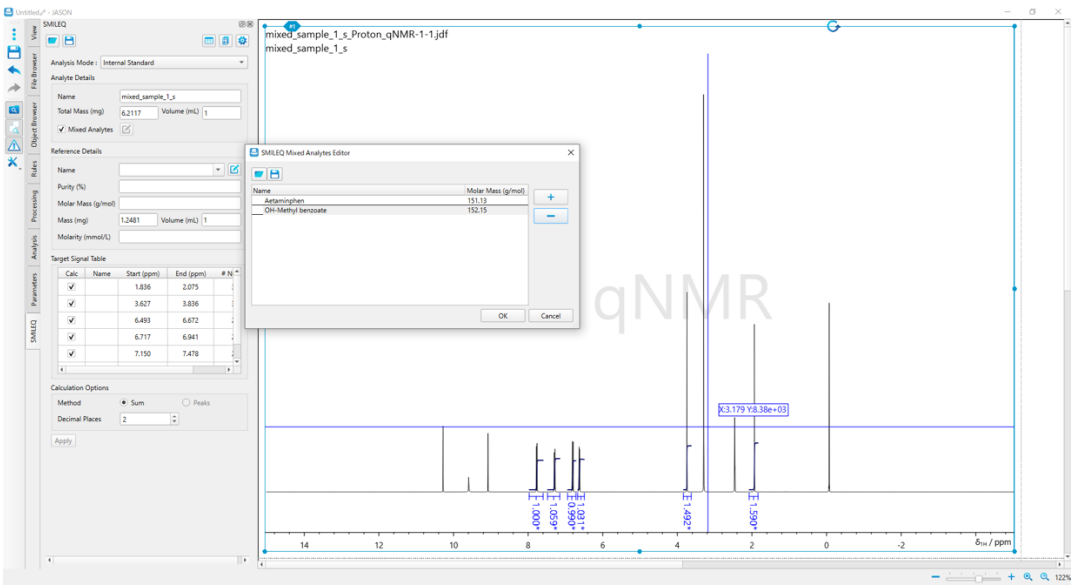


Figure 40 : Mixed Analyte and Mixed Analytes Editor

3. The SMILEQ widget and enter the required parameters in the sample information area.

Where the raw data (FID) has parameters such as mass or volume stored, these values will be set automatically in the appropriate fields on the SMILEQ widget. It is only compatible data that acquired by "qNMR seamless" function. (see 3.2.2)

4. Fill in the box at reference information area. Select the desired reference from the "**Name**" and enter the reference parameters required for the analysis (see section 2.2.)

5. Select the target analyte on column of Name that is the pulldown menu in "**Target Signal Table**".

6. After setting the calculation option, press the "**Apply**" button to view the result.

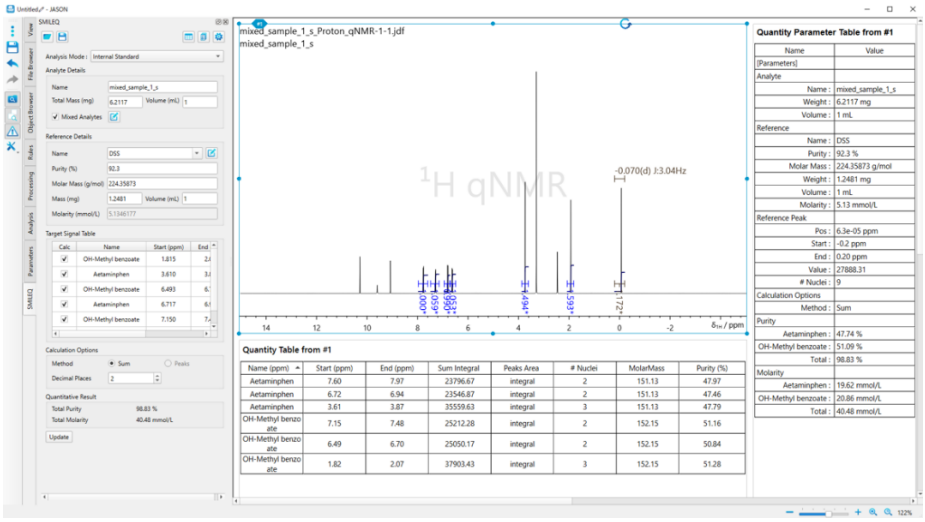


Figure 41: Mixture analysis

Mixture analysis is also available for Automatic analysis (with analytical file) and multiplespectrum analysis.

