TopSpin ERETIC 2

- Electronic to Access In-Vivo Concentration

User Manual

Version 001

Innovation with Integrity

NMR

This manual was written by

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1 General Information

The new module ERETIC2 is a quantification tool which replaces 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]:

$$C_{UNK} = kC_{ref}\frac{A_{unk}T_{unk}\Theta_{unk}n_{ref}}{A_{ref}T_{ref}\Theta_{ref}n_{unk}}$$

Where the *unk* and *ref* indices stand for unknown and reference respectively, A is the integral value, C is the concentration, T is the temperature, θ is the pulse length (for 90° or 30° pulse), 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.

ERETIC2 can be used with internal or external standard methods, and needs 1D spectra measured under “quantitative” condition : a relaxation delay equals to at least $5 \times T_1$ (for a 90° pulse) or $3 \times T_1$ for a 30° pulse, an acquisition time longer than $3 \times T_2$, and a sufficient signal to noise (at least 10:1).

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.

[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).

2 Quantification Procedure

2.1 Calibration with External Standard

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.

2.1.1 Acquisition Parameters Setting

- Insert the reference sample into the magnet.
- Prepare a new experiment using the **New** command.

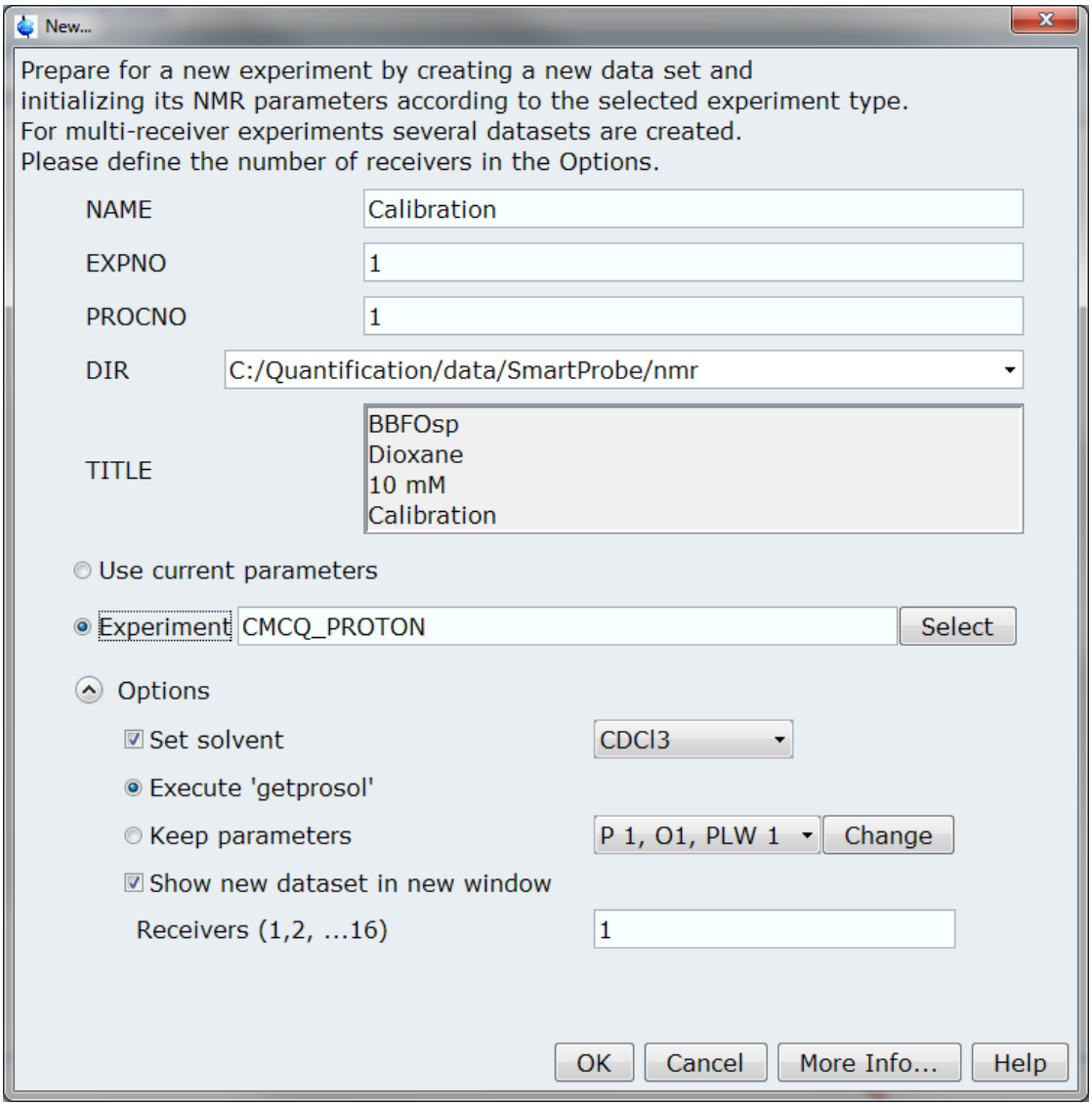


Figure 2.1: Preparing for a New Experiment

- Select the user and directory names, the experiment and processing numbers.
- Select the parameters set CMCQ_PROTON.

- Lock the magnetic field (lock **solvent**).
- Tune and match the probe (**atma exact**).
- Shim the sample (**topshim**).
- Calibrate the 90° pulse either manually or with the AU program `pulsecal`. Without option for proton, or option `sn opt`:
  - 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**) (optional).
- Start the experiment (**zg**).

2.2 Processing Parameters Setting, Reference Sample

- Select 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**).

2.3 ERECTIC2 Calibration

The reference sample is a 10 mM Dioxane solution in $CDCl_3$.

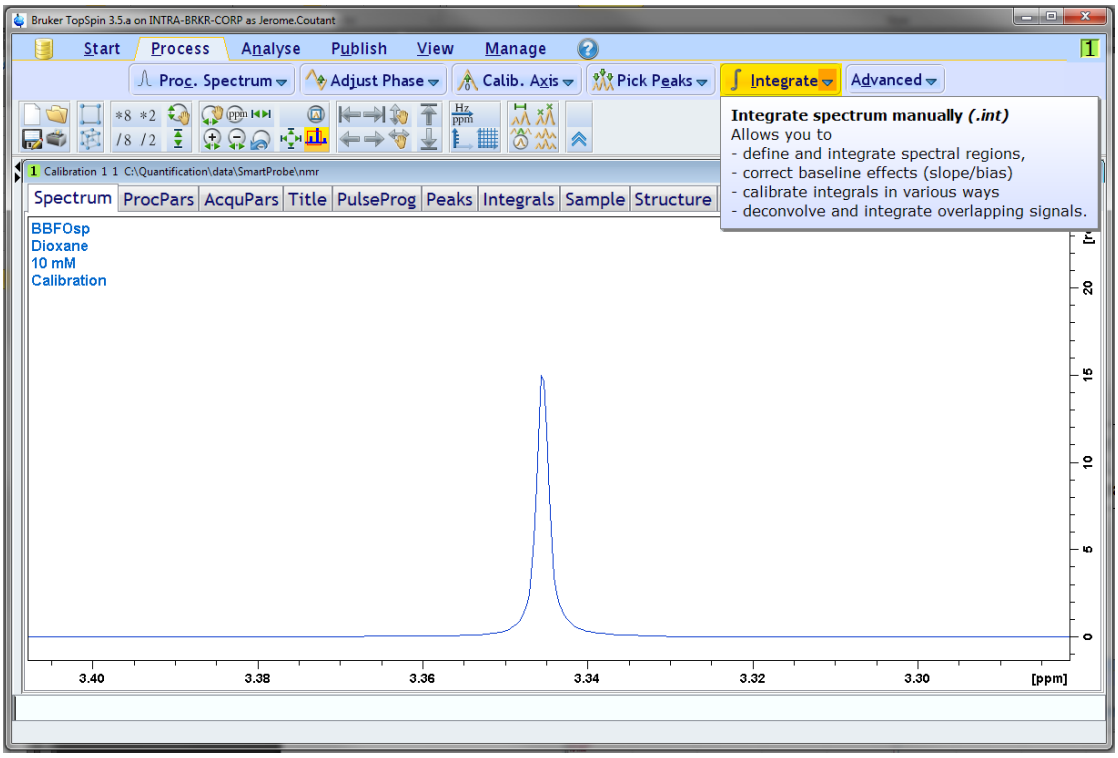


Figure 2.2: Integrating a Spectrum Manually

- In the reference sample, go into the integrate menu.

