KJM 5250 and KJM 9250

SHSQC and SHMBC Experiments

Version 4.1

Topspin 4.3

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AVneo400 SHSQC and SHMBC Experiments.
(This manual is about narrow, medium and wider F1 axis SHSQC and SHMBC experiments witch can be performed without using the "selinv" command and its complexities).

1.0 Introduction

aw coded **Neo-400 SHSQC** and **SHMB**C 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 should be determined before setting up S**HSQC** or **SHMBC** experiments. 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 **shsqc135** 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.

Constant time (CT) **aw** coded **shmbcct** parameter sets are set up with **min/max ^{1}J** coupling constants of **120 Hz** and **170** Hz respectively and an **8 Hz** ^{n}J selection filter .

1.1 Processing

SHSQC45 and SHQC135 experiments are phase sensitive experiments which should be manually phased before optionally using the **abs1** and **abs2** commands. Low level ^{2}J correlations and some correlations outside the set up ^{13}C SW (F1) range may be observed in S**HSQC** spectra.

Constant time **SHMBCCT** spectra acquired in echo-antiecho mode are transformed with **xfb** *and* **xf2m**. Phasing of these spectra is not required.

The ^{13}C axis resolution of **SHSQC** and **SHMBC** spectra acquired using 128 increments and linearly processed with **512 or 1024** ^{13}C axis points is typically 3-4 times greater than that of standard full window **HSQC** and **HMBC** spectra acquired with 160-256 or more increments.

2.0 SHSQC Experiments and Parameter Sets

The following ^{1}H detected **SHSQC** experiments and parameter sets have been set up on the **Neo-400** spectrometer.

2.1	**shsqc45**	narrow window experiment
2.2	**shsqc135**	narrow window experiment
2.3	**shsqc135pr**	with PR presaturation
2.4	**shsqc135es**	with ES peak suppression

2.5	**shsqc135.m**	medium window experiment
2.6	**shsqc135pr.m**	with PR presaturation
2.7	**shsqc135es.m**	with ES peak suppression
2.8	**shsqc135espr.m**	with ES + PR peak suppression
2.9	**shsqc135.w**	wider window experiment
2.10	**shsqc135pr.w**	with PR presaturation
2.11	**shsqc135es.w**	with ES peak suppression

3.0 SHMBC Experiments and Parameter Sets

The following ^{1}H detected **SHMBC** experiments and parameter sets have been set up on the **Neo-400** spectrometer.

3.1	**shmbcct**	narrow window experiment
3.2	**shmbcctpr**	with PR presaturation
3.3	**shmbcctes**	with ES peak suppression
3.4	**shmbcct.m**	medium window experiment
3.5	**shmbcctpr.m**	with PR presaturation
3.6	**shmbcctes.m**	with ES peak suppression
3.7	**shmbcctespr.m**	with ES + PR peak suppression
3.8	**shmbcct.w**	wider window experiment
3.9	**shmbcctpr.w**	with PR presaturation
3.10	**shmbcctes.w**	with ES peak suppression

4.0 Appendix How to offset ES from O1 in ESPR experiments

2.1 SHSQC45 Spectrum

Parameter set: **awshsqc45 (+ getprosol + pulsecal)**
Pulse program: **awshsqcetgpsisp2.2-45**
d24 is automatically calculated from **cnst2**
SW(^{13}C) = **15 ppm,** excitation band width is **8-10 ppm**

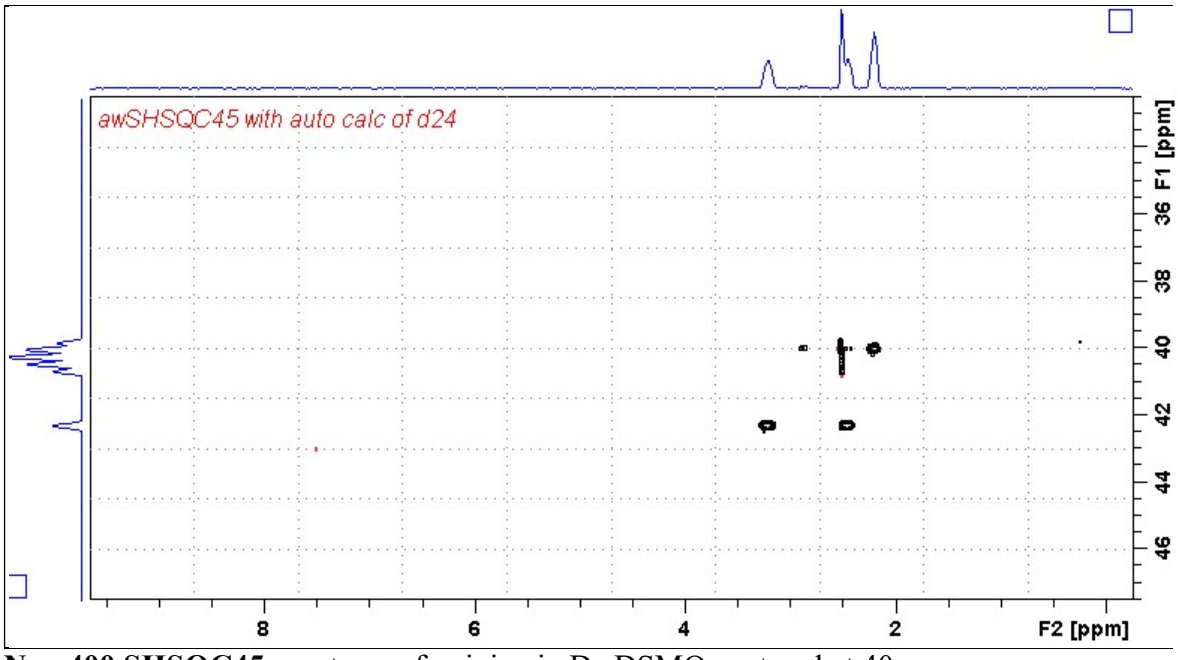
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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, including a prosol Table defined
p43:sp32 Q3_surbop.1 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 SHSQC45 spectrum of quinine in D_6-DSMO centered at 40 ppm.

2.2 SHSQC135 Spectrum

Parameter set: **awshsqc135 (+ getprosol + pulsecal)**
Pulse program: **awshsqc135**
d21 and **d24** are automatically calculated from **cnst2**
SW(^{13}C) = **15 ppm;** excitation band width is **8-10 ppm**

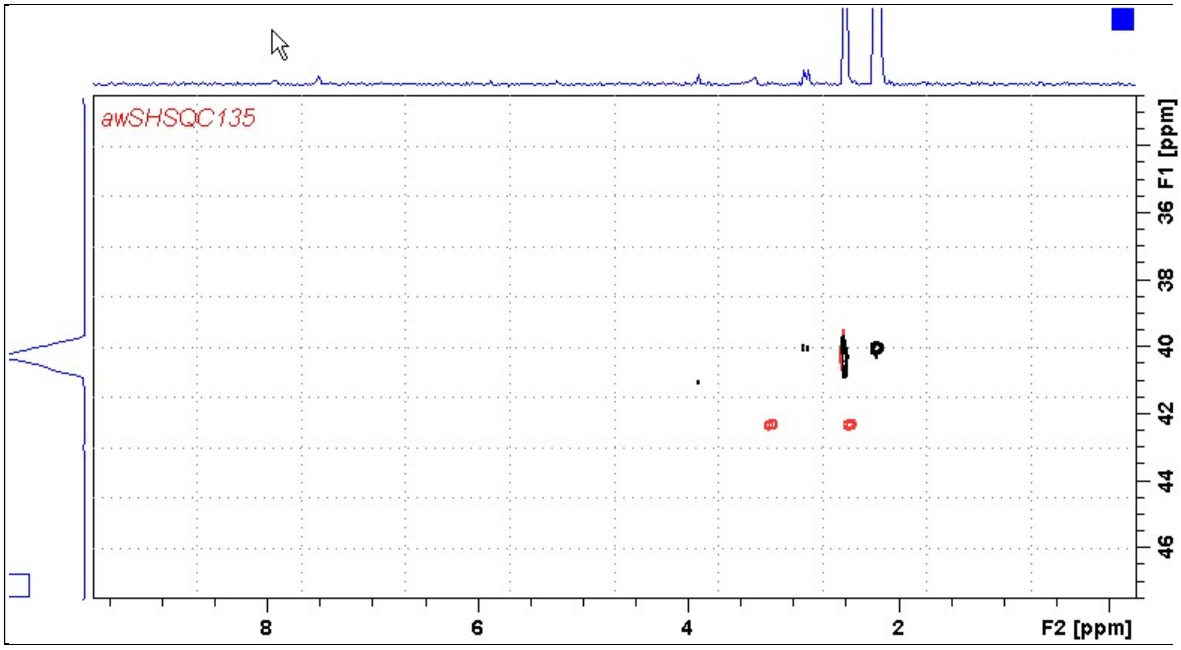
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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
p43:sp32 Q3_surbop.1 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 SHSQC135 spectrum of quinine in D_6-DSMO centered at 40 ppm. Correlations at the edges of the ^{13}C spectral window have reduced intensity**.**

