
KJM 9250

# $^{1}$H-$^{15}$N f3 Experiments on the AVI and AVII-600 Spectrometers

Version 4.0

Topspin 1.3 Windows XP AVI600
Topspin 2.1 Windows 7 AVII600


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## 1.1 Introduction

aw coded **$^{1}$H-$^{15}$N f3 HSQC, HSQC-DIPS**I2 and **HMBC** parameter sets on the AVI and AVII-600 spectrometers are set up with 2048 (2K) acquired and processed points acquired across a **12 ppm $^{1}$H** window centered at **4.7 ppm** and a **30 ppm $^{15}$N window (SW)** centered at **118 ppm (O3)**. The $^{1}$H and $^{15}$N NMR signals of peptides and microcystins typically occur in these windows. Different **SW** and **O3** setttings may (will) be required for other nitrogen containing compounds.

**D24** = **1/8J** (for all $NH_x$'s, x = 1 or 2) and **D26** = **1/4J** are auto calculated from **CNST4 =** $^{1}$***J*** **$^{1}$H-$^{15}$N** coupling constant = **90 Hz** in aw coded **f3 HSQC** parameter sets.

## 1.2 Processing

**f3 HSQC** and **HSQC-DIPSI2** experiments are phase sensitive experiments which should be phased *before* using the **abs1** and **abs2** commands.

**f3 HMBC** experiments are absolute value experiment. Phasing is not required.

# 2.0 Experiments and Parameter Sets

The following aw coded **$^{1}$H-$^{15}$N f3 HSQC, HSQC-DIPS**I2 and **HMBC** parameter sets are available on the **AVI-600** and AVII-600 spectrometers.

- **2.1** **$^{1}$H-$^{15}$N f3 HSQC** spectrum
- **2.2** **$^{1}$H-$^{15}$N f3 HSQC-DIPSI2** spectrum
- **2.3** **$^{1}$H-$^{15}$N f3 HMBCET** spectrum

## 2.1 $^{1}$H-$^{15}$N f3 HSQC spectrum

Parameter set: **awf3hsqc (+ getprosol)**
Pulse programme: **awf3hsqc**

Type **eda** (enter) and review the following default parameters:

**SW $^{1}$H** = 12 ppm**, SW $^{15}$N = 30** ppm (or other suitable values).
**TD $^{1}$H** = 2K, **TD $^{15}$N** = 128-160 (your choice).
**O1P = $^{1}$H** spectral window midpoint = 4.7 ppm other value of your choice.
**O3P = $^{15}$N** spectral window midpoint = 118 ppm other value of your choice.
**NS** = multiple of 8 or 16, **DS** = 16.

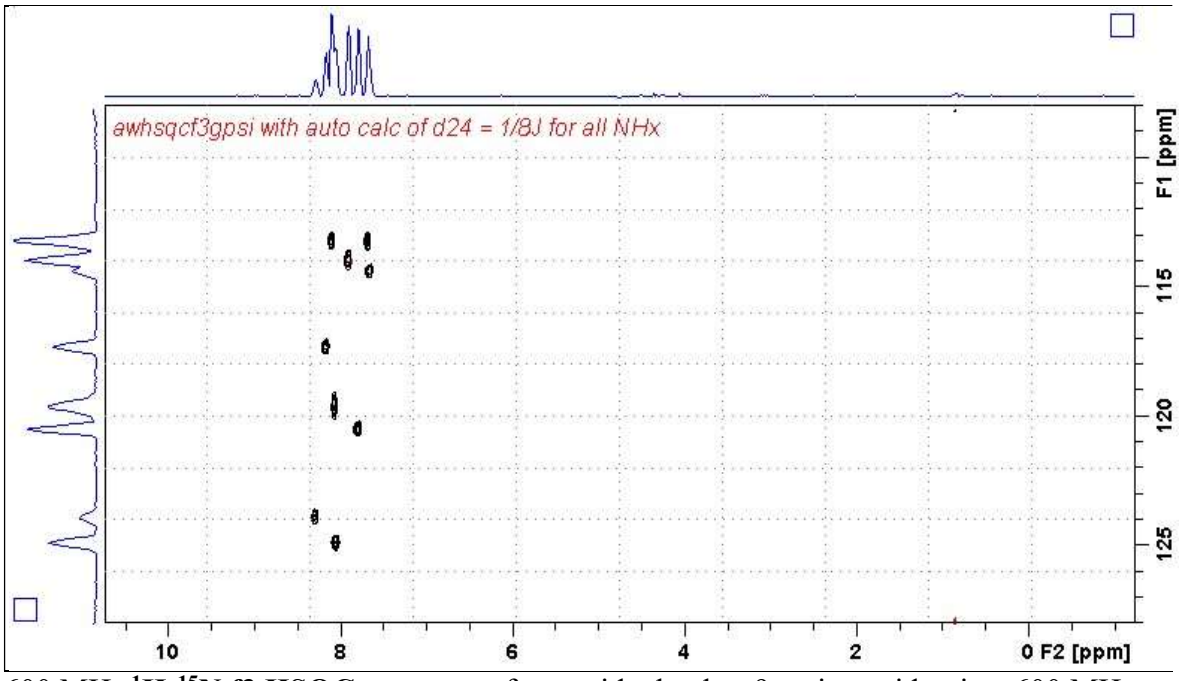
Type **ased** (enter) and review other parameters used in the job.