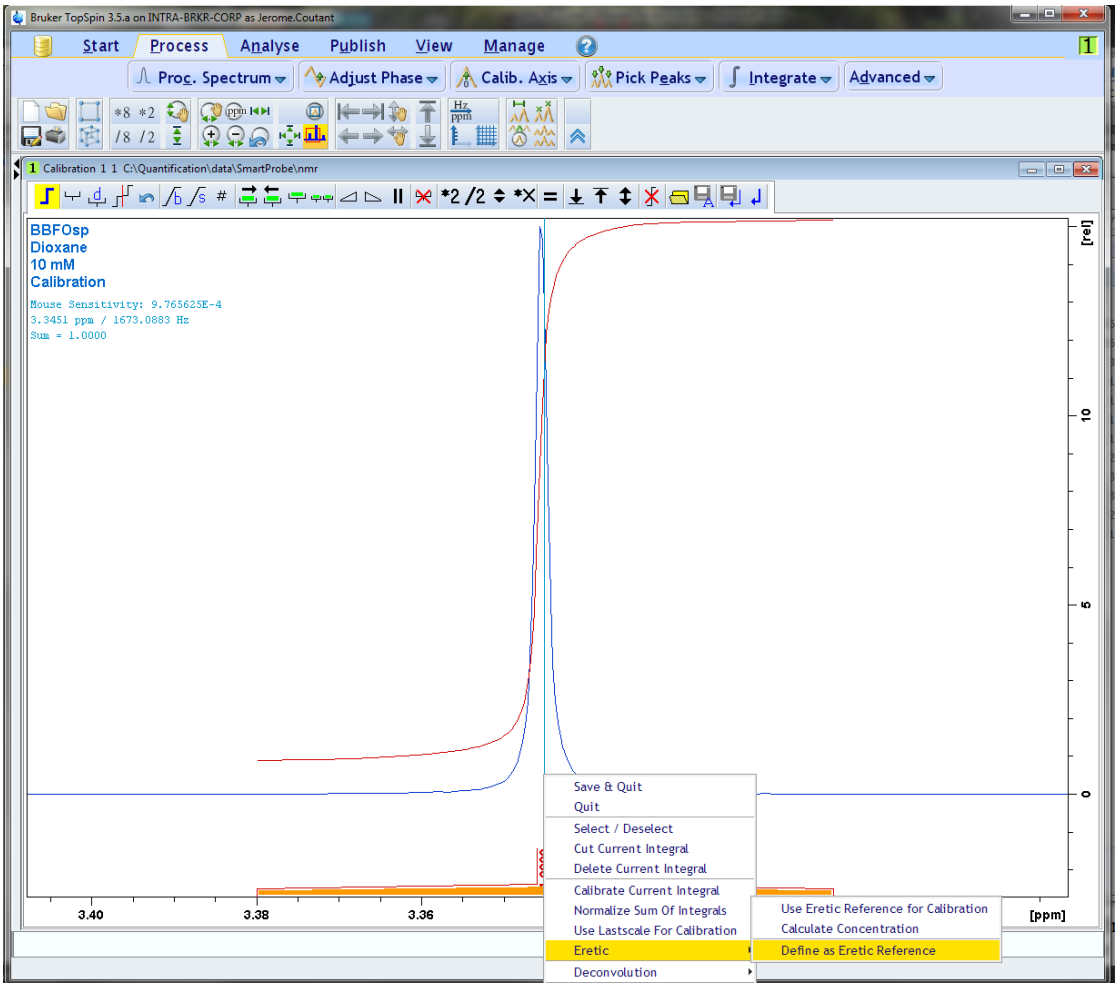


Figure 2.3: Defining the ERETIC Reference

- 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**.

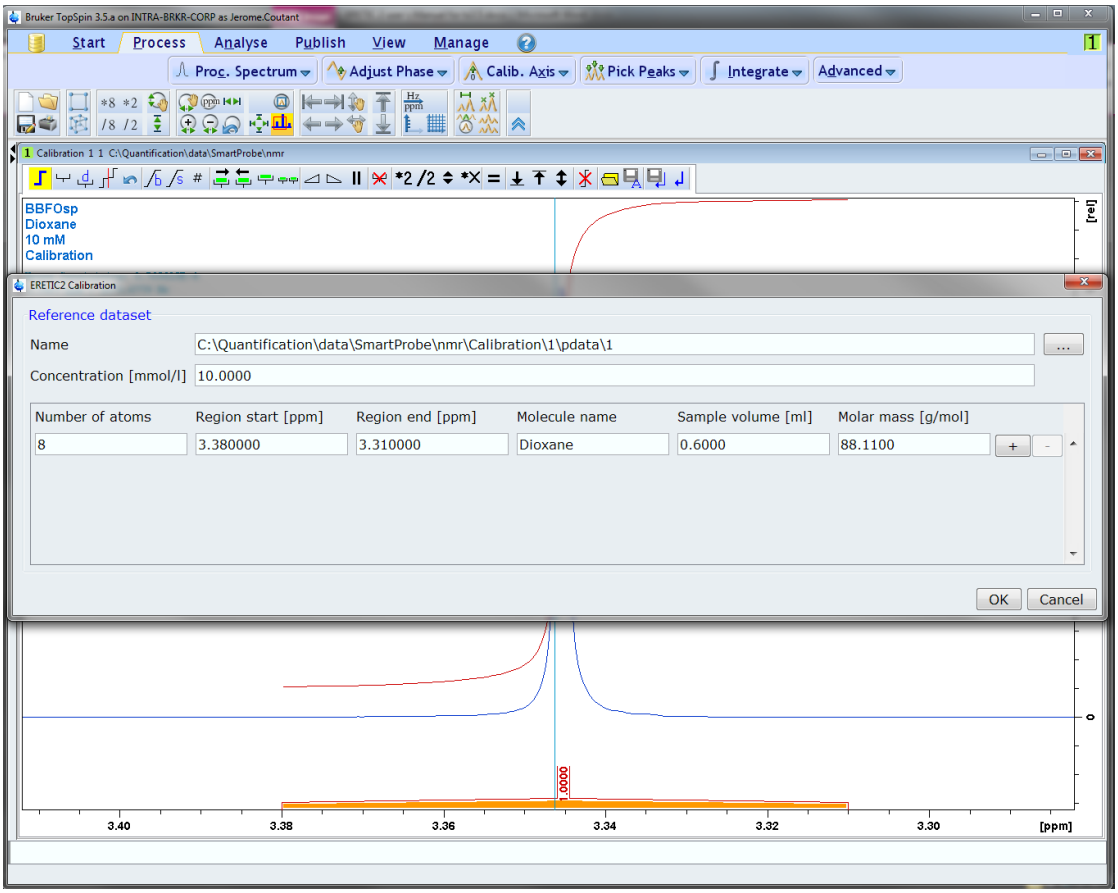


Figure 2.4: Defining the concentration of the reference sample (mM)

- Define the concentration of the reference sample (mM).
- Define the number of nuclei per signal.
- Molecule name, sample volume as well as molar mass could be defined.

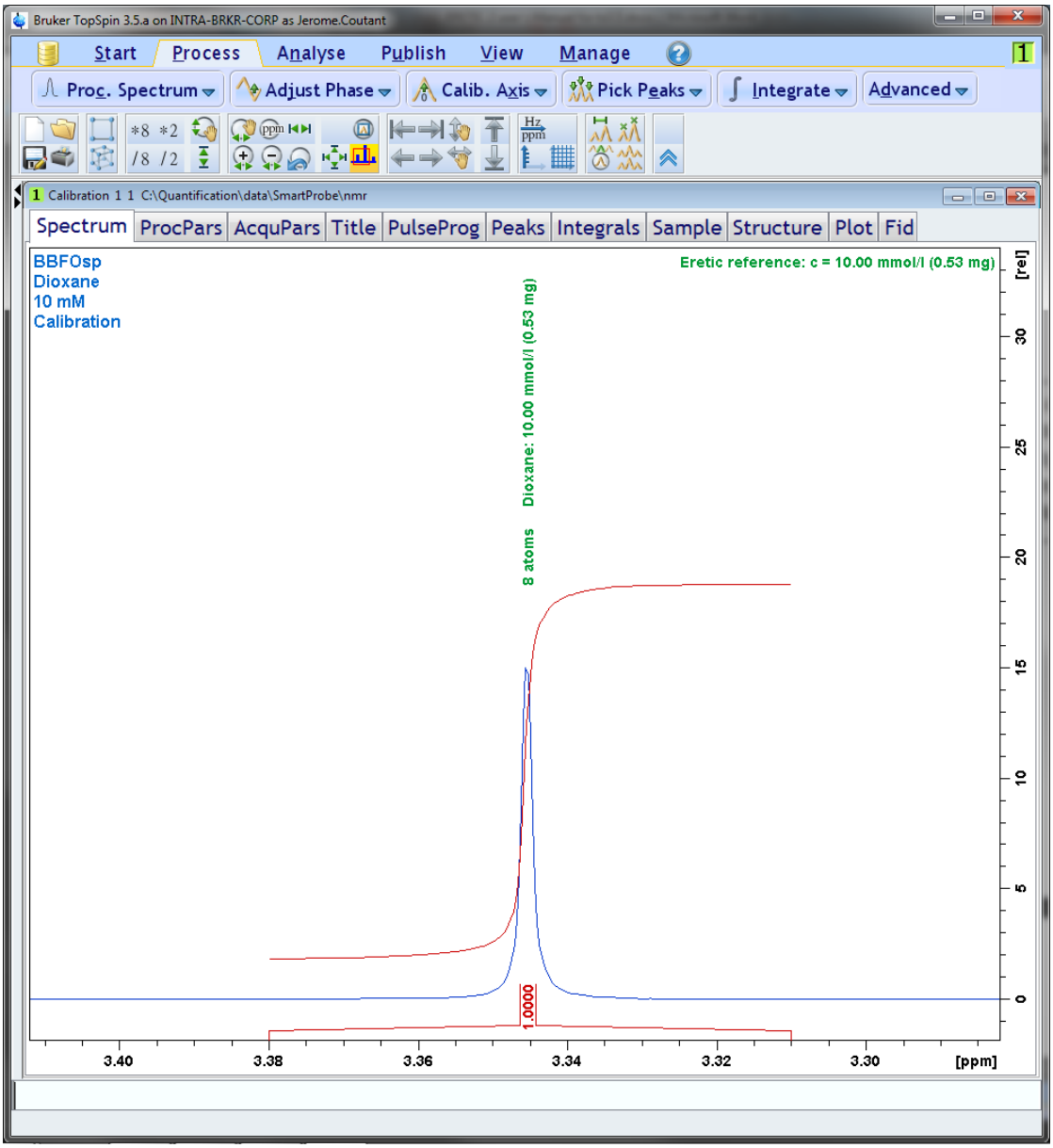


Figure 2.5: The Number of Nuclei, the Concentration and Corresponding Weight Appear on the Spectrum