5 Results

The analysis results can be output as individual reports, summary reports, uncertainty reports (only for internal standard method), and reference reports (only for external standards). This chapter explains each report.

5.1 Individual Report

To create individual reports showing the results for each spectrum by using the Apply button in the SMILEQ widget or the Multispectrum SMILEQ tool. (see 2.6 for details of necessary information) Both internal and external standard methods are supported. The individual reports include the spectrum and related parameters, and the analysis results.

The average, SD and RSD for the purity are calculated and displayed at the bottom of the results summary.

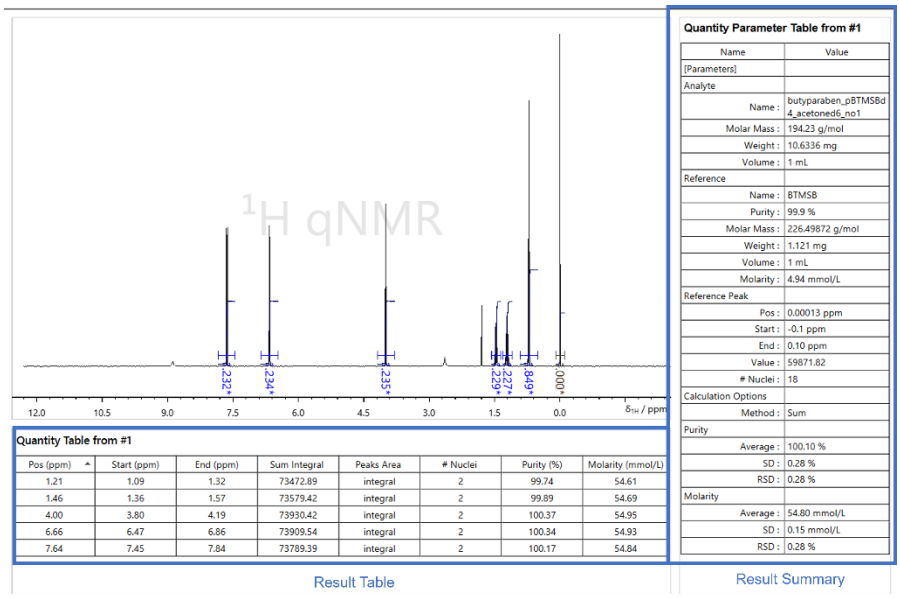


Figure 42 : Example of individual report

Results Table: The purity or molarity of each of the region is reported in this table.

Results Summary: A summary of the results for the analyte is reported here.

- If the regions of interest used in the quantification are defined using multiplet analysis in JASON, then the Peak Area estimate will be available. However, if the regions are defined as integrals only then it is not possible to use the peak area calculation.
- The result table can be changed to show or hide each parameter and display the color of the table by using the table tools panel that appears when the table is selected.
- The results table will only show the results for the regions defined in the multiplet details table that have the "**calc**" check box selected.