2.3 SHSQC135pr Spectrum

Parameter set: **awshsqc135pr (+ getprosol + pulsecal)**
Pulse program: **awshsqc135pr**
d21 and **d24** are automatically calculated from **cnst2**
SW(^{13}C) = **15 ppm,** excitation band width is **8-10 ppm**

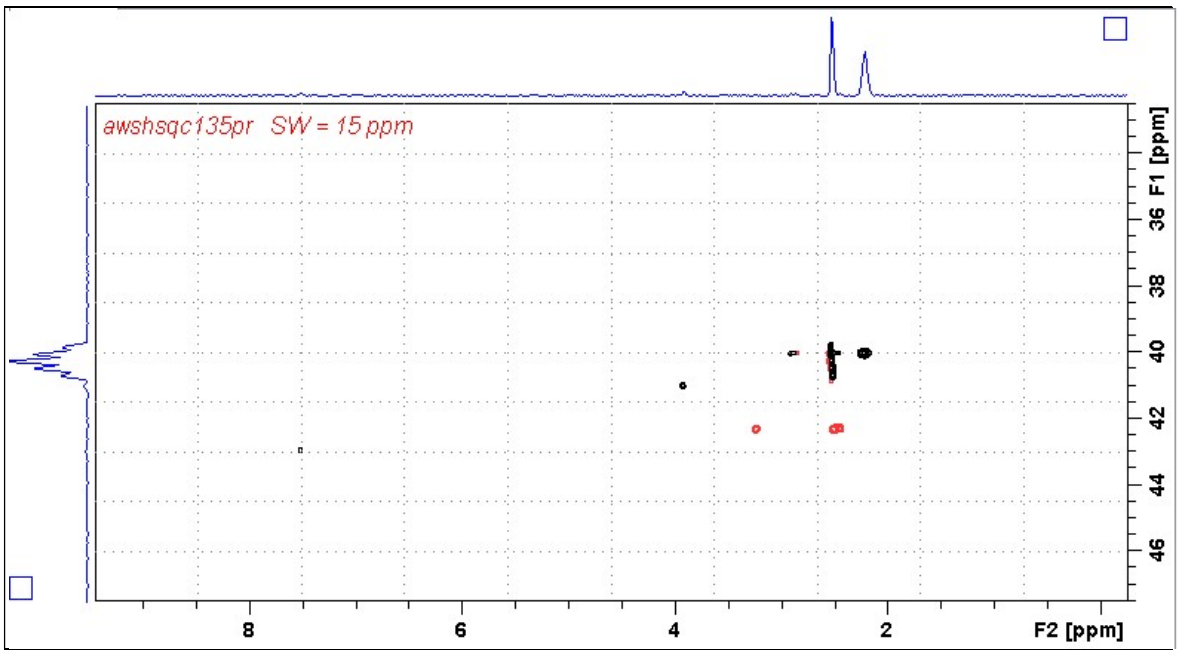
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **PR**).
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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

Check that gradients and shaped pulses are OK, including a prosol Table defined
p43:sp32 Q3_surbop.1 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 SHSQC135pr spectrum of quinine in D_6-DSMO centered at 40 ppm. Presaturation was applied at the HOD line frequency (3.35 ppm). Correlations at the edges of the ^{13}C spectral window have reduced intensity**.**

2.4 SHSQC135es Spectrum

Parameter set: **awshsqc135es (+ getprosol + pulsecal)**
Pulse program: **awshsqc135es**
d21 and **d24** are automatically calculated from **cnst2**
SW(^{13}C) = **15 ppm,** excitation band width is **8-10 ppm**

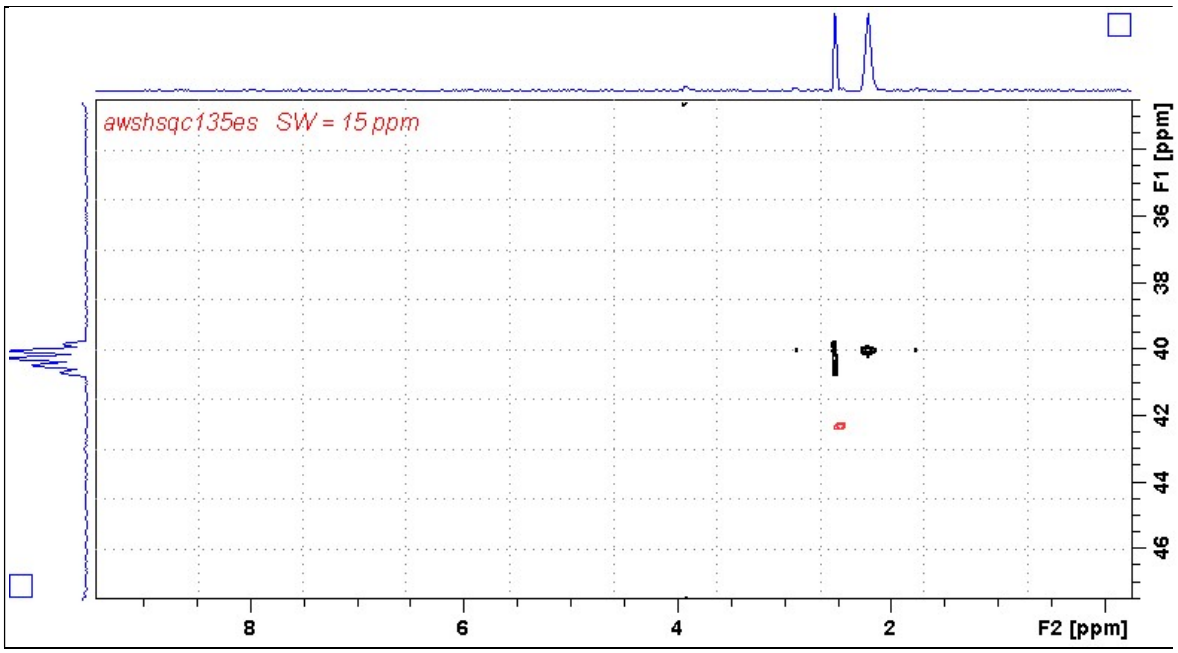
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **ES**).
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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 and a **p43:sp32 Q3_surbop.1** 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 SHSQC135es 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. Correlations at the edges of the ^{13}C spectral window have reduced intensity**.**

2.5 SHSQC135.m Spectrum

Parameter set: **awshsqc135.m (+ getprosol + pulsecal)**
Pulse program: **awshsqc135.m**
d21 and **d24** are automatically calculated from **cnst2**
SW(^{13}C) = **40 ppm;** excitation band width is ~ **30-34 ppm**

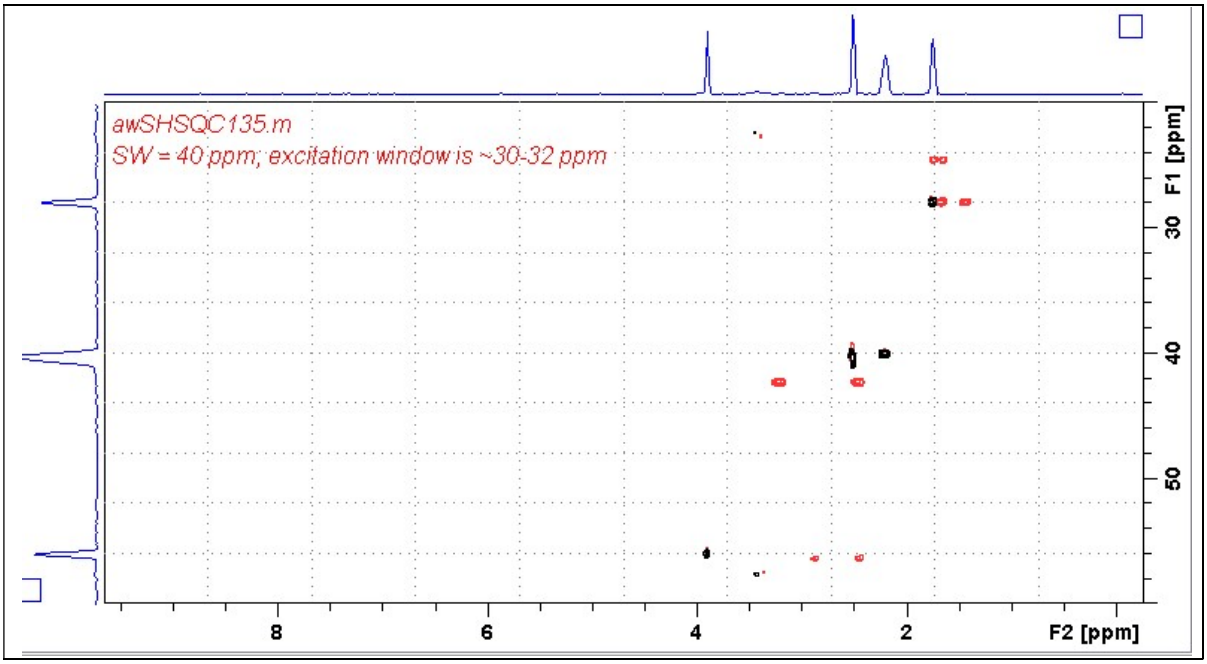
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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
p24:sp16 Q3_surbop.1 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 SHSQC135.m spectrum of quinine in D_6-DSMO centered at 40 ppm.
Correlations at the edges of the ^{13}C spectral window have reduced intensity.

