ADVANCED NMR TRAINING - VARIAN

Simple procedures for measuring homo- and heteronuclear 2D NMR spectra

It is strongly recommended to run 2D spectra in an experiment that has the experiment number greater than 1, for example Exp:2, Exp:3, etc. To create such an experiment type the command *cexp(#)*, where # represents the number of the new experiment, and press the return button on the keyboard. For example, the command *cexp(2)* will create the experiment with experiment number Exp:2. Alternatively, you can consecutively click the buttons Main Menu, Workspace and Create New using the left mouse button to create a new experiment.

To join the experiment with experiment number Exp:#, type the command *jexp(#)*, where # represents the number of this experiment, and press the return button on the keyboard. For example, by issuing the command *jexp(2)* you will join the experiment Exp:2, or alternatively you can successively click the buttons Main Menu, Workspace and Exp 2.

If the message "foreground processing active" appears on the screen after issuing the command *jexp (#)*, type the command *unlock(#)*,where # represents the number of the experiment you want to joint and press the return button on the keyboard.

Measurement of 2D homonuclear spectra

COSY

1. Acquire 1D ^{1}H spectrum, as described in the Varian basic training manual, in the experiment that has the experiment number Exp:1. It is adequate to measure this spectrum with only one transient (nt=1) if you do not wish to plot COSY spectrum with the corresponding 1D proton spectrum along its vertical and horizontal axes.

2. Create a new experiment as described above, for example the experiment Exp:2. Join the newly created experiment using the command *jexp(#)*, for example *jexp(2)*.

3. Type the command *mf(1,#)*, where # represents the number of the experiment which you joined and press the return button on the keyboard. For example, the command *mf(1,2)* will move 1D proton data from the experiment Exp:1 into the experiment Exp:2.

4. Type the command *cosy* and press the return button on the keyboard. Alternatively, you can consecutively click the buttons Main Menu, Setup, Sequence, 2D and COSY using the left mouse button. This will load all parameters needed for the measurement of COSY spectrum.

5. Adjust number of transients (parameter nt) correspondingly to the concentration of your sample. The number of transients must be a multiple of 4, e.g., nt=4, 8, 12, 16, 20, etc.

6. Type the command *au* and press the return button on the keyboard, or alternatively click the buttons Acquire and Automatic using the left mouse button. The experiment will proceed automatically to the end when the final 2D COSY spectrum will be plotted.
Any 2D experiment can also be started using the command *go* or alternatively, by clicking the buttons Main Menu , Acquire and Go . In this case the spectrum must be processed, phased, referenced, and plotted manually. *See the section 2D NMR Data Manipulation how to perform these tasks*.
Never start 2D experiment using the command *ga* or by clicking the buttons Main Menu , Acquire and Go, Wft.

7. To plot COSY spectrum with the corresponding 1D proton spectrum along both vertical and horizontal axes, type the command *plcosy(x,y,z)*, for example *plcosy(8,1.2,1)* and press the return button on the keyboard. The first number in the parentheses represents the number of cross-peak contours, the second number stands for the distance between contours and the third number denotes the experiment number of the experiment in which 1D proton spectrum was measured.

TOCSY

1. Follow the steps 1-3 in the setup of COSY experiment.

2. Type the command *tocsy* and press the return button on the keyboard. Alternatively, you can consecutively click the buttons Main Menu, Setup, Sequence, 2D, More 2D and TOCSY using the left mouse button. This will load all parameters needed for the measurement of TOCSY spectrum.

3. Adjust number of transients (parameter nt) correspondingly to the concentration of your sample. The number of transients must be a multiple of 8, e.g., nt=8, 16, 24, etc.

4. Type the command *au* and press the return button on the keyboard, or alternatively click the buttons Acquire and Automatic using the left mouse button. The experiment will proceed automatically to the end when the final 2D TOCSY spectrum will be plotted.

5. To plot TOCSY spectrum with the corresponding 1D proton spectrum along both vertical and horizontal axes, use the command *plcosy(x,y,z)*.

