Jan 24, 2023

Protocol for quantitative NMR analysis on AVIII600

Book time and log in computer with a user account

Procedure:

Open Topspin 3.6.5

A. Collect a routine ^{1}H NMR spectrum
   1. Create dataset (**edc**): name; exp #; directory (D:\group name\user name\MonthYear)
   2. Read in parameter set (**rpar Ah1** (30° flip angle pulse))
   3. Load sample in autosampler holder #; (**sx #1**)
   4. Lock the magnetic field (**lock**) and select the right solvent
   5. Tune the probe automatically (**atma**)
   6. no spinning
   7. Read in shim file (**rsh shim.current**); shim (**topshim**)
   8. Get probehead and solvent dependent parameters (**getprosol**)
   9. Automatic receiver gain optimization (**rga**)
   10. Check and adjust (**ns**), (**ds**), (**d1**)
   11. run (**zg**)
   12. **efp**;**apk**, adjust phase if necessary

B. Determine proton T1
   13. **edc** to create a new dataset, read in parameter set (**A_PROTONT1_BC**)
       Pulse program = **t1ir**
   14. Type **setlimits**, a window will pop up. Open the 1D proton NMR spectrum collected, zoom in the peaks of interest and leave ~1 ppm of baseline on both sides of the spectrum, then click OK to close the window
   15. Type **eda** to review default settings and adjust them if required, check **VDLIST** and click '**E**' button next to it to edit **t1delay_BC** values if necessary, then save, make sure TD(F1) matches the number of the delays
   16. Type **getprosol** then **rga**
   17. Run (**zg**)

C. Process and analyze the t1 data
   18. Type **rser 10** and **ef** to process the last spectrum with the longest delay, phase properly
   19. Type **edp** to check phase constants
   20. Type **xf2** to process the data and check if the phase constants are consistent
   21. Type **abs2** for baseline correction
   22. Open Topspin's **Analysis** and select **T1/T2** on the **Dynamics** button
   23. Click **FID**, a window will pop up
   24. Click **Spectrum**, then type **10** in the Slice Number dialog cell, and click **OK**

25. A 1D proton spectrum appears, click **Peaks/Range**
26. A window pops up, select **Manual integration**, **OK**
27. Integrate selected peaks per standard processing
28. Click **Save region as**…, and select **Export Regions to Relaxation Module and .ret.**
29. Click the **Relaxation** button, review any message that appears
30. Select **Area** and click "**>>**" button to perform fittings of all peaks
31. Use "**-**" or "**+**" to review individual fitting
32. Click **Report** to open a fitting report file
33. Click **File** and **save as** to save the report
34. The individual fitting image can be saved as pdf, jpg, or tif file as well.

D. Collect 1D proton spectrum using a 90° flip angle pulse

35. Create a new dataset (**edc**)
36. Read in parameter set (**rpar Ah1_90**)
37. Get probehead and solvent dependent parameters (**getprosol**)
38. Automatic receiver gain optimization (**rga**)
39. Determine proton 90 degree pulse
    a) Set the following parameters: **ns** = 1, **d1** = 4, **p1** = 5
    b) Acquire and phase the spectrum
    c) Select a single peak to display (not solvent peaks) and type (**dpl**) to save the display region
    d) Type (**paropt**) and enter the parameters:
       Parameter to modify: **P 1**
       Initial parameter value: **3** us
       Increment: **2** us
       Number of experiments: **18**
    e) Determine the value of **p1** for the null (**360º**)
    f) Repeat the process to refine the **p1** value by decreasing the increment
    g) Set **p1** = **p1 (360º)/4**
40. Check and adjust **d1** to be 5 times of the longest t1 values
41. Run (**zg**)
42. **efp**; **apk**, adjust phase if necessary
43. Perform baseline correction (**abs**), or manually polynomial baseline correction if necessary
44. Integrate the peaks corresponding to the internal reference and the target peaks
45. Calculate to convert the integral values into sample concentration or purity information

Key factors for successful application of quantitative NMR:

1. Appropriate internal reference
2. No overlapping target peaks to be analyzed from either internal reference or sample
3. Both sample and internal reference being stable
4. Samples and internal references being weighted accurately and precisely