

Heteronuclear Shift Correlation Spectroscopy: HETCOR

The HETCOR experiment provides correlation of resonances of two different spin 1/2 nuclei. Most often, the two nuclei observed are carbon and protons and they are used as our example here, but this technique can be applied to other spin 1/2 nuclei, for instance ^{31}P and ^{19}F nuclei could be involved. The experiment relies on scalar coupling between the different nuclei and is based on the INEPT experiment (from which the DEPT experiment is also derived).

The pulse sequence for the HETCOR experiment is shown below:

^1H :	D1-90- $t_{1/2}$ -90-D3-180- τ_1 -90- $t_{1/2}$ -D3-90- τ_2	BB
^{13}C :	180	90 FID

The various delays are as follows: D1 is the relaxation delay; $t_{1/2}$ is one-half evolution period (see Derome for more information on this delay); τ_1 delay is $1/2J$, where J is the average C-H coupling constant; and τ_2 delay is $1/4J$, where J is the average C-H coupling constant.

As with the DEPT experiment, we need to know the 90° pulses for both the proton and carbon transmitters, and the protons are pulsed with the transmitter usually used for decoupling. As of 4-3-00, the 90° pulses on the two transmitters are as follows:

^{13}C : 14.5 μs at $tpwr = 50$
 ^1H : 15.7 μs at $pplvl = 59$.

Some of the important parameters are as follows:

pw 90° pulse on ^{13}C (at the indicated transmitter power - *tpwr*)
pp 90° pulse on ^1H using the decoupler transmitter (at the indicated decoupler transmitter power - *pplvl*)
d1 the relaxation delay to allow system to return to equilibrium between pulses. This should be set to $1-3 \cdot T_1$, where T_1 refers to the T_1 's for protons attached to ^{13}C nuclei.
nt should be a multiple of 4.
j1xh the average one-bond ^{13}C -H coupling constant. Default is 140.
sw1 should be equal to the spectral width (*sw*) used to obtain the proton spectrum.
dof decoupler offset, should be set to the *tof* (transmitter offset) used to obtain the proton spectrum - since the decoupler is being used to pulse on protons.
ni the number of increments; i.e. the number of different FID's. This parameter controls the resolution in the proton dimension. The larger *ni* is the better the resolution, but the longer the experiment will take (to improve the apparent resolution try some zero-filling in the ^1H dimension (*fn1*)).
rfl1, rfl2, wp1, sp1 should be set to the corresponding parameters used to obtain the ^1H spectrum.

The parameters *sw1, dof, rfl1, rfl2, wp1, sp1* should all be set properly by the computer if you set-up the experiment as described below. You might just check these parameters anyway, especially *sw1* and *dof*, which must be set correctly before you start the experiment (the others are just processing and display parameters which could be changed any time - see the *Command and Parameters* manual for a description of these parameters).

Other parameters are available to be set to provide variations of this experiment to give other information. For instance, a version of this experiment provides correlation of long-range ^{13}C -H couplings - only two other parameters need to be set for this experiment (see the *Guide to NMR Experiments* manual for more information).

Step-by-Step Instructions

1. Acquire a routine proton spectrum in *exp1* with *nt* = 1. Expand around the peaks of interest and change *sw* with the *movesw* command. Re-acquire the ^1H spectrum with the new spectral window.
2. Phase the spectrum and set the scale reference. Set the vertical scale of the peaks to a reasonable level. Save the spectrum.
3. Join *exp2* and set-up the ^{13}C parameters. You have a choice here, you can run the HETCOR with the regular ^{13}C parameters (i.e. with the default *sw*) or you can change the *sw* to include only the peaks of interest. Since resolution usually is not an issue in the ^{13}C dimension (it is an issue in the ^1H dimension, however!) it is probably not that critical, but you can run a quick ^{13}C spectrum and then change the *sw* to include only those peaks of interest (use *movesw*), just as you did for the proton spectrum. This would give you better resolution along the ^{13}C dimension. I recommend doing this (if you are running this experiment along with a DEPT, you may have already acquired an appropriate carbon spectrum, which can be used – just load it into *exp2*). In any case, acquire a quick spectrum, phase, set the scale reference peak, and set the vertical scale of the peaks to a reasonable level; then save the fid.
4. Join *exp3*. Move the ^{13}C parameters you used to acquire the carbon spectrum from *exp2* to *exp3*. To do this enter *mp(2,3)* (*mp* is the move parameters command). Now, call up the HETCOR parameters “on top of” the carbon parameters you just transferred to this *exp*, and tell the computer where the ^1H parameters are that you used to acquire the ^1H -NMR spectrum (they are in *exp1*). To do this, enter *hetcor(1)*. The argument (1) tells the computer that the proton parameters to use for the HETCOR are in *exp1*. It takes a minute to load all the parameters.
5. Check to see that the parameters are set correctly (*dg* to get the parameters displayed). Most should be fine since they were set according to the ^1H and ^{13}C parameters for the regular 1D spectra, but you will have to decide on the number of scans (transients). Set *nt* = number of scans required to get a reasonable ^{13}C spectrum (must be a multiple of 4). Be as liberal with the number of scans as you have time for. A relaxation delay precedes each run through the pulse sequence (*d1*). Ideally this is set to $3 \times T_1$, set it to a bare minimum of 1 or 2 sec. More will help, depending on your time constraints. *ni* is the number of increments; that is, the number of different FID's. Set this for as many as you can stand. At the *very* least 64, and more gives you better resolution in the proton dimension.
6. Check the time required to do the experiment, enter *time*. The time can be controlled by changing *nt* and *ni*. *nt* controls the s/n in the ^{13}C dimension, while *ni* will determine the resolution in the ^1H dimension. For reasonable results, *ni* probably must be at least 64, depending on your ^1H *sw* and how close together your ^1H resonances are (256 may be required for some of our compounds).
7. If you would like your hetcor automatically saved to a file after the experiment is complete (recommended if you are going to be leaving the instrument!) you need correctly set the parameter *wexp*, which tells the computer what to do after the acquisition is complete.
 - a. Set *wexp* to save to a file by entering: *wexp='svf(n3)'*. This tells the computer to save the fid to a file designated by the string **n3**.
 - b. Now you just need to define **n3**. You will want **n3** to include the filename for your cosy, and you also need to tell the computer *exactly* where to save the data, so the whole, absolute path must be specified. So, define **n3** as follows: *n3='/export/home/your account(probably advlab)/your directory/filename'*. It is very important that you type these commands accurately, with the proper “punctuation”, i.e. the single quotes. If it is not all correct, the experiment will not be saved! However, the hetcor will still be stored in the experiment you are working in...until someone overwrites it.
8. Enter *au* to start the acquisition.