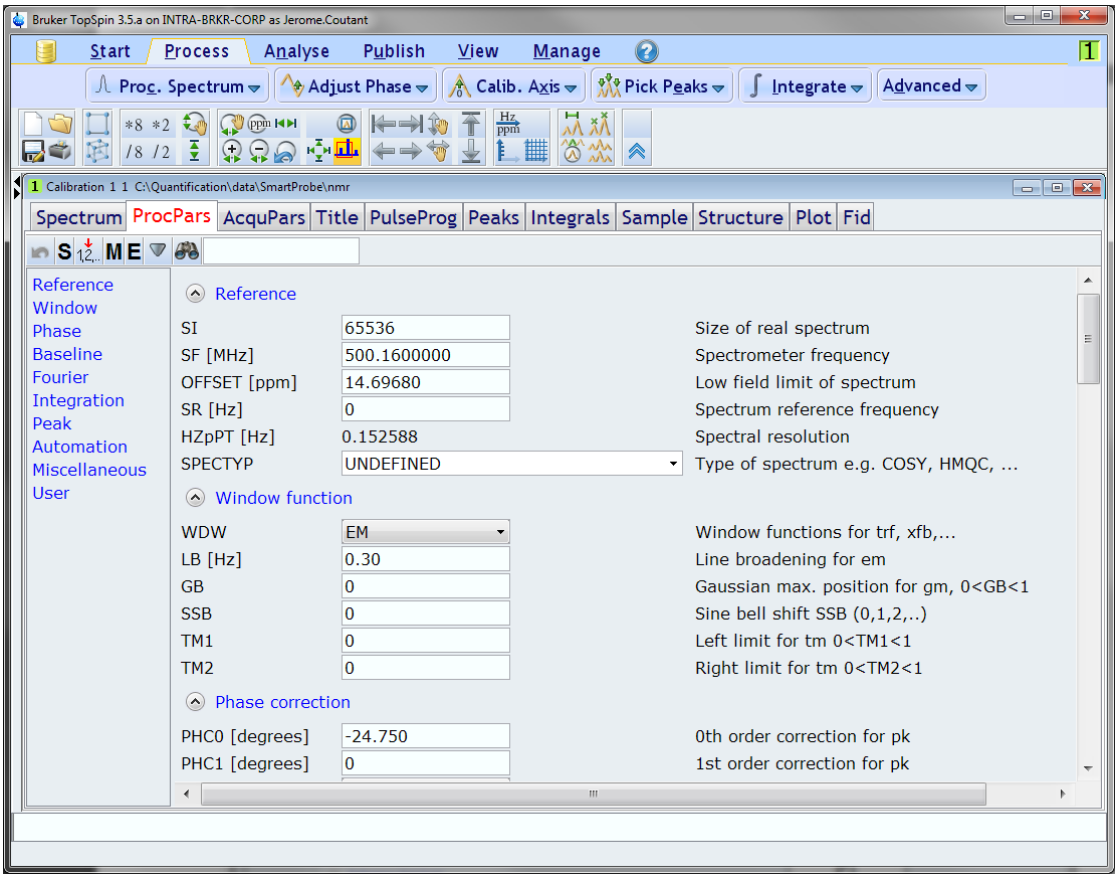


Figure 2.6: The E Button

In the processing menu, the **E** button leads to a sub-menu where you can find a summary of the acquisition parameters of the calibration experiment.

