KJM 5250 and KJM 9250

HSQC, HMBC and H2BC Experiments with CW (PR) and Excitation Sculpting Solvent Suppression on the AVneo400 Spectrometer.

Version 3.1

Topspin 4.3

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AVneo-400 HSQC, HMBC and H2BC Experiments

1.0 Introduction

aw coded TS4 **Neo-400 HSQC** and **HMBC** parameter sets are set up with 1K or 2K acquired ^{1}H points in F2 and 128 ^{13}C increments in F1.

^{1}H and ^{13}C spectral windows and their mid points can be adjusted if required. The **O1** frequency at which **PR** or **ES** is applied at should be determined accurately in **Hz**, rather than approximately in **ppm**.

Topspin's **getprosol** and **pulsecal** commands should be used to read in **prosol Table** stored **pulse times** and **powers** and adjust them based on your samples solvent and matrix /buffer effects. Optionally, for concentrated samples, but not low level samples, **pulsecal 13c** can be used to adjust ^{13}C pulse times and powers.

Neo-400 aw coded **hsqc135** pp's have been derived from Topspin's **hsqcedetgpsisp2.3** pp with the addition of auto-calculation of **d21** and **d24** from **cnst**2 (= the ^{1}J ^{13}C-^{1}H coupling constant: default value = 145 Hz) and the removal of an optional **p28** trim pulse.

1.1 Processing

HSQC experiments are phase sensitive experiments which should be manually phased before optionally using the **abs1** and **abs2** commands. Low level ^{2}J may be observed in **HSQC** spectra.

HMBC spectra are magnitude mode (QF) spectra (phasing is not required) and should be transformed with **xfb.**

The **H2BC** experiment is acquired in phase sensitive mode and transformed to afford an absolute value spectrum using the **xfb** and **xf2m** commands. Phasing of **h2bc** spectra is not required

2.0 HSQC Experiments and Parameter Sets

The following **HSQC experiments** have been set up on the **Neo-400** spectrometer.

2.1	**hsqc45**	not multiplicity edited, DEPT45 like
2.2	**hsqc135**	multiplicity edited, DEPT135 like
2.3	**hsqc135pr**	with CW presaturation
2.4	**hsqc135es**	with ES peak suppression
2.5	**hsqc135espr**	with ES and PR peak suppression

HMBC and H2BC spectra...next page

3.0 HMBC and H2BC Experiments and Parameter Sets

The following **HMBC and H2BC experiments** have been set up on the **Neo-400** spectrometer.

3.1	**hmbc**	with ^{n}J selection
3.2	**hmbcpr**	with CW presaturation
3.3	**hmbces**	with ES peak suppression
3.4	**hmbclp2**	with $^{1}J_{min/max}$ filter
3.5	**hmbc-cigar**	with ^{13}C decoupling
3.6	**hmbcct**	with min/max ^{1}J selection
3.7	**hmbcctpr**	with CW presaturation
3.8	**hmbcctes**	with ES peak suppression
3.9	**hmbcctespr**	with ES +PR presaturation
3.10	**h2bc**	for ^{2}J correlations

2.1 HSQC45 Spectrum

Parameter set: **awhsqc45 (+ getprosol + pulsecal)**
Pulse program: **awhsqcetgpsisp2.2-45**
d24 is automatically calculated from **cnst2**

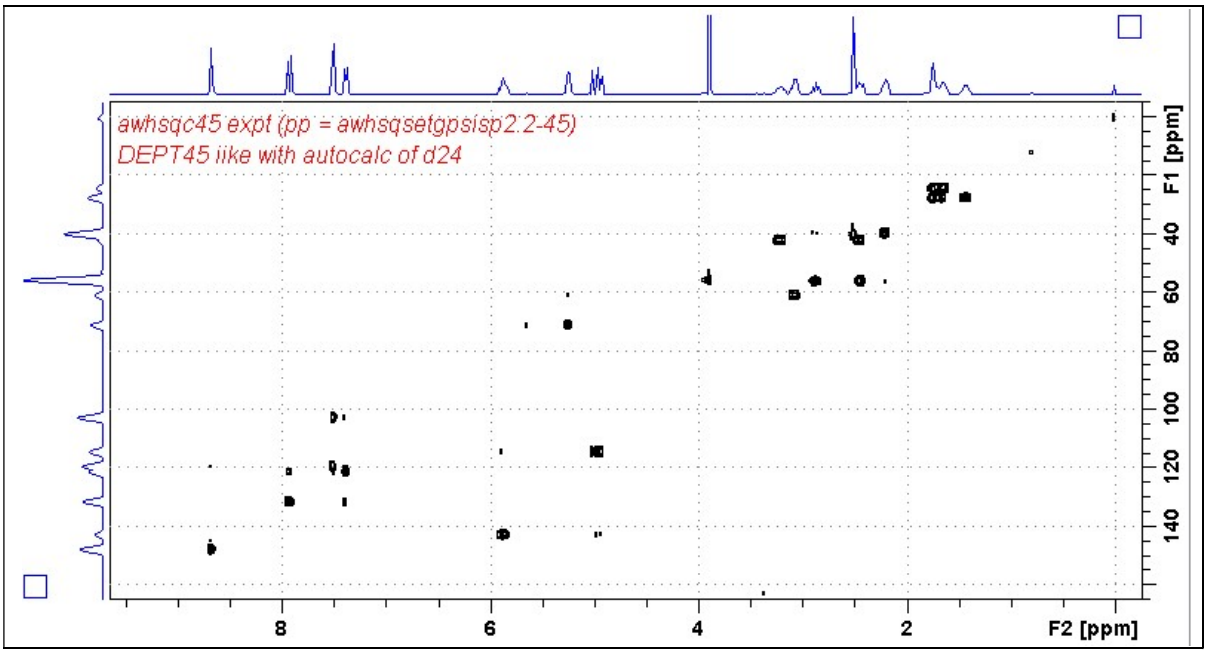
Type **eda** (enter) and enter **SW (^{1}H)** and **SW(^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST2 = ^{1}J coupling constant = **145 Hz** or other value of your choice (eg: 125-160 Hz).

Type **ased** (enter) and review parameters used in the job.
Check gradients and shaped pulses are OK.

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

Process with: **SI(F2) = 1K or 2K, SI(F1) = 512 or 1K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, manual phasing *and* **abs1 + abs2**



Neo-400 HSQC45 spectrum of quinine in D_6-DMSO.

