KJM 5250 and KJM 9250

HSQC, HMBC and H2BC Experiments NMR spectra with and without solvent peak pre saturation (PR CW) or Excitation Sculpting (ES) suppression on the AVneo800 spectrometer.

Version 5.1

Topspin 4.3

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AWneo800 HSQC, HMBC and H2BC Experiments

1.0 Introduction

aw coded TS4 **Neo-800 HSQC** and **HMB**C parameter sets are set up with 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-800 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.

2.0 Experiments and Parameter Sets

The following **HSQC, HMBC and H2BC experiments** have been set up on the **Neo-800** spectrometer.

2.1	**hsqcetgp**	not multiplicity edited, DEPT45 like
2.2	**hsqcedetgp**	multiplicity edited, DEPT135 like
2.3	**hsqc45**	not multiplicity edited, DEPT45 like
2.4	**hsqc135**	multiplicity edited, DEPT135 like
2.5	**hsqc135pr**	with CW presaturation
2.6	**hsqc135es**	with ES peak suppression
2.7	**hsqc135espr**	with ES and PR peak suppression
2.8	**hsqc-tocsy**	DEPT45 like with TOCSY correlations

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 HSQCETGP Spectrum

Parameter set: **awhsqcetgp (+ getprosol + pulsecal)**

Pulse programme: **hsqcetgp**

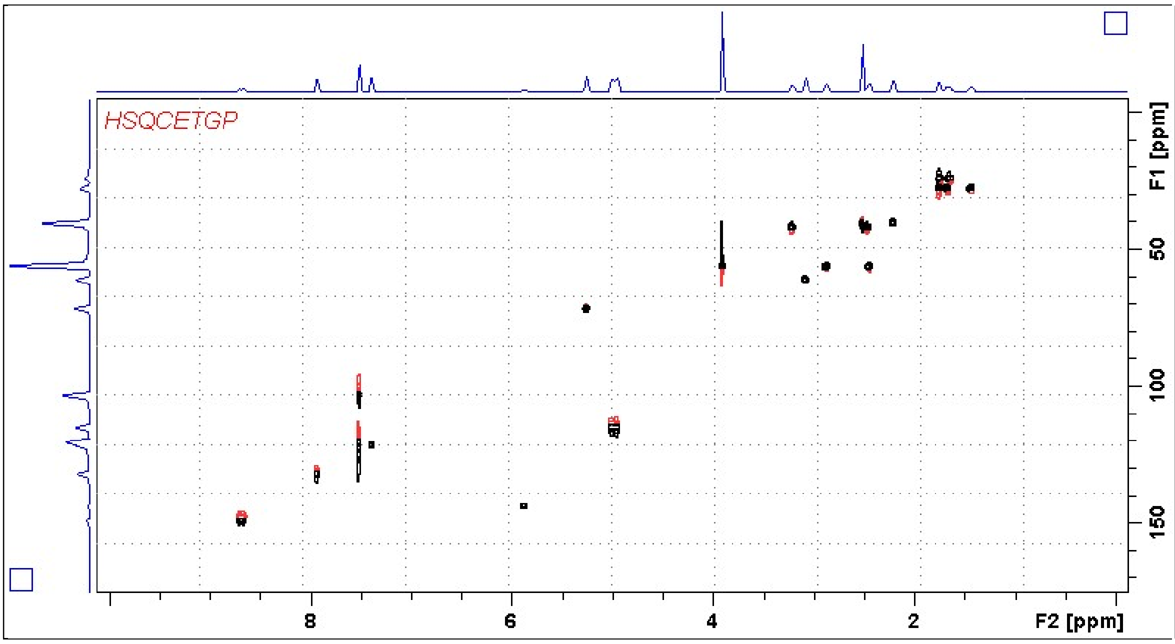
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 2K, **TD(F1)** = 128-256 (your choice).

NS = Multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST2 = ^1J 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) = 2K, SI(F1) = 1K** or **2K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, manual phasing and **abs1** + **abs2**



Neo-800 HSQCETGP spectrum of quinine in D_6-DMSO.

2.2 HSQCEDETGP Spectrum

Parameter set: **awhsqcedetgp-135 (+ getprosol + pulsecal)**
Pulse programme: **awhsqcedetgp-135**
d21 is automatically calculated from **cnst2**

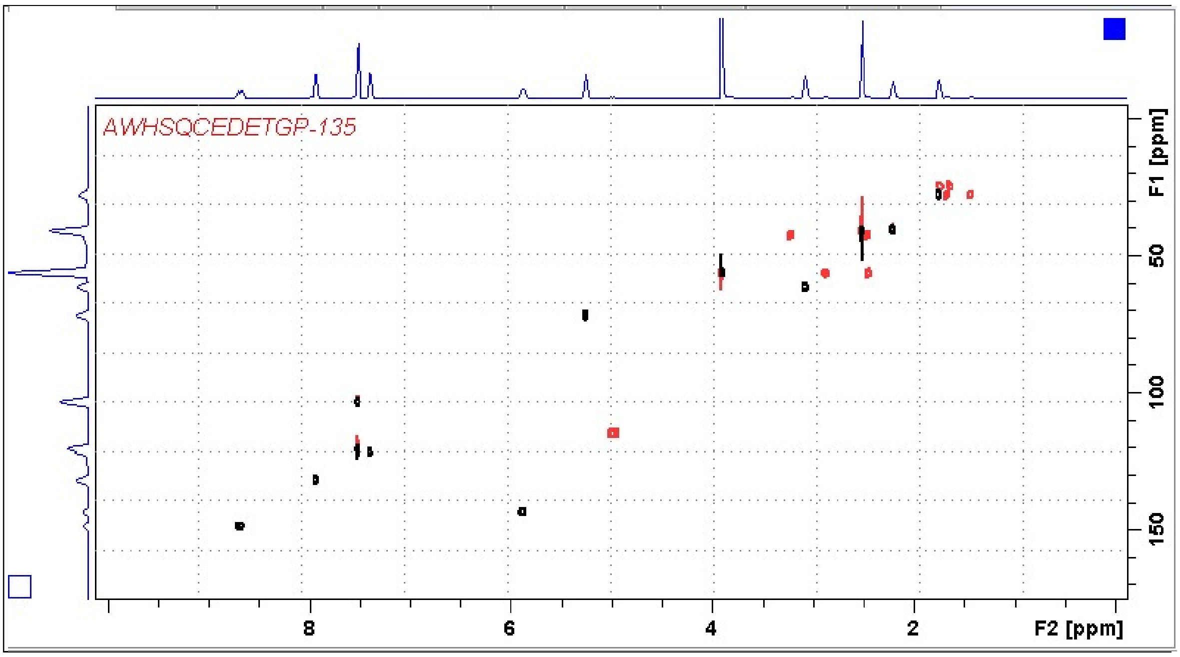
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)**in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 2K, **TD(F1)** = 128-256 (your choice).