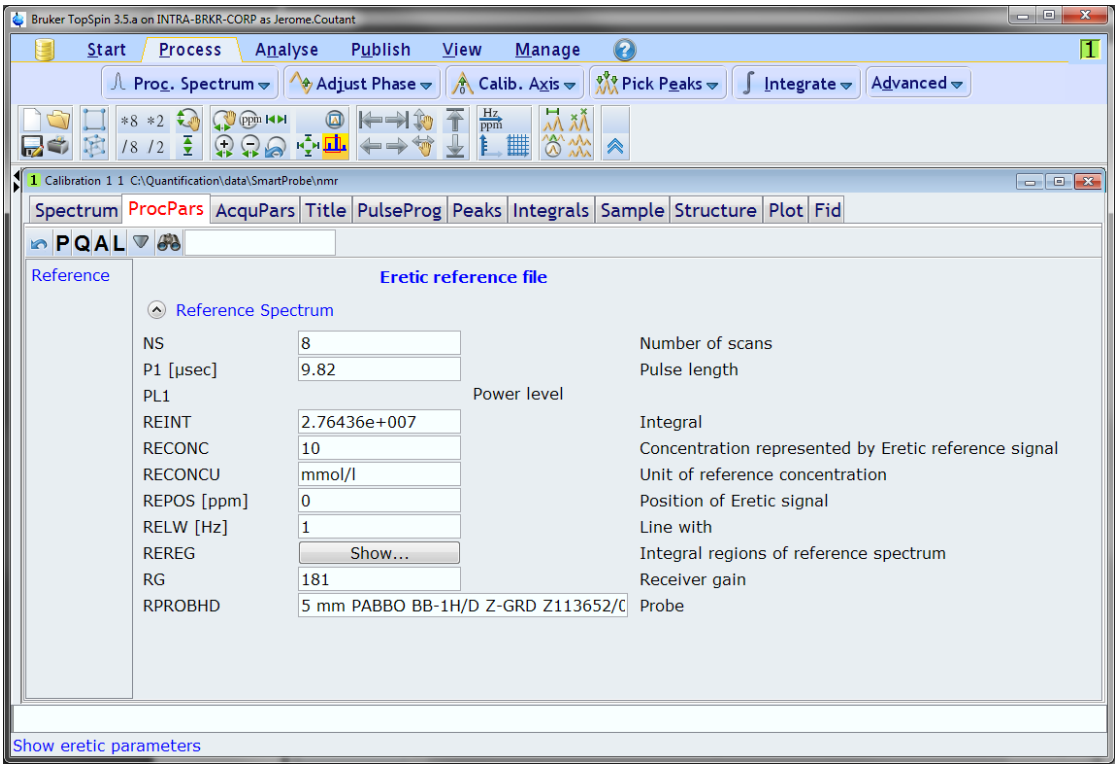


Figure 2.7: The "NS", "P1", "RG", and "PROBE" Parameters

Quantification Procedure

3 Quantification with External Standard

3.1 Acquisition

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

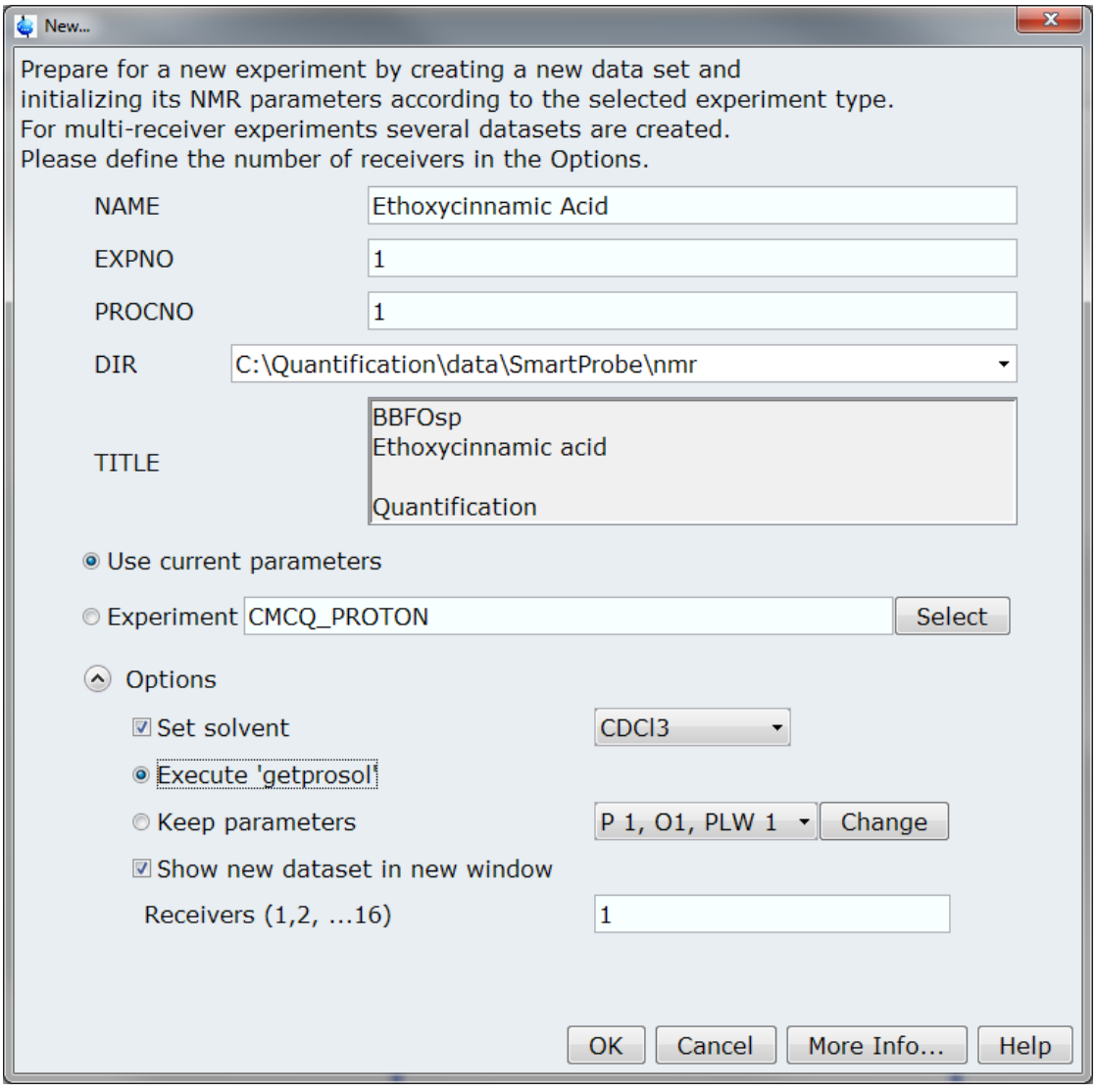


Figure 3.1: Preparing for a New Experiment

- Lock the magnetic field (lock **solvent**).
- Tune and match the probe (**atma exact**).
- Shim the sample (**topshim**).
- Calibrate the 90° pulse either manually or with the AU program pulsecal. Without option for proton, or option **sn opt**.
  - 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**) (optional).
- Start the experiment (**zg**).

3.2 Processing

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

3.3 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.

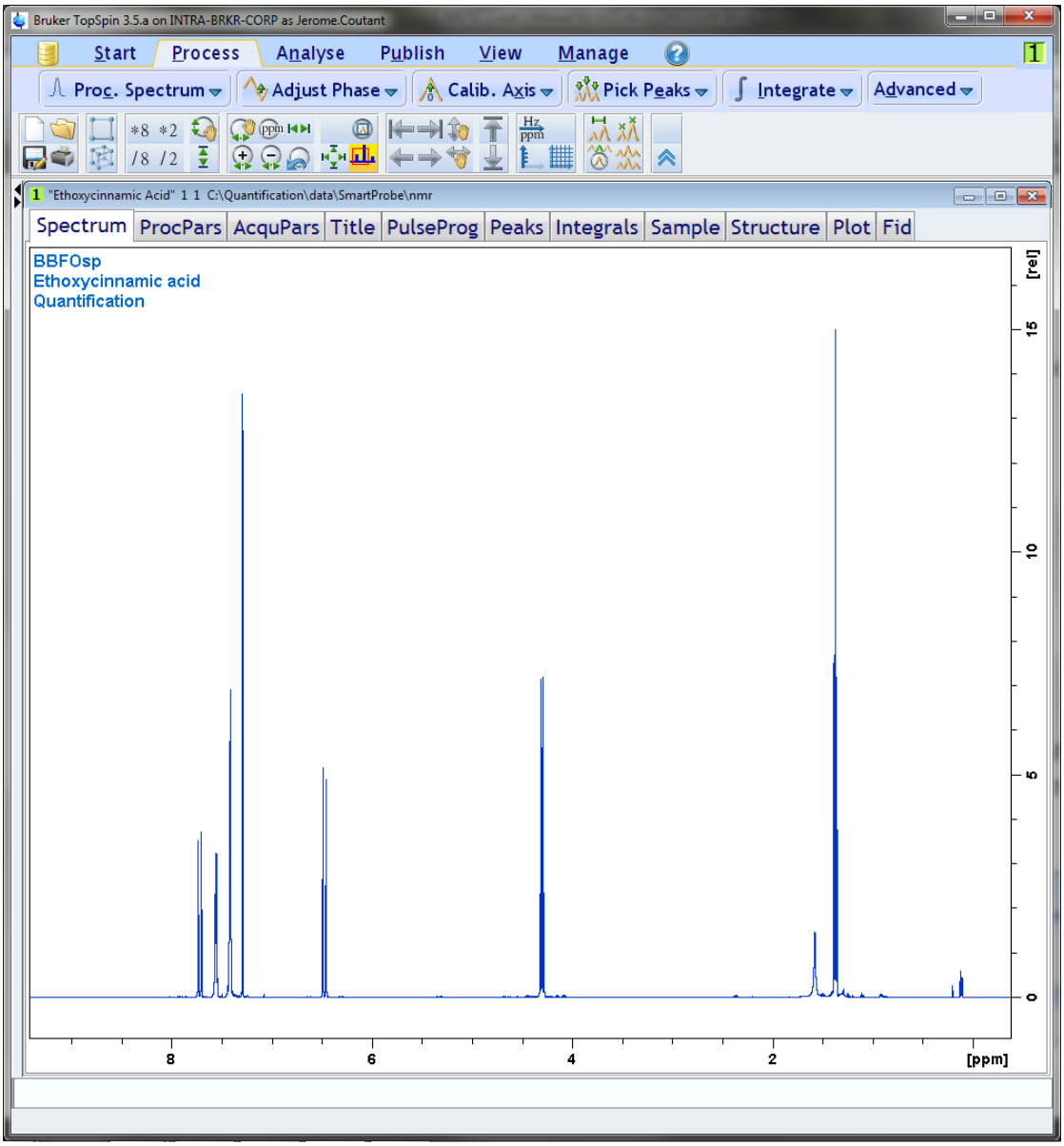


Figure 3.2: Quantification of the Sample

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 select the option **Calculate Concentration**.

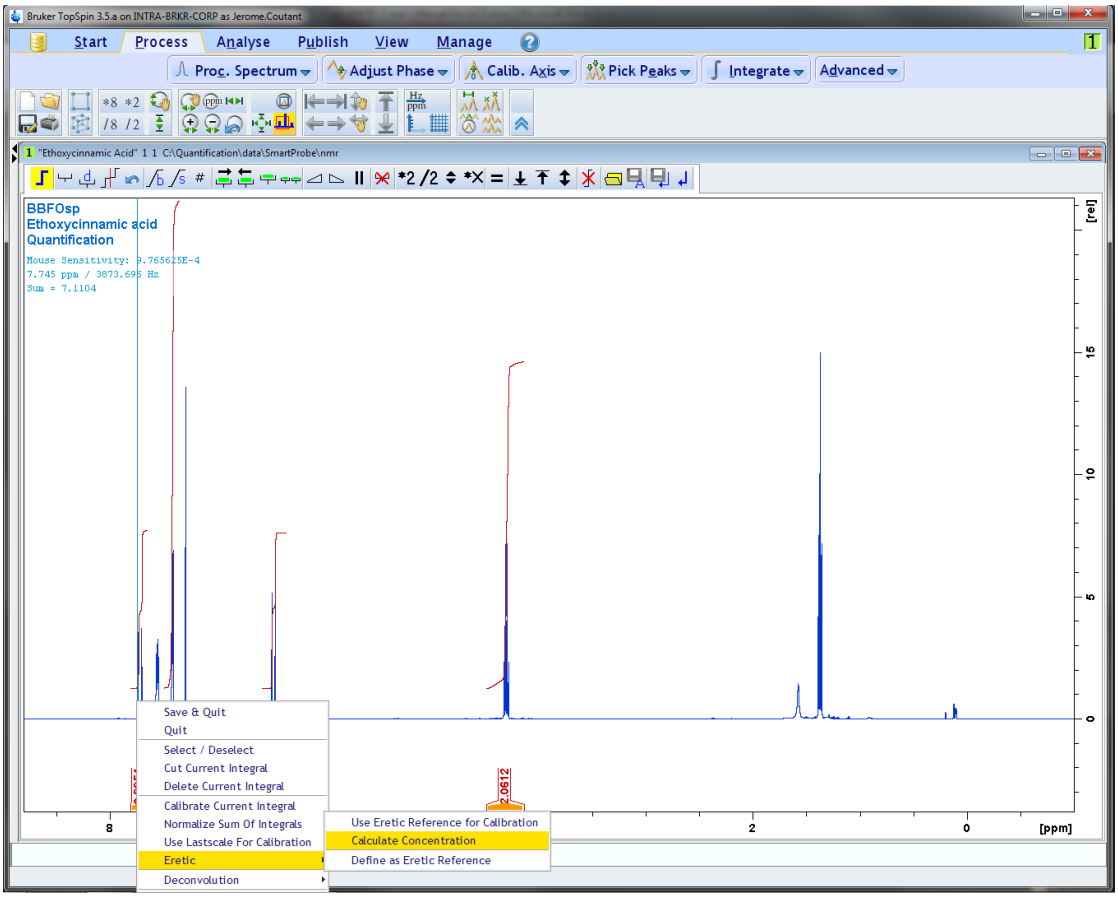


Figure 3.3: The Calculate Concentration Option

- Go into the integration menu, integrate the signals you want to quantify.
- Right click on the mouse, and select **Calculate Concentration**.