2.2 HSQC135 Spectrum

Parameter set: **awhsqc135 (+ getprosol + pulsecal)**
Pulse program: **awhsqcedetgpsisp2.3-135**
d21 and **d24** are automatically calculated from **cnst2**

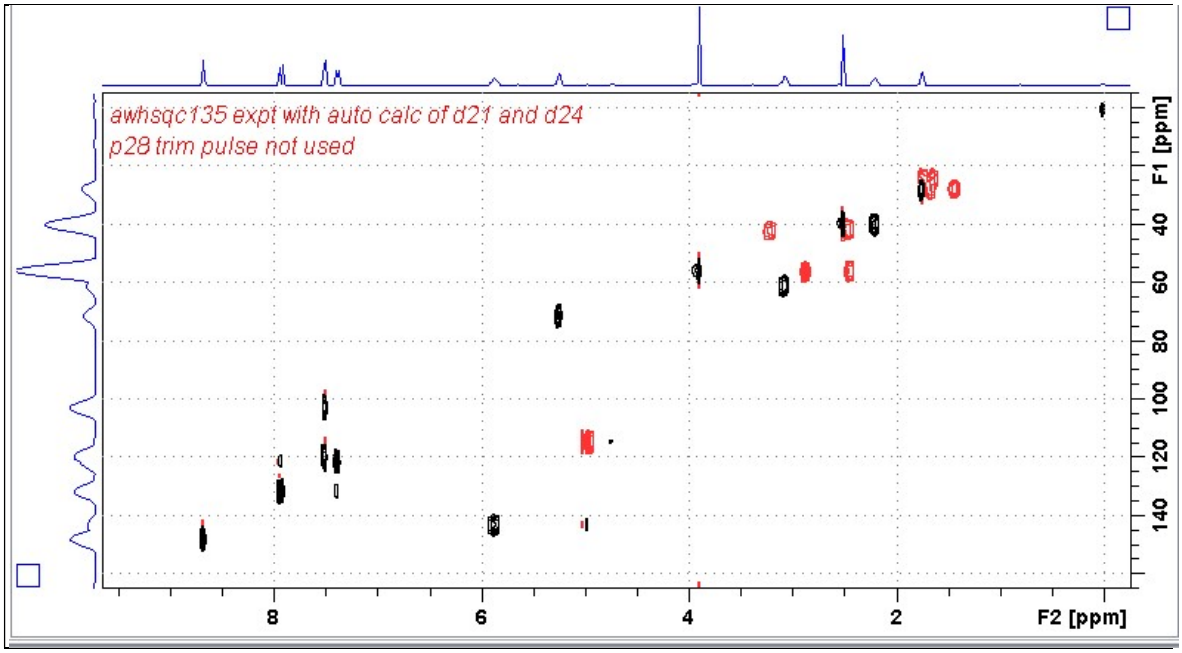
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time of your choice.
CNST2 = ^{1}J coupling constant = **145 Hz** or other value of your choice (eg: 125-160 Hz).

Type **ased** (enter) and review parameters used in the job.
Check gradients and shaped pulses are OK.

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

Process with: **SI(F2) = 1K or 2K, SI(F1) = 512 or 1K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, manual phasing and **abs1** + **abs2**



Neo-400 HSQC135 spectrum of quinine in D_6-DMSO plotted with positive CH andCH_3 correlations (black) and negative CH_2 correlations (red).

2.3 HSQC135pr Spectrum

Parameter set: **awhsqc135pr (+ getprosol + pulsecal)**
or **awhsqcedetgpsisp2.3-135pr (+ getprosol + pulsecal_**
Pulse program: **awhsqcedetgpsisp2.3-135pr**
d21 and **d24** are automatically calculated from **cnst2**

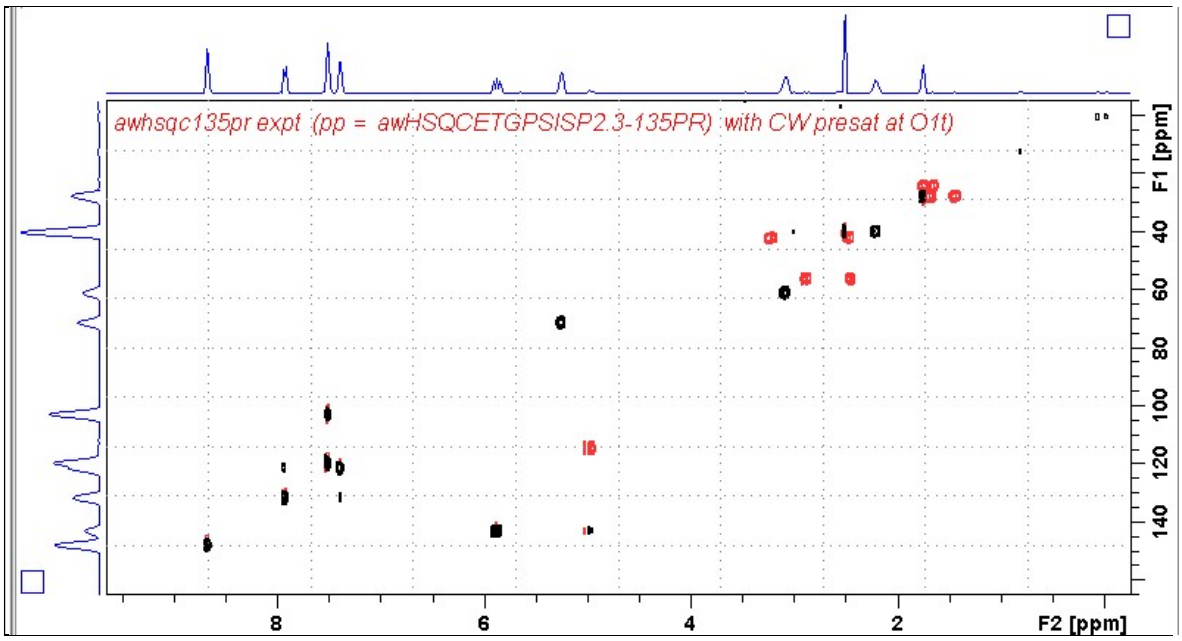
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **PR**).
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time of your choice.
CNST2 = ^{1}J coupling constant = **145 Hz** or other value of your choice (eg: 125-160 Hz).
PLW9(db) = **PR** power applied during **D1**. If required the **PR** power can be increased by *subtracting* 6 or 12 db from its prosol Table value.

Type **ased** (enter) and review parameters used in the job.
Check gradients and shaped pulses are OK.

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

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



Neo-400 HSQC135pr spectrum of quinine in D_6-DMSO with CW presaturation of the HOD line at 3.37 ppm. The spectrum is plotted with positive CH and CH_3 correlations (black) and negative CH_2 correlations (red).

2.4 HSQC135es Spectrum

Parameter set: **awhsqc135es (+ getprosol + pulsecal)**
Pulse program: **awhsqc135es**
d21 and **d24** are automatically calculated from **cnst2**

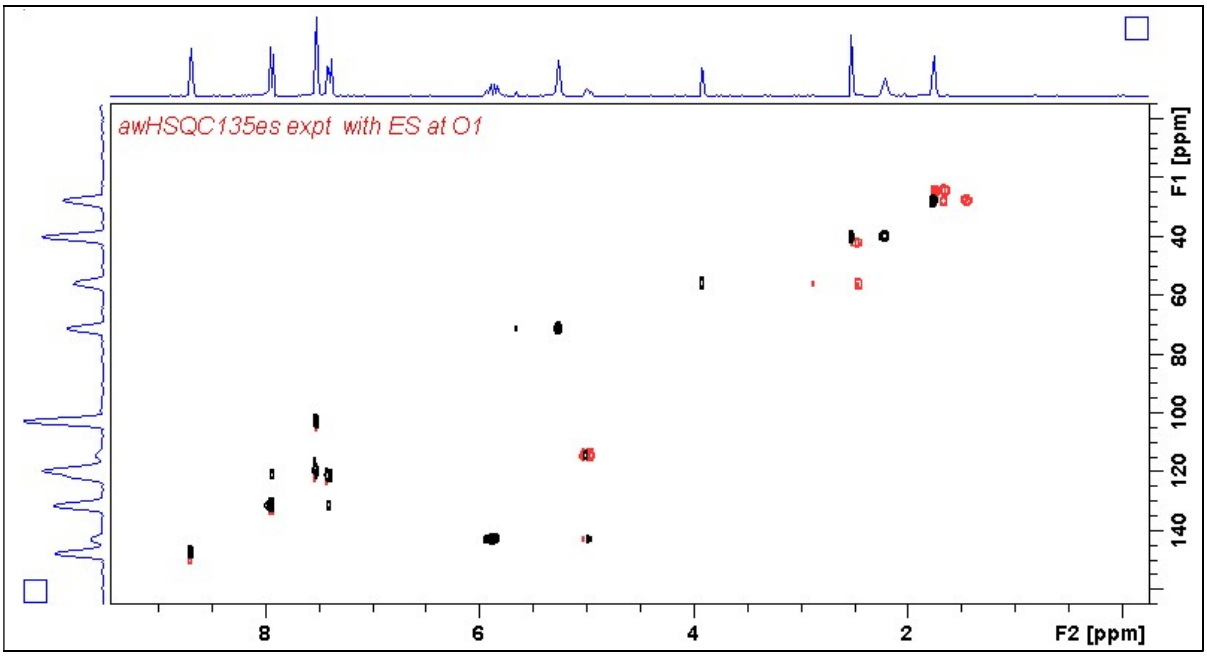
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **ES**)
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST2 = ^{1}J coupling constant = **145 Hz** or other value of your choice (eg: 125-160 Hz).