NS = Multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST2 = ^1J 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) = 2K, SI(F1) = 1K or 2K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, manual phasing and **abs1** + **abs2**



Neo-800 HSQCEDETGP spectrum (DEPT135-like) of quinine in D_6-DMSO plotted with CH and CH_3 positive (black) and CH_2 negative (red).

2.3 HSQC45 Spectrum

Parameter set: **awhsq45 (+ getprosol + pulsecal)**
or **awhsqcetgpsisp2.2-45 (+ getprosol + pulsecal)**

Pulse programme: **awhsqcetgpsisp2.2-45**
d24 is automatically calculated from **cnst2**

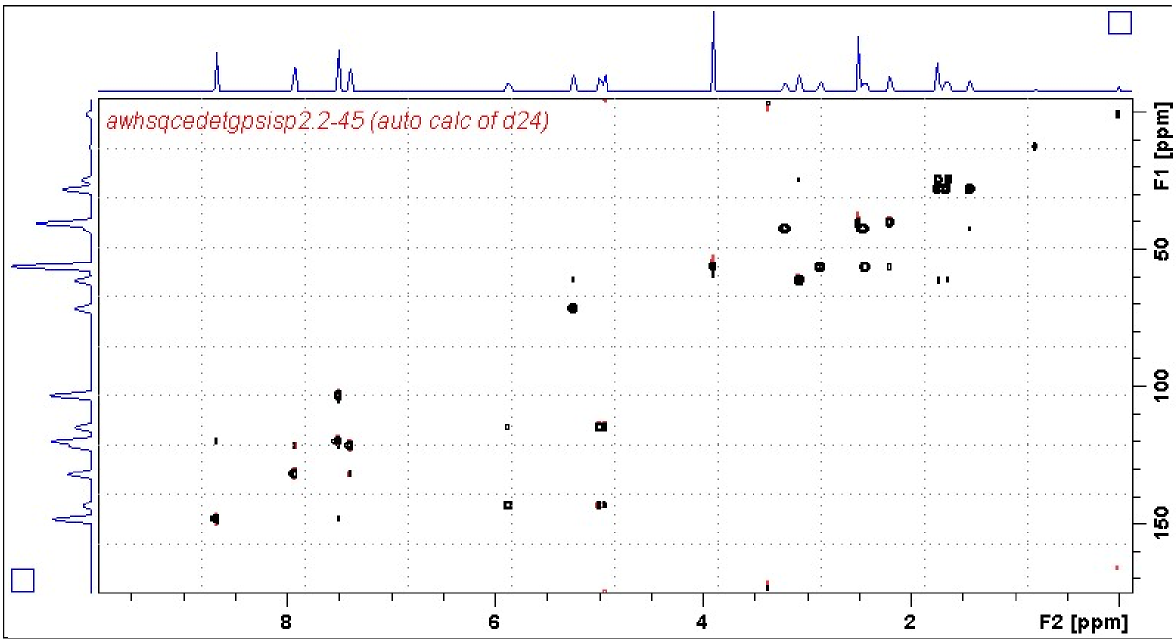
Type **eda** (enter) and enter **SW (^{1}H)** and **SW(^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

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



Neo-800 HSQC45 spectrum of quinine in D_6-DMSO.

2.4 HSQC135 Spectrum

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

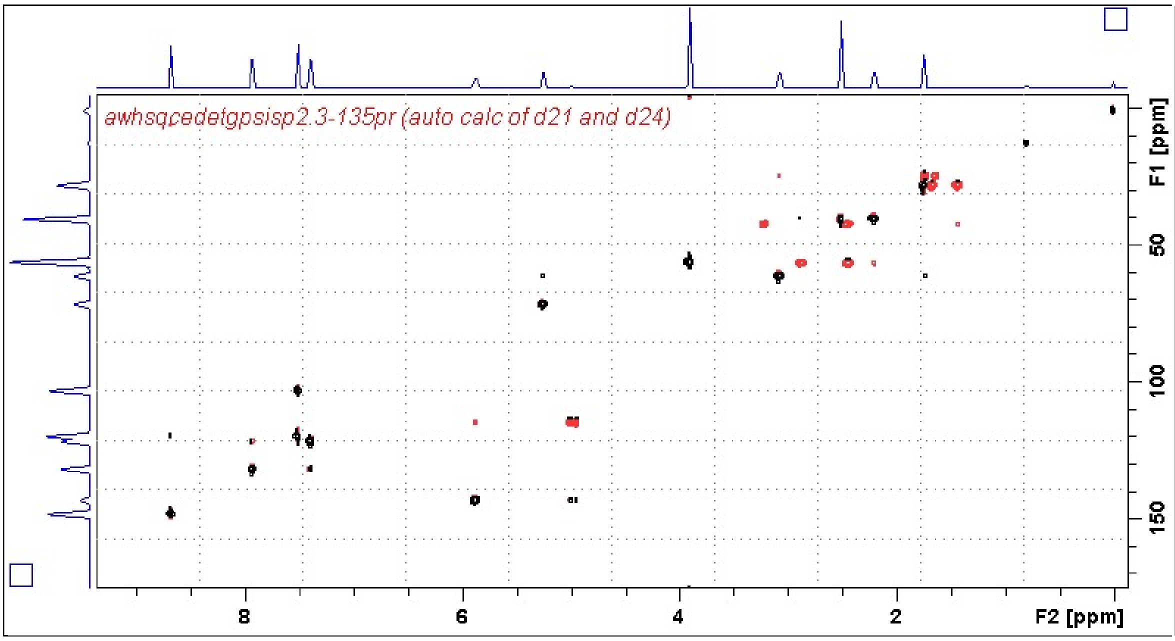
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time of your choice.
CNST2 = ^1J 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) = 2K, SI(F1) = 1K** or **2K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, manual phasing and **abs1** + **abs2**



Neo-800 HSQC135 spectrum of quinine in D_6-DMSO plotted with positive CH andCH_3 correlations (black) and negative CH_2 correlations (red).