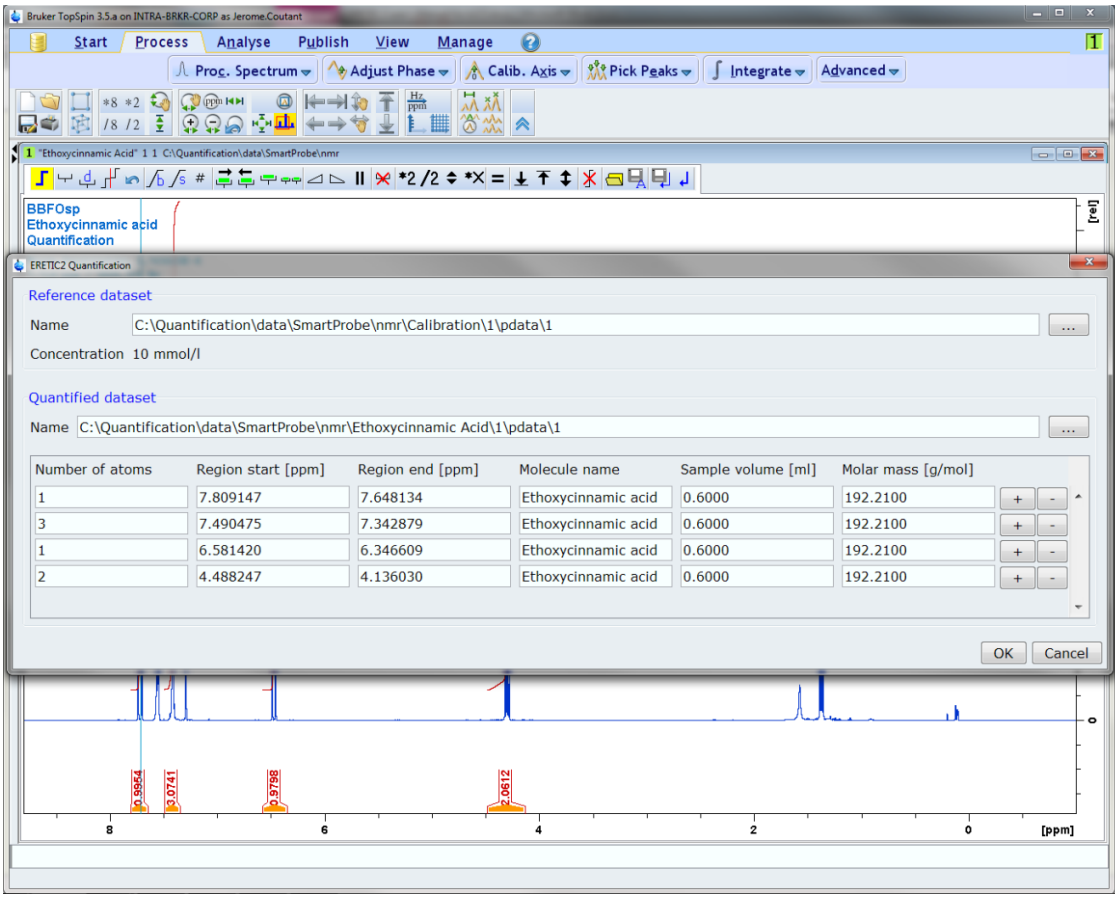


Figure 3.4: Defining the Reference File Used for Quantification

- Define the reference file used for quantification.
- Define the number of nuclei that are included in each integrals used for quantification.
- Molecule name, sample volume and molar mass can be defined as well, in order to get the amount (mg) of sample in the NMR tube.

Once this information has been entered in the window, the concentrations appear on the right. Then, click on **OK**. The results will now appear in the spectrum.

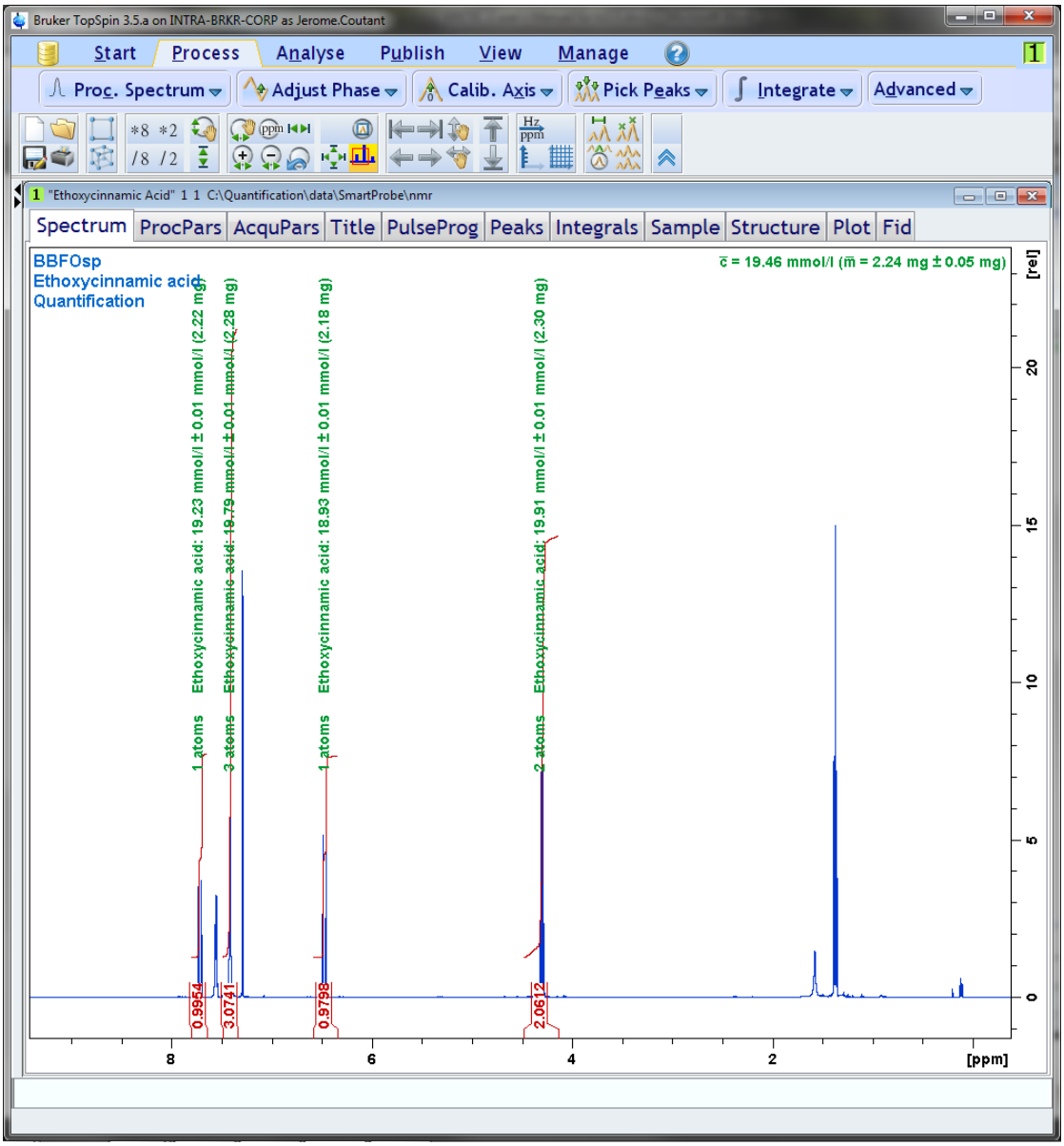


Figure 3.5: The Concentrations and Corresponding Weights Appear in the Spectrum

The concentrations and corresponding weights 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)**.

Spectrum | ProcPars | AcquPars | Title | PulseProg | Peaks | Integrals | Sample | Structure | Plot | Fid

Object	Integral [abs]	Integral [rel]	v(F1) [ppm]	Concentration (Eretic)	Atoms (Eretic)
Integral	26413726.35	0.9954	7.7286	19.2275	1
Integral	81573629.53	3.0741	7.4167	19.7935	3
Integral	25998715.32	0.9798	6.4640	18.9254	1
Integral	54694291.80	2.0612	4.3121	19.9070	2

4 Miscellanous

4.1 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 of manually defining the line width (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
- Concentration of the reference sample
- Probe
- Receiver gain

