KJM 9250

QQHSQC Experiments on the AVI-600, AVII-600 and AVIIIHD-800 Spectrometers

Version 5.0

Topspin 2.1 Windows XP AVI 600
Topspin 3.2 Windows 7 AVII 600
Topspin 3.5 Windows 7 AVIII HD 800

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1.0 Introduction

Prosol compatible adaptations of the **qqhsqc.hko** pp which ran on the **AVI-600** under Topspin 2.1 but was not prosol compatible and would not run on the **AVII-600** under Topspin 3.2 or the **AVIIIHD-800** under Topspin 3.5 have now been developed.

QQ-HSQC: a quick, quantitative heteronuclear correlation experiment for NMR spectroscopy

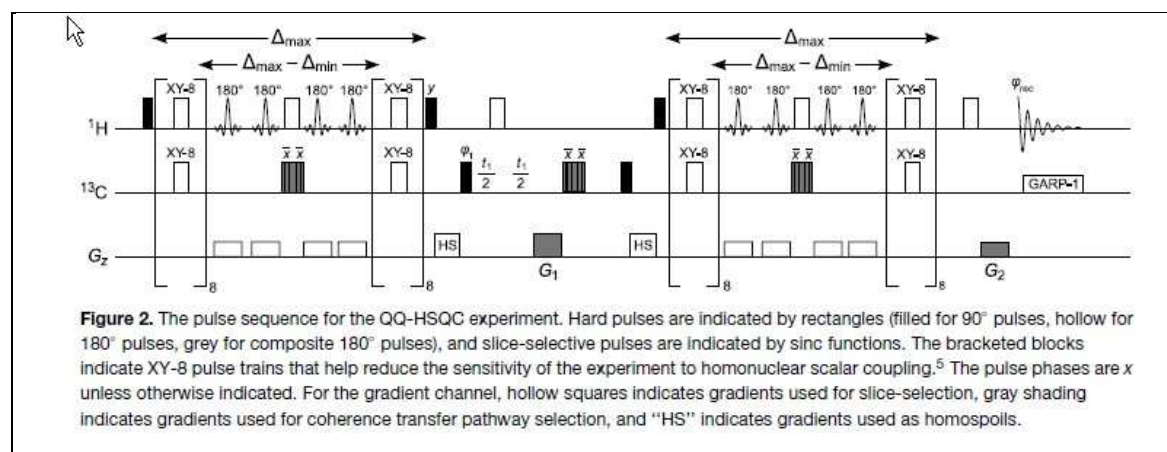Daniel J. Peterson and Nikolaus M. Loening*

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Quantitative heteronuclear single quantum coherence (Q-HSQC) is a variant of the HSQC experiment that provides quantitative peak areas. This is accomplished by combining signals acquired using four different INEPT delays. Consequently, the experiment requires four times as many scans as the HSQC experiment to achieve the same resolution in the indirect dimension. We have removed this drawback by modifying the Q-HSQC experiment so that signals corresponding to different INEPT delays are acquired simultaneously from different parts of the sample. This new experiment, which we call Quick, Quantitative HSQC (QQ-HSQC), has the quantitative properties of the Q-HSQC experiment but only requires as many scans as a conventional HSQC experiment. Copyright © 2007 John Wiley & Sons, Ltd.

QQHSQC paper's abstract.

Figure 2. The pulse sequence for the QQ-HSQC experiment. Hard pulses are indicated by rectangles (filled for 90° pulses, hollow for 180° pulses, grey for composite 180° pulses), and slice-selective pulses are indicated by sinc functions. The bracketed blocks indicate XY-8 pulse trains that help reduce the sensitivity of the experiment to homonuclear scalar coupling.[5] The pulse phases are x unless otherwise indicated. For the gradient channel, hollow squares indicates gradients used for slice-selection, gray shading indicates gradients used for coherence transfer pathway selection, and "HS" indicates gradients used as homospoils.

QQHSQC pulse sequence.

1.1 AVI-600 version under Topspin 2.1

The **awqqhsqc25-10** pp substitutes the AVI-600's prosol linked ROESY spin lock pulse time (**p25** = 200 usec) and TOCSY power level (**pl10**) for the pulse time (**p12**) and power level (**sp12**) respectively of the **BIP720,50,20.1** shaped pulse used in the **qqhsqc.hko** pp.

