

PotencyMR 2.0

- Streamlines potency (% w/w) determination with the internal standard method

User Manual
Version 003



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1 Introduction

PotencyMR is a workflow for potency determination offering the design of methods and a straightforward and automated application of them. The potency determination is streamlined from sample deposition in the sample changer to the potency report.

This documentation also includes important experimental aspects about sample preparation and NMR parameters for acquisition and processing.

This documentation is written as an introduction containing a description of all important steps in potency determination with NMR.

1.1 Potency Determination

Potency determination consists of the determination of the % weight/weight of an analyte in a given substance. In this release, the method with an internal standard is implemented. The basics of the method is that a precisely known amount of the internal standard and of the substance are dissolved in an NMR solvent.

The qNMR potency (P) of the analyte is determined by the following equation:

$$Pa = Ps \times \frac{Ia}{Is} \times \frac{Ns}{Na} \times \frac{MWa}{MWs} \times \frac{Ws}{Wa}$$

where Pa, Ps = potency of analyte/internal standard; Ia, Is = integral area of analyte/internal standard from the NMR spectrum; Na, Ns = number of nuclei of integrals; MWa, MWs = molecular weight of analyte/internal standard; Wa, Ws = weight of substance/internal standard.

The workflow applies mostly to active pharmaceutical ingredients (API) which usually have a potency of 95% or higher. In this manual, the molecule of the API is known as the analyte. In favourable cases, lower potency chemicals can also be determined with PotencyMR, where at least one NMR signal of the analyte is well separated from the signal of the internal standard and from other signals from this analyte. A NMR frequency of a minimum of 300 MHz is recommended, however higher NMR fields usually help to solve an overlap between signals.

1.2 Glossary of Technical Terms used in this Manual

Internal standard or standard - compound used as internal standard, e.g. 1,3,5-trimethoxybenzene

Substance – assayed powder or liquid

Analyte – assayed compound in the substance

SOP – Standard Operating Procedure

Sino – signal/noise ratio

PotencyMR terms:

Reference – internal standard

Reference Data – stored information for internal standard integration and quantification

Batch – stored information for internal standard with its potency

Analyte Data – stored information for analyte integration and quantification

Analyte weight - weight of the substance in the sample

Method – stored information for analyte integration and quantification with preset analyte integrals

1.3 Preferred Integral Regions stored in Reference and in Method

For flexibility and reusability of integral region definition, the information about the internal standard and the methods are stored separately.

PotencyMR offers two ways to analyze the analyte, one is an automatic spectrum interpretation and the other uses methods.



Although it is obvious for NMR users, it is worth mentioning that NMR signal positions differ drastically from one NMR solvent to another, so a reference or a method is only valid for a given solvent. This manual shows that the function “peak snappings” allows small changes in sample composition to be managed, but not switching from one solvent to another.

1.4 IconNMR for Acquisition and CMC-assist for Method Development

PotencyMR functionalities related to acquisition, definition and application of methods from SOPs are directly found in the IconNMR interface.

PotencyMR functionalities related to method development are found in the CMC-assist interface. In CMC-assist, the spectrum can be visualized and used for method development.

2 Sample Preparation

Following sample preparation, some information will be needed to setup the potency determination, and these are listed in section [Needed Information from Sample for the Potency Determination Experiment](#) [► 7].

See also

 [Needed Information from Sample for the Potency Determination Experiment](#) [► 7]

2.1 Needed Information from Sample for the Potency Determination Experiment

The following information from the sample preparation step will be needed when setting up the potency determination experiment:

- The solvent used will be needed to setup the acquisition in IconNMR.
- Name of the reference in PotencyMR, e.g. MA_DMSO which is an example name that could suit a definition for maleic acid in DMSO.

If the reference of the internal standard that is used is not already available in PotencyMR, then a new reference must be created, as described in section [Create a New Reference for an Internal Standard in the QNMR Settings Window](#) [► 20] (Note that all of chapter [PotencyMR Workflow in IconNMR](#) [► 11] is worth reading before actually creating a reference).

- Batch name in PotencyMR.
- If a batch of this reference is not already available in PotencyMR, then a new batch must be created and must contain: the name of the batch and its potency (% w/w) as described in section [Create a New Reference's Batch for an Internal Standard in the QNMR Settings Window](#) [► 21].
- The amount of internal standard and of the substance (substance is simply referred to as analyte in PotencyMR) in the sample (expressed in mg). Note that it is important that the ratio is correct, as can be seen in the potency formula at the beginning of this document.
- If automated analysis of the analyte is being used, then a .mol file of the analyte is needed.
- Method name in PotencyMR, if using an existing method, e.g. Ibuprofen_DMSO (which is an example name for ibuprofen as analyte in DMSO).

If the desired method is not already available in PotencyMR, then the user must create a new method, as described in section [Create a Method in the QNMR Settings Window](#) [► 22].



Only general guidelines for a successful sample preparation for potency determination are given in this documentation. A good practice is to describe the sample preparation in a standard operating procedure (SOP) and to validate it. With PotencyMR, an SOP can be written for a range of analytes using the automated analysis, or for a specific analyte using a method. Once an SOP is first used in PotencyMR, the required details (reference, batch and method) are used, these are then stored for subsequent re-use.

See also

 [Create a New Reference for an Internal Standard in the QNMR Settings Window](#) [► 20]

- ▢ PotencyMR Workflow in IconNMR [▶ 11]
- ▢ Create a New Reference's Batch for an Internal Standard in the QNMR Settings Window [▶ 21]
- ▢ Create a Method in the QNMR Settings Window [▶ 22]

2.2 Choosing the Internal Standard

The measurement of a proton spectrum of the analyte without an internal standard is needed when working with a new analyte, in order to identify an internal standard that has at least one signal which does not overlap with any signals due to the analyte. Overlaps must be avoided as they would cause significant errors in the determination of potency.

The chemical shifts of the protons of the internal standard are usually known (approximately) for any given solvent, so there is no need to measure them separately.

When a suitable internal standard is chosen for a given analyte then the only sample to prepare is the mixture of internal standard and analyte.

2.3 Weighing the Internal Standard and the Analyte

As a general guideline, weighing is carried out by taring as this tends to minimize errors i.e. starting with an empty vial and without removing it during subsequent addition of the internal standard and of the substance.

The error on weighing depends on the amount which is weighed. As an example, for a balance with a stated repeatability of 0.03 mg, it is recommended to weigh out at least 10 mg. Note that during operation, the balance door has to be closed properly in order to avoid air currents affecting the scale pan.

The manual of the balance and/or an accuracy calibration certificate – if available – are sources of information to properly calculate the error on weighing.

NMR grade deuterated solvent is then added in order to reach the desired approximate concentration that is designed to be well above the limit of quantification for the NMR method (see section [Considerations about Signal to Noise Ratio \(sino\)](#) [▶ 9] for more details). By visual operation, ensure complete dissolution and homogeneity of the solution before transferring to the NMR tube. Sealing of the tube by flame sealing the end of the NMR tube or using parafilm is recommended in cases the sample would have to be kept for a period of time – refer to validation documentation.

In general, if the compound being weighed is hygroscopic, the potency result will be affected by the water uptake of the compound, from the time during the weighing when it is exposed to moisture in the air, until it is dissolved in the NMR solvent and the container is closed. NMR solvents such as DMSO can also absorb moisture from the air but that would not affect the potency determination as it is the weighing before dissolution that matters. The total water content of the sample could affect the integration of signals and then have an effect on the potency determination. As described in this paragraph, it is generally noted that the sample preparation conditions and also its duration can affect the potency determination.



Proper operation of the balance is out of scope of this manual. Please refer to the manual and to available calibration documents of your balance in order to find out the error contribution of weighing to your potency determination.

See also

- ▢ Considerations about Signal to Noise Ratio (sino) [▶ 9]

2.4 Considerations about Signal to Noise Ratio (sino)

The amount of internal standard and of analyte directly affect the sino, so it is worth mentioning here considerations about the error on the potency determination.

As found in qNMR guidelines, a good sino is essential for accurate integration. For integration errors of < 1%, a S/N of at least 250:1 is required. We have estimated from simulations that errors of < 0.05% require 10000:1.

If the substance is not available in sufficient amount to achieve the recommended sino, then the number of scans would have to be increased. Another option would be to use an NMR probe with a higher proton sensitivity (e.g. inverse probe, cryoprobe).

2.5 Solvent's Adduct to Eliminate Signals from Internal Standard and Analytes's Exchangeable Protons

Broad peaks originating from exchangeable protons (protons attached to polar atoms like O, N and S) of the internal standard and/or the analyte can contribute to the integral of neighbouring signals in the spectrum. This is a common source of errors in potency determination. Even if the tail of the broad exchangeable peak has a low amplitude, it can contribute significantly to neighboring integrals.

The exchangeable protons cannot be used for quantification in most cases as their integral most often has a very significantly smaller value than expected from the structure, due to chemical exchange. The techniques of sample preparation described in this section can help to remove exchangeable in cases where there is a doubt that a peak is an exchangeable proton or not, or if they overlap with a region of interest for integration.

To displace the exchangeable protons signals in the spectrum from the internal standard and of the analyte, one can add a small molecule in a significant amount relative to molecules having the exchangeable protons.

Usually the exchange rate is fast compared to the NMR time scale such that the resultant spectrum has a single broad resonance representing all of the exchangeable protons.

There are two known ways of achieving this which are considered good practice: adding D2O or adding trifluoroacetic acid (TFA).

2.5.1 Adding D2O

Here a significant amount of D2O is added to the solvent (e.g. dDMSO, CDCl3). The Deuterium atoms in the D2O are exchangeable and will exchange with the exchangeable protons of the internal standard and/or of the analyte.

In practice, the amount of D2O to add is about two drops which represents approximately 50 µl of D2O in 600 µl of dDMSO, when the sample contains about 10 mg of internal standard and analyte.

The following chemical exchange reaction will occur:



X atom is N, O or S.

If the internal standard contains exchangeable protons, the same mechanism will apply, e.g. with maleic acid and benzoic acid:



As a consequence of this exchange, the signal from HDO or H2O, is typically moved to around 4.0 ppm in dDMSO and is larger and broader than before adding D2O. The rest of the spectrum is free from exchangeable protons.

One has to be aware that in the vicinity of the water signal, there will be more uncertainty for integrating internal standard and/or analyte signals.

2.5.2 Adding Trifluoroacetic Acid (TFA)

Here, a significant amount of protonated TFA is added. The acidic proton will exchange with the exchangeable protons of the internal standard and/or of the analyte.

As a consequence, through chemical exchange, only one resonance will be observed for exchangeable protons. As the chemical shift of the carboxylic proton of TFA has a very high shift value around 15ppm, the resulting signal will be at a high shift value, therefore freeing most of the spectrum from signals due to exchangeables.