NOESY

1. Follow the steps 1-3 in the setup of COSY experiment.

2. Type the command *noesy* and press the return button on the keyboard. Alternatively, you can successively click the buttons Main Menu, Setup, Sequence, 2D and NOESY using the left mouse button. This will load all parameters needed for the measurement of NOESY spectrum.

3. Adjust number of transients (parameter nt) correspondingly to the concentration of your sample. The number of transients must be a multiple of 8, e.g., nt=8, 16, 24, etc.

4. Type the command *au* and press the return button on the keyboard, or alternatively click the buttons Acquire and Automatic using the left mouse button. The experiment will proceed automatically to the end when the final 2D NOESY spectrum will be plotted.

5. To plot NOESY spectrum with the corresponding 1D proton spectrum along both vertical and horizontal axes, use the command *plcosy(x,y,z)*.

ROESY

1. Follow the steps 1-3 in the setup of COSY experiment.

2. Type the command *roesy* and press the return button on the keyboard. Alternatively, you can successively click the buttons Main Menu, Setup, Sequence, 2D and ROESY using the left mouse button. This will load all parameters needed for the measurement of ROESY spectrum.

3. Adjust number of transients (parameter nt) correspondingly to the concentration of your sample. The number of transients must be a multiple of 8, e.g., nt=8, 16, 24, etc.

4. Type the command *au* and press the return button on the keyboard, or alternatively click the buttons Acquire and Automatic using the left mouse button. The experiment will proceed automatically to the end when the final 2D ROESY spectrum will be plotted.

5. To plot ROESY spectrum with the corresponding 1D proton spectrum along both vertical and horizontal axes, use the command *plcosy(x,y,z)*.

Measurement of 2D heteronuclear spectra

HETCOR

1. Acquire 1D ^{1}H spectrum, as described in the Varian basic training manual, in the experiment that has the experiment number Exp:1. It is adequate to measure this spectrum with only one transient (nt=1) if you do not wish to plot HETCOR spectrum with the corresponding 1D proton spectrum along its proton axis.

2. Create a new experiment as described above, for example the experiment Exp:2. Join the newly created experiment using the command *jexp(#)*, for example *jexp(2)*. In this experiment, acquire 1D $^{13}C\{^{1}H\}$ spectrum as described in the NMR training manual. It is adequate to measure this spectrum with only one transient (nt=1) if you do not wish to plot HETCOR spectrum with the corresponding 1D carbon spectrum along its carbon axis.

3. Create another new experiment as described above, for example the experiment Exp:3. Join this experiment using the command *jexp(#)*, for example *jexp(3)*. Move 1D carbon data from the experiment in which you acquired them into this newly created experiment. For example, the command *mf(2,3)* will move 1D carbon data from the experiment Exp:2 into the experiment Exp:3.

4. Type the command *hetcor* and press the return button on the keyboard. Alternatively, you can successively click the buttons Main Menu, Setup, Sequence, 2D and HETCOR using the left mouse button. This will load all parameters needed for the measurement of HETCOR spectrum.

5. Adjust number of transients (parameter nt) correspondingly to the concentration of your sample. The number of transients must be a multiple of 4, e.g., nt=4, 8, 12, 16, 20, etc.

6. Type the command *au* and press the return button on the keyboard or alternatively click the buttons Acquire and Automatic using the left mouse button. The experiment will proceed automatically to the end when the final 2D HETCOR spectrum will be plotted.

7. To plot HETCOR spectrum with the corresponding 1D proton and carbon spectra along the respective proton and carbon axes, type the command *plhxcor(w,x,y,z)*, for example *plhxcor(6,1.2,1,2)* if the 1D proton and carbon spectra were measured in the experiments Exp:1 and Exp:2, respectively, and press the return button on the keyboard. The first number in the parentheses represents the number of cross-peak contours, the second number stands for the distance between contours, the third number denotes the experiment number of the 1D proton spectrum and the fourth number represents the experiment number of the 1D carbon spectrum.