Type **ased** (enter) and review parameters used in the job. Check that gradients and shaped pulses are OK, including a prosol Table defined **2000 usec p40:sp10 Sinc1.1000 ES** pulse.

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

Process with: **SI(F2) = 1K or 2K, SI(F1) = 512 or 1K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, manual phasing and **abs1** + **abs2**



Neo-400 HSQC135es spectrum of quinine in D_6-DMSO with ES suppression of the HOD line at 3.37 ppm. The spectrum is plotted with positive CH and CH_3 correlations (black) and negative CH_2 correlations (red). ^{1}H signals /correlations located 0.5-0.7 ppm either side of the **ES** suppressed line have reduced intensity.

2.5 HSQC135espr Spectrum

Parameter set: **awhsqc135espr (+ getprosol + pulsecal)**
Pulse program: **awhsqc135espr**
d21 and **d24** are automatically calculated from **cnst2**

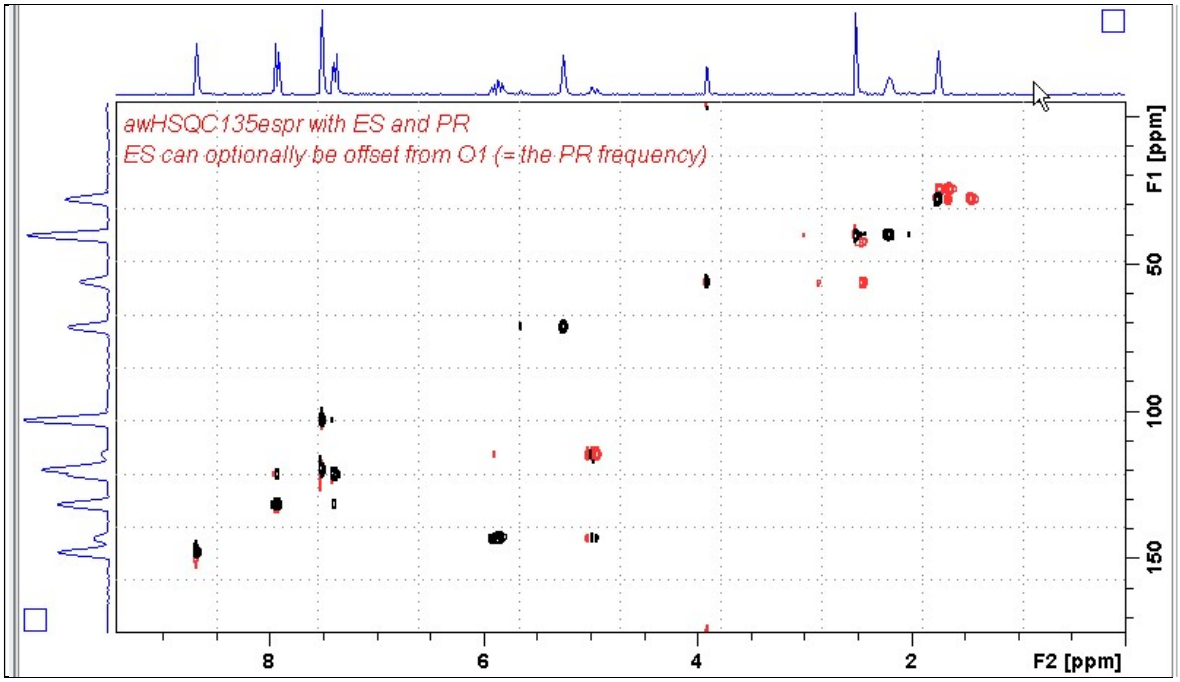
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **ES** + **PR**).
ES can optionally be offset from **PR** as described in the Appendix.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time of your choice.
CNST2 = ^{1}J coupling constant = **145 Hz** or other value of your choice (eg: 125-160 Hz).
PLW9(db) = **PR** power applied during **D1**. If required the **PR** power can be increased by *subtracting* 6 or 12 db from its prosol Table value.

Type **ased** (enter) and review parameters used in the job. Check that gradients and shaped pulses are OK, including a prosol Table defined **2000 usec p40:sp10 Sinc1.1000 ES** pulse.

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

Process with: **SI(F2) = 1K or 2K, SI(F1) = 512 or 1K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, manual phasing and **abs1** + **abs2**



Neo-400 HSQC135espr spectrum of quinine in D_6-DSMO. **ES** + PR was applied at 3.35 ppm (= the HOD line). ^{1}H signals /correlations located 0.5-0.7 ppm either side of the **ES** suppressed line have reduced intensity.

3.1 HMBC Spectrum

Parameter set: **awhmbc (+ getprosol + pulsecal)**
Pulse program: **hmbcgplpndqf**

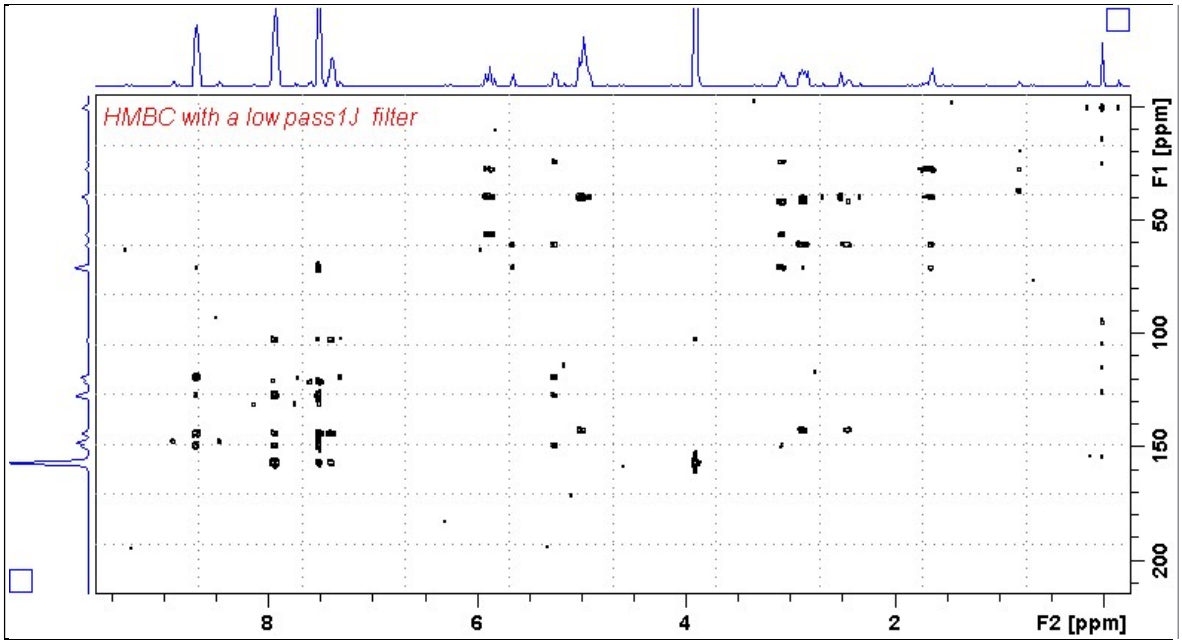
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST2 = ^{1}J coupling constant = **145 Hz** or other value of your choice.
CNST13 = ^{n}J selection filter = **8 Hz** or other value of your choice.