2.5 HSQC135pr Spectrum

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

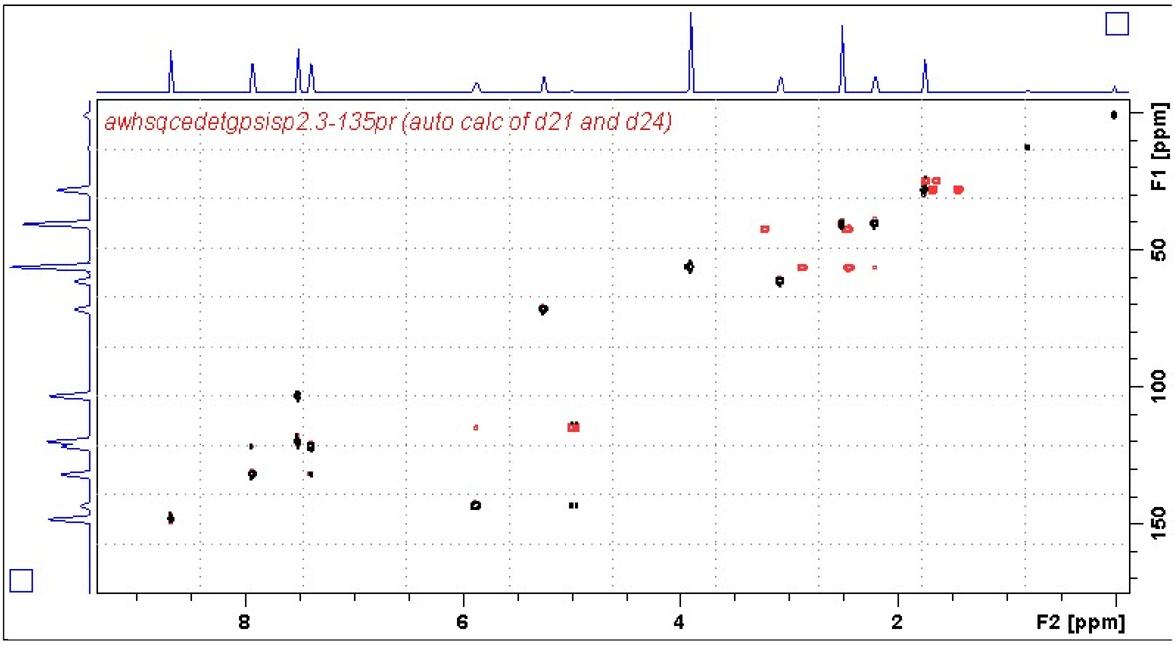
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **PR**).
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time of your choice.
CNST2 = ^1J 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** or **2K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, manual phasing and **abs1** + **abs2**



Neo-800 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).

2.6 HSQC135es Spectrum

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

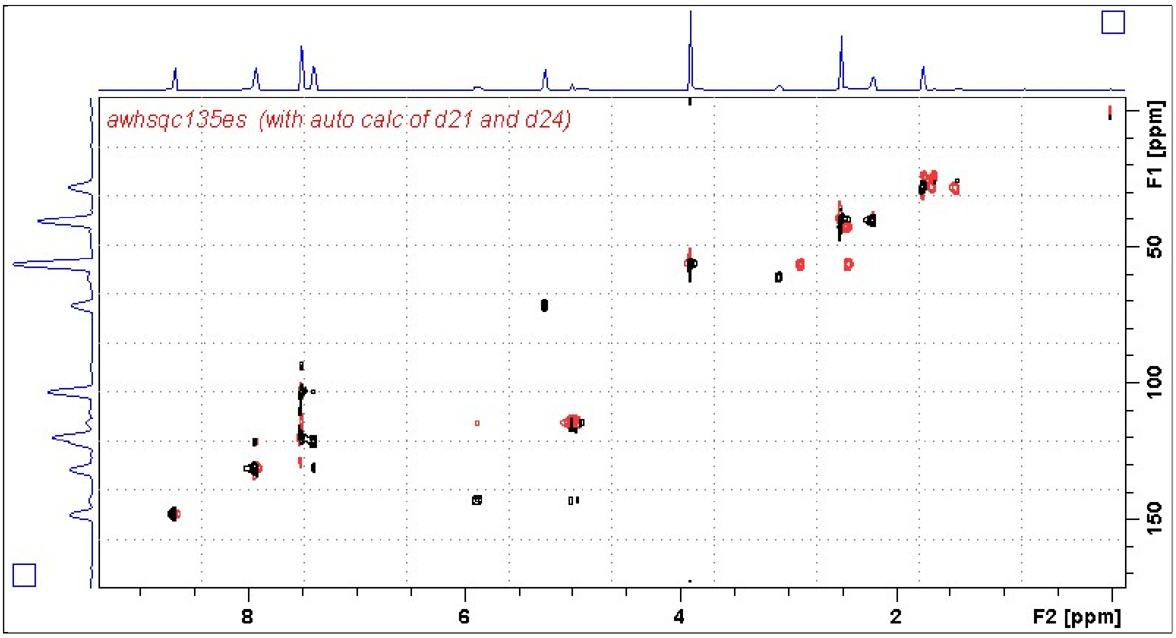
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **ES**)
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST2 = ^1J 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) = 2K, SI(F1) = 1K** or **2K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, manual phasing and **abs1** + **abs2**



Neo-800 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 0.2-0.3 ppm either side of the **ES** suppressed line have reduced intensity.

2.7 HSQC135espr Spectrum

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

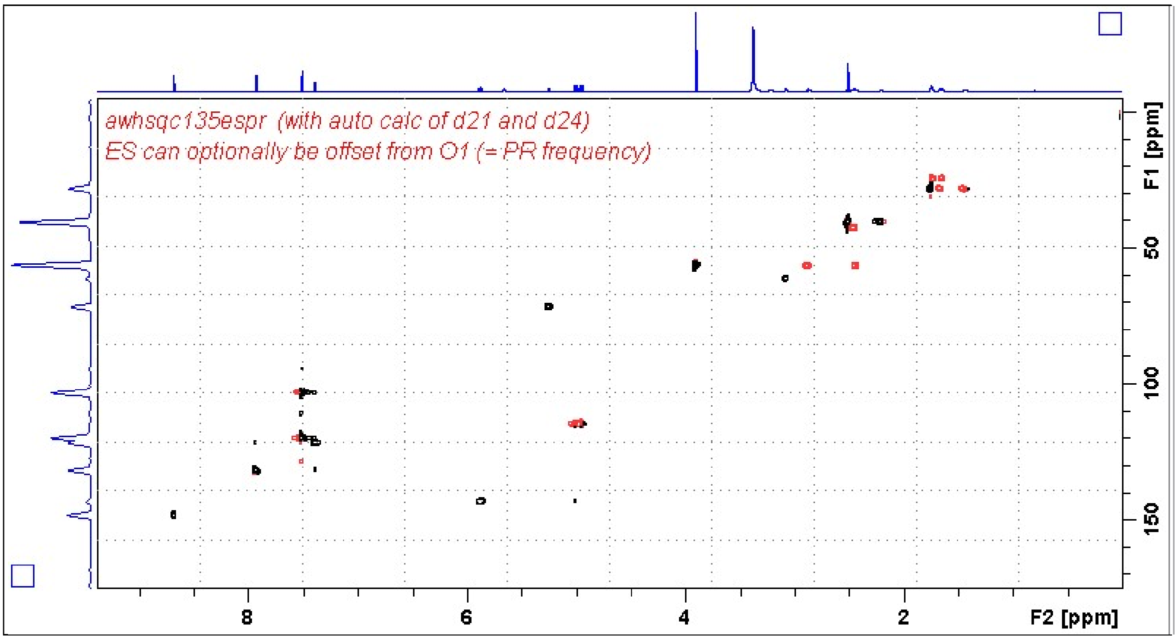
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **ES** + **PR**).
ES can optionally be offset from **PR** as described in the Appendix.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 2K, **TD(F1)** = 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 = ^1J 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) = 2K, SI(F1) = 1K** or **2K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, manual phasing and **abs1** + **abs2**



Neo-800 HSQC135espr spectrum of quinine in D_6-DSMO. **ES** + PR was applied at 3.35 ppm (= the HOD line). ^{1}H signals /correlations located 0.2-0.3 ppm either side of the **ES** suppressed line have reduced intensity.