On the **AVI** the **pl10** TOCSY spin lock power level can be substituted for the **sp12** power level by inserting the line **"sp12=pl10"** in modified versions of the **qqhsqc.hko** pp.
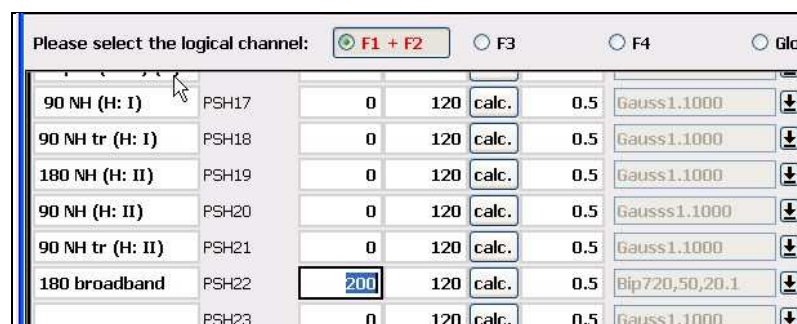
The AVI-600's prosol Table linked **p25** and **pl10** values are coincidently identical to the values which can be calculated for the **BIP720,50,20.1** shaped pulse's **p12** time and **sp12** power levels respectively.

While the choice of the **BIP720,50,20.1** shaped pulse is saved in aw coded **qqhsqc** experiment parameter files, efforts to prosol link this choice and its time + power level to a prosol Table line with this information (eg the PSH22 shaped pulse line for ^{1}H via Amp A2) have thus far failed. All possible shaped pulse ID's, sp1 to sp31, have been tried in trial runs of the **qqhsqc** pp.
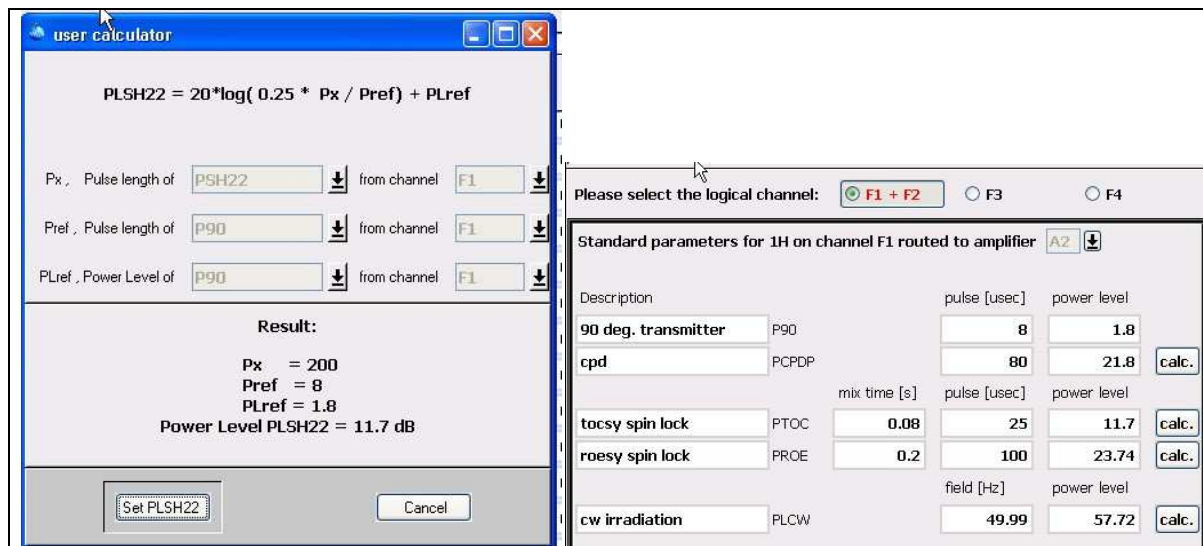
AVI-600's prosol Table

The AVI's prosol Table includes a greyed out (not available or active?) PSH22 line for a **BIP720,50,20.1 180 broadband** pulse with its time and power set to 0 and 120 db respectively.

If **200 usec** is entered as its time its calculated power level based on an 8.0 usec 90 degree ^{1}H pulse is **11.7 db** = exactly the same as the power level that Prof Rise originally deduced was OK for this shaped pulse – and coincidently it is the same as the prosol Table linked TOCSY spin lock power level.

PSH22 shaped pulse prosol Table line with 200 usec entered for its time.

Calculated power level (11.7 db) for a
200 usec **BIP720,50,20.1** pulse.

TOCSY spin lock power (**pl10**) = 11.7 db with a
8.0 usec 90 degree ^{1}H pulse time

The calculated **BIP720,50,20.1** power level can be saved in the prosol Table but thus far attempts to read this information into an experiment's parameter set via the **getprosol** command have failed (see above).

The **pl10** TOCSY spin lock power level may be **pulsecal** linked.