9. To look at the spectrum along the way, enter *wft2d*.
10. When the experiment has finished, save the HETCOR fid (if you didn't have it automatically saved as in step 7): enter *svf*, and provide a filename when prompted.

Processing and Plotting the Data

Processing and plotting is essentially the same for all 2D spectra, so the work-up is analogous to the work-up used for the COSY experiment. The data can be processed on the NMR computer or on the Mudd 169 PC's using Exceed.

There are many processing, display, and plotting options. Consult the "System Operation" and the "Commands and Parameters" manuals, for detailed information on displaying and plotting 2D spectra. Below is a "bare bones" displaying and plotting protocol.

1. Transform the data with the *wft2d* command. A 2D spectrum will appear and you will be manipulating the spectrum in the "Color Map" mode. Very often, the first spectrum that appears is larger than the display window (if it is the correct size, you will see all four borders around the 2D plot). If your spectrum appears larger than the window when you transform your spectrum, you can fix the plot size with the following sequence of mouse "clicks":

Main Menu → Display → Size → Full Screen

At any point, if you happen to "click" your way out of the **Color Map** mode, you can get back as follows:

Main Menu → Display → Color Map

In the **Color Map** mode you can expand and interact with the spectrum in a way similar to a normal 1D spectrum. Your right and left mouse buttons provide cursors to allow you to expand on regions of the spectrum. The middle mouse button controls the contour threshold and can be used to go up or down levels in the contour map. To do so, place the cursor on the **colored threshold level indicator on the right of the spectrum** and use the middle mouse button to go up or down contour levels. The threshold can also be input by typing using the *th* command (e.g. *th=12*). You can also change the vertical scale by typing in vertical scale settings (**vs2d=500** or any level of your choice) or, more conveniently, you can raise or lower the vertical scale in 20% increments using the **vs+20%** or **vs-20%** buttons. The current **vs2d** is displayed on the bottom of the spectrum window. If you type in a **vs** setting, you need to update the spectrum display with the new **vs2d** setting by clicking the **Redraw** button.

2. Play with the threshold and the vertical scale to display the data in an appropriate fashion. When you are satisfied with the display you can, if you wish, change from the color map to the contour map to display a "true" 2D contour display, comparable to what will be plotted on paper. Use the **Contour** button (this is not a necessary step).
3. You are now ready to plot your spectrum. The *plot2d* command will prompt you to enter the correct information to properly plot the HETCOR with the 1D proton and carbon spectra traces plotted along the axes of the HETCOR spectrum.

An alternative plotting macro is the *plhxcor* macro. To use this macro do the following: to plot the HETCOR with the 1D proton and carbon spectra traces plotted along the axes of the HETCOR spectrum, enter the following command: *plhxcor(7,2,1,2)*. The *plhxcor* command is the macro that plots the spectrum. The numbers in the parentheses (the "arguments") have the following meanings, from left to right: **7** tells the computer to print out seven contour levels; **2** is spacing used between levels; **1** is the *exp#* where the proton spectrum is located; **2** is the *exp#* where the carbon spectrum is located.

4. Obtain a printout of the HETCOR parameters, if desired. Enter the following: *printon dg dg1 printoff*.