

ERETIC

Eretic Software Operating & User Guide

Version 001



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Contents

	Contents	iii
1	General information	5
1.1	ERETIC Technique	5
2	Installation	7
2.1	The ERETIC Pack	7
2.2	Spectrometer hardware	7
2.3	Routing / Cabling	8
2.4	Files extraction	15
2.5	Test procedure	16
3	Users Guide	17
3.1	Calibration	17
	Quick Start	17
	Description	18
	Calibration parameters setup	19
3.2	Quantification	20
	Acquisition	20
	Processing	21
4	Appendix	23
4.1	ERETIC Pulse program (using 3 rd channel version)	23
4.2	Literature	24
	Figures	25

Contents

The ERETIC technique (Electronic Reference To Access In Vivo Concentration) is a quantification experiment. Its goal is to measure directly the concentration of one or more components in the NMR tube. The main advantage of this technique is that it uses an electronic signal as a reference, thus avoiding all problems inherent to other quantification methods. Absolute quantification using NMR is widely performed by using a chemical internal reference. This method suffers nevertheless from many disadvantages since the chemical that has been chosen as the reference has to meet many criteria in terms of solubility, stability, relaxation time, chemical shift, and non interaction with the sample being quantified. The ERETIC method which uses instead an electronic signal as the reference is good alternative since it alleviates all these problems. In order to help Bruker users performing ERETIC (proton) experiments, a so-called ERETIC software has been developed and this manual provides all related information.

The electronic signal is an exponential decay modulated at the desired frequency (^1H). This signal, recorded as a pseudo-FID during the acquisition of the NMR FID, is sent on the coil that is not used for the excitation. The Fourier transform of the exponential decay gives a lorentzian peak, the intensity and position of which are totally adjustable.

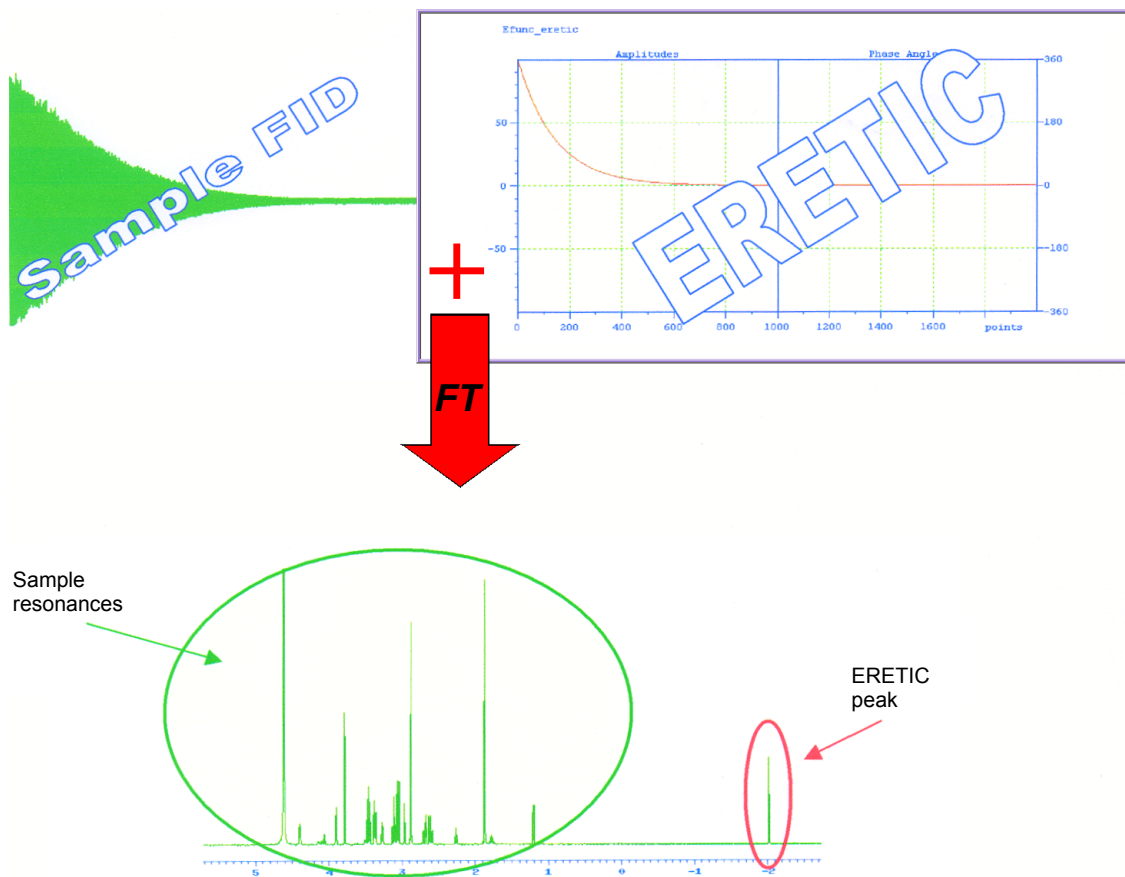
The ERETIC method consists in 2 major steps :

- a **calibration** step: where the ERETIC peak is calibrated against a standard solution, which concentration is exactly known.
- a **quantification** step: the ERETIC signal is used as a reference in order to quantify unknown solutions.

Specific pulse programs are needed in order to perform an ERETIC experiment (see "**Appendix**" on page 23). The ERETIC function has to be created *on the fly* since it depends on the spectral width of the proton spectrum.

General information

Figure 1.1. Principle of the ERETIC experiment



Installation

2

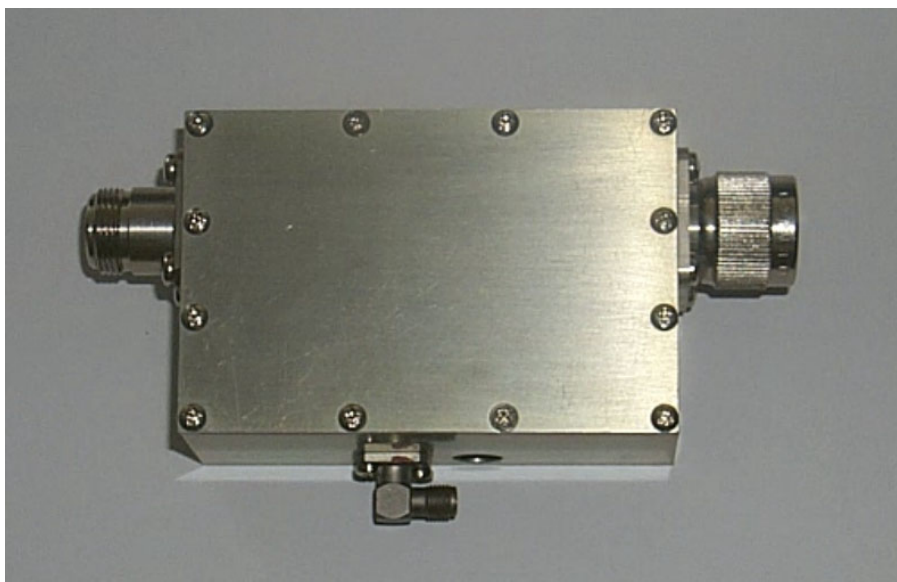
The ERETIC Pack

2.1

Description content:

- Installation CD Soft ERETIC (PN 1500325).
- ERETIC cable (PN W1101434).
- Directional coupler (PN W1301911).

