Potency Determination
Quick Start

Sample Preparation

Prepare a **sample** containing the **analyte** and the internal reference standard (**reference**). The following information will be needed when setting up the potency determination experiment:

- Purity of the reference
- Weight of reference and of analyte in mg
- Structure of the analyte (mol file)
- Sample's solvent. A tip to get rid of exchangable protons: add D_2O (50ul in 600ul dDMSO)

For each reference's integral region:

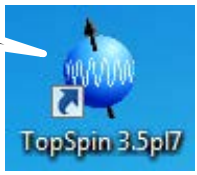
- The left and right borders in ppm
- The proton content
- The multiplicity (optional)

Optional: prepare and measure a ^1H spectra of the analyte and the reference separately, in order to identify regions with no overlapping between the analyte and the reference. Overlap would lead to large errors.

Automated Potency Determination: Measurement

Start TopSpin*:

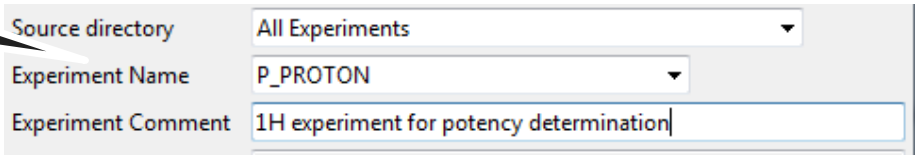
1



Start IconNMR with the TopSpin command: *iconc*

Add the P_PROTON experiment to the desired user:

2



Activate *FastLane* (in the *CMC-Verification*) tab

3

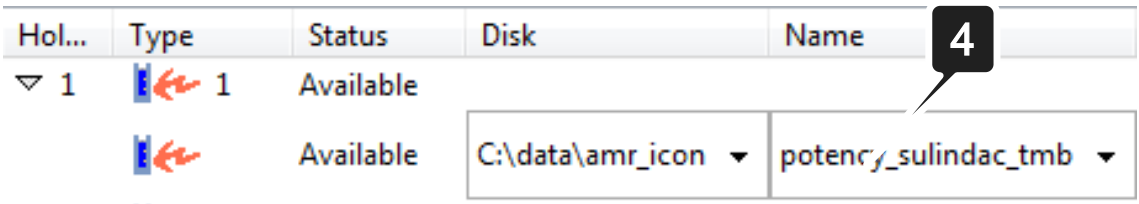
Note:

- Refer to IconNMR reference guide for more details
- Extra license is not required when working at the spectrometer

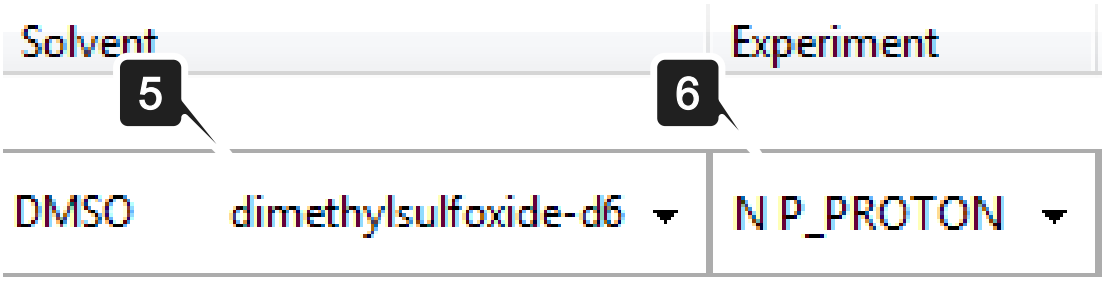
* TopSpin 3.5pl7 or above

Automated Potency Determination: Measurement

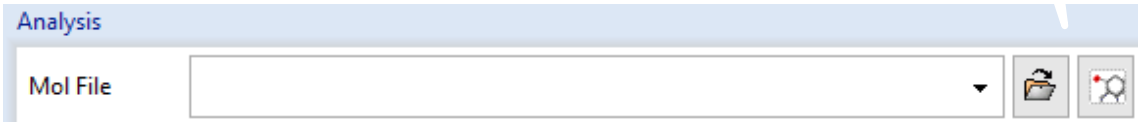
Create a new Experiment for the holder with the sample. Give an experiment name (use letters, numbers and no spaces)



Select the solvent and the P_PROTON experiment.