3 PotencyMR Workflow in IconNMR

3.1 Workflow

IconNMR is the interface for PotencyMR at the spectrometer. New users are referred to the IconNMR documentation ([TopSpin Help](#) | [Manuals](#) | [ICON-NMR Automation interface](#) or in IconNMR help)

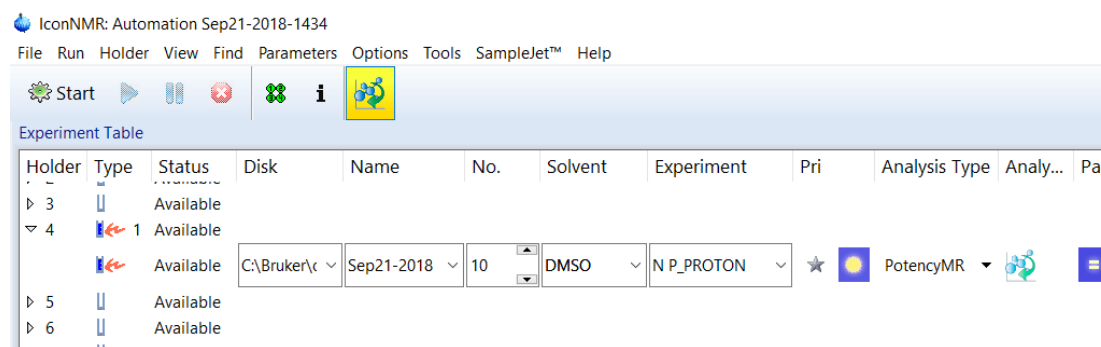
To setup a potency determination experiment, first create an experiment as described in section [Create a Potency Determination Experiment in IconNMR](#) [▶ 11] and secondly, enter the potency determination settings as described in section [Setup the Potency Determination Analysis Settings](#) [▶ 12].

See also

- ▶ Create a Potency Determination Experiment in IconNMR [▶ 11]
- ▶ Setup the Potency Determination Analysis Settings [▶ 12]

3.1.1 Create a Potency Determination Experiment in IconNMR

The first step in to create an experiment in IconNMR is in the row corresponding to the location of the sample in the sample changer. In the example shown below, the sample is in position 4.



Disk location and name of the dataset of the measured data are all set by the following columns: **Disk**, **Name**, and **No** (expno).

The solvent is selected from a list of solvents and in the case shown here, DMSO has been chosen as deuterated DMSO was the solvent used to prepare the sample. In case of adducts containing two drops of D2O for example, the solvent selected would still be the bulk solvent used by the method. Most of the common NMR solvents are already setup after instrument installation.

P_PROTON is the parameter set dedicated to potency determination and it is supplied with TopSpin standard build. Chapter NMR Parameters: Find and Modify NMR Parameters in the P_PROTON Parameter Set in this document deals with the acquisition parameters and the automation programs for acquisition and processing.

The button in the **Par** column allows to change NMR parameters for this experiment. Typically, the number of scans can be changed here. Adding a specific parameter like D1, in the Par can be done in [IconNMR configuration](#) | [User Settings](#) | [User Manager](#) | [User Specific Parameters Commands](#).



If the P_PROTON is not in the Experiment drop down menu then it should be added to the IconNMR configuration/User Settings/User Manager/Experiment List. For convenience, P_PROTON can be chosen as the default experiment in *IconNMR configuration/VerificationQuantification/PotencyMR*. This option is shown in [Potency Determination with Automatic Analyte Integration Using a Structure \[► 23\]](#) in another context.

See also

[Potency Determination with Automatic Analyte Integration Using a Structure \[► 23\]](#)

3.1.2 Setup the Potency Determination Analysis Settings

After having created an experiment in IconNMR, the potency determination settings can be easily adjusted using existing data about the reference, batch and analyte. With one more mouse click, the user can create new data using the **Edit Data** button.

3.1.2.1 Overview of the Analysis/Quantification Window

In the newly created IconNMR experiment, set the Analysis Type to PotencyMR (if not already done), and in the next column, click on the **Analysis/Quantification** button.

The window that appears contains a section **Reference Data** which is about the internal standard, a section **Analyte Data** which is about the analyte, and two options, namely **peak snappings** and **Repeat Experiment**.

In this window, the description of a sample is flexible by selecting the internal standard by its given reference name, and by its batch which has an identifier of the chemical actually used and its potency. Reference and batch are separated in order to easily switch between an internal standard of a given potency to another without recreating a copy of the reference.

Concerning the analyte, there are two ways to enter the integral and the proton number.

- Use integrals calculated by the analysis: this is a universal method for any analyte. A structure of the analyte needs to be provided as a .mol file or drawn.
- Use method: it is a method specific to a given analyte. The analyte's molecular mass, the integrals and their proton number are defined in the method.

This interface allows users to enter the weights of the internal standard and of the analyte.

The case shown below is an example of a valid automated analysis of the analyte:

Analysis/Quantification

Reference Data

Reference weight mg




Reference

Batch

Analyte Data

Analyte weight mg

☒ Use integrals calculated by the analysis ☐ Use method

Mol File   

☐ Peak snappings

☐ Repeat Experiment (Re-Insert Sample for this experiment/analysis)

Alternatively, a method can be used, as shown in the valid example below:

Analysis/Quantification

Reference Data

Reference weight mg

Reference

Batch

Analyte Data

Analyte weight mg

☐ Use integrals calculated by the analysis ☒ Use method

Method

☐ Peak snappings

☐ Repeat Experiment (Re-Insert Sample for this experiment/analysis)

Peak snappings checkbox: if ticked, integral regions defined for the reference will be snapped to the signal after measurement. This ensures that the integration region is centered on the desired singlet or multiplet, while keeping the width as defined. See [Peak Snappings](#) [33] in this manual for a description of the peak snappings function.

Repeat Experiment checkbox: if ticked, the sample will be ejected and reinserted for new tuning/matching and shimming steps. See [Repeat Measurements \[▶ 18\]](#) for a description of the repeat measurements.

See also

- Peak Snappings [▶ 33]
- Repeat Measurements [▶ 18]

3.1.2.2 Access to the QNMR Settings Window (by Clicking on Edit Data Button)

Clicking on the **Edit Data** button is possible if the user has the privilege of an IconNMR Supervisor user. This privilege can be given by a supervisor to another user in the **Icon Configuration | User Settings | User Manager/Permission**. The supervisor option is ticked in the example shown below.

Permissions

<input checked="" type="checkbox"/> Priority	<input checked="" type="checkbox"/> Parameter Edit
<input type="checkbox"/> Archive Data	<input checked="" type="checkbox"/> Exit (IconNMR)
<input checked="" type="checkbox"/> Supervisor	<input checked="" type="checkbox"/> Data Set Name Edit
<input type="checkbox"/> Essential Originator	<input checked="" type="checkbox"/> Originator
<input type="checkbox"/> Manual Lock/Shim	<input type="checkbox"/> Mail Spectrum PDF/PS
<input type="checkbox"/> Email Notification	<input type="checkbox"/> JDX DataMail
<input type="checkbox"/> JDX Copy	<input type="checkbox"/> Edit Lock/Shim/ATM
<input type="checkbox"/> ZIP Copy	<input type="checkbox"/> ZIP DataMail
<input type="checkbox"/> Print Spectrum	<input checked="" type="checkbox"/> Control Printing
<input type="checkbox"/> PDF Copy	<input type="checkbox"/> PNG Copy
<input checked="" type="checkbox"/> Verification/Quantification	

The reason for restricting access to the Edit Data window is generally to control who can create and delete reference, batch and methods.



The **Edit Data** availability is restricted to users with IconNMR supervisor privileges, in order to have two types of users, a user responsible of creating and deleting reference, batch and methods, and another user that would simply apply them (for example a users who is an operator in a quality control environment).

3.1.2.3 QNMR Settings Window

In the QNMR Settings window, and similarly the analysis/quantification window, the selection of already existing reference, batch and the method is possible and closing it using the **Apply** button will update the analysis/quantification window.

There are two additional features in this window that are not found in the example described above:

- activate the peak snappings for the current measurement.
- create and delete reference, batch and the method.

Please note that it is not possible to modify existing reference, batch and method in order to keep the possibility to reuse them as they were defined.

In the QNMR Settings window, the integral regions for both the internal standard and the method, are defined by left and right ppm borders and proton content. In the case of the internal standard, it is required to define all regions and to set them (indicated by the tick mark in the image below) if they are used, or not, for integration. For the method, only the integrals used for integration need to be defined.

In the example shown below, the method **MySulindac** is selected:

The QNMR Settings window is divided into two main sections: Reference Data and Analyte Data.

Reference Data:

- Weight: 10.3 mg
- Reference: TMB_DMSO
- Molecular Mass: 168.192 g/mol
- Batch: DFGHZTRE, potency 99.97%
- Integral table:

Identifier	Left ppm	Right ppm	Protons	Quant
Tmba	6.2200	5.9400	3	✓
Tmbb	3.8940	3.6130	9	

☒ Snap reference signals to integrals in spectrum

Analyte Data:

- Weight: 11.2 mg
- Method: MySulindac
- Molecular Mass: 356.41 g/mol
- Integral table:

Identifier	Left ppm	Right ppm	Protons
15_s	7.4545	7.3807	1
14_dd	7.0663	6.9575	1
10_m	6.7544	6.6449	1

Buttons: Apply, Cancel

In another example shown below, the selected method is **Use integrals calculated by the analysis** and the molecular mass of the analyte will be calculated from the .mol file provided:

The QNMR Settings window is divided into two main sections: Reference Data and Analyte Data.

Reference Data:

- Weight: 10.3 mg
- Reference: TMB_DMSO
- Molecular Mass: 168.192 g/mol
- Batch: DFGHZTRE, potency 99.97%
- Integral table:

Identifier	Left ppm	Right ppm	Protons	Quant
Tmba	6.2200	5.9400	3	✓
Tmbb	3.8940	3.6130	9	

☐ Snap reference signals to integrals in spectrum

Analyte Data:

- Weight: 11.2 mg
- Method: Use integrals calculated by the analysis
- Molecular Mass: Calculated from mol file
- Integral table: (Empty)

Buttons: Apply, Cancel

3.1.3 Comparison of Calculated Analyte's Integral and Method

As mentioned above, there are two ways in PotencyMR to analyze the analyte's integrals:

- With an existing method, or by creating one.
- Automatically calculated from a .mol file and the measured spectrum, with **Use integrals calculated by the analysis**

In the following table, the pros and cons of each approach are listed, in order to help the user make a choice depending on their expectations about the potency determination.

Characteristics of the two methods	Automatically calculated	Method
------------------------------------	--------------------------	--------

Signals selected are the same in replicate and repeat measurement	Most often no	yes, always
Development time for a new analyte	None if they are no known impurities in the sample because it takes some time to figure it out and to enter them	About 20 minutes, excluding measuring the spectrum
Symmetry and width of integrals	Usually not symmetric integrals and the width is mostly unrelated to the peak's width	As good as defined in the method
Peak snappings	Not available	Available as an option
The targeted accuracy on potency by the whole workflow from experiment to report.	About 2%	About 0.5% or better if the automated processing performs well
Signals of analyte's exchangeable protons are used for quantification	Best effort by the algorithm to avoid it, especially when exchangeable integral differs a lot, but this could happen and increase the error on potency	Not possible unless exchangeable are by error in the method

The calculated integrals are very useful when working with a new analyte, as a first step towards development on a method for that analyte. The result from an automated analysis is likely to have more errors on potency than with a method, but by using three replicates, the average potency usually gives a robust accuracy by averaging the integration of various analyte's integrals. Careful inspection of the integral regions used in the results is recommended.