1.2 AVII-600 version under Topspin 3.2

Neither the original **qqhsqc.hko** or the **awhsqc25-10** (see Section 1.1) versions of the **qqhsqc** experiment would run on the **AVII-600** under **Topspin 3.2**. Initially it was not clear why this was the case, however it eventually became apparent that Topspin 3.2 was not able to interpret one or more of the following pp lines:

```
define delay LONG
define delay SHORT

"SHORT=2.94m"
"LONG=5.92m"

"TAU1=SHORT/32"
"TAU2=(LONG/2-SHORT/2-p25*2-d17*4)/4"
"TAU3=(LONG/2-SHORT/2-p25*2-d17*4)/4-p3*2.2"
```

Replacing these lines by those below afforded a prosol compatible version with a **200 usec BIP720,50,20.1** shaped pulse time which appeared to be linked to a "*180° bradband*" line in the AVII-600's prosol Table. Possibly this line had been added by Bruker, Sweden (bradband = Swedish version of broadband).

```
"d30=2.94m"
"d31=5.92m"

"TAU1=d30/32"
"TAU2=(d31/2-d30/2-p44*2-d17*4)/4"
"TAU3=(d31/2-d30/2-p44*2-d17*4)/4-p3*2.2"
```
.

The **BIP720,50,20.1** *'180 bradband'* shaped pulse time and power is parameter set linked via the **getprosol** command as **p44 sp30,** as opposed to **p12 sp12** in the original **qqhsqc.hko** pp.

An **AVII-600** prosol Table linked **p44 sp30** version of the **qqhsqc** pulse programme has been saved as **awqqhsqc-TS3.**

The significance of the **d30** (SHORT) and **d31** (LONG) delays (2.94 msec and 5.92 msec respectively) is commented on in the **AVIIIHD-800** notes.

1.3 AVIIIHD-800 version under TS3.5

The **awhsqc-TS3** version implemented on the AVII-600 also ran on the **AVIIIHD-800** under **Topspin 3.5** and was fully prosol compatible, however the prosol Table linked **BIP720,50.1,20** shaped pulse time was **150 usec** rather **200 usec**.

While it would be possible to change the 800's prosol Table's time for this pulse there may be merit in leaving it at **150 usec** since in Hz terms since the 800's ^{1}H spectral window in ppm will be 33.3% wider in Hz than that of the same spectral window on the 600's. To match the 600's flat topped, coupling constant independent, modulated linear response range the 800's shaped pulse time would need to be significantly shorter than that for the 600's. Coincidently $800/600 = 200/150$.

The significance of the SHORT and LONG delays (2.94 msec and 5.92 msec respectively) in the TS2.1 version of the **qqhsqc** pp which are redefined as **d30** and **d31** respectively in the TS3.2/TS3.5 versions is not explained or commented on in the original **qqhsqc.hko** pp notes.
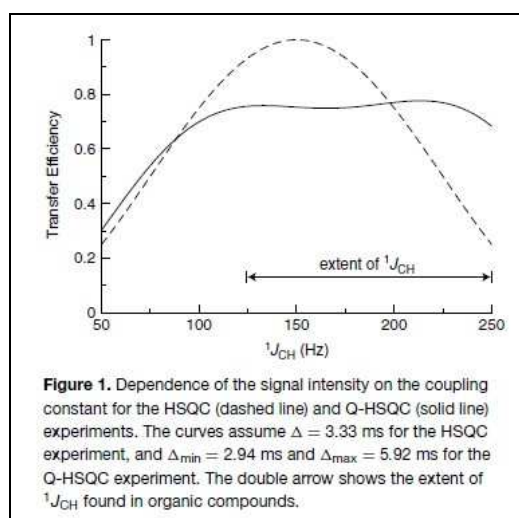
Literature searching revealed the significance of these delay terms which are calculated from the minimum and maximum ^1J coupling constants as below.

```
"d30=1s/(cnst21*2)"    ;1J (Hz) upper limit
"d31=1s/(cnst2*2)"     ;1J (Hz) lower limit
```

The **qqhsqc** pp is designed to afford modulated/uniform responses ex target signals irrespective of their varying ^1J coupling constants, provided they are within the defined upper and lower ^1J limits. While the default LONG (5.92 msec) and SHORT (2.94 msec) delay times correspond to J_{min} and J_{max} = 84.4 and 170 Hz respectively, the experimental linear response range of the **qqhsqc** pp with these delays is ~ 120-225 Hz.

1	QQHSQC Calculator	
2	1J (Hz)	d30 (d31) (msec)
3	80	6.25
4	100	5.00
5	120	4.17
6	140	3.57
7	160	3.13
8	180	2.78
9	200	2.50
10		
11	84.4	5.92
12	170	2.94

Figure 1. Dependence of the signal intensity on the coupling constant for the HSQC (dashed line) and Q-HSQC (solid line) experiments. The curves assume $\Delta = 3.33$ ms for the HSQC experiment, and $\Delta_{min} = 2.94$ ms and $\Delta_{max} = 5.92$ ms for the Q-HSQC experiment. The double arrow shows the extent of $^1J_{CH}$ found in organic compounds.

Calculated d30 (d31) delays.   Linear response range of the **qqhsqc** expt.

