

# Instructions for $^1\text{H}$ -, $^{13}\text{C}$ -, $^{19}\text{F}$ -, and $^{31}\text{P}$ -Spectra on the Varian Mercury-Vx-300

## Please note:

- Under no circumstances move the magnet or the automatic sampler table.
- Do not attempt to use the auto sampler (robot) unless given specific instructions to do so.
- The new Varian Vnmr software enables the user to easily access and change functions, options, and their properties. If you are not sure about a option, button or command ask the NMR staff for assistance.
- If you encounter problems at any time during locking, shimming, acquiring you should halt the experiment and reload the standard parameters for your experiment.
- In order for the automatic shimming to work well, samples should be filtered and prepared in good-quality tubes. Sample volume should be at least 0.7 mL. Do not use more than 0.1% TMS.

## Conventions in this guide

- Buttons are depicted with small caps, e.g. **ABORT ACQ**
- Commands entered in the command window are described in bold letters, e.g. **h1cdcl**, and executed by hitting the return key

## Important basic functions

- **ABORT ACQ** button -> aborts/halts aquisition
- **RESIZE** button -> enlarges/reduces spectrum window
- **FLIP** button -> flips the spectrum window over the display window and vice versa
- **jexp $x$**  command -> switches to experiment number  $x$  (1, 2, 3, 4, etc.)
- **ds** command -> switches back to interactive display mode

## Login / Logout

- In order to log in enter your user name followed by the password into the welcome screen.
- In order to log out click the **EXIT** button in the control panel on the bottom of the screen.

## Sample Preparation

- click the Vnmr icon to start the Vnmr software
- **e** (ejects existing sample)
- remove sample from spinner and replace it with your cleaned (use provided Kimwipes) sample
- **i** (inserts new sample)

## Acquisition of $^1\text{H}$ -NMR Spectra

- **jexp1** (go to experiment 1)
- **h1xxxx** (loads solvent-dependent parameters; xxxx = cdcl, acet, d2o, dmso, meth; for benzene use xxxx=cdcl)
- **acqi** (opens acquisition window)
- click **LOCK** (do not change Z0 and lockphase, the spin value should be 20)
- sample should be locked (do not try to maximize the lock level before shimming is completed)
  - if locklevel is above 70%, decrease lockpower
  - if sample is not locked, increase lockpower by 16, wait for lock, decrease lockpower stepwise by 4 until locklevel is below 70%
- click **SHIM**
- adjust **Z1C** and **Z2C** only (if the lock level exceeds 100, then decrease lockgain which can be altered in the very same window)
- click **CLOSE**
- **nt=xx** (sets number of scans; default is nt=8)
- **ga** (starts acquisition)
- wait for acquisition to finish (beep will sound)
- **aph** (automatic phasing, the new software does a very good job)
 

for manual phasing:

  - (a) click **PHASE**
  - (b) left click on highest peak
  - (c) with the left mouse button pressed move cursor up or down in order to adjust the phasing for this peak
  - (d) then right click on farmost peak and repeat phasing
  - (e) interactively phase these two peaks until phasing is completed
  - (f) **ds** (switches back to interactive display mode)
- **vsadj** (automatic adjustment of vertical scale -> highest peak adjusted to maximum of spectrum window)
  - for manual vertical scale adjustment: use the center mouse button, a left mouse click above the baseline causes the spectrum to scale up and a left click below the baseline causes the spectrum to scale down, the distance of the cursor from the baseline while clicking determines the multiplication factor of the in- and decrease
  - alternative vertical scale adjustment: **vs=xxx**, the current value of the vertical scale (vs) is displayed in the lower portion of the spectrum window
- expand spectrum:
  - (a) with left mouse button drag red left spectrum limit to its position (cr on bottom of spectrum = ppm)
  - (b) with right mouse button click and drag red right limit to its position (delta = difference in ppm)
  - (c) click **EXPAND** (expands spectrum to its set limits)

