KJM 5250 and KJM 9250

HSQC135 and HMBC Experiments with Excitation Sculptured (ES) Peak Suppression on the AVneo400 spectrometer.

Version 3.1

Topspin 4.3

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AVneo400 HSQC135 and HMBC Experiments with Excitation Sculptured (ES) Peak Suppression

1.1 Spectral Window Set up

The spectral window width (in ppm), centered around the peak to be **ES** suppressed should be determined in a standard ^{1}H NMR spectrum *before* setting up **a HSQCES** or **HMBCCTES** experiment. There should be no signals within 0.5 ppm of the upper or lower limits of the spectral window. The frequency of the peak to be **ES** suppressed should be determined **in Hz.**

The **getprosol** command will read in **prosol Table** saved pulse time, powers and types into an experiment. After using the **getprosol** command the **pulsecal** and **pulsecal 13C** commands can be used to adjust the ^{1}H and ^{13}C 90 degree pulse times and **prosol Table** linked pulse powers to take account of solvent and/or buffer matrix effects which influence a samples 90 degree pulse time.

1.2 ES set up

ES is applied at **O1 Hz** = the spectral window midpoint, or it can *optionally be offset* at **O1* Hz** where **SPOFFS1** (or **SPOFFS10** in some ES pp's) = **O1*-O1 Hz.** *The use of a 2000 usec Sinc1.1000 ES pulse suppresses signals ~ ± 0.7 ppm (~ 280 Hz) either side of its frequency. This band width is 2-3 times greater than that of PR presaturation.*

1.3 Processing

HSQC135ES experiments are phase sensitive experiments which should be phased **before** using the **abs1** and **abs2** (and optional **syma**) commands.

The **HMBCES** experiment in acquired in MC (magnitude) mode and does not require phasing

The **HMBCCTES** (constant time) 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

2.7 HSQC135ES with Excitation Sculpting

Parameter set: **awhsqc135es (+ getprosol)**
Pulse programme: **awhsqcedetgpsisp2.3es**
d21 and **d24** are automatically calculated from **cnst2**

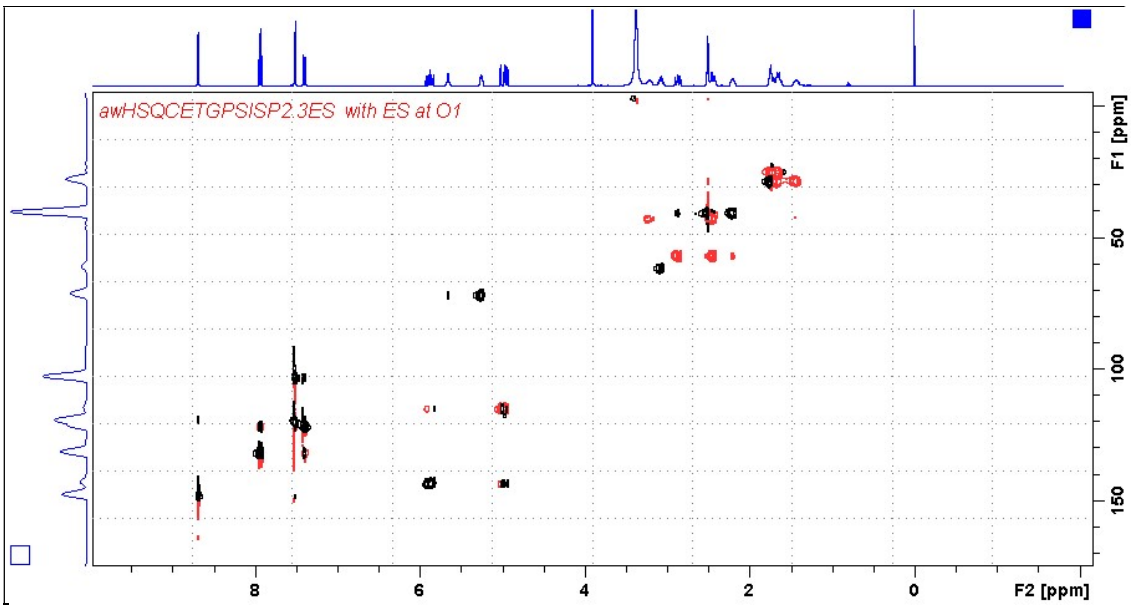
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 in Hz** of the signal to be **ES** suppressed
O1 = spectral window midpoint. Check **SW (^{1}H)** is wide enough.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 160, or 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = 1.5-**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.
Verify that a **2000 usec sinc1.1000** shaped pulse is used.
Check gradients are OK.

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

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



Neo400 HSQC135ES spectrum of quinine in D_6-DMSO with the OCH_3 signal at 3.9 ppm suppressed. If the HOD signal at 3.31 ppm was **ES** suppressed correlations located ± 0.7 ppm either side of the HOD peak would also have also suppressed.