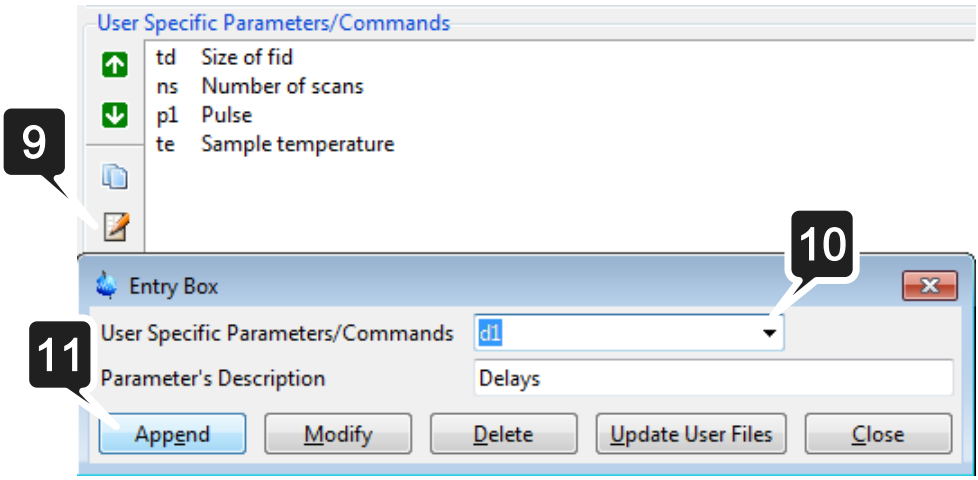


Browse for the analyte mol file or draw the structure

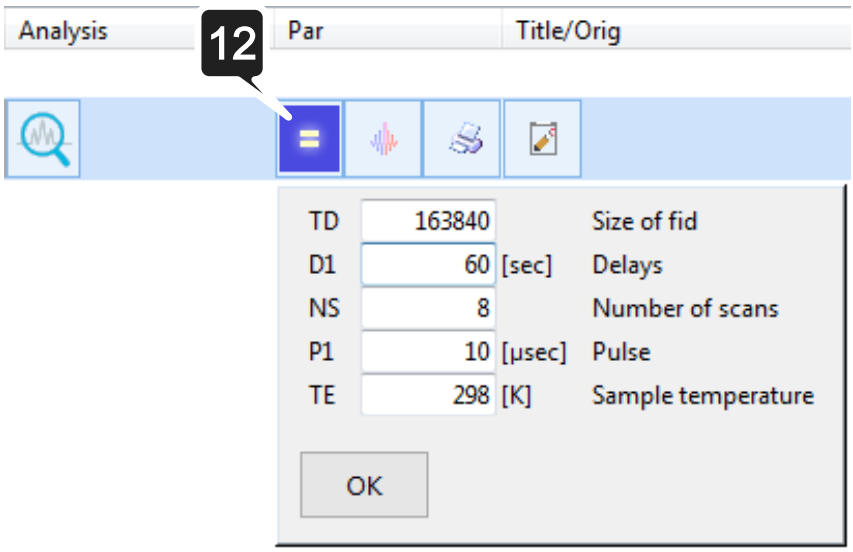


Change D1 Delay (default is 40s)

IconNMR configuration allows to define parameters that can be changed (**9**)