2.6 SHSQC135pr.m Spectrum

Parameter set: **awshsqc135pr.m (+ getprosol + pulsecal)**
Pulse program: **awshsqc135pr.m**
d21 and **d24** are automatically calculated from **cnst2**
SW(^{13}C) = **40 ppm;** excitation band width is **30-34 ppm**

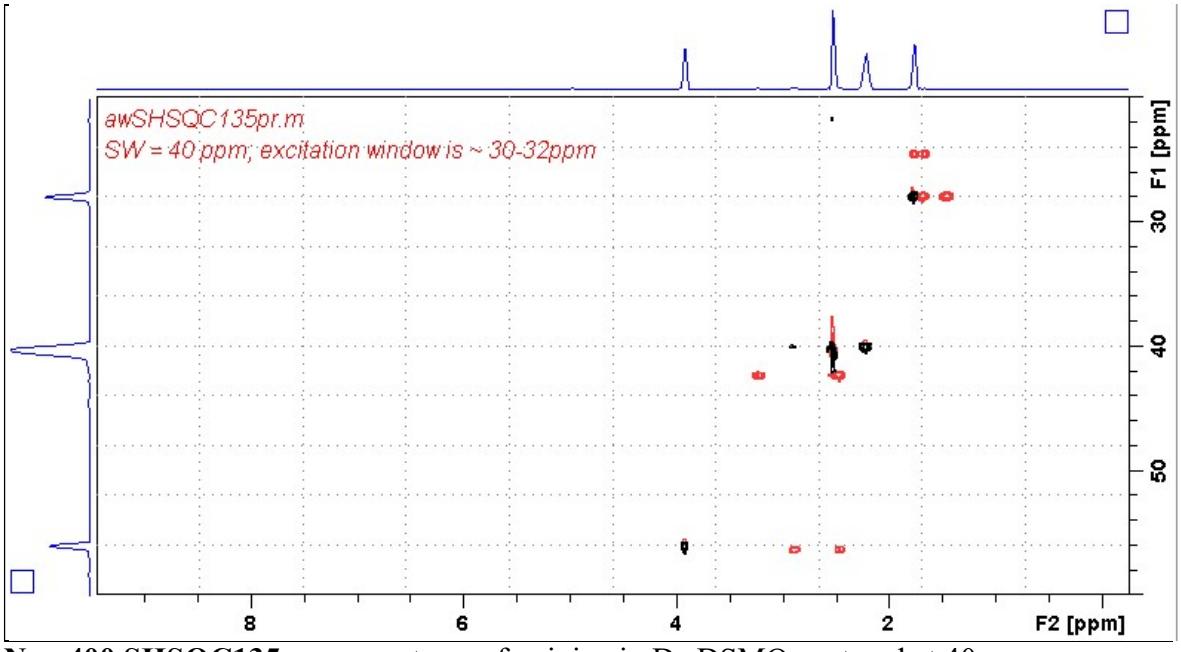
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **PR**).
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time 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.
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 **p24:sp16 Q3_surbop.1** 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 SHSQC135pr.m spectrum of quinine in D_6-DSMO centered at 40 ppm. Presaturation was applied at the HOD line frequency (3.35 ppm). Correlations at the edges of the ^{13}C spectral window have reduced intensity**.**

2.7 SHSQC135es.m Spectrum

Parameter set: **awshsqc135es.m (+ getprosol + pulsecal)**
Pulse program: **awshsqc135es.m**
d21 and **d24** are automatically calculated from **cnst2**
SW(^{13}C) = **40 ppm;** excitation band width is **30-34 ppm**

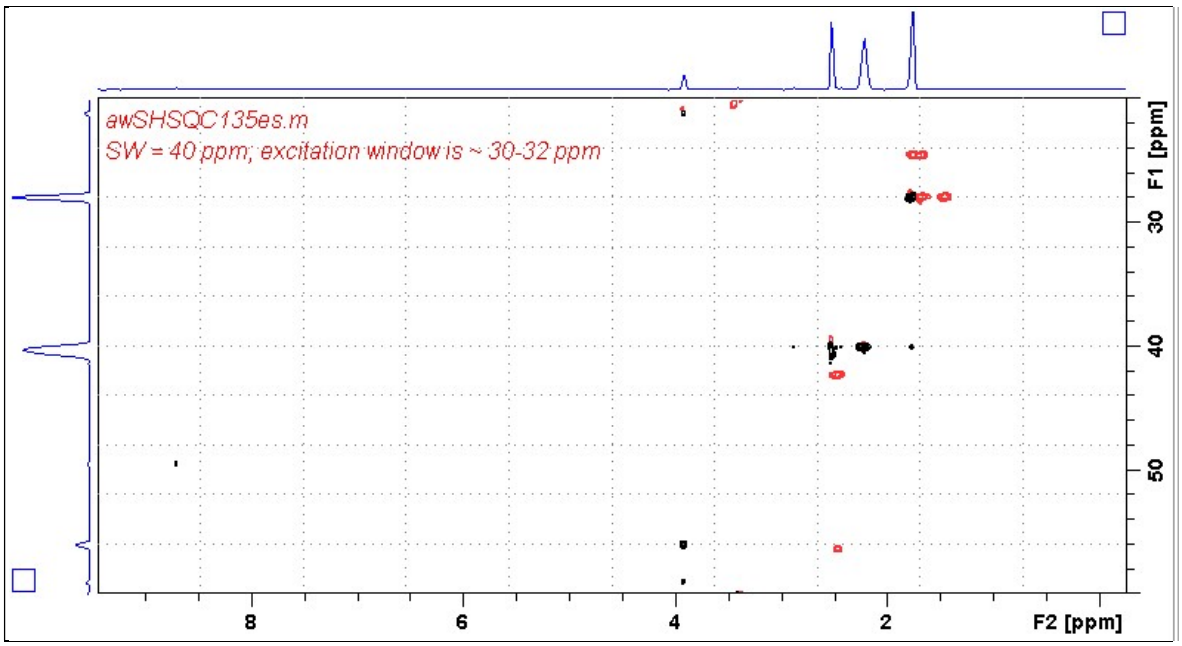
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **ES**).
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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 p12:sp1 Sinc1.1000 ES pulse and a **p24:sp16 Q3_surbop.1** 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 SHSQC135es.m 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. Correlations at the edges of the ^{13}C spectral window have reduced intensity**.**

2.8 SHSQC135espr.m Spectrum

Parameter set: **awshsqc135espr.m (+ getprosol + pulsecal)**
Pulse program: **awshsqc135espr.m**
d21 and **d24** are automatically calculated from **cnst2**
SW(^{13}C) = 40 ppm; excitation band width is **30-34 ppm**

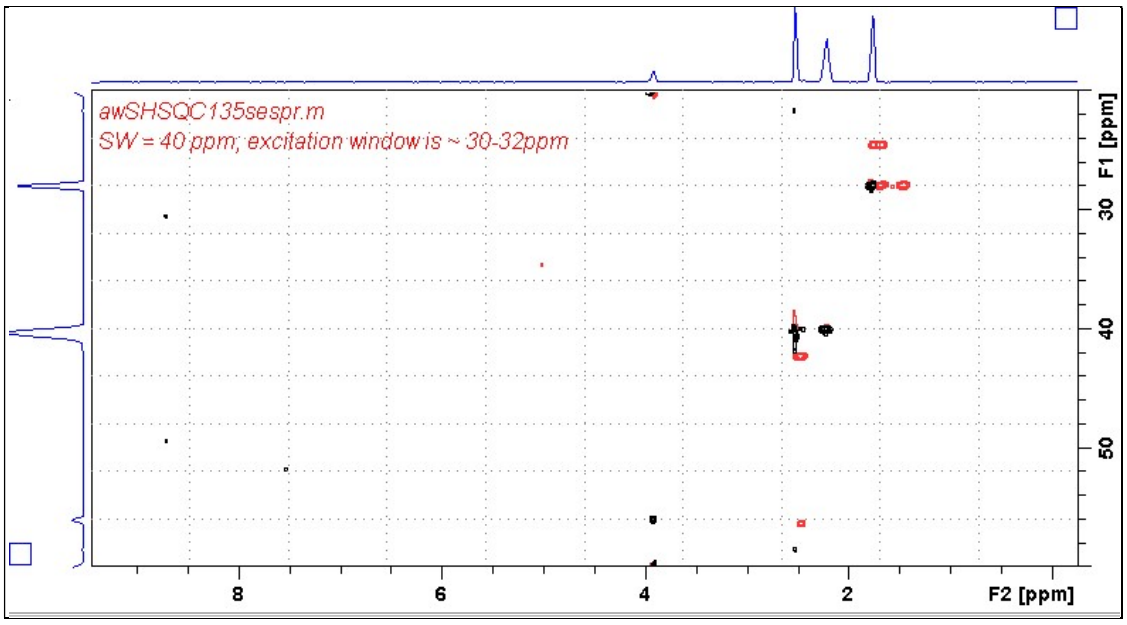
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **ES** + **PR**).
ES can optionally be offset from **PR** as described in the Appendix.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1)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

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 p12:sp1 Sinc1.1000 ES** pulse and a **p24:sp16 Q3_surbop.1** pulse.
ES can optionally be offset from **PR** at **O1** as described in the Appendix.