- integration:
  - (a) click **INTEGRATION** (the integration button consists of three sequential levels: **PART(ial) INTEGRAL**, **FULL INTEGRAL**, **NO INTEGRAL**; it shows not the actual integration mode, but the mode that can be accessed)
  - (b) click **PART INTEGRAL** (green integral trace appears; see also 'Adjustment of Integral Trace')
  - (c) **cz** (clears currently defined integral)
  - (d) click **RESETS**
  - (e) click the left mouse button closely to the left of the left-most peak
  - (f) click the left mouse button closely to the right of the left-most peak
  - (g) repeat for each peak or group of peaks across the spectrum (clicking the right mouse button at any time anywhere in the spectrum window causes an undo of the last integral section)
  - (h) click **FULL INTEGRAL**
  - (i) with the left mouse button position red vertical line on desired integral section
  - (j) click **SET INT**
  - (k) enter desired value, type return (the display window generates a list of integrals)
  - (l) click **NO INTEGRAL**
- set peak picking threshold:
  - (a) click **TH**
  - (b) drag the yellow line to the desired limit
  - (c) click **TH**
- **text('xxxxx')** (enters a title to be printed on top of your spectrum)
- **print, printp, printi** or **printpi** (i=with integration, p=with peak picking)
- if another experiment is required proceed with it, if not go to '**Finishing Acquisition of Spectra**'

### Acquisition of $^{13}\text{C}$ -NMR Spectra (note: $^1\text{H}$ -NMR must have been acquired first)

- **jexp2** (go to experiment 2)
- **c13xxxx** (loads solvent-dependent parameters; xxxx = cdcl, acet, d2o, dmso, meth; for benzene use xxxx=cdcl)
- if the lock level drops considerably, shim the sample as described for  $^1\text{H}$  spectra
- **nt=xx** (sets number of scans; default is 256)
- **ga** (starts acquisition)
- wait for acquisition to finish (beep will sound)
- **aph** (automatic phasing, the new software does a very good job - if the signal-to-noise ratio is good)

for manual phasing:

- (a) click **PHASE**
- (b) left click on highest peak
- (c) with the left mouse button pressed move cursor up or down in order to adjust the phasing for this peak
- (d) then left click on farthest peak and repeat phasing
- (e) interactively phase these two peaks until phasing is completed

- (f) **ds** (switches back to interactive display mode)
- **vsadj** (automatic adjustment of vertical scale -> highest peak adjusted to maximum of spectrum window)
  - for manual vertical scale adjustment: use the center mouse button, a left mouse click above the baseline causes the spectrum to scale up and a left click below the baseline causes the spectrum to scale down, the distance of the cursor from the baseline while clicking determines the multiplication factor of the in- and decrease
  - alternative vertical scale adjustment: **vs=xxx**, the current value of the vertical scale (vs) is displayed in the lower portion of the spectrum window
- expand spectrum:
  - (a) with left mouse button drag red left spectrum limit to its position (cr on bottom of spectrum = ppm)
  - (b) with right mouse button click and drag red right limit to its position (delta = difference in ppm)
  - (c) click **EXPAND** (expands spectrum to its set limits)
- set peak picking threshold:
  - (a) click **TH**
  - (b) drag the yellow line to the desired limit
  - (c) click **TH**
- **text('xxxxx')** (enters a title to be printed on top of your spectrum)
- **print** or **printp** (p=with peak picking)
- if another experiment is required proceed, if not go to section '**Finishing Acquisition of Spectra**'

### Acquisition of $^{13}\text{C}$ -DEPT Spectra (note: $^1\text{H}$ and $^{13}\text{C}$ spectra must have been acquired first)

The DEPT-45 experiment shows all of the protonated carbons with positive phase. The DEPT-90 experiment shows only the -CH- carbons, with positive phase. The DEPT-135 experiment shows -CH<sub>3</sub> and -CH- carbons with positive phase and -CH<sub>2</sub>- carbons with negative phase, quaternary carbons do not appear in any DEPT spectrum. The DEPT-45 experiment is useful because its sensitivity is about twice that of a standard  $^{13}\text{C}$  experiment, making DEPT-45 the  $^{13}\text{C}$  experiment of choice if only a limited amount of sample is available.

