ERETIC2

user's guide

I. General Information

The new module ERETIC2 is a quantification tool which will replace the ERETIC (Electronic to Access In Vivo Concentration) software.
This new tool is based on PULCON[1], an internal standard method which correlates the absolute intensities of two different spectra. Concentration measurements with PULCON use the principle of reciprocity which indicates that the lengths of a 90° or 360° pulse are inversely proportional to the NMR signal intensity[2,3]. Therefore, provided that the concentration of one of the samples is known precisely and that the 90° pulse of all the samples have been well calibrated, the unknown concentrations can be obtained using the following equation[1] :

$$C_{UNK} = kC_{REF}\frac{A_{UNK}T_{UNK}\theta_{90}^{UNK}n_{REF}}{A_{REF}T_{REF}\theta_{90}^{REF}n_{UNK}}$$

where the UNK and R indices stand for unknown and reference respectively, C is the concentration, T is the temperature, θ_{90} is the 90° pulse length, n is the number of transients used for the experiments, and k is a correction factor taking into account the use of different receiver gains for measurement of the reference and of the unknown samples, or incomplete relaxation.

This equation is valid when the experiments are recorded with the same NMR probe, tuned and matched.

ERETIC2 only needs a 1D spectrum measured on a sample of known concentration, under "quantitative" condition : a tuned and matched probe, a calibrated 90° pulse, a relaxation delay equals to at least 5*T1, an acquisition time longer than T2, and a sufficient signal to noise (at least 100:1). The correlation between this spectrum and those of unknown concentrations can be done very easily through a calibration step in the integration menu. This correlation can also be depicted by a digitally synthetized signal, whose integral value stands for one nucleus at the concentration of the reference sample. The linewidth and chemical shift of this synthetic signal can be adjusted at will via a dedicated window in the software.

Compared to the former ERETIC software, the main advantage of this new tool is that it doesn't require any additional hardware needed to generate the electronic signal used as reference. Hence, ERETIC2 allows the user to have more flexibility when choosing the 1D NMR experiment used for quantification.
ERETIC2 is also a good alternative to the classical internal standard method. Actually, this method suffers from many disadvantages since the chemical used as reference has to meet many criteria in terms of solubility, stability, relaxation time, chemical shift and non interaction with the sample being quantified.

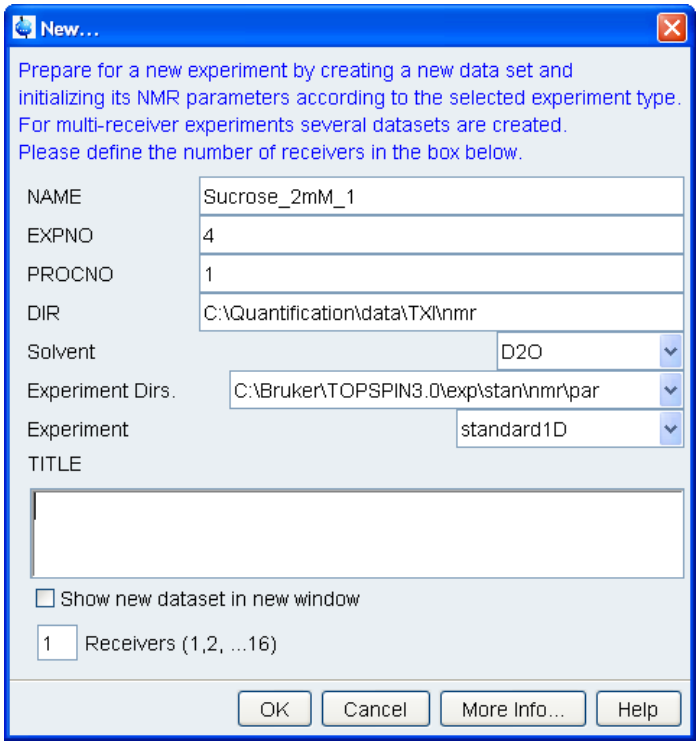
II. Quantification procedure

1. *Calibration*

Bruker does not provide any standard NMR samples for calibration. Nevertheless, it is recommended to use your own reference samples prepared in various solvents and with well known concentrations.

1.1 Acquisition parameters setting :

Insert the reference sample into the magnet
Prepare a new experiment using the edc command



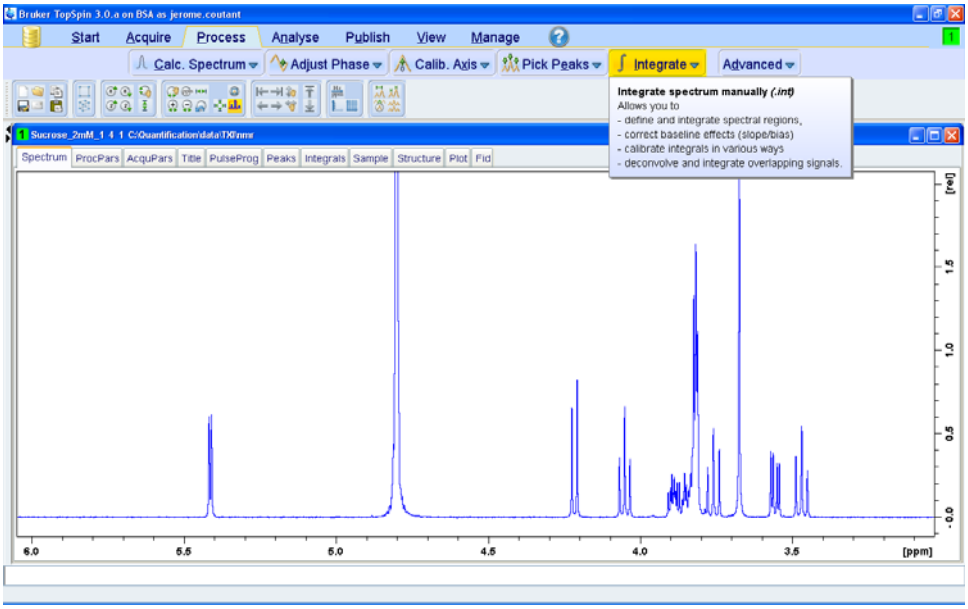
Choose the user and directory names, the experiment and processing numbers
Choose the parameters set
Lock the magnetic field (lock “solvent”)
Tune and match the probehead (“atma exact”)
Shim the sample (“topshim”)
Calibrate the 90° pulse either manually or with the AU program “pulsecal”:
without option for proton
Option “c13” for carbone
Option “f19” for fluorine
Option “p31” for phosphorus
In the acquisition window (eda) set the digitization mode to baseopt
Set D1 and NS according to your sample
Set the receiver gain (“rga”)
Start the experiment (“zg”)

1.2 Processing parameters setting, reference sample :

Choose an exponential window function (EM window), with an lb=0,3
Use the EF command to perform an exponential window multiplication and a fourier transform of the FID (em, ft)
For baseopt acquisition : automatic zero order phase correction with apk0
Otherwise : automatic zero and first order phase correction with apk
Base line correction without automatic integration (“absn”)

1.3 ERECTIC2 calibration :

The reference sample is a 2mM sucrose solution in D2O.