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 SHSQC135espr.m spectrum of quinine in D_6-DSMO centered at 40 ppm. **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. Correlations at the edges of the ^{13}C spectral window have reduced intensity**.**

2.9 SHSQC135.w Spectrum

Parameter set: **awshsqc135.w (+ getprosol + pulsecal)**
Pulse program: **awshsqc135.w**
d21 and **d24** are automatically calculated from **cnst2**
SW(^{13}C) = **60 ppm;** excitation band width is **45-50 ppm**

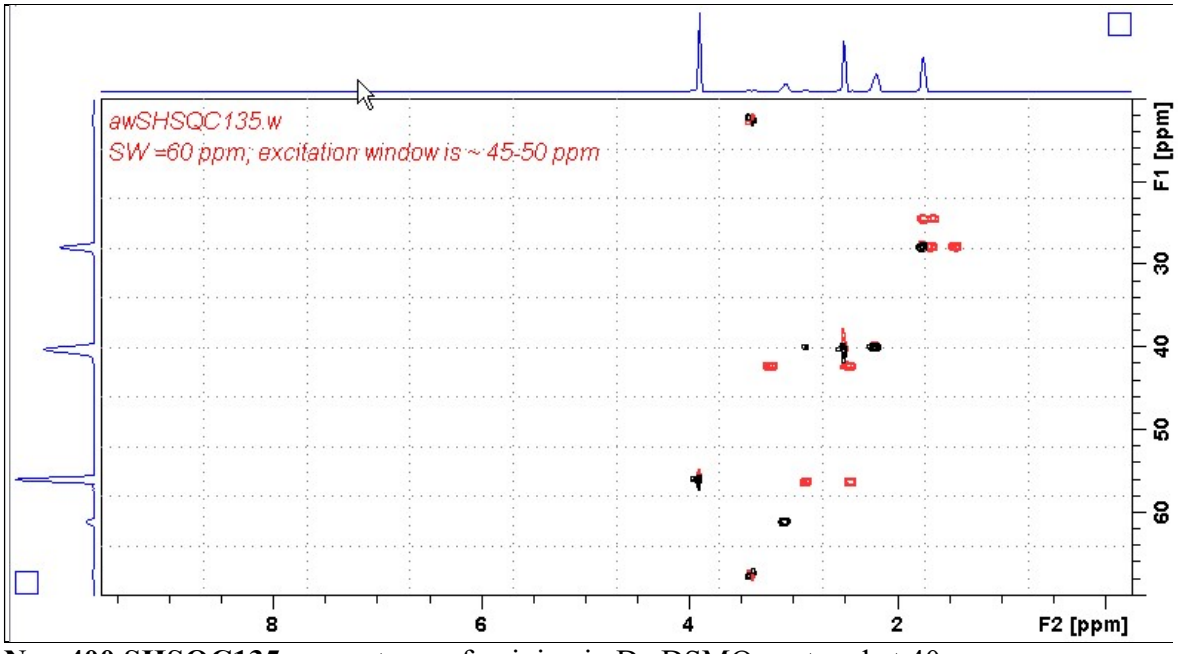
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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
p33:sp23 Q3_surbop.1 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 SHSQC135.w spectrum of quinine in D_6-DSMO centered at 40 ppm.
Correlations at the edges of the ^{13}C spectral window have reduced intensity.

2.10 SHSQC135pr.w Spectrum

Parameter set: **awshsqc135pr.w (+ getprosol + pulsecal)**
Pulse program: **awshsqc135pr.w**
d21 and **d24** are automatically calculated from **cnst2**
SW(^{13}C) = **60 ppm;** excitation band width is **45-50 ppm**

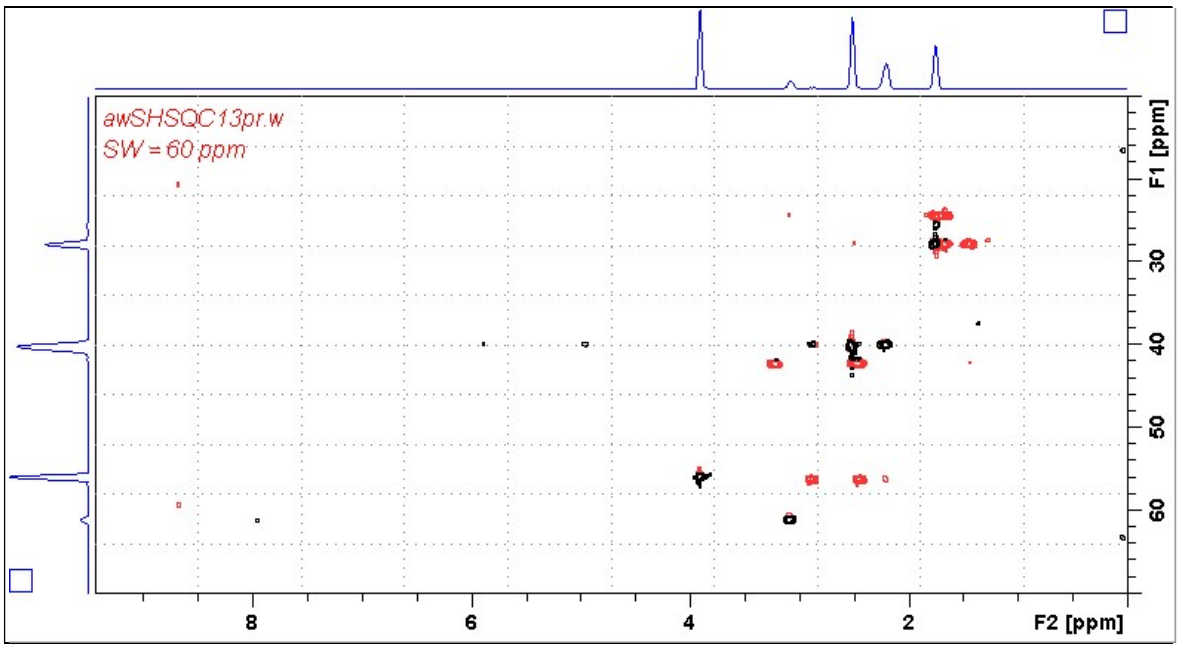
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **PR**).
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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

Check that gradients and shaped pulses are OK, including a prosol Table defined **p33:sp323** Q3 type 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 SHSQC135pr.w spectrum of quinine in D_6-DSMO centered at 40 ppm. Presaturation was applied at the HOD line frequency (3.35 ppm). Correlations at the edges of the ^{13}C spectral window have reduced intensity.

2.11 SHSQC135es.w Spectrum

Parameter set: **awshsqc135esw (+ getprosol + pulsecal)**
Pulse program: **awshsqc135es.w**
d21 and **d24** are automatically calculated from **cnst2**
SW(^{13}C) = **60 ppm;** excitation band width is **45-50 ppm**