An **awqqqhsqc-^{1}J** variant of the **qqhsqc pp** with the **d30** and **d31** values defined from user input $J_{min/max}$ coupling constants as **cnst2** and **cnst21** Hz respectively has been created and successfully run on the **AVIIIHD-800.**

2.0 AWQQHSQC parameter sets on the AVI-600, AVII-600 and AVIIIHD-800

The following aw coded prosol compatible parameter sets have been created on the 600 and 800 MHz spectrometers:

AVI-600   **2.1   awqqhsqc25-10**

                   2.2   awqqhsqc25-10pr

AVII-600   **2.3   awqqhsqc-TS3**

                   2.4   awqqhsqcpr-TS3

AVIIIHD-800   **2.5   awqqhsqc-TS3**

                   2.6   awqqhsqcpr-TS3

                   2.7   awqqhsqc-1J

2.1 AWQQHSQC25-10 Spectrum on the AVI-600

Parameter set: **awqqhsqc25-10**
Pulse programme: **awqqhsqc25-10**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.

TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).
NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time of your choice.

Type **ased** (enter) and review parameters used in the job.
Check gradients are OK (**gpz1 = 80%, gpz2 = 20.1%, gpz10 = 27.1%,**
  gpz11 = 62.1%, gpz12 = 0%).
Check **P25** = 200 **usec** and **SP12** is set to **11.7 db** or its current prosol Table value.
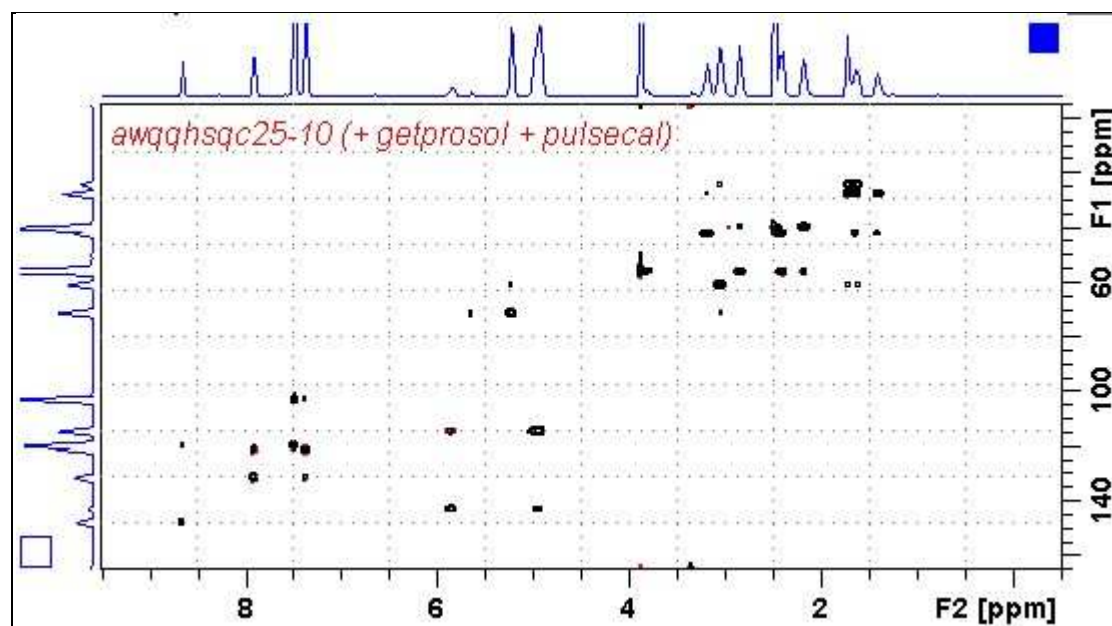 **SP12** (= **PL10**) will be updated by **pulsecal**.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = SI(F1) = 1K or 2K**
        WDW(F1) = WDW(F2) = QSINE
        SSB(F2) = SSB(F1) = 2
        xfb, abs1 and abs2

AVI-600 **AWQQHSQC25-10** spectrum of quinine in D$_6$-DMSO.

2.2 AWQQHSQC25-10PR Spectrum on the AVI-600

Parameter set: **awqqhsqc25-10pr**
Pulse programme: **awqqhsqc25-10pr**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 (Hz)** = frequency in **Hz** of the signal to presaturated.
$\qquad\qquad$ = ^{1}**H** spectral window midpoint. Check SW is wide enough.
Enter **O2P** = ^{13}**C** spectral window midpoint in ppm.

TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).
NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time of your choice.
PL9 = CW presaturation power applied during **D1**.