HMBC

A HMBC spectrum contains fewer artifacts when measured with a pulse sequence utilizing gradients. Such experiment can be run only on the 600 and 500. In the case of the 500, the indirect detection (ID) triple resonance probe must be used. Please, ask NMR facility personnel to install this probe. When the probe is installed, join the experiment Exp:1 by typing the command *jexp(1)* and pressing the return button on the keyboard.

1. In the experiment Exp:1 type the command *setexp_1H_solvent_bio*, where solvent is the name of deuterated solvent of your sample, for example, *setexp_H1_cdcl3_bio*, *setexp_H1_d2o_bio*, *setexp_H1_cd3od_bio, setexp_H1_dmso_bio, setexp_H1_acetonitrile_bio, setexp_H1_acetone_bio*, etc, and press the return button on the keyboard. All shim and lock parameter values will be updated accordingly to the used solvent and necessary acquisition, processing and plotting parameters will be loaded.

2. Put your sample in the magnet as described in the Varian basic NMR training manual. Lock and shim the sample and measure its 1D proton spectrum with 4 transients (nt=4). Phase and reference the spectrum.

3. Create a new experiment as described at the beginning of this manual, for example the experiment Exp:2. Join the newly created experiment using the command *jexp(#)*, for example *jexp(2)*. In this experiment, type the command *mf(1,#)*, where # represents the number of the experiment which you joined and press the return button on the keyboard. For example, the command *mf(1,2)* will move 1D proton data from the experiment Exp:1 into the experiment Exp:2.

4. Type the command *set_gHMBC* and press the return button on the keyboard. This will load all parameters needed for measurement of HMBC spectrum with a pulse sequence utilizing gradients.

5. Turn on a gradient amplifier by typing *pfgon='nny' su* and press the return button on the keyboard. Turning on the gradient amplifier will cause a level of the lock signal to go down. Adjust the lock signal to the level before turning on the gradient amplifier using shim Z1.

6. Adjust number of transients (parameter nt) correspondingly to the concentration of your sample. The number of transients must be a multiple of 4, e.g., nt=4, 8, 12, 16, 20, etc.

7. Start the experiment by typing the command *go* and press the return button on the keyboard or alternatively click the buttons Acquire and Go using the left mouse button.

8. Process and plot the spectrum as described in the section 2D NMR Data Manipulation.

2D NMR Data Manipulation

Processing

1. To convert (Fourier transform) array of time domain FID signals obtained with the acquisition command *go* into a 2D frequency spectrum, type the command *wft2da* and press the Return button on the keyboard. Alternatively, you can consecutively click the buttons Main Menu, Process and Full Transform using the left mouse button.

Phasing

1. Only phase sensitive spectra can be phase corrected, for example, spectra acquired with the parameter *phase* arrayed, e.g., *phase=1,2*. Magnitude spectra do not require any phase correction.
Set the value of the *pmode* parameter to *full*, e.g., type *pmode='full'* and press the Return button on the keyboard.
Use diagonal peaks to phase homonuclear spectra, COSY, DQF-COSY, TOCSY, NOESY, and ROESY. In heteronuclear spectra HETCOR, HSQC, HMQC, and HMBC cross-peaks must be used for phasing.

2. Display a full 2D spectrum by typing *dconi('dpcon',32,1.2) full* and pressing the Return button on the keyboard. Alternatively, you can click consecutively the buttons Main Menu, Display, Contour, and Full using the left mouse button.
If the horizontal and vertical axes do not have labels *F2(ppm)* and *F1(ppm)*, respectively, click consecutively the buttons Main Menu, Display, More, and F2 Mode using the left mouse button.