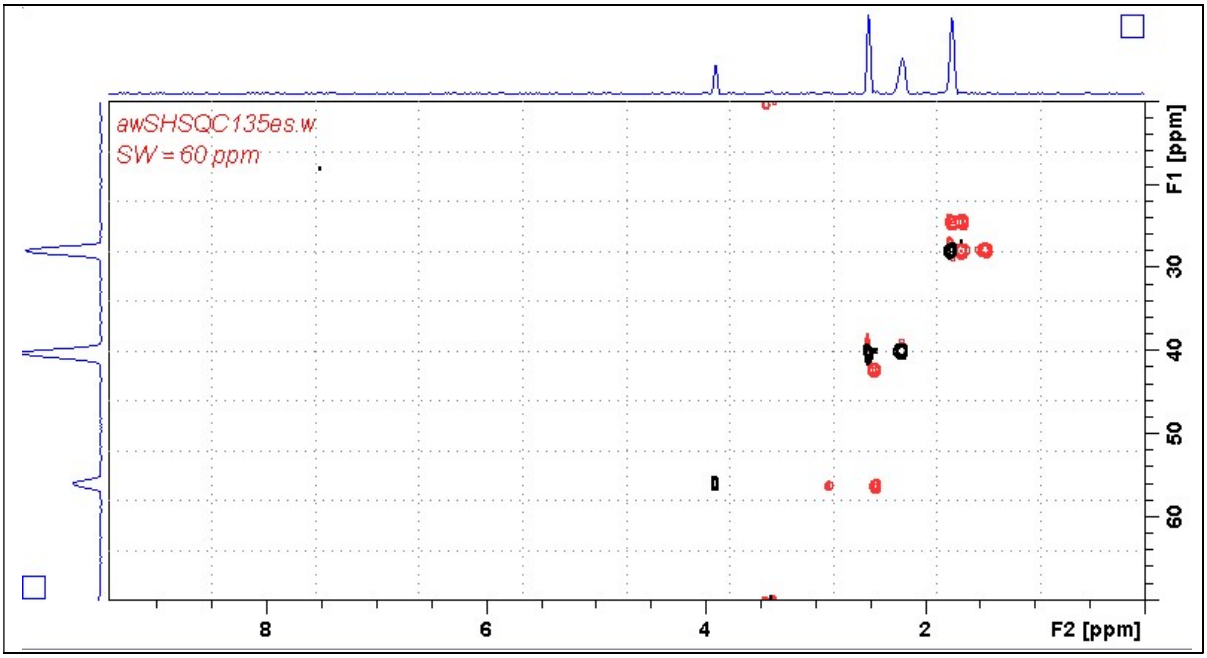
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **ES**).
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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 p12:sp1 Sinc1.1000 ES pulse and a **p33:sp23 Q3_surbop.1** 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 SHSQC135es.w 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. Correlations at the edges of the ^{13}C spectral window have reduced intensity**.**

3.1 SHMBCCT Spectrum

Parameter set: **awshmbcct (+ getprosol + pulsecal)**
Pulse program: **shmbcctetgpl2nd**
SW(^{13}C) = **15 ppm,** excitation band width is **8-10 ppm**

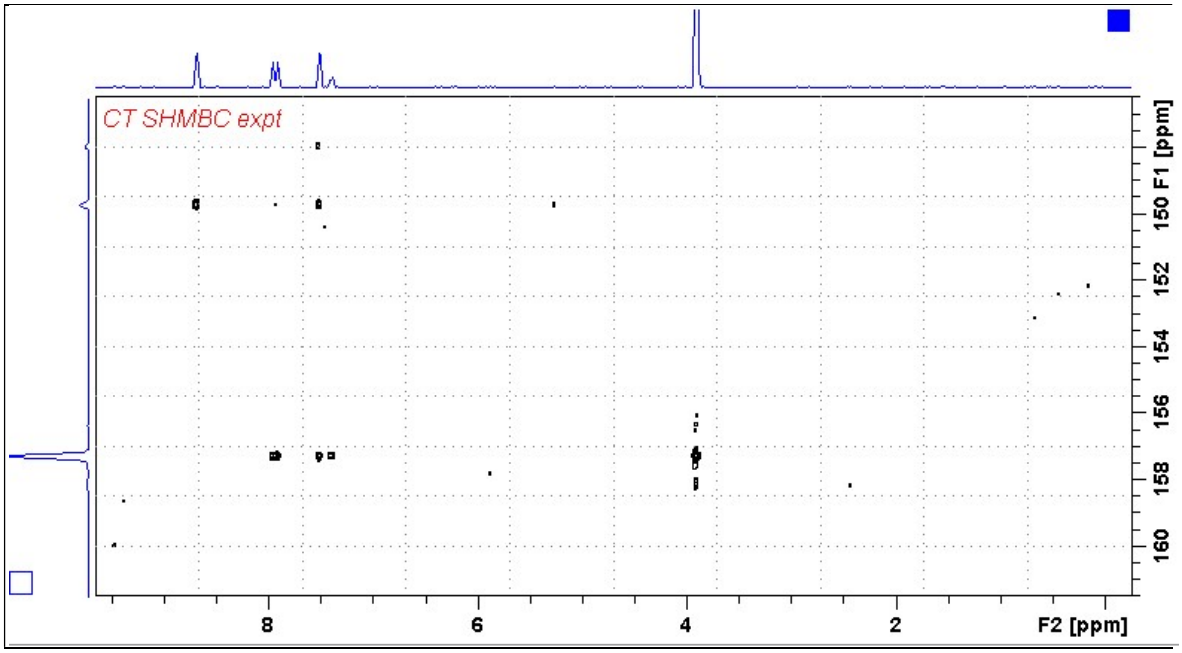
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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 **p43:sp32 Q3_surbop.1** 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 SHMBCCT spectrum of quinine in D_6-DMSO centered at 154 ppm.
Correlations at the edges of the ^{13}C spectral window have reduced intensity**.**

3.2 SHMBCCTpr Spectrum

Parameter set: **awshmbcctpr (+ getprosol + pulsecal)**
Pulse program: **awshmbcctpr**
SW(^{13}C) = **15 ppm,** excitation band width is **8-10 ppm**

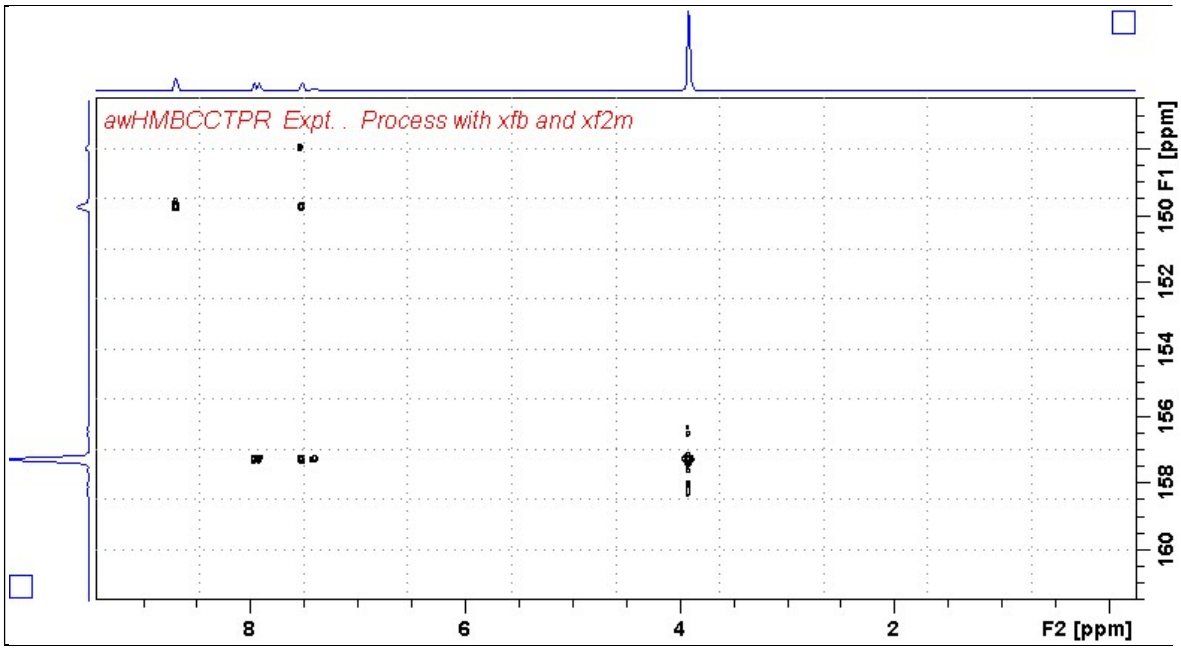
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **PR**).
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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.

Check that gradients and shaped pulses are OK, including a prosol Table defined
p43:sp32 Q3_surbop.1 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 SHMBCCTpr spectrum of quinine in D_6-DMSO centered at 154 ppm. Presaturation was applied at the HOD line frequency (3.35 ppm). Correlations at the edges of the ^{13}C spectral window have reduced intensity.

