Inverse 2D Heteronuclear Correlation Experiments

Step-by-step setup for inverse 2D heterocorrelation experiments:

1. Acquire a normal 1D proton spectrum in exp1.

   – Turn the spinner off, and tune both X (always first) and 1H channels of the probe.
   – Shim normally, including X and Y shims as required to achieve reasonable line shape.
   – Calibrate **pw**=**pw90** and check the setting of **gain**.
   – Set **d1** ≥ T_1 .
   – Reacquire the 1D ^1H spectrum, and optimize **sw** with ~10% of the spectrum as good baseline on both edges. Use **movesw ga↵** to effect the change. Do not exclude any solute peaks except –OH or –NH, etc.

2. **jexp2** MAIN MENU SETUP NUC,SOLV [C13 *solvent*]

   – **nt=1 ss=0 go↵ <wait for acquisition> dsx↵**

   The above will show a one-scan spectrum; if the solvent contains carbons, they will usually show, e.g., $CDCl_3$ at 77ppm, which you can then reference directly. **xref** is preferred for referencing (follow it with **rl**).

   – **movesw ga↵** ;sets up the X-nucleus 1D **sw** and **tof** which will become **sw1** and **dof** for the 2D hsqc/hmbc
   → 160p to 0p is usually ok for hsqc
   → 200p to 0p usually ok for hmbc unless keto carbons are further downfield
   → **cr=160p delta=160p** will set first range exactly (although eyeball is ok)

   This important step optimizes the sweepwidth in the indirect dimension, which optimizes the resolution for fixed experiment time; there should be ~10% baseline on either end of the spectrum, but no more if it can be avoided (err on the side of too large an **sw**, however, if you are uncertain about the range of chemical shifts).

3. **jexp3 mf(1,3) dsx** ;the ^1H spectrum is used for setting up hsqc-hmbc exp types

   Type in: **HSQCAD** (or **HSQC**) for best and **mult=2** spectra, or
   gHSQCAD (or **gHSQC**) for **mult=0 nt=1** spectra, or
   gHMBCAD (or **gHMBC)** for n-bond (long-range) data

   See the discussion at the end of this document for more about these variants.

   Answer **2** (for this example) when asked for the location of the ^{13}C experiment

   → does **sw** (exp2) → **sw1** (exp3)
   tof (exp2) → **dof** (exp3)
   and transfers referencing parameters

4. ***Important checks to perform every experiment:***

a) cables ^{1}H on observe (change needed on U500 only)
^{13}C on decouple (change needed on U500 only)
^{13}C filter in-line (the low-pass filter will not work!)

b) check **nt=2×j** ;**nt=2** is minimum for **HSQC**, **nt=1** is ok for **gHSQC** if mult=0)
;**nt=8** is the recommended minimum phase cycle for ***hmbc***

check **ni** ;**dres1=sw1/(2×ni)** need enough to resolve ^{13}C chem shifts

check **d1 ≥ 1×T_1** for ^{1}H of interest

check **mult** → = 0 all peaks are positive
= 2 $-CH_2-$ are inverted wrt –CH< and methyls

c) Check that the 1st row/spectrum; wait until FID gets to 2 in the STATUS panel, then:

dsx dc va

If you can see proton signals, then **nt** is OK; in fact, maybe 4× larger than necessary.

Other notes:

– check experimental time **time↵** ; **nt**, **ni** and **d1** are the primary factors here

– **ff** may be needed on 1st processing (**prun**)

– **dc** will keep 1D traces from going below bottom of screen

– **"waveform timing error"** often displays upon **go**; this warning is OK, that the waveform cannot be produced exactly (but it's close enough)

5. ***Processing:***

– **prun dqcon plot2dhr** are the simplest/best methods to process, display and plot

wft2da ; to transform data manually, see the **dqcosy** in VUG for details
dconi **;**to display or DISPLAY ...
dqcon ;gives a nicer contour plot, but best done only on expansions (can be slow)
pcon page **;**simplest command for plotting (**plot2dhr** can also be used)
plot2dhr ;queried macro for plotting 2Ds with high-resolution 1Ds as traces

– 2× [type **setLP1(2*ni)**] to 4× [**setLP1(4*ni)**] linear prediction in the indirect, F1 dimension should normally be applied during final processing of hsqc/hmbc type data; will improve sensitivity and resolution of the data. [use **prun(2)** or **prun(4)**]

☞ HETEROCORRELATION 2D EXPERIMENT VARIANTS

HSQC, HSQCAD, gHSQC, and **gHSQCAD,** as well as **HMQC** and **gHMQC,** are all similar experiments, providing chemical-shift correlations between ^{1}H and ^{13}C having one-bond *J*-coupling of ~110 to 170 Hz (approx. value set by **j1xh**). **gHMBC** and **gHMBCAD** provide chemical-shift correlations using multiple-bond *J*-coupling (set by **jnxh**, typically between 2 to 25 Hz); one-bond correlations are filtered out (at size **j1xh**).