Type **ased** (enter) and review parameters used in the job.
Check gradients are OK (**gpz1 = 80%, gpz2 = 20.1%, gpz 10 = 27.1%,**
$\qquad$ **gpz11 = 62.1%, gpz12 = 0%**).
Check **P25** = **200 usec** and **SP12** is set to **11.7 db** or its current prosol Table value.
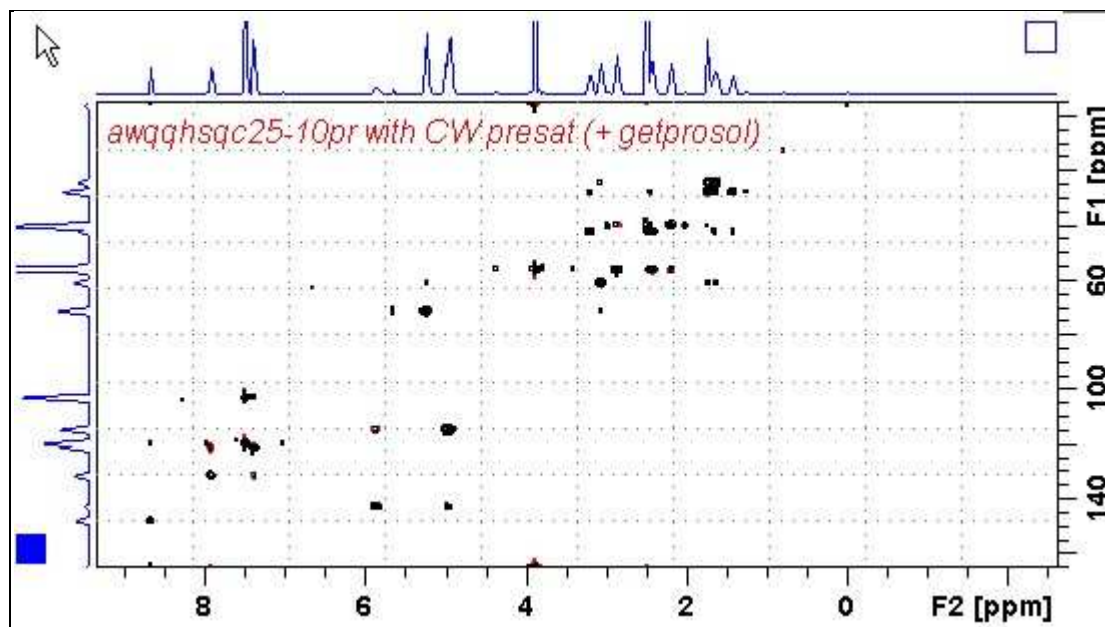$\quad$ **SP12** (= **PL10**) will be updated by **pulsecal**.

Set **receiver gain** using **RGA** *(Important!).*

Process with: $\quad$ **SI(F2) = SI(F1) = 1K** or **2K**
$\qquad\qquad\qquad$ **WDW(F1) = WDW(F2) = QSINE**
$\qquad\qquad\qquad$ **SSB(F2) = SSB(F1) = 2**
$\qquad\qquad\qquad$ **xfb, abs1 and abs2**

AVI-600 **AWQQHSQC25-10PR** spectrum of quinine in D$_6$-DMSO with presaturation of the HOD line at 3.37 ppm.

2.3 AWQQHSQC-TS3 Spectrum on the AVII-600

Parameter set: **awqqhsqc-TS3**
Pulse programme: **awqqhsqc-TS3**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.

TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).
NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time of your choice.

Type **ased** (enter) and review parameters used in the job.
Check gradients OK (**gpz1 = 80%, gpz2 = 20.1%, gpz10 = 27.1%, gpz11 = 62.1%, gpz12 = 0%**)
Check **P44 = 200 usec** and **SP30** is set to its current prosol Table value.
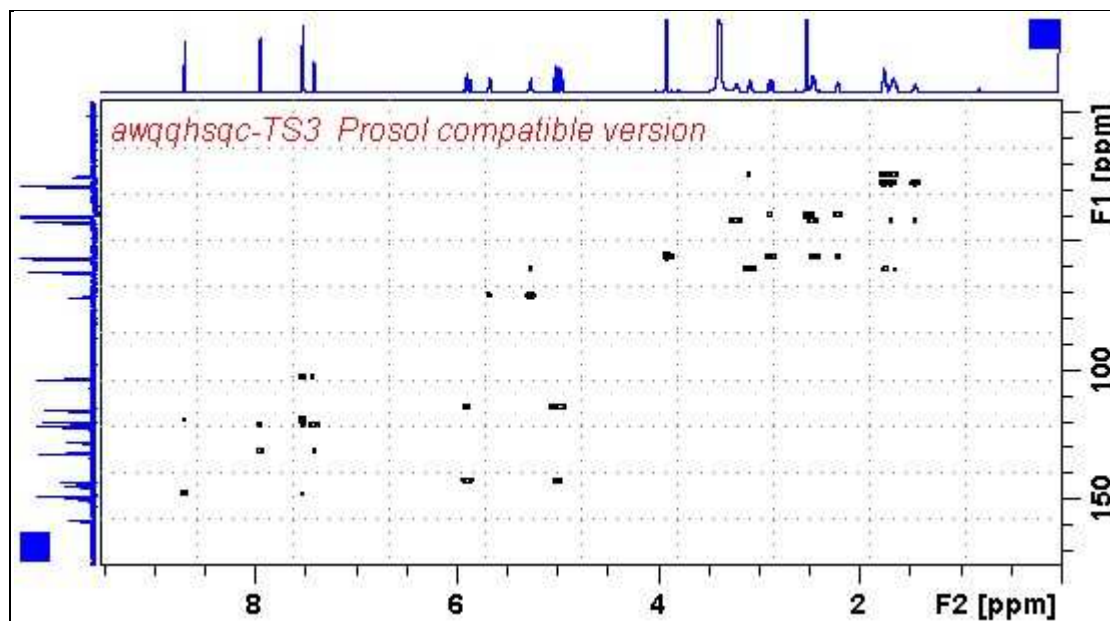   **SP30** will be updated by **pulsecal**.
Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = SI(F1) = 1K or 2K**
          WDW(F1) = WDW(F2) = QSINE
          SSB(F2) = SSB(F1) = 2
          xfb, abs1 and abs2

