

Technical Bulletin #C0060: gHSQC with CapNMR

This bulletin describes how to use the CapNMR probe for gHSQC (^1H - ^{13}C) on a Varian console/system.

Begin by loading an appropriate 'Proton' sequence. Make sure to use the appropriate power levels (see Figure 1). The solenoid microcoil in the CapNMR probe requires less transmitter power than traditional probes. The standard pulse width (pw90) is calibrated at installation for a transmitter power of 45 dB (tpwr=45).

Parameter	Varian Range	Bruker Range
Lock power	1 to 8	-55 to -45
Lock gain	34 to 48	110 to 130
Transmitter power level (dB)	40 to 45	15 to 20
90° Transmitter pulse width (µsec)	2.5 to 5	2.5 to 5
90° Decoupling pulse width (µsec)	5 to 12	7 to 12

Figure 1. Appropriate power levels for the CapNMR Probe

Once there is a good Proton spectrum, type the macro 'gHSQC' into the command prompt and this will load the gHSQC pulse sequence. You can view the pulse sequence with a dps command. The sequence should look like the sequence in Figure 2.

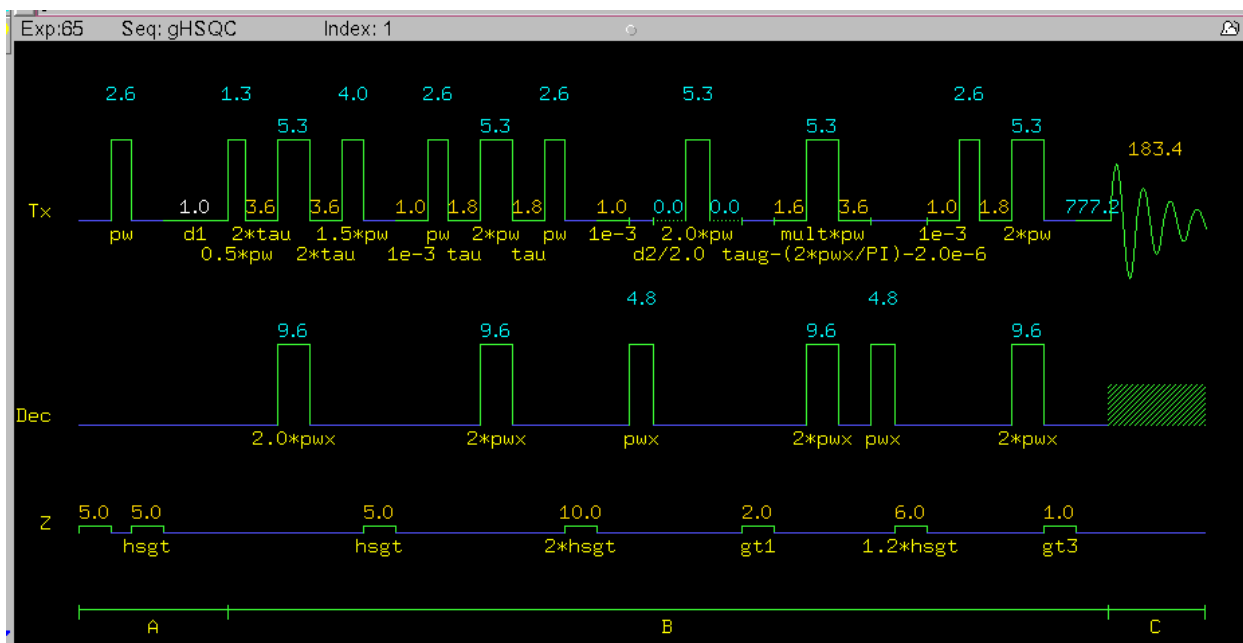


Figure 2. gHSQC Pulse Sequence

However, the pulse width is probe/console specific so your pulse width values (pw & pwx) should be different than the 2.6 and 4.8 values shown in Figure 2 and Figure 3. The pw90 for proton was calibrated with an array of the pw parameter in a Proton sequence. For tpwr=45, the pw90 for this probe was 2.6 microseconds. The pwx90 for carbon was calibrated with a pwxcal pulse sequence and for pwxlv1=45, the pwx90 for this probe was 4.8 microseconds.