Figure 2.1. Directional Coupler



Spectrometer hardware

2.2

In order to run a *proton* ERETIC experiment, two SGU's are needed: one for regular ^1H signal generation and observe (SGU2) and the other one for the ERETIC signal generation.

Two different concepts need to be differentiated: the cabling and the routing. The most important thing to remember is that the ERETIC signal never physically goes through any amplifier. Only in the routing (edasp), the SGU might be connected to an amplifier.

Several questions need to be answered before installing the ERETIC PACK:

- How many SGU's are available ?
- Is there a router in the console with an output (RO) that is not used ?

Installation

The following rules are then usually applied:

- The ERETIC signal is always generated on the last channel.
- If available, a free router output will be used in order to avoid de-cabling when running an ERETIC experiment (see next section to find out how to determine the RO number).
- If not, the SGU that is used to generate the ERETIC signal will be connected in **edasp** to an X amplifier (if several), and the router output number of this amplifier has to be determined.

Routing / Cabling

2.3

The routing corresponding to the specific hardware configuration on which the ERETIC package is installed will be saved in the ERETIC standard acquisition parameters after the AU-program `eretic_config` is done.



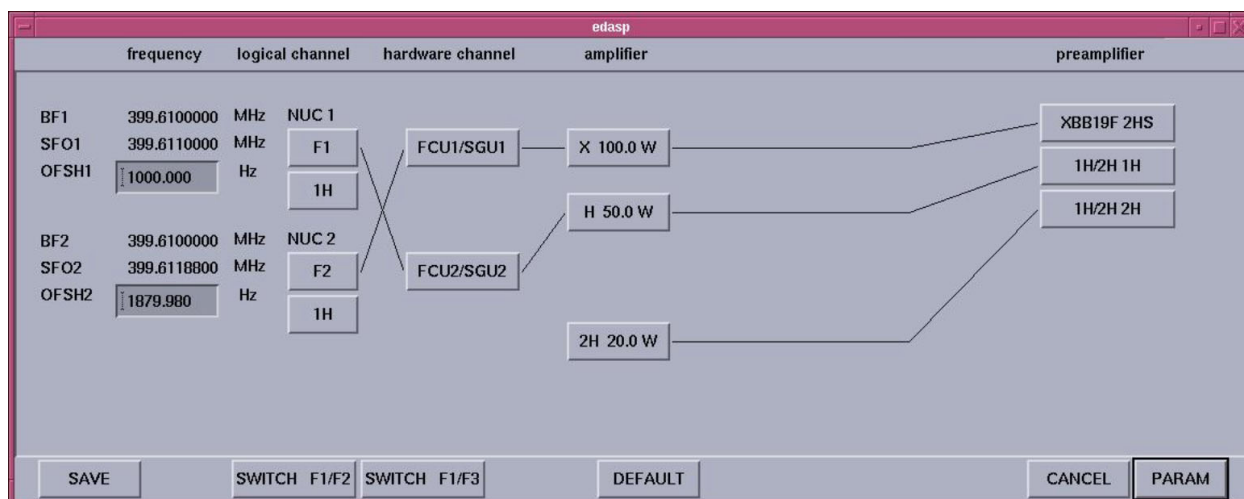
The following examples are a non-exhaustive list of typical configurations frequently encountered.

2 channels spectrometer with no router

This configuration is typical on 2 channels spectrometers equipped with a BLA2BB amplifier. In this case, no router is available, and de-cabling is necessary.

The ERETIC signal will be generated on channel 2 using SGU1, and the RO number is 1.

Figure 2.2. Routing for 2 channels spectrometer (no router)

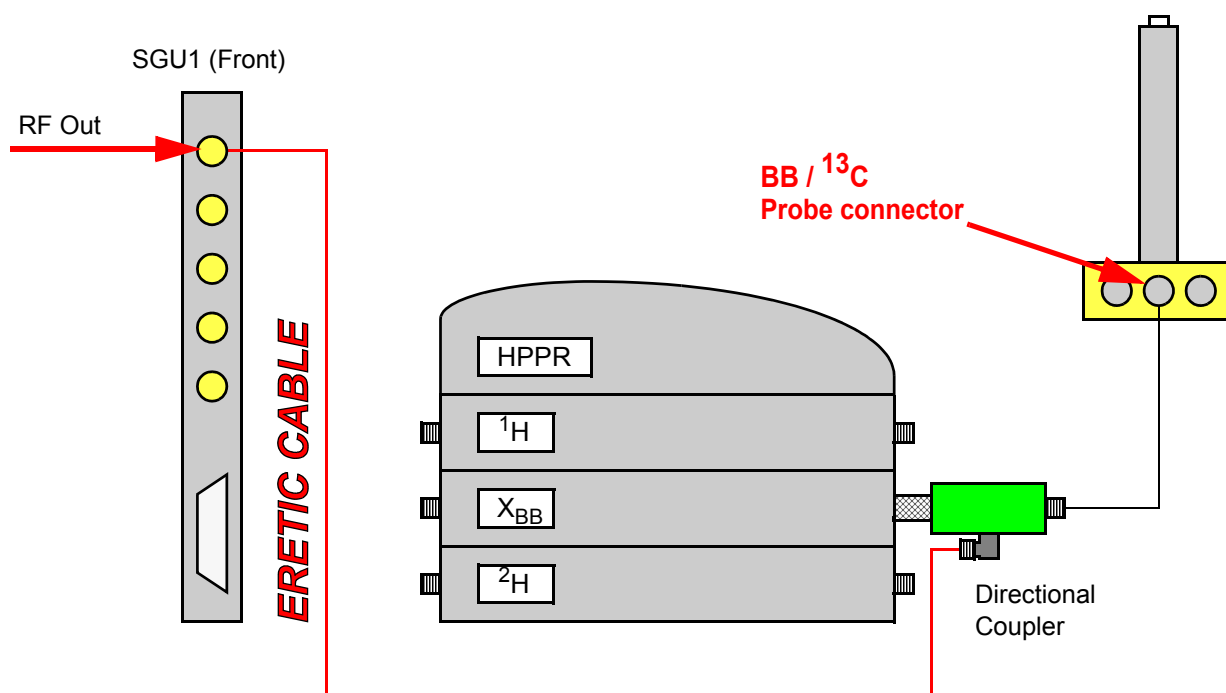


The wiring is described in the figure below.



REMEMBER: the ERETIC signal directly goes from the SGU to the probe, it never goes through the amplifier. The ERETIC cable has to be connected to the RF out output of the SGU1 and then connected to the directional coupler.