The versions starting with **g** use pulsed-field gradients (PFG) for *coherence selection*: the gradients reduce artifacts, but also reduce sensitivity by a factor of 2. Empirically, we find the higher sensitivity overcomes artifact noise in **HSQC** and **HSQCAD**, whereas artifact reduction is crucial to **gHMBC** and **gHMBCAD**; these four are the recommended experiments.

The versions ending in **AD** are relatively new experiments that improve data quality by compensating for mismatches in J-coupling (e.g., **j1xh** is unlikely to match all the 1-bond J_{CH} couplings in a sample), and by improving the X-nucleus [^{13}C] 180° pulses. Initial experiments with **HSQCAD** have demonstrated clear improvements over **HSQC** and **gHSQC**, especially for *edited* experiments (a DEPT-135 analog; when **mult**=2).

HSQCAD with **nt=2** (**mult**=0 or 2) *is the recommended 1-bond experiment*.

g**HSQC** (and **gHSQCAD**) *has a significant advantage when working with* **nt=1**, halving the time from an **nt=2** HSQC experiment. The sample concentration must of course be sufficiently high to allow good signal-to-noise with a 1-scan experiment (20mM is enough). For *edited* hsqc (with **mult=2** rather than the default **mult=0**), **nt=2** must be used, and **HSQCAD** is strongly recommended. *Edited* hsqc is analogous to a DEPT-135, in presenting $-CH_2-$ inverted with respect to $-CH<$ and $-CH_3$, with some loss in sensitivity compared to the **mult=0** experiment (which is analogous to DEPT-45).

HSQC (and **HSQCAD**) gives narrower lines than **HMQC** by removing ^{1}H–^{1}H J-couplings during the F1 (^{13}C) evolution.

HSQC is more sensitive to pulse-width errors than **HMQC**, since it contains more 180° pulses, and also gives a bit more phase distortion**. HMQC** is therefore preferred on older spectrometers (**HSQC** is preferred in our facility, even on the UNITY).

HSQC is better for high-MW (or similarly for low temperatures and/or viscous solvents) compounds since the internal times in the experiment are shorter than **HMQC**.

All six 1-bond experiments are processed with **gaussian** or **sqcosine** apodization.

All six 1-bond experiments can be processed with 2× (or 4×) linear prediction in the indirect, F1 dimension using **setLP1(2*ni)** [see also the PROCESS tab]. Linear prediction can provide significant sensitivity and resolution enhancements, but may also generate(enhance) artifacts.

gHMBCAD *is recommended for observing ^{1}H-^{13}C long-range J-couplings.*

Since delays that are based on the long-range couplings (set by **jnxh**) are large, the sequence length is kept as short as possible. More importantly, the inherent variation in long-range ^{1}H-^{13}C couplings in any compound prevents refocusing of antiphase magnetization using a single delay period. ^{13}C decoupling is therefore not possible, and all peaks will be ^{13}C-coupled in the ^{1}H (direct, F2) dimension. It is not uncommon that a **gHMBCAD** must be run twice, or even three times, to cover the range of possible couplings in a compound, e.g., with **jnxh** = 4, 9, and 16 (***not*** as an array, but in three separate experiments). The elimination of artifacts by use of gradients (PFG) in ***hmbc*** is crucial to the success of the experiment; because of this, there is no non-gradient version of **gHMBCAD**.

gHMBC is absolute value in both dimensions, and processed with **sinebell** apodization, 2× [**setLP1(2*ni)**] or 4× [**setLP1(4*ni)**] linear prediction in F1 (**proc1='lp'**; see the PROCESS tab) is recommended. [**prun** does **sinebell** by default; use **prun(2) or prun(4)** to perform 2× or 4× linear prediction]

gHMBCAD is a newer version of the experiment that improves data quality by improving the X-nucleus [^{13}C] refocusing pulse, and being phase-sensitive in F1 (remaining absolute value in F2).

2×F1 (to 4×F1) linear prediction is recommended, and is setup by typing:

setLP1(2*ni)

Apodization reset (*after* setting up lp) by typing:

sqsinebell('f2') sqcosine('f1') [use **prun** or **prun(2)**]