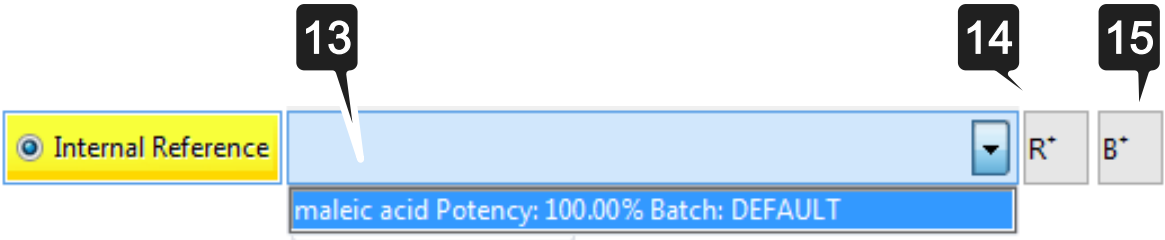
Then when setting up a new experiment, D1 is available by clicking on the *Par* button (**12**), together with the number of scans (NS):



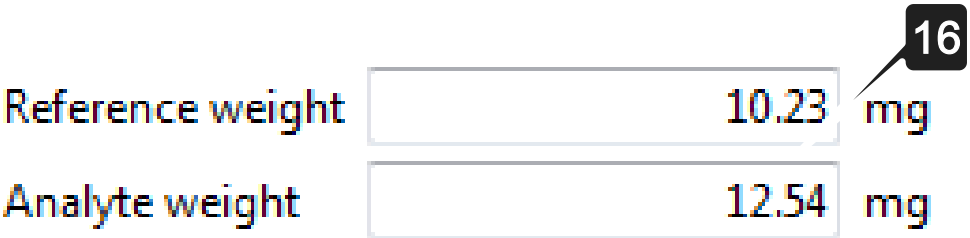
Automated Potency Determination: Measurement

Select the Internal Reference Standard Batch to be used (13)

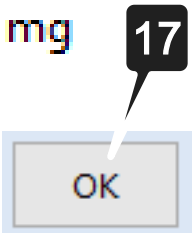
If maleic acid is not used as internal reference standard, then click on *R+* button (**14**) to create a new one. *B+* button (**15**) will create a batch for an already defined internal reference standard.



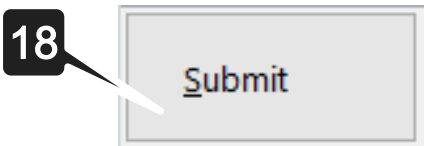
Enter the exact weight of analyte and internal reference standard in mg (**16**).



The experiment is set-up. Click *OK*



Submit the experiment

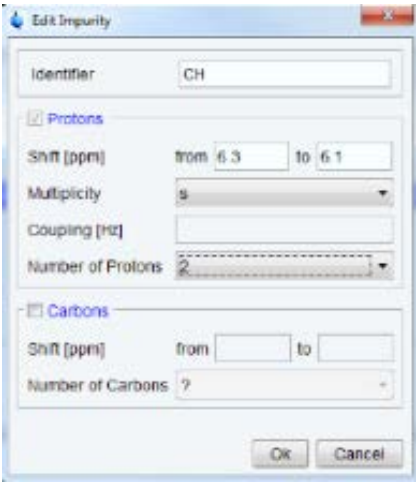


Automated Potency Determination: Measurement

Add a new reference by clicking on R+ (**14**)

Add the following information for the new internal reference:

- Name
- Molecular mass
- All reference integral regions by clicking on *New*

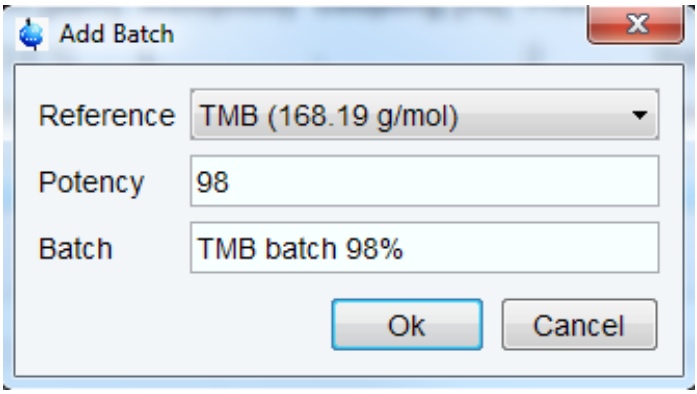


Select the integrals that will be used for

Automated Potency Determination: Measurement

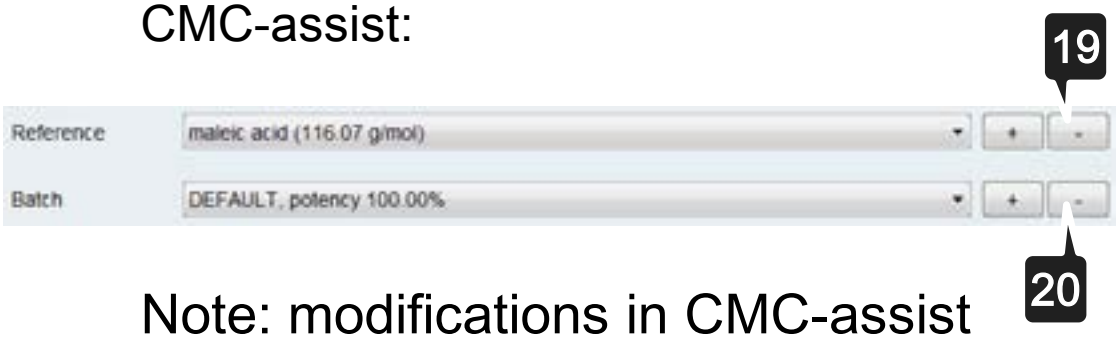
Add a new Batch by clicking on *B+* (**15**)

- Select the reference for the new batch
- Enter a batch name
- Enter the potency of the batch



The batch is then ready to be selected for a potency determination.

Deleting a reference (**19**) or a batch (**20**) is not possible in IconNMR but possible in CMC-assist:

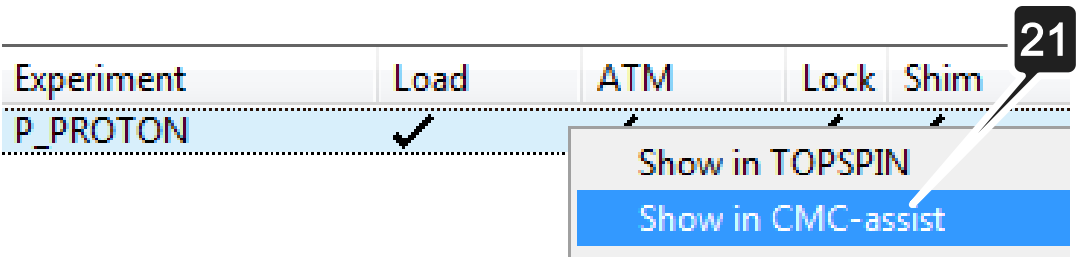


Note: modifications in CMC-assist qNMR settings can be discarded by clicking on *Cancel* and are stored only if the potency calculation is started