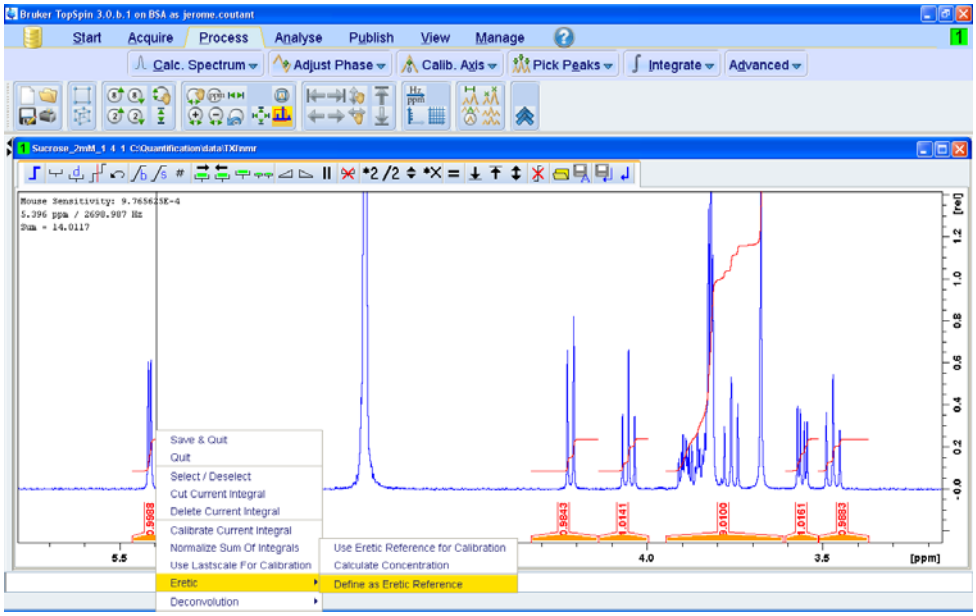


In the reference sample, go into the integrate menu

Integrate the reference signals

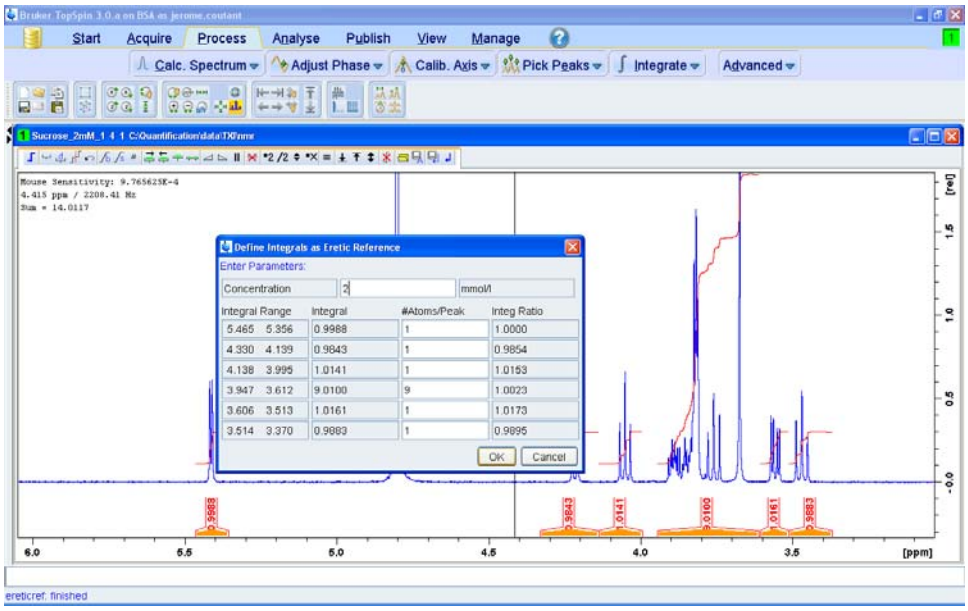
Select the signals you want to use for calibration

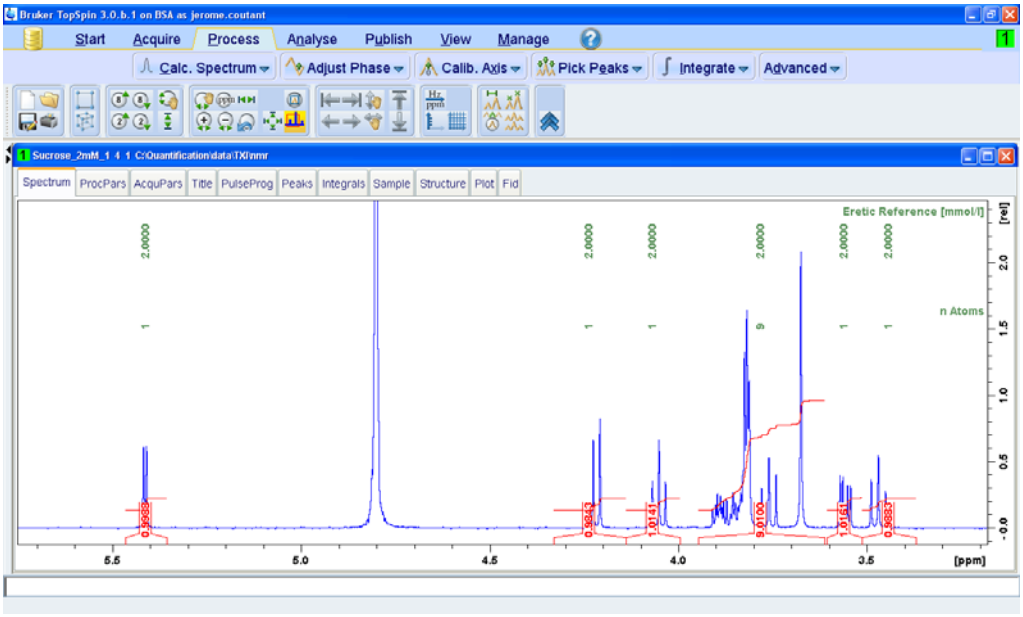
Click on the right mouse button, and choose the option “Define as Eretic Reference”



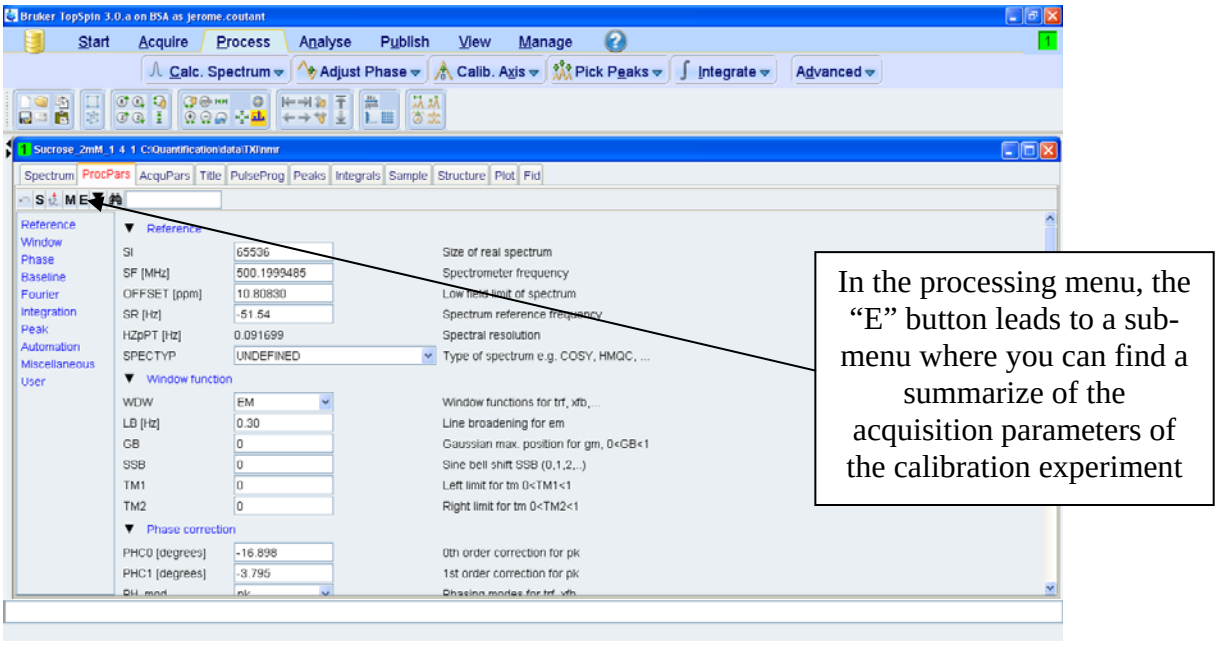
Define the concentration of the reference sample (in mM)

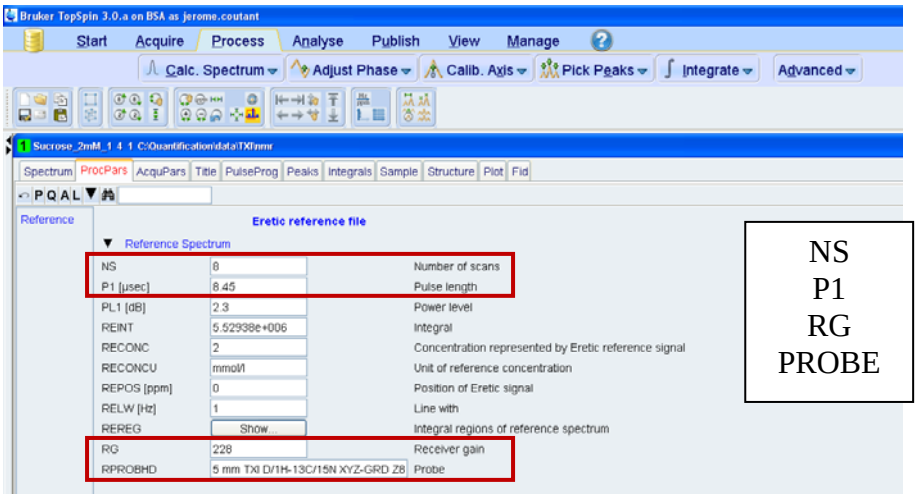
Define the number of nuclei per signal





The number of nuclei and the concentration appear on the spectrum

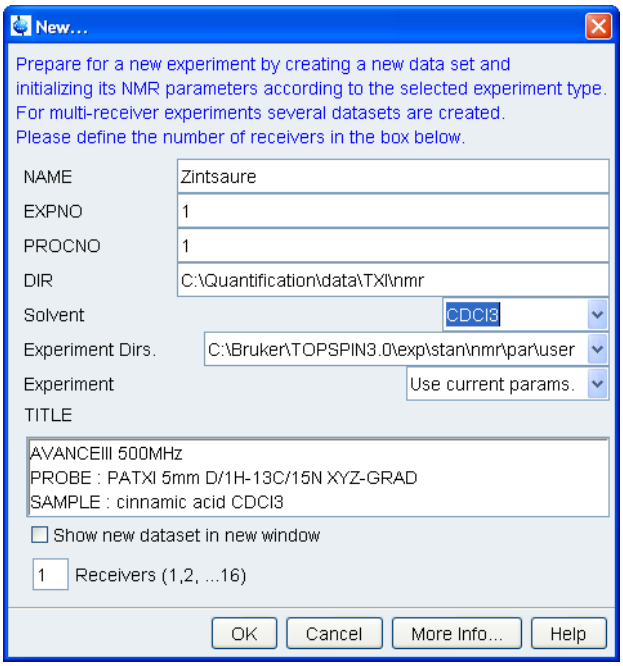




2. *Quantification:*

2.1 Acquisition

From the calibration spectrum, create a new experiment with the edc command. As the calibration and quantification should be as close as possible, use the option "use current params" in the experiment line