Type **ased** (enter) and review parameters used in the job.
Check gradients and shaped pulses are OK.

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

Process with: **SI(F2) = 1K or 2K, SI(F1) = 512 or 1K points**
WDW(F1) = WDW(F2) = SINE
SSB(F2) = SSB(F1) = 0
xfb *and* **abs1 + abs2**



Neo-400 HMBC spectrum of quinine in D_6-DMSO.

3.2 HMBCpr Spectrum

Parameter set: **awhmbcpr (+ getprosol + pulsecal)**
Pulse program: **awhmbcgplpndqfpr**

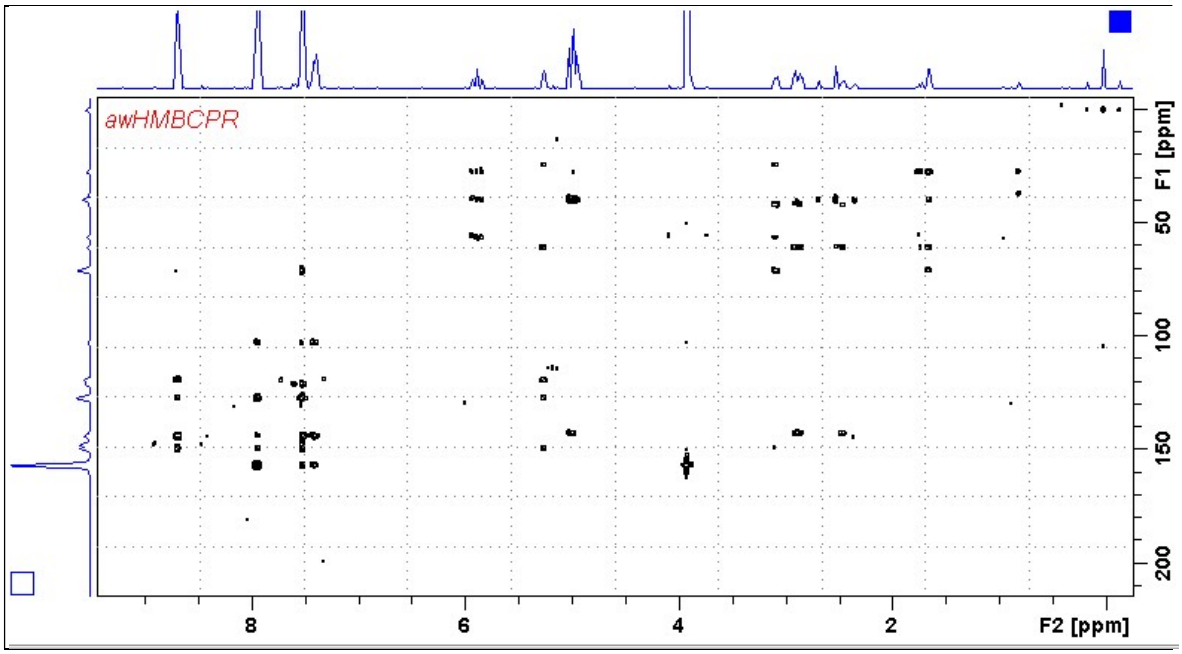
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in Hz (for **PR**)
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K,**TD(F1)** = 128-256 (your choice).

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 = ^{1}J coupling constant = **145 Hz** or other value of your choice.
CNST13= ^{n}J selection filter = **8 Hz** or other value of your choice.
PLW9(db) = **PR** power applied during **D1**. If required the **PR** power can be increased by *subtracting* 6 or 12 db from its prosol Table value.

Type **ased** (enter) and review parameters used in the job.
Check gradients and shaped pulses are OK.

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

Process with: **SI(F2) = 1K or 2K, SI(F1) = 512 or 1K points**
WDW(F1) = WDW(F2) = SINE
SSB(F2) = SSB(F1) = 0
xfb *and* **abs1 + abs2**



Neo-400 HMBCpr spectrum of quinine in D_6-DMSO with CW presaturation of the HOD signal at 3.37 ppm,

3.3 HMBCes Spectrum

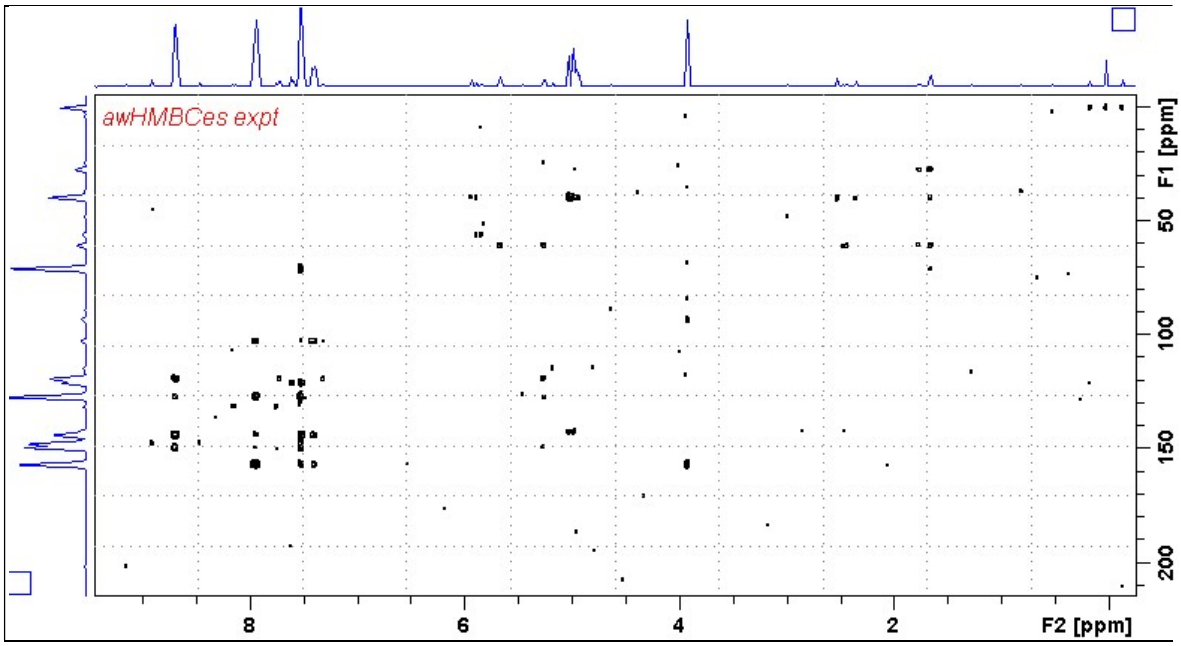
Parameter set: **awhmbces (+ getprosol + pulsecal)**
Pulse program: **awhmbces**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in Hz (for **ES**)
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K,**TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay =**1.5 sec** or other time of your choice.
CNST13 = ^{n}J selection filter = **8 Hz** or other value of your choice (eg: 6-14 Hz).