AVII-600 **AWQQHSQC-TS3** spectrum of quinine in D$_6$-DMSO.

2.4 AWQQHSQCPR-TS3 Spectrum on the AVII-600

Parameter set: **awqqhsqcpr-TS3**
Pulse programme: **awqqhsqcpr-TS3**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 (Hz)** = frequency in **Hz** of the signal to presaturated.
  = ^{1}**H** spectral window midpoint. Check SW is wide enough.
Enter **O2P** = ^{13}**C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time of your choice.
PL9 = CW presaturation power applied during **D1.**

Type **ased** (enter) and review parameters used in the job.
Check gradients are OK (**gpz1 = 80%, gpz2 = 20.1%, gpz10 = 27.1%,**
  gpz11 = 62.1%, gpz12 = 0%)
Check **P44** = **200 usec** and **SP30** is set to **11.7 db** or its current prosol Table value.
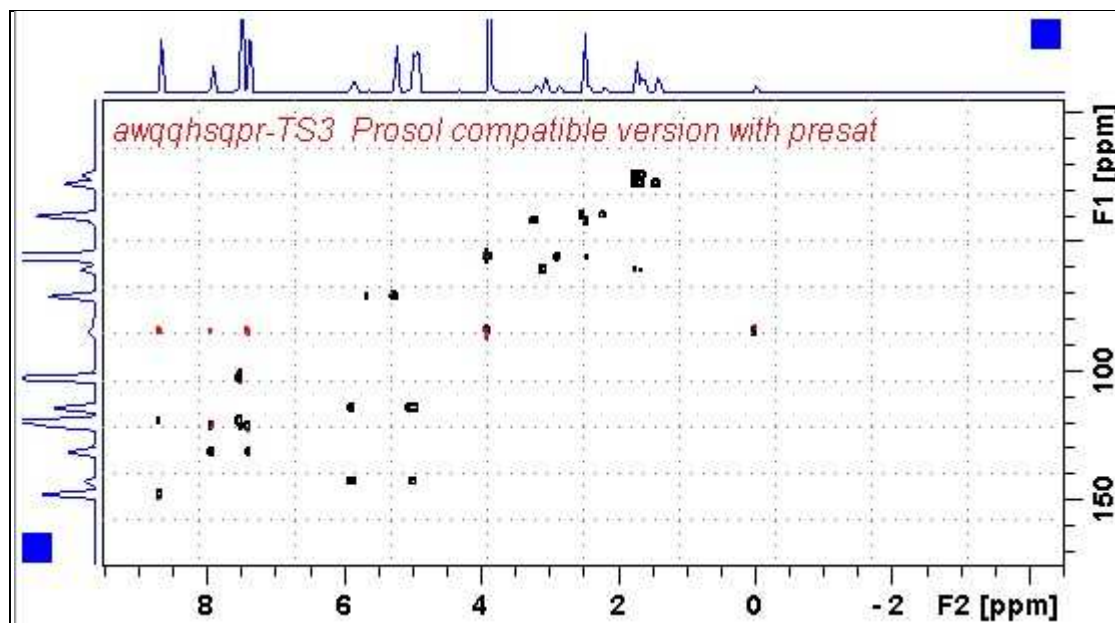 **SP30** will be updated by **pulsecal**.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = SI(F1) = 1K** or **2K**
      WDW(F1) = WDW(F2) = QSINE
      SSB(F2) = SSB(F1) = 2
      xfb, abs1 and abs2

AVII-600 **AWQQHSQCPR-TS3** spectrum of quinine in D$_6$-DMSO.

2.5 AWQQHSQC-TS3 Spectrum on the AVIIIHD-800

Parameter set: **awqqhsqc-TS3**
Pulse programme: **awqqhsqc-TS3**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.

TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).
NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time of your choice.

Type **ased** (enter) and review parameters used in the job.
Check gradients are OK (**gpz1 = 80%, gpz2 = 20.1%, gpz10 = 27.1%,
      gpz11 = 62.1%, gpz12 = 0%**)
Check **P44 = 150 usec** and **SP30** is set to its current prosol Table value.
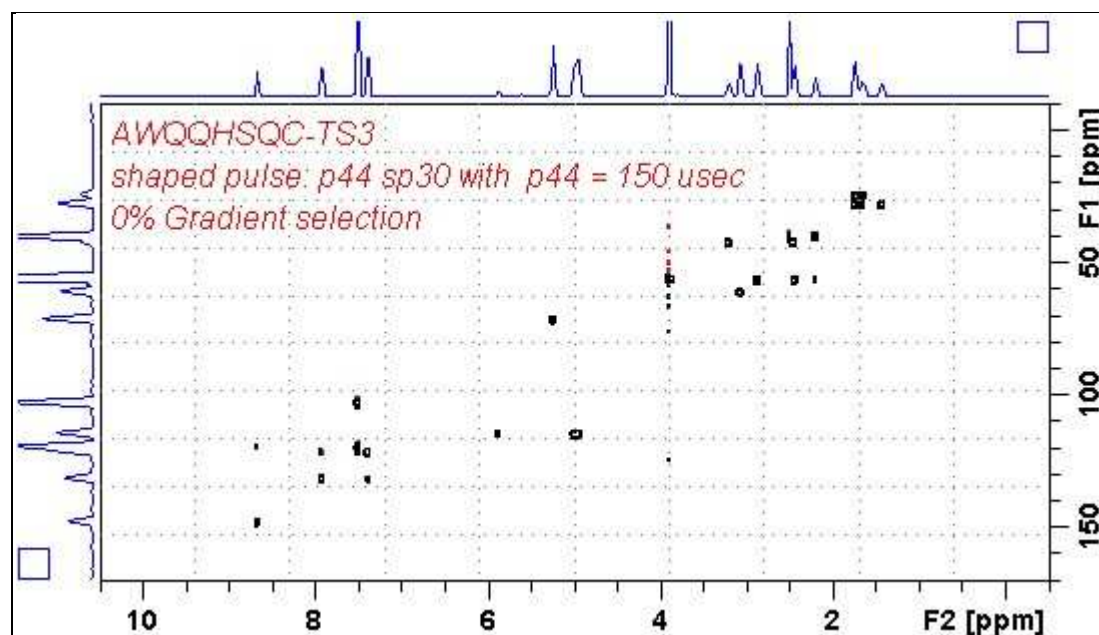   **SP30** will be updated by **pulsecal**.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = SI(F1) = 1K or 2K**
               WDW(F1) = WDW(F2) = QSINE
               SSB(F2) = SSB(F1) = 2
               xfb, abs1 and abs2

AVIIIHD-800 **AWQQHSQC-TS3** spectrum of quinine in D$_6$-DMSO.

2.6 AWQQHSQCPR-TS3 Spectrum on the AVIIHD-800

Parameter set: **awqqhsqcpr-TS3**
Pulse programme: **awqqhsqcpr-TS3**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 (Hz)** = frequency in **Hz** of the signal to presaturated.
          = ^{1}**H** spectral window midpoint. Check SW is wide enough.
Enter **O2P** = ^{13}**C** spectral window midpoint in ppm.

TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).
NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time of your choice.
PL9 = CW presaturation power applied during **D1**.