2.8 HSQC-TOCSY Spectrum

Parameter set: **awhsqc-tocsy (+ getprosol + pulsecal)**
Pulse programme: **awhsqc-tocsy**

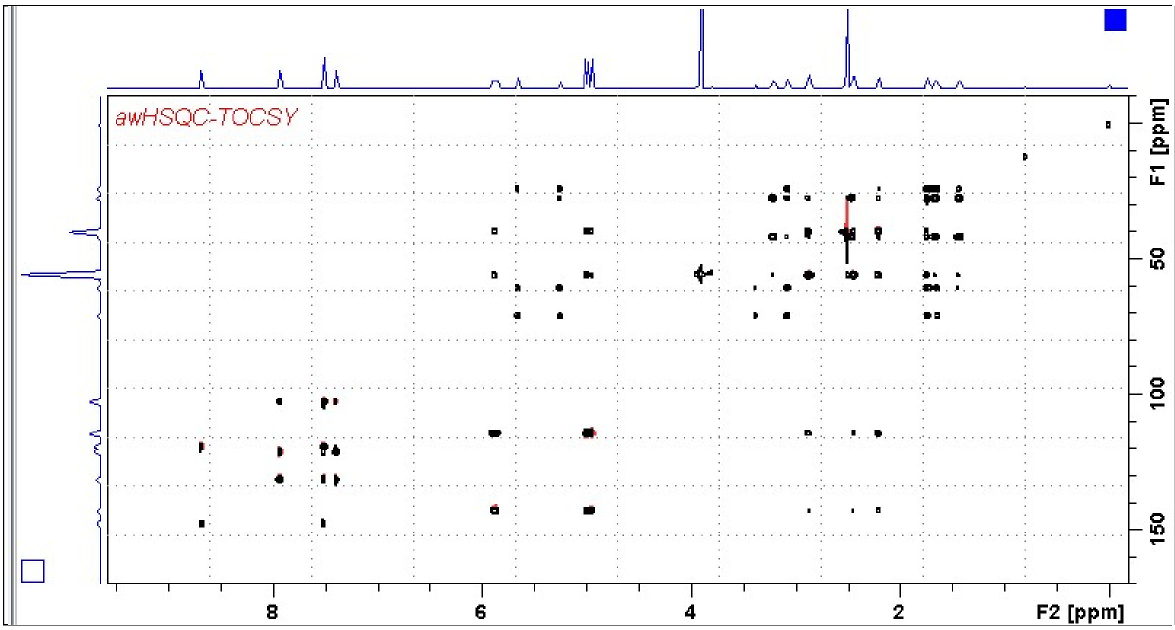
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
D9 = correlation time = **80 msec** or other value of your choice (6-240 msec).
CNST2 = ^1J 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) = 2K, SI(F1) = 1K** or **2K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, manual phasing *and* **abs1 + abs2**



Neo-800 HSQC-TOCSY spectrum of quinine in D_6-DMSO. HSQC and correlated TOCSY peaks are positively phased.

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3.1 HMBC Spectrum

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

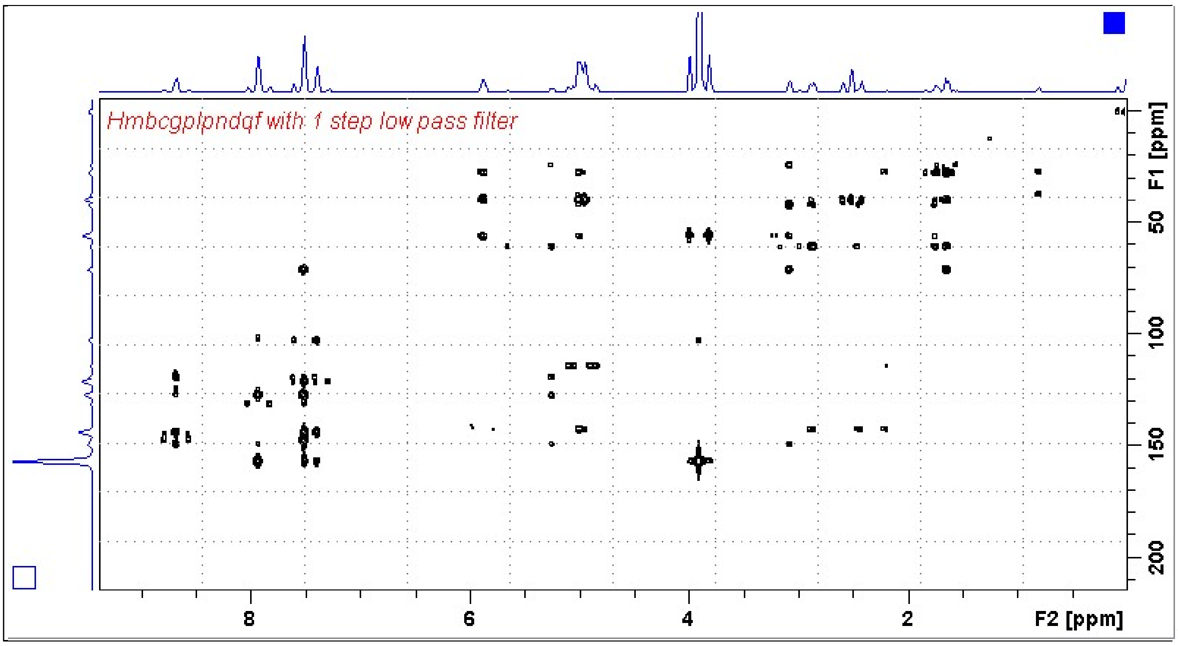
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

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 = ^1J coupling constant = **145 Hz** or other value of your choice.
CNST13 = ^nJ 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) = 2K, SI(F1) = 1K** or **2K points**
WDW(F1) = WDW(F2) = SINE
SSB(F2) = SSB(F1) = 0
xfb *and* **abs1 + abs2**



Neo-800 HMBC spectrum of quinine in D_6-DMSO.