3.1.4 Submit the Experiment and Access Results

Once measurements are setup, the experiments can be submitted by a single mouse click. The global IconNMR **Start** needs also to be clicked once, before or after submission. Please see the IconNMR manual if needed.

The output files are named *qnmrOutput.xls* and *qnmrOutput.csv* and can be accessed directly from IconNMR after the measurement has completed by right clicking on an experiment in the **Preceding Experiments** panel at the bottom of the IconNMR window. CMC-assist can be opened from the same place and the spectrum visualized. Please open with CMC-assist or TopSpin when the processing has been completed.

An example of an excel report is shown below (only half is shown here as the full image is in too large to fit this document):

Dataset										Preparation	Wt analyte [mg]	Wt IS [mg]	N IS* [mmol]	Potency IS [%]				
C:/Daten/videoSulindac/sulindac_tmb/sulindac_tmb/10/pdata/1										1	3	2	0.0119	99.97				
internal standard										TMB_DMSO								
* mmols of IS, corrected to its potency																		
** Areas are normalized by # of protons																		
Mw IS [g/mol]										168.192								
Mw analyte [g/mol]										356.41								
Potency = Potency IS * (Averaged Area analyte / Area IS) * (Wt IS / Wt analyte) * (Mw analyte / Mw IS)																		
Area** IS Left and Right Area** analyte Left and Right p1																		
Potency IS [%]	Tmba	from	to	10_m	from	to	14_dd	from	to	Averaged Area analyte	SD Area analyte	N analyte [mmol]	Potency [%]	SD Potency [%]	Warnings			
99.97	1.49657	6.2382	5.9582	1	6.7802	6.6767	1.00898	7.0857	6.977	0.99577	7.4226	7.3488	1.00158	0.00551	0.008	94.52	0.52	

Integration regions used for potency determination are reported: left and right ppm border and their normalized value to the number of protons. Deviations between integrals and potency are reported. Some warnings can appear in the report in case of overlap between the reference and the analyte's integrals. Large deviations are indicated the corresponding cells in this table report being filled in with red.

3.1.5 Replicates Measurements for a Combined Potency Report

In PotencyMR, replicate measurements corresponding to two or more samples that have been prepared and analysed in exactly the same way. For these replicates, only the weights of the internal standard and of the analyte will differ.

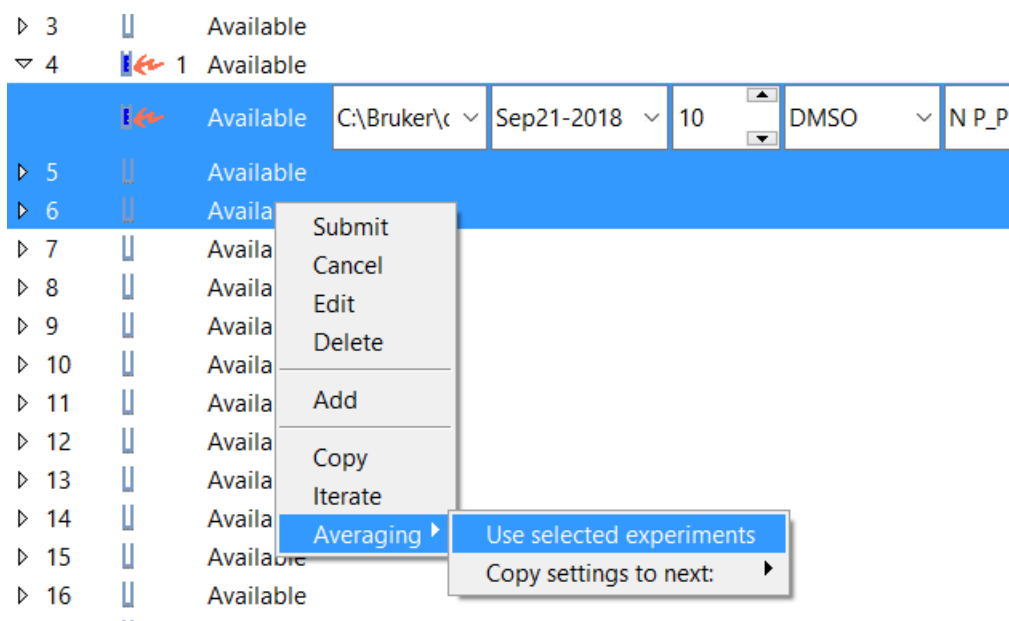
Replicate measurements allow the user to assess the precision of the potency determination, both in terms of sample preparation and in terms of the NMR measurement itself. The averaged potency and the residual between potency values are available in the combined report, which is generated automatically.

3.1.5.1 Setup Replicate Measurements

There is a convenient way to setup replicate measurements:

- Select the line of the first experiment that has been completely setup, and others lines where the other(s) replicate samples are, in the sample changer.
- Right click and select **Averaging / Use selected experiments** and - that will create new experiments in a manner that is very much like using the **Copy** in IconNMR but here, the experiments will be linked and a combined potency report will be automatically generated.

If the samples are in consecutive holders, then the other option of **Averaging / Copy settings to next** can also be used (see below).



3.1.5.2 Replicate Measurements Combined Report

This excel file and a csv report are automatically generated for each and every replicate measurement.

There is one line for each replicate with the same information as a single measurement (see [Submit the Experiment and Access Results \[▶ 16\]](#)). The average (98.01 in the case below) and the relative standard deviation (0.39) between replicate potency determinations are given in the right hand side of the report (also shown below).

Potency [%]	RSD Potency [%]
98.28	
98.28	
97.48	
98.01	0.39

The files are named *qnmrCombinedResults.xls* and *qnmrCombinedResults.csv* and can be accessed directly from IconNMR after the measurement has been completed by a right clicking on an experiment in the **Preceding Experiments** at the bottom of the IconNMR window.

See also

Submit the Experiment and Access Results [▶ 16]

3.1.6 Repeat Measurements

Repeat measurements consist of repeating entirely the measurement of a given sample to assess the precision of the NMR measurement itself. Between the measurements, the sample is ejected and re-inserted in the magnet and all of the measurement steps are reconducted: (tuning/matching, shimming) before the subsequent measurement.

In order to setup Repeat measurements:

- Setup all of the first experiment and tick **Repeat Experiment** in the **Analysis/Quantification** window (show below).

Analysis/Quantification

Reference Data

Reference weight mg

Reference

Batch

Analyte Data

Analyte weight mg

☐ Use integrals calculated by the analysis ☒ Use method

Method

☐ Peak snappings

☒ Repeat Experiment (Re-Insert Sample for this experiment/analysis)

Edit Data

OK

- Set the desired number of repeated measurements minus one, and click on Add in IconNMR. The added experiments will be exactly the same. Note that the expno is incremented as this value it is needed to record experiments in different folders.
- The repeat experiments, shown below, are ready to be submitted.

Holder	Type	Status	Disk	Name	No.	Solvent	Experiment	Pri	Analysis Type	Analysis/Quantification
3	U	Available								
4	U	Queued	C:\Bruker\Top	Sep25-2018	10	DMSO	dii N P_PROTON	★	PotencyMR	TMB_DMSO 99.97% DFGHZTRE 12.3 11.6 MySulindac
	U	Queued	C:\Bruker\Top	Sep25-2018	11	DMSO	dii N P_PROTON	★	PotencyMR	TMB_DMSO 99.97% DFGHZTRE 12.3 11.6 MySulindac
	U	Queued	C:\Bruker\Top	Sep25-2018	12	DMSO	dii N P_PROTON	★	PotencyMR	TMB_DMSO 99.97% DFGHZTRE 12.3 11.6 MySulindac
5	U	Available								

After having made repeat measurements, a combined report can be manually generated in order to record the repeat measurements results in a single excel report showing the relative standard deviation (RSD) between experiments. The combined report will look the same as for the combined report on replicates (as shown in [Replicate Measurements Combined Report \[18\]](#)). The manual combine report generation function is described in this manual in section [Generate a Combined Report Manually \[30\]](#).

The RSD reflects the precision of the whole NMR measurement including tuning, acquisition and processing and this is the recommended way to assess the precision of a method. To estimate the errors on automatic processing the datasets can be all reprocessed manually in TopSpin or CMC-assist and then the combined report can be regenerated manually.

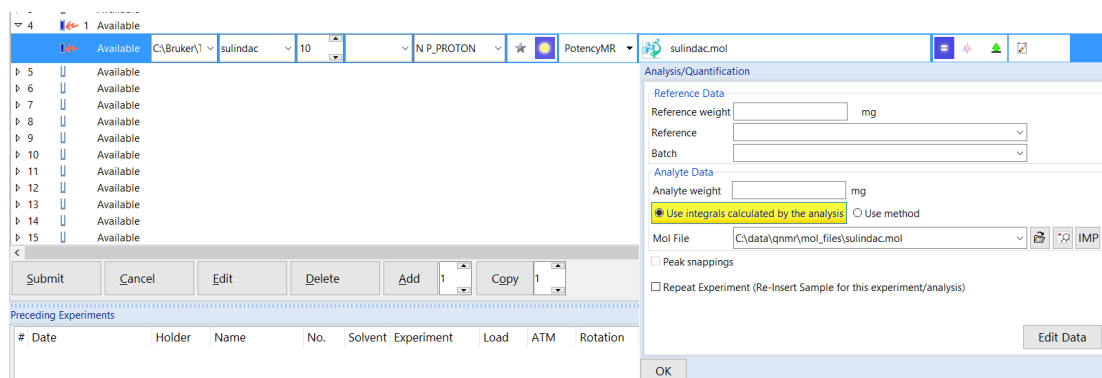
See also

- [Replicate Measurements Combined Report \[18\]](#)

Generate a Combined Report Manually [[▶ 30](#)]

3.1.7 Simplified Way to Handle .mol File in IconNMR

There is a way to automatically browse to the mol file. For this, the name of the experiment in IconNMR has to match an existing .mol file, as in the example shown below:

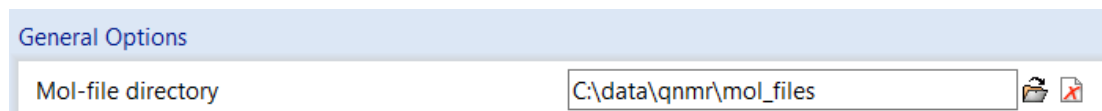


It is automatically filling the path to the .mol file in the **Analysis/Quantification** window. If the experiment name is sulindac, and if the file *sulindac.mol* exists in the molfile folder, then a path like the following one shown below will be automatically entered:

C:\data\qnmr\mol_files\sulindac.mol

The configuration of the path to the mol file folder is done in IconNMR:

IconNMR configuration/VerificationQuantification / General Options

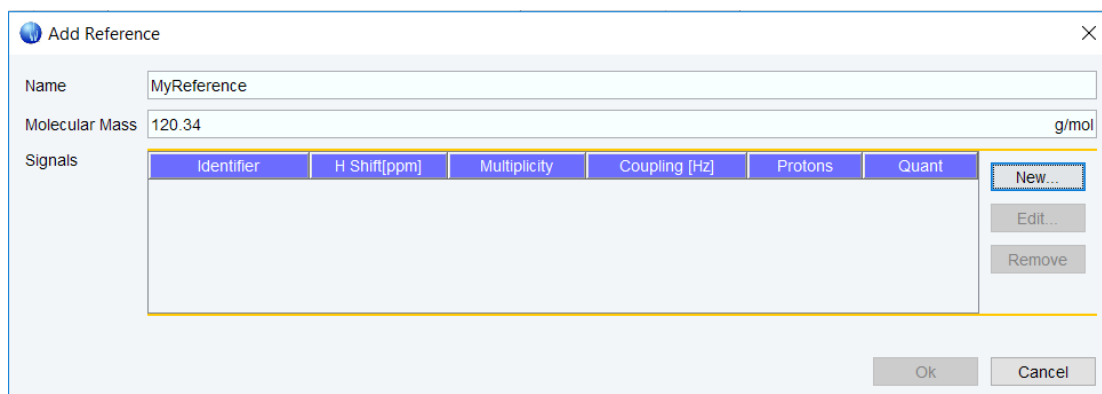


3.2 Create a New Reference for an Internal Standard in the QNMR Settings Window

The internal standard is named “reference” in PotencyMR. They can be created in the QNMR settings window from Edit Data in IconNMR or from Potency calculation in CMC-assist. It is possible to create a new reference by entering all values (click on the + button), or by cloning an existing reference (click on button with an arrow). The “-” button will delete the selected reference. If the window is closed using **Cancel**, then any changes will be discarded.



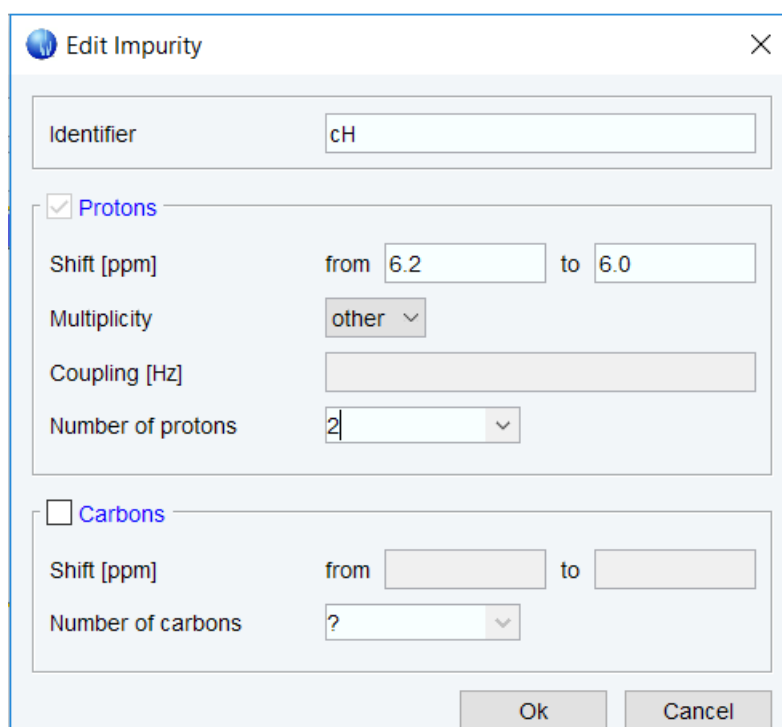
This action also requires the molecular mass of the reference, and for each of the integral regions the left and right borders (in ppm) and the proton number for each integral region. The integral regions used for quantification are selected by ticking, after that the region is created.



The 'Add Reference' dialog box contains the following fields and controls:

- Name:** MyReference
- Molecular Mass:** 120.34 g/mol
- Signals:** A table with columns: Identifier, H Shift [ppm], Multiplicity, Coupling [Hz], Protons, and Quant.
- Buttons:** New..., Edit..., Remove, Ok, and Cancel.

For each new integral regions entered using the **New...** button, the next window will need to be completed. Not all information is useful, only shifts and number of protons, the rest will not be used as it is a general dialog for impurities used for entering impurities in the automatic analysis of analyte in CMC-assist.



The 'Edit Impurity' dialog box contains the following fields and controls:

- Identifier:** cH
- Protons (checked):**
 - Shift [ppm]: from 6.2 to 6.0
 - Multiplicity: other
 - Coupling [Hz]:
 - Number of protons: 2
- Carbons (unchecked):**
 - Shift [ppm]: from to
 - Number of carbons: ?
- Buttons:** Ok and Cancel.

3.3 Create a New Reference's Batch for an Internal Standard in the QNMR Settings Window

New batches are created from an existing reference. Select the reference and click on the “+” button. The “-” button will delete the selected batch. If the window is closed using Cancel, then any changes will be discarded.

Batch Create or select a batch + -

After clicking on “+” a new window will appear asking for a batch name and, the batch potency.

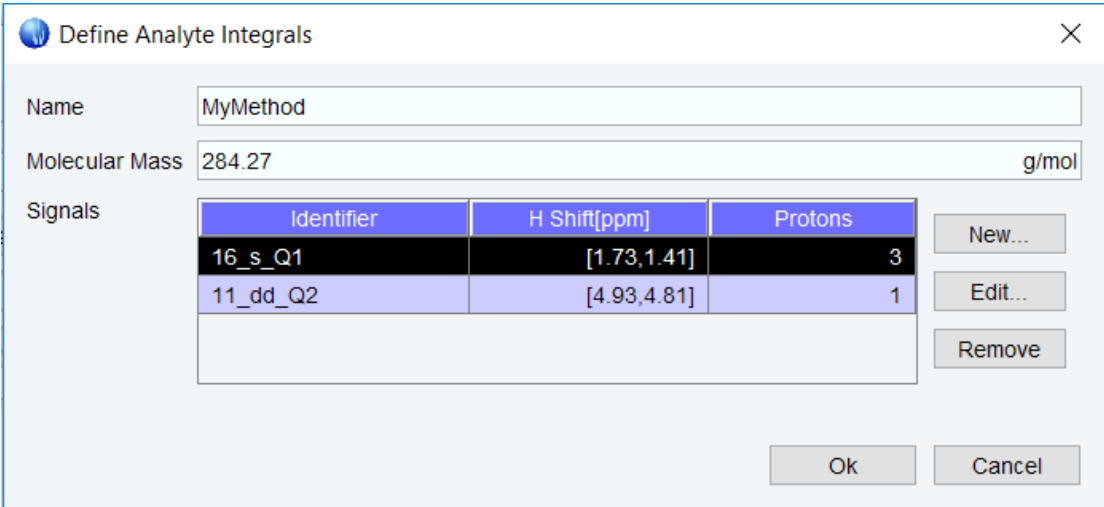
3.4 Create a Method in the QNMR Settings Window

This requires its molecular mass, and for each of the analyte’s integral regions: the left and right borders (in ppm) and the proton number for each integral region. Methods can be created by entering values directly, as shown below, or using an analyte spectrum in CMC-assist, as described in [Create a Method from Spectrum](#) [29].

Like for the reference described earlier, the new method is either entered with the “+” button or by cloning an existing method (button with arrow).

Method MySulindac + ▼ -

When clicking on “+”, a new window will allow the user to set the method name, to define integral region with proton number, and to enter the molecular mass of the analyte. An example is show below:



Define Analyte Integrals

Name:

Molecular Mass: g/mol

Identifier	H Shift[ppm]	Protons
16_s_Q1	[1.73,1.41]	3
11_dd_Q2	[4.93,4.81]	1

New... Edit... Remove

Ok Cancel



Method described in an existing SOP containing the integral regions to use for quantification can be directly entered as described in the corresponding section in IconNMR and used to measure in automation.

See also

[Create a Method from Spectrum](#) [29]

3.5 Potency Determination with Automatic Analyte Integration Using a Structure

Using this way of analyzing the analyte, the integral regions and their proton numbers of the analyte are automatically determined.

The automated analysis can also provide a consistency check if that option is selected in the *IconNMR configuration/Verification Quantification/PotencyMR*, as shown below:

PotencyMR



☒ PotencyMR
An automatic potency analysis is carried out after the measurement.
For more information click on this text

☒ Activate structure verification

☐ Set P_PROTON as default experiment

As shown in the example below, the structure can be entered by browsing to a .mol file or by drawing the structure by clicking on the icon representing a molecule.



☒ Use integrals calculated by the analysis ☐ Use method

Mol File   **IMP**

The IMP button allows users to define known impurities in the sample. That is needed if an impurity or an unusual solvent signal that has a peak position close to the position of an integral from the analyte. For convenience, the internal standard is automatically considered as an impurity in the automated analysis and there is no need to define it here. In section [On the Importance of Defining Known Impurities \[▶ 24\]](#), an important example of defining a known impurity for potency determination is shown.

Please refer to the CMC-assist manual chapter [IconNMR Configuration and Start \[▶ 37\]](#), for more details about the CMC automated analysis.

See also

-  On the Importance of Defining Known Impurities [▶ 24]
-  IconNMR Configuration and Start [▶ 37]

3.5.1 Consistency Check

The consistency check gives a consistent or inconsistent status about the structure provided. Open the measurement in CMC-assist to visualize the results and the assignments.

3.5.2 Number of Analyte's Integral that are Selected for Quantification

The automated analysis will select one to three integral regions for the analyte. When the algorithm is unsure about the consistency (i.e. the inconsistent case) then only one integral is selected for quantification. When the algorithm finds that it is a consistent structure then up to three integrals are selected.

3.5.3 On the Importance of Defining Known Impurities

When the amount of water in the sample is high enough and/or exchangeable protons are in the internal standard or the analyte, the water signal can shift significantly towards the left hand side of the spectrum. Such water signals are not handled by the automated analysis and would have to be defined as impurities.

A typical case where this is often needed is when applying a D2O adduct as described in section [Adding D2O](#) [9].

4 Method Development and Manual Potency Determination in CMC-assist

This manual has shown that PotencyMR within IconNMR enables the whole workflow of potency determination, from measurement to report.

CMC-assist offers additional possibilities:

- Manual potency determination on a measured spectrum
- Visualize the spectrum with the integral regions from a potency determination run by IconNMR
- Create a method from a spectrum



Concerning manual potency determination:

Only the green integral regions indicated in the spectrum matters for potency determination.

