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 acquisition
- RESIZE button -> enlarges/reduces spectrum window
- FLIP button -> flips the spectrum window over the display window and vice versa
- jexpx 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 $^1$H-NMR Spectra

• **jexp1** (go to experiment 1)
• **h1xxxx** (loads solvent-dependent parameters; xxxx = cdcl, acet, d2o, dmos, meth; for benzene use xxxx=cdcl)
• **acqi** (opens acquisition window)
• click **LOCK** (do not change $Z_0$ 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 13C-NMR Spectra** (note: 1H-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 1H 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 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 increase 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’

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**Acquisition of ¹³C-DEPT Spectra** (note: ¹H and ¹³C 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 -CH₃ and -CH- carbons with positive phase and -CH₂- 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 ¹³C experiment, making DEPT-45 the ¹³C 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 ¹H 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 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 increase 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 $^{19}$F-NMR and $^{31}$P-NMR Spectra (note: $^1$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 $^1$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 $^1$H-NMR …

### Finishing Acquisition of Spectra

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

- in order to change the chemical shift reference type \( r_1(x.xx * sfrq) \) 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 \( dpf \), 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 1H spectrum in experiment 1 (jexp1) using the standard parameters
- phase the 1H 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 1H spectrum in experiment 1 (jexp1) using the standard parameters
• phase the 1H 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 $^{13}$C spectrum in experiment 2. If time permits, expand, optimize, and re-run the $^{13}$C spectrum (note that if your spectrum has downfield quaternary carbons these do not need to be included in the expanded region)
• move the $^{13}$C fid from experiment 2 to experiment 3 with mf(2,3)
• go to experiment 3 (jexp3), do wft to check that the $^{13}$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