3.3 SHMBCCTes Spectrum

Parameter set: **awshmbcctes (+ getprosol + pulsecal)**
Pulse program: **awshmbcctes**
SW(^{13}C) = **15 ppm,** excitation band width is **8-10 ppm**

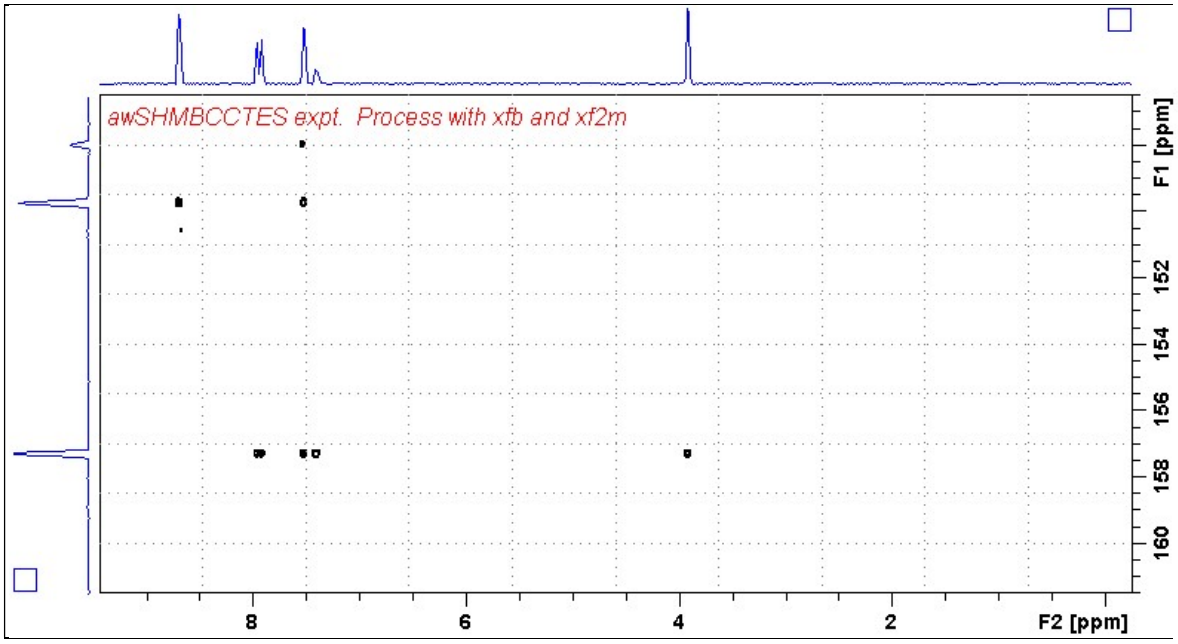
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **ES**).
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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 and a **p43:sp32 Q3_surbop.1** 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 SHMBCCTes spectrum of quinine in D_6-DSMO centered at 154 .. **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. Correlations at the edges of the ^{13}C spectral window have reduced intensity**.**

3.4 SHMBCCT.m Spectrum

Parameter set: **awshmbcct.m (+ getprosol + pulsecal)**
Pulse program: **awshmbcct.m**
SW(^{13}C) = **40 ppm,** excitation band width is **28-32 ppm**

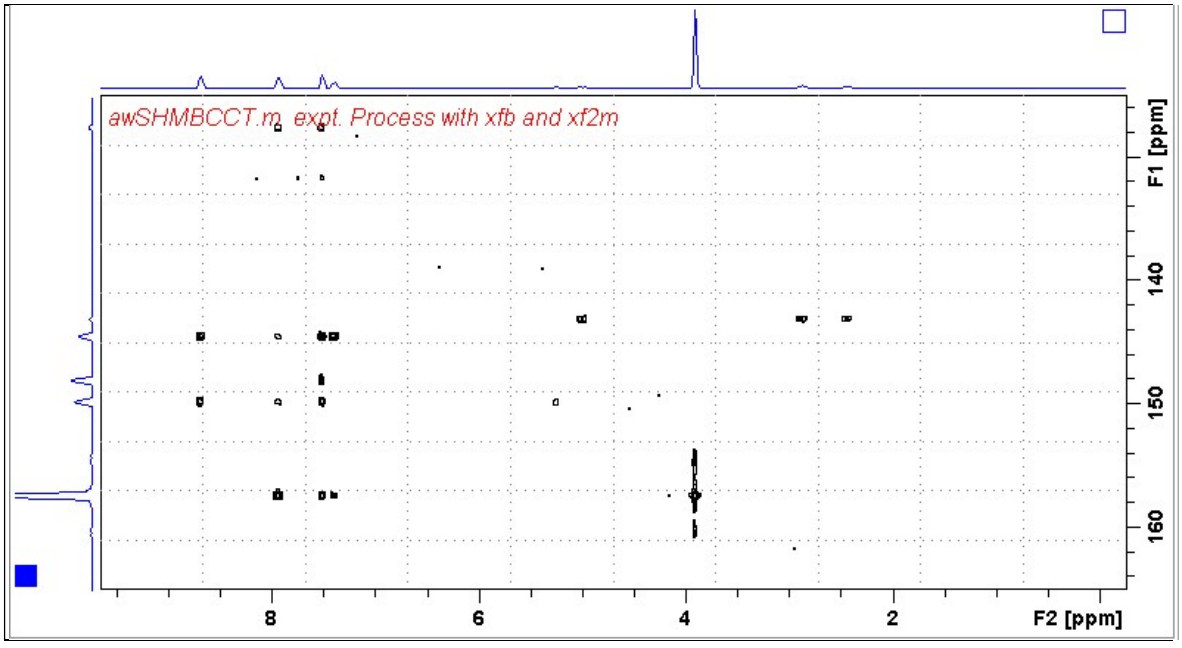
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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 **p24:sp16 Q3_surbop.1** 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 SHSQCCT.m spectrum of quinine in D_6-DSMO centered at 145 ppm.
Correlations at the edges of the ^{13}C spectral window have reduced intensity**.**
SHMBCCTPR (Constant time mode SHMBC experiment)

3.5 SHMBCCTpr.m Spectrum

Parameter set: **awshmbcctpr.m (+ getprosol + pulsecal)**
Pulse program: **awshmbcctpr.m**
SW(^{13}C) = **40 ppm,** excitation band width is **30-32 ppm.**

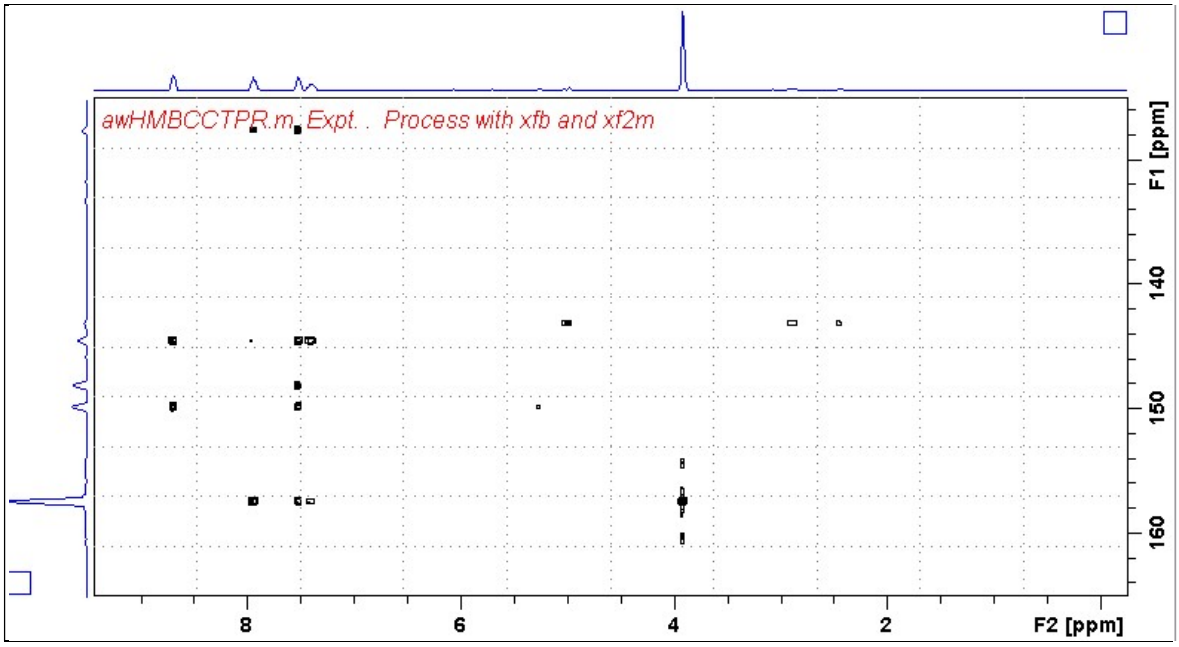
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **PR**).
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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 **p24:sp16 Q3_surbop.1** 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 SHMBCCTpr.m spectrum of quinine in D_6-DSMO centered at 145 ppm. Presaturation was applied at the HOD line frequency (3.35 ppm). Correlations at the edges of the ^{13}C spectral window have reduced intensity.