3. Display a pair of perpendicularly oriented red cursors by clicking the buttons Main Menu, Display, Contour using the left mouse button. Using the mouse put arrow cursor on a cross-peak (diagonal peak in the case of homonuclear spectrum) in the far upper right corner of 2D spectrum and click the left mouse button. Remember or write down an index number displayed at the top of command window, for example Index: 409, which corresponds to the position of the red horizontal cursor. Also, positions of the red cursors in ppm along the vertical *F1* (*cr1* value) and horizontal *F2* (*cr* value) axes will be shown below displayed 2D spectrum. Using the mouse put arrow cursor on a cross-peak (diagonal peak in the case of

homonuclear spectrum) in the far bottom left corner of 2D spectrum and click the left mouse button. Remember or write down an index number displayed at the top of command window, for example Index: 114, which corresponds to the position of the red horizontal cursor. Again, new positions of the red cursors in ppm along the vertical *F1* (*cr1* value) and horizontal *F2* axes (*cr* value) will be shown below displayed 2D spectrum.

4. Type *ds(first index number)*, e.g. *ds(409)*, and press the Return button on the keyboard to display 1D spectrum (first trace) corresponding to this index number. Click on the Phase button using the left mouse button and phase the peaks around the *cr* position of this spectrum that they have absorption profile (dispersive profile for COSY). Without exiting a phasing routine, type *ds(second index number)*, e.g. *ds (114)*, and press the Return button on the keyboard to display 1D spectrum corresponding to the second trace. Click on the Phase button using the left mouse button, place the arrow cursor at the position of the first *cr* value and click the left mouse button. Then move the arrow cursor to the position of the second *cr* value and phase the peaks around this position that they have absorption profile (dispersive profile for COSY). Exit the phasing procedure by clicking the Phase button with the left mouse button and display 2D spectrum either by typing *dconi('dpcon',32,1.2) full* and pressing the Return button on the keyboard or by clicking consecutively the buttons Main Menu, Display, Contour, and Full using the left mouse button.

5. Click consecutively the buttons Main Menu, Display, More, and F1 Mode using the left mouse button. Now horizontal and vertical axes will be labeled *F1(ppm)* and *F2(ppm)*, respectively. Repeat the phasing procedure described in the paragraphs 3 and 4 for this orientation of the axes. When the phasing is finished, 2D spectrum can be displayed as it is said in the paragraph 2.

Referencing

1. Use a diagonal peak to reference homonuclear spectrum.
Expand a region of the spectrum around a diagonal peak chosen for referencing. Click consecutively the buttons Main Menu, Display, Contour using the left mouse button. A pair of perpendicularly oriented red cursors will be displayed. Using the mouse place arrow cursor on the diagonal below the chosen diagonal peak and click the left mouse button to put intersection of the red cursors in the position of the arrow cursor, then move the arrow cursor on the diagonal above the chosen diagonal peak and click the right mouse button. The second pair of perpendicularly oriented red cursors will be displayed and the chosen diagonal peak will be inside a red rectangle. Click the Expand button in the second row of the command window using the left mouse button to expand area of the spectrum surrounded by the red rectangle.
2. Using the mouse place arrow cursor in the center of the chosen diagonal peak, then type *rl(abcp) rl1 (abcp)* and press the Return button on the keyboard, where *abc* represents chemical shift value of the selected diagonal peak in ppm in the both *F2(ppm)* and *F1(ppm)* dimensions and *p* stands for ppm units. Redisplay full spectrum by typing *dconi('dpcon',32,1.2) full* and pressing the Return button on the keyboard or by clicking consecutively the buttons Main Menu, Display, Contour, and Full using the left mouse button.
3. A cross-peak must be used for referencing of heteronuclear spectrum.
Expand region of the spectrum containing a cross-peak chosen for referencing in a similar manner as described in the paragraph 1.
4. Using the mouse place arrow cursor in the center of the chosen cross-peak, then type *rl(abcp) rl1(xyzd)* to reference spectrum in the *F2(ppm)* and *F1(ppm)* dimensions (axes), respectively, and press the Return button on the keyboard. *abc* and *xyz* represent chemical shift values of the selected cross-peak in ppm in

the corresponding *F2(ppm)* and *F1(ppm)* dimensions (axes), and *p* and *d* stand for ppm units in these dimensions.
Redisplay full spectrum by typing *dconi('dpcon',32,1.2) full* and pressing the Return button on the keyboard or by clicking consecutively the buttons Main Menu, Display, Contour, and Full using the left mouse button.