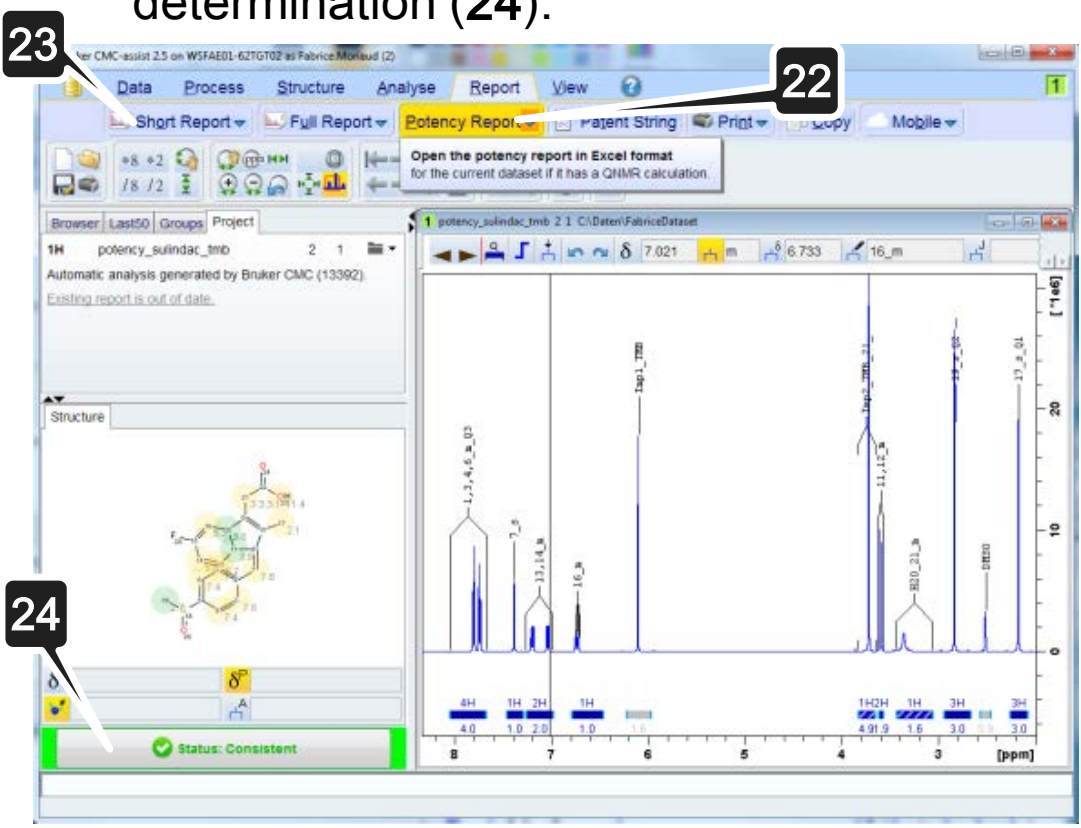
Automated Potency Determination: Analysis and Reporting

The analysis of the measured data is automatic

Open in CMC-assist with a right click in the *Preceding Experiments*



Get the Potency excel report (**22**), the consistency analysis Report (**23**) ,integral regions used for potency determination (**24**).