2.8 HMBCES with Excitation Sculpting

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

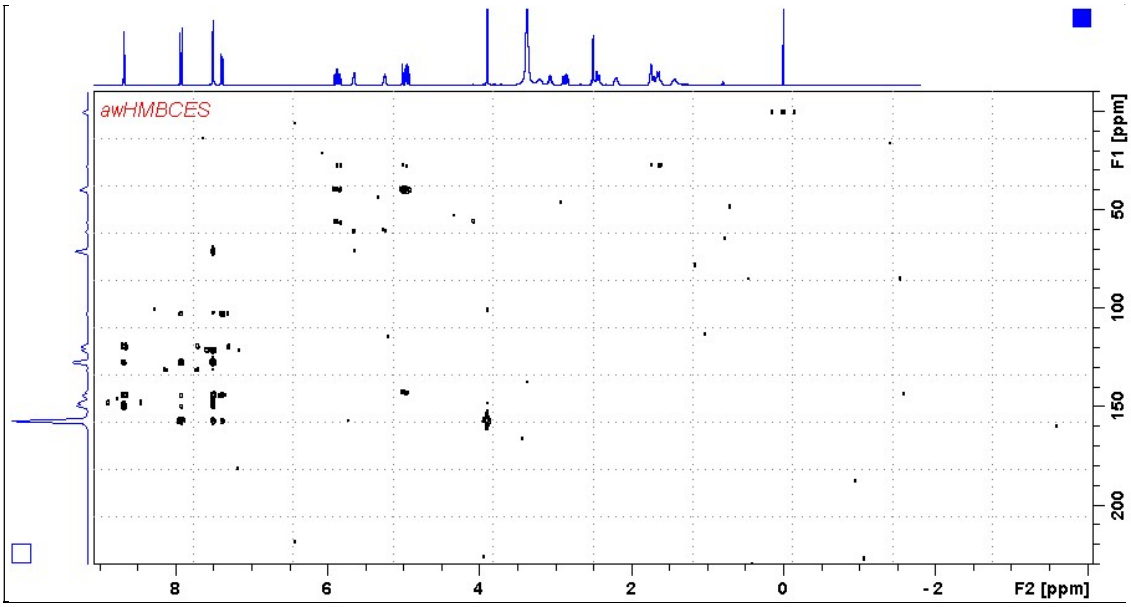
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 in Hz** of the signal to be **ES** suppressed
O1 = spectral window midpoint. Check **SW (^{1}H)** is wide enough.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 160, or 128-256 (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.
Verify that a **2000 usec sinc1.1000** shaped pulse is used.
Check gradients are OK.

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

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



Neo400 HMBCES spectrum of quinine in D6-DMSO with **ES** suppression of the DMSO signal at 2.52 ppm. Correlations located ± 0.7 ppm either side of the DMSO signal are also suppressed.

2.9 HMBCCTES with Excitation Sculpting

Parameter set: **awhmbcctes (+ getprosol)**
Pulse programme: **awhmbcctes**

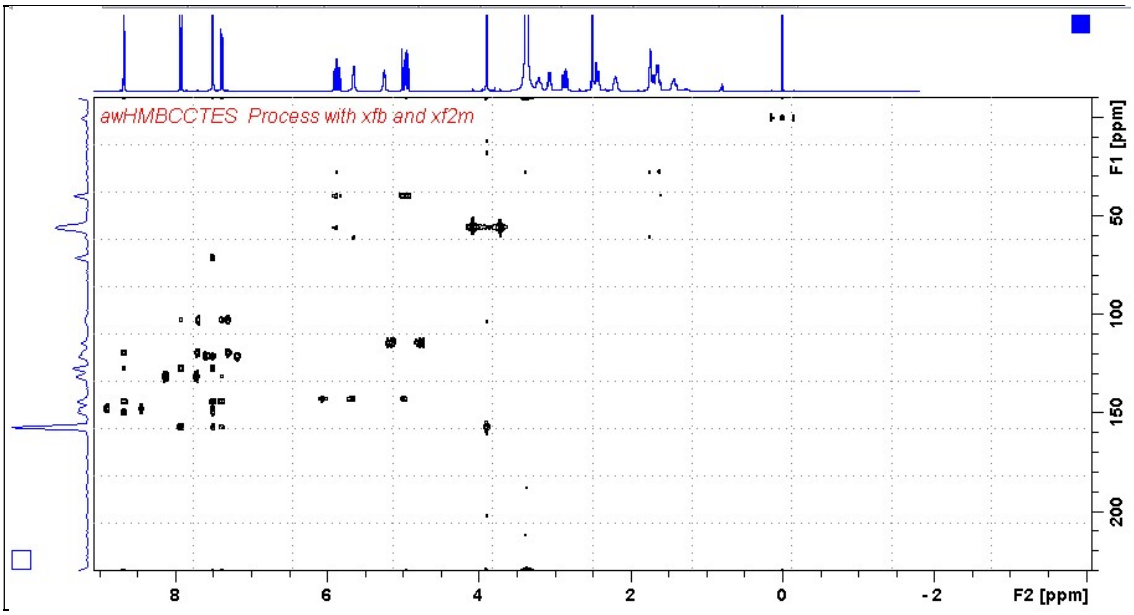
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 in Hz** of the signal to be ES suppressed
O1 = spectral window midpoint. Check **SW (^{1}H)** is wide enough.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST6 = min ^{1}J coupling constant = **120 Hz** or other value of your choice.
CNST7 = max ^{1}J coupling constant = **170 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.
Verify that a **2000 usec sinc1.1000** shaped pulse is used.
Check gradients are OK.

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

Process with: **SI(F2) = SI(F1) = 1K** or **2K**
WDW(F1) = WDW(F2) = SINE
SSB(F2) = SSB(F1) = 0
xfb, xf2m, abs1 and abs2



Neo400 HMBCCTES spectrum of quinine in D_6-DMSO with **ES** suppression of the DMSO signal at 2.52 ppm. Correlations located ± 0.7 ppm either side of the DMSO signal are also suppressed.