Explanation of Parameters

Result Table	
Name of Parameters	Description
Pos	Signal position (ppm)
Start	Start position of multiplet or integral region (ppm)
End	End position of multiplet or integral region (ppm)
Sum integral	Integral value by direction summation
Peak Area	When using multiplet, the value of area from peak deconvolution. In the case when the option is "sum", this parameter is shown as "integral".
Number of Nuclei	Number of Nuclei, e.g. protons, responsible for the signal
Purity	Purity value (%)
Molarity	Molarity value (mmol/L)

Result Summary	
Name of Parameters	Description
Analyte	Target Analyte
Name	Analyte name or anything is able to write
Molar mass	Molar mass (g/mol)
Mass	Mass (mg)
Volume	Prepared sample solution volume (mL) / this parameter will be calculated molarity.
Reference	
Name	Reference name that is prepared in Reference Editor
Purity	Possessed purity value of reference material (%)
Molar mass	Molar mass (g/mol)
Mass	Mass (mg)
Volume	Prepared sample solution volume (mL) / this parameter will be calculated molarity.
Molarity	Molarity (mmol/L)
Reference Peak	
Pos	Signal position (ppm)
Start	Start position of multiplet or integral region (ppm)
End	End position of multiplet or integral region (ppm)
Sum integral	Integral value by direct summation
Number of Nuclei	Number of Nuclei, e.g. protons, responsible for the signal
Method	Calculation option whether using Sum integral or Peak area from deconvolution
Delta Method	When qNMR seamless, the parameters are shown. qNMR analysis method, "Prescribed "means using template file for analysis

[Result]	
Name of Parameters	Description
Purity	
Average	Average value of purity (%)
SD	Standard deviation of purity value for all signals
RSD	Relative standard deviation of purity value for all signals
Molarity	
Average	Average value of molarity (mmol/L)
SD	Standard deviation of purity value for all signals
RSD	Relative standard deviation of purity value for all signals

5.2 Summary Report

To create summary reports in the report editor (see section 2.6 Report Editor for details of the necessary information). The statistical analysis results of each parameter using multispectrum are output as a report. Both the internal standard method and the external standard method are supported.

Summary Report (Purity)

Sample name	Pos (ppm)	Run 1 (%)	Run 2 (%)	Run 3 (%)	Avg. (%)	SD (%)	RSD (%)	Avg. (%)	SD (%)
butyparaben_pBT MSBd4_acetoned6 _no1	1.46	99.89	99.60	99.52	99.67	0.19	0.19	100.08	0.30
	4.00	100.37	100.43	100.40	100.40	0.03	0.03		
	6.66	100.34	100.31	100.27	100.30	0.03	0.03		
	7.64	100.17	100.27	100.12	100.19	0.07	0.07		
	1.21	99.74	100.00	99.80	99.85	0.14	0.14		
	Avg. (%)	100.10	100.12	100.02					
	SD (%)	0.28	0.33	0.36					
butyparaben_pBT MSBd4_acetoned6 _no2	1.46	99.65	99.53	99.69	99.62	0.09	0.09	100.11	0.32
	4.00	100.42	100.54	100.40	100.45	0.07	0.07		
	6.66	100.03	100.45	100.18	100.22	0.22	0.22		
	7.64	99.99	100.46	100.18	100.21	0.23	0.23		
	1.21	100.05	100.14	99.89	100.03	0.13	0.13		
	Avg. (%)	100.03	100.22	100.07					
	SD (%)	0.27	0.42	0.28					
butyparaben_pBT MSBd4_acetoned6 _no3	1.46	99.83	99.81	99.79	99.81	0.02	0.02	100.06	0.22
	4.00	100.21	100.16	100.21	100.19	0.03	0.03		
	6.66	100.20	100.30	100.21	100.24	0.06	0.06		
	7.64	100.02	100.60	100.00	100.21	0.34	0.34		
	1.21	99.99	99.81	100.10	99.97	0.15	0.15		
	Avg. (%)	100.05	100.14	100.06					
	SD (%)	0.16	0.34	0.18					

Summary Report (Purity) (continued)

Figure 43 : Example of Summary report (Purity)

5.3 Uncertainty Report

To create an uncertainty report in the report editor. Only the internal standard method is supported. The uncertainty report uses the calculation method of ISO24583. Settings such as coverage factor, number of digits displayed, and rounding method for values can be made in the dialog when you select creating uncertainty report in the Report Editor. (see section 2.6 Reference Editor for details of the necessary information).