- **dept135** (if error message appears enter **c13xxxx**, then **dept135**)
- **mult=x.x** (x.x= 1.5 for DEPT-135, 1.0 for DEPT-90, 0.5 for DEPT-45)
- if the lock level drops considerably, shim the sample as described for  $^1\text{H}$  spectra
- **nt=xx** (sets number of scans; default is 128)
- **ga** (starts acquisition)
- wait for acquisition to finish (beep will ring out)
- **aph** (automatic phasing)
  - for manual phasing:
    - (a) click **PHASE**
    - (b) left click on highest peak
    - (c) with the left mouse button pressed move cursor up or down in order to adjust the phasing for this peak
    - (d) then left click on farthest peak and repeat phasing
    - (e) interactively phase these two peaks until phasing is completed

- (f) **ds** (switches back to interactive display mode)
- **vsadj** (automatic adjustment of vertical scale -> highest peak adjusted to maximum of spectrum window)
  - for manual vertical scale adjustment: use the center mouse button, a left mouse click above the baseline causes the spectrum to scale up and a left click below the baseline causes the spectrum to scale down, the distance of the cursor from the baseline while clicking determines the multiplication factor of the in- and decrease
  - alternative vertical scale adjustment: **vs=xxx**, the current value of the vertical scale (vs) is displayed in the lower portion of the spectrum window
- expand spectrum:
  - (a) with left mouse button drag red left spectrum limit to its position (cr on bottom of spectrum = ppm)
  - (b) with right mouse button click and drag red right limit to its position (delta = difference in ppm)
  - (c) click **EXPAND** (expands spectrum to its set limits)
- set peak picking threshold:
  - (a) click **TH**
  - (b) drag the yellow line to the desired limit
  - (c) click **TH**
- **text('xxxxx')** (enters a title to be printed on top of your spectrum)
- **print135, print135p, print90, print90p, print45 or print45p** (p=with peak picking)

### Acquisition of $^{19}\text{F}$ -NMR and $^{31}\text{P}$ -NMR Spectra (note: $^1\text{H}$ -NMR must have been acquired first)

- **jexp4** (go to experiment 4)
- **f19cdcl** or **p31cdcl** (loads solvent-dependent parameters)
- if the lock level drops considerably, shim the sample as described for  $^1\text{H}$  spectra
- **nt=xx** (sets number of scans)
- **ga** (starts acquisition)
- wait for acquisition to finish (beep will sound)
- proceed following the exact procedure and using the same print commands as for  $^1\text{H}$ -NMR ...

### Finishing Acquisition of Spectra

- **e**
- reinsert cleaned (use provided Kimwipes) standard sample
- **i**
- **jexp1** (go back to experiment 1)
- **standard**
- **exit**
- log out as described on first page

## Supplementary instructions

- in order to change the chemical shift reference type **rl(x.xx\*sfrq)** after placing the cursor on peak of interest; x.xx is the desired value to be entered
- in order to observe a spectrum during acquisition type **wft** and process the spectrum as described above
  - if the number of scans was not satisfactory repeat **wft** after additional scans
  - if the spectrum is satisfactory type **aa** (abort acquisition) and process the spectrum
- **axis='h'** changes the axis unit to Hertz, **axis='p'** changes it to ppm
- if you would like to restart phasing with its initial values type **lp=0 rp=0**, redo the phasing
- in order to display the picked peaks type **dpr**, to remove the displayed chemical shifts type **ds**
- click **DSCALE** to show a scale displaying the chemical shift below the spectrum, click **DSCALE** to switch off the scale

## Adjustment of Integral Trace

The **LVL/TLT** button activates interactive zero and first-order baseline correction mode. The zero order correction is represented by the **LVL** parameter; the first order correction is represented by the **TLT** parameter.