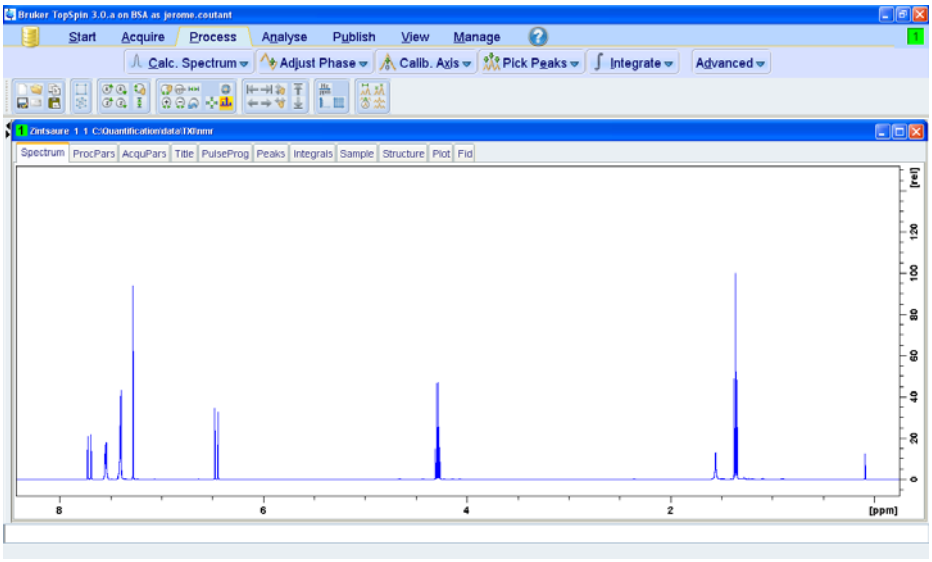
Lock the magnetic field (lock “solvent”)
Tune and match the probehead (“atma exact”)
Shim the sample (“topshim”)
Calibrate the 90° pulse either manually or with the AU program “pulsecal”:
without option for proton
Option “c13” for carbone
Option “f19” for fluorine
Option “p31” for phosphorus
In the acquisition window (eda) set the digitization mode to baseopt
Set SW, D1, NS and TD according to your sample
Set the receiver gain (“rga”)
Start the experiment (“zg”)
Lock « solvent »
Atma « exact »
Topshim
Pulsecal without option for proton
Option “c13” for Carbone
Option “f19” for fluorine
Option “p31” for phosphorus
D1 and NS Setting
rga
zg

2.2 Processing

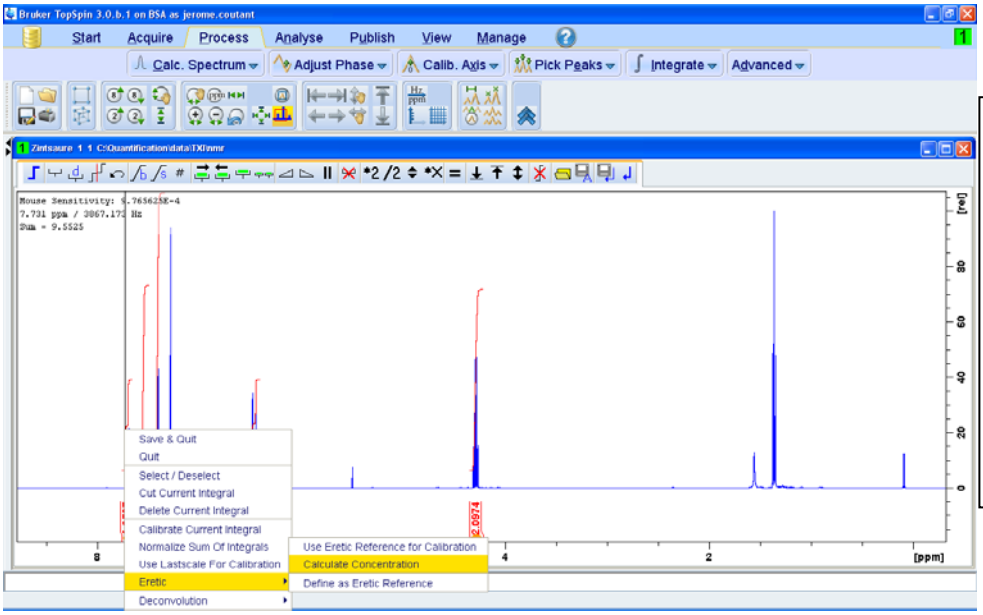
Keep the same processing parameters that the one used for the calibration experiment.

2.3 Quantification of the sample

The sample used in the example is a cinnamic acid solution in CDCl3. The 1D 1H spectrum (pulse sequence “zg”) of this sample is represented in the figure below.

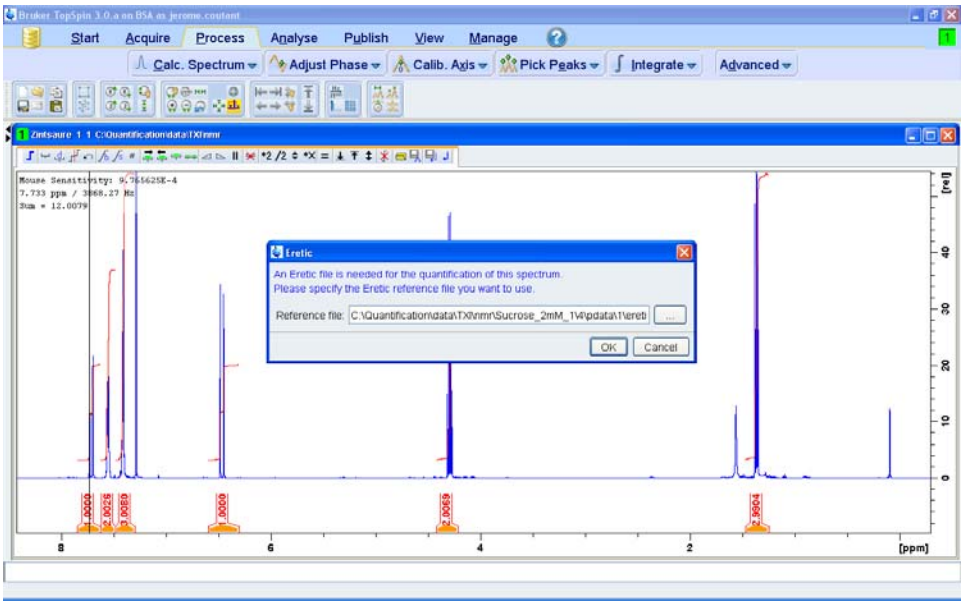


Once the spectrum has been recorded and processed, go into the integrate menu, integrate the signals to be quantified, select all integrals and then right click on the mouse and choose the option “Calculate Concentration”.

