

DCIF: How to calibrate the ^1H 90° Pulse on the Varian NMRs

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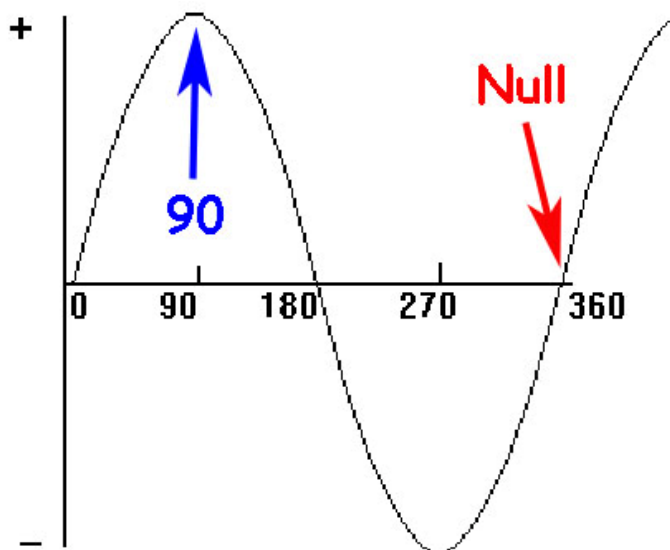
Background Information:

For many NMR experiments such as DEPT, TOSCY, NOESY, and HMBC, the pulse sequence requires that many specific pulses or a series of pulses (90° , 45° , 180° , etc.) be applied. Without properly calibrated pulses, many of these experiments will yield meaningless results, or most likely, fail outright.

Since each compound (and each nucleus) has a different chemical environment, each had a distinct 90° pulse width (pw90). The pw90 is defined as the duration, in microseconds, that the rf signal must irradiate your sample in order to tilt the magnetizations into the XY-plane, 90° away from the Z-axis of the NMR's magnetic field. Another way to think of it is how long you must pulse in order to tip all the spins into the XY plane. This pulse is often referred to as the $\pi/2$ pulse.

The 90° pulse width for proton NMR experiments is about 10-20 microseconds on most modern spectrometers. The exact value of the 90° pulse width depends on the sample (nucleus, solvent, etc.) as well as the instrument (probe, transmitter power, etc.). It may be 5 microseconds long, 17 microseconds, or 35 microseconds, or some other number determined experimentally. For this reason, it is necessary to measure the 90° pulse for every sample you need to perform 2D experiments on. Lucky for us, the proton 90° pulse is typically quite similar for all the protons in your sample.

Measuring the 90° pulse width is simple enough. Remember that the 90° pulse tilts the sample magnetization into the XY plane, which contains the detector. A simple pulse sequence of irradiate-observe (in Varian lingo this is called *s2pu*) should show a maximum for the pulse duration corresponding to a 90° pulse. Because it is difficult to discern maximum signal intensities by comparing similarly intense peaks (i.e. comparing an 89° , a 90° , and a 91° pulse.), we look at the 180° or the 360° pulse.



The 360° pulse corresponds to a 'null' - no signal is observed at this irradiation. Searching for this null is easier to determine and has the added advantage of minimizing the time required between pulses due to relaxation issues.

The Varian nitty gritty:

Please note that **all bold** print is input into the command window. Many steps are being skipped on the assumption that you already know how to acquire a simple 1D-proton spectrum.

- Tune the probe and obtain a well-shimmed ^1H spectrum.
- Use the cursors to zoom in on an area of interest and carefully phase this portion of the spectrum. If necessary, phase this portion by hand.
- Prepare the instrument by typing **nt=1 gain='y' ai vp=50** (number transients = 1, receiver gain set to value in stored experiment, set absolute intensity mode, spectrum vertical position = 50)
- Type **pw90?** and remember this value (write it down!). For this example, let's assume this value to be $8.65\mu\text{s}$ (microseconds).
- Create an array of values bracketing the 360° pulse width ($4 * \text{pw90}$). For example:
 - Let's assume $4 * \text{pw90}$ is $34.6\mu\text{s}$, but we of course would like to more precisely determine this value.
 - Type **array('pw',20,32.6,.2)** This will array pw in 20 increments starting at $32.6\mu\text{s}$ with a step size of $0.2\mu\text{s}$. (i.e., $\text{pw}=32.6,32.8,33.0,33.2,\dots$ to 36.2). If you are unsure of what to enter as an array, start with general array with fewer steps and larger increments, like **pw=28, 30, 32, 34, 36, 38**. or **array('pw',6, 28, 38, 2')**.
 - Type **pw[1]=8.65** (or whatever the value was when you queried the pw90 above)
 - To display the arrayed parameters type **da**. A listing will appear in the Text Window.
- Type **au** (submit the experiment to acquisition and process data)
- Type **wft(1)**, phase the spectrum (**aph**), then type **vsadj**
- When the experiment finishes, process the data by typing **wft dssh** (weight and Fourier transform data, display stacked spectra horizontally)
- On the screen, (with the exception of the first spectrum), you should see a series of spectra that start positive, pass through a null at 180° , become negative, and pass through a second null at 360° , similar to Figure 1. Estimate the point where the signal goes from negative values through zero then become positive. This is the location of your 360° pulse.
- Locate the array value that best describes the 'zero point'. For example:
 - Let's assume the 'zero point' (360° pulse width) corresponds to $\sim 34.9\mu\text{s}$.
 - Type **pw90=34.9/4** ($8.725\mu\text{s}$). Your pw90 for this sample is $8.725\mu\text{s}$.
- If you do not see a clear null at 360° , you may need to run your array again, adjusting the array's increments and/or step size.
- Similar procedures may be utilized to determine 90° pulses for other nuclei. For example, ^{13}C 90° pulses need to be determined for HMQC experiments. For this, the determination may require a longer time since $\text{nt}=1$ may be inadequate for obtaining sufficient S/N.

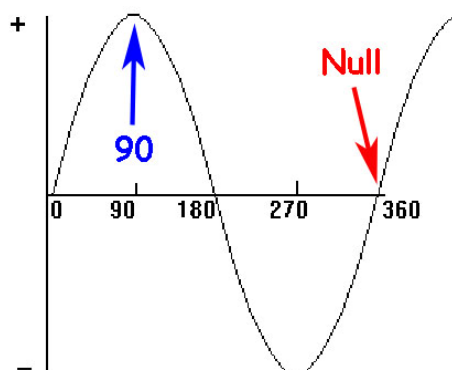


Figure 1