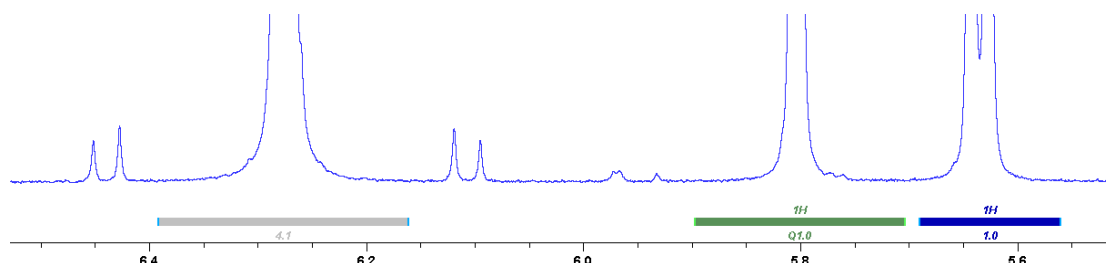
The integral regions for the internal standard are always taken from the reference definition, never from the impurity integral (grey in the spectrum). It is therefore useless to manually resize the internal standard/impurity/grey integral for a potency calculation.

Below, the PotencyMR relevant features of CMC-assist are described, please also read the CMC-assist manual for more detailed information.

4.1 Integral Regions in CMC-assist

In CMC-assist, integral regions are displayed with horizontal bars shown in the spectrum below. There are different types of integral and the bars have the following color coding:

- Blue: from analyte and not used for quantification
- Green: from analyte and used for quantification. A "Q" label is also added before the integral value.
- Grey: internal standard and impurities



Change the integral region by a right clicking on an integral bar and by selecting properties, the integral properties window will appear:

Edit Integral Properties

Status: Substance

Proton Content: 1.0

Integral value: 1.0203

Shift [ppm]: from: 5.8983 to: 5.7037

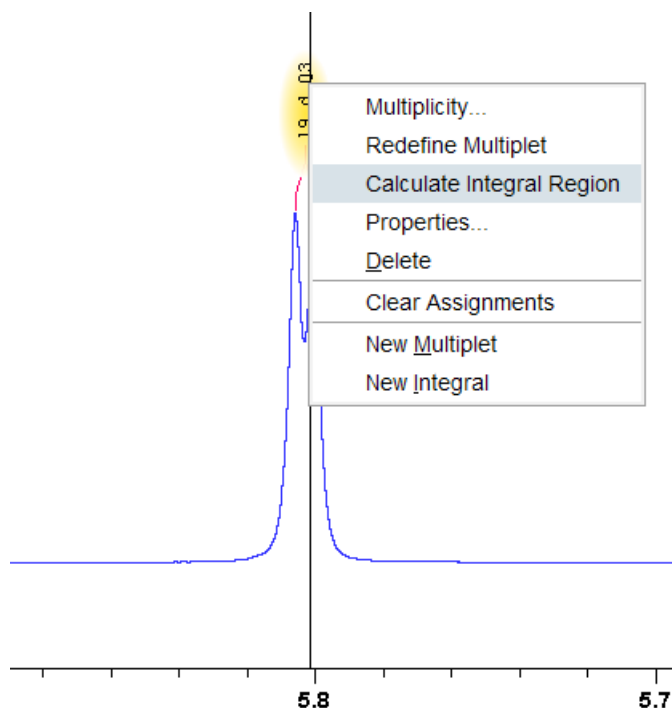
Annotation:

☒ Use this integral for quantification

OK Cancel

- The **Use this integral for quantification** check box determines if this analyte's integral region is used for potency determination.
- Set the left and right ppm values manually by typing in the text boxes, or use the spinner which has an increment of 0.05 ppm.
- Change the type (**status** in this window) of integral (**top bar** in this view) to choose if this integral region is from the analyte (**substance** in this window) or an impurity.
- Change the proton number (**proton content** in this window) of the integral as appropriate.

For an accurate integral definition, it is possible to define the left and right ppm positions by right clicking on a CMC-assist multiplet and by selecting **Calculate Integral Region**, as shown below. Please refer to the CMC-assist manual for creating a multiplet (As a hint: a right click in the spectrum display will allow the **New Multiplet** option).



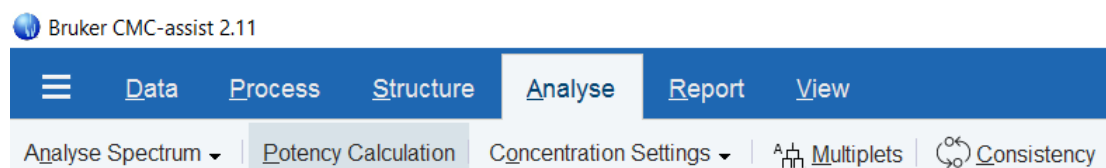
This functionality is based on calling a Python script that is opened for modification by the user. The version provided by default allows users to define an integration region by a multiplication factor of the full width at maximum, which is recommended good practice in potency determination. More information is given in section [Calculate Integral Regions in CMC-assist Using a Python Script \[33\]](#).

See also

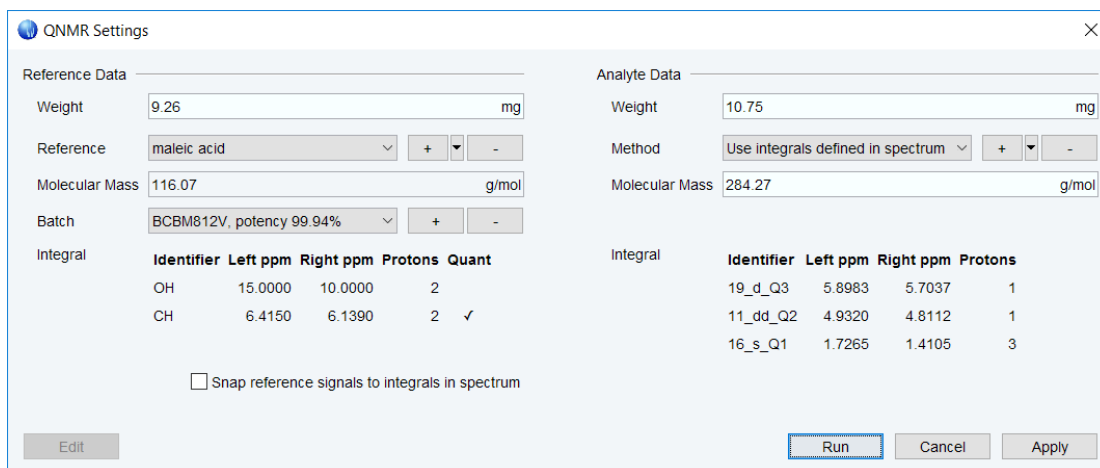
[Calculate Integral Regions in CMC-assist Using a Python Script \[33\]](#)

4.2 Manual Potency Calculation

The interface for manual potency determination can be opened by clicking on Potency Calculation as shown below:



Then the QNMR Settings window will open. It is the same QNMR Settings as described in [QNMR Settings Window \[14\]](#) above when clicking on Edit Data button in IconNMR, except that the option to use analyte's integral from the displayed spectrum is possible, as shown in the case below:



QNMR Settings

Reference Data

Weight: 9.26 mg

Reference: maleic acid

Molecular Mass: 116.07 g/mol

Batch: BCBM812V, potency 99.94%

Integral

Identifier	Left ppm	Right ppm	Protons	Quant
OH	15.0000	10.0000	2	
CH	6.4150	6.1390	2	✓

☐ Snap reference signals to integrals in spectrum

Analyte Data

Weight: 10.75 mg

Method: Use integrals defined in spectrum

Molecular Mass: 284.27 g/mol

Integral

Identifier	Left ppm	Right ppm	Protons
19_d_Q3	5.8983	5.7037	1
11_dd_Q2	4.9320	4.8112	1
16_s_Q1	1.7265	1.4105	3

Buttons: Edit, Run, Cancel, Apply

After clicking on **Run**, the potency report will be updated and the integral regions in the spectrum view will be automatically updated according to the QNMR Settings for both the internal standard and the analyte. The multiplets and the assignments that already exist remain unchanged.

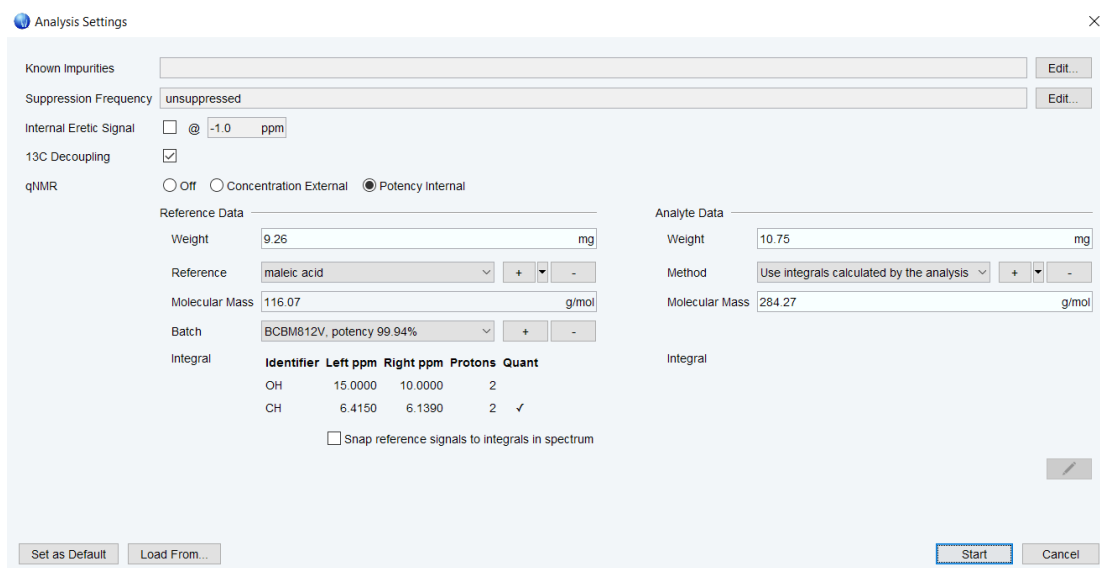
See also

 QNMR Settings Window [▶ 14]

4.3 Manual Potency Determination with Automatic Analyte's Integration Using a Structure

The automatic analysis of the analyte can be carried out in CMC-assist in the same way as from IconNMR as described in [Potency Determination with Automatic Analyte Integration Using a Structure](#) [▶ 23]. To access this functionality, open the CMC-assist menu **Analyse | Analyse Spectrum | Analysis Settings** and the following window will appear. Set the radio button **qNMR** to **Potency internal**.

The QNMR Settings are the same as from IconNMR, except that the known impurities are entered in the same window (see the top row in the image below).



Analysis Settings

Known Impurities: Edit...

Suppression Frequency: unsuppressed Edit...

Internal Eretic Signal: ☐ @ -1.0 ppm

¹³C Decoupling: ☒

qNMR: ☐ Off ☐ Concentration External ☒ Potency internal

Reference Data

Weight: 9.26 mg

Reference: maleic acid

Molecular Mass: 116.07 g/mol

Batch: BCBM812V, potency 99.94%

Integral

Identifier	Left ppm	Right ppm	Protons	Quant
OH	15.0000	10.0000	2	
CH	6.4150	6.1390	2	✓

☐ Snap reference signals to integrals in spectrum

Analyte Data

Weight: 10.75 mg

Method: Use integrals calculated by the analysis

Molecular Mass: 284.27 g/mol

Integral

Buttons: Set as Default, Load From..., Start, Cancel

See also

 Potency Determination with Automatic Analyte Integration Using a Structure [\[► 23\]](#)


4.4 Create an Internal Standard Definition (Reference) from a Spectrum

This is a different procedure than for the method because a reference integral cannot be directly read from a spectrum into the QNMR settings.