Type **ased** (enter) and review parameters used in the job.
Check gradients are OK (**gpz1 = 80%, gpz2 = 20.1%, gpz10 = 27.1%,**
      gpz11 = 62.1%, gpz12 = 0%).
Check **P44** = **150 usec** and **SP30** is set to its current prosol Table value.
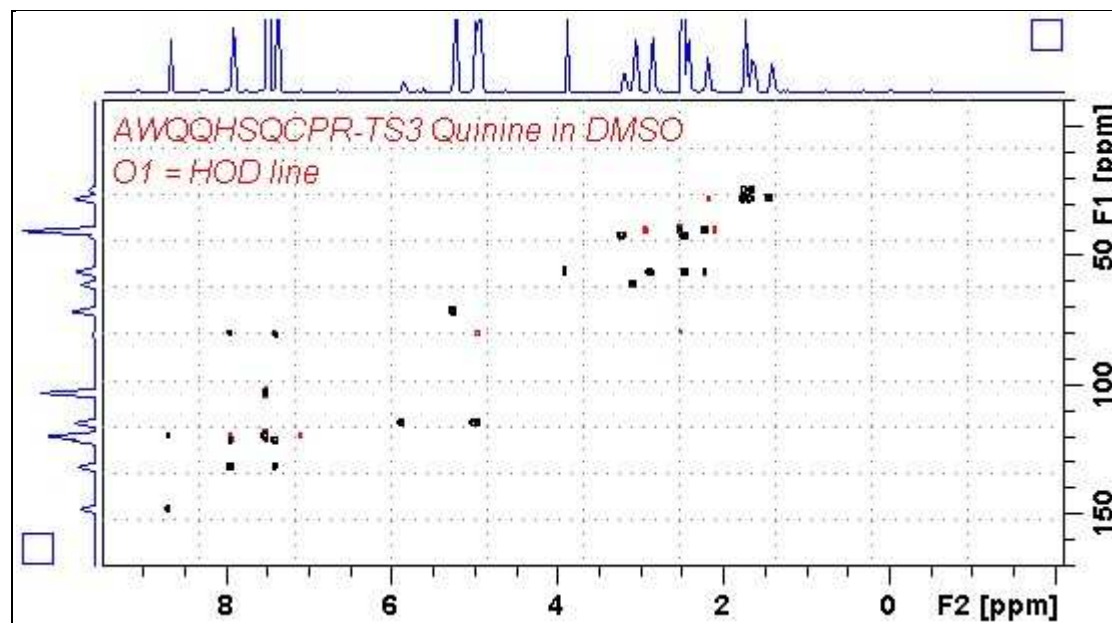 **SP44** will be updated by **pulsecal**.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = SI(F1) = 1K** or **2K**
          WDW(F1) = WDW(F2) = QSINE
          SSB(F2) = SSB(F1) = 2
          xfb, abs1 and abs2

AVIIIHD-800 **AWQQHSQCPR-TS3** spectrum of quinine in D$_6$-DMSO with presaturation of the HOD line at 3.37 ppm.

2.7 AWQQHSQC-1J Spectrum on the AVIIIHD-800

Parameter set: **awqqhsqc-1J**
Pulse programme: **awqqhsqc-1J**
   With user entry of min. and max. ^1J coupling constants.

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.

TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).
NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time of your choice.

CNST2 = minimum ^1J coupling constant (eg: 85-120 Hz).
CNST21 = maximum ^1J coupling constant (eg: 165-220 Hz).

Type **ased** (enter) and review parameters used in the job.
Check gradients OK (**gpz1 = 80%, gpz2 = 20.1%, gpz10 = 27.1%,**
        gpz11 = 62.1%, gpz12 = 0%).
Check **P44** = **150 usec** and **SP30** is set to its current prosol Table value.
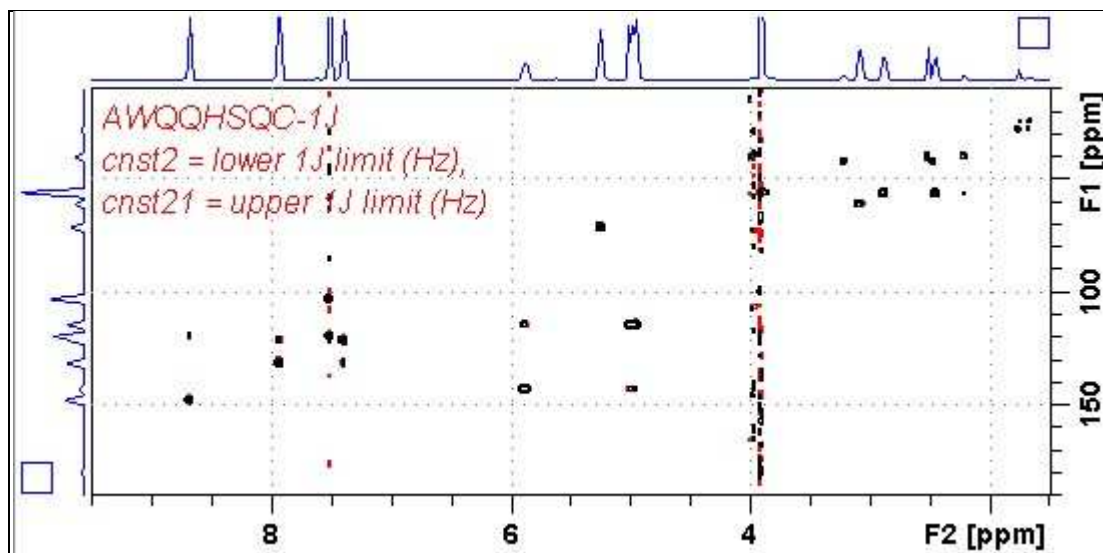   **SP30** will be updated by **pulsecal**.

Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = SI(F1) = 1K or 2K**
               WDW(F1) = WDW(F2) = QSINE
               SSB(F2) = SSB(F1) = 2
               xfb, abs1 and abs2

AVIIIHD-800 **AWQQHSQC-1J** spectrum of quinine in D$_6$-DMSO.