3.6 SHMBCCTes.m Spectrum

Parameter set: **awshmbcctes.m (+ getprosol + pulsecal)**
Pulse program: **awshmbcctes.m**
SW(^{13}C) = **40 ppm,** excitation band width is **28-32 ppm**

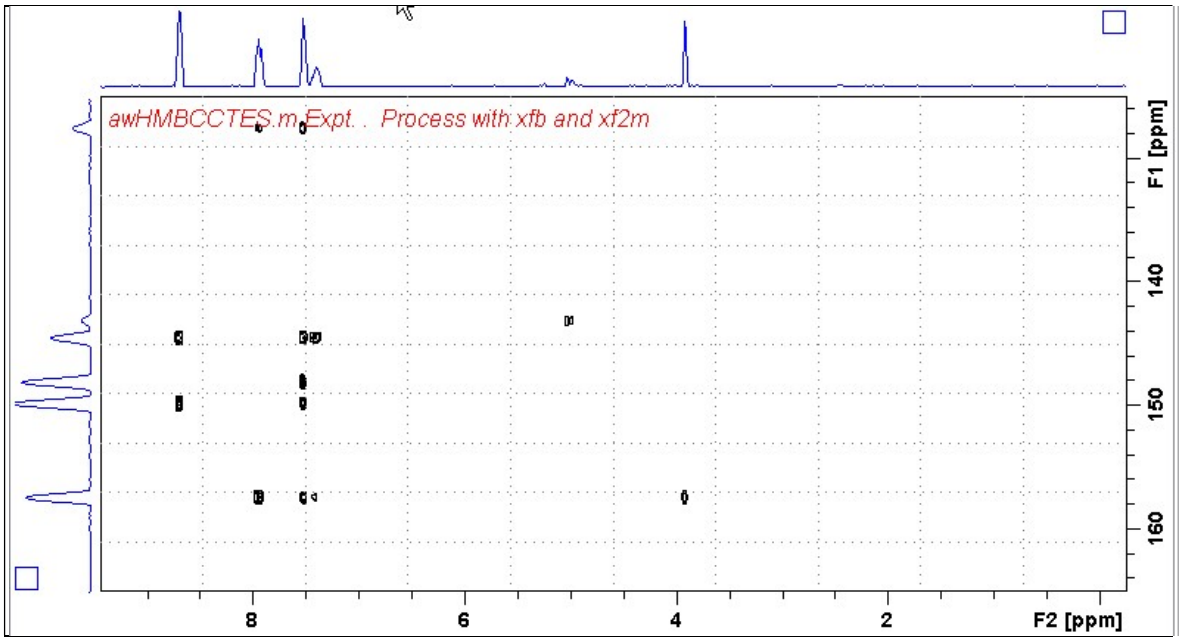
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **ES**).
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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 p12:sp1 Sinc1.1000 ES pulse and a **p24:sp16 Q3_surbop.1** 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 SHMBCCTes.m spectrum of quinine in D_6-DSMO centered at 145 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. Correlations at the edges of the ^{13}C spectral window have reduced intensity**.**

3.7 SHMBCCTespr.m Spectrum

Parameter set: **awshmbcctwespr.w (+ getprosol + pulsecal)**
Pulse program: **awshmbcctespr.w**
SW(^{13}C) = **40 ppm,** excitation band width is **30-32 ppm**

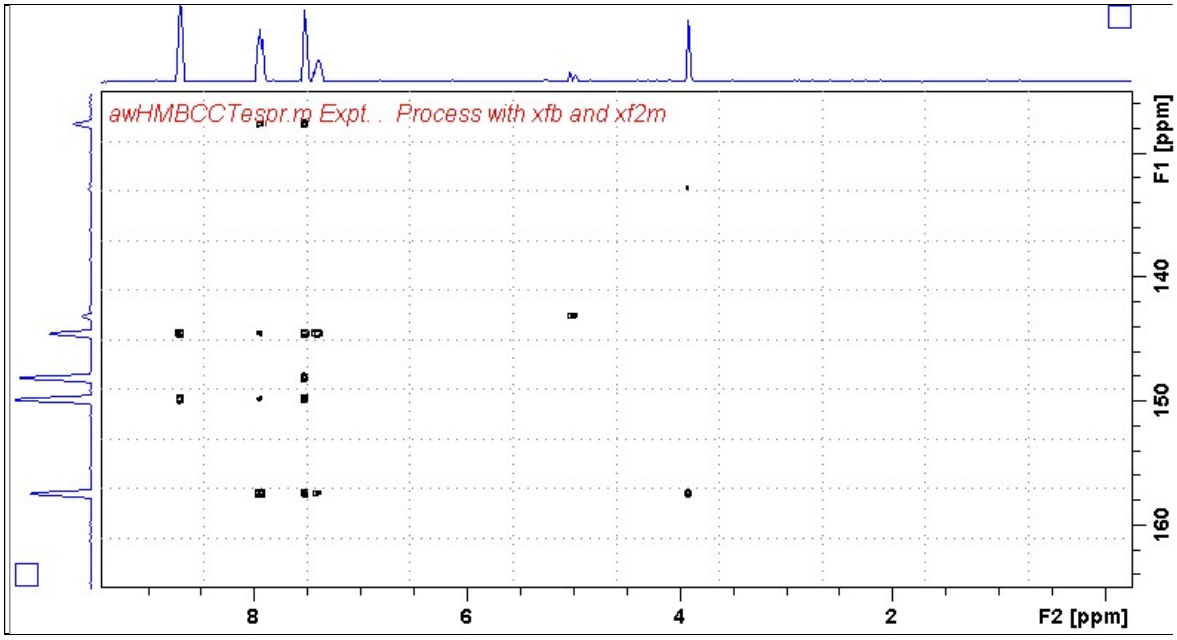
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **ES +PR**).
ES can optionally be offset from **PR** as described in the Appendix.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) =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

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 p12:sp1 Sinc1.1000 ES** pulse and a **p24:sp16 Q3_surbop.1** pulse.
ES can optionally be offset from **PR** at **O1** as described in the Appendix.

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

3.8 SHMBCCT.w Spectrum

Parameter set: **awshmbcct.w (+ getprosol + pulsecal)**
Pulse program: **awshmbcct.w**
SW(^{13}C) = **60 ppm,** excitation band width is **45-50 ppm**

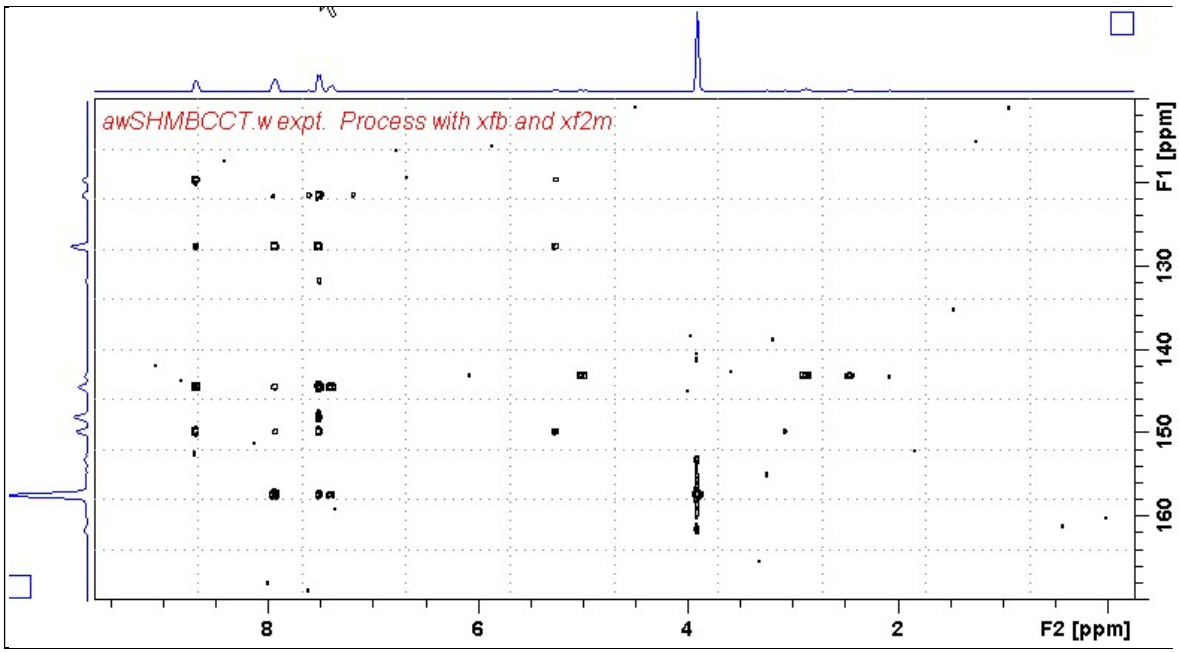
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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
p33:sp23 Q3_surbop.1 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 SHMBCCT.w spectrum of quinine in D_6-DSMO centered at 140 ppm.
Correlations at the edges of the ^{13}C spectral window have reduced intensity**.**

3.9 SHMBCCTpr.w Spectrum

Parameter set: **awshmbcctpr.w (+ getprosol + pulsecal)**
Pulse program: **awshmbcctpr.w**
SW(^{13}C) = 60 ppm, excitation band width is **45-50 ppm**