It is possible to do it, if needed, in three steps:

- define the integral region of the internal standard in the spectrum
- right click on the integral bar to read the left and right ppm borders (section [Integral Regions in CMC-assist \[► 25\]](#))
- create a new reference manually (section [Create a New Reference for an Internal Standard in the QNMR Settings Window \[► 20\]](#)).

See also

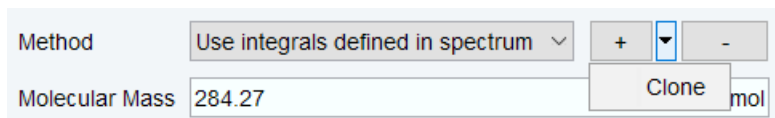
 Integral Regions in CMC-assist [\[► 25\]](#)

 Create a New Reference for an Internal Standard in the QNMR Settings Window [\[► 20\]](#)

4.5 Create a Method from Spectrum

With the integral region for quantification defined in the spectrum (i.e. they are shown in green), then use the **Use integral from spectrum** in the QNMR settings window.

Then a method can be created by clicking on the button with the arrow and this step will then clone the integral from the spectrum into a method:



Then a new window opens (the same as shown in section [Create a Method in the QNMR Settings Window \[► 22\]](#)), and the method's name has to be defined. The analyte's integral are then automatically filled with the integrals from the spectrum. Further manual modifications of integral regions are possible before clicking on **Run** or **Apply** that would both save the newly created method. Alternatively, click on **Cancel** to cancel all the modifications carried since this window was opened.



Methods defined in CMC-assist are available from IconNMR. This is the recommended way to create methods from an analyte's spectrum in CMC-assist and then to use them from IconNMR for further measurements using the method.

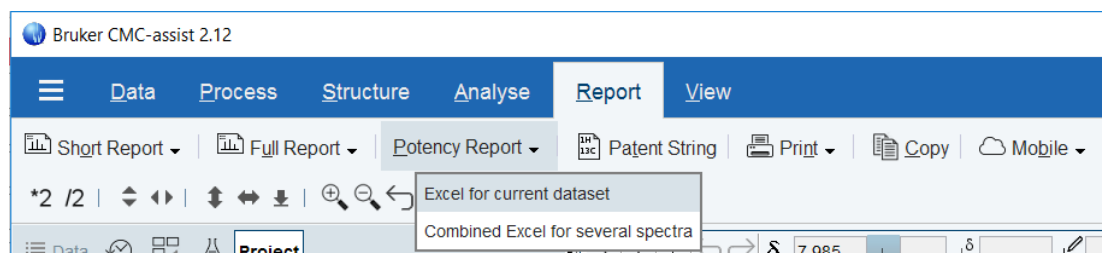
See also

 Create a Method in the QNMR Settings Window [\[► 22\]](#)

4.6 Potency Report

The potency excel report is regenerated each time a potency calculation is carried out. It is stored in the dataset as described in section [Submit the Experiment and Access Results \[▶ 16\]](#).

To open the excel potency report from the CMC-assist, click on the **Report** menu and then on the **Potency Report** submenu. As shown below, the report and the combined report can be opened. This simply opens the existing reports, it does not create the report, only a potency calculation would do that.



See also

Submit the Experiment and Access Results [▶ 16]

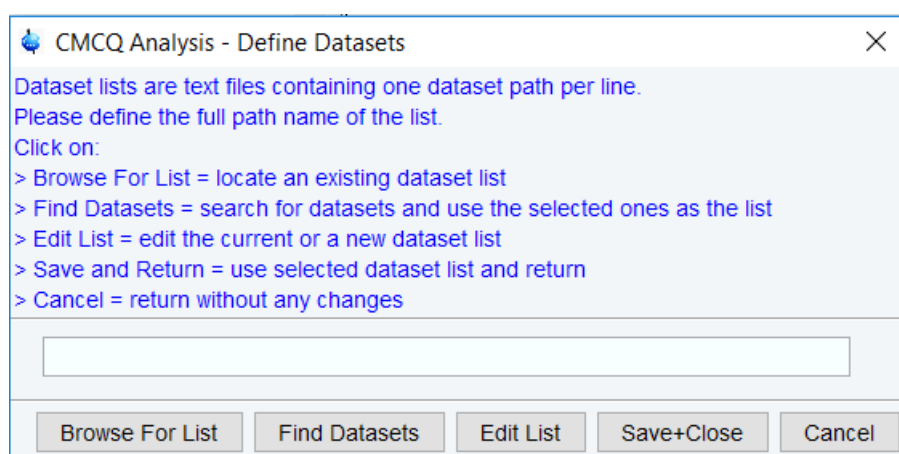
4.7 Generate a Combined Report Manually

The combined report is generated manually by using the following command that runs in TopSpin and CMC-assist:

qnmr combineResults

Please note that this command will only run if a dataset is open.

The following window will popup:



- The first option in this window is to Browse for a list of paths to the procno of desired experiments, also called a Serial Processing List (SPL) in TopSpin.
- The second option will call the TopSpin command **find** that will enable the user to build an SPL by exploring the disk. The **find** command is described in the TopSpin documentation **Help | Commands**.

- The third option will open a text editor to build the list manually by entering the paths to each of the procno of the experiments.
- **Save+Close** will generate the combine report in each of the paths in the SPL.
- **Cancel** will quit the combined report generation.

5 Description of Others PotencyMR Functionalities

5.1 Peak Snappings

The peak snapping functionality applies optionally to integral regions which are predefined for the reference and for the method. Peak snappings doesn't apply to the automatic analyte analysis.

The peak snappings is available for PotencyMR in IconNMR and in the CMC-assist.

Within the defined integral region, the centroid of the signal is detected and used as the new center of the snapped integral region. The width of the integral region is therefore not changed. This is important for integration as this width is likely to have been carefully chosen according to its full width at half maximum.

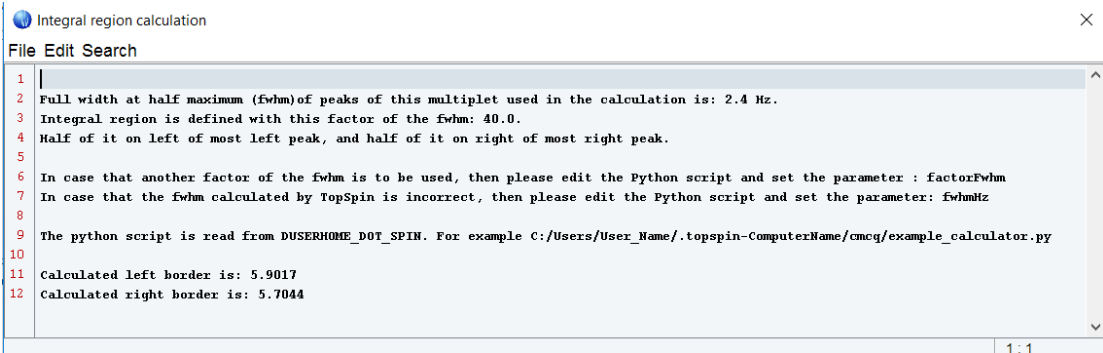
Peak snappings is offered as a way to run replicate measurement with the same method and the same reference for the internal standard. Only slight changes are expected in the signal due to different amounts of internal standard and of analyte in the sample, but they can be handled properly with the peak snapping with the general objective of achieving a higher accuracy and precision in the potency determination of replicates.

When using the same internal standard with different analytes, more obvious changes are expected in the position of the signals of the internal standard, therefore the peak snapping is highly recommended in such cases.

Changes of solvent are not handled by the peak snappings, as the signals are expected to drastically change their position in the spectrum.

5.2 Calculate Integral Regions in CMC-assist Using a Python Script

After the calculation of the integral using "Calculate integral region" as described in [Integral Regions in CMC-assist](#) [▶ 25], the integral is created in the spectrum view. Also, a report is displayed (as shown below). This report is generated by a python script and its content can be modified or not shown at all, by modifying the Python program. In this report, it is possible to check if the full width at half maximum was correctly determined by TopSpin as the value is given at the start of this report (see below).



```

1 Full width at half maximum (fwhm) of peaks of this multiplet used in the calculation is: 2.4 Hz.
2 Integral region is defined with this factor of the fwhm: 40.0.
3 Half of it on left of most left peak, and half of it on right of most right peak.
4
5
6 In case that another factor of the fwhm is to be used, then please edit the Python script and set the parameter : factorFwhm
7 In case that the fwhm calculated by TopSpin is incorrect, then please edit the Python script and set the parameter: fwhmHz
8
9 The python script is read from DUSERHOME_DOT_SPIN. For example C:/Users/User_Name/.topspin-ComputerName/cmcq/example_calculator.py
10
11 Calculated left border is: 5.9017
12 Calculated right border is: 5.7044
  
```

The python script is editable and located in the domain user's folder:

users document folder/.topspin/cmcq/example_calculator.py.

This file is created automatically when using **Calculate integral region** for the first time.

It is recommended to use this functionality by having the CMC-assist open, and the python script opened in a text editor such as notepad++ for example. That makes it easy to evaluate factors such as fwhm until a common value can be identified for the internal standard and for the analyte.

The spectrum points are available as an input so a more advanced analysis can be programmed if needed (please inspect the python script itself, as documentation is provided).



The full width at half maximum is from the same code as in TopSpin peak picking. Reliable results are only obtained with singlets or multiplets having a lorentzian lineshape. A user defined fwhm can be introduced directly in the python script.

See also

Integral Regions in CMC-assist [▶ 25](#)

5.3 TopSpin Integral Region Export/Import

This section refers to importing integral region, left and right ppm borders, not the values themselves.

Export to TopSpin of CMC-assist integral regions shown in the spectrum, can be useful to do a more advanced integration, for example with local baseline correction, than the integration implemented in PotencyMR. This process has the additional advantage that the TopSpin spectrum report can be used.

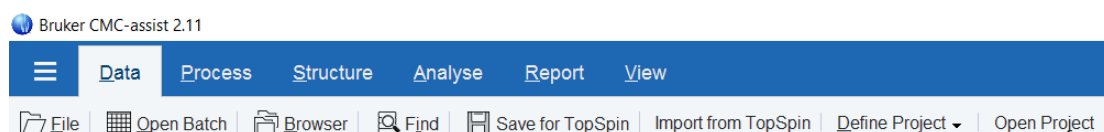
Import from TopSpin can be useful if integration regions are already available in TopSpin format, in order to create a method for PotencyMR in CMC-assist.