Position the cursor on an integral region of interest, about halfway vertically up the screen, and click the left mouse button. A horizontal line will intersect at the cursor and two vertical lines will be placed on either side of the cursor. Now moving the cursor above or below the horizontal line, but within the two vertical lines, and clicking the left or right mouse button will adjust the zero-order baseline correction parameter **LVL**. Placing the cursor right on the horizontal line and clicking the mouse button will restore the initial value of **LVL**.

Now move the cursor to another region of the spectrum, outside the vertical lines, and click the left mouse button again. A new horizontal line and two vertical lines will be displayed again and a single vertical line will be displayed in the middle of the region where **LVL** was being updated. The mouse will now control the first-order baseline correction parameter **TLT**. Clicking the left or right mouse button above or below the horizontal line will now increase or decrease **TLT**, and will also change **LVL** so that the total drift correction at the single vertical cursor in the middle of the previous region will be held constant. This process substantially reduces the necessity to iteratively adjust the two parameters **LVL** and **TLT**. As with the zero-order correction, clicking onto the horizontal baseline will restore the initial value of **TLT**.

Each time the cursor is moved outside the two vertical lines and the mouse button is clicked, a new vertical and horizontal line is displayed. The parameter adjustment alternates between adjusting **LVL** and adjusting **LVL** and **TLT**. The left and the right mouse button both adjust the baseline correction parameters and differ only in their sensitivity; changes with the left mouse button are eight times larger than changes caused with the right mouse button.

The middle mouse button adjusts the integral scale (height of integral trace). To exit the interactive baseline correction mode, click **LVL/TLT**.

# Instructions for Magnitude COSY Spectra on the Varian

## COSY data acquisition:

- acquire a <sup>1</sup>H spectrum in experiment 1 (**jexp1**) using the standard parameters
- phase the <sup>1</sup>H spectrum, then expand the spectrum such that the peaks of interest are displayed on the screen
- type **movesw** to set up an optimized acquisition window for your sample
- re-acquire the spectrum (**ga**), phase and display it. Check that it looks correct
- move your recently acquired FID to experiment 3 using the command **mf(1,3)**
- go to experiment 3 (**jexp3**) and to **wft** to display your spectrum. It does not need to be phased
- type **cosyjsh** to set up a magnitude COSY spectrum. The COSY takes about 30 minutes
- open the interactive acquisition window (**acqi**) and turn off the sample spinning. Check the lock level is acceptable
  - increase lock power and/or gain to give a lock level of at least 50%. Check the Z1 and Z2 shims
- type **au** to start the acquisition - DO NOT USE **ga**!

## COSY data processing and plotting:

- type **wft2d** to carry out a 2D Fourier transform and to display the 2D spectrum
- click the **MAIN MENU** button above the spectrum window, then click the **DISPLAY, SIZE, FULL SCREEN** buttons
- use the **VS-20%** and the **VS+20%** buttons above the spectrum to adjust the vertical scaling of the intensity image prior to plotting
- type **plcosy(8,1.5,1)** to plot the 2D spectrum along with the 1D spectrum along the axes
- expansions are done in the same fashion as for 1D spectra, with the left and right mouse buttons and the **EXPAND** menu button

## Finishing up:

- go back to experiment 1 (**jexp1**) and restart sample spinning
- proceed with other spectra or finish up as described earlier in this instruction book

# Instructions for Magnitude HETCOR Spectra on the Varian

## HETCOR data acquisition:

- acquire a  $^1\text{H}$  spectrum in experiment 1 (**jexp1**) using the standard parameters
- phase the  $^1\text{H}$  spectrum, then expand the spectrum such that the peaks of interest are displayed on the screen
- type **movesw** to set up an optimized acquisition window for your sample
- re-acquire the spectrum (**ga**), phase and display it. Check that it looks correct
- acquire a  $^{13}\text{C}$  spectrum in experiment 2. If time permits, expand, optimize, and re-run the  $^{13}\text{C}$  spectrum (note that if your spectrum has downfield quaternary carbons these do not need to be included in the expanded region)
- move the  $^{13}\text{C}$  fid from experiment 2 to experiment 3 with **mf(2,3)**
- go to experiment 3 (**jexp3**), do **wft** to check that the  $^{13}\text{C}$  spectrum appears correctly, then type **hetcorjsh(1)** to set up a magnitude-mode heteronuclear correlation spectrum
- check that the **nt** and **d1** values are appropriate for your sample (usually the defaults are fine)
- type **go** to start the acquisition - DO NOT USE **ga**!