**D1** = repetition delay = **1.0 sec** or other time of your choice.
**CNST4 = $^{1}J$ $^{15}$N-$^{1}$H** coupling constant = **90 Hz** or other value of your choice.
**D24** = **1/8J** (for all $NH_x$'s) and **D26** = **1/4J** are auto calculated from **CNST4**
**ZGOPTNS** = Not used.

Check gradient settings are OK for $^{15}$N.

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

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




600 MHz **$^{1}$H-$^{15}$N f3 HSQC** spectrum of a peptide that has 9 amino acid units. 600 MHz

## 2.2 $^{1}$H-$^{15}$N f3 HSQC-DIPSI2 spectrum

Parameter set: **awf3hsqc-dipsi2 (+ getprosol)**
Pulse programme: **awf3hsqdietf3gpsi**

Type **eda** (enter) and review the following default parameters:

**SW $^{1}$H** = 12 ppm**, SW $^{15}$N = 30** ppm (or other suitable values).
**TD $^{1}$H** = 2K, **TD $^{15}$N** = 128-160 (your choice).
**O1P = $^{1}$H** spectral window midpoint = 4.7 ppm other value of your choice.
**O3P = $^{15}$N** spectral window midpoint = 118 ppm other value of your choice.
**NS** = multiple of 8 or 16, **DS** = 16.

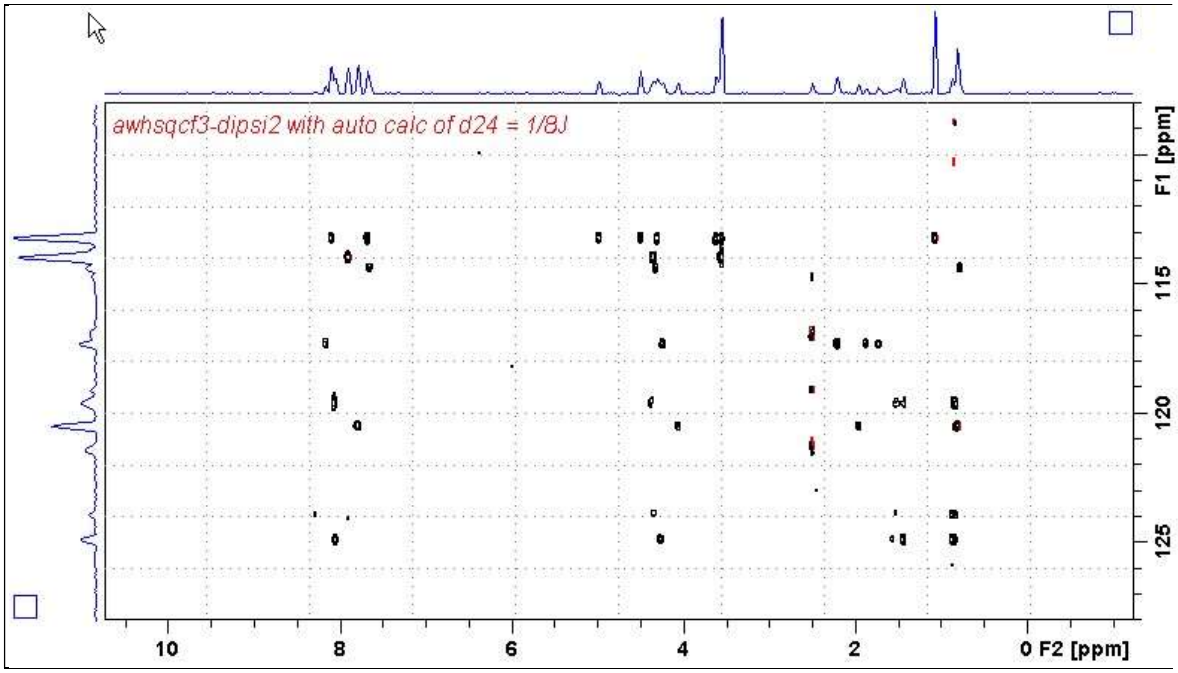
Type **ased** (enter) and review other parameters including:

**D1** = repetition delay = **1.0 sec** or other time of your choice.
**CNST4 = $^{1}J$ $^{15}$N-$^{1}$H** coupling constant = **90 Hz** or other value of your choice.
**D24** = **1/8J** (for all $NH_x$'s) and **D26** = **1/4J** are auto calculated from **CNST4**
**D9 = 80 msec** or other value of your choice (**20-160 msec**).
**ZGOPTNS** = Not used.

Check gradient settings are OK for $^{15}$N.

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

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




600 MHz **$^{1}$H-$^{15}$N f3 HSQC-DIPSI2** spectrum of a peptide that has 9 amino acid units. The spectrum was acquired with d9 = 120 msec (rather than 80 msec).

### 2.3 $^{1}$H-$^{15}$N f3 HMBCET spectrum

Parameter set: **awf3hmbcet+ getprosol)**
Pulse programme: **hmbcetf3gpnd**

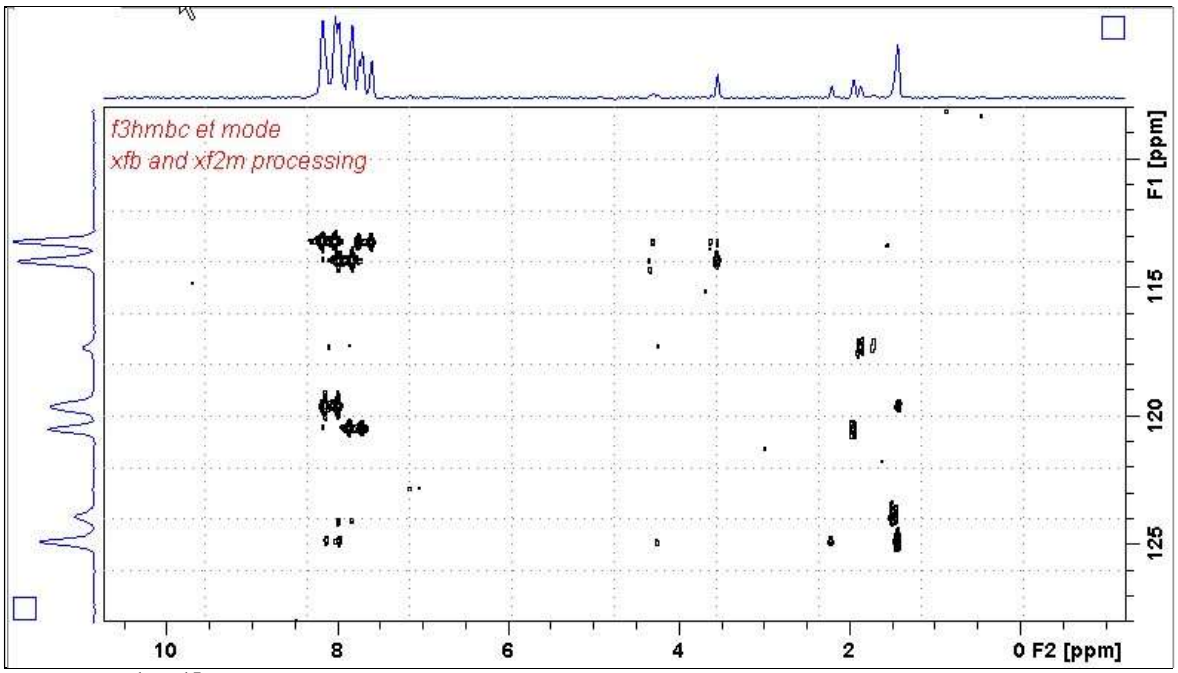
Type **eda** (enter) and review the following default parameters
**SW $^{1}$H** = 12 ppm**, SW $^{15}$N = 30** ppm (or other suitable values).
**TD $^{1}$H** = 2K, **TD $^{15}$N** = 96-160 (your choice).
**O1P = $^{1}$H** spectral window midpoint = 4.7 ppm other value of your choice.
**O3P = $^{15}$N** spectral window midpoint = 118 ppm other value of your choice.
**NS** = multiple of 8 or 16, **DS** = 16.