Figure 2.3. Wiring for a 2 channels system



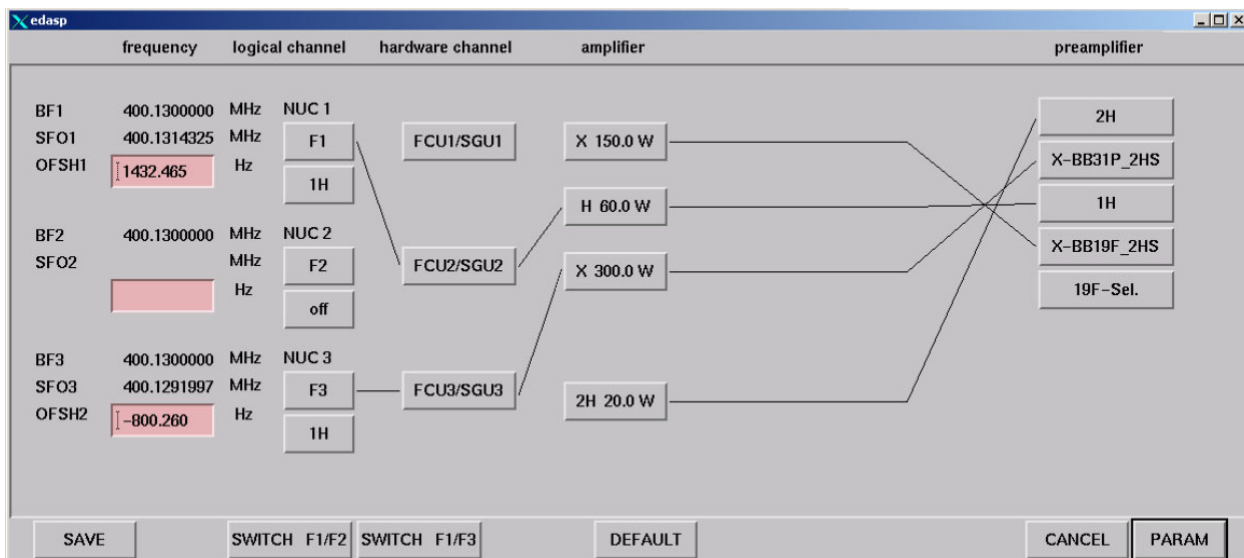
3 channels spectrometer with no router (or no router output available)

This configuration can be encountered on 3-channels spectrometers equipped with a BLA2BB and an external BLAX300. In this case, no router is available and de-cabling is also necessary.

The ERETIC signal will be generated on channel 3 using SGU3, and the RO number is 3.

Installation

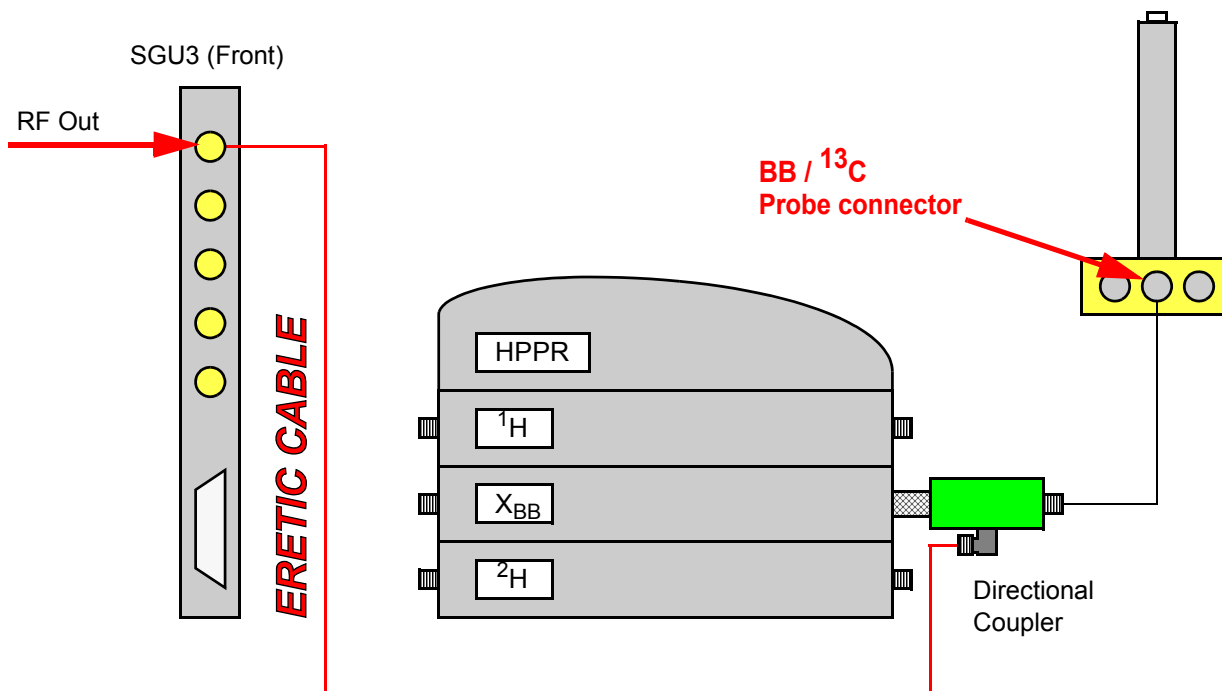
Figure 2.4. Routing for a 3 channels spectrometer (no router)



The wiring is described in the figure below.

The ERETIC cable has to be connected to the «RF out» output of the SGU3 and then connected to the directional coupler.

Figure 2.5. Wiring for a 3 channels system (no router)



3 channels spectrometer with a router output available

This configuration corresponds to most 3-channels spectrometers equipped with external amplifiers.

Routing

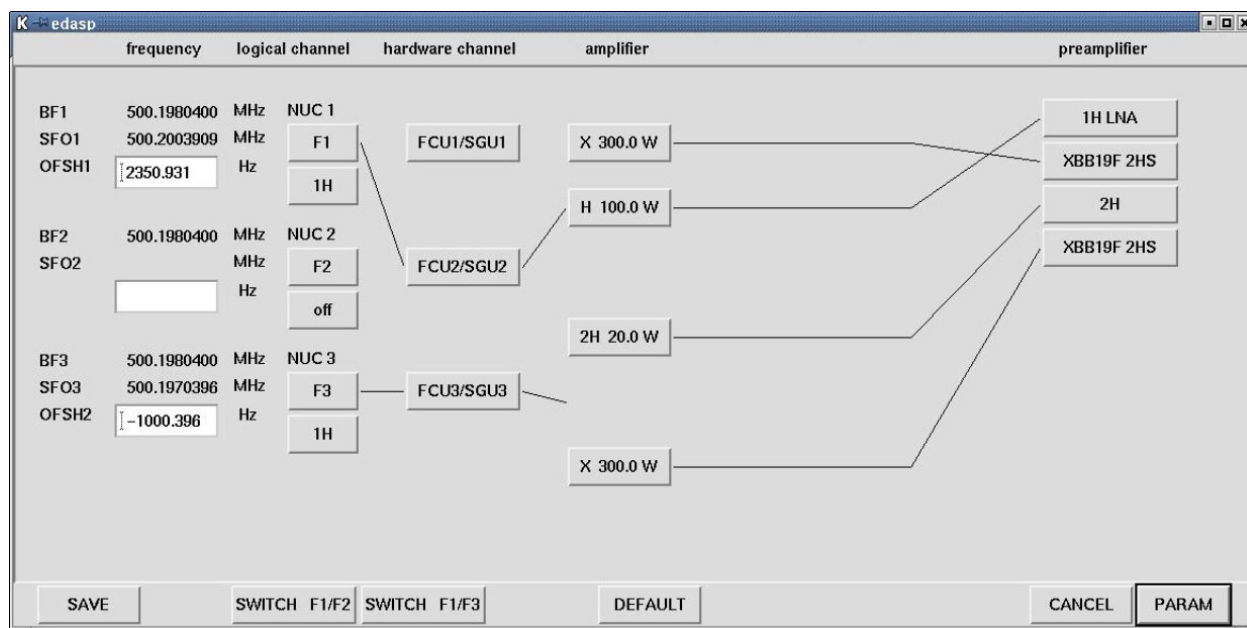
In the example shown below, we have the following configuration:

Amplifier	RO
X 300.0W	1
¹ H 100.0W	2
² H 20.0W	3
X 300.0W	5

This information can be found in <XWIN-NMR_HOME> /conf/instr/spect/uxnmr.info. The logical router outputs numbering in edasp is straightforward for a system with only one router (see figure 2.6).

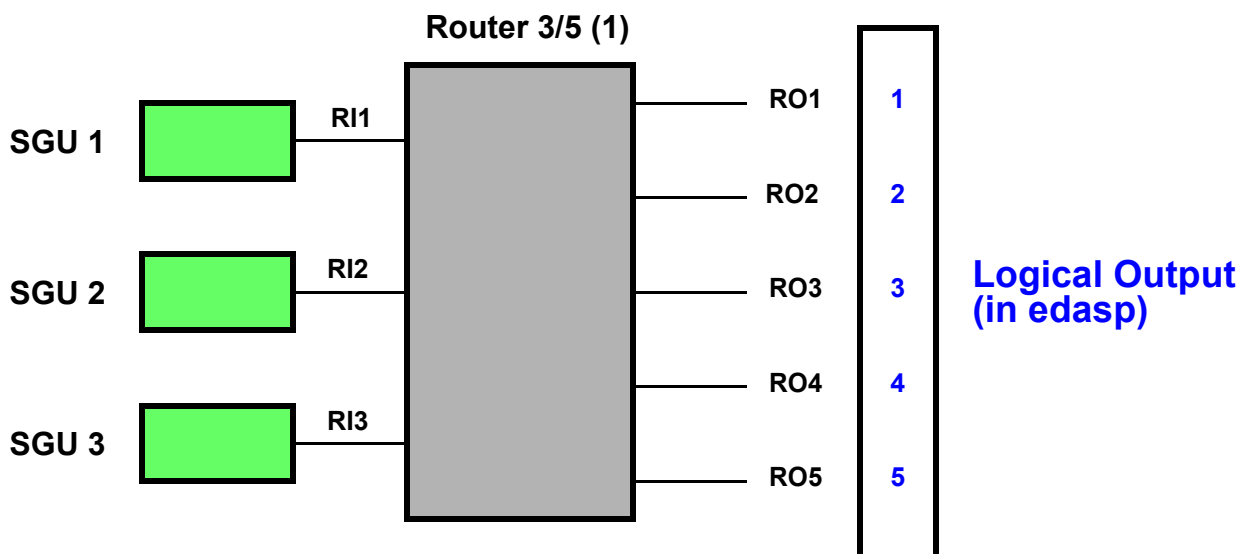
In this specific example, RO 4 is free and can be used to route the ERETIC signal.

Figure 2.6. Routing for a 3 channels spectrometer (1 router available)



This configuration cannot be manually set up in edasp, it is done by the eretic_config AU program.

Figure 2.7. Logical routing (1 Router system)



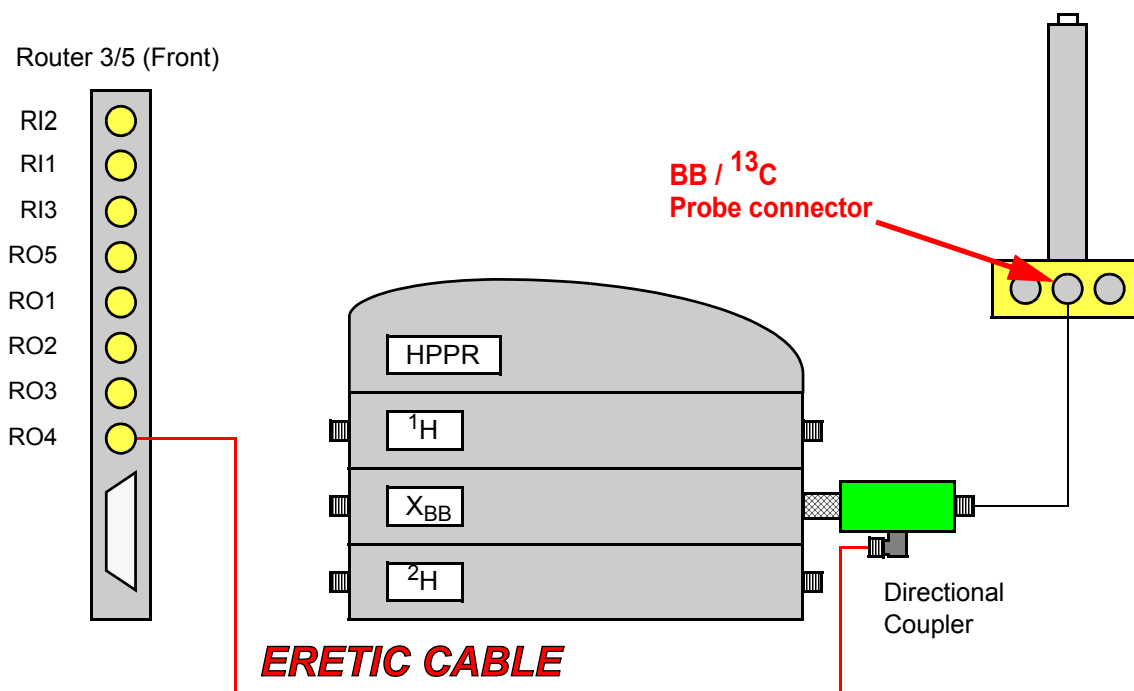
Wiring:

The wiring is described in the figure below.

The ERETIC cable has to be connected to the «RO 4» output of the router and then connected to the directional coupler.

The advantage of this configuration is that the ERETIC cable can be connected and then left at this position.