## HETCOR data processing and plotting:

- type **wft2d** to carry out a 2D Fourier transform and to display the 2D spectrum
- click the **MAIN MENU** button above the spectrum window, then click the **DISPLAY, SIZE, FULL SCREEN** buttons
- use the **VS-20%** and the **VS+20%** buttons above the spectrum to adjust the vertical scaling of the intensity image prior to plotting
- type **plhxcor(8,1.5,1,2)** to plot the 2D spectrum along with the 1D spectra along the axes
- expansions are done as for 1D spectra, with the left and right mouse buttons and the **EXPAND** menu button
- NOTE: multiple printouts with different vertical scale settings may be needed to allow all of the cross-peaks to be seen

## Finishing up:

- go back to experiment 1 (**jexp1**) and restart sample spinning
- proceed with other spectra or finish up as described earlier in this instruction book



# Instructions for NMR Laboratory - Chem 343

## 1D Polarization-Transfer and INEPT Experiments

- we will use the sealed  $\text{CHCl}_3/\text{CDCl}_3$  sample for these experiments
- acquire a  $^1\text{H}$ -NMR spectrum in exp 1. After acquiring with the standard parameters, expand the spectrum so that the main peak ( $\text{CHCl}_3$ ) and the two  $^{13}\text{C}$  satellite peaks take up approximately one-half of the total display width, then use the command **movesw** to set that displayed region to be the new spectrum parameters (the **tof** and **sw** parameters will change)
- acquire the  $^1\text{H}$  spectrum again using **nt=1** and **ss=0**. Set **axis='h'** and record the **tof** value in your notebook.
- Put the cursor in the center of the spectrum (there will be a glitch at this location) and measure the distances in Hz. between the center of the spectrum and each  $^{13}\text{C}$  satellite peak (using the two-cursor mode). Take these two distances and add (or subtract, if the peaks are upfield of the transmitter) each one to the **tof** value to obtain the  $^1\text{H}$  transmitter frequencies for each satellite peak. Write these values in your notebook, along with the value you measure for the JC-H coupling constant
- **text('xxxxx')** (include your names in the title)
- **print**
- set **axis='p'**
- **print**
- acquire a  $^1\text{H}$ -coupled  $^{13}\text{C}$ -NMR spectrum in exp 2 (**jexp2**) - use **nt=4**, **ss=0**, and **dm='n'** for this spectrum. Expand this spectrum in the manner described above. Set the following parameters manually: **d1=12**, **pw=13**, **tpwr=60**, **gain=33**, **nt=1**, **np=1024**, **fn='n'**, **vp=20**. Re-acquire the spectrum using the optimized parameters
- use **dof** to see the default  $^1\text{H}$  decoupler frequency, enter the value in your notebook
- After processing and displaying the spectrum, place the two cursors such that they bound a representative region of baseline noise (i.e., no extra peaks or glitches present between the cursors). Use the command **dsn** to display the signal-to-noise ratio for the tallest peak in the spectrum and record the s/n in your notebook
- **text('xxxxx')** (include your names and the s/n value in the title)
- **print**
- **mf(2,3)** (moves the  $^{13}\text{C}$ -FID from exp 2 to exp 3), then **jexp3** (switches to exp 3)
- we will now obtain the first polarization-transfer spectrum. Set the following parameters manually: **d2=0.01**, **dm='nyn'**, **dmm='c'**, **dpwr=0**. Set **dof** to be equal to one of the  $^1\text{H}$  satellite frequencies you determined earlier.
- click the **SEQUENCE** button on the right side of the window below the spectrum, then click the **DISPLAY PULSE SEQUENCE** button that shows up in the new window. This shows the pulse sequence for the experiment we just set up
- acquire the spectrum with **nt=1**
- record the s/n (cursors, **dsn**, as described above) in your notebook
- **text('xxxxx')** (include your names and the s/n value in the title)
- **print**