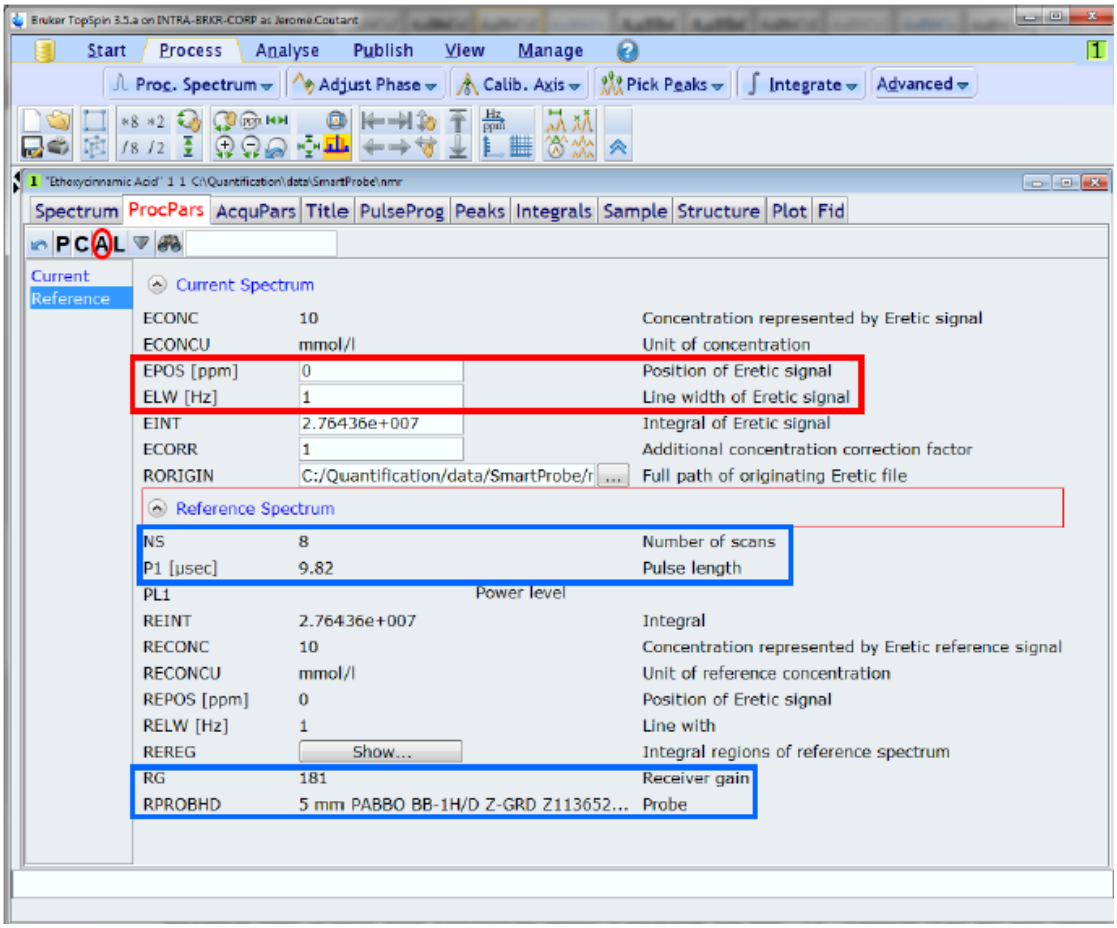


Figure 4.1: Inserting the Synthetic Signal in the Spectrum

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 weighted by the P90, NS and RG ratios between calibration and quantification.

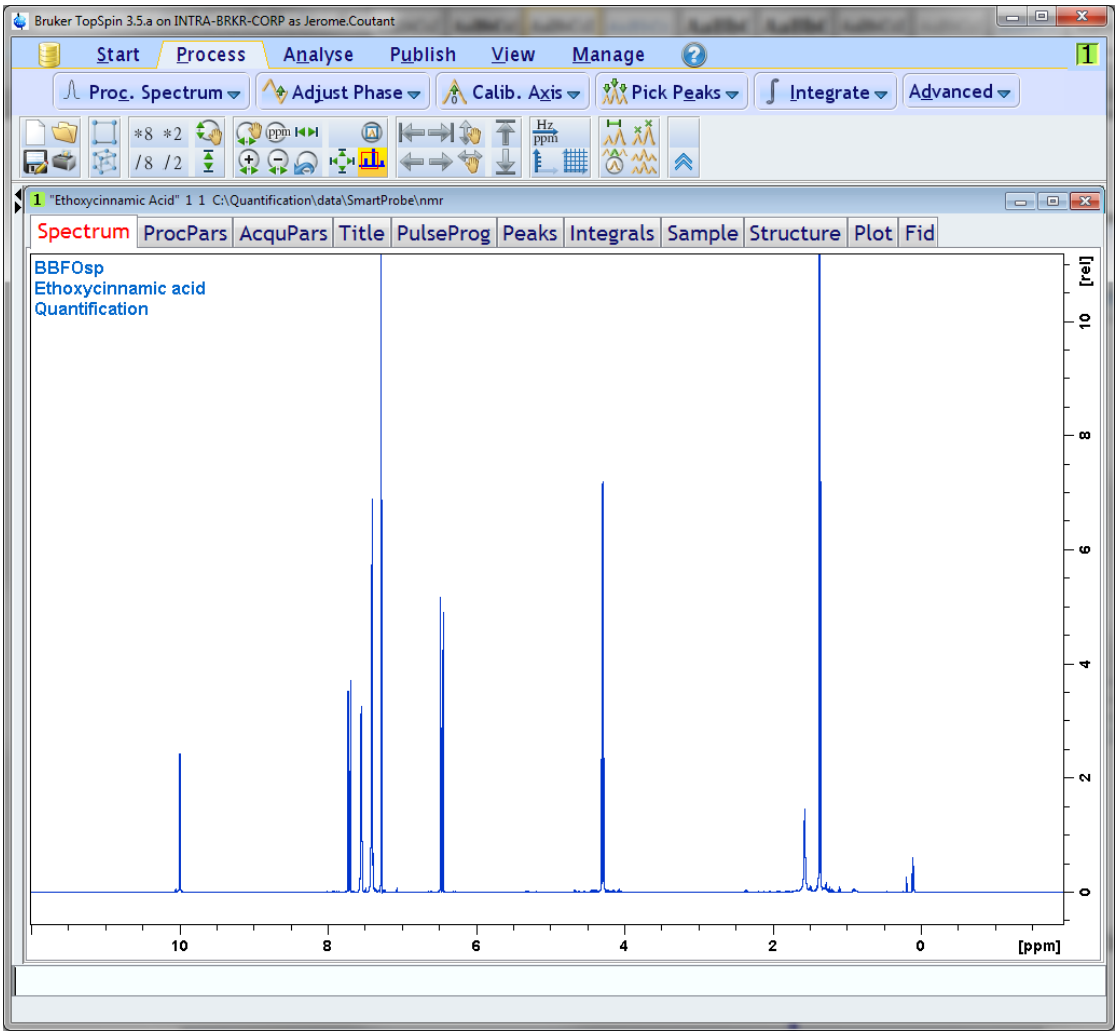


Figure 4.2: Eretic signal

4.2 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.