Figure 2.8. Wiring for a 3 channels system (1 Router available)



4 channels spectrometer with a router output (or more) available

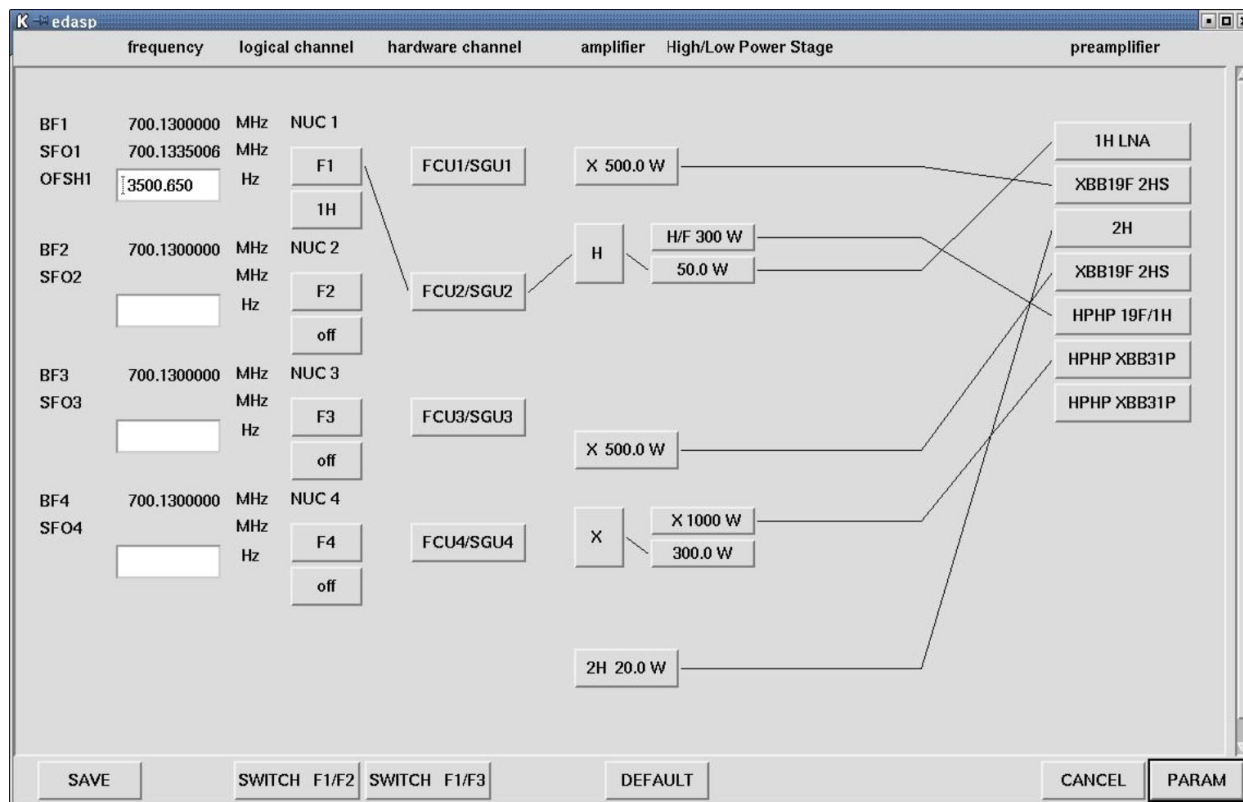
This configuration is usually found on high field instruments (4 SGU's and 2 routers).

In the example shown in the figure 2.9, we have the following configuration:

Amplifier	RO
X 500.0W	1
¹ H 300.0W	2
¹ H 50.0W	2
X 500.0W	5
X 1000.0W	6
X 300.0W	6
² H 20.0W	7

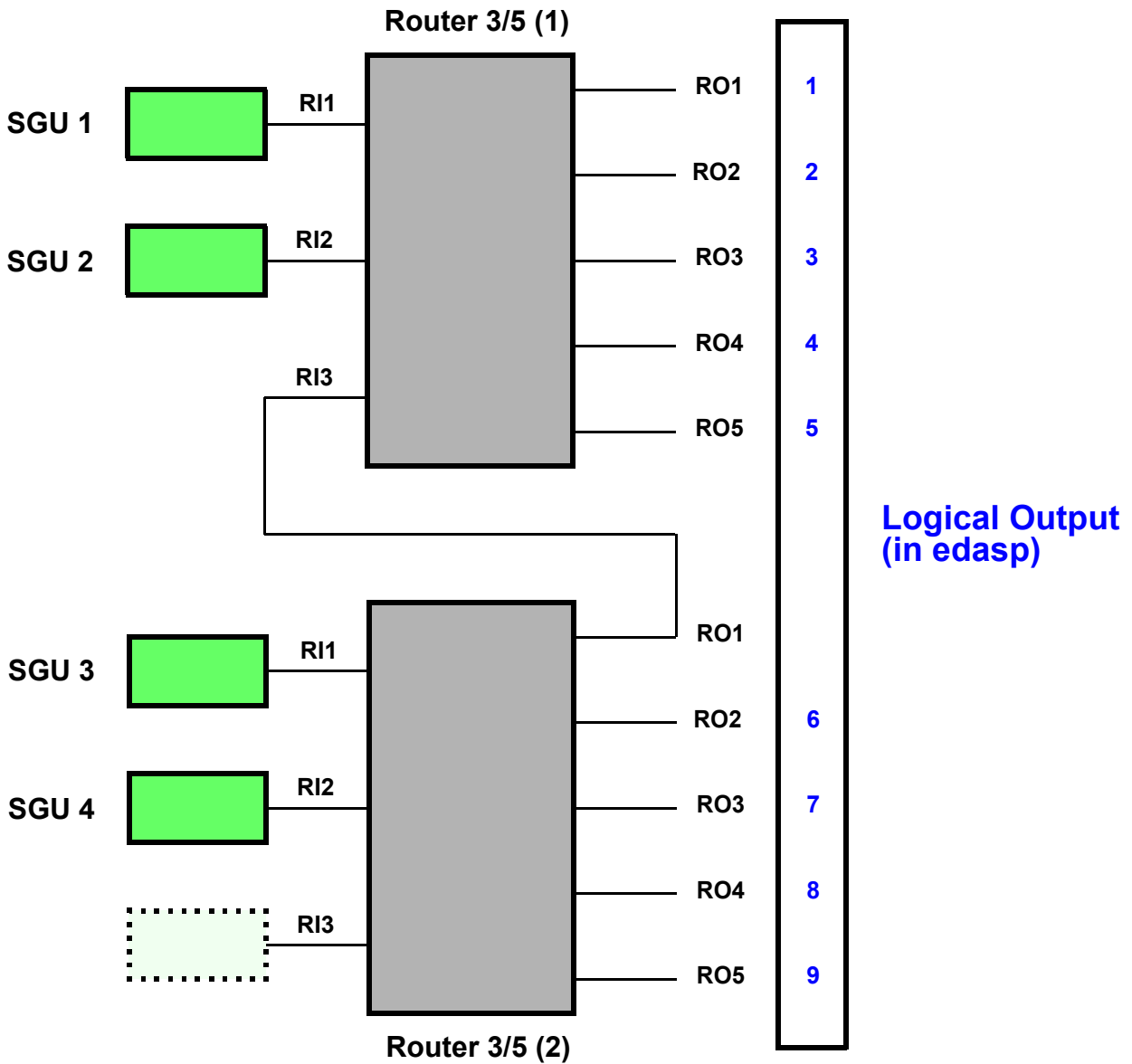
This information can be found in <XWIN-NMR_HOME> /conf/instr/spect/uxnmr.info. Each router has 3 entries and 5 outputs (RO's). Figure 2.10 shows how two routers are connected and also the correspondence between the physical router outputs and the logical outputs in edasp.