- repeat the above using the dof for the other satellite peak, **print** out the spectrum
- **mf(3,4), jexp4**
- we will now obtain a polarization-transfer spectrum using the INEPT pulse sequence. Use the command **ineptjsh** to set up the pulse sequence then enter the following parameters manually: **gain=33, d1=12, ss=0, nt=1**. Set **dof** to be equal to the default value you noted earlier. Set **j=xxx**, where xxx is the C-H coupling constant you measured earlier
- display the pulse sequence as described above
- acquire the spectrum and phase it appropriately
- record the s/n in your notebook
- **text('xxxxx')** (include your names and the s/n value in the title)
- **print**
- set **focus='y'** (allows the  $^{13}\text{C}$  doublet components to refocus prior to acquisition), re-acquire
- display pulse sequence
- set **dm='nny'** (turns on  $^1\text{H}$  decoupling during acquisition), re-acquire. Record the s/n
- **text('xxxxx')**
- **print**
- **mf(4,5), jexp5**
- set **mult=0** (this runs a normal  $^{13}\text{C}$  experiment – display the pulse sequence to see), re-acquire, record the s/n
- **text('xxxxx')**
- **print**

## 2D $^1\text{H}$ - $^{13}\text{C}$ Heteronuclear Correlation Experiment

- **mf(5,6), jexp6**
- use the command **hetcorjsh(1)** to set up a 2D heteronuclear correlation experiment. Enter the following manually: **ss=0, nt=4, ni=32, fn1=64, d1=3, j1xh=xxx** (xxx = C-H coupling constant). Check that **dof** and **sw1** are correct for the  $^1\text{H}$  dimension (compare to the  $^1\text{H}$  spectrum printed earlier)
- acquire with **go** (this will take less than ten minutes)
- when finished, type **wft2d** to do a 2D Fourier transform and display the spectrum
- click the **MAIN MENU** button above the spectrum window, then click the **DISPLAY, SIZE, FULL SCREEN** buttons
- use the **VS-20%** and the **VS+20%** buttons above the spectrum to adjust the vertical scaling of the intensity image prior to plotting
- **text('xxxxx')**
- **plhxcor(8,1.5,1,5)** (plots the 2D spectrum with the 1D spectra along each axis)
- click the **DISPLAY** button on the right side of the window below the spectrum window
- in the display window that is now present, set the following checkboxes: Scaling - Absolute, Display - Phased, WYSIWYG - off, Display Position - Center
- type **wft1d** to carry out a Fourier transformation on the F2 (acquisition,  $^{13}\text{C}$ ) dimension only

- type **dssa(1,32,1)** to display all 32  $^{13}\text{C}$  spectra
- type **ds** to display the first spectrum, expand as necessary with the cursors, then redisplay all spectra, check that the vertical scaling (**vs**) is OK (expand horizontally if desired)
- plot the 32 spectra in stacked format with **pl(1,32,1)\_pscale\_ppa\_page** (no returns between these commands)
- type **trace='f1', dcon1** to redisplay the 2D data with the F1 axis horizontal
- click the **TRACE** button above the spectrum, then select the most intense trace using the cursor and the left mouse button
- **ds** (displays selected trace by itself)
- expand the region of the trace where the signal is, if needed
- set **vp=40**, then print this interferogram using **pl\_pscale\_ppa\_page** (no returns between these commands)
- using the axis printed on the spectrum, record the period of the sinusoidal signal. From this calculate the frequency of the signal. Enter both values in your notebook
- Go back to the  $^1\text{H}$  spectrum in exp1 and determine the offset of the  $\text{CHCl}_3$  peak from the transmitter (using the method described above). Record this value in your notebook

### Finishing up:

- go back to experiment 1 (**jexp1**) and restart sample spinning
- proceed with other spectra or finish up as described earlier in this instruction book