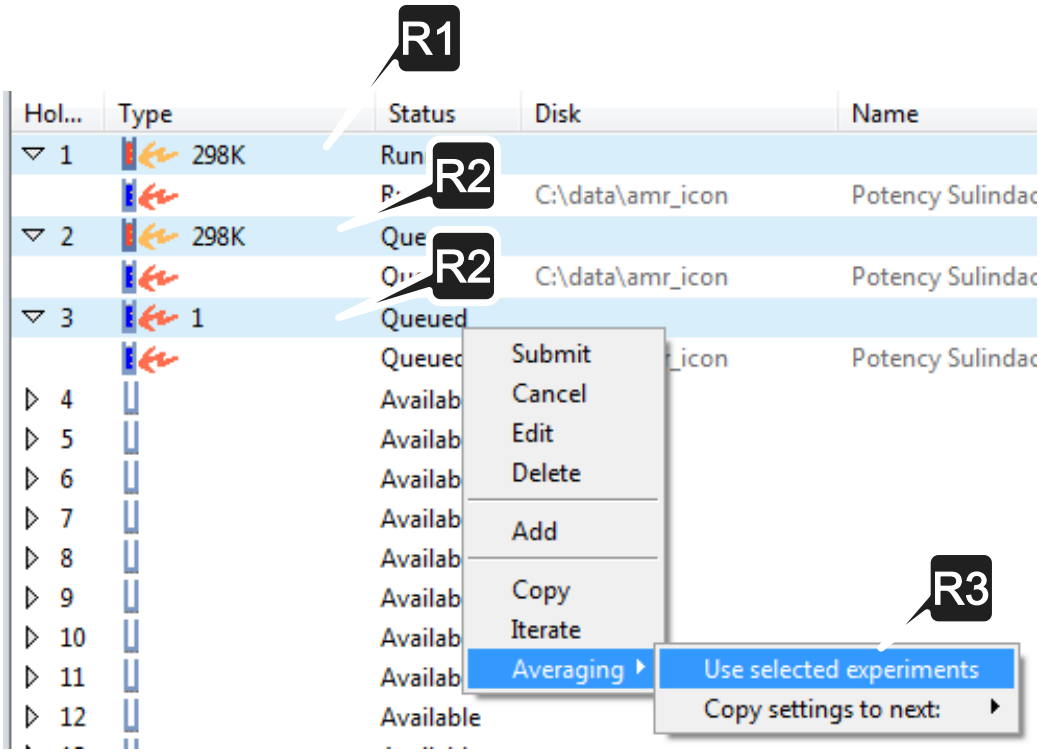
Automated Potency Determination: Replicates

The average potency of several samples prepared in duplicate, triplicate... can be automatically calculated by following the steps below:

Setup the first experiment as described before. Don't submit it.

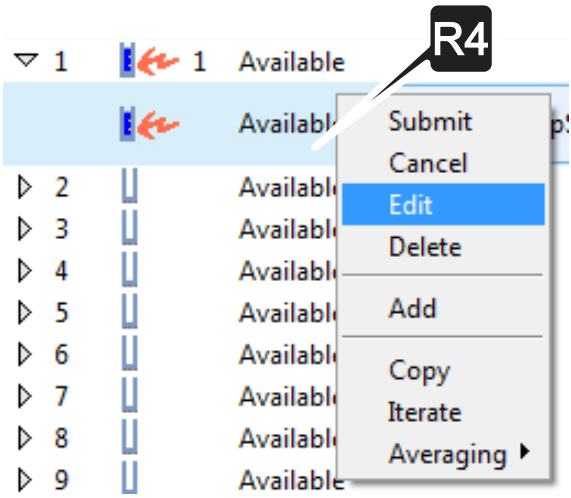
Select the replicate by selecting the first experiment with a left click (**R1**) and then, press *Ctrl key* and select the holders where the replicate samples are (**R2**).

Then right click, select *Averaging/Use selected experiments* (**R3**)



Automated Potency Determination: Replicates

Correct the analyte's and reference's weights by right click/*Edit* on each of the experiments and edit the weight as in (**16**).



Select all replicates and submit:

When all experiments are finished, open one of them in CMC-assist as in step (**21**).

Open the co

The average potency (**R7**)

Region 2	Averaged Area analyte	SD Area analyte	N analyte [mmol]	Potency [%]	RSD Potency [%]
1.00	1.00	0.00	0.02	98.05	
1.01	1.00	0.00	0.05	99.92	
1.00	1.00	0.00	0.02	99.70	
				99.23	0.84

R7

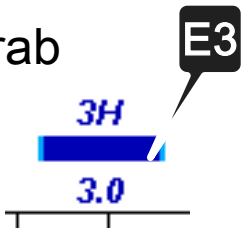
R8

Integral Region Adjustments and Manual Report Generation

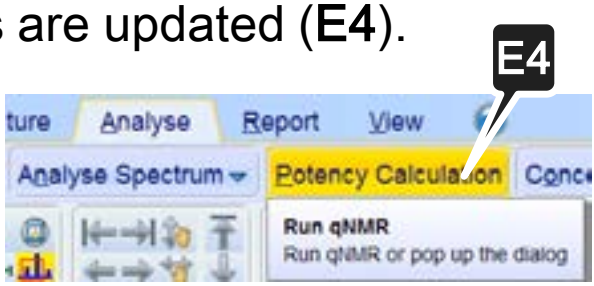
The automatically selected analyte integral regions can be modified manually and the reports updated.