Type **ased** (enter) and review parameters used in the job. Check that gradients and shaped pulses are OK, including a prosol Table defined **2000 usec p40:sp10 Sinc1.1000 ES** pulse.

Process with: **SI(F2) = 1K or 2K, SI(F1) = 512 or 1K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb *and* **abs1 + abs2**



Neo-400 HMBCes spectrum of quinine in D_6-DSMO. **ES** was applied at 3.35 ppm (= the HOD line). ^{1}H signals /correlations located 0.5-0.7 ppm either side of the **ES** suppressed line have reduced intensity.

3.4 HMBCL2 Spectrum

Parameter set: **awhmbcl2 (+ getprosol + pulsecal)**
Pulse program: **hmbcgpl2ndqf**

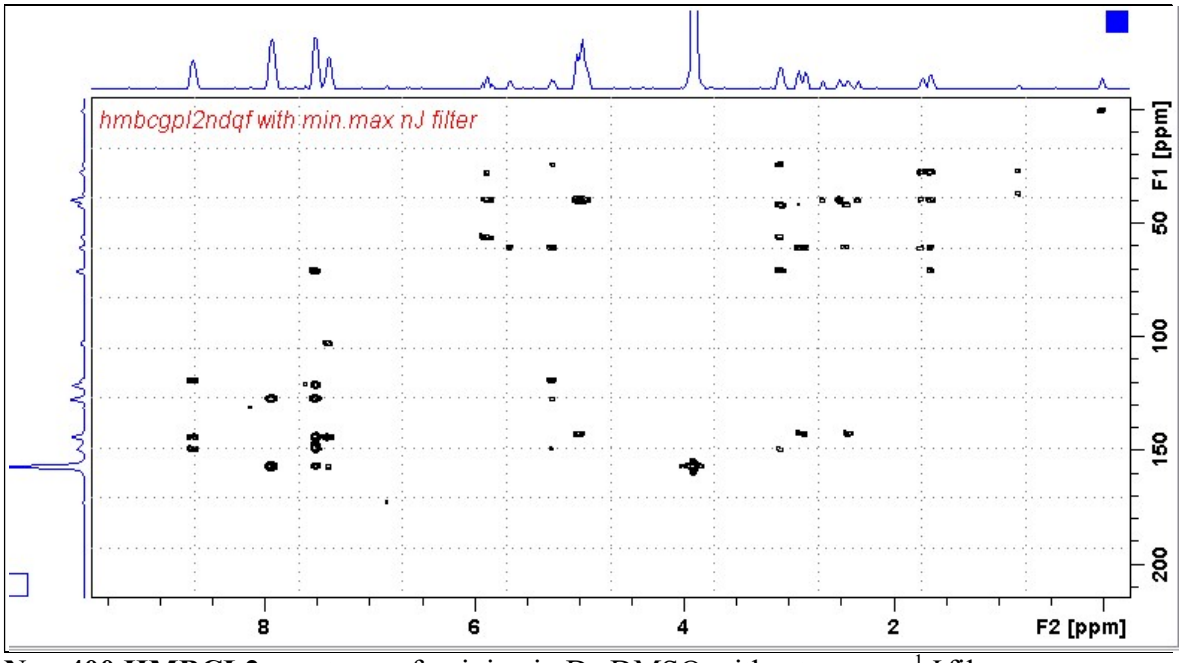
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay =**1.5 sec** or other time of your choice.
CNST6 = min.^{1}J coupling constant = **125** Hz or other value of your choice.
CNST7 = max.^{1}J coupling constant = **165** Hz or other value of your choice.
CNST13 = ^{n}J selection filter = **8 Hz** or other value of your choice

Type **ased** (enter) and review parameters used in the job.
Check gradients and shaped pulses are OK.

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

Process with: **SI(F2) = 1K or 2K, SI(F1) = 512 or 1K points**
WDW(F1) = WDW(F2) = SINE
SSB(F2) = SSB(F1) = 0
xfb *and* **abs1 + abs2**



Neo-400 HMBCL2 spectrum of quinine in D_6-DMSO with a two step ^{1}J filter.

3.5 HMBC-CIGAR Spectrum

Parameter set: **awhmbc-cigar (+ getprosol + pulsecal)**
Pulse program: **hmbcacgplpqf**
Spectrum is acquired with ^{13}C decoupling

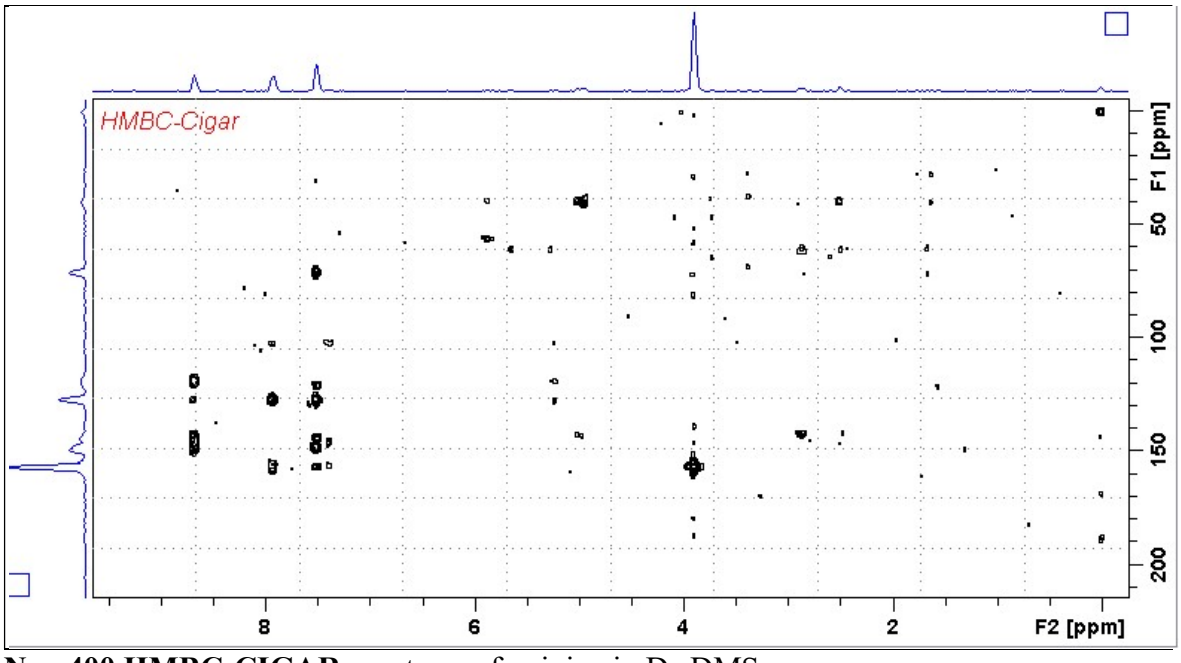
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST6 = 125 Hz, **CNST7** =160 Hz = min/max ^{1}J selection filter range.
CNST14 = 4 Hz**, CNST15** =12 Hz = min/max ^{n}J selection filter range.
CNST16 = 1.0 = *J* scale factor.