Figure 2.9. Routing for a 4 channels spectrometer (1 Router available)



Installation

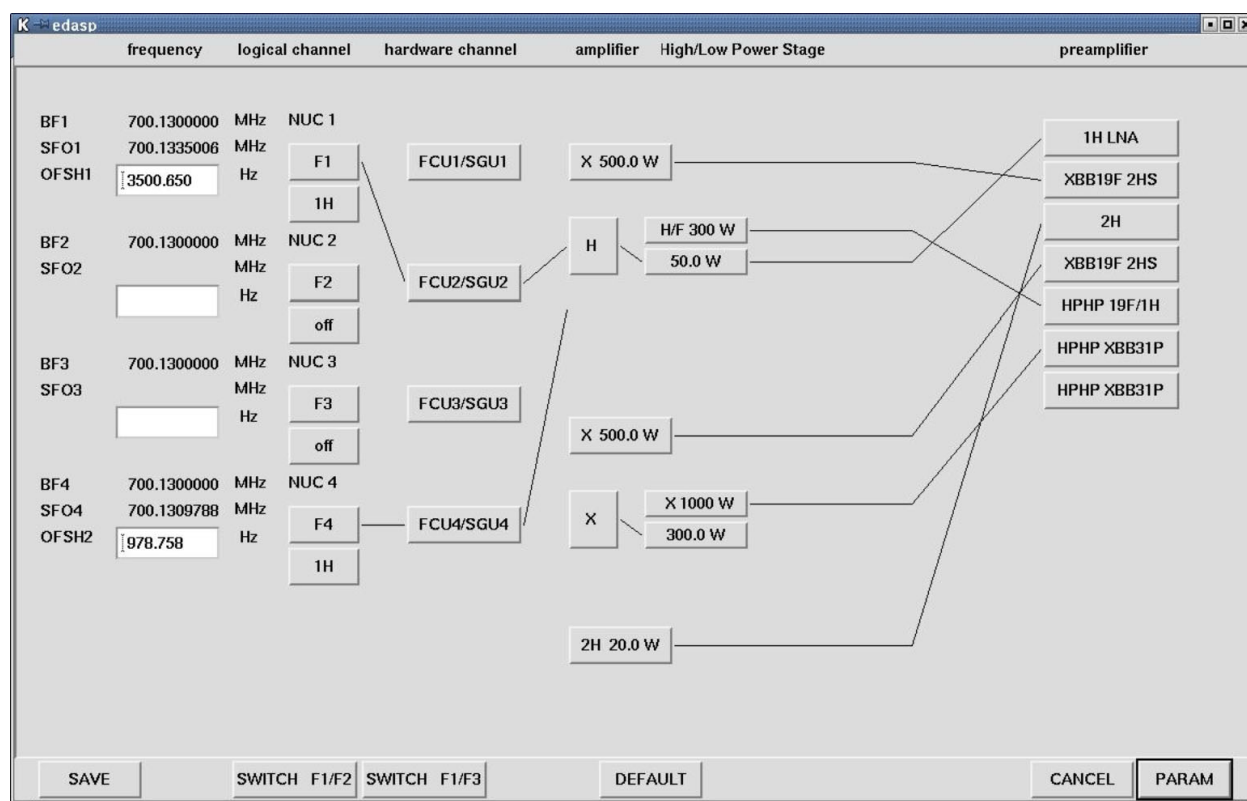
Figure 2.10. Logical Routing (2 Routers system)



This means that RO3, RO4, RO8 and RO9 are free and can be used to route the ERETIC signal.

This configuration cannot be manually set up in edasp, it is done by the eretic_config AU program. In the configuration represented in figure 2.11, RO3 has been used.

Figure 2.11. Routing for a 4 channels spectrometer (1 Router available)

**Wiring:**

The ERETIC cable has to be connected to the RO 3 output of the router number 1 and then connected to the directional coupler. If RO 8 had been chosen, the ERETIC cable would have had to be connected to the physical output RO 4 of the second router.

Files extraction**2.4**

Different files are needed depending on the channel used for the ERETIC signal generation.

The CD contains three directories: ERETIC.F2, ERETIC.F3, and ERETIC.F4. Each of the directory contains a unique file with the same name, and the extension .exe for WINDOWS system or .tar.gz for Linux systems.

Instructions:

1. Copy the appropriate file in the \$XWIN-NMR HOME directory.
2. For Windows systems: **double click** on the file in order to auto-extract it.
For Linux systems, make sure you are super user and type: **tar xvf file.tar**.
3. Login in XWIN-NMR as nmrsu.
4. Type *expinstall* in the XWIN-NMR command line.
5. Type *edhead*, and write down the probe numbers in the list that will be used with the software.
6. Type *xau eretic_config* and answer the different questions.

The following test can be performed in order to ensure that everything has been installed correctly.

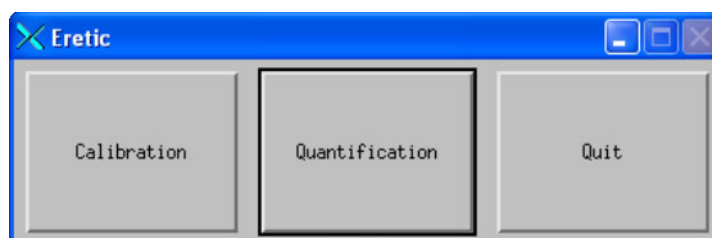
- Cable the spectrometer so that is ready to run an ERETIC experiment.
- Create a new dataset "eretic_test".
- Insert a sample, tune it, shim it. Type the following commands:
RPAR ERETIC ALL.
GETPROSOL
- Adjust specific parameters if necessary (TD, RG, NS, ...).
- **Check in edasp if the routing corresponds to the routing chosen during the installation.**
SP15 = 30dB
ZG
EFP
- When the acquisition is done, type **xau apktic**, and run the experiment again. The spectrum should be correctly phased now.

The absolute value of the ERETIC peak for a given initial electronic signal strongly depends on the coupling between the two coils. For this reason, the coil receiving the ERETIC signal has to be always tuned at the same frequency. The dielectric constant of the sample is also a factor which can have an influence on this coupling: this means that the sample used for calibration has to be as close as possible to the one being quantified. In order to allow several calibrations, the ERETIC software offers the possibility of choosing the NMR solvent when doing ERETIC experiments.

Standard Samples:

Bruker does not provide any standard NMR tubes for the calibration. Nevertheless, it is recommended to use standard solutions of 1,4 p-dio-xane in various solvents. All default parameters in the ERETIC calibration are set for 1,4 p-dioxane in D₂O, CDCl₃, DMSO, or C₆D₆, but it is possible to change them in order to use any chemical to calibrate the electronic signal (see section "**Calibration parameters setup**" on page 19).

Figure 3.1. ERETIC SOFTWARE Interface



- Put the reference sample in the magnet, tune it, shim it.



Important Notice for BB probes:

Since the ERETIC signal is sent on the BB coil, it is very important that this coil is always tuned to the same frequency for calibration AND quantification. It has been also observed that higher the frequency is, all the better it is. Thus, we recommend to tune the BB coil to the phosphorus frequency for example.