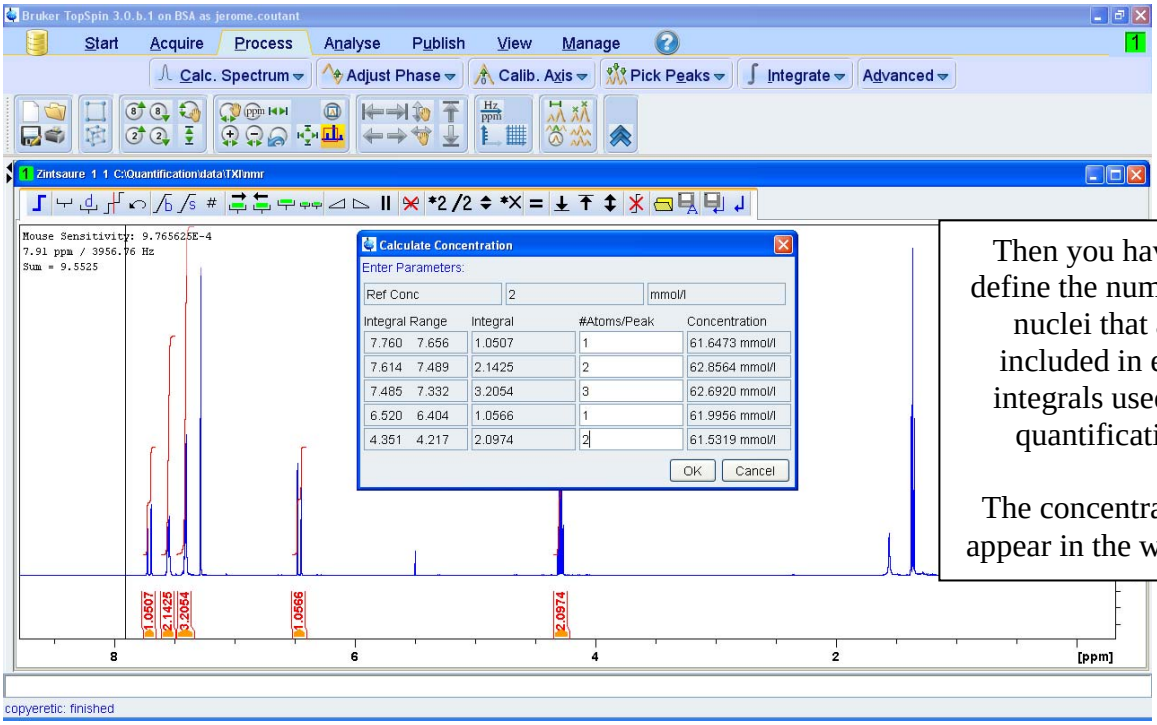


Go into the integration menu, integrate the signals you want to quantify

Right click on the mouse , and choose “Calculate Concentration”



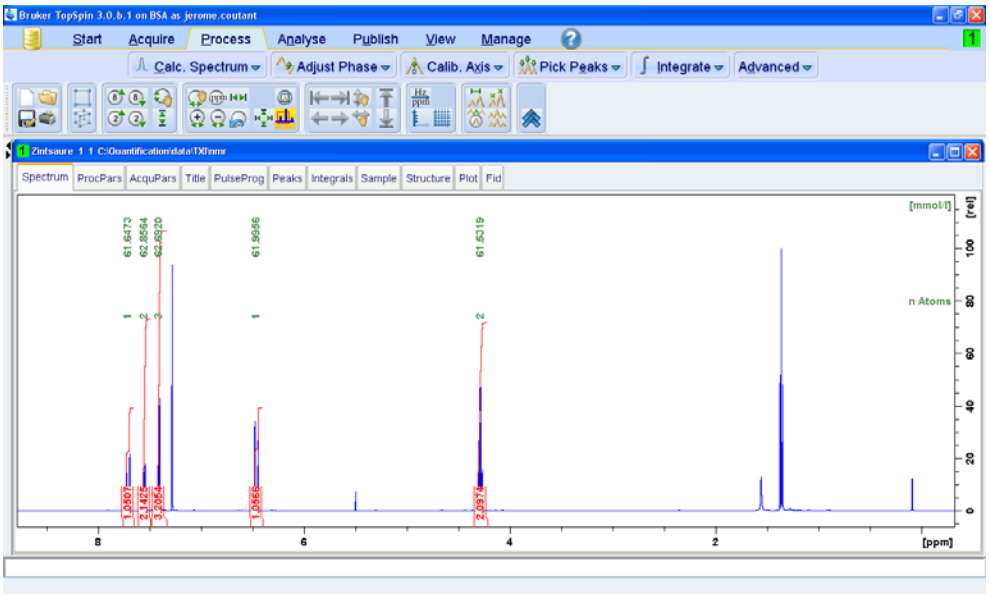
First, you will have to define the reference file used for quantification



Then you have to define the number of nuclei that are included in each integrals used for quantification

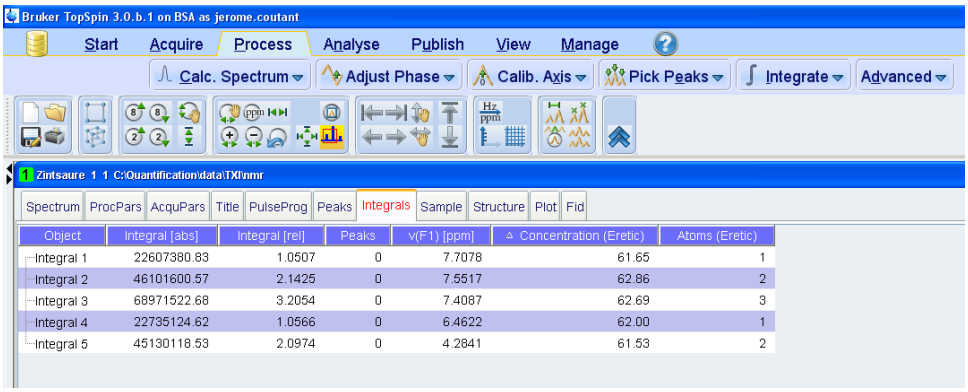
The concentrations appear in the window

Once these informations have been entered in the window, the concentrations appear on the right. Then, click on OK. The results will now appear in the spectrum



The concentrations also appear in the spectrum

You can also have a display of the quantification results in the integral tab : move the mouse in one of the cells of the first line of the table. Then click on the right mouse button, and select “concentration (eretic)” and “atoms (eretic)”.