Type **ased** (enter) and review parameters used in the job.
Check gradients and shaped pulses are OK.

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

Process with: **SI(F2) = 1K or 2K, SI(F1) = 512 or 1K points**
WDW(F1) = WDW(F2) = SINE
SSB(F2) = SSB(F1) = 0
xfb *and* **abs1 + abs2**



Neo-400 HMBC-CIGAR spectrum of quinine in D_6-DMS

3.6 HMBCCT Spectrum

Parameter set: **awhmbcct (+ getprosol + pulsecal)**
Pulse program: **hmbcctetgpl2nd**

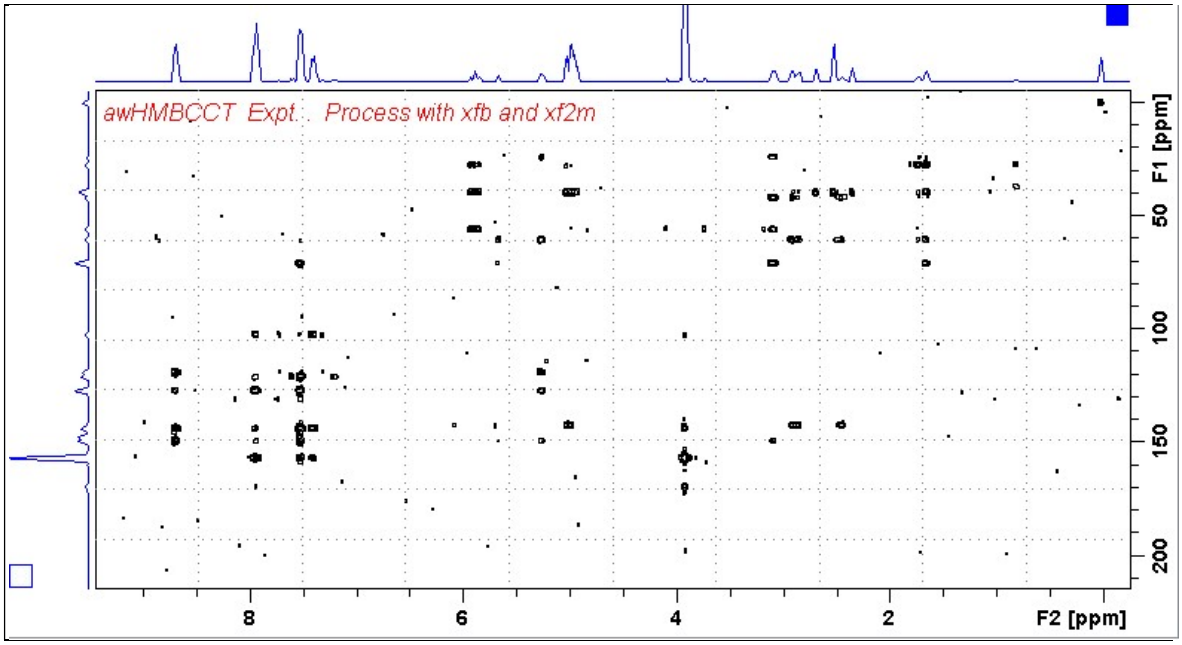
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay =**1.5 sec** or other time of your choice.
CNST6 = **120 Hz**, **CNST7 = 170 Hz** = min/max ^{1}J coupling constants.
CNST13 = ^{n}J selection filter = **8 Hz** or other value of your choice (eg: 6-14 Hz).

Type **ased** (enter) and review parameters used in the job.
Check that gradients and shaped pulses are OK

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

Process with: **SI(F2) = 1K or 2K, SI(F1) = 512 or 1K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb *and* **xf2m (***and* **abs1 + abs2)**



Neo-400 HMBCCT spectrum of quinine in D_6-DSMO centered at 145 ppm.
Correlations at the edges of the ^{13}C spectral window have reduced intensity**.**

3.7 HMBCCTpr Spectrum

Parameter set: **awhmbcctpr (+ getprosol + pulsecal)**
Pulse program: **awhmbcctpr**

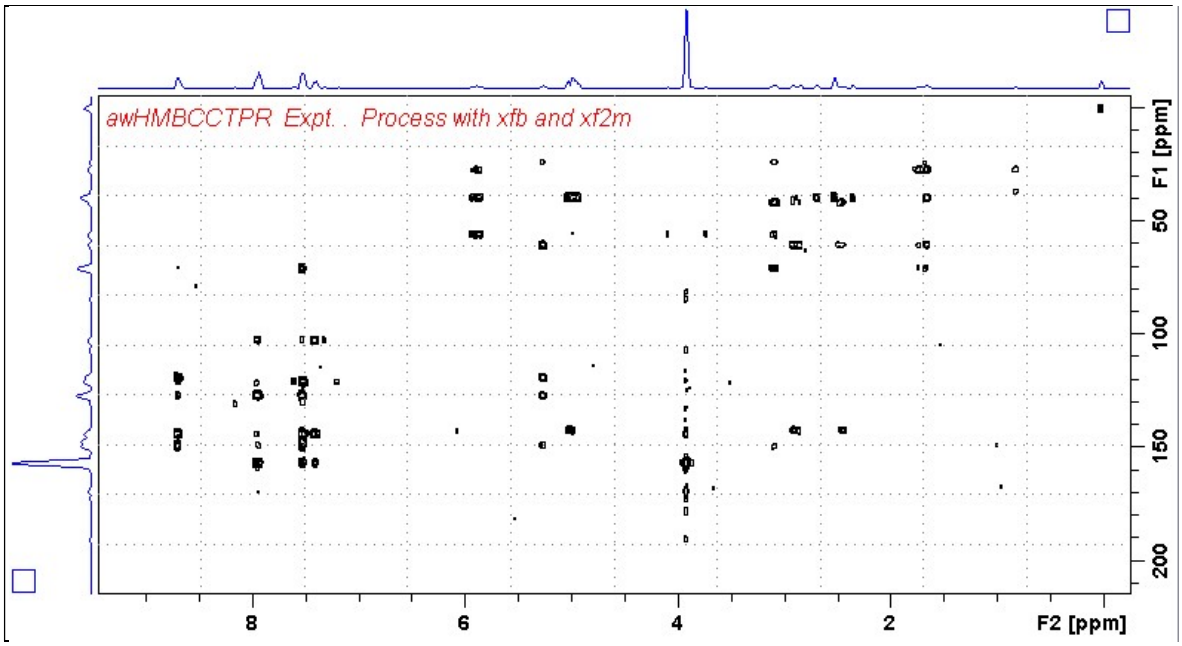
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in Hz (for **PR**)
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time of your choice.
CNST6 = **120 Hz**, **CNST7 = 170 Hz** = min/max ^{1}J coupling constants.
CNST13 = ^{n}J selection filter = **8 Hz** or other value of your choice (eg: 6-14 Hz).
PLW9(db) = **PR power** applied during **D1**. If required the **PR power** can be increased by *subtracting* 6 or 12 db from its prosol Table value.

Type **ased** (enter) and review parameters used in the job.
Check that gradients and shaped pulses are OK.

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

Process with: **SI(F2) = 1K or 2K, SI(F1) = 512 or 1K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb *and* **xf2m (***and* **abs1 + abs2)**



Neo-400 HMBCCTpr spectrum of quinine in D_6-DSMO centered at 40 ppm.
Presaturation was applied at the HOD line frequency (3.35 ppm).