- Start the ERETIC software by typing *eretic* in the XWIN-NMR command line. A window similar to the one shown in figure 3.1 pops up. Click on the **Calibration** button.
- Choose the solvent of the reference.
- Enter the proton concentration of the reference signal (mM).
- Click on **Apply and Continue**.

Description

3.1.2

The **Dataset** field indicates the dataset name and location from which the eretic program has been launched.

The **Update dataset name** button allows the user to update the Dataset Field. The **Solvent** Field allows the user to select the solvent of the standard solution that is going to be used as a reference.

Figure 3.2. Eretic Calibration

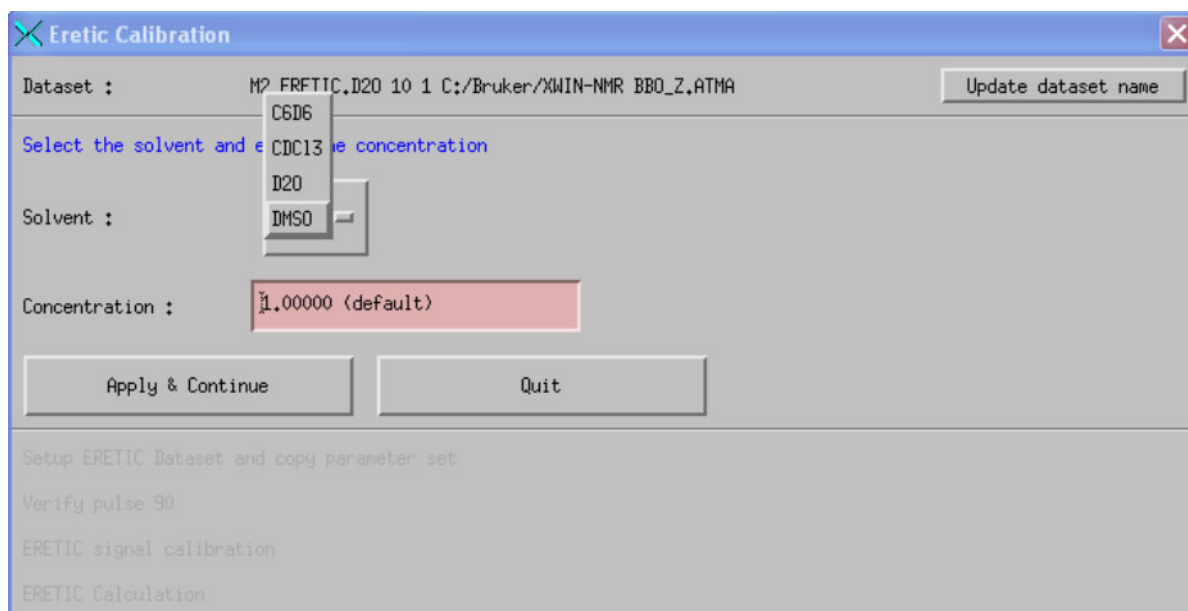


Figure 3.2 shows that clicking on the solvent name button opens a list containing different solvents. The default list of pre-defined solvents can be found in the following file:

```
<XWIN-NMR_HOME> /conf /instr /eretic /solvents.txt
```

The **Concentration** field allows the user to input the concentration value of the standard sample being used for the calibration.

After clicking on **Apply & Continue** the following operations are performed automatically by the software:

- a dataset called ERETIC.<solvent> is created in the current user directory.
- automatic determination of the 90° pulse.
- automatic determination of the phase (*phcor*) of the electronic signal.

- automatic determination of the amplitude of the electronic signal. This amplitude is calculated so that the ERETIC concentration is close to the concentration of the reference tube. The calculated value is stored in a file `cpd_etic.<probehead_id>` located in the `<XWIN-NMR_HOME> /conf /instr / eretic` directory. This value is common for all solvents and is just probe dependant. It is done only once for each probe. If needed, it is possible to remove manually this file so that this calibration is done again, or to edit and modify it if the user wants to work at a specific power.
- acquisition: 10 experiments are then performed (experiments 10 to 19) and processed.
- calculation of the ERETIC concentration. A calibration file is then created: `ERETIC.<SOLVENT>.<probehead_id>`, located in the `<XWIN-NMR_HOME> / conf /instr / eretic` directory.

Example of calibration file.

```
#ERETIC LOG FILE
#DATE 2003/11/14 17:05:59
DATASET=D:/ tests_141103_2 nmr ERETIC.D2O 999 1
P1=11.0747
SP15=19.000000000
Concentration=5.000000000mM
ConcentrationProton=40.000000000mM
Eretic Ratio (5.000000000mM)=4.663942814
Eretic Concentration=8.576434488mM
Standard deviation=0.0132948505
```

The ERETIC concentration is calculated as the average value of the 10 experiments.

Calibration parameters setup

3.1.3

The main parameter that needs to be changed when customizing the calibration experiment is the `intrng` file. This file contains the integration limits of the dioxane peak and the ERETIC peak.

For example, the `ERETIC.D2O intrng` file is by default:

```
A 1.0 #regions in PPM
# low field high field bias slope
3.90 3.4 -0 -0 # for region 1
-1.88 -2.12 -0 -0 # for region 2
```

The red values correspond to the integration region of the dioxane peak. If another chemical is used, these integration limits have to be changed.

When running the calibration experiment, a solvent dependant standard parameter set is retrieved (the command `RPAR ERETIC.solvent` is executed). From one parameter set to the other, only the parameter `D1` is different by default. The default `D1` value can be changed so that the T_1 value of the reference chemical is taken into account for a given solvent.

D1 can be changed in one parameter set by using the following XWIN-NMR commands:

```
RPAR ERETIC.<solvent>
```

```
D1=XX s
```

```
WPAR ERETIC.<solvent>
```

Quantification

3.2

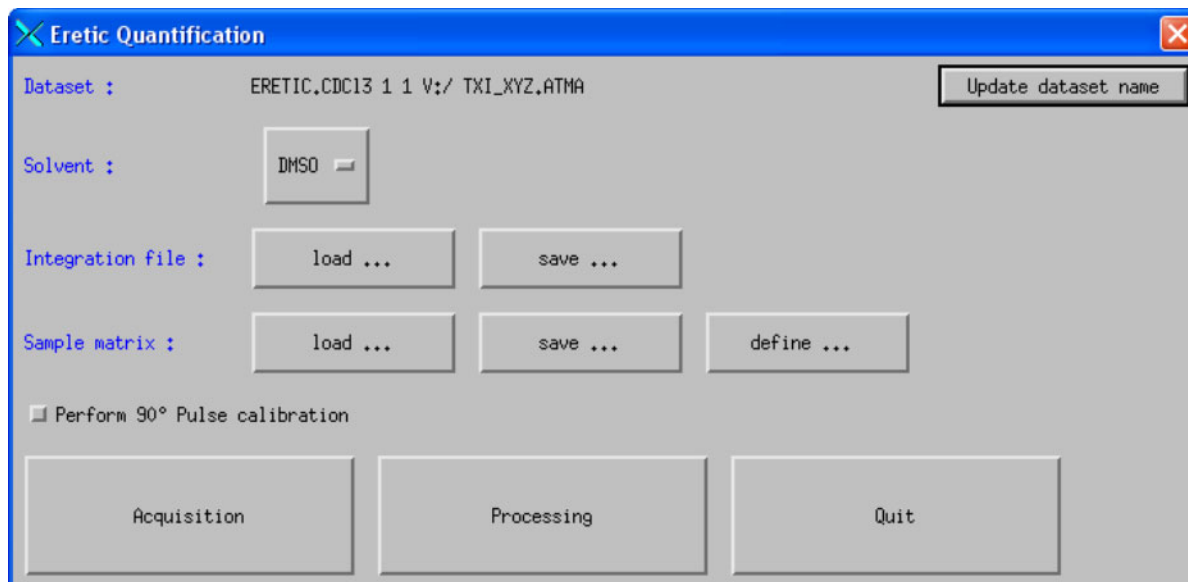
The ERETIC software needs a proton spectrum as a reference. This spectrum, recorded by the user has to be acquired in "quantitative conditions". This practically means that the relaxation time has to be long enough, and that the signal to noise ratio allows an accurate integration of the resonances of interest.