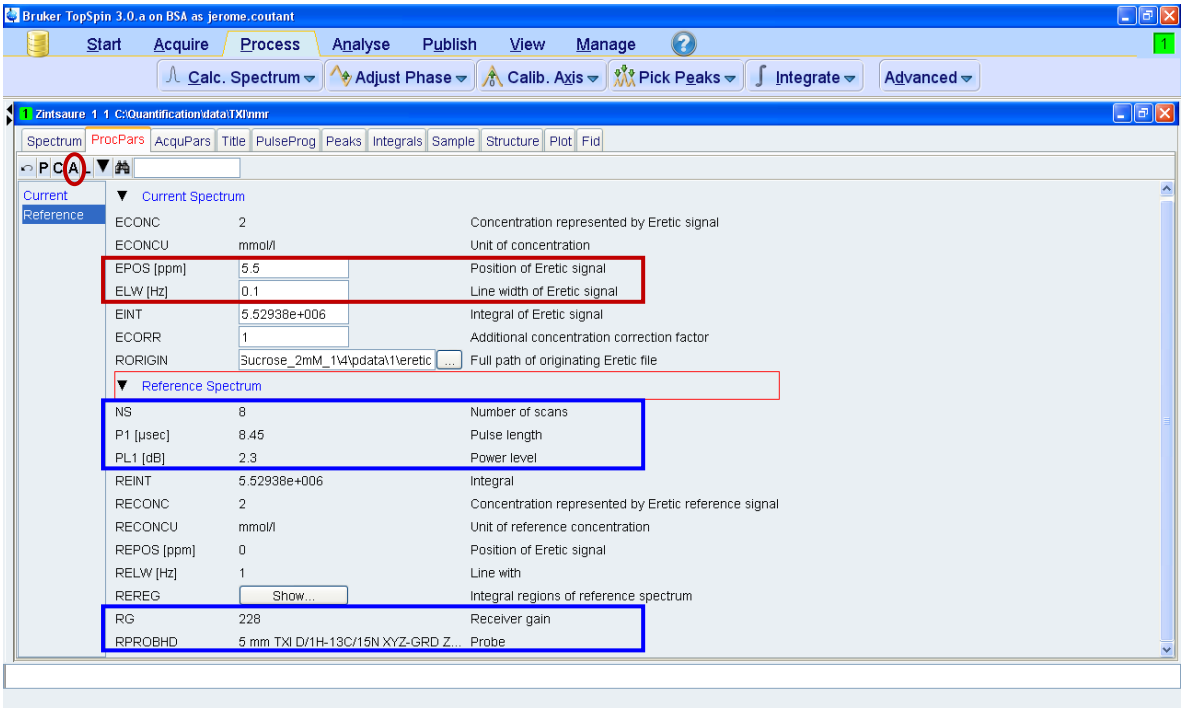


III. Miscellanous

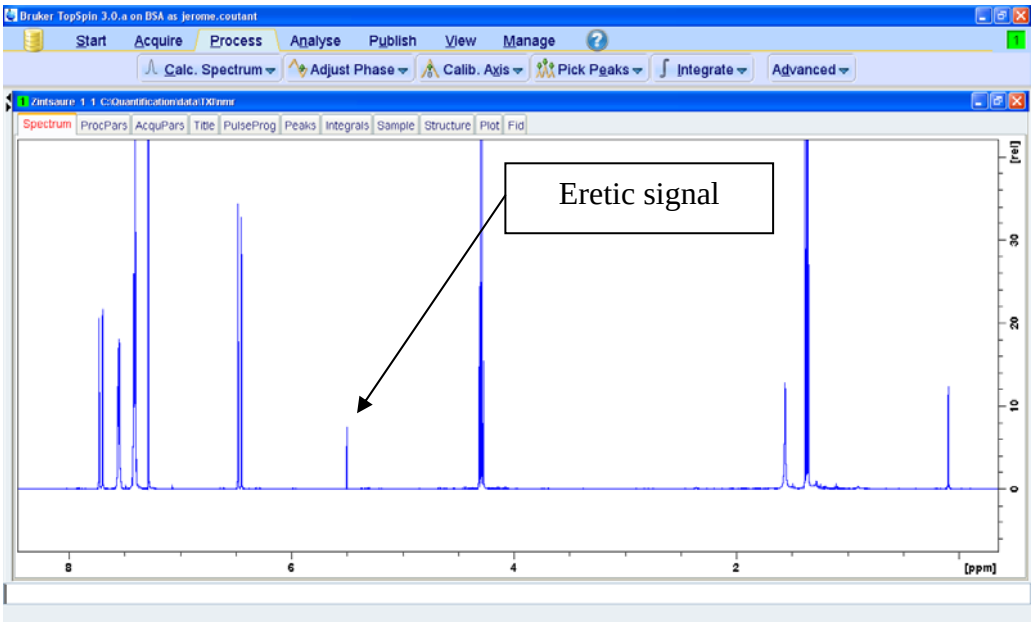
1. *ERETIC signal*

In the quantification procedure, ERETIC signal insert is not necessary. However, you still have the possibility to add this synthetic signal in your spectrum :
Go into the processing menu (edp) and click on the “E” button. In this sub-menu, you have the possibility to manually define the linewidth (ELW parameter) and chemical shift (EPOS parameter) of the ERETIC signal. You can also find some of the acquisition parameters of the reference spectrum (especially those used in the quantification calculation) :

Number of scans
Pulse length and power of the pulse
Concentration of the reference sample
Probe head
Receiver gain

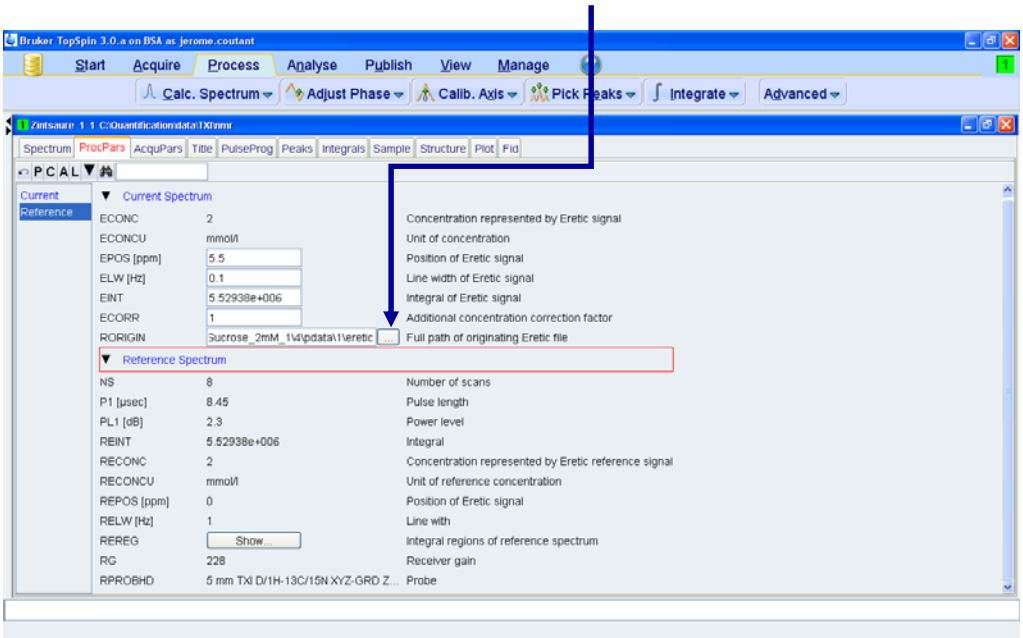


The “adderetic” command will insert the synthetic signal in the spectrum, with the user-defined line width and chemical shift. Clicking on the “A” button will do the same. It should be pointed out that the integral value of this signal is

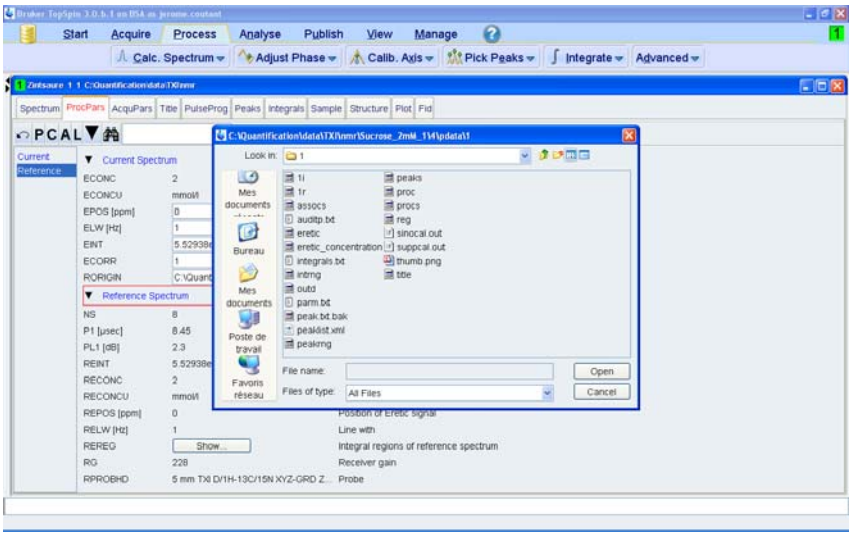


2. *Modification of the reference dataset*

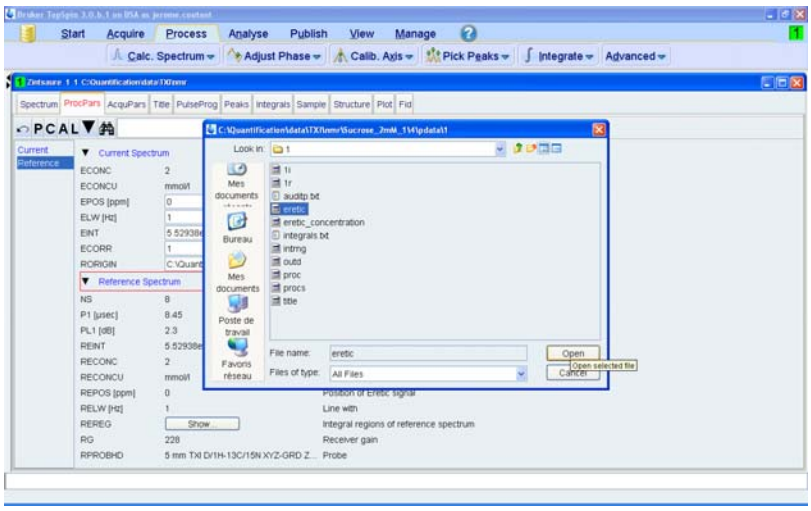
ERETIC2 offers the possibility to modify the dataset used as calibration reference. You simply have to use the "Full path of originating Eretic file" button



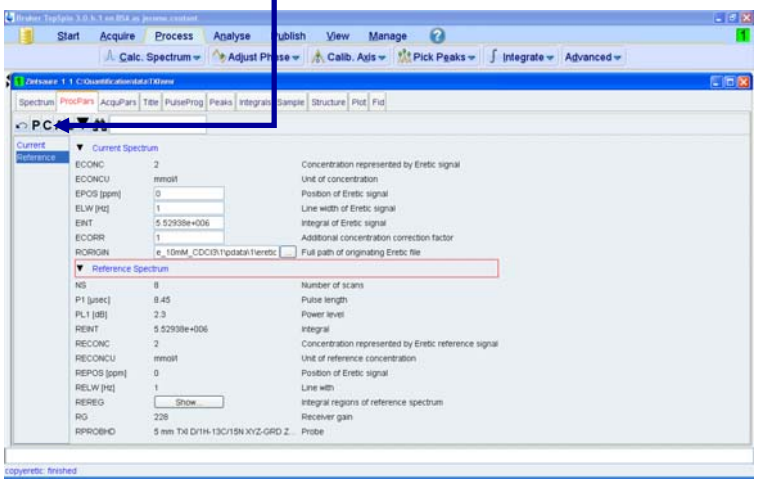
A new dialog window will open.