Uncertainty of the Purity (according to ISO 24583)

Sample name	Pos (ppm)	Run 1 (%)	Run 2 (%)	Run 3 (%)	Avg. (%)	SD (%)	Avg. (%)	SD (%)	Avg. (%)	SD (%)
butylparaben_pBT MSBd4_acetoned6 _no3	1.46	99.83	99.81	99.79	99.81	0.02	100.08	0.19	100.09	0.01
	4.00	100.21	100.16	100.21	100.19	0.03				
	6.66	100.20	100.30	100.21	100.24	0.06				
	7.64	100.02	100.60	100.00	100.21	0.34				
butylparaben_pBT MSBd4_acetoned6 _no2	1.21	100.05	100.14	99.89	100.03	0.13	100.11	0.31		
	1.46	99.65	99.53	99.69	99.62	0.09				
	4.00	100.42	100.54	100.40	100.45	0.07				
	6.66	100.03	100.45	100.18	100.22	0.22				
	7.64	99.99	100.46	100.18	100.21	0.23				
butylparaben_pBT MSBd4_acetoned6 _no1	1.21	99.74	100.00	99.80	99.85	0.14	100.08	0.31		
	1.46	99.89	99.60	99.52	99.67	0.19				
	4.00	100.37	100.43	100.40	100.40	0.03				
	6.66	100.34	100.31	100.27	100.30	0.03				
	7.64	100.17	100.27	100.12	100.19	0.07				

Uncertainty Budget (according to ISO 24583)

	Source of uncertainty	Unit	Value (%)	Standard uncertainty (%)	Sensitivity coefficient	Relative standard uncertainty (%)
NMR experiments	Measurement repeatability	%	100.21	0.34	1.00	0.34
	Variations from different signals selected	%	100.08	0.31	1.00	0.31
	Variations from qNMR sample solution preparations	%	100.09	0.01	1.00	0.01
Purity of standard	The purity of the internal standard used	%	99.90	0.3	1.00	0.25
					Combined standard uncertainty (%)	0.53
					Coverage factor	2
					Expanded uncertainty (%)	1.05

Figure 44: Example of Uncertainty report

5.4 Reference Report

To create a reference report in the report editor, only the external standard method is supported. The reference standard information and statistical calculation results for each parameter when using multiple spectra are output as a report.

External Reference

Sample name	Pos (ppm)	Start (ppm)	End (ppm)	Proton	Integral Value
BA_DMSOd6_JEOL	7.90	7.85	7.98	2	16994.18
	7.58	7.53	7.66	1	8536.85
	7.46	7.41	7.51	2	16953.30
BA_DMSOd6_JEOL	7.91	7.85	7.98	2	17159.91
	7.58	7.53	7.66	1	8598.82
	7.47	7.41	7.51	2	17118.98
BA_DMSOd6_JEOL	7.90	7.85	7.98	2	16927.25
	7.58	7.53	7.66	1	8505.30
	7.46	7.41	7.51	2	16900.09

External Reference Summary

Sample Name	Reference Integral Value	Proton	Solvent Integral Value	Mass (mg)	Volume (mL)	Molarity (mmol/L)	Area/Proton	CCF	DCCF
BA_DMSOd6_JEOL_	42484.34	5	0.00	2.00	1.00	16.38	8496.87	1.93e-06	0.00e+00
BA_DMSOd6_JEOL_	42877.71	5	0.00	2.00	1.00	16.38	8575.54	1.91e-06	0.00e+00
BA_DMSOd6_JEOL_	42332.64	5	0.00	2.00	1.00	16.38	8466.53	1.93e-06	0.00e+00
							Avg.	1.92e-06	0.00e+00
							SD	1.27e-08	0.00e+00
							RSD	0.66	nan

Figure 45:Example of reference report