3.2 HMBCpr Spectrum

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

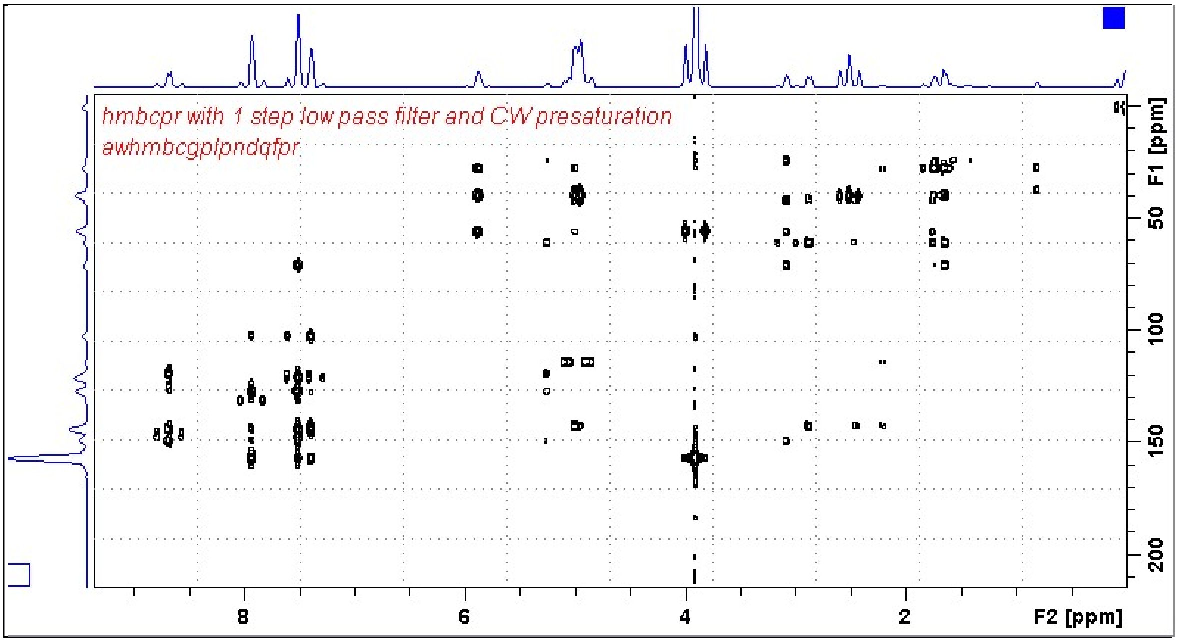
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in Hz (for **PR**)
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K,**TD(F1)** = 128-256 (your choice).

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



Neo-800 HMBCpr spectrum of quinine in D_6-DMSO with CW presaturation of the HOD signal at 3.37 ppm,

3.3 HMBCes Spectrum

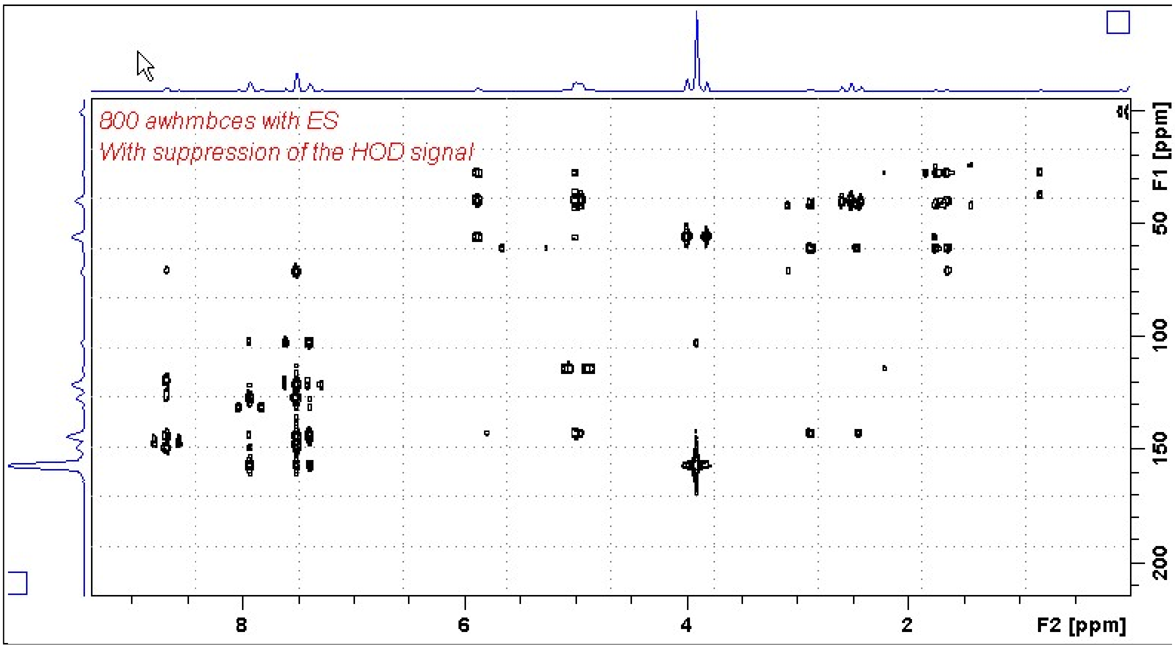
Parameter set: **awhmbces (+ getprosol + pulsecal)**
Pulse programme: **awhmbces**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{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 = ^nJ 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) = 2K, SI(F1) = 1K or 2K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb *and* **abs1 + abs2**



Neo-800 HMBCes spectrum of quinine in D_6-DSMO. **ES** was applied at 3.35 ppm (= the HOD line). ^{1}H signals /correlations located 0.2- 0.3 ppm either side of the **ES** suppressed line have reduced intensity.

3.4 HMBCL2 Spectrum

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

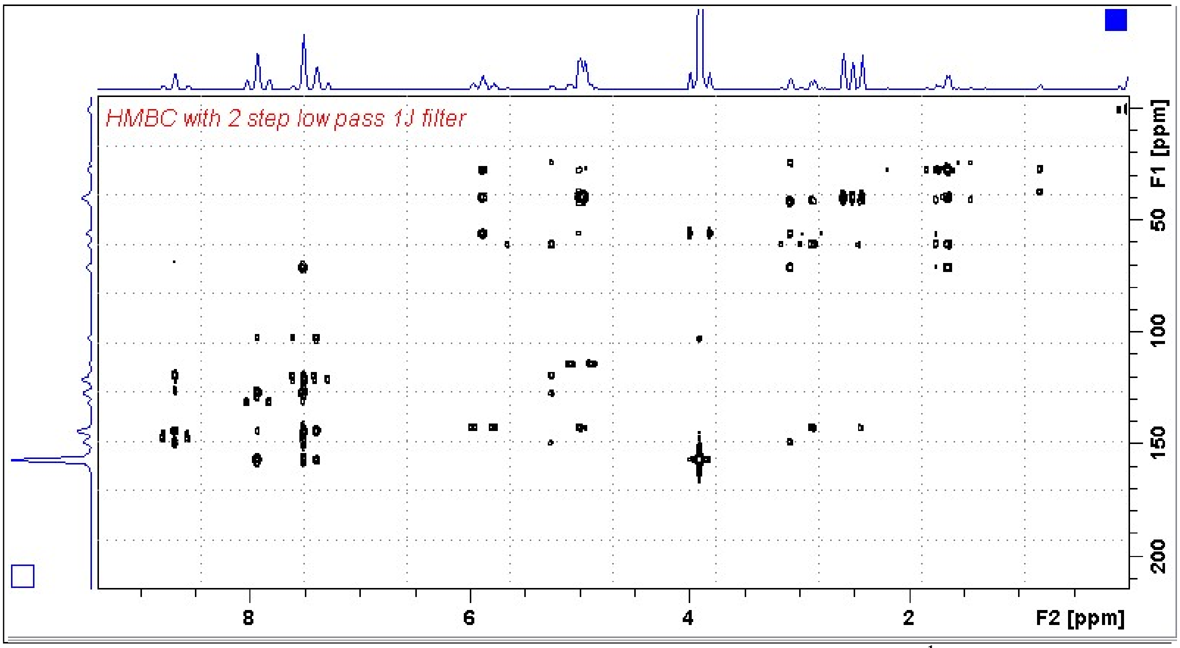
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