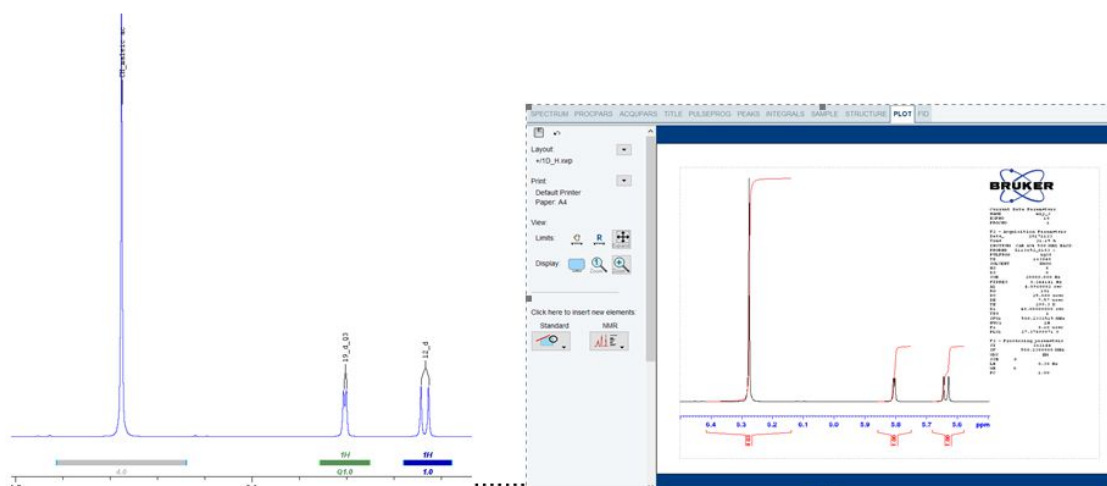
Bias and slope and local baseline correction in TopSpin is not supported in CMC-assist or in PotencyMR. All integral regions in PotencyMR relate to left and right positions, expressed in ppm.

5.3.1 Export to TopSpin

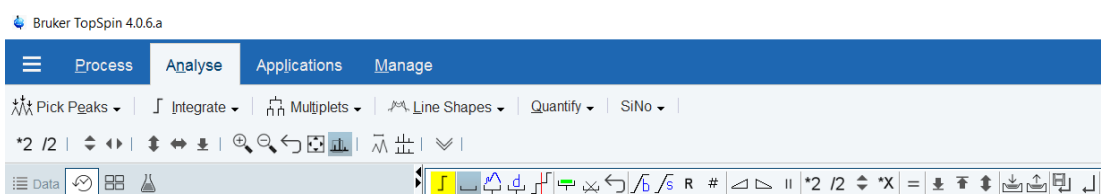
Integral regions defined in CMC-assist can be exported to TopSpin using **Data | Save for TopSpin**, as shown below. This simply writes text files into the dataset that can later be read by TopSpin when opening the dataset. The file responsible for the integral region display in TopSpin is *intrng* which contains left and right ppm borders of the integrals.



One usage would be to use the TopSpin reporting instead of the CMC-assist pdf reporting of the spectrum (as shown below).



Another use for this function is the integrate module (see below) in TopSpin as shown below.



The buttons **b** and **s** (in the above image, the 9th and 10th buttons from the left) allows use of bias and slope in the integral calculation. The third button from the left is used to create a new integral region with an automatic linear baseline correction from first point to last point.

The integrate module is described in TopSpin documentation ([TopSpin Help | Manuals | User Manual | section 12.3 Interactive integration](#)).

5.3.2 Import from TopSpin

Import from TopSpin is done using the CMC-assist data menu which is shown in section [Export to TopSpin \[► 34\]](#). The TopSpin *intrng* file is used to redefine integral region in the CMC-assist.

If bias and slope were defined in TopSpin, they are not imported in CMC-assist because they are not yet supported. In CMC-assist, it is currently not possible to change bias and slope, and not possible to visualize their effect in the integral trail whereas it is possible in TopSpin.

See also

[Export to TopSpin \[► 34\]](#)

6 IconNMR Configuration and Start

To start using PotencyMR from IconNMR, IconNMR needs to be configured using the TopSpin command **iconc**, and then to be started with the command **icona**.

Only an IconNMR superuser can open the IconNMR configuration, which then offers the following configuration possibilities:

- IconNMR user management. Access to P_PROTON or others parameter sets can be granted to specific existing users, as described in the note in section [Create a Potency Determination Experiment in IconNMR](#) ► 11].
- Supervisor privilege can be granted to specific existing users
- The language used in IconNMR can be set in **Options**.
- An important factor to configure is an optimal shimming for each system. This can be set in IconNMR configuration: **Automation/Lock/Shim Options/Solvent and Probe Dependencies**. Please read 3.11 and 3.12 of the IconNMR setup manual for proper instructions.

Please refer to the IconNMR documentation for more information about configuring IconNMR.

See also

- 📄 [Create a Potency Determination Experiment in IconNMR](#) ► 11]

7 NMR Parameters: The P_PROTON Parameter Set Family

NMR parameters for quantitative methods must be chosen with great care, because they can cause significant impact on the accuracy of the method. The internal standard method, used in PotencyMR, has the potential to reach a high degree of accuracy (trueness and precision – typically 0.5 %, although 0.1 % is possible).

Table “[Table 71](#) [▶](#) [39](#)” lists the parameters sets that have been included in the Bruker library for use with PotencyMR.

Name	Pulse Sequence	Description
P_PROTON	zg	¹ H-qNMR experiment with 90deg excitation pulse
P_PROTON30	zg30	¹ H-qNMR experiment with 30deg excitation pulse
P_PROTON_IG	zgig	¹ H{ ¹³ C}-qNMR experiment with 90deg excitation pulse
P_PROTON30_IG	zgig30	¹ H{ ¹³ C}-qNMR experiment with 30deg excitation pulse

Table 7.1: Bruker library parameter sets for use with PotencyMR



Parameters may need to be adapted to a given spectrometer and to a given sample/method in order to obtain optimal results with PotencyMR. The P_PROTON parameter set family has been optimized for room temperature probes and a spectrometer frequency of 300MHz or higher.

The parameters that are most important for quantitative NMR will be put into context below. They will be listed as they are named in TopSpin together with their default values.

7.1 Pulse Sequence

The default pulse sequences used by the P_PROTON parameters set family are listed in Table “[Table 71](#) [▶](#) [39](#)”.

7.1.1 Pulse Excitation Angle

The parameter sets with a 90deg excitation pulse (P_PROTON and P_PROTON_IG) will provide the best signal to noise. The 30deg pulse versions ((P_PROTON30 and P_PROTON30_IG) feature a broader excitation profile and hence, are more suited for qNMR methods where the internal standard and the analyte signal are strongly separated in the spectrum, especially at higher fields.

7.1.2 ¹³C Decoupling

For each excitation pulse, there is also a version with ¹³C decoupling. These versions can be used if ¹³C satellite signals from additional signals overlap with the integral regions (see [Figure 7.1](#) [▶](#) [40](#)). If this is not the case, the use of ¹³C decoupling is not recommended.

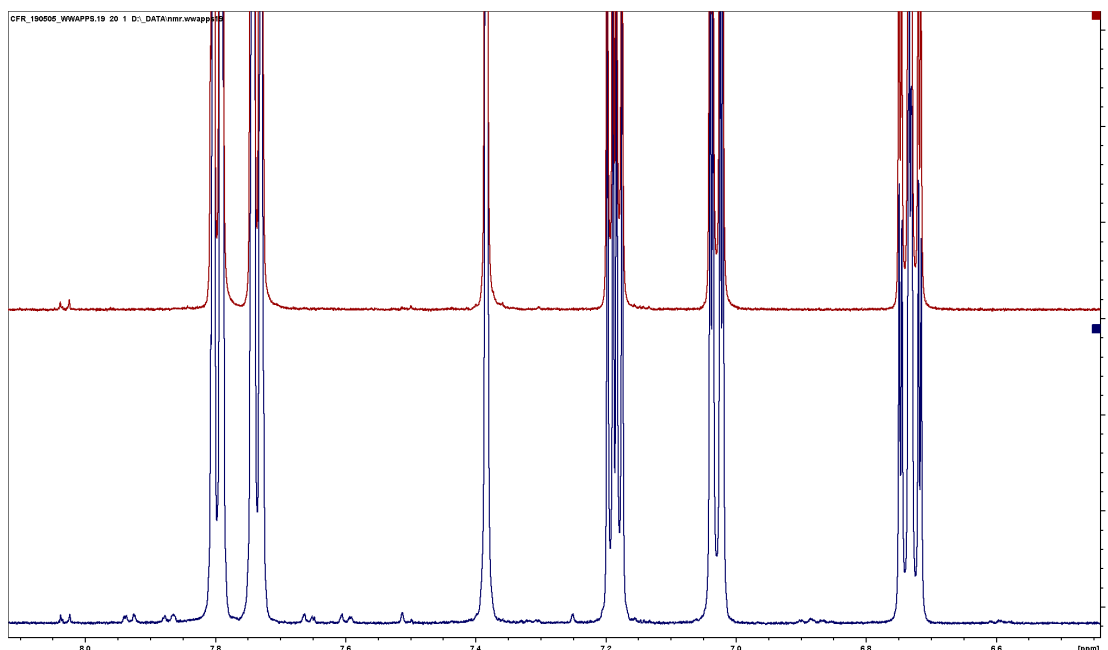


Figure 7.1: Aromatic region of the ^1H -NMR spectrum of sulindac (600 MHz). Bottom: standard ^1H -NMR spectrum. Top: ^1H -NMR spectrum with ^{13}C decoupling.

The ^{13}C decoupling is implemented with bi-level adiabatic composite pulse decoupling using a SmoothedChirp shape, which is automatically adjusted to the spectrometer frequency using WaveMaker. If the experiments are performed via IconNMR the WaveMaker command is automatically executed by the acquisition AU program (AUNM).

- Shape type: SmoothedChirp
- Pulse length: 2.5ms / 1.25ms
- Smoothing: 25%
- Q-Factor: 2.5
- Number of points: 4000
- CNST63: [240ppm] RF bandwidth of the adiabatic sweep. The default value of 240ppm will result in a qNMR compatible decoupler bandwidth of approximately 145ppm. The parameter CNST63 can be used to adjust the decoupler bandwidth to the analytical method. Note that increasing the decoupler bandwidth will increase the RF power with a potential to damage the NMR probe!

7.2 Relaxation

For the accuracy of qNMR experiments it is most important that all nuclei contributing to the NMR signals used for the analysis have relaxed to the state of full (i.e. $\geq 99,9\%$) equilibrium magnetization. This process is characterized by the T_1 relaxation time of a signal. It is strongly recommended to determine the maximum T_1 relaxation time of a sample as part of the method development process. See the Dynamics Center manual for further information.

- D1: [60s] Relaxation delay.

If this parameter is set to low the integrals obtained for the NMR signals may be underestimated, resulting in a systematic error of the method. As a guideline $D1 \geq 7 \times T_1$ is recommended for experiments with a 90deg excitation pulse.

7.3 Digital Resolution

In order to obtain reliable integral values from an NMR spectrum it is important that the spectra have been acquired and processed with sufficient digital resolution – in other words, there are enough data points to represent the signal. If the signals are narrow, more data points will be needed. If the experiments are performed via IconNMR the acquisition AU program (AUNM) will automatically adjust the parameters that will define the digital resolution. This calculation is based on the minimal FWHM that is expected in the spectrum.