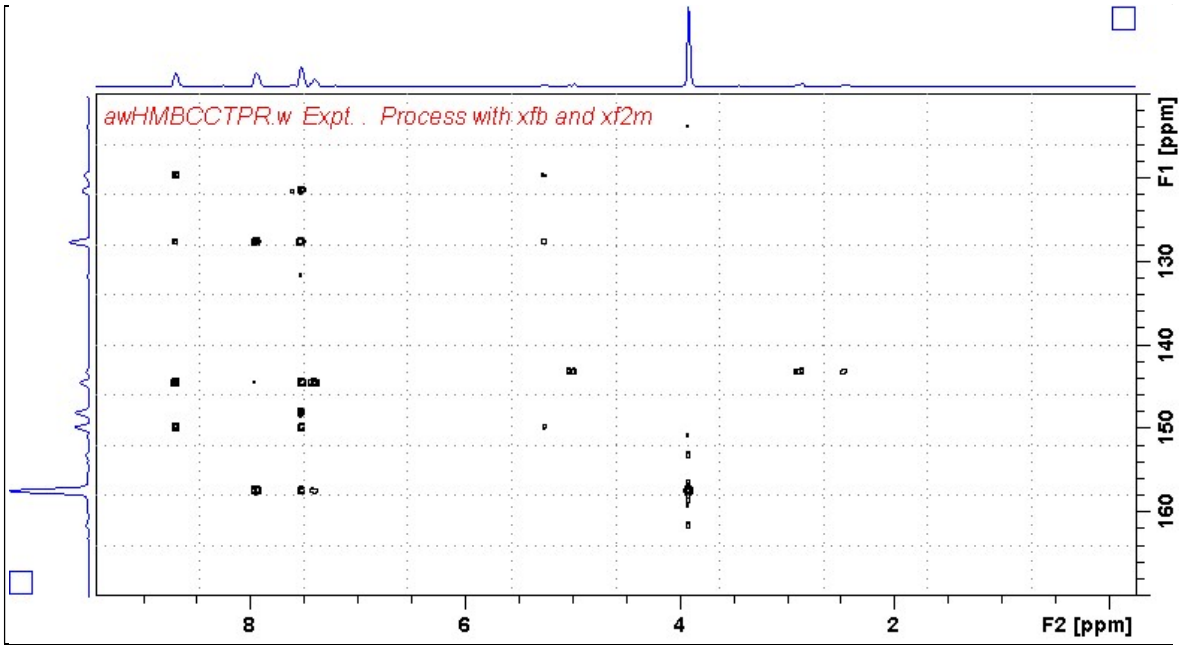
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **PR**).
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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 **p33:sp23 Q3_surbop.1** 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 SHMBCCTpr.w spectrum of quinine in D_6-DSMO centered at 140 ppm. Presaturation was applied at the HOD line frequency (3.35 ppm). Correlations at the edges of the ^{13}C spectral window have reduced intensity.

3.10 SHMBCCTes.w Spectrum

Parameter set: **awshmbcctes.w (+ getprosol + pulsecal)**
Pulse program: **awshmbcctes.w**
SW(^{13}C) = 60 ppm, excitation band width is **45-50 ppm**

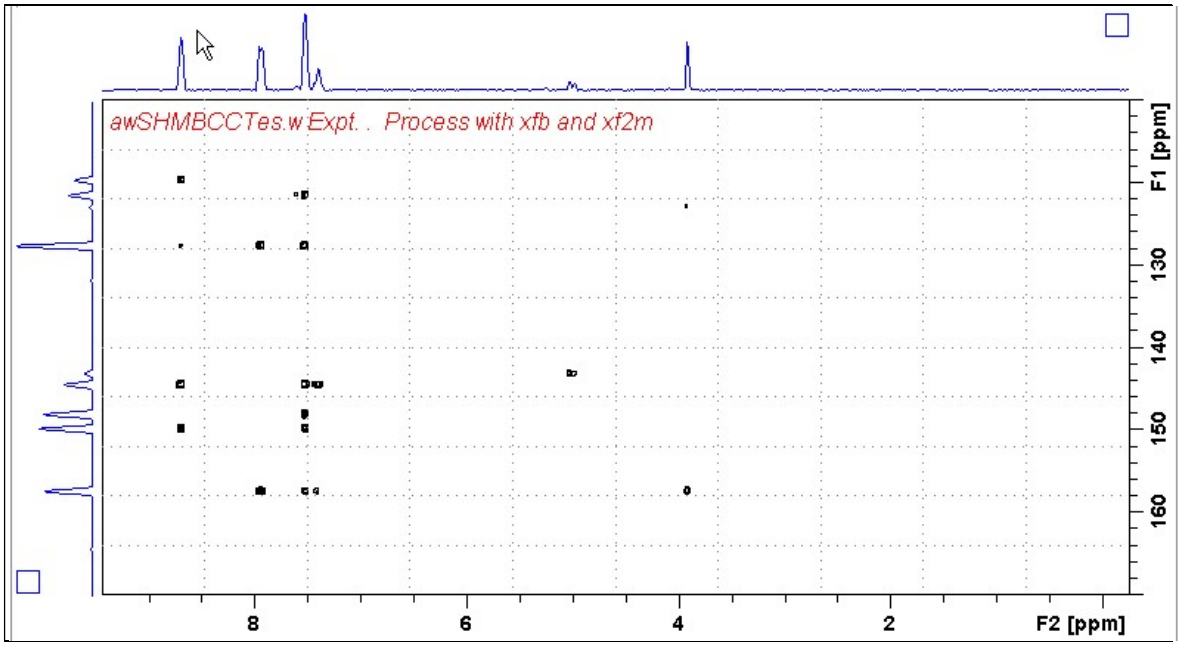
Type **eda** (enter) and enter **SW(^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** spectral window midpoint in **Hz** (for **ES**).
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, TD(F1) = 128 points.

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 p12:sp1 Sinc1.1000 ES pulse and a **p33:sp23 Q3_surbop.1** 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 SHMBCCTes.w spectrum of quinine in D_6-DSMO centered at 140 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. Correlations at the edges of the ^{13}C spectral window have reduced intensity**.**

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 **shmbc135espr.m** or **shmbcctespr.m** experiments is defined as an f1 (^{1}H) channel **2000 usec Sinc1.1000 p12:sp1** pulse.

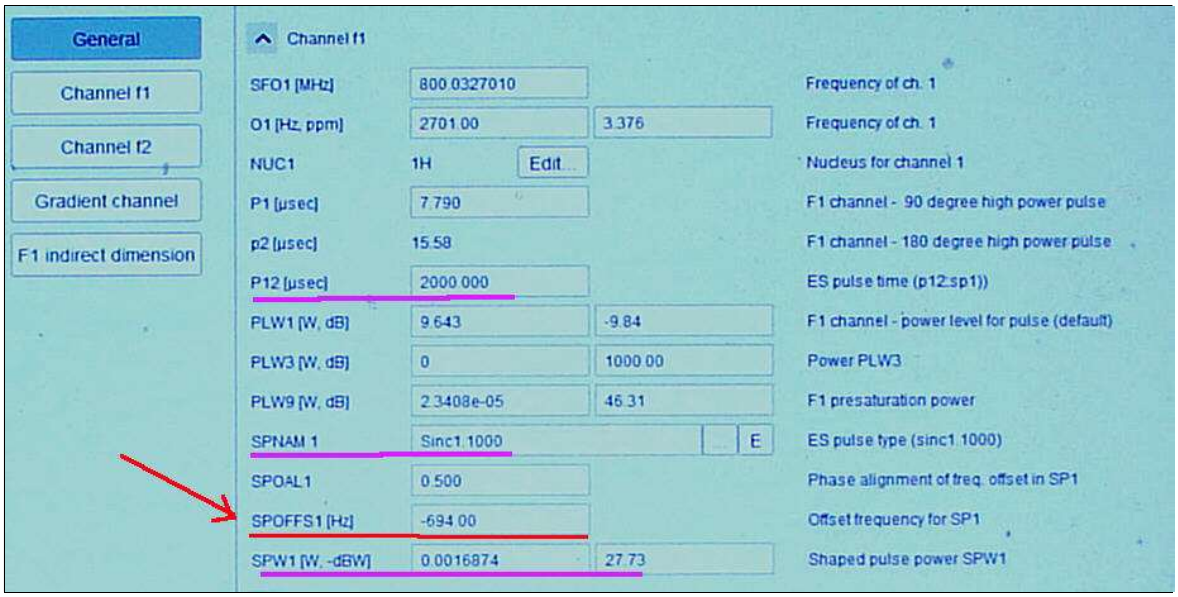
The frequency (in Hz) at which **ES** is applied can optionally be offset from **O1** (= the frequency PR is applied) so you can suppress two solvent lines by entering an **SPOFFS1(Hz)** offset value in its **ased** visible cell.

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**.



p12:sp1 (<triple> and <triple2>) **ES** pulse parameters. **The SPOFFS1(Hz)** offset value is red arrowed.

Footnote: **ES** pulses are defined as a **p12:sp1** pulses in pp's that run with *prosol relations = <triple> or <triple2>* or as **p40:sp10** pulses with *prosol relations = <default>* or *not shown/not included* as a pp line**.**