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.^1J coupling constant = **125** Hz or other value of your choice.
CNST7 = max.^1J coupling constant = **165** Hz or other value of your choice.
CNST13 = ^nJ 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) = 2K, SI(F1) = 1K** or **2K points**
WDW(F1) = WDW(F2) = SINE
SSB(F2) = SSB(F1) = 0
xfb *and* **abs1 + abs2**



Neo-800 HMBCL2 spectrum of quinine in D_6-DMSO with a two stage ^1J filter.

3.5 HMBC-CIGAR Spectrum

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

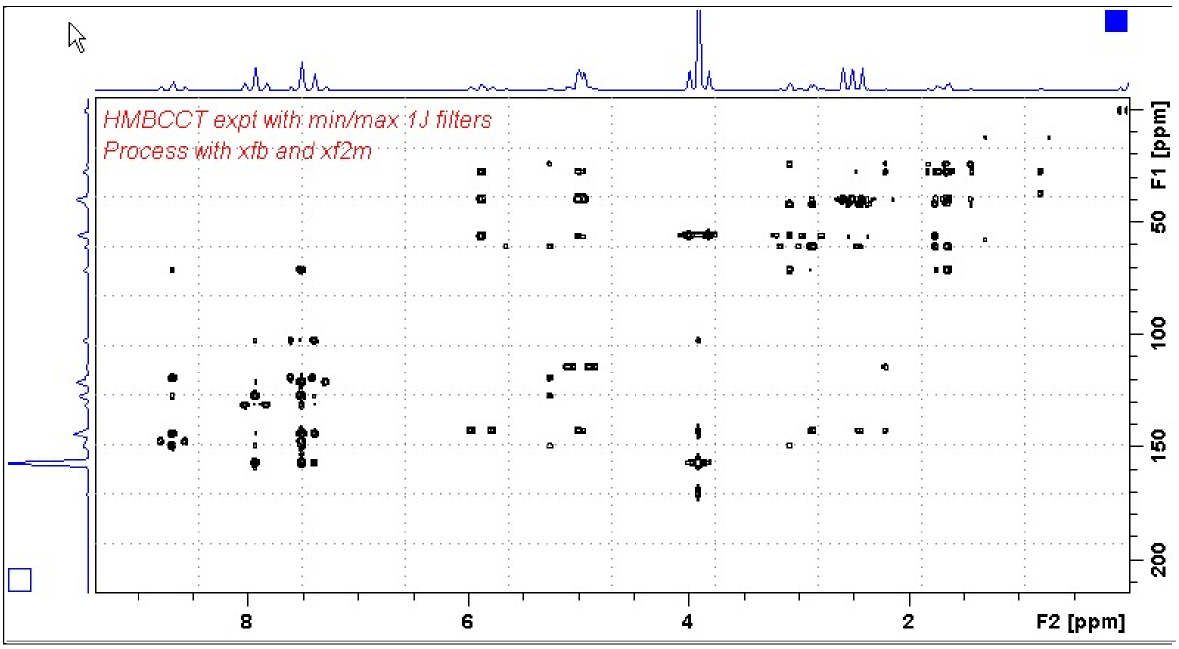
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

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 ^1J selection filter range.
CNST14 = 4 Hz**, CNST15** =12 Hz = min/max ^nJ 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) = 2K, SI(F1) = 1K** or **2K points**
WDW(F1) = WDW(F2) = SINE
SSB(F2) = SSB(F1) = 0
xfb *and* **abs1 + abs2**



Neo-800 HMBC-CIGAR spectrum of quinine in D_6-DMSO.

3.6 HMBCCT Spectrum

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

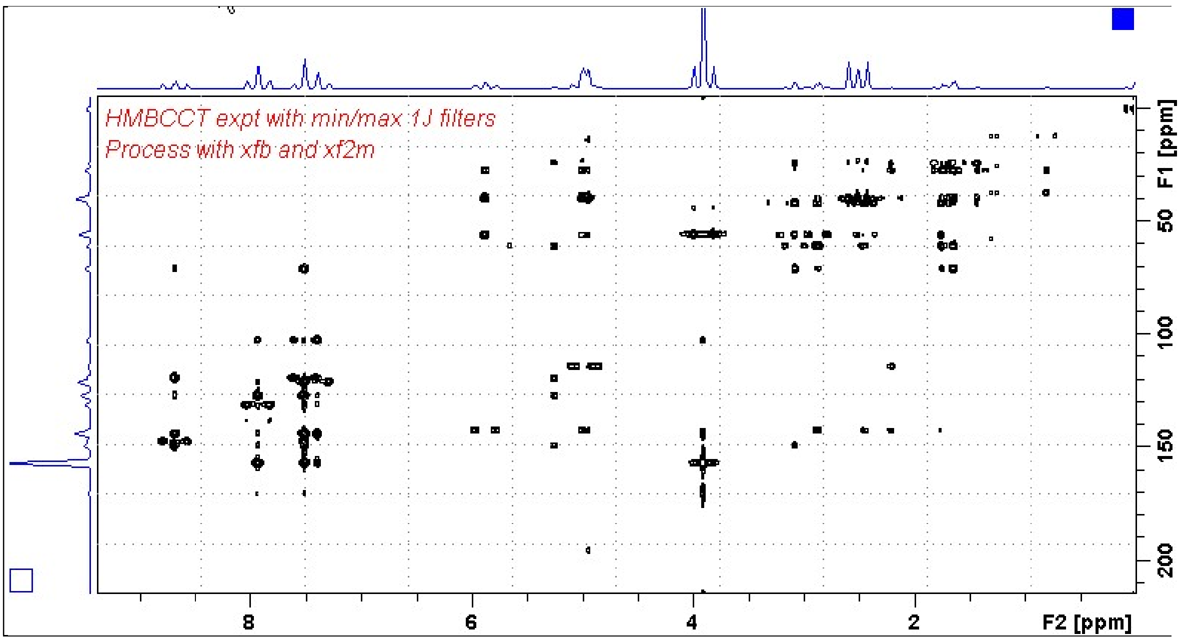
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

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



Neo-800 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 programme: **awhmbcctpr**