Here is an example of the parameter set used for the gHSQC pulse sequence. In order to view this window, click on Process and select Text Output and then type 'dg'.

	ACQUISITION	TRANSMITTER	HSQC	PROCESSING
sw	5584.6	tn	H1 jlxh	140.0 gf 0.085
at	0.183	sfrq	599.763	nullflg y gfs not used
np	2048	tof	-311.8	mult 2 fn 2048
bs	32	tpwr	45	GRADIENTS 2D PROCESSING
ss	32	pw	2.650	gzlv11 888 gfl 0.009
dl	1.000	DECOUPLER	gt1	0.002000 gfs1 not used
nt	44	dn	C13	gzlv13 444 procl lp
ct	44	dof	-2185.3	gt3 0.001000 fnl 2048
	2D ACQUISITION	pwxlv1	45	gstab 0.000500 pmode full
sw1	25641.0	pwx	4.800	hsglv1 888 SAMPLE
ni	128	dm	nmy	hsgt 0.005000 date Sep 14 2006
phase	arrayed	dmm	ccg	SPECIAL solvent CDC13
		dmf	22000	temp not used sample
		dpwr	23	spin not used

Figure 3. gHSQC Parameters for the CapNMR Probe

IMPORTANT: GRADIENT POWER LEVELS

The CapNMR probe has a much stronger z-gradient than most standard tube probes. Most common tube probes are 50-80 gauss/cm at 10 amps while the CapNMR is commonly 350-400 gauss/cm at 10 amps. The default gzlv11 and hsglv1 values from Varian are 2000. You may have seen these values as high as 4500 for some tube probes. However, due to a more efficient gradient coil, not as much gradient power (gzlv1) is required for the same gradient field.

A standard gradient value (gzlv11 & hsglv1) for the CapNMR probe is 700 to 900.

A standard gradient value (gzlv13) for the CapNMR probe is 350 to 450.

These gradient and homospoil gradient values are automatically calculated from the gcal value in the probe file. The gcal value is calibrated at installation with a profile sequence, which performs a z gradient echo, and is saved in the probe file. Make sure your probe is set correctly (check with 'probe?') and that the 'probegcal' value is correct.

Sample and Data Set:

The sample used was 10% (v/v) 2-ethyl-1-indanone in CDCl₃. Pure 2-ethyl-1-indanone is a clear, brown liquid with a T1 relaxation of approximately 0.8 seconds. This data set was taken on a **5- μ L of 10% 2-ethyl-1-indanone (519 μ g; 3.2 μ mol; v/v in CDCl₃)**, which was injected and then pushed into a 5 microliter flowcell with more CDCl₃ solvent. This data was taken on a Varian Inova at 600 MHz with a TXI (Triple Inverse ¹H, ²H, ¹³C, ¹⁵N) CapNMR probe with a 5 microliter enhanced flowcell in 4 hours.