- SI: Size of the Fourier transformed spectrum, that is the total number of points in the spectrum.
- TD: Number of points acquired in the FID. The number of complex data points is TD/2.
- SW: [20ppm] Spectral width, which is the width of the spectrum. It is recommended that peaks (including broad peaks) are completely included in the spectral width for better processing.
- AQ: Acquisition time. For experiments using ^{13}C decoupling a limit of $\text{AQ} \leq 2.5\text{s}$ will be set by the acquisition AU program (AUNM) in order to protect the NMR probe.

AQ, TD and SW are related by $\text{AQ} = \text{TD} / (2 \cdot \text{SWH})$. SWH (in Hz) corresponds to SW (in ppm).

- MINIMAL_FWHM: [0.5 Hz] Minimal FWHM that is expected for at least one signal in the spectrum. This parameter needs to be changed in the acquisition AU program (AUNM).

7.4 Signal-to-Noise

The signal-to-noise of the NMR spectrum will depend on the overall sensitivity of the instrument, the concentration of the sample and the excitation pulse angle.

- NS: [16] Number of scans. Adjust the number of scans of the experiment to ensure sufficient signal-to-noise for all signals used for quantification. In order to calculate the signal-to-noise ratio in a spectrum, use the TopSpin command “.sino”.

7.5 Automatic Data Acquisition

As already mentioned the automatic data acquisition with IconNMR is using an AU program to control the experiment.

- AUNM: [au_zg_qNMR] default acquisition AU for experiments without ^{13}C decoupling
 1. Adjust TD, AQ and SI for the required digital resolution.
 2. Perform automatic receiver gain adjustment (RGA) with reduced D1.
 3. Start acquisition.
- AUNM: [au_zgig_qNMR] default acquisition AU for experiments with ^{13}C decoupling
 1. Adjust TD, AQ and SI for the required digital resolution. Limit $\text{AQ} \leq 2.5\text{s}$.
 2. Call WaveMaker to calculate adiabatic shapes.
 3. Perform automatic receiver gain adjustment (RGA) with reduced D1 and AQ.
 4. Start acquisition.

7.6 Data Processing

Similar to the acquisition AU program IconNMR is using a second AU program AUNMP to control the automatic data processing.

- AUNMP: [cmc_proc1h_qNMR] default processing AU for all PotencyMR experiments

1. Perform exponential apodization (EM).
2. Calculate Fourier transform spectrum (FT).
3. Perform automatic zero-order phase correction (APK0).
4. Perform automatic phase correction (APK).
5. Perform automatic baseline correction (ABS).
6. Calculate Hilbert transform to restore imaginary part after baseline correction (HT).

7.6.1 Window Functions

Before the Fourier transformation the FID is usually multiplied with a window function as the first processing step. The default for the P_PROTON parameter sets is to use an exponential window function with a small line broadening factor LB. This will slightly increase the signal-to-noise and most importantly reduce sinc-wiggles in the foot the signals. Alternatively, a Gaussian window function may be used for certain applications.

- LB: [0.1 Hz] Line broadening factor of the exponential window function.

Please read the manual “TopSpin Processing Commands and Parameters” for more information.

7.6.2 Automatic Phase Correction

Several automatic phase correction algorithms are available in TopSpin. An automatic phase correction approach is recommended in order to avoid bias introduced by the operator. As shown above multiple automatic phase correction commands can be combined to improve the performance.

- APK0: robust command for 0th order phase correction.
- APK/APKS/APKM: alternative versions for 0th order and 1st order phase correction.
- APBK -f: 2nd generation algorithm that combines automatic phase and baseline correction.

Please read the manual “TopSpin Processing Commands and Parameters” for more information.

7.6.3 Automatic Baseline Correction

Make sure that baseline correction applied after the phase correction.

- ABS: Automatic baseline correction with a fifth order polynomial over the entire spectrum width.
- ABSF: Alternative to ABS that is only applied within the limits set by ABSF1 and ABSF2.

Please read the manual “TopSpin Processing Commands and Parameters” for more information.

7.7 How to Adjust Parameter Sets

To access the NMR parameters of e.g. P_PROTON, enter the TopSpin command **new** and read the parameter set P_PROTON. This will create a data set based on the stored parameters.

Create New Dataset - new

Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type. For multi-receiver experiments several datasets are created. Please define the number of receivers in the Advanced field.

Dataset

NAME: myQnmrMethodTemplate

EXPNO: 10

Directory: C:\Bruker\TopSpin4.0.6\examdata

☐ Open in new window

Parameters

☐ Use current parameters

☒ Read parameterset: P_PROTON [Select]

☐ Set solvent: DMSO

Additional action

☒ No additional action

☐ Execute getprosol

☐ Keep parameters: P 1, O1, PLW 1 [Change]

Advanced

Number of datasets (receivers): 1

Title:

OK Cancel More Info... Help

The acquisition parameters are found in the ACQUPARS tab, processing parameters in the PROCPARS tab, respectively. Alternatively typing a parameter name in the TopSpin console (e.g. SI) will pop up an editable text window.

Once the parameters have been adjusted accordingly a new parameter set may be written with the command **wpar <myQnmrMethod> all**.

Please read the TopSpin help/User manual for more information about parameter sets.

8 FILCOR Optimisation for a Data Processing

Optimising the FILCOR parameter is required to facilitate the phase correction of the measured spectra, in situations where processing is automatic or manual. It only makes sense when using a parameter set that has the acquisition parameter DIGMOD set to baseopt, which is the case for the P_PROTON.

It is increasingly important to do it with the sensitivity of the probe. A high sensitivity probe has a higher Q-factor and therefore has a longer ringing time, so that the damping of the pulse is finished. During this time, data acquisition is not possible and therefore the FID has missing points. These missing points are of course corrected automatically (although not perfectly way) and this correction is better when the FILCOR is optimized. As a consequence, the FILCOR correction effect is more obvious when using a cryoprobe compared with the situation when using a room temperature probes.



Filcor optimisation is an important precaution for improved processing of the data. It is important to set the parameter “pre-scan delay DE” correctly i.e. not too short and not too long in order to ensure that the spectrometer starts recording when the last pulse is just damped enough. DE is usually set during installation of the probe and applies to all parameter sets.

The protocol is carried out in TopSpin. Only a NMR superuser can change the FILCOR value. It is recommended to redo this FILCOR optimisation each time the probe and/or associated cables are changed. It is also recommended to re-do the FILCOR optimization when the solvent has a significantly different polarity, for example DMSO versus D2O. DMSO is recommended, but sometimes the analyte is not soluble in DMSO and D2O has to be used as the NMR solvent. The optimisation steps are:

- Insert a sample with signals across a wide chemical shift range, preferably using the solvent to be used for potency determination.
- Open the Spectrometer Parameters window with the TopSpin command **edscon** and set the FILCOR value to 0.
- Measure you sample with the P_PROTON in IconNMR. Simply use the Analysis Type set to No analysis.
- Open the spectrum in TopSpin. Reprocess the spectrum using the TopSpin command **ef**.
- Manually phase the spectrum using both zero and first order phasing. Record the first order phase value (positive value).
- Use the equation below to calculate the FILCOR value (FILCOR (filter delay correction); PHC1 (first order phase correction); DW (dwell time)).

$$\text{FILCOR } (\mu\text{sec}) = (\text{PHC1}(\text{degrees}) * \text{DW}(\mu\text{sec})) / 180$$

- Set the FILCOR value in the Spectrometer Parameters window (**edscon**).
- Re-measure the same sample in the same way, ideally the first order phase correction should not be needed anymore. The 0th order phase correction will still be needed.

9 Command Line QNMR

When executing PotencyMR from IconNMR using a method, a TopSpin command is used to determine the potency. It is possible to write scripts to perform potency determination in batch mode.

9.1 On any Dataset in TopSpin or CMC-assist

As an advanced option, it is possible to run a potency calculation via the command line. This can be especially useful when re-analyzing a batch of spectra that have already been measured. For this, it is needed to go a bit deeper in the PotencyMR implementation by describing where the reference, batch and method are stored and how to use them in the command line.

With a dataset open in TopSpin or CMC-assist, applying a method is possible using a command line. The UUID of the batch and of the method are required for this.

Each batch has a UUID (a unique identifier). The UUID for a batch can be found in the file: *internalStandardLibrary.xml*

Below is an example of the batch section of this xml file where the UUID for the batch is highlighted in red:

```
<internalStandardBatches>
<internalStandardBatch id="fde0d072-4540-492e-b53e-8cbb7a511205" potency="0.9997"
batchNumber="DFGHZTRE">
<internalStandard reference="../../internalStandards/internalStandard"/>
</internalStandardBatch>
</internalStandardBatches>
```

The UUID for the method can be found in the file:

presetAnalyteIntegralListLibrary.xml

This is an example of this xml file where the UUID for the method is highlighted in yellow:

```
<QnmrAnalyteMethod version="1.3">
<name>MySulindac</name>
<id>1c8f4df8-4190-47d8-a82a-5309cc61f278</id>
<molecularMass>356.41</molecularMass>
<integralList>
<QnmrIntRng>
<leftPpm>6.754449660425326</leftPpm>
<rightPpm>6.644920032659794</rightPpm>
<protonNumber>1</protonNumber>
<name>10_m</name>
</QnmrIntRng>
<QnmrIntRng>
<leftPpm>7.066258238632489</leftPpm>
```

```
<rightPpm>6.957491352007388</rightPpm>
<protonNumber>1</protonNumber>
<name>14_dd</name>
</QnmrIntRng>
<QnmrIntRng>
<leftPpm>7.454493479110592</leftPpm>
<rightPpm>7.380660136717115</rightPpm>
<protonNumber>1</protonNumber>
<name>15_s</name>
</QnmrIntRng>
</integralList>
</QnmrAnalyteMethod>
```

These two xml files are located in the users document folder – *folder/.topspin/cmcq*.

An example of a valid syntax of the command is:

```
qnmr potency analysis batchId fde0d072-4540-492e-b53e-8cbb7a511205 analyteId
1c8f4df8-4190-47d8-a82a-5309cc61f278 referenceWeight 10.1 analyteWeight 11.4
peaksnapping false
```

The weight of the reference and of the analyte have to be set according to the sample. Peak snappings can be used or not.

This command automatically generates the PotencyMR excel/csv report.

In case reprocessing is needed, with different LB, or phase and baseline correction for instance, please refer to the TopSpin Commands manual.

Commands can be grouped in a TopSpin macro, an “au” program or a python script. This is described in the TopSpin user manual.

9.2 On a Dataset Having Already Valid QNMR Settings

In some cases the qnmr settings were set before and are fine. The qnmr settings are stored in the dataset in the file:

QnmrUserInput.xml

The command **qnmr** does the potency calculation using the existing QNMR settings, and the excel report is updated.

The command **qnmr** only works in CMC-assist, not in TopSpin.

The typical use case of this command is when the dataset is reprocessed

10 Contact

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