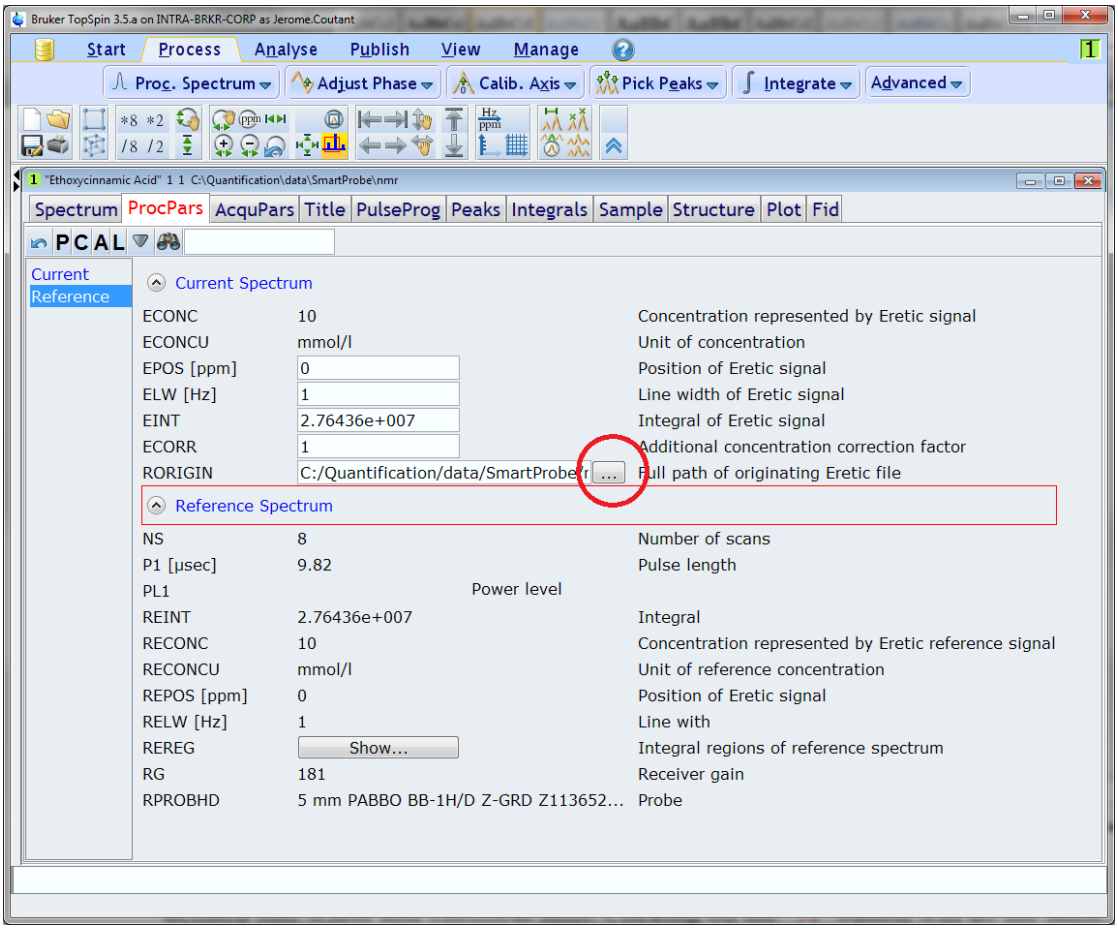
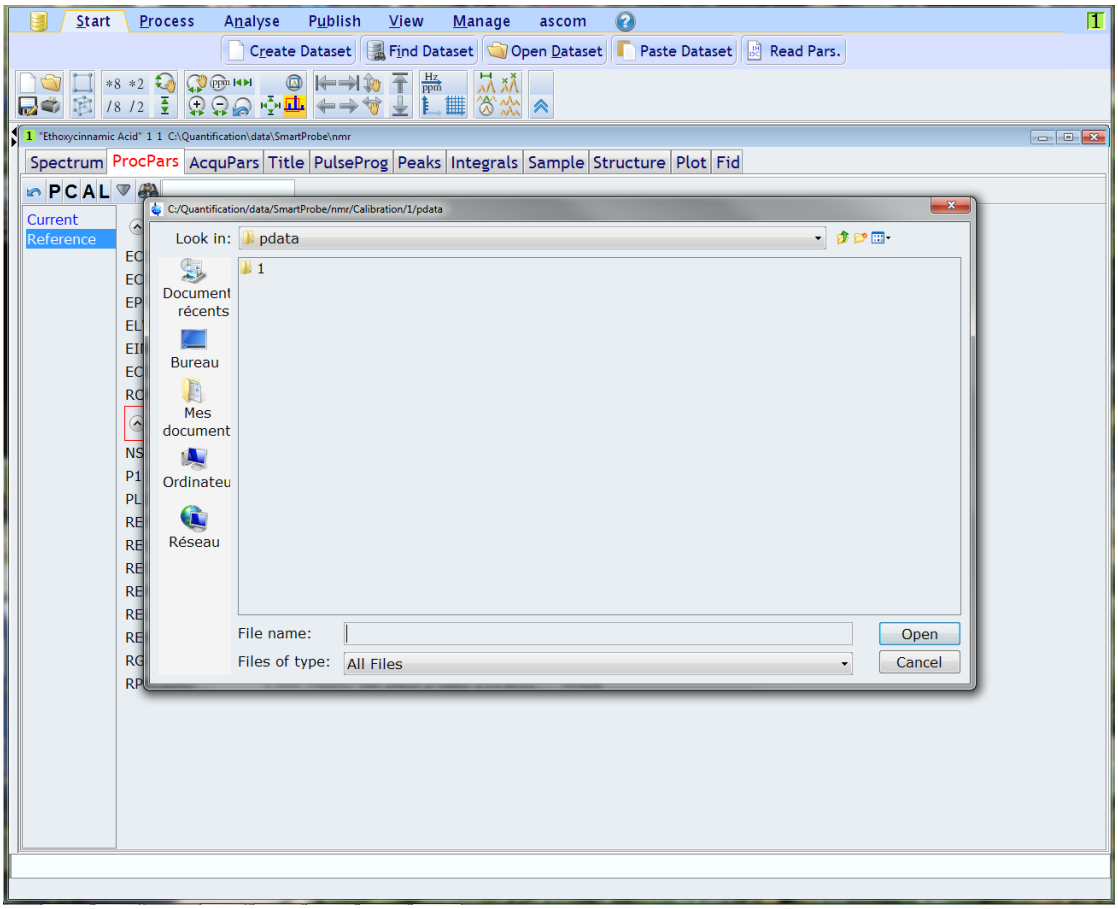
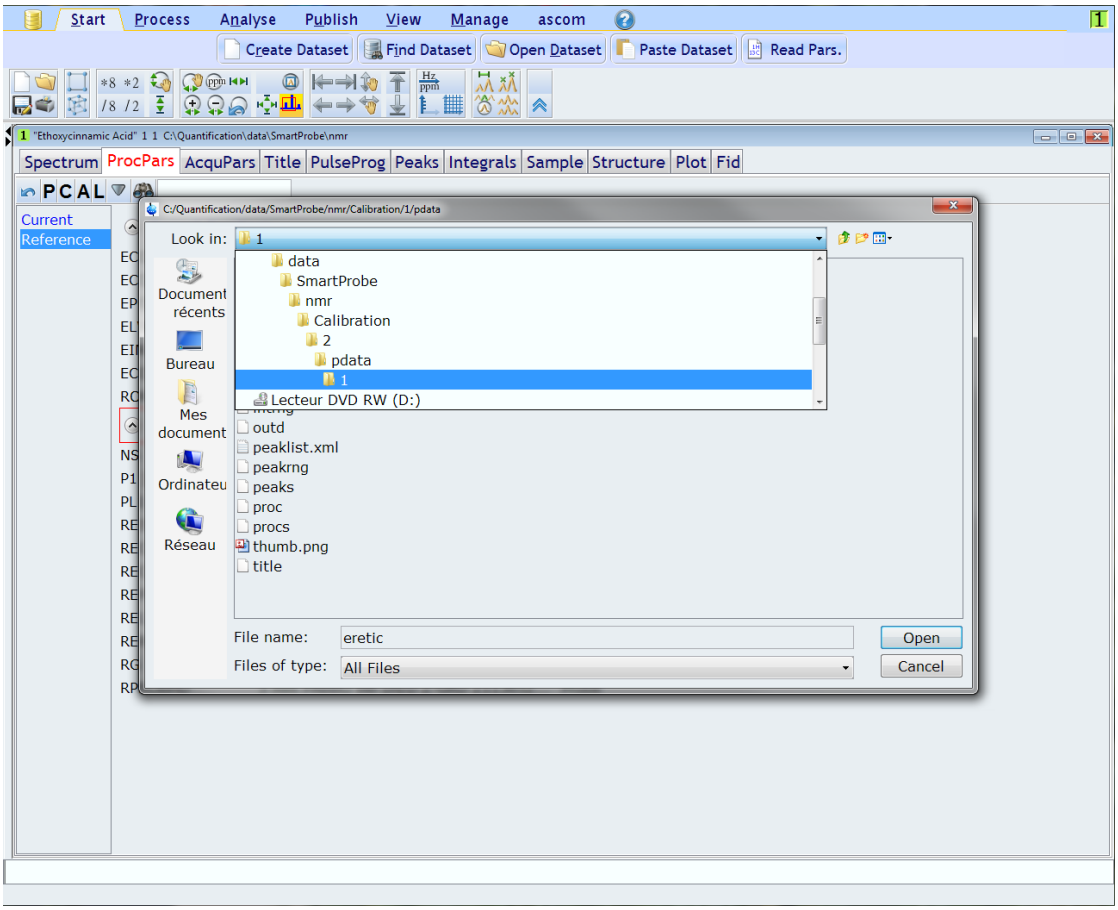


Figure 4.3: Using the Full Path of Originating ERETIC File Button

A new dialog window will open.



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


Start
Process
Analyse
Publish
View
Manage
ascom
Create Dataset
Find Dataset
Open Dataset
Paste Dataset
Read Pars.
Spectrum
ProcPars
AcquPars
Title
PulseProg
Peaks
Integrals
Sample
Structure
Plot
Fid
Current
Reference
C:/Quantification/data/SmartProbe/nmr/Calibration/1/pdata
Look in:
1
data
SmartProbe
nmr
Calibration
2
pdata
1
Lecteur DVD RW (D:)
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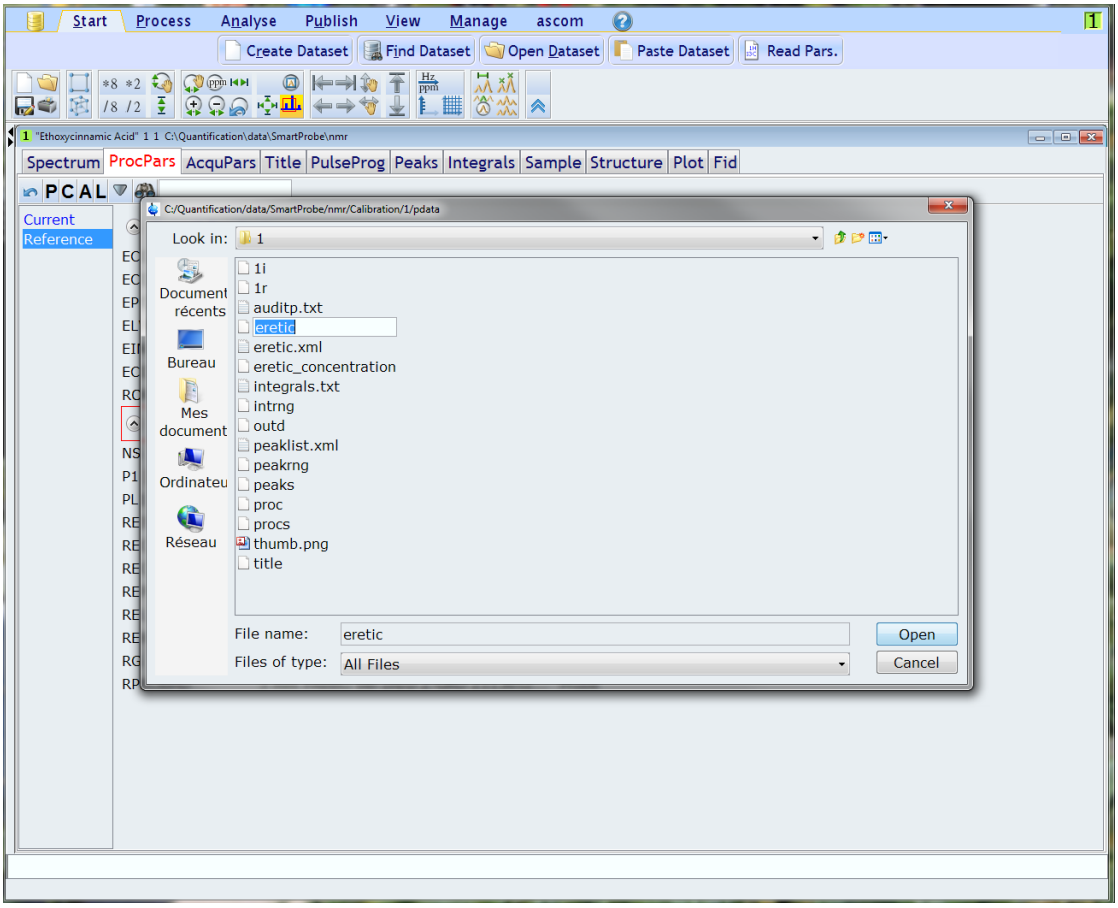
peaklist.xml
peakrng
peaks
proc
procs
thumb.png
title
Document récents
Bureau
Mes document
Ordinateu
Réseau
File name:
eretic
Files of type:
All Files
Open
Cancel