Type **ased** (enter) and review other parameters used in the job.
**D1** = repetition delay = **1.0 sec** or other time of your choice.
**CNST4 = $^{1}J$ $^{15}$N-$^{1}$H** coupling constant = **90 Hz** or other value of your choice.
**CNST13 = 6 Hz**

**ZGOPTNS** = Not used.
Check gradient settings are OK for $^{15}$N.

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

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



600 MHz **$^{1}$H-$^{15}$N f3 HMBCET** spectrum of a peptide that has 9 amino acid units

## 3.0 Appendix - Some Processing Options

### 3.1 Qfil mode processing

The vertical axis noise pattern often seen in 2D spectra when spectra are run in **9:1 $H_2O$:$D_2O$** or other solvents can by suppressed by setting up the experimen with its **O1 Hz**, or **O1P ppm** value (typically ~ 4.7 ppm), to that of the **HOD** line and processing the spectrum using the following **ProcPars (edp)** settings:

**BC_MOD = qfil**
**BCFW = 0.5 ppm** or other (smaller) suppresion band width value of your choice.
**COROFFS (Hz)** can be use to offset the center of the concealed region from O1

Default values are: **BC_MOD = quad** or **no, BCFW = 0 or 1.00000, COROFFS = 0**

Baseline correction

| | | | |
|---|---|---|---|
| ABSG | 5 | 5 | Degree of polynomial for abs (0..5) |
| ABSF1 [ppm] | 1000.00000 | 1000.00000 | Left limit for absf |
| ABSF2 [ppm] | -1000.00000 | -1000.00000 | Right limit for absf, abs1, abs2 |
| BCFW [ppm] | 0.50000 | 1.00000 | Filter width for bc (sfil/qfil) |
| COROFFS [Hz] | 0 | 0 | Correction offset for BC_MOD=spol etc. |
| BC_mod | qfil | no | Fid baseline modes for em, ft, xfb,... |

### 3.2 Linear prediction and STSI processing

Provided the s/n ratio of Fourier transformed **$^{15}$N** axis data points is reasonable **linear prediction** can be applied to improve the resolution of correlations in that axis.

Fourier transform

| | | | |
|---|---|---|---|
| TDeff | 0 | 0 | Number of fid data points used by ft |
| STSR | 0 | 0 | First output point of strip transform |
| STSI | 1024 | 0 | Total number of output points of strip transform |
| ME_mod | no | LPfc | Linear prediction for ft, xfb, ... |
| NCOEF | 0 | 32 | Number of LP coefficients |
| LPBIN | 0 | 256 | Number of output points for LP |
| TDoff | 0 | 0 | Number of back-predicted points |

**F1 axis** (2nd column) **$^{15}$N settings** can be set up as:
**ME_MOD = LPfc, NCOEF = 32, LPBIN** = twice the number of acquired increments.

Default linear predicion values when they are not used are:
**ME_MOD = no, NCOEF = 0, LPBIN = 0**

**STSI** can be used to *ONLY* display spectral data to the left hand side (higher ppm side) of a selected number of processed points. If, for example, a noisy residual $H_2O$/HOD line appeared in the vicinity of **4.7 ppm** ppm in a **2048 point** processed spectrum acquired with **SW = 12 ppm** and **O1 = 6 ppm**, it would not be visible in the **6-12 ppm** region view of the spectrum that would be displayed when it was processed with **STSI = 1024 points.**

The default value of **STSI** is **0**.