3.8 HMBCCTes Spectrum

Parameter set: **awhmbcctes (+ getprosol + pulsecal)**
Pulse program: **awhmbcctes**

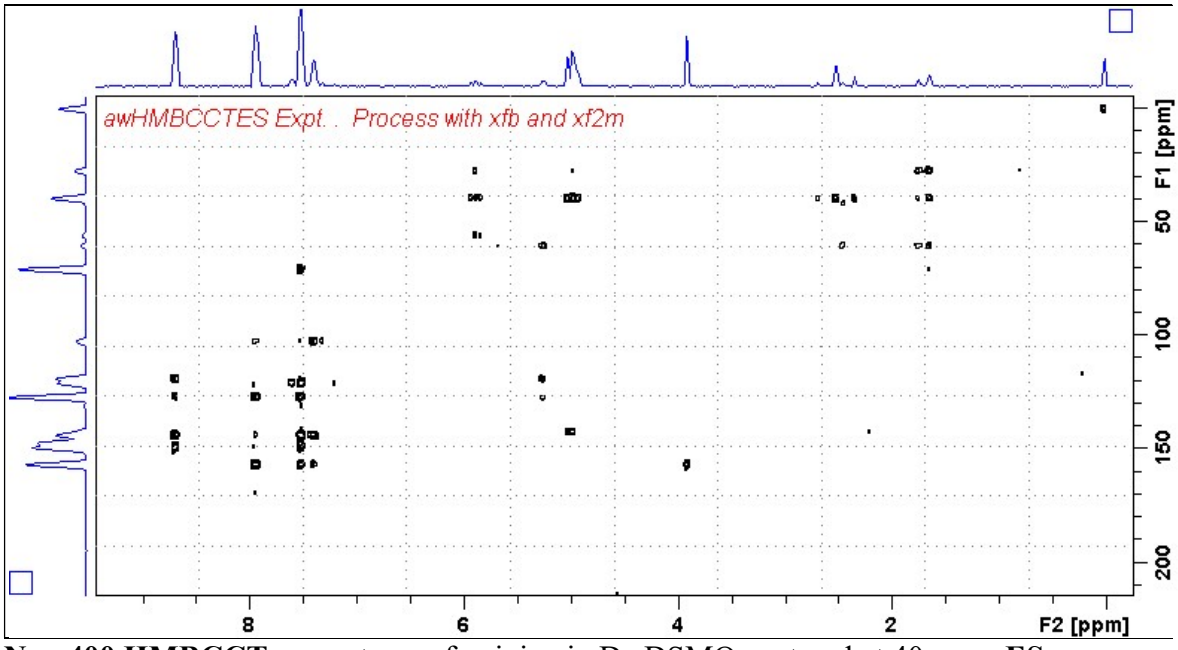
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in Hz (for **ES**)
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay =**1.5 sec** or other time of your choice.
CNST6 = **120 Hz**, **CNST7 = 170 Hz** = min/max ^{1}J coupling constants.
CNST13 = ^{n}J selection filter = **8 Hz** or other value of your choice (eg: 6-14 Hz).

Type **ased** (enter) and review parameters used in the job.
Check that gradients and shaped pulses are OK, including a prosol Table defined **2000 usec p40:sp10 Sinc1.1000 ES** pulse.

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

Process with: **SI(F2) = 1K or 2K, SI(F1) = 1K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb *and* **xf2m (***and* **abs1 + abs2)**



Neo-400 HMBCCTes spectrum of quinine in D_6-DSMO centered at 40 ppm. **ES** was applied at 3.35 ppm (= the HOD line). ^{1}H signals /correlations located 0.5-0.7 ppm either side of the **ES** suppressed line have reduced intensity

3.9 HMBCCTespr Spectrum

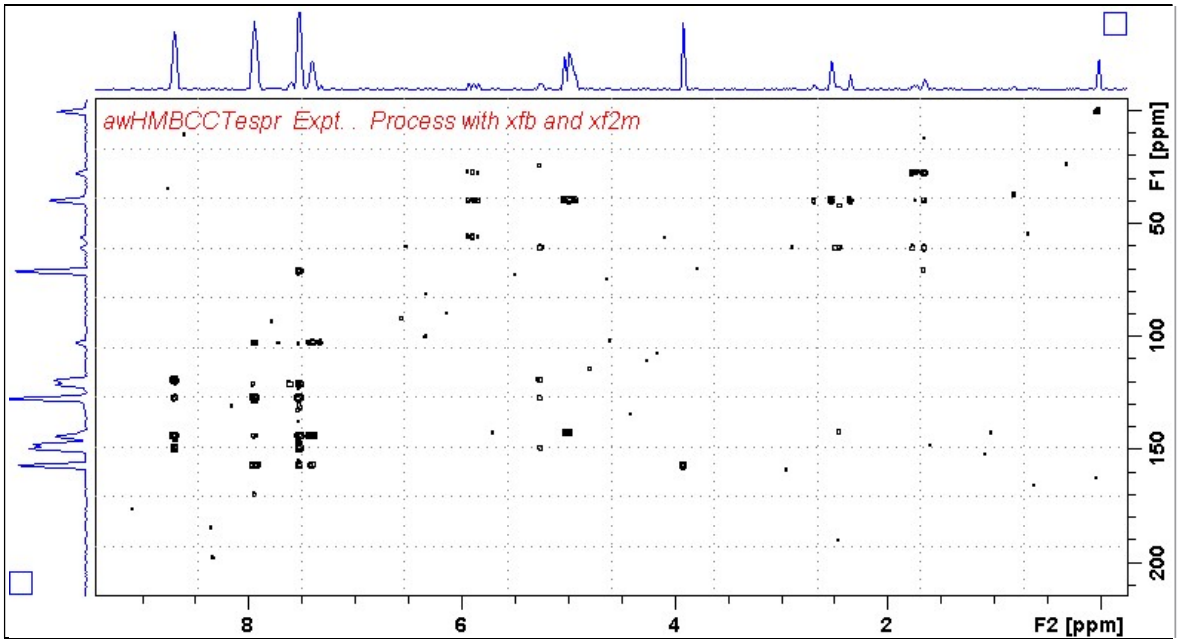
Parameter set: **awhmbcctespr (+ getprosol + pulsecal)**
Pulse program: **awhmbcctespr**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in Hz (for **ES** and **PR**).
ES can optionally be offset from **PR** as described in the Appendix.
TD(F2) = 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay =**2 sec** or other time of your choice.
CNST6 = **120 Hz**, **CNST7 = 170 Hz** = min/max ^{1}J coupling constants.
CNST13 = ^{n}J selection filter = **8 Hz** or other value of your choice (eg: 6-14 Hz).
PLW9(db) = **PR power** applied during **D1**. If required the **PR power** can be increased by *subtracting* 6 or 12 db from its prosol Table value.

Type **ased** (enter) and review parameters used in the job. Check that gradients and shaped pulses are OK, including a prosol Table defined **2000 usec p40:sp10 Sinc1.1000 ES** pulse.
.
Set **receiver gain** using **RGA** *(Important!).*

Process with: **SI(F2) = 1K or 2K, SI(F1) = 512 or 1K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb *and* **xf2m (***and* **abs1 + abs2)**



Neo-400 HMBCCTespr spectrum of quinine in D_6-DSMO. Combined **ES** + **PR** was applied at 3.35 ppm (= the HOD line). ^{1}H signals /correlations located 0.5-0.7 ppm either side of the **ES** suppressed line have reduced intensity.