Adjusting vertical scale

1. Type the command *nm2d* and press the Return button on the keyboard to set up automatically the parameters *vs2d* and *th*.
To redisplay full spectrum corresponding to new *vs2d* and *th* values type *dconi('dpcon',32,1.2) full* and press the Return button on the keyboard or click consecutively the buttons Main Menu, Display, Contour, and Full using the left mouse button.

2. Appearance of the spectrum (intensities of the peaks) can also be adjusted by altering value of each parameter (*vs2d* and *th*) individually, e.g., by typing *vs2d=new value* and/or *th=new value* and pressing the Return button on the keyboard. For example, typing *vs2d=1000 th=3* and pressing the Return button on the keyboard will change the values of the both parameters *vs2d* and *th*. Typing either *vs2d=1000* or *th=3* and pressing the Return button on the keyboard will only change the value of the corresponding parameter.
To redisplay full spectrum corresponding to the new *vs2d* and/or *th* values type *dconi('dpcon',32,1.2) full* and press the Return button on the keyboard or click consecutively the buttons Main Menu, Display, Contour, and Full using the left mouse button.

Plotting

1. 2D spectra are generally plotted as "contour plots". The size of the plotted spectrum is controlled by the parameters *sc*, *wc*, *sc2*, and *wc2*.
sc and *wc* represent the start of the plot and the width of the plot, respectively, along the horizontal axis with respect to the right edge of the plotter (paper). *sc2* and *wc2* then represent the start of the plot and the width of the plot, respectively, along the vertical axis with respect to the bottom edge of the plotter (paper). A maximum plot size for the 11'x 8' paper sheet is *sc=0, wc=220, sc2=0, wc2=170*.
Plot size must be set first, for example typing *sc=5, wc=160, sc2=5, wc2=160* and pressing the Return button on the keyboard will produce a square shape (16x16 cm) of a contour plot and its bottom right corner will be 5 mm from the both right and bottom edge of the paper.

2. A contour plot is produced by the command *pcon*. One can specify how many contour levels should be plotted, what spacing should be between the successive contour levels and whether the contours of positive and/or negative peaks should be plotted.
For example, typing *pcon('pos',12,1.2) page* and pressing the Return button on the keyboard will plot the positive peaks with 12 contours and spacing (relative intensity of successive contour levels) 1.2. Typing *pcon('neg',10,2.4)* will then plot the negative peaks with 10 contours and spacing 2.4.
The positive and negative peaks of a phase sensitive spectra can be plotted on a monochrome plotter simultaneously, each with a different number of contours. For example, typing *pcon('pos',16,1.4) pcon ('neg',4,3) page* and pressing the Return button on the keyboard will plot the positive peaks with 16 contours and spacing 1.4, and the negative peaks with 4 contours and spacing 3.

3. To plot the corresponding 1D spectra along the horizontal and vertical axes of a 2D spectrum click consecutively the buttons Main Menu, Display, Contour, Proj, Hproj(max), Plot, Vproj(max), Plot using the left mouse button and then type the commands *pcon(x,y) page* and press the Return button on the keyboard. x and y represent the number of contours and their spacing, respectively. After the corresponding projection is created and displayed on the screen one can adjust its vertical height using the middle mouse button in the same manner as the vertical height of spectral lines is adjusted in 1D spectrum.

4. 2D spectrum can also be plotted by clicking consecutively the buttons Main Menu, Display, Contour, Autoplot after proper adjustment of vertical scale of the spectrum. This procedure does not allow changing the preset plot size (15x15 cm).