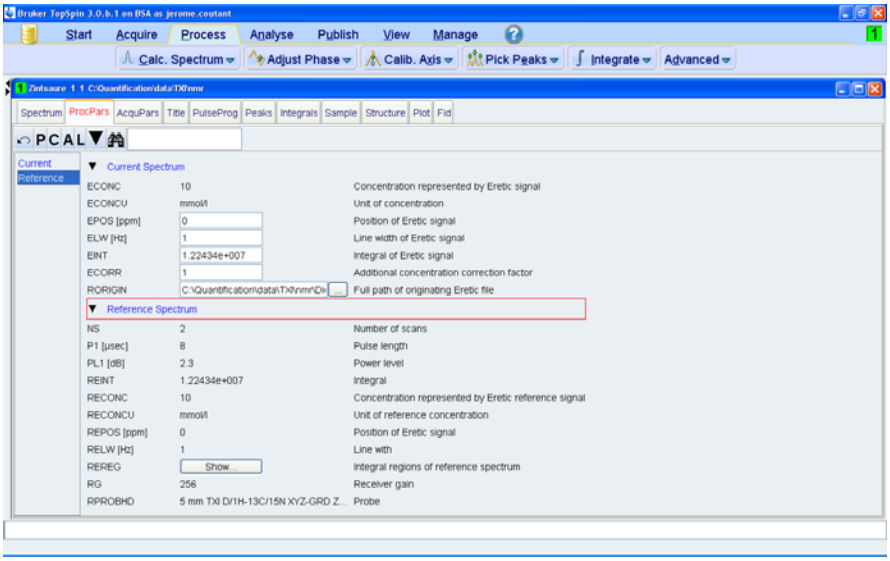
You will have to browse through the directory to the new calibration dataset, and load the “eretic” file.



Once the file has been changed, you have to reload the acquisition parameters of the new calibration dataset by clicking on the “**C**” button.



The acquisition parameters of the reference spectrum have been reloaded.

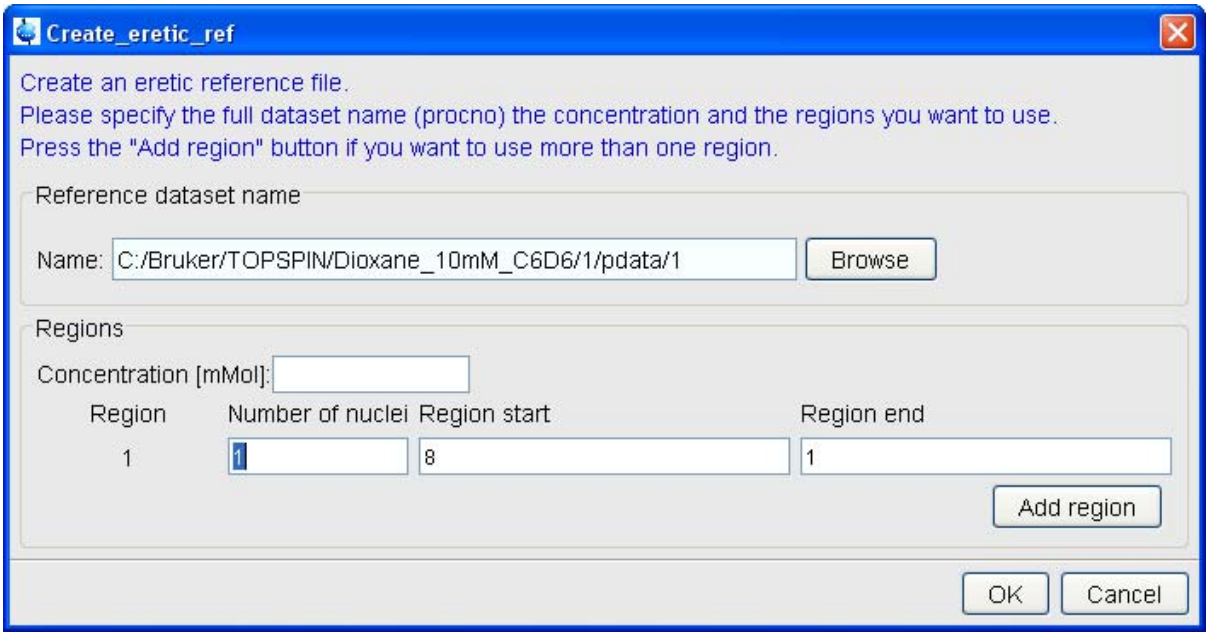


Remarque 2 : When you add an ERETIC peak in your spectrum this peak is weighted ratio of pulcon(P90, NS, RG)

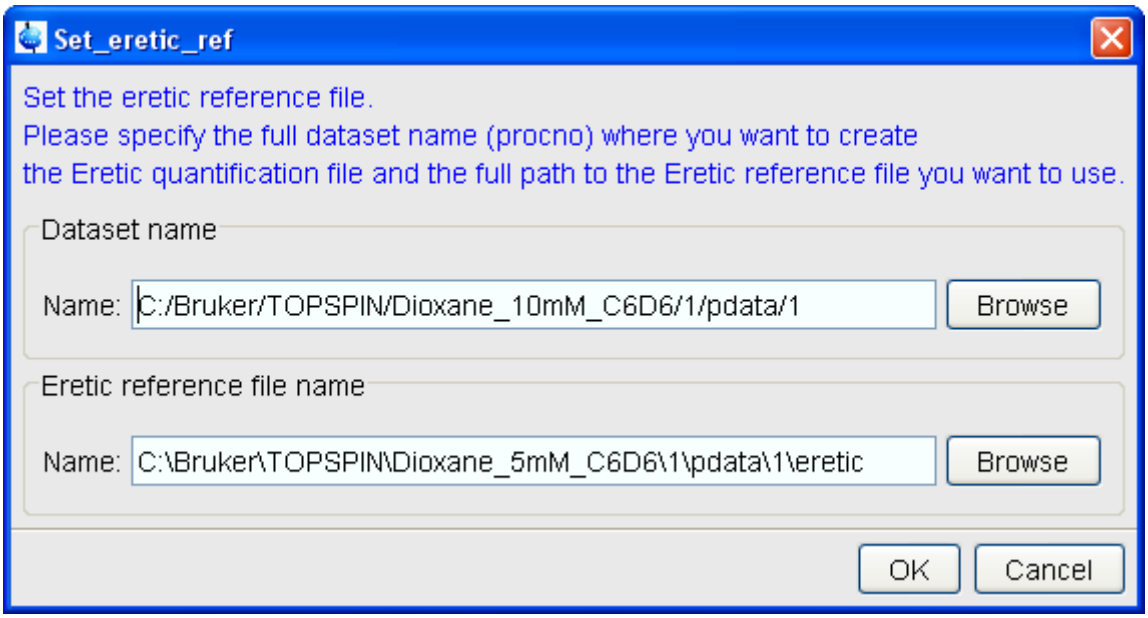
3. LIST OF COMMANDS

Adderetic : add an ERETIC peak in the current data set

Create_eretic_ref : Define the concentration (mM), the integral regions and nucleus/region used for the setting of the reference ERETIC peak



Set_eretic_ref : Calculate the eretic integral reference value and store the result in the defined dataset.



Create_eretic_cal : Define the integral regions and nucleus interested for the quantification.

Calc_eretic : Calculate the concentration in mM

4. AUTOMATION

4.1 Exemple of au_program

```
/*
 * to create an eretic reference, the command is:
 * XCMD("sendgui createereticref arg1|arg2|arg3") from AU program
 * Arguments: arg1: Dataset name (where the eretic ref file is created)
 *            arg2: Concentration
 *            arg3: Number of nuclei
 *            arg4: Integral region start
 *            arg5: Integral region end
 *                                                    .
 *                                                  .
 *                                                  .
 * (for multiple nuclei and integral regions repeat arg3 - arg5)
 * Arguments are separated with "|"
 */

XCMD("sendgui createereticref
C:/Users/wk/nmrdata/Dioxane_10mM_C6D6/1/pdata/1|10|8|3.757|3.071|1|0.817|0.208");

QUIT
    ……
    ……
/*
 * to calculate the concentration the command is
 * XCMD("sendgui calcconc arg1|arg2|arg3") from AU program.
```

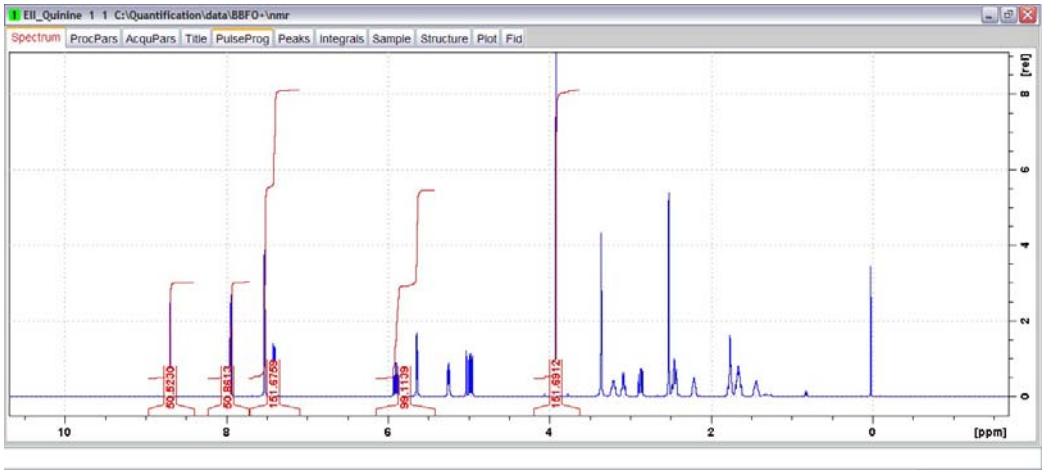
```
* Arguments: arg1: Dataset name
*            arg2: Number of nuclei
*            arg3: Integral region start
*            arg4: Integral region end
*                        .
*                        .
*                        .
* (for multiple nuclei and integral regions repeat arg2 - arg4)
* Arguments are separated with "|"
*/

XCMD("sendgui calcconc
C:/Users/wk/nmrdata/Dioxane_5mM_C6D6/1/pdata/1|8|3.757|3.071|1|0.180|-0.343");

QUIT
```