3.10 H2BC Spectrum

Parameter set: **awh2bc (+ getprosol + pulsecal)**
Pulse program: **h2bcetgpl3**

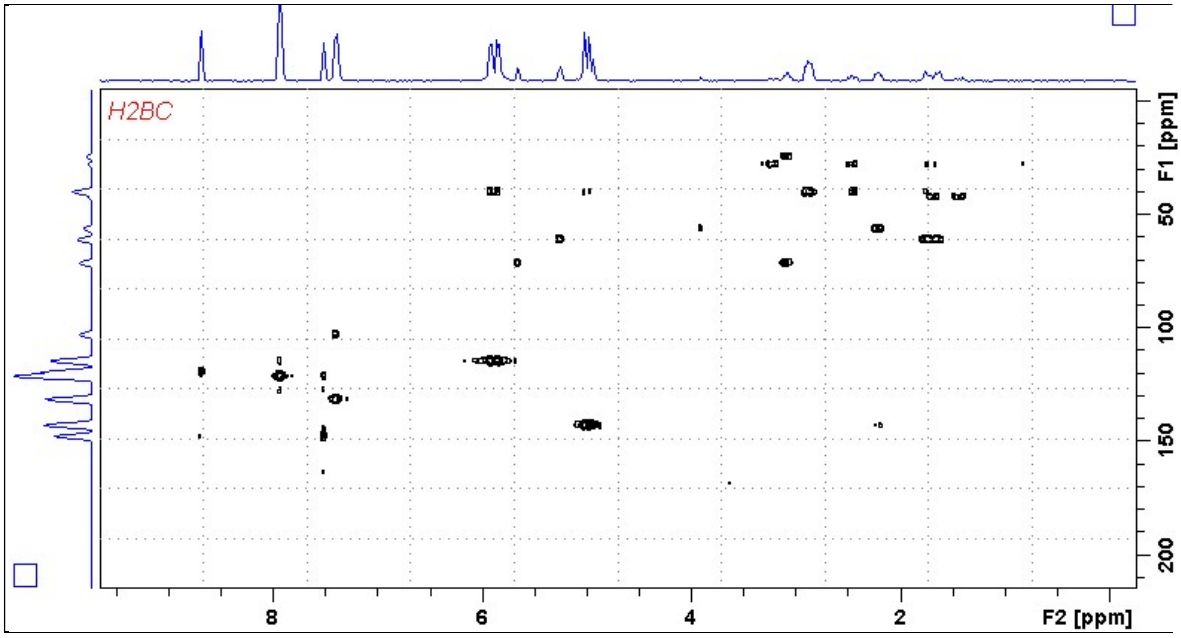
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay =**1.5 sec** or other time of your choice.
CNST6 = 125 Hz, **CNST7** =165 Hz = min/max ^{1}J selection filter range.

Type **ased** (enter) and review parameters used in the job.
Check gradients and shaped pulses are OK.

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

Process with: **SI(F2) = 1K or 2K, SI(F1) = 512 or 1K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb *and* **xf2m (***and* **abs1 + abs2)**



Neo-400 **H2BC** spectrum of quinine in D_6-DMSO.

4.0 How to offset ES from O1 in an ESPR experiment

By default **ES** and **PR** are applied at **O1** (Hz) frequency in aw coded **HSQC** and **HMBCCT ESPR** experiments. Combined (double) **ES + PR** can be used to suppress a large HOD or solvent peak.

The **ES** pulse in **hmbc135espr** or **hmbcctespr** experiments is defined as an F1 (^{1}H) channel **2000 usec Sinc1.1000 p40:sp10** pulse, rather than a **p12:sp1** pulse as used in **shsqc135espr** or **shmbcctespt** experiments.

The frequency (in Hz) at which **ES** is applied in **hsqc135** and **hmbcct** can optionally be offset from **O1** (= the frequency PR is applied) so you can suppress two solvent lines by entering an **SPOFFS10(Hz)** offset value in its **ased** visible cell. hsqc135

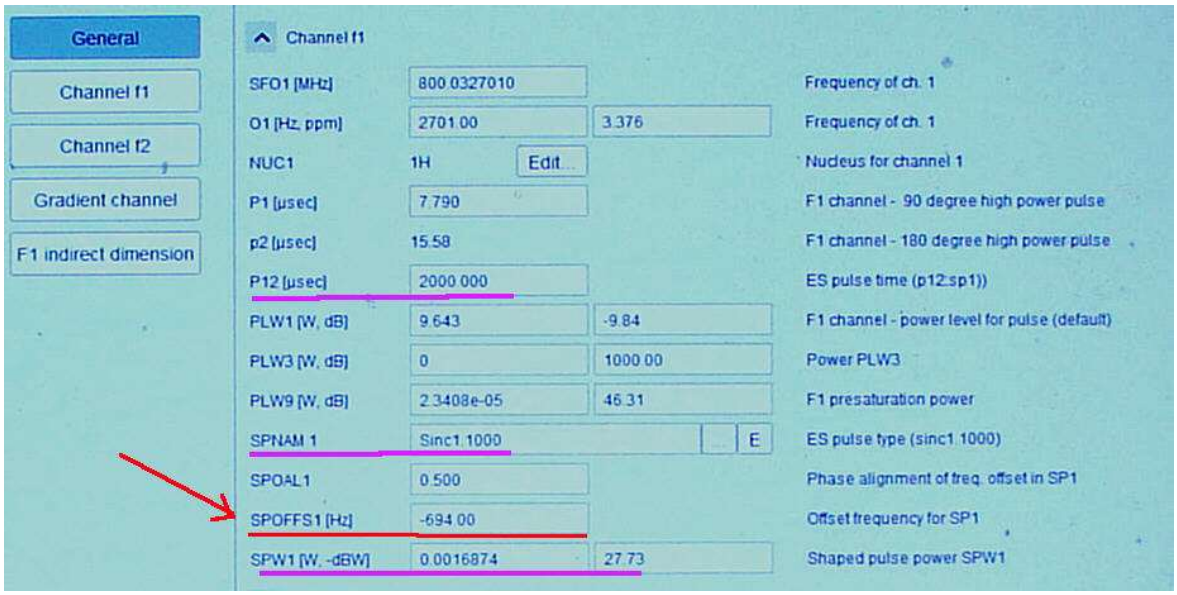
For example if, the **HOD line** occurs at **2701 Hz** and the **DMSO line** occurs at **2007 Hz**, the offset of the **DMSO line** (*to be ES suppressed*) relative to that of the **HOD line** (*PR suppressed at O1*) is calculated as:

SPOFFS1(Hz) = **ES** offset signal (Hz) - **O1** frequency (Hz)

ie **2007 Hz (DMSO) - 2701 Hz (HOD) = - 694 Hz**

The offset is negative in this case since the **DMSO line** occurs at **- 694 Hz *less*** than that of the frequency at which **PR** is applied to the **HOD line** at **O1 Hz**.

The setup of an **ES** pulse is illustrated below for a **p12:sp1** pulse as used in **shsqc135** and **shmbcct** experiments. The purple and red under lined cells will be replaced by **p40:sp10** cells/values when **ES** is used in **hsqc135** and **hmbcct** experiments.



ased view of **p12:sp1** ES pulse parameters. The red highlighted (arrowed) **SPOFFS1(Hz)** line will be replaced by a **SPOFFS10(Hz)** line when **a p40:sp10 ES** pulse is used in **HSQC135es** and **HMBCCTes** experiments.