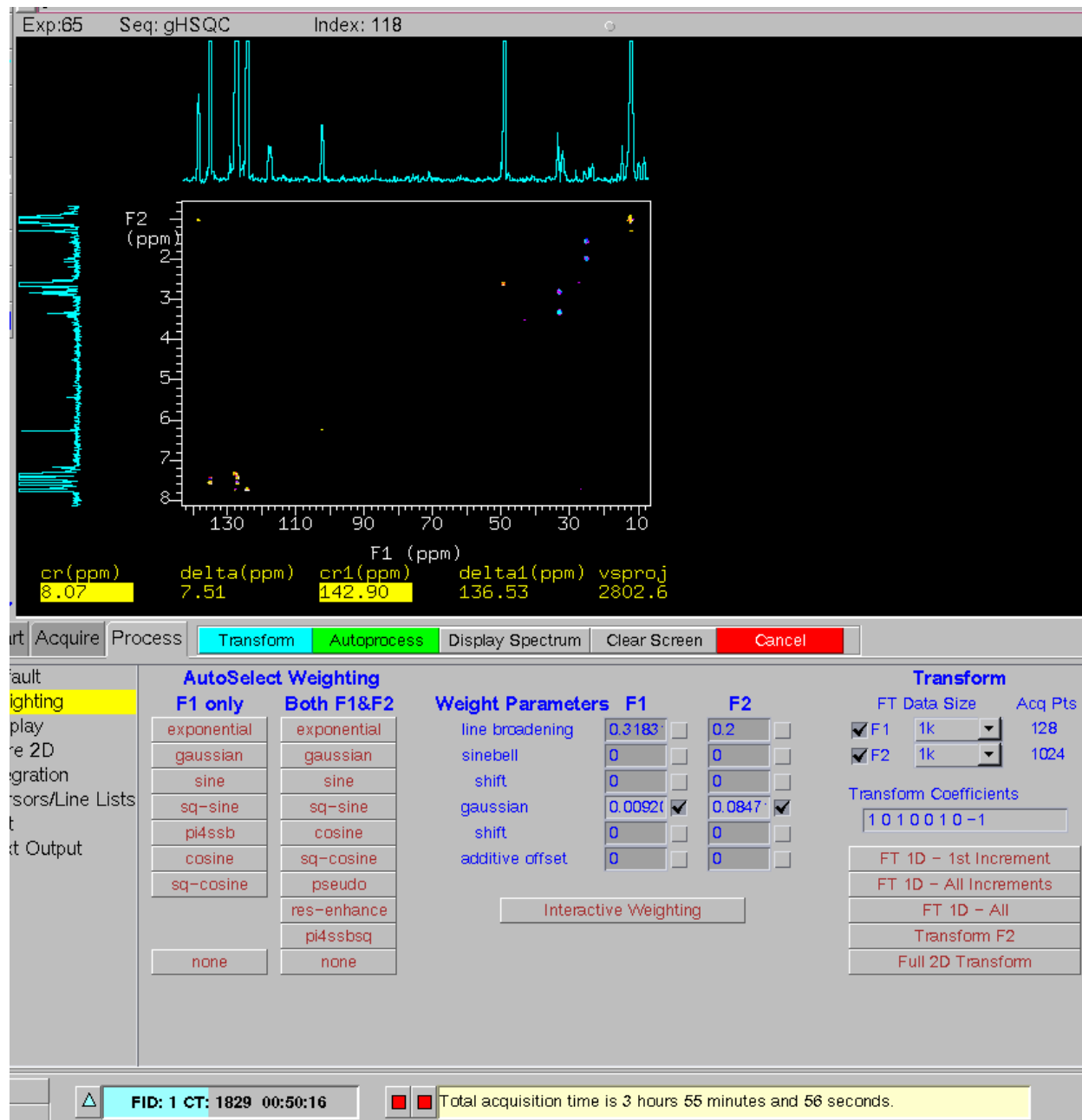


Figure 4. gHSQC Spectrum from 10% 2-ethyl-1-indanone

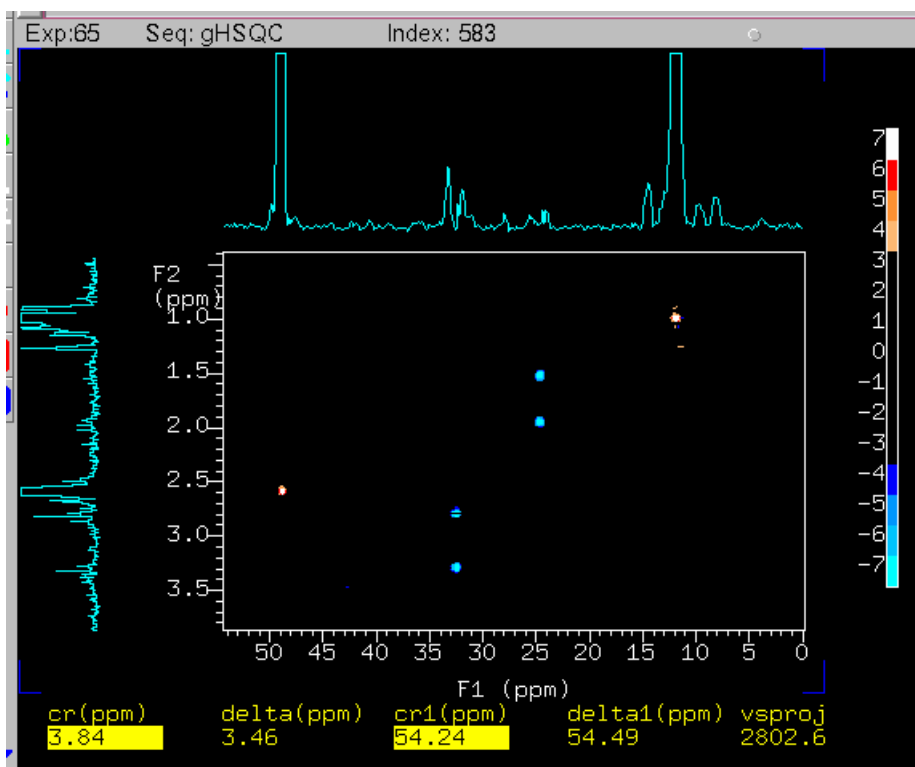


Figure 5. gHSQC Spectrum (Aliphatic Region) from 10% 2-ethyl-1-indanone

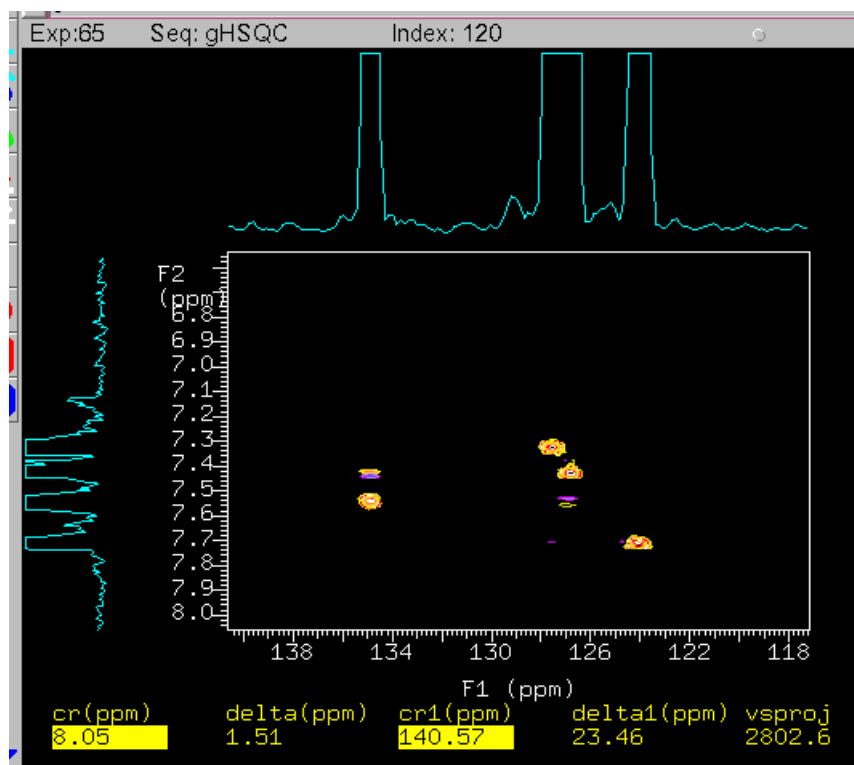


Figure 65. gHSQC Spectrum (Aromatic Region) from 10% 2-ethyl-1-indanone

Injection Method

A Hamilton Gastight 25- μ L syringe was filled with clean CDCl_3 solvent and used to rinse the probe. Then, 5- μ L of 10% 2-ethyl-1-indanone (519 μ g; 3.2 μ mol; v/v in CDCl_3) was drawn into the syringe and injected into the probe. Using the same syringe, 12 μ L of clean CDCl_3 was picked up and injected into the probe to push the 5- μ L sample into the NMR flow cell of the CapNMR probe. The 12- μ L Push Volume was calibrated in advance of sample injection.

Screenshots

All of the screenshots shown in this Technical Bulletin are from a Varian Inova console running Varian VnmrJ 2.1B software on a Unix workstation. The magnet was an Oxford 600 MHz magnet. (For more information on the Varian VnmrJ software, see <http://www.varianinc.com/cgi-bin/nav?products/nmr/>)