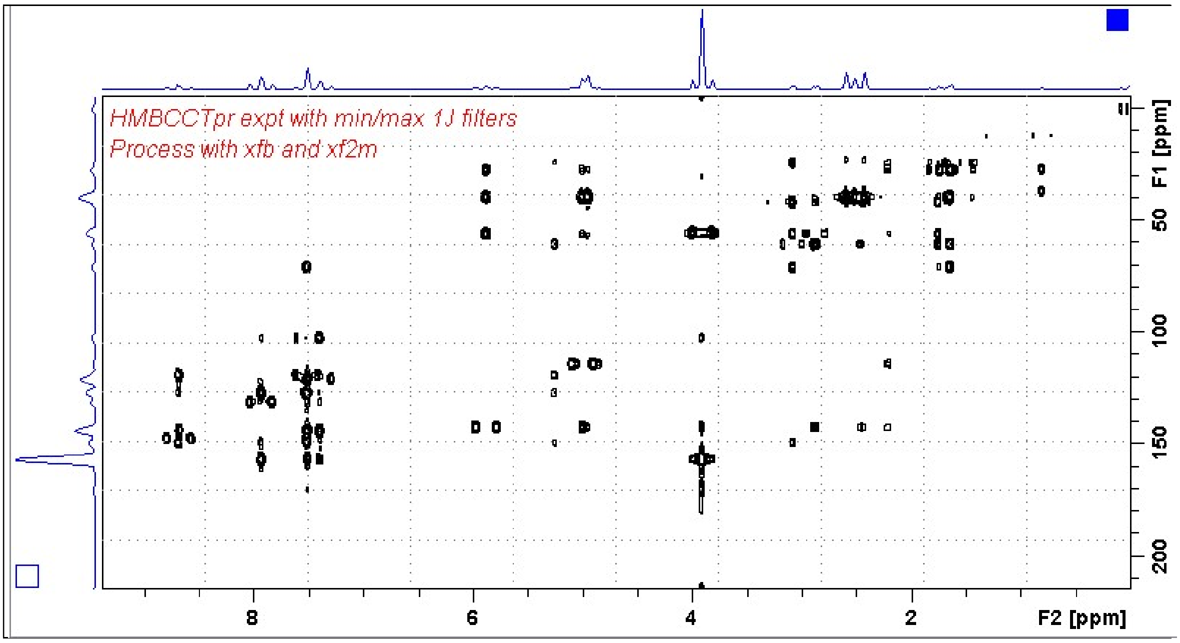
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1** = **^{1}H** spectral window midpoint in Hz (for **PR**)
Enter **O2P** = **^{13}C** spectral window midpoint in ppm.
TD(F2) = 2K, **TD(F1)** = 128-256 (your choice).

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 ^1J coupling constants.
CNST13 = ^nJ 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) = 2K, SI(F1) = 1K or 2K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb *and* **xf2m** (*and* **abs1 + abs2)**



Neo-800 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 programme: **awhmbcctes**

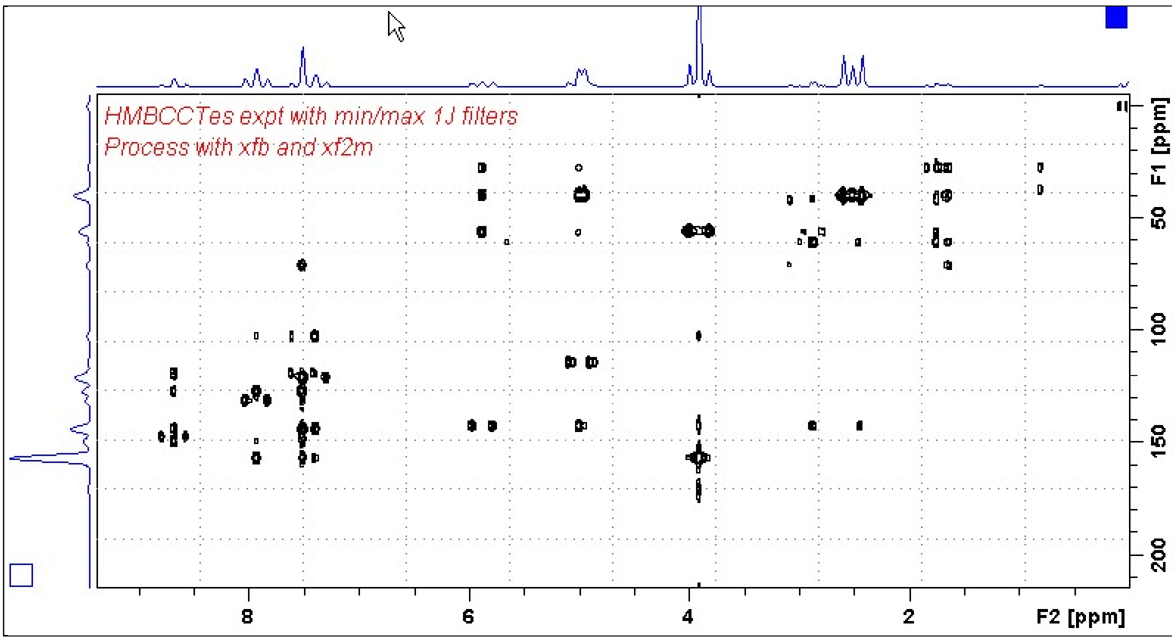
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in Hz (for **ES**)
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 2K, **TD(F1)** = 128-256 (your choice).

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 ^1J coupling constants.
CNST13 = ^nJ 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) = 2K, SI(F1) = 1K or 2K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb *and* **xf2m (***and* **abs1 + abs2)**



Neo-800 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 0.2- 0.3 ppm either side of the **ES** suppressed line have reduced intensity