4.2 ICONNMR

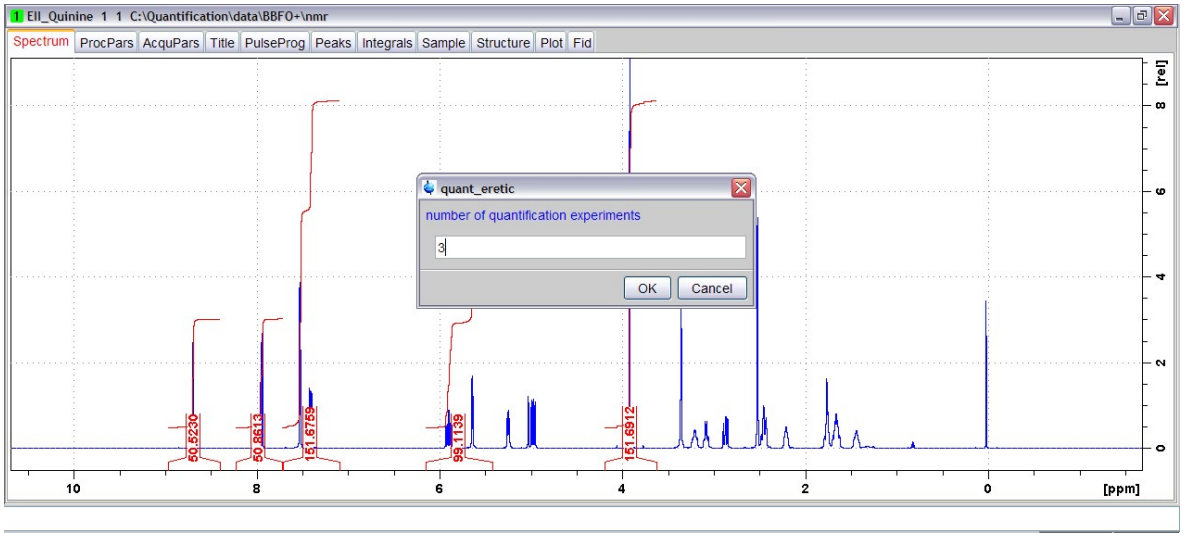
4.3 AU program to quantify a set of experiments

The AU program “quant_eretic” allows to quantify a set of experiments, provided that these spectra are recorded in a successive manner and that a valid calibration file already exists. This AU program provides an average concentration over the successive spectra, a standard deviation, the date and time at which the calculation has been performed, and the first and last dataset that have been used for the quantification. These informations appear in the title of the first experiment used for quantification, and are saved in a file in the pdata of this first experiment as well.

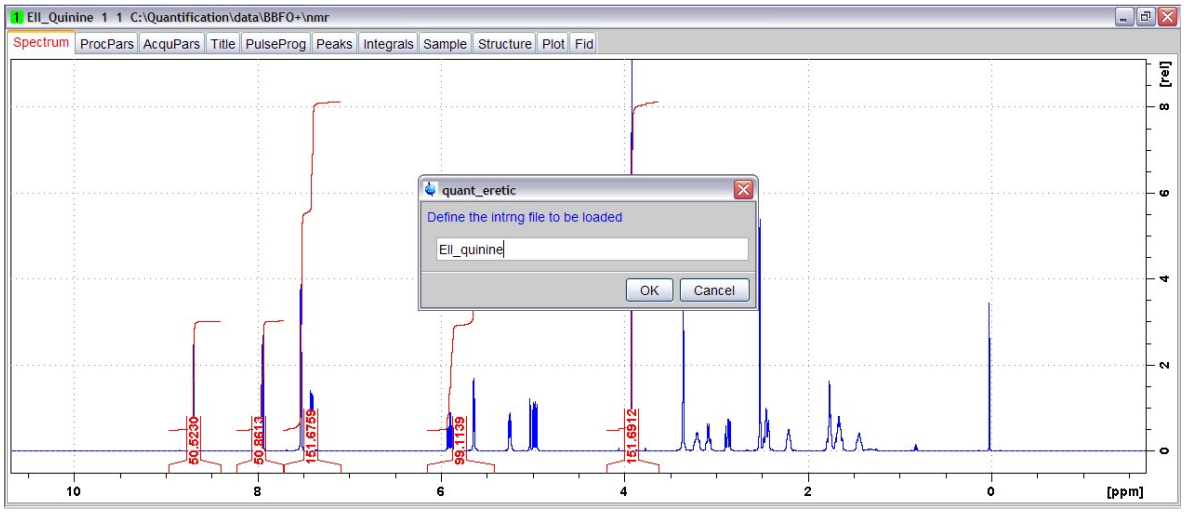
The first step is to process the experiments using the same parameters, and correct the baseline. An integration file must be created (if it does not already exist), that should only contain the regions that will be used during the quantification calculation. The AU program should be started in the first experiment of the set.
In the following, you will have an example illustrating the use of this automatic quantification tool. The sample used is a 50 mM Quinine sample in DMSO-d6. Three experiments (EXPNO 1 to 3) have been recorded and have to be quantified. The integration file name is EII_quinine, and a calibration file already exists.



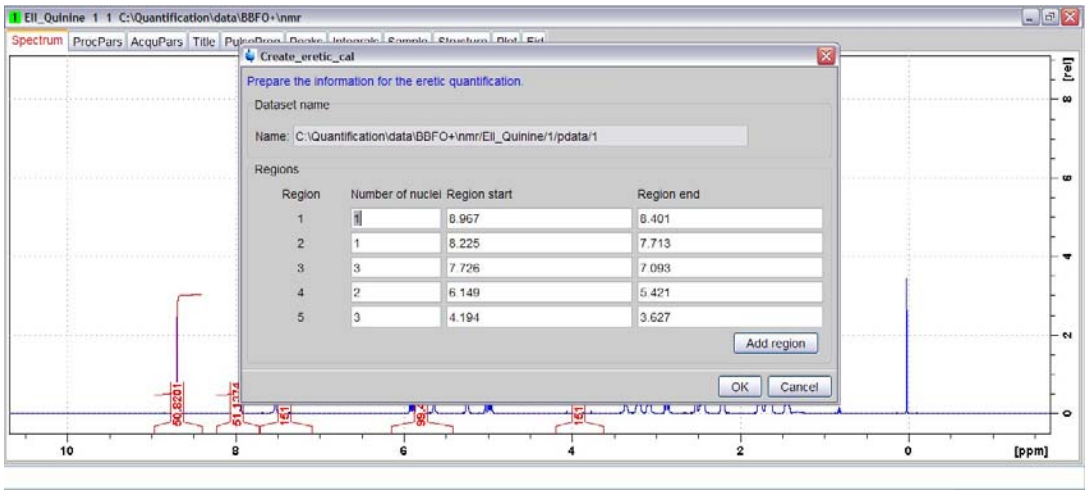
Use the command xau quant_eretic to start the AU program. First, the user has to define the number of experiments that will be quantified (3 experiments in this example).