Acquisition

3.2.1

The quantification step is only possible if a calibration has been performed first. After opening the ERETIC interface, click on the **Quantification** button. A window similar to the one shown in figure 3.3 pops up. Make sure that the field **Dataset** corresponds to the proton spectrum that is being used as the reference for the ERETIC experiment. If not, go to the correct dataset in XWIN-NMR and click on **Update dataset name**.

Figure 3.3. ERETIC Quantification



The Solvent Field allows the user to select the solvent of sample under study. Since the 90° pulse needs to be perfectly adjusted for ERETIC experiments, the software is designed to perform a 90° pulse calibration on the sample if needed. This calibration will be done only if the check button "Perform 90° Pulse Calibration" is checked.

- Perform 90° Pulse Calibration button unchecked:
No calibration is done. The 90° pulse used for the ERETIC experiments is taken from the P1 value in the original user's proton spectrum.
- Perform 90° Pulse Calibration button checked:
In order to perform this calibration, the user has to choose a resonance that will be used for the 90° pulse determination. The way to choose it is to open the integration menu, select the region by integrating it, and save it as an intrng file.



Note: for samples where solvent suppression is needed, always select the solvent resonance for the 90° pulse calibration.

Finally, click on **Acquisition**.

The following operations are then performed automatically by the software:

- a dataset called `<dataset>_ERETIC.<solvent>` is created in the current user directory, where "dataset" is the name of the dataset containing the 1D proton reference spectrum.
- automatic determination of the 90° pulse if chosen by the user.
- automatic determination of the phase (*phcor*) of the electronic signal.
- acquisition: 10 experiments are then performed (experiments 10 to 19) and processed. The number of scans and relaxation delay used are the ones taken from the reference spectrum.

Processing

3.2.2

Display the reference proton spectrum in XWIN-NMR.

In order to quantify the signals of interest, it is necessary to integrate them first in the integration menu and save the corresponding file as an intrng file.

The **Integration file** field offers two options: **Load** and **Save**. This is an easy and fast access to integration files when working on series of similar samples.

The **Sample Matrix** has to be defined by clicking on the **define** button. First, the number of components in the sample has to be entered. The sample Matrix consists in a matrix containing *i* rows and *j* column, where *i* is the number of components, and *j* is the number of integrated regions. Each element *H_{ij}* of the matrix represents the number of protons *H* of the compound *i* for the integral *j*. Once completed, click on **Save** and the **Quit**.

Automatic processing

Automatic integration of ERETIC spectra can be performed just by clicking on **Processing**. The result is automatically displayed, and written in a text file called `eretic_result` in the dataset directory. The content of this text file is also copied in the title of the dataset.

Manual processing

It is also possible to further optimize some processing parameters like phasing, baseline correction or integrals in each individual ERETIC spectrum.

For instance, if the original proton spectrum was in the dataset proton, just open the corresponding ERETIC dataset which normally is proton_ERETIC.<solvent>. The experiments numbers used for the measurements are expnos 10 to 19.

After such operation, the same procedure is used: just click on the **Processing** button.

```
;eretic
;avance AV version using f3 channel

prosol relations=<eretic>
#include <Avance.incl>

"d11=30m"
"d12=20u"
"p11=aq"
"d13=5u"

1 ze
2 d13 pl12:f3
d1 do:f3
d13 reset1:f1 reset1:f3
d13 reset2:f1 reset2:f3
d13 reset3:f1 reset3:f3
p1 ph1
d13 cpds3:f3 ph3:r
go=2 ph31
wr #0
d11 do:f3
exit

ph1=0 2 2 0 1 3 3 1
ph3=0 2 2 0 1 3 3 1
ph31=0 2 2 0 1 3 3 1

;p11 : f1 channel - power level for pulse (default)
;p112 :f2 channel - power level for eretic pulse
;p1 : f1 channel - high power pulse

;d1 relaxation delay; 1-5 * T1
;d11 : delay for disk I/O (30ms)
;d12 : delay for power switching (20us)
;cpds3 : decoupling according to sequence defined by cpdprg3
; cpdprg eretic :
;   #setphase
;   1 p11:sp15:0
;   jump to 1
;sp15= eretic shape pulse
;phcor3: phase difference
```

- Dispositif d'Analyse Quantitative Par Resonance Magnetique Nucleaire, L. Barantin, S. Akoka, A. Le Pape, French Pat. CNRS N° 9507651 (1995).
- Concentration Measurement by Proton NMR Using the ERETIC Method, S Akoka, L. Barentin, M. Trierweiler, Anal. Chem. 1999, 71, 2554-2557.
- Kinetic Constant Measurement by NMR Using a Calibrated Internal Signal (ERETIC), I. Billault, S. Akoka, Instrumentation Science & Technology, 28(3), 233-240 (2000).
- L. Barentin, A. Le Pape, S. Akoka, Magn. Reson. Med. 38, 179-182 (1997).

Figures

1	General information	5
Figure 1.1.	Principle of the ERETIC experiment	6
2	Installation	7
Figure 2.1.	Directional Coupler	7
Figure 2.2.	Routing for 2 channels spectrometer (no router)	8
Figure 2.3.	Wiring for a 2 channels system	9
Figure 2.4.	Routing for a 3 channels spectrometer (no router)	10
Figure 2.5.	Wiring for a 3 channels system (no router)	10
Figure 2.6.	Routing for a 3 channels spectrometer (1 router available) .	11
Figure 2.7.	Logical routing (1 Router system)	12
Figure 2.8.	Wiring for a 3 channels system (1 Router available)	12
Figure 2.9.	Routing for a 4 channels spectrometer (1 Router available)	13
Figure 2.10.	Logical Routing (2 Routers system)	14
Figure 2.11.	Routing for a 4 channels spectrometer (1 Router available)	15
3	Users Guide	17
Figure 3.1.	ERETIC SOFTWARE Interface	17
Figure 3.2.	Eretic Calibration	18
Figure 3.3.	ERETIC Quantification	20
4	Appendix	23

Figures