Select/UnSelect analyte integral regions: right click on a CMC-assist integral region (**E1**) and tick/untick (**E2**).

Change analyte integral regions: grab left or right borders with the mouse

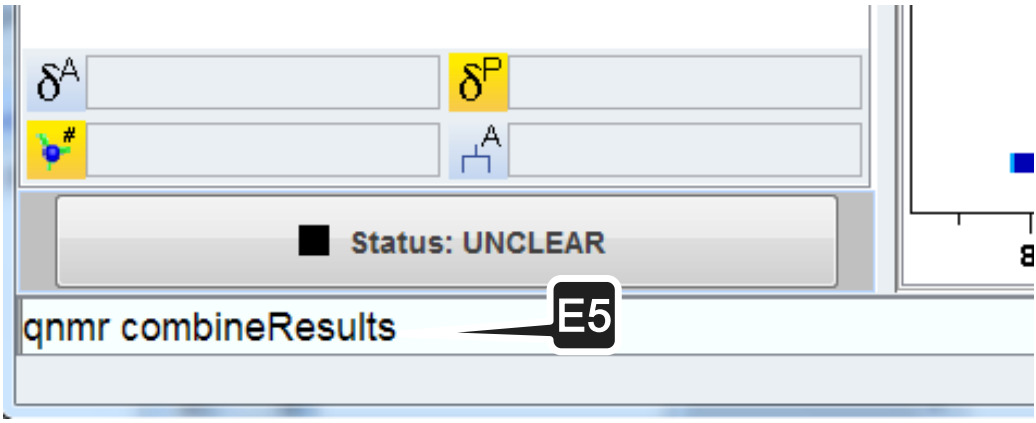


Recompute the potency, then the reports are updated (**E4**).