3.9 HMBCCTespr Spectrum

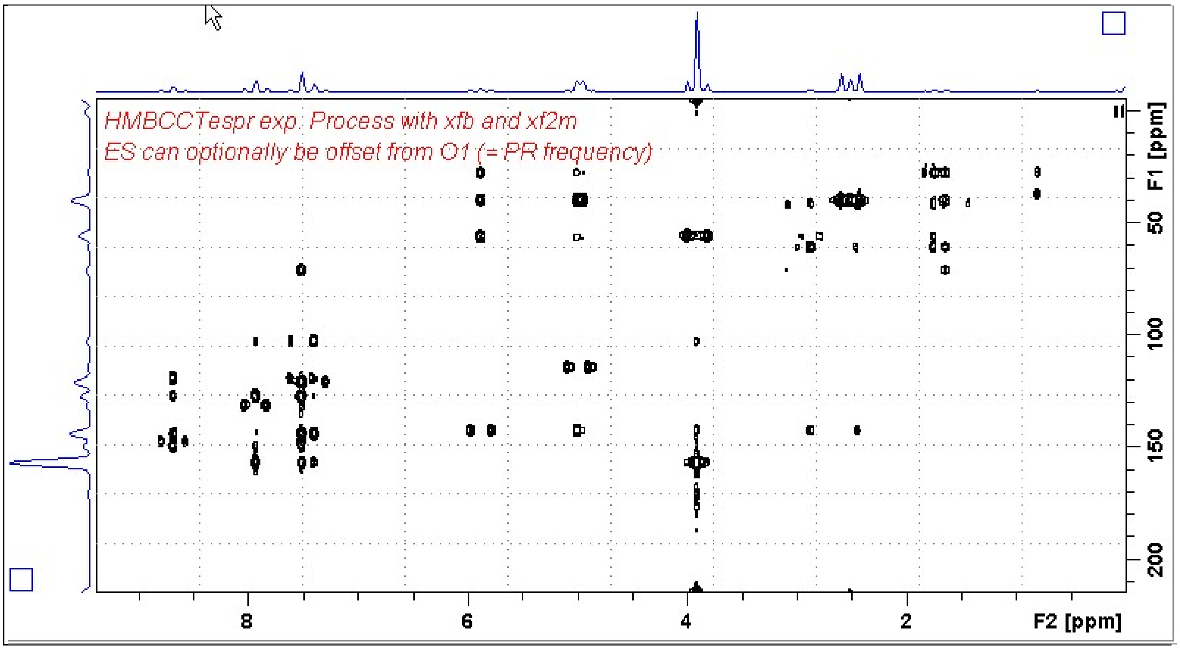
Parameter set: **awhmbcctespr (+ getprosol + pulsecal)**
Pulse programme: **awhmbcctespr**

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.
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 ^1J coupling constants.
CNST13 = ^nJ 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) = 2K, SI(F1) = 1K or 2K points**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb *and* **xf2m** (*and* **abs1 + abs2)**



Neo-800 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 0.2-0.3 ppm either side of the **ES** suppressed line have reduced intensity.

3.10 H2BC Spectrum

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

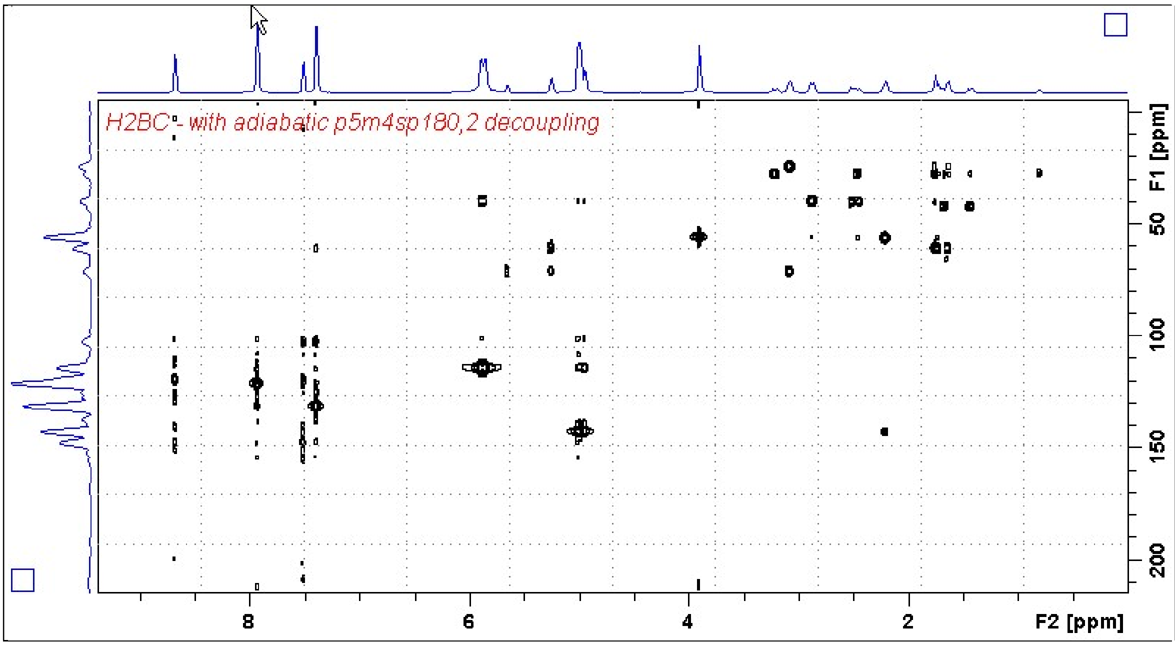
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

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



Neo-800 **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

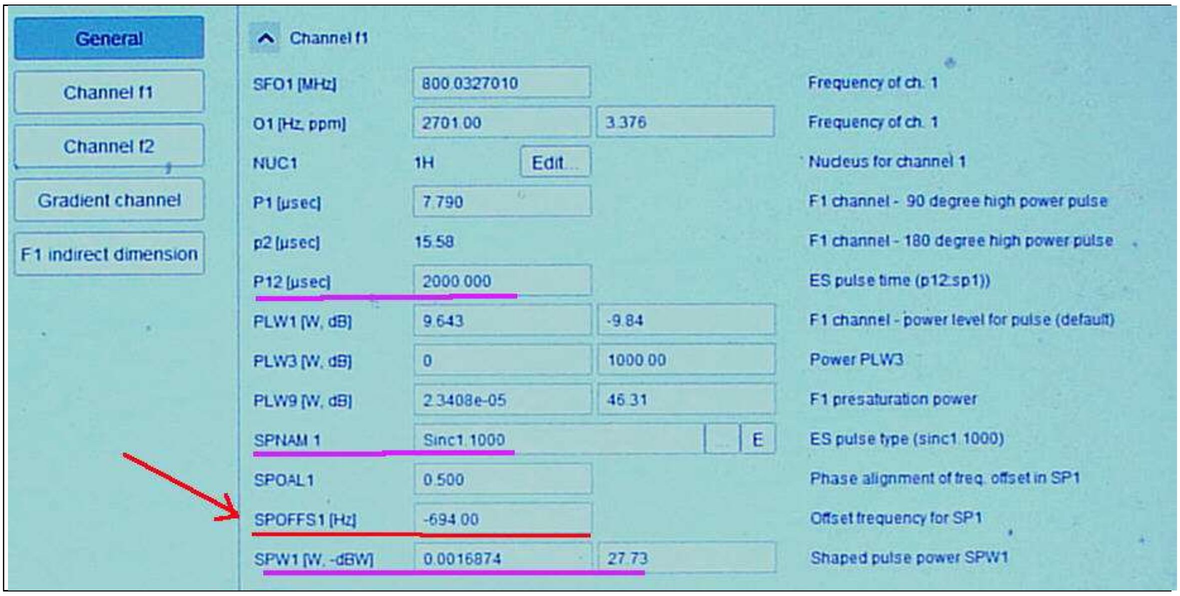
For example if, the **HOD line** occurs at **2701 Hz** and the **DMSO line** occurs at **2007 Hz**, the offset of the **DMSO line** (*to be ES suppressed*) relative to that of the **HOD line** (*PR suppressed at O1*) is calculated as:

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.