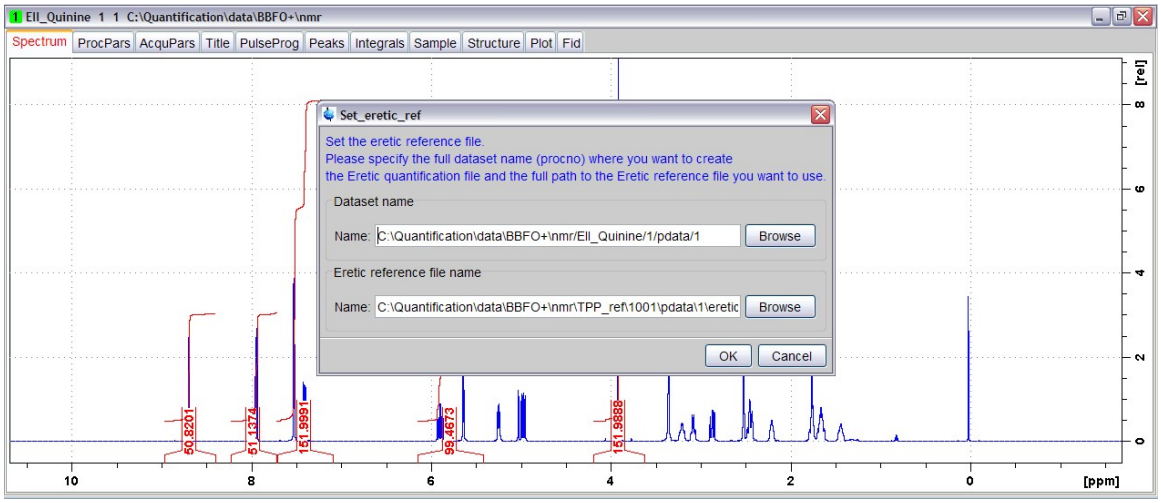
Then, the name of the integration file has to be defined (EII_quinine).



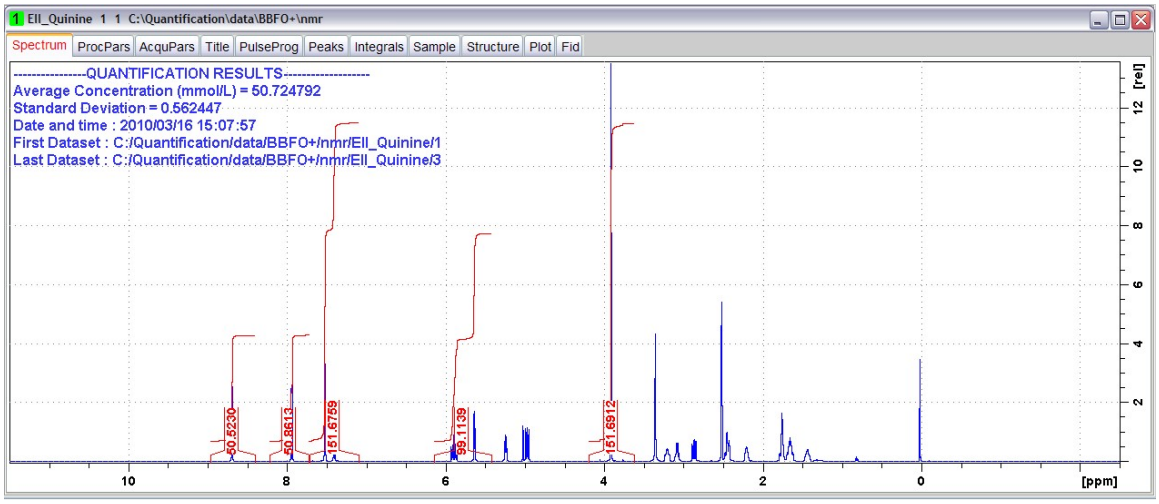
Once the integration file has been defined, the number of nuclei per region has to be set.



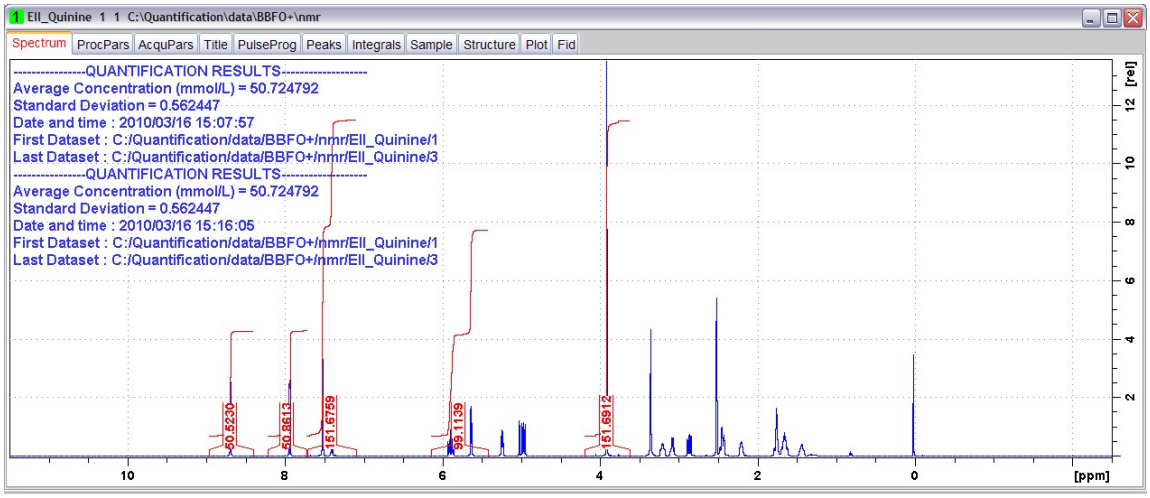
Finally, the full path to the eretic reference file must be set (the path corresponding to the directory of the last calibration is automatically set in the window). The dataset name (with EXPNO and PROCNO) where the eretic quantification file will be created has to be defined in this window as well.



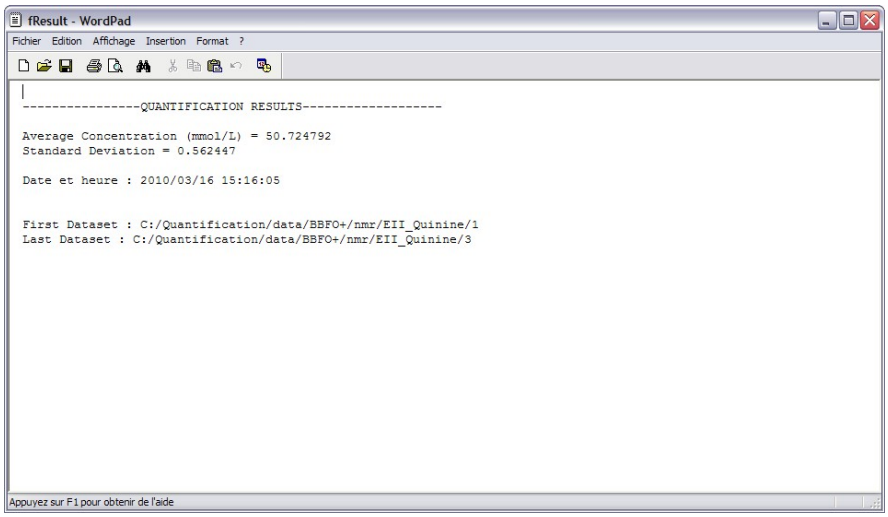
The quantification results are displayed in different ways.
First, these results are saved in the title of the first quantification experiment:



If a second calculation is run, the corresponding results append to the title :



The same informations are saved in a file called “fResult”, located in the pdata of the first experiment :



In the same directory, the file called “concentration_result” contains the quantification calculation for each experiment. The average concentration as well as the standard deviation are saved in this file. However, it should be pointed out that if a new quantification is performed on the experiments, the quantification results are replaced by the new ones.

concentration_result - WordPad

Fichier Edition Affichage Insertion Format ?

```
# Dataset: C:/Quantification/data/BBFO+/nmr/EII_Quinine/1/pdata/1
# nAtoms Integrated-Region Integral  Concentration

1   8.967000   8.401000   50.522999   50.649899
1   8.225000   7.713000   50.861301   50.989101
3   7.726000   7.093000   151.675903   50.685699
2   6.149000   5.421000   99.113899   49.681499
3   4.194000   3.627000   151.691193   50.690800

# Dataset: C:/Quantification/data/BBFO+/nmr/EII_Quinine/2/pdata/1
# nAtoms Integrated-Region Integral  Concentration

1   8.967000   8.401000   0.059300   50.457199
1   8.225000   7.713000   0.059500   50.604801
3   7.726000   7.093000   0.179100   50.802299
2   6.149000   5.421000   0.116800   49.709900
3   4.194000   3.627000   0.179100   50.807800

# Dataset: C:/Quantification/data/BBFO+/nmr/EII_Quinine/3/pdata/1
# nAtoms Integrated-Region Integral  Concentration

1   8.967000   8.401000   26.368700   51.521400
1   8.225000   7.713000   26.566299   51.907600
3   7.726000   7.093000   78.174004   50.914398
2   6.149000   5.421000   51.475601   50.288799
3   4.194000   3.627000   78.552200   51.160702

----------------QUANTIFICATION RESULTS-------------------

Average Concentration (mmol/L) = 50.724792
Standard Deviation = 0.562447

Date and time : 2010/03/16 15:16:05
```

Appuyez sur F1 pour obtenir de l'aide

REFERENCES

[1] : Wider G. & Dreier L., *J. Am. Chem. Soc.*, **2006**, 128 (2571-2576)
[2] : Hoult D. I. & Richards R. E., *J. Magn. Reson.*, **1976** (71-85)
[3] : Hoult D. I., *Concepts in Magn. Reson.*, **2000**, 12 (173-187)