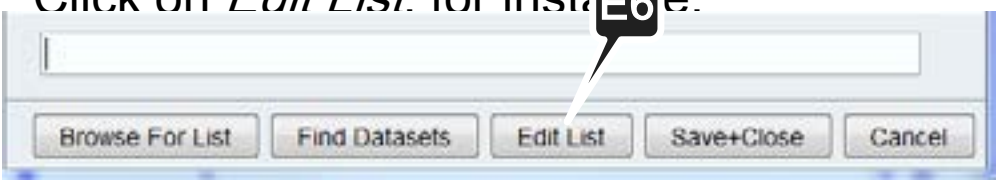


Integral Region Adjustments and Manual Report Generation

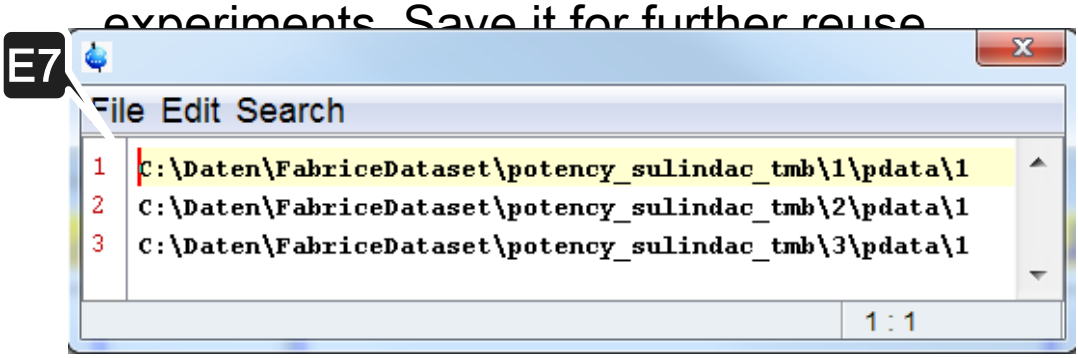
Enter command: *qnmr combineResults*



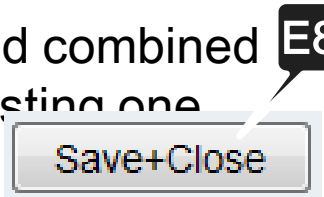
Click on *Edit List* for instance:



Copy/Paste there the paths to experiments. Save it for further reuse



Click to generate an updated combined report that override any existing one



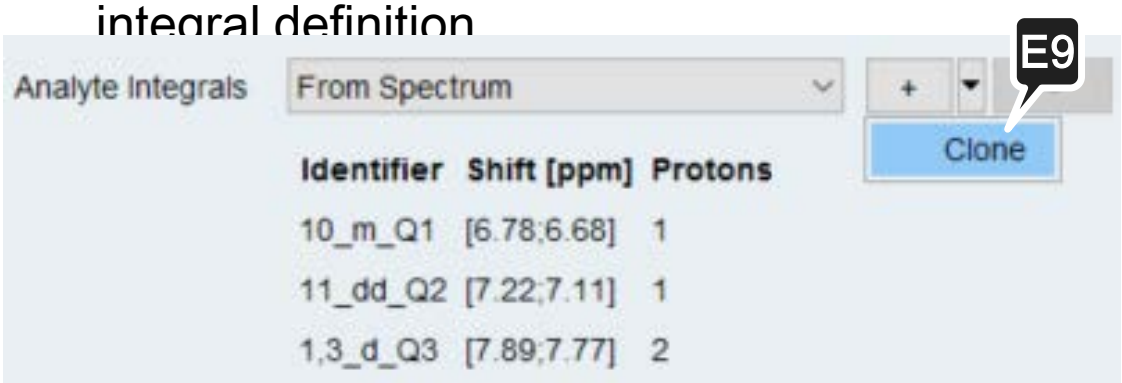
Define and Use Predefined Analyte's Integral Regions

Measure first with IconNMR as described before. Open the results with CMC-assist, modify the analyte integrals (**E1 E2 E3**) that were automatically chosen.

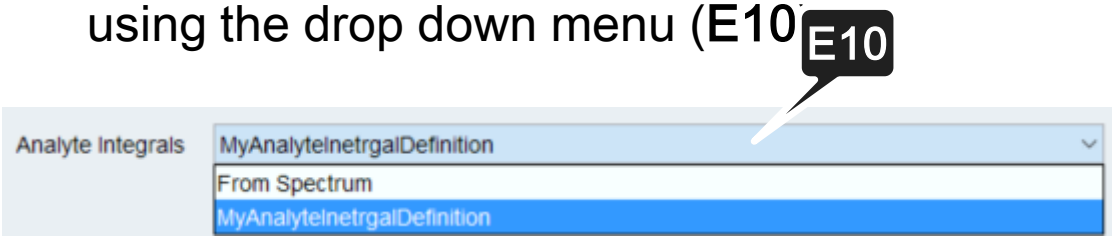
Open Potency Calculation (**E4**)

Click on the button to edit : 

The analyte's integral(s) from spectrum is the default. Selecting *Clone* (**E9**) allows to use them as a template to create and store a user defined analyte integral definition



The user defined analyte integral definition is stored and can be reused using the drop down menu (**E10**)



Potency Determination

Quick Overview

Useful Links

- ❑ License request for using another workstation (Demo CMC-assist license)

https://www.bruker.com/nmr_license_requests.html

Learn More

- ❑ Potency determination Bruker webpage

https://www.bruker.com/products/mr/nmr/nmr-software/software/qnmr/overview.html