Start
Process
Analyse
Publish
View
Manage
ascom
Create Dataset
Find Dataset
Open Dataset
Paste Dataset
Read Pars.
Spectrum
ProcPars
AcquPars
Title
PulseProg
Peaks
Integrals
Sample
Structure
Plot
Fid
Current
Reference
C:/Quantification/data/SmartProbe/nmr/Calibration/1/pdata
Look in:
1
1i
1r
auditp.txt
eretic
eretic.xml
eretic_concentration
integrals.txt
intrng
outd
peaklist.xml
peakrng
peaks
proc
procs
thumb.png
title
Document récents
Bureau
Mes document
Ordinateu
Réseau
File name:
eretic
Files of type:
All Files
Open
Cancel

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

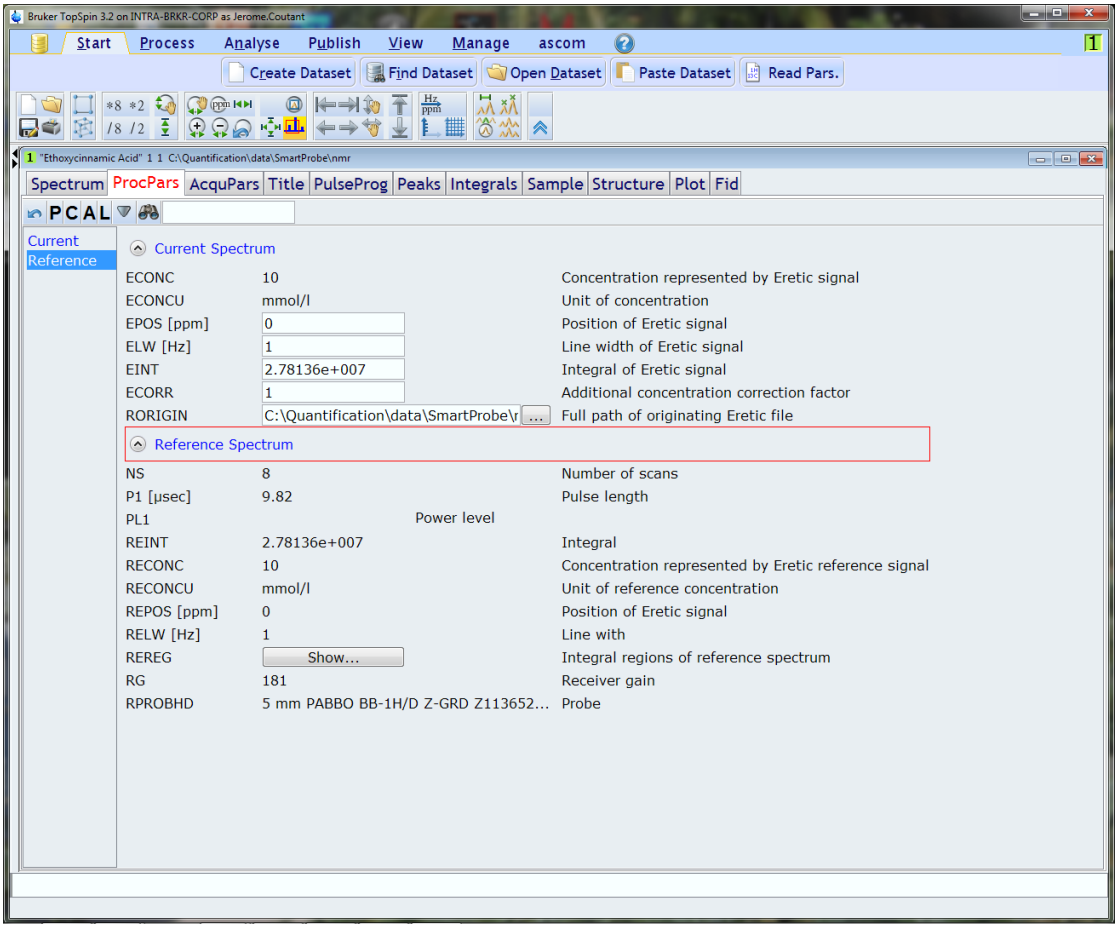


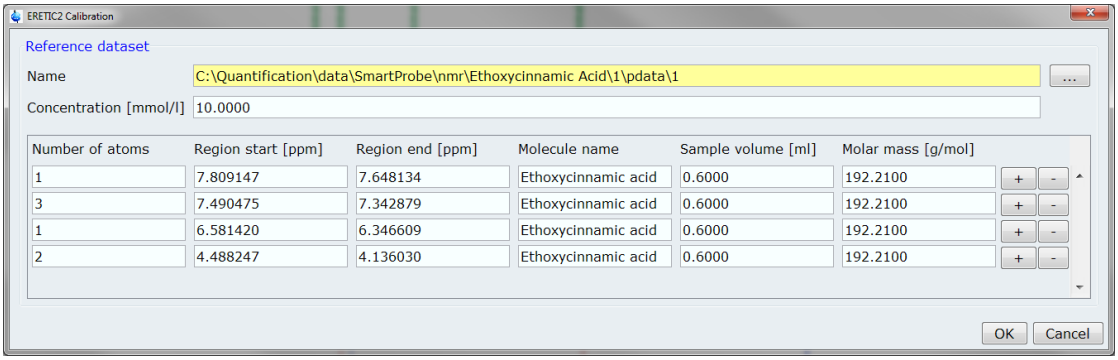
Figure 4.4: Reloading the Acquisition Parameters of the Calibration Dataset Using the "C" Button

The acquisition parameters of the new reference spectrum have been loaded.

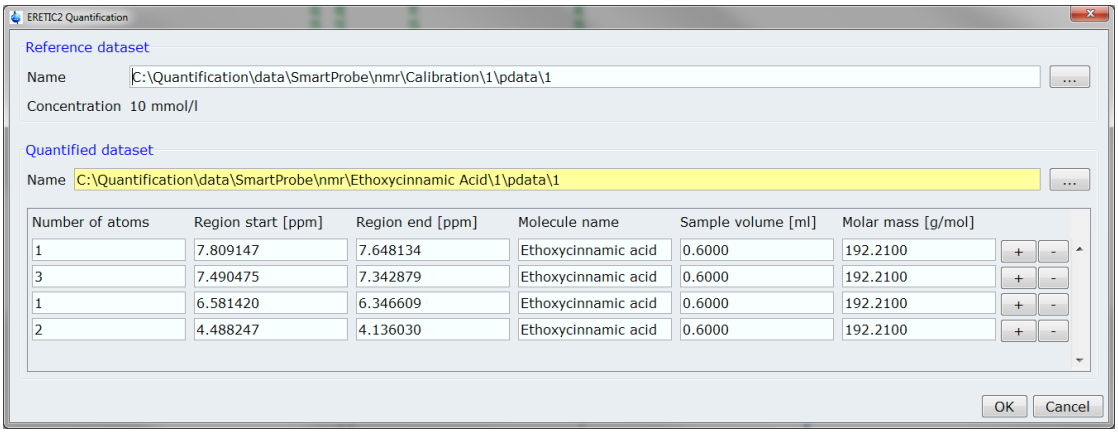
4.3 List of Commands

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.



Calc_eretic: Calculate the concentration (mM).



wpar eretic: If an eretic file has been created, the option **eretic** added to the command wpar will write the eretic file in the parameter set file.

rpar eretic: This command will write in the current dataset the eretic file contained in the parameter set file which has been read.

Miscellanous

5 Contact

Manufacturer

Bruker BioSpin GmbH
Silberstreifen 4
D-76287 Rheinstetten
Germany
http://www.bruker.com

WEEE DE43181702

NMR Hotlines

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